RESEARCH ARTICLE

Quantification of enamel decussation in gracile and robust capuchins (*Cebus, Sapajus*, Cebidae, Platyrrhini)

Russell T. Hogg[1] [iD] | Abdallah Elokda[2]

[1]Department of Rehabilitation Sciences, Florida Gulf Coast University, Fort Myers, Florida, USA

[2]Department of Biological Sciences, Florida Gulf Coast University, Fort Myers, Florida, USA

Correspondence
Russell Hogg, Department of Rehabilitation Sciences, Florida Gulf Coast University, 10501 FGCU Blvd South, Ft Myers, FL 33965, USA.
Email: rhogg@fgcu.edu

Abstract

Multiple behavioral and biomechanical analyses have demonstrated that capuchin monkeys (*Cebus* and *Sapajus*) are specialized for breaking down hard-object foods as compared to other cebid monkeys. In addition to a complex suite of craniodental adaptations, it has specifically been demonstrated that capuchins possess highly complex dental enamel, with extensive Hunter–Schreger banding and other decussation, that likely serve as an adaptation to resist crack propagation during hard-object feeding. Furthermore, it has been demonstrated that robust capuchins (*Sapajus* spp., formerly *Cebus apella*) demonstrate further adaptation for hard-object feeding than other capuchins, routinely breaking down extremely mechanically challenging foods. However, there has been no comparison of dental enamel complexity in robust versus gracile capuchins, to assess whether the dental enamel in *Sapajus* follows this same pattern of further specialization. Therefore, this study compares dental enamel complexity in images of dental thin sections from a sample of robust versus gracile capuchins using image compression ratio (ICR) analysis. ICR is a variable that correlates with enamel complexity, such that higher ICR values are indicative of increased complexity in the form of enamel decussation. We found no significant difference between robust and gracile capuchins when assessing all teeth in our sample together, however, we did find that robust capuchins have significantly higher ICR values than gracile capuchins for canine teeth, specifically. Our results support prior studies suggesting that robust capuchins are specialized to generate increased masticatory loads with their anterior dentition, specifically, as compared to gracile species.

KEYWORDS
enamel complexity, hard-object feeding, Hunter–Schreger bands, image compression ratio

1 | INTRODUCTION

It has long been recognized that hard-object feeding (also known as durophagy or obdurate feeding), frequently combined with destructive foraging strategies, is an adaptive suite that helps make capuchins (*Cebus* and *Sapajus*, Cebidae, Platyrrhini) stand out from their near relatives, and indeed plays a major role in their adaptive success (Anapol & Lee, 1994; Rosenberger, 1992;

Rosenberger & Kinzey, 1976; Wright, 2005). Extensive research has been performed into these particular feeding and foraging behaviors within this group, and into the craniodental morphology connected to them (e.g., Anapol & Lee, 1994; Daegling, 1992; Martin, 2003; Rosenberger, 1992; Rosenberger & Kinzey, 1976; Wright, 2005).

One important component of a durophagous feeding regime, whether relying on hard-object foods as a mainstay or a fallback

food, is the ability of the dentition to withstand the heavy loads required to break down these hard objects, as generated by the masticatory musculature. Dental enamel is an extremely hard and durable tissue, nevertheless, it is subject to fracturing when subjected to forces that exceed its loading threshold (Maas & Dumont, 1999; Rensberger, 2000). Moreover, dental enamel is fairly unique among vertebrate tissues in having zero capacity to heal injuries, such that fractures can lead to permanent, catastrophic tooth failure that has obvious consequences for the acquisition of nutrients and fitness. Therefore, we frequently find that primate dental enamel (and mammalian enamel in general) possesses specialized macro- and microstructural adaptations to species' particular dietary regimes (e.g., Pfretzschner, 1988; Popowics et al., 2004; Rensberger, 2000; Von Koenigswald et al., 1987).

