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Conference Abstract

Improved freshwater macroinvertebrate detection from environmental DNA through minimized nontarget amplification

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Abstract

Environmental DNA (eDNA) metabarcoding is a new, promising, and non-invasive method to detect biodiversity in aquatic environments. So far, it has mainly been used to screen for fish and amphibian diversity and rarely to detect macroinvertebrates. Typically, DNA metabarcoding relies on PCR amplification of a fragment of the mitochondrial cytochrome c oxidase I (COI) gene with degenerate primers. In comparison to other genes like 16S, COI has a greater taxonomic resolution and availability of an extensive reference database. Benthic stream invertebrates are of critical importance for regulatory biomonitoring, but when using universal primers on eDNA isolated from water, the number of reads and OTUs is “watered down”. This means the target taxa, macroinvertebrates, are underrepresented in comparison to other nontarget taxa, e. g. algae, bacteria, and fungi.

The aim of the project was to design an insect-specific primer, which minimizes nontarget amplification. Therefore, data from a time series of 15 months at the Kinzig (Hesse), a silica-rich low-mountain-range stream, which is part of the Rhine-Main-Observatory (LTER site) was generated using the universal primers BF2/BR2. With this data we identified the most abundant nontarget taxa and designed a new reverse primer (EPTDr2n) with 3’ -

specificity toward benthic invertebrate taxa. Primer specificity was validated in silico together with universal forward primer fwhF2 using available data from GenBank and BOLD. 20 eDNA samples from the Kinzig River and its tributaries were then used to test the new primer in situ together with primer fwhF2.

The new primer combination showed a much higher amplification of benthic invertebrates, insects in particular, than two other universal primer pairs for both, number of target reads (fwhF2/EPTDr2n: 99.6% versus BF2/BR2: 25.89% and fwhF2/fwhR2n: 39.04%; Fig. 1) and number of target species (fwhF2/EPTDr2n: 305 versus BF2/BR2: 113 and fwhF2/fwhR2n: 185). Additionally, the number of benthic invertebrate species exceeded even the number of 153 species identified by expert taxonomists at nearby sites across two decades of sampling. While several taxa reported, like a few trichopteran genera, flatworms, and some crustaceans, were not found, the primer shows greatly improved results for eDNA metabarcoding of benthic invertebrates(Leese et al. 2021).

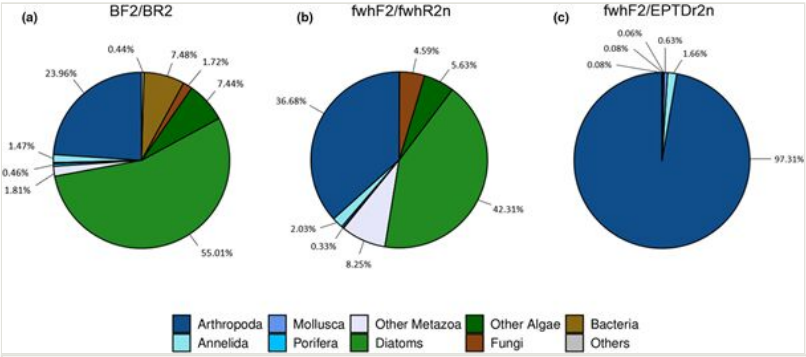

Figure 1. doi

In situ primer evaluation using the 20 eDNA samples from the RMO LTER site 54. Pie charts show proportion of reads assigned to phylogenetically distinct groups (see legend) using primer combinations BF2/BR2 (a), fwhF2/FwhR2n (b), and fwhF2/EPTDr2n (c)

Keywords

bioassessment, bioindication, biomonitoring, COI, eDNA, insects, LTER, metabarcoding, primer bias

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