The Ontogenetic Osteohistology of the Eureptile *Captorhinus aguti* (Reptilia: Captorhinidae) and the Community Histology of the Early Permian Fissure-Fill Fauna Dolese Quarry, Richards Spur, Oklahoma

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

Palaeohistological research has greatly enhanced our ability to draw conclusions about the physiology and growth of extinct vertebrates. A comprehensive histological growth study has never been undertaken in an Early Permian taxon at the initial stages of terrestrial vertebrate evolution. *Captorhinus aguti*, a common Early Permian eureptile from the fissure-fill locality near Richards Spur, Oklahoma, is the ideal taxon for a study of this type. *C. aguti* femora from all growth stages were measured and sectioned to compare bone structure through ontogeny. Representatives of other major taxa from the Richards Spur locality were also sectioned to compare histology across the palaeocommunity. Long bones of all sectioned Richards Spur taxa, including all growth stages of *C. aguti*, display slow growing parallel-fibered lamellar bone with poor vascularization. Captorhinids were the only taxon from this locality that lacked growth lines, implying that they were employing a different growth strategy from the other taxa that were preserved at this locality.
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Chapter 1
Introduction

The Early Permian was a critical time in the evolution of the earliest terrestrial vertebrates. Just prior to the start of the Permian, anamniote tetrapods were joined by two other major tetrapod groups: eureptiles (including extant reptiles and birds), and synapsids (including extant mammals), and together these three major groups completed the invasion of equatorial terrestrial environments at the beginning of the Permian (Sumida and Martin, 1997). Early tetrapod diversification and evolution is of interest to evolutionary biologists because the adaptations in a group’s life history impact the trajectory of that group. Yet, not many studies focus on the growth and life history of these early terrestrial tetrapods. By understanding the mechanics behind the growth strategies in early terrestrial vertebrates, we will further be able to understand the evolution of growth strategies in vertebrates through time, and this is what I hope to achieve in my research. To accomplish this goal, a histological approach is used.

Histology is the study of the structure of cells and tissues of organisms (Padian and Lamm, 2013). Specifically, bone histology – involving the sectioning and grinding down of bones until the microscopic structure of bone can be examined – is exceptionally useful in understanding the physiology (e.g. de Ricqlès, 1974; de Ricqlès, 1980), ecology (e.g. Cooper et al., 2008), and growth (e.g. Horner et al., 1999; Erickson and Tumanova, 2000) of extinct vertebrates, and is a popular approach used in palaeontology as a result. Due to extensive histological studies that have been performed with extant vertebrates (e.g. Enlow and Brown, 1956; 1957; 1958; Castanet et al., 2000; Köhler et al., 2012; Quémeneur et al., 2013), palaeontologists have a wide knowledge base in the literature to use as a basis of comparison when examining the microanatomy of fossil bone structure.

Inferences that can be made about an animal’s physiology and life history from histological studies of fossils are numerous. For instance, the type of bone seen in cross-section can indicate whether the individual was immature or an adult at the time of its death, as well as its relative rate of growth based on the amount of remodeling and vascularization in the bone (Padian and Lamm, 2013). Amprino (1947) first presented the concept of estimating the rate of
bone formation based on the type of bone present in a cross-section. For instance, lamellar bone with poor vascularization is an indication of slow bone formation as the ordered arrangement of the collagen fibres found in lamellar matrix requires more time to form, and consequently leaves little room for vascularization (Amprino, 1947). In contrast, the woven bone type with rich vascularization indicates fast bone growth as the collagen fibres within this bone type are arranged in generally less organized orientations, allowing for more vascularization (Amprino, 1947). “Amprino’s Rule” has been tested, advanced, and shown accurate in multiple studies, including de Ricqlès et al. (1991) and de Margerie et al. (2002; 2004).

Pauses in osteogenesis caused by limiting seasons that slow the metabolic rate of animals produce Lines of Arrested Growth (LAGs) from which an estimation of age and rate of growth can be determined (Erickson and Tumanova, 2000). Metabolic rate in vertebrates can potentially be assessed by the Osteocyte Lacunar Density (OLD) – a proxy for rates of cell proliferation – in which a high density of osteocyte lacunae would indicate a relatively high metabolism, and vice versa (Bromage et al., 2009; Stein and Werner, 2013). The lifestyle of an animal can also be inferred: the Relative Bone Thickness (RBT) – the thickness of the cortical bone relative to the diameter of the medullary cavity as determined from a cross-section – typically shows that thick cortices surrounding small – or non-existent – medullary cavities is characteristic of aquatic vertebrates (it has been suggested that the high bone density is used as a method for buoyancy regulation (Gray et al., 2007)), whereas terrestrial vertebrates tend to have relatively lower RBTs with a clearly distinguished medullary cavity (de Buffrénil and Mazin, 1990; Quémeneur et al., 2013). The ability to make such inferences of an extinct organism’s physiology and lifestyle is understandably very alluring to palaeontologists whom, prior to the induction of the histological approach, had to rely on other, arguably less reliable, means of making such interpretations. Histology is thus not only an essential and well-used technique in palaeontology, but it also acts as an important check when extrapolating about physiology and lifestyle, instead of relying solely on the external morphology of an organism (Peabody, 1961).

The primary drawback of a histological approach is obvious: this method involves sectioning precious, often irreplaceable fossils. Before sectioning and grinding down a fossil to make a thin section, permission must be granted by the owner of the fossil, and they are much more likely to do so if the element in question is abundant. Of course, having a large sample size – in addition to a large size range – is also ideal when executing histological growth studies.
Osteological changes and variation during development can interfere with the ability to accurately assess the growth dynamics of an individual, and thus a histological assessment of the life history of a taxon is not advisable on the basis of only a few individuals (Horner et al., 1999). In order to make valid conclusions about growth in extinct vertebrates, as well as to ensure easy access to specimens for sectioning, a common type species with lots of readily accessible specimens is preferable. One such species is the common basal eureptile *Captorhinus aguti* (Cope, 1882).

As a result of the large number of skeletal elements available for examination, the anatomy of *C. aguti* is well known and described (Price, 1935; Fox and Bowman, 1966; Holmes, 1977; 2003; Modesto, 1998). *C. aguti* is primarily distinguishable from other captorhinids in having multiple tooth rows, both on the maxilla and the dentary, that are oriented posteromedially at an oblique angle (Fox and Bowman, 1966; Heaton, 1979). Multiple tooth rowed captorhinids, including *C. aguti*, with wear facets on the cheek teeth thought to indicate propalinal jaw movement (in which the upper and lower dentitions slide across each other for oral processing of tough plant materials) have traditionally been regarded as high-fibre herbivores (Olson, 1955; Hotton et al., 1997; Reisz and Sues, 2000). However, more recent evidence suggests that *C. aguti* did not have a true grinding dental battery as the teeth of the maxilla and dentary lacked the extensive occlusion necessary for grinding high-fibre plant material (LeBlanc et al., 2015). Instead, *C. aguti* was likely more omnivorous: the presence of wear facets labially in the first premaxillary teeth of *Captorhinus* may indicate that these teeth were brought into contact with the ground during foraging, and this suggests that their diet may have also consisted of fructifications and/or animal carcasses (Modesto et al., 2007).

The *C. aguti* bone histology of a few different skeletal elements has been previously examined and described broadly by Enlow and Brown (1957) and Peabody (1961). In cross section, *Captorhinus* long bones display basic compact structure with some circumferential lamellae (Enlow and Brown, 1957; Peabody, 1961) – potentially indicating slowing growth during limiting seasons. Vascular canals are simple primary and are generally longitudinal in orientation; endosteal Haversian systems that are typically found in mature individuals are absent from all specimens (Enlow and Brown, 1957). A more thorough ontogenetic histological examination into *C. aguti* growth has never been undertaken, however.
For this study, a large sample size and range of *Captorhinus aguti* femora have been sectioned at mid-diaphysis to determine the ontogenetic growth trajectory of the species (Horner et al., 1999). All sectioned *C. aguti* specimens are from the Dolese Limestone Quarry, near Richards Spur, Oklahoma, USA, an upland locality that is incredibly rich and diverse in exceptionally well-preserved Early Permian (Artinskian) terrestrial vertebrates, of which *C. aguti* skeletons make up the vast majority of the total fossils found there (Modesto, 1998). This Richards Spur locality is the most diverse Palaeozoic faunal site in North America – more diverse than nearby lowland sites – because of its cave depositional preservation system (MacDougall and Reisz, 2015). Thousands of finely detailed disarticulated skeletal elements can be found within the extensive fissure-fill cave systems of the locality (Bolt, 1980). These elements are from a faunal assemblage that lived concurrently during the Artinskian Stage (age estimation from cave speleothems is 289-286 Ma) and would have experienced similar environmental conditions (MacDougall and Reisz, 2015). Examinations into the palaeosol of the locality, as well as the oxygen isotopes from speleothems located there, can provide insight into the climate record and environment of the area. The Richards Spur locality was terrestrial during the Early Permian, and therefore so were all the species that resided there; the locality was also equatorial at the time, but climate records indicate that the environment was semiarid and monsoonal, and there was thus a strong seasonality (Tabor et al., 2009; Woodhead et al., 2010). In cross-section, long bones of all Early Permian fauna from Richards Spur are then expected to have a relatively low RBT (a thinner cortex relative to a distinct medullary cavity) because the locality is terrestrial, and should also display LAGs because of the seasonality indicated in the climate records.

