First Report of Peripheral Nerves in Post-Cranial Elements of *Cacops* Williston, 1910, (Temnospondyli: Dissorophidae) from the Lower Permian Richards Spur, Oklahoma

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Abstract: Permian bone beds that extend across several states in the US have been known for over 200 years. Those beds have yielded the richest assemblage of the world's oldest amphibian tetrapod bones in the world. *Cacops* sp., a well-known amphibian tetrapod from these deposits, has been widely studied, however, no bone histology or bone decalcification has been conducted on these important animals. We report here on the results of decalcification of post-cranial limb elements of *Cacops* sp., donated by the Sam Nobel Oklahoma Museum of Natural History. Our results include the presence of nerve fibers that display the diagnostic crosshatch pattern known to enclose nerve fascicles and axons, and lipid droplets that exude under cover slip pressure.

Keywords: Permian, amphibian tetrapods, peripheral nerves, nerve fibers, lipids

Introduction

Early studies of Earth's oldest amphibian tetrapods were characterized by an initial flurry of work at the turn of the twentieth century by Americans who dug trails across the "American Permian" deposits, most notably in TX [1–5]. Terms like "Permian material," "Permian formation," "Permian beds," "Permian of northern Texas," "Texas Permian," "Texas red-beds," and "American Permian" characterized reports for a decade. Later studies suggested the use of "Permo-Carboniferous" as a concession to environmental change over time versus a strict stratigraphic interpretation of the beds [5,6]. Nevertheless, the Permian in America became widely studied and known. By 1918, it was recognized that beds bearing similar fauna elsewhere in America could be correlated to the famous Texas red-beds [6], and more importantly, the soon-to-be prodigious Oklahoma deposits [7–9]. Deposits in OK, NM, KS, IL, OH, PA, WV, and elsewhere, including Europe, are now considered Permian [7–14].

Previously, the richest diversity of Permian amphibian tetrapods (and most well-preserved specimens) in America had been unearthed at Richards Spur, OK [11–21]. A unique group of the early amphibians found there, the "armored dissorophoids," feature dorsal bones that arise from the vertebral column and serve as a bony protective shield within the dermis on the dorsal side. *Cacops sp.*, a famous dissorophoid, was first collected at the so-called "*Cacops* bone beds" (CBB) of TX and described in 1910 [3]. After the CBB was exhausted, new, un-described species of *Cacops* were discovered at Richards Spur, OK [11]. This animal has received much attention because "*Cacops* is part of a radiation that may have given rise to some or all of the living amphibians…" [11]. Over the years, many discoveries of *Cacops* from the Richards Spur locality have been reported [15–22].

Limited histology has been conducted on two related members of the temnospondyli [23–26], (the larger group that includes dissorophoids like *Cacops*). We note no reports of fixation, decalcification, ground sectioning, or other histology of *Cacops* material. The purpose of this study was to examine Permian limb bones from the Fissure Fills locality for peripheral nerve fibers.

Materials and Methods

Post-cranial limb elements (an ulna, a femur, and humeri) of *Cacops sp.* (Figure 1) were donated by William May of the Sam Nobel Oklahoma Museum of Natural History (OMNH), Lawton, OK. Limbs were given accession numbers, fixed in 10% formalin, washed in water, and air-dried. After dehydration, limbs were subjected to decalcification in 14% EDTA for 4 weeks. Sealed vials were examined daily using a dissecting microscope with fiber optic illumination. Fibers were removed via a Pasteur pipette, post-fixed in osmium, and air-dried. Wet mounts and whole mounts were made of collected fibers and were examined with polarized light microscopy with quarter and full wave-plates. Voucher specimens of nerves are deposited into the DSTRI, Inc. repository.

