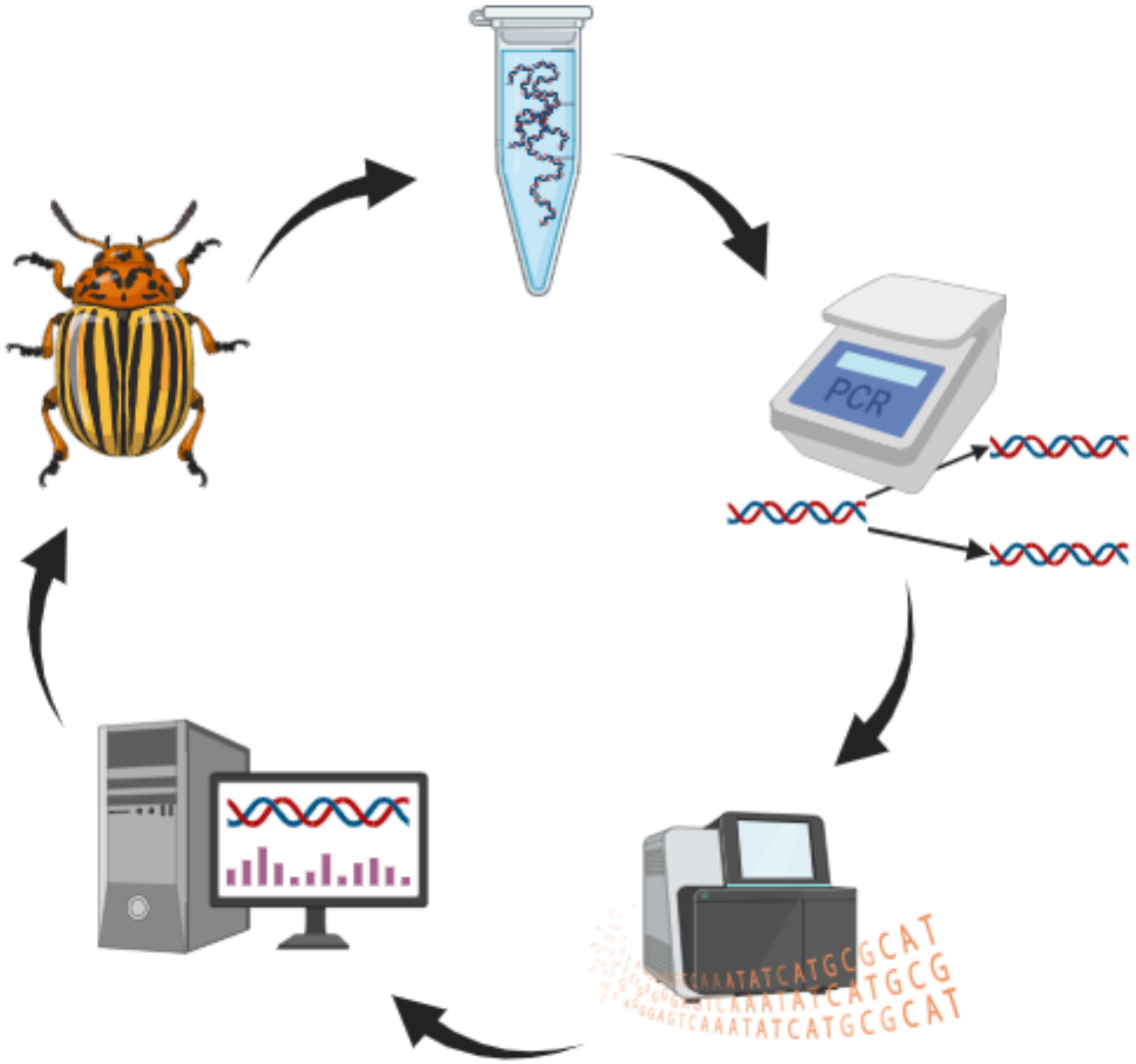
Metabarcoding and eDNA

- Course: Molecular Ecology
- Block 1: Genetic identifications in zoology
- Guest Teacher: Domagoj Gajski, VUK

Výzkumný ústav pro krajinu, v. v. i.

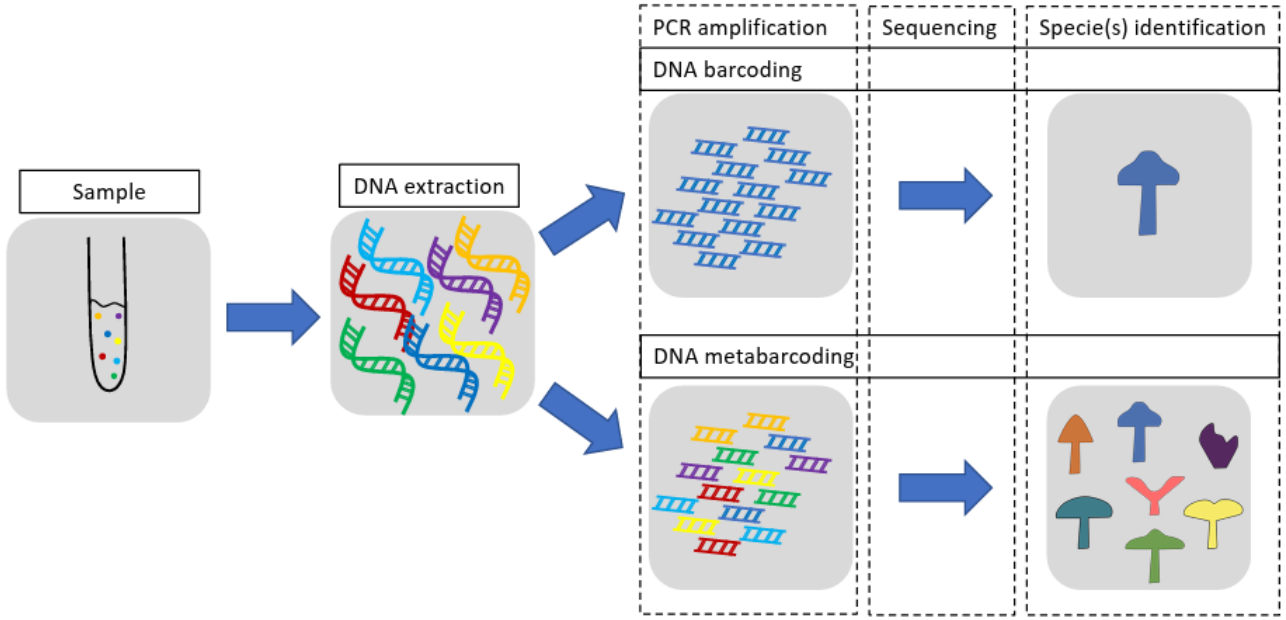
DNA barcoding

- Identifies species or confirms morphological species identification
- Barcodes deposited into large databases
- Getting cheap (5-10 $ per sample)



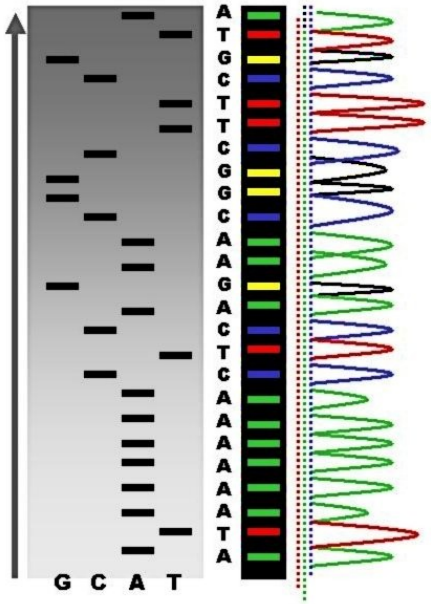
Metabarcoding - definition

- performing barcoding and identification of several to hundreads of different DNA sequences at once
- sequences come from the same gene(s), just from different species
- was only possible with the arival of 2nd generation sequencing (Ion Torrent and Illumina) and became even cheaper with 3rd generation sequencing (ONT

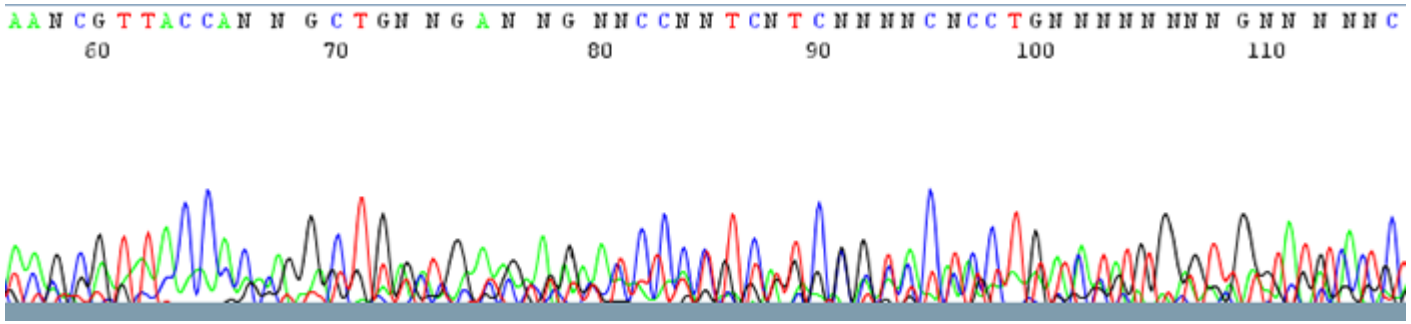


Taken from:
https://en.wikipedia.org/wiki/File:DNA_(meta)barcoding_differences.pdf

Why metabarcoding could not work with Sanger sequencing (1st generation sequencing):

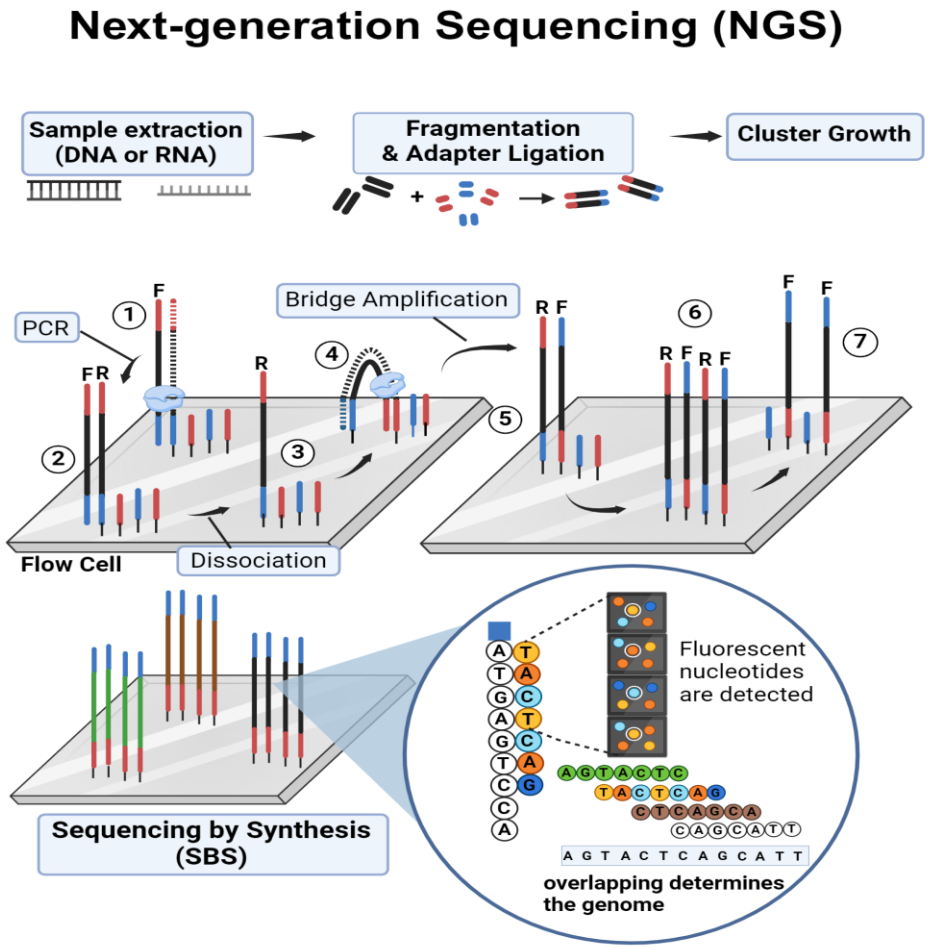


Regular barcoding: This is how it looks like when you run a sample with more than 99% of the same species DNA coming from a specific gene.

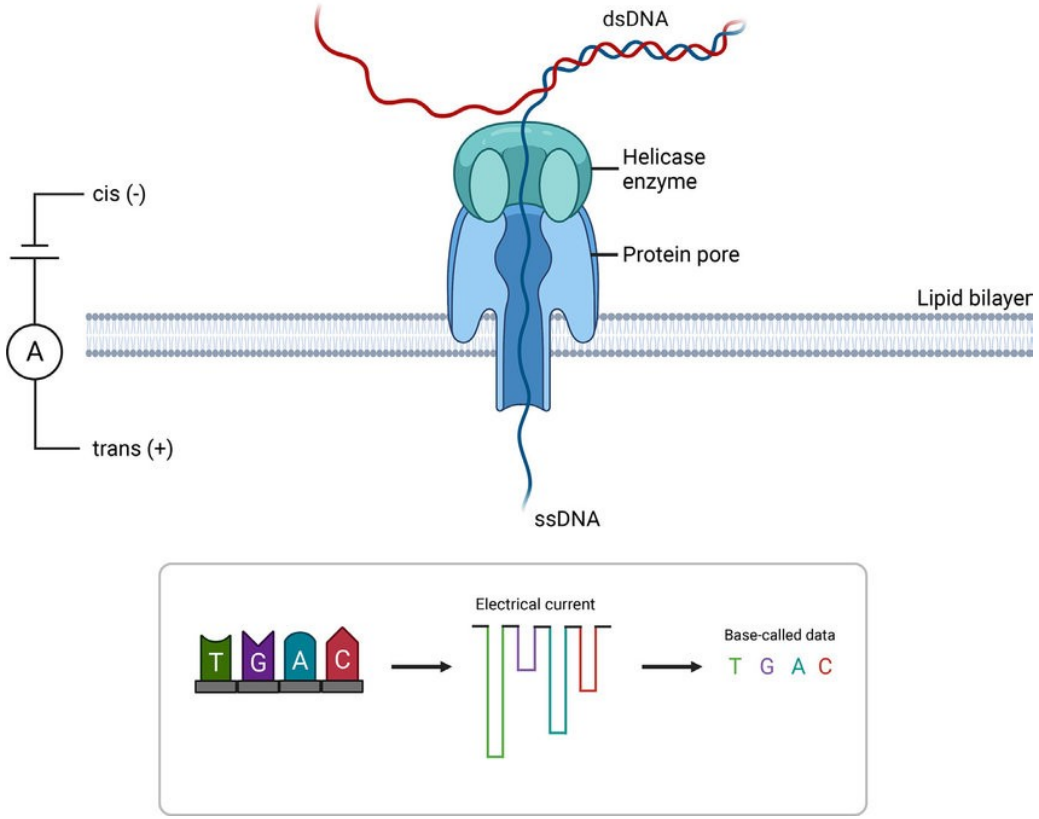


This is how it looks like if you would run a sample with many different versions of the same gene, coming from different species.

