Evaluating the Role of Diet, Taxonomy, Sex, and Geography of the Oral and Rectal Microbiome of Puerto Rican Bats

Collaboration with Dr. Michael Willig

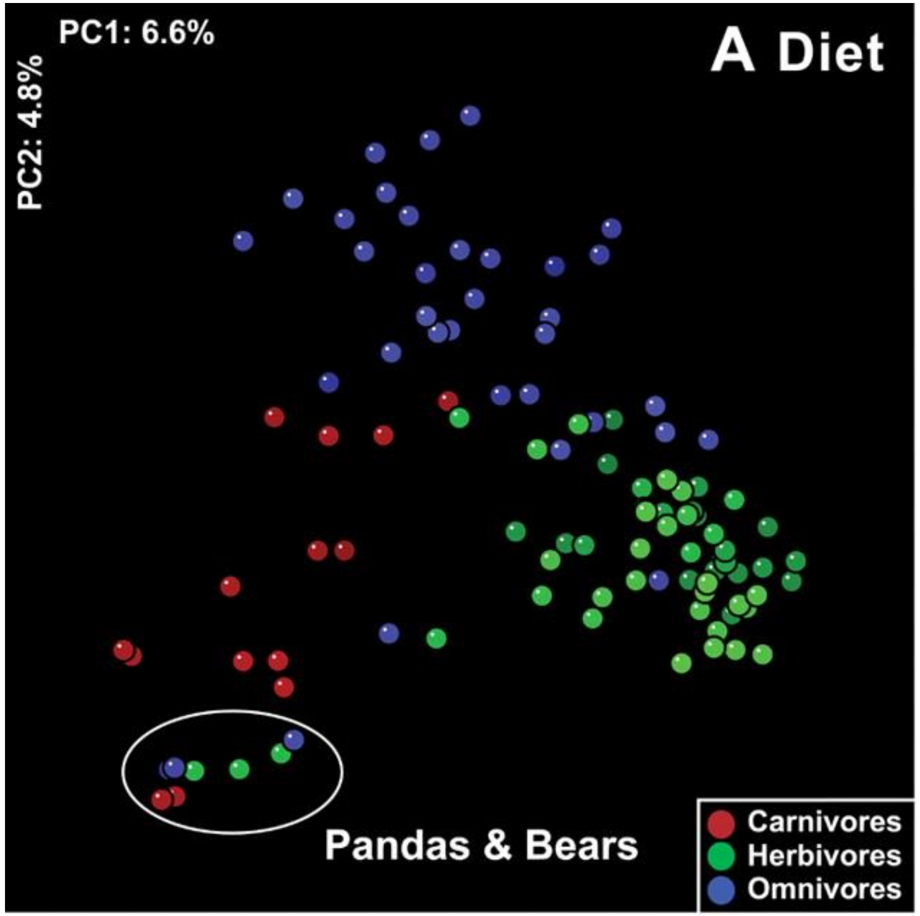
Ahmad Hassan

2019-11-08

Outline

- Background
- Why study bats?
- Methods
- Results
- Summary

“Evolution of Mammals and Their Gut Microbes”



Ley et al. (2008)

Background

Facts

- Only mammals capable of true flight
- Bats account for 20% of mammalian species
- They are found on every continent except Antarctica

Ecological and Economical Significance

- Bats act as natural pest control saving agriculture industry~ $3.7 billion per year
- Natural pollinators and seed dispersers
- Guano droppings are rich natural fertilizers

❖ **White-nose syndrome:** fungal infection caused by *Pseudogymnoascus destructans* that killed millions of bats in North America

https://msphere.asm.org/content/3/5/e00397-18#F1

Puerto Rican Bats

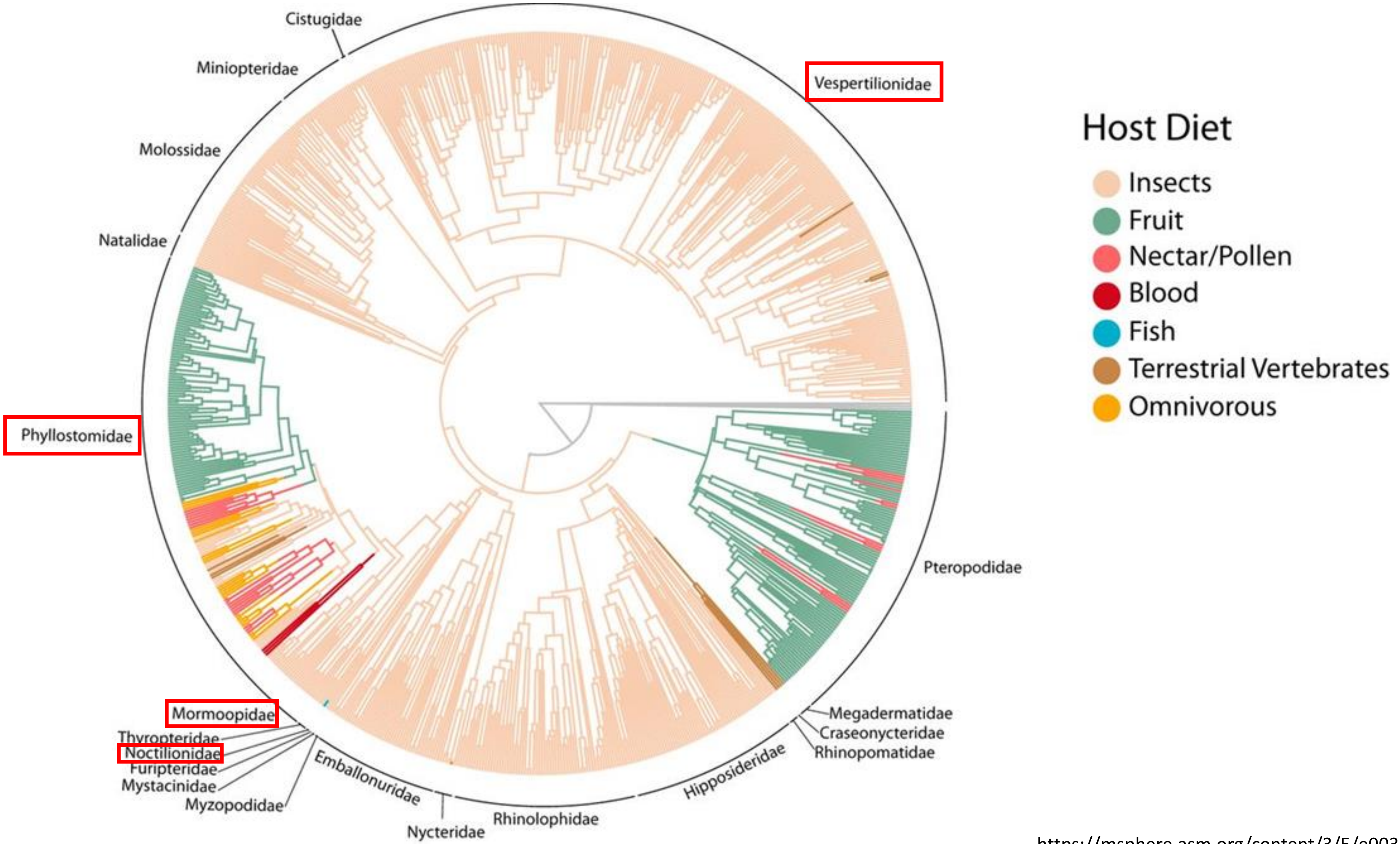
Why study bat microbiome?

- Harbor zoonotic pathogens
- Microbiome is understudied & poorly characterized
- Studying their microbiomes could help us answer:

❖What factors could shape the microbial communities in bats?

- Factors that influence the composition of microbiome:
  - Diet
  - Host taxonomy
  - Sex
  - Geography

https://www.google.com/search?biw=1275&bih=681&tbm=isch&sa=1&ei=hwW1W9XiCMKOgge1oqqIAQ&q=artebius+jamaicensis&oq=artebius+jamaicensis&gs_l=img.3...2953.10616..10725...7.0..0.102.1225.22j1......1....1..gws-wiz-img.......0j35i39j0i67j0i10i24j0i24j0i30.XOzVOVk4PY0#imgrc=iF9sQqO8gwzFrM:



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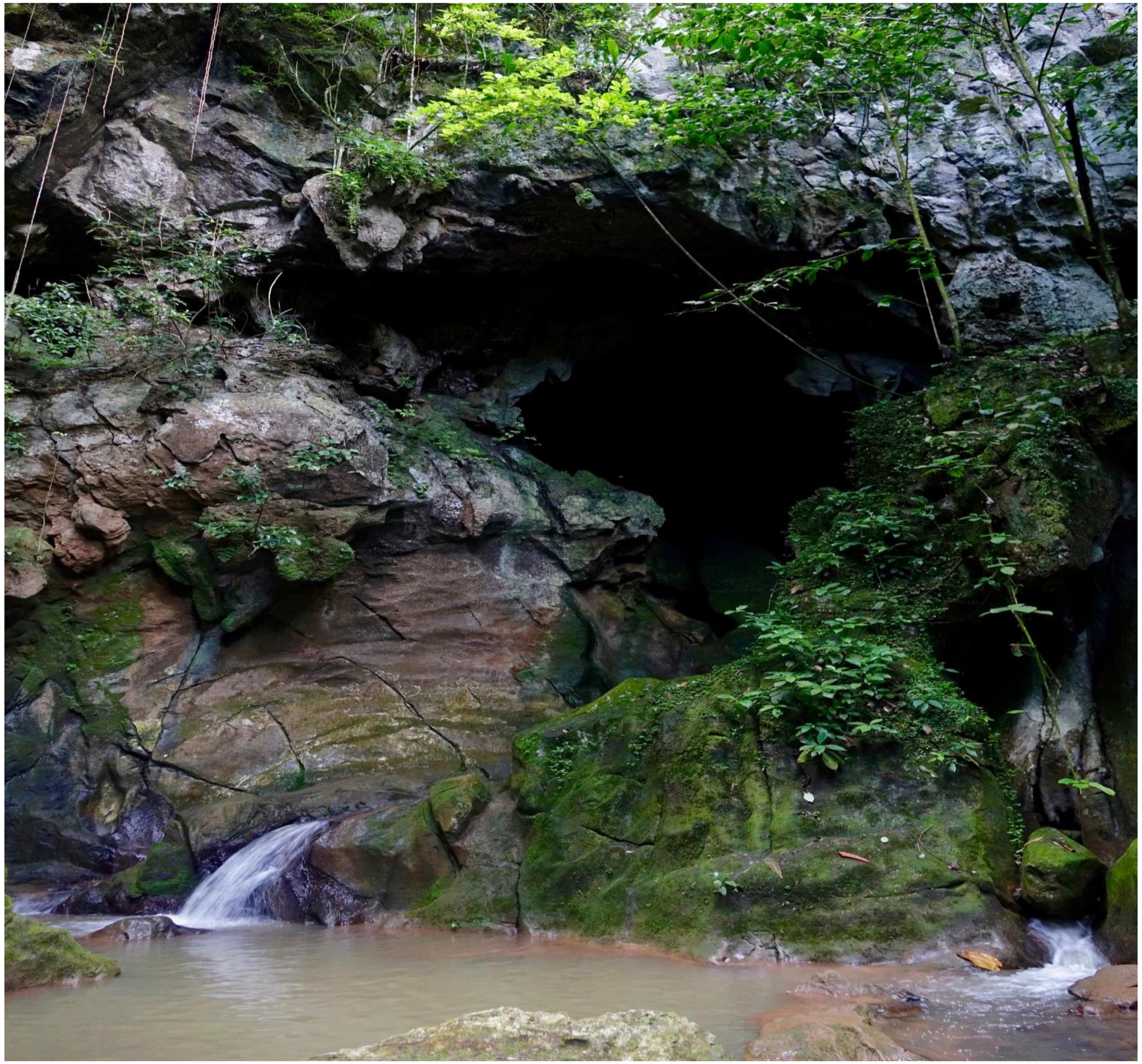
Previous Research Suggests:

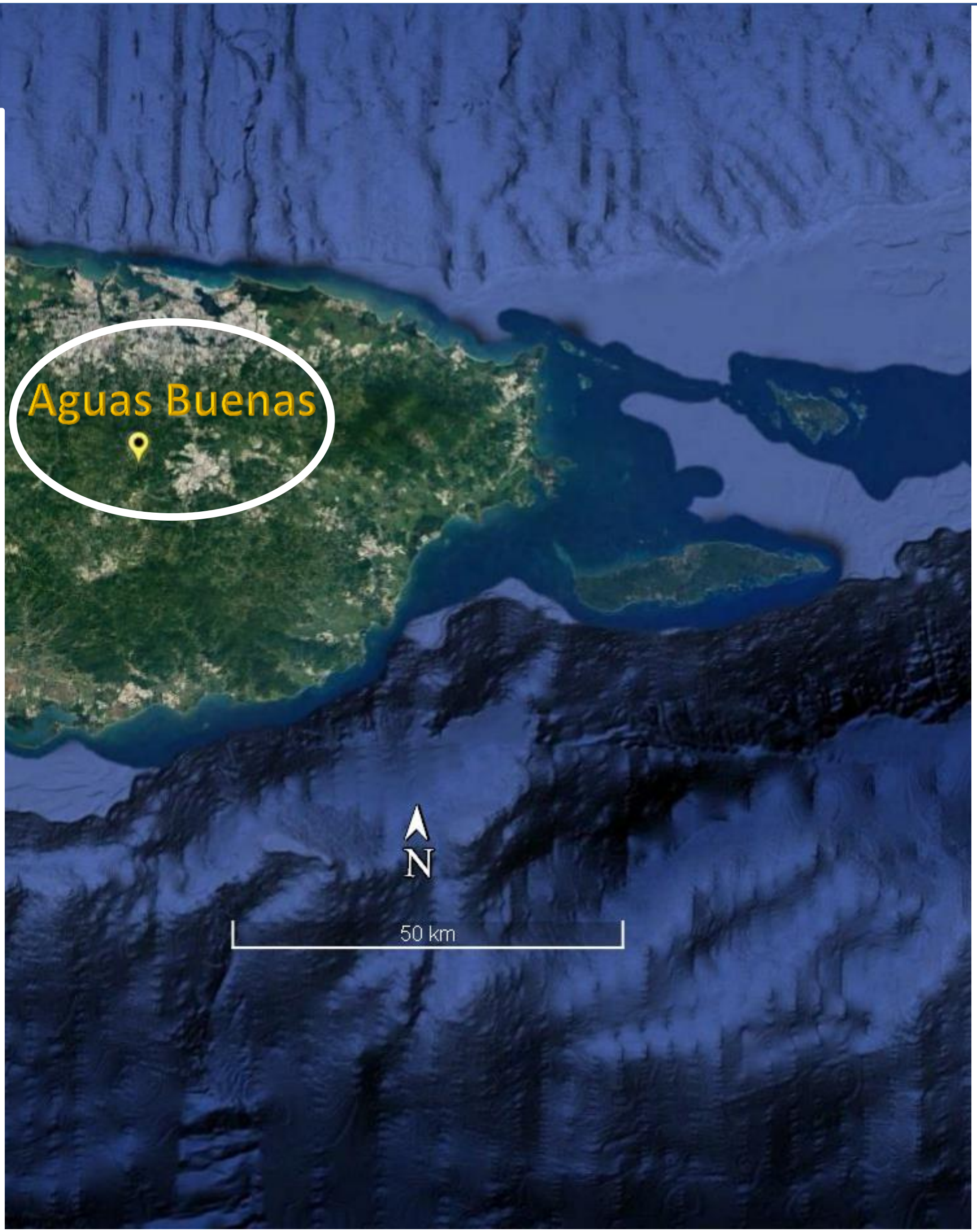
- Diet and host taxonomy have greatest effect on the fecal microbiome (Lee et al., 2008; Muegge et al. 2001).

