

ADN environnemental et biodiversité

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LECA
Laboratoire d'Ecologie Alpine



Environmental DNA and biodiversity

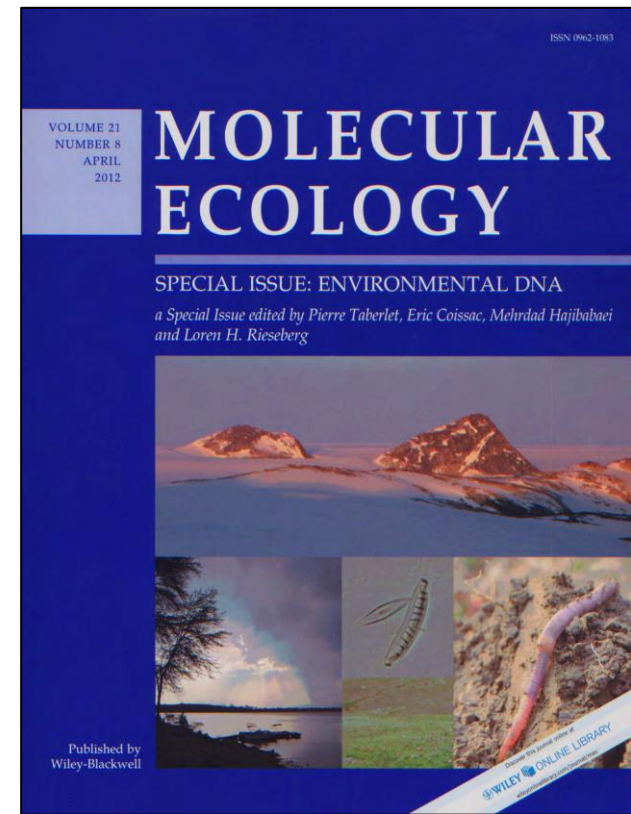
- Definitions and historical aspects
- Potential of DNA metabarcoding
 - For diet analysis
 - For biodiversity assessment
 - For tracking past communities
- Current limitations
 - Sampling difficulties
 - Amplification/Sequencing errors
 - Bias due to weak experimental design
- Conclusion

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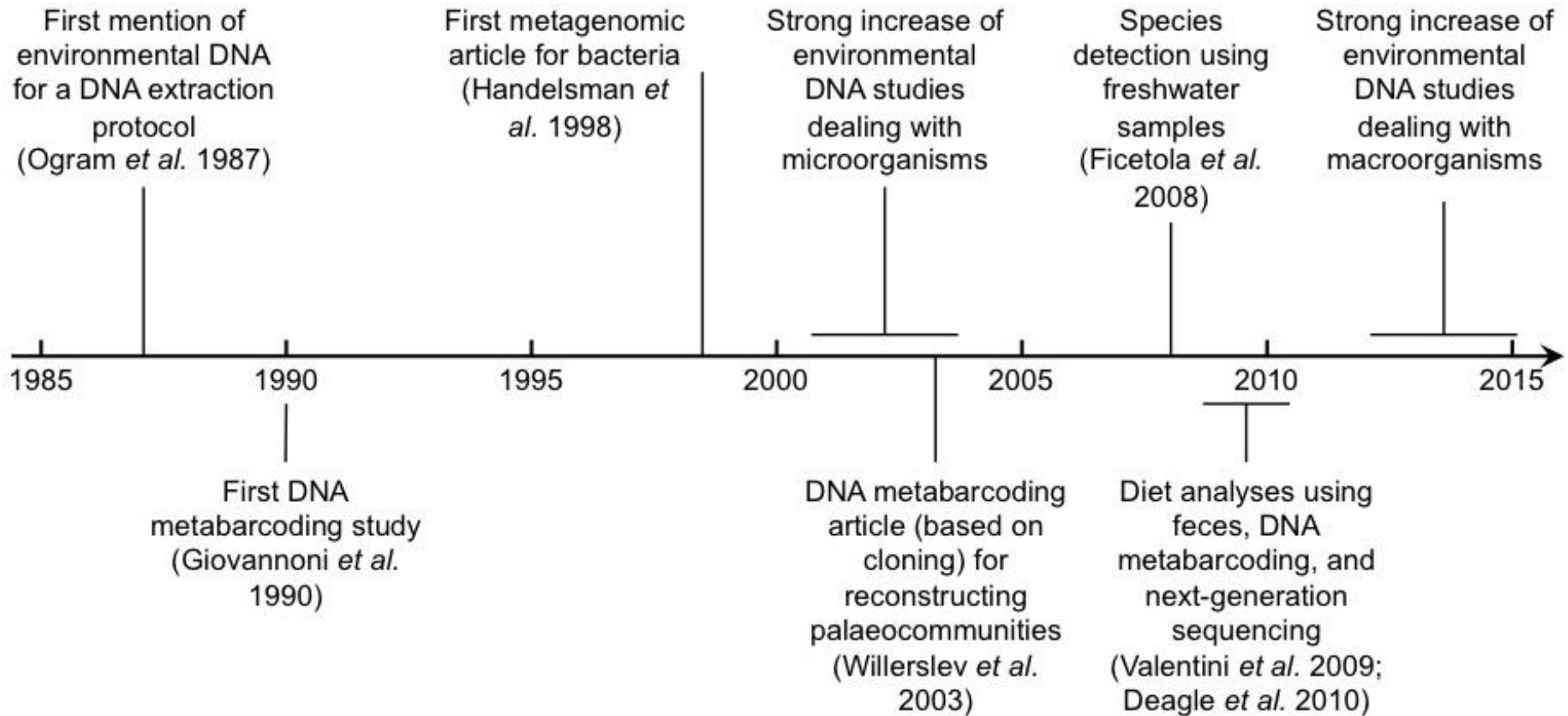
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Environmental DNA

