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

WABSI Workshop, 28th February 2024

DR NICOLE WHITE, TRACE AND ENVIRONMENTAL DNA LAB, CURTIN UNIVERSITY, PERTH, WA

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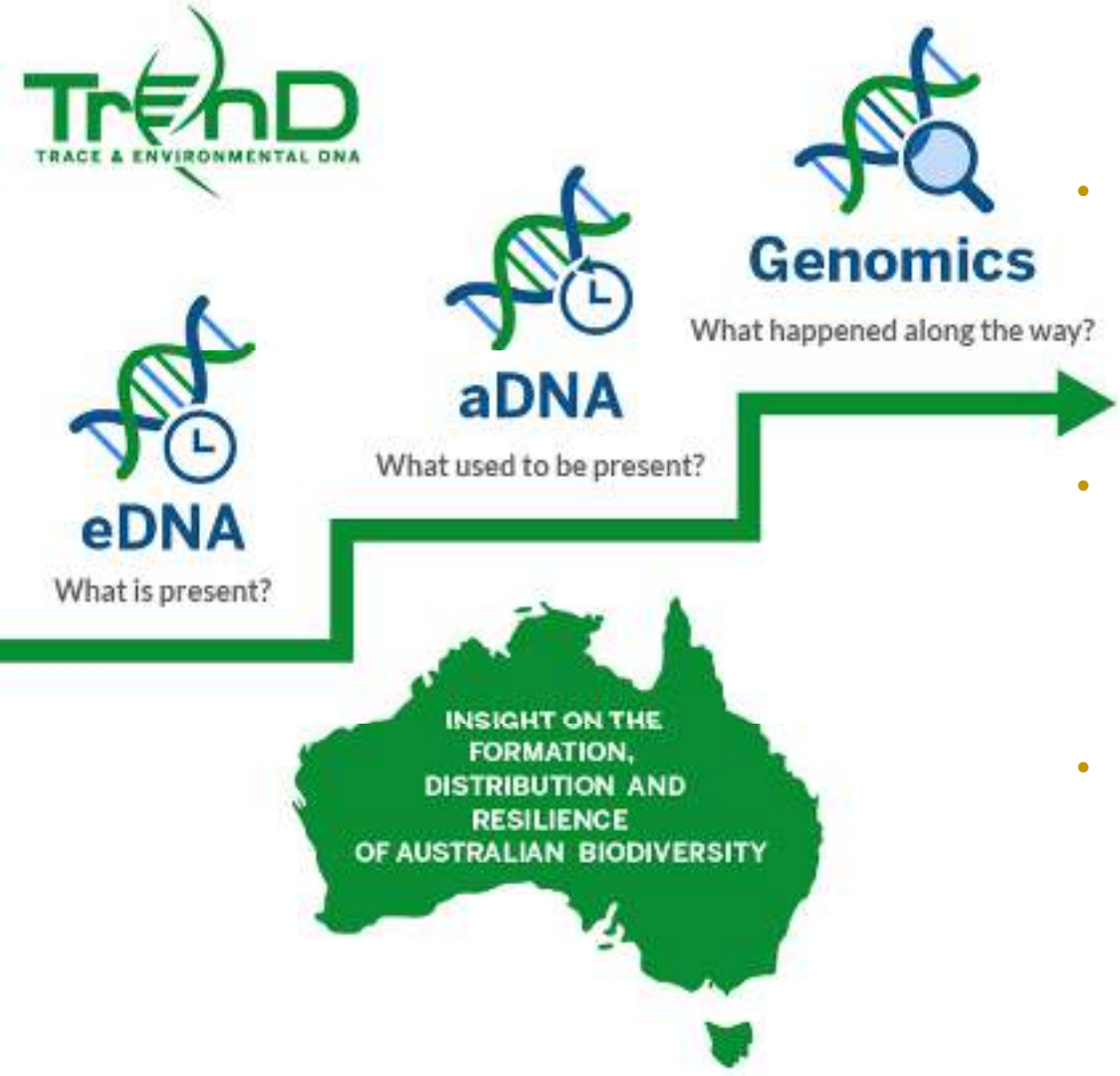
FIRST NATIONS ACKNOWLEDGEMENT

Curtin University acknowledges all First Nations of this place we call Australia and recognises the many nations who have looked after Country for more than 60,000 years.

We are honoured and grateful for the privilege to maintain campuses operating in Boorloo (Perth) and Karlkurla (Kalgoorlie) in Australia. We pay our respects to Elders past and present as Custodians and Owners of these lands. We recognise their deep knowledge and their cultural, spiritual and educational practices, and aspire to learn and teach in partnership with them.

Curtin also acknowledges First Nations peoples connected with our global campuses. We are committed to working in partnership with all Custodians and Owners to strengthen and embed First Nations' voices and perspectives in our decision-making, now and into the future.



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eDGES

- eDNA: What is present?
  - eDNA is ubiquitous in the environment and this property can be explored and utilized in numerous applications.
- aDNA: What used to be present?
  - Involves the isolation and analysis of *old* degraded DNA which can provide a window into the past (i.e. changes in biodiversity over time).
- Evolutionary Genomics: What happened along the way?
  - Sequencing and analyzing genomic datasets from selected species we gain knowledge on natural selection processes and the evolutionary impacts of habitat/climate change.

SuRGE

Subterranean Research & Groundwater Ecology Group

A small multidisciplinary team who specialize in eDNA approaches for biomonitoring, functional ecological studies, taxonomy and systematics.

