High-fidelity organic preservation of bone marrow in ca. 10 Ma amphibians

Maria E. McNamara, Patrick J. Orr — School of Geological Sciences, University College Dublin, Belfield, Dublin 4, Ireland
Stuart L. Kearns — Department of Earth Sciences, University of Bristol, Wills Memorial Building, Queen's Road, Bristol BS8 1RJ, UK
Luis Alcalá — Fundación Conjunto Paleontológico de Teruel-Dinópolis, Avenida Sagunto s/n, 44002 Teruel, Aragón, Spain
Pere Anadón — Consejo Superior de Investigaciones Científicas, Institut de Ciencies de la Terra "Jaume Almera", Lluís Solé i Sabarís s/n, 08028 Barcelona, Spain
Enrique Peñalver-Mollá — Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024-5192, USA

ABSTRACT

Bone marrow in ca. 10 Ma frogs and salamanders from the Miocene of Libros, Spain, represents the first fossilized example of this extremely decay-prone tissue. The bone marrow, preserved in three dimensions as an organic residue, retains the original texture and red and yellow color of hematopoietic and fatty marrow, respectively; moldic osteoclasts and vascular structures are also present. We attribute exceptional preservation of the fossilized bone marrow to cryptic preservation: the bones of the amphibians formed protective microenvironments, and inhibited microbial infiltration. Specimens in which bone marrow is preserved vary in their completeness and articulation and in the extent to which the body outline is preserved as a thin film of organically preserved bacteria. Cryptic preservation of these labile tissues is thus to a large extent independent of, and cannot be predicted by, the taphonomic history of the remainder of the specimen.

Keywords: taphonomy, organic preservation, bone marrow, Miocene, frogs, Spain.

INTRODUCTION

Blood and bone marrow are the most labile, decay-prone tissues in the vertebrate body (Custer, 1974), and therefore almost invariably degrade rapidly during the initial stages of decay. Most purported examples of fossilized red blood cells have proved controversial, and some have been reinterpreted subsequently as artifacts, e.g., pyrite framboids (Martill and Unwin, 1997; however, see Higby Schweitzer and Horner, 1999). Furthermore, high-fidelity preservation of extremely labile tissues almost always results from their replication in early diagenetic authigenic minerals (Briggs, 2003; Martill, 1988; Voigt, 1939). The sole exceptions are the unmineralized blood vessels and cell-like microstructures, possibly osteocytes, recovered by Schweitzer et al. (2005a, 2005b) after dissolving fragments of dinosaur bone; it was suggested that preservation of these structures may have resulted from "some kind of unknown geochemical replacement process" (Schweitzer et al., 2005a, p. 1955). Here we report the first example of fossilized bone marrow (in ca. 10 Ma amphibians from northeastern Spain) and provide a model of how extremely labile tissues such as these can be preserved with histological fidelity as three-dimensional organic residues (the terms "organic" and "carbonaceous" do not imply that the original biomolecular composition is unaltered, in whole or in part). Such tissues provide insights into the physiology of ancient organisms (Schweitzer et al., 2005a) and are potential targets for the recovery of biomolecules. Therefore, an understanding of the taphonomic processes responsible, specifically the environmental context and precise geochemical conditions under which such preservation occurs, is crucial to realizing the paleobiological potential of these organic remains.

GEOLOGICAL BACKGROUND

The Libros Basin forms the southern part of the Teruel graben in northeastern Spain; its infill is early Miocene to Pliocene age, varies from 300 to 500 m in thickness, and includes an early late Miocene (Vallesian, 11.2–8.7 Ma) gypsum-dominated 120-m-thick lacustrine sequence (Libros Gypsum; Ortí et al., 2003). Thermally immature organic-rich laminated mudstones (oil shales: total organic carbon, 1%–2.6% [de las Heras et al., 2003]) of the deep-water Libros Gypsum Unit crop out in the Barrio de las Minas near Libros (Fig. 1); these mudstones host exceptionally preserved fauna and flora that include salamanders, frogs (both adults and larvae), birds, snakes, insects, arachnids, and leaves (Navás, 1922). Adult and larval specimens of the frog *Rana pueyoi* and adult specimens of the salamander *Triton* sp. occur as articulated skeletons enclosed in a thin, dark brown, carbonaceous bacterial biofilm that defines part, or the entire outline, of their soft tissues. In addition, patches of dermal collagen fibers, morphologically identical to those in extant frogs, are replicated in calcium phosphate (Fig. 2A).