Results

EDTA solutions remained clear over the 4-week period, thus EDTA was exchanged infrequently. Bones did not slough away in clumps as readily in EDTA, nor was there discoloration of solutions (Figure 2a), as we have observed in our treatment of Cretaceous bones. Sub-periosteum, compact bone came away in long, thin blocks (Figure 2a) and often harbored fibers within them. Fibers were frequently located and collected from the undersides of displaced bone (Figure 2b) and emanating from canals (Figure 2c). When bones did cleave, deposits of clear calcite and iron pyrite were observed occupying medullary cavities (Figure 2d). Often, thin clear fibers lay isolated and free of surrounding material on the bottom of decalcification dishes and featured a "beads-on-a-string" appearance (Figure 2b).

Collecting and mounting several fibers at one time helped with permanent mounts by eliminating bubbles and allowing several fibers to be compared immediately and adjacent to one another (Figure 3). Once mounted, fibers were positively identified as peripheral nerves due to the diagnostic crosshatch pattern of epineurium and perineurium visible under polarized light (Figures 4–10). We mounted over 50 fibers from these four bones. Fibers lost their "plumpness" over time and contracted somewhat peripherally during slide curing. Osmium staining darkened fibers only slightly in most cases, but post fixation seemed to stabilize the pattern more rigidly, so crosshatching was preserved. Small spheres resembling lipid droplets were often observed within fiber sheaths or emanating from them under coverslip pressure (Figures 7, 10-red arrows, 11-red

Figure 1: Post-cranial limb elements of *Cacops sp.* decalcified in this study.

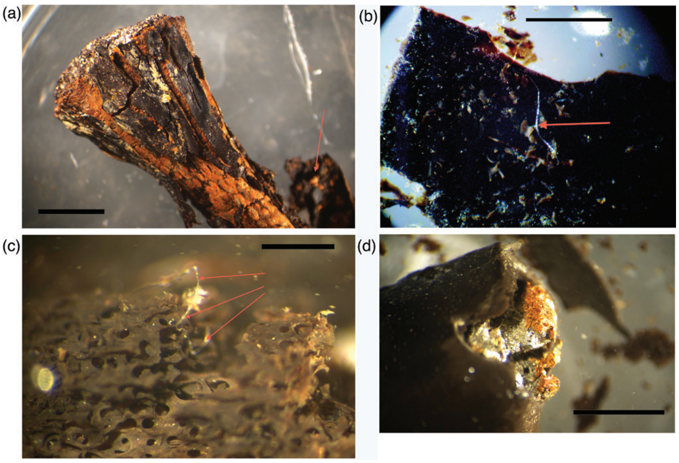

Figure 2: (a) Postcranial ulna of *Cacops sp.* Note clear EDTA solution, flaking of compact bone, and separated shard (arrow) during decalcification. Scale bar = 5 cm. (b) Inside surface of shard of compact bone from Figure 2a. Note thin, clear fiber attached (arrow). Scale bar = 0.5 cm. (c) Shard of cancellous bone from *Cacops sp.* ulna. Note fiber emanating from vessel canal (arrows). Scale bar = 0.8 cm. (d) Diaphysis of *Cacops sp.* ulna. Note wide presence of pyrite emanating from cancellous bone. Scale bar = 2.5 cm.

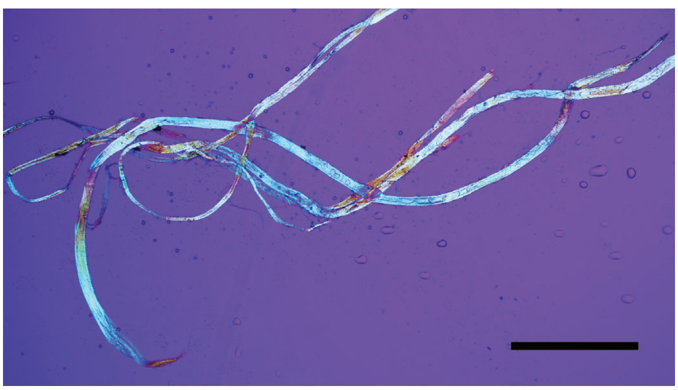

Figure 3: Grouping of nerve fibers collected from *Cacops sp.* post-cranial limb bones. Scale bar = 250 μm.

arrows, 12). The lipids in Figure 12, stained by osmium treatment, are hydrophobic and seen ascending in a trail to the surface of the polymer medium under cover slip pressure, as any lipid would.

