Analysis of fecal bacterial diversity in howler monkeys (*Alouatta*) through metagenomics

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INTRODUCTION AND GOALS

Howler monkeys are considered threatened by CITES (Convention of International 16 Threatened and Endangered Species) and vulnerable by IUCN (International Union for Conservation of Nature and Natural Resources). Knowing the eating habits of these animals in captivity and non-captive may contribute to a better adaptation in each habitat and, consequently, lead to better conservation practices for these species.

Figure 1: Howler monkey infant feeding (Fonte: G1-SP)

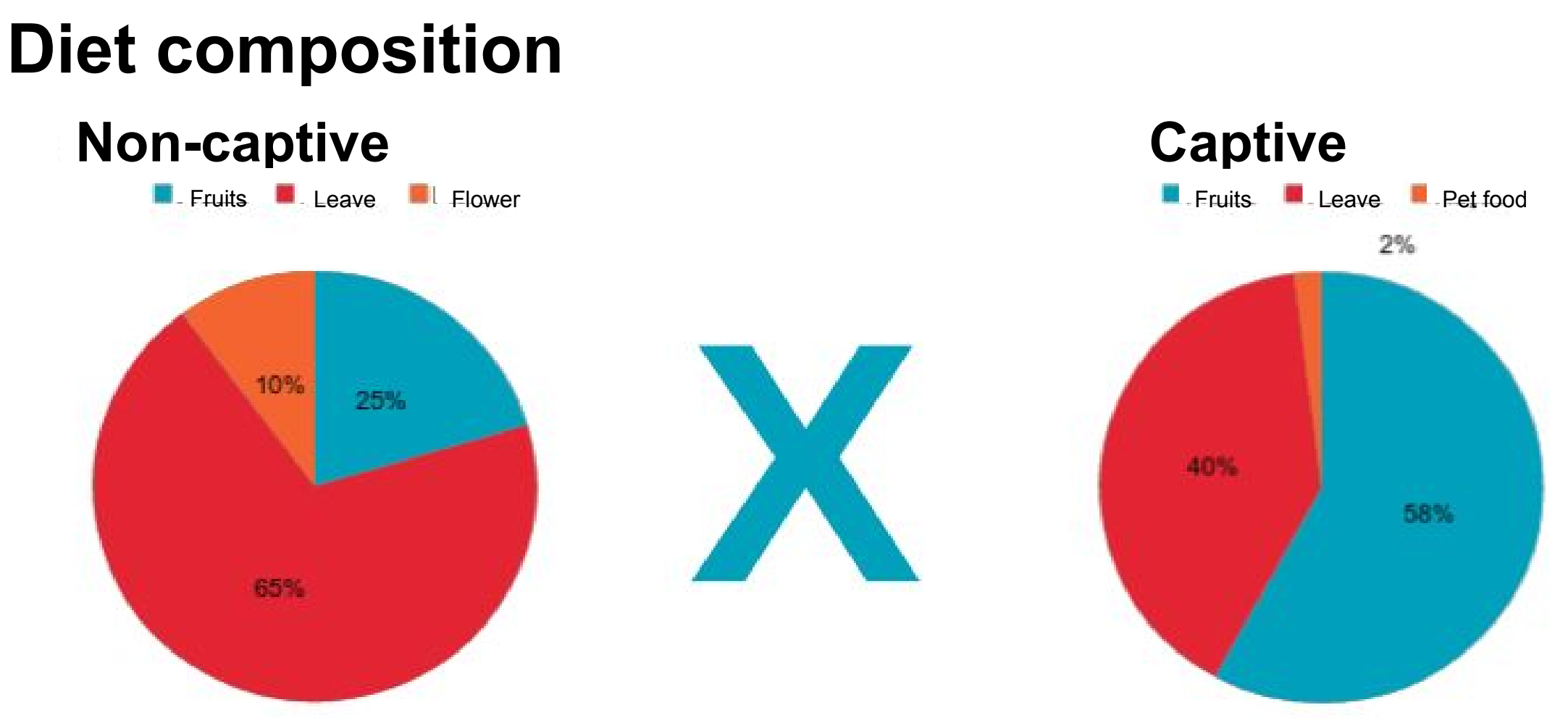
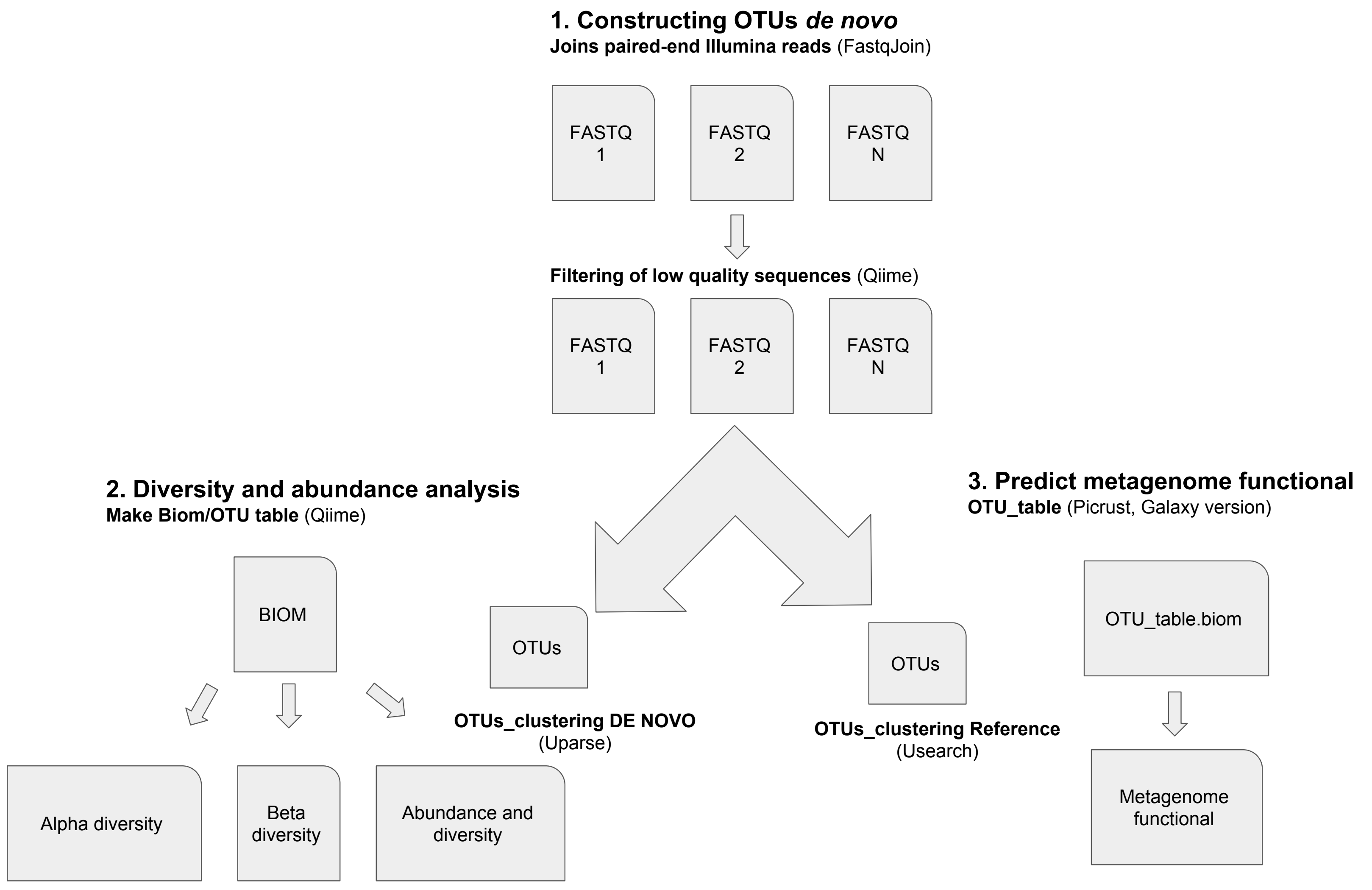


Figure 2: Diet comparison between captive and non-captive howler monkey (Source: MIRANDA, et al., 2004; Fundação Parque Zoológico de São Paulo.)