- Most bat microbiome studies characterized the fecal microbiome and showed intra-species variation in microbiome (Banskar S. et al., 2016).

- Our study design differs by collecting oral and rectal swabs, while most other studies focused on the fecal microbiome.

Sample Collection Done by the Willig Lab in Puerto Rico





Diets of Nine Bat Species Analyzed in this Study

https://www.google.com/search?biw=1275&bih=681&tbm=isch&sa=1&ei=hwW1W9XiCMKOgge1oqqIAQ&q=artebius+jamaicensis&oq=artebius+jamaicensis&gs_l=img.3...2953.10616..10725...7.0..0.102.1225.22j1......1....1..gws-wiz-img.......0j35i39j0i67j0i10i24j0i24j0i30.XOzVOVk4PY0#imgrc=hBrZHsHzCPSVAM:

Frugivore:

Fruits

- *Artibeus jamaicensis*

Euryphagous:

Fruits, Nectar, Insects

- *Brachyphylla cavernarum*
- *Erophylla sezekorni*

https://www.google.com/search?q=monophyllus+redmani&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiz-InV8-rdAhXqTN8KHbYWCSUQ_AUIDygC&biw=1275&bih=681#imgrc=IdO444vXW2ZIgM:

Nectarivore:

Nectar

- *Monophyllus redmani*

http://www.dailynews.lk/sites/default/files/news/2017/12/11/z_p04-Bats-01.jpg

Insectivore:

Insects

- *Mormoops blainvillii*
- *Eptesicus fuscus*
- *Pteronotus portoricensis*
- *Pteronotus quadridens*

https://www.google.com/search?biw=1275&bih=681&tbm=isch&sa=1&ei=XAu1W83oBYrr_Qb_saOIBw&q=Noctilio+leporinus++fish&oq=Noctilio+leporinus++fish&gs_l=img.3...88425.89328..89441.

Piscivore:

Fish

- *Noctilio leporinus*

Methods

Bats caught by harp traps and hand nets

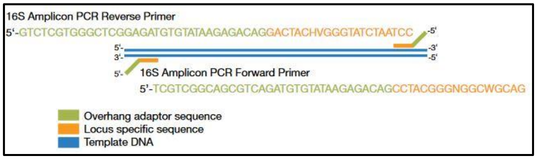
Oral and rectal swabs collected

DNA extracted using DNeasy PowerSoil kit/QIAcube

https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/dneasy-powersoil-kit/#orderinginformation

https://www.qiagen.com/us/shop/automated-solutions/sample-preparation/qiacube/#orderinginformation

PCR Amplify V4 16S rRNA gene



https://www.molzym.com/next-generation-sequencing/ngseq-16s-v3-v4

Sequence on illumina Miseq

https://assets.illumina.com/content/dam/illumina-marketing/images/systems/v2/systems-carousel/system-carousel-miseq-right.png

DADA2

https://user-images.githubusercontent.com/19511476/32180070-3a25cb94-bd4e-11e7-871a-d3d5237d06a6.png

Amplified Sequence Variants (ASVs)

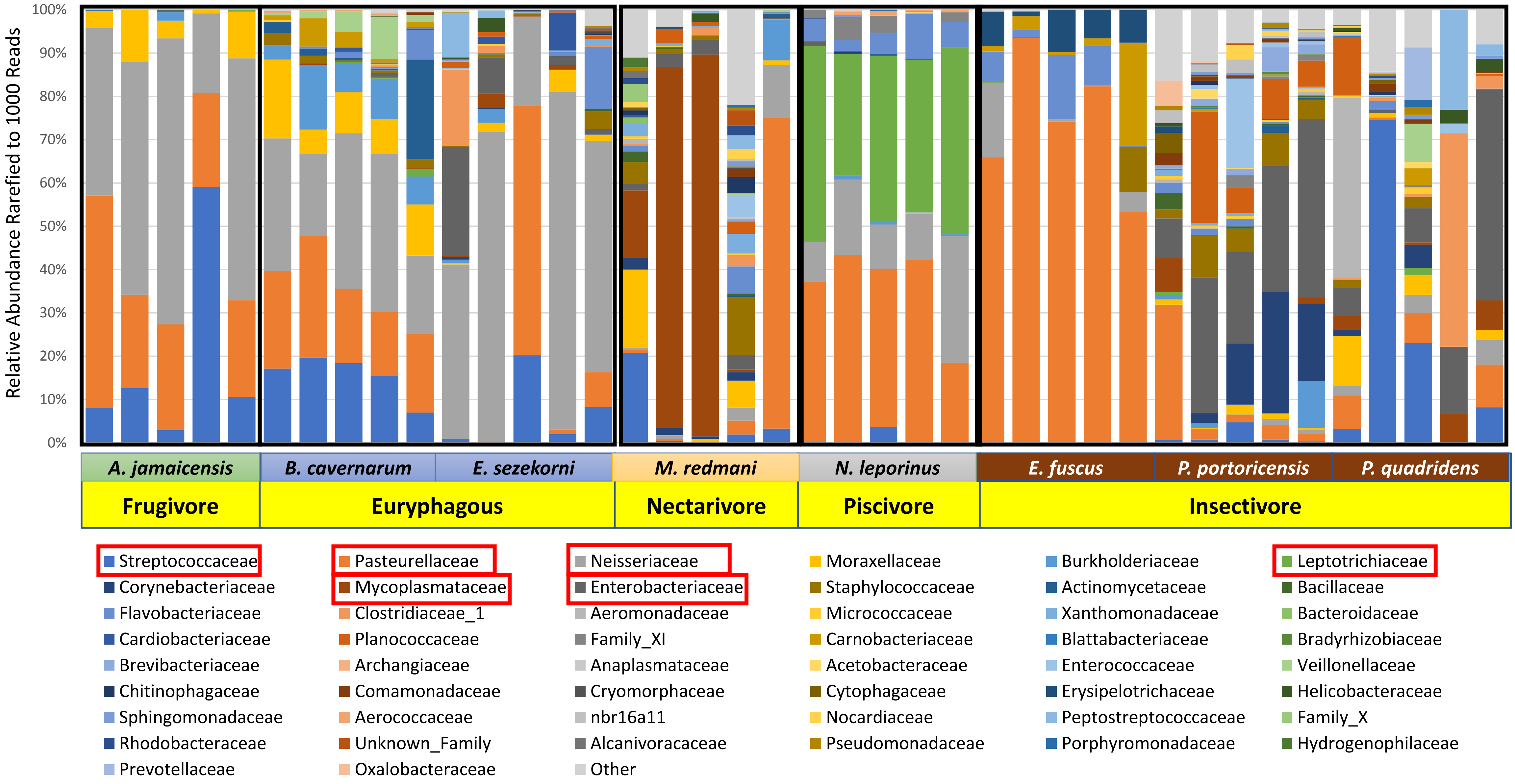
- Decontam
- phyloseq, microbiome, and vegan
- Samples rarefied to 1,000 reads
- 134 oral samples
- 155 rectal samples

Samples Rarefied to 1,000 Reads

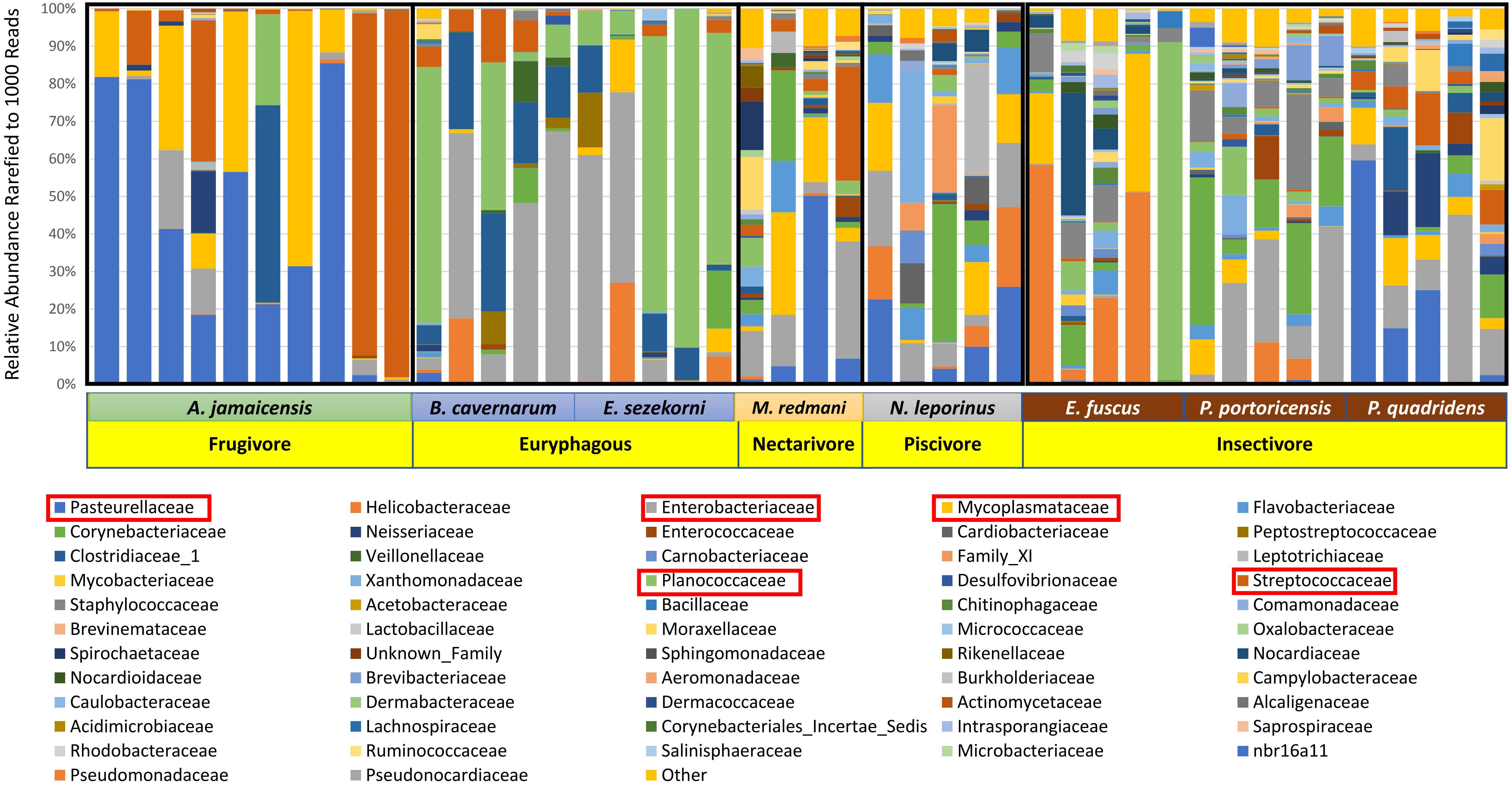
Species	**Oral**	**Rectal**
Artibeus jamaicensis	51	61
Brachyphylla cavernarum	20	19
Erophylla sezekorni	10	26
Monophyllus redmani	14	4
Mormoops blainvillii	2	5
Eptesicus fuscus	11	10
Noctilio leporinus	12	11
Pteronotus portoricensis	7	11
Pteronotus quadridens	7	7

Does diet influence the oral and rectal microbiome?