This problem was solved with 2nd and third generation sequencing – many reads sequenced at the same time

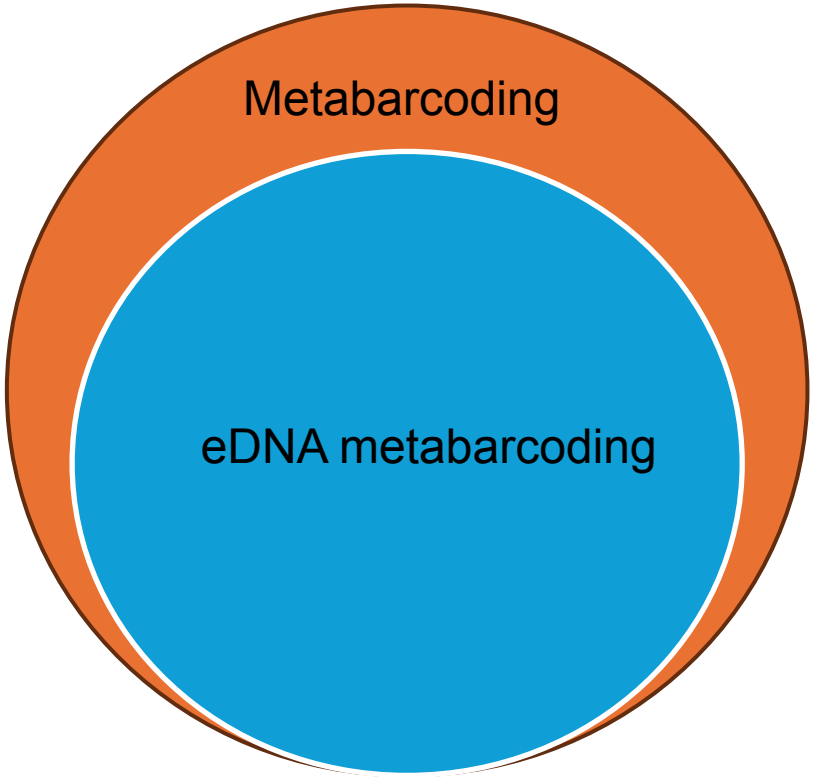


Illumina sequencing (2nd generation)

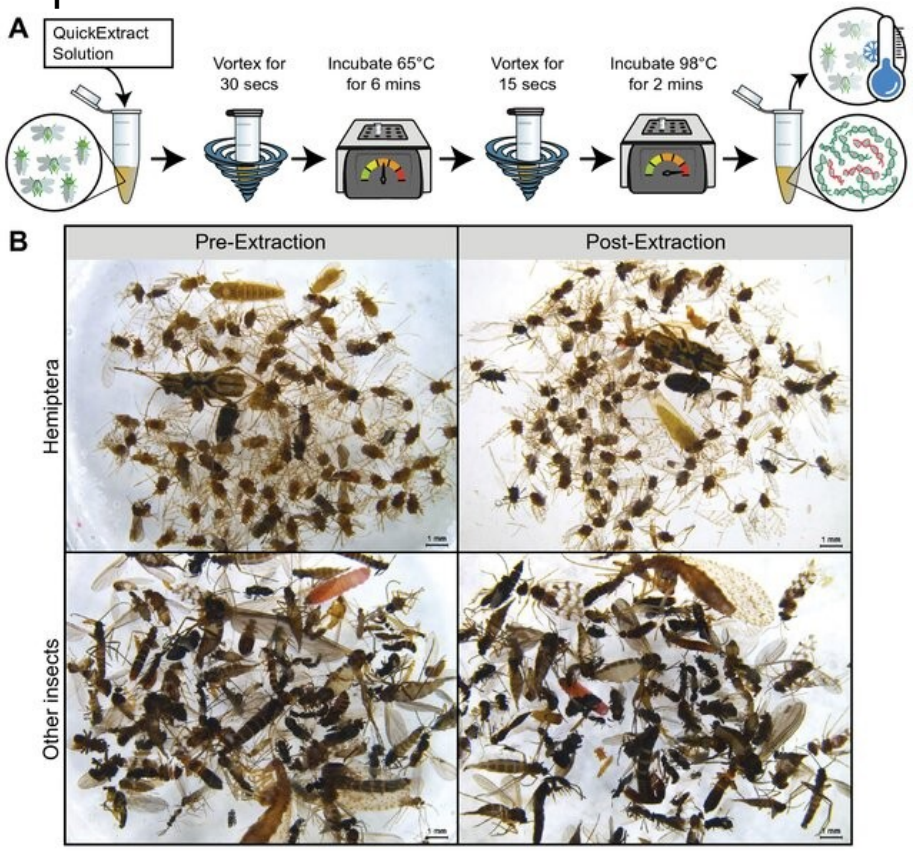


Nanopore sequencing (3rd generation)

Difference between metabarcoding and eDNA metabarcoding

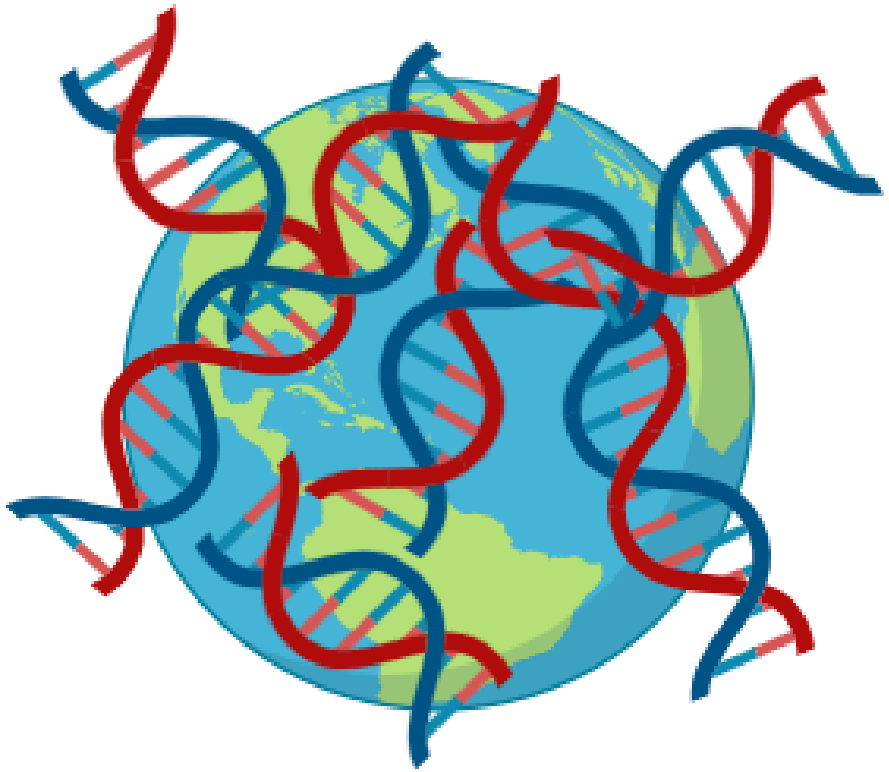


- Part of metabarcoding is also taking a large mix of specimes or a „soup” of specimens



eDNA metabarcoding

- genetic material present in environmental samples (eg. soil, water, air...)
- able to assess whole communities from a single environmental sample



Common sources of eDNA

Sources of eDNA: water

ELSEVIER

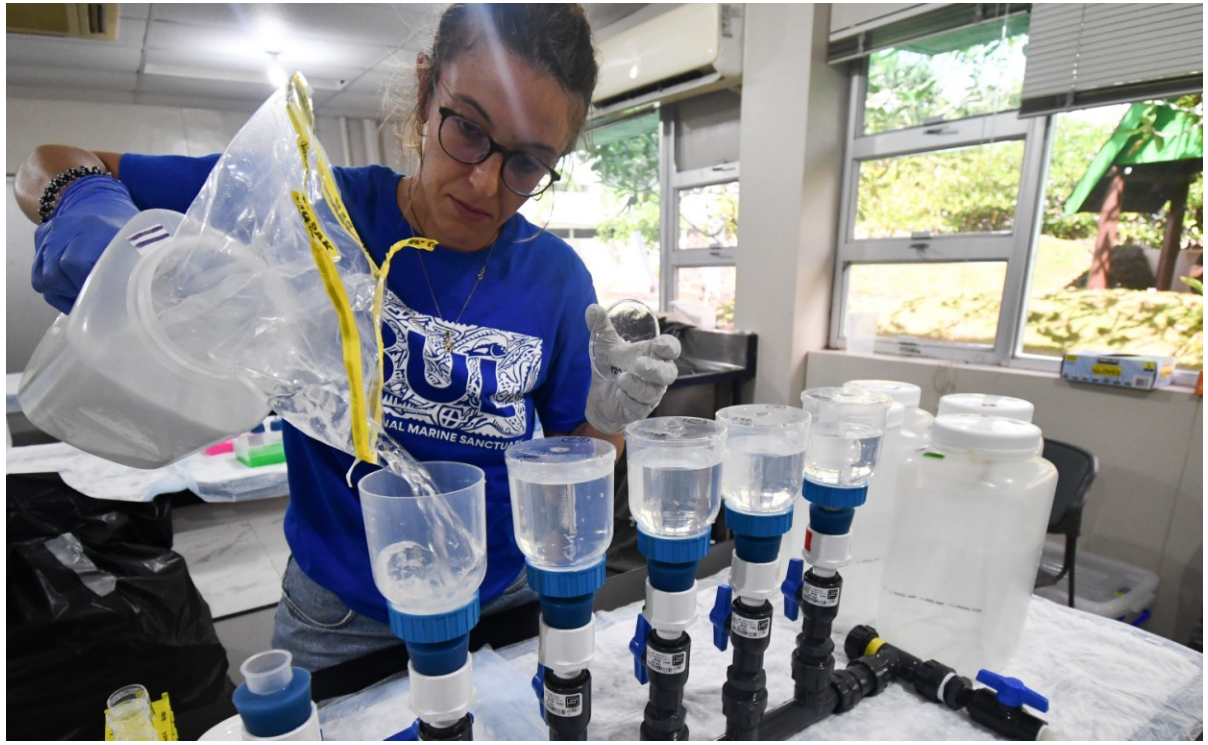
Science of The Total Environment

Volume 873, 15 May 2023, 162322

Review

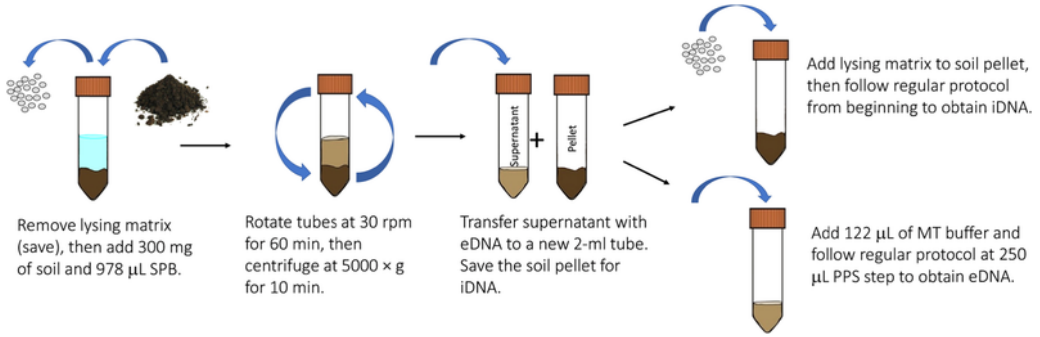
Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution

Miwa Takahashi [a b], Mattia Saccò [a 1], Joshua H. Kestel [a], Georgia Nester [a], Matthew A. Campbell [a], Mieke van der Heyde [a], Matthew J. Heydenrych [a c], David J. Juszkiewicz [a], Paul Nevill [a], Kathryn L. Dawkins [a], Cindy Bessey [d], Kristen Fernandes [a], Haylea Miller [b], Matthew Power [a], Mahsa Mousavi-Derazmahalleh [a], Joshua P. Newton [a], Nicole E. White [a], Zoe T. Richards [a], Morten E. Allentoft [a e]



407 studies in this review!

Sources of eDNA: soil



ELSEVIER

Ecological Indicators

Volume 174, May 2025, 113438

Review

Environmental DNA as a tool for soil health monitoring and unveiling new ecological frontiers

Yuan Zhang [a], Weijun Lu [a], Kaihang Xing [a], Fen Guo [a], Qingping Du [a], Xinfei Zhang [a], Fan Zhang [b], Zongyao Qian [c], Feilong Li [a]

Show more

700 mentioned studies in this review!

Sources of eDNA: air

nature ecology & evolution

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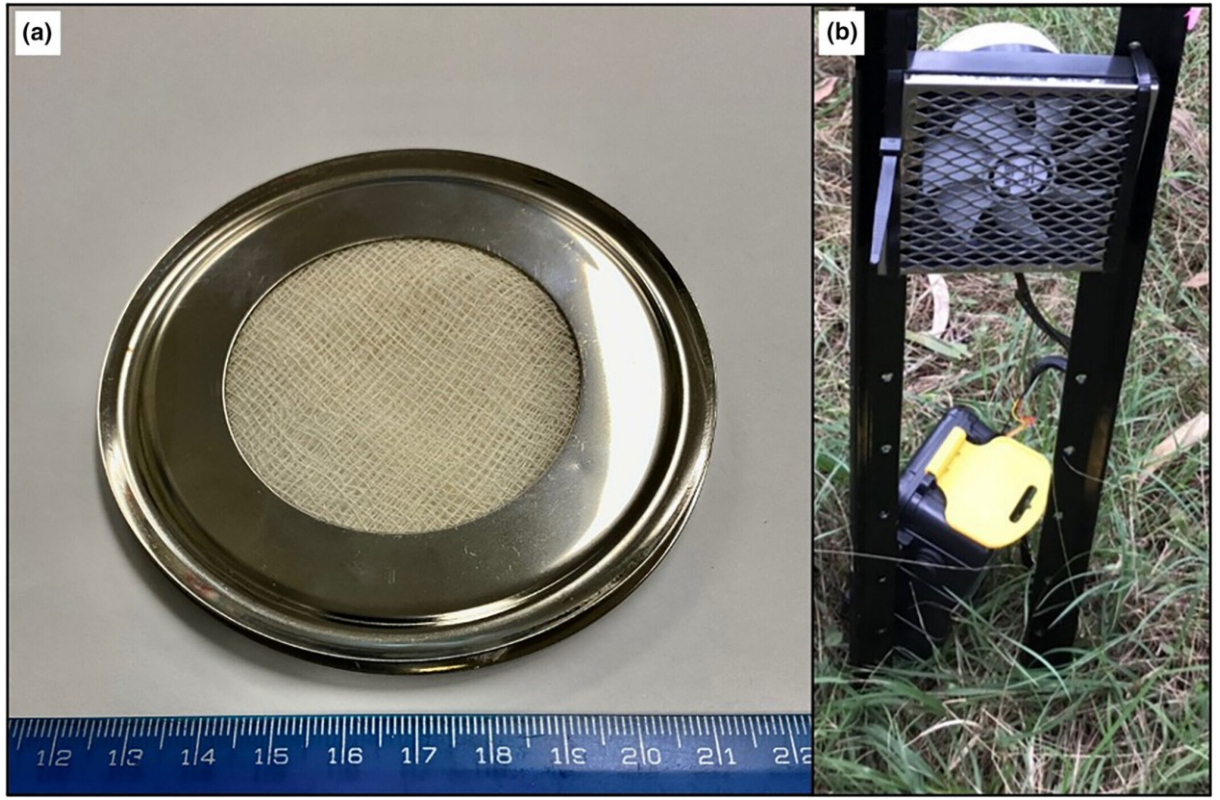
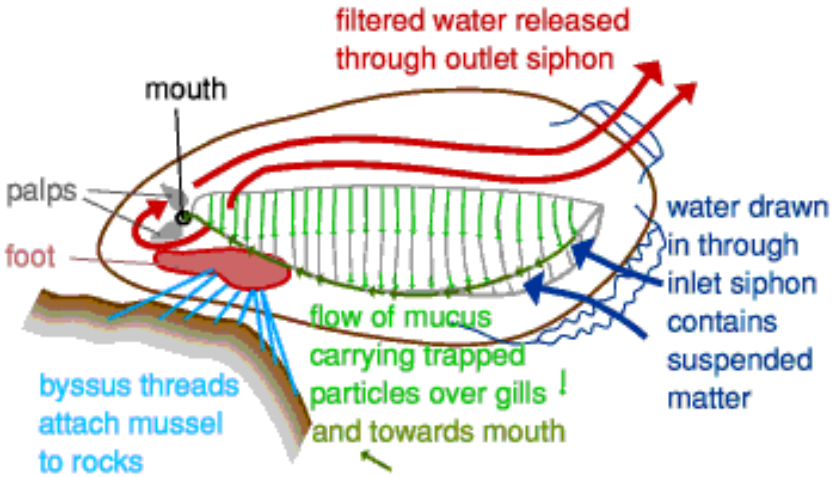
Article | Open access | Published: 03 June 2025

