An Upper Miocene marine turtle from panama that preserves osteocytes with potential DNA

Edwin-Alberto Cadena, Carlos De Gracia & Diego A. Combita-Romero

View supplementary material 🗗

Published online: 28 Sep 2023.

Submit your article to this journal 🗗

View related articles 🗗

View Crossmark data 🗗

Check for updates

ARTICLE

AN UPPER MIOCENE MARINE TURTLE FROM PANAMA THAT PRESERVES OSTEOCYTES WITH POTENTIAL DNA

EDWIN-ALBERTO CADENA, [*,1,2,3] CARLOS DE GRACIA, [2,4,5] and DIEGO A. COMBITA-ROMERO [6,7]

[1]Facultad de Ciencias Naturales, Grupo de Investigación Paleontología Neotropical Tradicional y Molecular (PaleoNeo), Universidad del Rosario, Bogotá, 111221, Colombia, edwin.cadena@urosario.edu.co;
[2]Smithsonian Tropical Research Institute, Box 0843–03092, Balboa, Ancon, Panama, Republic of Panama;
[3]Field Museum of Natural History, Chicago, IL, 60605, U.S.A.;
[4]Department of Palaeontology, Faculty of Earth Sciences, Geography and Astronomy, University of Vienna, Josef Holaubek Platz 2 – (UZA II) 2A323, 1090, Vienna, Austria, degraciac86@univie.ac.at;
[5]Vienna Doctoral School of Ecology and Evolution (VDSEE), University of Vienna, Djerassiplatz 1, 1030, Vienna, Austria;
[6]Departamento de Geociencias, Universidad Nacional de Colombia, Bogotá, 111221, Colombia, dacombitar@unal.edu.co;
[7]Museo Paleontológico de Villa de Leyva, Grupo de investigación en Paleobiología e Historia natural, Sede Bogotá-Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia

ABSTRACT—*Lepidochelys* is a genus of extant marine turtles that includes the critically endangered Kemp's Ridley turtle. The evolutionary history of this genus is poorly understood due to the lack of an undisputed fossil record for the group. Here we describe a partially preserved carapace from the Upper Miocene Chagres Formation of Panama, which represents the oldest fossil record of *Lepidochelys*. The specimen has rectangular, anteroposteriorly short pleural scutes, a characteristic shared with members of *Lepidochelys*. It is potentially closely related to *L. olivacea* because it shares a similar number of pleurals, but its precise taxonomic status remains uncertain. We discuss the ecological role that a marine turtle played in the paleoecosystem of the Chagres Formation. The new specimen exhibits exceptional preservation of bone sutures, sulci, sculpturing, and bone microstructure, including remains of blood vessels, collagen fibers, and osteocytes. This is the first time that a histochemical stain (DAPI) indicates preservation of a compound consistent with DNA in a fossil vertebrate outside Dinosauria. These data demonstrate the potential for DNA to persist in specimens that are both millions of years old and are from lower latitudes, which challenges traditional paradigms of biomolecular preservation.

SUPPLEMENTARY FILES—Supplementary files are available for this article for free at www.tandfonline.com/UJVP

INTRODUCTION

Lepidochelys Fitzinger 1843 is a genus of extant marine turtles that comprises two species. One of them, Kemp's Ridley turtle, is the most critically endangered of all marine turtles (Cáceres-Farias et al., 2022; Wibbels & Bevan, 2019). However, our understanding of the evolution of these turtles is hindered by a lack of undisputed fossils of the genus. Currently, the fossil record of *Lepidochelys* consists of two dentary bones from the Lower Pliocene Bone Valley Formation of Central Florida (Dodd & Morgan, 1992), the Lower Pliocene Yorktown Formation of North Carolina (Zug, 2001), and with less taxonomic certainty, a left femur from the Lower Miocene of New Zealand, referred to as ?*Lepidochelys waikatoica* Buckeridge, 1981.

Although there is no reliable and precisely calibrated molecular study on the divergences among cheloniid genera, some studies have suggested that the split between *Lepidochelys*

and *Caretta* occurred during the Early to Middle Miocene, around 20–12 million years ago (Naro-Maciel et al., 2008; Thomson et al., 2021). Furthermore, it has been suggested that the split between the two extant taxa of the genus, *L. olivacea* and *L. kempii*, occurred during the Late Miocene, approximately 7.5 million years ago (Torres-Vilaça et al., 2022), prior to the final closure of the Isthmus of Panama. It is in this region where we found a partially preserved carapace in the Chagres Formation over the present-day intertidal zone. The fossil locality is situated within the Chagres Sandstone Member and is dominated by volcaniclastic sandstone, coquinas, and intermittent mudstone horizons (Coates, 1999; Collins et al., 1996). Depending on the proxy used, this member is considered to be an outer platform–upper slope (Carrillo-Briceño et al., 2015; Collins et al., 1996; De Gracia et al., 2012) or a shallow-marine sequence (Pyenson et al., 2015; Stiles et al., 2022).

The Chagres Formation contains a diversity of fossil vertebrates, including shark teeth, large fish, cetaceans, and abundant fish otoliths and most of these fossils are from the Chagres Sandstone Member (Carrillo-Briceño et al., 2015; De Gracia et al., 2012, 2022a, b; Fierstine 1978; Perez et al., 2017;

Pyenson et al., 2015; Schwarzhans and Aguilera, 2013; Velez-Juarbe et al., 2015; Vigil & Laurito, 2014). The larger vertebrate fossils exhibit excellent external bone surface preservation, the enamel on the shark teeth shows exquisite preservation, and many fish fossils are articulated and preserved in three dimensions without crushing (De Gracia et al. 2022a, b; Fierstine 1978; Pyenson et al., 2015; Velez-Juarbe et al. 2015). The quality of macroscopic preservation of these fossils motivated us to explore, for the first time, the possible preservation of bone microstructural elements, such as cells (osteocytes) and blood vessels, in fossil turtle material preserved within the Chagres Sandstone Member. Bone and cartilage microstructural elements, including osteocytes, blood vessels, collagen fibers, osteoblasts, and chondrocytes, have been widely documented in different groups of fossil vertebrates, including dinosaurs (non-avian and avian), fishes, turtles, and sauropterygians (Bailleul et al., 2020; Cadena 2020; Schweitzer et al., 2013; Surmik et al., 2021, and references therein).

In this study, we describe a partially preserved turtle carapace found in a layer of the Chagres Sandstone Member of the Chagres Formation, which represents the oldest fossil record of *Lepidochelys*. Additionally, we discuss the evolutionary and paleoecological implications of this fossil finding, and explore the preservation of bone microstructural elements.

Institutional Abbreviations—**MUPAN-STRI**, Museo Paleontológico de Panamá, Panama City, Panama Republic; **QM**, Queensland Museum Herpetological Collection, Brisbane, Australia.

MATERIAL AND METHODS

We carefully extracted the specimen (MUPAN-STRI-39311) from the rock matrix using an air scribe and dental picks. Unfortunately, some extremely delicate impressions of the costal ribs were lost during this process. The fossil is housed in the Museo Paleontológico de Panamá (MUPAN). For close-up images of the bone surface sculpturing patterns, we used a Nikon SMZ1270 stereomicroscope coupled with a DS-Fi3 camera at the Core Lab of the Faculty of Natural Science at Universidad del Rosario. We compared the specimen with a variety of extant taxa (Supplementary Data 1 for list of extant specimens analyzed) and consulted previous literature for comparisons with fossil taxa.

For the study, we used small pieces of fresh bone ($\sim$5 mm^2), ensuring they were free of any consolidation resin used to stabilize the cracks within the specimen. We demineralized the bone pieces using 0.5 M disodium ethylenediaminetetraacetic acid (EDTA, pH 8.0 filtered-sterilized using a 0.22 μm filter) following previously used protocols (Cadena, 2020; Schweitzer et al., 2013, and references therein). We changed the EDTA every 48 hours, and after the fourth day, almost all the bone was disaggregated, allowing us to collect and mount some drops of the pellets on glass slides to be examined under transmitted light microscopy to search for bone microstructures using 40× and 60× objective lenses. We tested for the possible preservation of chemical markers consistent with deoxyribonucleic acid (DNA) in the preserved osteocytes, using 4**'**,6-diamidino-2-phenylindole, dihydrochloride (DAPI) from Thermo Fisher Scientific, following the protocols previously used for osteocytes and chondrocytes of dinosaurs (Bailleul et al., 2020; Schweitzer et al., 2013), summarized as follows: (1) we collected samples from the demineralizing solution in two 1.5 ml tubes, (2) centrifuged them at 400 relative centrifugal force (rcf) for 2 minutes and discharged the EDTA, washing them with 1× phosphate-buffered saline (PBS) three times, allowing cell pellets to form at the bottom of the tubes, (3) we removed the PBS from the tubes and added 100 μl of 300 nM DAPI, mixing the solution with the same tip that we

used to add the DAPI, (4) we covered the tubes with aluminum foil and left cells to incubate for 15 minutes in dark conditions, (5) we washed them three times using PBS, centrifuging the tubes every time as in step 2, (6) we transferred and mounted the cells in 6-well polytetrafluoroethylene 'PTFE' Printed Slides, (7) we observed and photographed the samples using a Nikon Eclipse 80i fluorescence microscope using 100× oil immersion objective at the Natural Science lab of the Universidad del Rosario, Bogotá, Colombia, (8) we used ImageJ 1.52q (Schneider et al., 2012) to merge DAPI stain fluorescent photos with the photos of the samples seen in bright-field.

GEOLOGICAL FRAMEWORK

The specimen (MUPAN-STRI 39311) comes from the F4 facies of the Chagres Sandstone Member, which is the upper unit of the Upper Miocene Chagres Formation, which is exposed on the Caribbean coast of Panama (Fig. 1A–C) (Stiles et al., 2022). This facies is a bioturbated fine-grained sandstone layer with abundant *Thalassinoides* ichnofossils. We found the fossil in a tidal flat at the South section of Piña beach (Locality 620012, Piña Sur, 9°16′20.04′′N, 80°3′20.28′′W). The fossil turtle was found very close to an articulated skull and a rostrum from a marlin billfish and 168 m southwest of the locality where the fossil cetacean *Nanokogia isthmia* was found (Velez-Juarbe et al., 2015). An abundant record of shark teeth, large fish and cetacean skeletal elements, as well as abundant fish otoliths, mollusks, trace fossils and wood remains have also been found in the Chagres Sandstone Member (Benites-Palomino et al., 2023; Carrillo-Briceño et al., 2015; De Gracia et al., 2012, 2022a, b; Fierstine 1978; Pyenson et al., 2015; Schwarzhans & Aguilera, 2013; Stiles et al., 2022, Velez-Juarbe et al., 2015).

The depth and conditions of accumulation of the Chagres Sandstone Member have been controversial. Rare Earth Elements (REE) isotopic analyses of in situ fossil marlins collected near the Piña Sur locality suggest accumulation in water between $\sim$175–300 meters in depth (Macfadden et al., 2015). Conversely, the abundance of the trace fossils *Thallasinoides* and *Ophiophaga* in the Chagres Sandstone Member has been presented as evidence to suggest shallow-marine conditions, which contrast with the deep-water accumulation hypothesized by the REE results (Stiles et al., 2022). Recent support for shallow marine conditions across the entire Chagres Formation comes from the discovery of a diverse assemblage of small-sized cetaceans (Benites-Palomino et al., 2023).

SYSTEMATIC PALEONTOLOGY

TESTUDINES Batsch 1788 sensu Joyce, Parham, Anquetin, Claude, Danilov, Iverson, Kear, Lyson, Rabi, & Sterli, (2020a)
CRYPTODIRA Cope 1868, sensu Joyce, Parham, Anquetin, Claude, Danilov, Iverson, Kear, Lyson, Rabi, & Sterli, (2020b)
CHELONIIDAE Cope 1867, sensu Joyce, Anquetin, Cadena, Claude, Danilov, Evers, Ferreira, Gentry, Georgalis, Lyson, Perez-García, Rabi, Sterli, Vitek, & Parham, (2021)
LEPIDOCHELYS Fitzinger 1843
LEPIDOCHELYS sp.
(Figs. 2, 3)

Referred Specimen—MUPAN-STRI-39311, partially preserved carapace.

Locality, Horizon, and Age—Piña beach, STRI locality 620012, 9°16′20.04′′N, 80°3′20.28′′W, Caribbean coast of
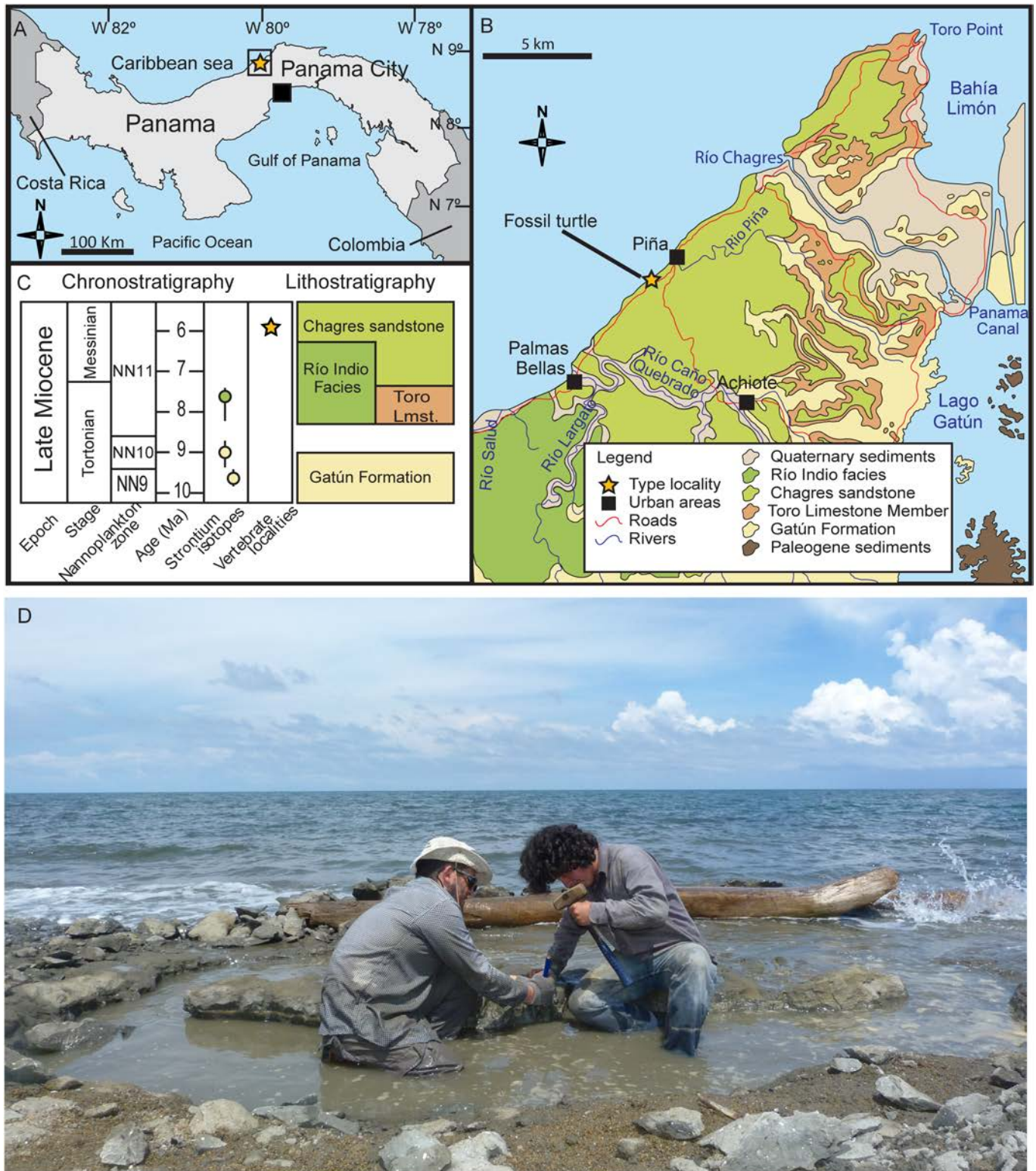
FIGURE 1. Maps, location, and stratigraphic context of the fossil. **A**, map of Panama with orange star indicates the region where *Lepidochelys* sp. (MUPAN-STRI 39311) was found; **B**, geological map and exact location where the fossil was found near the town of Piña; **C**, Upper Miocene stratigraphic units, indicating the vertebrate localities from the Chagres Sandstone Member of the Chagres Formation (modified from Pyenson et al., 2015); **D**, view of the Piña beach locality and at the Caribbean Coast of Panama. **Abbreviation**: **Lmst.**, limestone.
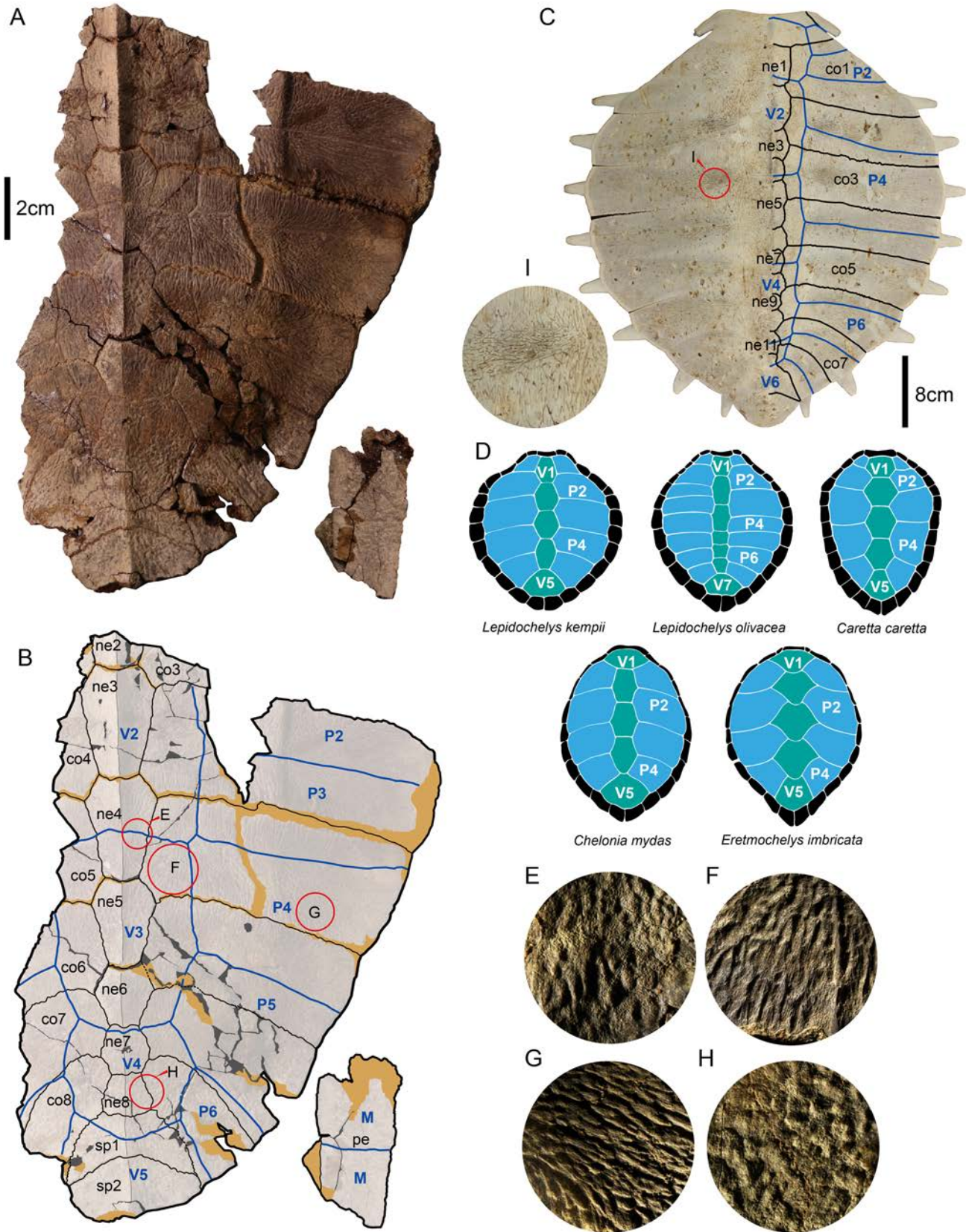
FIGURE 2. *Lepidochelys* sp. (MUPAN-STRI 39311) from the Upper Miocene of Panama, carapace views and comparisons. **A**, carapace in dorsal view; **B**, outline of MUPAN-STRI 39311 indicating sutures (black), sulci (blue), and bone surface sculpturing close-ups areas shown in (**E–H**); **C**, *Lepidochelys olivacea* (QM-J85565), carapace in dorsal view without the peripherals used for comparison with MUPAN-STRI 39311; **D**, carapace of five extant marine turtle taxa indicating the scutes number and patterns, redrawn from Wyneken (2001); **E–H**, different patterns of bone surface sculpturing in MUPAN-STRI 39311, red circles in (**B**). **I**, bone surface sculpturing in *Lepidochelys olivacea* (QM-J85565), red circle in (**C**). **Abbreviation**: **co**, costal bone; **M**, marginal scute; **ne**, neural bone; **P**, pleural scute; **pe**, peripheral bone; **sp**, suprapygal bone; **V**, vertebral scute. Scale bar for **A** and **B** equals 2 cm; for **C** equals 8 cm.

FIGURE 3. *Lepidochelys* sp. (MUPAN-STRI 39311) from the Upper Miocene of Panama in ventral view. **A**, **B**, carapace in ventral view before its extraction from rock showing the lateral costal ribs of costal bones; **C**, posterior region of the carapace in ventral view including the suprapygals 1 and 2, as well as the costals 8, showing strong costal ribs; **D**, *Lepidochelys olivacea* QM-J85565 specimen in ventral view showing similar pattern and shape of suprapygals, costals 8 and costal ribs of *Lepidochelys* sp. MUPAN-STRI 39311. **Abbreviations**: **co**, costal bone; **cor**, costal rib; **rom**, rock matrix; **sp**, suprapygal bone. Scale bar equals 2 cm.

