Histological insights into trait acquisition in non-mammalian synapsids

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Abstract

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The synapsid stem lineage is classically known to document a step-wise pattern of trait acquisition however, evidence of homoplasy in synapsids is common and increased scrutiny within synapsid clades and less readily apparent microanatomical characters may not follow classic step-wise patterns. This dissertation examines a broad taxonomic sampling of synapsid fossils with special attention to the histological details of their dentitions to test for patterns of trait acquisition.

In the first chapter, we describe the peculiar dentition of tapinocephalids, an early herbivorous group of synapsids. Tapinocephalids were the first synapsids to acquire mammal-like specializations such as precise tooth-to-tooth occlusion and a periodontal ligament while retaining ancestral features like alternating and rapid tooth replacement and wavy prismless enamel. The combination of traits that make up the tapinocephalid dentition are not represented in modern amniotes and appear to have been specialized to herbivory.

In the second chapter, I compare seasonal physiological patterns captured by the growth marks in the dentine of *Lystrosaurus* from South Africa and Antarctica. While South African *Lystrosaurus* dentine shows evidence of severe and punctuated physiological stress, Antarctic specimens reveal a more constant, but less extreme amount of stress. This may reflect different responses to seasonality where *Lystrosaurus* living in South Africa experienced intense periods of drought while those in Antarctica experienced prolonged periods of darkness due to its high latitudinal position. These differences, although significant, are relatively minor suggesting a level of flexibility in *Lystrosaurus* physiology that would be expected from endothermic metabolisms.

In the third chapter, we test for taphonomic proxies for tooth attachment in the proportion of empty tooth sockets in fossil synapsid jaws. We surveyed non-occluding jaws of most major clades and families of synapsids for the proportion of teeth retained in the jaw and found tooth attachment and replacement poorly explained proportion of teeth missing from a fossil jaw while enamel and diet were better predictors. We suggest function of the teeth may be influencing taphonomic preservation. We also discuss the significance of a lack of reliable correlations between traits and patterns of trait evolution as it pertains to the notion of acquiring “mammalness”.

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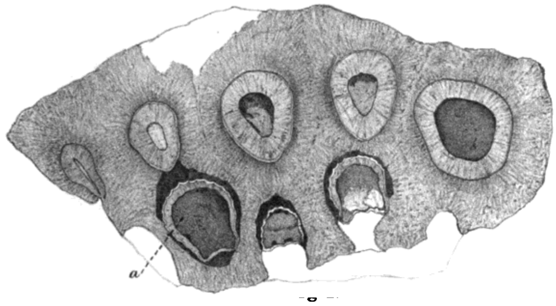
DEDICATION

"With regard to the Teeth, some of the most interesting and extraordinary modifications were peculiar in species that have long since passed away from the stage of animated existence; and, indeed, no comprehensive view could be obtained of the dental tissues without a knowledge of those intermediate conditions which they present in fossil teeth."
-Owen 1845



-Owen 1876

-BMNH 2018

My undergraduate advisor, Dr. Kristi Curry Rogers, was the first to show me the possibilities of fossil tissues in studying the biology of extinct animals. Without her support, guidance, and enthusiasm this dissertation would never have happened. I dedicate this body of work to Kristi, the reason I became a paleontologist.

Chapter 1. INTRODUCTION

Morphology is an essential data set for assessing evolutionary patterns in vertebrate history. The vertebrate form contributes to reconstructions of shared ancestry and divergence, can reveal instances of interesting convergent evolution, and responses to selective pressures acting on species. Importantly, morphology is a data set that facilitates comparisons between modern and extinct vertebrates since behavioral and genomic data is, more often than not, missing from fossils. The morphology of extant amniotes has historically given rise to a dichotomy between reptiles and mammals. Despite the fact that living species represent a fraction of time in the nearly 300 million year evolution of their respective lineages, this apparent dichotomy has often colored how fossil amniotes are interpreted. For instance, this dichotomy stereotypes mammals as having acquired a suite of novel traits throughout their evolutionary history whereas reptiles retained plesiomorphic traits, a narrative is particularly prevalent in discussions of growth and dentition.

Fossil synapsids provide a unique test of the dichotomy between extant reptiles and mammals and have played an important role in establishing this dichotomy. Nonmammalian synapsids have long served as an exceptional record of macroevolutionary processes, large-scale trends, and rates of morphological evolution (Olson, 1959; Hopson, 1969; Crompton and Jenkins, 1973; Sidor and Hopson, 1998; Rubidge and Sidor, 2001). The step-wise pattern of trait acquisition ‘away’ from plesiomorphic ‘reptile-like’ characters and ‘towards’ derived ‘mammal-like’ features, has long characterized interpretations of the synapsid lineage. This pattern has been detailed in studies of the middle ear, secondary palate, and limb posture (Hopson, 1950; Crompton and Jenkins, 1973; Rubidge and Sidor, 2001; Sidor, 2003). However, evidence of homoplasy in synapsids is common. Recent work has detailed instances of convergence along the synapsid line in features like protocones and pseudo-protocones (Luo 2007), the middle ear (Luo 2007), phalangeal formula (Hopson 1995), skull simplification (Sidor 2001), and in a permanent gomphosis (LeBlanc et al. 2016; 2018). While many of the macroanatomical traits along the spine of the synapsid lineage detail a step-wise transition, increased scrutiny within synapsid clades and less readily apparent microanatomical characters may not follow similar patterns (e.g. Sidor 2001).

The acquisition of the mammalian dentition serves as an exemplar system to study trends in synapsid evolution for two reasons. First, the remarkable diversity of extant mammals is often attributed to the complexity and specializations of their dentition (Crompton and Jenkins 1968; Crompton and Parker 1978; Hopson 1969; Luo et al. 2004, 2015; Luo 2007; Schultz and Martin 2014; Grossnickle 2017). The combination of traits that gave rise to mammalian dentition has historically been considered the most derived and specialized among vertebrate taxa (e.g. Seeley, 1888; Smith 1958; Gaengler and Metzler, 1992) and include single replacement (diphyodonty), stereotyped tooth-to-tooth occlusion for oral food processing, crown morphology specialization along the tooth row (heterodonty), prismatic enamel thought to resist crack propagation, and a soft tissue attachment to the jaw (gomphosis). The latter, however, also occurs in modern crocodylians (Poole 1961; Soule 1967; Berkovitz and Sloan 1979) and some non-mammalian synapsids (LeBlanc et al. 2018). The suite of derived features that comprise the mammalian dentition work in concert to function as an efficient and durable mechanical processing system for food, which is often tied to the origins of mammalian growth strategies and other characters like lactation and parental care (e.g. Kemp 1982; Luo et al. 2004; Hopson 2012). The pattern by which many of these features were acquired remain poorly understood and the synapsid fossil record archives details that can contribute to understanding patterns in the acquisition of the mammalian dentition.

Second, the dentition is a system is ideal for studying trends in evolution. Dental traits, individually and in concert, are under intense selective pressure due to the importance of feeding as a basic need. Dental traits have developmental plasticity that provides flexibility in form and function and are less fixed than other components of the vertebrate Bauplan (Kangas et al. 2004; Kavanagh et al. 2007). Dentitions are more strongly tied to diet in both capture and processing of food (Evans et al. 2007) and because of this, it is less likely that the morphology or acquisition of traits are the result of phylogenetic inertia and contain important functional information. Furthermore, the dentition is formed of tissues that are readily incorporated into the fossil record and preserve functional and life history information, sometimes at daily intervals. Teeth are generally composed of an outer capping hypermineralized enamel layer and inner softer dentine layer. Teeth are anchored to the jaw in a variety of geometries and via either a hard tissue attachment or soft tissue attachment. Furthermore, hard tissues act as a story book, depositing material appositionally such that somatic information about the individual is captured in chronological order. These tissues capture a variety of characters that have a variety of phenotypes for each of those characters. Given the functional, physiological and phenotypic variability in dentitions, the system serves as a critical way to study the biology of fossils as well as macroevolutionary trends and transitions.

Here, the dentition is used to study the acquisition of adaptations in both the physiology and dentition of non-mammalian synapsids as well as to comment on how trait linkage and combinations of traits within the dentition can inform evolutionary patterns along the synapsid line. In Chapter 1, I examine the peculiar dentition of early herbivorous synapsids, the tapinocephalids. I detail the unique combination of 'mammal-like' and 'reptile-like' features found in their dentition and describe this suite of features as adapted for herbivory rather than necessarily trending towards 'mammalness'. In Chapter 2, I pivot to examine adaptations in the physiology of Early Triassic *Lystrosaurus* from Antarctica. Using tusk histology, this chapter describes possible seasonal influences in the metabolism of these synapsids and potential implications in the origins of endothermy. Finally, in Chapter 3, I explore possible proxies for tooth attachment, correlations between dental features and macroevolutionary trends in synapsid dentitions and comment on how these results can be interpreted in the evolution of 'mammalness'.

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Chapter 2. HISTOLOGICAL AND DEVELOPMENTAL INSIGHTS INTO THE HERBIVOROUS DENTITION OF TAPINOCEPHALID THERAPSIDS

2.1 ABSTRACT

Tapinocephalids were one of the earliest therapsid clades to evolve herbivory. In acquiring derived tooth-to-tooth occlusion by means of an exaggerated heel and talon crown morphology, members of this family have long been considered herbivorous, yet little work has been done to describe their dentition. Given the early occurrence of this clade and their acquisition of a dentition with several derived features, tapinocephalids serve as an important clade in understanding adaptations to herbivory as well as macroevolutionary patterns of dental trait acquisition. Here we describe the histology of tapinocephalid jaws and incisors to assess adaptations to herbivory. Our results yield new dental characters for tapinocephalids including a peculiar enamel structure and reduced enamel deposition on the occlusal surface. These traits are convergent with other specialized herbivorous dentitions like those found in ornithischian dinosaurs and ungulates. Furthermore, these results demonstrate that while acquiring some specializations, tapinocephalids also retained plesiomorphic traits like alternate, continuous replacement. We interpret these findings as an example of how different combinations of traits can facilitate a derived and specialized dentition and then discuss their implications in the acquisition of a mammal-like dentition.

2.2 INTRODUCTION

Vertebrate herbivory is an integral feature of modern terrestrial ecosystems, evolving in sauropsid (reptile line), synapsid (mammal line), and early amniote lineages multiple times independently since its origin more than 300 million years ago in the late Carboniferous [1-3]. It was not until the late Permian (260 Ma), however, that herbivory became common among communities of terrestrial tetrapods [4]. Insects likely constituted most of the primary consumer trophic level during early to middle Permian times, although tetrapod herbivores were diversifying throughout that period [1-2, 5]. In addition to transitions on the trophic landscape, the middle Permian saw a synapsid faunal turnover resulting in a shift from a pelycosaur-dominated landscape to one dominated by therapsids [1, 5, 6] (Fig 1). This turnover was followed by diversification of therapsids, including several herbivorous clades and genera [1, 7]. These coinciding transitions give the middle Permian a unique set of trophic conditions in the history of vertebrate life on earth (Fig 1).

Synapsid herbivory in the early Permian was restricted to two clades of pelycosaur-grade synapsids, edaphosaurids and caseids (Fig 2.1), both of which show evidence of herbivory in their jaw musculature, barrel-shaped rib cage, and dentitions [4]. Their dentitions are similar to contemporaneous herbivorous sauropsids (i.e. captorhinids) with cutting or shearing crown morphologies and abundant palatal teeth [4]. By the middle Permian when therapsid synapsids began to dominate the landscape, edaphosaurids had gone extinct and only a single species of herbivorous caseid persisted [8]. The two clades with herbivorous therapsids during the middle Permian were the dinocephalians and anomodonts, both of which evolved dental specializations that differ from earlier occurring herbivorous amniotes.

Anomodonts have been recognized for their extremely derived dentitions and early instances of tooth-to-tooth occlusion, both of which have been related to efficient herbivorous processing [9-12]. Dicynodont anomodonts acquired a particularly derived dentition with a keratinized beak and often paired maxillary tusks that have been hypothesized for a variety of functions including foraging [13-15]. While anomodont adaptations to herbivory have been well documented [12-13, 16-17], dinocephalians have been comparatively overlooked by previous surveys of vertebrate herbivory during the Permian. Here we describe tapinocephalid dentition with special focus on microanatomical features that shed light on specializations acquired by these middle Permian therapsids.

2.2.1 *Background on tapinocephalids*

Dinocephalian systematics has received relatively little attention in comparison to other therapsid clades like anomodonts, therocephalians, and non-mammalian cynodonts, but two major subgroups were recognized early on by Hopson and Barghusen [18]. Anteosaurs were large-bodied carnivorous dinocephalians known from southern Africa, China, Russia, and Brazil and recently Kammerer [19] has developed a robust phylogenetic framework for the group including two major subfamilies and nine genera. The other major dinocephalian subgroup is the herbivorous Tapinocephalia. Rubidge and van den Heever [20] described four tapinocephalian families including estemmenosuchids, stryacocephalids, titanosuchids, and tapinocephalids. The Russian estemmenosuchids and South African stryacocephalids are each known from one genus and their relative placement within Dinocephalia has been of historical debate [11, 21]. By contrast, titanosuchids, and to a greater degree, tapinocephalids contain several named taxa. Most tapinocephalian families have the plesiomorphic condition seen in anteosaurs and other therapsids more broadly, where an enlarged canine separates the jaw into incisal and postcanine regions [22]. The herbivorous dentitions of most tapinocephalians include incisors with a heel, pronounced canines, and bulbous (in Stryacocephalidae) or leaf-shaped (as in Estemmenosuchidae and Titanosuchidae) postcanines [20, 23-24].

The noted exception to this generalized pattern is the most taxonomically diverse group of tapinocephalians, the tapinocephalids [23]. Tapinocephalids were a globally distributed, middle Permian group, with fossils known from Brazil, Russia, and southern Africa [7] and in contrast to other tapinocephalians, tapinocephalids are secondarily homodont with variation along the tooth row principally confined to a gradual reduction in crown size distally, towards the back of the jaw (e.g. Fig 2.2A). It is worth noting, however, that two potentially early diverging tapinocephalids, *Riebeeckosaurus* and *Tapinocaninus*, retain canines and *Riebeeckosaurus* possesses leaf-shaped postcanines similar to, albeit more bulbous than, other tapinocephalians [25].

The global distribution and peculiar shape of tapinocephalid teeth have been described in some detail [23, 25-30]. Tapinocephalid crowns retain the tapinocephalian incisor heel and talon morphology (Fig 2.2B) throughout the tooth row which facilitated precise tooth-to-tooth occlusion [23]. Tapinocephalids achieved precise occlusion by developing an elongated labial talon that intermeshes with the opposing and adjacent talons (Fig 2.2C-E) and a labiolingually expanded heel for opposing upper and lower teeth [23] (Fig 2.2G). In addition to their dentition, further evidence of herbivory in the clade includes a large barrel-shaped rib cage [31] and aspects of the jaw adductor musculature that suggest a crushing bite force [32]. These gross anatomical features, however, do not address other possible adaptations to herbivory that can be revealed by microanatomical analyses. For instance, recent histological work has described a permanent ligamentous tooth attachment (i.e. a gomphosis) in tapinocephalids that would have provided shock-absorbing support during occlusion [33].

Given the early phylogenetic and temporal occurrence of herbivory in tapinocephalids and their acquisition of derived, once considered uniquely mammalian features (i.e. a gomphosis and precise tooth-to-tooth occlusion), further examination of their dentition can reveal additional derived characteristics and alternative combinations of traits that would have facilitated efficient oral food processing early in the evolution of synapsid herbivory. In addition to describing and adding details about tapinocephalid dentition, we detail interesting possible convergences with other specialized herbivorous dentitions (i.e. ornithischian dinosaurs and ungulates) and discuss possible implications for the combination of traits tapinocephalids acquired in a broader understanding of the macroevolution of dental characters.

2.3 METHODS

2.3.1 *Institutional Abbreviations*

NHMUK, Natural History Museum, London, United Kingdom; **BP,** Evolutionary Studies Institute (formerly Bernard Price Institute), University of Witwatersrand, Johannesburg, South Africa; **NHCC**, National Heritage Conservation Commission, Lusaka, Zambia; **SAM**, Iziko:South African Museum, Cape Town, South Africa.

2.3.2 *Specimen collection and curation*

All necessary permits were obtained for the described study which complied with all relevant regulations. Excavation, temporary export, and research permits for Zambian fossils were obtained from the NHCC (Lusaka). Locality information is available to qualified researchers. Comparative material was examined with permission of the relevant curators at the

NHMUK, Evolutionary Studies Institute, SAM, the Field Museum of Natural History, and the American Museum of Natural History.

2.3.3 *Specimen selection and identification*

Specimens selected for this study include fragmentary dentaries and isolated teeth from the Madumabisa Mudstone Formation of the Mid-Zambezi Basin of southern Zambia [29]. Isolated teeth included in this study measured between 21-37 mm in labiolingual width and had heels with exaggerated labiolingual extensions (i.e. heel length: mesiodistal width > 1), comparable to those reported from similar localities [29-30]. Isolated teeth that did not show these proportions (i.e. heel length: mesiodistal width < 1) were not used as to avoid the inadvertent inclusion of titanosuchian incisors, which can be morphologically similar to more posterior tapinocephalid teeth. Dentaries were identified as belonging to tapinocephalids by their relatively large size compared to other vertebrate fossils from localities, the lack of pronounced canine alveoli [23], and oftentimes the lack of in situ teeth, which has been a noted feature of this clade (e.g. Fig 2.2A,F) [21, 23, 29].

In addition to these adult or sub-adult specimens, we included a very small partial left dentary, NHCC LB303, that provides data on early ontogeny in tapinocephalid dentition (Fig 2.2A). To our knowledge, this specimen represents the smallest tapinocephalid individual known and thus likely a very young juvenile. The dentary which measures approximately 54 mm long includes the symphysis as well as the first seven tooth positions. We estimate the total jaw length to have been 100-130 mm when complete. NHCC LB303 can be assigned to Tapinocephalidae primarily because of the gradation in tooth socket size moving mesially to distally (i.e. no indication of heterodonty, lack of an enlarged canine tooth position) [23]. Furthermore, the contribution of the splenial to the mandibular symphysis has been described in some titanosuchians [34] which is not apparent in NHCC LB303 where the symphysis is almost entirely composed of the dentary. Unfortunately, this specimen fails to preserve several anatomical features that have noted variation in tapinocephalids throughout ontogeny (e.g. cranial pachyostosis, [35]), which could provide alternative evidence of its ontogenetic stage.

The dentary of NHCC LB303 is edentulous, like most tapinocephalid fossils, but preserves a single nearly completely developed incisor that was close to eruption (Fig 2.2A). Micro-computed tomographic (μCT) scans of the specimen reveal that root formation was incomplete, but most of the crown development, including enamel deposition, was complete. The incisor has a well-developed heel and talon with a notch at the junction between heel and talon which is visible in lateral view (Fig 2.2B). The ratio of heel length: mesiodistal width (0.65) and presence of a notch is most similar to proportions observed in the isolated tapinocephalid teeth from specimen SAM-PK-323 (0.55) and BP/1/7287 (0.52), *Moschops* specimen AMNH 555 (0.53), and *Struthiocephalus* specimen SAM-PK-115911 (0.53). Interestingly, this is in contrast to the vast majority of isolated Zambian tapinocephalid incisors that are consistently at a ratio > 1 (e.g. Fig 2.2G). Due to the lack of material associated with NHCC LB303 as well as a dearth of characters diagnostic to genera within Tapinocephalidae, we are unable to assign this specimen to a genus.

2.3.4 *Microanatomical analysis*

Of the specimens selected for microanatomical analyses, five were thin sectioned, two were scanned under an electron microscope, and one was scanned using μCT (Table 2.1). Histological sectioning was conducted following the established methods described by Lamm [36] and quantitative and qualitative data were collected under a Nikon Eclipse POL100 polarized microscope in plain and cross-polarized light using NIS Elements software. Scanning electron microscopy (SEM) was used to examine enamel microstructure. Epoxy embedded specimens were surface etched with 10% HCl and gold sputter-coated images were collected using JEOL JSM-6610LV and carbon-coated images with JEOL JSM 7000F scanning electron microscopes. μCT images were collected using NSI X5000 scanner at 2K resolution with a 24μm voxel size.

2.3.5 *Frequency and pattern of tooth replacement*

The rate of tooth replacement, although not an exact measure of the number of replacement events through the lifetime of an animal, has been estimated in fossil taxa, mostly dinosaurs (e.g. [37-38]). These studies have employed methods developed by Erickson [39] that examine the difference in daily incremental growth lines between a functional tooth and its developing replacement.

However, given our current sample of tapinocephalid teeth, employing the replacement frequency techniques established by Erickson [39] would be unreliable. The edentulous nature of most tapinocephalid jaws, especially those available for thin sectioning in this study, render the sample such that none of the jaws had a developing tooth and its functional predecessor in the same position. Furthermore, the lack of rigorous ground-truthing for the effects of phylogeny or ontogeny on dentine growth make approximations in synapsids precarious.

To develop an alternative estimate for replacement frequency, we collected data on the proportion of teeth in a jaw at different stages of replacement in 31 tapinocephalid jaws as well as several other non-mammalian synapsids for comparison (92 pelycosaur-grade synapsids, 23 other dinocephalians, 27 gorgonopsians, 20 therocephalians, 23 non-mammalian cynodonts) (Appendix A). Although not a direct measure of replacement rate, these data provide generalizations about the relative rate of replacement in tapinocephalids compared to other fossil synapsids. For each jaw examined, we categorized each tooth position in one of the following four categories: 1) functional tooth present, 2) empty alveolus without preservation of the developing tooth, 3) replacement tooth present in its developing alveolus (i.e. crypt), and 4) replacement tooth in functional position but not fully erupted. All three latter categories were considered replacement events and compared to the total number of tooth positions in the jaw. Smaller, presumably sub-adult and juvenile specimens (e.g. NHCC LB303) were excluded because it is likely that they were replacing their teeth at a more rapid rate than adults, possibly making standardization across various groups of synapsids inconsistent.

2.4 RESULTS

2.4.1 *Dentine*

All sectioned teeth have thick dentine walls with dentine tubules and periodic depositional growth marks of both small-and large-scale increments. Mostly primary dentine was observed, with secondary dentine, classified by direction of dentine tubules, visible in a shed incisor (NHCC LB298; Fig 2.3A), confirming that this tooth had completed root formation and was in occlusion [40]. The amount of dentine deposited between incremental lines varied between 20 and 40μm and averaged about 34μm per depositional period, similar to the 5–10 day cycles seen in mammals [41-42].

2.4.2 *Enamel*

Very little has been published on the enamel of tapinocephalids, although Sidor et al. [29] noted the presence of wrinkled enamel similar what is seen in sauropod dinosaurs. Enamel caps tapinocephalid teeth except for the heel where dentine is exposed (Fig 2.3B), either due to wear or possibly congenitally (see *Development*). Enamel thickness varies along the long axis of the tooth with the thickest enamel observed mid-crown (approximately 0.4–0.59 mm) and tapering towards the cervical (i.e. towards the root) and occlusal ends (approximately 0.3–0.1 mm). There is also a slight difference in enamel thickness between the lingual and labial edges of the heel, with thicker enamel labially (approximately 0.4–0.5 mid-crown) than lingually (approximately 0.1–0.3 mm mid-crown) The enamel shows no evidence of wear on these surfaces, indicating that the variation in thickness between the labial and lingual sides of the teeth is a result of differential deposition. In thin section, regular incremental lines are visible in the enamel with a distance of about 6.5 μm between each line and as many as 60 lines at the thickest point (Fig 3C). We interpret these incremental lines as striae of Retzius, given their similarity to structures seen in other non-mammalian synapsids [43].

SEM imaging revealed a lack of organizational units (i.e. columns or prisms) in the crystallite structures of tapinocephalid enamel (Fig 2.3D-E). Instead, tapinocephalid enamel appears most similar to what has been described as coarse wavy enamel by Sander [44-45], Hwang [46], and Chen et al. [47] with crystallites set at oblique angles relative to the dentine-enamel junction (DEJ) and gradual changes in angles towards the outer enamel surface (OEJ) (Fig 2.3E). Although SEM imagery reveals similar structure to what has been described as wavy enamel, the distinct wavy pattern observed by Hwang [46] and Chen et al. [47] under cross-polarized light does not appear in the tapinocephalid material sampled here. SEM data suggest a relatively uniform crystallite structure throughout tapinocephalid enamel, with the exception of the thick Basal Unit Layer (Fig 2.3D), which is approximately 30 μm thick and made of finer wavy structural units.

2.4.3 *Crown morphology and development*

Thin sections of the jaws of tapinocephalids capture various stages of crown development providing new data on the underlying processes giving rise to the peculiar morphology. Our data suggest that the complex morphology of a heel and talon differs developmentally from mammalian crown formation. In mammals, complex crown shape is initiated prior to mineralized tissue deposition such that the shape of the interface between the inner enamel epithelium and papilla (where deposition will begin) corresponds to the future outline of cusps with multiple enamel knots [48]. Tapinocephalids initiate tissue deposition of both enamel and dentine in a standard bell shape formation contained within a crypt lingual to the functional tooth (Fig 2.4A). None of our thin-sections provide evidence of additional enamel knots developing to form the heel and thus, we propose that this initial bell stage deposition continues cervically to create the talon and subsequently extends lingually and cervically to form the heel (Fig 2.4B). The resorptive crypt of the developing tooth eventually meets the alveolus of the functional tooth during development with the developing tooth moving into its functional position (Fig 2.4C). Sidor et al. [29] and Simon et al. [30] previously noted a semicircular lingual erosion of the roots of isolated tapinocephalid teeth and suggested that this erosion was likely the result of a developing tooth initiating root resorption (e.g. Fig 2.4D). Coronal and sagittal thin sections of developing teeth as well as transverse sections of alveoli confirm this proposal: merging of the functional alveolus and developing crypt happens well before shedding of the functional tooth (Fig 2.4E). This is further evidenced by serial sections taken through a *Tapinocephalus* dentary (SAM-PK-12139) that show merging of the two alveoli and erosion of the functional tooth root (Fig 2.4F-H).

Interestingly, we have been unable to find substantive enamel deposition along the heel of any developing teeth that were μCT scanned or thin-sectioned. For example, the developing incisor of NHCC LB370 was sectioned in a sagittal plane to examine deposition of tissues during crown formation but before eruption and occlusion (Fig 2.5A). On the lingual (occlusal) side of the tip of the talon a poorly preserved, but very thin (<19μm) layer of enamel is apparent. This layer, however, extends cervically only briefly. The rest of the occlusal surface is formed by a thin (approximately 25μm) outer layer that can be differentiated from the dentine under polarized light by a granular band (Fig 2.5C-E). Contrary to the distinctive structure of enamel apparent along the rest of this tooth, this layer most similar to mantle dentine, which forms regularly near the DEJ just deep to where enamel normally forms [40].

