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Author for correspondence:

Aaron R. H. LeBlanc

e-mail: arl@ualberta.ca

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

Aaron R. H. LeBlanc^{1,2}, Kirstin S. Brink^{1,3}, Megan Whitney⁴,
Fernando Abdala^{5,6,7} and Robert R. Reisz^{1,8,9}

¹Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, Ontario, Canada L5 L 1C6

²Department of Biological Sciences, Faculty of Science, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

³Department of Oral Health Sciences, Faculty of Dentistry, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada

⁴Department of Biology and Burke Museum, University of Washington, Seattle, WA, USA

⁵Unidad Ejecutora Lillo, Conicet, Tucumán, Argentina

⁶Evolutionary Studies Institute and School of Geosciences, University of the Witwatersrand, Johannesburg, South Africa

⁷National Research Foundation, Centre of Excellence: Palaeosciences, Pretoria, South Africa

⁸Institute of Oral Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan, Republic of China

⁹DERC, Jilin University, Changchun, Jilin Province, People's Republic of China

ARHL, 0000-0002-2497-1296; KSB, 0000-0001-6630-1525

The mammalian dentition is uniquely characterized by a combination of precise occlusion, permanent adult teeth and a unique tooth attachment system. Unlike the ankylosed teeth in most reptiles, mammal teeth are supported by a ligamentous tissue that suspends each tooth in its socket, providing flexible and compliant tooth attachment that prolongs the life of each tooth and maintains occlusal relationships. Here we investigate dental ontogeny through histological examination of a wide range of extinct synapsid lineages to assess whether the ligamentous tooth attachment system is unique to mammals and to determine how it evolved. This study shows for the first time that the ligamentous tooth attachment system is not unique to crown mammals within Synapsida, having arisen in several non-mammalian therapsid clades as a result of neoteny and progenesis in dental ontogeny. Mammalian tooth attachment is here re-interpreted as a paedomorphic condition relative to the ancestral synapsid form of tooth attachment.

1. Introduction

The origin and evolution of the complex mammalian dentition from the modest heterodonty and continually replaced dentitions of non-mammalian synapsids is a major topic in vertebrate palaeontology [1–7]. In addition to having limited tooth replacement and complex cusp patterns, mammals are unusual among amniotes in having teeth that are not fused to the jaw but suspended in tooth sockets by periodontal ligaments (PDLs) [2,3,8,9]. The dental gomphosis (socketed, ligamentous tooth attachment, *sensu* [10,11]), unlike dental ankylosis (fused tooth attachment) in most other vertebrates, provides a cushion to resist the compressive and shear forces associated with chewing and a means through which teeth can maintain precise positioning and occlusion [12–15]. Paradoxically, among extant amniotes, the ‘mammalian’ mode of tooth attachment is elsewhere only seen in crocodylians [9,16–19]. The tissues forming the tooth attachment systems in mammals and crocodylians are identical, but the evolution of a gomphosis in the two groups was clearly a case of convergence,

64 given that early synapsids and reptiles had teeth that were
65 fused to the jaws [8,9,20,21].

66 Hypothetical transitional series have been used to explain
67 how the three-part tooth attachment system in mammals—
68 consisting of cementum, alveolar bone and PDL—might
69 have differentiated from an ancestral ‘bone of attachment’, a
70 single-tissue attachment system that fuses teeth to the jaws
71 in most non-mammalian vertebrates [9–11,22]. These
72 hypotheses treat the mammalian gomphosis as the most com-
73 plex form of tooth attachment and posit that it has increased
74 in complexity through evolutionary time in association with
75 dental occlusion, culminating in the mammalian condition
76 [10]. This view has been challenged more recently, because
77 even when teeth are fused to the jaws, all amniote teeth pos-
78 sess homologous tooth attachment tissues to those in
79 mammals [19,20,23]. These recent findings are re-framing
80 our understanding of amniote dental evolution, but they
81 still do not provide an evolutionary or developmental process
82 to explain how mammals evolved a gomphosis from the
83 ancestral synapsid condition of ankylosis. Further complicat-
84 ing this question, numerous studies have lauded the excellent
85 fossil record spanning the evolutionary transition towards
86 crown mammals [2,7,10,24], but none have pinpointed
87 when during the 300 Myr evolutionary history of Synapsida
88 this important shift in dental development occurred, and
89 thus it is often assumed that the gomphosis is a mammalian
90 synapomorphy [3,5,9]. Both the evolutionary origins and
91 mechanism explaining how mammals evolved this condition
92 can be empirically tested using histological data from the
93 fossil record of non-mammalian synapsids.

94 In order to trace and propose a novel mechanism to
95 explain the origins of the mammalian tooth attachment
96 system, we examined histological sections and micro-Computed
97 Tomography (μ CT) scans to document dental
98 ontogeny in a large sample of Permo–Triassic stem-mamma-
99 lian taxa (figure 1). These data allow us to assess the non-
100 mammalian homologues of cementum, alveolar bone and
101 PDL and derive a novel method for characterizing dental
102 evolution and ontogeny across synapsid phylogeny. We use
103 this method to test for fundamental shifts in the timing and
104 sequence of dental ontogeny in Paleozoic and Mesozoic
105 synapsids as a mechanism underlying the transition from
106 ankylosis in early synapsids to the mammalian tooth attach-
107 ment system.

