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Microscopic analysis of the developing dentition in the pouch young of the extinct marsupial *Thylacinus cynocephalus*, with an assessment of other developmental stages and eruption

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Abstract

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A pouch young of the extinct dasyuromorphian marsupial *Thylacinus cynocephalus* was examined histologically to study the stages of the developing dentition during the pre-eruptive period of development. Both deciduous and successional stages of tooth development were examined, and these were compared to later stages of development and eruption of the teeth in *Thylacinus* and with selected developmental stages of other dasyuromorphians. Our analysis shows that the development and eruption of teeth in *Thylacinus* is most similar to that of dasyurids with only two premolars, such as *Dasyurus* and *Sarcophilus*, rather than the dasyurids with three premolars, such as *Antechinus* and *Sminthopsis*.

Keywords *Thylacinus*, marsupials, Dasyuridae, deciduous dentition, dental development, pouch young, homologies

Institutional abbreviations

AM, Australian Museum, Sydney, Australia; AMNH, American Museum of Natural History, New York, New York, USA; CU, Cambridge University, Museum of Zoology, Cambridge, England; LAC, Laboratoire d'Anatomie Comparée, Paris, France; MN, Museum für Naturkunde, Berlin, Germany; NMV, Museums Victoria, Melbourne, Australia; SAM, South Australian Museum, Adelaide, Australia; TMAG, Tasmanian Museum and Art Gallery, Hobart, Tasmania, Australia; USNM, United States National Museum, Washington DC, USA, WAM, Western Australian Museum, Perth, Australia

Introduction

Although the last known living specimen of the marsupial *Thylacinus cynocephalus* died in the Hobart Zoo in Tasmania, Australia, on 7 September, 1936, frequent reports of sightings have continued to occur in Tasmania; (see Paddle, 2012, for a recent summary). However, no new living or dead specimens of the thylacine have been found since 1936, and most authorities accept that the thylacine is now extinct. An excellent overview on the biology of *Thylacinus* was provided by Joan Dixon (1989).

The earliest study on the developing dentition of *Thylacinus* known to us was conducted by William Flower (1867), who illustrated a juvenile pouch young with unerupted teeth. In lateral view, Flower showed three (of the four) developing incisors, the canine, three deciduous premolars, a developing successional third premolar (P3), and the developing M1 - 2 in both jaws. A reproduction of his figure is shown in fig. 1. The juvenile specimen was from the Museum of the Royal College of Surgeons of England and the Head Length (HL) measured 71.1 mm.

No teeth had yet erupted above the soft tissues of these jaws; however, Flower's dissection revealed the apex of a small tooth in both the maxilla and dentary, barely elevated above the level of the bone. These small teeth were identified as the third deciduous premolars (dP3), overlying the deeper and less developed third successional premolars (P3). In later stages of all marsupials then known, Flower noted that the underlying and larger successional third premolars would later displace and replace these deciduous predecessors in both jaws.

The tiny dP3 in the upper jaw measured only 2.54 mm in length and lacked distinct roots. The tiny dp3 in the dentary was slightly smaller and also lacked distinct roots. Following

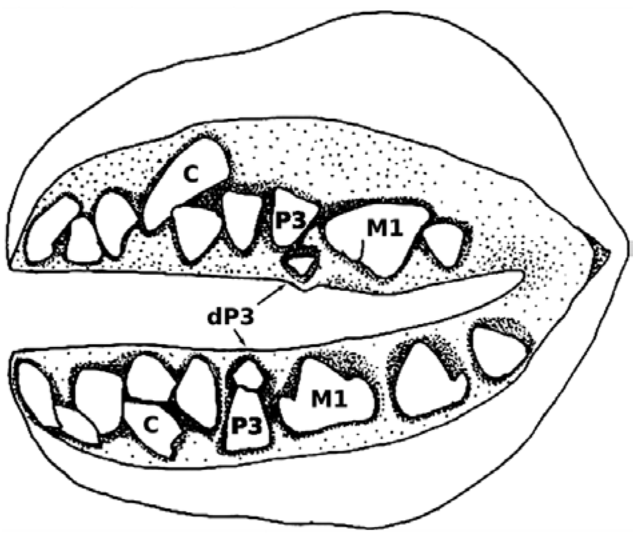

Figure 1. *Thylacinus* (71.1 mm Head Length) pouch young, with unerupted dentition; redrawn from Flower, 1867. C, upper and lower canine; dP3, upper and lower deciduous third premolar; M1, upper and lower first molar; P3, upper and lower successional third premolar.

his examination of *Thylacinus* and representatives of five other families of marsupials, Flower emphasised that the "peculiar condition of dental succession"(Flower, 1867: page 637) in marsupials differed greatly from that in eutherian mammals. This mode of dental development was characterised by tooth succession at only the third premolar position in all marsupials examined by him. Flower therefore considered this condition in marsupials to be a "rudimentary diphyodont condition"(Flower, 1867: page 638), with tooth replacement being confined to a single tooth position on each side of both jaws.

As a closing thought, Flower (1867) suggested that earlier developmental stages of marsupials might show additional evidence for deciduous predecessors at other tooth positions. Later studies by Oldfield Thomas (1887) revealed that variation occurred in dental development within the marsupial family Dasyuridae, with some genera having three premolars (*Thylacinus*, *Sminthopsis* and *Myrmecobius*), whereas others had only two premolars (*Dasyurus* and *Sarcophilus*). Curiously, Thomas called the posterior deciduous premolar and its successor "Pm4," rather than dP3 and P3, considering them to be homologous with the fourth premolars in eutherians. Numerous studies on the developing dentitions of marsupials and eutherians continued during the latter part of the 19th century, although none of these included additional specimens of *Thylacinus*.

Several authors began to use microscopic studies of the marsupial and eutherian dentitions in the 1890s. A significant study by Leche (1893) showed histological sections of early dental development in the dasyurid *Myrmecobius*, in which he described and illustrated the occurrence of small abnormal teeth, which he called "prelacteal" or pre-milk teeth, in the developing incisor and canine regions. Some of these abnormal teeth contained a prominent dentinal nodule and occurred labial to the normal developing first incisor and canine, which were in the late bell stage of development (his figs 1 and 2). This and other related studies on the developing dentition of marsupials were discussed in great detail by Wilson and Hill (1897), in their microscopic study of extensive samples of the developing dentition in the Australian peramelid *Perameles*. One of their major findings was to note that the third deciduous premolar in several genera of marsupials differentiated from the dental lamina contemporaneously with the so-called "prelacteal" or pre-milk teeth in the incisor and canine regions, and they concluded that these teeth belong to the same dental series and were homologous to the deciduous or milk series of eutherians.

In contrast, Wilson and Hill (1897) considered the two premolars anterior to dP3 in both jaws of marsupials to be homologous with the successional third premolars (i.e., to be P1 and P2), although they presented no developmental data to support this hypothesis. These anterior premolars develop later than dP3 in all marsupials studied to date, and they develop directly from the primary dental lamina, as do dP3. We are unaware of any developmental studies that show that these anterior deciduous premolars are replaced by successional teeth. Nevertheless, most studies of fossil and extant marsupials have continued to refer to these teeth as P1 and P2 [see Archer et al.(2016), Murray and Megirian (2006), and Yates (2014, 2015) as recent examples].

In Flower's (1867) study of the juvenile *Thylacinus*, he considered that it belonged in the family Dasyuridae, and he noted that it would be useful to compare its developing dentition with that of Dasyurus and other dasyurids. Numerous studies on dental development in dasyurids have been carried out since then (Archer, 1976; Luckett and Woolley, 1996), although *Thylacinus* was later placed in a separate family Thylacinidae. Today, both morphological and molecular studies group Thylacinidae, Dasyuridae and Myrmecobiidae within the marsupial order Dasyuromorphia (see Archer et al., 2016, and Westerman et al., 2015). Unfortunately, additional studies on dental development in *Thylacinus* were not carried out before the genus became extinct in 1936.

Material and methods

During a trip by one of us (WPL) to Melbourne, Australia, in 1992, to study dental development in a variety of marsupials, numerous specimens of juvenile dasyurids and other Australian marsupials were examined at Museums Victoria. This included several specimens of juvenile thylacines preserved in the alcohol collections of the Mammal Department. One case included a female thylacine and her four pouch young, which had been collected in Tasmania on 23 June, 1909 by W.M. McGowan. The four pouch young and the head of the mother were stored in alcohol. Fortunately, a testis had been sectioned from one of the pouch young by Dr Patricia Woolley of La Trobe University; this indicated that tissue preservation was relatively good. Following discussions between WPL and Joan Dixon, Curator of Mammals at

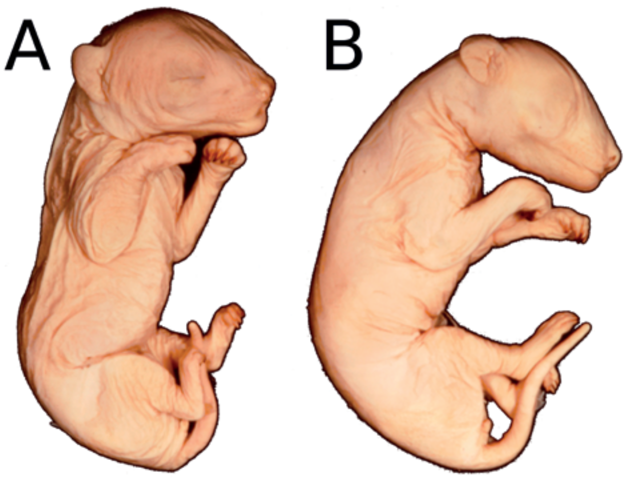

Figure 2. Sibling pouch young thylacines. A, NMV C5754, male specimen sectioned for histology images; B, NMV C 5757, female specimen, used by Feigin et al. (2018) for genomic analysis.

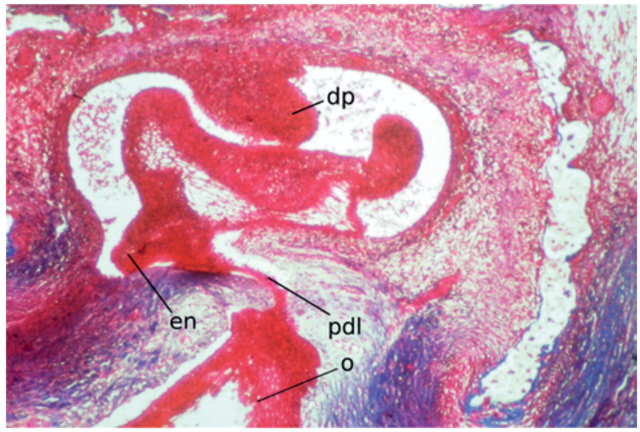

Figure 3. Histological section of I1 in *Thylacinus*, in middle bell stage. dp, dental papilla; en, epithelial nodule; o, oral epithelium; pdl, primary dental lamina.

Museums Victoria at the time, it was agreed that one of these valuable pouch young would be made available for histological investigation. The pouch young (PY) selected for study (NMV C 5754) was a male with a crown - rump length (CRL) of 77.8 mm and a head length (HL) of 34 mm (fig. 2a). The age of this pouch young litter was recently estimated to be 4.5 weeks old (Newton et al., 2018).

