# Applications in Paleohistology: Osteohistology of *Protostega gigas*

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## INTRODUCTION

*Protostega gigas* was a large sea turtle (Fig. 1) that lived in the Late Cretaceous Western Interior Seaway of North America (Fig. 2). *Protostega* fossils are found in the Smoky Hill Member of the Niobrara Formation in Kansas. Despite their abundance, little is known about how these turtles grew and aged. Osteohistology is the study of bone microstructure, and is used to study bone growth rates. Microstructure and growth rates directly relate to phylogeny (evolutionary relationships), ontogeny (growth stage), biomechanics (how a bone is used), and environment (Ricqles et al., 1991; Horner et al., 2000; Padian and Lamm, 2013).

The osteohistology of turtles, and sea turtles specifically, has not been studied extensively. Limited previous studies have focused on describing bone microstructure (Houssaye, 2012) and skeletochronology (Snover & Hohn, 2004; Chinsamy & Valenzuela, 2008), but little comparison and synthesis has been undertaken. The purpose of this study is to expand the knowledge of sea turtle osteohistology and determine the ontogenetic stage of the specimen at the time of death.


**Figure 1.** Reconstruction of *Protostega gigas*, a large sea turtle that lived in the Western Interior Seaway that covered the middle of North America during the Late Cretaceous.


**Figure 2.** Reconstruction of Western Interior Seaway ~83 million years ago.

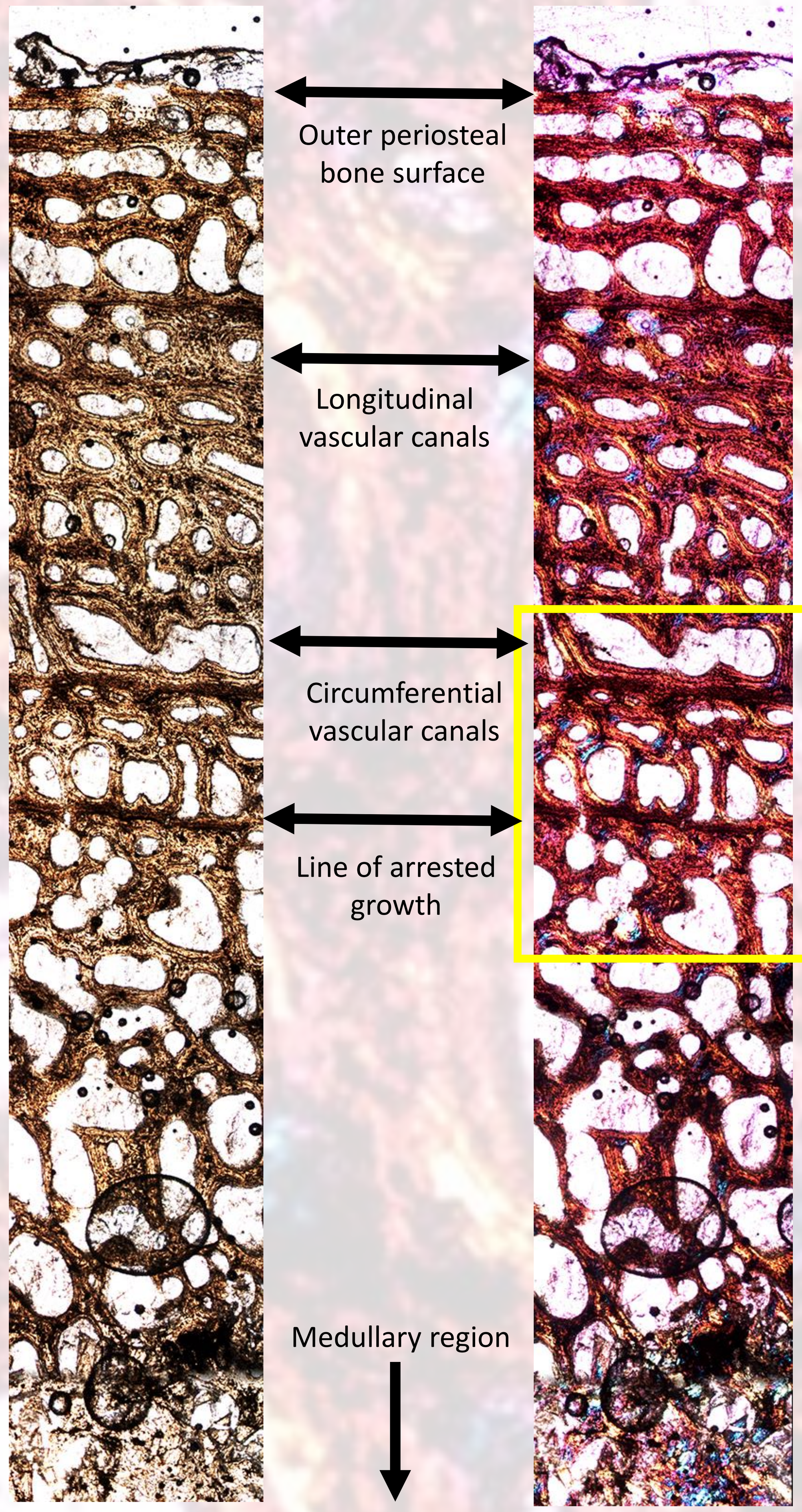
## MATERIALS & METHODS

One right femur from a *Protostega gigas* specimen housed at the Sternberg Museum of Natural History (FHSM VP-17979) was selected for histologic sampling. The specimen was photographed, molded, and casted prior to sectioning. Thin section preparation followed the procedure outlined by Lamm (2013). Slides were observed using an AmScope PZ300TC polarizing microscope and 10 MP camera. Images were edited in ImageJ.

