A Tale of 3 Mummies: A Microbiome Analysis of Life in the Peruvian Andes 1,000 Years Ago

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- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
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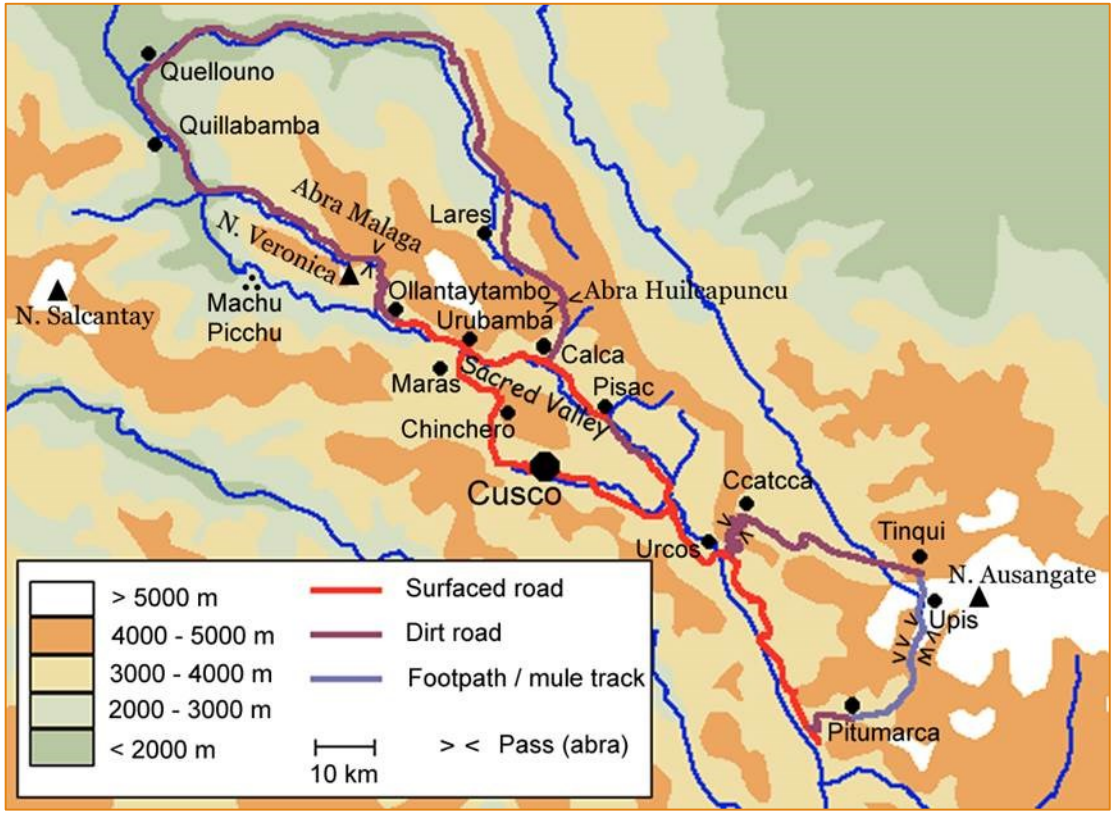
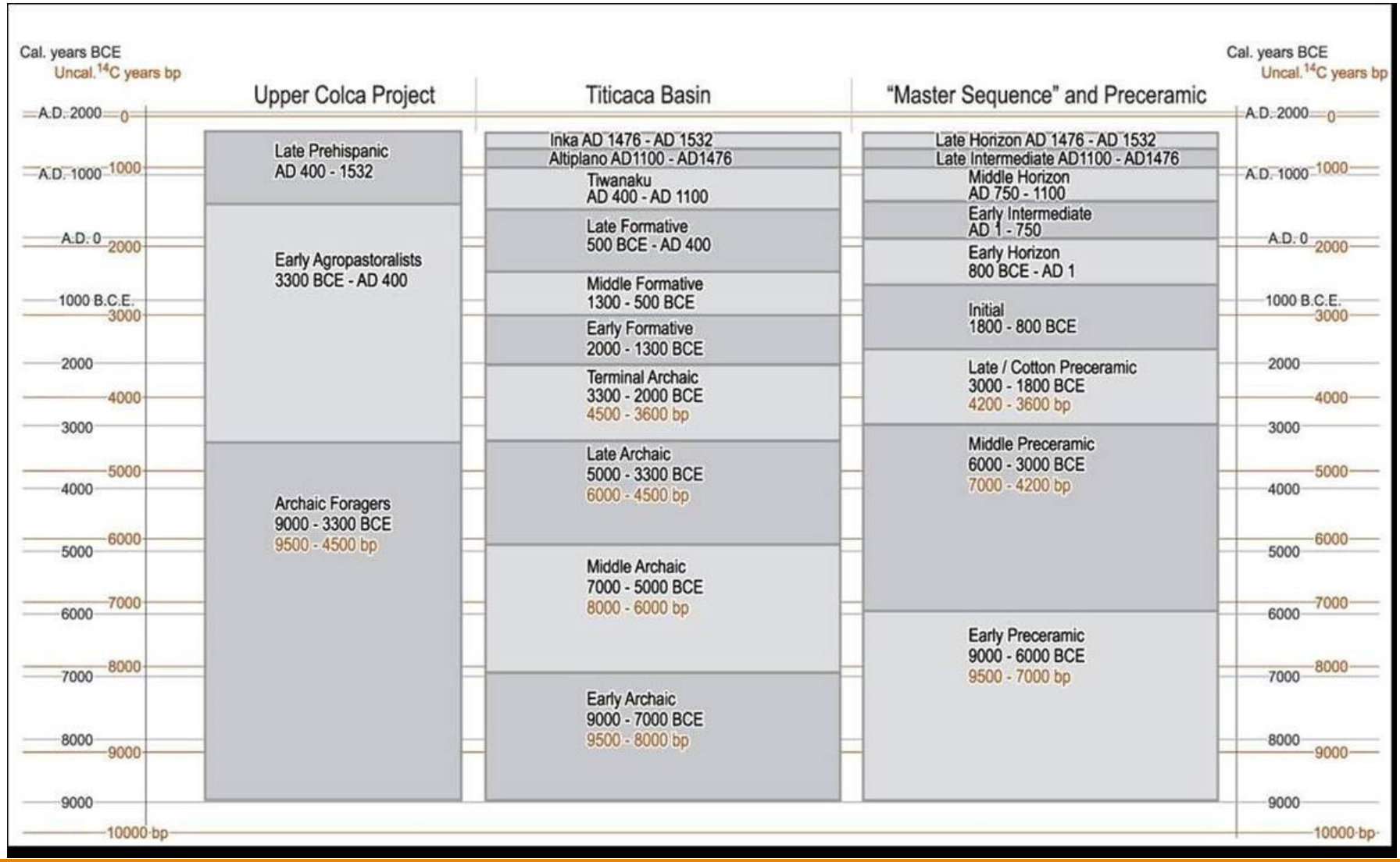
Cuzco, Peru — Geography



Collaborators and Funding

- Tasha Santiago-Rodriguez: Sample processing, data analysis, and MOST everything else
- Franco Rollo: My friend and gatekeeper
- Gino Fornaciari: Mummy Czar
- Stefania Luciani: Ancient DNA isolation
- Isolina Marota: Continue Franco Rollo's legacy (and lab)
- Gary Toranzos: Public health emphasis

Selected Andean Chronologies



Inca Accomplishments

- Excellent farmers, builders, and managers
- Roads and aqueducts
  - Built > 19,000 miles of roads (over mountains)
  - Built canals and aqueducts to carry water to dry areas
- Advancements in medicine and how to make them – use of medicinal plants
- Arts and science
- Accomplished travelers

Inca Diet

- Crops cultivated across the Inca Empire included quinoa, maize, beans, grains, potatoes, sweet potatoes, peppers, tomatoes, peanuts, cashews, squash, cucumber, cotton, carob, and avocado
- Livestock was llama and alpaca herds; meat eaten as charqui
- Drank chicha a lot! (so I'm told)

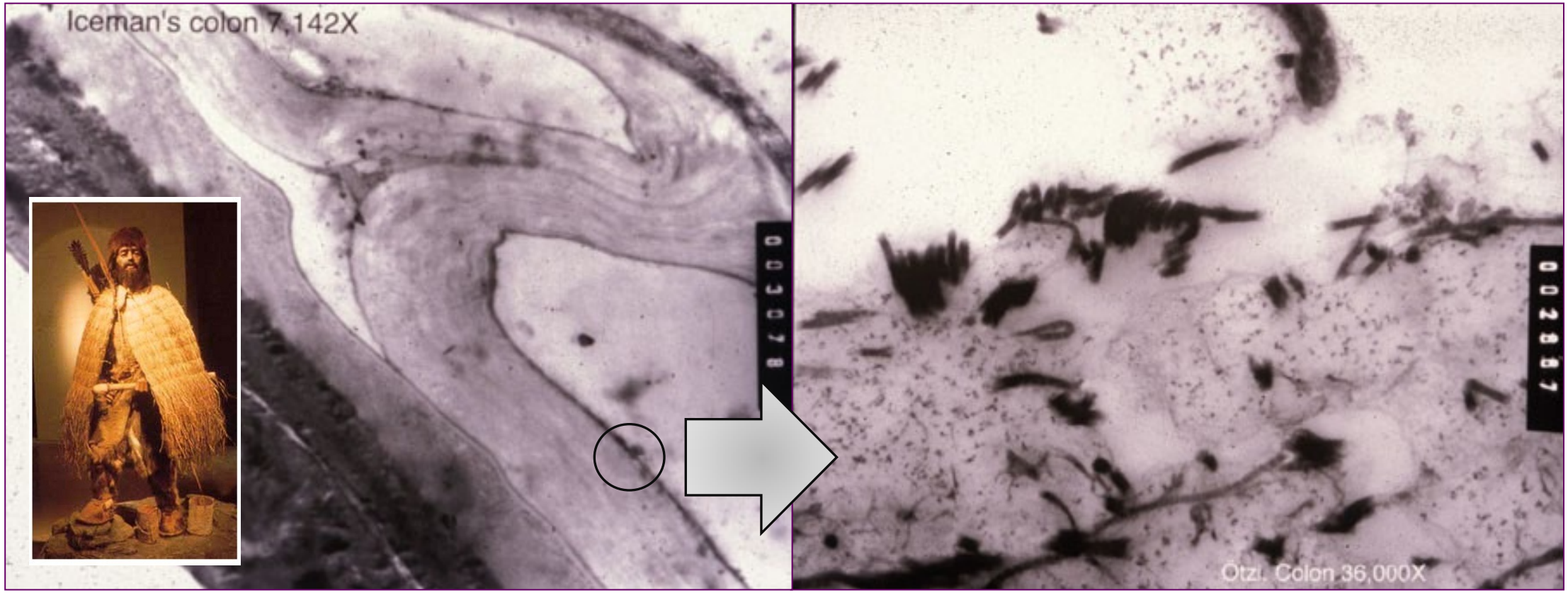
Inca qollqa (storehouses) used for storing grain and other foodstuffs

Questions

- Suitability for analysis
- Microbiome/metagenome structure
  - Microbial diversity
  - Metabolic diversity
  - Correlations?
- Medical and cultural aspects
  - AMR/MDR genotypes
  - Infectious diseases
  - Diet

What Got It All Started!

Iceman colon, circa 1998



Cast of Characters

The Protagonists: Three Natural Mummies

FI3

14th Century male
25-30 years old
Good preservation
Leishmaniasis

FI9

11th Century female
18-23 years old
Well preserved
Cardiomegaly

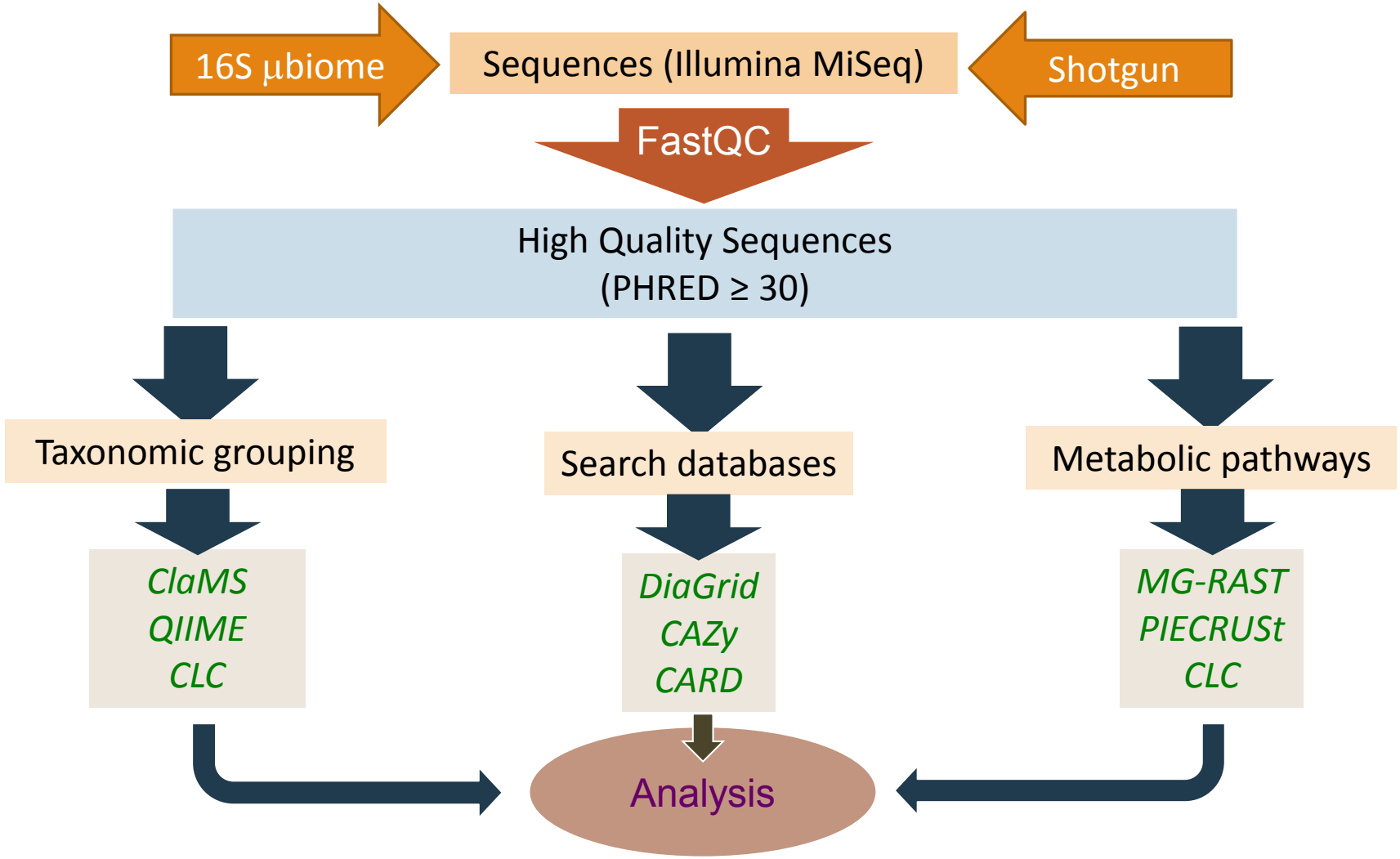
FI12

14th Century female
20-25 years old
Good preservation
Bronchopneumonia

Supporting Cast

	Medici Family			Aragonese Family		
Sample ID	NASD3	NASD12	CM40	NASD22	NADS27	NASD29
Personage	**Francesco I**	**Ferdinando I**	**Gian Gastone**	**Ferrante I d'Aragona**	**Luigi Carafa**	**Unknown**
Lifespan	1541-1587	1549-1601	1671-1737	1431-1494	1511-1576	16th Century
Comments	Grand Duke	Grand Duke	Gran Duke	King of Naples	Prince of Stigliano	¿?
COD	Malaria	Tertian fever	Small Pox	Colon carcinoma	Natural causes	Cirrhosis

