

Introduction à l'ADN environnemental

Pierre Taberlet

Laboratoire d'Ecologie Alpine, CNRS UMR 5553
Université Grenoble Alpes, Grenoble, France
et

The Arctic University of Norway, Tromsø Museum, Tromsø, Norway



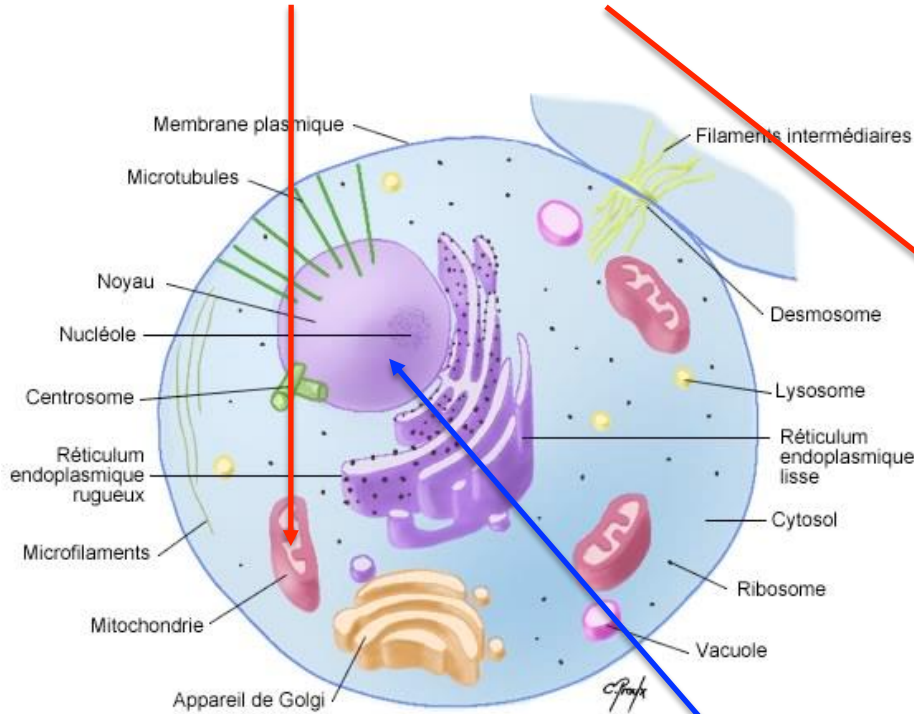
LECA
Laboratoire d'Ecologie Alpine



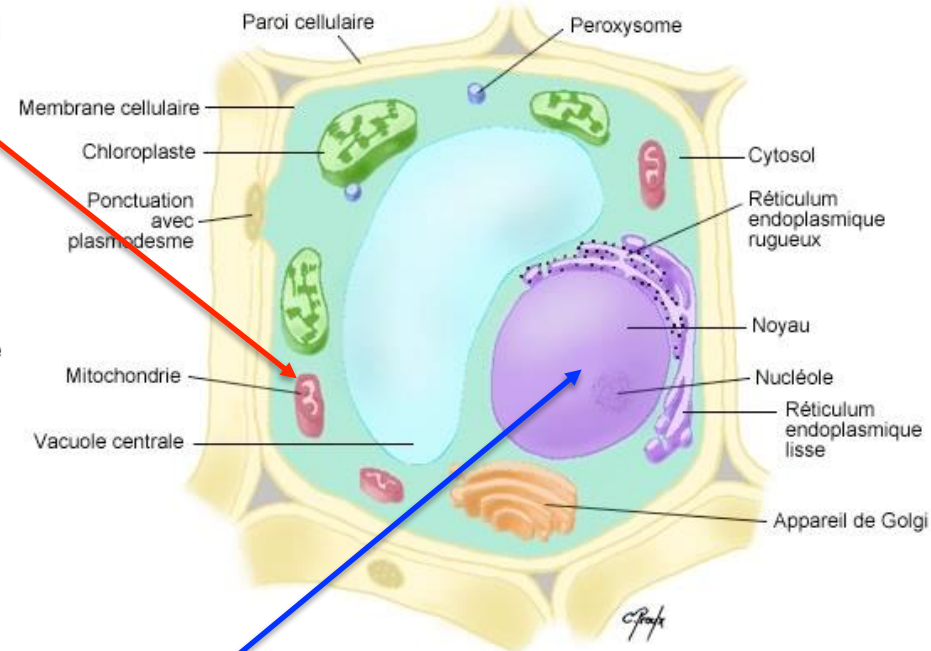
Paris, 28 Septembre 2023

L'ADN dans les cellules

ADN mitochondrial

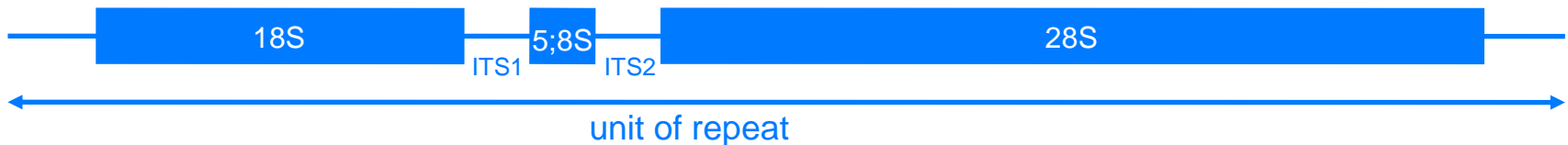


Cellule animale



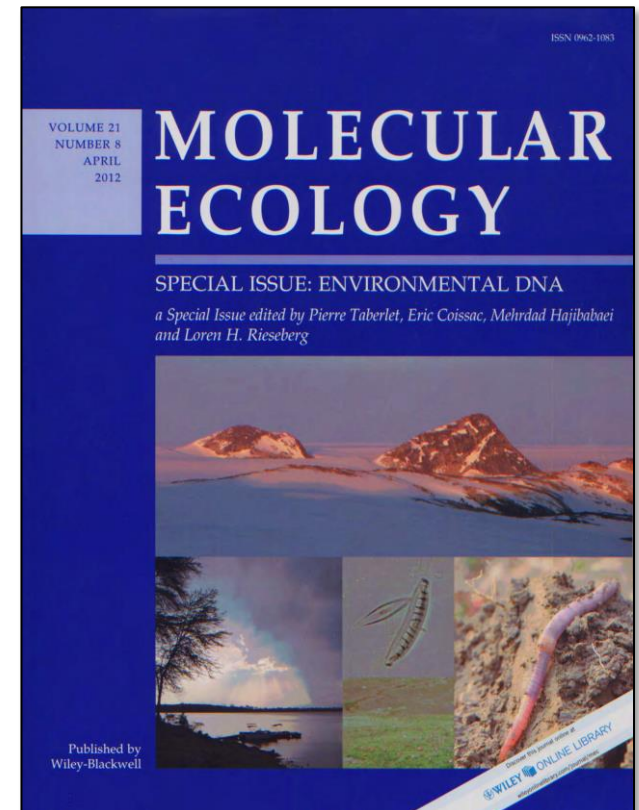
Cellule végétale

ADN nucléaire ribosomal DNA