We decided to have this valuable pouch young sectioned histologically by our colleague Professor Dr. Milan Klima at the Dr Senckenbergische Anatomie, J.W. Goethe-Universität in Frankfurt am Main, Germany, because of his extensive experience in the preparation and study of histological serial sections from mammalian foetuses and pouch young, including marsupials and whales. Dr. Gerhard Storch at the Senckenberg Natural History Museum in Frankfurt am Main agreed to assume responsibility for this valuable specimen during its preparation in Germany. Following photographs and X-rays, the head of the pouch young was removed and decalcified in 5% HNO_3. It was then embedded in celloidin-paraffin and sectioned serially at 10 um in a transverse (coronal) plane. The resulting 323 slides were stained alternately with azan trichrome, or with haematoxylin and eosin. As a consequence of the unknown fixation of the specimen in 1909, the resulting tissue preparation is only fair. Nevertheless, the histological detail is adequate for the recognition of most soft tissues, the developing dentition and the enamel matrix. There is some loss of bone and of dentin in the sections. In addition to our study of the developing dentition, we anticipated that other aspects of the developing cranium and postcranium might be studied by other collaborators, including Professor Klima. Following our examination of the slides for dental development, the slide series was returned to Museums Victoria.

After preparation of the histological series for dental development, we were assisted by our colleague Dr. Friedemann Schrenk, at the Landesmuseum, Darmstadt, Germany, in photographing the slides.There is some distortion in the specimens, but most details can be recognised. One of us (NHL) made some camera lucida reconstructions from the serial sections to better show the relationships between dP3 and P3 in both jaws. There was no eruption of any teeth in the developing pouch young. For comparison, we show X-ray analysis of an older pouch young (AM P 762) of the thylacine from the Australian Museum in Sydney, which shows very early signs of tooth eruption. Developmental stages and dental homologies follow those described in Luckett (1993a, b) and Luckett and Woolley (1996).

Results

Upper jaw

The first incisor (I1) is a moderately large tooth in the middle bell stage of development, with moderate development of the stellate reticulum (fig. 3). The outer and inner layers of the middle bell are artifactually separated; this may be due, in part, to the lack of dentin and enamel. Development of this tooth is considerably retarded or delayed compared with that of the other developing incisors. The primary dental lamina stalk is relatively intact and attached to the oral epithelium. The premaxillary alveolus for this tooth is relatively shallow, compared with that for I2. Bilaterally, there are tiny buccal epithelial nodules that may represent epithelial remnants of a rudimentary deciduous I1, as are known to occur in many other marsupials (see Leche, 1893). However, this is impossible to corroborate without access to younger developmental stages. Such rudimentary deciduous incisors, often containing dentin, are readily seen in the dasyurids *Sminthopsis virginiae* (Luckett and Woolley, 1996) and *Dasyurus viverrinus* (Luckett et al., unpublished research). For instance, in a *Dasyurus viverrinus* pouch young of 23 mm greatest length, there is a rudimentary dI1 with a distinct tiny epithelial knot associated with a moderately large successor I1 in the middle bell stage.

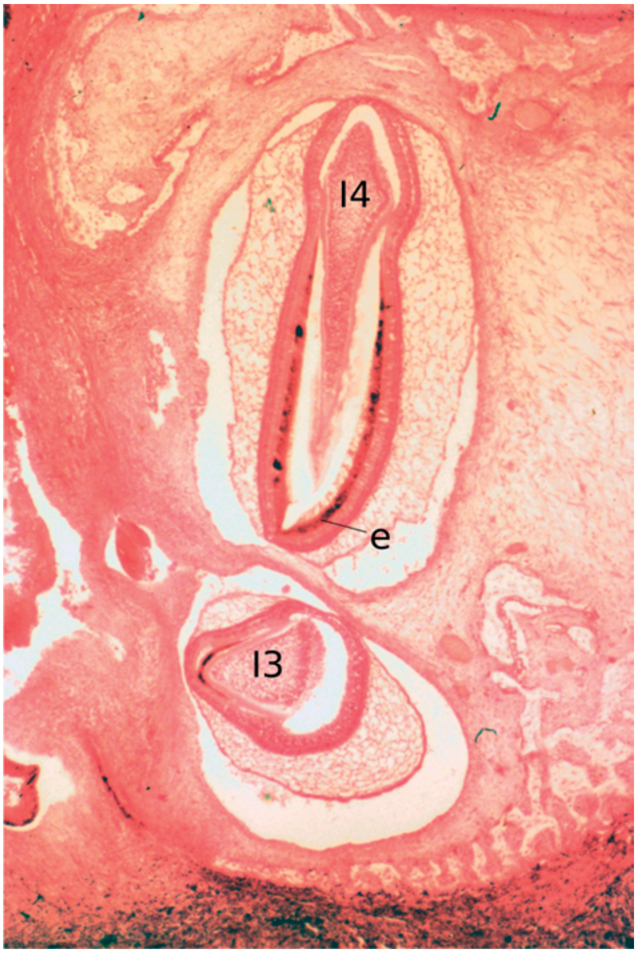

Figure 4. Longitudinal section through I4 and transverse section through smaller I3. e, disrupted enamel.

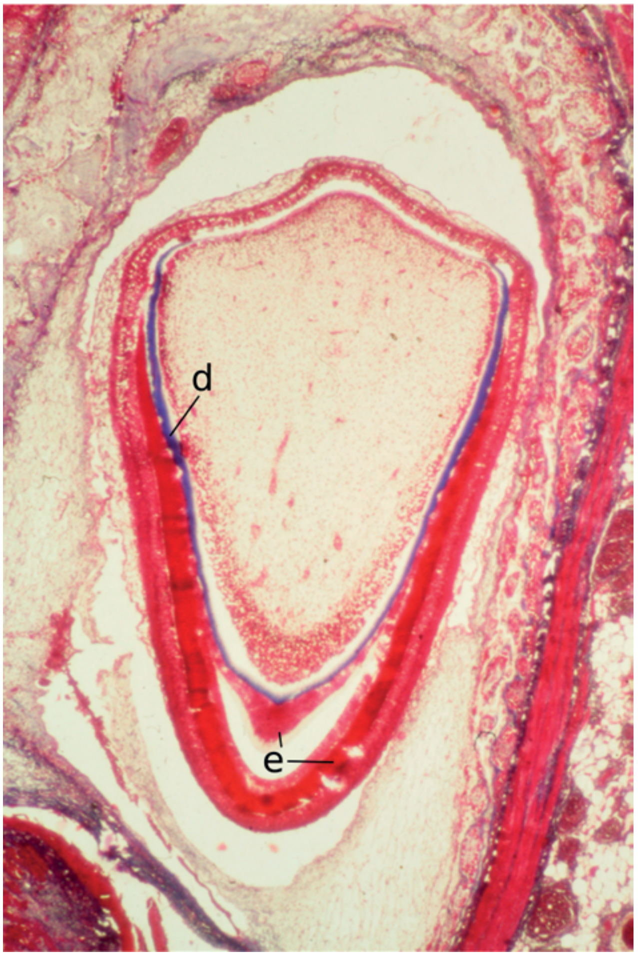

Figure 5. Longitudinal section through large successional upper canine, with disrupted dentin and well -developed enamel. d, dentin; e, enamel.

I2. A large tooth with moderately thick enamel. The dentin was probably moderately thick to thick; however, partial dissolution of dentin in this and other teeth makes this difficult to determine accurately. There are only scattered epithelial remnants of the primary dental lamina, with little if any connections to the oral epithelium. The alveolus for the developing tooth is much deeper than that for I1. There is no evidence of the remains of a rudimentary dI2.

I3. A large tooth, similar in development to I2, with moderately thick enamel and disrupted dentin. The tooth is somewhat procumbent and its enamel is thicker buccally than lingually (fig. 4). There are prominent buccal epithelial nodules on both sides of the jaw; these occur mesio-buccal to I3 and they could be remnants of a rudimentary dI3.

I4. A moderately-sized tooth, smaller than I2 and I3, with moderately developed to moderately thick enamel on its apex (fig. 4). There is no trace of a rudimentary dI4.

Canine. A large vertically implanted tooth, with moderately thick enamel and disrupted dentin (fig. 5). The tooth lies in a deep alveolus at the rostral extent of the maxilla. There are no traces of a rudimentary dC.

dP1. A moderately sized tooth, with relatively thick enamel and disrupted dentin. Its primary dental lamina stalk is detached from the oral epithelium. A short, flattened lingual successional lamina occurs at the mid-level of the tooth.

dP2. A moderately large tooth, with relatively thick enamel and disrupted dentin on the apex of the single cusp (fig. 6). The primary dental lamina stalk is also detached from the oral epithelium. A short flattened lingual successional lamina occurs along the mid-level of the tooth. The tooth lies anterior to the large orbit. A thin distinct layer of connective tissue separates the detached primary dental lamina stalk from the oral epithelium for both dP1 and dP2.

dP3. A small tooth, lying in a shallow alveolus beneath the anterior margins of the orbit. A layer of moderately thick to

Figure 6. Longitudinal section through dP2, with disrupted dentin and well - developed enamel. d, dentin; e, enamel.

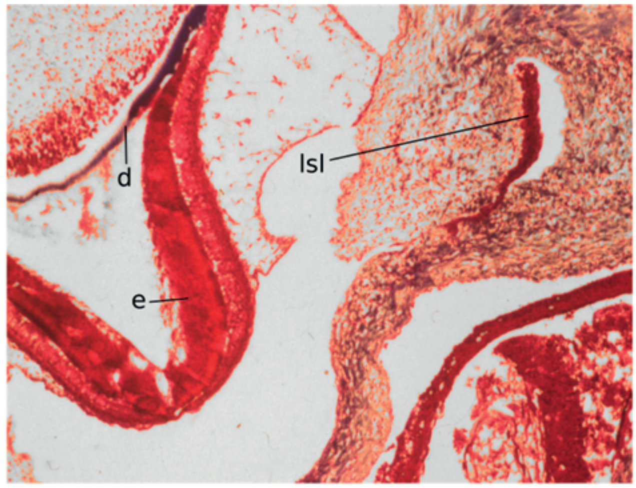

Figure 7. Longitudinal section through the apex of the small dP3, with its disrupted dentin and thick enamel. A segment of its lingual successional lamina is evident, but its attachment to the outer enamel epithelium is not evident in this section. d, dentin; e, enamel; lsl, lingual successional lamina.

