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Palaeoproteomics gives new insight into early southern African pastoralism

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Scientific Reports 10, Article number: 14427 (2020) Cite this article

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<u>Sci Adv.</u> 2021 Jan; 7(3): eabb9314. Published online 2021 Jan 15. doi: <u>10.1126/sciadv.abb9314</u>

Ancient protein analysis in archaeology

Jessica Hendy^{1,2}

Article Published: 25 November 2021

The earliest Denisovans and their cultural adaptation

<u>Samantha Brown</u>[™], <u>Diyendo Massilani</u>[™], <u>Maxim B. Kozlikin</u>, <u>Michael V. Shunkov</u>, <u>Anatoly P. Derevianko</u>, <u>Alexander Stoessel</u>, <u>Blair Jope-Street</u>, <u>Matthias Meyer</u>, <u>Janet Kelso</u>, <u>Svante Pääbo</u>, <u>Thomas Higham</u> & <u>Katerina Douka</u>[™]

Nature Ecology & Evolution 6, 28-35 (2022) Cite this article

6545 Accesses | 18 Citations | 870 Altmetric | Metrics

Species identification of ivory and bone museum objects using minimally invasive proteomics

CATHERINE GILBERT 💿 , VACLAV KRUPICKA, FRANCESCA GALLUZZI 💿 , ALEKSANDRA POPOWICH, KATELL BATHANY 💿 . STÉPHANE CLAVEROL 💿 , JULIE ARSLANOGLU

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SEDIMENTARY ANCIENT DNA, FLORA AND FAUNA



EVA CHOCHOLOVÁ

LABORATORY OF BIOLOGICAL AND MOLECULAR ANTHROPOLOGY DEPARTMENT OF EXPERIMENTAL BIOLOGY

SEDIMENTARY ANCIENT DNA

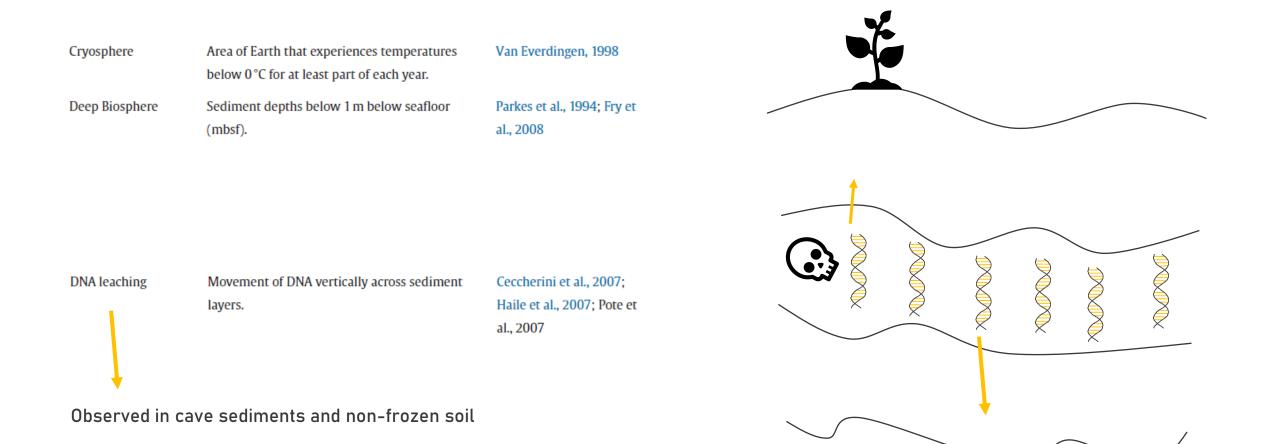
- Abbreviated to sedaDNA
- Proxies (indirect sources of information) e.g., fossil assemblages, indicator species, geochemical proxies...
- Before HTS mostly limited to fossil record (molluscs, diatoms, foraminifera...)
- Often for study of palaeoenvironment, climate change, biodiversity
- Greater resolutions compared to pollen analysis
- Best preservation in anoxic and cold environment, high clay, borate and organic content
- Organisms underrepresented in databases, missing references, unknown organisms

SEDIMENTARY ANCIENT DNA

Table 1. Terms commonly used in marine *sed*aDNA research and their definition. aDNA terms are listed hierarchically, all other terms are listed alphabetically.

aDNA Terms	Definition	References
Ancient DNA (aDNA)	Biomolecular analysis of non-modern genetic material preserved in a broad range of biological samples.	Shapiro and Hofreiter, 2012
Palaeoenvironmental DNA (PalEnDNA)	Disseminated genetic material found in ancient environmental samples such as sediment, soil, paleosols, coprolites, water and ice.	Rawlence et al., 2014
Environmental DNA (eDNA)	DNA isolated from environmental samples (e.g., soil, water, air), usually a complex mixture of genomic DNA from various organisms.	Taberlet et al., 2012a; Stat et al., 2017
Sedimentary aDNA (<i>sed</i> aDNA)	Ancient DNA isolated from sediments.	Willerslev et al., 2003; Jørgensen et al., 2012
Marine <i>sed</i> aDNA	Ancient DNA retrieved from marine sediment cores.	This review

SEDIMENTARY ANCIENT DNA



SOURCES

- Lakes and other freshwater sources
- Marine
- Cave
- Burials
- Settlements
- Permafrost
- (Ice, glacier)
- (Latrines)
- (Extraterrestrial sources)