111 2. Material and methods

113 (a) Thin sections

114 Thirty-six specimens of fossil stem and crown mammals were
115 sectioned at the ROM (Royal Ontario Museum, Toronto,
116 Canada) Palaeohistology Laboratory or at UW (University of
117 Washington, Seattle, WA, USA), including representatives of
118 most of the major lineages of ‘pelycosaur’-grade synapsids (Case-
119 idae, Varanopidae, Edaphosauridae, Sphenacodontidae) and
120 therapsids (Dinocephalia, Anomodontia, Gorgonopsia, Theroce-
121 phalia, Cynodontia) (electronic supplementary material, table
122 S1). Material from the Evolutionary Studies Institute (South
123 Africa) was loaned to A. LeBlanc and R. R. Reisz under
124 SAHRA permit ID 1945 for thin sectioning. Moulds and
125 casts were made of several specimens prior to sectioning.
126 Moulds were made using Blustar Silicones V-SIL 1062 silicone
and Hi Pro Green catalyst. Casts were made by pouring

Smooth-on-Smooth-Cast 321 or 322 liquid plastic into the
silicon moulds and placed under pressure until they had set.

Thin sections were made at the Royal Ontario Museum
palaeohistology laboratory and the University of Washington fol-
lowing standard procedures for sectioning fossil material. Each
specimen was embedded in Castolite AC polyester resin and
placed under vacuum. The hardened resin blocks were cut
using a Buehler Isomet low-speed wafer blade saw (between
225 and 300 rpm) and the cut surfaces were polished using
600-grit silicon carbide powder. The polished surfaces were
mounted on frosted plexiglass slides using cyanoacrylate glue
and cut again using the Buehler Isomet. The mounted wafer
was then ground to approximately 100 μ m using the Hillquist
grinding machine with a 240-grit grinding cup. Each specimen
was then hand ground to the preferred thickness using progress-
ively finer grits (600, 1000-grit) of silicon carbide powder and
polished using 1 μ m grit aluminium oxide powder. Thin section
images were taken using a Nikon DS-fi2 camera mounted to a
Nikon AZ-100 microscope using Nikon NIS-Elements imaging
software (Basic Research package).

113 (b) μ CT scans

μ CT scans of four skulls of the early cynodonts *Galesaurus* and
Thrinaxodon were also examined, including a juvenile (BP/1/
5372) and subadult (BP/1/7199) *Thrinaxodon* specimen. As per
Abdala *et al.* [6], scans of BP/1/5372 were made at the European
Synchrotron Radiation Facility (Grenoble, France) on beamline
ID19 in propagation phase-contrast mode (isotropic voxel size
of 20.24 μ m). BP/1/7199 was scanned on beamline ID19 using
the filtered white beam in a half-acquisition mode with a voxel
size of 30 μ m (see [6] for detailed scanning methodology). The
intermediate-sized (BP/1/4602) and large (BP/1/5064) skulls
of *Galesaurus* were μ CT scanned at the Evolutionary Studies
Institute (University of the Witwatersrand, South Africa) using
a Nikon Metrology XTH225/320 LC dual source CT system
[26]. BP/1/4602 was scanned at 130 kV and 185 μ A and BP/1/
5064 at 170 kV and 95 μ A. All scans are segmented in Aviso
6.3 (Visualization Sciences Groups, Merignac, France) and
VGStudio MAX 2.2 (Volume Graphics, Heidelberg, Germany).
All μ CT scan files used for these specimens in this study are
available in the Dryad Digital Repository [27].

113 3. Results

113 (a) Tooth attachment histology in synapsids

The resulting histological dataset consisted of nearly 120 thin
sections and 4 μ CT scans spanning at least 20 distinct synap-
sid taxa. Despite being fossilized specimens, all of the thin
sections revealed the presence of the mineralized attachment
tissues, cementum and alveolar bone (figure 1*b–l*) and in
comparable arrangements to those in extant mammals
(figure 1*m*). Cementum occurs in two forms: a thin acellular
band adjacent to the dentine of the tooth root and a thicker
outer layer with abundant cell lacunae (cellular cementum).
Alveolar bone is a highly vascularized bone layer forming
the tooth socket. Identifying the PDL in fossils is inherently
difficult, because the majority of this tissue is uncalcified
and therefore decays shortly after death. The collagen fibre
bundles of the PDL span the gap between the cementum
and the alveolar bone to suspend the tooth in place
(figure 2*a,b*). Fortunately, these collagen fibre bundles are
partially mineralized where they anchor into the alveolar
bone and cementum, forming Sharpey’s fibres that are visible
in extant and fossil material (figure 2). The presence of