## RESULTS

In the femur of VP-17979, the cortical bone is spongiose, with large networks of vascular canals and little or no open medullary cavity at the center of the bone (Fig. 3). The center of the bone has a more spongiose texture, there is no obvious decreases in vascular canal size toward the periosteal surface. Vascular canal orientation is dominated by longitudinal and circumferential canals organized in concentric layers. Vascular canals increase in circumferential organization towards the outer cortex (Fig. 3).

Parallel-fibered bone encircled the vascular canals, while woven bone is found between the vascular canals (Fig. 4). Three growth lines were observed in the bone, with vascular canals open to the periosteal surface. No lamellar bone was observed either along the internal or external margins of the bone.



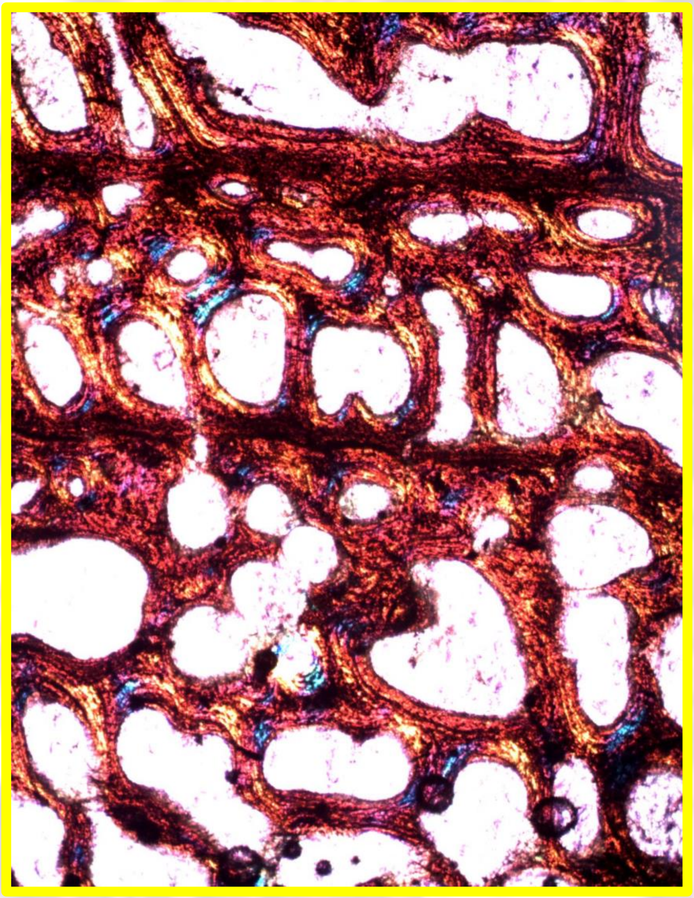



**Figure 4.** Close up of bone microstructure from the mid-cortex of VP-17979 under polarized light with a lambda plate. The large vascular canals are surrounded by well organized lamellar bone. Woven bone comprises the bone tissue between vascular canals.

**Figure 3.** Cross section through the femur of VP-17979 in plain (left) and polarized light with a lambda plate (right), showing general bone microstructure from medullary region to endosteal surface. Note the spongiose bone throughout the bone and lack of open medullary cavity. Vascular canals open to endosteal surface, showing that the turtle was still growing at the time of death.

## DISCUSSION

There has been very little previous research on sea turtle bone microstructure, and no other histology study has looked at *Protostega*, specifically. One study on the long bone histology of the sea turtle *Dermochelys* (leatherback sea turtle) found spongious bone through the entire cortex, with concentrically-organized longitudinal vascular canals (Houssaye, 2012). The *Protostega* bone analyzed in this study has a similar microstructure to that described for *Dermochelys*. However, *Protostega* has more circumferentially oriented vascular canals and a slightly less spongiose medullary region.

Skeletal maturity is marked by the reduction and cessation (or near cessation) in bone tissue deposition (de Ricqles et al., 1991; Horner et al., 2000; Woodward et al., 2013). This results in a deposit of well-organized poorly vascularized parallel-fibered (slow-growing) tissue along the periosteal surface. Because there are vascular canals open to the periosteal surface of VP-17979, with no indications of reduced vascularity and cessation in bone growth, this specimen is interpreted to have been a juvenile at the time of death. Histologic results contradict previous research interpreting VP-17979 as an adult (Everhart, 2012). The presence of three growth lines indicates the animal died in its third year while still in a rapid juvenile growth phase.

## FUTURE WORK

Given the paucity of research on turtle histology in general, and sea turtles specifically, more research on these animals is needed. More bones of VP-17979 should be sectioned to add to the understanding of bone growth within a individual. Comparisons can be made to other *Protostega* individuals to better understand sea turtle ontogeny, and comparisons to other species can shed light on how phylogenetic, function, and environmental factors affect sea turtle growth and development.

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