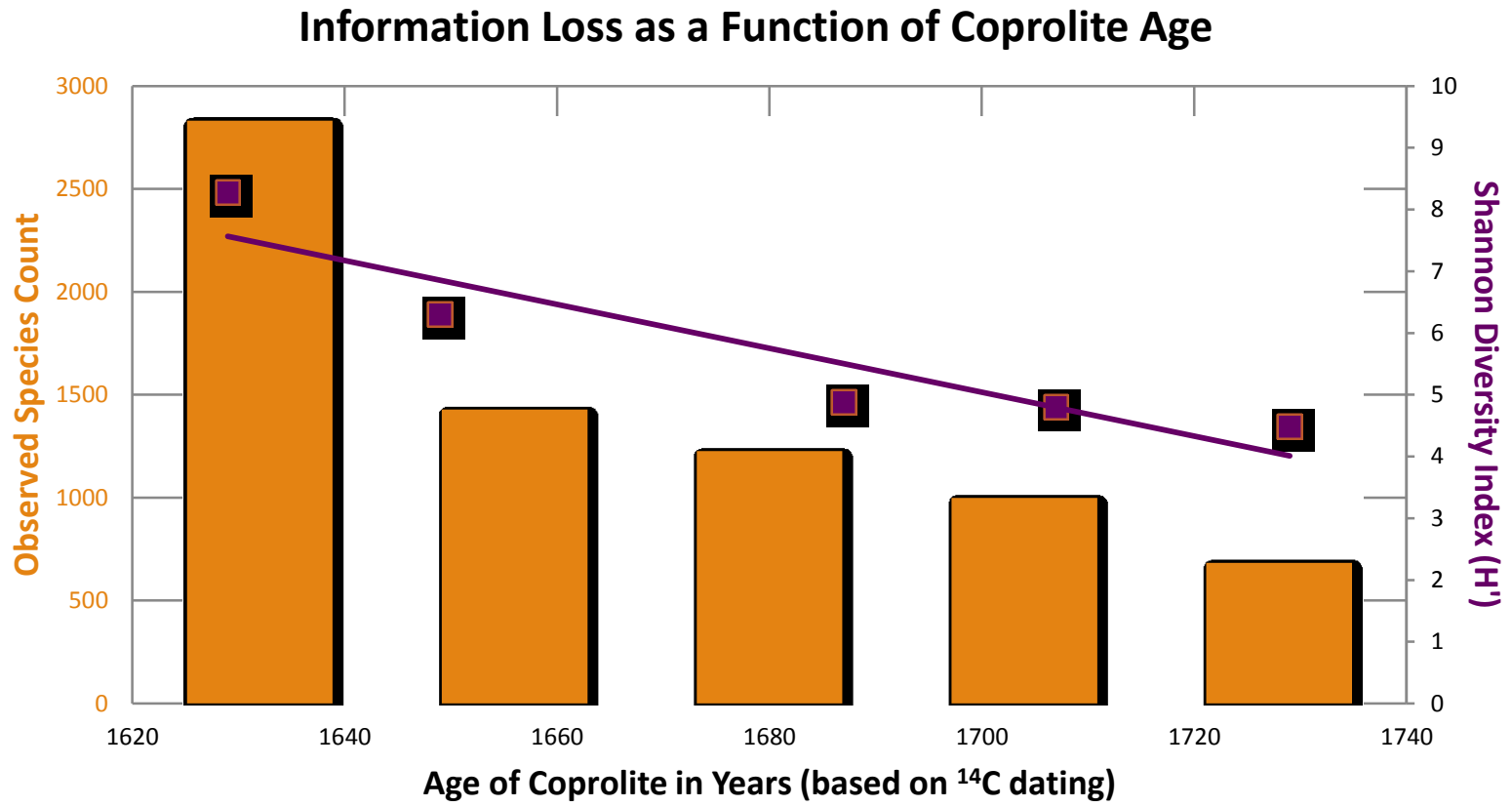
Analysis Workflow



QC/QA of Sequences

ANCIENT DNA MAY BE DAMAGED – SEQUENCES OF POOR QUALITY

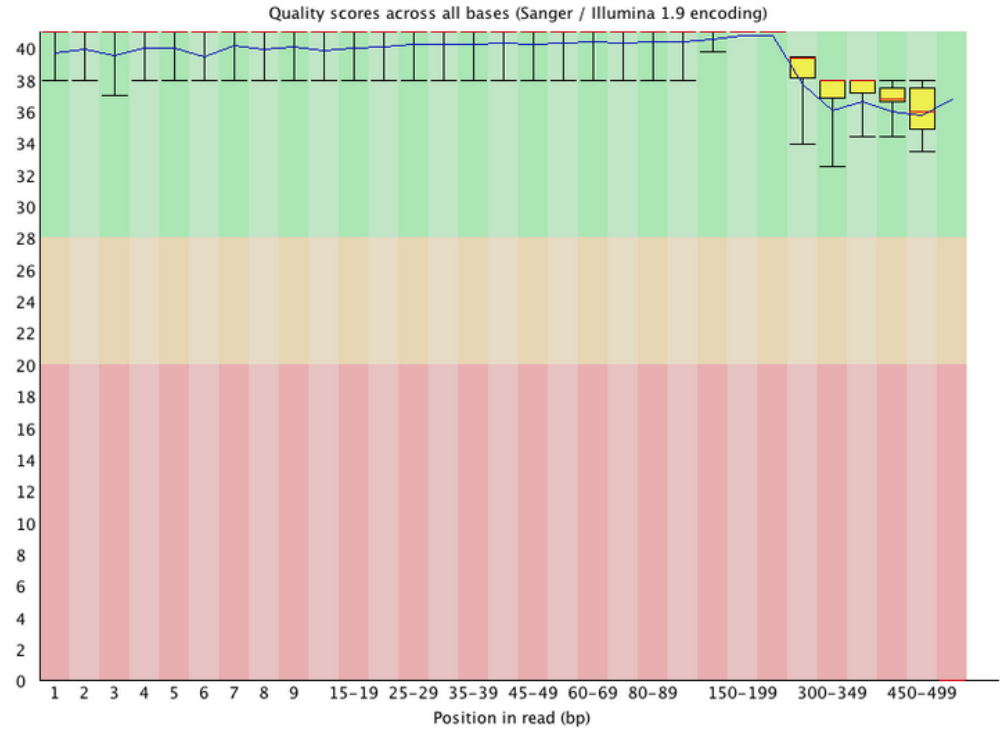
Genetic Information is Lost Over Time



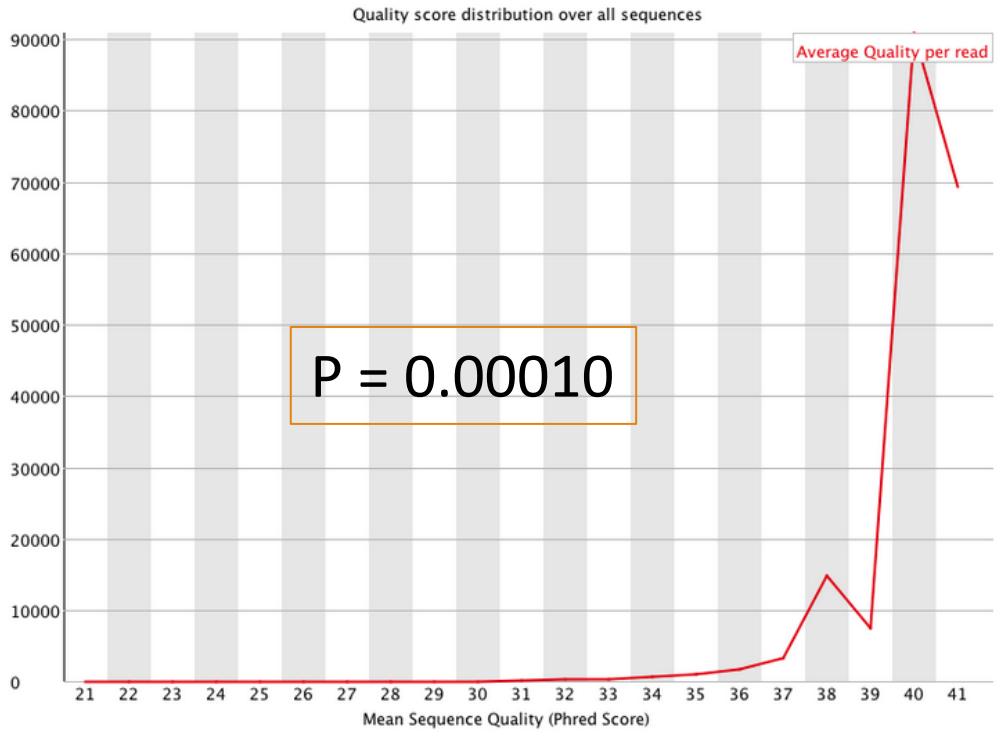
FastQC Results

Assurance of good NGS results

Per base sequence quality



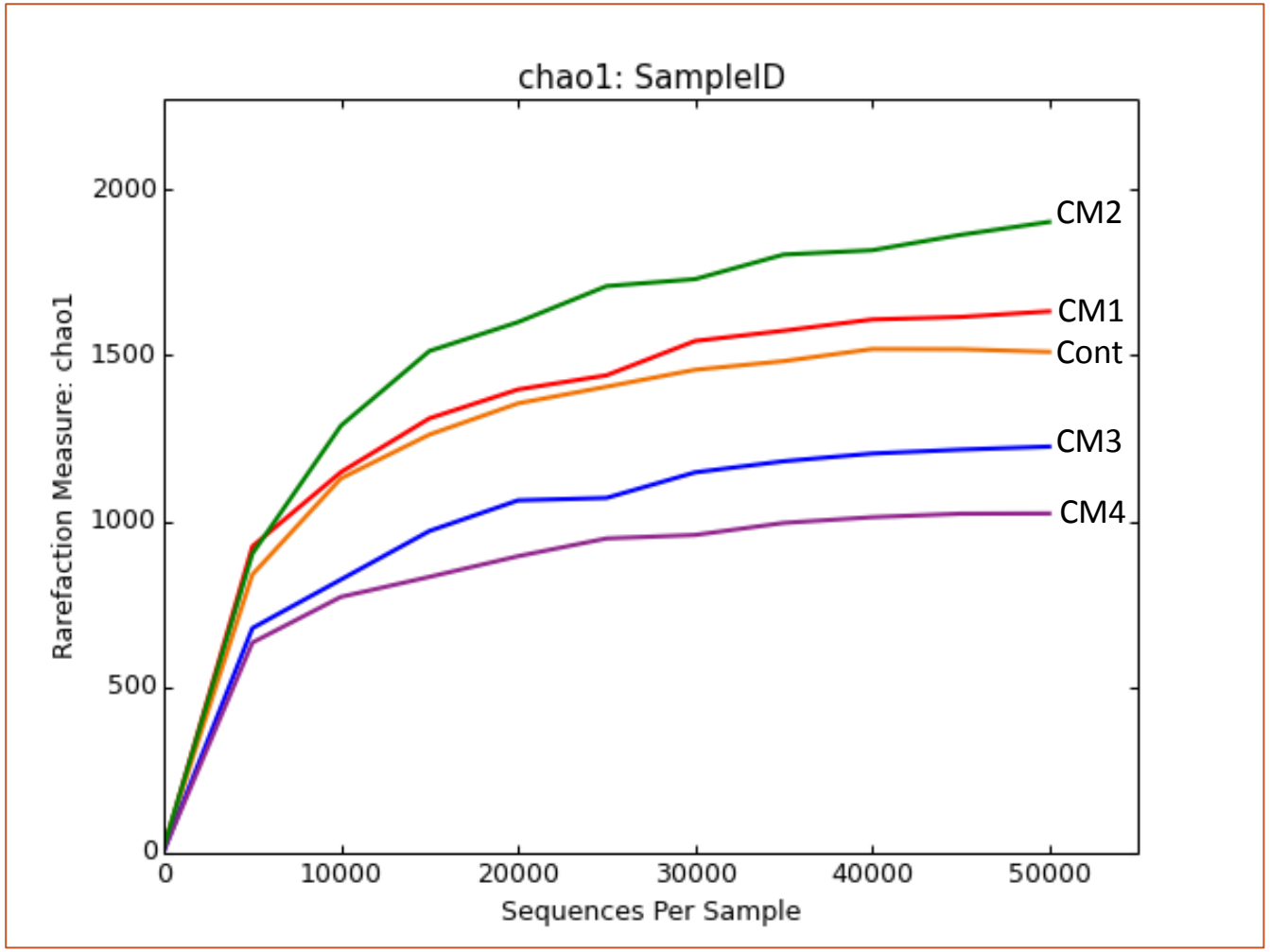
Per sequence quality scores



Sampling Depth

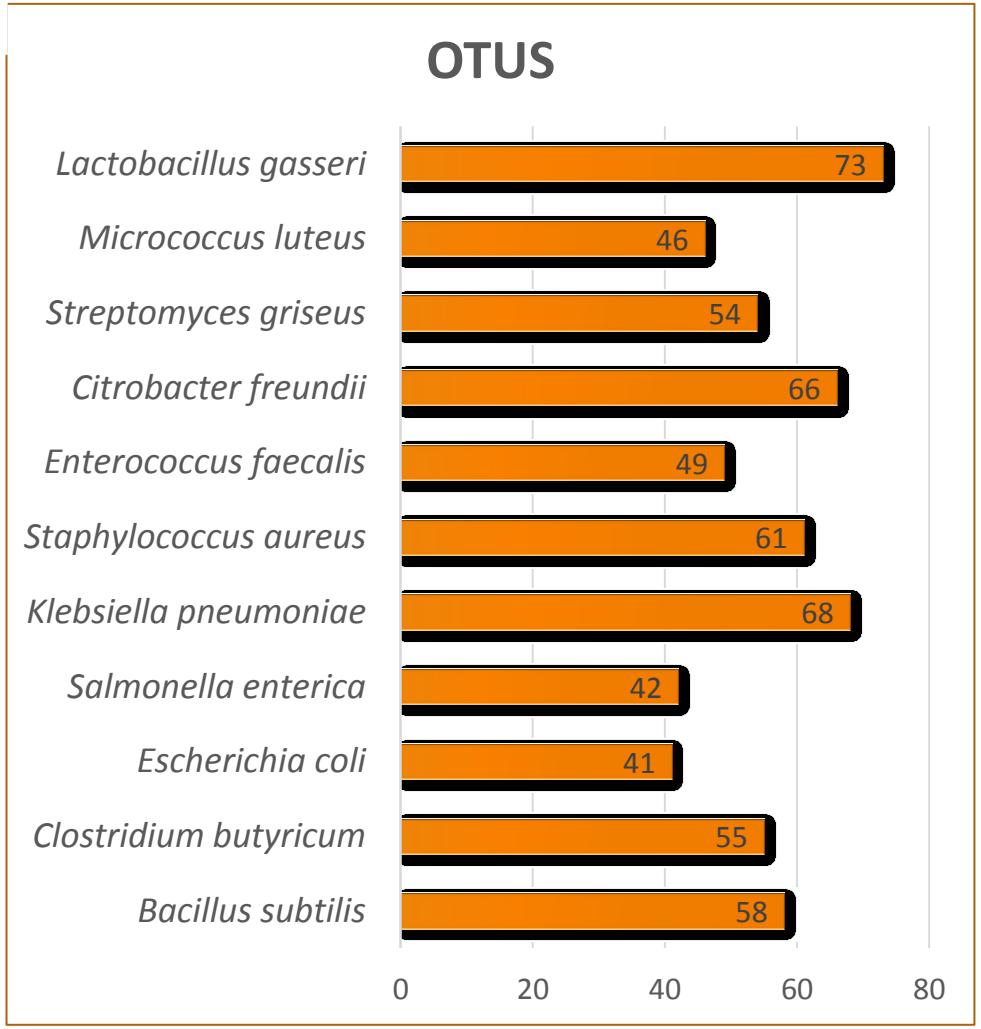
Rarefaction Curves

Assurance of sufficient sample size



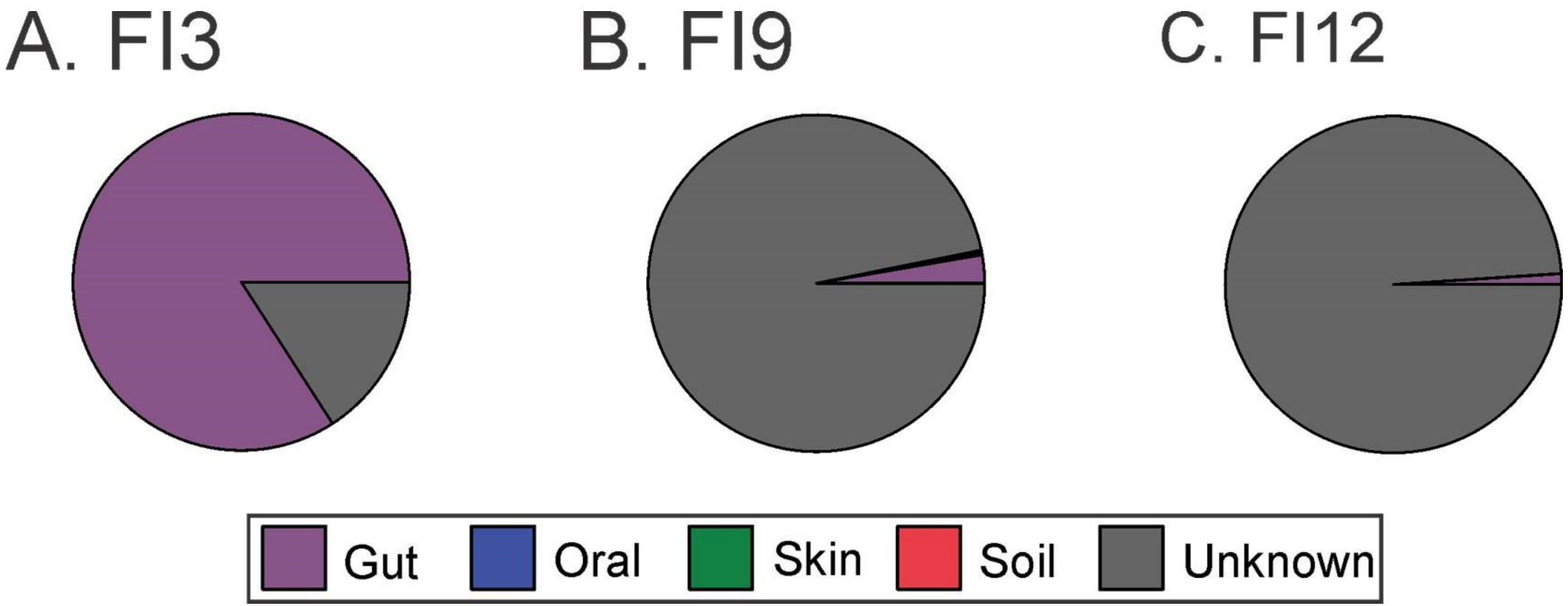
Mock Community for Assessing DNA Extraction

Taxon	ATCC® No.	Inoculum CFU/mL
Bacillus subtilis	6501™	1.00E+06
Clostridium butyricum	3627™	1.00E+06
Escherichia coli	11775™	1.00E+06
Salmonella enterica	8388™	1.00E+06
Klebsiella pneumoniae	13883™	1.00E+06
Staphylococcus aureus	12600™	1.00E+06