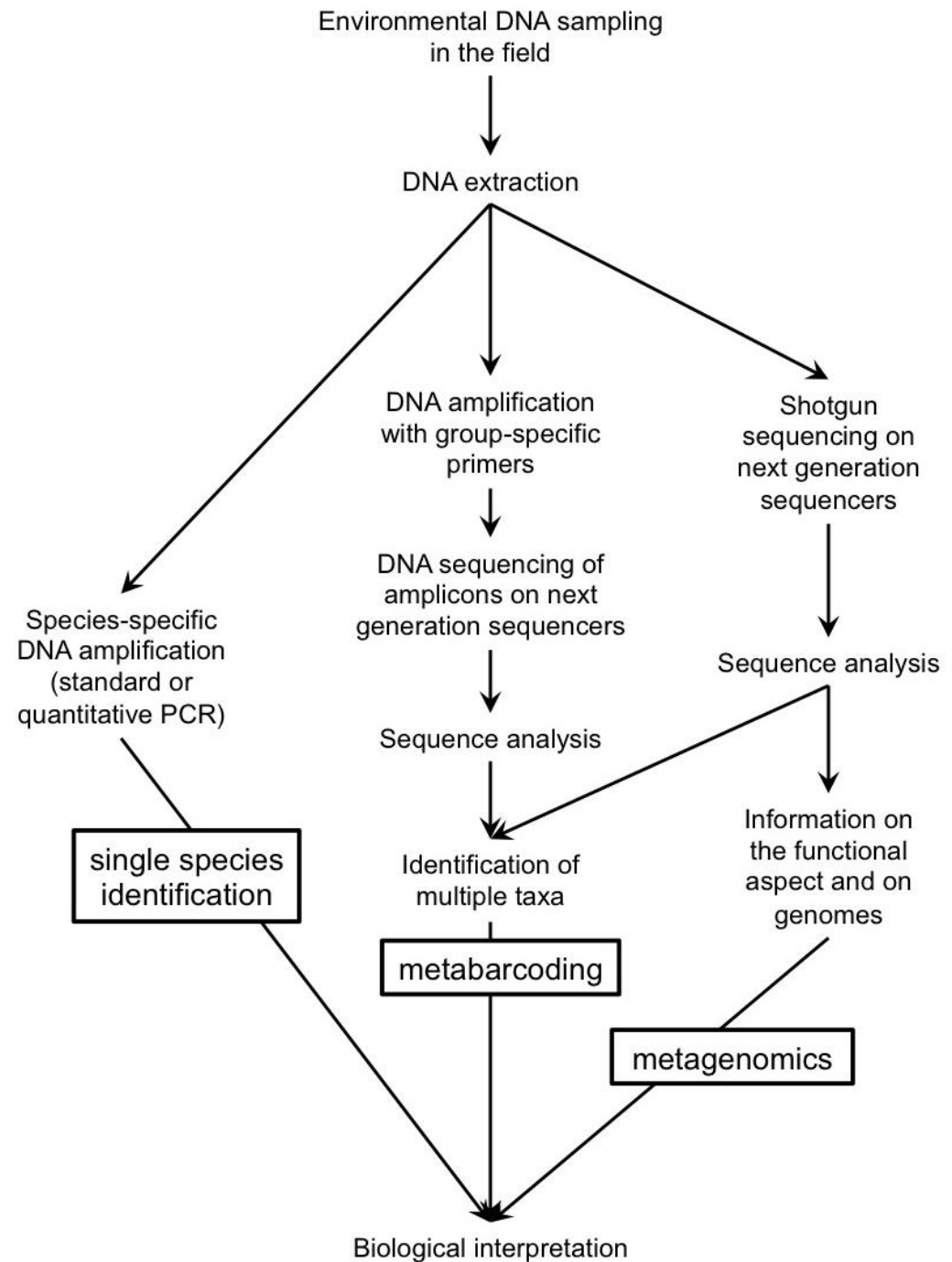
- First reference in 1987
- Microbiology: from 2000
- Plants and animals: from 2003
- Environmental DNA: DNA that can be extracted from environmental samples (such as soil, water, or air), without first isolating any target organisms
- Complex mixture of genomic DNA from many different organisms, possibly degraded
- Contains intracellular and extracellular DNA



