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ORIGINAL PAPER

Vestigial structures and variation in the evolution of the marsupial mammal dental development—a study of the woolly opossum *Caluromys philander*

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Abstract

The pattern of dental replacement in marsupial mammals has received much attention for its derived nature and potential relationship to the life history of the group. However, few species have been studied thoroughly, and little is known about the embryonic structures and their use in addressing issues of homology and dental evolution in general. We studied a developmental series of ten individuals of pouch young *Caluromys philander* to thoroughly document dental development with histological sections and 3D models of dental series. We report that the successor P3 arises from a lingual successional lamina from its predecessor dP3. The germs of vestigial, unerupted deciduous incisors and canines are present alongside their respective permanent successors. These discoveries demonstrate significant differences from the developmental patterns reported for *Didelphis* and *Monodelphis* and illustrate that an unsuspected diversity of dental ontogeny is not reflected in the adult pattern of mineralised, erupted or almost erupted teeth.

Keywords *Caluromys* · Didelphidae · Metatheria · Dentition · Ontogeny

Introduction

During the evolution of mammals, significant changes have occurred in the pattern of tooth replacement. Whereas other tetrapods, including early synapsids, had primarily several tooth generations, Mammalia – including the last common ancestor of monotremes, marsupials, placentals and all its descendants–is characterised by a diphyodont dentition, in which the incisors, canines and premolars are replaced, and molars are not (Luo et al. 2004). Tracing the evolution of this tooth replacement pattern, which has been tied to life history evolution (van Nievelt and Smith 2005a, b; Asher et al. 2017), has been a goal of palaeontology–one for which new tools of non-invasive imaging have been important in order to document mineralised tissues in fossils (Cifelli et al. 1996; Rose et al. 2018)—and of comparative anatomy, as revealed by dental development studies.

Studies of dental development that aim to establish the identity of teeth and their embryonic precursors, and of transient structures that do not erupt nor develop into easily recognisable tooth precursors, require developmental series that cover the critical times of development and proper histological or imaging methods (e.g., Hautier et al. 2016). These methodological and sampling limitations have meant that we still have much to learn about variation across mammals, including the fundamental dichotomy of the eutherian and metatherian dental patterns.

The plesiomorphic placental, and more generally eutherian (placentals and their stem forms) dentition is characterised by two generations (diphyodonty) of teeth at the incisor, canine and premolar loci, with one generation of teeth at the molar loci (Luckett 1993a; Luo et al. 2004). In contrast, marsupials have consistently been shown to reduce tooth replacement further, with two functional generations only observed to exist at the third premolar locus (Flower 1867; Archer 1974; van Nievelt and Smith 2005a, b). Specifically, members of the Didelphidae retain a metatherian adult dental formula of I 5/4, C 1/1, P 3/3, M 4/4, with replacement only occurring at the third premolar (P3/p3, henceforth

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referred to as P3 unless specifically referring to either of the lower p3 loci) locus (Astúa and Leiner 2008). This single replacement at the P3 locus is described as the most derived form of dental replacement in therian mammals by Luo et al. (2004), though some species may have lost replacement altogether, thus are functionally monophyodont (van Nievelt and Smith 2005a, b).

The pattern of dental replacement within marsupials, and wider Metatheria (the group including the stem forms of the clade), is well established, as it has been shown to have arisen by at least the Late Cretaceous (Cifelli et al. 1996). What is less clear, is the extent to which certain genera within this clade have retained unerupted, vestigial precursors to their established dentition.

Dental development – the case of Didelphidae and the study of *Caluromys philander*

As first described by Leche (1892, 1895) all developing teeth go through the same three stages—bud, cap and bell. Each of these stages can also be divided into early, middle and late stages (Luckett 1993b: fig. 1). The deciduous dentition and molars are initiated from the connection of the primary dental lamina to the oral epithelium, with the permanent dentition then arising from a lingual successional lamina of its corresponding deciduous predecessor (Jussila et al. 2014). Histological analysis of developmental series can use this pattern of development to determine the generational origin of teeth, that is to say whether the tooth within the adult dentition is a retained deciduous tooth or a true successor (Luckett 1993a; Luckett and Woolley 1996). This may also be used to determine the presence or absence of vestigial predecessor teeth of species previously known to have only one erupted generation. To be classified as vestigial structures rather than occasional atavisms, these teeth would need to occur regularly in the population and their ancestors, and likely only occur transiently in development (Peterkova et al. 2006).

Didelphis and *Monodelphis* have been extensively studied with regard to dental development (Berkovitz 1967; Tribe 1990; Luckett 1993a). Individuals within both didelphid genera have been reported to possess only one generation at each tooth locus, apart from the last premolar, P3. Fonseca and Alves (2006) found a secondary dental lamina to emerge from the epithelium of *Didelphis albiventris*, but in all cases it was broken down when it reached the bud stage, suggesting that this is a vestigial remnant of a previously-existing secondary dentition. Berkovitz (1978) observed undifferentiated lingual outgrowths from the lamina of developing teeth in *Didelphis virginiana*, but these do not appear to grow into adult teeth. Similarly to *Didelphis*, (van Nievelt and Smith 2005a, b) complete series of *Monodelphis domestica* found it to only ever possess one generation at all but the last premolar (P3), with no vestigial teeth present at any locus.