2.4.4 *Tooth Attachment*

Boonstra [23] was the first to note that tapinocephalid jaws were edentulous more frequently than those of other dinocephalians, which he postulated was related to differences in tooth attachment and replacement. Sidor [21] echoed these observations and broadened the comparison with other contemporaneous therapsids.

The tissues involved in tooth attachment in fossil vertebrates can be assessed by examining the microstructure of teeth and jawbone [49]. A fossil jaw with an ankylosis will display histological evidence of a connection between the bone forming the alveolus (alveolar bone) along the jaw and the cementum of the tooth root. A fossil jaw with a gomphosis will preserve alveolar bone, cementum lining the root of the tooth, and a periodontal space where the attaching ligaments once occupied. In fossils lacking soft tissue preservation, Sharpey's fibers in both the alveolar bone and cementum are inferred to represent the embedded mineralized ends of fibers of a ligamentous connection between the two hard tissues, congruent with what has been described by LeBlanc et al. [33].

Alveolar bone of NHCC LB369 and NHCC LB370 lines the empty tooth sockets and is characterized by woven bone that is highly vascularized (~58% vascularity) while the surrounding jaw bone is compact fibrolamellar bone with lower vascularization (~32%) (Fig 2.6A). Sharpey's fibers are abundant in the alveolar bone and arranged roughly parallel to the apical-cervical axis of the root (Fig 2.6B-C), although the lack of teeth in situ makes it difficult to determine whether the ligaments acted as a sling or radiated from the alveolar bone.

The cementum of all three of the teeth that were sectioned is generally poorly preserved. The sections of cementum that are preserved have an average thickness of approximately 70μm, however it is likely that the outermost portion of the cementum is incomplete given its outer wavy border. Tapinocephalid cementum has two layers with an acellular layer found adjacent to dentine and a superficial cellular layer with cell lacunae (Fig 2.6E). Visible in the cementum are Sharpey's fibers oriented perpendicular to the long axis of the tooth (Fig 2.6F).

Specimens available for sectioning did not include jaws with functional teeth to directly examine the relationship between the tooth roots, periodontal space, and alveolar bone. Unground serial sections of *Tapinocephalus* (SAM-PK-12139) preserve teeth in situ, and although the details of the tissues are not apparent, some positions preserve clear periodontal space surrounding the roots of functional teeth. Based on this specimen, we approximate the width of the periodontal space to be between 0.6mm and 1.7mm.

2.4.5 *Replacement*

Teeth were actively being replaced in all 31 tapinocephalid jaws examined and comparative data show that tapinocephalids replace proportionally more tooth positions than other groups of therapsids (Fig 2.7). Furthermore, the inferred rapid rate of tooth replacement in tapinocephalids is most similar to that in basal synapsids, including carnivorous sphenacodontids and herbivorous caseids (Fig 2.7).

Although much attention has been paid to understanding patterns of replacement in non-mammalian cynodont therapsids (e.g. [50-52]) dinocephalian patterns of tooth replacement are limited to brief observations of titanosuchians recorded by Boonstra [23]. The adult *Tapinocephalus,* SAM-PK-12139, and the very small tapinocephalid, NHCC LB303, provide some insight into patterns of replacement although interpretations are limited due to the lack of a corresponding side and entire tooth row. These specimens suggest that tapinocephalid replacement was similar to other non-mammalian synapsids in that replacement was alternate and continuous through life (Fig 2.4F-H).

2.5 DISCUSSION

2.5.1 *Specializations to herbivory*

The tapinocephalid specimens included in this study provide evidence of a dentition adapted for the processing of plant material. Histological data including comparatively thick and wavy-like enamel, significantly reduced enamel deposition on the heel, subsequent wear of this surface, and a possibly wide periodontal ligament space, are congruent with previously described gross anatomical features indicative of herbivory.

Herbivorous dentitions must adapt to the wear inflicted on teeth during the mechanical breakdown of plant matter. Alternating and rapid tooth replacement provides a mechanism to combat the wear tapinocephalid teeth encountered, providing a continuous source of unworn surfaces. Alternating pattern and frequent replacement, however, can result in periods of time where positions lack a functional tooth possibly interrupting precise occlusion and thus feeding.

In tapinocephalids, the retention of a functional tooth late into a single position's replacement likely mitigated this problem. Tapinocephalid teeth appear to have remained functional until replacement teeth were nearly erupted, minimizing the amount of time a tooth position was unavailable to assist with food processing. Evidence of this timing is apparent in the second tooth position of SAM-PK-12139, where replacement tooth is nearly entirely formed and in the functional position with the functional tooth not yet ejected (Fig. 2.4F-H). Retention of the functional tooth is likely aided by the lingual development and subsequent labial movement of the replacement tooth. In contrast to replacement that develops in the same position, labial drift of the replacement tooth allows the functional tooth to remain operative longer.

Our findings note a particularly wide periodontal space and confirm the LeBlanc et al. [33] result of a permanent gomphosis in tapinocephalids. Gomphosis would have provided a mechanism to maintain occlusion in a dentition that that was rapidly replacing in addition to cushioning the forces of mastication. Although SAM-PK-12139 may represent an unusually wide periodontal ligament space (especially at its maximum of 1.7mm), this is much larger than the values reported for humans (0.15 to 0.38 mm [42]), crocodylians (0.37 mm [53]), and even animals with comparable body sizes like bovines (0.40 to 0.65 mm [54]). A wide ligament space would, at least in part, help to explain previous observations of tapinocephalid jaws frequently being recovered edentulous.

A possible explanation for the acquisition of a wide ligament may relate to the response of this ligament to force. The periodontal ligament is particularly adept at force perception which allows it to maintain occlusion through either tooth wear or jaw growth [55-56] as well as preventing damage to the tooth through cushioning and avoidance of unfavorable forces. The anchoring periodontal ligament of a gomphosis has been shown to widen with increased stress on teeth and jaws in modern mammals [57]. Thus, the acquisition of a permanent gomphosis in tapinocephalids could have been acquired in a response to occlusion while the maintenance of a wide ligament space could represent a response to the high, repeated forces encountered through increased oral food processing.

Tapinocephalid enamel is strikingly similar to what has been reported for other grinding herbivorous dentitions in terms of its structure, development, and thickness (Table 2.2). A surprising finding of this study is the seemingly congenital lack of enamel on the heels of tapinocephalid incisors. Previous studies have noted this lack of enamel on the heel, but this absence has been interpreted as the result of wear during tooth-to-tooth occlusion (e.g.[29]). Our findings reveal that at least some tapinocephalids developed teeth without enamel on the heel and instead have an acellular tissue resembling mantle dentine capping the occlusal surface. Given the distribution of enamel around the rest of the tooth, it is most likely that the developmental shift resulting in this morphology was an initial formation of enamel-secreting ameloblasts, followed by an eventual termination of mineralization such that enamel did not form into its mineralized state. The lack of mineralization exclusively on the occlusal surface suggests an evolutionary modification akin to that seen in extant grazing mammals, in which ridges formed by enamel surround dentine valleys, thereby predisposing the tooth to wear into complex occlusal surfaces specialized for shearing and grinding [58-60].

A surprising result from these data is the presence of prismless wavy-like enamel in tapinocephalid teeth. Enamel that lacks even columnar structure is unexpected in tapinocephalids given the abundance of this feature in other therapsids. Synapsid columnar enamel (SCE) has been described in a variety of therapsid groups including anteosaur and titanosuchid dinocephalians [61]. Thus, the lack of columnar structure in tapinocephalids is, for the time being, best interpreted as being secondarily lost.

Furthermore, tapinocephalid enamel is potentially a remarkable case of convergence with previous descriptions of wavy enamel restricted to ornithopod dinosaurs [44, 46-47]. The lack of a wavy transmitted light pattern under cross-polarized light suggest caution in assigning this enamel type as wavy, however the crystallite structure does appear similar to previous descriptions of this enamel type and may have evolved under similar functional pressures. It has been proposed that wavy enamel may be an ornithopod specialization to shearing tough plant matter [45, 47], or resistance to crack propagation, analogous to the prisms of mammalian enamel [45]. In tapinocephalids, if wavy-structured enamel is considered in the context of the wide periodontal ligament and crushing heel morphology, this microstructural organization suggests it functioned in the prevention of crack propagation in the enamel. Yet, given the significant lack of enamel on the crushing heel, tapinocephalids were not subjecting their enamel to the repeated crushing forces suggested by Boonstra [64] and commonly used in descriptions [20, 27, 35, 66]. The lack of enamel on the basin of the heel however, does create an enamel ridge surrounding the heel that was likely useful in shearing plant matter. Thus, tapinocephalids may have evolved a specialized enamel to increase the efficiency of their food processing that allowed them to shear plant matter in addition to other adaptations that allowed them to crush their food.

2.5.2 *Combination of traits*

The data discussed here reveal an unexpected array of features in an early therapsid, providing an example of how descriptions of fossil taxa as having either a basal reptile-like or derived mammal-like dentition can be an oversimplification that masks surprising combinations of features. While polyphyodont replacement and prismless enamel are considered plesiomorphic traits, especially relative to mammalian dentitions, when the entire suite of traits comprising tapinocephalid dentition is considered, it is evident that these animals had a derived, alternative combination of features well-suited for herbivorous oral processing. The suite of features that comprise the tapinocephalid dentition are one combination of many that have evolved to enhance oral processing, often in taxa specialized for herbivory (Table 2.2). Individual traits do not operate in isolation and thus, the categorization of teeth as either "simple" or "complex" can be misleading about the specialization or effectiveness of an entire dentition. Furthermore, certain specializations may be more cryptic in fossil taxa (e.g. wavy enamel) and microanatomical analyses provide essential data that cannot be gleaned or can be incorrectly assigned from macroanatomical assessment alone.

2.5.3 *Caveats*

It is worth mentioning that the features discussed in this study are uniformly present within all sampled specimens. That said, we suspect some degree of variation within tapinocephalids likely exists. For instance, the tapinocephalid teeth included in this study have thicker enamel than previously reported for the tapinocephalid *Moschops* [65]. In addition, the present study is limited in two ways. First, the material available for thin-sectioning did not include features diagnosable to the genus-level, which limited our interpretations to Tapinocephalidae without narrower comparisons within the family. Second, the phylogeny of tapinocephalids is poorly resolved [66] and so it is unclear to what degree the features described here show taxonomically important systematic variation. A robust and detailed phylogeny for this clade with special attention to the characters of teeth and jaws would provide an important foundation upon which to make further conclusions about the evolution of dental features within Tapinocephalidae.

2.5.4 *The acquisition of derived dental features*

The acquisition of mammalian features has often been described as a step-wise pattern (e.g. 21, 32), including derived dental features (e.g. 52, 67). Tapinocephalids independently evolved several mammal-like dental traits and can therefore act as a test of the sequence by which mammals acquired a complex dentition. Our results show that tapinocephalids are characterized by frequent, alternating, and continuous replacement as well as prismless enamel, all of which are plesiomorphic. This suite of plesiomorphies suggests that the acquisition of some derived mammal-like features (i.e. precise occlusion and permanent gomphosis) are not necessarily tied to the acquisition of a derived diphyodont replacement or prismatic enamel.

Furthermore, our data on tapinocephalids suggest that the continuous, rapid replacement of teeth may mitigate the selective pressures to evolve greater crack resistance in enamel structure, which has been hypothesized to be a factor in the evolution of prismatic enamel [68]. We suggest that continued sampling of fossil synapsid and sauropsid dentitions could reveal combinations of traits not previously considered, which can continue to test the relationships between traits as well as the sequence in which these traits were acquired in derived dentitions.

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2.8 FIGURES AND TABLES

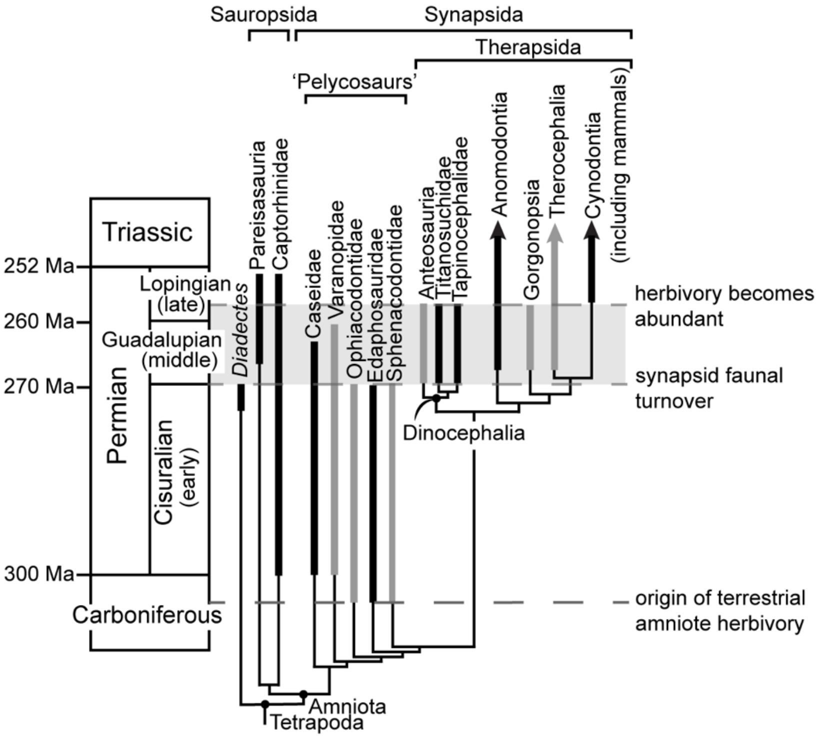

Figure 2.1. **Simplified cladogram of Permian herbivores and major synapsid clades**. The stratigraphic range of each taxon is represented by thick bars, with gray bar color characterizing carnivorous clades and black bar color signifying clades that include herbivorous taxa. The unique combination of trophic conditions (i.e. low abundance of terrestrial tetrapod herbivory and therapsid dominance) during the middle Permian is highlighted in gray.

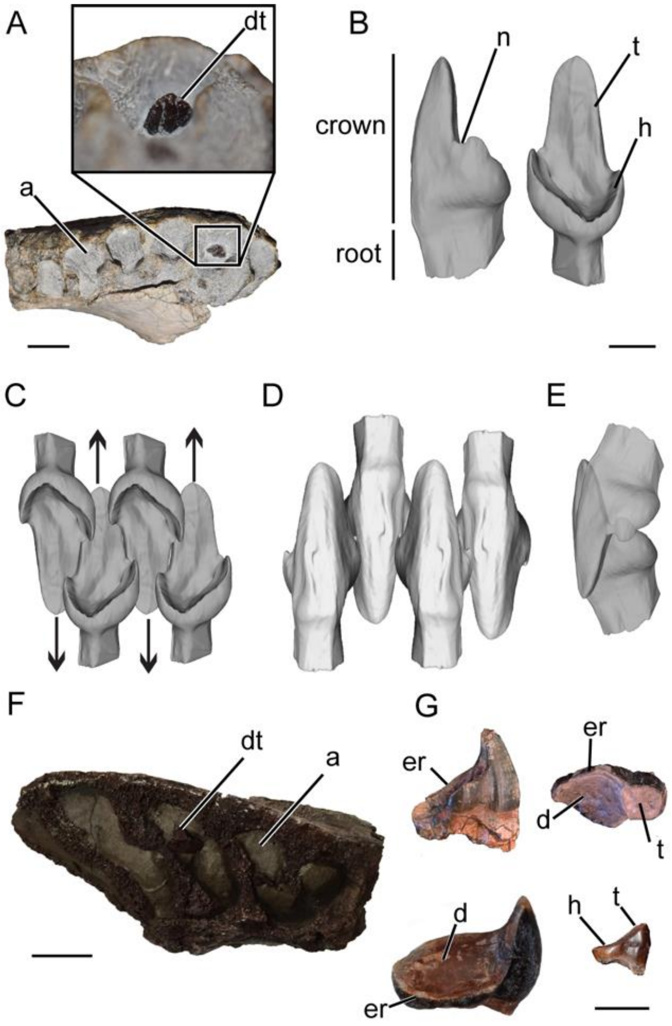

Figure 2.2. **Tapinocephalid occlusion and dental morphology**.

A) Occlusal view of NHCC LB303 the anterior left dentary of a very small juvenile with a developing tooth (inset); scale bar = 1 cm. (B) Segmented μCT scan of the developing tooth of NHCC LB303 in distal (left) and lingual (right) views; scale bar = 0.5 cm. Model of tapinocephalid tooth-to-tooth occlusion using μCT scan and segmented tooth NHCC LB303 in (C) lingual, (D) labial, and (E) side lateral views. (F) Occlusal view of edentulous right anterior dentary of NHCC LB370 that contains a developing tooth. Anterior to left and labial up, scale bar = 3 cm. (G) Examples of Madumabisa Mudstone Formation tapinocephalid teeth with examples of heels comprised of enamel ridges and dentine basins. NHCC LB125 at top left in mesial view (assuming the tooth is from the lower jaw), NHCC LB131 at top right in occlusal view, NHCC LB126 at bottom left in occluso-mesial view to demonstrate wear on heel (assuming the tooth is from the lower jaw), NHCC LB1007 at bottom right in mesial view (assuming the tooth is from the lower jaw); scale bar = 2 cm. Abbreviations: **a**, alveolus; **d**, dentine; **dt**, developing tooth; **er,** enamel ridge; **h**, heel; **n**, notch; **t**, talon.

Table 2.1. Specimens included in this study and analyses performed

Specimen #	Element	Analyses
NHCC LB653	Talon portion of tooth	Histological, SEM
NHCC LB733	Tooth with partial root	Histological, SEM
NHCC LB298	Ejected tooth with resorbed root	Histological
NHCC LB369	Partial edentulous dentary	Histological
NHCC LB303	Anterior left dentary	μCT
NHCC LB370	Right edentulous dentary in two parts:	
	Incisal region with erupting tooth	Histological
	Postcanine region with developing tooth	Histological

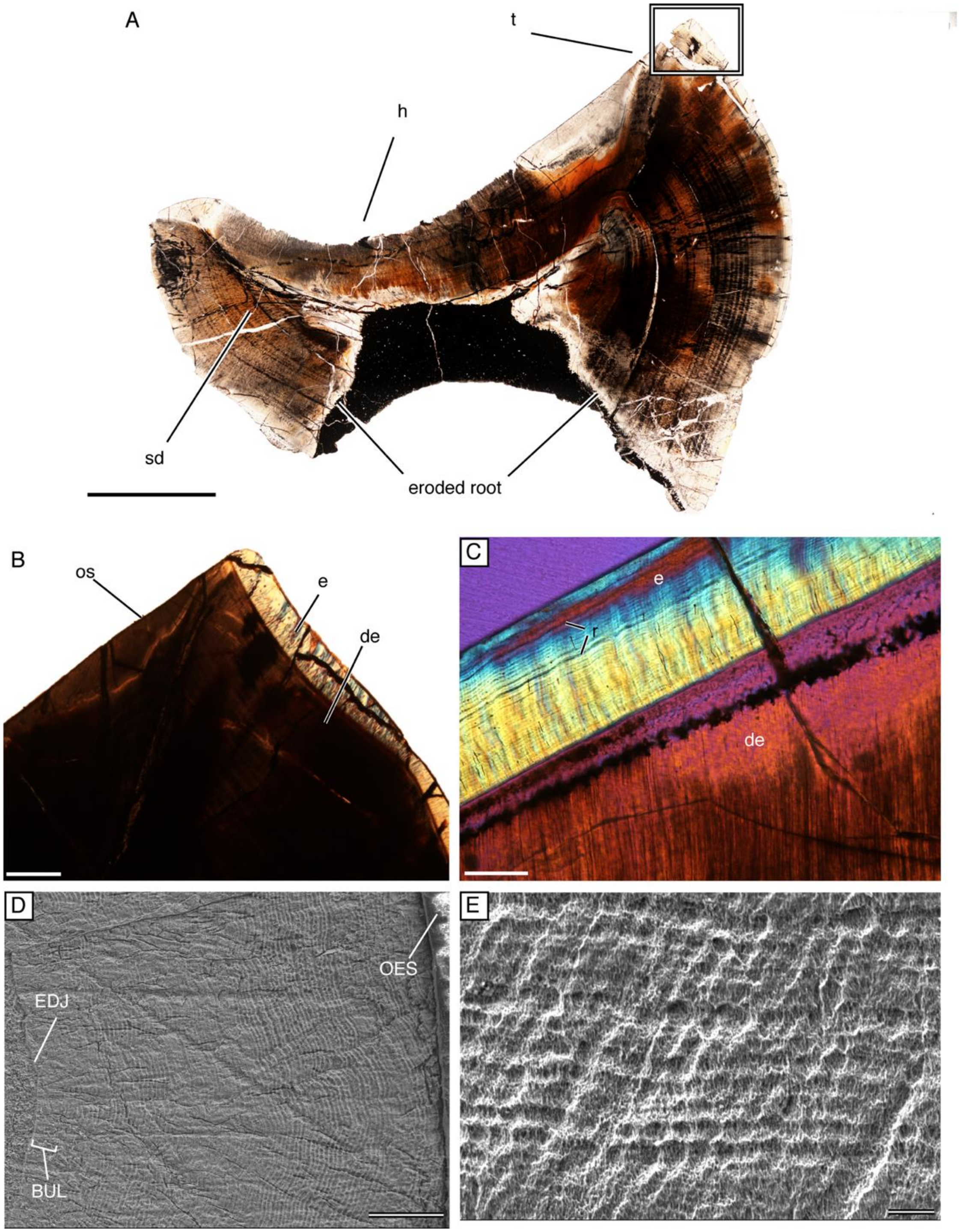

Figure 2.3. **Enamel microstructure of tapinocephalid teeth.**

(A) Longitudinal thin section of NHCC LB298 that was ejected from the jaw as evidenced by the eroded root and secondary dentine. Labial to the right and occlusal to the top of the page; scale = 5000 μm. (B) Histology under cross polarized light of the tip of the talon of NHCC LB298 with thick enamel on the labial edge and no enamel on the lingual, occlusal surface; scale = 1000µm. (C) Under cross polarized light and a lambda plate, a section of NHCC LB298 enamel with incremental lines of Retzius; scale = 100µm. (D) SEM image of NHCC LB653 with relatively uniform prismless, coarse wavy-like enamel from the DEJ to OEJ; scale = 100µm. (E) Close up SEM image of NHCC LB653 with coarse wavy-like enamel in helical arrangement; scale = 10µm. Abbreviations: **BUL**, Basal Unit Layer; **de**, dentine; **e**, enamel; **EDJ**, enamel-dentine junction; **h**, heel; **OES**, outer-enamel surface; **os**, occlusal surface; **r**, striae of Retzius; **sd**, secondary dentine; **t**, talon.

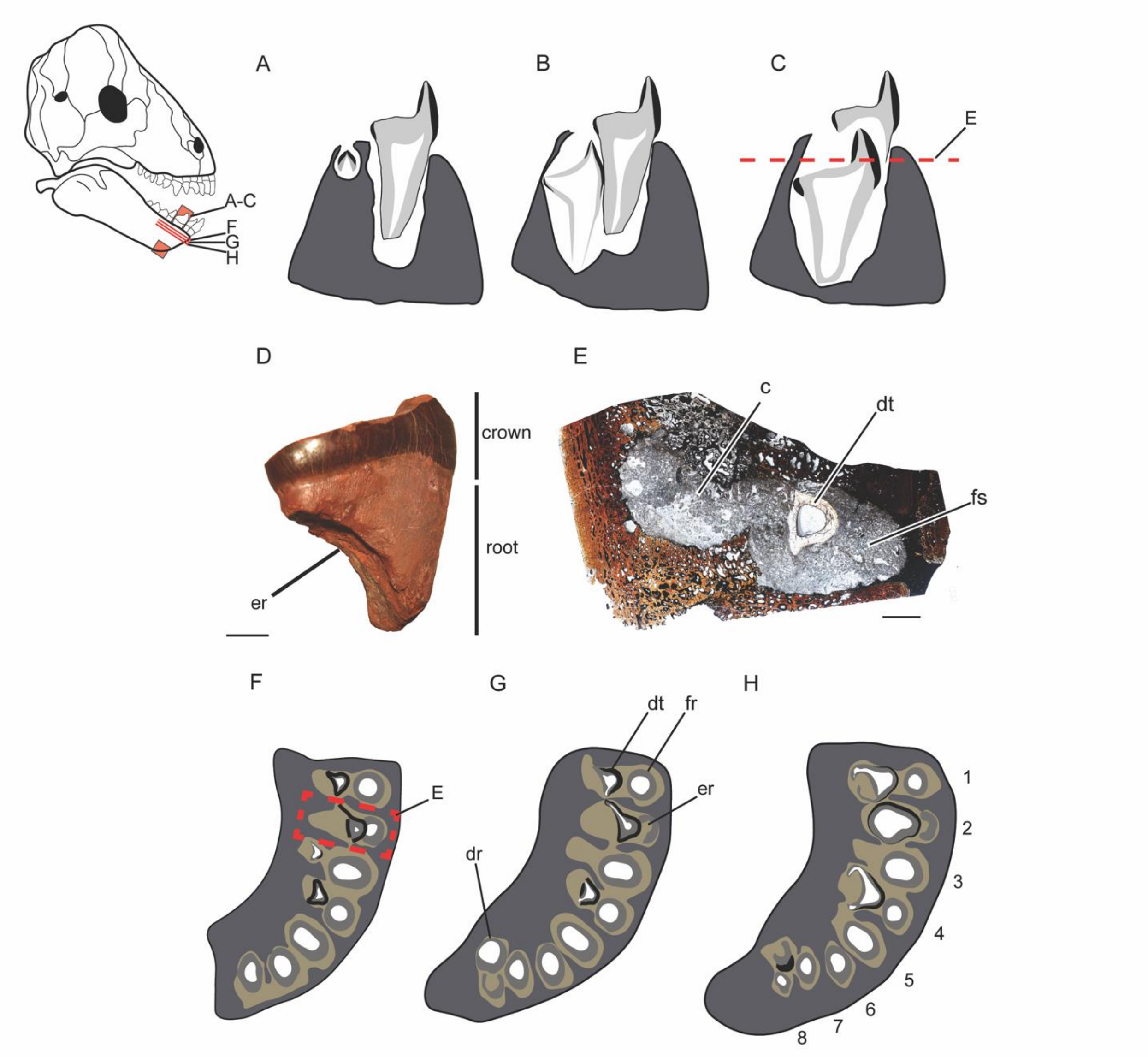

Figure 2.4. **Tapinocephalid tooth development and replacement**.