This project has as a main objective to investigate the gastrointestinal bacterial diversity in howler monkeys, *Alouatta guariba clamitans* e *Alouatta caraya*. And also compare the possible differences between the microbiota from captivite and non-captive individuals, considering the varation in their diet's composition.

METHODS

Stool samples were collected from six individulas: n=3 VL(non-captive) ; n=3 CAT (captive). All samples were collected at Fundação Parque Zoológico de São Paulo, then were processed and had their full DNA extracted, with the QIAamp DNA Stool kit, QIAGEN. The technic used for sequencing was the Next-Generation Sequencing, in the Miseq platform, Illumina and the analysis pipeline was run as it follows:



1. Constructing OTUs *de novo*
Joins paired-end Illumina reads (FastqJoin)

FASTQ 1
FASTQ 2
FASTQ N

Filtering of low quality sequences (Qiime)

FASTQ 1
FASTQ 2
FASTQ N

2. Diversity and abundance analysis
Make Biom/OTU table (Qiime)

BIOM

Alpha diversity
Beta diversity
Abundance and diversity

OTUs
OTUs_clustering DE NOVO
(Uparse)

OTUs
OTUs_clustering Reference
(Usearch)

3. Predict metagenome functional
OTU_table (Picrust, Galaxy version)

OTU_table.biom

Metagenome functional

RESULTS AND PERSPECTIVES

The preliminary results revealed that the abundance of different taxons varies between captive and non-captive individuals. And shows the dominance of Prevotellaceae family in both groups, but more abundance in NC group, according to Russell and Baldwin, 1979; Salyers, 1979, the Prevotella is common bacterial genus capable of degrading hemicellulose, pectin and simpler carbohydrates, such as those expected in fruits and low-complexity fibrous resources.

