The Western Australian Biodiversity Science Institute

Wednesday 28 February 2024
9:00am – 3:30pm

Integrating eDNA into bioassessment and monitoring of subterranean fauna in Western Australia

What will the future look like?

University of Adelaide – Dr Michelle Guzik

Building a custom barcode reference library for subterranean fauna living in the ancient Pilbara (Western Australia) landscape

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 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.

Curtin University - Trace and Environmental DNA lab (TrEnD) - Dr Nicole White

To validate or not to validate: is this really a question now for environmental DNA in subterranean systems?

The increased use of DNA assays with the advancement of sequencing technology to assess environmental samples for the detection of species has rapidly advanced over the last decade. These methods have proven to be useful to screen for pest species as part of biosecurity measures and risk management, to screen for threatened species as part of development requirements, or for biodiversity monitoring purposes for conservation management strategies.

To ensure that eDNA testing results are reliable, it is imperative to develop and validate DNA assays using quality standards that can minimise the potential for false negative and false positive results by; 1) defining the intended purpose of the DNA assay; 2) design and test the assay; 3) validate and optimise the assay; and 4) check specificity, sensitivity, repeatability and reproducibility of the assay. The robustness of DNA assays must be considered and explored extensively to determine how inhibiting and environmental factors affect the amplification of known eDNA in controlled lab validation experiments.

Curtin University - Subterranean Research and Groundwater Ecology Group (SuRGE) - Dr Mieke van der Heyde

Taking eDNA underground: detecting subterranean fauna in groundwater using environmental DNA

The impacts of anthropogenic climate change, extraction and pollution on groundwater pose major threats to groundwater ecosystem health, prompting the need for efficient and reliable means to monitor subterranean communities. Traditional survey techniques for subterranean fauna relies on trapping organisms and morphological identification, which can be biased, labour intensive, and often indeterminate to lower taxonomic levels. Environmental DNA (eDNA)-based methods have the potential to dramatically improve on existing stygofaunal survey methods in a large range of habitats and for all life stages, reducing the need for the destructive manual collection of often critically endangered species or specialized taxonomic expertise.

We tested various eDNA sampling methods on groundwaters on two locations in the Pilbara region of Western Australia, and compared these to conventional survey methods using nets and morphological identification of specimens. Our results revealed a number of major findings. First, morphological identification and eDNA detection were complementary but eDNA metabarcoding was able to detect soft-bodied taxa and fish often missed by nets. However, only seven of the nine stygofaunal crustacean orders identified from haul-net specimens were detected from eDNA.

Secondly, shallow water samples and sediment were observed to contain the greatest biodiversity, and passive samples (material submerged in groundwater - eliminating the need for filtration) also detected subterranean fauna.

Finally, development of a Pilbara barcode reference library improved taxonomic identification of subterranean fauna and aided the testing and development of new assays suitable for the detection of subterranean fauna using eDNA. The findings of this study demonstrate that eDNA metabarcoding of groundwater can substantially improve efficiency of subterranean faunal surveys.

Department of Water and Environmental Regulation – Ms Claire Stevenson

Current and future use of eDNA for assessment of impacts to subterranean fauna

eDNA for the detection of subterranean fauna in environmental impact assessment has been hoped to be the ‘silver bullet’ to resolving issues on detection and reducing survey requirements. Following the conclusion of the ARC Linkage Project we ask: What are the limitations that remain? What are the realistic outcomes of the research? And, how can we apply eDNA to assessments and monitoring?

In the presentation, I will outline the current process for assessing subterranean fauna and the processes for changes to EPA policy, followed by an open discussion exploring how eDNA can be implemented as a tool to better inform our understanding of this often difficult factor.

Helix Molecular Solutions - Dr Zoë Hamilton

Stygofauna of the Beetaloo Sub-Basin in the Northern Territory – conventional sampling enhanced by using an eDNA species-specific probe

The Beetaloo Sub-basin is located approximately 500 km southeast of Darwin. Potential onshore gas development in the region resulted in the NT government commissioning Biota Environmental Sciences and Helix Molecular Solutions to complete a baseline stygofauna survey. The NT has had limited sampling for stygofauna relative to other parts of Australia. However, a previous survey undertaken in the region by CSIRO provided initial data to inform the survey. As is typical of northern Australia, crustaceans strongly dominated the known fauna, with the Decapoda; specifically Parisia unguis (family Atyidae), being of particular interest.

We conducted a 2-phase survey of 66 groundwater bores in the area, stratified across the three hydrogeological units present. Conventional sampling was completed at each site, as well as groundwater sample collection for eDNA analysis. Decapod specimens were initially morphologically identified and sequenced for variation at mitochondrial genes, which also enabled the design and testing of a species-specific eDNA probe for Parisia unguis.

The use of eDNA methods significantly expanded the mapped distribution of Parisia unguis compared to conventionally collected specimen records alone. These additional data showed the taxon occurred in all three hydrogeological units, highlighting the value of eDNA sampling as an adjunct survey method. Due to their specificity, eDNA probes tend to be more sensitive to the detection of a target species than eDNA metabarcoding, and usually provide more conclusive determination of the presence of a target taxon in environmental samples. We acknowledge the benefits and disadvantages of both methods but advocate the use of real-time PCR and species-specific eDNA probes if surveys seek to detect a particular subterranean species.

Biologic - Dr. Joel Huey

Environmental DNA underground: applied use in environmental impact assessments and monitoring

Environmental DNA (eDNA) from subterranean habitats is a powerful tool to complement existing methods for undertaking environmental impact assessments (EIA) and monitoring. It is well suited to sampling environments where existing methods have low capture rates, providing an additional approach to improve our understanding of species' distributions. At Biologic Environmental Survey, in collaboration with Rio Tinto, we have used eDNA to detect stygofauna in several project areas throughout Western Australia. We will discuss three consecutive years of eDNA sampling to monitor and map the distribution of Blind Cave Eel (Ophisternon candidum, BCE) populations in the Robe Valley, with the application of two molecular approaches.

In this work we have extended the known distribution of the BCE, detected the species in eDNA studies not targeting the BCE, and explored how sampling methods and DNA degradation may influence the interpretation of results. As well as the BCE, we have undertaken targeted eDNA surveys for invertebrate taxa to support EIA and will share some of the results and insights from these projects. We will conclude by outlining current uncertainty in how eDNA work is interpreted, and what we would like to see for future applications of this technology in the environmental consultancy sector.