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Dental Topographic Analysis of Maxillary and Mandibular Phyllostomid Bat Dentitions: Implications for Dietary Prediction in the Fossil Record

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Dental Topographic Analysis of Maxillary and Mandibular Phyllostomid Bat Dentitions:

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Abstract

Mammalian dental anatomy has evolved in accordance with the physical properties of its diet, and multiple features on each tooth have specific functions related to the breakdown of food during mastication and ingestion. Tooth structure is under tight genetic control and much of the anatomical variation in dentition across species is related to adaptation to a specific dietary regime. This diet-dentition relationship can be exploited to reconstruct mammalian diets from fossil specimens through calculation of dental topographic metrics. To date, most studies of dietary reconstruction using dental topography have focused on mandibular molars; thus, this study seeks to test whether the dietary signal from maxillary molars is congruent with that of the mandibular dentition.

As a test case, an extant sample of maxillary and mandibular phyllostomid bat dentitions from Balta, Peru were collected and classified by dietary regime: frugivore, frugivore-nectarivore, insectivore-frugivore, and insectivore. The specimens were cast using epoxy material, after which second molars were excised, mounted on discs, and microCT-scanned at 13µm resolution. The resulting images were compiled to create a 3D surface model of the anatomical tooth crown, and topographic metrics were then calculated.

Paired t-tests of relief index (RFI), Dirichlet normal energy (DNE), and orientation patch count-rotated (OPCR) values of maxillary and mandibular molars within each dietary group demonstrated that there is a significant difference between maxillary and mandibular dental topographies across diets ($P<0.05$). Additionally, discriminant function analysis of maxillary and mandibular dental topography indicated that maxillary

second molars are as effective at predicting a species' diet as mandibular molars, and a combination of maxillary and mandibular dental topographic values predicts diet more effectively with an 65% success rate. Results from this study increase the dietary prediction accuracy for complete fossil specimens, expand paleontological dental topographic analysis to include maxillary molars, and demonstrate the potential of incorporating an occlusal approach to dental topography.

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List of Abbreviations

cytb	Cytochrome B
DFA	Discriminant function analysis
DNE	Dirichlet normal energy
F	Frugivore
FN	Frugivore-nectarivore
I	Insectivore
IF	Insectivore-frugivore
Mand	Mandibular
Max	Maxillary
OPCR	Orientation patch count-rotated
RFI	Relief index

Chapter 1: Introduction

Molars are the key to understanding what mammals eat, the environment in which they live, and also give clues about the evolution of species. Mammalian dental anatomy has evolved over time in accordance with the physical properties of its diet, and multiple features on each tooth have specific functions related to the breakdown of food during mastication and ingestion (Anderson and LaBarbera, 2008; Czarnecki and Kallen, 1980; Lucas, 2004; Rosenberger and Kinzey, 1976; Strait, 1993; Winchester et al., 2014). There are strong selective pressures among mammals to be efficient at both acquiring nutrients and pre-processing food in order to maximize the surface area upon which enzymes can act during digestion (Lucas, 2004; Santana et al., 2011; Ungar, 2016). For example, cows and horses exhibit relatively flat molars, which are suited for grinding plant cellulose for easier digestion. In contrast, mammals that eat hard-bodied insects exhibit tall, tapered cusps that break through the chitinous exoskeleton and propagate a crack in order to expose the soft insides for digestion (Strait, 1993). The advantage of tall cusps in these species lies in their ability to apply a large amount of masticatory force to a small area of exoskeleton in order to break through it, thus increasing an organism's chewing efficiency for a diet of hard-bodied insects (Evans and Sanson, 2006). All of these adaptations share the common feature of maximizing digestive efficiency for metabolic use in mammals.

Tooth enamel is the densest, hardest component in the mammal body (Cuy et al., 2002), and as a result, teeth are commonly preserved in fossil collections. Since tooth morphology is considered to be under tight genetic control, analyzing tooth structure is directly related to evolutionary adaptation (Anthony and Kay, 1993; Bunn et al., 2011; Lucas, 2004; Seligsohn and Szalay, 1978). Molariform teeth are predominantly used for mastication and breakdown of food for further digestion, and "diversity in functional demands on molar molars is roughly equivalent to diversity in material properties of different food items processed" (Boyer et al., 2010; Bunn and Ungar, 2009; Butler, 1972; Freeman, 1988; Kay, 1975; Lucas, 2004; Marshall and Butler, 1966). Studying extant mammalian dental topography expands our understanding of the molar form-function relationship, allowing for dietary (and thus ecological) reconstructions of related fossil mammals. The current research project is significant because it has the potential to infer the specific diets and subsequent ecologies of extinct mammals using isolated maxillary molars, whereas past studies have predominantly shown success using mandibular molars. In the field of paleontology, fossil specimens are often incomplete, so testing the efficacy of maxillary molars at predicting diet will be of great benefit to the field when mandibular molars are unavailable. A combined metric of maxillary and mandibular molar topography could also provide greater dietary prediction accuracy of complete fossil specimens.

Purpose

The purpose of this study is to determine the efficacy of maxillary second molar topography and combined maxillary and mandibular second molar topography at dietary prediction in a sample of phyllostomid bat dentitions.

Scope

Dental topography can include a number of different metrics such as shearing quotient, shearing ratio, molar length, relief index (RFI), Dirichlet normal energy (DNE), and orientation patch count-rotated (OPCR), all of which have been employed in dietary predictive analyses though mostly using mandibular second molars. This study aims to utilize RFI, DNE, and OPCR to determine dietary predictive success in a sample of phyllostomid bat dentitions using both maxillary and mandibular second molars.

Assumptions

1. Dental anatomy is under tight genetic regulation and is the result of selection for specific dietary regimes.
2. The anatomic variation in this sample can be attributed to dietary adaptation.

Hypothesis

Hypothesis 1: Dental topographic metrics will be similar between maxillary and mandibular second molars.

Hypothesis 2A: Maxillary second molar topography will predict species' diet as effectively as mandibular second molar topography.

Hypothesis 2B: Combined maxillary and mandibular second molar topography will have greater dietary predictive success than those of either maxillary or mandibular topographies alone.

Significance

In the field of paleontology, fossil specimen acquisition is highly variable. Thus, increasing the number and types of molars available to researchers to use in dietary prediction would be beneficial for reconstructing dietary regimes in the fossil record. In the event of discovering a complete specimen, researchers would be able to more accurately predict a species' diet using combined maxillary and mandibular dental topography in the fossil record.

Chapter 2: Review of Literature

Many techniques have been developed over time to quantify tooth structure within the field of mammalian paleontology, and they have been useful in reconstructing the dietary niches of fossil mammal species. Identification of the molar form-function relationship began with the utilization of linear dental metrics such as cusp height, buccal notch angle, and shearing ratios in conjunction with study of the physical properties of species' diets (Rosenberger and Kinzey, 1976; Strait, 1993). Shearing ratios have been employed in many different studies of molar form and have shown to be resilient to different methodological approaches to its calculation; it still appears to be an accurate predictor of species diet (Boyer et al., 2015; Allen et al., 2015). In recent years, dental topographic analysis has been the standard in quantifying tooth structure in mammals.

Most dental topographic studies utilize three specific metrics to determine topography: relief index (RFI), Dirichlet normal energy (DNE), and orientation patch count-rotated (OPCR). RFI was first proposed in a preliminary study of topographical analysis by Ungar and Williamson (2000) and is comprised of "a ratio of the three-dimensional surface area to the two-dimensional x-y area" of a tooth crown. Relief index allows for the inclusion of morphologically diverse taxa and is a sensitive and accurate predictor of diet (Boyer, 2008). Overall, the three-dimensional surface area of a tooth increases when the number of features on a given tooth increases, or individual cusps become more prominent on the tooth surface. In general, one would expect an insectivore to have greater relief than a frugivore since the tall, tapered cusps of the insectivore increase the three-dimensional surface area of the tooth crown compared to the flatter anatomy of a frugivore tooth crown. DNE was first introduced in 2011, and in short “measures the deviation of a surface from being planar” (Bunn et al., 2011). DNE correlates strongly with relief index but is less sensitive to the cropping process of three-dimensional topographic measurement. The peaked cusps of insectivorous molars would exhibit high DNE values due to their deviation from being planar. DNE provides insight into the potential for a given tooth structure to do work. One would anticipate that frugivore molars would exhibit lower DNE values since the work of mastication is spread across the entire occlusal surface rather than a few key areas as in insectivore molars. OPCR is a measure of tooth surface complexity calculated by grid points on the occlusal surface as they relate to eight compass directions (Evans and Jernvall, 2009). Groups of grid points on the tooth surface that lie in the same compass direction constitute a patch, and OPCR increases as the number of patches increases. OPCR differs from the other two metrics in that it measures surface complexity rather than topographic relief, meaning that molars with surface crenulations and microscopic ridges will have a higher OPCR value (Bunn et al., 2011). The more directional changes on a tooth surface, the higher the OPCR. Since frugivores have more complex surfaces than insectivores, it is anticipated that frugivores will have higher OPCR values than insectivores. On the other hand, DNE and RFI values increase with the presence of larger tooth features such as cusps and crests. Dental topographic analysis may also be used to compare morphology among similarly worn individuals from different species (Dennis et al., 2004). A computer program developed by Winchester et al. (2016) called MorphoTester calculates each of these metrics readily from a processed microCT image of a three-dimensional dental specimen.

