Rapid growth in Late Cretaceous sea turtles reveals life history strategies similar to extant leatherbacks (#78278)

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Rapid growth in Late Cretaceous sea turtles reveals life history strategies similar to extant leatherbacks

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Modern sea turtle osteohistology has been surprisingly well-studied, as it is used to understand sea turtle growth and timing of life history events, thus informing conservation decisions. Previous histologic studies reveal two distinct bone growth patterns in extant sea turtle taxa, with *Dermochelys* (leatherbacks) growing faster than the cheloniids (all other living sea turtles). *Dermochelys* also has a unique life history compared to other sea turtles (large size, elevated metabolism, broad biogeographic distribution, etc.) that is likely linked to bone growth strategies. Despite the abundance of data on modern sea turtle bone growth, extinct sea turtle osteohistology is virtually unstudied. Here, bone microstructure of the large, Cretaceous sea turtle *Protostega gigas* is examined to better understand itslife history. Long bone histology reveals bone microstructure patterns similar to *Dermochelys* with variable but sustained rapid growth through early ontogeny. Similarities between *Progostegea* and *Dermochelys* osteohistology suggest similar life history strategies like elevated metabolic rates with rapid growth to large body size and sexual maturity. Comparison to the more basal protostegid *Desmatochelys* indicates elevated growth rates are not present throughout the entire Protostegidae, but evolved in larger and more derived taxa, possibly in response to Late Cretaceous ecological changes. Given the uncertainties in the phylogenetic placement of the Protostegidae, these results either support convergent evolution towards rapid growth and elevated metabolism in both derived protostegids and dermochelyids, or a close evolutionary relationship between the two taxa. Better understanding the evolution and diversity of sea turtle life history strategies during the Late Cretaceous greenhouse climate can also impact current sea turtle conservation decisions.

Rapid growth in Late Cretaceous sea turtles reveals life history strategies similar to extant leatherbacks

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Abstract

Modern sea turtle osteohistology has been surprisingly well-studied, as it is used to understand sea turtle growth and timing of life history events, thus informing conservation decisions. Previous histologic studies reveal two distinct bone growth patterns in extant sea turtle taxa, with *Dermochelys* (leatherbacks) growing faster than the cheloniids (all other living sea turtles). *Dermochelys* also has a unique life history compared to other sea turtles (large size, elevated metabolism, broad biogeographic distribution, etc.) that is likely linked to bone growth strategies. Despite the abundance of data on modern sea turtle bone growth, extinct sea turtle osteohistology is virtually unstudied. Here, bone microstructure of the large, Cretaceous sea turtle *Protostega gigas* is examined to better understand its life history. Long bone histology reveals bone microstructure patterns similar to *Dermochelys* with variable but sustained rapid growth through early ontogeny. Similarities between *Progostegea* and *Dermochelys* osteohistology suggest similar life history strategies like elevated metabolic rates with rapid growth to large body size and sexual maturity. Comparison to the more basal protostegid

Desmatochelys indicates elevated growth rates are not present throughout the entire Protostegidae, but evolved in larger and more derived taxa, possibly in response to Late Cretaceous ecological changes. Given the uncertainties in the phylogenetic placement of the Protostegidae, these results either support convergent evolution towards rapid growth and elevated metabolism in both derived protostegids and dermochelyids, or a close evolutionary relationship between the two taxa. Better understanding the evolution and diversity of sea turtle life history strategies during the Late Cretaceous greenhouse climate can also impact current sea turtle conservation decisions.

Introduction

The timing of major life history events in sea turtle species is poorly understood because they spend most of their lives at sea (Bolten, 2003). This makes devising effective conservation measures particularly difficult. Because osteohistology can be used to assess age, growth rates, skeletal maturity, and sexual maturity, it plays an important role in sea turtle conservation biology. Consequently, the osteohistology of many modern sea turtle populations has been surprisingly well studied (Zug et al., 1986; Snover & Hohn, 2004; Snover & Rhodin, 2007; Avens & Goshe, 2007; Braun-Mcneill et al., 2008; Goshe et al., 2009; Snover et al., 2011; Petitet et al., 2015). Despite this wealth of knowledge regarding bone growth in modern taxa, the osteohistology of fossil sea turtles is virtually unknown. The purpose of this study is to examine the osteohistology of *Protostega gigas*, a large Late Cretaceous protostegid sea turtle, to better understand its growth dynamics. Framing analyses within the context of known bone microstructure, biology, and ecology in extant sea turtles help elucidate the timing of *Protostega* life history events. Additional comparisons to other extinct protostegid and non-protostegid taxa shed light on the evolution and phylogenetic distribution of sea turtle growth strategies in the Late Cretaceous with possible implications for conservation efforts.

Protostega gigas is the second largest known sea turtle taxon (behind its sister taxon *Archelon ischyros*), reaching a length of 3.4m with a flipper span of 4.7m (based on DMNH 1999). Like the modern leatherback sea turtle, *Dermochelys coriacea*, *P. gigas* had a reduced carapace and plastron. Specimens are found in Santonian to Campanian-aged marine rocks of the Western Interior Seaway and Atlantic coast, with the northern-most definitive specimen from the Pembina Member of the Pierre Shale in Manitoba, Canada (Nicholls, Tokaryk & Hills, 1990). Although the phylogenetic position of the Protostegideae in relation to other turtle groups is not clearly resolved (see discussion below), the genera included in and monophyly of the Protostegidae are fairly consistent (Hirayama, 1994, 1998; Hooks, 1998; Kear & Lee, 2006; Cadena & Parham, 2015; Evers & Benson, 2018). Within these phylogenetic frameworks, *Protostega* is considered one of the most derived protostegids and sister taxon to *Archeon*, who seemed to replace *Protostega* in late Campanian seas. Historically, several *Protostega* species have been named, including *P. gigas* (Cope, 1871), *P. potens* (Hay, 1908), *P. dixie* (ZANGERL, 1953), and *P. eaglefordensis*(ZANGERL, 1953). However, Hooks (1998) suggested removing *P. eaglefordensis* from the genus and synonymized all remaining *Protostega* species into *P. gigas*, making *Protostega* monospecific. Hooks's (1998) taxonomy is followed here.

Because bone growth patterns record the history of bone growth for that organism, and bone growth reflects phylogenic, ontogenic, biomechanic, and environmental factors, osteohistology studies can be used to infer life history strategies of extinct organisms (Cooper et al., 2008; Padian & Lamm, 2013; Marín-Moratalla, Jordana & Köhler, 2013). Histologic features like vascular canal density, vascular canal orientation, osteocyte lacunae shape and density, and college fiber orientation can be used to infer relative growth rates. Cyclical growth marks (CGMs; e.g., annuli and lines of arrested growth) are used to calculate absolute growth rates and the age at time of death (see Padian & Lamm, 2013 for overview). Changes in growth rates through the life of an organism are used to infer life history traits like metabolism, age at sexual maturity, and age at somatic growth (e.g., Padian & Lamm, 2013), making histology important for understanding vertebrate growth.