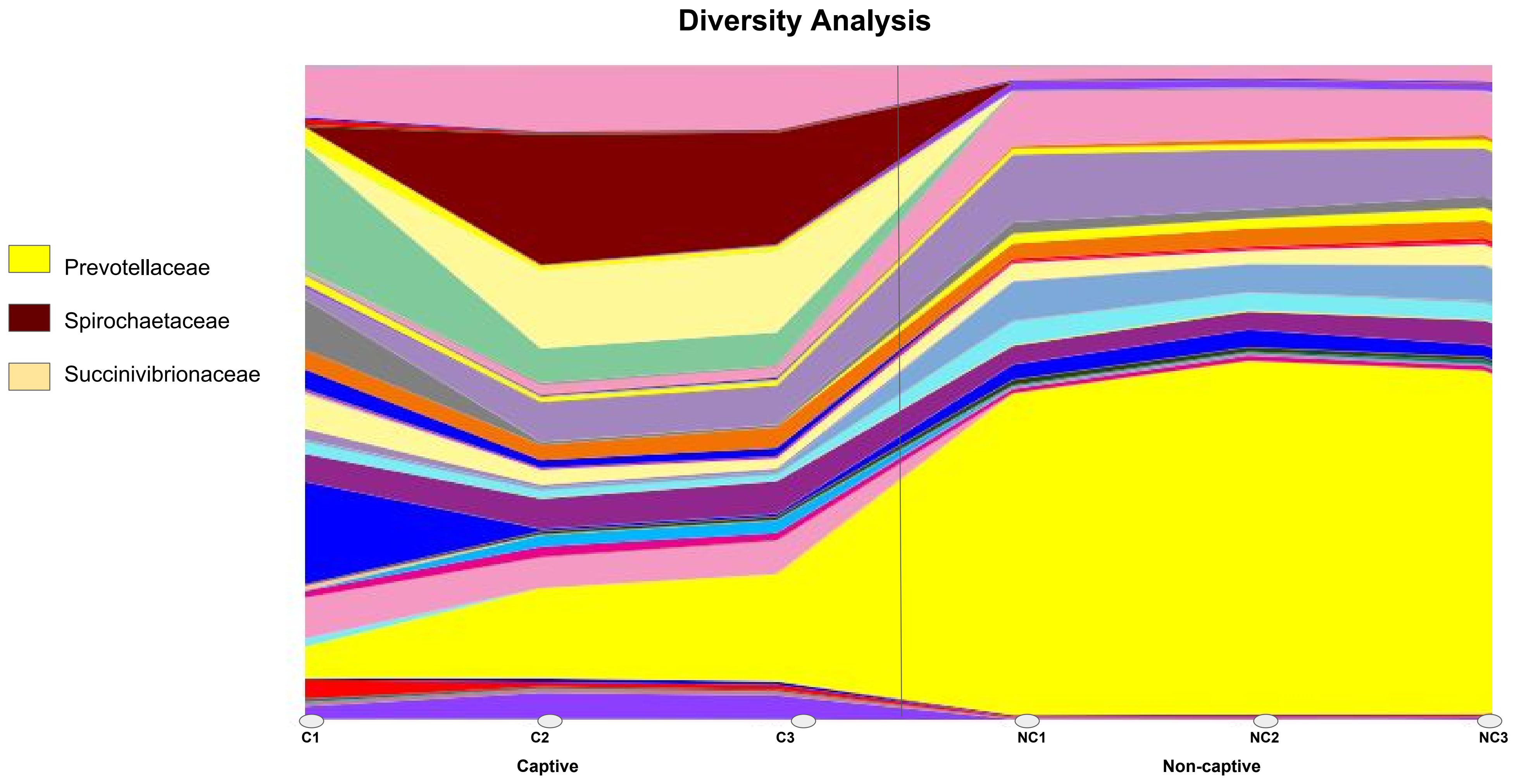


Figure 3: Genus level relative abundance in the different analysed samples: Captive 1, 2 and 3 (C1, C2 and C3) and Non-Captive 1, 2 and 3 (NC1, NC2 and NC3).