Caluromyines have previously been shown to diverge morphologically in many traits from all other didelphids (Flores et al. 2010). We made use of an exceptional developmental series of *Caluromys* to investigate dental development and address issues of homologies and variation. *Caluromys* has previously been shown to only exhibit replacement at the third premolar, with the tooth at this locus molariform in the deciduous generation, and premolariform in the permanent generation (Astúa and Leiner 2008). We examined the presence or absence of unerupted, vestigial precursors to the adult generation incisors and canines, previously shown to be absent in *Didelphis* and *Monodelphis*. We also used these specimens to investigate one further controversy surrounding the development of the marsupial dentition: whether the third permanent premolar P3 arises from a successional lamina of its deciduous predecessor dP3 (Luckett 1993a; Luckett and Woolley 1996; Luckett et al. 2019), or whether it arises from a separate primary dental lamina originating from the oral epithelium (Archer 1974, 1978). The former condition has been reported in *Didelphis* (Luckett 1993a), while the latter has been reported in *Monodelphis* (van Nievelt and Smith 2005a, b). Table 1 summarises the findings from a number of previous studies on the presence of any vestigial dentition in a range of metatherian species, and on their origin of the successor P3.

Materials and methods

We examined sectioned and stained material of pouch young of the bare-tailed woolly opossum, *Caluromys philander*, a highly arboreal member of the Didelphidae from northeastern and central South America (Eisenberg 1989: p. 23–25). It has an adult dental formula of I 5/4, C 1/1, P 3/3, M 4/4, with two functional tooth generations at the posterior premolar (P3) locus in both the upper and lower jaws. Specimens used in this study originated in a captive colony described by Atramentowicz (1995), who reported individuals typically weigh 300—400 g as adults. At birth, young weigh < 200 mg and are < 11 mm long. Upon exiting the pouch at around 80 days, young weigh 20 g, are 80 mm long, and begin to eat solid food. Young either travel with the mother or remain in the nest until they are fully weaned at about 120 days.

Our series of *Caluromys* documents a detailed sequence of dental development. Ten pouch young paraffin-embedded specimens were sectioned at 10, 15 or 20µ, and stained with Azan after Domagk. Head lengths (KL) in mm of the specimens examined are: KL6, KL7.5, KL10, KL13(D20), KL13(D30), KL22, KL24(D64), KL25(D77), KL26(D84) and KL26. The age of the specimen (days after birth) is in parentheses after the

Table 1 Metatherian dental characters concerning the presence/absence of any vestigial structures, the origin of the successor P3, and the adult dental formula, all as reported in the literature

Species	**Materials Studied**	**Vestigial Structures**	**Origin of Successor P3**	**Ancestral Marsupial Dental Formula? (I5/4, C1/1, P3/3, M4/4)**
Deltatheridium pretrituberculare (fossil methatherian)[1,2]	Fossil	—	Replacement occurs in marsupial-like manner, but no indication of origin	No - I4/3, C1/1, P3/3, M4/4
Alphadon (extinct)[3]	Partial skull	—	Lies lingual and deep to erupted dP3	Yes
Monodelphis domestica [4,5]	Live specimen, macerated skull & histological section	None	First appears as thickening of free edge of lamina between dp2 and dp3	Yes
Didelphis albiventris[6,7]	Macerated skull & histological section	No evidence of a vestigial, deciduous dentition	—	Yes
Didelphis virginiana[8,9,10]	Live specimen, macerated skull & histological section	No evidence of any non-functional teeth	Develops from dental lamina between P2/p2 and dP3	Yes
Caenolestes convelatus[11]	Macerated skull	—	Has a dp3 when originally thought not to, but no mention of the origin of P3	No - I4/3, C1/1, P3/3, M4/4
Isoodon macrourus[12]	Histological section	Lower i1 - abortive tooth germ and germ-like enlargement	—	No - I5/3, C1/1, P3/3, M4/4
Isoodon obesulus[13]	Histological section	Small tooth-like structure labial to i1, believed to be an abortive tooth germ	Upper - germ of P3 at deep and terminal portion of the lamina at the distal part of dP3 Lower - develops from same lamina parietally connected to p2	No - I5/3, C1/1, P3/3, M4/4
Perameles gunnii[13]	Histological section	Small tooth-like structure observed labial to I1	—	No - I5/3, C1/1, P3/3, M4/4
Perameles nasuta[14,15]	Macerated skull & histological section	Vestigial teeth at upper incisors and canine	Develops lingual to dP3	No - I5/3, C1/1, P3/3, M4/4
Antechinus stuartii[16]	Histological section	No traces of labial abortive tooth germs	Develops on the same lamina to which P2/p2 is parietally connected.	No - I4/3, C1/1, P3/3, M4/4
Sminthopsis crassicaudata[16]	Histological section	None observed	Dental lamina of P3 had no connection to second or deciduous premolar	No - I4/3, C1/1, P3/3, M4/4
Sminthopsis virginiae[17]	Macerated skull & histological section	A number of vestigial, deciduous structures at the incisors and canines, with di2 persisting the longest	Develops from lingual successional lamina of dP3	No - I4/3, C1/1, P3/3, M4/4
Sarcophilus harrisii[18,19]	Live specimen & macerated skull	None observed	—	No - I4/3, C1/1, P2/2, M4/4
Thylacinus cynocephalus[20]	Histological section & soft tissue	Buccal epithelial nodules may represent remnants of rudimentary dI1 and dI3	Develops from lingual successional lamina of dP3	No - I4/3, C1/1, P3/3, M4/4
Dasyurus viverrinus[21]	Macerated skull, histological section & soft tissue	Yes - replacement occurs at first three incisors and canine	Arises from lingual successional lamina of dP3	No - I4/3, C1/1, P2/2, M4/4
Trichosurus vulpecula[22]	Histological section	Non-functional enamel organ at P2, with three small enamel organs in lower jaw	Develops lingual to anterior part of dP3, but not certain of lamina origin	No - I3/2, C1/0, P2/1, M4/4
Macropus eugenii[23,24,]	Macerated skull & histological section	Three functionless incisors in each quadrant; maxillary functionless canine tooth germ	Arises from dental lamina between P2 and dP3	No - I3/1, C0/0, P2/2, M4/4