All images are oriented lingual to the left and for A–D, occlusal is to the top of the page. (A) Bell stage development in a crypt lingual to functional tooth. (B) Crown formation and joining of the crypt and functional socket. (C) Erosion of the functional tooth and movement of the developing tooth into the functional position. (D) NHCC LB827 was in the process of being replaced as evidenced by the lingual erosion of its root. Scale = 1 cm. (E) Histology of NHCC LB369 in horizontal section, with the talon of a developing tooth moving into the functional position. Red box in (F) details approximate location of socket. Scale = 5000μm. (F-H) Occlusal (F) to apical (H) schematics of sections taken of SAM-PK-12139 demonstrating different developmental stages of replacement as well as the alternating replacement pattern. Enamel is thickened black outline and dentine is gray in A–C and F–H. Abbreviations: **c**, crypt; **dr**, developing root; **dt**, developing tooth; **er**, eroded root; **fr**, functional root; **fs**, functional socket.

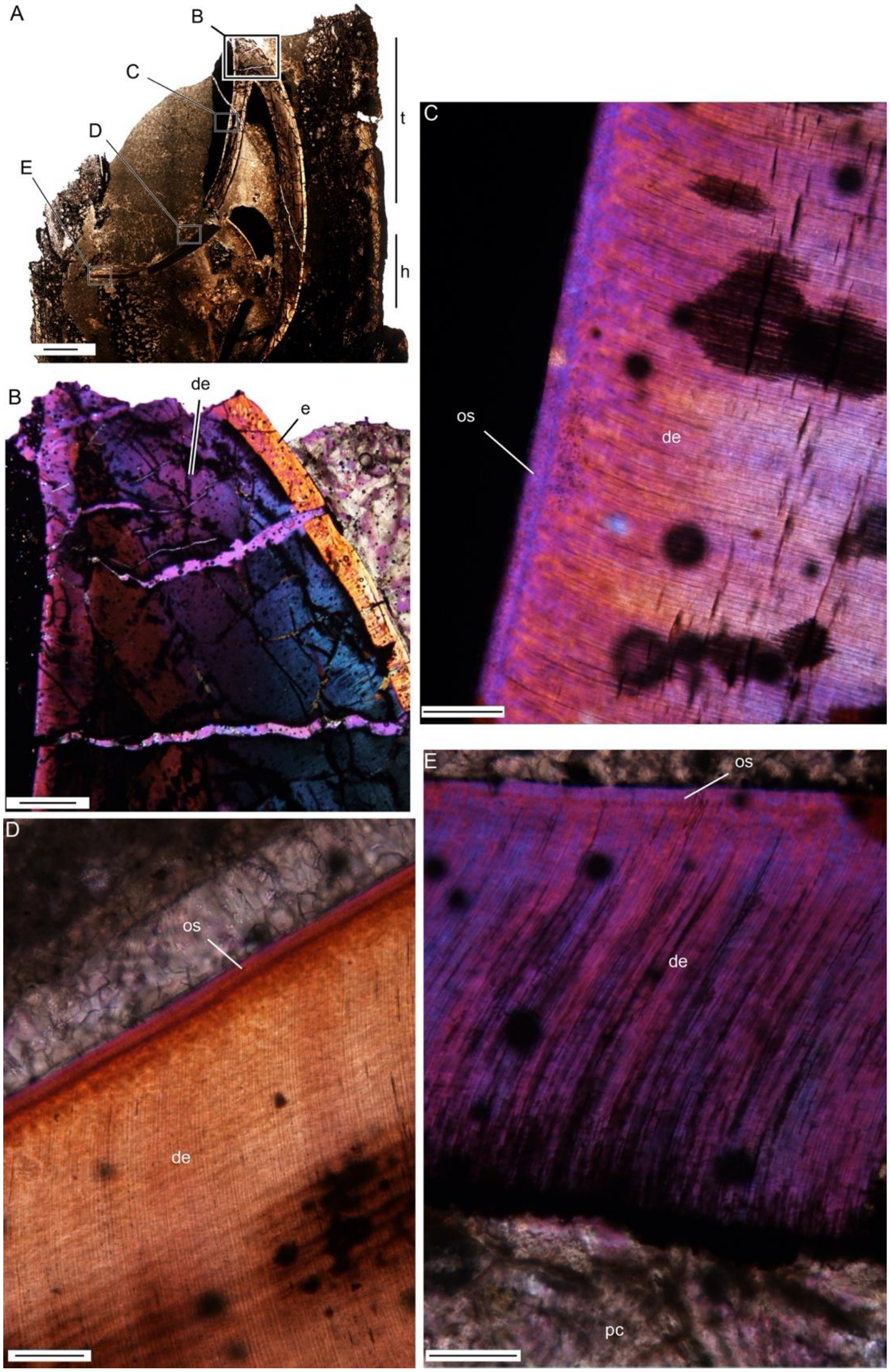

Figure 2.5. **Developing tooth in the second incisor position of NHCC LB370 with no enamel deposition along the occlusal surface.**

(A) Sagittal section through the tooth demonstrating heel and talon development, with locations of subsequent images noted. Scale = 5000µm. (B) Tip of the preserved talon with substantial enamel deposition labially and none lingually. Scale = 1000µm. (C-E) The dentine and occlusal surface along the talon (C), labial portion of the heel (D), and lingual most portion of the heel (E) that all lack enamel deposition. Scale = 100µm. Abbreviations: **de**, dentine; **e**, enamel; **h**, heel; **os**, occlusal surface, likely formed of mantle dentine; **pc**, pulp cavity; **t**, talon.

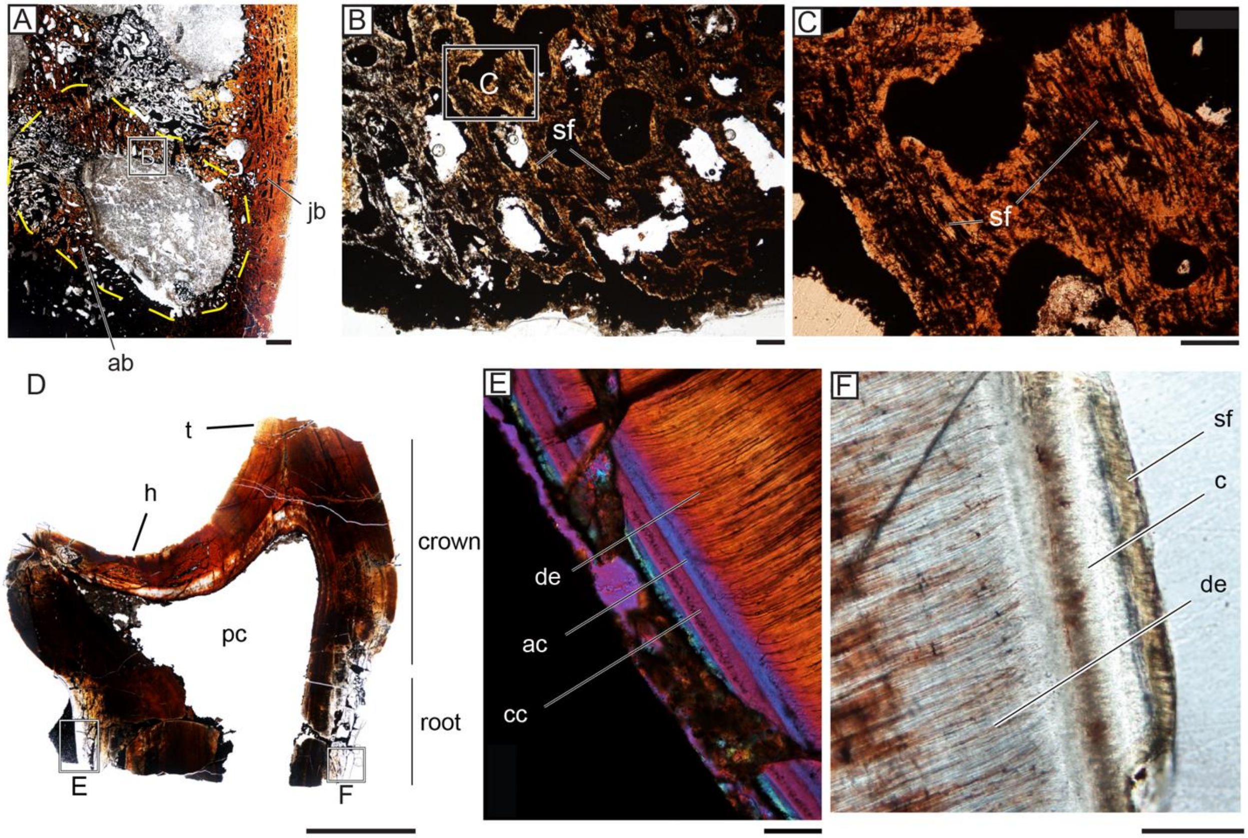

Figure 2.6. **Evidence of a permanent gomphosis in tapinocephalid jaws and teeth.** (A) Histology of NHCC LB369, an empty tooth socket with the alveolar bone outlined from jaw bone by yellow dashed line. Scale = 2000μm. (B) Alveolar bone from (A) with dense Sharpey's fibers. Scale = 200μm. (C) Close up of Sharpey's fibers from (B). Scale = 100μm. (D) Longitudinal thin section of NHCC LB733 that contains part of the root. Scale = 10000μm. (E) Close up of the lingual root of NHCC LB733 under polarized light with a lambda plate. Clear boundaries between the acellular and cellular cementum lining the tooth root are visible. Scale = 100μm. (F) Close up of the cementum of NHCC LB733 on the labial side of the root with Sharpey's fibers under polarized light. Scale = 100μm. Abbreviations: **ab**, alveolar bone; **ac**, acellular cementum; **cc**, cellular cementum; **de**, dentine; **h**, heel; **jb**, jaw bone; **pc**, pulp cavity; **sf**, Sharpey's fiber; **t**, talon.

Table 2.2. Selected herbivorous taxa highlighting details of their dentitions.

†indicates extinct taxa; -, not applicable field; x, unknown field; *suggested character state; [1][33, 51, 61]; [2][33, 51]; [3][59, 62];[4][53]; [5][44, 46]; [6][47]; [7][44, 63].

	Synapsida				Sauropsida			
					Diapsida			
						Dinosauria		
	†Tapinocephalidae	†*Diademodon*	†*Cotylorynchus*	*Equus*	*Uromastyx*	†*Iguanodon*	†*Changchunsaurus*	†*Captorhinus*
Enamel								
max thickness (∝m)	590	>100	>100	700	110	150	55	50
type	wavy-like	synapsid columnar	parallel crystallite	prismatic	prismatic	wavy	wavy	parallel crystallite
Attachment	gomphosis	gomphosis	ankylosis	gomphosis	ankylosis	gomphosis	gomphosis	ankylosis
Replacement								
pattern	alternating	sequential	alternating	sequential	-	x	alternating	horizontal
# of events	polyphyodont	determinate polyphyodont	polyphyodont	diphyodont	monophyodont	polyphyodont	polyphyodont	polyphyodont
frequency of events	rapid	low	rapid	-	-	rapid	rapid	rapid
Tooth Crown								
morphology	expanded occlusal surface	expanded occlusal surface	peg-like	expanded occlusal surface	leaf-shaped	expanded occlusal surface	leaf-shaped	peg-like
regionalization	homodont	heterodont	homodont	heterodont	homodont	homodont	homodont	homodont
occlusion	yes	yes	no	yes	no	yes	yes	no
tissues in occlusion	dentine, enamel	enamel	enamel	dentine, enamel, cementum	enamel posteriorly, dentine anteriorly	dentine, enamel	dentine, enamel	enamel
other herbivory specializations	wide periodontal ligament	-	palatal teeth	hypsodont	-	dental battery	-	Multiple marginal tooth rows; palatal teeth

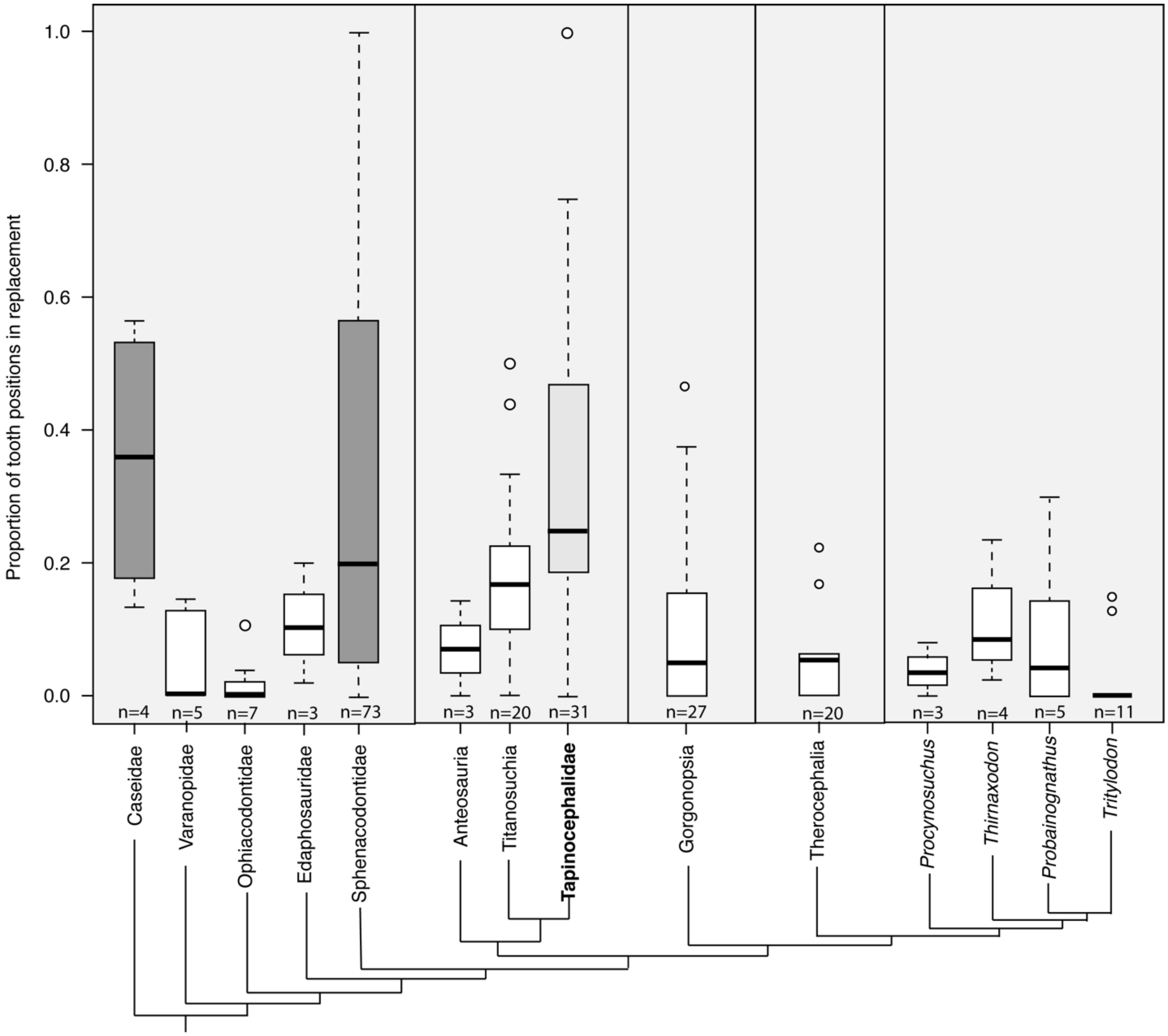

Figure 2.7. **Proportion of tooth positions in replacement in fossil synapsids.** Proportions are such that 0.0 represents no positions visibly in replacement and 1.0 represents all positions in visible replacement. With the exception of pelycosaurs and dinocephalians, synapsid clades generally have an average proportion below 0.1. Within pelycosaurs, caseids and sphenacodontids (dark grey boxes) have a significantly higher average proportion of teeth in replacement than do other pelycosaurs, and tapinocephalids (light grey box) have a higher average proportion than other dinocephalian clades. Caseids (*p*=0.868) and sphenacodontids (*p*=0.600) are the only groups to not have significantly fewer average number of teeth in replacement than tapinocephalids.

Chapter 3. COMPARATIVE RECORDED RESPONSES TO SEASONALITY IN THE TUSKS OF EARLY TRIASSIC *LYSTROSAURUS*

3.1 INTRODUCTION

Today, Antarctica is the coldest and driest continent with intense variation in light availability. These extreme conditions restrict vertebrate life to coastal regions, rendering most of the continent uninhabited (Convey et al. 2008). These environmental conditions, however, are anomalous for the history of life in Antarctica and flora and fauna have occupied large regions of this terrestrial setting placing it in stark contrast to the relatively restricted marine and coastal distribution of life today. Although more habitable with a warmer climate than today, the high latitudinal position of Antarctica for much of the Phanerozoic (Scotese et al. 1999) has subjected its inhabitants to intense photoperiod seasonality. In particular, fossils from the Early Triassic present a much different terrestrial ecosystem than what is known today. The climate of the continent was somewhat similar to a temperate deciduous forest or taiga (Retallack and Krull 1999) while the geographical location at a polar paleolatitude inflicted strong light seasonality that today is only found in much colder polar environments. This pronounced seasonality during the Triassic and preceding Permian Eras has been well-documented in both the paleosol (Retallack and Alonso-Zarza 1998) and paleobotanical records with pronounced growth rings in fossil wood (Taylor et al 1992; Taylor and Taylor 1993; Francis et al. 1993; Decombeix et al. 2018). Given the temperate temperatures hypothesized for Early Triassic Antarctica, it is likely that this pronounced seasonality is a record of the drastic changes in photoperiod.

Habitats with seasonally low photoperiods are characterized by regular bursts of nutrient accessibility during light seasons contrasted with minimal available energy during dark seasons (Chapin et al. 1975, 1980). Vertebrates, especially herbivores, living in these environments must adapt to pronounced seasonal energy influxes as well as times of energy reductions in the ecosystem compared to more temperate environments (Chapin et al. 1975). Thus, it is predicted that if Early Triassic Antarctic tetrapods were year-round residents of the geologic basins in which their fossils are found, they would have experienced and adapted to similar seasonal energy flux, especially when compared to other populations living at lower paleolatitudes.

Extant vertebrates living in seasonally variable climates have evolved a variety of mechanisms to curb the effects of regular intervals of stress including daily torpor, hibernation, brumation, migration, and increased time foraging (Geiser and Ruf 1995). These adaptive responses to seasonality often involve predictable seasonal changes to the hypothalamic-pituitary-adrenal axis that controls glucocorticoids, an important hormone in glucose metabolism (Reeder and Kramer 2005). While the mechanisms of seasonal changes to metabolic activity are similar in heterothermic vertebrates, the overall physiology (i.e. endothermic vs. ectothermic) plays an important role in the response to seasonality. Heterothermic ectotherms reduce their metabolic activity from more than half to nearly complete quiescent metabolic activity (Boutilier et al. 1997). Heterothermic endotherms, on the other hand, can enter a state of torpor generally reducing metabolic activity by at most a third although most reduce by no more than 10% of normal activity (Geiser and Ruf 1995). Generally, ectothermic heterotherms are not able to reactivate metabolic activity during unfavorable environmental conditions and enter times of burmation while endothermic heterotherms, even hibernators, frequently will come out of metabolic dormancy either daily, weekly, or monthly (Geiser 2013).

In fossil vertebrates, metabolic activity can be examined in hard tissue growth (Castanet et al. 2000; de Margerie et al. 2002; Montes et al. 2007; Olivier et al. 2017), and as such, seasonal growth patterns can be captured in fossil hard tissue histology in both bone (Mcgee-Lawrence et al. 2008; Köhler and Moyá-Solá 2009; Köhler et al. 2012) and teeth (Rinaldi and Cole 2004; Goodwin et al. 2005; Batavia et al. 2013). Furthermore, the nature of hard tissue deposition is appositional, which records seasonal stress in chronological order, essentially creating a diary of a vertebrate fossil's metabolism.

The earliest record of tetrapod life in Antarctica comes from the Early Triassic Fremouw Formation that was discovered by expeditions in the late 1960s and early 1970s (Barrett et al. 1968; Elliot et al. 1970; Kitching et al. 1972). The lower member of the Fremouw Formation contains a vertebrate fauna found 7-10 m above the underlying Buckley Formation that contains abundant coal and plant fossils but lacks vertebrate remains (Collinson et al. 2006). The fauna of the lower Fremouw Formation resembles that of the *Lystrosaurus* Assemblage Zone in South Africa with temnospondyl amphibians (Colbert and Cosgriff 1974; Sidor et al. 2007, 2008), *Thrinaxodon* cynodont material (Colbert and Kitching 1977), therocephalians (Huttenlocker and Sidor 2012), dicynodonts including *Lystrosaurus* (Colbert 1974) and *Myosaurus* (Hammer and Cosgriff 1981), as well as the reptiles *Prolacerta* (Colbert 1987) and *Procolophon* (Colbert and Kitching 1975). However, it is worth noting that increased sampling of the lower Fremouw has revealed more endemic taxa that differentiate between the two assemblages (Peecook et al. 2019). While there is evidence of seasonal stress in fossil trees, no such line of inquiries have been established for Early Triassic vertebrates.

Lystrosaurus has served as a vital study system to test comparative geographic and temporal questions surrounding the Permian, Triassic, and the Permo-Triassic mass extinction.

Lystrosaurus was a globally dispersed genus with Early Triassic *L. murrayi* from the Panchet Formation of India (Ray 2005), *L. curvatus, murrayi, and declivis* from the *Lystrosaurus* Assemblage Zone of South Africa (Botha-Brink and Smith 2007), *L. georgi* from the Vokhmian Gorizont of Russia (Surkov et al. 2005), *L. broomi, hedini, robusts* and *shichanggouensis* from the Jiucaiyuan Formation of China (Li et al. 2008), and *L. curvatus, murrayi* and *maccagi* from the Fremouw Formation of Antarctica (Colbert 1974; Cosgriff et al. 1982). *Lystrosaurus* is also one of the few tetrapod taxa with a Permian and a Triassic fossil record. The global dispersal and temporal extension of this genus has provided a key line of evidence examining trends in growth rate (Botha-Brink and Angielczyk 2010), extinction recovery dynamics (Botha and Smith 2007), and continental drift (Elliot et al. 1970).

Lystrosaurus also serves as an ideal study system for comparisons in Early Triassic physiology because of its paired maxillary tusks, which are composed of ever-growing dentine (Whitney et al. 2019). Dentine serves as a particularly good record of seasonality as it is not a regularly remodeled tissue (providing a more complete record than bone) and preserves evidence of regular incremental growth as well as intervals of stress.

Using specimens collected during 1985–86 and 2017–2018 field seasons, tusk dentine growth was examined in the dicynodont therapsid *Lystrosaurus* from the Fremouw Formation and compared to lower paleolatitude *Lystrosaurus* from South Africa (Fig. 1; Table 1). The estimated difference in paleolatitude between Antarctic and South African localities is about ten degrees which constrains population/species-level differences and aims to isolate the effects of photoperiod. For reference, South African localities were positioned similarly to modern-day southern Patagonia while Antarctic localities were positioned only slightly higher than their current position today both below the polar circle (66°33’S). Using quantitative and qualitative methodologies, we compare Karoo Basin South African and Antarctic Beardmore and Shackleton Glacier *Lystrosaurus* tusk histology, to test whether individuals living in more dramatic photoperiods (i.e. the Antarctic portion of southern Pangea) acquired specializations in their seasonal metabolism and whether or not those specializations are indicative of an ectothermic or endothermic physiology.

3.2 INSTITUTIONAL ABBREVIATIONS

NHMLA, Natural History Museum Los Angeles County, Los Angeles, California; **SAM**, Iziko: South African Museum, Cape Town, South Africa; **UWBM**, University of Washington Burke Museum, Seattle, Washington.

3.3 SYSTEMATIC PALEONTOLOGY

Synapsida Osborn, 1903

Therapsida Broom, 1905

Dicynodontia Owen, 1859

Lystrosauridae Broom, 1903

Lystrosaurus Cope, 1870

Lystrosaurus murrayi Huxley, 1859

Referred specimen. UWBM 118025, tusk-bearing right maxilla.

Locality. UWBM 118025 was recovered from Coalsack Bluff in the Beardmore Glacier region of the Transantarcitc Mountains within 30 meters of the base of the Fremouw Formation (Collinson and Elliot 1984).

Lystrosaurus mccaigi Seeley, 1898

Referred specimen. UWBM 118026, tusk-bearing right maxilla.

Locality. UWBM 118026 was collected approximately 3 meters below the Permo-Triassic boundary in the Bethulie District, Eastern Cape Province of South Africa.

Lystrosaurus declivis Owen 1860

Referred specimen. UWBM 118027, tusk-bearing right maxilla.

Locality. UWBM 118027 was collected 7 meters above the Permo-Triassic Boundary in the Namakwa District, Northern Cape Province of South Africa.

***Lystrosaurus* indet.**

Referred specimens. SAM-PK-K011603, tusk-bearing right maxilla; UWBM 118028, tusk-bearing left maxilla; UWBM 118022, isolated tusk; UWBM 118024, isolated tusk; UWBM 109730, isolated tusk, NHMLA COLHWLYST, portion of maxilla and tusk; NHMLA MCTUSK, isolated tusk.

Locality. SAM-PK-K011603 was collected approximately 22 meters above the boundary from the Nieu Bethesda District, Eastern Cape Province of South Africa. UWBM 118028 was collected 5 meters above a major sandstone package of the basal Katburg Formation in the Nieu Bethesda District, Eastern Cape Province of South Africa. UWBM 118022, UWBM 118024, UWBM 109730 were collected were recovered from Coalsack Bluff in the Beardmore Glacier region of the Transantarcitc Mountains within 30 meters of the base of the Fremouw Formation (Collinson and Elliot 1984). NHMLA COLHWLYST was collected at Collinson Ridge and NHMLA MCTUSK was collected at McIntosh Ridge.

3.4 METHODS

Following standard thin-sectioning protocol established by Lamm (2013) transverse and occasional longitudinal sections (see Fig. 2) of six Antarctic and four South African *Lystrosaurus* tusks were examined. Paleolatitude is estimated from tectonic plate reconstructions using paleomagnetic reference frame developed by Torsvik et al. (2012) and made available through the online calculator developed by van Hinsbergen et al. (2015).

