



**FLORIDA
MUSEUM**
OF NATURAL HISTORY™

BULLETIN

**SKELETOCHRONOLOGY: A METHOD FOR
DETERMINING THE INDIVIDUAL AGE AND GROWTH OF
MODERN AND FOSSIL TORTOISES (REPTILIA: TESTUDINES)**

Dana J. Ehret

Vol. 47, No. 2, pp. 49-72

2007

The **FLORIDA MUSEUM OF NATURAL HISTORY** is Florida's state museum of natural history, dedicated to understanding, preserving, and interpreting biological diversity and cultural heritage.

The **BULLETIN OF THE FLORIDA MUSEUM OF NATURAL HISTORY** is a peer-reviewed publication that publishes the results of original research in zoology, botany, paleontology, archaeology, and museum science. Address all inquiries to the Managing Editor of the Bulletin. Numbers of the Bulletin are published at irregular intervals. Specific volumes are not necessarily completed in any one year. The end of a volume will be noted at the foot of the first page of the last issue in that volume.

Richard Franz, *Managing Editor*

Cathleen L. Bester, *Production*

Bulletin Committee

Richard Franz, *Chairperson*

Ann Cordell

Sarah Fazenbaker

Richard Hulbert

William Marquardt

Susan Milbrath

Irvy R. Quitmyer

David Steadman, *Ex officio Member*

ISSN: 0071-6154

Publication Date: October 15, 2007

**Send communications concerning purchase or exchange
of the publication and manuscript queries to:**

Managing Editor of the BULLETIN
Florida Museum of Natural History
University of Florida
PO Box 117800
Gainesville, FL 32611-7800 U.S.A.
Phone: 352-392-1721
Fax: 352-846-0287
e-mail: dfranz@flmnh.ufl.edu

SKELETOCHRONOLOGY: A METHOD FOR DETERMINING THE INDIVIDUAL AGE AND GROWTH OF MODERN AND FOSSIL TORTOISES (REPTILIA: TESTUDINES)

Dana J. Ehret¹

ABSTRACT

Skeletochronology is a method used to estimate the individual ages of animals by counting lines of arrested growth (LAGs) within skeletal tissues. This method was applied to evaluate the ages of modern gopher tortoises, *Gopherus polyphemus*, from north central Florida and fossil tortoise species, *Gopherus laticuneus* and *Styemys nebrascensis*, from the White River Group in northwestern Nebraska. Different skeletal elements were tested for growth lines and the humerus was determined to be the most useful bone for analysis. LAGS in *Gopherus polyphemus* were correlated with alternative age estimations based on scute annuli counts, carapace lengths, and plastron lengths. While age estimates were similar in younger individuals, the alternative aging techniques did not accurately reflect the ages of older individuals. Scute wear, sexual dimorphism, and decreased growth with age are factors contributing to this discrepancy. Similarly, LAGS were found in the humeri of *Gopherus laticuneus* and *Styemys nebrascensis*. Fossil specimens range in age from 0 years (hatchling) to over 40 years old. Fossil samples provide insight into changing ecological conditions during the Eocene-Oligocene Transition. This critical period appears to show a shift from a *Styemys nebrascensis* to *Gopherus laticuneus* dominant ecosystem.

Key Words: *Gopherus polyphemus*, skeletochronology, *Styemys nebrascensis*, *Gopherus laticuneus*, incremental growth, Eocene, Oligocene.

TABLE OF CONTENTS

Introduction	50
Geological Context of the Nebraska Badlands.....	50
White River Group Fossil Tortoises.....	50
Paleoenvironment and Paleoclimate.....	52
Modern <i>Gopherus polyphemus</i>	53
Materials.....	53
<i>Gopherus polyphemus</i> Samples.....	53
Fossil Tortoise Samples.....	53
Determining Individual Ages of Tortoises.....	54
Modern <i>Gopherus polyphemus</i> For Baseline Data.....	54
LAGS in Fossil Tortoises.....	57
LAGS in <i>Gopherus polyphemus</i>	57
Resorption Data.....	58
Growth Analysis.....	61
<i>Gopherus polyphemus</i>	61
Fossil Tortoises.....	65
Discussion and Summary.....	65
Acknowledgements.....	69
Literature Cited.....	70

¹Florida Museum of Natural History, Dickinson Hall, PO Box 117800, University of Florida, Gainesville, Florida 32611-7800 <dehret@flmnh.ufl.edu>
Ehret, D.J. 2007. Skeletochronology: A Method For Determining The Individual Age and Growth of Modern and Fossil Tortoises (Reptilia: Testudines). Bull. Florida Museum Nat. Hist. 47(2):49-72.

INTRODUCTION

The task of aging long-lived chelonian species has been a problem for scientists as long as turtles have been studied. Many techniques are used to accurately estimate the probable age of wild and captive raised individuals. More popular methods include mark-release-recapture, scute annuli counts, carapace and/or plastron measurements, scute wear assessments, and changes in skeletal morphology (Zug 1991). While some methods are more successful than others, none consistently predict accurate age estimates for individuals. However, the precision of all methods of age estimation is dependent on having access to known-aged individuals for reference.

A promising method to estimate age for some reptilian and amphibian species is skeletochronology. This method counts lines of arrested growth (LAGs) in a cross-section of a long bone. While it has proven to be a reliable age indicator in some species, the method has never been used in *Gopherus polyphemus* Daudin 1802, or any fossil chelonian species.

In this study, I use skeletochronology to estimate the ages of *Gopherus polyphemus* and the fossil tortoise species *Gopherus laticuneus* (Cope) 1873 and *Styemys nebrascensis* Leidy 1851. Skeletochronological data collected in this study are also cross-referenced with three other aging techniques in the extant species to evaluate similarities and differences in the methods. While this method has been used in some amphibians, reptiles, dinosaurs, mammals, and even birds, skeletochronology has been largely overlooked for aging tortoises. Published studies using skeletochronology in various reptiles have shown that one LAG is equal to one year (Grubb 1971; Castanet & Cheylan 1979; Germano 1988; Castanet 1994). An accurate method for aging tortoises is extremely important in demographic and population studies. Information on age at sexual maturity, maximum age in the wild, and growth differences between sexes can all be inferred using skeletochronology.

The extinct tortoises *Gopherus laticuneus* and *Styemys nebrascensis* are common in rocks of the White River Group exposed across the North American Great Plains. Both species have a geologic range spanning the late Eocene and early Oligocene (Chadronian and Orellan North American land mammal ages, or NALMAS) (Hutchinson 1992, 1996; Prothero & Swisher 1992).

Gopherus polyphemus is found exclusively in the southeastern United States, ranging from southern South Carolina south to Dade County, Florida, and west to the eastern portion of Louisiana (Auffenberg & Franz 1982;

Franz & Quitmyer 2005). This species is intended to serve as a modern analog for the fossil species because of its phylogenetic relationship to the fossil *Gopherus* species, the relative similarity between the modern tortoise's environment and the inferred paleoenvironment of the fossil species, and the relative abundance and accessibility of materials.

GEOLOGICAL CONTEXT OF THE NEBRASKA BADLANDS

The White River Group consists of volcanoclastic fluvial, eolian, and lacustrine sediments that accumulated across the mid-continent of North America from the middle Eocene to the middle Oligocene, 38 to 29 million years ago (mya). These lithostratigraphic units are most commonly exposed in Nebraska, South Dakota, Colorado, Montana, and Wyoming (Larson and Evanoff 1998). Fossil tortoises occur throughout the White River Group which is divided into the Chamberlain Pass, Chadron, and Brule formations (LaGarry 1998, Terry 1998, Terry and LaGarry 1998). The type sections for the Chadron and Brule formations are located at Toadstool Park in Sioux county, Nebraska (Figure 1).

The Chadron Formation of northwestern Nebraska is divided into two distinct members. The lower unit is the Peanut Peak Member and the upper unit is the Big Cottonwood Creek Member (Terry 1998, Terry and LaGarry 1998). The Big Cottonwood Creek Member also coincides with the Eocene-Oligocene boundary (Swisher & Prothero 1990). It is in the Chadron Formation that *Styemys nebrascensis* first appears (Figure 2).

The lower unit of the overlying Brule Formation in northwestern Nebraska is the Orella member. Two lithotopes can be distinguished across the exposed outcrop (LaGarry 1998). The dominant lithotope consists of volcanoclastic clayey siltstones and silty claystones, sheet sandstones, and volcanic ashes. The secondary lithotope consists of single and multistoried channel sandstones. Historically, the Big Cottonwood Creek Member of the Chadron Formation, along with the Orella Member of the Brule Formation, was correlated to the Turtle-Oreodont zone of South Dakota based on the abundance of fossils of these taxa (Schultz & Stout 1955).

WHITE RIVER GROUP FOSSIL TORTOISES

Tortoises have a relatively extensive fossil record when compared to other chelonian groups; however, they are poorly studied (Auffenberg 1974; de Broin 1977; Hutchinson 1980; Joyce et. al 2004). Basal tortoises are known from the Eocene of North America and Europe. Specimens referred to the genus *Hadrianus* and

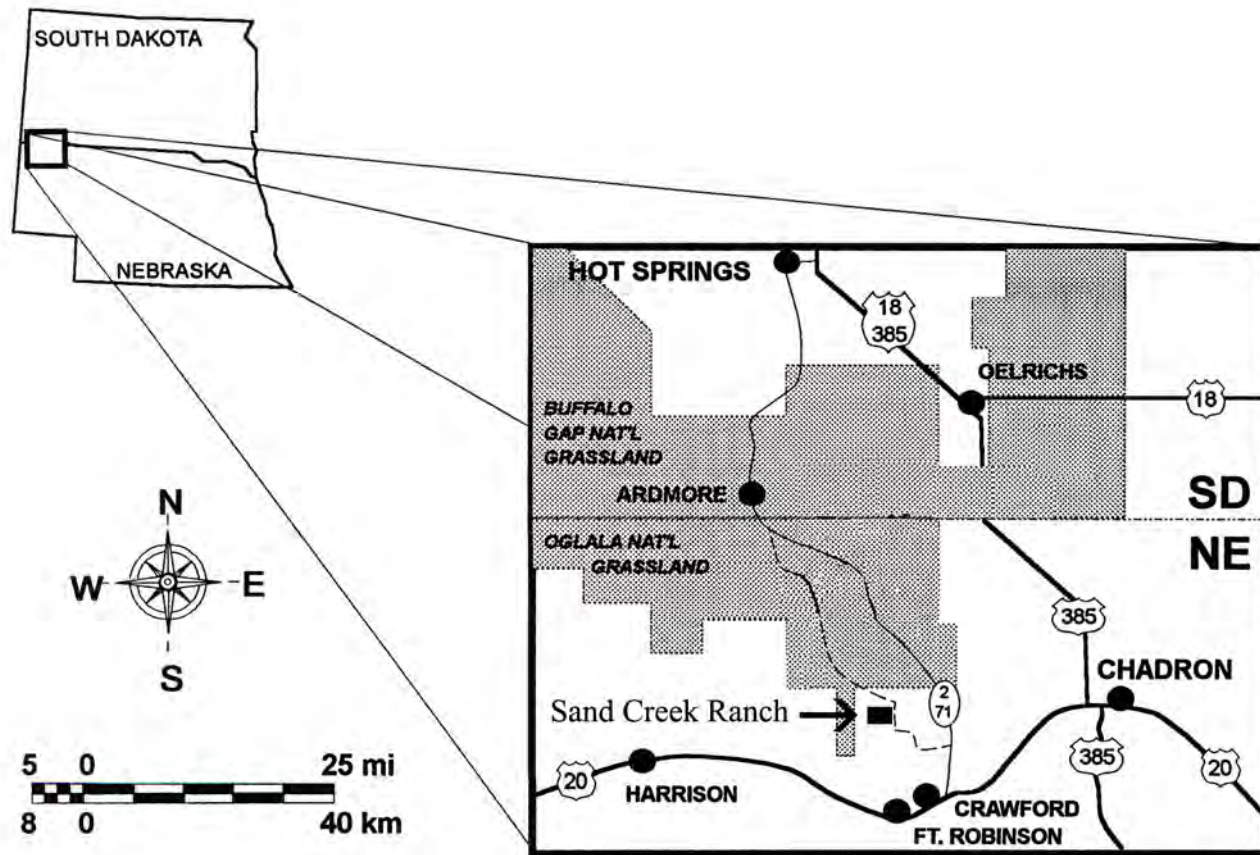


Figure 1. Map of northwestern Nebraska field area with permission from Terry and LaGarry (1998 p. 124). Reprinted with permission from Geological Society of America.

an undescribed species from the Paleocene of Asia are currently classified as the oldest members of the group (Joyce et. al 2004). While turtles in general are relatively abundant in the fossil record, complete specimens (including skulls, shells, and post-cranial elements) that allow precise identification are extremely rare. Therefore, the precise taxonomic placement for *Hadrianus* is unknown. By the late Eocene, *Stylemys* and *Gopherus* are recognizable as distinct genera (McCord 2002).