The Cheloniidae (which includes all extant sea turtle species except the leatherback) shows similar growth patterns. All sampled taxa have low global compactness, indicating overall spongiose bone (Nakajima, Hirayama & Endo, 2014). Loggerheads (*Caretta caretta*) (Zug et al., 1986; Snover & Hohn, 2004; Casale et al., 2011; Guarino et al., 2020), Kemp's ridleys (*Lepidochelys kempii*) (Goshe et al., 2009; Snover et al., 2011), olive ridleys (*Lepidochelys olivacea*) (Petitet et al., 2015), and green sea turtles (*Chelonia mydas*) (Snover et al., 2011) have a spongiose medullary area that grades into a more compact cortical bone towards the periosteal surface. Cortical bone is characterized by small, longitudinal vascular canals oriented in concentric rows. The size and density of vascular canals typically decrease towards the periosteal surface with the thickness of the dense periosteal cortical bone increasing with ontogeny (Snover & Hohn, 2004; Snover et al., 2011; Guarino et al., 2020). Secondary remodeling is present in older individuals (Zug et al., 1986; Snover & Hohn, 2004). While not all previous studies have noted the collagen fiber orientation associated with cheloniid bones (since many studies are focused on skeletochronology and samples are often decalcified), some authors note loggerheads have parallel-fibered bone (Zug et al., 1986; Houssaye, 2013).

Though not as well studied as some of the other extant taxa, leatherbacks have a distinctly different growth pattern. Global compactness profiles reveal an even greater degree of spongiose bone and vascularity compared to cheloniids (Kriloff et al., 2008; Nakajima, Hirayama & Endo, 2014; Houssaye, Martin Sander & Klein, 2016). Vascular canals are large and longitudinally oriented in concentric rows, but sampled individuals lack the denser cortical bone on the periosteal margin (Rhodin, 1985; de Ricqlès, Castanet & Francillon-Vieillot, 2004). Similar growth patterns are observed in the humerus, femur, and tibia, despite differences in function between the fore- and hindlimbs. Because studies have used bones either decalcified or micro-CT scanned bones, collagen fiber organization has not been noted. The difference in leatherback bone growth is particularly intriguing considering the unique biology and ecology of leatherbacks with rapid early ontogenetic growth, elevated body temperatures, gigantothermy, deep diving capabilities, and fully pelagic lifestyles (Lutcavage & Lutz, 1986; Paladino, O’Connor & Spotila, 1990; Spotila, O’Connor & Paladino, 1997; Bolten, 2003).

Within the Protostegidae, only the histology of *Archelon ischyros* (the sister taxon to *Protostega*) has been noted. Rhodin(Rhodin, 1985: 763) briefly described the microstructure of a phalange as “nearly identical to the pattern in the leatherback” with no clear transition between medullary and cortical regions and no compact cortical bone. No other extinct sea turtles have been histologically studied and changes in bone microstructure through ontogeny are not well understood for extant or extinct sea turtles. The lack of rigorous study leaves many questions regarding the osteohistologic patterns and their relationship to the life history strategies of protostegids, specifically, and extinct sea turtle taxa in general.

Materials & Methods

Institutional Abbreviations

CM – Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA; DMNH – Denver Museum of Nature and Sciences, Denver, Colorado, USA; FHSM – Fort Hays State University’s

Sternberg Museum of Natural History, Hays, Kansas, USA; KUVP – University of Kansas Museum of Natural History, Vertebrate Paleontology Collection, Lawrence, Kansas, USA

Materials

Fossil specimens histologically sampled for this study are listed in Table 1. Different sized *Protostega* specimens were selected with the goal of capturing multiple ontogenetic stages. Because studies on modern sea turtles show growth and life history differences between geographically separate populations (e.g., Seminoff et al., 2002; Bjorndal et al., 2003, 2013; Chaloupka, Limpus & Miller, 2004; Balazs & Chaloupka, 2004; Peckham et al., 2011; Ramirez et al., 2020; Avens et al., 2020), only *Protostega* specimens collected from the Smoky Hill Member of the Niobrara Formation of Kansas were used in this study.

Additional taxa were also sectioned for comparison. *Desmatochelys lowi* provides an example of growth in a more basal protostegid and *Toxochelys* is generally considered an outgroup to all other sea turtle clades (Kear & Lee, 2006; Cadena & Parham, 2015; Raselli, 2018; Scavezzoni & Fischer, 2018; Gentry et al., 2018; Evers & Benson, 2018; Evers, Barrett & Benson, 2019). Six previously sectioned *Dermochelys* individuals were loaned from Dr. Anders Rhodin (Chelonian Research Foundation) for analysis. See Rhodin (Rhodin, 1985) for slide preparation methods. Descriptions of extant cheloniids included in this study are based on previous publications.

Methods

Humeri and femora were thin sectioned to assess ontogenetic stage and growth rates (Fig. 1). Humeri were sectioned just distal to the deltopectoral crest, and femora were sectioned at the mid-diaphysis. Zug et al. (1986) sectioned multiple elements from Loggerhead cranial, axial, and appendicular skeletons to access suitability for osteohistology analysis. Of the bones sectioned (carapace, dentary, cervical vertebrae, phalanx, ulna, and humerus), the authors found the humerus most suitable due to the preservation of growth marks in periosteal bone, but also state that the femur is likely suitable, as well. The authors also show that the highest density of cortical bone in the humerus was located just distal to the deltopectoral crest. Subsequently, sectioning humeri just distal to the deltopectoral crest is common practice in sea turtle osteohistology studies (e.g., Zug et al., 1986; Snover & Hohn, 2004; Goshe et al., 2020) and was followed in this study (Fig.1). The FHSM VP-17979 femur was sectioned at the mid-diaphysis, as is typical in most tetrapod long bone ontogentic studies (e.g., Padian & Lamm, 2013).

All specimens were photographed, molded, casted, and 3D scanned prior to sectioning. Osteohistology methods followed Lamm (2013). A tile saw was used for most sectioning, except for the *Toxochelys*, FHSM-17979 femur, and CM-1393, which were sectioned with an Isomet Low Speed saw. Most bones were embedded in Silmar 41 with an MEKP catalyst; the FHSM-17979 femur was embedded in Buehler EpoThin epoxy resin and hardener. All sections were mounted to glass slides with either Devcon 2-Ton Epoxy or J.B. Weld ClearWeld. Sections were ground to optical clarity on a Buehler Ecomet lap wheel. Thin sections were only made and observed in transverse section.

As bone expands, primary bone is absorbed and remodeled into secondary bone, permanently obscuring the early ontogenetic record, including CGMs. Taphonomic alteration like crushing and bacterial invasion can also obscure the growth record, especially in the spongiose medullary region of sea turtle long bones. Consequently, qualitative retrocalculation was used to estimate missing CGMs from the inner regions of the sampled long bones to allow for a more accurate age estimate of individuals at the time of death. To estimate lost CGMs due to secondary remodeling and taphonomic alteration, smaller and larger humeri were appropriately scaled and the smaller specimen was transposed on the larger in Adobe Photoshop. The number of CGMs identified in the smaller humerus but missing for the larger was added to the number in the larger specimen to estimate the age of the larger individual at the time of death.