Panama. Chagres Formation, Chagres Sandstone Member, which is Messinian, between ~6.4 and 5.8 Mya based on microfossils (Collins et al., 1996).

Description—The specimen consists of a partial carapace that preserves neural series 3–8, the most posterior portion of neural 2, suprapygals 1 and 2, right costals 5–8, the most medial portion of right costal 3, and the most medial portion of left costals 3–8, as well as one isolated peripheral, possibly from the lateral half of the carapace (Fig. 2A, B). The specimen appears to have an elliptical shape, with a maximum length of 19 cm and a maximum width of 13.3 cm as preserved, suggesting that the general shape of the shell was longer than wide. Taphonomic crushing of the carapace has reduced the height of the specimen (Fig. 2B).

Neurals 3–5 are hexagonal, longer than wide, with shorter anterolateral edges. Neurals 6 and 7 are almost perfectly hexagonal, and neural 8 is slightly trapezoidal, being wider posteriorly. Along the midline of the neurals, there is a pronounced dorso-longitudinal keel, interrupted only by the sulci between vertebral scutes (Fig. 2A, B). The right costal bones 4–6 are nearly rectangular, slightly curved at their lateral edge. The right costal 7 is longer laterally than medially, and the right costal 8 is the smallest of the preserved costals; having a curved anterior margin and contacting neurals 7, 8 and both suprapygals medially (Fig. 2A, B). There are two suprapygal bones in the specimen, the first one having a convex boomerang-shape (suprapygal 1), while the second (suprapygal 2) is trapezoidal, missing its most posterior margins. An isolated and nearly complete peripheral bone is also preserved, and is assumed to be from the carapace due to its rectangular shape and the fact that it lacks a strong indentation at the lateral contact between the marginal scutes (Fig. 2A, B).

The carapace has deep and clearly defined sulci (Fig. 2A, B). The sulci indicate that there were at least five vertebral scutes. Sulci are only recognizable for vertebrals 2–5, due to the fact that the anterior portion of the carapace is missing. All of the vertebrals are narrower than the pleurals. The sulci of vertebral 2 suggest that this scute contacts vertebral 3 posteriorly and pleurals 2–4 laterally on its right side. Vertebral 2 has an almost straight lateral margin, with only a very narrow contact with pleural 4 (Fig. 2A, B). Vertebral 3 is also much longer than wide, slightly tapered posteromedially, and contacts pleurals 4 and 5 laterally with a sulcus between vertebrals 3 and 4 located right over the sutural contact between neurals 6 and 7 (Fig. 2A, B). Vertebral 4 is the smallest and narrowest of the vertebrals and has an almost hexagonal shape with its anterior and posterior margins slightly concave. Vertebral 4 contacts pleurals 5 and 6 laterally and its contact with vertebral 5 is positioned medially over suprapygal 1 (Fig. 2A, B). Vertebral 5 is the widest of all the vertebrals as indicated by its most anterolateral sulci, and it contacts pleural 6 laterally.

The sulci between the pleural scutes suggests there are at least six pleurals, considering that in the missing portion of the carapace, there was space to accommodate another pleural (pleural 1) (Fig. 2A, B). Pleural 2 covers half of costals 3 and 4. Pleural 3 is located on top of the sutural contact between costals 4 and 5, and only contacts vertebral 3 medially. Pleural 4 is slightly longer laterally and it contacts vertebrals 3 and 4 medially. Pleural 5 covers the most posterior region of costal 6 and almost entirely costal 7 and has a medial contact with vertebrals 4 and 5. Pleural 6 is the smallest of the pleurals and it covers most of costal 8 and a narrow posterior portion of costal 7. Pleural 6 contacts vertebral 5 medially. The sulci on the preserved peripherals indicate marginal scutes cover the peripherals (Fig. 2A, B).

On its dorsal surface, the carapace exhibits a variety of sculptured patterns (Fig. 2A, E–H). The neurals and suprapygals regions have a vermicular texture (Fig. 2E, F), while each costal bone has a radial-anastomosed sculpture near the sutures between each other. This pattern changes to slightly pitted-vermicular towards its center of each bone (Fig. 2G). A different sculpturing pattern is observed on neural 8 and the medial region of the right costal 8. The sculpture on these regions consists of a moderately pitted surface that changes to slightly anastomosed at its lateral portion (Fig. 2H).

On its ventral surface, the costal bones of MUPAN-STRI-39311 lack lateral ossifications, leaving the distal ends of the ribs exposed. This condition occurs in costals 3–5 (Fig. 3A, B), and continues in the posterior costals, including costal 8 (Fig. 3C), similar to that in *Lepidochelys olivacea* QM-J85565 specimen (Fig. 3D).

Comparative Remarks—MUPAN-STRI 39311 is referable to Cheloniidae based on the following characteristics: (1) two suprapygal bones, with suprapygal 1 being slightly larger than suprapygal 2 (Fig. 2A, B) and (2) the costal bones lack lateral ossification (ontogenetically conservative), allowing the dorsal exposure of the distal end of ribs in all costal bones (Cadena & Parham, 2015) (Fig. 3A, B). It differs from *Eretmochelys*, *Natator*, and *Chelonia* genera in having more than four pleural scutes (Wyneken, 2001) (Fig. 2C, D). The only two extant genera with five or more pleural scutes are *Lepidochelys* and *Caretta*. In *Caretta*, the pleurals are longer than wide, unlike in *Lepidochelys*, where the opposite is true, resulting in proportionally wider shells (Fig. 2C, D). MUPAN-STRI 39311 most closely resembles *Lepidochelys olivacea* in having an extra pleural scutes (more than five in each side), which causes some of the adjacent costals to exhibit sulci between pleurals (Fig. 2C, D). This feature is seen in *L. olivacea* (QM-J85565; Fig. 3C, D) and other specimens of this taxon that we directly examined (Supplementary File S1).

DISCUSSION

Taxonomic Identity

Although we have pointed out the similarity between MUPAN-STRI 39311 and extant *Lepidochelys olivacea*, there are four notable differences between the two. Firstly, the fossil specimen has eight neural bones (Fig. 2B), rather than 12–15 as in *L. olivacea* (Pritchard, 1988) (Fig. 2C). Secondly, the width of all vertebral scutes is almost invariant in the fossil, whereas the posteriormost vertebral scute (vertebral 6) of *L. olivacea* is significantly wider than the others. Thirdly, pleurals 3–5 cover half of two adjacent costals in MUPAN-STRI 39311 (Fig. 2B), whereas pleurals 3 and 4 in *L. olivacea* cover three costals (Fig. 2D). Fourthly, MUPAN-STRI 39311 has great variation of sculpturing patterns, including different types of sculptures on the same bone (Fig. 2B, E–H). In contrast, the bone surface of the carapace in *L. olivacea* exhibits a more homogeneous anastomosed sculpturing pattern (Fig. 2C, I).

At first glance, these differences seem sufficient to consider MUPAN-STRI 39311 as representing a new fossil species. However, due to the absence of other preserved parts of the skeleton (such as the skull, plastron, and limb bones), we refrain from doing so. Instead, we refer MUPAN-STRI 39311 to *Lepidochelys* sp., hoping that future discoveries of more complete fossils in the Chagres Formation support this possible new taxon.

