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HETEROCHRONY OF CRANIAL BONES IN AMNIOTA AND THE PHYLOGENETIC PLACEMENT OF TESTUDINES

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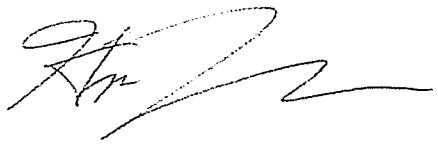

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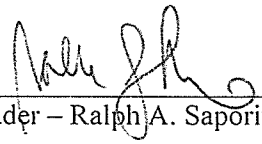

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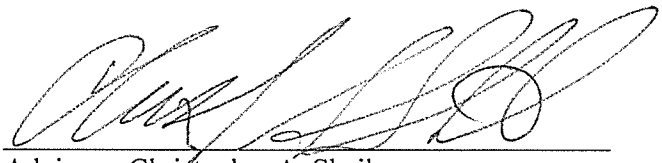

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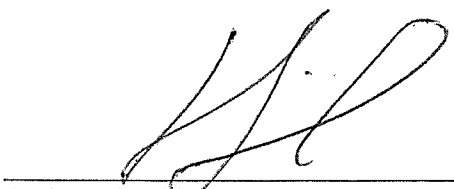

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TABLE OF CONTENTS

ABSTRACT

The study of developmental systems may help to resolve the disagreement between morphological data and molecular data when it comes to the placement of Testudines among Amniota. Among other unique morphological adaptations, turtles possess an anapsid (unfenestrated) condition of the temporal region of the skull. If turtles are descended from diapsids, as molecular data suggests, this implies a rapid transformation of the temporal region from the diapsid condition to the anapsid condition. This study specifically addressed temporal bone heterochony among amniotes using the methods of Continuous Analysis (Germain and Laurin 2009) and Parsimov-based Genetic Inference (Harrison and Larsson 2008) to analyze cranial ossification sequences from representative taxa of all major orders of amniotes. In addition to the use of Continuous Analysis (Germain and Laurin 2009), this study recorded the internodal heterochronies reconstructed with this method. A smaller, complete dataset was analyzed by Continuous Analysis and PGi so that a direct comparison of the methods could be made. A larger dataset with missing data was also analyzed by PGi. Each analysis had three iterations for the three supported placements of Testudines within Amniota. With the data used in this study, I was also able to empirically assess the hypothesis that endochondral bones shift more often during evolution than dermal bones. Endochondral bones were not found to shift any more often than dermal bones during the course of evolution. The results of the analyses of the smaller dataset do not support any particular placement of turtles over another. However, the results of the analyses of the larger dataset support Testudines as sister to all of Diapsida.

INTRODUCTION

Heterochrony

Ernst Haeckel (1880) suggested that change in the ontogeny of a species was only accelerative, and that descendant taxa had "added to" the developmental plan of their ancestor, such that juveniles of descendant taxa were representative of the adult state of the ancestral taxon. For example, the fish-like appearance of tetrapod embryos represented a recapitulation of their fish-like ancestor. This change in developmental plan between an ancestor and its descendants was termed "heterochrony", a reference to the change in timing of developmental events. Gould (1985) later synthesized the idea that heterochrony was not a purely accelerative phenomenon, and by its modern definition (Gould 1977, p. 222-234), heterochrony is recognized to operate under two basic mechanisms that can each occur through three patterns (or types) of shifts (Reilly et al. 1997: Fig. 1). The primary mechanisms include peramorphosis (i.e., accelerated development which is characterized by an extension in development relative to an ancestor) and paedomorphosis (characterized by a truncation in development relative to an ancestor). Both of these mechanisms can occur by changes in rate of development, or a shift in the timing of onset or offset of development, and in either scenario, these changes are suggested to occur between a common ancestor and a descendant taxon (Gould 1985; Reilly et al. 1997: Fig. 1). The most well-known examples of heterochrony are those that demonstrate paedomorphosis, or retention of juvenile or larval traits in a sexually mature adult organism (e.g., retention of larval gills in *Ambystoma mexicanum*), but heterochrony has also been demonstrated in the development of specific somatic organs, such as the loss of limbs in snakes (Cohn and Tickle 1999) and the development

of the ribs in turtles (Nagashima et al. 2009). Changes in ontogenetic or developmental patterns are thought to be primary drivers of evolution (Gould 1985).

Early studies of heterochrony focused on how this evolutionary process can affect gross morphology or the shape of specific organs, by quantifying developmental trajectories (Alberch et al. 1979; Reilly et al. 1997). However, recent studies of heterochrony have been generally applied to sequences of development of modular systems, such as the relative timing of appearance of structures associated with major portions of the basic tetrapod body plan (Werneburg and Sánchez-Villagra 2009) or specifically, bones in the skeleton (Mabee et al. 2000; Sánchez-Villagra et al. 2009; Harrington et al. 2013; Sheil et al. 2014; Koyabu et al. 2014). Renewed interest in collecting developmental data, combined with the publication of new methods to compare developmental events, has resulted in more comparative studies and discoveries of heterochrony in gross morphology and ossification sequences (Nunn and Smith 1998; Jeffery et al. 2002a; Schoch 2006; Werneburg et al. 2009; Werneburg and Sánchez-Villagra 2009; Harrington et al. 2013; Sheil et al. 2014; Koyabu et al. 2014).

Developmental sequences are empirical data that describe the entire ontogeny of an organism, and by comparting developmental sequences across taxa one can infer evolutionary changes in development in a comprehensive context, as opposed to the narrow focus of developmental trajectories. Ossification sequences are widely used in studies of heterochrony, because the timing of ossification of bones may correlate with the evolution of other structures in the body, such as the brain, and distinct life history strategies, such as the short gestation period of marsupials as compared to placental mammals (Nunn and Smith 1998; Maxwell and Larsson 2009; Harrington et al. 2013;

Koyabu et al. 2014).

Whereas some major patterns of heterochrony are now well-understood, more specific patterns of heterochrony are mostly hypothetical. For instance, it is hypothesized that structures that are reduced or "lost" (i.e., those that fail to develop in a descendant) do so through progressive delay (or post-displacement) in initiation of development, and may present a comparative reduction in size (Alberch 1979; Cohn and Tickle 1999; Maxwell and Larsson 2009). For example, flightless ratite birds have small forelimbs, the appearance of which seems to be correlated with relatively late ossification of the forelimb bones (Maxwell and Larsson 2009). Among temnospondyls, loss of skull bones appears to be preceded by a post-displacement of their timing in the developmental sequence, possibly due to a reduction in the overall relative size of these bones (Schoch 2002). Additionally, it has been hypothesized that within the skull, endochondral bones ossify later (Shaner 1926; Good 1995) and exhibit more variability in timing of ossification than dermal bones, and therefore may exhibit more heterochronic shifts (Smith 1997; Montero et al. 1999; Mabee et al. 2000; Sheil and Greenbaum 2005; Sánchez-Villagra et al. 2008). It is unclear if these observations are predictable patterns or even strict laws of heterochrony, and so they need to be empirically studied.

To study heterochrony in an evolutionary context, it is necessary to compare development among taxa. However, standard metrics of developmental progress,(e.g., the length of gestation, crown-rump or snout-vent length, or developmental progress in limb or head formation), vary widely among taxa, meaning the timing of developmental events must be scaled before comparisons are made in a phylogenetic context (Bininda-Emonds et al. 2002; Jeffery et al. 2002a). The common practice is to rank events in a

sequence, which reduces variability in measures to their timing relative to other events thereby removing any consideration of absolute timing and allowing for direct comparisons to be made among taxa (Velhagen 1997; Harrison and Larsson 2008; Germain and Laurin 2009). Developmental sequence data from extant organisms can be used to reconstruct ancestral developmental sequences in a process of mapping onto existing phylogenetic hypotheses (Germain and Laurin 2009; Harrison and Larsson 2008; Harrington et al. 2013; Sheil et al. 2014). Instances of heterochrony can then be discovered by comparing sequences of developmental events between taxa (i.e., between nodes or between nodes and terminal taxa), thereby identifying changes in timing of events between ancestors and descendant taxa.

When considering heterochrony in developmental sequences it is important to understand the modes by which an element may appear to shift (Fig. 1). For instance, the apparent switch in developmental timing of the maxilla and frontal bones can occur five ways; (1) the frontal has moved later relative to the maxilla, (2) the maxilla has moved earlier relative to the frontal, (3) they have both moved in opposite direction, (4) they have both moved to later positions but the frontal moved much later than the maxilla, or (5) they have both moved earlier, but moved much earlier (Fig. 1; Jeffery et al. 2002b). The mode of a shift in relative timing is important to understand because it demonstrates which elements are actually shifting, and in which direction. Ultimately, knowing which type of shift has occurred helps one to understand the general mobility of the elements, and in a larger context, the evolution of developmental sequences.

Although the mode of shifting is important to understanding heterochrony, not all methods for reconstructing sequence heterochrony are capable of making these

distinctions. Currently, the methods that exist for reconstructing sequence heterochrony have distinctly different approaches each with several strengths and weaknesses. The methods that exist to discover heterochrony include Event-pairing (Velhagen 1997); Parsimov (Jeffery et al. 2005); Parsimov-based Genetic Inference (Harrison and Larsson 2008); and Continuous Analysis (Germain and Laurin 2009). These methods are used to reconstruct the ancestral sequences of development and can be used to identify heterochronic shifts in the developmental sequences of descendant species, but only PGi provides actual sequences at ancestral nodes *and* identifies which of the five possible shifts (Fig. 1) must occur between ancestors and descendants.

Event-pairing (Velhagen 1997) operates by converting pairs of events in a developmental sequence into characters that are scored according to the relationship of the event pair, which can then be mapped onto existing phylogenetic trees to reconstruct ancestral sequences. For example, the relative timing of the ossification of the frontal (event A) and the maxilla (event B) would be represented by a character "AB" that would receive the state of "0" (the frontal ossifies before the maxilla), "1" (the frontal and maxilla ossify simultaneously), or "2" (the maxilla ossifies before the frontal). These data are then used to create a character matrix of event-pairs, which is optimized onto a phylogenetic tree, thereby reconstructing the ancestral sequences of development. Heterochrony is then identified as changes that occur between the ancestral and descendant taxa sequences. Parsimov (Jeffery et al. 2005) was developed as an automated method to find the most parsimonious explanation of heterochronies reconstructed with event-pairing data. It is a method that is considered to seek an explanation that minimizes the number of event shifts that could explain the distribution

of ossification sequences observed among terminal taxa, thereby identifying the distribution of ancestral sequences and shifts that requires the fewest possible instances of heterochrony. However, event-pairing as a method of inferring sequence heterochrony is problematic because it creates multiple characters for the same event, cannot incorporate missing data into its analyses, and is known to reconstruct ancestral sequences that are logically inconsistent. For example, Parsimov is known to reconstruct ancestral sequences in which element A appears before element B, B appears before element C, and C appears before A (Schulmeister and Wheeler 2004; Germain and Laurin 2009). Additionally, Parsimov assumes that event-pairs are independent, heritable characters that have biological meaning, and that these event pairs can be mapped on trees to reconstruct ancestral sequences.

Parsimov-based Genetic Inference (PGi; Harrison and Larsson 2008) avoids these paradoxical reconstructions among ancestors by analyzing entire sequences of ossification as complex characters. PGi uses a modified version of the Parsimov algorithm and a heuristic search method to reconstruct entire ancestral sequences, which avoids the issues of atomizing a sequence into event-pairs, and can reconstruct descendant sequences that require the fewest number of evolutionary steps. Additionally, PGi is appealing because it provides the mode of shifting, as per Jeffery et al. (2002b), and can be applied to ossification sequences for which data are missing for timing of appearance of some bones; however, PGi has not yet been updated to be used in conjunction with time-calibrated trees (Harrington et al. 2013).

Continuous Analysis (CA, Germain and Laurin 2009) also avoids the logical inconsistencies of event-pairing, but does so by treating developmental events (such as

timing of appearances of bones) as continuous characters that are scored on a continuum, rather than a discrete scale. Each developmental event can be analyzed through squared-change parsimony, which uses a quadratic formula to optimize the reconstructed value of each ancestral character; this is done by considering the values of its descendants and ancestral states (Maddison 1991). Confidence intervals for the reconstructed ancestral values are calculated from Felsenstein's Independent Contrasts (Felsenstein [1985] as modified by Garland and Ives [2000]), and descendant values that are shown to fall outside of the confidence intervals are considered to represent significant changes (instances of heterochronies; Germain and Laurin 2009). Additionally, Continuous Analysis could be considered appealing because it is a method that can incorporate branch-length data, a more realistic approach because it considers the amount of evolutionary time that has passed. A disadvantage of this method is that it may not be used to analyze datasets with missing sequence data without losing the capacity to reconstruct the affected heterochronies (Laurin and Germain 2011).

Event-pairing, Parsimov-based Genetic Inference, and Continuous Analysis have been applied to discover instances of heterochrony in organogenesis among major clades (Jeffery et al. 2002a; Weisbecker et al. 2008; Werneburg and Sánchez-Villagra 2009), as well as general patterns of heterochrony in post-cranial and cranial ossification (Sheil 2003; Schoch 2006; Germain and Laurin 2009; Werneburg et al. 2009; Harrington et al. 2013; Koyabu et al. 2014; Sheil et al. 2014; Werneburg and Sánchez-Villagra 2014). These studies have revealed potentially important links between changes in development and the evolution of new biological functions (e.g., endothermy in mammals [Jeffery et al. 2002a]).

Temporal Fenestration

The temporal region of the amniote skull is the site of origin for muscles related to jaw movement. Many amniotes have openings or fenestrae in the temporal region that allow for expansion of muscles during jaw adduction, thereby providing greater bite force (Frazzetta 1968). Several patterns of temporal fenestration exist among Amniota and are defined by the bones that border them (Frazzetta 1968; Carroll 1988) (Fig. 2). The anapsid condition presents a skull that lacks temporal fenestration and is considered the plesiomorphic condition for amniotes (Fig. 2A; Carroll 1988). This condition is common among the Parareptilia (Fig. 3). In the synapsid condition (which characterizes the clade Synapsida) there is a single, subtemporal fenestra, which is bordered by the postorbital, squamosal, and jugal, and sometimes the quadratojugal and quadrate bones (Fig. 2B). Within early Reptilia, some lineages possesed the diapsid condition instead of the plesiomorphic anapsid condition (Carroll 1988). The diapsid condition is the defining condition of the clade Diapsida, and is characterized by the presence of the supratemporal fenestra (which is consistently bordered by the parietal, postorbital, and squamosal bones) and the subtemporal fenestra (which is bordered by the postorbital, squamosal, and jugal, and sometimes the quadratojugal and quadrate bones) (Fig. 2D). However, the diapsid condition has been modified evolutionarily several times. For instance, the extinct group Euryapsida has lost the lower temporal opening (Fig. 2C), and the lower temporal bar (formed by the jugal and sometimes qudratojugal bones) is commonly lost among Squamata, resulting in ventrolateral emargination of the skull—this represents one of several highly modified conditions of the diapsid skull. Among extant amniotes, turtles are the only group that do not display temporal fenestration, which is frequently

used as evidence to demonstrate that they have the anapsid condition. However, recent evidence supports the hypothesis that this condition may be derived from the diapsid condition (Bever et al. 2015; Schoch and Sues 2015).

Fenestration may have evolved independently in the synapsid and diapsid lineages, as well as in some extinct early amniotes (Carroll 1988) and at least two hypotheses exist for the origin of these holes in the skull. The most widely accepted hypothesis of skull evolution proposes that temporal fenestration followed a shift in muscle attachment to concentrated areas of a vertically-expanding skull roof, and secondarily allowed for additional expansion of jaw musculature during jaw adduction (Frazzetta 1968, Carroll 1988). An alternative hypothesis proposes that fenestration allowed for a lighter skull, perhaps as a logical outcome of the transition to a terrestrial life, as air is a less physically supportive medium than water (Werneburg 2012). Although turtles lack fenestration, many clades have significant lateral and posterodorsal emargination of their skulls (Gaffney 1979; Müller 2003), which may be functionally comparable to the temporal fenestration of other amniotes (Frazzetta 1968, Werneburg 2012). The lateral emargination of turtles may be produced through the loss of the lower temporal bar, as it is in Squamata, however, the posterior emargination is most likely a unique modification of the skull related to neck retraction (Schoch and Sues 2015; Werneburg 2015).

The Evolutionary Origin of Turtles

Amniotes, united by the ability to lay a cleidoic egg (since lost in placental mammals), includes four major groups (Fig. 3): Mammalia (the mammals), Parareptilia (an extinct group of reptile-like amniotes), Archosauria (birds and crocodiles), and

Lepidosauria (squamates and tuatara). The largest unsolved mystery of amniote phylogeny includes one of the most interesting groups in amniotes, in terms of issues relating to development, the Testudines. Turtles are unique among extant amniotes in their possession of an apparently anapsid skull, a condition that is also prevalent in the extinct, paraphyletic, group of basal reptiles, Parareptilia (Fig. 2), and that may represent the plesiomorphic condition of the skull in the earliest amniotes and reptiles. However, the temporal bones of turtles (jugal, parietal, postorbital, quadratojugal, and squamosal) appear to have a different arrangement than most extinct anapsid reptiles, with regard to the relative positions of the jugal and quadratojugal (Müller 2003), and recent evidence suggests that their anapsid condition may represent yet another state derived from the diapsid condition (Bever et al. 2015; Schoch and Sues 2015). Turtles also possess a carapace and plastron, and pectoral and pelvic girdles have shifted (evolutionarily) beneath the ribcage, making statements of morphological homology between turtles and other reptiles difficult (Carroll 1988; Rieppel 1996; Lee 1997a; Wilkinson et al. 1997; Nagashima et al. 2013). The monophyly of the major clades of amniotes is well supported, and the relationships among them are well understood with the exception of the placement of Testudines relative to other reptiles (Reisz and Laurin 1991; deBraga and Rieppel 1997; Wilkinson et al. 1997; Rieppel and Reisz 1999; Lyson et al. 2012; Werneburg 2013). The transitional fossils that demonstrate putative intermediate forms between the anatomy of turtles and other reptile clades are inconclusive, and hypotheses for the placement of turtles have often relied heavily on inference from morphological and molecular data of crown-group taxa only. Because turtles are so highly derived, most phylogenetic hypotheses based on morphological data have placed them entirely outside

all other extant reptiles (Fig. 3, Scenario 1; Gauthier et al. 1988; Reisz and Laurin 1991; Lee 1997b; Werneburg and Sanchez-Villagra 2009). Contrary to most morphological studies, the majority of molecular-based phylogenetic hypotheses find support for the placement of turtles within Diapsida (Fig. 3, Scenarios 2 and 3; Hedges and Poling 1999; Iwabe et al. 2005; Chiari et al. 2012; Crawford et al. 2012; Lyson et al. 2012; Lu et al. 2013; Crawford et al. 2015), and support their placement either nested within, or as sister to, Archosauria (Fig. 3, Scenario 2; Kirsch and Mayer 1998; Hedges and Poling 1999; Kumazawa and Nishida 1999; Iwabe et al. 2005; Chiari et al. 2012; Fong et al. 2012; Lu et al. 2013; Field et al. 2014). Fewer molecular studies support the placement of turtles as related to Lepidosauria (Fig. 3, Scenario 3; Lyson et al. 2012). The results of Werneburg and Sánchez-Villagra (2009), which examines developmental data, support the placement of turtles as sister to Diapsida, but do not refute alternative placements within Diapsida.

Knowing the true placement of turtles within Amniota is key to understanding the evolutionary history and origin of various patterns of temporal fenestration in amniote skulls. The major implication of turtles as sister to archosauromorph or lepidosauromorph reptiles is that the anapsid condition of the skull would represent a secondarily-derived condition from a diapsid skull, and the pattern seen in turtles is therefore not plesiomorphic, but rather represents a highly derived diapsid condition. This scenario also implies that during the course of turtle evolution, the skull evolved from the typical diapsid condition to that of an apparent anapsid condition, and becoming extremely emarginated in some species (Gaffney 1979). Depending on where turtles are placed (Fig. 3), they either represent a plesiomorphic skull condition, or one of the most

derived examples of a modified diapsid skull (Fig. 2).

The disagreement on the phylogenetic placement of turtles based on results of analyses of molecular and morphological data may be resolved with input from developmental data. Despite the extensive investigation of amniote phylogeny, few studies employ developmental data to reconstruct phylogenetic history (Fucik 1991; Schoch 2006; Weisbecker et al. 2008; Werneburg and Sanchez-Villagra 2009), and all have difficulties in doing so. Fewer studies specifically address the temporal regions of amniote skulls beyond simply stating that alternate placements have implied alternate scenarios of temporal series evolution (Werneburg and Sánchez-Villagra 2009). A study of the morphology of tendons in the temporal region of amniotes (Werneburg 2013) supported a placement of turtles outside of Sauria (*sensu* deBraga and Rieppel 1997) (Fig. 3), but does not exclude the possibility that turtles are basal diapsids. Comparative studies of developmental sequences are lacking, mainly because the methods of comparison have only recently been developed (Harrison and Larsson 2008; Germain and Laurin 2009), and comparable new ossification data rarely are combined with existing data in the literature. As a consequence, large-scale comparative developmental sequence studies are relatively new, and few have specifically analyzed ossification sequences (Jeffery et al. 2002a; Schoch 2006; Werneburg et al. 2009; Werneburg and Sánchez-Villagra 2009; Harrington et al. 2013, Koyabu et al. 2014).

Hypotheses

Because the variable position of turtles within Amniota has different implications for the evolution of the skull, I hypothesize that the scenarios of skull bone heterochrony

will differ when the three alternate phylogenetic placements of turtles are considered (Fig. 3): Scenario 1) as sister to Diapsida (Gauthier et al. 1988; Laurin and Reisz 1995; Werneburg and Sánchez-Villagra 2009; Lyson et al. 2010); Scenario 2) sister to Archosauria (Kirsch and Mayer 1998; Meyer and Zardoya 1998; Hedges and Poling 1999; Kumazawa and Nishida 1999; Iwabe et al. 2005; Chiari et al. 2012; Fong et al. 2012; Lu et al. 2013; Schoch and Sues 2015) and therefore representing a modified diapsid condition; and Scenario 3) as sister to Lepidosauria (deBraga and Rieppel 1997; Rieppel and Reisz 1999; Li et al. 2008; Lyson et al. 2012) and therefore representing a modified diapsid condition. Herein, my preferred placement of turtles will be the one that requires the fewest number of evolutionary steps or the fewest reconstructed heterochronies.

I hypothesize specifically that there are identifiable instances of ossification sequence heterochrony in the formation of the temporal series bones (jugal, parietal, postorbial, quadratojugal, and squamosal) among crown-group amniotes, because there is a wide range of temporal modification in this clade, and that the ossification sequence of the temporal series of turtles should fit most parsimoniously in one of the three phylogenetic positions tested. Due to the reduction of the postorbital and jugal bones through emargination in turtles (Gaffney 1979), I would expect these bones to be delayed in ossification relative to other clades, but not necessarily among Lepidosauria, in which many clades exhibit reduction or loss of the jugal. The hypotheses of temporal bone evolution and heterochrony will be tested through Continuous Analysis (Germain & Laurin 2009) and Parsimov-based Genetic Inference (PGi: Harrison & Larsson 2008). I hypothesize that endochondral bones will exhibit significantly more shifts than dermal

bones, and I will test this with a Chi-squared test of the heterochronic shifts reconstructed with PGi.

