# Dental Microwear Texture Analysis of the Tasmanian Devil: Assessing Variability Among Teeth

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BRIEF. Here, we aim to assess dental microwear variability between teeth with similar morphology and inferred function.

ABSTRACT. Dental microwear, the microscopic wear left due to the processing of food, can reveal diets in living and fossil animals. However, recent work has demonstrated that different teeth are used for different functions and may capture dental microwear differently. Unlike most carnivorous mammals that are members of the order Carnivora, marsupial carnivores have multiple molars that are analogous to carnassials, a tooth with a sheering facet used for slicing flesh. Here, we clarify if the molars in Tasmanian devils (*Sarcophilus harrisii*) that appear similar in morphology yield similar dental microwear features. As nearly all dental microwear attributes useful for inferring relative bone consumption in carnivores have statistically indistinguishable mean values between different tooth positions in Tasmanian devils, these results suggest that morphologically similar teeth (i.e., lower second, third, and fourth molars) likely capture dietary information similarly. Further, our results suggest that any tooth position can be used in dietary studies of extinct or extant Tasmanian devils; however, future work is necessary to evaluate why such high variability exists in complexity values, as compared to complexity values of carnivores examined on an older confocal microscope.

## INTRODUCTION.

Tasmanian devils (*Sarcophilus harrisii*), which currently only exist in Australia on the island state of Tasmania, are the world's largest living carnivorous marsupial [1]. Tasmanian devils also have a diverse diet, having been known to consume primarily carrion of wallabies, wombats, sheep, and rabbits and typically scavenge or hunt at night [1-2]. The Tasmanian devil's broad diet, which includes scavenging and durophagy (i.e., bone processing), should leave evidence of such behavior via the microscopic wear patterns on their teeth. Microwear analysis on the Tasmanian devil's molars has been limited. To date, only Robson and Young [3] have examined their dental microwear, comparing the Tasmanian devil to the extinct Tasmanian tiger (*Thylacinus cynocephalus*) - a marsupial with potentially similar dietary habits to the Tasmanian devil. Although the study was primarily aimed at comparing the dental microwear between the Tasmanian devil and Tasmanian tiger, it also revealed the importance of compression in the shearing action of the Tasmanian devil's carnassial-like sheering teeth, as evidenced by numerous pits on Tasmanian devil molars.

Dental microwear remains one of the most effective methods of inferring dietary habits in extant and extinct mammals, including carnivorous mammals. Scanning electron microscopes were initially used to detect microscopic pits and scratches on teeth in carnivorous mammals [4-5]. The emergence of new technology has created a new form of microwear imaging, 3D dental microwear texture analysis [6-8]. While 2D imaging can be useful at determining dietary categories, differences in feature assignment (e.g., pits and scratches) from human observers can pose problematic. Therefore, 3D dental microwear texture analysis is more accurate for distinguishing between diverse diets, particularly in carnivorous mammals [9]. Based on previous microwear research of the carnassial tooth in extant African feliforms (i.e., cats and their relatives), relative degrees of bone consumption and durophagy can be inferred [9-11].

The carnassial, a specialized tooth used to shear flesh, is found in carnivorous mammals and varies in form and function. While carnivorous mammals that are members of the placental order Carnivora contain one lower carnassial molar, carnivorous marsupials like the extant Tasmanian devil and extinct Tasmanian tiger, contain multiple molars that are analogous to carnassial teeth, with the m3 and m4 as most similar when considering placement in the jaw relative to the condyle (i.e., the jaw joint) [12]. In feliforms, the carnassial is the most effective tooth to infer diet and is useful for differentiating between primarily soft tissue and brittle bone consumption - differentiating between cheetahs, African lions, and spotted hyenas [10-11]. In canids (i.e., dogs and their relatives), carnassial tooth microwear patterns indicate that the lower first molar ($m_1$) carnassial is used to shear meat while the second lower molar is used for bone crunching [13]. In ursids (i.e., bears), lower second molar ($m_2$) dental microwear is indicative of diet whereas lower first molar microwear is reflective of tool use and food processing, not diet [14]. Thus, dental microwear textural analysis can accurately identify diets of carnivorans in extant animals and is therefore effective at inferring diet in extinct animals [10-11]; however, it is important to have an understanding of whether different molars are used for different functions and/or record dental microwear in a predictive manner. Although Tasmanian devil's carnassial-like teeth are morphologically similar, we hypothesize that the microscopic textures recorded on each carnassial-like tooth will yield similar dental microwear textures consistent with their similar morphology and observed diet.

