

A Pelomedusoid Turtle from the Paleocene–Eocene of Colombia Exhibiting Preservation of Blood Vessels and Osteocytes

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ABSTRACT.—We describe a Paleocene–Eocene pelomedusoid turtle from tropical South America in a new fossiliferous locality, from Los Cuervos Formation, at the Calenturitas Coal Mine, Colombia. Although no further systematic precision beyond *Pelomedusoides incertae sedis* can be supported, the presence of an ischial scar positioned far anteriorly to the anal notch, and a strongly decorated ventral surface of the plastron, indicate that this specimen is potentially related to the bothremydid clade formed by *Puentemys*–*Foxemys*. We also demonstrate the potential organic preservation of osteocytes and blood vessels for this specimen, indicating that preservation of soft tissue such as vessels and bone cells in the fossil record is independent of bone type, fossil site, or locality; occurring in different lineages of vertebrates; and that it is even possible to occur in tropical depositional environments during a period of maximum global warming such as the Paleocene–Eocene.

RESUMEN.—Aquí describimos la primera ocurrencia de tortugas pelomedusoid del Paleoceno–Eoceno en la parte tropical de Suramérica, en una nueva localidad fósilífera, de la Formación Los Cuervos, en la mina de carbón Calenturitas, Colombia. Aunque, no mayor precisión sistemática puede ser soportada más que *Pelomedusoides incertae sedis*, la presencia de una cicatriz isquial posicionada muy anteriormente a la escotadura anal y una fuertemente decorada superficie ventral del plastron, indican que el espécimen está relacionado con el clado bothremydid formado por *Puentemys*–*Foxemys*. También demostramos la posible preservación orgánica de osteocitos y vasos sanguíneos para este espécimen; indicando que la preservación de tejidos blandos como vasos y células óseas en el registro fósil es independiente del tipo de hueso, sitio fósil o localidad, ocurriendo en diferentes linajes de vertebrados, y que es posible que ocurra en ambientes deposicionales tropicales, durante periodos de máximo calentamiento global como el Paleoceno–Eoceno.

Pelomedusoids represent a large group of fossil and extant Side-Necked Turtles. The most diverse pelomedusoid clades are Bothremydidae (Early Cretaceous–Early Eocene) sensu Gaffney et al. (2006) and Podocnemidae (Paleocene–Present) sensu Cadena et al. (2012). Despite the vast and relatively continuous fossil record of pelomedusoids, gaps still remain, particularly in the Neotropics. Such is the case for the Paleocene–Eocene (~56 Ma), a time period marked by intense global warming including rapid thermal events like the Paleocene–Eocene Thermal Maximum or PETM (DeConto et al., 2012; Foreman et al., 2012, references therein). Although effects of these events on turtles have been documented in high latitudes (Holroyd et al., 2001), nothing is known for turtles that inhabited the Neotropics.

Here we report a new record of Paleocene–Eocene turtles from tropical South America, represented by a complete imprint of a plastron. The pelomedusoid turtle that we describe here also constitutes the first report of fossil vertebrates at the Los Cuervos Formation, Cesar-Rancheria Basin, Colombia. Exceptional preservation of blood vessels and osteocytes are also documented in this fossil. Microstructures similar in location, size, and morphology to vertebrate osteocytes have been documented in Late Cretaceous dinosaurs, Pleistocene mammoths and mastodons (Pawlicki et al., 1966; Pawlicki, 1978; Schweitzer et al., 2005, 2007, 2013; Schweitzer, 2011), and more recently in Mesozoic and Cenozoic turtles (Cadena and Schweitzer, 2012). Likewise, still-soft tissue vessels have been reported in multiple taxa from varying ages and depositional environments including long bones from several Late Cretaceous nonavian dinosaurs (Schweitzer et al., 2007) and bone marrow from 10-Ma fossil amphibians (McNamara et al., 2006); however, this is the first time that preservation of blood vessels are reported in fossil turtles.