Enamel thickness is one such adaptation that has been assessed in capuchins, with regard to obdurate feeding (Martin, 2003; see below). Another is the microanatomy of the matrix making up the overall enamel, also known as the *schmeltzmuster* (Von Koenigswald, 2000; Von Koenigswald et al., 1992). Aprismatic enamel is generally considered the simplest type of *schmeltzmuster*; in this category, hydroxyapatite crystallites of the enamel form a relatively homogenous matrix (Boyde, 1989). Prismatic enamel, the next category, is more organized in that crystallites are arranged into long cylindrical bundles called enamel prisms, which are readily identifiable at low power magnification (e.g., ×50–100). In turn, prismatic enamel occupies a continuum of organization from the fairly simple to the highly complex. In *radial enamel*, the simplest type of prismatic enamel, the prisms take a relatively direct, straight trajectory between the enamel surface and the enamel–dentine junction (EDJ), in parallel with each other (Boyde, 1989; Maas & Dumont, 1999; Risnes, 1998; Figure 1). *Decussating enamel* is more complex, and in this category the enamel prisms are seen to take more sinuous trajectories through the enamel matrix in such a manner that they can cross paths at fairly steep angles to each other (Boyde, 1989; Maas & Dumont, 1999; Risnes, 1998; Tomes, 1850). Hunter–Schreger bands are alternating groups (fascicles) of decussating prisms wherein the prisms of each fascicle share a common geometry, but the fascicles themselves exhibit alternating, complex pathway differences when compared to each other. Hunter–Schreger bands stand out as the most extreme representation of enamel decussation, such these alternating fascicle bundles can even be seen unaided, with no magnification, in longitudinal tooth sections of many mammal species (Boyde, 1989; Maas & Dumont, 1999; Noyes & Thomas, 1921; Risnes, 1998). See Lynch et al. (2010) for a summary of research into Hunter–Schreger banding.

In mammal species where abrasive wear acts as the main threat to structural integrity and function of the overall tooth, radial enamel tends to be the predominant type, due to the fact that radial enamel resists abrasive wear well (Rensberger, 2000). This occurs because the hydroxyapatite crystallites making up the bulk of the enamel matrix best resist abrasive wear against vectors that are parallel to their long axis. Radial enamel takes advantage of this property by orienting the enamel prisms (and therefore crystallites) to be

FIGURE 1 Varying complexity of decussation in primate dental enamel, as imaged in CPL. Images of teeth with more complex enamel display more alternations between different colors when viewed in this imaging modality. (a) Radial (simple) enamel in a squirrel monkey (*Saimiri boliviensis*). The relatively homogenous color of the enamel represents minimal changes in prism orientation. (b) Fairly broad, simple Hunter–Schreger bands in a gracile capuchin (*Cebus capucinus*) P_3; note alternating lighter and darker banding. (c) More complex Hunter–Schreger banding in a canine of a robust capuchin (*Sapajus* sp./*C. apella*). Note the highly organized, repeated alternating band colors (between blue and orange). Field width: a = 203 μm, b = 507 μm, and c = 490 μm

uniformly parallel to the primary abrasion vector, making radial enamel patterns well-adapted to minimizing abrasive wear (Rensberger, 2000; Von Koenigswald, 2000). However, radial enamel is more subject to the propagation of cracks, since the relatively linear boundary between enamel prisms facilitates the direct travel of a crack between the tooth surface and the EDJ; this means that radial enamel is more prone to catastrophic tooth failure (Rensberger, 2000).

Decussation reduces the risk of catastrophic tooth failure substantially, by placing a physical barrier to crack propagation within the enamel matrix. Since decussating prisms take complex paths between the tooth surface and the EDJ, any crack propagating within a tooth is likely to eventually encounter a sudden change in prism orientation, and therefore be interrupted; this failsafe can potentially save the enamel as a functional unit (Bajaj & Arola, 2009; Pfretzschner, 1988; Popowics et al., 2004; Rensberger, 2000). Therefore, where we see mammal species exhibiting more complex enamel decussation patterns, these also tend to be the species that exert greater loading forces on their tooth enamel, such as hyenas with teeth adapted to crush bone—as opposed to howler monkeys, which have radial enamel representing a lower-load, folivorous dietary regime (Hogg, 2010; Lucas et al., 2008; Rensberger, 2000). Furthermore, there are variations in complexity possible; for

example, Hunter–Schreger band packing density (i.e., frequency) has been shown to be higher in tooth regions undergoing greater loading (Lynch et al., 2010). Here, we define "enamel complexity" to signify any microanatomical feature of dental enamel that increases the heterogeneity of enamel crystallite orientations, including width and frequency of Hunter–Schreger bands, the geometry of Hunter–Schreger bands, and degree of sinuosity/direction changes of enamel prisms both within and outside of Hunter–Schreger bands.

