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ULTRASTRUCTURAL VARIATION IN ENAMEL OF AUSTRALIAN MARSUPIALS

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Abstract

This paper initiates a survey of the enamel of fossil and extant Australian marsupials by scanning electron microscopy. Enamel was examined from 17 extant and 11 extinct marsupials. Assessment was made of prism packing pattern, prism course, tubule presence, tubule size and distribution. Values calculated were: prism diameter; prism axis ratio; cross-sectional prism area; cross-sectional ameloblast area; and numerical prism density.

Three different prism packing arrangements were found for extant and fossil marsupials within the classical Pattern 2. The Pattern 1 arrangement found in three extant species was relatively unexpected given the general acceptance of Marsupialia as having Pattern 2 enamel. Attention is drawn to the variable loss of prism demarcation towards the outer enamel surface. The majority of both extant and fossil marsupials exhibited a simple radial prism course. Prism diameters were small ranging from 1.4 μm to 3.9 μm and prism densities were high, compared to those for human and multituberculate enamel.

A significant inter-species variation was noted in the presence and size of enamel tubules. The absence of enamel tubules in the incisors of *D. optatum*, *N. tedfordi* and *T. rostratus* and the molar of *W. wakefieldi* was confirmed. Large bulbous spaces were found either along or at the termination of enamel tubules in some teeth of five fossil species: these spaces may represent the resting place of an ameloblast.

We have found: a greater variation in prism packing patterns; a greater difference in characteristics studied between incisor and molar teeth; and a greater variety of tubule morphology than anticipated. There are signs that useful enamel ultrastructural characters are emerging to help ultimately with taxonomic investigations of Australian marsupials.

KEYWORDS: Enamel Ultrastructure, Marsupials, Fossil Marsupials, Phylogeny, Prism Pattern, Enamel Tubules.

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Introduction

The analysis of enamel ultrastructure as a possible aid to testing phylogenetic hypothesis is attracting continued attention (e.g., Fosse et al., 1978, 1985; Sahni, 1979, 1985, 1987; Boyde and Martin, 1984a; Carlson and Krause, 1985; Grine et al., 1987; Krause and Carlson, 1987). Two recent studies have drawn attention to the potential variability in fossil and extant marsupial enamel characters at the ultrastructural level (Lester et al., 1987; Lester et al., 1988a). It is our intention with this paper to initiate a wider survey of the enamel of fossil and extant Australian marsupials by scanning electron microscopy. We believe that the information itself, even at a substantially qualitative level, is of intrinsic interest and overdue. That the information might eventually help elucidate the largely obscure phylogenetic relationships of Australian marsupials (see Aplin and Archer, 1987) is, at this stage, a hope which further analysis and quantitation might sustain.

Materials and Methods

Enamel was examined from the teeth of 17 extant marsupials (9 families) and 11 extinct marsupials (9 families) (Table 1). For the extant marsupials, the species names used are as given in "A Complete Book of Australian Mammals" (Strahan, 1983) and the classification used for all species is as given in "Possums and Opossums" (Aplin and Archer, 1987).

A total of 62 teeth was sampled: 26 incisors; 2 canines; 31 molars and 3 premolars (see Acknowledgements). Many of the specimens, particularly the fossils, were both rare and valuable. Where availability of material permitted, our preferred option was a polished transverse section exposing the whole width of the enamel and including transversely sectioned prisms (Figs. 1a, b); this allowed an assessment of prism packing pattern, prism course, tubule presence, tubule size and distribution. Where possible, a longitudinal polished section was also prepared. The teeth of *Tarsipes rostratus* were embedded in resin solely as a support medium prior to sectioning because their very small size (a coronal width of ca. 0.1 mm for the upper incisors) made them otherwise difficult to handle.

TABLE 1

ORDER	SUBORDER	FAMILY	SPECIES	TOOTH	AGE OF FOSSIL
DIPROTODONTIA	Vombatiformes	Diprotodontidae	*Diprotodon optatum**	I_1, I^1, LM	Pleistocene
			*Zygomaturus trilobus**		late Pleiocene
		Palorchestidae	*Palorchestes parvus**		latest Oligocene
			*Ngapakaldia tedfordi**	I, M	mid-Miocene
		Thylacoleonidae	*Thylacoleo carnifex**	I^1, PM	Pleistocene
		Vombatidae	*Vombatus ursinus*	I_1, PM_1, M^1, M_3	
			*Warenja wakefieldi**	M	mid-Miocene
	Phalangerida	Phalangeridae	*Phalanger vestitus*	M_3	
		Ektopodontidae	*Ektopodon serratus**	M^5	mid-Miocene
		Potoroidae	*Ekaldadeta ima**	M	mid-Miocene
		Macropodidae	*Macropus eugenii*	I_1, M_1	
			Macropus parryi	I_1, M_1	
			Petrogale penicillata	I_1, M_1	
			*Protemnodon chinchillaensis**	I, M	Miocene
		Petauridae	*Petauroides volans*	I_1, M_2	
		Tarsipedidae	*Tarsipes rostratus*	I_1, I^1, C, M^1	
		Acrobatidae	*Acrobates pygmaeus*	I_1, M_1	
			Distoechurus pennateus	M^4	
DASYUROMORPHIA		Dasyuridae	*Dasyurus maculatus*	C_1, M_1	
			Dasyurus viverrinus	I_1, M_1	
			Sminthopsis murina	I_3, M_2	
			Antechinus stuartii	I^1, M^1	
			Sarcophilus harrisii	I_1	
NOTORYCTEMORPHIA		Notoryctidae	*Notoryctes typhlops*	I_1, M_1	
			Unnamed notoryctid*	I, M	Miocene
PERAMELEMORPHIA		Peramelidae	*Perameles nasuta*	I_1, M^1	
			Isoodon macrourus	I, M_1	
			Unnamed peramelid*	M	Miocene

Listing of the species and teeth studied with indication of age of fossils.
C - canine; I - incisor; LM - lower molar; M - molar; PM -premolar
* Fossil species

All sections were polished, ("Metaserv" hand grinder and rotary polisher, Metallurgical Services, Betchworth, Surrey, England) etched with 0.5-1.0% H_3PO_4 for 5-10 secs, sputter-coated with gold and examined in a JEOL 840 scanning electron microscope (SEM) at 15 kV. In addition, some specimens were treated with a 50% dilution of a stock solution of NaOCl (12% available chlorine) for 2 mins to remove organic residue from tubule spaces before gold coating. As a result of

observations made and in order to produce casts of the enamel tubules, samples of *Diprotodon optatum* were refluxed in chloroform/methanol for 6 h, embedded in London White acrylic resin, sectioned, polished and etched in 2% H_3PO_4 for 20 secs.

Quantitative analysis

We measured or calculated the following values in order to make some comparison with the data collected by others for a variety of enamels (Fosse, 1968a, 1968b; Boyde, 1969; Fosse et al., 1973; Sahni, 1979; Krause and Carlson, 1986).

i. Prism diameter (PDx)
ii. Ratio between axes of cross-sectional prism profile (Dx/Dy)
iii. Cross-sectional prism area (PA)
iv. Cross-sectional ameloblast area (AA)
v. Numerical prism density per mm^2 (PDe)

Initially, we attempted to use a fully automated image analysis system integrated with the SEM for this morphometric survey. However, because prism boundaries were often too indistinct for registration for automated analysis, we reverted to an interactive system utilising bitpad and cursor to trace prism outlines in conjunction with a computer program which calculated areas of irregular figures. The values for PDe and AA were calculated using formulae derived by Fosse (1968a) and Fosse et al. (1985).

Because of the variations which have been shown to occur in prism parameters at varying levels within a tooth (Fosse et al., 1973; Boyde and Martin, 1982, 1984a, 1984b; Carlson and Krause, 1985; Grine et al., 1987), all our measurements were obtained wherever possible from the middle of the enamel layer and at the level indicated (Figs. 1a, b). SEM lenses were cleared before photography to eliminate magnification errors caused by magnetic hysteresis and instrument magnification was calibrated with a magnification standard.

Results

Extant Species

Prism packing pattern. In the areas examined and with the etching technique used, the majority of prisms appeared to have complete boundaries. Despite this, we compared the arrangements found (Fig. 1c) with those depicted by Boyde (1964, 1965) (Fig. 2) for incomplete horse-shoe shaped prisms (also see Discussion).

Three main variations or types of Pattern 2 prism packing were observed.

i. A Pattern 2 arrangement with parallel rows of prisms separated by well-defined inter-row sheets, the prisms close-packed within a row with relatively little inter-prism phase (cf. Pattern 2A, Boyde (1964) in Fig. 2), was found in all the extant macropodoids (Fig. 3) and perameloids examined and in *N. typhlops* and *V. ursinus*.

ii. A Pattern 2 arrangement with a readily identifiable inter-prism component (cf. Pattern 2, Boyde (1964) in Fig. 2) was found in *P. volans* (Fig. 4) and *P. vestitus*. In *P. volans*, the inter-prismatic leaves produce an arcade effect across the prism rows.

iii. A Pattern 2 arrangement with flattened ovoid prisms in transverse section and with maximal development of the inter-prismatic phase was found in the dasyurids (Figs. 5, 6).