Fig. 3.1 Examples of (a) researchers sampling an accessible cave sediment profile in the field; (b) a freshly subsampled lake core, where the rectangular areas represent discarded sediment surface for access to underlying pristine sediment, and the circular holes correspond to the material that was sampled for DNA analysis. Alternative core sectioning and subsampling strategies can be used for higher resolution analyses; (c) subsamples that were taken in a non-cleanroom space; and (d) a subsample to be used as input for DNA extraction in a cleanroom space. Images are copyright of Dr. Richard G. Roberts (a), Dr. Peter D. Heintzman (b, c), and the Max Planck Institute for Evolutionary Anthropology (d)

Heintzman et al., 2023 DOI: 10.1007/978-3-031-43799-1_3

METHODOLOGICAL CONSIDERATIONS

	Fresh cores recommended	Contamination potential	Suitable for		Further
Group			Amplicon	Metagenomics ^d	reading
Prokaryotes	Yes	High	No ^b	Yes	Chapters 4, 5
Microbial eukaryotes	Yes	High	No ^b	Yes	Chapters 6, 7
Fungi	Yes	High	Yes ^c	Yes	Chapter 6
Wildlife ^a	No	Low	Yes ^c	Yes	Chapters 8-11
Domesticated/ farmed taxa ^a	No	Intermediate	Yes ^c	Yes	Chapters 10, 11
Human	Yes	High	No	Yes	Chapter 11

Table 3.1 Taxon-specific methodological considerations for the *sed*aDNA workflow, with signposts to other chapters of this volume

aWildlife and domesticated/farmed taxa include both animal and plant taxa

bAmplicon methods may be appropriate for taxa that are unable to have colonised the sediment after collection or be present in the modern sampling or laboratory environment cData are prone to contamination and so need to be interpreted carefully. See also Chap. 11 dThe feasibility of metagenomics may be limited by the availability of reference genome data for certain taxonomic groups

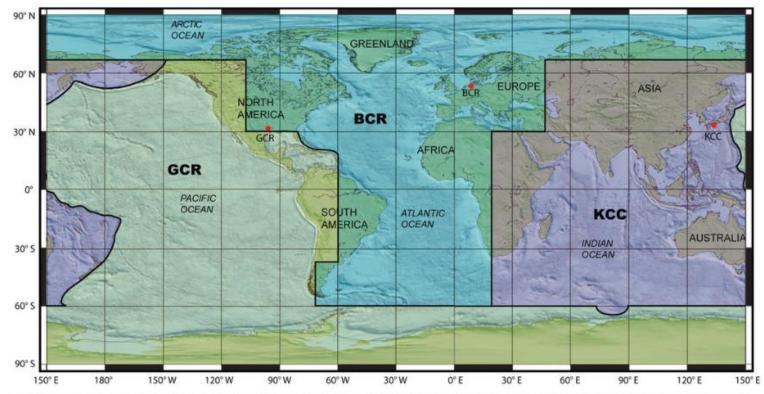
SEDIMENT CORING

- Cold storage, subsampling ideally on board in case of marine sediments
- Horizontal (accessible profiles permafrost, soil, cave)
 - from bottom to top to prevent cross-contamination
- Vertical (inaccessible profiles marine, lake, ice)
- Contamination monitoring by addition of exotic DNA or synthetic tracers
- Archiving in specialized facilities, e.g., LacCore facility in Minnesota, Permafrost ArChives Science (PACS) Laboratory at the University of Alberta, International Ocean Discovery Program

Geographic Assignment of Core Samples to Repositories

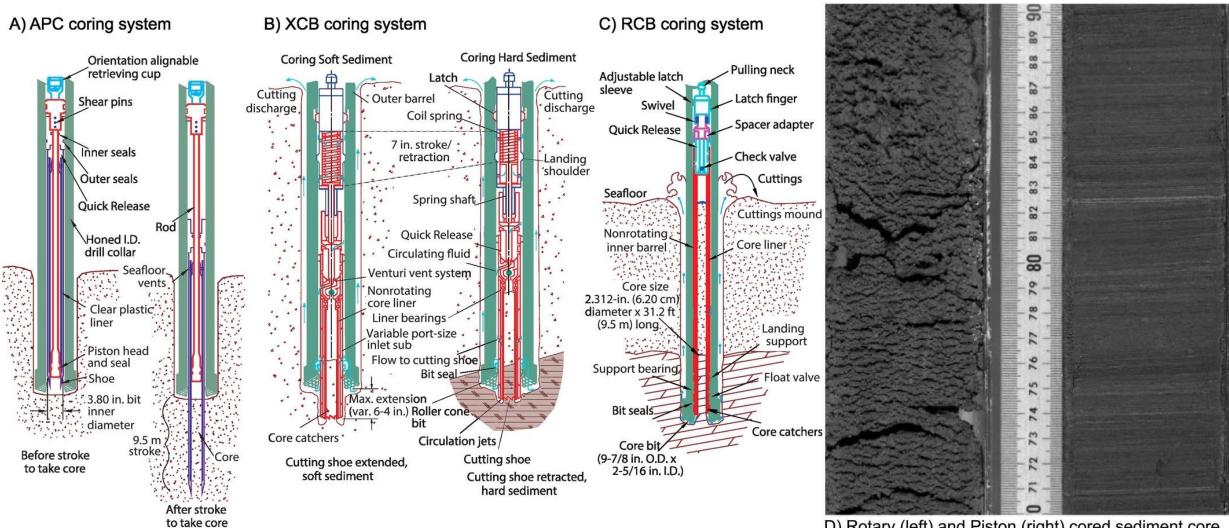
Repository	Institution	Amount of Core	Program(s) Generating Core	Geographic Location
GCR	Texas A&M University	152 km	DSDP, ODP, and IODP	Pacific (Pacific plate east of western boundary); Caribbean Sea and Gulf of Mexico; Southern Oceans (S of 60° except Kerguelan Plateau)
BCR	University of Bremen	185 km	DSDP, ODP, and IODP	Atlantic and Arctic Oceans (north of Bering Strait); Mediterranean, Black, and Baltic Seas
ксс	Kochi University / JAMSTEC	149 km	DSDP, ODP, and IODP	Pacific (west of western boundary of Pacific plate); Indian Ocean (N of 60°S), all of Kerguelan Plateau, and the Bering Sea
Rutgers/NJGS	Rutgers / NJGS	4.1 km	ODP Leg 150X & 174AX	Land-based New Jersey and Delaware cores