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SYSTEMATIC PALEONTOLOGY

TESTUDINES Batsch, 1788

PANPLEURODIRA Joyce, Parham, and Gauthier, 2004.

PELOMEDUSOIDES Cope, 1868 sensu Cadena et al. (2012).

Incertainae sedis.

Material Referred.—CCMFC01 (Calenturitas Coal Mine, fossils collection, Jagua de Ibirico, Cesar Department, Colombia). Complete imprint of the plastron, preserving portions of bone at the most anterior edge of the anterior lobe and on the lateral regions of the bridge.

Locality.—Calenturitas Coal Mine, La Jagua de Ibirico town, Cesar Department, Colombia. (Fig. 1A).

Horizon and Age.—Upper segment of Los Cuervos Formation. Late Paleocene–Early Eocene, based on pollen and spores (Colmenares and Teran, 1993). The specimen was found in a massive sublithic arenite (sandstone) layer interpreted as belonging to deltaic channel deposits (Fig. 1B).

Description.—CCMFC01 is represented by an imprint of a plastron with a maximum length of 58 cm, the entire dorsal surface of which is preserved as a hard, thin layer (4 mm average thickness) of hematite. The ischial and pubic scars, sulci, and sutural contacts between scales and bones are visible and contribute to the diagnosis of the specimen. Plastral bones are still in place at the most anterior edge of the anterior lobe and both lateral bridge regions. The anterior plastral lobe is slightly shorter than the posterior at the midline of the plastron (Fig. 1C). Both ischial scars are triangular in shape, slightly separated from the midline of the plastron, and located anterior to the anal notch. The pubic scars are elongate, with the long axis parallel to the midline of the plastron. The entoplastron has a diamond shape, slightly wider than long. The sutural contacts between bones are also visible, indicating that the hypoplastron and hyoplastron have almost the same length medially. The mesoplastron is pentagonal in shape, slightly wider than long. On the ventral surface of the portion of bone preserved at the