The genus *Stylemys* encompasses a number of species that span from the late Eocene through the Miocene (40 to 10 mya; McCord 2002). *Stylemys nebrascensis* is one of the most common fossil turtles in North America. It was the first fossil chelonian described from North America (Leidy 1851). Specimens have been found in North Dakota, South Dakota, Wyoming, Colorado, and Nebraska for over 150 years (Prothero and Whittlesey 1998). The geologic range of this species extends from the Chadron Formation through the Orella Member of the Brule Formation, a period of 4 to 5 million years (Hutchinson 1996).

Stylemys is recognized by a number of diagnostic

characteristics. One of the features used for identification is the normal neural formulae (4-6-6-6-6-6-6 or 4-8-4-6-6-6-6) for the species. In the neural formula, each number indicates the number of sides per neural bone starting with the first neural. In all specimens the posterior epiplastral excavation is shallow or absent (Auffenberg 1964). The cervical scale is longer than it is wide. The anterior lobe of the plastron is wider than it is long. The shape of the humeral head in *Stylemys* is also compressed dorso-ventrally in adults (Auffenberg 1964). A proportionately thicker and more rounded shell is diagnostic of the species (Hay 1908; Hutchinson 1996). The carapace of these individuals may reach, or exceed, lengths of 530 mm. Finally, the square, boxed-off shape of the gular projection differs from that of *Gopherus*.

The first reported appearance of the genus *Gopherus* in the fossil record is from the late Eocene (~ 34 Ma) with *Gopherus laticuneus*. Although it is unknown if the genus is descended from *Stylemys* or from a common ancestor, the genus shares a number of morphological characteristics with *Stylemys*

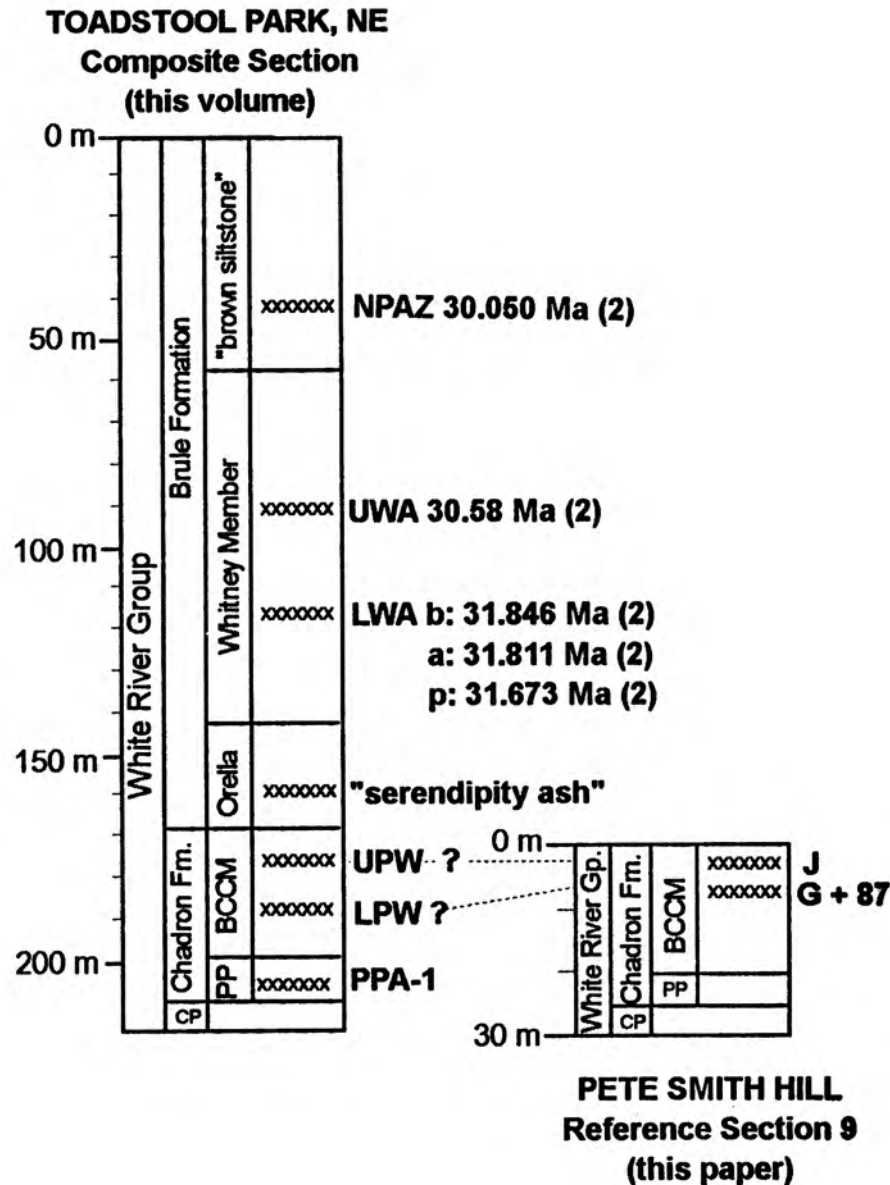


Figure 2. Geologic Section of the White River Group at Toadstool Park, NE, from Terry and LaGarry (1998 p. 133). Reprinted with permission from Geological Society of America.

nebrascensis (Hay 1908; Auffenberg 1964). Previously, these species were closely linked because they share the synapomorphy of a premaxillary ridge in their upper jaw (Crumly 1994; McCord 2002). *Gopherus laticuneus* is the most primitive described species of the genus, which includes four extant species (*G. polyphemus*, *G. flavomarginatus*, *G. berlandieri*, and *G. agassizii*). Given the basal phylogenetic position of this taxon within *Gopherus*, some have placed it in a separate taxon, *Oligopherus* (Hutchinson 1996, McCord 2002).

The characteristics that diagnose *Gopherus*

laticuneus includes the normal neural formulae 6-6-4-6-6-6-6-6 or 4-8-4-6-6-6-6-6, posterior epiplastron excavation relatively shallow, nuchal scale rather short and wide, the shell generally thinner, overly pronounced and toothed epiplastral extensions, and an extended and toothed xiphiplastra (Hutchinson 1996).

PALEOENVIRONMENT AND PALEOCLIMATE

The use of skeletochronology relies on seasonal cycles to preserve lines of arrested growth (LAGs) and annuli. Therefore, information on the paleoclimate during the Eocene-Oligocene transition is important to the

context of this study. Seasonal climatic variation is very important for accentuation of LAGs (Castanet & Smirina 1990; Chinsamy-Turan 2005). The Eocene-Oligocene (34.36 +/- 0.11 Ma) transition shows a major shift in climatic conditions (Terry and LaGarry 1998). Paleosols from the Big Cottonwood Creek member (late Chadronian, North American Land Mammal Age) shows a gradual change from humid, forested conditions to more seasonal, semi-arid conditions occurring through the Late Eocene (Terry 2001). This change was originally interpreted as the “Terminal Eocene Event” when it was first detected. After redefinition of the Eocene-Oligocene boundary, the event has now been termed the “Early Oligocene Event” or the Eocene-Oligocene Transition (EOT) (Miller 1992, Prothero & Heaton 1996; Terry 2001, Kohn et al. 2004).

It was suggested that the temperature during the White River Group deposition in the early Oligocene was ~ 16 °C, which is a decrease from an Eocene greenhouse of ~ 25 °C (Wolfe 1992, Berggren & Prothero 1992). As mentioned previously, *Styemys nebrascensis* appears during the middle to late Eocene, but does not become common until the Eocene-Oligocene boundary. *Gopherus*, on the other hand, did not appear until the latest Eocene and can be found in the upper Chadron (latest Eocene) and lower Brule (early Oligocene) Formations. Bramble (1971) and McCord (2002) suggested that *Styemys* is a more mesic-adapted species, while *Gopherus* is a more xeric-adapted species. This would account for a shift in species abundance as the climate became cooler and drier (Terry 2001).

Based on paleosols, the White River Group represents a transition from fluvial to eolian sediments that occur near the top of the Orella Member at Toadstool Park, Nebraska (LaGarry pers. comm., Terry 2001). Paleosols show a transition from forested/slightly forested conditions in the late Eocene to more open prairie-like conditions in the Oligocene (Terry 2001.) River systems also shifted during this period from meandering to braided systems with periods of seasonal deposition.

MODERN *GOPHERUS POLYPHEMUS*

Gopher tortoises (*Gopherus polyphemus*) are found most often in sandy upland areas of pine (*Pinus* spp.) and oak (*Quercus* spp.) with an understory of wiregrass (*Aristida* spp.), beach scrub, oak hammocks, or pine flatwoods (Auffenberg & Franz 1982; Ernst et al. 1994). Annual precipitation levels over the range of *G. polyphemus* is between 1162-1593 mm (Germano 1994). They are avid burrowers and may keep several burrows active at any given time. Active and aban-

doned burrows are used by the tortoise and other vertebrate and invertebrate species. In Florida, tortoises are active most of the year, retreating to their burrows at night and only coming out for portions of the day. When they are above ground, *G. polyphemus* spends most of its time basking and searching for food or feeding (Smith 1992; Ernst et al. 1994). Although there is little information on the longevity of *G. polyphemus*, captive raised *Gopherus berlandieri* Agassiz 1857 (the Texas tortoise) have been documented to exceed 52 years of age (Judd & McQueen 1982).

MATERIALS

GOPHERUS POLYPHEMUS SAMPLES

The shells and skeletons of *Gopherus polyphemus* were obtained by Richard Franz and the author throughout north central Florida under the FLMNH Florida Fish and Wildlife Conservation Commission collection permit #WS01058 to salvage mortally wounded specimens on roads, killed by predators, or burned in wildfires. Gopher tortoises were sampled from several localities in north central Florida. Samples were collected from High Springs, Rattlesnake Island in Fort Matanzas National Monument near St. Augustine, and Melbourne.

FOSSIL TORTOISE SAMPLES

Fossil tortoises (*Gopherus laticuneus* and *Styemys nebrascensis*) were collected in the late Eocene-Oligocene beds of northwestern Nebraska in the summer of 2001 with the aide of Bruce MacFadden and volunteers that were assisting the annual Pony Express trip. Specimens were collected from the Sand Creek Ranch and on Forest Service land near Toadstool Park north of Crawford, Nebraska (Figure 1). Specimens designated with FLMNH (UF) numbers are housed in the Nebraska collection at the museum (Table 1). The main sites of collection for the summer 2001 collections include: Horse Hill Low (NE 008), Turkey Foot East High (NE 001) Figures 3 and 4, Sagebrush Flats (NE 016), Bald Knob High (NE 004) Figure 5, and the Pettipiece ranch.

As mentioned previously, all fossil materials are from the Chadronian and Brule formations of the White River Group (Figure 6). Most samples come from the “turtle-oreodont” zone in the boundary area between the Chadron Formation and the Orellan member of the Brule Formation. All fossils were collected well below the Whitneyan-Orellan boundary, based on local lithostratigraphy. Given the geochronology of this interval (see above), these fossils occur within an interval between 34 and 32 mya.

Table 1. Fossil tortoise specimens, identifications, and localities.