Evolutionary History of *Lepidochelys*

The oldest putative fossil record of *Lepidochelys* is an isolated left femur from the Lower Miocene of New Zealand (Buckeridge, 1981). The taxonomic placement of that specimen as belonging to *Lepidochelys* is based on similarities of this femur
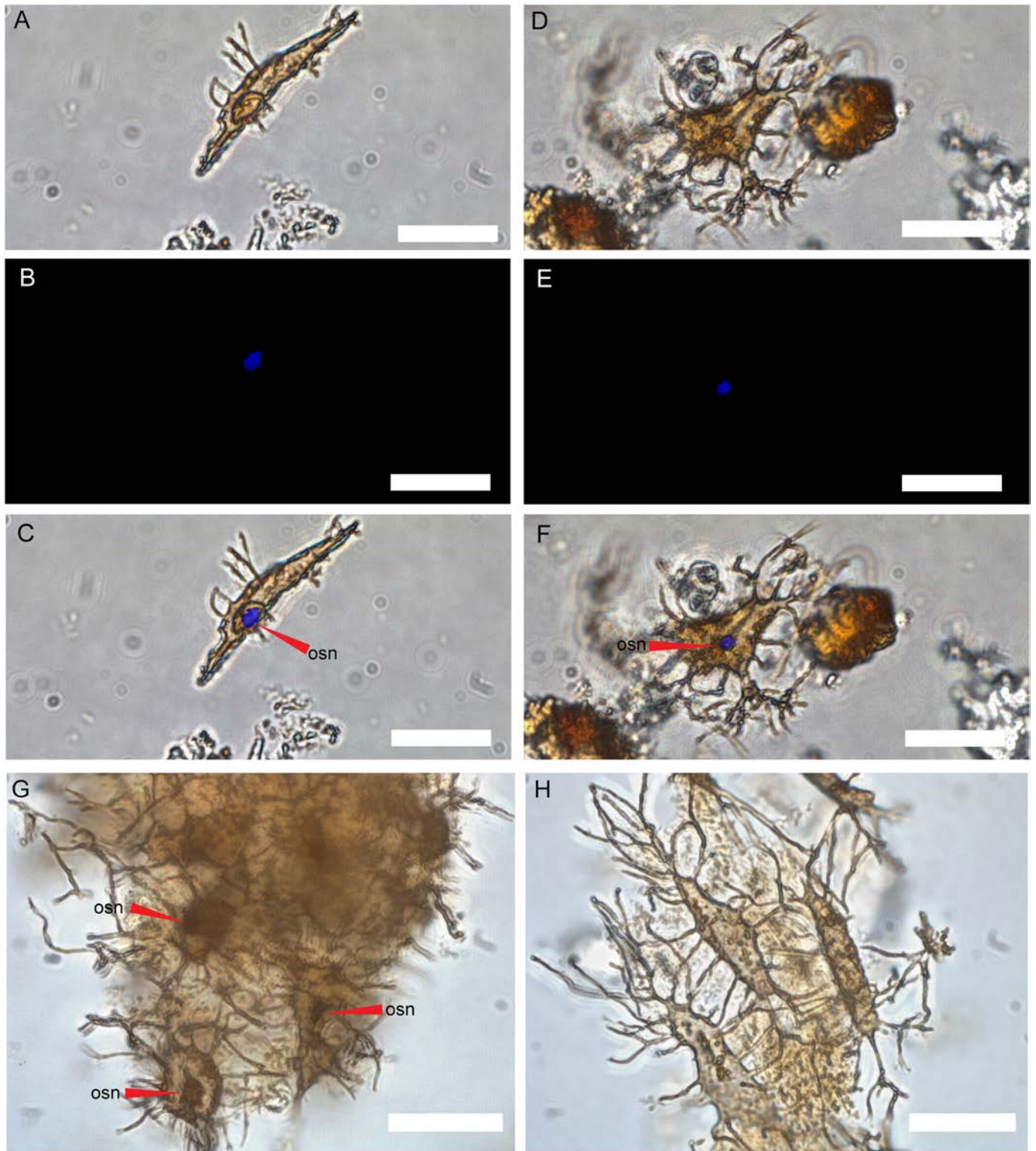
FIGURE 4. Osteocytes of *Lepidochelys* sp. (MUPAN-STRI 39311) from the Upper Miocene of Pamana showing DAPI (DNA staining) fluorescent reaction. **A–C**, one of the osteocytes showing a 'nucleus-like' internal structure in: **A**, brightfield illumination; **B**, DNA traces in the 'nucleus-like' structure DAPI stain; **C**, overlay photo between the fluorescent DAPI stain and the brightfield photos. **D–F**, another of the osteocytes showing a 'nucleus-like' internal structure in: **D**, bright light; **E**, DNA traces in the 'nucleus-like' structure DAPI stain; **F**, overlay photo between the fluorescent DAPI stain and the brightfield illumination photos. **G**, group of osteocytes showing 'nucleus-like' internal structure but not reaction to DNA DAPI stain; **H**, group of osteocytes lacking 'nucleus-like' internal structures with no reaction to DNA DAPI stain. **Abbreviation**: **osn**, osteocyte 'nucleus-like' internal structure. Scale bars equal 10 μm.
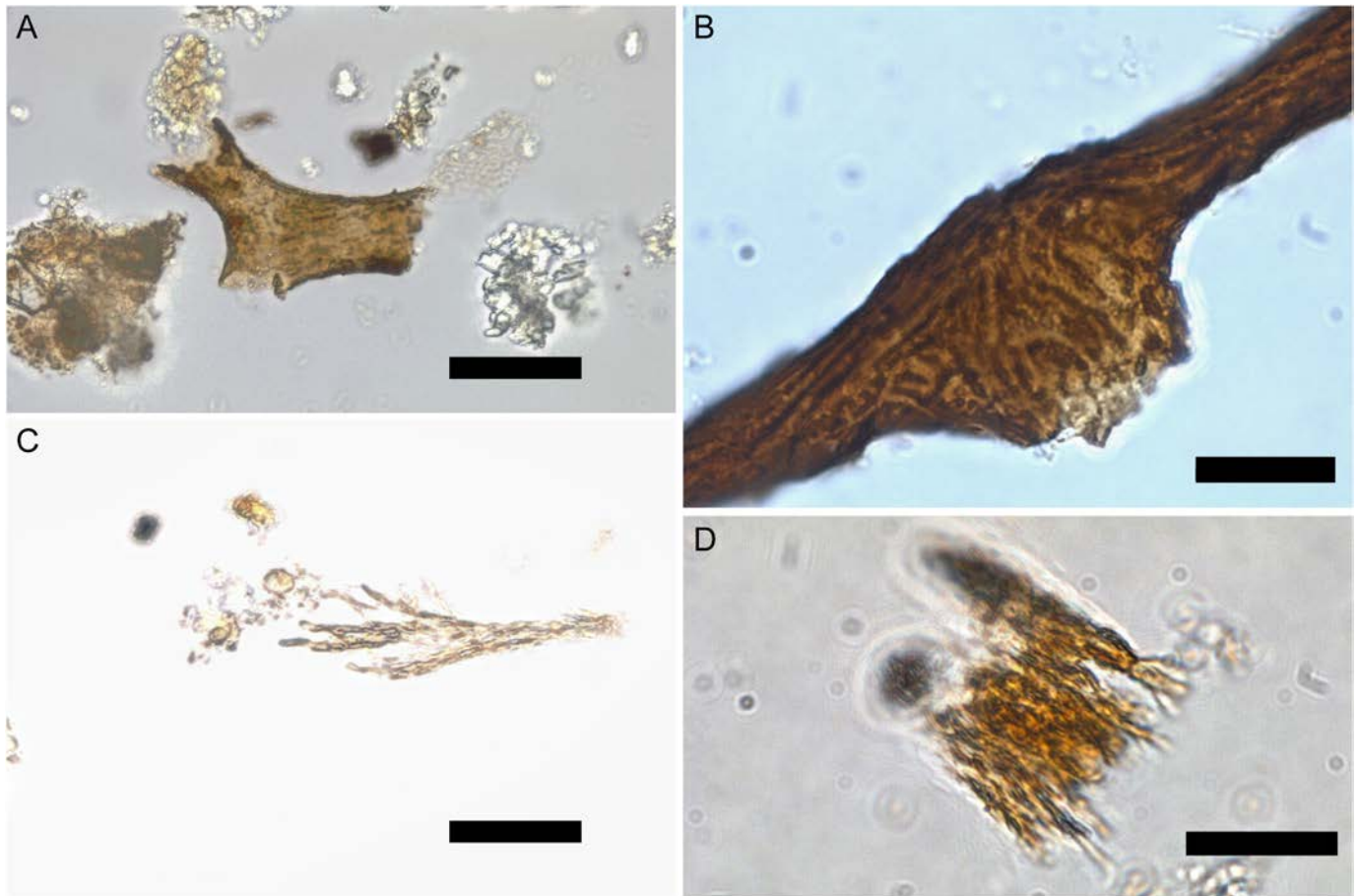
FIGURE 5. Blood vessels and collagen fibers of *Lepidochelys* sp. (MUPAN-STRI 39311) from the Upper Miocene of Panama. **A**, **B**, blood vessels in brightfield illumination. **C**, **D**, collagen fibers in brightfield illumination. Scale bars equal 10 μm.

