

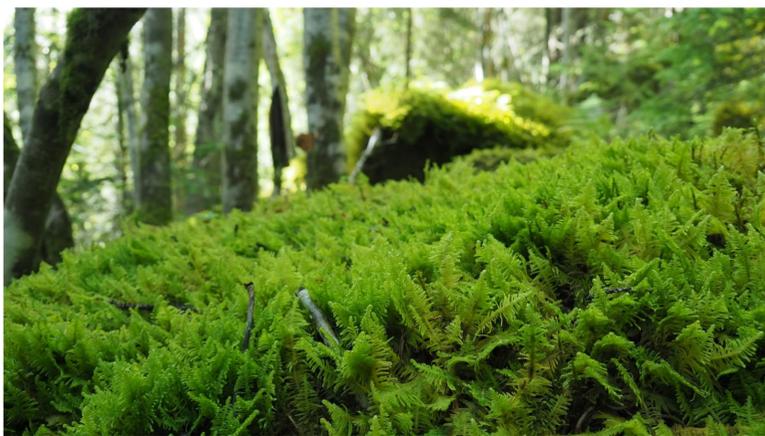
Barcoding Norwegian water bears (Tardigrada)

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Background

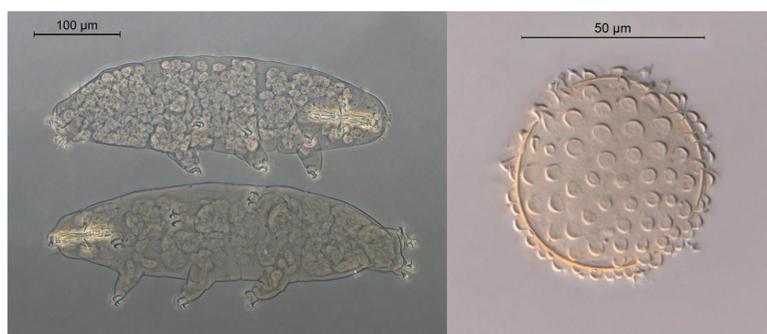
Tardigrades are microscopic animals inhabiting the great majority of ecosystems throughout the world. Essentially they are aquatic, since they need water to move and reproduce. In terrestrial habitats they are particularly numerous in moist moss and lichens. Many species are able to survive extreme conditions by temporally suspending their metabolism thanks to an adaptive strategy called cryptobiosis.



The knights plume moss (*Ptilium crista-castrensis*) is a favorable habitat for many tardigrade species.

Challenges

-  Small size of the animals makes it challenging to obtain DNA, identify species, and recover voucher skins.
-  Some species lack type material.
-  There are several cryptic and semi-cryptic species complexes.
-  Eggs and multiple specimens from the same population is often needed for species ID.



Species identification often require paragenophores from the same population. Many closely related species are separated by egg morphology.

Conclusion

Although time consuming, single-specimen DNA barcoding of tardigrades can contribute significantly to the Barcode of Life reference library. The small size of the animals makes recovery of voucher cuticles difficult and lowers sequencing success. Therefore, it is crucial to preserve paragenophores from the same population and analyse more specimens from each species than normal for macroinvertebrates.

Acknowledgements

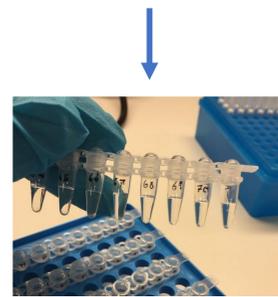
Thanks to The Norwegian Biodiversity Information Centre (Artsdatabanken) for funding the workshop on which these results originate.

Methods

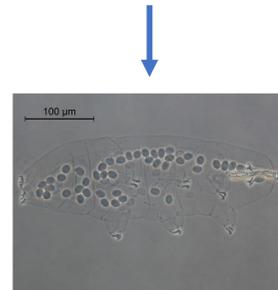
We tested a protocol to retrieve DNA and COI barcodes from single specimens, while at the same time recover vouchers:



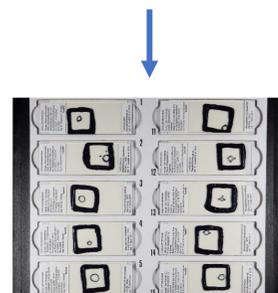
Extracting tardigrades. Sorting, preliminary ID, photo.



Isolation of DNA using Quick Extract, pipette out extract after spinning down skin. PCR, sequencing at CCDB.



Recovery of tardigrade skin from extract tube.



Slide mounting in Hoyer's solution. Identification by comparison with specimens from same sample.

Results

-  62 of 84 specimens received sequences.
-  31 sequences barcode compliant.
-  6 sequences contaminated, 25 with poor sequence quality.
-  52% voucher recovery.
-  18 BINs.
-  7 named species so far.