A comprehensive comparative study of vertebrates living concurrently in a palaeocommunity could achieve two key insights: first, it could enlighten the variability in the physiologies and lifestyles at this early stage of tetrapod evolution, and this in turn could provide insight into the palaeoenvironment of the locality. Most palaeohistological studies focus on one taxon for examination at a time; a histology study on a (palaeo)community-wide scale has never been undertaken. Because the Dolese Quarry, Richards Spur locality has such a rich and diverse faunal assemblage of well preserved fossils, it is the ideal locality from which to perform a community-wide study, as well. Representatives from all major Early Permian tetrapod taxa are found there, including anamniote tetrapods (both temnospondyls and lepospondyls), synapsids
(eupelycosaurs), and reptiles (parareptiles and captorhinomorphs) (MacDougall and Reisz, 2012). For this second part of my research, representatives of all the major taxa of Richards Spur have been reviewed and compared in a palaeohistological survey.

Most palaeohistological studies have focused on dinosaurs and other Mesozoic reptiles (e.g. de Buffrénil and Mazin, 1990; Chinsamy et al., 1995; de Ricqlès et al., 2003; Gren, 2010; Scheyer et al., 2010); this research would be the first to examine histological growth during the earliest stages of terrestrial tetrapod diversification and evolution. Experimental research in palaeontology is always mensurative, but that does not mean that hypotheses cannot be made and tested, and valid conclusions drawn. Because the histological growth studies of early tetrapods are limited, this research relies on comparisons to the long bone histology of other taxa. For example, to determine whether *Captorhinus aguti* is a good representative of early reptiles from which to draw conclusions for this research, comparisons to long bone sections of other early reptile taxa can be made. To determine what effect – if any – this particular locality has on the growth of early tetrapods, comparisons could be made between early tetrapods from different localities. When exploring what osteological characteristics are unique to amniotes, differences between bone structure in anamniote tetrapods (i.e. amphibians) and amniotes could be evaluated. Additionally, a common practice in palaeontology is to use extant analogues of known physiology and life history to draw conclusions about lesser-known extinct fauna and this is particularly beneficial in this case when so little is known about the growth dynamics of these early terrestrial tetrapods. The only limitation when it comes to making thorough assessments and accurate inferences based on comparisons to other taxa is access to the required specimens. The histological sectioning of rare specimens is contingent on acquiring permission from the holders of those bones.

Palaeohistological studies are valuable in making inferences relating to the growth, physiology, and life history of extinct vertebrates, and this information is central not only to the research of palaeobiologists, but to evolutionary biologists as well. The field of evolutionary biology strives to explain the underlying causes and effects of speciation and adaptation in major taxa through time. The proposed research explores these questions as they relate to terrestrial tetrapod evolution. In examining growth dynamics and physiology at a critical period of early tetrapod diversification and evolution, I have investigated questions that can also be applied to the study of life history traits of vertebrates in general.
Literature Cited


Chapter 2

The Ontogenetic Osteohistology of the Eureptile *Captorhinus aguti* (Reptilia: Captorhinidae)

Abstract

Palaeohistological growth studies of Mesozoic tetrapods are numerous, but a comprehensive histological growth study has never been undertaken in a Permian taxon at the initial stages of terrestrial vertebrate evolution. *Captorhinus aguti*, a common and well-studied Early Permian eureptile from the fissure-fill locality near Richards Spur, Oklahoma, is the ideal taxon for a histological growth study of this type due to the prevalence of bones to section of a wide variety of growth stages. Twenty *C. aguti* femora ranging in length from 15 mm to 34 mm were measured to examine relative change in shape through ontogeny, as well as histologically sectioned at mid-diaphysis to compare bone structure through ontogeny. The Osteocyte Lacunar Density (OLD) – potentially a proxy for metabolic rate – was also determined from femora at a range of sizes. To estimate an approximate life span for *C. aguti*, an adult maxilla was sectioned and the lines of von Ebner – which are formed by daily dentine deposition – were counted. Reduced Major Axis regression analyses show positive allometry in dimensions associated with major muscles relative to femur length in *C. aguti*. Thin sections of *C. aguti* femora at all growth stages display parallel-fibered lamellar bone with little vascularization, an indication of a relatively slow-growing animal. The OLD in *C. aguti* increases through ontogeny and the OLD of the largest sectioned *C. aguti* individual is on par with that of extant non-varanid squamates. None of the sectioned *C. aguti* femora display fully circumferential Lines of Arrested Growth (LAGs) despite the fact that the Richards Spur locality was monsoonal during the Early Permian and despite the fact that von Ebner line counts reveal that *C. aguti* lived through at least two limiting seasons. Out of all the taxa that have been sectioned from the Early Permian Richards Spur locality, *C. aguti* is the only taxon that lacks LAGs, implying that *C. aguti* was employing a different growth strategy from the other taxa.
**Introduction**

Research into the bone histology of fossils has greatly enhanced our ability to draw conclusions about the physiology (e.g. de Ricqlès, 1974; de Ricqlès, 1980; Chinsamy, 1990; Köhler and Moyà-Solà, 2009) and growth (e.g. Chinsamy, 1993; Erickson and Tumanova, 2000; Horner et al., 2000; Botha and Chinsamy, 2001) of extinct vertebrates. The ability to make these inferences about an extinct organism’s biology is understandably very alluring to paleontologists whom, prior to the induction of the histological approach, had to rely on other, arguably less reliable means of making such interpretations. Histology is thus not only an essential and well-used technique in palaeontology, but it also acts as an important check when extrapolating about life history and lifestyle, instead of relying solely on the external morphology of an organism (Peabody, 1961). Histological assessments into the growth patterns and skeletochronology of Mesozoic tetrapod vertebrate taxa are numerous (e.g. de Buffrénil and Mazin, 1990; Sander, 1999; de Ricqlès et al., 2003; Steyer et al., 2004), but a comprehensive histological growth study has never been undertaken for an Upper Palaeozoic taxon during the earliest stages of terrestrial vertebrate evolution. Understanding the growth patterns of terrestrial vertebrates at this critical period of early amniote diversification is valuable not only in our interpretation of the growth and physiology of early amniotes, but also in key lineages evolving from this ancestral state.

An effective histological interpretation of the life history of a taxon is often impeded by studies that utilize only a few individuals to analyze the growth dynamics of the entire taxon (Horner et al., 1999). Among extinct vertebrates it is not uncommon that there be limited permissible material available for histological examinations, and changes and variations in the bone during development can interfere with making valid conclusions about the growth dynamics of a taxon through ontogeny (Horner et al., 1999). It is for this reason that large sample sizes of elements at various growth stages are ideal and this is why the common Early Permian amniote *Captorhinus aguti* is the perfect type species for a study on the histological growth of early amniotes.

*C. aguti* was a small-bodied basal eureptile, primarily distinguished from other captorhinids in having multiple tooth rows oriented at an oblique angle posteromedially across both the maxilla and dentary (Fox and Bowman, 1966; Heaton, 1979). Most *C. aguti* specimens are from the Dolese Brothers Limestone Quarry near Richards Spur, Oklahoma, U.S.A., a fissure-fill locality that is incredibly rich in exceptionally well-preserved Early Permian (Artinskian) terrestrial
vertebrates, of which *C. aguti* skeletons make up the vast majority of the total fossils found there (Modesto, 1998). As a result of the large number of remarkably detailed specimens, the osteology of *C. aguti* is also well known and described (Price, 1935; Fox and Bowman, 1966; Holmes, 1977; 2003; Modesto, 1998) and this in turn has led to some ideas about lifestyle and diet. Traditionally thought to be herbivorous due to the multiple-rowed dentition with evidence of wear facets in the cheek teeth (Hotton et al., 1997), the consensus now is that *C. aguti* was likely omnivorous as it lacked the extensive occlusion between maxilla and dentary necessary for the grinding of tough plant materials (LeBlanc et al., 2015). Signs of wear facets in the first premaxillary teeth may indicate that these teeth were brought into contact with the ground during foraging, suggesting that fructifications and/or animal carcasses may have also been apart of the diet of *Captorhinus* (Modesto et al., 2007).

The goal of this study is to describe the bone histology of *Captorhinus aguti* through ontogeny, interpret what the bone structure indicates about its growth and physiology, and compare this interpretation with what has already been postulated about the biology of *C. aguti* based on its morphology and the environment in which it lived. This is the first comprehensive histological growth study to examine a Permian taxon during the earliest stages of terrestrial vertebrate evolution.
Materials and Methods

All specimens are from the Early Permian (Artinskian) Dolese Quarry locality near Richards Spur, Oklahoma, U.S.A., and are the property of R.R. Reisz. Twenty *C. aguti* femora were measured, photographed, and scanned using NextEngine 3D Laser Scanner prior to histological sectioning. For a thorough study of growth through ontogeny in *C. aguti*, femora ranging in length from 15 mm to 34 mm were measured. Note that there are potentially larger outliers, as well as smaller juvenile sizes, of the species that exist; the femora included for measurements comprised of the greatest size range available to the authors.

