



# Taking eDNA underground: transforming assessment of subterranean ecosystems

M. T. Guzik, M. van der Heyde; N. White, S. J. B. Cooper, A. D. Austin, P. Nevill, M. Harvey, R. King, Pinder, A.



# ARC Linkage Project 2019

## The project

- Industry: Rio Tinto, BHP, Chevron
- Biodiversity stakeholders: DBCA, and WABSI
- Regulators: DWER
- University of Adelaide and Curtin University



Photo: James Nankivell



# ARC Linkage Project 2019

## The project

- 1) develop a framework of knowledge on the unique faunal diversity of the Pilbara region that has direct implications for conservation management;
- 2) develop a novel environmental DNA (eDNA) approach using new assays and rigorous sampling methods for accurate, cost effective and reproducible monitoring of groundwater fauna





# ARC Linkage Project 2019

## New approaches to bioassessment in subterranean ecosystems – primary outcomes

- Tested sampling approaches for eDNA metabarcoding
- Verified existing and new eDNA metabarcoding assays
- Developed a best-practice framework for a Barcode Reference Library (BRL) for subterranean species
- Expanded eDNA research into conservation genomics (Drs Nicole White and Mattia Sacco)
- Continued taxonomy (Drs Giulia Perina and Danielle Stringer)



# ARC Linkage Project 2019 publications - to date

- van der Heyde, M., Alexander, J., Nevill, P., Austin, A. D., Stevens, N., Jones, M., & Guzik, M. T. (2023). Rapid detection of subterranean fauna from passive sampling of groundwater eDNA. *Environmental DNA*, 5(6), 1706-1719. <https://doi.org/10.1002/edn3.491>
- Saccò, M., Mammola, S., Altermatt, F., Alther, R., Bolpagni, R., Brancelj, A., Brankovits, D., Fišer, C., Gerovasileiou, V., Griebler, C., Guareschi, S., Hose, G. C., Korbel, K., Lictevout, E., Malard, F., Martínez, A., Niemiller, M. L., Robertson, A., Tanalgo, K. C., Guzik, M.T., ... Reinecke, R. (2023). Groundwater is a hidden global keystone ecosystem. *Global Change Biology*, 30, e17066. <https://doi.org/10.1111/gcb.17066>.
- Perina, G., Camacho, A. I., Danks, M., White, N., & Guzik, M. T. (2023). Two new species of *Atopobathynella* (Parabathynellidae, Bathynellacea) from the Pilbara region, Australia. *Systematics and Biodiversity*, 21(1), 2228326. <https://doi.org/10.1080/14772000.2023.2228326>
- van der Heyde, M.; White, N., Nevill, P., Austin, A.D, Stevens, N., Jones, M., Guzik, M.T. (2023) Taking eDNA underground: factors affecting eDNA detection of subterranean fauna in groundwater. *Molecular Ecology Resources*. <https://doi.org/10.1111/1755-0998.13792>.
- Stringer, D. N., King, R. A., Austin, A. D., & Guzik, M. T. (2022). *Pilbarana*, a new subterranean amphipod genus (Hadzioidea: Eriopisidae) of environmental assessment importance from the Pilbara, Western Australia. *Zootaxa*, 5188(6), 559-573. <https://doi.org/10.11646/zootaxa.5188.6.4>.
- Saccò, M., Blyth, A.J., Douglas, G., Humphreys, W.F., Hose, G.C., Davis, J., Guzik, M.T., Martínez, A., Eberhard, S.M. and Halse, S.A., (2022). Stygofaunal diversity and ecological sustainability of coastal groundwater ecosystems in a changing climate: The Australian paradigm. *Freshwater Biology*, 67(12), 2007-2023. <https://doi.org/10.1111/fwb.13987>.
- Saccò, M., Guzik, M.T., van der Heyde, M.; Nevill, P., Cooper, S.B.J. Austin, A.D, Coates, P.J., Allentoff, A. E. and White, N.E. (2022) eDNA in subterranean ecosystems: applications, technical aspects and future prospects. *Science of the Total Environment*. 820: 153223. <http://dx.doi.org/10.1016/j.scitotenv.2022.153223>.