Specimen	Species	Locality	Locality Number
UF 226256	<i>G. laticuneus</i>	Horse Hill Low	NE 008
UF 226257	Unknown	Horse Hill Low	NE 008
UF 226258	<i>G. laticuneus</i>	Horse Hill Low	NE 008
UF 226259	<i>S. nebrascensis</i>	Horse Hill Low	NE 008
UF 226260	<i>S. nebrascensis</i>	Horse Hill Low	NE 008
UF 226261	Unknown	Horse Hill Low	NE 008
UF 226262	<i>S. nebrascensis</i>	Bald Knob High	NE 004
UF 226263	<i>G. laticuneus</i>	Bald Knob High	NE 004
UF 226264	Unknown	Sagebrush Flats	NE 016
UF 226265	<i>G. laticuneus</i> c.f.	Sagebrush Flats	NE 016
UF 226266	<i>G. laticuneus</i>	Sagebrush Flats	NE 016
UF 226267	<i>S. nebrascensis</i>	Sagebrush Flats	NE 016
UF 226268	<i>S. nebrascensis</i>	Sagebrush Flats	NE 016
UF 226269	<i>S. nebrascensis</i>	Turkeyfoot East High	NE 001
UF 226270	<i>S. nebrascensis</i>	Pasture 33B Low	NE 035
UF 226271	<i>S. nebrascensis</i>	Pasture 33B Low	NE 035
UF 226272	<i>G. laticuneus</i> c.f.	Pasture 33B Low	NE 035
UF 191470	<i>S. nebrascensis</i>	Turkeyfoot East High	NE 001
UF 201906	<i>G. laticuneus</i>	Turkeyfoot East High	NE 001
UF 209750	<i>G. laticuneus</i>	Unknown	N/A
UF 226273	<i>S. nebrascensis</i>	Bald Knob East Butte	NE 004
UF 226274	<i>S. nebrascensis</i>	Turkeyfoot	NE 003
UF 226275	Unknown	Turkeyfoot above PWL	NE 003
UF 226276	<i>G. laticuneus</i>	Turkeyfoot	NE 003
UF 226277	<i>S. nebrascensis</i>	Turkeyfoot East	NE 001
UF 226278	<i>G. laticuneus</i>	Pettipiece West Basin	N/A

DETERMINING INDIVIDUAL AGES OF TORTOISES

MODERN *GOPHERUS POLYPHEMUS* FOR BASELINE DATA

Skeletochronology data were collected from *Gopherus polyphemus* individuals to determine if the technique would be a viable method before destructive analysis of rare fossil specimens. *Gopherus polyphemus* was chosen for the following reasons: 1) it is closely related to the fossil species, *Gopherus laticuneus*, used in this project (Bramble 1971), 2) in a previous study a closely related species (*Gopherus agassizii*) provided positive results for age determination (Germano 1988), and 3) skeletons of *Gopherus polyphemus* are relatively abundant in museum collections. The specimens used in this study are from the Florida Museum of Natural History (UF) and The Chelonian Research Institute (PPC).

Different studies have recommended the use of

long bones (e.g. humerus and/or femur) for skeletochronology, while others use vertebrae or sclerotic rings (Zug 1991). Therefore, I examined different skeletal elements to determine which would be most advantageous for these tortoises. Bones were taken from both the left and right sides of these individuals. Bones from the same side of the body should be used; however, most specimens in my sample were incomplete and alternative bones had to be substituted. Data suggest that this did not bias the analysis, because similar bones grow at similar rate, regardless of which side of the body it comes from (G. Erickson pers. comm). This assertion was tested on one specimen; UF 143426, in which the left and right humeri were sectioned and both consistently showed the same number of LAGs.

Sets of bone elements from individual *G. polyphemus* were sectioned. Humeri, femora, scapulae, ilia, and vertebrae were selected for histological preparation and examined for LAGs. I initially predicted



Figure 3. Erosional surfaces of Chadron and Brule formations at Turkey Foot East Badlands, looking westward toward the pine ridge, Toadstool Park and Roundtop, at Sand Creek Ranch, Sioux County, Nebraska. Most of the rough-textured sediments on the lower slopes represent the turtle-oreodont zone of the older literature (photography by Shelley E. Franz).

that bones from the pelvic or shoulder girdle might prove better for this research because those elements are preserved more regularly in fossil specimens. Articulated girdles tend to remain within the shell of dead specimens, thus providing for a better chance for preservation. Upon examination of the scapula and ilium, I found that LAGs could be observed, but bone samples had undergone more reconstruction than other bones. Vertebrae also show a high amount of reconstruction and a majority of the LAGs are not visible. The humerus and femur proved the most reliable in maintaining LAGs with the least amount of reconstruction in *G. polyphemus*.

Based on this preliminary assessment, I selected the humerus as the optimal skeletal element to be exam-

ined. The decision between using the humerus or the femur is based on the availability of material. In both modern and fossil samples, there are more humeri than femora available in the sample. Counts of LAGs in all bone samples are included in Table 2.

An advantage of using the modern gopher tortoise, *G. polyphemus*, is the possibility for parallel age estimates obtained from other methods (Halliday & Verrell 1988). The most beneficial specimens would have been individuals of known-age (Castanet & Smirina 1990). Unfortunately no such samples of *G. polyphemus* were located and other aging techniques had to be employed.

Scute annuli counts have been used (quite) extensively in research involving chelonians. They are thought to be annual in most species and can be viewed and counted with relative ease (Legler 1960). The drawbacks, however, include: loss of annuli due to excessive wear (Figure 7), false annuli being counted as true annual increments, and the difficulty of aging older individuals because of the closer spacing of the annuli on the scute (Germano 1988). However, annuli can provide a method to evaluate and compare the age estimations as determined by LAGs.

Scute annuli were counted for all but one of the specimens examined. One large female proved to be too old and its scute annuli were too worn to count. Due to this problem, evaluated age estimates were compared with other methods that were used regularly in study of chelonian species, i.e. measuring straight-line carapace and/or plastron length to correlate size and age (Landers et al. 1982; Mushinsky et al. 1994). There are a number of drawbacks and restrictions however, that should be addressed.

Given that chelonians are ectotherms, size can vary based on the environment in which different populations live. Factors including average temperature, rainfall, vegetation levels, and nutrition can all influence the growth rates of individuals (Gibbons 1976). Therefore, carapace and plastron lengths will vary from population to population throughout a given range. Plastron lengths in gopher tortoises are very limiting for another reason: the epiplastral extension (gular region) of the gopher tortoise is a highly variable feature (Figure 8). Males tend to have a longer gular projection than females, although this is not a rule. Plastron length can be measured, but its use in estimating age is likewise equivocal (Mushinsky et al. 1994).

Carapace lengths tend to be more precise than plastron length for age reconstruction however; estimates are still relative and not absolute. Straight-line carapace length is a popular method among researchers. The



Figure 4. Eroded cliffs of Chadron and Brule formations at Turkey Foot East Badlands, looking northwest toward Sand Creek Road, at Sand Creek Ranch, Sioux County, Nebraska. Upper P.W.L. ash forms a prominent white layer, adjacent to the grass, in the bottom of the valley (photography by Shelley E. Franz).



Figure 5. Eroded surfaces of the Chadron and Brule formations at Bald Knob Badlands, looking southwest toward the pine ridge, at Sand Creek Ranch, Sioux County, Nebraska. The upper P.W.L. ash layer forms a prominent white shelf about mid-slope (photography by Shelley E. Franz).



Figure 6. A moderate-sized specimen of gopher tortoise, *Gopherus laticuneus*, excavated from the Chadron Formation, just above the upper PWL ash layer, at Horse Hill Badlands, Sand Creek Ranch, Sioux County, Nebraska (photography by Shelley E. Franz).

same environmental factors mentioned above also apply to carapace length therefore individuals from different populations should not be compared to one another unless they share a common geographic range or environment.

LAGS IN FOSSIL TORTOISES

Based on the results for *G. polyphemus*, I chose the humerus as the appropriate bone element for determining the age of White River fossil tortoises because of the high number of humeri present and the observable LAGs. The fossil specimens were prepared in the Vertebrate Paleontology lab at the Florida Museum of Natural History, Gainesville (FLMNH). Table 1 shows all samples and the localities from which they came. Samples were embedded, cut, and polished following the methods of Chinsamy and Raath (1992) and examined for LAGs. The fossil specimens showed growth marks similar to those documented in *G. polyphemus*. The LAGs analyzed within the long bones of the fossils were correlated to plastron and carapace lengths since keratinous scutes typically do not preserve in the fossil record. Measurements of shell dimensions were compared; however, there are no known shell length-age classes for these species.

In *G. laticuneus* and *S. nebrascensis* specimens, the plastron tends to be better preserved in the fossil record than the carapace. Post-mortem deformation and distortion during fossilization tends to compress and misshape the carapace in fossil tortoises. In many cases, shell length estimates for White River fossils are affected by taphonomic factors. As a result, available shell fragments were compared with specimens that were better preserved. Therefore, a majority of fossil tortoise shell length estimates are not exact, but provide sufficient information to allow interpretation.

LAGS IN *GOPHERUS POLYPHEMUS*

All individuals were cleaned and prepared before bones were measured and sectioned. Most of the carcasses had to be skeletonized. Individuals were then rinsed in a weak solution of industrial strength soap and bleach and then scrubbed with a soft brush to remove any remaining tissue. Bones were dried under a heat lamp. All individual bones were measured for length and width to the nearest millimeter. I also measured the diameter of the shaft of the humerus because of its importance when estimating resorption of growth lines in thin section. However, there is no correlation between individual bone size or length and age (Castanet &

Table 2. *Gopherus polyphemus* identification, sex, and visible LAG counts.

Specimen	Sex*	Humerus	Femur	Scapula	Ilium	Vertebra
UF 143424	F	18	21	17	21	10
UF 143425	M	10	11	7	8	11
UF 143426A	F	9	13	6	6	8
UF 143426B	F	9	13	6	6	8
UF 143427	M	7	6	7	7	6
UF 143428	J	3	0	0	0	0
UF 144653	J	6	7	6	8	N/A
UF 144657	J	6	8	4	7	N/A
UF 144659	F	8	7	5	7	4
UF 144655	J	7	N/A	6	7	9
UF 150177	F	7	6	6	5	N/A
UF 143430	J	10	7	5	4	N/A
PPC 6669	M	12	14	15	14	N/A
PPC 6674	M	17	21	18	21	16
PPC 3510	H	0	N/A	N/A	N/A	N/A

* M = Male, F = Female, J = Juvenile and H = Hatchling

Cheylan 1979, Chinsamy-Turan 2005).

For preparation of the thin sections, all samples were sent to Matson's Laboratory, LLC of Milltown, Montana. Following standard procedures, bones were cut, decalcified, embedded in paraffin, and stained with hematoxylin dye (Castanet & Cheylan 1979; Zug et al. 1986; Chinsamy & Raath 1992, Chinsamy-Turan 2005). The dye is an important aide in making the annual growth marks more distinguishable. The sections were then embedded in plastic and mounted on microscope slides (also see www.matsonslab.com). For each specimen, the actual age was withheld until the author also had a chance to make an independent age estimate. This was done to evaluate the precision of the estimates.

I counted the LAGs using a compound microscope; counts were repeated two times. The average of the two counts provided the age estimates for all individuals. Professional technicians at Matson's Laboratory also provided counts. The two sets of LAG counts varied no more than 1-2 LAGs, suggesting consistency in the interpretation. In previous studies, the occurrence of two LAGs per year has been observed (Castanet & Smirina 1990). These non-periodic lines, which can result from a double annual growth cycle, were not found in specimens analyzed during this study. In addition to counting the number of LAGs, the width of all increments was also measured. These measurements were used to estimate resorption of early LAGs.

RESORPTION ESTIMATION

In order to measure the LAGs and account for resorption, the average layer-thickness calculation was used. All counts and measurements involved the LAGs found on the ventral side of the bone. Due to biomechanical function and resorption, LAGs are not equally spaced around the circumference of the humerus (Parham & Zug 1997; Chinsamy-Turan 2005). When looking at a bone in thin section, the LAGs persist longer on the dorsal and ventral sides of the bone (also known as the short axis). Therefore, the radius of the humerus was recorded as half the diameter of the resorption core plus the sum of the LAGs on the ventral half of the bone.

