Demineralization of Phalanges from the Permian Vertebrate *Eryops* Reveals a Pincushion of Vessels and Fibers

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Abstract: Vertebrate Permian bones have been the subject of histological study for decades. However, no such studies have been conducted on phalanges, even though these important bones must certainly have played a role in retaining balance, maneuverability, reproduction, and certain behaviors in Permian terrestrial vertebrates. We report the results of thin-sectioning and decalcification on the terminal phalanges of *Eryops*, a well-known Permian tetrapod. Toe bones responded quickly to decalcification and revealed the presence of vessels, pillar clots, and clear fibers that we interpret as Sharpey's fibers. These vessels, clots, and fibers jutted out from phalanges even after millimeters of bone had been removed. Red-stained osteocytes with robust filipodia were also liberated.

Keywords: Permian vertebrate phalanges, *Eryops*, histology, decalcification

Introduction

There is certainly no shortage of histological studies on the skeletal and dermal bones, tooth-like projections, and teeth of Paleozoic terrestrial vertebrates [1–21]. Such studies elucidate valuable clues regarding growth rhythms and rates, for example, arrested lines of growth, annuli, primary and secondary osteons, and erosion cavities, as well as preferred habitats and periods of change or stasis in paleoenvironments and ecosystems. Documentation of changes in bone can infer abundance or absence of food and water resources, predator activity, competition from other vertebrates, and environmental disasters.

Most histological studies on ancient terrestrial vertebrates have been performed on Mesozoic dinosaurs [5]. Nevertheless, paleohistology of Paleozoic vertebrates has been reported with a particular focus on postcranial limb and vertebral bone architecture. These details elucidate variations in bone structure (parallel-fibered, plywood-like-fibered, laminated, thickened, and lightly or heavily vascularized bone) and lines of growth. It is significant, however, that histological reports of toe bones or phalanges of Permian vertebrates are essentially nonexistent. Some work has concentrated on terminal phalanges and mobility of Paleozoic hands and feet (manus and pes), however, these studies include no histology [22–24]. Morphology of toes and claws are also inferred from Permian vertebrate trackways (ichnofossils), first identified in the 1800s, and are found within sedimentary deposits across 21 countries in six continents [25–35]. Permian phalanges are also well represented on Paleozoic sales websites and in museum collections, yet these bones are rarely sectioned or otherwise studied with destructive techniques, including demineralization. It could be that workers do not consider decalcification of Permian bone a valid methodology for the study of these specimens since none seems to have been reported. Workers might also resist destructive testing (and resort to CT scans [19] and such) because Permian terrestrial vertebrate fossils are rare, thus precious to collectors, museums, investigators, and sales outlets.

Intuition would dictate that toes play a significant role in maintaining balance, maneuverability, reproduction, and social/survival behaviors for any vertebrate. They certainly are weight- and pressure-bearing structures and must respond capably at a moment's notice under varying environmental conditions to ensure viability over time. Therefore, it is lamentable that they remain largely unstudied histologically or via demineralization.

We report here the results of decalcification experiments on Permian toes of *Eryops*, a well-known Paleozoic terrestrial vertebrate.

Materials and Methods

Phalange specimens of *Eryops*, an early Permian amphibian (Figures 1 and 2) were donated by William May of the Sam Nobel Oklahoma Museum of Natural History (Lawton, OK) for destructive histological and decalcification testing. Toes were immersed in 10% formalin upon receipt and after washing and drying were either infiltrated with polymer and polymerized for 24 hours under vacuum or decalcified in 14% EDTA. Polymerized blocks were cut and affixed to glass slides, and non-coverslipped 40µm ground sections were made. All images were collected with a Canon T6s 24MP camera attached to a ZEISS Jena, Jenaval-Contrast microscope.

Results

Most *Eryops* toes used in this study retained an outer periosteal layer of bone (Figures 2 and 3), but others did not, possibly an environmental burial or erosion artifact. Surprisingly, toes were demineralized quickly (within 2 hours) in EDTA (Figures 3–6). Therefore, specimens were removed after short intervals to record the progress of decalcification and note the structures that were uncovered by this process. Only one toe was decalcified completely. Vascular canals were exposed and eroded rapidly (Figures 7–9), revealing the presence of vessels and other structures. Vessels were white or tan

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Figure 1: Brightfield image of *Eryops* terminal phalanges.

