Ophiacodon long bone histology: the earliest occurrence of FLB in the mammalian stem lineage

Christen Shelton, P. Martin Sander

The origin of mammalian endothermy has long been held to reside within the early therapsid groups. However, shared histological characteristics have been observed in the bone matrix and vascularity between Ophiacodontidae and the later therapsids (Synapsida). Historically, this coincidence has been explained as simply a reflection of the presumed aquatic lifestyle of *Ophiacodon* or even a sign of immaturity. Here we show, by histologically sampling an ontogenetic series of *Ophiacodon* humeri, as well as additional material, the existence of true fibrolamellar bone in the postcranial bones of a member of 'Pelycosauria'. Our findings have reaffirmed what previous studies first described as fast growing tissue, and by proxy, have disproven that the highly vascularized cortex is simply a reflection of young age. This tissue demonstrates the classic histological characteristics of true fibrolamellar bone (FLB). The cortex consists of primary osteons in a woven bone matrix and remains highly vascularized throughout ontogeny providing evidence to fast skeletal growth. Overall, the FLB tissue we have described in Ophiacodon is more derived or "mammal-like" in terms of the osteonal development, bone matrix, and skeletal growth then what has been described thus far for any other pelycosaur taxa. Ophioacodon bone histology does not show well-developed Haversian tissue. With regards to the histological record, our results remain inconclusive as to the preferred ecology of Ophiacodon, but support the growing evidence for an aquatic lifestyle. Our findings have set the evolutionary origins of modern mammalian endothermy and high skeletal growth rates back approximately 20 M.Y. to the Early Permian, and by phylogenetic extension perhaps the Late Carboniferous.

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Abstract: The origin of mammalian endothermy has long been held to reside within the early 11 12 therapsid groups. However, shared histological characteristics have been observed in the bone 13 matrix and vascularity between Ophiacodontidae and the later therapsids (Synapsida). 14 Historically, this coincidence has been explained as simply a reflection of the presumed 15 aquatic lifestyle of *Ophiacodon* or even a sign of immaturity. Here we show, by histologically 16 sampling an ontogenetic series of Ophiacodon humeri, as well as additional material, the 17 existence of true fibrolamellar bone in the postcranial bones of a member of 'Pelycosauria'. 18 Our findings have reaffirmed what previous studies first described as fast growing tissue, and 19 by proxy, have disproven that the highly vascularized cortex is simply a reflection of young 20 age. This tissue demonstrates the classic histological characteristics of true fibrolamellar bone 21 (FLB). The cortex consists of primary osteons in a woven bone matrix and remains highly 22 vascularized throughout ontogeny providing evidence to fast skeletal growth. Overall, the 23 FLB tissue we have described in Ophiacodon is more derived or "mammal-like" in terms of 24 the osteonal development, bone matrix, and skeletal growth then what has been described thus 25 far for any other pelycosaur taxa. Ophioacodon bone histology does not show well-developed

Haversian tissue. With regards to the histological record, our results remain inconclusive as to
the preferred ecology of *Ophiacodon*, but support the growing evidence for an aquatic
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phylogenetic extension perhaps the Late Carboniferous.

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32 Key words: Pelycosauria, Therapsida, Brinkman

33 1. Introduction

34 Ophiacodon (Marsh, 1878), which means "snake tooth", is a eupelycosaur that 35 belongs to Ophiacodontidae (Nopcsa, 1923) and was considered a primitive pelycosaur by 36 Romer and Price (1940). However, Ophiacodon has shown to be more derived osteologically 37 than varanopids, eothyridids, and caseids (Brinkman & Eberth, 1985; Reisz, 1986; Kemp, 38 2007). The earliest known synapsid fossils, dating back to the Carboniferous and found in 39 Nova Scotia, the Czech Republic, and the USA, are assigned to Ophiacodontidae (Reisz, 40 1972; Reisz 1975). Ophiacodon is considered a more derived member of the clade that existed 41 between the Late Carboniferous and the Early Permian. Most of the fossil remains have been 42 found throughout the Southwest of the United States, specifically New Mexico, Kansas, 43 Texas, and Oklahoma (Romer & Price, 1940; Vaughn, 1966; Vaughn 1969, Reisz, 1986). 44 Several gross- and micro-anatomical features of *Ophiacodon* have been suggested as evidence 45 for an amphibious or (semi-) aquatic lifestyle. These include an elongated narrow cranium, 46 lack of a fully fused braincase, lack of claws, disparity in hind limb and fore limb lengths, a 47 poorly ossified endochondral skeleton, and highly vascularized cortices in the long bones (Romer & Price, 1940; de Ricglès, 1974a; de Ricglès, 1974b; Reisz, 1986; Huttenlocker & 48 49 Rega, 2012; Felice & Angielczyk, 2014). However, it should be noted that Romer and Price (1940) did observe well ossified epiphyses in a few large Ophiacodon specimens. Felice and 50

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Angielczyk (2014) suggested that further histologic analysis of the pelycosaur-grade
synapsids is necessary to understand this delayed ossification phenomenon and how it could
reflect the lifestyle of *Ophiacodon*.

54 The early histologic studies of *Ophiacodon* long bones based ony scanty material all 55 concur that the histology differs by sharp contrast in vascularity, matrix organization, and 56 presence of growth marks from that observed in other 'pelycosaurs' (Enlow & Brown, 1957; 57 Enlow & Brown, 1958; Enlow, 1969; de Ricglès, 1974a; de Ricglès, 1974b). These authors invoked the proposed aquatic or amphibious lifestyle (Romer & Price, 1940) as an 58 explanation of these differences. Germain and Laurin (2005) addressed the ecology enigma by 59 60 quantifying the cortical porosity and comparing it to extant animals. Their results were 61 inconclusive. Enlow (1969), however, noted that the characteristics of Ophiacodon bone 62 tissue reflected fast skeletal growth, but he suggested this could just be the juvenile condition 63 as adequate detailed ontogenetic comparisons were lacking for pelycosaurs.

65 1.1 Brinkman's morphological stages of *Ophiacodon* humeral development.

66 Brinkman (1988) demonstrated that morphological ontogenetic stages (MOS) in 67 pelycosaurs can be defined on the basis of degree of ossification of the limb bones. Size alone 68 cannot be used as a proxy for reconstructing ontogenetic age in pelycosaur taxa because 69 individuals of the same size may represent more than one stage of development. However, 70 because of the delayed ossification observed in the epiphysis of Ophiacodon (Romer & Price, 71 1940), Brinkman was restricted as to what elements to use to test his hypothesis that the 72 ossification of the limb bones is a better means of interpreting relative age of an individual 73 than size. He chose the humerus because the complex articulations in the epiphyses exhibited 74 more than two stages of ontogeny.

75 Originally, Brinkman (1988) used 27 Ophiacodon humeri to denote five distinct 76 morphological ontogenetic stages; bone length was used as a proxy for size. All material came from localities in the Nocona Formation (Artinskian, formerly known as the Admiral 77 78 Formation), which includes the Rattlesnake Canyon (RSC) and the Briar Creek Bonebed 79 (BCBB) localities, as well as various localities in the Petrolia Formation (formerly known as 80 Belle Plains Formation) (Hentz, 1988). Here we summarize the criteria Brinkman (1988) used 81 to establish his five MOS (Fig. 1): 1) Three specimens were scored as MOS I, where both 82 proximal and distal ends of the humerus are concave, and the unfinished bone of the supinator process is confluent with the distal articular surface; 2) Five humeri were scored as MOS II, in 83 84 which finished bone separates the supinator process from the distal articular surface; 3) Eight 85 humeri were scored as MOS III, where the radial-ulnar surface is convex, although the 86 ectepicondyle and entepicondyle are concave; 4) Five humeri were scored as MOS IV, where 87 the radial condyle is well formed, and the proximal articular surface, the ectepicondyle, and 88 the entepicondyle are convex; 5) Six humeri were scored as MOS V, where it was observed 89 that finished bone separates the radial condyle from the ectepicondyle, the ulnar articular 90 surface from the entepicondyle, and the pectoralis process from the proximal articular surface 91 (Fig. 1).

As noted earlier, Brinkman (1988) used humeri from both RSC and the BCBB. He noted that although both bonebeds are in the same geological formation (Nocona Formation), he observed most of the humeri from the BCBB are between MOS II and MOS V and differ considerably in size from those of RSC (Fig. 2). This led Brinkman to suggest the presence of two populations of *Ophiacodon* in the same stratigraphic interval.

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98 1.2 Purpose of this study

99 In this study we have set out to obtain histological data by sectioning the *Ophiacodon* 100 humeral growth series figured by Brinkman (1988) (Fig. 1) and to compare the results to 101 isolated *Ophiacodon* bones from various localities ranging in age from the Late Carboniferous 102 to the Early Permian. Most importantly, we want to investigate what previous studies have 103 called "fast growing" tissue (Enlow & Brown, 1957; Enlow, 1969) as a possible overlooked 104 earliest occurrence of fibrolamellar bone in the tetrapod skeleton of basal synapsids. Finally, 105 the quantitative histologic results will be combined with the morphometric data to evaluate the hypothesis that Ophiacodon was semiaquatic (Felice & Angielczyk, 2014). 106

Institutional Abbreviations: IPBSH, Palaeohistology collection, Steinmann Institute of
Geology, Mineralogy, and Palaeontology, University of Bonn, Bonn, Germany; MCZ,
Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA; MSU,
Geology Department, Midwestern State University, Wichita Falls, TX, USA; OMNH, Sam
Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK, USA;
UMMP, Museum of Paleontology, University of Michigan, Ann Arbor, MI, USA.