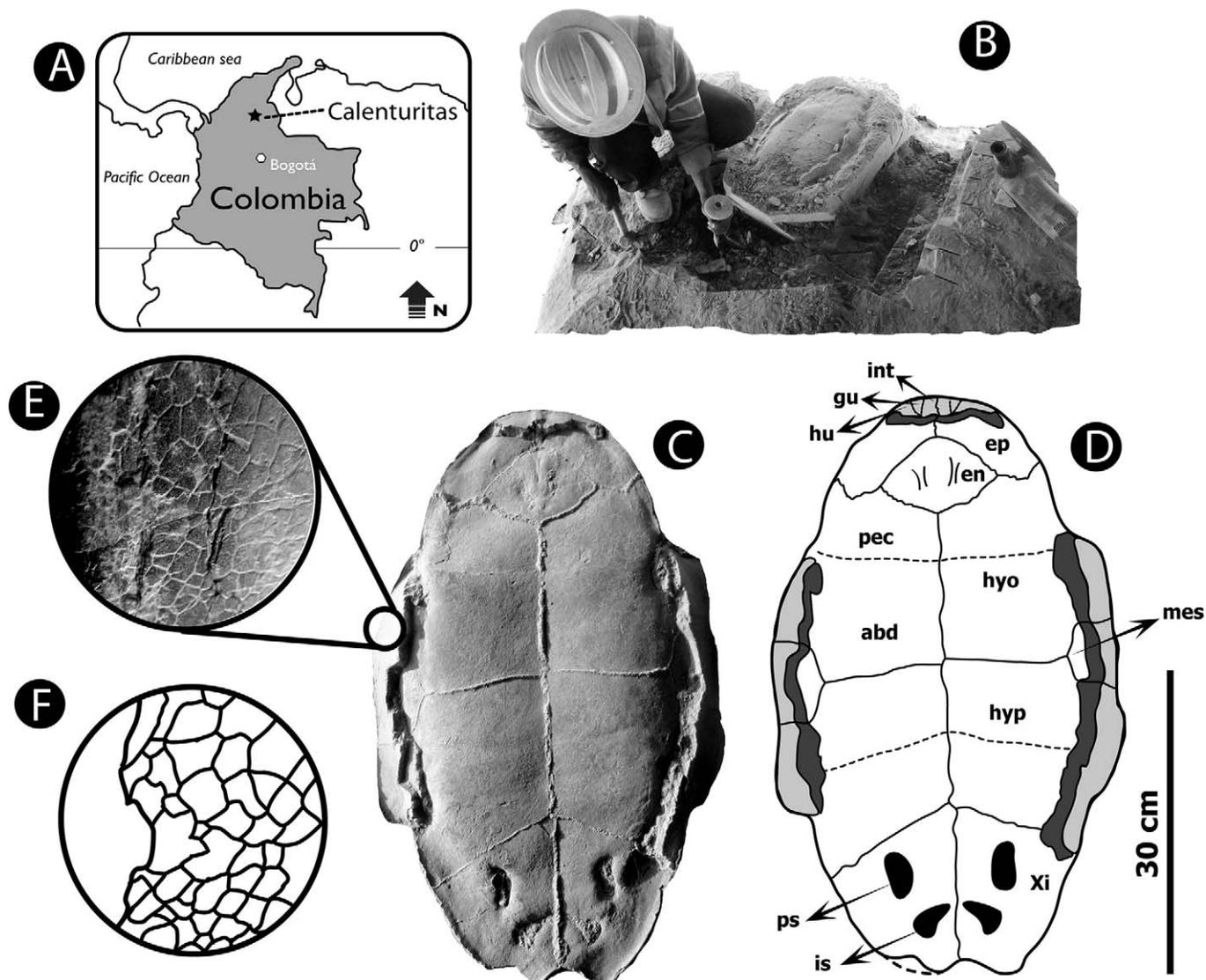


FIG. 1. (A) Location of Calenturitas Coal Mine, La Jagua de Ibirico town, Cesar Department, Colombia, where the CCMFC01 pelomedusoid turtle was discovered. (B) Process of extraction of the fossil from the massive block of sandstone in which it was preserved. (C–D) CCMFC01 *Pelomedusoides incertae sedis*, plastron in ventral view. (E–F) Pattern of decoration on the ventral surface of the right hyoplastron. Abbreviations: abd, abdominal scale; en, entoplastron bone; ep, epiplastron bone; gu, gular scale; hu, humeral scale; hypo, hyoplastron bone; hyp, hypoplastron bone; int, intergular scale; is, ischial scar; mes, mesoplastron; pec, pectoral scale; ps, pubic scar; xi, xiphoplastron bone.

anterior plastral lobe, the sulci between intergular and gulars are visible. The gulars are triangular in shape and restricted to the epiplastra. On the portion of bone preserved at the bridge level, the sculpturing of the plastron is well preserved, exhibiting a pattern of irregular polygons separated by sulci of 0.7-mm width (Fig. 1D–E).

Remarks.—Strong ischial and pubic scars show that the pelvis was sutured strongly to the plastron. This places CCMFC01 in Panpleurodira (Character 125; Joyce, 2007). It is attributed to *Pelomedusoides incertae sedis* because of the ischial scar positioned far anteriorly to the anal notch, a characteristic shared by two bothremydids: *Puentemys mushaisaensis* Cadena et al. (2012a) from the Middle–Late Paleocene of Colombia and *Foxemys mechinorum* Tong et al. (1998) from the Late Cretaceous of Europe. A plastron with a strongly decorated ventral surface is shared by CCMFC01 and most of the bothremydids; however, the arrangement of sulci exhibited by CCMFC01 appears to be unique to this specimen, and is preserved without any

diagenetical alteration based on the absence of crushing or cracking of the bone surface.