METHODS

Cranial Ossification Sequence Data

Cranial ossification sequence data were compiled from published literature for 70 unique species (Table 1) from five major clades within Amniota (38 Mammalia, 11 Testudines, 10 Squamata, 8 Aves, and 3 Crocodilia) and newly-collected data are reported for *Lepidochelys olivacea* and *Eretmochelys imbricata* (Tables 1 and 2). These data summarize the sequence of appearance of bones through ontogeny. At a minimum, each order of amniotes was represented by one species. Amphibians were not included as an outgroup because their skulls are highly derived and do not possess many of the bones that are present in amniotes; including Amphibia would reduce the pool of bones that are common across all taxa, and therefore the number of bones that could be used in the analyses. Because of the study's focus on the placement of turtles within Reptilia, Mammalia were treated as the outgroup in all analyses for the purpose of reconstructing ancestral developmental sequences at the base of Amniota. Snakes were not included in the dataset because their skulls are highly derived and therefore might skew reconstructions of ancestral sequences within Squamata (Werneburg and Sánchez-Villagra 2014). The postorbital and quadratojugal bones were not included in any analysis, because of low representation in the literature. The sequence for *Eretmochelys imbricata* (Sheil 2013) was recorded from the original specimens and original notes, rather than the published table of data, because discrepancies exist in the original

published descriptions. New, original data were obtained for *Lepidochelys olivacea* (Table 2), based on 53 specimens ranging from Stage 20 to Stage 31 (Crastz 1982; Miller 1985; Appendix 1). Embryos were staged based on reference to external anatomy according to Miller (1985), with reference to Crastz (1982). Embryos were cleared and double-stained with Alzarin Red and Alcian Blue to indicate the presence and timing of ossification of bone and cartilage, respectively (Taylor and Van Dyke 1985; Sheil 1999). The ossification sequence was inferred by examination of dissected embryos with a dissecting microscope (Leica MZ125, Leica Microsystems Ltd., Switzerland).

Considerations of the Dataset

The wide range of taxa included in this study introduced some complexity to assessment of ossification sequences because different identities and names have been applied to homologous elements across some taxa (Table 1 legend). For example, the incus in mammals is homologous to the quadrate in reptiles. The complete set of metadata was optimized individually for each of the analyses run (either CA or PGi), and was analyzed on each of the three scenarios for the phylogenetic placement of turtles (Fig. 3). For each analysis, a dataset was constructed from the metadata set that eliminated bones and/or taxa to construct datasets that maximized the number of taxa and bones, while considering the constraints of an analysis (e.g., CA cannot reconstruct heterochronies with missing data). Inclusion of bones in every generated dataset was prioritized over inclusion of taxa, as the exclusion of bones increases the chance that a bone will erroneously not appear to move, because the analyses are reconstructing the relative motion of bones within a sequence.

Analysis	Number of Taxa	Number of Bones
Continuous Analysis	20	15
PGi (complete)	20	15
PGi (with missing data)	39	15

Sequences with poor resolution (i.e., a great number of "ties" in appearance of bones) are, at a minimum, unresolved (Velhagen 1997; Bininda-Emonds 2002). Lack of sequence resolution increases the likelihood of Type I error, because the greater the number of events that are "tied," the more uncertain the actual timing of these events becomes and the higher the chance that they will erroneously appear to shift (see Discussion). Therefore, species with a sequence resolution (i.e., number of developmental stages or ranks) of 3 or less were excluded from all datasets, with the exceptions of *Ornithorhynchus anatinus* (de Beer and Fell 1936; de Beer 1937) and *Sphenodon punctatus* (Howes and Swinnerton 1901), which were left in the analyses because these are critical, basal taxa among Mammalia and Lepidosauria, respectively (Table 1).

Finally, in recording ossification sequences from the existing literature, the criteria for what qualifies as first appearance of a bone were recorded for each sequence. Most studies (including this one) recognize first appearance of bone as the first stage at which 100% of the embryos retain Alizarin stain in the bone in question. However, some studies label first appearance based on the observation of bone-like texture of tissue that preceeds retention of Alizarin stain in bone. When appearance of texture and retention of Alizarin stain were recorded in the same study, the data given for retention of Alizarin stain was taken for use in this study.

Phylogenetic Hypotheses

The phylogenetic placement of turtles is contentious (Gauthier et al. 1988; Reisz and Laurin 1991; Lee 1997b; Kirsch and Mayer 1998; Hedges and Poling 1999; Kumazawa and Nishida 1999; Rieppel and Reisz 1999; Iwabe et al. 2005; Li et al. 2008; Werneburg and Sanchez-Villagra 2009; Chiari et al. 2012; Fong et al. 2012; Lyson et al. 2012; Lu et al. 2013; Field et al. 2014; Schoch and Sues 2015), and three possible placements (Scenario 1, 2, and 3) have been proposed by molecular and morphological data (Fig. 3). To examine the impact of these competing hypotheses, turtles were placed at these positions on the tree for all analyses: Scenario 1) as sister to Diapsida (Gauthier et al. 1988); Scenario 2) sister to Archosauria (Hedges and Poling 1999); and Scenario 3) sister to Lepidosauria (deBraga and Rieppel 1997). For the rest of this paper these phylogenetic hypotheses will be referred to as Scenario 1, Scenario 2, and Scenario 3, respectively.

Trees used for the reconstruction of ancestral states through mapping were pared down from the existing literature (Novacek 1992; Thomson and Shaffer 2009; Barley et al. 2010; Kimball et al. 2013; Pyron et al. 2013; Wang et al. 2013; Shinohara et al. 2014). Time-calibration of the trees (used by CA) was based primarily on information from TimeTree.org in December 2014 (Hedges et al. 2006) using the "Expert" divergence date, unless a discrepancy occurred between the divergence dates and the phylogenetic hypothesis, in which case the "Median" date was used. In other words, if the TimeTree.org "Expert" date would require a clade to be in an incorrect position, then the "Median" date was used instead. The divergence date for turtles and all other reptiles (Scenario 1) of 282 Mya was taken specifically from Pereira and Baker (2006), as

TimeTree.org does not yield different results when comparing Testudines to either Diapsida or any subset of Diapsida (e.g., Lepidosauria, Archosauria, Squamata, etc.).

Continuous Analysis of the Complete Dataset

Continuous Analysis (Germain and Laurin 2009) of a pared dataset with 20 taxa (7 turtles, 4 squamates, 6 birds, 1 crocodilian, and 2 mammals; Table 3), 15 bones, and no missing data (i.e., a complete dataset) was performed as a complement to a PGi analysis (below). In their original paper, Germain and Laurin (2009) compared ossification sequences from terminal taxa to the reconstructed sequence of the root node and used this comparison to infer instances of heterochrony; however, this type of comparison precludes the ability to identify on which exact branch a significant instance of heterochrony occurred. Herein, adjacent nodes were compared so that significant shifts could be said to occur definitively between a particular ancestor and its immediate descendant. Sequences from terminal taxa were also compared to the root node to complement and compare to the node-to-node approach. Lastly, terminal taxa within Testudines were compared to their last common ancestor with all diapsids, archosaurs, or lepidosaurs, for Scenarios 1, 2, or 3, to see the differences in reconstructed heterochrony among all phylogenetic hypotheses.

In each of the 20 ossification sequences (Table 3), the 15 bones were scored according to their position in the sequence of ossification of the cranium, following the ranking protocols for CA (Germain and Laurin 2009). The first and last bone(s) to appear were scored as “0” and “1,” respectively, and every bone appearing between these extremes was scored proportionally according to the formula:

$$y = (x_i - x_{min})/(x_{max} - x_{min})$$

where y is the assigned value, x_i is the rank value of the bone, x_{min} is the lowest rank in the sequence, and x_{max} is the highest rank in the sequence. For example, if there are "n" events (appearances of bones), the interval from "0 to 1" is divided into n–1 segments at equal intervals. Given the focus of this study on turtles and the temporal region, bones generally not found in turtles (e.g., nasals and lacrimals) were excluded from this analysis, whereas some temporal bones (e.g., jugal) were mandatory in the sequence for a taxon to be included in the analysis (Table 3). Ancestral nodal sequences were reconstructed using squared-change parsimony (Maddison 1991) on a time-calibrated phylogeny in Mesquite v2.75 (build 564) (Maddison and Maddison 2011) for each of Scenarios 1, 2, and 3 for the placement of turtles (Fig. 3). Confidence intervals for every event in an ancestral sequence were calculated with Felsenstein's Independent Contrasts (Felsenstein 1985), using the PDAP module v1.16 of Mesquite (Midford et al. 2010), following the protocols of Maddison (1991). Heterochrony was inferred by identifying instances in which difference in the relative timing of ossification of a descendant taxon fell outside of the 95% CI of the ancestral value; these significant shifts were considered to be instances of heterochrony, *sensu* Germain and Laurin (2009) and Laurin and Germain (2011).

Parsimov-based Genetic Inference of the Complete Dataset

The dataset with no missing data (Table 3) was analyzed with three iterations (Scenarios 1, 2, and 3) for the placement of turtles within Reptilia using PGi. This permitted direct comparison of the results of PGi and CA. Each of the 15 cranial bones

in each of the 20 sequences was scored according to their position in the sequence of ossification, following the ranking protocols of PGi (Harrison and Larsson 2008). The bones were assigned a numerical rank for positions 1 through 9, and an alphabetical rank beyond that, due to constraints of the program (e.g., 10 = A, 11 = B, etc.). All analyses were run using a Parsimov edit-cost function, and a semi-strict superconsensus with 30 cycles and 2,000 replicates.

Parsimov-based Genetic Inference of the Dataset with Missing Data

A Parsimov-based Genetic Inference (PGi) analysis of a larger dataset that incorporated missing data was run and allowed for an analysis of a larger set of taxa. Analyses were run for all three scenarios for the placement of turtles (Fig. 3) to address the hypotheses of temporal bone heterochrony in amniotes. The dataset pared from the metadata had 15 bones and 39 taxa (9 turtles, 4 squamates, 6 birds, 1 crocodilian, and 19 mammals; see Table 4). Individual bones had a maximum of 40% missing data across taxa (coded as Z), individual taxa had a maximum of 20% missing data, and overall missing data for the entire dataset was 6.2%. Cranial bones in every sequence were scored as described above. All analyses were run using a Parsimov edit-cost function, and a semi-strict superconsensus with 30 cycles and 2,000 replicates.

Chi-Squared Test

A Chi-squared test was run to test the hypothesis that endochondral bones shift more often in the course of evolution than dermal bones. The shifts reconstructed with both PGi analyses were used as the count data. The number of reconstructed shifts in

endochondral bones was compared to the number of reconstructed dermal bones, per scenario of turtle placement. The null hypothesis was that endochondral bones and dermal bones would exhibit an equivalent number of shifts.

RESULTS

Cranial Ossification Sequence for *Lepidochelys olivacea*

The sequence of cranial ossification for *Lepidochelys olivacea* (Table 2) describes the appearance of 26 bones resolved over 13 stages. Nine stages captured a single instance of ossification, and four stages captured two or more ossification events. Some variability was observed in the relative timing of ossification of the exoccipital, supraoccipital, basioccipital, prootic, and opisthotic bones as indicated by variable presence in embryos at earlier stages. All cranial bones initiated ossification by the time of hatching. Timing of appearance of bones was based on the criterion of first appearance prior to stage 26, because of the low numbers of specimens and (in some instances) moderate bleaching led to weak indication of staining with Alizarin Red. Timing of appearance in Stages 25 and beyond was based on 100% retention of Alizarin, except in the case of the prootic and opisthotic bones, which consistently ossified together but had only 67% retention by Stage 30 (Sheil et al. 2014). The decision was made to treat the prootic and opisthotic bones as ossifying before the articular bone, because the articular did not retain Alizarin until Stage 30, whereas the prootic and opisthotic first retained Alizarin at late Stage 28.

Continuous Analysis of the Complete Dataset

Ancestral sequences were reconstructed with minor differences among Scenarios 1, 2, and 3 for the placement of turtles. For example, the pterygoid and squamosal bones in Archosauria occupy the 4th and 5th positions, respectively (Scenario 1), but occupy the 5th and 4th positions, respectively, in Tables 6 and 7 (for Scenarios 2 and 3, respectively). Similarly, one-position shifts were also found in Lepidosauria, Testudines, and Amniota for the maxilla and pterygoid bones, pterygoid and palatine bones, and premaxilla and pterygoid bones, respectively (Tables 5–7). The ancestral sequences reconstructed on Scenario 2 were not unique and were either identical to those reconstructed on Scenario 3 or Scenario 1. Node-to-node comparison of reconstructions for all three phylogenetic hypotheses yielded an early shift of the prootic bone in *Coturnix coturnix* only, and no other significant heterochronic shifts were found. Some differences in reconstructed heterochrony were found among the comparisons of terminal branches for Testudines relative to their three different hypothetical ancestors (e.g., an early ossification of the pterygoid bone in *Chelydra, Eretmochelys,* and *Phrynops* in Scenario 1 but not Scenario 2 or 3; and significant shifts of the jugal bone in *Eretmochelys, Apalone,* and *Phrynops* in Scenarios 2 and 3 but not Scenario 1) (Table 8). There were no differences in reconstructed heterochrony among Scenarios 1-3 when comparing terminal taxa to the root node (Table 9). In all scenarios, when comparing ossification sequences for terminal taxa to the root node, late ossification of the supraoccipital bone was found in 7 of the 19 non-mammalian taxa, late ossification of the pterygoid bone was found in both mammalian taxa (*Ornithorhynchus* and *Loris*) and early ossification of the pterygoid bone was reconstructed for 8 of the remaining 19 taxa (Table 9). There were no

differences in reconstructed among Scenarios 1–3 when comparing terminal taxa to the ancestor of Testudines, and all scenarios reconstructed a late ossification of the jugal bone in *Macrochelys* and *Apalone* (Table 10). The dentary bone of *Lepidochelys* ossifies late in all scenarios, when compared with the ancestor of Testudines, and in Scenarios 2 and 3 when compared to the shared ancestor with Archosauromorpha and with Lepidosauromorpha (Table 10). All scenarios and node-to-tip comparisons reconstructed an early ossification of the parietal bone in *Chelydra* and *Eretmochelys* (Tables 8–10).

Comparing a terminal taxon to more than one ancestor yields some variability in the reconstructed heterochronies. For example, compared to the root node of all amniotes (Table 9), *Chelydra serpentina* has relatively early ossification of the frontal, parietal, and pterygoid bones. Compared to the alternate ancestors (Scenarios 1–3; Table 8), *C. serpentina* has relatively early ossification of the frontal, parietal, premaxilla, pteryoid, and squamosal bones (Scenario 1), or relatively early ossification of the frontal, parietal, premaxilla, and squamosal bones (Scenario 2 and 3). Compared to the ancestor of Testudines, *C. serpentina* has relatively early ossification of the frontal, parietal, and premaxilla bones. Compared to its immediate ancestor, *C. serpentina* has no significant shifts. In all comparisons, the frontal and the parietal bones are shifted early. However, the other heterochronies do not reconstruct in every comparison. All of the species of Testudines exhibited similar variability when compared to the various ancestors (Tables 8–10).

Parsimov-based Genetic Inference of the Complete Dataset

The PGi analysis of the data used for CA (Table 3) reconstructed 124 (56 early and

68 late), 122 (62 early and 60 late), and 123 (52 early and 71 late) shifts for Scenarios 1, 2, and 3, respectively (Table 11). The maxilla exhibited the greatest number of shifts (34 total) and the palatine exhibited the fewest number of shifts (10 total) among Scenarios 1–3 (Table 11). However, the basioccipital, basisphenoid, dentary, and supraoccipital bones shifted the most (11 shifts) in Scenario 1, and the palatine the fewest (3 shifts). The jugal exhibited the most shifts (13) in Scenario 2, and the palatine the fewest (3 shifts). The maxilla exhibited the most shifts (12) in Scenario 3, and the prootic the fewest (1 shift). Notably, the jugal shifted only 6 times in Scenario 1, but 13 and 8 times in Scenarios 2 and 3, respectively. Ancestral sequences reconstructed with PGi using this dataset had minor differences among all scenarios (Tables 5–7). The general pattern of temporal bone heterochrony progressing from the ancestor of Reptilia (Figs. 4–6 internode 4; Table 11 row 4) to the ancestor of Cryptodira (Figs. 4–6 internode 7; Table 11 row 7) is similar across all scenarios. Between the ancestor of Reptilia and the ancestor of Cryptodira, the palatine exhibited no shifts in any scenario (Table 11, Figs. 4–6); but 22 heterochronies were reconstructed across all three scenarios, regardless of the placement of Testudines (Table 11). Of particular interest are the late shift of the parietal and squamosal bones in Cryptodira, because they did not move as hypothesized (Table 11).

Parsimov-based Genetic Inference of the Dataset with Missing Data

PGi reconstructed differences in ancestral sequences among Scenarios 1–3 (Table 12). The PGi analysis of the dataset with missing data (Table 4) yielded 127 (62 early and 65 late), 264 (147 early and 117 late), and 290 (162 early and 128 late) shifts in

timing for Scenarios 1, 2, and 3, respectively. The nasal exhibited the most shifts (55 total) and the dentary exhibited the fewest shifts (28 total) among all scenarios (Table 13). However, the nasal shifted the most (13 shifts) in Scenario 1, and the dentary and parietal the fewest (5 shifts). The pterygoid exhibited the most shifts (23) in Scenario 2, and the dentary the fewest (10 shifts). The premaxilla exhibited the most shifts (27) in Scenario 3, and the supraoccipital the fewest (10 shifts). Notably, the parietal shifted only 5 times in Scenario 1, but shifted 18 and 23 times in Scenarios 2 and 3 respectively. Between Reptilia (Figs. 7–9 internode 38; Table 13 row 38) and Cryptodira (Figs. 7–9 internode 43; Table 13 row 43) the reconstructed heterochronies are not similar across scenarios. That is, across Scenarios 1–3 the placement of turtles produced a mostly unique set of heterochronies (Table 13). The only congruence between the scenarios was a lack of shifts in the maxilla between the Reptilia ancestor and the Cryptodira ancestor (Table 13, Figs. 7–9). Eighteen heterochronies were reconstructed across Scenarios 1–3, regardless of the placement of Testudines (Table 13).

Comparison of PGi and Continuous Analysis

The ancestral sequences reconstructed using CA and PGi on the same dataset were similar, overall, but sometimes reconstructed larger differences. For example, in the Amniota ancestor in Scenario 1, the pterygoid reconstructed first with CA in a 15-event sequence, but second-to-last using PGi in a 4-event sequence (Table 5). In the ancestor of Iguania and Anguimorpha in Scenario 2, the squamosal reconstructed fifth with CA in a sequence of 15 events but fifth with PGi in a sequence of 8 events. The early shift of the prootic in *Coturnix* found with CA was not found in any scenario using PGi (Table

11). There was no overlap in reconstructed heterochronies for *Eretmochelys* or *Lepidochelys* between the PGi analysis and Continuous Analysis. Heterochronies overlap between the PGi analysis and Continuous Analysis for the rest of the species of Testudines. The early shift of the opisthotic in *Pelodiscus* was the only heterochrony to reconstruct in all analyses and comparisons (Tables 8-11).

Chi-Squared Test

None of the Chi-squared tests of shifts in endochondral versus dermal bones for either set of PGi analyses (the complete dataset or the dataset with missing data) were significant. The lowest p-value among the Chi-squared tests was 0.58, for Scenario 2 of the PGi analysis of the complete dataset. The highest p-value among the Chi-squared tests was 1.00, for Scenario 3 of the PGi analysis of the complete dataset.

DISCUSSION

The Placement of Testudines

Ossification sequence heterochrony of skull bones was reconstructed differentially among scenarios of turtle placement, supporting the hypothesis that the phylogenetic position of turtles may influence reconstructions of skull bone evolution. Each set of analyses reconstructed a different set of heterochronies, and therefore a different set of hypotheses of skull evolution (below). The preferred scenario of placement of turtles according to the PGi analysis of the dataset with missing data is as sister to all other diapsids, given the 137 fewer shifts reconstructed in that scenario (Table 13). However, the PGi analyses of the complete dataset reconstructed similar numbers of shifts across

scenarios, so I cannot reject Scenarios 2 and 3 as possibilities (Table 11). My preferred scenario of turtles as sister to Diapsida contradicts the recent finding of Sues and Schoch (2015) who support turtles as nested with Lepidosauria based on morphological evidence. However, it does not preclude the possibility that turtles are diapsids, a possibility supported by Bever et al. (2015) who re-examined *Eunotosaurus africanus,* a good candidate for a close ancestor of turtles (Lyson et al. 2010). They found *E. africanus* to be potentially transitional between the diapsid condition and anapsid condition of the temporal region, as the juveniles of *E. africanus* appear to have a supratemporal fenestra which is closed in adults, possibly an ontogenetic analog of the evolutionary closure of that fenestra. Indeed, our findings are consistent with the hypothesis that the placement of Testudines within Diapsida requires more changes in the skull, as that scenario requires that the anapsid condition of the turtle skull arose from the diapsid condition, and the results of the PGi analysis of the dataset with missing data reconstructed a much greater number of shifts in Scenarios 2 and 3 (the scenarios which place turtles within Diapsida) than in Scenario 1 (Table 13).

Continuous Analysis of the Complete Dataset

Continuous Analysis finds differences in ancestral sequences depending on the hypothesis of turtle placement (Scenarios 1–3). However, there were only differences in reconstructed heterochronies among the three scenarios when comparing to the three hypothetical, common ancestors of all Diapsida, Archosauromorpha, or Lepidosauromorpha (Table 8, Figs. 4–6 Node 3). For example, the pterygoid reconstructs as shifting early in Scenario 1 in *Chelydra,* but not in Scenario 2 or 3, and

the jugal reconstructs as shifting late in Scenarios 2 and 3 in *Apalone,* but not in Scenario 1 (Table 8). Additionally, heterochronies reconstructed in Scenarios 2 and 3 were more similar to each other than to those reconstructed in Scenario 1. This suggests that the divergence date of turtles was more influential than the position of turtles within the tree, because Scenarios 2 and 3 had the same divergence date (262 Mya), whereas Scenario 1 required an earlier divergence date (282 Mya) (Figs. 4–6).

Changing the phylogenetic position of Testudines (Scenarios 1–3) had only minor effects on reconstructed heterochronies, because of the nature of the analysis. Continuous Analysis infers the values of the root node from the terminal nodes, and changing positions within the tree doesn't change the pool of observations for the terminal taxa, demonstrating that the values of the root node are more dependent on the data for the terminal taxa than the relationships within the tree.

Although the differences between the scenarios of turtle placement are minor and the biological relevance is obscure, the reconstructions of the supraoccipital, pterygoid, and jugal are interesting. Late ossification of the supraoccipital in 7 out of 19 non-mammalian terminal taxa (as compared to the root node) may support the finding of Koyabu et al. (2014) of early supraoccipital timing in mammals being correlated with encephalization—later ossification of the supraoccipital (Table 9) may reflect the comparatively smaller brain size in non-mammalian taxa. The early ossification of the pterygoid in *Chelydra, Eretmochelys*, and *Phrynops* in Scenario 1 (but not in Scenarios 2 and 3) may reflect the larger palate of turtles as compared to other Reptilia (Table 8). Crocodilians have expanded palates (i.e., the secondary palate), however, only one crocodilian (*Caiman*) was included in the analysis. If additional crocodilians were

included, the pterygoid might not be reconstructed as ossifying earlier in Testudines, or it might appear to ossify late in Archosauria. The ossification sequences for *Alligator mississipiensis* (Rieppel 1993) and *Crocodylus cataphractus* (Müller 1967) were not included because they lacked records for key bones.

