Three-dimensional molar enamel distribution and thickness in *Australopithecus* and *Paranthropus*

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Thick molar enamel is among the few diagnostic characters of hominins which are measurable in fossil specimens. Despite a long history of study and characterization of *Paranthropus* molars as relatively 'hyper-thick', only a few tooth fragments and controlled planes of section (designed to be proxies of whole-crown thickness) have been measured. Here, we measure molar enamel thickness in *Australopithecus africanus* and *Paranthropus robustus* using accurate microtomographic methods, recording the whole-crown distribution of enamel. Both taxa have relatively thick enamel, but are thinner than previously characterized based on two-dimensional measurements. Three-dimensional measurements show that *P. robustus* enamel is not hyper-thick, and *A. africanus* enamel is relatively thinner than that of recent humans. Interspecific differences in the whole-crown distribution of enamel thickness influence cross-sectional measurements such that enamel thickness is exaggerated in two-dimensional sections of *A. africanus* and *P. robustus* molars. As such, two-dimensional enamel thickness measurements in australopiths are not reliable proxies for the three-dimensional data they are meant to represent. The three-dimensional distribution of enamel thickness shows different patterns among species, and is more useful for the interpretation of functional adaptations than single summary measures of enamel thickness.

Keywords: hominin evolution; microtomography; enamel distribution

1. INTRODUCTION

Thick molar enamel is among the few diagnostic characters of hominins observable in the fossil record

(e.g. Martin 1985; Strait *et al.* 1997). Relatively thick enamel distinguishes hominins from thin-enamelled African apes, and enamel thickness is discussed in the seminal diagnosis of nearly every newly described hominin taxon (e.g. Leakey *et al.* 2001; Senut *et al.* 2001; Brunet *et al.* 2002). Thick molar enamel is often associated with the mastication of hard or abrasive foodstuffs, although recent evidence indicates that enamel thickness may not relate directly to the primary dietary strategies of hominins (Ungar *et al.* 2008). Although much is said about thick enamel in hominins, few fossil molars have been measured using standardized techniques. Previous studies employed tooth fragments or cross sections produced manually or via medical CT (e.g. Beynon & Wood 1986; Grine & Martin 1988; Macho & Thackeray 1992; Schwartz *et al.* 1998). These cross sections were designed to yield proxy measures of the entire tooth crown, since medical imaging techniques were insufficient to record the whole-crown measurements at the time of their development (Martin 1985). These two-dimensional methods prohibit the examination of enamel thickness distribution over the entire molar crown (*sensu* Kono 2004; Olejniczak *et al.* 2008*a*).

Recently, non-destructive three-dimensional micro-CT techniques have been applied to the study of enamel thickness in several taxa, including recent and fossil hominoids (Kono 2004; Tafforeau 2004; Olejniczak *et al.* 2008*a,b*), recent humans (Suwa & Kono 2005), Neandertals (Olejniczak *et al.* 2008*c*) and fossil *Homo sapiens* (Smith *et al.* 2006, 2007). The australopith fossil record, however, has not been systematically studied using modern techniques (e.g. Macchiarelli *et al.* 2004). Here, we measure molars of two australopith taxa (*Australopithecus africanus* and *Paranthropus robustus*), with the aim of documenting fossil hominin enamel thickness using a whole-crown, three-dimensional technique. We also compare two-dimensional sections (analogous to those of previous studies) with three-dimensional data, to assess the impact of methodological differences on the enamel thickness measurements.

2. MATERIAL AND METHODS

(a) *Study sample*

Fully formed and unworn fossil teeth are rare; but three-dimensional measurements require that the entire volume of the enamel cap is present with only minor breakage and attrition. From the Transvaal Museum and University of the Witwatersrand, 18 molars were suitable for measurement (table 1). In some cases, molars from the same individual were included (e.g. STW 421a and STW 421b). Digital reconstructions of broken cervical enamel were made when necessary. Scans were produced with a Sky scan 1172 at 100 kV and 100 mA, with aluminium and copper filters. Voxel dimensions for each specimen were 13.98 µm^3. Enamel and dentine were digitally segmented; virtual models depicting the three-dimensional distribution of enamel were created (figure 1). Comparative data for extant primates derive from Olejniczak *et al.* (2008*b*); modern human and Neandertal data are from Olejniczak *et al.* (2008*c*).

(b) *Three-dimensional measurements*

Enamel thickness data were recorded for each molar as follows: enamel volume (mm^3), coronal dentine volume (mm^3) and enamel–dentine junction (EDJ) surface area (mm^2). Two indices were calculated as follows: average enamel thickness (three-dimensional AET; the volume of the enamel cap divided by the surface area of the EDJ, yielding the average straight-line distance between the EDJ and the outer enamel surface); and relative enamel thickness (three-dimensional RET; three-dimensional AET divided by the cube root of coronal dentine volume, then multiplied by 100, yielding a scale-free enamel thickness index suitable for intertaxon comparisons).

Table 1. Two-dimensional and three-dimensional enamel thickness data for each australopith specimen.

| taxon	tooth	accession	enamel volume (mm^3)	dentine volume (mm^3)	EDJ surface area (mm^2)	three-dimensional average enamel thickness (mm)	three-dimensional relative enamel thickness	mesial section enamel area (mm^2)	mesial section dentine area (mm^2)	mesial section EDJ length (mm)	mesial section average enamel thickness (mm)	mesial section relative enamel thickness
A. africanus	mandibular LM2	STW 3	540.81	711.18	363.86	1.49	16.65	43.48	44.18	20.89	2.08	31.32
A. africanus	mandibular LM2	STW 421B	503.57	589.82	409.39	1.23	14.67	31.75	61.82	24.41	1.30	16.54
A. africanus	mandibular LM3	STW 412B	345.47	531.32	353.52	0.98	12.07	25.57	44.36	21.43	1.19	17.91
A. africanus	mandibular LM3	STW 529	554.49	561.71	347.45	1.60	19.34	42.57	51.64	22.10	1.93	26.81
A. africanus	mandibular LM3	STW 560B	551.06	777.22	394.43	1.40	15.20	39.96	63.04	24.03	1.66	20.94
A. africanus	mandibular RM1	STS 9	468.27	486.31	227.88	2.05	26.13	36.01	42.59	19.87	1.81	27.76
