Supplementary Material

Dental ontogeny in extinct synapsids reveals a complex evolutionary history of the mammalian tooth attachment system

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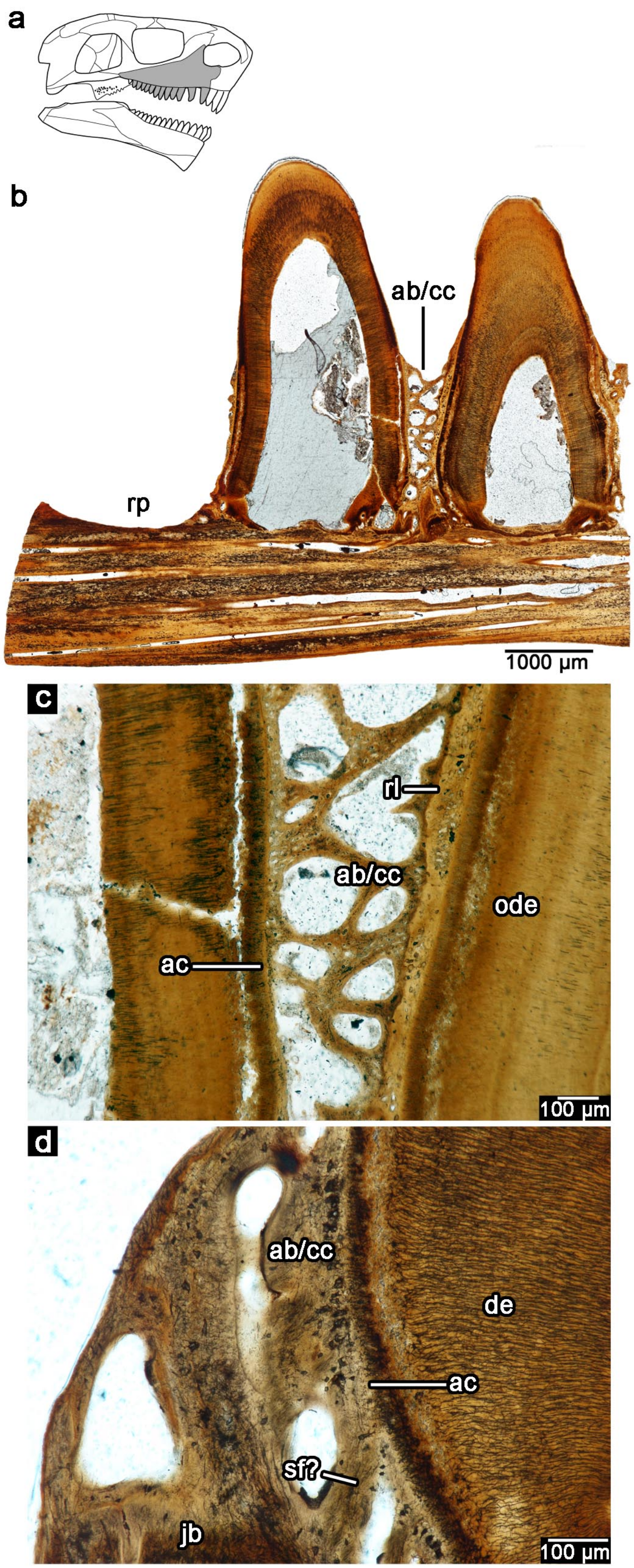
Supplementary Information S1: Histological data

Taxon I	Taxon II	Genus/Species	Specimen	Section description	Cat. No.	ROM thin section no.
“Pelycosauria”	Caseidae	*Oromycter*	Partial maxilla	Transv. sec. of 2 teeth x3; long. sec. of 2 teeth x1	ROM 67604	00532, 00533, 00550, 00877
“Pelycosauria”	Varanopidae	Indet	Partial dentary	Transv. sections x3	ROM 66866	00441, 00477, 00193
“Pelycosauria”	Varanopidae	Indet	Maxilla fragment	Long. section x2	ROM 67513	00144, 00145
“Pelycosauria”	Varanopidae	Indet	Dentary	Transv. sec. of 5 teeth x3	ROM 67514	00132, 00158
“Pelycosauria”	Edaphosauridae	*Edaphosaurus*	Dentary	Transv. sec. of 2 teeth x7; coronal sec. thru 1 tooth x1	NHB 2041070	00524, 00531, 00534, 00535, 00555, 00805, 00875, 00876
“Pelycosauria”	Sphenacodontidae	*Secodontosaurus*	Jaw fragment	Transv. sec. of 2 teeth x2	ROM 6027	00410, 00411
“Pelycosauria”	Sphenacodontidae	*Sphenacodon ferocior*	Jaw fragment	Transv. sections x9	ROM 66105	00097, 00098, 00099, 00100, 00104, 00105, 00106, 00117, 00127
“Pelycosauria”	Sphenacodontidae	*Sphenacodon ferocior*	Partial maxilla	Transv. sec. of 4 teeth x5; coronal sec. thru 1 tooth x2	CM 89931	00885, 00886, 00890, 00891, 00893, 00894, 00902
“Pelycosauria”	Sphenacodontidae	*Dimetrodon limbatus*	Partial dentary	Transc. sec. of 4 teeth x4	StIPB R-602	00119, 00118, 00131, 00594
“Pelycosauria”	Sphenacodontidae	*Dimetrodon limbatus*	Partial maxilla	Transv. sec. of 4 teeth x5	StIPB R-601	00113, 00124, 00129, 2xN/A
“Pelycosauria”	Sphenacodontidae	*Dimetrodon grandis*	Partial right maxilla	Transv. sec. of 2 teeth x7	ROM 6039	00140, 00141, 00153, 00160, 00166, 00178, 00195
Therapsida	Dinocephalia	Indet.	Partial dentary	Transv. sec. of 7 teeth x1	BP/1/6854	00950
Therapsida	Dinocephalia	Indet.	Ant. fragment of dentary	Transv. sec. of 3 teeth x3	BP/1/4851	00904, 00916, 00931
Therapsida	Dinocephalia	Indet.	Ant. fragment of left dentary	Transv. sec. of 5 teeth x1; coronal sec. thru anterior tooth x1	BP/1/5417	00955, 00956
Therapsida	Tapinocephalidae	Indet.	Anterior dentary fragment (with no teeth)	Transverse section of empty tooth socket	NHCC LB370	N/A
Therapsida	Tapinocephalidae	Indet.	Isolated incisor	Coronal section of whole tooth	NHCC LB733	N/A
Therapsida	Anomodontia	*Diictodon* “A”	Partial skull	Coronal sec. of both teeth x3	ROM 52624	00708, 00709, N/A

Therapsida	Anomodontia	*Diictodon* “B”	Partial skull	Coronal sec. of both teeth x1; transv. thru tooth x1	ROM 52624	00028, N/A
Therapsida	Anomodontia	*Diictodon* “C”	Partial maxilla	Coronal sec. of tooth x5	ROM 52624	00508, N/A 00706 00707, N/A
Therapsida	Anomodontia	*Lystrosaurus*	Partial maxilla	Transv. sec. thru tusk	UWBM 109908	N/A
Therapsida	Gorgonopsia	Indet.	Partial dentary	Transv. sections of left dentary x2	BP/1/784	01002, 01003
Therapsida	Gorgonopsia	Indet.	Partial skull	Transv. sections of right maxilla x2	BP/1/2395a	00967, 00975, 00983
Therapsida	Gorgonopsia	Indet.	Anterior jaw fragment	Transv. sections	NMT RB404	N/A
Therapsida	Gorgonopsia	Indet.	Jaw fragment	Transv. sections	NHCC LB367	N/A
Therapsida	Therocephalia	Indet.	Partial dentary	Transv. sec. of 8 teeth x4	BP/1/7257	00887, 00892, 00895, 00903
Therapsida	Therocephalia	Indet.	Partial skull	Transv. sec. of 6 teeth x3; coronal sec. thru 1 tooth x3	BP/1/172	00988, 00989, 00990, 00997, 0998, 09999
Therapsida	Therocephalia	*Bauria*	Nearly complete dentary	Coronal sec. of tooth x4; transv. sec. thru teeth x4	BP/1/2523	00830, 00835, 00837 00838, 00843, 00870, 00871, 00872
Therapsida	Cynodontia	?*Cynognathus*	Partial dentary	Transv. sec. of 2 teeth x5	BP/1/6097	00831, 00832, 00839, 00844, 00874
Therapsida	Cynodontia	*Diademodon*	Nearly complete maxilla	Coronal sec. of indivual teeth x4; transv. sec. thru teeth x4	BP/1/4652	00833, 00834, 00836, 00840, 00841, 00842, 00867, 00873,
Therapsida	Cynodontia	*Galesaurus*	Skull + mandibles	CT scans of a subadult individual	BP/1/4602	N/A
Therapsida	Cynodontia	*Galesaurus*	Skull + mandibles	CT scans of an older individual	BP/1/5064	N/A
Therapsida	Cynodontia	*Thrinaxodon*	Skull + mandibles	CT scans of a young individual	BP/1/5372	N/A
Therapsida	Cynodontia	*Thrinaxodon*	Skull + mandibles	CT scans of an older individual	BP/1/7199	N/A
Therapsida	Mammalia	*Hyopsodus*	Partial dentary	2 long. Sec. of 2 teeth; 2 coronal sec. thru 1 tooth	USNM 595273	00579, 00580, 00603, 00604
Therapsida	Mammalia	*Equus*	Partial dentary	Transv. sec. of 2 teeth x1; coronal sec. x1	ROM 33036	00197, 00203

Table S1. Synapsid specimens sectioned and CT scanned in this study. Abbreviations: Cat. No., Catalogue Number; ROM thin section no., Royal Ontario thin section number (only applicable to samples sectioned at the Royal Ontario Museum); BP, Evolutionary Studies Institute (South Africa); CM, Carnegie Museum (Pittsburgh, U.S.A.); NHCC (National Heritage Conservations Commission (Lusaka, Zambia); NMT National Museum of Tanzania (Dar es Salaam, Tanzania); UWBM, Burke Museum of Natural History (Seattle, U.S.A); ROM, Royal Ontario Museum (Toronto, Canada); StIPB, Steinmann Institute for Geology, Mineralogy, and Paleontology (Bonn, Germany); USNM, National Museum of Natural History (Washington, U.S.A.)



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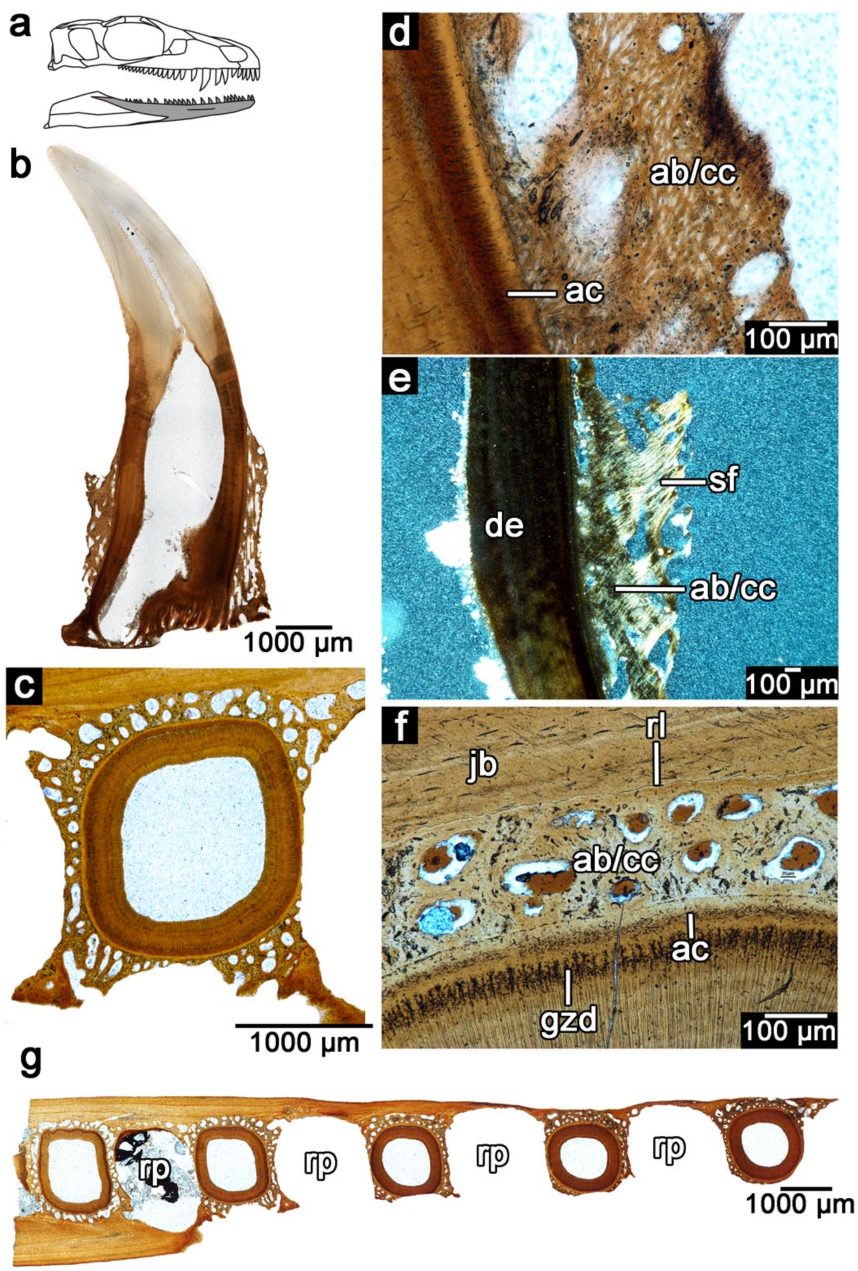
Supplementary Figure 1. Tooth attachment tissues in the Early Permian caseid *Oromycter*. **a,** skull drawing of a caseid highlighting the elements sampled in this study (modified from[1]. **b,** longitudinal section of a partial maxilla (ROM 67604) showing teeth that were shed and those that are ankylosed to the jaw. **c,** closeup of tooth attachment tissues between two teeth, note that the alveolar bone of the younger tooth (left) directly attaches to the alveolar bone of the older tooth (right). **d,** closeup image of the tooth attachment tissues in coronal aspect. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; jb, jawbone; ode, dentine of older tooth; rl, reversal line; rp, replacement pit; sf, Sharpey's fibers.