115 2. Materials

The sample base for this study consists of a growth series of *Ophiacodon retroversus* humeri
from the RSC locality figured by Brinkman (1988), a single *Ophiacodon uniformis* humerus,
a single *Ophiacodon* sp. humerus, and four *Ophiacodon* femora from different localities (see
Table 1).

- 120 **2.1** Stratigraphic provenance of sampled material
- 121 2.1.1 Ophiacodon Humeri
- 122 2.1.1.1 Growth Series of Ophiacodon Humeri from RSC

123 Brinkman (1988) illustrated examples of the five stages of development seen in the 124 Ophiacodon humeri from an ontogenetic series of bones collected from RSC (Lower Permian, 125 Artinskian, Nocona Formation) that are housed in the MCZ collection (Fig. 1) (Hentz, 1988; 126 Sander, 1989). We obtained permission for sampling of the specimens representing MOS II to 127 MOS V (MCZ-5926, MOS II; MCZ-2819, MOS III; MCZ-4816, MOS IV; MCZ-1486, MOS 128 V) (Table 1; Fig. 1; Appendix 1A-E). MOS I, represented by humerus MCZ-1435 (Fig. 1A) 129 could only be measured but not histologically sampled. All specimens in Brinkman's study 130 are of Ophiacodon retroversus. Morphological terminology is based on Romer and Price (1940) and Brinkman (1988).

2.1.1.2 Briar Creek humerus

IPBSH-62: This left humerus pertains to O. uniformis (R. R. Reisz personal communication) and was obtained during a 2010 IPBSH excavation at the BCBB (Lower 136 Permian, Artinskian, Nocona Formation) (Table 1; Appendix 1F) (Case, 1915; Hentz, 1988). 137 A scapulocoracoid, probably of the same individual, was associated with this humerus. Based 138 on the criteria of Brinkman (1988), this humerus is an early MOS V even though unfinished 139 bone is still present on the edge of the entepicondyle, the ectepicondyle, and the tip of the 140 pectoralis process. The ulnar and radial surfaces are both well ossified, and finished bone 141 separates the pectoralis process from the proximal articular surface as defined by Brinkman (1988) for MOS V (See for example Fig. 1). 142

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144 2.1.1.3 Seminole humerus.

145 *OMNH-73698*: This right humerus is of *Ophiacodon* sp. and collected in Seminole,
146 OK, from the Pennsylvanian Vamoose Formation which is equivalent to the Lower Markley

Formation of Texas (May et al., 2011). The specific site is OMNH-V1518, nicknamed the
"Fixico Site" (Olson, 1977; Kissel & Lehman, 2002). It is difficult to assign a MOS to this
humerus based on the criteria set by Brinkman (1988) because its distal end is damaged;
however, what remains of the entepicondyle is highly rugose. The proximal end of the
humerus is convex, and the pectoralis process is separated from the proximal articular surface
by unfinished bone. Based on these observations, OMNH-73698 is a late MOS IV or early
MOS V (Table 1; Appendix 1G).

2.1.2 Ophiacodon Femora

OMNH-55234: This distal fragment of a right femur measures 100 mm in preserved length. The identification on the label as *O. mirus* is suspect due to the fragmentary nature of the bone. This specimen comes from the Upper Carboniferous Ada Formation (also equivalent to the Lower Markley formation mentioned earlier) in Seminole County, Oklahoma, from site OMNH-V1005, Site 4 (Table 1; Appendix 2B) (Olson, 1977; Kissel & Lehman, 2002; May et al., 2011).

OMNH-35389: This left femur is only identified as *Ophiacodon* sp. on the label and
 comes from the Lower Permian Garber Formation in Tillman County, Oklahoma, from
 OMNH-V716, Site 6 (Olson, 1967; Olson, 1977; Sander, 1989). Total length measures 221
 mm (Table 1; Appendix 2D). Due to the great size of this bone, it most likely belongs to *O*.
 retroversus, or a larger unknown species.

MSU uncatalogued: The only record accompanying this left femur is "Waurika Site",
a well known site in the Wellington Formation of Jefferson County, Oklahoma (Olson, 1977).
The overall length of this bone measures 115 mm, and the mid-diaphysis circumference is 55
mm. The adductor crest is damaged; thus, the circumference would have been larger in life.

We identified the specimen as *O. uniformis* based on Romer and Price (1940) and Reisz
(1986) (Table 1; Appendix 2C).

IPBSH-46: This right femur was obtained during the 2011 IPBSH excavation at the
BCBB (Lower Permian, Artinskian, Nocona Formation). The total length is 78 mm. Based on
this criterium alone we would have identified this specimen as *O. uniformis* (Romer & Price,
1940; Brinkman 1988) (Table 1; Appendix 2A). However, the size of the specimen suggests
that the femur belongs to a very immature individual of a larger species. For our purposes, we
simply identify it as *Ophiacodon* sp. due to the early ontogenetic stage. The only other
contemporaneous *Ophiacodon* species was the larger *O. retroversus*, which is known from the
Artinskian bone beds.

3. Methods

3.1 The Full Mid-diaphysis Cross-sectioning Method

The mid-diaphysis is the region of a long bone where the most complete record of growth is preserved. It also corresponds to the area of the smallest shaft circumference 185 (Francillon-Vieillot et al., 1990; Currey, 2002). Silicon molds (Provil NovoTM putty, Heraeus 186 Kulzer Technique) of all long bone diaphyses were created before sectioning to record the 187 original shape of the shaft region and aid in later reconstruction of the specimen. To prevent 188 splintering of the outer cortex, the region to be sectioned was encased in an epoxy resin putty 189 (Technovit Universal TM liquid and Technovit TM 5071 powder, Heraeus Kulzer 190 Technique). After curing of the putty, the bone was sectioned transversely in two cuts with a 191 rock saw equipped with a standard diamond-tipped blade, removing a slice of the diaphysis. 192 Humeral midshaft sections bisect the area where the medial head of the triceps muscle inserts, giving the cross section a distinct shape (Fig. 3A). Femoral sections bisect the area of the 193 194 adductor muscle attachment (Fig. 3B) (Romer & Price, 1940; Romer, 1969). After removal of 195 the slice, the epoxy resin putty is dissolved with acetone, and the bone is reconstructed by

filling in the mold with plaster, thus preserving the morphological and anatomical features of
the original material. After sawing, sections were ground to approximately 35 to 50 µm by
hand on a glass plate with wet grit (600 and 800) and sealed with a cover slip using UV
activated resin (Verifix TM LV 740, Bohle). The following specimens were sectioned using
this method: MCZ-5926, MCZ-2819, MCZ-4816, MCZ-1486, and IPBSH-62 (all humeri) and
OMNH-55234, IPBSH-46, and the MSU uncatalogued (all femora).

3.2 The Miniaturized Coring Method

This method is a miniaturized version of the coring method for histological sampling described by Stein and Sander (2009). While a full cross-section of the mid-diaphysis is preferred, coring increases sample size and permits access to more valuable specimens and those encased in large aggregations of matrix. It is crucial for the coring method that homologous locations are sampled in the different bones (Stein and Sander 2009). Material available for sampling while onsite at the OMNH was core drilled dorsally at the mid-211 diaphysis allowing for minimal damage to the specimen (humerus OMNH-73698 and femur 212 OMNH-35389) (Figs. 11 & 17). The direction of the long bone axis was marked on the bone 213 surface at the sample location by a line so that sample orientation could be maintained. Two 214 sizes of diamond-tipped coring bits were used (3 mm and 5 mm), and attached to a Dremel-215 type variable speed rotary tool mounted on a hand operated miniature drill press. Water was 216 used to lubricate the drill bit to reduce friction and prevent damage of the outer periosteal 217 tissue. The lubricant water was contained by building a small reservoir using plasticine (Fig. 218 4A). The core hole was later infilled with plasticine.

Cores were thin-sectioned in the Steinman Institute's paleohistology laboratory. Each
core was imbedded in a translucent Araldite 2020 epoxy-resin (Bodo Möller Chemie) and

221 allowed to harden for 24 hours before being sectioned by cutting the core perpendicular to the 222 long axis of the original long bone orientation (Fig. 4), as indicated by the mark drawn prior 223 to coring. The plane of section is thus the same as in the full transverse section of the mid-224 diaphysis. Thin-sectioning and slide preparation follow the same procedure as that described 225 above for bones cut in full cross-section.

Thin sections were permanently cover slipped and imaged in normal transmitted light and polarized transmitted light using a Leica DM2500LP Polarizing Microscope configured with a 360 degree rotating stage and polarizing and lambda filters. Digital images were acquired with a Leica DFC420 color camera and edited using the 2007 Leica IMAGE ACCESS EASYLAB 7 software (Leica, Wetzlar, Germany) (see Petermann & Sander, 2013). Overview images of thin sections were obtained in normal light with an EPSON V750 highresolution transmitted light scanner. Bone histological terminology follows Francillon-Vieillot et al. (1990) and Shelton et al. (2013).

Most slides are reposited at the IPBSH. Additionally, thin sections of all OMNH material are reposited at that institution.

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237 **3.3 Measurements**

238 It is common practice to perform morphometric analysis of the individual bone being 239 sampled preceding any histological work to procure the raw metric data before the bone is 240 damaged in any way (e.g., Sander & Klein, 2005; Sander et al., 2006; Klein & Sander, 2007). 241 Total length and minimal diaphysis circumference was recorded for each bone using standard 242 analytical calipers and a metric measuring tape (Table 1). Length was taken as the total 243 distance between the termination of the proximal and distal ends. Circumference was taken at 244 the mid-diaphysis (see Fig. 3). The ratio of the length and circumference was also calculated 245 for interspecific variation (Table 1).