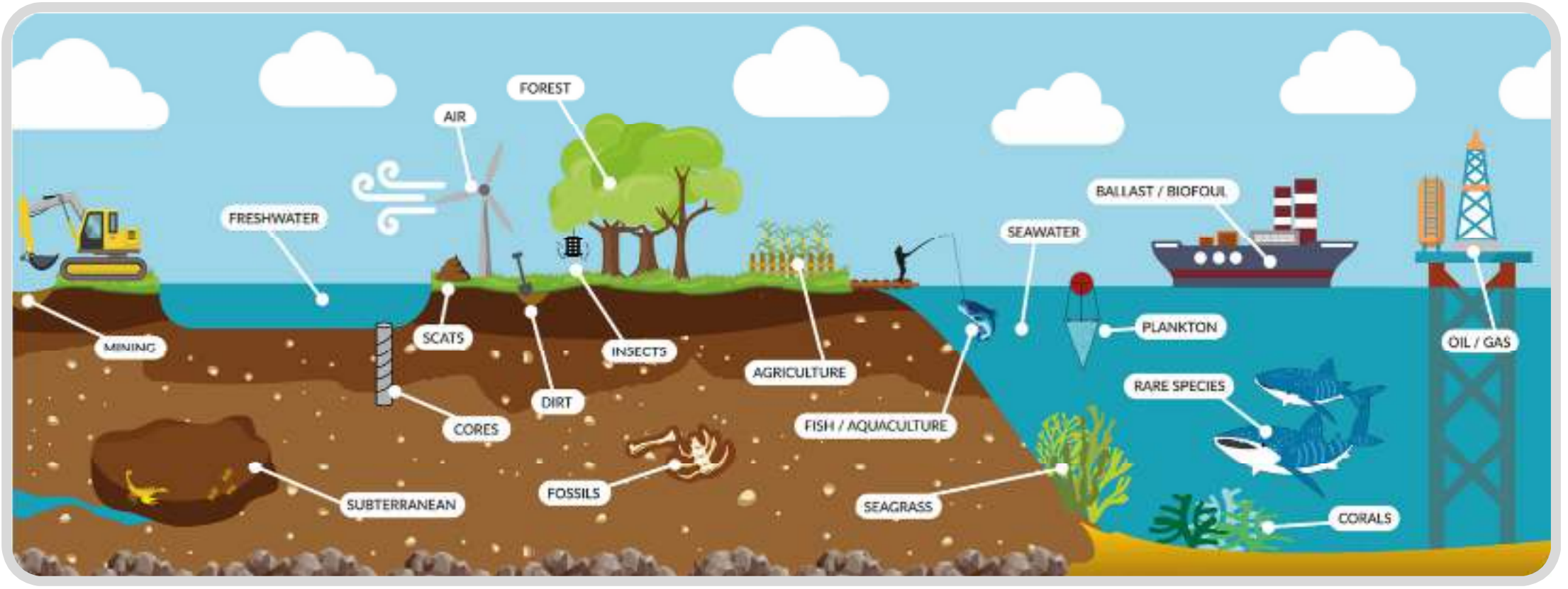
Dr Mattia Sacco

Dr Nicole White

Dr Mieke van der heyde

Dr Giulia Perina

A new way to monitor the environment



eDNA Assay Validation

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ORIGINAL ARTICLE | Open Access

A validation scale to determine the readiness of environmental DNA assays for routine species monitoring

Bettina Thalinger, Kristy Deiner, Lynsey R. Harper, Helen C. Rees, Rosetta C. Blackman, Daniela Sint, Michael Traugott, Caren S. Goldberg, Kat Bruce

First published: 16 March 2021 | https://doi.org/10.1002/edn3.189 | Citations: 69

SHORT COMMUNICATION | Full Access

Robust environmental DNA assay development and validation: A case study with two vulnerable Australian fish

Jackson Wilkes Walburn, Meaghan L. Rourke, Elise Furlan, Joseph D. DiBattista, Matt K. Broadhurst, Ashley M. Fowler, Julian M. Hughes, Stewart Fielder

First published: 03 April 2022 | https://doi.org/10.1002/aqc.3809 | Citations: 2

eDNA Best Practice and Standards

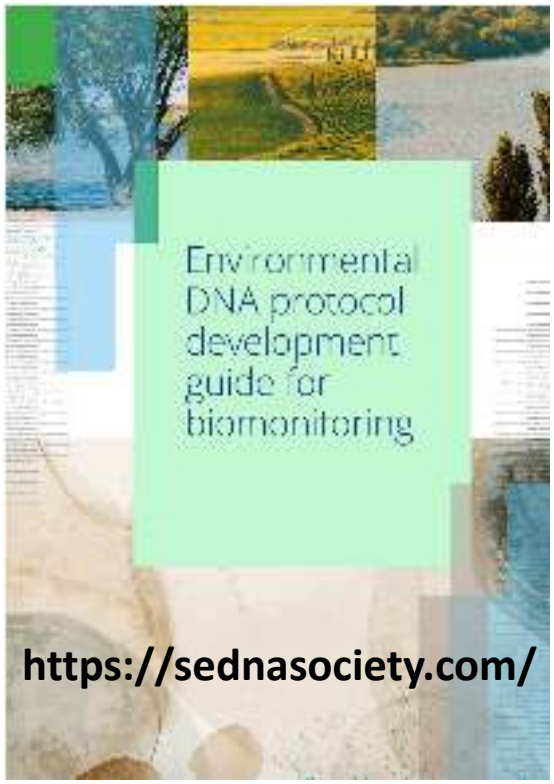
Mission

Promoting science and industry collaboration across Australia and New Zealand to advance best practice eDNA methods and adoption in government, private and community sectors.

SOUTHERN eDNA SOCIETY

De Brauwer M, Chariton A, Clarke LJ, Cooper MK, DiBattista J, Furlan E, Giblot-Ducray D, Gleeson D, Harford A, Herbert S, MacDonald AJ, Miller A, Montgomery K, Mooney T, Noble LM, Rourke M, Sherman CDH, Stat M, Suter L, West KM, White N, Villacorta-Rath C, Zaiko A & Trujillo-Gonzalez A (2022). **Environmental DNA test validation guidelines.** National eDNA Reference Centre, Canberra.

eDNA Best Practice and Standards



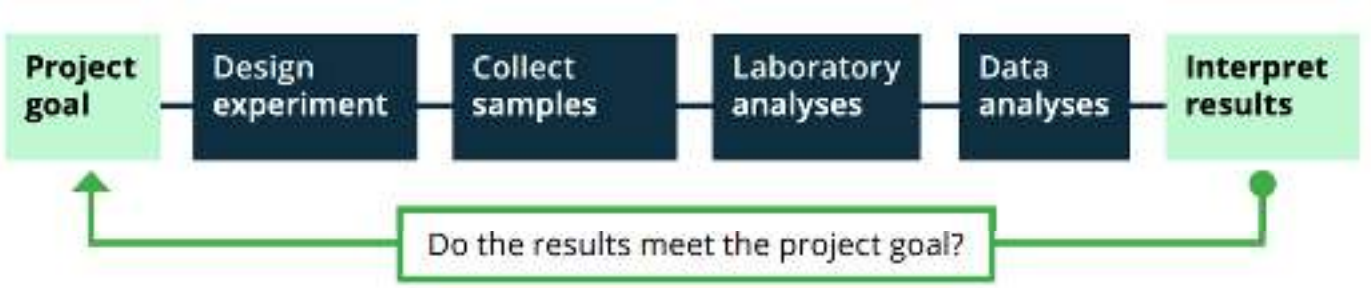
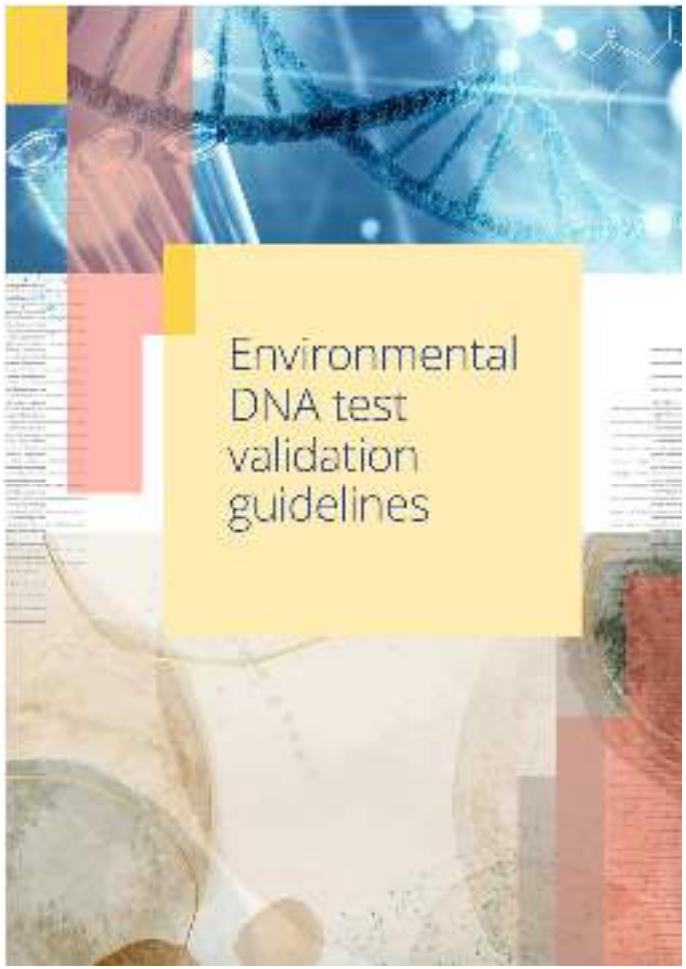


Figure 1 Steps of an eDNA project, and how they must consider the project goal if they are to be fit for purpose

De Brauwer M, Chariton A, Clarke LJ, Cooper MK, DiBattista J, Furlan E, Giblot-Ducray D, Gleeson D, Harford A, Herbert S, MacDonald AJ, Miller A, Montgomery K, Mooney T, Noble LM, Rourke M, Sherman CDH, Stat M, Suter L, West KM, White N, Villacorta-Rath C, Zaiko A & Trujillo-Gonzalez A (2022). **Environmental DNA protocol development guide for biomonitoring.** National eDNA Reference Centre, Canberra.