To return to the capuchins specifically, Martin (2003) first noted that *Cebus apella* (*sensu lato*) has relatively thicker dental enamel compared to most other platyrrhines, with the exception of pithecines that are also hard-object feeders. Since it has been repeatedly demonstrated that relatively thicker enamel is related to the ability of a tooth to bear greater bite force (e.g., Constantino et al., 2012; Lucas et al., 2008), this is good evidence that capuchin teeth have indeed adapted to break apart mechanically resistant food items. In further support of this notion, Constantino et al. (2012) demonstrated in a nanoindentation study that *C. apella* teeth are highly durable compared to most other primates. With regard to *schmeltzmuster*, Martin (2003) also observed consistently complex enamel in *C. apella*, with well-defined Hunter–Schreger bands. Hogg (2010) identified extensive Hunter–Schreger bands across multiple capuchin species, including *C. apella* (*sensu lato*), *C. albifrons*, *C. capucinus*, and *C. olivaceus*, in contrast to the simple, radial enamel the study identified in all other groups of cebids (with the exception of the tree-gouging marmosets).

All of these studies support the notion that capuchin teeth are indeed specialized to deal with the high loading requirements of a diet containing obdurate foods, as compared to their non-obdurate feeding relatives. However, one area that has not been adequately assessed is the degree to which different groups of capuchins differ from each other with regard to dental adaptations for hard-object feeding, considering that the robust (tufted) capuchins (*Sapajus*, formerly grouped into *C. apella sensu lato*; see Lynch Alfaro et al. (2012), for a thorough treatment of capuchin taxonomy) are well-demonstrated to be even further specialized for hard-object feeding than the gracile (untufted) capuchins (*C. albifrons, capucinus*, and *olivaceus*) (Daegling, 1992; Terborgh, 1983; Terhune et al., 2015; Wright, 2005; Wright et al., 2009). This is especially the case with comparisons of enamel-specific adaptations among capuchins, which are essentially unstudied to date. Comparisons of morphological masticatory adaptations among the capuchins have focused on musculoskeletal anatomy before now, having demonstrated that, as compared to gracile capuchins, robust capuchins have (1) more robust mandibular corpora (especially at the symphysis) to resist parasagittal bending, wishboning, and other anterior masticatory forces; (2) adaptations to create greater bite forces in the anterior teeth, such as more anteriorly positioned masseter and temporalis; and (3) greater cranial integration (Daegling, 1992; Makedonska et al., 2012; Wright, 2005).

One reason for the lack of systematic comparison among *schmeltzmusters* of different capuchins has been the difficulty of quantifying enamel decussation and enamel complexity. However,

Hogg and Richardson (2019) have recently demonstrated that analysis of Image Compression Ratios (ICR), a technique developed by Evans et al. (2007) for assessing tooth crown complexity, provides a reliable basis for quantitative comparison of enamel complexity in histological images as well. To summarize, the variable of ICR compares total file sizes, in bytes, of digital images taken in uncompressed file formats (e.g., .tif, .bmp) to the size of the same images stored in compressed file formats that have been reduced using standard computer algorithms (e.g., .jpg or .png) (Cover & Thomas, 1991; Evans et al., 2007). The method takes advantage of the fact that simpler images can be more fully compressed in terms of file size by the computer algorithms that build the compressed image file formats. As demonstrated by Hogg and Richardson (2019), therefore, digital images of complex enamel have higher compression ratios than images of simpler enamel, following the calculation: *ICR = compressed file size/uncompressed file size*. This allows statistical comparison of ICR to access quantitative information about relative tooth complexity, versus the qualitative comparisons that have been the norm to date.