This study intends to demonstrate that dental topography of maxillary second molars can add to the greater picture of molar occlusion and could allow for dietary predictions based on isolated maxillary molar specimens in the fossil record. Rather than narrowing the analysis to the anatomy of a single tooth, observing aspects of the molar occlusion of small mammals can paint a larger picture of how efficiently an animal is able to break down food and maximize its caloric potential. A study by Santana et al. (2011) observed maxillary and mandibular molar complexity (OPC-orientation patch count) of microbats. Overall, the topography of maxillary and mandibular second molars was relatively simplistic for insectivores and omnivores and more complex for the puncture-crush tooth function of frugivores. Maxillary molars tended to be more complex than mandibulars but did not vary amongst dietary groups (Santana et al., 2011). Additionally, a study by Allen et al. (2015) observed maxillary and mandibular first molar relief (RFI) and shearing quotient (SQ) of a sample of platyrrhine primates. They concluded that maxillary and mandibular relief were significantly different among species, and the dietary predictive success was similar between maxillary and mandibular relief. Combined maxillary and mandibular relief index did increase the dietary predictive success compared to individual first molars. This research project builds on the work of Allen et al. (2015) and Santana et al. (2011) by including more specimens with higher dietary variation as well as using a more complete topographic analysis that includes DNE in addition to relief index (RFI) and complexity (OPCR).

Among mammals that can be used for dental topographic analysis, microchiropterans are ideal for inferring diet from maxillary and mandibular molar morphology. Chiropterans (bats) are an ideal study sample due to their high degree of species diversity and dental morphological variation within small geographic areas (Dumont, 1999; Freeman, 2000; Gutzwiller and Hunter, 2015). Phyllostomidae, or New World leaf-nosed bats are one of the most ecologically diverse mammalian families ranging from southern North America to South America, reaching as far south as Argentina. Phyllostomid species can have diets categorized as frugivorous, nectarivorous, insectivorous, omnivorous, and even carnivorous. Fossil evidence suggests that this chiropteran family can be traced back to the Oligocene, and phylogenetic analysis estimates the family to be roughly 30 million years old (Rojas et al., 2016). Phyllostomids forage at night, relying on smell and echolocation for identifying food sources. Since most phyllostomid species echolocate nasally, their leaf-shaped noses are thought to provide amplification and direction to their calls. Echolocation is especially important for insectivorous species that need to rapidly locate flying prey, whereas frugivorous species rely more heavily on smell to locate food sources (Bogdanowicz et al., 1997).

Applying dental topographic analysis to microbats has the potential to provide further insight into dietary categorization using dental morphology, and past studies have shown that analysis of mammalian mandibular second molars can provide valuable insight to diet prediction in the fossil record (Boyer, 2008; Bunn et al., 2011; Ledogar et al., 2013; Pampush et al., 2016; Prufrock et al., 2016; Ungar, 2004). However, few studies have examined dental topography of both maxillary and mandibular molars. Occlusion allows for a dramatic increase in the level of oral processing in early tetrapods, and it develops independently in each species according to evolutionary pressure and diet (Reisz, 2006; Terhune et al., 2015). As such, dental topographical analysis of solely mandibular second molars demonstrate only half the evidence when it comes to topographical analysis.

Chapter 3: Methodology

Specimen Acquisition

This study was conducted on a phyllostomid museum sample (LSU Museum of Natural Science) from the Balta community of Peru. Balta lies deep in the Peruvian rainforest and is home to many different species of microbat. Figure 1 depicts examples of maxillary and mandibular second molars of specimens included in each dietary category analyzed in this study. This sample of paired maxillary and mandibular dentitions (N=99 individuals, 198 (99 maxillary, 99 mandibular) isolated molars) was collected by Dr. Laura Stroik in May 2012 and is detailed in Table 1. Figure 2 depicts the phylogenetic tree containing each species included in this study and is derived from the phylogeny of Rojas et al., 2016. Although not ideal for all statistical analyses, the sample size proposed here is the best available, and like all similar studies of dental material, this research acknowledges the limitations and assumptions built into the analysis of small samples.

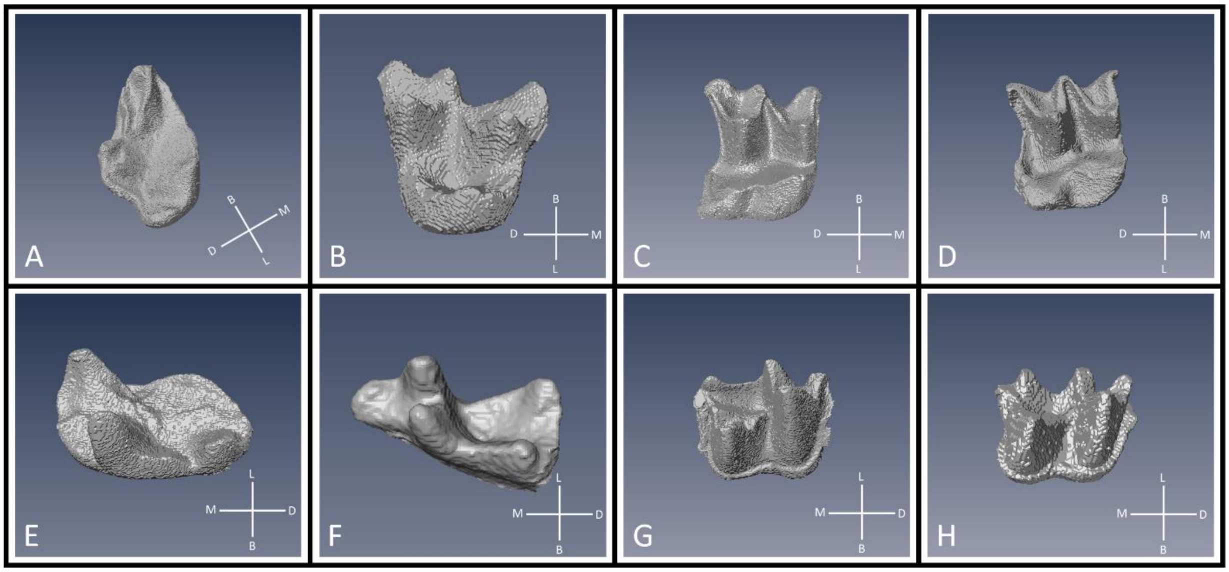

Figure 1. Frugivore *Artibeus obscurus* maxillary (A) and mandibular (E) second molars, frugivore-nectarivore *Anoura caudifer* maxillary (B) and mandibular (F) second molars, insectivore-frugivore *Lophostoma silvicolum* maxillary (C) and mandibular (G) second molars, insectivore *Macrophyllum macrophyllum* maxillary (D) and mandibular (H) second molars. M=mesial, D=distal, B=buccal, L=lingual.

Table 1. Balta, Peru specimens included in this study. Dietary group assignments are as follows: F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. N=number of individuals (2 molars (1 maxillary, 1 mandibular) per individual).

Species	Subfamily	Dietary Group	N
Artibeus cinereus	Stenodermatinae	F	4
Artibeus obscurus	Stenodermatinae	F	5
Artibeus planirostris	Stenodermatinae	F	4
Carollia brevicauda	Carolliinae	F	3
Carollia castanea	Carolliinae	F	4
Chiroderma villosum	Stenodermatinae	F	5
Mesophylla macconnelli	Stenodermatinae	F	3
Platyrrhinus brachycephalus	Stenodermatinae	F	3
Platyrrhinus helleri	Stenodermatinae	F	3
Rhinophylla pumilio	Rhinophyllinae	F	5
Uroderma bilobatum	Stenodermatinae	F	3
Uroderma magnirostrum	Stenodermatinae	F	5
Anoura caudifer	Glossophaginae	FN	4
Anoura geoffroyi	Glossophaginae	FN	1
Choeroniscus minor	Glossophaginae	FN	2
Glossophaga soricina	Glossophaginae	FN	4
Hsunycteris thomasi	Lonchophyllinae	FN	4
Sturnira lilium	Stenodermatinae	FN	3
Sturnira tildae	Stenodermatinae	FN	4
Lophostoma silvicolum	Phyllostominae	IF	4
Micronycteris megalotis	Micronycterinae	IF	3
Trinycteris nicefori	Glyphonycterinae	IF	1
Phyllostomus elongatus	Phyllostominae	IF	5
Tonatia saurophila	Phyllostominae	IF	4
Macrophyllum macrophyllum	Phyllostominae	I	4
Mimon crenulatum	Phyllostominae	I	4
Trachops cirrhosus	Phyllostominae	I	5
TOTAL			**99**