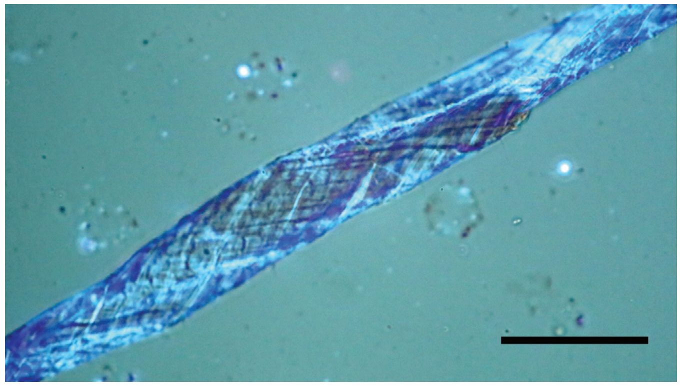

Figure 4: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note diagnostic crosshatch pattern of epineurium and perineurium connective tissue. Scale bar = 40 μm.

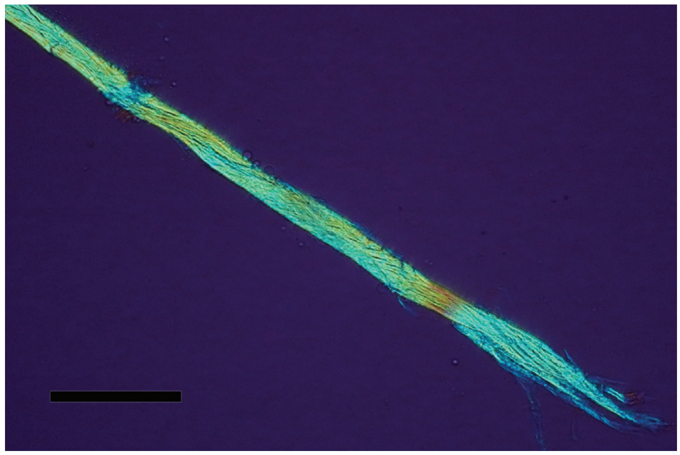

Figure 5: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note diagnostic crosshatch pattern of epineurium and perineurium connective tissue. Scale bar = 80 μm.

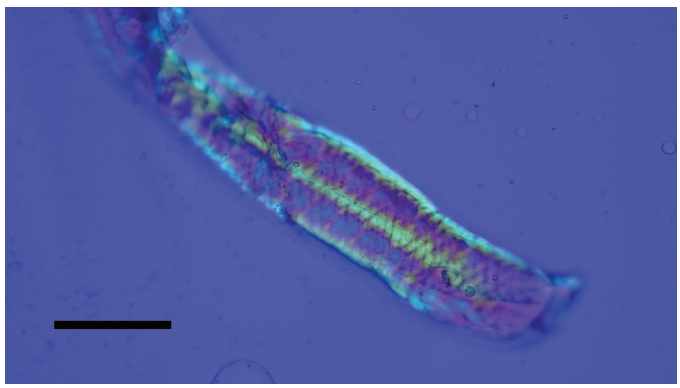

Figure 6: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note diagnostic crosshatch pattern of epineurium and perineurium connective tissue. Scale bar = 25 μm.