to those of extant marine turtles, and the differences with other genera, with the primary distinction being the oval rather than circular shape of the femoral head (Buckeridge, 1981). The oval femoral head is indicative of marine adaptation, as it facilitates the vertical mobility of the hindlimb within the water column (Zangerl, 1953). Thus, this feature serves to identify fully marine-adapted turtles but lacks any further taxonomic specificity. Taking this into account, the femur specimen described by Buckeridge (1981), considered as ?*Lepidochelys waikatoica* is unreliable and should be considered Pan-Chelonioidea indet. In contrast, MUPAN-STRI 39311 is a fossil of the genus *Lepidochelys* due to the presence of the same scute patterns, not reported for any other pan-chelonioid. Therefore, this fossil from the Late Miocene represents the oldest record of *Lepidochelys* and has implications for understanding the evolution of this genus.

Considering that the age of the upper part of the Chagres Sandstone Member is Messinian, between ~6.4 and 5.8 Ma based on microfossils (Collins et al., 1996), the new specimen of *Lepidochelys* sp. supports molecular studies (Naro-Maciel et al., 2008; Torres-Vilaça et al., 2022) that infer the existence of *Lepidochelys* during the Late Miocene in lower latitude tropical conditions, similar to the current distribution of the extant *L. olivacea* (Turtle Taxonomy Working Group, 2021). Thus, the morphological differences between the two extant species of *Lepidochelys* and MUPAN-STRI 39311 suggest a higher diversity of species along the evolutionary history of the genus.

Paleoecological Implications

As the extant representatives, extinct marine turtles should have played a significant role in the interspecific interactions of the marine ecosystems they inhabited (Bjorndal & Jackson, 2003). They function as consumers, prey, competitors, hosts for parasites and pathogens, substrates for epibionts, nutrient transporters, and landscape modifiers. They transfer energy within and amongst ecosystems and consume species to a greater extent than other organisms (Bjorndal & Jackson, 2003).

In order to establish the possible paleoecological significance of the new *Lepidochelys* sp. specimen (MUPAN-STRI 39311) within the tropical ecosystem it inhabited, we used its potential closest living relative, *Lepidochelys olivacea*, as the most accurate analog. *L. olivacea* individuals are tertiary consumers, opportunistic foragers that primarily prey on crustaceans, jellyfish, and fish; with fewer consumption records of mollusks, fish eggs, and pyrosomes (urochordates) (Carpena-Catoira et al., 2022). Based on this analogy, we hypothesize that the new *Lepidochelys* sp. specimen likely had a similar diet and ecological role in tropical waters of Panama during the Late Miocene. It was likely an opportunistic forager that migrated from open oceans to shallow waters.

Lepidochelys sp. would have potentially been able to feed on small or juvenile individuals of bentopelagic and pelagic fishes that existed in this paleoecosystem, such as Squaliformes, Pristiophoriformes, Lamniformes, Carcharhiniformes, Myctophiformes (lanternfish), istiophorids (billfishes) and the shark *Isistius* sp.

(Carrillo-Briceño et al., 2015; De Gracia et al., 2012, 2022a, b; Schwarzhans & Aguilera, 2013). It likely consumed crustaceans and other kinds of invertebrates, following the coastal upwellings, similar to its extant relatives. On the other hand, *Lepidochelys* sp. from the Chagres Sandstone Member may have faced competition from larger, more active third-level consumers such as billfishes, Kogiid sperm whales, delphinoids, and smaller sharks found in the paleoecosystem. *Lepidochelys* sp. may also have been susceptible to predation by large sharks.

Bone Microstructural Preservation

The bone microstructural elements in *Lepidochelys* sp. from Panama are exquisitely preserved and include osteocytes that vary between 20–30 μm in greatest diameter (Fig. 4), as well as some blood vessels (Fig. 5A, B) and collagen fibers (Fig. 5C, D). Some of the osteocytes exhibit an oval to circular internal structure resembling the nucleus, and some of them react to DAPI: BLUE staining (Fig. 4A–F). This is similar to the reaction observed in osteocytes from extant mice (Dallas & Moore, 2020), and previously reported osteocytes from two dinosaur species, *Tyrannosaurus rex* and *Brachylophosaurus canadensis* (Schweitzer et al., 2013), as well as chondrocytes of *Hypacrosaurus stebingeri* (Bailleul et al., 2020). No reaction to DAPI was observed outside the 'nucleus-like' structures of the osteocytes, supporting the potential endogenous origin of these traces. However, not all osteocytes that have this 'nucleus-like' internal structure show reaction to DAPI (Fig. 4G). This may be because the cell body is completely sealed, with no evidence of cracks along their membrane surface or broken edges, making it difficult for DAPI to reach or concentrate at the internal body of the cell. Inorganic elements, such as iron and manganese, may have contributed to this sealing, as they commonly occur in osteocytes from other fossil turtles (Cadena, 2020, and references therein). Additionally, it is also possible that DNA remains are not preserved in all cells or have been highly degraded, preventing them from reacting with DAPI. Furthermore, the 'nucleus-like' structure is not present in all preserved osteocytes (Fig. 4H).

The occurrence of potential DNA in the osteocytes of *Lepidochelys* sp. from Panama represents the first report of this biomolecule in a fossil vertebrate millions of years old other than dinosaurs, and from a completely different sedimentological environment (shallow marine) and age (Miocene, ~6 Ma) than for previous reports of ancient DNA. Future work with other stains, for example PI (propidium iodide) can be used to test our results. The excellent preservation of bone microstructures in the new specimen of *Lepidochelys* sp. (MUPAN-STRI 39311) suggests that the Chagres Formation fauna could be a good lower latitude site for molecular paleontology studies. This could help explore not only the preservation of these microstructures in other vertebrates such as fishes and mammals but also their potential original endogenous constituents (DNA and proteins) in conditions different from those found at higher latitudes. This could challenge traditional hypotheses that consider the preservation of these biomolecules in deep-time tropical environments (Demarchi et al., 2016; Orlando et al., 2013, and references therein) as extremely unlikely.

