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Redescription of teeth and epithelial plates from the platypus (*Ornithorhynchus anatinus*): morphological and evolutionary implications

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Redescription of teeth and epithelial plates from the platypus (*Ornithorhynchus anatinus*): morphological and evolutionary implications

by

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Thesis

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Dedication

To my mother and grandfather.

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Abstract

Redescription of the teeth and epithelial plates from the platypus (*Ornithorhynchus anatinus)*: morphological and evolutionary implications

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The evolutionary history of mammals, when including extinct taxa, is mainly reconstructed using tooth morphology and employs terminology based on non-monotreme mammals. Although adult monotremes are edentulous, juvenile platypuses have teeth that can be compared with extinct monotremes, but terminology can be a barrier to efficient comparison to non-monotreme mammals. Deciduous teeth and thickened epithelial plates of the extant platypus, *Ornithorhynchus anatinus*, are sparsely figured in the literature. New imagery of those teeth and plates from high-resolution x-ray computed tomography and scanning electron microscopy contribute to the understanding of mammal evolution and the unique morphology of platypus teeth. The teeth of the juveniles are highly variable, but early-forming features (major cusps and transverse valleys) are stable enough for comparison. Transverse lophs on monotreme teeth contain complexity not reflected in cusps alone, unlike therian mammals. These differences reinforce the need for caution when applying dental terminology that originally was produced for therian mammals. New imagery highlights potential phylogenetically informative morphology in the pulp cavity and roots.

As the roots of the juvenile teeth degenerate, the epithelium below the teeth thickens into epithelial plates. Structures in the epithelial plates are broadly similar to those found in the keratin plates. New images of the epithelial plates offer insight into a series of tubes concentrated under the juvenile teeth. The tubes are a continuous conduit to the plate surface and may serve a sensory function or result from the ever-growing nature of the epithelial plate.

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INTRODUCTION

Extant mammals are split in two sister groups, the Monotremata and Theria. All extant marsupial and placental mammals are therians, but, there are relatively few monotremes. The only two extant clades within Monotremata are Tachyglossidae, including echidnas, and Ornithorhynchidae, including the platypus. There are four species of extant echidna. The short-beaked echidna, *Tachyglossus aculeatus*, is endemic to Australia, and the three species of long-beaked echidna (*Zaglossus bruijnii*, *Zaglossus attenboroughi*, and *Zaglossus bartoni)* are found only in New Guinea (Flannery et al. 1995, Musser 2003, Baillie et al. 2009). In contrast to the echidna clade, there is only a single platypus species, *Ornithorhynchus anatinus* Shaw 1799*,* limited to mainland Australia and Tasmania. All extant adult monotremes are edentulous, but they develop oral keratin which performs some of the functions of teeth (Griffiths et al. 1991, Grant 2007). Echidnas entirely lack erupting enameled teeth, but juvenile *Ornithorhynchus anatinus* have long been known to possess them (Poulton 1888a). Juvenile platypuses are the only living monotremes with teeth, and thus connect extinct monotremes represented by fossilized teeth with molecular data from living mammals. Platypuses are therefore important to phylogenetic analyses of mammal relationships that use either type of data.

Teeth and jaws are the most commonly identified fossilized parts from mammals - frequently to the exception of other skeletal elements. Tooth and jaw characters are numerous in datasets for morphological phylogenetic analysis including extinct mammals, and often are the only characters scored for some extinct taxa (Luo et al. 2001, 2005, Rowe et al. 2008). Therefore, the functional unit of jaw mastication often is the only available comparative entity for extinct mammals.

The current fossil record of monotremes is not immune to the preservation bias in therian mammals because most identified fossil remains of monotremes are teeth and jaws. Monotremes lack many tooth features commonly used when comparing therian mammals. Nevertheless, monotreme dental morphology is sometimes described in datasets using characters written specifically for teeth from therian mammals. Although researchers struggled to find an appropriate dental terminology for platypuses and other monotremes, and often found therian dental terms lacking (Simpson 1929, Green 1937, Archer et al. 1985, Archer et al. 1993, Woodburne et al. 2003, etc.), there is no consensus on appropriate terminology for monotremes.

The teeth of platypus have long been a challenge for researchers, and come with their own set of difficulties including, but not limited to, availability of specimens, terminology, homology, and evolutionary derivation. Additionally, these teeth are deciduous and fall out early in the life of the platypus (Poulton 1888a) so characters related to tooth wear also are not applicable. Much research has concentrated on dental formation in the platypus, looking for hints to a more complete dentition. The tone of frustration with this line of research is evident in the literature - “The determination of a complete dental formula for *Ornithorhynchus* is impossible (Simpson 1929:2).”

New fossil discoveries and techniques such as x-ray computed tomography and scanning electron microscopy can be applied to the study of evolutionary dynamics of monotremes. Better imagery resulting from these types of analyses makes the limited dentition of extant platypus increasingly accessible. This new look at the formation and transition from teeth to keratin plates provides clues to the evolution and derivation of this important early branch of mammals. I present relevant data elucidating aspects of the evolutionary transition from adult functional teeth in fossils of monotremes to the ephemeral teeth of the extant juvenile platypus and keratin plates of the adults. I use imagery to clarify further the problematic tooth anatomy, and I propose an appropriate terminology.

Fossils and biogeography

It was not until 1971, nearly 100 years after the discovery of juvenile teeth in the platypus (Poulton 1888a), that fossil remains of monotremes were first unearthed and identified (Woodburne et al. 1975). The isolated teeth from the Late Oligocene (Woodburne et al. 1994) were dubbed *Obdurodon insignis*, and identified as monotreme based on similarities with teeth of the extant platypus (Woodburne et al. 1975). Later, an older opalized dentary identified as belonging to a new Early Cretaceous taxon, *Steropodon galmani*, was uncovered at Lightning Ridge and assigned to Monotremata based on similarities with the teeth of *Obdurodon insignis* (Archer et al. 1985). At that point, *Obdurodon insignis* was known only from teeth, but soon *Obdurodon dicksoni* confirmed the diagnosis with a nearly complete skull bearing close resemblance to the extant *Ornithorhynchus anatinus* (Archer et al. 1992). Additionally, the holotype of *Obdurodon dicksoni* was found in close proximity to isolated teeth strikingly similar to those from *Obdurodon insignis* (Archer et al. 1992). Then, for the first time a fossil was found outside of the previous Australian range. *Monotrematum sudamericum*, from Argentina, is represented by isolated teeth (Pascual et al. 1992) and the distal end of a femur (Forasiepi et al. 2003). *Teinolophos trusleri*, from the Early Cretaceous, is represented entirely by jaws and teeth and although originally thought to be a placental mammal (Rich et al. 1997, 1999), it is now hypothesized to be another early monotreme (Rich et al. 2001). Most recently, *Obdurodon tharalkooschild* was named from a single m1 found near *Obdurodon dicksoni*, also of Middle Miocene age (Pian et al. 2013). The tooth from *Obdurdon tharalkooschild* is larger and has simpler cusp morphology than teeth of *Obdurodon dicksoni* and *Obdurdon insignis* (Pian et al. 2013). Another taxon of questionable affinity with monotremes is *Kollikodon ritchiei* with bizarre bunodont lower (Flannery et al. 1995), and upper (Kilean-Jaworowska et al. 2004) jaws. To date, all monotremes have been found in the Southern Hemisphere. The generally poor fossil record of toothed monotremes (*Obdurodon dicksoni*, *Obdurodon insignis*, *Obdurodon tharalkooschild, Steropodon galmani*, *Monotrematum sudamericanum,* and *Teinolophos trusleri)* supports a past Gondwanan distribution (Pascual et al. 1992).

Murky Molecular Dates and Troublesome Fossils within Monotremata

The fossil record and molecular data are two important sources of information for the current understanding of evolution of monotremes. Nevertheless, those relationships remain unclear in part due to a sparse fossil record, a current lack of molecular calibration points, inconsistent molecular dates, paucity of specimens for study, and few living species to substantiate characters within Monotremata. However, substantial recent work increased understanding of this radiation.

Before the introduction of cladistics, platypus, the “taxonomist’s nightmare” (Macintyre 1967:835), was shuffled around various Mammalian and therapsid groups (Gill 1872, Poulton 1888a, MacIntyre 1967). Evidence against the homology of anatomical features used to diagnose monotremes, and the advent of cladistics both retired prior mammalian group names from use (Rowe 1988, Wible 1991), but the most recent classification hypotheses for the basal split in mammals (Monotremata + Theria vs. Marsupionta + Placentalia) merits further discussion.

The Marsupionta hypothesis, articulated by Gregory (1947), proposed that monotremes evolved from a lineage of Australasian marsupials. Under that hypothesis, marsupials and monotremes are more closely related to each other than either is to placental mammals (Figure 0.1 A). Marsupionta was supported by morphological (Kühne

1973) and, more recently, molecular data (Penny et al. 1997, Killian et al. 2001, Janke et al. 2002), that have since been disputed (Luckett et al. 1989, Kullberg et al. 2008).

The majority view holds that Monotremata is the sister taxon to the rest of extant mammals (grouped within Theria; Figure 0.1 B). These clades are supported by fossil and genetic data (Marshall 1979, Rowe 1988, Rougier et al. 1996, Van Rheede et al. 2006, Rowe et al. 2008, Warren et al. 2008, Luo et al. 2001). The genomic data variously place the split of Monotremata from the rest of Mammalia at around 116 Ma (Van Rheede et al. 2006), 125 Ma (Kullberg et al. 2008), or 186 Ma (Phillips et al. 2009) (Figure 0.2). Other authors also support a Late Triassic-Early Jurassic split (McKenna et al. 1997, Messer et al. 1998, Woodburne 2003).

Understandably, molecular data alone cannot reconstruct relationships among long-extinct organisms; therefore, taxa represented exclusively by fossils are, of necessity, excluded from molecular analyses. However, the minimum divergence ages of clades are based on the age and proposed taxonomic affinities of fossils. Recent molecular divergence estimates between Monotremata and Theria are consistent to a degree; however dates of divergence between platypus and echidna are not.

Molecular dates for the divergence of Tachyglossidae and Ornithorhynchidae, the two extant monotreme clades, range widely from 17 Ma to 180 Ma (Rowe et al. 2008). Oddly, the divergence estimates rely on the affinity of the fossil *Teinolophos*, only known from teeth and dentaries containing a relatively large mandibular canal. Evidence of that canal morphology in *Teinolophos*, *Ornithorhynchus anatinus*, *Obdurodon dicksoni*, and *Steropodon galmani* was used to suggest a closer relationship between *Teinolophos* and platypuses than with echidnas (Rowe et al. 2008). That resulted in the oldest date of divergence between the extant clades (Rowe et al. 2008). That date met with some controversy among molecular phylogeneticists whose dates converged towards a more recent divergence of Tachyglossidae and Ornithorhynchidae (Phillips et al. 2009). Support for shorter dates comes from two arguments: the proposed overweighting of the enlarged mandibular canal, and extreme changes in the rate of evolution from stem to crown monotremes associated with older divergence dates (Phillips et al. 2009). When some mandibular canal characters are removed from the analysis, the divergence date estimate for crown monotremes contracts from 112.5 Ma (Cretaceous, Aptian, *Teinolophos trusleri*) to 32 Ma (Miocene, *Obdurodon dicksoni*; Phillips et al. 2009). With a divergence age as postulated by Rowe et al. (2008), rates of molecular evolution in stem monotremes would be among the highest in any land vertebrate, but evolution in the crown clade would be unusually slow (Phillips et al. 2009). The rate shift between stem and subsequent crown clades would be abnormally disparate (Phillips et al. 2009). The debate is unresolved (Camens 2010, Phillips et al. 2010).

New techniques and information may help clarify the date. For example calibration of molecular rates using the genetic divergence between platypus populations on mainland Australia and Tasmania (Furlan et al. 2010) may provide a better proxy of evolutionary rates. The More characters concerning the degree of homology behind the absence of teeth in adult monotremes could come from genetic analyses of tooth reduction and signaling pathways (Jernvall et al. 2012). The mechanism controlling tooth loss would show the degree of homology in the absence of teeth in adult monotremes, which would help determine if tooth loss occurred independently in the two clades. Finally, better comparison of existing characters in monotreme dentition and jaws with therian mammals, potentially aided by new fossils, would elucidate character-based analysis.

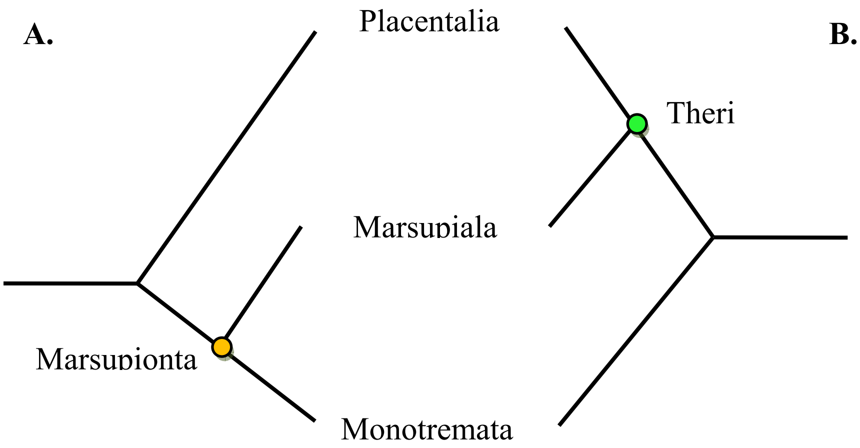

Figure 0.1. Two trees showing a simplified schematic diagram of competing hypothesis of monotreme phylogeny within Mammalia. A. The Marsupionta hypothesis places marsupials and monotremes as sister to Placentalia. B. The current, popular view sets monotremes apart as sister to Theria.

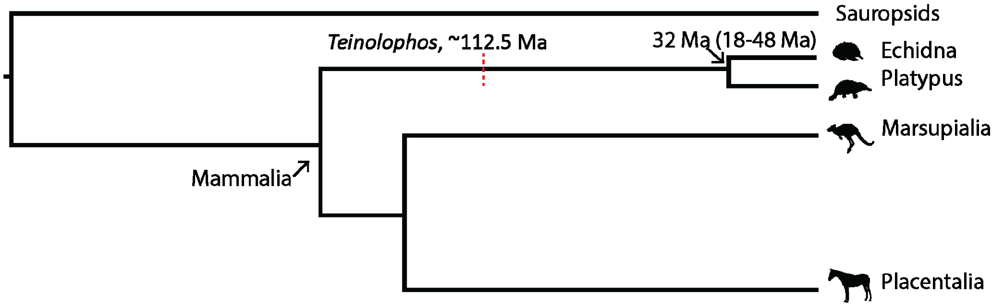

Figure 0.2. Divergence date estimates of Monotremes (shaded box) showing a recent divergence of platypus and echidna. Divergence of platypus and echidna from Phillips et al. (2009) is indicated by the black arrow. Divergence time from Rowe et al (2008) at the date of the fossil *Teinolophos* is indicated at the dotted red line. Modified from Phillips et al. (2009).

CHAPTER 1: DESCRIPTION OF THE TEETH OF *ORNITHORHYNCHUS*

INTRODUCTION

Teeth often are the only identified remains of extinct mammal species in the fossil record. Thus, an understanding of extant mammal teeth is necessary to compare extinct lineages with extant taxa. Teeth of the juvenile platypus (*Ornithorhynchus anatinus*) are the only material available for comparison with the teeth of extinct monotremes because no extant adult monotremes have mineralized teeth. In most mammals, dentitions found in juveniles are different from, and have a greater range of variation than, adult teeth (Hillson 2005, Ungar 2010), so juvenile platypus teeth must be interpreted with care. Nevertheless, the information offered by juvenile platypus teeth is important for understanding the transitions in form and relationships between extinct monotremes and the extant juvenile platypus.

I utilize High Resolution x-ray Computed Tomography (HRCT) and Scanning Electron Microscopy (SEM) to evaluate the morphology and formation of platypus teeth. I use CT imagery to link prior histological lamina descriptions and tooth development phases with erupted teeth. I also describe the teeth with respect to formation, eruption, wear, loss, roots, pulp cavity, replacement, and variation. Additionally, I discuss the support for classifying these teeth by tooth family (incisor, canine, premolar, or molar), applying descriptive terminology, and appropriate character homology.

Discovery of Platypus Teeth and Past Research

The mineralized teeth of *Ornithorhynchus anatinus* were first discovered by Poulton (1888b), as indicated by Latter (1889). The tooth morphology was recognized as reduced from a hypothetical tooth-bearing mammalian ancestor (Poulton 1888a), but thought to be too variable for determination of cusp homology with other mammals (Poulton 1888b, Simpson 1929). A statement by Thomas in 1889(:128-129, footnote) highlights the reduced nature and variation of platypus teeth, “A considerable amount of individual variation is to be expected in the case of organs in such a state of decadence as are the teeth of *Ornithorhynchus*.” Subsequent research on the teeth of juvenile platypuses was complicated by their variable morphology, which resulted in unclear homology of features and many researchers to apply different terminology to the teeth they examined (Poulton 1888, Simpson 1929, Archer et al. 1992). This confusion is due in part to variability and rarity of specimens, but also, to difficulties applying terminology developed for therian mammals. However, morphology of juvenile platypus teeth is adequate to identify monotremes in the fossil record (Woodburne et al. 1975); therefore, there are useful shared features at least between monotremes.

Dental studies of fuvenile platypus have taken two forms: dental lamina formation from histological sections and descriptions of erupted teeth (Table 1.1). There is a general consensus that three teeth erupt above and below (Thomas 1889, Stewart 1891, Simpson 1929, Green 1937, Woodburne et al. 2003), but the dental formulae from teeth forming along the dental lamina is less consistent (Table 1.1; Latter 1889, Wilson et al. 1907, Poulton 1888b, Broom 1935, Green 1937). The most complete examination of tooth formation was by Green (1937). In that study, sequential histological sections through the jaws of eleven juvenile platypuses (Table 1.2) show incipient teeth in association with an epithelial band, the dental lamina, from which the teeth grow. Morphology of unerupted teeth is fairly consistent (Green 1937) but crown features of erupted teeth are not (Simpson 1929).

Table of Dental Formulae

First description, damaged specimens.						Total
1888	Poulton					4/4
Formulae: Erupted Teeth						Total
1889	Thomas					3/3
1891	Stewart					3/3
1929	Simpson			P ?	M 2/2	3/3
1937	Green			P 1/0	M 2/3	3/3
This study	Latimer			P 1/0	M 2/3	3/3
Formulae: Unerupted Teeth, Dental Lamina						Total
1907	Wilson et al.			P 2/2	M 3/3	5/5
1935	Broom	I 0/2	C 0/1	P 4/4	M 2/2	6/9
1937	Green	I 0/5	C 1/1	P 2/2	M 3/3	6/11

Table 1.1: Table of juvenile platypus tooth dental formulae and tooth family. Designations were derived from unerupted and erupted teeth from this and other studies, cited above. Abbreviations: I - incisor, C - canine, P - premolar, M –molar. Upper tooth number is above the slash, lower tooth number below.

Specimens Numbers for Juvenile Platypus (Green 1937)

This study	**Green 1937**	**SL**	**DCL**
1W	1-Platypus W (Wilson).	16.5 mm	28mm
2W	2-Platypus WW (Hill).	16.5 mm	28mm
3X	3-Platypus X (Hill).		56 mm
4D	4-Platypus Delta (Wilson).		80 mm
5XXVII	5-Platypus XXVIII B (Wilson).		122 mm
6HN	6-Platypus H.N. (Harrison).		140 mm
7HJ	7-Platypus H.J. (Harrison).		170 mm
8HP	8-Platypus H.P. (Harrison).		200 mm
9HQ	9-Platypus H.Q. (Harrison).		225 mm
10B	10-Platypus Beta (Wilson).		250 mm
11HX	11-Platypus H.X. (Harrison).		295 mm

Table 1.2: Specimen numbers of platypus teeth in the dental lamina study by Green (1937). Numbers are simplified and shown with relative lengths of specimens. Abbreviations: SL – straight length, DCL – dorsal contour length.

Tooth Family

Platypus teeth were described originally as part of the molar series of "higher mammals (Poulton 1888:354)." Subsequent early researchers avoided designating a tooth family for juvenile platypus teeth (Simpson 1929). Since then several tooth family assignations have been proposed (Table 1.1), most notably by Green (1937). Differentiated and distinct molars and premolars (cheek teeth) are a diagnostic characteristic of Mammalia (Kielan-Jaworowska et al. 2004), and identification of tooth family is required to compare fossils of mammalian teeth. Cheek teeth are identified as molars or premolars based on dental characteristics that were applied to stem Theria, but were not designed to accomodate monotremes. No known monotremes have incisors or canines.