Family Level Microbial Community Relative Abundance of Oral Samples

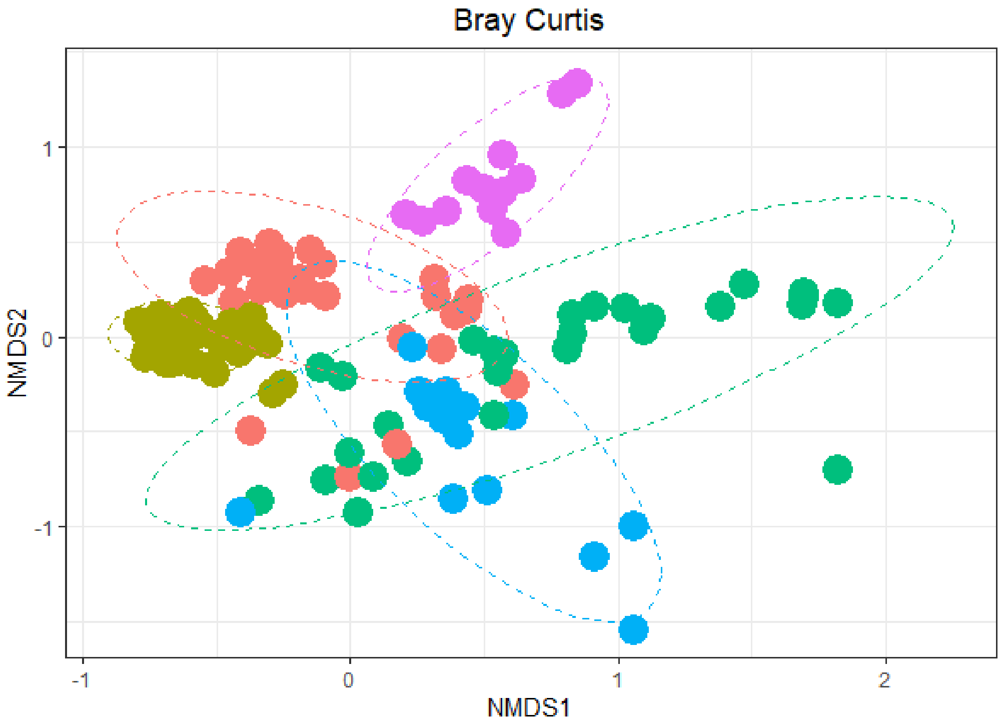


Family Level Microbial Community Relative Abundance of Rectal Samples



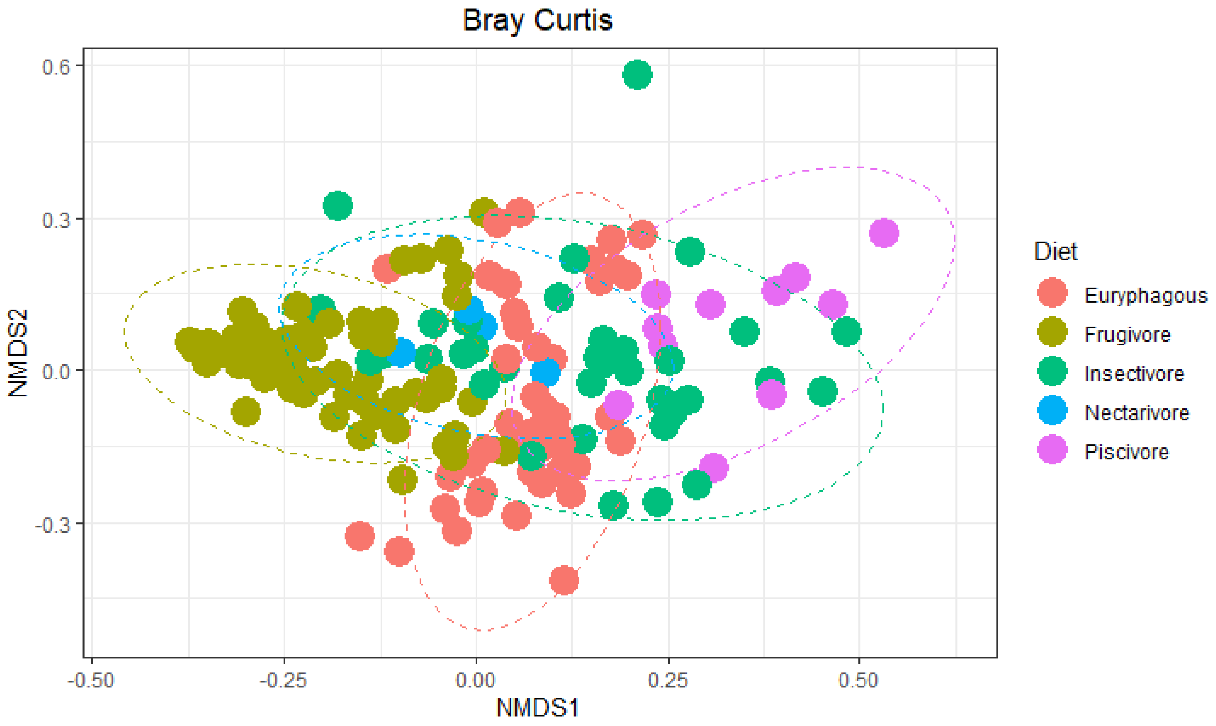
Influence of Diet on the Microbiome

NMDS Oral Samples



PERMANOVA = 0.001
R^2 = 0.404

NMDS Rectal Samples

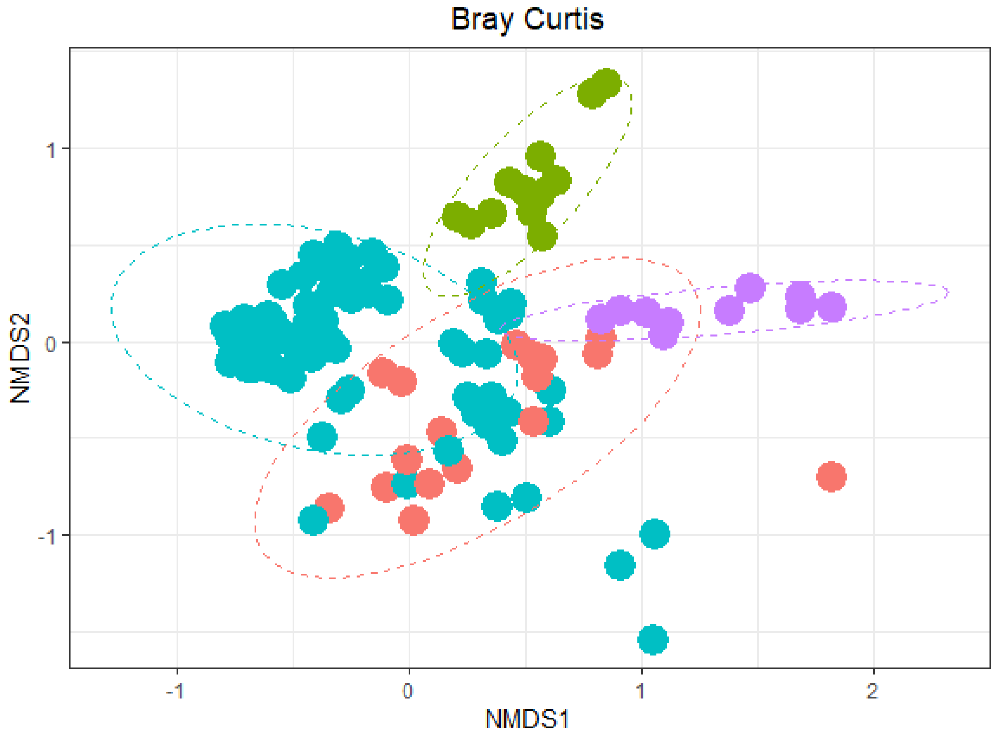


PERMANOVA = 0.001
R^2 = 0.165

Does host taxonomy affect the microbiome?

Influence of Host Family on the Microbiome

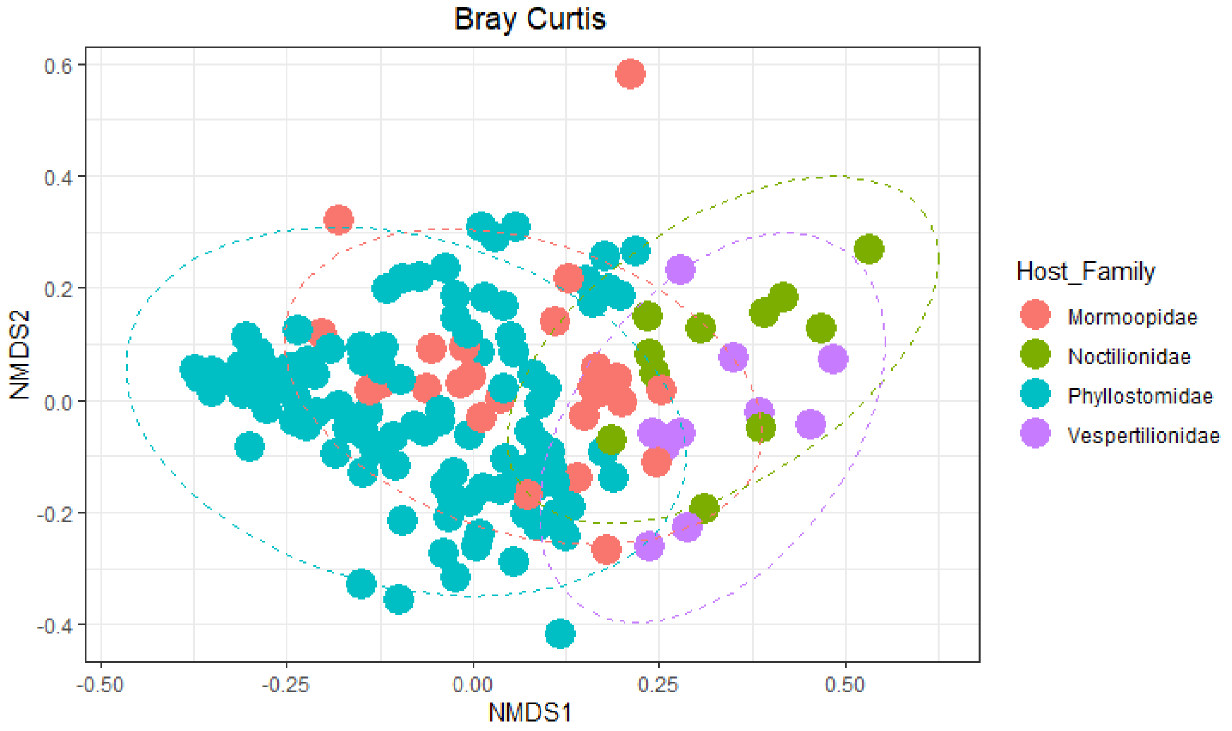
NMDS Oral Samples



PERMANOVA = 0.001

$R^2 = 0.237$

NMDS Rectal Samples

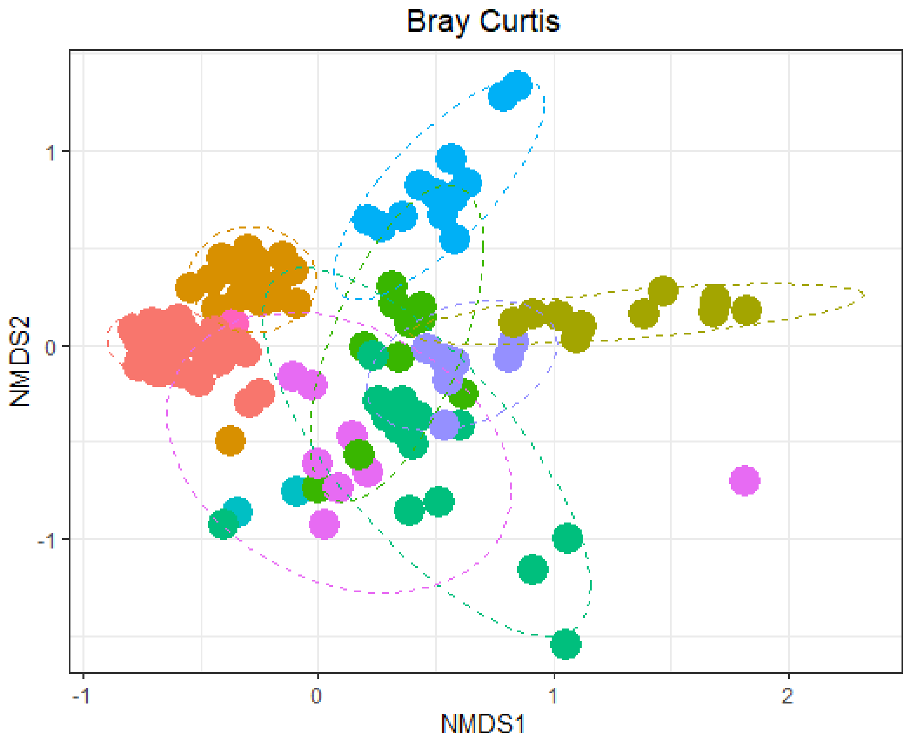


PERMANOVA = 0.001

$R^2 = 0.081$

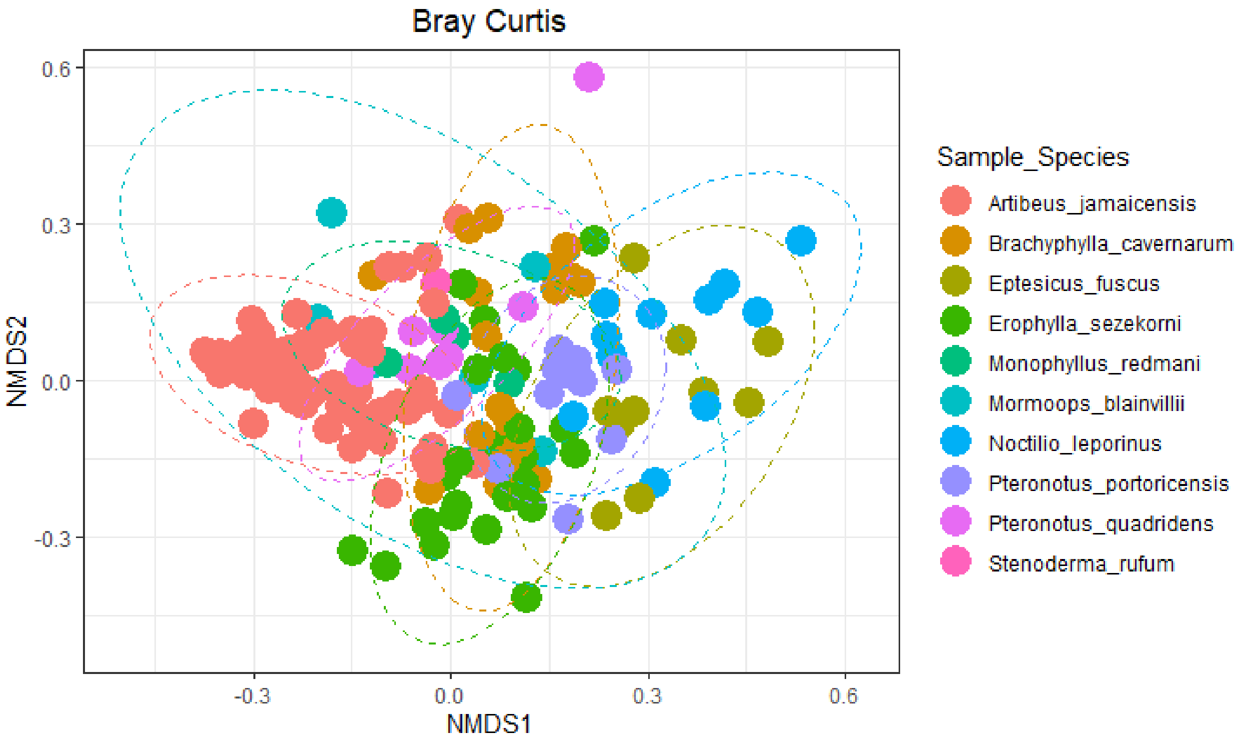
Influence of Host Species on the Microbiome