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eDGES

Species-specific assay development and validation 12

Figure 1 Steps of an eDNA project, and how they must consider the project goal if they are to be fit for purpose

Assay purpose and selection

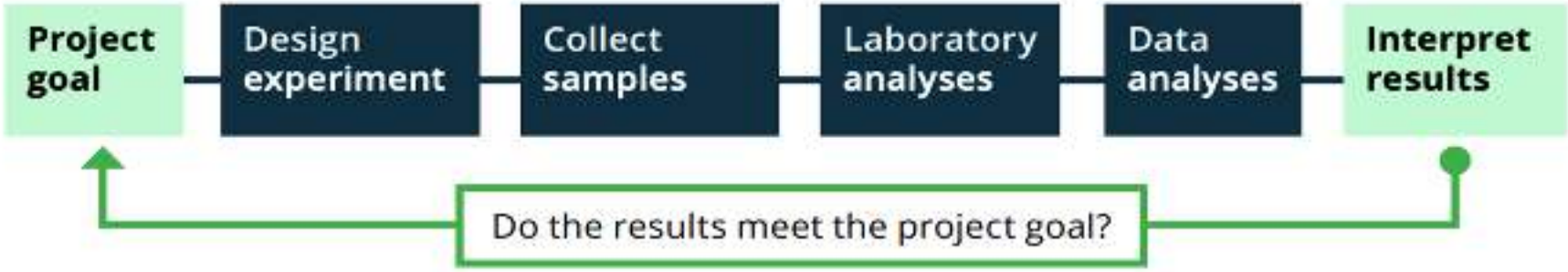
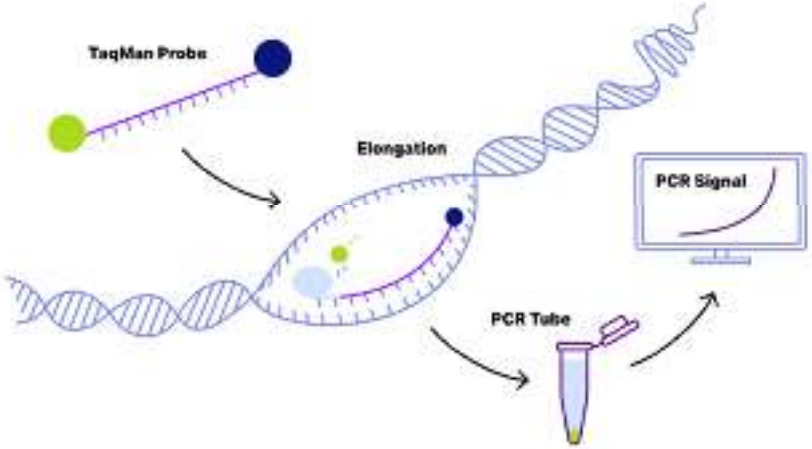
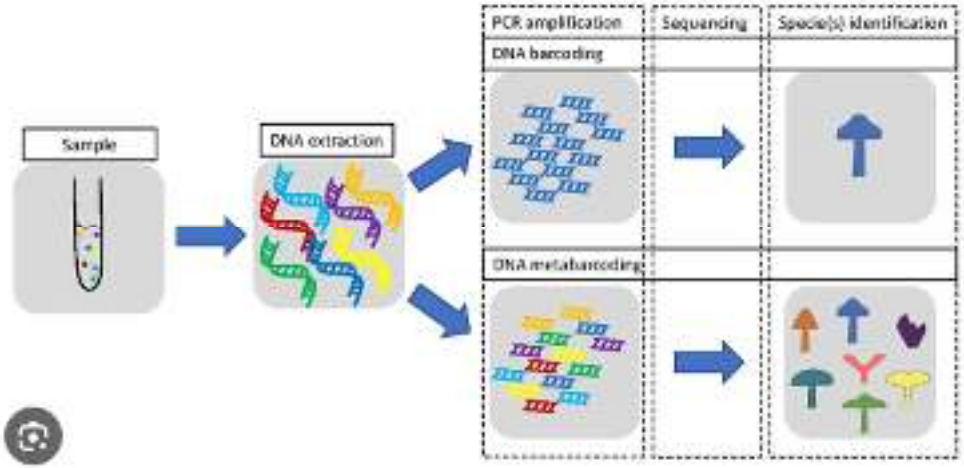


Figure 1 Steps of an eDNA project, and how they must consider the project goal if they are to be fit for purpose

1) The purpose of the assay is defined at the outset (Experimental Design).
2) Determine whether a species-specific, metabarcoding or combined approach is appropriate.
3) Develop and validate the assay to ensure it is fit for purpose.
4) Ensure that results are appropriate are relevant to management or regulatory authorities.