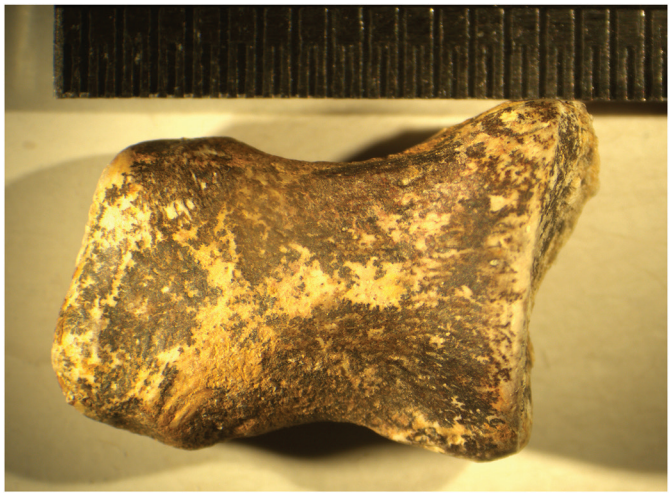

Figure 2: Brightfield image of *Eryops* terminal phalange showing thin periosteal dark bone. Scale bar is in 0.5 millimeter divisions.

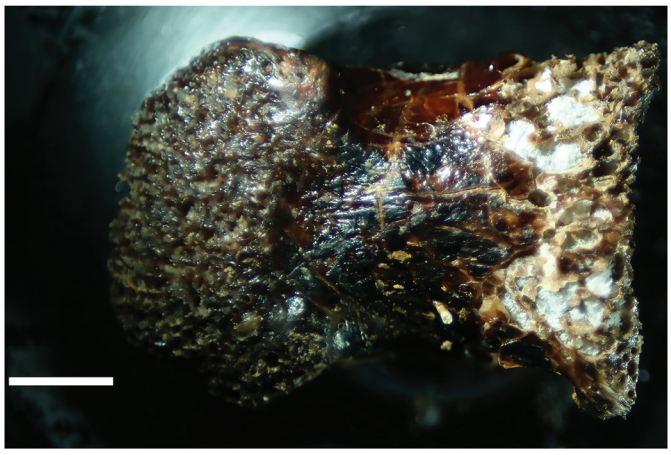

Figure 3: Brightfield image of phalange in Figure 2, after light demineralization. Scale bar = 3.5mm.

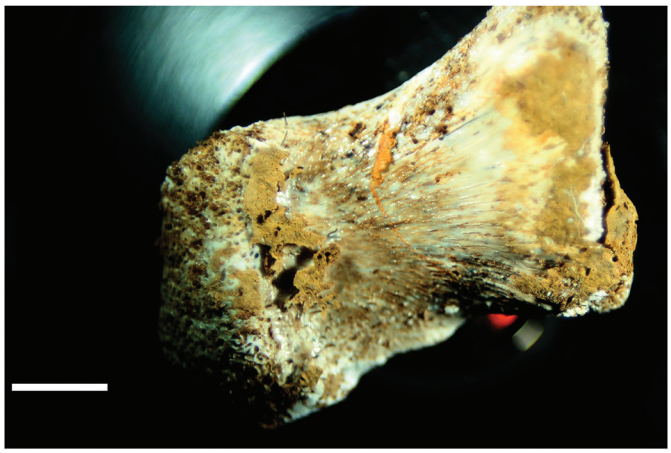

Figure 4: Brightfield image of *Eryops* terminal phalange after light demineralization. Scale bar = 3.5mm.

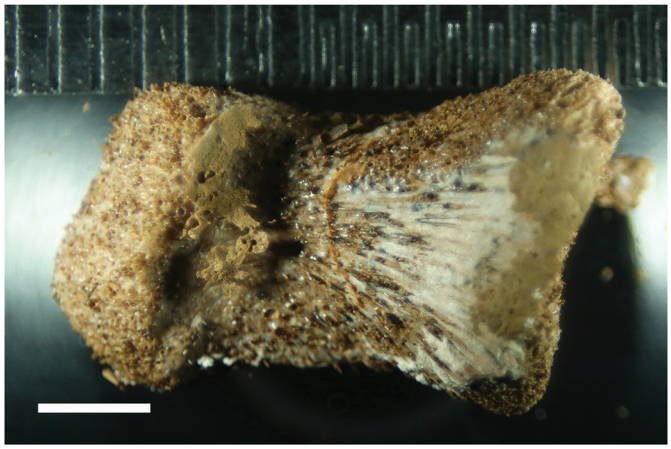

Figure 5: Brightfield image of phalange in Figure 4, after moderate demineralization. Scale bar = 3mm.