Table 1 (continued)

Species	Materials Studied	Vestigial Structures	Origin of Successor P3	Ancestral Marsupial Dental Formula? (I5/4, C1/1, P3/3, M4/4)
Macropus giganteus[25,26]	Live specimen, macerated skull, histological section & soft tissue	dI1, dI2, dI3, C1 and di2 were minute and did not erupt. Lower i1, P1 and p1 were vestiges and barely developed beyond the bud stage	From the lamina between second premolar and dP3	No - I3/1, C0/0, P2/2, M4/4
Setonix brachyurus[27]	Histological section	Non-functional canine and premolar in upper jaw, with one non-functional tooth in lower jaw	From dental lamina between P2 and dP3, not from lingual downgrowth of dP3	No - I3/1, C0/0, P2/2, M4/4
Osphranter robustus[28]	Macerated skull	A pair of minute incisiform teeth on premaxilla external to erupting third incisors	—	No - I3/1, C0/0, P2/2, M4/4

[1]Rougier et al. (1998); [2]Velazco et al. (2022); [3]Cifelli et al. (1996); [4]van Nievelt and Smith (2005a, b); [5]van Nievelt and Smith (2005a); [6]Fonseca and Alves (2006); [7]Chemisquy and Martin (2016); [8]Berkovitz (1967); [9]Berkovitz (1978); [10]Petrides (1949); [11]Luckett and Hong (2000); [12]Fosse and Risnes (1972a, b); [13]Fosse and Risnes (1972a); [14]Wilson and Hill (1897); [15]Luckett (1993a, b); [16]Fosse (1969); [17]Luckett and Woolley (1996); [18]Guiler and Heddle (1974); [19]Landy et al. (2021); [20]Luckett et al. (2019); [21]Luckett et al. (2021); [22]Berkovitz (1968); [23]Berkovitz (1972); [24]Nasrullah et al. (2022); [25]Kirkpatrick (1978); [26]Poole (1982); [27]Berkovitz (1966); [28]Tate (1947)

head length, if it is known. Pictures of some of the specimens are reproduced in Sánchez-Villagra et al. (2002).

In developing teeth, we recognise the following standard developmental stages: thickening of the dental lamina (tdl), bud, early cap, late cap, early bell, late bell with only dentine present (abbreviated as "dentine") and late bell with enamel and dentine present ("enamel"). The characteristic features of each stage are to be found in sections that pass through the part of the tooth that is the most advanced (i.e., began development first) and are best seen in the coronal plane (Luckett 1993b).

The developmental sequence of vestigial teeth was determined by the relative positions of the tooth germs and their attachment to the dental lamina. Typically in mammals the deciduous tooth germ forms with a proliferation of cells in the dental lamina, resulting in a lingual extension of the lamina (Luckett 1993b).

3D reconstruction

TIFF stacks for five of the specimens were imported into the 2015 distribution of Fiji (Schindelin et al. 2012) and then aligned via the automated analysis of consecutive slices within TrakEM2 (Cardona et al. 2012). Coloured areas of individual structures in the upper dentition were produced using a graphics tablet, with different colours used for different tooth germ regions at the same locus. The 'Show in 3D' function was then used to generate 3D reconstructions of the tooth germs of each specimen, with the subsampling rate always set to one to prevent data loss. Specimens KL13(D20) and KL22 were imaged within the 3D viewer of TrakEM2, whereas the smaller specimens KL6, KL7.5 and KL10 were exported as Wavefront.obj files and imaged within Blender (Community 2018). The dental laminae were coloured only in specimen KL22 so as to prevent the obstruction of vestigial tooth germs in the smaller specimens.