The variability among these reconstructions in the ossification of the jugal between Scenario 1 and Scenarios 2 and 3, may reflect the implied differences in temporal bone evolution. Interestingly, these differences only exist between the placement of turtles within crown-group Diapsida, or outside of crown-group Diapsida. Early ossification of the jugal in *Eretmochelys* may reflect the comparative lack of temporal emargination in that species (Gaffney 1979). The early ossification of the jugal in *Phrynops* may be related to its contribution to the unique pleurodiran structure of the postorbital wall (Gaffney 1979). Late ossification of the jugal in *Macrochelys* and *Apalone*, as compared to the common ancestor of all Testudines may reflect temporal emargination and consequent reduction of the jugal, supporting my hypothesis that ossification of the jugal will be delayed in Testudines.

This study's finding that node-to-node comparisons using the Continuous Analysis method do not produce significant heterochronies except under extreme circumstances (e.g., the early ossification of the prootic in *Coturnix*), would indicate that, at least used in this way, Continuous Analysis is a highly conservative method of heterochrony reconstruction. This also means that Continuous Analysis is not a comparable method to PGi, in that Continuous Analysis cannot recreate the exact internodal branches on which heterochronies occur, and therefore cannot be used to quantify heterochronic shifts the way that PGi can. Continuous Analysis is best used to generalize about heterochronic

change and for estimating the rate at which a bone shifts (part of the calculation in Felsenstein's Independent Constrasts; Felsenstein 1985).

Some of the heterochronies reconstructed with Continuous Analysis may be artifacts of poor sequence resolution. For example, the sequence of *Coturnix* has lower resolution than the other bird sequences used in these analyses (Table 1), which may explain the reconstruction of a significant early shift of the prootic. In particular, 7 of the 15 bones used in the analysis were tied for the same position in the *Coturnix* sequence. Similarly, the reconstruction of a late-ossifying dentary in *Lepidochelys* may be the consequence of there being 7 ties with other bones for the second position (Table 3 and 8). If the dentary truly ossifies in the second position, for example, its value (as assigned by Continuous Analysis) is inflated. Continuous Analysis may also have deflated the values of the parietal in *Chelydra* and *Eretmochelys,* both of which exhibit the parietal as tied for the first position with 7 and 6 bones, respectively (Table 3), thereby explaining their significantly early ossification. For example, consider a sequence of events involving seven bones. If the sequence is fully resolved, the bones will be scored 0, 0.17, 0.33, 0.5, **0.67**, 0.83, and 1, in order (see Methods for the formula). However, if the first five bones are tied, they will be scored 0, 0, 0, 0, **0**, 0.75, and 1, respectively. Note that whichever bone is in the fifth position (indicated with bold) has its value reduced by more than half the interval when tied with other bones. Additionally, the value of the sixth bone is deflated. It could be argued that Continuous Analysis is particularly sensitive to ties in sequences, and the more bones tied for a position, the worse the distortion of their true values.

Parsimov-based Genetic Inference of the Complete Dataset

In the set of analyses using PGi and the dataset without missing data, there weas no preferred scenario of turtle placement, as all Scenarios 1, 2, and 3 reconstructed 124, 122, and 123 shifts respectively, a difference too small to dismiss any scenario as a plausible placement for Testudines. Among results of analyses for all three scenarios, the jugal reconstructed with fewer shifts in Scenario 1 than in either Scenario 2 or 3, which suggests that Scenario 1 may afford the most parsimonious explanation for the evolution of temporal bone development. Overall, the placement of Testudines did not seem to have a large influence on ancestral sequences or the general patterns of reconstructed heterochrony, particularly in the temporal bones (Table 11).

For the complete dataset, some ancestral sequence reconstructions generated with PGi produced unusual patters of ossification that are not typical of most extant taxa, that fall into four categories: 1) the dentary, maxilla, or premaxilla bones are not the first to ossify; 2) the dentary and/or maxilla appear in the fourth position or later in the sequence; 3) the exoccipital, basioccipital, or basisphenoid bones ossifying first or second in the sequence; or 4) the premaxilla ossifies within five positions from the end of the sequence in sequences that exhibit a resolution greater than 7 events. Scenario 1 reconstructed 12 of these unusual timings, whereas Scenario 2 reconstructed 13 of these events, and Scenario 3 reconstructed 11 of these events (Tables 5–7). These unusual patterns of ossification may be artifacts of the poor resolution of many sequences in the dataset, as a result of numerous ties and/or poor specimen sampling across development.

The maxilla exhibited the most shifts across all three scenarios which may be a result of the apparent lability of bones related directly to feeding (Bever 2009; Curtis et

al. 2011; Harrington et al. 2013). The low number of shifts in the palatine across all three scenarios is not surprising given the consistency with which it ossifies early (Table 3). These counts also suggest that endochondral bones are not more evolutionarily flexible in timing than dermal bones, as endochondral bones and dermal bones shifted roughly the same amount (Tables 4–6).

The heterochronies reconstructed in all three scenarios of turtle placement suggest that the evolution of the turtle skull involved an early- (then late) shift of the jugal, a late- (then early) shift of the parietal, and an early shift of the squamosal. The late shift of the jugal supports our hypothesis of lateral emargination causing a delay in the timing of the jugal. The late timing of the parietal, however, does not support the hypothesis that larger bones will have an earlier onset of ossification, and the early shift of the squamosal could be related to its final size, or to the development of jaw musculature. Late shifts of the parietal and squamosal in Cryptodira (Table 11) are interesting, because they do not support the hypothesis of final bone size being linked to the timing of onset of ossification. However, the late shift of the parietal may be related to the posterior emargination of the skull, similar to the hypothetical delay in ossification of the jugal, which is due to lateral emargination.

Parsimov-based Genetic Inference of the Dataset with Missing Data

In the set of analyses using PGi and the dataset with missing data, Scenario 1 presents the scenario of turtle placement that requires the fewest number of heterochronic shifts in bones, requiring approximately half the number of shifts (Table 13). Scenario 1 requires 127 shifts, whereas Scenario 2 and 3 require 264 and 290, respectively.

Additionally, the parietal reconstructs the fewest (5) shifts in Scenario 1, whereas Scenarios 2 and 3 require an average number of shifts (18 and 23 respectively), suggesting that Scenario 1 may also be the most parsimonious scenario of temporal bone evolution. The heterochronies and ancestral sequences reconstructed in each scenario in this set of analyses differed more widely than in either of the other two sets of analyses (Tables 5–13), most likely because of the inclusion of missing data and the larger size of the dataset.

For the dataset that included missing data, some of the ancestral sequences that were generated with PGi yielded unusual patterns of ossification sequences that were not typical of patterns seen among extant taxa, that fall into four categories: 1) the dentary, maxilla, or premaxilla bones are not the first to ossify; 2) the dentary and/or maxilla appear in the fourth position or later in the sequence; 3) the exoccipital, basioccipital, or basisphenoid bones ossifying first or second in the sequence; or 4) the premaxilla ossifies within five positions from the end of the sequence in sequences that exhibit a resolution greater than 7 events. These unusual patterns of ossification may be artifacts of the poor resolution of many sequences in the dataset, as a result of numerous ties and/or poor specimen sampling across development. Scenario 1 reconstructed 12 of these questionable timings, whereas Scenario 2 reconstructed 17, and Scenario 3 reconstructed 40 (Table 12). The greater number of questionable timings in Scenario 3 may weaken support for turtles as sister to Lepidosauria as compared to the other scenarios.

The fact that the nasal shifted the most across scenarios may be a consequence of the high variability in timing of the nasal across amniotes as well as its absence in Testudines (Table 4). For instance, in Squamates the nasal ossifies relatively late

whereas in Archosauria it ossifies relatively early, and in Mammalia there appears to be no trend (Table 4). The low number of shifts in the dentary across scenarios may reflect the consistent early ossification of this bone (Table 4).

The heterochronies reconstructed in Scenario 1 suggest that the evolution of turtles involved an early shift of the parietal, and a late shift of the jugal and supraoccipital. The early shift of the parietal may be related to the relatively large size of the parietal in turtles, whereas the late shift of the jugal is congruent with my hypothesis that emargination would cause a delay in the onset of ossification of the jugal. The late shift of the supraoccipital, however, does not support the hypothesis that earlier ossification may result in larger final size of a bone—the supraoccipital in turtles is relatively expanded, and thus its large final size may be produced through a change in the rate of development, i.e., the bone grows faster, rather than a change in the timing of ossification, meaning the bone has more time to grow.

Comparison of PGi and Continuous Analysis

The degree of congruence between Continuous Analysis and Parsimov-based Genetic Inference was small, indicating that the different approaches of the two methods may not produce similar results and therefore will infer different histories of heterochrony to explain observed sequences of ossification. The first point of difference between the methods is with the reconstructed ancestral sequences, which consequently exacerbates differences in the reconstructed heterochronies because they are based on a comparison with the ancestral sequence or values—if the ancestral sequences are different to begin with, the recovery of identical heterochronies by both methods is much

more unlikely. Continuous Analysis seems more sensitive to ties in ossification data, and does not produce different results when taxa are rearranged on the tree (e.g., Scenarios 1–3).

Comparison of PGi using Complete or Incomplete Datasets

The PGi analysis of the dataset with missing data reconstructed greater differences among the three scenarios of turtle placement (Table 13). This might imply that key taxa were included in the dataset with missing data that were not included in the complete dataset. Alternatively, the PGi analysis of the complete dataset had fewer differences in reconstructed heterochronies among scenarios of turtle placement, which might imply that PGi is not greatly affected by rearrangements of the tree unless there are gaps in the data.

Chi-Squared Test

The results of the Chi-squared tests failed to reject the null hypothesis of equivalent number of ossification sequence shifts through evolution. The implication is that the timing of ossification of endochondral bones is not necessarily more evolutionarily variable than the timing of ossification of dermal bones, and so evolutionary modifications of the skull involving endochondral bones are not more likely than modifications involving dermal bones. However, the test does not address the issue of intraspecific variation in timing, for which there is evidence that endochondral bones are more variable than dermal bones (Rieppel 1994; Smith 1997; Mabee et al. 2000; Sheil and Greenbaum 2005; Sheil et al. 2014). It is possible that the observed intraspecific

variability in the ossification endochondral bones does not translate to an increase in interspecific variability in timing.

On the Nature of Ossification Sequence Data

Although most studies of heterochrony necessarily treat developmental events as independent (Schoch 2006; Werneburg and Sánchez-Villagra 2009; Harrington et al. 2013; Koyabu et al. 2014), they are not (Bininda-Emonds et al. 2002). This is of special concern in the study of skull bone ossification, where the complexity of the skull obscures the developmental relationships of the structures within it; heterochrony in the ossification of skull bones cannot be fully understood without knowledge of the non-independence of these data. Although some studies find no evidence for modularity of bone ossification in the skull (Goswami 2007; Koyabu et al. 2011), the idea that sets of skull bones belong to developmental modules is supported by common developmental origin (e.g., endochondral vs. dermal) and observations of sets of bones whose ossification appears to be linked (e.g., the facial bone series) (Hanken and Thorogood 1993; Rieppel 1994; Mabee et al. 2000; Schoch 2006; Piekarski et al. 2014). In fact, the data compiled and collected by this study supports the observation that dermal bones typically ossify before endochondral bones (Shaner 1926; Rieppel 1994; Good 1995; Abdala et al. 1997; Montero et al. 1999). The implication of modularity for studies of heterochrony is that shifts in timing of individual bones may be correlated among bones that represent discrete, developmentally-linked modules (e.g., the mandibular-, palatal-, circumorbital-, skull roof-, and neurocranial-modules; Schoch 2006). The data and reconstructed ancestral sequences in this study seem to show a relationship between the

maxilla and dentary bones, which often ossify together and first, the basioccipital, basisphenoid, exoccipital, and supraoccipital bones, which often ossify last, and the prootic and opsithotic bones, which often together and late (Tables 5–7 and 12). Interestingly, the vomer (which is dermal) often ossifies late and with the basioccipital, basisphenoid, exoccipital, and supraoccipital. Additionally, bones of the skull are known to have at least two distinct developmental origins: cranial neural crest cells and mesoderm. Furthermore, the neural crest cells are known to produce distinct mandibular, hyoid, and branchial neural crest cell streams (Piekarski et al. 2014) and these bones might then respond by shifting earlier or later in development as a consequence of representing derivatives of one stream of cells. If the cells of the neural crest progenitor region are delayed in development, then we might expect to see a correlated shift in timing of ossification or appearance of these bones. Whether the cellular origin of bones creates modularity has yet to be shown conclusively, but at a minimum, bones of the skull are known to be derived from related tissues, and modularity should continue to be explored in studies of heterochrony. Observed patterns or shifts in timing of ossification and heterochrony might provide insights into the early formation and determination of neural crest cells and cells of the lateral mesoderm.

The existence of modularity is related to the problem of ties in a sequence of ossification through the possible simultaneous appearance of two or more bones that are treated as separate, when in reality they may actually represent a single bone. For example, two or more bones may be inextricably linked developmentally, and therefore may appear to ossify at the same time, thereby producing an observed "tie" (Maxwell 2008). This was observed in the prootic and opisthotic bones in *Lepidochelys olivacea,*

in which eight late-stage embryos had initiated ossification of both bones, and no embryos were observed with either the prootic or opisthotic only. If true ties such as this do exist, it would be erroneous to treat the bones in question as tied for timing of appearance. Instead, they should be treated as a single element or event.

Another consideration when using ossification sequence data is whether or not to include the timing of other developmental events. For instance, Smith (1997) compared placental mammals and marsupials and found an early ossification of the facial bones in marsupials relative to the development of the central nervous system, a unique developmental strategy that is only observed in a context larger than ossification of the skeleton. It does seem that a broader context is generally more useful, especially given the ambiguous phylogenetic signal of ossification sequences (Sánchez-Villagra 2002; Schoch 2006; Maxwell et al. 2010; Laurin and Germain 2011; Werneburg and Sánchez-Villagra 2014). In addition to expanding the developmental sequences to include key non-ossification events, it has been proposed that the focus of heterochrony studies should also include the study of heterotopy, the evolutionary change in the spatial arrangement of structures and/or gene expression (Schoch 2014; Hanken 2015).

CONCLUSIONS

Herein, PGi provided greater utility than Continuous Analysis in terms of the results that it generated. PGi was considered more useful than CA, primarily because the latter was so insensitive to changes in the movement of turtles across each of Scenarios 1–3 that it could not reconstruct differences in ancestral sequences or instances of heterochrony no matter where turtles were placed; the only exception was in the case of

comparing the alternate ancestors of turtles and their potential sister group. Additionally, CA does not provide explicit reconstructions of ancestral sequences for specific nodes internodes, nor does it map exactly where heterochrony occurred on each internode; therefore, hypotheses of evolution based on the results of CA could not be specific about when and where specific changes occurred, nor could it provide information about trends of movement in individual bones. This problem is compounded by the fact that only one instance of heterochrony was reconstructed when adjacent nodes were compared (the early ossification of the prootic in *Coturnix*).

The result that endochondral bones are not more evolutionarily variable than dermal bones might indicate that timing of ossification is not a selectable trait. However, the data used for the Chi-squared tests were the results of an analysis of changing in timing of skull bone ossification relative to other skull bones, and modularity of the skull bones was not considered here. If the developmental sequences incorporated timing of appearance of organs and non-skeletal structures, and also considered the potential modularity of skull bones, then endochondral bones might appear to be more evolutionarily variable.

In a very general sense, there is some indication of modularity in these results, as dermal bones were generally found to begin ossification before endochondral bones (Shaner 1926; Rieppel 1994; Good 1995; Abdala et al. 1997; Montero et al. 1999), a pattern that likely is linked to their membrane versus cartilage origins, respectively. From the results of this study, there also appear to be links between the timing of ossification of the dentary and maxilla, the prootic and opisthotic, and the basioccipital, basisphenoid, exoccipital, and supraoccipital bones. In the case of the prootic and

opisthotic, their ossification was observed to be consistently simultaneous in *Lepidochelys olivacea,* indicating that rather than treating them as tied for appearance, their appearance should be treated as a single event.

It is intriguing that when turtles are placed as sister to all other reptiles (Fig. 1, Scenario 1), PGi infers fewer than half the number of instances of heterochrony (127 shifts) than when placed in Scenario 2 (264 reconstructed shifts) or Scenario 3 (290 reconstructed shifts). Placement of turtles in Scenario 1 seems to fit with historical notions of Testudines as an extant lineage of reptiles that exhibit an anapsid skull. Though a strict application of parsimony as a system of choosing among competing hypotheses would indicate that Scenario 1 is the preferred placement of turtles (i.e., turtles as sister to all other reptiles) because it would require the fewest number of events of heterochrony, the majority of recent phylogenetic analyses provide compelling evidence that turtles are the sister to Archosauria (Fig. 3, Scenario 3) and that they are not sister to all other crown reptiles (Scenario 1) or sister to Lepidosauria (Scenario 2) (Kirsch and Mayer 1998; Meyer and Zardoya 1998; Hedges and Poling 1999; Kumazawa and Nishida 1999; Iwabe et al. 2005; Chiari et al. 2012; Fong et al. 2012; Lu et al. 2013; Schoch and Sues 2015). Placement of turtles in either Scenario 2 or 3 (i.e., within Diapsida) then requires that the anapsid skulls observed in extant turtles represents an example of a highly modified diapsid skull—in short, preference for either Scenarios 2 or 3 suggests that turtles are members of Diapsida that have secondarily lost the diapsid condition. Placement of turtles as sister to Archosauria (or perhaps as a member of unkonw placement among Archosauromorpha) would require radical changes in the overall appearance of the skull, and the larger number of instances of heterochrony

required of Scenario 3 may be consistent with type of changes that would be required of evolving such derived, highly modified anapsid skull from an ancestral anapsid condition.

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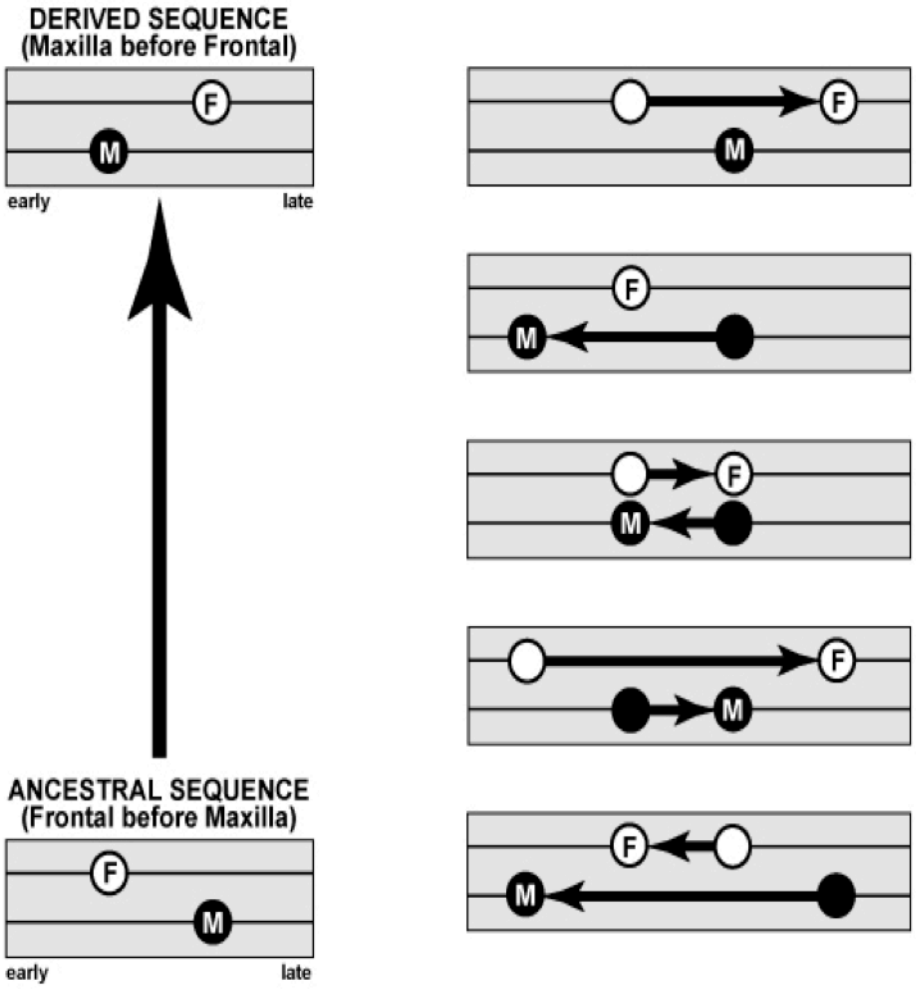

Figure 1. – Modified from Jeffery et al. (2002b: Fig. 2). A depiction of the modes by which two elements may appear to switch position of relative timing in a sequence via heterochrony. F, frontal; M, maxilla.

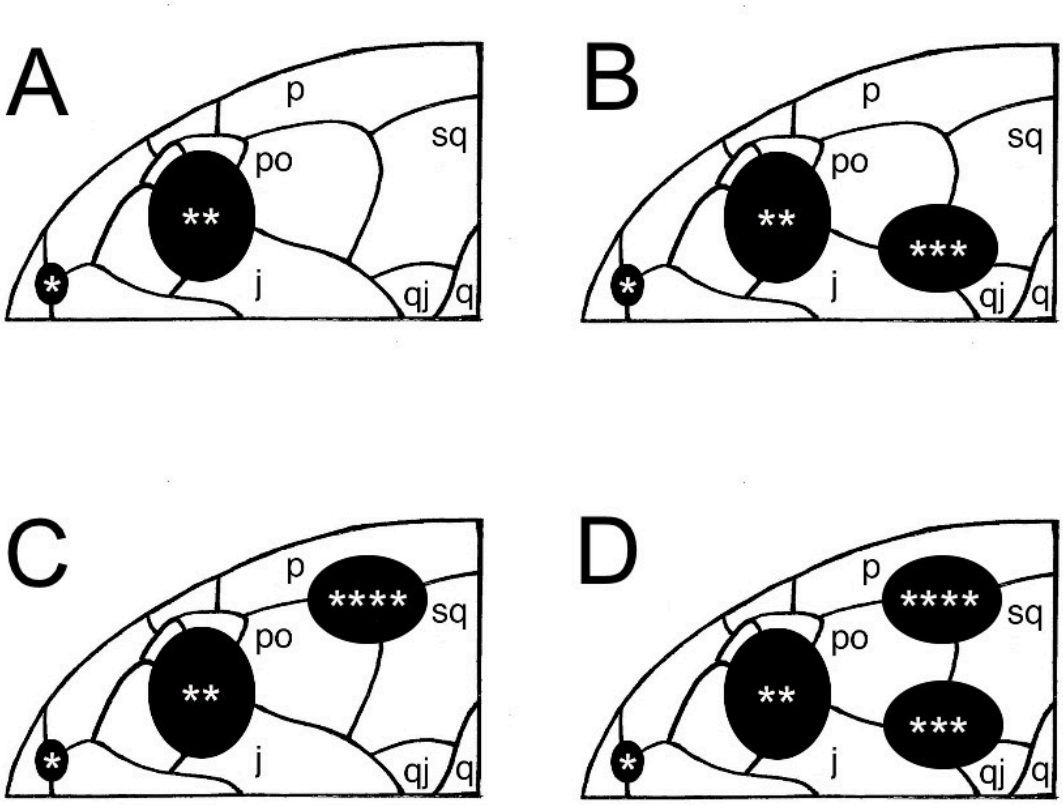

Figure 2 – A representation of the four main patterns of temporal fenestration in amniotes. **A:** anapsid; **B:** synapsid; **C:** euryapsid; **D:** diapsid. (p, parietal; po, postorbital; sq, squamosal; j, jugal; qj, quadratojugal; q, quadrate; *, nares; **, orbit; ***, subtemporal fenestra; ****, supratemporal fenestra).