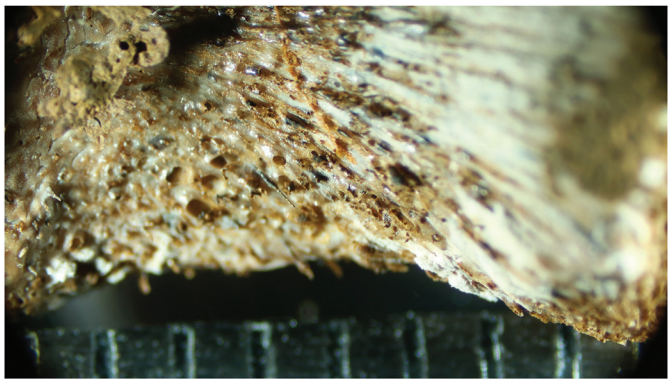

Figure 6: Close-up image of phalange in Figure 4 displaying tan-colored vessels and dark pillar clots. Scale bar is in millimeters.

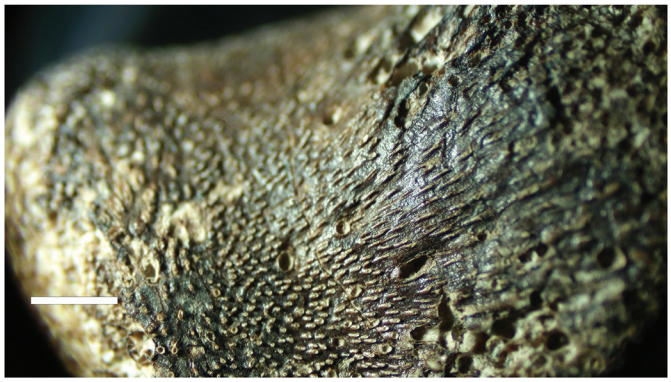

Figure 7: Brightfield image of *Eryops* terminal phalange after light demineralization. Scale bar = 2mm.

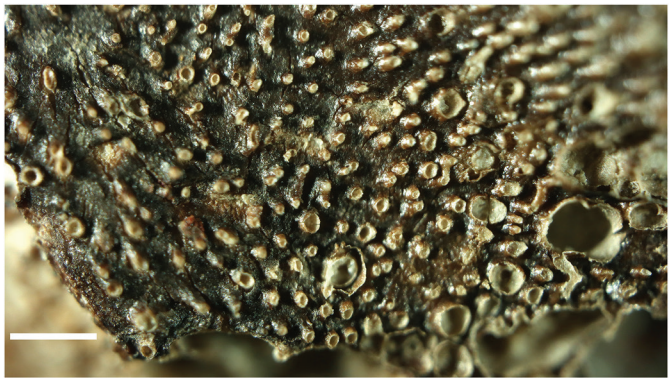

Figure 8: Close-up image of phalange in Figure 7 after moderate demineralization showing vessels emanating from canals. Scale bar = 1mm.

Figure 9: Brightfield image of an *Eryops* terminal phalange after extensive demineralization showing pillar clots and fibers. Scale bar = 2mm.

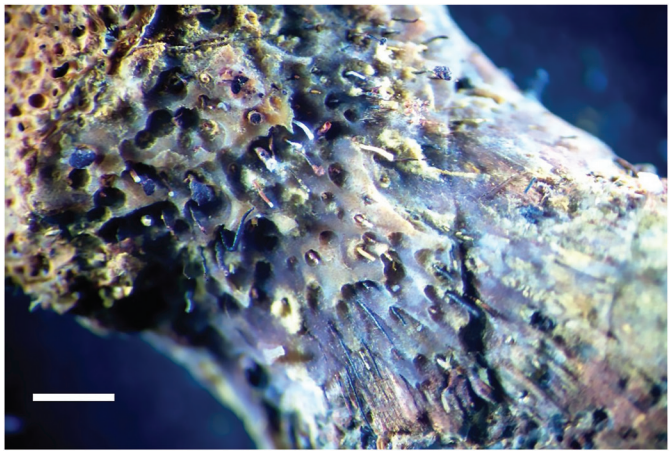

Figure 10: Brightfield image of an *Eryops* terminal phalange after extensive demineralization showing pillar clots and fibers. Scale bar = 2mm.

