



DNA BARCODING AND GENOMICS IN THE MEGADIVERSE AMAZON ALTITUDE FIELDS

by GUILHERME OLIVEIRA, GISELE NUNES, RAFAEL VALADARES, RONNIE ALVES, and SANTELMO VASCONCELOS

Environmental Genomics Group, Instituto Tecnológico Vale, Belém, Brazil

Scientists are contributing to the most profound molecular representation of biodiversity in any Brazilian environment.

Industrial activities in the Brazilian Amazon are highly regulated by governmental agencies. However, the lack of knowledge about megadiverse areas is a problem for the establishment of best conservation practices; this is the case for mining operations in the eastern Amazonian Carajás, a region comprised of a mosaic of national parks, indigenous peoples' conservation areas, and nature reserves.

Of particular interest are the ferruginous altitude fields known as the Canga. Our floristic survey described the presence of 1,094 species from just over 200 previously known¹. The lack of biodiversity data is even more significant for the ferruginous caves where only around 10 invertebrate species are identified to the species level. Unfortunately, this is typical for the Amazon basin.

To provide reliable scientific data that contributes to the implementation of best conservation practices, Instituto Tecnológico Vale is developing DNA barcode reference libraries for the flora, cave invertebrates, and bats of the region, and providing deeper genomic references for species that are endangered or difficult to identify. To achieve this goal, we established the necessary infrastructure to conduct DNA sequencing using Sanger, Illumina, and PacBio technologies, coupled with high-performance computing, artificial intelligence algorithms, and highly trained personnel.

To date, over 8,575 barcodes for 3,548 specimens of plants and invertebrates have been produced, while a large number of species remain to be identified by morphological attributes. Morphological specimen determination is conducted by in-house specialists, as

The entrance of a ferruginous cave at the Bocaina mountain in Pará State, Brazil
PHOTO CREDIT: João Marcos Rosa

well as by an extensive network of specialists in universities and museums across Brazil and abroad.

For this purpose, nuclear and chloroplast or mitochondrial markers as well as low coverage to whole genome sequencing or restriction site-associated DNA sequencing (RADSeq) are being employed to unravel the vast genetic diversity of the biota of Carajás²⁻⁴. For several endemic plants, such as species of Asteraceae, Melastomataceae⁵, Convolvulaceae⁶, and Isoetaceae², diversity analyses, based on next-generation sequencing, aim to characterize the genetic variability among and within populations, as well as the identification of markers under selective pressure.

These methods also contribute to the understanding of population structure and the process of gene flow between populations affected by natural factors

Botanists collecting samples in a temporary lake in ferruginous altitude fields in the Carajás region, Pará State, Brazil.

PHOTO CREDIT: João Marcos Rosa



and industrial operations. Models of environmental distribution, including parameters sensitive to climate change, were determined for several taxonomic groups, including plants and bats⁷.



Specimens of the pinheiro-da-canga (Paepalanthus fasciculoides) highly adapted to inhabit the canga (ancient ferruginous rock outcrops) at the altitude fields in the Carajás region, Pará State, Brazil.

PHOTO CREDIT: João Marcos Rosa

We are also establishing eDNA methods, as well as metagenomics and metaproteomics data for environmental monitoring of ferruginous fields phytophysiologicals, areas under rehabilitation processes, and caves⁸. Together these data constitute the most profound molecular representation of any environment in Brazil. We have contributed a total of 3,072 specimens to the Barcode of Life Data System (BOLD) comprising 398 genera (291 new) in addition to the 408 different genera collected through the national effort for angiosperms in Brazil. We have also provided 571 cave fauna specimens.

It is important to highlight that all of these data generated are being provided to the public and its use will be critical to the conservation of such a unique collection of species.

Online:

<https://ibol.org/barcodebulletin/research/dna-barcoding-and-genomics-in-the-megadiverse-amazon-altitude-fields/>

References:

1. Brazil Flora Group (2018) Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia* 66(4): 1085–1113. <http://dx.doi.org/10.1590/2175-7860201566411>
2. Nunes GL, Oliveira RRM, Guimarães JTF, Giuliatti AM, Caldeira C, Vasconcelos S, et al., (2018) Quillworts from the Amazon: A multidisciplinary populational study on *Isoetes serracarajensis* and *Isoetes cangae*. *PLoS ONE* 13(8): e0201417. <https://doi.org/10.1371/journal.pone.0201417>
3. Oliveira RRMO, Vasconcelos S, Pires ES, Pietrobon T, Prous X and Oliveira G (2019) Complete mitochondrial genomes of three troglophile cave spiders (Mesabolivar, pholcidae), *Mitochondrial DNA Part B* 4(1): 251–252. <https://doi.org/10.1080/23802359.2018.1547139>
4. Ramalho AJ, Zappi DC, Nunes GL, Watanabe MTC, Vasconcelos S, Dias MC, Jaffé R, Prous X, Giannini TC, Oliveira G and Giuliatti AM (2018) Blind testing: DNA barcoding sheds light upon the identity of plant fragments as a subsidy for cave conservation. *Frontiers in Plant Science* 9:1052. <https://doi.org/10.3389/fpls.2018.01052>
5. Carvalho CdS, Lanes ECM, Silva AR, Caldeira CF, Carvalho-Filho N, Gastauer M, Imperatriz-Fonseca VL, Nascimento W, Oliveira G, Siqueira JO, Viana PL, Jaffé R (2019) Habitat loss does not always entail negative genetic consequences. *bioRxiv* 528430. <https://doi.org/10.1101/528430>
6. Lanes ÉC, Pope NS, Alves R, Carvalho Filho NM, Giannini TC, Giuliatti AM, Imperatriz-Fonseca VL, Monteiro W, Oliveira G, Silva AR, Siqueira JO, Souza-Filho PW, Vasconcelos S and Jaffé R (2018) Landscape genomic conservation assessment of a narrow-endemic and a widespread morning glory from Amazonian Savannas. *Frontiers in Plant Science* 9:532. <https://doi.org/10.3389/fpls.2018.00532>
7. Costa WF, Ribeiro M, Saraiva AM, Imperatriz-Fonseca VL, Giannini TC (2018) Bat diversity in Carajás National Forest (Eastern Amazon) and potential impacts on ecosystem services under climate change. *Biological Conservation* 218: 200–210. <https://doi.org/10.1016/j.biocon.2017.12.034>
8. Gastauer M, Vero MPO, de Souza KP, Pires ES, Alves R, Caldeira CF, Ramos SJ, Oliveira G (2019) A metagenomic survey of soil microbial communities along a rehabilitation chronosequence after iron ore mining. *Scientific Data* 6:190008. <https://doi.org/10.1038/sdata.2019.8>