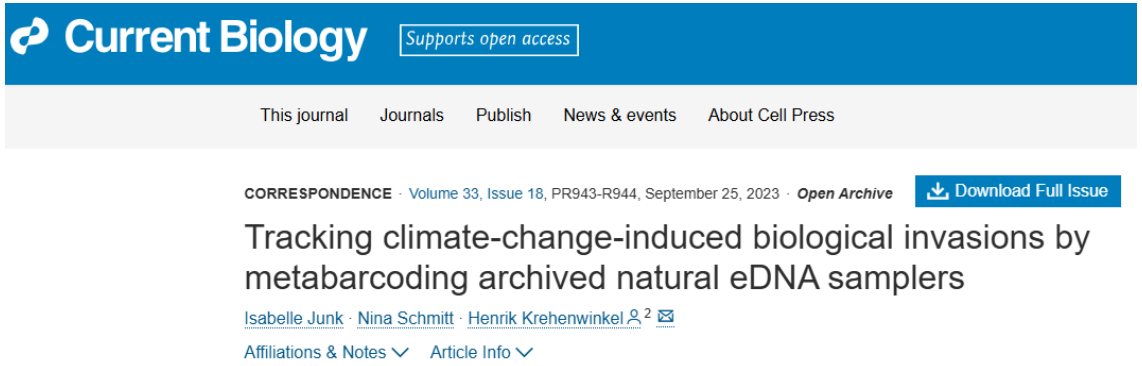
Shotgun sequencing of airborne eDNA achieves rapid assessment of whole biomes, population genetics and genomic variation

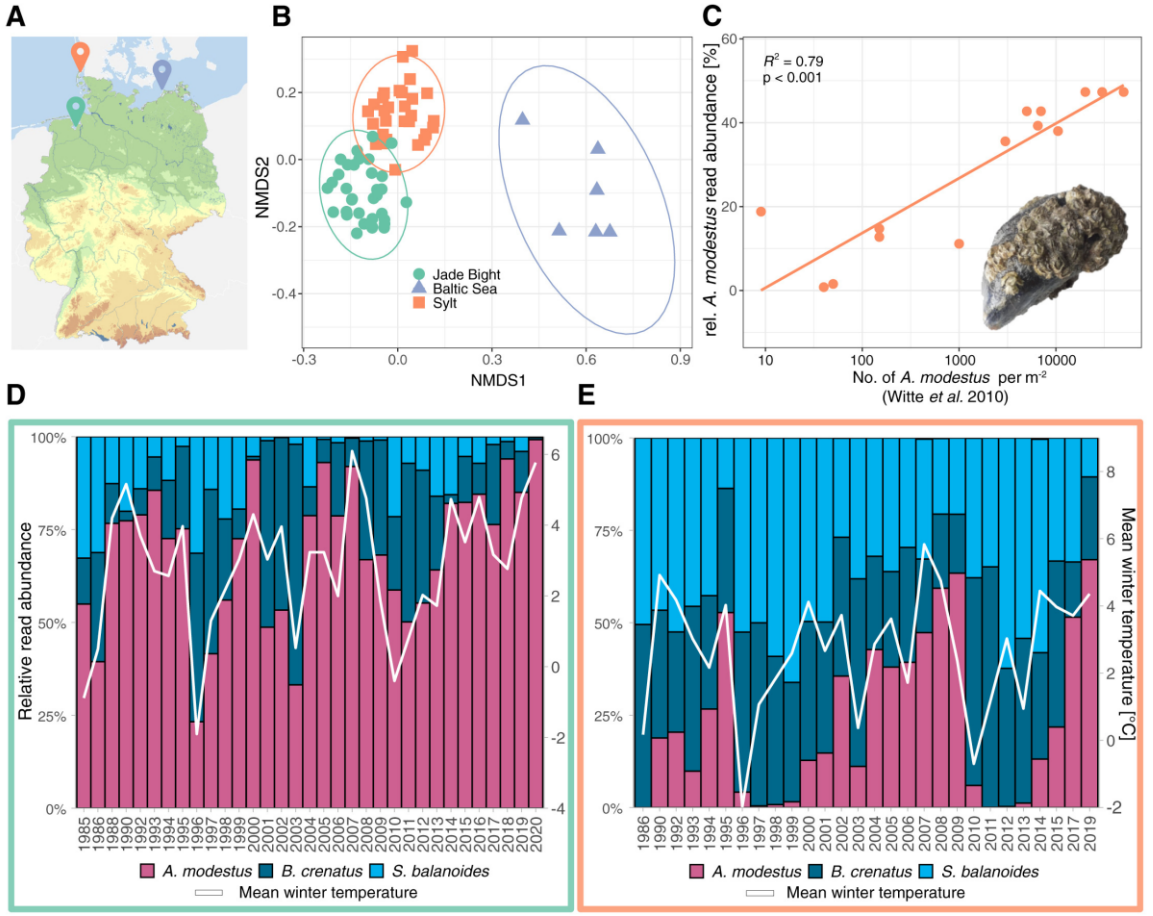
Orestis Nousias, Mark McCauley, Maximilian R. Stammnitz, Jessica A. Farrell, Samantha A. Koda, Victoria Summers, Catherine B. Eastman, Fiona G. Duffy, Isabelle J. Duffy, Jenny Whilde & David J. Duffy



Sources of eDNA: mussels







Sources of eDNA: spider webs

iScience

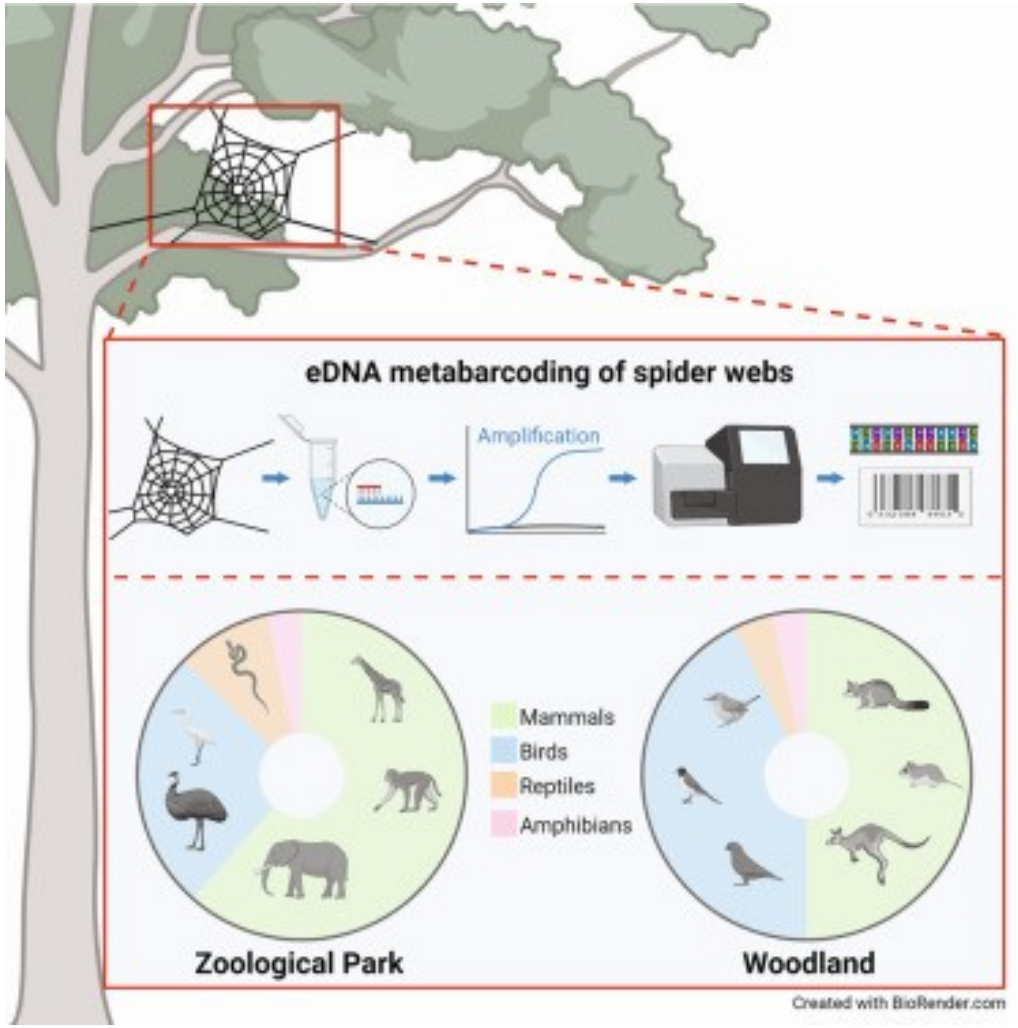
CellPress

Volume 27, Issue 2, 16 February 2024, 108904

Article

Spider webs capture environmental DNA from terrestrial vertebrates

Joshua P. Newton [1 2 5], Paul Nevill [1 2], Philip W. Bateman [2 3], Matthew A. Campbell [1], Morten E. Allentoft [1 4]



Study 5

Sources of eDNA: fecal matter

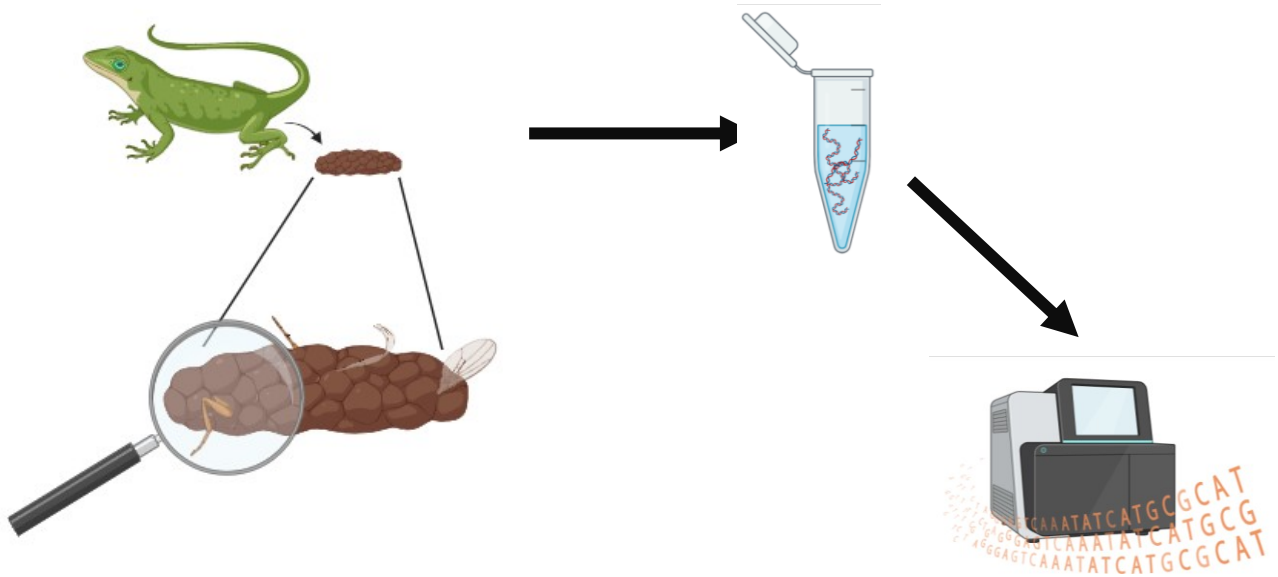
30 September 2023

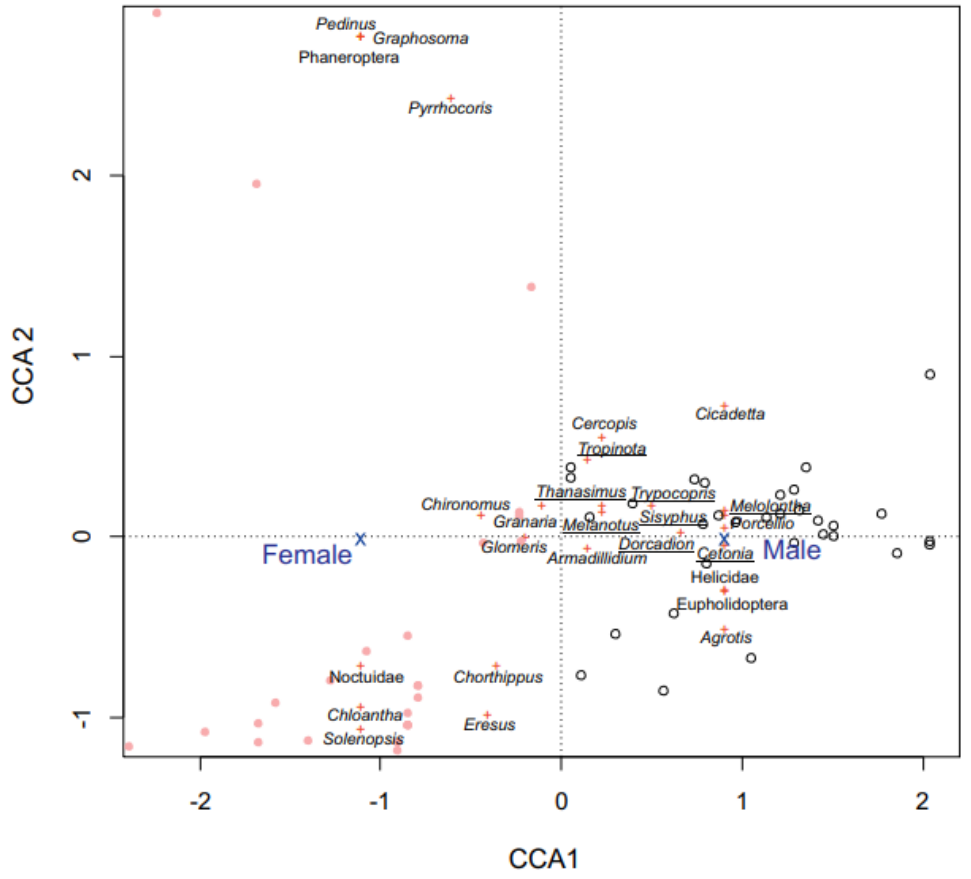
Natural Diet of European Green Lizards, *Lacerta viridis* (Squamata: Lacertidae): A Comparison of Macroscopic and Molecular Identification Methods

Stano Pekár, Domagoj Gajski, Tamara Mifková, Radovan Smolinský, Tomislav Gojak, Martina Martišová

Author Affiliations +

Herpetologica, 79(3):135-143 (2023). https://doi.org/10.1655/Herpetologica-D-23-00017





Sources of eDNA: gut conten

nature ecology & evolution

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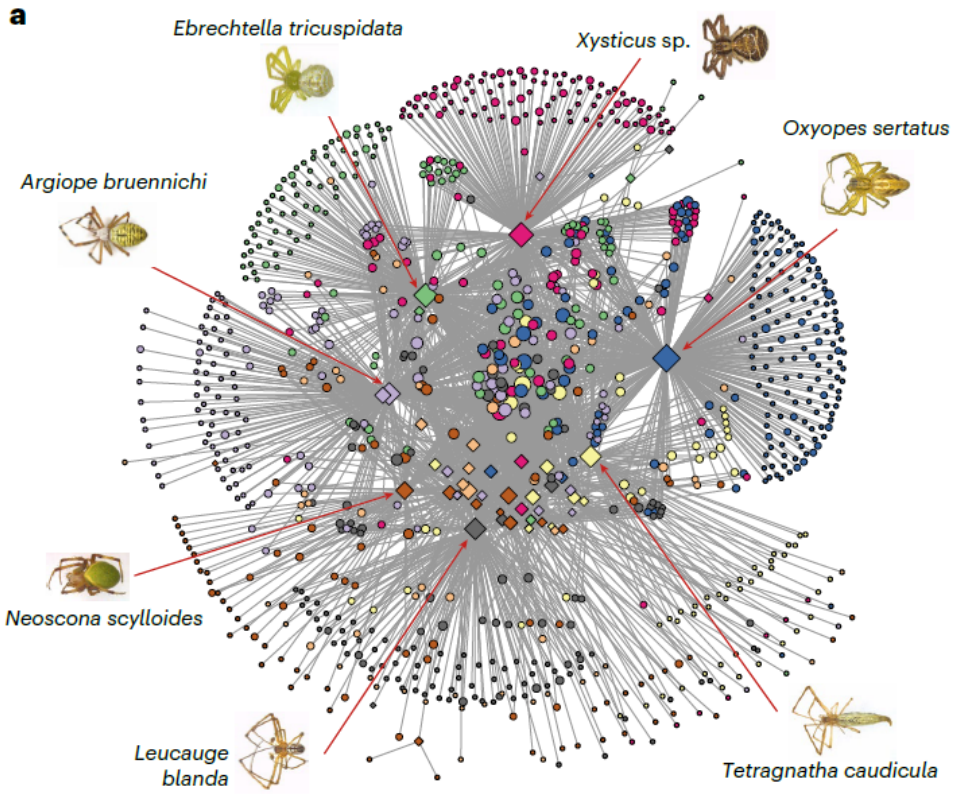
Article | Published: 17 July 2023

