AN ABSTRACT OF THE THESIS OF

Nicholas A. Thurber for a Master of Science in Physical Sciences
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A Review and Evaluation of the Scientific Literature on the Early Evolution of Turtles

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Turtles are unique organisms within the animal kingdom. Unlike any other living reptile, their skulls have neither an upper nor lower opening in the temporal region. All other living reptiles and birds are diapsid, possessing both temporal openings on each side of the skull. For the turtle shell to form, primitive turtles must have experienced several changes, including the rib cage surrounding the shoulder blades and the flattening and extending of the ribs. Because of these unique features, it has been difficult to place turtles into a taxonomic group, a debate that has been going on since Reptilia was first named in 1768. Despite the great amount of study on living and fossil turtles, there are many controversies remaining on their origins and on how they developed their unique features. Each new fossil discovery and idea proposed requires one to look back with a new perspective and reinterpret old literature. The purpose of this thesis is to review and summarize current and past research regarding the origin and early evolution of turtles, as well as their relationship with other animal groups. In this study I am also analyzing the information collected in order to determine the most likely hypotheses regarding these topics.

Keywords: Turtles, Testudines, Origin, Evolution, Shell

A REVIEW AND EVALUATION OF THE SCIENTIFIC LITERATURE ON THE EARLY EVOLUTION OF TURTLES

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Chapter 1: Introduction

Turtles are unique organisms within the animal kingdom (Figure 1). Unlike any other living tetrapod (four-footed) vertebrates, their skulls have neither an upper nor lower opening in the temporal region, a condition called anapsid (Figure 2). This type of skull is reminiscent of the now extinct Anapsida tetrapods of the Paleozoic, including captorhinomorphs, procolophonids and pareiasaurs (Figure 3). All other extant reptiles (Diapsida: lizards, snakes, crocs, tuataras (Figure 4)) have diapsid skulls, which possess both upper and lower temporal openings on each side of the skull (Figure 2). Not only is turtle skull morphology unique among living reptilians, but their shell (Figure 5) is like nothing else in the animal kingdom, both anatomically and physiologically (Jackson, 2011). In order for the turtle shell to form, early turtles must have incorporated the shoulder blades inside the rib cage (Cordero, 2017). Because of these and other unique features, it has been difficult to place turtles into a taxonomic group and to hypothesize how they originated. It has recently been proposed that turtles are not true anapsids, but are actually modified diapsids, in which the upper and lower temporal openings have secondarily closed (Lyson *et al*., 2010). This is one of many hypotheses that will be discussed in this paper. The controversies surrounding where turtles came from and how they originated their unique features has been debated throughout the scientific community for many years and has resulted in only limited consensus.

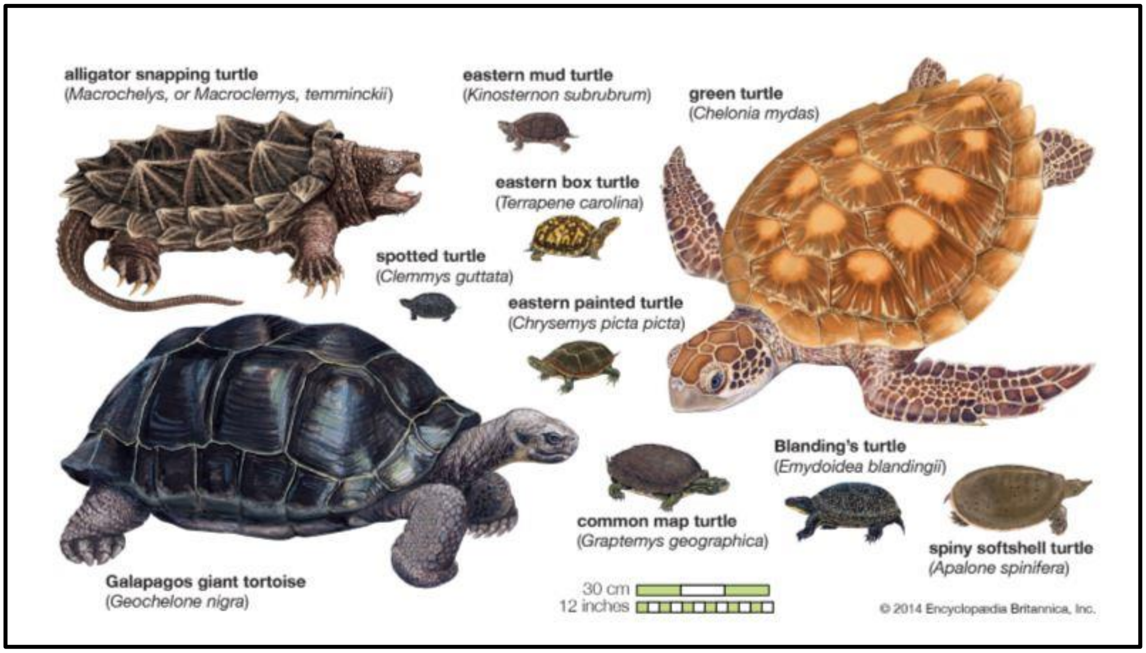


Figure 1. Representative turtle species (order Testudines). From *Encyclopædia Britannica*, 2014

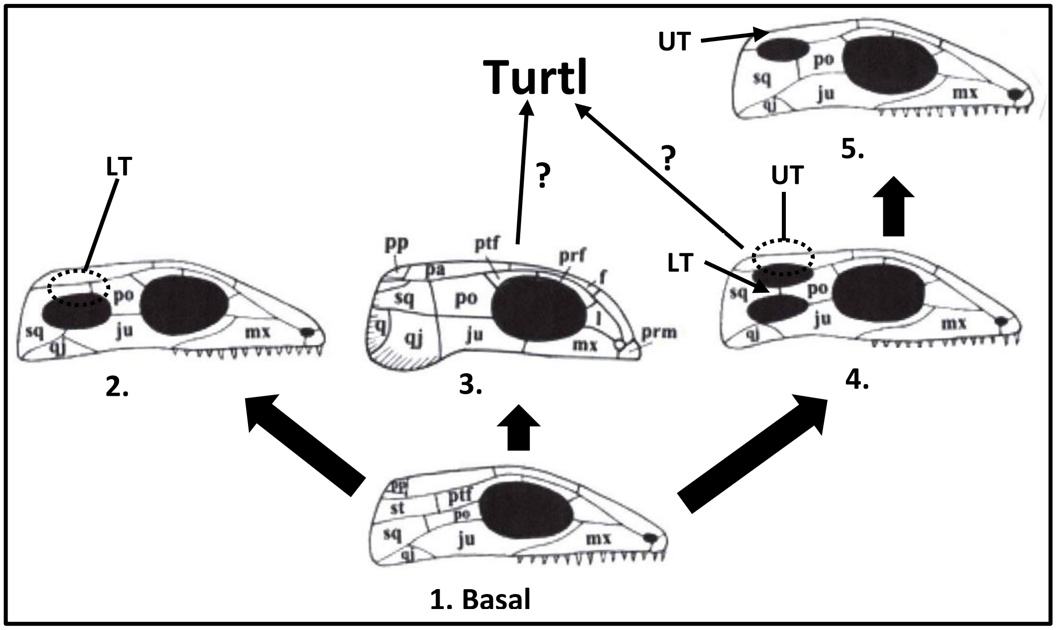


Figure 2. Amniote skull types with possible evolutionary pathways. (1) Basal amniotes have neither an upper nor lower temporal fenestra as seen in early Paleozoic stem-reptiles. (2) Synapsid skulls have only the lower temporal opening as seen in mammals. (3) Anapsid skulls have no temporal fenestrae and the reduction of certain skull bones as exhibited in turtles. (4) The diapsid skull type has an upper and lower temporal fenestra as seen in modern reptiles. (5) Euryapsid skull types have only the upper temporal fenestra and include ichthyosaurs, plesiosaurs, nothosaurs, and placodonts. Skull features: (pp) postparietal, (pa) parietal, (UTA) upper temporal arch, (st) supratemporal, (ptf) postfrontal, (prf) prefrontal, (f) frontal, (prm) premaxillary, (sq) squamosal, (LTA) lower temporal arch, (po) postorbital, (l) lacrimal, (q) quadrate, (qj) quadratojugal, (ju) jugal, (mx) maxilla. Modified from Benton, 2005

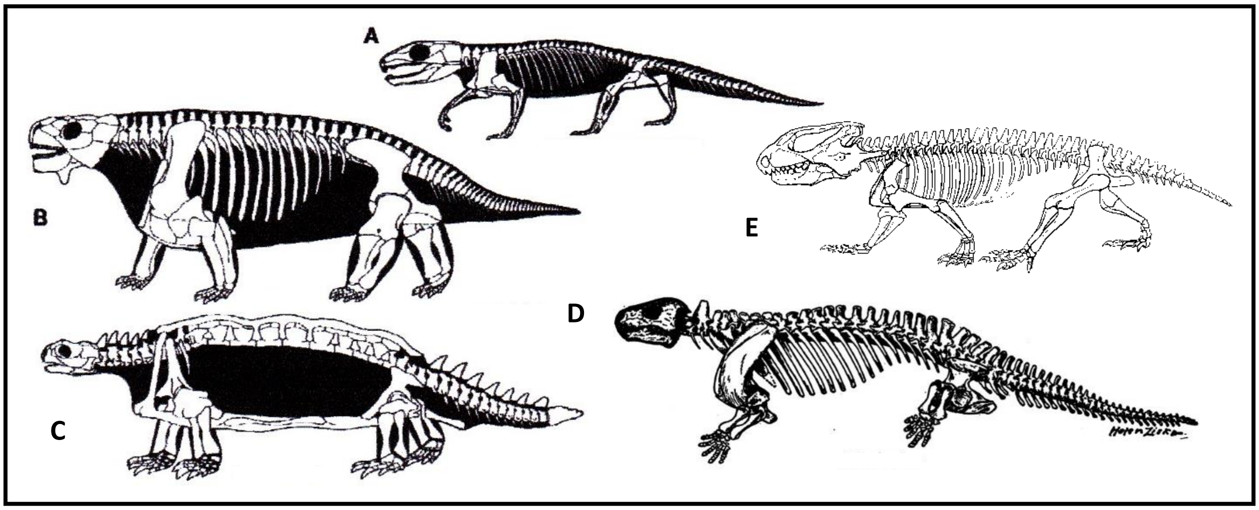


Figure 3. Outline skeletal reconstructions of anapsid animals. A. *Captorhinus* of Captorhinidae (from Heaton and Reisz, 1986), B. *Bradysaurus* of Pareiasaur (from Lee, 1997), C. *Proganochelys* (basal turtle) (from Gaffney, 1990), D. *Diadestes* of Diadectomorpha, reptile-like amphibians (once believed to belong to Cotylosauria) (from Gregory, 1946), E. *Kapes bentoni* of Procolophonidae (modified from Zaher, 2019).

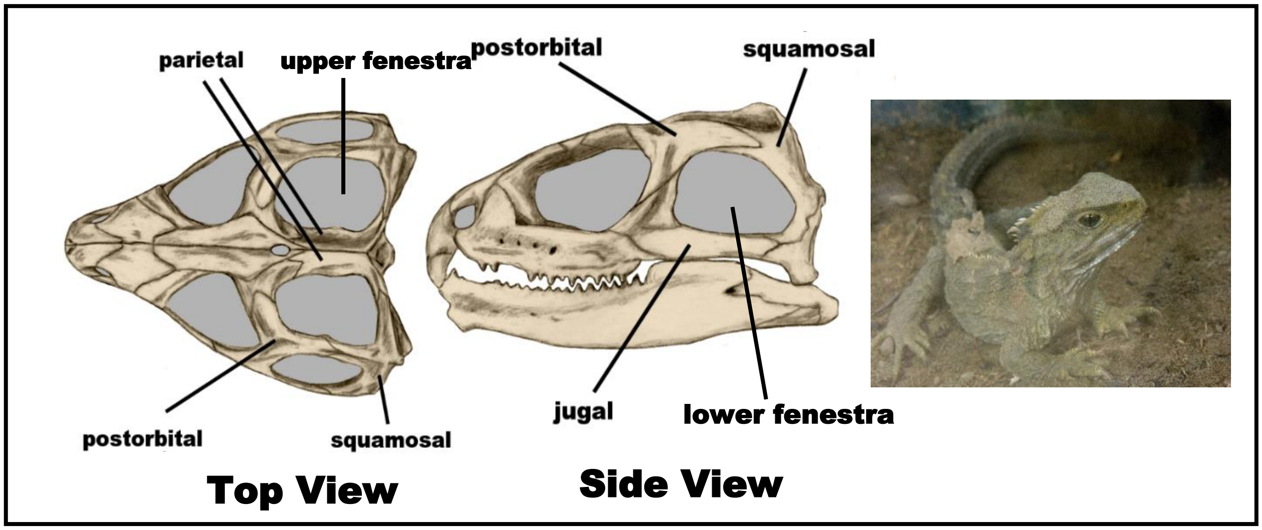


Figure 4. Representative Tuatara (genus *Sphenodon*), a basal reptile. Modified from Tamura, 2007a and PhillipC., 2007

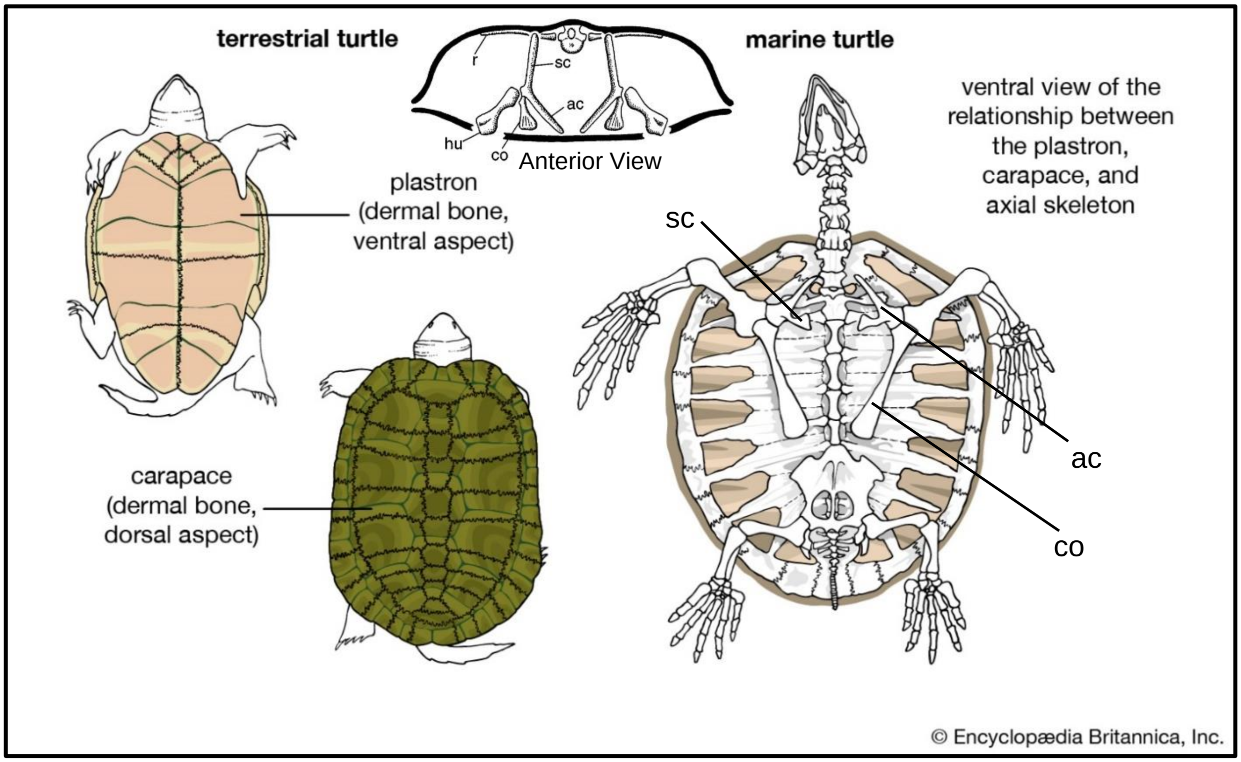


Figure 5. Turtle plastron (lower shell) and carapace (upper shell composed of ribs and dermal bone) in relation to pectoral girdle (within shell). Pectoral girdle is composed of (sc) scapula, (ac) acromion process of scapula, and (co) coracoid (with the scapula forms the scapulocoracoid). Also shown (hu) humerus and (r) ribs. From *Encyclopædia Britannica*, 2014, with modified top center figure from Rieppel and Reisz, 1999

The purpose of this study was to thoroughly review and summarize current and past research, published and unpublished, regarding the origin, early evolution, and phylogeny of turtles, and to analyze this information so that the most likely hypotheses regarding these topics can be determined. This process will result in the identification of any scientific gaps in what has been interpreted concerning turtle evolution, and the best course of action should be going forward in this field of study.

Despite the great amount of study done on living and fossil turtles, there are many controversies remaining on their origins and on how they developed their unique features. Information on each controversy will be read and reported on and by presenting lines of evidence used to support all sides of each controversy. These controversies can be characterized by the following questions:

1. Turtles are reptiles; therefore, how have they been classified within Reptilia?
2. From which reptile group did turtles evolve?
3. In regards to turtle phylogeny, are morphological and molecular studies currently in agreement?
4. In which habitat did true turtles and their immediate ancestors utilize, terrestrial or aquatic?
5. Were endoskeletal or exoskeletal components used to construct the turtle shell?
6. What role, if any, does the fossil *Eunotosaurus* play in the origin of turtles?

These questions were chosen based on the controversial nature of the topics involved and in some part due to the significance they have on the hypotheses surrounding early turtle evolution. These topics often appear in the scientific literature when turtle evolution is discussed, and due to the impact they have on turtle history, the debates are often very heated. These debates can initiate solid research, but they often polarize the scientific community and leave little agreement. A summary of each controversy is presented in chronological order to provide a historical prospective.

Chapter 2: Methods

Literature Search

All pertinent literature, published and unpublished, was searched using search engines such as JSTOR, Google Scholar, and any sources Emporia State library services provide, including Academic Search Complete and ScienceDirect. Literature summaries by well-known scientists in the field of turtle evolution were also utilized in order to get an overview of the concepts that involve turtle evolution (e.g., *Turtles as Hopeful Monsters* by Olivier Rieppel (2017)). An attempt was made to gather only primary sources for this paper, with the use of secondary sources only when the primary source could not be found or was unable to be translated from its original language. Bibliographies of these sources were mined in order to acquire additional sources. PDFs of the sources or digital copies were stored in multiple locations including a university computer, a personal laptop, and on multiple flash drives. The goal was to create a complete bibliography of all literature pertinent to the questions stated in the previous chapter.

After a large number of scientific papers and books was amassed, a total of 155, the literature was critically evaluated and digital notes and summaries of each article were created. For each question mentioned in Chapter 1, the main hypothesis or hypotheses, their supporting evidence, and any counter arguments was determined. The notes and summaries were saved with their corresponding article in their own folder with multiple locations so that they were backed up, readily available, and able to be reviewed at any time.

Once the bibliography was nearly complete, the previous work chapter was composed. In this writing, an objective reporting method was utilized where summaries of the information were provided without any analysis or critique. The ideas were presented in their corresponding chapters based on the topics discussed in the article. Within each chapter the ideas were presented in chronological order with the oldest information presented first and the most recent presented last. By the time the previous work chapters have been read, the reader will have a solid idea of all the work done on early turtle evolution since it was first discussed in the mid-1800s up until the late 2010s. Furthermore, the complete skeleton of a Giant Asian pond turtle, *Heosemys grandis*, was purchased and studied in order to gain further insight on the skeletal features that are unique only to turtles and were mentioned heavily in the scientific literature.

Analysis and Conclusions

In the analysis portion of this thesis, investigation and interpretation of the information and hypotheses was found in the literature by comparing the various hypotheses and the evidence used to support them. The most likely hypothesis to answer each question mentioned in Chapter 1 was determined using logic, parsimony (simplest method), a weighting of the evidence according to understanding of the literature, and double checking the facts presented in the scientific papers. The methods and procedures that were used in this study were selected because these methods minimized bias and error. No results of one researcher were favored over another due to the recentness of the paper or the number of times that paper is cited in other papers thereby avoiding a bias of authority. The goal was to determine the best picture of early turtle evolution, by answering the various questions posed in Chapter 1. The conclusion section includes short summaries of the analyses and presents ideas for future work in early turtle evolution.

Chapter 3: Previous Work

Question 1. Turtles are reptiles; therefore, how have they been classified within Reptilia?

One cannot dive into the evolution of the turtle without first understanding their placement in the larger tree of life. Due to the novel (anapsid) nature of the turtle skull and the unique nature of turtles in general, the controversy surrounding what clade they came from dates back to the early naming of the group and its place inside Reptilia (Laurenti, 1768). Reptilia is within the clade Amniota (Haeckel, 1866), animals that produce eggs containing an amnion. Amniota lies within the clade Tetrapoda (Linnaeus, 1758), vertebrates possessing four limbs; therefore, Reptilia also falls under Tetrapoda (Figure 6). Turtles reproduce by a shelled egg that has an amnion and they have four limbs.

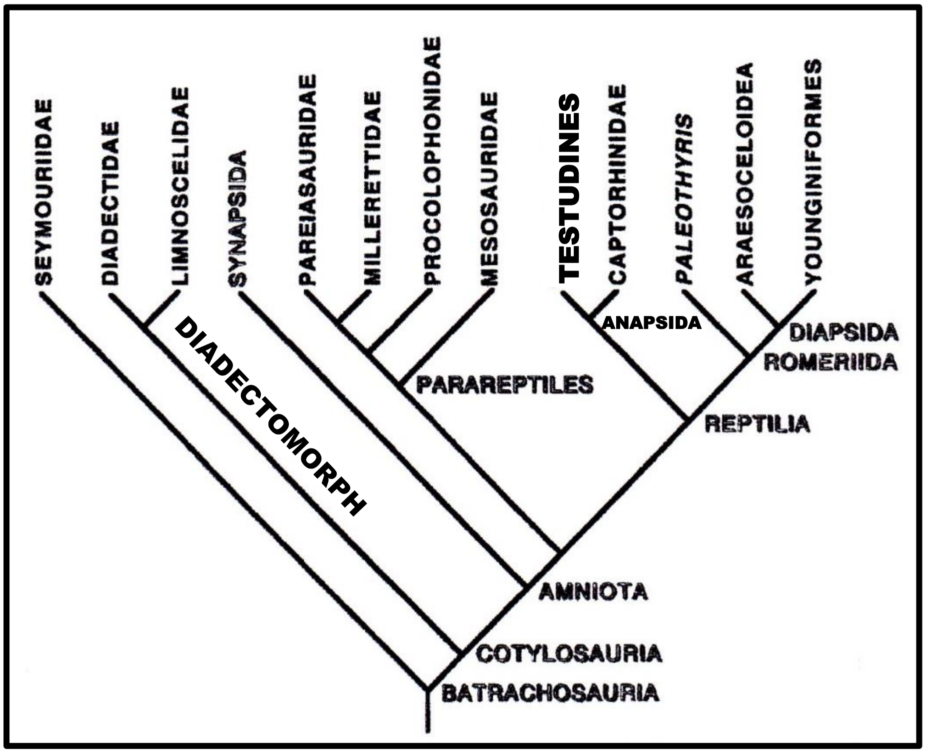


Figure 6. General phylogenetic tree of the clade, Amniota. Notice the placement of Testudines (turtles), sister to Captorhinidae in the clade Anapsida. The original cladogram is from Gauthier *et al.,* 1988, but this iteration was modified from Laurin and Reisz, 1995

There is currently a broad consensus about the animals that encompass Amniota (Figure 6), despite the early incorrect placement of microsaurs (Carroll and Gaskill, 1978) and seymouriamorphs (White, 1939) within the clade. It is generally defined as a crown-group bounded by mammals, testudines, and diapsids (Figure 2, p. 2) (Gauthier *et al*., 1988). In addition to these extant taxa, Amniota is believed to also contain pareiasaurs, procolophonids, captorhinids (Figure 3, p. 3) and several other extinct taxa (Laurin and Reisz, 1995). Amniotes closest relatives are believed to be diadectomorphs (Gauthier *et al.*, 1988). Skull morphology, specifically the number of temporal fenestrae, has long been used to distinguish clades within Amniota.

Linnaeus defined and named the order Testudines (1758) as tetrapods with a shell composed of an upper carapace and a lower plastron. In 1768, Laurenti coined the name Reptilia to include various “creeping” tetrapods including a combination of reptiles, amphibians, and select turtles. Of the fourteen synonymous names proposed for turtles, only Chelonia, Testudinata, and Testudines have remained prevalent to this day (Laurin and Reisz, 1995). Because the group that comprises turtles was first named Testudines, it will be the only proper name used to describe this group in this paper. The Testudines is defined by forty-one autapomorphies (shared derived characters) (deBraga and Rieppel, 1997). The most significant character for classification has been an anapsid skull type. Some other significant autapomorphies include the loss of teeth, loss of the fourth femoral trochanter, unique scapula morphology (possessing an acromion process and coracoid) and location, and the acquisition of skeletal and dermal elements into a shell (Figure 5, p. 4).

The discussion of how to classify amniotes by the possession or lack of temporal fenestrae or openings started when Günther (1867) correctly identified that an early tetrapod lizard-like animal, *Sphenodon*, possessed the lower temporal arch (LTA) seen in many agamid lizards (Figure 2, p. 2). Common agamid lizards include bearded dragons, frilled lizards, and Chinese Water Dragons. In the following years, Baur (1889, 1895) and Cope (1892) independently developed a classification based on fenestration of amniote skulls. Baur (1895) compared patterns of skull emargination or reduction seen in turtles to that of diapsid reptile skulls (from Rieppel, 2001). Some turtles have an upward emargination of the left and right ventral side of the skull, which resembles the upper temporal arch (UTA) (Figure 2, p. 2). He concluded that even though the side of the skull may resemble an upper temporal arch of diapsids, the process of reduction is still fundamentally different from the temporal fenestration observed in diapsids (from deBraga and Rieppel, 1997). Cope (1892) concurred with Baur (1889, 1895) and was the first to compare the Testudines (he used Testudinata) directly to another group, the Sauropterygia, a group of extinct marine reptiles with a modified diapsid skull type (Figure 7). He based his comparison on the belief that both groups possessed a subtemporal fossa produced by temporal emargination from the lower portion of the skull (from Rieppel, 2001). During this time Parker (1868), Cope (1892), Baur (1887) and Lydekker (1889) highlighted similarities between the Plesiosauria, a member of Sauropterygia (Figure 7), and the Testudines (from Osborn, 1903).

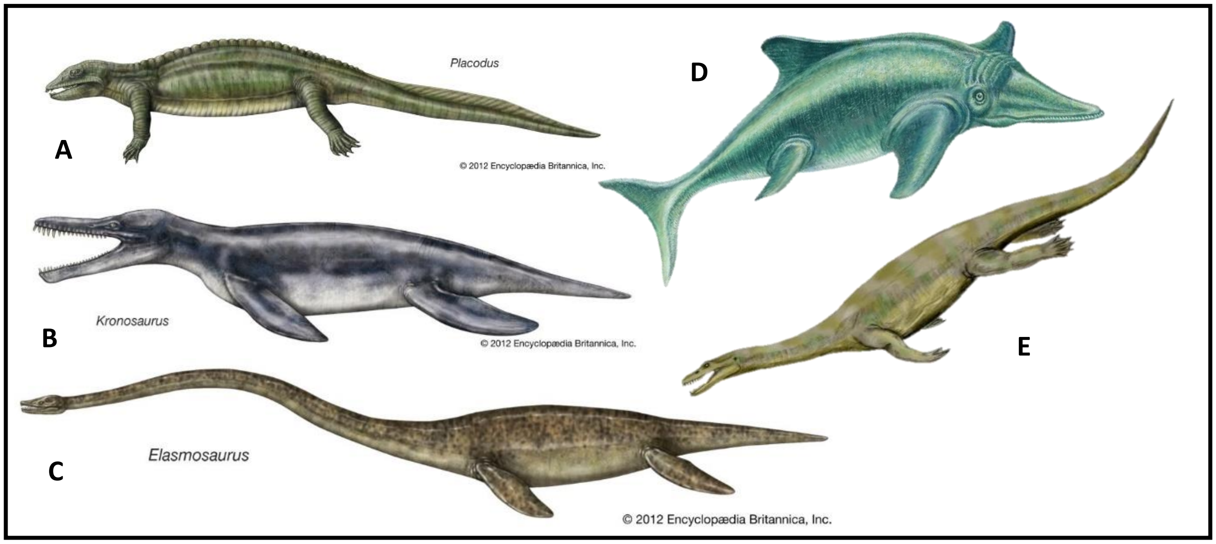


Figure 7. Representatives of order Sauropterygia. A. *Placodus* of Placodontia (from *Encyclopædia Britannica,* 2012) B. *Kronosaurus* a short-necked plesiosaur (from *Encyclopædia Britannica*, 2012) C. *Elasmosaurus* a long-necked plesiosaur (from *Encyclopædia Britannica,* 2012) D. Ichthyosaur (from *Encyclopædia Britannica*, 2009) E. Nothosaur (from Tamura, 2007b).

Although Baur (1887) and Smith Woodward (1898) discussed the possibility of subdividing Reptilia based on the number of fenestrae located on the temporal region of the skull, neither of them proposed names. It should be noted that Broom (1901) placed phylogenetic values on the single-arched and twin-arched groups, but he did not name them. Osborn (1903) designated formal subdivisions within Reptilia, those with a single temporal arch or undivided temporal arches he named the subclass Synapsida, and those with double or separate temporal arches he called the subclass Diapsida (Figure 2, p. 2). Within Synapsida, Osborn (1903) placed Cotylosauria (a group of basal reptiles now known as Captorhinidae), Anomodontia (superorder), that included Theriodontia, Dicynodontia and Placodontia, Testudines and Sauropterygia (Figure 7). Testudines was placed in Synapsida based on the undivided temporal arches characteristic of the clade. Osborn (1903) also noted that Broom

(1902) first recognized the vomer and prevomer as having phylogenetic significance. Osborn used Broom's study to conclude that the presence of a large, median vomer is characteristic of Synapsida, with the exception of the Plesiosauria and the Cotylosauria, in which the vomer is either reduced or considered small by Broom (1902). Broom viewed a small vomer as being characteristic of an anapsid skull condition (no temporal openings). Osborn (1903) stated that prevomers are small in most Synapsida, but are large in Cotylosauria and present in Plesiosauria. On the other hand, the prevomers of Diapsida are large, except in certain Diapsida orders. Osborn also used the phalangeal count as another character supporting a Synapsid classification of Testudines. He showed that all the primitive Synapsids (Cotylosauria, Anomodontia, and Testudinata) have a phalangeal formula of 2, 3, 3, 3, 3, which is identical to that of mammals. Osborn placed the remaining clades of Reptilia in Diapsida. Both Synapsida and Diapsida possess the bony elements which encompass the upper and lower temporal arches: squamosals, parietals, postfrontals, postorbitals, quadratojugals and jugals (Figure 2, p. 2) (Benton, 2005). The most significant difference between the two is that Synapsida has arches that are undivided, whereas Diapsida has lower arches that are separated by the formation of the upper temporal fenestra (Osborn, 1903). It was also at this time that Jaekel (1902, from deBraga and Rieppel, 1997) showed that Cotylosauria, specifically the Placodontia (Figure 7), exhibit shared characteristics with the Testudines, going so far as to propose placodonts, specifically *Placochelys placodonta,* as a turtle ancestor (Jaekel, 1907). Broom (1924) used Jaekel's (1907) description of *Placochelys placodonta* to show similarities between the occiput and jugal arch of turtles and of placodonts and to support a hypothesized placodont origin.

Goodrich (1916) was the first to use toe bone structure and heart structure to determine turtle relationships. He specifically mentioned the hooked-shape of the fifth metatarsal bone. It was generally accepted at that time that the hooked-shaped element was a modified fifth metatarsal (Sewertzoff, 1907 from Goodrich, 1916). Goodrich concluded that turtles belong to the Sauropsida branch of Reptilia, a branch created by Huxley in 1871 that was meant to showing similarities between crocodiles, dinosaurs, and birds. This branch, Huxley hypothesized to have led to birds, was characterized by the retention of a hooked-shaped metatarsal and a heart partially subdivided by a vertical septum that does not completely separate the left and right atria (Figure 8). Although all the clades within it possess two temporal fenestrae, Goodrich stated that the prior two traits also characterize turtles and

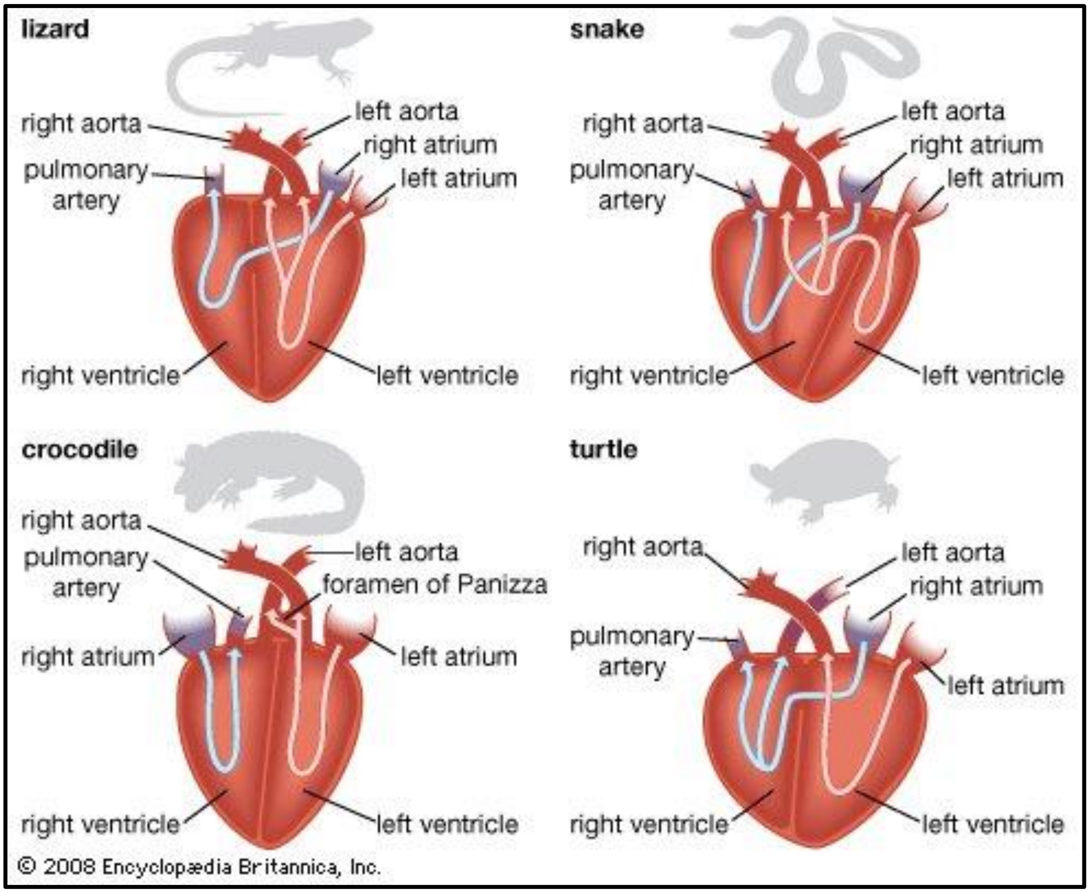


Figure 8. Diagram showing types of reptile hearts. From *Encyclopædia Britannica*, 2008

therefore make turtle's sauropsidans, even though they lacked both temporal fenestrae. Goodrich stated that turtles split from the Sauropsida branch when the heart and foot were already specialized, but before the skull was secondarily closed. These findings do not correlate to Osborn's (1903) findings, but they are nonetheless significant for the new traits that were studied.

Shortly after Goodrich's (1916) findings, Williston (1917) expanded upon the work done by Osborn (1903) by categorizing reptiles with a completely roofed temporal region of the skull into a new group known as Anapsida. He placed Cotylosauria and Testudines into this new group. Williston (1917) cited von Huene (1912) when he stated that the skull's cranial and temporal regions are the most conservative and least likely to experience homoplastic duplication, similarity due to non-phylogenetic factors. Williston concluded that all further studies should emphasize these regions of the skull, countering the work of Goodrich (1916) who emphasized all anatomical features. In any case, a cotylosaur relationship with turtles subsequently became widely accepted in the scientific community, but their exact placement within Anapsida remained controversial (deBraga and Rieppel, 1997).

Question 2. From which reptile group did turtles evolve?