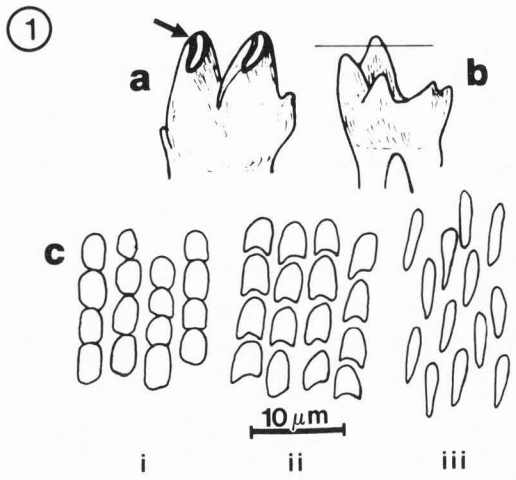

Fig. 1.

a, b: representation of two marsupial teeth showing the preferred areas for obtaining transverse sections for SEM.

c: represents tracings of the three main variations of Pattern 2 prism packing found in extant marsupial enamels. These tracings were made directly from micrographs. Type (i) is found in macropodoids, perameloids and *N. typhlops*, type (ii) in phalangers and petauroids, and type (iii) in dasyurids.

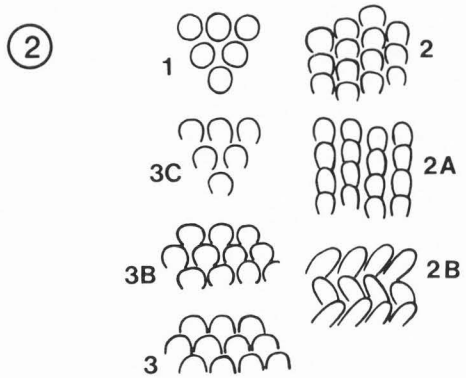

Fig. 2. Representation of the major enamel prism packing patterns depicted by Boyde (1964).

Individual rows of prisms are correspondingly less well defined.

These three variations are represented diagrammatically in Fig. 1c as tracings made directly from sample micrographs.

Two other significant packing pattern-related differences were observed. In *N. typhlops* and the perameloids, an extensive zone of poorly defined prisms merging with aprismatic enamel extends one-half to one-third of the thickness of the enamel surface beyond the clearly defined Pattern 2-packed prisms (Fig. 7). A Pattern 1 or a distinct tendency to Pattern 1 prism packing was found in the enamel of *D. pennateus* (Fig. 8) and *A. pygmaeus* and in the enamel of all teeth examined of *T. rostratus* except the lower incisor. The

teeth of *T. rostratus* are extremely small and the enamel correspondingly thin (Figs. 9, 10). We have previously reported the discontinuous but aprismatic enamel of the lower functional incisor of *T. rostratus* (Lester et al., 1988a).

Prism course. The extant marsupials exhibited a simple radial prism course in their enamel with the exception of: macropodoid incisors which have an abrupt change of prism orientation in the labial enamel of lower incisors (Fig. 11) (also see Beier, 1984) and a more gradual change in upper incisors (also see Gilkeson, 1986; Lester et al., 1987); and *V. ursinus*, which was found to have parazones and diazones in the enamel of all teeth examined (Fig. 12).

Tubules. A significant inter-species variation was found in the presence and size of enamel tubules (Table 2). Overall, tubules were found in the enamel of all extant species with the exception of *V. ursinus* and the anterior teeth of *T. rostratus*. Tubules were most numerous in the enamel of the macropodoids and of *I. macrourus* and least numerous in the molar enamel of *T. rostratus*. Tubule size varied from a minimal 0.2 - 0.5 µm diameter range in the dasyurids to a maximal 2.5 µm diameter in the outer enamel of *P. penicillata*.

In the macropodoids (*M. eugenii*, *M. parryi*), tubules (≤ 1.5 µm diam.) were clearly discernible and distributed throughout the molar enamel (Fig. 13). Tubule size was similar in the incisor enamel, but assessment of distribution was difficult. This is because the change in prism course produces a marked obliquity of the prisms and tubules in the outer layer and means that inner and outer areas are not readily comparable in our particular sections (Fig. 11). *P. penicillata* differed from the other macropodoids in that there was an increase in maximum tubule diameter from 1.5 µm in the inner enamel to 2.5 µm in the outer enamel - light microscopy sections proved this to be a gradual increase in tubule diameter towards the outer surface. Although the tubules in the enamel of *P.vestitus* and *P. volans* ranged up to 1.5 µm in diameter, there were many more very small tubules in these species than in the macropodoids and overall tubule density was much greater near the enamel-dentine junction than in the outer enamel. *V. ursinus* enamel was, as is known, atubular (for example, Tomes, 1849; Lester and Boyde, 1968).

All the dasyurids, as well as *A. pygmaeus* and *D. pennateus*, have very small tubules (≤0.5 µm in diam.) which made assessment of tubule distribution difficult (Fig. 14). There were no tubules in the mandibular or maxillary incisors or in the maxillary canine of *T. rostratus*, although a few vestigial tubules (≤ 0.5 µm diam.) penetrated a short distance from the enamel-dentine junction in the molars.

Inter-generic variation in enamel tubule size and distribution occurred in the bandicoots, *P. nasuta* and *I. macrourus*, with tubules in the former being both smaller and fewer. While most tubules in *I. macrourus* were ≤ 1.5 µm in diameter, some ranged up to 2.0 µm. Tubule density decreased in the outer half of the enamel where prisms were not well defined.

Enamel tubules in *N. typhlops* were relatively small (≤ 1.0 µm diam.) and distributed unevenly throughout the enamel, although generally being less evident in the outer half.

Fossil Species

Prism packing pattern. All the fossil teeth displayed Pattern 2 enamel, with Pattern 1 prism packing present only in the outer layer of the *W. wakefieldi* molar. The fossil enamels generally showed a greater variation both in prism size and in width of inter-prism than the extant enamels; nonetheless, the same three types of Pattern 2 prism packing could be identified (refer Fig. 1c).

i. The enamel of *E. ima* and *P. chinchillaensis* has well-defined inter-row sheets with little obvious inter-prism component (Fig. 15).

ii. The enamel of *D. optatum*, *N. tedfordi*, *E. serratus* and the notoryctid and peramelid fossils has strong inter-row sheets and definable inter-prism components (Fig. 16).

Figs. 3-28 are scanning electron micrographs unless otherwise indicated. In all micrographs of enamel, the outer enamel surface is to the top.

Figs. 3-14 are extant enamels.

Fig. 3. Transverse section of *M. eugenii* molar showing ovoid prisms (p), parallel inter-row sheets (s) and little inter-prism in a Pattern 2 type (i) packing. Several tubules (t) are visible. Bar = 10 µm.

Fig. 4. Transverse section of *P. volans* molar showing ovoid and horse-shoe shaped prisms (p) aligned in rows separated by inter-row sheet (s) and inter-prism (i). The prisms are separated by a more easily identifiable inter-prism component in this Pattern 2 type (ii) packing than in the macropodoids or the perameloids (cf. Fig. 3). Small tubules (t) are present. Bar = 10 µm.

Fig. 5. Transverse section of *S. harrisii* incisor with elongated prisms (p) fully surrounded by inter-row sheet (s) and inter-prism (i) in Pattern 2 type (iii) packing. Although in generalized Pattern 2 packing, the prisms are not well aligned in rows (t - tubules). Bar = 10 µm.

Fig. 6. Transverse section of *S. murina* with elongated prisms (p) separated by inter-prism (i) and inter-row sheets (s). Row formation is not clearly defined and the prisms have a higher axis ratio than those in *S. harrisii* (cf. Fig. 5). Tubules are present but are visible only at higher magnification (see Fig. 13) because of their small size. Bar = 10 µm.

Fig. 7. Transverse section of *N. typhlops* incisor showing the gradation from the enamel-dentine junction (j) and clearly prismatic zone (p) to the outer zone (o) in which prisms are not well demarcated. Bar = 10 µm.

Fig. 8. Transverse section of *D. pennateus* molar showing rounded prisms (p) surrounded by inter-prism (i) in a Pattern 1 packing arrangement. Organic casts of tubules (t) remain in relief after acid etching of the surface. Bar = 10 µm.