A. africanus	mandibular RM1	STW 421A	483.31	668.33	368.77	1.31	14.99	30.32	60.37	24.88	1.22	15.69
A. africanus	maxillary LM1	STS 57	382.29	491.08	240.58	1.59	20.14	33.93	46.61	22.05	1.54	22.54
A. africanus	maxillary LM3	STW 179	480.39	573.15	288.14	1.67	20.07	42.23	55.78	22.08	1.91	25.60
P. robustus	mandibular RM1	DNH 60B	383.29	387.34	283.16	1.35	18.57	31.10	38.50	20.09	1.55	24.95
P. robustus	mandibular RM1	DNH 67	469.41	383.89	201.39	2.33	32.07	33.21	36.49	18.77	1.77	29.29
P. robustus	mandibular RM1	SK 3974	563.39	445.73	303.17	1.86	24.33	40.77	44.26	20.12	2.03	30.47
P. robustus	mandibular RM2	DNH 60C	485.52	410.35	312.07	1.56	20.94	39.93	36.30	18.57	2.15	35.68
P. robustus	maxillary LM1	SK 102	591.98	498.18	248.18	2.39	30.09	49.81	56.79	23.10	2.16	28.61
P. robustus	maxillary LM1	SK 832	642.42	544.84	381.78	1.68	20.60	50.72	54.75	23.61	2.15	29.03
P. robustus	maxillary LM3	SK 41	574.60	620.92	419.66	1.37	16.05	47.18	67.07	23.40	2.02	24.62
P. robustus	maxillary RM3	SK 31	624.15	783.59	320.62	1.95	21.12	48.50	75.04	24.90	1.95	22.49
P. robustus	maxillary RM3	TM 1517C	682.82	493.60	336.20	2.03	25.70	61.54	69.90	24.28	2.53	30.31
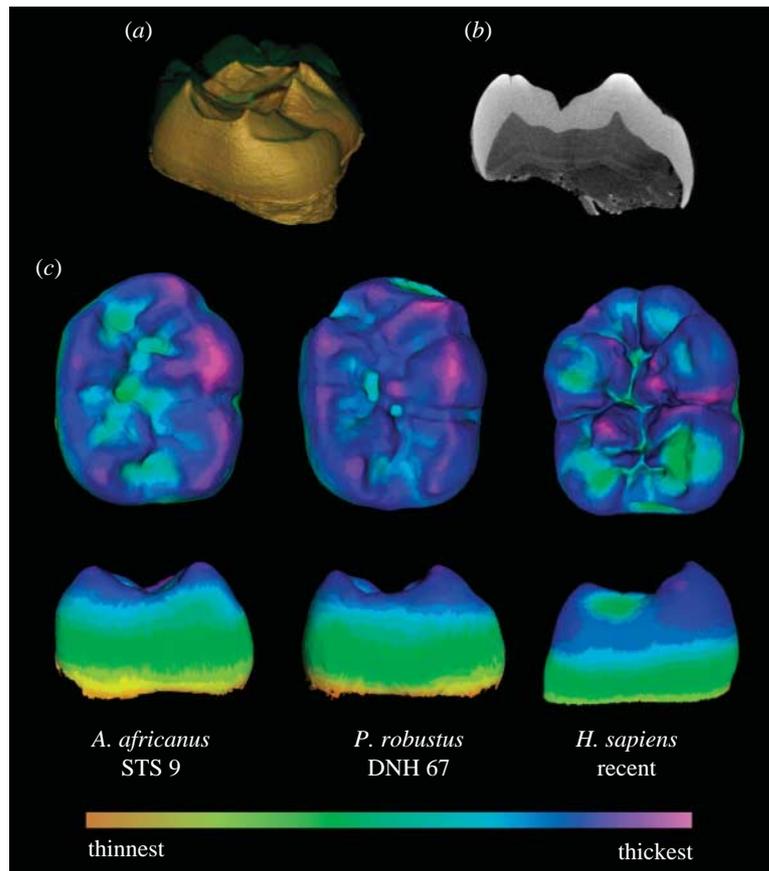
Figure 1. Micro-CT reconstructions are as follows: (*a*) segmentation of enamel and dentine; (*b*) section through the mesial cusps of an *Australopithecus* molar; enamel over cusp tips in this section is thicker than the lateral enamel; (*c*) enamel thickness distribution in mandibular molars; the scale of enamel thickness is relative to each tooth. *Paranthropus* and *Australopithecus* molars have thicker enamel at the cusp tips than at the lateral aspect of the cusps.
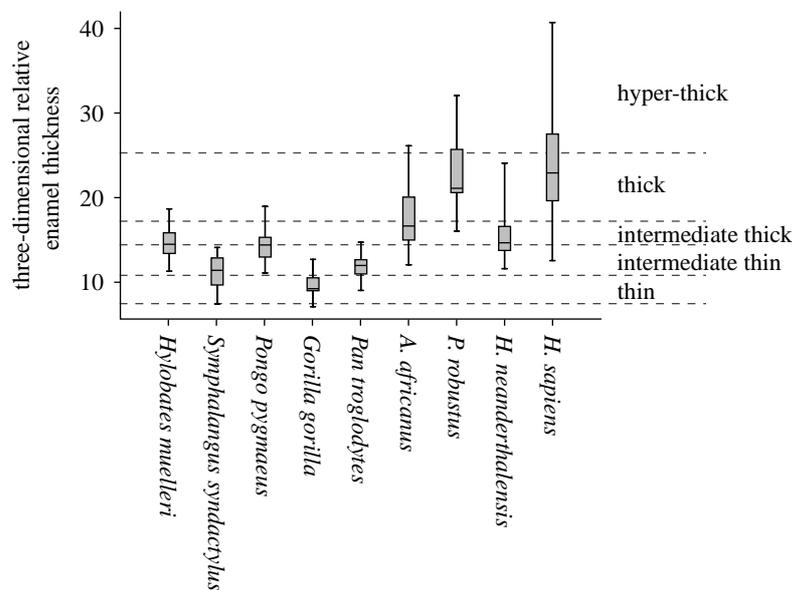