Enterococcus faecalis	19433™	1.00E+06
Citrobacter freundii	8090™	1.00E+06
Streptomyces griseus	23345™	1.00E+06
Micrococcus luteus	4698™	1.00E+06
Lactobacillus gasseri	33323™	1.00E+06



Source Tracker Results (from QIIME 1.9.0)

Assessing Environmental Contamination



Mummy FI9

Peruvian Mummy from the Museum of Anthropology

- A Peruvian natural mummy of a young woman 20±3 years old from Cuzco (Peru), housed in the Museum of Anthropology and Ethnology of Florence, was autopsied
- The funerary equipment is typical of the Andean highlands, Late Intermediate Period (1000-1476 A.D.)

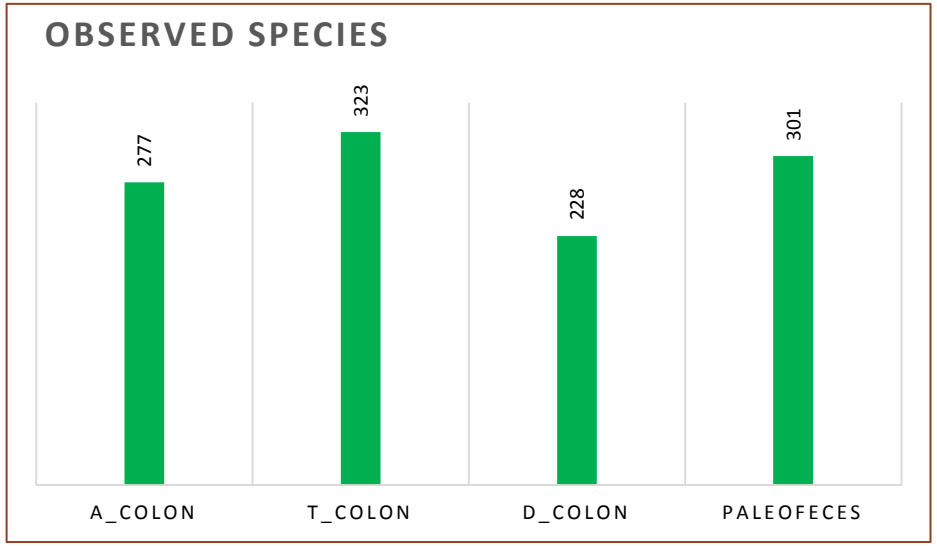
After preliminary X-rays showing good preservation of the body, the mummy was then autopsied through the posterior thorax to avoid damage

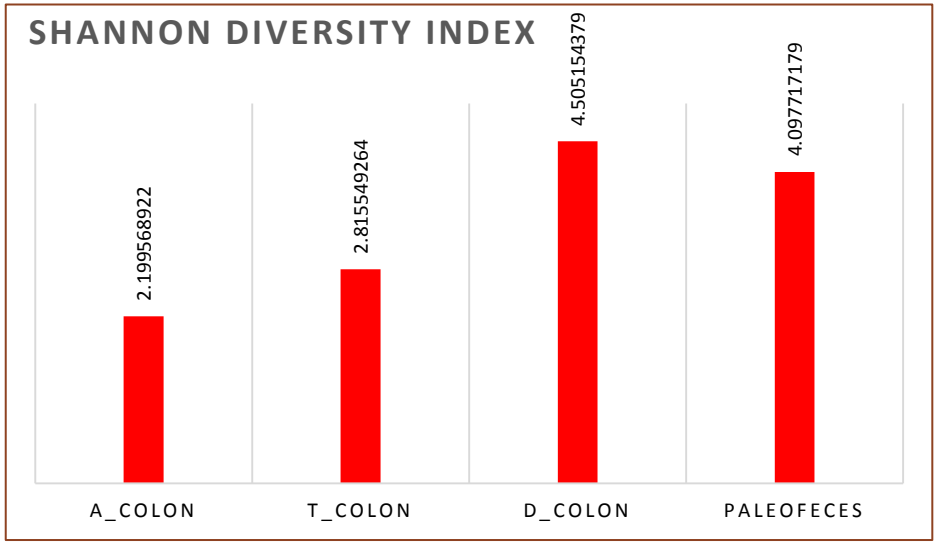
The mummy in its shroud, in strict fetal position