Slides were analyzed using an Olympus BX53M microscope, and photographs were taken with an Olympus SC180 camera. Images were edited using Olympus Stream Essentials and Adobe software. 3D surface scans of sectioned specimens are reposited on Morphosource (Project ID: 000418396); high resolution images of thin sections are reposited in MorphoBank (Project 4289).

Results and Discussion

Protostega long bone osteohistology has never been studied, so detailed descriptions of sampled specimens are provided in the Supplemental Material. In general, similar histologic patterns are observed in all *Protostega* bones analyzed in this study. Well-vascularized spongiose bone with abundant, round osteocyte lacunae, mixed woven, parallel-fibered, and lamellar bone, and widely spaced CGMs provide evidence of sustained, rapid growth during all sampled ontogenetic stages (Figs. 2, S1, S2). End fundamental system (EFS) are characterized by closely spaced CGMs, low vascularity, flattened osteocytes, and/or lamellar bone are observed at the periosteal surface, and indicate somatic maturity. No EFSs are observed in any sampled bones, meaning that even the older *Protostega* individuals (CM-1421 and KUVP-1208) had not reached skeletal maturity in their ninth year (Fig. 2E, F, S2). Because sexual maturity and decreased growth rates (and consequently body size) are closely correlated in sea turtles (Wood & Wood, 1980; Price et al., 2004; Casale et al., 2009; Avens & Snover, 2013; Bjorndal et al., 2014; Omeyer, Godley & Broderick, 2017; Turner Tomaszewicz et al., 2022), no *Protostega* individuals had likely reached sexual maturity at the time of death either. Despite this, the humerus grew to over 35cm in length by age eight and doubled in length between ages four and eight (Table 1). One of the largest recorded *Protostega* humeral lengths is 42 cm from a Mooreville Chalk specimen (Renger, 1935; Danilov et al., 2022). If this specimen represents a skeletally (and thus sexually) mature individual, then KUVP 1208 (the largest specimen in this sample set) is 85% of maximum humeral length. With sustained growth rates, it is possible that Protostega reached skeletal and sexual maturity within 10 years.

Previous studies reveal two bone growth strategies in extant sea turtle populations. All sampled cheloniid taxa have low global compactness, indicating overall spongiose bone (Nakajima, Hirayama & Endo, 2014). Leatherbacks display extremely spongiose bone throughout the cortex with no clear separation between the medullary cavity and cortical bone (Rhodin, 1985; de Ricqlès, Castanet & Francillon-Vieillot, 2004; Snover & Rhodin, 2007; Kriloff et al., 2008; Nakajima, Hirayama & Endo, 2014; Houssaye, Martin Sander & Klein, 2016) (Figs. 3C, S4). Cheloniids also have low global compactness (Nakajima, Hirayama & Endo, 2014), but the outer cortex is denser with lower vascularity even in earlier ontogeny (e.g. Zug et al., 1986; Goshe et al., 2009; Casale et al., 2011; Snover et al., 2011; Petitet et al., 2015; Şirin & Başkale, 2021). When compared to modern sea turtle long bones, *Protostega* bone microstructure is more similar to leatherbacks, with no distinguishable medullary cavity and highly vascularized bone extending to the periosteal surface. Even in the oldest individuals sampled, spongiose bone is evidence through the entire cross section, with the denser cortical bone observed in cheloniids lacking.

The similarities between *Protostega* and leatherback bone growth invites comparison in life history strategies. Leatherbacks differ from other sea turtles in their large body size, completely pelagic ecology, migration into cold arctic waters, deep diving, and continuous swimming (Paladino, O'Connor & Spotila, 1990; Spotila, O'Connor & Paladino, 1997). One of the most notable leatherback life history characteristics is their elevated resting metabolic rates and ability to hold a body temperature above the surrounding water temperature (Paladino, O'Connor & Spotila, 1990; Spotila, O'Connor & Paladino, 1997). While they are not considered endothermic, the term 'gigantothermy' was first used to describe the adult leatherback's elevated metabolism (Paladino, O'Connor & Spotila, 1990); although, it should be noted that smaller-bodied juvenile leatherback also have elevated resting metabolic rates and unique behaviors like constant swimming (Lutcavage & Lutz, 1986). Some of these life history strategies are reflected in bone microstructure. For example, rapid leatherback growth and elevated metabolic rates are denoted in the highly vascularized bone with widely-spaced CGMs. A pattern also observed in *Protostega* (Figs. 2, S1, S2).

Studies on leatherback appendicular bones also reveal unique surficial features. The epiphyseal articular surface of leatherback bones has a rough, dimpled subchondral surface, that is evidence of highly vascularized epiphyseal cartilage (Rhodin, Ogden & Conlogue, 1981; Rhodin, 1985; Snover & Rhodin, 2007). This unique chodro-osseous characteristic likely reflects high vascularization of cartilage related to rapid growth to large body size (Rhodin, Ogden & Conlogue, 1981; Rhodin, 1985; Snover & Rhodin, 2007). Because this feature is missing in the large, extinct, freshwater *Stupendemys* (Rhodin, 1985), it cannot be attributed to large size alone.

While this chondro-osseous growth pattern is not seen in other living sea turtles, it has been identified in the derived protostegid *Archelon* (Rhodin, 1985). Although vascularized epiphyseal cartilages was originally note as absent in *Protostega* (Snover & Rhodin, 2007), the present study provides evidence that these epiphyseal rugosities are, in fact, present in large *Protostega* humeri (Fig. 2G). These rugosities are absent in the more basal *Desmatochelys*, an observation in agreement with previous reports (2007). Snover and Rhodin (2007) suggest that the presence of this character possibly supports a close phylogenetic relationship among protostegids and leatherbacks. While this has yet to be widely supported (see phylogenetic discussion below), similarities in osteohistological and chondro-osseous growth patterns between *Protostega* and leatherbacks support similar growth and life history patterns—specifically, rapid growth to large body size with elevated metabolic rates.