"Pelycosauria": Caseidae

Thin sections of a partial maxilla of the early Permian caseid *Oromycter* were made in transverse, longitudinal, and coronal aspects (Suppl. Fig. 1, Suppl. Table 1). The teeth of *Oromycter* are found either fully ankylosed to the jaws or in the process of erupting, the latter teeth only being preserved as resorption pits along the tooth row. Tooth ankylosis is also clearly seen along broken teeth in the holotype of *Cotylorhynchus* (OMNH 04327) and *Ennatosaurus tecton* (PIN 1580). The thin sections of *Oromycter* confirm that each tooth is ankylosed to the jaw (Suppl. Fig. 1) and show that the dentine of each tooth is coated in a thin, cloudy band of acellular tissue that bears resemblance to the fibrillar, acellular cementum in captorhinids [2]. As in captorhinids, it is unclear if the thin fibrils within this tissue are of intrinsic or extrinsic origin, but this acellular cementum layer is distinct from the underlying dentine and the overlying bone tissue. A microcancellous bone tissue extends from the tooth root surface to the jaw, or to neighbouring teeth and firmly anchors each tooth in place (Suppl. Fig. 1b–d). This bone tissue is composed of thin bone trabeculae and large, simple vascular spaces and sparse primary osteons, but no secondary osteons. Large, globular cell lacunae line the areas closest to the acellular cementum, whereas most of the cell lacunae along the outer fringes of this bone tissue and the bone of the jaw are smaller and more elongate. The trabeculae forming the bone anchoring the teeth to the jaws contain numerous coarse, dark fibers that mainly extend towards the tooth roots. These fibers probably represent the mineralized collagen fibers of the periodontal ligament, entombed in cementum and alveolar bone.

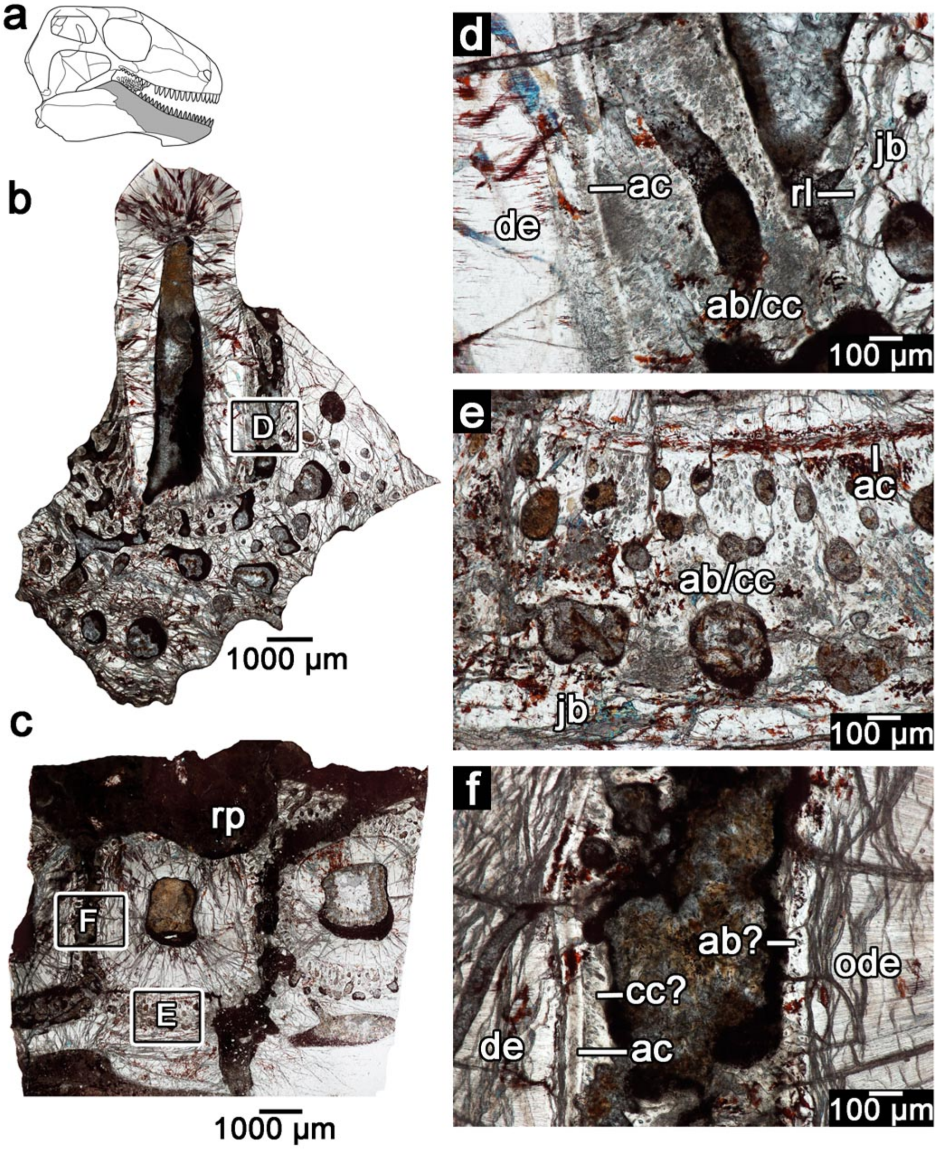


Supplementary Figure 2. Tooth attachment tissues in an Early Permian varanopid. **a,** skull drawing of a varanopid highlighting the elements sampled in this study (modified from[3]. **b,** wholeview of a tooth sectioned in coronal aspect (ROM 67513). **c,** wholeview of a dentary tooth sectioned in transverse aspect (ROM 66866). **d,** closeup of bone-like attachment tissue that ankyloses varanopid teeth to the jawbone in coronal section. **e,** closeup of bone-like attachment tissue under cross-polarized light, note the prevalence of Sharpey's fibers. **f,** closeup image of attachment tissues in transverse section. **g,** wholeview of a varanopid dentary sectioned in transverse aspect (ROM 66866). Note that teeth are either absent due to replacement, or are fully ankylosed to the jaws. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; gzd, globular zone of dentine; jb, jawbone; rl, reversal line; rp, replacement pit; sf, Sharpey's fibers.

"Pelycosauria": Varanopidae

Two partial dentaries and a partial maxilla of a mycterosaurine varanopid from the Early Permian Richards Spur locality were sectioned for this analysis. All of the teeth were either in the process of erupting or were fully ankylosed to the jaws by microcancellous bone tissue (Suppl. Fig. 2). The outer layers of dentine in each tooth consist of thick layers of highly branched dentine tubules, corresponding to the globular zone of dentine reported in other amniotes. A thin band of light-coloured tissue coats the dentine of each tooth root and is histologically consistent with acellular cementum (Suppl. Fig. 2d, f).

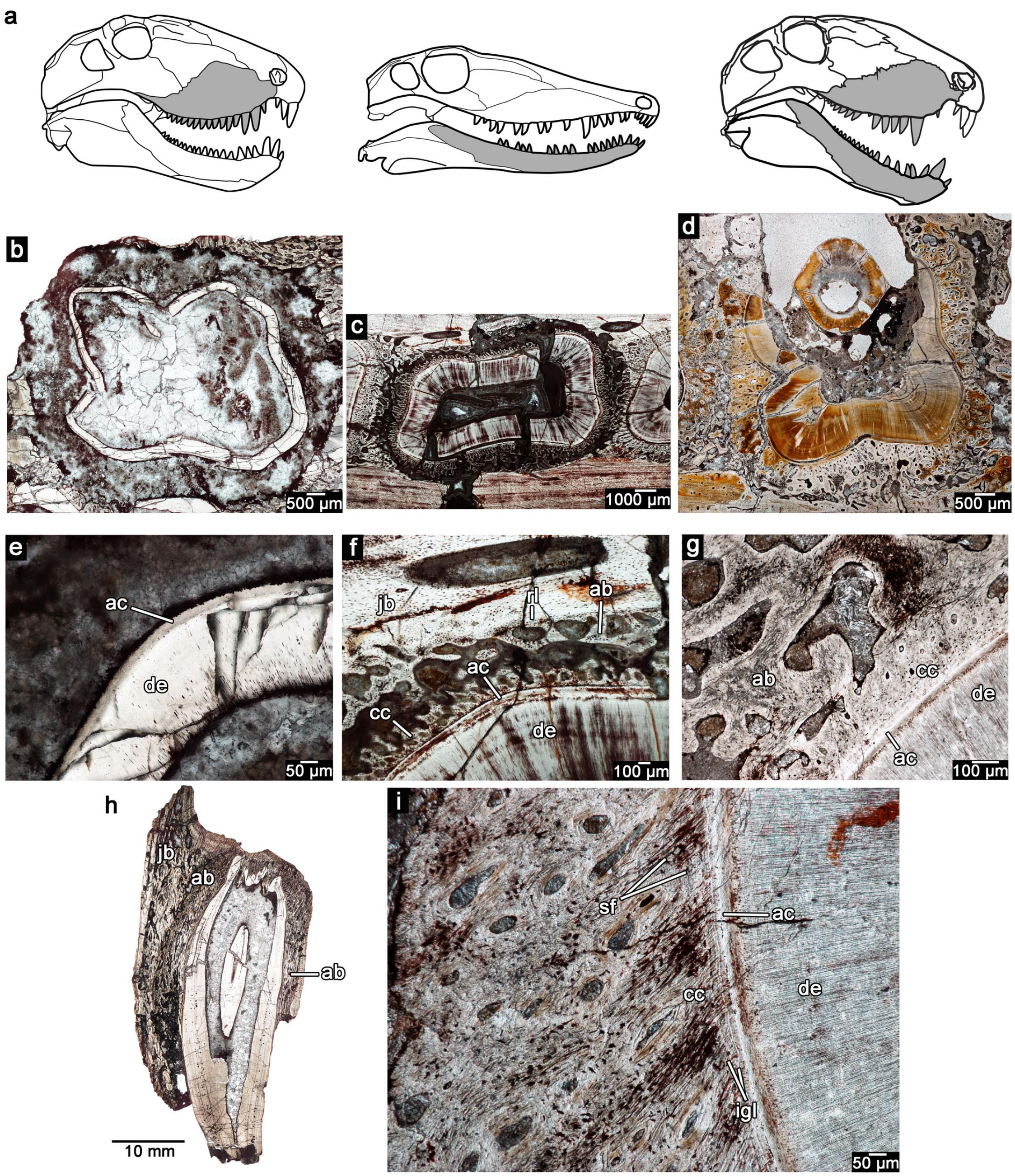
The acellular cementum is in turn directly connected to a microcancellous bone tissue consisting of primary, woven bone trabeculae. Most of the simple vascular spaces and primary osteons in this tissue are oriented parallel to the apical-occlusal axis of each tooth, whereas the vascular spaces in the surrounding jawbone are typically oriented perpendicular to this axis (Suppl. Fig. 2c, f, g). The osteocyte lacunae within the microcancellous bone tissue are much larger and more disorganized than those within the jawbone. A clear reversal line separates the microcancellous bone surrounding each tooth from the jawbone, indicating that this bone tissue is resorbed and redeposited with each tooth, suggesting that it is one of the periodontal tissues that form anew with each new tooth (Suppl. Fig. 2d, f). These features are consistent with either cellular cementum or alveolar bone, depending on the growth direction of this tissue. Unfortunately, lack of preservation of intermediate stages of periodontal tissue formation makes it impossible to tell if this tissue grew centripetally (alveolar bone) or centrifugally (cellular cementum). In coronal section, this spongy bone tissue extends from the alveolar margin down to the base of the tooth root (Suppl. Fig. 2b). At higher magnifications, this tissue has a fibrous texture that is oriented perpendicular to the long axis of the tooth and consists of abundant, roughly parallel Sharpey's fibers under cross polarized light (Suppl. Fig. 2e), indicating the presence of a completely calcified periodontal ligament.



Supplementary Figure 3. Tooth attachment tissues in *Edaphosaurus* (NHB 2041070). **a,**skull drawing of *Edaphosaurus* highlighting the elements sampled in this study (modified from[1]. **b,** wholeview of a dentary tooth sectioned in coronal aspect. **c,** wholeview of three teeth sectioned in transverse aspect. **d,** closeup of dental attachment tissues that ankylose the teeth to the jaws in coronal view. **e,** closeup of dental attachment tissues in transverse view. **f,** closeup of dental attachment tissues between two teeth in transverse view, potentially showing intermediate stage of development. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; ode, dentine of older tooth; jb, jawbone; rl, reversal line; rp, replacement pit.

“Pelycosauria”: Edaphosauridae

Thin sections through a partial dentary of *Edaphosaurus* (Suppl. Fig. 3) show similar fusion of the teeth to the surrounding jawbone as in caseids and varanopids. Each tooth is anchored in place by spongy, primary bone tissue. As in caseids and varanopids, the dentine of each tooth root is coated in a well-defined band of acellular cementum (Suppl. Fig. 3d–f), which in turn directly contacts the surrounding, spongy bone. The acellular cementum contains numerous parallel fibrils, similar to the acellular cementum of captorhinids [2], varanopids, and caseids. The numerous vascular spaces within the surrounding bone tissue are all oriented parallel to the apico-occlusal axis of the tooth and have larger diameters towards the outer fringes of the bone tissue. The cell lacunae within this tissue are large and round, differing markedly from the small, flat lacunae within the jawbone. Unfortunately, the poor preservational quality of the material precludes any detailed view of the histology of the bone tissues. However, the presence of large cell lacunae, high vascularity, and a persistent reversal line between the outer layers of this bone and the surrounding hard tissues (Suppl. Fig. 3d, e) indicate that this tissue formed rapidly and in association with each new tooth. From these data, it is clear that each tooth was firmly ankylosed to the jaw and to neighbouring teeth prior to being replaced (Suppl. Fig. 3). The rapid formation of this tissue and the lack of preservation of intermediate stages of tooth tissue formation make it difficult to assign the surrounding bone tissue to cellular cementum or to alveolar bone, although there is some evidence for two growth directions for this tissue (Suppl. Fig. 3e, f).



