

How much primer choice affects the perceived biodiversity? A case study of bat diet according to three COI gene regions.

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Abstract

With the advent of the metabarcoding strategy, the study of diet components and niche overlap has reached an unprecedented taxonomic resolution, especially concerning the Arthropoda phylum. The cytochrome c oxidase subunit I (COI), widely adopted as marker for Metazoa, presents distinct variation levels at distinct gene regions, making the primer choice essential to amplification success of targeted taxa. The bat diet recovered from guano eDNA amplified by three distinct COI primer pairs was compared between each primer pair dataset in terms of taxa recovery, Arthropoda taxonomic coverage, and community structure response to the spatial component according to diversity indexes and beta-diversity patterns. The guano samples were collected in eight caves located in Amazon biome and one cave sampled along one year in Caatinga biome, totalizing 13 sample units. The P23 short primer pair (130bp) recovered mostly Chiroptera reads (56%), presented the lowest Arthropoda recovery, but the most even proportion of Arthropoda orders. The P34 primer pair, which amplifies the longest and more variable amplicons (370bp), recovered the largest proportion of Arthropoda reads (57%), 73% of it recruited by Diptera and Lepidoptera orders. The P56 primer pair, amplifying a 350bp COI amplicon originally described as the most conserved region, showed the narrowest taxonomic coverage, with over 95% of Arthropoda reads assigned to Lepidoptera. Both P34 and P56 long amplicons recovered the largest portion of reads from Caatinga cave, showed better Arthropoda recovery and resolution than P23, and presented a nested beta-diversity structure, probably as a result from the narrow taxonomic coverage and the time-series sampling design. Even so, the three primer pair datasets shared similar compositional responses to spatial scale, with most part of the variance explained by sample units, followed by regions and biomes. Since there was a spatial-ecological shared response between the datasets, mostly between P34 and P56, their Arthropoda community of reads could be complementary, potentially improving the niche breadth detected. The amplicon variability observed was substantially different from described by the literature, reinforcing the critical importance of local ecological and biological attributes in primer choice and expected outcome.

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Link to Video: