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Consequences of Specialization in Neotropical Bats: Not All Foods Are Created Equal

Author

Hall, Ronald Parker

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Consequences of Specialization in Neotropical Bats: Not All Foods Are Created Equal

DISSERTATION

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In

Quantitative and Systems Biology

by

Ronald Parker Hall

Dissertation Committee:

Elizabeth Dumont (Advisor)

Jessica Blois

Sora Kim

Justin Yeakel

The dissertation of Ronald Parker Hall is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Elizabeth Dumont

Jessica L. Blois

Sora Kim

Justin Yeakel

Table of Contents

List of Figures

List of Tables

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Curriculum Vitae

Education

PhD University of California - Merced, Environmental Systems Dec 2024

Dissertation: Consequences of Specialization in Neotropical Bats: Not All Foods Are Created Equal

Advisor: Elizabeth R Dumont

BS William Marsh Rice University May 2017

Bachelor of Arts in Biochemistry and Philosophy

Publications

- PDB entries 6D2V and 6D34
- Clinger, J.A., Elshahawi, S.I., Zhang, Y., **Hall, R.P.**, Liu, Y., Thorson, J.S., Phillips Jr., G.N., *TerB and TerC mediated dehydration of polyporic acid displays a novel function for NTF-2 type enzymes, ACS Chemical Biology* 16.12 (2021): 2816-2824.
- Laurel R. Yohe; Matteo Fabbri; Daniela Lee; Kalina T.J. Davies; Thomas P. Yohe; Miluska K.R. Sánchez; Edgardo M. Rengifo; **Ronald P. Hall**; Gregory

Mutumi; Brandon P. Hedrick et al, *Ecological constraints on highly evolvable olfactory receptor genes and morphology in neotropical bats,* Evolution, (2022)

- Michael N. Dawson; Bailey J. Carlson; Sam R. Fellows; **Ronald P. Hall**; Bianca E. Salazar; Corey M. Shaver *"How the fraying fabric was woven: a pocket guide to the changing world." Frontiers of Biogeography* 13.2 (2021).
- Gregory L. Mutumi, **Ronald P. Hall**, Brandon P. Hedrick, Laurel R. Yohe, Alexa Sadier, Kalina TJ Davies, Stephen J. Rossiter, Karen E. Sears, Liliana M. Dávalos, Elizabeth R. Dumont, *Disentangling mechanical and sensory modules in the radiation of noctilionoid bats, American Naturlist (2022)*
- Neal Anthwal, **Ronald P. Hall**, Frederick Aneudy de la Rosa Henandez, Michael Koger, Laurel R. Yohe, Brandon P. Hedrick, Kalina T. J. Davies, Gregory L. Mutumi, Charles C. Roseman, Elizabeth R. Dumont, Lilina M. Dávalos, Stepehn J. Rossiter, Alexa Sadier, Karen E. Sears, *Cochlea development shapes bat sensory system evolution,* Anatomical Record (2023)
- **Ronald P. Hall**, Gregory L. Mutumi, Alexa Sadier, Kalina TJ Davies, Brandon P. Hedrick, Liliana M. Dávalos, Stephen Rossiter, Karen Sears, and Elizabeth R. Dumont, *Find the Food First: An Omnivorous Sensory Morphotype Predates Biomechanical Specialization for Plant Based Diets in Phyllostomid Bats*, Evolution, 75.11 (2021): 2791-2801.

Conference Presentations

- **Ronald P. Hall**, Gregory L. Mutumi, Alexa Sadier, Kalina TJ Davies, Brandon P. Hedrick, Liliana M. Dávalos, Stephen Rossiter, Karen Sears and Elizabeth R. Dumont, *How is Relative Sensory Organ Volume Related to Diet in Phyllostomids?,* Poster presented at NASBR 2018, Puerto Vallarta, Mexico
- **Ronald P. Hall**, Gregory L. Mutumi, Alexa Sadier, Kalina TJ Davies, Brandon P. Hedrick, Liliana M. Dávalos, Stephen Rossiter, Karen Sears and Elizabeth R. Dumont, *Ancestral Generalization as a Gateway to Rapid Dietary Divergence.* Talk given at NASBR 2019, Kalamazoo, Michigan.
- **Ronald P. Hall**, Gregory L. Mutumi, Alexa Sadier, Kalina TJ Davies, Brandon P. Hedrick, Liliana M. Dávalos, Stephen Rossiter, Karen Sears and Elizabeth R. Dumont, *Ancestral Generalization as a Gateway to Rapid Dietary Divergence.* Talk given at SICB 2020, Austin, Texas
- **Ronald P. Hall**, Gregory L. Mutumi, Alexa Sadier, Kalina TJ Davies, Brandon P. Hedrick, Liliana M. Dávalos, Stephen Rossiter, Karen Sears and Elizabeth R. Dumont, *Do Plant Visiting Neotropical Bats Show a Different Pattern of Sensory Investment?*, Twitter Presentation at the first World Bat Twitter Conference, 2020.

- **Ronald P. Hall**, Elizabeth R. Dumont, *Structural Constraints Induced by Feeding Performance May Shape Evolutionary Trajectories of Sensory Structures,* Presented at SICB+ 2022
- **Ronald P. Hall**, Elizabeth R. Dumont, *Island Endemic Frugivores and Nectarivores Show Opposite Patterns of Extremity,* Talk given at NASBR/IBRC 2022, Austin, Texas
- **Ronald P. Hall**, Phil Oelbaum, Kenneth Welch, Elizabeth R. Dumont, *Do Extreme Morphologies Limit Niche Breadth in Phyllostomidae,* Talk given at NASBR 2023, Winnipeg, Manitoba, Canada
- **Ronald P. Hall,** Elizabeth R Dumont, *Structural Constraints and Functional Demands Shape the Cochleae in Neotropical Noctilionoid Bats,* Talk given at NASBR 2024, Guadalajara, Mexico.

Public Talks

- **Ronald P. Hall,** Phil Oelbaum, *Bats of Jamaica,* Talk given to the Jamaican Naturalist Society July 2023
- **Ronald P. Hall,** *Bats of California and Beyond!,*Talk given to the public at a local event (Science on Tap) in Mariposa, California.

Grants Received

- Theodore Roosevelt Grant (2022) from the American Museum of Natural History, New York City, New York

Lab Experience

PhD student in the Dumont Lab (August 2019-Present)

- Stable Isotope Prep and Analysis
- 3D Geometric Morphometrics
- Phylogenetic Comparative Methods

Junior Morphology Specialist in the Dumont Lab (June 2018 -2019)

- Performed various statistical analyses using R scripts and Excel
- Collected data on soft tissues from CT scans for morphometric analyses
- Performed digital morphometric analyses

Lab Tech in the Phillip's Lab (August 2017 - May 2018)

- Performed protein extraction and purification (Nickel and SEC FPLC)
- Performed expression and solubility tests
- Set up protein crystallization screens and optimization trays

Field Experience

Sites:

- Lamanai Outpost, Belize (November 8-14, 2021) and (May 1-8, 2022)
- Sylvan Field Station, Costa Rica (February 21 – March 14, 2023)
- Jamaica (various caves) (March 22 – April 4, 2023)

Skills

- Catching bats in mist nets and harp traps
- Field identification
- Wing Punch Biopsies
- Hair Sample Collection
- Pit tagging
- Field Morphometrics

Teaching Experience

Teaching Fellow – Opportunities in Data Science

- Curriculum Development (Assignments and Course Content)
- Lectures

Teaching Assistant

- Biology for Non-Majors
- Ecosystems of California

Outreach and Service

Radio Bio – a science communication podcast run by graduate students at UC Merced focused on outreach to the local community

- Conducted interviews with researchers
- Storyboarded and produced podcasts
- Chair of Events Committee 2020-2021
- Member of Events Committee 2019-2020
- President 2022-2023, 2023 – 2024

Abstract

Neotropical leaf-nosed bats are an incredible example of adaptive radiation within mammals. This family of bats exhibits a wider range of dietary habits than any other mammalian family and they possess a high level of morphological variation to accommodate their dietary habits. With carnivorous, nectarivorous, frugivorous, and even sanguivorous species, they make an exceptional study system to understand patterns in the evolution of diet and morphology. In particular, the morphological spectrum between incredibly long-faced nectar specialists and very short-faced fruit specialists allows for an investigation of the limits and consequences of specialization. In Chapter 2, I outline the relationship between different measures of specialization within this family using stable isotope analysis of the hair of wild caught individuals. These stable isotope ratios are compared with morphological data from museum specimens and dietary data from previous studies. Supplement 1 contains data for all museum specimens used for morphometric data throughout this dissertation. In Chapter 3, I examine biogeographic patterns in specialization within two different dietary groups to understand the limitations of specialization. Palate aspect ratio, a morphological proxy for diet, is compared between Caribbean endemic and continentally distributed frugivorous and nectarivorous bats to identify different patterns of specialization in different evolutionary contexts. Lastly, in Chapter 4 I use geometric morphometrics to examine the relationship between the shape of the cochlea, the shape of the skull, and diet within these bats. These analyses reveal a complicated network of impacts on the shape of the cochlea in this family of bats. Overall, I describe different metrics of specialization, identify different ecological consequences of specialization on different resources, and possible structural trade-offs driven by high levels of specialization under ecological and structural constraint.

Chapter 1 : Background and Introduction

The concept of diversity is central to both ecology and evolutionary biology [Schluter 2000, Brooks and McLennan 2002, Daly et al 2018]. Increased diversity, measured in various ways, is associated with increased resilience and ecosystem function. This includes phylogenetic diversity [Srivastava et al 2012, Lean and Maclaurin 2016], species richness [Potter and Woodall 2014], and functional diversity [Tilman 2001] to name a few. As we face previously unseen levels of habitat destruction and climate change, understanding and maintaining biodiversity is critical [Anderson and Ferree 2010, Bellard et al 2012].

Evolution is not strictly a process of the past, it continues in the present and bringing evolutionary biology into a conservation context is needed [Crandall et al 2000, Stockwell et al 2003, Kinnison and Hairston 2007, Mace and Purvis 2007]. Understanding evolvability can give key insights for conservation biology [Milot and Maris 2020]. Furthermore, developing our comprehension of the guiding principles of evolution, diversification, and specialization more generally will help contextualize biodiversity risks globally. Specialization in particular plays an important role in shaping the evolution of diverse functions and forms, as is the case in adaptive radiations [Schluter 2000, Monteiro and Nogueira 2010, Stroud and Losos 2016]. However, ecological processes inherent in the process of specialization leave organisms with a narrower niche [Futuyma and Moreno 1988, Devictor et al 2010]. The characteristic dependence on a smaller set of resources leaves specialized species across a range of lineages vulnerable to extinction, by anthropogenic factors or otherwise [McKinney 1997, Meyer et al 2008, Ofori et al 2017, Griffis-Kyle et al, 2018, Hossain et al 2018].

This research project aims to identify potential consequences of specialization within a unique and diverse lineage: family Phyllostomidae. Phyllostomidae is highly speciose and contains immense ecological and morphological variation [e.g. Freeman 2000, Monteiro and Nogueira 2011, Dumont et al 2012, Hedrick et al 2020, Santana et al 2024]. This family has been the focus of studies investigating habitat change [Willig et al 2007, Meyer and Kalko 2008], evolution of morphology and disparity [Monteiro and Nogueira 2011, Hedrick et al 2020, Mutumi et al 2023], and the relationship between morphology and diet [Freeman 2000, Dumont 2004, Hedrick and Dumont 2018]. Evolutionary analyses have connected specialization to increased levels of speciation and trait evolution within this family [Shi and Rabosky 2015] and identified alternation between generalization and specialization as central to the evolution of this family [Dumont et al 2012, Hall et al 2021, Shi et al 2021].To continue investigating the history and impact of dietary specialization in this unique lineage, the project sets out to do the following:

Primary Research Objective

1. Characterize specialization within phyllostomids by integrating observational data, morphology and isotope ecology.
2. Identify possible differences in vulnerability between specialists and generalist phyllostomids
3. Assess ecological and morphological trade-offs within phyllostomids

Project Overview

Chapter 2: Integrating Three Metrics of Dietary Preference in a Phyllostomid Bat Assemblage Reveals a Complex Picture of Specialization

Abstract: Neotropical Leaf-Nosed Bats (Family: Phyllostomidae) are remarkably speciose and morphologically diverse. Though they show a broad geographic distribution from North to South America, including the Caribbean, they are also present in highly diverse assemblages in geographically local areas. We utilize morphometrics, isotope ecology, and compiled observational data from the literature to assess specialization in a community of Phyllostomids in Costa Rica. With hair samples from 31 species and morphometrics from 29 species, we are able to assess the relationship between morphological specialization, dietary specialization, and isotopic niche breadth. Overall, we find that while carbon and nitrogen isotope ratios pair well with morphological and dietary data to describe feeding ecology, isotopic niche breadth is not explained by morphology or dietary specialization. We advocate for the use of additional isotopes in future studies and the careful interpretation of isotopic analyses in the face of dietary complexity.

Chapter 3: The Caribbean Archipelago Fosters Specialization for Frugivory and Limits Specialized Nectarivory in Phyllostomid Bats

Abstract: The Caribbean is an important region for the diversification of many lineages, and the neotropical leaf-nosed bats (Family: Phyllostomidae) are no exception. This family contains diverse diets and morphologies, including specialized frugivores, nectarivores, and generalists. Phyllostomids, particularly phytophagous taxa, have colonized the islands of the Caribbean several times and the islands are known to have fostered diversification within the Stenodermatine clade of frugivores. However, the

archipelagos are highly disturbed on an evolutionary time scale and so we hypothesized that specialization for fruit and flowers would be reduced on the islands compared to taxa with a strictly continental distribution. Overall, we identify reduced levels of specialization and colonization for nectarivorous taxa but increased specialization in frugivorous taxa. We elaborate on key differences between frugivory and nectarivory that may explain this disparity.