Premolars generally are simple teeth with single roots found anterior to more complicated teeth. Platypus premolars consist of an erupting upper anterior calcified tooth (Simpson 1929) in addition to a lower anterior calcified nodule that never erupts (Figure 1.1; Wilson et al. 1907, Green 1937). There is disagreement in the literature over the assignation of some premolars as molars because they are probably not replaced (Kühne 1977), but like all platypus teeth, premolars are currently thought to have no replacement (Luckett et al. 1989), and the tooth family for the premolars in *Obdurodon dicksoni* is much more clear because of their morphology. The upper premolar is probably shed earlier than molars (Simpson 1929), but the lower premolar degenerates and never erupts (Green 1937). The reported morphology of the erupted upper premolar is variable; in some specimens the premolar is a single cone which may have some accessory cuspules (Simpson 1929, Poulton 1888b), but in others it is a low, multicuspate tooth (Stewart 1891).

Most mammals have two sets of incisors, canines, and premolars; but they have only one set of molars. Molars are traditionally defined as permanent, multi cuspate teeth found posterior to the last premolar and possessing multiple roots (Owen 1845, Stewart 1981); however, there are exceptions among derived mammals (Jernvall et al. 2012). All molars, by definition, belong to the adult dentition as they lack deciduous precursors (Ungar 2010). Molars are neither shed nor replaced, and are relatively larger and more complex than other teeth (Osborn 1907). Proposed developmental definitions of molars include a buccal shift in thickness of the dental epithelium during the bell stage of tooth formation (Luckett 1993), and lack of later tooth replacement (Kühne 1973, 1977). Several of these definitions can be applied to fossils, although definitions based on ontogeny, generation, and replacement can be problematic. Some definitions of molariform teeth are untested outside extant therian mammals, such as a buccal shift in thickness during formation (Luckett 1993). The juvenile platypus could be assessed for ontogenetic traits but they are outside the scope of this work.

Cusp terminology

With the influence of Darwinian evolution, crown morphology of therian teeth was classified using the 'tritubercular theory.' The tritubercular theory includes four parts, but, simply put, states that mammalian teeth began with a simple, conic reptilian tooth with the addition of two cusps, creating tricuspate teeth (the protocone, metacone, and paracone). Those original cusps became rearranged over the course of evolution and the teeth became more complex, leading to the forms found in all other mammals (Osborn 1907). Cusps were named for their interpreted development time, the protocone being first, followed by the metacone and paracone. Although our understanding of timing of cusp development has changed, the names have been retained to retain continuity with prior terminology (Ungar 2010). Following tritubercular cusp rearrangement, teeth became tribosphenic with the addition of a basined heel (Simpson 1936). Tribosphenic terminology is widely used to describe therian mammal teeth, but the applicability of that terminology to monotremes is questionable (Woodburne et al. 2003). The extinct monotremes *Teinolophos trusleri* (Rich et al. 1997, Rich et al. 1999) and *Steropodon galmani* (Archer et al. 1985) were interpreted to express tribosphenic characters, but they are currently viewed now as most probably non-homologous and independently derived (Rich et al. 2001, Kielan-Jaworowska et al. 1987, Luo et al. 2001). In this paper, tooth terminology for the juvenile platypus is written as pretribosphenic; monotremes likely did not inherit tribosphenic tooth morphology from other mammals, but most probably do share cusp homology.

Cusp interpretations for teeth were in flux when platypus teeth were first being described, complicating the interpretation of older literature written under previous paradigms on platypus teeth (see Woodburne et al. 2003 for a discussion of the cusp reassignments). I follow the terminology proposed by Green (1937) and re-interpreted by Woodburne et al. (2003). Pretribosphenic terminology applied here is descriptive and does not imply homology of the cusps with those of therian mammals, again following Woodburne et al. (2003).

Replacement

Replacement data were used to support several past phylogenetic assignations of monotremes, with premolars playing the largest role. The thickened oral epithelium of adult platypuses, termed cornules and discussed in Chapter 2, are not considered replacement teeth here. Although there are no erupting replacement teeth in living platypus, researchers have suggested the presence of a partially formed but unerupting replacement premolar (Wilson et al. 1907, Green 1937). That replacement premolar, hinted at by the dental lamina of juvenile platypus (Wilson et al. 1907, Green 1937), would have suggested a close phylogenetic relationship between monotremes and marsupials (Kühne 1973), but no evidence for that replacement tooth was found in subsequent studies of platypus growth series (Luckett et al. 1989).

Institutional abbreviations

AMNH – American Museum of Natural History, New York, New York; MNHM - Muséum National d'Histoire Naturelle, Paris, France; MZS – Musée Zoologique de la Ville de Strasbourg; TMM – Vertebrate Paleontology Laboratory at The University of Texas at Austin, Austin, Texas; USNM - National Museum of Natural History, Washington D.C.

MATERIALS

Due to the small sample size and high frequency of destructive research undertaken on platypus teeth by previous authors, few remain accessible in collections (specimens missing or destroyed: Poulton 1888a, 188b, Simpson 1929, Broom 1935, Green 1930, Green 1937; Luckett et al 1989, specimens damaged since publication: Woodburne et al. 1975). I rely on much previously published literature, and a limited sample of three juvenile and one adult platypus to describe the differences in growth and ontogeny of the jaws and teeth (Table 1.3).

Specimens			
Relative age	Specimen	Skull length	Material in this study
(Juvenile) 1	USNM 221109	36mm	Head, CT
(Juvenile) 2	Isolated teeth	Unknown	Teeth, HRCT
(Juvenile) 3	AMNH 252512	61mm	Skull, HRCT
(Adult) 4	TMM M-5899	91mm	Skull, cornules, HRCT

Table 1.3: Specimens used in this study in order of relative age. Age is approximated by relative skull length and amount of wear on the teeth.

METHODS

All available specimens were scanned with HRCT. Stage of development approximations for juvenile platypus in this study are based on specimen size. There are two different measurements associated with preserved specimens in the literature. The first measure, straight length (SL), is a straight measurement from the tip of the snout to the tail, but this measurement does not take into account the curved posture of the specimens. The dorsal contour line (DCL) measurement does account for the curvature; the measurement begins the tip of the snout, follows along the spine to the end of the tail, and is thereby longer than the straight measurement (Table 1.2). None of the specimens used in this study (Table 3) had associated body size information, and only isolated teeth, skulls, and heads were available.

The specimen USNM 221109 is a juvenile platypus, and only CT data were available for this project. It was scanned at a resolution of x=0.036mm y=0.036mm z=0.036mm parameters by Bhart-Anjan Bhullar at a resolution of .6mm^3. Only the head was scanned, with a skull length along a horizontal plane of approximately 36mm (Inset of Figure 1.1). The full length of the body would be over 200mm in length, assuming the skull is approximately 17% of the body. The skull ratio is derived from measuring specimen skull and body lengths from figures 15-19 by Green (1937). The skulls of juvenile platypuses increase a small amount in proportion with body size, from 16% and 17%. The stage of development of USNM 221109 is between that of specimens 8HP and 9HQ illustrated by Green (1937). There is no dental lamina cross section for a specimen of that size in Green (1937), so I compared it with specimens 7HJ, 8HP, 6HN, and 9HQ (Table 1.2). USNM 221109 is closest to the size of specimen 7HJ in Green (1937), but is developmentally different because 7HJ has resorbed the anterior lower tooth. Specimens 8HP, 9HQ, and 6HN are included here because they have better developed lowermost anterior teeth. Additionally I compared USNM 221109 to a histological photograph of a later growth stage from specimen 11HX (figure 81; Green 1937). Calcification is not represented in histological dental lamina line diagrams except by an outline of the developing tooth, so correspondence with CT data is approximate.

Isolated teeth from a specimen in the possession of Dr. Tom Grant were scanned at The University of Texas High Resolution X-Ray Computed Tomography Facility, Austin, Texas with an Xradia scanner. The voxel size is 4.51 cubic microns at 16-bit pixel resolution. The number of slices per specimen is as follows: M1A 912, M1B 933, M2A 756, M2B 931. Each tooth was photographed in six standard views using a Leica EZ 4D digital microscope at 16x magnification at several focal lengths, and the photos were assembled using Heliconfocus. I hypothesize these teeth were newly erupted and are unworn.

A whole skull of a single juvenile platypus with some dried soft tissue in place, AMNH 252512, is 6.2cm long from the premaxilla to the end of the occipital condyle. AMNH 252512 was scanned at The University of Texas High-Resolution X-ray CT Facility with 1024x1024 16-bit pixel resolution and a field of reconstruction of 33 mm. There are 1740 slices with a slice thickness of 0.03485 mm and inter-slice thickness 0.03485 mm. Photographs were taken after the teeth were prepared by smoking with ammonium chloride; and the teeth were photographed at several depth planes and reconstructed using Photoshop CS6. The teeth are worn and post-eruption, so it is the oldest specimen in this study.

SEM images were acquired using a Quanta 650 SEM on low vacuum, 7-11mm focus distance, 5kv, spot 4, chamber pressure 3 torr. The loose teeth and cornules were mounted on carbon tape, but the skull was not mounted.

VGStudio Max version 2.1 was used to render and process the data. Those teeth which are not represented in these specimens, notably the premolars and third lower molar, are figured from other specimens in the literature. The scan data are available from DigiMorph.org and 3D PDFs were created by exporting surfaces from VG studio, converting to .u3d in MeshLab, and importing to a PDF.

Teeth compared in this study were figured using a wide range of media, as has been done in the past. The literature includes drawings from camera lucida, hand drawn images of dried teeth, photographs of in situ teeth, histological sections, line drawings of dental lamina, sketches of lamina, and tooth models. From this study new CT images, photographs, and line drawings were produced. Differences between specimens could be the result of stage of ontogeny, individual variation, or medium of representation in the literature. Stage of ontogeny is estimated based upon the series of teeth published by Simpson (1929) and Green (1937) and those provided a guide to size, complexity, and shape through ontogeny. Individual variation is discussed below, and interpretation is not greatly affected by method of specimen representation. Comparing specimens across these media was done with careful consideration of the techniques used.

Terminology

For group names, ‘monotreme’ includes extant platypus and echidna as well as *Teinolophos trusleri*, *Steropodon galmani*, *Monotrematum sudamericanum*, *Obdurodon insignis*, *Obdurodon dicksoni*, and *Obdurodon tharalkooschild*. Theria includes the most recent common ancestor of marsupials and placentals and all that ancestor’s descendents.

Only cheek teeth are present in the juvenile platypus, so cheek tooth terminology is employed (buccal, anterior/posterior) as opposed to terminology for an arched dental arcade (mesial/distal and labial). The orientation term for the tooth face adjacent to the cheek is buccal; the side facing the tongue is lingual. The surface of cheek teeth facing the front of the mouth is anterior and the face behind is posterior. The external surface of a tooth is the occlusal surface, and the root side is apical.

Following the convention for dental lamina studies, unerupted teeth and their cusps in the dental lamina are described following terminology of Wilson et al. (1907), Green (1937), and Luckett et al. (1989). The tooth rudiments have different names from the erupted teeth. During formation the upper three main rudiments are called, from anterior to posterior, $\underline{w}$, $\underline{x}$, $\underline{y}$, and $\underline{z}$ while the lower are w, x, y, and z. The upper rudiments are underlined to show they are above the occlusal plane, lower rudiments are not. This terminology differs from the original convention where the lower rudiments have lines above letter (w,x,y, and z) showing they are below the occlusal plane (Wilson et al. 1907) because there is no convenient way to show that in the current typeface.

Terminology and labeling of dental formulae and tooth family in the descriptive portion of this study follow general conventions developed initially for therian mammals and stem therians. Dental formulae follow the labeling scheme of the tooth family (also known as tooth types: incisor, canine, premolar, molar; I C P M, respectively) followed by the number of teeth in the upper or lower jaw (P Upper/Lower). Individual teeth are indicated by tooth family abbreviation capitalized for upper teeth and lowercase for lower teeth (Hopson et al. 1969) followed by tooth number (M1 or m1). Based on conclusions reached by Woodburne et al. (2003) and Simpson (1929), I consider the teeth in the platypus to be P, M1, M2 and m1, m2, and m3, as explained in the discussion.

For clarity, here the terms 'deciduous,' 'unreplaced,' and 'adult' are used independently. 'Deciduous' refers only to teeth not retained in the adult. 'Unreplaced' describes teeth with no erupting successors and no evidence of dental lamina replacement (when dental lamina studies have been done). 'Adult' refers to teeth that are retained in adults, but also for tooth families that are part of that dentition, such as molars. The term ‘neomorphic cusps’ is used in figures to maintain consistent terminology with figures by Woodburne et al. (2003), these are the same as ‘cuspules’ mentioned elsewhere.

RESULTS: DESCRIPTION OF THE JUVENILE TEETH

Cusp Formation

The erupted dentition of the juvenile platypus consists of an upper premolar and two molars (M2 and M3), as well as three lower molars: m1, m2, and m3. In the following section there is discussion of a lower premolar that does not erupt; it is, therefore, not included in the erupted dentition in Table 1.1.

There is evidence of only four teeth in the CT data from 221109; they are identified as the upper and lower posterior-most premolars and the anterior-most molars. In past publications, premolars were considered to be present in specimens in which the molars are incompletely calcified, but not in larger and presumably older specimens in which the teeth are erupted and worn (Simpson 1929, Green 1937). The premolars of USNM 221109 are similar to those found in specimens smaller and larger than USNM 221109, presumably younger and older specimens respectively, but not the specimen closest in size, 7HJ (Green 1937). The unerupting teeth are more variable during formation. The anterior upper premolar, w̲, is a single smooth cusp inclined and inflected anteriorly. The anterior lower premolar, w in Figure 1, is a rounded cusp without inclination.

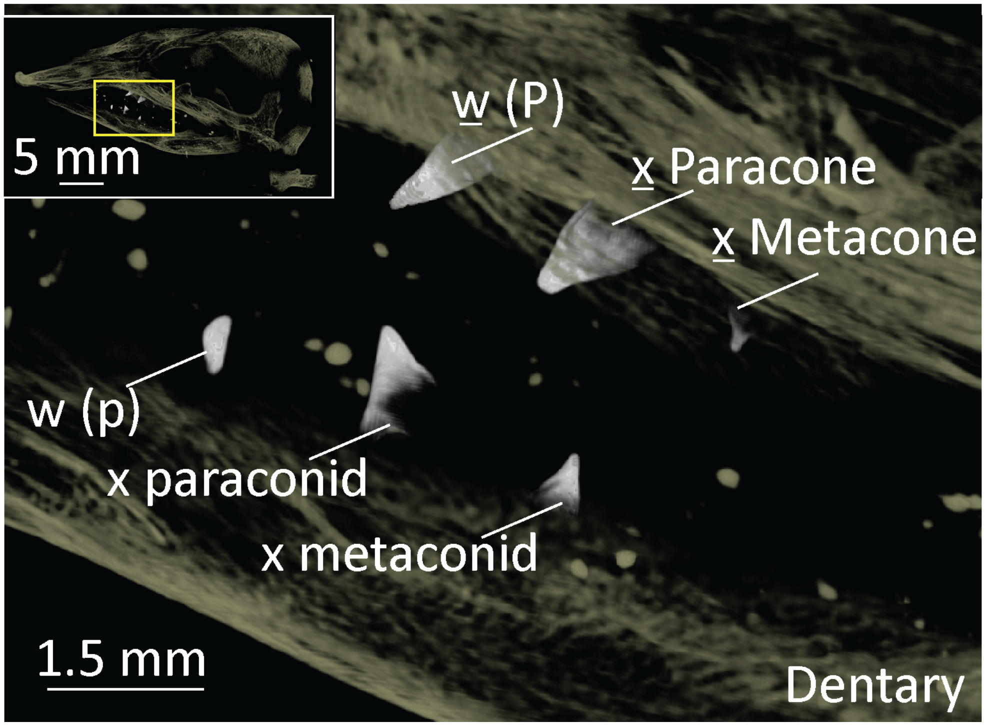

Figure 1.1. A CT image of the developing teeth in juvenile platypus USNM 221109. Calcified parts of teeth, in light grey, come from the region highlighted in a gold box (inset). Cusps figure labeling follows Green (1937) for specimens 6HN and 7HJ. Darker dots are from extra material within the oral cavity, not from dental rudiments.

The M2 labeled as x by Green (1937) in dental lamina diagrams and Figure 1.1, has two calcified cusps. The anterior cusp of x, the paracone, has a broad base and is deflected downward and anteriorly. The second cusp of x, the metacone, is smaller and less calcified than the paracone, and has a distinct indentation along the anterior calcified edge and a thin cusp tip. This indentation is present in Figure 1.2 on specimen 7HJ. The lower molar x has two calcified cusps; the first is an anteriorly directed paraconid, the second metaconid is a smaller cusp with an indentation on the anterior surface similar to the corresponding metacone of x. The two cusps of tooth x and x are not connected by calcified tissue. Spacing between cusps is consistent across specimens observed by Green (1937) and USNM 221109.

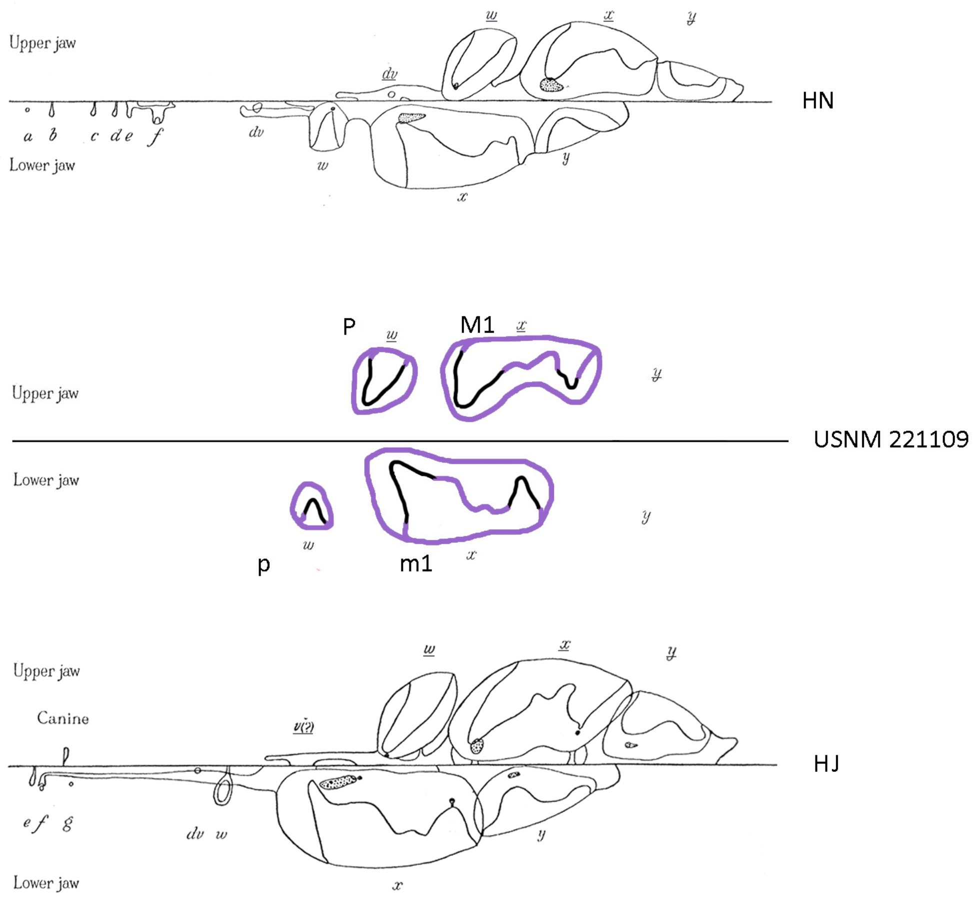

Figure 1.2. A reconstruction of USNM 221109 alongside 6HN and 7HJ. A relative growth series showing the relationship between the cusps in the CT image of Figure 1.1 with those still growing from the dental lamina illustrated by Green (1937: figure 6HN and figure 7HJ). Horizontal black lines indicate separation between upper and lower jaws. In USNM 221109, black lines outline calcified teeth from CT data, purple lines are estimates based on lamina diagrams published by Green (1937) to show association of cusps. Anterior is left, the middle image was traced from a straightened and resized version of Figure 1.1 but is otherwise the same view. Abbreviations for tooth rudiments: w/p - lower premolar, x/m1 - lower first molar, y - lower second premolar, w/P - upper first premolar, x/M1 - first upper molar, y - upper second premolar.

