

# *One ring to rule them all*

*Constructing pipeline for mass  
barcoding of biodiversity in Ukraine*

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# Peculiarities of biodiversity research in Ukraine

- Traditionally strong, “old school”, but chronically underfunded
- Absence of modern approaches, lag comparing to the Europe, “Sanger” stage skipped
- Long logistics shoulder, taxes and custom regulations due to being outside of EU (outsourcing and commercial sequencing is not possible)

# Challenges and opportunities

- High demand (biodiversity crisis, understudied biodiversity)
- Shortage of resources
- Need to perform leap from first generation sequencing to third generation sequencing technology, simplify and make preparation cheap (invention by subtraction), move from individual

# Lysis C or Hot Shot extraction in plates



- <https://dx.doi.org/10.17504/protocols.io.ewo1c2m1r2/v1>

# All around ONT

- Compact and mobile device, simplistic library preparation
- Low entrance costs
- Possibility to multiplex



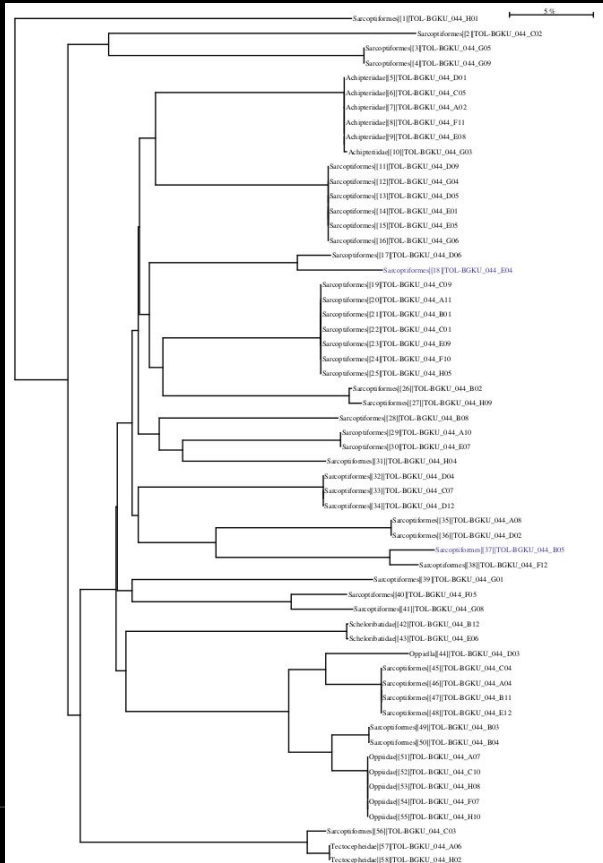
# Difficult taxonomic groups, Dark biodiversity

- Oribatida soil mites – diverse group of soil arthropods
- Small size, identification using permanent slides (DNA barcoding OR traditional taxonomy)



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# Difficult taxonomic groups, Dark biodiversity

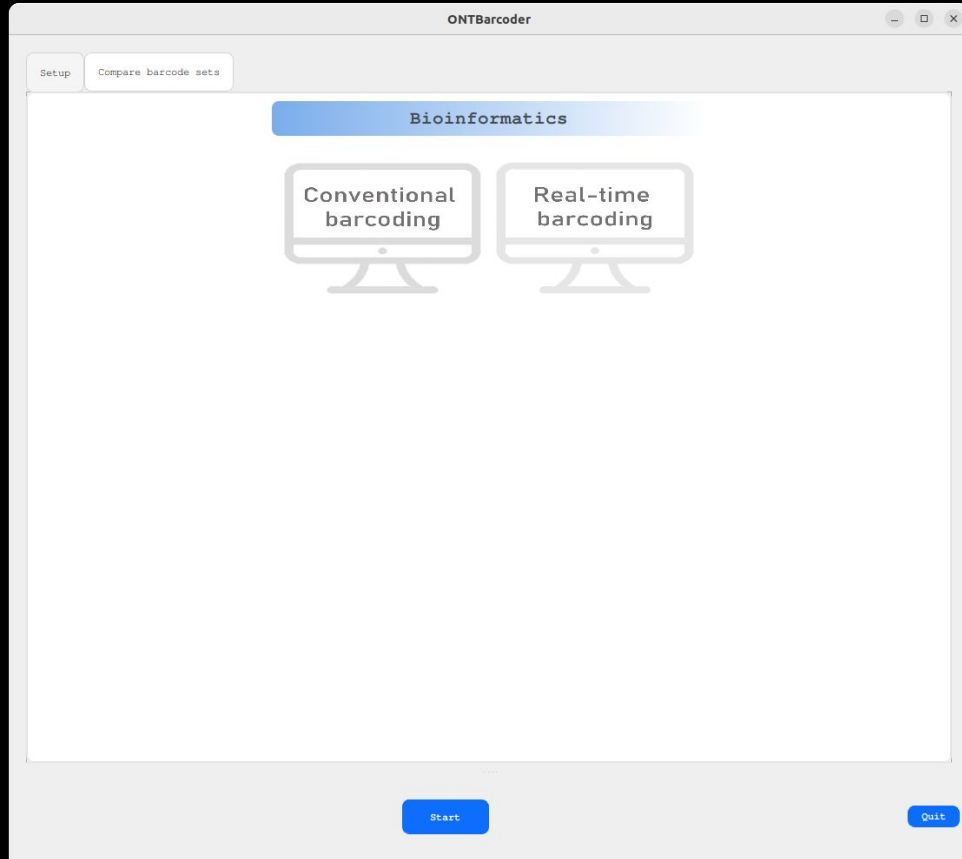


- We will use non-destructive approach to extract DNA in order to barcode AND provide morphological description

- Using 24x24 universal indexed primers it is possible to multiplex 576 samples
- Two stage PCR with tailed primer pairs allows additional inclusion of barcodes of different nature (COI, ITS, 16S ...) and combining them in the same run
- Native barcoding kit or similar barcoding preparation of *libraries*

may further increase efficiency of ONT8

# ONTbarcoder or custom pipeline



- Srivathsan, A., Lee, L., Kato, K. et al. ONTbarcoder and MinION barcodes aid biodiversity discovery and identification by everyone, for everyone. BMC