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Cover Page Footnote

We thank the Arkansas Game and Fish Commission for issuing the scientific collection permit to SET (no. 020520182).

Bilateral Diaphyseal Chondrodysplasia and Polymorphic Osteodysplasia of the Tibiofibulas in a Southern Leopard Frog, *Lithobates sphenocephalus* (Amphibia: Anura: Ranidae)

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Running Title: Tibiofibula Osteochondrous Dysplasia in a Southern Leopard Frog

Much attention has been focused on limb malformations in anurans following the startling discovery in 1995 of major limb deformities in Northern Leopard Frogs (*Rana pipiens*) from Minnesota in 1995 (Ouellett *et al*. 1997; Ouellet 2000; Blaustein and Johnson 2003; Lannoo 2008). Ouellet (2000) and Lannoo (2008) provided in-depth summaries on the widespread occurrence of anuran limb malformations in the United States. Blaustein and Johnson (2003) described the complexity of these malformations. The numerous causes for these malformations can be attributed to a number of natural phenomena, or they can be considered as being manmade (Lannoo 2008).

In Arkansas, Thigpen *et al.* (2014) documented limb abnormalities in a population of Fowler's Toads (*Anaxyrus fowleri*). They also provided a short description of the complexity of each observed abnormality. McCallum (1999) reported on the malformed Southern Leopard Frogs (*Rana sphenocephala = Lithobates sphenocephalus*) in Illinois and summarized the literature on amphibian abnormalities to date. McCallum and Trauth (2003) reported the Arkansas distribution and growing frequency of abnormalities among Blanchard's Cricket Frogs (*Acris blanchardi*) over four decades. However, the frequency of amphibian abnormalities in Arkansas and the species afflicted is an understudied segment of their ecology. Below, we report on a previously undescribed type of limb abnormality (Meteyer 2000) in the Southern Leopard Frog (*Lithobates sphenocephalus*) from Arkansas.

While road cruising on Turkey Pond Loop (35.2147195N, 92.7567921W) on the night of 21 September 2018 in Conway County, SET collected 5 *Lithobates sphenocephalus* (Dodd 2013) specifically for histological study of the urogenital anatomy in this species. All frogs were returned to Trauth's histo-herpetology laboratory in Morrilton and sacrificed using a dilute chloretone solution. Upon closer examination of the frogs prior to fixation in 10% neutral buffered formalin (NBF), SET observed that one female (SVL = 63 mm) exhibited unusual swellings in the diaphyseal region of both tibiofibulas. This frog was tagged with a personal identification tag (SET 4625) and immediately photographed (Fig. 1). (Later, this specimen was re-tagged with an Arkansas State University Museum of Zoology tag—ASUMZ 3370).

We removed the swollen portion from the diaphyseal region of the left tibiofibula of our specimen along with the tibiofibula proper into 10% NBF. Then, we treated the tissue with a decalcifying solution (1% hydrochloric acid) for 48 h. Next, we prepared the tissue for light microscopy and staining using standard histological methods (Presnell and Schreibman 1997). We dehydrated the tissue mass in a graded series of ethanol solutions, cleared the mass with xylene, and infiltrated the mass in paraffin in a 56°C oven overnight. We then placed the tissue into an embedding mold for hardening, which was followed by block trimming and mounting for sectioning using a rotary microtome. We histosectioned the tissue into 10 µm serial ribbons and adhered these ribbons to microscope slides with Haupt's adhesive. We transferred the slides through staining dishes containing hematoxylin (eosin counterstaining) —H&E to reveal general cytology or Pollak trichrome stain (Pollak) to enhance connective tissue components. SET photographed tissue images using a Leica M80 stereomicroscope and/or a Leica DM 2000 LED light microscope. All slides are currently in the possession of the senior author.