Chapter 4: Cochlea Shape in Noctillionoid Bats is Influenced by Diet, Structural Constraint, and Evolutionary History

Abstract: The cochlea is a sensory organ that is highly conserved across mammals. In most bats, it serves a vital role in enabling echolocation. Most bats are aerial insectivores, and echolocation is central to their survival. Plant visiting bats, however, may have different echolocation demands – paleotropical frugivores, for instance, have lost the ability to echolocate altogether. In the neotropics, frugivorous and nectarivorous bats still echolocate and are close relatives with echolocation specialists. We assess the morphology of the cochlea in a highly diverse lineage of neotropical bats (superfamily Noctilionoidea) and the relationship between cochlea morphology, diet, and skull shape. Our results suggest that the evolution of specialized echolocation in mormoopid bats as well as the evolution of omnivory in phyllostomid bats both play a central role in shaping the evolution of the shape of the cochlea. Furthermore, unique aspects of the cochlea shape are independently impacted by lineage, diet, and cranial morphology suggesting a complicated picture of functional and structural constraint on the evolution of cochlea shape in bats.

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Chapter 2 : Integrating Three Metrics of Dietary Preference in a Phyllostomid Bat Assemblage Reveals a Complex Picture of Specialization

Introduction

Classifying species as generalists or specialists is central to ecological research. ‘Generalist’ refers to species that are able to feed on or utilize various habitats or resources whereas ‘specialist’ refers to species we identify as requiring specific resources and conditions to survive [Futuyma and Moreno 1988, Devictor et al 2010]. In other words, generalists are organisms with broad niches while specialists are organisms with narrow niches. Across a variety of taxa, the level of specialization of an organism has implications for extinction vulnerability [McKinney 1997, Vázquez et al 2002, Clavel et al 2011, Reed and Tosh 2019], their role in ecosystem stability [Richmond et al 2005, Dennis et al 2011, Dehling et al 2021], and even evolution and diversification [Buress 2015, Ebel et al 2015, Reynolds et al 2016, Martinossi-Allibert et al 2017, Sexton et al 2017].

Because animals must eat, dietary niche is a frequent focus for ecological studies of animals [eg. Kartzinel et al 2015, Shipley et al 2019, Felice et al 2019]. Predictions about the dietary habits of vertebrates are often generated based on relevant morphological traits, particularly of the skull [Samuels 2009, Santana and Dumont 2012, Figueirido et al 2013, Powder et al 2015, Law et al 2018, Felice et al 2019]. While we often think of functional aspects of morphology as being adaptive, and often they are, they also delimit what sorts of resources are available to an animal. Features such as bite force, snout length, gape angle, and cranium size limit resource availability in some ways while allowing access to particular resources [Emerson 1985, Dumont and Herrel 2003, Christiansen and Wroe 2007, Nogueira et al 2009, Kendrick and Hyndes 2015, Maestri et al 2016]. For this reason, when empirical dietary data are scarce cranio-facial morphology can be a helpful starting point for understanding the dietary ecology of an animal.

Stable isotope analysis (SIA) offers a complementary method for assessing the diet of an animal [Tykot 2004, Shoeninger 2010, Katzenberg and Waters-Rist 2018]. Though isotopic niches are not identical to the ecological niche of an organism, they can represent certain elements of the ecological niche of an organism. By leveraging natural differences in the ratios of stable isotopes of Carbon, Nitrogen, and other elements, we can gain insight into the ecology of animals. These isotopic axes represent key features of an organism's diet including trophic position [Post 2002], canopy vs understory foraging [Bonafini et al 2013], and energy source at the base of the food chain [Rounick 1986]. SIA also allows for straightforward quantification of niche overlap and uniqueness for identifying potential competition or partitioning [Young et al 2010, Swanson et al 2015, Shaner 2022].

In this study, we compare isotopic niches with morphological and observational dietary data in a hyper-diverse group of neotropical bats, the family Phyllostomidae. Phyllostomids represent a considerable mammalian radiation into diverse diets and morphologies [Freeman 2000, Dumont 2004, Dumont et al 2012, Hedrick and Dumont 2018]. Palate aspect ratio and its associated relationship to feeding performance are central to the ecological radiation of this family [Monteiro and Nogueira 2011, Hedrick et al 2020, Mutumi et al 2023]. Our primary hypothesis is that species with very low and very high palate aspect ratios, features thought to be indicative of dietary specialization within this family, will have smaller isotopic niche breadths, more unique isotopic niches, and increased levels of specialization as quantified by observational dietary data than species with intermediate palate morphologies. On the other hand, for species with intermediate morphologies, thought to be indicative of dietary generalization, we expect broader niches and reliance on more kinds of food resources.

Methods

Morphometric data was collected at the American Museum of Natural History in New York City, New York, by Dr. Elizabeth Dumont and Ronald Hall. Specimen measurements and museum record numbers are reported in Supplement 1. Palate aspect ratio is calculated as the length of the hard palate divided by the width of the palate at the widest point across the molars. Species averages are used for analyses. To represent different levels of morphological extremity, palate aspect ratios are used to bin taxa into 4 categories: extremely short faced, short faced, long faced, and extremely long faced. These categories are defined by the four standard quartiles along the distribution of palate aspect ratios. We lack morphometrics for two taxa for which we have dietary data; those taxa are excluded from statistical analyses.

Dietary data are drawn from data compiled by Rojas et al 2011. This compilation assigns a degree of reliance on particular food resources (invertebrates, vertebrates, fruit, and nectar). The scale is: 0 (none of a resource), 1(some of a resource), 2 (predominantly that resource) to 3 (exclusively that resource). We lump insects and vertebrates into one feeding category as in Hall et al 2021 because carnivores can be regarded as big insectivores rather than a unique foraging guild [Giannini and Kalko 2005]. Dietary categories are assigned based on the resource a genus relies on the most (at least a 2 on the dietary reliance scale). Our dietary categories are Frugivore, Animalivore, Nectarivore, and Generalist. The generalist category encompasses taxa which have a dietary reliance score of 2 in more than one food category. To generate a specialist–generalist spectrum that is diet agnostic, we assigned each taxa a level of specialization by assigning any genera that rely heavily on a single food type the 'specialist' category, any genera that rely equally on two food types to an 'intermediate' category, and taxa that rely equally on three food types to the 'generalist' category. We perform analyses with individual dietary item reliance scores, dietary categories, and specialization level.

Hair samples for stable isotope analysis were collected across three field excursions to the Sylvan Ecolodge in Puntarenas Province, Costa Rica. This study incorporates hair samples from 339 individuals across 31 taxa. Five taxa do not have an n=3 or more and are excluded from statistical analyses, though their species means are reported. The first sampling effort was in June of 2022, followed by trips in February/March of 2023 and 2024. Field capture and handling of bats was done with permission of the Ministerio de Ambiente y Energía (MINAE) Comisión Nacional para la Gestión de la Biodiversidad (R-014-2022-OT-CONAGEBIO). Handling and sampling procedures were approved by the Animal Care Committee of the University of Toronto (AUP #20012113) and University of California Merced (IAUCUC D16-00791, A4561-1), and following the guidelines of the American Society of Mammalogists [Sikes et al. 2016].

The $\delta^{13}C$ and $\delta^{15}N$ values and elemental carbon and nitrogen contents of all samples were measured in the Stable Isotope Ecosystem Laboratory at the University of California, Merced. Hair samples were washed in a solution of 2:1 Chloroform:Methanol and allowed to dry prior to isotope analyses. Samples were weighed into tin capsules and combusted in a *Costech 4010 Elemental Analyzer* coupled with a *Delta V Plus Continuous Flow Isotope Ratio Mass Spectrometer*. Carbon and nitrogen isotope compositions were corrected for instrument drift, mass linearity and standardized to the international VPDB ($\delta^{13}C$) and AIR ($\delta^{15}N$) scales using the USGS 41a and USGS 40 standard reference materials. Mean measured isotope compositions for reference materials pooled across all runs are USGS 40 = -26.39±0.08‰ (n= 109) and USGS 41a = 36.55±0.11‰ (n=65) for $\delta^{13}C$ and USGS 40 = -4.52±0.14‰ (n= 109) and USGS 41a = 47.54±0.18‰ (n=65) for $\delta^{15}N$. Elemental carbon and nitrogen contents were determined via linear regression of CO_2 and N_2 sample gas peak areas against the known carbon and nitrogen contents of USGS 40, USGS 41a, and EA acetanilide. All isotope compositions are expressed in standard delta notations where:

$\delta = (R_{sample}/R_{standard} - 1) \times 1000$ and R represents the ratio of heavy to light isotope.

To investigate the relationship between morphological specialization and observed dietary specialization, two separate one-way ANOVAs are performed, one for palate aspect ratio against dietary category and one for palate aspect ratio against specialization level. Reliance on each dietary item – animal prey, fruit, and nectar – is also compared with trophic level ($\delta^{15}N$ range) via one-way ANOVAs. We also used one-way ANOVAs to assess the relationship between isotopic niche breadth and specialist level as well as between isotopic niche breadth and morphological categories. All ANOVAs use the aov() function and post-hoc tests are performed with TukeyHSD in R version 4.3.1 [R Core Team 2023]. Plots are generated with the ggplot2 package in R [Wickham 2016].

We used niche breadths and overlap probabilities generated in the nicherOVER package in R [Lysy et al 2023] to assess niche size and overlap. Niche breadths are calculated with a 95% threshold. 'Overlap probability' is a one-way metric representing the probability that a species' niche occurs in the range of another species' niche or, in other words, the proportion of a species' niche that exists within the niche of another species. This probability is calculated pair-wise for each species occurring in the niche range of each other species. Overlap probabilities of 50% are used as a cutoff to identify a probable overlap. For each species, the number of other niches its niche is found within is reported and analyzed. ANOVAs are performed on regressions of number of other species' niches a species is overlapped by against morphological bins while linear regressions are used to compare the number of other niches occupied against niche breadth.

Results

Animalivores and generalists occupy trophically higher positions with increased values of $\delta^{15}N$ (Figure 2-1). Degree of reliance on fruit (F=3.69 (1,29), p=0.07) and nectar (F=0.009 (1,29), p=0.93) are not associated with trophic level. In contrast, increasing degree of reliance on animal prey is strongly associated with increased trophic level (F=14.63 (1,29), p<0.001). Interestingly, *Centurio senex*, the least animalivorous frugivore, has a higher trophic level (higher $\delta^{15}N$) than any other frugivore. Two frugivores, *Carollia sowelli* and *C. castanea*, have substantially lower $\delta^{13}C$ values than other taxa in this data, indicating reliance on a different carbon source than the rest of the bat community. This source may be plants in the genus *Piper*, an understory plant that is common in disturbed habitats whose fruit *Carollia sp.* are known to prefer [Thies and Kalko 2004]. *Carollia perspicillata* plots closer to other frugivores indicating a possible reduced reliance on *Piper*. Anecdotally, we observed C. *perspicillata* eating bananas at our field site – a behavior consistent with its isotopic shift away from other *Carollia* species in the analysis.

Palate aspect ratio is significantly associated with diet category (F=16.58 (3,25), p<0.001). Nectarivores have substantially higher aspect ratios than other dietary categories, frugivores and generalists have low aspect ratios, and animalivores occupy an intermediate range (Figure 2-2). Aspect ratio is not significantly related to level of specialization (specialist, intermediate, generalist) across all taxa (F=0.875 (2,26), p=0.429). There is a significant relationship between aspect ratio and degree of frugivory (F=37.99 (1,27), p<0.001) and degree of nectarivory (F=5.066 (1,27), p<0.05) (Figure 2-3,4). Tukey post hoc comparisons of aspect ratio among degrees of frugivory identified significant difference between occasional (1) and predominant (2) frugivores (p<0.001). Strict frugivores (3) have an even lower aspect ratio, but a sample of one. Tukey post-hoc comparison of aspect ratio among degrees of nectarivory find no significant difference between any pair of taxa, likely due to the large range of morphologies exhibited by predominant (2) nectarivores (Figure 2-3).

While morphology and diet are associated, a different pattern emerges for isotopic niche breadth and dietary data. There is no relationship between isotopic niche breadth and diet category (F=0.089 (3,22), p=0.97), niche breadth and level of specialization (F=0.1 (2,23), p=0.9), or niche breadth and morphology categories (F=0.642 (4,21), p=0.639). There is, however, a significant relationship between palate aspect ratio and the number of other niches occupied (F=3.734 (3,25), p=0.024, Figure 2-5). Species with more generalist morphologies, quartiles 2 and 3, occupy fewer of other species' niches – higher uniqueness – while more specialized morphologies show increased occupancy of other species' niches – lower uniqueness.

Discussion

Previous isotopic studies of phyllostomid communities have assessed partitioning based on foraging height [Rex et al 2011], trophic level [Herrera et al 1998, Rex et al 2011], diet seasonality [Shipley and Twining 2020], and community structure [Oelbaum et al 2019]. The tight relationship between morphology and dietary preference [Freeman 2000, Dumont et al 2012, Santana and Dumont 2012] offers an opportunity to explicitly examine the link between isotope ecology and craniofacial morphology within this family. We aimed to fill this gap by compiling morphological, observational, and isotopic data to connect interpretations of specialization across methodologies.

We affirm the link between palate aspect ratio and diet within Phyllostomidae by comparing morphology with dietary data and isotopic niche breadth, location in isotopic niche space, and the number of other species' niches occupied. Increasing reliance on fruit is associated with reduced palate aspect ratios, and increasing reliance on nectar is associated with increased palate aspect ratios, though post hoc assessments do not find significant differences between any pair of categories. While not all predominant nectarivores (dietary reliance score of 2) have long palates, all of the bats with long palates are predominantly nectarivorous. Many predominant nectarivores in this data set are also partially frugivorous (dietary reliance score of at least a 1). The mechanical requirements of frugivory may be in conflict with the development of more elongated palates in these taxa.