Assay selection: Probe vs Metabarcoding





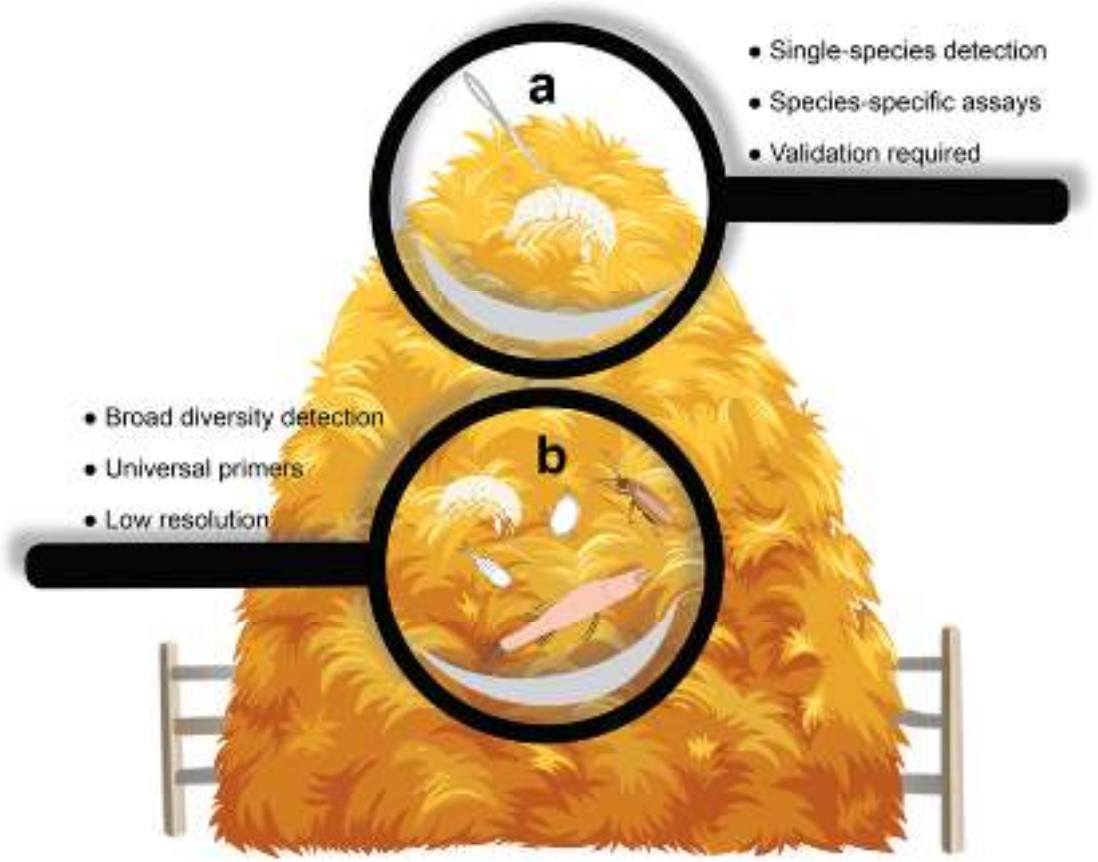
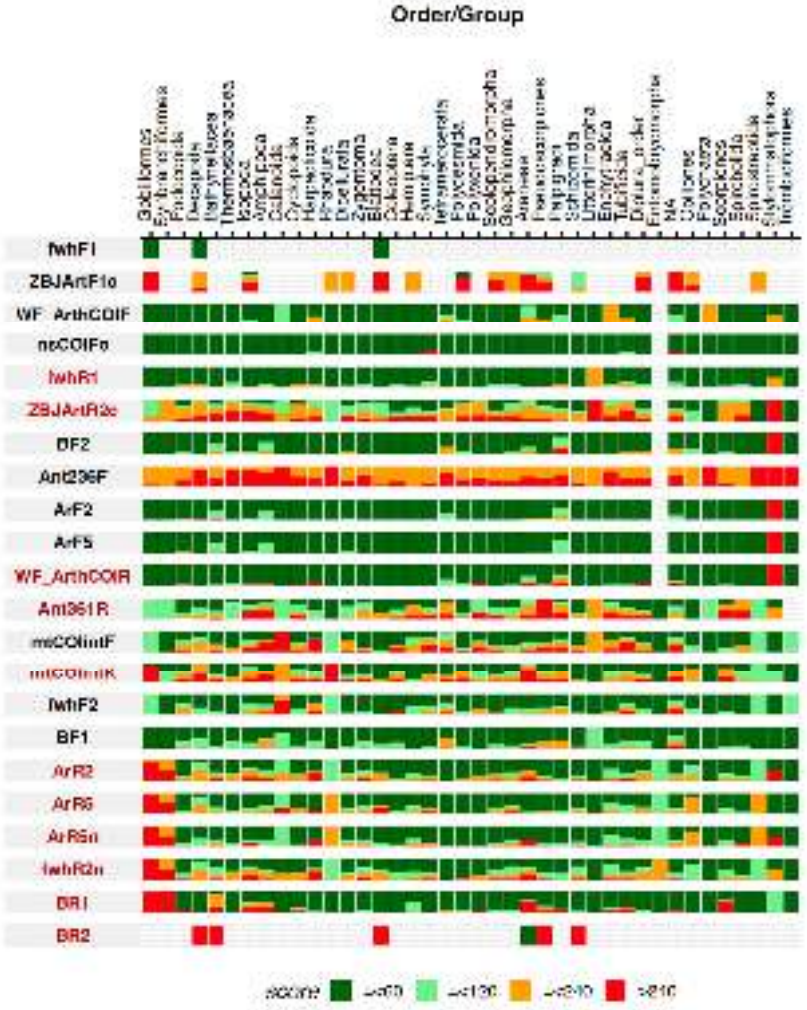
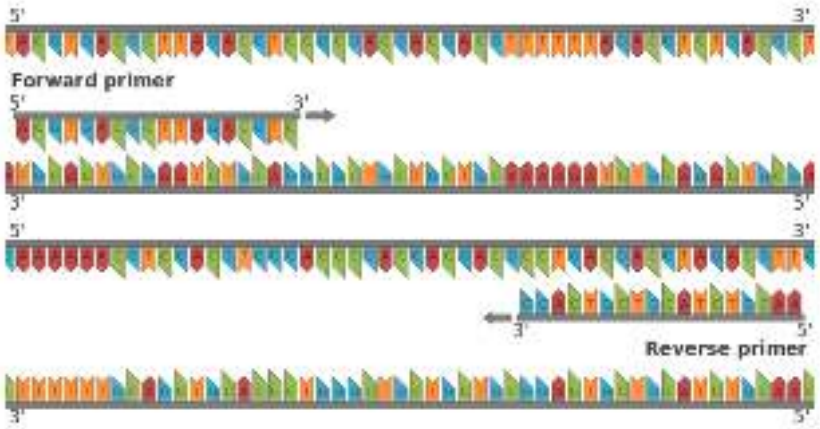
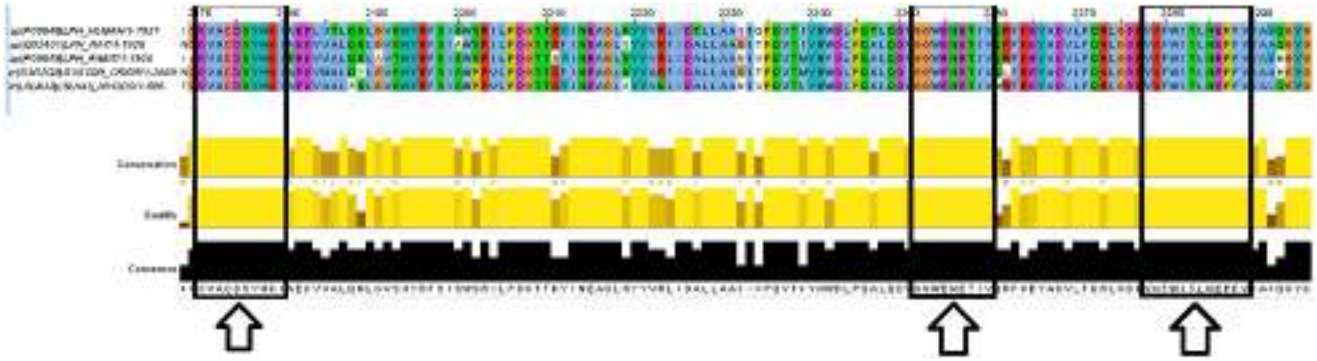


Diagram depicting the "needle vs the haystack" design and the main characteristics of each approach. (a) Singular species approach ('needle') and (b) ecological community based study design ('haystack').