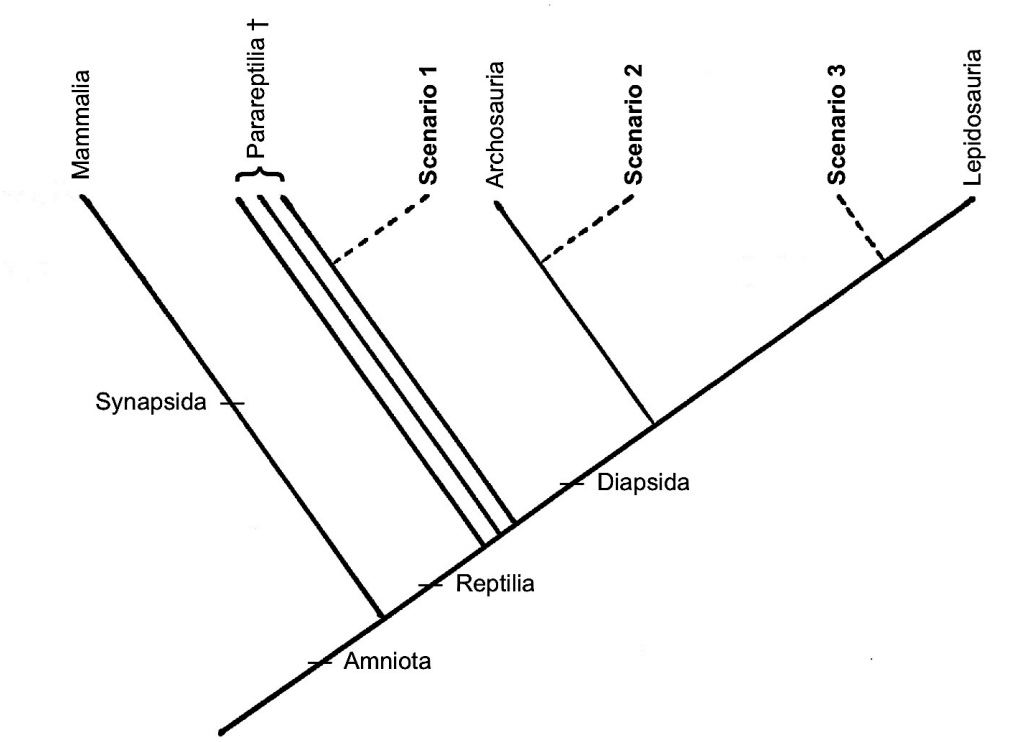

Figure 3 – The three competing placements of turtles within Amniota. **Scenario 1)** Sister to Diapsida representing an extant, anapsid parareptilian; **Scenario 2)** Sister to Archosauria representing a modified diapsid condition; **Scenario 3)** Sister to Lepidosauria representing a modified diapsid condition. † Extinct.

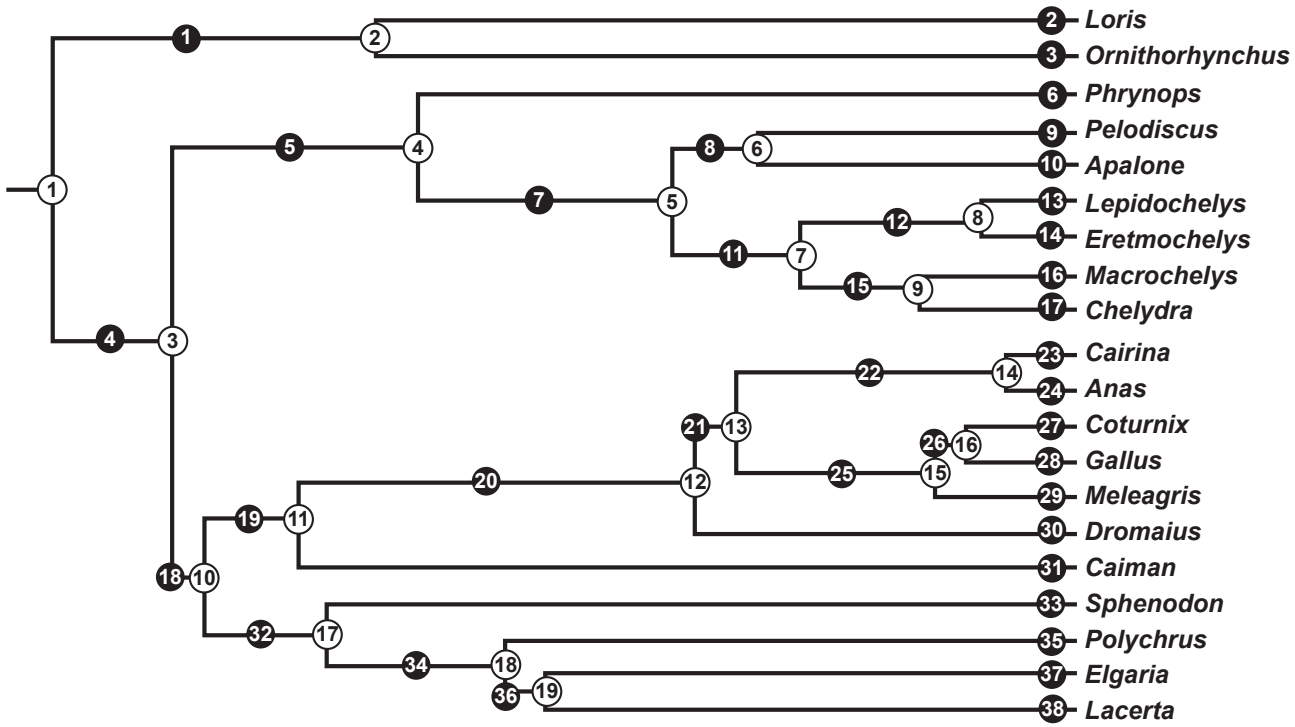

Figure 4 – Phylogeny used for Scenario 1 (Turtles as sister to Diapsida) for the Continuous Analysis and PGi analysis of the Continuous Analysis dataset. Branch lengths are equivalent to divergence dates. Branch lengths are considered in Continuous Analysis but not yet in Parsimov-based Genetic inference. Black circles indicate internode branches and white circles indicate nodes. Black circles reference Table 11 and white circles reference Table 5.

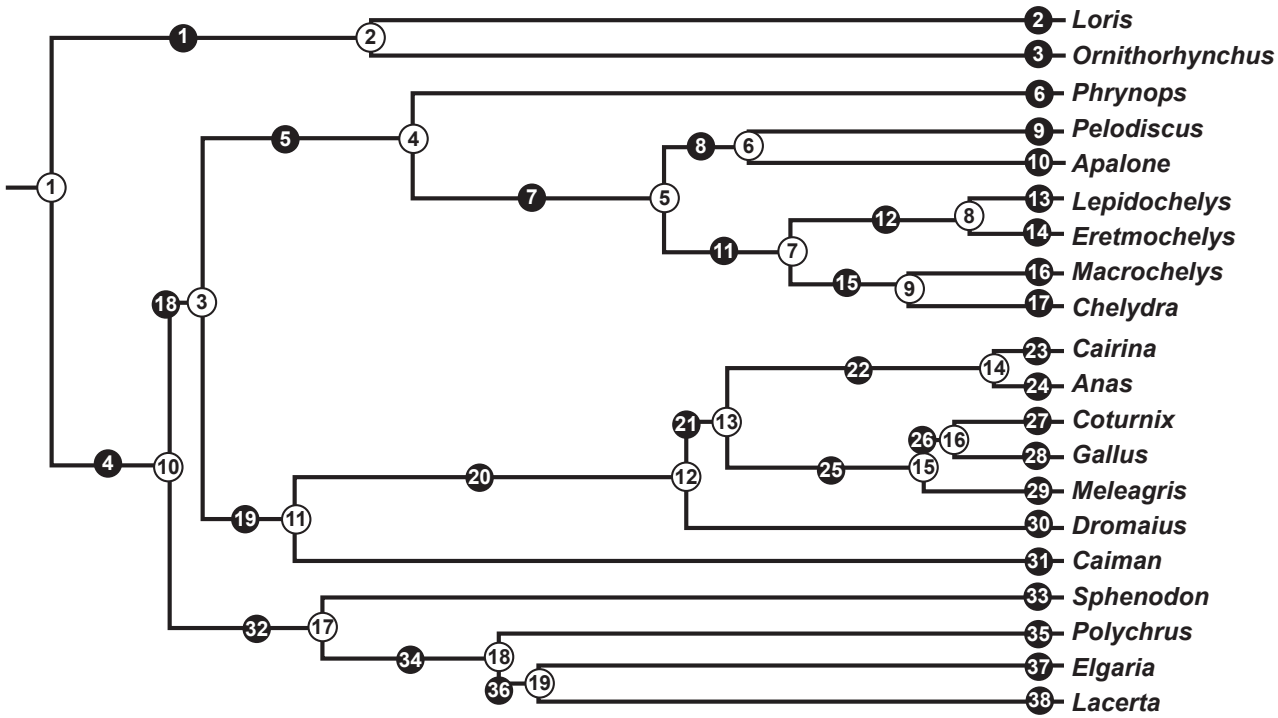

Figure 5 – Phylogeny used for Scenario 2 (Turtles as sister to Archosauria) for the Continuous Analysis and PGi analysis of the Continuous Analysis dataset. Branch lengths are equivalent to divergence dates. Branch lengths are considered in Continuous Analysis but not yet in Parsimov-based Genetic inference. Black circles indicate internode branches and white circles indicate nodes. Black circles reference Table 11 and white circles reference Table 6.

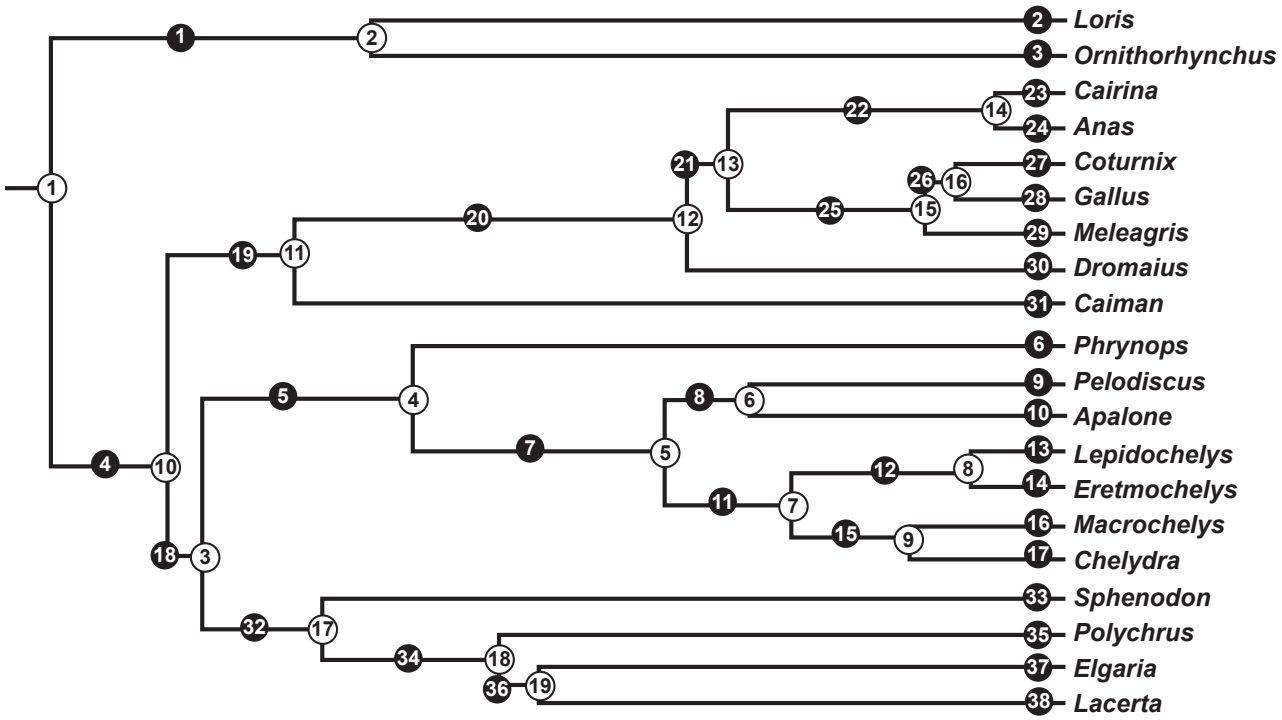

Figure 6 – Phylogeny used for Scenario 3 (Turtles as sister to Lepidosauria) for the Continuous Analysis and PGi analysis of the Continuous Analysis dataset. Branch lengths are equivalent to divergence dates. Branch lengths are considered in Continuous Analysis but not yet in Parsimov-based Genetic inference. Black circles indicate internode branches and white circles indicate nodes. Black circles reference Table 11 and white circles reference Table 7.

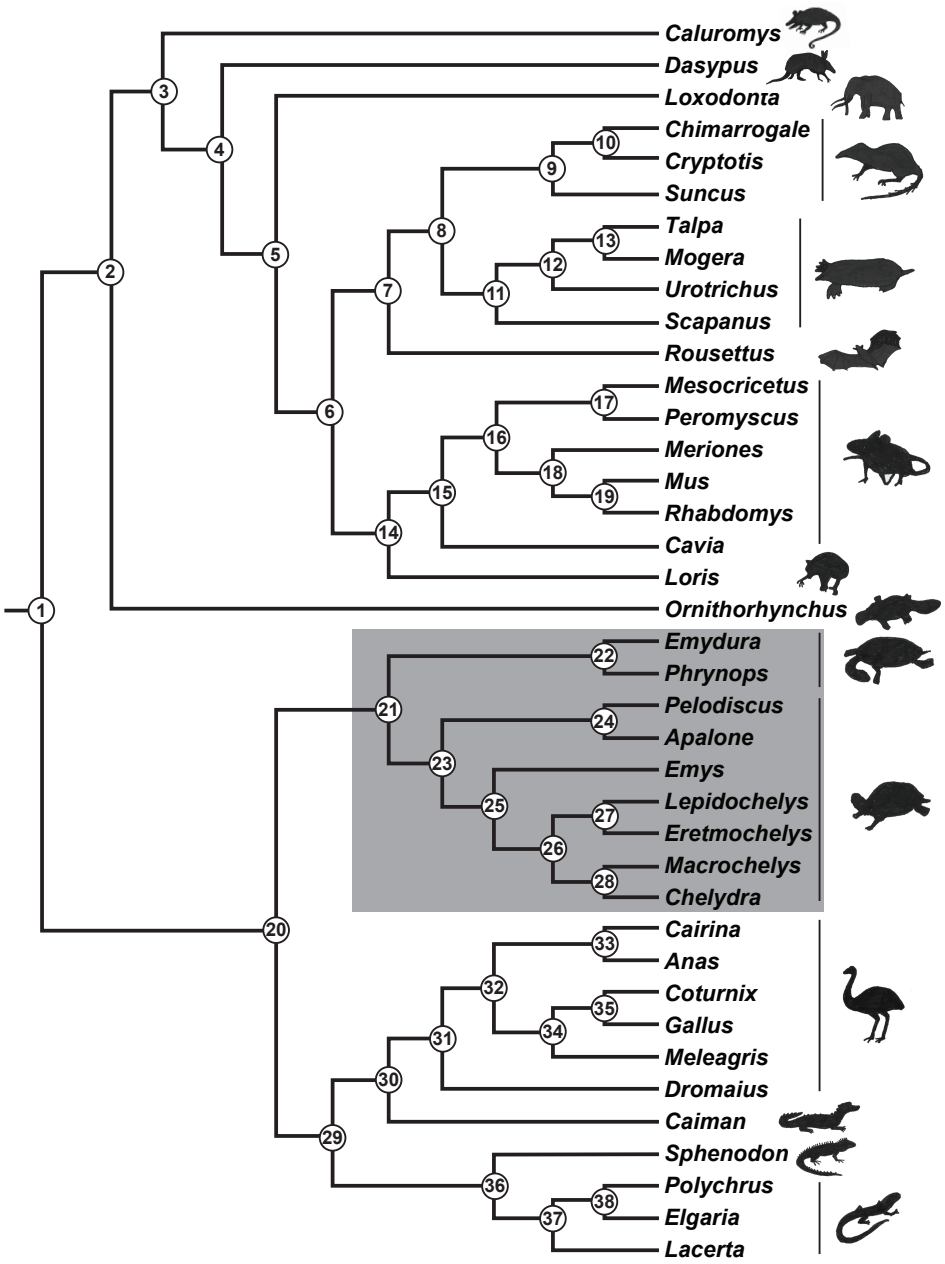

Figure 7 – Phylogeny used for Scenario 1 (Turtles as sister to Diapsida) for the PGi analysis of the dataset containing missing data. White circles indicate nodes and the numbers refer to Table 12, black circles indicate internodes and the numbers refer to Table 13.

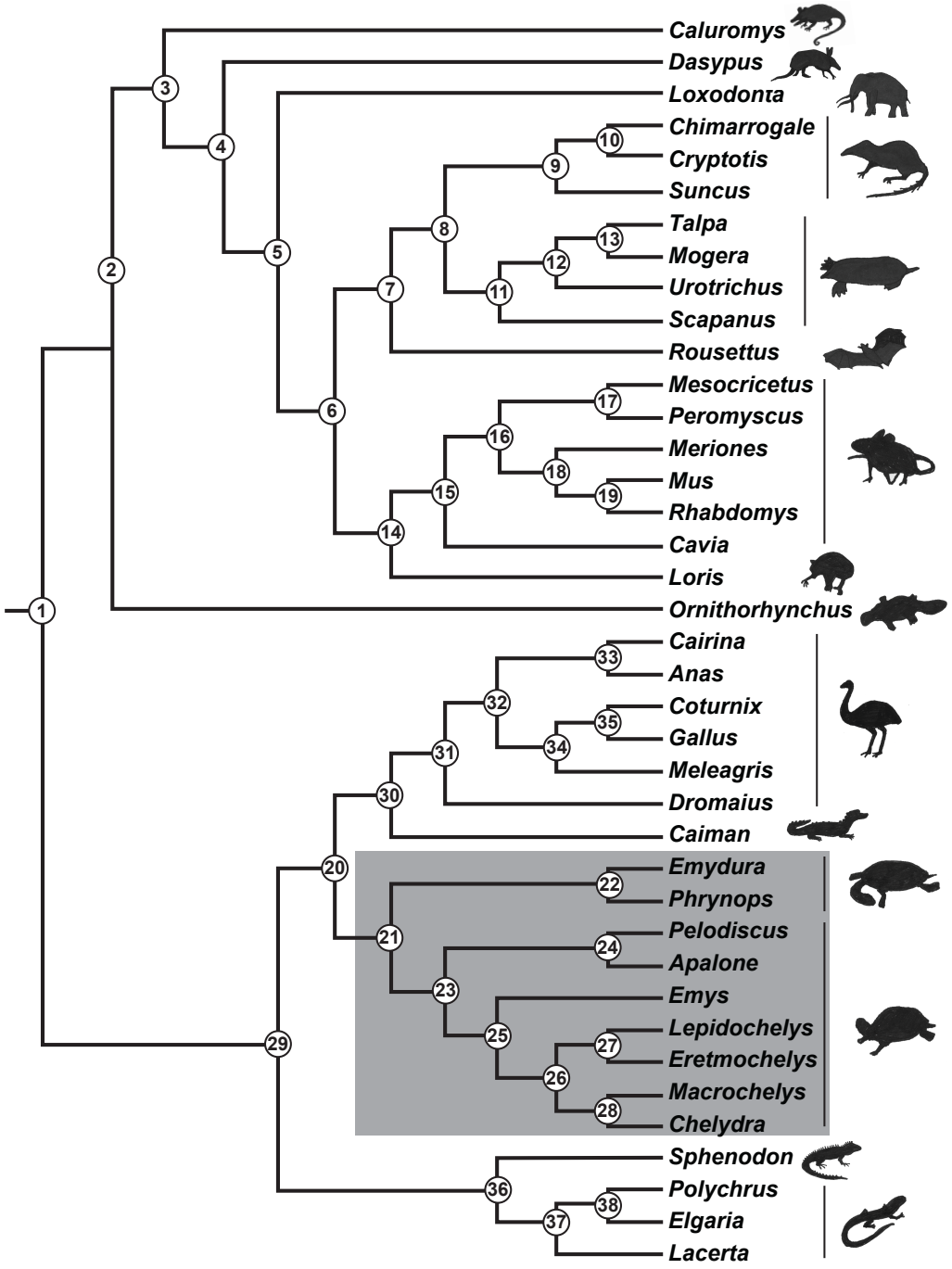

Figure 8 – Phylogeny used for Scenario 2 (Turtles as sister to Archosauria) for the PGi analysis of the dataset containing missing data. White circles indicate nodes and the numbers refer to Table 12, black circles indicate internodes and the numbers refer to Table 13.

