How Accurate is Species Detection with Metabarcoding
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Introduction:
Metabarcoding has the potential to allow rapid determination of the species present in an entire community, which in turn allows monitoring and greatly increased power to compare communities in space or time. However, taxonomic identification of metabarcodes requires the use of a reference library of known sequences and building libraries with sufficient taxonomic coverage takes a great deal of time and taxonomic expertise. Here we test 3 different reference libraries to determine the importance of reference library choice.

We used two groups of planktonic taxa to determine how accurate metabarcode species assignment is with different reference libraries.

The inclusion of both barcodes and metabarcodes in a phylogenetic tree allowed the assessment of metabarcode taxonomic assignment.

Methods:
• Pteropoda (Mollusca, Gastropoda) and Hyperiidea (Arthropoda, Amphipoda) were chosen as test cases because we have many unpublished barcodes available.
• Collected all available (many unpublished) zooplankton barcodes and metabarcodes assigned to species using 1. RDP Classifier using the Midori curated reference library, 2. BLASTn-GenBank using all GenBank sequences, and 3. BLASTn-StreamCode using our own curated library.
• Sequence alignment using MAFFFT in Geneious v2021.1.1.
• Tree building using RAxML, with 1000 replicates, GTR+GAMMA model, with partitions, run on the SI/HPC.
• Analyzed metabarcodes species assignment in each clade and categorized them as “Yes”, “No Clear Evidence”, and “No” based on their position on the tree.

Conclusion:
Including metabarcodes and all reference barcodes in a tree is a useful way to assess metabarcode taxonomic assignments.

Taxonomic assignment of metabarcodes has been shown to be highly dependent on the representation of each taxonomic group within a reference library. Likely the difference in reference library impact was because pteropods are well represented in all of these databases, while hyperiids are not well represented only in the StreamCode reference library. Based on this, a complete reference library is the most important thing for metabarcoding to be an effective tool.

I recommend focusing effort on improving the reference libraries by increasing the amount of good quality barcodes.

References:

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