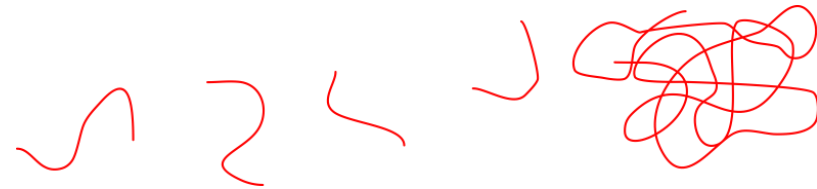
Qu'est-ce que l'ADN environnemental ?

- Il s'agit de l'ADN qui peut être extrait d'un échantillon environnemental sans isoler *a priori* d'organismes cibles
- Exemple d'échantillons environnementaux:
 - Sol
 - Eau
 - Sédiment
 - Air
 - Fèces

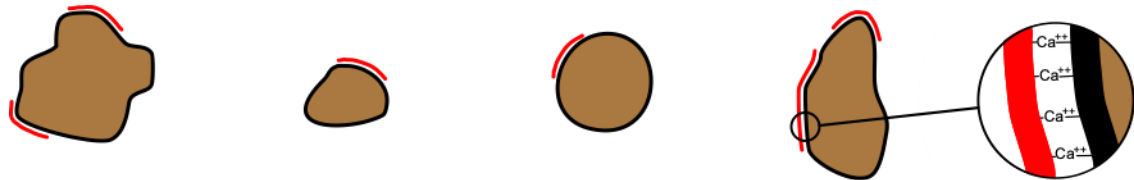


Trois formes différentes pour l'ADN environnemental

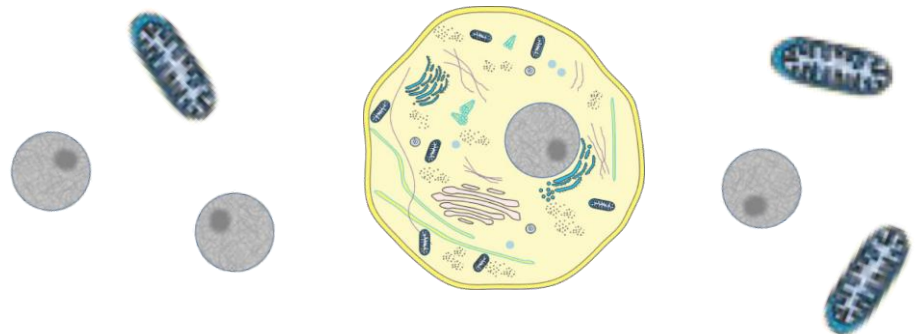
- ADN libre



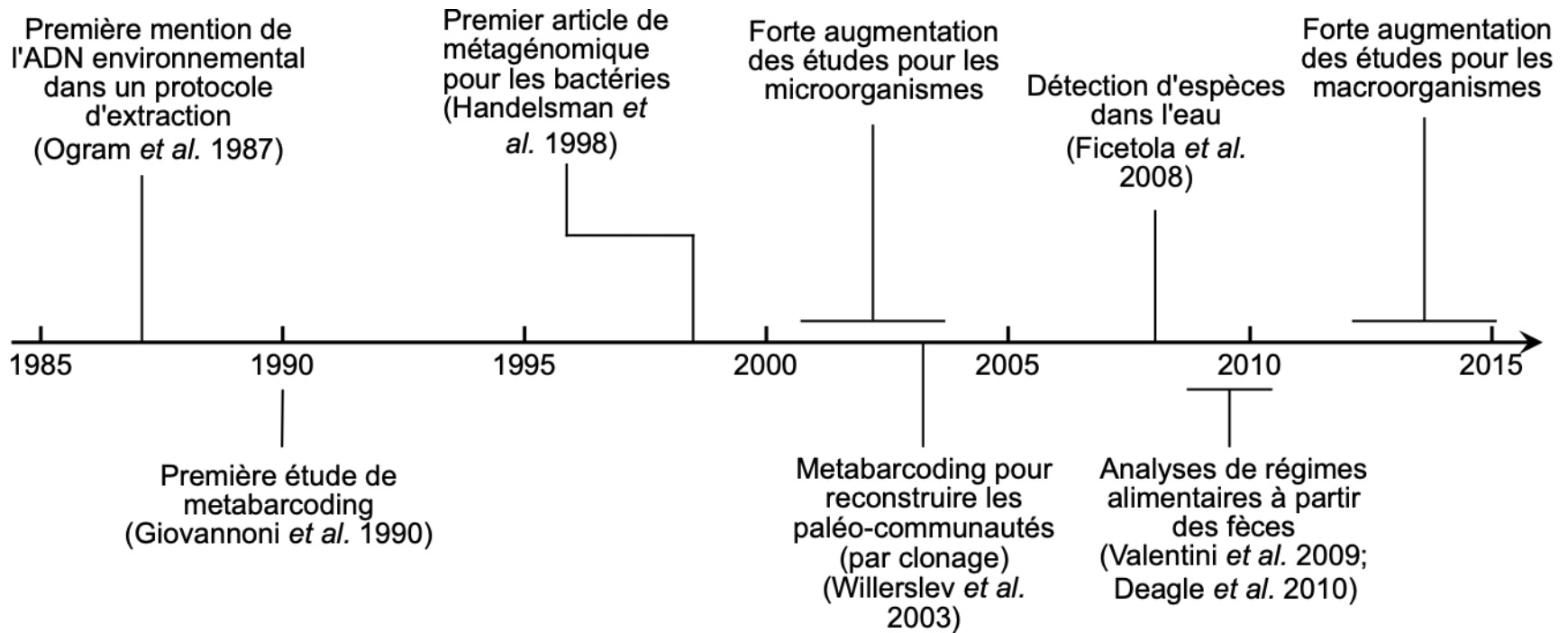
- ADN adsorbé sur des particules minérales ou organiques



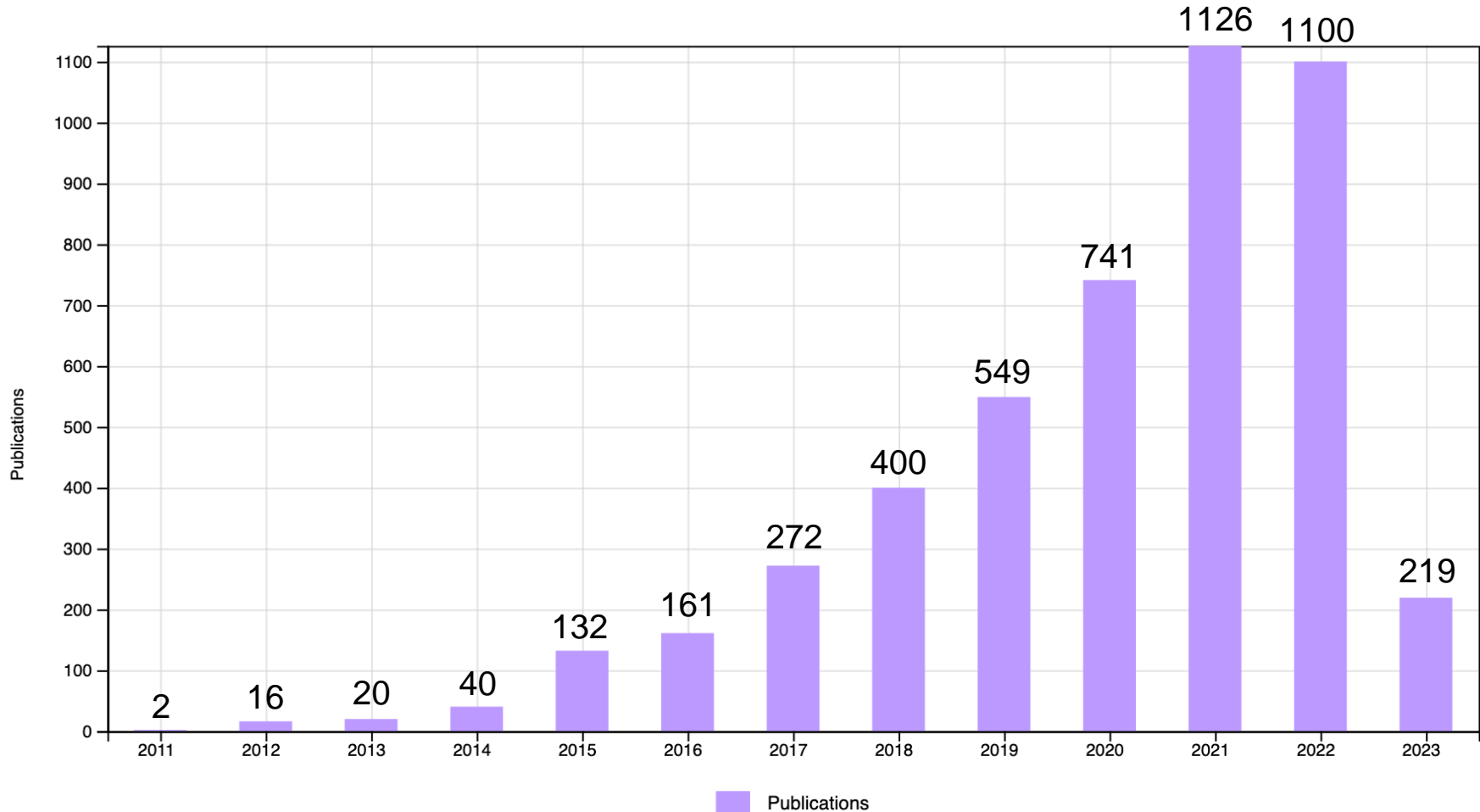
- Cellules ou débris cellulaires



Emergence de l'ADN environnemental

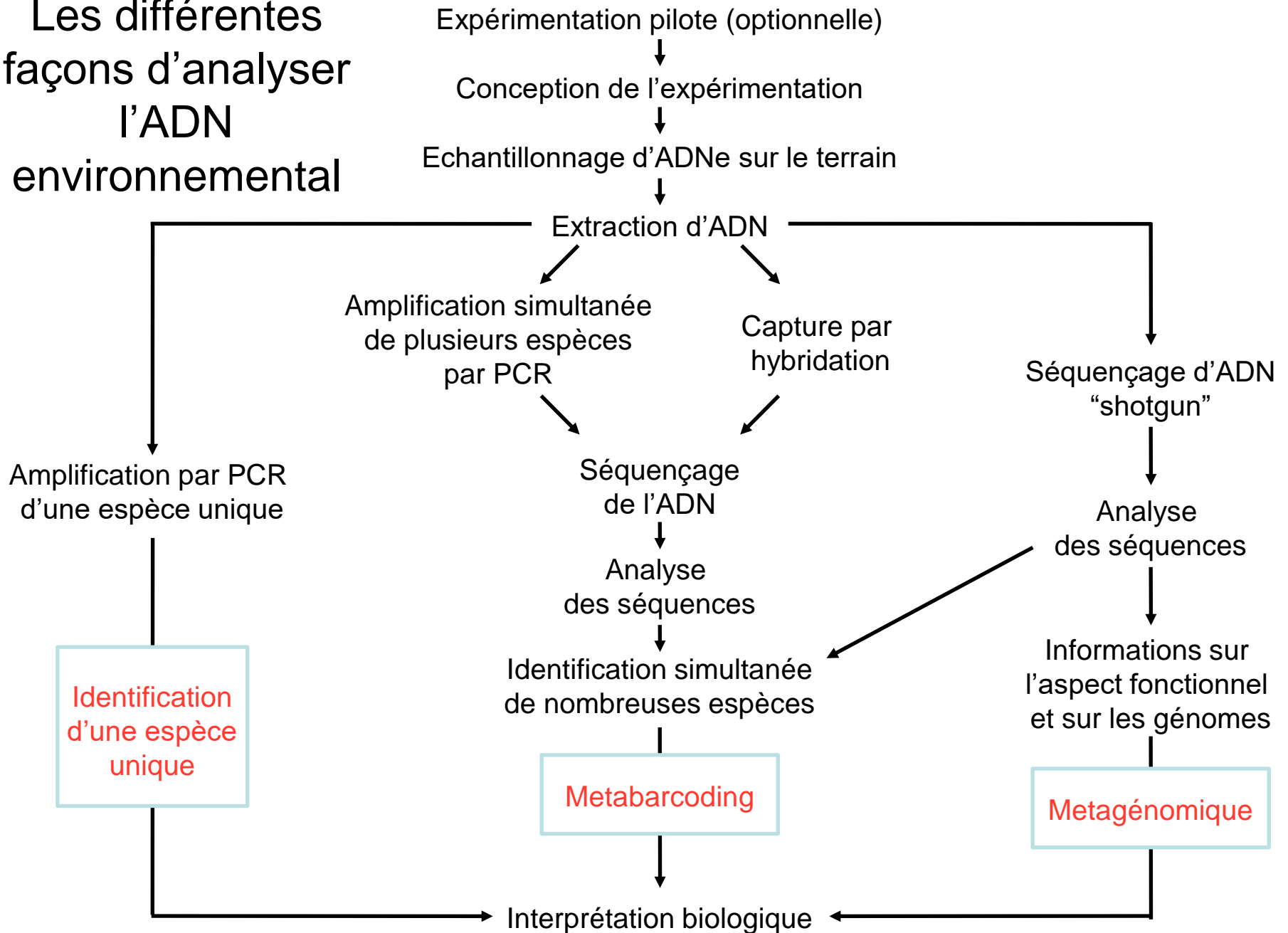


Emergence de l'ADN environnemental

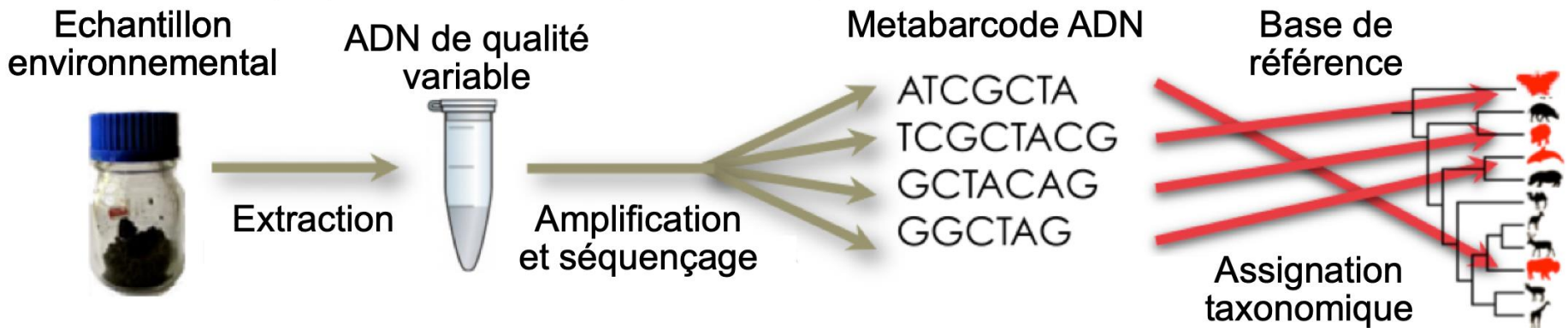


Source: "metabarcoding" dans le Web of Science, 19 Mars 2023

Les différentes façons d'analyser l'ADN environnemental



Metabarcoding: identification simultanée de plusieurs espèces à partir de leurs ADN



Le paradoxe du metabarcoding:

- Chaque étape est très simple au niveau technique
- Mais globalement, c'est difficile de concevoir une expérimentation solide

Régimes alimentaires

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

ALICE VALENTINI,[†] CHRISTIAN MIQUEL,^{*} MUHAMMAD ALI NAWAZ,^{‡§} EVA BELLEMAIN,^{*} ERIC COISSAC,^{*} FRANÇOIS POMPANON,^{*} LUDOVIC GIELLY,^{*} CORINNE CRUAUD,[¶] GIUSEPPE NASCETTI,[†] PATRICK WINCKER,[¶] JON E. SWENSON,^{‡**} and PIERRE TABERLET^{*}

^{*}Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble cedex 9, France, [†]Dipartimento di Ecologia e Sviluppo Economico Sostenibile, Università degli Studi della Tuscia, via S. Giovanni Decollato 1, I-01100 Viterbo, Italy, [‡]Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Post Box 5003, NO-1432 Ås, Norway, [§]Himalayan Wildlife Foundation, 01, Park Road, Sector F-8/1 Islamabad 44000, Pakistan, [¶]Genoscope – CNS, 2 rue Gaston Crémieux, BP 5706, F-91057 Evry cedex, France, ^{**}Norwegian Institute for Nature Research, NO-7485 Trondheim, Norway

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.