Therefore, our objective in the present study is to apply the ICR technique to assess differences in enamel complexity among robust versus gracile capuchins. Based on the results of prior studies and the "extra" specialization of robust capuchins for durophagy, we have two hypotheses to examine: (1) robust capuchins will exhibit an overall pattern of higher ICR, and therefore more complex enamel, compared to gracile capuchins, and (2) the difference in ICR between the two groups will be greater for anterior as compared to posterior teeth, considering prior demonstrations that the robust capuchin masticatory apparatus is specially adapted to generate and resist higher anterior masticatory forces specifically (Daegling, 1992; Wright, 2005).

2 | METHODS

This study complied with protocols approved by the FGCU (Florida Gulf Coast University) Institutional Animal Care and Use Committee (IACUC) and adhered to the legal requirements of the United States and the State of Florida. The research adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-human Primates. All specimens included in this study were gathered from pre-deceased animals housed in museum and university collections, therefore no living animals were harmed or otherwise affected by this study. All data are available from the authors upon reasonable request.

This study was conducted at Florida Gulf Coast University between March and August of 2020; the images were originally collected at New York University College of Dentistry from 2006 to 2008.

We incorporated a total of 113 images of thin sections from three gracile capuchin species (*C. albifrons, C. capucinus*, and *C. albifrons*) and from unidentified species of robust capuchins, *Sapajus* spp. (11 individuals in all; see Table 1). We were unable to verify species

designation for our robust capuchins, as they were previously recorded as *C. apella* (*sensu lato*) under the old capuchin taxonomy scheme, without further designation (as reported in Hogg, 2010). All images were taken from the image databank generated for Hogg (2010); further provenance for the specimens is provided there.

As outlined by Hogg (2010), all specimens were prepared and mounted identically, being first embedded in polymethyl-methacrylate (PMMA) resin, cured under ultraviolet light. Cured blocks were next sectioned using an Isomet® (Buehler Ltd.) slow-speed diamond wafering saw with a 3-in diameter blade. Sections for molars were taken in the mesiodistal plane through the apex of the highest cusp as a reference point; sections for premolars and canines were taken in the buccolingual plane (also through the apex of the highest cusp) due to differences in tooth shapes. Sections were next mounted onto glass microscope slides using Permatex® Windshield Repair Kit (Permatex Inc.), a methacrylate-based adhesive designed to bond to the glass. Sections were ground and polished to a thickness of approximately 100 μm using a graded series of emery papers and a 1 μm diamond polish (in water suspension) on a nylon pad on an Ecomet® (Buehler Ltd.) rotary polisher to maintain consistent section thickness and plane-parallel polishing, which were verified following the protocols of Bromage et al. (2003). All images are of mandibular teeth.

Images were captured as *X–Y* montages in an uncompressed file format (.tif) via Syncroscopy Montage Explorer (Synoptics Ltd.), using

TABLE 1 Specimens incorporated in this study

Category	Specimen	Genus	Species	Teeth included
Robust	AMNH 133901	*Sapajus*	sp. (formerly *C. apella*)	C, P_2, P_4, M_1, M_2, M_3
	MNRJ 446	*Sapajus*	sp. (formerly *C. apella*)	C
	NYU female[a]	*Sapajus*	sp. (formerly *C. apella*)	C
	Yerkes 15	*Sapajus*	sp. (formerly *C. apella*)	P_3, P_4, M_1, M_2
Gracile	AMNH 62838	*Cebus*	*albifrons*	C
	AMNH 78504	*Cebus*	*albifrons*	C
	USNM[a]	*Cebus*	*albifrons*	M_2
	Rose[a]	*Cebus*	*capucinus*	P_3, M_1, M_2
	USNM[a]	*Cebus*	*capucinus*	P_2, P_3, P_4, M_1
	AMNH 42419	*Cebus*	*olivaceus*	C, P_2, P_3, P_4, M_1, M_2, M_3
	Rosenberger[a]	*Cebus*	sp.	C, P_2, M_1, M_2