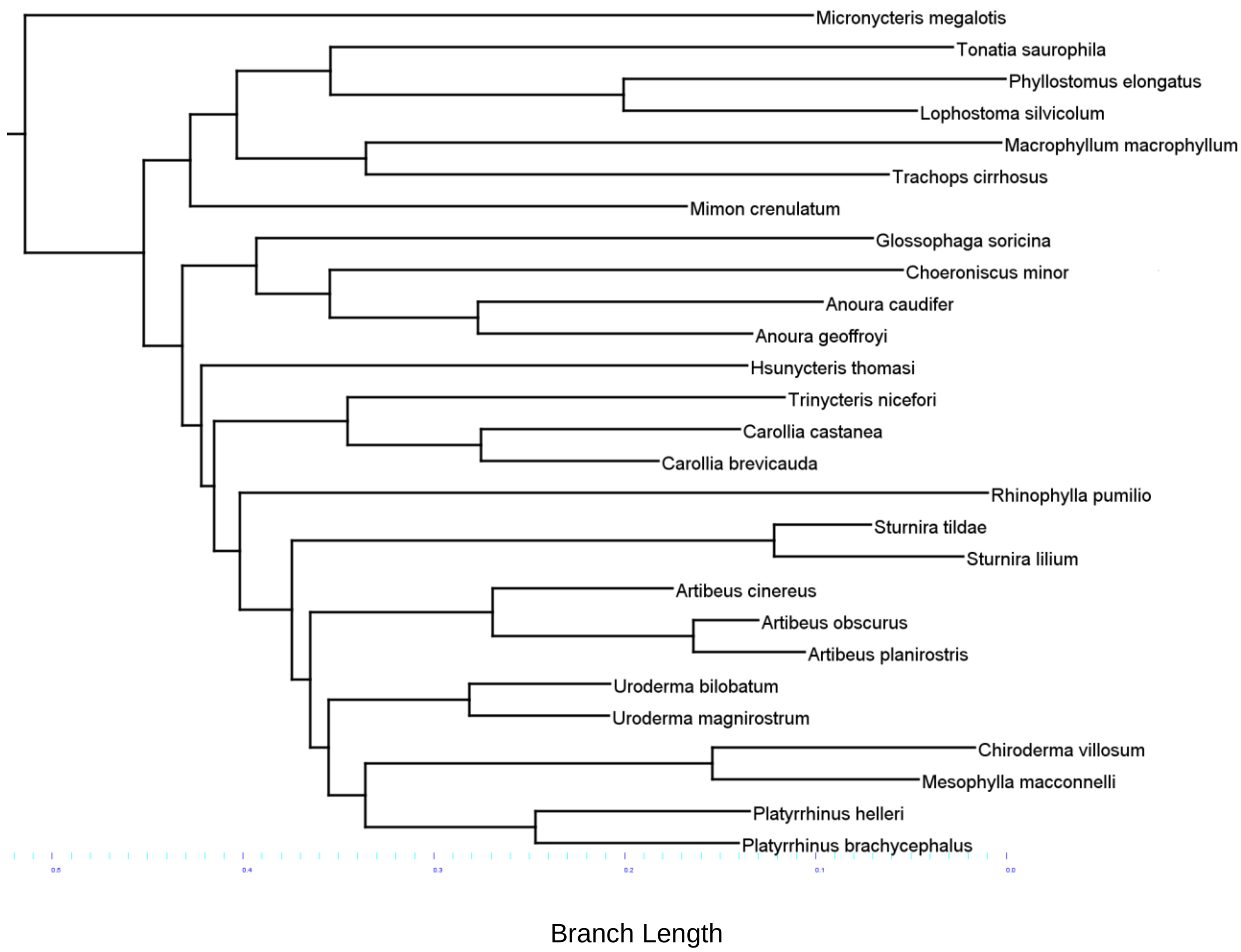

Figure 2. Phylogeny of species (N=27) included in this study, derived from *cytb* gene. Data was sourced from the phylogeny of Rojas et al., 2016.

Specimen Preparation

Dental impressions were taken of both maxillary and mandibular dentitions of each museum specimen by Dr. Laura Stroik. Each mold was used to create a cast of each tooth row using an epoxy material (EPO-TEK 301-1). Maxillary and mandibular second molars were excised from the cast rows of molars using a Buffalo Dental 4-speed micro-motor handpiece and diamond cutting disk. The casts were arranged on 1 in. diameter wafers in groups to save costs during the μCT scan process.

μCT-Scanning

Each of the wafers containing the specimens were shipped out to the Duke University Shared Materials Instrumentation Facility (SMiF) to be scanned using a Nikon XT H 225 ST micro x-ray computed tomography scanner (μCT). μCT-scanning is necessary to produce three-dimensional images of microbat molars due to their small size, which requires scanning at a high resolution (13μm). This scanner provides high resolution images of the interior and exterior surfaces of an object by projecting an x-ray beam onto the sample and creating a radiographic image of the interaction. It has been used for surface studies on small dental specimens due to its ability to create precise, high resolution topographic images.

Scan Processing and MorphoTester

Using the Amira software (version 5.2.0), the μCT scan files were rendered, cropped, and smoothed into a series of three-dimensional Tiff files that were used to reconstruct the three-dimensional surface of each tooth (Figure 3). These surface files (one for each molar) were then analyzed by the MorphoTester software (Winchester et al., 2016), which output DNE, RFI, and OPCR values for each molar specimen.

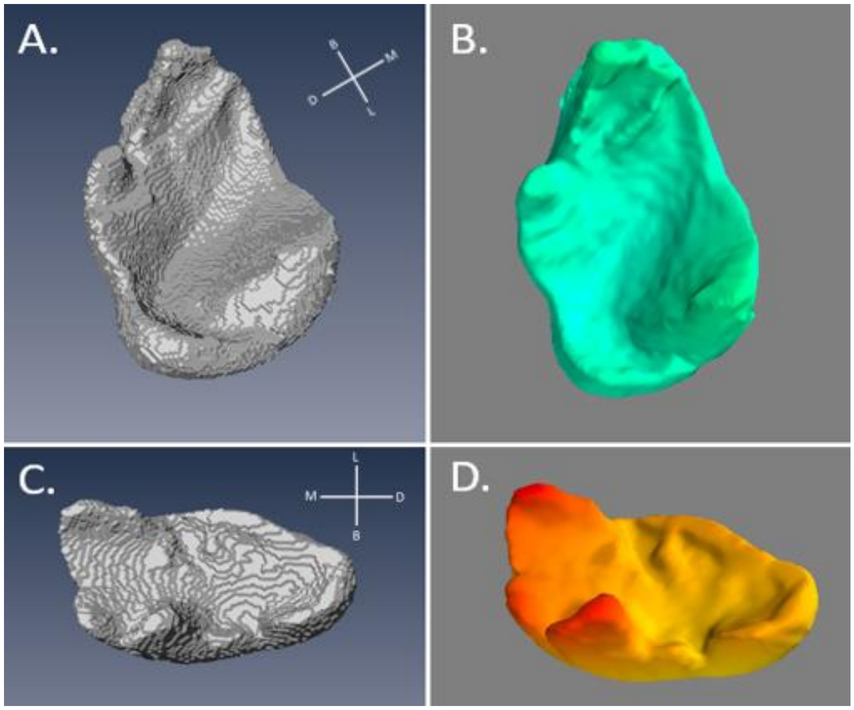

Figure 3. *Artibeus cinereus* maxillary (A,B) and mandibular (C,D) molars in Amira (A,C) and MorphoTester (B,D). Areas of higher relief are depicted in Morphotester by warm colors, and areas of lower relief are depicted by cool colors. M=mesial, D=distal, B=buccal, L=lingual.

Statistical Analyses

All statistical analyses were performed using the statistical package for the social sciences (SPSS) software. Before each statistical analysis was conducted, the data values for maxillary and mandibular molars were tested for normality for each topographic metric. The Shapiro-Wilk test of normality determined that RFI, DNE, and OPCR across all dietary categories were non-normal, thus a non-parametric Wilcoxon matched-pairs signed-ranks test was conducted on each topographic metric separately wherein the maxillary and mandibular molars of each individual forms a pair (N=99 pairs). The purpose of this analysis was to determine if each topographic measure results in similar values in occluding (paired) maxillary and mandibular second molars in microbats, and thus is the most appropriate analysis for directly testing Hypothesis 1.

Using the dental topographic values and known species diet categories collected from the literature, the topographical results of each measure (DNE, RFI, and OPCR) and all measures combined (DNE+RFI+OPCR) of maxillary and mandibular molars separately (Hypothesis 2A) and together (Hypothesis 2B) were tested for their efficacy at predicting species dietary niche, resulting in 12 total analyses: 4 analyses (DNE, RFI, OPCR, DNE+RFI+OPCR) each for maxillary molars, mandibular molars, and both maxillary and mandibular molars combined. The ability of the dental topographic variables to predict diet (using the dietary categories given in Table 1) was assessed using discriminant function analysis with cross-validation using jack-knifing, which has been employed by many researchers in this field testing similar hypotheses (i.e., the ability of dental metrics to predict diet): e.g., Boyer et al., 2008, Bunn et al., 2011, Stroik, 2014, Winchester et al., 2014. This analysis assigns groups based on discriminant functions and allows misclassification rates (in this case, percent of specimens misassigned to each dietary category) to be calculated to test the accuracy of the classification rules (in this case, the dietary predictive success based on the dental topographic input variables) (Khattree and Naik, 2000). Assessing the predictive value of each dental topographic measure is directly applicable to the accuracy of dietary reconstructions using molar morphology in the fossil record, the ultimate goal of studies of extant species.

Chapter 4: Results

Maxillary and Mandibular Topographic Similarity (Hypothesis 1)

Relief index was significantly different ($p<0.05$) between maxillary and mandibular second molars across all dietary categories. DNE and OPCR values of maxillary and mandibular molars were significantly different for the frugivore-nectarivore and insectivore dietary categories ($p<0.05$), whereas they were similar for the frugivore and insectivore-frugivore groups ($p>0.05$) (Table 2). Across the entire sample (N=99) with all dietary categories included, the RFI, DNE, and OPCR values for maxillary and mandibular molars were significantly different for each topographic metric evaluated (Table 2).

Comparison boxplots were created to illustrate the difference between maxillary and mandibular second molars for each topographic measure. Across all dietary categories (F=frugivore, FN=frugivore-nectarivore, IF=insectivore-frugivore, I=insectivore), mandibular second molars appeared to have higher RFI than maxillary second molars, which reinforces the significant difference found between maxillary and mandibular RFI in Table 2 (Figure 4). Similarity between maxillary and mandibular frugivore and insectivore-frugivore DNE values is evident based on their closely associated means (maxillary F=301.5, mandibular F=301.1, maxillary IF=383.7, mandibular IF=391.4) in Figure 5. A similar trend was present in frugivore and insectivore-frugivore maxillary and mandibular OPCR values (maxillary F=145.8, mandibular F=146.0, maxillary IF=137.8, mandibular IF=132.2), which further supports the lack of significant difference between the maxillary and mandibular values of those two dietary categories (Table 2; Figure 6). Maxillary and mandibular OPCR values had the greatest range in the frugivore group (maxillary=252.4, mandibular=157.125) when compared to the other three dietary categories (Figure 6), and this difference in variation between the frugivore and non-frugivore dietary categories will be further evaluated below.