Histological examination of the tissue mass (Figs. 2, 3) revealed a complete disruption of the normal diaphyseal bone structure in this adult frog. The tibiofibula was separated into two poorly ossified and mostly fragmented bony shaft regions (Fig. 2A) on opposite sides of the lesion. These peripheral segments of compact bone were surrounded by hypertrophic regions of hyaline cartilage intermingled with

complexes of dysplastic bone (Fig. 2B). Polymorphic bone is the name we have chosen for this unusual dysplastic bone type, because of its aberrant physical appearance and configuration. This abnormal bone type shares some structural features with cancellous (spongy) bone of caudates (Castanet *et al.* 2003) by exhibiting a

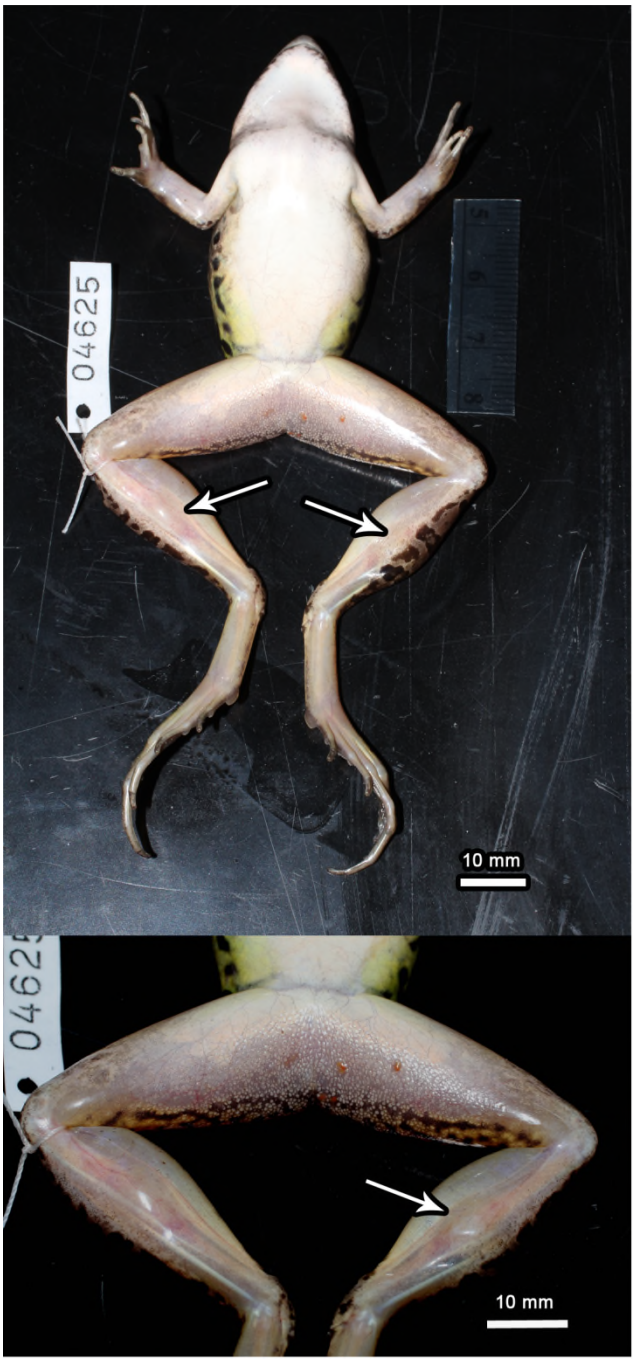

Figure 1. A. Ventral view of female *Lithobates sphenocephalus* (ASUMZ 33750) possessing bilateral diaphyseal dysplasia (arrows) of the tibiofibulas. B. Magnification of the hindlimbs of A with arrow pointing to the bulbous enlargement of the left tibiofibula.