LAGs and periosteal layers lost into the cancellous core of the bone are a major problem in age estimation (Francillon-Vieillot et al. 1990, Chinsamy-Turan 2005). In bone growth, early periosteal layers tend to be tightly packed followed by a number of layers that are more widely spaced. These widely spaced layers continue until the animal reaches sexual maturity, at which point they tend to become closely spaced again. The variation in growth line width may lead to an exaggeration in the number of total LAGs (both present and re-absorbed) when only the mean width of all LAGs is calculated.

The average layer-thickness back-calculation uses the mean width of the three existing innermost layers,

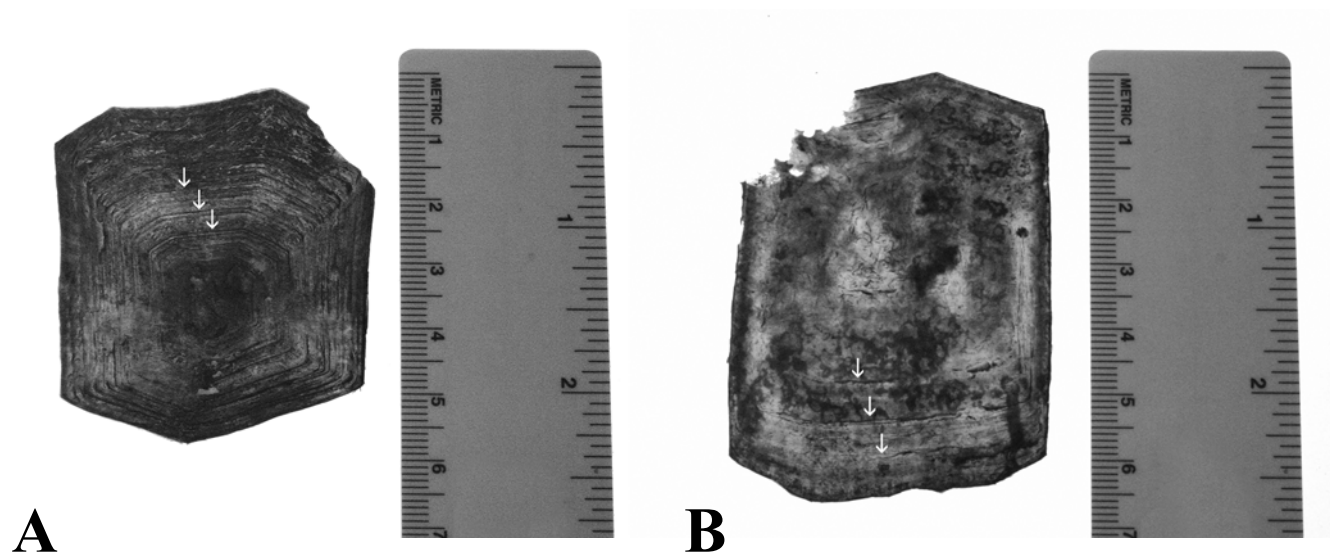


Figure 7. (A) Scute of young individual showing well-defined annuli (B) Scute of an older individual showing wear and loss of annuli.

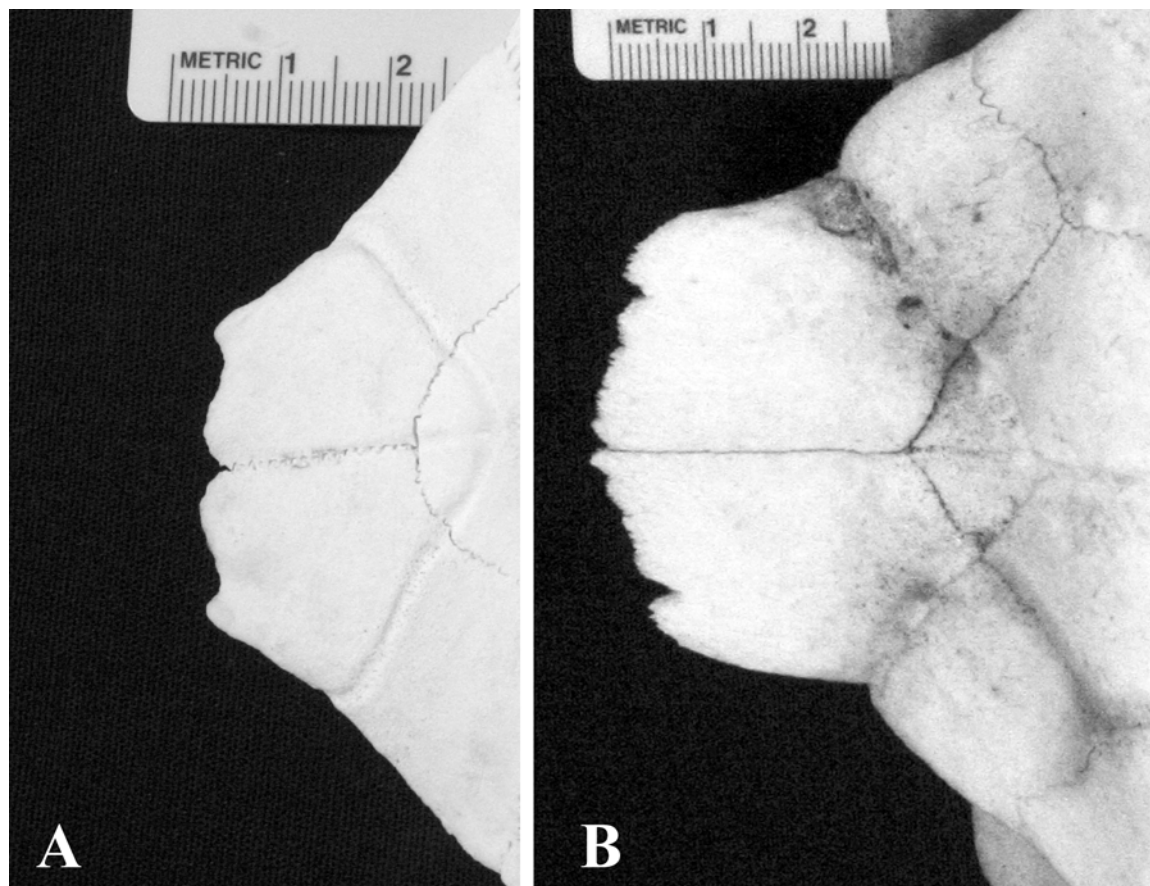


Figure 8. Differences in gular projections of *Gopherus polyphemus* (A) Juvenile (B) Adult male.

which is then divided into the radius of the bone's short axis, to provide an estimate of the number of resorbed marks (Castanet & Cheylan 1979; Zug et al. 1986; Castanet & Smirina 1990; Parham & Zug 1997; Erickson & Tumanova 2000). While most research indicates this to be an appropriate protocol, Parham and Zug (1997) caution that this equation may also yield an overestimate of LAGs. (The latter authors' work on skeletochronology in sea turtles uses an alternate method to recount for resorption however it is beyond the intended scope of this paper to make comparisons in methods.)

As an independent age estimate, scute annuli measurements were also collected and correlated with those derived from skeletochronology. On the shells of many chelonians, concentric growth increments form on each individual scute. It has been found that these rings are annual in some species and can be positively correlated with age up until a given point, usually 20 years (Cagle 1946; Sexton 1959; Castanet & Cheylan 1979; Judd & Rose 1983; Galbraith & Brooks 1987; Germano 1988). The second costal scute of the carapace was chosen to count annuli following Germano (1988). This scute was chosen for two reasons: the carapace receives much less wear than the plastron, allowing for preservation of scute annuli. Also, the second costal is much more even-sided than others, making the annuli easier to distinguish.

True annual rings (annuli) were distinguished from false or double annuli based on descriptions by Legler

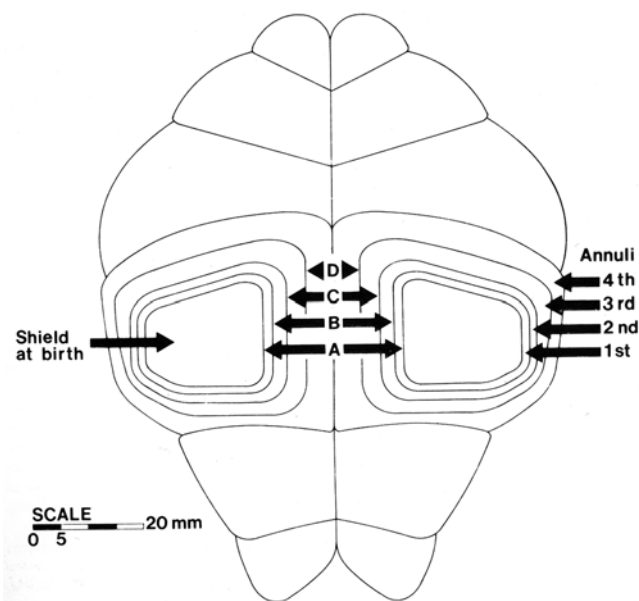


Figure 9. Measurement of scute annuli on the plastron of *Gopherus polyphemus* from Landers et al. (1982 pg. 84). Reprinted with permission from FLMNH.

(1960) and Landers et al. (1982.) These authors defined true annuli as those rings that formed a deep groove around the entire scute (Figure 9). Two counts were made on (two) separate occasions, and the average (of the two counts) was used as the age estimate. These results were then matched with the LAG counts taken from long bones.

The other set of measurements taken from *G. polyphemus* specimens include shell dimensions. Measurements taken included: straight-line carapace length (SCL), straight-line plastron length (PL) along suture, and the length of hyoplastron at the suture. Some studies have shown that carapace and/or plastron length are suitable for age/size relationships (Landers et al. 1982; Mushinsky et al. 1994). The latter measurement (hyoplastron) was taken in order to check the accuracy of calculating carapace length based on plastron elements published in Franz and Quitmyer (2005).

Straight-line carapace and plastron lengths were recorded by placing large calipers at either end of the tortoise shell at the midline. This length was taken instead of an over-the-shell measurement using a measuring tape, because of the increased chance of error in broken or misshapen shells. The length of the hyoplastron was also taken down the midline suture, as per Franz and Quitmyer (2005).

I prepared all specimens to recover humeri, took shell measurements, and identified individuals to species when possible. Preparations were done using a dremel tool, dental picks, an air scribe, and various adhesives in the prep lab at the FLMNH with the help of Russ McCarty.

Before sectioning, fossil bones were measured in the same way as modern samples, specifically, the lengths and widths of bones or the remaining portions of bones. No discrimination was made as to whether the right or left humerus was used due to the extreme rarity of fossil tortoise limb bones. Rough cuts were made on a rock saw prior to embedding to produce a clean surface

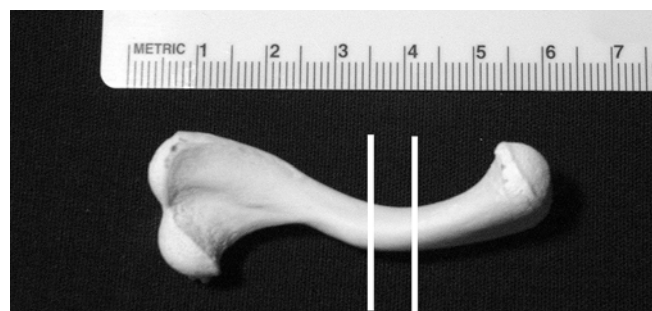


Figure 10. A mid-diaphyseal cut made on the humerus.

in the mid dyaphseal shaft (Figure 10). Sections are cut from the mid-shaft to avoid remodeling that may occur near the proximal or distal end of the long bone (Parham & Zug 1997, Chinsamy-Turan 2005). Humeri were then put into molds and embedded in Por-a-Kast® clear plastic produced by the Synair Corporation. Samples were then allowed to cure for a few days to ensure hardening.

A slow speed Buehler Isomet saw was used to cut 1-3 mm sections from the humeri. The Por-A-Kast® plastic used in the embedding process did not allow for finer cuts, as section warping was visible in thinner samples. These sections were then mounted on petrographic slides using a two-part epoxy manufactured by Logitech. Slides were then allowed to cure for 2-3 days before grinding.