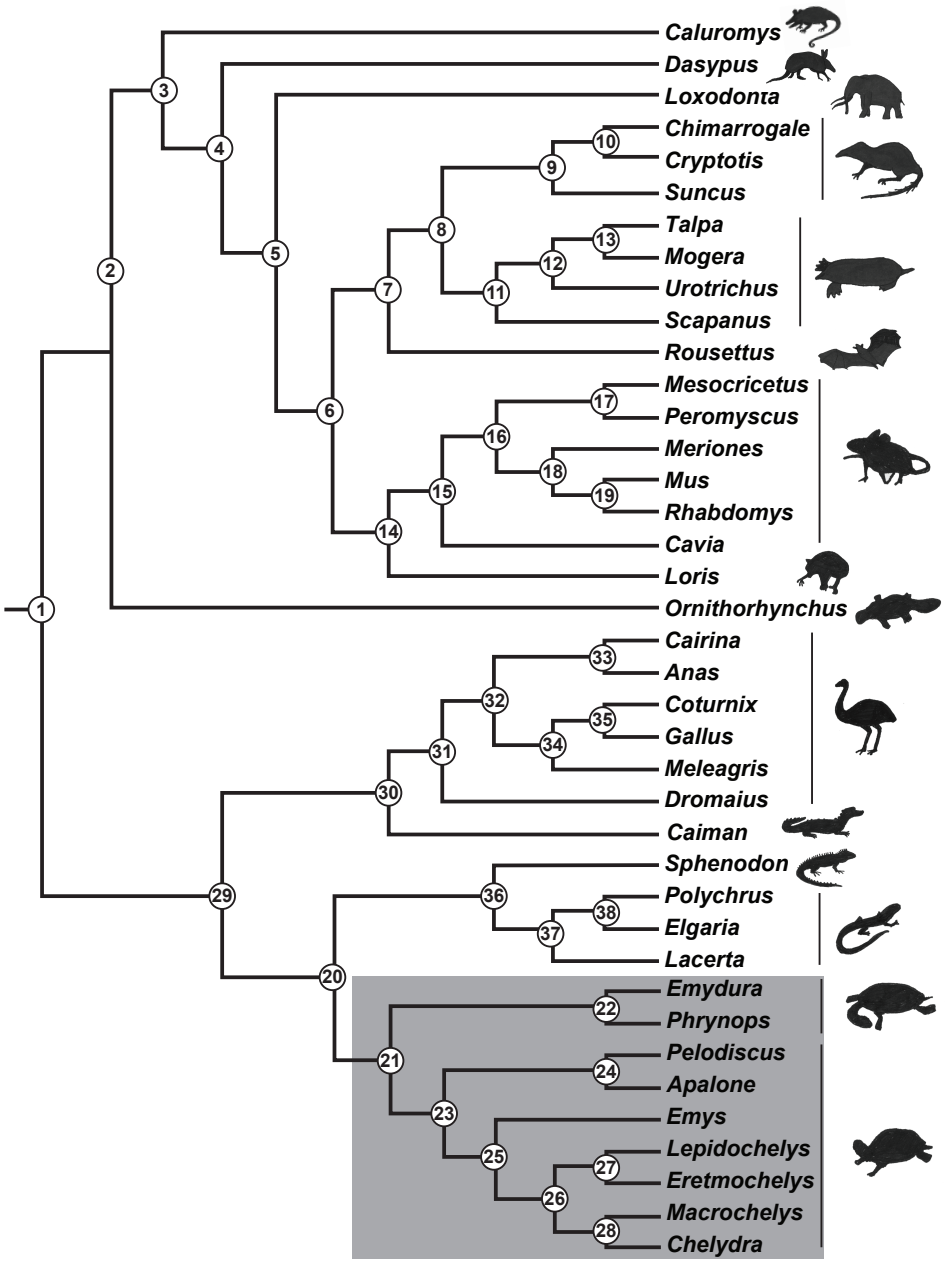

Figure 9 – Phylogeny used for Scenario 3 (Turtles as sister to Lepidosauria) for the PGi analysis of the dataset containing missing data. White circles indicate nodes and the numbers refer to Table 12, black circles indicate internodes and the numbers refer to Table 13.

Table 1. Ossification sequences collected from the citations listed below, for 52 bones across 73 species. Resolution refers to the sequence resolution (i.e., the number of events in the sequence). All data used in the analyses was taken from this set. **Ali**, alisphenoid; **Ang**, angular/ectotympanic; **Art**, articular/malleus; **Bo**, basioccipital; **Bps**, basiparasphenoid; **Bs**, basisphenoid; **Col**, columella/stapes; **Cor**, coronoid/complementary; **D**, dentary; **Ecpt**, ectopterygoid; **Epo**, epiotic; **Ept**, epipterygoid; **Ex**, exoccipital; **F**, frontal; **Fpa**, frontoparietal; **Ipar**, interparietal; **J**, jugal; **L**, lacrimal; **Ls**, laterosphenoid; **Ma**, mastoid; **Max**, maxilla; **Me**, mesethmoid; **N**, nasal; **Op**, opisthotic; **Os**, orbitosphenoid; **Pal**, palatine; **Par**, parietal; **Pat**, prearticular; **Pet**, petrosal; **Pf**, postfrontal; **Pm**, premaxilla; **Por**, postorbital; **Pot**, periotic; **Prf**, prefrontal; **Pr**, prootic; **Prs**, presphenoid; **Ps**, parasphenoid; **Pt**, pterygoid; **Pv**, pre **Parsimov-based Genetic Inference of the Complete Dataset**; **Q**, quadrate/incus; **Qj**, quadratojugal; **Sa**, suprangular; **Sco**, scleral ossicles; **Sm**, septomaxilla; **So**, supraoccipital; **Sp**, splenial; **Sq**, squamosal; **St**, supratemporal; **Sur**, surangular; **Tv**, transversum; **Tym**, tympanic; **V**, vomer. Homologies were assumed as follows, where **R** indicates Reptilia and **M** indicates Mammalia: Angular (**R**) and Ectotympanic Lamina (**M**), Articular (**R**) and Malleus (**M**), Columella (**R**) and Stapes (**M**), Coronoid (**R**) and Complementary (**M**), Gonial (**R**) and Prearticular (**R**) and Malleus Anterior Process (**M**), Pleuroccipital (**R**) and Exoccipital (**R** and **M**), Quadrate (**R**) and Incus (**M**) (Williston 1898; Shaner 1926; Luo 2011).

Species	Citation	Resolution	Ali	Ang	Art	Bo	Bps	Bs	Col
Chelydra serpentina	Sheil & Greenbaum 2005	6	-	6.5	-	25.5	20.5	20.5	20.5
Macrochelys temminckii	Sheil 2005	6	-	8	-	24.5	21.5	16	16
Eretmochelys imbricata	Sheil 2013	7	-	7	-	22	-	19	19
Lepidochelys olivacea	This study	7	-	13	25	20	-	18	17
Apalone spinifera	Sheil 2003	6	-	13.5	20.5	20.5	-	20.5	-
Pelodiscus sinensis	Sánchez-Villagra et al. 2009	9	-	9.5	26.5	22.5	-	9.5	13.5
Trionyx spp.	Fucik 1991	3	-	-	-	-	-	-	-
Emys orbicularis (lutaria)	Kunkel 1912; Fucik 1991	5	-	10.5	18.5	-	-	22	2
Chrysemys (picta) marginata	Shaner 1926	3	-	11	23	23	-	23	23
Aldabrachelys dussumieri	Gerlach 2012	5	-	-	22	18.5	-	14.5	14.5
Testudo hermanni	Fucik 1991	4	-	-	-	-	-	-	-
Phyrnops hilarii	Bona & Alcade 2009	6	-	7.5	30	23	-	17	17
Emydura subglobossa	Werneburg et al. 2009	6	-	8	25	18.5	-	18.5	18.5
Ptychoglossus bicolor	Hernández-Jaimes et al. 2012	3	-	-	-	13	-	13	-
Tupinambis merianae	Arias & Lobo 2006	4	-	10	29.5	10	-	27	29.5
Mabuya (Trachylepis) capensis	Skinner 1973	4	-	13	31.5	23.5	-	31.5	31.5
Lacerta agilis exigua	Rieppel 1994	7	-	15	15	23	-	23	30
Lacerta agilis	Fucik 1991	5	-	-	-	-	-	-	-
Polychrus acutirostris	Alvarez et al. 2005	9	-	12.5	23.5	27	-	23.5	-
Liolaemus quilmes	Abdala et al. 1997	4	-	12	31.5	25.5	-	25.5	25.5
Liolaemus scapularis	Lobo et al. 1995	4	-	9.5	31	23.5	23.5	-	23.5
Amphisbaena darwini heterozonata	Montero et al. 1999	4	-	7.5	-	17.5	17.5	-	17.5
Elgaria coerulea	Good 1995	12	-	26.5	35	21	-	30.5	-
Sphenodon punctatus	Howes & Swinnerton 1901	4	-	5	-	24.5	-	24.5	24.5
Gallus gallus	Maxwell 2008; Maxwell et al. 2010	16	-	2.5	17	21	-	21	-
Meleagris gallopavo	Maxwell 2008; Maxwell et al. 2010	19	-	2	-	21	-	18	-
Coturnix coturnix	Maxwell 2008; Maxwell et al. 2010; Mitgutsch et al. 2011	9	-	4	-	-	-	17.5	-
Taeniopygia guttata	Mitgutsch et al. 2011	3	-	9.5	-	16	-	-	-
Anas platyrhynchos	Maxwell et al. 2010; Mitgutsch et al. 2011	14	-	2.5	27	24	-	21	-
Cairina moschata	Maxwell et al. 2010	14	-	1	26	21.5	-	18.5	-
Larus argentatus	Maxwell et al. 2010	5	-	1	-	-	-	18.5	-
Dromaius novaehollandiae	Maxwell et al. 2010	21	-	1	28	23	-	18	-
Alligator mississippiensis	Rieppel 1993b	6	-	3.5	-	-	-	-	-
Caiman yacare	Lima et al. 2011	5	-	11.5	28	11.5	-	11.5	-
Crocodylus cataphractus	Müller 1967	8	-	-	-	-	-	-	-
Mus musculus	Smith 1997; Nunn & Smith 1998	4	9.5	-	-	5	-	9.5	-
Mus (Rattus) norvegicus albinus	Strong 1925	5	19	-	-	7.5	-	16	-
Meriones unguiculatus	Sánchez-Villagra et al. 2008	5	12.5	-	-	5	-	12.5	-

Species	Cor	D	Ecpt	Epo	Ept	Ex	F	Fpa	Ipar	J	L	Ls	Ma	Max	Me	N	Op	Os
Chelydra serpentina	15.5	6.5	-	-	25.5	20.5	6.5	-	-	13	-	-	-	6.5	-	-	28	-
Macrochelys temminckii	10	5	-	-	27	21.5	16	-	-	16	-	-	-	1.5	-	-	24.5	-
Eretmochelys imbricata	7	7	-	-	-	19	19	-	-	7	-	-	-	7	-	-	24.5	-
Lepidochelys olivacea	-	4	-	-	-	21	-	8.5	-	4	-	-	-	1	-	-	23.5	-
Apalone spinifera	13.5	4	-	-	-	20.5	7.5	-	-	13.5	-	-	-	1.5	-	-	24.5	-
Pelodiscus sinensis	9.5	1.5	-	-	26.5	22.5	17.5	-	-	9.5	-	-	-	1.5	-	-	22.5	-
Trionyx spp.	-	-	-	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-
Emys orbicularis (lutaria)	18.5	10.5	-	-	-	-	10.5	-	-	10.5	-	-	-	2	-	-	-	-
Chrysemys (picta) marginata	11	2.5	-	23	23	23	11	-	-	11	-	-	-	2.5	-	-	23	-
Aldabrachelys dussumieri	-	6.5	-	-	-	14.5	6.5	-	-	6.5	-	-	-	6.5	-	-	18.5	-
Testudo hermanni	-	-	-	-	-	-	-	-	-	4.5	-	-	-	-	-	-	-	-
Phyrnops hilarii	23	7.5	-	28	-	17	7.5	-	-	7.5	-	-	-	7.5	-	7.5	23	-
Emydura subglobossa	18.5	1.5	-	-	-	18.5	8	-	-	8	-	-	-	1.5	-	14	18.5	-
Ptychoglossus bicolor	-	3	13	-	13	13	13	-	-	3	21.5	-	-	3	-	13	13	21.5
Tupinambis merianae	10	10	10	-	10	22.5	10	-	-	10	22.5	-	-	10	-	22.5	-	-
Mabuya (Trachylepis) capensis	13	4	23.5	-	-	13	13	-	-	13	23.5	-	-	13	-	23.5	31.5	-
Lacerta agilis exigua	15	5	15	-	23	23	5	-	-	5	26	-	-	5	-	15	28	-
Lacerta agilis	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-
Polychrus acutirostris	12.5	3	12.5	-	12.5	12.5	12.5	-	-	3	28.5	-	-	12.5	-	21.5	25.5	-
Liolaemus quilmes	12	2	12	-	25.5	12	12	-	-	12	25.5	-	-	12	-	12	25.5	31.5
Liolaemus scapularis	9.5	9.5	9.5	-	23.5	23.5	9.5	-	-	9.5	23.5	-	-	9.5	-	23.5	23.5	31
Amphisbaena darwini heterozonata	7.5	7.5	7.5	-	-	7.5	7.5	-	-	-	-	-	-	7.5	-	21	-	-
Elgaria coerulea	21	12	21	-	21	21	12	-	-	3	28.5	-	-	12	-	26.5	32.5	34
Sphenodon punctatus	15	5	-	-	24.5	24.5	15	-	-	15	-	-	-	5	-	5	24.5	-
Gallus gallus	-	6.5	-	26	-	18.5	14	-	-	6.5	11.5	-	-	6.5	28	10	26	26
Meleagris gallopavo	-	9.5	-	25.5	-	19	16	-	-	9.5	14	23	-	9.5	25.5	14	24	-
Coturnix coturnix	-	9	-	-	-	17.5	14	-	-	3	9	-	-	9	-	9	-	-
Taeniopygia guttata	-	3	-	-	-	16	16	-	-	-	-	-	-	9.5	-	9.5	-	-
Anas platyrhynchos	-	12	-	30	-	21	16	-	-	12	7.5	-	-	7.5	28.5	7.5	25.5	28.5
Cairina moschata	-	10.5	-	28	-	21.5	16	-	-	10.5	4	-	-	4	29	4	25	27
Larus argentatus	-	10.5	-	-	-	18.5	18.5	-	-	10.5	15	-	-	4	-	10.5	-	-
Dromaius novaehollandiae	-	3	-	26	-	21	18	-	-	8.5	11.5	-	-	13.5	24.5	15	27	22
Alligator mississippiensis	14	3.5	-	-	-	-	9.5	-	-	9.5	9.5	-	-	3.5	-	15	-	-
Caiman yacare	22	2.5	22	28	-	28	22	-	-	2.5	11.5	22	-	2.5	-	11.5	28	-
Crocodylus cataphractus	-	-	-	-	-	-	8	-	-	3	11	-	-	3	-	13.5	-	-
Mus musculus	-	1.5	-	-	-	5	5	-	-	9.5	-	-	-	5	-	-	-	-
Mus (Rattus) norvegicus albinus	-	2	-	-	-	7.5	2	-	16	13	13	-	-	2	-	13	-	-
Meriones unguiculatus	-	2	-	-	-	8	2	-	-	12.5	12.5	-	-	5	-	8	-	16

Species	Pal	Par	Pat	Pet	Pf	Pm	Por	Pot	Prf	Pr	Prs	Ps	Pt	Pv	Q	Qj	Sa	Sco
Chelydra serpentina	6.5	6.5	15.5	-	-	6.5	6.5	-	6.5	25.5	-	20.5	6.5	-	20.5	15.5	-	-
Macrochelys temminckii	16	16	10	-	-	10	1.5	-	5	24.5	-	-	5	-	16	16	-	-
Eretmochelys imbricata	7	7	7	-	-	15	7	-	7	24.5	-	-	7	-	19	15	-	-
Lepidochelys olivacea	13	8.5	13	-	-	4	13	-	8.5	23.5	-	-	13	-	19	16	-	-
Apalone spinifera	7.5	7.5	13.5	-	-	13.5	13.5	-	4	20.5	-	-	7.5	-	13.5	13.5	-	-
Pelodiscus sinensis	9.5	4.5	17.5	-	-	17.5	17.5	-	4.5	22.5	-	-	9.5	-	13.5	17.5	-	-
Trionyx spp.	-	5.5	-	-	-	-	2.5	-	-	-	-	-	2.5	-	7.5	5.5	-	-
Emys orbicularis (lutaria)	10.5	10.5	10.5	-	10.5	10.5	-	-	10.5	-	-	18.5	10.5	-	22	18.5	10.5	-
Chrysemys (picta) marginata	11	11	11	-	11	11	-	-	2.5	-	-	23	11	-	23	11	-	-
Aldabrachelys dussumieri	6.5	6.5	-	-	-	6.5	6.5	-	6.5	18.5	-	-	6.5	-	21	14.5	-	-
Testudo hermanni	-	4.5	-	-	-	-	2	-	-	-	-	-	2	-	7.5	6	-	-
Phyrnops hilarii	7.5	17	23	-	-	17	7.5	-	7.5	23	-	23	7.5	-	23	-	-	28
Emydura subglobossa	8	8	23.5	-	-	8	8	-	8	-	-	-	8	-	18.5	-	-	-
Ptychoglossus bicolor	-	13	-	-	-	13	-	-	3	13	-	-	3	-	13	-	-	-
Tupinambis merianae	10	10	-	-	22.5	10	22.5	-	22.5	27	-	-	10	-	10	-	-	-
Mabuya (Trachylepis) capensis	4	4	4	-	13	13	23.5	-	4	31.5	-	23.5	4	-	23.5	-	4	-
Lacerta agilis exigua	5	15	5	-	15	5	15	-	15	28	-	-	5	-	23	-	-	-
Lacerta agilis	-	2.5	-	-	6	-	4	-	-	-	-	-	1	-	8	-	-	-
Polychrus acutirostris	3	12.5	-	-	-	12.5	12.5	-	21.5	25.5	-	-	1	12.5	12.5	-	12.5	-
Liolaemus quilmes	12	12	-	-	25.5	12	12	-	12	25.5	-	-	2	-	12	-	-	-
Liolaemus scapularis	9.5	9.5	-	-	23.5	9.5	9.5	-	9.5	23.5	-	-	1	23.5	23.5	-	-	-
Amphisbaena darwini heterozonata	7.5	7.5	-	-	-	7.5	-	-	7.5	17.5	-	-	7.5	-	17.5	-	-	-
Elgaria coerulea	3	5	12	-	12	12	12	-	6.5	32.5	-	21	1	-	28.5	-	-	21
Sphenodon punctatus	5	15	-	-	15	15	5	-	15	29	-	15	5	-	24.5	15	5	-
Gallus gallus	6.5	18.5	-	-	-	14	-	-	-	23	-	11.5	6.5	-	16	1	6.5	-
Meleagris gallopavo	5.5	17	27	-	-	9.5	-	-	-	22	-	9.5	5.5	-	14	1	3.5	-
Coturnix coturnix	9	16	-	-	-	9	-	-	-	-	-	9	9	-	15	1.5	9	-
Taeniopygia guttata	9.5	16	-	-	-	3	-	-	-	-	-	3	3	-	16	9.5	3	-
Anas platyrhynchos	7.5	14.5	23	-	-	12	-	-	7.5	25.5	-	18.5	7.5	-	14.5	2.5	2.5	-
Cairina moschata	7.5	17	21.5	-	-	10.5	-	-	-	24	-	14	7.5	-	18.5	4	10.5	-
Larus argentatus	4	18.5	18.5	-	-	10.5	-	-	-	-	-	10.5	4	-	18.5	10.5	4	-
Dromaius novaehollandiae	6	20	16	-	-	8.5	-	-	-	29	-	11.5	6	-	18	3	3	-
Alligator mississippiensis	-	16	-	-	-	3.5	9.5	-	9.5	-	-	-	1	-	-	9.5	-	-
Caiman yacare	11.5	22	-	-	-	11.5	2.5	-	11.5	28	-	-	11.5	-	22	11.5	11.5	-
Crocodylus cataphractus	11	16	-	-	5.5	8	-	-	11	-	-	15	1	-	-	5.5	-	-
Mus musculus	-	5	-	-	-	1.5	-	12	-	-	-	-	-	-	-	-	-	-
Mus (Rattus) norvegicus albinus	7.5	7.5	-	-	-	7.5	-	-	-	-	19	-	7.5	-	-	-	-	-
Meriones unguiculatus	8	12.5	-	-	-	2	-	-	-	-	-	-	5	-	-	-	-	-

Species	Sm	So	Sp	Sq	St	Sur	Tv	Tym	V
Chelydra serpentina	-	25.5	-	6.5	-	15.5	-	-	6.5
Macrochelys temminckii	-	24.5	-	5	-	5	-	-	16
Eretmochelys imbricata	-	23	-	7	-	7	-	-	15
Lepidochelys olivacea	-	22	-	8.5	-	4	-	-	4
Apalone spinifera	-	24.5	-	1.5	-	4	-	-	20.5
Pelodiscus sinensis	-	25	-	4.5	-	4.5	-	-	17.5
Trionyx spp.	-	7.5	-	2.5	-	-	-	-	-
Emys orbicularis (lutaria)	-	22	-	2	-	-	-	-	4
Chrysemys (picta) marginata	-	23	-	2.5	-	11	-	-	11
Aldabrachelys dussumieri	-	18.5	-	6.5	-	-	-	-	6.5
Testudo hermanni	-	7.5	-	2	-	-	-	-	-
Phyrnops hilarii	-	28	7.5	7.5	-	7.5	-	-	7.5
Emydura subglobossa	-	18.5	-	8	-	8	-	-	23.5
Ptychoglossus bicolor	-	13	-	13	-	-	-	-	13
Tupinambis merianae	-	27	10	10	10	10	-	-	10
Mabuya (Trachylepis) capensis	23.5	31.5	13	23.5	13	-	23.5	-	13
Lacerta agilis exigua	-	28	-	15	15	5	-	-	-
Lacerta agilis	-	-	-	6	2.5	-	-	-	-
Polychrus acutirostris	-	28.5	12.5	12.5	12.5	-	-	-	-
Liolaemus quilmes	-	25.5	12	12	12	2	-	-	25.5
Liolaemus scapularis	-	31	9.5	9.5	9.5	9.5	-	-	-
Amphisbaena darwini heterozonata	7.5	17.5	-	-	-	-	-	-	7.5
Elgaria coerulea	6.5	30.5	12	21	21	3	-	-	12
Sphenodon punctatus	-	24.5	15	5	-	-	-	-	15
Gallus gallus	-	21	14	2.5	-	-	-	-	24
Meleagris gallopavo	-	20	9.5	3.5	-	-	-	-	-
Coturnix coturnix	-	-	-	1.5	-	-	-	-	-
Taeniopygia guttata	-	-	9.5	9.5	-	-	-	-	9.5
Anas platyrhynchos	-	21	18.5	2.5	-	-	-	-	17
Cairina moschata	-	21.5	14	4	-	-	-	-	14
Larus argentatus	-	-	10.5	4	-	-	-	-	10.5
Dromaius novaehollandiae	-	24.5	13.5	6	-	-	-	-	10
Alligator mississippiensis	-	-	9.5	-	-	9.5	-	-	-
Caiman yacare	-	22	11.5	11.5	-	-	-	-	11.5
Crocodylus cataphractus	-	-	-	3	-	-	13.5	-	8
Mus musculus	-	-	-	9.5	-	-	-	-	-
Mus (Rattus) norvegicus albinus	-	19	-	7.5	-	-	-	16	7.5
Meriones unguiculatus	-	-	-	12.5	-	-	-	-	-