NMDS Oral Samples



PERMANOVA = 0.001
$R^2 = 0.544$

NMDS Rectal Samples



PERMANOVA = 0.001
$R^2 = 0.226$

Diet and Host Taxonomy Effect on the Microbiome

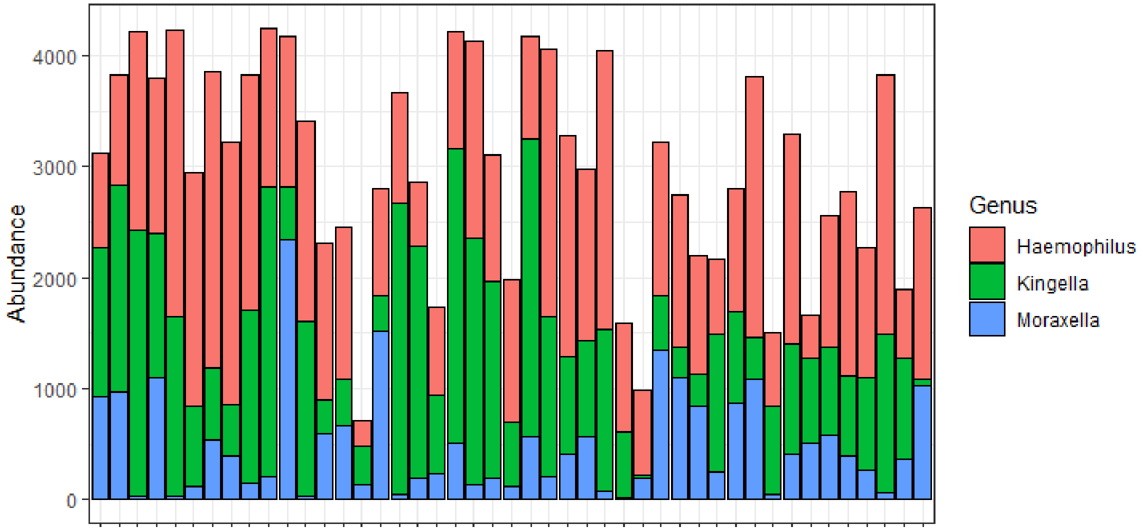
- Diet contributed to 40.4% of variation in the oral microbiome and 16.5% in the rectal microbiome.
- Host species contributed to 54.4% of variation in the oral microbiome and 22.6% of variation in the rectal microbiome.

Most Prevalent Taxa in Five Species Analyzed

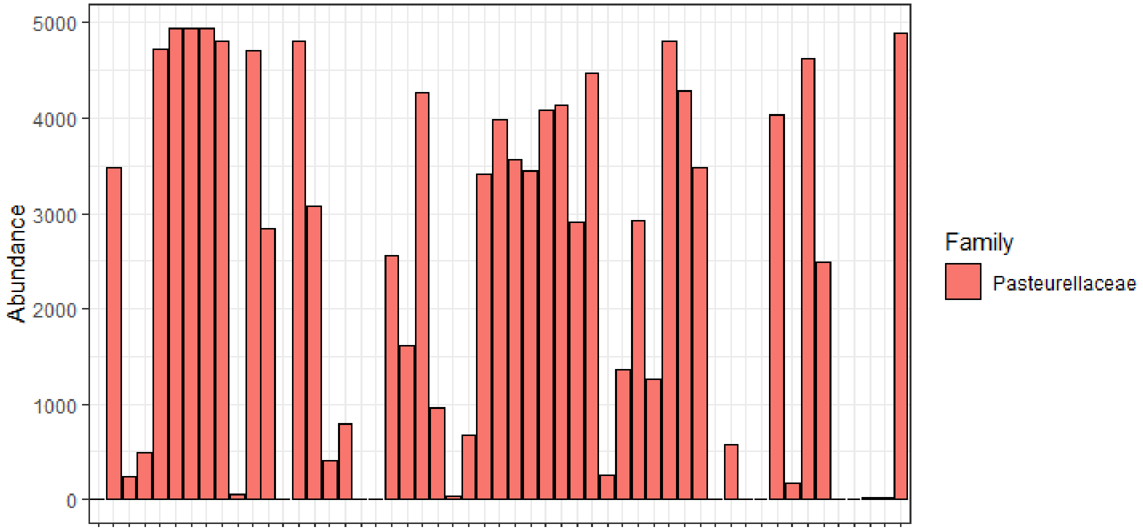
Species	**Diet**
Artibeus jamaicensis	Frugivore
Brachyphylla cavernarum	Euryphagous
Monophyllus redmani	Nectarivore
Noctilio leporinus	Piscivore
Eptesicus fuscus	Insectivore

Frugivore *Artibeus jamaicensis* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 5,000 Reads

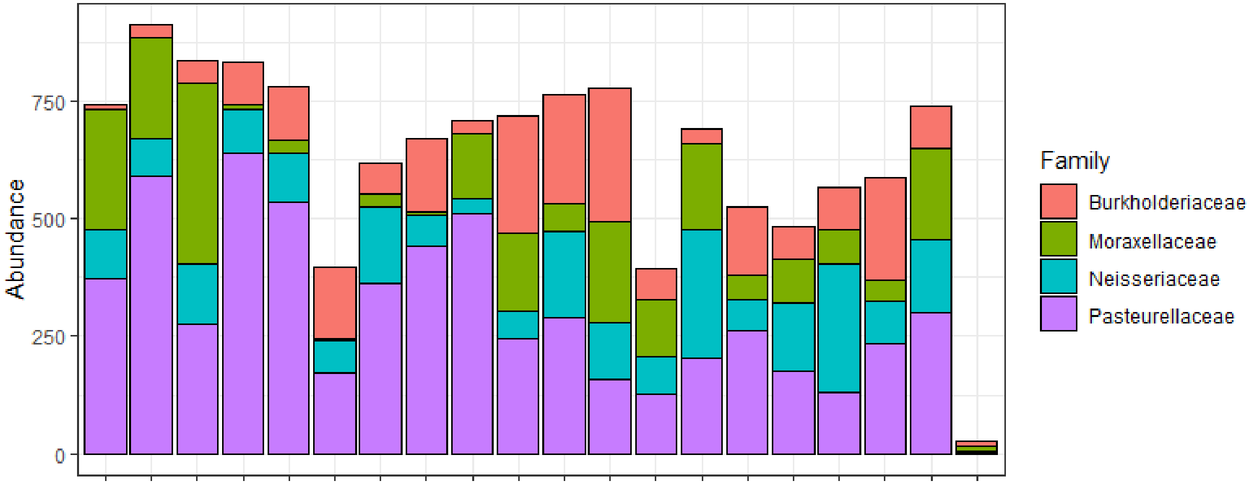


Rectal Samples Rarefied to 5,000 Reads

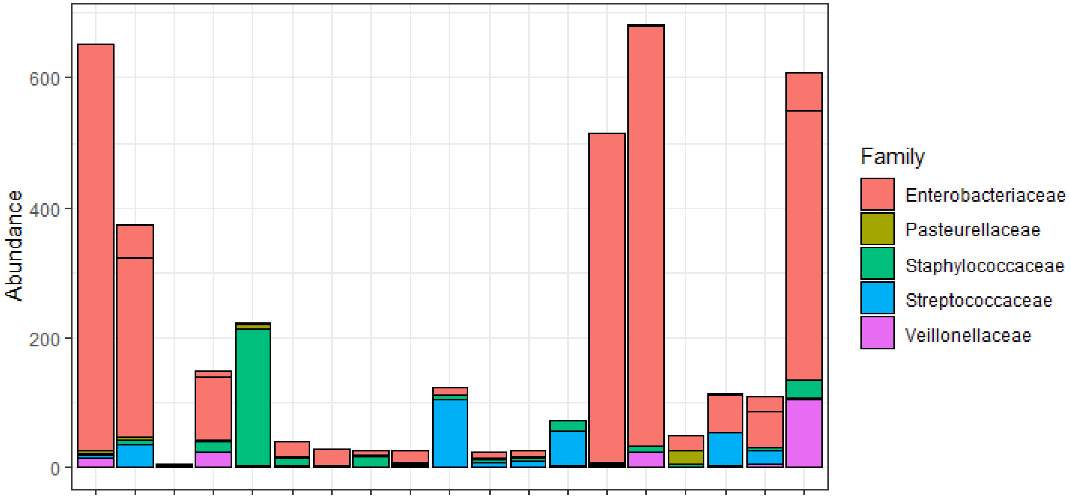


Euryphagous *Brachyphylla cavernarum* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 1,000 Reads

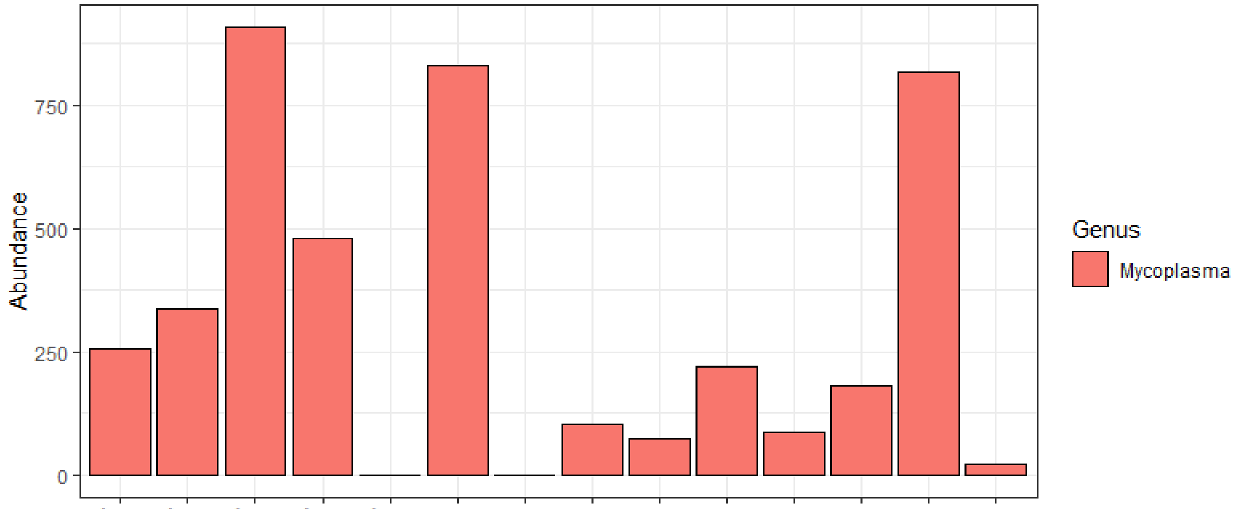


Rectal Samples Rarefied to 1,000 Reads

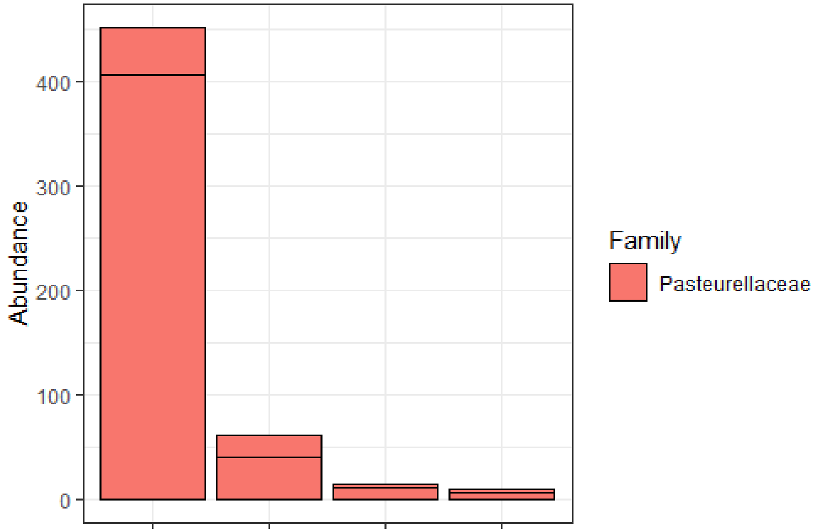


Nectarivore *Monophyllus redmani* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 1,000 Reads