## **Building a custom barcode reference library for subterranean groundwater fauna living in the ancient Pilbara landscape.**

M. T. Guzik, J. Thornhill, D. N. Stringer, S. J. B. Cooper, A. D. Austin, W. F. Humphreys, M. J. Hillyer, A. M. Hosie, L. Kirkendale, J. A. Huey, M. van der Heyde, N. White



# Problem

## Identification of subterranean fauna to species is critical to Environmental Impact Assessment

- Undertaken by non-specialists
- Time consuming
- Expensive
- Inconsistent
- Most species are unknown



# eDNA for biomonitoring of subterranean fauna

#OTU ID	Blast of OTU Seq	Type	Sample 1
OTU14	Calanoida	Stygofauna	0
OTU1	Cyclopidae	Stygofauna	7
OTU24	Cyclopidae	Stygofauna	0
OTU3	Harpacticoida	Stygofauna	0
OTU23	Macrostromidae	Stygofauna	0
OTU15	Naididae	Stygofauna	0
OTU41	Parabathynellidae	Stygofauna	0
OTU28	Plectidae	Stygofauna	0
OTU5	Seriata	Stygofauna	0
OTU30	Stenostomidae	Stygofauna	0
OTU13	Tubificina	Stygofauna	0



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eDNA in subterranean ecosystems: Applications, technical aspects, and future prospects

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<sup>e</sup> Evolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide 5000, SA, Australia

<sup>f</sup> Bedford Institute of Oceanography, Fisheries and Oceans Canada, 1 Challenger Drive, 1006, Dartmouth, Nova Scotia B2Y 4A2, Canada

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# Aim

## Develop custom Barcode Reference Library (BRL) for Pilbara subterranean fauna

- Findable, Accessible and Reusable database (FAIR)
- Homologous DNA sequences that correspond to metabarcoding assay regions
- Metadata
- Unified nomenclature
- Phylogenetic backbone





# DATABASES



## Public Data Portal

A data retrieval interface that allows for searching all 1.3M public records in BOLD using multiple search criteria including, but not limited to, geography, taxonomy, and depository. Search results can be summarized, plotted on high-res maps, and downloaded.



## BIN Database

A searchable database of Barcode Index Numbers (BINs), sequence clusters that closely approximate species. This system allows for rapid validation and use of barcode data where taxonomic data are lacking or unverified.



## Publications

A collection of barcode publications and publications that have utilized barcode records.



## Primer Database

A comprehensive registry of primers used in the generation of barcode sequences. The registry is maintained by users of BOLD.

### DATABASES

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[Public Data Portal](#)  
[Taxonomy Browser](#)

### RESOURCES

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- Michelle T Guzik <
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## Record List - DS-SUBFAUNA

### Specimens

4976 Specimens

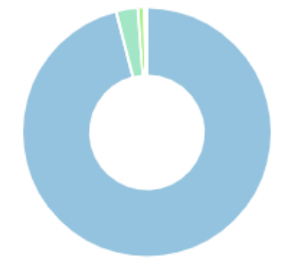
- GPS: 1060 / 4976
- Country: 1352 / 4976
- Images: 0 / 4976
- Barcode Compliant: 2 / 4976

### Sequences

5910 Sequences

- COI-5P: 4454/4976
- 16S: 202/4976
- 18S: 595/4976
- 28S: 466/4976
- 12S: 137/4976
- COI-3P: 56/4976

### Taxonomy



- Arthropoda (phylum): 4779
- Annelida (phylum): 140
- Chordata (phylum): 41
- Mollusca (phylum): 13
- Platyhelminthes (phylum): 3

### Issues

- Seqs lacking successful traces**
  - 12S [137], 16S [202], 18S [595], 28S [463], COI-3P [4452]
- Seqs with stop codons**
  - 0
- Contaminated seqs**
  - 0
- Problematic records flagged**
  - 26