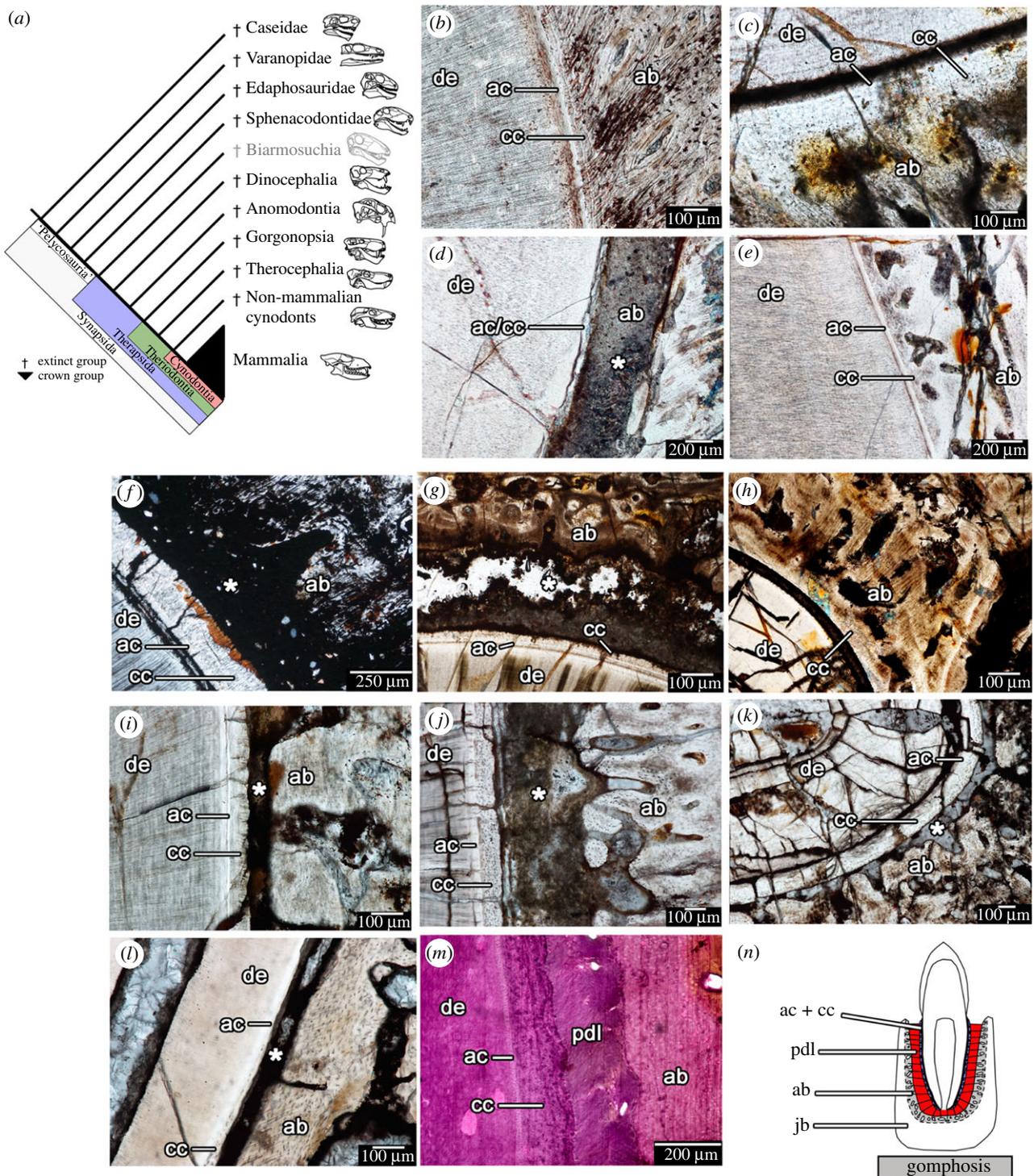


Figure 1. Tooth attachment in stem and crown mammals (Synapsida). (a) Cladogram of the evolutionary relationships of the major taxa within Synapsida (modified from [5,20,25]). (b–l) thin sections showing tooth attachment tissues in major synapsid groups. (b) The sphenacodontid *Dimetrodon* (ROM 6039). (c) An indeterminate dinocephalian (BP/1/4851). (d) The anomodont *Diictodon* (ROM 52624). (e) A second individual of *Diictodon* (ROM 52624). (f) An indeterminate gorgonopsian (NMT RB404). (g) An indeterminate therocephalian (BP/1/7257). (h) The same therocephalian, but an adjacent tooth position. (i) The derived therocephalian *Bauria* (BP/1/2523). (j) The cynodont *Cynognathus* (BP/1/6097). (k) The cynodont *Diademodon* (BP/1/4652). (l) The extinct ungulate mammal *Hyopsodus* (USNM 595273). (m) A stained section of the periodontium in an extant badger (*Taxidea*) (n) illustrated the arrangement of the attachment tissues of the gomphosis in Synapsida. ab, alveolar bone (grey); ac, acellular cementum (blue); cc, cellular cementum (blue); de, dentine; pdl, periodontal ligament (red). Asterisks indicate spaces formerly occupied by periodontal ligament in life.

Sharpey's fibres and a sediment or mineral-filled gap between the tooth root and the socket bone are therefore strong indicators of the presence of a PDL in a fossil vertebrate [20,23,28].

All of the 'pelycosaur'-grade synapsids, exemplified by the sail-backed carnivore *Dimetrodon*, exhibited dental ankylosis (figure 1b), where the soft tissue comprising the PDL

has completely calcified [19,20]. Partially mineralized Sharpey's fibre bundles radiate around the tooth roots within the alveolar bone in these ankylosed teeth and extend to the cellular cementum coating the tooth roots, indicating the presence of a soft tissue attachment prior to complete mineralization [20]. Nearly all of the sectioned teeth were either erupting into the oral cavity (usually preserved only