METHODS

Fossilized Bone Marrow

Samples of bone marrow were extracted using sterile tweezers; no mechanical separation from the bone or dehydration was necessary. The solid, brittle samples were mounted on aluminum stubs for scanning electron microscopy (SEM) without further preparation.

For SEM, the sample illustrated (Figs. 2D–2F) was gold coated and examined with a Hitachi S-3500N variable pressure microscope equipped with an EDAX energy dispersive X-ray spectrometer (EDS) running Genesis software. Observations were made at an accelerating voltage of 15 kV, with acquisition times of 60 s for EDS spectra.

For transmission electron microscopy (TEM), samples were washed in ethanol 3 times, each for 30 min. The sample of fossil bone marrow was then impregnated with TAAB EM resin under vacuum in the following resin:ethanol mixtures, each for 2 h: 10%,

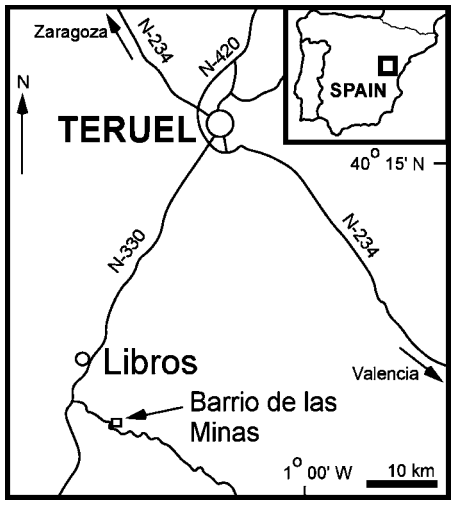

Figure 1. Map showing location of Barrio de las Minas near Libros, Teruel. Inset—position of Teruel province within Spain.

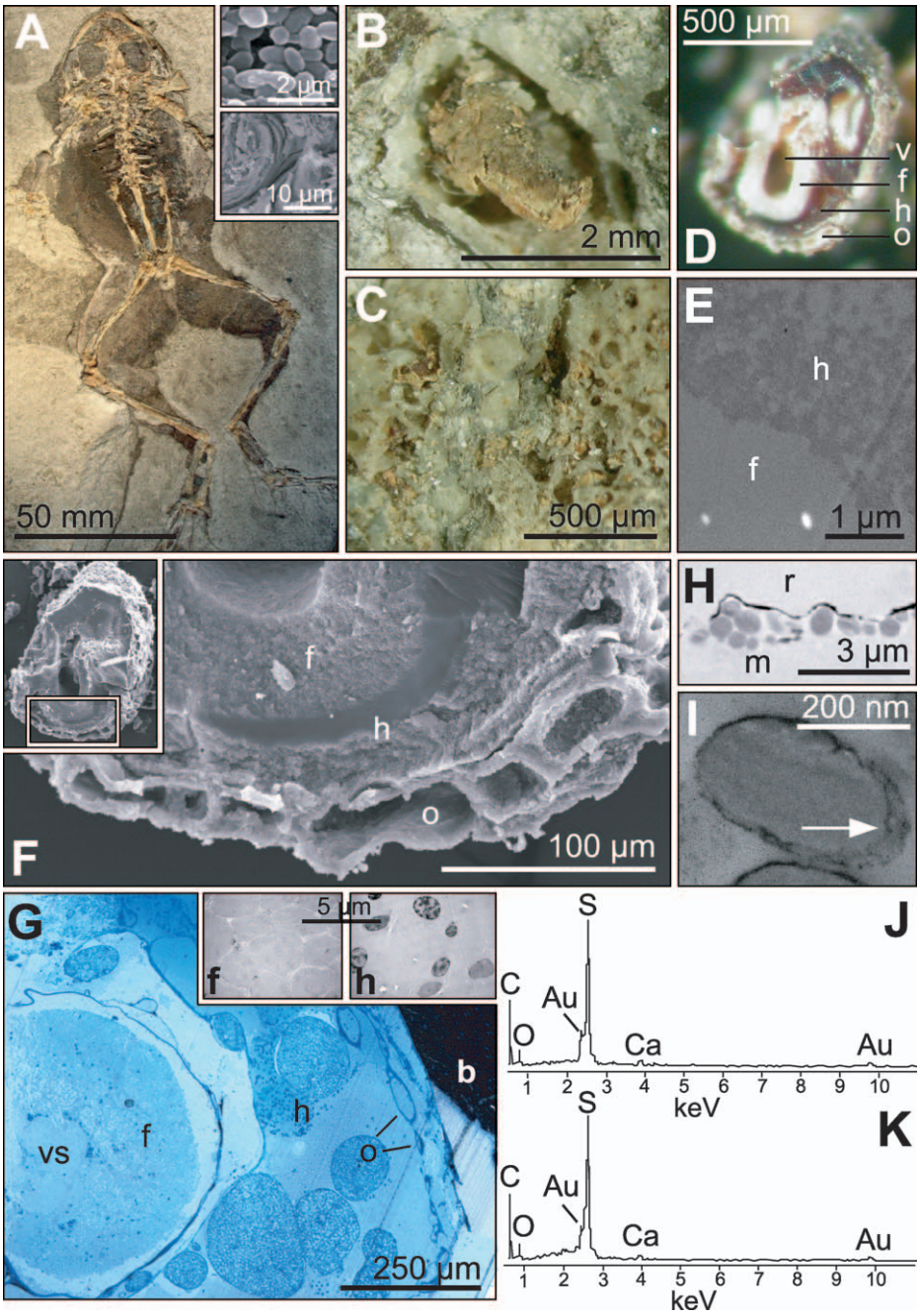

Figure 2. Bone marrow from fossil (A–F, H–I) and extant (G) amphibians. A: *Rana pueyoi* (specimen 26217, Museu de Geologia del Seminari, Barcelona) with outline of soft tissues preserved as bacterial biofilm. Insets: secondary electron micrographs of fossilized bacteria (upper) and collagen fibers (lower). B–D: Light micrographs of marrow within costal marrow cavity (B); spongy bone of femural epiphysis (C) extracted from vertebra (D). E: Transmission electron micrograph (TEM) of junction between zones of hematopoietic (h) and fatty (f) marrow. F: Secondary electron micrograph of marrow; position indicated in inset. G: Light micrograph of semithin stained section of vertebral bone marrow from extant frog, with inset TEMs of fatty (f) and hematopoietic marrow (h). H: TEM of thin layer of ovoid bacteria coating surface of marrow cylinder. Thin electron-dense line between marrow and embedding resin is gold coating. I: TEM of fossil bacterium exhibiting tripartite division of electron-dense margin (at arrow). Qualitative compositional analyses (energy dispersive X-ray spectrometer spectra) of fossil marrow (J) and bacteria on surface of marrow cylinder (K). Abbreviations: b, bone; f, fatty marrow; h, hematopoeitic marrow; m, marrow; o, moldic osteoclasts; r, resin; v, vascular structure; vs, central vascular sinus (now resin infilled).

30%, 50%, 70%, 90%, 100%, 100%, 100% resin. Unstained ultrathin (80–90 nm thick) microtome sections were placed on Cu grids and examined using a JEOL 2000TEMSCAN operating at 80 kV and using an objective aperture of 10 µm diameter.