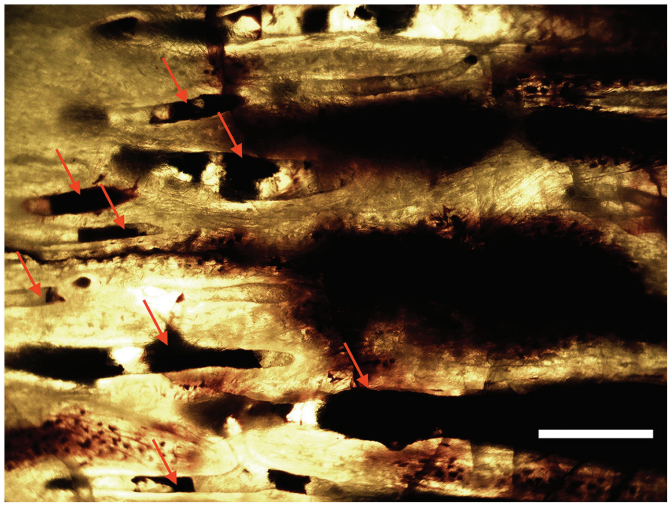

Figure 11: Brightfield image of a thin section of *Eryops* terminal phalange showing clots in vessels (red arrows). Scale bar = 100 microns.

in color and sometimes clear (Figures 6, 8–9). They contained dark (reddish brown to black) pillars of compacted occluding material within the lumen (Figures 9–11), and many of these pillars had no vessel structure around them. This could suggest that vessels had already disintegrated or were removed during processing or at some time during burial or removal. The vessels and pillars projected out of the bone in a perpendicular angle much like pins from a pincushion (Figures 9–11) for a millimeter or more (Figures 6, 9–10), and both structures seemed to extend from below the bone removed and may be anchored by a vessel or the hydroxyapatite itself. Within sectioned material, *in situ* occluded vessels were identified and reacted to UV (365nm) autofluorescence (Figures 11 [red arrows], 12, and 13). Thin sections revealed how vessels were also invaded by intrusions of calcite (Figure 14A), however, the intrusion did not dislodge the occlusions in the canal, which were reactive to UV (Figure 14B).

Clear and white fibers were similarly exposed (Figures 9, 10, 15, and 16) and in most cases were as long as the vessels and pillars uncovered. Most of these structures remained in place during EDTA processing. Furthermore, in many cases, the terminal end of the clot pillars extended into a widening canopy of dark material, possibly where vascular capillaries may have intersected with soft tissue surrounding the phalange (Figure 17). Vessel fragments containing these dark brown to black occlusions were collected by pipette from the bottom of