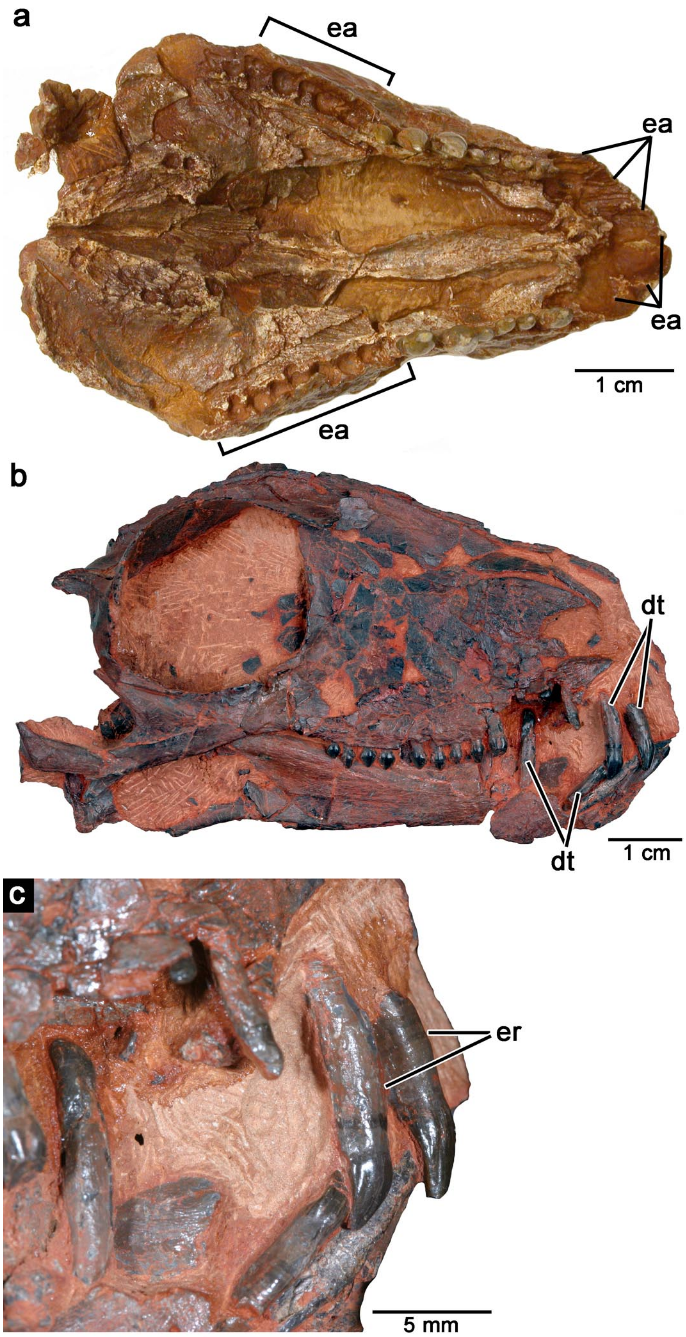
Supplementary Figure 4. Tooth attachment tissues in Sphenacodontidae. **a,** skull drawings of *Sphenacodon*, *Secodontosaurus*, and *Dimetrodon* highlighting elements sampled in this study (modified from[4]. b, closeup of a single maxillary tooth root of *Sphenacodon* that is in the process of erupting (CM 89931). Note the replacement pit that encircles the developing tooth. **c,** closeup of a single dentary tooth root of *Secodontosaurus* that is in an intermediate stage of attachment tissue development (ROM6027). **d,** closeup of a single dentary tooth root of *Dimetrodon* at an advanced stage of development (StIPB R-602). The tooth is completely ankylosed to the jaws and is in the process of being replaced. **e,** closeup of the unerupted tooth in b showing early development of acellular cementum, but no other attachment tissues. **f,** closeup of the erupted tooth in c showing the development of the attachment tissues. Alveolar bone develops along the fringes of the replacement pit and grows centripetally, whereas the spongy cellular cementum develops from the root surface and grows centrifugally. **g,** closeup of a maxillary tooth of *Dimetrodon* showing complete ankylosis (ROM 6039), when the alveolar bone and cellular cementum meet and close off the periodontal space. **h,** wholeview of the maxillary caniniform tooth of *Sphenacodon* exhibiting complete ankylosis (CM 89931). **i,** closeup image of dental attachment tissues of the same *Dimetrodon* tooth as in (G). Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; igl, incremental growth lines; jb, jawbone; rl, reversal line; sf, Sharpey's fibers.

"Pelycosauria": Sphenacodontidae

Several specimens of three sphenacodontid genera were sectioned (Suppl. Table 1) and nearly all of the teeth exhibited complete ankylosis to the surrounding jawbone and to neighbouring teeth (Suppl. Fig. 4). Transverse sections of some of the larger individuals show that the teeth of sphenacodontids were ankylosed to the jaw, but the surrounding bony tissue exhibited a high degree of differentiation (Suppl. Fig. 4d, g, i). The dentine of the tooth roots is coated in thin bands of avascular, acellular tissue (Suppl. Fig. 4i). This layer corresponds to the acellular cementum of mammals and other amniotes [2,5]. In the larger specimens of *Dimetrodon* and *Sphenacodon*, incremental bands of cellular, bone-like tissue line the acellular cementum and represent the cellular cementum of the tooth root (Suppl. Fig. 4i). This tissue is also perforated by numerous, well-organized Sharpey's fibers that extend through the cellular cementum layers and nearly reach the acellular cementum band. The bony tissue that forms the bulk of the attachment of the tooth to the jaw is highly vascularized and occurs in two distinct zones. The inner zone, closest to the tooth, is composed of smaller vascular spaces and contains the well-organized network of Sharpey's fibers that extend towards the tooth root. The outer portion of the periodontium is a bony tissue with much larger vascular spaces and an irregular arrangement of Sharpey's fibers, with very little directionality to the fibers within the bone matrix when compared to the more internal layers. This tissue consists of rapidly deposited woven bone and is separated from the bone of the jaw by a reversal line, indicating that this tissue was deposited after resorption of the previous tooth tissues (Suppl. Fig. 4f).

None of the individual jaw fragments exhibited enough stages of hard tissue formation to reconstruct the ontogenetic stages of tooth attachment or the growth directions of the individual tissues for a given sphenacodontid taxon. The most common condition was for teeth to be either preserved as partially erupted crowns with incipient roots (Suppl. Fig. 4b), or as completely ankylosed teeth (Suppl. Fig. 4g–i). However, in rare instances, teeth were preserved nearly erupted and sometimes exhibiting early stages of periodontal tissue formation (Suppl. Fig. 4f). Combining sections from different specimens permits a reconstruction of the ontogenetic sequence of tooth attachment in sphenacodontids, which appears to be consistent across taxa, based on gross histology.

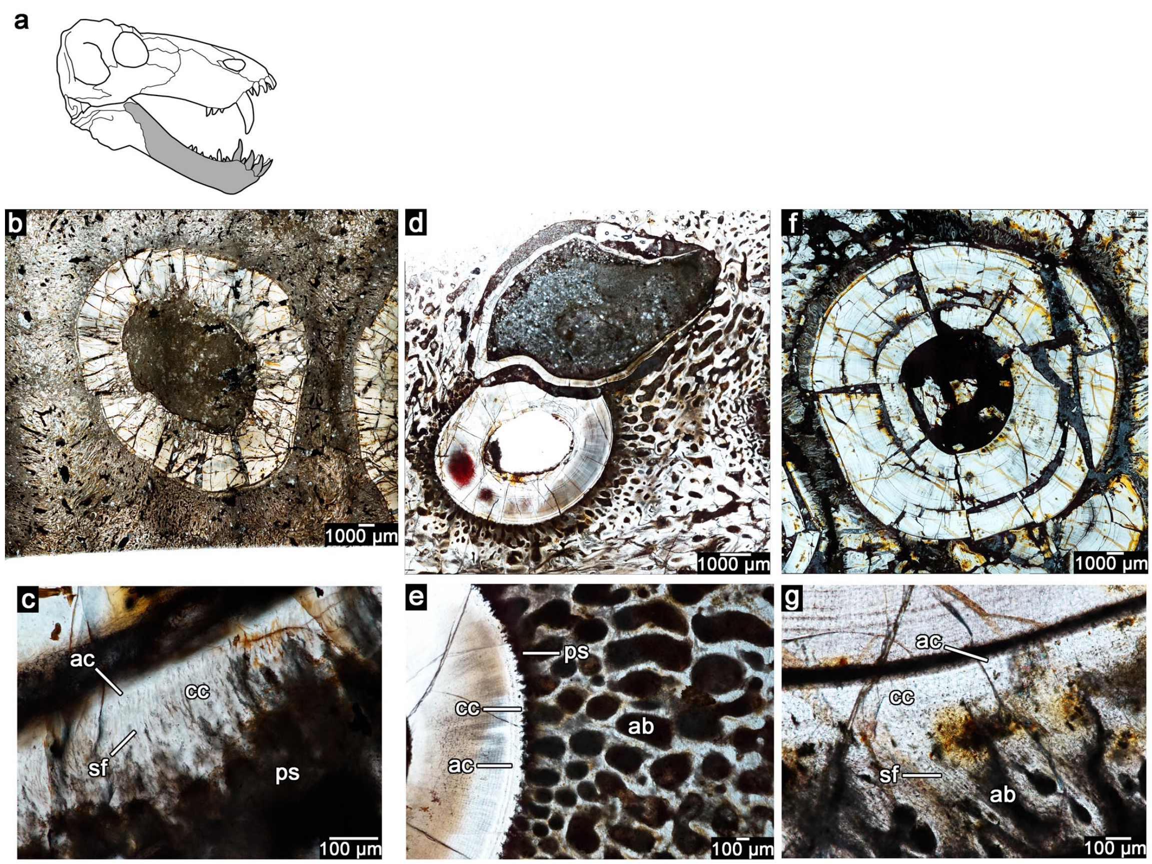
Teeth that were nearly erupted consisted of dentine, an enamel cap, and a thin band of acellular cementum coating the root. A transverse section through a partial maxilla of *Sphenacodon* shows a post-caniniform maxillary tooth at this stage. Sections through the four-lobed root show that no cellular cementum or alveolar bone was present at this early stage of eruption and the surrounding hard tissues had been partially resorbed to accommodate the new tooth (Suppl. Fig. 4b). The subsequent stage involved the development of alveolar bone and cellular cementum, once the tooth had fully erupted. In sphenacodontids, there are two growth directions to the vascularized bone tissues that surround each tooth: the tissue coating the tooth root, which contains small vascular spaces appears to have grown centrifugally, whereas the more vascularized bone tissue surrounding the entire tooth root grew centripetally. These two tissues appear to have joined together very rapidly once the tooth had erupted, given the rarity of teeth in our sample from stages between eruption and complete ankylosis. However, based on a transverse section of a partial jaw of *Secodontosaurus* there was a brief point at which cellular cementum and alveolar bone were separate tissues that grew towards each other (Suppl. Fig. 4f). The growth direction and histological features of the outer bone layer are consistent with alveolar bone [6,7]. The more internal vascularized layer is consistent with cellular cementum. Cellular cementum appears to have been quite extensive and probably met the surrounding alveolar bone far from the surface of the tooth root (Suppl. Fig. 4f, g). The alveolar bone and cellular cementum were both perforated by Sharpey's fibers (Suppl. Fig. 4i) indicating that an unmineralized periodontal ligament was present during the brief intermediate stage between tooth eruption and complete ankylosis. By the time a new tooth had begun to form lingual to its predecessor, the functional tooth was completely ankylosed to the jaws (Suppl. Fig. 4d). The alveolar bone and cementum of functional teeth at this stage were still composed of primary tissue (i.e., no remodeling was observed), however layers of lamellar bone, a characteristic of primary osteon and cementeon development [8–10], fringe the vascular spaces in the alveolar bone and cementum.



Supplementary Figure 5. Tooth attachment in the biarmosuchian *Niaftasuchus* (images provided by D. Scott with permission from R. Reisz). **a,** palatal view of the skull of *Niaftasuchus* (PIN 3717/36) showing extensive post-mortem tooth loss, which is indicative of ligamentous tooth attachment. **b,** lateral view of the skull of *Niaftasuchus* (PIN 162/63) showing displaced teeth, which is indicative of ligamentous tooth attachment. **c,** closeup of premaxillary teeth in b showing exposed roots of displaced teeth. Abbreviations: ea, empty alveoli; dt, displaced tooth; er, exposed root.

Therapsida: Biarmosuchia

No histological data were available for Biarmosuchia. However, the nature of preservation of some of the cranial material of *Niaftasuchus* Ivachnenko, 1990 (No.3717/36) provide clear insights into tooth attachment in this member of the clade. *Niaftasuchus* is a poorly known, presumably omnivorous biarmosuchian (R. Reisz, pers. obs.) from Russia. The teeth possess long roots and elaborate incisiform and postcaniniform crowns. In both specimens, several teeth have been displaced post-mortem or were lost completely (Suppl. Fig. 5). The sheer number of teeth that were lost in one skull (Suppl. Fig. 5a) indicates that the teeth fell out of each alveolus after death rather than being in the process of being replaced. Furthermore, most of the teeth were clearly not in the process of being shed at the time of death, given the presence of small replacement pits lingual to the functional alveoli. Normally, post-mortem tooth loss can only occur if the teeth were ligamentously attached to the alveoli in life. The displaced teeth in the second skull (Suppl. Fig. 5b, c) show that the roots of each tooth were not fused to the alveolar margin. Without histological data, it is not possible to confirm that the teeth did not become ankylosed later in ontogeny, but it is clear that the teeth were attached by gomphosis for a significant duration of the life of each tooth, unlike the condition in “pelycosaurs”.