Anapsids as Turtle Ancestors: Pareiasaurs vs. Placodonts

Naming of the group Anapsida and the placement of turtles within it gave many scientists studying testudines an opportunity to propose different anapsids as turtle ancestors. One of the first to do so was Jaekel (1907), who had stressed the anapsid skull structure, which he called stegal, as a defining character of the turtle clade. He used it to place the turtle ancestor in the group Placodontia, within Sauropterygia (Figure 7, p. 11) (from deBraga and Rieppel, 1997). Jaekel (1907) went so far as naming the placodont, *Placochelys placodonta,* as a possible turtle ancestor, by showing similarities between the shell of this species and that of turtles. However, in 1915, Jaekel described *Stegochelys dux* and stated that the small marginal teeth of pareiasaurs, another anapsid group (Figure 3, p. 3), is more similar to the small teeth found in the maxilla and dentaries' of *Stegochelys dux* than the larger teeth of placodonts (from Gregory, 1946). *Stegochelys dux* was later found to be a junior synonym for *Triassochelys dux* (Jaekel, 1918), which even latter was found by Gaffney (1990) to be the junior synonym of *Proganochelys quenstedti* (Baur, 1887) (Figure 3, p. 3).

The view that *Stegochelys* was closer to pareiasaurs than placodonts would later be adopted and expanded upon by Gregory (1946). Gregory was also the first to identify that pareiasaurs and turtles both had an acromion process on the lower end of the anterior edge of the scapular blade (Figure 5, p. 4), a trait that diadectids (Figure 3, p. 3), a previously proposed turtle ancestor, did not possess. Diadectids, within Cotylosauria (Cope, 1880), were hypothesized to be turtle ancestors by Cope (1898) and Case (1905) based on skull features such as the otic notch. This view was sustained by Williston (1917), Hay (1905), and von Huene (1936).

In Gregory's pivotal 1946 paper, he compared many turtle skeletal features to those of the leading hypothesized cotylosaurian clades and to the sauropterygian placodont (Figure 7, p. 11), *Henodus chelyops* (von Huene, 1936), both belonging to Anapsida and believed by past scientists to have led to turtles (Jaekel, 1902; Jaekel, 1915; Broom, 1924). The cotylosaurian clades he used in his study included *Diadectes phaseolinus* (Cope, 1880), pareiasaurs, captorhinomorphs, and seymouriamorphs. The majority of the paper focused on turtle, pareiasaur, and diadectid relationships (Gregory, 1946). He ultimately concluded that turtles most closely resemble pareiasaurs more than any other group of early anapsid reptiles. Gregory (1946) identified several characters that the recently described basal turtle genus, *Triassochelys*, shared with pareiasaurs, including conical protuberances around the rear borders of the skull, shared features on the occiput, the thoracic region (resembling pareiasaurs more than *Diadectes* (Figure 3, p. 3)), location of the columella (small auditory bone), and similar humeri. On the basis of the skull, he concluded that "the turtle skull owes both its basic plan and its peculiarities to descent with modification from the cotylosaurian stock with special affinity to the pareiasaurs" (Gregory, 1946, p. 294). He did acknowledge that *Henodus*, the placodont, possesses a carapace and plastron and has a very turtle-like appearance, however it still retains several nothosaurian characters as well as many significant differences from true turtles. Some of these differences include dorsal supratemporal fenestrae, monimostylic quadrates (united to the skull via suture; Figure 9), a plesiosaur-like pectoral girdle and well-developed gastralia (ventral bones found between the sternum and pelvis in certain reptiles) (von Huene, 1936; 1938).

Shortly after Gregory's paper, Olson (1947) suggested the formation of a new group, named the Parareptilia, comprised of turtles, diadectomorphs, procolophonids, and pareiasaurs (Figure 3, p. 3), based on his reevaluation of the cotylosaurian clade. Up to this point the Cotylosauria clade was defined by the anapsid condition of the skull and the broad neural arches of the vertebrae. Olson proposed that these traits are primitive for reptiles and, therefore, cannot be the sole critieria to define a reptile clade. He placed Seymouriamorpha,

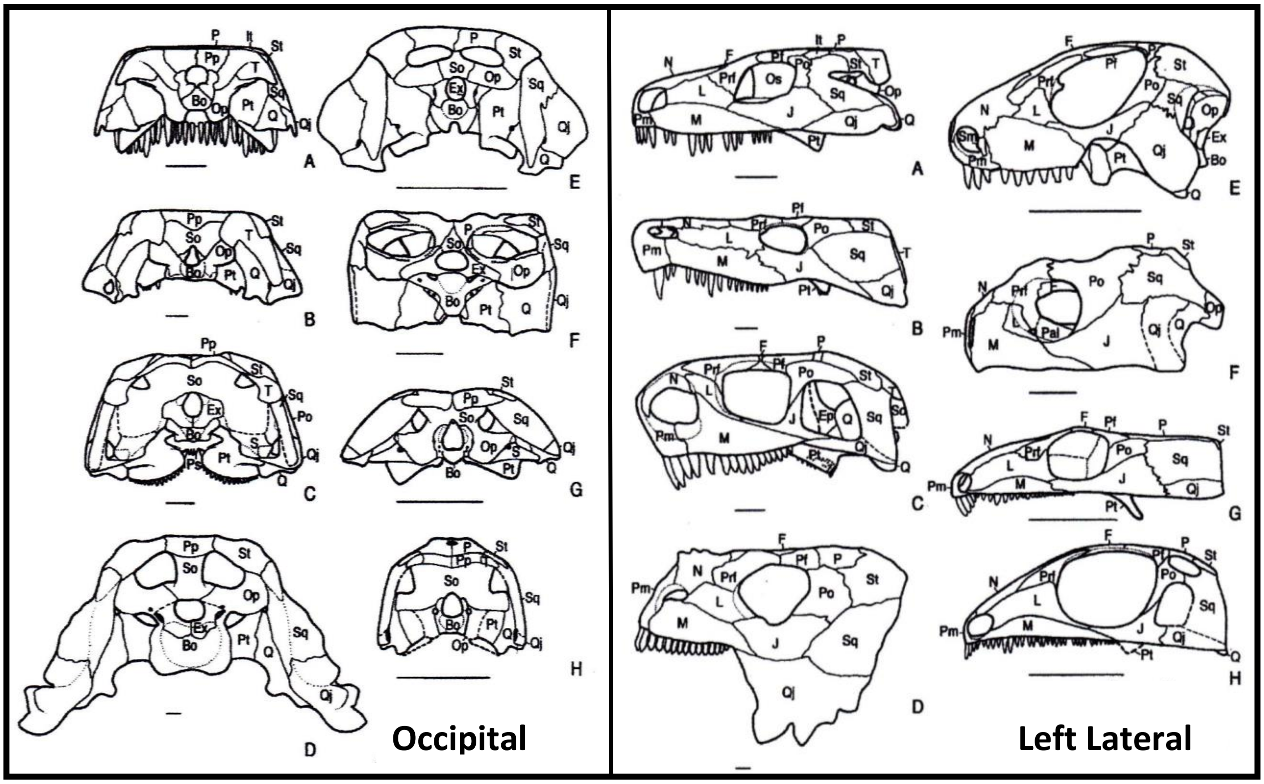


Figure 9. Skulls in occipital and lateral views representing basal reptiles. A. Seymouriamorpha, a stem tetrapod (*Seymouria*) B. Diadectomorpha (*Limnoscelis*) C. Cotylosauria (*Cotylorhynchus*) D. Pareiasauria (*Scutosaurus*) E. Procolophonids (*Procolophon*) F. basal turtles (*Proganochelys*) G. Captorhinids (*Captorhinus*) H. basal diapsids (*Petrolacosaurus*). Skull features: (Pp) postparietal, (P) parietal, (It) intertemporal bone, (St) supratemporal, (T) temporal, (Pf) postfrontal, (Prf) prefrontal, (F) frontal, (N) nasal, (Sm) septomaxilla, (Pm) premaxillary, (Sq) squamosal, (Po) postorbital, (L) lacrimal, (Q) quadrate, (Ep) epipterygoid, (Qj) quadratojugal, (J) jugal, (M) maxilla, (Pt) pterygoid, (S) stapes, (Ps) parasphenoid, (So) supraoccipital, (Op) opisthotic, (Ex) exoccipital, (Bo) basioccipital, (Pal) palatine. Scale bars = 1 cm. Modified from Laurin and Reisz, 1995

Diadectomorpha, Pareiasauria, and Procolophonia in the order Diadecta. This group is then placed with the order Chelonia (Testudines) in the proposed subclass Parareptilia, characterized by an anapsid skull and a strong otic notch. It should be noted that Olson placed turtles originating from the common ancestor to Diadectomorpha based primarily on similar palate (pertaining to the roof of the mouth) structure (shape and disposition). This is contrary to what Gregory (1946) stated, but Olson reported that Gregory used primarily postcranial features, and that they can be misleading. A diadectid relationship with turtles was further supported by Watson (1954) and Romer (1956).

Later, Olson (1965) redefined the Parareptilia as only including the procolophonids and their possible descendants, the pareiasaurs. He also stated that turtles may be derived from either the pareiasaurs or the procolophonids. Even with this partial support for a pareiasaur turtle ancestry, there were still those who considered turtles to be a derived form of a diadectomorph, such as Romer (1964, 1968). In his serious of papers noting the similarities in the palatal structure of the early fossil turtle *Proganochelys* (= *Triassochelys,* = *Stegochelys*) and the diadectid *Nyctiphruretus* (Figure 3, p. 3).

Anapsids as Turtle Ancestors: Captorhinids

Gregory (1946) stated that the skull, jaws and dentition of captorhinomorphs (at the time containing *Labidosauris* and *Captorhinus*) are more primitive than those of Testudines, but do not show convincing evidence to support a relationship to turtles compared to pareiasaurs. The first to resurrect a captorhinid origin of turtles was Clark and Carroll (1973 from Laurin and Reisz, 1995). Their characters uniting turtles and captorhinids (Figure 3, p. 3) are the anapsid skull condition, large post-temporal shallow depressions (fossae) separated by a narrow supraoccipital, and the paroccipital process being braced against the squamosal (Clark and Carroll, 1973 from Laurin and Reisz, 1995) (Figure 10). The latter two characters have since been considered inconclusive (Laurin and Reisz, 1995), and the anapsid skull is a primitive character. The large post-temporal fossae is found in all reptiles, and many other clades have a narrower supraoccipital than captorhinids including pareiasaurs, procolophonids, and

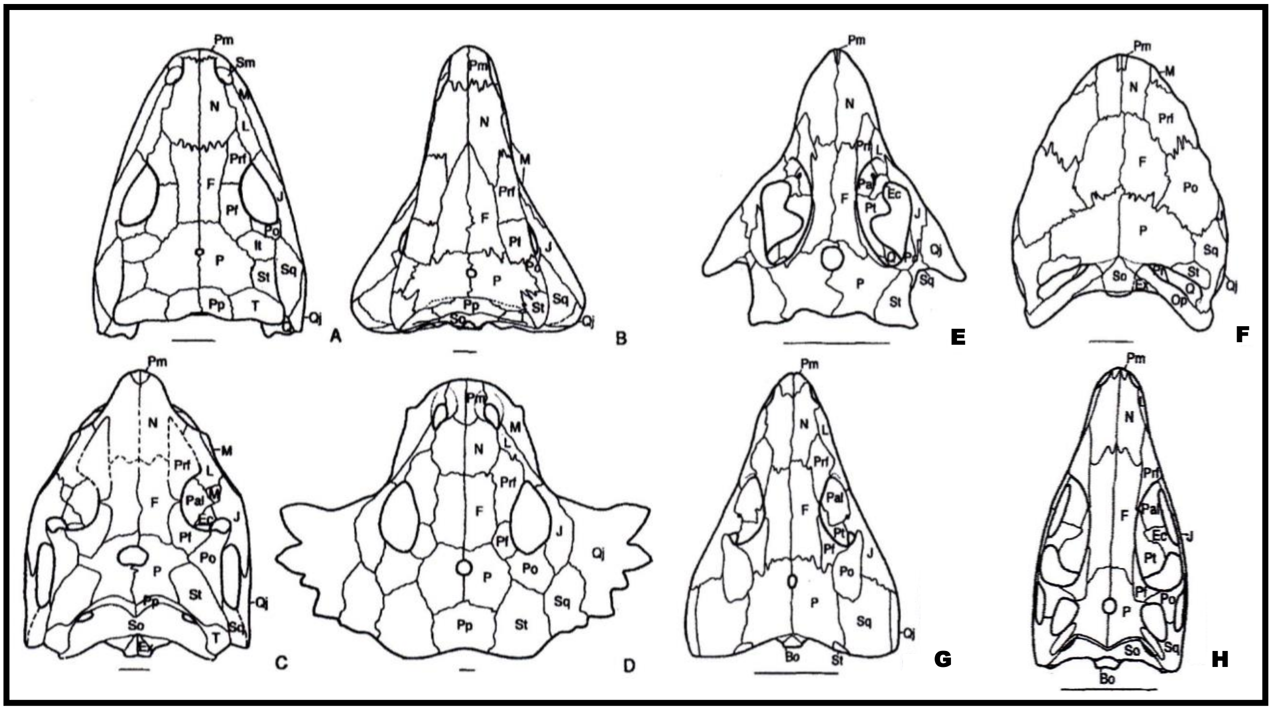


Figure 10. Skulls in dorsal view representing basal reptiles. A. Seymouriamorpha, a stem tetrapod (*Seymouria*) B. Diadectomorpha (*Limnoscelis*) C. Cotylosauria (*Cotylorhynchus*) D. Pareiasauria (*Scutosaurus*) E. Procolophonids (*Procolophon*) F. basal turtles (*Proganochelys*) G. Captorhinids (*Captorhinus*) H. basal diapsids (*Petrolacosaurus*). Skull features: (Pp) postparietal, (P) parietal, (It) intertemporal bone, (St) supratemporal, (T) temporal, (Pf) postfrontal, (Prf) prefrontal, (F) frontal, (N) nasal, (Sm) septomaxilla, (Pm) premaxillary, (Sq) squamosal, (Po) postorbital, (L) lacrimal, (Pr) paroccipital, (Q) quadrate, (Ep) epipterygoid, (Qj) quadratojugal, (J) jugal, (M) maxilla, (Pt) pterygoid, (So) supraoccipital, (Op) opisthotic, (Ex) exoccipital, (Ec) ectopterygoid, (Bo) basioccipital, (Pal) palatine. Scale bars = 1 cm. Modified from Laurin and Reisz, 1995

Testudines (Laurin and Reisz, 1995). Not only is the bracing of the paroccipital to the squamosal fundamentally different in turtles (it's a boney suture) and captorhinids (it's a cartilaginous extension), but the paroccipital process is braced against the squamosal in *Paleothyris*, a basal anapsid reptile, and diapsids. Therefore this type of bracing is not unique to captorhinids and turtles (Laurin and Reisz, 1995).

Even with the noted flaws in Clark and Carroll's (1973) theory, it was nevertheless supported by many workers who followed (Gaffney and McKenna, 1979; Gaffney, 1980; Gaffney and Meeker, 1983; Gaffney and Meylan, 1988; Gauthier *et al.,* 1988; Gaffney, 1990).

Gaffney and McKenna (1979) and Gaffney (1980) proposed two captorhinid hypotheses: (1) Permian family Captorhinidae is a sister taxon to turtles, and (2) Captorhinidae plus turtles are sister clade to all other amniotes (i.e., Diapsida and Synapsida). They drew this comparison based on both Captorhinidae and turtles lacking ectopterygoids and tabulars and both possessing a basipterygoid (basisphenoid) articulation on the skull (Figure 11). It wasn't until Gaffney and Meylan (1988) and Gauthier *et al*. (1988) that the presence of an alary process (wing shaped projection) of the jugal (Figure 10) and an orbitonasal foramen (opening between the orbit and nasal; Figure 10), as well as the features discussed above, were considered synapomorphies (shared derived characters) for the clade including turtles and captorhinids. It should be noted that at this time phylogenetic tree analysis software was starting to become more accessible and utilized in the scientific community, especially in the field of cladistics. Gauthier *et al.* (1988) was the first to use this software to publish the first large-scale cladistic

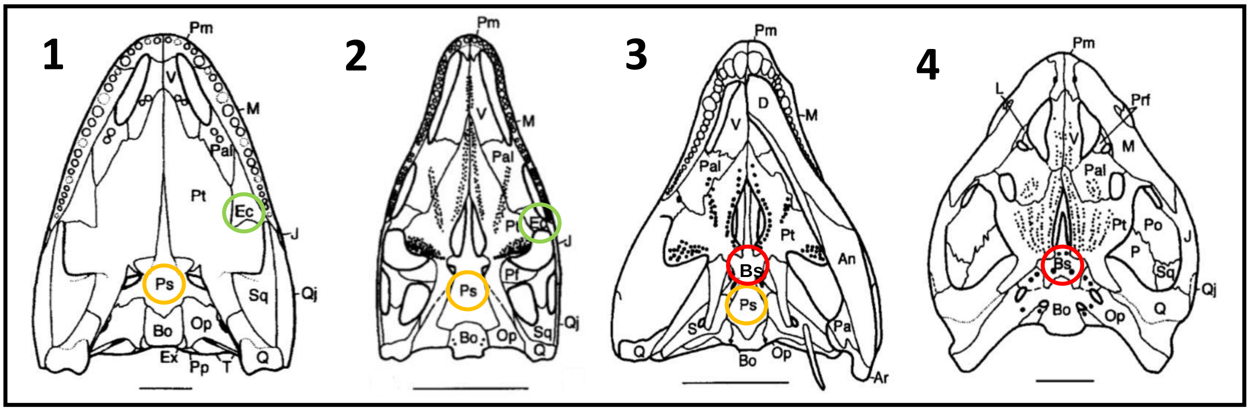


Figure 11. Generalized palates of basal reptiles. 1. A basal tetrapod (*Seymouria*), 2. A basal diapsid (*Petrolacosaurus*), and 3. A Captorhinid (*Captorhinus*), and 4. A basal turtle (*Proganochelys*). Skull features: (Pm) premaxillary, (M) maxilla, (D) dentary, (An) angular, (Ar) articular, (Pa) prearticular, (V) vomer, (L) lacrimal, (Prf) prefrontal, (Pal) palatine, (Pt) pterygoid, **(Ec)** ectopterygoid (green), (J) jugal, (Po) postorbital, (P) parietal, (Sq) squamosal, (Qj) quadratojugal, (Q) quadrate, (Op) opisthotic, (T) temporal, (Pp) postparietal, (Ex) exoccipital, (Bo) basioccipital, **(Ps)** parasphenoid (orange), **(Bs)** basisphenoid (red), (S) stapes, (Q) quadrate. Scale Bars = 1 cm. Modified from Laurin and Reisz, 1995

analysis of early amniotes using a large data matrix that was then analyzed by the software, specifically Swofford's (1984) PAUP (Phylogenetic Analysis Using Parsimony) (Figure 6, p. 8). This software and many like it were used by many of the scientists in the 1990s to either support their position of turtle relationships or counter those who didn't support their ideas. Two other important results from Gauthier *et al.* (1988) study was the exclusion of diadectomorphs from Amniota, supporting its sister relationship to Amniota, and the reconfiguration of the clade Parareptilia (Olson, 1947) to include mesosaurs (anapsid aquatic reptile), millerosaurs (also known as milleretids), pareiasaurs and procolophonoids (Figure 6, p. 8). However, Laurin and Reisz (1995) stated that due to the large nature of the database compiled by Gauthier *et al.* (1988), it required them to rely heavily on outdated and inadequate descriptions of taxa. Gauthier *et al*. (1988) admitted that they had little faith in their parareptile clade, but the use of a cladogram and the publication of the data matrix allowed others to evaluate and reach their own conclusions on the study. In Gaffney's (1990) description of the basal turtle *Proganochelys quenstedti*, he used captorhinids as an accepted outgroup for turtles (Figure 3, p. 3).

Anapsids or Diapsids as Turtle Ancestors: Procolophonids vs. Pareiasaurs vs. Lepidosaurs

Reisz and Laurin (1991). Largely in a response to Gauthier *et al.* (1988), Reisz and Laurin (1991) proposed a new theory, placing turtles within the parareptiles, with procolophonoids as their closest relative. This study utilized a description of a basal procolophonian, *Owenetta*, to find many synapomorphies with turtles and procolophonoids. These synapomorphies include (Figures 9-13):

1) cultriform (blade-like) process of the parasphenoid greatly reduced in length (Figure 11)
2) loss of teeth on transverse flange of pterygoid
3) a distinctly shaped anterodorsal expansion of the maxilla (Figure 11)
4) the prefrontal and palatine are massively buttressed against each other
5) dorsal process of the quadrate is exposed laterally, the edge of the well-developed tympanic (relating to the tympanum) notch is formed by the squamosal and the enlarged quadratojugal
6) the slender stapes (middle ear bone) has lost the dorsal process and foramen
7) the anterior edge of the splenial is not present between the angular and suprangular (Figure 12)
8) dorsal surface of retroarticular process (formed by articulate, angular and prearticular) is broad and concave (Figure 12)
9) the post-parietal is greatly reduced or lost
10) the entepicondylar foramen of the humerus is lost (Figure 13)

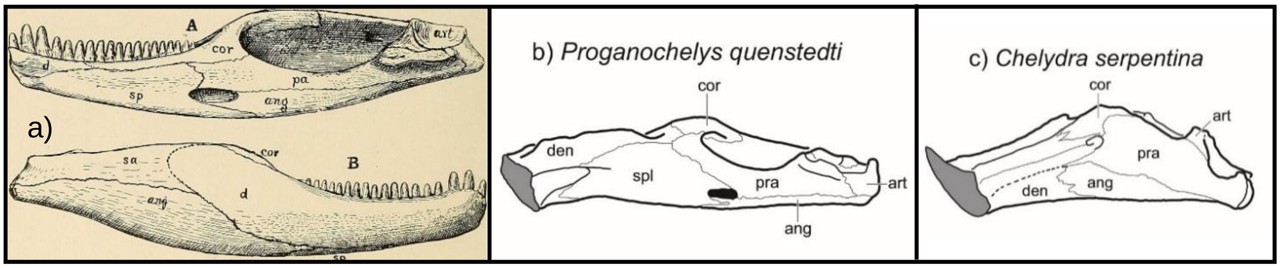


Figure 12. Representative mandibles. a) Inner and outer mandible of *Labidosaurus hamatus*, showing features of primitive reptile mandible. b) Inner mandible of basal turtle, *Proganochelys quenstedti* (redrawn from Gaffney, 1990). c) Inner mandible of the common snapping turtle (redrawn from Gaffney, 1972). Abreviations: (d/den) dentary, (cor) coronoid, (art) articulate, (sp/spl) splenial, (pa/pra) prearticular, (ang) angular, and (sa) suprangular. Modified from Williston, 1925; Joyce, 2007

The most significant shared traits are the presence of a large post-temporal fenestrae and ventral emargination of the cheek, which is seen in some turtles but not in primitive anapsids or synapsids. The loss of the entepicondylar foramen is the only post-cranial synapomorphy (Figure 13).

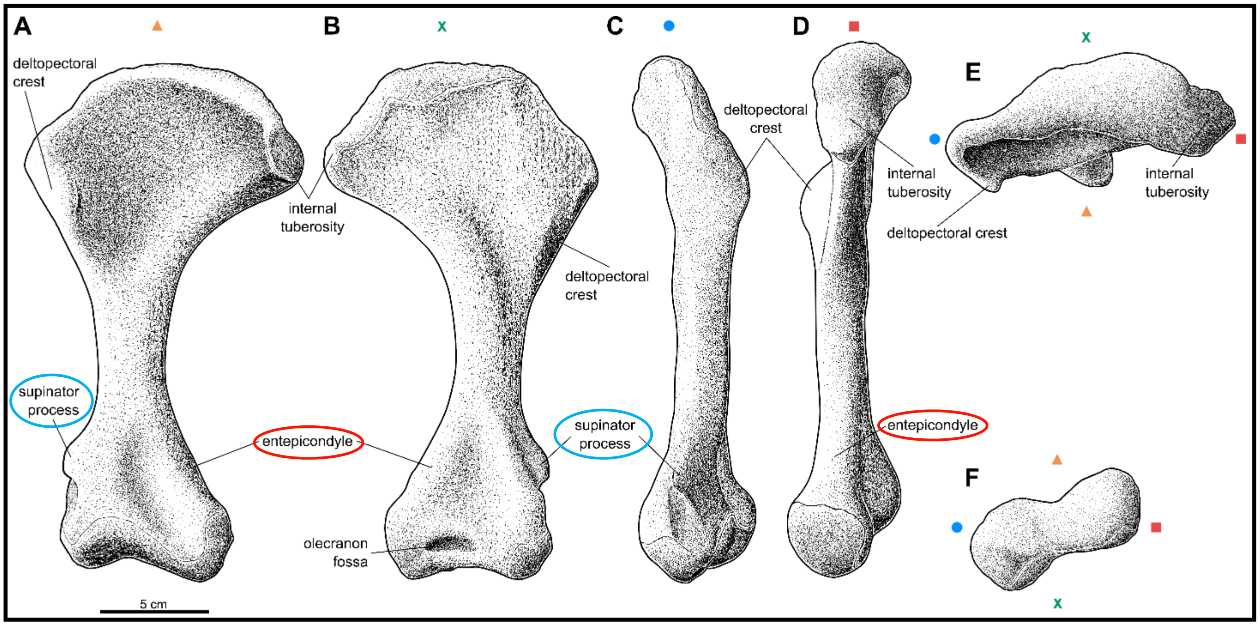


Figure 13. Reconstruction of the right humerus. It is of the Late Triassic archosaur, *Stagonolepis olenkae* in (A) Ventral view. (B) Dorsal view. (C) Lateral view. (D) Medial view. (E) Proximal view. (F) Distal view. Special interest in entepicondyle foramen and supinator process. Symbols attached to pictures show which surface is exposed in the drawing, with (X) for the dorsal, (▲) for the ventral, (■) for the medial, and (●) for the lateral, and how the surfaces are oriented in proximal and distal view. Modified from Dróżdż, 2018

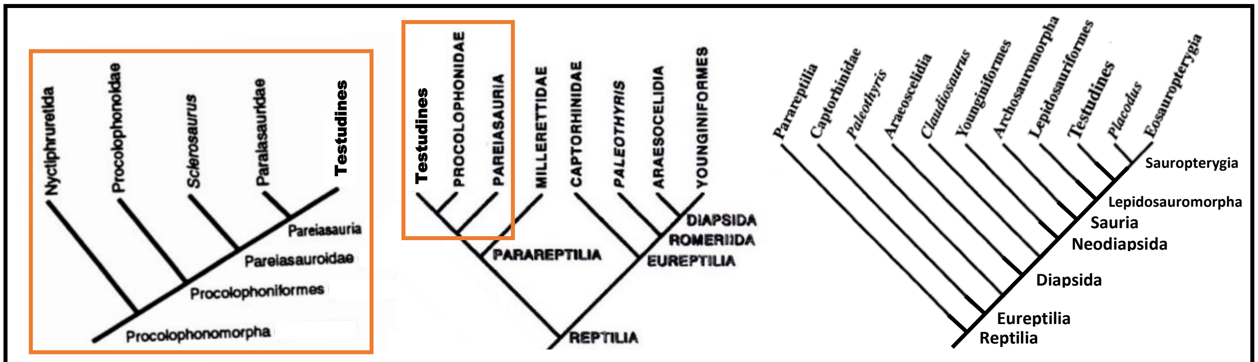
In total there are ten synapomorphies that support a procolophonoid relationship with turtles, whereas the captorhinid-turtle hypothesis was supported by four synapomorphies, two of which (absence of the tabular and the presence of the orbito-nasal foramen) are found in other reptiles, such as procolophonids (Reisz and Laurin, 1991). A procolophonid origin of turtles also shortens the time gap of the clade's origin to the late Permian. In the early 1990s, the earliest turtle was *Proganochelys quenstedti* (Baur, 1887), which was dated to the Late Triassic, around 210 million years ago (Figure 3, p. 3). If a captorhinid origin is to be believed, then turtles must have arisen at least as early as the early Permian and possibly the late Carboniferous. This implies an unusually long gap in the fossil record of more than 100 million years (Reisz and Laurin, 1991). One last thing a procolophonid origin of turtles would result in, and perhaps the most significant, is a new diagnosis of the turtle clade, because a rod-like imperforate stapes and the distinctive morphology of the quadrate-squamosal-quadratojugal complex have been used to diagnose turtles (Gaffney and Meylan, 1988), but these features are also present in procolophonids (Reisz and Laurin, 1991).

Michael Lee (1993a). Counter to Reisz and Laurin (1991), Lee proposed a relationship between turtles and Pareisauridae using sixteen characters. He also found nine other features that link the primitive reptile *Sclerosaurus*¸ which some believed was an early procolophonid (von Huene, 1902), to turtles and pareiasaurs. Several of these characters were initially suggested by Gregory (1946) when he attempted to connect pareiasaurs and turtles (as presented in Laurin and Reisz, 1995). Lee (1993a) acknowledged the work by Reisz and Laurin (1991), especially where it came to their counter argument to the captorhinid hypothesis. They stated that of the five synapomorphies proposed to unite turtles and Captorhinidae (Gaffney and Meylan, 1988), three have been found in many other primitive amniotes including pareiasaurs (Reisz and Laurin, 1991). The three are the absence of the tabular bone, the absence of the supinator process (groove on the end of the humerus, Figure 13), and the presence of the foramen orbitonasal. That being said, Lee ended up dismissing most of the characters used by them to support a procolophonid origin. He stated that of the ten synapomorphies that support a procolophonid ancestry, only one is valid, that being the otic ("tympanic" in Reisz and Laurin, 1991) notch bordered by an enlarged quadratojugal (1993a).

He did state that this condition is approached in some millerosaurs (Lee, 1993a citing Gow, 1972).

Lee (1993a) stated that the only postcranial character, the loss of the entepicondylar foramen, is not the general condition in procolophonoids, and some pareiasaurs also lack the foramen (Lee, 1993a citing Colbert and Kitching, 1975; See Figure 13). Lee noted that the remaining traits used to connect turtles to procolophonoids are seen in many primitive amniotes and reptiliomorph amphibians. He also expressed that the development of the unique turtle Bauplan (body plan) included the gradual accumulation of stages in a precise order as evidenced by a large number of traits that characterize the turtle clade appear during embryological development both before and after the shell (1993a). He did this by comparing the known traits that define recent Testudines to the traits of the earliest known turtle at the time, *Proganochelys,* and found that some of the traits are present in *Proganochelys* and some are not. Lee claimed that previously it was thought that the features that define the turtle clade evolved all at the same time and represented a huge evolutionary leap.

Lee (1993a) concluded that turtles and Pareiasauridae have a common ancestor and the sister group would include Procolophonoidae, Nyctiphruretida and *Sclerosaurus* (Figure 14). Pareisauria and *Proganochelys* share frontals excluded from the orbital margin, bosses above orbits and nares, the thick braincase floor, the pleurosphenoid ossification, a prootic foramen enclosed by bone, the anterodorsally directed dorsum sellae, the loss of the prootic-opisthotic suture, and the dorsal lump at the apex of the retroarticular process. Lee used this hypothesized connection between turtles and pareiasaurs to make conclusions about the development of the shell. He concluded that because both groups have heavy dermal armor, this trait must be homologous, even though dermal armor has evolved independently many times in reptiles (Romer, 1956).



1. Pareiasaur Relationship (Modified from Lee, 1993a)

2. Procolophonid Relationship (Laurin and Reisz, 1995)

3. Lepidosaur Relationship (Modified from Rieppel and deBraga, 1996)

Figure 14. Representative cladograms from the major views during the 1990s. Orange boxes represents clade containing turtles, procolophons, and pareiasaurs.

Lee (1993a) took it one step further and stated that because pareiasaurs demonstrate a fusion of dermal ossification with unmodified neural arches and ribs (Owen, 1849) that this means that the turtle shell arose in a similar fashion (Figure 15). He also concluded that a pareiasaur origin supports a less accepted hypothesis (Watson, 1914; Parsons and Williams,

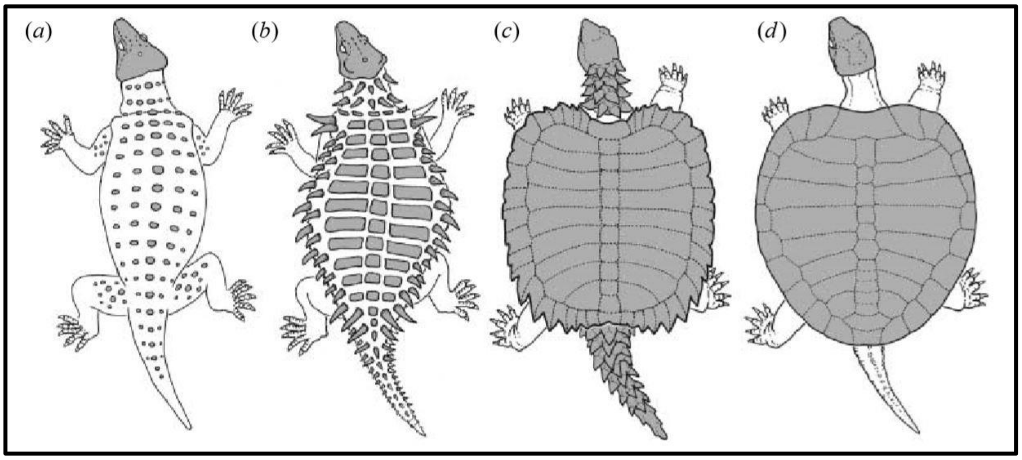


Figure 15. Proposed evolutionary scenario. Reconstruction of two hypothetical turtle ancestors that exhibit (*a*) initial and (*b*) enlarged amounts of isolated, osteoderms that are organized in rows, (c) the well-preserved Late Triassic turtle, *Proganochelys quenstedti*, and (*d*) modern turtle shell, as first seen in the Early Jurassic turtle *Kayentachelys aprix* showing the fusion of dermal ossifications leading to complete turtle shell as proposed by Lee (1993a, 1996, 1997). All dermal armor is highlighted in grey. From Joyce *et al*., 2009

1961) on how the scapula ended up within the rib cage in turtles. Watson (1914) suggested that the flat carapace composed of laterally flaring ribs first evolved behind the scapulocoracoid (Figure 5, p. 4), and then the scapulocoracoid migrated posteriorly. Lee (1993a) stated that in pareiasaurs, the shoulder girdle is much more narrow than the rib cage because the clavicles and coracoids do not form wide ventral plates, which therefore show a similar morphology as predicted by Watson (1914), that of a narrow shoulder girdle anterior to a wide, flat carapace. Lee also stated that the vertebral count also supports Watson's hypothesis (1914), with procolophonoids having five cervical and 20 dorsal vertebrae (Colbert and Kitching, 1975), *Sclerosaurus* and pareiasaurs having five cervicals and 14 or 15 dorsals, and all turtles having eight cervicals and 10 dorsals (1993a). Lee concluded that the most parsimonious evolutionary scenario is that five or six dorsals were lost in the lineage leading to *Sclerosaurus*, pareiasaurs, and turtles, and that a further modification increased the number of cervicals. Lee hypothesized that cervicals number six to eight in turtles are modified dorsals.

Laurin and Reisz (1995). Laurin and Reisz published a comprehensive paper in 1995 supporting the Parareptilia group. This group was composed of pareiasaurs and a clade they named Testudinomorphs, made of the last common ancestor of procolophonids and testudines and all its descendants. Testudinomorphs was supported by 17 synapomorphies and was the most well supported clade of the analysis. They created a cladogram based on 13 taxa, including outgroups, and 124 characters. The characters came from Gauthier *et al.,* (1988), Lee (1993a), Berman *et al.* (1992) and characters of their own choosing (Figure 14). They also defined Reptilia as the most recent common ancestor of diapsids and all its descendants.

Laurin and Reisz (1995) correlated their tree stratigraphically, showing that procolophonids originated in the Early Triassic and turtles in the Middle Triassic, which would be a closer span of time than for an origin from pareiasaurs in the Lower Permian (1995) (Figure 14, p. 26). They also disputed Lee's (1993a) pareiasaur hypothesis using the phalanges count on the hind foot, which Lee believed pareiasaurs shared with turtles. Laurin and Reisz (1995) showed that the turtle phalanges formula is 2 3 3 **3** 3 (originally stated by Osborn, 1903) and not 2 3 3 **4** 3 as seen in pareiasaurs. They also pointed out that the phalanges of pareiasaurs are shaped like a disc, suggesting modifications due to a heavy body mass, whereas the phalanges of testudines are moderately long, possibly for digging or swimming (1995). They also showed that some of the characters used by Lee (1993a) may not be homologous between turtles and pareiasaurs, namely the attachment of the chevrons to the anterior caudal centra and the presence of the acromion process. Some other characters (a massive horizontal paroccipital process sutured to the squamosal and supratemporal, a basisphenoid-basioccipital suture, a fully ossified medial wall of the prootic) were cited by Laurin and Reisz (1995) as being problematic due to Lee (1993a) not showing the correct distribution in amniotes and their close relatives. Laurin and Reisz (1995) also explained that a host of characters Lee (1993a) used to unite pareiasaurs and *Sclerosaurus* to turtles are also procolophonid synapomorphies, including thick dermal armor, a greater trochanter and a tall, narrow scapular blade. Laurin and Reisz (1995) also noted that the presence of an ectepicondylar foramen (relating to the groove on the condyle located at the distal end of the humerus) appears to be primitive for parareptiles because the foramen is present in all parareptiles (i.e. millerettids, mesosaurs, pareiasaurs and testudines). Other concerns Laurin and Reisz had of Lee's conclusions included his interpretation that the plastron may represent modified gastralia (ventral bones) or tissues giving rise to gastralia and not simply the loss of gastralia. With these observations and a plethora of other minor concerns about the characters used be Lee (1993a), Laurin and Reisz (1995) countered Lee's hypothesis.