Figure 1 shows the six measurements that were recorded for each femur in order to assess any relative changes to shape through ontogeny: i) length of femur from proximal-most tip to distal-most tip; ii) anterior-posterior diameter at mid-shaft of femur; iii) proximal width of femur from anterior-most point of the proximal articular surface to posterior-most point; iv) distal width of femur from anterior-most tip of the fibular facet to the posterior-most tip of the tibial facet; v) dorsal-ventral diameter at mid-shaft of femur; and vi) height of the trochanter, measured from the proximal view of the femur from the dorsal edge to the ventral edge of the trochanter.

Measurements were logarithmically transformed and regression analyses were calculated using the reduced major axis (RMA) method – employed by RMA v.1.17 software (Bohonak and van der Linde, 2004) – comparing length to the five other femur measurements. Correlation coefficients, slopes, intercepts, and 95% confidence intervals of the slopes were recorded for each variable relative to femur length. The relative size increase is considered to be positively allometric when the 95% confidence interval of the slope is greater than 1, negatively allometric when the 95% confidence interval of the slope is less than 1, and isometric when the 95% confidence interval of the slope includes 1 (Evans, 2010; Brown and Vavrek, 2015).
Figure 1: Line drawings of a *Captorhinus aguti* femur in ventral view on the left, followed by the anterior view, and then the proximal view on the right, showing the six measurements that were recorded across twenty femora of a range of growth stages to assess relative changes to femur shape through ontogeny. The six measurements were: i) length of femur from proximal-most tip to the distal-most tip; ii) mid-shaft diameter of femur from the ventral view (anterior-posterior width); iii) proximal width of femur from the anterior-most point of the proximal articular surface to the posterior-most point of the proximal articular surface; iv) distal width of femur from the anterior-most tip of the fibular facet to the posterior-most tip of the tibial facet; v) dorsal-ventral diameter at mid-shaft of femur; and vi) trochanter height, measured from the proximal view of the femur from the dorsal edge to the ventral edge of the trochanter.
Standard histological sectioning practices were carried out at the Royal Ontario Museum’s histology laboratory. All specimens were imbedded in polyester resin (Castolite AP), vacuumed, and left to cure for 24 hours. Specimens were sectioned transversely at mid-diaphysis using a low-speed wafer blade saw (Buehler Isomet 1000) before the cut surfaces were mounted onto frosted plexiglass slides, affixed with a cyanoacrylate adhesive (Scotch Weld CA40). Resin blocks were then ground down to a thickness of approximately 150 µm using a grinding cup (Hillquist 1010) before being further ground by hand using progressively finer silicon carbide grits. Specimens were then polished using a one-micron aluminum oxide grit. Thin sections were photographed using a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope, fitted with crossed-polarizing and lambda filters. Image processing was performed using Nikon NIS-Elements (Basic Research) v. 4.10 imaging software.

The Osteocyte Lacunar Density (OLD) was determined in twelve thin sections of Captorhinus femora of a range of sizes as a means of estimating metabolic rate (Stein and Werner, 2013). OLDs in each thin section were measured following the methods of Cullen et al. (2014) with some modifications, and are as follows: thin sections of the cortical bone were examined under 40X magnification, with a field of view (FOV) of 360 µm x 360 µm. The thickness, or height, of the FOV was calculated by capturing images in 2 µm increments for a total depth of 36 µm and combining these images into a single z-stacked image; the thickness of 36 µm was then multiplied by the refractive index of the resin used (1.6) to find the height of the FOV. Osteocyte lacunae were counted within the total FOV at three different, random locations in the cortical bone, and then averaged before being divided by the total FOV to find the density of osteocyte lacunae. To test whether there is any correlation between size or ontogenetic stage and OLD in C. aguti, Pearson’s correlation coefficient was calculated.

Of the histologically sectioned specimens, four representatives of femora at four different growth stages, numbered I (smallest) to IV (largest) are presented here in this paper (Figure 2). These femora were chosen for presentation in the paper because they represented a roughly equidistant spread of available femora sizes in C. aguti and histologically, they were the clearest thin sections for imaging. The bone structure in the thin sections of these four representative C. aguti femora (I-IV) were then examined and compared.
Figure 2: Ventral views of the four representative femora at different growth stages, numbered I (smallest) to IV (largest), prior to being imbedded in resin and sectioned. The black line cutting across the mid-shaft of the smallest femur (I) is the approximate location on each femur where histological sectioning was executed.
Captorhinid representatives from other Early Permian localities outside of Richards Spur, Oklahoma, were also histologically sectioned, using the aforementioned practices, for comparative purposes. Among these were: OMNH 752, labeled Captorhinus, a fragment of the distal end of a femur from Northeast Frederick, Oklahoma, of the Upper Garber Formation; OMNH 1702, labeled Protocaptorhinus, a fragment of the proximal end of a femur from Orlando, Oklahoma, of the Wellington Formation; and OMNH 641, labeled Captorhinus, a fragment of the distal end of a femur from Oscar, Oklahoma, from a “small pond deposit” in the Wellington Formation. All these specimens are from the personal collection of W. May and were generously donated for sectioning.

To estimate an approximate life span for *C. aguti*, a method used by Erickson (1996) to find the tooth replacement rate by counting the lines of von Ebner (which are created by daily dentine formation) was appropriated for this study. As Erickson (1996) details, the difference between the number of von Ebner lines in the functional tooth of a dinosaur and the number of von Ebner lines in the unerupted replacement tooth underneath is equivalent to the approximate tooth replacement rate for that dinosaur. *C. aguti* is known for having multiple tooth rows within their maxillae and dentaries in which new teeth are added lingually and shed labially in a sideways conveyor-belt motion (de Ricqlès and Bolt, 1983; LeBlanc and Reisz, 2015). Thus the assumption here is that the tooth replacement rate in *C. aguti* can be determined by finding the differences in the von Ebner lines between adjacent teeth, rather than between the erupted functional tooth and the unerupted replacement tooth in a dinosaur. Using the same histological sectioning practices as above, a thin section of an adult *C. aguti* maxilla (ROM 66861) cut cross-wise labially-lingually across three rows of teeth was examined with a Nikon AZ-100 microscope under cross-polarized light to illuminate the lines of von Ebner. By adding up the differences in von Ebner line numbers between each adjacent tooth, the minimum age in days of that individual captorhinid was determined. Counts of von Ebner lines in each tooth in the thin section were performed three times and the average was recorded for this paper.
Results

The measurements of the twenty *C. aguti* femora, along with the logarithmically transformed data, are recorded in Appendix 1. Table 1 shows the results of the RMA regression analyses, recording the correlation coefficient ($R^2$), intercept, slope, and 95% confidence interval of the slope for each variable relative to femur length. All measured variables (anterior-posterior diameter, proximal width, distal width, trochanter height, and dorsal-ventral diameter) exhibit a close correlation relative to femur length (Table 1). The anterior-posterior diameter at mid-shaft of the femur in *C. aguti* is the only variable that grows isometrically relative to length (Table 1). All other femoral variables show a positively allometric size increase relative to length as the lower and upper limits of the 95% confidence interval of the slopes are all greater than 1 (Table 1).

**Table 1.** Reduced major axis regression analyses of the five femoral measurements of *Captorhinus aguti* relative to femur length. Recorded here are the correlation coefficients ($R^2$), y-intercept values, slopes, lower and upper confidence intervals (lCI and uCI) of the slopes (95%), and the corresponding growth patterns of each variable (I: isometric; PA: positively allometric).

<table>
<thead>
<tr>
<th>Variable</th>
<th>$R^2$</th>
<th>Intercept</th>
<th>Slope</th>
<th>lCI</th>
<th>uCI</th>
<th>Growth Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior-Posterior Diameter</td>
<td>0.966</td>
<td>-0.929</td>
<td>1.095</td>
<td>0.994</td>
<td>1.211</td>
<td>I</td>
</tr>
<tr>
<td>Proximal Width</td>
<td>0.989</td>
<td>-0.843</td>
<td>1.204</td>
<td>1.143</td>
<td>1.273</td>
<td>PA</td>
</tr>
<tr>
<td>Distal Width</td>
<td>0.985</td>
<td>-0.741</td>
<td>1.211</td>
<td>1.149</td>
<td>1.296</td>
<td>PA</td>
</tr>
<tr>
<td>Trochanter Height</td>
<td>0.979</td>
<td>-0.966</td>
<td>1.259</td>
<td>1.172</td>
<td>1.357</td>
<td>PA</td>
</tr>
<tr>
<td>Dorsal-Ventral Diameter</td>
<td>0.964</td>
<td>-1.086</td>
<td>1.152</td>
<td>1.070</td>
<td>1.257</td>
<td>PA</td>
</tr>
</tbody>
</table>
Table 2 shows the results of the Osteocyte Lacunar Densities (OLDs), a potential indication of metabolic rate, of twelve *C. aguti* thin sections at a range of sizes. OLDs were then plotted relative to the anterior-posterior mid-shaft diameters of the thin sections (Fig. 3). There is a significant positive correlation between diameter and OLD ($r = 0.846, n = 12, p = 0.000521$), indicating that the density of osteocyte lacunae at mid-diaphysis increases with increasing femur size in *C. aguti*.