LL X-ray of the mummy

Autopsy by posterior breach

Alpha Diversity: FI9



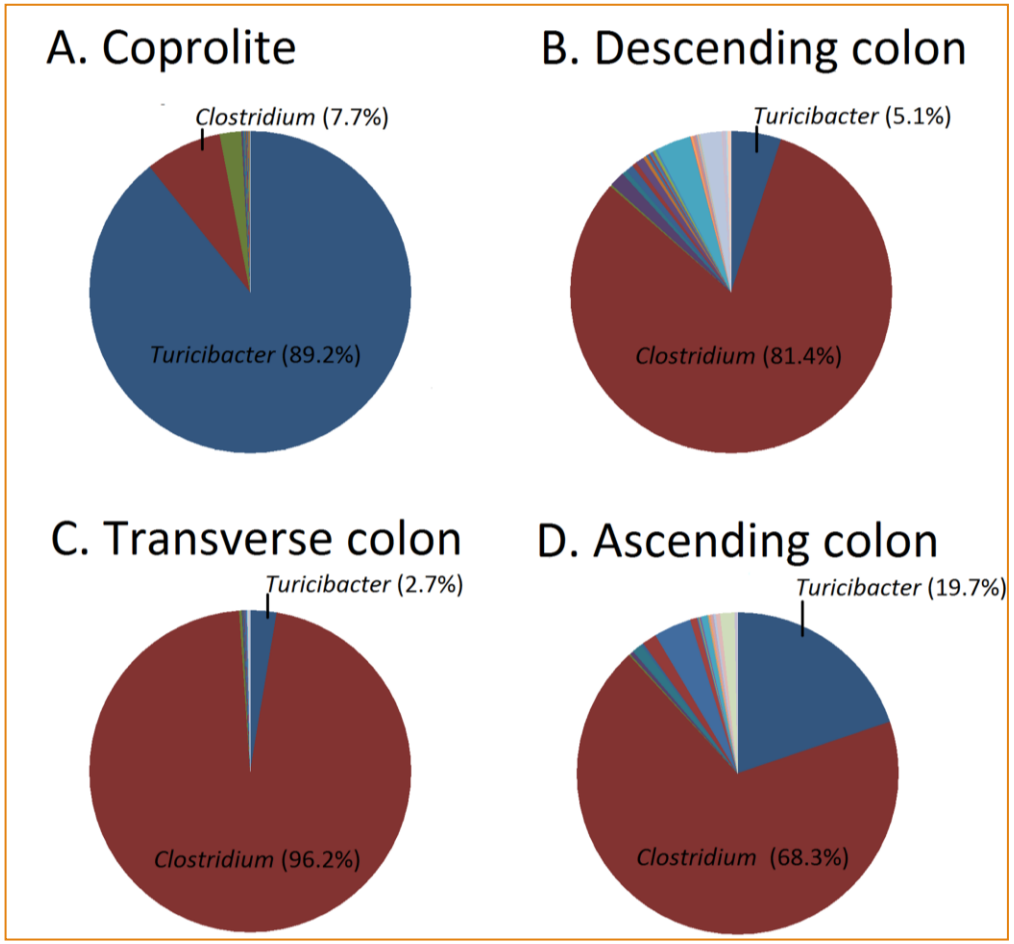


Taxonomic Diversity

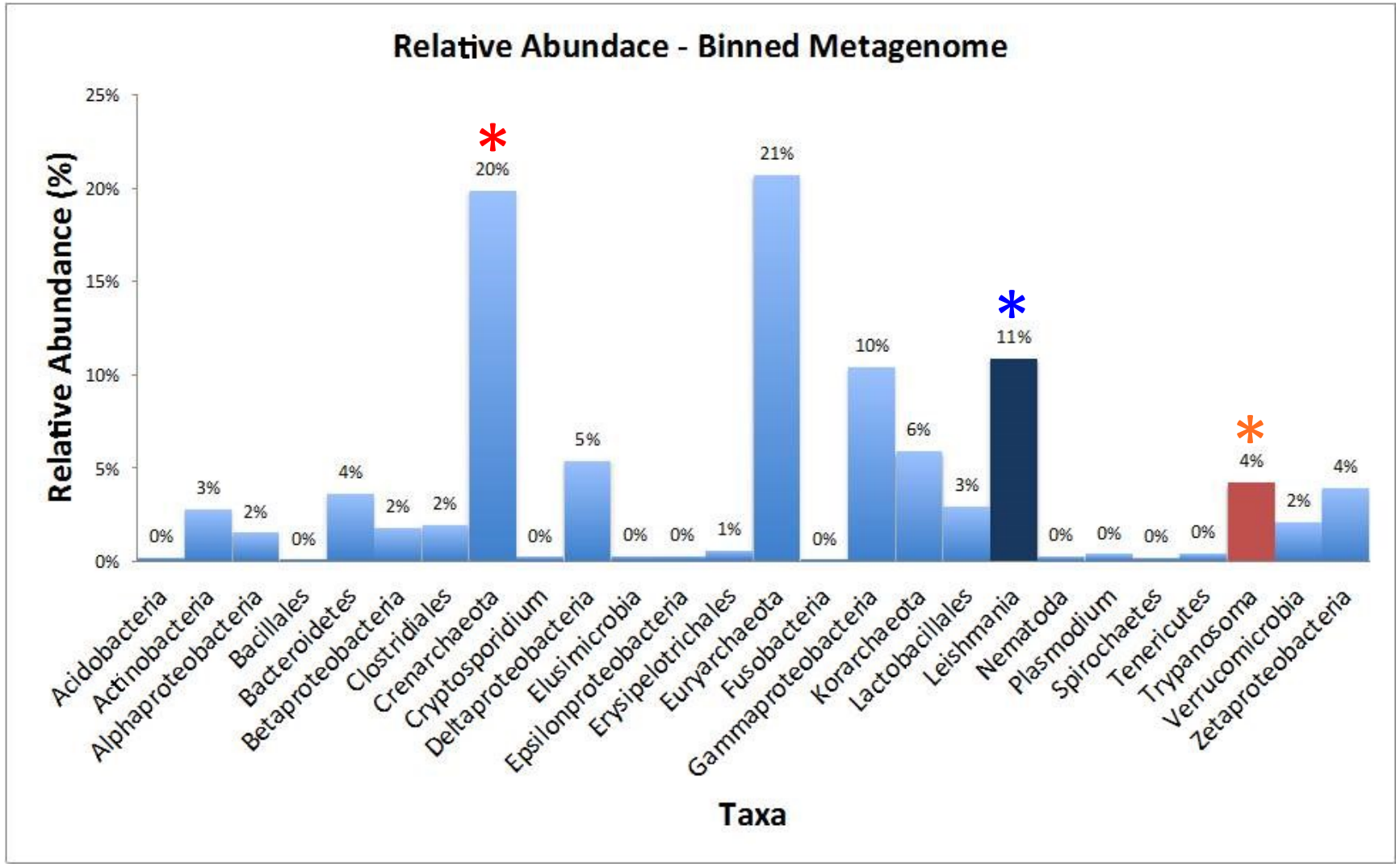
Circa 1999: TRFL-P + clone and sequence

BLAST ID	No. Clones
Clostridium botulinum E	18
Clostridium botulinum G	2
Clostridium perfringens	4
Clostridium sp.	6
Clostridium algidicarnis	1
Eubacterium pectinii	2

Circa 2014: NGS + QIIME



Metagenome Binning (ClaMS) – FI9



Paleopathology of Peruvian Mummies

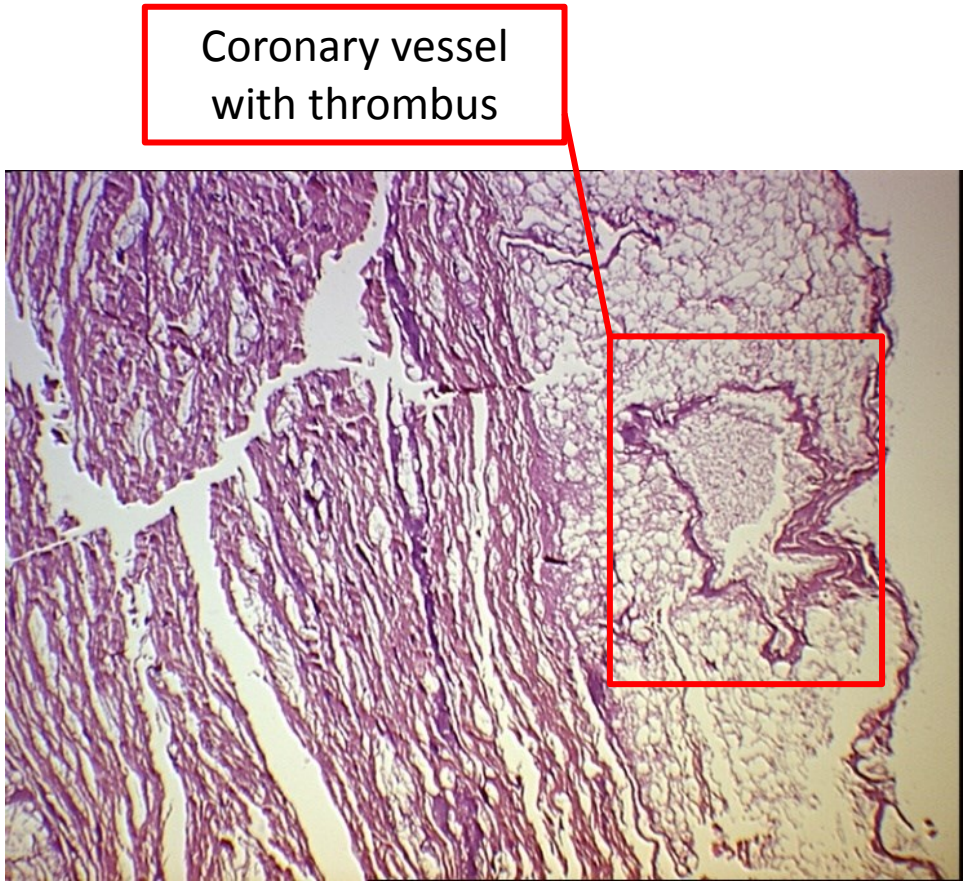
Macroscopically, we (Gino) found a megavisceral syndrome in the form of cardiomegaly, megaesophagus, gastric ectasia, and megacolon, with enormous amounts of feces

Megacolon with abundant feces

Cardiomegaly

- Light microscopy showed massive fat substitution, fibrosis of the myocardium, and coronary thrombosis

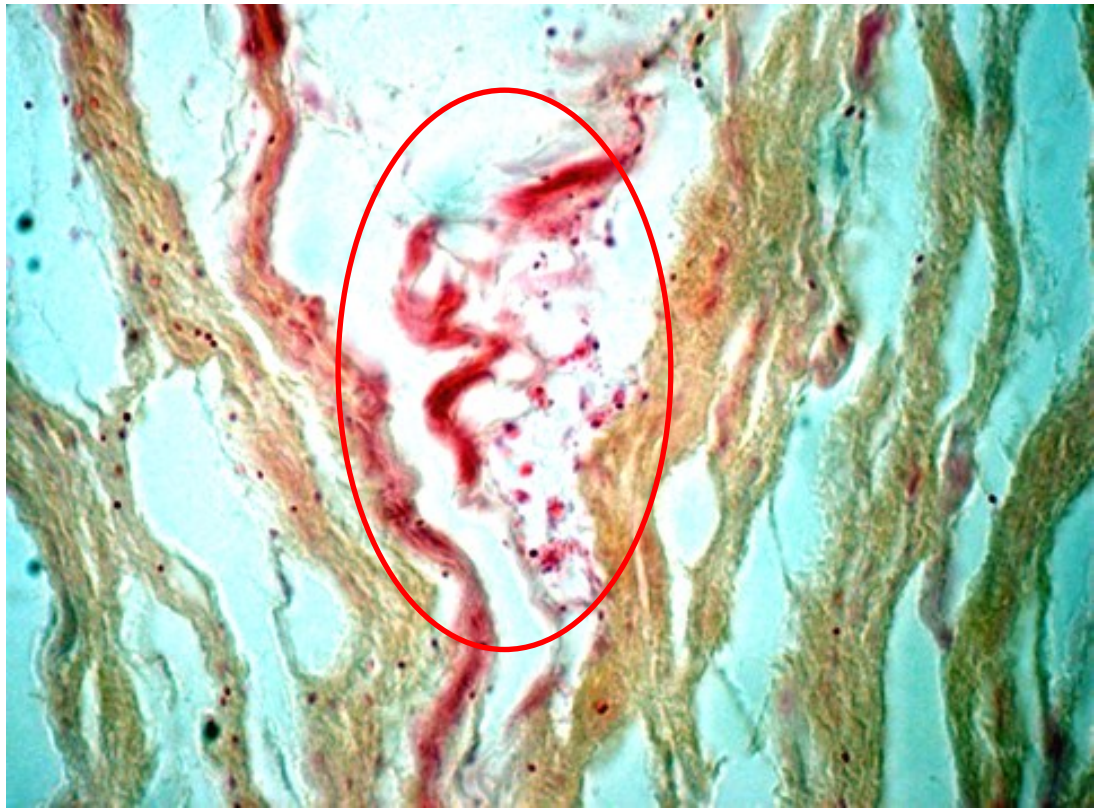
- The mummy mega-visceral syndrome strongly suggested a case of Chagas' disease, chronic phase, caused by the protozoan parasite *Trypanosoma cruzi*



Fat substitution and fibrosis of myocardium (EE, 50X)

- Giemsa staining evidenced rare roundish intratissutal nests, about 15-20 µm large, in the myocardium, containing several ovular formations (1-2 µm) with small nuclei

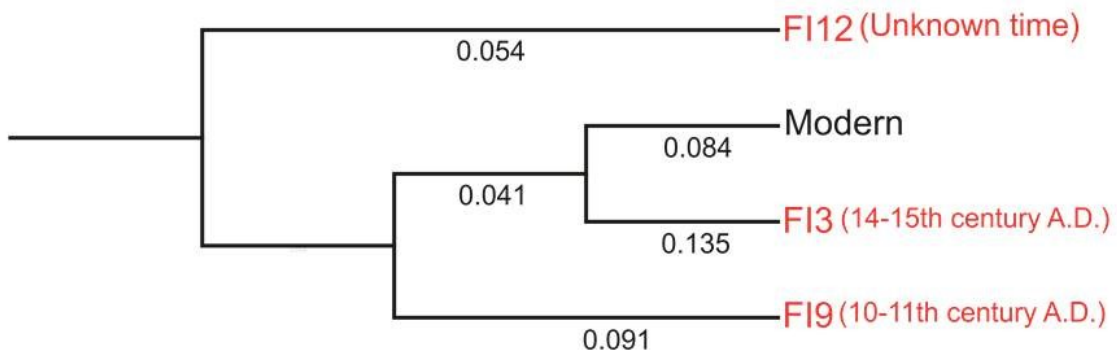
- The findings correspond morphologically to intratissutal nests of amastigotes of *Trypanosoma cruzi*



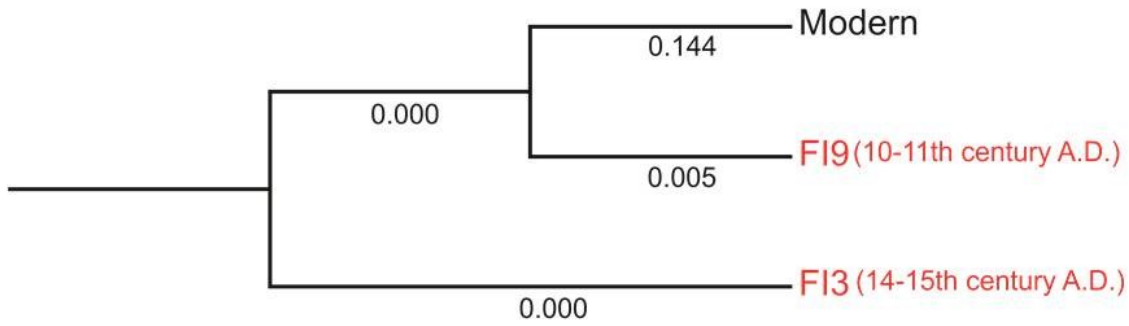
An intramyocardial nidus of amastigotes of *Trypanosoma cruzi* (Giemsa, 1000X)