We find that nectarivorous bats separate poorly from insectivorous bats and frugivores in isotope space, a similar result to another recent study of a similar central American Phyllostomid assemblage [Oelbaum et al 2019]. We also find that increased reliance on fruit is associated with reduced isotopic trophic level, while increased reliance on animal prey is associated with higher isotopic trophic level. While many nectarivorous species are generalist foragers, we find that even our most specialized nectarivores are not isotopically distinct from various generalist, animalivorous, and frugivorous taxa. This is in part because nectarivores likely rely on insects for protein supplementation [Clair et al 2014, Ingala et al 2019, Oelbaum et al 2019] and thus isotopically resemble non-nectarivorous omnivores. Nectar as a resource is perhaps indistinguishable from fruit using carbon and nitrogen isotope ratios alone. Observational diet data of the family overall [Rojas et al 2011] and fecal metabarcoding from similar systems [Ingala et al 2019] suggest that frugivorous phyllostomids also consume some insect prey, yet here they show markedly lower $\delta^{15}N$ ratios than generalists, nectarivores, and insectivorous taxa. This supports other findings that show frugivores may be attaining dietary protein from the fruit they eat while nectarivores primary resource – nectar – is protein poor and requires more supplementation [Herrera et al 2001, Mancina and Herrera 2010, Voigt et al 2011]

Despite the strong association between morphology and diet, we find that neither diet nor morphology are associated with isotopic niche breadth. Isotopic methods do recover ecological information that is consistent with known dietary preferences of the taxa examined here. Taking these data together, we find that morphology and isotope ecology are both indicative of what kinds of food a phyllostomid relies on, but that variability within the diet of a species is poorly captured with Carbon and Nitrogen isotope ratios. Individuals within a species may consume a wide variety of foods that look similar isotopically but require different feeding abilities, such as fruit and nectar from the same tree species – anecdotally, some bat species will consume banana flower nectar and banana fruit while others can only feed on one or the other. Conversely, an animal might consume foods that require the same morphology and feeding ability but have large isotopic differences, such as nectar from C3 and CAM flower species [Newsome et al 2007, Martínez del Rio et al 2009, Chakravarty et al 2023].

Our overlap analyses unexpectedly found that increased morphological specialization is associated with being overlapped by many other species' isotopic niches. This suggests that morphological specialization in these taxa is enabling resource partitioning in a way carbon and nitrogen isotope ratios do not capture well. Phyllostomids are known to partition resources based on fruit species [Munin et al 2012, Ingala et al 2019], flower shape [Nicolay 2001, Muchhala et al 2007], and acoustic parameters [Tuttle et al 1995], none of which have a direct bearing on isotope ratios. High levels of niche overlap within this family also seem to be the norm [Oelbaum et al 2019, Stevens 2022] and thus resource partitioning may be either too fine scale for isotopic methods or non-existent outside of resource bottlenecks [Tschapka et al 2008, Dumont et al 2009, Bobrowiec et al 2014]. Conversely, many different taxa with intermediate palate morphologies exist in more disparate isotopic niches showing this generalist morphology is capable of processing a wide variety of food resources. These intermediate palate morphologies are thus likely differentiating along alternative trait axes such as sensory specialization [Dammhahn et al 2015, Miller 2019, Leisser-Miller and Santana 2020, Thiagavel et al 2020].

Conclusion

We found that quantifying the diets of phyllostomids using stable isotopes is a complicated and nuanced undertaking. Virtually all phyllostomids are omnivorous to some extent [Rojas et al 2011, 2018] even taxa thought to be exclusively insectivorous have recently been found to be frugivorous, at least opportunistically. Specifically in the case of *Lophostoma evotis*, our isotope data corroborates findings that the species feeds frugivorously [Ingala et al 2021]. At the same time, a bat species regarded as the pinnacle of frugivory, *Centurio senex*, seems to be feeding trophically higher than all other frugivorous taxa in our data set indicating either a confounding nitrogen input or hidden insectivory. We found no relationship between morphological extremity and isotopic niche size, though we did uncover an interesting pattern of isotopic niche differentiation among species with similar, generalist morphologies. While even differences among insect type can be detected using stable isotopes in morphologically similar bat species [Dammhahn et al 2015], it is possible that phyllostomid bats are foraging in diverse ways that stable isotope ratios of carbon and nitrogen cannot fully capture. Increased dimensions of isotopic analyses, particularly the addition of oxygen and hydrogen isotope ratios, could show niche differentiation beyond what is detectable by carbon and nitrogen alone, and we advocate for the use of these higher-dimensional analyses going forward.

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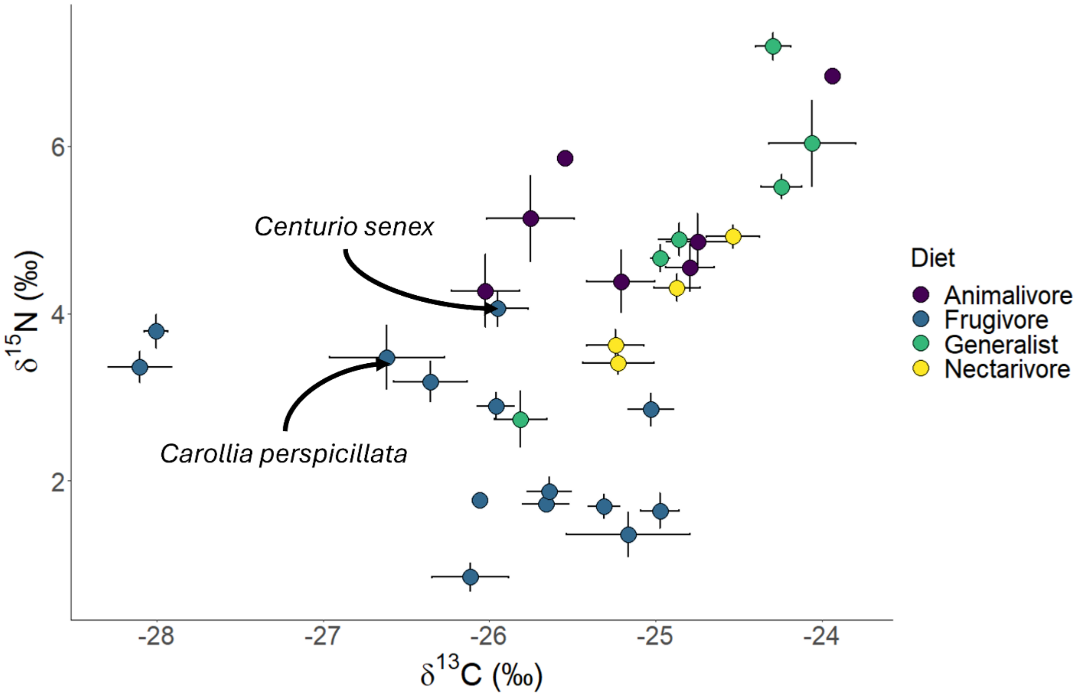
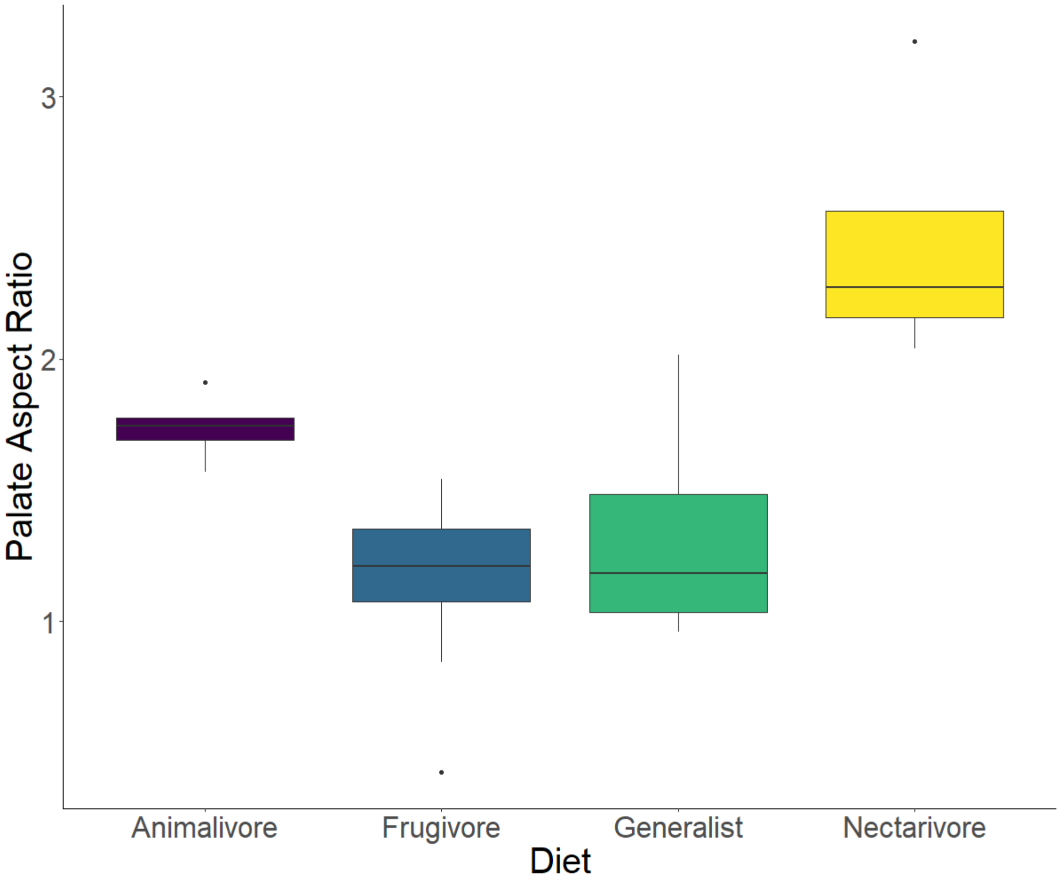
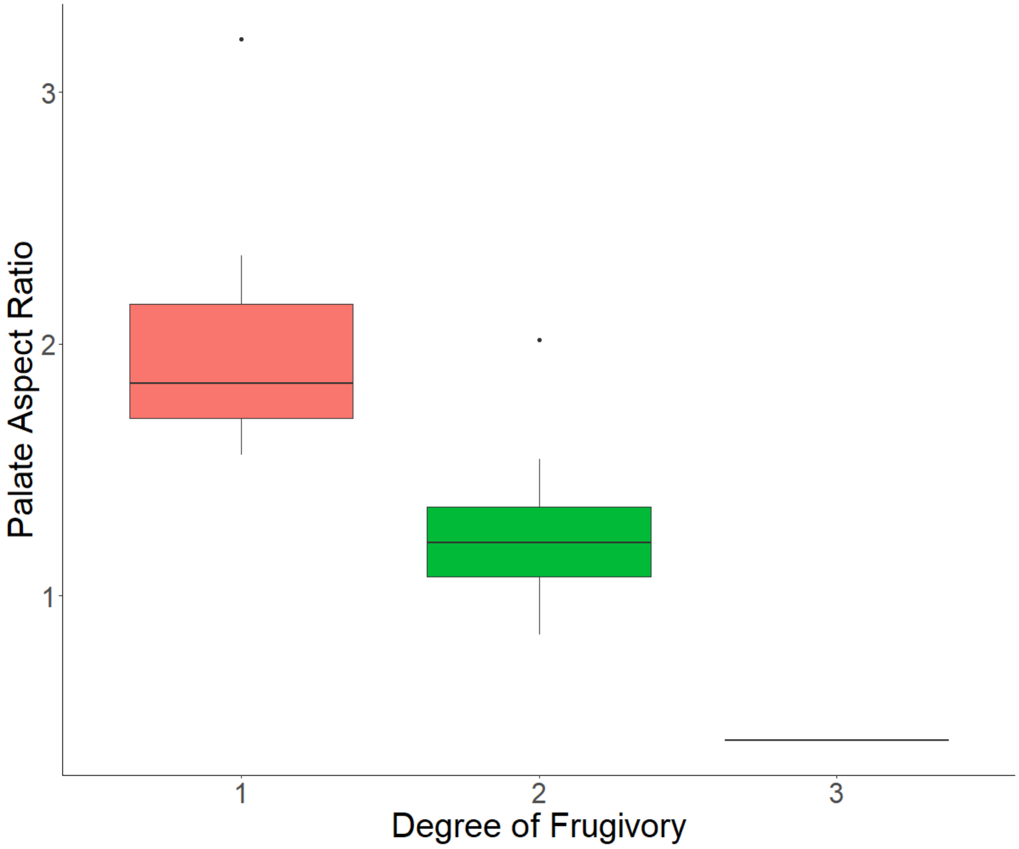
Figures



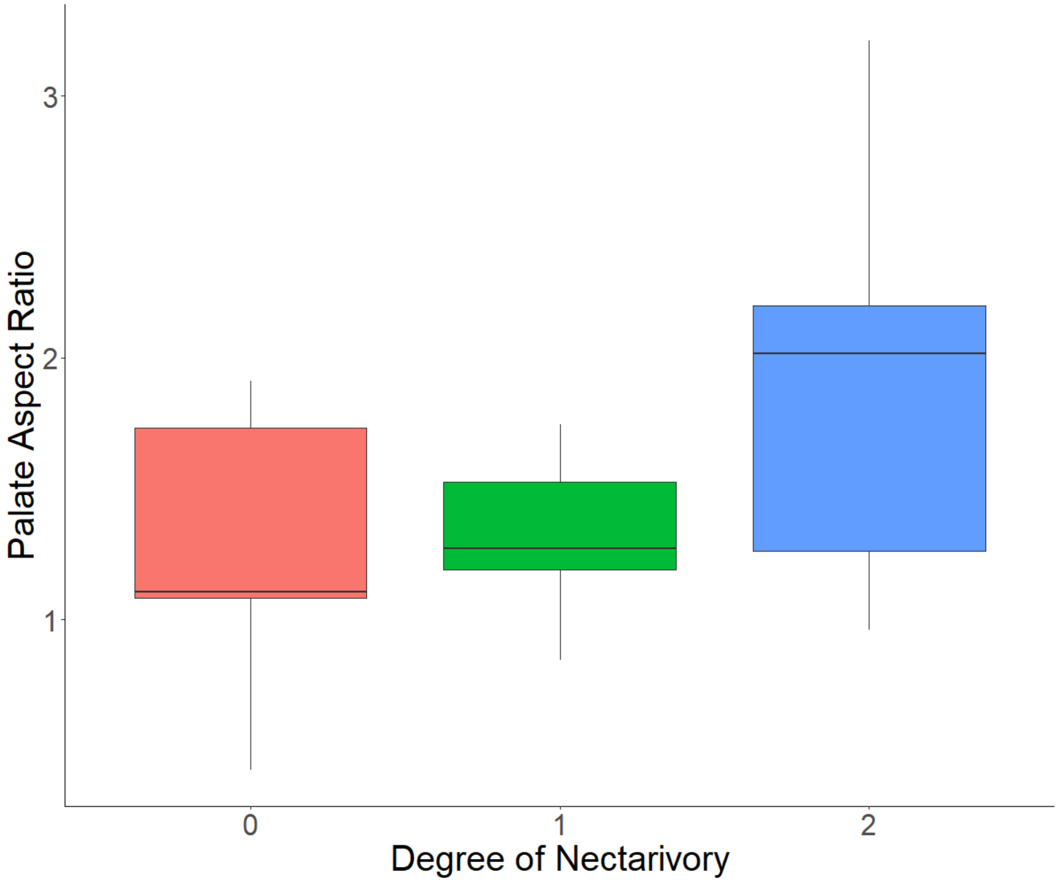
2-1 $\delta^{13}C$ and $\delta^{15}N$ values for the species in this study. Circles represent species means and error bars illustrate standard errors. Species represented by less than three individuals lack error bars.