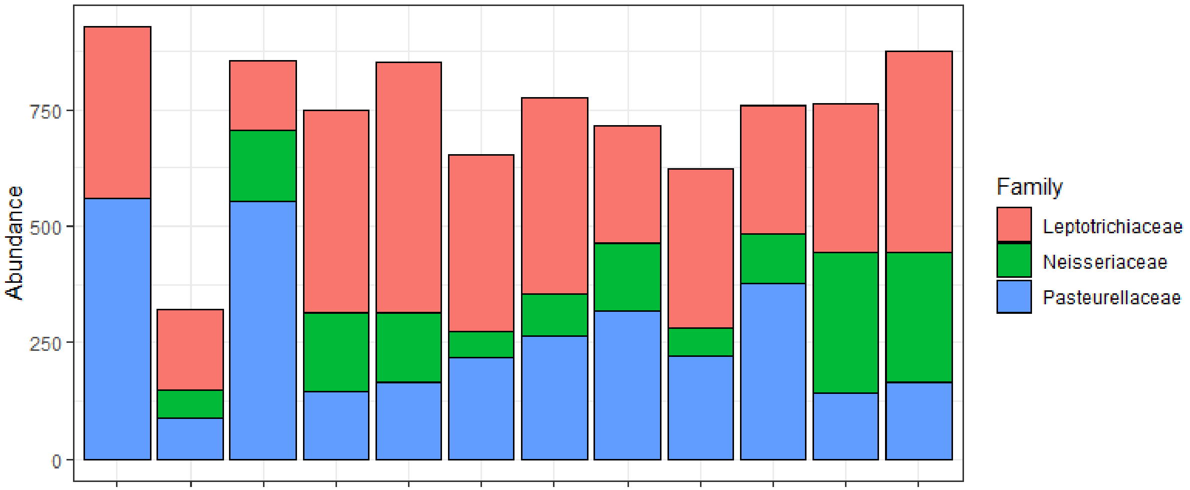


Rectal Samples Rarefied to 1,000 Reads

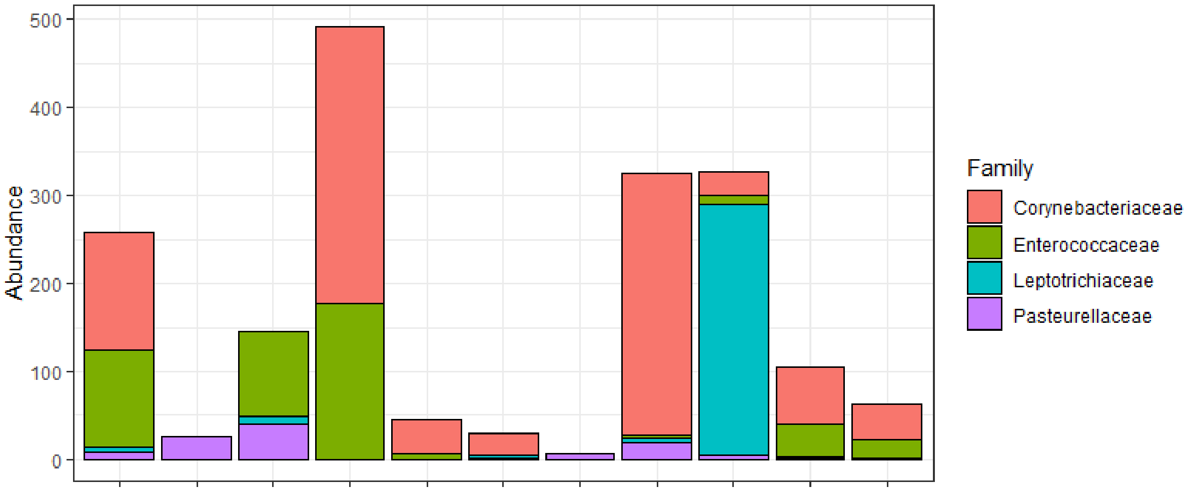


Piscivore *Noctilio leporinus* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 1,000 Reads

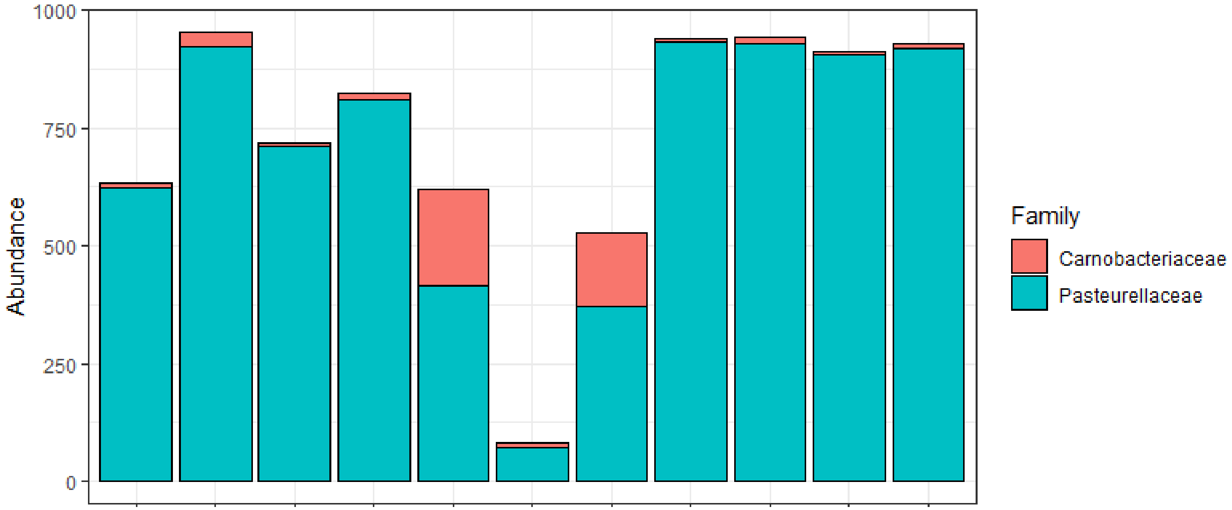


Rectal Samples Rarefied to 1,000 Reads

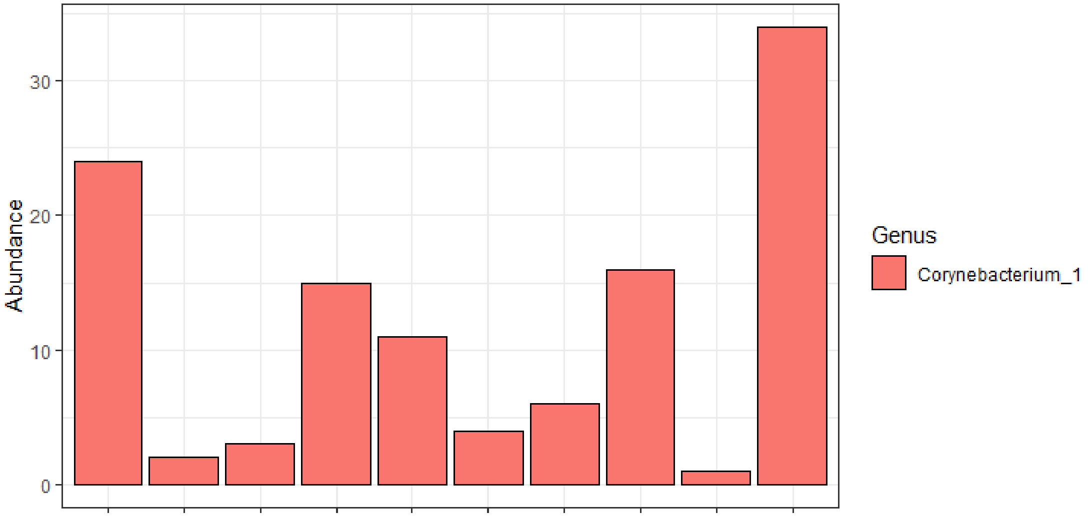


Insectivore *Eptesicus fuscus* Relative Abundance of Most Prevalent Taxa

Oral Samples Rarefied to 1,000 Reads



Rectal Samples Rarefied to 1,000 Reads



How Do Sex and Geography Influence the Microbiome?

- Are there cave specific taxa and where are these taxa found, oral or rectal microbiome?

- Are there differences between male and female bat microbiomes?

Presence of ASVs Across Three Caves

Taxa/ASVs **# of samples**	**Aguas oral** **32**	**Mata oral** **74**	**Rio oral** **30**	**Aguas rectal** **28**	**Mata rectal** **92**	**Rio rectal** **37**
Haemophilus	22	35	22	13	36	15
Kingella	22	33	21	14	32	14
Aggregatibacter	22	11	14	4	3	0
Pasteurellaceae	7	14	7	21	36	21
Moraxella	22	46	21	7	31	11
Enterobacteriaceae	4	23	10	10	47	8
Escherichia/Shigella	6	26	12	13	44	21
Lysinibacillus	4	17	1	10	38	22

Is there an Effect of Sex and Cave on the Microbiome

Oral Samples

Diversity Index	***Artibeus jamaicensis***				***Brachyphylla cavernarum***		***Erophylla sezekorni***		***Monophyllus redmani***		***Noctilio leporinus***				***Pteronotus quadridens***	
	Sex		**Cave**		**Sex**		**Sex**		**Sex**		**Sex**		**Cave**		**Sex**	
	P-value	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**
Bray Curtis	0.471	0.018	0.001	0.119	0.023	0.103	0.719	0.058	0.722	0.057	0.551	0.082	0.503	0.162	0.544	0.138
Jaccard	0.526	0.018	0.001	0.096	0.039	0.089	0.796	0.060	0.784	0.060	0.516	0.084	0.451	0.171	0.555	0.140

Is there an Effect of Sex and Cave on the Microbiome

Rectal Samples

Diversity Index	***Artibeus jamaicensis***				***Brachyphylla cavernarum***		***Erophylla sezekorni***		***Monophyllus redmani***		***Noctilio leporinus***				***Pteronotus quadridens***	
	Sex		**Cave**		**Sex**		**Sex**		**Sex**		**Sex**		**Cave**		**Sex**	
	P-value	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**
Bray Curtis	0.109	0.027	0.042	0.059	0.001	0.146	0.731	0.028	0.266	0.268	0.044	0.155	0.054	0.251	0.251	0.164
Jaccard	0.093	0.025	0.044	0.054	0.011	0.113	0.623	0.033	0.266	0.244	0.035	0.136	0.059	0.234	0.201	0.156

Summary

- Host species and diet had greatest effect on the oral microbiome.
- Only *B. cavernarum* and *N. leporinus* showed a difference between male and female microbiome.
- Geography had minimal effect on the microbiome. There was a significant difference in the microbiome of *A. jamaicensis* among three caves.
- Our results show a greater effect of diet and host taxonomy than previous studies probably because we used oral and rectal swabs.