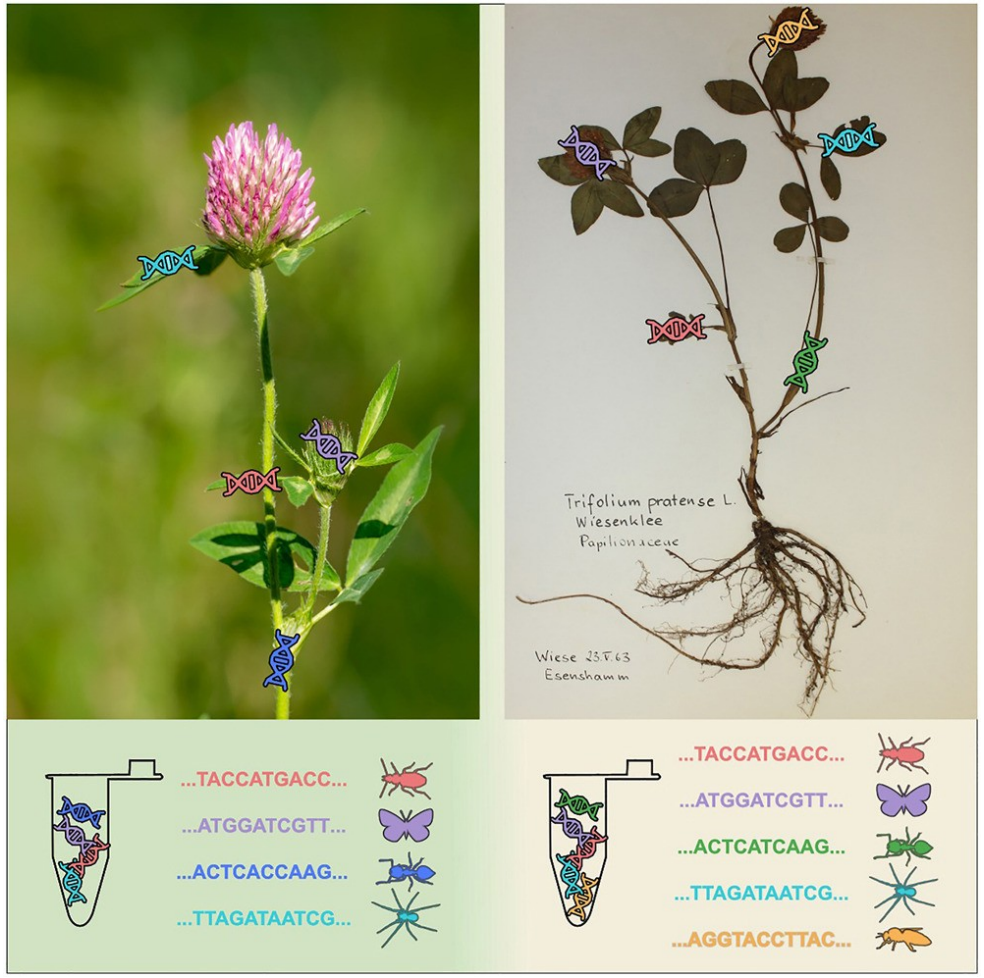
Dynamics of species-rich predator–prey networks and seasonal alternations of core species

Sayaka S. Suzuki, Yuki G. Baba & Hirokazu Toju

Nature Ecology & Evolution **7**, 1432–1443 (2023) | Cite this article



Sources of eDNA: plants and herbaria



REPORT · Volume 34, Issue 18, P4318-4324.E6, September 23, 2024 · *Open Access* Download Full Issue

Recovering plant-associated arthropod communities by eDNA metabarcoding historical herbarium specimens

Manuel Stothut [1,4] · Lisa Mahla [1,4] · Lennart Backes [1] · … · Amirmohammad Avazzadeh [3] · Majid Moradmand [1,3,5] · Henrik Krehenwinkel [1,5,6] … Show more

Affiliations & Notes · Article Info

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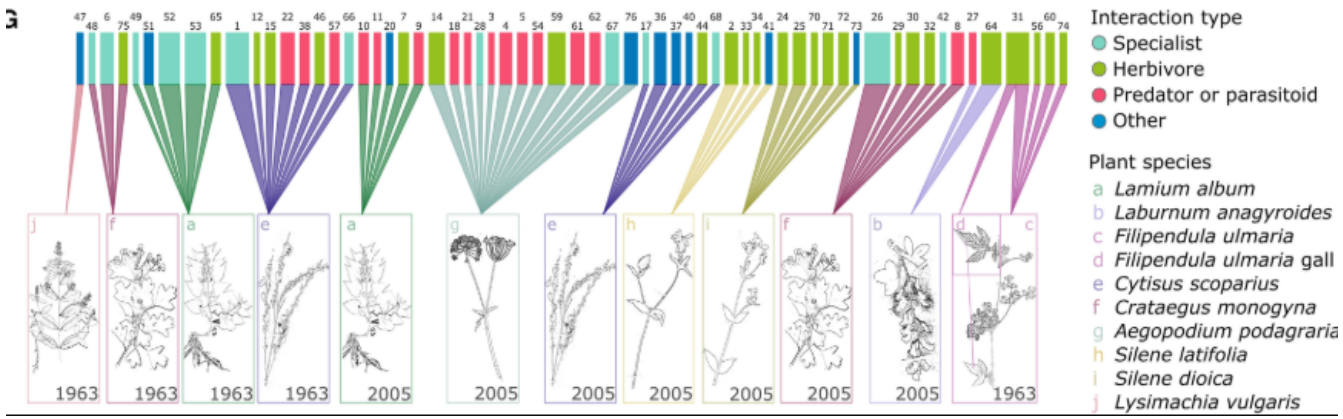
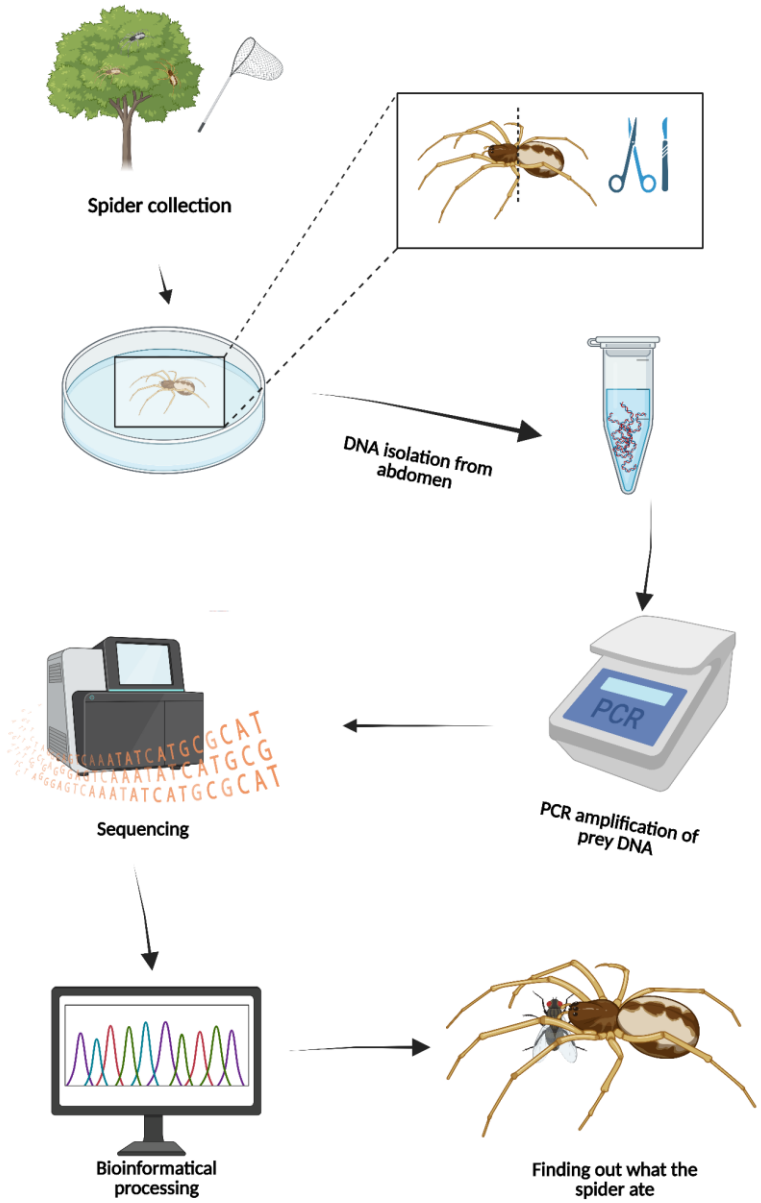
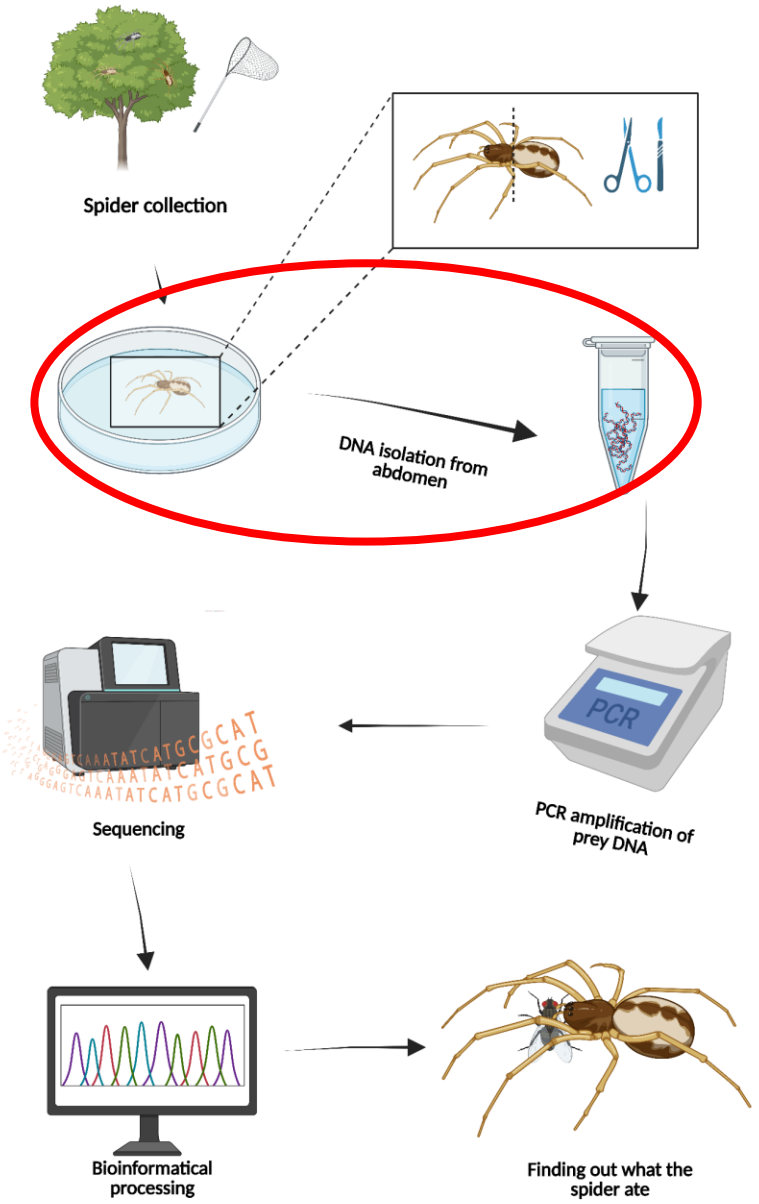


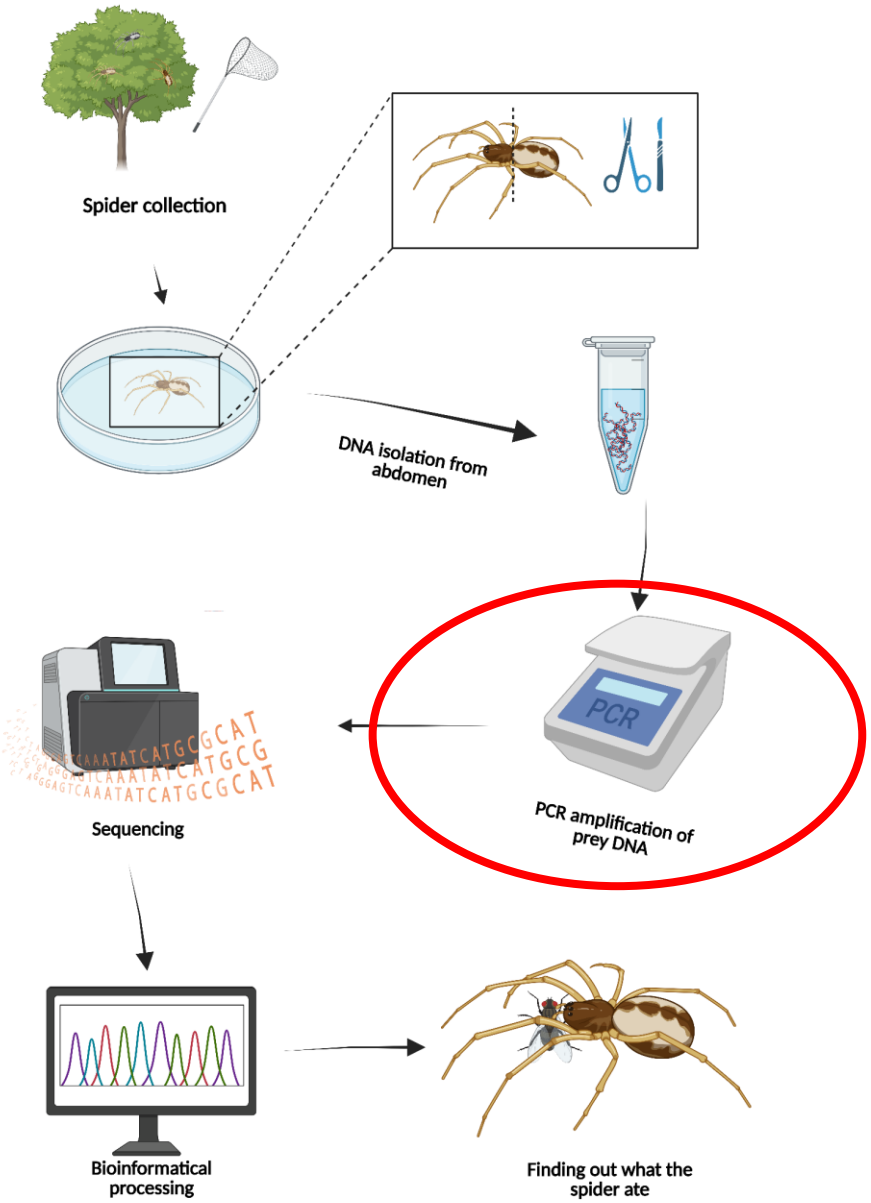
General metabarcoding protocol – explained through spider gut content analysis


Spider collection
DNA isolation from abdomen
PCR
PCR amplification of prey DNA
Sequencing
Bioinformatical processing
Finding out what the spider ate



DNA isolation

- chemicals used for isolation highly depend on the type of sample you have
- the amount of sample is highly variable (several liters of water compared to e.g. a small spider abdomen)
- eDNA half-life varies in every sample (in guts it can degrade in few days, while it can stay safe for weeks in soil or water)