Limb length disparity (LLD) has been used in earlier studies to interpret a semiaquatic habitat for *Ophiacodon* (Romer & Price, 1940; Kemp, 1987), however Felice & Angielczyk (2014) have shown this is unreliable. Regardless, the LLD ratio, specifically femur length divided by humerus length (femur/humerus), was calculated from a combined data set of *Ophiacodon* for the purpose of intraspecific comparison to other pelycosaur clades. These data were obtained from the literature (Romer & Price, 1940) and combined with measurements taken by the authors from specimens of different localities in the collections of the CFM, MCZ, and AMNH (Table 2).

3.4 Growth Curve

The Brinkman humeral growth series (Fig. 1) was used to construct a growth curve. Each cross section was hand drawn with a camera lucida and then traced onto clear translucent sheets. Subsequent overlays were correlated by matching cycle boundaries of successive MOS. This allowed for age estimates of the individual specimens at time of death, and the results are quantified in a growth curve (Fig. 5). This method is similar to that described by Bybee, Lee & Lamm (2006) to reconstruct growth curves from *Allosaurus* bones.

- 265 4. Results
- 266 4.1 Morphometry

Length and circumference data for each bone used in this study is compiled in Table 1. The average length to circumference (L/C) ratio of the sampled *Ophiacodon* humeri was 1.96 and for the femora was 1.91 (Table 1). However, the L/C ratio of the MOS V humerus from RSC (MCZ-1486) is different from the L/C ratio of the BCBB humerus (IPBSH-62) of the

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271 same stage. MCZ-1486 is 152 mm in length and 83 mm in circumference with a ratio of 1.83 272 (Table 1). IPBSH-62 is 82 mm in length and 37 mm in circumference with a ratio of 2.22. The 273 RSC humerus is 46% longer and the circumference is 55% larger than the BCBB humerus. 274 The average LLD ratio for *Ophiacodon* humeri and femora is 1.20 (Table 2). 275 Estimated lengths for the complimentary stylopodia have been calculated by multiplying 276 humerus length by the LLD ratio (1.20) and dividing femur length by the LLD ratio (1.20) 277 (Table 1). Most striking is the corresponding measurements of the smallest femur and humerus. The corresponding sizes for the largest humerus and femur were off by at least four 278 centimeters (see Table 1). It is unlikely the bones would have grown this much further in 279 length after reaching skeletal maturity. Measurements of additional articulated specimens will refine this ratio.

4.2 **Ophiacodon** humerus histology

The histology of all transversely sectioned humeri (Table 1) is described below by increasing MOS. All histology is described from the mid-diaphyseal section bisecting the triceps muscle insertion, a prominent feature in *Ophiacodon* (Romer, 1969) (Figs. 1 & 3).

288 4.2.1 Brinkman's ontogenetic series of RSC O. retroversus humeri

289 4.2.1.1 Histology of MCZ-5926 (MOS II)

290 MCZ-5926 (Fig. 2) is a right humerus 76.2 mm in length (Appendix 1A) with

unossified epiphyses exhibiting calcified matrix vesicles (Fig. 6B) that functioned to transport

292 hydroxyapatite crystals to facilitate the ossification of the hypertrophied cartilage (Anderson,

293 1969; Hall, 2005; Nair & Jagannathan, 2013), and a smooth cortical surface. In thin section,

the mid-shaft cortex is relatively thick and consists of parallel-fibered bone (PFB) and woven

bone (WB), prevalent dorsally and ventrally. Vascularization in the cortex is dense and

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consists of longitudinal and radial canals. In the dorsal and ventral regions of the outermost cortex, anastomosis is strongest between the longitudinal canals making the vascularity more of a reticular pattern in this area. The overall radial organization of the canals gives the cortical bone a "bicycle wheel" pattern (Fig. 6C & D). The vascular canals have varying degrees of lamellar bone (LB) infilling. Some have none while others are immature to fully formed primary osteons (Fig. 6E & F). By definition (Francillon-Vieillot et al., 1990), the combination of primary osteons set in a woven bone matrix identifies this tissue as fibrolamellar bone (FLB). Osteocyte lacunae (OL) are plump to sub-angular and appear to be randomly oriented in between the osteons, while others that follow the circumferential layering of the LB are flat.

The cortical bone contains a record of cyclical growth. Most notable is the division of the cortex by the neonatal line (NL) (Fig. 6A & C). This indicates the time when the animal hatched. The NL appears as an annulus with three corresponding lines of arrested growth (LAGs), indicating a period where growth slowed down dramatically. In this specimen, we 310 refer to the postnatal area beyond the NL as the outer cortex, and the prenatal area inside the 311 NL towards the medullary margin, the inner cortex (Fig. 6C). The average width of the 312 vascular canals inside the NL appears to be slightly larger than in the outer cortex. Two 313 previous cycles of slowed growth can be seen within the prenatal area of embryonic bone in 314 proximity to the boundary of the medullary cavity. These obviously formed while the animal 315 was in ovo, but the significance of these growth marks is unknown. One true LAG appears 316 near the outer bone surface, approximately 3 mm beyond the NL (Table 1). Sharpey's fibers 317 (SF) were not observed. This animal died very shortly after completing its first life cycle (Fig. 318 5).

The medullary region is distinguished from the cortex by small to medium-sized erosional cavities (EC), some of which are lined by a thin layer of lamellar bone. The EC extend into the inner cortex but do not go beyond the NL. Endosteal lamellar bone is presentin the form of primary, but mostly secondary, trabecular bone in the medullary cavity.

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324 *4.2.1.2 Histology of MCZ-2819 (MOS III)*

325 MCZ-2819 (Fig. 2) is a left humerus 97.22 mm in length with unossified epiphyses 326 (Appendix 1B). The calcified matrix vesicles (first observed in MCZ-5926, MOS II) are only 327 visible as darkened rings on the proximal articular surface (not pictured). The outer surface of the bone is also smooth. In the transverse thin section, PFB is prevalent on the posterodorsal 328 side, as well as in the outermost cortex at the triceps muscle attachment. WB is seen throughout the cortex, especially surrounding the vascular canals. Vascularity consists of small longitudinal canals from the mid to outer cortex, and the "bicycle wheel" pattern persists. In the deeper cortex, vascularity is more radial and canals are more open (Fig. 7C). The vascular canals have varying degrees of LB infilling; they are thus incipient to fully formed primary osteons (Fig. 7B). OL are plump to sub-angular as well as flat and oriented 335 parallel to the bone surface in the slower growing tissue.

The cortical bone contains two cycle boundaries (Table 1) marked by annuli. The most recent cycle is visible in the darkly stained area of the outer cortex (Fig. 7C). The NL is no longer visible as it has been removed by the resorption front. SF are located mostly on the anterior side and visible at the triceps muscle attachment (Fig. 7B). By correlating the earliest LAG with the first cycle boundary of MCZ-5926; MCZ-2819 is estimated to have died shortly after completing its second year of life (Fig. 5).

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344 The medullary cavity is occluded by a network of secondary trabeculae. EC with a345 lining of lamellar bone are abundant, having formed by expansion of the medullary cavity,

altering the pre- and neonatal areas observed in humerus MCZ-5926 (Fig. 6). Small to
medium-sized ECs mark the outer most boundary of the medullary region (Fig. 7C & D).

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349 4.2.1.3 Histology of MCZ-4816 (MOS IV)

350 MCZ-4816 (Fig. 2) consists of a pair of associated humeri that presumably came from 351 the same individual (Appendix 1C & D). Both are damaged at the mid-diaphysis, and the right 352 humerus is encrusted with a hematitic or limonitic matrix, which incorporates small bones from another animal (Fig. 8A & B). The two humeri themselves look yellowed and weathered 353 with a worn surface. Epiphyses look roughened and black. The left humerus, which was figured by Brinkman (1988), has a length of 122.06 mm. The right humerus is 124.61 mm in length. Histology of the two humeri is identical, reaffirming that they are from the same individual, and will be described together. Unfortunately, preservation of the histology is not optimal (Fig. 8C & E); most of histological detail has been lost due to the effects of weathering and diagenesis. Diagenetic staining has darkened the tissue, making LAGs, OL, 360 and even bone matrix nearly impossible to distinguish. LB can still be seen infilling some of 361 the vascular canals. However, immature or fully formed primary osteons cannot be 362 differentiated (Fig. 8E & F); the canals appear ragged and degraded taphonomically (Fig. 8C). 363 The "bicycle wheel" vascularity pattern is still visible in the cortex (Fig. 8D). PFB is 364 distinguishable in some patchy areas. WB could not be observed. Vascularity appears to 365 consist of small densely concentrated radial and longitudinal canals. The outermost cortex has 366 even thinner radial canals.

367 The preserved growth record consists of two cycle boundaries (Table 1) marked by annuli368 within the medullary region and inner cortex, mostly visible only in polarized light (Fig 8D).

369 By correlating the earliest annulus with the second growth mark of MCZ-2819, this animal is

370 estimated to have died between the third and fourth year of life (Fig. 5).

The medullary margin consists mainly of large ECs stretching into the cortex as they appear to follow the orientation of the vascular network. Interstitial primary cortical bone is incorporated as trabeculae due to the formation of large and small EC lined by lamellar bone (Fig. 8C & D).