3.4.1 *Dentine growth mark terminology*

The use of tusks as a reliable comparative tool for seasonality in fossils was pioneered in *Mastodon* tusks by Fisher (1987, 1996, 2001) with correlation of dentine growth and seasonality ascertained from isotopic evidence (Fisher et al. 2003). Generally, there are three kinds of growth marks recognized in dentine: (1) daily increments that correspond to a circadian rhythm called lines of von Ebner, (2) 6–10 day increments called Andresen lines, and (3) periods of stress where dentine deposition essentially ceases creating particularly thick growth marks (Dean 1998; 2000). While these three marks are recognized in primates, especially humans, there is little investigation into the consistency of this hierarchy outside of mammals. This is in large part due to the range of reported distances between these growth marks. Dean (1998) reviewed the discrepancies in both reported short-periods between lines of von Ebner (varying from 1.7 - 20μm) and long-periods between Andresen lines (varying from 4-20μm) of which values overlapp. Erickson (1996) was one of the few to experimental test for daily lines of von Ebner in a non-mammalian vertebrate, alligators. Erickson, however only reported that lines of von Ebner are less than 20μm and does not discuss the variation of space between lines or whether or not there was a distinction between short- and long-term periods. Furthermore, that study was conducted on juvenile alligators that were rapidly growing and thus, reported distances between growth marks may not be truly comparable to the adult primates previously studied or representative of adult dentine growth more generally.

Given the lack of modern ground-truthing and ability to experimentally test the temporal associate of dentine growth in *Lystrosaurus*, Dean's (1998) terminology of short-term and long-term periods growth marks are both described here as incremental lines, assigning no specific amount of time, but rather representation of routine dentine deposition (Fig. 2c). The more pronounced growth marks are simply referred to as stress marks, removing specific assignment of duration or time of year of the metabolically stressful event (Fig. 2b). With this terminology, general patterns of growth and stress in *Lystrosaurus* populations are compared rather than assigning specific amounts of time (i.e. daily growth, yearly growth, etc.).

3.4.2 *Quantitative analysis*

An additional aim of this study was to develop a more quantitative and objective method for measuring and counting growth marks, especially in fossil material where visuals can be impaired by taphonomic alteration. Using NIS Nikon Elements software ROI Measurement Tool, we ran transects across live images of tusks that collected measurements on light intensity (Fig. 3). The output of these transects records objective dips and rises in light intensity that can be confirmed in the live image as growth marks and spaces between growth marks. Relative dips in light intensity are associated with incremental and stress lines whereas relative increases in light intensity represent times of growth and tissue deposition. The space between regular increments as well as distance between and thickness of stress intervals were captured in these light transects. None of the specimens included here had pristine preservation of incremental lines and thus, average distance between incremental lines was used to reconstruct transects where stress lines were evident, but the incremental lines in between were not measured. Statistical differences in these readings were determined using an unpaired Welch Two Sample t-test assuming variance was unequal.

3.5 RESULTS

Measurement of stress and incremental lines were examined as both averages for specimens (Fig. 4a-d) and individual data points (i.e. line; Fig. 4e-g). There were consistent differences between Antarctic and South African specimens and data points, although sample size in specimen averages were limited and statistically insignificant. Stress lines are significantly more abundant in Antarctic specimens (32.76% of all lines counted) than in South African specimens (11.81% of all lines counted) ($p = 0.01674$) (Fig. 4d). These stress lines, as individual data, are more closely spaced in Antarctic specimens (on average 38.22μm apart compared to 116.49μm apart) suggesting shorter periods of uninterrupted growth ($p = 6.292\text{e-}06$) (Fig. 4a,e). Surprisingly however, South African specimens record thicker stress lines (average of 50.23μm compared to 24.03μm; $p = 1.746\text{e-}07$) suggesting that each individual periods of stress was longer in duration (Fig. 4b,f). The incremental lines are only somewhat differently spaced suggesting slight differences in "regular" resting metabolic rates. Antarctic specimens have a slightly higher average of 17.85μm between consecutive stress lines compared to South African specimens that average around 15.77μm for the same measurement ($p = 0.02266$) (Fig. 4c,g).

The reconstructed transects (Fig. 5) provide qualitative confirmation of these observations. Generally, Antarctic specimens appear to experience more frequent stress without prolonged periods of regular growth while at least some South African specimens experience more pronounced intervals of stress (i.e. thicker lines). The remarkably thick lines noted in the South African sample appear to be restricted to a single specimen, UWBM 118027, that also had by far the longest transect. South African datasets of comparative length to the Antarctic transects (i.e. UWBM 118026 and UWBM 118028) still have thicker lines, but are more similar to Antarctic specimens UWBM 118025, 118024, and 118022 (Fig. 5). Importantly, these transects reveal the variance between specimens both in the amount of available data (i.e. line counts) and the stress recorded. It is also worth noting a complete transect was only available for UWBM 118027 and at any given point along the transect of a similar length as what was available for other specimens (i.e. only 120 or less lines) was exclusively counted, the data could be biased towards times of stress or times growth.

3.6 DISCUSSION

3.6.1 *Comparative growth of Lystrosaurus tusks*

The significant differences between *Lystrosaurus* tusk growth suggest that these populations may have been living under different environmental stressors that required specific alterations to their metabolisms. Interestingly, the signals differ in ways that were not initially predicted. Both populations experienced stress, but the responses to stress appear different. Antarctic *Lystrosaurus* appears to have experienced more continuous but less pronounced stress than South African populations. This might reflect the distinct seasonal patterns in South Africa and Antarctica. Seasonal variation in photoperiod is a gradual environmental variable in polar regions. In modern vertebrates, changes early in or even prior to reduced photoperiod initiate metabolic pathways to reduce body temperature and energy requirements (Garland and Adolph 1991). Thus, vertebrate metabolic response to photoperiod seasonality is largely anticipatory and sustained for extended periods.

In drought conditions like those interpreted for the Karoo Basin, dramatic variability in available nutrients were more episodic and punctuated. The recovery facies of the Karoo above the Permo-Trassic mass extinction boundary described by Smith and Botha-Brink (2014) is composed of thick beds with planar or gently undulating contacts as well as massive maroon siltstone that is loessic, indicating a semi-arid to dry environment punctuated by intense and brief wet seasons. This drought-ridden environment may have placed strong, episodic strains on the metabolism of its inhabitants rather than gradual, more constant stress like those likely experienced in Antarctica. The Fremouw Formation, on the other hand, is interpreted as being much warmer than the underlying swampy and seasonally freezing Buckley Formation, but not as arid as the Karoo Basin (Retallack 2005). Instead, paleosol and paleobotany data suggest a warmer woodland environment on a seasonally wet floodplain (Retallack 2005).

The physiological differences observed here may reflect acclimatization Antarctic *Lystrosaurus* populations incurred. The Fremouw Formation vertebrate fauna, including *Lystrosaurus*, are relatively small-bodied taxa with an abundant tetrapod burrow record (Miller et al. 2001; Sidor et al. 2008). These lines of evidence suggest that these taxa were not seasonally migrating during the Early Triassic and instead lived year-round in their seasonally stressed environment (Fröbisch et al. 2010). The metabolic indicators for *Lystrosaurus* indicate a level of torpor was experienced by Antarctic *Lystrosaurus* with sustained reductions in metabolic activity in comparison to South African populations. Torpor is typically categorized as either hibernation (i.e. multi-day torpor) or daily torpor where the organism experiences reductions in metabolic activity at short intervals rather than prolonged periods (Geiser 2013). Unfortunately there is no ubiquitous physiological signal for hibernation in modern tissues, however the pattern of stress in Antarctic tusks currently appears most similar to daily torpor when compared to South African specimens. Daily torpor is a physiological response of endothermic vertebrates since it requires reactivation of the animal's metabolism regardless of the sustained environmental stressors (Geiser 2013). The suggestion of endothermy in *Lystrosaurus* has been detailed in the osteohistology (Botha-Brink and Angielczyk 2010) and maxilloturbinal reconstructions (Laaβ et al. 2011) of South African populations, and latitudinal comparisons of tusk histology may provide an additional line of evidence in support of this claim.

Alternatively, the differences observed could be artificial, with a limited sample size both in quantity of tusks and preservation of lines in thin-section biasing the data. These results were more similar than originally predicted and thus, it is possible that with increased sampling distinctions between Antarctic and South African *Lystrosaurus* tusks would become less significant. In all likelihood, both differences in environmental setting and sampling play a role in the results described here.

3.6.2 *Antarctica as a refugium*

Antarctica has a noted sample of Early Triassic taxa that are missing from other contemporaneous localities such as the Karoo Basin. *Lystrosaurus mccagi* is found in the latest Permian of South Africa with only one identifiable Early Triassic specimen having been recovered low in the Fremouw Formation (Collinson et al. 2006). The dicynodont *Kombuisia* and the diapsid *Palacrodon browni* first appear low in the Fremouw Formation and are only found in the Middle Triassic of South Africa in spite of the extensive sampling of this basin. Fröbisch et al. (2010) suggest these data support the hypothesis that Antarctica acted as a refuge from the global climate warming that is a widely accepted explanation for the Permo-Triassic Mass Extinction (Erwin et al. 2002; Royer 2006). Antarctica may have been a habitat with extreme seasonality in light availability, however its relatively temperate climate could have acted as a “Goldilocks Zone” for terrestrial vertebrates through the extinction boundary marked by global climate change towards warming and hypoxia.

The way in which Antarctica may have served as a refugium, however, is uncertain due the lack of vertebrate fossils from the Permian-aged Buckley Formation. There are two hypotheses for this dearth of fossils: 1) there were no vertebrates occupying the landmass prior to the extinction event and the rock record accurately reflects this or 2) there were in fact vertebrates inhabiting Antarctica, but were not preserved in the limited rock outcroup that has been sampled. Acting under the first hypothesis Antarctica can be viewed as a locality that served as a novel ecosystem for vertebrates providing a haven from the rising temperatures and hypoxia predicted for the end of the Permian, which has been a trend observed across tetrapod taxa (Bernardi et al. 2018). If *Lystrosaurus* migrated during the mass-extinction event, populations would have had to acclimatize their physiology to the seasonal photoperiod variation giving rise to the data presented here.

If, on the other hand, *Lystrosaurus* occupied the land mass prior to the extinction (i.e. the second hypothesis), this acclimatization would have occurred in the Permian. Although a colder environment (Retallack et al. 2005), the dramatic shift in environment across the Permo-Triassic boundary observed in the Karoo may not have played out similarly in Antarctica. Whereas South African fluvial morphology was altered from high- to low-sinuosity streams across the extinction boundary (Ward et al. 2000), sequences in Antarctica are more consistently defined by low-sinuosity streams across this boundary (Collinson et al. 2006). It is possible that more consistency in the Antarctic environment provided a selective advantage for vertebrates such as *Lystrosaurus maccaigi* to survive across the boundary if they already occupied the locality during the Permian.

Whether Antarctica acted as an environmental haven for migration or pre-existing endemic tetrapods, the populations of *Lystrosaurus* occupying the landmass during the Early Triassic appear to have experienced seasonal stress differently than those living in the Karoo Basin of South Africa. This demonstrates a level of flexibility in their physiology to maintain year-round activity that, today, is only known in endothermic birds and mammals (Geiser 2013). Osteohistological comparisons between other taxa shared by both South Africa and Antarctica (e.g. *Procolophon, Prolacerta, Thrinaxodon*) may help to delineate the means by which the continent may have served as a refuge.

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3.9 TABLES AND FIGURES

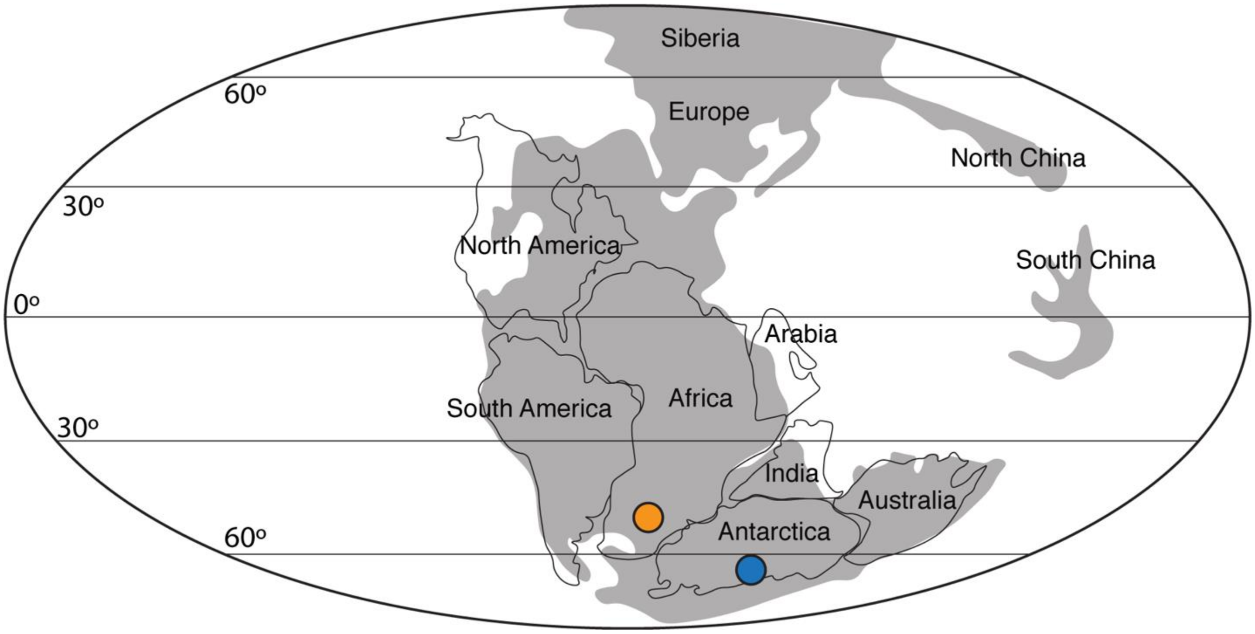

Figure 3.1. **Global schematic with inferred continental position and specimen localities.** Orange circle represents South African specimens and blue circle represents Antarctic specimens. Note the polar position of Antarctic localities.

Table 3.3. List of specimens and locality info with amount of data collected for each specimen. Inferred paleolatitudes were generated by paleolatitude.org (van Hinsbergen et al. 2015).

Specimen #	Formation	Inferred Paleolatitude	# of regular lines	# of stress lines
UWBM 118022	Fremouw	72° S	40	55
UWBM 118024	Fremouw	72° S	86	35
UWBM 118025	Fremouw	72° S	75	30
UWBM 109730	Fremouw	72° S	25	4
MCTUSK	Fremouw	73° S	38	19
NHMLA COLHWLYST	Fremouw	71° S	17	20
UWBM 118026	Teekloof	61° S	158	15
UWBM 118027	Katburg	58° S	320	45
UWBM 118028	Katburg	62° S	77	26
SAM-PK-K011603	Balfour	62° S	50	4

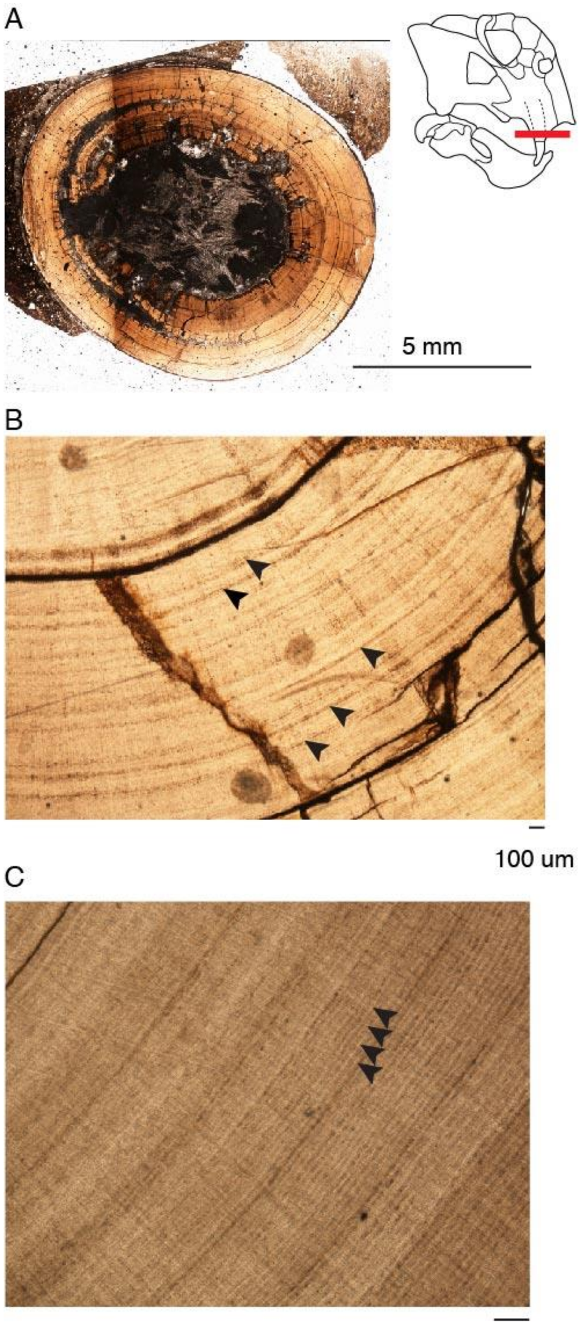

Figure 3.2. **Representation of different lines measured.**

A) Cross section through UWBM 118028. B) Zoomed in image with arrows indicating stress lines. C) High resolution image of well preserved “regular” lines of incremental growth indicated by arrows. Scale bar = 100 um.

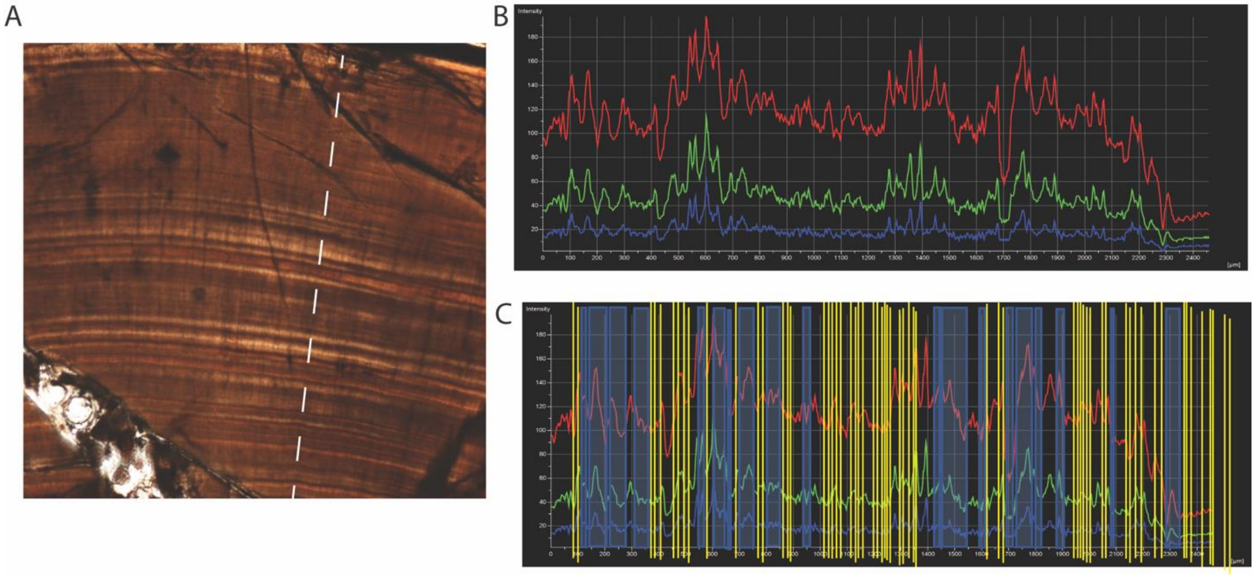

Figure 3.3. **Representation of light transect workflow.**

A) Live image with transect running through well-preserved stress and regular lines. B) Output from light transect with light intensity on the y-axis and distance from start of transect on the x-axis. Red, green and blue colors are simply outputs of color variation. C) Yellow lines represent regular, incremental lines and blue boxes are stress lines where the width of the box indicates the width of the line.

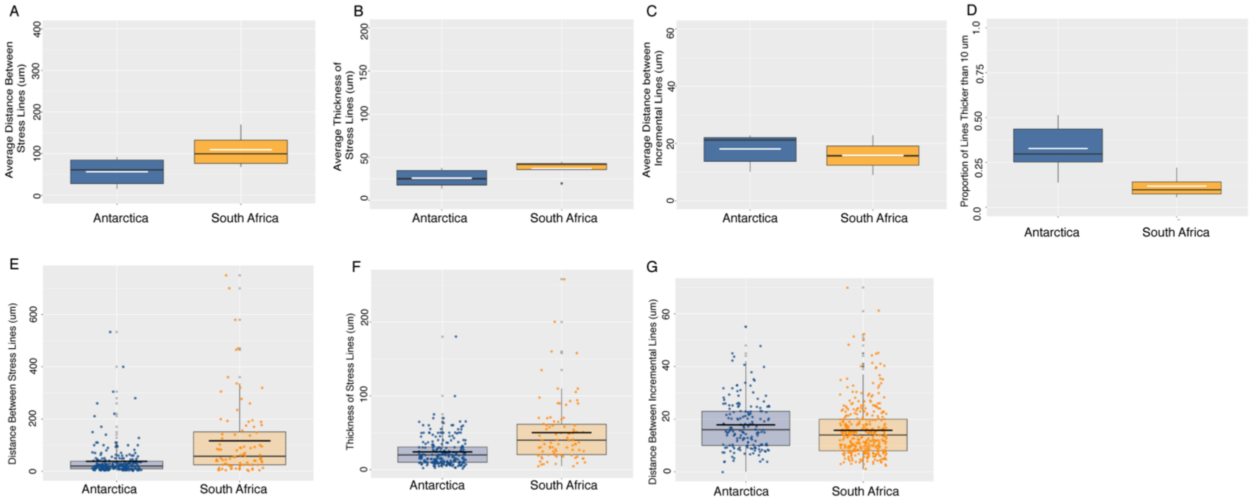

Figure 3.4. **Results from line measurements by specimen (A-D) and by lines as individual data points (E-G).**

The small sample size in specimens per locality likely contribute to the lack of significant differences in (A-C) and when released from individual specimen averages, individual lines follow similar and significant differences (E-G). A,E) Antarctic stress lines are closer together than South African ones indicating shorter periods without stress. B,F) Antarctic specimens have thinner stress lines, indicating that a period of stress lasts for less time than in South African specimens. C,G) Small difference was observed in regular incremental growth marks between Antarctic and South African specimens, however this difference is only about 2µm. D) Given that different tusks had different amounts of available data, the proportion of very thick lines (>10µm) was measured. Antarctic specimens have a higher proportion than South African specimens.

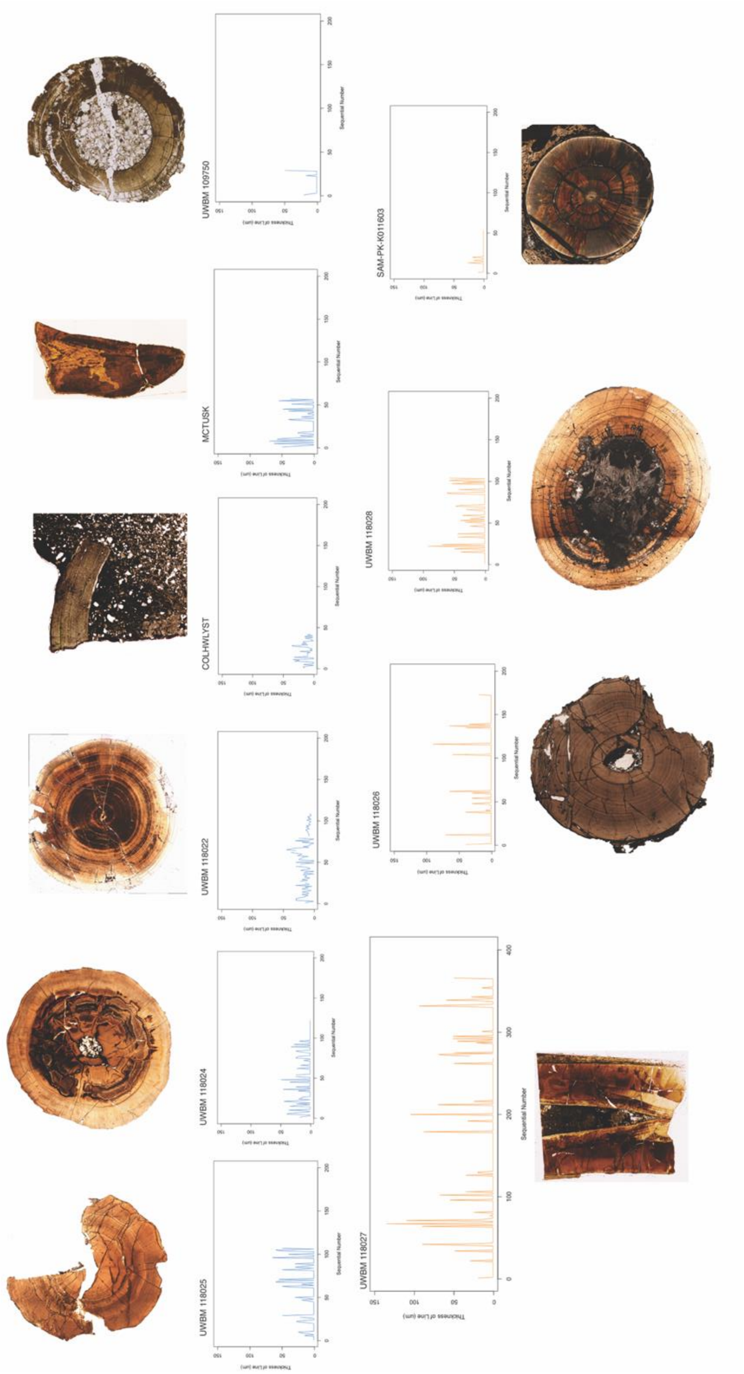

Figure 3.5. **Reconstructed transects for specimens studied here with photographs.** Antarctic specimens are denoted in blue lines and South African specimens in orange. The x-axis is sequential number (i.e. 1 being where the transect begins) and the y-axis is thickness of line with regular incremental lines having a uniform thickness of 1 μm. Note, images not to scale.

Chapter 4. TOOTH ATTACHMENT IN NON-MAMMALIAN SYNAPSIDS: TAPHONOMIC PROXIES, PATTERNS OF TRAIT ACQUISITION AND COEVOLUTION OF DENTAL TRAITS

4.1 INTRODUCTION

The periodontal ligament (PDL) is a distinct connective tissue that anchors teeth to alveolar bone of the tooth socket. The ligament is unique in its functional capabilities in that it can erode alveolar bone, perceive physical forces, and has rapid and adaptive regenerative properties. As such, the ligament serves as a critical tissue in vertebrate dentition facilitating tooth drift to maintain tooth-to-tooth occlusion, and in preventing overloading a tooth by both force detection and cushioning. Unlike other connective tissues, the PDL cannot develop from fibroblasts other than the ectomesenchymal fibroblasts of the dental papilla, making it developmentally specialized, and has properties that are unique such as high alkaline phosphatase activity, an enzyme notable for its role in the mineralization process (Beersten et al. 1997).

Because of its regenerative properties and importance in dentistry and orthodontics, human, pig, and mouse PDLs have been extensively studied, providing a wealth of observational and experimental data (Beersten et al. 1997). However, discussions of such a unique tissue in an evolutionary context is often lacking. This is in large part because of the assumption that the ligament was acquired as part of a specialized mammalian dentition (e.g. Seeley 1888; Smith 1958; Gaengler and Metzler 1992), which in extant vertebrates is largely true, with one exception, crocodylians (Kvam 1960; Soule 1967; Miller 1968; Berkovitz and Sloan 1979).

Investigations into the homology of the crocodilian and mammalian PDL have revealed developmental and morphological similarities between these tissues, while noting some differences. Two of these differences (viz. mammals have a cribriform plate whereas caiman alveolar bone is much less vascularized and PDL tissue is reused by multiple generations of caiman teeth; Brekovitz and Sloan 1979) can be explained by differences in the geometry of tooth replacement. While replacement teeth in mammals develop in individual crypts that eventually replace the entire socket of the predecessor (i.e., tooth, PDL, and alveolar bone), crocodilian teeth develop and erupt within the same socket. Caiman PDLs also differ in having fewer fibroblasts with fewer intracytoplasmic organelles (Brekovitz and Sloan 1979), resulting in slower tissue turnover than the mammalian PDL, and more mineralization foci than mammals, giving rise to a partially mineralized PDL (McIntosh et al. 2002).

McIntosh et al. (2002) suggested the mineralized caiman PDL serves as a glimpse into the "intermediate" phase of acquiring a mammal-like periodontium from an ankylosed ancestor. However, recent work examining fossil dentitions has shifted this paradigm. Under traditional hypotheses, mammals acquired a novel PDL tissue while the tissues of an ankylosis were plesiomorphic for vertebrates. LeBlanc et al. (2016) detailed evidence of an alternative pattern of acquisition where rather than acquiring a new tissue, mammals evolved a gomphosis through heterochronic shifts of preexisting tissues. Histological observations of fossil dentitions suggest that the teeth of many non-mammalian synapsids develop and move into functional position in a gomphosis and the plesiomorphic character state is to subsequently ankylose the tooth to the jaw. In mammals, teeth retain the PDL not only throughout their development, but also into their adult, functional position. Mammals can experience dental ankylosis. However, it is a pathological condition resulting from damage to the PDL, either through changes in local metabolism, excessive force, or genetic malfunctions (Andersson et al. 1984).

In light of the evolutionary history of the mammalian PDL, the requirements for PDL homeostasis in preventing ankylosis and responding to force reception from the tooth make sense. Cells of the PDL can secrete a variety of osteoblastic and osteoclastic regulatory molecules that can remodel or deposit bone regionally around the tooth root (McCulloch et al. 2000). Perhaps because of the demands of this tissue to counteract the evolutionary program to eventually ankylose, the PDL tissue has a remarkably high rate of collagen production, remodeling and turnover, more than bone, gingiva, skin, or other connective tissues (Carneiro and Fava de Moraes 1965; Sodek 1977; Beersten et al. 1997). Furthermore, the ligament is sensitive to somatic nutrient deficiencies (Goldman 1954; Waerhaug 1958). Given the reduced metabolic rate of modern crocodiles compared to mammals, it is possible that a partially mineralized PDL provides a mechanism to maintain the tissue with a slower metabolism. Under this scenario, the caiman's partially mineralized PDL can be reinterpreted to reflect the physiological demands of this tissue to maintain a gomphosis rather than an intermediate phenotype.

Adding complexity to the evolutionary history of the PDL are recent studies examining fossil taxa that have found evidence of a permanent gomphosis in some fossil synapsids (Jasinoski and Chinsamy 2012; LeBlanc et al. 2018; Whitney et al. 2019; Whitney and Sidor 2019) and sauropsids (Fong et al. 2016). Together, these studies document the independent evolution of a permanent gomphosis in a variety of amniotes despite the limited occurrence of this character state in modern vertebrates. The convergence of a permanent gomphosis in synapsids adds to the documentation of wide-spread homoplasy in synapsid evolutionary history (Olson 1941, 1959; Hopson 1991, 1995; Sidor and Hopson 1998; Sidor 2001). Convergence and homoplasy are evolutionary reactions to similar selective pressures. The selective pressures that gave rise to a permanent PDL remain elusive in large part because of a lack of systematic taxonomic sampling for synapsid tooth attachment that no doubt results from the destructive nature that is often required for tissue-level analysis.

A proxy for the presence of a permanent PDL would be a preferable alternative to histological sectioning however, no tests for such proxies have been conducted. Boonstra (1962) and Sidor (2000) both observed the frequent lack of teeth in tapinocephalid dinocephalian jaws and suggested that the preservation of edentulous jaws was probably related to the mode of tooth attachment in the group. Here, we test whether taphonomic indicators (i.e. empty or filled sockets) can predict tooth attachment strategy or other aspects of the dentition. Specifically, we consider replacement frequency, enamel type, enamel thickness, and diet as possible alternative explanations for loss or retention of functional teeth during fossilization. Finally, we apply data collected here to discuss correlations between traits and detail trends in the synapsid dentition that may shed light on the acquisition of a mammal-like dentition including a gomphosis.

4.2 METHODS

4.2.1 *Specimen-based data*

Individual jaws of pelycosaur-grade, dinocephalian, gorgonopsian, therocephalian and cynodont synapsids were examined for proportion of teeth lost as well as proportion of tooth positions with evidence of replacement. In total, 457 jaws from 404 specimens from 20 families and 58 genera of synapsids were included giving rise to 30 terminal taxa for analyses (see Supplemental). The proportion of empty sockets in the upper and lower jaws of fossil synapsids included only marginal teeth and excluded sockets that lacked clear preparation. Empty tooth positions were accounted for in the incisor, canine, and postcanine regions, except in homodont dentitions (Caesidae, Varanopidae, Ophiacodontidae, Edaphosauridae, Tapinocephalidae) where the canine position was not included as a distinct category in the analysis (i.e., the jaw was considered to include only incisor and postcanine regions). Specimens with upper and lower jaws in occlusion were not included in the analyses due to their taphonomic predisposition to retain teeth and thus inflating tooth retention in taxa that are frequently recovered as such small-bodied cynodonts.

The lack of specimens preserving dentitions in Biarmosuchia and the highly specialized, thus difficult to compare, dentition of Anomodontia precluded the inclusion of these two clades in this analysis. Juvenile specimens were included in data collection, however, they were removed from subsequent analyses to account for any changes in tooth retention that may be associated with ontogeny. Singleton taxa were removed from the analysis, with one exception, *Galesaurus* which was included because it has a known permanent gomphosis (LeBlanc et al. 2018). Tooth positions that were under replacement (i.e. a replacement tooth had moved into functional position and the functional tooth could have been ejected rather fallen out) were excluded when calculating the proportion of empty tooth sockets. Lastly, in addition to the proportion of empty tooth positions, the proportion of positions under replacement was also collected. Detailed in Chapter 1 (Whitney and Sidor 2019), the updated data here counts all stages of replacement as replacement events.

4.2.2 *Taxonomically assigned data*

Categorical assignment of character states (enamel type, enamel thickness, mode of tooth attachment, diet) were assigned by taxa to the specimens examined in this study (see Supplemental). Using data and categories established by Sander (1997), enamel type (prismless, synapsid columnar enamel) and enamel thickness (very thin: <10μm; thin: 10-50μm;

intermediate: 50-100 μm; thick >100μm) were assigned to taxa that were included in specimen-based data collection. Whitney and Sidor (2019) provided enamel data for tapinocephalids as they were not included in the Sander (1997) survey.

For each taxon, mode of tooth attachment was assigned by histological interpretations from this study as well as others. Attachment in pelycosaur-grade synapsids, akidognathids, baurioids, *Galesaurus, Cynognathus* were based on the observations of LeBlanc et al. (2018), while tapinocephalid gomphosis (LeBlanc et al. 2018; Whitney and Sidor 2019), *Diademodon* gomphosis (Hopson 1971), *Thrinaxodon* ankylosis (Crompton 1963; Abdala et al. 2013) and *Tritylodon* gomphosis (Jasinoski and Chinsamy 2012) were assigned from previous publications. This current study provides histological and tentative anatomical tooth attachment data for Anteosauria, Titanosuchia, gorgonopsians, scylacosaurids, *Procynosuchus*, *Cynosaurus*, *Trirachodon*, *Cricodon*, *Scalenodon*, *Exaeretodon, Aleodon* and *Pachygenelus* (Appendix A). Broad diet categories were assigned by tooth shape (e.g. transversely expanded crows suggesting herbivory, serrations indicating carnivory, etc.).

4.2.3 *Data analysis*

Statistical test of variance was conducted with a one-way ANOVA for taxon-specific proportion of positions empty and in replacement. Post-hoc Tukey multiple comparisons were utilized to compare means between taxa. For pairwise differences in mode of attachment (2 factors), Welch Two Sample t-tests were run assuming variances were not equal and for replacement, enamel type, enamel thickness, and inferred diet (all 3 factor variables), we ran a one-way ANOVA and a post-hoc pairwise Tukey multiple comparisons of means at 95% family-wise confidence level. Regressions were run under a linear model and associations were tested using Kendall’s and Spearman’s rank correlation tests. Correlations between categorical data sets were determined with a Pearson’s Chi-squared test.

4.3 PROPORTION OF EMPTY TOOTH POSITIONS

4.3.1 *Results*

The proportion of empty tooth positions yielded significant differences among the 30 terminal taxa studied (F = 9.324; p <2e-16). In particular, the data confirms observations by Boonstra (1962) and Sidor (2001) that tapinocephalid jaws typically have a greater proportion of empty sockets (0.655) than other therapsids (Fig. 1). Significant differences from tapinocephalids include most pelycosaurs, other dinocephalians, gorgonopsians, some therocephalians, some basal cynodonts, some gomphodont cynodonts, and most probainognathian cynodonts (see Supplemental) some of which have an ankylosis and some of which have a gomphosis (Fig. 1). Gorgonopsians, on average, had the lowest proportion of empty sockets (0.024) which was significantly lower than tapinocephalids, most, but not all, gomphodont cynodonts, and the probainognathian cynodont *Aleodon* (see Supplemental).

On average, higher proportions of empty positions were observed in specimens with a permanent gomphosis (0.372) than those with an ankylosis (0.126) (p = 7.036e-15; Fig. 2), however, many specimens from taxa with a gomphosis had a proportion more similar or even less than those in an ankylosis (Fig.1). Taxa with a gomphosis showed significantly greater variation in the proportion of empty tooth socket than those in an ankylosis ($p < 2.2e-16$). Furthermore, significant differences within attachment strategy was observed. Of those that have an ankylosis, ophiacodontids have a higher proportion of empty sockets than varanopids (p = 9.855e-04), *Thrinaxodon* (p = 5.916e-03), sphenacodontids (p = 0.011), edaphosaurids (p = 0.024), caesids (p = 8.44e-05). Among those with a gomphosis, tapinocephalids have a significantly higher proportion than akidognathids (*p* = 3.653e-04), gorgonopsians (*p* = 9.000e-07), and tritylodontids (*p* = 1.500e-06) while, in addition to tapioncephalids, gorgonopsians are significantly lower than *Cynognathus* (*p* = 0.0442).

4.3.2 *Utility of a taphonomic proxy for attachment*

Loss of teeth does not always indicate mode of attachment. In taxa with an ankylosis, proportions rarely exceed more than 0.25 edentulous positions. Synapsids with a gomphosis on average do have a higher proportion missing, however taxa like gorgonopsians, *Pachygenelus*, and *Tritylodon* lose few teeth and plot similarly or even below taxa with known ankylosis. We conclude that observing retention of teeth in fossils does not accurately reflect the mode of tooth attachment. While the range of proportions of missing teeth in ankylosed data do not vary dramatically, there is intriguing variation in taxa that have a gomphosis with some terminal taxa having a wide range of proportions and others remaining restricted. Rather than a proxy for attachment alone, we investigate other possible explanations for the significant variation in the proportion of teeth missing.

4.4 ALTERNATIVE EXPLANATIONS TO PROPORTION OF EMPTY TOOTH POSITIONS

If tooth attachment strategy cannot alone explain the retention of loss of teeth, there are other possible biotic and abiotic factors that may help to explain these data. We examined the possible effects of factors that we categorized as phylogenetic (i.e. evolutionary trend), functional, or taphonomic. In all likelihood a combination of factors gives rise to the patterns observed in tooth loss during fossilization, yet we aim to delineate the degree to which each of the following factors may have contributed.

4.4.1 *Phylogenetic signal*

Given that many traits in synapsid evolution show gradual, step-wise trends, proportion of teeth in the jaw of fossil synapsids may be influenced by similar patterning. We examined the mean proportion of teeth missing with the 30 terminal taxa assigned in Fig.1 as well as major clades of synapsids that have typically served as bins in examining trends along the synapsid line (e.g. Hopson 1969, Rubidge and Sidor 2003; Sidor 2003; Fig. 3). These groups include ‘pelycosaurs’, non-cynodont therapsids, non-eucynodont cynodonts, cynognathian cynodonts and probainognathian cynodonts. At a course scale, a general increase in proportion of missing teeth is observed with both groups of eucynodonts having higher averages than non-eucynodont cynodonts and other synapsids. This trend, however, has noted exceptions (e.g. tapinocephalids, probainognathian cynodonts) that suggest caution in assigning a broad trend.

Intraclade variation in proportion of teeth lost exists in taxa with an ankylosis as well as those with a gomphosis. Cynognathian cynodonts included in this study are uniformly described as having a gomphosis except for the current unknown character state for *Massetognathus*. Slight variation exists between taxa (F = 2.697; p = 0.0182) with no significant pair-wise comparisons. However, compared to other groups of synapsids, cynognathians appear to have a high degree of variation within terminal taxa (Fig. 1). Variation is also observed between taxa where ankylosis is the uniform character state for tooth attachment. Sphenacodontids were one of the few well-sampled families in these data with many specimens identified to the genus-level, rendering the group useful for intraclade analysis at a particularly fine scale. There is slight variation between clades (F = 5.243; p = 0.0023) with *Sphenacodon* having slightly lower proportion of empty sockets than *Dimetrodon* (p = 0.026) and *Secodontosaurus* (p = 1.109e-03) (Fig. 4b). It is worth noting, however, that these differences in a biological context are relatively minor where the differences in proportion of missing teeth would be by only a socket or two.

We conclude that while generally, there is an increase in proportion of teeth missing in synapsid fossils, extensive variation exists at many levels of clade classification. While this variation does not support a common trend, it may in fact, provide more detail about how this preservational feature can serve as a proxy for dental features. As such, we explored functional correlates that may help to explain the loss of teeth in fossil jaws.

4.4.2 *Functional signal*

A possible explanation for the discrepancy in proportion of teeth lost may be that a functional aspect of the dentition is driving the loss of teeth. Tooth attachment can explain some, but not all of the variation observed in these data. While taxa with an ankylosis are relatively uniform in proportion of empty tooth positions, those in a gomphosis are notably varied. Thus, in taxa with a known permanent gomphosis, we examined frequency of replacement, enamel thickness, enamel microstructure, diet, and PDL width as possible alternative explanatory variables.

Proportion of positions in replacement. —To test for the effects frequency of replacement on proportion of missing teeth, both sets of specimen-based data were compared to one another. Overall there is a slightly positive relationship between proportion of empty sockets and proportion of positions under replacement (p = 2e-16), however replacement explains very little of the variation in retention of functional teeth in the jaw ($R^2 = 0.001885$) and is poorly correlated (Kendall’s: p = 0.3787; Spearman’s: p = 0.3971; Fig 5a). When analyzed relative to attachment, taxa with an ankylosis do not have a significant relationship between replacement and retention ($R^2 = 0.0005662$; p = 0.7733) and are poorly correlated (Kendall’s: p = 0.6107;

Spearman's: $p = 0.602$) while taxa with a gomphosis have a slightly positive relationship ($p = 0.006318$; $R^2 = 0.05769$) (Fig. 5b). Furthermore, in taxa with a gomphosis these two traits are correlated (Kendall's: $p = 0.000785$; Spearman's: $p = 0.001329$).

Replacement alone has a weak relationship with proportion of empty sockets, explaining very little of the variation in proportion of teeth lost. It is interesting to note, however that delineating traits like attachment may reveal significance in this relationship. A significant relationship in taxa with a gomphosis exists with more empty sockets increasing the number of positions in replacement although generally, increases in the proportion of positions in replacement are only partially explained by proportions of empty tooth positions.

Enamel Thickness.—Enamel thickness, as categorized by Sander (1997), has significant differences in the average proportion of empty sockets (F = 15.46; $p = 1.37\text{e-}09$) (Fig.6a). Specimens of taxa with thick enamel had the highest average proportion of empty sockets (0.390) compared to intermediate (0.037; $p = 0.00028$) and thin enamel (0.172; $p = 1.0\text{e-}07$). Although thin enamel appeared to have a slightly higher proportion than intermediate enamel, this difference is insignificant ($p = 0.381$).

Enamel Type.—Three types of enamel have been recorded in the taxa included in this analysis: prismless, synapsid columnar enamel (SCE), and prismatic. Of note is that only one taxon (*Pachygenelus*) had prismatic enamel and only seven specimens were available. As such, comparisons between prismatic enamel and other categories must be considered tentative. Overall variation in proportion of empty sockets between groups was significant (F = 17.34; $p = 1.13\text{e-}10$) with the highest proportion in prismless enamel (0.450) which is significantly different than SCE (0.166; $p = 1.0\text{e-}10$) and prismatic (0.076; $p = 0.008$) (Fig. 6b). There was no statistical significance between SCE and prismatic ($p = 0.852$).

Inferred diet.—Three broad diet categories (herbivore, carnivore, and omnivore, although only three taxa were assigned to the latter) determined by crown morphology, showed significant variation in the proportion of empty positions (F = 23.46; *p* = 2.01e-10) (Fig. 6c). Omnivores had a higher average proportion (0.412) that is significantly different from that of carnivores (*p* = 1.1e-06) but not herbivores (*p* = 0.107). Carnivores had the lowest average proportion (0.143) that was also significantly different from herbivores (0.308; *p* = 1.0e-07).

PDL width.—A hypothesis, put forward in Chapter 1 (Whitney and Sidor 2019) is the width of the periodontal space, could influence the ease with which a tooth might fall out once the anchoring soft-tissue of the PDL was degraded. Experimental tests on the PDL in modern species have demonstrated changes in width in response to force experienced by the tooth. Increased occlusal force results in widening of the PDL as the result of increased production of bone resorbing factors like osteoclasts and osteopontin (Ramfjord and Kohler 1955; Wentz et al. 1958; Kaku et al. 2005). When functional loads are less frequent, the width of the PDL is reduced occasionally resulting in ankylosis (Sringkarnboriboon et al. 2003). Thus, PDL width differs with the force applied. Sustained occlusal forces result in wider widths whereas intermittent force does not experimentally show as much resorption of bone resulting in a wider PDL width (Kaku et al. 2005).

If these experimental data are applicable to function of the dentition (i.e. repeated vs. intermittent occlusal force), PDL space, as potentially ascertained from proportion of teeth lost, in fossils may serve as a novel proxy for function. Preliminary data collected here suggest a tentative positive relationship between PDL space in fossil synapsids and proportion of missing teeth ($R^2 = 0.4924$; *p* = 0.0161; Fig. 8). Although both rank correlation results yield slightly insignificant results (Kendall's test: $p = 0.0866$; Spearman's test: $p = 0.09364$), these preliminary data are intriguing for continued investigation.

If PDL space is associated with function, an additional possible difference may be seen along the tooth row and may be able to act as an additional test for the relationship between function, PDL width and proportion of empty tooth sockets. Our data yield consistent differences between diet categories with herbivores and omnivores losing a higher proportion of teeth than carnivores in all regions (Fig. 9d-f), however differences in the three regions of the jaw are insignificant when separated by diet category (Fig. 9a-c). These preliminary results suggest that diet may influence retention of teeth in a fossil jaw, but that regional differences are insignificant.

4.4.3 *Taphonomic signal*

An abiotic explanation for the preservation of teeth in fossil jaws is the geological setting of each specimen (i.e. formation and deposition). Although generally a limited subset of these data, two tests were conducted inspecting 1) differences among taxa that were recovered from a single formation and 2) similar taxa that were recovered from different formations. The first test was run using specimens from the middle Permian Abrahamskraal Formation of the Karoo Supergroup of South Africa. This formation contains multiple taxa that employed both a gomphosis (i.e. tapinocephalids) and an ankylosis (i.e. anteosaurs, scylacosaurids, and titanosuchians) and provide a test for consistency of results from the combined data in a subset of the data from the same formation. Although a small sample was available (and no statistical significance in variation $F = 2.537$; $p = 0.0761$), the high proportions observed in tapinocephalids and low proportions in the other groups remain consistent within a formation (Fig. 7a). The second test was run with sphenacodontids which are known from several formations (Fig. 7b).

No significant variation was recovered (F = 1.358; p = 0.211) also suggesting little systematic effect, if any on depositional setting and retention of teeth in fossil jaws. However, the current sampling is limited, especially for a taxon with a known gomphosis from several formations, such that additional testing of the effect of depositional setting is desirable.

4.4.4 *Significance of proportion of empty tooth positions*

The significant differences observed in proportion of empty tooth sockets in fossil synapsid jaws is likely the product of several factors. Here, we demonstrate weak phylogenetic and taphonomic signals and instead find greater support for correlations with functional aspects to the dentition, in taxa with a permanent gomphosis. Diet seems to have some effect with herbivores and omnivores losing more teeth than carnivores. Enamel also correlates with tooth loss in that thick prismless enamel has higher average proportions of teeth missing. These data combined suggest herbivorous/omnivorous taxa (tapinocephalids, *Diademodon*, *Trirachodon*, *Cricodon*, *Scalenodon*, *Exaeretodon*, *Aleodon*, *Tritylodon*) with thick (tapioncephalids, *Tritylodon*), primsless (tapinocephalids) enamel are more likely to lose teeth during fossilization.

These relationships are further supported by the possible connection between the frequency of force and PDL width as it relates to retention of teeth in the jaw. Under this hypothesis, strictly carnivorous taxa that did not apply repeated force to their teeth may acquire a gomphosis, but maintain a narrow PDL that cushioned piercing. Grinding herbivorous taxa would be expected to have wide PDLs to accommodate the sustained force imposed upon these teeth, albeit at a significant metabolic cost given the physiological demands required for PDL homeostasis. As such, post mortem retention of teeth could act as a proxy for function with herbivorous grinding or chewing dentitions more likely to lose teeth post-mortem than those that intermittently experienced force.

4.5 CORRELATION AND EVOLUTIONARY PATTERNS OF DENTAL CHARACTERS

In compiling data to test for explanations for proportion of teeth lost, several critical, additional questions can be tested surrounding the co-evolution and trends in trait acquisition along the synapsid line. Data on proportion of tooth positions in replacement were tested similarly to proportions in empty tooth sockets to test for relationships with diet and enamel. Furthermore, limited, but curious correlations between diet and enamel were explored as well as individual and combined trait patterns in the dentition of synapsids.

4.5.1 *Proportion of positions in replacement*

The proportion of positions in replacement was less varied than when comparing proportions of empty sockets, but was nonetheless significantly varied between taxa (F = 4.804; *p* = 1.2e-12; Fig. 10). Significantly high proportional replacement was recorded in tapinocephalids and sphenacodontids when compared to some eucynodont taxa, which tended to have less evidence of replacement events. There is a general reduction in proportion of positions in replacement at course and fine phylogenetic scales suggesting an overall shift towards reduced replacement in therocephalians and cynodonts (Fig. 11) similar to observations made by Kermack (1956). Within the well-sampled sphenacodontids, replacement yielded no significant variation (F = 2.644; *p* = 0.0552; Fig. 12). *Dimetrodon* replacement does appear to have peculiar specimens with high proportion of positions in replacement. It is the only genus among all synapsids studied here to have a specimen with all of its tooth positions in some form of replacement (see Fig. 1). Whether the statistical insignificance recorded here results from these specimens truly representing outliers or a small sample size requires additional testing.

Replacement frequency and number of replacements in the lifetime of an individual is a particularly challenging trait to study in fossil taxa, especially when an ontogenetic series is not available. While proportion of positions in replacement is not a direct proxy for either feature, it can serve as at least a baseline comparison between a wide range of taxa with varying sample sizes. There are many caveats to using this measure to study replacement frequency, most significantly the possibility of replacement that occurs in a position other than lingual to the functional position. Alternatives include horizontal replacement where teeth are added posteriorly (Domning and Hayek 1984; Rodrigues and Sumbera 2015) and crocodilian-style replacement that happens in the same tooth socket (Poole 1961), both of which would not be readily apparent without histological investigation. Horizontal replacement has been reported for some traversodonts (Hopson 1971) and some tritylodonts (Cui and Sun 1987), however the dynamics of replacement are difficult to confirm without internal observations of the jaw. Yet, even in taxa that do to not have horizontal replacement (e.g. *Aleodon*), low rates of replacement were still recorded.

Functional signals were generally significant, yet minimal in their effects on the proportion of positions in replacement. A statistically significant difference between the average proportion of positions in replacement between specimens with an ankylosis (0.219) compared to those with a gomphosis (0.133) (p = 0.001757; Fig. 13a). Enamel thickness reported significant variation (F = 13.76; p = 1.8e-08) (Fig. 13b), yet pairwise differences were not significant (p-vals: thick – intermediate = 0.337; thin – intermediate = 0.683; thick – thin = 0.195). Replacement was varied with enamel type (F = 13.03; p = 4.66e-08) with only the means between prismless (0.275) and SCE (0.175) having significant differences (p = 0.016) and prismatic (0.057) having no significant differences perhaps due to sample size (p val: – SCE =

0.530; – prismless = 0.077; Fig. 13c). Finally, while slightly significant (p = 0.0487), low variation (F = 3.051) and lack of significant differences between categories (p-val: H – C = 0.191; O – C = 0.08; O – C = 0.43) fail to support a relationship between diet and replacement (Fig. 13d).

There is a significant difference among taxa in the proportion of tooth positions under replacement. While statistically this difference is significant, when framed in a biological context the difference is minimal. Sphenacodontids and tapinocephalids have the highest average proportion of teeth in replacement (approximately 0.23) that is significantly different from the rest of taxa sampled here (approximately 0.14). This means that if given a tooth row of ten tooth positions, the difference between the highest and lowest replacement would be the difference of about one position. By contrast, under the same scenario when looking at differences between proportion of empty sockets (Fig. 1), the highest would be about four empty positions compared to the lowest average of about one empty position. Given this lack of variation in replacement, it is unsurprising that diet, enamel thickness, and enamel type have less significant differences than those observed in retention.