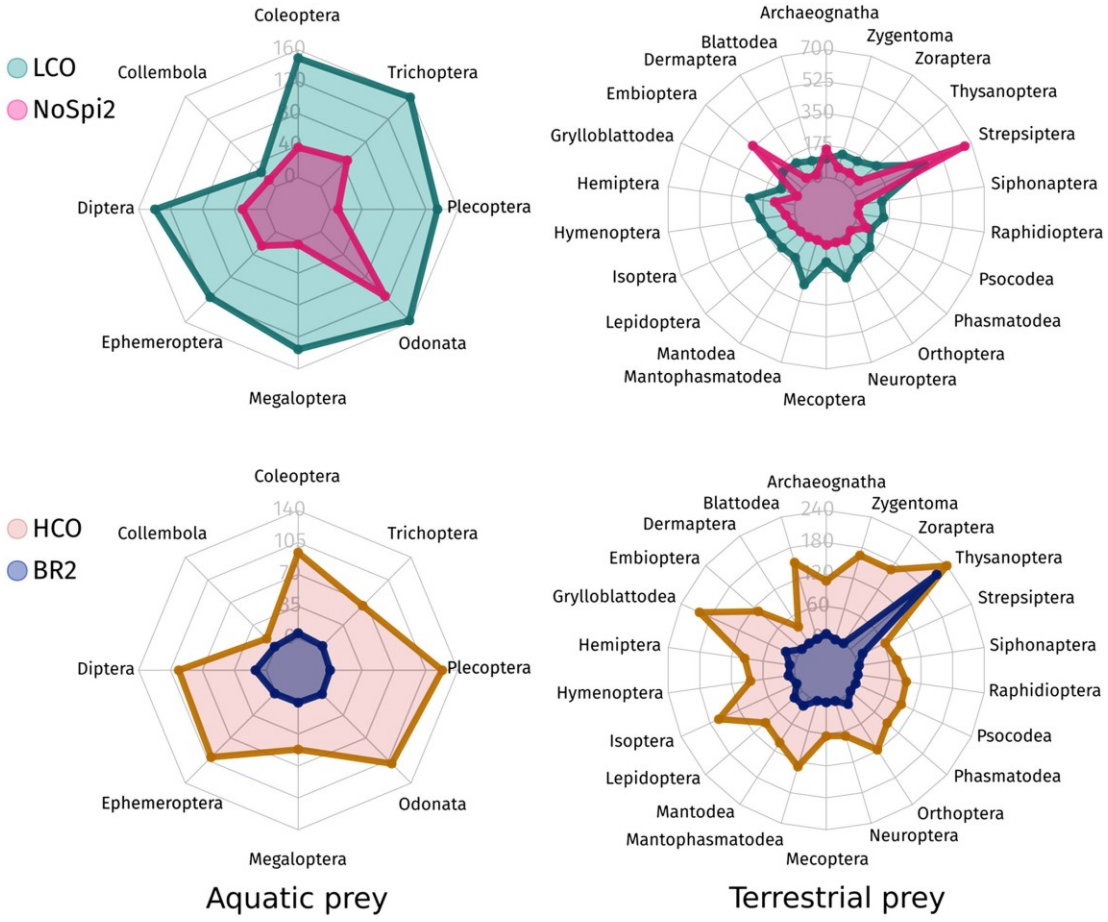


PCR amplification

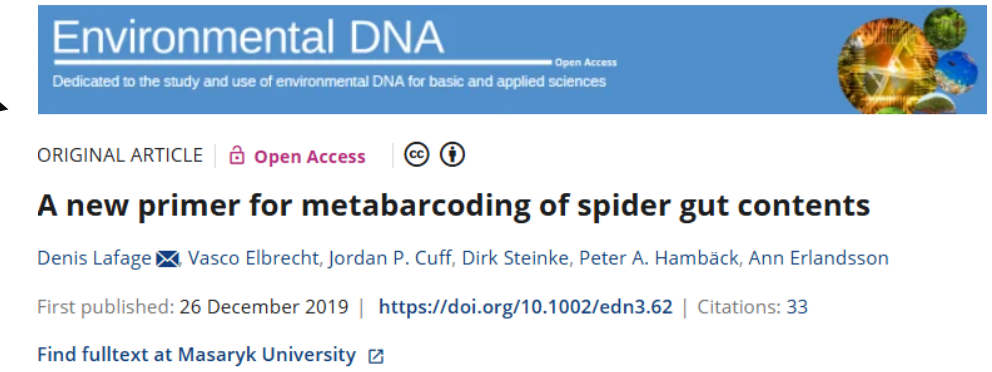
- Amplify fragments of gene regions that are common in large barcode databases (e.g. NCBI, BOLD):
- animals – COI
- plants – rbcL
- fungi – ITS
- bacteria – 16S

- A need for universal primers!

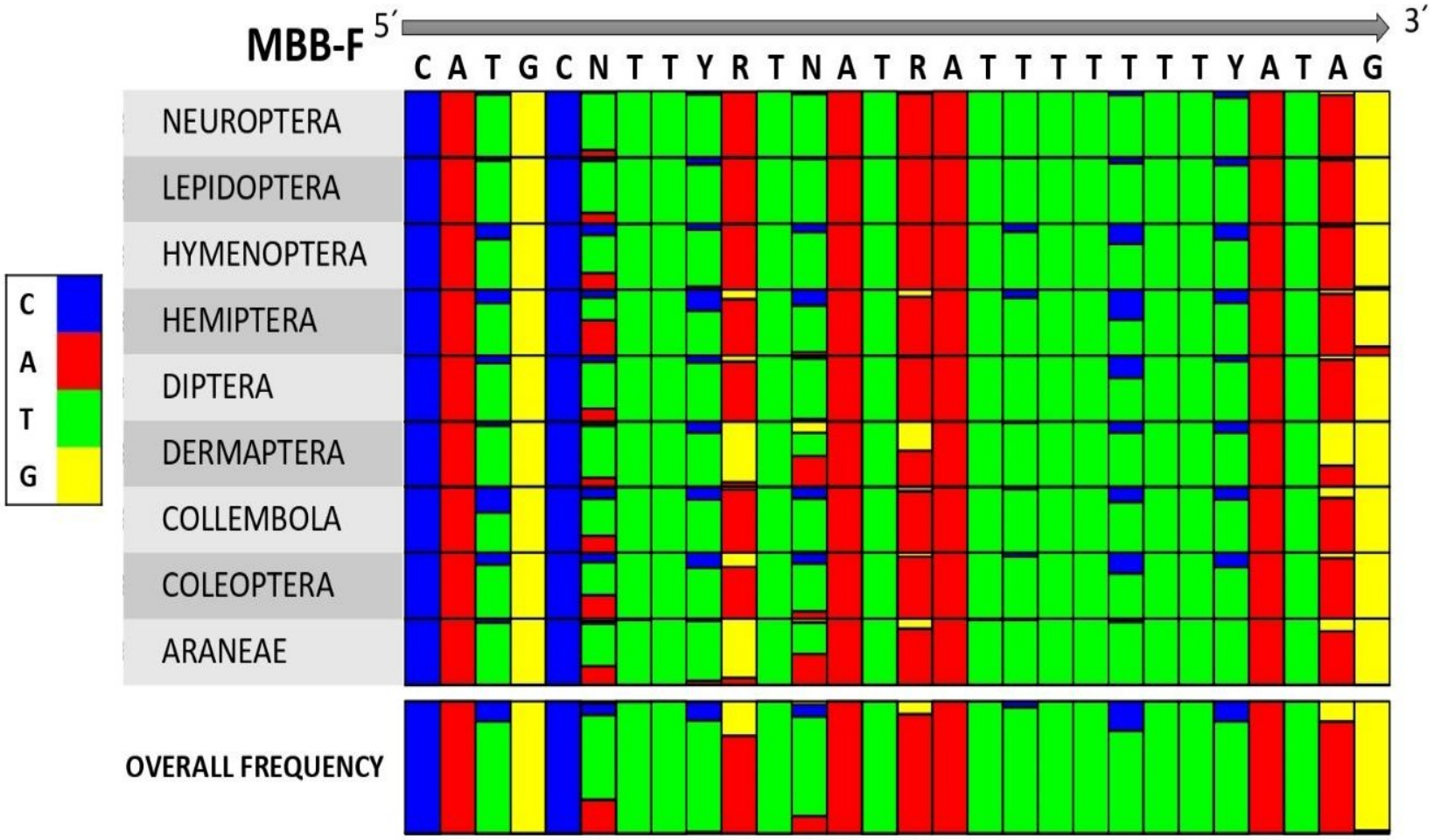
Selectivity of universal primers



- Universal primers are rarely amplifying all taxa, even inside genera or families.
- Good approach is to use several universal primers that amplify different groups of taxa



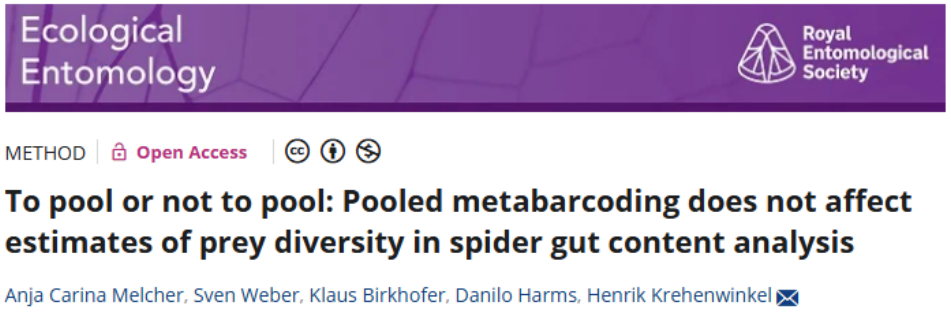
Universal primer



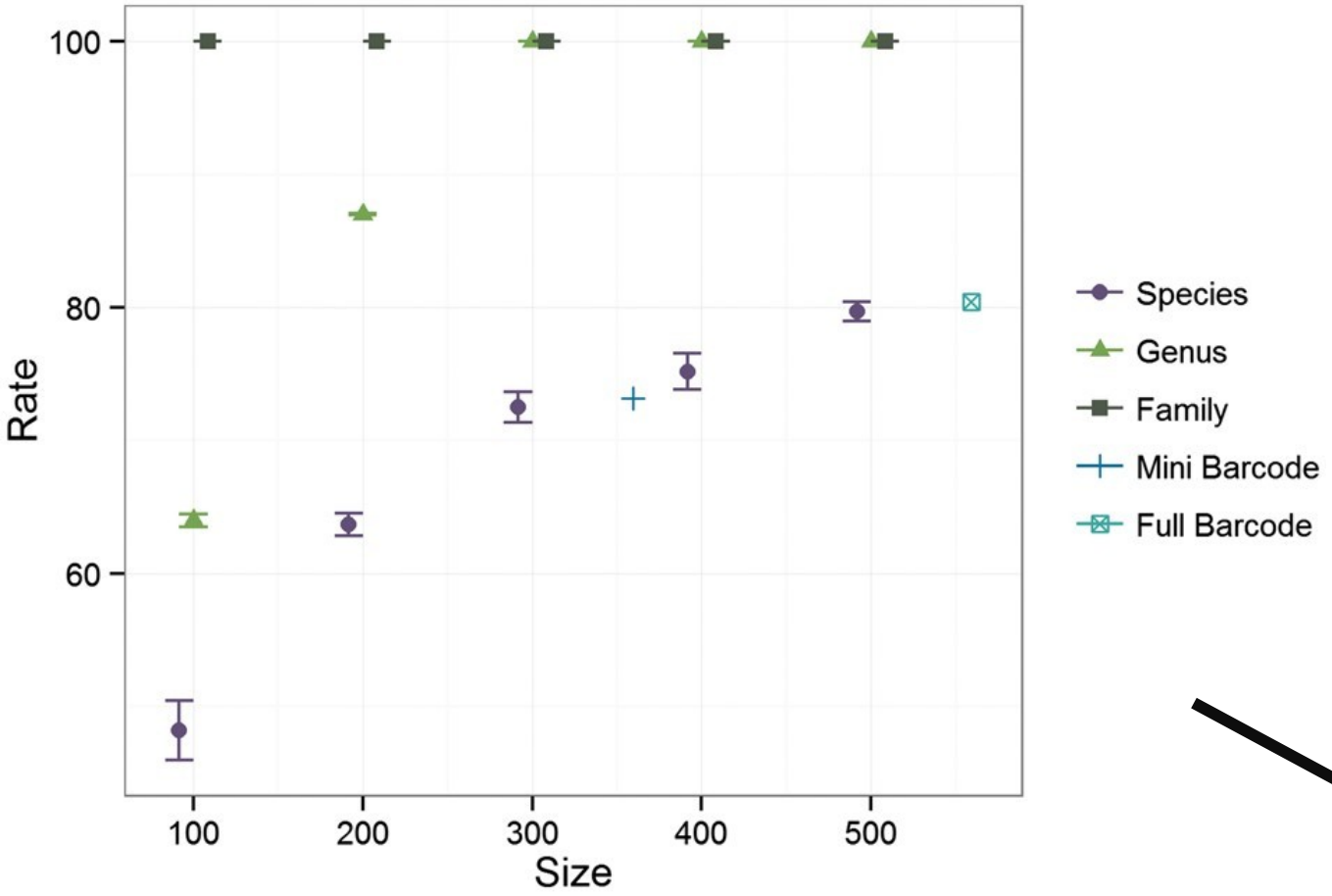
- Primers often can't be too universal. They will never amplify everything in your interest.
- Useful to use several primers tha amplify other organisms

Selective universal primer

- Amplifies DNA of some some organisms of interest, but blocks amplification of other organisms
- The missmatch is usually at the 3’ of the primer, where it has the greatest chances of nullyfing amplification
- Good when there is a lot of DNA from unwanted organisms



Size of the primer amplicon



- eDNA usually degrades fast and the largest fragments that stay stable for some time are 100-600 bp large
- The primer pairs must amplify the smallest possible fragment that will still give you informative results when searched in databases

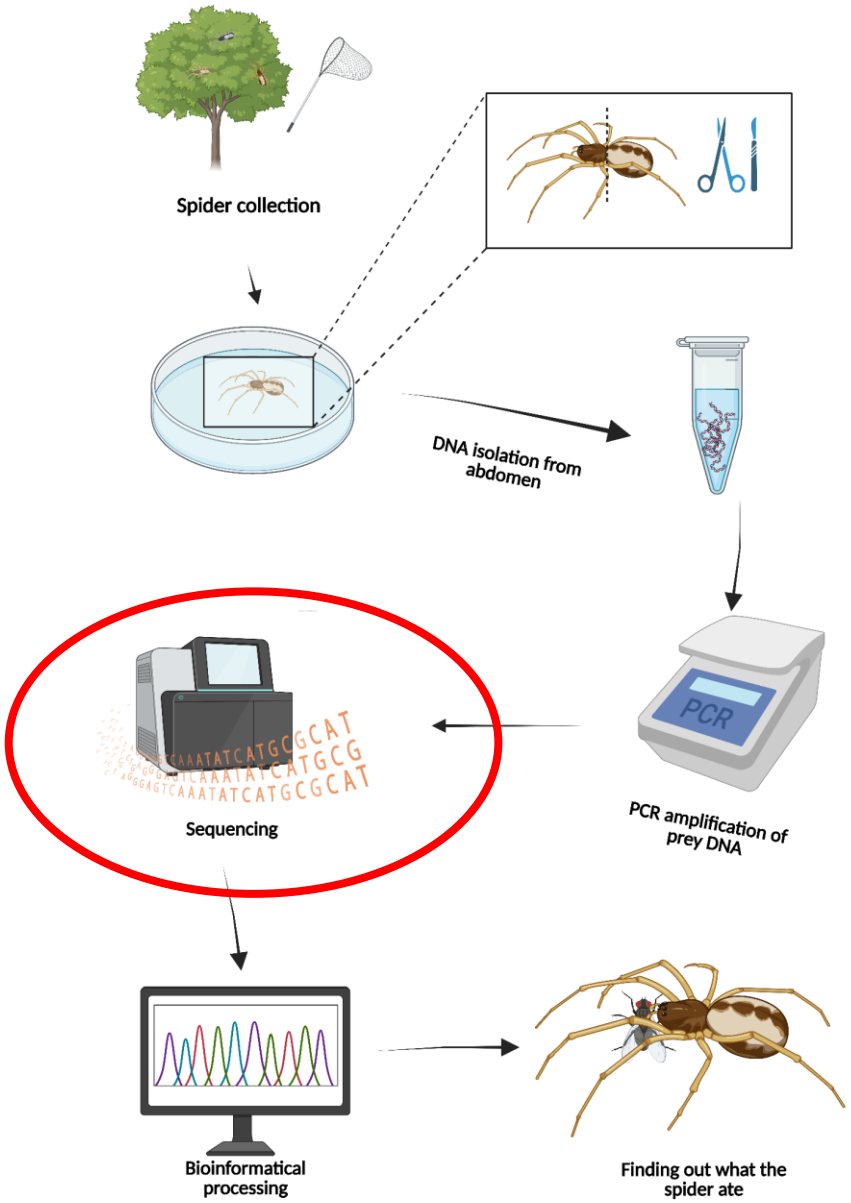
JOURNAL ARTICLE

Reconstructing a herbivore's diet using a novel *rbc*L DNA mini-barcode for plants