Michael Lee (1996). Lee responded to Laurin and Reisz's (1995) assertions by publishing a preliminary paper in 1996 followed by another paper in 1997. In the first paper, he reiterated that a procolophonid origin to turtles represents a sudden evolutionary change or saltation, while a pareiasaur origin represents evolution in a correlated progression, meaning evolutionary changes in one feature progresses the evolution of another feature. Lee stated that through this method turtles developed their shell. In the 1996 study, Lee included each pareiasaur taxon as its own unit, and did not consolidate them into one taxon as in Lee (1993a) and Laurin and Reisz (1995). He included all characters he considered valid from his previous analyses as well as new informative characters that were primarily postcranial in nature. The full analysis wasn't published until 1997. However, he did give some of the results in the earlier paper. He again stated that pareiasaurs were the nearest relative to turtles. Pareiasaurs that were close to turtles had cranial bosses, and the closest pareiasaurs to turtles were the dwarf forms, *Anthodon serrarius* and *Nanoparia pricei*.

Rieppel and deBraga (1996). Before Lee could publish his 1997 study, Rieppel and deBraga (1996) published a large-scale cladistic analysis using a broad range of extinct (especially Mesozoic) and extant taxa. Their study concluded that turtles have a closer relationship to diapsids than parareptiles. They state that previous cladistic analyses have only used Paleozoic taxa with *Petrolacosaurus* (Order: Aracoscelidia) being the only stem group diapsid included (Lee, 1993a; Laurin and Reisz, 1995; Gaffney 1990). These previous studies used the predetermined assumption that turtles are truly anapsid derived from an early Paleozoic anapsid due to their skull type. Because of this assumption, the authors produced their cladograms using primarily anapsids or proposed anapsid ancestors. Rieppel and deBraga (1996) used 33 taxa and 168 osteological characters to produce two equally parsimonious trees, both showing a sister relationship between turtles and Sauropterygia, with these two clades being sister to Lepidosauriformes within Sauria (a group that contains Lepidosauriformes and Archosauromorpha and all their descendants) (Figure 14, p. 26). These two cladograms have fewer steps than others that have been presented, but they are lower in terms of consistency index, retention index, and bootstrapping support. Rieppel and deBraga stated that these three factors indicate a higher degree of homoplasy, but this is an expected result due to the large number of characters and taxa.

After compiling characters comprising all modern day diapsids (lizards, snakes, crocs, and birds), Rieppel and deBraga (1996) indicated that the most significant characters that turtles and Lepidosauriformes share are several developmental features including the early ossification of postorbital (temporal) bones in the skull and, during the early stages of ossification, the jugal shows a crescent shape without a posterior process. Other important developmental features include the lack of a true radiale in the carpus, the pattern of early ossification of the hooked fifth metatarsal, and the development of a single proximal tarsal cartilage (a character uniquely derived among Amniota (Rieppel, 1993b) in Rieppel and deBraga, 1996).

Michael Lee (1997). Lee’s 1997 study presented very little in terms of new information on his pareiasaur hypothesis. He used a small portion of the paper’s text countering Rieppel and deBraga's 1996 paper. Lee (1997) stated that several of their pareiasaur characters were, in his opinion, incorrectly coded. However, he did not explicitly state what those characters were and how they were incorrectly coded. Lee wrote that once he corrected the characters and re-ran the analysis, he found that the results showed a pareiasaur relationship with turtles. However, the data and analysis specifics was never published. In fact, in the same issue of the same journal, deBraga and Rieppel (1997) published a more in depth account of their 1996 paper, including summaries of past anapsid and diapsid hypotheses and detailed taxonomic descriptions. One such taxonomic description was that of Testudines being defined by being the most recent common ancestor of the basal turtles, *Proganochelys, Australochelys,* and Casichelydia and all their descendants in 1997 by deBraga and Rieppel. They included in the discussion section of this 1997 paper several developmental characters that they had not previously discussed. For example, turtles have a truly unique order of carpal ossification in which the most distal elements of the carpus ossify prior to the most proximal elements. This order is not shown in either archosaurs or lepidosaurs. However, some of the developmental characters used by deBraga and Rieppel (1997) to support a turtle archosaur relationship include a semi-lunate jugal, the development of the interclavicle and clavicles, the development of the tarsale proximale (a cartilaginous precursor that ossifies into the astragalus and calcaneum). DeBraga and Rieppel also noted that the presence of a hooked fifth metatarsal is found in archosaurs, lepidosaurs, and turtles, but in turtles and lepidosaurs ossification starts where the hooked portion of the fifth metatarsal articulates with the lateral side of the proximal head of the fourth metatarsal. DeBraga and Rieppel (1997) counted the number of steps it took to get from the derived section of the clade down to the most recent common ancestor of Lee's Procolophoniformes (1995) clade and Laurin and Reisz's Procolophonia (1995) clade (the hypothesized clades that turtles lie within). They then used this number over the total number of steps required to resolve the respected trees in total to get a ratio clade steps to entire cladogram steps. Laurin and Reisz (1995) required 32 steps out of 323 total steps for a ratio of 9.9%, and Lee (1995) required 18 steps out of a total of 80 for a ratio of 22.5%. According to deBraga and Rieppel, these ratios showed that Lee's cladogram and character selection are more heavily biased toward those characters that will help resolve the relationships in his cladogram. They also state that this view is supported by the high confidence limits for Lee's data set (0.80 CI) compared to Laurin and Reisz's (0.669 CI). DeBraga and Rieppel (1997) also expressed that the geologic time gap between earliest known turtles and earliest known Sauropterygia (~20 mya) is the smallest amount of time between any of the relationships previously proposed.

Rieppel and Reisz (1999). It was not until Rieppel and Reisz (1999) that an attempt was made to combine the methods of Rieppel and deBraga (1996) and Lee (1996, 1997). They modified Rieppel and deBraga's (1996) dataset in accordance with recent criticisms (Lee, 1997) and reanalyzed the data using PAUP 3.1. and McClade version 3. They did not take all of Lee's recommendations and implemented only valid characters that appeared relevant to a more extensive, global analysis. They did not add any of new characters proposed by deBraga and Rieppel (1997). They found that turtles were still sister to Placodontia/Eosauropterygia (stem group of Sauropterygia) and nested within lepidosaurs similar to the tree of Rieppel and deBraga (1996) (Figure 14, p. 26). Also similar to Rieppel and deBraga (1996), their trees had a high homoplasy index, suggesting wide spread convergence. It should be noted that when they removed Sauropterygia from their analysis, turtles were placed next to pareiasaurs, but the additions of ichthyosaurs placed turtles next to parareptiles once again. They undertook a rigorous evaluation of primary homology for four characters that have played a key part in the discussion of turtle origins: dermal armor (the carapace), the acromion process of the scapula, the astragalus-calcaneum complex, and the hooked fifth metatarsal (Figure 16). They concluded that the turtle carapace is morphologically unique and morphogenetically distinct from other amniotes (i.e., it is autapomorphic for turtles). The acromion process (Figure 5, p. 4) is shorter in *Proganochelys* than in other turtles and has a unique location on the scapula which may indicate that it evolved within the clade. The astragalus-calcaneum complex lacks a foramen for the perforating artery that passes between the astragalus and calcaneum, a trait seen in

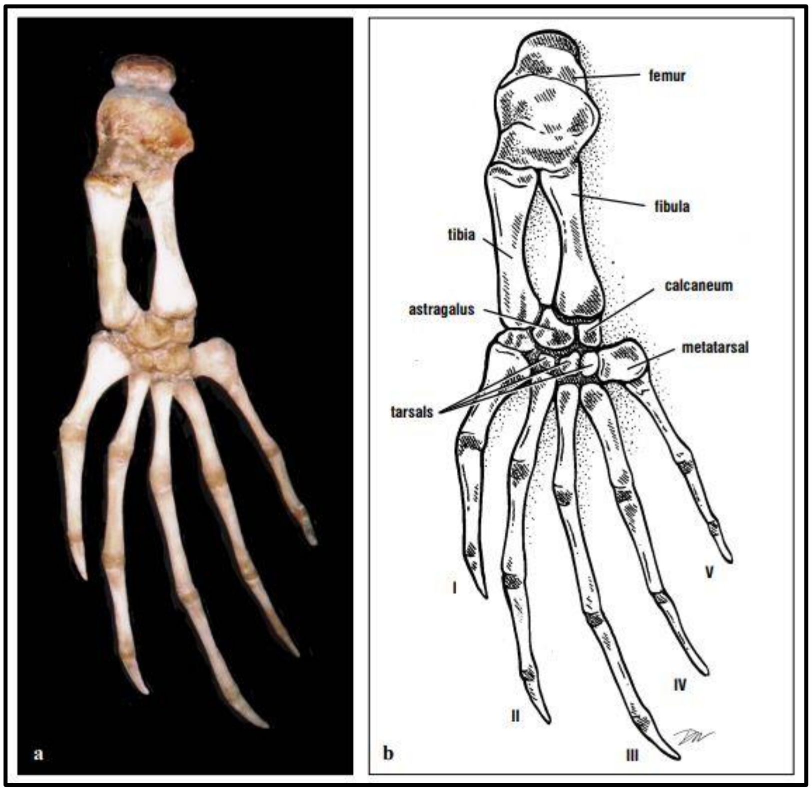


Figure 16. Dorsal view of the hind limb of a leatherback sea turtle. Digits labeled I-V. The astragalus and calcaneum have been fused in most extant non-marine turtles (known as the astragalus-calcaneum complex). The hooked element, labeled here as a metatarsal, has been identified by some to be a distal tarsal 5 (Sheil and Portik, 2008; Fabrezi *et al*., 2009). Image modified from Wyneken, 2001 with illustration by Dawn Witherington

lepidosaurs and not pareiasaurs. The hooked fifth metatarsal as well as a mesotarsal joint are found in turtles and squamates suggesting that these features were inherited by turtles form their common ancestor with lepidosaurs.

***Odontochelys*.** The discovery of a new basal turtle, *Odontochelys semitestacea* (Li *et al.,* 2008)*,* that had only a plastron and no osteoderms on its dorsal side of the body, was a key component of future debate (Figure 17). If *Odontochelys* is believed to be an ancestral turtle, then the absence of dorsal osteoderms indicates that the carapace did not evolve through the fusion of ancestrally present osteoderms (Li *et al.,* 2008) as stated by Lee (1996, 1997). This discovery led to a study done by Scheyer and Sander in 2009. This study compared the armor plates of three common Permian pareiasaurs (*Bradysaurus, Pareiasaurys,* and *Athodon*) to that of the osteoderms from a total of nine extinct and extant taxa including lepidosaurs (*Pseudopus apodus, Tiliqua scincoides*), turtles (*Hesperotestudo*), crocodylomorphs (*Steneosaurus, Alligator mississippiensis, Diplocyndon*) and ankylosaurs (*Ankylosauridae, Nodosauridae*). The histology of the osteoderms collected in this paper were then compared to the histology of the turtle shell and osteoderms described in a previous paper (Scheyer and Sander, 2007). In this earlier paper Scheyer and Sander tested extant terrestrial turtles *(Geochelone pardalis, Terrapene carolina, Cuora picturata*), extant aquatic turtles (*Chelydra serpentine, Chelus fimbriatus, Caretta caretta*), and fossil turtles (*Proganochelys quenstedti and Proterochersis robusta*). *Proterochersis*, described by Fraas in 1913, is as primitive as or more primitive than *Proganochelys*, and possesses a complete shell (carapace and plastron). They concluded that *Proganochelys* and *Proterochersis* share a shell bone histology with the terrestrial turtles that they tested. Based on this conclusion, Scheyer and Sander (2009) used the carapace osteoderms of the extant giant tortoise, *Hesperotestudo,* as a modern analogue for the basal turtle shell. They used a computer program named Bone Profiler to determine bone compactness and overall vascularization. They discovered that all three of the pareiasaurs have a very similar bone histology to one another, and thus shared a common mode of skeletogenesis.

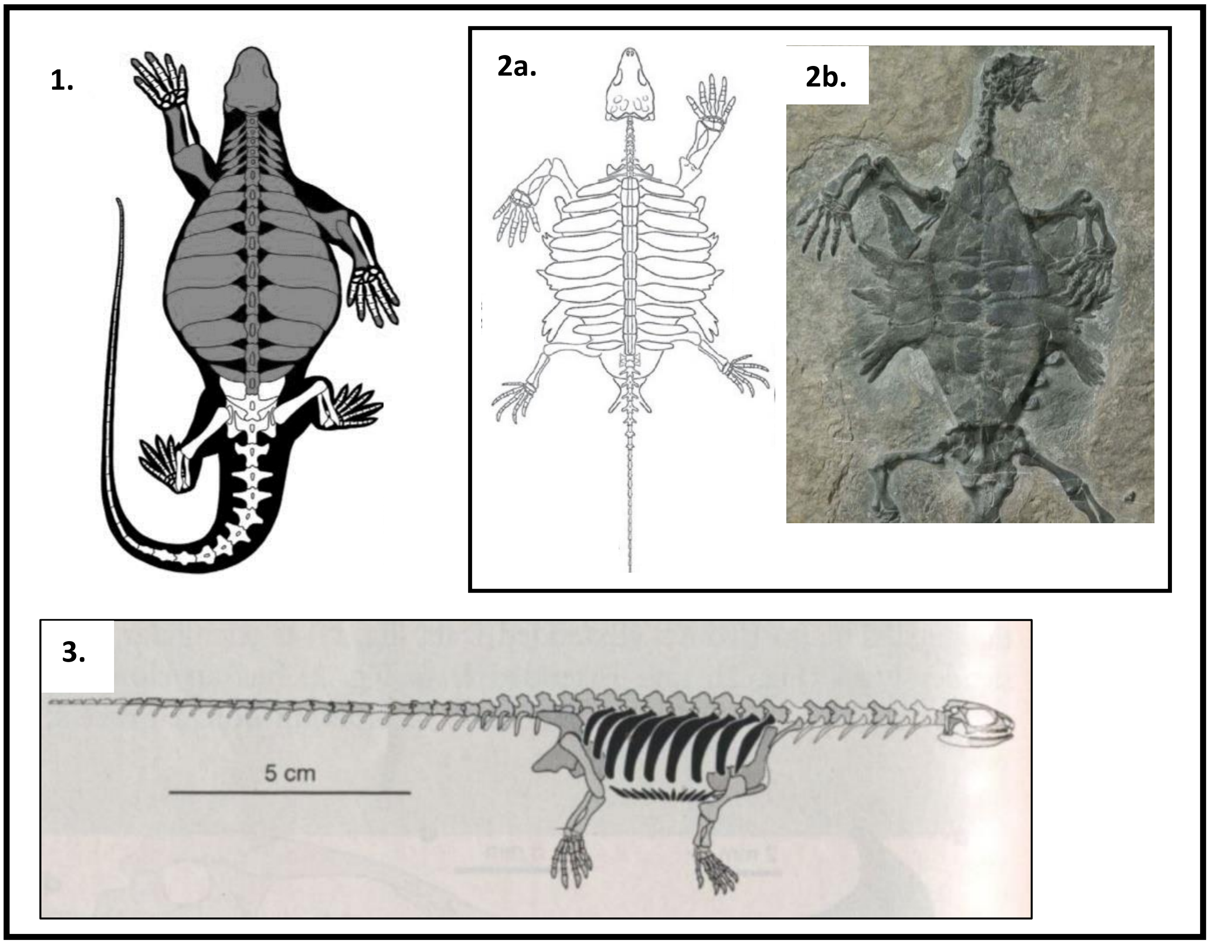


Figure 17. Skeletal reconstructions of proposed turtle ancestors. 1. *Eunotosaurus* in dorsal view. Gray represents discovered elements (From Lyson *et al*., 2016). 2. a) *Odontochelys* in dorsal view (Lyson *et al.,* 2010). b) Specimen (IVPPV-13240) in ventral view showing fully formed plastron (Modified from Li *et al*., 2008). 3. *Pappochelys* in lateral view. White elements have not been found. Preserved bones in gray. Trunk ribs and gastralia highlighted in black (from Schoch and Sues, 2015). Images at different scales.

Scheyer and Sander (2009) concluded that there are few characters that pareiasaur osteoderms (e.g., the presence of parallel-fibered bone tissue) share with the microstructure and anatomical details of turtle shell bones and turtle osteoderms (2009). Turtle shell bones do not show the pareiasaur features of ornamental bosses, radial ridges, radial growth, and vascularization patterns. Furthermore, pareiasaur osteoderms do not have turtle features, such as diploe structures (the spongy substance between bone plates), scute sulci, and a clear distinction between internal and external cortical (outer most layer) bone tissue. All of the given turtle characters are possible histology-based synapomorphies for turtle shell bones (Scheyer, 2007). Using Bone Profiler, Scheyer and Sander determined that pareiasaur osteoderms generally have a lower compactness than all other amniote osteoderms.

Diapsids as Turtle Ancestors: Lepidosaurs vs. Archosaurs

Rieppel and deBraga were not the first to propose a diapsid origin of Testudines. DeBeer (1937), Hofsten (1941), Lovtrup (1985), Ax (1984), and Gardiner (1993) all proposed a diapsid origin, specifically an archosaur relationship to turtles. All of these scientists used extant taxa to define their (neontological) characters. DeBeer (1937) used the parachordal developmental of the occipital condyle in turtles, crocodiles, and birds as opposed to the hypochordal development in squamates and an embryonic connection of the columella auris (a bony or cartilaginous rod connecting the tympanic membrane with the inner ear) with the posterior part of the Meckel’s cartilage (this was also used by Lovtrup, 1985) to show an archosaur relationship. Hofsten (1941) used the presence of a secondary subclavian artery to show this relationship (from Rieppel, 1999). Lovtrup (1985) listed an epipterygoid joining the parietal, thyroid, and cricoid cartilages, the absence of a cartilaginous disc embedded in the lower eyelid, the attachment of the tendon from the nictitating membrane to a pyramidalis muscle, the presence of a ciliary processes in the eye (also used by Gardiner, 1993), the presence of a nonlobed thymus, a sinus cavernosus (a network of veins in the head), a retroperitoneal position of the adrenal (glands on the kidneys), and vascular compact bone as synapomorphies of turtles and crocodiles. Ax (1984) stated that the reduction (as in crocodiles and birds) or the complete loss (as in turtles) of the Jacobson's organ unites these clades (from Rieppel, 1999). Hofsten (1941), Lovtrup (1985), and Gardiner (1993) all used a single penis with erectile tissue as a character uniting turtles and crocodiles (from Rieppel, 1999). Hofsten (1941), Ax (1984), and Lovtrup (1985) used the secondary subclavian artery as another character (from Rieppel, 1999). Gardiner (1993) also used the presence of pore canals in the egg membrane as a special similarity between turtle and archosaur eggs (from Rieppel, 1999).

Olivier Rieppel (1999) reviewed these neontological characters which supposedly gave support to an archosaur turtle relationship and found that most, but not all, were invalid or show weak support. He found that the only valid character with strong support was the differentiation of a pyramidalis muscle for the tendon of the nictitating membrane (Lovtrup, 1985; Gardiner, 1993; Gauthier *et al*., 1988). These neontological characters will not be discussed further in this thesis. Several molecular studies supported the idea of a turtle-archosaur clade (Hedges and Poling, 1999; Kumazawa and Nishida, 1999; Chiari *et al*., 2012), but also studies that supported a lepidosaur-turtle clade (Hedges *et al*., 1990; Lyson *et al*., 2011). This topic will be discussed in Question 3.

In 1990, Gaffney re-described *Proganochelys quenstedti* from a near complete specimen. He identified a pair of dorsoventrally tall, flat ossifications that articulate with the prootic and basisphenoid on each side of the anterior region of the braincase (Figure 18).

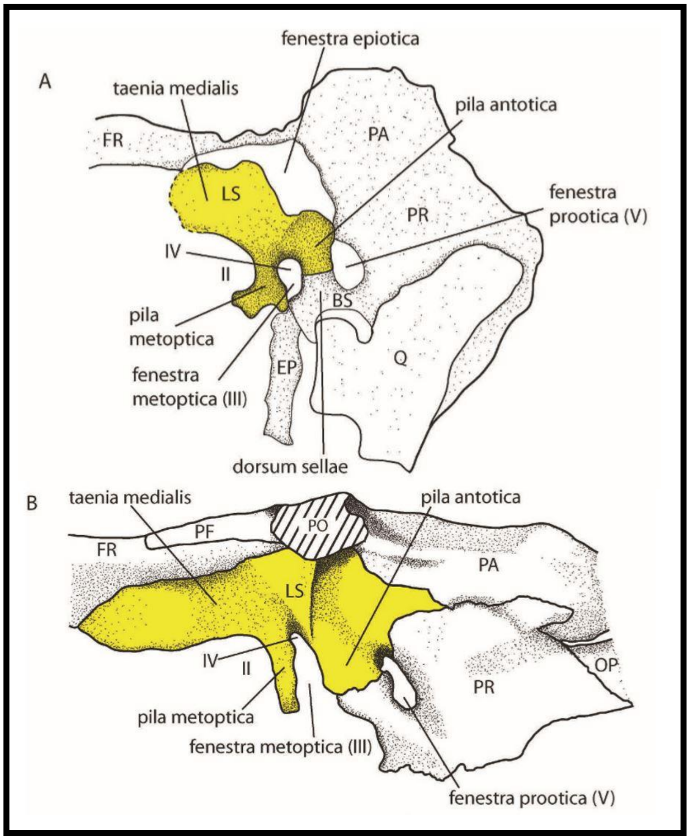


Figure 18. Representative laterosphenoids. A. left laterosphenoid of *Proganochelys quenstedti* in lateral view (after Gaffney, 1990) and B. right laterosphenoid of *Proterosuchus fergusi,* an early member of Archosauriformes, in lateral view (reflected after Clark *et al*., 1993). Abbreviations: (BS) basisphenoid, (FR) frontal, (EP) epipterygoid, (LS) laterosphenoid in yellow, (OP) opisthotic, (PA) parietal, (PF) postfrontal, (PO) postorbital, (PR) prootic, and (Q) quadrate. Modified from Bhullar and Bever, 2009

Gaffney (1990) named them "pleurisphenoids" with the quotation marks indicating probable non-homology with Archosauriformes. Clark *et al*. (1993) reinterpreted these as laterosphenoids and that the only difference between the bone found in *Proganochelys'* and the laterosphenoid found in Archosauriformes is that the latter retains an open suture with the skull roof (Figure 18). Therefore Clark *et al.* concluded that "pleurisphenoids" in *Proganochelys* are actually laterosphenoids, an opinion that Bhullar and Bever (2009) agreed with.

Kayentachelys aprix (Gaffney *et al.,* 1987) also has laterosphenoids, but its morphology has not been described in great detail (Sterli and Joyce, 2007). Odontochelys has a skull that is dorsoventrally crushed obscuring the relevant region (Li *et al*., 2008), and modern turtles have ventral downgrowths of the parietals which articulate directly with the prootic and thus obliterate any remnant of laterosphenoids. Not all stem archosaurs including protorosaurs, rhynchosaurs, and *Trilophosaurus* (Dilkes, 1998; Sues, 2003; Modesto and Sues, 2004) have laterosphenoids, but all Archosauriformes (composed of *Euparkeria* and *Proterosuchas*) do, making those bones synapomorphic for the Archosauriformes (Clarke *et al.,* 1993).

The position of the sphenethmoid in extinct anapsids is taken over by the laterosphenoid in the turtle/archosaur group, and the sphenethmoid is lost in diapsids (deBraga and Rieppel, 1997). The posterior-most interorbital region, notably the base of the pila antotica (Figure 18), is ossified only in archosaurs and turtles (Gaffney, 1990; deBraga and Rieppel, 1997). Lee (1993a, 1995, 1997) stated that the extinct anapsid sphenethmoid and the turtle laterosphenoid are homologous, suggesting a pareiasaur relationship. DeBraga and Rieppel (1997) stated that *Proganochelys* and pareiasaurs have sphenethmoids based on the complete enclosure of the optic nerve foramen, but Bhullar and Bever (2009) stated that this definition of a sphenethmoid is not present in *Proganochelys*, but it is present in pareiasaurs. However, their definition of a laterosphenoid, a more posterior ossification which is contacting the prootic, is present in *Proganochelys* (Figure 18) (Bhullar and Bever, 2009).

Bhullar and Bever (2009) performed a preliminary phylogenetic analysis using the 144 character matrix created by Dilkes (1998), a very comprehensive archosauromorph matrix, and after adding three new characters (laterosphenoid sutured to parietal, skull wedge-shaped or tall and mediolaterally narrow, the presence of mid-dorsal region ossifications), found that turtles ended up most closely to Archosauromorpha, but not sister to Archosauriformes. This indicated a duel origin for the laterosphenoid. After a second analysis constraining *Proganochelys* to Archosauriformes to determine potential synapomorphies in the case of a single origin of the laterosphenoid, Bhullar and Bever found that the *Prolacerta* and *Trilophosaurus* jump down to more primitive positions, which is in line with more traditional view, and the new characters did not muddle the broad-scale topology of the tree (Figure 19).

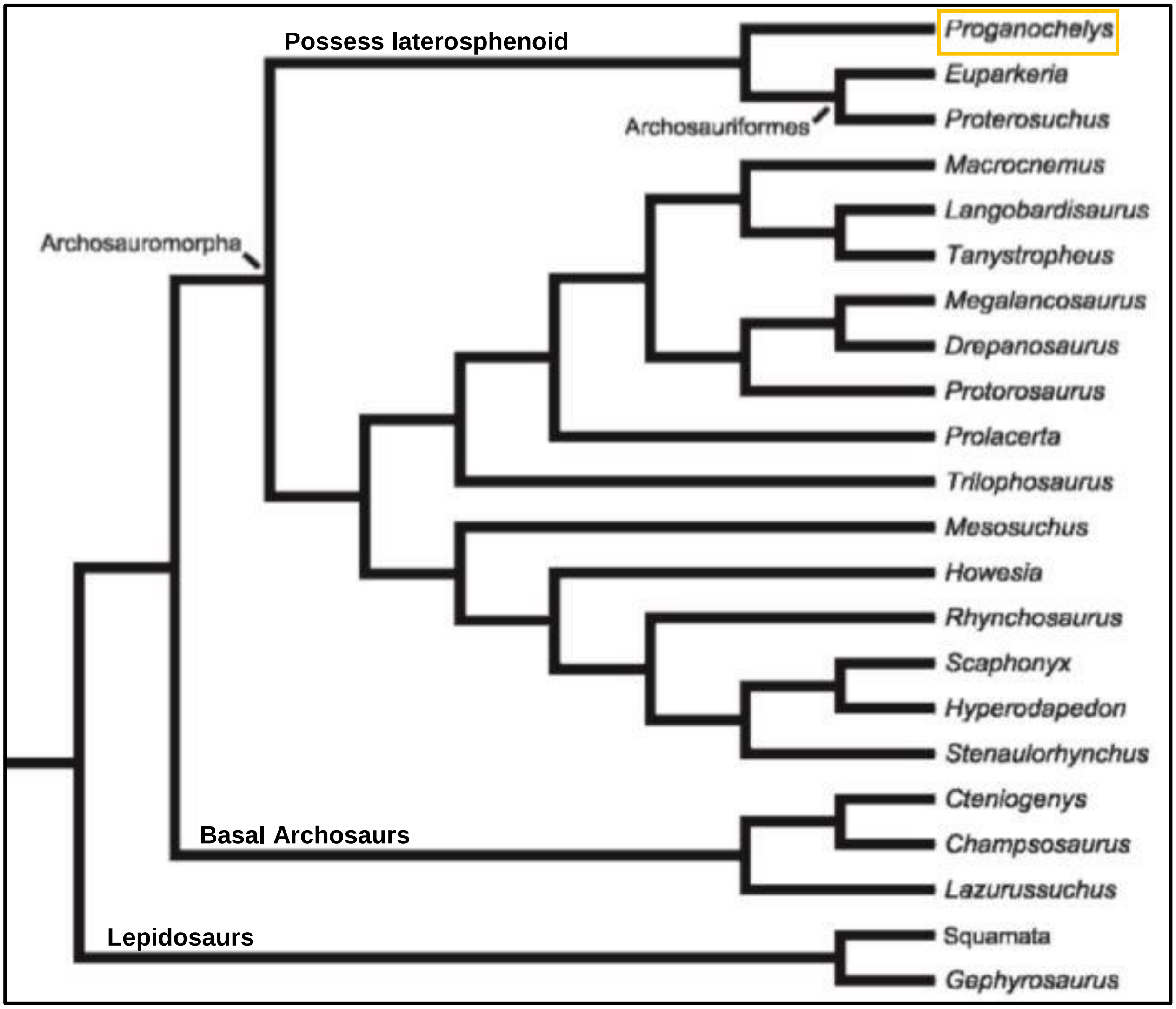


Figure 19. Most parsimonious tree after constraining *Proganochelys* (indicated by orange box) to Archosauriformes. *Proganochelys* is representative of the order Testudines. This is indicative of a single origin of the laterosphenoid. Modified from Bhullar and Bever, 2009

It is also at this point in time that a character that had been around since Diapsida was redefined by Goodrich (1916) was brought into question. The character is the hooked element in toe V or the hooked fifth metatarsal as it was known (Figure 16, p. 33). Although its presence in *Youngina* and other primitive diapsids remains a controversial issue (Goodrich, 1916; Lee, 1997; Rieppel and Reisz, 1999), it is assumed by most authors that it represents at least a synapomorphy of the clade Sauria (common ancestor of Lepidosauria and Archosauria and all its descendants) of Diapsida (Gauthier *et al*., 1988; Rieppel and deBraga, 1996; deBraga and Rieppel, 1997; Rieppel and Reisz, 1999) (Figure 14, p. 26). Recent studies have proposed that the hooked fifth toe is a modified metatarsal in both lepidosaurs (Fabrezi *et al*., 2007) and some archosaurian reptiles (Müller and Alberch, 1990). The majority of scientists have held that this is true in turtles as well (Goodrich, 1916; deBraga and Rieppel, 1997; Lee, 1997; Rieppel and Reisz, 1999; Rieppel, 1993a). In all extant reptiles the hooked bone extends from both rows of distal tarsals and metatarsal; however, in lepidosaurs it predominantly extends with the row of metatarsals whereas in turtles it predominantly extends with the distal tarsals (Fabrezi *et al*., 2009). Sheil and Portik in 2008 proposed the hypothesis that the hooked metatarsal in turtles is in fact a distal tarsal 5 that elongates and becomes hook-shaped to form the curved metatarsal 5, in order to articulate with the lateral margin of an enlarged distal tarsal 4. They were the first to state that the hooked fifth metatarsal (per Goodrich, 1916; Gaffney, 1980) should be referred to as a hooked distal tarsal 5.

Fabrezi *et al*. (2009) discussed several other features that identify this element as a distal tarsal in turtles and support a non-homologous relationship with lepidosaurs. The muscle insertion in turtles shows that the hooked element of the fifth toe is a distal tarsal in turtles

(2009). The endochondral ossification of the hooked element and the timing in which it develops in turtles provide the two strongest arguments for the element to be identified as a distal tarsal 5, and not a distal metatarsal as seen in lepidosaurs (Sheil, 2005; Fabrezi *et al*., 2009). It should be noted that Fabrezi *et al*. (2009) used only pleurodire turtles, but they stated that these patterns were observed in other turtles as well. Their reasoning for the use of only pleurodire turtles is that the present knowledge of limb variation in turtles is based mostly on Cryptodire taxa (Goodrich, 1916; deBraga and Rieppel, 1997; Lee, 1997; Rieppel and Reisz, 1999; Hill, 2005).

Joyce *et al*. (2013) countered the hypothesis presented by Fabrezi *et al*. (2009) and Sheil and Portik (2008). Joyce *et al*. stated that since all extant reptiles have a hooked fifth metatarsal and do not have a fifth distal tarsal, the question is whether turtles show the more derived condition similar to modern reptiles (hooked fifth metatarsal) or a more basal condition (hooked fifth tarsal). They also chose to use the term “ansulate bone,” as opposed to hooked element, to remain homology neutral (to not show bias towards the hooked fifth metatarsal of reptiles). Joyce *et al*. used several criteria to assess the primary homology of the ansulate bone: muscle attachment, mode of ossification, and the fossil record. They state that of the ten muscles that attach to the ansulate bone, all but two (muscle 11 and 33) attach to the hooked element found in reptiles. They concluded that the muscle attachment of the only turtle used in the study performed by Fabrezi *et al*. (2009), the pleurodire *Podocnemis unifilis,* is autapomorphic for the taxon and is the reason why Fabrezi *et al*. stated that the hooked element in reptiles is non-homologous with turtles.

Fabrezi *et al*. (2009) stated that because distal tarsals generally ossify endochondrally (i.e., through replacement ossification) and that the ansulate bone has been shown to ossify endochondrally, than it is a distal tarsal. Joyce *et al*. (2013) agreed that the ansulate bone ossifies endochondrally, but they stated that other distal elements have been known to ossify endochondrally (i.e., the distal elements of the paddles of ichthyosaurs (Caldwell, 1997)). They also use the fact that they have witnessed the ansulate bone in early stages be comprised of two separate, tightly connected anlagen they believe to represent a composite of distal tarsal 5 and metatarsal 5, to explain how there can be endochondral ossification and perichondral ossification as seen in metatarsal development. The endochondral ossification only occurs on the tarsal half of the bone, and perichondral ossification occurs on the metatarsal half. Joyce *et al*. concluded that the muscle attachments, embryological data, and topological data are consistent with the hooked element found in turtles being a fusion of the fifth distal tarsal and metatarsal 5. They state that available phylogenetic and fossil data are currently insufficient to clarify the homology of this element in Reptilia; however, this didn’t stop them from discussing turtles fifth metatarsal being closer in appearance to a crocodiles fifth metatarsal than it is to squamates.

Question 3. In regards to turtle phylogeny, are morphological and molecular studies currently in agreement?

Early Molecular Studies

Molecular studies of ancient organisms’ phylogeny have always been problematic. The issue is simple. There is very little molecular data for organisms that lived hundreds of millions of years ago (Gauthier *et al*., 1989; Carroll, 2013). Uncontaminated DNA lasts around 6.8 million years (Allentoft *et al*., 2012). This setback has not deterred scientists from using the results found from extant taxa's DNA to extrapolate phylogeny. An early molecular cladistic analysis (Hedges *et al*., 1990) trying to determine tetrapod relationships using 18S and 28S ribosomal RNA (rRNA) sequences showed that turtles group either with archosaurs or lepidosaurs depending on the use of maximum parsimony or neighbor-joining analyses. However, the study of Hedges *et al*. also gave strong support to a bird-mammal clade. Birds and mammals have long been shown to be distantly related groups (Gauthier *et al*., 1988). It wasn't until another analysis was later run removing the 18S rRNAs that favored the bird-mammal grouping that turtles consistently grouped with crocodiles (Hedges and Poling, 1999). Hedges (1994) was another early study using comparative DNA technology to study relationships within Diapsida. He found that 15 nuclear genes significantly support a bird-crocodile clade, also known as the archosaurs. In 1999, Hedges partnered with Laura L. Poling to test the placement of turtles, tuataras, squamates, crocodiles, and birds using two new genes, alpha enolase and 18*S* rRNA. For phylogenetic and timing analyses, these new sequences were added to 340 available protein and DNA sequences representing 24 nuclear and 9 mitochondrial genes. They used rodent and primate DNA as the outgroup.

Hedges and Poling (1999) found that turtles grouped with one or both of the archosaurs, bird and crocodile, used in individual analyses of all 15 genes used. Nine of the genes paired turtles with crocodiles and one gene paired turtles with squamates. A combined analysis using all nuclear proteins resulted in significant support (>97% confidence) for a turtle-crocodile clade on the basis of an interior branch test and bootstrapping of neighbor-joining, maximum likelihood, and maximum parsimony trees. Hedges and Poling (1999) used 23 nuclear genes and 2 mitochondrial regions (9 genes) to determine divergence times and found that turtles diverged from crocodiles 207±20.5 million years ago. They used the fossil divergence time between mammals and reptiles (including birds) at 310 mya as the calibration. 207±20.5 million years correlates to the age of the earliest fossil turtles.

Some of the results of this study do not correlate to the fossil or morphological record. Six of the 8 genes used to test tuatara relationships paired them with a group containing turtles, crocodiles, and birds (1999) (Figure 4, p. 3). Hedges and Poling's study also showed that birds diverged from the clade containing crocs and turtles at 228±10.3 million years. They used the same calibration as stated above. This is much earlier than the earliest known occurrence of birds (149±3 million years). Unlike this 1999 molecular study, there had been little support for a turtle-crocodile clade based on morphology up until that point in time (Rieppel and deBraga, 1996; deBraga and Rieppel, 1997). These issues were highlighted in Rieppel's (1999b) study on the morphological differences between crocodiles and turtles.