Note: All teeth are permanent mandibular teeth.
Abbreviations: AMNH, American Museum of Natural History; MNRJ, Museu Nacional do Rio de Janeiro; NYU, Department of Anthropology Collection, New York University; Rose, Rose Primate Collection; Rosenberger, private collection of Dr. Alfred Rosenberger; USNM, United States National Museum; Yerkes, Yerkes Primate Collection.
[a]Accession numbers not available.

a JVC KYF55B color video camera. The optical configuration employed a Leica-Leitz DMRX/E Universal Microscope fitted with a Marzhauser motorized stage, phase contrast, and circularly polarizing filters. Leica PL Fluotar ×10, ×20, and ×40 objective lenses were used. No particular standardized field of view was employed, as the original purpose for this sample was to provide measurements of enamel growth increments (Hogg, 2010). However, the image sample is still useful as an opportunistic sample for our study here because the original montages captured large regions of the tooth enamel in the longitudinal sections (with field widths of multiple millimeters), and because all images were originally captured using circularly polarized light (CPL; Bromage et al., 2003; Slayter & Slayter, 1992). Hogg and Richardson (2019) found that differences in objective magnification had no significant impact on ICR results, and also found that ICR results were similar for both standardized fields of view as well as unstandardized montage images. Therefore, since the specimens in our study were all prepared following the same protocols, these prior results signify that this image sample is reliable for ICR analysis.

Following Hogg and Richardson (2019), the use of CPL in our optical system is an indispensable component in the generation of our images for this ICR method. In CPL, the waves of light passing through the specimen have been polarized around two perpendicular axes instead of just one axis (linearly polarized light, LPL). In consequence, light waves in CPL possess a helical structure in three dimensions, instead of a sinusoidal structure in two dimensions (Slayter & Slayter, 1992). This is vital to ICR analysis because tooth enamel matrix is birefringent, and therefore polarized light can be used to provide information about differing crystallite orientations, which will show up in the final image as regions of differing color (i.e., polarized light provides color contrast for regions of differing enamel crystal patterns; see Bromage et al., 2003; Hogg, 2010). CPL is further advantageous as compared to LPL, in that it reveals refractive interference around 360° (Rochow & Tucker, 1994), and therefore can provide an essentially full representation of the crystallite geometry in a section, which is not possible in LPL (Bromage et al., 2003; Hogg, 2010). Therefore, the CPL imaging modality inherently creates an image that contains a color-coded map of enamel complexity in a tooth section, exactly of the sort necessary for applying the image compression ratio method of Evans et al. (2007); it also provides a much more complete representation of that complexity as compared to LPL. CPL also allows one to control for differences in orientation of the specimen or the polarization filters that would act as an additional, undesired variable in the ultimate ICR analysis. It can do this precisely because it captures birefringent properties of the matrix around a 360° axis, such that no information is lost by rotating the specimen, and also because it standardizes the configuration of the polarizing filters by definition (Rochow & Tucker, 1994; Slayter & Slayter, 1992). This would include eliminating variability in relative widths of Hunter–Schreger bands and the regions between them. Since CPL is a transmitted-light optical modality in which the light beam is traveling perpendicular to the *XY* axes of the specimen, it also eliminates any variability in this regard that might be created by

inconsistent angles of incidence when using reflected-light microscopy (as used, e.g., in Lynch et al., 2010).

Source.tif images were digitally processed using the eraser tool of GNU Image Manipulation Program (GIMP) 2.10.6 (https://www.gimp.org/) to subtract all dentine, cementum, and artifacts that would affect the final image compression ratio. These edited.tif images were then converted to a compressed.jpg format at 80% quality using IrfanView (http://www.irfanview.com/), which allows batch image conversion, following the protocols of Evans et al. (2007). The compressed.jpg file size, in bytes, was next divided by the original.tif size to provide the compression ratio (Evans et al., 2007).

To test our hypotheses, we compared ICR across our images using standard non-parametric Kruskal–Wallis and Mann–Whitney tests; we opted for nonparametric analyses as they are less subject to influence by outliers, and also do not assume a normal distribution. All tests were conducted using IBM SPSS Statistics v. 25.0 (IBM Corp.).