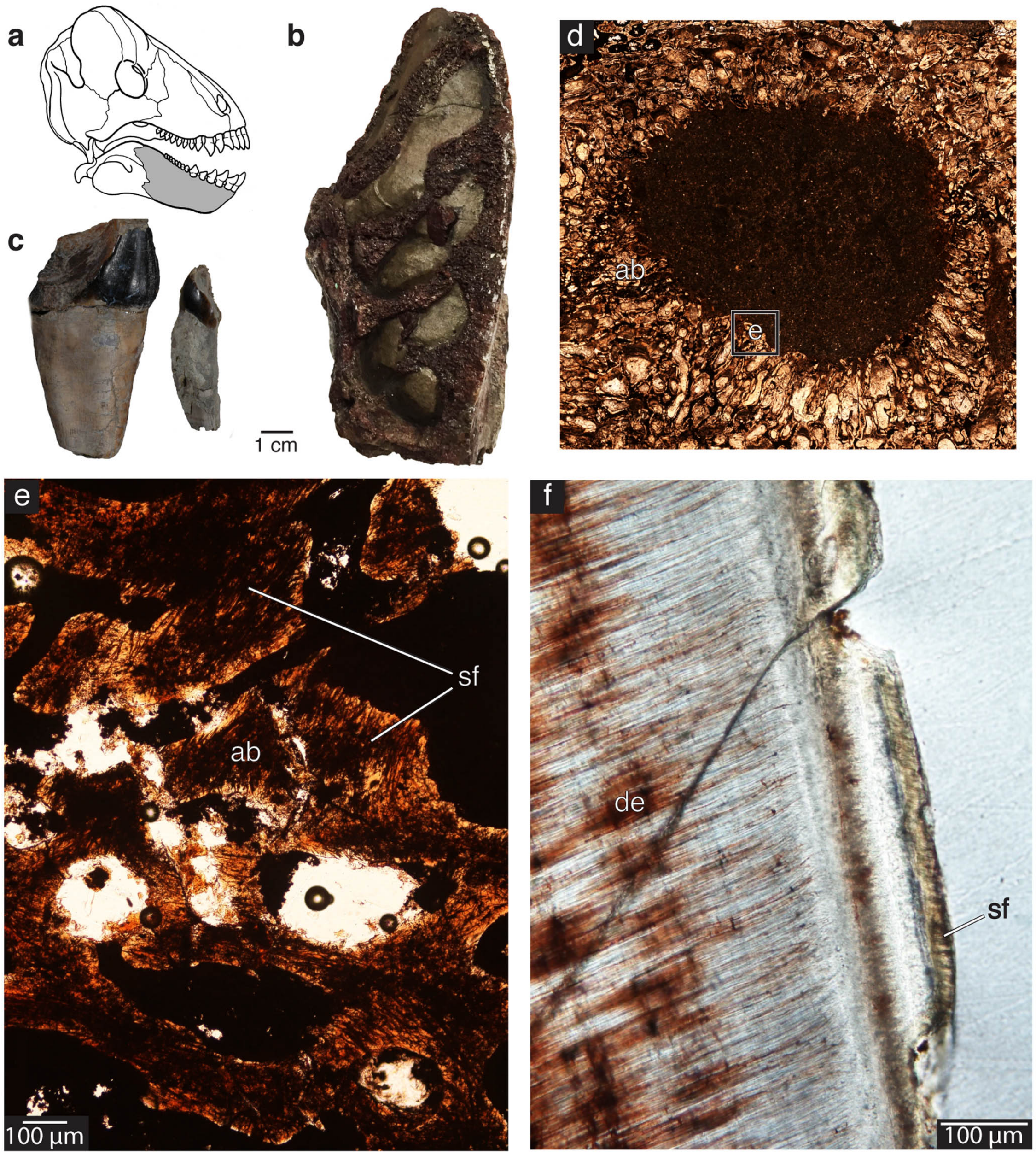


Supplementary Figure 6. Tooth attachment tissues in indeterminate dinocephalians. **a,** skull drawing highlighting elements sampled for this study (modified from[11]. **b,** closeup of a single, fully ankylosed tooth in a large dinocephalian (BP/1/5417). **c,** closeup of the attachment tissues in a neighbouring tooth in BP/1/5417 showing thick layers of cellular cementum and Sharpey's fibers from the periodontal ligament. This tooth is not ankylosed to the jaw. **d,** closeup of a single, partially ankylosed tooth of a dinocephalian (BP/1/6854) that was in the process of being replaced. Note the size discrepancy between the old and replacement teeth. **e,** closeup of the attachment tissues of the partially ankylosed tooth in d. **f,** closeup of a single, partially ankylosed dentary tooth of a dinocephalian (BP/1/4851). **g,** closeup of attachment tissues of the same tooth as in f, showing extensive cellular cementum and Sharpey's fibers. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; ps, periodontal space; sf, Sharpey's fibers.

Therapsida: Dinocephalia Indet.

Three indeterminate dinocephalians from the Beaufort Group of South Africa were sampled, one of which was described in detail previously [12]. The teeth of these specimens are at various stages of gomphosis and ankylosis (Suppl. Fig. 6). A thin, featureless band of acellular cementum and a thicker, outer layer of cellular cementum immediately surround the dentine of each tooth. Overall, the boundary between cellular cementum and alveolar bone is much clearer in therapsids compared to "pelycosaurs". The cellular cementum in these dinocephalians contains cell lacunae entombed in an avascular, calcified matrix that are arranged into concentric layers (Suppl. Fig. 6c, e, g). In some specimens, (e.g., BP/1/6854) Sharpey's fibers extend across the cellular cementum in plain-polarized light, indicating the attachment sites of the periodontal ligament (Suppl. Fig. 6c, g). The Sharpey's fibers are highly organized and extend parallel to each other across the cellular cementum, similar to the case in modern mammals. The cellular cementum comes in direct contact with the surrounding alveolar bone in some teeth, whereas other teeth were clearly suspended within the socket (Suppl. Fig. 6f).

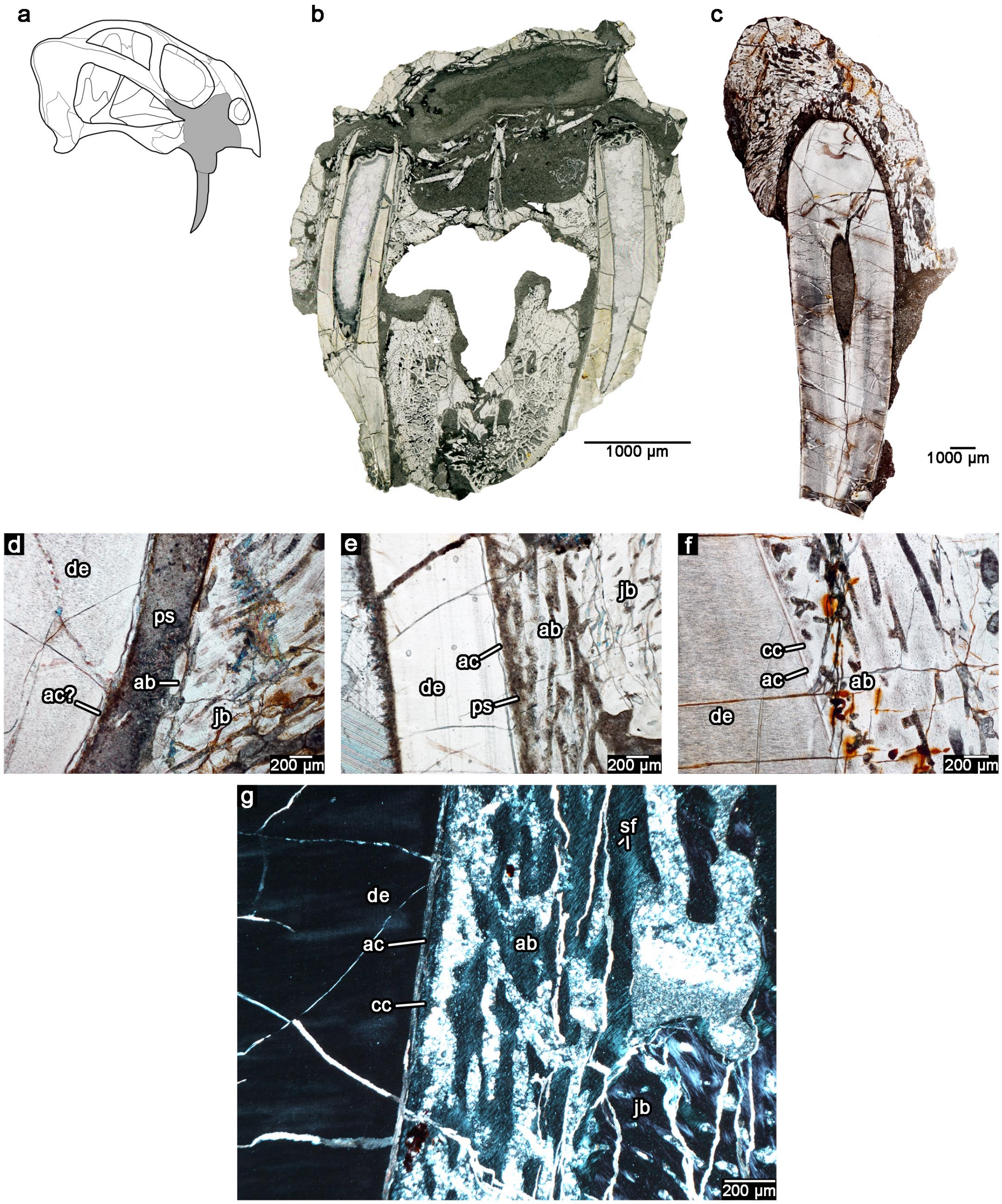
The surrounding alveolar bone is a micro-cancellous bone tissue with a woven matrix under cross-polarized light, suggesting rapid bone deposition. Some of the tooth positions record teeth that are only partially ankylosed to the surrounding alveolar bone, presumably representing intermediate stages between full gomphosis or ankylosis. The surrounding alveolar bone nearly makes contact with the cellular cementum in some areas of these teeth, whereas it clearly makes contact with the cementum in other areas. The cellular cementum coating these teeth has a frayed external surface, indicative of irregular mineralization along the cementum surface (Suppl. Fig. 6d, e). From these data, the erupted teeth of these dinocephalians probably were initially attached by periodontal ligament to the socket margins only to later become ankylosed to the alveolar bone via mineralization of the ligament. At all stages, however, cementum, alveolar bone, and periodontal ligament are visible in thin section.



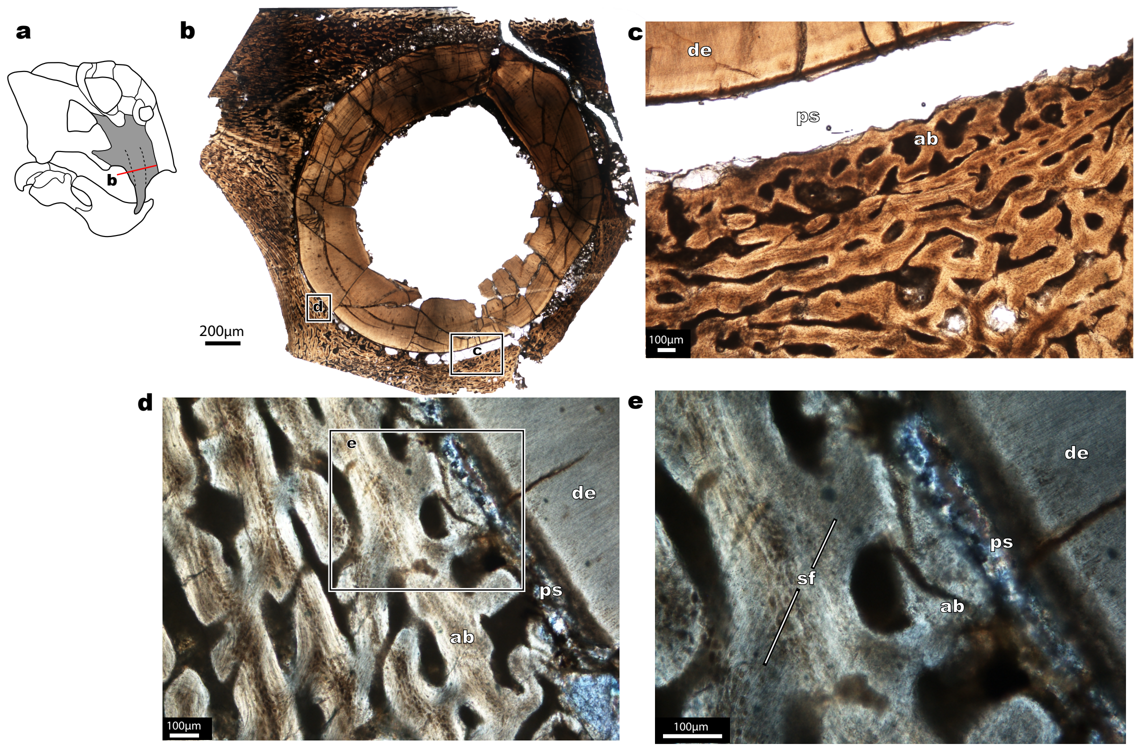
Supplementary Figure 7. Tooth attachment tissues in tapinocephalid dinocephalians. **a,** skull drawing highlighting elements sampled for this study (modified from[11]. **b,** example of an edentulous partial anterior tapinocephalid dentary (NHCC LB370). **c,** isolated teeth with intact roots (NHCC LB734, LB735). **d,** transverse section of an empty tooth socket from the posterior portion of dentary (NHCC LB370). **e,** closeup of alveolar bone surrounding tooth socket with Sharpey's fibers. **f,** coronal section of an isolated incisor (NHCC LB733) with Sharpey's fibers in the outer cementum layer of the root. Abbreviations: ab, alveolar bone; de, dentine; sf, Sharpey's fibers.

Therapsida: Dinocephalia: Tapinocephalidae

Several tapinocephalid dinocephalians from the Lower Madumabisa Formation of Southern Zambia were sectioned. None of the jaws recovered from this area preserve teeth in-situ and as such, edentulous jaws (Suppl. Fig. 7) and isolated teeth (Suppl. Fig. 7) were thin-sectioned to examine tissues related to attachment. The teeth reveal a layer of cementum lining the roots although the preservation does not provide details on the the composition and anatomical order of cellular/aceullar cementum. However, preserved in the cementum are densely organized Sharpey's fibers that run parallel to one another indicating the attachment of a periodontal ligament (Suppl. Fig. 7). The empty sockets of tapinocephalid jaws are lined by alveolar bone that is highly vascularized primary woven bone (Suppl. Fig. 7). Embedded within the alveolar bone just adjacent tooth sockets are abundant Sharpey's fibers, often organized into bundles where fibers run parallel to each other. The appearance of Sharpey's fibers in both cementum and alveolar bone, as well as the prevalence of toothless jaws and isolated teeth with intact roots in the tapinocephalid fossil record provide evidence of a permanent gomphosis in these dinocephalians.



Supplementary Figure 8. Tusk attachment tissues in the dicynodont (Anomodontia) *Diictodon*. **a,** skull drawing highlighting the elements sampled in this study (modified from[13]. **b,** wholeview of a coronal section through the maxillary tusks and lower jaw of *Diictodon* ("*Diictodon* A", ROM 52624). **c,** wholeview of a coronal section through a maxillary tusk of *Diictodon* ("*Diictodon* C", ROM 52624) showing a gomphosis attachment. **d,** closeup image of same tooth as in c showing extensive periodontal space between the tooth root and surrounding alveolar bone (crown tip is towards the top). **e,** closeup of tooth attachment tissues of same section as in b showing partial closure of periodontal space by alveolar bone (crown tip is towards the top). **f,** closeup image of a completely ankylosed tooth in a third specimen (“*Diictodon* B”, ROM 52624) (crown tip is towards the top). **g,** tooth attachment tissues in the same ankylosed tooth under cross-polarized light (crown tip is towards the top). Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; jb, jawbone; ps, periodontal space; sf, Sharpey’s fibers.