Some of the different approaches used by the molecular scientists during this time were using (1) only mt rRNA (Zardoya and Meyer, 1998), (2) complete mtDNA sequences of lepidosaurs, including a green sea turtle, (Kumazawa and Nishida, 1999), (3) nuclear-encoded proteins and ribosomal RNAs (Hedges and Poling, 1999), and (4) DNA-DNA hybridization data (Kirsch and Mayer, 1998). All of these studies supported an archosaur-turtle relationship as opposed to a lepidosaur-turtle relationship. Cao *et al*. (2000) decided to update the data by Kumazawa and Nishida (1999) and the nuclear sequences used by Hedges and Poling (1999) and run an analysis that utilized both data. They found that a cladogram of turtles and crocs as sister to birds was best supported (Figure 20; Tree-3), but that a tree with crocs and birds sister to one another, and that group being sister to turtles (Figure 20; Tree-2), had enough support that it cannot be rejected. Tree-3 was statistically rejected by Kumazawa and Nishida (1999), but there were several errors with their data, including log-likelihood differences in their analyses that were slightly overestimated due to errors in the matrix used.

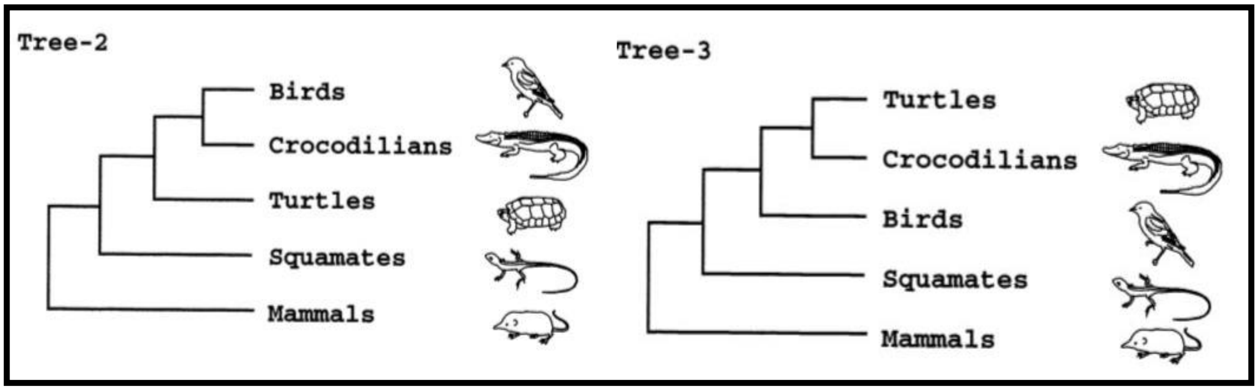


Figure 20. Two cladograms showing turtle relationships by Cao *et al.* (2000). Both with varying degrees of support. Modified from Cao *et al.,* 2000

After noticing that different results were reached using either mitochondrial or nuclear genomes (Zardoya and Meyer, 1998; Kumazawa and Nishida, 1999; Hedges and Poling, 1999; Kirsch and Mayer, 1998), Cao *et al.* (2000) stated that molecular analyses are prone to biases and errors due to the selection of the gene and gene sequences. While these studies all showed archosaur affinities to turtles, they could not agree whether birds or crocs were sister to turtles. The differing results can also be attributed to subtle difference in alignment of gene sequences, the choice of outgroup taxa, and different sampling of ingroup taxa. This view of molecular analysis lasted for much of the 2000s. It wasn't until the ability to categorize large amounts of genomic data came about in 2007-2008 that molecular studies with a focus on genomes started showing more clear results.

More Recent Molecular Studies

The first large scale genomic dataset showed similarities between archosaurs and turtles, but without strong support (Shedlock *et al*., 2007). Lee *et al*. (2008) discussed the complications facing molecular studies, and they expanded upon the arguments of Cao *et al*., (2000). These complications include composition bias (by choosing 18S or 23S rRNA), short sequences (e.g., nuclear amino acid residues), inappropriately fast substitution rates (e.g., in mitochondrial genes) and potential paralogues (genetic synapomorphy in DNA sequences) or pseudogenes (imperfect copies in mitochondrial DNA sequences). Even with these problems, almost every molecular study placed turtles as sister to archosaurs or within them, thus giving the turtle-archosaur argument strong support (Hedges and Poling, 1999; Kumazawa and Nishida, 1999; Zardoya and Meyer, 2001). Hedges and Poling (1999) reported a tree with squamates as the most basal branch leading to turtles, tuataras, crocodiles and birds (Figure 21). Lee *et al*. (2008) suggested that the issue may be in the placement of the squamates clade and not necessarily the positon of turtles, because the turtle-tuatara-crocodile-bird relationship remained constant in Hedges and Poling’s study (Figure 21). Lee *et al*. stated that studies have shown that nuclear genetic evolution occurs much faster in squamates than in other reptiles

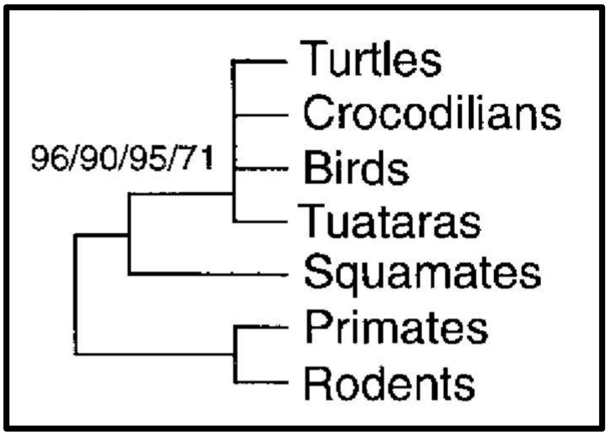


Figure 21. Cladogram composed by Hedges and Poling (1999). It uses combined sequences from four nuclear protein-coding genes (785 amino acids). Confidence values (%) supporting individual nodes are separated by slash marks and are based on interior-branch test and bootstrap analyses of neighbor-joining, maximum likelihood, and maximum parsimony. Modified from Hedges and Poling, 1999

(Hughes and Mouchiroud, 2001). Mammalian rates do not appear to be any slower than those of typical reptiles (crocodiles, tuatara, and birds) as reported on by Kumazawa and Nishida (1999). Lee *et al*. hypothesized that the longest branches of the cladogram are those leading to squamates and the mammal outgroup. They stated that the reason for this was because of the rapid divergence between squamates and the other reptiles (crocodiles, tuataras, and birds) and the long temporal gap between the common ancestors of the clade Mammalia and Reptilia. Long branch attraction within the phylogenetic program could artificially force squamates toward the base of the reptile tree (Lee, 2001; Lee *et al*., 2008). The elevated evolutionary rates throughout the nuclear genome of most squamates and the mitochondrial genome of at least some could, therefore, cause multiple genetic data to converge on the same tree (Lee *et al*., 2008).

Counter to Lee *et al*. (2008), Lyson *et al*. (2011) stated that the issue isn't deciding inter-relationships, but rather determining polarity, i.e., choosing which characters are ancestral or derived. Lyson *et al*. used the presence or absence of specific mircoRNAs (the genes that encode approximately 22 nucleotide non-coding regulatory RNAs) to root their tree. They state that since miRNAs are continually added to metazoan genomes through time and, once added, are rarely lost in most metazoan taxa, they make ideal phylogenetic characters (Wheeler *et al*., 2009; Sperling and Peterson, 2009). miRNAs also show extreme nucleotide conservation of the mature sequence and structural considerations based on the requirement to fold into the canonical miRNA hairpin structure make convergence highly unlikely, resulting in little homoplasy (Wheeler *et al*., 2009; Sperling and Peterson, 2009). Lyson and company extracted RNA from a turtle (*Chrysemys picta bellii*), a lizard (*Anolis carolinensis*), and an alligator

(*Alligator mississippiensis*) and compared it to already extracted data from miRBase of mammals (platypus, opossum, human), birds (chicken and zebrafinch) and an amphibian (frog). They performed a maximum parsimony analysis using PAUP 4.0b10 with all characters given equal weight and using the branch and bound search algorithm. A total of 282 miRNAs were coded, belonging to 186 miRNA families.

The analysis run by Lyson *et al*. (2011) resulted in a single most parsimonious tree. Alligators and birds share one miRNA, supporting their monophyly. All living reptiles studied share miR-1677. Turtles and lizards share four of the 77 unique miRNA gene families identified in *Anolis*, representing support for a turtle-lizard sister group relationship. All other nodes were each supported by one or more unique miRNA. They proposed the name Ankylopoda for the group made of Lepidosauria and Testudines and suggested that the molecular studies that gave archosaur affinities to turtles in the past may have been caused by the long-branched lizards attracting towards the outgroups as stated by Lee *et al*., (2001; 2008). Becker *et al*. (2011) also found a lepidosaur-turtle relationship when studying the full length cDNA sequence of the polypeptide hormone precursor proopiomelanocortin (POMC).

Shen *et al*. (2011) published the first genomic study that gave strong support to a turtle-archosaur clade using the multiple genome alignment resources from the University of California–Santa Cruz Genome Browser. Tzika *et al*. (2011) published a study based on reptile brain transcriptomic data that found a large number of genes are shared between turtles and archosaurs. Chiari *et al*. (2012) used a dataset comprising 248 nuclear protein-coding genes from 16 vertebrates, including four species of turtles (representing the two suborders), a caiman, two lizards, a snake, two birds, an alligator and a lungfish. They found strong support

(Bayesian, ML, and bootstrap) for the phylogenetic position of turtles as a sister group to Archosauria. Molecular dating analyses using nucleotides (CAT-GTR + G model) showed the divergence between turtles and archosaurs around the Permian-Triassic boundary at a mean of 255 mya (274-233 mya) with six fossils as calibration points as used in Benton *et al.* (2009). This result conflicts with that of Lyson *et al*. (2011) which utilized miRNAs. Chiari *et al*. (2012) reported that this is not the first time that studies that use miRNAs and sequence-based phylogenetic studies have conflicting results (Philippe *et al*., 2011, Sempere *et al*., 2007). Chiari *et al*. suggested caution when using miRNA as it might not be free from homoplasy, as thought by Lyson *et al*. (2011), and secondary loss of multiple families of miRNAs have been reported in tunicates (Fu *et al*., 2008), counter to what was reported by Lyson *et al*. (2011).

Crawford *et al*. (2012) was also skeptical of the findings of Lyson *et al*. (2011) and stated that if a relationship between turtles and lepidosaurs exists, then there should be evidence in the genomes of both organisms. They tested this by using the ultraconserved elements (UCEs) of DNA, the genomes that are conserved between evolutionary distant taxa (i.e., identical nucleic acid sequences) (Bejerano *et al*., 2004) and their flanking sequences from a tuatara and two species each of crocodiles, squamates, and turtles (Figure 22). The Bayesian analysis of linked alignments of DNA and species-tree analysis of 1145 independent gene histories showed turtles to be the sister lineage of extant archosaurs with complete support. Crawford *et al*. (2012) found no support for turtles being sister to lepidosaurs. They concluded that the turtle-archosaur relationship is unlikely to be caused by long-branch attraction as suggested by Lyson *et al*. (2011) due to the taxonomic sampling, the genome-wide scale, and the robust results obtained, regardless of analytical method. The cladogram obtained by Crawford *et al*. (2012)

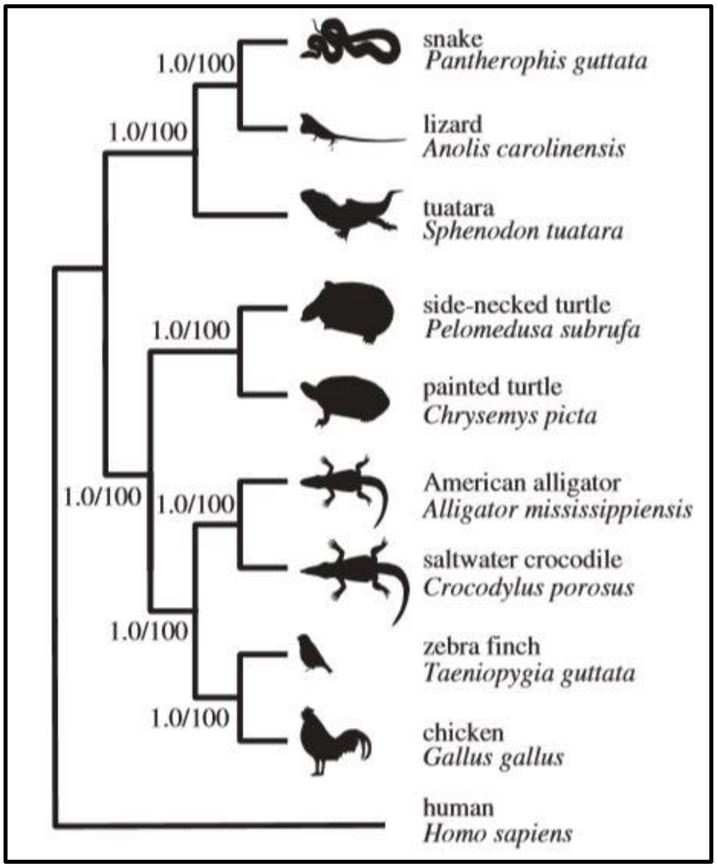


Figure 22. Cladogram composed by Crawford *et al.* (2012). It uses 1145 ultra-conserved loci. Node labels indicate posterior probability/bootstrap support. From Crawford *et al.,* 2012

was the first well-resolved reptile cladogram to include tuatara and multiple loci (single-copy nuclear locations on a genome) (Figure 22). Their reasoning for the results found by Lyson *et al*. (2011) were the use of miRNA sequences collected from miRNA expression libraries which they considered a biased sampling process, and that the four miRNA families found in lizards and turtles may be present in the other reptile taxa studied, but aren't yet sequenced. Crawford *et al*. (2012) stated that in preparing and sequencing libraries, like the ones used by Lyson *et al*. (2011), the detection probability for specific targets is variable, causing some miRNAs to be more likely to be detected than others.

Applying a 'genes as characters' approach, Lu *et al*. (2013) argued that the turtle-archosaur hypothesis could be the result of large, concatenated (linked) alignments overburdened by gene heterogeneity. This is likely the largest source of systematic error in phylogenomic analyses (Jeffroy *et al*., 2006; Salichos and Rokas, 2013). Lu *et al*. (2013) took it one step further and stated that many of the cladograms supporting a turtle-archosaur sister group relationship may be under positive selection, where new advantageous genetic variants sweep a population, or positive selection plays an important functional role in the genomes showing a turtle-archosaur relationship.

Field *et al*. (2014) partnered with many scientists including Tyler Lyson and Jacques Gauthier to retest turtle relationships using miRNA. Their test differed from Lyson *et al*. (2011) in that it was a much larger miRNA dataset and employed more rigorous criteria for miRNA annotation (= naming or identification). They state that the study completed by Lyson *et al*. (2011) did not meet the minimal criteria established for miRNA annotation (Kozomara and Griffiths-Jones 2011; Tarver *et al*., 2012), especially because none of the four miRNAs shared between lizards and turtles (as stated by Lyson *et al*., 2011) exhibited expression of both arms (= sides) of the hairpin. miRNA are regions of RNA transcripts that fold back in on themselves forming a loop structure named the hairpin. The issues exhibited by Lyson *et al*. can be solved with deep phylogeny; however, Field *et al*. (2014) stated that it is problematic to use miRNA to answer questions of deep phylogeny as was done by Lyson *et al*. To counter any discrepancies, Field *et al*. took the near-complete miRNA repertoire from a turtle, snake, alligator, and bird using both small RNA library reads and genomic sequences. They also used previously published lizard (Lyson *et al*., 2012) and bird (Kozomara and Griffiths-Jones, 2011) data.

The results of Field *et al*. (2014) fully supported an archosaur affinity with turtles. They stated that the original miRNAs identified by Lyson *et al*. (2012) appeared to be misleading, and that the study of Field *et al*. (2014) demonstrated several miRNAs shared between archosaurs and turtles. They stated that their conclusion is strongly supported by a Bayesian phylogenetic analysis of 238 precursor miRNA sequences. Field *et al*. stated that the different results compared to Lyson *et al*. is not due to the decision to use miRNAs to determine relationships, as suggested by Chiari *et al*. (2012), because turtles do have slow rates of miRNA evolution and minimal secondary miRNA gene loss.

Question 4. In which habitat did true turtles and their immediate ancestors utilize, terrestrial or aquatic?

Watson (1914) was the first to mention the environmental context of the earliest turtle ancestor when he predicted that the hypothetical turtle ancestor evolved on land and had an anapsid skull with many primitive reptile features including a primitive palate and teeth. He used the fact that earlier turtle forms such as *Proterochersis robusta* (Fraas, 1913)*,* from the Triassic, have more shell elements, specifically plastron elements, than modern aquatic forms. He stated that the fact that aquatic forms of Testudines almost always show a degeneration of the shell to imply that his hypothetical ancestor must have been land-based because *Proterochersis* has a more complete shell. Watson (1914) predicted several features for his hypothetical ancestor of turtles, including possessing more shell elements, eight cervical vertebrae, a primitive reptilian palate, and a pectoral girdle overlying the first dorsal ribs. He also stated that the early reptile, *Eunotosaurus africanus* (Seeley, 1892), shares the most characters with that of the predicted turtle ancestor.

Lee (1993a) picked up where Watson (1914) left off, but, instead of proposing a relationship with *Eunotosaurus*, he stated that pareiasaurs share the most characters with

Watson's hypothetical turtle ancestor. Watson's proposed hypothetical ancestor had a narrow shoulder girdle anterior to a wide, flat carapace. Lee stated that this morphology is comparable to that of pareiasaurs. Pareiasaurs are all terrestrial organisms, which implies that the turtle ancestor was also land based. This wasn't directly stated until the study by Lee (1996). Once this pareiasaur-turtle hypothesis became heavily argued against by Laurin and Reisz (1995), Rieppel and deBraga (1996) and deBraga and Rieppel (1997), so did the terrestrial habitat of the turtle ancestor.

Rieppel and Reisz (1999) gave several lines of evidence to support the origin of turtles in an aquatic habitat of turtles as opposed to a terrestrial one. The primary mode of respiration in modern terrestrial turtles is greatly inhibited due to the fact that the ribs are fused together to form the shell. In a generalized tetrapod reptile, aspiration of air is driven by passive recoil of the body walls that are supported by ribs and/or by compression of the lungs as a result of active compression of the rib cage. However, in the case of land turtles, respiration depends on volume changes of the thoracic-peritoneal cavity (location of the lungs, anterior to the abdominal cavity,) inside the shell, which is achieved by altering the position of the limb flanks through the activity of anterior and posterior muscles (Gans and Hughes, 1997), i.e. they move their limbs to change the volume of the body cavity. A study of aquatic turtles and land tortoises found that the aquatic turtles change from inhalation and exhalation due to limb muscle output to hydrostatic pressure (Gaunt and Gans, 1969). Rieppel and Reisz (1999) stated that it seems easier for an aquatic turtle ancestor to evolve a method of respiration and locomotion independent of one another than it is for the two methods to co-evolve with one another on land. In an aquatic environment, inhalation and exhalation may be passive using only gravity and hydrostatic pressure. An aquatic environment also provides buoyancy which greatly facilitates both body support and locomotion.

Lastly, Rieppel and Reisz (1999) stated that the formation of a plastron is best explained by adding protection to the ventral surface, which is more likely to be exposed to predators in an aquatic environment. In addition to protection, they stated that the dermal armor could be used as an osmotic barrier. A study done by Bentley (1976) showed that slider turtles (*Trachemys scripta*) with a well ossified carapace and plastron gained significantly less water (in fresh water) or loss of water (in sea water), as opposed to soft-shelled turtles (*Apalone spiniferus*) with a less ossified shell (from Rieppel and Reisz, 1999). The type of rock that early turtle forms are found in also indicates an aquatic environment. Rieppel and Reisz (1999) state that the occurrence of a proganochelyid (*Proganochelys*-like) turtle in Late Triassic marine deposits indicates that this clade of early turtles, if not including marine members, at least pursued an amphibious mode of life. This form of life includes living in delta systems or lake systems close to the sea shore. These latter types of habitat are indicated in the geologically younger rock that *Proganochelys quenstedti* (Baur, 1887) is found in the Late Triassic (middle Norian) Stubensandstein (Gaffney, 1990).

In 2003, Joyce and Gauthier attempted to quantify turtle forelimb morphology in order to determine its relationship to the turtle's ecology and use it to predict paleoecology from fossil turtle specimens. Modern turtle forelimbs generally reflect their ecology (Ernst and Barbour, 1989). Joyce and Gauthier recorded measurements from *Proganochelys quenstedti*, another basal turtle, *Palaeochersis talampayensis* (Rougier *et al.,* 1995), and 77 skeletons representing 71 species of extant turtles (2003). These species were selected so as to ensure a comprehensive estimation of ancestral conditions for all major turtle clades. *Proganochelys quenstedti* was chosen because it has been described as semi-aquatic (Gaffney 1990; Gaffney and Kitching 1994; Rieppel and Reisz 1999), whereas *P. talampayensis* has been described as a terrestrial form (Gaffney and Kitching 1994; Rougier *et al.,* 1995).

Using morphometric data, Joyce and Gauthier (2003) concluded that all testudinids (tortoises) are short-handed and terrestrial, and all chelonioids (sea turtles) are long-handed and aquatic. However, they found that the remaining clades cover a wide range of habitats and morphospace. Their results showed that at least four turtle lineages invaded land independent of one another when their data is compared to the current molecular and morphological phylogenies at the time (Gaffney and Meylan 1988; McCord *et al*. 2000; Feldman and Parham 2002). The four clades were terrestrial tortoises (Testudinidae), *Cuora* (Asian box turtles), *Rhinoclemmys* (Central American pond and wood turtles) and *Terrapene* (North American box turtles). All four clades exhibit short hands compared to the aquatic forms, showing that Joyce and Gauthier's (2003) results are not accidental, but rather causal in nature (Figure 23). The aquatic bottom walking turtles, musk turtles and snapping turtles, still exhibited aquatic front limb lengths. The hind limbs did not show as strong of a correlation to a terrestrial or aquatic environment. Both *Proganochelys quenstedti* and *Palaeochersis talampayensis* exhibited terrestrial front limb lengths (Figure 23). Joyce and Gauthier reported on the other characters that *P. quenstedti* possess that indicate a terrestrial life style, including osteoderms on the limbs, reduced phalangeal count, and a tail club. It should be noted that the reduced phalangeal count was used by Lee (1994) to state that *P. quenstedti* is terrestrial. Lastly, Joyce and Gauthier (2003) stated that it is conspicuous that stem turtle fossils, which are found in quarries across

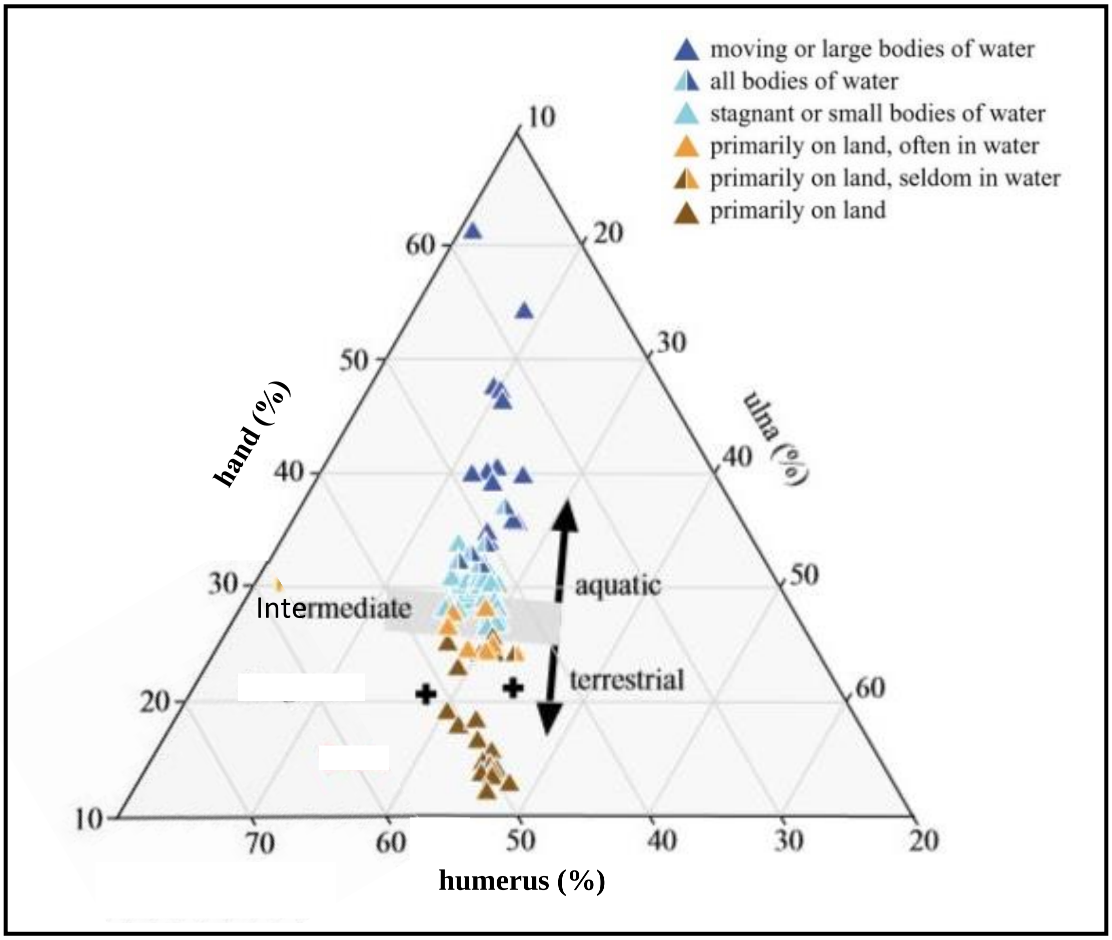


Figure 23. Distribution of turtle taxa and habitat preference on a ternary plot. It uses turtle forelimb measurements. Habitat preference is defined by color using the key in the upper right corner. Percentage of forelimb length that is the hand (left side), ulna (right side), and humerus (bottom side) is shown along sides of ternary plot. The intermediate field shows turtles that are both aquatic and terrestrial. Basal turtles *Proganochelys* and *Palaeochersis* are marked with (**+**). Modified from Joyce and Gauthier, 2003

the globe (Lucas *et al*., 2000), are never associated with aquatic faunas, but rather with other terrestrial vertebrates, especially when you consider the high fossilization potential of the turtle shell.

The study done by Joyce in 2007 to test the timing of the separation of Cryptodira and Pleurodira from the clade Testudines corroborated the results of Joyce and Gauthier (2003) for a terrestrial origin of turtles. The topography of the cladogram of turtles produced by Joyce

(2007) displays a completely terrestrial stem. *Proganochelys, Palaeochersis talampayensis*/*Australochelys africanus,* and *Proterochersis robusta* would lead to all other extinct and extant turtles in the cladogram composed by Joyce. These organisms have all been proposed to inhabit land (Rougier *et al.,* 1995; Joyce and Gauthier, 2003). He stated that this conclusion could explain why basal turtles are far less common in the fossil record than their derived, aquatic relatives.

Scheyer and Sander (2007) noticed the work done by Joyce and Gauthier (2003) and that *Proterochersis robusta* could not be tested due to the lack of limbs on the specimen. Scheyer and Sander sought to test the terrestrial vs aquatic habitat of early turtles, especially the specimens that lack limbs, by using shell bone microstructures. This is assuming that modern shell morphology is similar to extinct turtle shell morphology. They tested three terrestrial extant turtles and three aquatic extant turtles, as well as the extinct *P. robusta* and *Proganochelys quenstedti.* Joyce and Gauthier determined that characteristics of terrestrial turtle shells are compact diploe structure (the spongy internal structure of bone) of shell bone, no homogenization of cortical (the outer most layer) and cancellous (the inner most layer) bone, and no to low reduction of vascularization of the internal cortex (Figure 24). Whereas, aquatic shells possess a non-compact diploe structure, low to high homogenization of cortical and cancellous bone, and moderate to strong reduction of internal cortex and vascularization of the internal cortex (Figure 24). The two fossil turtle species they tested showed compact diploe structure, no homogenization of cortical and cancellous bone, and low to absent reduction and vascularization of the internal cortex. Scheyer and Sander (2007) concluded that *P. robusta* and *P. quenstedti* exhibit a bone microstructure consistent with a terrestrial lifestyle, mainly a

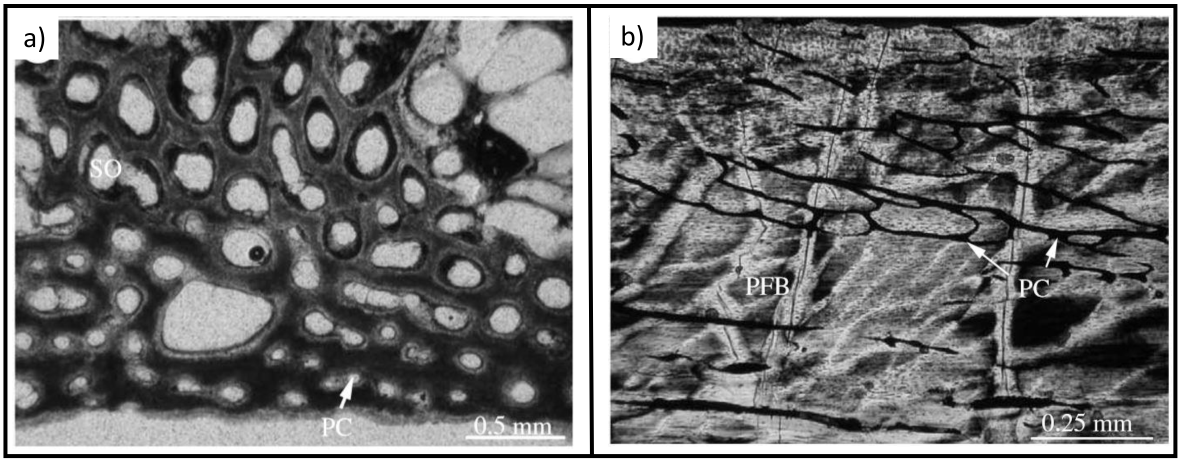


Figure 24. Close up of internal cortex of turtle shell. a) left costal of the aquatic loggerhead sea turtle (*Caretta caretta*) showing high vascularization by circular canals and b) a plastron fragment of *Proterochersis robusta* showing vascularization by thin vascular canals in parallel-fibred bone seen in polarized light. (SO) secondary opening; (PC) primary vascular canal; (PFB) parallel-fibred bone. Modified from Scheyer and Sander, 2007

robust diploe structure with well-developed internal cortical bone layers. Later studies using similar methods to the aforementioned study showed that most Jurassic stem turtles, including *Condorchelys antiqua, Eileanchelys waldmani,* and *Heckerochelys romani*, show an adaption to an aquatic habitat (Scheyer *et al*., 2014), and more derived stem turtles, such as solemydids and meiolaniforms, show terrestrial adaptations (Scheyer *et al*., 2015).

Some new insights were reached with the discovery of *Odontochelys* by Li *et al*. (2008), an early reptile with only a plastron and lateral bridge, but no carapace. Due to its possession of marginal teeth rather than a beak, free sacral ribs, and a long tail, Li *et al*. concluded that this animal represents a primitive turtle form. Li *et al*. did the same proportion analysis done by Joyce and Gauthier (2003) and found that *Odontochelys* compares to living turtles that inhabit stagnant or small bodies of water.

Reisz and Head (2008) took another approach to explain *Odontochelys'* peculiar morphology. They hypothesized that there was indeed a carapace present at one point, but it was reduced due to a shift to an aquatic environment. This shift resulted in the lack of ossification of some of *Odontochelys'* dermal components. This morphological change can also be seen in extant soft-shelled turtles, which have a greatly reduced bony shell (carapace and plastron) and have lost the dermal peripheral elements (marginal) of the carapace. Extant sea turtles and snapping turtles also have greatly reduced ossification of the dermal components of the carapace. Reisz and Head (2008) drew upon the conclusion of Li *et al.* (2008) that *Odontochelys* lived in an aquatic environment, along with the information discussed above, to conclude that the absence of the dermal carapace is a secondary loss associated with the move from a terrestrial habitat to an aquatic habitat, and it is not a primitive condition as inferred by Li *et al.* (2008). Reisz and Head (2008) stated that the similarities between *Odontochelys* morphology and early growth stages in living turtle embryos represents a simple truncation of carapace ossification, in which adults retain juvenile features (paedomorphosis) and could be the cause of the lack of ossification of the carapace. An extensive study of shell reduction through paedomorphosis in extant turtles by Kordikova (2000, 2002) showed that this reduction often is accompanied by a reduction in plastral ossification. *Odontochelys* shows no such reduction of the plastron except for a narrow midline fontanelle, which could have been caused by postmortem dissociation as concluded by Kordikova (2000, 2002).

The discovery of the oldest known turtle from North America, *Chinlechelys tenertesta,* by Joyce *et al*., (2009) provided further support for a terrestrial origin of turtles. *Chinlechelys*, from the Upper Triassic terrestrial deposits of New Mexico, is known for having the thinnest fully ossified shell (plastron and carapace), although it may be a juvenile. Joyce *et al*. interpreted that *Chinlechelys* the size of the specimen (35 cm, slightly smaller than the smallest

Proganochelys) paired with the lack of sutures present on the carapace make it at least sub-adult in age and has not yet terminated growth. Joyce *et al*. also speculated that the reason why there hasn't been an abundance of Triassic turtles found in aquatic deposits is because turtles originated on land. The thin shell of *Chinlechelys* may have been typical of other early terrestrial turtles, and it may be the reason why there hasn't been a large amount of turtle fossils found in Triassic terrestrial deposits (Lucas *et al.,* 2000; Joyce *et al.,* 2009).

The discovery of the most complete Middle Jurassic turtle, *Eileanchelys waldmani*¸by Anquetin *et al*. in 2009 fills the temporal gap between the most basal turtles and the post-Middle Jurassic diversification of the turtle crown group proposed by Joyce (2007) and Scheyer and Anquetin (2008). *Eileanchelys* displays an intermediate morphology between these two clades, specifically in the vomer region. Anquetin *et al*. (2009) concluded that this turtle inhabited aquatic environments, making it one of the earliest known aquatic turtles (Figure 25). In 2010, Anquetin revealed skepticism for the aquatic nature of *Odontochelys* stated by Li *et al*.

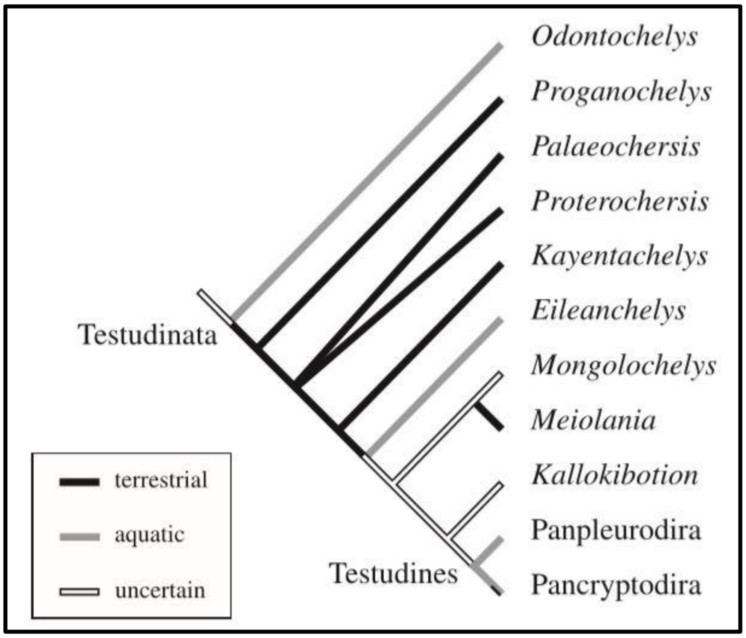


Figure 25. Cladogram showing palaeoecology of early turtles. It was composed by Anquetin *et al.* (2010). Relationships of *Odontochelys* and *Eileanchelys* according to Li *et al.* (2008) and Anquetin *et al.* (2009), respectively. Within Pancryptodira, some groups secondarily adapted to terrestrial habitats. From Anquetin *et al.*, 2010

(2008) based on the phalanges of the manus and pes being short and more similar to the limb proportions of extant turtles that live in terrestrial habitats than aquatic turtles.

Further support was given to a terrestrial origin of the turtle clade after the reemergence of the hypothesis that *Eunotosaurus*, an early terrestrial reptile, is a possible turtle ancestor (Lyson *et al*., 2010; 2013; 2014). This theory was only further supported when it was found that Eunotosaurus is a modified diapsid (Bever *et al*., 2015). One of the only studies to counter this terrestrial hypothesis, for one that demonstrates a more diverse range of ecotypes in the stem turtles, was suggested by Benson *et al*. (2011). In this study they used a three-parameter geometric model of the shell curve in anterior view to determine the paleoecology of *Proterochersis robusta* (Fraas, 1913) and other extant turtles. They chose *Proterochersis* because it is the only stem turtle with a well preserved shell that is not been distorted in any way. This type of study is similar to the limb proportion study done by Joyce and Gauthier (2003). Benson *et al*. (2011) used the data collected from 110 extant turtle shells encompassing 12 families, excluding soft shelled turtles, to define three types of shells, terrestrial, semiaquatic, and aquatic. Benson *et al*. used the detailed descriptions of extant turtle habitats found in Ernst and Barbour (1989) and Bonin *et al*. (2006). These descriptions include aquatic (representing turtles that never or rarely go on land), semiaquatic (those that swim well and feed in water, but spend long times on land), and terrestrial (exclusively found on land). Benson *et al*. (2011) used three-parameter geometric model to categorize each species' carapace that was tested and checked against their actual habitat. The three parameters were R = height/width, p = perimeter measurement, k = flatness/curvature of the plastron. They concluded that *Proterochersis* is not terrestrial, as previously stated by Scheyer and Sander (2007), but rather semiaquatic. Benson *et al*. (2011) stated that the basal positon of this taxa implies that the ecological diversification of stem-group turtles may have been rapid or that a substantial period of currently unknown diversification preceded the first appearance of the stem turtle lineage.