**Table 2**: Osteocyte Lacunar Densities (OLDs) of *Captorhinus aguti* at a range of growth stages (based off the anterior-posterior diameter of the thin section at mid-diaphysis of the femur). The number of osteocyte lacunae (#OL) within the field of view (FOV) was counted at three different locations in the cortex and averaged before being divided by the total FOV to find the osteocyte lacunar density. Specimen numbers refer to the corresponding resin block and thin section number that were used to determine the OLDs.
Figure 3: The Osteocyte Lacunar Density (OLD) relative to anterior-posterior diameter at mid-diaphysis in *Captorhinus aguti* femora of a range of sizes. There is a significant positive correlation between diameter and OLD (r = 0.846, n = 12, p = 0.000521), indicating that the density of osteocyte lacunae at mid-diaphysis increases with increasing femur size in *C. aguti*.

Thin sections from the four representative femora sizes (I-IV) are imaged in Figure 4. At all growth stages, *Captorhinus aguti* displays parallel-fibered lamellar bone with low vascularity and some primary osteons within the cortical bone that surrounds a relatively large medullary cavity (Fig. 4). There is some evidence of a growth line in femur III along the inner region of the dorsal cortex close to the medullary cavity, but complete circumferential growth lines are absent at all femoral growth stages. The overall shape of the femoral mid-shaft cross-section becomes progressively less round through ontogeny; figure 4 shows the ventral edge of the cortical bone becoming more angular, particularly anteriorly where the fourth trochanter forms. Porosity in the cortex seems to become more prominent with each successive growth stage as the bone remodels with age, particularly in the inner cortical region near the medullary cavity.
<table>
<thead>
<tr>
<th>I</th>
<th><img src="image" alt="Image I" /></th>
<th><img src="image" alt="Image II" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td><img src="image" alt="Image III" /></td>
<td><img src="image" alt="Image IV" /></td>
</tr>
</tbody>
</table>

Scale bars: 1 mm for I, II, III, and 100 μm for IV.
**Figure 4:** Thin section images from the four *Captorhinus aguti* representative femora sizes (I-IV), imaged with a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope at a magnification of 2X on the left and corresponding close-ups of trochanter development at a magnification of 20X on the right. At all growth stages, *C. aguti* displays parallel-fibered lamellar bone within the cortex that surrounds a relatively large medullary cavity. All growth stages show low vascularity with some primary osteons. The overall shape of the mid-shaft of the femur becomes less round through ontogeny as the fourth trochanter forms in the anterior-ventral corner. Femur III displays some evidence of a growth line along the inner region of the dorsal cortex close to the medullary cavity, but complete circumferential growth lines are absent at all femoral growth stages.

Mid-diaphysis femoral thin sections from the three non-Richards Spur captorhinid representatives are imaged in Figure 5. Much like their captorhinid counterparts from the Dolese Quarry locality near Richards Spur, these non-Richards Spur captorhinids also display parallel-fibered lamellar bone that has a low amount of vascularity within the cortex, as well as a relatively large medullary cavity (Fig. 5). None of the sectioned femora from the Northeast Frederick (Fig. 5a), Orlando (Fig. 5b), and Oscar (Fig. 5c), Oklahoma localities display any evidence of circumferential growth lines within the cortex.
Figure 5: Thin section images from the three non-Richards Spur captorhinid femoral representatives (a. OMNH 752, *Captorhinus* from Northeast Frederick, Oklahoma; b. OMNH 1702, *Protocaptorhinus* from Orlando, Oklahoma; c. OMNH 641, *Captorhinus* from Oscar, Oklahoma), imaged with a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope at a magnification of 2.5X on the left and corresponding close-ups at a magnification of 25X on the right. Like the Richards Spur captorhinids, the non-Richards Spur captorhinids display parallel-fibered lamellar bone within the cortical bone surrounding a relatively large medullary cavity, and no evidence of any circumferential growth lines within the cortex.
The lines of von Ebner, which are incremental lines of dentine that are deposited daily in teeth, were counted within adjacent teeth in an adult *C. aguti* individual and the corresponding thin section is imaged in Figure 6. *C. aguti* has multiple rows of teeth positioned obliquely across both the maxilla and dentary, and replacement teeth and added lingually and shed labially (de Ricqlès and Bolt, 1983; LeBlanc and Reisz, 2015). In a labial-lingual thin section across three rows of teeth, the oldest (labial) tooth had an average count of 603 lines of von Ebner, the adjacent and younger medial tooth had an average count of 384 lines of von Ebner, and the youngest (lingual) tooth had an average count of 299 lines of von Ebner (Fig. 6). If the oldest tooth is about 603 days old (because von Ebner lines are deposited daily), the medial tooth appeared about 219 days later (603 von Ebner lines minus 384 von Ebner lines equals 219 days difference), and the youngest tooth appeared about 85 days after that (384 von Ebner lines minus 299 von Ebner lines equals 85 days difference), then this particular *C. aguti* individual must have been at least 907 days old at the time of its death (Erickson, 1996). Thus a conservative estimate of the life span of the early eureptile *C. aguti* indicates that individuals lived at least 2 years.
Figure 6: Thin section of an adult *Captorhinus aguti* maxilla (ROM 66861) sectioned labially-lingually across three rows of teeth on the left, and a close up view under cross-polarized light of the lines of von Ebner, which are created by daily dentine formation, on the right. The side of the jaw labeled “La” is the labial side, where the oldest tooth is located and will be shed, and the side of the jaw labeled “Li” is the lingual side, where replacement teeth are added and thus is the position of the youngest tooth in the multiple tooth rows. The numbers above each individual tooth refer to the number von Ebner lines counted in each tooth.
Discussion

The regression analyses indicate that growth in the femur is largely positively allometric in *Captorhinus aguti* (Table 1). The anterior-posterior diameter at mid-shaft is the only parameter that grows isometrically with the length of the femur, perhaps because there is no direct association with a major muscle with this particular dimension. The dorsal-ventral diameter at mid-shaft, however, does grow at an increased rate relative to the length of the femur, potentially because this dimension includes the growth of the adductor crest, which curves down the midline of the ventral side of the femur and is the site of attachment of the adductor femoris muscle (Holmes, 2003). The proximal end of the femur is also the site of another major femoral muscle – the puboischiofemoralis externus – responsible for adducting the hindlimb (Fishbeck and Sebastiani, 2012), and so it makes sense that the closely associated proximal width and trochanter height variables also grow allometrically relative to femur length. The hindlimb provides the main power of the locomotion in modern lizards (Snyder, 1954; Urban, 1965) and this may be why typically thigh muscle mass increases with body mass (positive allometry; Garland, 1985). Though the muscle mass of an extinct animal cannot be calculated, the indication that the dimensions of the *C. aguti* femur associated with major muscles increase with femur length, in addition to the knowledge that this is commonplace in extant analogues, implies that femur musculature may increase allometrically as *C. aguti* grows, perhaps corresponding to an increase in locomotion speed.

Comparing the mid-diaphyseal thin sections of *C. aguti* through ontogeny, the most obvious change between each growth stage besides size is the overall shape of the cross section. The smallest and youngest *C. aguti* femur (I) has the most smoothly round shape in cross-section and this shape becomes progressively less round through ontogeny (Fig. 4). The cause of this shape change is due to muscle growth and the corresponding bone remodeling due to this stress (Rayfield, 2007). The fourth trochanter, which is the site of insertion of the coccygeofemoralis, the primary muscle responsible for the retraction of the hindlimb (Holmes, 2003), becomes progressively more acuminate through ontogeny as it begins to form in the antero-ventral corner in the femoral cross-sections (Fig. 4). Muscle stress is an important factor in the remodeling of bone throughout an individual’s growth as osteocytes near the site of insertion detect the strain and signal cells to secrete, remove, or realign collagen fibres (Currey, 2002), and this is seen
clearly in *C. aguti* as the most intense remodeling in the bone tissue occurs at the site of the trochanter where one of the critical hindlimb muscles inserts (Fig. 4).