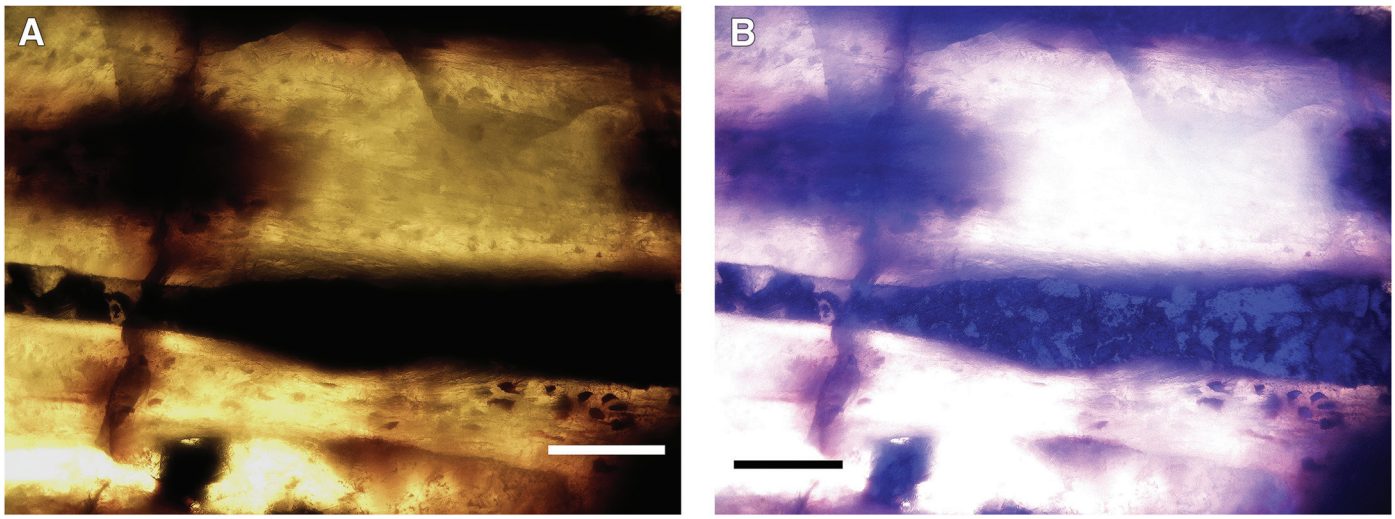

Figure 12: A, close-up brightfield, and B, UV auto fluorescence images of vessels in Figure 11 showing iron-auto fluorescence of iron in vessel clot. Scale bars = 80 microns.

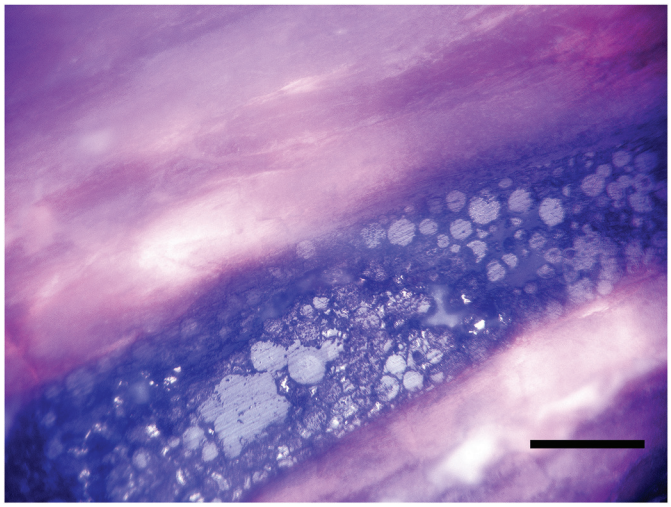

Figure 13: UV image of a thin section of *Eryops* terminal phalange showing spherical iron oxide framboids in vessel clot reacting to UV. Scale bar = 40 microns.

demineralization vials and were also positive for iron under examination with 365nm UV epifluorescence (not shown). Osteocytes, stained red to brown, were also liberated during demineralization and feature multi-branching extended filipodia (Figures 18A and 18B).

Discussion and Conclusions

We were surprised that *Erypos* phalanges demineralized rapidly. One toe was allowed to remain in EDTA until completion and left an amorphous, unrecognizable mass of debris in the vial, but most toes retained their structure during decalcification. We interpret the dark pillars and the material occluding vessels to be the remains of clotted blood [13,36] still present as a result of an asphyxiation event during or just prior to burial. The presence of clots in thin-sectioned Permian bone has been noted previously [13,36], therefore it was not unexpected to see them in these specimens.

Reports of vessels within the bones of Permian terrestrial vertebrates are absent from the literature to date, making this the first such report. Whether the vessels are per-mineralized is yet to be investigated, but their presence is remarkable and suggests incomplete or no per-mineralization took place, especially in the case of the dark clot pillars. The surrounding vessel material may be missing because it was labile and was lost in processing or was not preserved in burial.

We interpret the semi-clear fibers as Sharpey's fibers. At higher magnification (Figure 16), they reveal a speckled inner core with discreet dark dots across the fiber, as if it is reinforced for stress loads applied to the toe in life.

The fact that Permian bone can be removed via standard decalcification suggests that they are only lightly mineralized if at all. The vessels, clot-pillars, and fibers within these phalanges likewise must also be minimally affected by environmental conditions, thus more study (including destructive study) must be undertaken to fully understand the preservation of Paleozoic bones.

Acknowledgements

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References

[1] M Vecoli et al., *Geological Society, London, Special Publications* 339 (2010) https://www.lyellcollection.org/doi/full/10.1144/sp339.1.

[2] S Sanchez et al., *Geological Society, London, Special Publications* 339 (2010) https://www.lyellcollection.org/doi/abs/10.1144/SP339.12.

[3] F Witzmann and R Soler-Gijón, *Acta Zool* 91 (2010) https://doi.org/10.1111/j.1463-6395.2008.00385.x.