The growth pattern observed in *Protostega* bones is in strong contrast to the more basal protostegid *Desmatochelys* (Figs. 3A, S4A-C). *Desmatochelys* humerus microstructure is more similar to cheloniids and the extinct *Toxochelys* (Fig. 3B, S3D-F), having a discernable cortical bone with reduced vascularization. These histologic patterns indicate prolonged growth with a later ontogenetic attainment of sexual maturity at smaller body size. Consequently, at some point between *Desmatochelys* and *Protostega*, protostegids evolved rapid growth rates to larger size. While it is always difficult to ascertain the biotic and abiotic pressures leading to evolutionary novelties, large body size is a successful defense against predation (e.g. Reimchen, 1991; Chase, 1999; Isbell, 2005). To this point, adult leatherbacks have few non-human predators, owing to their large body size (James, Myers & Ottensmeyer, 2005). Though both *Protostega* and *Desmatochelys* are in the Protostegidae and share an evolutionary history, *Desmatochelys* is a more basal taxon that evolved in the late Early Cretaceous (Barremian) tens of millions of years prior to *Protostega*, with *D. lowii* occurrences dating to the Cenomanian and Turonian (López-Conde et al., 2019). This puts the origin of *Desmatochelys* prior to the evolution of mosasaurs and polycotylid plesiosaurs. On the other hand, the appearance of *Protostega* in the Santonian parallels the evolution of large pelagic tylosaurid mosasaurs like *Tylosaurus prorigor*. Organismal and theoretical studies suggest that rapid growth evolves in response to predation pressure (e.g. Arendt, 1997; Arendt & Reznick, 2005; Cooper et al., 2008; Woodward et al., 2015), allowing prey taxa to reach a large body size faster and avoid predation at various ontogenetic stages. In the case of Late Cretaceous protostegids, changes in ecosystem structure between the evolution of *Desmatochelys* and *Protostega* may have resulted in selective pressures favoring the evolution of faster growth rates and larger body size. The evolutionary timing of rapid growth to large body size observed in derived protostegids supports a hypothesis that this growth strategy provided an advantageous evolutionary response to the evolution of large open ocean predators. Increased growth rate would be particularly important for sea turtles where the timing of sexual maturity is correlated with body size(Omeyer, Godley & Broderick, 2017).

Despite overall sustained rapid growth, most individuals in this study show variable bone deposition rates between CMGs. This is most notable in FHSM VP-19797, in which the femur and humerus both preserve similarly uneven bone apposition rates. In this individual, the first and second CGMs are much more closely spaced than the second and third. Modern sea turtles are known to have irregular growth rates with closely-spaced CGMs in early ontogeny followed by more widely spaced CGMs later in ontogeny (e.g., Snover & Hohn, 2004). Growth plasticity would increase survival during periods of harsh environmental conditions (abnormal temperatures, low food availability, etc.) (Kohler et al., 2012) and would have given Protostega an evolutionary advantage over animals with more rigid growth strategies unable to adjust for environmental stress. This phenomenon has been observed in other archosauromorphs (e.g., Cullen et al., 2014; Zanno et al., 2019; Woodward et al., 2020), and is now proposed for *Protostega*, perhaps contributing to the overall evolutionary success of sea turtles in general.

Analysis of *Protostega* osteohistology also has interesting phylogenetic implications. While several studies have addressed fossil sea turtle phylogenetics (e.g. Hooks, 1998; Kear & Lee, 2006; Joyce, 2007; Cadena & Parham, 2015; Raselli, 2018; Evers & Benson, 2018; Evers, Barrett & Benson, 2019), consensus regarding the phylogenetic placement of various sea turtle taxa, including the Protostegidae, is lacking. Most of the recent analyses place *Toxochelys* outside, and basal to, the Chelonoidea, which includes all extant sea turtles and the Protostegidea (Kear & Lee, 2006; Cadena & Parham, 2015; Gentry et al., 2018; Raselli, 2018; Scavezzoni & Fischer, 2018; Evers, Barrett & Benson, 2019; but see Gentry, Ebersole & Kiernan, 2019). While leatherbacks are generally regarded as a separate evolutionary lineage from the Cheloniidae, the relationship among leatherbacks, cheloniids, and protostegids is not clear. Most studies resolve *Dermochelys* and the Protostegidae as sister groups (Hirayama, 1998; Kear & Lee, 2006; Cadena & Parham, 2015; Scavezzoni & Fischer, 2018) or as a single lineage (Gentry et al., 2018), but some align *Dermochelys* more closely with the Cheloniidae (Raselli, 2018; Gentry, Ebersole & Kiernan, 2019; Evers, Barrett & Benson, 2019) leaving protostegids a more distant lineage. A few studies resolve the Protostegidae further removed from other sea turtles as a more basal eucryptodire lineage (Joyce, 2007; Anquetin, 2012). Most studies focused specifically on protostegid phylogentics support Protostegidae and Dermochelyidae as sister groups (Hooks, 1998; Hirayama, 1998; Kear & Lee, 2006; Cadena & Parham, 2015; Scavezzoni & Fischer, 2018).

If protostegids and dermochelids are sister taxa (Hirayama, 1998; Kear & Lee, 2006; Cadena & Parham, 2015; Scavezzoni & Fischer, 2018), then highly spongiose bone and rapid growth until sexual maturity either evolved convergently in sea turtles or were shared by a common ancestor and *Desmatochelys* secondarily lost this character. Because these bone microstructure patterns are also paired with the presence of vascularized cartilage and rugosities marking the epiphesial surface of the proximal humerus, and this morphological pattern is seen in leatherbacks, *Archelon*, and *Protostega* but not more basal protostegids (Rhodin, 1985; Snover & Rhodin, 2007; this study), it is likely that other basal protostegids lack the bone growth patterns of *Protostega* and leatherbacks. Consequently, bone microstructure and macrostructure better support the hypothesis that rapid growth strategies are convergent between derived protostegids and *Dermochelys*. Likewise, if *Protostega* and *Dermochelys* are more distantly related (Joyce, 2007; Anquetin, 2012; Raselli, 2018; Evers & Benson, 2018; Gentry, Ebersole & Kiernan, 2019; Evers, Barrett & Benson, 2019), then the osteohistologic patterns seen in these two taxa must be convergent, as other taxa do not share their bone growth pattern (e.g. *Toxochelys*, cheloniids). Alternatively, at least one study has hypothesized that the Dermochelyidae is within the Protostegidae (Snover & Rhodin, 2007; Gentry et al., 2018). In this case, the similar histologic patterns could be explained by a single evolutionary innovation inherited from a common derived protostegid ancestor. These hypotheses can be tested with more sampling from fossil Dermochelidae and basal Protostegidiae taxa, in addition to refining phylogenetic analyses.

Conclusions

Extant sea turtles display two bone growth patterns that appear to relate to life history strategies (Bolten, 2003; Snover & Rhodin, 2007) with leatherback having evolved sustained rapid growth to large body size and an elevated metabolism compared to cheloniids. When compared to extant sea turtle osteohistology, the bone microstructure of the Late Cretaceous protostegid *Protostega gigas* more closely resembles leatherbacks than sampled members of the Cheloniidae. Consequently, histological evidence supports a hypothesis that *Protostega* likely shared life history traits with leatherbacks, such as elevated early ontogenetic growth and possibly elevated resting metabolic rates. This is corroborated by the first evidence of vascularize cartilage on the epiphysial surface of the *Protostega* humerus, a character also associated with rapid growth (Rhodin, Ogden & Conlogue, 1981; Rhodin, 1985; Snover & Rhodin, 2007). Results from this study illustrate that *Protostega* could reach 85% of the body size of the largest known individual within nine years of hatching.