- 375
- 376 4.2.1.4 Histology of MCZ-1486 (MOS V)

MCZ-1486 (Fig. 2) is a right humerus 152 mm in length with fully ossified epiphyses
(Appendix 1E). The outer surface is smooth with much rugosity in the epiphyses. In the midshaft thin section, the cortical bone is very thin (Fig. 9A & C) and vascularized by radial and longitudinal canals radially arranged, again forming the "bicycle wheel" pattern (Fig. 9C, D, E). WB is concentrated in the dorsal and posterior areas. PFB is seen throughout the cortex. Blackened SF appear in the anterior region. OL are smaller and more angular than in MCZ-2819. Primary and, rarely, secondary osteons are observed in the deep cortex (Fig. 9F)

The cortical bone contains a growth record consisting of four zones separated by four 385 LAGs (Table 1), as well as an external fundamental system (EFS) in the outer cortex (Fig. 9B, 386 C, D, E). The EFS itself represents at least eight years of growth and is clearly visible in this 387 specimen (Fig. 9B). Overlapping the growth cycles of this specimen with those of the next 388 smaller, MCZ-4816, and adding the record contained in the EFS, this animal lived at least 16 389 years (Fig. 5). The reduction of vascularity in the outermost cortex and the presence of the 390 EFS unequivocally indicate that the animal was sexually mature and had begun to slow down 391 in skeletal growth. The zones in the cortex are heavily vascularized by small longitudinal 392 canals that consist of primary osteons. The zone preceding the EFS has a reduced vascularity 393 and consists of very thin radial canals with almost no LB infilling (Fig. 9D).

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As noted, medullary expansion has nearly reached the surface. ECs as well as EC 395 lined with lamellar bone persist throughout most of the medullary region, forming trabeculae 396 of interstitial primary cortical bone (Fig. 9F).

397

398

4.2.2 Isolated Ophiacodon uniformis humerus IPBSH-62 (MOS V)

399 IPBSH-62 (MOS V; Fig. 2) is a left humerus 82 mm in length (Appendix 1F). The 400 cortex consists of WB and PFB. Vascularity consists of radial and longitudinal canals 401 exhibiting anastomosis (Fig. 10E). Immature and fully formed primary osteons are present in the outer cortex (Fig. 10E & F). Primary osteons and the more rare secondary osteons are observed in the deeper cortex. Interstitial primary cortical bone remains in the medullary margin as secondary trabeculae similar to what is observed in MCZ-4816 (Fig. 10A & B). Vascularity is more radial in the deeper cortex whereas the vascular canals near the cortical surface are more longitudinal (Fig. 10C). OL are plump to sub-angular in the areas with WB and more flat and oriented parallel to the cortical surface in the areas with PFB. There are very few SF, and those are only visible under polarized light (Fig. 10F).

409 Three annuli are visible in the mid to outer cortex (Fig. 10D), the inner two of which 410 contain LAGs (Fig. 10C & D). The earliest LAG is essentially the boundary between the 411 medullary region and the cortex (Fig. 10C & F), as ECs do not extend beyond this area. This 412 individual died before reaching skeletal maturity because there is no EFS. Thick LB lines the largest ECs in the center of the bone. The histology described here is similar to that described 413 414 for MCZ-4816 (MOS IV) (Fig. 2).

415

Isolated Ophiacodon sp. humerus OMNH-73698 (MOS V) 416 4.2.3

417 OMNH-73698 (MOS V) is a right humerus 113 mm in length (APPENDIX 1G) of

418 Ophiacodon sp. The histology described here is from a core drilled through the dorsal and 421 The cortex is thin, presumably because of the location of the sample away from the 422 midshaft. Vascularity appears to be reduced, consisting mainly of small primary osteons and 423 thin radial canals. These are similar to what is seen in the outer cortex of MCZ-1486. The 424 dorsal cortex is mostly PFB with flat OL oriented parallel to the bone surface and reduced vascularity (Fig. 11B). The ventral cortex is WB, and OL are large and round with a dense 425 concentration of SF (Fig. 11C). 426

The cortical bone contains an EFS visible in the outermost cortex (Fig. 11B & C); no other growth marks are visible. The medullary cavity consists of secondary trabecular bone with very little interstitial primary cortical bone (Fig 11A). There are ECs with and without a lining of lamellar bone in the medullary region that extend very close to the outer bone surface (Fig. 11B & C) because of the proximal location of the sample.

434

435 4.3 **Ophiacodon** femur histology

436 The *Ophiacodon* femora (Table 1) are described in order of increasing length (Fig. 3). 437 Overall shape of the cross section is affected by the increasing development of the adductor 438 crest with size, but no MOS have been erected for femora.

439

440 4.3.1 Isolated Ophiacodon sp. femur IPBSH-46

IPBSH-46 is a right femur 78 mm in length (APPENDIX 2A). This specimen is the 441 442 smallest femur sampled. The outer surface is smooth, and the epiphyses are unossified. The 443 bone is dorsoventrally crushed along the diaphysis (Fig. 12A) and distal end. Histology is

444 similar to what is seen in humerus MCZ-5926 (MOS II). The deep cortical bone consists of 445 embryonic or prenatal bone, which is mostly WB with large wide radial and longitudinal 446 vascular canals (Fig. 12B & E). The onset of PFB in the mid-cortex marks the NL (Fig. 12B 447 & E). Just beyond this in the outermost cortex is the start of a zone of fast growth with a WB 448 matrix and thin reticular vascular canals. The nutrient canal is located in the dorsal region 449 (Fig. 12A) of the shaft cross section. It extends from the prenatal cortex and the MC to the 450 outermost cortical layer (Fig. 12B). The periosteal bone is well vascularized in the outer 451 cortex by longitudinal and reticular canals. The posterior region, where the adductor crest is located, consists mostly of radial canals (Fig. 12A & C). The vascular pattern is longitudinal 452 and reticular in the postnatal area, but the vascularity of the prenatal area is more radial (Fig. 12E).

The vascular canals themselves have varying degrees of LB infilling in that most represent incipient primary osteons in the postnatal cortex, but there are many fully formed primary osteons in the prenatal areas (Fig. 12C & F). OL shape in prenatal WB are large and plump while those in the postnatal PFB are more flat and oriented parallel to the cortical surface. The tissue in the deep cortex thus is best called embryonic fibrolamellar bone.

The growth record contained in the cortex indicates that the individual most likely died within the first year of its life, shortly after hatching (Fig. 12B). The MC is obscured due to deformation; trabecular bone is not evident, but a few small ECs appear around the medullary cavity (Fig. 12A).

464

465 4.3.2 Isolated Ophiacodon mirus femur OMNH-55234

466 OMNH-55234 is an incomplete right femur (100 mm in length) (Appendix 2B). The
467 adductor crest is damaged (Fig. 13A). The cortical bone consists of WB and PFB alternating
468 as zones and annuli (Fig. 13D). The vascularity is radially arranged, similar to what is

observed in the humeri, forming an overall "bicycle wheel" pattern in the cortex (Fig. 13C &
D). Vascularity remains radial until it reaches the first LAG and then it changes to a more
reticular pattern (Fig. 13D & F). Primary osteons are present throughout the cortex (Fig. 13E
& F). OL are large and plump in the zones and more flat in the annuli (Fig. 13E).
The cortex contains a growth record of three zones separated by two LAGs set in
annuli (Fig. 13C & D), suggesting that the animal dies in its third year of life. It is worth

noting that the amount of WB decreases from the inner to the outer cortex (Fig. 13D). Also, in
the dorsal region of the outer cortex, perpendicular to the bone surface, the lining of the
nutrient canal extends deep into the medullary region (Fig. 13B).

The medullary region is occluded by secondary trabeculae and bound by large ECs in the process of complete resorption of the primary cortex and formation of secondary trabecular bone (Fig. 13A). A few ECs have appeared in the deep cortex. Endosteal resorption/redeposition is strongest in the posterior region. A free medullary cavity is present unlike what is observed in the humeri.

485

486 4.3.3 Isolated Ophiacodon sp. femur MSU uncatalogued

The MSU specimen is a left femur, 115 mm in length (Appendix 2C) with a damaged adductor crest (Fig. 14A). In the transverse thin section, it can be seen that the cortical bone consists of WB and PFB alternating as zones and annuli (Fig. 14B). The vascularization is reticular and longitudinal with sparse thin radial canals (Fig. 14C & D). OL range in shape from round to flat (Fig 14B). Incipient and fully formed primary osteons are visible throughout the cortex, but secondary osteons are concentrated near the borders of the medullary region (Fig. 14E & F). 494 An extensive growth record is contained in the periosteal bone. At least eight growth 495 cycle boundaries are marked by annuli (Fig. 14C & D). The cortex contains one large annulus 496 at the medullary boundary, but it is in the process of being resorbed. This could be the first 497 complete cycle of growth; although earlier growth marks could have been lost to resorption. ECs have already formed and extended to the fifth annulus. The nutrient canal in the dorsal region of the outer cortex is similar to those in OMNH-55234 and IPBSH-46 (Fig. 14A, C, D).

The medullary margin is bound by many ECs with and without a lining of lamellar bone that extend to the mid cortex. The MC is occluded by secondary trabecular bone (Fig. 13A). The concentration of secondary and primary osteons at the border of the medullary region is considered here to be a poorly defined Haversian tissue (Fig. 14E & F).

4.3.4 Isolated Ophiacodon retroversus femur OMNH-35389

OMNH-35389 is a left femur with an overall length of 221 mm (Appendix 2D); this is 508 the largest femur sampled. The histology described here is from a core drilled in the dorsal 509 side of the midshaft. The cortical bone consists of WB and PFB with alternating zones and 510 annuli (Fig. 15A & B). Vascularization is mainly restricted to the zones and is found in 511 reticular, radial, and longitudinal patterns, decreasing in size from the deep inner cortex to the 512 outer cortex (Fig. 15C). Vascular canals are more radial in the deeper primary cortex and 513 become more reticular towards the outer surface (Fig. 15A). OL are plump in the deep cortex 514 and in the zones, but they are flat in the annuli and EFS (Fig. 15C). Primary osteons are 515 mostly incipient in the outer cortex, but fully formed osteons are observed in the deeper 516 cortex.