Table 2. Results of Wilcoxon matched-pairs signed-ranks test for similarity between maxillary and mandibular second molars. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Topography was found to be similar in frugivore and insectivore-frugivore DNE and OPCR (p>0.05).

p-Values	F	FN	IF	I	All Dietary Groups
RFI	<0.001	0.004	<0.001	0.007	<0.001
DNE	0.975	0.002	0.619	0.003	0.004
OPCR	0.579	<0.001	0.246	0.006	0.006

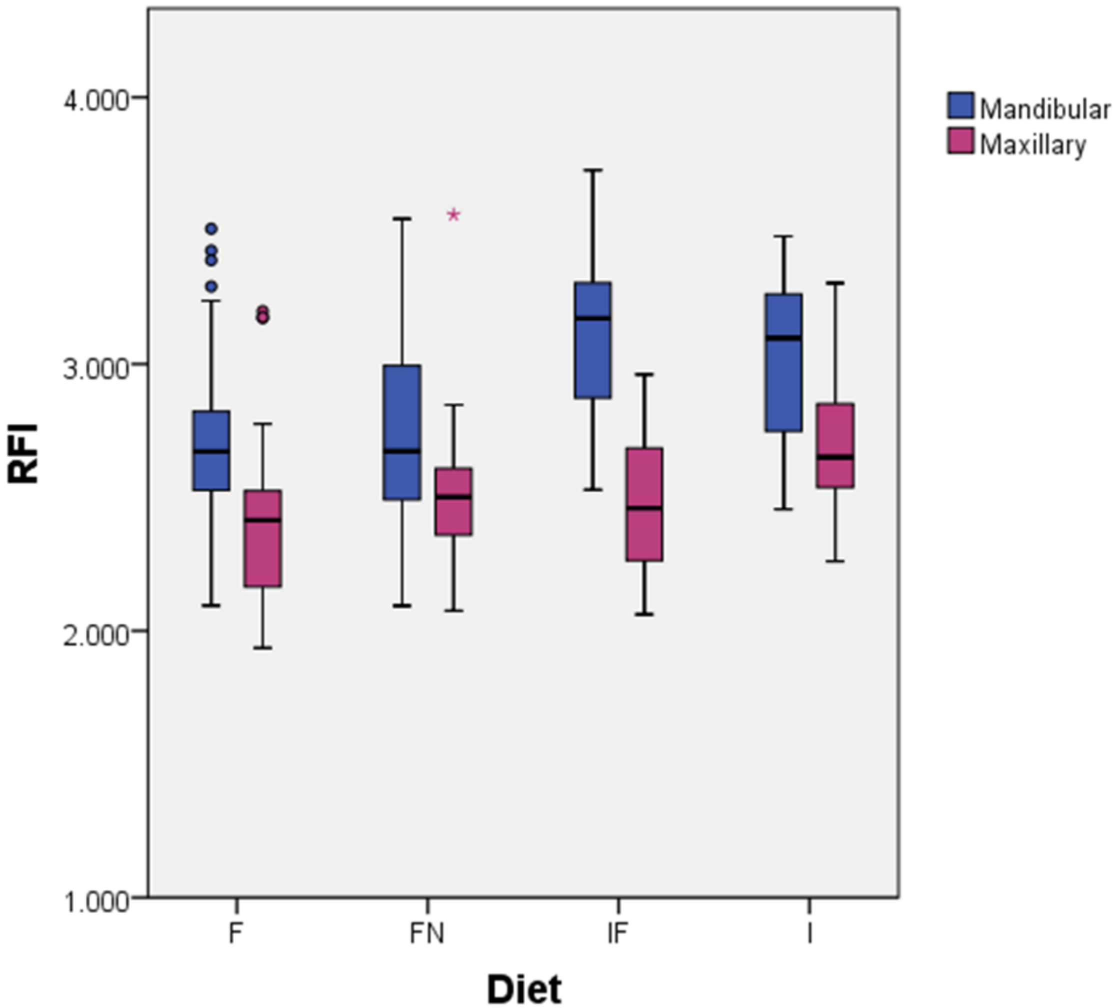

Figure 4. Boxplots of maxillary and mandibular relief index (RFI) values for each dietary group: F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore.

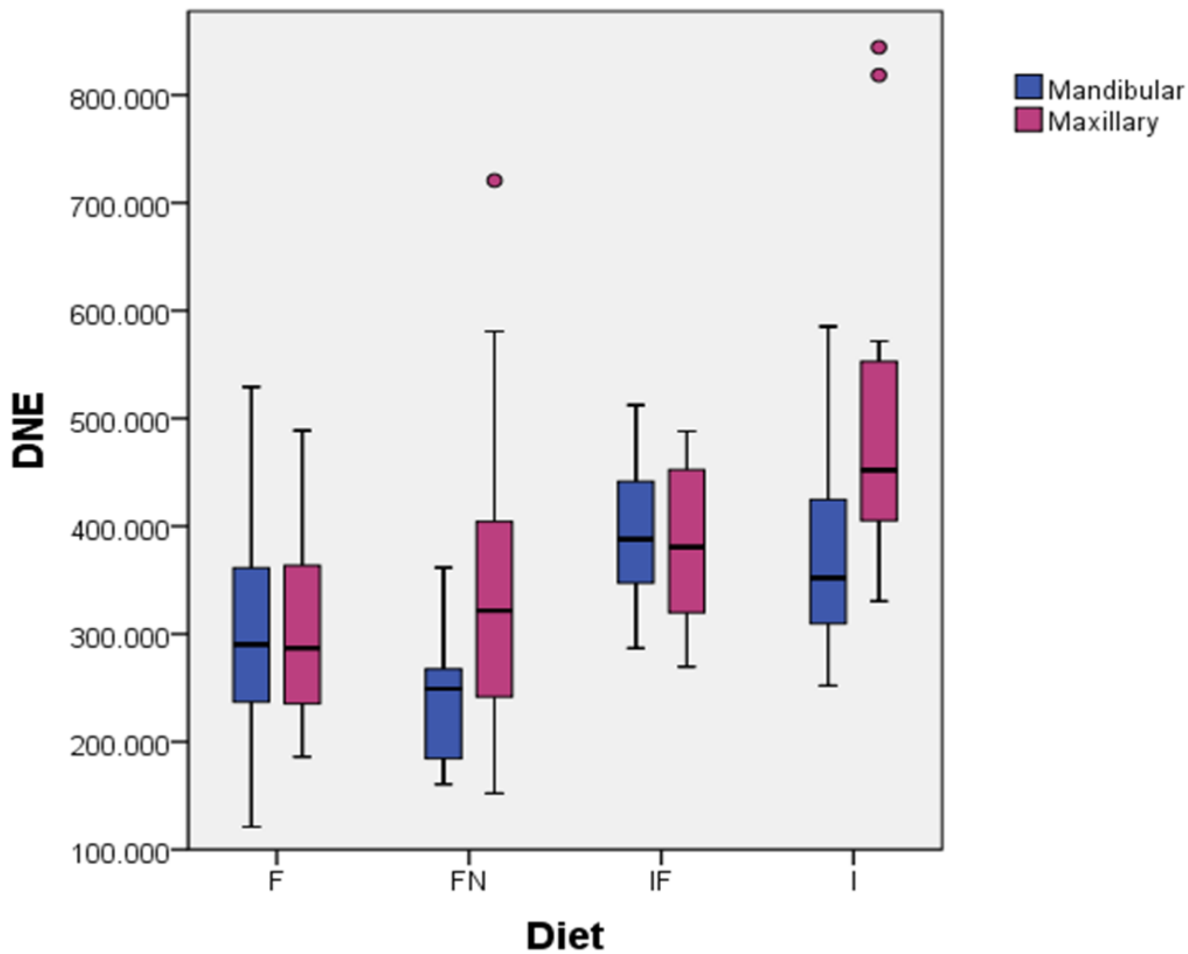

Figure 5. Boxplots of maxillary and mandibular Dirichlet normal energy (DNE) values for each dietary group: F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore.