The mid-diaphysis cross sections of *C. aguti* femora at all growth stages consist of parallel-fibered lamellar bone in the cortex with relatively little vascularization (Fig. 4). Lamellar matrix is an indication of a slow rate of bone formation as the ordered arrangement of the collagen fibres found in lamellar cortical bone requires more time to form, and consequently allows little room for vascularization (Amprino, 1947). *C. aguti* was thus likely a relatively slow growing animal when compared to other amniotes like dinosaurs and birds that have fast growing fibrolamellar cortical bone, in which the collagen fibres are arranged in generally less organized orientations, allowing for more vascularization (de Margerie et al., 2004; de Buffrénil et al., 2008). This inference that *C. aguti* was a slow growing animal seems to be confirmed by the Osteocyte Lacunar Densities (OLDs) that were calculated from the thin sections (Table 2). Because the density of osteocyte lacunae acts as a proxy for rates of cell proliferation, OLDs may act as an indication of metabolic rate (Bromage et al., 2009). *C. aguti* has at its highest (in the largest sampled individual, IV, specimen JX-TS00848) an OLD of 22,371 (Table 2), which is on par with the OLDs found in modern non-varanid squamates, a group of animals that is known to have relatively low metabolic rates, and is a much lower density of osteocyte lacunae than is found in animals with known high metabolic rates such as birds and small mammals (Stein and Werner, 2013). Because *Captorhinus* is at a relatively basal position in the early stages of terrestrial vertebrate evolution, this implication that *C. aguti* was a slow growing animal with a relatively low metabolic rate is to be expected. There is a significant positive correlation between diameter at mid-diaphysis and OLD in *C. aguti* (Fig. 3), indicating that the metabolic rate – assuming that OLD is an accurate proxy for rates of cell proliferation – increases ontogenetically in *Captorhinus*.

The relatively thin cortex that surrounds a distinct medullary cavity also confirms that the lifestyle of *C. aguti* at the Richards Spur locality was terrestrial (Polley and Reisz, 2011; Quémeneur et al., 2013). In addition to the knowledge that the locality is terrestrial, it has also been determined by oxygen isotope and palaeosol studies of the cave deposits of the locality that the Early Permian Oklahoma environment had a strong seasonality and was potentially monsoonal, despite its equatorial location at the time (Tabor et al., 2009; Woodhead et al., 2010). The fauna of the Dolese Quarry, Richards Spur locality, including *C. aguti*, are thus expected to
display circumferential Lines of Arrested Growth (LAGs), which are produced when limiting seasons slow vertebrate metabolism which results in a pause in osteogenesis (Padian and Lamm, 2013). When estimating the life span of an adult *C. aguti* individual from the von Ebner line counts of daily dentine deposition in teeth (Erickson, 1996), the sectioned *C. aguti* maxilla revealed an individual that was at least two years old at its death (Fig. 6) and thus would have lived through at least two limiting seasons at the Richards Spur locality. It is therefore a bit puzzling that LAGs are almost completely absent in the mid-diaphyseal cross-sections of *C. aguti* at all growth stages, with only an incomplete segment of a LAG found near the interior region of the dorsal cortex in femur III (Fig. 4).

The reason that LAGs are either obstructed or altogether missing near the medullary cavity of the mid-shaft thin sections is likely because of the way matrix is deposited and resorbed through bone growth. As individuals grow, the endosteal surface of the cortex surrounding the marrow cavity is eroded away as bone is resorbed to make way for the outwardly expanding medullary cavity, resulting in the erasure of the part of the growth record that is closest to the periphery of the cavity (Padian and Lamm, 2013). The medullary expansion and erasure of the innermost cortical bone does not explain why no other LAGs are found anywhere else in the cortex at any growth stage in *C. aguti*, however. Also missing in even the largest of *C. aguti* individuals is an External Fundamental System (EFS), a series of closely placed annuli of avascular lamellar bone in the outermost cortex that indicates somatic maturity and the cessation of growth (Cormack, 1987), which likely indicates that *C. aguti* experiences indeterminate growth like most other reptiles (Vitt, 2013).

There are a number of potential reasons why *C. aguti* does not have any LAGs in the main bulk of the mid-diaphyseal cortical bone as would be expected in fauna that experience seasonal variation like those living in the Early Permian Dolese Quarry, but a firm conclusion cannot be made without further research. Osteohistological examinations of the other fauna found in the same fossil assemblage confirms that LAGs can be found in all other major groups of tetrapods besides *C. aguti* at the Richards Spur locality (see Ch. 3). Additionally, no LAGs were found in any of the other sectioned captorhinids from Early Permian localities outside of Richards Spur (Fig. 5) indicating that this may be a feature common to all small captorhinids, regardless of locality.
A possible explanation as to why *C. aguti* and other small captorhinids are different from the rest of the faunal assemblage from the Dolese Quarry could be that *C. aguti* experiences a continuous rate of growth year round, unlike most limbed vertebrates, including the other fauna of Richards Spur, that experience a slowing or pause in osteogenesis in limiting seasons. Perhaps the multiple-tooth-rowed *C. aguti* was a hardy generalist that managed to maintain a consistent metabolic rate in all seasons or else had a continuous food source no matter the conditions it endured. *C. aguti* may have been unique among the other fauna of Richards Spur as it was omnivorous. There are few herbivorous representatives found in the Early Permian of Richards Spur perhaps because the locality was an arid, upland environment with little vegetation (Reisz and Sutherland, 2001), and other faunal representatives of Richards Spur may have been insectivorous (Modesto et al., 2009). The capacity for omnivory in *C. aguti* may have been a major advantage over the rest of the faunal assemblage at the Richards Spur site and may be why *C. aguti* is found in such abundance there, as well. Further research into the osteohistology of other, perhaps larger, species of captorhinomorphs at other localities and comparisons to extant analogues may provide some insight into this hypothesis. All that can be stated with certainty at this time is that *C. aguti* was employing a different growth strategy than the other Early Permian terrestrial tetrapods at the Richards Spur fissure-fill locality.

**Acknowledgements**

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Literature Cited


Chapter 3

Community Histology of the Early Permian Fissure-Fill Locality Dolese Quarry, Richards Spur, Oklahoma

Abstract

Most palaeohistological studies focus on one taxon for examination at a time; a histology study on a (palaeo)community-wide scale has never been undertaken. A comprehensive comparative study of vertebrates living concurrently in a palaeocommunity could enlighten the physiologies and lifestyles of these extinct animals, which in turn could provide insight into the palaeoenvironment of the locality. The Early Permian fissure-fill locality of Dolese Quarry, near Richards Spur, Oklahoma is an ideal locality from which to perform a community-wide study as there is a great diversity of taxa available for sectioning from this site. Nine representatives from all major early terrestrial tetrapod taxa from the locality were included to be sectioned for this study. Thin sections of the sampled Richards Spur fauna showed that all taxa have slow-growing parallel-fibered cortical bone with poor vascularization and remodeling. There are some relative differences between taxa, however: in general, there is an increasing trend in vascularization and Osteocyte Lacunar Density (OLD) – a potential indication of metabolic rate – from the anamniote tetrapods to the reptiles to the synapsids of the Richards Spur locality. Lines of Arrested Growth (LAGs) are found in all sectioned taxa except for the captorhinid reptiles. The histological findings confirm what has already been posited about the palaeoclimate and palaeoenvironment of the Early Permian Richards Spur locality. Unlike the differences in bone tissue structures found among modern vertebrate groups, this early terrestrial vertebrate community does not show a great amount of variability in the life history strategies of its fauna; research into the community histology of another Early Permian locality could be beneficial in determining whether these patterns are seen in all palaeocommunities at this critical stage of early terrestrial tetrapod diversification and evolution.
Introduction

The fissure-fill fossil locality of the Dolese Brothers Quarry, near Richards Spur, Oklahoma, USA, is one of the most exceptional Early Permian tetrapod localities due to the prevalence of finely detailed bones of numerous terrestrial tetrapod species found in an extensive cave preservational system (Bolt, 1980). Of the thousands of disarticulated bones found at the Richards Spur site, the large majority of them are attributable to Captorhinus aguti, a multiple-tooth-rowed early eureptile (Modesto, 1998), but representatives from all major Early Permian tetrapod taxa are found there as well, including anamniote tetrapods (both temnospondyls and lepospondyls), synapsids (eupelycosaurs), and other reptiles (parareptiles and captorhinomorphs) (Macdougall and Reisz, 2012). These disarticulated elements are from a faunal assemblage that lived concurrently during the Artinskian Stage (age estimation from cave speleothems is 289-286 Ma) and would have experienced similar environmental conditions (MacDougall and Reisz, 2015).

Because it has such a rich and diverse faunal assemblage of well preserved fossils, the Richards Spur site is the ideal locality from which to perform a community-wide study. A comprehensive comparative study of tetrapod species living concurrently in this community could enlighten the physiologies and lifestyles of these early terrestrial tetrapods, which in turn could provide insight into the palaeoenvironment of the locality. Various aspects of the environment of the Richards Spur locality are already known or postulated. Because of the rarity of herbivores found at the site, it has been proposed that the palaeoenvironment was one of an arid, upland ecosystem with scarce vegetation (Reisz and Sutherland, 2001). Oklahoma was located near the equator near the end of the Palaeozoic (Fig. 1), but despite its equatorial position, palaeosol and oxygen isotope studies of cave deposits from the site indicate that the climate in Oklahoma during the Lower Permian had a strong seasonality and was monsoonal (Tabor et al., 2009; Woodhead et al., 2010).
Palaeohistology has proved a valuable resource in our understandings of the life histories and physiologies of extinct vertebrates (e.g. de Ricqlès, 1974; Chinsamy, 1990; Sander, 1999; Erickson and Tumanova, 2000; Horner et al., 2000) and such an osteohistological study of the fauna of the Richards Spur locality could act as a check to confirm what has been posited about the palaeobiologies of the fauna, as well as the palaeoenvironment of the locality. Most histological studies focus on one species or taxon for examination at a time; this study is the first to examine histology on a community-wide scale as representatives of all the major taxa of Richards Spur were reviewed and compared. A histological survey of an Early Permian community like Richards Spur is especially valuable as most palaeohistological studies have focused on dinosaurs and other Mesozoic reptiles (e.g. de Buffrénil and Mazin, 1990; Chinsamy et al., 1995; de Ricqlès et al., 2003; Gren, 2010; Scheyer et al., 2010). Thus this inspection of the bone structure of the fauna at Richards Spur intends to enlighten the histological growth dynamics of less-studied vertebrates at the earliest stages of tetrapod diversification and evolution. There are thus two questions that this study aims to answer. Firstly, what can a community-wide study on the bone histology of the local fauna tell us about the environment of that (palaeo)community (i.e. the palaeocommunity of Early Permian (Artinskian) Richards Spur)? Secondly, what is the variability in the life history strategies in the earliest terrestrial vertebrate communities?