Mollusques

Deroceras reticulatum (1)

Arion rufus (1)

Helix aspera (1)



Insectes

Gonfophocerippus rufus (2)

Chorthippus biguttulus (1)



Oiseaux

Tetrao urogallus aquitanicus (4)

Tetrao urogallus major (2)



Mammifères

Ursus arctos (12)

Marmota caudata (12)

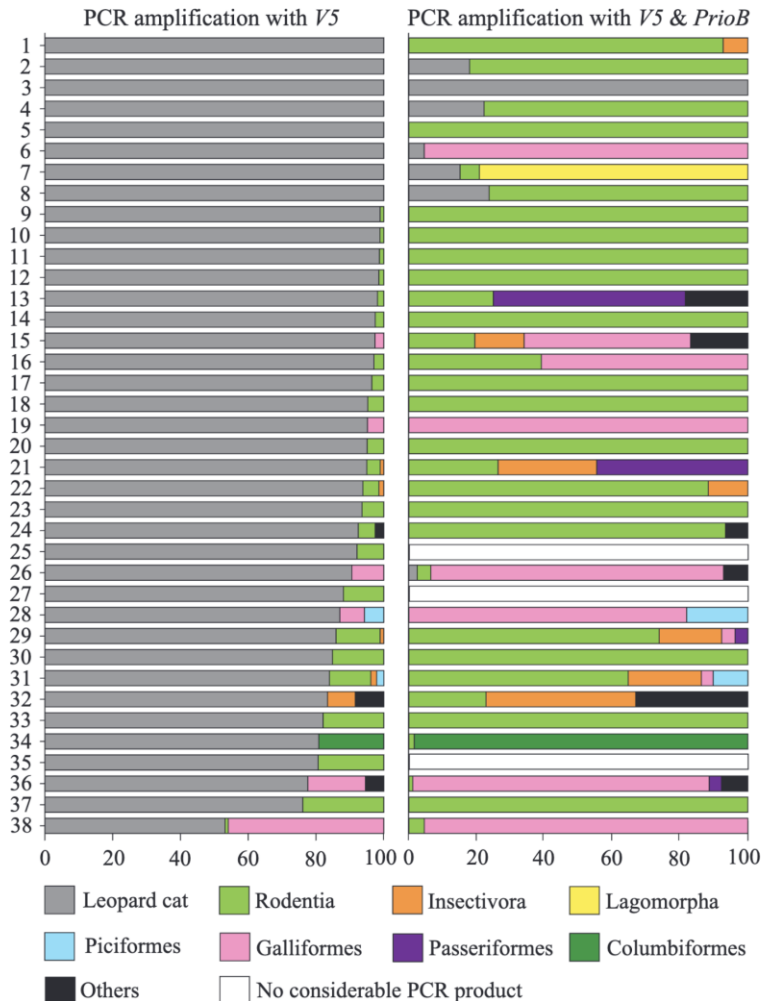
Carnivore diet analysis based on next-generation sequencing: application to the leopard cat (*Prionailurus bengalensis*) in Pakistan

WASIM SHEHZAD,* TIAYYBA RIAZ,* MUHAMMAD A. NAWAZ,*+ CHRISTIAN MIQUEL,* CAROLE POILLOT,* SAFDAR A. SHAH,‡ FRANÇOIS POMPANON,* ERIC COISSAC* and PIERRE TABERLET*

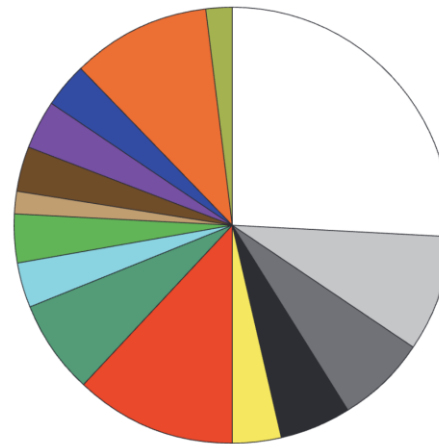
*Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France, †Snow Leopard Trust (Pakistan Program), 17-Service Road North, I-8/3, Islamabad, Pakistan, ‡Wildlife Department, Khyber Pakhtunkhwa, Pakistan



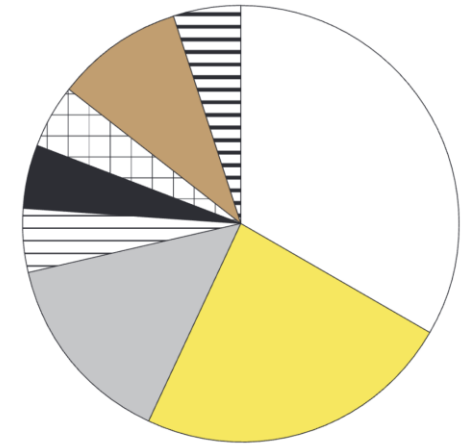
Régime alimentaire du chat léopard



(a) Ayubia National Park



(b) Chitral Gol National Park



Mammals

House rat
(*Rattus rattus*)

Himalayan wood mouse
(*Apodemus rusiges*)

Murree vole
(*Hyperacrius wynnei*)

House mouse
(*Mus musculus*)

Kashmir flying squirrel
(*Eoglaucomys fimbriatus*)

Forest dormouse
(*Dryomys nitedula*)

Asiatic white toothed shrew
(*Crocidura pullata*)

Cape hare
(*Lepus capensis*)

Kalij pheasant
(*Lophura leucomelanos*)

Chicken
(*Gallus gallus*)

Koklass pheasant
(*Pucrasia macrolopha*)

Chukar partridge
(*Alectoris chukar*)

Babbler
(*Timaliidae*)

Jungle crow
(*Corvus macrorhynchos*)

Woodpecker
(*Dendrocopos* sp.)

Rock pigeon
(*Columba livia*)

Amphibian

Murree hill frog
(*Paa vicina*)

Fish

Cat fish
(*Siluriformes*)

Analyse de l'eau

Species detection using environmental DNA from water samples

Gentile Francesco Ficetola^{1,2,*}, Claude Miaud², François Pompanon¹ and Pierre Taberlet¹

¹Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université Joseph Fourier, BP 53, 38041 Grenoble Cedex 09, France

²Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université de Savoie, 73376 Le Bourget du Lac Cedex, France

*Author and address for correspondence: Dipartimento di Scienze dell'Ambiente e del Territorio, Università Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy (francesco.ficetola@unimi.it).