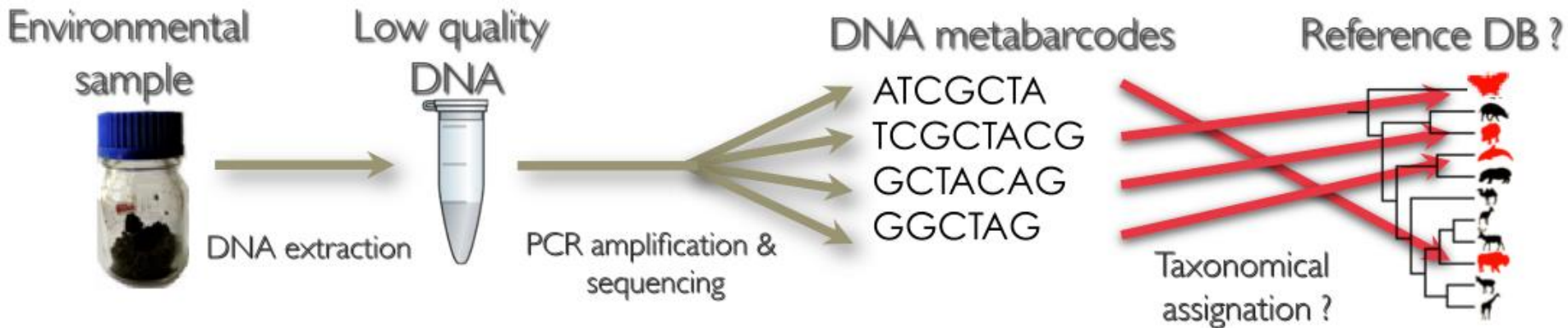
Overview of the emergence of eDNA studies



The main steps of an eDNA study, showing the three possible approaches: single-species identification, metabarcoding, and metagenomics