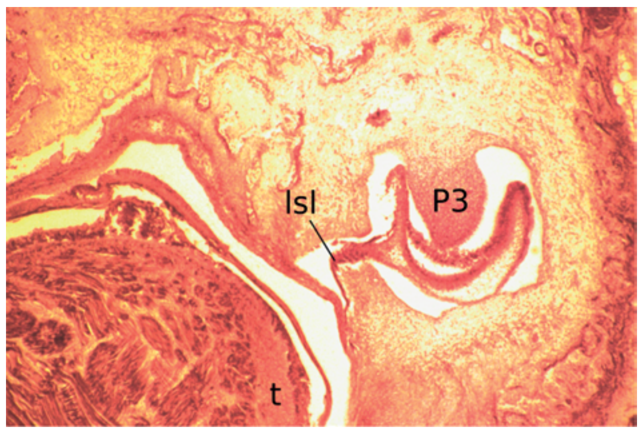

Figure 8. Late bell stage successor P3. This tooth lies anterior to dP3 and it lacks dentin and enamel. lsl, short segment of lingual successional lamina; t, tongue.

thick enamel and disrupted dentin overlies the small ovoid cusp (fig. 7). There are only slender fragmented remnants of the detached primary dental lamina stalk. A slender epithelial strand connects the outer enamel epithelium (OEE) of dP3 to its lingual successional lamina at a level near to the middle of the paracone elevation. At this level, the successional lamina appears relatively short in the sections; however, it is continuous with the lamina extending anteriorly to the developing successor P3 (see camera lucida reconstructions of the same relationships between dp3 and p3 in the lower jaw; fig. 11a, b).

Successor P3. A large late bell stage tooth with moderately well-developed stellate reticulum lies immediately anterior to the smaller dP3. There is no distinct evidence for dentin or odontoblasts on the apex of the cusp (fig. 8). The larger P3, although less differentiated than its deciduous predecessor, extends deeper into its developing alveolus than the more superficial disto-buccal and smaller dP3. Only a short segment of the lingual successional lamina is evident in fig. 8. This successional lamina runs parallel to the oral epithelium but is not attached to it. Instead, it extends posteriorly through the sections to its origin on the lingual successional lamina of dP3, in the same manner as it occurs in the lower jaw (see camera lucida fig. 11b).

M1. A large tooth overlapping the distal end of dP3 extends deeper into the jaw than dP3. Only isolated remnants of the primary dental lamina stalk are evident mesially. A moderately elevated mesio-buccal paracone is in the late bell stage but lacks dentin and odontoblasts (fig. 9b). A small spherical epithelial nodule of stratum intermedium cells is detached over the distal extent of the paracone elevation (fig. 9a), similar to the condition that occurs in some dasyurids and *Didelphis*. Detachment of this small epithelial nodule over the paracone, but not over the metacone or protocone, has been suggested to be correlated with the earlier development and

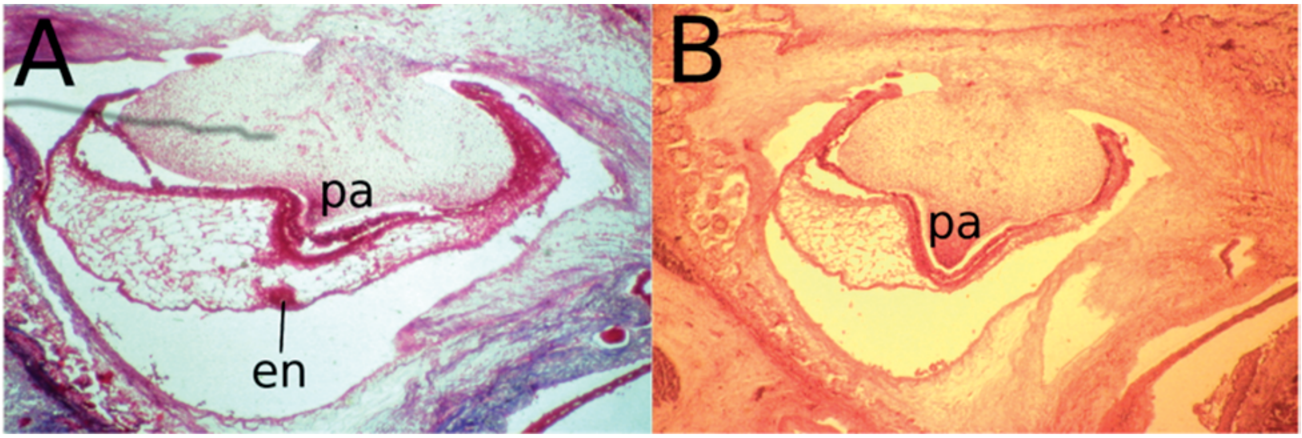

Figure 9. Section of M1, at level of paracone. A, paracone with overlying epithelial nodule; B, paracone at more central level, lacking the epithelial nodule; en, epithelial nodule; pa, paracone apex.

calcification of the taller metacone, in relation to the shorter and later developing paracone in didelphids and some dasyurids (Luckett et al., unpublished research). Presumably, a similar developing condition is also occurring in *Thylacinus*.

A short flat stylar shelf occurs buccally at the paracone level; a broader flat lingual shelf is also present at this level for the future development of a protocone, which is still lacking at this stage. A moderately tall disto-buccal metacone shows greater development, with moderately thick dentin and enamel (fig. 10). In contrast with the paracone, there is no evidence of a detached epithelial nodule over the taller metacone.

M2. Moderately large early-middle bell stage with a moderately developed central epithelial knot developing distal to M1. This tooth occurs beneath the distal 1/3 of the eye. The dental lamina ends distal to this tooth with no trace of a primordium for M3 or M4.

Lower jaw

i1. A large tooth, somewhat procumbent, with moderately thick enamel and dentin. Only isolated remnants of the dental lamina stalk are evident. There is no trace of rudimentary elements for a deciduous precursor for this or other lower incisors.

i2. A large tooth with moderately thick enamel and disrupted dentin.

i3. A moderately large tooth with relatively thick enamel and disrupted dentin.

canine. A very large tooth with moderately thick dentin and enamel, similar to the condition in the upper canine.

dp1.A moderate sized tooth with relatively thick enamel and disrupted dentin on its apex. There is a short flattened lingual successional lamina on both dp1 and dp2, as occurred in the upper jaws.

dp2.A larger tooth with moderately thick enamel; this may be slightly thinner than that on dp1, but this is difficult to determine.

dp3. A small and somewhat elongate tooth, with relatively thick enamel and disrupted dentin on the apex of the tooth (fig. 11a, b). The mesial end of the tooth overlaps the more lingually situated, and larger, successor p3. A nearly intact slender strand of the primary dental lamina stalk extends from the apex of the tooth near its mesial end towards the oral epithelium. Then, larger epithelial islands of the fragmented primary dental lamina stalk continue towards the oral epithelium. There is no direct connection with the oral epithelium, but the intermittent epithelial island remnants make this earlier connection evident (see camera lucida fig. 11b).

A single moderately elevated cusp (probably the protoconid) is evident on the tooth; this becomes reduced distally. There is a slight suggestion of two additional cusps distal to the protoconid, but these are not very distinct. At the level of the middle of the protoconid, a nearly complete slender epithelial strand extends between the greatly thinned outer enamel epithelium of dp3 and the fragmented proximal portion of the lingual successional lamina (see fig. 11b). At this level there are fewer remnants of the primary dental lamina stalk attached to dp3; these are better developed along the mesial third of the tooth. Epithelial strands of the lingual successional lamina continue mesially towards the large developing successor p3.

successor p3. A large, late bell stage tooth, with thin to moderately developed dentin on its apex (fig. 12). The tooth lies lingual to, and somewhat mesial to, the smaller, more superficial dp3 and it extends deeper into the jaw. Ameloblasts are polarised over the cusp, but there is no distinct evidence of enamel. Epithelium of the lingual successional lamina is attached to the apex of p3 and the epithelium runs parallel to the oral epithelium but is not attached to the latter (fig. 11a). The larger p3 extends somewhat mesial to the smaller dp3. The intermittent, and nearly complete successional lamina attachment between p3 and dp3 is best developed on the right side of the jaw (see fig. 11b).

m1. A very large tooth with thick enamel and disrupted dentin on the apex of the tall protoconid. There is no trace of a lingual paraconid mesially. While there are other slight swellings of the epithelium, there is no distinct evidence for other developing cusps.

m2. A large tooth with moderately thick enamel and dentin on the tall mesio-buccal protoconid. There is no evidence for a

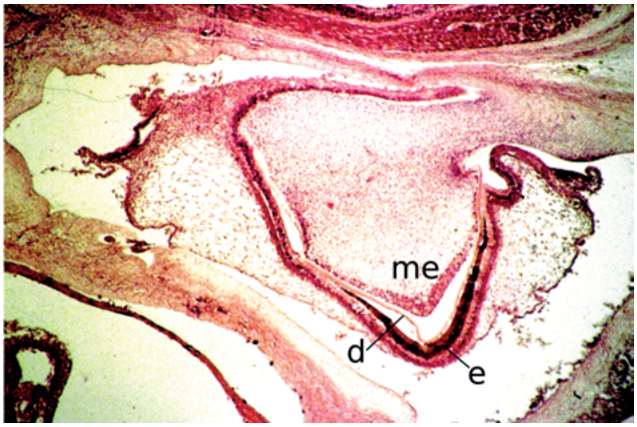

Figure 10. Section through the level of the metacone on M1, with thick enamel on its apex and disrupted dentin; d, dentin; e, enamel; me, metacone apex.

paraconid elevation mesially, or for other developing cusps. There is a short residual lingual lamina at the level of the protoconid.

m3. A moderately large tooth in the middle- late bell stage. The dental lamina disappears distal to this tooth with no evidence of a developing m4.

Other developing stages of the dentition in *Thylacinus*

There has been little, if any, attempt to describe early or later developmental stages of the dentition in *Thylacinus*. One of the few examples was the study by Heinz Moeller (1968), in which he described and illustrated the erupting dentition in a juvenile skull of *Thylacinus*. We have included this specimen in a larger sample of developing dentitions in juvenile and subadult thylacines, collected by one of us (WPL) from several museums in Australia, North America, France and Germany.

The recent discovery of four juvenile thylacine pouch young in the collections of the Charles University in Prague, Czech Republic (Sleightholme et al., 2012), that are even younger than our specimen, raises hopes for the possible further assessment of early development of the rudimentary deciduous incisors and canines in *Thylacinus*, as well as other aspects of their developmental biology. The specimens shown in their photos suggest the possibility that these pouch young are in a poorer state of preservation than our pouch young, and it is unclear as to whether the authors plan to attempt a histological study of one of their pouch young. We hope that they will attempt such a project.

During the preparation of our manuscript, a paper published by Newton et al. (2018) presented X-ray computed tomography scans of five thylacine pouch young specimens, representing all of the known PY litters of *Thylacinus* (see their fig. 2). The emphasis in their study was on growth changes in the cranial and postcranial skeleton; only minimal data were provided concerning the developing dentition in these PY. Their youngest specimen, from the Prague collection (DZCU 8021), was estimated to be 1.5 weeks old (10–11 days). For this pouch young , the authors noted that "two to three tooth sockets are visible in each of the jaw quadrants"(Newton et al., 2018: page 9). They made no attempt to identify or locate the specific developing teeth.