Because the more basal protostegid *Desmatochelys* lacks the same rapid growth patterns as *Protostega*, this evolutionary character likely evolved along the protostegid lineage and is not plesiomorphic to the clade. This has phylogenetic implications regarding the single or multiple origin of rapid growth patterns in large-bodied sea turtles, depending on the phylogenetic placement of *Dermochelys* with respect to the Protostegidae. Regardless, rapid early ontogenetic growth to large body size provides an evolutionary advantageous for these sea turtles sharing a pelagic habitat with large predators like sharks, ichthyodectids, plesiosaurs, and mosasaurs.

Although more research is needed, studying the evolution, growth strategies, and biodiversity of extinct sea turtles has implications for extant sea turtle conservation, particularly in light of warming ocean temperatures. Numerous sea turtles taxa thrived in Late Cretaceous oceans under greenhouse conditions, providing a model of the future through the lens of deep time experiments. Exploring the diversity of sea turtle growth strategies, possible environmental stressors leading to evolutionary innovations, and survivability of taxa (with an understanding of their life history strategies) across space and time has the potential to inform sea turtle conservation efforts.

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Figure 1(on next page)

Protostegid sea turtle long bones histologically sampled.

One long bone from the basal protostegid *Desmatochelys lowii* and four long bones from the derived protostegid *Protostega gigas* were thin sectioned for analysis and comparison. Red lines indicate where samples were taken. See Table 1 for absolute bone sizes and text for institutional codes and sampling protocols.

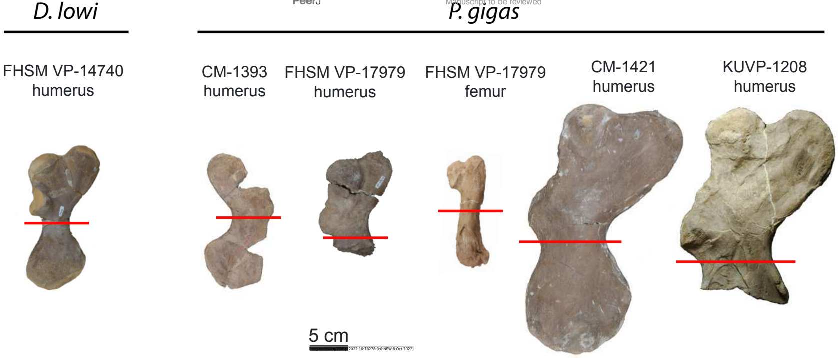
D. lowi
P. gigas
FHSM VP-14740
humerus
CM-1393
humerus
FHSM VP-17979
humerus
FHSM VP-17979
femur
CM-1421
humerus
KUVP-1208
humerus
5 cm

Figure 2

Micro- and macrostructures observed in *Protostega gigas* long bones.

Small *P. gigas* humerus FHSM VP-17979 in (**A**) plane light and (**B**) polarized light with a lambda filter. Small *P. gigas* femur FHSM VP-17979 in (**C**) plane light and (**D**) polarized light with a lambda filter. Note the irregularly spaced cyclical growth marks (pink arrows) in both the humerus and femur. Large *P. gigas* humerus KUVP 1208 in (**E**) plane light and (**F**) polarized light with lambda filter with pink arrows highlighting CGMs. (**G**) Epiphysial surface of large *P. gigas* humerus CM 1421 showing rugosities associated with fast-growing vascularized cartilage. Periosteal surface to the right in (**A–F**). Scale bars on (**A–F**) is 1mm; scale bar on (**G**) is 1cm.

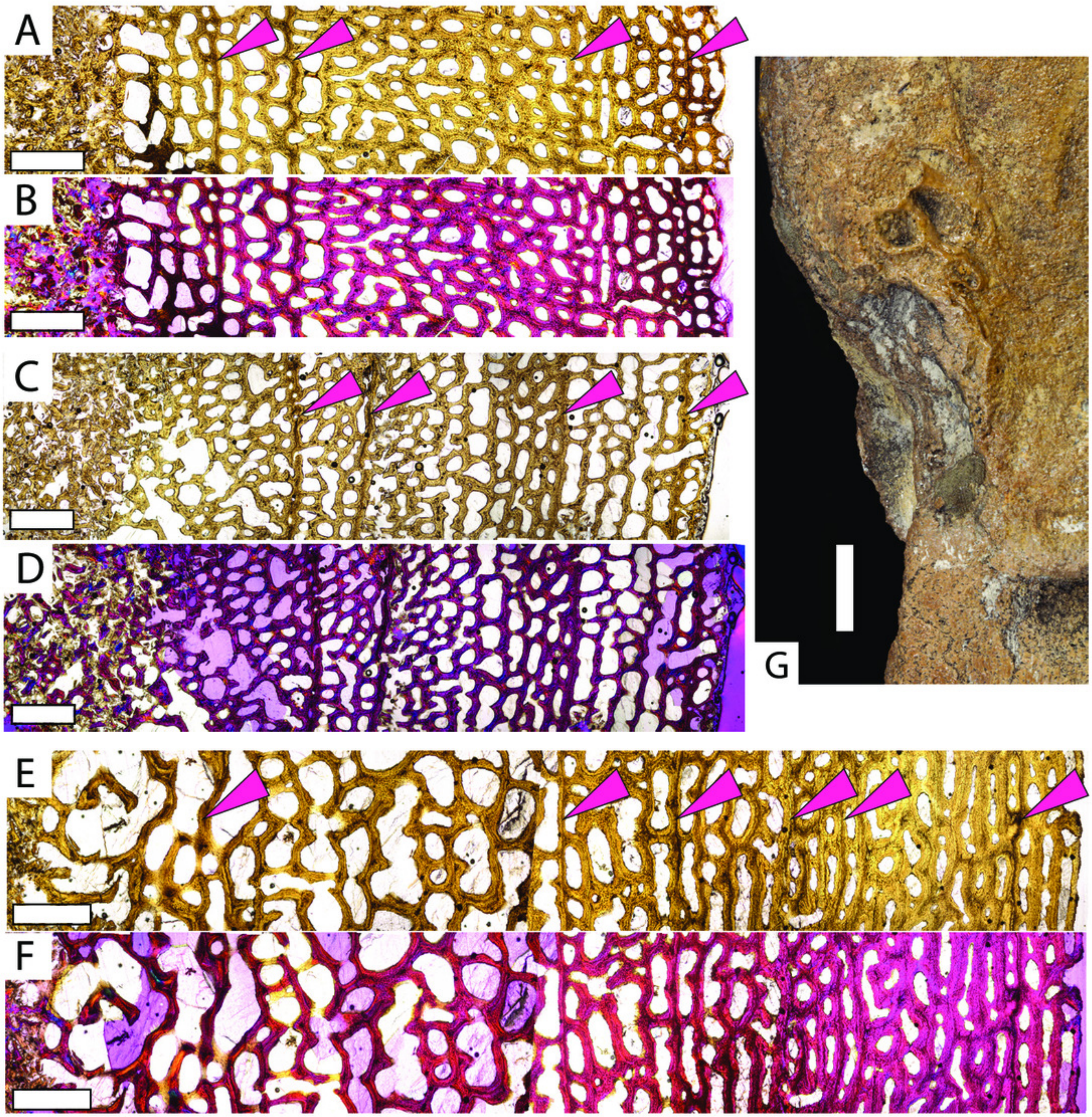
A
B
C
D
G
E
F

Figure 3

Microstructures observed in non-*Protostega* sea turtle long bones for histologic comparison.