CONCLUSIONS

A new specimen of *Lepidochelys* sp. (MUPAN-STRI 39311) found in the Upper Miocene Chagres Formation in Panama differs significantly from the extant *Lepidochelys olivacea*. These differences include the number of neural bones, the width of vertebral scutes, and bone sculpturing. However, due to the absence of other preserved parts of the skeleton, we refrain from naming it as a new species of *Lepidochelys*. The fossil represents the oldest record of *Lepidochelys*, and its morphological differences from the extant species suggest a potentially higher diversity of species along its evolutionary history. We hypothesize that *Lepidochelys* sp. played a key ecological role in the past tropical ecosystem represented by the Chagres Formation fauna, similarly to extant representatives of the genus in the Caribbean Sea today. This could have included opportunistic foraging on demersal fish, crustaceans, and other kinds of invertebrates, as well as competing with other large third trophic level consumers that were more active, such as billfishes, Kogiid sperm whales, delphinoids, and smaller sharks. We also demonstrate here the exceptional preservation of bone microstructures in the new specimen, including blood vessels, collagen fibers, and osteocytes, as well as traces of material consistent with identification as DNA inside 'nucleus-like' structures within osteocytes. This is the first time that likely DNA traces (older than 5 Ma) are documented in a fossil turtle and in a non-dinosaurian vertebrate.

AUTHOR CONTRIBUTIONS

E-AC and C.D-G designed the project. E-AC and D. A.C-R gathered, analyzed the data, and wrote the first draft of the manuscript. All authors edited the manuscript.

SUPPLEMENTARY FILES

Supplementary Data _1.docx: List of fossil and extant specimens of marine turtles directly examined.

ORCID

Edwin-Alberto Cadena ![ORCID] http://orcid.org/0000-0003-3038-567X
Carlos De Gracia ![ORCID] http://orcid.org/0000-0003-0637-3302
Diego A. Combita-Romero ![ORCID] http://orcid.org/0000-0002-3640-1178

LITERATURE CITED

Bailleul, A. M., Zheng, W., Horner, J. R., Hall, B. K., Holliday, C. M., & Schweitzer, M. H. (2020). Evidence of proteins, chromosomes and chemical markers of DNA in exceptionally preserved dinosaur cartilage. *National Science Review*, 7(4), 815–822.

Batsch, A. J. G. C. (1788). Versuch einer Anleitung zur Kenntniss und Geschichte der Thiere und Mineralien. Erster Theil. Allgemeine Geschichte der Natur; besondre der Säugthiere, Vögel, Amphibien und Fische. Jena: Akademische Buchhandlung.

Benites-Palomino, A., Vélez-Juarbe, J., De Gracia, C., & Jaramillo, C. (2023). Bridging two oceans: small toothed cetaceans (Odontoceti) from the Late Miocene Chagres Formation, eastern Caribbean (Colon, Panama). *Biology Letters*, 19, 20230124. https://doi.org/10.1098/rsbl.2023.0124.

Bjorndal, K. A & Jackson, J. B. C. (2003). Roles of sea turtle in marine ecosystems: reconstructing the past. *In*: Lutz, P. L., Musick, J. A., and Wyneken, J. *The biology of sea turtles* (pp. 259–270). CRC Press.

Buckeridge, J. S. (1981). A marine turtle (Cheloniidae) from the lower Miocene of Port Waikato, New Zealand. *New Zealand Journal of Geology and Geophysics*, 24(3), 435–437.

Cáceres-Farias, L., Reséndiz, E., Espinoza, J., Fernández-Sanz, H., & Alfaro-Núñez, A. (2022). Threats and vulnerabilities for the globally distributed Olive Ridley (*Lepidochelys olivacea*) sea turtle: A historical and current status evaluation. *Animals 12*, 1837. https://doi.org/10.3390/ani12141837.

Cadena, E-A. (2020). In situ SEM/EDS compositional characterization of osteocytes and blood vessels in fossil and extant turtles on untreated bone surfaces; different preservational pathways microns away. *PeerJ 8*, e9833.

Cadena, E-A., & Parham, J. F. (2015). Oldest known marine turtle? A new protostegid from the Lower Cretaceous of Colombia. *PaleoBios*, 32, 1–45.

Carpena-Catoira, C., Ortega-Ortiz, C. D., Liñán-Cabello, M. A., Olivos-Ortiz, A., & Elorriaga-Verplancken, F. R. (2022). Foraging ecology of the olive ridley sea turtle (*Lepidochelys olivacea*) from the Mexican Central Pacific based on stable isotopes. *Regional Studies in Marine Science*, 52, 102296.

Carrillo-Briceño, J. D., De Gracia, C., Pimiento, C., Aguilera, O. A., Kindlimann, R., Santamarina, P., & Jaramillo, C. (2015). A new Late Miocene chondrichthyan assemblage from the Chagres Formation, Panama. *Journal of South American Earth Sciences*, 60, 56–70.

Coates, A. G. 1999. Lithostratigraphy of the Neogene strata of the Caribbean coast from Limon, Costa Rica, to Colon; pp. 17–38. *In*: L. S. Collins and A. G. Coates (eds.). *A Paleobiotic Survey of the Caribbean Faunas from the Neogene of the Isthmus of Panama*. Bulletins of American Paleontology 357.

Collins, L. S., Coates, A. G., Berggren, W. A., Aubry, M.-P. & Zhang, J. (1996). The late Miocene Panama isthmian strait. *Geology*, 24, 687–690.

Cope, E. D. (1867). On *Euclastes*, a genus of extinct Cheloniidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 19, 39–42.

Cope, E. D. (1868). On the origin of genera. *Proceedings of the Academy of Natural Sciences, Philadelphia*, 20, 242–300.

Dallas, S.L., & Moore, D. S. (2020). Using confocal imaging approaches to understand the structure and function of osteocytes and the lacuno-canalicular network. *Bone*, 138, 115463.

De Gracia, C., Carrillo-Briceño, J., Schwarzhans, W., & Jaramillo, C. (2012). An exceptional marine fossil fish assemblage reveals a highly productive deep-water environment in the Central American Seaway during the late Miocene. *Geological Society of America Abstracts with Programs*, 44(7), 164.

De Gracia, C., Correa-Metro, A., Carvahlo, M., Velez-Juarbe, J., Přikryl, T., Jaramillo, C., & Kriwet, J. (2022a). Towards a unifying systematic scheme of fossil and living billfishes (Teleostei, Istiophoridae). *Journal of Systematic Palaeontology*, 20(1), 2091959.

De Gracia, C., Rodriguez, F., Aguilera, O. A., Jaramillo, C. & Kriwet, Jürgen. (2022b). Fossil evidence for earlier radiation in istiophorid billfishes (Teleostei, Istiophoriformes) uncovered by comparative morphology of the caudal vertebrae. *Journal of Vertebrate Paleontology*, 42(1), e2100709.