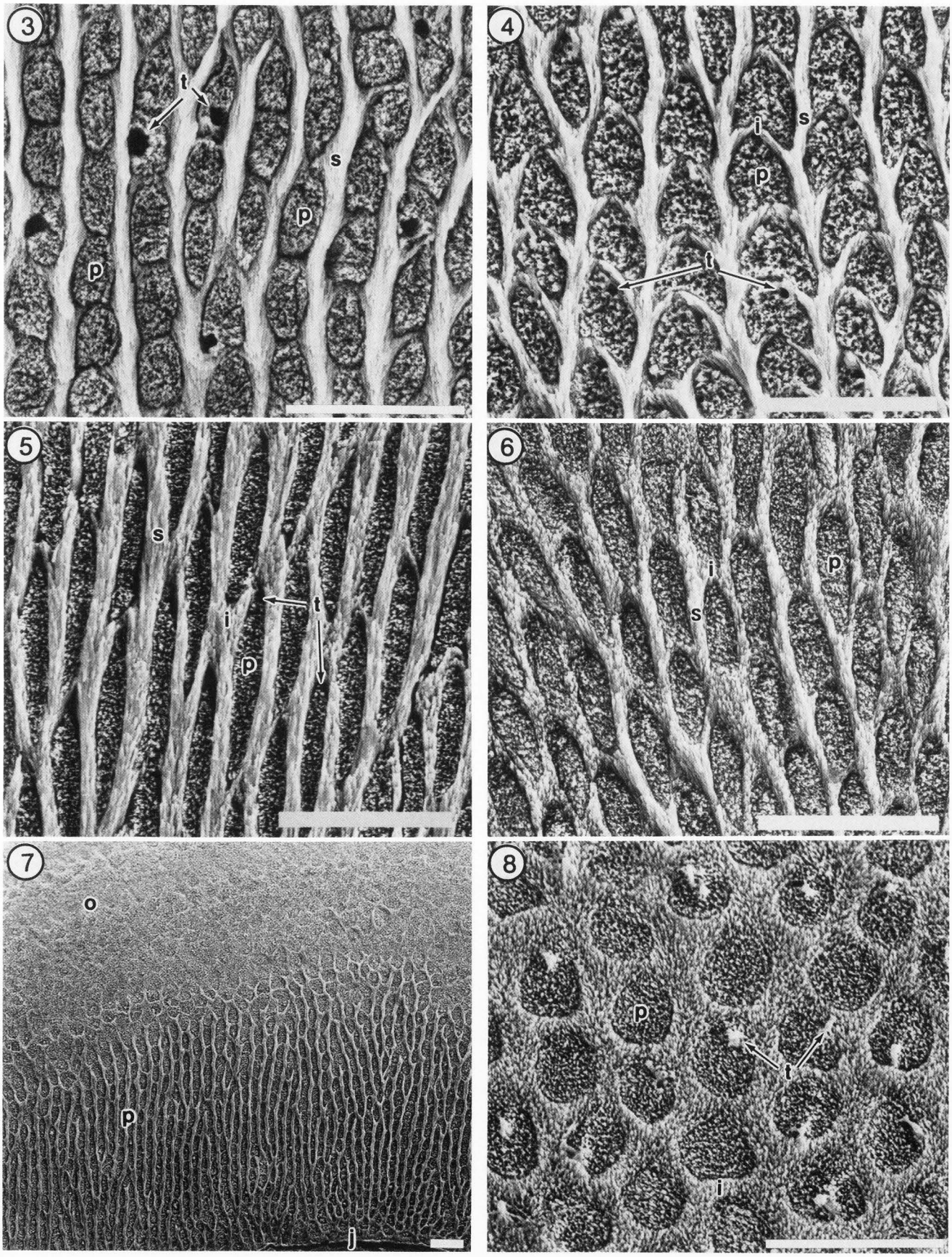
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TABLE 2

ABSENT -	ISOLATED +	MODERATE ++	ABUNDANT +++
*Diprotodon optatum** (incisor) *Ngapakaldia tedfordi** (incisor) *Tarsipes rostratus* (lower incisor) *Vombatus ursinus* *Warenja wakefieldi**	*Tarsipes rostratus* (molar)	*Acrobates pygmaeus* *Antechinus stuartii* *Dasyurus maculatus* *Dasyurus viverrinus* *Distoechurus pennateus* *Notoryctes typhlops* Unnamed notoryctid* Unnamed peramelid* *Perameles nasuta* *Petauroides volans* *Phalanger vestitus* *Sarcophilus harrisii* *Sminthopsis murina* *Thylacoleo carnifex** *Zygomaturus trilobus**	*Diprotodon optatum** (molar) *Ekaldadeta ima** *Ektopodon serratus** *Isoodon macrourus* *Macopus eugenii* *Macropus parryi* *Ngapakaldia tedfordi** (molar) *Protemnodon chinchillaensis** *Petrogale penicillata*

An assessment of the relative distribution of tubules in the species studied. Qualitative assessment of tubules was difficult in those species with very small tubules (the dasyurids, *A.pygmaeus*, *D.pennateus* and *T.rostratus*). The species are in alphabetical order in each column.

* Fossil species.

iii. *T. carnifex* enamel alone has maximal development of the inter-prismatic phase together with less clearly defined row formation (Fig. 17), a similar arrangement to the extant dasyurid enamels (cf. Figs. 5, 6).

The *W. wakefieldi* molar showed mostly Pattern 2 type (ii) enamel in the inner two-thirds with a middle layer of Pattern 2 type (i) beneath an outer Pattern 1 layer. For quantitation purposes, the prism parameters for *W. wakefieldi* were measured in the Pattern 2 type (i) region because the zonation in the Pattern 2 type (ii) region did not allow large enough areas of transversely sectioned prisms for analysis. *P. parvus* and *Z. trilobus* were not measured as we were unable to obtain transverse sections; however, the existence of an inter-row sheet and of parallel rows of prisms in longitudinal sections of *P. parvus* clearly indicated a Pattern 2 prism packing. The fragment of *Z. trilobus* enamel available also allowed confirmation of a Pattern 2 packing but was too small for further assessment of the type.

In the outer enamel of *P. chinchillaensis* (Fig. 18), the inter-row sheets increase in thickness relative to the prisms and an inter-prism component becomes obvious (cf. Fig. 15). Significantly, the notoryctid and peramelid fossils have an outer zone of poorly prismatic to aprismatic enamel comparable to *N. typhlops* and the extant peramelids.

Prism course. The majority of the fossil enamels exhibited a simple radial prism course. A gentle curve in an otherwise radial prism course occurs in the inner enamel of the *E. serratus* molar. Irregular parazones and diazones are present in the inner two-thirds of the incisor (Fig. 19) and premolar enamel of *T. carnifex*. More regular parazones and diazones extend through the width of the enamel up to the worn occlusal surface in the *W. wakefieldi* molar (Fig. 20).

Fig. 9. A longitudinal section of the crown of the maxillary canine of *T. rostratus*. The enamel thickness is a little over 100 µm and the prisms are Pattern 1 packed (d - dentine; e - enamel; j - enamel-dentine junction). Bar = 100 µm.

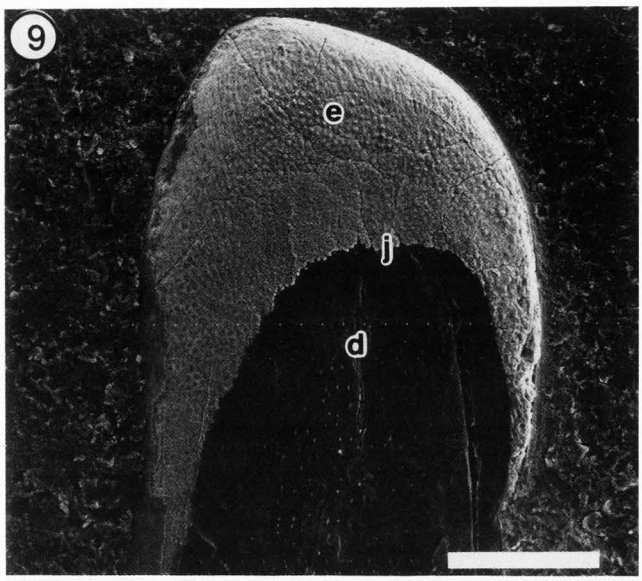
Fig. 10. A transverse section of a maxillary molar of *T. rostratus* showing the minimal thickness enamel (30 µm) (j - enamel-dentine junction; o - outer enamel surface; p - prism). Bar = 10 µm.

Fig. 11. Transverse section of *M. eugenii* lower incisor illustrating the acute change in prism course characteristic of the labial enamel of macropodoid lower incisors - the dentine (d) and enamel-dentine junction (j) are at bottom. Bar = 10 µm.

Fig. 12. Parazones (pz) and diazones (dz) in a longitudinal section of enamel of *V. ursinus* incisor. The zones extend from just beyond the enamel-dentine junction (j) almost to the outer surface (o). Bar = 10 µm.

Fig. 13. Transverse section of inner enamel of *M. eugenii* molar showing even distribution of tubules (t). All tubules are located at the border of prisms (p) with inter-row sheet (s) or inter-prism. Bar = 10 µm.