517 The cortical bone contains eight growth cycles ending in annuli (Fig. 15A) and one 518 very prominent LAG marking the start of the EFS in the outermost cortex (Fig. 15C). Any

growth cycles earlier than the one ending in the innermost annulus have been resorbed (Fig. 15B). This is the only femur to exhibit an EFS; however, the exact number of growth cycles in the EFS remains unknown due to the diagenetic staining (Fig. 15C). The EFS indicates that the animal had reached skeletal maturity. The medullary cavity appears to be open with extensive ECs extending into the third growth cycle. Secondary trabeculae are present. There is much more endosteal resorption and redeposition of cortical bone in contrast to what was observed in the humeri.

4.4 Ontogenetic age and growth curve

Growth mark count and age estimate for each of the four O. retroversus humeri studied by Brinkman (1988) are provided in Table 1. The first fully completed growth cycle is still visible in the smallest humerus sampled (MCZ-5926; MOS II) (Fig. 6C). This cycle is the area between the neonatal line (NL) and the first growth mark in the outermost cortex. By using a method similar to that of Bybee, Lee & Lamm (2006), we estimate that this growth 533 series represents seven years of growth between the time of hatching and the year skeletal 534 maturity was achieved. At least another eight cycles were visible in the external fundamental 535 system (EFS) of MCZ-1486 (see Fig. 9B). Assuming the cycles in the EFS are annual, this 536 means that the largest Ophiacodon in our sample grew substantially for half of its life. 537 However, we cannot assume that we sampled the oldest individuals. The estimated total 538 lifespan represented by this growth series is at least 16 years. However, due to preservation 539 and shape discrepancies observed throughout humerus ontogeny, a proper figure using the 540 Bybee, Lee & Lamm (2006) method was not possible. This method revealed a discrepancy 541 between MOS IV (MCZ-4816) and MOS V (MCZ-1486) because of the uncertain matching 542 of the absent fourth cycle in MOS IV, and the third cycle boundary in MOS V, which is 543 assumed to have been resorbed. This results in a margin of error of at least a one year. The

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time represented by Brinkman's (1988) *O. retroversus* growth series (Fig. 1) is consistent
with the growth record preserved in some of the *Ophiacodon* femora (MSU uncatalogued and
OMNH-35389) (see Figs. 14 & 15).

547

548 5. Discussion

549 In this study we focus on the long bone histology of the basal synapsid Ophiacodon from Texas and Oklahoma localities of various ages in order to investigate what previous studies have called "fast growing" tissue as a possibly overlooked earliest occurrence of fibrolamellar bone in the lineage leading to mammals. Included in this sampling is Brinkman's (1988) figured Ophiacodon humeral growth series from RSC. The sectioning of this ontogenetic series served two purposes: first, to answer the call for proper ontogenetic sampling of pelycosaur material as to ascertain data relevant for explaining the dense vascularity of the cortical bone. Additionally, this sample set allowed us to test Brinkman's (1988) hypothesis of morphological ontogenetic stages for a single species, O. retroversus. If 558 the material represents a growth series of a single species, then histology needs to correlate 559 with the morphologic stages of development assigned to these bones (Figs. 1 & 2). Finally, an 560 attempt was made to constrain the ecology of *Ophiacodon* using qualitative parameters of 561 bone histology.

562

563 5.1 Synthesis of histology

In general, the bone tissue of *Ophiacodon* is a mixture of WB and PFB alternating as zones and annuli. LB is restricted to the osteons and trabecular bone. The cortical bone is well vascularized. All humeri, including the latest Carboniferous-age femur OMNH-55234 and the prenatal cortex of IPBSH-46 consists of radially arranged longitudinal and radial canals. The organization of the canals gives the cortical bone a "bicycle wheel" pattern (See Figs. 6-10 &

23

14). The other femora sampled, including the postnatal cortex of IPBSH-46, consisted of longitudinal and reticular vascular canals with varying degrees of LB infilling. True primary osteons are prevalent in all bones. A few secondary osteons were only observed in the deep cortex of the largest sampled humerus (MCZ-1486, Fig. 9F), but they are not present in the largest femur (OMNH-35389). Dense secondary osteons, the best evidence of Haversian tissue, were seen in the smaller femur from MSU (Fig. 14E & F).

575 Osteocyte lacunae in the primary tissue of Ophiacodon are mostly plump while others that follow the circumferential layering of the LB are basically flat. The medullary cavity of 576 the humeri is completely occluded with secondary trabeculae in contrast to the open MC of the femora. Mostly, the humeri and femora differ in resorption patterns, which affects the preserved growth record. In humeri, erosional cavities follow the radial organization of the vascular network and incorporate areas of primary cortical bone into the medullary region as trabeculae bound by lamellar bone. Femora completely resorb the primary cortex and develop a more open MC. Resorption and endosteal deposition seem to be occurring at a much slower 583 rate than the periosteal deposition, resulting in a better preservation of the growth record in 584 femora. In addition, as observed in humeri, primary and, more rarely, secondary osteons 585 develop within the vascular canals in the deeper isolated parts of the primary cortex. One 586 feature that seems consistent in the *Ophiacodon* femora, regardless of geologic age or size, is 587 the dorsal position of the nutrient canal (Figs. 12-14). This has not yet been consistently 588 observed in the other pelycosaur taxa. Presence of the nutrient canal in the transverse sections 589 reaffirms the accuracy of our sampling location which was chosen on the premise that the 590 minimal diaphyseal circumference (Fig. 5) is the neutral zone where growth started. 591 The histology of the Ophiacodon retroversus humeral growth series does indeed

correlate with the MOS assigned by Brinkman (1988) and contains histological evidence of
progressive growth from a juvenile stage to fully grown adult (Figs. 2 & 5). The smallest

sampled humerus MCZ-5926 (MOS II) (Table 1, Fig. 1) contains a neonatal line recording the event of hatching. All humeri sampled, as noted earlier, contain a highly vascularized cortex of woven and parallel-fibered bone incorporating numerous primary osteons. Through the progressive stages of growth, the medullary region expands through resorption, and secondary trabeculae form from remodeling of primary cortex. The largest humerus in the growth series, MCZ-1486 (MOS V) (Fig. 2), contains an external fundamental system, indicating that the animal had reached skeletal maturity.

However, it should be noted that humerus IPBSH-62, which also is MOS V and thus is also is in the late stages of full ossification, lacks an EFS. Therefore, this animal died slightly before reaching skeletal maturity, indicating that MOS V has a lower resolution for indicating skeletal maturity than does bone histology. This is consistent with the observation of Brinkman (1988) that bones of different length are classified in the same MOS (Fig. 2).

The growth curve constructed from the *O. retroversus* growth series supports the hypothesis that these animals grew relatively fast compared to other pelycosaur (Shelton 2014) and modern squamates (Fig. 5). The life history of *Ophiacodon* seemingly consisted of a period of relatively fast growth followed by a period of equal or greater length were only minimal or no growth took place. Relatively fast growth is also suggest be the nature of the growth cycle boundaries which are mainly developed as annuli, with LAGs being only seen in the latest cycles. Thus, growth may have been too fast in *Ophiacodon* to produce true LAGs before the animals were well advanced in age.

614

615 5.2 Fibrolamellar bone in *Ophiacodon*

616 Based on our sampling we can affirm that true fibrolamellar bone exists in

617 Ophiacodon and that the dense vascularized woven bone is not just a simple reflection of

618 immaturity, but was laid down during the entire period of growth. Our results corroborate the

619 findings of Enlow and Brown (1957) and Enlow (1969) that the histology of *Ophiacodon* long
620 bones is indicative of rapid skeletal growth.

Fibrolamellar bone is defined as woven bone matrix in which the vascular canals are filled in centripetally by lamellar bone, forming primary osteons. Enlow (1969) concluded the specimen he analyzed must have been a juvenile because of the extreme difference from the other pelycosaurs; however, at the time, proper ontogenetic sampling of this group had not yet occurred in order to support this statement. De Ricqlès (1974a, p. 63) compared *Ophiacodon* cortical bone to that of therapsids, noting a similarity in the dense vascularization, but he concluded that this histology must reflect the aquatic lifestyle of *Ophiacodon*, further noting a similarity to crocodiles and plesiosaurs.

However, ours is not the first time that FLB has been described pelycosaurs.
Huttenlocker, Angielczyk & Lee (2006) and Huttenlocker and Rega (2012) suggested that
FLB occurs in *Sphenacodon ferocior* (Synapsida: Sphenacodontidae), without providing
conclusive evidence, however. Shelton et al. (2013) noted incipient FLB in *Dimetrodon natalis*. FLB was also described in the neural spines of sphenacodontids and edaphosaurids by
Huttenlocker and Rega (2012), Huttenlocker, Rega & Sumida (2010), and Huttenlocker,
Mazierski & Reisz (2011). Previously, the earliest unequivocal occurrence of true FLB in
synapsid was found in the basal therapsid clade Dinocephalia (de Ricqlès, 1972; de Ricqlès,
1974a).

638

- 639 5.3 Comparison of long bone histology of *Ophiacodon* spp. and *Dimetrodon natalis*
- *Ophiacodon* is not as basal among Synapsida as once thought but is the sister clade to
 Edaphosauridae and the clade consisting of Sphenacodontidae and Therapsida or the like,
- 642 (Romer & Price, 1940; Kemp, 1987, 2007). Remarkably, *Ophiacodon* is histologically more
- 643 derived along the path to fast growth than the sphenacodontid *Dimetrodon*. In

644 sphenacodontids, there is an incipient form of FLB where osteons remain immature 645 throughout ontogeny and the cortex contains more PFB than in Ophiacodon (Shelton et al., 646 2013). Otherwise, *Dimetrodon* and *Ophiacodon* humeri differ mainly in the patterns of 647 resorption and remodeling in which primary bone is incorporated as trabeculae in the 648 Ophiacodon MC, but in Dimetrodon primary bone is completely resorbed during the MC 649 expansion process. Also, while the "bicycle wheel" vascular pattern has been described in 650 both the humerus and femur of *Dimetrodon* (Shelton et al., 2013), it consistently only occurs in the humerus in the Ophiacodon. Only two femora show it: the Carboniferous one (OMNH-651 55234) and the embryonic tissue of IPBSH-46. In comparison to the growth trajectory of D. natalis (Shelton et al., 2013, Fig. 10), it is clear that O. retroversus reached skeletal maturity much earlier, but the life expectancy of both seems to have been similar (Fig. 5).

5.4 **Ophiacodon** ecology: terrestrial, amphibious, or aquatic?

We cannot infer an aquatic habitat for Ophiacodon based solely on histologic results. 658 Previous studies suggested that the dense vascularization of the periosteal bone is evidence of 659 an amphibious lifestyle (de Ricqlés, 1974a; de Ricqlés, 1974b; de Ricqlés 1976; Germain & 660 Laurin, 2005). However, the specific "bicycle wheel" pattern formed by the radially arranged 661 longitudinal and radial vascular canals has been noted in the limb bones of both extinct and 662 extant aquatic, semi-aquatic, and terrestrial amniotes. These include but are not limited to the following taxa: Sphenacodontidae (Shelton et al., 2013), Varanopsidae (Huttenlocker & Rega, 663 664 2012), and the therocephalian therapsids Notosllasia and Theriognathus (de Ricqlès, 1975; 665 Huttenlocker & Botha-Brink, 2014). Also, this pattern has been recognized in several taxa in 666 the marine reptile clade Sauropterygia, specifically in some Placodontia and some 667 Eusauropterygia, where this pattern is specific only to certain morphotypes (see Klein, 2010; 668 Krahl et al., 2013). The "bicycle wheel" vascular pattern is also prevalent in large modern

varanids such as *Varanus komodoensis* (Reid, 1984) and the semi-aquatic Nile monitor lizard, *Varanus niloticus* (de Buffrénil & Francillon-Vieillot, 2001; de Ricqlès, Castanet &
Francillon-Vieillot, 2004). The "bicycle wheel" pattern has also been observed in *Edaphosaurus* humeri (Shelton 2014). The partial *Ophiacodon* radius described by de Ricqlès
(1974b, 1978) does not possess this "bicycle wheel" pattern; instead longitudinal primary
osteons are randomly arranged within a PFB matrix.

675 Huttenlocker and Rega (2012) suggested that the organization of Ophiacodon bone histology is most likely a reflection of both the growth pattern and lifestyle. We suggest additional geochemical and isotope analyses of Ophiacodon teeth to definitively resolve the preferred habitat enigma (for example see Fischer et al., 2013). De Margerie et al. (2004) point out that radial vascularization is most resistant to shear forces. Perhaps the "bicycle wheel" vascular pattern is a response to specific biomechanical forces acting upon the stylopodium of Ophiacodon given that these bones have the largest muscle attachment points of any of the pelycosaurs. The exception is the edaphosaurid femur, which shares an extended adductor crest with Ophiacodon (Romer & Price, 1940). Further analysis is required to assess 684 the significance of the pattern of cortical vascularity, including further sectioning of the 685 zeugopodium and autopodium because it appears to vary in vascularity from the "bicycle 686 wheel" pattern found in the stylopodium.

687

688 6. Conclusion

By sampling Brinkman's (1988) *Ophiacodon* ontogenetic series (Figs. 1 & 2), as well as additional material (Appendix 1 & 2), we have confirmed the presence of true fibrolamellar bone in the postcrania of pelycosaurs which Enlow and Brown (1957, 1958) first described as "fast growing tissue". The highly vascularized cortex is not simply a reflection of immaturity nor is it direct evidence for an aquatic lifestyle. Thus, our study does not resolve the questionof a potentially (semi-) aquatic habitat of *Ophiacodon*.

Furthermore, the tissue we describe here possesses the classic histological characteristics of the text book definition of FLB (Francillon-Vieillot et al., 1990). In general, the compacta consists of primary osteons in a woven bone matrix. Overall, the radially vascularized FLB tissue we have described in *Ophiacodon* is more derived in terms of the osteonal development, bone matrix, and skeletal growth then what has been described thus far in any other pelycosaur taxa, pushing back the origin of FLB in the Synapsida by 20 million years from its previous oldest occurrence in basal therapsids.

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- 864
- 865
- Table 1: Dimensions and growth mark count of the sectioned *Ophiacodon* long bones.
- 867 Abbreviations: C=circumference; EFS= external fundamental system; HOS= histological

35

868 ontogenetic stage; L= length; LAG= line of arrested growth; LLD= limb length disparity;

869 NL= neonatal line; MOS= morphological ontogenetic stage.

870

Table 2 Measured specimens used to calculate the femur/humerus ratio (1.20) taken from the

- 872 literature and combined with data collected by the authors at the CFM, MCZ, and AMNH
- 873
- 874

Figure 1 Morphology and histology of ontogenetic series of *Ophiacodon* humeri figured by 875 875 876 877 878 878 879 880 881 Brinkman (1988) and used in this study A) Ontogenetic series of Ophiacodon humeri from the Rattlesnake Canyon locality in Archer County, Texas, from Brinkman (1988) illustrating his morphological ontogenetic stages (MOS) I to V. Each humerus is shown in ventral and distal view and is a right one except when noted otherwise. I = MCZ-1435, II = MCZ-5926, III = MCZ-2819 (reversed), IV = MCZ-4816 (reversed), V = MCZ-1486. B) Photographs of the specimens figured by Brinkman (1988) with the exception of MCZ-1435 (MOS I) which 882 was not available for this study. The white area in the mid-diaphysis represents the volume of 883 bone sectioned for histological analysis and reconstructed with plaster. Each bone is shown in 884 ventral and distal view. C) Corresponding thin sections from the mid-diaphysis of the four 885 humeri of MOS II to V. Abbreviations: ect= ectepicondyle; ent= entepicondyle; pect= 886 pectoralis crest; rad= radial articular surface; sup=supinator process; tri= triceps muscle 887 insertion.

888

Figure 2 Graph showing the relationship between size and MOS of *Ophiacodon* humeri from
the Briar Creek Bonebed (black diamonds) and other localities (open diamonds) from the
Petrolia and Nocona formations. The latter includes the sectioned Rattlesnake Canyon humeri.
These humeri, as well as IPBSH-62, are marked on the graph. The remaining data points

cannot be labeled because Brinkman (1988) did not identify specimens used in the plot. Note
the arrow indicating that IPBSH-62 (MOS V) and MCZ-4816 (MOS IV) both have a similar
histology with the exception of better developed primary osteons in MCZ-4816. Graph is
inverted and modified from Brinkman (1988, figure 7).

897

Figure 3 Illustration of an *Ophiacodon retroversus* humerus (A) and femur (B) with
indications of how and where the minimal diaphysis circumference (plane of section) and
total length were measured. (Modified from Reisz 1986).

Figure 4 Miniaturization of the coring process. A) The miniature hand-operated drill press is set up with a variable speed rotary tool. It is important to keep the core bit lubricated with water to prevent drill bit overheating. Arrow is pointing to a water bath constructed around the drilling site with plasticine. B) Operation of the drill press while drilling a pelycosaur bone. Light pressure is applied to the drill while in operation to facilitate a slow, steady pace. 907 It is important to drill into the cortex slowly because the outer cortex may be sheared off if 908 drilled too quickly. C) The results of the coring operation on femur OMNH-35389. The core 909 was drilled on the dorsal side of the midshaft at the minimal diaphysis circumference. This 910 core sample is further processed in the lab. Arrow is directed from drill site to the resulting 911 core sample is indicated by the oval. D) The final core sectioned transversely after being 912 embedded in a clear epoxy and before being processed into a thin section.

913

914 Figure 5 Ophiacodon retroversus growth curve, with specimens representing each MOS,

915 constructed by plotting overall bone length and individual age at time of death for each

916 humerus. Age was calculated by correlating subsequent overlays of each MOS and matching

917 successive cycle boundaries, resulting in an asymptotic growth curve. MCZ-1435 (MOS I)

918 was included as the earliest morphological stage of the ontogenetic series (black diamond); 919 however, it could not be sectioned. Based on the histology of the next larger specimen, we assumed that the bone is from a young animal less than one year old that died shortly after hatching. All other humeri represented by white diamonds were sectioned and recorded in Table 1. Included here is the percentage increase of length and minimal diaphysis circumference between each successive MOS. Length seems to increase steadily throughout ontogeny, but circumference increases quite rapidly, peaking at 38 percent between MOS III and MOS IV. MCZ-2819 (MOS II) is a humerus from an individual that died shortly after completing its first life cycle marked by an annulus. The neonatal line (NL) is still visible in the deep cortex, but was destroyed by resorption in the higher MOS. MCZ-5926 (MOS V) contains many LAGs but no annuli, which correspond to the slowed growth. This is also evident by the reduction of vascularization and the presence of an external fundamental system (EFS) in the outermost cortex. The EFS indicates that this individual had reached skeletal maturity and lived at least for another eight years.