David L. Erickson, Elizabeth Reed, Padmini Ramachandran, Norman A. Bourg, William J. McShea, Andrea Ottesen Author Notes

AoB PLANTS, Volume 9, Issue 3, May 2017, plx015, https://doi.org/10.1093/aobpla/plx015

Published: 21 April 2017 **Article history**

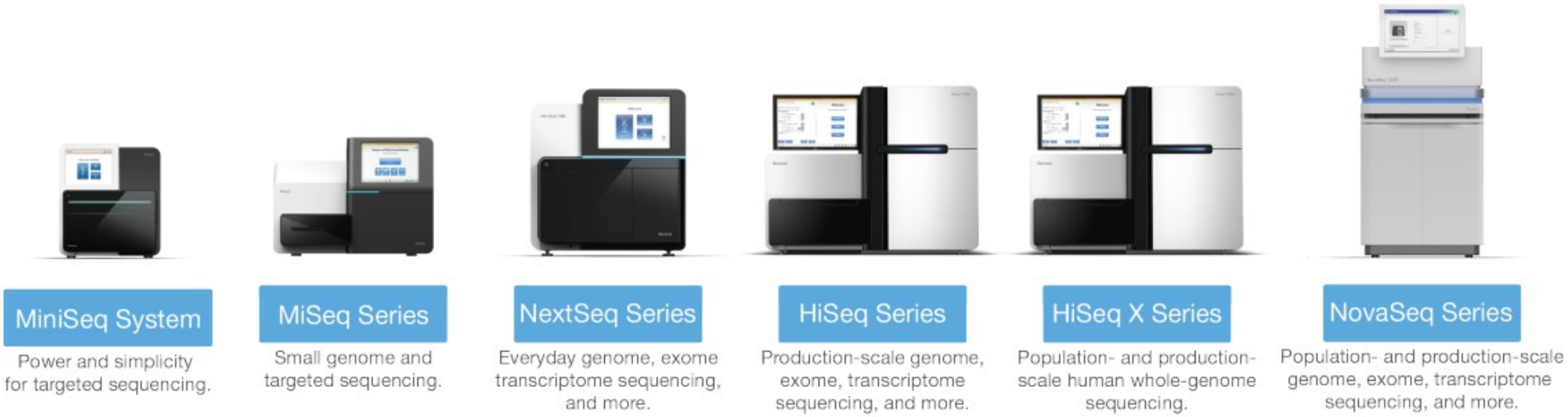


Sequencing

- Still, most commonly done on a Illumina sequencer, but more and more often on ONT Nanopore.
- It is important to have a sequencing depth of at least 10,000 reads per sample, to obtain a large diversity of results per sample

- Example: Common Illumina sequencers sequence around 20,000,000 reads total – 20,000,000 / 10,000 = 2000 samples.

Sequencing platforms - Illumina



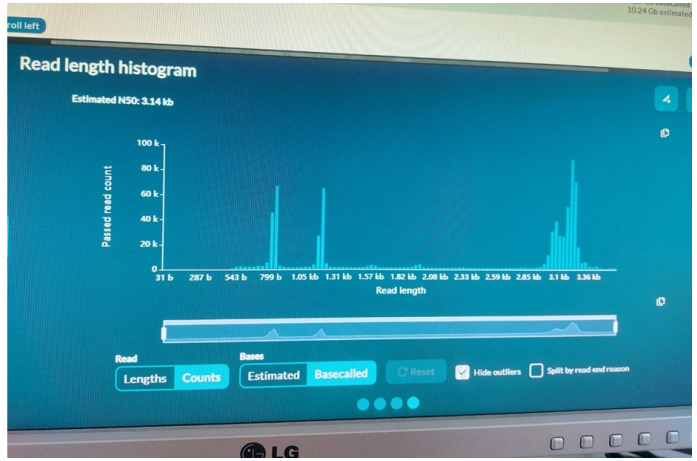
- Prices usually range between 10$ and 20$ per sample
- Library prep is the most expensive part – need for two PCRs:

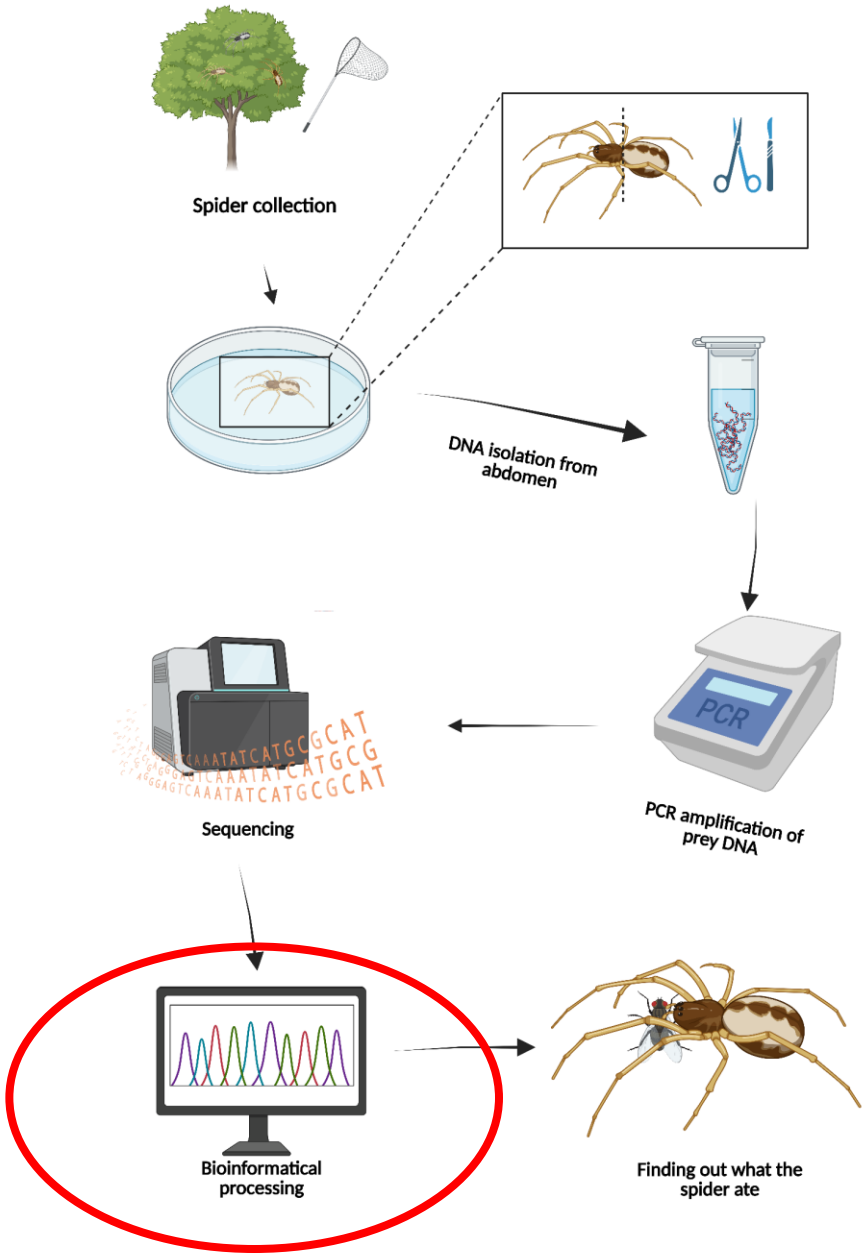
1) Regular amplicon PCR amplification + barcodes
2) Annealing of large Illumina adapters

Sequencing platforms – ONT Nanopore

Nanopore device

- Library prep is simpler, only one PCR reaction needed
- Costs around 5-10 per sample
- Still prone to larger sequencing errors than Illumina





Bioinformatical processing

- Still no standard procedure, can be done using python, R (dada2) or linux (bash) scripts,or by user-friendly software (Geneious)
- Main steps:

a) quality filtering

b) grouping of very similar sequences (MOTUS or AVS)

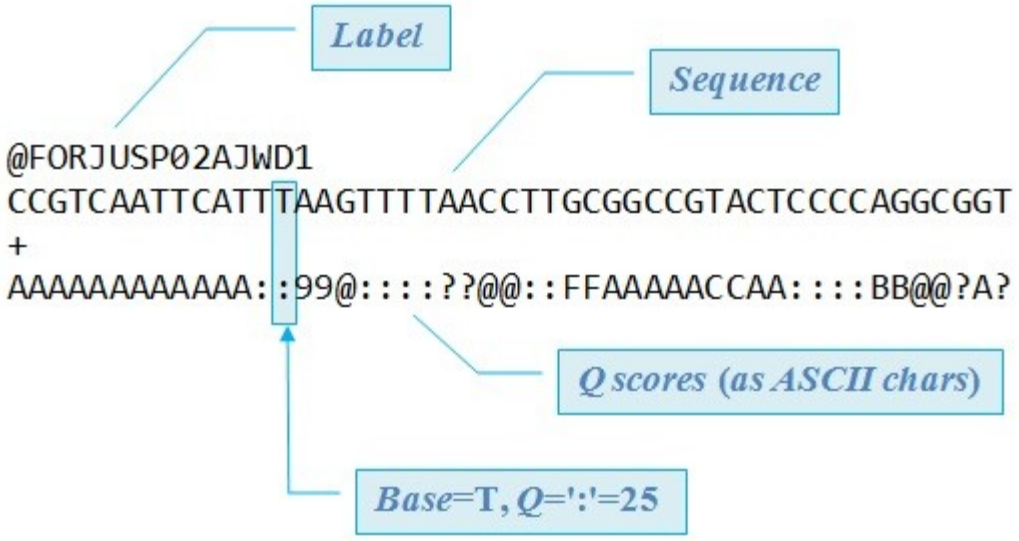
c) BLAST-ing through databases

d) cleaning possible contaminations

Quality filtering

Filtering out all the reads that are worse than 20 or 30 in Phred quality score!

fastQ file:



Phred quality scores are logarithmically linked to error probabilities

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Info about quality score indexing: https://help.basespace.illumina.com/files-used-by-basespace/quality-scores

Assembling (grouping) of very similar sequences

- Most common ways how to assemble similar sequences is by MOTUs or ASVs

- MOTU:

- molecular operational taxonomic unit
- clusters sequences based on a threshold of similarity (often 3%)

- ASV:

- amplicon sequence variant
- clusters very similar sequences, taking into account potential sequencing errors based on an algorithm

BLAST-ing sequences through databases

- BLAST:

- basic local alignment search tool
- algorithm for searching similar sequences in the database

- Common search databases:

BOLD SYSTEMS

unite community

NCBI

Cleaning possible contaminations

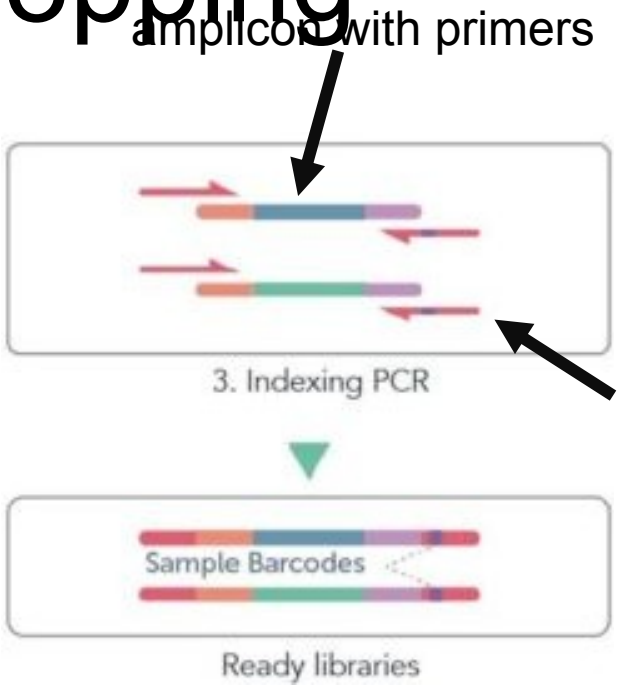
- **Always have negative controls!**
- Negative controls:

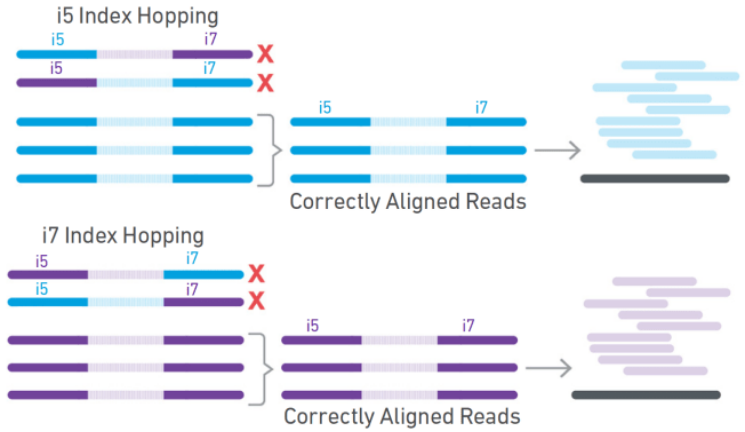
- samples that don’t have any DNA, just pure water

- they go through the whole metabarcoding protocol and you treat them as a sample

- if you find some reads in the negative controls, you usually remove those results from the samples

Cleaning possible contaminations – Index hopping





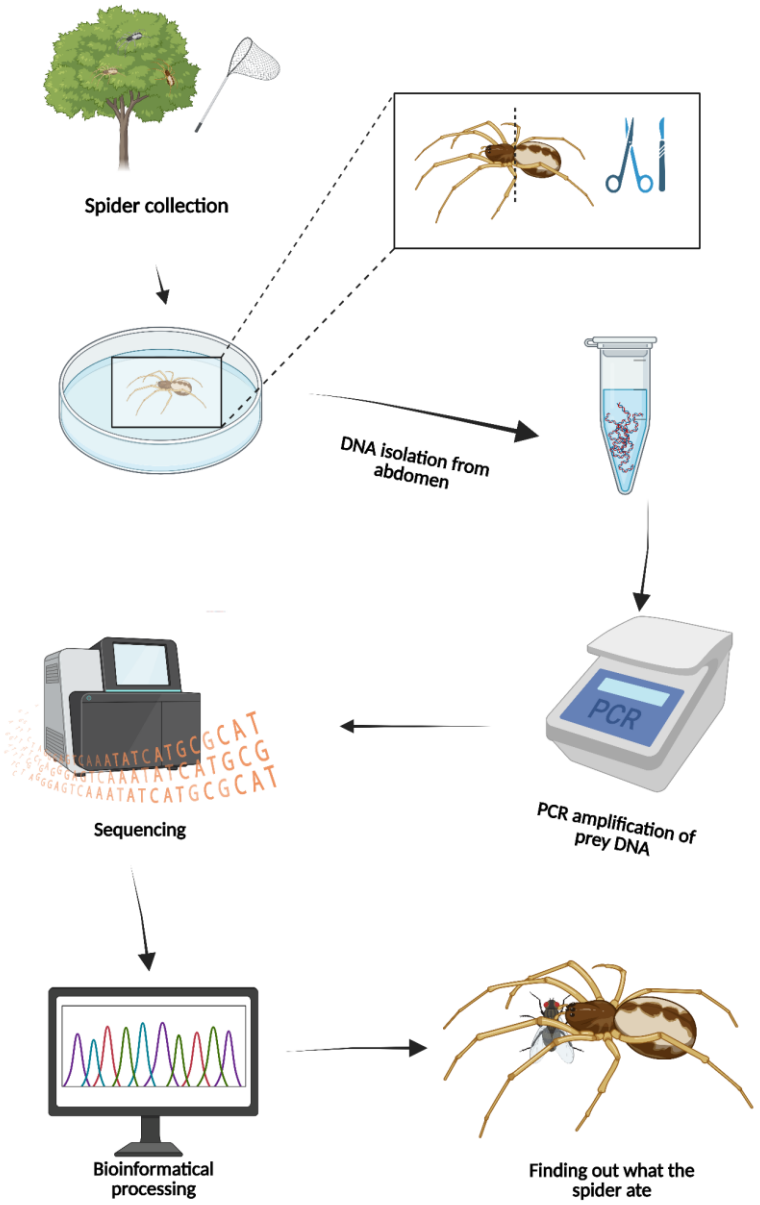
Index (also called sample barcode) – a sequence of bases (usually 5-20 bp long) that is used for identification of every investigated sample