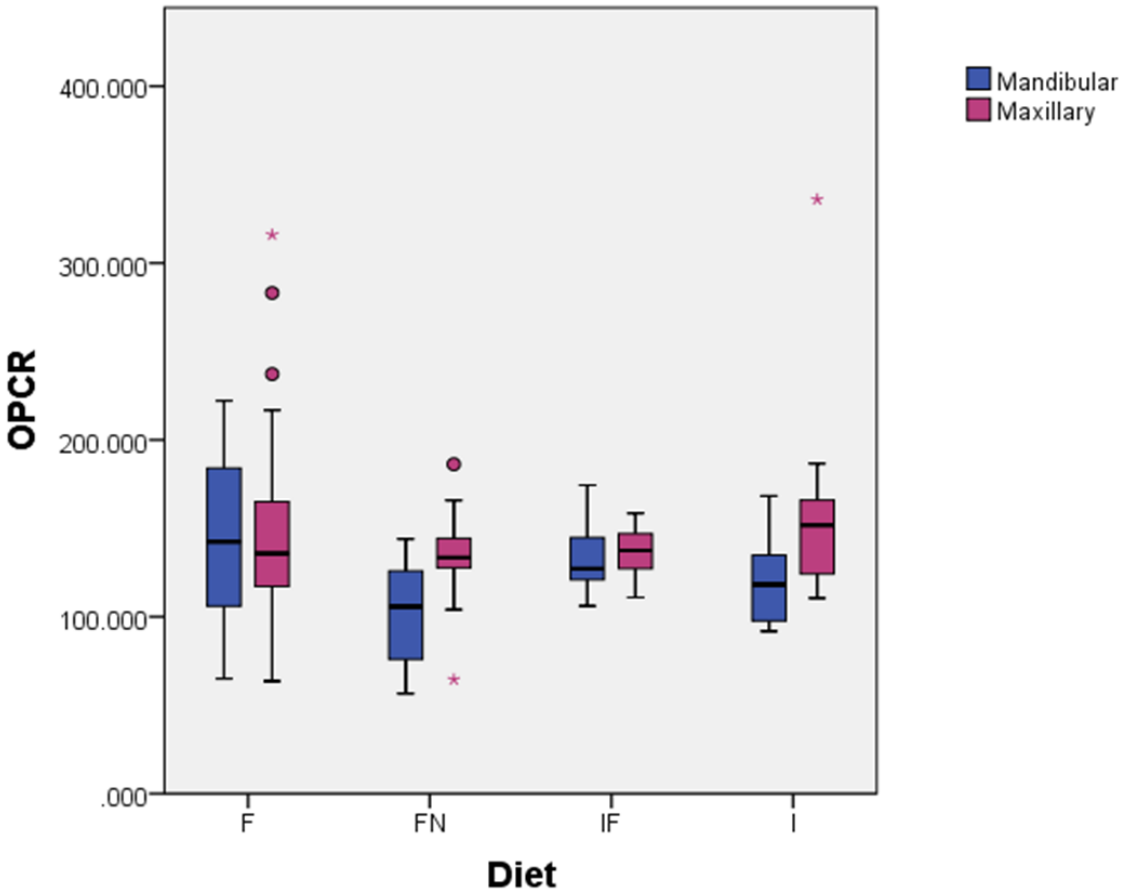

Figure 6. Boxplots of maxillary and mandibular orientation patch count-rotated (OPCR) values for each dietary group: F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore.

Maxillary and Mandibular Topography and Diet Prediction (Hypothesis 2A)

Table 3. Success of classification (%) for the total sample (N=99) from discriminant function analysis across all metrics.

	Maxillary	Mandibular
RFI	47.5	50.5
DNE	52.5	49.5
OPCR	47.5	47.5
RFI, DNE, OPCR	55.6	52.5

Table 4. Success of classification into dietary groups using cross-validated discriminant function analysis using relief index (RFI) for maxillary second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Maxillary			Classified Group			
			F	FN	IF	I
Original Group	F	N	44	0	0	3
		%	93.6	0.0	0.0	6.4
	FN	N	21	0	0	1
		%	95.5	0.0	0.0	4.5
	IF	N	10	0	0	3
		%	88.2	0.0	0.0	11.8
	I	N	15	0	0	2
		%	76.9	0.0	0.0	23.1

Table 5. Success of classification into dietary groups using cross-validated discriminant function analysis using relief index (RFI) for mandibular second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Mandibular			Classified Group			
			F	FN	IF	I
Original Group	F	N	42	0	5	0
		%	89.4	0.0	10.6	0.0
	FN	N	20	0	2	0
		%	90.9	0.0	9.1	0.0
	IF	N	9	0	8	0
		%	52.9	0.0	47.1	0.0
	I	N	8	0	5	0
		%	61.5	0.0	38.5	0.0

With an overall predictive success of 47.5% (Table 3), the likelihood of successful classification using maxillary RFI alone isn't an ideal success rate for accurate dietary prediction. Predictive success of maxillary second molar RFI was extremely low across each dietary category, save frugivores (93.6%) (Table 4). Mandibular second molar RFI dietary predictive success was also quite low across all dietary categories with the exception of frugivores; however, mandibular second molar RFI did show greater discriminatory capability in the insectivore-frugivore group (47.1% success) when compared to maxillary insectivore-frugivore RFI (0.0%) (Table 5), indicating that mandibular second molar RFI is more effective at insectivore-frugivore discrimination than maxillary second molar RFI. Maxillary RFI did exhibit greater discriminatory capability in the frugivore and insectivore categories than mandibular RFI, though only by 4.2% and 23.1%, respectively.

Table 6. Success of classification into dietary groups using cross-validated discriminant function analysis using Dirichlet normal energy (DNE) for maxillary second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Maxillary			Classified Group			
			F	FN	IF	I
Original Group	F	N	47	0	0	0
		%	100.0	0.0	0.0	0.0
	FN	N	19	0	0	3
		%	86.4	0.0	0.0	13.6
	IF	N	17	0	0	0
		%	100.0	0.0	0.0	0.0
	I	N	8	0	0	5
		%	61.5	0.0	0.0	38.5

Table 7. Success of classification into dietary groups using cross-validated discriminant function analysis using Dirichlet normal energy (DNE) for mandibular second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Mandibular			Classified Group			
			F	FN	IF	I
Original Group	F	N	37	5	5	0
		%	78.7	10.6	10.6	0.0
	FN	N	16	6	0	0
		%	72.7	27.3	0.0	0.0
	IF	N	11	0	6	0
		%	64.7	0.0	35.3	0.0
	I	N	10	0	3	0
		%	76.9	0.0	23.1	0.0

Discriminant function analysis using Dirichlet normal energy for maxillary second molars yielded classification success rates at 0% for frugivore-nectarivores and insectivore-frugivores, while frugivore and insectivore classification success was 100% and 38.5%, respectively (Table 6). The overall predictive success was 52.5%, which was the highest achieved for an individual metric on an individual tooth in this sample (Table 3). Mandibular second molar DNE had an overall predictive success of 49.5%, but had greater discriminatory capability for categorizing frugivore-nectarivores and insectivore-frugivores compared to maxillary second molar DNE (Tables 7,3).

Table 8. Success of classification into dietary groups using cross-validated discriminant function analysis using orientation patch count-rotated (OPCR) for maxillary second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Maxillary			Classified Group			
			F	FN	IF	I
Original Group	F	N	46	0	0	1
		%	97.9	0.0	0	2.1
	FN	N	22	0	0	0
		%	100.0	0.0	0.0	0.0
	IF	N	17	0	0	0
		%	100.0	0.0	0.0	0.0
	I	N	12	0	0	1
		%	92.3	0.0	0.0	7.7

Table 9. Success of classification into dietary groups using cross-validated discriminant function analysis using orientation patch count-rotated (OPCR) for mandibular second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Mandibular			Classified Group			
			F	FN	IF	I
Original Group	F	N	36	11	0	0
		%	76.6	23.4	0.0	0.0
	FN	N	11	11	0	0
		%	50.0	50.0	0.0	0.0
	IF	N	17	0	0	0
		%	100.0	0.0	0.0	0.0
	I	N	9	4	0	0
		%	69.2	30.8	0.0	0.0

Discriminant function analysis using maxillary OPCR exhibited the least amount of discriminatory capability among the three topographic metrics used. Maxillary OPCR had a 97.9% classification success rate for the frugivore group, and the other three categories were at or near 0.0% predictive success (Table 8). Mandibular second molar OPCR exhibited a lower predictive success rate for frugivores but higher predictive success for the frugivore-nectarivore group compared to maxillary OPCR (Tables 8,9). Overall predictive success for both maxillary and mandibular OPCR was 47.5% (Table 3).

Table 10. Success of classification into dietary groups using cross-validated discriminant function analysis for all topographic variables together (total topography) for maxillary second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications highlighted in blue.

Maxillary			Classified Group			
			F	FN	IF	I
Original Group	F	N	46	0	0	1
		%	97.9	0.0	0.0	2.1
	FN	N	17	2	0	3
		%	77.3	9.1	0.0	13.6
	IF	N	12	3	0	2
		%	70.6	17.6	0.0	11.8
	I	N	5	1	0	7
		%	38.5	7.7	0.0	53.8

Discriminant function analysis (DFA) of maxillary molar topography had a near 50% overall classification success rate for each topographic metric individually, and a near 56% overall success rate using all three topographic metrics together (total topography) (Table 3). In the total topography (RFI+DNE+OPCR) DFA for maxillary second molars, frugivore specimens had the highest classification success in the entire sample at 97.9%, followed by insectivores at 53.8%. Predictive success for the frugivore-nectarivore and insectivore-frugivore dietary categories were much lower at 9.1% and 0.0%, respectively (Table 10).

Table 11. Success of classification into dietary groups using cross-validated discriminant function analysis for all topographic variables combined for mandibular second molars. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Mandibular			Classified Group			
			F	FN	IF	I
Original Group	F	N	33	11	1	2
		%	70.2	23.4	2.1	4.3
	FN	N	12	9	0	1
		%	54.5	40.9	0.0	4.5
	IF	N	8	0	9	0
		%	47.1	0.0	52.9	0.0
	I	N	5	1	6	1
		%	38.5	7.7	46.2	7.7

Classification success for total topography (RFI+DNE+OPCR) of mandibular molars was greatest in the frugivore and insectivore-frugivore groups at 70.2% and 52.9%, respectively, and success was greater for the frugivore-nectarivore and insectivore-frugivore groups in mandibular molars than maxillary total topography (Tables 10,11). Mandibular second molar topography dietary predictive success was also near 50% for each individual metric and approximately 53% for total topography (Table 3) indicating that a combination of topographic metrics can more accurately predict diet than individual metrics alone.

Combined Maxillary and Mandibular Topography and Diet Prediction (Hypothesis 2B)

Table 12. Success of classification (%) for the total sample (N=99) from discriminant function analysis across all metrics.

	Combined
RFI	53.5
DNE	56.6
OPCR	49.5
RFI, DNE, OPCR	64.6

Discriminant function analysis was utilized to assess the dietary category predictive success of each topographic metric for maxillary and mandibular molars combined. Dietary predictive success for maxillary and mandibular second molar topography was greater using all 3 analyses combined rather than each metric individually (Tables 3,12).