DSDP: Deep Sea Drilling Program ODP: Ocean Drilling Program IODP: Integrated Ocean Drilling Program / International Ocean Discovery Program



U. Röhl adapted from Firth, JV, Gupta, LP and Röhl, U (2009) New focus on the Tales of the Earth - Legacy Cores Redistribution Project Completed. Scientific Drilling, 7. 31-33. doi:10.2204/iodp.sd.7.03.2009. [Map Mar 15, 2016]. Retrieved from http://www.marum.de/en/Cores_at_BCR.html

https://www.iodp.org/resources/core-repositories



D) Rotary (left) and Piston (right) cored sediment core

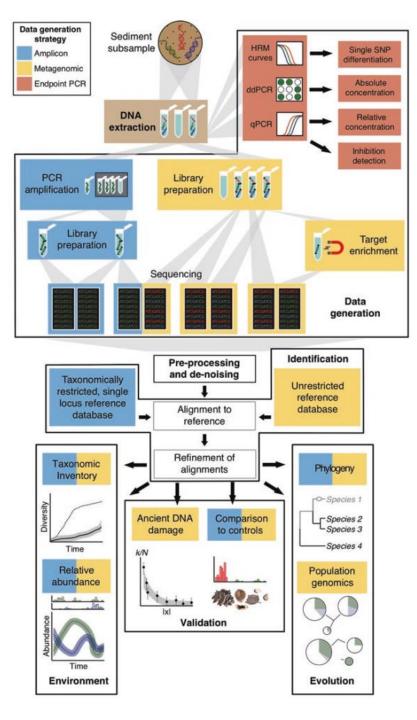
Fig. 2. Overview of IODP coring systems. A) Advanced piston coring system (APC), shown before and after stroking; only small volumes of drill fluid can enter the space between the core barrel and collar from above after stroking, greatly reducing the risk of contamination. B) Extended core barrel system (XCB) and C) Rotary core barrel system (RCB); both containing circulation jets at the bottom of the core barrel through which drill-fluid enters and removes coring debris by transporting it upwards within the drill hole to the surface. D) Comparison of rotary and piston cored sediments demonstrating the well-preserved lamination in Piston cored material. Figure adapted from <u>Sun et al. (2018)</u> and IODP (iodp.tamu.edu/tools/index.html)

Armbrecht et al., 2019 DOI: 10.1016/j.earscirev.2019.102887

Impact subject Impact score Modern DNA in seawater *** Sediment coring Contaminant DNA in drilling fluid Core pipe Surface sediment DNA pushed downwards during coring Recommended contamination control Core liner measures: seawater control sample, with core application of chemical tracers Decontaminated Decontaminated core-liner core-cutter aDNA ** Human DNA from analysts sample Sediment core subsampling · Contaminant DNA present in working (core centre) area Cross-contamination • Recommended contamination control measures: reduction of airflow, sampling under cold conditions, decontamination of Chemicá Chemical tracer at tracer samples core-liners, removal of exposed surface core-(core centre sediments, air controls and lab swabs periphery and periphery) * Samples Negative control Contaminant DNA present in reagents aDNA extraction · Contaminant DNA present in laboratory Cross-contamination 0 0 Recommended contamination control measures: working in low-DNA environment, personal protective equipment, negative controls aDNA preparation for sequencing * Metagenomic library preparation Samples Negative control (reagent contaminants) ** PCR and amplicon library preparation (PCR bias) ð ð ð Recommended contamination control measures: working in low-DNA environment, personal protective equipment, negative controls aDNA data analysis *** Rigorous data filtering and quality ACGTACGT control ACGTACGT Recommended contamination control measures: analysis of negative controls alongside samples

Fig. 1. Schematic showing the key steps involved in acquiring deep marine <u>sediment</u> <u>cores</u>, subsampling, DNA extraction, aDNA preparation for sequencing and data generation. Indicated are sources of potential contamination and reduction in data quality, as well as recommended precautions to be considered and/or controls to be taken. An impact score (1–3 stars) is given to indicate the severity of potential contamination or the impact that impaired data would have on the results at each step in the process. Schematic graphics are not to scale.

Armbrecht et al., 2019 DOI: 10.1016/j.earscirev.2019.102887



(a) Metabarcoding (b) Metagenomics (c) Target-capture DNA fragment size 🙀 DNA fragment size DNA fragment size not prescribed prescribed not prescribed **M** Authentication not Authentication Authentication possible possible possible Cost-effective Deep sequencing Cost-effective sequencing required sequencing

genomics. (a) Metabarcoding is the amplification and analysis of equally sized DNA fragments from a total DNA extract. (b) Metagenomics is the extraction, amplification, and analysis of all DNA fragments independent of size. (c) Target-capture describes the enrichment and analysis of specific (chosen) DNA fragments independent of size from a total DNA extract.