As we will discuss later, *Dasyurus viverrinus* appears to be very close in its pattern of dental development to the conditions in the thylacine, and we have examined two 10–day old PY of *D. viverrinus*, in order to obtain an estimate of the likely dental developmental conditions in the Prague pouch young of 10 –11 days old (Luckett et al., unpublished research). There is evidence of two to three developing teeth in the dasyurid PY, similar to the condition noted for the Prague PY. In the upper jaw of the *Dasyurus* PY, there is a well-developed dP3 in the early–middle cap stage, and a tiny spherical epithelial knot for dC, that is associated with a distinct lingual successional bud for C. There is also a less distinct nodular cap anteriorly for dI2 or dI3, with a disto-lingual successional early bud for I2 or I3. Hopefully, these data would be useful when and if it is possible to examine one of the Prague PY histologically.

The next older pouch young examined by Newton et al. (2018) was estimated to be 35–37 days old (see their Fig. 5) and has an 89 mm CRL (TMAG A931). This PY was considered by the authors to be similar in its development to the Museums Victoria specimens. This would include our 34 mm HL PY and its sister (NMV C5755), examined by them (see their Fig. 2b). Newton et al. chose to examine and describe the TMAG specimen rather than the Museums Victoria PY, because of its apparent better state of preservation. The authors noted the occurrence of several deciduous teeth in both jaws, without further comment or illustrations. However, we were able to examine some aspects of this and the older dentitions of the thylacine PY studied by Newton et al. (2018) due to their inclusion of extensive electronic supplementary material that they made available publicly for study of the dentition and the cranio-skeletal morphology.

Our brief examination of Newton et al.'s (2018) supplementary material for TMAG A931, which is slightly older than our sectioned thylacine PY, revealed the presence of tiny, unerupted dP3 in both jaws, and the apparent very early eruption of the protoconid of m1 above the alveolar margins in the lower jaw (fig. 13a). There was no distinct evidence for any tooth eruption in the upper jaw. The unerupted canine and dP1-3 were evident in both jaws. However, we were unable to see any distinct evidence for the successor P3 in either jaw. We suspect that this is correlated with the development of little or no dentin on the P3 as yet, similar to the condition in our sectioned PY.

The next older pouch young examined by Newton et al. (2018) was a male (TMAG A930), estimated to be about 9.5 weeks old (66 - 67 days), and illustrated in their fig. 6. The authors noted that this specimen contained several unerupted teeth in both jaws but gave no further description of the dentition. Our examination of their supplementary material revealed the presence of tiny dP3 in both jaws; these appear to be erupted above the alveolar margins of both jaws (fig. 13b). These images also show the presence of the unerupted successor P3 in both jaws, in close proximity and immediately anterior to the smaller erupting dP3. The lower dp3 is somewhat more erupted than the upper one, and its successor p3 is also anterior to, but not as closely apposed, to dp3 as is the upper P3.

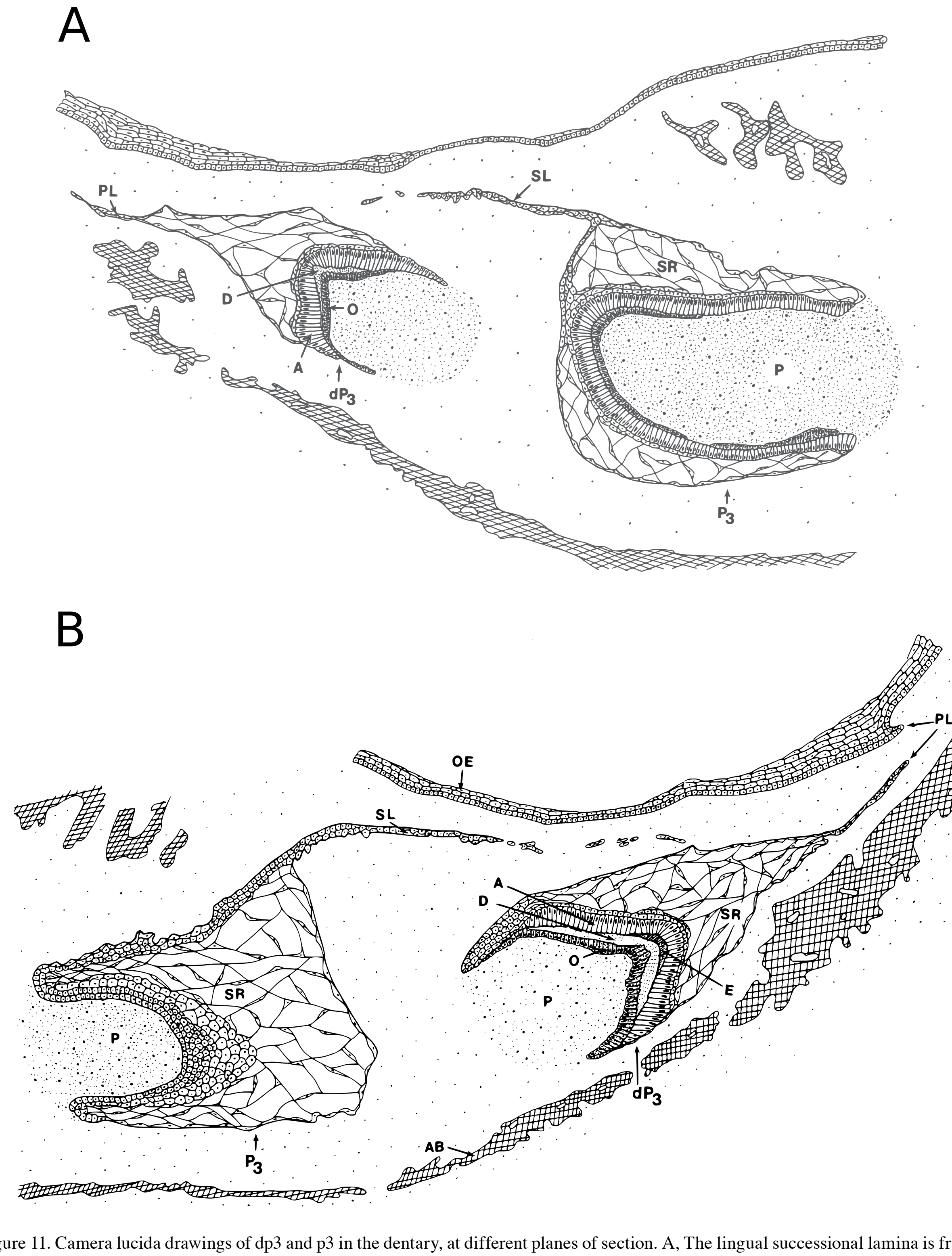

Figure 11. Camera lucida drawings of dp3 and p3 in the dentary, at different planes of section. A, The lingual successional lamina is fragmented, but still largely intact, between the small dp3 and its larger successional p3; B, The small dp3 is evident with its dentin and enamel, and its primary dental lamina connection to the oral epithelium is fragmented but still evident. The section through the successor p3 is not central, but it shows the fragmented lingual successional lamina between the two teeth. A, ameloblasts; AB, alveolar bone; D, dentin; dp3, deciduous third premolar; E, enamel; O, odontoblasts; OE, oral epithelium; P, dental papilla; p3, successor third premolar; PL, primary dental lamina; SL, lingual successional lamina; SR, stellate reticulum.

Figure 12. Section through dp3 and its successor p3. Only a small fragment of the lingual successional lamina is evident. Compare this single section with the camera lucida drawings in Figure 11. dp3, deciduous third premolar; lsl, lingual successional lamina; p3, successional third premolar.

Clearly, the complete dentitions of these thylacine PYs should be carefully described and illustrated, and we hope that Newton et al. (2018) will do so in a future publication.

A later stage of tooth eruption in *Thylacinus* available to us is from a pouch young in the Australian Museum in Sydney. This specimen (AM P 762) measured 80.5 mm HL and was collected in Tasmania during 1866 (fig. 14). This PY was recently estimated to be 12 weeks old (Newton et al., 2018). It was not possible to section this young, but we were able to examine the head using X-rays with the help of Dr Lucjan Sych, from the School of Dentistry at the University of Melbourne. The images of the head made by Dr Sych showed that the small spherical dp3 was erupted bilaterally above the alveolar margins in the dentary, with only minimal suggestions of distinct roots (fig. 14a, c). The unerupted, but larger, successor p3 lies immediately anterior to the smaller dp3, as it was in our younger sectioned pouch young and in the TMAG young described above. The apex of dp1 was just slightly above the alveolar margins, whereas the larger dp2 was at or just below the alveolar margins (see fig. 14c). The lower m1 protoconid is in an early stage of eruption above the alveolar margins; m2 is evident within its alveolus but is not yet erupting, as is the less developed m3.

The upper jaw was more difficult to interpret in our X-rays, and it was especially difficult to identify the dP3 and its state of eruption. Fortunately, the recent publication by Newton et al. (2018) on the available thylacine pouch young specimens included X-ray computed tomography scans of AM P 762, and we were able to examine this specimen in greater detail, thanks to their inclusion of extensive supplementary material. We noted that both of the tiny dP3 in the upper and lower jaws were at least partly erupted, with greater eruption in the lower jaw (fig. 15). The unerupted but well-developed successor P3 are also clearly seen immediately anterior and deeper in both jaws, as they were in our younger developing pouch young.

A later stage of dental eruption in *Thylacinus* was described and illustrated by Moeller (1968). We were not able to examine this specimen (CU A6 7/10) from the Cambridge University, Museum of Zoology, but instead relied on the careful description and figures supplied by Moeller (1968). He showed that, in this specimen, I2 - 3 were erupting in the upper jaw, whereas I1 and I4 were unerupted, but evident in their alveoli. This is consistent with our observation of a delayed development of I1 in our sectioned pouch young. Moeller also showed that dP1 and dP2 were in early eruption, but there was no sign of dP3, except for an alveolus that contained the unerupted P3. It is unclear whether the poorly rooted dP3 (probably erupted) was lost or damaged during preparation of the skull. The M1 was unerupted.

In the lower jaw, the canine is unerupted, and dp1 and dp2 are partly erupted, with dp1 being more erupted. A small spherical dp3 is erupted anterior to an erupting m1 (see our reproduction of Moeller's fig. 32a in fig. 16a). The apex of the unerupted p3 is evident in an alveolus anterior to the small dp3. Clearly, Moeller's specimen is only slightly more advanced than our pouch young (AM P 762) from the Australian Museum. A slightly later stage of *Thylacinus* (USNM 115365; skull length = 87.75 mm) shows that dp3 has been lost in the dentary and its successor p3 is in early eruption (fig. 16b). The m1 is almost completely erupted, and m2 is in early eruption. The dp1 is almost completely erupted, whereas the larger dp2 is in an earlier phase of eruption.

Our stages for these and later development and eruption in the upper and lower jaws of *Thylacinus* are presented in Tables 1 and 2. Included in specimens from the dentary we have presented some samples of fossil *Thylacinus cynocephalus* from caves in Western Australia. Radiocarbon analysis of charcoal samples from the Henschke Fossil Cave suggests that the cave was filled in between 32,000 and 40,000 years ago, trapping the thylacines and many other marsupial species within (Pledge, 1990). Although these dentitions are in most cases somewhat smaller than the more recent Tasmanian thylacines, the developmental stages appear to be identical in both groups. For instance, both p3 and m3 are erupting at about the same time in the lower jaw.