Specimen USNM 221109 shows similarities with specimens 6HN and 7HJ used by Green (1937; Figure 1.2). The cusp shape of each tooth from USNM 221109 is surprisingly consistent with thin section morphology of 6HN and 7HJ described by Green (1937). The HRCT imagery does not show the soft tissue correspondence between calcified cusps of a single tooth that is immediately obvious in a histological section (compare Figure 1.1 to Figure 1.2); however, cusp morphology in HRCT is consistent with the dental lamina figured by Green (1937) so cusp relationships are discernable. The close match between USNM 221109 and specimens figured by Green (1937) corroborate the estimation of size outlined in the Methods. These new data show CT data and dental lamina histological sections of platypus teeth can be compared, but each provides independent information.

Upper Erupted Molars

Each tooth appears to have a repeating motif (Figure 1.3) of two approximate trigons. Each trigon includes a major cusp, two crests, and at least two opposing cuspules. All trigons include an acute-angled loph beginning at an anterior cuspule, running up to the apex of a major cusp and back down to a more posterior cuspule. The trigon shape is composed of a major cusp and two lophs; accessory cuspules may or may not be present, depending on the tooth examined.

The M1 and M2 of *Ornithorhynhcus* have two major cusps on the lingual side, and cuspules on the buccal size (Figure 1.3). Two troughs separate the main features. The first trough antero-posteriorly separates the major cusps from accessory cuspules buccally. The second, called the transverse valley (Figure 1.3), is a deep bucco-lingual trough separating the anterior trigon composed of a major cusp and its associated accessory cuspules from the posterior cusp and its cuspules. The major cusps of the upper molars each have an acute-angled, crescentic transverse loph (Figure 1.3). These lophs may be more or less distinct on some specimens, varying from near-invisible to pronounced. The loph traces an acute angle beginning along the antero-buccal edge of the major cusp, continuing up the long axis of that cusp (buccal-lingual) to the peak, and then returning to a more posterior buccal cuspule. The buccal ends of the loph do not appear to be directly associated with a cuspule; they terminate before meeting them. Cuspules may be independent and defined, or the margins of the teeth may appear only crenulated (Thomas 1889). The cuspules form a shelf or cingulum. The cuspules are highly variable and are discussed further in the section on variation, but they cover a similar region between corresponding teeth even if individual cuspules are inconsistent.

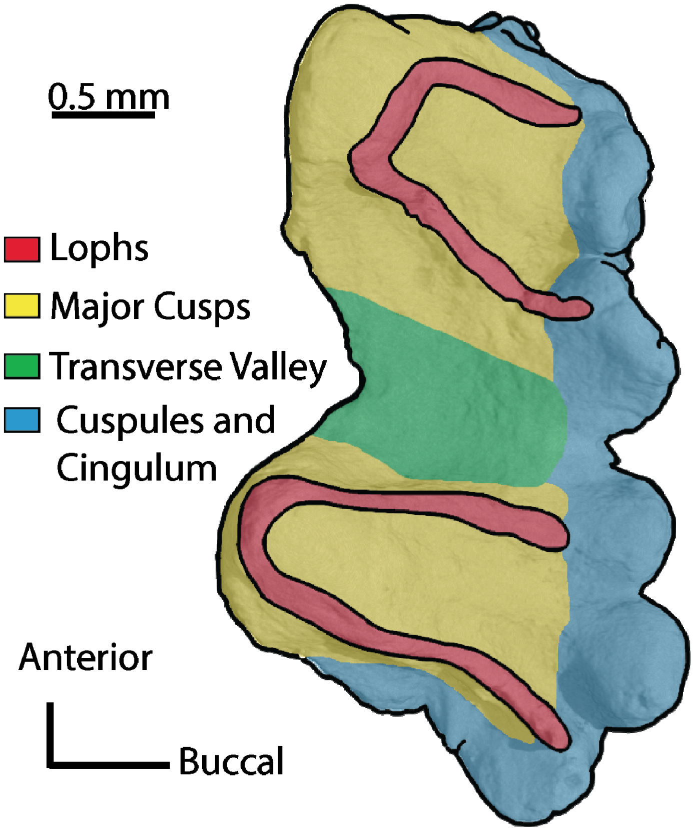

Figure 1.3. Terminology for molariform platypus teeth. Each upper tooth has a repeating motif of major cusp, loph, and cuspules. The Lophs only are found on the upper teeth, but the other three regions are found on all platypus molariform teeth. Tooth anatomy illustrated here does not imply homology.

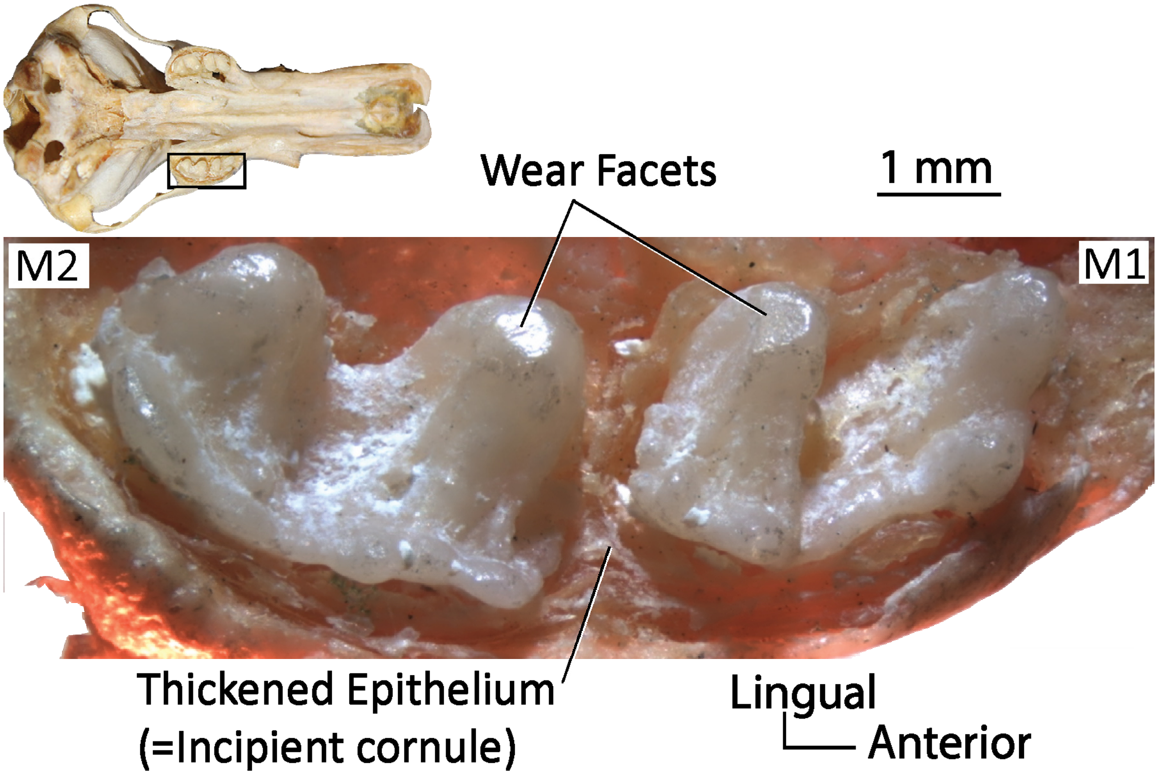

Figure 1.4. Photograph of upper left dentition of AMNH 252512. Teeth are in situ in the jaw, shown in inset skull, above. Wear facets on M1 and M2 are visible on the major cusps.

The erupted upper molars are 3 to 4 mm in length, and no more than 3 mm wide. On the anterior M1 (Figure 1.4) the two equally-sized major cusps point antero-lingually, the anterior -most cusp is inclined to a greater degree than the second. The cuspules on the anterior tooth wrap around the buccal side of both large cusps with a short interruption at the transverse valley; they wrap around the posterior margin of the posterior cusp, terminating in the postero-lingual corner. The transverse valley of M1 is shallow on the anterior edge with a gentle gradient from the anterior-most cusps; however it ends at a steep incline before the second major cusp.

M2 is slightly more triangular (Figure 1.4) than M1; M2 is broader anteriorly than along the posterior edge. The anterior cusp of M2 is comparable to the cusps of M1, but the second major cusp is small. Although they sometimes form equal in size, the anterior major cusp in some specimens is smaller. The cuspules of M2 also cover the anterior and buccal tooth margins. The anterior shelf extends along half to two-thirds of the extent of the anterior-most cusp. When the tooth is held in anatomical position with the two major cusps on the lingual side, the transverse valley of M2 is asymmetrical, deflecting posteriorly in the buccal direction. The general topography running from the apex of the anterior major cusp to the apex of the posterior major cusp is the same as M1: a shallow descent, flat valley, and a steep climb.

Lower Erupted Molars

The lower dentition of *Ornithorhynchus* is different from the upper; it lacks a premolar and instead has three molars. Like the reduced anterior premolar of the upper dentition, the lower dentition has a reduced posterior molar. The two large anterior molars occlude with the upper molars. Like the upper teeth, each lower tooth has two major cusps and associated accessory cuspules, however the major cusps are located buccally, the opposite of the upper teeth. Most significantly the lower teeth lack the well developed crescentic lophs of the upper teeth and the major cusps may be less defined. Cusp triangulation is not as clear as on the upper molars.

The m1 is the most irregularly shaped of all the teeth and has a highly variable number of associated cuspules. Because the first major cusp points anteriorly, the outline of m1 is triangular. There may be a few accessory cuspules associated with the anterior cusp, from zero to four, depending on the tooth, and cuspules are sometimes no more than light crenulations in the margin (Thomas 1889). The second cusp more resembles those of the other teeth because it has mesial cuspules. A deep transverse valley separates the major cusps, but is less defined among the cuspules on the lingual border. Molar m1 has no anterior cingulum, instead the cingulum on m1 is limited to the lingual side of the tooth, usually associated with the second major cusp.

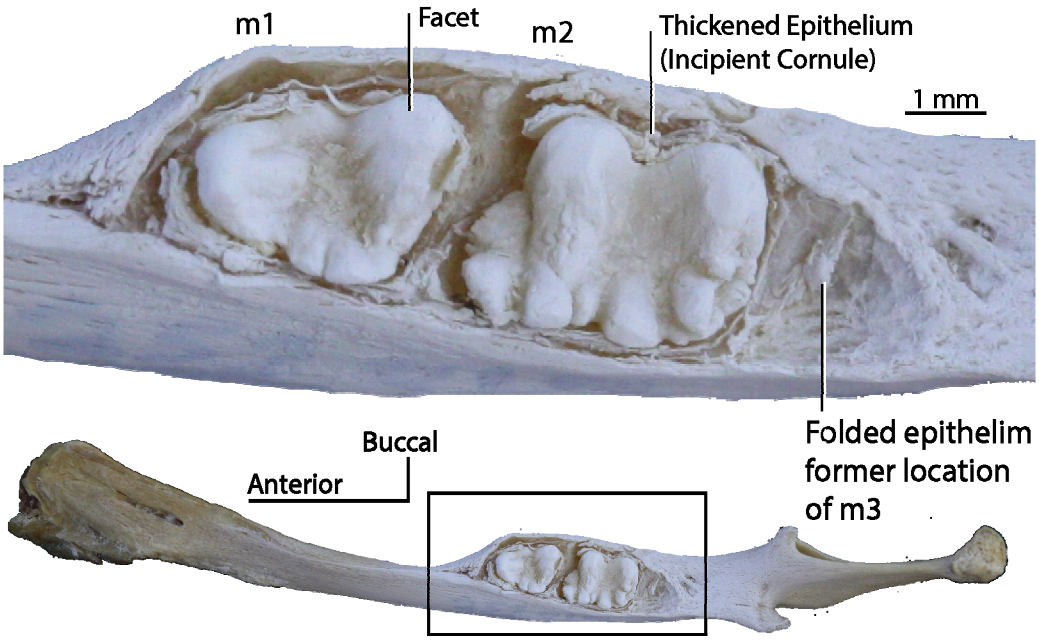

Figure 1.5. Photos of a lower right jaw of the juvenile platypus AMNH 252512. Dentary and teeth smoked with ammonium chloride, a rectangle shows the region expanded above. A close up of right m1 and m2 highlights a wear facet at the cusp apex, and surrounding the tooth is thickened epithelium which will become a cornule, discussed in chapter 2. An artifact of the smoking technique produced a whitened fold in the epithelium that is not a tooth, a fact confirmed with SEM (Figure 1.6).

The rectangular posterior m2 resembles M2, it has two buccally located cusps pointed roughly antero-buccally, a pronounced transverse valley bisecting the tooth between the major cusps, and a variable number of cuspules associated with each major cusp. The major cusps of m2 are less peaked and lower-crowned than those of M2, and was sometimes described as elongate (Simpson 1929, Woodburne et al. 1975). Each cusp tends to run transversely at its maximum height for a greater distance than the more pointed cusps of the upper molars; that was illustrated prominently in figure 4b of

Woodburne et al. (1975). Each trigonid has approximately 2 to 3 well developed cuspules. The cingulum on m2 extends along the anterior, posterior, and lingual sides. The lower first molar (Figure 1.8) shows no evidence of a metaconid by the protoconid. The m2 has multiple anterior cuspules where the models by Green (1937) show only one, therefore the identity of the metaconid as identified by Woodburne et al. (2003) is unknown if present.

The third posterior tooth figured by Green (1937) is not visible in CT scans. A small amorphous lump is visible posterior to the tooth row in AMNH 252512 (Figure 1.4 C, Figure 1.8), this is not a tooth, as shown in Figure 1.9, only some curled epithelium. No specimens in my sample have that tooth.

Cusp terminology

Only the major cusps are present consistently enough to justify names. The two upper molars are similar, and have no protocone. The anterolingual upper major cusp is a paracone, and the metacone is posterior. Cingular cuspules line the buccal side of the upper molar and lingual side of the lower molars; they appear neomorphic compared to the teeth of any extinct monotremes. The anterior and posterior major cusps of the lower molars are a protoconid, and hypoconid respectively. The hypoconulid and potential metaconid identified by Woodburne et al. (2003) are not consistent between specimens, and are thus not recorded.

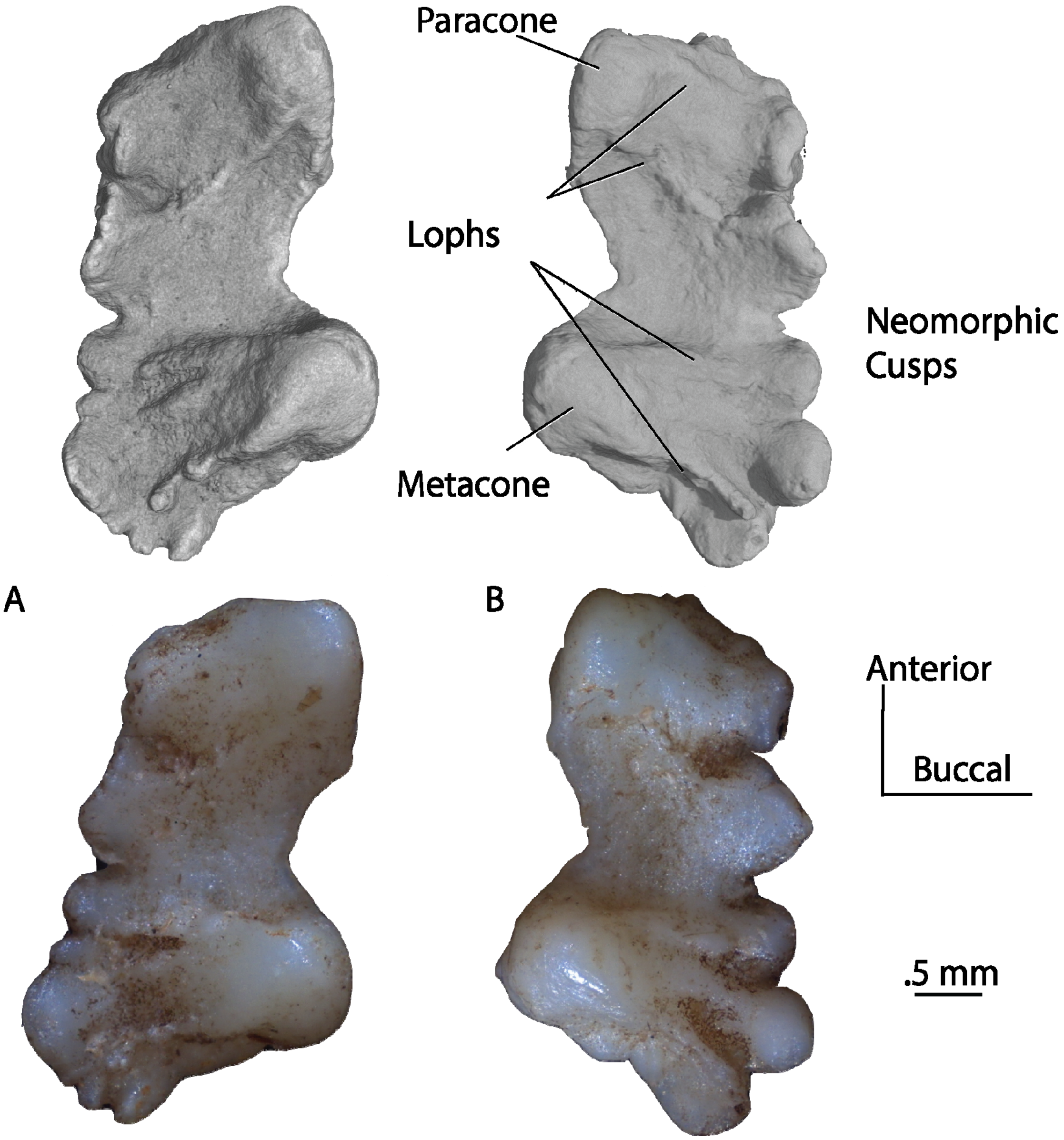

Figure 1.6. Upper left (A) and right (B) M1 from the Grant specimen. Terminology describing features here follows Woodburne et al. (1975, 2003). Note variability and anterior tilt of the major cusps. CT imagery shown above and photographs are below, directions apply to B, A is from the opposite side of the jaw.

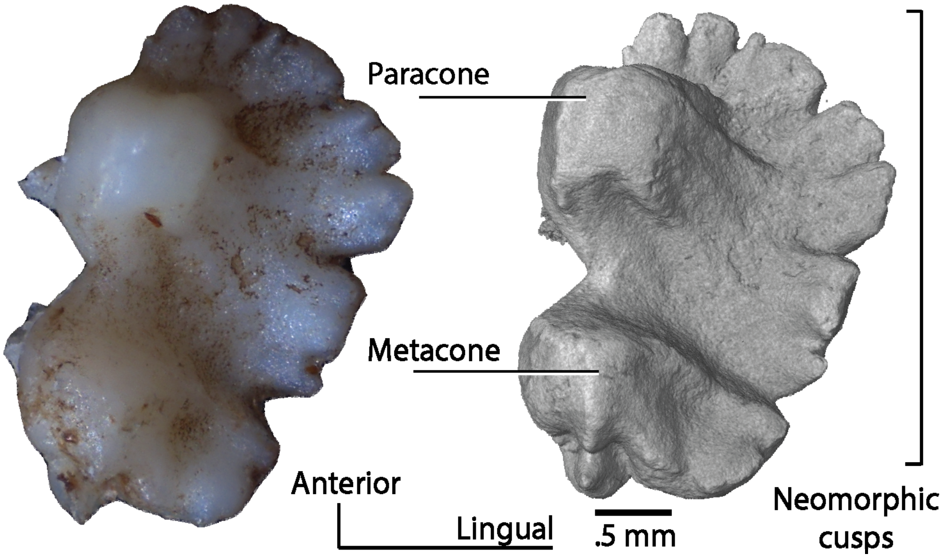

Figure 1.8. Left M2 of the Grant specimen. Terminology follows Woodburne et al. (1975, 2003). Photograph on the left, and labeled CT image on the right.