FIGURE 2. Schematic of different methodological approaches in modern and ancient marine

Hypothetical changes in

paleo-communities through time

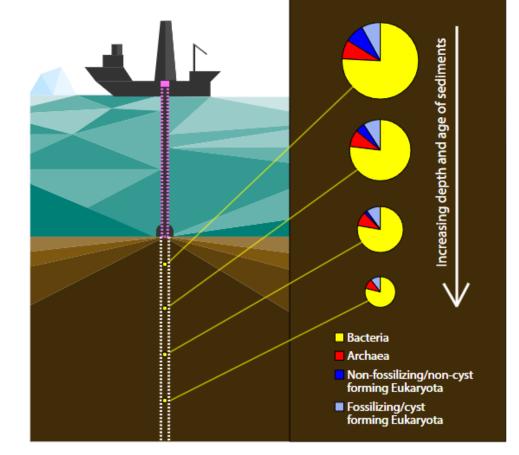
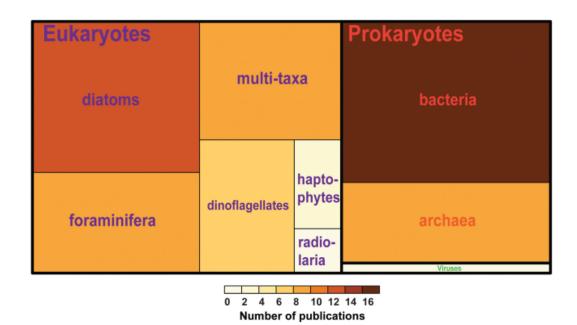


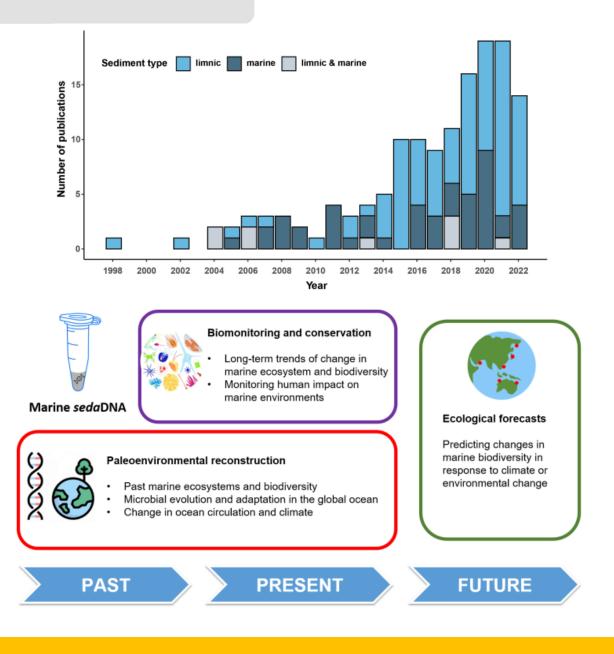
FIGURE 1. Schematic of a drilling vessel recovering a sediment core for sedaDNA analysis and hypothetical past marine community composition. The pink dashed line indicates the use of a chemical tracer for contamination tracking during coring. The white dashed line depicts the sediment core. Small yellow circles indicate theoretical sedaDNA sampling intervals, corresponding to pie charts on the right. Pie charts represent hypothetical paleo-communities detectable from sedaDNA shotgun analysis, where the majority (~75%, see text and Figure 3c) of the recovered sedaDNA sequences originate from bacteria, and where sedaDNA from fossilizing/cyst-forming taxa increases relative to non-fossilizing/non-cyst-forming taxa with subseafloor depth (assuming that sedaDNA of fossilizing/ cyst-forming taxa preserves better than that of non-fossilizing/non-cyst-forming taxa). The decreas-Ing size of the pie charts with subseafloor depth indicates an expected decrease in sedaDNA. Schematic not to scale.

Armbrecht et al., 2020 DOI: 10.5670/oceanog.2020.211

MARINE sedaDNA

- Research always multidisciplinary geology, organic and inorganic chemistry, geomorphology, palaeoceanography, micropaleontology...
- More dynamic environment than freshwater systems





MARINE sedaDNA

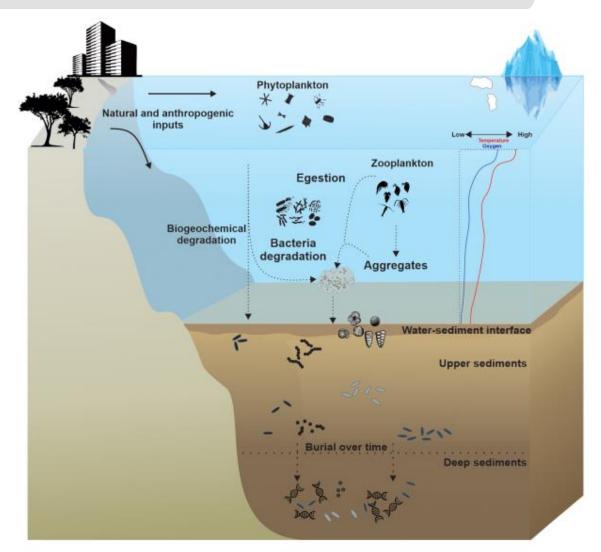
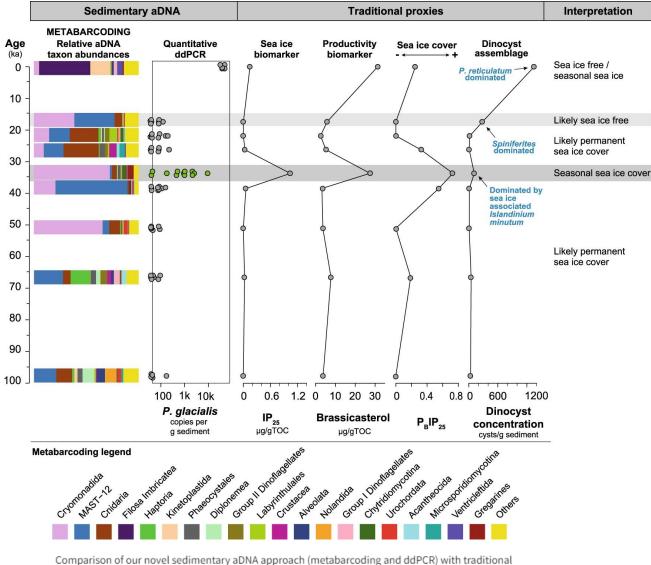