Fig. 14. Transverse section of *S. murina* molar showing natural organic casts of small tubules (t), the lower ones crossing the enamel-dentine junction (j) from the dentine (d). These tubules are extremely difficult to visualize in anorganic specimens. Bar = 1 µm.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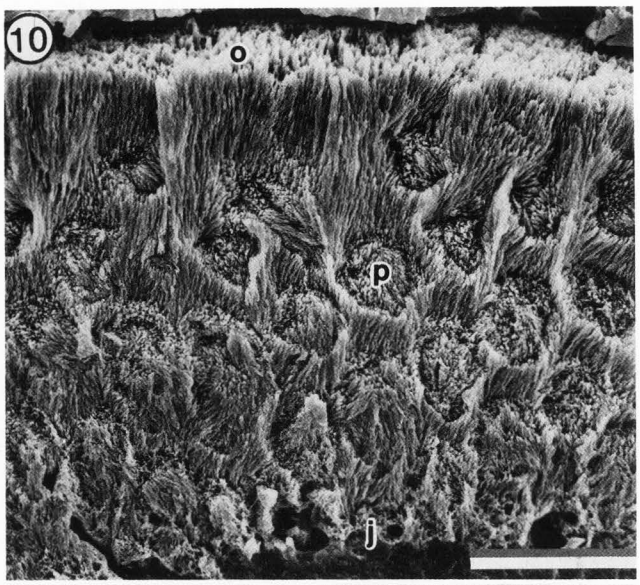
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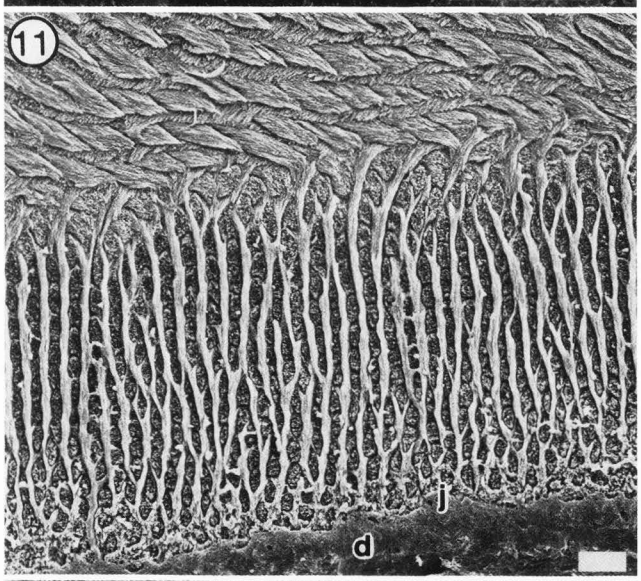
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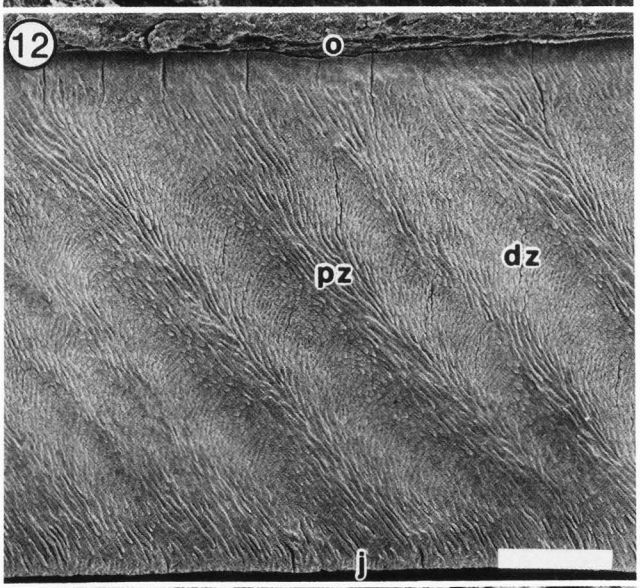
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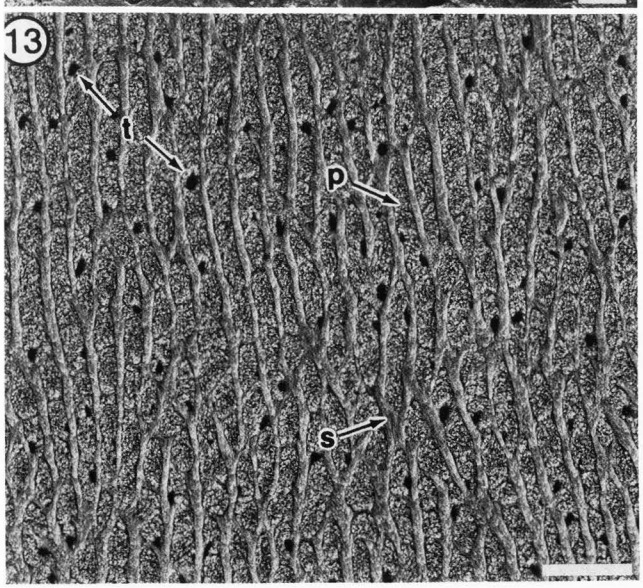
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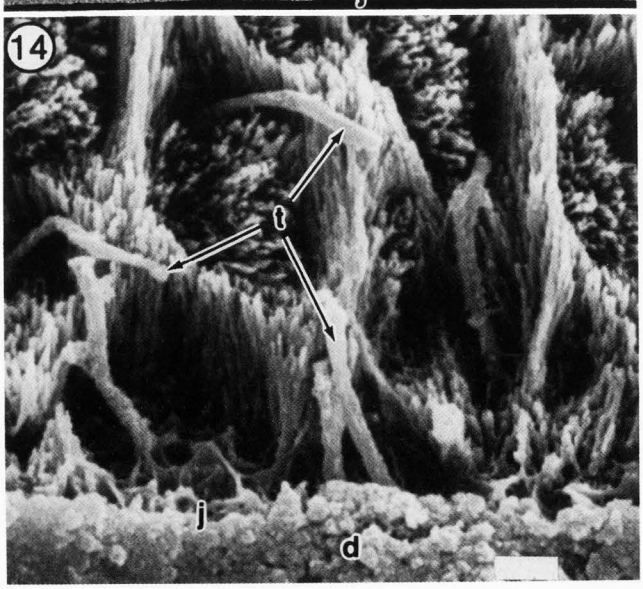
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Tubules. No enamel tubules were observed in the incisors of *D. optatum* and *N. tedfordi* or in the molar of *W. wakefieldi*. The molars of *P. chinchillaensis*, *E.ima*, the notoryctid fossil and the peramelid fossil, together with the very small fragment of *Z. trilobus* incisor available to us, showed small tubules only (≤ 1.5 µm diam.).

Large empty spaces of uncertain origin were initially observed in transverse sections of *D. optatum* (Fig. 21). Subsequent checking of longitudinal sections by light microscopy (Fig. 22) and SEM showed these spaces to be terminal expansions of tubules (Fig. 23) which could be clearly demonstrated as matching positives in resin-infiltrated material (Fig. 24). The majority of tubules were confined to the inner one-quarter to one-third of the enamel with no tubules in the outer third enamel. Large spaces were also observed in the molar enamel of *E. serratus* by SEM; by light microscopy, multiple bulbous expansions were found along the tubules (Fig. 25) rather than single expansions restricted merely to the tubule endings as in *D. optatum*. Although large tubule spaces were also observed in *N. tedfordi* premolar and *P. parvus* molar enamel by SEM, insufficient material was available to us to allow confirmation of the type. The *T. carnifex* premolar showed fine tubules following the course of the prisms, some with small bulbous terminations (Fig. 26).

Quantitative Analysis

The quantitative results are presented in Table 3: the major findings can be summarised as follows.

Prism diameters (PDx) range from 1.4 µm to 3.9 µm with the most marked differences being between the extant Pattern 2 type (iii) enamels ($\bar{x}$ = 1.6 µm, range 1.4 - 1.8 µm) and the extant Pattern 1 enamels ($\bar{x}$ = 3.5 µm, range 3.2 - 3.9 µm). Members of the Dasyuridae collectively possess the prisms with smallest diameter, with prisms of *S. harrisii* and *D. viverrinus* averaging 1.4 µm. All the Dasyuridae displayed Pattern 2 type (iii) prism packing. The largest prism diameters were found in *T. rostratus* (molar - 3.9 µm) and *A. pygmaeus* (3.6 µm) both of which have Pattern 1 prism packing. A further correlation here is that the Tarsipididae and the Acrobatidae have an axis ratio at or close to 1 proclaiming their round shape as opposed to the more common horse-shoes of Pattern 2 (*S. harrisii* has the smallest axis ratio and therefore the most compressed transverse section at a value of 0.15). Prism cross-sectional areas (PA) in the extant enamels are generally smaller (5.7 - 14.0 µm^2) than the prism areas of the fossil enamels (7.9 - 24.5 µm^2). The largest prism cross-sectional areas were found in the fossils *D. optatum* (24.5 µm^2) and *N. tedfordi* (21.0 µm^2). The smallest prism cross-sectional areas were found in the extant peramelid *I. macrourus* (5.7 µm^2). Similarly, the range for ameloblast secretory areas (AA) in the extant enamels (11.0 - 25.5 µm^2) were found to be less than that in the fossil enamels (17.1 - 41.8 µm^2). The large ameloblast secretory area of *T. carnifex* incisor (35.5 µm^2) compared to its prism area (12.5 µm^2) indicates the large inter-prism component present. While small variations in parameters exist between molars and incisors in extant enamels, a significant variation was found between the *D. optatum* molar and incisor: ameloblast and prism areas of the incisor were more than twice those of the molar. Prism densities (PDe) were found to range from 23,917 to 90,090 prisms/mm^2: the relatively high prism densities confirming the characteristically small size of marsupial enamel prisms (Boyde, 1969; Fosse et al., 1973) compared to those in human (ca. 28,000 - 33,000 prisms/mm^2 (Fosse, 1968b)) and in multituberculate enamel (1,866 - 51,534 prisms/mm^2 (Krause and Carlson, 1986)).