Figure 1: Line drawing of the approximate location of Richards Spur, Oklahoma, USA during the Early Permian.
Materials and Methods

All sampled specimens are from the fissure-fill deposits of the Lower Permian (Artinskian) Dolese Brothers Limestone Quarry near Richards Spur, Oklahoma and are either the property of R.R. Reisz or else generously provided by R. Cifelli, W. May, and the Sam Noble Oklahoma Museum of Natural History. Faunal representatives of all major taxa were included for study. The complete faunal list is recorded in Table 1 and includes long bones from three anamniote tetrapods (two dissorophids of opposing sizes and one microsaur), three eureptiles (two species of captorhinids, as well as one unidentified reptile), and three synapsids (two mycterosaurine varanopids and one varanodontine varanopid). All specimens are incomplete fragments of long bone elements, but sufficient diagnostic details are available in each element to identify a higher taxon, if not any lower taxonomic ranks.

Table 1: Faunal list of sectioned specimens from the Early Permian fissure-fill deposits of the Dolese Brothers Quarry near Richards Spur, Oklahoma.

<table>
<thead>
<tr>
<th>Anamniote Tetrapods</th>
<th>Sectioned Specimens</th>
<th>Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temnospondyli</td>
<td>Dissorophidae</td>
<td>Cacops sp.</td>
</tr>
<tr>
<td>Lepospondyli</td>
<td>Microsauria</td>
<td>Doleserpeton sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiocephalus sp.</td>
</tr>
<tr>
<td>Reptiles</td>
<td>Captorhinidae</td>
<td>Captorhinus aguti</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Captorhinus magnus</td>
</tr>
<tr>
<td></td>
<td>Unidentified reptile</td>
<td></td>
</tr>
<tr>
<td>Synapsids</td>
<td>Varanopidae</td>
<td>Mycterosaurine I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mycterosaurine II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Varanops sp.</td>
</tr>
</tbody>
</table>
Standard histological sectioning practices were carried out at the Royal Ontario Museum’s histology laboratory. All long bones were imbedded in Castolite AC polyester resin, vacuumed, and then left to harden for 24 hours. Resin blocks were then sectioned at the mid-shaft of the elements using a Buehler Isomet 1000 low-speed wafer blade saw. The cut surfaces were then affixed onto frosted plexiglass slides using Scotch Weld CA40 cyanoacrylate adhesive. The resin on the slides was then ground down to an approximate thickness of 150 µm using a Hillquist 1010 grinding cup, and then further ground by hand using progressively finer silicon carbide grits, before being polished using a 1-micron aluminum oxide grit. Thin sections were imaged using a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope. Images were processed using Nikon NIS-Elements (Basic Research) v. 4.10 imaging software.

The composition and structure of the matrix and vascularization of all Richards Spur sectioned specimens were then examined and compared. The Osteocyte Lacunar Density (OLD) of all specimens were determined as an approximation of the metabolic rate of each taxon (Bromage et al., 2009; Stein and Werner, 2013). OLDs were determined following the procedure of Cullen et al. (2014) with some modifications, and are as follows: cortical bone of each taxon was examined under 40X magnification using a Nikon AZ-100 microscope with a field of view (FOV) of 360 µm (length) x 360 µm (width). The height of the FOV was calculated by capturing images in each thin section in 2 µm increments for a total depth of 36 µm, combining these images into a single z-stacked image, and then multiplying the thickness of 36 µm by the refractive index of the resin (1.6X). Osteocyte lacunae in each taxon were counted within the total field of view at three different, random locations in the cortex, averaged, and divided by the total FOV to find the osteocyte lacunae density.
**Results**

All histological thin sections of the sectioned taxa from Dolese Quarry, Richards Spur, display parallel-fibered lamellar bone throughout the cortical bone at mid-diaphysis. Vascularization is generally poor in all sections, as is bone remodeling, though the amount and orientation of the vascularization varies slightly from one taxon to another.

The mid-shaft cross-sections of anamniote tetrapods *Cacops* sp., *Doleserpeton* sp., and *Cardiocephalus* sp. are shown in Figure 2. These anamniote tetrapods share a common tear-drop shape in mid-shaft cross-sections of the femur; the adductor crest is a prominent feature along the ventral side of the shaft, though it is overall sharper in the dissorophid temnospondyls, *Cacops* sp. and *Doleserpeton* sp., than it is in the microsaurian lepospondyl, *Cardiocephalus* sp. (Fig. 2). Note that *Cacops* sp. is about 10 times larger in size than *Doleserpeton* sp. despite belonging to the same family, and this is not the only difference between the two. The sectioned *Cacops* sp. has a very thin cortex and a large medullary cavity, resulting in a ratio of cortex thickness to overall diameter that is smaller than any other sectioned taxa at the Richards Spur locality (Fig. 2a). Within the thin cortex, the sectioned *Cacops* sp. displays some primary osteons and the beginnings of some secondary osteons (Fig. 2a). Three Lines of Arrested Growth (LAGs) can be seen within the cortical bone of *Cacops* sp., and there were potentially others before the vastly expanded medullary cavity erased the growth record. The sectioned *Doleserpeton* sp. and *Cardiocephalus* sp. cortices are both nonvascular (Fig. 2b,c). However, *Doleserpeton* sp. displays numerous growth lines spaced almost equidistantly throughout the cortical bone whereas *Cardiocephalus* sp. has only a few growth lines located sparsely in the cortex (Fig. 2b,c).
**Figure 2:** Mid-diaphyseal transverse sections of anamniote tetrapods from Dolese Quarry, Richards Spur: a. *Cacops* sp. femur cross-section viewed under 1X magnification (left) and close-up view of the dorsal region of the cortex under 15X magnification (right); b. *Doleserpeton* sp. femur cross-section viewed under 10X magnification (left) and close-up view of the dorsal region of the cortex under 35X magnification (right); c. *Cardiocephalus* sp. femur cross-section viewed under 15X magnification (left) and close-up view of the dorsal region of the cortex under 35X magnification (right). Thin sections were imaged with a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope. All sectioned anamniote tetrapods display parallel-fibered bone with poor or no vascularization and very minor to no bone remodeling. White arrows indicate growth lines in the cortical bone.

Figure 3 shows the mid-shaft cross-sections of the sampled reptiles from the Richards Spur locality: two captorhinids (*Captorhinus aguti* femur and *C. magnus* humerus) and one unknown reptile femur that could not be further identified to a lower taxonomic rank. The sectioned *C. aguti* femur has a distinct shape due to the pointed internal trochanter that is located at the anteroventral corner at mid-diaphysis (Fig. 3a). The other femur belonging to the unknown reptile has a notably different shape in cross-section, one that is altogether more smoothly round, with a crest that occurs centrally on the ventral side of the mid-shaft (Fig. 3c). Vascularization and remodeling is relatively limited in the reptiles as it across all sectioned fauna from Richards Spur. The sectioned *C. aguti* has the beginnings of some primary osteons forming in the cortex and a range of different vascular orientations including simpler longitudinal and radial canals and some more complex reticular canals (Fig. 3a). There are some secondary osteons surrounded by cement lines in the cortical bone of *C. magnus*, but otherwise it is generally less vascularized than *C. aguti* and most vascular canals are longitudinal (Fig. 3b). The unidentified reptile femur is the most poorly vascularized of the three reptiles, with only some occasional longitudinal canals to be seen throughout the parallel-fibered matrix (Fig. 3c). This unidentified species is however the only specimen of the three reptiles to display growth lines in the cortical bone at mid-shaft (Fig. 3c).
Figure 3: Mid-diaphyseal transverse sections of reptiles from Dolese Quarry, Richards Spur: a. *Captorhinus aguti* femur cross-section viewed under 2X magnification (left) and close-up view of the dorsal region of the cortex under 10X magnification (right); b. *Captorhinus magnus* humerus cross-section viewed under 2.5X magnification (left) and close-up view of the dorsal region of the cortex under 10X magnification (right); and c. unidentified reptile femur cross-section viewed under 4X magnification (left) and close-up view of the dorsal region of the cortex under 15X magnification (right). Thin sections were imaged with a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope. All sectioned reptiles display parallel-fibered bone within the cortical bone at mid-shaft with poor to moderate vascularization and minor bone remodeling. White arrows indicate growth lines in the cortex of the unidentified reptile; there are no growth lines found in the sectioned captorhinids.