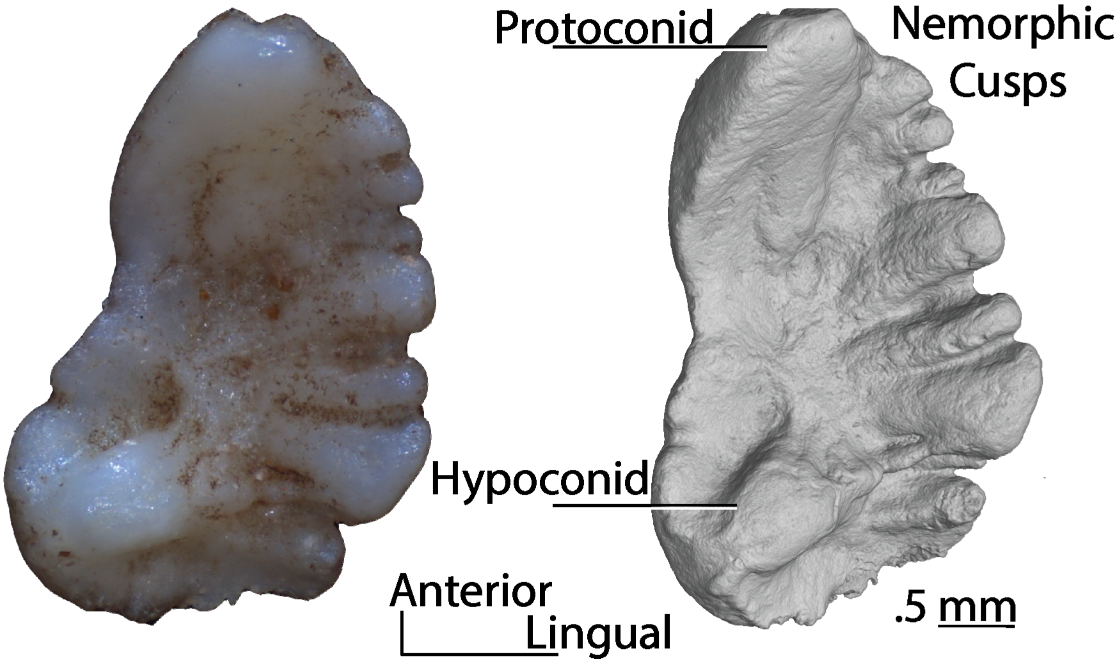

Figure 1.8. Left m1 from the Grant specimen. Terminology follows Woodburne et al. (1975, 2003). Photograph on the left, and CT imagery on the right

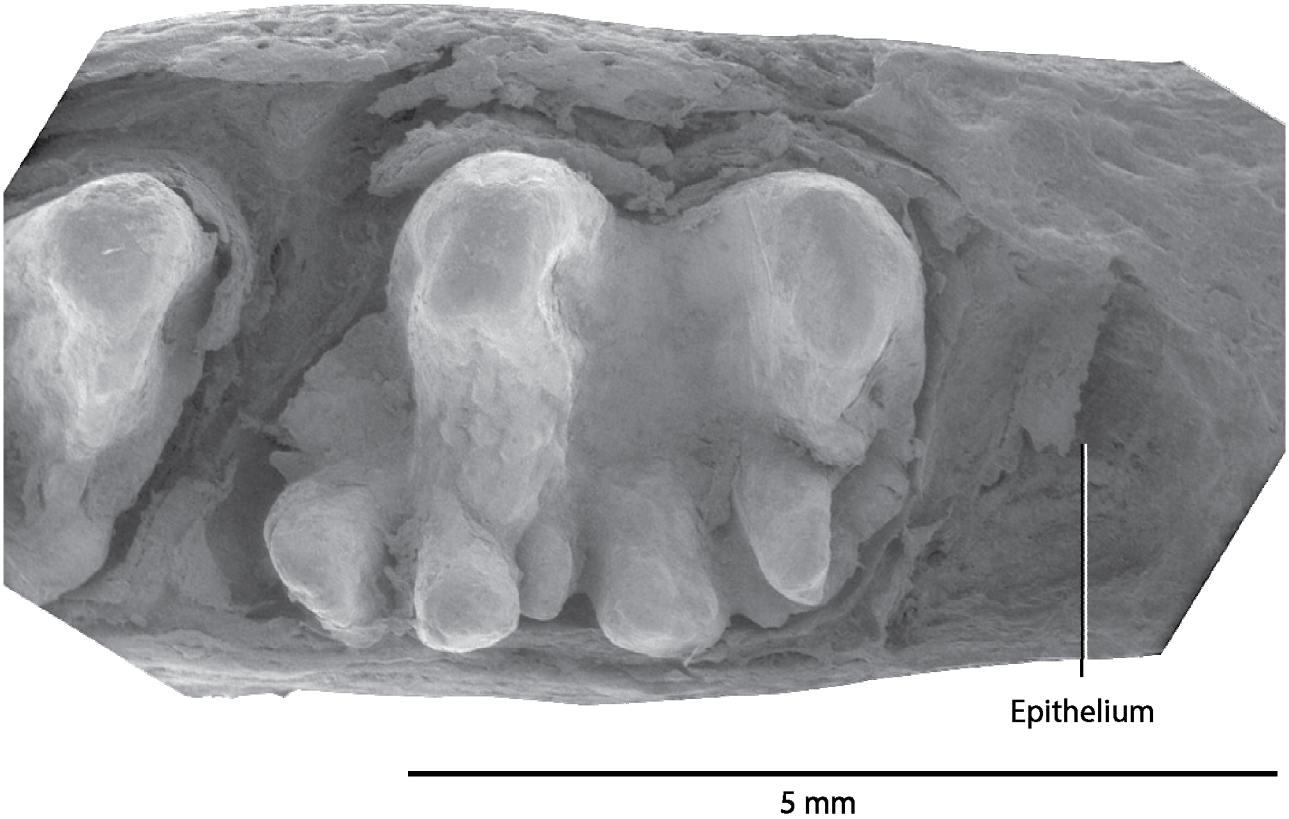

Figure 1.9. SEM image of the lower right jaw of AMNH 252512. Same view as Figure 1.5, zoomed in on m2 and posterior epithelium. No m3 is present.

Pulp Cavity and Roots

The pulp cavity is thin and discontinuous, often meshlike, and enters most cusps and cuspules. The external surface of the tooth is similar in shape to the internal pulp cavity. The pulp cavity in each tooth is concentrated in two separate regions, one under each trigon and becomes more discontinuous as it reaches the basin between the anterior and posterior large cusps. This is particularly clear in view B of Figure 1.10. There are root canals that extend under the main concentrations of the pulp cavity, but main root canals never extend below with little pulp cavity. There is always a main canal extending under each major cusp, however the position of other canals appears variable. There is evidence for three canals per trigon of the upper molar, suggesting six roots per upper molar.

The roots of the juvenile platypus are resorbed as the tooth nears ejection. Those figured by Stewart (1891) show well-defined roots (Figure 1.10 F, G). The roots of the specimens here are different across the left and right teeth of a single specimen, and are each in different states of resorption.

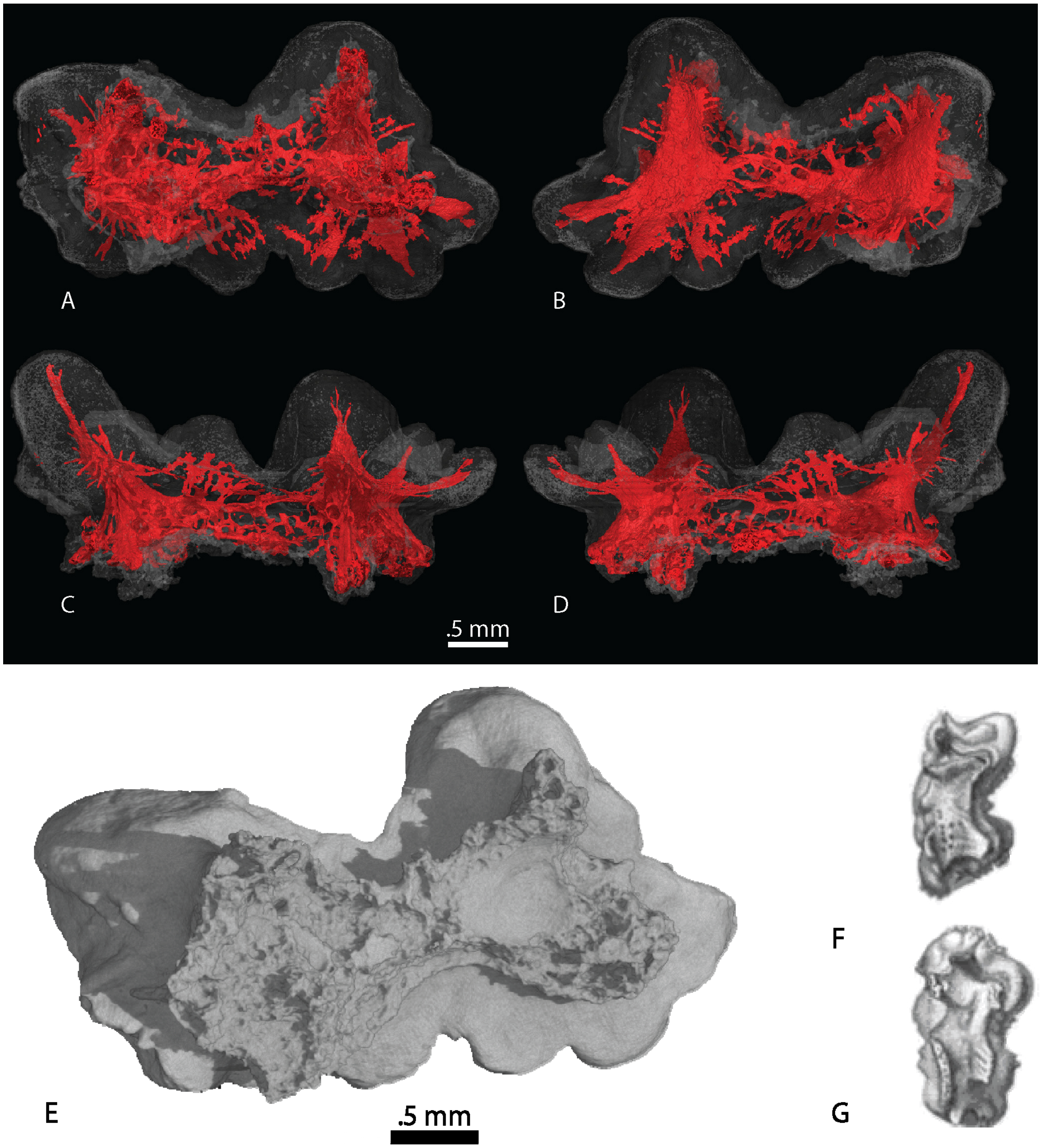

Figure 1.10. The pulp cavity and partially degenerated roots in multiple views (A-E). More profound and well developed roots on a right M1 (F) and M2 (G) from Stewart 1891. CT images of the right M1 from the Grant specimen. M1 was figured because it had the most sub-crown structure remaining. Apical view (A) anterior left, buccal down. Orientations: crown view (B) anterior left, buccal down; lingual view (C) anterior left; buccal view (D) anterior right; apical view (E).

Structure and Wear

The teeth are composed mainly of dentine with a thin layer of enamel (Figure 1.11) on the crown. Enamel has been worn along the tips of all the major cusps of AMNH 252512 (Figure 1.9). The wear on the cusps is probably a function of the specimen age and time since tooth eruption. The evidence of enamel wear on the teeth of the platypus is slight yet visible to the naked eye (Figures 1.3), and clear under SEM (Figure 1.12). The wear is not damage from the way the teeth were stored or from motion in the container because the facets are inclined, not parallel with the storage box surface. There are obvious, shiny facets (Figure 1.4 D) on the major cusps of each tooth with the exception of the anterior-most cusp of m1. Although strikingly clear on the upper molars, the wear is least obvious on the anterior cusp of m1. Juvenile platypus jaw motion is largely unrestricted and, thus, microwear is oriented in many directions. Wear in this specimen is further evidence that these teeth erupt and are functional for a period of time.

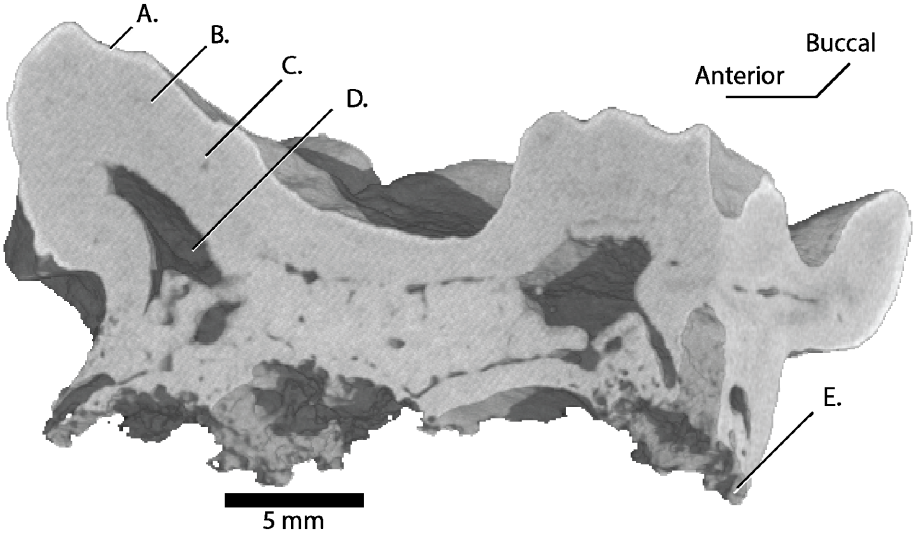

Figure 1.11. A sliced CT image of the right M1 of the Grant Specimen in lingual view, occlusal surface to the top of the page. The clipping plane cuts the lingual and posterior sides. Enamel (A.) is light grey surrounding dentine (B.) in darker grey. The pulp cavity is visible in the central vacuities (D.) and the dark spots in the clipping plane (C.). The tooth is in the same view as Figure 1.10 C. Grant Specimen.

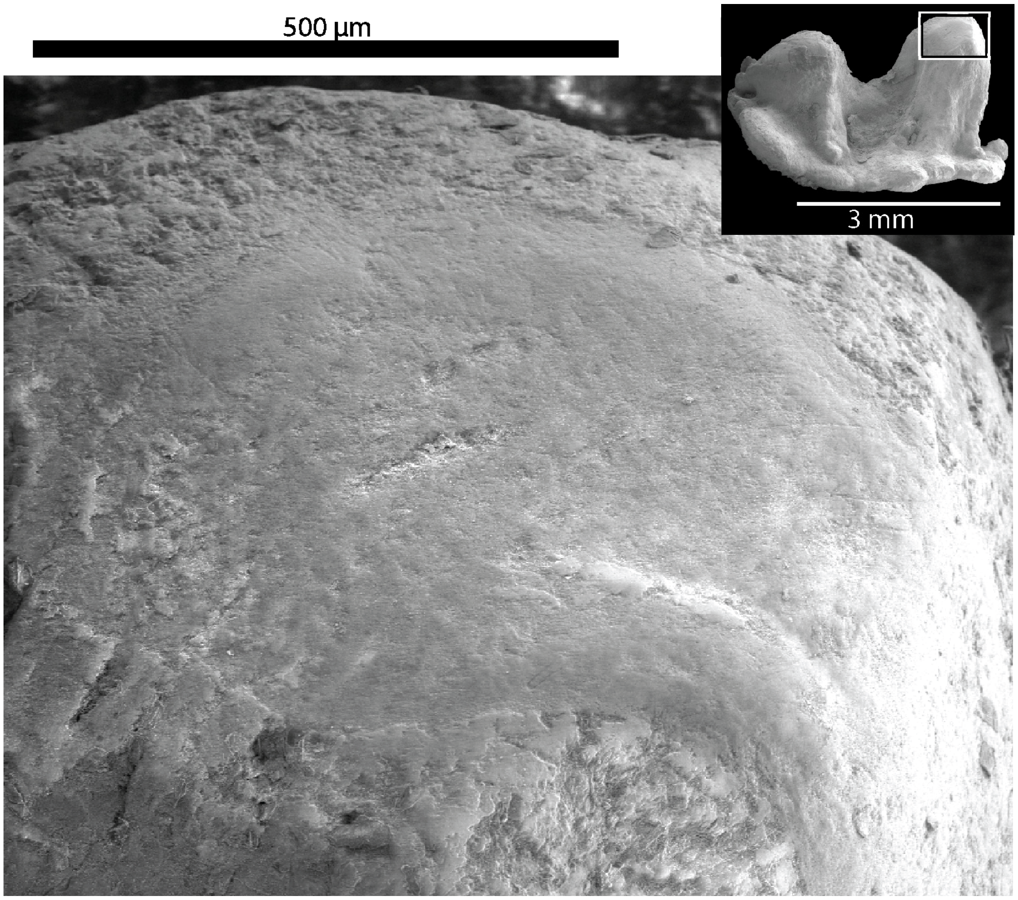

Figure 1.12. SEM image of a wear facet on the anterior cusp of the right M2 of the Grant Specimen. Visible area of smoothed enamel on the tip of the cusp surrounded by the more typical enamel texture. Grant specimen.

Occlusion

I found the occlusal pattern of the platypus in juveniles with unerupted teeth to be consistent with that of adults, as diagrammed by Simpson (1929), rather than that by Green (1937; Figure 1.13). In the juvenile USNMH 222209 (Figure 1.14 B), the anterior-most molar is the m1, with the anterior cusp of m2 in the basin of m1. The occlusal diagram by Simpson (1929; Figure 1.1 lower) follows this pattern. Direct occlusion between the teeth in specimen AMNH 252512 is impossible; the teeth will not connect when the dentary condyle is in place. The dentary of AMNH 252512 (Figure 1.14 A) has been warped by drying, and the teeth have probably sunken post mortem, however the specimen is in approximate orientation as seen in the USNMH 221109 in Figure 1.14. All major cusps except the anterior cusp of m1 show wear, supporting the occlusal diagram (Figure 1.13) where m1 is the anterior-most cusp with P as the opposing surface.

