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Building a custom barcode reference library for subterranean groundwater.

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There are high stakes in regulatory biodiversity assessments especially for those associated with mineral exploration and resource developments. For such assessments there is a clear need for accurate, fast and consistent species identification with new molecular methods potentially offering a significant alternative to traditional morphological approaches. Use of environmental DNA (eDNA) metabarcoding as a tool for detecting subterranean species inhabiting groundwater ecosystems associated with mineral deposits is quickly gaining momentum in the area of environmental impact assessment. However, in subterranean ecosystems invertebrate dark taxa with ancient and genetically diverse evolutionary lineages dominate, making meaningful taxonomic assignment of eDNA metabarcoding Operational Taxonomic Units (OTU) extremely patchy. Therefore, a custom library of reference sequences is essential. Using one of the oldest hydrogeological environments on earth, Western Australia's Pilbara region, we have collaborated with taxonomic specialists and industry partners to generate extensive, verified taxonomic data to establish a custom reference library of sequences, which will be expanded over time to meet future needs. Here we present our current subterranean fauna reference library and demonstrate its effectiveness when we query it with eDNA metabarcoding OTU from Pilbara groundwater. We also identify the importance of taxonomic metadata in developing custom reference libraries.

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