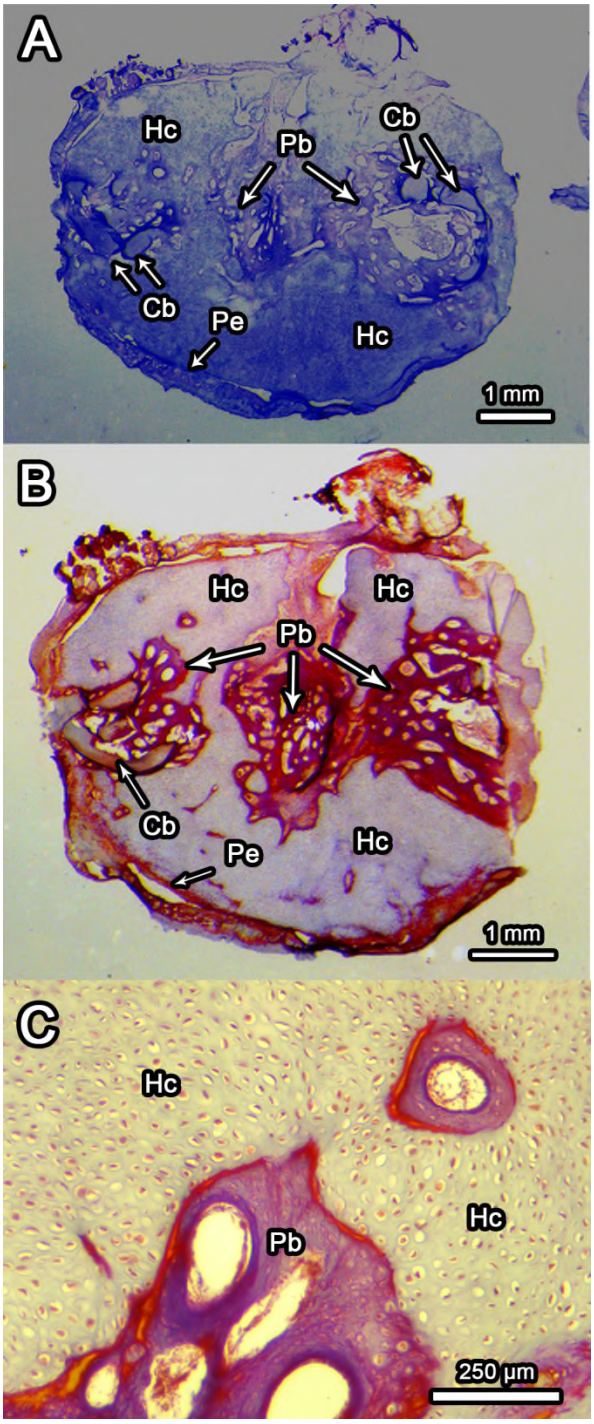

Figure 2. A. Transverse section through the bulbous tissue mass of the left tibiofibula of ASUMZ 33750 revealing original diaphyseal bone shaft fragments of compact bone (Cb) along with a preponderance of hyaline cartilage (Hc) interspersed around regions of polymorphic bone (Pb). Pe = perichondrium. H&E. B. Transverse section of bulbous mass showing the circumferential arrangement of hyaline cartilage (Hc) surrounding three regions of polymorphic bone (Pb). The perichondrium (PE) lines the exterior of the hyaline cartilage. Pollak. C. Magnification of hyaline cartilage and polymorphic bone. A Pb nodule is isolated at the upper right. Pollak.