The assessment of species distribution is a first critical phase of biodiversity studies and is necessary to many disciplines such as biogeography, conservation biology and ecology. However, several species are difficult to detect, especially during particular time periods or developmental stages, potentially biasing study outcomes. Here we present a novel approach, based on the limited persistence of DNA in the environment, to detect the presence of a species in fresh water. We used specific primers that amplify short mitochondrial DNA sequences to track the presence of a frog (*Rana catesbeiana*) in controlled environments and natural wetlands. A multi-sampling approach allowed for species detection in all environments where it was present, even at low densities. The reliability of the results was demonstrated by the identification of amplified DNA fragments, using traditional sequencing and parallel pyrosequencing techniques. As the environment can retain the molecular imprint of inhabiting species, our approach allows the reliable detection of secretive organisms in wetlands without direct observation. Combined with massive sequencing and the development of DNA barcodes that enable species identification, this approach opens new perspectives for the assessment of current biodiversity from environmental samples.

*Lithobates
catesbeianus*



Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding

ALICE VALENTINI,* PIERRE TABERLET,†‡ CLAUDE MIAUD,§ RAPHAËL CIVADE,¶ JELGER HERDER,** PHILIP FRANCIS THOMSEN,†† EVA BELLEMAIN,* AURÉLIEN BESNARD,§ ERIC COISSAC,†‡ FRÉDÉRIC BOYER,†‡ COLINE GABORIAUD,* PAULINE JEAN,* NICOLAS POULET,†‡ NICOLAS ROSET,§§ GORDON H. COPP,¶¶*** PHILIPPE GENIEZ,§ DIDIER PONT,¶ CHRISTINE ARGILLIER,††† JEAN-MARC BAUDOIN,††† TIPHAINE PEROUX,††† ALAIN J. CRIVELLI,††† ANTHONY OLIVIER,††† MANON ACQUEBERGE,§§§ MATTHIEU LE BRUN,¶¶¶ PETER R. MØLLER,**** ESKE WILLERSLEV†† and TONY DEJEAN*



Analyse des sols

FROM THE COVER

DNA from soil mirrors plant taxonomic and growth form diversity

N. G. YOCCOZ,* K. A. BRÅTHEN,* L. GIELLY,+ J. HAILE,‡§ M. E. EDWARDS,¶ T. GOSLAR,** H. VON STEDINGK,¶ A. K. BRYSTING,++ E. COISSAC,+ F. POMPANON,+ J. H. SØNSTEBØ,++ C. MIQUEL,+ A. VALENTINI,+ F. DE BELLO,+‡‡ J. CHAVE,§§ W. THUILLER,+ P. WINCKER,¶¶ C. CRUAUD,¶¶ F. GAVORY,¶¶ M. RASMUSSEN,‡ M. T. P. GILBERT,‡ L. ORLANDO‡ C. BROCHMANN,++¹ E. WILLERSLEV,‡¹ and P. TABERLET,+¹

**Department of Arctic and Marine Biology, University of Tromsø, NO-9037 Tromsø, Norway, †Laboratoire d'Ecologie Alpine, CNRS UMR 5553, Université Joseph Fourier, BP 43, F-38041 Grenoble Cedex 9, France, ‡Centre for GeoGenetics, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark, §Murdoch University, Perth, Western Australia 6150, Australia, ¶University of Southampton, Geography and Environment, Southampton SO17 1BJ, UK, **Faculty of Physics, Adam Mickiewicz University, ul. Umultowska 85, 61-614 Poznan, Poland, ++National Centre for Biosystematics, Natural History Museum, University of Oslo, PO Box 1172, Blindern, N-0318 Oslo, Norway, ‡‡Institute of Botany, Czech Academy of Sciences, Dukelská 135, CZ-379 82, Třeboň, Czech Republic, §§Laboratoire Evolution et Diversité Biologique, CNRS UMR 5174, Université Paul Sabatier, F-31062 Toulouse, France, ¶¶Genoscope, CEA, CNRS, UMR 8030, 2 rue Gaston Crémieux, BP 5706, F-91057 Evry cedex, France*

Yoccoz *et al.* (2012) *Molecular Ecology*, **21**, 3647–3655.

Relevés botaniques traditionnels versus metabarcoding sur le sol



Relevés botaniques traditionnels

Avenella flexuosa



Poa sp.



Taraxacum sp.



Anthoxanthum nipponicum



Carex sp.



Deschampsia sp.



Festuca sp.



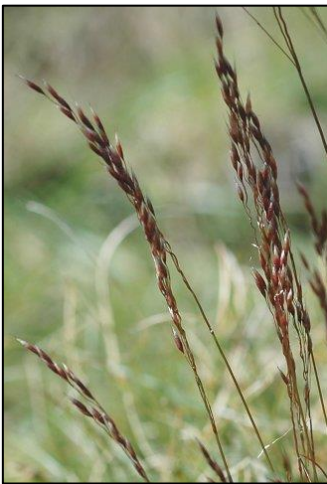
Rumex sp.



Calamagrostis sp.



Metabarcoding sur le sol



Bistorta vivipara



Salix sp.



Alchemilla sp.



Viola biflora



Deschampsia sp.



Calamagrostis sp.

Equisetum sp.