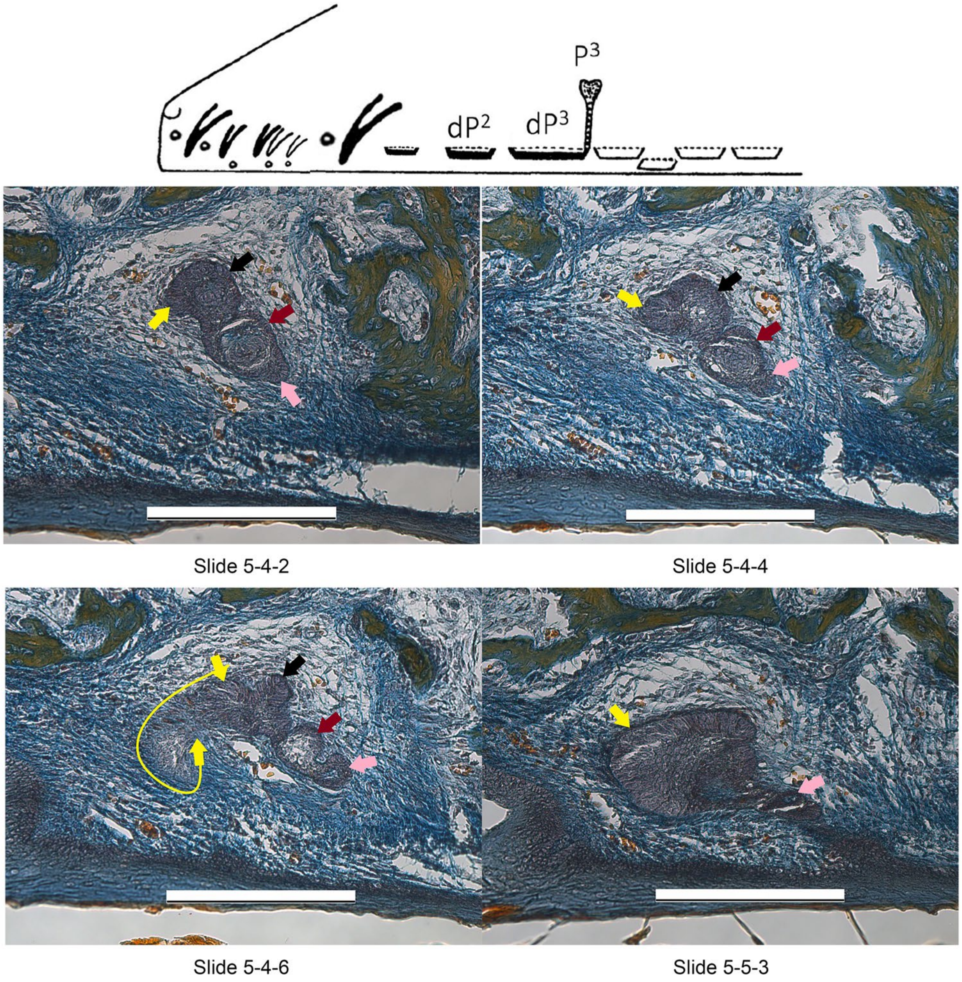
Results

Development of the functional dentition in *Caluromys philander* is well documented by our series. Most of the functional teeth can be traced from bud stage to late bell with enamel.

Vestigial structures

We found incontrovertible evidence for unerupted, vestigial tooth germs in the incisor (Fig. 1) and canine regions (Fig. 2) of the KL7.5, KL10, KL13 (D20), and KL13(D30) specimens. These loci demonstrate the functional tooth germ alongside condensations of cells which potentially represent more than one vestigial generation. What remains as of yet unclear is the

Fig. 1 **Above**. Schematic of the developing dentition in the upper jaw of *Caluromys philander* (adapted from Hopewell-Smith and Tims 1911; Nasrullah et al. 2022). **Below**. Developing dentition in *Caluromys philander* at upper left I2 in specimen KL13(D20). Yellow arrow points to developing permanent generation I2, while black, maroon, and pink represent anlage of different, non-erupting, vestigial dentition. Scale bar equals 0.2 mm



number of generations present at some of the incisor loci. In the labelled figures below, the colour yellow represents the developing permanent generation. We document the anlage of vestigial dentition, indicating how different condensations of cells and structures may represent different generations. We hypothesise that each of these colours could represent a transient structure, each belonging to a different generation of vestigial dentition. Modelling of these sections using TrakEM2 allows for a 3D visualisation of the described structures (Fig. 3).

The last premolar, P3

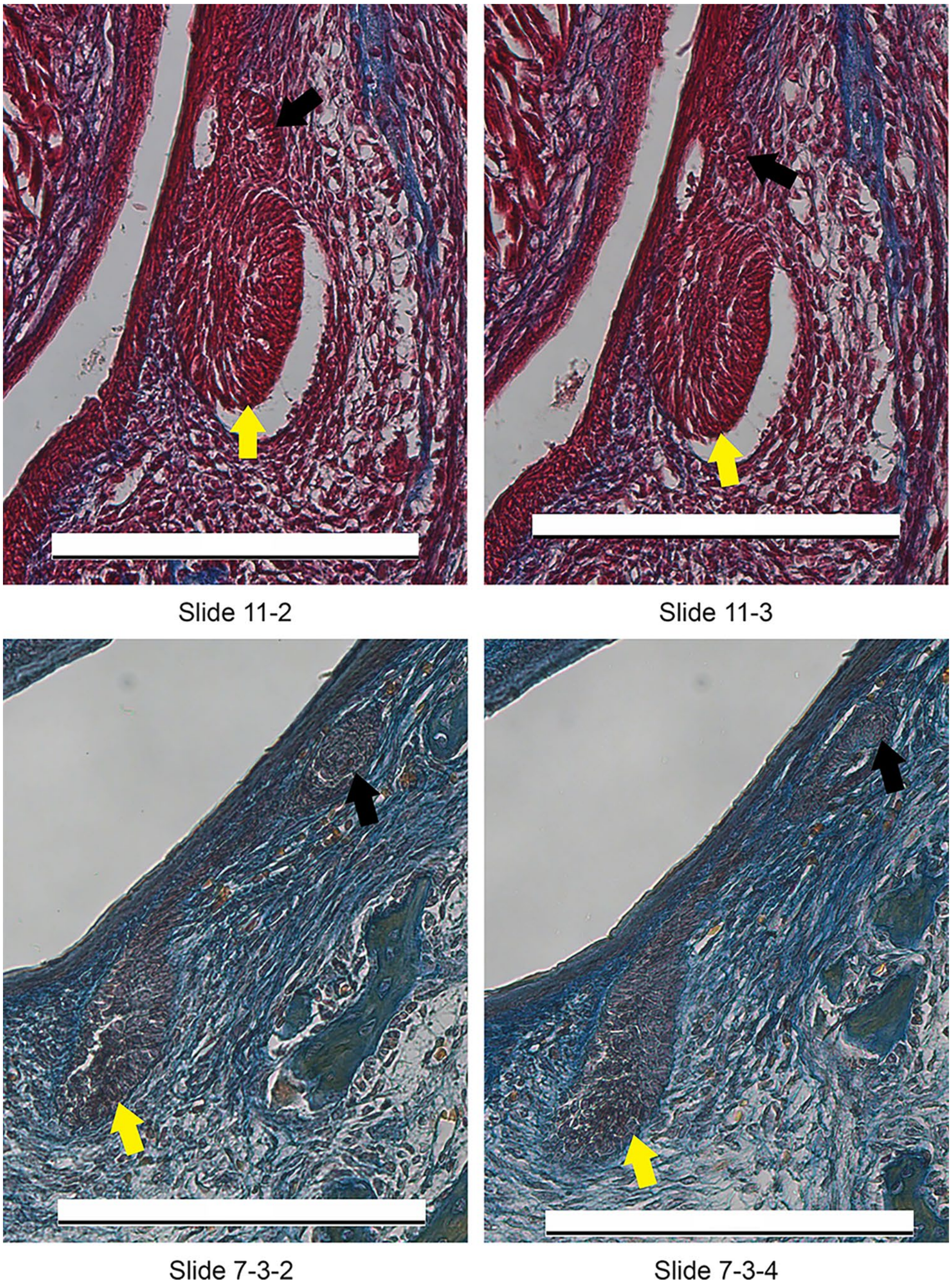
The successor of P3 develops from the lingual successional lamina of dP3 in both the upper and lower jaws, rather than from a separate primary dental lamina originating from the oral epithelium between dP3 and M1. This is the same as the condition reported in *Didelphis* (Luckett 1993a) and is in contrast to reports of the latter condition by Archer (1974, 1978) for a diverse range of marsupials, including *Antechinus flavipes*.

From lesser to greater developed individuals, we note a progression from lingual ridge (Fig. 4a), to lingual successional lamina (Fig. 4b), to a developing bud at the terminal end of the successional lamina (Fig. 5). Although both the connection of the successional lamina and the primary dental lamina are at times fractured (Fig. 5), there is never a connection between what we describe as the successional lamina and the oral epithelium. A description of the development of the post-incisor dentition of the specimens studied is available in Online Resource 1.