3 | RESULTS

As was the case with Hogg and Richardson (2019), a variety of objective lenses were used to generate our images, so it is first necessary to examine any potential influence of objective lens power on our results. In line with the results of Hogg and Richardson (2019), we found no significant difference in ICR values among images taken with different objective lenses as demonstrated by a standard Kruskal–Wallis test, and found no significant correlation between objective lens and ICR.

With regard to our first hypothesis, we assessed ICR inclusive of all tooth categories, comparing the robust capuchins (Sapajus spp./C. apella sensu lato) to the gracile capuchins (C. albifrons, C. capucinus, C. olivaceus) using Mann–Whitney U tests (see Tables 2 and 3 and Figure 2). Surprisingly, we found no significant difference in ICR between robust and gracile capuchins across all teeth ($p = 0.35$). Likewise, we found no significant difference in ICR for all tooth categories when all gracile capuchin species were examined separately rather than assessed as one category (Kruskal–Wallis; $p = 0.48$). A comparison of canines, premolars, and molars against each other including the total capuchin sample showed no significant difference among the three tooth types in the total data set ($p = 0.36$).

With regard to the second hypothesis, we separately compared anterior (canines) and posterior teeth (premolars and molars) for robust versus gracile capuchin sample sets. Given the results of the analyses above, it is not surprising that once again we found no difference in ICR between robust and gracile capuchins for the posterior dentition ($p = 0.28$). However, the canine sample did display a significant difference between the two groups, with robust capuchins demonstrating higher ICR values and therefore more complex enamel ($p = 0.04$; Tables 2 and 3 and Figures 3 and 4).

4 | DISCUSSION

Our results suggest a lack of difference in enamel complexity between robust and gracile capuchins when the whole mandibular toothrow (exclusive of incisors) is examined together, and this finding is at first glance quite surprising. The list of morphological specializations in robust capuchins for generating higher masticatory

TABLE 2 Descriptive statistics

Sample	N (images)	Mean ICR	Min. ICR	Max. ICR	Std. dev.	Skewness	Kurtosis
Whole sample	113	0.016	0.0002	0.075	0.132	2.720	8.119
Robust, all teeth	40	0.017	0.0002	0.075	0.015	2.428	6.623
Gracile, all teeth	73	0.015	0.001	0.072	0.124	2.976	9.963
All canines	23	0.014	0.003	0.054	0.013	2.205	4.040
All premolars	48	0.015	0.001	0.075	0.012	2.957	12.155
All molars	41	0.016	0.0002	0.072	0.015	2.866	8.196
Robust canines	9	0.023	0.002	0.054	0.019	0.810	−1.017
Gracile canines	14	0.008	0.004	0.014	0.002	0.377	0.608
Robust posterior	26	0.015	0.0002	0.075	0.129	3.641	16.433
Gracile posterior	59	0.016	0.001	0.072	0.013	2.671	7.806

Note: Relatively higher ICR values are indicative of increased enamel complexity, in terms of decussation.

Abbreviation: ICR, image compression ratio.