Acknowledgments

Graf Lab

Dr. Joerg Graf

Dr. Lidia Beka

Dr. Jacquelynn Benjamino

Dr. Jeremiah Marden

Todd Testerman

Susan T. Janton

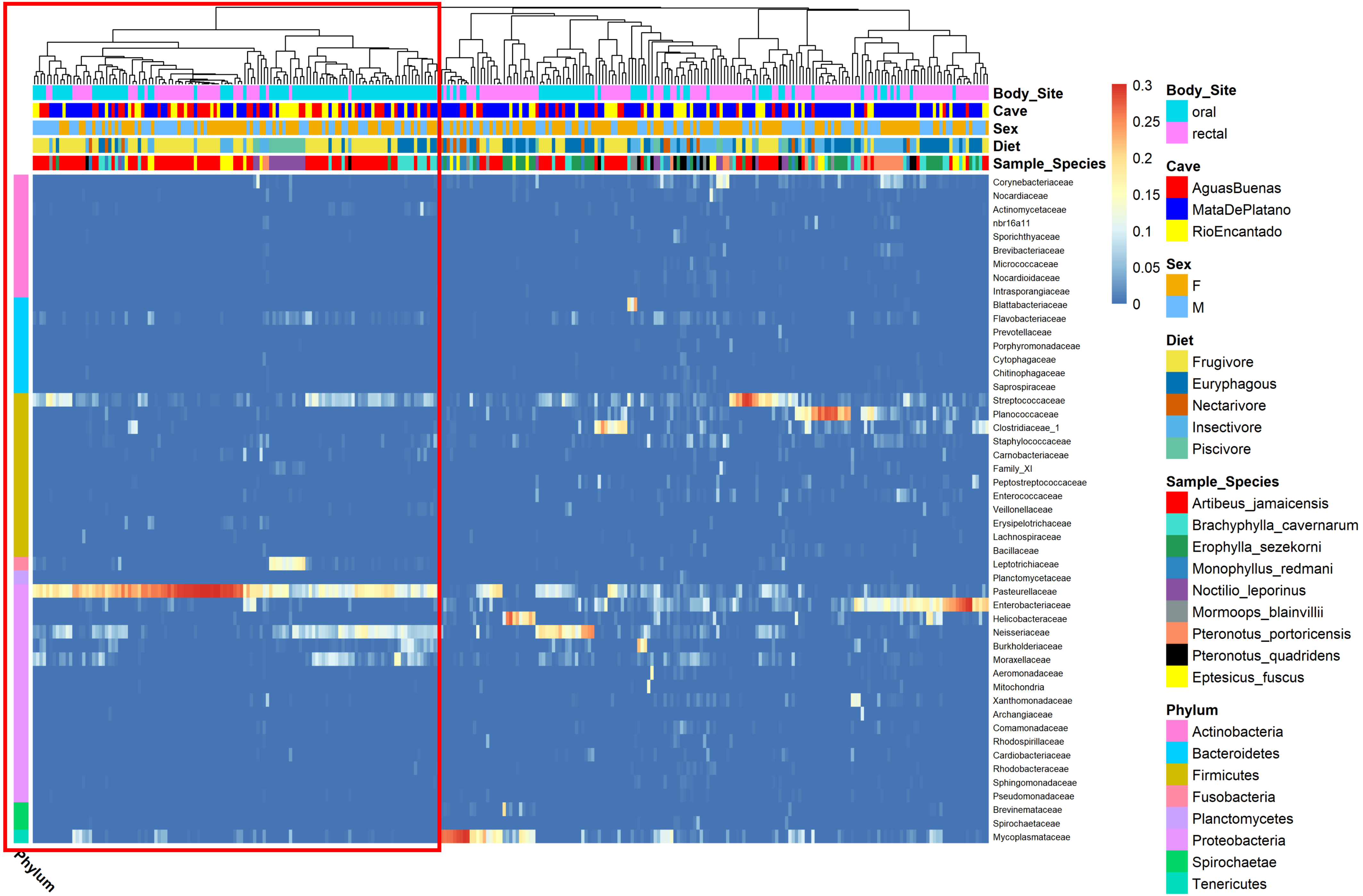
Michael Willig Lab

Dr. Michael Willig

Dr. Steven Presley

Anna R. Sjodin

This study was funded by UConn Provost Microbiome Research Excellence Award


Body_Site
Cave
Sex
Diet
Sample_Species
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Nocardiaceae
Actinomycetaceae
nbr16a11
Sporichthyaceae
Brevibacteriaceae
Micrococcaceae
Nocardioidaceae
Intrasporangiaceae
Blattabacteriaceae
Flavobacteriaceae
Prevotellaceae
Porphyromonadaceae
Cytophagaceae
Chitinophagaceae
Saprospiraceae
Streptococcaceae
Planococcaceae
Clostridiaceae_1
Staphylococcaceae
Carnobacteriaceae
Family_XI
Peptostreptococcaceae
Enterococcaceae
Veillonellaceae
Erysipelotrichaceae
Lachnospiraceae
Bacillaceae
Leptotrichiaceae
Planctomycetaceae
Pasteurellaceae
Enterobacteriaceae
Helicobacteraceae
Neisseriaceae
Burkholderiaceae
Moraxellaceae
Aeromonadaceae
Mitochondria
Xanthomonadaceae
Archangiaceae
Comamonadaceae
Rhodospirillaceae
Cardiobacteriaceae
Rhodobacteraceae
Sphingomonadaceae
Pseudomonadaceae
Brevinemataceae
Spirochaetaceae
Mycoplasmataceae
Phylum
0.3
0.25
0.2
0.15
0.1
0.05
0
Body_Site
oral
rectal
Cave
AguasBuenas
MataDePlatano
RioEncantado
Sex
F
M
Diet
Frugivore
Euryphagous
Nectarivore
Insectivore
Piscivore
Sample_Species
Artibeus_jamaicensis
Brachyphylla_cavernarum
Erophylla_sezekorni
Monophyllus_redmani
Noctilio_leporinus
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Pteronotus_quadridens
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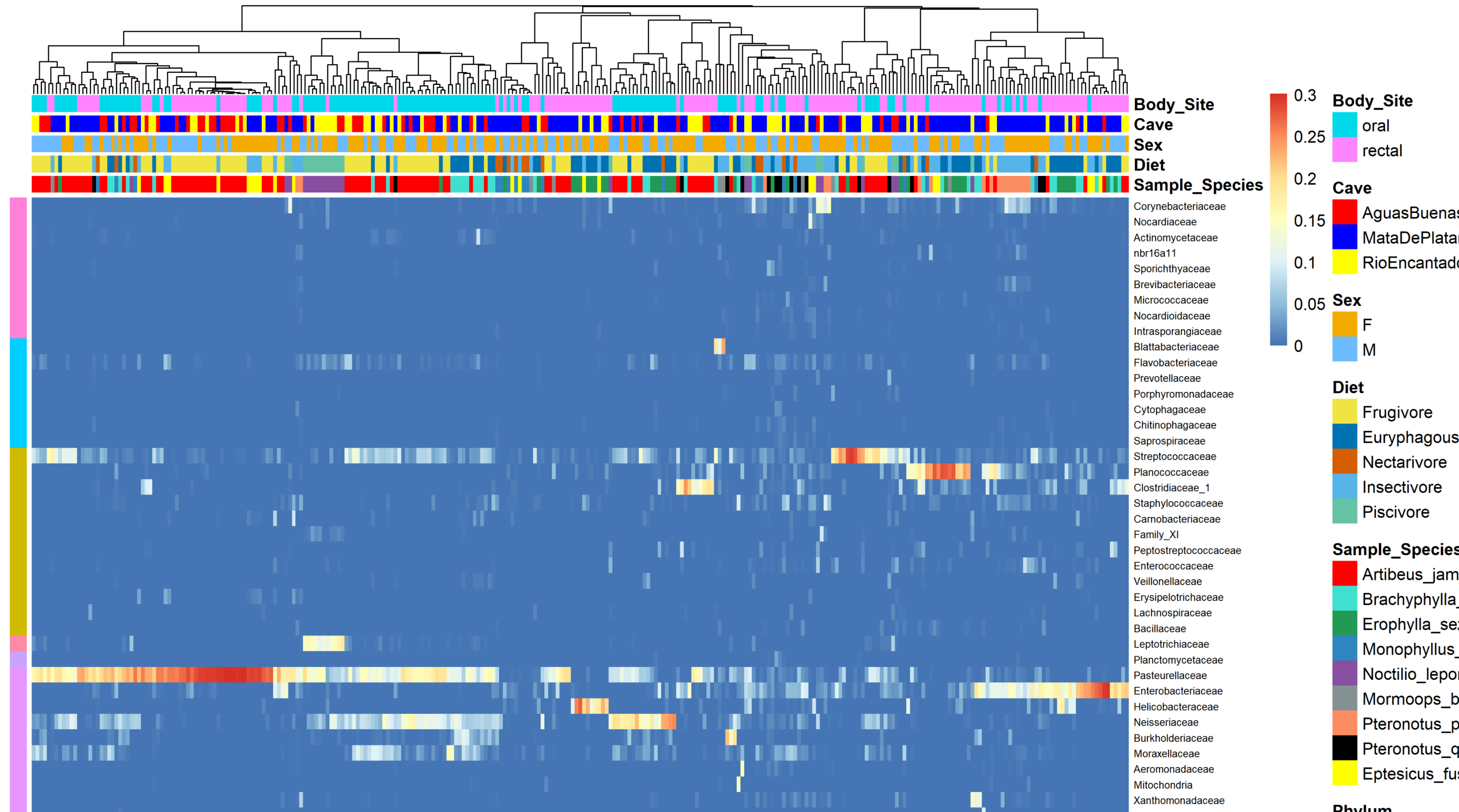
Bacteroidetes
Firmicutes
Fusobacteria
Planctomycetes
Proteobacteria
Spirochaetae
Tenericutes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Moraxellaceae
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Xanthomonadaceae
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rectal
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AguasBuena
MataDePlata
RioEncantad
Sex
F
M
Diet
Frugivore
Euryphagous
Nectarivore
Insectivore
Piscivore
Sample_Species
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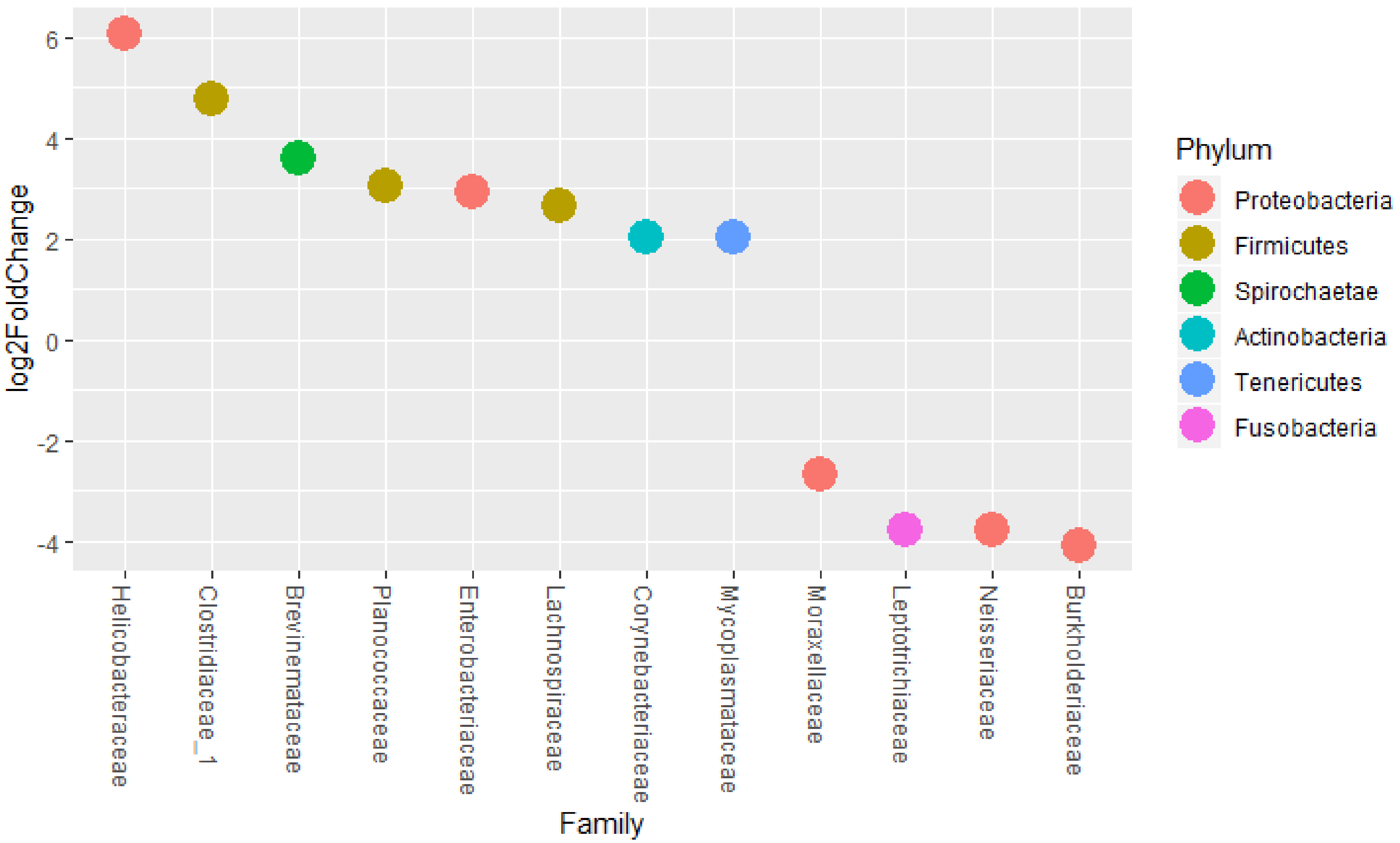
Brachyphylla_
Erophylla_se
Monophyllus_
Noctilio_lepo
Mormoops_b
Pteronotus_p
Pteronotus_q
Eptesicus_fu
Phylum

Differential Abundance-Rectal Vs Oral



Skin Microbiome Can Aide in the Fight Against *Pseudogymnoascus destructans*

Bacteria Isolated from Bats Inhibit the Growth of *Pseudogymnoascus destructans*, the Causative Agent of White-Nose Syndrome

Joseph R. Hoyt, Tina L. Cheng, Kate E. Langwig, Mallory M. Hee, Winifred F. Frick, A. Marm Kilpatrick

Published: April 8, 2015 • https://doi.org/10.1371/journal.pone.0121329

Abstract

Emerging infectious diseases are a key threat to wildlife. Several fungal skin pathogens have recently emerged and caused widespread mortality in several vertebrate groups, including amphibians, bats, rattlesnakes and humans. White-nose syndrome, caused by the fungal skin pathogen *Pseudogymnoascus destructans*, threatens several hibernating bat species with extinction and there are few effective treatment strategies. The skin microbiome is increasingly understood to play a large role in determining disease outcome. We isolated bacteria from the skin of four bat species, and co-cultured these isolates with *P. destructans* to identify bacteria that might inhibit or kill *P. destructans*. We then conducted two reciprocal challenge experiments *in vitro* with six bacterial isolates (all in the genus *Pseudomonas*) to quantify the effect of these bacteria on the growth of *P. destructans*. All six *Pseudomonas* isolates significantly inhibited growth of *P. destructans* compared to non-inhibitory control bacteria, and two isolates performed significantly better than others in suppressing *P. destructans* growth for at least 35 days. In both challenge experiments, the extent of suppression of *P. destructans* growth was dependent on the initial concentration of *P. destructans* and the initial concentration of the bacterial isolate. These results show that bacteria found naturally occurring on bats can inhibit the growth of *P. destructans in vitro* and should be studied further as a possible probiotic to protect bats from white-nose syndrome. In addition, the presence of these bacteria may influence disease outcomes among individuals, populations, and species.