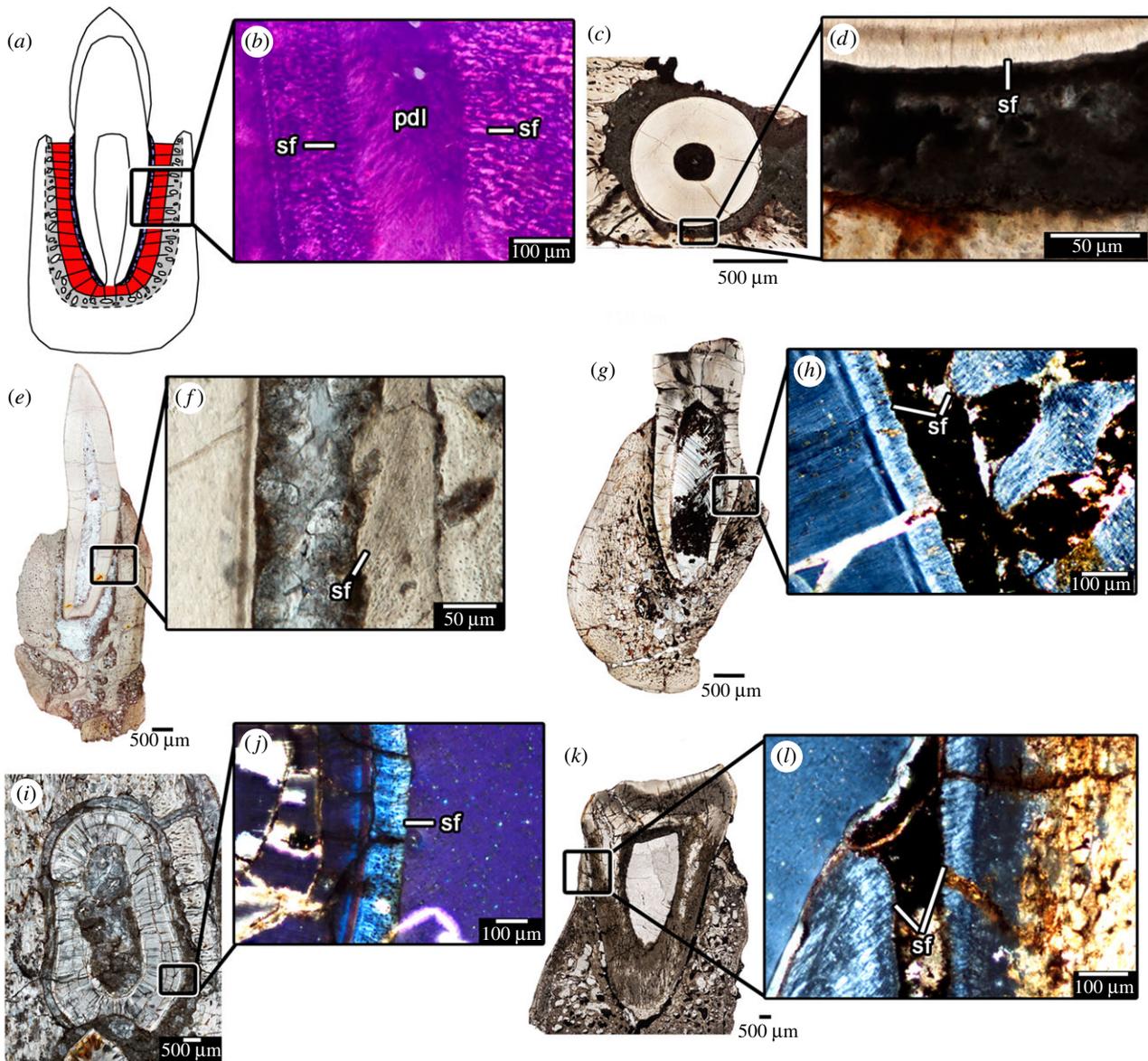


Figure 2. 'Mammal'-like tooth attachment in non-mammalian therapsids. (a) Illustration of the tooth attachment tissues in a gomphosis. (b) Closeup of the tooth attachment tissues in an extant badger under cross-polarized light, showing the Sharpey's fibres of the PDL in the cementum and alveolar bone. (c) Transverse section of a tooth of a gorgonopsian (BP/1/784). (d) Closeup of the periodontal tissues in (c). (e) Coronal section of a tooth of an indeterminate therocephalian (BP/1/172). (f) Closeup of periodontal tissues in (e). (g) Coronal section of a tooth in the therocephalian *Bauria* (BP/1/2523). (h) Closeup of periodontal tissues in *g* under cross-polarized light. (i) Transverse section of a tooth in the cynodont *Cynognathus* (BP/1/6097). (j) Closeup of the periodontal tissues in (i) under cross-polarized light. (k) Coronal section of a tooth in the cynodont *Diademodon* (BP/1/4652). (l) Closeup of the periodontal tissues in *l* under cross-polarized light. pdl, periodontal ligament; sf, Sharpey's fibres.

as replacement pits in the jaws), or completely ankylosed to the jaws, with only four teeth in the entire 'pelycosaur' sample being incompletely ankylosed to the jaws (figure 1*b*; electronic supplementary material, Information S1).

Unlike the condition in 'pelycosaurs', numerous non-mammalian therapsids, including some dinocephalians, therocephalians and the early cynodont *Thrinaxodon* show evidence of ankylosis and gomphosis-type tooth attachment in the same taxon or even the same individual, indicating prolonged ligamentous tooth attachment prior to complete ankylosis (figure 1*c,d,e,g,h*; electronic supplementary material, Information S1). Some of the teeth in these therapsids show evidence of the centripetal growth of the surrounding alveolar bone, indicating that it is the alveolar bone that extends towards the tooth root through dental ontogeny, eventually encasing the PDL in bone (figure 1*d,e,g,h*).

(b) 'Mammal'-like tooth attachment in several non-mammalian synsids

Several non-mammalian therapsids had teeth that were exclusively attached to the socket by an uncalcified PDL, including tapinocephalid dinocephalians, gorgonopsians, bauriid therocephalians and numerous, but not all cynodont genera (figures 1*f,i,j,k*; 2; electronic supplementary material, Information S1). Among non-mammalian cynodonts, only the early cynodont *Thrinaxodon* and some tritheledontids show evidence of dental ankylosis in older generations of teeth [6,29,30]. All other cynodonts we and others [31] examined show evidence of well-developed cementum, PDL (indicated by a mineral or sediment filled space between the tooth root and the socket) and alveolar bone without ankylosis. This gomphosis-type of tooth attachment in these therapsid groups is characterized by smooth outer margins