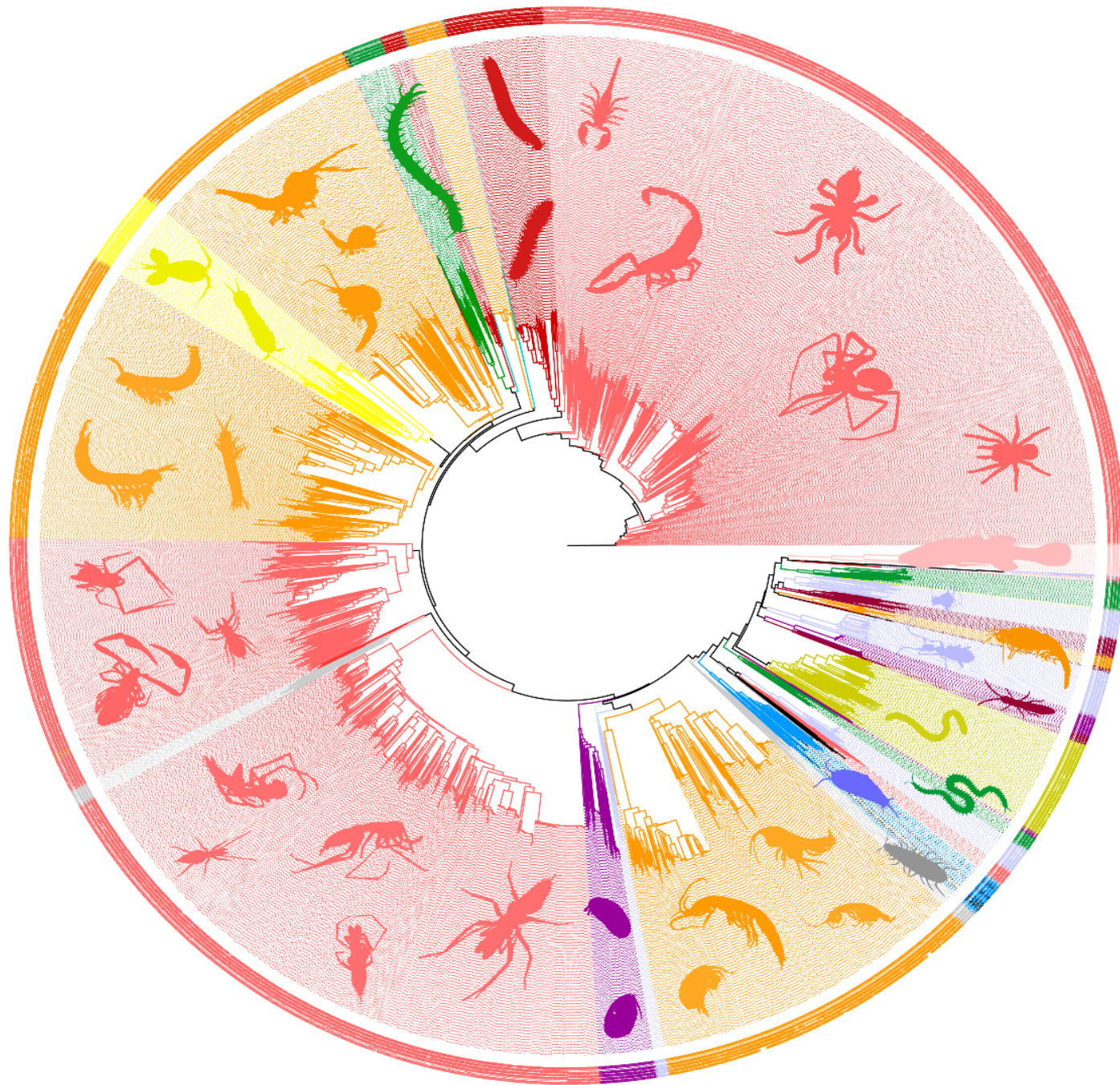


100 records per page

Search:

Select	Identification	Specimen Page	Sequence Page	Extra Info	BIN	Record Flags					Bases [Ambig]					Tags		
						📍	📷	📄	📊	✖	✖	🚫	COI-5P	16S	18S		28S	12S
<input type="checkbox"/>	Armadillidae	MZ427859	GBMND86385-21		BOLD:AEG6572	📍	0	0				687[0n]	0	0	0	0	0	
<input type="checkbox"/>	Armadillidae	MZ427868	GBMND86384-21		BOLD:AEG6572	📍	0	0				687[0n]	0	0	0	0	0	
<input type="checkbox"/>	Naididae	MW021288	GBMNC20216-20		BOLD:AEG2949		0	0				676[0n]	0	0	0	0	0	
<input type="checkbox"/>	Armadillidae	MZ427865	GBMND86382-21		BOLD:AEG6572	📍	0	0				687[0n]	0	0	0	0	0	
<input type="checkbox"/>	Gnaphosidae	MW621140	GBMND59095-21		BOLD:AEI6977		0	0				658[0n]	0	0	0	0	0	
<input type="checkbox"/>	Missulena sp. 1 JAH-2021a	MZ462254	GBMNE24664-21		BOLD:AEM7572		0	0				658[0n]	0	0	0	0	0	





- Actinopterygi
- Arachnida
- Chilopoda
- Clitellata
- Collembola
- Copepoda
- Diplopoda
- Diplura
- Gastropoda
- Insecta
- Malacostraca
- Ostracoda
- Pauropoda
- Polychaeta
- Symphyla

0.08



# Impact

## Advance new technologies in biomonitoring of subterranean fauna

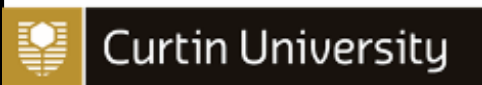
- Established information resources that follow international principals of Findable Accessible Interoperable and Reusable (FAIR) data management
- Developed a best practice protocol for improving information resources available to industry and environmental stakeholders, and the scientific community
- Promoted a unified nomenclature for taxonomic OTU assignment that is stable and repeatable
- Continue to progress taxonomy







Gorgon Barrow Island  
Net Conservation Benefits Fund  
[www.gorgon-ncb.org.au](http://www.gorgon-ncb.org.au)



Australian Government  
Australian Research Council



- ARC Linkage Project (LP190100555) (Adelaide and Curtin University)
- ARC Linkage Project (LP14010055) (Adelaide University)
- Net Conservation Benefits Fund (Western Australian Museum)