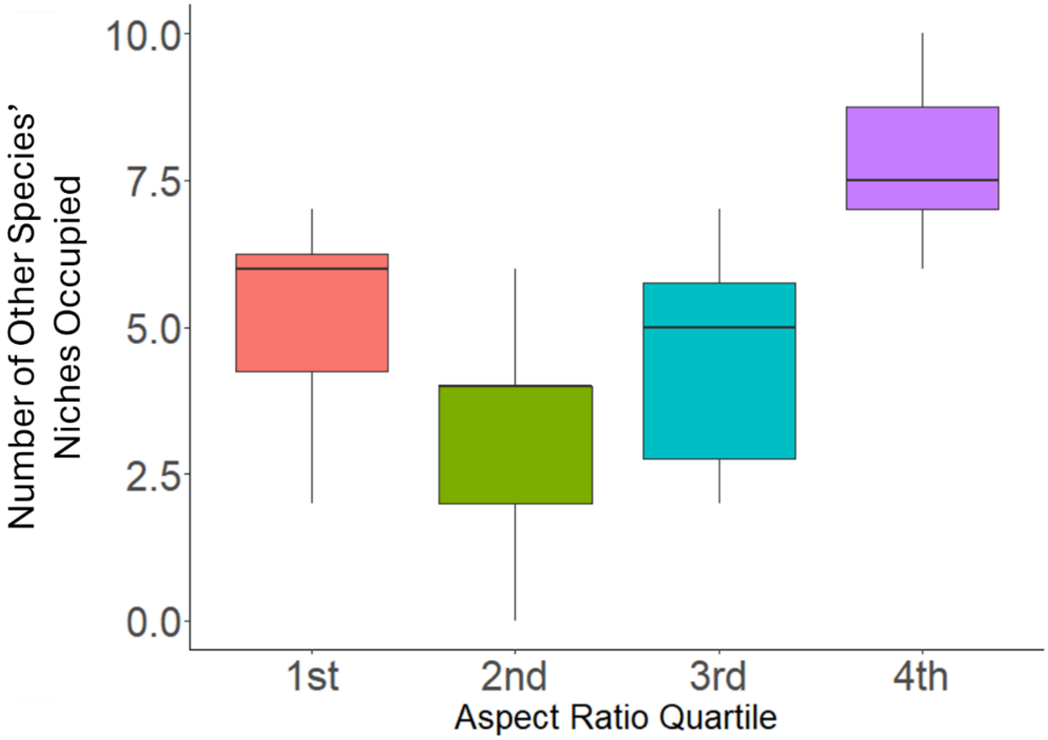
2-2 Palate Aspect Ratio by Dietary Category. Vertical lines represent the extent of the 1st and 4th quartile while the boxes encompass the 2nd and 3rd quartile. Box colors correspond to colors used in Figure 2-1



2-3 Palate aspect ratio and degree of frugivory: 1 (consumes fruit occasionally), 2 (relies predominantly on fruit), and 3 (eats exclusively fruit). Increased reliance on fruit is strongly associated with reduced palate aspect ratios. Vertical lines represent the extent of the 1st and 4th quartile while the boxes encompass the 2nd and 3rd quartile.



2-4 Palate aspect ratio and degree of nectarivory: 0 (never consumes nectar), 1 (consumes nectar occasionally), and 2 (relies predominantly on nectar). Vertical lines represent the extent of the 1st and 4th quartile while the boxes encompass the 2nd and 3rd quartile.



2-5 Number of other species' niches occupied by taxa in each aspect ratio quartile bin. A lower number of other niches occupied indicates a higher level of niche uniqueness, while occupying many other niches indicates low niche uniqueness.

Chapter 3 : The Caribbean Archipelago Fosters Specialization for Frugivory and Limits Specialized Nectarivory in Phyllostomid Bats

Introduction

While Neotropical leaf-nosed bats (Family: Phyllostomidae) have their origins in continental South America, the Caribbean served as an important region for the diversification of the family[Rojas et al 2016, Velazco 2013, Davalos 2009]. In fact, recent evidence suggests that back-colonization from Caribbean lineages contributed to the diversity of phyllostomids in South America [Tavarez 2018, Rojas et al 2016]. There are 23 species of phyllostomids found in the Caribbean and nine are endemic to the islands in the region [Kurta and Duran 2023]. Phyllostomids arose around 35 million years ago [Kurta and Duran 2023, Rojas et al 2016, Teeling et al 2005] and have been present in the Caribbean for at least 15 mya [Rojas et al 2016]. The family has colonized the island archipelagos multiple times, and at least three species have not speciated from their continental populations [Greenbaum and Baker 1976, Carstens et al 2004, Kurta and Duran 2023]. The Caribbean islands are disturbed, and occasionally devastated, by hurricanes and volcanic activity and this is thought to drive founding events and population structure across the archipelagos [Carstens et al 2004, Pederson 1996, 1997, 1998, 2001]. In this paper we ask if this level of disturbance limits the development of specialized feeding strategies in the Caribbean.

Neotropical leaf-nosed bats (Family: *Phyllostomidae*), unlike all other families of bats in the Americas, are largely omnivorous [Rojas et al 2011, Fleming et al 2020, Clare and Oelbaum 2023] and noted for both hyper-specialization and broad generalization when it comes to food resources [Freeman 2000, Katja et al 2010, Rojas et al 2011, Hedrick and Dumont 2018, Clare and Oelbaum 2023]. Changes in dietary specialization in this family are linked to increased rates of diversification [Hedrick et al 2020, Dumont et al 2012, Mutumi et al 2023]. Phyllostomid bats possess an incredible array of morphologies and dietary habits from short-faced fig-specialists to sharp-toothed vampires, all the way to long-tongued nectar specialists [Freeman 2000, Dumont et al 2012, Fleming et al 2020]. Ecological opportunity coupled with the alignment of morphological and phylogenetic capacity has allowed the phyllostomids to become the 3rd most speciose family of bats and to feed upon a larger variety of resources than any other mammalian lineage [Freeman 2000, Shi and Rabosky 2015, Fleming et al 2020, Hedrick et al 2020].

Frugivorous and nectarivorous phyllostomids each have hallmark morphologies that indicate specialization for particular food resources. Among nectarivorous phyllostomids, pollination syndromes of varying severity are characterized by elongated rostra, reductions in dentition, and metabolic and morphological specializations for hovering

[Fenster 2004, Welch et al 2008, Rosas-Guerrero et al 2014, Ashworth et al 2015, Dellinger 2020, Camacho et al 2024]. Frugivorous phyllostomids, and particularly the *Stenodermatinae*, are instead characterized by short, broad faces which allow them to eat figs and other hard fruits that demand high bite forces [Dumont et al 2012, Santana and Dumont 2012]. While frugivory is a defining feature of most members of the family Phyllostomidae, a doubling down on frugivorous specialization generated increased speciation and further development of the frugivorous morphotype [Hall et al 2021].

Biogeographic theory predicts that generalists tend to be better able to colonize islands while specialists may be excluded from colonizing events [MacArthur & Wilson 2001, Warren et al. 2015, Gillespie et al 2017]. Essentially, if the colonizer's niche is sufficiently broad, the likelihood of finding a suitable niche on a new island is higher. However, over time evolutionary processes begin to outweigh initial colonization bias, and adaptation, alongside allopatric and sympatric speciation, can generate diverse and specialized island species [MacArthur & Wilson 2001, Warren et al. 2015, Gillespie et al 2017]. Thus, we would expect older island lineages to have had more time to specialize, all else being equal, than newer island colonists. It is possible that the longstanding relationships with particular food resources needed to generate hyper-specialization cannot exist in the Caribbean because any potential coevolutionary pairings that may begin to form are disrupted by the annual hurricane cycle [Pedersen 1996, Fleming and Murray 2009, Zamora-Gutierrez 2021], volcanism [Pedersen 1997, 2001], and cycles of sea level rise and fall [Rojas et al 2016] . For these reasons, high degrees of specialization for frugivory and nectarivory might be rarer in Caribbean phyllostomids than among their continental relatives even in lineages that have had more time to specialize in the island context.

In this study, we investigate whether Caribbean plant-visiting phyllostomid bats are more or less specialized than their continental counterparts. Palate aspect ratio, the ratio of the length of the palate to the width of the palate, is an important parameter for assessing feeding performance for both nectarivores and frugivores – short broad palates increase the bite force and gape angle for frugivores, while long slender palates facilitate nectar extraction in nectarivores [Nogueira et al 2009, Dumont et al 2012, Santana and Dumont 2012, Gonzalez-Terrazas et al 2012]. Because theory suggests that island colonization is biased toward generalists and due to the potential for disruption of coevolution by devastating hurricanes, we hypothesize that continental nectarivores and frugivores will be more specialized than those of the islands. Continental frugivores (subfamily: S*tenodermatinae)*, will have shorter, broader palates (low aspect ratio) while for continental nectarivores (subfamily: *Glossophaginae*) will have longer, narrower palates (high aspect ratio). We also hypothesize that if there are specialized island lineages, they will be present in lineages that have been evolving on the islands for longer because over long periods of time evolutionary pressures are expected to overtake colonization bias and perhaps produce specialists on islands.

Methods:

Species and Morphometric data

Morphometric data was collected by hand with calipers by Betsy Dumont and Ronald Hall from museum specimens at the AMNH (Supplement 1). Palate aspect ratio is calculated as the length of the palate divided by the width of the palate (both measured at distal edge of the last molar). Species averages of palate ratio are used in phylogenetic comparisons and species sample sizes range from 1 to 11. The aspect ratio spectrum across phyllostomid bats is represented in Figure 3.1. Because this study focuses on fruit and nectar specialization, and most phyllostomids in the Caribbean belong to two major frugivore and nectarivore clades – *Stenodermatinae* and *Glossophaginae* respectively – we leave the predominantly insectivorous *Macrotus waterhousii* and *Micronycteris buriri* out of morphometric analyses but do consider their colonization timing. Continental lineages of *Phyllostomidae* that represent independent frugivorous and nectarivorous evolutions are also excluded

Categorization as an 'island species' is based on distributions presented in the *Bats of the West Indies* and the IUCN redlist species distribution data. Only three phytophagous phyllostomids distributed in the Caribbean also occur in the mainland, and as such are excluded from statistical analyses (due to power issues driven by low sample size) but they are discussed qualitatively.

Phylogeny and Diet

The phylogeny is trimmed from a consensus tree developed by Rojas et al (2016) using the Phytools package in R [Revell 2024]. We manually added *Glossophaga antillarum*, a recently split species endemic to Jamaica whose closest relative in the data set is *G. soricina*, with an estimated divergence time of ~1.8 million years [Calahorra-Oliart et al 2021]. All descendants of the most recent common ancestor (MRCA) of *Glossphaga soricina* and *Anoura caudifer* are treated as nectarivores or nectarivorous generalists and comprise the subfamily G*lossophaginae.* All descendents of the MRCA of *Sturnira lilium* and *Artibeus jamaicensis* are considered frugivores and comprise the subfamily S*tenodermatinae*. Figure 3.2 shows the entire phylogeny for species represented in this analysis. Heatmap phylogenies are generated using the ContMap() function in Phytools [Revell 2024]. Heatmaps use fast ancestral reconstruction to estimate ancestral trait states. These ancestral estimates are used for qualitative comparisons of morphology through time but are not statistically evaluated beyond their use as a visual aid.

Phylogenetic signal is calculated using the phylosig() function in Phytools [Revell 2024] and is represented with Blomberg's K statistic [Blomberg et al 2003]. Values close to 0 represent near independence from phylogenetic structure, a value of 1 represents perfect Brownian evolution of a trait, and values greater than 1 represent higher phylogenetic structure in a trait than expected under Brownian evolution.

Analysis of Variance

Palate aspect ratio within stenodermatine bats (frugivores) and within glossophagine bats (nectarivores) were compared in 1-way ANOVAs to assess differences in morphological specialization between island and mainland taxa. To control for phylogenetic effects, the same comparisons were made using phylogenetic ANOVAs. The nectarivore clade's ANOVA is carried out once with and once without *Monophyllus redmani* included as it is a substantial outlier morphologically. All analyses are carried out in R version 4.3.1 [R Core Team 2023] using the package phytools [Revell 2024].