OSTEOCYTE AND BLOOD VESSEL PRESERVATION

We demineralized a small (1 × 0.5 cm) fragment of bone corresponding to the left hyoplastron using filter-sterilized (0.22- μ m pore size) ethylenediaminetetraacetic acid (EDTA; 0.5 M pH 8.0) with changes every 2 days. After 5 days, we placed 5 μ l on Teflon-coated glass slides (Cat. N 63421-8), added coverslips, and observed them with a Zeiss Axioskop 2 plus biological epifluorescence microscope using $\times 40$ plain and $\times 63$ oil immersion lenses. We recovered 20 isolated osteocytes, exhibiting the three different morphologies previously described (Cadena and Schweitzer, 2012); two variants of flattened osteocytes, FO1 (Fig. 2A) and FO2 (Fig. 2B), and stellate osteocytes (SO); (Fig. 2C–F). We identified the morphology of each osteocyte while the osteocyte was floating in the EDTA, avoiding a false interpretation of its morphology because of

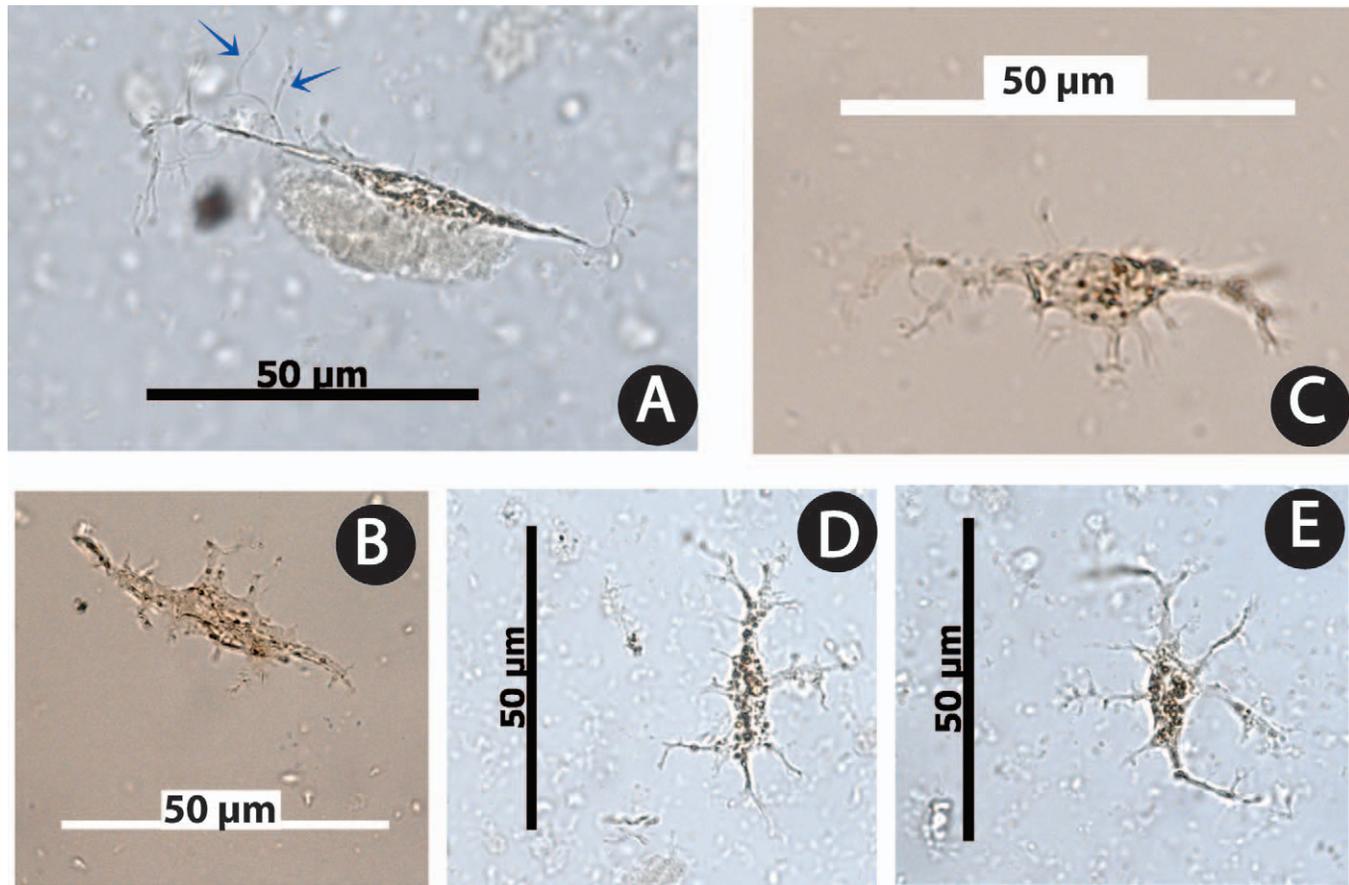


FIG. 2. Osteocytes isolated from a fragment of plastral bone from CCMFC01 *Pelomedusoides incertae sedis*, after demineralization. All photographs were taken under transmitted light and a $\times 63$ oil lens. Osteocyte morphology following Cadena and Schweitzer, 2012. (A) Flattened osteocyte (FO1), arrows indicate tertiary ramification of filipodia. (B) Flattened osteocyte (FO2), slightly shorter and wider than FO. (C–E) Stellate osteocyte (SO).

changes in orientation of the main axis of the cell. Some of the osteocytes exhibited tertiary ramifications of the filipodia (Fig. 2A, arrows). Maximum length of osteocytes varied between 48–60 μm and 5–10 μm in maximum width. In addition to osteocytes we also recovered soft, semi-transparent blood vessels (Fig. 3) showing dual ramification to a 180° angle. The diameter of the blood vessels was measured from photographs using ImageJ 1.43U (Rasband, 2011). We took 50 different measurements for each vessel segment between ramifications (Fig. 3D), the lowest and highest obtained values defined as the range of variation which, for CCMFC01, was between 10–23 μm . To explore variations in vessel diameter in other taxa, we applied the same method of measurement for blood