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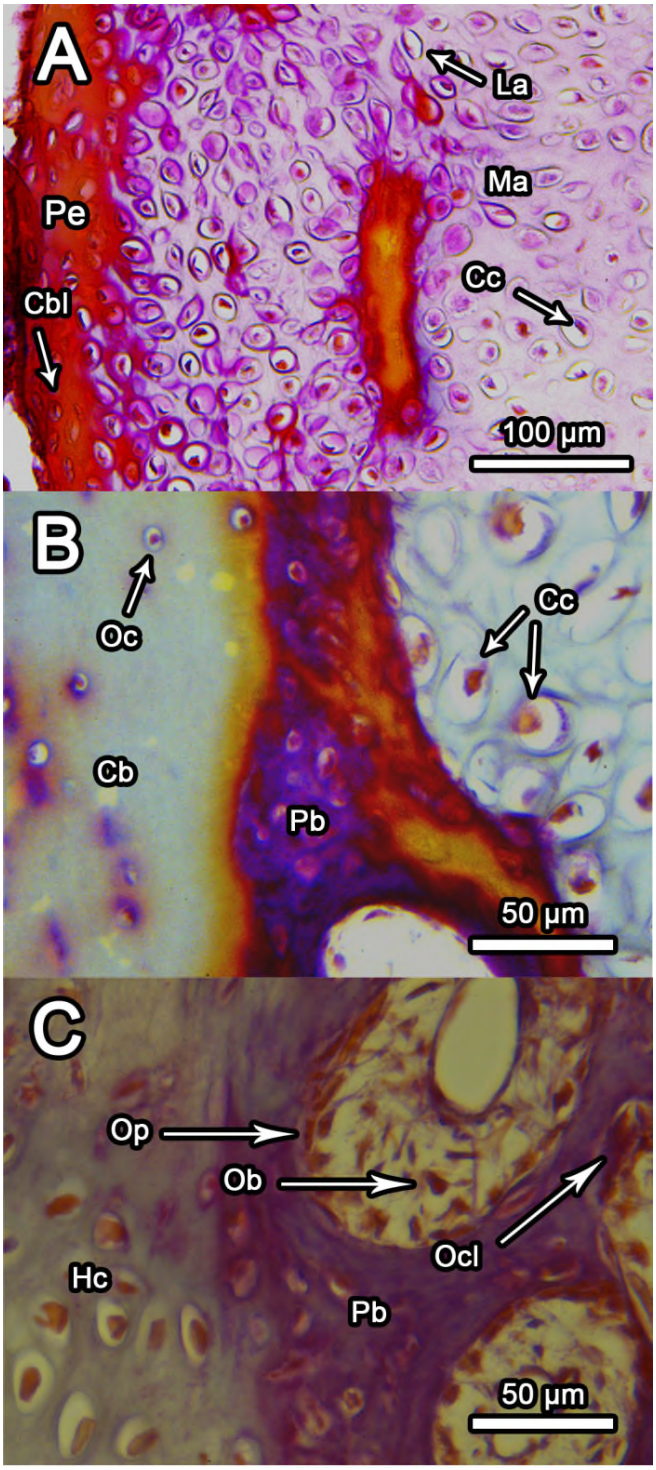

Figure 3. A. Magnification of the perichondrium (Pe) shown in Fig. 2B illustrating the proliferation of chondrocytes of hyaline cartilage. A chondrocyte (Cc) can be seen within its lacuna. Pollak. La = lacuna; Ma = matrix; Cbl = chondroblast. B. Magnification of the compact bone /hyaline cartilage interface of Fig. 2B separated by a zone of polymorphic bone. Abbreviations as before. Pollak. Oc = osteocyte. C. Magnification of a hyaline cartilage/polymorphic bone boundary zone showing an osteoprogenitor cell (Op), an osteoblast (Ob), and an osteoclast (Ocl) within pocket-like nodules. Pollak.

trabeculae-like fretwork whose inner linings are comprised of endosteal cell layers of osteoprogenitor and osteoblast cells (Fig. 3C); however, these trabeculae lack any evidence of extensive calcification even though osteoprogenitor cells appeared to be scattered about within the dark-staining matrix. Also, the trabeculae of polymorphic bone exhibited signs of continuous remodeling as evidenced by the numerous pocket-like nodules (Fig. 2C) scattered throughout much of the chondrodysplastic regions that dominate this tumor-like growth. The presence of osteoclasts supports this hypothesis (Fig. 3C).

We observed three major polymorphic bone aggregates (Fig. 2B). The overall design of these osteogenic regions can best be described as an arachnoid-like patchwork of numerous pockets, channels, spaces, and nodules separated by trabeculae containing a matrix embedded with subperiosteal bone cells.

Mizgireuv *et al.* (1984) reported a high incidence of tumor-like osteochondrous dysplasia affecting the hindlimbs of the Inkiapo Frog, *Rana chensinensis*, and the lesions were associated with larval development in paper factory and sewage effluents. The authors concluded that the anomalies in adult frogs were attributed to the cytotoxic effect of teratogenic agents.

At present, we are unaware of any environmental conditions that could account for the osteochondrous dysplasia in our specimen. Moreover, the remarkable bilateral placement of the 2 lesions in our specimen of *Lithobates sphenocephalus* suggests the possibly of a genetic factor leading to a pairing of hindlimb developmental anomalies during embryonic bone growth.

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