933 Figure 6 Humerus MCZ-5926, MOS II, of O. retroversus. A) Scan of transverse section 934 through the mid-diaphysis in normal light. Notice the "bicycle wheel" pattern formed by the 935 radial arrangement of the vascular canals. Note that the radial canals are in the plane of 936 section and extend from the medullary cavity to the outer cortex. The medullary cavity is 937 occluded with mostly secondary (some primary) trabeculae, and erosional cavities (EC) are 938 present in the medullary margin area. Note that EC do not extend past the neonatal line (NL) 939 (indicated by arrow), which is a line of slowed growth marking the time of hatching, forming 940 the boundary between the prenatal embryonic bone inside, and the postnatal periosteal bone 941 beyond. Also, the size of the vascular canals is larger in the prenatal area and smaller in the 942 postnatal area. B) Close-up of the distal epiphysis with exposed calcified matrix vesicles. C)

943 Photomicrographs of the mid-diaphyseal cortex viewed in normal transmitted light magnified from an area marked by a box in (A). The large arrow indicates the NL. Note the two prenatal growth mark just beyond the medullary cavity. The small arrow indicates the LAG approximately three millimeters beyond the NL. D) Same view as in (C) but in polarized transmitted light. Vascular canals are more distinctive in polarized light. The "bicycle wheel" pattern is very prominent cause by the extinction pattern of the lamellar bone infilling. Also, EC are clearly distinguishable. Parallel-fibered and woven-bone matrix are found throughout the cortex. The arrow indicates the position of the NL. E) Microscopic close-up of the NL indicated by the boxed area in (C) in normal transmitted light. Notice the three closely spaced growth marks within the NL (indicated by arrows). At MOS II, EC have not yet crossed the NL. Note the plump shape and high density of the OL. F) Same view as in (E) but in polarized transmitted light. Note the mix of incipient and fully formed primary osteons set in a wovenbone matrix, thus forming fibrolamellar bone. Abbreviation: tri= triceps muscle insertion.

957 Figure 7 MCZ-2819, MOS III, O. retroversus humerus. A) Scan of transverse section through 958 the mid-diaphysis in normal light. The NL has been completely resorbed by expansion of the 959 medullary cavity. Radial and longitudinal vascular canals remain consistent with the "bicycle 960 wheel" pattern. The EC have greatly increased throughout the medullary margin. Growth 961 marks are preserved in the darkly iron-stained areas of the cortex. B) Photomicrograph of the 962 outer cortex in normal transmitted light. Arrow points to Sharpey's fibers. C) Microscopic 963 view of the mid-diaphysis cortex in normal transmitted light. Note that the expansion of the 964 medullary cavity has erased all traces of the prenatal bone. Also, the vascular canals appear to 965 decrease in size towards the outer cortex. The approximate location of the two annuli are 966 denoted by the letter "A". D) Same view as (C) but in polarized transmitted light. The "bicycle

967 wheel" pattern is better visible given the extinction pattern of the lamellar bone infilling in 39

contrast to the WB matrix. E) Microscopic view of the outer cortex in normal transmitted
light. The darker iron-staining preserved growth marks just below the cortical surface,
marking the end of the second growth cycle in the *Ophiacodon* humeral growth series. F)
Same view as (E) in polarized transmitted light. Note the inner annulus of the first year
growth cycle. Also the annulus corresponding with the visible growth marks in (E) marks the
second year growth cycle. Abbreviations: A= annulus; tri= triceps muscle insertion.

Figure 8 MCZ-4816, left and right humerus of a MOS IV O. retroversus individual. Both of 975 these bones have been affected by recent physical and chemical weathering. A) Scan of the transverse section through the mid-diaphysis of the left humerus. Mid-shaft is damaged. The cortical bone is thinner and the EC are much larger than they were in the previous stage of development, stretching across the cortex. The radial arrangement of vascular canals is seen even in the bone last deposited. B) Scan of the transverse section through the mid-diaphysis of the right humerus. This bone was also incrusted with hematitic or limonitic matrix that 982 incorporated long thin bones from another animal (visible on the left). Mid-shaft is also 983 damaged. C) Photomicrograph of the mid-diaphysis cortex as indicated by the boxed area in 984 (A) in normal transmitted light. The EC appear to be following the pattern of the vascular 985 canals. As the EC develop, they isolate areas of primary cortex that are being incorporated as 986 trabeculae in the medullary cavity and are bounded by LB. D) Same view as (C) but in 987 polarized transmitted light. The interstitial cortical bone is better visible in polarized light. But 988 because of the poor preservation, the polarized light image gives a false positive for WB as 989 most of the affected matrix remains dark due to the alteration of the bone apatite crystallites. 990 This has caused areas of PFB and most areas of LB to be obscured. Arrow marks the second 991 year cycle boundary that was resistant to resorption. Also, the third year cycle boundary is visible as a thick annulus in the middle cortex marked by "A". E) Magnified view of the 992

cortex indicated by the boxed area (C), in normal transmitted light. The bone matrix is
obscured by taphonomic staining rendering proper identification of the specific type of bone
matrix difficult; however, it is most likely WB and PFB. F) Same view as in (E) but in
polarized transmitted light. Again, most of the LB of the primary osteons lacks birefringence
because of poor preservation. Abbreviations: A= annulus; tri= triceps muscle insertion.

999 Figure 9 MCZ-1486, MOS V, O. retroversus humerus. A) Scan of the transverse section 01000 through the mid-diaphysis. Overall shape of this section is affected by the prominent triceps 1001 1002 1003 1004 1005 muscle insertion. Vascularity is highly reduced in the outer cortex but still maintains the "bicycle wheel" pattern of longitudinal and radial canals. B) Photomicrograph of the boxed area in (A) showing the EFS in normal transmitted light with at least eight growth cycles. C) Microscopic view of the mid-diaphysis cortex in normal transmitted light. While retaining a radial orientation, the vascularity diminishes from the medullary cavity to the outer cortex where it is almost nonexistent in the EFS. ECs have not yet reached the outer cortex, but they 1006 1007 seem to be following the same pattern, transforming the cortex into cancellous bone with 1008 interstitial primary compact bone. D) Same view as (C) in polarized transmitted light. The 1009 outwards decreasing vascularity is better observed in this light. ECs are lined by a thin layer 1010 of LB. E) Photomicrograph of the outer cortex as indicated by the boxed area in (A) in normal 1011 transmitted light. The growth record preserved here consists of four cycles ending in LAGs, 1012 representing years 4 to 7. Note that most of the LAGs are double. Skeletal maturity was 1013 reached after the seventh life cycle. F) Photomicrograph of boxed area indicated in (A) in 1014 polarized transmitted light with lambda filter. This area is just beneath the triceps muscle 1015 insertion. Primary woven cortex is persists, and OSL are large and numerous. Abbreviations: 1016 EC= erosional cavity; EFS= external fundamental system; IPO= incipient primary osteons;

PO= primary osteons; ECL= erosional cavity with a thin lining of lamellar bone; SO=
secondary osteon; tri= triceps muscle insertion.

1019

1020 Figure 10 IPBSH-62 MOS V, O. uniformis humerus. A) Scan of the transverse section 1021 through the mid-diaphysis. The triceps muscle insertion is much smaller than what is seen in 1022 O. retroversus at MOS V. ECs are in the mid to lower cortex but do not yet cross the 1023 innermost LAG. Histology is similar to what was described in MCZ- 4816. Note the lack of ¹⁰²⁴ an EFS. B) Photomicrograph of boxed area indicated in (A) viewed in polarized light and a 1025 1026 1027 1028 1028 lambda filter. Large areas of interstitial primary cortex of WB are incorporated into secondary trabecular bone. Primary osteons are present here in the oldest areas of the cortex. C) View of the boxed area in (A) in normal transmitted light. The "bicycle wheel" pattern is present in the cortex, consisting of radial and longitudinal canals with a varying degree of anastomosis. Arrow is pointing to the LAG. D) Same view as (C) in polarized light. Arrows indicate annuli. E) Photomicrograph of boxed area indicated in (C) viewed in polarized light and a lambda 1030 1031 filter. Note the combination of both incipient and primary osteons. Arrow points to the LAG. 1032 F) Photomicrograph of boxed area in (C) in polarized transmitted light. Arrows indicates 1033 Sharpey's fibers and the LAG. Overall the cortical matrix consists of WB. Abbreviations: A= 1034 annulus; IPO=incipient primary osteon; PO= primary osteon; tri= triceps muscle insertion; Z= 1035 zone.

1036

Figure 11 OMNH-73698, MOS V, *Ophiacodon* sp. humerus. A) Scan of a transverse core section drilled through the dorsal and ventral sides of the diaphysis close to the proximal metaphysis. The cortex is thin and vascularity is highly reduced in the dorsal cortex. The ventral cortex contains small primary osteons and larger incipient osteons. Notice the EFS and that the vascular canals are smaller on the dorsal side and near the EFS. The medullary cavity is occluded by secondary trabeculae. B) Photomicrograph of the EFS in the dorsal cortex in
polarized transmitted light. The cortical bone matrix of the dorsal side is mostly PFB with a
strong LB affinity. C) Magnified view of the ventral cortex in normal transmitted light. This
area is highly concentrated with Sharpey's fibers. Osteocyte lacunae are rounder and
randomly oriented. The cortical bone matrix below the EFS on the ventral side is WB.
Abbreviations: EFS= external fundamental system; PO= primary osteon.