Discussion

We report for *Caluromys philander* that the successor P3 arises from a lingual successional lamina from its predecessor dP3, and that vestigial, unerupted deciduous incisors and canines are present alongside their respective, permanent successors. Vestigial teeth are consistently present in all upper and lower incisor and canine loci. This consistent

Fig. 2 Developing dentition in *Caluromys philander* at the lower left canine locus area in specimens KL7.5 (above, two slides) and KL13(D20) (below, two slides). Yellow arrow indicates tooth bud which will go on to form the canine in the permanent dentition. Buccal black arrow points to the vestigial, deciduous canine (**dc**). Scale bar equals 0.2 mm



presence at a number of developmental stages confirms that these structures are vestigial rather than occasional atavisms. These discoveries demonstrate significant differences with the developmental patterns reported for *Didelphis* and *Monodelphis* and illustrate that an unsuspected diversity of dental ontogeny is not reflected in the adult pattern of mineralised, erupted or almost erupted teeth. They also posed a nomenclatural challenge, as many loci of the adult dentition of marsupials may represent either deciduous or permanent teeth. Before making a definitive proposal on this matter, it would be important to first have a more thorough understanding of dental development across marsupials.

Vestigial teeth across marsupials and beyond

Other didelphid genera studied so far, i.e., *Didelphis* and *Monodelphis,* have been reported to possess no vestigial teeth in the incisor and canine regions (Berkovitz 1978; van Nievelt and Smith 2005a, b; Fonseca and Alves 2006). In contrast, species such as *Dasyurus viverrinus* (Hill and Osman Hill 1955; Luckett et al. 2021) have consistently been reported as having unerupted, vestigial structures in these regions across the different levels of dental development described by Luckett (1993b). This variation in the presence of vestigial structures also characterises other species

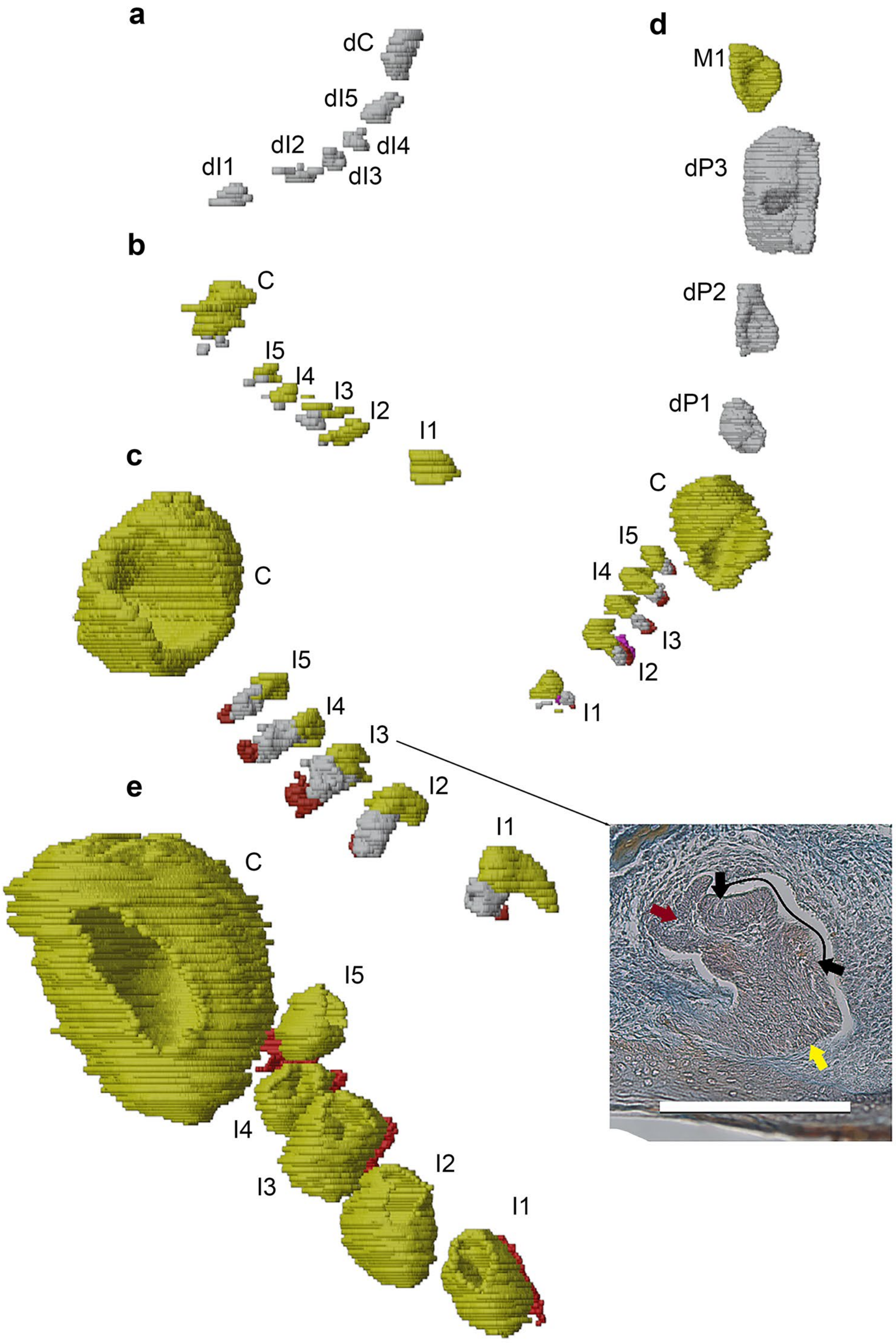
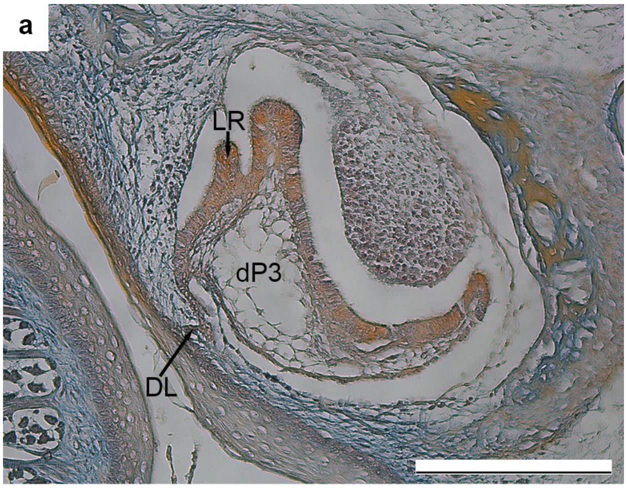
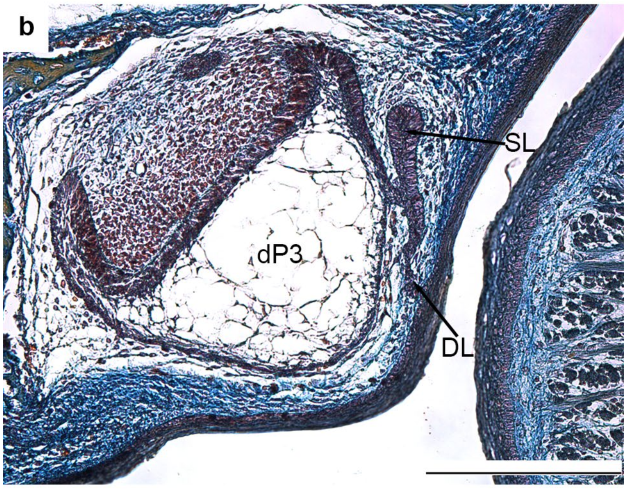