## MATERIALS AND METHODS.

### *Dental microwear texture analysis.*

Extant Tasmanian devil (*Sarcophilus harrisii*) specimens from throughout their current geographic range were sampled from museum specimens from the American Museum of Natural History (AMNH) and Museum Victoria (MV) Mammalogy collections. All lower molars (first through fourth molars, $m_1$-$m_4$) preserving antemortem microwear were cleaned with cotton swabs soaked in acetone (to remove preservative). These teeth were then molded with a polyvinylsiloxane dental impression material (President's Jet regular body, Coltène-Whaledent Corp., Cuyahoga Falls, OH, USA) while at each museum. Tooth replicas were prepared at Vanderbilt University using Epotek 301 epoxy resin and hardener (Epoxy Technologies Corp., Billerica, MA, USA).

Dental microwear texture analysis (DMTA) was performed on all replicas with sufficient wear. The enamel region of the lower "carnassial" shearing facet of the $m_2$, $m_3$, and $m_4$ were analyzed. As mentioned above, Tasmanian devils have numerous molar teeth that are analogous to "carnassials" in placental carnivorans. In all cases, the first molars were too worn and unable to be analyzed; however, in most cases the lower second through fourth molars could be analyzed using 3D confocal profilometry and scale-sensitive fractal analysis (SSFA). All specimens were scanned in three dimensions in one field of view, representing a sampled area of 95×127 $\mu m^2$. In contrast to prior methods that scan multiple areas and analyze median values of variables used to quantify tooth surfaces, here we attempted to increase the number of specimens and teeth examined by reducing the number of scans per tooth. All scans were analyzed using SSFA software (ToothFrax and SFrax, Surfract Corp., www.surfrait.com) to characterize tooth surfaces according to the following variables: (*i*) Complexity (*Asfc*), change in surface roughness with scale - used to distinguish taxa that consume hard, brittle foods from those that eat softer ones (for example, we expect higher values in carnivorous mammals that engage in bone consumption); (*ii*) Anisotropy (*epLsar*), the degree to which features are oriented in a similar direction, such as lots of parallel striations as can be formed by meat slicing; and, (*iii*) Textural fill volume (*Tfv*), a measure of difference in volume filled by large (10 μm) and small (2 μm) diameter square cuboids (high values indicate many deep features between these sizes) [6-8, 10]. Based on the work by Schubert and colleagues

[10], extant African carnivores have greater complexity and textural fill volume, and lower anisotropy with increased durophagy.

*Statistical analyses.*

Non-parametric statistical tests were used to compare all data, as the majority of DMTA variables for each tooth category were not normally distributed (Shapiro-Wilk tests). We primarily used Wilcoxon signed-rank tests (the non-parametric equivalent of paired Student's t-tests) to compare dental microwear variables between different tooth positions (i.e., $m_2$ vs. $m_3$, $m_3$ vs. $m_4$, and $m_2$ vs. $m_4$). All statistical comparisons were performed using XLSTAT and comparisons were considered significant if p-values were < 0.05.

RESULTS.