Results

Analysis of Variance

The pattern of specialization on islands and among continental species differs between frugivores and nectarivores. While there are a few mainland frugivores with very short palates, most island frugivores have aspect ratios less than 1. Conversely, no island frugivores in this data set have an aspect ratio greater than 1.2 while many mainland frugivores bats have longer palates. Island nectarivores, like island frugivores, tend to have shorter rostra than their mainland counterparts. However, the implications of rostrum length for specialization are reversed in this lineage, with longer rostra indicating higher levels of specialization for nectarivory. We find significant differences (Figure 3.3) in palate aspect ratio between island and mainland frugivores (F=8.3 (2,37), $p<0.05$). The nectarivore ANOVA does not yield a p value lower than 0.05 (F= 3.6 (1,21), p=0.07). This borderline result is driven largely by *Monophyllus redmani*, with the highest aspect ratio among island nectarivores. Removing it from the analysis returns a significant difference in palate aspect ratio between island and mainland nectarivores (F = 5.65 (1,21), $p<0.05$).

When phylogenetic signal is considered, the pattern changes. Phylogenetic ANOVAs indicate no significant differences between island and mainland species. Blomberg's K for aspect ratio within the nectarivorous glossophagine clade is K = 0.8 and within stenodermatines it is K=1.05 while with the two clades combined it is K=1.33. This indicates high levels of phylogenetic signal within each lineage. A value substantially greater than 1 for both lineages together indicates the associations between lineage and morphology are stronger than expected by chance – dietary specialization as well as island colonization is largely clade restricted within our sample.

Arrival dates and specialization / diversity:

The extant, island endemic nectarivores and nectarivorous generalists predate modern frugivorous lineages considerably. The oldest island endemic lineage (~20mya) is the most speciose (phyllonycterines, 6 extant species) but also the least specialized morphologically (Figure 2). The next oldest lineage (~12mya to ~ 20mya) is the most morphologically extreme nectarivore lineage but is among the least diverse including only two species *Monophyllus redmani* and *M. plethodon*. It is possible that the phyllonycterines and *Monophyllus* are the result of only one past colonization event [Rojas et al 2016]. In that case, this ancestral lineage would have yielded a higher diversity of species than any other island colonization event and yet no living descendants in the Caribbean would exhibit extreme morphologies.

Meanwhile the more recent lineage of island endemic frugivores are more diversified and specialized. The stenodermatini clade arrived ~8mya and became quite morphologically extreme. The only bats on the continents that meet or surpass their extremely short palates are their close relatives which back-colonized the continents after evolving in the Caribbean. Surprisingly, this is the second most speciose lineage in the Caribbean despite being more recent colonists than genus *Monophyllus*.

There are several recent colonists that are recognized as endemic species but remain morphologically similar to other members of their genera. The nectarivorous *Glossophaga antillarum* (~2mya) was recently recognized as an island endemic species in Jamaica [Calahorra-Oliart et al 2021]. Four frugivorous taxa are also recognized. *Sturnira paulsoni* and *S. angeli* both independently colonized the Caribbean within the last five million years [Kurta and Duran 2023]. *Chiroderma improvisum* (~3mya) [Lim et al 2020] is another recent arrival. *Artibeus shwartzi* is considered the product of reticulate evolution from a past population of *Artibeus planirostris* from South America and Caribbean populations of *Artibeus jamaicensis* [Larsen et al 2007, Kurta and Duran 2023].

There are 3 species in our dataset that occur in the islands as well as on the continents: two closely related frugivores (genus *Artibeus*) and a nectarivore (genus *Glossophaga*). *Artibeus jamaicensis* maintains a considerable degree of genetic exchange with continental populations and likely has for the duration of its tenure in the Caribbean [Larsen et al 2007]. *Artibeus lituratus* is known from only 4 accounts in the southern Lesser Antilles and is likely a vagrant as *A. lituratus* is a wide-ranging, strong flier [Kurta and Duran 2023]. *Glossophaga longirostris* is a predominantly South American bat with a population present on St. Vincent [Kurat and Duran 2023]. There are two phyllostomids from insectivorous lineages that have colonized the Caribbean. *Macrotus waterhousii* is an insectivorous omnivore that is not recognized as a distinct species from its continental counterpart. The insectivorous *Micronycteris buriri*, found only on St. Vincent, is separated from its closest continental relative (*Micronycteris megalotis*) by less than 1 million years [Larsen et al 2011]

Discussion

We found significant differences between the palate morphologies of island endemic and continentally distributed bats in the family Phyllostomidae (Figure 3-2:6). Differences in palate aspect ratio has implications for the kinds of food resources these bats can access (Figure 3-1) [Freeman 2000, Dumont et al 2012]. Among nectarivores, the palates of the island endemic lineages are shorter and broader than those of their continental relatives, indicating increased ability to incorporate fruits and insects into the diet and reduced specialization for deep-corolla flowers [Nicolay 2001, Santana et al 2010, Muchhala et al 2024]. Conversely, island endemic frugivores have very short, highly specialized palates indicating increased capacity for durophagy (Figure 3-1) [Dumont et al 2009, Santana et al 2010]. Inclusion of the endemic island frugivores that we were unable to sample (2 *Sturnira*, 1 *Chiroderma,* and 1 *Artibeus* species) would skew the island average to a less extreme morphology. However, all eight frugivorous phyllostomid hyper-specialists are included in this analysis and they are all descended from the hyper-specialists that evolved in the Caribbean [Rojas et al 2016]. Further sampling of frugivores with continental distributions would only inflate the continental frugivore palate aspect ratios.

We found some support for the biogeographic null hypothesis that older lineages would be both more speciose and contain more specialized taxa (Figure 3-2). The oldest lineage, diversifying in the Caribbean for upwards of 20 million years, is the most diverse as expected. Contrary to our null expectation, this lineage contains both nectarivorous generalists (genera *Erophylla* and *Phyllonycteris*) and frugivorous generalists (genus *Brachyphylla*) but no specialists and no extremely long-faced bats. These three genera co-occur throughout the islands while species within each genus are largely allopatric. This pattern is consistent with an ecological radiation into available niches across the archipelago followed by isolation [MacArthur and Wilson 1967, Schluter 2000, Stroud and Losos 2016]. The relatively young Stenodermatini lineage speciated throughout the Caribbean and developed the most extreme morphologies amongst frugivores. Increased rates of trait evolution and speciation are found within the stenodermatine lineage more broadly [Dumont et al 2012, Shi and Rabosky 2015, Hall et al 2021;], which aligns with the fact that the high species count and extreme morphology developed in such a short time span.

Our results suggest colonization barriers in the Caribbean for nectarivorous bats. There are at least 13 distinct Caribbean colonization events within Phyllostomidae, and substantially more if other insectivorous families of bats are considered. Of these 13 island phyllostomid lineages, four remain at least partially nectarivorous, three are predominantly insectivorous, and the remaining six are primarily frugivorous. Of the four nectarivore lineages, only *Monophyllus* rivals continental nectarivores in morphology (Figure 3-2,3) and all 4 lineages are dietary generalists [Rojas et al 2011, Hall et al 2021]. Frugivores colonize more frequently and have high levels of morphological specialization upon colonization, ruling out that specialization per se is a barrier. One possible explanation for this colonization barrier is a difference in dispersal of the bats and of their food. If plant-pollinator fidelity is high enough, then the geographic distribution of plants will restrict the geographic range of pollinators [Gómez-Ruiz and Latcher 2019, Bedford et al 2012]. If seeds of bat pollinated plants colonize islands ahead of bats, they will not be able to propagate effectively. If nectar specialists colonize islands ahead of their flowers of choice, they will have no food. Conversely, frugivorous bats function as seed dispersers for their food and their bite force may allow them to forage for more kinds of food [Castro-Luna and Galindo-González 2012, Charles-Dominique and Cockle 2001, da Silva et al 2024]. This means that while the first wave of colonists may find no fruit trees, subsequent waves of colonizing frugivores likely will.

The different dynamic experienced between seed dispersing frugivores and flower dependent nectar specialists may explain more than colonization bias. Because the Caribbean is highly disturbed on a evolutionary timescale by sea level change, hurricanes, and volcanism [Pedersen 1996,1997,1998,2001; Vandemeer et al 2000, Canham et al 2010], island vegetation is frequently destroyed. Storms strong enough to destroy a crop of flowers are much more frequent than storms strong enough to destroy entire forests [Vandemeer et al 2000, Canham et al 2010]. Nectarivorous mutualisms may thus be interrupted more frequently than frugivore-seed disperser mutualisms on islands. In the event of an island-wide catastrophe, frugivorous bats tend to be bigger and need less frequent meals than nectar specialists [Welch et al 2008, Camacho et al 2024] therefore they can fly further and withstand resource scarcity longer. Nectarivores may only survive by being dietary generalists in a crisis. Further, after an island slate is wiped clean, a few wayward fig carrying bats could start a new patch of fig trees for future bats to feed on, but a wayward nectarivore can only carry pollen to a location where there are no flowers waiting. In this way, fruit eating bats may stabilize forests against disturbance and maintain geographic distributions of their food on evolutionarily relevant time scales in a way that pollinators cannot.

Conclusion

Leveraging the relationship between morphology and diet elaborated on in Section 2, Section 3 illustrates that hyper-specialized nectarivores have not been able to endure on the Caribbean islands. Conversely, the archipelago seems to have fostered increased levels of frugivory and morphological specialization among those taxa [Rojas et al 2016]. While back-colonization of frugivores from the archipelagos to the continents explains the presence of short-faced bats such as *Centurio senex*, all extremely long-faced bats evolved exclusively on the continents. Although an island origin for glossophagine diversity is not totally refutable [Rojas et al 2016], it is unlikely. Even if glossophagine bats did originate on the islands, none of the resulting back-colonized species were or became extreme nectarivores. Furthermore, a different origin of nectarivory on the continents gave rise to extreme morphologies in the lonchophylline subfamily [Datzmann et al 2010, Rojas et al 2011]. In fact, the brachyphylline subfamily bats radiated across the Caribbean spanning nearly the breadth of morphologies that the rest of phyllostomidae encompass. While we cannot confirm with certainty that disturbance in the Caribbean plays a causal role, the pattern of morphology across the examined taxa supports the hypothesis that due to high levels of disturbance in the Caribbean, highly-derived nectarivorous morphotypes are not tenable and that dietary generalism is the prevailing strategy for nectarivorous bats in the Caribbean. The pattern of morphological variation is not explicable solely by biogeography or phylogeny.

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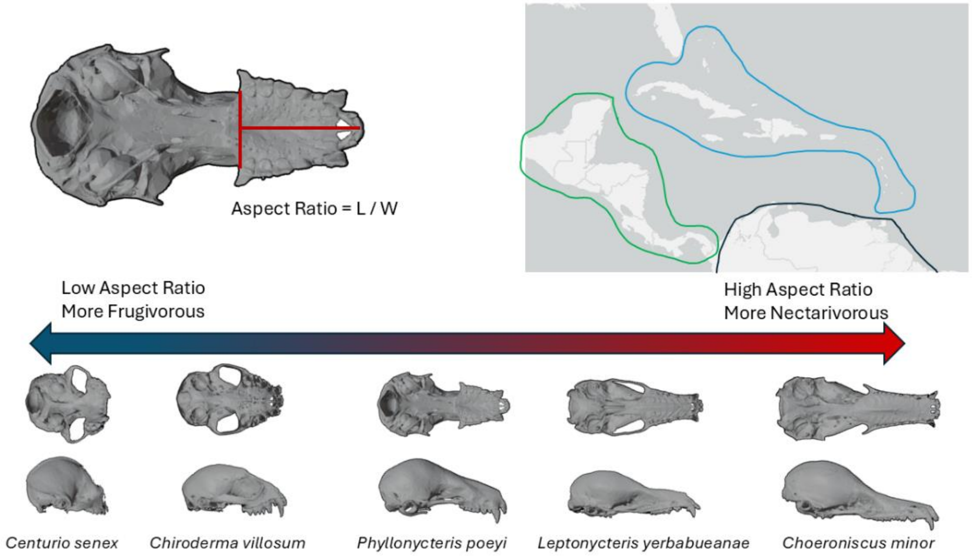
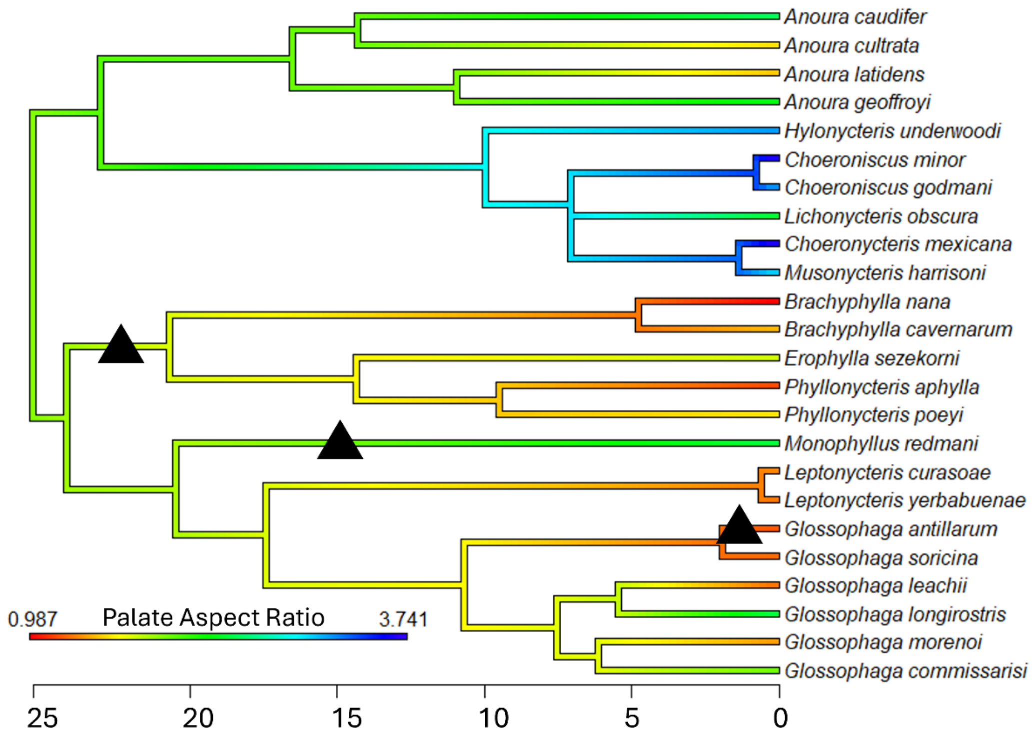
Figures