The discovery of another possible turtle ancestor from Upper Triassic marine deposits, *Eorhynchochelys sinensis* (Li *et al*., 2018), further demonstrates the variability of paleoecology of basal turtles. In this species the astragalus and calcaneum bones are not fused, which is a characteristic of turtles living in an aquatic environment. However, *Eorhynchochelys* exhibits stout limbs, prominent condyles on the humerus and femur, moderately developed olecranon process (a curved protuberance at the distal end) of the ulna, and enlarged claws. These traits are characteristic of a fossorial or burrowing lifestyle similar to that proposed by Lyson *et al*. (2016) as the lifestyle of *Eunotosaurus.* Li *et al*. (2018) hypothesized that the animal may have inhabited coastal waters, foraging on the land as well as in the water.

Question 5. Were endoskeletal or exoskeletal components used to construct the turtle shell?

Early Morphological Studies

The turtle shell is a unique morphological feature (Jackson, 2011). It utilizes both endoskeletal features (mainly the ribs and neural arches of the vertebrae) and exoskeletal or dermis (sub-skin) components (the osteoderms). Endoskeletal bones are elements of the internal structure of an organism that start as cartilaginous elements that are than replaced by bone later in development and differ from exoskeletal bones in that they have no association with the dermis or dermal cells. Exoskeletal bones often form from ossification centers within the dermis. The question posed in this section is whether exoskeletal components played a role in the formation of the shell or were they a secondary external addition to a shell already formed by endoskeletal components. One way to answer this question is by looking at embryological development of turtles to see how and from where the shell components form. The study of turtle embryos is often dependent on the technology available at the time and is a hotly contested science riddled with misidentifications.

In 1800, Cuvier first proposed that the bones of the turtle carapace were expanded ribs and vertebral elements (from Burke, 1989). In 1818 (from Burke, 1989) Geoffroy-St. Hilarie agreed with Cuvier on the carapace elements and stated that the plastron was the endoskeletal sternum in its greatest state of development. During the mid-1800s, there was much discussion in the biology community about whether the shell formed from endo- or exo- skeletal features. Carus (1834) was the first to say certain carapace plates belong to the dermis layer. Carus stated that the dermal plates and ribs developed independently and were later fused.

In 1848, Rathke was the first to look at turtle embryos in order to determine how the shell developed. He concluded that the carapace developed through the endoskeleton and the plastron was made from exoskeleton elements. In order to test the hypothesis proposed by Rathke, Owen (1849) compared turtle embryos to bird and crocodilian embryos. He was able to draw conclusive evidence on the origins of the parts of the carapace and plastron. He found that the neural osteoderms are homologous to the median dermal osteoderms of a crocodilian and the marginal osteoderms are fully dermal. He further stated that the lateral parts of the plastron (hyoplastron and hypoplastron) are fused with dermal osteoderms and the front part of the plastron (entoplastron and perhaps the epiplastron) are homologous to the sternum (Figure 26). Owen concluded that the turtle shell is composed of the fusion of dermal osteoderms and endoskeletal elements, and that the neural osteoderms are not developed independently of the neural spines but are continuously ossified with them.

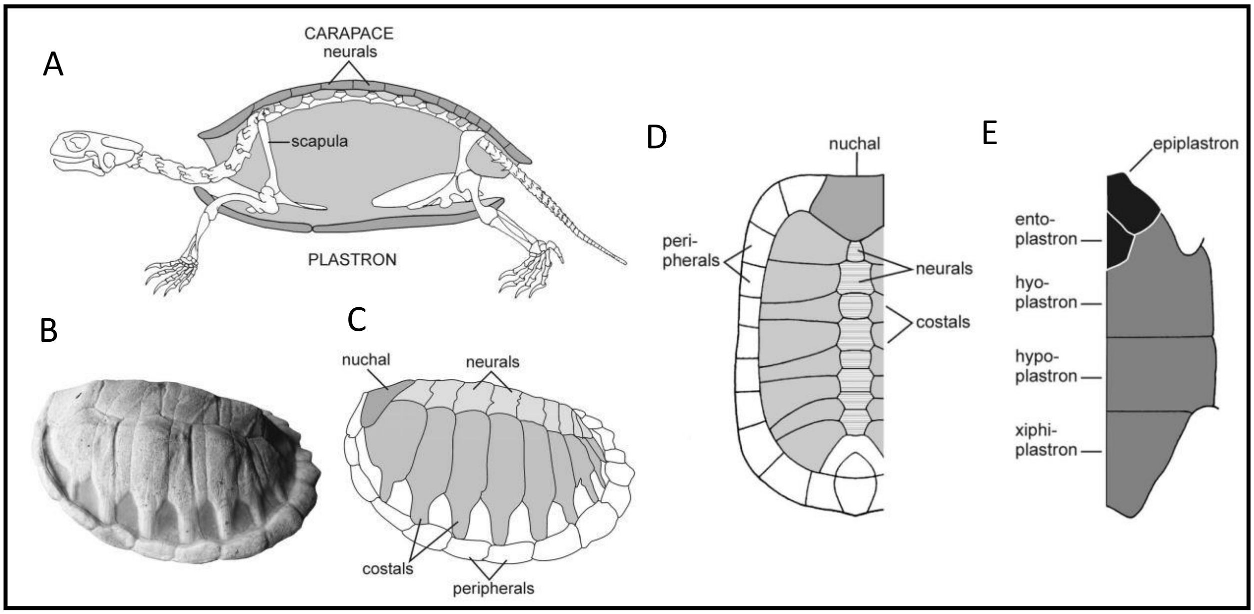


Figure 26. Features of the turtle shell. A, cross-section through the skeleton of a pond turtle (*Emys orbicularis*); B–C: bony carapace of *Chelydra serpentina* in B, oblique dorsolateral view and C, as an outline drawing with different bony components indicated by different shadings; Shell elements of a tortoise (*Testudo*): D, carapace in dorsal view; E, plastron in ventral view. From Schoch and Sues, 2019

According to Baur (1888), the discovery of an unknown extant tortoise among museum specimens of *Testudo leithii,* with large osteoderms on each forelimb, gave support to the origin of the shell from dermal elements. Baur described how the dermal armor of the carapace would form after small, isolated ossicles (small osteoderms) in the skin become larger and touched one another and eventually fused along their sutures. When the fused dermal armor or shield, connected to the endoskeleton, it would be better supported, and many of its sutures would disappear. Therefore, many of the individual osteoderms would disappear and end up aligned with the placement of the endoskeletal ribs. He concluded that this is the way in which costal osteoderms formed that part of the shell.

Baur (1888) also stated that turtle dermis derived clavicles and interclavicles were expanded and fused, forming a solid shield, thus the clavicles and interclavicles were transformed into the front part of the plastron (epiplastron and entoplastron). Baur stated that the dermal osteoderms of the belly coalesced to become the abdominal ribs (gastralia), which expanded later to become the central and posterior parts of the plastron (hyoplastron, hypoplastron, and xiphiplastron; Figure 26). He hypothesized that the plastron is of dermal origin. Baur mentions that the leatherback sea turtle exhibits the condition in which the carapace and plastron form by the dissolution of their shell elements into isolated ossicles.

It was in 1899 that Goette investigated the ontogeny of a sea turtle, *Eretmochelys imbricate*, and stated that the nuchal (neck) bone, pygal (tail) bone, and peripheral bones are true cutaneous (dermal) ossifications from the neural and costal bones, which are derived from the endoskeleton (Figure 27). This view was soon challenged when Zangerl (1939) highlighted the dual nature of shell formation (involving endo and exo-skeletal elements) when he noticed that the endoskeletal elements (the neural arches and ribs) enter the dermis as they develop.

Embryological Studies

Older Studies. Many accepted Baur's hypothesis for the exoskeletal origin of the carapace including Hay (1898), Versluys (1914), and Watson (1914). The first two authors proposed that the turtle shell formed from the gradual fusion of dorsal osteoderms, as seen in leatherback sea turtles. These early scientists thought that the shell of the leatherback sea turtle, made of scattered isolated osteoderms in the dermis, was a primitive character of

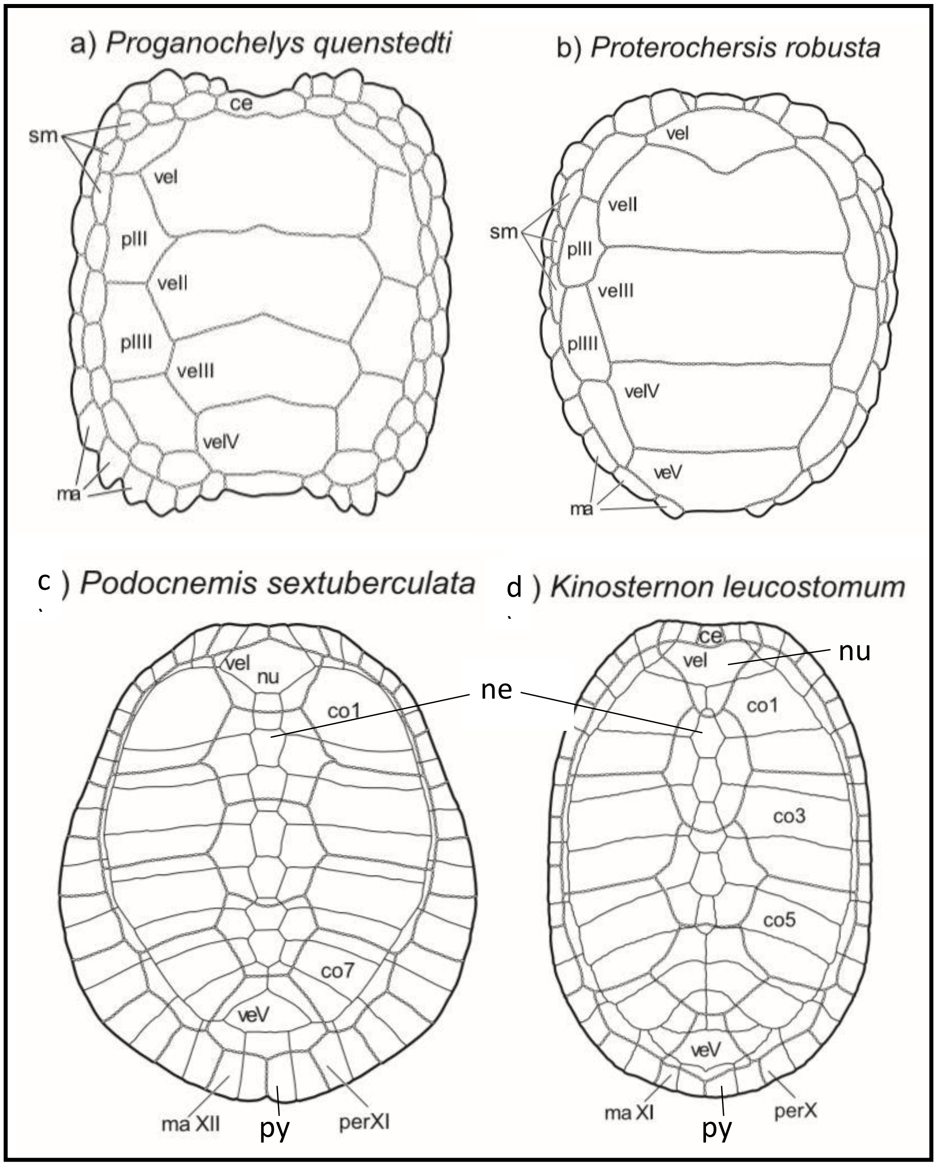


Figure 27. Dorsal views of turtle carapaces. a) *Proganochelys,* b) *Proterochersis,* c) *Podocnemis sextuberculata* redrawn from Boulenger (1889), and d) *Kinosternon leucostomum* redrawn from Boulenger (1889). Abbreviations: (ce) cervical scute, (co) costal bone, (ma) marginal scute, (ne) neural bone, (nu) nuchal bones, (pl) pleural scute, (per) peripheral bone, (py) pygal bone, (sm) supramarginal scute, and (ve) vertebral scute. Roman numeral or number represent the order. All scutes overlie the bone elements. Modified from Joyce, 2007

Testudines. The modern view is that the leatherback sea turtle's ancestors were fully shelled and that this turtle experienced a reduction of its shell due to an aquatic lifestyle (Rieppel, 2001). Watson (1914) looked at another key aspect of the endo- verses exo-skeletal debate, i.e., that being the evolutionary sequence by which the pectoral girdle comes to lie within the ribcage. That is, did the pectoral girdle stay in its primitive position and the ribs enveloped it, or did the girdle migrate posteriorly to lie within the ribcage? Rathke (1848) did not describe the process for which the pectoral girdles got within the shell. Watson (1914) stated that the turtle arrangement was caused by the gradual migration of the pectoral girdle backwards paired with the forward growth of the ribs, which are in the process of becoming part of the carapace. Ruckes (1929) looked at turtle embryos and agreed with Watson (1914) that the carapace does experience a unique growth. However, he observed not only anterior-posterior growth of the carapace but also lateral-ventral compressional growth. Ruckes (1929) concluded that the pectoral girdle remained stationary as the anterior-posterior growth of the ribs extended over the pectoral girdle and the lateral-ventral growth encased them from the dorsal-ventral side. He hypothesized that the enclosure of the girdles was accomplished long before the carapace and plastron bones ever appear embryological.

Ruckes (1929) was also able to identify on a snapping turtle embryo (*Chelydra*) what he called a condensation of dermis cells of the mesenchymatous type that would grow radially on top of the ribs and elements that make up the bridge and plastron. This collection of dermis cells would later be called the carapacial ridge (CR) (Burke, 1985). Ruckes (1929) would go on to state that this is not to say that the formation of the shell is wholly dermal in origin. The ribs are from the very early stages of embryonic development in close association with the dermis cells that bring about the carapace. The dermal layer is in such close proximity to the ribs that the normal intercostal and cutaneous musculature fail to develop. In fact, Ruckes noticed that in turtle embryos whichever direction the dermis grew, so too did the ribs. He observed that in the very early embryonic stages the ribs appear similar to a normal tetrapod, but during the growth of the dermal carapace, the dermal cells will envelop the ribs in a thin covering. This is, to Ruckes, what inhibits normal rib growth and causes the ribs to grow in more lateral direction. Due to the close association between the ribs and dermal cells, Ruckes concluded that the ribs must play some part in the formation of the shell, but it is the dermis that is the initiator. This research by Ruckes would lay the ground work for many embryological studies to come. It wasn't until Zangerl in 1969 that the plastron elements were shown to not have any cartilaginous precursors thus by definition belonged to the exoskeleton, proving half of Rathke's original theory (from Burke, 1989).

Modern Studies. In 1989 Ann Campbell Burke utilized autoradiographic and immunofluorescence (using colored antibodies to identify molecules) techniques to expand upon Ruckes's study. She worked with snapping turtle embryos, *Chelydra serpentina*. She postulated that the accumulation of carapacial cells results from a novel epithelial-mesenchymal interaction (i.e., interactions between the thin tissue lining the body and the connective tissue that derives from the mesoderm). She was able to confirm the presence of epithelial-mesenchymal interactions in the body wall initiating carapace growth because of a mass of adhesion molecules observed using autoradiography (X-ray photos of embyros). She used the term carapacial ridge to describe the collection of mesenchyme tissue of the dermis and overlying the ectoderm between dorsal and ventral flank mesoderm. This is where the ribs will eventually terminate (Figure 28).

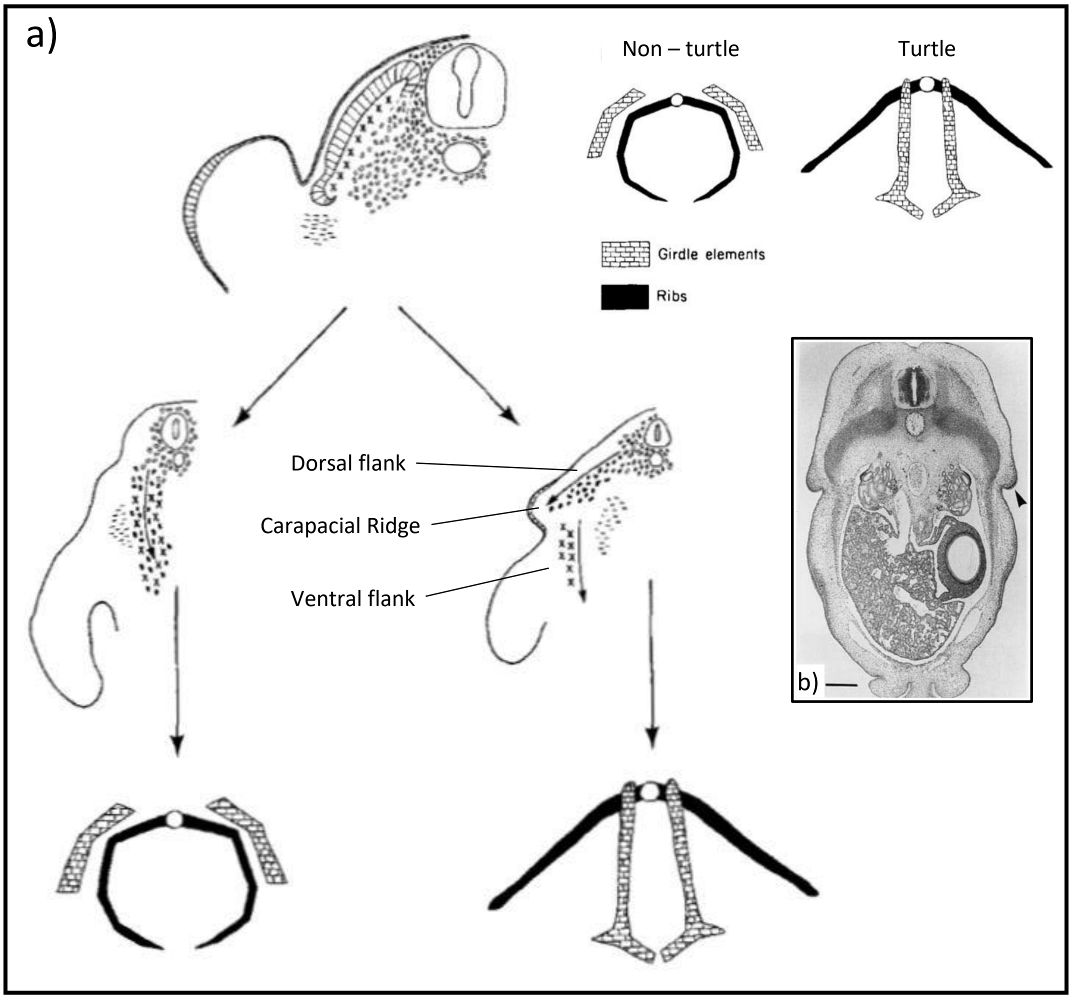


Figure 28. Formation of the carapace. **a)** Schematic illustrating the different migration pathways of prospective body wall cells in non-turtle and turtle embryos. In the non-turtle, costal and myogenic cells follow common route ventrally into the ventral flank. In the turtle, myogenic cells migrate ventrally, while the costal cells follow a lateral pathway into the expanding somatic dermis (carapacial ridge). (●) represents costal cells, (x) represents myogenic/muscle tissue cells, (dashed lines) represent scapular cells. **b)** Mid-trunk cross section through stage 15 that shows rib condensation (dark areas). The carapacial ridge (arrowhead) is fully developed overhanging the superficial remnant boundary between dorsal and ventral flanks. Scale bar = 300 μm. Modified from Burke, 1989

Normally the presumptive costal (rib) and myogenic (muscle tissue) cells migrate from the dorsally positioned somite to the ventral aspect of the embryo; however, in turtles, Burke (1989) reported that the costal cells never enter the ventral flank but follow a dorsolateral route into the expanding somatic dermis (Figure 28).

Burke (1989) stated that patterns of cell proliferation and the distribution of N-CAM (neural-cell adhesion molecules) and fibronectin (a glycoprotein involved in cell substrate adhesion (Yamada *et al*., 1984)) in the carapacial ridge are consistent with patterns seen in other structures initiated by epithelial-mesenchymal interactions, such as feathers and limb buds. Morphology of the carapacial ridge tissue is typical of areas that experience this interaction, and this e-m interaction is common in vertebrate development (Sawyer and Fallon, 1983). E-m interactions are characterized by the formation of an ectodermal (epithelial) thickening underlain by a condensation in the mesenchyme (a mesodermal embryonic tissue). The e-m interaction is a localized phenomenon that occur at all stages of development and is thought to be responsible for the induction of such structures as sensory organs (Jacobson, 1963), limbs (Saunders, 1948), and a wide variety of integumental structures such as scales, teeth, feathers, hair, glands, and dermal bone (Kresja, 1979). However, Burke (1989) concluded that a simple morphological comparison was not enough evidence to support her claim. She found in the carapacial ridge the presence of N-CAMs in the dense mesenchyme, high mitotic activity, and fibronectin. These molecules can also be found in the dermal mesenchyme of feather embryonic tissues (Chuong and Edelman, 1985) and in early limb bud mesenchyme (Tomasek *et al*., 1982).

As a source of developmental potential, epithelial-mesenchymal interactions can be vehicles for evolutionary novelty (Maderson, 1983 from Burke, 1989). Burke concluded by stating that this interaction, that primitively produced only integumental features, was modified and expanded to affect the deeper elements of the endoskeleton, primarily the ribs. She stated that this may be the result of a shift in the timing of onset of the induction. Finally, she also noticed that a large amount of trunk neural crest cells migrate during the body wall formation stages, but no further study was done to determine their role in the morphogenesis of the carapace (Burke, 1989).

Between Burke's work and the start of the twenty-first century, the formation of the shell through the fusion of osteoderms had been used as an evolutionary scenario to unite turtles with procolophonids (Laurin and Reisz, 1995) and pareiasaurs (Lee, 1996). Not much had been done on turtle embryology until the early part of the twenty-first century. Part of the focus of these more recent studies was to determine the role of trunk neural crest cells that were discussed by Burke (1989). Gilbert *et al*. (2001) stated that since the neural and costal plates ossify from and continuous with the outer surface of the endoskeletal bone that they are associated with, they are therefore endoskeletal components. If this is correct, then there would be no osteoderms involved in the formation of the shell, making the hypotheses proposed by Lee (1996) and Laurin and Reisz (1995) null and void. Cebra-Thomas *et al*. (2005) proposed a two-step method for carapace formation in turtle embryos. According to them, firstly fibroblast growth factors (FGFs) made in the carapacial ridge attract rib-precursor cells into the dermis, which ultimately coordinate the expansion of the dorsal dermis and ribs. This confirmed the conclusions of Burke (1991) that the carapacial ridge is providing chemotactic factors needed for the continued lateral growth of the ribs. Secondly, bone morphogenetic proteins (BMPs), secreted by the rib as it undergoes endochondral ossification, induce the dermis to ossify. Cebra-Thomas *et al*. (2005) stated that the key innovation in forming the shell was getting the ribs into the dermis. They hypothesized that if the ribs and carapacial ridge can produce a positive feedback loop, then they could co-ordinate rib and carapace growth. When the ribs undergo normal endochondral ossification, the BMPs would induce the rib bones that form the plate of the carapace at the site of the carapacial ridge. This mechanism would then allow the displacement of tissue to induce structures at new locations. They concluded that the lateral development of the turtle ribs appears to be directed by the carapacial ridge, and in the absence of the carapacial ridge, these ribs grow ventrally and enter the lateral plate, like the ribs of non-chelonian vertebrates. Cebra-Thomas *et al*. (2005) were able to stop the development of the carapacial ridge by using tantalum foil barriers between the somite and the lateral plate mesoderm.

Clark *et al*. (2001) demonstrated that each of the plastron bones from a 50-day turtle embryo (*Trachemys scripta*), near the time of hatching, stained positively with the HNK-1 (human natural killer-1, sulfated carbohydrate epitope) antibody and with antibodies directed against PDGFR*a* (the alpha subunit of the platelet-derived growth factor receptor). These are two markers of skeletogenic neural crests. Cebra-Thomas *et al*. (2007) examined earlier stage slider turtle (*Trachemys scripta*) embryos and demonstrated the existence of a population of late-forming cells, arising from the dorsal roof of the neural tube, that stain positively for HNK-1, FoxD3 (Forkhead Box D3, the neural crest-specifying transcription factor), and p75 (the low-affinity neurotrophin receptor). While neither HNK-1 nor PDGFRa are entirely specific for neural crest cells, according to Gilbert *et al*. (2007), the combination of HNK-1, p75, and FoxD3 are specific to neural crest cells.

Gilbert *et al*. (2007) decided to test if the plastron's bone-forming cells also contain these antibodies. They found that the HNK-1+ cells of the plastron also stain positive for p75+ and FoxD3+. This gives significant support to a skeletogenic neural crest cell origin to the plastron. They also found that the nuchal bone of the carapace tested positive for HNK-1 and PDGFRa, showing that it too is derived from neural crest cells. The neural crest cells appear to be from the trunk neural crest cells, which are not supposed to be able to form bone (Couly *et al*., 1993; Smith and Hall, 1993). According to McGonnell and Graham (2002), the neural crest of the trunk can gain skeletogenic abilities after being in culture for two weeks. McGonnell and Graham cultured midbrain neural cells in media (dexamethasone, ascorbic acid, and Beta-glycerophosphate) commonly used for growing bone and cartilage cells and after four to five weeks in culture the midbrain neural cells gave rise to bone and cartilage cells. Gilbert *et al*. (2007) hypothesized that turtles either delay the timing of the production of trunk neural crest cells, or they have the trunk neural crest cells produced at a normal time, but have them wait in a staging area in order to become skeletogenic. In conclusion these studies show that the formation of the plastron and parts of the carapace may have more endoskeletal influence than previously thought.

Modern Morphological Studies

Scheyer and Sander (2007) showed that the dorsal portions of the costal and neural bones show signs of residual structure of the dermis, based on the external cortex of the bones which consist of interwoven structural collagen fibres. This would later be used by Joyce *et al*. (2009) to conclude that costal and neural bones are the result of ossification of the dermis, supporting the notion that these elements are true composites.

The discovery of a basal turtle with the least sutured shell, *Chinlechelys tenertesta* (Joyce *et al*., 2009), brought new insights into the formation of the shell. In this taxa the thoracic ribs connect very poorly with the overlying costal bones and the neck and tail region are covered in osteoderms. Joyce *et al*. (2009) concluded that these traits, in addition to the findings of Scheyer and Sander (2007), support a composite origin of the turtle shell by fusion of both endoskeletal and exoskeletal elements with ribs that are tightly associated with the costal osteoderms. Joyce *et al*. (2009) agreed with Scheyer and Sander (2007) that the costal and neural bones are dermal in nature, further supporting the theory that that the ribs of turtles grow mediolaterally into the dermis towards the carapacial ridge, as seen in embryological studies (Burke, 1989; Kuraku *et al*., 2005). Scheyer *et al*. (2008) reported that after the ribs grow into the dermis the costal and neural elements, believed to be osteoderms, form onto the rib during the later developmental phase. Unlike other tetrapods with osteoderms, turtles do not have to form independent ossification centres within dermis because the ribs induce ossification. Joyce *et al*. (2009) concluded that the origin of the turtle shell is ultimately one of simplification, where through time, endoskeletal bone established itself as the precipitation surface of dermal bone, and fully armored turtles gave rise to less armored turtles (i.e. the leatherback sea turtle).

In 2017, the classification of *Chinlechelys* was put into question by Joyce. He concluded that *Chinlechelys* is actually a species of *Proganochelys* which he named *Proganochelys tenertesta*. This analysis was based on the holotype specimen containing a cervical spine that greatly resembles that of *Proganochelys.* This character was not originally described at the time of the original description. Joyce (2017) concluded that what was used to define *Chinlechelys* as a separate species, mainly the vertical orientation of the dorsal ribs and separation of the ribs from the overlying metaplastic portion of the carapace (i.e., least sutured shell), is less significant than previously thought. *Proganochelys* also possesses thin, vertically oriented ribs.

Joyce did not specify what caused the separation between the ribs and the carapace in the *Chinlechelys* specimen. Both *Proganochelys* and *Chinlechelys* have neck spines. It was also in Joyce (2017) that it was said that Joyce *et al.* (2009) was accepted long before the appearance of the description of *Odontochelys* in Li *et al.* (2008), which countered the view by Joyce *et al.* (2009) that the ribs formed from the fusion of exoskeletal and endoskeletal components.

The structure of *Odontochelys semitestacea* (Li *et al*., 2008), with only a plastron but no carapace, mimics the appearance of the plastron before the carapace in turtle embryology (Rieppel, 1993a; Sheil and Greenbaum, 2005). This discovery led others to hypothesize about the origin of the turtle carapace. Nagashima *et al*. (2009) studied the embryos of Chinese soft-shelled turtle, *Pelodiscus sinensis*, and compared them to mouse and chicken embryos. They found further evidence that the carapacial ridge develops through turtle-specific regulation of cells in the flank (Moustakas, 2008; Kuraku *et al*., 2005) and later grows anteriorly and posteriorly to form a circle that differentiates into the carapacial margin (CR). Nagashima *et al*. (2009) reported that the turtle scapula partially overlies the first rib, but that this rib does not extend or participate in carapace formation (Figure 29). They concluded that in the turtle embryos, the scapula primarily arises anterior to the ribs, excluding the first rib. Because the turtle ribs are confined to the axial domain (because of the axial arrest of ribs), they can only grow laterally and anteriorly to cover the scapula dorsally, while the AS (the anterior serratus muscle associated with the scapula) rotates to follow the scapula as hypothesized by Nagashima *et al*. (2007). By looking at the orientation of the AS muscle in turtle embryos, Nagashima *et al*. (2009) concluded phylogenetically that the scapula was always morphologically outside the prospective rib cage (Figure 29). The carapacial ridge does not

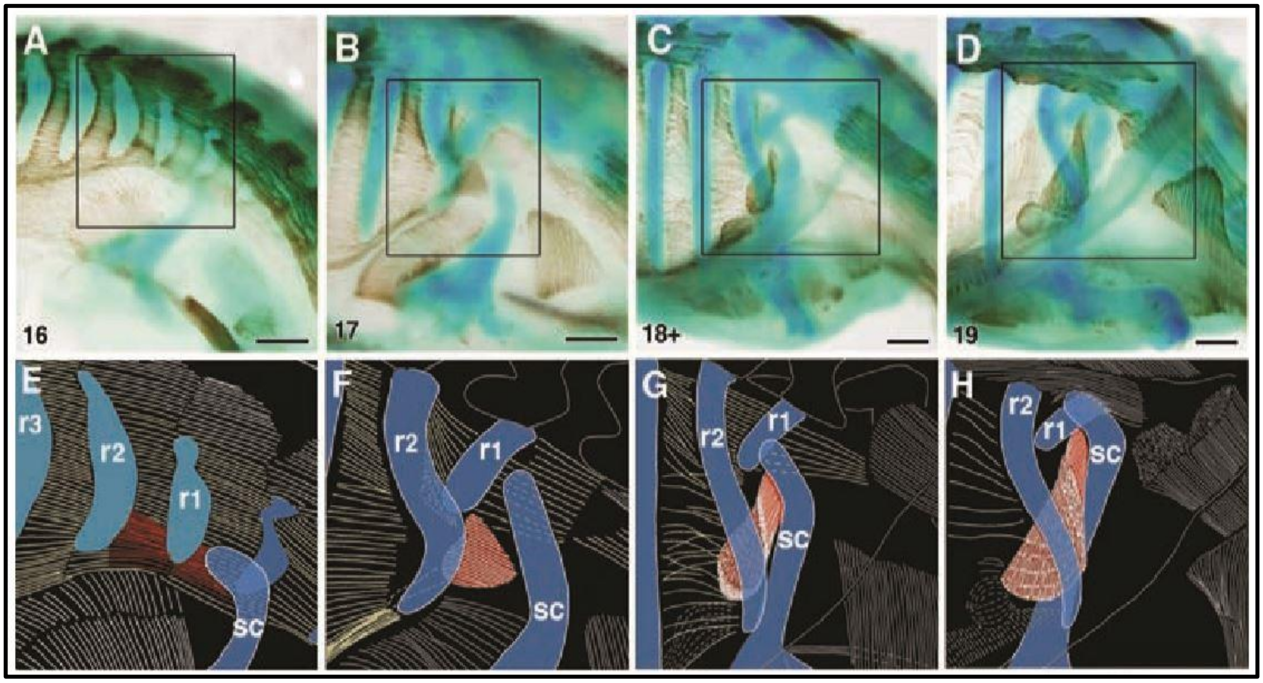


Figure 29. Developmental twisting of the anterior serratus (AS) muscle. Here seen in the embryo of a Chinese soft-shelled turtle (*Pelodiscus sinesis*). (A to D) shows stages 16 to 19 stained with alcian blue to show ribs and scapula (blue) and muscles were immunochemically stained with MF20 (brown). (E to H) show magnified schematic view of ribs (r1 to r3) and (sc) scapula (blue) and isolated AS muscle (red). Scale bar = 500 μm. Modified from Nagashima *et al.,* 2009

appear to induce the axial arrest of the ribs as suggested by Burke (1991) and Cebra-Thomas *et al*. (2005), but rather it functions in the flabellate (fan-shaped) expansion of the turtle ribs in late development (Nagashima *et al*., 2009).

Nagashima *et al*. (2009) stated that this type of development is consistent with *Odontochelys* not having fan-like ribs, but rather ribs that point to the medial most rib (Figure 30). They concluded that the ribs of *Odontochelys* appear to be already axially arrested because they never bend strongly ventrally. They hypothesized that the carapacial ridge has already formed in the flank (i.e., the lateral bridge present), but the carapacial did not persist and encircle the carapacial margin as in modern turtles because the fan-shape arrangement of the second to eighth ribs is not apparent in *Odontochelys* (Figure 30). Nagashima *et al*. (2009)

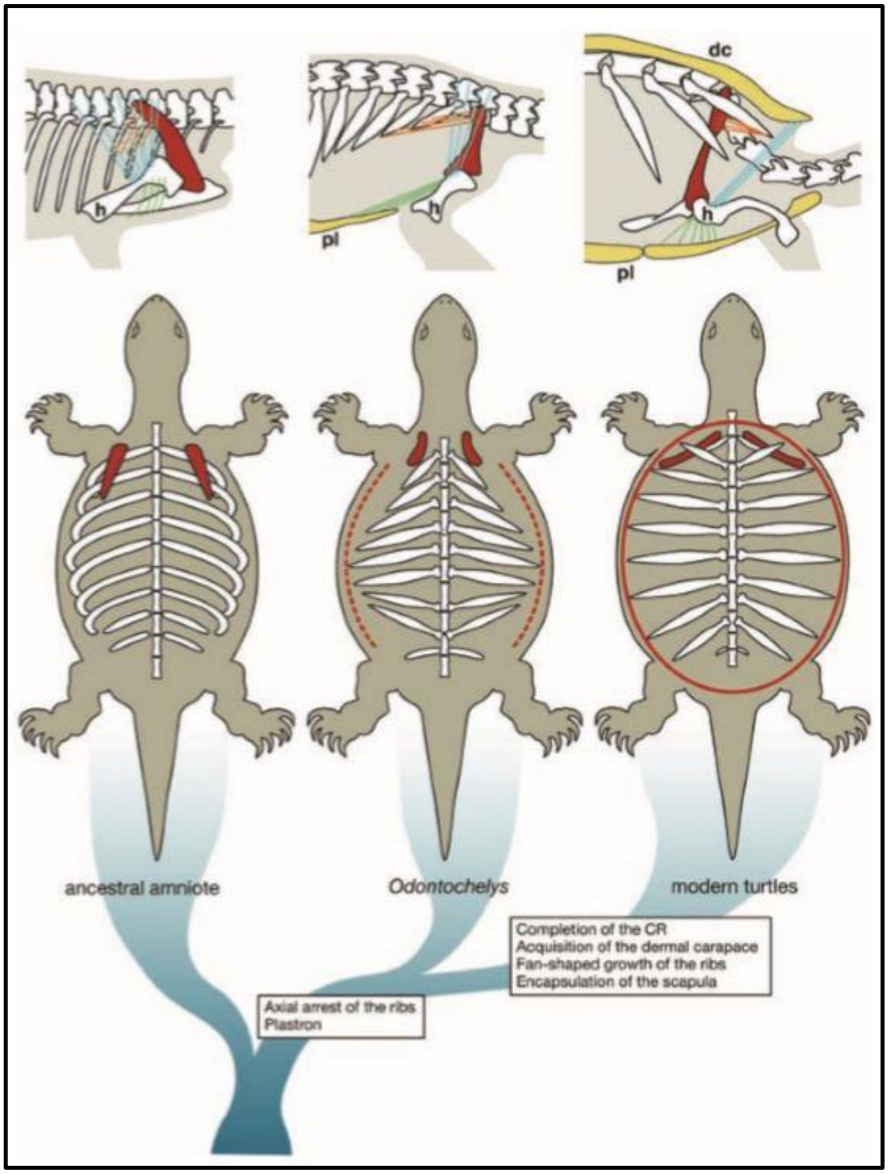


Figure 30. Hypothetical evolution of the turtle body plan by Nagashima *et al.* (2009). **(Top)** A hypothetical sequence of changes in musculoskeletal connectivities. In *Odontochelys,* the AS would have connected the scapula and distal tips of the anterior ribs anterior-posteriorly. The pectoralis would have connected the humerus and the plastron. Scapula (red), AS muscle (orange), latissimus dorsi muscle (blue), pectoralis muscle (green), and (dc) dermal carapace, (h) humerus, and (pl) plastron. **(Bottom)** A hypothetical phylogenetic scenario. In *Odontochelys*, the CR (red broken lines) may have developed only temporarily and incompletely in the embryo. In modern turtles, the CR (red solid line) forms a complete circle, inducing the fan-shaped growth of the ribs. Modified from Nagashima *et al.,* 2009

hypothesized that extant turtles may have their unique body plan by passing through an *Odontochelys*-like ancestral state during embryologic development, and that the formation of the shell is only induced in modern turtles after the completion of the carapacial ridge (Figure 30). They concluded that the true novelty in turtles is in the axial arrest of the rib growth.