Tracking earthworm communities from soil DNA

FRIEDERIKE BIENERT,* SÉBASTIEN DE DANIELI,† CHRISTIAN MIQUEL,* ERIC COISSAC,*
CAROLE POILLOT,* JEAN-JACQUES BRUN† and PIERRE TABERLET*

**Laboratoire d'Ecologie Alpine, CNRS-UMR 5553, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 9, France,*

†*Mountain Ecosystems Research Unit, Cemagref Grenoble, 2, Rue de la Papeterie, BP 76, 38402 Saint-Martin-d'Hères, France*



Analyse des sédiments

ARTICLE

Received 21 Mar 2013 | Accepted 7 Jan 2014 | Published 3 Feb 2014

DOI: 10.1038/ncomms4211

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

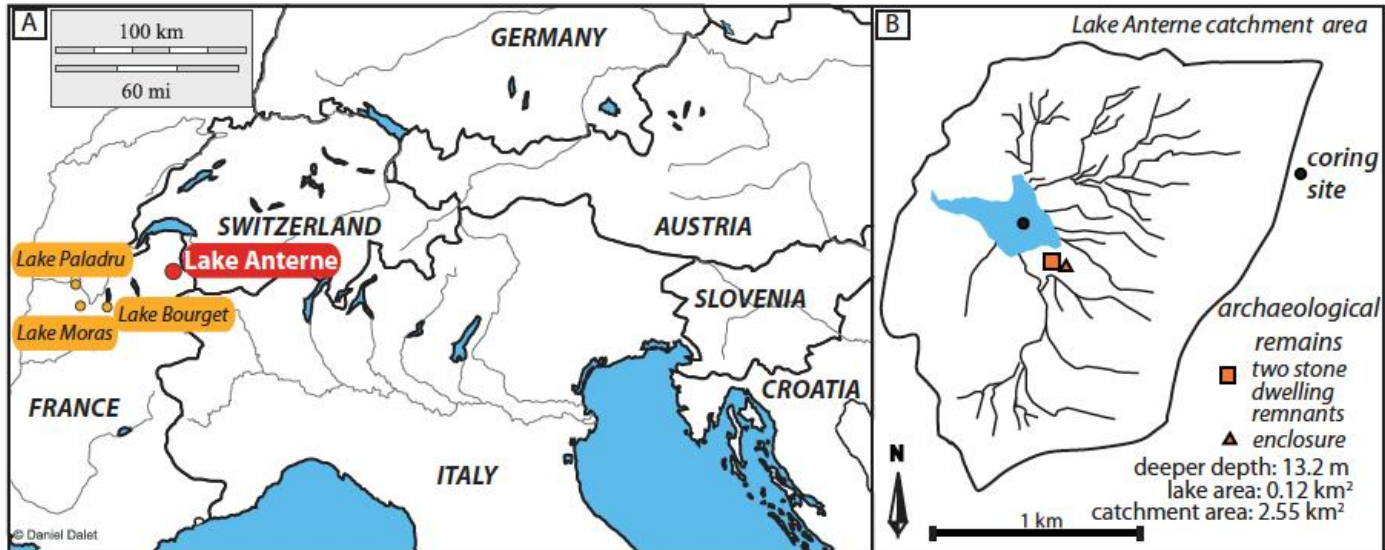
Charline Giguet-Covex^{1,2,*}, Johan Pansu^{1,*}, Fabien Arnaud², Pierre-Jérôme Rey², Christophe Griggo², Ludovic Gielly¹, Isabelle Domaizon³, Eric Coissac¹, Fernand David⁴, Philippe Choler^{1,5}, Jérôme Poulénard² & Pierre Taberlet¹

Reconstructing long-term human impacts on plant communities: an ecological approach based on lake sediment DNA

JOHAN PANSU,*†¹ CHARLINE GIGUET-COVEX,*†‡§¶¹ GENTILE FRANCESCO FICETOLA,*†
LUDOVIC GIELLY,*† FRÉDÉRIC BOYER,*† LUCIE ZINGER,** FABIEN ARNAUD,‡§¶ JÉRÔME
POULENARD,‡§¶ PIERRE TABERLET*† and PHILIPPE CHOLER*†††‡‡

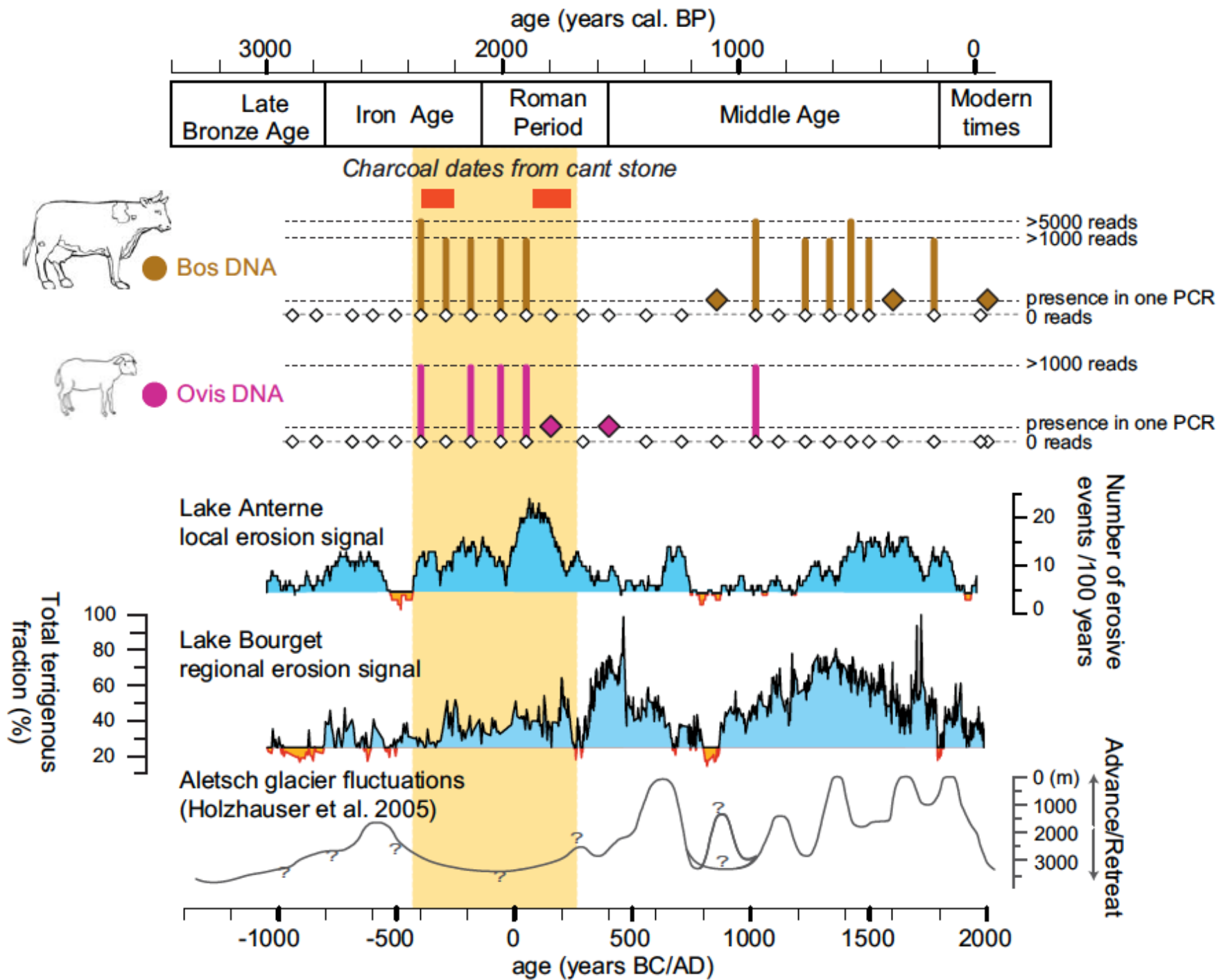
*Univ. Grenoble Alpes, LECA, F-38000 Grenoble, France, †CNRS, LECA, F-38000 Grenoble, France, ‡Univ. Savoie Mont
Blanc, EDYTEM, F-73000 Chambéry, France, §CNRS, EDYTEM, F-73000 Chambéry, France, ¶Ministère de la Culture et de la
Communication, EDYTEM, F-73000 Chambéry, France, **Université Toulouse 3 Paul Sabatier, CNRS, ENFA, UMR 5174 EDB,
F-31062 Toulouse, France, ††Univ. Grenoble Alpes, SAJF, F-38000 Grenoble, France, ‡‡CNRS, SAJF, F-38000 Grenoble, France