Discussion

We have been fortunate to be involved with the identification of fossil mammalian teeth found recently in Australia at the Riversleigh site by Archer and co-workers (Archer et al., 1988; Lester et al., 1988a). The first and essential question in such an exercise and one which continues to fascinate zoologists, is that of the possible placental versus non-placental origin of Australian fossil materials (Archer and Clayton, 1984). In this respect, there is emerging from the Riversleigh discovery increasingly clear evidence of a complex variety of previously unappreciated anatomical form amongst Australian fossil marsupials. The subsequent question then arises of proper taxonomic classification but, because of the lack of substantial evolutionary lineages, the

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Figs. 15-26 are of fossil enamel.

Fig. 15. Transverse section of *E. ima* molar. The inter-row sheet (s) is approximately the same width as the prisms (p) (Pattern 2, type (i)). Small tubules (t) are evenly distributed throughout the enamel. Bar = 10 µm.

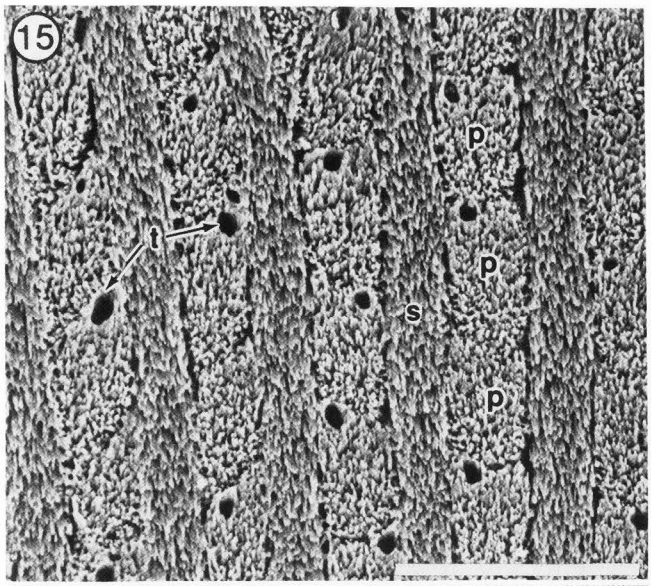
Fig. 16. Transverse section of *D. optatum* incisor showing ovoid or arcade-shaped prisms (p) separated by an easily definable inter-prism component (i) and inter-row sheet (s) in a Pattern 2 type (ii) packing arrangement. Bar = 10 µm.

Fig. 17. Transverse section of *T. carnifex* premolar. The prisms (p) are in a Pattern 2 type (ii) arrangement and are fully enclosed by inter-prism (i) and a wide inter-row sheet (s). Bar = 10 µm.

Fig. 18. Transverse section of the outer enamel of *P. chinchillaensis* molar showing a wide inter-row sheet (s) and some inter-prism (i). The middle layer of enamel, from which the measurements for Table 3 were obtained, exhibited both a narrower inter-row sheet and a less definable inter-prism (p - prism). Bar = 10 µm.

Fig. 19. Irregular parazones (pz) and diazones (dz) in the inner two-thirds from the enamel of a longitudinal section of *T. carnifex* premolar. Enamel-dentine junction (j). Bar = 10 µm.

Fig. 20. Longitudinal section of *W. wakefieldi* molar: the parazones (pz) and diazones (dz) extend through the full width of the enamel to the worn occlusal surface (o). Bar = 10 µm.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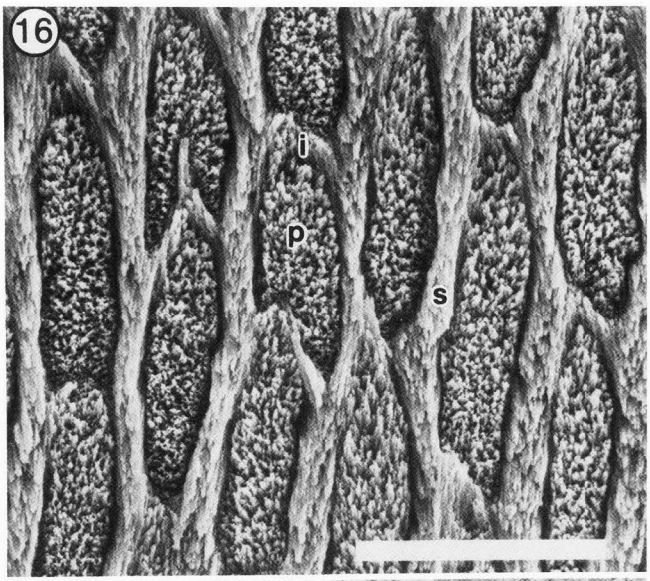
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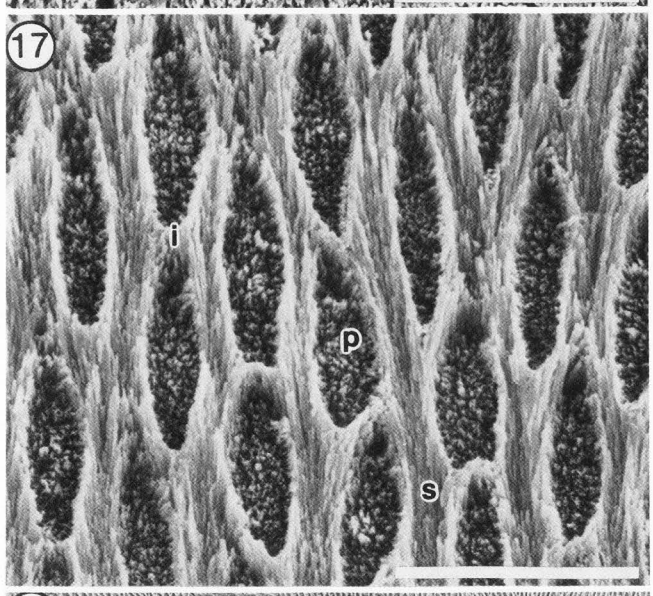
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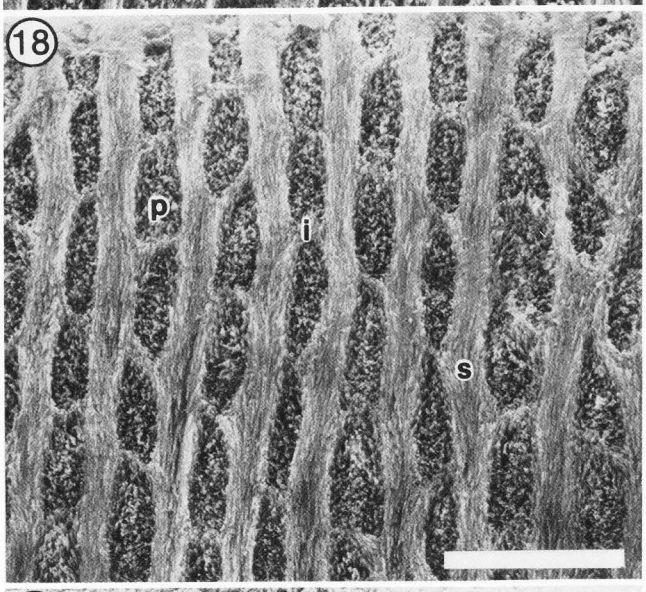
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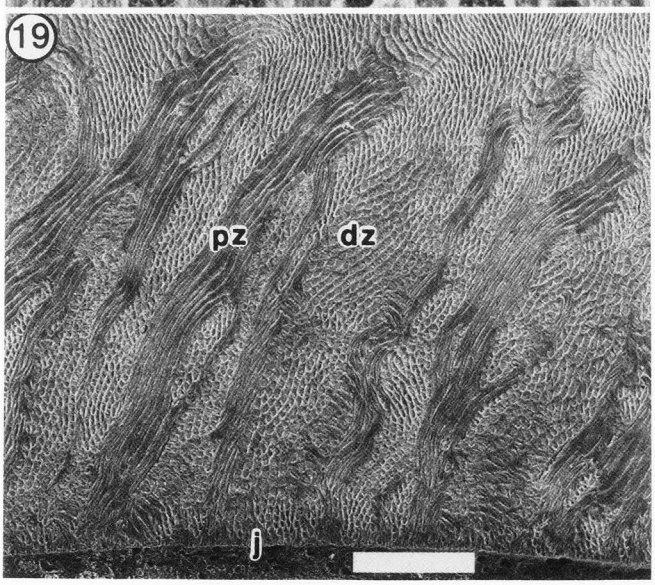
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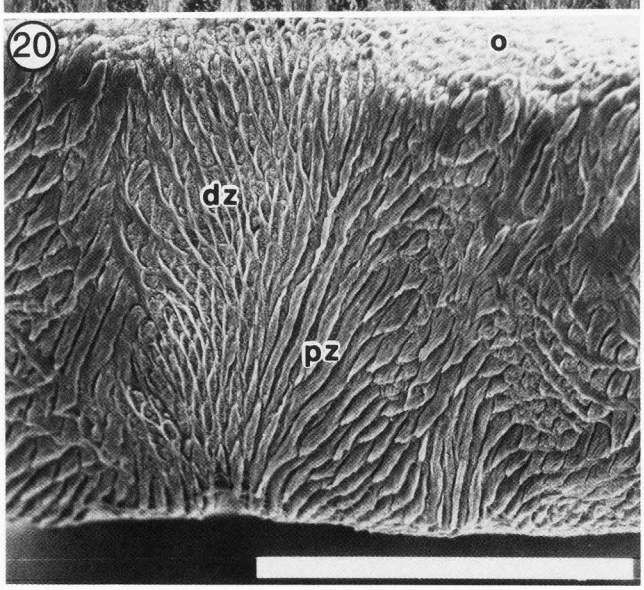
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phylogenetic relationships of Australian marsupials remain largely obscure (Aplin and Archer, 1987). As dental histologists, we are aware of a generalized lack of useful decriptions of marsupial enamel ultrastructure, and certainly of a lack of useful markers, apart from the well known and widely accepted combination of characters of tubules and Pattern 2 prism packing for all except the wombat (for example, Tomes, 1849; Boyde and Lester, 1967; Lester and Boyde, 1968; Ferreira et al., 1985; Lester et al., 1987).