Sample	Analysis	N (images)	p value
Robust versus gracile, all teeth	Mann–Whitney U	113	0.35
Individual species (4 total), all teeth	Kruskal–Wallis	113	0.48
Canine versus premolar versus molar, total species sample	Kruskal–Wallis	113	0.36
Robust versus gracile, canines	Mann–Whitney U	23	0.04
Robust versus gracile, posterior teeth	Mann–Whitney U	85	0.28
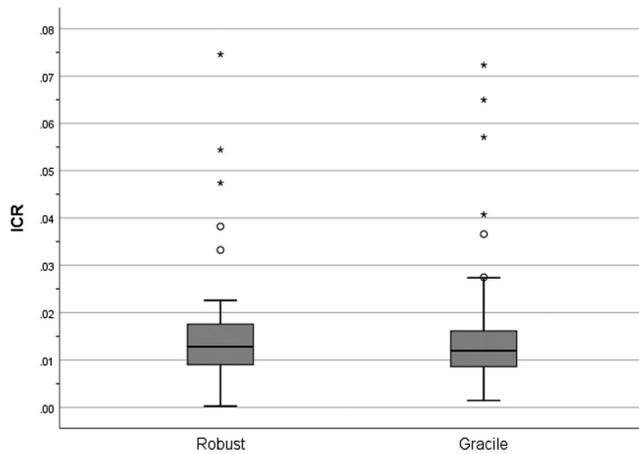
FIGURE 2 Boxplot of ICR values for the entire robust capuchin image sample, compared to the gracile capuchin image sample. Mann–Whitney U, $p = 0.35$. ICR, image compression ratio
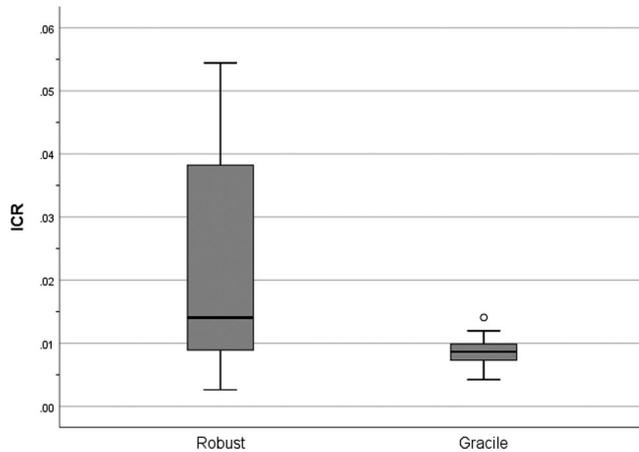

FIGURE 3 Boxplot of ICR values comparing canines only for robust versus gracile capuchins. Mann–Whitney U, $p = 0.04$. ICR, image compression ratio

FIGURE 4 Visual demonstration of canine enamel complexity; both images demonstrate mid-imbricational enamel. For the purpose of the figure, dentine was not edited out of the image. (a) Canine enamel from a gracile capuchin (*Cebus* sp.). Hunter–Schreger bands can be seen as alternating bands of gray-black and yellow color shifts. (b) Canine enamel from a robust capuchin (*Sapajus* sp.). More distinct color and shade contrasts can be seen between alternating light and dark regions of Hunter–Schreger bands. Approximate field width: a = 600 μm and b = 550 μm

loads than their gracile cousins is quite long, and includes the presence of sagittal crests, greater cranial integration, more robust mandibular morphology, as well as larger canine and postocclusal dental surface area, etc. (Anapol & Lee, 1994; Daegling, 1992; Wright et al., 2009). Based on this as well as the wealth of behavioral data and biomechanical data demonstrating that robust capuchins use their teeth to break down more mechanically challenging foods than other capuchins (Terborgh, 1983; Terhune et al., 2015; Wright, 2005; Wright et al., 2009) one would anticipate that enamel complexity across all teeth would be higher in the robust group.

However, enamel complexity is not the only factor determining fracture resistance in teeth; other factors such as enamel thickness also play a role. For example, Schwartz et al. (2020) recently advanced a variable that they refer to as "absolute crown strength" (ACS), which accounts for both enamel thickness and crown shape to describe tooth durability. While the authors did not account for enamel complexity in their study, their point remains that tooth crown mechanics are reliant on multiple variables. With regard to our study, therefore, one would expect robust capuchins to not only have more complex enamel than gracile capuchins, but also thicker enamel based on the increased mechanical challenge presented by their foodstuffs. However, a comparison of molar thickness between *C./S. apella* and *C. olivaceus* found no significant difference (Wright, 2005). The fact that neither complexity nor thickness seem to differ between robust and gracile capuchins needs explanation, based on

the above-cited observations of increased durophagy in robust capuchins. One potential hypothesis is that there may be some mitigating factor, such as tool use (e.g., Wright et al., 2009), that lessens the need for extreme complexity for resisting enamel fracture in robust capuchins. Alternatively there is potentially some epigenetic, developmental, or other limitation on the degree of complexity that capuchin teeth can attain. Yet another explanation could be that all capuchins have primitively evolved as a group to exceed a certain threshold of complexity that is satisfactory to meet the mechanical demands of their diet, which has been maintained by selection through time in each lineage; in other words, gracile capuchins may be retaining a primitively "overbuilt" morphology. However, robust capuchins have been observed to have a surprisingly low rate of usage of their molars for hard-object breakdown (Thiery & Sha, 2020), making all of these hypotheses seem less likely to be true.