4.5.2 *Correlations between discrete phenotypes*

Generally, there is little correlation between dental traits in the taxa studied here (Fig. 14). Importantly, in reference to the PDL, a gomphosis co-occurs with every phenotype of the other dental traits. Perhaps the only linked traits may be enamel and diet which is intuitive. Although limited in sample size, preliminary analyses between enamel and diet (reduced to herbivores and carnivores due to small omnivore samples) find tantalizing correlations that may be applicable in understanding the linkage between the suite of traits that give rise to a dentition. Correlations between phenotypes assigned to each taxa are statistically weak, but currently it is uncertain if this result is representative or an artifact of sampling, considering enamel data was only available for a handful of taxa. There does appear to be a tendency for herbivores to have thick prismless enamel, yet this conclusion is still preliminary (Fig. 15).

4.5.3 *Evolutionary patterns in the dentition*

A hallmark of synapsid paleontology has been the evolutionary trends synapsid fossils have revealed. To test for evolutionary patterns in the traits studied here, we can establish three hypothetical trends 1) step-wise where either individual traits evolved gradually or a suite of traits evolved in lockstep resulting in a combination of derived phenotypes, 2) wholesale shifts in individual traits and suites of traits would be expected, and 3) no pattern where there is no phylogenetic trend in trait acquisition (Fig. 16). Using the data compiled here we can assess trends in both the individual and combination of traits that make up synapsid dentitions.

The dentition is comprised of a suite of features with varying data availability for the taxa studied here. We subsampled terminal taxa to include only those who had known attachment and enamel type phenotypes as well as proportion of replacement data. The result for dentition-wide pattern analyses includes 14 nonmammalian synapsids that were scored for character states. In this framework we assigned low character values to the plesiomorphic stereotyped reptilian condition and high character values to the derived mammalian condition (see Supplemental). The result at coarse and fine taxonomic scales appears most similar to a step-wise pattern where incremental increases in the “derived score” occur along the synapsid lineage ultimately giving rise to the high score in mammals (Fig. 17). Interestingly, although the broad trend appears directional (i.e. mainly increases in score), the relative homogeneity of increases (6), decreases(3) and stasis (4) in scores at a finer taxonomic scale suggest this trend may be passive (McShea 1994). Although interpretations from this analysis are limited, these three dental traits do appear to give rise to the step-wise pattern commonly ascribed to synapsid evolution.

We constructed additional visualizations of trends by isolating each trait to test for patterns individually, again reducing terminal taxa that lacked assigned discrete phenotypes (Fig. 18). Enamel type and replacement pattern appear to have at least some degree of step-wise patterning (Hypothesis 1) with interesting exceptions like prismless enamel in *Procynosuchus* and Tapinocephalidae that are best interpreted as having secondarily lost the columnar structure ascribed as an intermediate between prismless and prismatic enamel (Sander 1997). The mechanism of step-wise patterning in either trait as gleaned from constructed trend lines, however, appears slightly different. Enamel type is characterized by long periods of stasis (10 steps) with occasional increases (4) and decreases (3) in evolutionary trends towards the mammal condition. Replacement, on the other hand, is characterized by equal increases (10) and decreases (13) with only a few moments of stasis (3) suggesting a passive trend toward reduced replacement frequency. Enamel thickness and proportion of empty sockets do not appear to follow any trend along the synapsid lineage (Hypothesis 3) suggesting flexibility in the character state that may be influenced by the acquisition of other characters.

Finally, attachment reveals an interesting combination of trends that change at different taxonomic scales. At the coarse taxonomic scale, trends show a general increase (6 steps) towards a gomphosis with extended periods of stasis (20) and only a few decreases away from the derived character state (2), suggesting whole-sale (Hypothesis 2) directional changes. These dips are restricted to non-eucynodont cynodonts which is peculiar and worth further investigation. At a finer taxonomic scale, a permanent gomphosis was acquired and subsequently fixed independently in the derived members of major therapsid clades (e.g. in dinocephalians, therocephalians, and eucynodonts) with no reversions to an ankylosed dentition (except in early cynodonts as previously mentioned) suggesting this shift was directional not only at the broad synapsid scale, but the finer clade-level scale as well.

These patterns reveal two important conclusions. First, the dentition of synapsids appears to have evolved in a step-wise trend not necessarily because of the acquisition of new phenotypes, but because of the fixing of certain phenotypes, some directionally and some passively, that were more variable in therapsids. Traits like a permanent gomphosis, reduced replacement, and enamel structure had a variety of character states that in eucynodonts become relatively uniform. Second, these data demonstrate the importance of considering evolutionary trends within therapsid clades as many character states vary at this scale and thus, assigning a character state to an entire clade of therapsids oversimplifies patterns.

4.5.4 *Relationship to Correlated Progression*

Kemp (2007) suggested that synapsid evolution was governed by a principle termed correlated progression. Under this hypothesis, selection acts on integrated suites of traits and no one structure is paramount. Thus, small changes in traits occur which differs from patterns expected by a series of key innovations giving rise to the mammal form. Under correlated progression, at any given time, only a small number of phenotypes are under directional selection while the others are under stabilizing selection. If traits are flexibly integrated such that small variation does not affect fitness, then these traits are considered neutral and variation in phenotype can be fixed by genetic drift. Thus, a variety of phenotypes can exist in a variety of combinations. Changes made possible by flexible integration and variation of phenotypes may eventually stiffen links between traits such that no further change is possible without reducing the fitness of the system.

The dental data presented here, while preliminary, support Kemp's hypothesis. It is evident that while some traits may be correlated once additional data are added, the overall pattern is that there are many unique combinations of traits that make up synapsid dentitions. Replacement and enamel type along the synapsid line appear to change such that they become relatively fixed (although some variation does occur in modern mammals). Similarly, gomphosis eventually becomes a fixed trait in derived taxa, at the intra- and interclade scale. The temporal and/or phylogenetic order in which these traits become fixed may be able to shed light on the linkage between dental features. For instance, reducing frequency of replacement is likely predicated on selective pressures to build a durable tooth that can function for extended periods. Thus, it would be no surprise that crack-resistant prismatic enamel and a gomphosis providing cushioning and the mobility to maintain occlusion become fixed with reduced replacement. Worthy additional lines of investigation are the relationship between these dental features and growth patterns, especially as it relates to hypothesized origins of synapsid endothermy in therapsids, cynodonts, and mammals.

Trends and patterns of dental character evolution can inform the idea of "mammalness" and acquiring mammal-like characters. Luo (2007) described key innovations such as the middle ear and molar morphology as being the product of parallel evolution and iterative acquisition of these traits early in mammal evolution. He noted that temporal moments of diversification were accompanied by increased instances of homoplasy. For early mammals, their Mesozoic diversification was riddled with homoplasy and it is possible that homoplasies early in therapsid evolution were governed by similar principles. Pelycosaur-grade synapsid morphology is generally low in disparity compared to therapsids, suggesting ecomorphological diversification during the middle-late Permian (Rubidge and Sidor 2001; Lungmus and Angielczyk 2019).

Diversification of therapsid clades operating under properties of correlated progression may work in concert give rise to the patterns observed here. If considered in a temporal context, Permian synapsids had diverse combinations of traits, some of which were lost in the Triassic (Fig. 19). This general reduction in combinations could be the result of merely time with the tightening of linkage between certain traits or could have been influenced by the Permo-Triassic mass extinction that placed selective pressures on specific traits and/or combinations of traits. If the patterns taxonomically and temporally continue to support the hypothesis of correlated progression, "mammalness" as it refers to the dentition is not necessarily the acquisition of a suite of novel traits, but rather the product of 300 million years of dental traits evolving together with increased linkage between phenotypes.

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4.8 FIGURES AND TABLES

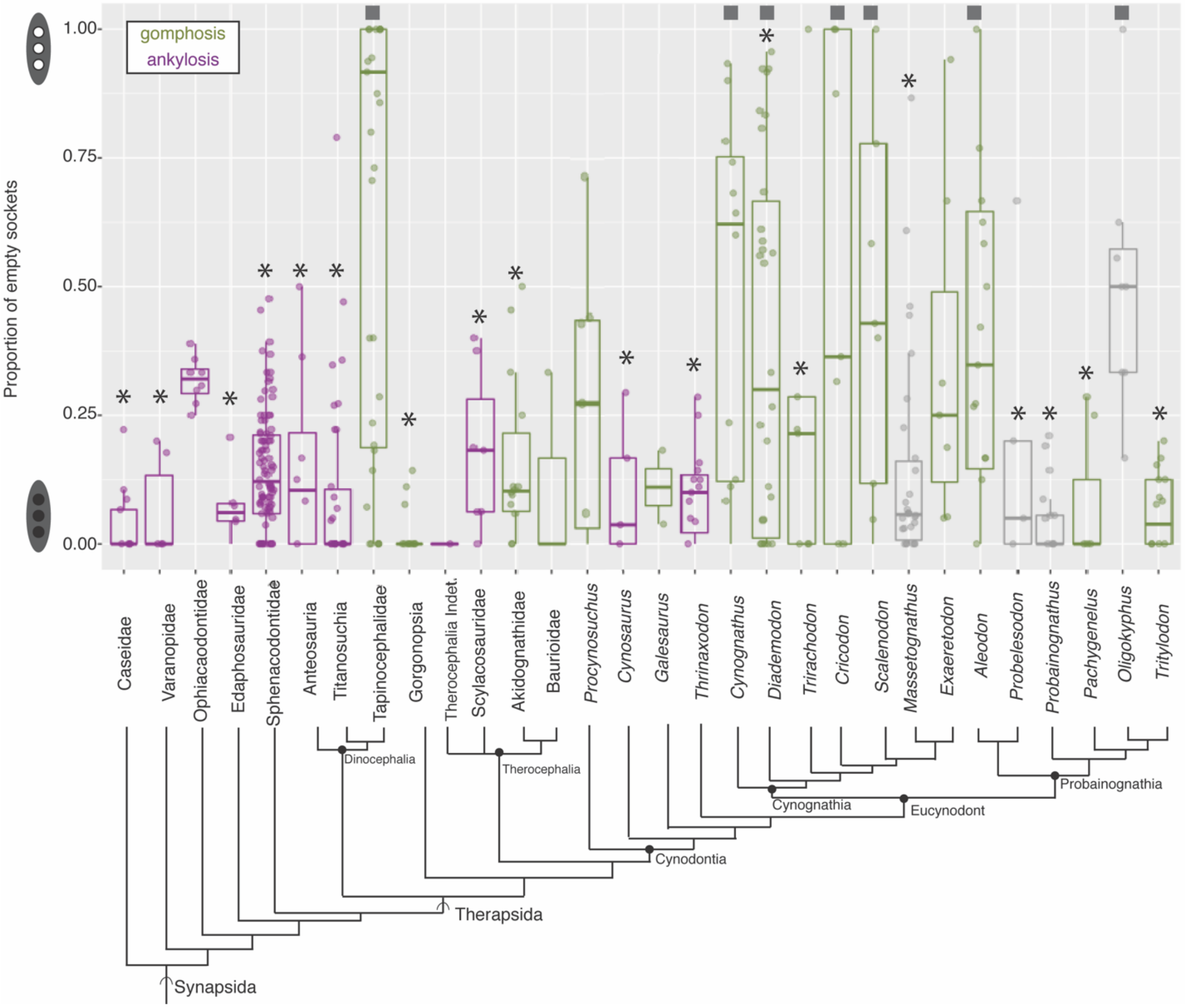


Figure 4.1. **Proportion of empty tooth positions by taxa.**

Colors indicate attachment with greyed taxa in unknown attachment. Asterisk indicate taxa that have a significantly lower mean than tapinocephalids and squares indicate taxa that have a significantly higher mean than gorgonopsians.

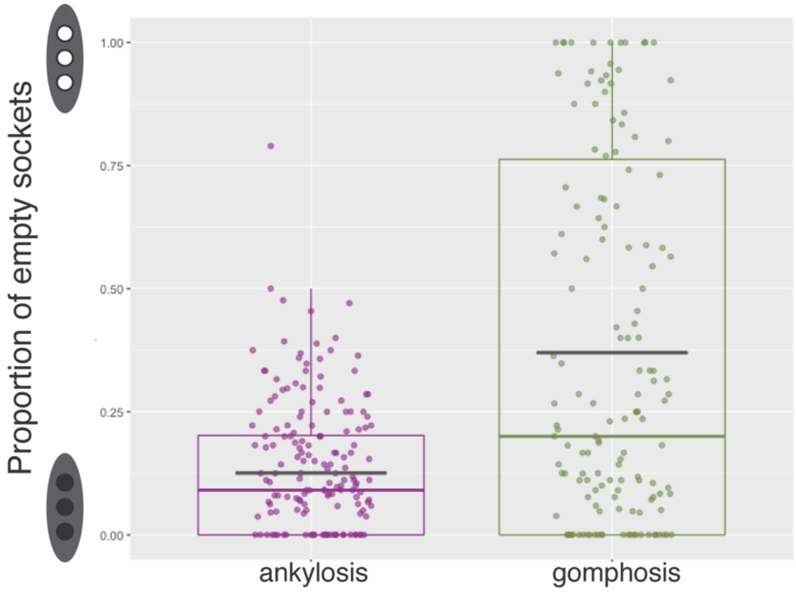


Figure 4.2. **Proportion of empty sockets as a proxy for tooth attachment.**

Significant influence of tooth attachment on proportion of empty sockets with specimens in a gomphosis having a higher average proportion empty (p = 7.036e-15). Black bars represent mean values for each category.

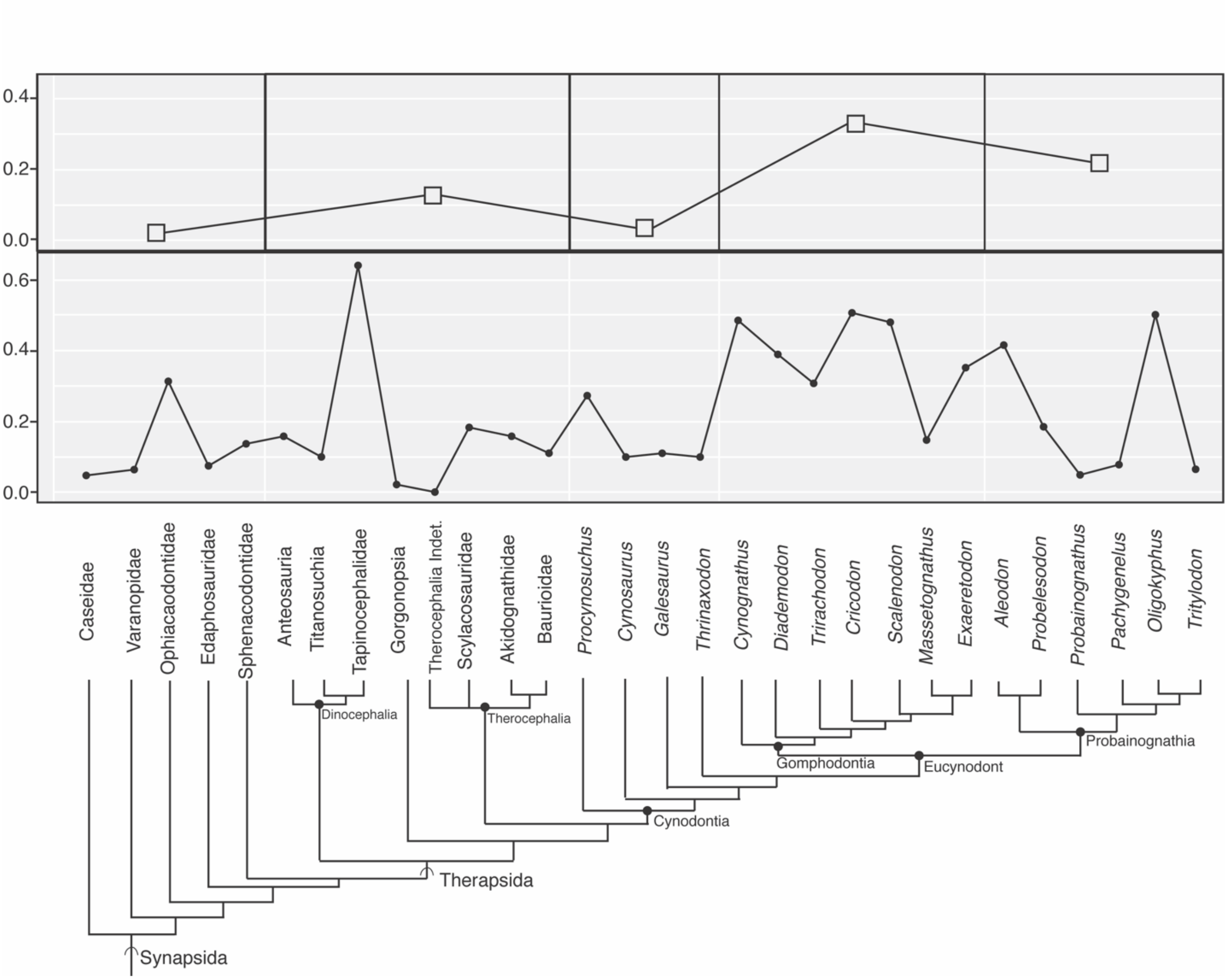


Figure 4.3. **Evolutionary trends in proportion of tooth attachment.**

Although average proportions missing increase along the synapsid phylogeny (top), averages in terminal taxa are highly variable (bottom). Taxonomic bins used in top trend line moving left to right are pelycosaur-grade, therapsid, non-eucynodont cynodont, cynognathian probainognathian groups.

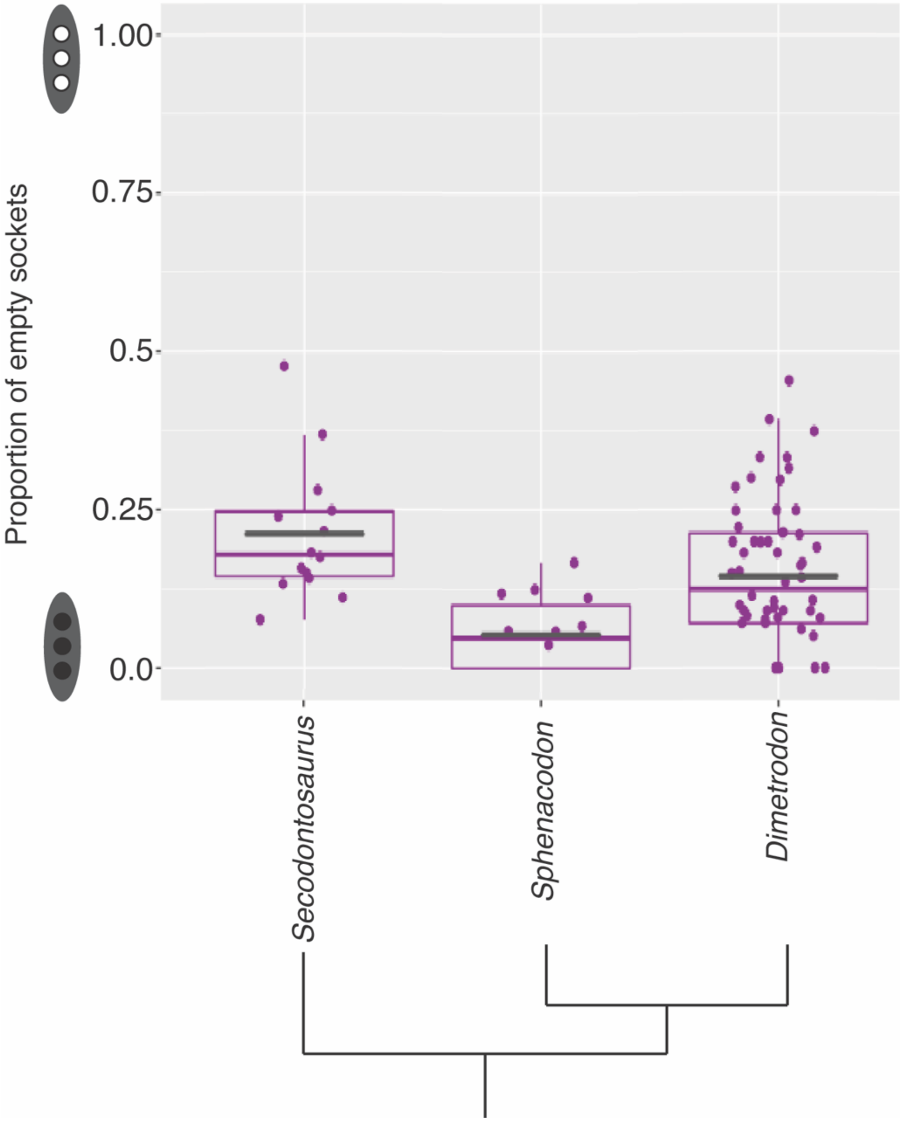


Figure 4.4. **Intraclade variation in the well-sampled family Sphenacodontidae for proportion of empty sockets.**

Sphenacodon had a significantly lower average proportion of empty sockets than *Dimetrodon* ($p = 0.026$) and *Secodontosaurus* ($p = 1.109e-03$), although differences in means are relatively small in a biological context.

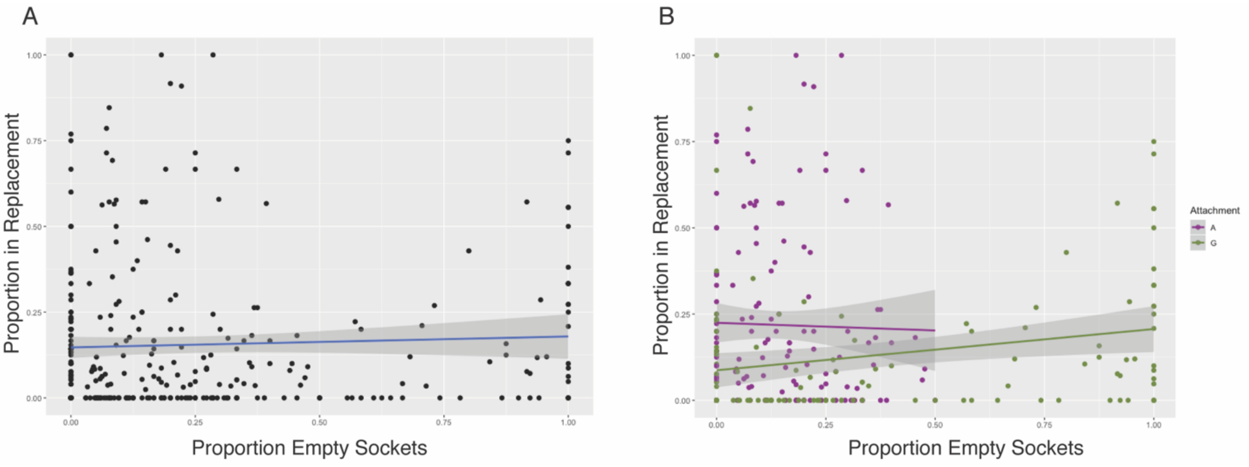


Figure 4.5. **Relationship between proportion of empty tooth positions and in replacement.** (A Proportion in replacement explains very little of the proportion of sockets that were recorded with missing teeth and is insignificantly correlated. B) When the data is separated by attachment strategy, ankylosed specimens have no significant relationship, while specimens with a gomphosis have a slightly positive relationship (R^2= 0.05769; p = 0.006318) that is correlated (Kendall's: p = 0.000785; Spearman's: p = 0.001329).

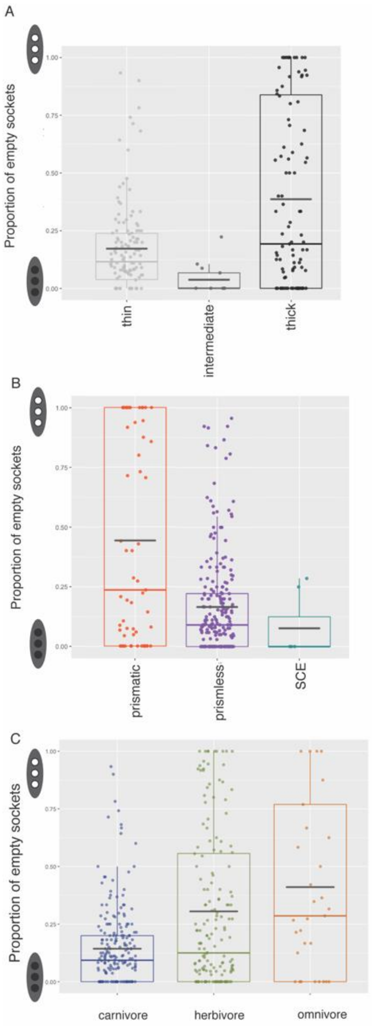


Figure 4.6. **The relationships of functional variables to proportion of empty tooth positions.** A) Variation in empty sockets was significantly varied among specimens with different enamel thicknesses (F = 15.46; p = 1.37e-09) with thick enamel having the highest proportion of empty sockets compared to intermediate (p = 0.00028) and thin (p = 1.0e-07) enamel. B) Enamel types were significantly varied in the proportion of empty tooth positions (F = 17.34; p = 1.13e-10) with prismless having a higher mean proportion than SCE (p = 1.0e-10) and prismatic enamel (p =0.008). C) Significant differences between diet categories were recorded with omnivores and herbivores preserving more empty sockets than carnivores (p = 1.1e-06; p = 1.0e-07). Black bars represent group means.