Other scientists (Kuratani *et al*., 2011; Sanchez-Villagra *et al*., 2009) looking at turtle embryos noticed similar histological novelties to those reported by Nagashima *et al*. (2009). *Odontochelys* prompted Sanchez-Villagra *et al*. (2009) to state that the sequence of evolutionary events derived from the fossil record largely mirrors the sequence of ossifications in turtle embryos. Kuratani *et al*. (2011) went so far as to write that the position of the scapula anterior to the ribcage in *Odontochelys* supports the idea that turtle's evolved from an ancestor with the scapula not within the ribcage, but rather positioned anterior to it. This view would later gain further support when Lyson *et al*. (2013) hypothesized the early reptile *Eunotosaurus* with expanded ribs and a scapula that is also positioned anterior to the ribcage.

The discovery of a Sauropterygian with turtle-like features, *Sinosaurosphargis yunguiensis*, by Li *et al*. in 2011 put doubt into the endoskeletal hypothesis that was gaining ground prior to the discovery. This new taxa had transversely broadened ribs creating a rib basket covered in dorsal osteoderms. This is the only feature that unites this genus with Testudines. Li *et al*. stated that a discovery like this exhibits turtle shell formation through the fusion of osteoderms as discussed by Versluys (1914) and Lee (1996; 1997). The lack of osteoderms on *Odontochelys* conflicts with this view, but Li *et al*. explains this by stating that *Odontochelys* may have a reduced carapace, including osteoderm elements, because of skeletal paedomorphosis as an adaption to an aquatic mode of life as postulated by Reisz and Head

(2008) and Burke (2009). *Sinosaurosphargis* does differ from other turtles by having an elongated and distally expanded transverse processes of the dorsal vertebrae, while the transverse processes are very short in *Odontochelys.*

A later developmental study done by Hirasawa *et al*. in 2013 demonstrated that the costal and neural bones of the turtle carapace are hypertrophied (abdominally large) ribs and vertebrae, indicating that the major part of the turtle carapace evolved solely by the modification of the endoskeleton. The costal and neural osteoderms were long thought to be of exoskeletal origin, but in recent studies have been shown to exhibit an endoskeletal origin (Nagashima *et al*., 2009; Sanchez-Villagra *et al*., 2009; Kuratani *et al*., 2011;). Unlike the study done by Scheyer *et al*. (2008), Hirasawa *et al*. (2013) witnessed the ribs cause the metaplastic ossification of the overlying dermal tissue as opposed to the surrounding tissue. They placed turtles and *Sinosaurosphargis* sister to Sauropterygia, the placodonts of which also exhibits this completely endoskeletal formation of their shell-like armor. In modern time, this view has been promoted by Rieppel and deBraga in 1996, Müller and Tsuji (2007), and Li *et al*. (2008).

Question 6. What role, if any, does the fossil *Eunotosaurus* play in the origin of turtles?

Eunotosaurus africanus (Seeley, 1892) has played a major yet controversial role in our modern understanding of turtle evolution. Seeley proposed a relationship between it and Testudines because of its flattened ribs, but this view was not generally accepted due to the lack of a skull on any of the specimens. It wasn’t until the late 1900s when a skull was finally discovered. This section will discuss not only its relevance to turtle evolution, but also the reason why it was not initially accepted in the scientific community.

The first five specimens of *Eunotosaurus* were found and named in 1892 at Beaufort West, Cape Colony, South Africa by H. G. Seeley. It is a small sized reptile of Permian age origin with posterior-anteriorly enlarged ribs causing the body to appear inflated (Figure 31). None of the specimens found by Seeley had a skull. There were a total of seven dorsal vertebrae (shown to be ten by Watson, 1914) of an elongated hour-glass form, as stated by Seeley. He described the specimen's vertebrae in form and number to be similar to a Testudine type. For example, the ribs being attached closely to the sides of the neural arches of the vertebrae resembling that of turtles. The ribs are very massive, long, and convexly curved. They appear as wide as the vertebrae are long. Seeley stated that the ribs also resemble that of the ribs and costal plates of turtles, but they are free at the margins unlike turtles. In this study *Eunotosaurus* was placed in the primitive clade *Mesosauria* based on a notch at the external posterior border of the pubis.

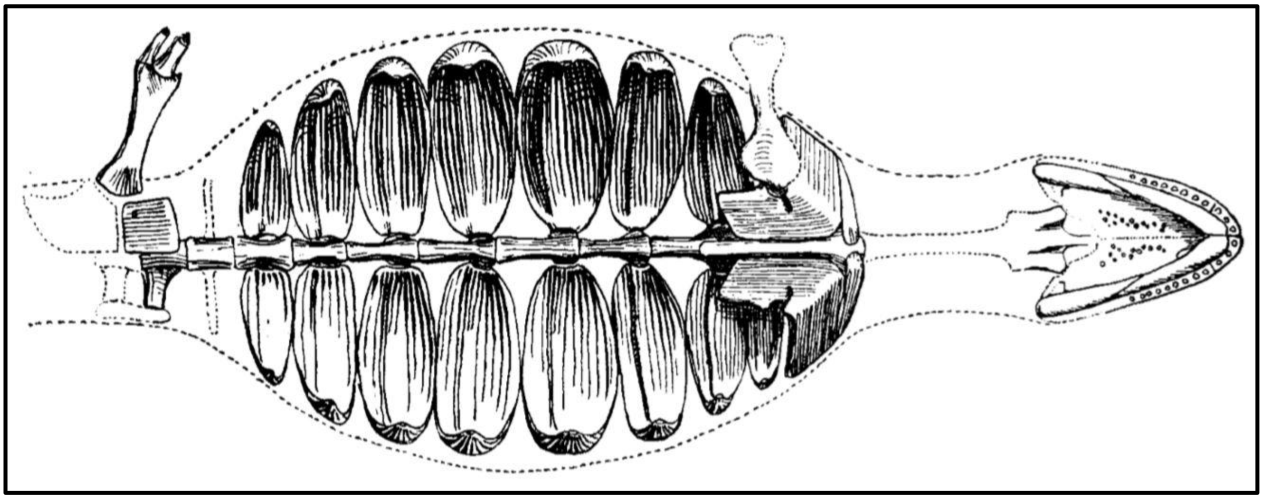


Figure 31. Skeletal reconstruction of *Eunotosaurus africanus* in ventral view. Composed by Watson (1914) from five specimens. From Watson, 1914

In 1914, D.M.S. Watson re-described the five specimens used by Seeley (1892). This is the first time *Eunotosaurus* was mentioned as an ancestor species of turtles. The specimens were in bad shape, but the pectoral girdle did resemble that of procolophonids, by having a large scapula with no acromion process fused with a small coracoid (Figure 5, p. 4). He was able to uncover some parts of the upper jaw, including the palate from one of the specimens, but the rest of the skull is missing. Watson (1914) proposed that *Eunotosaurus* was a possible transition species he termed “Archichelon” between procolophonids and turtles. He discussed other features that *Eunotosaurus* and Testudines share, including the capitular facets being far forward on the centrum of the vertebrae, the anterior positon of the neural arches on each dorsal centrum, and the loss of a definite tuberculum on the dorsal ribs is suggesting the loss of articulation in turtle ribs. The possession of a short, powerful ulnar crest on the humerus and the slight upturning of its head are also similar between the two groups.

This idea was elaborated when Williston (1917) named Anapsida and placed Cotylosauria and its descendant Testudines within it. Broom (1924) expressed that nothing was known about the skull of *Eunotosaurus*, and the other features used to connect them with turtles, mainly the enlarged ribs, could also be found in *Mesosaurus* and other aquatic forms. Therefore, he ruled that the conclusion of *Eunotosaurus* being a turtle ancestor was inconclusive. This led the way for Parsons and Williams (1961) to observe that the endochondral ribs of modern turtles are significantly narrower than the overlying metaplastic bone. This is most apparent in many juvenile turtles and various aquatic turtles, where the distal portions of the ribs are not covered by metaplastic bone. Joyce (2015) stated that Parsons and Williams (1961) incorrectly presumed that the broadened elements of *Eunotosaurus* consists of only endochondral rib bone and not metaplastic (perichondral) outgrowths. Parsons and Williams stated that the lack of metaplastic outgrowths, paired with an endochondral rib that is much narrower proportionally to that of Eunotosaurus, shows a lack of a relationship between the two. Lyson *et al*. (2013) revealed that the expanded ribs of *Eunotosaurus* consists of vertically oriented endochondral rib and horizontally expanded perichondral outgrowths of the ribs, a character previously thought to be unique to turtles (Scheyer *et al*., 2008b.). Joyce (2015) would go on to say, "This assessment (Parsons and Williams, 1961) was not only incorrect, but also had far reaching effects, as most following authors (Cox, 1969) disregarded the *Eunotosaurus* hypothesis completely."

The first and only good skull of *Eunotosaurus* was described by Keyser and Gow (1981). In this study, *Eunotosaurus* was placed from the order Testudines into the order Cotylosauria, as was the view of Cox (1969) based on postcranial features. Keyser and Gow (1981) reported on the hardness of the fine-grained, mudstone matrix the fossil was in and that chemical methods of preparation were ineffective. Unlike what was reported by Watson (1914), the skull does have premaxillary bars, unlike turtles. Keyser and Gow (1981) conclude that the skull is of the primitive anapsid type. They concluded that *Eunotosaurus* is more primitive than turtles based on the skull possessing primitive features such as supratemporals, postfrontals, lacrimals (small bones forming the eye socket), pineal foramen (relating to the pineal eye) and a premaxillary bar (Figure 32). They reported that the open lateral temporal region (later reported as a lower temporal fenestra by Gow, 1997) is seen in other cotylosaurs such as millerettids and the procolophonid, *Owenetta.* Romer (1956) placed *Eunotosaurus* into the suborder Eunotosauria of the order Testudines, but Keyser and Gow (1981) stated that there is no turtle affinities present in *Eunotosaurus*, and that this suborder be placed in the order Cotylosauria. Lee (1993a) used this new skull description to place *Eunotosaurus* as a basal synapsid when he was arguing that turtles were advanced pareiasaurs.

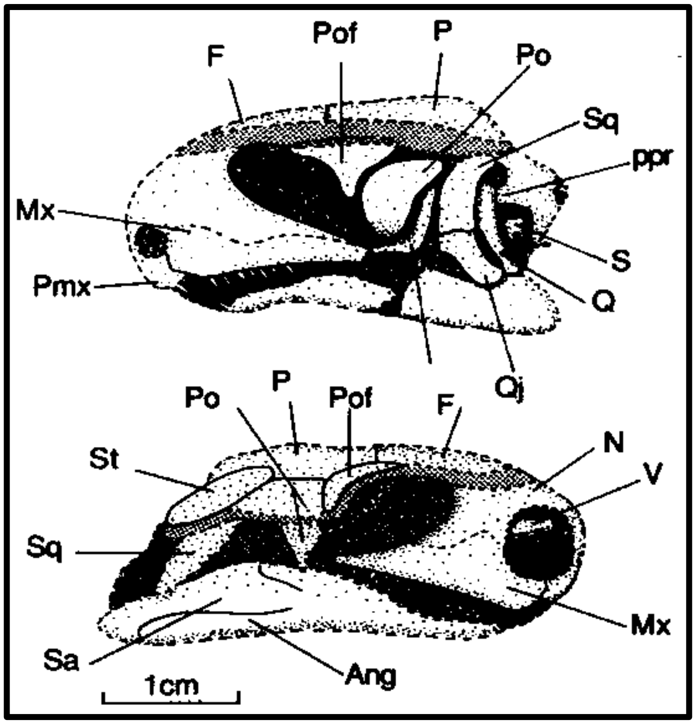


Figure 32. Lateral views of *Eunotosaurus* skull. Black is matrix. Dotted texture indicates epoxy cement. Abbreviations: (Pmx) premaxilla, (Mx) maxilla, (F) frontal, (Pof) postfrontal, (P) parietal, (Po) postorbital, (Sq) squamosal, (ppr) paroccipital process, (S) stapes, (Q) quadrate, (Qj) quadratojugal, (St) supratemporal, (N) nasal, (V) vomer, (Ang) angular, and (Sa) suprangular. Modified from Gow, 1997

The discovery of yet another skull *Eunotosaurus* specimen prompted Gow (1997) to do a full re-description of *Eunotosaurus* in its entirety. His interpretations were built primarily on one nearly complete large, dorso-ventrally crushed specimen with a skull, pectoral girdle, and anterior trunk all in articulation. The preparation was reported by Gow to be far from ideal, with most surface detail, such as the ornamentation of the dermal bones of the skull, being lost due to the preparation. He found that *Eunotosaurus* did have a lower temporal fenestra that experienced secondary emargination and, therefore, is open on the ventral side. The temporal fenestra is bounded dorsally by the postparietal, parietal, and supratemporal. He found that three of the seven autapomorphies of Parareptilia support *Eunotosaurus* as a parareptile. Those three are the temporal emargination formed by the quadratojugal and squamosal, paroccipital process contacting the supratemporal, and the iliac blade dorsally expands into a fan like structure. He placed *Eunotosaurus* as sister to Millerettidae with both groups sister to

Ankyramorpha (deBraga and Reisz, 1996). Ankyramorpha was defined by deBraga and Reisz to include the most recent common ancestor of Procolophonomorpha and Lanthanosuchoidea and all its descendants. Gow (1997) reported that *Eunotosaurus* shares only one derived character with Ankyramorpha, namely the anteroposteriorly expanded paroccipital process. He stated that *Eunotosaurus* has a low presacral (cervical and thoracic) vertebral count (fewer than 20), which matches the low presacral count found in turtles and pareiasaurs, (at least 18).

It wasn't until 2010 when Lyson *et al*. included *Eunotosaurus* in a phylogenetic study about turtle origins. They placed *Eunotosaurus* and *Proganochelys* in the unaltered dataset that Li *et al*. (2008) used to reach the conclusion that turtles are sister to a lizard-tuatara clade. Given that both major amniote matrices converge on a subgroup of parareptiles as stem turtles, Lyson *et al*. (2010) also placed the newly discovered *Odontochelys semitestacea* (Li *et al*., 2008) and *Proganochelys* in the most recent parareptile dataset (Müller and Tsuji, 2007). Lyson *et al*. (2010) used PAUP 4.0b10 with all characters unordered and unweighted, as was done in an early cladistic analysis (Rieppel and deBraga, 1996). The analysis of both datasets (lizard-tuatara dataset and the parareptile dataset) nested turtles within parareptiles as the extant sister to Diapsida (Figure 33). Lyson *et al*. (2010) stated that *Odontochelys* and *Proganochelys* possess all six unequivocal synapomorphies listed by Tsuji and Müller (2009) that diagnose Parareptilia. *Eunotosaurus* and Testudines share T-shaped (in cross-section), ribs abutted to each other that taper to finished points (indicating cessation of growth), 10 elongated dorsal vertebrae, cranial tubercles and a wide body form (Watson, 1914; Cox, 1969; Gow, 1997). Lyson *et al*. (2013) later showed more turtle affinities with *Eunotosaurus*, mainly paired gastralia that lack both lateral and median elements, the loss of intercostal muscles

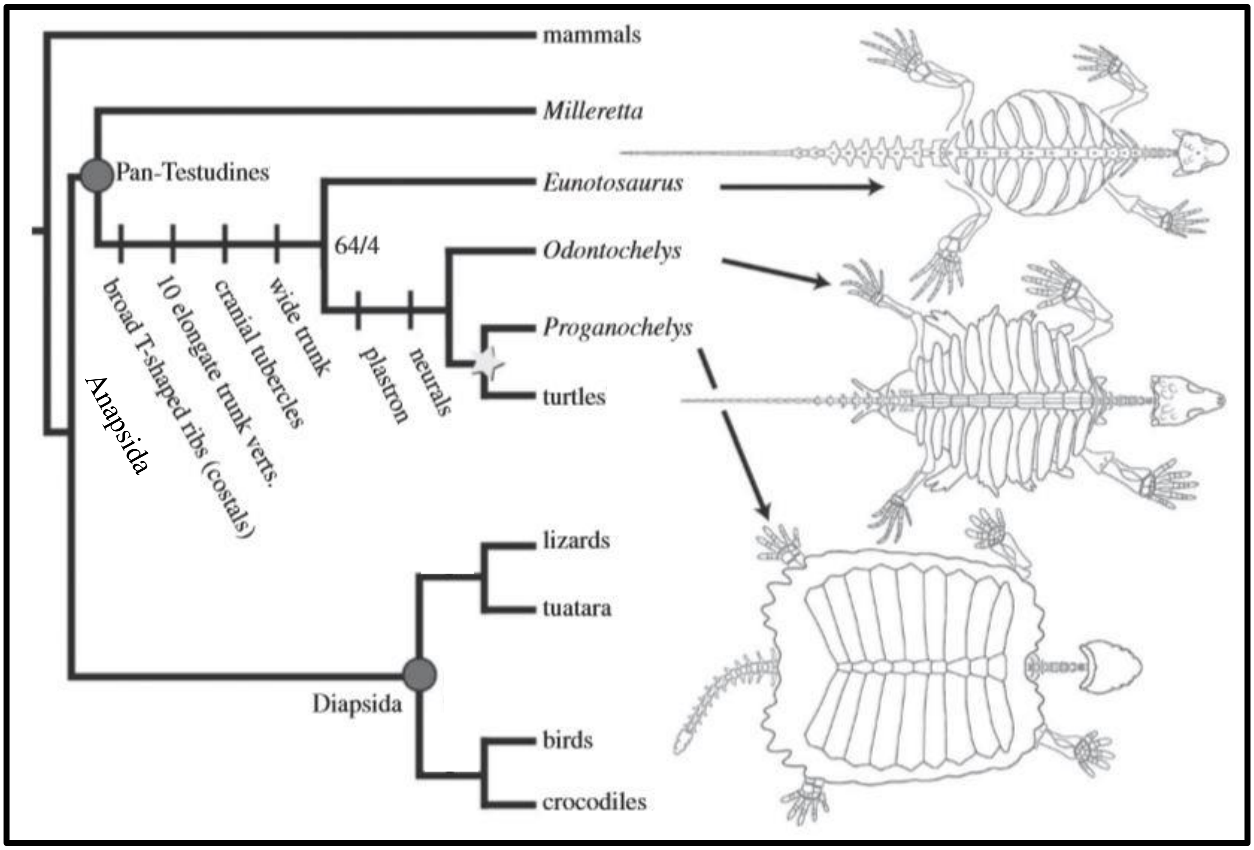


Figure 33. Cladogram composed by Lyson *et al*. (2010). It supports turtles outside of Diapsida. Uniquely derived features of significant nodes are given. Bootstrap and Bremer support values are given (separated by a slash) for *Eunotosaurus* clade. Star indicates complete shell. Modified from Lyson *et al*., 2010

(Sharpey's fibers only present on ventral surface of ribs), dermal outgrowth of bone from the perichondral/periosteal collar of the developing ribs, and these features encompassing both embryologic and adult morphology (Figure 34). Lyson *et al*. (2013) also concluded that the

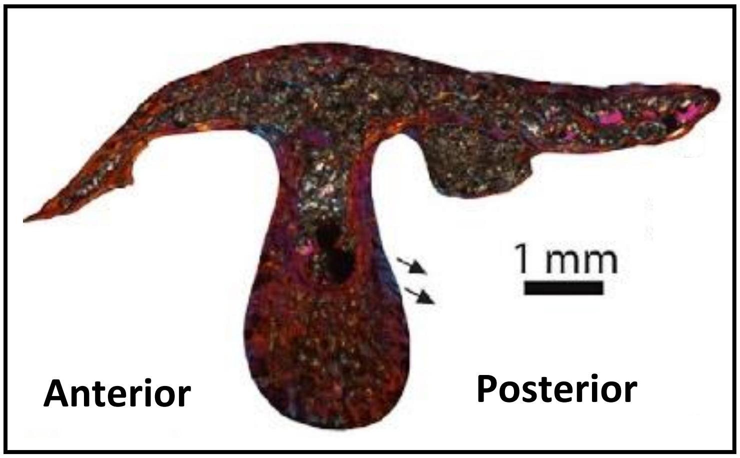


Figure 34. Histological section of T-shaped ribs of *Eunotosaurus* in polarized light. This is a cross-section of one of the ribs. Black arrows indicate the presence and orientation of Sharpey's fibers on ventral shaft. Modified from Lyson *et al*., 2013

relationship between *Eunotosaurus* and *Odontochelys* is consistent with the view of Nagashima *et al*. (2009) and Kuratani *et al*. (2011) that the early stages of the turtle shell was marked by a scapula positioned in front of the rib-cage.

The view of Lyson *et al*. (2013) gave heavy support to a turtle relationship with basal Diapsida, which until that point had taken a backseat to the archosaur-lepidosaur debate that consumed much of the 2000s. Up until this point turtles had been proposed as sister to Archosauria (Cao *et al*., 2000; Hugall *et al*., 2007), Lepidosauria (Rieppel and Reisz, 1999; Li *et al*., 2008), and Diapsida in general (Lee *et al*., 2008; Werneburg and Sanchez-Villagra, 2009). The Archosauria hypothesis had large support in the molecular community, while the lepidosaur hypothesis had support in the morpho-paleo community. Lyson *et al*. (2010, 2013) took the interpreted anapsid condition of *Eunotosaurus* to show a relationship between turtles and Anapsida. These studies nested *Eunotosaurus* and turtles within parareptiles, sister to procolophonids and pareiasaurs (Figure 33). Anapsid analyses of *Eunotosaurus* that exclude turtles because they were thought to be diapsid, nested *Eunotosaurus* with other parareptiles (Tsuji *et al*., 2012). Needless to say, the conclusions of Lyson *et al*. (2010) were met with great skepticism. Lee (2013), who promoted a parareptile origin of turtles but with pareiasaur affinities, commented on the instability (i.e., weak Bayesian and bootstrap support) of the Lyson *et al*. (2010) clade that had turtles paired with *Eunotosaurus*. Lee ran a series of cladistic analyses utilizing scaffolding (a backbone phylogenetic constraint using other phylogenies as a guide; Springer *et al*., 2001), that mirrors what genomic data was indicated at the time which was that turtles are sister to archosaurs (Chiari *et al.*, 2012; Crawford *et al.*, 2012). Lee found that when this scaffold was used it did not affect the support of the other clades in Diapsida, but when a scaffold that united turtles to *Eunotosaurus* was used, then the other clades became weakly supported in Diapsida. In short, if *Eunotosaurus* does have a relationship with turtles, then all archosaur or lepidosaur studies would make that relationship very weakly supported.

Bever *et al*. (2015) used computed tomography (CT) to locate what they considered an upper temporal fenestra (UTF) of two adult *Eunotosaurus* skulls as well as emarginated lower temporal fenestra (LTF). One of the skulls was the same as the one described by Keyser and Gow (1981) and later by Gow (1997), the second partial skull was previously undescribed. Gow (1997) considered the first skull to only have a lower temporal fenestra. Bever *et al*. (2015) performed observational studies on a third juvenile specimen, but they were unable to CT scan it. In both adult and juvenile specimens the lower temporal fenestra is ventrally open. Using CT technology, they were able to locate the upper temporal fenestra closed by an elongated supratemporal bone in the adult stage. Digital removal of the supratemporal reveals that the UTF has a reduced diameter due to the expansion of the surrounding elements, primarily the postorbital and squamosal (Figure 35). They hypothesized that the relatively late ontogenetic expansion of these elements reduced the circumference of the LTF and modified its rounded shape. Bever *et al*. stated that the ventrally open nature of the LTF doesn’t agree with *Eunotosaurus*’s parareptile classification, but rather with a basal diapsid classification where an open LTF is known only in conjunction with a more conservative UTF. Bever *et al*. stated that the covering of the UTF is secondarily derived because the supratemporal bone fully overlaps the postorbital, whereas plesiomorphically (ancestrally) these two elements are touching or overlapping at their edges only. Bever *et al*. concluded that the ecological context for the closure of the UTF is unclear, but it may have to do with the masticatory (chewing) apparatus. This study is significant in supporting the view that *Eunotosaurus* is a diapsid that experienced secondary closure of the UTF. Furthermore, they stated that if *Eunotosaurus* is related to turtles, then turtles too may be of diapsid origin, as has been reported by genomic data (Wang *et al*., 2013, Field *et al*., 2014) (see Figures 19, 20, 21).

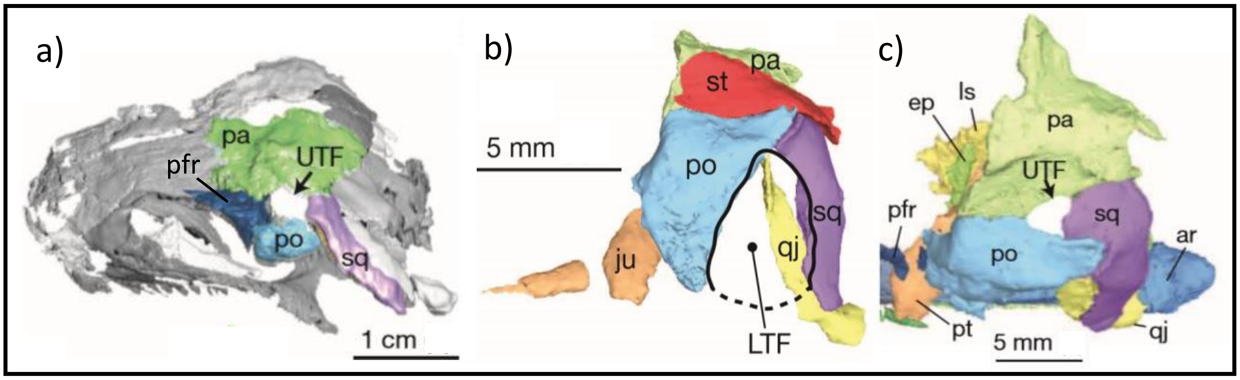


Figure 35. Digitally rendered photos of *Eunotosaurus* skulls. a) Adult skull of *Eunotosaurus* (CM777) in dorsolateral view after digital removal of (st) supratemporal bone reveals (UTF) upper temporal fenestra. b) Digitally rendered and reflected right lateral view of the temporal region of *Eunotosaurus* (CM86-341) with (st) supratemporal bone still in place showing ventrally open (LTF) lower temporal fenestra. c) Digitally rendered adult skull of *Eunotosaurus* (CM86-341) in dorsal view after the digital removal of the (st) supratemporal bone revealing (UTF) upper temporal fenestra. Abbreviations: (pa) parietal, (pfr) postfrontal, (po) postorbital, (sq) squamosal, (st) supratemporal, (ju) jugal, (qj) quadratojugal, (ep) epipterygoid, (ls) laterosphenoid, (pt) pterygoid, (ar) articular, (UTF) upper temporal fenestra, and (LTF) lower temporal fenestra. Modified from Bever *et al.,* 2015

The discovery of the new possible basal turtle, *Pappochelys rosinae*, by Schoch and Sues (2015) demonstrated a rib shape morphology that is intermediate between *Eunotosaurus* and *Odontochelys*. The ribs of *Pappochelys* are anteroposteriorly broad, T-shaped in cross section, and contain dorsal sculpturing (Figure 36). The T-shaped ribs are a shared trait of *Pappochelys, Odontochelys,* and *Eunotosaurus.* The plastron region of *Pappochelys* features large paired normal gastralia similar to *Eunotosaurus*, as opposed to a fully formed plastron (Figure 36). Several of the larger gastralia appeared to have formed by the fusion of several successive

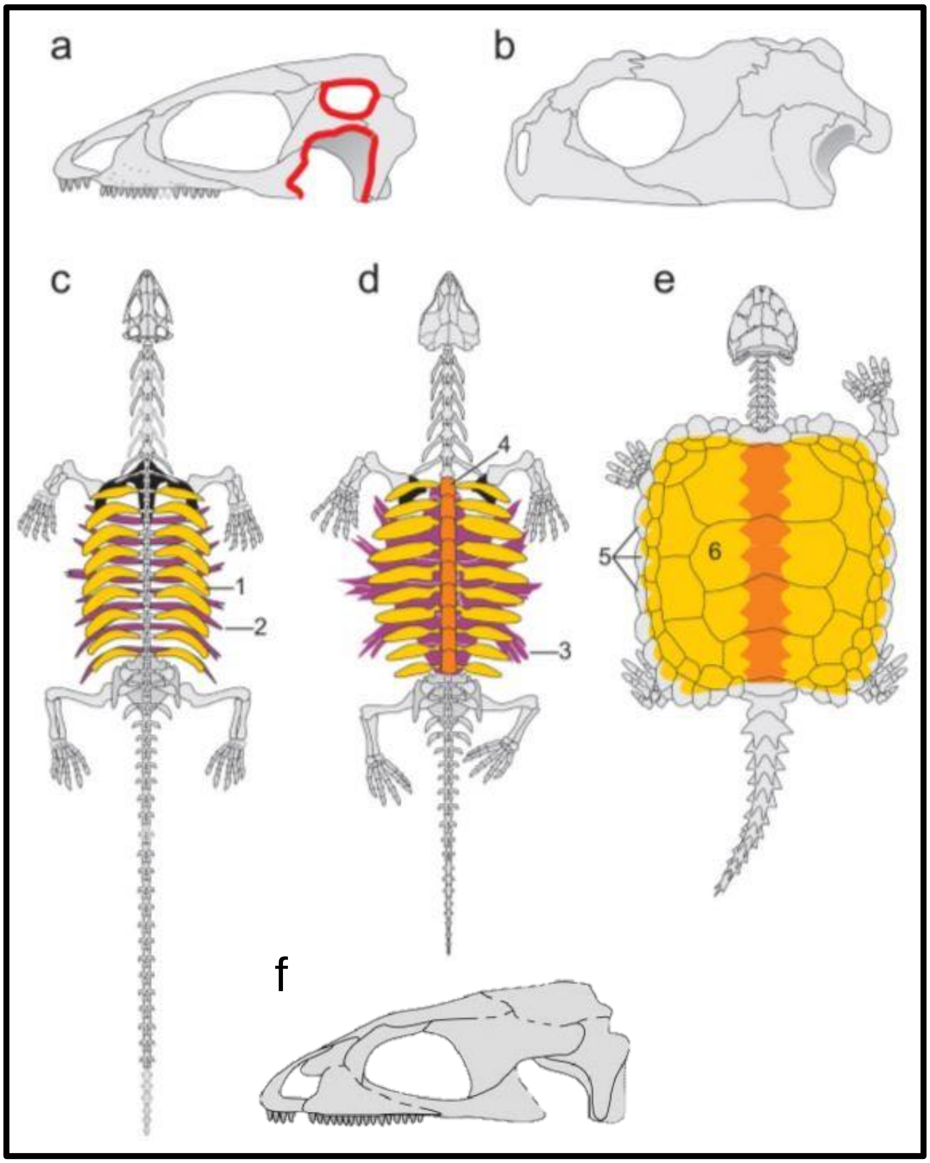


Figure 36. Skulls and bodies of *Pappochelys*, *Odontochelys*, and *Proganochelys.* Dorsolateral views of the skulls of a) *Pappochelys* and b) *Proganochelys*. Fenestrae marked with red. Dorsal view of c) *Pappochelys* d) *Odontochelys*, and e) *Proganochelys*. f) Dorsolateral view of the skulls of *Odontochelys.* Gastralia in purple. Neural bones in orange. Costal bones and other marginal elements in yellow. Modified from Schoch and Sues, 2018, 2019

gastralia based on the forked nature of their distal ends. Schoch and Sues stated that this fusion represents the increased ossification of the ventral region as seen in turtles. The lateral ends of the plastral elements of *Odontochelys* form spine-like projections (Li *et al*., 2008) that resemble the distal ends of the gastralia in *Pappochelys* in alignment and texture. The pubis has a distal lateral process where it contacts the plastron as seen in *Odontochelys.* The outline of the ilium closely resembles that of *Proganochelys.* However, the coracoid of *Pappochelys* is not as expanded and the acromion process is shorter than that of *Proganochelys*. The ventrally open lower temporal opening of *Pappochelys* is also shared with *Eunotosaurus* (Bever *et al*. 2015, 2016). Later Bever *et al*. (2016) stated that due to the size of *Pappochelys'* lower temporal fenestra, intermediate between the juvenile and adult *Eunotosaurus* specimens, paired with the unfused nature of *Pappochelys*' scapulacoracoid (Figure 5, p. 4) (Schoch and Sues, 2015), the holotype of *Pappochelys* may be skeletally immature, representing a juvenile stage.

In 2016, Lyson *et al*. compared the broadened ribs of *Eunotosaurus* to extant taxa (gopher tortoise, giant anteater, mole, badger) that specialize in a fossorial, digging lifestyle. They postulated that the broad ribs would provide a stable base to operate digging mechanisms as well as additional stability to the vertebral column. This digging mechanism is similar to the gopher tortoise which use their head and neck to brace their bodies as their forelimbs dig. Lyson *et al*. (2016) used recently discovered sclerotic rings in a *Eunotosaurus* skull to determine the eye sockets optical ratio ((internal diameter of the sclerotic ring)^2/(orbital length X external diameter of the sclerotic ring)). They calculated it to be 0.0209, which indicates an eye with low sensitivity to light (Bramble, 1982,). Compared to the eyes of only fossorial animals (Hildebrand, 1985), Lyson *et al*. (2016) stated that the relatively large eyes of *Eunotosaurus* (~10 mm) are similar to fossorial animals that dig for shelter but habitually forage above ground (i.e., gopher tortoise). In fossorial limbs the manual ungula phalanges are both wider and longer than those in non-fossorial taxa. Lyson *et al*. further reported that many stem turtles, *Eunotosaurus, Odontochelys, Proganochelys,* and *Palaeochersis*, have this characteristic and used it (and other characters discussed by Lyson) to create a hypothesized cladogram (Figure 37). Lastly, Lyson *et al.* (2016) concluded that a fossorial lifestyle may have been a result of environmental stresses and may have helped *Eunotosaurus* to survive the end-Permian extinction. Burrowing has been hypothesized to be a behavioral strategy used in response to environmental stresses (Fernandez *et al*., 2013) and has been hypothesized to allow taxa to survive the end-Permian extinction (Botha, 2003).