Fig. 3 3D reconstructions of the developing dentition in *Caluromys philander* generated in TrakEM2. Specimens studied are: **a.** KL6; **b.** KL7.5; **c.** KL10; **d.** KL13(D20); and **e.** KL22. Also shown is a histological section of slide 5-3-3 at the upper right I3 locus in specimen KL10. Yellow structures and arrows indicate the developing permanent generation, while black, maroon, and pink represent anlage of different, non-erupting, vestigial dentition. KL13(D20) and KL22 are shown to scale, while KL6, KL7.5 and KL10 are magnified 2x. Scale bar equals 0.2 mm

across the metatherian tree; of the 21 species described in Table 1, 12 possessed vestigial structures representative of the greatly reduced deciduous dentition, no vestigial structures were observed in six species, and the material was not available to potentially observe these structures in three species. Histological analysis was not carried out on four of the nine species that did not present vestigial structures, so further analysis could potentially elucidate the presence of unerupted, vestigial structures. A systematic investigation of many specimens for ontogenetic series of several taxa with a similar approach can provide a definitive assessment of the variation in vestigial structures. The study of Nasrullah et al. (2022) on the tammar wallaby *Macropus eugenii*, a diprotodontian with two procumbent lower incisors diagnostic of the clade, provides a recent and significant contribution in this discussion. The documentation produced



KL10 slide 10-5-2



KL13 slide 12-4-3

Fig. 4 Developing dentition in *Caluromys philander*. **a.** A lingual ridge (**LR**) is demonstrated at the upper left P3 locus in specimen KL10, while **b.** lingual successional lamina (**SL**) illustrated at upper right P3 locus in KL13(D20). Abbreviations: **DL** = primary dental lamina. Scale bar equals 0.2 mm

Fig. 5 Presence of a developing permanent p3, arising from a lingual successional lamina (**SL**) from the deciduous dp3 precursor in *Caluromys philander*: Slide 35–3-1 at the lower left p3 locus in specimen KL26. Developing tooth at the second premolar locus labelled following the criteria of Luckett (Luckett 1993a). Scale bar equals 0.5 mm

using iodine staining and microCT scanning (diceCT) of embryos and pouch young provides an alternative and more economical approach to classical histology as presented by us in our study of *Caluromys philander*. To gain a complete documentation of the early development of incisor anlage, embryonic materials may be needed given what we know of the timing from wallabies (Berkovitz 1972; Nasrullah et al. 2022). Examining reports for other groups of mammals is also worthwhile (Popa et al. 2016).