Species	Citation	Resolution	Ali	Ang	Art	Bo	Bps	Bs	Col	Cor	D	Ecpt
Rhabdomys pumilio	Wilson et al. 2010	12	15	-	-	8	-	12	-	-	3	-
Mesocricetus auratus	Kanazawa & Mochizuki 1974	8	12	-	-	12	-	6.5	-	-	1	-
Peromyscus melanophrys	Sánchez-Villagra et al. 2008	5	13	-	-	6	-	9.5	-	-	2	-
Cavia porcellus	Wilson et al. 2010	12	5	-	-	10.5	-	15	-	-	5	-
Tupaia javanica	Smith 1997; Nunn & Smith 1998	5	9	-	-	9	-	11	-	-	2	-
Tarsius spectrum	Nunn & Smith 1998	8	9	-	-	10.5	-	10.5	-	-	1	-
Loris tardigradus	Ramaswami 1957	7	17.5	-	24	6.5	-	17.5	24	-	1.5	-
Homo sapiens	Noback & Robertson 1951	11	-	-	10	15.5	-	17	20	-	1	-
Felis domesticus	Smith 1997; Nunn & Smith 1998	7	9	-	-	9	-	11	-	-	2	-
Sus scrofa	Smith 1997; Nunn & Smith 1998	8	7	-	-	10	-	11	-	-	1	-
Manis javanica	Smith 1997; Nunn & Smith 1998	6	9	-	-	9	-	11	-	-	1	-
Cryptotis parva	Sánchez-Villagra et al. 2008; Koyabu et al. 2011	13	18	13.5	-	13.5	-	17	-	-	2	-
Chimarrogale platycephala	Koyabu et al. 2011	8	17.5	14	-	13	-	15	-	-	1	-
Suncus murinus	Koyabu et al. 2011	11	17.5	16	-	7	-	15	-	-	2.5	-
Condylura cristata	Koyabu et al. 2011	9	20	14	-	6.5	-	15	-	-	6.5	-
Scapanus orarius	Koyabu et al. 2011	7	21	12.5	-	17.5	-	15	-	-	5.5	-
Urotrichus talpoides	Koyabu et al. 2011	8	19.5	19.5	-	10	-	12.5	-	-	4.5	-
Mogera wogura	Koyabu et al. 2011	8	21	17.5	-	13	-	19	-	-	2.5	-
Talpa europaea	Koyabu et al. 2011	7	21	17.5	-	10	-	15	-	-	4	-
Talpa occidentalis	Koyabu et al. 2011	10	20	16	-	5	-	17	-	-	5	-
Rousettus amplexicaudatus	Sánchez-Villagra et al. 2008	7	14.5	-	-	12	-	14.5	-	-	1.5	-
Erinaceus europaeus	Koyabu et al. 2011	7	14	18	-	14	-	9.5	-	-	3	-
Erinaceus amurensis	Koyabu et al. 2011	9	10.5	18	-	10.5	-	10.5	-	-	2.5	-
Loxodonta africana	Hautier et al. 2012	5	8.5	-	-	14	-	16	-	-	2	-
Bradypus variegatus	Hautier et al. 2011	4	14	-	-	11.5	-	16	-	-	5	-
Cyclopes didactylus	Hautier et al. 2011	2	13	-	-	6	-	-	-	-	6	-
Tamandua tetardactyla	Hautier et al. 2011	4	7.5	-	-	7.5	-	15	-	-	7.5	-
Dasypus novemcinctus	Hautier et al. 2011	8	13	-	-	12	-	16	-	-	5.5	-
Monodelphis domestica	Smith 1997; Nunn & Smith 1998	7	10	-	-	8.5	-	11	-	-	2	-
Caluromys philander	Sánchez-Villagra et al. 2008	6	9	-	-	13	-	13	-	-	2	-
Macropus eugenii	Smith 1997; Nunn & Smith 1998	6	8.5	-	-	10	-	11	-	-	2	-
Dasyurus viverrinus	Smith 1997; Nunn & Smith 1998	8	8	-	-	10	-	11.5	-	-	2	-
Perameles nasuta	Smith 1997; Nunn & Smith 1998	6	9	-	-	9	-	11	-	-	2	-
Trichosurus vulpecula	Sánchez-Villagra et al. 2008	4	8.5	-	-	14	-	14	-	-	-	-
Ornithorhynchus anatinus	de Beer & Fell 1936 & de Beer 1937	4	17.5	-	-	17.5	-	17.5	-	-	7.5	-

Species	Epo	Ept	Ex	F	Fpa	Ipar	J	L	Ls	Ma	Max	Me	N	Op	Os	Pal	Par	Pat	Pet
Rhabdomys pumilio	-	-	9	3	-	-	13	14	-	-	6.5	-	10	-	16	3	3	-	-
Mesocricetus auratus	-	-	6.5	6.5	-	16	16	19	-	-	2.5	-	12	-	20	6.5	6.5	-	-
Peromyscus melanophrys	-	-	9.5	6	-	-	13	13	-	-	2	-	13	-	16.5	6	6	-	-
Cavia porcellus	-	-	13	5	-	-	5	13	-	-	5	-	10.5	-	16.5	5	5	-	-
Tupaia javanica	-	-	9	5.5	-	-	5.5	-	-	-	2	-	-	-	-	-	5.5	-	-
Tarsius spectrum	-	-	8	5	-	-	5	-	-	-	2.5	-	-	-	-	-	7	-	-
Loris tardigradus	-	-	13	6.5	-	-	6.5	-	-	-	1.5	26	13	21	17.5	6.5	6.5	17.5	-
Homo sapiens	-	-	13.5	-	6	11.5	6	15.5	-	-	2	-	11.5	-	-	6	13.5	-	18
Felis domestica	-	-	9	4	-	-	5.5	-	-	-	2	-	-	-	-	-	5.5	-	-
Sus scrofa	-	-	7	5	-	-	9	-	-	-	3	-	-	-	-	-	7	-	-
Manis javanica	-	-	9	3.5	-	-	3.5	-	-	-	3.5	-	-	-	-	-	6.5	-	-
Cryptotis parva	-	-	13.5	5	-	-	-	13.5	-	21	2	-	9	-	19	5	7.5	16	20
Chimarrogale platycephala	-	-	7	7	-	-	-	17.5	-	20.5	7	-	7	-	17.5	7	7	17.5	20.5
Suncus murinus	-	-	10	5	-	-	-	13	-	21.5	2.5	-	13	-	19	7	2.5	17.5	20
Condylura cristata	-	-	6.5	6.5	-	-	-	17.5	-	16	6.5	-	6.5	-	19	6.5	6.5	17.5	21.5
Scapanus orarius	-	-	12.5	5.5	-	-	-	5.5	-	17.5	5.5	-	5.5	-	19	5.5	5.5	16	21
Urotrichus talpoides	-	-	11	4.5	-	-	-	19.5	-	14.5	4.5	-	14.5	-	16	4.5	4.5	19.5	19.5
Mogera wogura	-	-	14.5	8.5	-	-	-	14.5	-	16	2.5	-	8.5	-	17.5	8.5	2.5	8.5	21
Talpa europaea	-	-	13	4	-	-	-	15	-	15	4	-	10	-	19	4	4	17.5	21
Talpa occidentalis	-	-	5	10.5	-	-	-	13	-	13	5	-	10.5	-	18.5	5	5	18.5	21
Rousettus amplexicaudatus	-	-	12	4.5	-	-	4.5	8.5	-	-	4.5	-	8.5	-	17	8.5	4.5	-	-
Erinaceus europaeus	-	-	14	3	-	-	-	18	-	21	3	-	9.5	-	14	7	9.5	18	21
Erinaceus amurensis	-	-	10.5	5	-	-	-	17	-	21.5	2.5	-	10.5	-	16	10.5	2.5	19	20
Loxodonta africana	-	-	8.5	8.5	-	-	8.5	8.5	-	-	2	-	8.5	-	15	8.5	8.5	-	-
Bradypus variegatus	-	-	11.5	5	-	-	5	11.5	-	-	5	-	5	-	16	11.5	5	-	-
Cyclopes didactylus	-	-	6	6	-	-	-	6	-	-	6	-	6	-	13	6	6	-	-
Tamandua tetardactyla	-	-	7.5	7.5	-	-	7.5	7.5	-	-	7.5	-	7.5	-	16	7.5	7.5	-	-
Dasypus novemcinctus	-	-	14	5.5	-	-	5.5	5.5	-	-	5.5	-	5.5	-	15	5.5	5.5	-	-
Monodelphis domestica	-	-	4.5	4.5	-	-	6.5	-	-	-	2	-	-	-	-	-	8.5	-	-
Caluromys philander	-	-	9	5	-	-	5	13	-	-	2	-	9	-	15.5	9	5	-	-
Macropus eugenii	-	-	5.5	5.5	-	-	5.5	-	-	-	2	-	-	-	-	-	8.5	-	-
Dasyurus viverrinus	-	-	5.5	4	-	-	5.5	-	-	-	2	-	-	-	-	-	8	-	-
Perameles nasuta	-	-	5	5	-	-	5	-	-	-	2	-	-	-	-	-	9	-	-
Trichosurus vulpecula	-	-	8.5	4	-	-	4	8.5	-	-	1.5	-	8.5	-	14	8.5	14	-	-
Ornithorhynchus anatinus	-	-	17.5	7.5	-	-	17.5	-	-	-	7.5	-	7.5	24	24	7.5	7.5	7.5	-

Species	Pf	Pm	Por	Pot	Prf	Pr	Prs	Ps	Pt	Pv	Q	Qj	Sa	Sco	Sm	So	Sp	Sq	St
Rhabdomys pumilio	-	6.5	-	17	-	-	-	-	3	-	-	-	-	-	-	-	-	11	-
Mesocricetus auratus	-	2.5	-	-	-	-	18	-	-	-	-	-	-	-	-	12	-	6.5	-
Peromyscus melanophrys	-	2	-	16.5	-	-	-	-	6	-	-	-	-	-	-	-	-	13	-
Cavia porcellus	-	5	-	16.5	-	-	-	-	13	-	-	-	-	-	-	-	-	5	-
Tupaia javanica	-	2	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	5.5	-
Tarsius spectrum	-	2.5	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-
Loris tardigradus	-	6.5	-	-	-	21	21	-	13	13	24	-	-	-	-	13	-	6.5	-
Homo sapiens	-	6	-	-	-	-	-	-	-	-	19	-	-	-	-	6	-	6	-
Felis domestica	-	2	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-
Sus scrofa	-	3	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
Manis javanica	-	3.5	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	6.5	-
Cryptotis parva	-	2	-	-	-	-	22	-	7.5	-	-	-	-	-	-	10.5	-	10.5	-
Chimarrogale platycephala	-	7	-	-	-	-	22	-	7	-	-	-	-	-	-	7	-	7	-
Suncus murinus	-	2.5	-	-	-	-	21.5	-	10	-	-	-	-	-	-	7	-	10	-
Condylura cristata	-	6.5	-	-	-	-	21.5	-	6.5	-	-	-	-	-	-	6.5	-	13	-
Scapanus orarius	-	5.5	-	-	-	-	21	-	5.5	-	-	-	-	-	-	12.5	-	12.5	-
Urotrichus talpoides	-	4.5	-	-	-	-	19.5	-	4.5	-	-	-	-	-	-	12.5	-	9	-
Mogera wogura	-	2.5	-	-	-	-	21	-	8.5	-	-	-	-	-	-	8.5	-	8.5	-
Talpa europaea	-	4	-	-	-	-	21	-	10	-	-	-	-	-	-	10	-	10	-
Talpa occidentalis	-	5	-	-	-	-	22	-	15	-	-	-	-	-	-	5	-	13	-
Rousettus amplexicaudatus	-	8.5	-	16	-	-	-	-	12	-	-	-	-	-	-	-	-	1.5	-
Erinaceus europaeus	-	3	-	-	-	-	21	-	3	-	-	-	-	-	-	14	-	9.5	-
Erinaceus amurensis	-	2.5	-	-	-	-	21.5	-	10.5	-	-	-	-	-	-	10.5	-	10.5	-
Loxodonta africana	-	2	-	17	-	-	-	-	8.5	-	-	-	-	-	-	-	-	8.5	-
Bradypus variegatus	-	5	-	16	-	-	-	-	5	-	-	-	-	-	-	-	-	5	-
Cyclopes didactylus	-	6	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-
Tamandua tetardactyla	-	7.5	-	17	-	-	-	-	7.5	-	-	-	-	-	-	-	-	7.5	-
Dasypus novemcinctus	-	5.5	-	17	-	-	-	-	11	-	-	-	-	-	-	-	-	5.5	-
Monodelphis domestica	-	2	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	6.5	-
Caluromys philander	-	2	-	17	-	-	-	-	15.5	-	-	-	-	-	-	-	-	9	-
Macropus eugenii	-	2	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	5.5	-
Dasyurus viverrinus	-	2	-	11.5	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-
Perameles nasuta	-	2	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-
Trichosurus vulpecula	-	1.5	-	14	-	-	-	-	8.5	-	-	-	-	-	-	-	-	4	-
Ornithorhynchus anatinus	-	1	-	24	-	24	24	7.5	17.5	17.5	-	-	-	-	7.5	17.5	-	7.5	-

Species	Sur	Tv	Tym	V
Rhabdomys pumilio	-	-	-	-
Mesocricetus auratus	-	-	16	12
Peromyscus melanophrys	-	-	-	-
Cavia porcellus	-	-	-	-
Tupaia javanica	-	-	-	-
Tarsius spectrum	-	-	-	-
Loris tardigradus	-	-	6.5	-
Homo sapiens	-	-	-	6
Felis domestica	-	-	-	-
Sus scrofa	-	-	-	-
Manis javanica	-	-	-	-
Cryptotis parva	-	-	-	5
Chimarrogale platycephala	-	-	-	7
Suncus murinus	-	-	-	13
Condylura cristata	-	-	-	6.5
Scapanus orarius	-	-	-	5.5
Urotrichus talpoides	-	-	-	4.5
Mogera wogura	-	-	-	8.5
Talpa europaea	-	-	-	4
Talpa occidentalis	-	-	-	5
Rousettus amplexicaudatus	-	-	-	-
Erinaceus europaeus	-	-	-	6
Erinaceus amurensis	-	-	-	10.5
Loxodonta africana	-	-	-	-
Bradypus variegatus	-	-	-	-
Cyclopes didactylus	-	-	-	-
Tamandua tetardactyla	-	-	-	-
Dasypus novemcinctus	-	-	-	-
Monodelphis domestica	-	-	-	-
Caluromys philander	-	-	-	-
Macropus eugenii	-	-	-	-
Dasyurus viverrinus	-	-	-	-
Perameles nasuta	-	-	-	-
Trichosurus vulpecula	-	-	-	-
Ornithorhynchus anatinus	-	-	7.5	7.5

Table 2. The sequence of cranial ossification for *Lepidochelys olivacea* as based on 53 specimens ranging 10 stages (Appendix 1). Tied bones in each stage are listed in alphabetical order. Bones in bold are endochondral in origin, bones unbolded are dermal in origin.

Stage	Bone
22	Maxilla
23	Dentary, Jugal, Premaxilla, Surangular, Vomer
24	Frontal, Parietal, Prefrontal, Squamosal
25	Angular, Coronoid, Palatine, Postorbital, Prearticular, Pterygoid
26 early	Quadratojugal
26	**Columella**
27 early	**Basisphenoid**
27	**Quadrate**
27 late	**Basioccipital**
28	**Exoccipital**
29	**Supraoccipital**
30	**Opisthotic, Prootic**
31	**Articular**

Table 3. The ranked ossification sequence data for 15 bones and 20 taxa used in Continuous Analysis. Taxa and bones were selected to maximize representation of taxonomic groups (Table 1). **Bo**: basioccipital; **Bs**: Basisphenoid; **D**: dentary; **Ex**: exoccipital; **F**: frontal; **J**: jugal; **Max**: maxilla; **Op**: opisthotic; **Pal**: palatine; **Par**: parietal; **Pm**: premaxilla; **Pr**: prootic; **Pt**: pterygoid; **So**: supraoccipital; and **Sq**: squamosal. Citations for the ossification sequences are listed in Table 1.

Species	Bone														
	Bo	Bs	D	Ex	F	J	Max	Op	Pal	Par	Pm	Pr	Pt	So	Sq
Chelydra	13	10.5	4.5	10.5	4.5	9	4.5	15	4.5	4.5	4.5	13	4.5	13	4.5
Macrochelys	13.5	8	3	11	8	8	1	13.5	8	8	5	13.5	3	13.5	3
Eretmochelys	12	10	4	10	10	4	4	14.5	4	4	8	14.5	4	13	4
Lepidochelys	11	10	5.5	12	5.5	5.5	1	14.5	5.5	5.5	5.5	14.5	5.5	13	5.5
Apalone	11.5	11.5	3	11.5	5.5	8.5	1.5	14.5	5.5	5.5	8.5	11.5	5.5	14.5	1.5
Pelodiscus	12.5	6.5	1.5	12.5	9.5	6.5	1.5	12.5	6.5	3.5	9.5	12.5	6.5	15	3.5
Phyrnops	13	9.5	4	9.5	4	4	4	13	4	9.5	9.5	13	4	15	4
Lacerta	11	11	4	11	4	4	4	14	4	8.5	4	14	4	14	8.5
Polychrus	14	11	3	7.5	7.5	3	7.5	12.5	3	7.5	7.5	12.5	1	15	7.5
Elgaria	10	12.5	6.5	10	6.5	2.5	6.5	14.5	2.5	4	6.5	14.5	1	12.5	10
Sphenodon	12	12	3	12	7.5	7.5	3	12	3	7.5	7.5	15	3	12	3
Gallus	12	12	4	9.5	7.5	4	4	15	4	9.5	7.5	14	4	12	1
Meleagris	13	10	5.5	11	8	5.5	5.5	15	2.5	9	5.5	14	2.5	12	1
Coturnix	11	12.5	4	8	9	4	4	14	4	12.5	4	10	4	15	4
Anas	13	11	6	11	9	6	3	14.5	3	8	6	14.5	3	11	1
Cairina	12	10	6	12	8	6	1.5	15	3.5	9	6	14	3.5	12	1.5
Dromaius	12	8.5	1	11	8.5	5.5	7	14	3	10	5.5	15	3	13	3
Caiman	6.5	6.5	2	14	11	2	2	14	6.5	11	6.5	14	6.5	11	6.5
Loris	6	13	1.5	11	6	6	1.5	14.5	6	6	6	14.5	11	11	6
Ornithorhynchus	10.5	10.5	4.5	10.5	4.5	10.5	4.5	14.5	4.5	4.5	1	14.5	10.5	10.5	4.5

Table 4. The ranked ossification sequence data used in the PGi analysis to address temporal bone heterochrony. Z represents missing data. Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Max: maxilla; N: nasal; Pal: palatine; Par: parietal; Pm: premaxilla; Pt: pterygoid; So: supraoccipital; Sq: squamosal; and V: vomer. Citations for the ossification sequences are listed in Table 1.

Species	Bone														
	Bo	Bs	D	Ex	F	J	Max	N	Pal	Par	Pm	Pt	So	Sq	V
Chelydra	13	11	1	11	1	10	1	Z	1	1	1	1	13	1	1
Macrochelys	13	6	2	12	6	6	1	Z	6	6	5	2	13	2	6
Eretmochelys	13	10	1	10	10	1	1	Z	1	1	8	1	14	1	8
Lepidochelys	12	11	2	13	6	2	1	Z	9	6	2	9	14	6	2
Apalone	10	10	3	10	4	8	1	Z	4	4	8	4	14	1	10
Pelodiscus	12	5	1	12	9	5	1	Z	5	3	9	5	14	3	9
Emys	Z	11	4	Z	4	4	1	Z	4	4	4	4	11	1	3
Phrynops	14	10	1	10	1	1	1	1	1	10	10	1	15	1	1
Emydura	11	11	1	11	3	3	1	10	3	3	3	3	11	3	15
Lacerta	11	11	1	11	1	1	1	8	1	8	1	1	14	8	Z
Polychrus	13	12	2	5	5	2	5	11	2	5	5	1	14	5	Z
Elgaria	10	14	5	10	5	2	5	13	2	4	5	1	14	10	5
Sphenodon	12	12	1	12	7	7	1	1	1	7	7	1	12	1	7
Gallus	12	12	2	10	8	2	2	7	2	10	8	2	12	1	15
Meleagris	14	11	4	12	9	4	4	8	2	10	4	2	13	1	Z
Coturnix	11	12	1	9	10	1	1	1	1	12	1	1	15	1	14
Anas	15	12	6	12	10	6	2	2	2	9	6	2	12	1	11
Cairina	13	12	6	13	10	6	1	1	4	11	6	4	13	1	9
Dromaius	14	10	1	13	10	5	8	9	2	12	5	2	15	2	7
Caiman	4	4	1	15	12	1	1	4	4	12	4	4	12	4	4
Mus	4	14	1	4	1	12	1	12	4	4	4	4	15	4	4
Meriones	4	10	1	7	1	10	4	7	7	10	1	4	Z	10	Z
Rhabdomys	8	12	1	9	1	13	6	10	1	1	6	1	Z	11	Z
Mesocricetus	10	4	1	4	4	14	2	10	4	4	2	Z	10	4	10
Peromyscus	4	9	1	9	4	11	1	11	4	4	1	4	Z	11	Z
Cavia	9	13	1	11	1	1	1	9	1	1	1	11	Z	1	Z
Loris	3	14	1	10	3	3	1	10	3	3	3	10	10	3	Z
Cryptotis	12	14	1	12	4	15	1	9	4	7	1	7	10	10	4
Chimarrogale	13	14	1	2	2	Z	2	2	2	2	2	2	2	2	2
Suncus	6	14	1	9	5	Z	1	12	6	1	1	9	6	9	12
Scapanus	14	13	1	10	1	Z	1	1	1	1	1	1	10	10	1
Urotrichus	10	12	1	11	1	Z	1	14	1	1	1	1	12	9	1
Mogera	12	14	1	13	5	Z	1	5	5	1	1	5	5	5	5
Talpa	1	14	1	1	10	Z	1	10	1	1	1	13	1	12	1
Rousettus	10	13	1	10	3	3	3	7	7	3	7	10	Z	1	Z
Loxodonta	12	13	1	4	4	4	1	4	4	4	1	4	Z	4	z
Dasypus	11	13	1	12	1	1	1	1	1	1	1	10	Z	1	Z
Caluromys	11	11	1	7	4	4	1	7	7	4	1	13	Z	7	Z
Ornithorhynchus	10	10	2	10	2	10	2	2	2	2	1	10	10	2	2

Table 5. Ancestral sequences reconstructed with PGi and Continuous Analysis for **Scenario 1** (Figure 3) using the dataset without missing data. Bolded and highlighted bones represent a section of the sequence that is unique when compared with the other phylogenetic hypotheses. Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Max: maxilla; Op: opisthotic; Pal: palatine; Par: parietal; Pm: premaxilla; Pr: prootic; Pt: pterygoid; So: supraoccipital; Sq: squamosal.