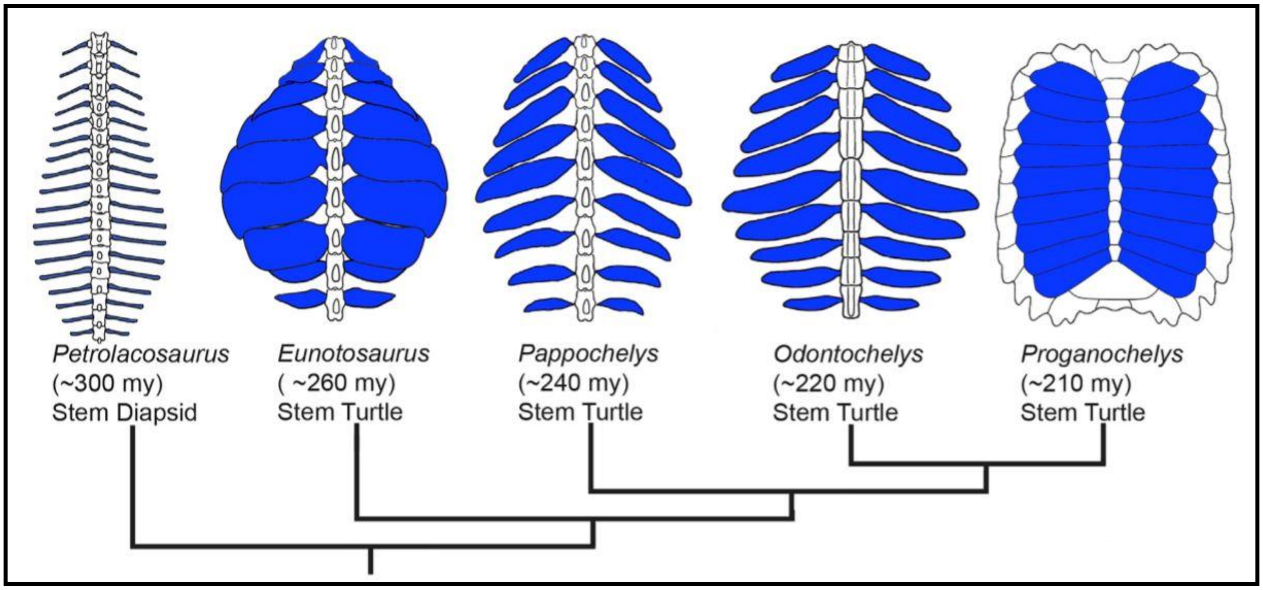


Figure 37. Cladogram proposed by Lyson *et al.* (2016). It shows anteroposterior broadened ribs and proposed progression of the formation of the shell in stem turtles. Stem turtles are placed within Diapsida. Approximate ages of the fossils have been given. Rib elements in blue. From Lyson *et al.,* 2016

Chapter 4: Analysis and Conclusion

Analysis

In this chapter, evidence, interpretations, and conclusions that were presented in each of the papers discussed so far are critically examined and answers are provided to the questions presented in Chapter 3. The answers to the questions are based on current understanding and critical review of the literature. Some of the questions are addressed together due to their interrelatedness. Question 1 is covered in the context of Question 2. Question 6 is discussed in the evaluation of *Eunotosaurus* within Question 2. Each critique is in reference to the question asked, and if a specific study is not mentioned, it is because it was considered not pertinent to the question. The answers appear at the end of each section of this text designated to a question or series of questions.

Question 1. Turtles are reptiles; therefore, how have they been classified within Reptilia?

Question 2. From which reptile group did turtles evolve?

Cladistic Analysis

Due to turtles being vertebrates with four limbs and the presence of an amnion within turtle eggs, it is obvious that Testudines lie within Amniota (Haekel, 1866) and Tetrapoda (Linnaeus, 1758). Amniotes have long been classified by the number or lack of temporal fenestrae on the skull. These skulls types have been named and in some cases used to define clades (Figure 2). The skull types fall into two categories, descriptive use of the term or a cladistic, phylogenetic use of the term. The differential diagnostic features of turtles include an anapsid skull, the use of skeletal and dermal elements to form a shell (carapace, plastron, and lateral bridge), and a scapula with a fan-shaped coracoid and uniquely shaped acromion process lying within the ribcage. Turtles have also been characterized by a loss of teeth. Reptilia is currently defined in a cladistic methodology as the most recent common ancestor of diapsids and all its descendants (Laurin and Reisz, 1995). If this definition is to be upheld, then turtles with an anapsid skull would lie outside of Reptilia. The question that has been plaguing the position of turtles in the tree of life is whether their anapsid skull condition is secondarily derived from a diapsid skull, or whether it represents the condition outside of Reptilia as defined above. In this section the morphology of turtles and their proposed ancestral groups will be discussed, with attention given to specific, individual taxa that have been proposed as transition species. Molecular studies are discussed in the next question.

The early idea was that the process of closure or emargination seen in the lower temporal region of turtle skulls did not resemble that of diapsids (Baur, 1889, 1895; Cope, 1892), and therefore turtles were placed in different clades outside of Reptilia, and in some cases even having groups created specifically for them. Cope (1892) and Baur (1895) concluded that the emargination in turtles occurs from below, i.e., from the margin of the subtemporal fossa, and from behind, i.e., from the margin of the posttemporal fossa, rather than by fenestration as seen in other reptiles (from deBraga and Rieppel, 1997). Cope (1892) suggested that emargination from the lower temporal part of the skull also occurs in Sauropterygia. Some of the groups turtles were placed in or sister to include the sauropterygian Plesiosauria (Cope, 1892; Baur, 1887; Lydekker, 1889) and Placondontia (Jaekel, 1902; 1907), Synapsida (Osborn, 1903), Sauropsida (Goodrich, 1916), Anapsida (Williston, 1917), Pareiasaurs (Jaekel, 1915; Gregory, 1946), and Parareptilia (Olson, 1947).

There was a time that a captorhinid origin was proposed for turtles based on both possessing the anapsid skull condition, large post-temporal shallow depressions (fossae) separated by a narrow supraoccipital, and the paroccipital process being braced against the squamosal (Clark and Carroll, 1973). The only features that today would unite turtles and captorhinids would be an anapsid skull type, lack of ectopterygoids, and a basisphenoid (Figure 9), with all other features being proposed as invalid (Reisz and Laurin, 1991; Laurin and Reisz, 1995).

These studies led to the debate in the 1990s between a procolophonid origin (Reisz and Laurin, 1991; Laurin and Reisz, 1995), a pareiasaur origin (Lee 1993, 1996, 1997), and a lepidosaur origin (Rieppel and deBraga, 1996; Rieppel and Reisz, 1999). The first two hypotheses went back and forth with both scientists' proposing characters and cladistics to support their turtle grouping. It is difficult to determine which hypothesis has the most support because the issue lies in each scientist having a different view on the primary homology of certain traits, that is to say whether a trait is derived from a common ancestor or an example of convergent evolution. For example, Reisz and Laurin (1991) stated that the anterior expansion of the maxilla is unique to a turtle and procolophonids, but Lee (1993a) stated that it is also found in many taxa including pareiasaurs, pelycosaurs, and basal diapsids (for example, *Coelurosaurawus* and *Youngina*). Lee concluded that this trait is primitive for amniotes. The outcomes of these papers are so reliant on the personal opinions of the authors, when it comes to primary homology, that it is difficult to determine from them which hypothesis is the most well supported. Therefore, no conclusion can be reached regarding this issue.

Rieppel and deBraga (1996) stated that turtles are in fact diapsids with a secondary closure of the temporal fenestrae. This idea was based on cladistic analyses performed by Rieppel (1994) and Rieppel and deBraga (1996). They found that Testudines pair with Sauropterygia, and both lie sister to Lepidosauriformes within Sauria (=Reptilia). All the features used to support this relationship are developmental and are difficult to discern in the fossil taxa. In their 1996 paper they did not discuss how the anapsid turtle skull formed from a diapsid skull. Placodonts most closely resemble the appearance of extant turtles (Figure 38). However, the appearance of placodonts and turtles is most likely an example of convergent evolution, possibly parallel evolution if turtles came out of the same branch as nothosaurs, from living in similar habitats because the appearance of proposed turtle ancestors (i.e., *Eorhynchochelys, Pappochelys*) do not resemble that of placodonts, but rather nothosaurs. It is easy to understand why these earlier scientists believed turtles came out of Sauropterygia.

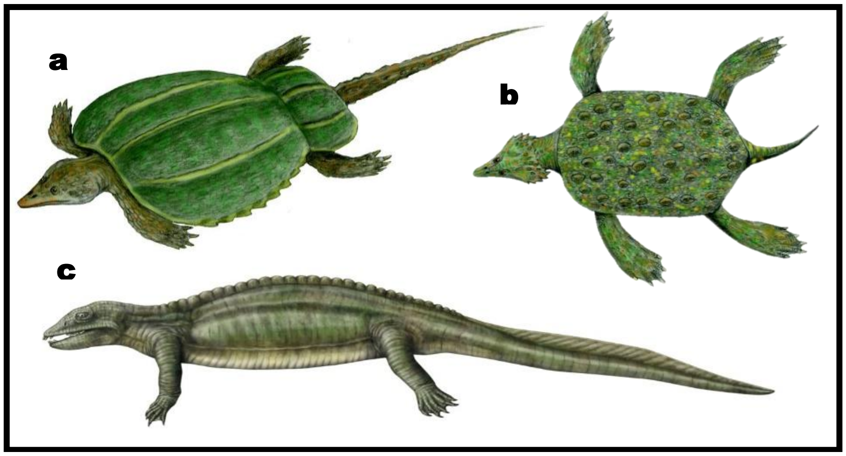


Figure 38. Representative placodonts. **a.** *Psephoderma alpinum*, a placodont from the Late Triassic of Europe (Tamura, 2004). **b.** *Placochelys placodonta,* a placodont from the Late Triassic of Europe (Tamura, 2007c). **c.** *Placodus gigas*, a placodont from the Middle Triassic of Germany (*Encyclopædia Britannica,* 2012).

DeBraga and Rieppel (1997) gave evidence against turtles being a part of Anapsida. The turtle skull has a very tall quadratojugal with a squamosal with a ventral surface that never goes beneath the eye socket (see Figure 9, p. 17). They reported that no other anapsid skull has this orientation of squamosal. They stated that the loss of the lower temporal fenestra has been demonstrated in the diapsid clade (Araeoscelidia) and must have occurred at least one other time in Eosauropterygia (all sauropterygians except placodonts). The cladogram proposed by Rieppel and deBraga (1996) were supported by another cladistic analysis performed by Rieppel and Reisz (1999). They showed good evidence that the structure of the carapace part of the shell and the location of the acromion process of the scapula are unique for the turtle clade as well as showing that the fused astragalus-calcaneum complex is similar between turtles and lepidosaurs.

Clark *et al.* (1993) showed that Gaffney (1990) misidentified the presence of laterosphenoids in his redescription of *Proganochelys quenstedti*. The presence of laterosphenoids unites living turtles and archosaurs (crocodiles and birds). This trait can also be found in *Proganochelys* and *Kayentachelys aprix* (Gaffney *et al.,* 1987) as stated by Sterli and Joyce (2007). Bhullar and Bever (2009) ran a cladistic analysis using an archosauromorph matrix (from Dilkes, 1998) and found that turtles grouped more closely with Archosauromorpha (the more inclusive group) than Archosauriformes (the less inclusive group).

There has been much recent discussion on the hooked fifth metatarsal in turtles. All extant reptiles have a hooked element in toe V, so the question is whether the hooked element in turtles represents one possible derived condition (i.e., is a derived distal tarsal) or another derived condition similar to extant reptiles (i.e., is a distal metatarsal). In lepidosaurs this element predominately extends with the row of metatarsals and in archosaurs it extends from the row of tarsals (Fabrezi *et al.*, 2009). The idea is that curved toe element is convergent between lepidosaurs and archosaurs. In turtles the endochondral ossification of the hooked element and the timing in which it develops provide the two strongest arguments for the element to be identified as a distal tarsal 5 as in archosaurs, and not as a distal metatarsal 5 as in lepidosaurs (Fabrezi *et al.*, 2009). Joyce *et al.* (2013) countered this idea by concluding that the distal toe element of turtles is a mixture of endochondrally (like tarsals) and perichondrally (like metatarsal) ossified bone. According to Joyce *et al.* (2013) the distal element is a composite of both tarsal and metatarsal bone. Using this conclusion three possible evolutionary scenarios have been proposed and will be discussed later in this section.

In conclusion, the only synapomorphies with strong support that unite turtles with lepidosaurs are neontological features as presented by Rieppel and deBraga (1996) and the fused astragalus-calcaneum complex. There have been a plethora of neontological features to support an archosaur relationship with turtles (see DeBeer (1937), Hofsten (1941), Lovtrup (1985), Ax (1984), and Gardiner (1993) in Chapter 3), but many have been countered or disputed (Rieppel, 1999). The only neontological feature that showed strong support is the differentiation of a pyramidalis muscle for the tendon of the nictitating membrane (Rieppel, 1999). However, this character is impossible to identify in fossils. The only morphological feature that gives strong support for a turtle-archosaur clade is that of the presence of laterosphenoids (Sterli and Joyce, 2007). Possible turtle outgroups include captorhinids, procolophonid, pareiasaur, lepidosaurs, sauropterygians (i.e., placodonts and nothosaurs) and archosaurs, in order of least likely turtle grouping to most likely turtle grouping. The diapsid hypothesis has been accepted based on the molecular data. Therefore, the anapsid hypothesis will no longer be discussed.

Three positive cladistic scenarios based on the neontological feature of the hooked fifth toe are presented in Figure 39. These scenarios all have turtles as sister to Archosauria because of the molecular evidence that supports this pairing. The first scenario assumes that the primitive condition of Lepidosauria and Archosauria is that the hooked toe V is derived from a composite of both metatarsal and tarsal bones, which is the condition seen in extant Testudines. This scenario is not strongly supported because the diapsid ancestor has separate tarsal V and metatarsal V bones. This scenario implies a reversal back to the toe V being derived from either the tarsal or metatarsal bone. This scenario is extremely unlikely from an evolutionary point of view; therefore, it is not present in Figure 39 and will not be discussed further.

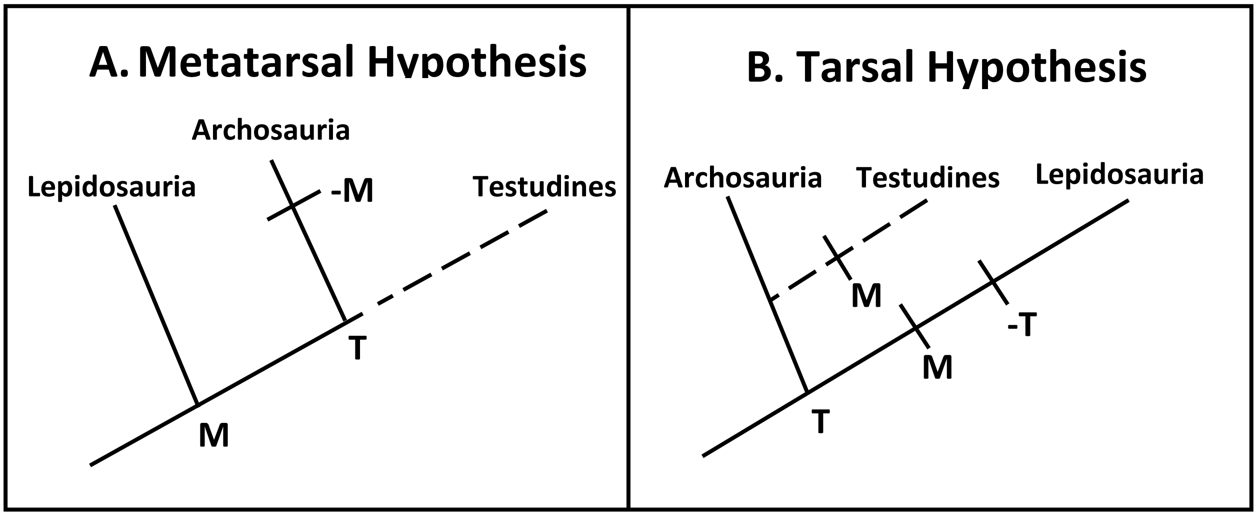


Figure 39. Two hypotheses of turtle relationships. Cladograms representing possible cladistic scenarios based on the neontological characteristics of the fifth toe and that Turtles and Archosauria are sister taxa based on molecular studies. **A** Primitive condition: hooked toe V derives from a metatarsal. **B** Primitive condition: hooked toe V derives from a tarsal. **M** Hooked toe V derives from a metatarsal. **T** Hooked toe V derives from a tarsal. **-M** Loss of hooked toe V being derived from metatarsal. **-T** Loss of hooked toe V being derived from a tarsal. Fossil data is discussed in the Sister Taxon section.

The second scenario (Figure 39 B) implies that the primitive condition for diapsids is a hooked toe V that is derived from a tarsal bone. This condition subsequently gains a metatarsal component in turtles. In lepidosaurs the tarsal component was lost and a metatarsal component was gained. This scenario implies that the metatarsal component to the hooked toe V was gained two times independently in the diapsid tree.

The first scenario (Figure 39 A) is the most parsimonious. The primitive condition of this scenario is the hooked toe V being derived from a metatarsal. This leads to lepidosaurs and a clade containing archosaurs and turtles. The tarsal component is added in the turtle-archosaur clade. Later, in archosaurs the metatarsal component is lost. The independent addition of the metatarsal component in turtles and lepidosaurs (Figure 39 B) is less parsimonious than if this character was added once (Figure 39 A). The first scenario (Figure 39 A) is the most likely cladistic scenario because it is the most parsimonious of the scenarios and the second scenario requires the metatarsal component of the distal toe V to be gained twice (Figure 39 B).

The Sister Taxon

This next section will include a critical look at each of the proposed turtle ancestors that have been discovered starting with the three oldest (Late Triassic or older) true turtles *Palaeochersis*, *Proganochelys*, and *Proterochersis*. The most significant traits that define Testudines are an anapsid skull type, a scapula with an elongated acromion process sutured to a fan-shaped coracoid, and a shell comprised of a carapace, plastron, and lateral bridge. It is very difficult to determine where Testudines came from without determining their sister group. That is why the classification and description of these taxa are very critical. Table 1 is a chart listing the oldest true turtles and proposed turtle ancestors with the features that define Testudines. The other features that have been used to unite the proposed turtle ancestors with Testudines will also be critiqued in this section.

Genera	Skull Type?	Complete Shell? (carapace, plastron, lateral bridge)	Scapula with Acromion Process and Coracoid?	Proposed Habitat
Palaeochersis	anapsid	Yes	Acromion process (elongated) and coracoid (fan-shaped)	Terrestrial
***Proganochelys* (incl. *Chinlechelys*)**	anapsid	Yes	Acromion process (elongated) and coracoid (fan-shaped)	Terrestrial
Proterochersis	anapsid	Yes	Acromion process (elongated) and coracoid (fan-shaped)	Terrestrial
Odontochelys	anapsid	only plastron and lateral bridge	No acromion process and coracoid (fan-shaped)	Aquatic-terrestrial
Eorhynchochelys	anapsid?	No	Acromion process? (not-elongated) and coracoid (fan-shaped)	Terrestrial-aquatic
Pappochelys	diapsid	No	Acromion process (not-elongated) and coracoid (not fan-shaped)	Terrestrial-aquatic
Eunotosaurus	diapsid?	No	No acromion process and coracoid (not fan-shaped)	Terrestrial (fossorial)

Table 1. Diagnostic features and proposed habitats of proposed turtle ancestors. Taxa are in order of youngest (top) to oldest (bottom). Habitats: Terrestrial (lives only on land); Aquatic-terrestrial (spends majority of time in water, but may go on land); Terrestrial-aquatic (spends majority of time on land, but may go in water); Aquatic (lives only in water) (Data from Gaffney, 1990; Rougier *et al.,* 1990; Szczygielski and Sulej, 2016; Li *et al.*, 2008; Li *et al.*, 2018; Schoch and Sues, 2015; Lyson *et al.*, 2010).

***Palaeochersis* & *Proganochelys* & *Proterochersis*.** *Palaeochersis talampayensis* (Rougier *et al.,* 1995) from Upper Triassic deposits of Argentina has a full turtle shell (plastron, carapace, and lateral bridge) (Figure 40). *Palaeochersis* has been proposed as an intermediate form between *Proganochelys* and *Proterochersis* (Rougier *et al.*, 1995; Scheyer and Sander, 2007), but the current understanding is that it is more derived than the two (Joyce, 2017). It differs from

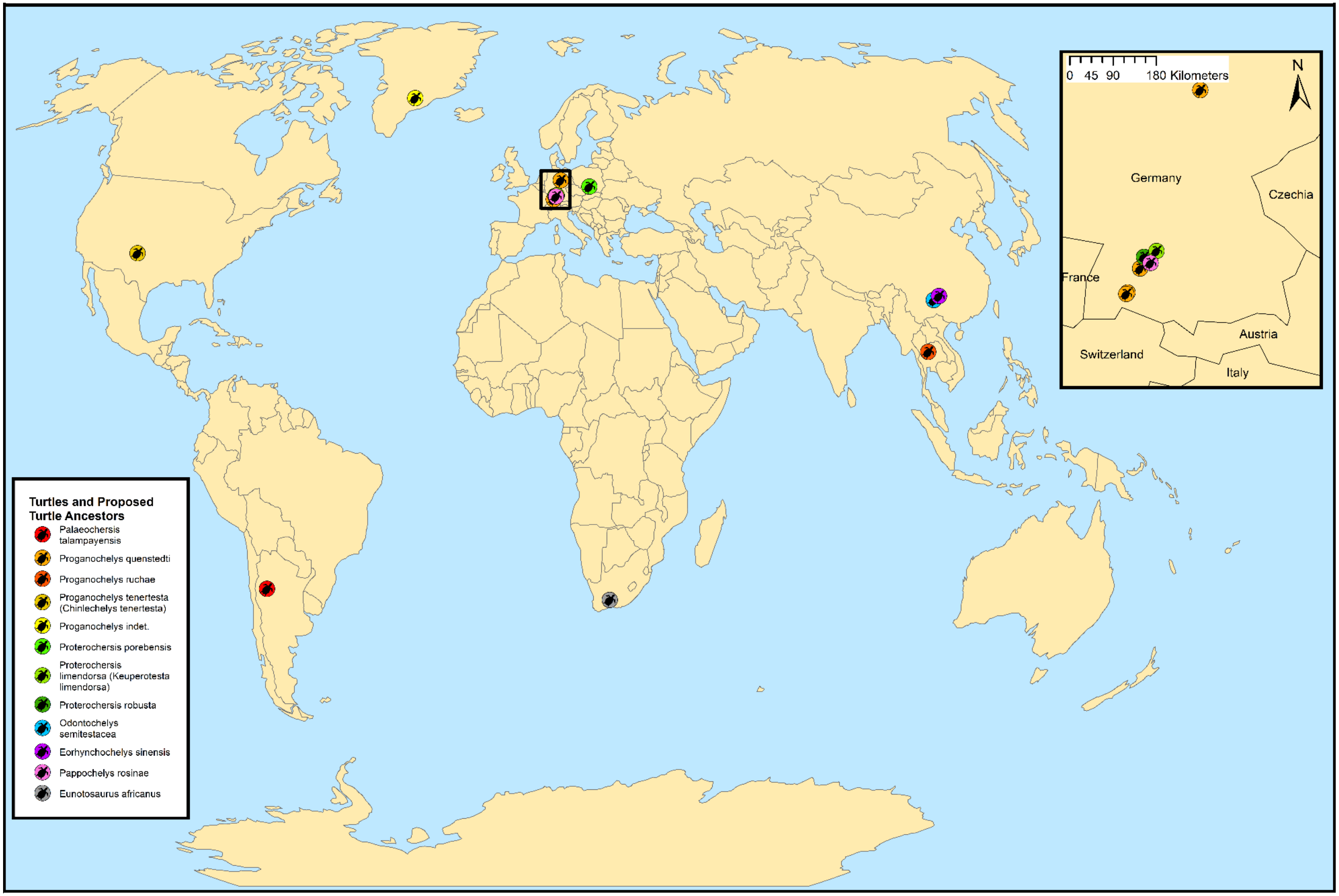


Figure 40. Global distribution of earliest true turtles and proposed turtle ancestors based on fossil localities.

Proganochelys in many aspects including lacking vomer teeth, having a sutured basipterygoid and paroccipital articulations, and lacking osteoderms on the neck, tail, and limbs. It differs from *Proterochersis* by exhibiting anterior supramarginals and lacking post-anal scutes (Joyce, 2017). *Proganochelys* (= *Stegochelys dux* (Jaekel, 1915) and *Triassochelys dux* (Jaekel, 1918)) from upper Triassic deposits of Germany, Poland, the United States, Thailand, and Greenland has a complete shell (Figure 40 also see Figures 3, 36; p. 3, 90). Joyce (2017) gave strong evidence to support that *Chinlechelys tenertesta* is a species of *Proganochelys. Proterochersis robusta* (Fraas, 1913) from the upper Triassic sediments of Germany and Poland also has a full shell (Figure 40 also see Figure 27, p. 67). This genus has been proposed to be as old as *Proganochelys* by Joyce (2017) and in some cases older after a study and reevaluation of the Norian age rocks the specimens were found in (Szczygielski and Sulej, 2016). *Proterochersis* and *Palaeochersis* both resemble *Proganochelys* in that they both have fully formed high-domed shells, turtle-like heads, short tails and stout limbs. These three taxa represent the oldest true Testudines, named Testudinata by Joyce (2017).

Odontochelys. The next oldest proposed turtle ancestor is *Odontochelys semitestacea* (Li *et al.*, 2008) (Figure 41). The age of the rock that *Odontochelys* came out of has been estimated to be around 220 million years in age by Li *et al.* (2008), and is located in southwestern China (Figure 40). The skull is relatively intact of the holotype of *Odontochelys*. Li *et al.* reported that the temporal region is not fenestrated although they mention that the pterygoid does show a transverse process that may have separated a subtemporal fenestra from a suborbital fossa. The specimen contains a lateral bridge and a fully formed plastron with

Figure 41. Reconstruction of *Odontochelys semitestacea*. Illustrated by Adrienne Stroup, the Field Museum, Chicago, Illinois. From fieldmuseum.org, 2018

epiplastron and entoplastron, one pair of hyoplatron, two pairs of mesoplatron, one pair of hypoplastron, and one pair of xiphiplastron (see Figure 17, p. 35). The pubis and ischium greatly resemble that of *Eorhynchochelys* (Figure 42). Characters that mark it as primitive to *Proganochelys* are no acromion process on the scapula, broadened dorsal ribs that articulate in facets located at the middle of the centrum and are T-shaped in cross section, a long tail, and only the neural plates being ossified. The well persevered nature of the holotype and paratype specimens leads to the conclusion that these descriptions are accurate. The lack of a complete shell leads to the conclusion that *Odontochelys* is not a member of Testudines. The presence of near complete plastron and lateral bridge, broadened dorsal ribs, and an anapsid skull leads to the conclusion that *Odontochelys* represents a hypothetical transition species between Testudines and a non-turtle forms, similar to the conclusion of Li *et al.* (2008).

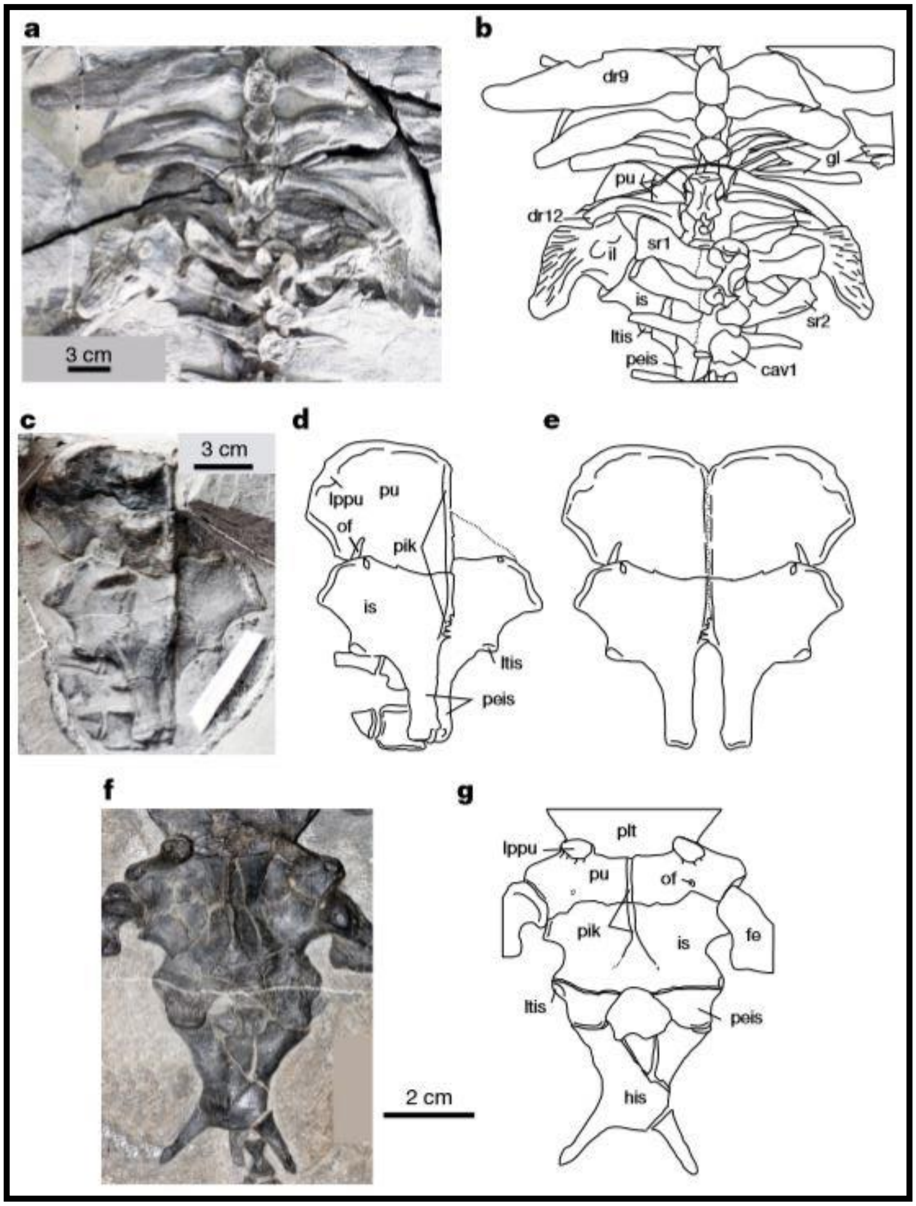


Figure 42. Photographs and interpretative drawings of *Eorhynchochelys* and *Odontochelys*. **a-b.** the holotype of *Eorhynchochelys sinensis* (SMMP 000016) in dorsal view, **c-d.** pubis and ischium of *Eorhynchochelys* in ventral view, and **f-g.** pubis and ischium of the paratype of *Odontochelys semitestacea* (IVPP V 13240) in ventral view. **e.** reconstruction of pubis and ischium of *Odontochelys.* Abbreviations: cav1, caudal rib 1; dr9, dr12, dorsal ribs 9, 12; fe, femur; gl, gastralia; his, hypoischium; il, ilium; is, ischium; lppu, lateral process of pubis; ltis, lateral tubercle of ischium; of, obturator foramen; peis, posterior elongation of pubis; pik, puboischiadic keel; plt, plastron; pu, pubis; sr1, sr2, sacral ribs 1, 2. From Li *et al.,* 2018

***Eorhynchochelys*.** The next oldest proposed turtle ancestor is *Eorhynchochelys sinensis* (Li *et al.* 2018), which is from Late Triassic rock (Carnian age) of around 228 million years from the Hainan Province of China (Figure 40, 43). The skull is of a modified anapsid condition with the upper temporal fenestra being closed by the contact of the parietal with the postorbital and postfrontal and the lower temporal region partially emarginated, but possibly being open ventrally. The posterior region of the skull has been crushed, leaving many features difficult to ascertain. It does not have a supratemporal bone, which true turtle possess. Dorsal ribs one through ten are anterior-posteriorly broadened, T-shaped in cross section, and have gently tapered distal ends. The ribs do not contact or overlap one another. There is not a carapace or plastron present, and in the place of a plastron there is a rigid puboischiadic (relating to the pubis and ischium) plate, which Li *et al.* stated is related to the plastron of *Odontochelys*. Li *et al.* (2018) also stated that there is a slight acromion process on the scapula, but after reviewing

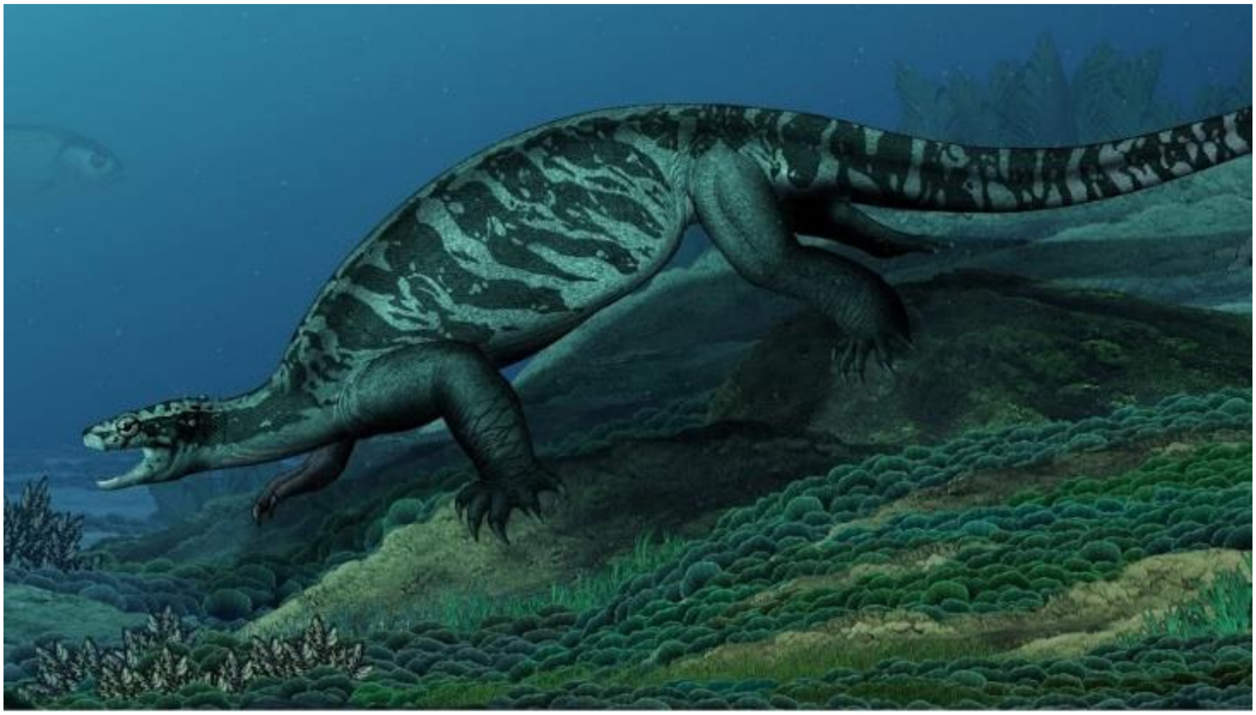


Figure 43. Reconstruction of *Eorhynchochelys sinensis*. Illustrated by Yu Chen, Institute of Vertebrate Paleontology and Paleoanthropology. *Chinese Academy of Sciences*, 2018

the photos, this process is very marginal and is difficult to identify (Figure 44). According to Li *et al.* (2018), parts of the pelvis (ischium, ilium, and pubis) resemble *Odontochelys* in that the ilium has a pronounced post-acetabular process, the ventral view of the ischium is of very similar shape, the posterior elongation of the ischium terminates in a blunt tip, and the pubis and ischium meet in a suture along the ventral midlines (Figure 42). Unlike in *Odontochelys*, the calcaneum and astragalus are not fused and there is no evidence of an ossified epipubic process. It is difficult to determine these similarities due to the preservation of the specimen, but the pubis and ischium are relatively similar to that of *Odontochelys'* (Figure 42).

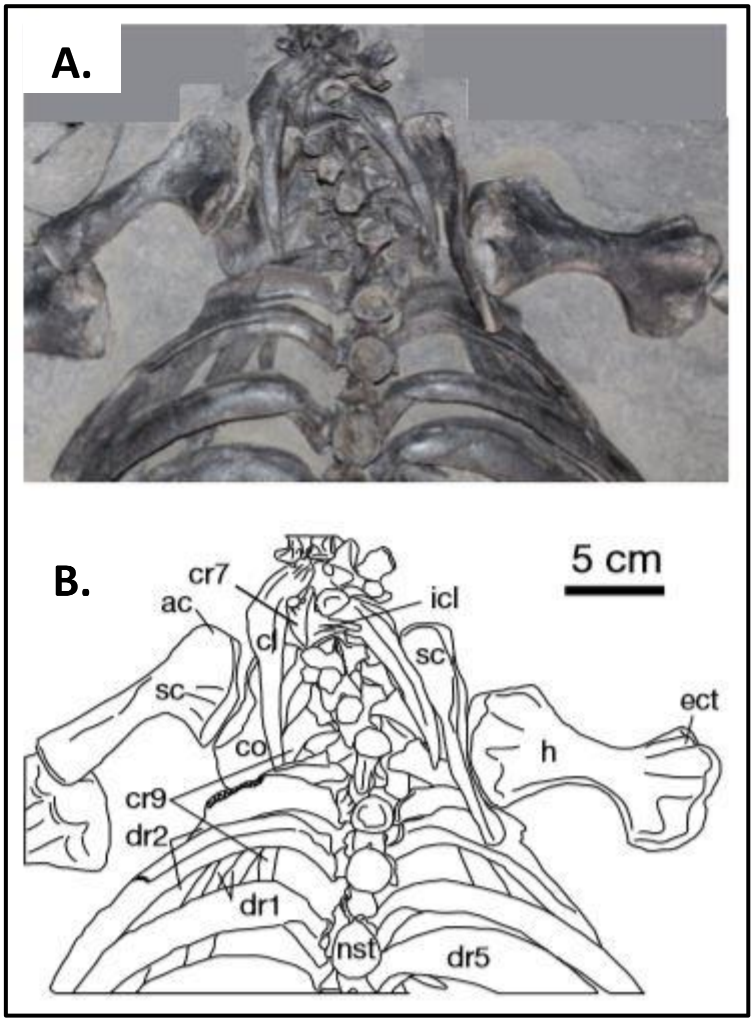


Figure 44. Photograph and interpretative drawing of *Eorhynchochelys* in dorsal view. **A.** Photograph and **B.** interpretative drawing of anterior axial skeleton and pectoral girdle of the holotype of *Eorhynchochelys*. Abbreviations: ac, acromion; cl, clavicle; co, coracoid; cr9, cervical rib 9; dr1, dr2, dr5, dorsal ribs 1, 2, 5; ect, ectepicondylar groove; h, humerus; icl, interclavicle; nst, neural spine table; sc, scapula. Modified from Li *et al.*, 2018

Lack of fully developed plastron and non-elongated acromion process in *Eorhynchochelys* make it more primitive than *Odontochelys. Eorhynchochelys* lies outside of Testudines because of it lacking a fully developed shell. Due to the nature of the skull, ribs, and, most significantly, the pubis and ischium elements do give strong support that *Eorhynchochelys* represents a lineage that could have led to *Odontochelys.* In conclusion, it is not accurate to call *Eorhynchochelys* a member of Testudines.