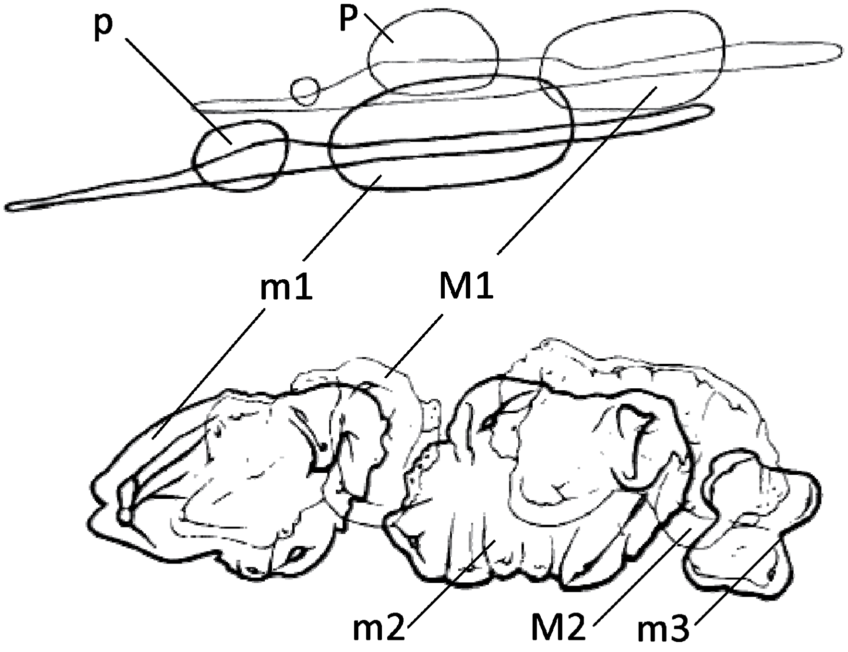

Figure 1.13. Occlusal diagrams by Green (1937; above, lateral view) and Simpson (1929; below, occlusal view) comparing relative location of the first molar. This is due in part to age of the sampled specimens (Green 1937). (Abbreviations: p, lower premolar; m1 lower first molar; m2, lower second molar; m3, lower third molar; P, upper Premolar; M1, upper first molar; M2 upper second premolar)

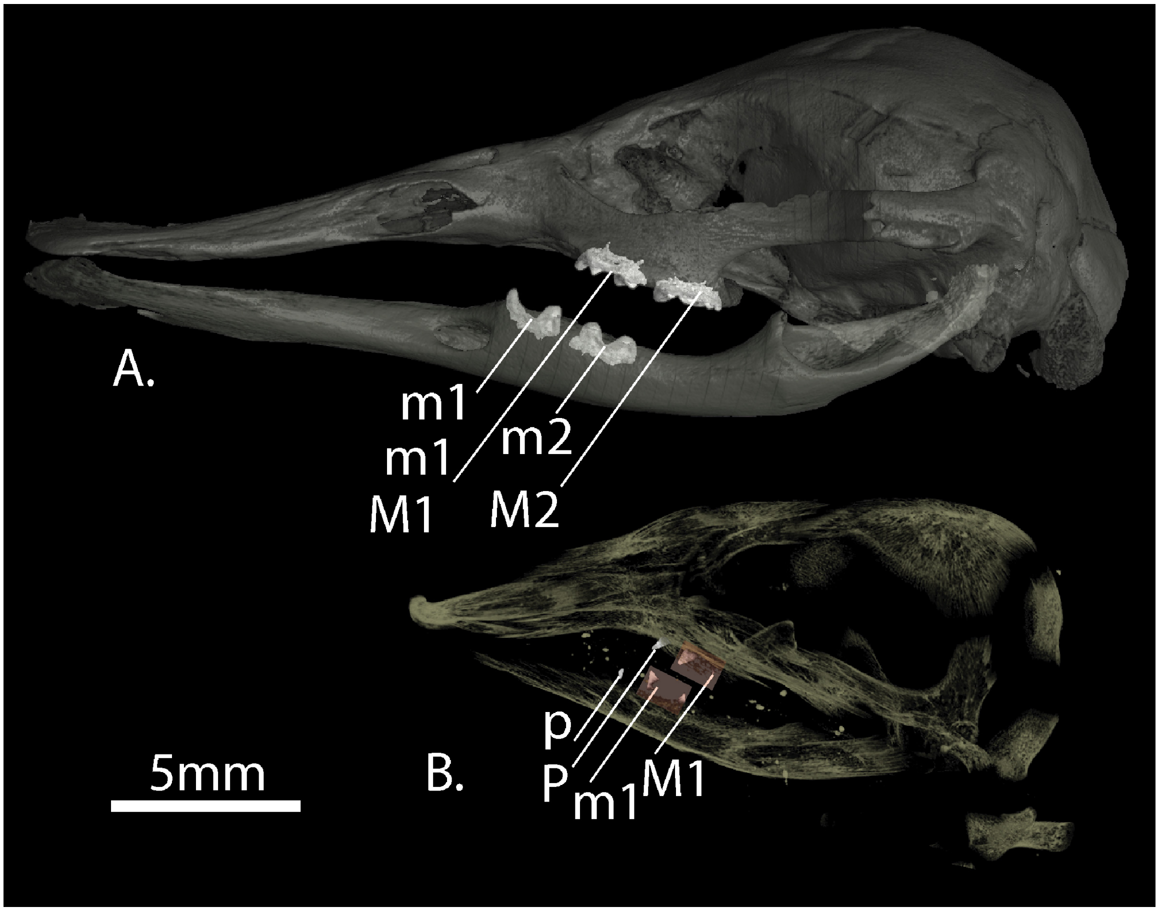

Figure 1.14. CT imagery of juvenile platypus specimens showing occlusal tooth relationship in left lateral view. Jaw position in A is reconstructed based on the articulated specimen B, occlusal figure 2 in Simpson (1929), and condyle position. The anterior cusp of m1 precedes M1 and most likely is the anterior premolar if present. Specimen A is AMNH 252512, Specimen B is USNM 221109. (Abbreviations: p, lower premolar; m1 lower first molar; m2, lower second molar; P, upper Premolar; M1, upper first molar; M2 upper second premolar)

DISCUSSION

Variation

There are only three sources for tooth variation in the juvenile platypus that I could asses with the given data: ontogeny, wear, and damage. There are no studies of sexual dimorphism or population scale differences. Unlike the skeleton, tooth crowns are not remodeled during the life of an organism, and only damage and wear may reshape them after eruption (Jernvall et al. 2012). The slight wear does not impact the features discussed for variation. Breakage is minimal and is concentrated on the roots; it does not appreciably change the cusp morphology of the teeth in my sample. Therefore, for this sample, once the juvenile teeth are erupted, variation is not a result of age-related remodeling post eruption. There is some disparity in the literature dealing with pre-eruption age-related differences in platypus teeth (Simpson 1929), and age does not appear to be a related factor for tooth complexity in erupted teeth. Therefore, I examined differences in cusp morphology of erupted teeth across my sample assuming that wear and breakage do not affect the surface morphology to a great degree, and age of the platypus post-eruption is not a factor changing the tooth crown morphology.

The major features of the upper teeth of platypuses are the cuspules composing the cingula, major cusps, transverse valley, and lophs of the teeth (Figure 1.2). Lower teeth have all the same features except lophs. Tooth outline and size are partially dependent on these. These features of platypus teeth, with the exception of major cusps, differ from one specimen to the next and even the corresponding left and right teeth vary greatly, but not all the same way. These differences were noted previously in most papers dealing with the dentition of *Ornithorhynchus* (Simpson 1929, Green 1937, Luckett et al. 1989). Some features of the tooth are more variable than others. The two major cusps of each molar are the most stable features; however, tooth size, outline, and the number of cingular cuspules have greater variability. Some features, notably cuspules and lophs, are absent in some specimens although prominent in others.

The cuspules vary in location, size, and number across specimens, between left and right of the same specimen, and presumably through ontogeny of a tooth (figure one in Simpson 1929). The cuspules sometimes appear as a crenulated cingulum with variable demarcation between peaks. The cingulum in some regions may have no definition, or may have a high number of cuspules. A single stretch of this crenulated margin, for example around the posterior cusp of M2, can vary from a single large cusp covering a rough quarter of the length to six small, well-defined cuspules (Simpson 1929). When cuspules do form, they do not appear to be in the same places or of the same relative size. These features are not consistent across specimens, and resemblance of cuspules across specimens is more a coincidence of small sample size. Occasionally there is a cuspule located interior from the margin, away from the crenulations but these are entirely inconsistent, even across left and right of one specimen.

Lophs are crescentic ridges that are found on each upper major cusp. Lophs appear more distinct in younger specimens, but this may be an artifact of sample size. More often than not, the lophs of the upper major cusps appear to terminate at a cuspule, however this is not consistent across all upper teeth. Presence of pronounced lophs on teeth from *Obdurodon*, similar to those seen on the upper teeth of juvenile platypus, contributed to the identification of this fossil as a monotreme (Woodburne et al. 1975). Although the first tooth and tooth direction are incorrect in the original description, identification of the fossil tooth as belonging to a monotreme is consistent with subsequent fossils (Archer et al. 1992).

Major cusps are the most stable features across teeth. They are always present on the large molars. Major cusps are the first to calcify (Figure 1.1) and their morphology is highly consistent across specimens in early formation before eruption (Figure 1.2). Major cusps on a tooth may be proportionally different from those of another tooth, however they remain in the same relative location and they point in the same relative directions. They are clearly defined on the upper molars, there appears to be a greater range of variation on the lower molars. The anterior cusp of m1, which is only in occlusion with the highly transient upper premolar, is consistently unworn and has the least consistent morphology. Major cusps and the transverse valleys are the most stable features, and therefore the ones that are easiest to name and use consistently in phylogenetic analysis.

Tooth families reviewed

More than the teeth of the juvenile platypus, teeth from extinct monotremes resemble tooth families in therian mammals. Those fossils include premolars (*Obdurodon dicksoni*) and molars (*Steropodon galmani, Teinolophos trusleri, Monotrematum sudamericanum, Obdurodon insignis, Obdurodon dicksoni, Obdurodon tharalkooschild*; Woodburne et al. 1975, Archer et al. 1985, Archer et al. 1992, Rich et al. 1997, Kielan-Jaworowska et al. 2004, Pain et al. 2012). Although extinct monotremes are known to have adult teeth, as of yet there is no evidence for a juvenile dentition in fossils of that clade. In an interesting switch, the teeth of the juvenile platypus are unreplaced by enameled teeth, and are not permanent. If the adult dentition of extinct monotremes is to be meaningfully compared to the juvenile dentition of extant platypus, they must represent the same tooth generation. Because molars, by definition, belong to adult dentitions, their presence in the juvenile platypus suggests that it is a reduced adult dentition. Additionally, the teeth labeled as premolars also are unreplaced (Luckett et al. 1989) suggesting that only the adult dentition similar to extinct monotremes is expressed. Here, for the sake of continued comparison, the juvenile teeth of *Ornithorhynchus anatinus* are considered homologous and to belong to the same tooth generation as the adult dentition of extinct monotremes.

Previous tooth family designations of the juvenile platypus are not supported based on character diagnosis, but rather general similarity. The upper anterior-most erupted tooth (P) in the juvenile platypus is small, reduced, and variable; however, it is still less complex than the posterior teeth. The roots are yet unknown for the erupted upper premolar, however the placement of that tooth is consistent with the double-rooted premolars described in the extinct platypus *Obdurodon dicksoni* (Archer et al. 1993). The fossilized premolars of *Obdurodon dicksoni* are cone-shaped with a single, large cusp surrounded by a cingulum, similar to the teeth in the juvenile platypus.

The only feature of the upper premolar that is inconsistent with premolars is it's lack of replacement. Lamina studies can provide information on replacement teeth in *Ornithorhynchus anatinus* that may not erupt. There was disagreement over replacement in the dental lamina of rudiment 'w,' corresponding to the anterior-most rudiment here labeled a premolar (Kühne 1973, Luckett et al. 1989). Replacement of rudiment 'w' (Kühne 1973) is unlikely, because 'w' shows no signs of a successor lamina (Luckett et al. 1989). Furthermore, rudiment 'w' is consistent with an unreplaced premolar because it differentiates before the rest of the dentition and the lamina shows no signs of replacement (Luckett et al. 1989). CT imagery of specimen USNM 221109 also shows no evidence for a replacement tooth.

With the exception of the upper first tooth hypothesized to be a premolar, erupting platypus teeth fit every physical definition of molars. The posterior teeth are analogous to molars at least in form, position, and function, but platypus teeth are difficult to classify because of their deciduous and degenerate nature (Simpson 1929). In studies of the dental lamina, the teeth 'w' and 'x' have no replacement excluding keratinous pads (Chapter 2), and no evidence of precursors or successors in the lamina.

The teeth are located at the rear of the jaw and have multiple roots, although the roots are later resorbed (Stewart 1891). The root reduction is a derived characteristic, becuase all molars from extinct monotremes are multi-rooted. They have increased complexity over the premolars and the fossil record provides good evidence in

Obdurodon dicksoni that well-differentiated molariform teeth are present in extinct monotremes (Musser et al. 1998). The third lower tooth is reduced in size and complexity especially for a molar, displaying only a single cusp and root (Stewart 1891). Nevertheless, the posterior position of the tooth indicates a molar affinity, and is congruent with the presence of a lower third molariform tooth in *Steropodon* (Archer et al. 1985), *Teinolophos* (Rich et al. 2001), and an undescribed third molar of *Obdurodon* that is highly reduced (R. Pian 2013, pers. comm. Nov 2). I propose the definition of molar to include deciduous molars found in the juvenile platypus. Molars, then, should be defined with all the previously discussed characteristics except permanence.

The molar label for platypus teeth might be irrelevant if these teeth are not part of the adult dentition, becuase only adult molar morphology is described in phylogenetic analysis. There are some characteristics of the juvenile platypus and other monotremes that could suggest these teeth are either juvenile or adult. Those features are changes in complexity, enamel thickness, loss after eruption, root reduction, and the fossil record. Increased complexity, enamel thickiness, and loss after eruption are characteristics commonly associated with juvenile dentitions (Ungar 2010), and all are present in the juvenile platypus. Increased complexity may be due to reduced importance of precise occlusion in platypus, and the loss may be indicative of a peramorphic trait. It is possible that monotremes have only a single set of erupting teeth, but it cannot be substantiated by the fossil record due to small sample size, and lack of any ontogenetic data. If an ontogenetic series of a monotreme was found in the fossil record, and it had only a single set of teeth, it would support the hypothesis that the dentition of the juvenile platypus is a reduced adult dentition. If they are found with two sets of teeth it would be another commonality with other mammals, but the juvenile teeth would be inappropriate for phylogenetic analysis. Nothing more can be said either way with the present data.

CONCLUSION

Previous dental lamina studies relied on histological sections and light microscopy (Poulton 1888a, 1888b, Broom 1935, Green 1937 etc.). Histological sections include soft tissues that are not shown in CT data. Comparison of CT images of specimen USNM 221109 (Figure 1.1) and histological sections of 7HJ, a platypus with similar size and maturity figured by Green (1937), reveals comparable tooth morphology. Therefore, CT data can be productively compared to dental lamina diagrams from light microscopy. The stage of dental development of a specimen may be estimated by comparison of CT data with studies of dental lamina. However, comparison is imprecise because the degree of calcification is not necessarily reflected in line drawings of soft tissue. The unclear distinction between tissues is problematic for comparing growth stages of mammals (Luckett 1993), but there is promise for discerning growth stage of developing platypus young using CT data. Spatial relationships between cusps are consistent; and diagnostic features in the calcified tissue can be imaged in detail. In the erupted teeth, features such as the pulp cavity and surface detail can be imaged, and CT opens the way for comparison of new, potentially phylogenetically important features of rare teeth in non-destructive ways.

Here I clarifies and confirms many points confused in the literature. There are three teeth per jaw that erupt, wear, and are shed. Like other mammals, each tooth has roots, dentine, enamel, and a pulp cavity. Both premolars and molars are present, and there is no evidence of diphyodonty in platypus. All the teeth in the juvenile platypus likely belong to the equivalent of the adult dentition in other extant mammals. They can still be described juvenile teeth because their enamel is reduced, but they are not a juvenile dentition. Stable features including the major cusps and transverse valleys are consistent enough for use in phylogenetic analysis, whereas cuspules and lophs are highly variable and probably less informative. The early calcifying cusps of the molars are the least variable features, further supporting those features as most likely useful in phylogenetic analysis.

Juvenile platypus teeth are derived, reduced, deciduous, unreplaced, cheek teeth. These teeth may represent an ancestrally adult dentition that is now only expressed in the juvenile, making way for a new state beyond the first (Chapter 2), due to the presence of molars and unreplaced premolars. This dentition is likely an example of a peramorphic trait, a set of adult characters expressed in juveniles making way for new states in the adult.

CHAPTER 2: EPITHELIAL PLATES: CORNULES

INTRODUCTION

The common name for *Ornithorhynchus anatinus,* the ‘duck-billed platypus,’ comes from its unique feeding apparatus shaped superficially like a duck’s bill. Beyond the bill's bizarre form, exceptional sensory and masticatory systems make it even more exceptional. The soft and pliable surface is closer to the rhinarium of a dog than the hard keratin beak of a bird (Wilson et al. 1893). The bill is covered in a thin layer of epidermis peppered inside and out with mechanosensory organs to enhance tactile sensation (Wilson et al. 1893) and electroreceptors to sense electric fields from living prey (Scheich et al. 1986). In the bill proper, platypuses have no mineralized teeth; but behind the bill in the oral cavity mineralized juvenile teeth are replaced by functionally analogous epithelial plates in adults. Those epithelial plates have been given many names such as cornules (used here; Figure 2.1; Thomas 1889), as well as ‘teeth,’ horny plates, and grinders (Home 1800, 1802).

Here I review thickened oral epithelium in monotremes and compare it with other instances of oral keratin. I describe the structure and formation of cornules in the adult platypus in relation to the juvenile teeth. I present new imagery of the cornules and associated structures from CT imagery and SEM, discuss innervation, and I asses the relationship between of feeding mechanism and cornule morphology.

Other Thickened Oral Epithelium in Monotremes

Thickened oral epithelium is common in extant monotremes. The platypus has two ‘tongue teeth-‘ laterally projecting keratin struts located at the base of the tongue, in addition to anterior longitudinal epithelial ridges (secateuring ridges, Musser et al. 1998), and semi-circular ridges on the bony palate (Figure 2.2). All echidnas have thickened oral epithelium at the base of their tongues and the on the posterior roof of their palates. *Zaglossus* further has thickened 'tongue teeth' on the anterior length of its tongue and thickened epithelium along the anterior portion of the palate (Griffiths et al. 1991). The beak epithelium of platypus is covered in electroreceptors inside and out, whereas those receptors are only external on the beak of the echidna (Wilson et al. 1893).

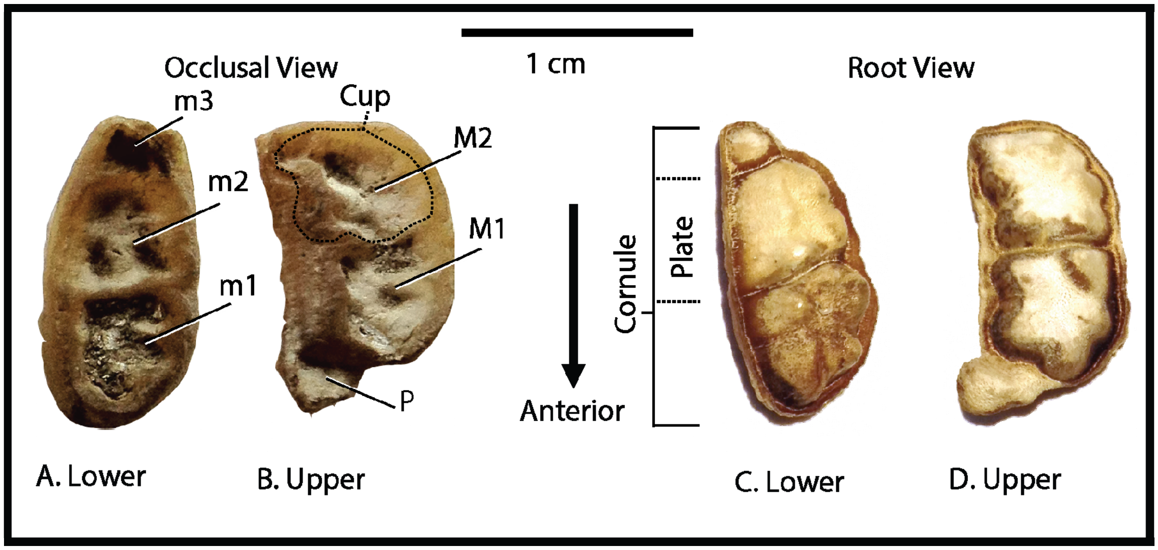

Figure 2.1. Occlusal surfaces of the lower cornule (A) and upper cornule (B) from specimen MZS Mam 4540. Apical/root view of lower cornule (C) and upper cornule (D) from specimen TMM M-5899. Debris fill the indention on the anterior end of A. Tooth abbreviations are for corresponding juvenile teeth: P - upper premolar; M1 - upper first molar; M2 - upper second molar; m1 - lower first molar; m2 - lower second molar; m3 - lower third molar Anterior is down.

Fossils of extinct monotremes show evidence for thickened oral epithelium in specific features seen in their preserved skulls, although the epithelium itself is not preserved. There are longitudinal maxillary troughs in the extinct platypus *Obdurodon dicksoni* similar to those hosting the secateuring ridges (Figure 2.2, Figure 2.3) in extant platypus (Archer et al. 1992), however *Obdurodon dicksoni*, lacks the bony understructure of palatal ridges seen in extant platypus and echidna. The fossilized remains of the echidna *Megalibgwilia* do have palatal ridges similar to those in the extant platypus and echidna suggesting the presence of thickened palatal epithelium and its use in the extinct monotremes at least in the Pleistocene (Griffiths et al. 1991). There is not, however, a fossil record for cornules in extinct monotremes, becuase all fossil finds (excepting *Ornithorhynchus* and echidna) have teeth.