Humeri of basal protostegid *Desmatochelys lowii* FHSM VP-17470 (**A**) and non-protostegid sea turtle *Toxochelys latiremis* FHSM VP-700 (**B**) in plane light. Humerus of modern *Dermochelys corticea* CRF (Chelonian Research Foundation) 4911 (**C**) in plane light. CRF 4911 is a female of unknown age with curved carapace length of 135 cm. Periosteal surface is to the right in all figures. Scale bar in all figures is 1mm.

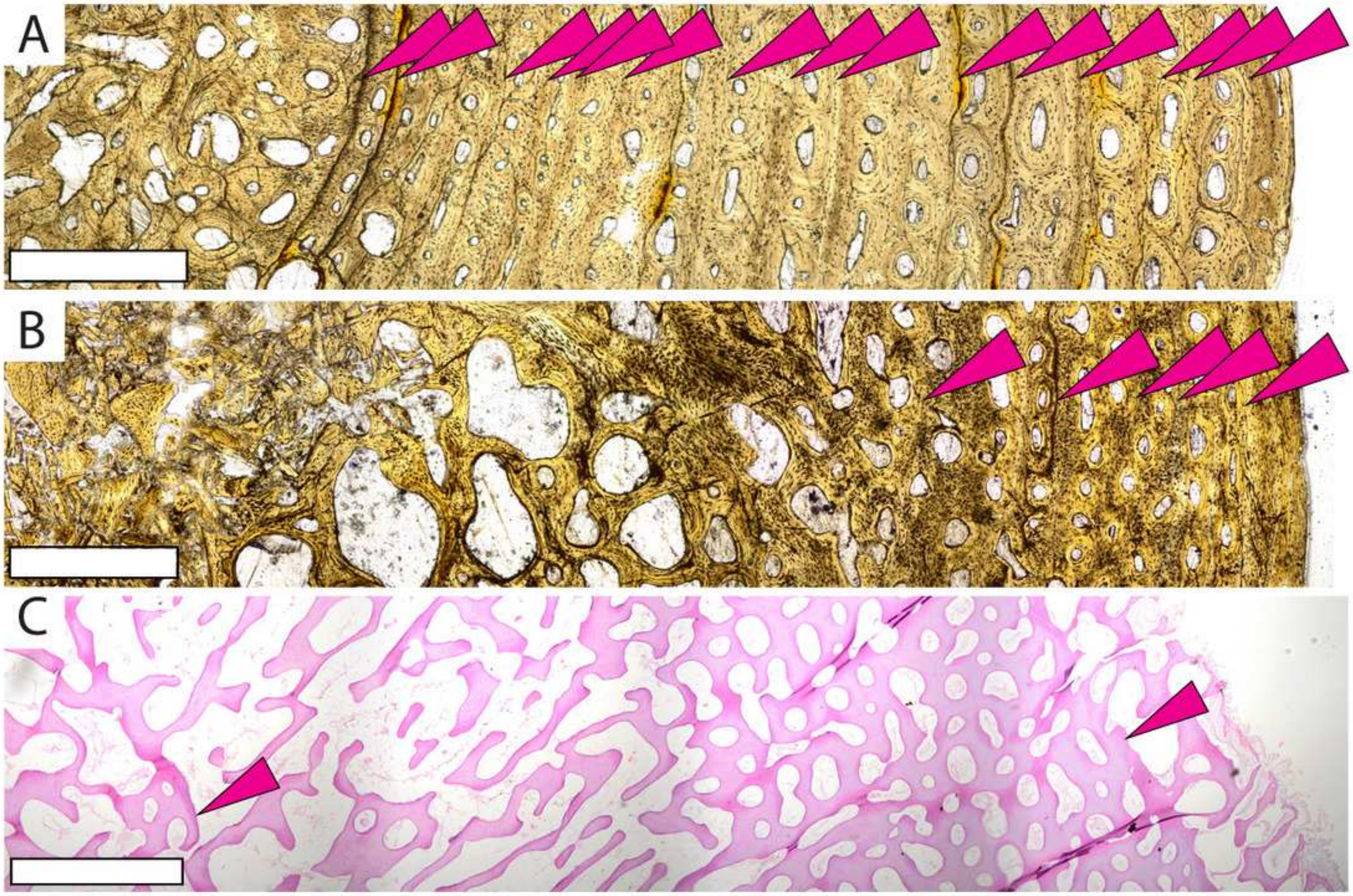

Table 1(on next page)

Fossils used forosteohistologicanalysis in this study.

Table 1. Fossils used for osteohistologic analysis in this study.

Taxon	Museum Number	Element	Length (cm)	CGMs
Protostega				
	FHSM VP-17979	Humerus	18.0	4
	FHSM VP-17979	Femur	14.2	4
	CM 1393	Humerus	17.7	2
	CM 1421	Humerus	33.8	8*
	KUVP 1208	Humerus	35.0	8*
Desmatochelys				
	FHSM VP-17470	Humerus		15+
Toxochelys				
	FHSM VP-700	Humerus	14.5	5+

* Total CGMs estimated by retrocalculation.

Supplementary Materials for

Title: Rapid growth in Cretaceous sea turtles reveals life history strategies similar to leatherbacks

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This PDF file includes:

Supplementary Text
References
Figs. S1 to S4

Supplementary Text: Histologic descriptions

FHSM VP-17979, *Protostega gigas* humerus and femur (Fig. 2A-D; Fig. S1A-F)

Although bone shape and size vary between bones, similar histologic patterns are observed in both the femur and humerus of FHSM VP-17979. Bones lack a distinct medullary cavity with well-vascularized, spongiosa bone continuing through the entire section. Longitudinally-oriented primary osteons dominate both the femur and humerus, with some canals stretched radially or circumferentially in different parts of the section. Vascular canals are surrounded by parallel-fibered bone with some woven tissue between osteons. The vasculature and spongiosa nature of *Protostega* long bones are similar to the leatherback sea turtle (Rhodin, 1985; de Ricqlès, Castanet & Francillon-Vieillot, 2004; Kriloff et al., 2008; Houssaye, Martin Sander & Klein, 2016) (Figs. 3C, S4). No secondary remodeling is observed.