Supplementary Figure 9. Maxillary tusk histology of *Lystrosaurus* (UWBM 109908) with evidence of a permanent gomphosis. **a**, skull representing where sections were taken for this study. **b**, transverse section of tusk with surrounding alveolar bone. **c**, close-up of the lateral margin of the tusk with a wide periodontal space evident between the dentine of the tusk and the alveolar bone of the jaw. **d**, close up of a more mesial/distal margin of UWBM 109908 under cross polarized light with a narrower periodontal space. **e**, closeup of **d** under cross polarized light with Sharpey's fibers embedded in the alveolar bone. Abbreviations: ab, alveolar bone; de, dentine; ps, periodontal space; sf, Sharpey's fibers.

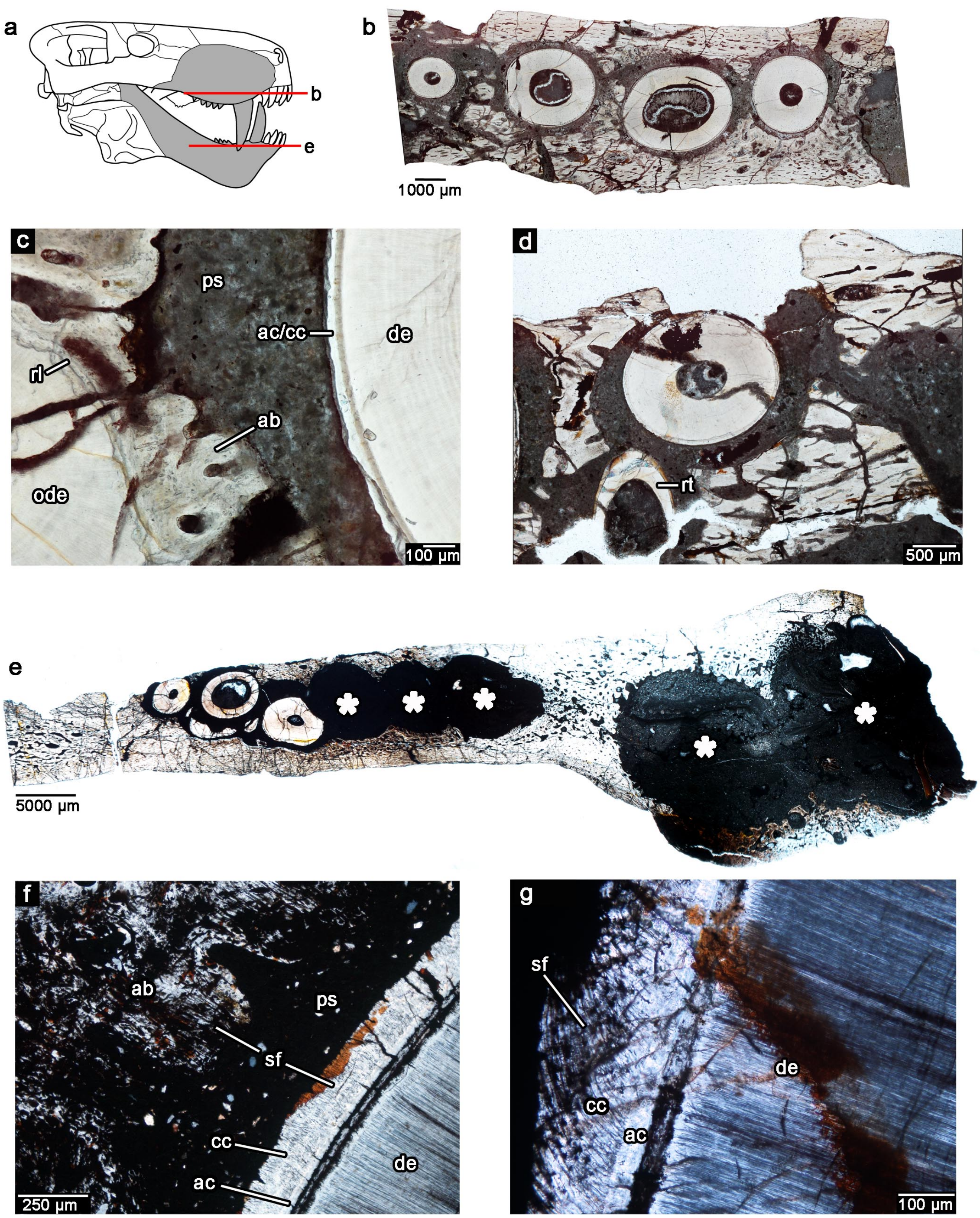
Therapsida: Anomodontia

Thin sections of the dicynodonts *Diictodon* and *Lystrosaurus* were made for comparisons of two dicynodont anomodonts. Early anomodonts possessed highly specialized dentitions and exhibited sophisticated patterns of dental occlusion, whereas dicynodont jaws were commonly almost edentulous [1]. In place of an occluding dentition, dicynodonts possessed keratinous beaks and at least one pair of maxillary tusks, which may not have been involved in feeding [13].

Several longitudinal sections of the tusks of three specimens of the Late Permian dicynodont *Diictodon* were used for this study (Suppl. Fig. 8; Suppl. Table 1). These tusks were deeply implanted, with long roots and enamel-covered crowns, similar to the enlarged canines of many mammals. Thin sections of the tusks in multiple specimens reveal two types of tooth attachment in *Diictodon*: tusks that are deeply implanted and ankylosed to the maxilla (Suppl. Fig. 8b) and those that are seemingly floating in a deep alveolus (Suppl. Fig. 8c). In both types of implantation, the dentine of the tooth root is coated in a very thin layer of acellular cementum.

In the ankylosed teeth, the acellular cementum band contacts a layer of highly vascularized woven bone. Most of the vascular spaces of this bone tissue extend parallel to the apical-occlusal axis of the tooth. This tissue also contains abundant Sharpey's fibers, best viewed under cross-polarized light, which are angled towards the tooth root (Suppl. Fig. 8g). These fibers represent the mineralized portions of the periodontal ligament and are present throughout. Most of this bone tissue is here considered to be alveolar bone, because it forms the entire alveolus and is separated from the fibrolamellar bone of the jaw by a reversal line. Other specimens of *Diictodon* possess a periodontal space, where the cementum of the tooth root does not come into contact with the surrounding alveolar bone (Suppl. Fig. 8c–e). Sharpey's fibers along the alveolar bone and cementum of the tooth root mark the points of insertion of the ligament. The presence of a periodontal space in erupted teeth such as these indicates that an unmineralized periodontal ligament was present, making this a true gomphosis, prior to the teeth becoming fused to the jaws. The width of the periodontal space also appears to vary in the three specimens that were examined. The surrounding alveolar bone layers probably grew centripetally during the ontogeny of each tooth, eventually contacting a very thin layer of cementum coating the tooth root (Suppl. Fig. 8f). These results suggest that *Diictodon* teeth progressed through successive stages of gomphosis, mineralization, and ultimately ankylosis, all of which are visible in thin section.

The sections of a maxillary tusk of *Lystrosaurus* (UWBM 109908) show a true gomphosis (Suppl. Fig. 9). Each tooth is seemingly floating in a socket made of woven alveolar bone. A network of Sharpey's fibers that mark the insertion points of the periodontal ligament perforates this surrounding bone layer. The root of the tooth in *Lystrosaurus* is coated in a thin, dark band of tissue that is distinct from the underlying dentine, but diagenetic alteration makes it difficult to determine whether or not this is acellular or cellular cementum.



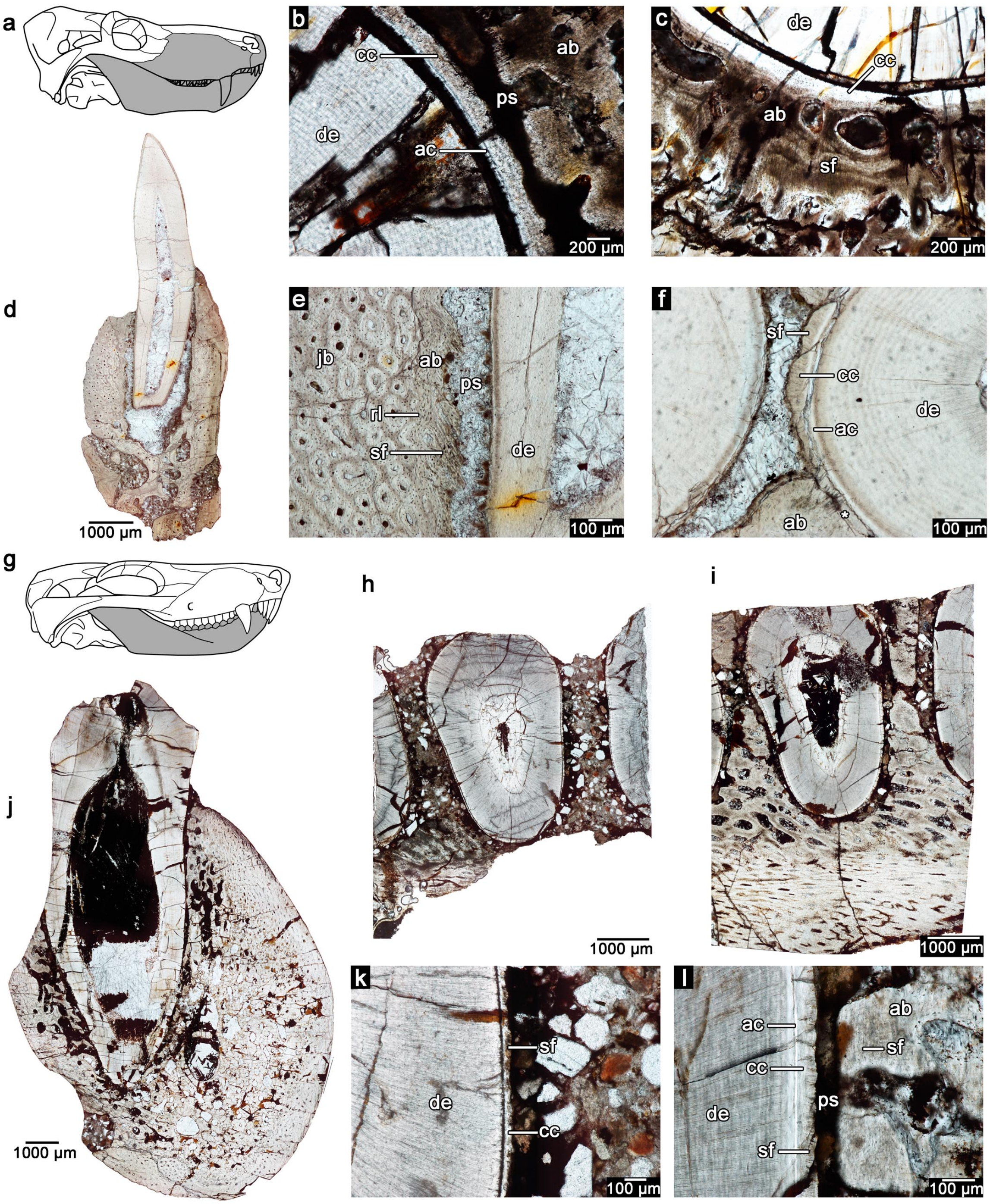
Supplementary Figure 10. Tooth attachment tissues in Gorgonopsia. **a,** skull drawing of a gorgonopsian highlighting elements sampled in this study (modified from[14]. **b,** wholeview of transverse section through a partial maxilla (BP/1/2395a), showing that none of the teeth were ankylosed to the jaw. **c,** closeup of the tooth attachment tissues in a maxillary tooth of BP/1/2395a. Note the presence of a fragment of an old tooth root that has become embedded in the socket wall. **d,** closeup of a single maxillary tooth in BP/1/2395a showing a mesially displaced replacement tooth. Teeth that were in the process of being replaced were still attached by gomphosis, indicating that this was a permanently ligamentous tooth attachment. **e,** wholeview of a gorgonopsian dentary (BP/1/784) sectioned in transverse aspect. Asterisks mark positions where teeth were lost post-mortem. **f,** closeup of tooth attachment tissues in NMT RB404 under plain-polarized light **g,** closeup of NMT RB404 with abundant Sharpey's fibers in the cellular cementum of the tooth root. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; jb, jawbone; oab, older generation of alveolar bone; ps, periodontal space; rl, reversal line; rt, replacement tooth; sf, Sharpey's fibers.

Therapsida: Gorgonopsia

A partial skull (BP/1/2395a) and a nearly complete dentary (BP/1/748) of two gorgonopsians from the middle Permian of South Africa, as well as two partial jaws from the late Permian of Tanzania (NMT RB404) and likely late Permian of Zambia (NHCC LB367) were sectioned for this study. All of the teeth were attached by gomphosis to the alveoli (Suppl. Fig. 10). One tooth position in a section through a maxilla preserved a developing successional tooth that was positioned mesially and lingually to the functional tooth (Suppl. Fig. 10d). This indicates that the functional tooth at this position was nearing the end of its lifespan. Functional teeth at comparable stages in "pelycosaur"-grade synapsids and dinocephalians show complete ankylosis. In gorgonopsids, gomphosis appears to have been a permanent state for each tooth, which is similar to mammals.

Each post-caniniform tooth root is circular in cross-section and is coated in two layers of cementum. The most internal layers is a thin featurless band of acellular cementum that is devoid of Sharpey's fibers or cementocyte lacunae, whereas the external layer is much thicker, consists of abundant circumferential growth lines, Sharpey's fibers, and cementocyte lacunae, consistent with cellular cementum (Suppl.

Fig. 10f, g). The presence of a non-mineralized periodontal ligament can be inferred by the presence of a space between the tooth roots and the surrounding alveolar bone, as well as Sharpey's fibers in the cementum and alveolar bone (Suppl. Fig. 10f, g). Clear instances of post-mortem tooth loss (Suppl. Fig. 10e) also support the presence of a ligamentous tooth attachment. Several of the teeth in the dentary sections are missing, but the surrounding alveolar bone is well developed and contains dense networks of Sharpey's fibers, marking the insertion points of the periodontal ligament. These teeth were clearly lost after decay of the periodontal ligament and were not in the process of being replaced. Alveolar bone in gorgonopsians consists of a well-vascularized, primary woven bone matrix that is separated by the surrounding hard tissues by a reversal line, indicating resorption prior to deposition of new layers of alveolar bone (Suppl. Fig. 10c, f, g). Along one tooth position, the alveolar bone has partially resorbed the root of an old tooth, leaving behind a fragment of dentine between two tooth positions (Suppl. Fig. 10c).