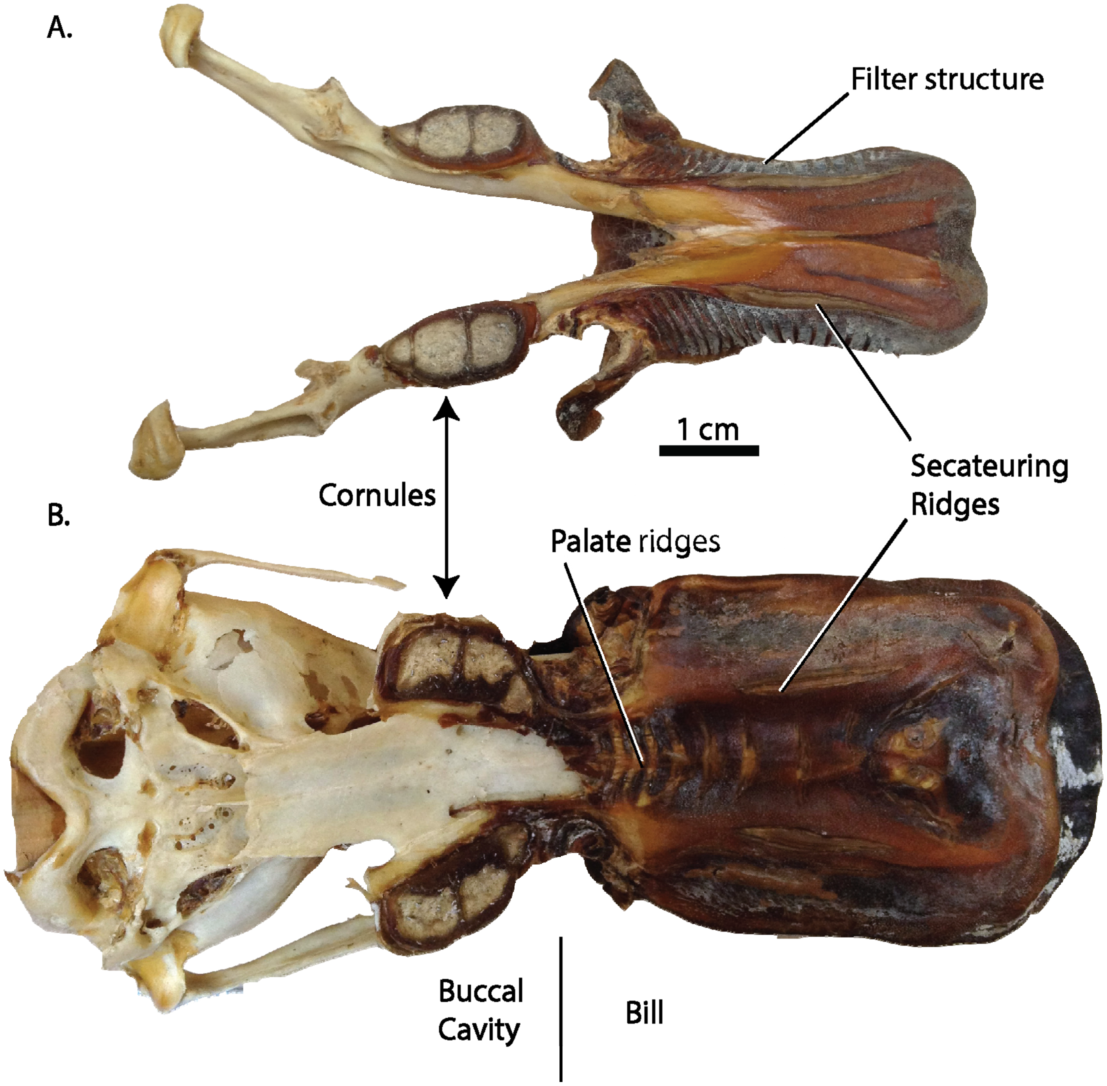

Figure 2.2. MNHN 2007-390 Photographs of a platypus bill dried on a skull, dorsal view of the lower bill (A) and a ventral view of the upper bill (B). The skull and beak are lined up with the cranial mandibular joint, in occlusal position. The Cornules, indicated by arrows, are located posterior of the bill proper inside the oral cavity, roughly below the orbits. This corresponds to stage F in Figure 2.4

Cornule Identity Matters

There has been little discussion of the cornules in the literature of late, however, the structure and formation of cornules has been something of a puzzle for over 100 years. At their discovery, platypuses were considered edentulous. Then, the cornules were later interpreted as grinding teeth (Home 1800, 1802, Seeley 1888). Eventually, after the discovery of the juvenile teeth, the cornules were again considered only functional analogs for teeth (Thomas 1889, Poulton 1888a). Cornules are either teeth or something different, and this question is essential to the anatomy of the cornules, homology of tooth generations, identity of the juvenile dentition and associated phylogenetic analysis, and relevant anatomical comparisons between keratins and teeth. Specific to juvenile platypus teeth, if the cornules are teeth, the cornules would take the place of the second set of dentition in mammals. If this were the case, it would strengthen the case that the juvenile teeth are truly a juvenile dentition and are therefore inappropriate for phylogenetic comparison with adult dentitions. Although this may appear a semantic argument, proper characterization in morphological phylogenetic analysis is dependent on word choice and interpretation, thus, character description can have far reaching consequences in proper scoring of organisms and our understanding of evolution. Furthermore, if the cornules are reduced teeth or some new form of teeth, then they should be compared with terminology appropriate for teeth. Away from the semantic definitions, anatomical comparison relies on homology, so if the cornules are not teeth then a more proper comparison should be found. The characteristics of teeth, and their proposed evolutionary precursors, odontodes, if applicable, would suggest the cornules formed from teeth. Other appropriate characterizations of the cornules may be found among gene homologs of keratins, or in structural similarities with keratins expressed elsewhere among mammals. If they are teeth, it would also expand our definition of teeth, and effect how researchers choose to compare teeth.

Terminology

I use the term 'cornule' to refer to the composite structure of thickened epithelial plates found in each jaw of the platypus. The term is borrowed from Thomas (1889) and encompasses the entire structure rather than the constituent parts. Each cornule is composed of the three smaller units, plates. Special reference is made to indentations, referring to the hollow on each plate left by juvenile teeth (Figure 2.1).

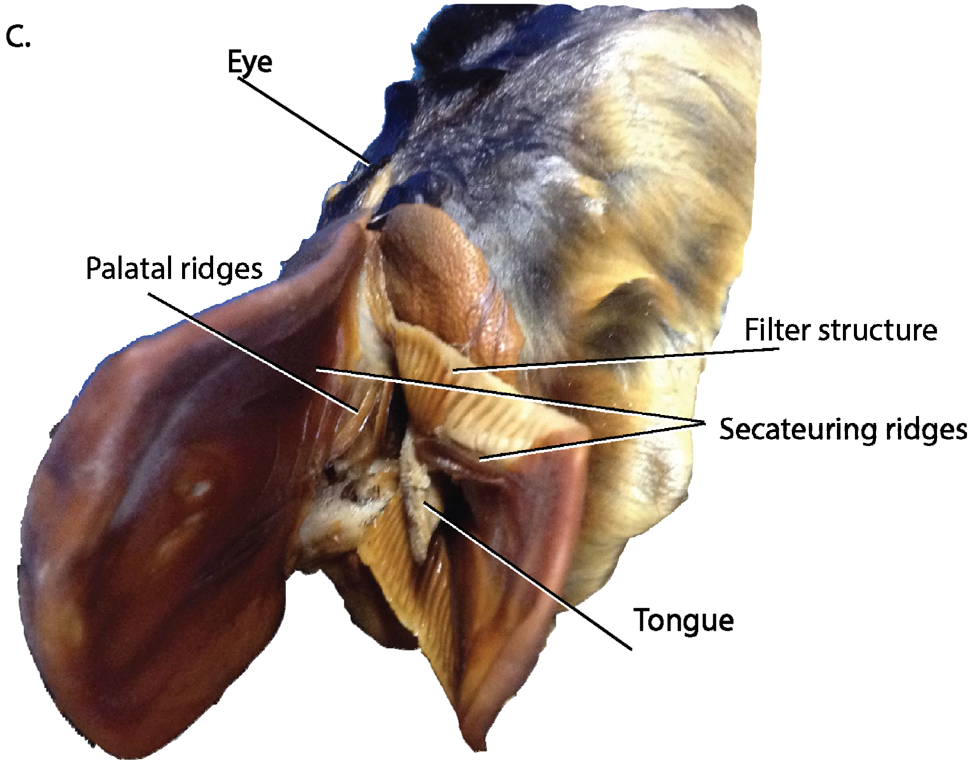

Figure 2.3. View inside the mouth of a pickled platypus MZS SM4, the dentary is broken. Other oral keratin used in mastication includes secateuring ridges, palate ridges, and filter structures.

Development

The cornules form from epithelium after the eruption of the teeth found in the juvenile platypus (Figure 2.4). Juvenile platypuses are any sub-adult platypuses that have not reached full size. Prior to shedding the teeth, the epithelium around and below each thickens, growing from the surrounding gums (Thomas 1889). This growing epithelium (Figure 2.5) surrounds each tooth and then grows below it, eventually growing under and around the roots (Thomas 1889). That epithelium makes up the cornule, and is split in three subunits, the plates (Figure 2.1). Each plate has a depression on the occlusal surface corresponding to a tooth of the juvenile (Figure 2.1). The size of a plate composing a cornule and indentations on it are relative to the size of the tooth above or below; a larger tooth (first or second molar) will leave a larger indentation, whereas a smaller tooth (premolar, third molar) will leave a smaller indentation. The depressions left behind by the juvenile tooth roots form the surface topography of the cornule, and correspond to the shape of the cornule in root view as well (Thomas 1889 ; Figure 2.1).The teeth are partially embedded in the cornules until they are either worn away or shed, leaving a series of three small epithelial plates (Thomas 1889). The plates then grow together as the juvenile jaw grows in length, making more room for the adult-sized cornule. The plates grow upward continuously through the life of the individual (Grant 1985, Tom Grant Pers. Com.).

As discussed in Chapter 1, the platypus begins its dental development as most mammals do, forming teeth in the gums from dental lamina and proceeding through normal mammalian stages of development to eruption and wear (Figure 2.4 A.-E. ; Thomas 1889). The erupted dental formula for the juvenile platypus is I 0/0 C 0/0 P 1/0P M 2/3. The teeth are variable and serve reduced function in the juvenile, enduring minimal wear before eventually falling out. They are superseded by something altogether unconventional in mammalian mouths, the cornules.

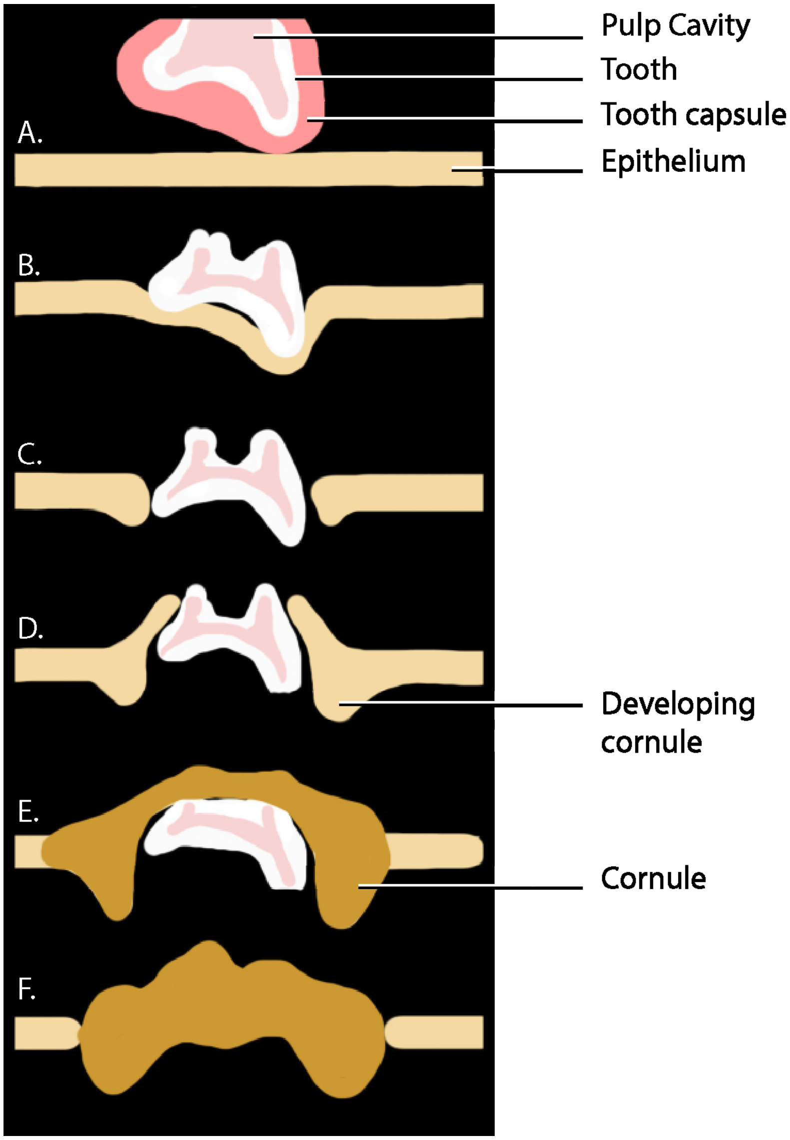

Figure 2.4. Schematic diagram of cornule development: (A.) tooth forms in capsule below gums, (B.) tooth erupts through epithelium, (C.) epithelium begins to thicken around the tooth as the tooth is subject to wear on major cusps, (D.) epithelium begins to grow under the tooth, probably concurrent with root reduction, (E.) Epithelium meets under and overtops tooth, cornule is differentiated from the surrounding epithelium, (F.) Tooth is lost, cornule thickens and grows. Redrawn and relabeled from Thomas 1889.

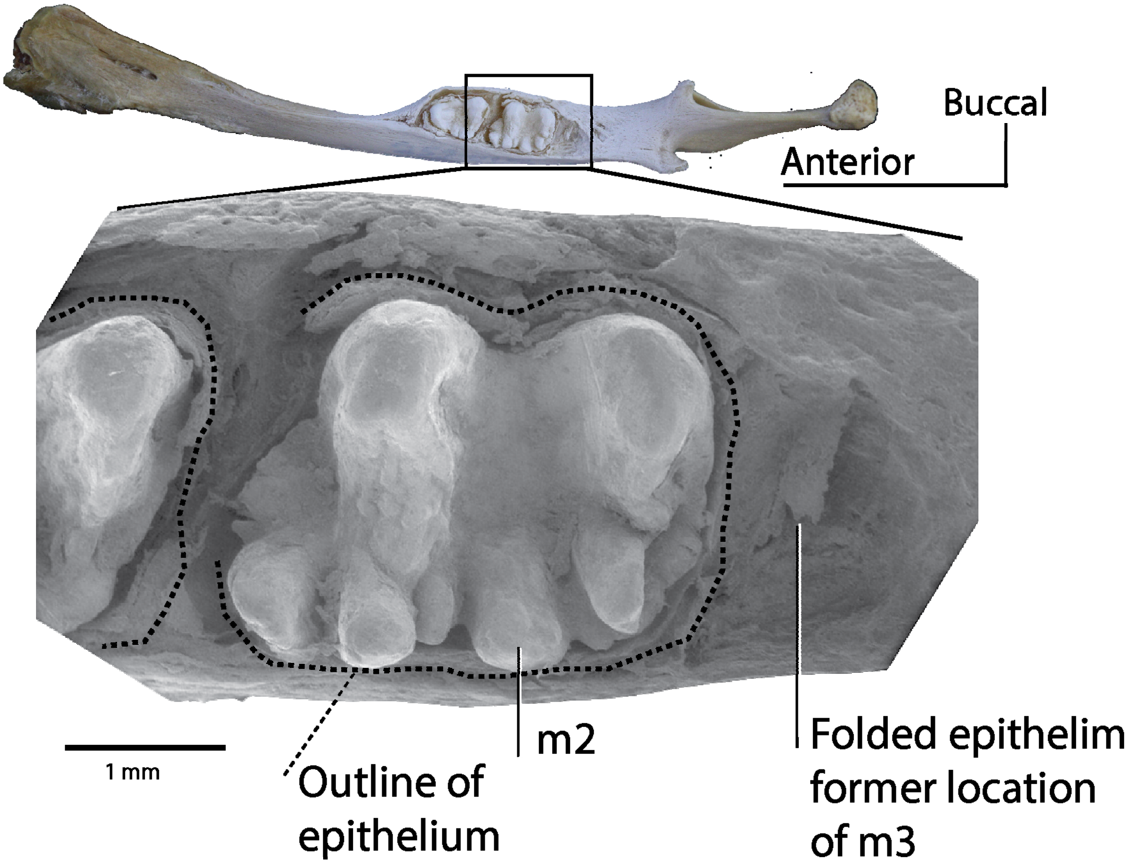

Figure 2.5. Upper: Photo image of the lower right dentary of AMNH 252512. Lower: SEM image of epithelium surrounding the lower second tooth, SEM. This corresponds to Figure 2.4 C.

MATERIAL

Institutional abbreviations

AMNH – American Museum of Natural History, New York, New York; MNHM - Muséum National d'Histoire Naturelle, Paris, France; MZS – Musée Zoologique de la Ville de Strasbourg; TMM – Vertebrate Paleontology Laboratory at The University of Texas at Austin, Austin, Texas.

Specimens

Several adult platypus specimens were photographed from the MNHM and MZS. A list of specimens examined is provided in Table 1.2.1.

Table 1.2.1: Adult Specimen List

Specimen Number	Specimen Contents
TMM M-5899	Skull with cornules (CT data available)
MNHM 1933.222	Bisected skull with dentary
MNHM 1962-2146	Skull with cornules, dentary, atlas
MNHM 1985-1795	Skull, kertainous beak, cornules, dentary
MNHM 2007-390	Skull with cornules and beak, dentary
MNHM A.12.821.	Skull and dentary
MNHM A.7128	Hinged skull and dentary
MZS Mam04441	Four bodies in alcohol
MZS Mam04536	Skull, dentary, dried flesh
MZS Mam4535	Skull missing left anterior mx, pmx, dentary
MZS Mam4537	Two males, two lower jaws, three upper alveoli, four spurs
MZS Mam4538	Skull, two pairs of dentaries
MZS Mam4539	Skull, denary, four cornules
MZS Mam4540	Cornules, two lower, one upper
MZS Mam4541	Anterior dentaries, upper left mx, pmx, cornules
MZS SM4	Mx, pmx, dentaries- alveoli and anterior, damaged cornules

Table 2-1: List of adult specimens examined for this study. Mx - maxilla; pmx - premaxilla.

METHODS

One specimen in the collections at the Vertebrate Paleontology Laboratory at the University of Texas at Austin was investigated using CT, SEM, and conventional photography. The skull of an adult platypus, TMM M-5899, was scanned at The University of Texas High-Resolution X-ray CT Facility with 1024x1024 16-bit pixel resolution in a field of reconstruction of 43 mm. Each of the 1998 slices has a slice and inter-slice thickness of 0.04529 mm. Tubules per plate were counted on 2d printouts of cross-sectional CT data through the indentations of plates. Areas of least density corresponding to tubules were manually counted in groups of 10 per plate and summed. The range of tubules comes from tubules on the upper and lower dried cornules of an adult platypus TMM M5899.

Parameters for tooth scans from the juvenile specimen (Grant Specimen) were provided in in Chapter 1. Those teeth were artificially overlain on the cornules of TMM M-5899.

Imaging Methods

Scanning electron microscopy (SEM) images are from a Quanta 650 scanning electron microscope; applicable scan parameters are provided in the figure captions. Loose cornules from TMM M-5899 were mounted on carbon tape. Permission to coat the sample was denied, and resulted in lower quality scans and artifact; however several features can still be identified. Charging is visible in many of the pictures, appears as bright streaks, and contributes to washed-out portions of images, and some poor focus. The cornules are imperfectly preserved, with damage visible at several scales. Larger scale damage is cracking, surface contamination, and large-scale damage although the smaller scale includes peeling of the epithelial layers and fraying around the edges, similar to poorly preserved baleen (Young 2012). These are not to be mistaken for morphological features of a fresh cornule.

Features that could not be observed using these methods (as opposed to comparative work using histology and light microscopy) are variations in color across the boundary between tubules and intertubular matrix, and relative thickness of the structures composing tubules around the hollow medulla. Comparisons of histology of other keratins with available CT data of platypus cornules are discussed as far as possible, and limits are discussed with respect to the methods.

RESULTS

Each cornule is composed of two large and one small plate (Figure 2.1). In both occlusal and root view the cornules of the adult are oval and taper in the direction of the smallest plate. In cross section, the anterior-most large plate is the thickest in each cornule, shown in cross section in Figure 2.6 C, D. The upper cornules have the smaller plate situated mesially and medially on the cornule under where a premolar (Figure 2.1 P, Figure 2.7) sits in the juvenile. The lower cornules have the smallest indentation positioned posteriorly (Figure 2.1 m3) and centrally on the plate replacing the reduced third molar (Thomas 1889). The smallest plate is most variable in size and shape.