Three cyclical growth marks (CGMs) are clearly observed in both the femur and the humerus, with a fourth visible along portions of the periosteal margin of the femur. However, taphonomic alteration (crushing, microbial invasion, etc.) prevent clear analysis of the periosteal-most surface in sectioned specimens. The first and second GCMs are visually similar to the depositional cycles Curry (1999) observed in the early juvenile *Apatosaurus* scapula. These growth marks lack a clear line delineating a full cessation of growth, as seen in lines of arrested growth (LAGs). CGMs marked by changes in deposition without a clear LAG are also typical of extant sea turtles early in ontogeny, like the Kemp's ridley sea turtle (Snover & Hohn, 2004). Skeletochronology studies on known-aged sea turtles indicate that these marks should be considered annual an can be used to age individuals (Snover & Hohn, 2004). Similarities in marks and relative distance between CGMs in the humerus and femur also support that they represent annual cycles. Although there is some internal crushing, there is no evidence to suggest that there are missing CGMs due to taphonomy or resorption. Based on histological evidence, FHSM VP-17979 was in its fifth year of growth at the time of death.

CM 1393, *Protostega gigas* humerus (Fig. 2E-F; Fig. S1G-I)

CM-1393 has a significant amount of crushing that prevents analysis of a complete cross section. The best-preserved areas show a similar pattern to FHSM VP-17979 with large vascular spaces extending to the periosteal surface. The effects of crushing are likely amplified by the cancellous nature of the bone. Longitudinal primary osteons are surrounded by parallel-fibered and lamellar tissue with some woven bone between osteons, and they are organized in concentric layers. No secondary remodeling is observed.

Despite crushing, three CGMs can be identified near the periosteal margin of the bone. It is unknown if additional CGMs were present in the inner cortex prior to crushing, but CM 1393 was at least in its fourth year of growth at the time of death. Overall, this age estimate is consistent with what is expected considering similarities in size between FHSM VP-17979 and CM 1393 (Fig. 1; Table 1).

CM 1421, *Protostega gigas* humerus (Fig. S2A-C)

Despite being almost twice as large as FHSM VP 17979 and CM 1393, the bone microstructure patterns observed in CM 1421 are strikingly similar. CM 1421 is comprised of spongiosa bone that extends to the periosteal surface with no clearly delineation medullary cavity. Vascular patterns are similar to those observed in smaller *Protostega* specimens, with large longitudinally-oriented primary osteons arranged in concentric layers; vascular canals increase in organization towards the periosteal surface. Likewise, parallel-fibered bone is found around vascular canals with small pockets of woven bone between vascular areas. Minimal secondary remodeling is evident by the formation of large erosion rooms throughout the bone cross section.

Despite crushing that obscures some areas of the inner-most bone, five CGMs are identified in the outer bone tissue extending to the periosteal margin. Retrocalculation performed by scaling and superimposing the smaller FHSM VP-17979 to the larger CM 1421 indicates that multiple CGMs were lost due to secondary remodeling and taphonomic alteration of CM 1421. Although estimates could be skewed due to the variable nature of CGM deposition in sea turtles (illustrated by the inconsistency in bone apposition rates between CGMs of FHSM VP-17979), it is estimated that three CGMs could have been lost. This indicates that CM 1421 was possibly in its ninth year of life at the time of death. No end fundamental system (EFS) was identified on along the periosteal margin of the bone, indicating this individual was still actively growing at the time of death.

KUVP 1208, *Protostega gigas* humerus (Fig. 2E-F; Fig. S2D-F)

KUVP 1208 is the largest specimen in this study, though only slightly larger than CM 1421. Unsurprisingly given the similar sizes, the bone microstructures of KUVP 1208 and CM 1421 are also quite similar. Like all the humeri in this study, post-depositional crushing obscures the innermost bone tissue. However, KUVP 1208 does preserve spongiosa bone throughout the humerus with the size and density of vascular canals decreasing towards the periosteal surface. Although bone is denser moving periosteally, it is still more spongiosa than living hard shelled sea turtles, the more basal *Desmotochelys*, and *Toxochelys* (see below). Like other *Protostega* elements sampled in this study, vascularization is organized circumferentially. Viewing the bone tissue under polarized light shows a mixture of parallel-fibered and woven collagen fiber orientation with paralleled-fibered bone concentrated around vascular canals.

Six CGMs can be identified in the humeral cross-section. However, retrocalculation using FHSM VP-17979 identify at least two CGMs lost due to crushing and/or remodeling. This means the individual was at least in its night year of life at the time of death, similar to CM-1421. As in other specimens, CMG are not evenly spaced. Also similar to CM 1421, KUVP 1208 is lacking an EFS and has vascular canals that open to the periosteal surface indicating KUVP 1208 was still growing at the time of death.

FHSM VP-17470, *Desmatochelys lowii* humerus (Fig. 3A; Fig. S3A-C)

Desmatochelys FHSM VP-17470 has a spongiosa medullary region that gradually transitions into denser cortical bone periosteally. The cortical region is well vascularized with longitudinal primary osteons surrounded by parallel-fibered tissue and organized in concentric rows. Vascular canals are stretched circumferentially in some regions. The cortex of *Desmatochelys* does not have the spongiosa texture of *Protostega*, but has more compact cortical bone with smaller and fewer vascular canals. Fifteen CGMs are observed and are significantly more closely spaced than CGMs observed in *Protostega* specimens. There is extensive secondary remodeling within the inner cortex and where the cortex transitions to the medullary region, but it is not clear if any or how many GCMs were removed by remodeling. Consequently, FHSM VP-17970 was at least in its 16th year at the time of death. Vascularized bone tissue continues to the periosteal margin with no evidence of an EFS, indicating the individual was still growing at the time of death.

FHSM VP-700, *Toxochelys latiremis* humerus (Fig. 3B; Fig. S3D-F)

Bone microstructure patterns in *Toxochelys* FHSM VP-700 are more similar to *Desmatochelys* than *Protostega*. Like *Desmatochelys*, a spongiosa medullary area transitions into denser cortical bone towards the periosteal surface. Longitudinal and circumferential vascular canals are small but abundant and become more circumferentially oriented towards the periosteal surface. Parallel-fibered and woven bone are observed through the section. Large erosion rooms are present in the inner part of the bone and are often surrounded by lamellar bone. Although the number of CGMs preserved varies throughout the bone depending on the distribution of spongiosa bone and secondary remodeling, at least nine CGMs are observed. However, it is unknown how many CGMs may be lacking due to endosteal resorption and secondary remodeling. An avascular layer with smaller osteocyte lacuna and parallel to lamellar bone does surround the periosteal surface of the humerus. This is possibly an EFS, indicating the FHSM VP-700 individual had reached somatic growth at the time of death.