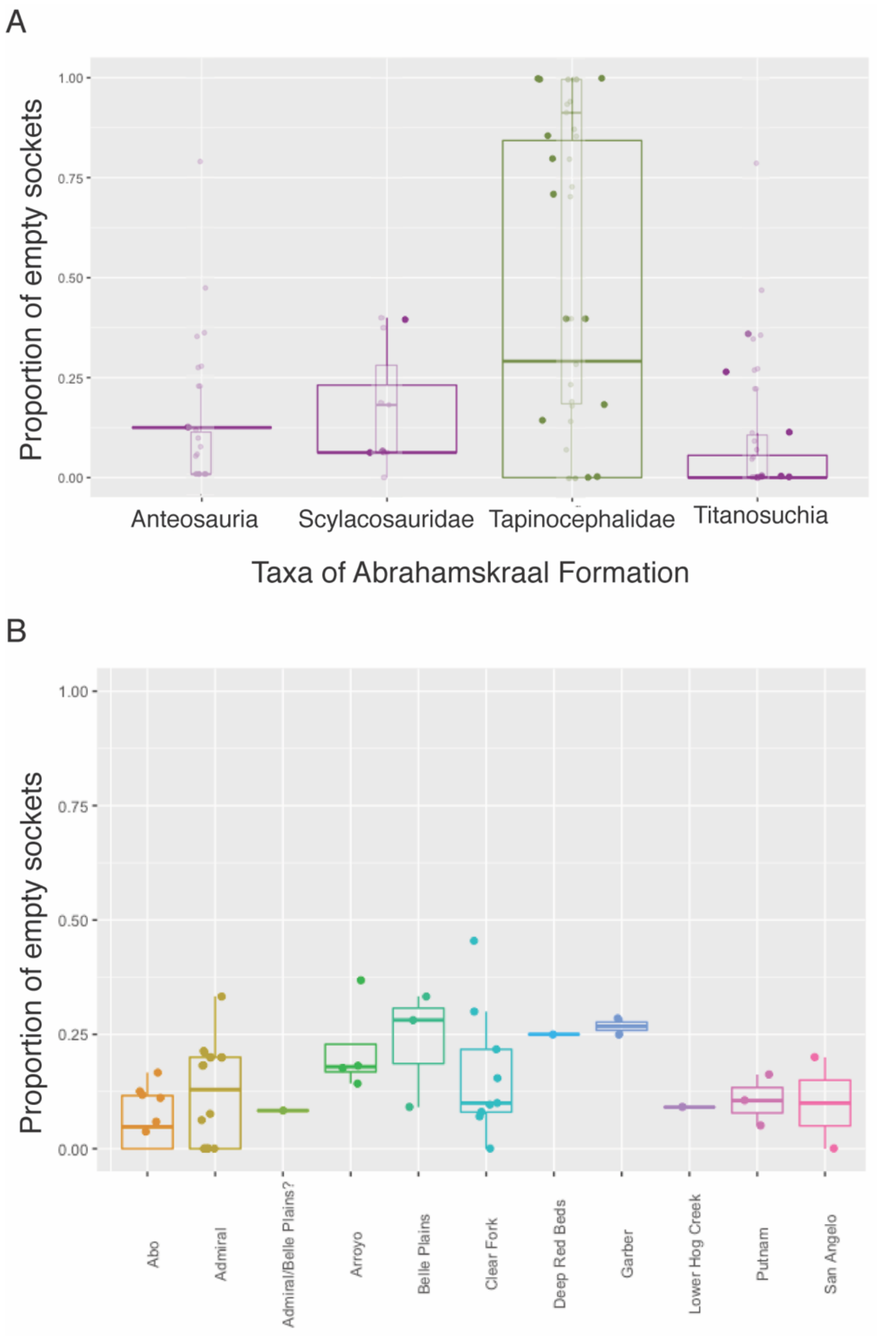


Figure 4.7. **The effect of abiotic, geological variables on proportion of empty sockets.**

A) In a single formation, Abrahamskraal Formation, taxa vary similarly to how they do in other formations (i.e. transparent box plots) and B) single taxon (sphenacodontids) in a variety of formations yielded no significant difference.

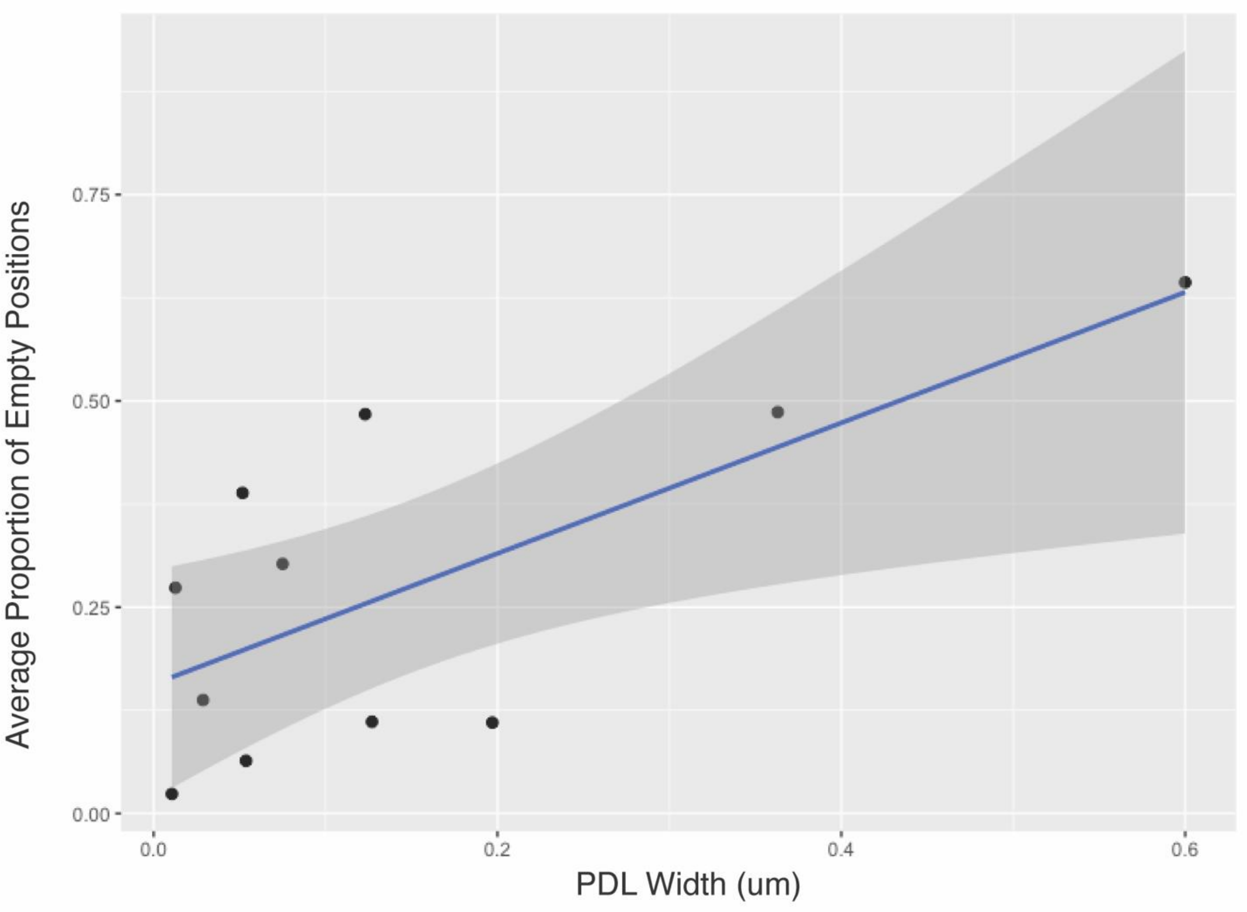


Figure 4.8. **Relationship of periodontal space (inferred PDL width) and proportion of empty tooth positions.**

A slightly significant positive relationship ($R^2 = 0.4924$; $p = 0.0161$) between PDL space and proportion of missing teeth. The small sample size presently available likely influences the slightly insignificant correlation between these two variables (Kendall's test: $p = 0.0866$; Spearman's test: $p = 0.09364$).

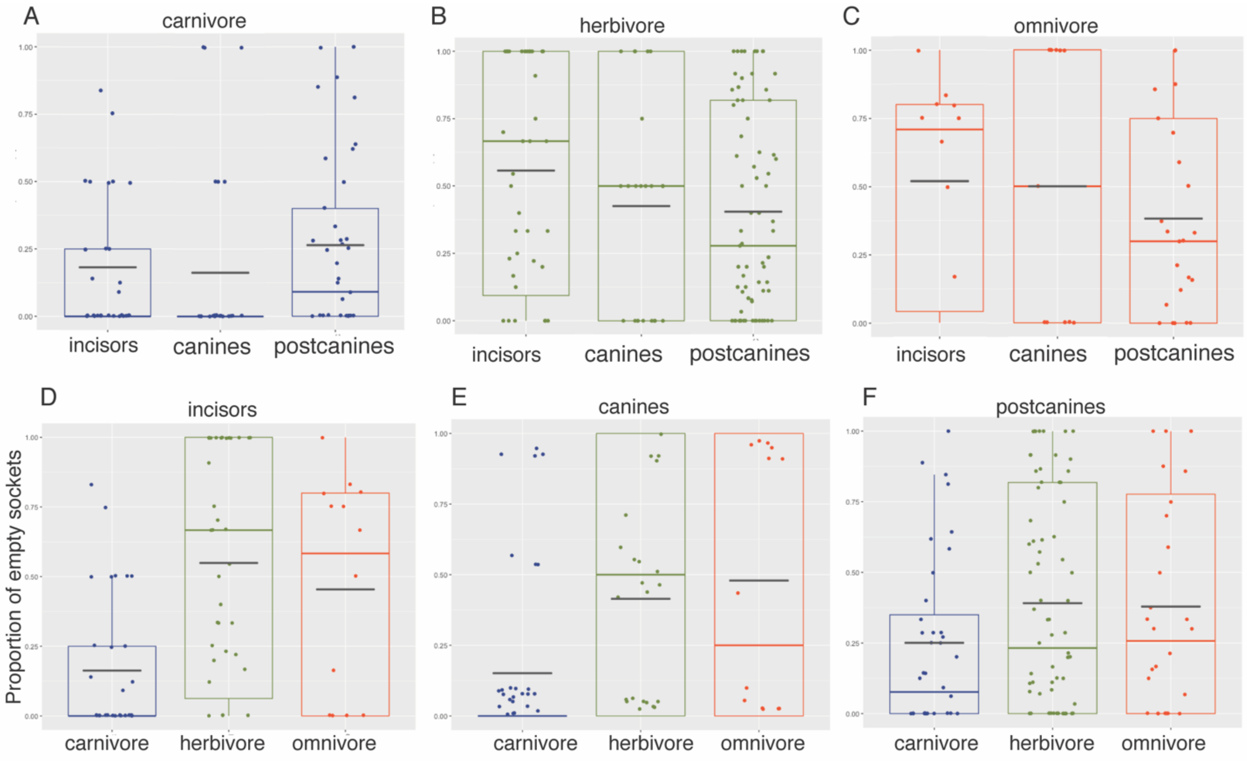


Figure 4.9. **Tests for functional differences in proportion of empty tooth positions in taxa with known gomphosis.**

A) Carnivore regionalization (F = 1.14; p = 0.324), C) herbivore regionalization (F = 2.322; p = 0.101) and D) omnivore regionalization (F = 0.502; p = 0.608) all lack significant variation in proportion of empty sockets. D) Incisors are more frequently missing teeth in herbivores (p = 1.63e-05) and omnivores (p = 0.0284) than carnivores. E) Canine positions are empty more often in herbivores (p = 0.0144) and omnivores (p = 0.0069) than carnivores. F) Variation in postcanine empty sockets is insignificant (p = 0.0966). Black bars represent group means.

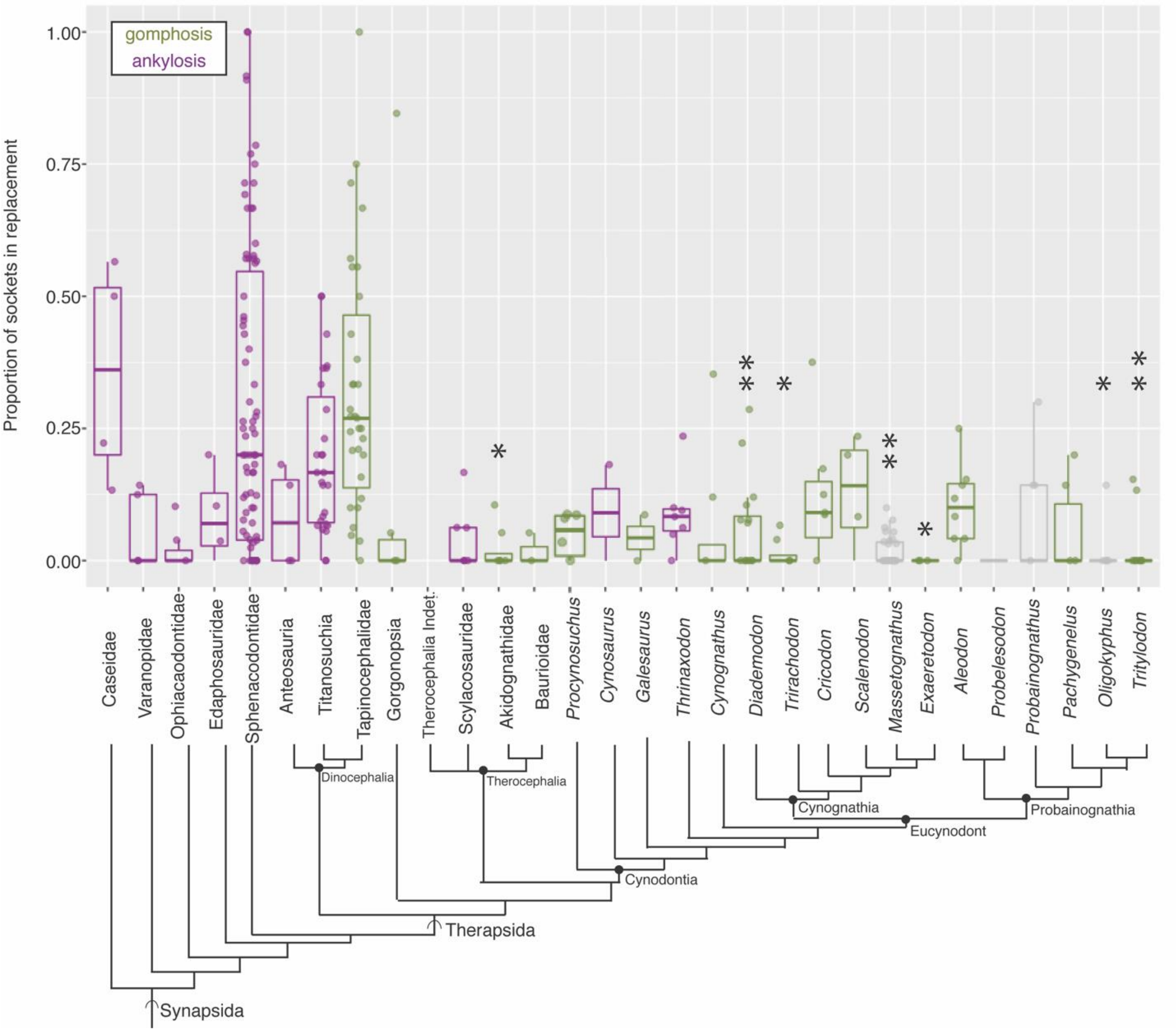


Figure 4.10. **Proportion of tooth positions in replacement by taxa.**

Attachment strategy indicated by color with grey taxa representing unknown attachment. Single asterisk indicates taxa that have a significantly lower mean proportion than tapinocephalids and two asterisks indicate taxa that have a significantly lower mean than tapinocephalids and sphenacodontids.

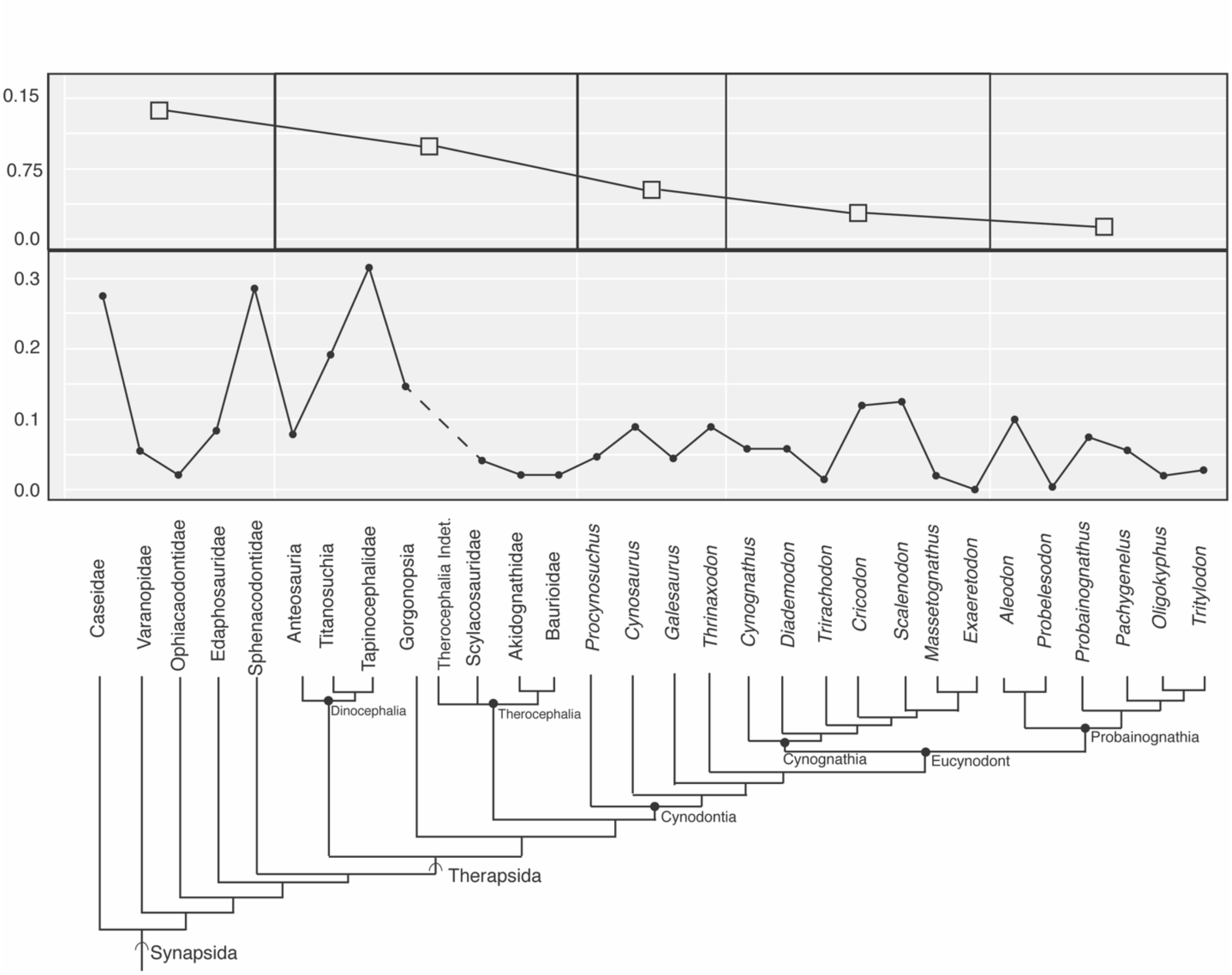


Figure 4.11. **Evolutionary trends in proportion of tooth positions under replacement.**

A general decrease in the average proportion under replacement is observed at both the coarse (top) and finer (bottom) taxonomic trends. Taxonomic bins used in top trend line moving left to right are pelycosaur-grade, therapsid, non-eucynodont cynodont, cynognathian probainognathian groups.

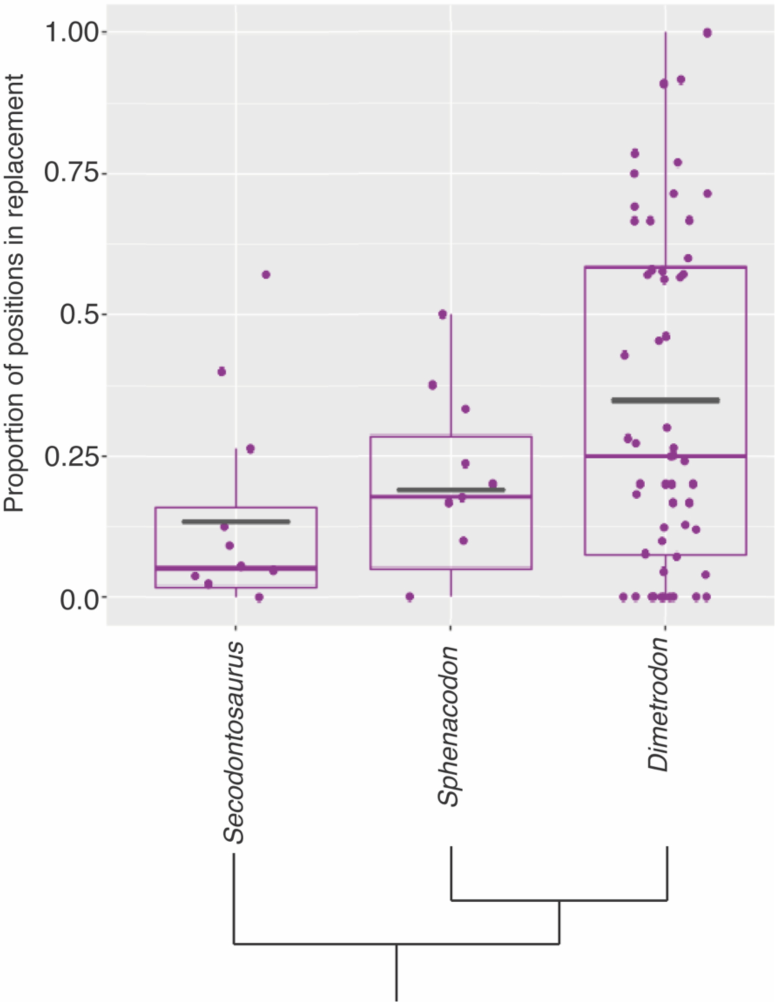


Figure 4.12. **Taxonomic differences in proportion of positions in replacement in Sphenacodontidae.**

No significant variation was observed in replacement (F = 2.644; p = 0.0552), however noted variation in *Dimetrodon* is peculiar.

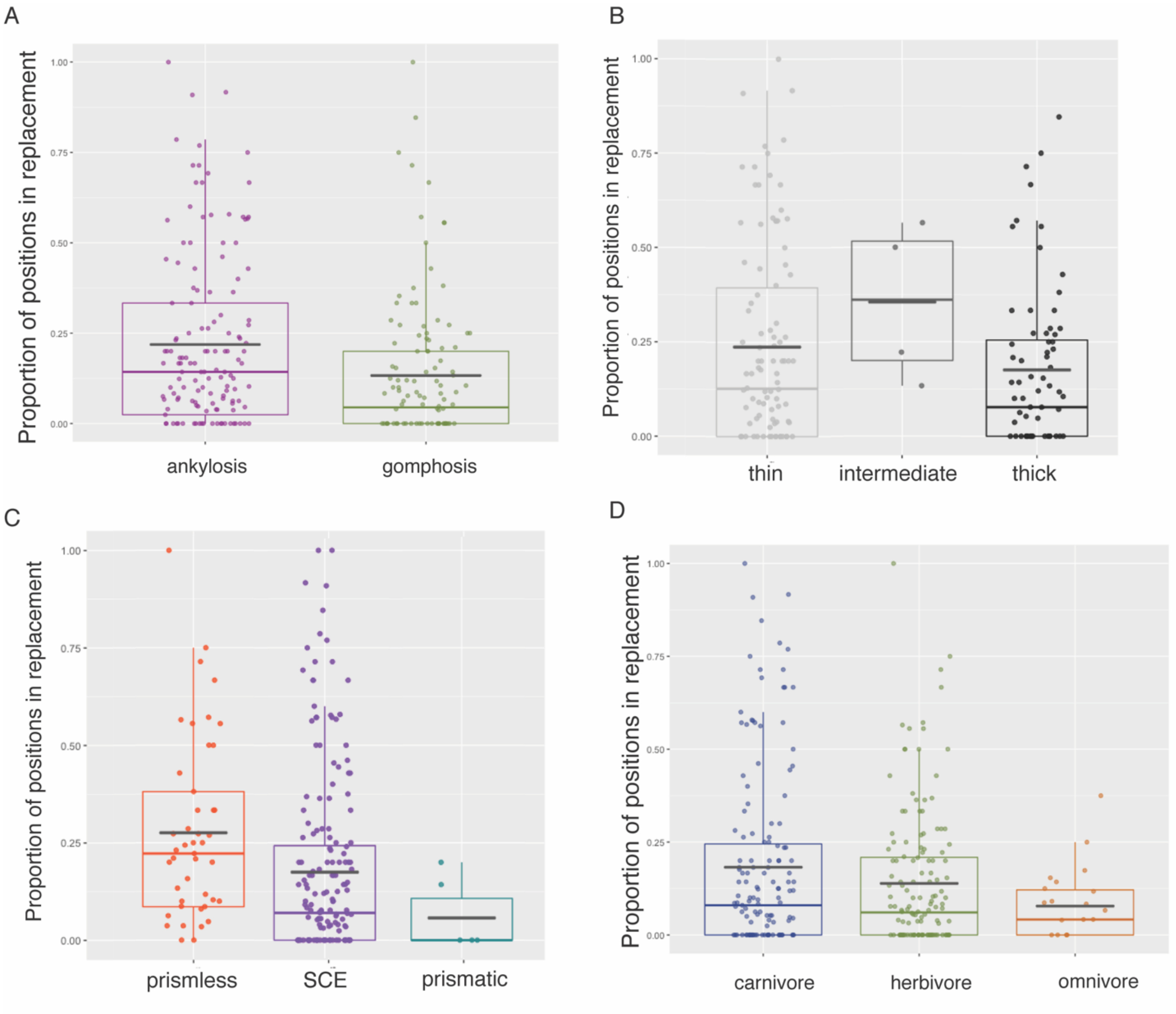


Figure 4.13. **Functional variables effect on proportion of positions in replacement.**

A) Proportion of positions in replacement was significantly higher in taxa in an ankylosis than those in a gomphosis (p = 0.001757). B) No statistical difference in replacement was reported for enamel thickness. C) Significant differences in the variation between enamel type in proportion of positions under replacement was recorded (F = 13.76; p = 1.8e-08) however, pairwise comparisons between means were insignificant. Significant variation between enamel structure and replacement were recorded (F = 13.03; p = 4.66e-08) yet, prismless enamel and SCE yielded the only significant difference in means (p = 0.016). D) No pairwise significance was recovered for differences in replacement between diet categories although overall the variation between categories was slightly significant with particular variation noted in carnivore dentitions (p = 0.0487). In all tests, black bars represent group means.