[4] M Danto et al., *PLoS ONE* 11 (2016) https://doi.org/10.1371/journal.pone.0152586

[5] EJ Richards, MS Thesis, Univ. Toronto (2016) https://utoronto.scholaris.ca/server/api/core/bitstreams/512a1e92-fc2c-446d-84e2-21824298a2a1/content.

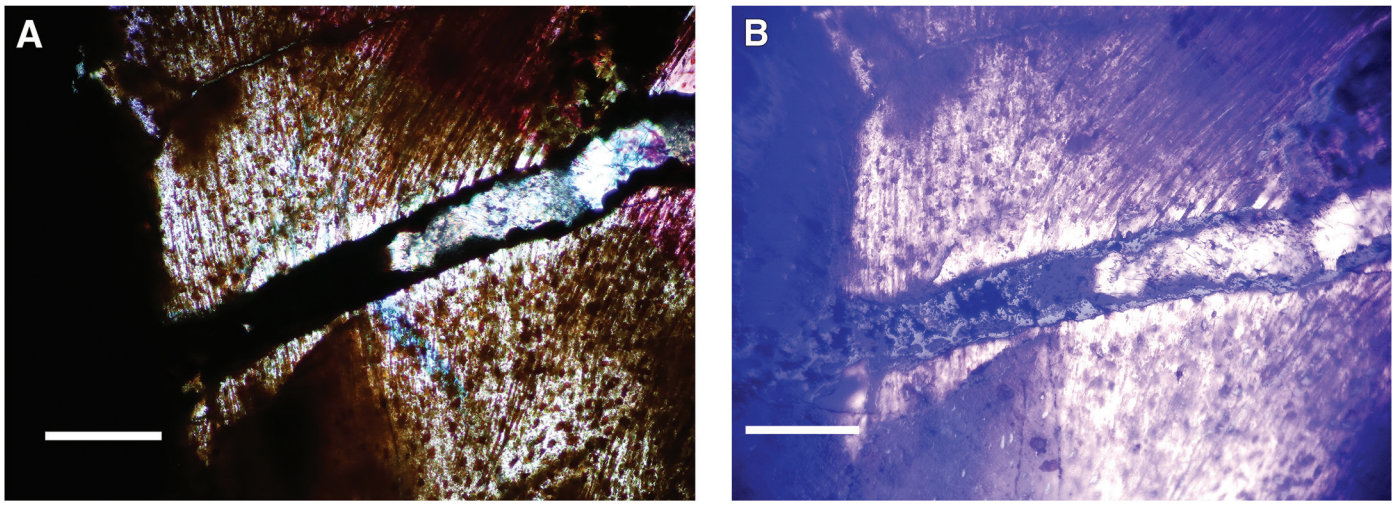

Figure 14: A, brightfield, and B, UV auto fluorescence images of a thin-section of *Eryops* terminal phalange showing iron-auto fluorescence in vessel clot. Note intrusion of calcite into right side of vessel, which did not dislodge the clot. Scale bars = 40 microns.

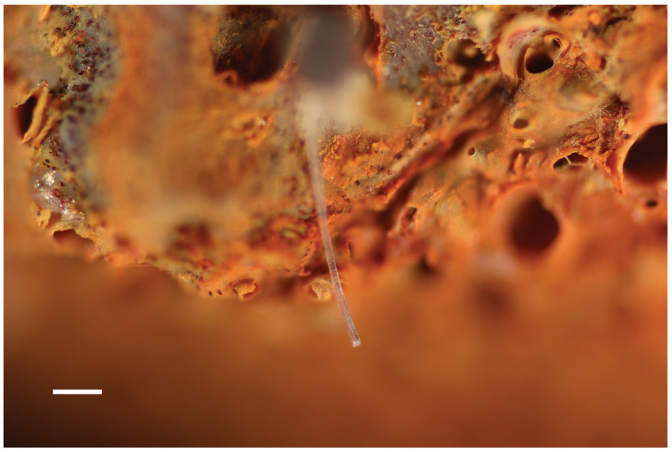

Figure 15: Brightfield image of a Sharpey's fiber jutting from bone of *Eryops* terminal phalange. Scale bar = 20 microns.