vessels obtained from a plastron of the extant freshwater cryptodire turtle, *Trachemys scripta*, and from taxa figured in Schweitzer et al. (2007:fig. 2B): *Dinornis maximus* (Moa); *Mammuthus columbi* (Mastodon, fig. 2L); *Tyrannosaurus rex* (fig. 3N); *Brachylophosaurus canadensis* (fig. 3s); and from Schweitzer et al. (2009) *Struthio camelus* (Ostrich, fig. 1G) and *Brachylophosaurus canadensis* (fig. 1F). Results are reported in Table 1.

The preservation of both blood vessels and osteocytes in CCMFC01 share similar features; walls are composed of a thin, orange-brownish layer which, in some regions, accumulates tiny spherical grains, giving the vessel walls a more-crystalline aspect. Under polarized microscope, the orange-brownish layer retains color and does not evidence birefringence, indicating

that blood vessels and osteocytes from CCMFC01 are still potentially organic in nature.

DISCUSSION

CCMFC01 *Pelomedusoides incertae sedis* from the Paleocene–Eocene Los Cuervos Formation described here and the pelomedusoids from the Middle–Late Paleocene Cerrejón Formation (Cadena et al., 2010, 2012a,b) show that this group of turtles was already geographically widespread and diverse in tropical South America, as is the case of the extant genera *Podocnemis* and *Peltocephalus*. Although no further systematic precision beyond *Pelomedusoides incertae sedis* can be supported strongly for CCMFC01 at this point, the presence of an ischial scar positioned far anteriorly to the anal notch and a strongly decorated ventral surface of the plastron indicate that this specimen is potentially related to a bothremydid clade formed by *Puentemys–Foxemys* (Cadena et al., 2012a). CCMFC01 (a potential bothremydid) and *Taphrosphyini incertae sedis* from the Eocene of Peru (see Gaffney et al., 2006:110) indicate that pelomedusoids, particularly bothremydid turtles, inhabited tropical South America during most of the warming event of the Paleocene–Eocene. However, bothremydids did not survive after the Eocene, potentially suggesting that this climatic event or the associated ecological or areal drainage changes worked as the triggers for their extinction.