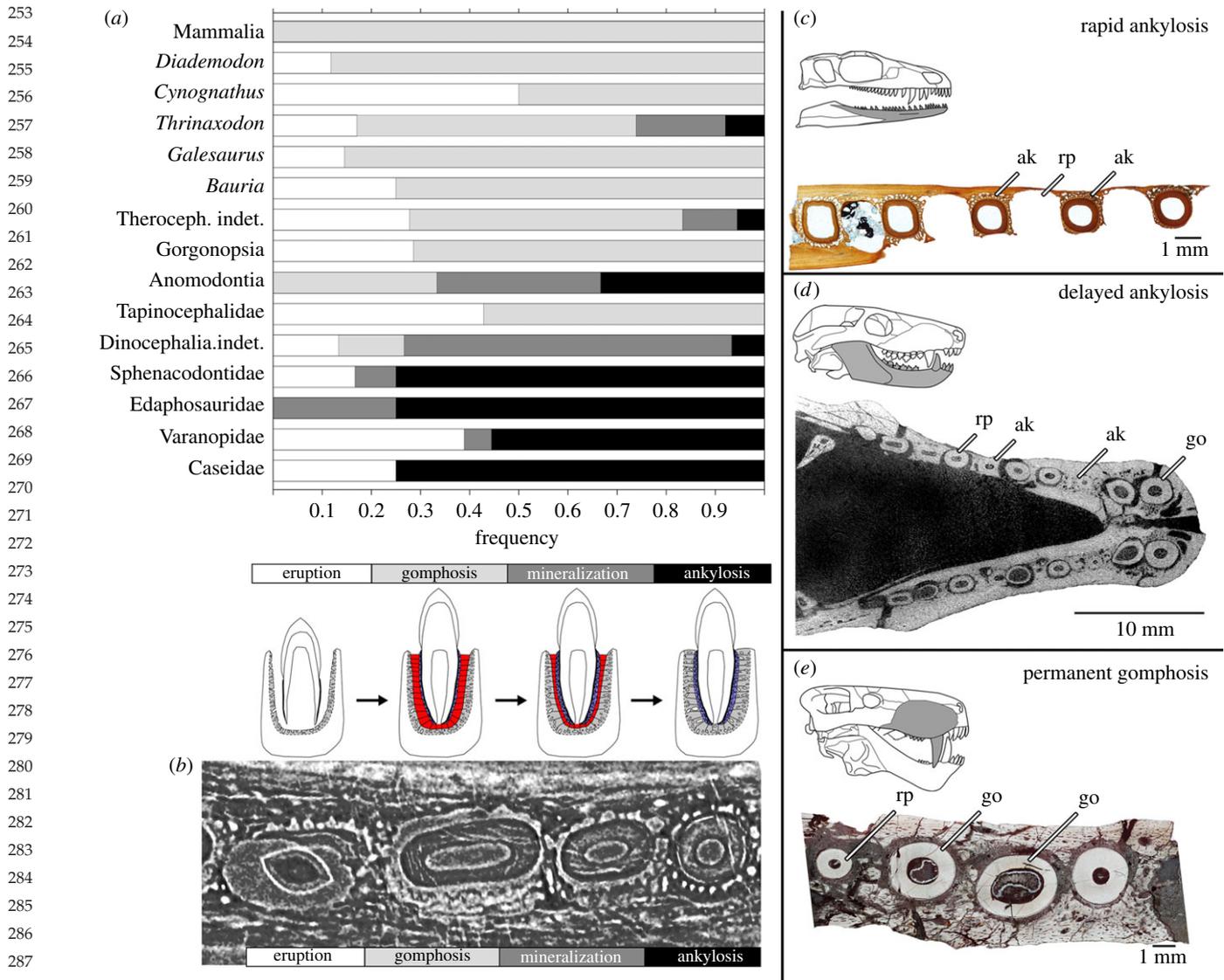


Figure 3. Tooth attachment and relative frequency of tooth attachment stages. (a) The frequency of teeth at the eruption, gomphosis, mineralization and ankylosis stages in thin sections and CT scans of fossil synapsids, reflecting the proportion of time teeth spend in the respective stages (note: the mammal samples used here all had erupted permanent dentitions). (b) Digital transverse section through four postcanines of *Thrinaxodon* (BP/1/5372) showing successive stages of eruption, gomphosis, mineralization and ankylosis (from left to right). (c) varanopid dentary transverse section showing teeth at either the eruption stage or completely ankylosed (rapid ankylosis) (ROM 66866). (d) CT image of the lower jaws of the cynodont *Thrinaxodon* (BP/1/7199) showing teeth at eruption, gomphosis and ankylosis stages (delayed ankylosis). (e) gorgonopsian maxilla (BP/1/2395a) transverse section showing teeth either at the eruption or gomphosis stages (permanent gomphosis). ak, ankylosis; go, gomphosis, rp, resorption pit/erupting tooth. (Online version in colour.)

of the cementum layers with abundant Sharpey's fibres that radiate around the tooth root (figure 2). The surrounding alveolar bone is also perforated by Sharpey's fibres that have complimentary orientations to those in the cementum. This condition is identical to that in extant mammals (figure 2).