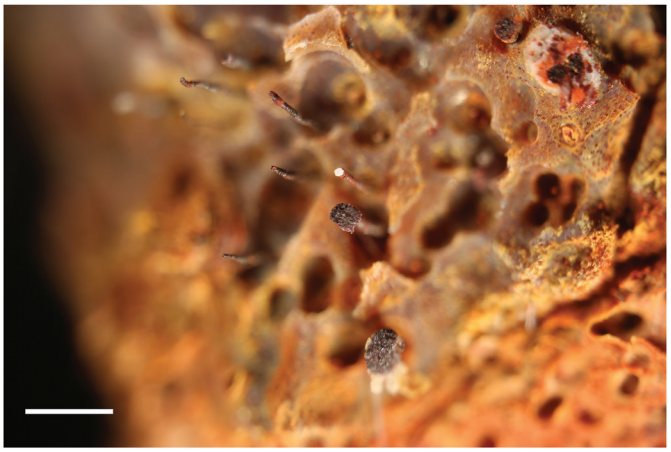

Figure 17: Brightfield image of clot-pillars with broadened canopy of wider clot uncovered by demineralization. Scale bar = 40 microns.

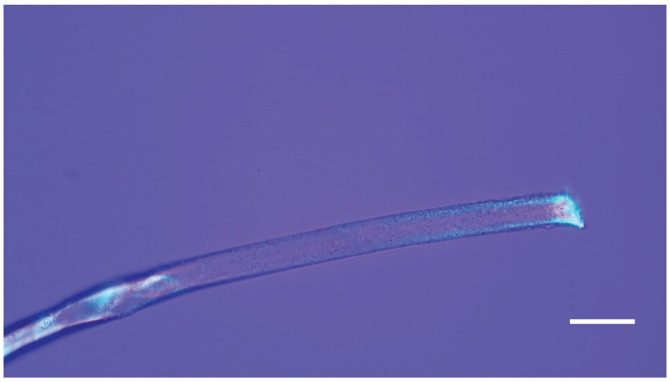

Figure 16: Brightfield image of a Sharpey's fiber under cross-polarization with wave plate. Note structural dots in matrix of fiber. Scale bar = 15 microns.

[6] BM Gee et al., *PeerJ* 5 (2017) https://doi.org/10.7717/peerj.3727.
[7] K Davis, *The Lower Permian Vertebrates of Waurika, Oklahoma*, D&D Fossils, Wakefield, UK, 2018. https://www.nhbs.com/the-lower-permian-vertebrates-of-waurika-oklahoma-book.
[8] KS Brink et al., *Sci Nature* 106 (2019) https://doi.org/10.1007/s00114-018-1598-1.
[9] Y Haridy et al., *Biol Lett* 15 (2019) https://doi.org/10.1098/rsbl.2019.0514.
[10] KD Bazzana et al., *PeerJ* 8 (2020) https://doi.org/10.7717/peerj.8698.
[11] AK Huttenlocker and CD Shelton, *Phil Trans Royal Soc B* 375 (2019) http://doi.org/10.1098/rstb.2019.0142.
[12] BM Gee et al., *Ecol Evol* 10 (2020) https://doi.org/10.1002/ece3.6054.
[13] MH Armitage, *Microsc Today* 30 (2022) https://doi.org/10.1017/S1551929521001565.