FIGURE 2

Schematic illustration of eDNA taphonomic processes in the marine environment. These processes involve eDNA distribution, degradation, and/or preferential preservation during the transition from the pelagic to the benthic zones, and from the water-sediment interface into subsurface sediment.

MARINE sedaDNA



Comparison of our novel sedimentary aDNA approach (metabarcoding and ddPCR) with traditional proxies (biomarkers and palynology) for sea ice reconstructions over the last ~100,000 years at Site GS15-198-38 in the Greenland Sea

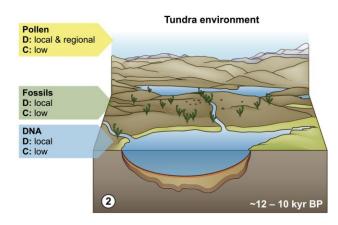
LAKE sedaDNA

- Accumulation of both aquatic and terrestrial environmental components
- Lower disturbance, temperature stability
- Preferencial winter sampling



A full bodysuit, shoe cover, hairnet, facemask, hood, gloves and sleeve guards are necessary during DNA extraction from a sediment core for ancient DNA (aDNA) analyses in an aDNA laboratory (a). During subsampling in a clean laboratory, surface contamination from the sediment core is removed with sterilized razors (b), and non-contaminated material from within the intact cores is extracted for DNA extraction (c).

(a) Pro-glacial environment Di local & regional C: very low Prossils D: local C: very low DNA D: local C: low D = Distribution C = Concentration

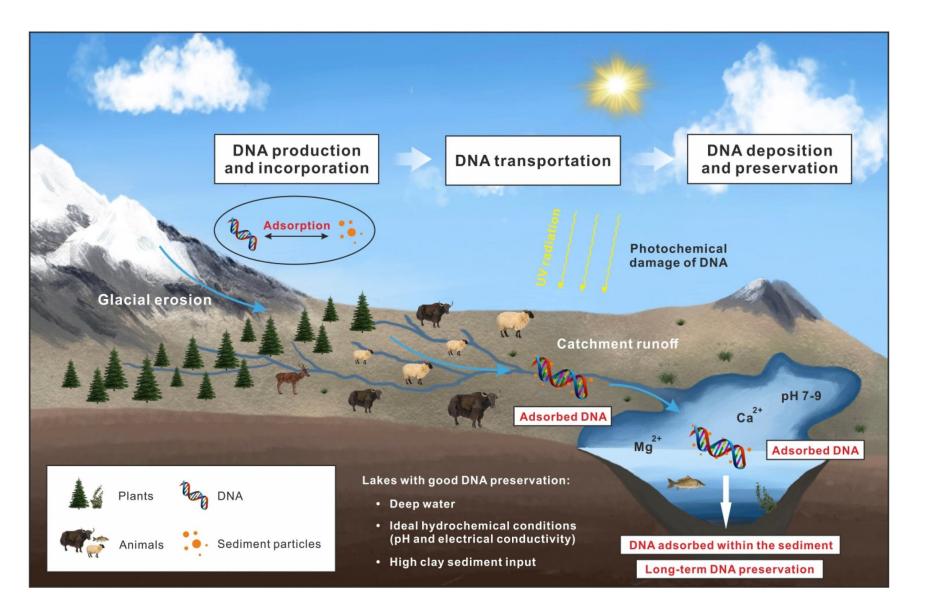


Boreal environment Temperate environment Pollen Pollen D: local & regional D: local & regional C: high C: medium Fossils Fossils D: local D: local C: medium C: medium DNA DNA D: local D: local C: medium C: high (C) 3 4 ~10 – 8.0 kyr BP ~8.0 kyr BP – present (b) (c) (d) (e) **Resulting DNA** DNA Mass Distance taxon profile Fossils Pollen Detectable taxa Fossil state Young Temperate environment **Boreal environment** Tundra environment Pro-glacial environment Ag PIC High wolium <u>80</u> No

Biotic palaeoenvironmental proxies in lake sediments. (a) Sequential environmental development for a temperate region, in which the lake sediments start to accumulate as the glacial ice retreats, incorporating glacially eroded debris and the sparse pioneering biota (1), which later is replaced by a tundra-steppe community (2); then the boreal forest establishes (3) before eventually being replaced by a temperate forest (4). By identifying organisms detectable by DNA, macro- and microfossils accumulated and preserved in the lake sediments (b), it is possible to reconstruct the environments through time (c). It is important to note that the rate of degradation is strongly correlated with the age of the sediments and that the input concentration (d) varies in different climatic environments from these three proxies. In addition, the resulting DNA profile (e), as well as macro- and microfossils, are influenced by taphonomic processes, such as differences in biomass production and the distance from source to deposit. This is why a combination of all three proxies makes a more robust palaeoenvironmental reconstruction. kyr up, thousand years before present.