Discussion and Conclusions

This is the first report demonstrating peripheral nerves in Permian material from the Richards Spur locality. Our results prompted us to reexamine decalcified material from our study of *Dimetrodon* material from the OMNH V173 Lower Permian

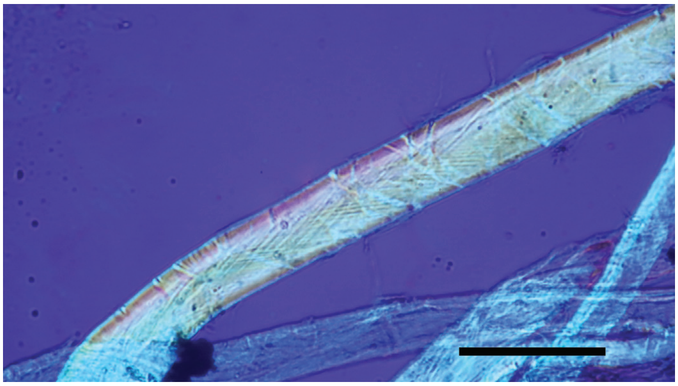

Figure 7: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note diagnostic crosshatch pattern of epineurium and perineurium connective tissue. Scale bar = 40 μm.

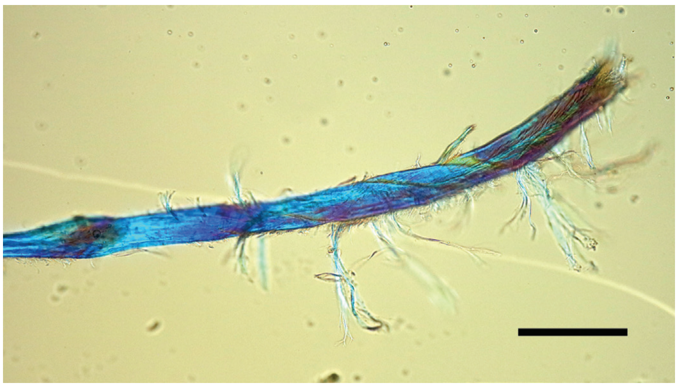

Figure 8: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note diagnostic crosshatch pattern of epineurium and perineurium connective tissue. Scale bar = 60 μm.

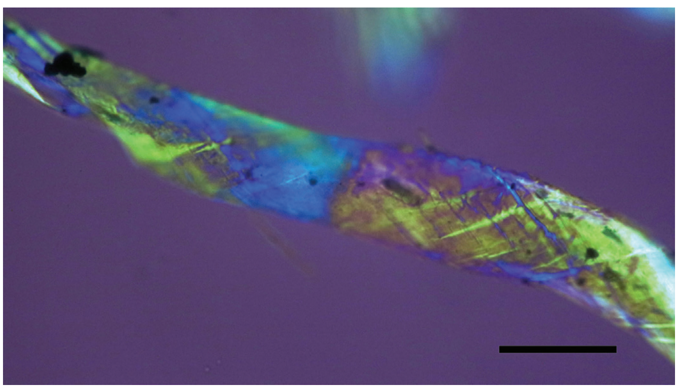

Figure 9: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note diagnostic crosshatch pattern of epineurium and perineurium connective tissue. Scale bar = 40 μm.

N.E. Frederick fossil site in OK [27], and we found similar nerve fibers (Figure 13). Thus, both the Richards Spur locality and site V173 have now yielded peripheral nerves from Permian bones. Moreover, these nerves closely resemble those we recovered from Cretaceous bones at Hell Creek, MT [28]. However, their diameters are about half of the 40-micron wide *Triceratops* nerves.

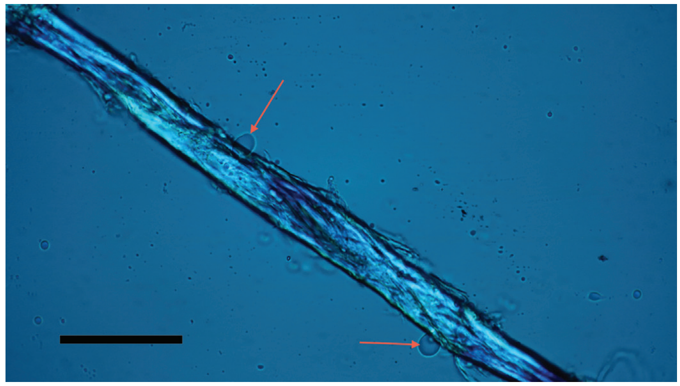

Figure 10: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note lipid droplets exuding from nerve (arrows). Scale bar = 80 μm.