Figure 2. Plot depicting three-dimensional RET ranges. Line inside the box represents the mean, ends of boxes are 25th and 75th percentiles and whiskers represent the range. Martin's (1985) thickness categories (and Grine & Martin's 1988 hyper-thick category) are shown to the right, and as dashed lines.

(c) *Two-dimensional measurements*

Virtual mesial-cusp planes of section mimicking the physically produced sections of earlier studies (figure 1) were created using VoxBlast software (Vaytek, Inc.); the accuracy of virtual sections and the compatibility of virtual and manually produced sections have been established elsewhere (Olejniczak & Grine 2006). Following Martin (1985), two-dimensional measurements include: enamel cap area (mm^2), coronal dentine area (mm^2) and EDJ length (mm). Average enamel thickness (two-dimensional AET) is the area of the enamel cap divided by the length of the EDJ, yielding the average straight-line distance from the EDJ to the outer enamel surface. Relative enamel thickness (two-dimensional RET) is two-dimensional AET divided by the square root of coronal dentine area, then multiplied by 100, yielding a scale-free index suitable for interspecies comparisons.

Table 2. Two-dimensional and three-dimensional enamel thickness measurements (mean) in fossil hominins and recent hominoids (ordered on three-dimensional relative enamel thickness).

	H. sapiens[a]	P. robustus	A. africanus	H. neanderthalensis[b]	Hylobates muelleri[c,d]	Pongo pygmaeus[c,e]	Pan troglodytes[c,e]	Symphalangus syndactylus[c,d]	Gorilla gorilla[c,e]
three-dimensional sample size	39	9	9	29	11	12	26	17	9
enamel volume (mm³)	218.91	557.51	478.85	225.47	26.37	197.91	137.00	62.29	372.01
dentine volume (mm³)	226.83	507.60	598.90	345.65	36.88	336.05	166.05	119.60	1023.18
EDJ surface area (mm²)	162.56	311.80	332.67	211.60	53.80	199.75	182.67	116.21	375.89
three-dimensional AET (mm)	1.43	1.83	1.48	1.08	0.49	1.01	0.75	0.55	0.98
three-dimensional RET	23.97	23.27	17.70	15.55	14.72	14.49	11.80	11.15	9.77
Two-dimensional sample size	257	9	9	42	11	41	40	17	15
Enamel area (mm²)	24.19	44.75	36.20	21.97	4.44	23.42	14.63	7.30	29.37
dentine area (mm²)	38.73	53.23	52.27	41.65	8.47	50.93	36.95	18.50	79.29
EDJ length (mm)	19.60	21.87	22.42	20.75	10.13	21.34	19.47	13.53	28.25
two-dimensional AET (mm)	1.22	2.03	1.63	1.06	0.44	1.10	0.75	0.54	1.04
two-dimensional RET	20.06	28.38	22.79	16.44	15.27	15.49	13.23	12.58	11.68

[a] Two-dimensional data from Smith *et al.* (2006).
[b] Data from Olejniczak *et al.* (2008c).
[c] Three-dimensional data from Olejniczak *et al.* (2008b).
[d] Two-dimensional data from A. J. Olejniczak (2006, unpublished Ph.D. dissertation).
[e] Two-dimensional data from Smith *et al.* (2005).