Modern Bone Marrow

A vertebra of the extant frog *Rana temporaria* was dissected using sterile tools and was fixed and treated as follows: osmium tetroxide (60 min), buffer (10 min), 70% ethanol (10 min, twice), 90% ethanol (10 min, twice), 100% ethanol (20 min, twice).

The fixed and dehydrated sample (histological sections) was prepared as follows: propylene oxide (15 min, twice), 50:50 propylene oxide: TAAB embedding resin (60 min), 100% resin (120 min at 37 °C), 100% resin (18 h at 60 °C). To maximize resin impregnation, the sample was extracted from the resin and the marrow was mechanically separated from the bone using sterile tools. The marrow was then placed in an aluminum mold with fresh 100% resin and polymerized a second time at 60 °C. Semithin (2 µm thick) sections were cut with a glass knife and stained using Toluidine blue.

For TEM, ultrathin (80–90 nm) microtome sections were placed on Cu grids, stained with uranyl acetate and lead citrate, and examined as above.

FOSSIL BONE MARROW

We examined 15 salamanders, 56 adult frogs, and 79 larval frogs from Libros. Three-dimensional fragments of fossilized bone marrow (each ~0.5–2 mm^3) were identified within both cortical (compact) and trabecular (spongy) bone of the limbs, vertebrae, ribs, and craniums of both adult frogs and salamanders (Figs. 2B, 2C); each of these bones is marrow bearing in the modern amphibian skeleton. The marrow is present in 10% of adult frog specimens and one salamander specimen; because its presence can only be confirmed where bone is fractured and the interior visible, this figure is almost certainly an underestimate. Rare small fragments (each ~0.1–0.5 mm^3) of bone marrow were identified in the vertebrae of a single premetamorphous (Gosner [1960] stage 40–41) larval frog.

The example of bone marrow illustrated (Figs. 2D–2F) was extracted from the vertebral body (the ventral cylindrical portion of a vertebra: essentially a hollow cylinder of cortical bone with a large, central, marrow lacuna [Sano-Martins et al., 2002]) of a specimen of *Rana pueyoi* (ANF-0016, Museu Nacional de Ciencias Naturales, Madrid). As in extant frogs (Fig. 2G) (Custer, 1974), yellow fatty marrow surrounds a central vascular structure, and is surrounded by translucent red hematopoietic marrow (Fig. 2D). The fatty marrow

would have almost exclusively comprised fat cells (Campbell, 1970), and thus is now uniformly electron lucent; the more variable electron contrast of the hematopoietic marrow is consistent with its having had a more diverse cellular composition in vivo (Baron, 2002) (cf. Figs. 2E, 2G). A train of flattened cavities (each 20–80 μm long and 15–20 μm wide) forms a semicontinuous layer around the periphery of the marrow (Fig. 2F). The cavities mark the former position of osteoclasts, i.e., flattened cells, each as long as 100 μm, found locally at the bone-marrow interface in modern vertebrates (Fig. 2G) and responsible for bone resorption (Baron 2002). The cavities are each defined by 4–5-μm-thick walls (Fig. 2F) derived from some or all of the cell membrane, the cell surface glycoproteins (Oursler et al., 1991), and the contractile protein-rich cytoskeleton immediately internal to the cell membrane (Lee et al., 1999). In vivo the cell membrane forms a sealing zone at the bone-marrow interface (Väänänen and Horton, 1995); upon death the cytoskeletal proteins contract, and the cell detaches from the endosteal surface (Baron, 2002).