The blood vessels and osteocytes in CCMFC01 demonstrate once again that the preservation of soft tissue vessels and bone

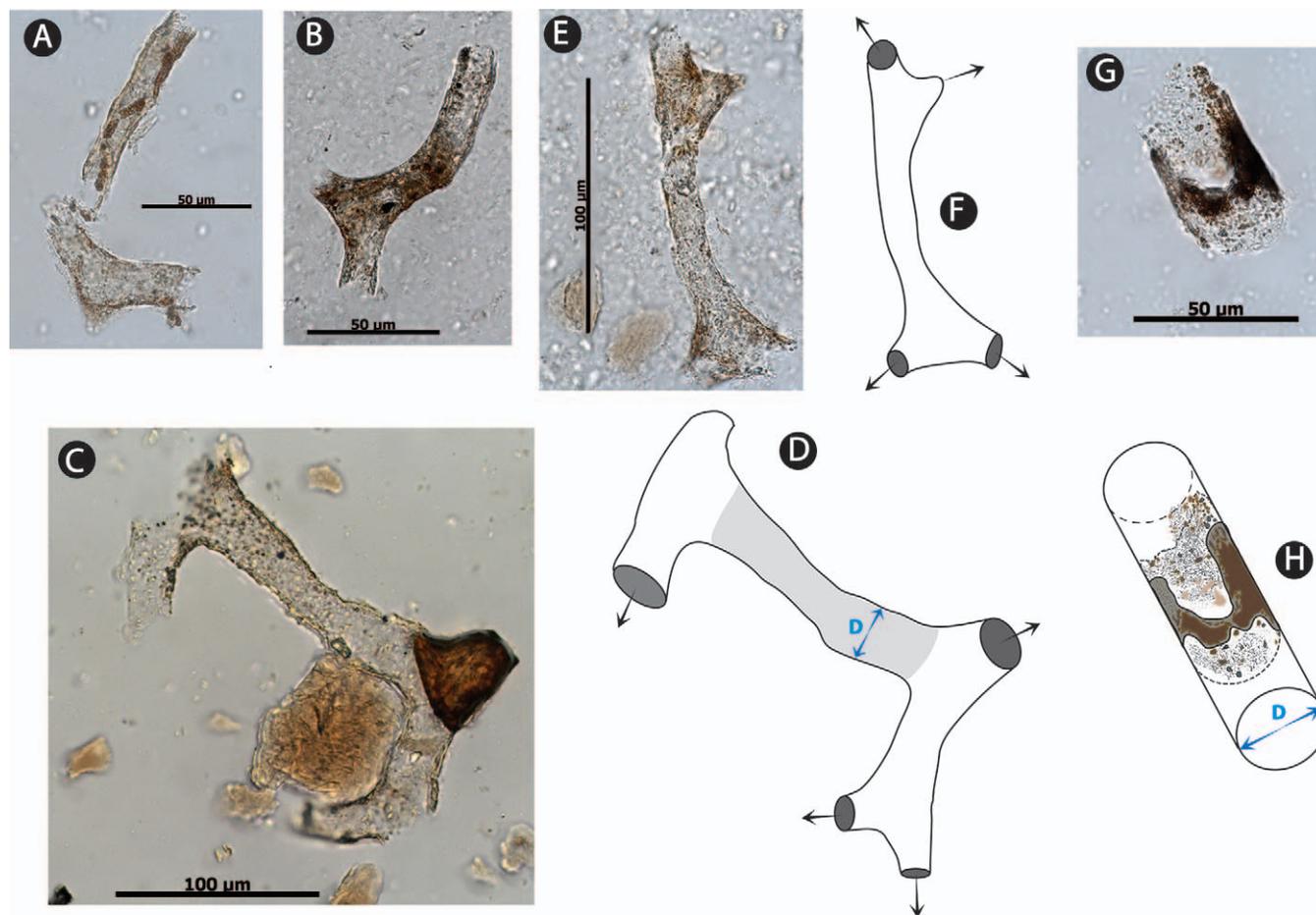


FIG. 3. Blood vessels isolated from a fragment of plastral bone from CCMFC01 *Pelomedusoides incertae sedis*, after demineralization. All photographs were taken under transmitted light and $\times 40$, except G, taken with a $\times 63$ oil lens. (A–B) Blood vessels; (C) blood vessel showing three points of ramification; (D) reconstruction of blood vessels shown in C; (E) blood vessel showing three points of ramification; (F) reconstruction of blood vessels showed in E; (G) fragment of blood vessel preserving its three dimensional shape; (H) reconstruction of blood vessels showed in G, showing the process of disintegration of the orange-brown wall in tiny grains. In blue is the diameter considered for the measurements, grey shadow region represents the segment between ramifications considered in the measurements of diameter, black arrows represent potential direction of the blood vessel. Abbreviation: D, diameter of the blood vessel.

cells in the fossil record occurs in different lineages of vertebrates (adding turtles to the list) and that it is independent of the sedimentary rock type where the fossils were preserved as, for example in this case, a sublithic arenite (sandstone). CCMFC01 also indicates that the preservation of blood vessels and osteocytes occurs in different fossiliferous localities and that it is even possible to occur in low latitudes with tropical conditions, as is shown by the vegetation reconstruction for the Paleocene–Eocene tropical South America (Jaramillo et al., 2010). Additionally, the preservation of blood vessels in CCMFC01 allow us to conclude that it is independent of bone type; for example in turtle plastral bones with an intramembranous origin and long bones with an endochondral origin, as in the case of dinosaurs and in some mammals as shown previously by McNamara et al. (2006). However, the mechanism of preservation and endogeneity of the blood vessels and osteocytes from CCMFC01, as well as the molecular constituents of these materials, remains to be investigated. Future studies, including elemental analysis, immunohistochemistry, and mass spectrometry should be undertaken to resolve these questions. However, potential mechanisms for such preservation have been proposed and are being tested experimentally (Schweitzer, 2011; Schweitzer et al., 2013).