A much more likely alternative answer for our findings, as well as the finding of Wright (2005) regarding molar enamel thickness, is that highly complex enamel may be specialized for certain teeth in the arcade that are the prime organs used in breaking apart highly difficult foodstuffs, such that other teeth may not need to be as complex and mask the adaptive signal when all teeth are analyzed together. As demonstrated by multiple studies (Daegling, 1992; Makedonska et al., 2012; Wright, 2005; Wright et al., 2009), robust capuchins do possess craniodental specializations allowing them to generate more bite force in the *anterior* masticatory apparatus in particular when compared to gracile capuchins. Moreover, behavioral evidence does indicate that robust capuchins have a preference for using canines and premolars to break open hard objects (Visalberghi et al., 2008), and also that they have a surprisingly low incidence of using molars to break down hard objects, as noted above (Thiery & Sha, 2020). So while typically one would consider molars to be more ideally suited to crushing hard objects due in part to their position closer to the fulcrum of the mandibular lever system, morphological and behavioral data indicate that robust capuchins seem to have specialized their apparatus in an unusual way, emphasizing heavy loads in anterior teeth. This of course seems odd, but a possible explanation lies in a trade-off between overall force and pressure that is set in place by the morphology of the teeth themselves. Given the low apical surface area of canines (i.e., their "pointiness") compared to more posterior teeth, typical cone-shaped canines should be able to exert more pressure (in terms of force per unit area) than other teeth, all else being equal. This feature may make canines in a number of primate species better at puncturing, fracturing, or tearing certain hard-object/durable foods than the posterior teeth, even though those teeth are able to exert more overall force due to their greater mechanical advantage. This ability of the canines, therefore, may have driven adaptations favoring their use in obdurate feeding within the robust capuchins and perhaps other primate species as well, such as the pitheciines (e.g., Martin, 2003). An alternative explanation—though not a mutually exclusive one—is that robust capuchins must use their canines more to break open obdurate foodstuffs because their chosen food items are more often too large to be accommodated between the rear teeth, given gape size limitations. In any case, our finding that dental enamel in canine teeth of robust capuchins exhibits greater complexity than that of gracile capuchins is further evidence in favor of the hypothesis that the need to break apart obdurate foodstuffs with the anterior—as opposed to posterior—teeth has driven much of the specialized craniodental anatomy seen in robust capuchins. The posterior teeth, by contrast, retain enamel thickness and complexity that is likely primitive for capuchins in general.

In conclusion, our ICR results indicate that premolars and molars among gracile and robust capuchins exhibit similar degrees of enamel complexity, but that canines in robust capuchins display increased complexity reflecting high specialization in this group for hard-object feeding in the anterior masticatory apparatus. One area that further sampling would help elucidate is the degree to which different species of robust capuchins may differ from each other. Based on available information for the individuals in our data set, which had all been gathered from collections before the recent taxonomic revision of capuchins and hence were all merely identified as "*C. apella*," we had no recourse but to include all robust capuchins in our analysis together in one subsample. It would be interesting to assess differences among the robust species, considering previous studies suggesting that tool use in some species may lessen the need for the generation of high anterior bite forces (e.g., Wright et al., 2009). Likewise, a larger sample set would allow us to better parse out differences among particular teeth, and to determine whether the enamel adaptation to heavy loading is restricted to the canines or also includes the incisors. In future studies, it would also be beneficial to compare capuchins to other primate hard-object feeders such as the pitheciines and *Paranthropus*, the latter of which would be particularly useful for assessing patterns of hard-object feeding in hominin evolution.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Russell T. Hogg http://orcid.org/0000-0002-9096-4289

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