The external surface of the marrow cylinder is covered in a semicontinuous thin (<5 μm thick) layer of ovoid bacteria (Fig. 2H); occasional bacteria also line the inner face of some of the cavities. The electron-dense ~20-nm-wide periphery of each bacterium can show a tripartite subdivision (at arrow in Fig. 2I) corresponding to the outer membrane, internal plasma membrane, and intervening, less-electron-dense, periplasmic space of the bacterial cell envelope (Matias et al., 2003). Similar cell wall detail has been reported from other fossil bacteria (Toporski et al., 2002; Westall et al., 1995). The marrow and bacteria are organically preserved: the only prominent peaks in SEM-EDAX spectra of each are for carbon and sulfur (Figs. 2J, 2K). There are no significant peaks for calcium or other elements (e.g., Fe, Mg, Mn, Sr, Ba) that would indicate the carbon is from a carbonate mineral. The sulfur is part of an organo-sulfur compound: SEM images show no textural evidence that the sulfur is present in elemental form, and elemental maps show that the carbon and sulfur exhibit identical continuous distributions over the area analyzed. The extremely low electron contrast of the marrow and bacteria in TEM images is consistent with an organic composition (e.g., Fig. 2H; cf. with the higher electron contrast of the thin gold coating [Hunter, 1993]).

PRESERVATION OF BONE MARROW

Only a subset of the specimens exhibits marrow; this is considered to be due in large part to the fortuitous fracture of bones (and subsequent exposure of marrow) during collection and archival processes. The presence of fossil marrow does not correlate with any other aspect of the taphonomy of the specimens. Notably, it is not restricted to better preserved specimens. Marrow has been identified in specimens in which skeletal elements (typically phalanges and tarsals) are disarticulated and/or absent. The areal extent of both the dark-colored body outline and phosphatized dermis varies greatly between specimens; either or both may be absent. We propose that the preservation of the marrow and not other, less labile, nonbiomineralized tissues reflects its location inside bone. The bone acted as a physical barrier, retarding the rate at which soft tissues inside became microbially infested (fossilized bacteria are restricted to the periphery of the bone marrow). Nonvascular osteal porosity comprises proteoglycan-filled spaces (15–50 nm diameter) and matrix micropores (5–12 nm diameter) (Thomas et al., 2005) that are too small to facilitate bacterial infiltration of the marrow lacuna but sufficiently large for ionic diffusion (see following). Access by bacteria to spaces inside bone, including the marrow lacuna, is easiest via the one or two principal arteries that perforate the bone. Bone in larval ranids is not fully ossified until Gosner (1960) stage 42 (at younger developmental stages, osteal porosity is visible to the naked eye), and is thus a less efficient barrier to bacterial infiltration. Marrow is only present in 1 of the 79 fossil frog larvae; it is at an advanced developmental stage and ossification of its skeleton is nearly complete.

The high degree of morphological fidelity of the bone marrow, and other organically preserved labile tissues, does not require their original biomolecular composition to be preserved. In their study of the taphonomy of late Paleocene–Miocene *Metasequoia* leaves, Yang et al. (2005) observed that the preservation of original morphological detail correlated positively with the extent to which labile biomolecules (in particular polysaccharides) were preserved. Gupta and Pancost (2005), however, observed significant morphological deterioration of *Arbutus* leaves before appreciable decay of their labile biomolecules (including proteins and carbohydrates). In the thermal maturation experiments of Stankiewicz et al. (2000), using nonbiomineralized arthropod cuticle, a high level of physical detail was retained at the micron scale (except in experiments using extremely rapid heating rates, 10 °C/min), but the aliphatic compounds and labile polymers were polymerized in situ into an insoluble, kerogen-like organic residue. The last of these examples confirms that there are known, albeit incompletely understood, diagenetic processes by which labile nonbiomineralized tissues can be organically preserved with a high degree of morphological fidelity.