In-silico Testing of Assay



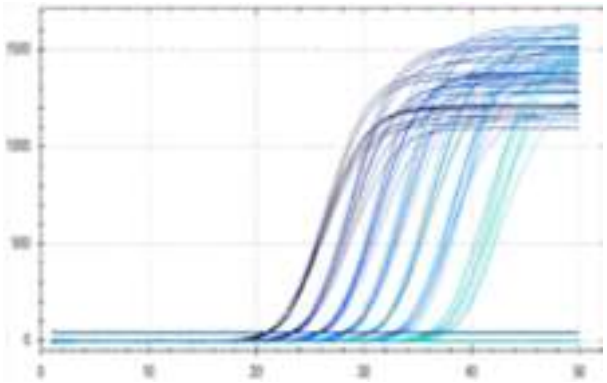


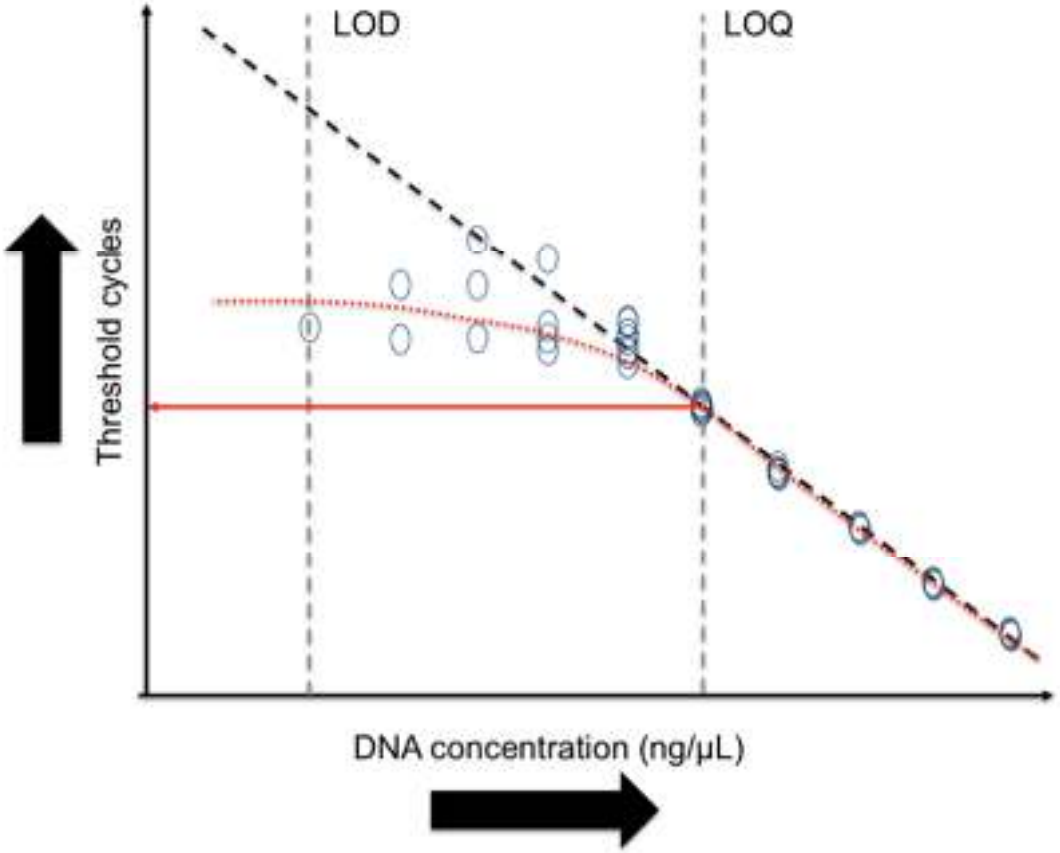


In-vitro Testing of Assay

Limit of Detection (LOD): the ***smallest concentration*** of DNA in a test sample that can easily be ***distinguished from zero*** (Blank or NTC).

Limit of Quantification (LOQ): The ***smallest concentration*** of DNA in a test sample that can be determined with ***acceptable repeatability and accuracy***.





In-vitro Testing of Assay

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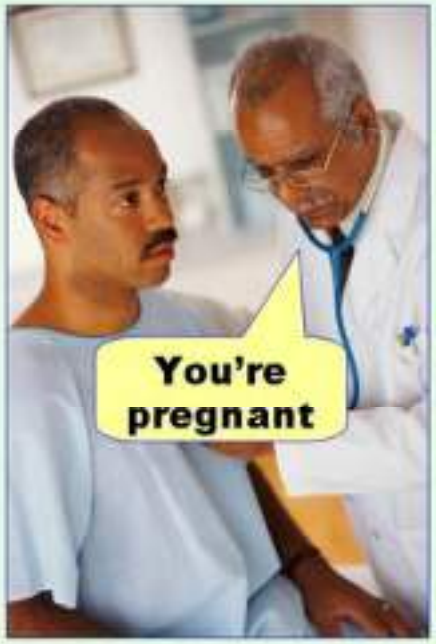
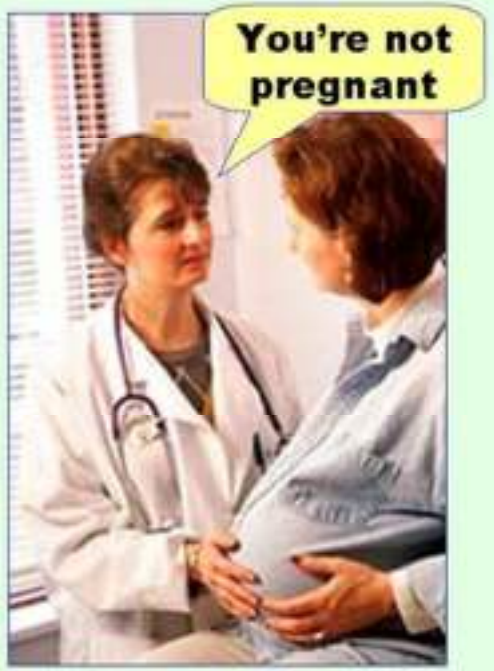
	Test says you don't have it	Test says you do have it
You really don't have it	TRUE NEGATIVE	FALSE POSITIVE
You really do have it	FALSE NEGATIVE	TRUE POSITIVE

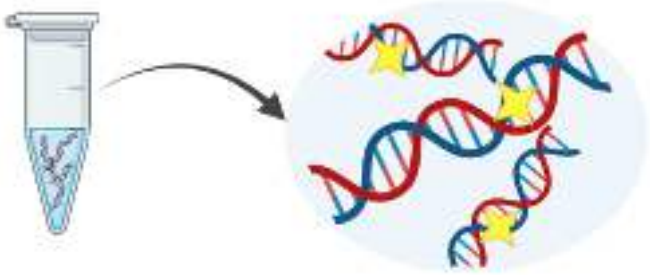
Type I error
(false positive)



Type II error
(false negative)



PCR Inhibition and Degraded DNA



Degraded DNA

Larger segments of DNA cannot be recovered when DNA molecules have fragmented into small pieces (caused by heat, water, or bacteria)

RESEARCH ARTICLE

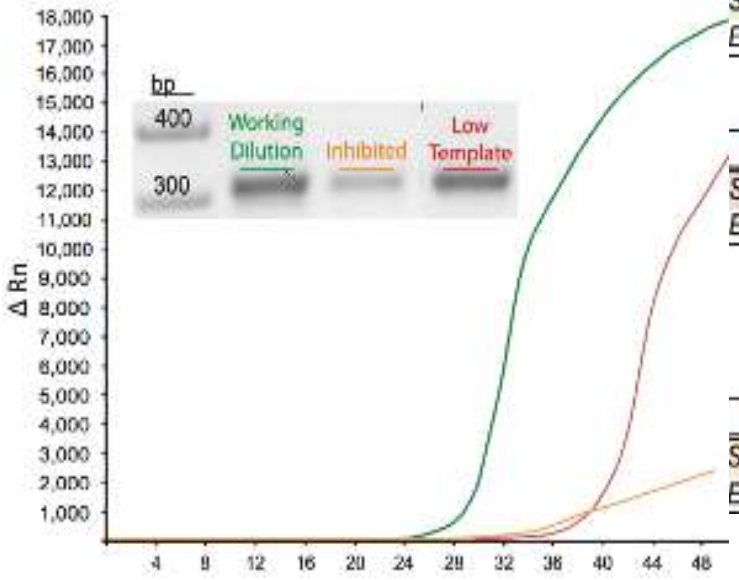
From Benchtop to Desktop: Important Considerations when Designing Amplicon Sequencing Workflows