Illumina adapter – a sequence of bases (usually between 20 and 35 bp long) that is used for attaching to the Illumina platform for sequencing

Index hopping:

- a sequence is assigned to the wrong sample because of error during sequencing of the index
- could give you false results for a sample
- error rate is usually around 0.3% of all reads of a sample, so one can remove them by removing identifications that the have a number of reads that is less than 0.3% of the total reads of that sample

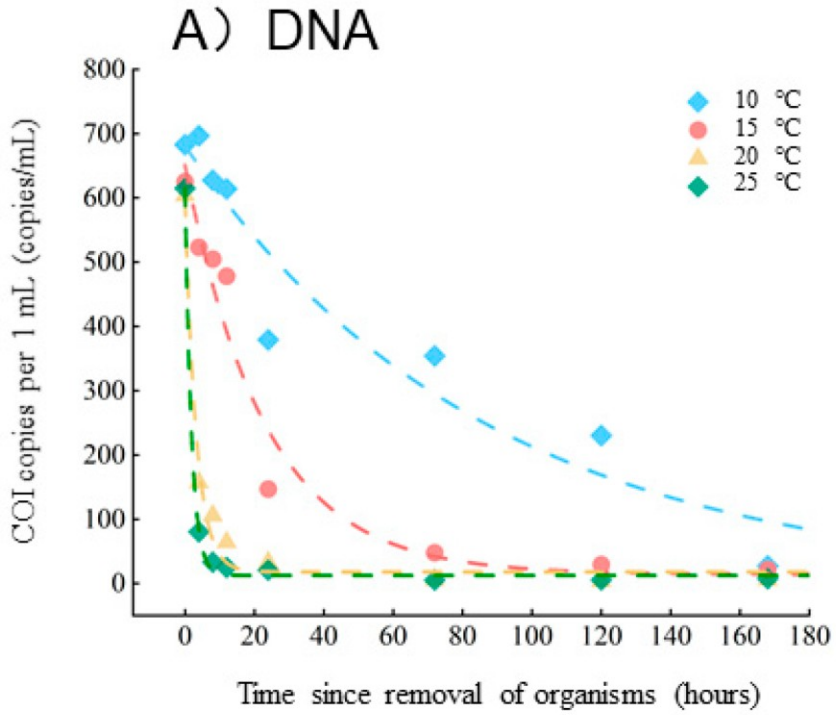
Congrats, you reached the end of the protocol! Enjoy your results!



Main challenges (disadvantages) of metabarcoding

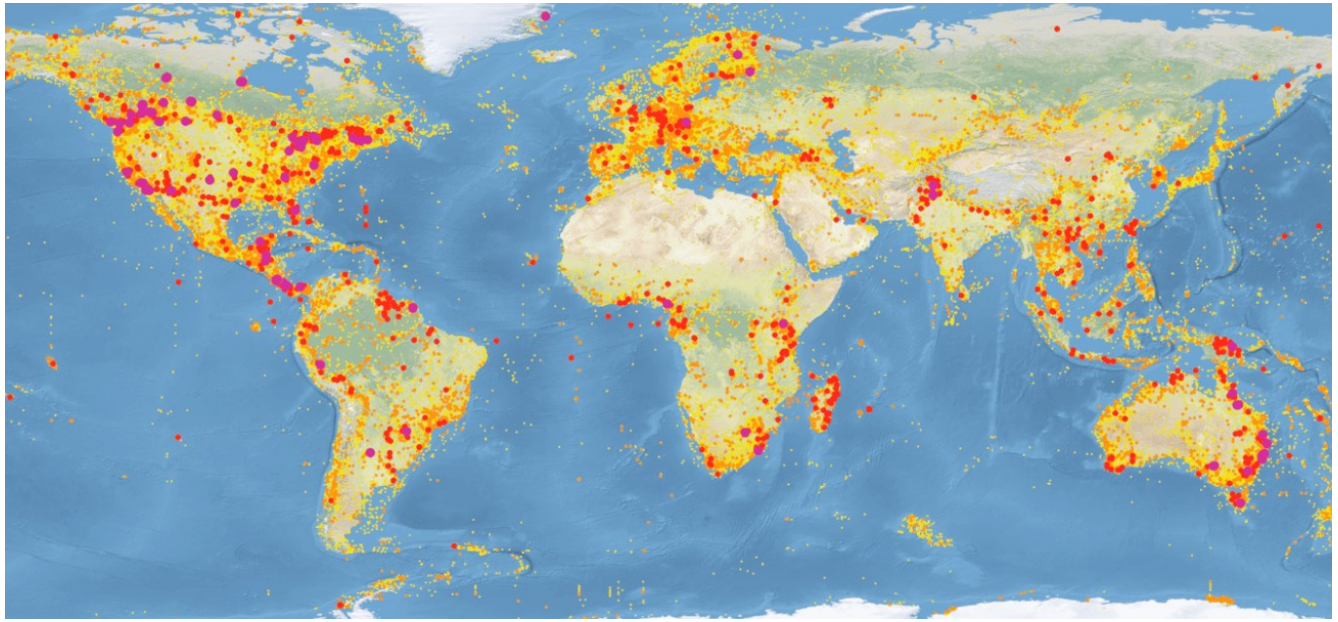
eDNA degradation and half-life

- eDNA half-life depends on many factors such as temperature, pH, humidity, microbial activity, UV radiation, enzime activity in stomach...
- might miss some diverse taxa in a sample because it was there few weeks ago at that spot, and the eDNA got degraded
- comparison between samples from different sites is harder because of different habitat characteristics that cause different eDNA half-lifes



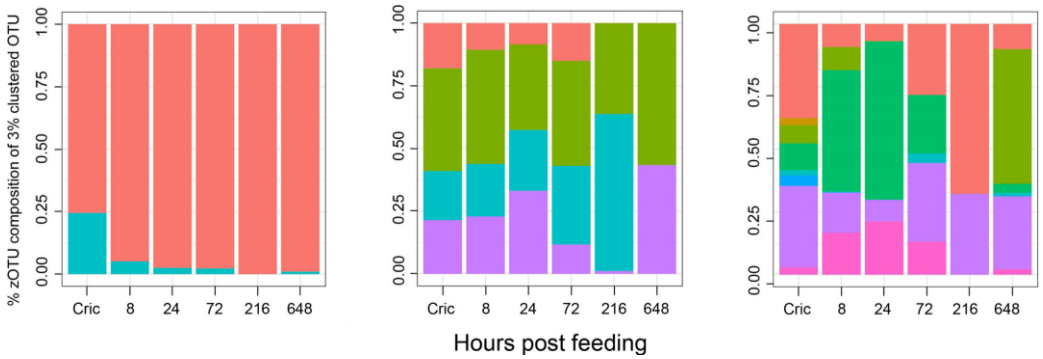
Availability of reference sequences for species at some specific area

- Not all areas of the world are well documented with barcode sequences of the present species
- BLAST results in such regions give you often a very low certainty that you have the right species/genera (<95%).



Data interpretation – qualitative, not quantitative

- Metabarcoding results cannot tell us reliably how many specimens of a species there are in a sample, only if a species is there or not (presence/absence; yes/no)
- sometimes (like for bacterial communities) they can tell us what are the ratios in abundance/mass between different bacterial species/communities, based on relative amount of sequencing reads.



Main applications of metabarcoding

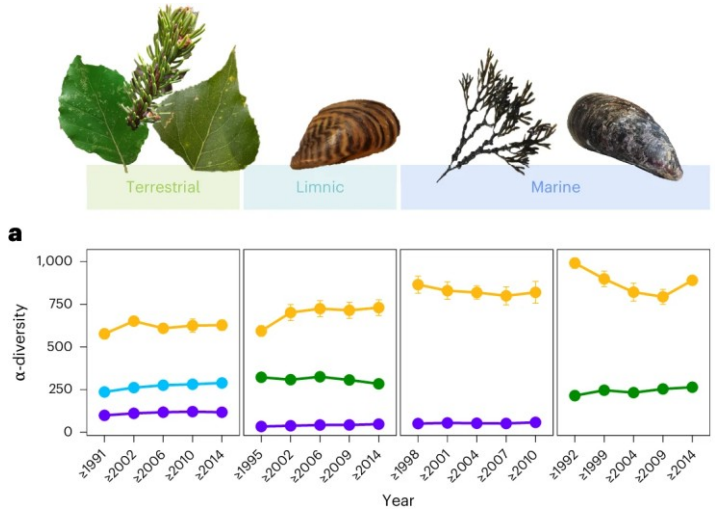
Use of metabarcoding: to track biodiversity change through space and time

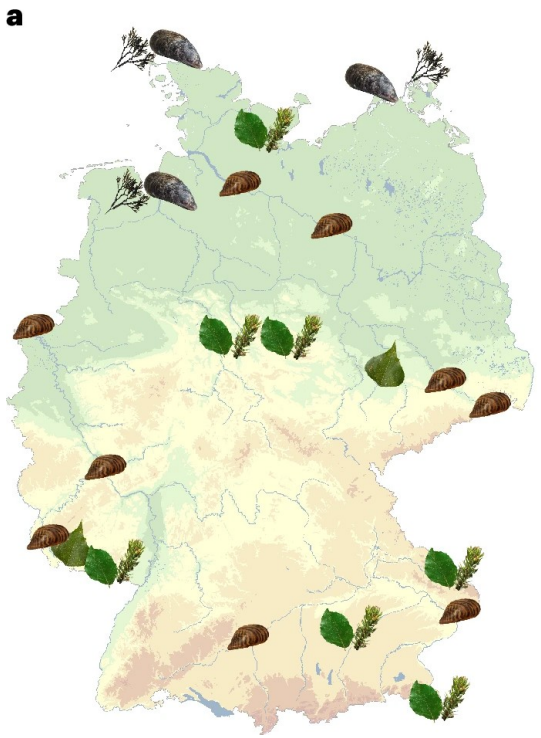
nature ecology & evolution

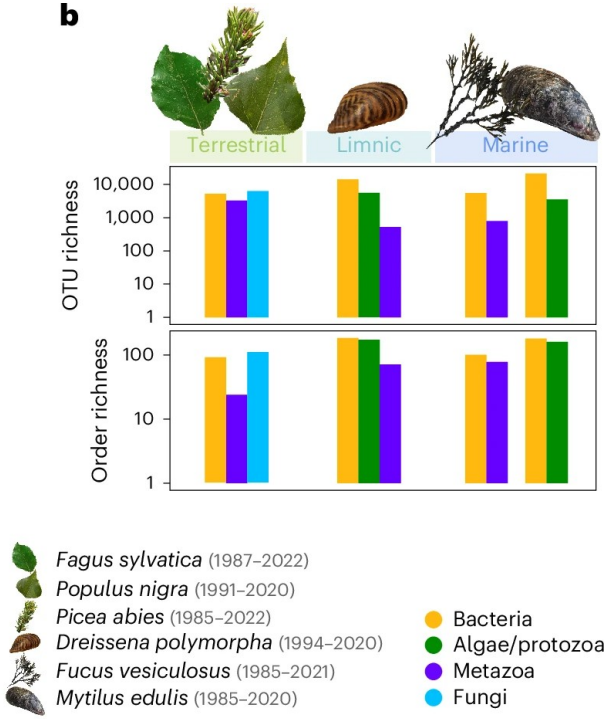
Article | Open access | Published: 01 August 2025

Archived natural DNA samplers reveal four decades of biodiversity change across the tree of life

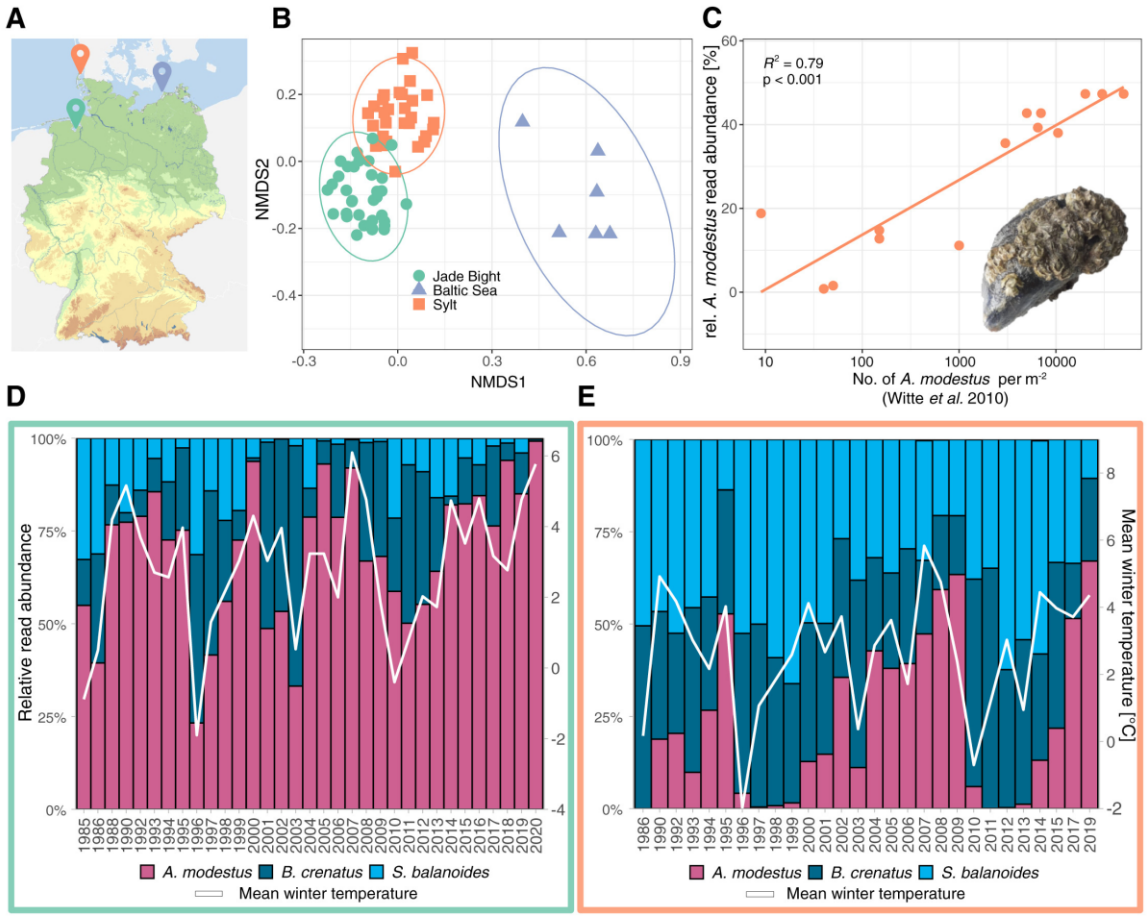
Isabelle Junk, Julian Hans, Benoît Perez-Lamarque, Manuel Stothut, Sven Weber, Elisabeth Gold,







Tracking the presence of rare/invasive species



CORRESPONDENCE · Volume 33, Issue 18, PR943-R944, September 25, 2023 · *Open Archive*