Although the number of fossil vessels reported and figured in the literature is still very low in terms of being considered statistically representative for each taxon, preliminary differences are recognized here in terms of diameter of blood vessels among different taxa. For example, the extant turtle *Trachemys scripta* and the fossil CCMFC01 pelomedusoid turtle described here share similar range values of blood vessel diameter for the plastron and are within the range of those figured for

TABLE 1. Comparisons between the diameter of blood vessels for different taxa figured in Schweitzer et al. (2007, 2009) and the pelomedusoid fossil turtle described here. Measurements were taken from photographs using ImageJ 1.43U (Rasband, 2011).

Taxa	Diameter range (μm)
CCMFC01 pelomedusoid turtle	10–23
<i>Trachemys scripta</i> (Pond Slider Turtle); extant	12–27
<i>Dimornis maximus</i> (Moa)	42–78
<i>Mammuthus columbi</i> (Mastodon)	50–72
<i>Struthio camelus</i> (Ostrich); extant	21–40
<i>Brachylophosaurus canadensis</i> (Schweitzer et al., 2007)	27–42
<i>B. canadensis</i> (Schweitzer et al., 2009)	25–39
<i>Tyrannosaurus rex</i> (Schweitzer et al., 2009)	12–21

Tyrannosaurus rex. Higher values of blood vessel diameters are present in two different samples of *Brachylophosaurus canadensis* and *Struthio camelus* (Ostrich). *Dinornis maximus* (Moa) and *Mammuthus columbi* (Mastodon) exhibit the highest range of blood vessel diameter from observed taxa. Future studies, including more specimens per species for extant and fossil vertebrates, could demonstrate if the variations among blood vessel diameter among different taxa described here persist and if they can be attributed to differences in metabolic rates as has been proposed and tested for blood vessels passing through long bone foramina (Seymour et al., 2012). Other aspects of blood vessels with potential physiological–evolutionary implications should be also explored; for example, number of vessels per volume of bone and levels of ramification according to bone type, among others.

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