Table 13. Success of classification into dietary groups using cross-validated discriminant function analysis using relief index (RFI) for maxillary and mandibular second molars combined. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Combined			Classified Group			
			F	FN	IF	I
Original Group	F	N	41	1	3	2
		%	87.2	2.1	6.4	4.3
	FN	N	19	1	2	0
		%	86.4	4.5	9.1	0.0
	IF	N	8	0	7	2
		%	47.1	0.0	41.2	11.8
	I	N	7	0	2	4
		%	53.8	0.0	15.4	30.8

Table 14. Success of classification into dietary groups using cross-validated discriminant function analysis using Dirichlet normal energy (DNE) for maxillary and mandibular second molars combined. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Combined			Classified Group			
			F	FN	IF	I
Original Group	F	N	38	3	6	0
		%	80.9	6.4	0.0	12.8
	FN	N	13	7	0	2
		%	59.1	31.8	0.0	9.1
	IF	N	11	0	6	0
		%	64.7	0.0	35.3	0.0
	I	N	6	0	2	5
		%	46.2	0.0	15.4	38.5

Table 15. Success of classification into dietary groups using cross-validated discriminant function analysis using orientation patch count-rotated (OPCR) for maxillary and mandibular molars combined. Number (N) and percent (%) of species classified into each dietary group listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Combined			Classified Group			
			F	FN	IF	I
Original Group	F	N	37	9	0	1
		%	78.7	19.1	0.0	2.1
	FN	N	11	11	0	0
		%	50.0	50.0	0.0	0.0
	IF	N	17	0	0	0
		%	100.0	0.0	0.0	0.0
	I	N	8	4	0	1
		%	61.5	30.8	0.0	7.7

Combined maxillary and mandibular RFI did confer greater discriminatory capability than maxillary or mandibular RFI alone in the discriminant function analyses (DFA), and predictive successes were greater within each dietary category in the combined maxillary-mandibular DFA than they were for either maxillary or mandibular DFAs (Tables 4,5,13). Overall predictive success using combined maxillary and mandibular RFI increased by 6.0% and 3.0%, respectively, compared to individual maxillary and mandibular second molar RFI predictive success (Tables 3,12). Combined maxillary and mandibular DNE increased overall dietary classification success by 4.1% for maxillary DNE and 7.1% for mandibular DNE. Combined DNE also showed greater dietary category discrimination with increased and more evenly distributed predictive successes across all dietary categories when compared to individual second molar DNE discriminant function analyses (Table 14). Using a combined maxillary and mandibular OPCR discriminant function analysis, overall predictive success increased by 2.0% compared to both maxillary and mandibular individual second molar OPCR DFAs (Tables 3,12). The combined OPCR DFA showed a similar trend of predictive success to mandibular second molars alone in that predictive success was higher in the frugivore and frugivore-nectarivore groups (Table 15).

Table 16. Success of classification into dietary groups using cross-validated discriminant function analysis for all topographic variables of maxillary and mandibular second molars combined. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Combined			Classified Group			
			F	FN	IF	I
Original Group	F	N	37	6	2	2
		%	78.7	12.8	4.3	4.3
	FN	N	8	12	1	1
		%	36.4	54.5	4.5	4.5
	IF	N	8	0	8	1
		%	47.1	0.0	47.1	5.9
	I	N	3	2	1	7
		%	23.1	15.4	7.7	53.8

Table 17. Success of classification into dietary groups using cross-validated discriminant function analysis for all topographic variables of maxillary and mandibular second molars – excluding frugivores. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Combined			Classified Group		
			FN	IF	I
Original Group	FN	N	21	0	1
		%	95.5	0.0	4.5
	IF	N	2	13	2
		%	11.8	76.5	11.8
	I	N	3	2	8
		%	23.1	15.4	61.5

Combined maxillary and mandibular classification success was also analyzed using discriminant function analysis of all 6 topographic variables for each individual: maxillary and mandibular RFI, DNE, and OPCR. Across each topographic metric, analyzing maxillary and mandibular molars together resulted in greater predictive success than individual molar topography (Table 3,12). Predictive success within the discriminant function analysis for the combined total topography was 65%, and the highest predictive success was in the frugivore and frugivore-nectarivore categories at 78.7% and 54.5%, respectively. The combined analysis showed the most evenly distributed predictive success rates across all dietary categories when compared to the individual second molar analyses (Table 16).

Table 18. Success of classification (%) for the total sample (N=99) from discriminant function analysis using combined RFI, DNE, and OPCR, excluding frugivores(F).

	RFI, DNE, OPCR - No F
Maxillary	50.0
Mandibular	71.2
Combined	80.8

The largest overlap of a single dietary category of the DFA plot of this sample was exhibited by the frugivore group (Figure 7). To assess the extent which frugivore diversity was affecting the discriminatory ability of the sample, a DFA was performed on the remaining three dietary categories alone. Predictive success increased sharply for the remaining three categories (frugivore-nectarivore, insectivore-frugivore, insectivore) when compared to the analyses including frugivores (Table 17). Overall predictive success decreased by 5.6% for maxillary, increased by 18.7% for mandibular, and increased by 16.2% for maxillary and mandibular combined when frugivores were excluded from the DFA (Table 18). This could be attributed to highly variable dental morphologies of fruit-eating bats used in this sample.

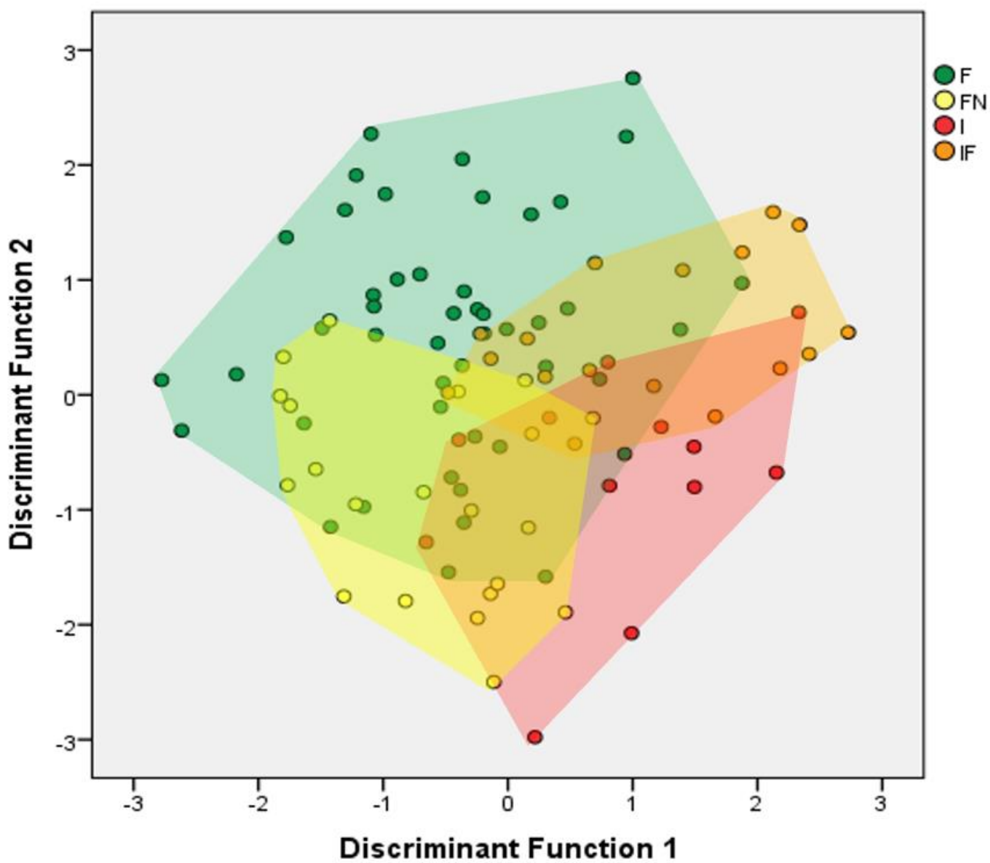

Figure 7. Plot of discriminant functions for maxillary and mandibular second molar topography (RFI, DNE, and OPCR) combined. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore.