The presence or absence of sulfur (confirmed by the EDAX spectra of both marrow and bacteria; Figs. 2J, 2K) may be a proxy for the biomolecular fidelity of organically preserved tissues: the incorporation of sulfur into organic matter is known to enhance the preservation potential of organic compounds such as lipids and carbohydrates on geologic time scales (Sinninghe Damsté et al., 1998). In their study of Late Jurassic kerogens, Riboulleau et al. (2002) identified amino acids only in kerogen fractions attributed to sulfurization, some of which were formed in oxic settings. Sulfurization occurs very early in diagenesis, even at the sediment-water interface (Wakeham et al., 1995), i.e., potentially before microbial degradation of extremely labile tissues can be completed. Sulfurization processes may have been integral to preservation of the original coloration of the red marrow: the hemoglobin components (containing heme, the most important red pigment in blood) of many amphibians and reptiles polymerize via the formation of intermolecular disulfide bonds (Tam et al., 1993). This can occur in the absence of external sources of sulfur, but the latter is known to augment the polymerization process (Riggs et al., 1964). An external source of sulfur (as sulfide) can be generated by the activities of sulfate-reducing bacteria; unless taken up rapidly by any reactive iron species present, the hydrogen sulfide and polysulfides (including HS_4^-, HS_5^-, S_4^{2-}, S_5^{2-}) produced can be incorporated into organic matter (Kok et al., 2000). Because the Libros oil shales were deposited under strongly reducing, iron-poor conditions (de las Heras et al., 2003; Ortí et al., 2003), H_2S generated by sulfate-reducing bacteria was abundant in the monimolimnion and porewaters (Anadón et al., 1992) and would have been available for incorporation into organic compounds during diagenesis.

WIDER IMPLICATIONS

The depositional context and mode of preservation of the Libros amphibians are similar to those of numerous other late Paleozoic to Holocene vertebrate faunas, including the Early Cretaceous Jehol Biota, Liaoning Province, China, from which an important fauna of dinosaurs and early birds is known (Zhou et al., 2003), and the Eocene Grube Messel (Wuttke, 1992) and Enspel (Toporski et al., 2002) faunas. This similarity favors the Libros amphibians being additional sources of very labile tissues. Cryptic preservation inside the protective microenvironment provided by bone is an intrinsic part of the taphonomic history of the other known occurrence of high-fidelity, three-dimensional, organic preservation of extremely labile tissues (Schweitzer et al., 2005a). These tissues are from a specimen of *Tyrannosaurus rex* preserved as an association of disarticulated elements in a soft, well-sorted estuarine sandstone (Schweitzer et al., 2005a, 2005b). The two examples occur in different depositional settings; specimens exhibit exten-

sive to complete (Libros) or complete (Schweitzer et al., 2005a) degradation of the other nonbiomineralized tissues, and fragmentation and disarticulation of the skeleton, i.e., the preservation of extremely labile tissues in the amphibians and dinosaurs, is decoupled from the taphonomy of the material as a whole. Further, in contrast to other mechanisms of exceptional preservation that are constrained by variables such as depositional environment, pH, Eh, and ionic concentrations, this mode of high-fidelity organic preservation does not require a very specific set of environmental conditions. Therefore, high-fidelity organic preservation of extremely labile tissues is likely to be more common in the fossil record than has been hitherto realized.

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REFERENCES CITED

Anadón, P., Rosell, L., and Talbot, M.R., 1992, Carbonate replacement of lacustrine gypsum deposits in two Neogene continental basins: Sedimentary Geology, v. 78, p. 201–216, doi: 10.1016/0037-0738(92)90020-R.

Baron, R., 2002, Anatomy and ultrastructure of bone-histogenesis, growth and remodeling, *in* Arnold, A., ed., Diseases of bone and calcium metabolism: http://www.endotext.org (February 2005).

Briggs, D.E.G., 2003, The role of decay and mineralisation in the preservation of soft-bodied fossils: Annual Review of Earth and Planetary Sciences, v. 31, p. 275–301, doi: 10.1146/annurev.earth.31.100901.144746.

Campbell, F.R., 1970, Ultrastructure of the bone marrow of the frog: American Journal of Anatomy, v. 129, p. 329–356, doi: 10.1002/aja.1001290306.