(c) Ancestral character state reconstruction

These histological comparisons show that simply mapping the presence of 'ankylosis' and 'gomphosis' as alternative states across synapsid phylogeny fails the homology test of conjunction [32,33], because some individuals exhibit both states along the same jaw. We did, however, note changes in the relative duration of a standardized sequence of four phases of dental ontogeny (figure 3). Tooth staging schemes are most frequently used to map tooth replacement patterns in vertebrates [34–36]; however, we devised a novel staging scheme to encompass the variation in tooth attachment

modes seen in synapsids. After a tooth is shed, all new teeth pass through an eruption stage where the surrounding hard tissues are resorbed and the developing tooth starts to form enamel and dentine (figures 3 and 4a). Upon erupting into the mouth, teeth were attached to alveolar bone by a PDL forming a dental gomphosis (figures 3 and 4b). The gomphosis phase is often followed by gradual inward growth of alveolar bone, which entombed the PDL in bone (figures 3 and 4c). This mineralization phase is characterized by incomplete ankylosis of the tooth root to the socket, with some areas around the tooth exhibiting contact between the cementum and alveolar bone, whereas others are still separated by an unmineralized gap (figures 3 and 4d). The final phase is ankylosis, where the surrounding alveolar bone has completely calcified the PDL and contacts the cementum all around the tooth root.

We tabulated the number of teeth represented in each phase along all of our thin sections and CT scans (electronic supplementary material, table S2) and treated these as being

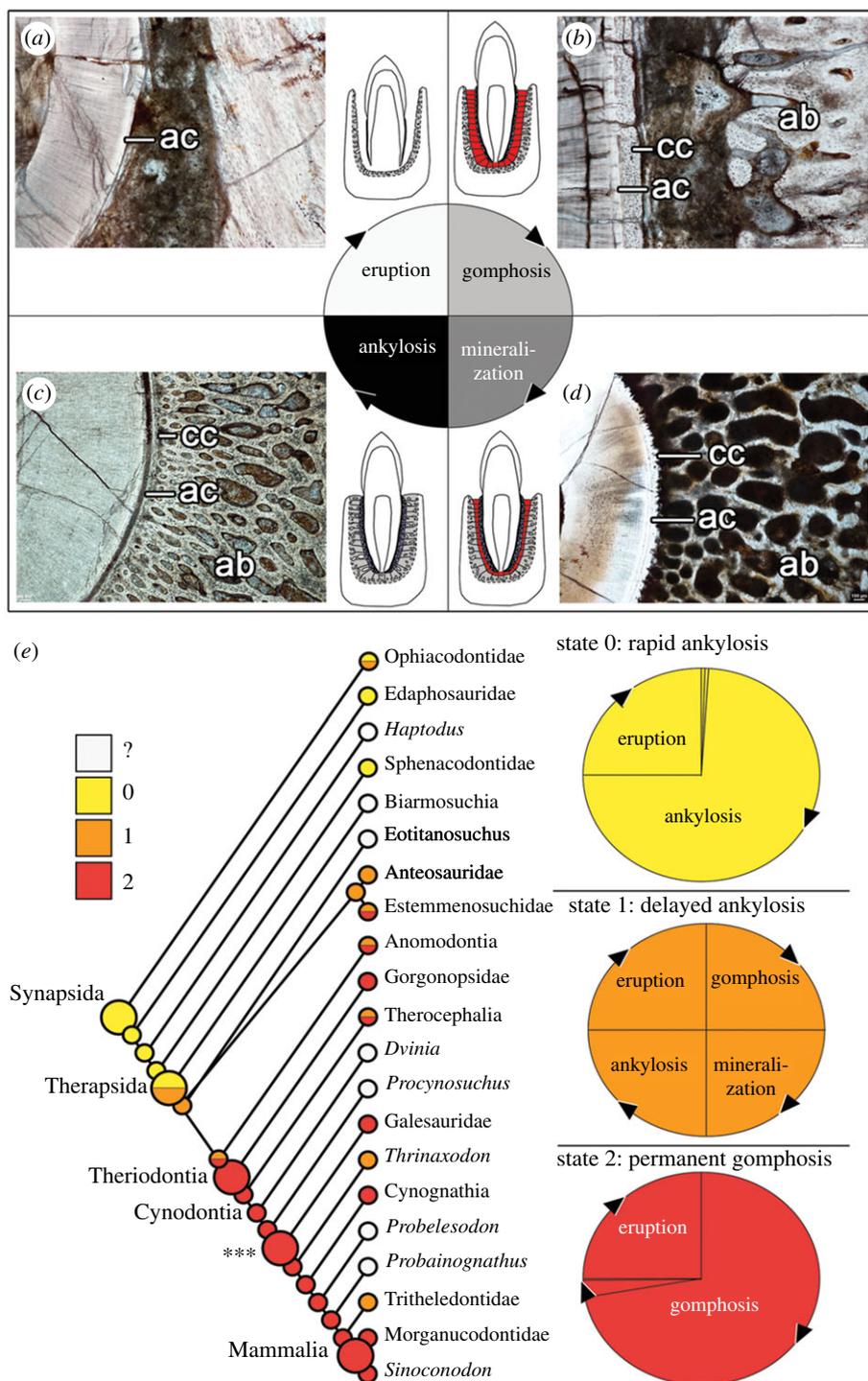


Figure 4. Heterochrony and the evolution of synapsid tooth attachment. (a) Eruption stage after the previous tooth is shed involves remodelling of tissues and formation of the tooth crown and early root tissues (image of erupting tooth of the cynodont *Cynognathus*) (BP/1/6097). (b) Gomphosis stage occurs when all three periodontal tissues are fully formed in a functional tooth (image of an erupted tooth root of *Cynognathus*) (BP/1/6097). (c) Mineralization stage occurs as alveolar bone begins to calcify centripetally. Minor centrifugal mineralization of cementum may also occur (image of a dinocephalian tooth root) (BP/1/6854). (d) Ankylosis stage occurs when alveolar bone and cementum meet and completely entomb the periodontal ligament (image of the tooth root of *Dimetrodon*) (ROM 6039). (e) Ancestral character state reconstruction for the major synapsid clades using a three-state character and the phylogeny of Sidor and Hopson [5]. Larger circles indicate the nodes for Synapsida, Therapsida, Theriodontia and Mammalia. Asterisks and associated large circle indicate earliest cynodont node for which histological data was actually observed. Small circles at terminal branches indicate character state codings for each OTU (Operational Taxonomic Unit). ab, alveolar bone; ac, acellular cementum; cc, cellular cementum.