Phylogenetic Studies – Mixed Infections

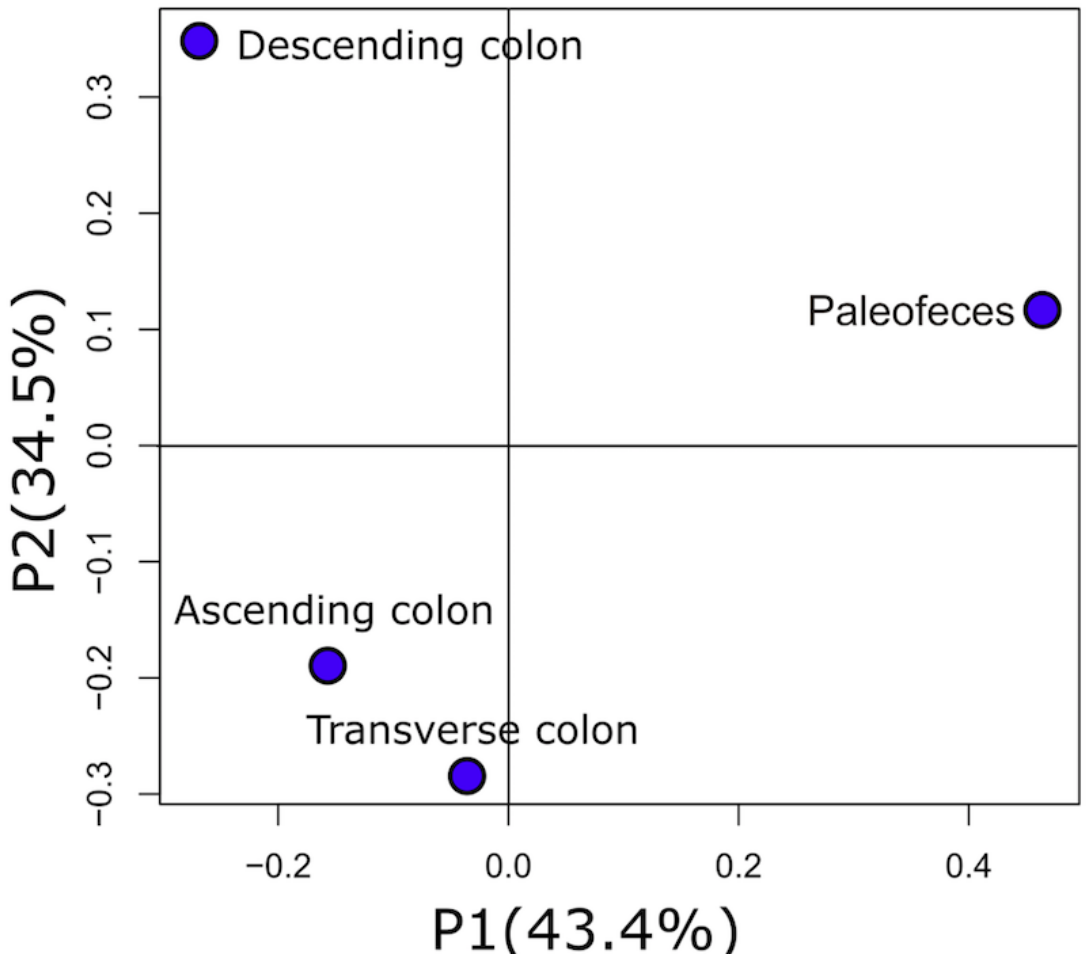
A. *Trypanosoma cruzi*

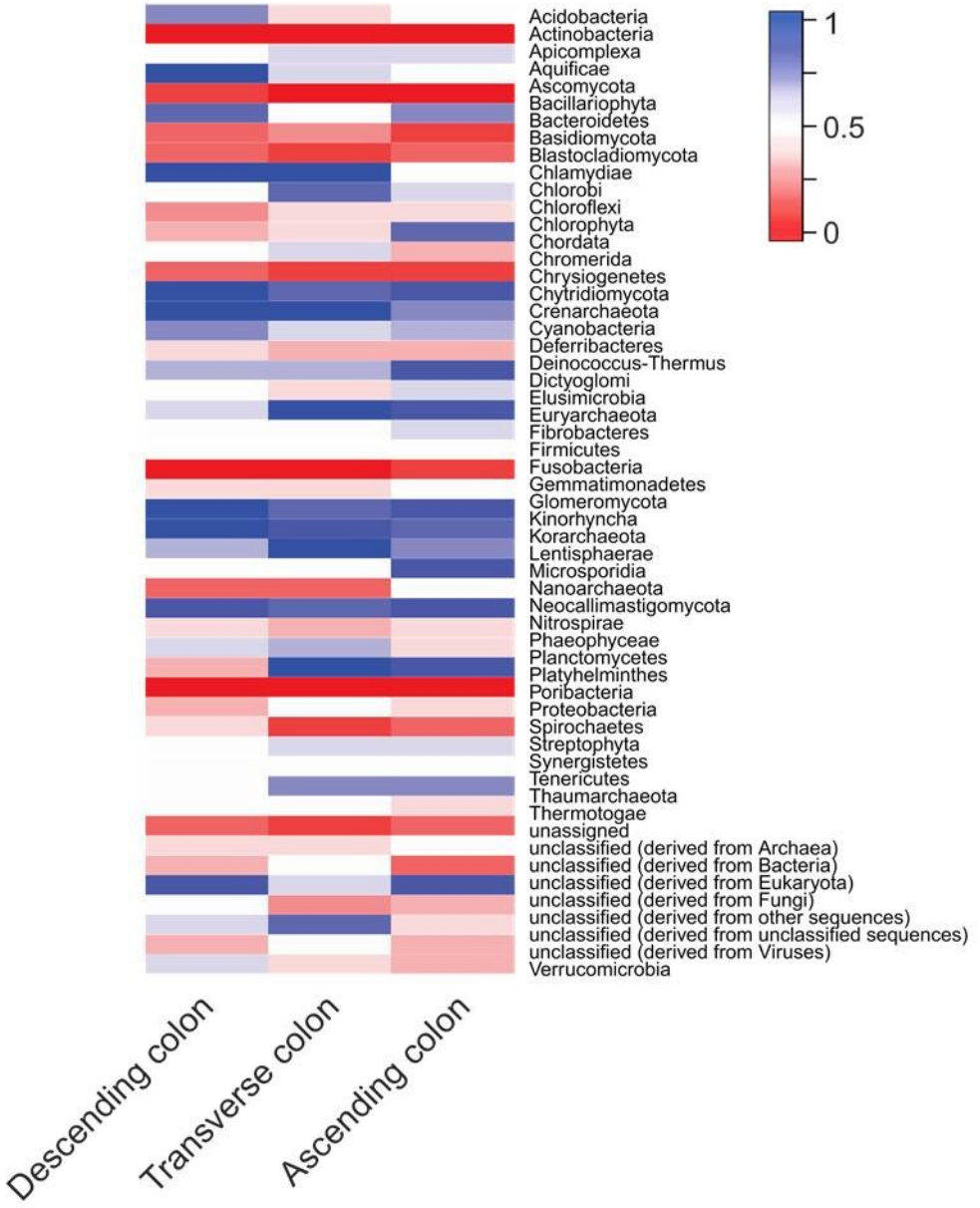


B. *Leishmania donovani*



PCoA and Heatmap: FI9



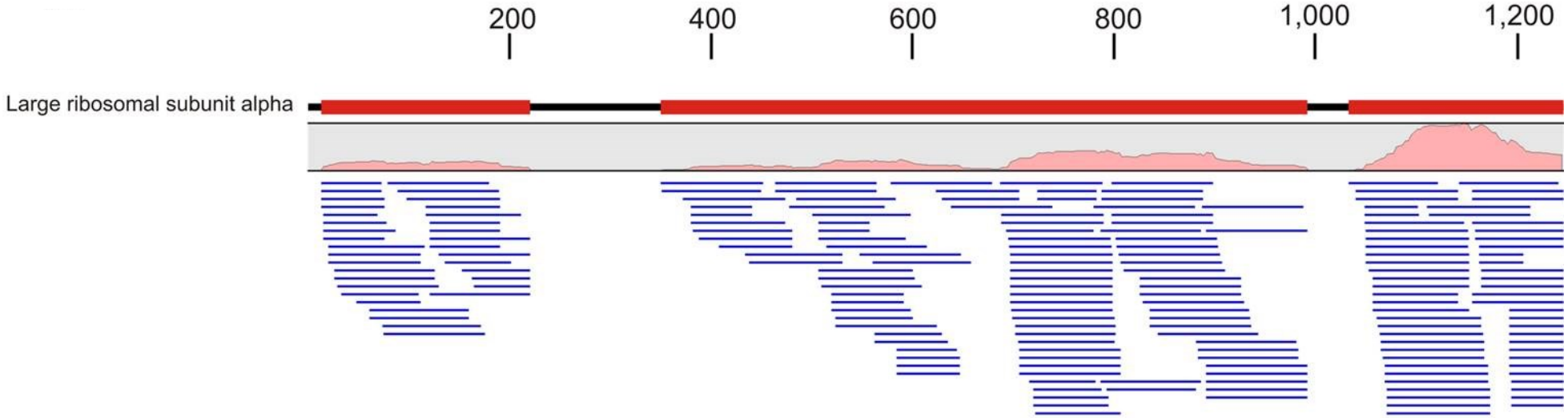


Principal Pathogens Detected

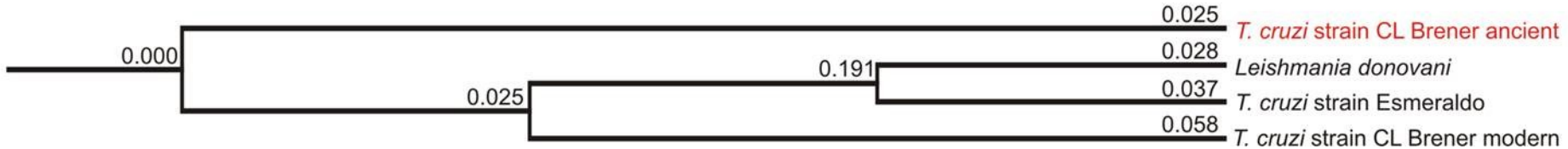
- *Trypanosoma cruzi* (Chagas disease)
- *Leishmania donovani* (Leishmaniasis)
- *Clostridium*
  - *botulinum*
  - *difficile*
- Human papillomavirus
  - HPV-21
  - HPV-49

Trypanosoma cruzi

A. *T. cruzi* large subunit



B. *Phylogenetic comparisons*

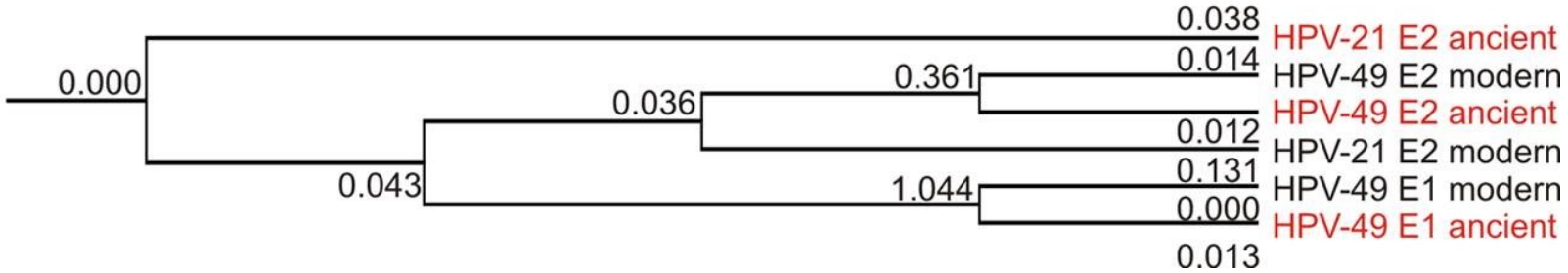


HPV – Sequence Homology and Phylogeny

A. Sequence homology

	1	2	3	4	5	6
HPV-21 E2 ancient	100.00	78.21	65.38	65.38	40.08	40.08
HPV-49 E2 modern	78.21	100.00	63.68	63.68	38.46	38.87
HPV-49 E2 ancient	65.38	63.68	100.00	97.44	34.82	35.22
HPV-21 E2 modern	65.38	63.68	97.44	100.00	35.63	36.03
HPV-49 E1 modern	40.08	38.46	34.82	35.63	100.00	98.76
HPV-49 E1 ancient	40.08	38.87	35.22	36.03	98.76	100.00