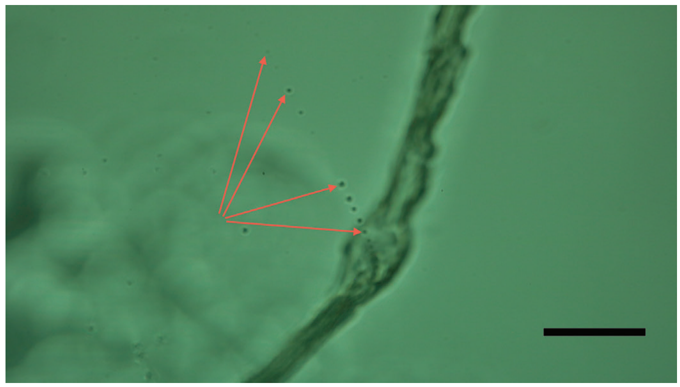

Figure 11: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note lipid droplets exuding from nerve (arrows). Scale bar = 40 μm.

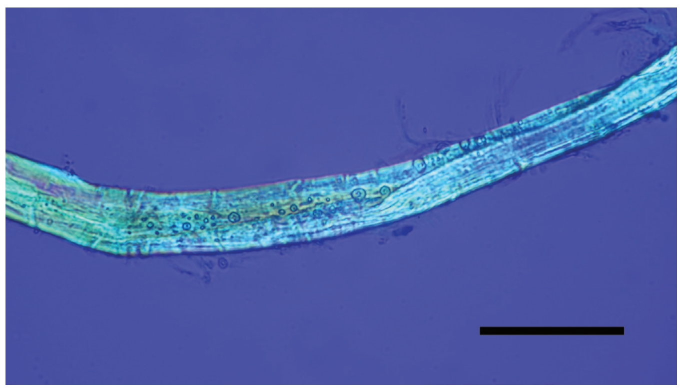

Figure 12: Nerve fiber collected from *Cacops sp.* post-cranial limb bones. Note spherical lipid droplets inside nerve sheath. Scale bar = 40 μm.

The excellent preservation typical of Richards Spur specimens [11,12,17,20] might suggest that nerve filaments are plentiful and better preserved than those found in Cretaceous bones from MT. The presence of what clearly appear to be osmium-stained lipids within the connective tissue sheets surrounding these nerves is astonishing. To date we have not observed such lipid droplets in any previous preparations of decalcified ancient material.

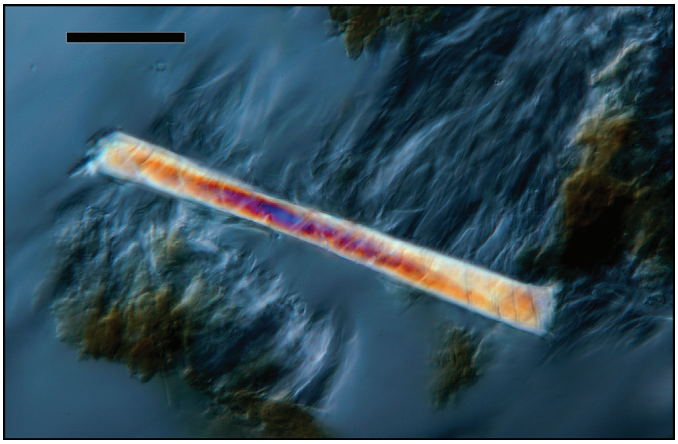

Figure 13: Nerve fiber collected from *Dimetrodon* post-cranial limb bones. Scale bar = 60 μm.

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