proportional to the relative duration of each phase in dental ontogeny in a given taxon (following the rationale of [37]). This method revealed three distinct states of synapsid dental ontogeny, based on the relative duration of the gomphosis and ankylosis phases: (0) Teeth pass rapidly to ankylosis after erupting (gomphosis not observed); (1)

teeth pass through a gomphosis stage more slowly, followed by ankylosis (gomphosis and ankylosis observed); and (2) teeth retain a permanent gomphosis (ankylosis not observed) (figures 3c–e and 4e). These character states were then mapped across the synapsid phylogeny of Sidor & Hopson [5] using Mesquite's 'trace character

379 history' function (electronic supplementary material,
380 Information S2).

381 The resulting comparisons of dental ontogeny and charac-
382 ter state distributions show that 'pelycosaurs' have a very
383 short-lived gomphosis phase, because teeth were either in
384 the process of erupting or were ankylosed to the jaws (state 0)
385 (figures 3a and 4e). Therapsids all display proportionally
386 longer-lasting ligamentous phases of tooth attachment rela-
387 tive to 'pelycosaurs' (states 1 or 2) (figures 3a and 4e). We
388 also identified several occurrences of a permanent gomphosis
389 (state 2) in tapinocephalids, gorgonopsians, therocephalians
390 and several non-mammalian cynodonts (figures 3a and 4e).
391 Given the relatively coarse resolution of this phylogeny, sev-
392 eral of the terminal taxa had to be coded as polymorphic for
393 this character, including anomodonts and therocephalians.
394 Ancestral character state reconstruction suggests that the
395 ancestral state for therocephalians was to have a permanent
396 gomphosis (state 2), with a reversal to delayed ankylosis
397 (state 1) in some therocephalian taxa. The resulting character
398 state distributions also indicate that a permanent gomphosis
399 (state 2) is a symplesiomorphy for mammals, with the tran-
400 sition to a permanent gomphosis characterizing the more
401 inclusive group, Theriodontia (Gorgonopsidae, Therocephalia
402 and Cynodontia). These results also highlight a reversal
403 to delayed ankylosis (state 1) in the early cynodont *Thrinaxo-*
404 *don* and potentially in tritheledontid cynodonts [29,30]
405 (figure 4e), and the convergent evolution of a permanent
406 gomphosis (state 2) in some herbivorous dinocephalians.

409 4. Discussion

410 (a) Heterochrony and the evolution of the mammalian 411 tooth attachment system

412 Our data do not support the classical hypotheses for the ori-
413 gins of the mammalian tooth attachment system, which
414 invoke evolutionary increases in dental tissue complexity
415 from an ancestral 'bone of attachment' to a three-tissue
416 tooth attachment system in crown mammals [9–11]. Whereas
417 finer within-clade comparisons are required to optimize this
418 ontogenetic character, the differences in tooth attachment
419 across Synapsida clearly do not relate to increases in tooth
420 tissue complexity, but to differences in the timing and
421 sequence of dental ontogeny. Teeth are unique organs in
422 that their ontogeny can be examined separately from the
423 ontogeny of the animal itself, because in most vertebrates,
424 the teeth are continually replaced. Sectioning the jaws of
425 non-mammalian synapsids therefore provides a window
426 into synapsid dental development and evolution: each sec-
427 tion reveals multiple generations of teeth progressing
428 through a repeating sequence of developmental stages from
429 initiation, tissue differentiation, calcification, to the shedding
430 of a functional tooth [20,38] (figure 3). Comparing dental
431 ontogeny across such a wide range of extinct synapsids
432 reveals that the ancestral condition for Synapsida is for
433 teeth to pass through a gomphosis phase early in dental ontog-
434 eny and to rapidly form a stable ankylosis by the extensive
435 alveolar bone formation that fixes the tooth in place (figures 3
436 and 4) [20].

437 Using our method for characterizing dental ontogeny and
438 the 'pelycosaur' condition as the ancestral synapsid state, we
439 propose that the observed differences in tooth attachment

440 across Synapsida are due to a neotenic (*sensu* [39]) delay in
441 the onset of ankylosis (a transition from state 0 to 1 in our
442 new dental character) and to progenesis (*sensu* [39]), or trun-
443 cation of the mineralization and ankylosis stages in dental
444 ontogeny in the stereotypically mammalian condition of a
445 permanent dental gomphosis (a transition from state 1 to
446 2). Functional teeth that were attached by a ligament,
447 previously thought to characterize cynodont or strictly mam-
448 malian tooth attachment within Synapsida [5,10,22,40,41],
449 appear across all therapsid clades (figures 1c–l and 2). More-
450 over, the permanent gomphosis in tapinocephalids,
451 gorgonopsians, bauriids and the majority of cynodonts
452 (including mammals) is therefore not the result of repeated,
453 de novo evolution of a PDL from a primordial 'bone of
454 attachment', but to delayed or a lack of calcification of the
455 PDL, which is plesiomorphically present in all synapsids
456 [20]. This heterochronic shift in the timing of mineralization
457 and subsequent ankylosis phases results in a paedomorphic
458 (*sensu* [39]) form of tooth attachment in these taxa. Mammals
459 and several other therapsid groups simply possess functional
460 teeth that remain at an earlier ontogenetic stage compared to
461 'pelycosaur'-grade synapsids (figures 3 and 4).