Plates are composed of two main material densities, a solid border and perforate interior (Poulton 1888b) that show clearly at the surface in SEM (Figure 2.8) and throughout the thickness of the cornule in CT imagery (Figure 2.6, Figure 2.9). The denser material surrounds the indentations on the occlusal surface of the plates (Poulton 1888b). The less dense, perforate material lies topographically lower in the plate than the margins, approximately below the previous locations of the juvenile teeth (Figure 2.7). The smallest plate appears perforate throughout; it does not appear to have an imperforate perimeter (Figure 2.5).

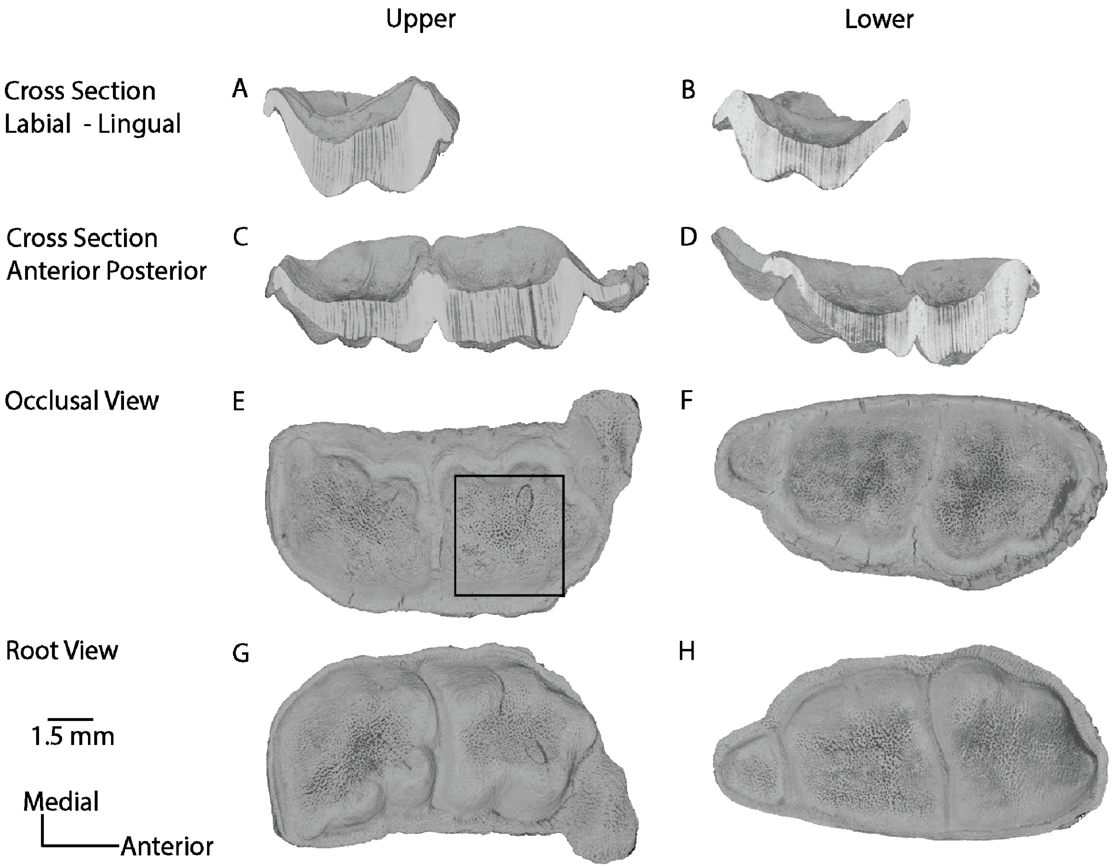

Figure 2.6. CT images of cornules, in cross section, occlusal view, and root view. Rectangle in E corresponds to rectangle in Figure 2.8. Specimen: TMM-5899.

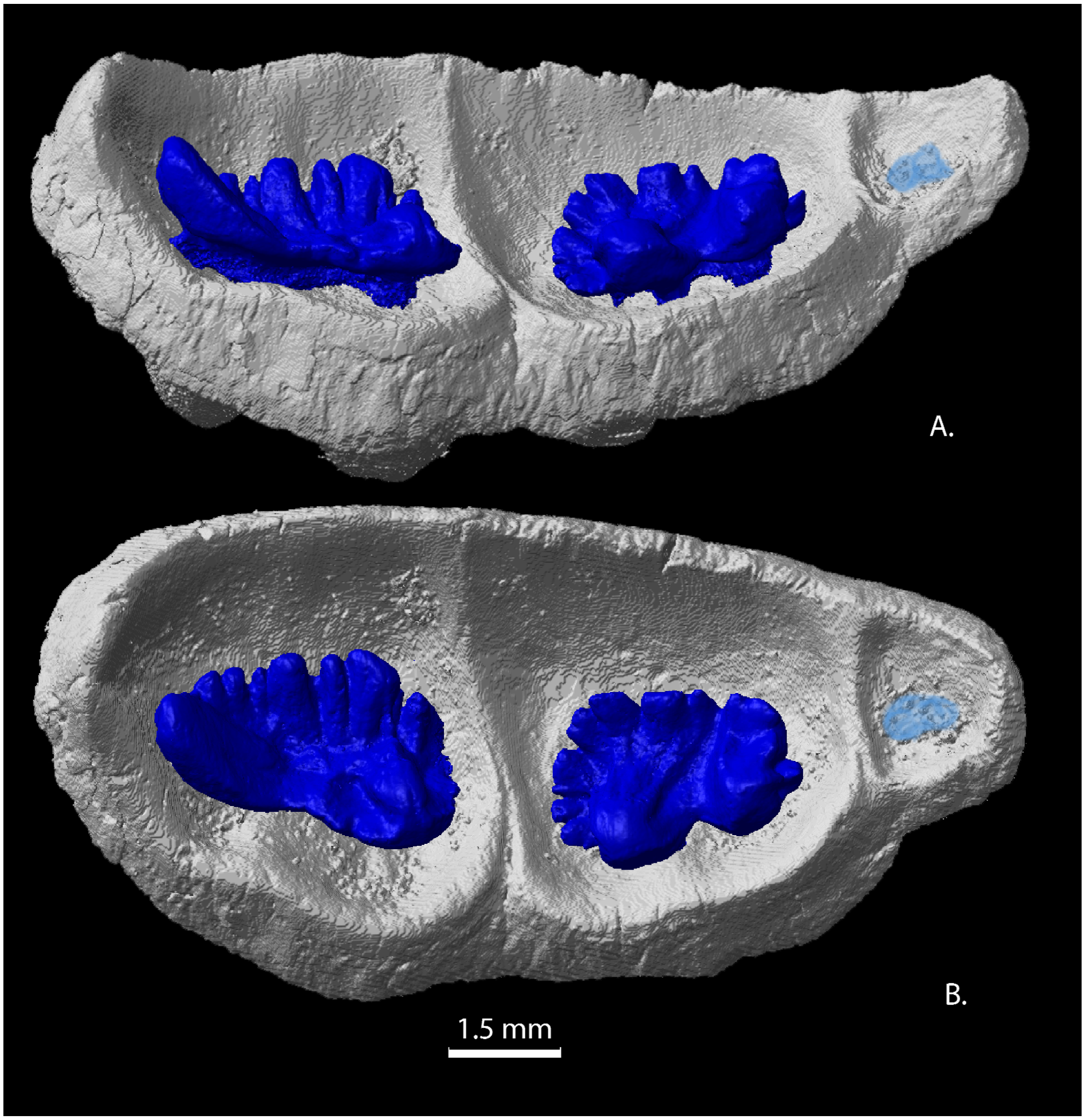

Figure 2.7. CT of composite of multiple individuals (teeth are from the Grant Specimen and cornules are from TMM M-5899) showing lower juvenile teeth in dark blue over a lower left adult cornule in grey, to scale. An approximate reconstruction of the third lower molar is shaded in light blue. The juvenile teeth were inverted to fit the plate, because no complete side was represented across specimens. A. Lateral view, anterior to the left; 2. Occlusal View, anterior to the left. Specimen: TMMM-5899.

Plates are composed of two main material densities, a solid border and perforate interior (Poulton 1888b) that show clearly at the surface in SEM (Figure 2.8) and throughout the thickness of the cornule in CT imagery (Figure 2.6, Figure 2.9). The denser material surrounds the indentations on the occlusal surface of the plates (Poulton 1888b). The less dense, perforate material lies topographically lower in the plate than the margins, approximately below the previous locations of the juvenile teeth (Figure 2.7). The smallest plate appears perforate throughout; it does not appear to have an imperforate perimeter (Figure 2.5).

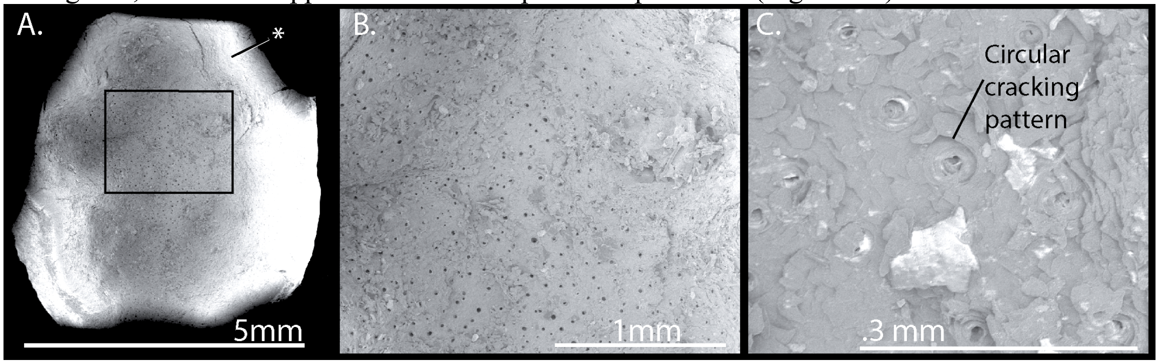

Figure 2.8. SEM images of upper keratin plate surface of TMM M-5899. Each image is zoomed in on an area of the former. (A.) Corresponding to rectangle in Figure 2.6, The perforate middle indentation of a plate is bordered by a dense imperforate region indicated with an asterisk (*). The surface of the perforated region (B.) shows holes of different sizes, magnified rectangle in A. Those holes are visible at higher magnification (C.), which also reveals a flakey texture to the plates partially a result of poor preservation. SEM parameters are as follows: detection is LFD, voltage was 3 kV, and working distance is 12.7 mm in A., 13mm in B. and C.

The perforations are tubules that penetrate the plate (Figure 2.5) and are visible holes at the surface (Figure 2.6). There are between 1200 and 1500 tubules per large pad, with a density of approximately 90 tubules per square millimeter in the central region of a plate. Tubules are loosely packed and vary in size ranging from 5 μm to 16 μm. There is no apparent pattern to the distribution of the tubules.

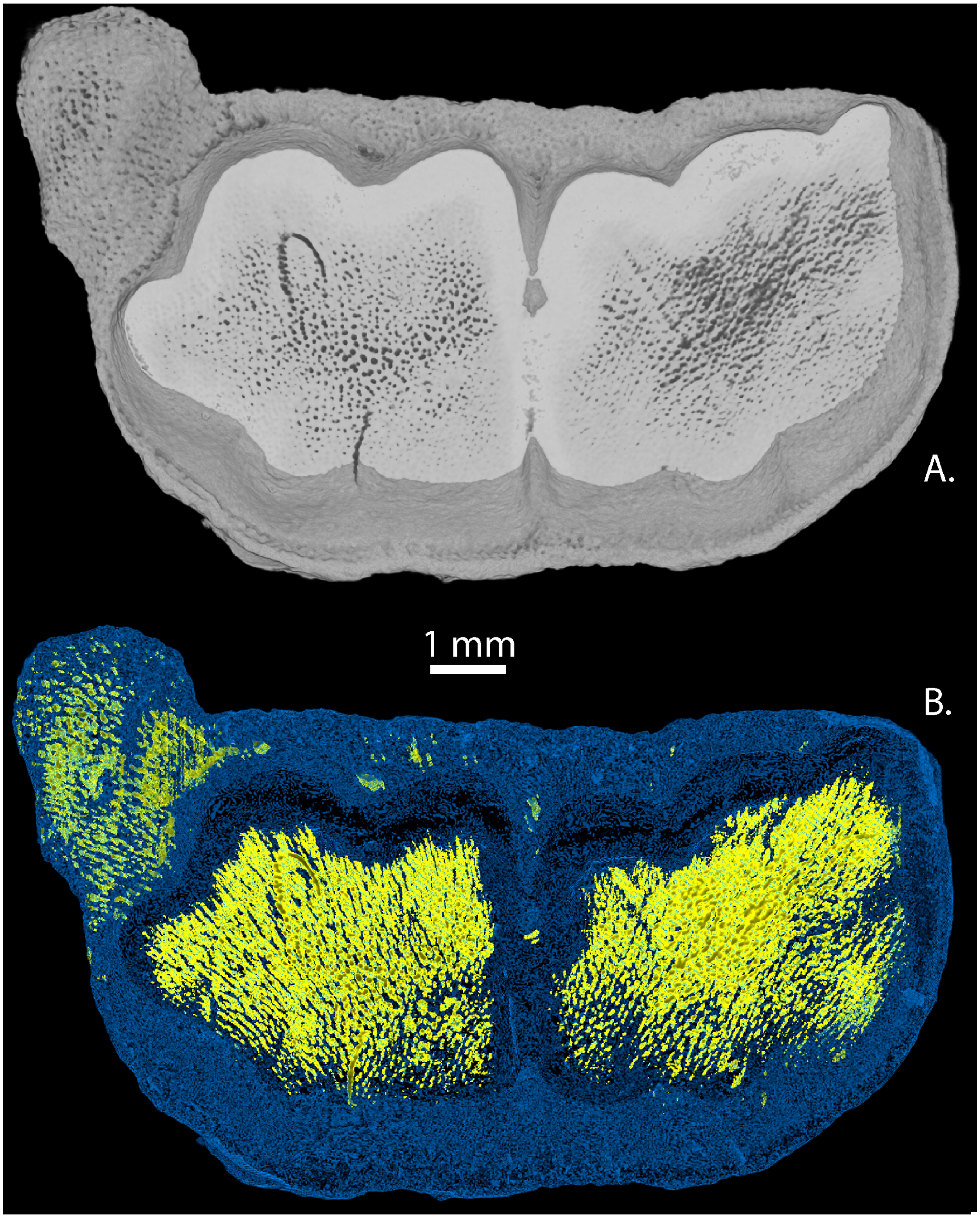

Figure 2.9. CT cross sections of an upper right cornule of TMM M-5899, showing (A) tubules as seen on a single plane, (B) the location of hollow spaces in the plate. The majority of the hollow spaces are centrally located and correspond to the tubules seen in A. Other hollow spaces are wrinkles in the epithelium or joints between plates. Specimen: TMMM-5899.

In an effort to provide some terminological continuity with keratinized structures, cornules are here compared with other keratin structures. Cornules, as far as keratinized structures go, share structural similarities with other mammalian keratins including hair, baleen, and rhinoceros horn. Strands of mammalian hair, tubules in baleen (Young 2012), and tubules in rhinoceros horn (Hieronymus et al. 2006) all have three layers, the cortex, cuticle, and medulla. The circular cracking pattern in cornule keratin surrounding the hollow tubules (Figure 2.8) and the tube cross-sections drawn from thin section by Poulton (1888b; Figure 2.10) are reminiscent of structural differences seen in other keratins, suggesting the same three layers. The hollow space, tubules, here would correspond to the medulla (as in baleen) and the cortex and cuticle would surround it, differentiated by the darker pattern in cross section. There is a differentiation of a homogenous white layer externally, followed by darker rings, and finally a hollow section internally (Figure 2.10; Poulton 1888b). Rhinoceros horn is also of epidermal origin, composed of irregular hair-like filaments of keratins packed rather densely with a filled intermedullary space between the strands holding them together (Hieronymus et al. 2006). A similar pattern is seen in most baleen; the medulla commonly is hollow or filled with cellular debris (Young 2012), similar to the hollow tubules in cornules, and once again with strand-like features held together with a solid intermedullary space. Comparing the CT data of tubule structure (Figure 2.11) in cornules with rhinoceros horn reveals additional similarities. Both are characteristics of an intertubular matrix, irregular medulla size, broadly heterogeneous composition, and a characteristic irregular packing pattern. The tubule packing pattern is more dispersed in the platypus than in rhinoceros horn, and serves a different function. The microstructure of the cornules (Figure 2.13) has a superficially similar configuration to non-linear ridges in baleen (Young 2012). There is more room for structural comparative histology of these tissues; however I here use the structural terminology to describe the structures (Figure 2.10) seen in the keratin plates here for greater clarity than that found in previous literature.

Damage under SEM

Specimen TMM M-5899 had damage to the cornules visible under SEM that was similar to that reported previously in baleen (Young 2012), including cracking, peeling, and sheeting (Figure 2.13).

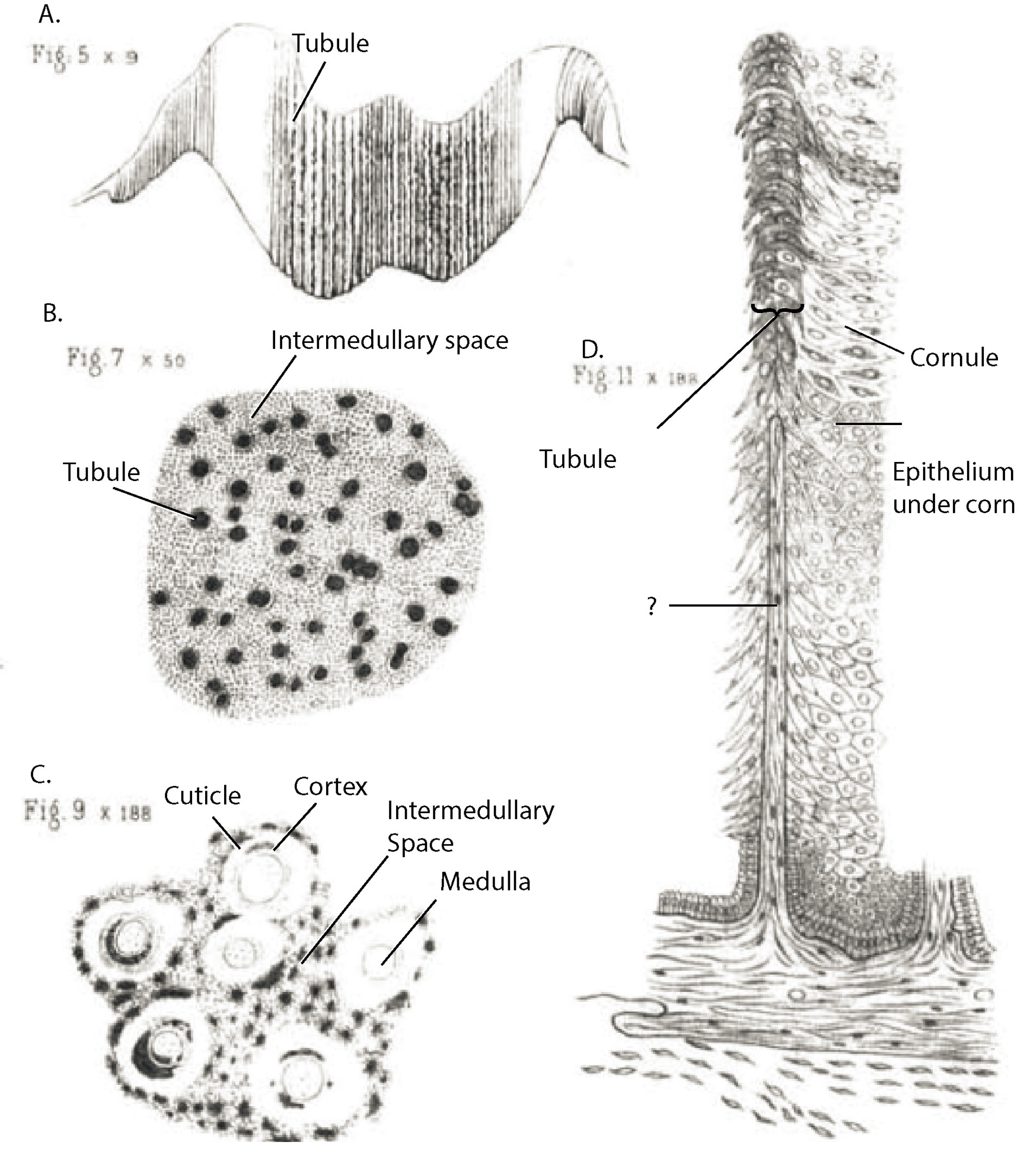

Figure 2.10. (A) Cross-section of tubules through cornules. (B) Horizontal cross-section. (C) High magnification drawing of the tubules in cross-section. (D) Cross-section through an upper rear epithelium. Figures modified from Poulton 1888b, original figure numbers retained.