Comparison of the thylacine pouch young with similar developmental stages in dasyurids

In comparing our single specimen of the thylacine pouch young with comparable developmental stages of dasyurids, presumably one of its closest relatives within the order Dasyuromorphia, it was interesting to note the occurrence of both similarities and differences within the family Dasyuridae. Unfortunately, we were unable to find a similar developmental stage of *Myrmecobius* (the single representative of Myrmecobiidae) in our studies.

In Table 3, the thylacine pouch young has the successor P3 in the late bell stage, and M2 is in the early - middle bell stage in the upper jaw. The closest similar developmental stage in our dasyurid sample is *Dasyurus viverrinus*, a species in which dP2 has been lost in both jaws (as in all species of *Dasyurus* examined by us). The *Dasyurus* PY has a 13.5 mm HL and its P3 is in the late bell stage, with a thin layer of dentin. Its M2 is in the middle - late bell stage. Given the slightly older specimen of *Dasyurus*, it is quite similar in its developmental stage to our

Table 1. Development and eruption of the upper postcanine dentition in *Thylacinus cynocephalus*, (HL = Head Length).

Stage	dP^1	dP^2	dP^3	P^3	M^1	M^2	M^3	M^4
NMV C 5754 34 mm HL pouch young; no teeth erupting; estimated 31 - 32 days	Moderately thick enamel on apex; flat, short lingual successional lamina	Moderately developed to moderately thick enamel on apex; short lingual successional lamina	Tiny tooth, with moderately thick enamel; fragmented lingual successional lamina extends anteriorly	Large, late bell stage, no odontoblasts; lingual and mesial to tiny dP3	Large tooth, with moderately thick enamel on tall metacone	Moderately large tooth, in early -middle bell stage	No trace	No trace
71.1 mm HL pouch young (Flower, 1867) Museum of the Royal College of Surgeons	Tooth calcified; not erupting	Tooth calcified; not erupting	Tiny, rootless tooth; apex just above alveolar margins (2.54 mm in length)	Tooth deep in jaw; beneath and slightly lingual to tiny dP3	Well calcified tooth; not erupting	Partly calcified tooth; not erupting	Not evident	Not evident
60 mm HL TMAG 930 Estimated 66 –67 days	Calcified tooth; not erupting	Calcified tooth; not erupting	Tiny tooth erupted	Tooth evident but not erupting; anterior to dP3	Tooth not erupting; close to alveolar margins	Tooth not erupting	Not evident	Not evident
80.5 mm HL AM P 762 Haired pouch young; estimated 84 days	Apex of tooth erupting slightly above alveolar margins	Apex of larger tooth just below alveolar margins; not erupting	Tiny tooth erupted	Apex of unerupted tooth evident mesio-lingual to tiny dP3	Tooth evident but not erupting	Tooth evident but not erupting	Not evident	Not evident
Skull length = 80 mm Cambridge Univ. A6 7/10	Tooth erupting	Tooth erupting	Tiny tooth not evident; probably lost	Tooth evident in alveolus, but not erupting	Tooth not erupting	Not evident	Not evident	Not evident
USNM 115365 Juvenile female (skull length = 87.75 mm)	Tooth almost completely erupted	Moderately large; about 2/3 erupted (less erupted than dP1)	No trace	Large tooth; very early erupting, just above alveolar margins	Large tooth; early erupting (about 1/4)	Not evident	Not evident	Not evident
MN Berlin An 13914 Juvenile (skull length = 129.4 mm)	Tooth erupted	Tooth erupted	No trace	Large tooth; almost completely erupted	Large tooth; erupted	Large tooth erupting (about 3/4)	Partially calcified tooth; evident deep in alveolus	Not evident
SAM M1958 Juvenile male (skull length = 130 mm)	Erupted	Erupted	No trace	Erupted	Large tooth; erupted	Large tooth almost completely erupted	Not evident	Not evident
NMV C 5744 Juvenile male (skull length = 157 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Evident in alveolus; not erupting	Not evident
AM P 778 Juvenile (skull length = 159.4 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Early emergence above alveolar margins	Not evident

Stage	dP^1	dP^2	dP^3	P^3	M^1	M^2	M^3	M^4
SAM M1956 Juvenile (skull length = 149 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Early emergence above alveolar margins	Not evident
NMV C 5743 Juvenile (skull length = 153.5 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Early erupting (less than 1/3)	Not evident
NMV C 5600 Juvenile female (skull length = 153.8mm); born in zoo; 18 months old	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Early erupting (about 1/4)	Not evident
AM P778 Juvenile (skull length = 159.4 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Early emergence above alveolar margins	Not evident
MN Berlin A 1745 Juvenile (skull length = 168 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupting (about 1/2)	Not evident
AMNH 77701 Juvenile (skull length = 191.2 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Almost completely erupted	Not distinct; alveolus only
WA F6358 Subadult fossil (skull length = 138.2 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Region damaged; uncertain
AM S1180 Subadult; skull length = 180 mm	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Early eruption (about 1/4)
AM 775 Subadult; skull length = 182 mm (collected 1866)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	About 1/3 erupted
LAC A 3298 Adult; skull length = 192 mm	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Erupted
Other adult skulls, with all teeth erupted; with skull lengths from 192 mm to 253mm								

Table 2. Development and eruption of the lower postcanine dentition in *Thylacinus cynocephalus*, (HL = Head Length).

Stage	dp_1	dp_2	dp_3	p_3	m_1	m_2	m_3	m_4
MV C 5754 34 mm HL pouch young; no teeth erupting; sectioned histologically estimated 31 - 32 days old	Moderately thick enamel on apex; short flat lingual successional lamina	Moderately thick enamel on apex; short flat lingual successional lamina	Tiny, elongate tooth, with moderately thick enamel; lingual successional lamina extends mesial to larger P3	Large, late bell, with moderately developed dentin; lingual and mesial to smaller dp3	Large tooth, with thick enamel on tall protoconid	Large tooth, with moderately thick enamel on tall protoconid	Moderately large, middle-late bell stage; dental lamina ends distal to it	No trace
71.1 mm HL pouch young (Flower, 1867)	Calcified tooth; not erupting	Calcified tooth; not erupting	Tiny, rootless tooth; apex just above alveolar margins	Tooth deep in jaw; beneath and slightly lingual to tiny dp3	Well calcified tooth; not erupting	Partly calcified tooth; not erupting	Partly calcified tooth; not erupting	Not evident
TMAG A930 60 mm HL; estimated 66–67 days old	Probably not erupting; region damaged	Apex of large tooth near alveolar margins	Tiny tooth erupted	Unerupted tooth apex evident mesial to tiny dp3	Tooth at alveolar margins, not erupting	Tooth evident but not erupting	Apex evident but not erupting	Not evident
AM P 762 80.5 mm HL Haired pouch young; estimated 84 days old	Apex of tooth erupting just above alveolar margins	Apex of larger tooth just above alveolar margins	Tiny spherical tooth; erupted above the alveolar margins	Apex of unerupted tooth evident mesio-lingual to tiny dp3	Protoconid of large tooth in early eruption	Apex of tooth evident just below alveolar margins, but not erupting	Partly calcified tooth deep in jaws, not erupting	Not evident
Cambridge University A6 7/10 80 mm skull length (Moeller, 1968)	Tooth almost completely erupted	Tooth partially erupted (about 1/3)	Tiny tooth erupted above alveolar margins	Apex of unerupted tooth evident mesio-lingual to tiny dp3	Protoconid of large tooth in early eruption	Protoconid apex just below alveolar margins; not erupting	Tooth not erupting	Not evident
WAM 72.1.1149 Juvenile fossil right dentary, Murray Cave, Western Australia	Alveolus only; at least partially erupted	Early erupting (about 1/3)	No trace	Apex of unerupted tooth just below alveolar margins	Alveolus only (probably erupted)	Early erupting; slightly less than that of dp2	Not evident	Not evident
USNM 115365 Juvenile female; skull length = 87.75 mm	Tooth erupted	Tooth erupting (about 1/2)	No trace; possible shallow buccal alveolus for lost tooth	Early eruption just above alveolar margins	Large tooth; erupted	Large tooth; early eruption (1/3)	Unerupted tooth in alveolar crypt	Not evident
SAM P 33482 Juvenile fossil dentary, Henschke Fossil Cave, South Australia	Partial alveolus only	Double rooted alveolus only for erupting (or erupted) tooth	No trace	Erupting tooth (about 1/2)	Alveolus only for erupted tooth	Alveolus only for erupting (or erupted) tooth	Erupting tooth (about 1/3)	Not erupting
WAM 61-2-26 Juvenile fossil left dentary, Mammoth Cave, Western Australia	Region missing	Tooth erupted	No trace	Tooth erupting (about 3/4)	Alveolus only for erupted tooth	Alveolus only for erupted tooth	Erupting tooth (about 1/2)	Not distinct

Stage	dp_1	dp_2	dp_3	p_3	m_1	m_2	m_3	m_4
Roaches Rest Cave, fossil 67-3-21	Region missing	Tooth erupted	No trace	No trace; probably erupted	Tooth erupted	Tooth erupted	Erupting tooth (about 2/3)	Unerupted, in deep alveolus
Berlin An 13914 Juvenile skull and dentary (skull length = 129.4 mm)	Erupted	Erupted	No trace	Almost completely erupted	Erupted	Erupted	Partially erupted (about 1/2)	Partially calcified; unerupted, deep in alveolus
SAM 1958 Juvenile skull and dentary (skull length = 130 mm)	Erupted	Erupted	No trace	Completely erupted	Erupted	Erupted	Partially erupted (about 1/2)	Not evident
MV C 5744 Juvenile skull and dentary (skull length = 157.1 mm) male	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Completely Erupted	Early emergence of protoconid above alveolar margins
SAM M1956 Juvenile skull and dentary (skull length = 149 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Very early eruption above alveolar margins
LAC, Paris 1883-352 Juvenile skull and dentary damaged (Collected in 1844)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Very early eruption of protoconid
MV C 5743 Juvenile female (skull length =153.5 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Early eruption (about 1/4)
AM P 778 Juvenile (skull length = 159.4mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Early erupting (about 1/4)
MN Berlin A 1745 Subadult (skull length = 168 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Erupting (about 3/4)
WAM F6358 Subadult fossil (skull length = 138.2 mm) Nullabor Plain, Western Australia	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Erupting (about 3/4)
AMNH 77701 Subadult (skull length = 191.2 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Almost completely erupted
AM 775 Subadult (skull length = 182 mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Erupted (upper M4 still erupting)
MV C 5748 Adult female (skull length = 192mm)	Erupted	Erupted	No trace	Erupted	Erupted	Erupted	Erupted	Erupted (all teeth erupted in both jaws)

