

TITLE: FROG FOAM-NESTS MICROBIOME

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ABSTRACT:

Some frog species have developed a breeding strategy in which they deposit their eggs in stable foams nests in order to protect the eggs and larval development and allowing independence from aquatic environment. Foam nests are hypothesized to have many functions such as oxygen supply, rate improvement and protection against ultraviolet rays and dehydration. The entire environment of the foam nests comprising eggs, larvae, water, secretions, air bubbles and microbiota represents a unique ecosystem. In spite of the fascinatingly uniqueness of the frog foam nests only few studies have been published concerning its biochemical composition, structure and evolution. The findings so far have demonstrated that the foam nests are mainly composed by proteins, carbohydrates and that they have an associated microbial community. Taking this into account, this study aims to access the microbiome associated with the foam nests of the frogs *Leptodactylus vastus* and *Physalaemus cuvieri*. The biofoams were collected in Ceará, Brazil, during the rainy season with proper regard to Brazilian legislation. The total DNA were isolated by commercial kit and the V4 region of rRNA 16S were amplified and, subsequently, sequenced by the MiSeq Illumina. The obtained sequences were clustered in OTUs using UCLUST program with a score similarity higher than 97% for species identification. Relative abundance above 1% was considered for dominant groups. It was achieved 195,889 reads, distributed in 949 OTUs, clustered in 23 phyla, 60 classes, 89 orders, 130 families and 152 genders, including Bacteria and Archaea domains. In the foam nest of *L. vastus*, the Proteobacteria phylum stands out with 92% of relative abundance, mainly represented by Gammaproteobacteria, with high abundance of sequences assign to the genus *Acinetobacter*. *P. cuvieri* foam nests are equally colonized by Proteobacteria (47%), mostly Gammaproteobacteria and Alphaproteobacteria, and Bacteroidetes (45%), comprising Flavobacteriia and Sphingobacteriia, with higher abundance of the genus *Sphingobacterium*. Our data showed that the frog foam nests display distinct microbiomes, suggesting the frog species play a key role in the microbial composition. Further studies are needed to decipher the contribution of the microbial composition in the nest architecture and larval development.

Keywords: foam nests, frog, metagenome, NGS sequencing.

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