Outside Metatheria, for example, within Rodentia, mice possess only one generation of incisors and a greatly reduced dental formula, but embryos exhibit anlage from different teeth, providing homology criteria for the adult teeth in this group (Peterkova et al. 2006). Remnants of dental structures lost in evolution in some lineages are also present in some extant species outside Mammalia. Despite losing their teeth between 100–80 million years ago, there are signs of dental lamina in birds, with neural-crest transplantation experiments in mice and chicks suggesting that the oral epithelium in the chick has retained the molecular signals required to induce odontogenesis (Mitsiadis et al. 2006; Sire et al. 2008).

The vestigial tooth anlage we report for *Caluromys philander* are remnants of a dentition that has not been functional since the Cretaceous (Cifelli et al. 1996), or even earlier in the Mesozoic if we consider vestiges beyond the second generation and thus stem synapsids that predated the origin of diphyodonty (Luo et al. 2004). Alongside the functional tooth germ, we found condensations of cells, representative of deciduous tooth germs, in four of ten specimens: KL7.5, KL10, KL13(D20), and KL13(D30) (Figs. 1, 2 and 3). It is not possible to establish with certainty the generational nature of each of these condensations of cells. These condensations may belong to the same vestigial, deciduous generation, thus only one vestigial generation precedes the permanent dentition. Alternatively, each condensation of cells may represent a different, vestigial generation. Therefore, depending on the locus, there is the potential for between one and three vestigial generations at different loci. Perhaps examining these cellular condensations at a molecular level could discern the number of generations present. Coupling a comparative embryological approach with experimental methods such as those used in mice to identify mutations affecting tooth morphology and dental formula (e.g., Line 2003) could provide more clarity on this matter.

The presence of vestigial structures may be an example of developmental instability, a situation in which variation in development arises in response to environmental and genetic perturbations – an example being wide individual variation in the skeleton of the vestigial wing of the flightless kiwi (Richardson 2022). Clearly, the variation in the presence of structures we document here for *Caluromys philander* is ontogenetic, as we examined one individual for each of the ages studied. To further examine developmental instability in *Caluromys* – a hypothesis on the mechanisms behind the variation observed – one would require several individuals of the same age and a more systematic and quantitative examination of patterns of variation across phylogeny in dental development in populations with known environmental context of diverse species.

Origin of the permanent third premolar

The extensive nature of our histological series of *Caluromys* also provided the opportunity to investigate the origin of the permanent third premolar, P3. Namely, whether it arises from an independent primary lamina originating from the oral epithelium (Archer 1974, 1978), or from a successional lamina of a deciduous predecessor dP3 (Luckett 1993a; Luckett and Woolley 1996). Our results for *Caluromys* support the second hypothesis, that similar to *Didelphis* (Luckett 1993a), the successor P3 arises from the lingual successional lamina of dP3 in both jaws. Development at the P3 locus in our histological series follows the series of steps expected in marsupials, as previously illustrated by Luckett et al. for *Dasyurus viverrinus* (2021: fig. 2) and *Perameles nasuta* (Luckett 1993a: fig. 13.11): as we progress from our least to most developed specimens, we observe a progression from a thickened lingual successional ridge (Fig. 4a), to a lingual successional lamina continuous with the outer enamel epithelium of dP3 (Fig. 4b), to a bud stage P3 at the terminal end of the successional lamina (Fig. 5). At this final stage there is no obvious connection of the dental lamina to the oral epithelium, rather what appears to be a disrupted connection of the successional lamina to the outer enamel epithelium of dP3. Furthermore, in none of our specimens do we observe a connection of the lingual successional lamina to the oral epithelium, this being the only factor that could have contradicted what we report. Observing a connection of this nature would be a prerequisite for supporting Archer (1974) hypothesis that the permanent P3 arises from its own primary dental lamina connection to the oral epithelium.

We conclude that in *Caluromys philander* the successor P3 develops from the lingual successional lamina of dP3 in both jaws, rather than from a separate primary dental lamina originating from the oral epithelium between dP2 and M1. There is until now unsuspected variation on how P3 develops across didelphids.

Conclusions

Our exceptional histological series of pouch-young *Caluromys philander* specimens indicates a number of cellular condensations, illustrative of an unerupted, vestigial dentition, representing an undetermined number of generations. Investigation of the region of the third premolar locus supports the hypothesis that the tooth belonging to the permanent generation of teeth at this locus arises from a lingual successional lamina of the deciduous dP3 predecessor.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10914-022-09638-0.

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Author contributions CJM studied and documented histological sections, developed interpretations of the findings, generated 3D models, produced the figures, and co-wrote the manuscript. WPL studied histological sections and developed interpretations of the findings. MRSV conceived the study, studied and documented histological sections, developed interpretations of the findings, procured funding, and co-wrote the manuscript.

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Data availability Digitalized TIFF stacks of histological specimens studied are available at: https://doi.org/10.5061/dryad.jm63xsjdd

Declarations

Competing interest All authors declare no conflicts of interest.

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