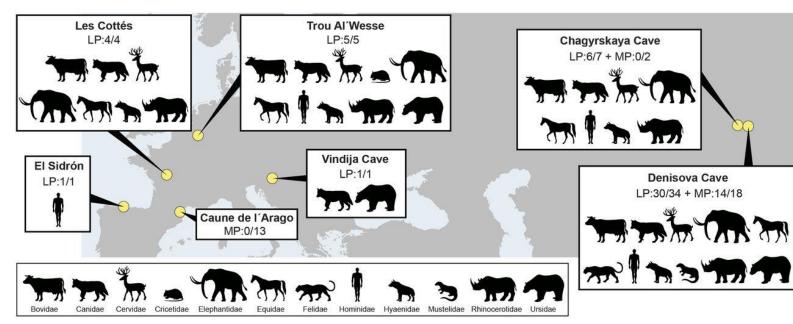
Parducci et al., 2017 DOI: 10.1111/nph.14470

Fig. 2. Conceptual model of the taphonomic processes of extracellular DNA from lake sediments. After having been released into the environment, extracellular DNA can be incorporated into soil or sediment particles by adsorbing to, for example, clay minerals and humic substances, which is the primary mechanism responsible for longterm DNA preservation. Adsorbed DNA can subsequently be transported into the lake through catchment runoff. Most large lakes on the Tibetan Plateau are expected to have high upstream DNA inputs because of diverse landscapes and well-developed hydrographical networks in their catchments. However, high-level ultraviolet (UV) radiation on the Tibetan Plateau might photochemically damage DNA over long transport distances. During the deposition stage, terrestrial adsorbed DNA is expected to be well preserved in deep lakes with neutral to slightly alkaline water pH (7–9), intermediate water conductivities (100–500 μ S cm⁻¹; suitable cation concentrations), and high clay sediment input. On the other hand, DNA from aquatic organisms (e.g., fish and macrophytes) might still be preserved in many Tibetan lakes characterized by high water pH (\geq 9) and conductivity (>1000 μ S cm⁻¹). After sediment particles are finally deposited and buried in the lake bottom, DNA is adsorbed within the sediment and stored in a cold and anoxic environment that limits bioturbation, sediment reworking, and microbial activity, which favors long-term DNA preservation. To conclude, we infer that some deep glacial lakes with freshwater and high clay sediment input, such as those from the southern and southeastern Tibetan Plateau, may have a high potential for sedimentary ancient DNA studies in the future.



MORE ON sedaDNA

- Burials
 - Contamination control (comparison of samples and sedaDNA)
 - Non-destructive for remains
 - Have to be sampled early
 - Potentially human DNA, pathogens, parasites, diet, ...
- Caves!



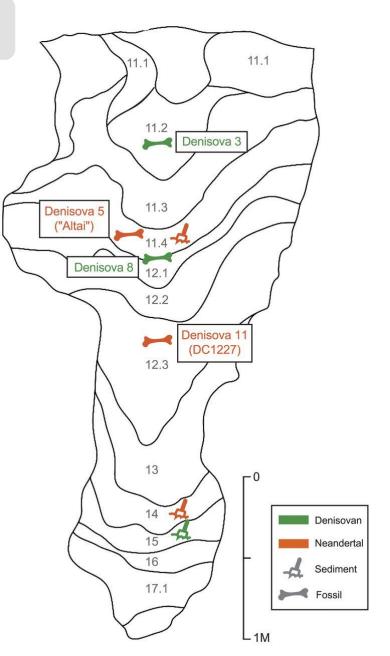


Fig. 1 Ancient taxa detected in Late Pleistocene (LP) and Middle Pleistocene (MP) sediment samples from seven sites.

For each time period, the fraction of samples containing DNA fragments that could be assigned to a mammalian family and authenticated to be of ancient origin is indicated. The shaded symbols representing each family are not to scale. Slon et al., 2017; DOI: 10.1126/science.aam9695

Slon et al., 2017; DOI: 10.1126/science.aam9695

MORE ON sedaDNA

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 - Mostly megafauna and hominins

MORE ON sedaDNA

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 - Have to be sampled early
 - Potentially human DNA, pathogens, parasites, diet, ...
- Caves!
 - Mostly megafauna and hominins
 - Microstratigraphy

Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments

Diyendo Massilani^{a,1}, Mike W. Morley^{b,1}, Susan M. Mentzer^{c,d}, Vera Aldeias^e, Benjamin Vernot^a, Christopher Miller^{c,d,f}, Mareike Stahlschmidt⁹, Maxim B. Kozlikin¹, Michael V. Shunkov^h, Anatoly P. Derevianko^h, Nicholas J. Conard^{c,d}, Sarah Wurz^{f,i}, Christopher S. Henshilwood^{f,i}, Javi Vasquez^j, Elena Essel^a, Sarah Nagel^a, Julia Richter^a, Birgit Nickel^a, Richard G. Roberts^{k,i}, Svante Pääbo^{a,1}, Viviane Slon^{a,m,n,o}, Paul Goldberg^{d,k}, and Matthias Meyer^{a,1}