DNA metabarcoding



MOLECULAR ECOLOGY

Molecular Ecology (2012) 21, 3647–3655

doi: 10.1111/j.1365-294X.2012.05545.x

FROM THE COVER

DNA from soil mirrors plant taxonomic and growth form diversity

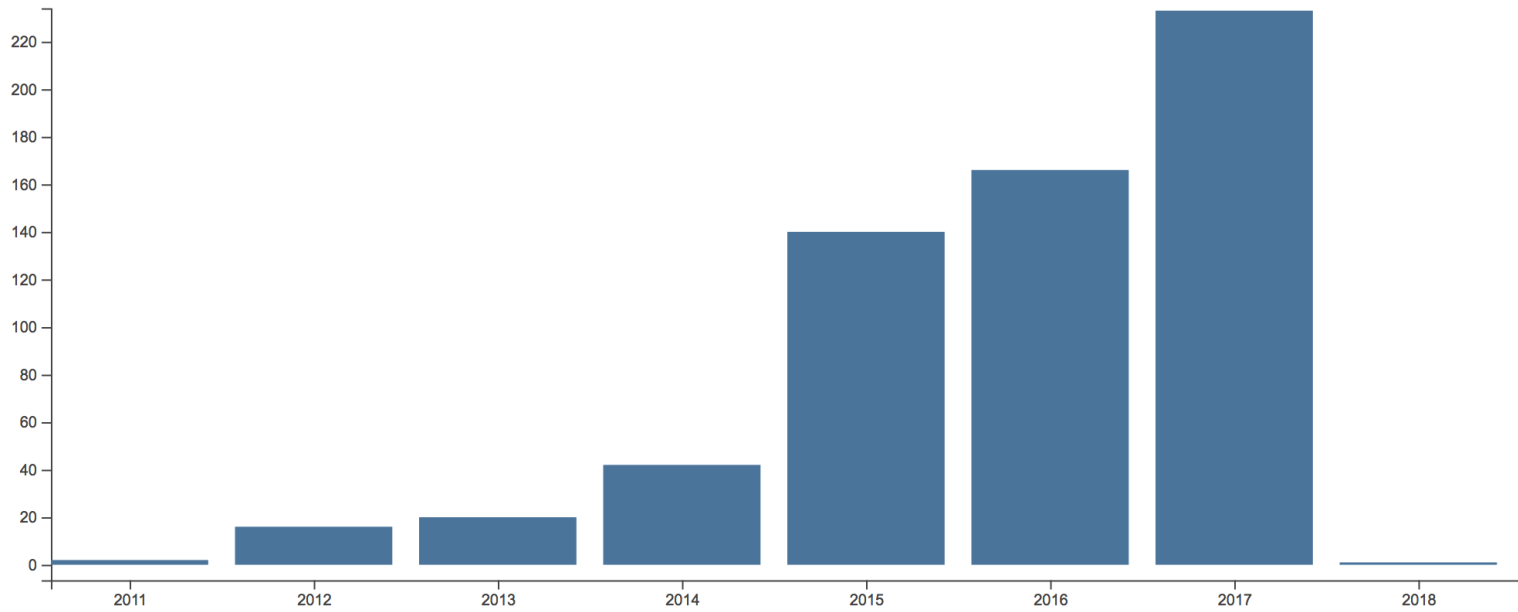
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metabarcoding

Total Publications

620

number of
papers



Year of publication

Web of Science, 16 October 2017

The metabarcoding approach: bioinformatics, field, bench, bioinformatics

- *In silico* analysis: design and test the most efficient metabarcodes for the target group
- Sampling in the field to obtain a DNA extract representative of the local biodiversity
- DNA amplification and sequencing
- Sequence analysis and taxa identification
 - OBITools (metabarcoding.org/obitools)
 - Problem of amplification/sequencing errors

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New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trnL* approach

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Abstract

The development of DNA barcoding (species identification using a standardized DNA sequence), and the availability of recent DNA sequencing techniques offer new possibilities in diet analysis. DNA fragments shorter than 100–150 bp remain in a much higher proportion in degraded DNA samples and can be recovered from faeces. As a consequence, by using universal primers that amplify a very short but informative DNA fragment, it is possible to reliably identify the plant taxon that has been eaten. According to our experience and using this identification system, about 50% of the taxa can be identified to species using the *trnL* approach, that is, using the P6 loop of the chloroplast *trnL* (UAA) intron. We demonstrated that this new method is fast, simple to implement, and very robust. It can be applied for diet analyses of a wide range of phytophagous species at large scales. We also demonstrated that our approach is efficient for mammals, birds, insects and molluscs. This method opens new perspectives in ecology, not only by allowing large-scale studies on diet, but also by enhancing studies on resource partitioning among competing species, and describing food webs in ecosystems.