B. Phylogenetic relationships



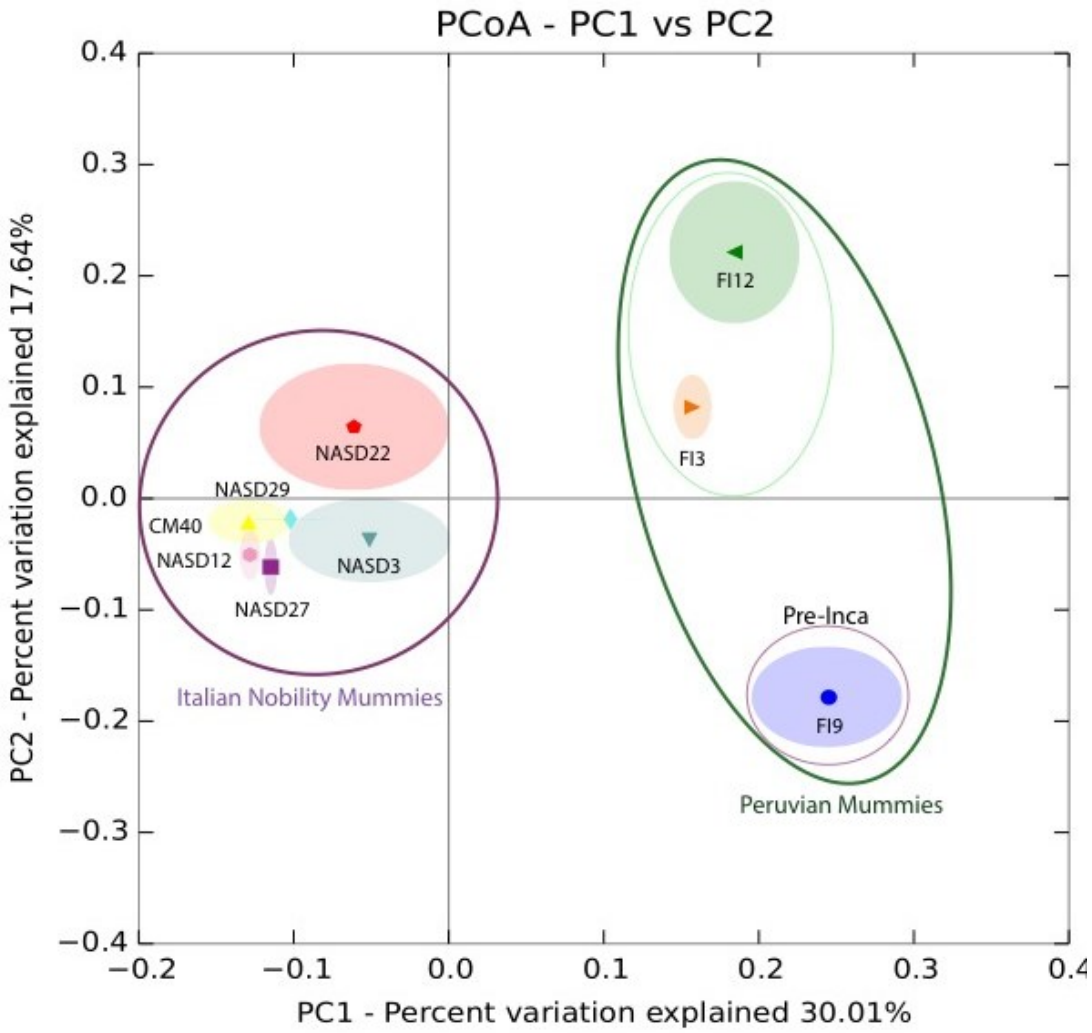
Diet and the Microbiome

Significant “Hits” in Peruvian Mummies WGS

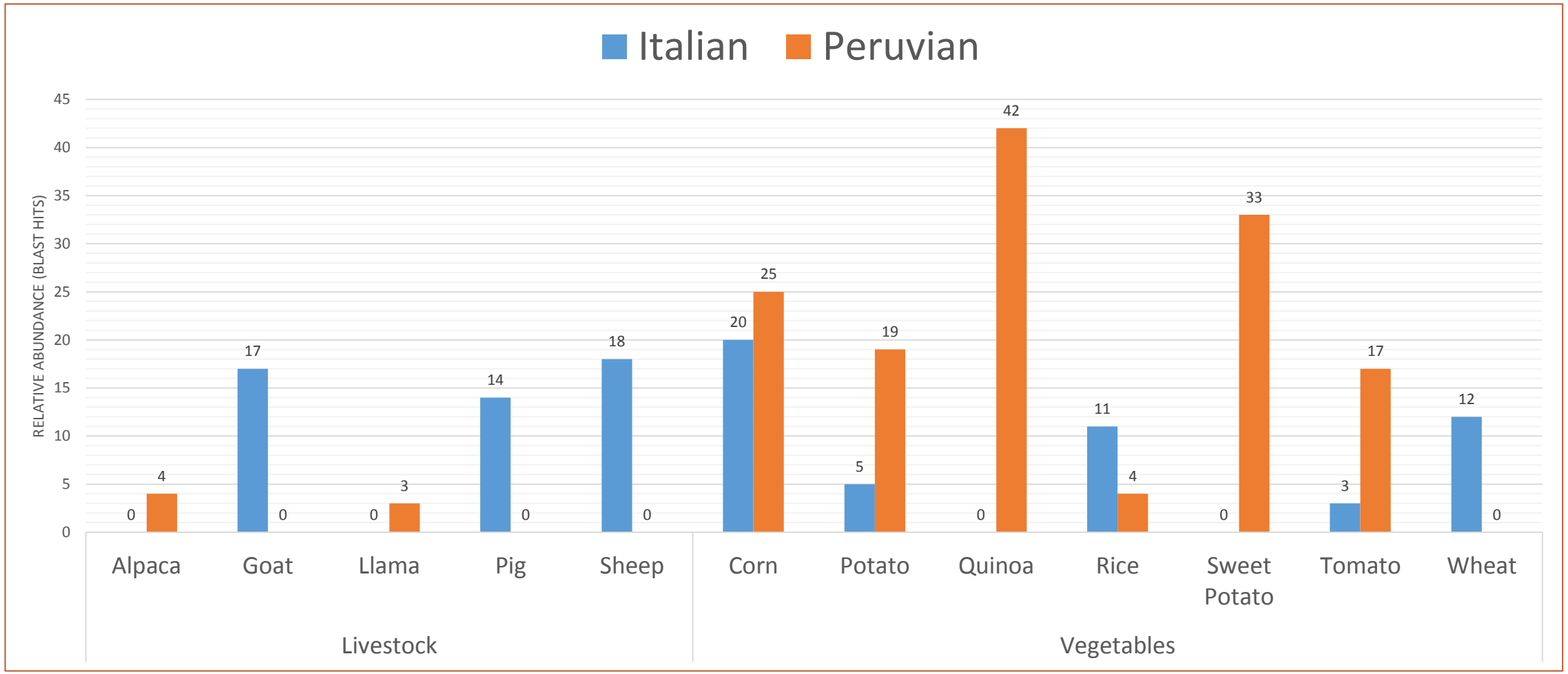
Crop plant	Number of hits
Coca	517
Beans	1122
Potatoes	865
Peanuts	912
Pepper	877
Tomatoes	922
Corn	589
Rice	435
Avocado	911
Quinoa	339
Sweet potatoes	673