Dáithí C. Murray, Megan L. Coghlan, Michael Bunce*

Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, Western Australia, Australia

* michael.bunce@curtin.edu.au

The importance of DNA input



Percentage Abundance

	GS Junior	
Taxon	Rep. 1	Rep. 2
Sardinops	93.5	85.4
Engraulis	6.5	14.6

	GS Junior	
Taxon	Rep. 1	Rep. 2
Sardinops	64.8	100
Engraulis	35.2	0

	GS Junior	
Taxon	Rep. 1	Rep. 2
Sardinops	100.0	99.3
Engraulis	0.0	0.7

PLOS ONE | DOI:10.1371/journal.pone.0124671, April 22, 2015

Environmental DNA metabarcoding

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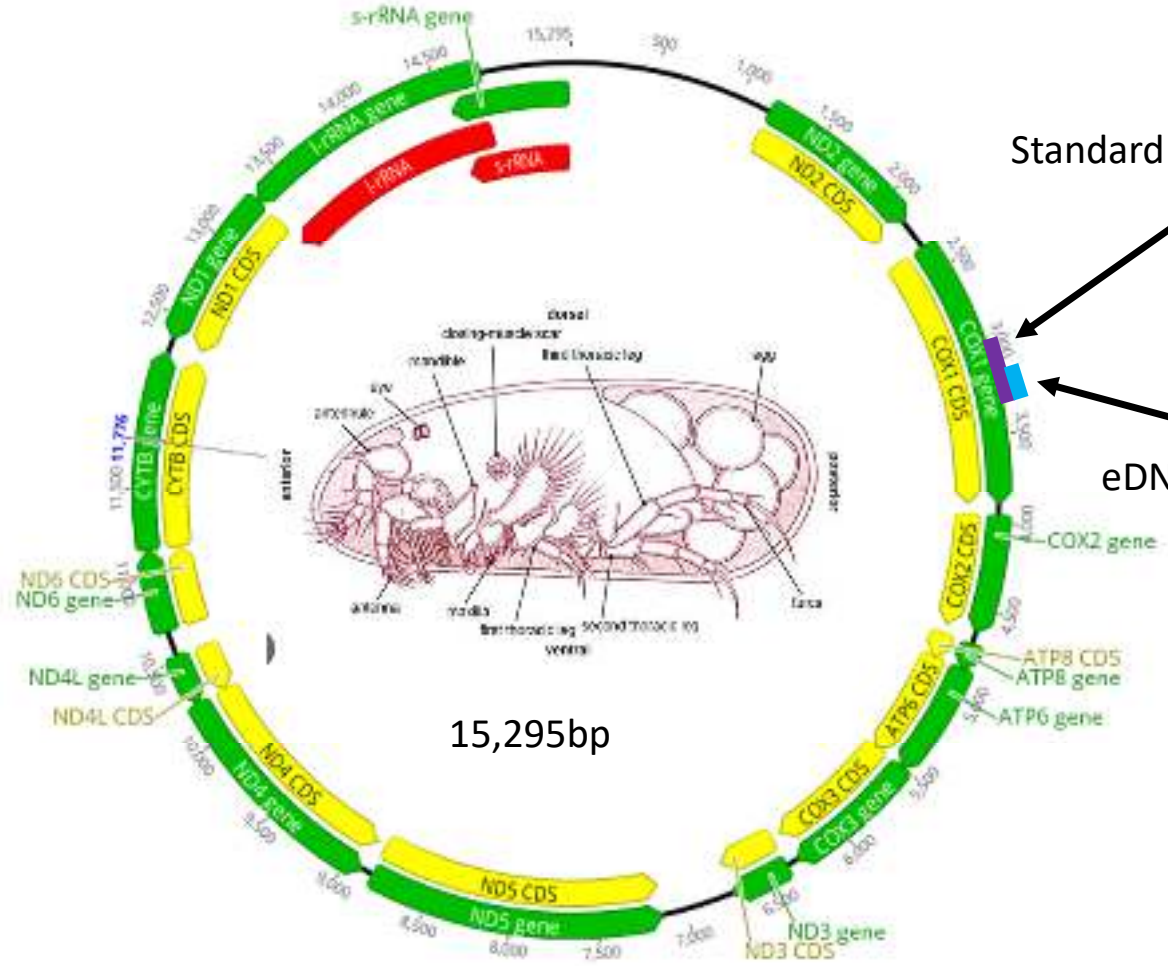
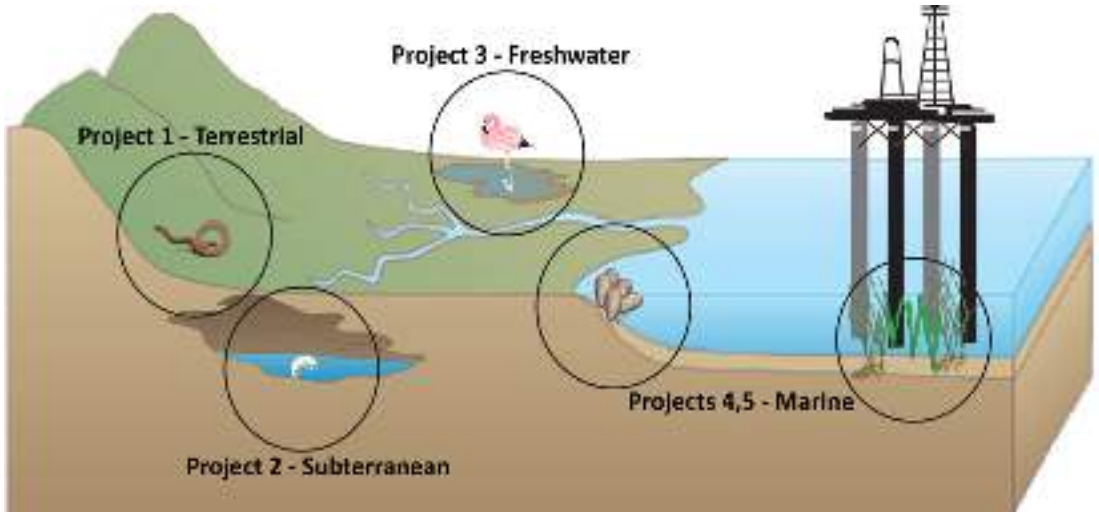
eDGES



Standard barcode region is ~4% of the genome (~600bp)