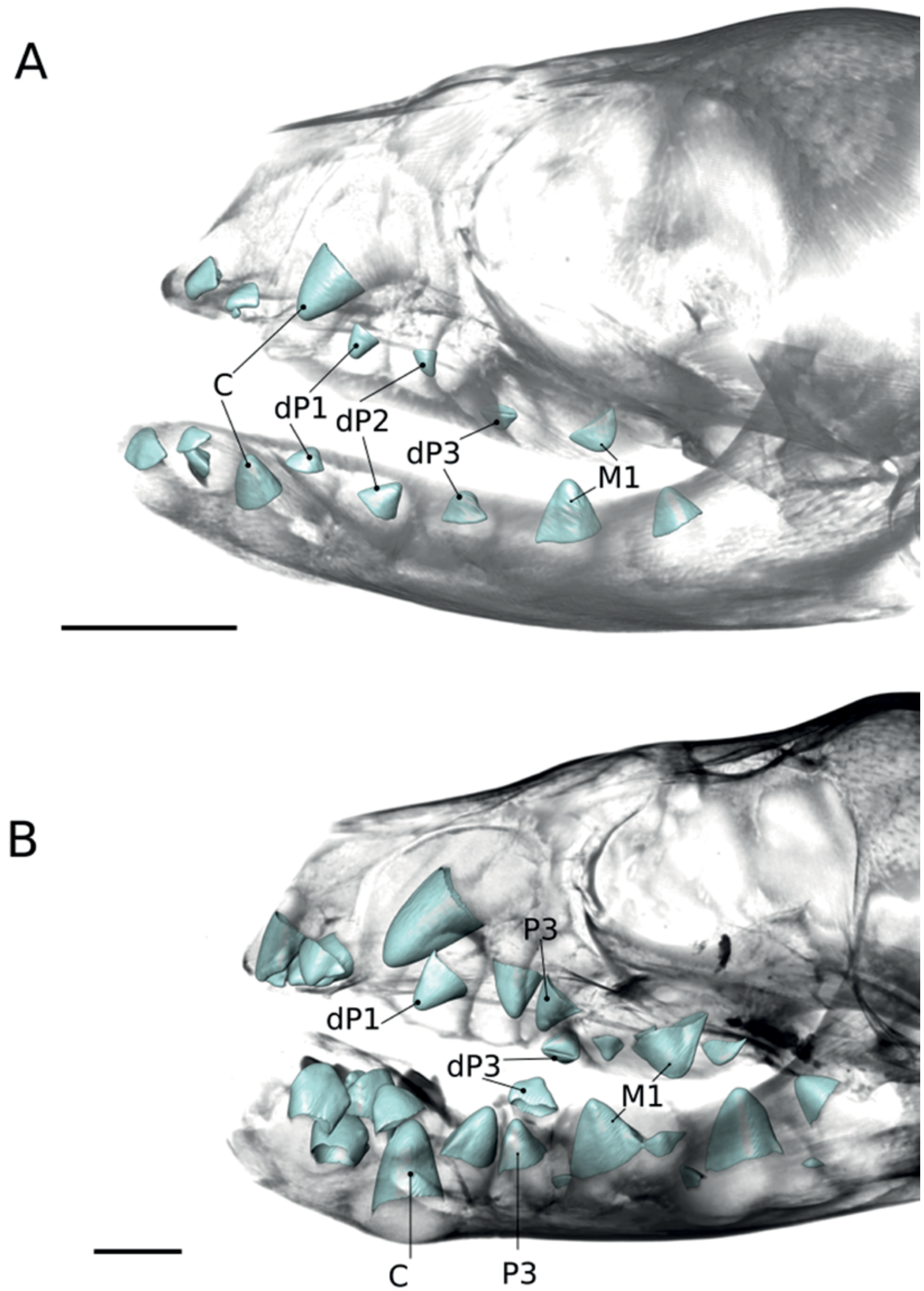

Figure 13. Computed tomography images from the supplementary data of Newton et al. (2018). A, section of the skull and dentition from TMAG A931, a thylacine pouch young of 35 - 37 days old; B, Section of the skull and dentition from TMAG A930, a thylacine pouch young of 66 - 67 days old. Scale bars are 5 mm. C, canine; dP1, deciduous first premolar; dP2, deciduous second premolar; dP3, deciduous third premolar; M1, first molar; P3, successional third premolar.

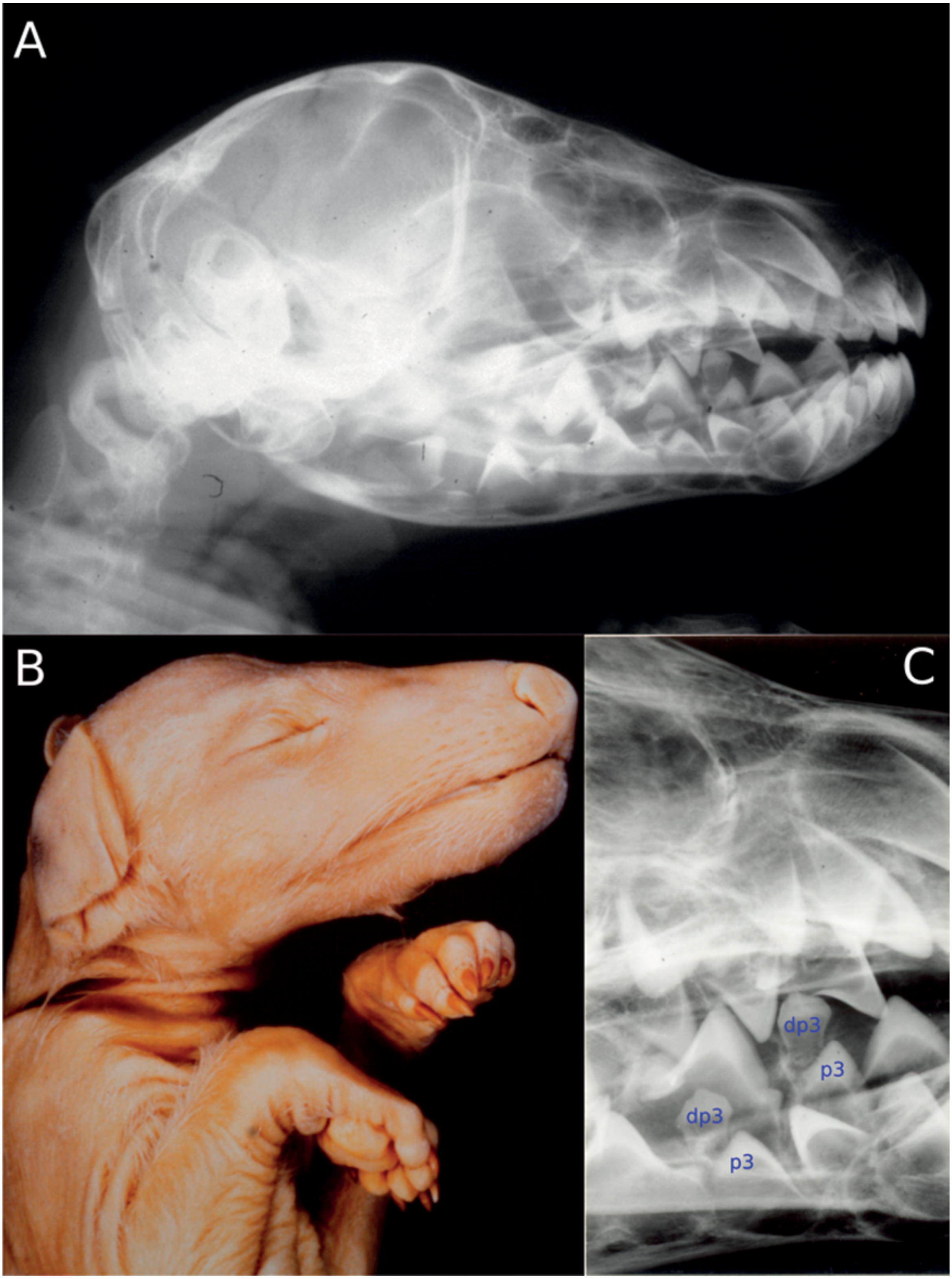

Figure 14. Images of the head, skull, and dentition of the thylacine pouch young from the Australian Museum (AM P 762). A, X-ray of the skull, showing deciduous and successional teeth in varying stages of development and early eruption; B, Head and upper body of the pouch young, prior to X-ray analysis; C, Higher magnification of a portion of the X-ray shown in figure A, with emphasis on the erupted lower dp3, and the unerupted but larger successional p3 immediately anterior to it.

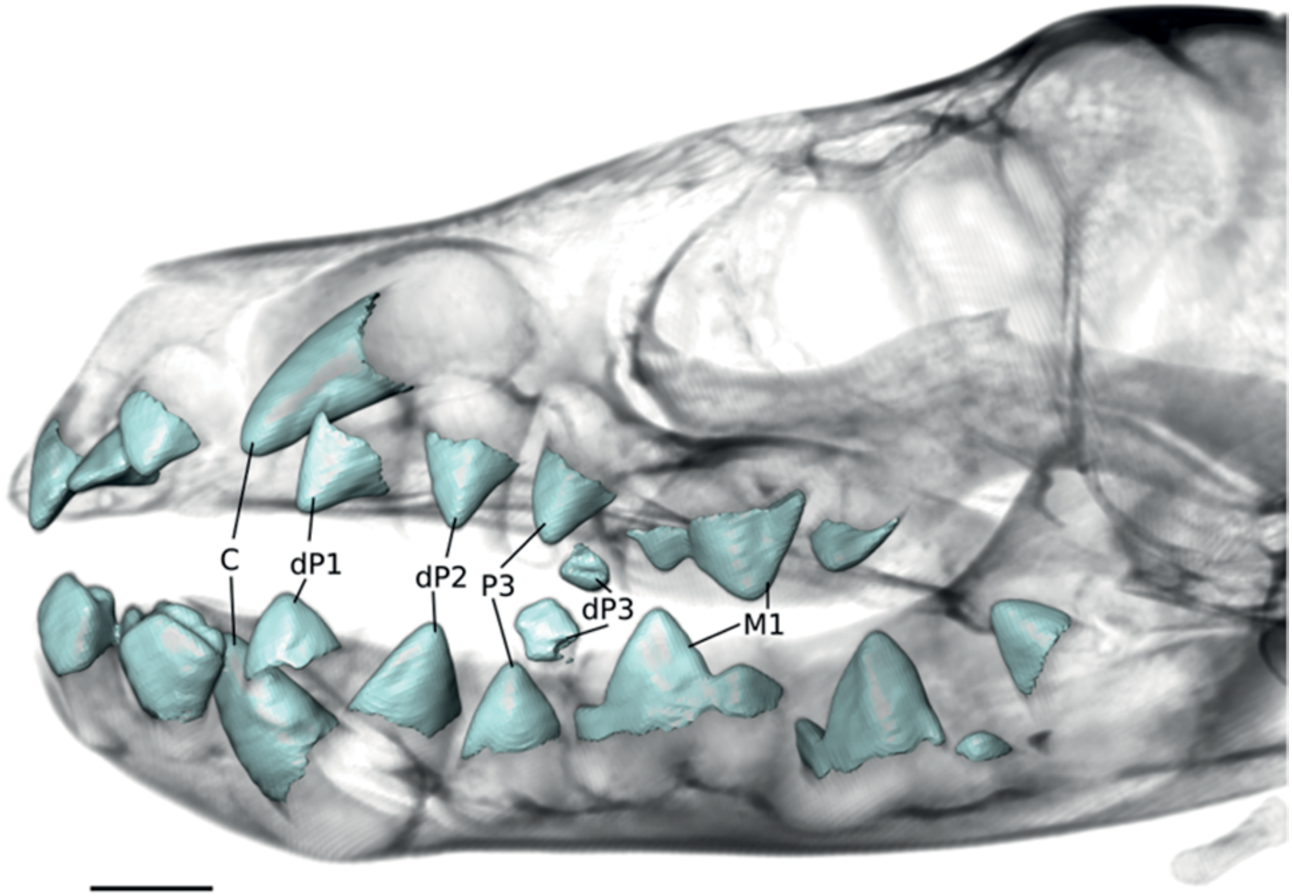

Figure 15. Computed tomography image of the skull and dentition of AM P 762, taken from the supplementary data of Newton et al. (2018). The figure clearly shows the erupted dP3 in both jaws, and the unerupted successor P3 immediately anterior to their predecessors. Scale bar equals 5 mm. Other early erupting and unerupted teeth are clearly labeled. C, successional canine; dP1, deciduous first premolar; dP2, deciduous second premolar; dP3, deciduous third premolar; M1, first molar; P3, successional third premolar.