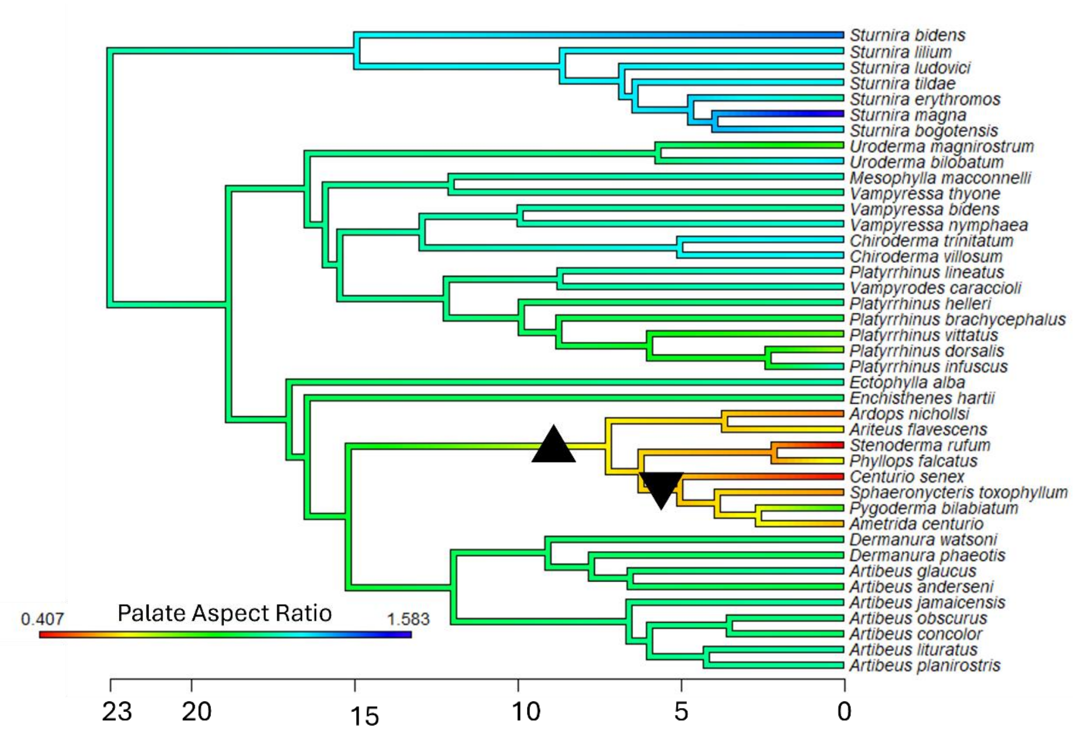


3-1 Top left panel indicates the measurements taken and the calculation for palate aspect ratio (Length / Width). Top right panel shows the Caribbean (blue) with Central (green) and South (Black) America. The bottom panel shows five skulls of taxa included in the analysis representing the gradient from low aspect ratio to high aspect ratio.

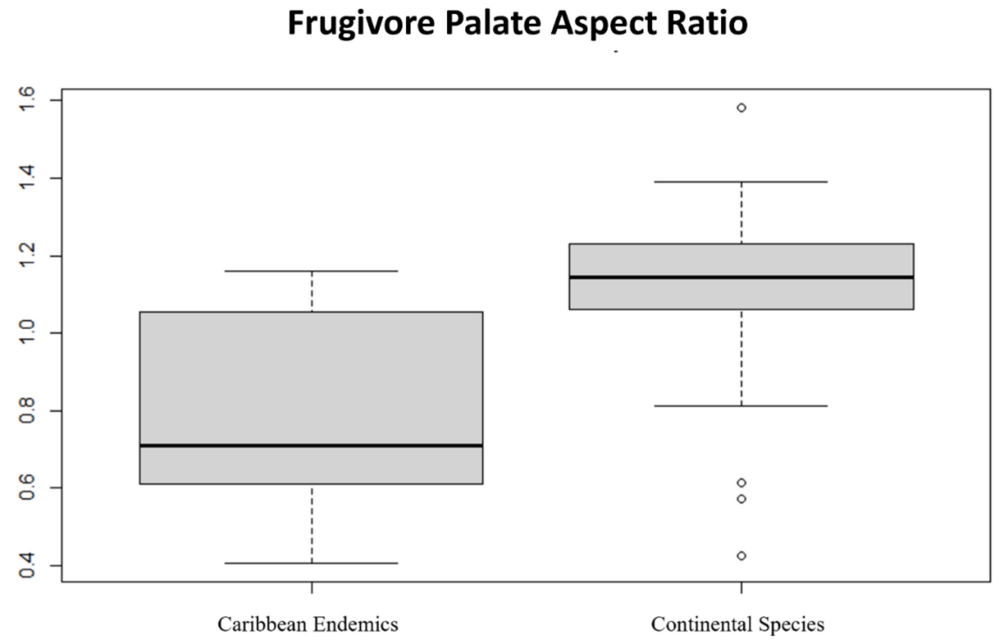
3-2 Time calibrated phylogeny of the frugivorous *Stenodermatinae* (below the dividing line) and nectarivorous *Glossophaginae* (above the dividing line) subfamilies. The color gradient represents log adjusted palate aspect ratio from low aspect ratio (red) to high aspect ratio (blue). Point-up triangles represent island colonization events and the point-down triangle represents re-colonization of South America from the Antilles.



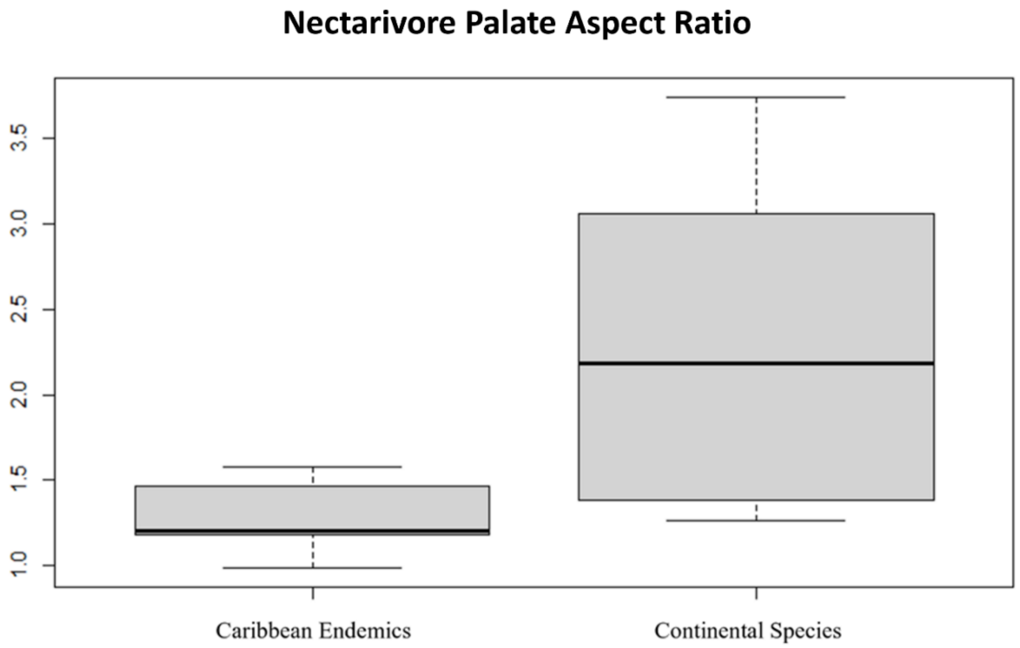
3-3 Time calibrated phylogeny for the nectarivorous glossophagine clade. The color gradient represents unadjusted palate aspect ratio from low aspect ratio (red) to high aspect ratio (blue). Point-up triangles represent island colonization events



3-4) Time calibrated phylogeny for the frugivorous stenodermatine clade. The color gradient represents unadjusted palate aspect ratio from low aspect ratio (red) to high aspect ratio (blue). Point-up triangles represent island colonization events



3-5 Box plots for comparison between Caribbean endemics and continentally distributed species. Boxes represent the 2nd and 4th quartile while the whiskers represent the 1st and 4th quartiles. The frugivorous stenodermatine bats in the Caribbean have much lower aspect ratios than continentally distributed species. The 3 low outliers for the frugivorous continental species are the result of back-colonization from the Caribbean lineage.



3-6 Box plots for comparison between Caribbean endemics and continentally distributed species. Boxes represent the 2nd and 4th quartile while the whiskers represent the 1st and 4th quartiles. The nectarivorous Caribbean endemics have much shorter palates than their continental counterparts.

Chapter 4 : Cochlea Shape in Noctillionoid Bats is Influenced by Diet, Structural Constraint, and Evolutionary History

Introduction

Often cast as an adaptive radiation [Monteiro and Nogueira 2010, Monteiro and Nogueira 2011, Shi and Rabosky 2015, Rossoni et al 2017, Leiser-Miller and Santana 2021, Grossnickle et al 2023], the neotropical leaf-nosed bats (family Phyllostomidae) are well known for their impressive diversity and morphological disparity (Figure 1) [Monteiro and Nogueira 2011, Dumont et al 2012, Dumont et al 2014, Shi and Rabosky 2015, Hedrick et al 2020, Mutumi et al 2023]. A large body of work supports the idea that the exploitation of novel niche space, enabled by the development of omnivory, facilitated the diversification of the family [Freeman 2000, Dumont et al 2012, Dumont et al 2014, Grossnickle et al 2023]. From an omnivorous ancestor, species diversified into niches that include frugivory, nectarivory, sanguivory, carnivory, and various degrees of omnivory [Hall et al 2021, Mutumi et al 2023]. Many studies focus on the role of biomechanical adaptations for feeding as a key factor in the radiation of the phyllostomids [Monteiro and Nogueira 2010, Santana et al 2011, Dumont et al 2012, Dumont et al 2014, Hedrick and Dumont 2018], and recent work found further evidence that supports the role of adaptation in the evolution of sensory structures used for foraging for various food resources [Hall et al 2021, Mutumi et al 2023, Yohe et al 2021, Sadier et al 2021].

Species within different feeding guilds have sensory profiles that fit their foraging needs. Insectivorous phyllostomids, and the insectivorous outgroups to phyllostomids, have increased cochlear volumes suggesting increased sensory function [Kössl and Vater 1985, Davies et al 2013]. All plant-visiting bat species rely on olfaction, vision and, to an extent, echolocation when foraging for flowers and fruits [Muchhala and Serrano 2015, Miller 2019, Brokaw et al 2021, Hall et al 2021]. Coevolution has produced fruit and flower adaptations that take advantage of, and work with, the sensory systems phyllostomids possess [Muchalla et al 2003, Hodgkison et al 2013, Simon et al 2021]. Fruit feeding phyllostomids have radically increased olfactory bulb and eye volumes, specializations in color perception and potentially advantageous changes to the structure of nasal turbinate bones [Yohe et al 2021, Sadier et al 2018, Davies et al 2020].

In addition to strong evidence for functional adaptation among phyllostomids, there are known functional trade-offs. Highly specialized, long-faced nectarivorous bats can reach and drink nectar from flowers easily but are unable to produce the bite force required for feeding on hard, fracture-resistant fruits and arthropods, while short-faced hard-food specialists are unable to effectively sip nectar out of deep flowers [Santana et al 2010, Dumont et al 2012, Dumont and Herrel 2003, Dumont et al 2009]. Some aspects of phyllostomid sensory morphology may also be explained as the consequence of trade-offs rather than direct adaptation [Hall et al 2021]. Nectar feeding phyllostomids have small eyes and olfactory bulbs, for their size, while these organs are quite large in frugivorous lineages [Thiagavel et al 2018, Hall et al 2021]. Additionally, nectarivores have significantly smaller cochlea than anticipated [Hall et al 2021]. Because there is considerable variation in cranial morphology within phyllostomids, it is possible that within these lineages, the size and shape of sensory structures may be explained better by structural influences than by functional adaptation.

In this study, we focus on the cochlea to assess differential impact of functional demands and structural constraint. The cochlea is the organ within the skull responsible for hearing and, in bats, echolocation. The shape of the cochlea is complex and highly conserved across mammals with primary differences being between echolocating and non-echolocating species, even among bats [Vater and Kössl 2011, Koppl and Manley 2019, Thiagavel et al 2018]. Both the shape and size of the cochlea have functional implications for echolocation [Vater 1985, Vater and Kössl 2011, Davies et al 2013, Dickinson et al 2023]. This suggests that the shape of the cochlea may be functionally constrained and under strong selection [Thiagavel et al 2018, Manley et al 2018, Koppl and Manley 2019]. The vestibular system, important for flight and balance, is also affixed to the cochlea and housed in the same bony structure [Davies et al 2013] and may compete with the cochlea for space. Finally, the cochlea is located within the cranium, potentially putting it in direct competition for space against the brain and other sensory systems.

Here we test two primary hypotheses about cochlea shape and size in neotropical leaf-nosed bats and their close outgroup families. The first hypothesis is that the shape and/or size of the cochlea is driven by dietary preference – the varied echolocation demands of insectivory, frugivory, and nectarivory may be linked to morphological variation in the cochlea. This would suggest adaptive and functional drivers of cochlea shape. The second hypothesis is that the shape of the cochlea is not related to diet and instead correlated only with variation in cranium shape and size. Support for this hypothesis would suggest non-sensory reasons for the shape and size of the cochlea and instead indicate that the cranium and cochlea are structurally entangled. Support for both hypotheses is possible: while the cranio-facial morphology of phyllostomids is tightly correlated with diet [Freeman 2000, Dumont 2004, Dumont et al 2012, Hedrick et al 2020], there is considerable morphological and dietary variation within each of the dietary guilds (Figure 4-1) [Rojas et al 2011, Clare and Oelbaum 2024].