Included in the Following Collections

Slice of PLOS: The Awesomeness of Bats
Editor's Picks: Microbiology

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Volume 97, Issue 6
5 December 2016

Article Contents

Abstract

Diet choice in frugivorous bats: gourmets or operational pragmatists? FREE

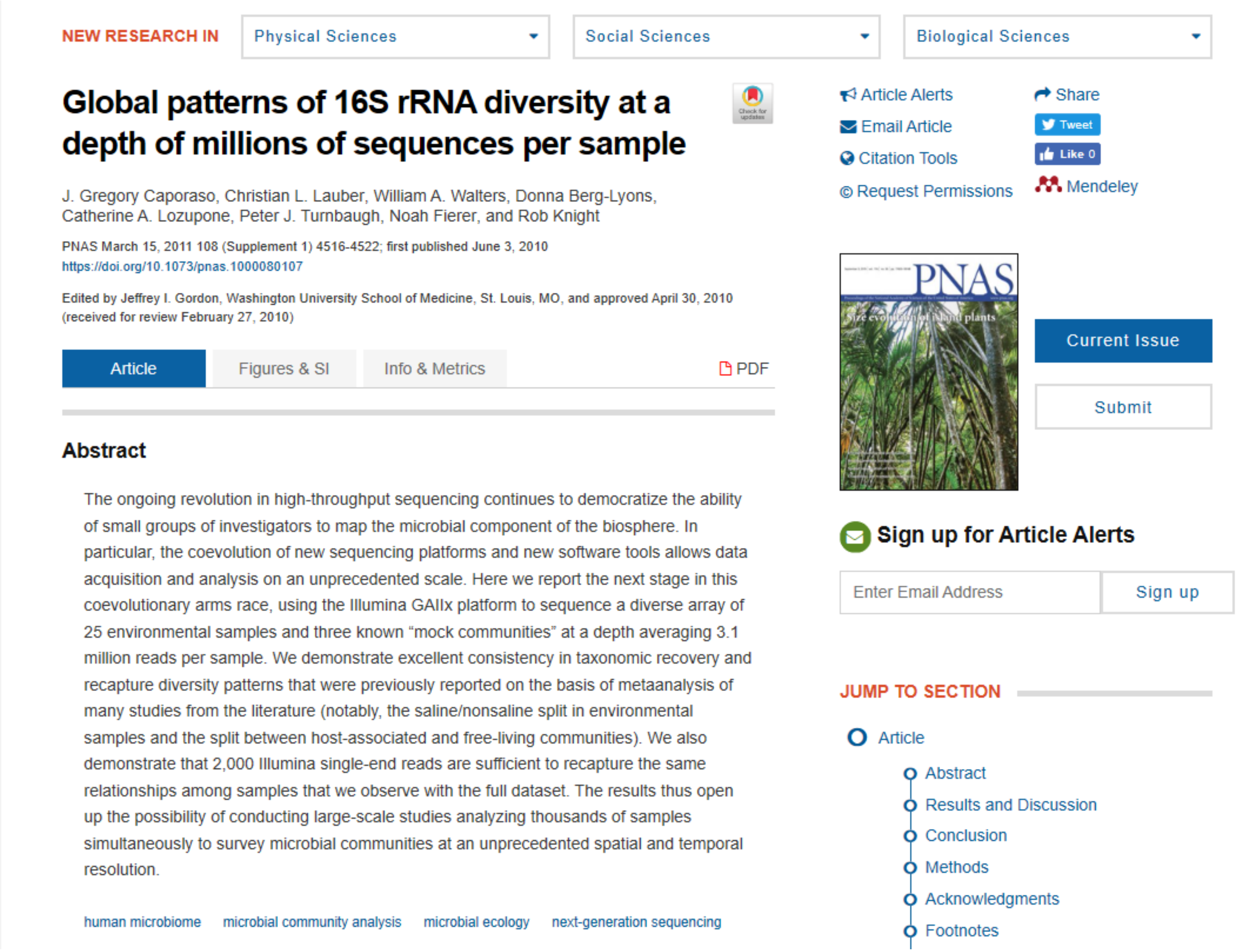
Teri J. Orr, Jorge Ortega, Rodrigo A. Medellín, Caitlin D. Sánchez, Kimberly A. Hammond

Journal of Mammalogy, Volume 97, Issue 6, 5 December 2016, Pages 1578–1588, https://doi.org/10.1093/jmammal/gyw122

Published: 26 July 2016 **Article history** ▾

Received: 24 July 2015
Revision received: 03 July 2016
Accepted: 05 July 2016
Published: 26 July 2016

Why Rarefy to 2000 Reads



Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample

J. Gregory Caporaso, Christian L. Lauber, William A. Walters, Donna Berg-Lyons, Catherine A. Lozupone, Peter J. Turnbaugh, Noah Fierer, and Rob Knight

PNAS March 15, 2011 108 (Supplement 1) 4516-4522; first published June 3, 2010
https://doi.org/10.1073/pnas.1000080107

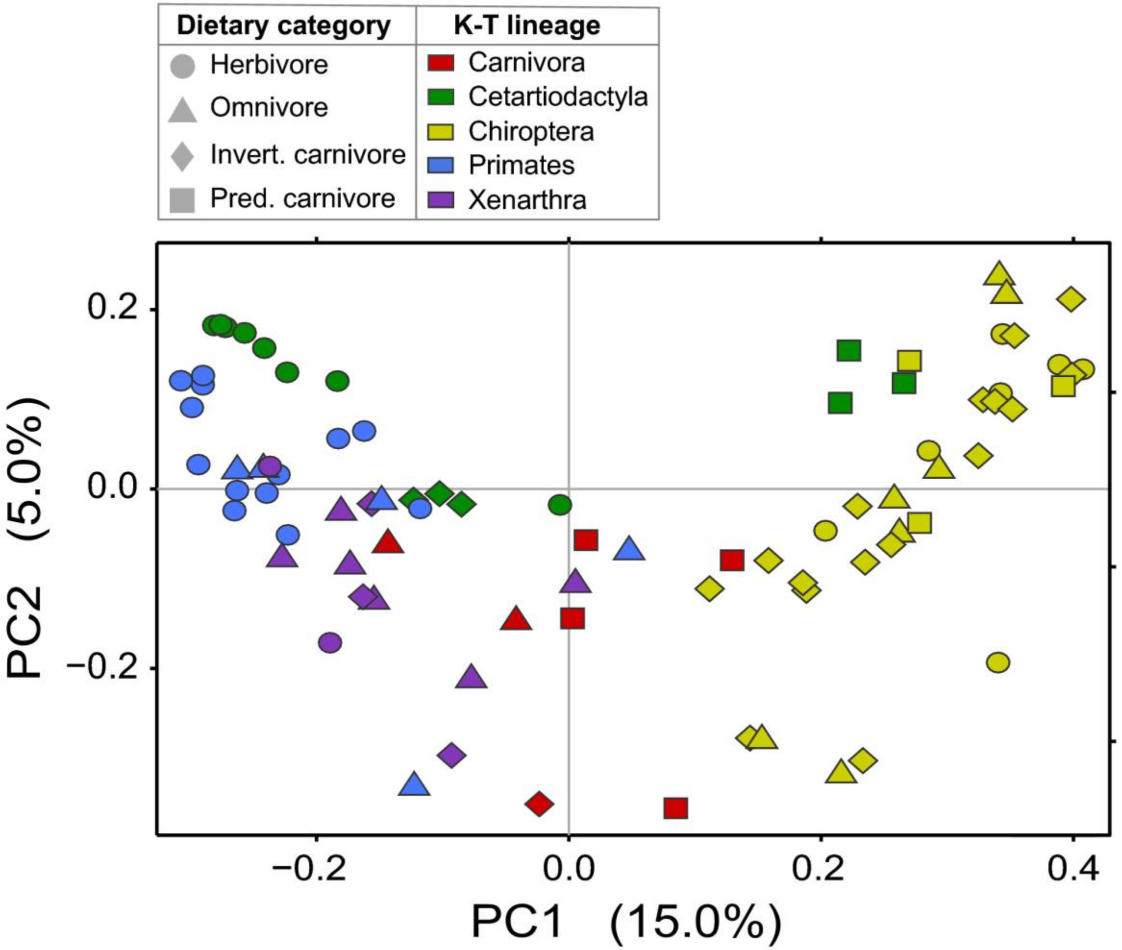
Edited by Jeffrey I. Gordon, Washington University School of Medicine, St. Louis, MO, and approved April 30, 2010 (received for review February 27, 2010)

Abstract

The ongoing revolution in high-throughput sequencing continues to democratize the ability of small groups of investigators to map the microbial component of the biosphere. In particular, the coevolution of new sequencing platforms and new software tools allows data acquisition and analysis on an unprecedented scale. Here we report the next stage in this coevolutionary arms race, using the Illumina GAIIx platform to sequence a diverse array of 25 environmental samples and three known "mock communities" at a depth averaging 3.1 million reads per sample. We demonstrate excellent consistency in taxonomic recovery and recapture diversity patterns that were previously reported on the basis of metaanalysis of many studies from the literature (notably, the saline/nonsaline split in environmental samples and the split between host-associated and free-living communities). We also demonstrate that 2,000 Illumina single-end reads are sufficient to recapture the same relationships among samples that we observe with the full dataset. The results thus open up the possibility of conducting large-scale studies analyzing thousands of samples simultaneously to survey microbial communities at an unprecedented spatial and temporal resolution.

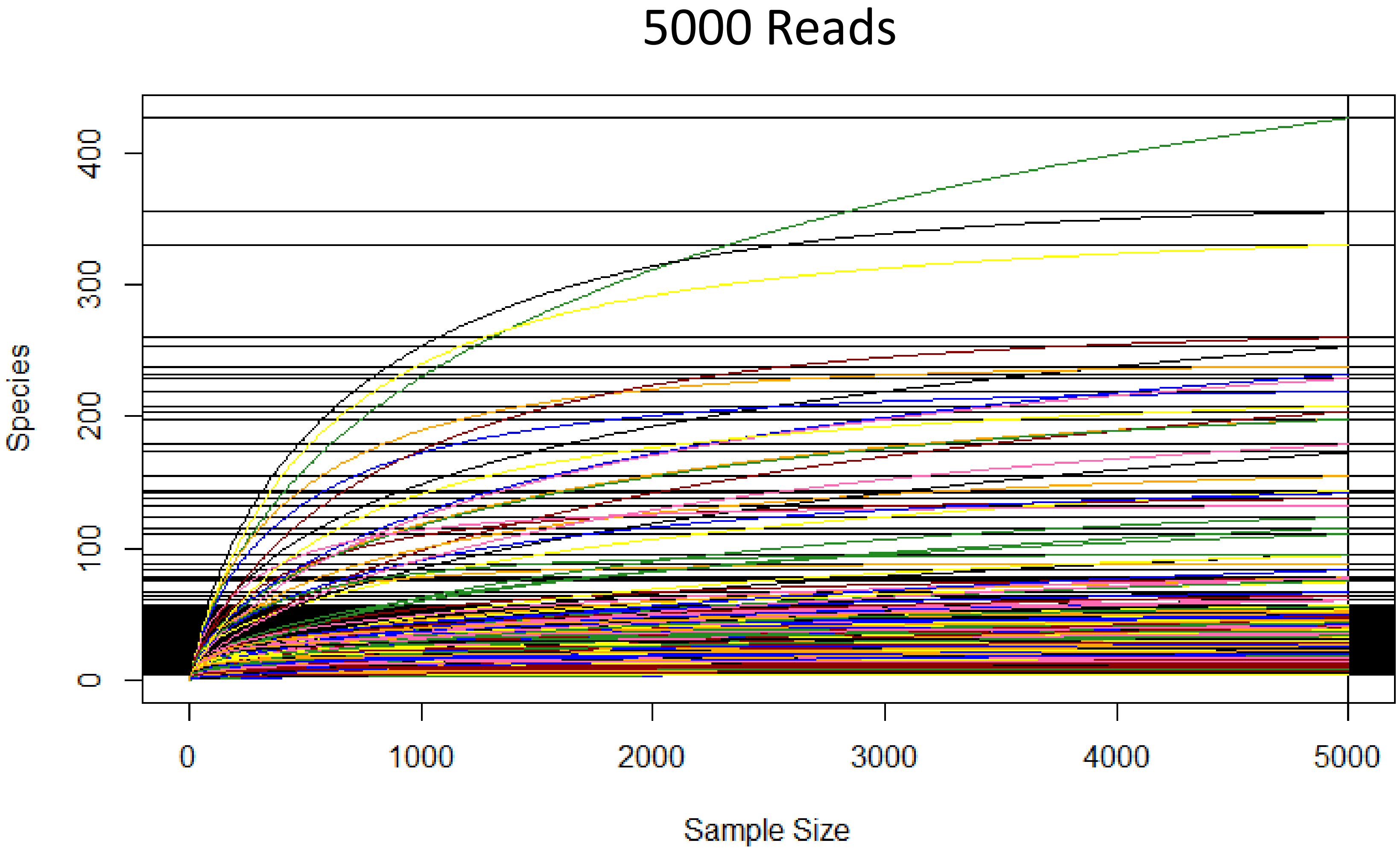
human microbiome microbial community analysis microbial ecology next-generation sequencing

“Rates of gut microbiome divergence in mammals”

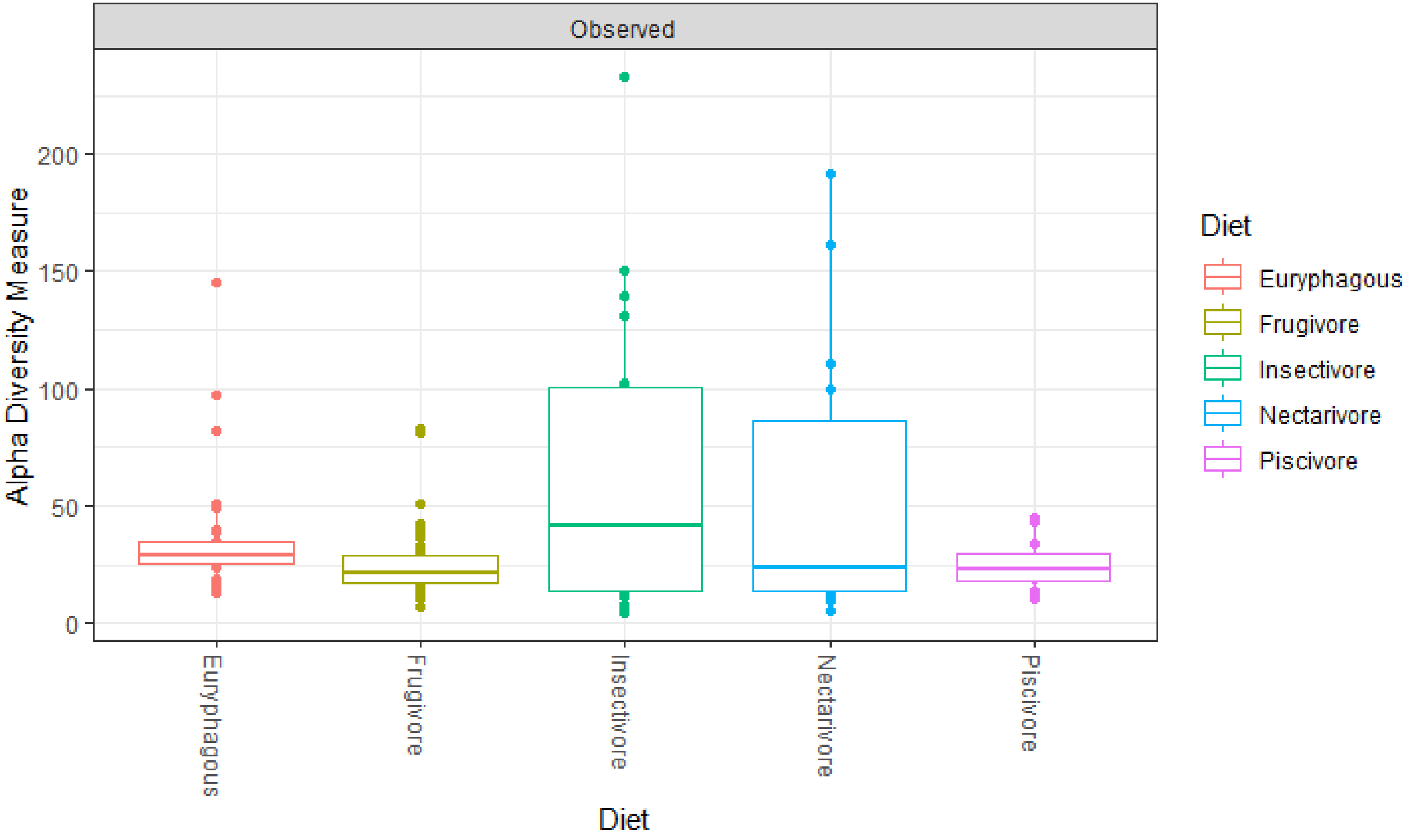


Nishida, AH, Ochman, H. (2018)