Thylacinus PY. In the lower jaw, *Dasyurus viverrinus* is also more similar in its developmental stages of the dentition with *Thylacinus* than with the dasyurids with three premolars, *Antechinus* and *Sminthopsis* (see Table 4). Note that there is an accelerated phase of dental development in the lower jaw, compared with that in the upper jaw for both *Thylacinus* and *Dasyurus*. Thus, the lower p3 is most similar with m3 in its developmental stage, rather than with m2.

In contrast, the two dasyurid genera with three premolars that we examined (*Antechinus* and *Sminthopsis*), are quite different and considerably delayed or retarded in the development of their successional P3, compared with *Thylacinus* and *Dasyurus*. Thus, in *Antechinus*, the successor P3 is only suggested by the slight swelling of the lingual successional lamina of dP3, and *Sminthopsis* has P3 in a very early bud stage in the upper jaw (Table 3). However, the M2 of both genera with three premolars are in a similar middle - late bell stage. Note that the differences occur only in the development of the premolars, and not the molars.

Although early developmental stages of *Myrmecobius* were not available to us for comparison with the thylacine 34 mm HL PY, we were able to compare later stages of development of *Myrmecobius* with comparable stages of *Thylacinus* from our Tables 1 and 2. In the upper jaw of a juvenile *Myrmecobius* (WAM M 19214, with skull length = 41.66 mm), P3 was about half erupted and M2 was almost completely erupted. In the lower jaw, p3 was about 3/4 erupted and m3 was erupting. These relationships are most similar to those of a juvenile *Thylacinus* from the Berlin Museum (MN 13914; skull length = 129.4 mm). In the upper jaw of the Berlin thylacine, P3 is almost completely erupted and M2 is about 3/4 erupted (see Table 1). In the lower jaw, p3 is almost completely erupted and m3 is about 2/3 erupted (see Table 2). These relationships suggest that *Myrmecobius* is most similar in its dental development to the dasyurids with two premolars and with *Thylacinus*, rather than with the dasyurids with three premolars, despite the fact that *Myrmecobius* has three premolars in both jaws.

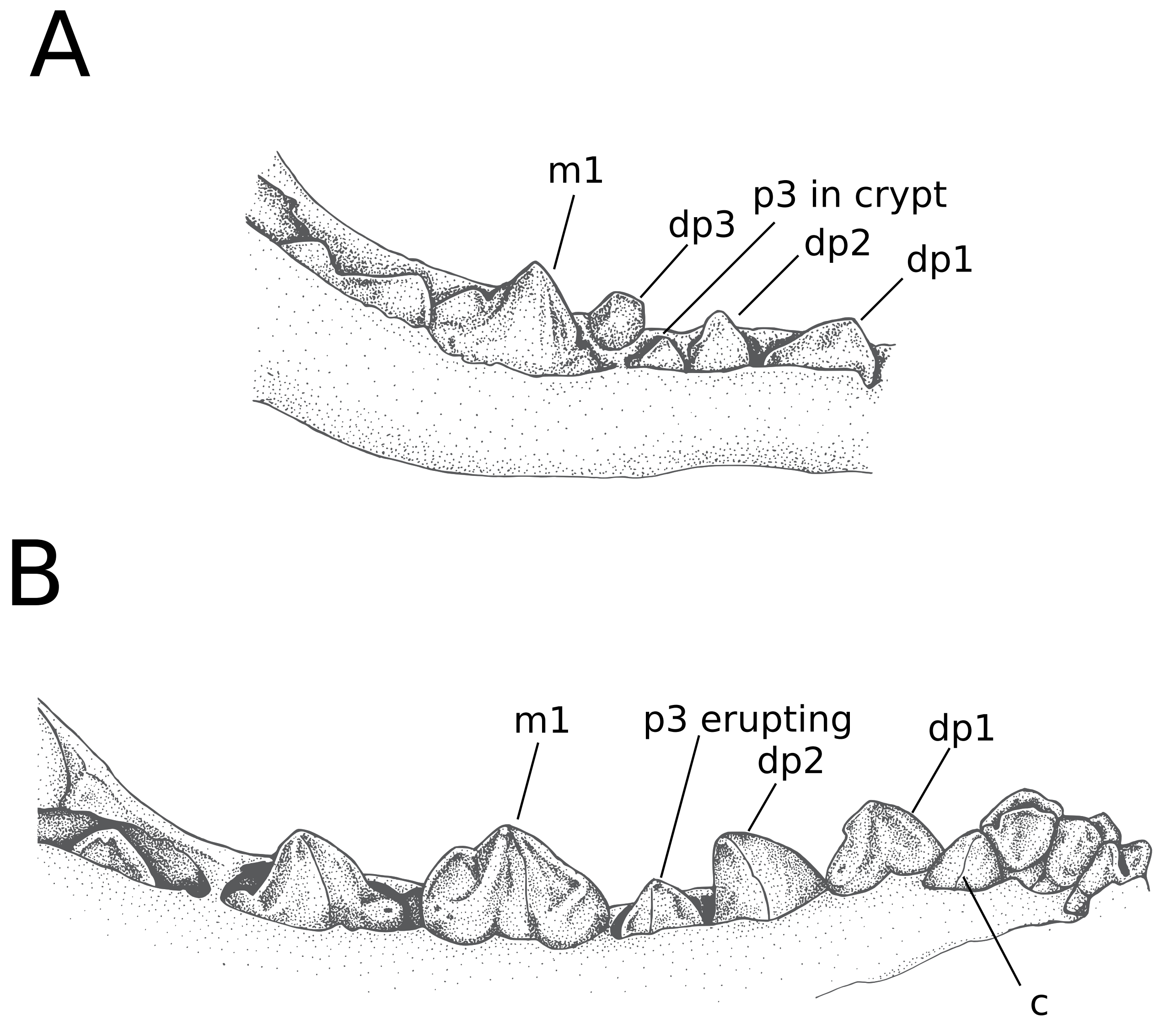

Figure 16. Later stages of early eruption in *Thylacinus* showing the presence and early loss of dp3 in the dentary. A, Part of the dentary (CU A6 7/10), redrawn from Moeller (1968), showing the erupted dp3, the unerupted successor p3 in its alveolar crypt, immediately anterior to dp3, and the erupting m1. The erupting dp1 and dp2 are also labeled; B, A slightly later stage of eruption in the dentary (USNM 115365) shows that the dp3 has been lost, and successor p3 is in early eruption. The m1 is now almost completely erupted. c indicates lower successional canine in B.

We were also able to examine a similar later stage of development in *Sarcophilus harrisii* (AMNH 65674), a dasyurid with two premolars, for comparison with *Thylacinus*. As in other dasyurids with two premolars, and in the thylacine, dental development is accelerated, with dP1, P3, and M1 - 2 erupted in both jaws. In the lower jaw, m3 is almost completely erupted. Although most authors have concluded that it is dP3 (and P3) that is the missing tooth in *Sarcophilus* (Archer, 1976; Tate, 1947; Thomas, 1877; Wroe, 1999), our developmental studies have demonstrated the presence of dP3 and P3 in both jaws of *Sarcophilus*, as well as the absence of a developing dP2 in both early and later stages, similar to the condition in *Dasyurus* (Luckett et al., unpublished research).

For an additional comparison, we examined one specimen of Peramelidae (*Perameles sp.*) and one Didelphidae (*Monodelphis domestica*). Both were similar in developmental stages to the dasyurids with three premolars (see Tables 3, 4). The successor P3 for *Perameles sp.* (16 mm HL) was in the early bud stage, and M2 was in the late bell stage with early odontoblasts in the upper jaw. In the lower jaw, *Perameles sp.* was in the early bud stage for p3, and m3 was in the late bell stage, with early odontoblasts. In *Monodelphis domestica* (14.5 mm HL), P3 was in the early - middle bud stage and M2 was in the late bell stage in the upper jaw. In the lower jaw, p3 was in the late bud - early cap stage and m3 was in the middle bell stage. Note that the molars are in similar developmental stages for all taxa examined, whereas it is the successional P3 that varies in both jaws.