The survey presented here of 17 extant and 11 fossil marsupials, covering 9 families in each, reveals a surprising diversity of enamel ultrastructure in all features examined: prism packing pattern; tubule course; zonation; prism shape and size; prism density; and tubule presence and size. We are properly wary of a simplistic allocation of descriptive features implying taxonomic significance (see Krause and Carlson, 1986, 1987; Grine et al., 1987) and aware too of the relative incompleteness of the present coverage of multiple representatives (where existing) of all marsupial families. Nevertheless, the present material represents the largest sample of Australian marsupials past and present yet examined in this way and we draw attention to a number of potentially useful findings.

Three different packing arrangements are described for Australian marsupials (Fig. 1c) within the classical Pattern 2 depicted by Boyde (1964, 1965 - Fig. 2). The majority of extant families represented in our material displayed type (i) (Fig. 3). Type (iii) was identified for only one extant family, the dasyurids (Fig. 5), and one fossil family, the thylacoleonids (Fig. 15).

Tomes (1849) first recognized inter-ordinal differences in prism arrangements; Shobusawa (1952) subsequently illustrated prism patterns by light microscopy, and these patterns were further defined by electron microscopy by Boyde (1964, 1965). Boyde described three subsidiary arrangements within Pattern 2: the Pattern 2 of primate enamel; the Pattern 2A of marsupials, ungulates and lagomorphs; and the Pattern 2B of hystricomorph rodents (Fig. 2). Our type (i) is most similar to Boyde's Pattern 2A and our type (ii) to his Pattern 2, there being no homologue for our type (iii). Beier (1984) described seven families of Australian marsupials which he suggested displayed one of two types of prism packing: a "primitive" type with inter-prismatic material surrounding the prisms (our type (ii)), and a more specialized type with prisms arranged in rows separated by strong inter-row sheets (Pattern 2 enamel as described by Boyde (1964) and our type (i)). It is well recognized that prism shape, completeness of outline and arrangement can alter through the thickness of enamel (Boyde and Martin, 1984a, 1984b, 1987; Martin and Boyde, 1984). The etching process, too, can exaggerate major boundary planes and influence perception of completeness of prism outline. Nevertheless, with definition and comparability of site, we remain firmly convinced that prism packing pattern remains a useful tool in recognition of an unknown enamel sample (for further discussion also see Lester et al., 1988b).

Attention is also drawn here to the loss of prism demarcation towards the outer enamel surface of marsupial teeth. As in most mammals (Boyde, 1964), the majority of the marsupials studied here had a thin layer of aprismatic enamel at the outer surface with the amount varying depending on tooth wear. In the extant and fossil notoryctids and perameloids however, an extensive zone including aprismatic enamel and poorly defined prisms extends one-third to one-half of the way from the occlusal surface. Boyde (1980) and Ishiyama (1987) found a similar zone of enamel in odontocete whales, and Ishyama (1987) attributed this to a "degenerating" prism structure. It is worth pointing out that techniques for producing tangential sections as recommended by Carlson and Krause (1985) in rare fossil materials would not yield useful information in these specimens because of the extent of this zone. The aprismatic enamel of the lower functional incisor of *T. rostratus* (also see Lester et al., 1988a) is only 15 µm thick and the entire dentition is in a secondarily reduced state.

The existence of a Pattern 1 arrangement of prisms in the enamel of *D. pennateus* and *A. pygmaeus*, and most teeth of *T. rostratus*, was relatively unexpected given the general acceptance of the notion of Marsupialia having Pattern 2 enamel. However, Pattern 1 packing has been observed in SEM images of the enamel of both *Didelphis nudicaudata* (Boyde - personal communication) and *Didelphis virginiana* (von Koenigswald - personal communication); and Kozawa (1984) has described round, complete prisms in some unspecified living marsupials. Interestingly, Aplin and Archer (1987) recently, as part of a proposed syncretic classification, placed these three species in one superfamily on the basis of morphological and immunological evidence.

Fig. 21. Large spaces (arrowed) in the transversely sectioned Pattern 2 enamel of the molar of *D. optatum*. These were found to be large, irregular bulbous terminations of enamel tubules. Bar = 10 µm.

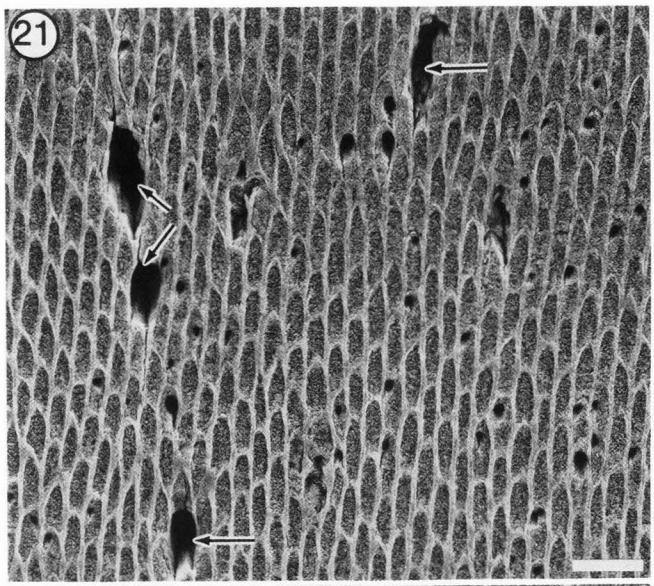
Fig. 22. Light micrograph of a longitudinal section of *D. optatum* enamel showing the bulbous terminations (arrowed) which occur about one-third of the way out from the enamel-dentine junction. Section thickness = 40 µm. Bar = 100 µm.

Fig. 23. A longitudinal section of *D. optatum* molar enamel showing at higher magnification a bulbous termination of a tubule (arrowed) (cf. Fig. 22). Bar = 10 µm.

Fig. 24. A resin cast of a tubule termination (arrowed) in a longitudinal section of *D. optatum* molar enamel (cf. Fig. 22). Bar = 10 µm.

Fig. 25. Light micrograph of a longitudinal section of *E. serratus* molar enamel shows bulbous expansions (arrowed) along the length of the tubules. Bar = 100 µm.