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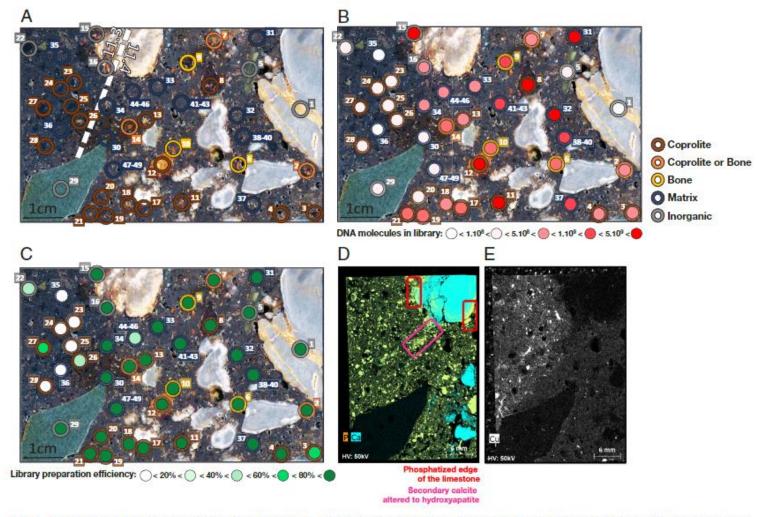


Fig. 3. Targeted sampling of microfeatures from block DCE5C. (A) Surface scan with sampling locations and layer designations. (B) Number of library DNA molecules recovered from each sample. (C) Library preparation efficiencies. (D) μ XRF surface scan for P (orange) and Ca (aqua) produces a distribution map of calcium phosphate (yellow) that indicates fragments of hydroxyapatite from bone and coprolite as well as phosphatized limestone (red frames) and secondary calcite (magenta frame). (E) μ XRF surface scan for Cu (white).

PLANTS

- Domestication!
- Greatest genome size, polyploidization allows subfunctionalization and neofunctionalization, transposable elements crucial
- aDNA offers insight into timing, number and locality of domestication events, processes behind domestication (human behaviour, genome changes)
- Research of wild and early-domesticated ancestors, adaptation to pathogens and environmental changes
- Palaeoecology
- Sources: seeds, herbarium specimens, wood, pollen, sediments, various plant fragments

Species	Dating ^a	Site	Sample	Characterization ^b	Extraction ^c	Reference
Oak	500-9800 BP	Europe	Waterlogged wood	NGS	TrisHCL SDS CaCl ₂ EDTA DTT PK/Phchlo/Column	[142]
Japanese cedar	3600 BP	Japan	Buried tree	PCR/sequencing	Column	[133]
Maize	1100-6000 BP	New World	Desiccated cob	PCR/sequencing	CTAB TrisHCL NaCl EDTA/ Chlo	[111] [112
Maize	360-1320 BP	New World	Desiccated cob	PCR/sequencing	Column	[114]
Maize	670-5280 BP	New World	Desiccated cob	Capture, NGS	SDS DTT PK/Phchlo/Column	[115]
Maize	650-4300 BP	New World	-	PCR/sequencing	SDS DTT PK/Phchlo/EDTA PTB/Column	[113]
Maize	4700 BP	Chile, Peru	Charred and non- charred grain	PCR/sequencing	SDS DTT PK/Phchlo	[110]
Maize	5310 BP	Mexico	Desiccated grain	NGS	TrisHCL SDS CaCl ₂ EDTA DTT PK/Phchlo/Column	[116]
Maize	5300 BP	Mexico	Desiccated cob	NGS	-	[117]
Sunflower	3100 BP	USA	Desiccated disk fragment, pericarp, kernel	NGS	TrisHCI NaCl SDS CaCl ₂ EDTA DTT PK/Phchlo/Column	[119]
Radish	350-550 AD	Egypt	Desiccated seed	Chemical analysis/ PCR/sequencing	CTAB TrisHCL NaCl EDTA/Chlo	[124]
Sorghum	2800 BP	Egypt	Desiccated seed	PCR/sequencing	-	[125]
Rice	1200-2400 BC	China	Desiccated seed and chaff	PCR/sequencing	Magnetic beads	[127]
Grape	1600-2500 BP	Europe	Waterlogged and charred pip	PCR/sequencing	DTAB /Chlo/CTAB	[120]
Grape	7 th -15 th century AD	Italy	Waterlogged pip	PCR	SDS DTT PK/Phchlo/Column	[121]
Grape, maize, olive, dogwood, cotton	400-2400 BP	New World, Europe	Non-carbonized remain	PCR/sequencing	SDS DTT PK/Phchl	[122]
Barley	6200-5800 BP	Israel	Desiccated seed	NGS	CTAB TrisHCL PVP BME/Phchlo/ Column	[104]
Barley	3000 BP	Egypt	Desiccated grain	PCR/sequencing	SDS DTT PK EDTA PTB/Column	[103]
Barley, wheat	150-5250 BC	Spain	Charred, partially charred, waterlogged seed	PCR/sequencing	TrisHCL SDS EDTA PK/Phchlo or Column	[105]
Wheat	700 AD-8400 BP	Anatolia	Charred grain	PCR/sequencing	CTAB TrisHCL NaCl EDTA/Chlo	[109]
Wheat	340-3500 BP	Spain	Charred and desiccated seed	PCR/sequencing	Tris EDTA CTAB βME/Column	[108]
Cotton	750-3750 BP	Brazil, Peru, Egypt	Desiccated seed	NGS	CTAB/Column	[132]
Arabidopsis	300 BP	USA	Herbarium	NGS	CTAB or PTB DTT/Column	[131]

Summary of ancient plant nuclear DNA recovery listing species, dating, location site, sample type, characterization method, extraction protocol, and associated references. [®]Datings are referenced as in the publication concerned using BC, AD, or BP. [®]*PCR/sequencing* polymerase chain reaction and sequence capture, *NGS* next-generation sequencing. ^CDDT dithiothreitol proteinase K, *Phchlo* phenol-chloroform, *Ph* phenol, *Chlo* chloroform, *SDS* sodium dodecyl sulphate, *EDTA* ethylene-diamine-tetraacetic acid, *CTAB* cetyltrimethylammonium-bromide, *PTB* phenacylthiazolium bromide, *DTAB* dodecyltrimethylammonium bromide, *βME* β-mercaptoethanol, *Tris* tris(hydroxymethyl) aminomethane, *PK* proteinase K