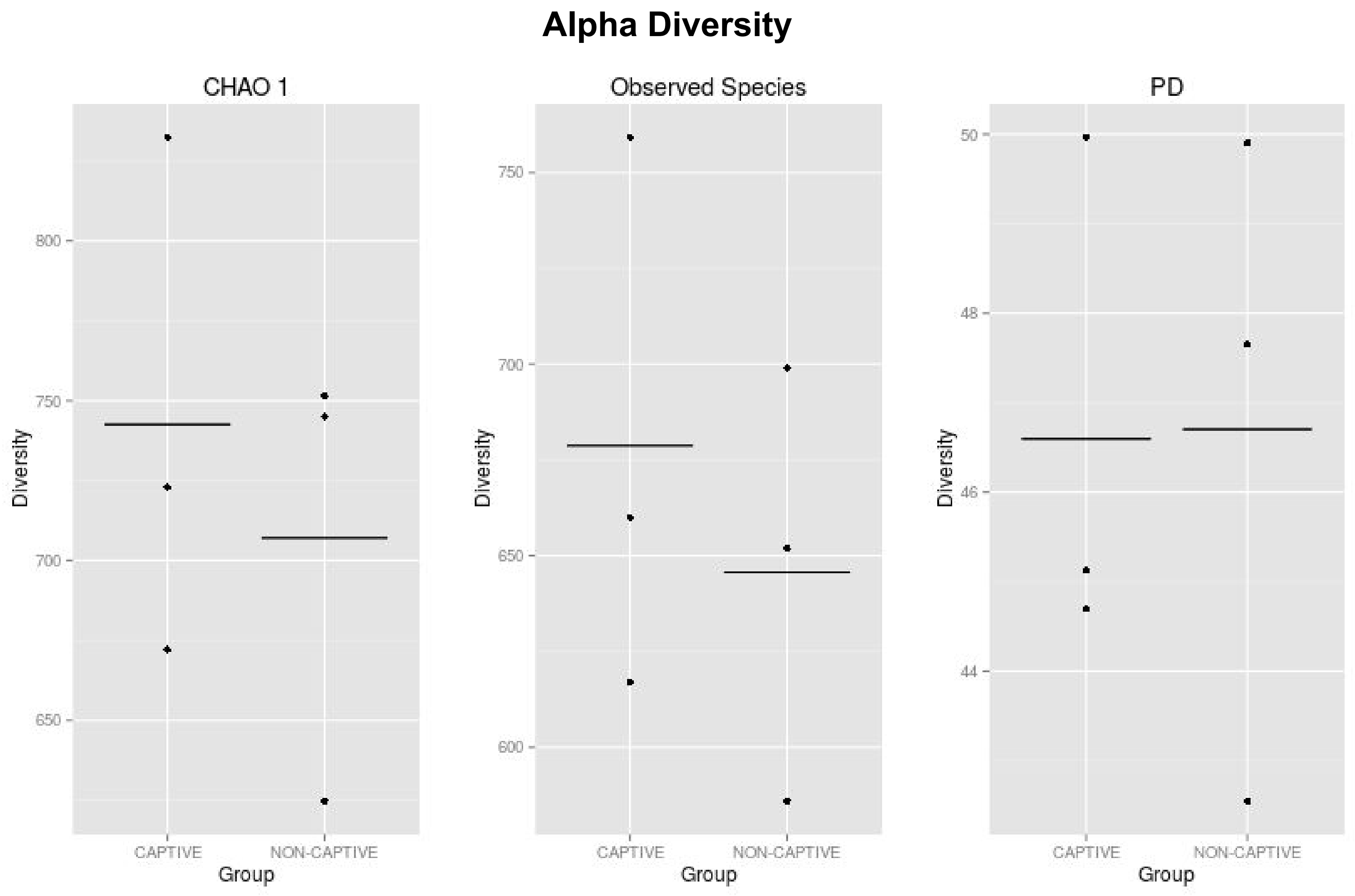


Figure 4: Graphics showing different alpha-diversity metrics, such as estimated abundance (CHAO1), observed abundance (Observed Species) and phylogenetic distance (PD).

Furthermore, the genus spectrum in figure 3 shows that the captive individuals have more genus represented here than the non-captive and that the captive's genus are more well-distributed than the non-captive's (the genus represented in yellow is disproportionally more present than the others). Looking at figure 4, it can be noticed that both the estimated abundance (CHAO1) and the observed abundance (Observed Species) are more elevated in the captive group. The phylogenetic distance is, aparrently, very similar for both groups (in the non-captive group it is slightly higher). In summary, the captive indivuals apparently have a more diverse gatrointestinal microbiota than the non-captive individuals. Analyses by functional profiles revealed no apparent differences between groups, but among the profiles observed, the most distinctive are involved in metabolic pathways. New analysis are meant to be done, but now the sampling will occur in autumn, and a more refined functional analysis will be conducted.

REFERENCES

RUSSEL *et al.* APPLIEDANDENVIRONMENTALMICROBIOLOGY (Mar.1979)
AMATO *et al.* The ISME Journal (2013)
MIRANDA *et al.* 2004. Revista Brasileira de Zoologia (2004)
LANGILLE *et al.* Nature Biotechonology (2013)
CAPORASO ***et al.*** Nature Methods (2010)

Support