Supplementary Figure 11. Tooth attachment in Therocephalia. **a,** skull drawing of a carnivorous therocephalian highlighting elements sampled in this study (modified from[15]. **b,** closeup of the tooth attachment tissues in an indeterminate therocephalian (BP/1/7257) exhibiting a gomphosis. **c,** closeup of tooth attachment tissues in a neighbouring tooth of the same specimen as b showing complete ankylosis via inward growth of alveolar bone. **d,** wholeview of a single therocephalian dentary tooth sectioned in coronal aspect showing a gomphosis (BP/1/172). **e,** closeup of dental attachment tissues of same tooth in d. **f,** closeup of tooth attachment tissues between two teeth in the same specimen (BP/1/172) but in transverse view. Asterisks indicates apparent ankylosis, but this is probably due to post-mortem displacement of the tooth. **g,** skull drawing of *Bauria* highlighting element sampled in this study (modified from[15]. **h,** closeup of a single dentary tooth root of *Bauria* sectioned in transverse aspect just above the jaw margin (BP/1/2523). **i,** closeup of same tooth as in h sectioned far below the alveolar margin. Note the presence of a clear periodontal space. **j,** wholeview of a dentary tooth of BP/1/2523 showing gomphosis-type tooth attachment in coronal aspect. **k,** closeup of root cementum and Sharpey's fibers of the tooth imaged in h. **l,** closeup of root cementum and Sharpey's fibers of the tooth imaged in i. The cellular cementum is significantly thicker towards the root apex. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; de, dentine; jb, jawbone; ps, periodontal space; rl, reversal line; sf, Sharpey's fibers.

Therapsida: Therocephalia indet. + *Moschorhinus kitchingi*

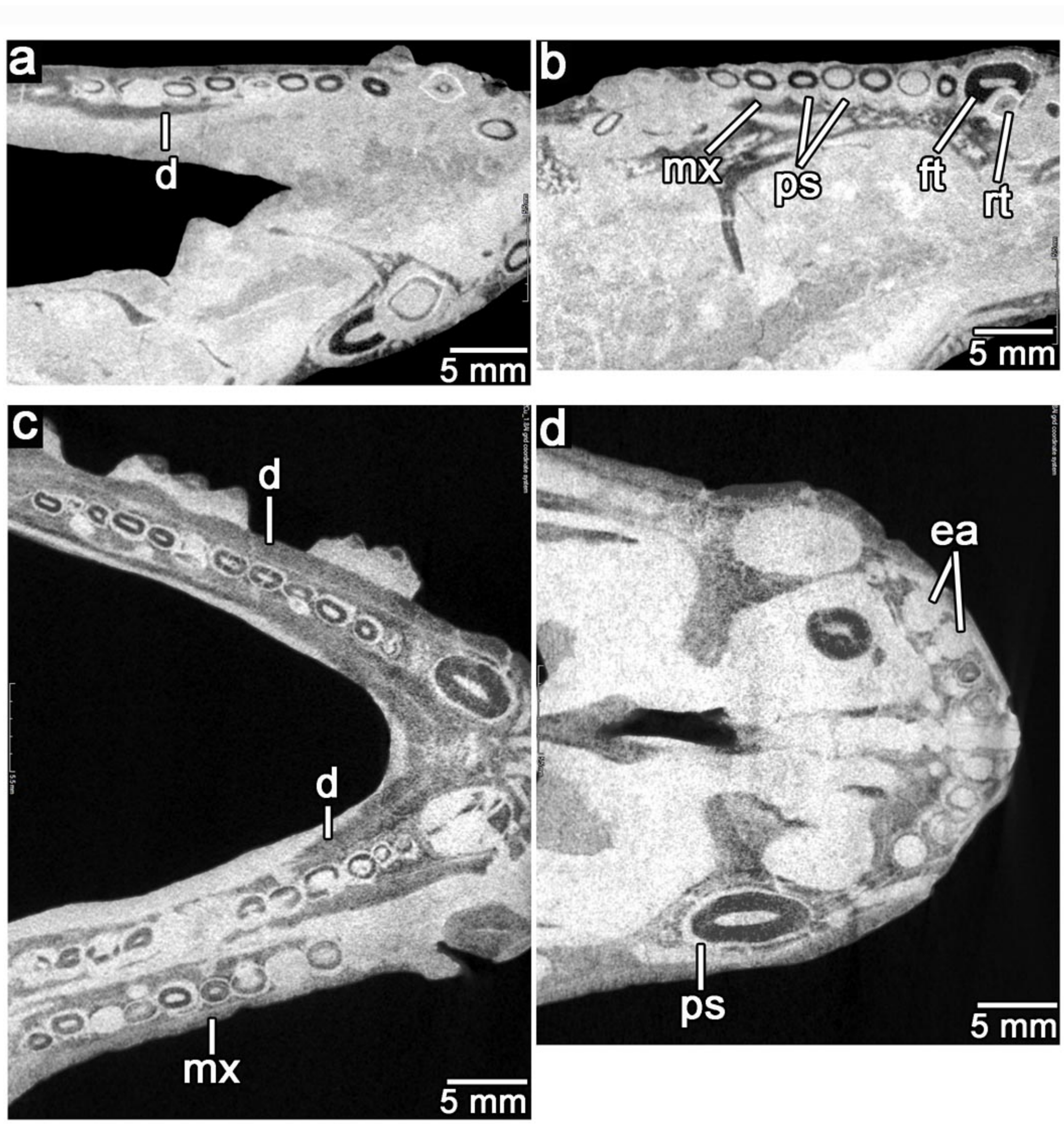
We sampled two indeterminate therocephalians from the Beaufort Group of South Africa (Suppl. Fig. 11), and examined a cut and polished skull preserving the canines of *Moschorhinus kitchingi* (BP/1/2788). Two of these specimens (BP/1/7257 and BP/1/2788) possessed both teeth that were ankylosed to the jaws as well as teeth that were attached by gomphosis (Suppl. Fig. 11b, c; Suppl. Table 2). Teeth that were ankylosed showed regional differentiation of the surrounding bony tissue. The layers immediately surrounding the acellular cementum of the tooth root possess concentric growth lines and well-stratified layers of cell lacunae (Suppl. Fig. 11b) and it is histologically identical to cellular cementum in other taxa. The cellular cementum in BP/1/7257 is a different colour from the surrounding spongy bone (Suppl. Fig. 11c), providing a clear demarcation between cementum and the surrounding alveolar bone in ankylosed teeth. Based on the different stages of tissue development along the jaws of this specimen, it is clear that teeth were initially attached by gomphosis, only to later become ankylosed by centripetal growth of alveolar bone [12].

The other indeterminate therocephalian specimen (BP/1/172) exhibits a different form of tooth attachment. All of the teeth in both dentaries were attached by gomphosis to the socket margins (Suppl. Fig. 11d–f). The dentine of each tooth is coated in a thin band of acellular cementum and a thicker layer of cellular cementum. The alveolar bone consists of a thin layer of woven bone that is separated from the vascularized bone of the jaw by a reversal line, indicating the extent of resorption of the previous generations of alveolar bone (Suppl. Fig. 11e). A well-defined periodontal space as well as extensive, parallel Sharpey's fibers in the alveolar bone and cellular cementum indicates that each tooth was attached by periodontal ligament (Suppl. Fig. 11e, f). Some of the tooth positions show more extensive centripetal growth of the alveolar bone than others, but none of them were ankylosed.

Therapsida: Therocephalia: *Bauria*

Several coronal and transverse sections were made through a complete dentary of the derived, herbivorous therocephalian *Bauria* (Suppl. Fig. 11g–l; Supplementary Table 1). These sections revealed that every tooth along the jaw was attached by gomphosis to the surrounding alveolar bone. Each tooth root was coated in a thin band of acellular cementum beginning at a level above the alveolar margin. Serial sections of the tooth roots revealed that cellular cementum coats the acellular cementum further apically and becomes progressively thicker towards the root apex, as it does in mammals [12]. More apical sections show that the cellular cementum contains a series of growth lines, which extend parallel to the root surface. The cellular cementum is perforated by Sharpey's fibers, marking the insertion points of the periodontal ligament. Sharpey's fibers with similar orientations to those found in the cementum of the root are also found in the surrounding alveolar bone further apically. The alveolar bone is always separated from the cellular cementum of the tooth root by a periodontal space in *Bauria* (Suppl. Fig.

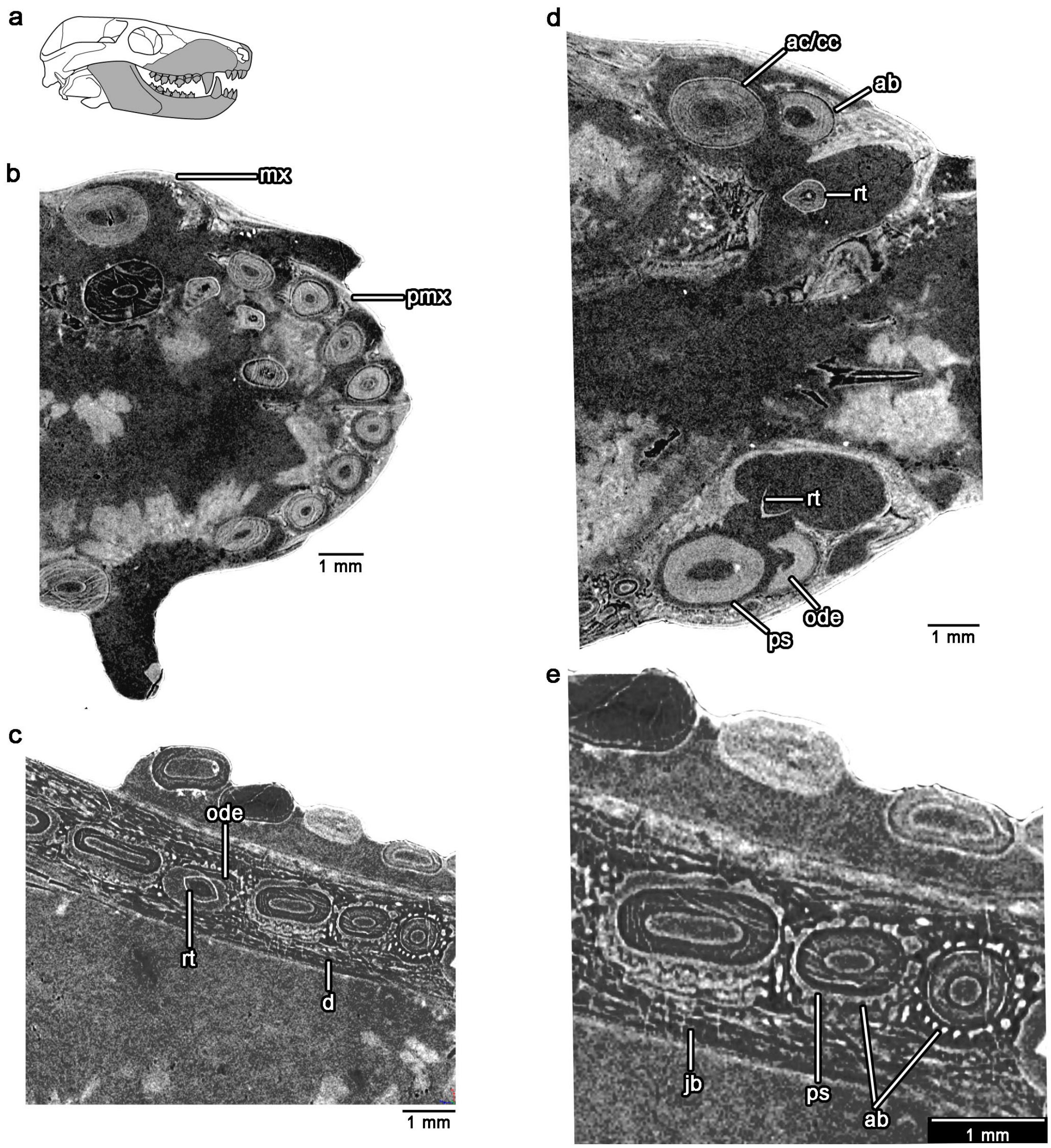
11h–l). These findings indicate that *Bauria* possessed a periodontium that was nearly identical to that of modern mammals.



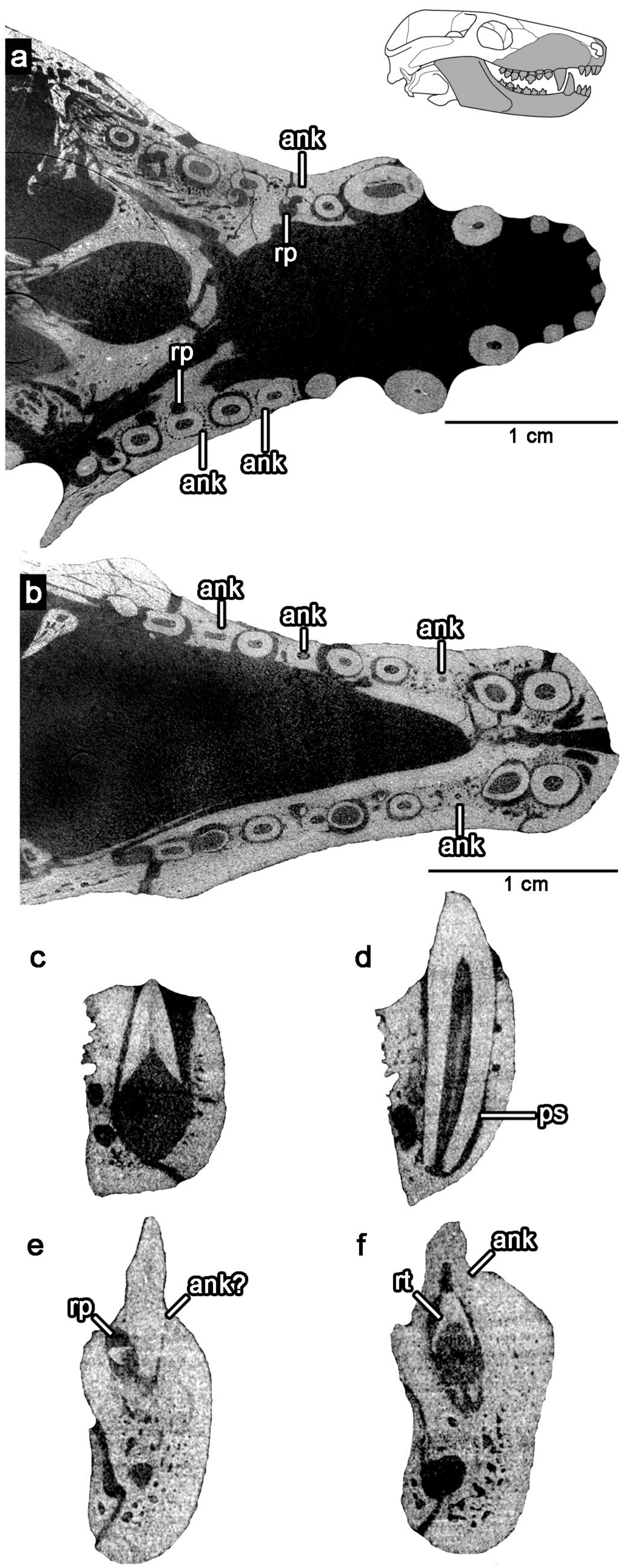
Supplementary Figure 12. Ontogeny of tooth attachment in the cynodont *Galesaurus*. **a,** dentary teeth in a subadult specimen (BP/1/4602) showing no evidence of dental ankylosis. **b,** left maxilla of BP/1/4602, showing no evidence of dental ankylosis. **c,** dentary adult specimen (BP/1/5064). All teeth were attached by gomphosis. **d,** premaxillary and anterior maxillary teeth of BP/1/4602 showing gomphosis at each tooth position.

Therapsida: Cynodontia: *Galesaurus*

We examined CT scans of a presumed subadult (BP/1/4602) and a presumed adult (BP/1/5064) of the Early Triassic cynodont *Galesaurus* (Suppl. Fig. 12) (see [16] for skull measurements and ontogenetic status assignment). These specimens preserve all of the upper and lower dentition thus providing a glimpse into possible variation in tooth attachment mode. Despite numerous documented ontogenetic changes to the skull in *Galesaurus* [16], the tooth attachment mode between subadult and adult individuals appears to have been the same. Although we cannot confirm the presence and types of root cementum present due to the scanning resolution, each tooth root is surrounded by a clear periodontal space that separates the surrounding spongy alveolar bone from the tooth root surfaces (Suppl. Fig. 12). The presence of periodontal spaces and numerous empty alveoli are indicative of a ligamentous tooth attachment. In both the subadult (Suppl. Fig. 12a, b) and adult (Suppl. Fig. 12c, d) specimens, even functional teeth that were in the process of being replaced show no evidence of ankylosis. As such, each tooth was probably attached to the alveolar bone by an uncalcified periodontal ligament throughout dental ontogeny, similar to modern mammals.



Supplementary Figure 13. Ontogeny of tooth attachment in a small individual (BP/1/5372) of the early cynodont *Thrinaxodon*. **a,** skull drawing of *Thrinaxodon* highlighting elements examined in this study (modified from[14]. **b,** CT scan image of the premaxillary and caniniform tooth roots of BP/1/5372. **c,** closeup of CT scan image of lower postcaniniform tooth roots in BP/1/5372. **d,** deeper slice through the premaxillary and caniniform tooth roots. Note that most of the teeth are separated from the alveoli by periodontal spaces, except for those that are being replaced. **e,** closeup of same image as c showing teeth at successive stages of attachment tissue development. Alveolar bone develops centripetally to enclose the periodontal space and ankylose older teeth. Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular cementum; d, dentary; jb, jawbone; mx, maxilla; ode, dentine of older tooth; pmx, premaxilla; ps, periodontal space; rt, replacement tooth.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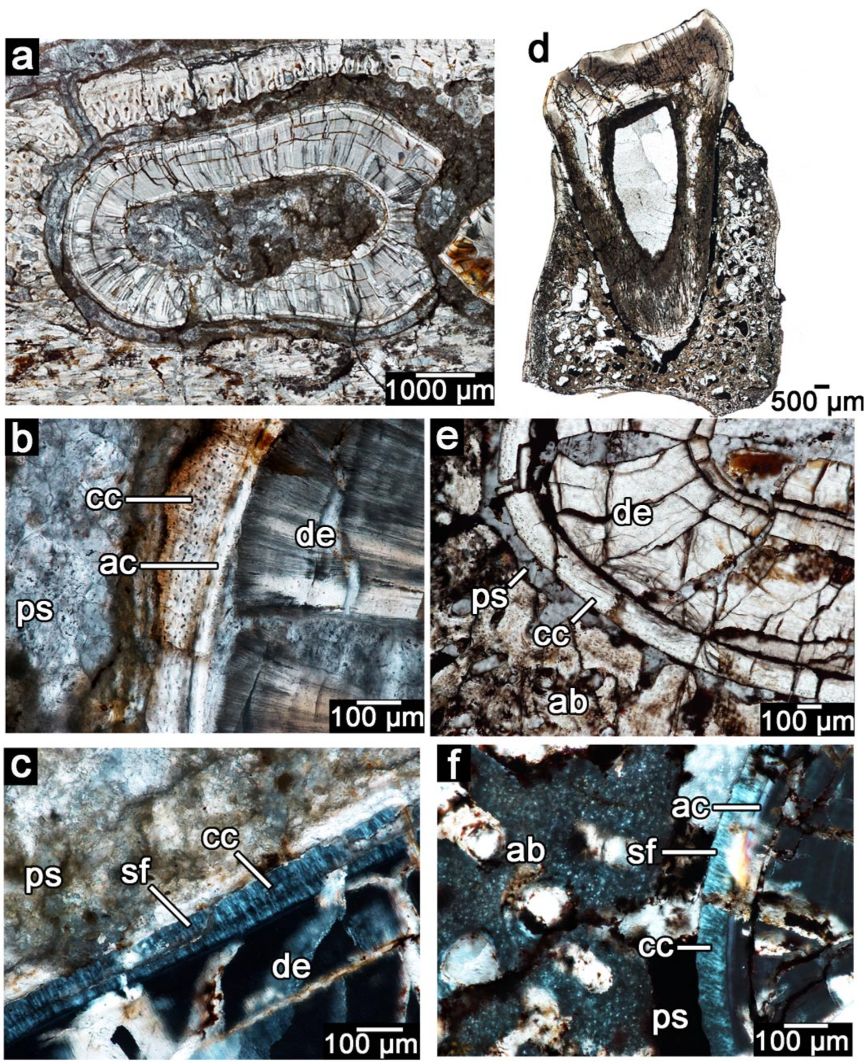
Supplementary Figure 14. The ontogeny of tooth attachment in a large individual (BP/1/7199) of the early cynodont *Thrinaxodon*. **a,** CT scan image of tooth attachment in the upper dentition of BP/1/7199. **b,** CT scan image of tooth attachment in the lower dentition of BP/1/7199. Some of the teeth possess periodontal spaces, whereas others are ankylosed to the jaws. **c,** coronal view of an erupting anterior dentary tooth. **d,** coronal view of an erupted anterior dentary tooth that is attached by gomphosis to the alveolus. **e,** coronal view of a posterior dentary tooth that is possible ankylosed to the jaws and exhibits an early stage of replacement. **f,** coronal view of a posterior dentary tooth that is at an advanced stage of replacement but is clearly ankylosed to the jaws. Abbreviations: ank, ankylosis; ps, periodontal space; rp, replacement pit; rt, replacement tooth.

Therapsida: Cynodontia: *Thrinaxodon*

CT scans of the upper and lower dentition of a small individual (BP/1/5372; Suppl. Fig. 13) of the Early Triassic cynodont *Thrinaxodon* revealed that nearly all of the teeth were separated from the surrounding alveolar bone by a periodontal space, indicating the presence of a ligament that held the teeth in place in life. Cementum coated the root portions of the teeth, but the nature and relative thicknesses of the acellular and cellular cementum could not be determined from the scans (Suppl. Fig. 13b–e). Some sections of the jaws show teeth at different stages of tissue development and clearly show that the spongy alveolar bone gradually extended centripetally and in some cases, contacted the cementum of the tooth root to form a stable ankylosis (Suppl. Fig. 13c, e). Areas in which teeth were actively being replaced by a new tooth showed that the older tooth was sometimes ankylosed to the surrounding alveolar bone. Some of the tooth positions, however, show ankylosis without any signs of a replacement tooth, suggesting that ankylosis occurred earlier in the functional life of the tooth, prior to the onset of root resorption (Suppl. Fig. 13c, e).

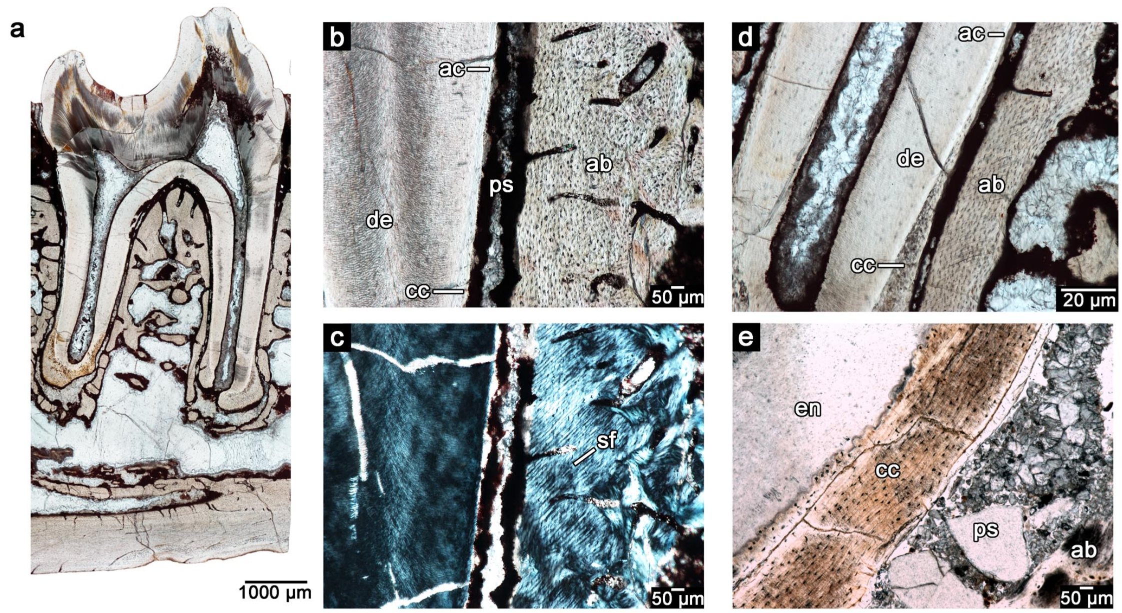
CT scans of a larger specimen (BP/1/7199) show similar occurrences of ankylosis and gomphosis (Suppl. Fig. 14a, b). Most of the erupted teeth in this specimen possess a periodontal space (and therefore a ligament) between the tooth roots and the surrounding alveolar bone. Teeth that were in the process of being replaced show signs of centripetal growth of alveolar bone and ankylosis (Suppl. Fig. 14a–f), again indicating that most of the functional teeth were ankylosed prior to being replaced. This was not universal along the jaw, however, as some postcanine teeth were still attached by gomphosis at an advanced stage of being replaced (Suppl. Fig. 14a, b). Even larger individuals show the same condition, where teeth are ankylosed immediately prior to being replaced [17]. These results suggest that ankylosis occurred at late ontogentic stages for individual teeth at many tooth positions, regardless of the age of the individual.



Supplementary Figure 15. Tooth attachment tissues in the cynodonts *Cynognathus* and *Diademodon*. **a,** closeup of a single tooth in a transverse section through a partial dentary of *Cynognathus* (BP/1/6097). Deeper sections through this tooth reveal that it was in the process of being replaced (see Suppl. Fig. 17), suggesting that older teeth in this taxon were still attached by gomphosis. **b,** closeup of the root cementum in a showing extensive cellular cementum. **c,** closeup of the root cementum in a under cross-polarized light, showing the Sharpey's fibers of the periodontal ligament. **d,** wholeview of a coronal section through a nearly complete maxilla of *Diademodon* (BP/1/4652). The tooth is separated from the alveolar wall by a periodontal space, indicating a ligamentous tooth attachment. **e,** closeup of the attachment tissues in a transverse section through BP/1/4652. **f,** closeup of same tooth in e under cross-polarized light, showing Sharpey's fibers of the periodontal ligament.

Therapsida: Cynodontia: *Cynognathus* and *Diademodon*

It was possible to section a partial jaw tentatively assigned to the non-mammalian eucynodont *Cynognathus* based on the morphology of the postcanine teeth and a nearly complete maxilla of the closely related form *Diademodon* with in-situ postcanines (Suppl. Fig. 15; Supplementary Table 1). Additional thick sections through a skull of *Diademodon* (BP/1/1171) were also examined (Supplementary Table 2). The teeth of all three specimens are all suspended in the sockets and are not fused to the alveolar margins. The tooth roots of *Cynognathus* and *Diademodon* are all coated in a well-defined, thin band of clear, acellular cementum, and multiple layers of cellular cementum (Suppl. Fig. 15b–f). Each layer of cellular cementum is separated by a growth line and contains rows of flattened cementocyte lacunae. The cellular cementum contains an abundance of Sharpey's fibers that are best viewed under cross-polarized light, indicating the presence of a periodontal ligament (Suppl. Fig. 15c, f). A mineral-filled space persists around all of the tooth roots in both specimens. The surrounding bone is a primary, well-vascularized bone matrix that is perforated by Sharpey's fibers with similar orientations to those in the cellular cementum. This bone is separated from the surrounding bone of the jaw by a reversal line. These observations indicate that, unlike the condition in *Thrinaxodon*, but similar to *Galesaurus*, the teeth of *Cynognathus* and *Diademodon* were attached by gomphosis. None of the teeth in *Diademodon* or *Cynognathus* showed evidence of dental ankylosis, indicating that ligamentous attachment in these cynodonts was probably permanent, similar to mammals.



Supplementary Figure 16. Mammalian tooth attachment tissues. **a,** closeup of a single tooth in a longitudinal section through the partial jaw of the condylarth *Hyopsodus* (USNM 595273). Note the presence of a periodontal space, which housed the periodontal ligament in life. **b,** closeup of the attachment tissues in USNM 595273. **c,** Same image as b under cross-polarized light, highlighting the Sharpey's fibers of the periodontal ligament. **d,** closeup image of the attachment tissues in an extracted human molar (ROM R9245). **e,** closeup image of the coronal cementum of an in-situ horse tooth (ROM 33036). Abbreviations: ab, alveolar bone; ac, acellular cementum; cc, cellular (and coronal) cementum; de, dentine; en, enamel; gzd, globular zone of dentine; ps, periodontal space; sf, Sharpey's fibers.