(d) Statistical analysis

Species differences in three-dimensional AET and three-dimensional RET were tested using the rank-based Kruskal–Wallis test with *post hoc* comparisons. Bootstrap resampling was performed on species mean values of three-dimensional AET and three-dimensional RET to determine confidence intervals (electronic supplementary material (esm)).

3. RESULTS AND DISCUSSION

Among hominins, *P. robustus* has the thickest enamel on average (two-dimensional AET and three-dimensional AET). Interspecies overlap in three-dimensional RET is abundant (figure 2), but statistically significant differences are apparent. *Paranthropus robustus* has significantly greater three-dimensional RET than all taxa except *H. sapiens*; *A. africanus* has significantly thinner three-dimensional RET than other hominins except *Homo neanderthalensis* (esm). The *P. robustus* molars derive from three localities and (potentially) three different geological ages, but relative enamel thickness does not appear to differ substantially among localities (table 1). The australopiths evince no clear differences in three-dimensional AET and three-dimensional RET among tooth positions, although small sample sizes prevent rigorous intrataxon analyses.

Measurements in two and three dimensions yield different results (table 2). Three-dimensional RET shows that *H. sapiens* and *P. robustus* have similarly thick enamel, while *A. africanus* and *H. neanderthalensis* have thinner enamel. However, two-dimensional RET is greatest in *P. robustus*, followed by *A. africanus*, *H. sapiens* and *H. neanderthalensis*, in rank order. Enamel distribution maps show that australopith molar enamel is thickest over cusp tips, while recent humans have thicker enamel surrounding cusp bases (figure 1). Previous studies employed cross sections deliberately made through cusp tips; hence the thickness of molar enamel is exaggerated in those sections. Our two-dimensional results are similar to those of Grine & Martin (1988), but such cross-sectional proxy measures do not reliably predict three-dimensional enamel thickness in these australopiths. The 'hyper-thick' category devised to accommodate *P. robustus* (Grine & Martin 1988) is not warranted when three-dimensional data are considered (figure 2).

Categories erected by Martin (1985) classify hominin molar enamel as relatively thick compared with that of African apes; this dichotomy is widely employed in studies of late Miocene and early Pliocene taxa (e.g. White *et al.* 1994; Suwa *et al.* 2007). Our data partially support a 'thin' versus 'thick' dichotomy, although overlap between species limits the diagnostic use of three-dimensional RET (figure 2; esm). A range of intermediate thickness encompasses the mean values of *H. neanderthalensis* and *P. troglodytes*, and parts of the *H. sapiens* and *A. africanus* ranges. This intermediate range also overlaps with the values for *Pongo*, *Hylobates* and *Symphalangus*. Species overlap and ambiguous character polarity (see Kono 2004) render summary enamel thickness indices such as three-dimensional RET ineffective in distinguishing species precisely.

While enamel thickness is not necessarily useful for australopith taxonomy, the pattern of molar enamel distribution may yield valuable data for the

interpretation of molar adaptation to diet. Among taxa studied in three dimensions, thick enamel over cusp tips is unique to these australopiths and *Gigantopithecus* (Olejniczak *et al.* 2008a); these taxa also exhibit relatively short dentine horns (ESM). Thick cuspal enamel coupled with short dentine horns results in a tabular occlusal surface; this molar configuration probably increases molar longevity in response to abrasive diets in small-object feeders, and prevents the formation of cracks at the enamel–dentine junction in large-object feeders (Lucas *et al.* 2008). Moreover, our observation of thicker enamel at cusp bases in *H. sapiens* may relate to the distribution of masticatory forces (e.g. Macho & Spears 1999). Although the australopiths studied here share a similar pattern of enamel thickness distribution, differences in their overall enamel thickness are consistent with previous studies reporting the dietary differences between these species (e.g. Scott *et al.* 2005).

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