

Scanning electron microscopy image of *Diploechiniscus oihonneae*
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THE CELEBRITIES OF THE MICROCOSMOS AREN'T ALWAYS EASY TO FIND: DETECTING TARDIGRADES IN ENVIRONMENTAL DNA

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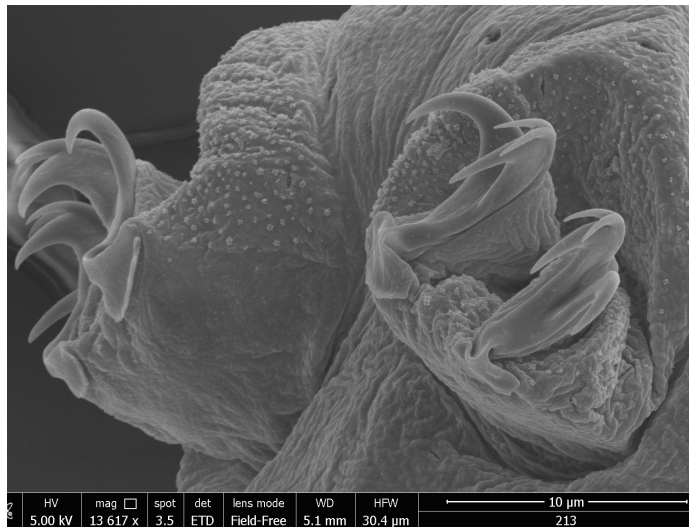
The hidden diversity of tardigrades is being uncovered in Norwegian forests using DNA barcoding and metabarcoding

Found across every continent on Earth, to now potentially living on our moon, tardigrades are some of the most resilient microorganisms we know of. But despite our fascination with these microscopic water bears, there is still much to discover. Our study is exploring the applicability of using environmental DNA to facilitate the examination of tardigrade diversity.

The popular narrative that tardigrades can withstand anything – from -272 degrees Celsius to as high as 150 degrees Celsius, 6,000 times the atmospheric pressure, extreme radiation, and vacuum – has earned them celebrity status of the microcosmos. However, tardigrades are more than just superstars. They constitute their own phylum of life, ranked at the same tax-

onomic level as arthropods (insects and spiders), and currently hold around 1,270 described species. Many of these species fulfill ecologically important roles related to the breakdown of organic material in the soil. Other species are found in freshwater streams, sediments, mosses, lichens, and leaf-litter, occurring in most ecosystems throughout the world. As with other tiny taxa, telling tardigrade species apart can be challenging. Confident identifications of many species depend on the presence of both adult specimens and eggs. Additionally, tardigrade taxonomy is traditionally based on a limited set of morphological traits. This has resulted in several complex species groups, comprising morphologically inseparable, but genetically distinct species.

DNA barcodes offer a solution to these impediments by generating unique genetic characteristics for each of these species. In recent years, there has been an increase in the use of molecular tools on tardigrades, but currently, only a small portion of the known species have barcodes deposited in public databases. Such reference sequences are essential if tardigrades are to be included in large-scale biomonitoring methods such as metabarcoding of environmental DNA (eDNA). Our study is the first to compare the applicability of eDNA-based metabarcoding of tardigrade diversity with morphologically identified communities.



The claws of one of the species in the *Macrobiotus hufelandi* group. These species are often inseparable based on morphology, but clearly distinct species based on the COI gene.

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We extracted tardigrades and eggs from samples of moss, lichens, and leaf-litter and identified them using morphology. The 3,788 recorded tardigrade specimens and eggs were identified as 40 morphologically distinct species, of which 24 were successfully sequenced for the gene cytochrome c oxidase I (COI). These were represented by 151 successfully sequenced individuals. Interestingly, the barcodes revealed 32 genetically distinct lineages among the 24 morpho-species, showing high levels of hidden diversity.

Next, we extracted eDNA from the same environmental samples and sequenced two fragments of the COI marker and one fragment of the 18S marker using the Illumina MiSeq next-generation sequencing platform. This method recovered 57 species of tardigrades compared to the 40 species detected by conventional methods. Mostly, the two methods identified the same species (Figure 1), yet, metabarcoding detected cryp-



Collection of lichen samples during fieldwork in Southern Norway

PHOTO CREDIT: Torbjørn Ekrem

tic species elusive to morphological identification. This indicates that metabarcoding of eDNA successfully captures tardigrade diversity.

However, the credibility of such records needs to be evaluated thoroughly. While the COI marker distinguishes well between tardigrade species, the 18S marker might not be as useful as there is not sufficient sequence variation between species (a so-called barcode gap). Furthermore, the 18S marker detected *Acutuncus antarcticus* in two of the samples, a species endemic to Antarctica. This species is likely not found in Norway and highlights the danger of blindly trusting marker-based identifications without carefully evaluating taxonomic assignments and possibilities of contamination.

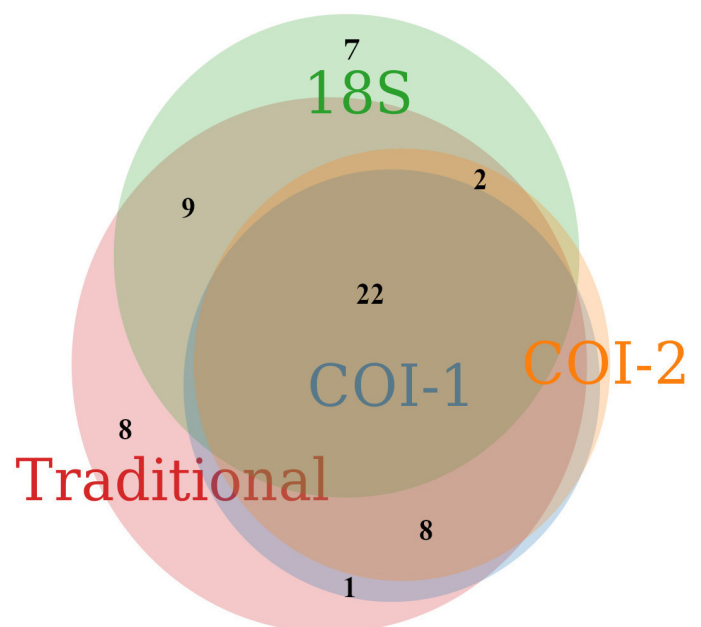


Figure 1. Overlap in species recovery by the different methods.

Our findings were dependent on our barcode reference library of locally sampled species and the use of multiple markers. As only a small portion of tardigrade species are deposited with reference sequences in public databases, both the COI and 18S markers are limited in their ability to detect species of tardigrades as most sequences will go unmatched. We demonstrate that metabarcoding is applicable for large-scale biomonitoring of tardigrades, but highlight the need for better reference libraries for tardigrade species

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Online:

<https://ibol.org/barcodebulletin/research/the-celebrities-of-the-microcosmos-arent-always-easy-to-find-detecting-tardigrades-in-environmental-dna/>