Node	Node Name	PGi sequence	CA sequence
	Scenario 1 (T+D)		
1	**Amniota**	[D,F,Max,Pm],[Pal,Par,Sq],[Bo,Bs,Ex,J,Pt,So],[Op,Pr]	Pt,Pal,J,D,Max,Pm,F,Par,Sq,Ex,Bs,Bo,So,Op,Pr
2	**Mammalia**	[D,Max,Pm],[Bo,F,Pal,Par,Sq],[Bs,Ex,J,Pt,So],[Op,Pr]	Pt,Pal,D,J,Max,Pm,F,Sq,Par,Ex,Bs,Bo,So,Op,Pr
3	**Reptilia**	[D,F,J,Max,Pm],[Pal,Pt,Sq],Par,[Bs,Ex,So],[Bo,Op,Pr]	D,Pal,Pt,Max,J,Sq,Pm,F,Par,Bs,Ex,Bo,So,Op,Pr
4	**Testudines**	Max,[D,F,J,Pal,Pt,Sq],[Bs,Ex,Par,Pm],[Bo,Op,Pr],So	Max,D,Sq,Pt,Pm,Pal,Par,F,J,Bs,Ex,Bo,So,Pr,Op
5	**Cryptodira**	Max,Sq,[Bs,D,J,Pal,Par,Pt],[F,Pm],Ex,[Bo,Pr],[Op,So]	Max,D,Sq,Pt,Pal,Par,J,Pm,F,Bs,Ex,Bo,So,Pr,Op
6	**Trionychidae**	Max,[D,Sq],[Bs,J,Pal,Par,Pt],[F,Pm],[Bo,Ex,Pr],[Op,So]	Max,D,Sq,Pt,Pal,Par,Pm,J,F,Bs,Ex,Bo,So,Pr,Op
7	**Chelonioidea**	Max,Sq,[Bs,D,J,Pal,Par,Pm,Pt],[Ex,F],Bo,[Op,Pr,So]	Max,D,Sq,Pt,Par,Pal,J,F,Pm,Bs,Ex,Bo,Pr,Op,So
8	**Cheloniidae**	Max,[D,J,Pal,Par,Pm,Pt,Sq],[Bs,Ex,F],Bo,So,[Op,Pr]	Max,D,Sq,Pt,Pal,Par,J,F,Pm,Bs,Ex,Bo,Pr,So,Op
9	**Chelydridae**	Max,Sq,[Bs,D,F,J,Pal,Par,Pm,Pt],Ex,[Bo,Op,Pr,So]	Max,D,Sq,Pal,Pt,J,F,Par,Pm,Bs,Ex,Bo,Pr,So,Op
10	**Diapsida**	[D,F,J,Max,Pm],[Pal,Pt,Sq],Par,[Bs,Ex,Op,So],Bo,Pr	Sq,Max,Pal,Pt,D,J,Pm,F,Par,Bs,Ex,So,Bo,Pr,Op
11	**Archosauria**	[D,J,Max],[Bs,Pal,Pm,Pt,Sq],[F,Par],Ex,Bo,[Op,Pr],So	Sq,Pal,Pt,D,Max,J,Pm,F,Ex,Par,Bs,Bo,Pr,So,Op
12	**Aves**	D,[Pal,Pt,Sq],[J,Pm],Max,F,Par,Bs,Ex,Bo,Op,[Pr,So]	Sq,Pal,Pt,D,Max,J,Pm,F,Ex,Par,Bs,Bo,Pr,So,Op
13	**Neognathae**	Sq,[Pal,Pt],[J,Pm],D,Max,[Ex,F],Par,[Bo,Bs],Pr,Op,So	Sq,Pal,Pt,D,Max,J,Pm,F,Par,Bs,Ex,Bo,So,Pr,Op
14	**Anseriformes**	[Max,Sq],[Pal,Pt],[J,Pm],D,F,Par,Bs,[Ex,So],Bo,Pr,Op	Sq,Pal,Pt,D,Max,J,Pm,F,Par,Bs,Ex,Bo,So,Pr,Op
15	**Galliformes**	Sq,[Pal,Pt],[D,J,Max,Pm],Ex,F,Par,Bo,[Bs,Pr],Op,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Bo,Ex,So,Pr,Op
16	**Gallus + Coturnix**	Sq,[D,J,Max,Pal,Pm,Pt],Ex,F,Par,Pr,Bo,Bs,Op,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Bo,Ex,So,Op,Pr
17	**Lepidosauria**	Pt,[D,F,J,Max,Pal,Par,Pm],Sq,[Bs,Ex,So],Bo,[Op,Pr]	D,Max,Pm,Pal,Sq,F,Par,J,Pt,Bo,Ex,Bs,So,Op,Pr
18	**Squamata**	Pt,[D,F,J,Max,Pal,Pm],[Par,Sq],[Bs,Ex],[Bo,Op,Pr],So	D,Max,Pal,Sq,J,Pt,Pm,F,Par,Bs,Bo,Ex,So,Op,Pr
19	**Iguania + Anguimorpha**	Pt,[D,J,Pal],[Ex,F,Max,Par,Pm,Sq],Bs,[Op,Pr],Bo,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Bo,Ex,So,Op,Pr

Table 6. Ancestral sequences reconstructed with PGi and Continuous Analysis for **Scenario 2** (Figure 3) using the dataset without missing data. Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Max: maxilla; Op: opisthotic; Pal: palatine; Par: parietal; Pm: premaxilla; Pr: prootic; Pt: pterygoid; So: supraoccipital; Sq: squamosal.

Node	Node Name	PGi sequence	CA sequence
	Scenario 2 (T+A)		
1	**Mammalia**	[D,Max],[Pal,Par,Pm,Pt,Sq],[Bo,F],[Ex,J],[Bs,So],[Op,Pr]	Pt,Pal,J,D,Max,Pm,F,Par,Sq,Ex,Bs,Bo,So,Op,Pr
2	**Amniota**	[D,Max],[F,Pal,Par,Pm,Sq],[Bo,Bs,Ex,J,Pt,So],[Op,Pr]	Pt,Pal,D,J,Max,Pm,F,Sq,Par,Ex,Bs,Bo,So,Op,Pr
3	**Archosauromorpha**	[Bo,Bs,D],[Max,Pal,Pm,Pt,Sq],[F,J,Par],Ex,[Op,Pr,So]	D,Pal,Pt,Max,J,Sq,Pm,F,Par,Bs,Ex,Bo,So,Op,Pr
4	**Testudines**	D,Bs,[F,J,Max,Pal,Par,Pt,Sq],Pm,[Bo,Ex,Pr],[Op,So]	Max,D,Sq,Pt,Pm,Pal,Par,F,J,Bs,Ex,Bo,So,Pr,Op
5	**Cryptodira**	Sq,[D,Par],[Bs,F,Max,Pal,Pt],[J,Pm],Ex,[Bo,Pr],[Op,So]	Max,D,Sq,Pt,Pal,Par,J,Pm,F,Bs,Ex,Bo,So,Pr,Op
6	**Trionychidae**	[Max,Sq],[D,Par],[Bs,F,Pal,Pt],[J,Pm],[Bo,Ex,Pr],[Op,So]	Max,D,Sq,Pt,Pal,Par,Pm,J,F,Bs,Ex,Bo,So,Pr,Op
7	**Chelonioidea**	[D,F,Max,Pal,Par,Pm,Pt,Sq],[Bs,J],Ex,[Bo,Pr,So],Op	Max,D,Sq,Pt,Par,Pal,J,F,Pm,Bs,Ex,Bo,Pr,Op,So
8	**Cheloniidae**	[D,F,J,Max,Pal,Par,Pm,Pt,Sq],Bs,Bo,Ex,So,[Op,Pr]	Max,D,Sq,Pt,Pal,Par,J,F,Pm,Bs,Ex,Bo,Pr,So,Op
9	**Chelydridae**	[D,F,Max,Pal,Par,Pm,Pt,Sq],[Bs,J],Ex,[Bo,Pr,So],Op	Max,D,Sq,Pal,Pt,J,F,Pm,Par,Bs,Ex,Bo,Pr,So,Op
10	**Diapsida**	Bo,D,[Max,Pal,Pm,Pt,Sq],[F,J,Par],[Bs,Ex],[Op,Pr,So]	Sq,Max,Pal,Pt,D,J,Pm,F,Par,Bs,Ex,So,Bo,Pr,Op
11	**Archosauria**	Bo,D,[Bs,Pal,Pm,Pt,Sq],[J,Max],[F,Par,So],Ex,[Op,Pr]	Sq,Pal,Pt,D,Max,J,Pm,F,Ex,Par,Bs,Bo,Pr,So,Op
12	**Aves**	D,[Pal,Pm,Pt,Sq],J,Max,F,Par,Bs,Ex,Bo,So,[Op,Pr]	Sq,Pal,Pt,D,Max,J,Pm,F,Ex,Par,Bs,Bo,Pr,So,Op
13	**Neognathae**	[Max,Pal,Pm,Pt,Sq],D,Ex,F,Par,[Bs,J],Bo,[Op,Pr],So	Sq,Pal,Pt,D,Max,J,Pm,F,Par,Bs,Ex,Bo,So,Pr,Op
14	**Anseriformes**	[Max,Pt,Sq],Pal,[D,Pm],F,Par,[Bs,Ex,J,So],Bo,[Op,Pr]	Sq,Pal,Pt,D,Max,J,Pm,F,Par,Bs,Ex,Bo,So,Pr,Op
15	**Galliformes**	[D,J,Max,Pal,Pm,Pt,Sq],Ex,F,Par,Pr,Bs,Bo,Op,So	D,Max,Pal,Sq,Pt,J,Pm,F,Par,Bs,Bo,Ex,So,Pr,Op
16	**Gallus + Coturnix**	[D,J,Max,Pal,Pm,Pt,Sq],Ex,F,Pr,[Bs,Par],Bo,Op,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Bo,Ex,So,Op,Pr
17	**Lepidosauria**	[Bo,D,J,Max,Pal,Pt,Sq],[F,Par,Pm],[Bs,Ex],[Op,Pr,So]	D,Max,Pm,Pal,Sq,F,Par,J,Pt,Bo,Ex,Bs,So,Op,Pr
18	**Squamata**	Pt,[D,J,Pal],[Bo,F,Max,Par,Pm,Sq],[Bs,Ex],[Op,Pr,So]	D,Max,Pal,Sq,J,Pt,Pm,F,Par,Bo,Bs,Ex,So,Op,Pr
19	**Iguania + Anguimorpha**	Pt,[D,J,Pal],[F,Max,Par,Pm,Sq],Ex,Bs,[Op,Pr],Bo,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Bo,Ex,So,Pr,Op

Table 7. Ancestral sequences reconstructed with PGi and Continuous Analysis for **Scenario 3** (Figure 3) using the dataset without missing data. Bolded and highlighted bones represent a section of the sequence that is unique when compared with the other phylogenetic hypotheses. Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Max: maxilla; Op: opisthotic; Pal: palatine; Par: parietal; Pm: premaxilla; Pr: prootic; Pt: pterygoid; So: supraoccipital; Sq: squamosal.

Node	Node Name	PGi sequence	CA sequence
	Scenario 3 (T+L)		
1	**Mammalia**	Pm,[D,Max],Sq,[F,J,Pal,Par],[Bo,Ex,Pt],[Bs,So],[Op,Pr]	Pt,Pal,J,D,Max,Pm,F,Par,Sq,Ex,Bs,Bo,So,Op,Pr
2	**Amniota**	Pm,[D,Max],[F,J,Pal,Par,Sq],[Bo,Ex,Pt,So],Bs,[Op,Pr]	Pt,Pal,D,J,Max,Pm,F,Sq,Par,Ex,Bs,Bo,So,Op,Pr
3	**Lepidosauromorpha**	D,[F,J,Max,Pal,Pm,Sq],Par,[Bo,Bs,Ex,Pt],So,[Op,Pr]	D,Pal,Max,Pt,J,Sq,Pm,F,Par,Bs,Ex,Bo,So,Op,Pr
4	**Testudines**	D,[F,J,Max,Pal,Pt],[Bs,Ex,Par,Pm],Sq,[Bo,So],[Op,Pr]	Max,D,Sq,Pt,Pm,Pal,Par,F,J,Bs,Ex,Bo,So,Pr,Op
5	**Cryptodira**	[Max,Sq],D,[F,J,Pal,Par,Pt],[Bs,Pm],Ex,[Bo,So],[Op,Pr]	Max,D,Sq,Pt,Pal,Par,J,Pm,F,Bs,Ex,Bo,So,Pr,Op
6	**Trionychidae**	[Max,Sq],D,[Bs,F,J,Pal,Par,Pt],Pm,[Bo,Ex,Pr],[Op,So]	Max,D,Sq,Pt,Pal,Par,Pm,J,F,Bs,Ex,Bo,So,Pr,Op
7	**Chelonioidea**	Pal,[Max,Sq],[D,F,J,Par,Pt],Pm,Bs,Ex,So,[Bo,Op,Pr]	Max,D,Sq,Pt,Par,Pal,J,F,Pm,Bs,Ex,Bo,Pr,Op,So
8	**Cheloniidae**	[D,F,J,Max,Pal,Par,Pt,Sq],Pm,Bs,Bo,Ex,So,[Op,Pr]	Max,D,Sq,Pt,Pal,Par,J,F,Pm,Bs,Ex,Bo,Pr,So,Op
9	**Chelydridae**	[Max,Pal],[D,F,Par,Pm,Pt,Sq],J,Bs,[Ex,So],[Bo,Op,Pr]	Max,D,Sq,Pt,Pal,J,F,Pm,Par,Bs,Ex,Bo,Pr,So,Op
10	**Diapsida**	[D,J],[Bs,Pal,Pm,Pt,Sq],Max,F,Par,[Bo,Ex],So,[Op,Pr]	Sq,Max,Pal,Pt,D,J,Pm,F,Par,Bs,Ex,So,Bo,Pr,Op
11	**Archosauria**	[D,J],[Bo,Bs,Pal,Pt,Sq],[Max,Pm],F,Par,Ex,So,[Op,Pr]	Sq,Pal,Pt,D,Max,J,Pm,F,Ex,Par,Bs,Bo,Pr,So,Op
12	**Aves**	D,[Pal,Pm,Pt,Sq],J,Max,F,Par,Bs,Ex,So,Bo,Op,Pr	Sq,Pal,Pt,D,Max,J,Pm,F,Ex,Par,Bs,Bo,Pr,So,Op
13	**Neognathae**	[D,Max,Pal,Pm,Pt],[Ex,J],F,Par,Bs,[Op,Sq,So],Bo,Pr	Sq,Pal,Pt,D,Max,J,Pm,F,Par,Bs,Ex,Bo,So,Pr,Op
14	**Anseriformes**	Sq,[Max,Pal,Pt],[D,J,Pm],F,Par,[Bs,Pr,So],[Bo,Ex],Op	Sq,Pal,Pt,D,Max,J,Pm,F,Par,Bs,Ex,Bo,So,Pr,Op
15	**Galliformes**	[D,J,Max,Pal,Pm,Pt,Sq],Ex,F,Par,Bs,So,Bo,Pr,Op	D,Max,Pal,Sq,Pt,J,Pm,F,Par,Bs,Bo,Ex,So,Pr,Op
16	**Gallus + Coturnix**	[D,J,Max,Pal,Pm,Pt,Sq],Ex,F,Par,Bs,Pr,Bo,Op,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Bo,Ex,So,Op,Pr
17	**Lepidosauria**	[D,F,J,Max,Pal,Pm,Pt,Sq],Par,[Bo,Bs,Ex],[Op,Pr],So	D,Max,Pm,Pal,Sq,F,Par,J,Pt,Bo,Ex,Bs,So,Op,Pr
18	**Squamata**	[D,F,J,Max,Pal,Pm,Pt],[Par,Sq],[Bo,Bs,Ex],[Op,Pr],So	D,Max,Pal,Sq,J,Pm,Pt,F,Par,Bo,Bs,Ex,So,Op,Pr
19	**Iguania + Anguimorpha**	Pt,[D,J,Pal],[Max,Op,Par],[Ex,F,Pm],Sq,[Bo,Bs,],Pr,So	D,Max,Pal,Pt,Sq,J,Pm,F,Par,Bs,Ex,Bo,So,Op,Pr

Table 8. Heterochronies inferred with Continuous Analysis between turtles and their three hypothetical ancestors (associated with Scenarios 1, 2, and 3, respectively), indicating shifts to "earlier" (early) or "later" (late) positions in a particular reconstructed sequence. *heterochronies that are unique to a specific reconstruction; highlighted heterochronies were reconstructed in two of the three scenarios. Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Op: opisthotic; Pal: palatine; Par: parietal; Pm: premaxilla; Pt: pterygoid; So: supraoccipital; Sq: squamosal. No significant heterochronies were reconstructed for the maxilla and prootic.

Species	Reptilia-to-Turtles	Archosauromorpha-to-Turtles	Lepidosauromorpha-to-Turtles
Chelydra serpentina	**Early:** F, Par, Pm, Pt*, Sq	**Early:** F, Par, Pm, Sq	**Early:** F, Par, Pm, Sq
Macrochelys temminckii	**Late:** Bo, J, Pal, So	**Late:** Bo, J, Pal, So	**Late:** Bo, J, Pal, So
Eretmochelys imbricata	**Early:** Par, Pt*, Sq	**Early:** J, Par, Sq	**Early:** J, Par, Sq
Lepidochelys olivacea		**Late:** D	**Late:** D
Apalone spinifera	**Early:** Sq **Late:** So	**Early:** Sq **Late:** J, So	**Early:** Sq **Late:** J, So
Pelodiscus sinensis	**Early:** Bs, Op **Late:** So	**Early:** Bs, Op, Par **Late:** Pm, So	**Early:** Bs, Op, Par **Late:** Pm, So
Phrynops hilarii	**Early:** F, Op, Pt*, Sq **Late:** So	**Early:** Ex, F, J, Op, Sq **Late:** So	**Early:** Bs*, Ex, F, J, Op, Sq **Late:** So

Table 9. Heterochronies inferred with Continuous Analysis between the root node and the terminal taxa following the methods of Germain and Laurin (2009). Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Op: opisthotic; Pal: palatine; Par: parietal; Pm: premaxilla; Pt: pterygoid; So: supraoccipital; Sq: squamosal.

Species	Early Shifts	Late Shifts
Chelydra serpentina	F, Par, Pt	
Macrochelys temminckii		Bo, Pal, So
Eretmochelys imbricata	Par, Pt	
Lepidochelys olivacea		
Apalone spinifera		So
Pelodiscus sinensis	Bs, Op	So
Phrynops hilarii	F, Op, Pt	So
Lacerta agilis	F, Pt	So
Polychrus acutirostris	Op, Pt	Bo, Max, So
Elgaria coerulea	Pt	D, Max, Sq
Sphenodon punctatus	Op, Pt	
Gallus gallus		
Meleagris gallopavo		
Coturnix coturnix	Ex, Pr, Pt	Par, So
Anas platyrhynchos		Bo
Cairina moschata		
Dromaius novaehollandiae		Max
Caiman yacare	Bo, Bs	Ex, F, Par
Loris tardigradus	Bo	Bs, Pt
Ornithorhynchus anatinus		J, Pt

Table 10. Heterochronies inferred with Continuous Analysis between the Testudines ancestor and its terminal taxa following the methods of Germain and Laurin (2009). Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Op: opisthotic; Pal: palatine; Par: parietal; Pm: premaxilla; Pt: pterygoid; So: supraoccipital; Sq: squamosal.

Species	Early Shifts	Late Shifts
Chelydra serpentina	F, Par, Pm	
Macrochelys temminckii		Bo, J, Pal
Eretmochelys imbricata	Par	
Lepidochelys olivacea		D
Apalone spinifera		J
Pelodiscus sinensis	Bs, Op	
Phrynops hilarii	F, Op	

Table 11. Shifts reconstructed with PGi of the complete dataset without missing data. Internode numbers refer to Figures 4–6.

		Scenario 1		Scenario 2		Scenario 3	
Internode #	**Internode Name**	**Early**	**Late**	**Early**	**Late**	**Early**	**Late**
1	**Mammalia**		F	F	Pt	So	
2	***Loris***	J	Bs,Pm	Bo,J	Bs	Bo	Pm
3	***Ornithorhynchus***		Bo,D,Max	Pm	D,Max	Bs	D,J,Max
4	**Reptilia**	J	Bo,Par	Bo,J	Max,Par,So	Bs,J,Pt	
5	**Testudines**		D,F,J,Par,Pm,So	F,Pr	Bo,Pm	Pt	Bo,Par,Pm,Sq
6	***Phrynops***		Max	Ex,J,Op	Bs,D	Sq	D,So
7	**Cryptodira**	Bs,Par,Sq	Ex,F,Op	Par,Sq		Max,Par,Sq	Ex
8	**Trionychidae**	D	Ex	Max		Bs,Pr	
9	***Pelodiscus***	D,Op,Par		D,J,Max,Op	F	D,Max,Op,Par	F
10	***Apalone***	F	Bs,J		Bs,Par		Bs,J
11	**Chelonioidea**	Pm	Pr	Ex,Pm,So	Bs,D,Par,Sq		D
12	**Cheloniidae**	So	Bs,Sq	Bo,J	Pr	Bo	Max,Pal,Sq
13	***Lepidochelys***	F	Ex	Max		Max,Pm	
14	***Eretmochelys***		Max,Pm	Ex	F,Pm	Ex	F
15	**Chelydridae**	F	Bo				J,Sq,So
16	***Macrochelys***	D,Pm,Pt		Max,Op	F,Pal,Par,Pm		F,Pal,Par
17	***Chelydra***		Bs,J,Max,Op,Sq		Bs	Pm	Bs,Max,Op,Pal
18	**"Diapsida"/-Morpha**	Bo,Op		Bs	J	F,Max	Bs,J,Pt
19	**Archosauria**	Bs	F,Op,Pm,So	J,So	Max	Bo	
20	**Aves**	F	Bs,J,Max,Pm,Pr		Bo,Bs,So	Op	Bo,Bs,J
21	**Neognathae**	Ex,Pr,Sq	D	Ex,Max	D,J,So	Ex,Max,Op	D,Sq
22	**Anseriformes**	Bs,Max,So	Ex	So	Ex,Pal,Pm	Sq	Bs,D,Ex,Pm
23	***Cairina***	Bo,D		Bo,Bs,J	Op,Pt	Max	Op,So
24	***Anas***	D,Op	Bs,F,Max	J	F,Max,Pt	Ex	F
25	**Galliformes**	D,Max		D,J,Pr		J,Sq	

		Scenario 1		Scenario 2		Scenario 3	
Internode #	**Internode Name**	**Early**	**Late**	**Early**	**Late**	**Early**	**Late**
26	***Gallus+Coturnix***	Pr	Pal,Pt				Bo,So
27	***Coturnix***		Par,Sq	Bo			Bs,Par
28	***Gallus***	So	Bo,Ex,Pm,Pr	Sq,So	Bs,Ex,Pm,Pr	Bo,Sq,So	Ex,Pm
29	***Meleagris***	Bs,So	Ex	Pal,Pt,Sq,So	Ex,Pr	Pal,Pt,Sq	Ex
30	***Dromaius***	Bs,So		Bs,Op	Pm	Bo,Bs	Pm
31	***Caiman***	Bo,So	Ex	J,Max	Bo,Ex	Max	Ex,F,Par
32	**Lepidosauria**	Pal,Par,Pt	Op		D,Pm	Pt	D,So
33	***Sphenodon***	Bo,D,Max,Op,Pal,Sq		Op,Sq,So	Bo,J	Op,So	F,J,Pm
34	**Squamata**	Bo	Par,So		Max		
35	***Polychrus***			Ex		Sq	Bo,Max,Op,Par
36	**Iguania+Anguimorpha**	Ex	Bo,F,Max,Pm	Pt	Bo,So	Ex,Op,Pt	F,Pm
37	***Elgaria***	Bo,Par,So	D,Ex,Sq	Bo,Par,So	D,Sq	So	D,Ex,Max,Op
38	***Lacerta***	So	Pt	F,Max,Pm		So	Par

Table 12. Ancestral sequences reconstructed with PGi. Node numbers reference Figures 4-6. Bo: basioccipital; Bs: Basisphenoid; D: dentary; Ex: exoccipital; F: frontal; J: jugal; Max: maxilla; N: nasal; Pal: palatine; Par: parietal; Pm: premaxilla; Pt: pterygoid; Sq: squamosal; So: supraoccipital; V: vomer.