Thin-section and slide grinding was performed at the laboratory of Dr. Gregory Erickson at Florida State University Tallahassee, Fl. Slides were sanded on table-top grinders using different grits in a fining up sequence. A coarser paper (600 grit) was used initially to remove excess material, and finer papers (800-1200 grit) were used in preceding succession to remove any coarse grooves or imperfections left behind. For fossil slides, most were sanded down to a thickness of about 100 micrometers or less. Slides were then viewed under a compound microscope (using polarized light) to count and measure LAGs. Measurements were taken in an identical manner to those described for *G. polyphemus*.

LAGs were more difficult to discern in fossil specimens due to the ineffectiveness of staining in fossils and

also the required increased thickness of the prepared thin-sections. Mineral replacement and diagenesis also destroyed some bone microstructure, which made counts difficult.

Both extant and fossil tortoise LAG counts were correlated and analyzed. Modern *G. polyphemus* LAGs were matched with scute annuli counts in order to test the validity of both methods. Plastron and carapace lengths were also matched with LAG estimates in both fossil and extant species to compare size-age relationships. Published size-age correlations were used to estimate ages of the tortoise specimens (Landers et al. 1982; Mushinsky et al. 1994).

GROWTH ANALYSIS

GOPHERUS POLYPHEMUS

LAGs were visible in some of the skeletal elements analyzed for *Gopherus polyphemus* (n=15). Table 2 shows the sex and the LAG counts for each skeletal element. The number of visible LAGs was not uniform in all elements for each individual. Those elements that did not show LAGs, or showed fewer LAGs, were either too remodeled or the individual was under 1 year of age. While both the femur and humerus retain the most visible LAGs, the humerus was chosen based on availability of specimens.

Resorption and age estimates were calculated using the average layer-thickness back-calculation (Table 3). The average thickness of the innermost three LAGs (in millimeters) was used to calculate those LAGs that

Table 3. *Gopherus polyphemus* age estimates including resorption calculation.

Specimen	Avg. Width of Inner 3 Annuli (mm)	Resorbed Annuli	Age (yrs)
UF 143424	0.10	18	36
UF 143425	0.35	1	11
UF 143426A	0.30	4	13
UF 143426B	0.22	4	13
UF 143427	0.10	1	8
UF 143428	N/R	0	3
UF 144653	N/R	0	6
UF 144657	N/R	0	6
UF 144659	0.34	6	14
UF 144655	N/R	0	7
UF 150177	0.35	4	11
UF 143430	0.23	5	15
PPC 6669	0.26	5	17
PPC 6674	0.14	8	25
PPC 3510	N/R	0	0

were resorbed. Specimens marked N/R showed no resorption of LAGs in my calculations. The numbers of resorbed LAGs, where present, are listed for all specimens. Older individuals have more resorbed LAGs as a result of enlarged medullar cavities. The combination of resorbed and visible LAGs was then added together to determine an age for each tortoise. Ages range from 0 years, for a hatchling, to 36 years, for a large, gravid female. The ages listed are ± 1 year, because the season of death is not known for most individuals.

Scute Annuli. The scute annuli were counted for separate age estimations in all specimens (Table 4). Two specimens did not have annuli, in UF 143424 the scutes were worn smooth, and PPC 3510 only had a natal plate visible. The skeletochronology age estimates were compared to the scute annuli age estimates using the Wilcoxon signed rank test. This is a non-parametric test used to test the median difference in paired data. This was done to determine if there is a significant difference between the two age estimations. A two-sided P-value was generated using age estimations and was calculated at $P < 0.002$. Obtaining a P-value smaller than $P < 0.05$ is considered statistically insignificant therefore the medians of the two age estimates are significantly different. Based on the problematic conditions concerning scute annuli, this test provides evidence that scute annuli counts are not accurate for *Gopherus polyphemus*.

Shell Allometry. Other methods that are used to estimate ages in chelonians include determinations of carapace and plastron lengths. Landers et al. (1982) published a study linking plastron length to age in *G.*

polyphemus (Figure 11). Although their tortoise population was from southern Georgia, the climate is similar enough to north central Florida to allow for comparison with the specimens studied here. For individuals where an accurate plastron measurement is available ($n=13$), Table 5 compares plastron length ages estimates based on the growth curve published in Landers et al. (1982). The actual plastron lengths can be seen in Table 6. Plastron age estimates were then compared with skeletochronology age estimates. A Wilcoxon signed rank test comparing this data yielded a two-sided P-value of $P < 0.002$. As with the scute annuli age estimates, this result shows that the medians of the two different age estimates vary significantly. I believe this low P-value indicates that plastron length is not a good indicator of age in *G. polyphemus*. Sexual dimorphism in the form of epiplastral extensions, tend to exaggerate plastron lengths in many specimens making length vs. age estimations suspect (Mushinsky et al. 1994).

Carapace measurements were also taken from all specimens where it was possible ($n=13$). Unfortunately, many skeletons, when collected, were disarticulated and exact straight-line carapace lengths had to be approximated. Shells were pieced together for measuring and the calculation methods of Franz and Quitmyer (2005) were implemented to validate measurements. Franz and Quitmyer found that the hyoplastron bone length along the midline suture scales allometrically to body size. This allometric relationship can be described using a straight-line regression that they derived: $\text{Log } y = a + b (\text{log } X)$.

Table 4. *Gopherus polyphemus* scute annuli counts and skeletochronology age estimates.

Specimen	Scute Annuli Count	Skeletochronology Age (yrs)
UF 143424	Worn Smooth	36
UF 143425	11	11
UF 143426	12	13
UF 143427	9	8
UF 143428	5	3
UF 144653	7	6
UF 144657	10	6
UF 144659	8	14
UF 144655	10	7
UF 150177	13	11
UF 143430	12	15
PPC 6669	11	17
PPC 6674	16	25
PPC 3510	0	0

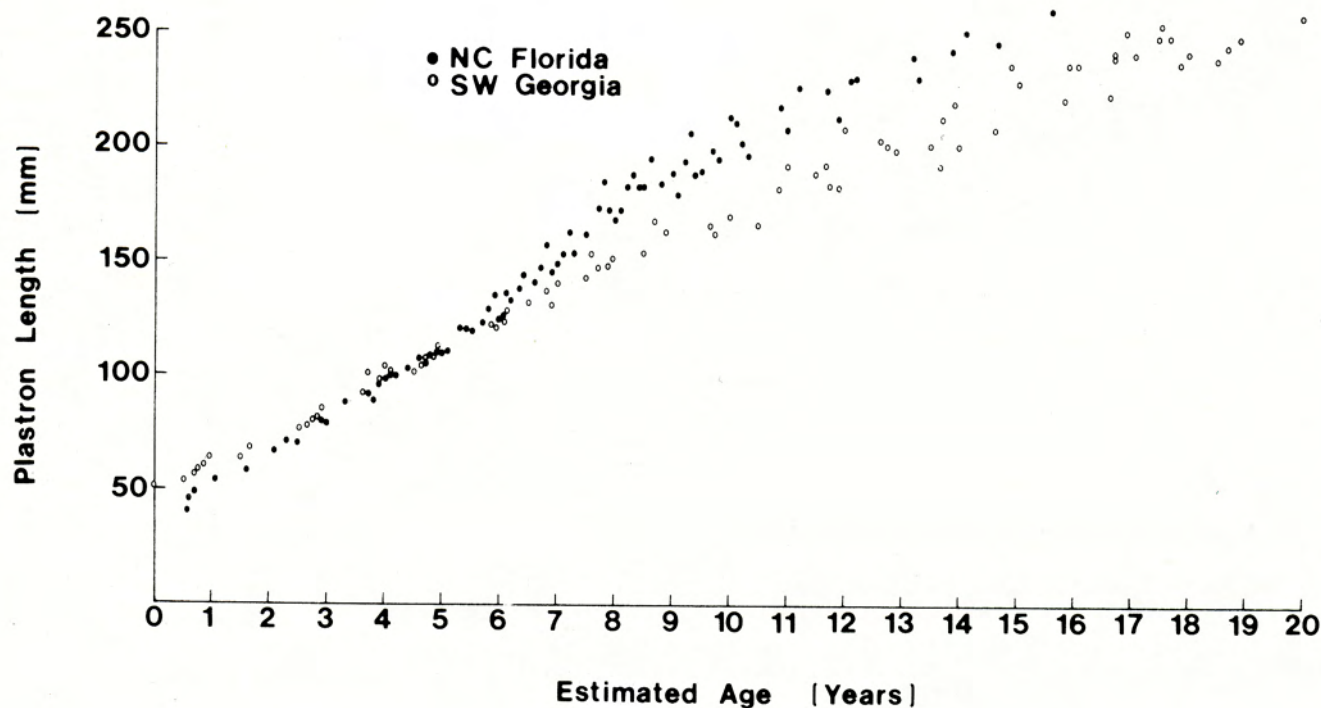


Figure 11. *Gopherus polyphemus* age estimations based on plastron lengths taken from Landers et al. (1982 pg. 101). Reprinted with permission from FLMNH.

Table 5. *Gopherus polyphemus* plastron and skeletochronology age estimates.

Specimen	Age Estimate Using Plastron Length (yrs) ^a	Skeletochronology Age (yrs)
UF 143424	15	36
UF 143425	9	11
UF 143426	13	13
UF 143427	11	8
UF 143428	6	3
UF 144653	5	6
UF 144657	8	6
UF 144659	10	14
UF 144655	7.5	7
UF 150177	12	11
UF 143430	N/A	15
PPC 6669	10.5	17
PPC 6674	10.5	25
PPC 3510	0	0

^a after Landers et al. (1982)

Table 6. *Gopherus polyphemus* shell allometry^a.

Specimen	Carapace	Hyoplastron	Carapace Estimate	Plastron
UF 143424	278.00	63.70	225.50	266.00
UF 143425	224.00	60.10	215.85	210.00
UF 143426	265.00	65.40	229.98	244.00
UF 143427	258.00	64.80	228.39	227.00
UF 143428	150.00	30.80	130.74	128.00
UF 144653	130.00	33.70	139.87	116.00
UF 144657	174.00	42.00	164.98	167.00
UF 144659	218.00	60.10	215.85	199.00
UF 144655	187.00	47.80	181.79	169.00
UF 150177	220.00	49.30	186.05	210.00
UF 143430	242.00	N/A	N/A	N/A
PPC 6669	244.00	65.10	229.18	223.00
PPC 6674	234.00	59.30	213.69	224.00
PPC 3510	45.50	N/A	N/A	46.50

^a after Franz and Quitmyer (2005)

Where b = the slope of the line

a = the y intercept

x = the independent variable (hyoplastron length along suture)

y = the dependent variable (estimated body size/ carapace length)

Based on this formula, Franz and Quitmyer found that a = 1 and a slope of the line (b) = 0.75 in modern gopher tortoises can be used. Using this equation, straight-line carapace estimations for disarticulated shells were obtained. Table 6 shows my estimated straight-line carapace lengths, hyoplastron lengths, and carapace lengths based on Franz and Quitmyer's work. The actual carapace measurements compared favorably with those that were estimated using Franz and Quitmyer's regression.