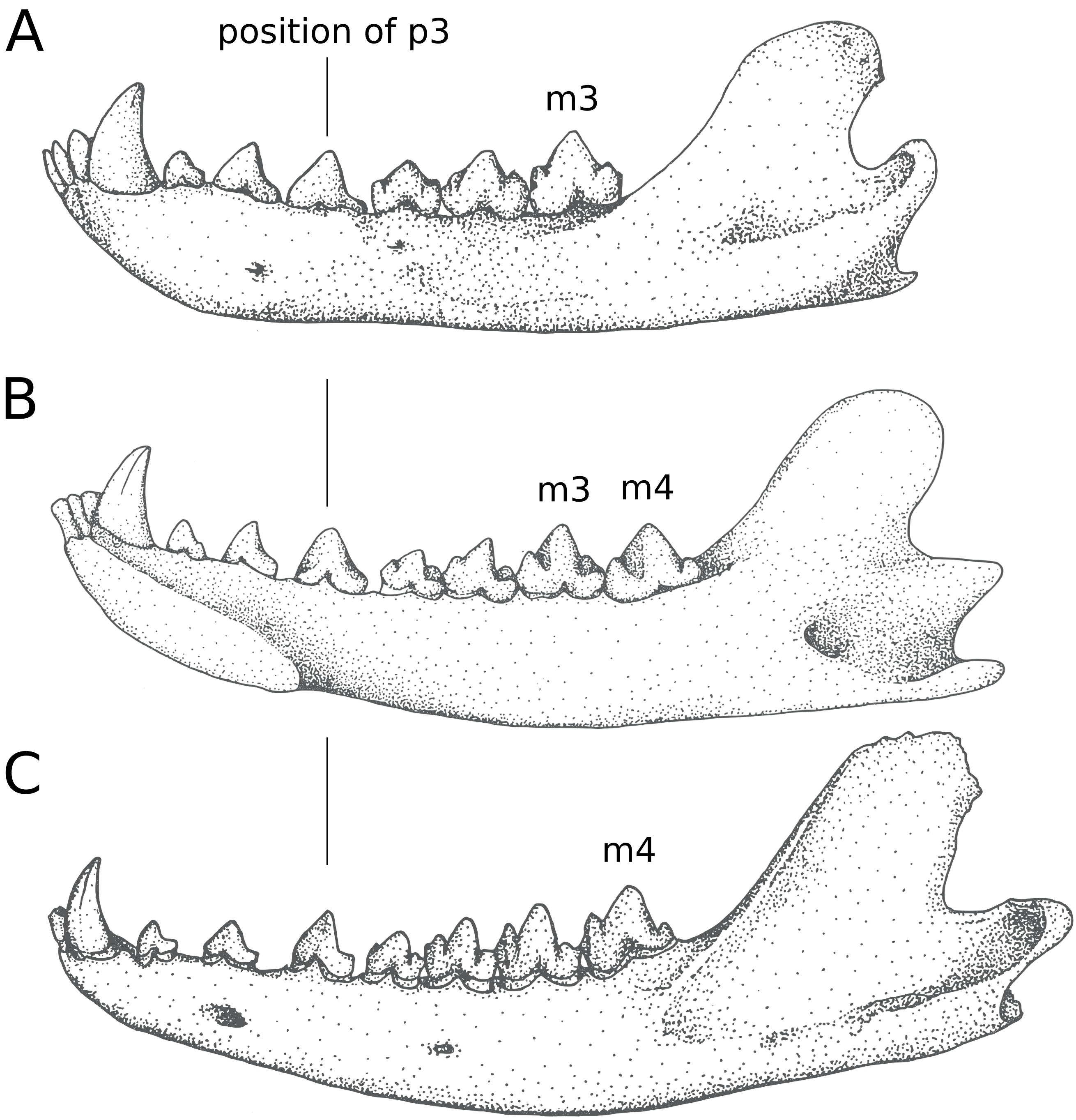

Figure 17. Selected dentaries of *Thylacinus* showing differences in the diastemata between the premolars as the effect of increasing age. A, subadult (in labial view) with m3 erupted, but not m4. Only slight suggestions of diastemata are evident between the premolars; B (lingual view) and C (labial view), showing later stages of m4 eruption and the increase of diastemata in adults.

Relationship of paracone and metacone in thylacines and other marsupials

In *Thylacinus cynocephalus*, the metacone is larger than the paracone on M1 - 3. A similar condition has been found also in numerous Late Oligocene–Miocene fossil thylacinids, including *Nimbacinus* (Muirhead and Archer, 1990), *Badjcinus* (Muirhead and Wroe, 1998), *Wabulacinus* (Muirhead, 1997), *Ngamalacinus* (Muirhead, 1997) and *Mutpuracinus* (Murray and Megirian, 2006). It is likely that a similar condition to that discovered in *Thylacinus* and didelphids, in which the apical

Table 3. Comparison of development in *Thylacinus* and Dasyuridae upper postcanine dentitions, (HL = Head Length).

Taxon	dP^1	dP^2	dP^3	P^3	M^1	M^2	M^3	M^4
Thylacinus (34 mm HL) estimated 31 - 32 days	Moderately thick enamel	Moderately developed to moderately thick enamel	Tiny tooth; moderately thick to thick enamel; shallow bony alveolus	Large, late bell stage; no odontoblasts; deeper bony alveolus	Large tooth; moderately thick dentin and enamel on tall metacone	Moderately large, early - middle bell stage	No trace	No trace
Dasyurus viverrinus (13.5 mm HL) About 35 days	Moderately thick dentin and enamel	No trace	Small tooth; moderately thick dentin; thin enamel; shallow bony alveolus	Late bell stage; thin dentin on apex	Large tooth; moderately thick dentin, thin enamel on tall metacone	Moderately large, middle -late bell stage	No trace	No trace
Antechinus stuartii (9.5 mm HL) 34 days	Moderately thick dentin; moderately developed enamel	Middle - late bell stage	Moderately thick dentin and enamel	Slight swelling of lingual successional lamina	Moderately thick dentin; moderately developed enamel	Middle - late bell stage	No trace	No trace
Sminthopsis virginiae (8.8 mm HL) 30 days	Moderately developed dentin; thin enamel	Late bell stage	Moderately thick dentin and enamel	Early bud stage	Moderately thick dentin; moderately developed enamel	Late bell stage	Early bud stage	No trace
Perameles sp. 16 mm HL	Moderate sized, late bell; possible early odontoblasts	Moderate sized, late bell stage; thin dentin	Moderate sized, late bell; moderately developed dentin	Small early bud stage	Large tooth, with moderately thick dentin and moderately developed enamel	Large, late bell with thin dentin	Small, early - middle bud	No trace
Monodelphis domestica (14.5 mm HL)	Well-developed dentin and enamel	Well-developed dentin and enamel	Large tooth; welldeveloped dentin and enamel	Small early –middle bud	Large tooth; moderately thick dentin and enamel	Moderately large late bell, with moderately developed dentin	Early bud stage	No trace

epithelial nodule is detached from the developing paracone but not from the metacone, is a causative factor in this relationship. It would be interesting to know whether some Oligocene - Miocene thylacinids also had a more molariform and functional dP3, with a tall metacone, than in *Thylacinus cynocephalus*. Unfortunately, we are unaware of any findings of a dP3 in these earlier fossil thylacinids .

Some comments and criticisms concerning similarities and differences between thylacines and other dasyuromorphians and with marsupials in general

It is often noted that *Thylacinus cynocephalus* differs from other thylacinids because of the pronounced diastema between the premolars in its adult dentitions. Indeed, in some cases this has been used in character analyses within thylacinids and other dasyuromorphians (see Muirhead and Wroe, 1998; Yates, 2014). This, however, is simply a factor that increases with age in *Thylacinus cynocephalus*. If we examine some younger juvenile to subadult stages, as shown in Figure 17a, there are few, if any, spaces separating dp1 dp2, and p3. In a juvenile (NMV C 5744; skull length = 157.1 mm) in which dP1 - 2, P3 and M1 - 2 are erupted in both jaws, and m3 is erupted in the lower jaw, the diastema in the upper jaw measured 2.0 mm between dP1 and dP2, and also between dP2 and P3. In the lower jaw, dp1 and dp2 are separated by a diastema of 1.5 mm, as are dp2 and p3. With increasing age and completion of eruption of M4 in both jaws, there is an increase in the length of the diastemata (Fig. 17b, c). We have examined and measured the diastema in a large number of subadult and adult thylacines (measuring192 mm to 253 mm skull length for the adults with all teeth erupted), and this clearly demonstrates that these diastemata increase with age, between the premolars, but not between the molars. As an example, in the oldest (i.e, largest) adult skull examined by us (AM P 767, measuring

Table 4. Development of *Thylacinus cynocephalus* and dasyurid lower postcanine dentitions, (HL = Head Length).

Taxon	dp_1	dp_2	dp_3	p_3	m_1	m_2	m_3	m_4
Thylacinus cynocephalus NMV C 5754 34 mm HL; estimated 31 - 32 days	Moderate sized, thick enamel and disrupted dentin	Moderately large, with thick enamel and disrupted dentin	Tiny tooth, with thick enamel and disrupted dentin; single distinct cusp	Large, late bell stage; thin to moderately developed dentin	Large tooth; thick dentin and enamel on protoconid	Large tooth; moderately thick dentin and enamel on protoconid	Moderately large, middle - late bell stage	Not developed
Dasyurus viverrinus 23 - 25 mm GL (35 days)	Moderately developed dentin, thin enamel	No trace	Tiny abnormal tooth; moderately thick dentin; thin enamel	Early - middle bell; mesial to abnormal dp3	Moderately thick dentin; moderate developed enamel	Moderately thick dentin; thin enamel	Middle - late bell stage	Not developed
Sminthopsis virginiae 10 mm HL (35 days)	Moderately thick dentin, thin enamel	Thin dentin; no enamel	Moderately thick dentin, thin enamel	Slight swelling of lingual successional lamina	Moderately thick dentin; thin enamel	Moderately thick dentin; thin enamel	Middle - late bell stage	Not developed
Antechinus stuartii 9.5 mm HL (34 days)	Moderately thick dentin, moderately developed enamel	Middle-late bell stage	Moderately thick dentin, moderately developed enamel	Slight swelling of lingual successional lamina	Thick dentin, moderately thick enamel	Moderately thick dentin, moderately developed enamel	Middle - late bell stage	Not developed
Perameles nasuta 35 mm GL (16 mm HL)	Moderately large, late bell; very thin dentin	Moderately large, late bell; early odontoblasts	Small tooth; late bell; thin dentin	Lingual early bud	Large tooth; dentin and moderately developed enamel on protoconid	Large tooth; dentin and thin enamel on protoconid	Moderately large, late bell, with early odontoblasts	Not developed
Monodelphis domestica 49 mm CR (16.5 mm HL)	Moderately sized tooth; moderately thick dentin and enamel	Large caniniform tooth; thick dentin and enamel	Large tooth; thick dentin and enamel on tall protoconid	Small late bud - early cap; mesio-lingual to dp3	Large tooth; thick dentin and enamel on tall protoconid	Large tooth; thick dentin and enamel on protoconid	Moderately large tooth, in middle bell stage	Not developed

about 253 mm skull length), the teeth were heavily worn, and the diastema in the upper jaw measured 3.3 mm between dP1 and dP2, and 4.9 mm between dP2 and P3. In the lower jaw, the diastema was 5.2mm between dp1 and dp2, and 5.8mm between dp2 and p3. Considerable variation occurred between the sizes of the diastemata in both jaws of all adults examined.

We suggest that the presence, absence and size of diastemata between premolars should be used with caution in assessing the phylogenetic relationships among fossil thylacinids, especially when only one or two specimens are known for any fossil species.

Another comment refers to the continued use of the terms P1 and P2 in the adult jaws of fossil and extant thylacinids, and in other marsupials in general. In all marsupials that have been examined histologically from early developmental stages up to the eruption of all teeth, there is no evidence known to us for the replacement of dP1 or dP2 by a successor P1 or P2 in the upper or lower jaws of any fossil or extant marsupial. This has been emphasised previously for both fossil (Cifelli et al., 1996) and extant (Luckett, 1993a, b; Luckett and Woolley, 1996) marsupials. Authors who continue to use the terms "P1 and P2" should present evidence for the replacement of their deciduous predecessors in marsupials, as is the case for dP3 and P3. Similar usage of the term "P1" instead of dP1 within fossil and extant eutherians has been discussed recently in a study on Eocene juvenile perissodactyls (Rose et al., 2018).

A final, very positive, observation on thylacine biology and evolution. We were pleased to see the publication on development of the immune system (Old, 2015) in the pouch young of *Thylacinus* that was based on the histological sections of NMV C 5754 from Museums Victoria. Hopefully, other investigators will study additional aspects of the cranial and postcranial biology in this specimen. We are also happy to note the recent publication of the thylacine genome (Feigin et al., 2018), thanks to the use of material from the sister (NMV C 5757) of our sectioned pouch young in the Museums Victoria collection (see fig. 2b).

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