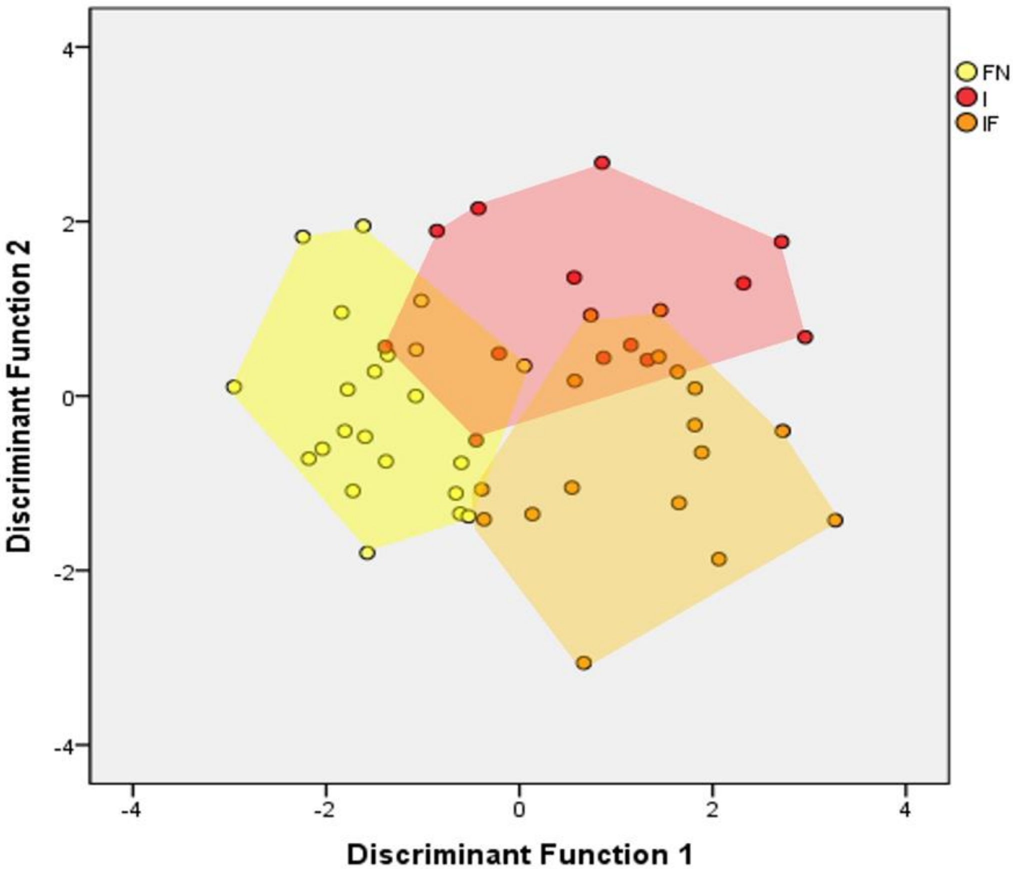

Figure 8. Plot of discriminant functions for maxillary and mandibular second molar topography (RFI, DNE, and OPCR) combined, excluding frugivores. FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore.

Plots of the first two discriminant functions for maxillary and mandibular combined topography were created to visualize the dietary category grouping within this sample. In Figure 7, the green frugivore category is quite large compared to the other 3 dietary categories and has a lot of overlap with the other categories included in this study. The plot of the first two discriminant functions excluding the frugivore category shows increased separation among the remaining dietary groups (Figure 8). Increased separation among dietary groups confers greater predictive accuracy in the discriminant function analysis.

Table 19. Success of classification into dietary groups using cross-validated discriminant function analysis for all topographic variables of maxillary and mandibular second molars combined with equal dietary group sizes. Number (N) and percent (%) of species classified into each dietary group are listed. F=Frugivore, FN=Frugivore-nectarivore, IF=Insectivore-frugivore, I=Insectivore. Correct classifications are highlighted in blue.

Combined			Classified Group			
			F	FN	IF	I
Original Group	F	N	9	3	0	1
		%	69.2	23.1	0.0	7.7
	FN	N	1	11	1	0
		%	7.7	84.6	7.7	0.0
	IF	N	3	2	6	2
		%	23.1	15.4	46.2	15.4
	I	N	1	2	0	10
		%	7.7	15.4	0.0	76.9

Since the number of frugivorous species in this sample is greater than each of the other dietary categories, a final DFA was run to assess whether utilizing equal group sizes would have a marked effect on the discriminatory capability of maxillary and mandibular combined RFI, DNE, and OPCR. Using 13 individuals from each group selected at random (N=52 individuals, 104 molars), predictive success increased for the frugivore-nectarivore and insectivore dietary categories when compared to the maxillary and mandibular RFI, DNE, and OPCR discriminant function analysis for the total sample (Tables 16,19). Overall predictive success for the equal group size DFA was 69.2%, compared to 64.6% for the total sample DFA, indicating a 4.6% increase. This is an improvement but still not a drastic change in overall predictive success for this sample.

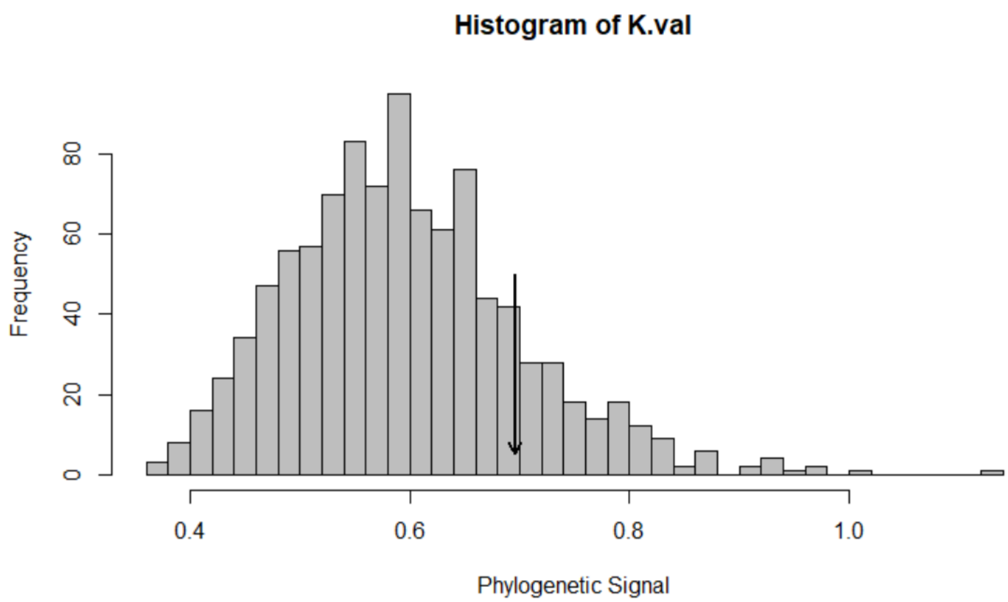

Figure 9. Histogram of K statistic for estimating phylogenetic signal within this sample. The arrow indicates the K statistic value for this sample.

To assess whether there might be phylogenetic patterning within the data obscuring dental variation based on diet, a K-statistic was employed. High-dimensional multivariate traits such as those used in dental topographic analysis can be analyzed along with the sample phylogeny to expound the phylogenetic relationships contained therein (Adams, 2014). The phylogeny for this sample was adapted from Rojas et al. (2016) via *cytb* sequencing (see Fig. 2). Results from this analysis showed there was no significant phylogenetic signal detected within the data ($K=0.6957$, $p=0.157$) (Figure 9). These results indicate that any variation in topographic values for both maxillary and mandibular second molars are most likely the result of adaptation to specific dietary regimes and not carryover from shared evolutionary history.

Chapter 5: Discussion and Conclusion

Observing and analyzing the form-function relationship of dental morphology and diet in extant species allows one to interpret the function from the form in extinct species. The overarching purpose of this study was to investigate the efficacy of maxillary second molar topography at dietary prediction in this sample, and by proxy, the fossil record.

In this study, it was hypothesized that maxillary second molar topography would be similar to mandibular second molar topography, but results indicated that maxillary and mandibular topographies were significantly different across most metrics and dietary categories. Visually, the maxillary molars of the species in this study are quite different from the matching mandibular molars, though this does not indicate that they couldn't have similar topographic values. Both maxillary and mandibular second molars could confer similar topographic values if there are areas of similar relief on different areas of the tooth crown. The topography of maxillary and mandibular second molars in this sample of phyllostomid bats were significantly different from each other, indicating that there are not similar areas of relief on the tooth crown for these species. This could be due to maxillary and mandibular teeth having specialized, independent functions and thus different molar surface morphologies where both assist in the breakdown of a specific diet. This variation between maxillary and mandibular second molar topography indicates that maxillary and mandibular molars each have unique structural features that play a specific role in the breakdown of food that are independent of each other but work commensally in the mastication and breakdown of particular diets. The geometry of blade shape for molariform teeth is different for maxillary and mandibular teeth since they do not directly occlude with each other as two blocks coming into contact might. Molar occlusion is offset, and blades or shearing crests on one margin of a mandibular molar could come into contact with complementary shearing crests on the opposite margin of an occluding molar in some cases (Evans, 2003). More specifically, if one half of a mandibular molar occludes with only half of a maxillary molar, the other halves of both the maxillary and mandibular molar would occlude with different molars and have different molar topographies. Molar occlusion is not always a direct relationship so differences in occluding dental topography are plausible.

The task of bringing the mandibular molars into occlusion with the maxillary molars is not a simple up and down motion. The mandible is hinged at the temporomandibular joint (TMJ) so the act of biting brings the mandible into occlusion with the maxilla in an upward swing motion. This motion could affect the dental topography of tooth crowns, resulting in the variation between maxillary and mandibular second molar topography within this study. Although the maxillary and mandibular second molar dental topographies are different, they still convey the same predictive success in this sample. Maxillary and mandibular second molars may have different roles in the process of mastication within this sample, but the same foodstuffs are being consumed and broken down between the two molars. The variation between maxillary and mandibular second molars was relatively proportional in each metric and dietary category. There are some variations between maxillary and mandibular second molars, which have been studied previously.

Maxillary second molars exhibit a talon that is present in many different dietary categories and serves as a basin for the crushing function of mastication. The presence of the upper molar talon has been previously shown to decrease maxillary relief index due to its low, flat molar area on the tooth crown (Gutzwiller and Hunter, 2015). This could have had a negative impact on dietary category discrimination especially in the insectivore group, resulting in the low predictive success for maxillary RFI in the insectivore-frugivore and insectivore groups specifically within this sample. Crushing aspects of mastication are not as important in insectivores as the puncture-shear mechanisms of their molar morphology (Lucas, 2004), so the presence and inclusion of the talon on the insectivore teeth could have adversely impacted the discriminatory capability of maxillary RFI. The other metrics included in this study are not as sensitive to molar area as relief index.