Based on my carapace length calculations, ages were determined using the growth curve published by Mushinsky et al. (1994). These curves were based on studies of tortoise populations in central Florida (Figure 12) which are similar geographically to individuals in this study and are considered appropriate correlations. These age estimates were compared to skeletochronology age estimates which can be seen in Table 7. A Wilcoxon Signed Rank Test performed on this data yielded a two-sided P-value of $P < 0.0332$. As stated previously, there are a number of factors that make carapace length age

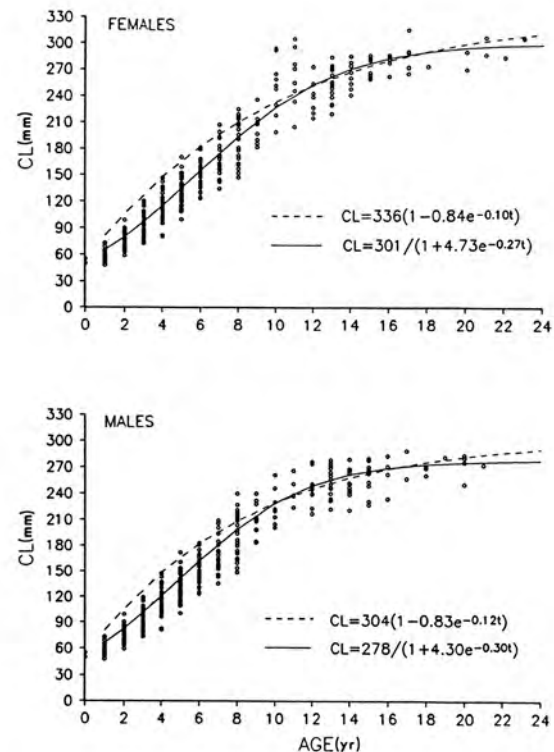


Figure 12. *Gopherus polyphemus* age estimates based on carapace lengths, from Mushinsky et al. (1994 pg. 122). Reprinted with permission of H. Mushinsky.

Table 7. *Gopherus polyphemus* carapace and skeletochronology age estimates.

Specimen	Age Estimate Using Carapace Length (yrs) ^a	Skeletochronology Age (yrs)
UF 143424	16	36
UF 143425	9	11
UF 143426	13	13
UF 143427	14	8
UF 143428	5	3
UF 144653	5	6
UF 144657	7	6
UF 144659	10	14
UF 144655	8	7
UF 150177	11	11
UF 143430	12	15
PPC 6669	12	17
PPC 6674	11	25
PPC 3510	0	0

^a after Mushinsky et al. (1994)

estimates suspect. Therefore, the low P-value obtained shows that skeletochronology age estimates are more precise than carapace lengths. This fact becomes accentuated after the animal has reached sexual maturity and growth slows.

FOSSIL TORTOISES

Based on the results from the *Gopherus polyphemus* specimens, the humeri of the two fossil tortoise species (n=21) were sectioned and examined for LAGs (Table 8). A hatchling sized specimen (UF 226265) yielded no rings because of it was under 1 year old. Another specimen, UF 226272 yielded no rings due to an advanced age. This specimen had a very high amount of remodeling in its bones, also known as Haversian reconstruction, that is most likely attributed to fatigue repair (Figure 13). The reason for this reconstruction is not well known; however, it makes aging of the individual impossible (Chinsamy-Turan 2005). It has been determined, based on the aging of other individuals that this specimen was well over 50 years.

Age estimates for fossil specimens were also compared to carapace and plastron lengths. Most of the shell measurements are based on estimates from the portions of the shells that were collected. Taphonomic factors caused a majority of the shells to be distorted and to break apart. Distortion is much more apparent in the carapace because it is much more curved than the plastron. For this reason, plastron length estimates are

more accurate than carapace estimates (Dodd 1995). The morphometric analyses used on the modern *G. polyphemus* could not be applied to fossil specimens due to differences in size and growth. Therefore, shell lengths were estimated by comparing partial shells with complete specimens housed in the FLMNH. Table 9 shows carapace and plastron lengths compared to age estimates for all specimens. As predicted, smaller tortoises yielded younger ages, while older specimens were larger in size. Growth curves were not speculated upon in this study because I believe more specimens of different age classes are needed, particularly hatchling sized or very young individuals.

DISCUSSION AND SUMMARY

A suite of different skeletal elements were taken from *G. polyphemus* specimens for sectioning (Figure 14). In all samples, the scapula and ilium were inconsistent in contrast to the number of LAGs found in the humerus and femur. With regard to vertebrae, there is a very high degree of resorption and remodeling in tortoise vertebrae that is unparalleled in other elements. Chinsamy-Turan (2005) noted that organization of a bone tissue composition and geometry is dependent on the weight of the animal and the biomechanical properties of that bone. Long bones, such as the humerus and femur, with their more cylindrical shafts tend to have slower and steadier rates of reconstruction and resorption (Klinger & Musick 1992). The humerus was primarily used be-

cause of the abundance of elements in the fossil collection. The relative abundance of humeri as opposed to femora seems to result from the protection offered by the tortoises' shells. When a tortoise tucks into its shell, the front limbs can be tucked more completely and tightly into the shell.

Skeletochronology estimates were compared to three other aging techniques that have been used in previous studies however Wilcoxon Signed Rank tests show that estimates were not comparable. Scute annuli counts are not consistent after the animal reaches its early twenties, at which point scute wear and condensation of ring makes for unreliable age estimates (Germano 1988, 1992; Mushinsky et al. 1994; Aresco & Guyer 1998). False annuli can also over inflate age estimations. Using plastral lengths to age tortoises can yield inaccurate ages as well. The epiplastral extension in the gular region of the plastron is misleading when aging individuals, therefore this measurement should not be used for aging *G. polyphemus* (Mushinsky et al. 1994). Skeletochronology also appears to be more accurate than carapace lengths as changes in resources and environment can greatly affect carapace growth in individual tortoises.

Caution should also be used when comparing shell

dimensions from different populations. Separate populations across a given species range may have different body dimensions due to changes in habitat, food availability, and climatic conditions (Gibbons 1976; Mushinsky et al. 1994). To determine ages of individuals from carapace and plastron lengths in this project, previously published growth trajectories were used from a geographically similar population.

Interestingly, UF 143424 yielded an age around 15-16 years based on shell dimensions and, an age of 36 years based on skeletochronology. This discrepancy may be attributed to the fact that it was a gravid female with two eggs present when the individual was necropsied. Additionally, the scutes on the shell were worn smooth, and no annuli could be counted. In a number of reptile species, gravid females utilize mineral deposits from bone to produce eggshell (Wink & Elsey 1986; Wink et al. 1987). After the eggs are produced, the mineral deposits are restored within the bone, which leads to a high amount of reconstruction. Thin-sections of this specimen revealed large, open vacuities that may have been a result of calcium and phosphate utilization (Figure 15). No other gravid specimens examined were gravid, and no other specimens (male or female) exhibited similar

Table 8. Fossil tortoise age estimates including resorption calculation.

Specimen	Avg. Width of Inner 3 Annuli (mm)	Resorbed Annuli	Age (yrs)
UF 226256	0.46	6	31
UF 226257	N/R	0	8
UF 226258	0.30	7	29
UF 226259	0.28	9	19
UF 226262	N/R	0	8
UF 226263	0.33	12	41
UF 226265	N/R	0	0
UF 226266	0.32	2	17
UF 226267	N/R	0	8
UF 226268	0.22	10	40
UF 226271	N/R	0	9
UF 226272	N/A	N/A	UNKNOWN
UF 191470	N/R	0	5
UF 201906	N/R	0	8
UF 209750	0.19	11	28
UF 226273	N/R	0	8
UF 226274	0.21	2	17
UF 226275	0.41	6	17
UF 226276	0.22	9	25
UF 226277	N/R	0	9
UF 226278	0.53	1	30

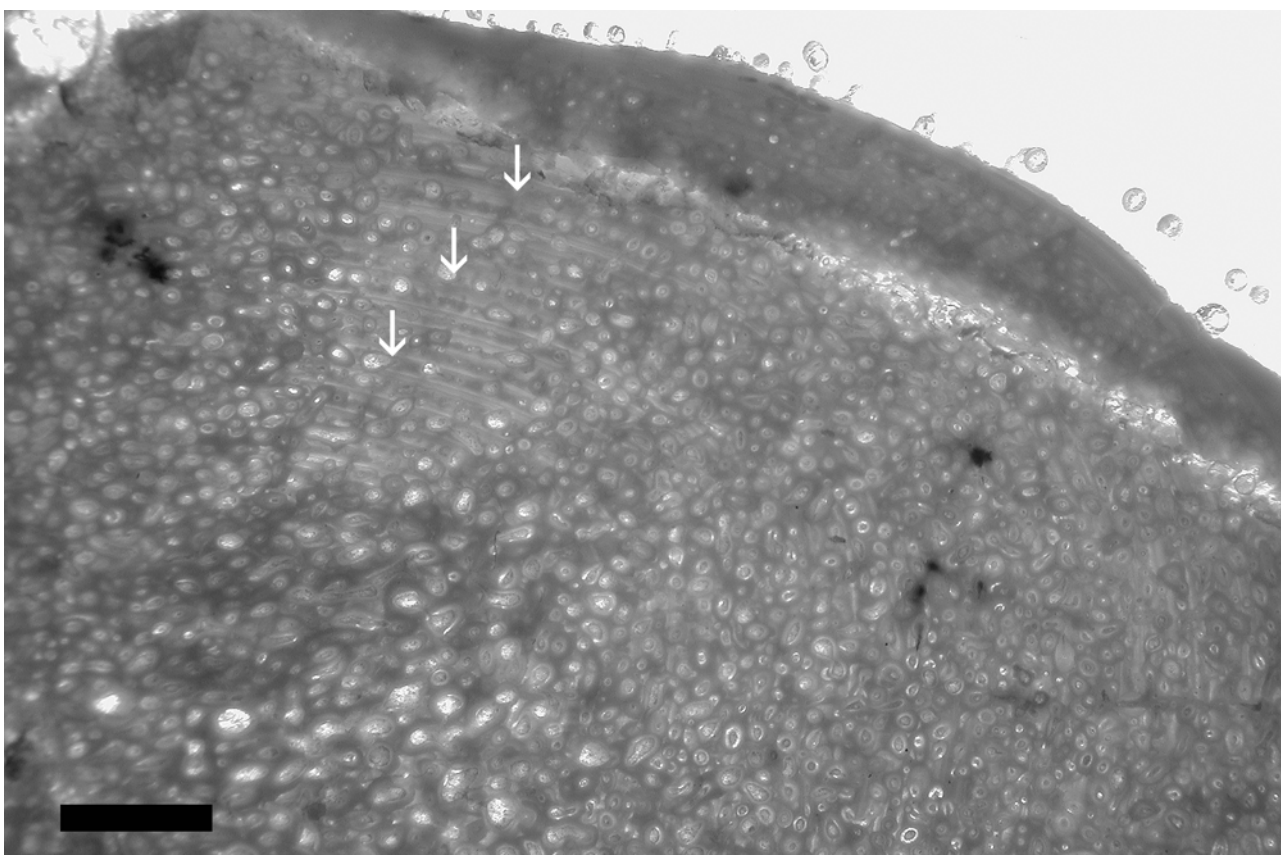


Figure 13. UF 226272 humerus cross-section showing high level of reconstruction (scale bar = 1mm).

Table 9. Carapace and plastron lengths of fossil tortoises compared to skeletochronology age estimates.

Specimen	Carapace Length (mm)	Plastron Length (mm)	Age (yrs)
UF 226256	~560	~480	31
UF 226257	~95	~80	8
UF 226258	~530	~450	29
UF 226259	~260	~240	19
UF 226262	~230	~210	8
UF 226263	562	481.5	41
UF 226265	~85	~90	0
UF 226266	~307	~282	17
UF 226267	240	~210	8
UF 226268	566	500	40
UF 226271	~165	~145	9
UF 226272	~600	~530	UNKNOWN
UF 191470	97.9	82	5
UF 201906	124	111.5	8
UF 209750	366	360	28
UF 226273	158	~142	8
UF 226274	~350	~340	17
UF 226275	~425	~405	17
UF 226276	~425	~405	25
UF 226277	156	132	8
UF 226278	~430	~410	30

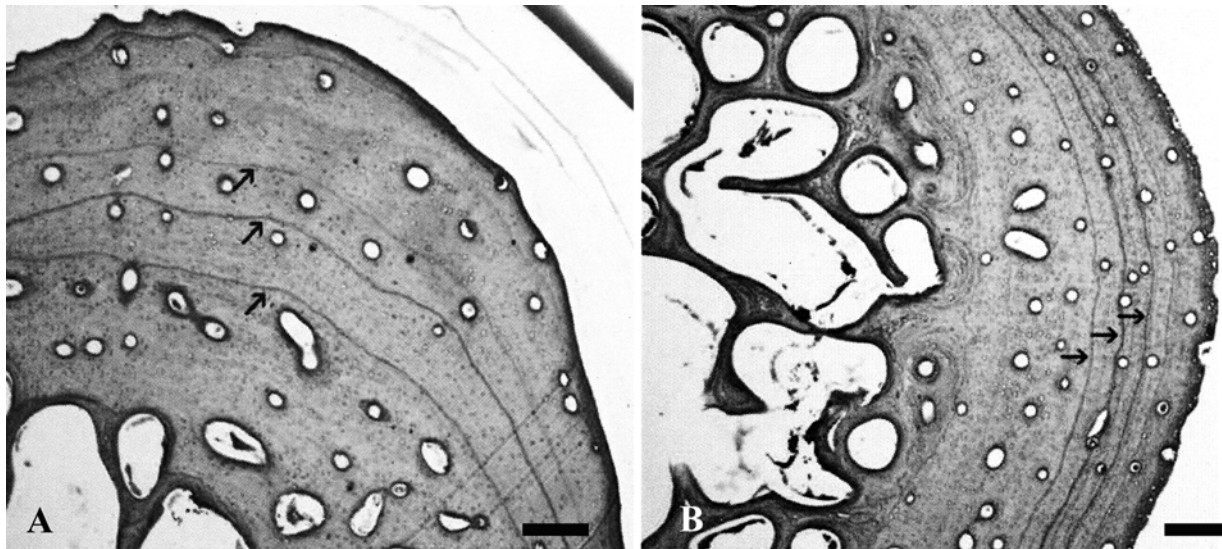


Figure 14. Slides depicting ilium (A) and scapula (B) cross-sections. Arrows show visible LAGs (scale bar = 1mm).

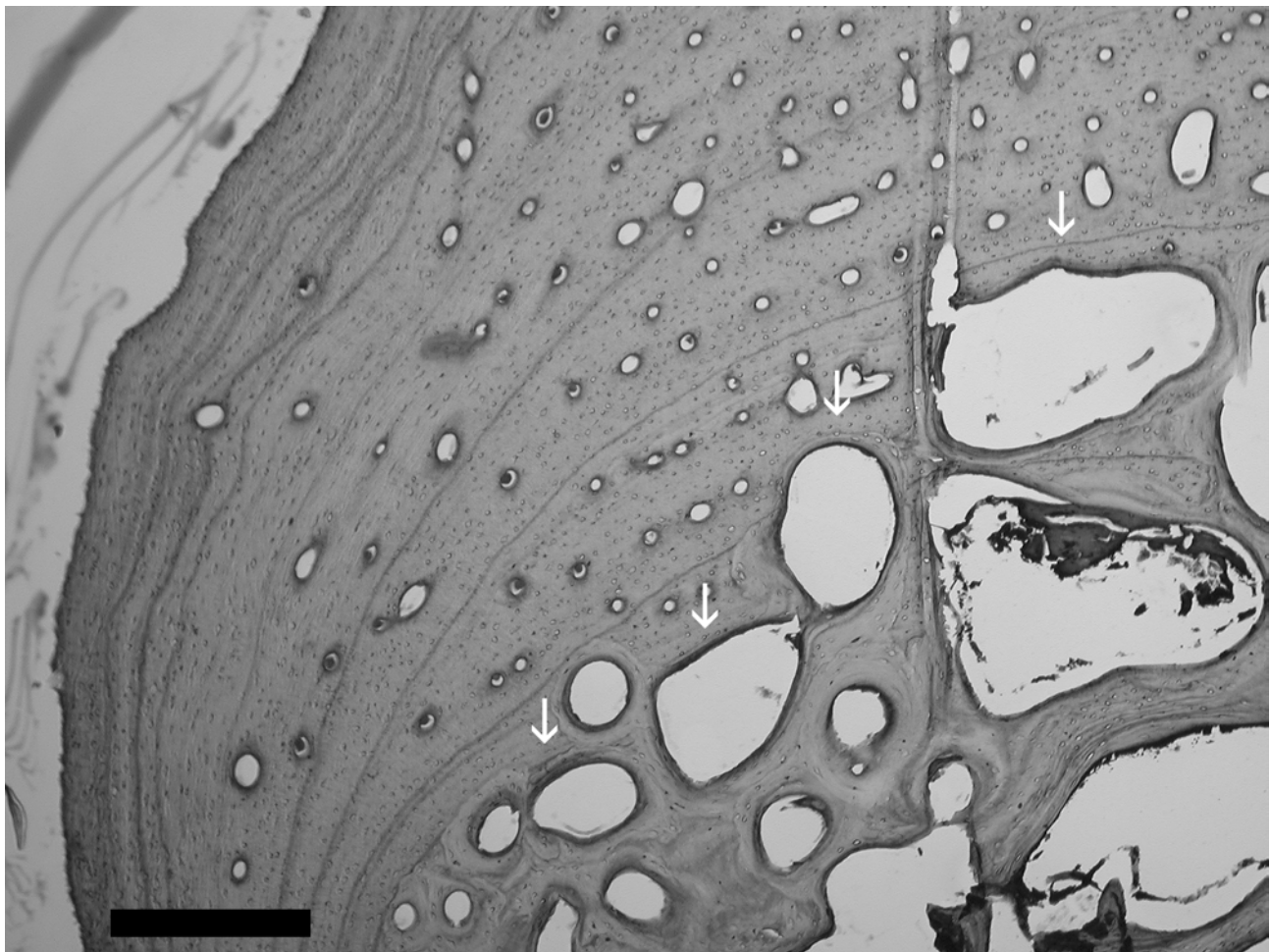


Figure 15. UF 143424 humerus cross-section showing open vacuities (scale bar = 1mm).

vacuities. I believe that this individual was likely much older than shell dimension age estimates showed, based on its size and advanced scute wear. However, potentially gravid females should be regarded with caution when using skeletochronology to determine the age these animals. More information on mineral utilization of reptiles as suggested in Wink and Elsey (1986) and Wink et al. (1987) should also be gathered.

LAGs in *S. nebrascensis* and *G. laticuneus* were visible in most specimens (Figure 16). With a larger sample size, growth rates are attainable for the two fossil tortoise species reviewed in this study. In order to predict growth rates, a growth series including specimens ranging from hatchlings and juveniles up to large adults is needed. Complete growth series for the two fossil species identified were not available. Instead, a composite growth series involving individuals from two separate species is represented. This does not provide enough data points for growth rate predictions for the two species. However, based on specimen sizes and age calculations, it can be inferred that growth rates were very similar for both species. Based on shell measurements, it appears that both species attained adult sizes over 0.5 m in length in and could have taken as long as 40 years to attain this size.

These findings are of particular importance for the gopher tortoise in the southeastern United States. Popula-

tion declines throughout its range have prompted state and federal protection throughout a majority of its range. In areas where tortoises have been exterminated due to human development, disease, or natural disaster, skeletochronology performed on carcasses can be an invaluable tool for determining the group structure, the carrying capacity of the habitat, information regarding sexual maturity, and individual longevity within the population. Where relocation of tortoises is possible, this information can be used for developing more precise repatriation goals. In areas where relocation is not feasible, this data will preserve historical records for the future when repatriation may be a viable option.

Use of fossil tortoise material for skeletochronology is also an acceptable method for studying the paleoecology of extinct species. The results reported are due, in part, to the excellent preservation of fossil materials. Historically, the badlands of South Dakota and Nebraska have been known for the excellent preserved fossils found in that region, and the fossil tortoise specimens studied here were no exception.

Most of the fossil specimens appear to be in or about the same age class. This artifact is a result of two factors: 1) smaller individuals do not preserve as well in the fossil record, and 2) larger individuals are more difficult to collect and transport. Therefore, it is difficult to ascertain demographic information for *S. nebrascensis* and *G. laticuneus* based on these initial findings.

However, population information gathered is important for examining the shift in genera during the EOT. As discussed previously, the climatic conditions and habitat of the region change over this period in time. It appears that the genus *Styemys* was in decline as *Gopherus* began to become more abundant in the fossil record (R. Franz, pers. comm.). Information on the age and structure of the fossil tortoise species can provide insight into why this change occurred. The genus *Gopherus* appears to be more suitably adapted for living in the badlands habitat but we still do not know why. Faster growing *Gopherus* individuals, shorter time to reach sexual maturity, or longer lifespans are all viable scenarios that can now be tested using skeletochronology.

ACKNOWLEDGEMENTS

This paper represents the work completed for the M.S. degree in Geological Sciences at the University of Florida. I thank my committee, especially Bruce J. MacFadden and Dick Franz, for all of their support and guidance. I also thank Greg Erickson at Florida State University for the use of his lab and discussions about skeletochronology and methods. Several individuals

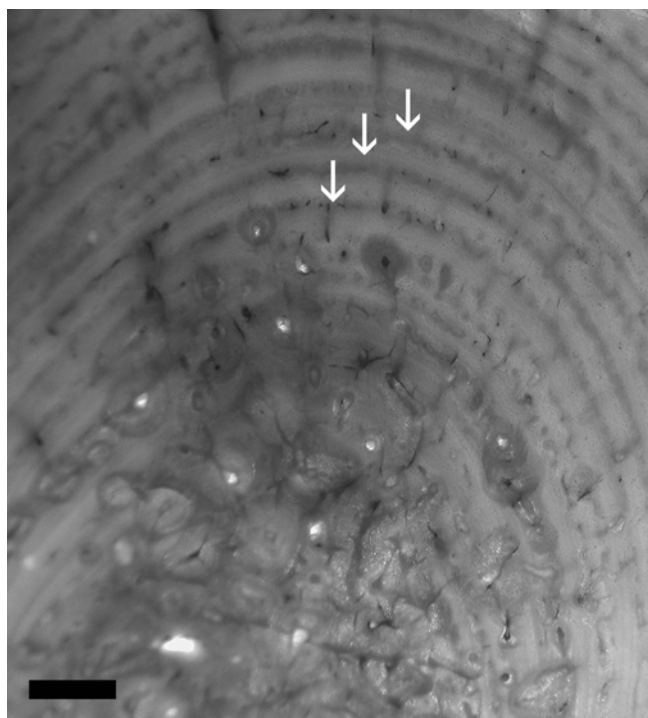


Figure 16. UF 226278 humerus cross-section with arrows showing well defined LAGs (scale bar = 1mm).

donated specimens for my research: Ray Ashton (Ashton Biodiversity Research and Preservation Institute, Inc.), Peter Pritchard (Chelonian Research Institute), Karen Frutche (University of Central Florida), Boyd Blihovde (Wekiwa Springs State Park), Joan Berish (Florida Fish and Wildlife Conservation Commission), Craig Guyer (Auburn University), Dave Parker (Ft. Matanzas National Monument #FOMA-2002-SCI-0001). I would also like to thank Barbara, Reed and Jim Toomey for their hospitality; Marcia Wright, Helen Cozzini, and members of the 2001 Pony Express collecting trip for their field assistance; and G. Erickson, Walter Joyce, and H. E. LaGarry for reviewing the manuscript. Special thanks also go to Russ McCarty for his help with fossil preparation and moral support. I thank Marisol Amador and Shelley Franz for assistance with images. For their financial support I thank the Gopher Tortoise Council, the Southwest Florida Fossil Club, and the Lucy Dickinson Fellowship from the Florida Museum of Natural History. Publication costs were supported by the Toomey Foundation for the Natural Sciences and the Vertebrate Paleontology Fund at the Florida Museum of Natural History. This is University of Florida Contribution to Paleobiology 597.