All primary DMTA data are noted below in Table 1. Complexity (*Asfc*) is not significantly different between any two tooth positions. Mean complexity values (standard deviations noted in parentheses, n-1) and minimum (min.) and maximum (max.) values are, as follows: $m_2$ = 29.51 (37.81), min.=1.39, max.=130.08; $m_3$= 37.34 (45.46), min.=2.92, max.=148.80; $m_4$= 40.25 (32.02), min.=5.59, max.=82.49. Based on these minimum and maximum values, the fourth molar appears less variable (total range of 76.91) than second (128.69) and third (145.88) molars in complexity values. However, it should be noted that no values above 25 have yet to be reported on any carnivore analyzed, using a different confocal microscope; thus, further methodological work is necessary to work out the cause of this extreme variation (as it also occurs on other taxa using newer microscopes, unpublished data). Similarly, anisotropy (*epLsar*) is not significantly different between any two tooth positions. Mean anisotropy values (standard deviations noted in parentheses, n-1) and minimum (min.) and maximum (max.) values are, as follows: $m_2$ = 0.0025 (0.0018), min.=0.0002, max.=0.0058; $m_3$= 0.0025 (0.0020), min.=0.0007, max.=0.0069; $m_4$= 0.0023 (0.0018), min.=0.0008, max.=0.0057. These values are similar to previously analyzed Tasmanian devil anisotropy values on a different confocal microscope and are characterized by similar levels of variability between tooth positions (m2=0.0056, m3=0.0062, m4=0.0049). Similarly, textural fill volume are not significantly differences between any two tooth positions. Mean textural fill volume values (standard deviations noted in parentheses, n-1) and minimum (min.) and maximum (max.) values are, as follows: $m_2$ = 7435 (4741), min.=841, max.=17562; $m_3$= 4771 (5757), min.=0, max.=16020; $m_4$= 5845 (5283), min.=244, max.=15149.

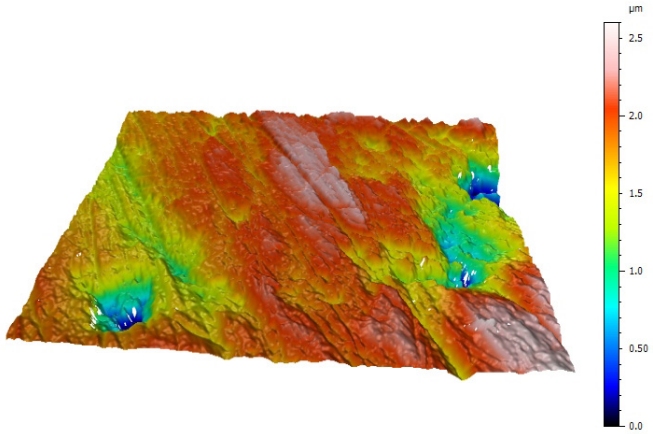


**Figure 1.** Example of 3D microwear of *Sarcophilus harrisii* (AMNH 65673, right lower second molar).

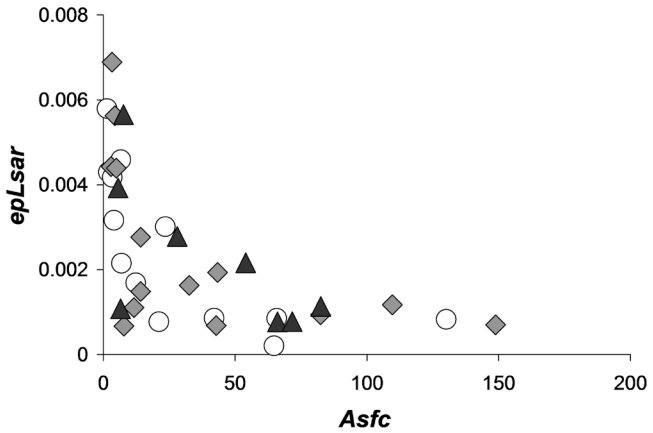


**Figure 2.** Bivariate plot of complexity (*Asfc*) and anisotropy (*epLsar*) of second molars (white circles), third molars (gray diamonds), and forth molars (black triangles).