Node	Node Name	Scenario 1 (T+D)	Sscenario 2 (T+A)	Scenario 3 (T+L)
1	Amniota	[D,J,Pm],Max,[F,Pal],[Bo,Pt,Sq],[N,Par],[Bs,Ex],So,V	[Max,N],Par,[J,Pal,Pm],[D,F,Sq],Ex,Bo,[Bs,Pt,So],V	Ex,D,[J,Max,Pm],N,[F,Pal,Par,Sq],Pt,[Bo,Bs],So,V
2	Mammalia	Pm,[D,Max],J,[F,Par],[N,Pal,Sq],Bo,[Bs,Pt],Ex,[So,V]	Max,Pm,[D,F,N,Pal,Par,Sq],Ex,Bo,[Bs,J,Pt,So],V	D,[J,Max,Pm],[Ex,F,N,Pal,Par,Sq],[Bo,Bs,Pt],So,V
3	Monotremes+Eutheria	[D,Max,Pm],[F,J],Par,[N,Pal,Sq],[Bo,Ex],Bs,Pt,[So,V]	[D,Max],[F,J,Pal,Par,Pm],[Ex,N,Sq],[Bo,Bs,Pt],V	[D,Max],J,[Ex,F,N,Pal,Par,Pm,Sq],[Bo,Bs],[Pt,So],V
4	Eutheria	[D,Max,Pm],Sq,[F,Par],[Ex,N,Pal],[Bo,J,Pt,V],[Bs,So]	[D,Max],[F,J,N,Pal,Par,Pm,Pt,Sq],Bo,Ex,Bs,V	[D,Max],[F,J,N,Pal,Par,Pm,Sq],[Pt,So],[Bo,Bs,Ex,V]
5	Eutheria-Dasypus	[D,Max,Pm],[F,J,Par,Sq],Ex,[N,Pal],V,Pt,Bo,[Bs,So]	[D,Max,Pm,Sq],[F,J,N,Pal,Par,Pt],Bo,Ex,[Bs,So],V	[D,F,J,Max],N,[Pal,Par,Pm,Sq],[Pt,So],Ex,V,Bo,Bs
6	Laurasia.+Euarch.	D,[F,Max],[J,Par,Sq],[Pal,Pm],N,[Bo,Ex,Pt,V],[Bs,So]	[D,Max,Pm,Sq],[Bo,F,J,N,Pal,Par],[Ex,Pt],[Bs,So],V	[D,F,J,Max],[Bo,N],[Pal,Par,Pm],[Pt,So],Sq,Ex,V,Bs
7	Laurasiatheria	[D,Sq],[Max,Par],J,[F,Pm],N,[Pal,V],[Ex,Pt],Bo,[Bs,So]	[D,Max,Pm,Pt,Sq],[F,J],[Bo,N,Pal],[Ex,Par],[Bs,So],V	[D,F,J,Max],[N,Pal,Pm],So,Par,[Pt,Sq],Bo,Ex,V,Bs
8	Talpidae+Soricidae	D,Max,J,[Par,Pm],F,[N,V],Pal,[Ex,Sq],Bo,Pt,[Bs,So]	[D,Max,Pm,Pt],F,[Bo,Pal],[Ex,Par,Sq],[Bs,So],V,N,J	[D,F,Max,Pm],[J,Pal],So,Par,[Pt,Sq],Bo,Ex,[N,V],Bs
9	Soricidae	[D,Max],J,[Par,Pm],F,V,Pal,N,[Bo,Pt,Sq],[Ex,So],Bs	[D,Max,Pm],F,[Bo,Pal,V],[Ex,Par,Pt,Sq],N,[Bs,So],J	[D,Max,Pm],[F,Pal],[Bo,So],Par,[Pt,Sq],Ex,[N,V],Bs,J
10	*Chimarrogale* + *Cryptotis*	[D,Max],[Par,Pm,V],[F,Pal],Pt,N,Sq,So,[Bo,Ex],Bs	Bs,[D,Max,Pm],[F,Pal,V],[Par,Pt],N,[Sq,So],[Bo,Ex,J]	[D,Max],[F,Pal,V],Par,N,[Pm,Pt,Sq,So],[Bo,Ex],Bs,J
11	Talpidae	[D,F,Max,Pal,Par,Pm],N,V,[Ex,Pt,Sq],Bo,[Bs,So]	[D,Max,Pal,Par,Pm,Pt,V],F,Bo,[Ex,Sq],[Bs,So],N,J	[D,F,Max,Pal,Par],[J,N],[Bs,Pm,Pt,So],Sq,Bo,Ex,V
12	Talpinae	[D,Max,Pal,Par,Pm],F,[Bo,N],Sq,[Pt,V],Ex,[Bs,So]	[D,Max,Pal,Par,Pm,Pt,V],[F,Sq],[Bo,Ex],[Bs,So],N	[D,F,Max,Pal,Par],J,[Pt,So],Sq,[Bo,Bs,N],[Ex,Pm],V
13	*Talpa* + *Mogera*	[D,Max,Par,Pm,So],Pal,[Bo,F,N],Sq,V,Ex,Pt,Bs	[D,Max,Pal,Par,Pm],F,[N,Sq],[Pt,V],[Bo,So],Ex,Bs	[D,Max,Pal,Par],J,[F,N,So],Sq,[Bo,Pt],[Ex,Pm],Bs,V
14	Euarchontoglires	[D,Max],F,[J,Pal,Par,Pm,Sq],Bo,N,[Ex,Pt],V,Bs	[D,Max,Pm,Sq],[Bo,F,J,Pal,Par],[Ex,Pt],[Bs,N,So,V]	[D,J,Max],[Bo,N],[F,Pal,Par,Pm],[Pt,So],[Bs,Ex,Sq],V
15	Rodentia	[D,F,Max,Pm],Pal,[Bo,N,Par,Sq],J,[Ex,Pt],V,Bs	[D,F,Max,Pm,Sq],[Bo,J,Pal,Par],[Ex,Pt],[Bs,N,So,V]	[D,F,J,Max,Pm],[Bo,N],[Pal,Par],[Ex,Pt],Sq,Bs,V
16	Muridae+Cricetidae	[D,F,Pm],[Max,Pal],Bo,[Ex,J],[Par,Sq],V,Bs,So,N	[D,F,Max,Pm],[Bo,Pal,Par,Pt,Sq],Ex,[Bs,J,N,So,V]	[D,F,Max,Pm],Bo,[Pal,Par,Pt],Ex,N,[J,Sq],Bs,So,V
17	Cricetidae	[D,Max,Pm],[Bo,F,Pal],[Par,Sq],Ex,Bs,[So,V],[J,N]	[D,Max,Pm],[F,Pal,Par,Sq],[Bo,Bs,Ex],[J,N,So,V]	[D,Max,Pm],[F,Pal,Par],Ex,[Bo,N,Pt,So],Bs,[J,Sq],V
18	Muridae	[D,F,Pm],[Max,Pal,Pt],Bo,Ex,N,[Par,Sq],J,Bs	[D,F,Pm],[Bo,Max,Par,Pt,Sq],[N,Pal],Ex,[Bs,J,V],So	[D,F],[Bo,Max,Pm],[Ex,Pal,Pt],N,[J,Sq],[Bs,Par],[So,V]
19	*Mus* + *Rhabdomys*	[D,F,Pal,Par,Pt],Max,[Bo,Pm],Ex,N,Sq,J,Bs	[D,F,Max],Pm,[Bo,Ex,Pal,Par,Pt,Sq,V],N,Bs,[J,So]	[D,F,Par],Max,[Bo,Ex,Pal,Pm,Pt],[J,N],Sq,Bs,[So,V]
20	"Diapsida"	Bo,D,[J,Pt],Max,[F,Pal,Pm],Sq,N,Par,Ex,Bs,So,V	[D,J,Max,N],Par,[Pal,Pm,Sq],[F,Pt],Ex,Bo,[Bs,V],So	Ex,D,[J,Pm],Max,N,F,Par,Pt,[Bs,Sq],[Bo,V],Pal,So
21	Testudines	[D,Max],[F,Pal,Pt,Sq],Bo,[J,N,Par],Pm,[Bs,Ex],So,V	[D,Max,N,Sq],Par,[Bs,F,J,Pal,Pm,Pt],Ex,[Bo,So],V	[D,Max],Par,Pt,Sq,[F,J,N,Pal],[Bs,Ex,Pm,V],[Bo,So]
22	Pleurodira	D,[F,J,Max,Pal,Pt,Sq],[N,Par],Pm,[Bs,Ex],Bo,So,V	[D,Max,N,Pal,Pt,Sq],[Bs,Ex,F,J,Par,Pm],Bo,[So,V]	[D,Max],[F,J,N,Pal,Pt],[Bs,Ex,Par,Pm,Sq],[Bo,V],So
23	Cryptodira	Max,Sq,[D,Par],[F,Pal,Pt],J,Pm,[Bs,Ex],N,So,V	[D,Max,Sq],Par,[Bs,J,Pal,Pm,Pt],[Ex,F,V],[Bo,So]	[D,Max],Par,Pt,Sq,V,[F,J,N,Pal,Pm],[Bs,Ex],[Bo,So]
24	Trionychidae	Max,[D,Sq],[Bo,Par],[Bs,F,Pal,Pt],J,Pm,V,Ex,So	[D,Max,Sq],Par,[Bs,J,Pal,Pm,Pt],F,[Bo,Ex,V],So	Sq,[D,Max],Par,[F,Pal,Pt],[J,N,Pm],[Bs,Ex,V],[Bo,So]
25	Durocryptodira	Max,D,J,[Par,Pm,Sq,V],[Pal,Pt],F,Ex,Bs,So	[D,Max,Sq],[Par,V],[J,Pal,Pm,Pt],[Ex,F],[Bo,Bs,So]	Max,[D,Pt],Sq,V,[F,J,Pal,Par,Pm],Bs,[Bo,So],N
26	Chelonioidea	Max,D,[Par,Sq,V],[Pm,Pt],[F,J],Pal,Bs,[Bo,Ex],So	[D,J,Max,Pt],[Par,Sq,V],Pm,[Bs,F,Pal],Ex,[Bo,So]	Max,[D,Pt,V],Sq,Pm,[F,J,Pal,Par],[Bs,Ex],[Bo,So],N
27	Cheloniidae	[D,Max],J,[Par,Sq],[Pal,Pm,V],F,[Bs,Pt],Bo,Ex,So	[D,J,Max,Pt],[Pm,V],[Par,Sq],Pal,[Bs,F],Bo,Ex,So	Max,[D,J,Par,Pt],[F,Sq],[Pal,Pm],[Bs,V],[Bo,Ex],N,So
28	Chelydridae	Max,[D,Sq],[F,Par,Pt,V],Pm,[J,Pal],[Bs,Ex],[Bo,So]	Max,[D,Pt,Sq,V],[N,Par,Pm],[F,J,Pal],[Bs,Ex],[Bo,So]	Max,[D,Pt,Sq,V],Pm,[F,J,Pal,Par],[Bs,Ex],[Bo,So],N
29	SCENARIOS	D,[J,Max,Pt],[Bo,Pm],Pal,[N,Sq],F,Par,Ex,[Bs,So],V	[D,Max,N],[Par,Sq],[Bs,Pal,Pm,Pt],F,J,Ex,Bo,V,So	[Bs,D],J,Ex,[F,Max,Pm],Par,[N,Pt],Sq,[Bo,V],Pal,So
30	Archosauria	[D,Max],[J,Pal,Pt],Pm,F,[Par,Sq],N,Ex,Bs,[Bo,So],V	[D,Max],[Bs,N,Pal,Pm,Pt,Sq],F,J,[Ex,Par],Bo,V,So	D,Ex,Pal,[Bo,J,Pm,Pt],Max,[N,Sq],F,Par,Bs,V,So
31	Aves	D,[Max,Pal,Pt],Pm,J,[N,Sq],[Ex,F],Par,Bs,Bo,[So,V]	[D,Max],[Ex,Pal,Pt,Sq],N,F,[J,Pm],Par,[Bo,Bs],V,So	Ex,[D,Sq],[Pal,Pt],[J,Pm],[Bo,Max],N,[Bs,F],Par,V,So

32	Neognathae	Sq,[Max,Pal,Pt],[D,Pm],[J,N],[Ex,F],Par,Bs,So,Bo,V	[Max,Sq],[D,Ex,Pal,Pt],N,[J,Pm],Par,F,[Bo,Bs,So],V	Ex,Sq,[Pal,Pt],[D,J,Max],N,F,[Par,Pm],Bs,[Bo,V],So
33	Anseriformes	Sq,Max,[N,Pal,Pt],[D,J,Pm],F,Par,V,Bs,[Ex,So],Bo	Sq,[Max,N,Pal,Pt],[D,J,Pm],[Par,V],F,[Bo,Bs,Ex,So]	Ex,Sq,[Max,N,Pal,Pt],[D,J,Pm],F,[Par,V],Bs,[Bo,So]
34	Galliformes	Sq,[Pal,Pt],[D,J,Max],Pm,N,Ex,F,Par,Bs,So,Bo,V	[D,J,Max,Pal,Sq],Ex,[N,Pt],[F,Pm],[Bs,Par],[Bo,So],V	Sq,[J,Pal,Pt],[D,Max],Pm,N,F,[Bs,Par],Ex,V,So,Bo
35	Gallus+Coturnix	[D,J,Max,Pt],Ex,Pm,[N,Pal,Sq],F,Bo,[Bs,Par],So,V	[D,J,Max,Pal,Pt,Sq],Ex,[F,N,Pm],[Bs,Par],[Bo,So],V	Sq,[D,J,Max,Pal,Pt],Ex,N,[F,Pm],Par,[Bo,Bs],V,So
36	Lepidosauria	[D,Max,Pt],[J,Pal],[N,Pm,Sq],[Bo,F,Par],[Bs,Ex,So,V]	[D,J,Max,N,Pal,Pt],[Ex,F,Par,Pm,Sq],Bs,[Bo,V],So	[Bs,Pt],[D,Ex],[J,Pal],[F,Max,Par,Pm,V],[N,Sq],[Bo,So]
37	Squamata	Pm,J,[D,Max,Pt],[F,Pal],[N,Par,Sq],[Bs,Ex],Bo,V,So	[D,F,J,Max,Pal,Pm,Pt,V],[N,Par,Sq],[Bs,Ex],Bo,So	Pt,J,[D,F,Pal,Par],[Max,Pm,V],[Bs,N],[Bo,Ex,Sq],So
38	Iguania+Anguimorpha	Pt,J,D,[F,Pal,Par,Pm],Max,N,Sq,Bs,[Bo,Ex],V,So	Pt,[D,F,J,Max,Pal,Pm,V],[Ex,Par,Sq],N,Bs,Bo,So	Pt,[J,Pal],Par,[D,F,Max,Pm,V],N,[Bo,Ex,Sq],[Bs,So]

Table 13. Heterochronies reconstructed using PGi and the dataset with missing data. Internode numbers match to Figures 5A-C. Bones across a row in red indicate a heterochrony that reconstructs in all scenarios, bones across a row in bold indicate a heterochrony that reconstructs in two of the three scenarios.

		Scenario 1 (T+D)		Scenario 2 (T+A)		Scenario 3 (T+L)	
Internode #	Internode Name	Early	Late	Early	Late	Early	Late
1	Mammalia		J,Pt		J,N,Pal,Par	Bs	Ex,N
2	Monotremata+Eutheria		Pt	D,J,Pt	Pm		Pm,Pt
3	*Caluromys*			Pm	Pal	F,Par,Pm	
4	Eutheria		J	Bo,N,Pt,Sq			Bo,Ex
5	*Dasypus*		Ex		D,Max,Pt		D,Max
6	Eutheria-Dasypus			Pm,Sq		Ex,F	
7	*Loxodonta*	Pt		Ex	Sq	Ex,Pm,Pt	F,J,N
8	Laurasia.+Euarch.		Pm	Bo	Pt	Bo	Sq
9	Laurasiatheria		F,Pal	F,J,Pt	Par		Bo,N,Par,Pt
10	Soricidae+Talpidae		Sq		J,N,Sq	Pm	J,N
11	Soricidae			N,V	Pt	Bo	F,J
12	*Chimarrogale+Cryptotis*	Pt		Bs	Bo,Ex,F,Sq	N,V	Bo,Pm,So
13	*Chimmarrogale*	Ex,N,Sq,So	F,Max	Ex,N,Par,Pt,Sq,So	Bs,Max,Pm	Ex	F,Max,N,Pal,Par,V
14	*Cryptotis*		J		Bs,J	Pm,Pt	
15	*Suncus*			Par,So	V	Ex,Par	Pal
16	Talpidae			Pal,Par,V		Bs,N,Par	
17	Talpinae		N	Sq		Pal	Pm
18	*Talpa+Mogera*	So	F,Pal	N,So	Pt		F
19	*Talpa*	Ex,Pal		Bo,Ex,N,So		Bo,Ex,Pm,So,V	
20	*Mogera*	Pt	So	Pt	F,Pal,V	Pm,Pt,Sq,V	Pal
21	*Urotrichus*		N			Pm,Pt,V	N,So

22	*Scapanus*	So		F,N,So	Bo	Ex,N,Pal,Pm,Pt,Sq,V	Bs
23	*Rousettus*		Max	Par	Bo,Max,Pm,Pt	Bo,D,Ex,Par,Sq	
24	Euarchontoglires			V	N	Bs	
25	Rodentia			F		Pm	
26	Cricetidae + Muridae		Max,N	Pt	Sq	Pt	J,N
27	Cricetidae	Max	F	Bs	F	Bs,So	Bo,F,Pt
28	*Mesocricetus*	D	Bo	Bs,D,Ex	Bo,J	Bs,D,Ex,Sq,V	
29	*Peromyscus*		Sq		Sq	Bo,Bs,Pt	N
30	Muridae	N		N	Max,So	Ex	J,Max,Par
31	*Meriones*	Bs	Pal	Ex	Pal,Par,Sq	N,Pt	Sq
32	*Mus + Rhabdomys*	Par,Pt	Pm	Ex,Max,V	J,Pm	J,Par	Bo,Pm
33	*Mus*	J,Max,Sq	Pal,Par,Pt	J	Pm	Max,Sq,V	Bo,Par
34	*Rhabdomys*			Bo,Pal,Par,Pt	Max,Sq	Pal,Pm,Pt	J
35	*Cavia*			N,Pal,Par		Pal,Par,Sq	
36	*Loris*			So	Pm,Sq	So,V	D,Ex,J,Max
37	*Ornithorhynchus*	V	Bo,D,J,Max	V	Bo,Ex,Max	Sq	Bo,J,N
38	Reptilia			Bs,D,Sq**,**V		F	Pal,Sq**,**So
39	Testudines	Par	**J,Pm,**So	F,J,Sq,So		Pal	Bs,D,Ex,F,**J,**N,**Pm**
40	Pleurodira			Ex,Pal,Pt	**Par,**So	Bo	**Par,**Pt,Sq
41	*Emydura*	Max,Pm,**So**		**So**	Bs,Ex,**N,**Pal,Pt,Sq	Bo,Par,Pm,**So**	**N**
42	*Phrynops*	N,**V**		F,J,**V**		**V**	D,Max
43	Cryptodira	Max,Par	N	**V**		Pm,**V**	
44	Trionychidae	Bs,N,**Sq**,V	Bo	Bo		F,Pal,**Sq**	V
45	*Pelodiscus*	D,Par	**F**	V	Pm,**Sq**	Bo,Bs,J,V	**F,Sq**
46	*Apalone*		**Bs**	F,Pal,Pt	**Bs**,D	Bo,Max	Par
47	Durocryptodira	Pm,**V**		**V**	Bs		D,N,Par
48	*Emys*				**D,**Par		Bs,**D,**Max,Pt
49	Chelonioidea			Bs,J,Pt	Sq	Pm,V	

50	Cheloniidae	**J,Pal**	Pt	Bo,**Pal,**Pm,V		F,**J,Pal,**Par	So,V
51	*Lepidochelys*		**D,J**	F	**D,J,**Pt	Pm,V	Ex,Par,Pt
52	*Eretmochelys*	**Ex,**Pt		**Ex,**Pal,Par,**Sq**		Pal,**Sq**	F,Max
53	Chelydridae				Bs,J,Pt	Sq	
54	*Macrochelys*	**Bs**		**Bs**	D,V	**Bs**	V
55	*Chelydra*		**Max**	**F,Pal,Par,Pm**	D,**Max**	**F,Pal,Par,Pm**	**Max**
56	Hypotheses 1, 2, or 3		**Bo**	**Bs**,Pt	J	**Bs**,F	**Bo**,Ex,N,Pm
57	Archosauria	Bs	Bo		N,Par	Pal,Pt,Sq	
58	Aves	**N**	J	Ex	Bs,**N**,Pm	Sq	
59	Neognathae	**Sq**		**Sq**,So	F		Bo,Bs,D,Pm
60	Anseriformes	**V**	**Ex**	**V**	D,**Ex**,Max	Max,N,Pm,**V**	
61	*Cairina*	**N,V**		Bs,Max,**N**	Par	Max,**N,V**	Ex
62	*Anas*		Bs,F		Bo,V	Par,So	Ex
63	Galliformes			Bs,F,**J**,Pal		D,**J**,Pm	Ex
64	*Coturnix + Gallus*	**Bo**	Bs	Pt		**Bo**,Ex,Max	Pm
65	*Coturnix*	**Bs**,N	**So**	**Bo,N,Pm**	**So**	**Bo,Bs,N,Pm**	Sq
66	*Gallus*	**Sq**	**Bo,Ex**	**Sq**	**Bs,Ex**	So	**Ex**
67	*Meleagris*		**Ex**	Pal,Pm,Pt,Sq	Bo,Bs,**Ex**		J
68	*Dromaius*	**Bs,V**	**Max**	**Bs,V**	**Ex**,F,**Max**,N	**V**	Bo,**Ex**,Sq
69	*Caiman*	**V**	**F**	Bo,**J**,Par,So,**V**		Bs,**J**,Max,N,Pm,**V**	Bo,Ex,**F**,Par
70	Lepidosauria	Pm	**Bo,D**	Bs,F,**Pal,Pt**	Par	**Pal**,Par,**Pt**,V	**Bo,D**
71	*Sphenodon*	N,**V**	**Bs,J,**Pm	**Sq,V**	Bo,**Bs**,Ex,**J**	D,Max,N,Pal,**Sq**	**Bs**,Ex,**J**
72	Squamata	**F**,J	So	**F**,Pm,V	**Ex**,N	Bo,Par	Bs,**Ex**
73	Iguania + Anguimorpha	**Pt**	Pm	Ex,**Pt**	N		
74	*Polychrus*	**Ex**			F,Max,Pm	Bs,D,**Ex**,Sq	Par
75	*Elgaria*		**Bs,N**	Bo,J,Pal,Par	**Bs**		**N**
76	*Lacerta*			Bo		Bs,Sq	J,Par,Pt

Appendix 1. Stages of the 53 specimens of *Lepidochelys olivacea* used to infer the ossification sequence for cranial bones. Specimens were obtained from the Carnegie Museum of Natural History. Staging was based on Miller (1985) with reference to Crastz (1982).

Stage	CMNH Specimen Numbers
20	108800
20/21	108901A
22	108901B
23	108832, 108854A
Late 23	108943A
Early 24	108943B
24	108854B, 108859
Late 24	108942
Early 25	108860
25	108831, 108893, 108919
Early 26	108876
26	108855, 108875, 108877, 108878, 108879, 108952
Early 27	108844, 108880
27	108792, 108794, 108795, 108864, 108881, 108926, 108950
Late 27	108872, 108930, 108939
28	108809, 108810, 108830, 108975
Late 28	108850, 108923, 108924, 108936
29	108803, 108851, 108886, 108912, 108970
30	108903, 108905, 108962
31	108838, 108921, 108954, 108963