Demarchi, B., Hall, S., Roncal-Herrero, T., Freeman, C. L., Woolley, J., Crisp, M. K., Wilson, J., Fotakis, A., Fischer, R., Kessler, B. M., Rakownikow Jersie-Christensen, R., Olsen, J. V., Haile, J., Thomas, J., Marean, C. W., Parkington, J., Presslee, S., Lee-Thorp, J., Ditchfield, P., Hamilton, J. F., … Collins, M. J. (2016). Protein sequences bound to mineral surfaces persist into deep time. *Elife*, 5, e17092.

Dodd, C. K. J. & Morgan, G. S. (1992). Fossil Sea Turtles from the Early Pliocene Bone Valley Formation, Central Florida. *Journal of Herpetology*, 26(1), 1–8.

Fierstine, H. L. (1978). A new marlin, *Makaira panamensis*, from the Late Miocene of Panama. *Copeia*, 1, 1–11.

Fitzinger, L. J. (1843). Systema Reptilium. Fasciculus Primus: Amblyglossae. Vindobona [Vienna]: Braumüller und Seidel.

Joyce, W. G., Parham, J. F., Anquetin, J., Claude, J., Danilov, I. G., Iverson, J. B., Kear, B., Lyson, T. R., Rabi, M., & Sterli, J. (2020a). Testudines. *In*: de. K. Queiroz., P. D. Cantino., & J. A. Gauthier (Eds.), *Phylonyms – A Companion to the PhyloCode* (pp. 1049–1052). CRC Press.

Joyce, W. G., Parham, J. F., Anquetin, J., Claude, J., Danilov, I. G., Iverson, J. B., Kear, B., Lyson, T. R., Rabi, M., & Sterli, J. (2020b). Cryptodira. *In*: de. K. Queiroz, P. D. Cantino., & J. A. Gauthier (Eds.), *Phylonyms – A Companion to the PhyloCode* (pp. 1061–1063). CRC Press.

Joyce W. G., Anquetin J., Cadena E. -A., Claude J., Danilov I. G., Evers S. W., Ferreira G. S., Gentry A.D., Georgalis G. L., Lyson T. R., Perez-García A., Rabi M., Sterli J., Vitek N. S., and Parham J. F. (2021). A nomenclature for fossil and living turtles using phylogenetically defined clade names. *Swiss Journal of Palaeontology*, 140(5).

MacFadden, B. J., C. Symister, N. Cannarozzi, C. Pimiento, & C. De Gracia. (2015). Comparative diagenesis and Rare Earth Element variation in Miocene invertebrate and vertebrate fossils from Panama. *The Journal of Geology*, 123, 491–507.

Naro-Maciel, E., Le, M., FitzSimmons, N. N., & Amato, G. (2008). Evolutionary relationships of marine turtles: A molecular phylogeny based on nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution*, 49(2), 659–662.

Orlando, L., Ginolhac, A., Zhang, G., Froese, D, Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Petersen, B., Moltke, I., Johnson, P. L., Fumagalli, M., Vilstrup, J. T., Raghavan, M., Korneliussen, T., Malaspinas, A. S., Vogt, J., Szklarczyk, D., Kelstrup, C. D., Vinther, J., … Willerslev, E. (2013). Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. *Nature*, 499, 74–78.

Perez, V. J., C. Pimiento, A. Hendy, G. González-Barba, G. Hubbell, and B. J. MacFadden. 2017. Late Miocene chondrichthyans from Lago Bayano, Panama: functional diversity, environment and biogeography. *Journal of Paleontology*, 91, 512–547.

Pritchard, P. C. H. (1988). A survey of neural bone variation among recent chelonian species, with functional interpretations. *Acta Zoologica Cravoviensia*, 31, 626–686.

Pyenson, N. D., Velez-Juarbe, J., Gutstein, C. S., Little, H., Vigil, D., & O'Dea, A. (2015). *Isthminia panamensis*, a new fossil inioid (Mammalia, Cetacea) from the Chagres Formation of Panama and the evolution of 'river dolphins' in the Americas. *PeerJ*, 3, e1227.

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). "NIH Image to ImageJ: 25 years of image analysis". *Nature Methods*, 9, 671–675.

Schwarzhans, W. & Aguilera, O. (2013). Otoliths of the Myctophidae from the Neogene of Tropical America. *Palaeo Ichthyologica*, 13, 83–150.

Schweitzer, M. H., Zheng, W., Cleland, T. P., & Bern, M. (2013). Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules. *Bone*, 52, 414–423.

Stiles, E., Montes, C., Jaramillo, C., & Gingras, M. K. (2022). A shallow-water depositional interpretation for the upper Miocene Chagres Formation (Caribbean coast of Panama). *GSA Bulletin*, 134(11–12), 2971–2985.

Surmik, D., Dulski, M., Kremer, B., Szade, J., & Pawlicki, R. (2021). Iron-mediated deep-time preservation of osteocytes in a Middle Triassic reptile bone. *Historical Biology*, 33(2), 186–193.

Thomson, R. C., Spinks, P. Q., & Shaffer, H. B. (2021). A global phylogeny of turtles reveals a burst of climate-associated diversification on continental margins. *Proceedings of National Academy of Science*, 118(7), e2012215118.

Torres-Vilaça, S. T., Hahn, A. T., Naro-Maciel, E., Abreu-Grobois, F. A., Bowen, B. W., Castilhos, J. C., Ciofi, C., FitzSimmons, N. N., Jensen, M. P., Formia, A., Limpus, C. J., Natali, C., Soares, L. S., de Thoisy, B., Whiting, S. D., & Bonatto, S. L. (2022). Global phylogeography of ridley sea turtles (*Lepidochelys* spp.): evolution, demography, connectivity, and conservation. *Conservation Genetics*, *23*(6), 995–1010.

Turtle Taxonomy Working Group [Rhodin, A. G. J., Iverson, J. B., Bour, R., Fritz, U., Georges, A., Shaffer, H. B., & van Dijk, P. P.]. 2021. Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status (9th Ed.). *In*: Rhodin A. G. J., Iverson J. B., van Dijk P. P., Stanford C. B., Goode E. V., Buhlmann K. A., & Mittermeier R. A. (Eds.). Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. *Chelonian Research Monographs*, *8*, 1–472. https://10.3854crm.8.checklist.atlas.v9.2021

Velez-Juarbe, J., Wood, A. R., De Gracia, C., & Hendy, A. J. W. (2015). Evolutionary patterns among living and fossil kogiid sperm whales: Evidence from the Neogene of Central America: *PLoS One*, *10*, 1–29.

Vigil, D. I., & Laurito, C. A. (2014). Nuevos restos de un Odontoceti fósil (Mammalia: Cetacea, Physeteroidea) para el Mioceno tardío de Panamá, América Central. *Revista Geológica de América Central*, *50*, 213–217.

Wibbels, T. & Bevan, E. (2019). *Lepidochelys kempii* (errata version published in 2019). The IUCN Red List of Threatened Species 2019: e.T11533A155057916. http://doi.org/10.2305/IUCN.UK.2019-2.RLTS.T11533A155057916.en

Wyneken, J. (2001). The anatomy of sea turtles. *U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC*, *470*, 1–172.

Zangerl, R. 1953. The vertebrate fauna of the Selma Formation of Alabama. Part IV. The turtles of the family Toxochelyidae. *Fieldiana Geology*, *3*, 135–277.

Zug, G. R. (2001). Turtles of the Lee Creek Mine (Pliocene: North Carolina). *Smithsonian Contributions to Paleobiology*, *90*, 203–218.

Handling Editor: Tyler Lyson.