Fig. 26. Light micrograph of *T. carnifex* premolar enamel - the tubule course reflects the prism course. Some tubules have bulbous terminations (arrowed). Bar = 100 µm.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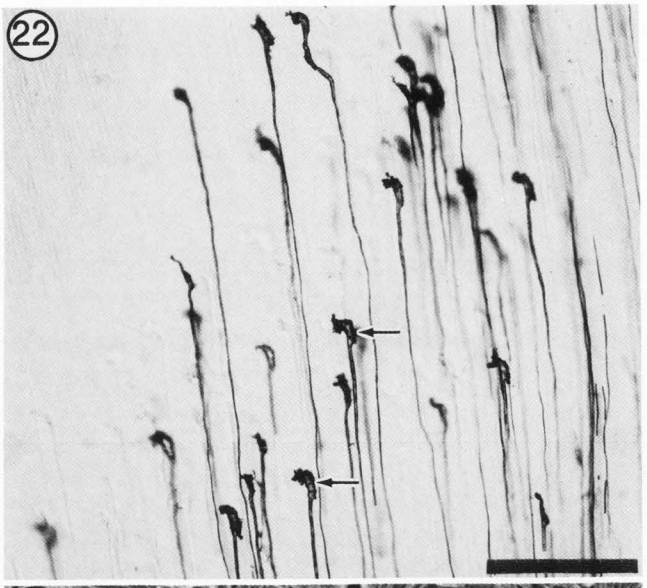
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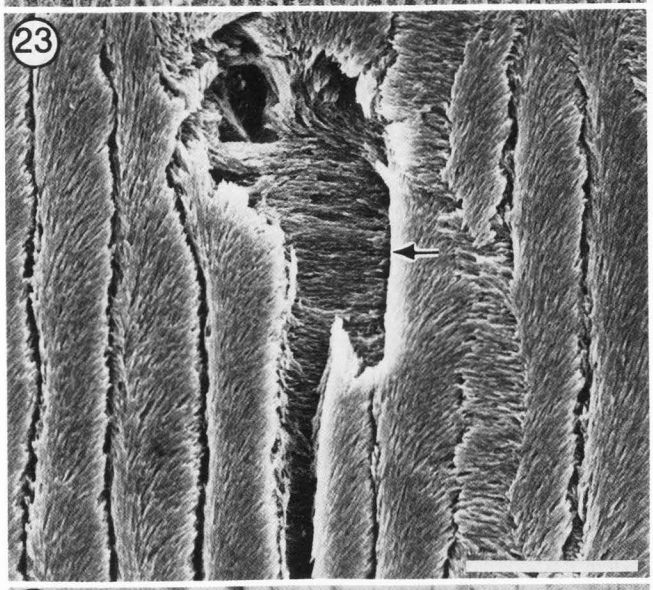
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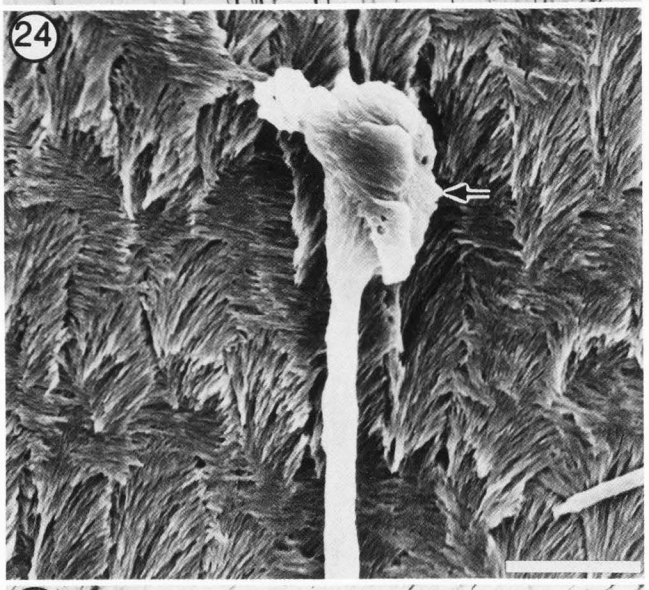
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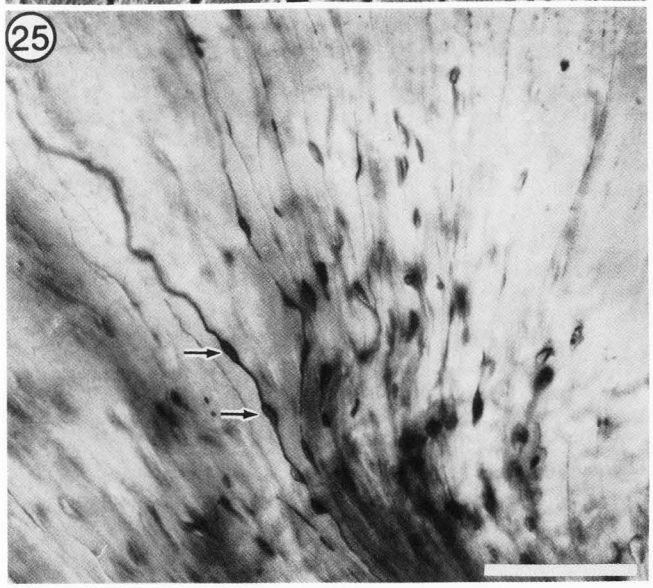
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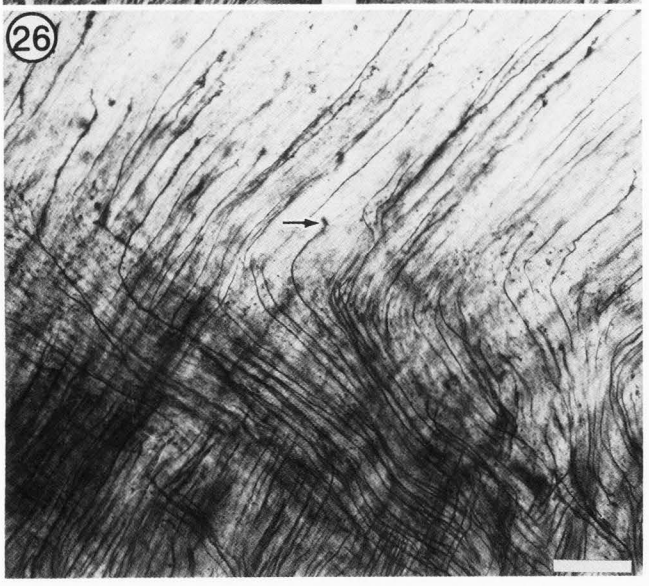
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TABLE 3

ORDER	FAMILY	SPECIES	TOOTH	PDx (μm)	SD	Dx/Dy	PA (μm^2)	AA (μm^2)	PDe
DIPROTODONTIA	Diprotodontidae	*D.optatum**	I_1	3.1	0.36	0.32	24.5	41.8	23,917
			M	2.2	0.28	0.39	11.6	20.3	49,226
	Palorchestidae	*N.tedfordi**	M	3.1	0.26	0.39	21.0	36.2	27,612
	Thylacoleonidae	*T.carnifex**	I^1	2.0	0.26	0.28	12.5	35.5	28,153
	Vombatidae	*V.ursinus*	M^1	1.8	0.19	0.33	8.6	15.8	63,206
		*W.wakefieldi**	M^1	3.5	0.38	0.80	13.1	21.2	47,100
	Phalangeridae	*P.vestitus*	M_3	2.6	0.33	0.40	14.0	25.5	39,151
	Ektopodontidae	*E.serratus**	M^5	1.5	0.30	0.25	7.9	18.4	54,179
	Potoroidae	*E.ima**	M	2.5	0.28	0.56	11.2	22.7	45,934
	Macropodidae	*M.eugenii*	I_1	2.2	0.22	0.63	6.9	11.2	89,163
			M_1	2.3	0.17	0.56	10.2	15.4	64,943
		M.parryi	I	2.1	0.37	0.49	7.2	12.0	83,204
		P.penicillata	I_1	2.1	0.48	0.65	7.2	13.1	76,275
			M_1	2.8	0.31	0.67	10.4	17.7	56,499
		*P.chinchillaensis**	M	2.5	0.25	0.51	10.7	17.1	58,321
	Petauridae	*P.volans*	M_2	2.7	0.46	0.60	11.4	19.1	52,221
	Tarsipedidae	*T.rostratus*	C^1	3.4	0.32	1.00	10.2	21.8	45,777
			M^1	3.9	0.32	0.93	13.4	28.3	35,442
	Acrobatidae	*A.pygmaeus*	M_1	3.6	0.25	1.08	10.6	21.9	45,714
		D.pennateus	M^4	3.2	0.29	1.03	10.2	22.1	45,317
DASYUROMORPHIA	Dasyuridae	*D.maculatus*	M_1	1.6	0.16	0.18	9.7	14.3	69,090
		D.viverrinus	I_1	1.5	0.21	0.21	8.7	21.3	46,783
			M_1	1.4	0.23	0.18	9.3	12.8	78,125
		S.murina	I_3	1.6	0.28	0.34	6.5	11.0	90,090
			M_2	1.7	0.23	0.35	6.2	13.1	76,099
		A.stuartii	I^1	1.6	0.28	0.29	6.9	18.8	53,205
			M^1	1.8	0.23	0.32	7.7	15.4	64,816
		S.harrisii	I_1	1.4	0.20	0.15	11.6	24.8	40,364
NOTORYCTEMORPHIA	Notoryctidae	*N.Typhlops*	I_1	2.1	0.29	0.43	9.1	18.3	54,734
		Unnamed notoryctid*	M	2.3	0.30	0.41	7.4	17.4	57,636
PERAMELEMORPHIA	Peramelidae	*P.nasuta*	I_1	1.7	0.25	0.39	6.9	15.9	62,907
			M^1	1.8	0.22	0.39	8.0	14.2	70,678
		I.macrourus	I	2.3	0.26	0.39	5.7	19.2	52,139
			M_1	1.8	0.28	0.49	5.9	12.5	80,190
		Unnamed peramelid*	M	2.3	0.20	0.44	9.1	20.2	49,423

Table 3 Quantitative parameters for marsupial enamel, extant and fossil*

PDx - Prism diameter in μm
Dx/Dy - Ratio between axes of cross-sectional prism profile
SD - Standard deviation
PA - Prism area in μm^2
AA - Ameloblast cross-sectional area in μm^2

Previously, *T. rostratus* had been regarded as the sole survivor of an early radiation and was originally placed in a superfamily of its own (Archer and Clayton, 1984; Strahan, 1983).