| | | *Asfc* | | | *epLsar* | | | *Tfv* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ID | | $m_2$ | $m_3$ | $m_4$ | $m_2$ | $m_3$ | $m_4$ | $m_2$ | $m_3$ | $m_4$ |
| AMNH134974 | r | 65.78 | 42.80 | 82.49 | 0.0009 | 0.0007 | 0.0011 | 15227 | 0 | 244 |
| AMNH150211 | l | 1.39 | 2.92 | 6.56 | 0.0058 | 0.0044 | 0.0011 | 6644 | 0 | 4805 |
| AMNH65670 | l | 12.29 | 4.36 | 5.59 | 0.0017 | 0.0056 | 0.0039 | 17562 | 0 | 5387 |
| AMNH65673 | r | 6.86 | 7.80 | | 0.0022 | 0.0007 | | 841 | 0 | |
| AMNH65674 | r | 21.08 | 14.18 | | 0.0008 | 0.0015 | | 3486 | 6932 | |
| AMNH65677 | l | 4.03 | 11.65 | | 0.0032 | 0.0011 | | 4519 | 2630 | |
| AMNH69544 | r | 42.01 | 82.41 | | 0.0009 | 0.0009 | | 5577 | 11499 | |
| MV36422 | r | 23.49 | 148.80 | 28.01 | 0.0030 | 0.0007 | 0.0028 | 10091 | 4102 | 15149 |
| MV36423 | l | 1.87 | 14.11 | 66.04 | 0.0043 | 0.0028 | 0.0008 | 5510 | 5370 | 1009 |
| MV6241 | r | 64.75 | 4.87 | | 0.0002 | 0.0044 | | 6725 | 2730 | |
| MV6254 | l | 130.08 | 109.64 | 71.66 | 0.0008 | 0.0012 | 0.0008 | 8803 | 1093 | 999 |
| MV6257 | r | 6.61 | 32.56 | 54.00 | 0.0046 | 0.0016 | 0.0022 | 8706 | 15842 | 8757 |
| MV8857 | r | | 3.29 | 7.63 | | 0.0069 | 0.0057 | | 16020 | 10409 |
| MV8858 | r | 3.38 | 43.30 | | 0.0042 | 0.0019 | | 2960 | 574 | |

**Table 1.** All DMTA data for all Tasmanian devil specimens examined, organized by tooth position (r, right; l, left; m2-m4, second through fourth lower molars) and DMTA variables (*Asfc*, *epLsar*, and *Tfv*, see Materials and Methods for descriptions).

DISCUSSION.

Prior work has shown that dental microwear texture analysis can accurately infer diets in both herbivorous and carnivorous mammals [9]. While previous data on the Tasmanian devil has been sparse, new confocal imaging methods allowed us to closely examine dental wear on each individual Tasmanian devil "carnassial-like" tooth to compare microscopic wear patterns between molars that are morphologically similar. Dental microwear texture analysis results demonstrate high complexity and textural fill volume values and low anisotropy values, all consistent with durophagy in carnivorous mammals [10-11]. However, the mean values of molars from different tooth positions were statistically indistinguishable in all dental microwear attributes, which allows us to infer that each "carnassial-like" tooth captures dietary information similarly. Since all Tasmanian devil "carnassial-like" teeth show no perceivable difference in wear, their function may be similar, especially the third and fourth molar which are located closer to the jaw joint and are capable of exerting similar bite forces [12]. Typically, third molars have been suggested for dental microwear analysis of marsupial carnivores because they typically exhibit some degree of wear, in contrast to fourth molars that often have little to no wear at the time of death in Tasmanian devils [3]. In contrast to placental carnivores, which can record disparate dental microwear signals depending on the position of the tooth examined, marsupial carnivores are less susceptible to these issues and the analysis of different combinations of teeth should be possible for DMTA.

The extreme variation we observe in complexity values is of concern and may result from differences in the capabilities of the 3D profilers used to acquire DMTA data; specifically, advances in technology may now allow us to see greater detail and thus yield vastly different measurements. While anisotropy and textural fill volume measurements are relatively consistent with prior analyses of Tasmanian devils (unpublished data), previous analysis of carnivores shows no complexity values exceeding 25 [10-11, 13-14]. While our analysis of only one scan per tooth may lead to increased variation (as opposed to using only median values of four scans), the overwhelming number of scans with complexity values exceeding 25 is inconsistent with prior analyses of either medians or individual scans. Thus, future work is necessary to clarify methodological discrepancies.

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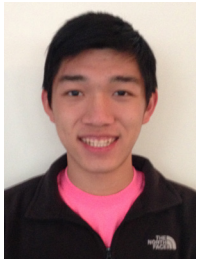

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