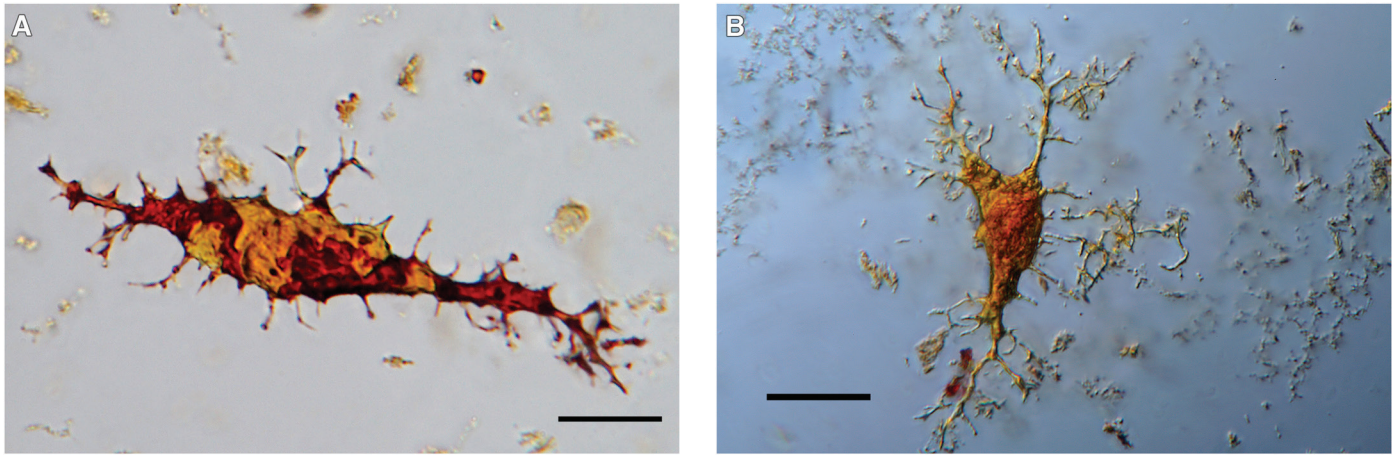

Figure 18: A and B, *Eryops* osteocytes with branching filipodia liberated during demineralization. Scale bars = 5 microns.

[14] V de Buffrenil et al., eds., *Vertebrate Skeletal Histology and Paleohistology*, CRC Press, Boca Raton, 2021, https://www.routledge.com/Vertebrate-Skeletal-Histology-and-Paleohistology/deBuffrenil-deRicqles-Zylberberg-Padian/p/book/9780367700867.

[15] T Maho and RR Reisz, *J Anat* 241 (2022) https://doi.org/10.1111/joa.13715.

[16] N Bowler et al., *J Vert Paleontol* 42 (2022) https://doi.org/10.1080/02724634.2022.2144338.

[17] T Maho et al., *Nat Commun* 13 (2022) https://doi.org/10.1038/s41467-022-32621-5.

[18] MR Whitney et al., *Commun Biol* 5 (2022) https://doi.org/10.1038/s42003-022-04079-0.

[19] T Maho et al., *PeerJ* 11 (2023) https://doi.org/10.7717/peerj.14898.

[20] S Kalita et al., *J Anat* (2025) https://doi.org/10.1111/joa.14197, in press.

[21] MH Armitage, *Microsc Today* 33 (2025) https://doi.org/10.1093/mictod/qaae107.

[22] RN Melchor and WAS Sarjeant, *Ichnos* 11 (2004) https://doi.org/10.1080/10420940490428814

[23] HC Maddin and RR Reisz, *Can J Earth Sci* 44 (2007) https://doi.org/10.1139/E06-076.

[24] SB Kümmell and E Frey, *PLoS ONE* 9 (2014) https://doi.org/10.1371/journal.pone.0113911.

[25] G Fontanarrosa et al., *J Vert Paleontol* 38 (2018) https://doi.org/10.1080/02724634.2018.1491404.

[26] S Voigt et al., *J Vert Paleontol* 27 (2007) https://doi.org/10.1671/0272-4634(2007)27[553:FWTAOP]2.0.CO;2.

[27] S Voigt and SG Lucas, in *The Permian Timescale*, SG Lucas and SZ Chen, eds., Geological Society, London, https://doi.org/10.1144/SP450.10.

[28] SG Lucas et al., *Ann Carnegie Museum* 83 (2016) https://doi.org/10.2992/007.083.0404.

[29] AN Kuznetsov, *Ameghiniana* 57 (2020) https://doi.org/10.5710/AMGH.15.05.2020.3285.

[30] L Marchetti et al., *82nd Annual Meeting Soc Vert Paleontol* (2022) https://www.researchgate.net/publication/366722664.

[31] RR Schoch, *Fossil Rec* 24 (2021) https://doi.org/10.5194/fr-24-49-2021.

[32] M Jansen et al., *XIX Ann Conf Euro Assoc Vert Paleon (PalaeoVertebrata)*, 1, (2022) https://eavp_2022_abstract volume.pdf.

[33] G Calabková et al., *Fossil Imprint* 79 (2023) https://doi.org/10.37520/fi.2023.001.

[34] SG Lucas, in *Vert Ichnol*, SG Lucas et al., eds. Elsevier, 2025, https://doi.org/10.1016/B978-0-443-13837-9.00014-7.

[35] L Marchetti et al., in *Vert Ichnol* SG Lucas et al., eds. Elsevier, 2025, https://doi.org/10.1016/B978-0-443-13837-9.00007-X.

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