1048

<mark>ي</mark>1049 Figure 12 IPBSH-46, Ophiacodon sp. femur, 78 mm in length. A) Scan of the transverse 1050 1051 1052 1053 1054 (above) and longitudinal section (below) of the mid-diaphysis. Even though the mid-shaft is crushed, there is a marked distinction between the prenatal and postnatal cortex. Arrow indicates the nutrient canal on the dorsal side. B) Photomicrograph of the dorsal cortex in normal transmitted light. Arrow indicates the nutrient canal that extends into the prenatal cortex. Vascular canals in the prenatal bone are larger than those in the postnatal bone. Vascularity consists of longitudinal, radial and reticular canals. Vascularity in the deep 1055 1056 prenatal cortex somewhat resembles the "bicycle wheel" pattern seen in the humeri. However, 1057 this is not the case in the postnatal cortex as the vascularity is more reticular. This animal was 1058 within the first year of its life when it died. C) Photomicrograph of boxed area in (A) of the 1059 longitudinally sectioned adductor crest in polarized transmitted light. Note the high 1060 concentrations of PO in the WB matrix. D) Photomicrograph of boxed area in (A) of the 1061 longitudinally sectioned diaphysis in polarized transmitted light. Note the extreme vascularity 1062 throughout the bone tissue. E) Photomicrograph of boxed area in (B) in polarized transmitted 1063 light. Most striking is the sudden change from the PNW in the inner cortex and the PNP in the 1064 mid- to outer cortex. Incipient primary osteons are prevalent in the outer cortex. Note the high 1065 vascularity in the prenatal bone. F) Photomicrograph of the boxed area in (C) in polarized 1066 transmitted light. Note the large primary osteons and OSL set within a WB matrix. This tissue

is characteristic of embryonic fibrolamellar bone. Abbreviations: ac= adductor crest; PNP=
postnatal parallel-fibered bone; PNW= prenatal woven bone.

1069

1070 Figure 13 OMNH-55234 O. mirus femur 100 mm in length. A) Transverse section through 1071 the mid-diaphysis. The midshaft is damaged and the adductor crest is incomplete 1072 (reconstruction of the missing area is general and not an accurate morphological 1073 reconstruction). Arrow is pointing to the location of the nutrient canal. B) Photomicrograph of ഗ1074 the dorsal cortex in normal transmitted light. Arrow is pointing to lining of the nutrient canal. 1075 1076 1077 1078 1078 1079 1080 Surrounding vascularity is reticular C) Microscopic view of the mid-diaphysis cortex in normal transmitted light of the boxed area in (A). Double-headed arrow is pointing to the LAGs. D) Same view as in (C) but in polarized transmitted light. Note the radial and longitudinal canals form the same "bicycle wheel" pattern as that seen in the humeri below the inner LAG. Small reticular vascular canals are also present above this LAG. E) Photomicrograph of the boxed area in normal transmitted light. OSL appear flat and generally 1081 oriented parallel to the cortical surface within the annulus. F) Same view as in (E) in polarized 1082 transmitted light. Note the primary osteons set in a WB matrix, i.e., fibrolamellar bone. 1083 Abbreviation: ac = adductor crest.

1084

Figure 14 Uncatalogued MSU femur, *O. uniformis*, 115 mm in length. A) Scan of the transverse section of the mid-diaphysis. The midshaft is damaged and the adductor crest is incomplete (reconstruction of the missing area is general and not an accurate morphological reconstruction). Arrow indicates the nutrient canal. B) Photomicrograph of the boxed area in (C) in normal transmitted light. Alternating zones and annuli can be observed. C) Magnified view of the dorsal cortex in normal transmitted light. Arrow indicates the nutrient canal that extends into the deep cortex. Note how the cortex was affected during ontogeny to continually incorporate this permanent structure throughout the life of the animal. The cortical growth
record consists of eight annuli, some of which are associated with LAGs. The oldest annulus
(A1) is the boundary between the cortex and the medullary region. The inner cortical
vascularity is longitudinal. The mid cortex is a combination of longitudinal and reticular
canals. In the outermost cortex, there are thin zones with one to two rows of longitudinal
primary osteons. Secondary trabeculae occlude the medullary cavity. D) Same view as in C)
but in polarized transmitted light. Annuli are better visible in polarized light. Notice that the
vascularization does not form the same "bicycle wheel" pattern as that seen in OMNH-55234
E) Photomicrograph of the boxed area in (C) in normal transmitted light just below the
nutrient canal. This is the best example of Haversian tissue, though poorly defined. Note the
combination of primary and secondary osteons.) F) Same view as in (E) in polarized
transmitted light. Abbreviations: A= annulus; ac= adductor crest; Z= zone.

Figure 15 OMNH-35389 *O. retroversus* femur 221 mm in length. A) Scan of a transverse core section drilled into the dorsal sides of the mid-diaphysis. A distinctive EFS can be seen in the outer cortex. Vascularity consists of longitudinal and reticular canals. In addition to the EFS, eight growth cycles can be seen in the cortex. Earlier growth cycles may have been resorbed. ECs do not extend beyond the third cycle. B) Photomicrograph of (A) in polarized transmitted light with a lambda filter. The annuli separating the zones are more distinctive. C) Photomicrograph of the outer cortex as indicated by bracket in (A). Vascularity has greatly decreased towards the EFS and just below the LAG. Abbreviations: EFS = external fundamental system; LAG = line of arrested growth; MC = medullary cavity.

1115 Appendix 1:

- 1116 Photographs of the seven sampled *Ophiacodon* humeri used in this study, dorsal (top) and
- 1117 ventral (bottom) views, scale is 10 mm. A) MCZ-5926 (right) B) MCZ-2819 (left) C) MCZ-
- 1118 4816 (left) D) MCZ-4816 (right) E) MCZ-1486 (right) F) IPBSH-62 (left) G) OMNH-73698
- 1119 (right).
- 1120

1 Appendix 2:

Photographs of the four sampled *Ophiacodon* femora used in this study, dorsal (top) and ventral (bottom) views, scale is 10 mm. A) IPBSH-46 (right) B) OMNH-55234 (right) C) Uncatalogued MSU specimen (left) D) OMNH-35389 (left).

Table 1

						Complimentary			
Specimen				Length	Circumference	stylopodium	L/C	Growth	
Number	Species	Bone	MOS	(mm)	(mm)	length (mm)	ratio	Cycles	Notes
MCZ-5926	O. retroversus	Right Humerus	2	76	44	91.20	1.73	1	NL
MCZ-2819	O. retroversus	Left Humerus 🗄	3	97	50	116.40	1.94	2	
MCZ-4816	O. retroversus	Left Humerus 🚡	4	122	69	146.40	1.77	2	Inc
MCZ-4816	O. retroversus	Right Humerus	4	125	71	150.00	1.76	2	Inc
MCZ-1486	O. retroversus	Right Humerus	5	152	83	182.40	1.83	4	EFS (+8GC)
OMNH-73698	<i>Ophiacodon</i> (sp)	Right Humerus	4-5	113	50	98.40	2.26	2	EFS (?)/core
IPBSH-62	O. uniformis	Left Humerus	5	82	37	135.60	2.22	3	
IPBSH-46	<i>Ophiacodon</i> (sp)	Right Femur	NA	78	37	65.00	2.11	0	NL
OMNH-55234	O. mirus	Right Femur	NA	100	60	83.33	1.67	3	Inc
MSU Specimen	O. uniformis	Left Femur	NA	115	55	95.83	2.09	8	Inc
OMNH-35389	O. retroversus	Left Femur	NA	221	145	184.17	1.52	8	EFS (?)/core

Table 2

	Femur Length	Humerus			
Specimen ID	(mm)	Length (mm)	Ratio	<i>Ophiacodon</i> (spp)	Notes
WM-454	125	107	1.17	Ophiacodon hilli	Romer & Price 1940
WM-671	125	102	1.23	Ophiacodon mirus	Romer & Price 1940
UMMP-UC-241	102	84	1.21	Ophiacodon mirus	Measured by C. Shelton
UMMP-UC-241	106	84	1.26	Ophiacodon mirus	Measured by C. Shelton
UMMP-UC-671	117	111	1.05	Ophiacodon mirus	Measured by C. Shelton
UMMP-UC-671	122 🕁	108	1.13	Ophiacodon mirus	Measured by C. Shelton
UR-632	82	64	1.28	Ophiacodon mirus	Measured by C. Shelton
AM-4777	119 😈	101	1.18	Ophiacodon navajovicus	Romer & Price 1940
WM-458	167 💾	130	1.28	Ophiacodon retroversus	Romer & Price 1940
YP-1103	135	117	1.15	Ophiacodon retroversus	Measured by C. Shelton
MCZ-1121	149	130	1.15	Ophiacodon retroversus	Romer & Price 1940
MCZ-1121	149	120	1.24	Ophiacodon retroversus	Measured by C. Shelton
MCZ-1121	151	127	1.19	Ophiacodon retroversus	Measured by C. Shelton
MCZ-1121	144	127	1.13	Ophiacodon retroversus	Measured by C. Shelton
UMMP-UC-458	164	140	1.17	Ophiacodon retroversus	Measured by C. Shelton
WM-690	115	103	1.12	Ophiacodon uniformis	Romer & Price 1940
MCZ-1366	121	85	1.42	Ophiacodon uniformis	Romer & Price 1940
MCZ-1366	123	95	1.29	Ophiacodon uniformis	Measured by C. Shelton
MCZ-1366	119	95 Peerl PrePrints L https://dx.doi.org/1/	1.25	Ophiacodon uniformis	Measured by C. Shelton





Figure 2:

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Figure 7



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C)



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APPENDIX 1



Ventral

APPENDIX 1 continued

