

DNA metabarcoding unravels unknown diversity and distribution patterns of tropical freshwater invertebrates

ABSTRACT

tropical freshwater invertebrate species are becoming extinct without being described, and effective conservation is hampered by a lack of taxonomic and distribution data. DNA metabarcoding is a promising tool for rapid biodiversity assessments that has never been applied to tropical freshwater invertebrates across large spatial and taxonomic scales. Here we use DNA metabarcoding to comprehensively assess the benthic freshwater invertebrate fauna of the Perak River basin, Malaysia. Specific objectives were to: (1) assess performance of two DNA metabarcoding protocols; (2) identify gaps in reference databases; (3) generate new data on species diversity and distribution; and (4) draw conclusions regarding the potential value of DNA metabarcoding in tropical freshwater conservation. Organisms were collected by hand and net at 34 sites and divided into small (retained in 0.5-mm but passing through 1-mm mesh) and large (retained in 1-mm mesh) fractions, and a 313-bp cytochrome c oxidase subunit I fragment amplified and sequenced using general Metazoa primers. Bioinformatic analysis resulted in 468 operational taxonomic units (~species) from 12 phyla. Only 29% of species could be assigned binominal names through matches to public sequence libraries, indicating varying levels of library completeness across Orders. Extraction of small-fraction DNA with a soil kit resulted in a significantly higher species count than with a general kit, but this was not even across taxa. Metabarcoding (amplification) success rate, estimated via comparison to morphological identifications of the large-fraction specimens, was high in most taxa analysed but low, for example, in ampullariid and viviparid gastropods. Conversely, a large proportion of species-site records for Decapoda and Bivalvia came from metabarcoding only. Species richness averaged 29 ± 16 species per site, dominated by Diptera, Annelida, and Odonata, and was particularly high in tributaries of the mountainous Titiwangsa Range. At least eight species are new records for Malaysia, including the non-natives *Ferrissia fragilis* (Gastropoda) and *Dugesia notogaea* (Platyhelminthes). Our study showed that DNA metabarcoding is generally more effective in detecting tropical freshwater invertebrate species than traditional morphological approaches, and can efficiently improve knowledge of distribution patterns and ranges of native and non-native species. However, current gaps in reference databases, particularly for bioindicator taxa, such as the Plecoptera, Ephemeroptera, and Coleoptera, need to be addressed urgently.