462 Whereas our understanding of the development and
463 maintenance of the mammalian PDL is steadily improving
464 [42–44], the role that phylogenetic history plays in this pro-
465 cess has only recently been explored [20]. Partial
466 mineralization of the PDL in modern caiman led McIntosh
467 *et al.* [17] to conclude that the crocodylian PDL was interme-
468 diate between the ankylosis type attachment in most other
469 reptiles and the gomphosis in mammals. Our results provide
470 a clearer depiction of a phylogenetically 'intermediate' con-
471 dition between the earliest synapsids and crown mammals:
472 the gomphosis in many non-mammalian therapsids is
473 replaced by complete ankylosis, with the PDL eventually
474 becoming entombed in the surrounding alveolar bone and
475 forming a stable ankylosis, a condition exemplified by
476 many non-mammalian therapsids [20] (figures 3 and 4).
477 Where mammals, tapinocephalids, gorgonopsians, many
478 therocephalians and cynodonts differ is in the decrease in
479 an alveolar bone deposition, decreased calcification of the
480 PDL and the maintenance of a non-mineralized region
481 between the tooth root and the alveolus.

482 (b) Functional implications

483 The evolution of the gomphosis has been historically linked
484 to complex dental occlusion in stem mammals [3,9], because
485 the widely accepted role of the PDL is to dissipate the forces
486 of occlusion [14,38,45]. However, for the stress-dissipating
487 hypothesis for the origin of the PDL to be supported, it
488 should have arisen in clades that exhibit extensive occlusion
489 or high bite forces and such fossil evidence has been lacking
490 up to this point [10]. Within Synapsida, some non-mamma-
491 lian clades may have independently evolved a gomphosis
492 in association with comparatively simple dental occlusion
493 (as in tapinocephalids and bauriids), but this could not possi-
494 bly apply to all groups. For example, the blade-like teeth of
495 gorgonopsians and the shearing teeth of *Galesaurus* exhibit
496 permanent gomphosis, whereas some of the teeth of the
497 early cynodont *Thrinaxodon* show evidence of ankylosis [6]
498 (figure 3; electronic supplementary material, Information
499 S1). Moreover, all 'pelycosaurs' show complete and rapid
500 ankylosis of teeth, irrespective of tooth function (figure 3;

electronic supplementary material, Information S1). Outside of Synapsida, many archosaurs (including all dinosaurs and extant crocodylians) exhibit a gomphosis, but many do not exhibit dental occlusion [17,28,46], whereas other extinct reptiles exhibit simple occlusion, but have ankylosed teeth [47,48]. The evolution of a permanent ligamentous tooth attachment in synapsids is therefore not uniquely associated with occlusion and may have arisen in association with another factor that many therapsid lineages share to the exclusion of the earlier 'pelycosaurs'.

We hypothesize that a reduction in the tooth replacement rate across the transition from 'pelycosaurs' to therapsids may at least explain why all therapsids show a prolonged (but not always permanent) ligamentous tooth attachment relative to 'pelycosaurs' (figures 3 and 4). We found evidence for extensive tooth migration and size discrepancies between functional and replacement teeth, indicating that a significant amount of time had passed between replacement events in several therapsid groups (electronic supplementary material, Information S3). A slower tooth replacement rate would mean that teeth spent proportionally more time in the oral cavity. A prolonged ligamentous phase of tooth attachment would mediate the re-positioning of teeth within the jaws through ontogeny, which is a key function of the mammalian PDL [10,13]. This prolonged ligamentous phase may have later been co-opted into its shock-absorbing function in the occluding teeth of mammals and several other therapsid lineages.

5. Conclusion

By comparing tooth tissue formation over a 300 Myr span of synapsid evolution, we can conclude that the mammalian periodontium is not the result of an evolutionary increase in dental complexity, but an example of pedomorphosis: namely a slower calcification of the PDL (neoteny) in some therapsids, and to truncation of the mineralization and ankylosis phases of dental ontogeny (progenesis) in others, including mammals. Heterochrony has played a prominent role in the diversification of mammalian teeth, including evolutionary changes to eruption timing and the evolution of high-crowned (hypsodont) teeth [49,50], but it has played a further role in the evolution of the dental gomphosis that characterizes all mammal teeth. Evolutionary changes to

the timing of calcification of the tooth attachment tissues were not restricted to synapsids, however, as phenomena have been proposed to explain the occurrences of a ligamentous tooth attachment in some extant and extinct squamates [23]. These similarities between synapsid and squamate dental ontogeny and evolution suggest that heterochrony may be an important driver of dental attachment tissue evolution in amniotes. These findings show that the classical divide between the mammalian gomphosis and reptilian ankylosis is not a distinction between complex and simple teeth, but two ends of a spectrum in the evolution and development of amniote tooth attachment.

Ethics. SAHRA (permit ID 1945) authorized the exportation and preparation of the specimens from the Evolutionary Studies Institute in Johannesburg, South Africa. These specimens and resulting thin sections have because been returned to their collections. Thin sections of extant mammals were taken from slide collections at the University of Alberta and did not require ethical approval.

Data accessibility. CT scan data for this study are available on Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.v23v5d2> [27].

Authors' contributions. A.R.H.L. conceived the study. A.R.H.L., K.S.B., M.W., F.A. and R.R.R. collected and contributed histological and CT scan data. A.R.H.L., K.S.B., M.W., F.A. and R.R.R. interpreted the data. A.R.H.L. wrote the manuscript. K.S.B., M.W., F.A. and R.R.R. edited the manuscript.

Competing interests. The authors have no competing interests to declare.

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