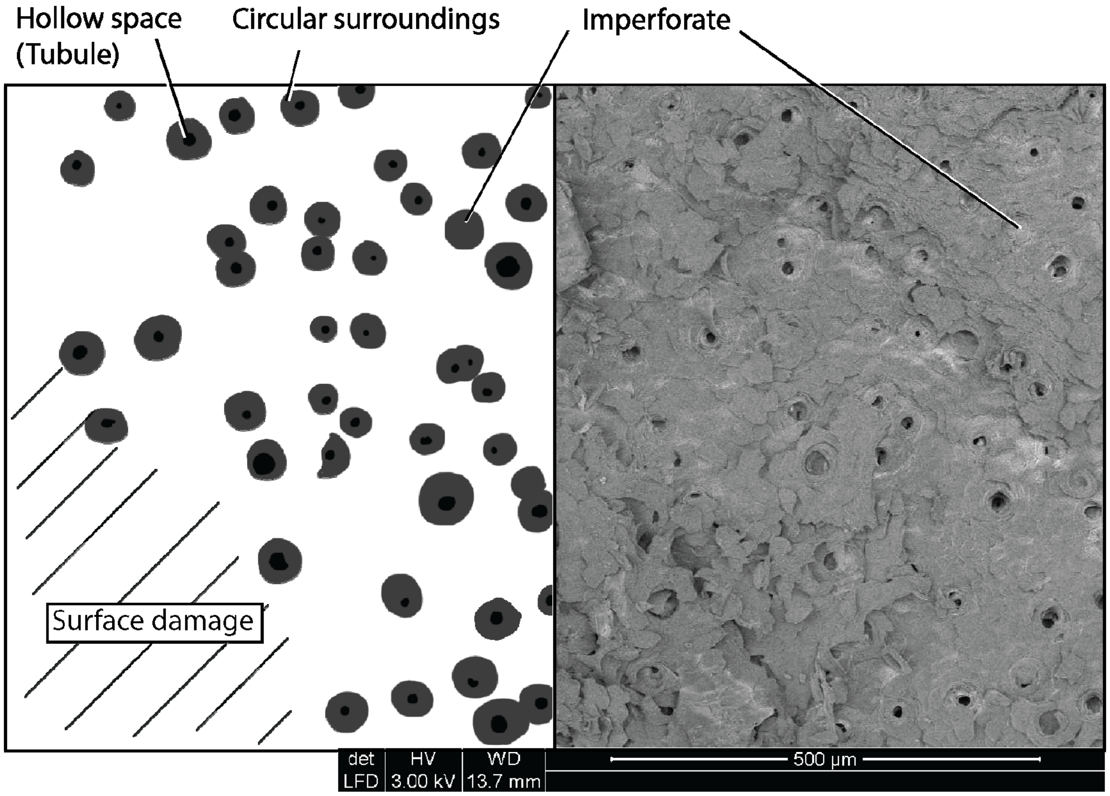

Figure 2.11. Distribution and packing pattern of tubules on the cornule surface of TMM M-5899. Left: dark grey circles correspond to the cuticle and cortex, black circled to the medulla from Figure 2.10. Right: the region where the tubules are located. Zoom from Figure 2.8.

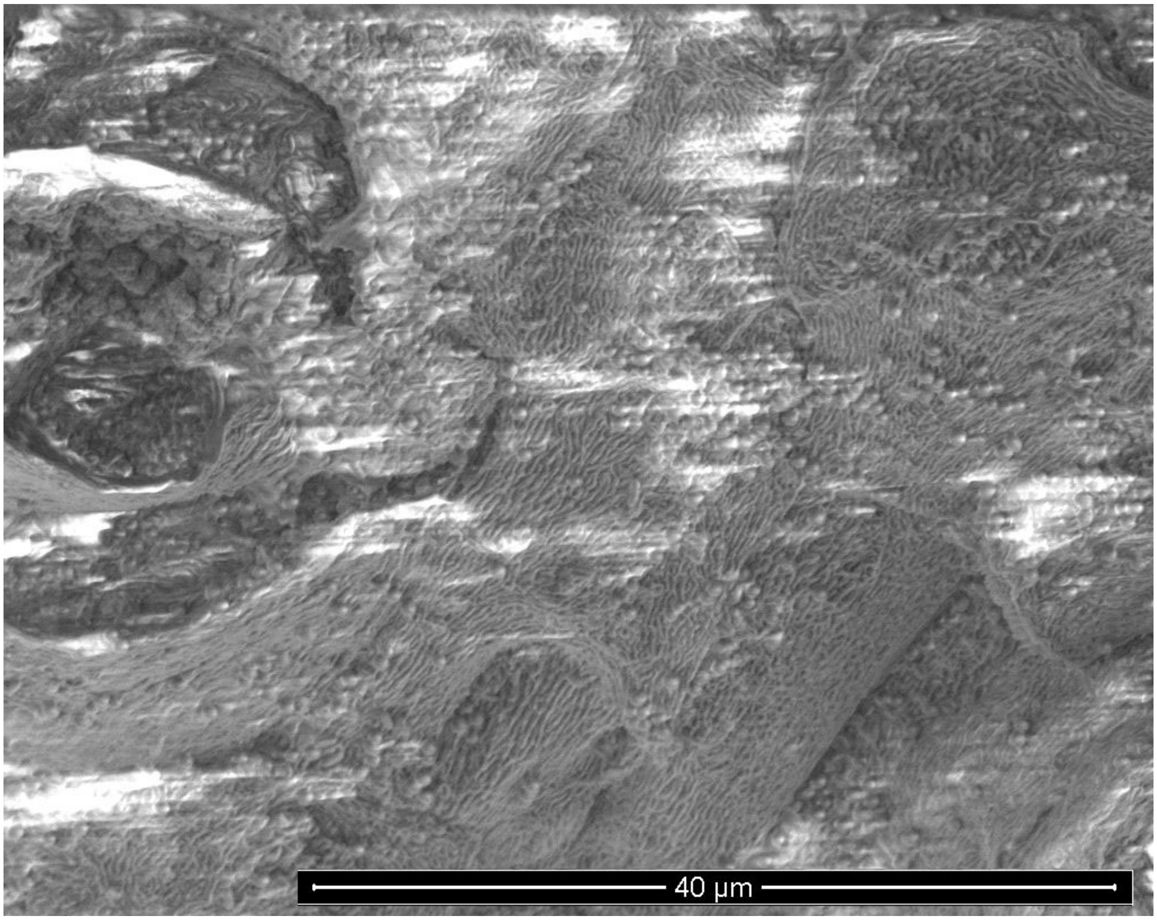

Figure 2.12. Close up of the surface of the cornule from TMM M-5899. This image is blurry because of charging, the specimen was uncoated. Tubule structure is visible to the left. SEM parameters: Working distance is 12.6mm, voltage 3kV. Specimen: TMMM-5899.

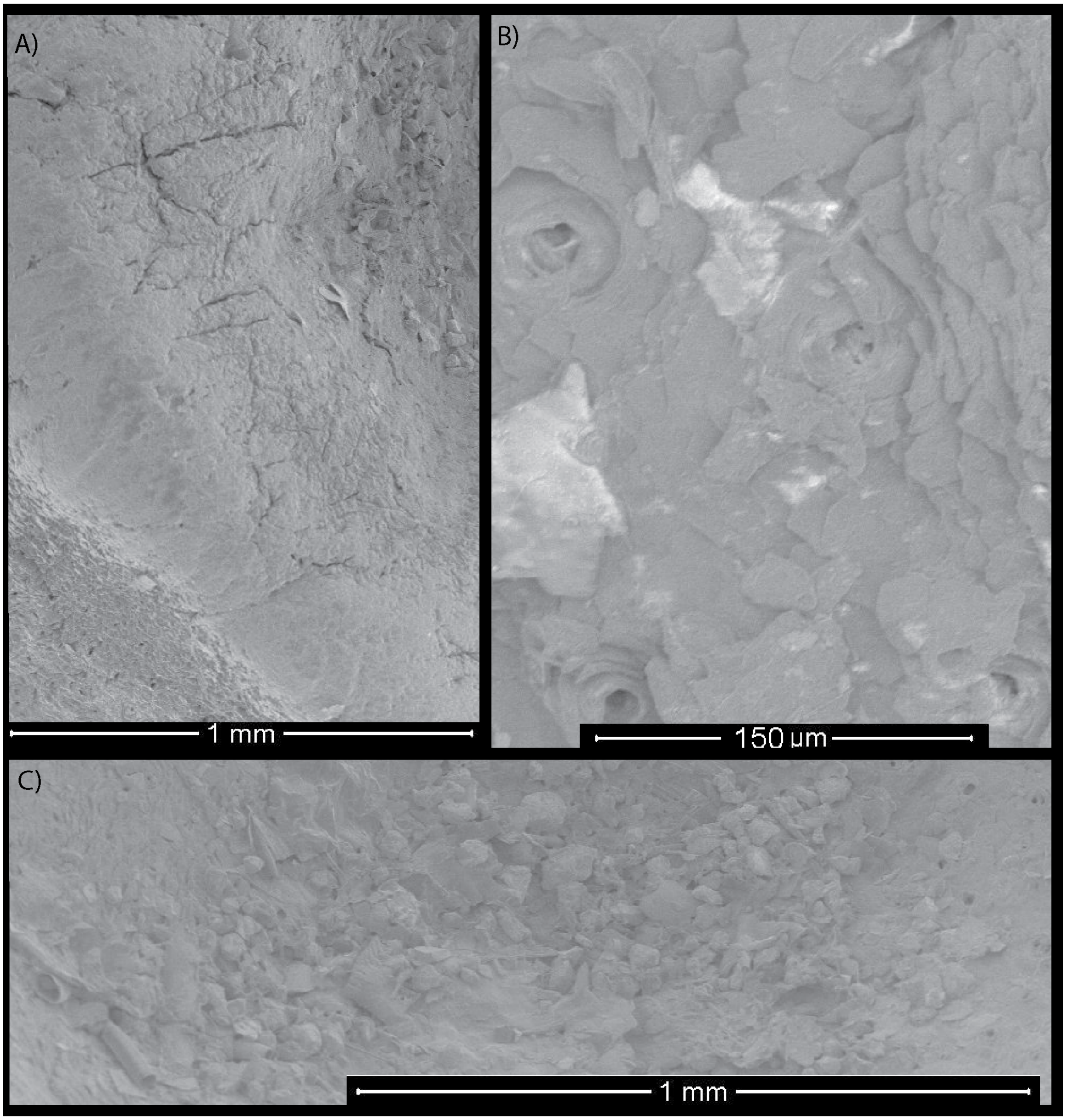

Figure 2.13. SEM images showing poor preservation of cornules of specimen TMM M-5988. (A.) Cracking visible at working distance 12.6mm, (B.) peeling, visible at working distance of 13mm (C.) surface contamination visible at working distance of 13mm. Each was scanned at a voltage of 3.00kV.

DISCUSSION

Sensory potential of the tubules/ Structure and innervation

The purpose or function of the tubes in the cornule is unknown, and the question has been largely neglected in the 21st century. Histology of both the cornule tubes and bill mechanoreceptors was illustrated in cross section by Poulton (1888b, 1894), showing similar structures below the tubules and within the epidermis of the bills. Comparison of these drawings have been interpreted as evidence of enervation, specifically mechanosensory ‘push-rods’ (Wilson et al. 1893). The oral keratin of the platypus was proposed to serve a sensory function (Wilson et al. 1893) because cells in the tubules of the epithelial plates of Poulton (1888b) are similar to those in the bill epidermis (Poulton 1884), which are now known to have a sensory function (Scheich et al. 1986). Differences between those systems were emphasized by Poulton (1894), who cited his own work from 1884, stating the tubules and sensory cells on the bill are not the same (Poulton 1894). Despite a strong observational similarity between the organs of the bill and cornules, sensory capabilities of the cornules seem unlikely because the putative push receptors are located at the topographic low of the indentations on the cornule, and the indentations are found packed full of debris (dirt and exoskeletons) as sometimes described in the literature (Thomas 1889) and seen in museum specimens during this study (Figure 2.1 A.). Tooth loss in monotremes may be related to several aspects of the bill. Increased sensory systems in the bill may have competed for space with tooth roots, or tooth loss may have may have given the opportunity for enervation in the cornules. Additionally, as investigated later, the teeth may be lost through evolution from to extinct monotremes to today due to a need for ever-growing teeth to deal with high sediment load

Derivation of the cornules

An understanding of teeth and odontodes, potentially the evolutionary precursors to teeth, is necessary to know the identity of the cornules. In order to determine if they are teeth in any way, they must meet some defining criteria of teeth; they must show some similarity in characteristics of structural composition, formation, or evolutionary continuity, including innervation.

In the most basic sense, teeth require few things: an attachment to bone, dentine, and sometimes enamel (Fraser et al. 2010). Additionally tooth structure generally also includes a pulp cavity. Odontodes, generally considered an evolutionary tooth precursor, require development from an undivided dental papilla with epithelium adjacent to a dental organ, consist of dentine or dentinous tissue, frequently possess enameloid, form from the upper level of the cornium, generally do not carry out the same functions as teeth, and are generally smaller than teeth (Ørvig 1977). These characteristics are missing from the epithelial plates. Compared to teeth they are not biomineralized epidermal tissues (Poulton 1888b, Ungar 2010 p.163), they lack a pulp cavity, are not connected to bone (Poulton 1888b), and have neither dentine nor enamel. They also are dissimilar from odontodes as described by Ørvig (1977) because cornules consists of neither dentine nor enamel. In so far as is known, cornules do not form from dental papilla. Cornules do carry out tooth function, are larger than the teeth of the juvenile platypus, and probably maintain continuity of enervation, which may support a tooth origin. The only similarity with odontodes is formation from the upper level of the cornium. Structurally, cornules do not resemble teeth or odontodes.

Tooth formation is induced by interaction of epithelial (endoderm or ectoderm) cells with neural crest cells (Fraser et al. 2010). If there are unreplaced premolars and adult molars present in the dentition of juvenile *Ornithorhynchus anatinus* (discussed in Chapter 1), it is likely an adult dentition. It is thought that the cornules grow only from the epithelium around and under the molars and premolar of the juvenile platypus. There is potential for further research in the genetics behind cornule formation and the involved tissues, but it is unlikely that cornule formation parallels teeth.

Shared evolutionary origin is most difficult to assess. Without any indication of replacement in extinct monotremes, the ancestral state for the number of tooth generations is unknown. The minimum number of likely tooth generation is one, suggested by the monotreme record which contains no evidence of replacement. A probable maximum of two is suggested by the ancestral state for tooth number in fossils from extinct therian mammals (Luo et al. 2004). A larger number of ancestral monotreme tooth generations is possible given the polyphyodont replacement of non-mammalian cynodonts (Luo et al. 2004), it is, however, unlikely. It is doubtful that cornules represent a changed replacement set of teeth, as is discussed above in addition to an argument that the teeth from the juvenile platypus belong to an adult dentition (discussed in Chapter 1). No other mammal has a replacement set of teeth subsequent to adult molars, and no adult molars have dental precursors. If some of the teeth from the juvenile are molars as hypothesized here, and monotremes have similar constraints on replacement as therian mammals, the molars in the juvenile platypus would have no tooth replacements. Cornules probably represent something without known analog in the dentitions of mammals, and are most likely not homologous to other teeth by shared evolutionary origin.

In addition to the previous criteria, continuity of innervation is occasionally used to support homology. As is well known, innervation of teeth on the dentary is accomplished through the mandibular branch of the trigeminal nerve, and this appears consistent with the juvenile platypus. In platypus, the mandibular branch continues anteriorly to Innervate the lower portion of the bill. After the teeth of the juvenile are lost, the cornules may be innervated by the same nerve. The debate outlined previously concerning the innervation of the cornules suggests a possibility of some sensory function associated with the tubules and underlying structures. This requires further study, and is mentioned here as another possible test for homology.

As was stated in the past, the fact that cornules develop directly from the surrounding epithelium, combined with the presence of a mineralized precursor, suggests the epithelial plates are unlikely to represent degenerate teeth (Thomas 1889, Stewart 1891). However the cornules take over the function and position of the juvenile teeth, and are potentially innervated by the same nerve, so more research is needed.

Feeding and cornule use

Food processing in the platypus is as unique among mammals as the platypus bill shape. Their main food sources are arthropods and crustaceans, and although they seem to take most anything they can catch (Grant 1982), they do display prey preferences (McLaughlan-Troup et al. 2010). They do not use the bill for extended food processing, instead they use it to locate, capture, and probably kill prey before moving food items to the buccal cavity (Grant 1892). After catching prey, the items are stored in cheek pouches and processed at the water surface by the cornules contained in the buccal cavity (McLachlan-Troup et al. 2010) presumably with aid of the tongue, ‘tongue teeth,’ and ridges on the palate. The platypus’ main food sources are relatively soft: arthropods, annelids, and crustaceans (Grant 1982), but platypus may use sediments to help process exoskeletons, because they tend to prefer sandy river substrates for foraging (Grant 2007). The filter structure on the bottom jaw outside the buccal cavity is then used to selectively remove unwanted bits of exoskeleton (McLaughlan-Troup et al. 2010). Interestingly, juvenile platypus in captivity chew mealworm food, but often need help to remove the exoskeletons (Booth et al. 2008).

Platypuses are commonly found with cheek pouches (Grant 1982) and cornules (Thomas 1889, this paper, Figure 2.1) containing sediment. In such high-sediment feeding environments, teeth would be subject to intense wear, in some ways similar to mammals that forage on silicarich or sandy plants. Most mammals are limited to two sets of teeth (Luo et al. 2004), and those feeding in adverse conditions circumvent the limitation with high tooth crowns or ever-growing teeth. Extant platypuses circumvented the restriction of a limited dentition with a unique solution. Instead of developing higher teeth to grind, platypuses get rid of teeth and have ever-growing keratin plates in their place. The habitat provides the material to aid mastication; water and sand probably contribute to chewing in adult platypuses.

CONCLUSION

The cornules of the adult platypus are a puzzle unto themselves, but an important part of the evolution *Ornithorhynchus*. Juvenile platypus teeth are involved with the development of the epithelial plates as the epithelium forms around the teeth and the teeth leave an impression on the epithelium, but the precise interaction has not been studied. The epithelial plates differ in density across their surface. The epithelial plates still hold secrets concerning their internal structure and innervation. They represent a new way of circumventing the limited dentition of mammals, on par with the evolutionary solutions found among durophagous animals such as horses, cows, elephants, rodents and lagomorphs. The reduction of the enameled dentition in platypus is a circumvention of the limits of an enameled dentition, rather than a reduction in the need for mastication. This way, the platypus can continue to eat in high-sediment environments without losing chewing power because of sediment abrasion to teeth. The evolutionary derivation of ever-growing cornules in platypus suggests an increased reliance on masticating food in association with sediments, consistent with a change to an increasingly aquatic lifestyle, or a shift in diet in a previously aquatic lifestyle from free-swimming to substrate-dwelling prey.

For future work

Comparing monotreme keratins to those from better-studied mammals may yield new insights on the functions of the cornule structures. Thin sections of keratins such as baleen

(Young 2012), mammal hair, and rhinoceros horn (Hieronymus et al. 2006) show structures similar to the tubules and sheets in cornules. In this study cornule thin sections could not be made for permission and logistical reasons. However, the features, such as the structure in the medulla, and similarities under light microscopy to other keratin in color and structure, that could be observed from thin sections or histological study in fresh platypus would elucidate the disputed functions of features. Additionally, because genetic pathways for tooth formation are highly conserved within vertebrates (Jernvall et al. 2012), an examination of the genetic basis of cornule development would test the developmental homology of cornules with teeth.

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