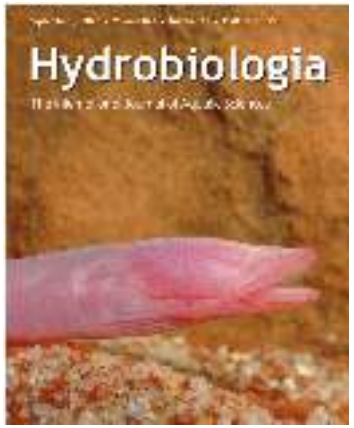
eDNA: metabarcoding region is ~1.3% of the genome

eDNA for Global Environment studies



- Developing and apply eDNA techniques to address biodiversity loss and sustainability
- Translate knowledge into outcomes
- Funded by BHP's Social Investment Framework
- Builds and expands eDNA's utility as a biological monitoring tool

eDGES Project 2: eDNA for subterranean fauna detection and conservation



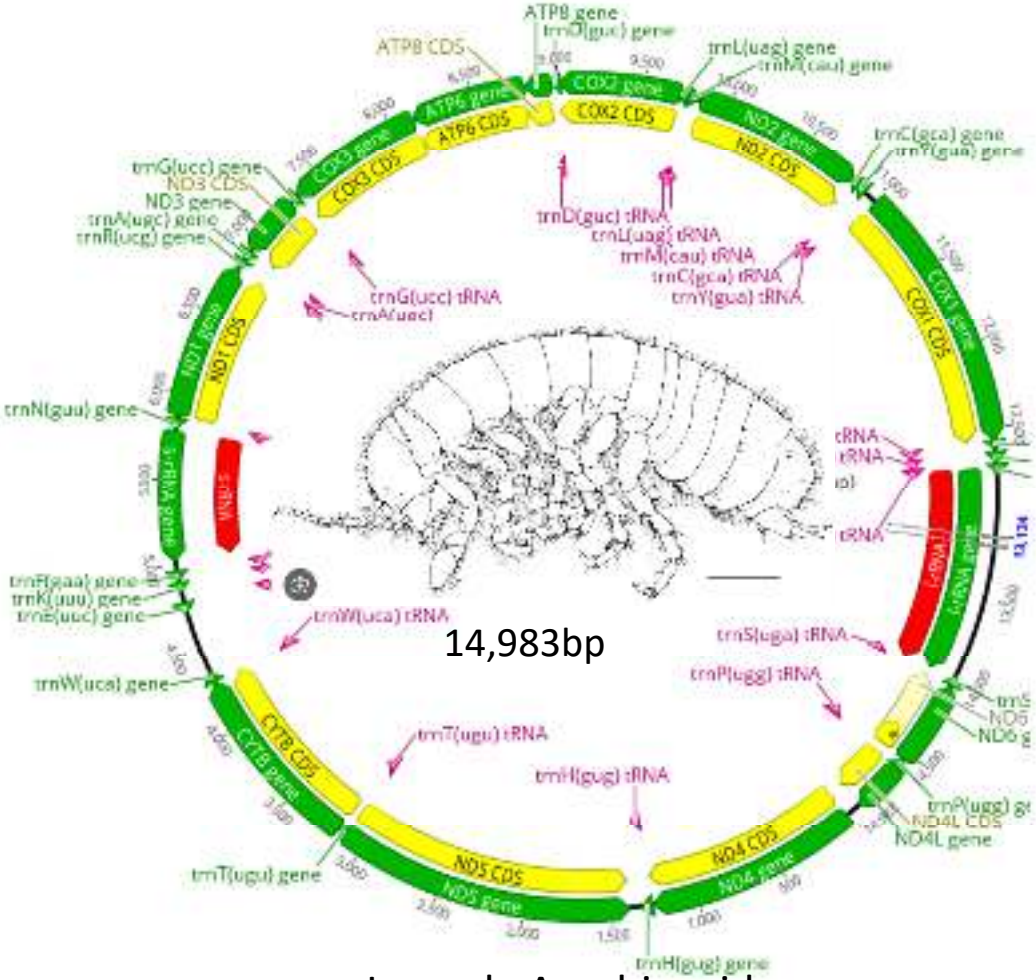
Aims:

- Develop new eDNA tools to describe subfauna biodiversity (What's beneath our feet?)
- Quantify how genetically different the organisms are between sites (i.e. understand short range endemism)

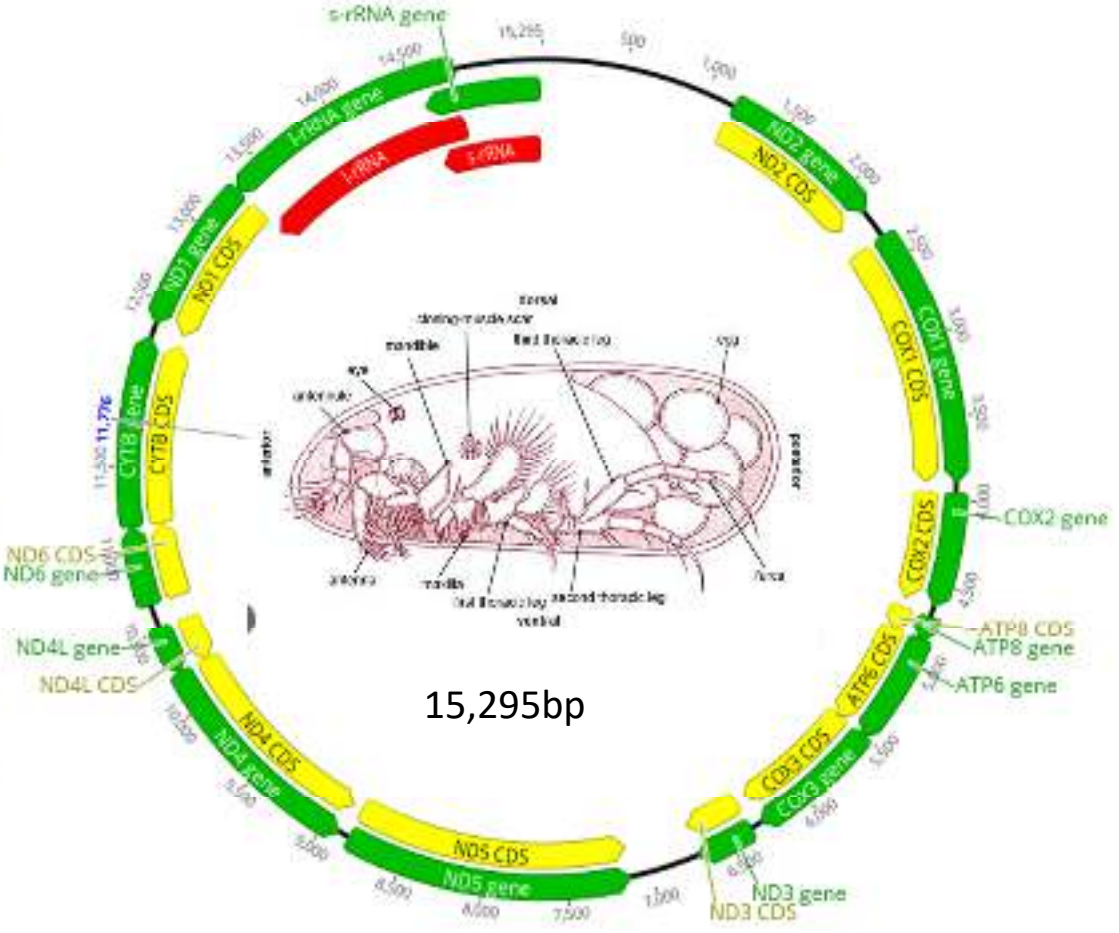
How:

- Sequence mitochondrial genomes (Beyond the barcode) to provide the foundation for new eDNA markers to be developed and applied

eDGES Project 2: Moving Beyond the Barcode

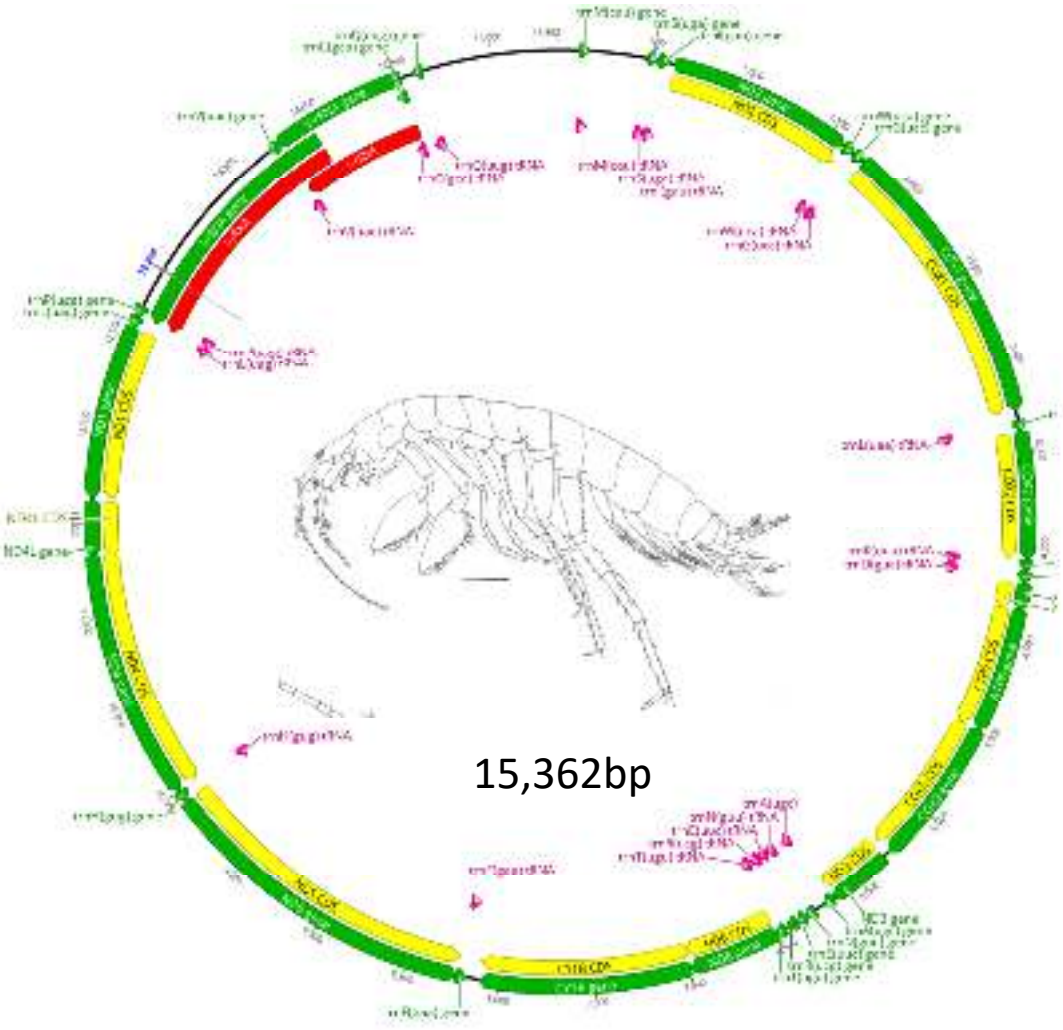


Isopoda Amphisopidae

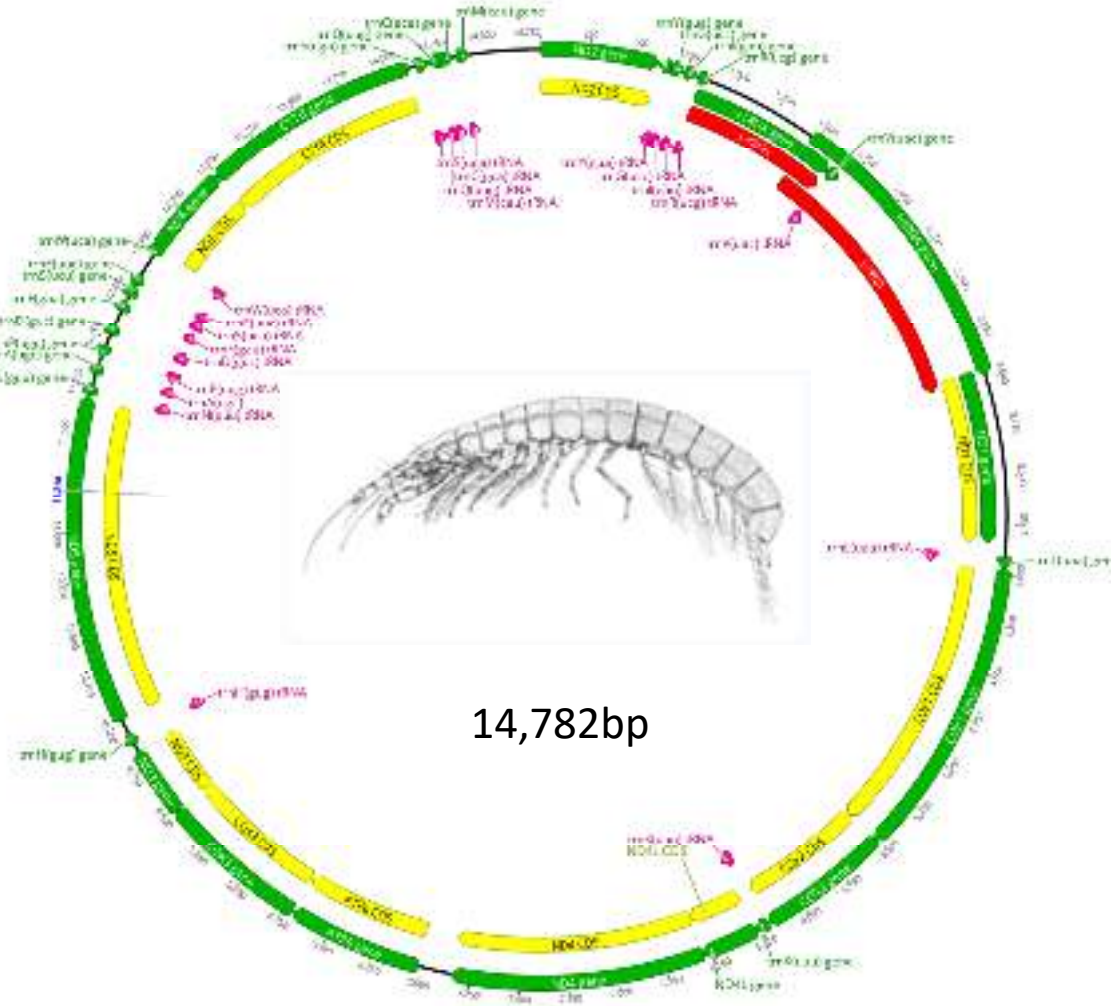


Ostracoda

eDGES Project 2: Moving Beyond the Barcode



Amphipoda *Chydaekata acuminata*



Syncarida *Brevisomabathynella pilbarensis*

Acknowledgements: Dr Michelle Guzik, Dr Nick Murphy, Prof. Morten Allentoft, Dr Mieke van der heyde, Dr Mattia Sacco and the TrEnD lab

Thank you

Make tomorrow better.

BHP | Curtin University | TrEnD | Arabana Aboriginal Corporation | La Trobe University | Adelaide University Australia