Methods

Taxon Sampling, Phylogeny, and Diet

We have 3D CT scans of cochlea for 47 species, and skull morphometrics from 36 of the same specimens. Seven are outgroup taxa within the superfamily noctilionoidea. The remaining 29 are representative of all major clades of Phyllostomidae. For phylogenetic comparative analyses we used the phylogeny developed by Rojas et al 2016, trimmed to include only the species for which we have cochlea and cranium data. Dietary data were taken from Rojas et al 2011 (Figure 4-1). Dietary data are binned here as they are in Chapter 2 of this document. Specimens and measurements are in compiled in Supplement 1.

Cranial Morphometrics

Linear measurements of the cranium (cranium length, width, and height) were collected with the caliper tool in IDAV Landmark [Wiley 2006]. We calculated two cranium shape parameters, the sphericity and aspect ratio of the cranium, to capture spatial efficiency in three and two dimensions, respectively. The geometric mean (the n^{th} root of the product of a set of n values) of the length, width, and height measurements is used as a proxy for cranium size. Here, that is $\sqrt[3]{LxWxH}$.

Cochlea Landmarks

We placed landmarks on the cochlear coils (Figure 4-2) with the goal of capturing turn radius, coil width, coil length, and distance between coils. Six landmarks were placed on the vestibular system to capture the relative size and orientation of the vestibular system. Using cochlea segmented from 3D CT scans [Hall et al 2021], we placed a total of 156 landmarks on 47 cochleae in IDAV landmark software (Figure 4-2) [Wiley 2006]. We adapted the landmarking regime from Costeur et al 2018 to accommodate the limitations of working with such small cochlea. While Costeur et al were able to landmark the internal side of the coil all the way to the tip, the resolution of the ct scans and tightness of the cochlea coil allowed us to landmark the internal side of only the first turn. Fifteen landmark curves, each consisting of 10 evenly spaced landmarks, were placed around the entire outer centerlines of the main coils and the inner centerline of the main coil for the first turn. Files containing landmark coordinates were exported as .pts files and manually altered to fit .tps file format.

To control for size and align all landmark point clouds, generalized procrustes analysis was performed on all 47 cochleae using the gpagen() function in geomorph package [Baken et al 2021] in R version 4.3.1 [R Core Team 2023]. The prcomp() function in geomorph [Baken et al 2021] was used to derive principal components of shape. In total, there were 46 principal components of shape variation, the first 10 are used for univariate post-hoc analyses.

Phylogenetic Multivariate Regressions

GIC values for Brownian Motion (BM) and Ornstein-Uhlenbeck models of evolution for the cochlea are compared to identify the best model of evolution for multivariate cochlea shape regressions in mvMORPH [Clavel et al 2024] .Using the best model for the whole shape of the cochlea (OU), we performed phylogenetic multivariate regressions on the landmark coordinates by treating the x,y, and z values of each landmark as 3 distinct traits per landmark, resulting in 458 ‘traits’ for cochlea shape. Cochlea shape was regressed against cranial size and shape metrics, cochlea size, dietary category, and specific diet preference data. For all multivariate regressions, we used function mvgls() in mvMORPH [Clavel et al 2024] with model = ‘OU’, method = ‘PL-LOOCV’, and upper=1000. Significance tests were performed with manova.gls() using 500 permutations.

Principal Component and Post-Hoc Investigations

As a post-hoc investigation to identify specific univariate aspects of shape driving significant trends, the first 10 principal components were regressed against variables found to have a significant association with multivariate cochlea shape with pgls() in the ouch package [King et al 2015] and phylANOVA() in the phytools pacakge [Revell 2024]. These PGLS regressions used maximum likelihood estimates for tree topology parameters and are not constrained to specifically ‘BM’ or ‘OU’ models of evolution. To further investigate the relationships, phylogenetic signal measured with Blomberg’s K [Blomberg et al 2003] was calculated with the phylosig() function from the phytools package [Revell 2024].

We do not further adjust p values for multiple tests and instead advocate for further research to verify and validate our findings. The purpose of Bonferroni and other multiple test corrections is to control type-I errors, but these come at the expense of an increased rate of type-II errors [Cabin and Mitchell 2000]. We believe that while type-I errors in this kind of study are certainly possible, the increased type-II error rate would lead to no identifiable trends due to the low signal-to-noise ratio. Furthermore, it is highly unlikely that a full null hypothesis is true (that the shape of the cochlea is totally unrelated to any dietary or structural parameters). Due to the exploratory nature of this study, we do not believe that further statistical correction is necessary. These results should be viewed as comparisons between the relative influence of various parameters on the shape of the cochlea and not as definitive confirmation of causal mechanisms.

Results

The PCA of the cochlea landmarks results in 46 principal components of shape. Component 1 is 46.5% of the variation, component 2 is 15.8%, and component 3 is 8.9% of the variation. The first 10 components make up 89% of the variation. Plots generated using ggplot2 [Wickham 2016] showing PC1-4 are shown in Figure 4-3. Results for all statistical analyses are summarized in Table 1. For the landmark data, the OU model has a much lower GIC (-141911.9) than the BM model (-136290.9). Thus, OU is the best-supported evolutionary model and is used for all multivariate shape analyses.

Dietary category is not significantly correlated with any variables describing cranium shape, cranium size, or cochlea volume. Cochlea shape is not significantly associated with dietary category though it is borderline ($t = 2.8$, $p= 0.052$). However, when diet is broken into proportion of individual food items on a categorical scale of 0 to 3 (none of a resource to only that resource), there is a significant relationship between whole cochlea shape and animalivory ($t = 2.85$, $p = 0.008$), frugivory ($t = 2.84$, $p = 0.01$), and nectarivory ($t =1.89$, $p = 0.03$).

Phylogenetic multivariate shape regressions reveal significant relationships between cochlea shape and cranium shape (sphericity and aspect ratio) as well as cranium size (Table 1). Cranium size is correlated with cochlea shape with p values < 0.05 (geometric mean, $t = 0.9647$, $p = 0.002$). Cochlea shape is also correlated with measures of cranial shape (sphericity, $t = 0.95$, $p = 0.03$; aspect ratio, $t = 0.97$, $p = 0.002$). Cochlea shape is also significantly associated with cochlear volume, indicating signal for shape allometry ($t = 0.96$, $p= 0.01$). Unlike cochlear shape, cochlear volume is not related to either cranial shape parameter (aspect ratio $p=0.15$; sphericity $p=0.38$)

Results of PC-by-PC investigations are summarized in Table 1. PGLS regressions show that cranium size is significantly associated with PC9 (geometric mean R^2 = 0.14, p = 0.012). Sphericity shows no significant relationship with any of the first 10 PCs. Cranium aspect ratio is significantly correlated with PC 2 (R^2 = 0.10, p = 0.032). Cochlea volume is correlated with PC4 (R^2 = 0.11, p = 0.03). Note that the aspects of allometric shape-size scaling within the cochlea (PC 4) are distinct from the aspects of shape that vary along cranium size (PC 9).

The degree of nectarivory is not significantly associated with any shape PCs while Animalivory and Frugivory are both significantly correlated with PC 3 (Phylogenetic ANOVAS, $F = 12.3$, $p =0.018$; $F = 12.3$, $p= 0.014$, respectively). Post-hoc tests show this trend is driven almost entirely in the difference between bats which eat no fruit and those that eat any, and bats that eat only animals (insects or vertebrates) and omnivores (Figure 4-4).

Phylogenetic signal (Blomberg's K [Blomberg et al 2003]) representing deviation from phylogenetic null expectation is reported for the first 10 PCs of cochlea shape variation as well as cranial morphometrics (Table 2). $K= 1$ is the phylogenetic null expectation, $K<1$ indicates a lower phylogenetic signal than expected and a $K>1$ indicates more phylogenetic signal than expected under Brownian motion evolution along the tree topology. Only 1 PC of shape (PC3) has a phylogenetic signal greater than 1. Animalivory and Frugivore also have $K>1$. For these traits, Phylogenetic history explains more of the variation than expected under Brownian motion - consistent with OU evolution of the cochlea.

Discussion

We found support for both hypotheses – diet and skull shape, independent of one another, are strongly related to cochlea shape. This reveals independent influences of diet and cranial morphology on the shape of cochlea in phyllostomids and their close relatives. This is indicated by post-hoc analyses that show distinct components of shape variation within the cochlea are related to dietary variables and cranial morphometrics (Table 1). We did not find a relationship between cochlea shape and dietary guild, which implies that guilds do not capture relevant aspects of echolocation strategy. Nectarivory and cranium sphericity are significantly related to cochlea shape overall, but none of the first 10 components of shape variation explain this relationship. This could be due to the multivariate regression being sensitive to shape variation that represents small fractions of overall shape variation (PCs < 10), or due to multivariate interactions that individual regressions against principal components cannot recover. Surprisingly, the first principal component, accounting for nearly 50% of shape variation, is not related to any dietary or morphometric variable and has a low phylogenetic signal. The lack of explanation for PC1, along with the potential for type-I errors from multiple tests, motivates future work to consider alternative ecological and structural parameters such as environmental clutter, echolocation call complexity, and frequency.

Investigation of principal components of shape variation reveals relationships between cochlea shape, reliance on particular dietary items (but not dietary guild), and phylogeny. The strength of phylogenetic signal for animalivory, frugivory, and PC3 suggests fundamental differences in the echolocation of the outgroup family Mormoopidae and Phyllostomidae (Figure 4-3,4). Figure 4.3B shows mormoopid bats cluster uniquely in the PC3 PC4 morphospace. Figure 4-4 illustrates that the dietary differences along PC3 recapitulate the mormoopid-phyllostomid distinction as mormoopid bats are all exclusively insectivorous (Figure 4-4A), while no Phyllostomids eat no fruit (Figure 4-4B). Mormoopids are obligate insectivores and many employ sophisticated and demanding constant frequency echolocation which explains this shape difference [O'Farrell and Miller 1997, Mora et al 2013]. Increasing degrees of frugivory amongst phyllostomids is not correlated at all with cochlea morphology, likely due to a shift toward alternative foraging modalities [Hall et al 2021, Santana et al 2024]. Nectarivory, unlike frugivory and animalivory, shows low phylogenetic signal. Unique morphological features of nectarivorous cochlea may pertain to identifying static shapes against complex backgrounds [Muchhala and Serrano 2015, Muchhala et al 2024].

We also found that aspects of cochlea shape that are related to cochlea volume, cranium size, and cranium shape (aspect ratio and sphericity) are all distinct from one another. Allometric scaling of cochlea shape as cochlea volume increases is described by PC 4 while allometric shape scaling with cranium size is described by PC 9. Further, changes in the shape of the cranium both in aspect ratio and sphericity are related to changes in cochlea shape. While no components in the first 10 explain the relationship between sphericity and cochlea shape, PC 2 and 8 explain the relationship between aspect ratio and cochlea shape. Aspect ratio of the cranium therefore has a more prominent impact on the shape of the cochlea than diet or phylogeny. Narrowing of the cranium, regardless of diet, is possibly driving shape changes to improve packing efficiency while maintaining echolocation functionality.

While the components of shape change in the cochlea driven by nectarivory, frugivory, and aspect ratio are distinct, the aspect ratio of the cranium is relevant to specialization for frugivory and nectarivory [Nogueira et al 2009, Hedrick and Dumont 2018, Hedrick et al 2020]. Frugivore skulls tend to be shorter and stouter while nectarivore skulls tend to be more elongated and gracile [Freeman 2000, Nogueira et al 2009, Hedrick and Dumont 2018, Hedrick et al 2020]. Spheres and circles optimize volume and area for a given surface area and perimeter, respectively. Because tubes are less space efficient than spheres (3D), and rectangles are less space efficient than circles (2D), highly specialized nectarivores may also be incurring trade-offs driven by extreme morphologies found in pollination syndromes [Muchhala 2003, Fenster 2004, Rosas-Guerrero et al 2014, Dellinger 2020]. Embryological data shows the cochlea deformed from its typical shape until very late in development in a nectarivorous bat while at the same stages the cochlea is in its final shapes [Anthwal et al 2023] indicating intracranial space competition during development as well. We suggest that in addition to possible diet-driven changes in the shape of the cochleae and vestibular system of nectarivores, longer, narrower skulls which allow bats to feed on deeper flowers reduce the spatial efficiency of the cranium. Frugivores on the other hand may have developed a synergistic suite of traits enabling larger sensory structures [Thiagavel et al 2018, Hall et al 2021], larger brains [Thiagavel et al 2018], and higher bite forces [Dumont et al 2012, Dumont et al 2014].

Conclusion

Our effort to investigate the relationship between form and function of the cochlea and cranium of phyllostomids and closely related bats revealed surprising results. We confirmed that the shape of the cochlea is quite different between Phyllostomids and their nearest outgroup, the Mormoopidae. Further, aspects of the shape of the cochlea are strongly tied to the aspect ratio of the cranium, suggesting that packing efficiency can impact the morphology of the cochlea. Independent of skull shape and phylogeny, nectarivory is also correlated with changes in cochlea shape. The morphology of the cochlea is closely related to echolocation parameters [Kössl and Vater 1995, Kössl et al 1999], and recent work has shown relationships between aspects of cochlea morphology and foraging behaviors [Dickinson 2023]. It is not entirely surprising that dietary category, as defined, may do a poor job of dividing bats into ecomorphological bins that pertain directly to echolocation. For instance, there is likely a wide array of echolocation strategies among insectivores that is erased by binning them together. What is particularly interesting is that there may be some aspects of nectarivory that demand specific echolocation capabilities. Additionally, while not all nectarivores have dramatically increased cranial aspect ratios, many do and highly specialized nectarivores may incur cochlea shape and size trade-offs as indicated in Hall et al 2021 and Anthwal et al 2023.