LITERATURE CITED

- Aresco, M., and C. Guyer. 1998. Efficacy of using scute annuli to determine growth histories and age of *Gopherus polyphemus* in southern Alabama. *Copeia*, 4:1094-1100.
- Auffenberg, W. 1964. A redefinition of the fossil tortoise genus *Stylemys* Leidy. *Journal of Paleontology*, 38:316-324.
- Auffenberg, W. 1974. Checklist of fossil land tortoises (Testudinidae). *Bulletin of the Florida State Museum*, 18 (3):121-251.
- Auffenberg, W., and R. Franz. 1982. The status and distribution of the gopher tortoise (*Gopherus polyphemus*.) In: Bury, R. ed., North American tortoises: conservation and ecology. Wildlife Reserve Report 12:95-126.
- Berggren, W., and D. Prothero. 1992. Eocene-Oligocene climatic and biotic evolution: an overview. Pp. 1-28. in D. Prothero and W. Berggren, eds. Eocene-Oligocene climatic and biotic evolution. Princeton University Press, 568 p.
- Bramble, D. M. 1971. Functional morphology, evolution, and paleoecology of gopher tortoises. Ph.D. dissertation. University of California, Berkeley. 341 p.
- Cagle, F. R. 1946. The growth of the slider turtles, *Pseudemys scripta elegans*. *American Midland Naturalist*, 36:685.
- Castanet, J. 1994. Age estimation and longevity in reptiles. *Gerontology*, 40:174-192.
- Castanet, J. and M. Cheylan. 1979. Les marques de croissance des os et des écailles comme indicateur de l'âge chez *Testudo hermanni* et *Testudo graeca* (Reptilia, Chelonia, Testudinidae). *Canadian Journal of Zoology*, 5: 1649-1665.
- Castanet, J. and E. Smirina. 1990. Introduction to the skeletochronological method in amphibians and reptiles. *Annales des Sciences Naturelles, Zoologie*, 11:191-196.
- Chinsamy, A. and M. Raath. 1992. Preparation of fossil bone for histological examination. *Palaeontologia Africana*, 29:39-44.
- Chinsamy-Turan, A. 2005. The microstructure of dinosaur bone. The Johns Hopkins University Press, Baltimore, Maryland, 195 p.
- Crumly, C. R. 1994. The phylogenetic systematics of North American tortoises (genus *Gopherus*): Evidence for their reclassification. Pp. 7-32 in R. Bury and D. Germano, eds. *Biology of North American tortoises*. Fish and Wildlife Research no. 13, U.S. Dept. of the Interior National Biology Survey, Washington, D.C., 204 p.
- de Broin, F. 1977. Contribution a l'étude des Chèloniens: Chèloniens continentaux du Crétacé et du Tertiaire de France. *Mémoires des Muséum National d'Histoire Naturelle*, 39: 366 p.
- Dodd, C. K. 1995. Disarticulation of turtle shells in north-central Florida: How long does a shell remain in the woods? *American Midland Naturalist*, 134:378-387.
- Erickson, G. M., and T. Tumanova. 2000. Growth curve of *Psittacosaurus mongoliensis* Osborn (Ceratopsia: Psittacosauridae) inferred from long bone histology. *Zoological Journal of the Linnean Society*, 130:551-566.
- Ernst, C. H., J. Lovich, & R. Barbour. 1994. *Turtles of the United States and Canada*. Smithsonian Institution Press, Washington and London, 578 p.
- Francillon-Vieillot, H., V. de Buffrenil, J. Castanet, J. Geraudie, F. Meunier, J. Sire, L. Zylberberg, & A. de Ricqlès. 1990. Microstructure and mineralization of vertebrate skeletal tissues Pp. 471-530 in J. Carter, ed. *Biom mineralization: Patterns and evolutionary trends*. New York: Van Nostrand Rheinhold, New York, 797 p.
- Franz, R., and I. Quitmyer. 2005. A fossil and zooarchaeological history of the gopher tortoise (*Gopherus*) in the southeastern United States Pp. 179-200 in R. C. Hulbert Jr., G. S. Morgan, & J. A. Baskin, eds. *Cenozoic vertebrates of the Americas: Papers to honor S. David Webb*. Florida Museum Bulletin,

- 4:125-562.
- Galbraith, D. A., and R. Brooks. 1987. Addition of annual growth lines in adult snapping turtles (*Chelydra serpentina*.) *Journal of Herpetology*, 23 (4):359-363.
- Germano, D. J. 1988. Age and growth histories of desert tortoises using scute annuli. *Copeia*, 4:914-920.
- Germano, D. J. 1994. Growth and age at maturity of North American tortoises in relation to regional climates. *Canadian Journal of Zoology*, 72:918-931.
- Gibbons, J. W. 1976. Aging phenomena in reptiles Pp. 454-475 in M. Elias, B. Eleftheriou, & P. Elias, eds. Special Review of experimental aging research/progress in biology. *Experimental Aging Research*, Bar Harbor, Me, 481 p.
- Grubb, P. 1971. The growth, ecology and population structure of giant tortoises on Aldabra. *Philosophical Transactions of the Royal Society, London*, 260:327.
- Halliday, T. R., and P. Verrell. 1988. Body Size and age in amphibians and reptiles. *Journal of Herpetology*, 22 (3):253-265.
- Hay, O. P. 1908. The fossil turtles of North America. *Carnegie Institute, Washington*, 75, 555 p.
- Hutchinson, J. H. 1980. Turtle stratigraphy of the Wildwood Formation, Wyoming: preliminary results. *University of Michigan Papers in Paleontology*, 24:115-118.
- Hutchinson, J. H. 1992. Western North America reptile and amphibian record across the Eocene/Oligocene boundary and its climatic implications Pp. 451-463 in D. Prothero and W. Berggren, eds. *Eocene-Oligocene climatic and biotic evolution*. Princeton University Press, 568 p.
- Hutchinson, J. H. 1996. Testudines Pp. 337-353 in D. Prothero and R. Emry eds., *The terrestrial Eocene-Oligocene transition in North America*. Cambridge University Press. Cambridge, U.K., 688 p.
- Joyce, W. G., J. Parham, & J. Gauthier. 2004. Developing a protocol for the conversion of rank-based taxon names to phylogenetically defined clade names, as exemplified by turtles. *Journal of Paleontology*, 78 (5):989-1013.
- Judd, F., and J. McQueen. 1982. Notes on longevity of *Gopherus berlandieri* (Testudinidae). *The Southwestern Naturalist*, 27:230-232.
- Judd, F., and F. Rose. 1983. Population structure, density, and movements of the Texas tortoise *Gopherus berlandieri*. *The Southwestern Naturalist*, 28 (4):387-398.
- Klinger, R., and J. Musick. 1992. Annular growth layers in juvenile loggerhead turtles (*Caretta caretta*.) *Bulletin of Marine Science*, 51 (2):224-230.
- Kohn, M. J., J. Josef, R. Madden, R. Kay, G. Vucetich, & A. Carlini. 2004. Climate stability across the Eocene-Oligocene transition, southern Argentina. *Geology*, 32 (7):621-624.
- LaGarry, H. E. 1998. Lithostratigraphic revision and redescription of the Brule Formation (White River Group) of northwestern Nebraska Pp. 63-92 in D. Terry, H. E. LaGarry, & R. Hunt Jr. eds., *Depositional environments, lithostratigraphy, and biostratigraphy of the White River and Arikaree Groups*. GSA Special Paper 325, 216 p.
- Landers, J. L., W. McRae, & J. Garner. 1982. Growth and maturity of the gopher tortoise in southwestern Georgia. *Bulletin of the Florida State Museum*, 27 (2):82-110.
- Larson, E. E., and E. Evanoff. 1998. Tephrostratigraphy and source of the tuffs of the White River sequence Pp. 1-14 in D. Terry, H. E. LaGarry, & R. Hunt Jr. eds., *Depositional environments, lithostratigraphy, and biostratigraphy of the White River and Arikaree Groups*. GSA Special Paper 325, 216 p.
- Legler, J. M. 1960. Natural history of the Ornate Box Turtle, *Terrepene ornata ornata* (Agassiz.) *University of Kansas Publication Museum of Natural History*, 11:527.
- Leidy, J. 1851. On a new species of fossil tortoise. *Proceedings of the Academy of Natural Sciences, Philadelphia*, 5:172-173.
- McCord, R. D. 2002. Fossil history and evolution of the gopher tortoises Pp. 53-66 in T.R. Van Devender ed. *The Sonoran Desert Tortoise natural history, biology, and conservation*. University of Arizona Press, 388 p.
- Miller, K. G. 1992. Middle Eocene to Oligocene stable isotopes, climate, and deep-water history: the Terminal Eocene Event Pp. 160-177 in D. Prothero and W. Berggren, eds. *Eocene-Oligocene climatic and biotic evolution*. Princeton University Press, 568 p.
- Mushinsky, H. R., D. Wilson, & E. McCoy. 1994. Growth and sexual dimorphism of *Gopherus polyphemus* in Central Florida. *Herpetologica*, 50 (2): 119-128.
- Parham, J., and G. Zug. 1997. Age and Growth of Loggerhead Sea Turtles (*Caretta caretta*) of coastal Georgia: An assessment of skeletochronological age-estimates. *Bulletin of Marine Science*, 61 (2):287-304.
- Prothero, D. R., and T. Heaton. 1996. Faunal stability during the Early Oligocene climatic crash. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 127: 257-283.
- Prothero, D. R., and C. Swisher III. 1992. Magnetostratigraphy and geochronology of the terrestrial Eocene-Oligocene transition in North America

- Pp. 46-73 in D. Prothero and W. Berggren, eds. Eocene-Oligocene climatic and biotic evolution. Princeton University Press, 568 p.
- Prothero, D. R., and K. E. Whittlesey. 1998. Magnetic stratigraphy and biostratigraphy of the Orellan and Whitneyan land-mammal "ages" in the White River Group Pp. 39-61 in D. Terry, H. E. LaGarry, & R. Hunt Jr. eds., Depositional environments, lithostratigraphy, and biostratigraphy of the White River and Arikaree Groups. GSA Special Paper 325, 216 p.
- Schultz, C. B., and T. Stout. 1955. Classification of Oligocene sediments in Nebraska. University of Nebraska State Museum Bulletin, 4:17-52.
- Sexton, O. J. 1959. A method for estimating the age of painted turtles for use in demographic studies. *Ecology*, 40 (4):716-718.
- Smith, L. L. 1992. Nesting ecology, female home range and activity patterns, and hatchling survivorship in the gopher tortoise (*Gopherus polyphemus*.) Master's thesis. University of Florida, Gainesville. 106 p.
- Swisher, C. III, and D. Prothero. 1990. Single-Crystal $^{40}\text{Ar}/^{39}\text{Ar}$ Dating of the Eocene-Oligocene Transition in North America. *Science*, 249:760-762.
- Terry, D. O. Jr. 1998. Lithostratigraphic revision and correlation of the lower part of the White River Group: South Dakota to Nebraska Pp. 15-37 in D. Terry, H. E. LaGarry, & R. Hunt Jr. eds., Depositional environments, lithostratigraphy, and biostratigraphy of the White River and Arikaree Groups. GSA Special Paper 325, 216 p.
- Terry, D. O. Jr. 2001. Paleopedology of the Chadron Formation of Northwestern Nebraska: Implications for paleoclimatic change in the North American mid-continent across the Eocene-Oligocene boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 168:1-38.
- Terry, D. O. Jr., and H. LaGarry. 1998. The Big Cottonwood Creek Member: A new member of the Chadron Formation in northwestern Nebraska Pp. 117-141 in D. Terry, H.E. LaGarry, & R. Hunt Jr. eds., Depositional environments, lithostratigraphy, and biostratigraphy of the White River and Arikaree Groups. GSA Special Paper 325, 216 p.
- Wink, C. S., and R. Elsey. 1986. Changes in femoral morphology during egg-laying in *Alligator mississippiensis*. *Journal of Morphology*, 189:183-188.
- Wink, C. S., R. Elsey, & E. Hill. 1987. Changes in femoral robusticity and porosity during the reproductive cycle of the female alligator (*Alligator mississippiensis*.) *Journal of Morphology*, 192:317-321.
- Wolfe, J. A. 1992. Climatic, floristic, and vegetational changes near the Eocene/Oligocene boundary in North America Pp. 421-436 in D. Prothero and W. Berggren, eds. Eocene-Oligocene climatic and biotic evolution. Princeton University Press, 568 p.
- Zug, G. 1991. Age Determination in Turtles. Society for the Study of Amphibians and Reptiles. *Herpetological Circular* 20. 28 p.
- Zug, G., A. Wynn, C. Ruckdeschel. 1986. Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth of the skeleton. *Smithsonian Contribution to Zoology*, 427:1-3