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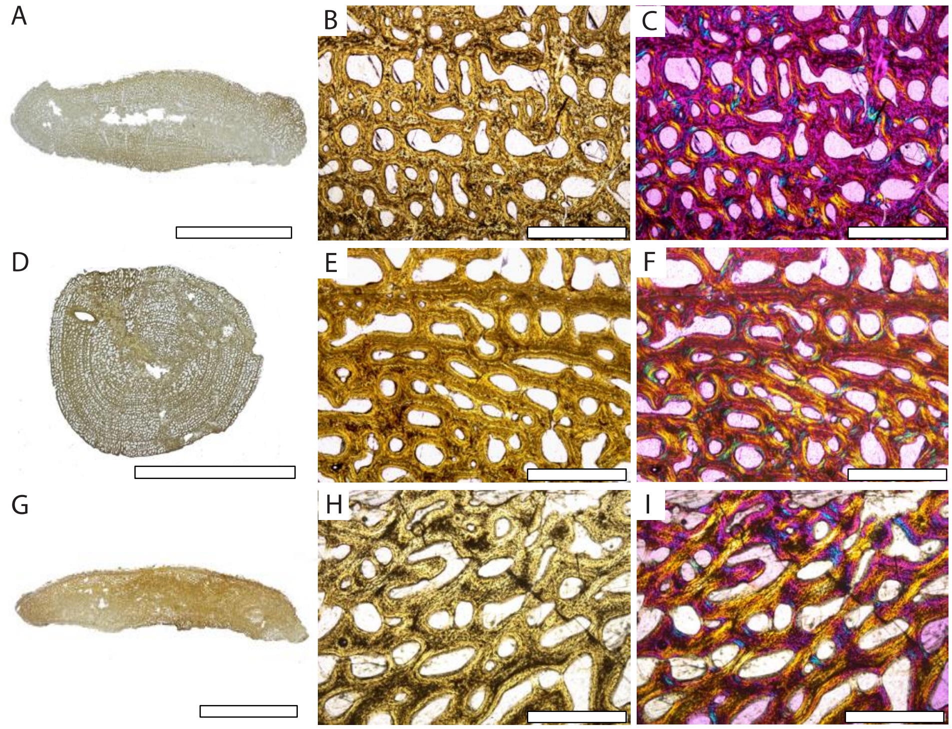

Fig. S1. Long bone microstructure of small *Protostega* specimens. *P. gigas* FHSM VP-17979 humerus full section in plane light (**A**), and close-up of bone microstructure in plane (**B**) and polarized light with a lambda filter (**C**). *P. gigas* FHSM VP-17979 humerus full section in plane light (**D**), and close-up of bone microstructure in plane (**E**) and polarized light with a lambda filter (**F**). *P. gigas* CM 1393 humerus full section in plane light (**G**), and close-up of bone microstructure in plane (**H**) and polarized light with a lambda filter (**I**). Scale bar on A, D, and G is 2cm; scale bar on B, C, E, F, H and I is 1mm.

Commented [TS1]: Unfortunately the resolution of the overview images in the supplement do not allow seeing any details – maybe this is because the file is for review purposes only?

If not, the resolution of especially the overview images should be increased and it would be great if these overview images would be provided also as page-width full scale images to allow counting of growth marks by the reader

Commented [TS2]: should be femur given the cross-section

just by looking at this image I think I can count 7 to 8 CGMs in this femur cross-section, which would be twice the number reported in Table 1 in the main manuscript

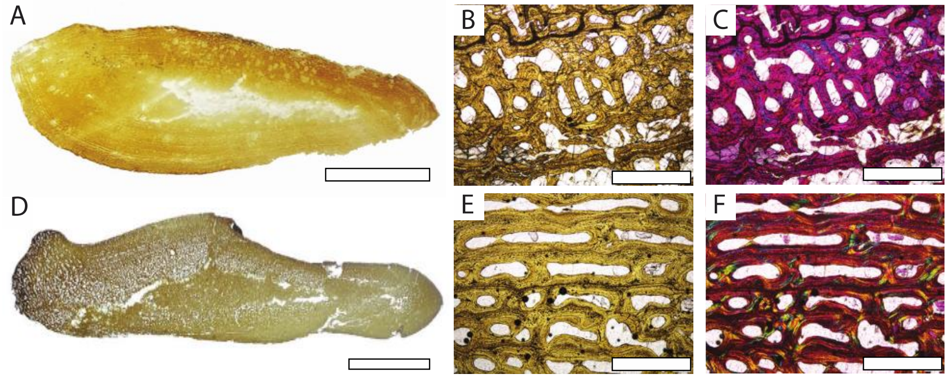

Fig. S2. Humerus microstructure of large *Protostega* specimens. *P. gigas* CM 1421 full section in plane light (**A**), and close-up of bone microstructure in plane (**B**) and polarized light with a lambda filter (**C**). *P. gigas* KUVP 1208 full section in plane light (**D**), and close-up of bone microstructure in plane (**E**) and polarized light with a lambda filter (**F**)**.** Scale bar on A and D is 2cm; scale bar on B, C, E, and F is 1mm.

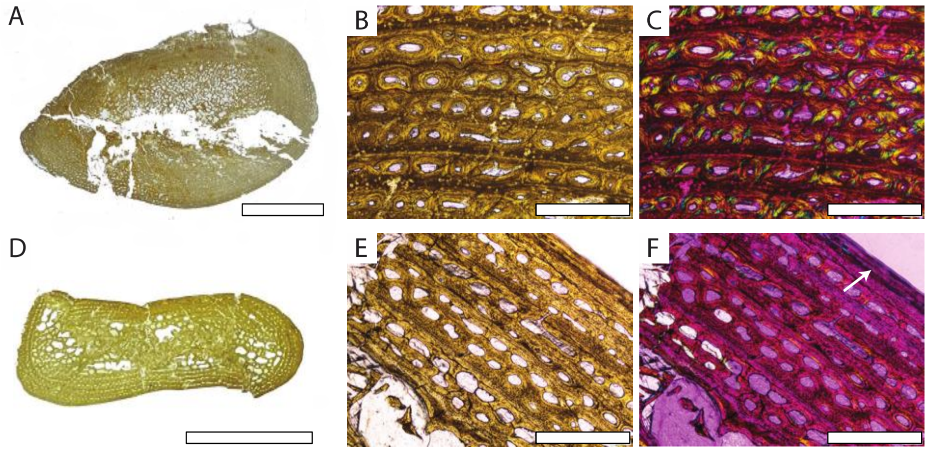

Fig. S3. Humerus microstructure of non-Protostega sea turtles for comparison. Basal protostegid *Desmatochelys lowii* FHSM VP-17470 full section in plane light (**A**), and close-up of bone microstructure in plane (**B**) and polarized light with a lambda filter (**C**). Non-protostegid basal sea turtle *Toxochelys latiremis* FHSM VP-700 full section in plane light (**D**), and close-up of bone microstructure in plane (**E**) and polarized light with a lambda filter (**F**)**.** White arrow marks a possible EFS along the periosteal surface of FHSM VP-700. Scale bar on A and D is 1cm; scale bar on B, C, E, and F is 1mm.

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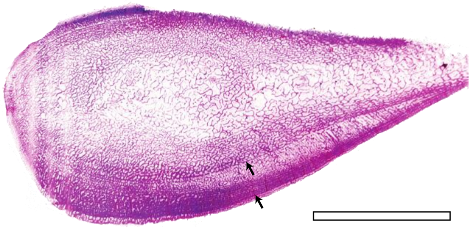

Fig. S4. Full section of *Dermochelys coriacea*, the modern leatherback sea turtle. Specimen CRF 4911 was a female with a curved carapace length of 135 cm at the time of death. Growth marks are indicated by black arrows. Scale bar is 2cm.