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Conference Abstract

Challenges in assessing the Neotropical fishes using DNA metabarcoding

Izabela S Mendes[‡,§], Heron O Hilário[§], Daniel F Teixeira[‡,§], Daniel Cardoso de Carvalho[§,‡]

‡ Federal University of Minas Gerais, Belo Horizonte, Brazil
§ Conservation Genetics Lab, Postgraduate Program in Vertebrate Biology, Pontifical Catholic University of Minas Gerais, Belo Horizonte, Brazil

Corresponding author: Izabela S Mendes (izabelasantosmendes@hotmail.com)

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Abstract

Species richness is a metric of biodiversity usually used in fish community assessment for monitoring programs. This metric is often obtained using traditional fisheries methods that rely on capture of target organisms, resulting in underestimation of fish species. DNA metabarcoding has been recognized as a powerful noninvasive alternative tool for fish biomonitoring and management. Despite the increasing popularity of this method for the assessment of aquatic megadiverse ecosystems, its implementation for studying the highly diverse Neotropical ichthyofauna still presents some challenges. One of them is to devise what primer set could reliably amplify the DNA of all fish species from a megadiverse river basin and have enough resolution to identify them. In order to identify and overcome these drawbacks, we have investigated the efficiency of the metabarcoding approach on Neotropical fishes using a mock sample containing genomic DNA of 18 fish species from the Jequitinhonha River basin, Eastern Brazil. We compared three primer sets targeting the 12S rRNA gene: two universal and widely used markers for fish metabarcoding [MiFish (~170bp) and Teleo_1 (~60bp)], and NeoFish (~190bp), recently developed by our research group specifically for the identification of Neotropical fishes (Milan et al., 2020). Two samples amplified using three primers were sequenced in a single multiplexed Illumina MiniSeq run, using normalized and non-normalized pools. Bioinformatic analyses were performed using a DADA2/Phyloseq based pipeline to perform filtering steps and to assign Amplicon Sequence Variants (ASVs). We used a custom 12S reference sequence

database that included 190 specimens representing 101 species and 70 genera from the Jequitinhonha and São Francisco river basins. A total of 187 ASVs were recovered: 79, 66 and 42 for NeoFish, MiFish and Teleo_1, respectively. ASVs of unexpected species were identified for both pools (Fig. 1), though each of these ASVs had an abundance of less than 50 copies. In addition, species of the *Hoplias* and *Prochilodus* genera could not be identified at the species level, due to identical sequences within each genus, possibly because of the insufficient variation within the 12S region recovered by these primers' amplicons. Unexpectedly, although a single individual of each species was placed in the pools, more than one ASV was identified for some species, likely caused by PCR biases. Overall, all primer sets displayed similar taxonomic resolution for the DNA pools and recovered all species, except for NeoFish, which could not detect *Steindachneridion amblyurum* due to an incompatibility in the 3' of the NeoFish forward primer and Teleo_1, which could not identify *Steindachnerina elegans*. These results highlight the need of reliable databases in order to enable the full assignment of ASVs and OTUs to species level, and the importance of calibrating the DNA metabarcoding approach with mock samples to identify weaknesses and pivotal steps prior to the application on large scale DNA based biodiversity evaluation, that can help with the complex task of conserving the megadiverse Neotropical ichthyofauna.

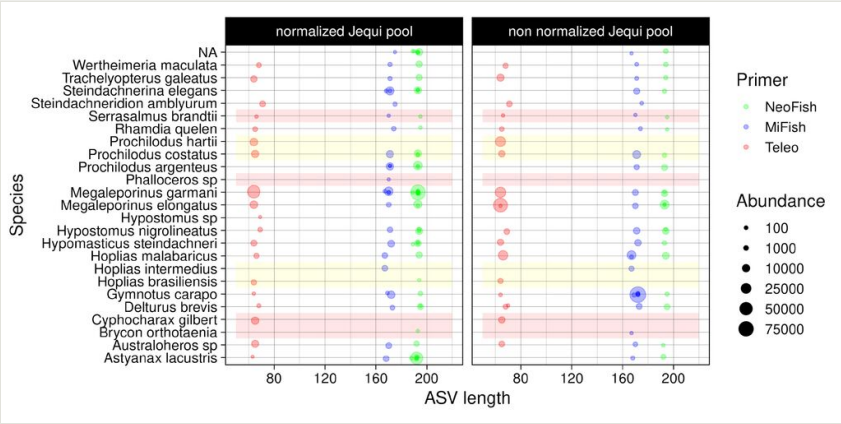

Figure 1. doi

Size distribution of ASVs and filtered abundance, considering libraries of normalized and non-normalized DNA pools. Each sphere represents a single ASV, size is proportional to the number of copies and colors correspond to each primer: NeoFish (green), MiFish (lilac) and Teleo_1 (red). Species highlighted by red and yellow bars correspond to unexpected ones and those which have identical sequences, respectively.

Keywords

metabarcoding, neotropical region, freshwater fish, 12S

Presenting author

Izabela S Mendes

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