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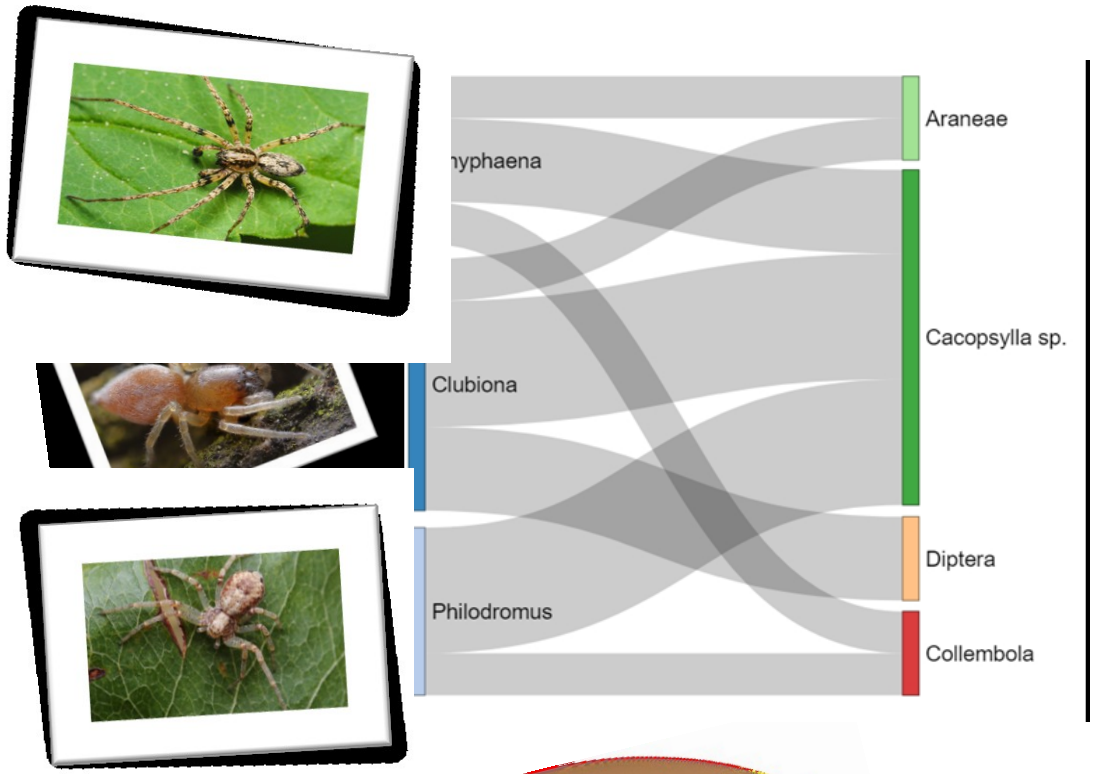
Tracking climate-change-induced biological invasions by metabarcoding archived natural eDNA samplers

Isabelle Junk · Nina Schmitt · Henrik Krehenwinkel [2]

Affiliations & Notes · Article Info

Study the diet of organisms – for pest management



Brace yourselves, winter is coming: the winter activity, natural diet, and prey preference of winter-active spiders on pear trees

Original Paper | Published: 02 March 2023

Volume 97, pages 113–126, (2024) Cite this article

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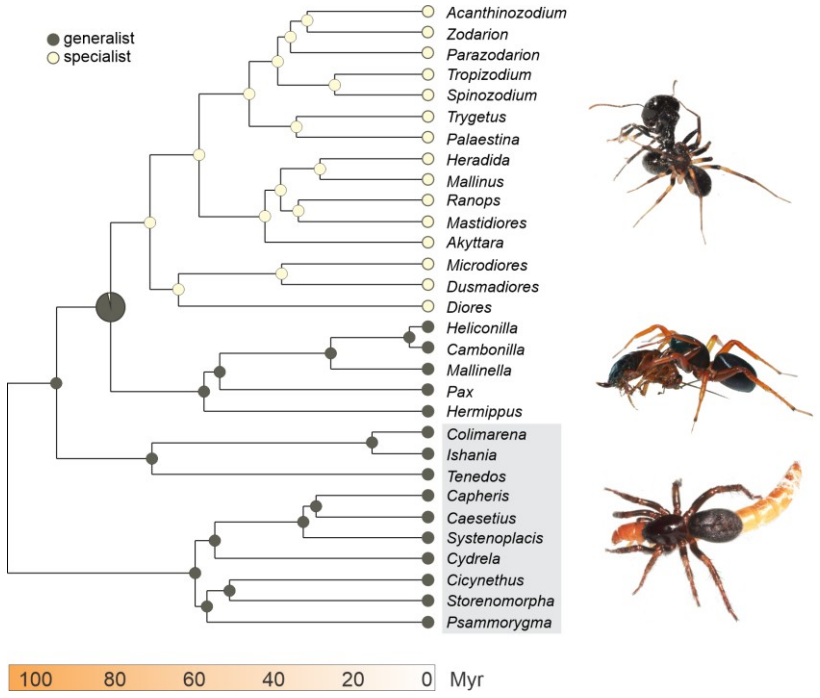
Domagoj Gajski, Tamara Mifková, Ondřej Košulič, Ondřej Michálek, Liliya Štarhová Serbina, Radek Michalko & Stano Pekár

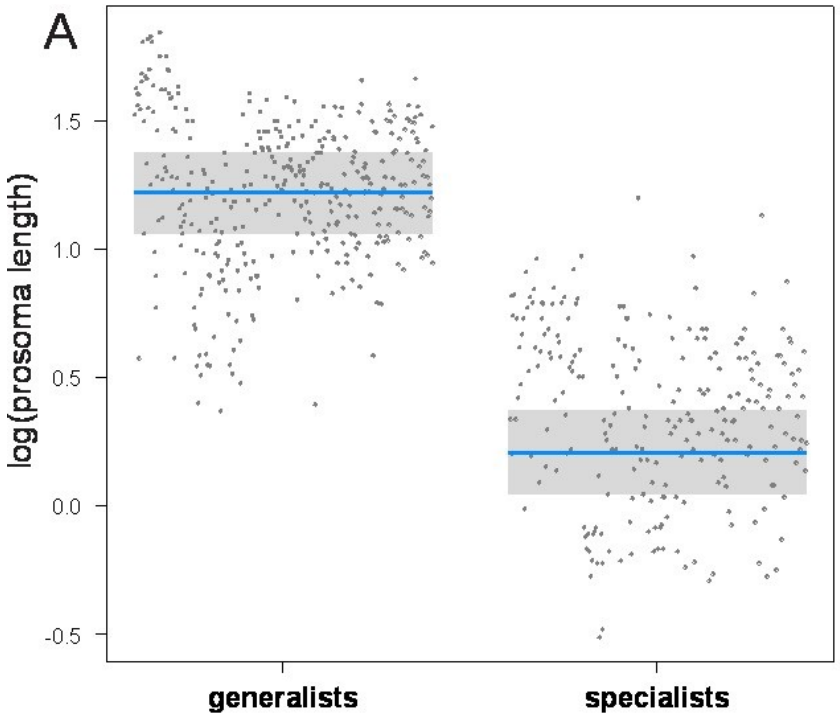
Cacopsylla pyri L. – a pest in pear orchards

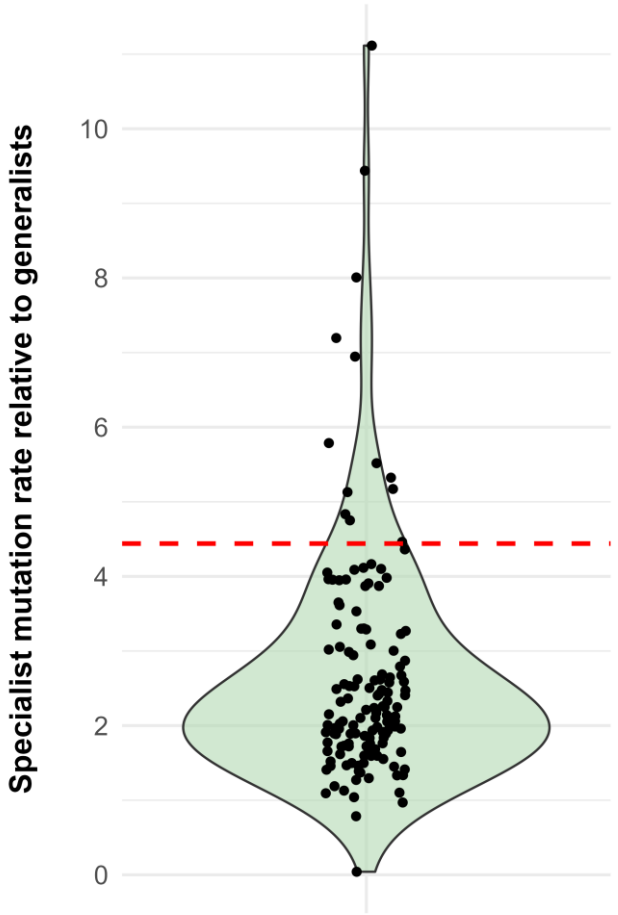
Study the evolution of diet specialisation

- **Specialisation towards dangerous prey leads to miniaturisation and accelerated evolution**

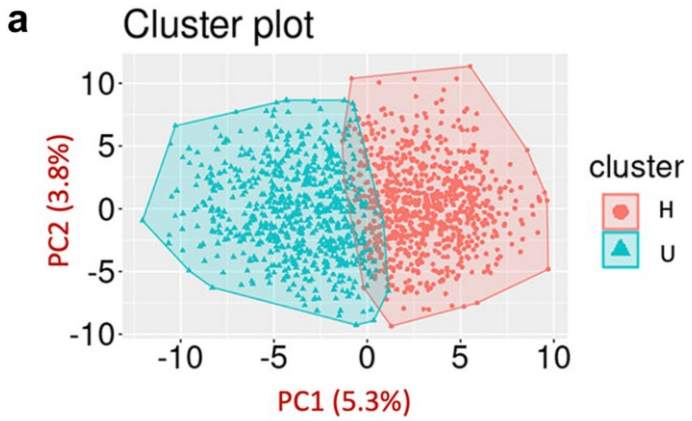
Domagoj Gajski[1,2], Stano Pekár[1], Vera Opatova[3], Tamara Wijacki[1,4], Ondřej Košulič[5], Charles Haddad[6], David Ortiz[1,7]







Gut microbiome and impact on human health



Association between gut microbiome profiles and host metabolic health across the life course: a population-based study

Ruolin Li [a,b,k] · Alexander Kurilshikov [c,k] · Shuyue Yang [d] · Julie A.E. van Oortmerssen [d] · Arno van Hilten [e,f] · Fariba Ahmadizar [d,g,j] · et al.

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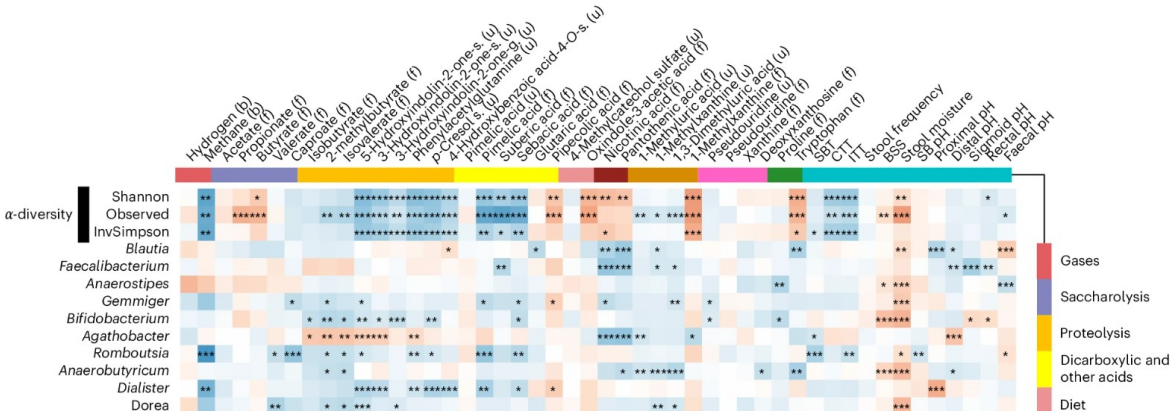
Fig. 5: Associations between metabolites, bacterial genera and gut environmental factors.

From: Gut physiology and environment explain variations in human gut microbiome composition and metabolism

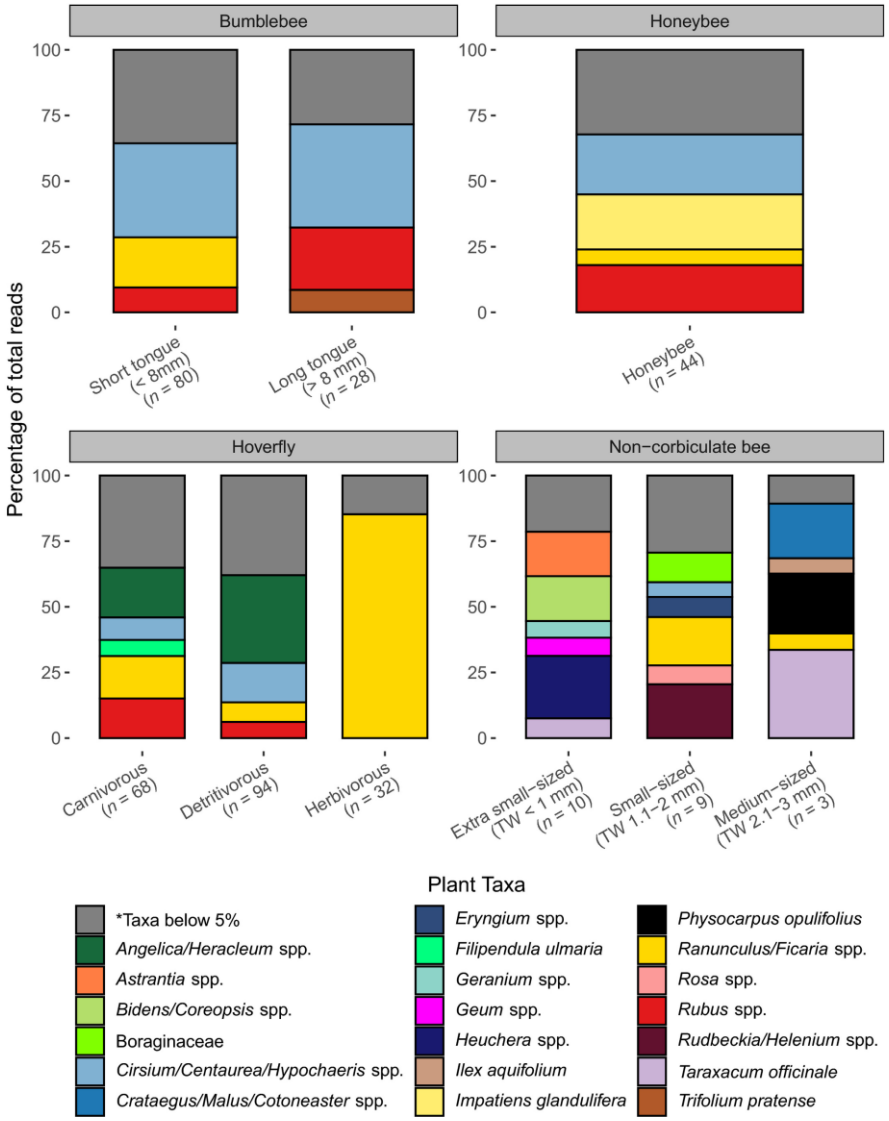


Article | Open access | Published: 27 November 2024

Gut physiology and environment explain variations in human gut microbiome composition and metabolism

Nicola Procházková, Martin F. Laursen, Giorgia La Barbera, Eirini Tsekitsidi, Malte S. Jørgensen, Morten A. Rasmussen, Jeroen Raes, Tine R. Licht, Lars O. Dragsted & Henrik M. Roager

Plant-pollinators interactions



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Seasonal progression and differences in major floral resource use by bees and hoverflies in a diverse horticultural and agricultural landscape revealed by DNA metabarcoding

Abigail Lowe, Laura Jones, Georgina Brennan, Simon Creer, Natasha de Vere

Thank you for your attention!!!

Contact:
molekularac2013@gmail.com

Metabarcoding and eDNA

- Course: Molecular Ecology
- Block 1: Genetic identifications in zoology
- Guest Teacher: Domagoj Gajski, VUK

Výzkumný ústav pro krajinu, v. v. i.