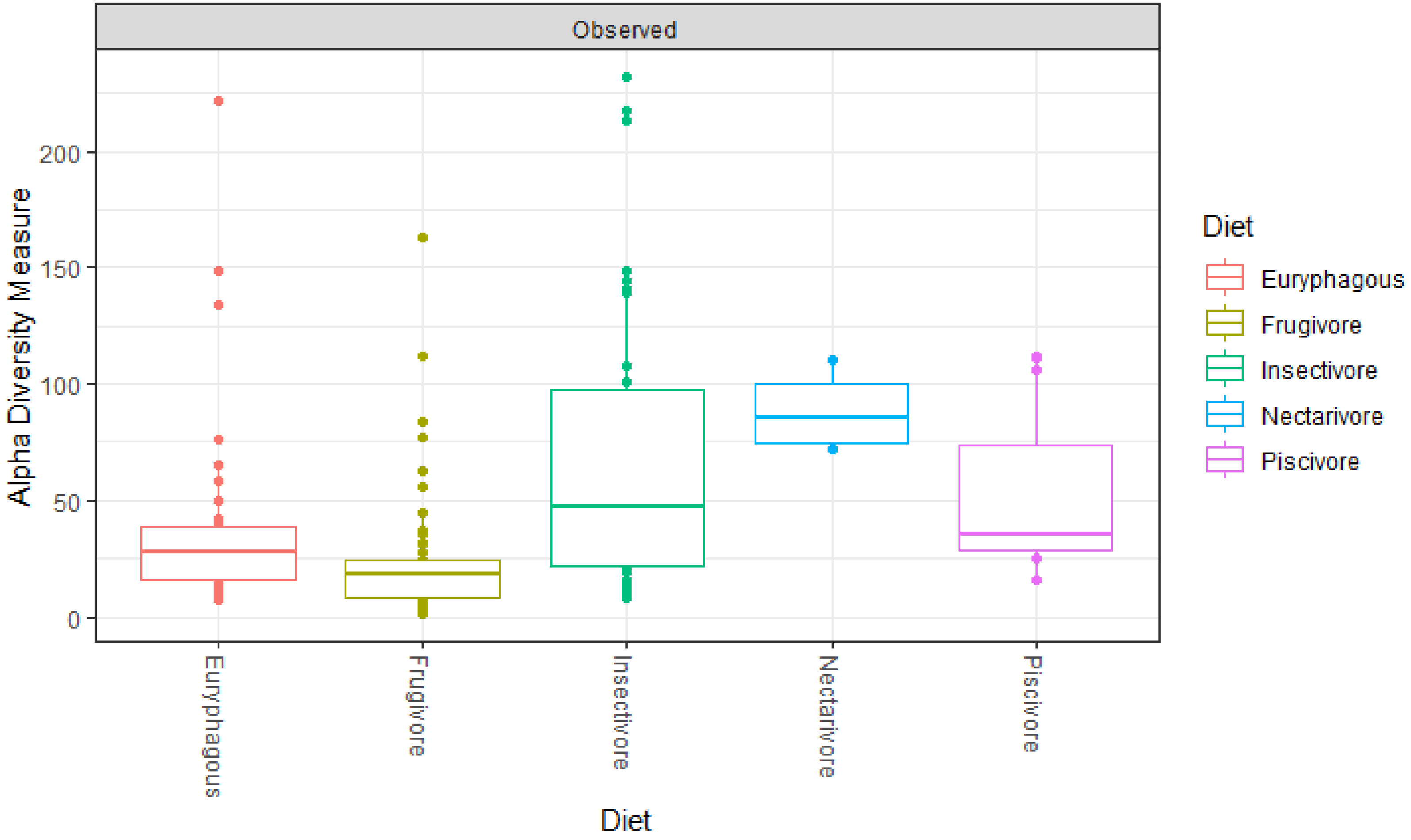
Rarefaction Curve



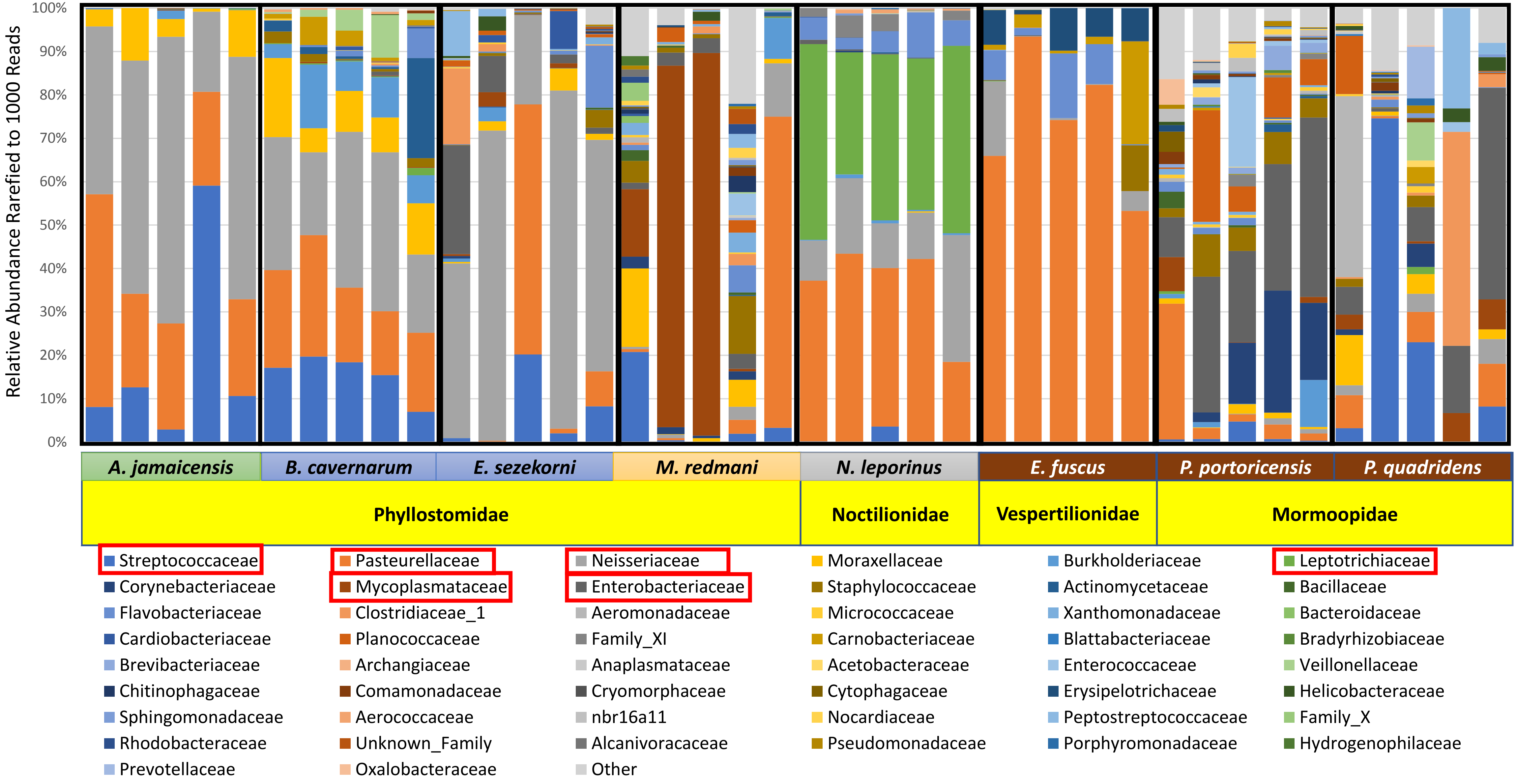
Alpha Diversity-Oral Samples



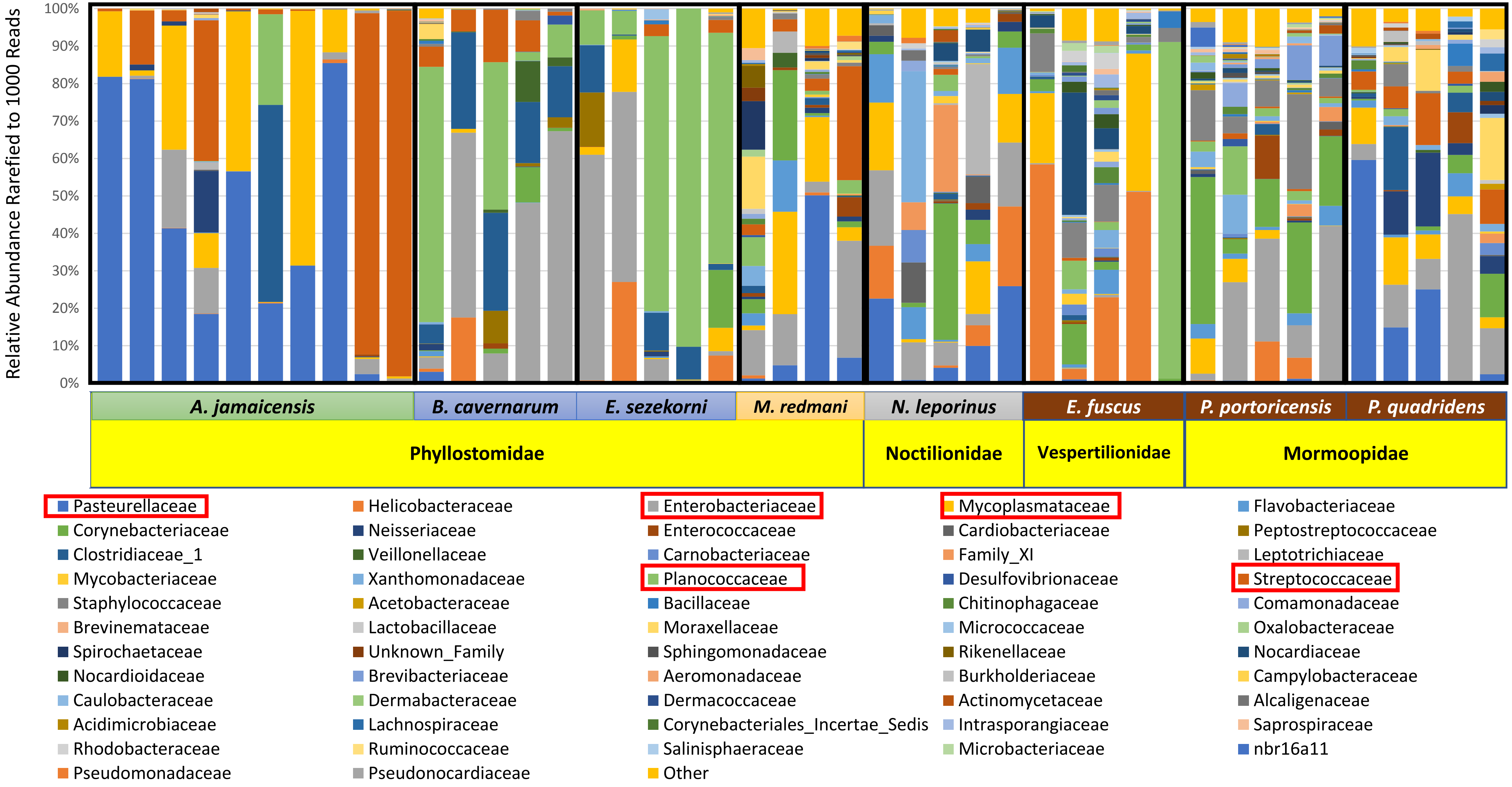
Alpha Diversity-Rectal Samples



Family Level Microbial Community Relative Abundance of Oral Samples



Family Level Microbial Community Relative Abundance of Rectal Samples



Artibeus jamaicensis- ASVs across three caves

Taxa/ASVs	**Aguas oral 22**	**Mata oral 14**	**Rio oral 15**	**Aguas rectal 23**	**Mata rectal 19**	**Rio rectal 19**
Haemophilus	22	14	15	13	15	9
Kingella	22	14	15	14	10	10
Aggregatibacter	22	10	14	4	3	0
Pasteurellaceae	6	2	4	21	18	16
Moraxella	21	14	15	7	8	8
Enterobacteriaceae	2	2	1	9	8	4
Escherichia/Shigella	4	1	3	10	2	11
Lysinibacillus	3	1	0	9	4	10

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