Animals	Number of hits
Alpaca	618
Llama	459
Chimney swift	155
Bony fishes	377

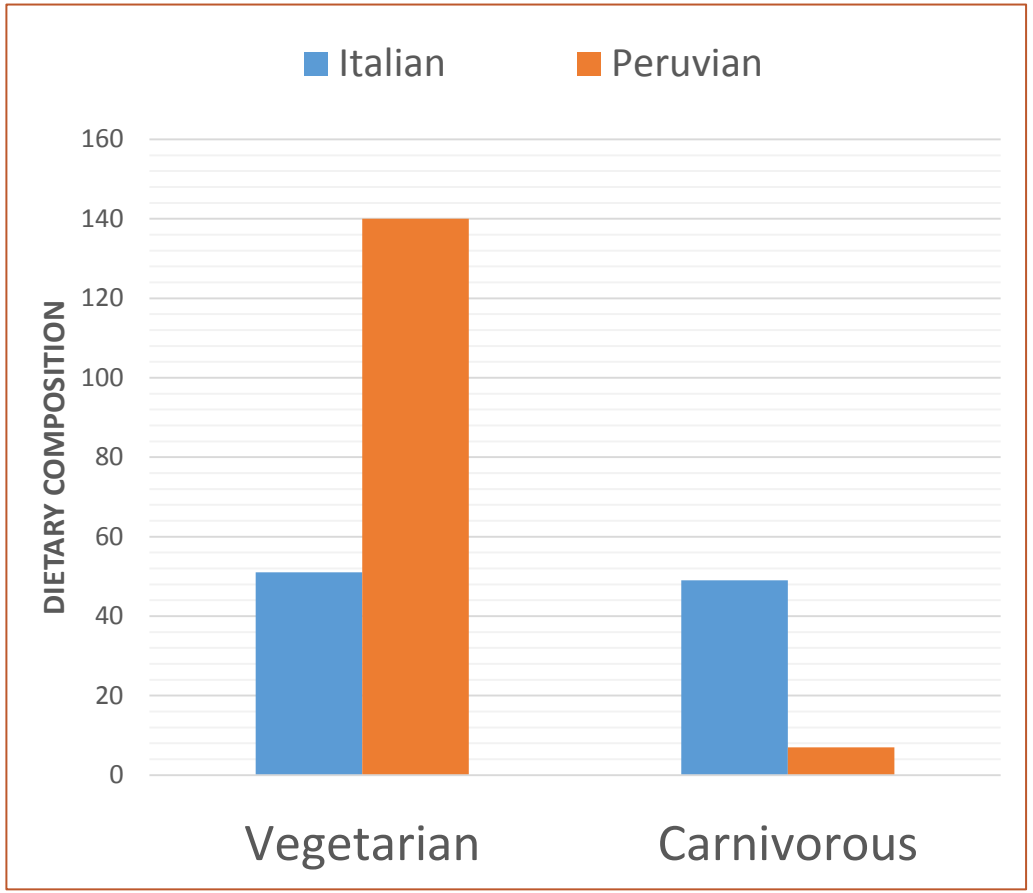
Gut Microbiome Comparisons: Two Diets

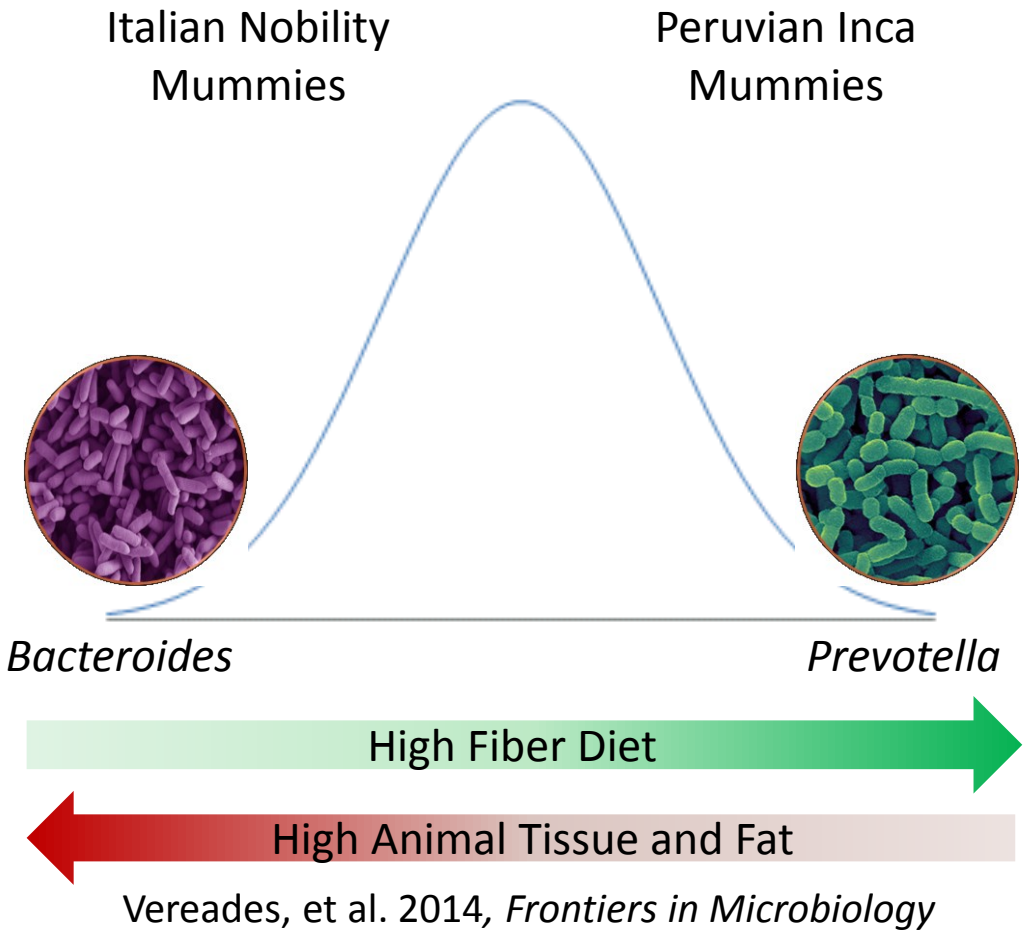


Principal Dietary Composition – Comparative Study



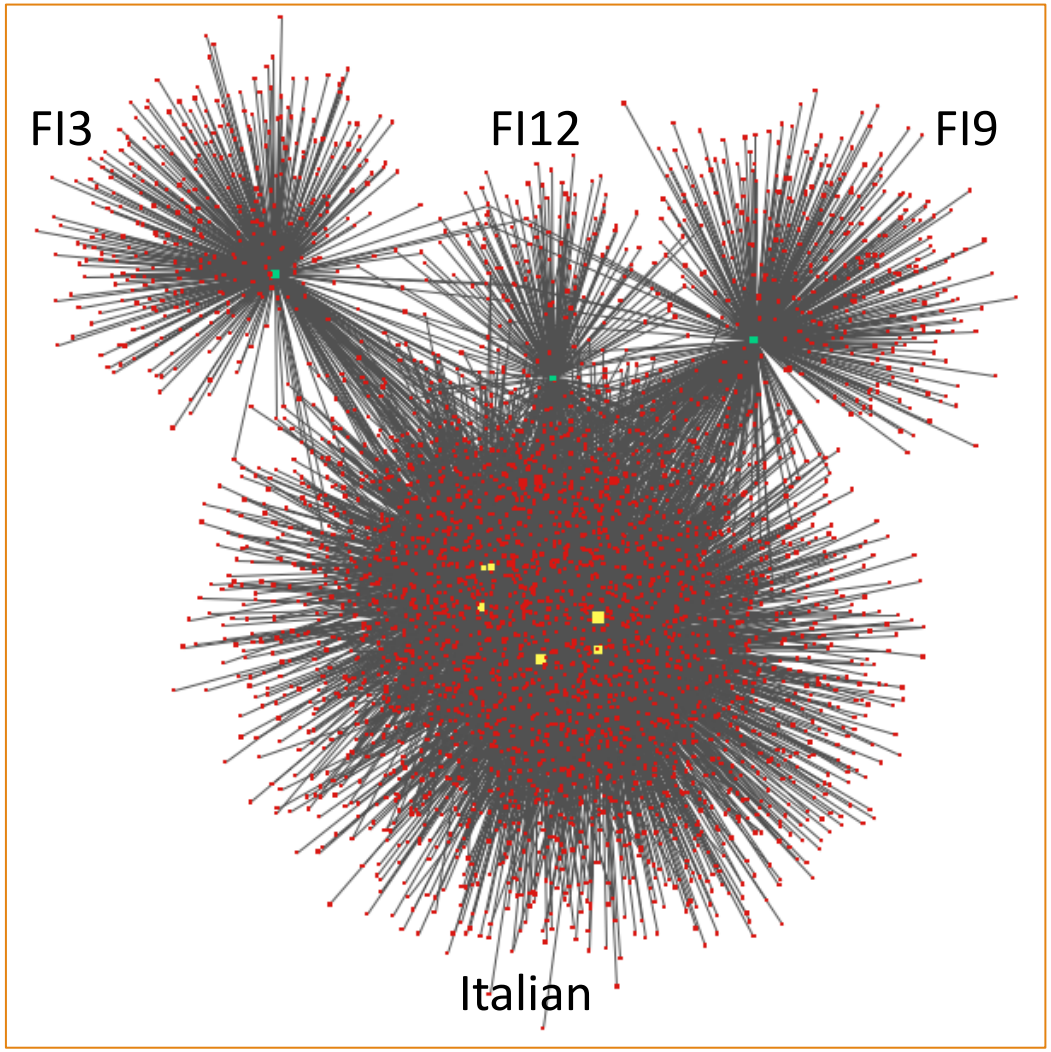
Dietary Impact on Microbial Prevalence

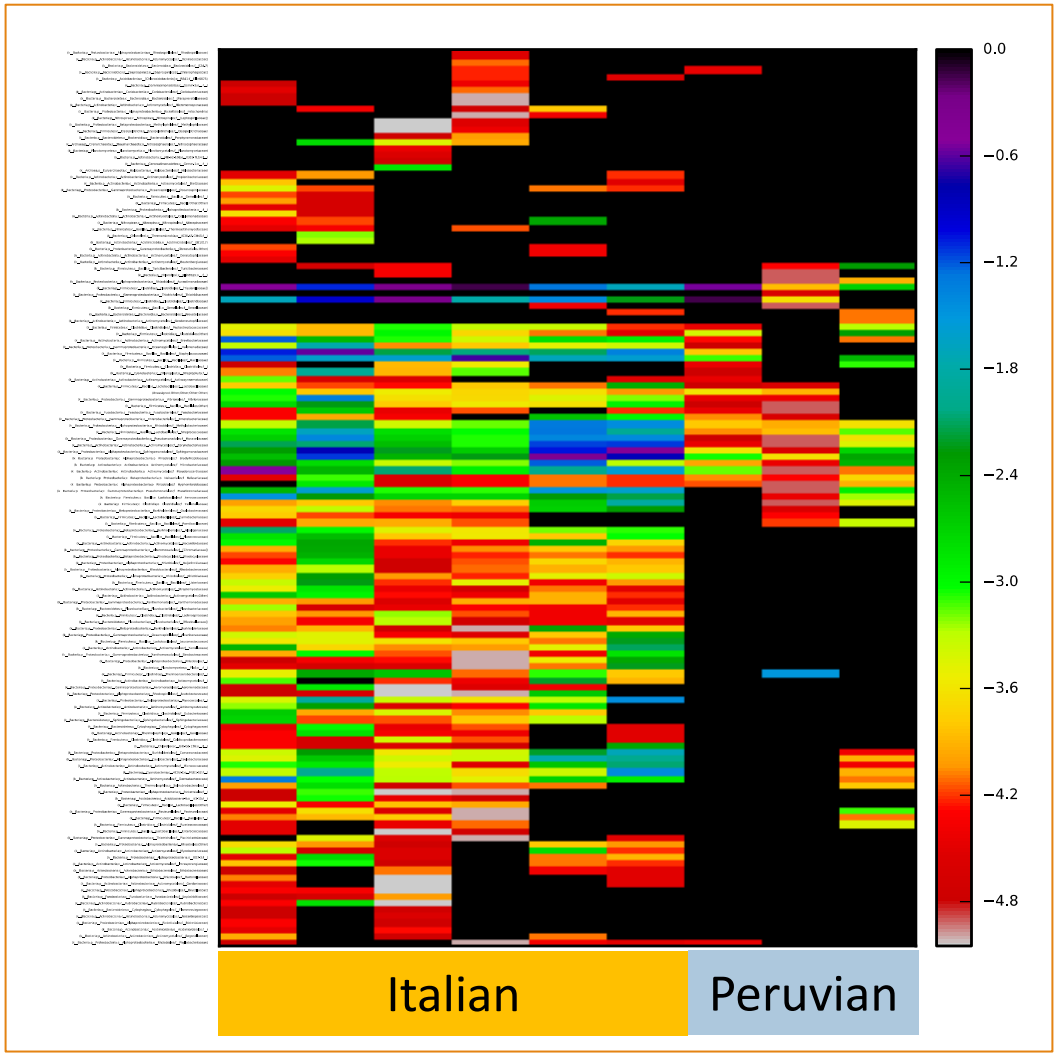




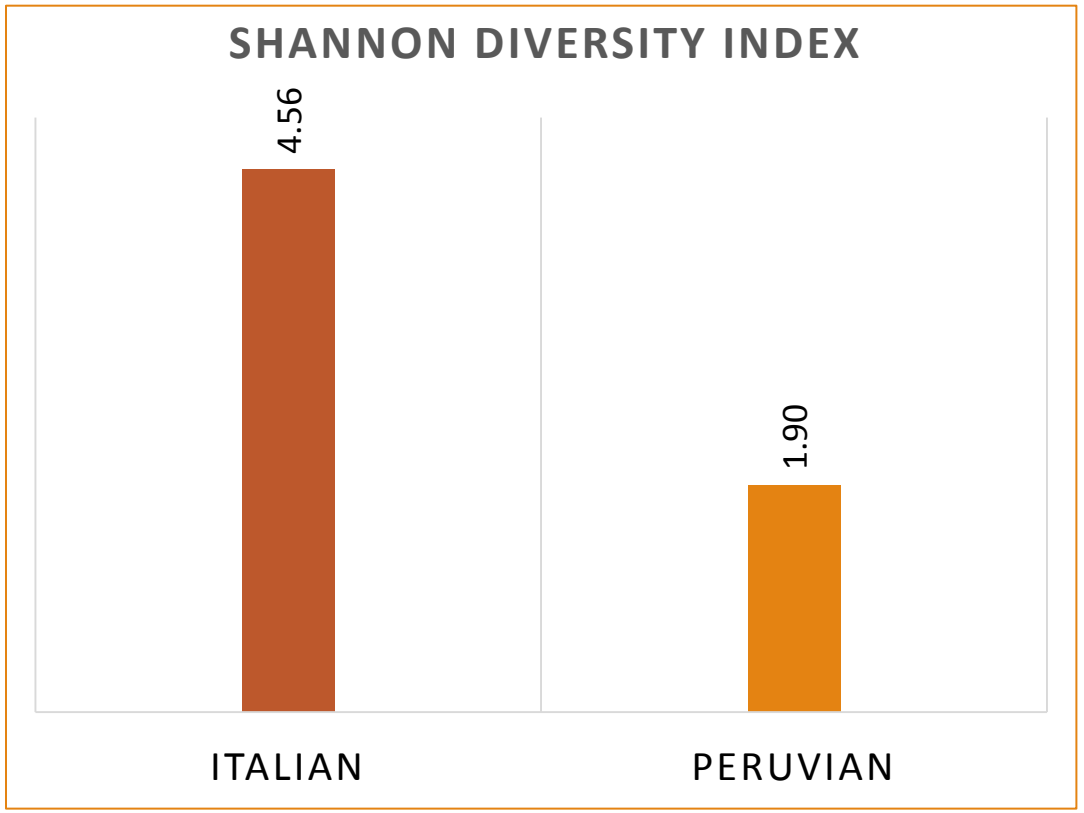
Vereades, et al. 2014, *Frontiers in Microbiology*

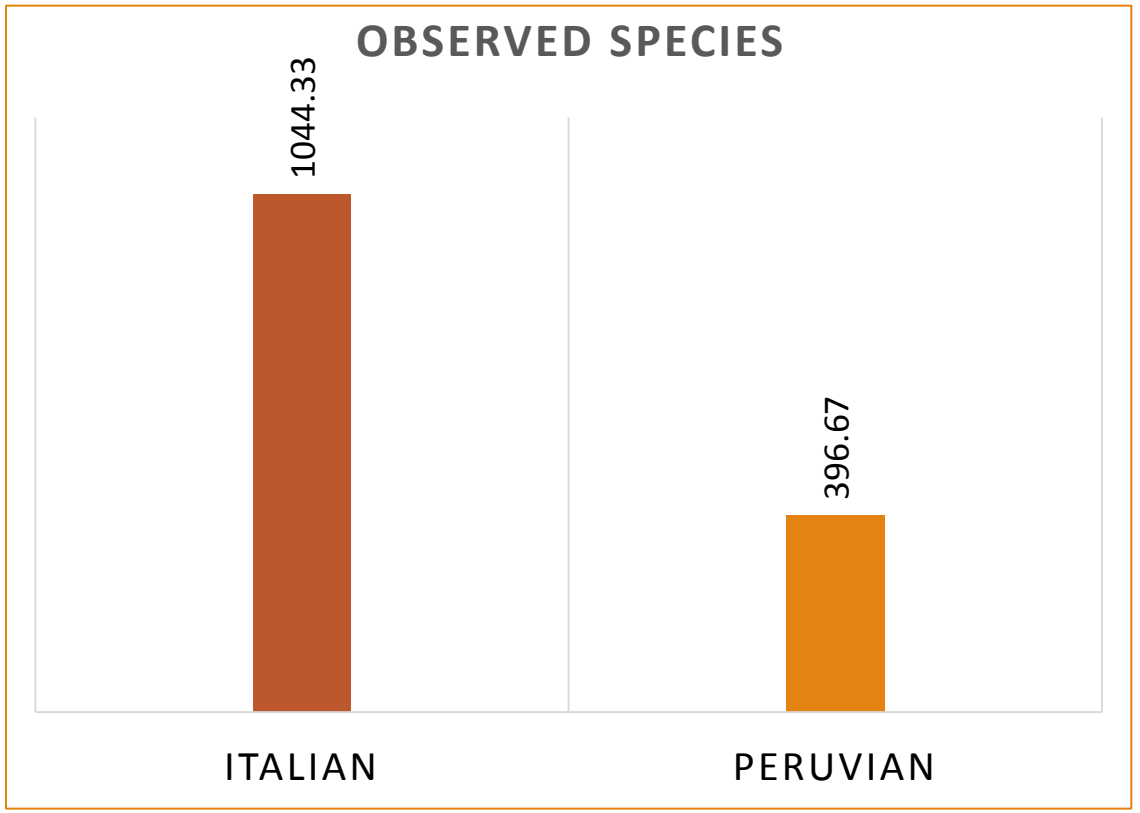
Dietary Impact on Microbial Prevalence … Continued





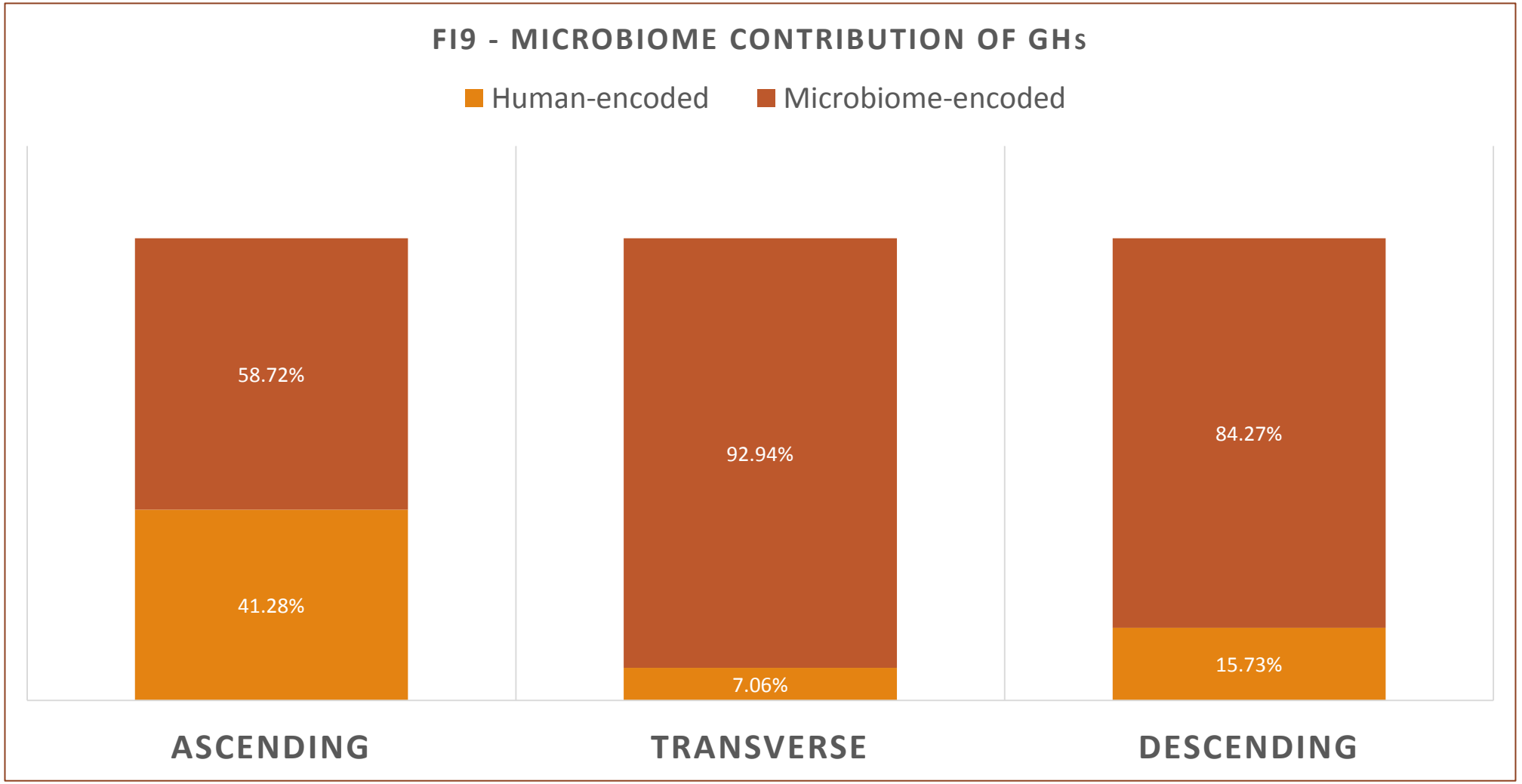
Alpha Diversity: Italian v. Peruvian Mummies