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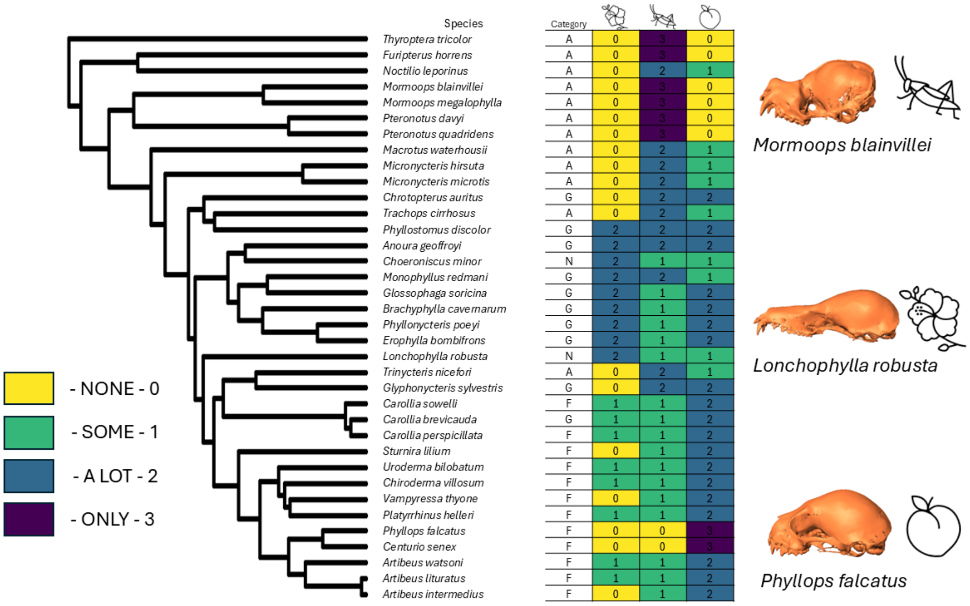
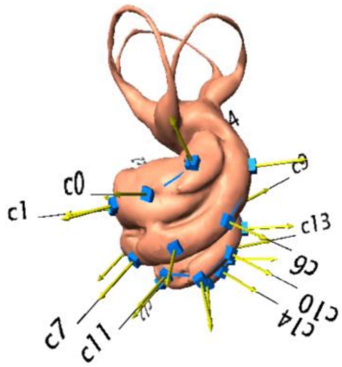
Figures



Species	Category	🌺	🦗	🍎
Thyroptera tricolor	A	0	3	0
Furipterus horrens	A	0	3	0
Noctilio leporinus	A	0	2	1
Mormoops blainvillei	A	0	3	0
Mormoops megalophylla	A	0	3	0
Pteronotus davyi	A	0	3	0
Pteronotus quadridens	A	0	3	0
Macrotus waterhousii	A	0	2	1
Micronycteris hirsuta	A	0	2	1
Micronycteris microtis	A	0	2	1
Chrotopterus auritus	G	0	2	2
Trachops cirrhosus	A	0	2	1
Phyllostomus discolor	G	2	2	2
Anoura geoffroyi	G	2	2	2
Choeroniscus minor	N	2	1	1
Monophyllus redmani	G	2	2	1
Glossophaga soricina	G	2	1	2
Brachyphylla cavernarum	G	2	1	2
Phyllonycteris poeyi	G	2	1	2
Erophylla bombifrons	G	2	1	2
Lonchophylla robusta	N	2	1	1
Trinycteris nicefori	A	0	2	1
Glyphonycteris sylvestris	G	0	2	2
Carollia sowelli	F	1	1	2
Carollia brevicauda	G	1	1	2
Carollia perspicillata	F	1	1	2
Sturnira lilium	F	0	1	2
Uroderma bilobatum	F	1	1	2
Chiroderma villosum	F	1	1	2
Vampyressa thyone	F	0	1	2
Platyrrhinus helleri	F	1	1	2
Phyllops falcatus	F	0	0	3
Centurio senex	F	0	0	3
Artibeus watsoni	F	1	1	2
Artibeus lituratus	F	1	1	2
Artibeus intermedius	F	0	1	2

4-1 A phylogeny of the phyllostomids and outgroup families. Category indicates the dietary category the taxa are assigned. A – Animalivore, G – Generalist, N – Nectarivore, F – Frugivore. The colors and numbers indicate how much of the taxon's diet is comprised of the given food resources: nectar, insects, and fruit. 0/yellow indicates none of the diet, 1/green indicates some of the diet, 2/blue indicates a large portion of the diet, 3/ purple indicates all of the diet. Skulls on the right represent typical morphologies of the respective dietary specialists and taxonomic groups.



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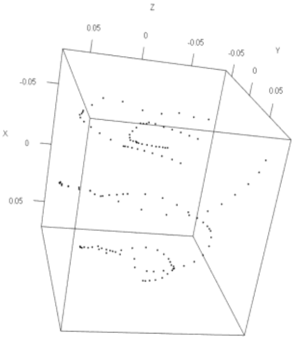

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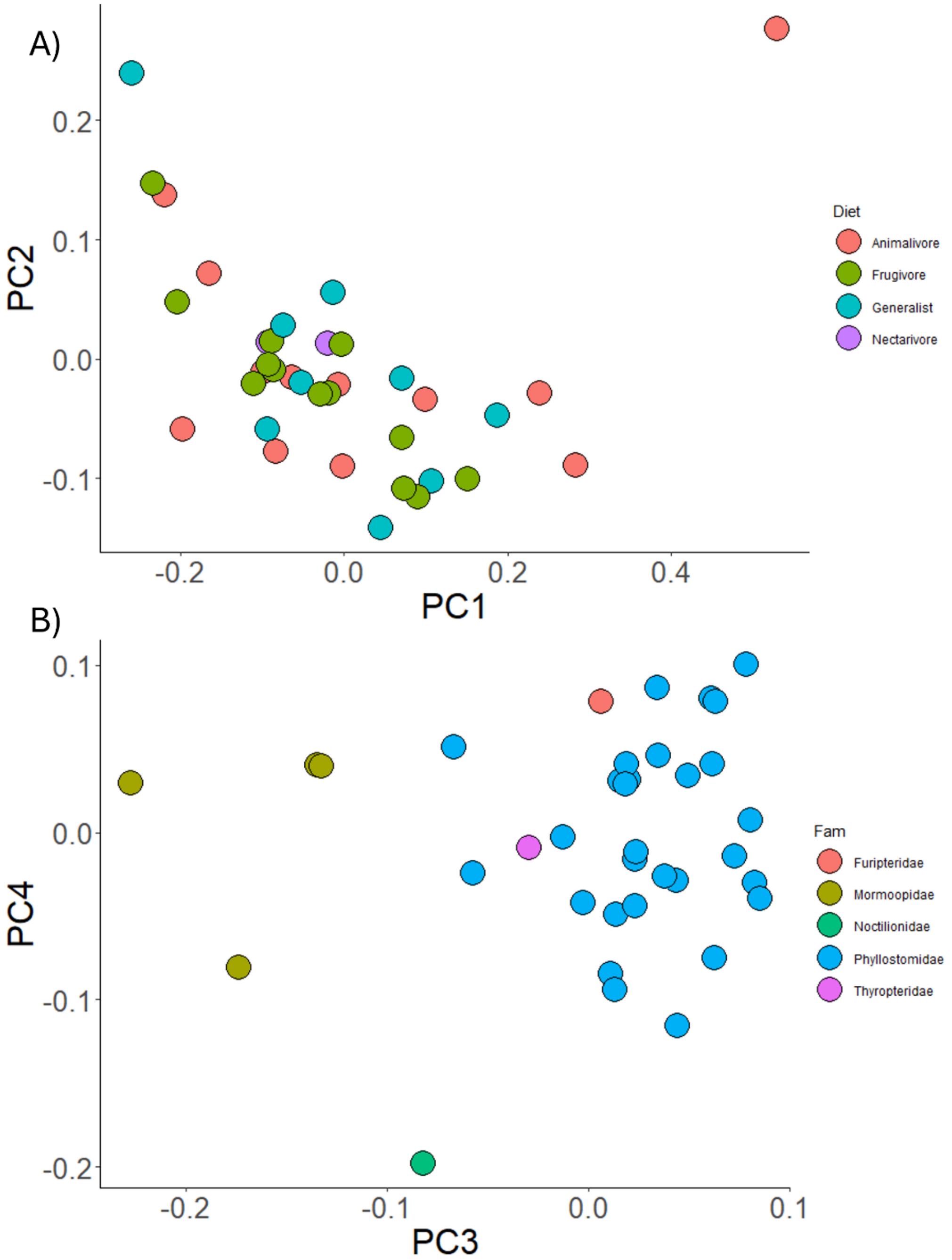
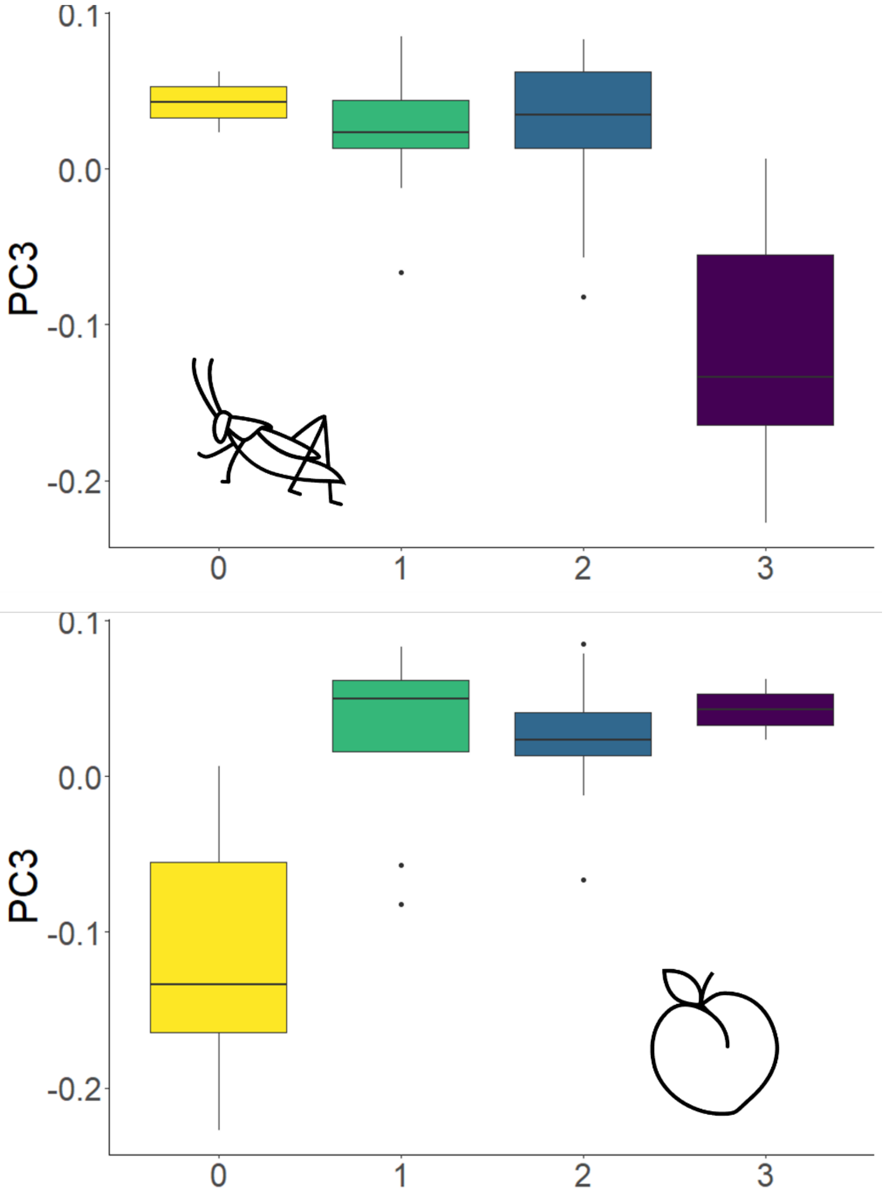


4-3 A) Relationship between PC1 and PC2. Circles represent individuals and are color coded by dietary category, B) relationship between between PC3 and PC4. Circles represent individuals and are color coded by family.



4-4 Relationship between the 3rd principal component of cochlea shape and degree of animalivory (top panel) and frugivory (bottom panel). The x axis is a scale from 0 (no consumption) to 3 (strict consumption).

Table 4-1 A summary of regression results across all analyses. Squares colored green and labeled ‘yes’ indicate a significant relationship (p<0.05) between variables. Exact test statistics are reported in the text as not all tests are further directly comparable.

	Diet Category	🦗	🍑	🌺	Cranium Sphericity	Cranium Aspect	Cranium Size	Cochlea Volume
Cochlea Shape	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PC 1	No	No	No	No	No	No	No	No
PC 2	No	No	No	No	No	Yes	No	No
PC 3	No	Yes	Yes	No	No	No	No	No
PC 4	No	No	No	No	No	No	No	Yes
PC 5	No	No	No	No	No	No	No	No
PC 6	No	No	No	No	No	No	No	No
PC 7	No	No	No	No	No	No	No	No
PC 8	No	No	No	No	No	No	No	No
PC 9	No	No	No	No	No	No	Yes	No
PC 10	No	No	No	No	No	No	No	No

Table 4-2 Phylogenetic signal (K) values for cranial morphometrics and diet (left) and principal components of cochlea shape (right).

Metric	Kappa	Component	Kappa
Cranium Size	1.07	PC1	0.25
Cranium Aspect Ratio	1.03	PC2	0.24
Cranium Sphericity	0.98	PC3	1.57
Nectarivory	0.62	PC4	0.32
Frugivory	3.56	PC5	0.44
Animalivory	3.72	PC6	0.54
		PC7	0.36
		PC8	0.42
		PC9	0.61
		PC10	0.49