The results from hypothesis one show that the two molar topographies are not interchangeable, and maxillary molars cannot be assumed to be effective dietary predictors based on similarity to mandibular topography alone. However, these results did show that frugivore and insectivore-frugivore maxillary and mandibular DNE and OPCR were similar. This is at odds with the results of Santana et al. (2011), which found that maxillary and mandibular OPCR were significantly different across the dietary categories they assessed (frugivory, insectivory, omnivory) in their sample of phyllostomid bats. These differences could be attributed to the fact that Santana et al. (2011) used complete molar tooth rows (first through second or third molars when available), and this sample utilized second molars exclusively. Additionally, Santana et al. (2011) used orientation patch count (OPC), whereas orientation patch count-rotated (OPCR) was employed in this study. The two metrics differ in that OPCR accounts for orientation in three-dimensional space by averaging the patch counts taken in five to six degree rotations of the patch boundaries (Bunn et al., 2011).

Given that the maxillary and mandibular second molars have significantly different topographies, predictive models were employed to test their efficacy at dietary niche prediction. The classification success rates for relief index (RFI) in this sample are consistent with the results of Allen et al. (2015) in that maxillary and mandibular second molar RFI values confer similar overall predictive success rates of around 50%. RFI depends largely on molar surface area, which makes it especially sensitive to the cementoenamel junction cropping process, and it must be noted that variations in cropping could increase the variation of RFI within each dietary category and result in less than favorable predictive success rates using a discriminant function analysis (Bunn et al., 2011). Consistent cropping techniques must be employed within a sample in order to mitigate this source of variation, as was done in this study. In all, maxillary RFI was just as effective at dietary prediction as mandibular RFI.

Dirichlet normal energy (DNE) was the most effective topographic metric for dietary prediction in this sample for individual maxillary and mandibular second molars among the three topographic metrics analyzed. Before DNE was available as a viable indicator of diet in molariform teeth, researchers had difficulty differentiating insectivores from folivores (Kay, 1975; Boyer, 2008). DNE correlates strongly with other topographic metrics and can accurately distinguish among multiple dietary categories (Bunn et al., 2011). DNE is less dependent on molar surface area than RFI and is more affected by surface angularity than both of the other topographic methods tested. As such, DNE is less sensitive to the virtual cropping process at the cementoenamel junction than RFI (Bunn et al., 2011). DNE increases with sharp angles on a tooth surface where surface energy is high. Surface angularity increases in areas such as the interproximal space where virtual cropping takes place to isolate the anatomical tooth crown. It is possible that second molars that required significant cropping at the interproximal space could exhibit greater DNE values than would normally be expected. It is highly recommended that second molar isolation be completed prior to scanning the specimens to avoid laborious interproximal cropping methods in any surface rendering software. Abnormally high DNE values for second molars in this sample, which required a lot of isolation within the interproximal space could have had a negative impact on variation within the dietary categories, causing lower than expected predictive success rates. Despite these limitations, overall predictive success rates for individual maxillary and mandibular DNE were similar, indicating that maxillary second molar DNE is as effective at dietary prediction as mandibular second molar DNE in this sample.

Orientation patch count-rotated (OPCR) had the least amount of discriminatory capability within this sample compared to the predictive success of RFI and DNE for individual second molars. Surface complexity is predominantly high on phyllostomid frugivorous teeth since they require channels and crenulations for adequate processing of fruit pulp (Santana et al., 2011). Predictive success rates were highest in the frugivore and frugivore-nectarivore categories for both maxillary and mandibular second molar OPCR in this sample. Since frugivores differed significantly from both insectivores and omnivores within the analyses of Santana et al. (2011), and insectivores and omnivores did not differ from each other, it follows that the highest predictive success rates in this sample would be in differentiating frugivorous species from the other dietary categories analyzed. The results of this study follow the results of Santana et al. (2011).

Overall predictive success for individual maxillary and mandibular second molar OPCR were identical, adding to the indication that maxillary second molar topography is just as effective at dietary prediction as mandibular second molar topography.

Thus, maxillary second molar topography was able to predict species diet just as effectively as mandibular second molar topography in the majority of analyses in this study. Even though maxillary and mandibular topography differed significantly, they both conferred a similar dietary signal in the majority of discriminant function analyses. This could indicate that maxillary second molars have different dental topography compared to mandibular second molars which have inverse areas of relief and complexity on mandibular second molars that additively contribute to the trituration of specific diets. Maxillary second molars aside, the predictive success rates for individual and combined metrics in this sample for mandibular second molars follow the same trend as Bunn et al. (2011) in that individual metrics confer predictive successes at around 45-55%, and all metrics together (RFI+DNE+OPCR) resulted in higher predictive success rates than individual metric DFAs.

Further analyses utilizing maxillary and mandibular topographic metrics combined were conducted to assess dietary prediction accuracy for complete (maxillary + mandibular) specimens. This analysis achieved dietary predictive success similar to Allen et al. (2015) for maxillary and mandibular RFI combined at around 54%, though Allen et al. (2015) achieved greater combined predictive success when incorporating other linear aspects of molar morphology such as shearing quotient and molar length in addition to relief index. Shearing quotients are beneficial to include in studies of molar morphology since they represent a more specific feature on the tooth crown that is directly involved in food processing, whereas other metrics of dental topography include the “unimportant” areas such as sidewall curvature which do not directly participate in food trituration (Allen et al., 2015). Molar length is beneficial to include in these types of analyses since it confers size information for each specimen and can potentially enhance predictive success rates (Allen et al., 2015, Boyer, 2008). The analysis of Allen et al. (2015) builds on the results of Boyer (2008) by including more linear metrics in the dietary predictive analysis in addition to relief index, as well as including maxillary second molars topography. Assessing linear metrics such as shearing quotient and molar length in addition to dental topography on these specimens would be a logical next step in attempting to increase the dietary predictive success for these four categories.

Maxillary and mandibular combined DNE more accurately predicted species diet within this sample than individual second molar DNE alone, further supporting the prediction that combined maxillary and mandibular topography would confer greater predictive success than individual topography. Similar to DNE and RFI, combined maxillary and mandibular OPCR increased overall predictive success of this sample compared to individual second molar OPCR predictive success. These combined maxillary and mandibular DNE and OPCR results are the first tested for efficacy in dietary prediction within an extant mammalian community, and results indicate that combining maxillary and mandibular topography grants increased dietary predictive success. Utilizing these metrics, one can perform analyses of extinct species and determine properties of their ancient diets. In this sample, overall dietary predictive success was greatest when utilizing all three topographic metrics for maxillary and mandibular molars combined, which is recommended for use when complete specimens are available. Maxillary and mandibular combined RFI, DNE, and OPCR predictive success was greater than individual second molar RFI, DNE, and OPCR, indicating that maxillary and mandibular teeth, which form an occlusal unit, give a more accurate picture of diet than individual teeth. This is also the first known study to offer a complete topographic analysis using occluding maxillary and mandibular second molars, and combined maxillary and mandibular RFI, DNE, and OPCR resulted in the highest overall dietary prediction accuracy within this sample.

Due to the high level of variation within the frugivore category and increased misclassification rates for the frugivore group, it was hypothesized that the high number of frugivorous species within this sample were confounding the discriminant function analysis by creating the majority of the prediction rules. A separate DFA using the same sample sizes for each dietary category yielded a nominal improvement upon the original analysis using every individual in the sample. In future studies, it is recommended that equal sample sizes for each discriminatory category are used for a more accurate prediction model. Similar dental morphological traits between species resulting from carry-over from a shared evolutionary history could be causing a lack of separation among species of different dietary categories. The K-statistic for phylogenetic signal within this sample yielded no patterning within this data, indicating that the dietary signal is trumping the phylogenetic signal. However, although the Stenodermatinae and Carolliinae subfamilies have a shared evolutionary background and have only undergone adaptive radiation in recent evolutionary history (Freeman, 2000), they have significant differences in both maxillary and mandibular dental topography, which seems to be increasing the variation within the frugivore dietary category. The most plausible explanation for variation between these two subfamilies is adaptation to different fruit diets. Different fruits have different physical properties, and adaptation to a particular type of fruit may not confer the same topographic values as a specimen adapted to a different type of fruit. Exclusion of the frugivore group resulted in increased classification success when compared to discriminant function analysis including frugivores, suggesting that frugivore topographic variation was a limiting factor in the discriminatory power of the discriminant function analysis for maxillary and mandibular combined DNE, RFI, and OPCR.

Alternatively, overall predictive accuracy in this sample could be helped through subcategorization of the frugivore group into hard- and soft-fruit feeding bats in order to increase the separation of discriminant function category centroids. Species adapt to the physical properties of specific dietary regimes, and there are many diverse fruits available within the Balta rainforest. An experimental study by Dumont (1999) analyzed dietary preference of frugivorous phyllostomid bats and fruit hardness preference on two of the subfamilies that are included in this sample. Evidence indicated that Stenodermatinae species more efficiently processed the hard figs than the soft papayas, whereas the Carolliinae species exhibited the opposite behavior. Thus, subdivision of the frugivore category into hard- and soft-fruit feeding bats could have a positive impact on dietary discrimination by reducing variation within the broad frugivore category. Future research should also be targeted to increase dietary prediction accuracy by including more frugivore-nectarivore, insectivore-frugivore, and insectivore specimens to increase the sample size and statistical power of analyses utilizing those dietary categories.

Increasing the number of molars available for dietary predictive analyses in the field of paleontology is beneficial due to the scarcity of complete specimens. Many researchers discover isolated mandibles and maxillae, which is limiting for performing classification analyses, so including maxillary second molars in these analyses increases the range of data that is useful for dietary prediction. The study of extant mammalian dental morphology from observed morphology and function allows one to determine the function from the form in extinct species where observed function has never been recorded. In the event that complete specimens are discovered, combined maxillary and mandibular topography results in more accurate dietary classification and offers a more complete analysis through the inclusion of occluding maxillary and mandibular second molars.

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