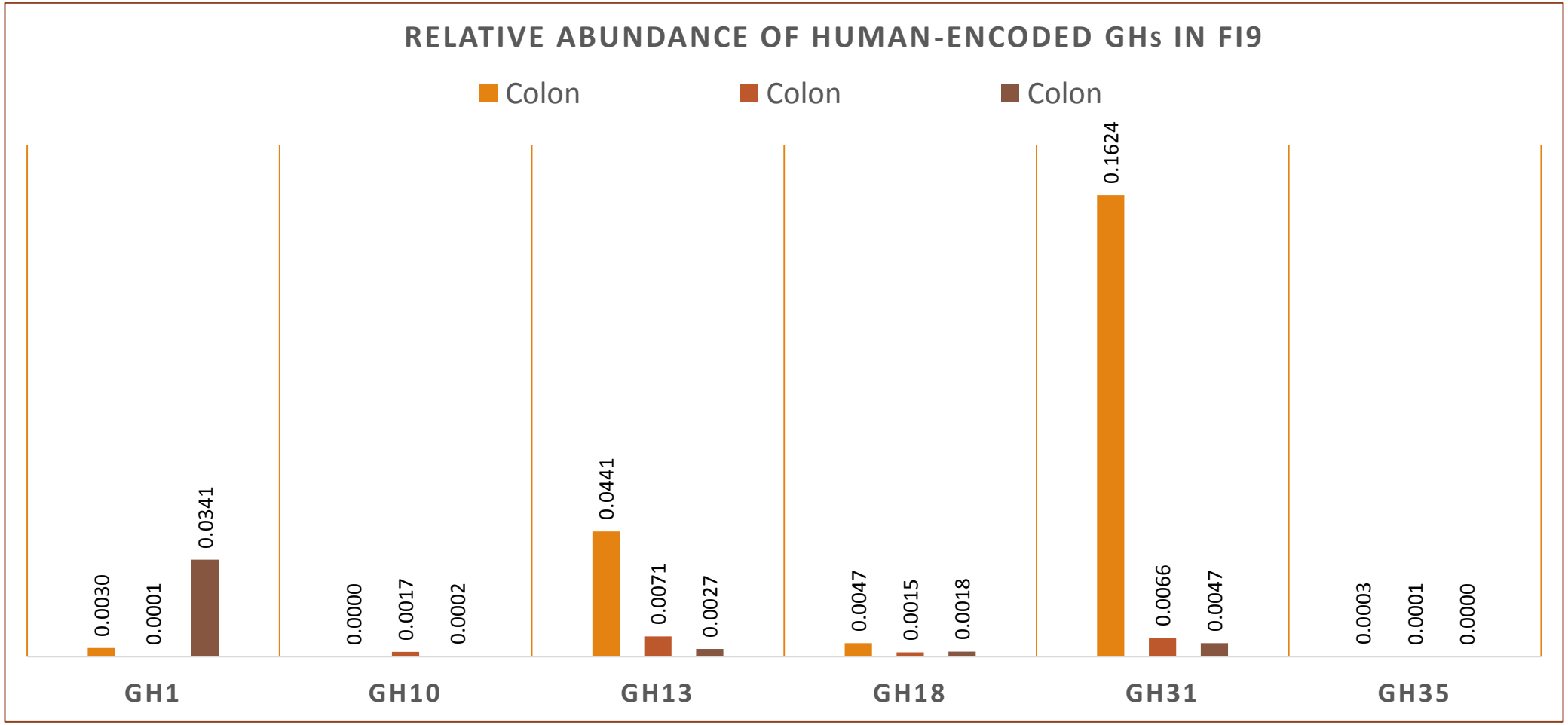


Carbohydrate Metabolism

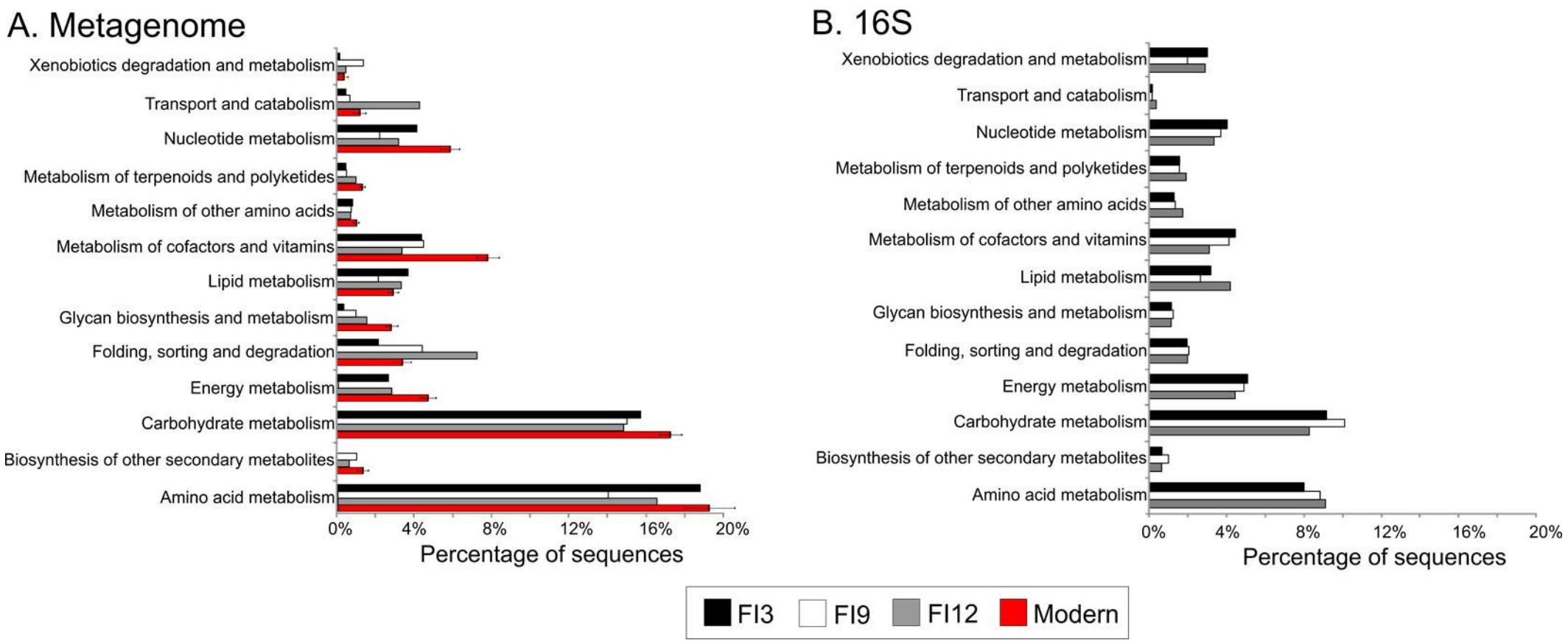
Human-Encoded GH: By Anatomical Site of FI9



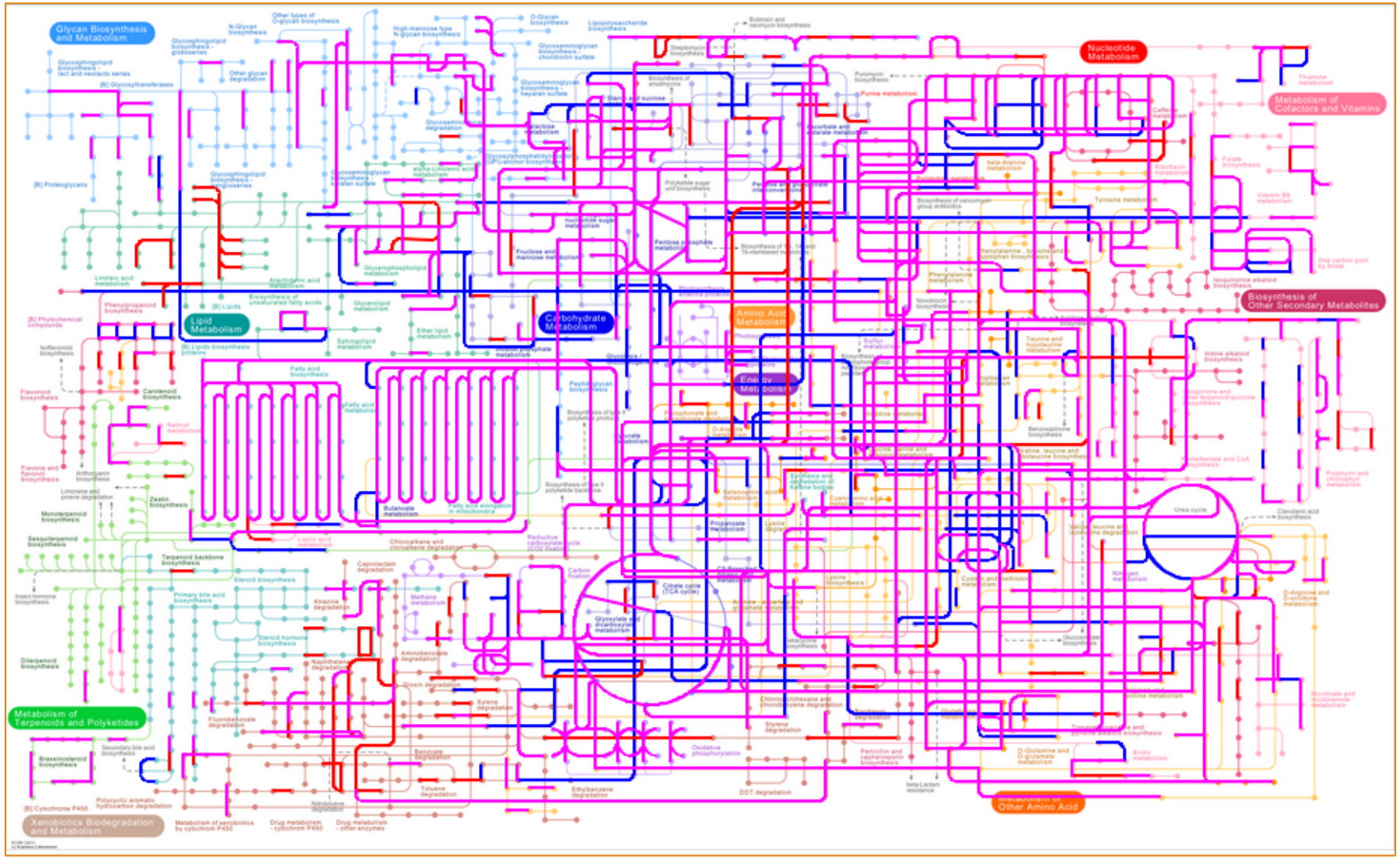
Human-Encoded GH: By Anatomical Site



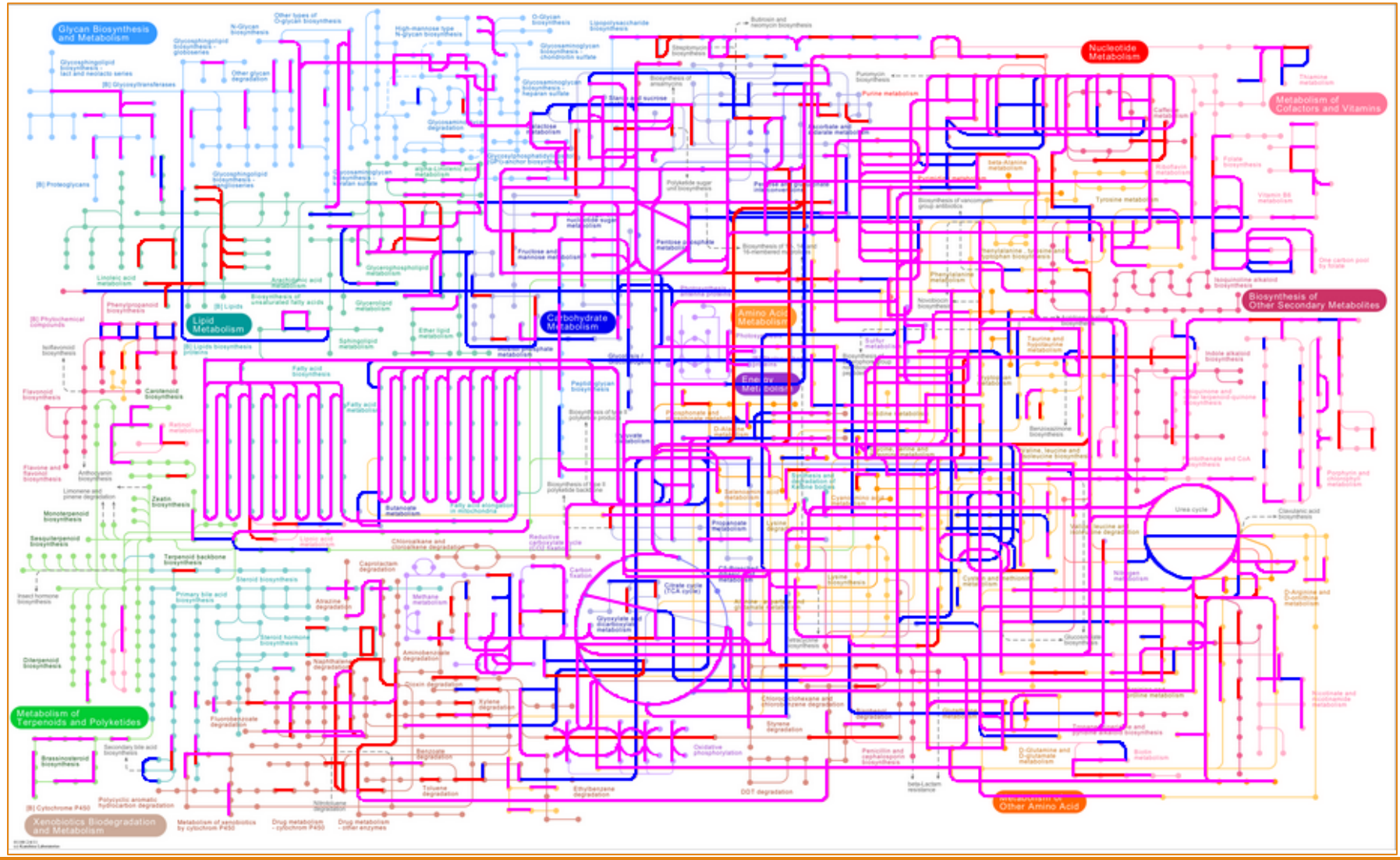
Metabolism (BlastX v PIECRUSt)



FI3 v. NASD27


Glycan Biosynthesis and Metabolism
Lipid Metabolism
Carbohydrate Metabolism
Amino Acid Metabolism
Nucleotide Metabolism
Metabolism of Cofactors and Vitamins
Biosynthesis of Other Secondary Metabolites
Metabolism of Terpenoids and Polyketides
Xenobiotics Biodegradation and Metabolism

NASD 22 v. NASD 27



Final Comments (Finally!)

- Ancient DNA must be suitable for analysis
  - Sample collection and processing
  - Taphonomy
  - Damage analysis recommended
- Can help answer questions by providing a time reference
  - Mindful of information loss
  - Ask the correct and appropriate questions

Thank you for your interest

Questions?

Thank you for joining today!

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- **April 21, 2016**
  10:00 AM, 3:00 PM EST
  Steven Budd, M.S., M.B.A., *Product Line Business Specialist*, ATCC
  Best Practices in Cryopreservation

- **April 28, 2016**
  10:00 AM, 3:00 PM EST
  Frank Simione, M.S., *Director, Standards, Standards Resource Organization*, ATCC
  The ATCC Story: A Ninety Year Celebration

Please email additional questions to:

tech@atcc.org

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