Custer, R.P., 1974, An atlas of the blood and bone marrow: Philadelphia, Pennsylvania, WB Saunders Company, 562 p.

de las Heras, F.X.C., Anadón, P., and Cabrera, L., 2003, Biomarker record variations in lacustrine coals and oil shales: Contribution from Tertiary basins in NE Spain, in Valero Garcés, B. L., ed., Limnogeology in Spain: A tribute to Kerry Kelts: Madrid, Spain, Spanish Research Council [Consejo Superior de Investigaciones Científicas (CSIC)], p.187–228.

Gosner, K.L., 1960, A simplified table for staging anuran embryos and larvae with notes on identification: Herpetologica, v. 16, p. 183–190.

Gupta, N.S., and Pancost, R.D., 2005, Biomolecular and physical taphonomy of angiosperm leaf during early decay: Implications for fossilization: Palaios, v. 19, p. 428–440.

Higby Schweitzer, M., and Horner, J.R., 1999, Intravascular microstructures in trabecular bone tissues of *Tyrannosaurus rex*: Annales de Paléontologie, v. 85, p. 179–192, doi: 10.1016/S0753-3969(99)80013-5.

Hunter, E.E., 1993, Practical electron microscopy: A beginner's illustrated guide (second edition): Cambridge, Cambridge University Press, 185 p.

Kok, M.D., Schouten, S., and Sinninghe Damsté, J.S., 2000, Formation of insoluble, nonhydrolyzable, sulfur-rich macromolecules via incorporation of inorganic sulfur species into algal carbohydrates: Geochimica et Cosmochimica Acta, v. 64, p. 2689–2699, doi: 10.1016/S0016-7037(00)00382-3.

Lee, M., Christopherson, I.P., Lehman, J.M., Bennett, C.J., and Cheung, H.T., 1999, Comparison of bone marrow extracellular matrices: Biochimica et Biophysica Acta, v. 1428, p. 300–304.

Martill, D.M., 1988, Preservation of fishes in the Cretaceous Santana Formation of Brazil: Palaeontology, v. 31, p. 1–19.

Martill, D.M., and Unwin, D.M., 1997, Small spheres in fossil bones: Blood corpuscles or diagenetic products?: Palaeontology, v. 40, p. 619–624.

Matias, V.R.F., Al-Almoudi, A., Dubochet, J., and Beveridge, T.J., 2003, Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*: Journal of Bacteriology, v. 185, p. 6112–6118, doi: 10.1128/JB.185.20.6112-6118.2003.

Navás, L., 1922, Algunos fósiles de Libros (Teruel): Boletin del Sociedad Ibérica de Ciencias Naturales, v. 21, p. 52–61.

Ortí, F., Rosell, L., and Anadón, P., 2003, Deep to shallow lacustrine evaporites in the Libros Gypsum (southern Teruel Basin, Miocene, NE Spain): An occurrence of pelletal gypsum rhythmites: Sedimentology, v. 50, p. 361–386, doi: 10.1046/j.1365-3091.2003.00558.x.

Oursler, M.J., Li, L., and Osdoby, P., 1991, Purification and characterization of an osteoclast membrane glycoprotein with homology to manganese superoxide dismutase: Journal of Cellular Biochemistry, v. 46, p. 219–233, doi: 10.1002/jcb.240460305.

Riboulleau, A., Mongenot, T., Baudin, F., Derenne, S., and Largeau, C., 2002, Factors controlling the survival of proteinaceous material in Late Tithonian kerogens (Kashpir Oil Shales, Russia): Organic Geochemistry, v. 33, p. 1127–1130, doi: 10.1016/S0146-6380(02)00081-5.

Riggs, A.F., Sullivan, B., and Agee, J.R., 1964, Polymerization of frog and turtle hemoglobins: National Academy of Sciences Proceedings, v. 51, p. 1127–1134.

Sano-Martins, I.S., Dabrowski, Z., Tabarowski, Z., Witkowska-Pelc, E., Spadacci Morena, D.D., and Spodaryk, K., 2002, Haematopoiesis and a new mechanism for the release of mature blood cells from the bone marrow into the circulation in snakes (Ophidia): Cell and Tissue Research, v. 310, p. 67–75, doi: 10.1007/s00441-002-0557-4.