We report here significant differences in prism course and in the degree of zonation amongst the species studied. Beier (1981, 1984) reported the presence of "Hunter-Schreger" bands in extant

Dasyuridae, Petauridae, Vombatidae and Macropodidae, but our observations would suggest that, with the exception of *Vombatus*, these "bands" occur only in outer areas of gnarled enamel at cusps and fossae. The greatest degree of zonation occurs in *T. carnifex*, a fossil carnivorous marsupial, and draws an interesting parallel with the fossil sabre-toothed tiger (*Smilodon*) and the extant domestic cat (*Felis catus*), the enamel of both being characterized by a strong definition of parazones and diazones - marked Hunter-Schreger bands being a well known characteristic of carnivora.

In reporting the presence of tubules in the enamel of all recent marsupials except *Vombatus*, Tomes (1849) commented that in *T. rostratus* the tubules were "reduced to small limits". Lester et al. (1988a) have described the absence of enamel tubules in the incisors of *D. optatum, N. tedfordi* and *T. rostratus* and the relative lack of tubules in the incisor of the fossil *Y. coheni*. Molar enamel of *W. wakefieldi* is also atubular suggesting that the lack of tubules in the extant *Vombatus* is not a recent development. It is clear that the loss of tubules may affect some teeth of one species and not others. It is interesting to note also that *T. carnifex* displays both enamel tubules and "decussation" with the tubules clearly following the course of the prisms (Fig. 26) - Osborn (1974) has suggested that tubules do not occur where "decussation" is present citing *Vombatus* as an example. Tubules, their nature and formation, remain a fascinating subject. There have been many studies on marsupial enamels since Tomes (1849) which have concentrated mainly on the origin, nature and contents of enamel tubules (for example, Mummery, 1914; Moss and Applebaum, 1963; Boyde and Lester, 1967; Fosse, 1969; Lester, 1970; Fosse and Holmbakken, 1971; Osborn, 1974; Risnes and Fosse, 1974). Our observations here are focussed on the presence, size and type of tubules and, with the diversity found, suggest it is perhaps an over-simplification to think of marsupial enamel tubules as representing merely the retention of a primitive characteristic.

The large spaces either along or at the termination of enamel tubules in some teeth of *D. optatum, E. serratus, N. tedfordi, P. parvus* and *T. carnifex* are intriguing. Similar endings to enamel tubules were described in the lower incisor of the extant *Bettongia lesueur* by Mummery in 1914 and that description has perhaps been largely forgotten. The addition of expanded endings to tubules raises the question of their origin. It is tempting to see the terminal bulbs as representing the resting place of an ameloblast - the dimensions are certainly of the right order. This would then add to the list of mineralized tissues: bone; dentine; and cementum which retain (for however long, to whatever degree and in whatever state) a parent formative cell. It is not too difficult to imagine that the surrounding ameloblasts would re-assemble around the expired, incarcerated ameloblast and carry on with enamel production. Explanation of the bulbs along the length of tubules is more difficult but presumably a temporary increase in size could see a wider stream of cytoplasm left to appear ultimately as a dilatation of the tubule. The foregoing presumes of course that one accepts the origin of tubules from the cytoplasm of the formative ameloblast (Lester, 1970): in any event, the features certainly provide yet another distinguishing characteristic to assist in the identification of marsupial enamel.

Fosse et al. (1973) calculated prism densities for a number of extant marsupials and obtained a lower prism density ($\bar{x}$ = 37,825 prisms/mm^2 for outer enamel surfaces and $\bar{x}$ = 42,022 prisms/mm^2 for inner enamel surfaces) than we have for our extant specimens ($\bar{x}$ = 61,445 prisms/mm^2). This observed difference may reflect the fact that all our measurements for extant species were taken from the middle enamel layer, not the outer or inner enamel as for their study. No comparable results have been published on Australian fossil marsupials, however, quantitative data obtained on multituberculates by Fosse et al. (1973, 1978, 1985), Sahni (1979), Carlson and Krause (1985) and Krause and Carlson (1986) indicate a much greater variation in prism densities (for example, 1,866 - 51,534 prisms/mm^2, $\bar{x}$ = 11,097 prisms/mm^2, Krause and Carlson, 1986)) than we obtained for the fossil marsupials (23,917 - 58,321 prisms/mm^2, $\bar{x}$ = 44,150 prisms/mm^2). This large variation in prism density in the multituberculates reflects the existence of two distinct populations within that group: those with gigantoprismatic enamel and those with normal prism size.

We have tried to provide here the beginnings of a series of templates by which any Australian marsupial enamel might be assessed and compared with other enamels. Although tempted, we have not jumped to conclusions of phylogenetic significance, especially as we are acutely aware that with many of the fossils our samples were either fragmented or from areas not easily compared or identified. As more material becomes available, we hope to expand our information base and see these data as a useful starting point. In general terms, we have found: a greater variation in prism packing patterns than anticipated; a greater difference in characteristics studied between incisor and molar teeth than anticipated; and a greater variety of tubule morphology than anticipated. There are encouraging signs that useful characters are emerging to help ultimately with taxonomic investigations of Australian extant and fossil marsupials utilizing enamel ultrastructure as visualized by SEM.

Acknowledgements

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Discussion with Reviewers

G. Fosse: If the bulbous endings (cavities) of some enamel tubules represent sites where single ameloblasts have died before the conclusion of amelogenesis, why have they not been seen in atubular enamels?

Authors: Our observations on Diprotodon and Ektopodon and the observations by Mummery (1914) on *Bettongia lesueur* certainly indicate rounded spaces at the terminations of some of the tubules within the enamel. Why this phenomenon should occur in these three species and not in other tubular or atubular enamels we do not know. Certainly, in Diprotodon the tubules are large and, if our theory of tubule formation is correct (Lester, 1970), one could easily imagine the ameloblast cell body tending to be somewhat restricted or anchored in its normal centrifugal path by the long embedded cytoplasmic process of the ameloblast.

M. Ishiyama: This paper deals with a variety of enamel structure in both living and fossil marsupials. Fig. 7 is very interesting to me: does the marked aprismatic region appear commonly in the incisor enamel of *N. typhlops*?

Authors: We have examined two incisors and one molar of *Notoryctes typhlops*: all of these teeth have exhibited a marked loss of prism demarcation in the outer enamel.

L. Moss-Salentijn: Is it your opinion that the tubules form in much the same way as do dentinal tubules: i.e., some of the secretory ameloblasts leave behind a cell process in the newly deposited enamel? This would allow the interpretation of the terminal bulbs as representing the (final) resting place of ameloblasts. I continue to have some difficulty with this interpretation and wonder if the authors would care to comment (further) on the possibility that these structures represent "outsized" spindles. Enamel spindles in eutherian enamel, formed most likely by odontoblastic processes, often possess bulbous terminals and they occur with higher frequency in molar cuspal enamel than in incisor enamel. If enamel tubules and enamel spindles were to be related, the latter might be considered as "vestigal" tubules.

Authors: The answer to the first part of the question is yes: we still believe that enamel tubules result from the extension of ameloblast cytoplasm within the forming enamel in much the same way as an odontoblast process becomes embedded in the forming dentine (Lester, 1970).

As to the second part of the question, we are also a little puzzled by the differences between spindles and tubules given that many authors describe both structures from different teeth of different species examined by different means. Strictly speaking, the difference rests with the degree of dentinal contribution to the structure (see Boyde and Lester, 1967 for discussion). In the absence, however, of high quality electron microscopic data on the dentinal component of spindles, similar to that which we have provided for tubules, the question remains largely unanswered. A careful correlative TEM and SEM study for a particular site in a particular tooth of a particular species known to exhibit both would go a long way to resolving the question.