Sediments du lac d'Anterne

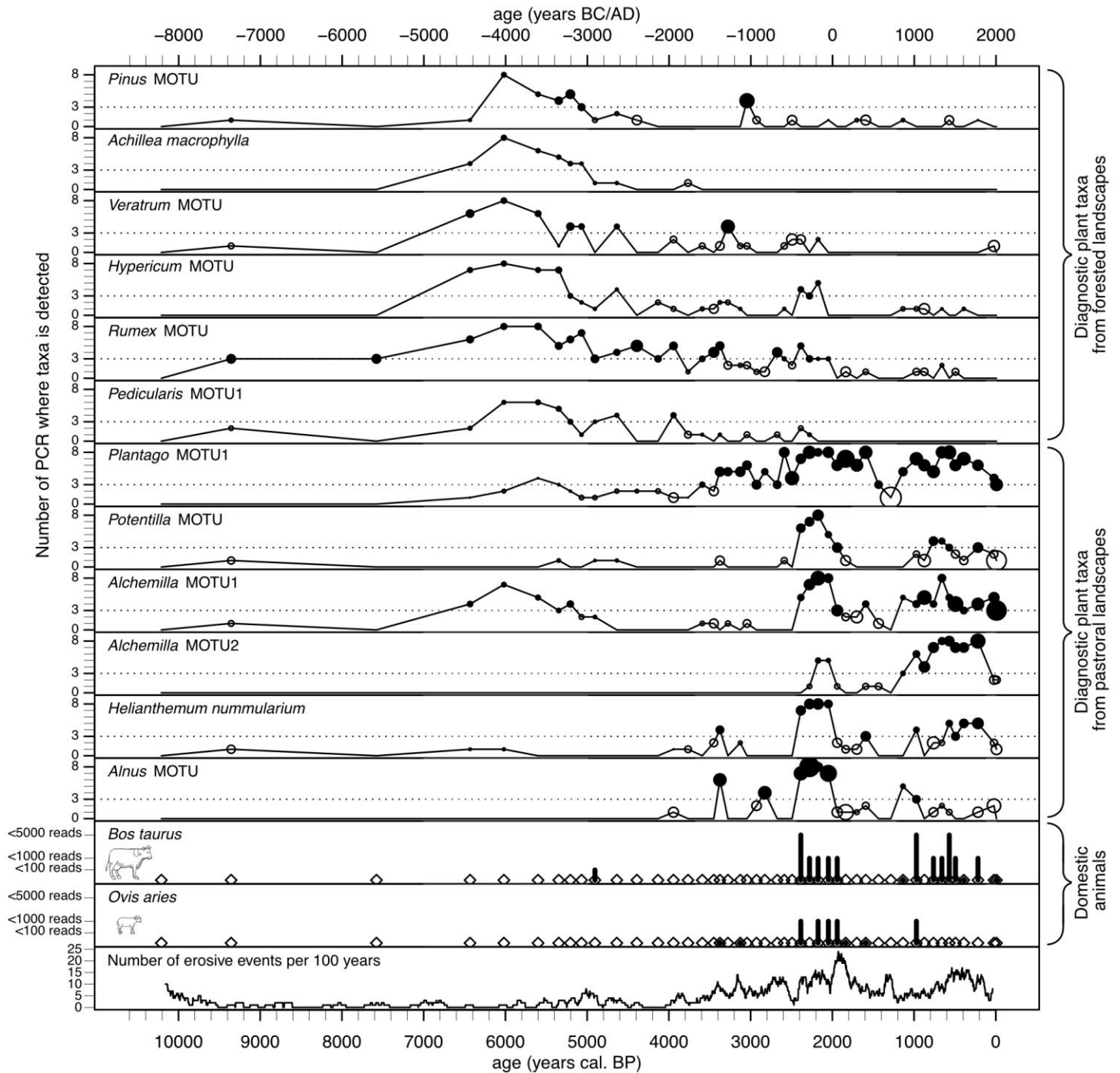


Sediments du Lac d'Anterne

- Analyse de 47 tranches d'une carotte de 20 m correspondant aux derniers 10.000 ans
- Marqueurs ADN utilisés
 - Intron du *trnL* (ADN chloroplastique) pour les plantes (amorces Sper01)
 - Court fragment du gène 16S (ADN mitochondrial) pour les mammifères (amorces Mamm02)
- 2 extractions d'ADN par tranche
- 4 PCRs par extraction



eDNA from lake Anterne





Merci pour votre attention