All three sectioned synapsid femora, two mycterosaurine varanopid species and one larger varanodontine varanopid species (*Varanops* sp.), are more roundly shaped in cross-section at mid-shaft than any of the other sectioned femora that had distinctive ridges indicating adductor crests and internal trochanters for muscle attachment (Fig. 4). However, the *Varanops* sp. specimen does display rugosity in the cortical bone antero-ventrally, where the trochanter is located (Fig. 4c). The three synapsids are undoubtedly the most vascularized specimens out of all the Richards Spur fauna that were sectioned (Fig. 4). All three have the beginnings of some primary osteons within the cortex, but no secondary osteons can be seen (Fig. 4). The two mycterosaurine species have largely longitudinally-oriented vascular canals within the internal cortical bone close to the endosteal surface and largely radially-oriented vascular canals within the external cortical bone close to the periosteal surface (Fig. 4a,b). In contrast, *Varanops* sp. has extensive radial canals throughout the cortex, though presumably the earlier growth record showing the vascular orientation more internally in the cortex has been erased (Fig. 4c). All sectioned synapsids have LAGs and many annuli, with the larger varanopid species having the most of any of the sampled taxa from Richards Spur (Table 2).
**Figure 4:** Mid-diaphyseal transverse sections of synapsids from Dolese Quarry, Richards Spur: a. mycterosaurine I femur cross-section viewed under 1.5X magnification (left) and close-up view of the postero-ventral region of the cortex under 5X magnification (right); b. mycterosaurine II femur cross-section viewed under 1.5X magnification (left) and close-up view of the postero-ventral region of the cortex under 10X magnification (right); and c. *Varanops* sp. femur cross-section viewed under 1X magnification (left) and close-up view of the postero-ventral region of the cortex under 5X magnification (right). Thin sections were imaged with a Nikon DS-Fi1 camera mounted onto a Nikon AZ-100 microscope. All sectioned synapsids have parallel-fibered bone within the cortex at mid-shaft and the highest amount of vascularization of all the sampled taxa from Richards Spur. White arrows indicate Lines of Arrested Growth.

**Table 2:** Number of Lines of Arrested Growth (LAGs) observed in each of the sectioned fauna of the deposits of Dolese Brothers Quarry, Richards Spur.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Element</th>
<th>LAGs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anamniote Tetrapods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cacops</em> sp.</td>
<td>femur</td>
<td>3</td>
</tr>
<tr>
<td><em>Doleserpeton</em> sp.</td>
<td>femur</td>
<td>7</td>
</tr>
<tr>
<td><em>Cardiocephalus</em> sp.</td>
<td>femur</td>
<td>3</td>
</tr>
<tr>
<td><strong>Reptiles</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Captorhinus aguti</em></td>
<td>femur</td>
<td>0</td>
</tr>
<tr>
<td><em>Captorhinus magnus</em></td>
<td>humerus</td>
<td>0</td>
</tr>
<tr>
<td>Unidentified reptile</td>
<td>femur</td>
<td>2</td>
</tr>
<tr>
<td><strong>Synapsids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycterosaurine I</td>
<td>femur</td>
<td>3</td>
</tr>
<tr>
<td>Mycterosaurine II</td>
<td>femur</td>
<td>4</td>
</tr>
<tr>
<td><em>Varanops</em> sp.</td>
<td>femur</td>
<td>10</td>
</tr>
</tbody>
</table>
The Osteocyte Lacunar Densities (OLDs), used as an approximation of metabolic rate, of the sectioned faunal assemblage of Richards Spur are recorded in Table 3. The synapsids have the highest average OLD, followed by the reptiles, and then the anamniote tetrapods. However, there are a few specimens that stand out within their larger group: the cross-sections of *Cacops* sp. and *C. aguti* had distinctly higher OLDs than the other anamniote tetrapods and the other reptiles within their groups, respectively (Table 3).

**Table 3**: Osteocyte Lacunar Densities (OLDs) of sectioned fauna of the fissure-fill deposits of Dolese Quarry, near Richards Spur, Oklahoma. The number of osteocyte lacunae (#OL) within the field of view (FOV) was counted at three different, random locations in the cortical bone and averaged before being divided by the total FOV to find the density of osteocyte lacunae.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Element</th>
<th>Field of View (L) (µm)</th>
<th>Field of View (W) (µm)</th>
<th>Field of View (H) (µm)</th>
<th>Total FOV (µm³)</th>
<th>Total FOV (mm³)</th>
<th>Av. #OL in FOV</th>
<th>OLD (#/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anamniote Tetrapods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cacops</em> sp.</td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>121</td>
<td>16,209</td>
</tr>
<tr>
<td><em>Doleserpeton</em> sp.</td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>77</td>
<td>10,314</td>
</tr>
<tr>
<td><em>Cardiocephalus</em> sp.</td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>72</td>
<td>9,645</td>
</tr>
<tr>
<td>Reptiles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Captorhinus aguti</em></td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>167</td>
<td>22,371</td>
</tr>
<tr>
<td><em>Captorhinus magnus</em></td>
<td>humerus</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>115</td>
<td>15,405</td>
</tr>
<tr>
<td>Unidentified reptile</td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>121</td>
<td>16,209</td>
</tr>
<tr>
<td>Synapsids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycterosaurine I</em></td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>190</td>
<td>25,452</td>
</tr>
<tr>
<td><em>Mycterosaurine II</em></td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>185</td>
<td>24,782</td>
</tr>
<tr>
<td><em>Varanops</em> sp.</td>
<td>femur</td>
<td>360</td>
<td>360</td>
<td>57.6</td>
<td>7464960</td>
<td>0.00746</td>
<td>156</td>
<td>20,898</td>
</tr>
</tbody>
</table>
Discussion

All histological thin sections of the sampled Richards Spur fauna consist of parallel-fibered lamellar compact bone at mid-diaphysis (Fig. 2, 3, 4). This type of bone is an indication of a slow rate of bone growth because it takes more time to arrange collagen fibres in the orderly arrangements to which they are seen in lamellar matrices (Amprino, 1947). Additionally, while there are some variations in the vascularization and remodeling of the bone between taxa, all sectioned Richards Spur fauna share relatively poor vascularity with minor bone remodeling compared to what is seen in the bone of dinosaurs, birds, and mammals, and this implies relatively lower metabolic rates, which is as one would expect of the early tetrapods of the time. Furthermore, the fibrolamellar bone type – alternating layers of woven and lamellar bone – that is commonly seen in dinosaurs and other animals that grow their bones quickly (Padian and Lamm, 2013) is absent from all sampled thin sections from Richards Spur. The absence of this key bone type from these Early Permian fauna likely indicates that fibrolamellar bone has yet to have evolved at this early stage in tetrapod evolution, though more studies from other Early Permian localities would be needed for confirmation.

Table 4 summarizes the bone types and variations across the three major taxa at Richards Spur, the anamniote tetrapods (Cacops sp., Doleserpeton sp., and Cardiocephalus sp.), the reptiles (Captorhinus aguti, C. magnus, and an unidentified reptile species), and the synapsids (two mycterosaurine varanopids and Varanops sp., a varanodontine varanopid). While all Richards Spur fauna display poor amounts of vascularization and remodeling, there are some relative differences between these major groups. There is a general increasing trend in vascularity from the anamniote tetrapods to the reptiles to the synapsids (Table 4). The smaller anamniote tetrapods (Doleserpeton sp. and Cardiocephalus sp.; Fig. 2b,c) display avascular bone, a bone type that lacks a vascular network within the bone matrix and therefore also lacks primary osteons, and is most commonly associated with fish and other lower vertebrates (Padian and Lamm, 2013). The large temnospondyl Cacops sp. however, does have some longitudinally-oriented vascular canals present within its cortical bone, but it is still pretty poorly vascularized (Fig. 2a). The unidentified reptile has similarly poor vascularity (Fig. 3c), but the captorhinids display relatively higher vascularity with largely longitudinal canals, but also some instances of more complex reticular canals (Fig. 3a,b). The synapsids undoubtedly have the highest amount of vascularity of the Richards Spur fauna (Fig. 4) and each sectioned taxon displays some
radially-oriented vascular canals, which are an indication of relatively faster bone deposition than longitudinal canals (de Margerie et al., 2004). The relative Osteocyte Lacunar Densities – which can potentially be used as a general proxy for metabolic rate (Bromage et al., 2009) – also seem to confirm that Richards Spur synapsids have the highest rate of growth and the highest metabolic rate, followed by the reptiles, and then the anamniote tetrapods (Table 4). It should be noted, however, that some caution should be applied when using OLD as an indication of metabolic rate; the density of osteocyte lacunae can vary between individuals and between different elements of a single individual (Skedros et al. 2005; Carter et al., 2013; Cullen et al., 2014).

Table 4: Summary of bone structure findings for the sectioned anamniote tetrapods, reptiles, and synapsids of the Dolese Quarry, Richards Spur.