Valentini *et al.* (2009) *Molecular Ecology Resources*, **9**, 51-60.

Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan

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Tracking earthworm communities from soil DNA

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Species detection using environmental DNA from water samples

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Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding

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Valentini *et al.* (2016) *Molecular Ecology*, **25**, 929–942.

ARTICLE

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Long livestock farming history and human landscape shaping revealed by lake sediment DNA

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Fifty thousand years of Arctic vegetation and megafaunal diet

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Although it is generally agreed that the Arctic flora is among the youngest and least diverse on Earth, the processes that shaped it are poorly understood. Here we present 50 thousand years (kyr) of Arctic vegetation history, derived from the first large-scale ancient DNA metabarcoding study of circumpolar plant diversity. For this interval we also explore nematode diversity as a proxy for modelling vegetation cover and soil quality, and diets of herbivorous megafaunal mammals, many of which became extinct around 10 kyr BP (before present). For much of the period investigated, Arctic vegetation consisted of dry steppe-tundra dominated by forbs (non-graminoid herbaceous vascular plants). During the Last Glacial Maximum (25–15 kyr BP), diversity declined markedly, although forbs remained dominant. Much changed after 10 kyr BP, with the appearance of moist tundra dominated by woody plants and graminoids. Our analyses indicate that both graminoids and forbs would have featured in megafaunal diets. As such, our findings question the predominance of a Late Quaternary graminoid-dominated Arctic mammoth steppe.

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Sampling difficulties

- An appropriate sampling design is crucial for the success of any ecological study
- The samples must be representative of the ecosystem under study
- Requirement for biological replicates

Amplification/Sequencing errors

- Always more MOTUs than true species
- α diversity difficult to estimate via DNA metabarcoding
- Reliable estimates of β diversity
- It is possible to deal with errors by building a comprehensive reference database

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Is DNA metabarcoding ready for monitoring?

- The experimental protocol must be standardized from the sampling to the sequence analysis
- Two solutions for coping with errors
 - Comprehensive database for the target taxonomic group
 - Large set of reference localities (taxonomy-free approach)
- Difficulty at the moment for choosing the appropriate metabarcode (COI vs other markers)

In press
(February 2018)

Preface
Chapter 1: Introduction to environmental DNA (eDNA)
Chapter 2: DNA metabarcode choice and design
Chapter 3: Reference databases
Chapter 4: Sampling
Chapter 5: DNA extraction
Chapter 6: DNA amplification and multiplexing
Chapter 7: DNA sequencing
Chapter 8: DNA metabarcoding data analysis
Chapter 9: Single-species detection
Chapter 10: Environmental DNA for functional diversity
Chapter 11: Some early landmark studies
Chapter 12: Freshwater ecosystems
Chapter 13: Marine environments
Chapter 14: Terrestrial ecosystems
Chapter 15: Palaeoenvironments
Chapter 16: Host-associated microbiota
Chapter 17: Diet analysis
Chapter 18: Analysis of bulk samples
Chapter 19: The future of eDNA metabarcoding
References
Appendix 1: Examples of primer pairs available for DNA metabarcoding
Appendix 2: 384 tags of 8 nucleotides, with at least 3 differences among them
Appendix 3: Checklist when designing a PCR-based DNA metabarcoding experiment

PIERRE TABERLET | AURÉLIE BONIN | LUCIE ZINGER | ERIC COISSAC

ENVIRONMENTAL

DNA

For Biodiversity Research and Monitoring

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Thank
you for
your
attention

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