***Pappochelys*.** *Pappochelys rosinae* (Schoch and Sues, 2015) is from late Middle Triassic (around 240 million years) German claystone (Figure 40, 45). The description focuses on two specimens, the holotype is a partially articulated postcranial skeleton and the other is a disarticulated skeleton with an incomplete skull. The disarticulated components are in good enough shape that a reasonable skeletal reconstruction could be made (see Figure 17, p. 35). According to the reconstruction of the skull, the upper temporal fenestra is bounded by the squamosal and parietal. The lower temporal fenestra is open ventrally similar to *Eorhynchochelys.* This would be the first diapsid to be a hypothesized turtle ancestor. The ribs are anterior-posteriorly broad and T-shaped in cross-section. There are large paired gastralia on the dorsal region. Schoch and Sues (2015) reported a short 'acromial' process on the scapula, but this is difficult to determine from the images provided. They also reported that the pelvis closely resembles that of *Odontochelys* and *Proganochelys* (and through extension *Eorhynchochelys*), but the ischium is not fused to the pubis as in these taxa. The ilium also has a long post-acetabular process similar to *Eorhynchochelys.*

Figure 45. Reconstruction of *Pappochelys rosinae*. Illustrated by Gabriel N. Ugueto. From abrielugueto.com/paleoart/, 2019

Pappochelys possess little in the way of features that unite it with Testudines. The pelvis does resemble that of *Odontochelys,* but without an anapsid skull or fully formed plastron and carapace, it is difficult to state that *Pappochelys* is a turtle or turtle ancestor. Schoch and Sues (2015) concluded that the paired gastralia present on the dorsal region of *Pappochelys* is indicative of the increased ossification seen in *Odontochelys.* Dorsal paired gastralia are present in lepidosaurs, archosaurs, and sauropterygian taxa therefore they cannot necessarily be used as a link between Testudines and *Pappochelys*. Disarticulated gastralia were present below the dorsal ribs of *Eorhynchochelys* (Li *et al.*, 2018), but it was not possible to tell if there was any fusion occurring. The strongest evidence that unites *Pappochelys* with the other proposed turtle ancestors is the broadened ribs that are T-shaped in cross section. In conclusion, this does not provide sufficient enough evidence to propose that *Pappochelys is* a representative of the lineage that led to Testudines.

Question 6. What role, if any, does the fossil *Eunotosaurus* play in the origin of turtles?

Eunotosaurus africanus (Seeley, 1892) is from Late Permian South African rock (around 260 million years) (Figure 40). The most recent work to hypothesize that *Eunotosaurus* is a proposed turtle ancestor is Lyson *et al.* (2010). This conclusion is based on it having broad T-shaped abutting ribs that taper to points (see Figure 33, p. 86), ten elongate trunk vertebrae (a reduced count), cranial tubercles (small rounded protuberances), and a wide trunk (Figure 46). The cranial tubercles were used by Gow (1997) to show a millerettid relationship with *Eunotosaurus* within Parareptilia, and therefore conclude that they are not synapomorphies for *Eunotosaurus* and turtles. The study done by Lyson *et al.* (2010) placed *Eunotosaurus* outside of Diapsida as a parareptile, therefore implying that it is anapsid. Lyson *et al.* also stated that the expanded ribs of other taxa are not similar to the ribs of *Eunotosaurus* and the other

Figure 46. Reconstruction of *Eunotosaurus africanus.* Illustration by Andrey Atuchin. A herd of pareiasaurs (Bradysaurus) are in the background. From livescience.com, 2016

hypothesized turtle taxa in that they are not T-shaped in cross-section, but rather biconvex in cross-section (Jenkins, 1970). It should be noted that Jenkins only used extant mammal taxa in his study (i.e., anteaters, armadillos, and primates). No fossil reptiles were discussed. Lyson *et al.* (2013) gave more evidence to support the axial arrest (loss of the muscles between the ribs) of the body of *Eunotosaurus*. Bever *et al.* (2015) would show that adult *Eunotosaurus* represents a modified diapsid that has developed a synapsid-like skull stage. The lower temporal opening is ventrally open in adult *Eunotosaurus* and a laterally expanded supratemporal bone covers the upper temporal opening. It is very uncommon for one skull bone to cover another, but since this covering was shown in two separate adult *Eunotosaurus* specimens, then it is difficult to dismiss these results. According to Bever *et al.* (2015), both upper and lower temporal fenestrae are present in a juvenile specimen (Figure 47). After looking at Figure 47, the fossil preparation work appears to have exaggerated and altered the shape of the upper fenestra present in the juvenile specimen. The difficulty to identify temporal bones of the skull, the inability to identify true boney edges (sutures are not clear), and the photo not being taken at a high resolution all support this conclusion.

It is difficult to say with any certainty that *Eunotosaurus* is a representative of the lineage that led Testudines. Now that *Eunotosaurus* is thought to be a modified diapsid (Bever *et al.*, 2015), the strongest evidence presented is the axial arrest of the body and the broadened T-shaped ribs. Other reptiles, including Placodontia within Sauropterygia, also have axially arrested body plans supported by broadened ribs. The T-shaped cross-section of the ribs in *Eunotosaurus* gives the best support for a turtle relationship. It is present in all proposed turtle ancestors, and there is a reduced version in extant turtles. The T-shape is most prominent

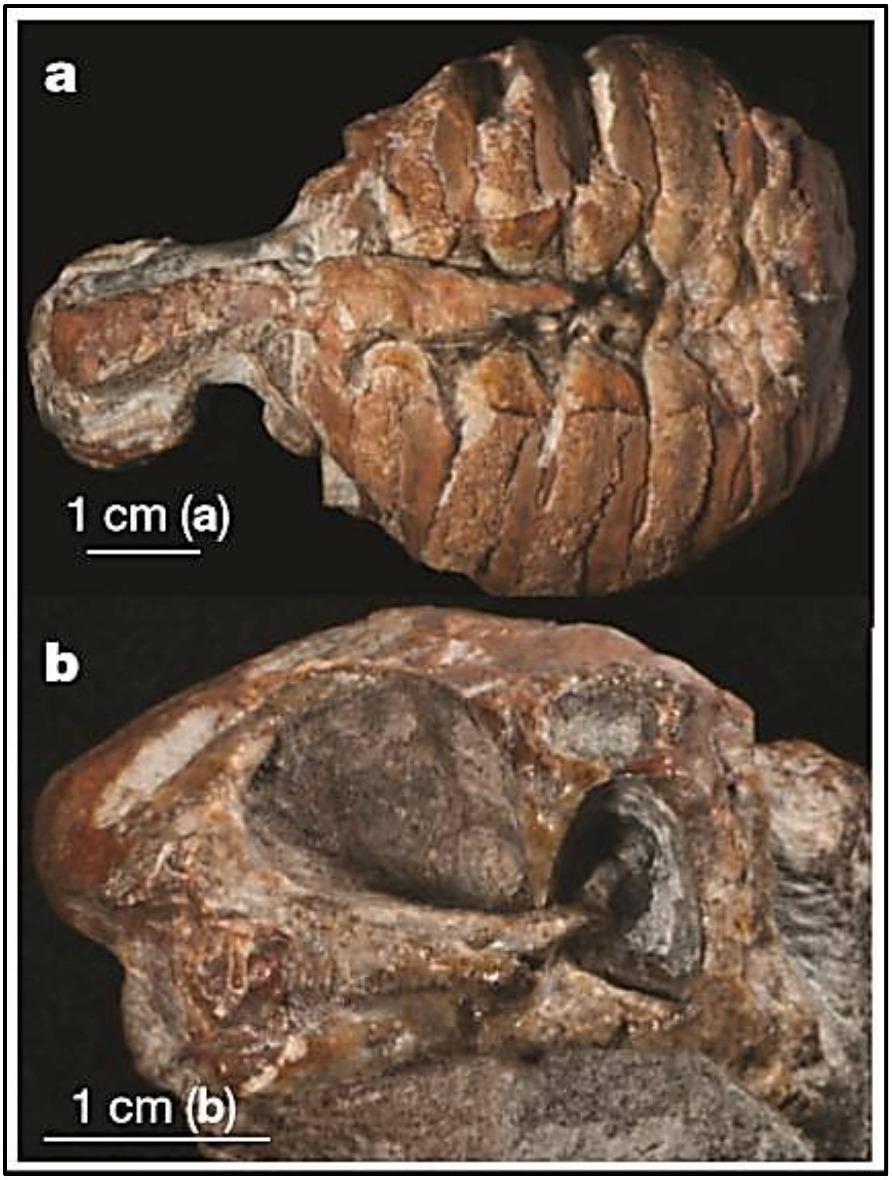


Figure 47. a. The postcranium and **b.** skull of a juvenile *Eunotosaurus.* Specimen seen in dorsal and right lateral (reflected) views, respectively. Notice the lower and upper temporal fenestrae present on the skull. Modified from Bever *et al.*, 2015

in *Eunotosaurus* (Figure 48). It is not present in sauropterygian ribs (Klein *et al.*, 2019). The T-shape may provide biomechanical support for the broadening and expanding of the rib. This character may be dependent on the expansion of the rib, and therefore what appears to be two characters may only be one. The fact that extant archosaurs and lepidosaurs do not possess T-shaped ribs indicates that this character is not indicative of Diapsida. This adds to the idea that this trait is specific to broadened ribs in turtles. Without the characters that define the ribs of *Pappochelys* and *Eunotosaurus*, it is difficult to give strong support to any of the proposed turtle

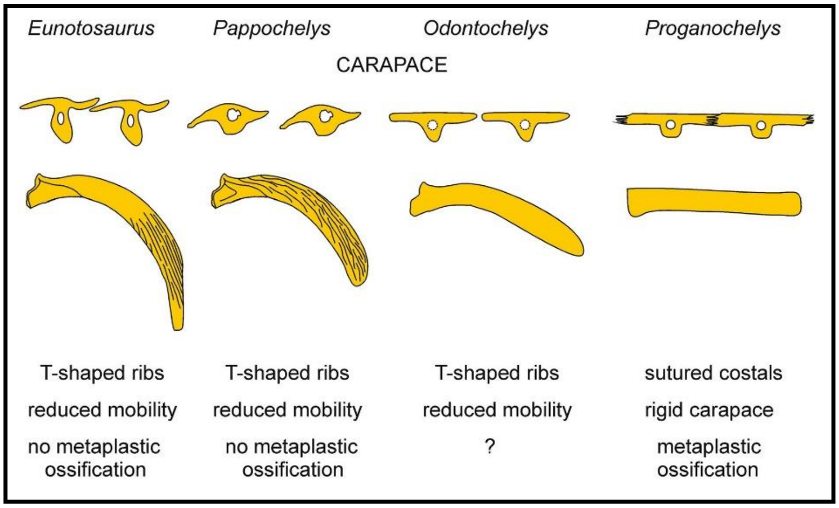


Figure 48. The ribs of select proposed turtle ancestors and *Proganochelys*. Seen in cross-section showing proposed T-shape and front view. Curvature of rib, body mobility, and metaplastic ossification are indicated. Modified from Schoch and Sues, 2019

synapomorphies. At best the proposed turtle-like features are evidence of convergent evolution. More fossil specimens of proposed turtle ancestors need to be discovered in order to strongly support T-shaped ribs in cross-section as a synapomorphy of the group that includes all proposed turtle ancestors. Without this support it is difficult to conclude that *Eunotosaurus* is a member of Testudines or on the lineage leading to Testudines and therefore the hypotheses discussed by Lyson *et al.* (2010; 2013; 2016) are inconclusive.

Question 3. In regards to turtle phylogeny, are morphological and molecular studies currently in agreement?

The most significant source of error when it comes molecular studies is their use of the DNA and RNA of extant taxa to determine evolutionary relationships. If there are only extant taxa to test, then the results will show relationships of extant taxa, but not possible relationships of extinct taxa. All molecular studies to date have been done on living turtles, crocodilians, lizards, snakes, tuatara, and birds plus an outgroup such as mammals. It is impossible to do molecular studies on any of the extinct parareptiles or extinct early diapsids.

The vast majority of molecular tests on turtle phylogenetics support an archosaur-turtle relationship. These tests have used only mt rRNA (Zardoya and Meyer, 1998), complete mtDNA sequences (Kumazawa and Nishida, 1999), nuclear-encoded proteins and rRNAs (Hedges and Poling, 1999), DNA-DNA hybridization data (Kirsch and Mayer, 1998), and mtDNA and rRNA (Cao *et al.*, 2000). Cao *et al.* (2000) noticed that many previous molecular analyses were having difficulty determining if turtles are more closely related to crocodiles or birds. This led them to state that molecular analyses are prone to biases and errors due to gene selection, differences in gene sequence alignments, the choice of outgroup, and different sampling of ingroup taxa. This paired with not being able to test extinct taxa are major problems in molecular studies of this kind.

At least one molecular study, however, supports the lepidosaur-turtle hypothesis. Lyson *et al.* (2011) used miRNAs to determine primitive and derived genes in order to root their tree. They found four common miRNAs between lizards and turtles, showing support for a lepidosaur-turtle clade. Chiari *et al.* (2012) stated that miRNAs are not as stable as suggested by Lyson *et al.* (2011) and cautioned those who use them in that way. Technology started to allow the sequences of full genomes allowing molecular scientists such as Shen *et al.* (2011), Tzika *et al.* (2011), and Chiari *et al.* (2012) to use large scale genomes to conclude that turtles are sister group to Archosauria. Crawford *et al.* (2012) used the lack of support in the genomic data of a lepidosaur relationship to counter the conclusion of Lyson *et al.* (2011) as well as countering the other points presented by Lyson *et al.* Crawford *et al.* (2012) also found an archosaur relationship after performing their own analysis using ultraconserved elements of DNA. The last straw in refuting the conclusions by Lyson *et al.* (2011) is when Lyson was a member of the study by Field *et al.* (2014) to fully counter their argument in favor of the full support of an archosaur turtle clade. With Lyson abandoning his earlier position, currently the archosaur-turtle hypothesis has no viable alternative in molecular studies.

These modern molecular studies only use extant taxa classified within Archosauria, Lepidosauria, and Testudines. The fact they cannot use extinct taxa makes it less clear of the ancient relationships of these taxa. What should have been stated in these studies is that a relationship between Archosauria and Testudines implies a relationship with the older and broader group Archosauromorpha, and the same applies to Lepidosauria and Lepidosauromorpha. Without stating this, the timing of when turtles separated from the

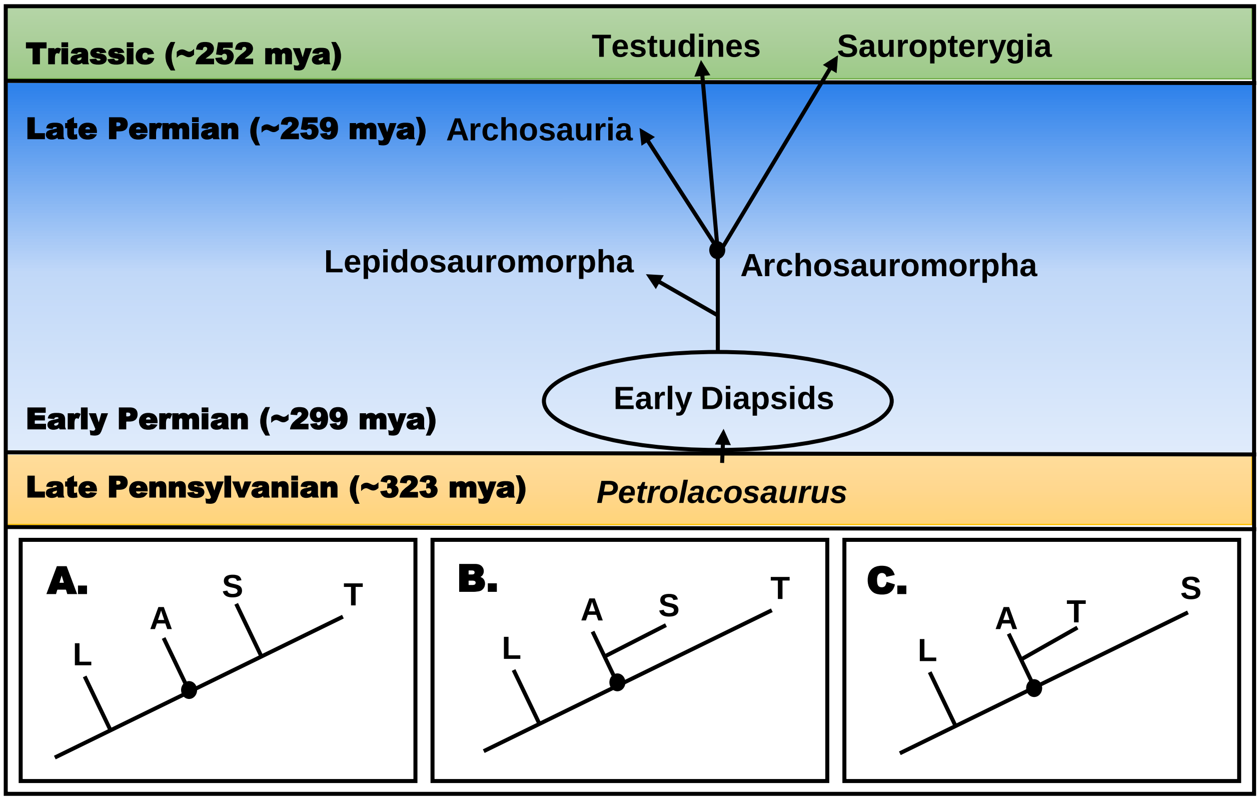


Figure 49. Possible cladistic scenarios of Archosauria, Testudines, and Sauropterygia. *Petrolacosaurus* is the earliest known diapsid. **A-C** Possible scenarios that resolve the trichotomy. (L) Lepidosauromorpha; (A) Archosauria; (T) Testudines; (S) Sauropterygia; (Dot = ●) Archosauromorpha

evolving Archosauromorpha lineage appears younger than when it probably occurred. With this in mind, Figure 49 shows the possible cladistic scenarios of an Archosauromorpha origin of turtles.

Representatives of Archosauromorpha first arose in the Middle to Late Permian, probably similar timing for Lepidosauromorpha, from the early diapsid group of the Early Permian. The main lineage of Lepidosauromorpha is classified as Lepidosauria, likewise, the main lineage of Archosauromorpha is classified as Archosauria. Using molecular data, there is strong support for the idea that turtles arose from a lineage of early archosauromorphans. Sauropterygia also probably arose from a lineage of Archosauromorpha. The earliest true fossil turtles (Testudines) is from the Late Triassic and the earliest Sauropterygia from the Early Triassic. The first archosaurian arose during the Late Permian or Early Triassic.

Based on the timing of these clades, the most likely cladistic scenario is that of Figure 49 A, with Archosauria being the first to come out of the trichotomy, followed by sauropterygians and turtles. Sauropterygia is most likely the next to branch off of the evolving lineage because the earliest ones are from the Early Triassic. This scenario indicates a closer turtle relationship to Archosauria than Lepidosauria, as indicated by molecular studies. It also indicates that turtles are within Diapsida, which more recent morphological studies have shown based on material of *Pappochelys* and *Eunotosaurus* (Schoch and Sues, 2015; Bever *et al.*, 2015; Lyson *et al.,* 2016). However, skepticism remains for the conclusions of these studies as stated above. Figure 49 A also indicates a relationship between turtles and Sauropterygia, which past and modern studies have also shown (Rieppel, 1994; Rieppel and deBraga, 1996; Schoch and Sues, 2015).

In conclusion, molecular and morphological studies are currently not in agreement in terms of early turtle evolution. There has been very strong support for a diapsid origin of turtles, specifically within Archosauromorpha, within the molecular community since the early 1990s. It is difficult calling turtles diapsid based on *Eunotosaurus* and *Pappochelys* having a diapsid skull type because they are not representatives of the turtle lineage as stated above. More Early and Middle Triassic fossils of early turtles, archosaurs, and sauropterygians will help to clarify these relationships, as could embryological studies. Currently, there is no strong morphological evidence within the paleontological field to support that turtles are diapsid with the only proposed traits being the presence of laterosphenoids or a fused astragalus-calcaneum complex. The neontological morphological data (i.e. hooked distal toe V) indicates that turtles are diapsid with more characters showing a Lepidosauria relationship than an archosaurian relationship (Rieppel and deBraga, 1996), counter to the results of molecular studies.

Question 4. In which habitat did true turtles and their immediate ancestors utilize, terrestrial or aquatic?

The habitats in which the earliest turtles and their ancestors lived can be divided into two main categories and two categories that are transitional between the two main categories. The two main categories are terrestrial (live only on land) and aquatic (live only in water). The two transitional categories are aquatic-terrestrial (spends majority of time in water, but may go on land) and terrestrial-aquatic (spends majority of time on land, but may go in water). The oldest true turtles and their immediate ancestors will now be placed into categories by looking at functional morphology, environment of deposition from rock type, and physiological studies.

Functional Morphological

I conclude that *Proganochelys* is terrestrial based on the fossil possessing neck and tail spikes, osteoderms on the limbs, and, in some specimens, a tail club (Joyce and Gauthier, 2003). *Palaeochersis* lacks osteoderms on the neck, tail, and limbs, but it does have a reduced phalangeal count on the front and rear foot that support a terrestrial habitat (Rougier *et al.,* 1995). Scheyer and Sander (2007) concluded that *Proganochelys* and *Proterochersis* have shells that closely resemble extant terrestrial turtles based on shell microstructure possessing a compact diploe structure, no homogenization of the outer most layer and inner most layer of bone, and no to low reduction of vascularization of the internal cortex (see Figure 24, p. 59). This comparison with extant turtle shells is supported by *Proganochelys* and *Proterochersis* both lying within Testudines. Benson *et al.* (2011) used a three-parameter (*R, p, k*) geometric model to conclude that the curvature of the shell of *Proterochersis* more closely resembles that of extant semi-aquatic turtle shells. Unfortunately Benson *et al.* did not give clear definitions of each of the parameters, therefore it makes it difficult to replicate or support his data. The conclusions of the study by Scheyer and Sander (2007) outweigh the conclusions of Benson *et al.* (2011) because of the stronger evidence presented (i.e. close up photos of discussed structures). In conclusion, *Proterochersis* is solely terrestrial.

Joyce and Gauthier (2003) did attempt to quantify habitat by comparing limb dimensions of modern turtles to that of fossil turtles. This method lends itself to the use of fossils as it allows morphological comparisons between modern turtles and their habitats with extant turtles and their proposed habitats. Similar to the studies performed by Scheyer and Sander (2007) and Benson *et al.* (2011), this analysis uses the assumption that patterns seen in extant turtles are the same for extinct turtles. This study showed that *Proganochelys* and *Proterochersis* have front limb dimensions closest to extant terrestrial turtles, however the placement of the dividing line (intermediate stage) between aquatic and terrestrial habitats by Joyce and Gauthier was completely subjective (see Figure 23, p. 57). The only way to determine habitat preference with strong support from their ternary plot is if *Proganochelys* and *Proterochersis* fall within their intermediate stage or at the poles of the plotted data. They fall just outside of the intermediate stage, in the terrestrial zone, and therefore the results presented are inconclusive.

The forelimb proportions of *Odontochelys* (Joyce and Gauthier, 2003) match those of living turtles that inhabit small bodies of water, however, this was countered by Anquetin (2010) based on the phalanges of the front and rear foot being short and more similar to the limb proportions of extant turtles that live in terrestrial habitats than aquatic turtles. The presence of only a plastron better supports that this animal lived and swam in water than any limb proportions. A plastron supplies armor to the dorsal region that is exposed when swimming in water (Rieppel and Reisz, 1999). It is difficult to discern from one specimen of *Odonotochelys* whether the traits associated with an aquatic habitat are a secondary adaption to an aquatic environment from a terrestrial turtle ancestor as proposed by Reisz and Head (2008). In conclusion, *Odontochelys* is aquatic-terrestrial.

The stout limbs, prominent condyles on the humerus and femur, moderately developed olecranon of the ulna, and enlarged claws indicate that *Eorhynchochelys* was predominately terrestrial (Li *et al.,* 2018). However, the astragalus and calcaneum are not fused, similar to extant marine turtles. This taxon was primarily terrestrial, but it lived near bodies of water. The thick paired gastralia, broadened ribs, and long whip-like tail support *Pappochelys* utilizing an aquatic to terrestrial-aquatic habitat, but the stout limbs suggest it had the ability to go on land. It fits within the terrestrial-aquatic habitat. *Eunotosaurus* lacks obvious aquatic adaptations and is only known from terrestrial sediments (Gow, 1997). Lyson *et al.* (2016) stated that *Eunotosaurus* exhibited characteristics of a fossorial lifestyle. Some of these characters include reduced anterior-posteriorly broadened ribs, a short spade-shaped skull with a broadened occipital region, short robust cervical vertebrae, and stout shoulder and limb bones with many processes for muscle attachment.

Environment of Deposition from Rock Type

Proganochelys specimens have been found in a variety of rock types including sandstone, claystone, and shale (Gaffney, 1990). *Proterochersis* shows similar variability in the rock type it came out of, having specimens come out of sandstone, claystone, and mudstone (Szczygielski and Sulej, 2016). *Palaeochersis* came out of extensive paleodunes (sandstone) and sporadic wadi deposits (siltstone) caused by a change in stream velocity. Rougier *et al.* (1995) proposed that the layer that yielded *Palaeochersis* may have been a mud flow covering a short-lived river channel. This type of deposit may not be representative of the environment of deposition of *Palaeochersis,* but rather caused by secondary deposition after death; however, the near complete articulation of the skeleton counters this assumption. All these rock types are inconclusive or support the conclusion of a terrestrial habitat for these true turtles.

A rock type was not given for *Odontochelys,* but it was found in the same member as *Eorhynchochelys,* the Wayao Member of the Falang Formation (Li *et al.,* 2008). From the photos (see Figure 38), the rock appears to be a dark gray shale, mudstone, or claystone. Li *et al.*,

(2008) did report that the sediment was marine in nature, with anoxic conditions and abundance of driftwood and other plant remains indicating a relative proximity to coastal waters. This evidence agrees with the conclusion that *Odontochelys* is aquatic-terrestrial. It was reported that *Eorhynchochelys* was found in marine black shaly marlstone (combination of clay and silt) (Li *et al.*, 2018). This rock type agrees with the conclusion that *Eorhynchochelys* lived in a terrestrial-aquatic habitat. It is difficult to determine whether the type of rock that these fossils were preserved in is indicative of the environment these animals inhabited or was caused by secondary transport from a location further inland.

Schoch and Sues (2015) reported that *Pappochelys* is from dark gray lake-deposited claystone, along with fishes, stem-amphibians, and terrestrial diapsid reptiles. *Pappochelys* is the most common reptile in the lake deposits it was found in (at least twenty specimens) which Schoch and Sues used to conclude that it lived along the lakeshore and frequently entered the lake. It should be noted that one skeleton is partially articulated and the other is disarticulated, which may indicate secondary deposition after death. That being said, the rock type paired with the morphology of *Pappochelys* led to the distinction of a terrestrial-aquatic habitat for this taxa. The first specimens of *Eunotosaurus* were found in sandstone nodules (Seeley, 1892), with all other specimens found in terrestrial rock types (Lyson *et al.*, 2016). This is indicative of the terrestrial classification of the habitat of *Eunotosaurus.*

Physiological

Rieppel and Reisz (1999) used a study by Bentley (1976) showing that more ossified extant turtles (with both carapace and plastron) gain significantly less water in fresh water or loss of water in salt water to conclude that a plastron could be used as an effective osmotic barrier. This study did highlight another advantage of a plastron, besides dorsal armor in an aquatic habitat. Rieppel (2013) hinted at the idea that the plastron could perform a hydrostatic function as a bone ballast. He stated that having the plastron on the dorsal side of the body is also the most effective placement for buoyancy control.

I conclude that the origin of true turtles was linked to a habitat that included a body of water, even if they spent time both in the water and on land. All fossils that are representative of the lineage that leads to turtles (excluding *Pappochelys* and *Eunotosaurus*) have been linked to an aquatic lifestyle in one way or another. Both *Odontochelys* and *Eorhynchochelys* were found in marine sediments showing a possible preference to oceanic environments in the lineage leading to turtles. As seen in modern marine turtles, early marine turtle ancestors most likely spent some time on land in order to lay their eggs. Strong evidence for a plastron developing in any way, but for armored protection, an osmotic barrier or bone ballast while swimming, has yet to be proposed.

Question 5. Were endoskeletal or exoskeletal components used to construct the turtle shell?

Endoskeletal bones are elements of the internal structure of an organism that start as cartilaginous elements that are than replaced by bone later in development. They differ from exoskeletal bones in that these bones form from ossification centers within or associated with the dermis or dermal cells. Many scientists have proposed turtle relationships based on the assumption that the shell formed first through the fusion of exoskeletal components, primarily dorsal and ventral osteoderms, and later incorporated the ribs (Gauthier, 1994; Laurin and

Reisz, 1995; Lee, 1996) (see Figure 15, p. 26). However, just as many researchers, if not more, have proposed the exact opposite, not through the fusion of osteoderms, but through expansion of the ribs, endoskeletal components, first and the subsequent incorporation of the ribs into the dermis (deBraga and Rieppel, 1997; Rieppel and Reisz, 1999; Hill, 2005). These scientists highlight the significance of the ribs and other endoskeletal bones in the formation of the shell, but it wasn't until the work of embryologists in the early part of the 21st century that a more duel nature of shell formation was revealed.

Determining which parts of the shell form from endoskeletal or exoskeletal bones can help to determine whether osteoderms were involved in any way in its construction. Gilbert *et al.* (2001) deduced from the embryos of a red-eared slider turtle and a snapping turtle that the neural (spine) and costal (rib) components of the shell are endoskeletal in nature (see Figure 26, p. 65). Cebra-Thomas *et al.* (2005) also looked at the embryonic stages of a red-eared slider and determined that the structural precursor to the carapace, the carapacial ridge (CR), induces the lateral growth of the ribs and causes their incorporation into the dermis. Burke (1989) stated that the CR is a collection of dermis cells that experience mesoderm interactions (see Figure 28, p. 70). Given these papers, both the ribs and the dermis, through interactions of the CR, form the turtle shell. Cebra-Thomas *et al.* (2005) stated it best when they described that the CR and the ribs form a positive feedback loop, whereas one grows so does the other. Scheyer and Sander (2007) stated that the overlying portions of the costal and neural bones show signs of residual structure of the dermis. This could very well be residue from when the ribs were enveloped by the dermis, but there is no evidence at this time to support this.

Scheyer *et al.* (2008) concluded that the ribs cause the ossification of the surrounding dermis, but Hirasawa *et al.* (2013) modified this to include only the overlying dermal tissue. Nagashima *et al.* (2009) also concluded that the carapacial ridge does not induce the axial arrest of the ribs (loss of the muscles between the ribs) as suggested by Burke (1991) and Cebra-Thomas *et al.* (2005), but instead the CR induces the expansion of the ribs in late development.

I conclude that the only component that solely arises from an exoskeletal or endoskeletal bone is the plastron, which has long been considered to be constructed of exoskeletal components ever since Zangerl (1869) deduced that there are no cartilaginous precursors to the plastron elements. This view was later supported by Burke (1985, 1989) when she concluded that the plastron arises from the dermal layers along the lateral flank of the turtle embryo. It is clear that the origin of the carapace is a more complex than the plastron because it involves both endo- and exo-skeletal components working together to form a truly unique feature. It is unclear whether the formation of the carapace was induced by the ribs expanding into the dermis (Nagashima *et al.*, 2009) or the dermis moving down to surround the expanded ribs (Cebra-Thomas *et al.*, 2005). Modern embryological studies are still not in agreement. Turtle embryos may hold the answer to this question, but it will take further studying before it is finally revealed and accepted.

Conclusion

Turtles are highly unique organisms, not only does their pectoral girdle lie within the confines of their rib cage, but their skull is anapsid. The purpose of this thesis was to discuss and evaluate, based on the pertinent literature, some of the major controversies involving turtles. After reading 155 scientific papers and book chapters and analyzing the facts and interpretations there in, I have come to several conclusions:

Question 1 and Question 2

It is difficult to discern whether turtles are primitive parareptiles or modified diapsids, but based on molecular studies and morphological studies of living reptiles, turtles are most likely modified diapsids. Based on the timing of the major diapsid clades, the turtle ancestor arose from a lineage of the early Archosauromorpha, one that was sister to either Sauropterygia or Archosauria.

The oldest true turtles are all from the Late Triassic (around 210 mya), and they are *Palaeochersis, Proganochelys*, and *Proterochersis.* The synapomorphies that make them true turtles are an anapsid skull, the use of skeletal and dermal elements to form a shell (carapace, plastron, and lateral bridge), and a scapula with a uniquely shaped acromion process sutured to a fan-shaped coracoid. Also from the Late Triassic, *Odontochelys* (around 220 mya) and *Eorhynchochelys* (228 mya) possess enough turtle synapomorphies to be considered representatives of the lineage leading to true turtles. These characters are an anapsid skull, a fan-shaped coracoid, and ossification of the dorsal region.

Question 3

Paleontological data supports either an anapsid origin or, to varying degrees of support, a diapsid origin. Neontological data supports a diapsid origin, but with more support towards turtles being sister to Lepidosauria. Molecular data gives strong support to an Archosauria relationship, with more recent studies having a common ancestor between Testudines and a group that contains crocodilians and birds. Currently, molecular and morphological studies are not in agreement; however, there have been some morphological studies that have a shown a diapsid origin of turtles.

Question 4

Early turtles evolved in water based on the evidence that *Odontochelys* and *Eorhynchochelys* inhabited aquatic environments. Living in association with water is significant to the early evolution of turtles, with marine environments possibly being a preference to early turtle ancestors. Speculatively, a fully aquatic turtle ancestor may be found in Middle Triassic, possibly marine, rock containing a modified diapsid skull, possibly showing signs of a synapsid skull type (i.e., closed upper temporal fenestra), a fully formed plastron without lateral bridge or a greatly reduced plastron formed by gastralia, an unfused astragalus-calcaneum complex, a reduced acromion process, a fan-shaped coracoid, and ribs that are only slightly broadened.

Question 5

The formation of the turtle shell is caused by both endo- and exoskeletal components and processes working together. The plastron is derived completely from endoskeletal components, whereas the carapace and lateral bridge is a mixture of endo- and exoskeletal components.

Question 6

The oldest proposed turtle ancestors are *Pappochelys* from the Middle Triassic (around 240 mya) and *Eunotosaurus* from the Late Permian (around 260 mya). These two taxa are not representative of the lineage leading to true turtles because they do not possess an anapsid skull, a complete turtle shell, and a scapula with an extended acromion process that is sutured to a fan-shaped coracoid. The traits used to group *Eunotosaurus* with turtles are their real or supposed diapsid skull type, their expanded ribs, T-shaped cross-section of the ribs, reduced vertebral count, and cranial tubercles. These traits are not valid because they are found in other groups (i.e., sauropterygians and millerettids), are indicative of environment (i.e., reduced vertebral count being an adaptation to a fossorial lifestyle), or a trait being dependent of another trait (i.e., the T-shape of the ribs cross-section being caused by the broadening of the ribs).

Significant progress won't occur until more fossils related to turtle origins are discovered from Early and Middle Triassic strata. However, the determination of whether or not turtles are modified diapsids may rest more with embryological studies of turtle skull development than fossil finds.

Suggested Future Research

Further work can be done in a variety of ways in many different fields. Embryological studies can be done on the skulls of living turtles to determine if it is a true diapsid skull type or a modified version. Paleontologists can search Middle and Early Triassic rock deposits that show lake, river, or coastal environments for turtle or turtle-like fossils. More studying can be done to determine the distribution of the T-shaped cross-section of the ribs of extant and extinct taxa. Ribs that are not broadened may also be observed for this trait, therefore a variety of broadened and narrow ribs should be studied. A re-examination of *Eunotosaurus* and *Pappochelys* should take place in order to make a more convincing argument that they are truly diapsid. Further work needs to be done using embryology in order to determine which method of carapace development is correct, the ribs growing into the dermis or the ribs being pulled into the dermis by dermal cells.

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