Schweitzer, M.H., Wittmeyer, J.L., and Horner, J.R., 2005a, Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*: Science, v. 307, p. 1952–1955, doi: 10.1126/science.1108397.

Schweitzer, M.H., Wittmeyer, J.L., Horner, J.R., and Toporski, J.K.W., 2005b, Gender-specific reproductive tissue in ratites and *Tyrannosaurus rex*: Science, v. 308, p. 1456–1460, doi: 10.1126/science.1112158.

Sinninghe Damsté, J.S., Kohnen, M.E.L., and Horsfield, B., 1998, Origin of low-molecular-weight alkylthiophenes in pyrolysates of sulphur-rich kerogens as revealed by micro-scale sealed vessel pyrolysis: Organic Geochemistry, v. 29, p. 1891–1903, doi: 10.1016/S0146-6380(98)00166-1.

Stankiewicz, B.A., Briggs, D.E.G., Michels, R., Collinson, M.E., Flannery, M.B., and Evershed, R.P., 2000, Alternative origin of aliphatic polymer in kerogen: Geology, v. 28, p. 559–562, doi: 10.1130/0091-7613(2000)028<0559:AOOAPI>2.3.CO;2.

Tam, L.T., Hanning, D., Cox, D.J., and Riggs, A.F., 1993, The hemoglobins of the bullfrog *Rana catesbeiana*. Deoxygenation-linked association of tetrameric components B and C to form the trimer BC_2: Sedimentation analysis and oxygen equilibria: Journal of Biological Chemistry, v. 268, p. 26972.

Thomas, C.D.L., Feik, S.A., and Clement, J.G., 2005, Regional variation of intracortical porosity in the midshaft of the human femur: Age and sex differences: Journal of Anatomy, v. 206, p. 115–125, doi: 10.1111/j.1469-7580.2005.00384.x.

Toporski, J.K.W., Steele, A., Westall, F., Avci, R., Martill, D.M., and McKay, D.S., 2002, Morphologic and spectral investigation of exceptionally well-preserved bacterial biofilms from the Oligocene Enspel Formation, Germany: Geochimica et Cosmochimica Acta, v. 66, p. 1773–1791, doi: 10.1016/S0016-7037(01)00870-5.

Väänänen, H.K., and Horton, M., 1995, The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure: Journal of Cell Science, v. 106, p. 2729–2732.

Voigt, E., 1939, Red blood corpuscles found in a lizard from the middle Eocene lignite of the Geiseltal near Halle: Research and Progress, v. 5, p. 53–56.

Wakeham, S.G., Sinninghe Damsté, J.S., Kohnen, M.E.L., and de Leeuw, J.W., 1995, Organic sulphur compounds formed during early diagenesis in Black Sea sediments: Geochimica et Cosmochimica Acta, v. 59, p. 521–533, doi: 10.1016/0016-7037(94)00361-O.

Westall, F., Boni, L., and Guerzoni, E., 1995, The experimental silicification of microorganisms: Palaeontology, v. 38, p. 495–528.

Wuttke, M., 1992, Conservation-dissolution-transformation, On the behaviour of biogenic materials during fossilization, *in* Schaal, S., and Ziegler, W., eds., Messel—An insight into the history of life and of the Earth: Oxford, Clarendon, p. 265–275.

Yang, H., Huang, Y., Leng, Q., LePage, B.A., and Williams, C.J., 2005, Biomolecular preservation of Tertiary *Metasequoia* fossil Lagerstätten revealed by comparative pyrolysis analysis: Review of Palaeobotany and Palynology, v. 134, p. 237–256, doi: 10.1016/j.revpalbo.2004.12.008.

Zhou, Z., Barrett, P.M., and Hilton, J., 2003, An exceptionally preserved Lower Cretaceous ecosystem: Nature, v. 421, p. 807–814, doi: 10.1038/nature01420.

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