<table>
<thead>
<tr>
<th></th>
<th>Anamniote Tetrapods</th>
<th>Reptiles</th>
<th>Synapsids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone type</td>
<td>parallel-fibered</td>
<td>parallel-fibered</td>
<td>parallel-fibered</td>
</tr>
<tr>
<td>Vascularity</td>
<td>avascular to poor</td>
<td>poor to moderate</td>
<td>relatively high</td>
</tr>
<tr>
<td></td>
<td>(some longitudinal)</td>
<td>(longitudinal, some reticular)</td>
<td>(longitudinal, some radial)</td>
</tr>
<tr>
<td>Remodeling</td>
<td>none / very minor</td>
<td>minor (some primary</td>
<td>minor (some primary</td>
</tr>
<tr>
<td></td>
<td>(few primary osteons)</td>
<td>osteons, few secondary</td>
<td>osteons)</td>
</tr>
<tr>
<td>Growth Lines</td>
<td>3 - 7</td>
<td>0 - 2</td>
<td>3 - 10</td>
</tr>
<tr>
<td>Osteocyte Lacunar Density</td>
<td>low (~10,000 – 15,000/mm³)</td>
<td>moderate (~15,000 – 22,000/mm³)</td>
<td>relatively high (~21,000 – 25,000/mm³)</td>
</tr>
</tbody>
</table>

Bone remodelling is quite poor across all sectioned Richards Spur taxa (Table 4). The small anamniote tetrapod taxa, *Doleserpeton* sp. and *Cardiocephalus* sp., have no evidence of remodelling throughout the cortex at mid-diaphysis (Fig. 2b,c), but the much larger *Cacops* sp. has the beginnings of a few primary osteons (Fig. 2a). The sectioned reptiles and synapsids of Richards Spur similarly have a few primary osteons within their cortices, but no major bone remodelling besides that (Fig. 3, 4). The sectioned eureptile *C. magnus* is the only taxon from Richards Spur to display a few instances of secondary osteons that are surrounded by cement lines (Fig. 3b). Extensive occurrences of mature Haversian bone are absent from all sampled taxa. True Haversian systems are associated with extensive secondary remodelling and are usually found in older, more mature individuals (Padian and Lamm, 2013). Absence of this
mature bone type could potentially mean these early terrestrial tetrapods have shorter lifespans that would not accommodate large amounts of bone reworking.

Growth lines are variable across the Richards Spur taxa and there are no clear patterns (Table 2). Circumferential Lines of Arrested Growth (LAGs) actually represent an absence of growth and are thus laid down in the bone matrix during limiting seasons in which the slowing of a vertebrate’s metabolism results in a pause in osteogenesis (Padian and Lamm, 2013). Even though the Dolese Quarry locality was equatorial during the Early Permian (Artinskian), oxygen isotopes studies of speleothem deposits as well as paleosol studies have demonstrated that the fauna still would have been experiencing a kind of seasonality in the form of monsoons (Tabor et al., 2009; Woodhead et al., 2010), and this explains why LAGs can be seen in all sectioned Richards Spur taxa (except the captorhinids). While there may be other factors that can affect the appearance of LAGs, growth marks tend to be the sharpest in vertebrates that live in environments that have a strong seasonality (Castanet and Baez, 1991; Castanet et al., 1993). The large sectioned Varanops sp. femur was the taxon that showed the most LAGs in the mid-shaft cortex – at least ten LAGs can be seen and there were potentially more in the matrix prior to the expansion of the medullary cavity (Fig. 4c, Table 2). This would put the age of this Varanops individual at a minimum of ten years, assuming that one zone of active growth followed by a LAG represents one growth cycle, which is normally one year (Castanet, 1975; Castanet et al., 1993).

The captorhinid reptiles, C. aguti and C. magnus, are the only taxa that are completely lacking in any LAGs from the Richards Spur locality, though there are a few annuli – an zone of slower bone growth – that can be observed in the bone cortex (Fig. 3a,b). Because LAGs are a common feature of bone growth in extinct terrestrial tetrapods that live through at least one limiting season, it is peculiar that they would be completely absent in the captorhinids of the Dolese Quarry. Richards Spur captorhinids were evidently employing a different growth strategy than the other fauna of the locality. It may be that Captorhinus was unique among the other fauna of Richards Spur as it was likely omnivorous (Modesto et al., 2007; LeBlanc et al., 2015) and being such a generalist could have been beneficial in maintaining continuous growth, even through limiting seasons, and this may be also why Captorhinus is found in such abundance at this arid, upland site.
This community-wide study on the bone histology of the local fauna provides insight into the environment of the palaeocommunity of Richards Spur. The growth lines, be they LAGs or annuli, that were found in every sectioned taxon confirmed what had been previously determined about the palaeoclimate of the Early Permian locality – that despite being equatorial in position, the Dolese Quarry near Richards Spur evidently still had some seasonality at the time. Additionally, the relatively thin cortices surrounding distinct medullary cavities in all sectioned fauna are indicative of terrestrial vertebrates (Quémeneur et al., 2013). Though this information about the Early Permian fissure-fill locality of Richards Spur is not new, it is nonetheless a good example of how palaeohistology can act as a supportive confirmation when drawing conclusions about the climate and environment of palaeocommunities.

Given that all disarticulated elements of fauna of the Richards Spur site were living contemporaneously and experiencing the same environmental conditions, this community-wide study is also beneficial in providing insight into the variability in the life history strategies in the earliest terrestrial vertebrate palaeocommunities. Among modern groups of vertebrates, extensive variation exists in tissue types and bone structure (Enlow and Brown, 1956; 1957; 1958), but in this early terrestrial tetrapod community all sectioned specimens displayed slow-growing parallel-fibered lamellar cortical bone at mid-diaphysis, with little to no vascularization and little to no bone remodeling (Table 4). Any variability seen between taxa is relative. For example, even though vascularization is fairly poor in all Richards Spur taxa, especially compared to dinosaurs and mammals, there is a relative increase in vascularity from the anamniote tetrapods to the reptiles to the synapsids (Table 4). There is also an associated increase in osteocyte lacunar density from anamniote tetrapods to reptiles to synapsids at the Richards Spur locality (Table 3). More research into the palaeocommunity histology of another Early Permian locality could be beneficial in determining whether these patterns are seen in all palaeocommunities at this critical stage of early terrestrial tetrapod diversification and evolution.
Acknowledgments

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Literature Cited


Bolt, J. R. (1980). New tetrapods with bicuspid teeth from the Fort Sill locality (Lower Permian, Oklahoma). *Neues Jahrbuch für Geologie und Paläontologie, Monatshefte, 8*, 449-459.


## Appendix 1

Table of measurements for twenty *Captorhinus aguti* femora from smallest (1) to largest (20), along with the logarithmically transformed data. Measurements are described in Figure 2.1.

<table>
<thead>
<tr>
<th>Side</th>
<th>Length (mm)</th>
<th>Log Length</th>
<th>Anterior-Posterior Diameter (mm)</th>
<th>Log Anterior-Posterior Diameter</th>
<th>Proximal Width (mm)</th>
<th>Log Proximal Width</th>
<th>Distal Width (mm)</th>
<th>Log Distal Width</th>
<th>Trochanter Height (mm)</th>
<th>Log Trochanter Height</th>
<th>Dorsal-Ventral Diameter (mm)</th>
<th>Log Dorsal-Ventral Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>left</td>
<td>15.27</td>
<td>2.47</td>
<td>0.3927</td>
<td>4.11</td>
<td>0.6138</td>
<td>5.26</td>
<td>0.7210</td>
<td>3.47</td>
<td>0.5403</td>
<td>1.93</td>
<td>0.2856</td>
</tr>
<tr>
<td>2</td>
<td>right</td>
<td>15.29</td>
<td>2.50</td>
<td>0.3979</td>
<td>3.92</td>
<td>0.5933</td>
<td>5.11</td>
<td>0.7084</td>
<td>3.63</td>
<td>0.5599</td>
<td>2.02</td>
<td>0.3054</td>
</tr>
<tr>
<td>3</td>
<td>left</td>
<td>17.16</td>
<td>2.50</td>
<td>0.3979</td>
<td>4.62</td>
<td>0.6646</td>
<td>6.04</td>
<td>0.7810</td>
<td>3.95</td>
<td>0.5966</td>
<td>2.07</td>
<td>0.3160</td>
</tr>
<tr>
<td>4</td>
<td>right</td>
<td>17.26</td>
<td>2.50</td>
<td>0.3979</td>
<td>4.30</td>
<td>0.6335</td>
<td>5.60</td>
<td>0.7482</td>
<td>3.84</td>
<td>0.5843</td>
<td>2.12</td>
<td>0.3263</td>
</tr>
<tr>
<td>5</td>
<td>left</td>
<td>17.62</td>
<td>2.52</td>
<td>0.4014</td>
<td>4.34</td>
<td>0.6375</td>
<td>5.56</td>
<td>0.7451</td>
<td>3.80</td>
<td>0.5798</td>
<td>2.15</td>
<td>0.3324</td>
</tr>
<tr>
<td>6</td>
<td>right</td>
<td>17.78</td>
<td>2.78</td>
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