Mammalia

We examined thin sections of a fossil condylarth *Hyopsodus* (USNM 595273) and a horse, *Equus sp.* (ROM 33036) (Suppl. Fig. 16). In nearly all mammals, the dentine of the tooth root is coated in several layers of acellular cementum [18]. Unlike the condition in non-mammalian synapsids, acellular cementum is not always covered by cellular cementum and the former tissue may form the principal attachment site for the periodontal ligament [18] (Suppl. Fig. 16a–c). By comparison, in non-mammlian synapsids, and in amniotes in general, this role is accomplished by the cellular cementum. Cellular cementum in mammals is typically restricted to the apical third of the root and is only a minor contributor to periodontal ligament attachment (Suppl. Fig. 16d). However, a major exception to this occurs in ever-growing and high-crowned (hypsodont) teeth (Suppl. Fig. 16e). In both cases, extensive layers of cellular cementum, perforated by parallel Sharpey's fibers, form the principal sites of attachment to the periodontal ligament.

Alveolar bone in mammals is variably woven, parallel-fibered, or lamellar bone, depending on the age of the tooth and the age of the individual [18]. The bone layer closest to the periodontal space always consists of a primary bone matrix perforated by coarse Sharpey's fiber bundles from the periodontal ligament and is thus termed the bundle bone layer. These Sharpey's fibers are best viewed under cross-polarized light and are neatly arranged into parallel groups extending towards the periodontal space (Suppl. Fig. 16c). In fossil and modern material, the periodontal ligament decays and its position is marked by a space between the cementum of the tooth root and the bundle bone of the alveolus (Suppl. Fig. 16b–e).

Supplementary Information S2: Character construction and ancestral character state reconstruction

Three character states for a single new character were constructed, based on the observed variations in the proportion of time teeth of a given taxon were found in one of the four stages of dental ontogeny (main text Figure 3). The following character states were used:

<u>State 0:</u> Functional gomphosis absent and ankylosis present. Teeth are almost always found in section as completely ankylosed or in the process of erupting. Extremely rare functional gomphosis and no incidences of post-mortem tooth loss. Teeth also very rarely found in the intermediate mineralization stage (i.e. incompletely ankylosed to the jaws).

<u>State 1:</u> Functional gomphosis and ankylosis present. Teeth found in all four stages. Some teeth found ankylosed, some possibly lost post-mortem. Can see all stages across a thin section of a jaw.

<u>State 2:</u> Functional gomphosis present and ankylosis absent. Teeth only found in thin section as gomphosis attachment or in the process of erupting. Ankylosis occurs only pathologically. Mineralization of periodontium is very limited, and typically associated with incremental cementum deposition. Post-mortem tooth loss very common.

Taxon	**Replacement**	**Gomphosis**	**Mineralization**	**Ankylosis**	**Character state for terminal taxon (0, 1, 2)**
Oromycter (ROM 67604)	1	0	0	4	**Caseidae**
Oromycter (ROM 67605)	1	0	0	2	**0**
Varanopid indet. (ROM 67514)	3	0	0	6	**Varanopidae**
Varanopid indet. (ROM 66866)	4	0	1	4	**0**
Edaphosaurid (NHB 2041070)	0	0	1	3	**Edaphosauridae 0**
Secodontosaurus (ROM 6027)	0	0	1	2	**Sphenacodontidae**
Secodontosaurus (UMMP 9714)	0	0	0	2	
Sphenacodon ferocior (ROM 66105)	1	0	0	2	

Sphenacodon ferocior (CM 89931)	1	0	0	3	**0**
Dimetrodon limbatus (StIPB R-602)	0	0	1	4	
Dimetrodon limbatus (StIPB-R601)	2	0	0	3	
Dimetrodon grandis (ROM 6039)	0	0	0	2	
Dinocephalia indet. (BP/1/6854)	2	1	4	0	**Dinocephalia indet.** **1**
Dinocephalia indet. (BP/1/4851)	0	1	2	0	
Dinocephalia indet. (BP/1/5417)	0	0	4	1	
Tapinocephalidae indet. (NHCC LB 370)	1	2	0	0	**Tapinocephalidae** **2**
Tapinocephalidae indet. (NHCC LB 369); jaw figured	2	2	0	0	
Diictodon "A" (ROM 52624)	0	0	2	0	**Anomodontia** **1 & 2**
Diictodon "B" (ROM 52624)	0	0	0	2	
Diictodon "C" (ROM 52624)	0	1	0	0	
Lystrosaurus (UWBM 109908)	0	1	0	0	
Gorgonopsia indet. (BP/1/784)	3	4	0	0	**Gorgonopsia** **2**
Gorgonopsia indet. (BP/1/2395a)	1	4	0	0	
Gorgonopsia indet. (NMT RB404)	0	1	0	0	
Gorgonopsia (NHCC LB 367)	0	1	0	0	
Therocephalia indet. (BP/1/7257)	2	4	1	1	**Therocephalia indet.** **1 & 2**
Therocephalia indet. (BP/1/172)	3	5	0	0	
Moschorhinus kitchingi (BP/1/2788)	0	1	1	0	

Bauria (BP/1/2523)	3	9	0	0	***Bauria* 2**
Galesaurus (BP/1/4602)	4	13	0	0	***Galesaurus***
Galesaurus (BP/1/5064)	4	34	0	0	**2**
Thrinaxodon (BP/1/5372)	7	22	10	2	***Thrinaxodon***
Thrinaxodon (BP/1/7199)	8	28	6	5	**1**
?Cynognathus (BP/1/6097)	1	2	0	0	**Cynognathia**
Diademodon (BP/1/1171)	2	6	0	0	**2**
Diademodon (BP/1/4652)	0	9	0	0	
Tritylodon (SAM-PK-K407) (Taken from Jasinoski and Chinsamy, 2012)	0	3	0	0	**Probainognathia 2**
Hyopsodus (USNM 595273)	0	3	0	0	**Mammalia**
Equus (ROM 33036)	0	2	0	0	**2**

Table S2. Frequencies of tooth stages observed in sectioned and CT-scanned synapsid specimens. Two additional specimens were added to the dataset from personal observations of cut and polished specimens (*Moschorhinus* [BP/1/2788] and *Diademodon* [BP/1/1171]). The colours correspond to the character states in main text figure 4.

The topology used for character state reconstruction was the strict consensus tree from Sidor and Hopson[14]. Varanopids and caseids were not added to the original data matrix due to ongoing uncertainty in their phylogenetic positions[14,19]. We ran the data matrix from Sidor and Hopson[14] under a heuristic search and the parsimony optimality criterion using PAUP* [20] ver.4.0a162. We were able to generate the same strict consensus tree from three most parsimonious trees as Sidor and Hopson[14], but with slightly different tree scores (TL: 369; CI: 0.661; RCI: 0.565).

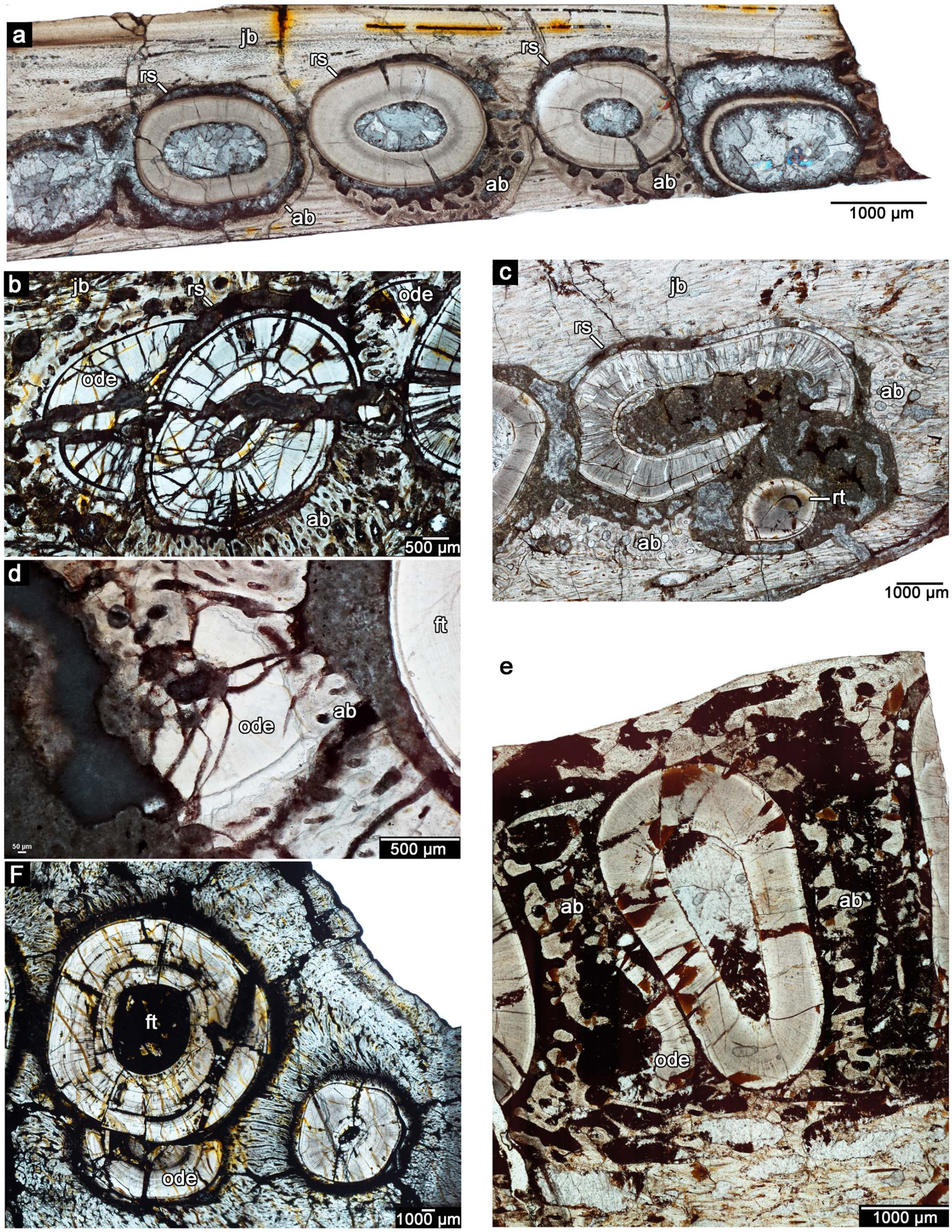
The new character (character 182) was then added and treated as unordered under parsimony and was examined using the "Trace Character History" function in Mesquite using the taxon-character matrix and strict consensus topology from Sidor and

Hopson[14]. Character coding for "Ophiacodontidae" was taken from descriptions by Edmund[21] who noted that the teeth of *Ophiacodon* and *Varanosaurus* were ankylosed at maturity, but might have spent more time in a non-ankylosed state than those of sphenacodontids. Due to the fact that we did not have histological or CT scan data for any biarmosuchian, "Biarmosuchia" was coded as "?" (however, see the anatomical observations of tooth attachment in the biarmosuchian *Niaftasuchus* [Supplementary Information 1]). "Estemmenosuchidae" was treated as the lineage that includes tapinocephalids and "Anteosauridae" was treated as the group of dinocephalians represented by the thin sections of non-tapinocephalid dinocephalians examined in this study, given that the dental morphology of the sampled specimens was consistent with omnivorous or carnivorous dinocephalian taxa. Codings for "*Probainognathus*", "*Probelesodon*", were left as "?" and "Tritheledontidae" was coded as state "1" based on descriptions of tooth attachment in several members of the group [22,23]. Observed character states for *Cynognathus* and *Diademodon* were used to code the OTU "Cynognathia". It is worth noting that the probainognathan *Tritylodon* probably exhibited a permanent gomphosis (state "2") [24], but other probainognathans probably exhibit state "1" [22,23]. This character was left as "?" for *Probainognathus* in the phylogeny of Sidor and Hopson [14].

Supplementary Information S3: Evidence for reduced tooth replacement rates in Therapsida

We found evidence that prolonged periods of time had passed between tooth replacement events in therapsids compared to "pelycosaurs" (Suppl. Fig. 17). In two of the therocephalians and some cynodonts sampled for this study, the functional teeth show evidence of extensive tooth migration (towards the upper left corner of the figure). In these instances, non-ankylosed teeth along the dentaries are surrounded by alveolar bone that is extremely unequal in thickness (Suppl. Fig. 17a, b, c). Thick layers of spongy alveolar bone define the trailing edge of the migrating tooth and tooth socket, whereas the leading edge is resorptive, with only a thin veneer of alveolar bone forming the alveolus.

This type of tooth migration has been documented in mammals and is mediated by a periodontal ligament [25]. Such tooth migration occasionally produced an offset between the functional tooth and its replacement tooth, resulting in slightly uneven root resorption (Suppl. Fig. 17c). Large fragments of dentine are also preserved in between functional teeth in gorgonopsians, dinocephalians, and therocephalians (Suppl. Fig. 17b, d, e, f), which are all indicators of extensive tooth migration prior to replacement. Lastly, in some instances, the replacement tooth is significantly larger than the previous tooth generation (Suppl. Fig. 17f), further suggesting an extended period of time has passed between replacement events.



Supplementary Figure 17. Evidence for extensive tooth drift and reduced tooth replacement rates in non-mammalian therapsids. **a,** whole view of a transverse section through the dentary of a therocephalian (BP/1/172) where each tooth has drifted towards the top left of the image. **b,** close-up of a single tooth in transverse section of a therocephalian dentary (BP/1/7257) in which the tooth has migrated towards the top left of the image, resorbing significant portions of an older, ankylosed tooth in the process. **c,** close-up of a dentary tooth of *Cynognathus* (BP/1/6097) that has migrated towards the top left of the image. As a result, the developing replacement tooth is distally offset from the functional tooth and does not resorb the middle of the older root. **d,** close-up of a remnant of an old tooth root that is located in between two tooth positions in a transverse section of a gorgonopsian maxilla (BP/1/2395a), which is a clear indication of extensive mesiodistal drift of the teeth over ontogeny. **e,** close-up of a single dentary tooth root of the therocephalian *Bauria* (BP/1/2523) in transverse section that is flanked on the left by a remnant of an old tooth root. This is indicative of extensive mesiodistal drift of the two generations of teeth. **f,** close-up of a single, functional dentary tooth of a dinocephalian (BP/1/4851) and its older, smaller predecessor in transverse section. The size discrepancy between the two generations of teeth indicates an extended period of growth in between tooth replacement events. Abbreviations: ab, alveolar bone; ft, functional tooth; jb, jawbone; ode, dentine of older tooth; rs, resorptive surface; rt, replacement tooth.

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