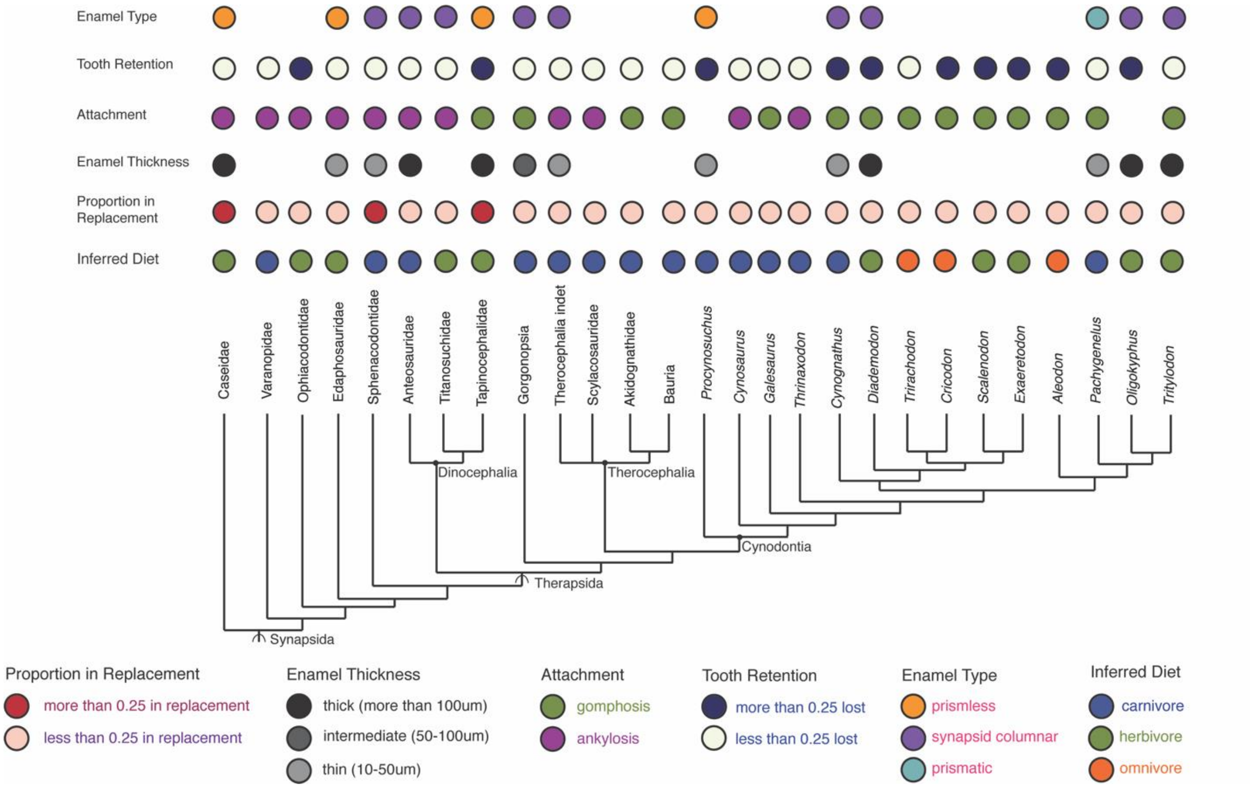


Figure 4.14. **Mapped characters and phenotypes on sample in a phylogenetic context.** Note the lack of co-occuring combinations of traits.

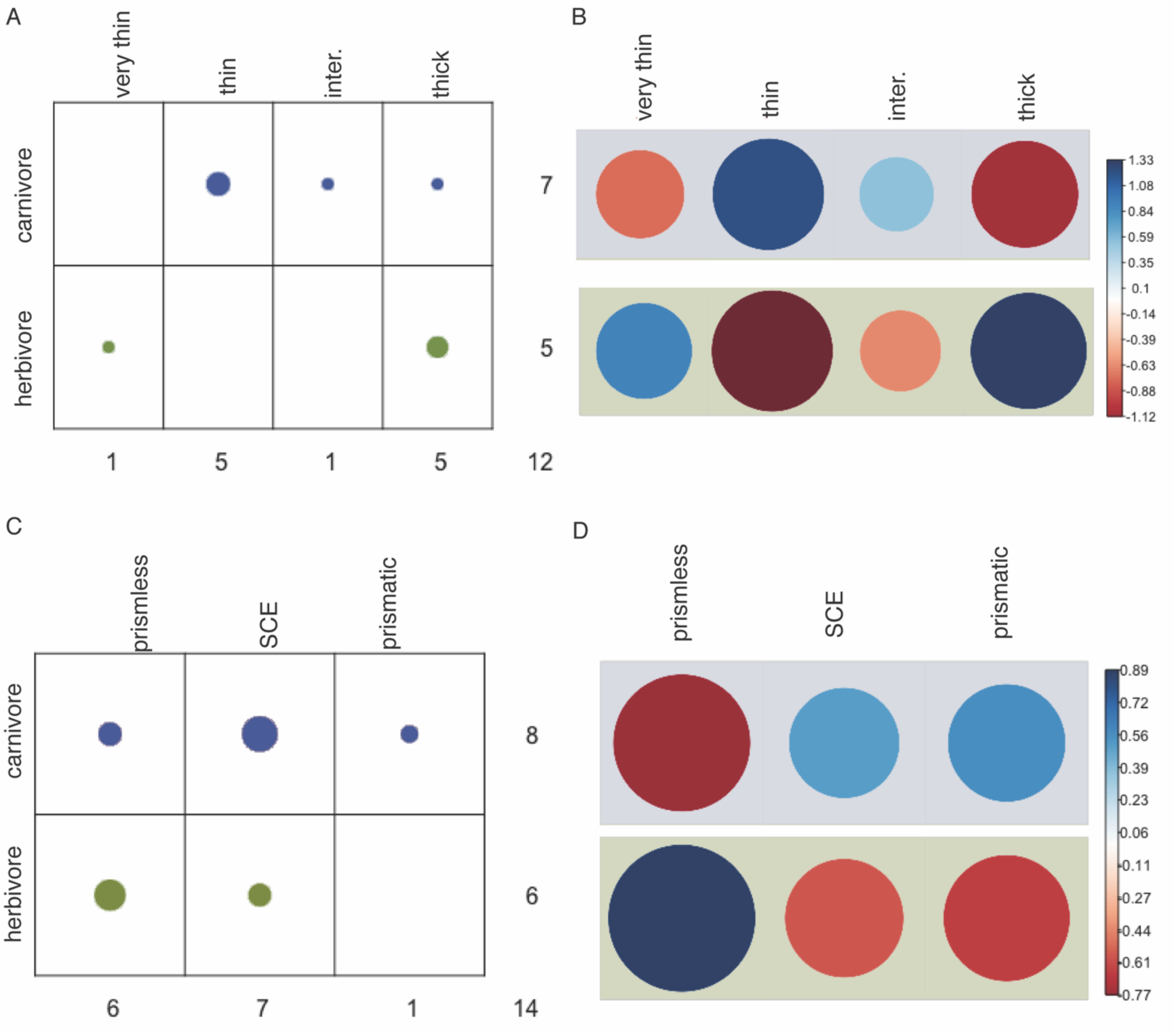


Figure 4.15. **Correlations between diet and enamel.**

Raw distribution of data represented in (A, C) and correlation matrices (B,D) denote details about the correlation. In (B) and (D) positive correlations are in blue and negative correlations are in red and both the color intensity and size of circle are proportional to the correlation coefficients. Enamel thickness (A-B) is not significantly correlated with diet in this preliminary data set ($p = 0.3483$) however, it is worth noting herbivores tend to have thicker enamel. Enamel type (C-D) has some significant correlation ($p = 0.03343$) with herbivore strongly correlated with prismless enamel.

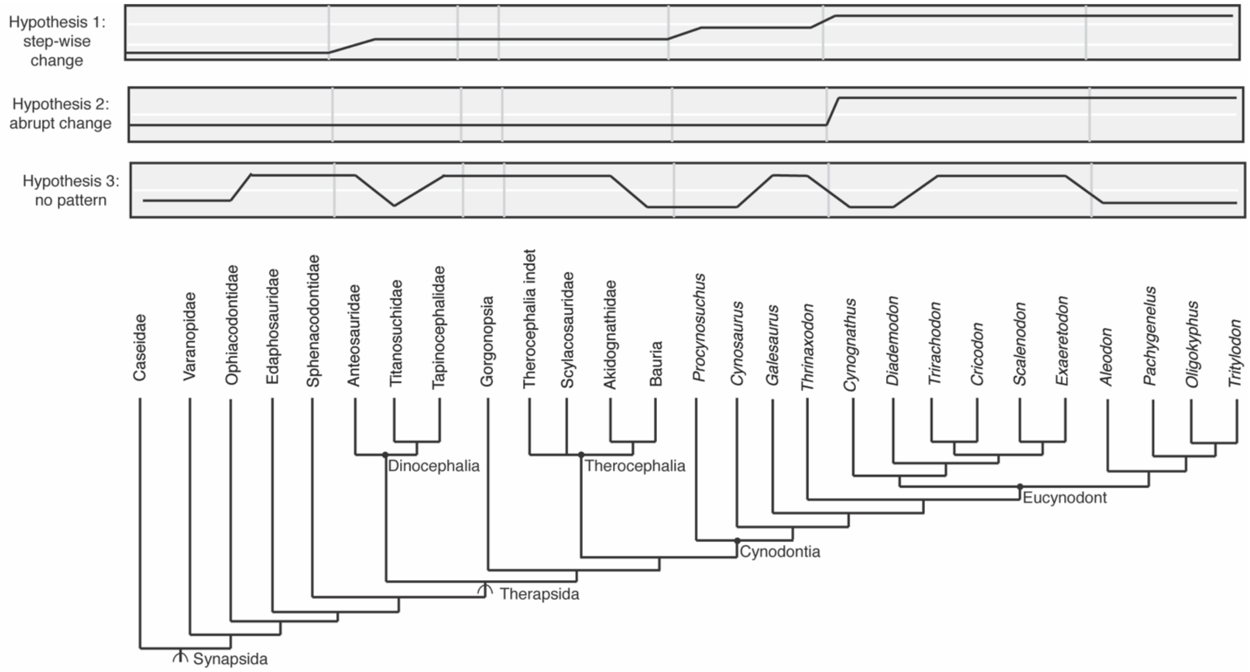


Figure 4.16. **Hypotheses for patterns of trait acquisition along sampled taxa.**

Hypothesis 1 would be the expected change in a character if it follows a gradual step-wise transition from the plesiomorphic to the derived phenotype. Hypothesis 2 would be the expected change if the trait is operating as a key innovation where once acquired, becomes fixed. Finally, Hypothesis 3 is that the trait does not operate under a recognizable pattern with not phylogenetic signal for phenotype.

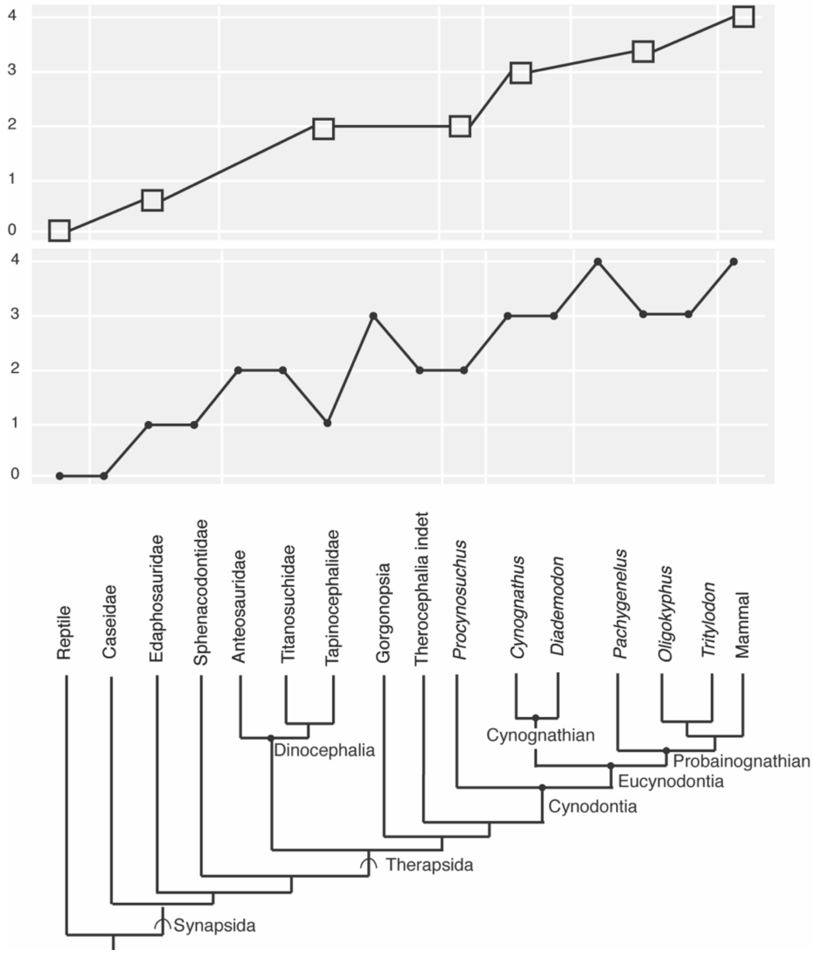


Figure 4.17. **Evolutionary trends in the dentition of synapsids.**

Using attachment, enamel type, and proportion of positions in replacement, character states for terminal taxa were scored relative to the stereotypical reptile and mammal conditions. The result in both the coarse (top) and fine (bottom) taxonomic scales is an upward, stepwise trend. At the coarse scale, the trend appears directional towards a mammal-like dentition with upward movement (i.e. no decreases in score) while at the fine taxonomic scale, the pattern appears more passive with roughly equal score increases, decreases, and points of stasis.

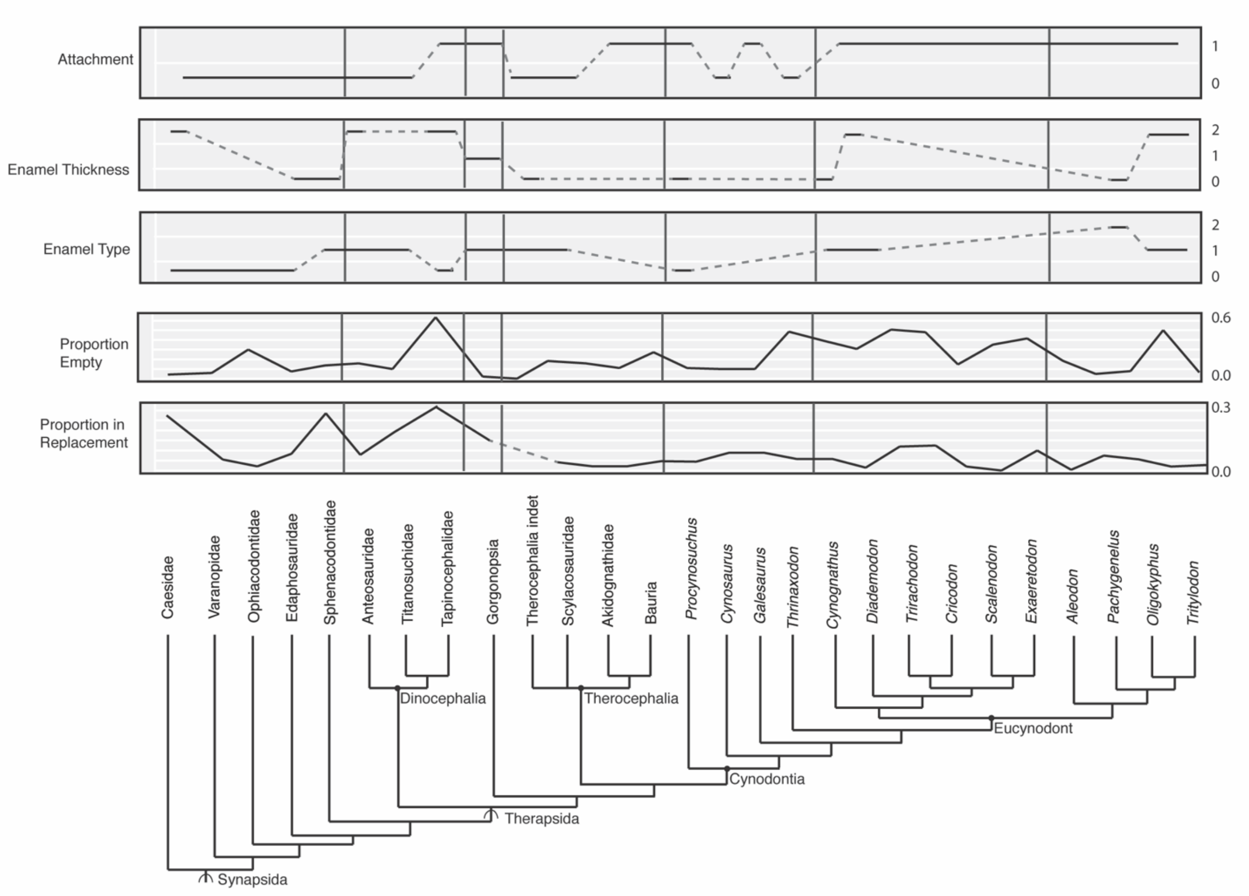


Figure 4.18. **Evolutionary trends in isolated traits of the synapsid dentition.**

Proportion of replacement and proportion of empty positions were both scaled on a continuous plot while the remaining categorical variables were scored. For traits with apparent evolutionary trends (i.e. enamel type and attachment) lower values represent ancestral phenotypes and higher ones representing derived phenotypes. Enamel type: 0 = prismless, 1 = SCE, 2 = prismatic; attachment: 0 = ankylosis, 1 = gomphosis. Enamel thickness does not relate to an evolutionary trend and was scored by thickness: 0 = thin, 1 = intermediate, 2 = thick.

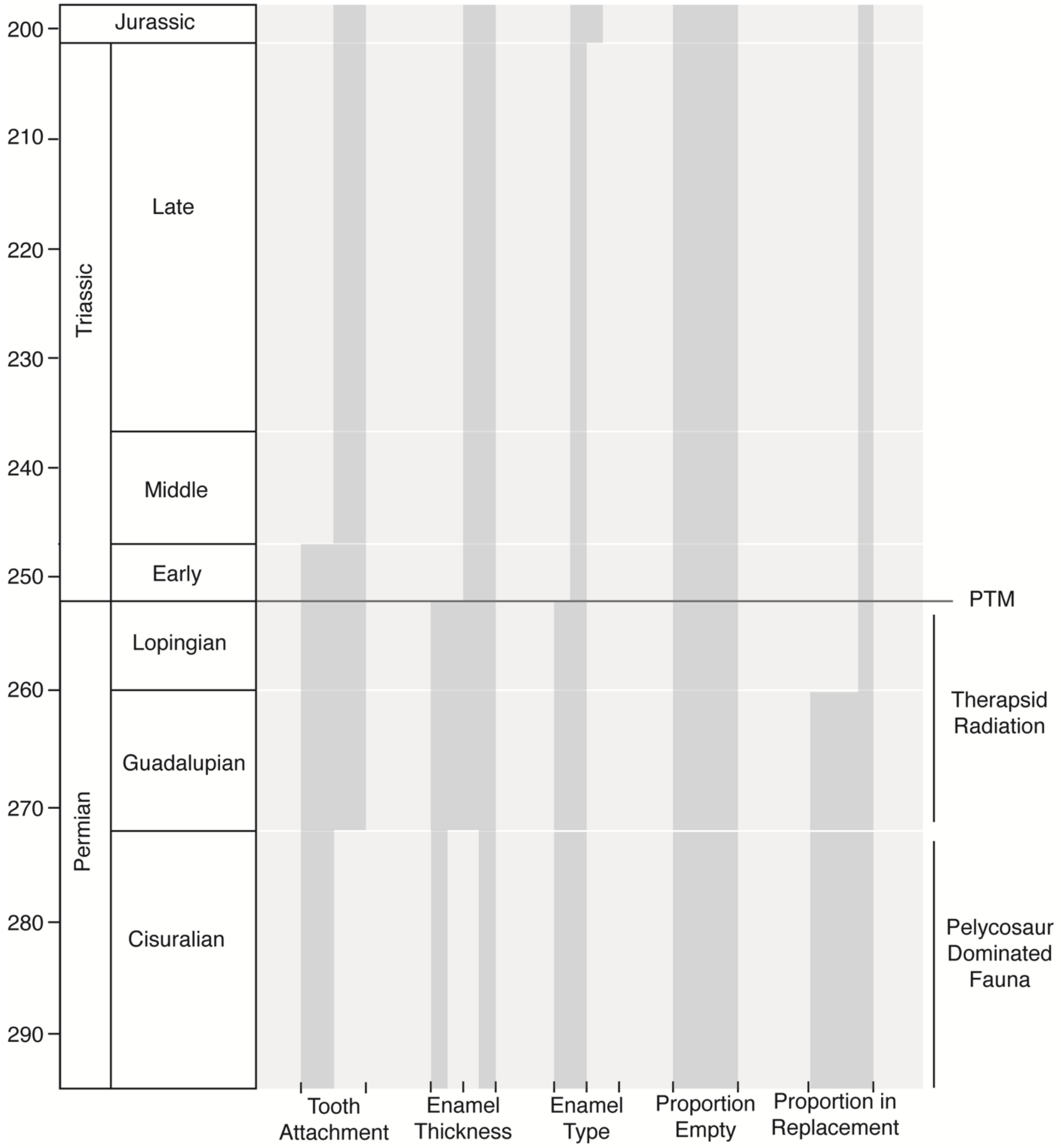


Figure 4.19. **Evolution of synapsid dental features through time.**

Width of box for each character represents the phenotypes of traits represented at given time intervals. Widening of some boxes co-occur with the Guadalupian therapsid radiation and narrowing of boxes occurs leading up to or just before the Permo-Triassic mass extinction (PTM). Subsequently, little variation is recorded suggesting the fixing of phenotypes.

Chapter 5. CONCLUSIONS

This dissertation examines histological and dental data to investigate patterns of trait acquisition in the physiology and dentition of non-mammalian synapsids. The synapsid dentition reveals combinations of features not present in modern vertebrates, patterns that in some ways follow suit to classic patterns in synapsid evolution and in other ways differ. Fossil synapsids studied here also shed light on the acquisition of mammalness in terms of growth and dentition. Here, I summarize conclusions from each chapter of this dissertation.

Chapter 2

- Tapinocephalid dinocephalians acquired some mammal-like and some reptile-like features in their dentition.
- Their dentition on the whole, however, appears to have been highly specialized to herbivory with prismless enamel, frequent replacement, precise occlusion, and a complex occlusal surface that consisted of an enamel shearing ridge and a dentine crushing basin.
- Many of these specializations are convergent with other herbivorous dentitions suggesting that dichotomizing independent dental traits as being either derived or ancestral can fail to recognize the specialization of an entire dentition.

Chapter 3

- Significant differences in dentine stress marks in the ever-growing tusks of Early Triassic *Lystrosaurus* suggest South African populations were experiencing stress differently than those in Antarctica.

- Antarctic *Lystrosaurus* dentine indicates stress that is more constant but less severe than the stress endured by South African *Lystrosaurus*.
- Significant, albeit relatively inconsequential, higher average regular growth was recorded for Antarctic populations.
- The differences recorded in South African and Antarctic specimens may be reflective of the kinds of seasons experienced in each locality. While indictors in tree growth and paleosol data suggest that Antarctica experienced photoperiod seasonality, geological indictors of episodic drought have been described in South Africa. The difference between long, predictable seasonal stress due to photoperiod compared to short, but dramatic periods of drought may be able to explain the patterns observed in *Lystrosaurus* tusk histology.
- The adaptability of *Lystrosaurus* to maintain metabolic activity throughout different kinds of seasonal stress suggest a level of physiological flexibility that is commonly ascribed to endothermic vertebrates. Comparative seasonal physiology may be able to shed light on the origins of endothermy in synapsids.

Chapter 4

- The proportion of empty tooth positions cannot reliably determine the mode of tooth attachment in synapsid fossils.
- This taphonomic artifact is more reliably explained by functional aspects of the dentition including diet and enamel type and thickness which are correlated.
- Enamel type and thickness are tentatively correlated to diet, however correlations are generally poorly supported by this analysis. We determine that the lack of consistent

correlations demonstrates that many combinations of dental features have made up synapsid dentitions.

- These combinations, however, do decrease especially after the Permo-Triassic mass extinction. Eventually, these traits become fixed such that by the Middle-Late Triassic the mammal-like condition in most of the dental traits studied here are present and persist.
- Linkage between these traits, where fitness is dependent on a combination of features, may be an explanation for how these dental traits become fixed.
- Trends in synapsid dentition along the spine of the lineage appear stepwise, however at a finer taxonomic scale and when trends in individual traits are examined, trends are varied and less consistent.

APPENDIX A

EVIDENCE FOR ATTACHMENT

Akidognathidae UC2468

Aleodon R10048

10.12.2017

Anteosauria SAM-PK-12082

Cricodon BP/1/5159

Cynosaurus BP/1/1563

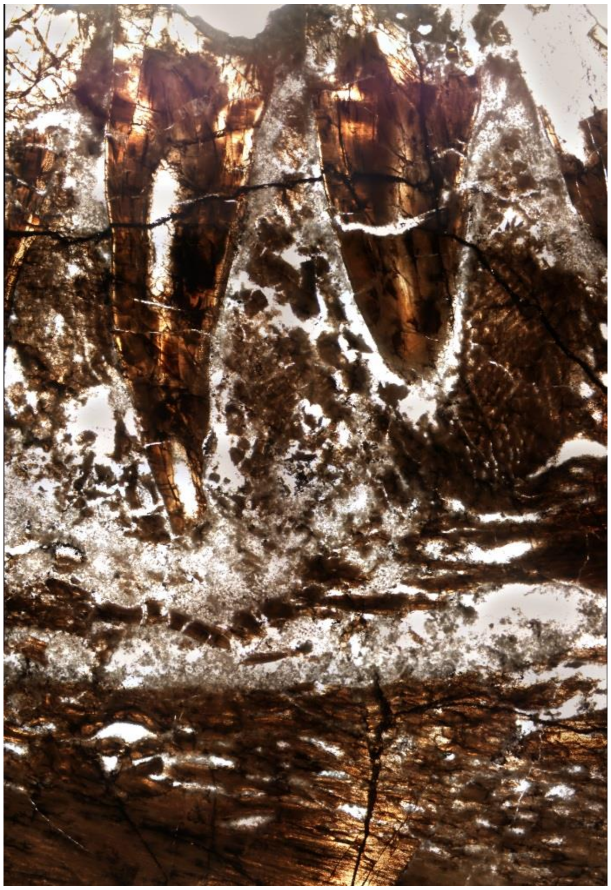
Jonkeria SAM-PK-9123

Oligokyphus T.1237

Pachygenelus R4091

Pristerognathus UR2470

Procynosuchus NMT RB3



Scalenodon Z81

Trirachodon SAM-PK-K171

Trirachodon SAM-PK-K10411

Exaeretodon BP/1/5295

VITA

M. Whitney is a paleontologist and paleohistologist from a small coastal town in Maine. She earned her B.A. in Biology at Macalester College under the supervision of Dr. Kristina Curry Rogers who gave her a glimpse into working with fossil tissues. After graduation, M. Whitney was hired to run the Macalester College paleohistology lab before beginning her PhD in the Biology Department at the University of Washington where she worked with Dr. Christian Sidor.