FAUNA

- Domestication!, introgression, loss of diversity, migrations and contact
- Domestication commensal, directed, prey
- Wild animals, extinct species such as mammoth

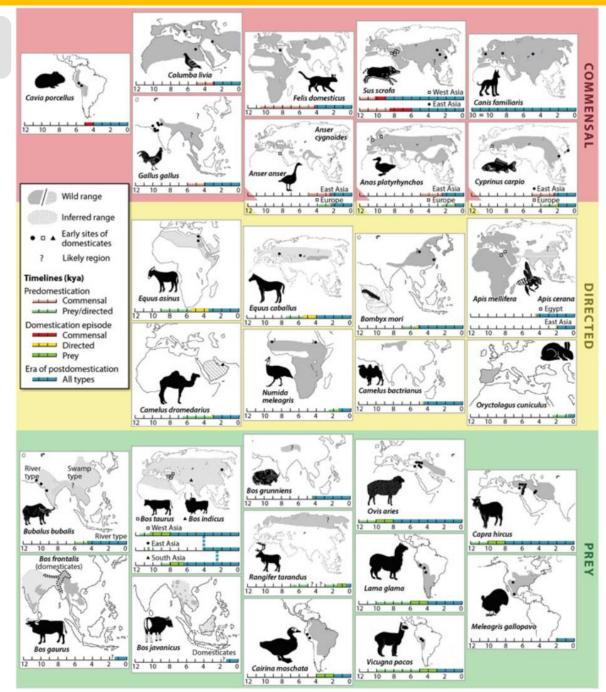


Fig. 1 Geographical/chronological time frame of domestication and potential pathways for major domestic animals. The timelines are in ky (1,000 years) increment. Adapted after Larson and Fuller (2014)

Irving-Pease et al., 2018; DOI: 10.1007/13836_2018_55,

CONSERVATION AND aDNA

- Biodiversity levels:
 - Diversity of ecosystems
 - Species diversity
 - Genetic diversity
- Loss of biodiversity usually in presented in lost species, genetic diversity often unknown

REVIEWS AND SYNTHESES 🛛 🔂 Open Access

Estimated six per cent loss of genetic variation in wild populations since the industrial revolution

Deborah M. Leigh 🔀, Andrew P. Hendry, Ella Vázquez-Domínguez, Vicki L. Friesen

First published: 07 May 2019 | https://doi.org/10.1111/eva.12810 | Citations: 131

CONSERVATION AND aDNA

- Biodiversity levels:
 - Diversity of ecosystems
 - Species diversity
 - Genetic diversity
- Loss of biodiversity usually in presented in lost species, genetic diversity often unknown
- Museum collections and sedaDNA

ORIGINAL RESEARCH article

Front. Ecol. Evol., 03 March 2020 Sec. Paleoecology Volume 8 - 2020 | https://doi.org/10.3389/fevo.2020.00037 This article is part of the Research Topic Applied Uses of Ancient DNA View all 14 Articles >

Unveiling the Ecological Applications of Ancient DNA From Mollusk Shells



limit for shell DNA recovery to ≥100,000 years

CONSERVATION AND aDNA

- Biodiversity levels:
 - Diversity of ecosystems
 - Species diversity
 - Genetic diversity
- Loss of biodiversity usually in presented in lost species, genetic diversity often unknown
- Museum collections and sedaDNA
- Ancient and historical DNA can help:
 - Set baselines for genetic diversity
 - Assess introgression, identify source populations
 - Assess evolutionary impacts of past events
 - Study effective population size, subpopulations and their distinctness

• ...

Atlantic cod (Gadus morhua)1

Analysis of temporal samples indicated that the rapid decline in age of maturation is not primarily caused by fishing-induced selection. This suggests that, in this case, the demographic impacts of fisheries are greater than the evolutionary ones, informing conservation efforts to focus on reducing fishing pressure to let populations recover.

Laysan duck (Anas laysanensis)⁸

Analysis of bones found on atolls outside of the current species range helped resolve the historical distribution. This was used to inform reintroductions to places where the species had gone locally extinct.

Galapagos giant tortoises (Chelonoidis sp.)⁷

Historical specimens were used to identify ancestry of hybrids between extinct and living species. Now, a captive breeding program exists to breed these hybrids to repopulate Floreana Island, where the parental species had gone extinct. Marsh orchid (Anacamptis palustris)² Museum specimens were used to determine the

genetic composition of extinct populations. This can be used to inform the choice of genetic resources for future reintroductions.

African elephants (Loxodonta sp.)⁶

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Analysis of living and extinct elephants clarified the evolutionary history of the genus *Loxodonta* and relationships between savanna and forest populations, confirming that they are separate species. This has led to updated taxonomy and conservation statuses of "Endangered" and "Critically Endangered", respectively.

Chinese giant salamander (Andrias sp.)³

Historical specimens identified the baseline phylogeographic structure of the species, identifying instances of human-mediated translocations, as well as various critically

endangered species. This can inform future translocation and restoration efforts.

Tiger (Panthera tigris)⁴

Genetic analyses comparing historical and contemporary specimens revealed a reduction in genetic variation and an increase in population structure in tigers on the Indian subcontinent. This highlights the importance of restoring connectivity among populations.

Lord Howe Island stick insect (Dryococelus australis)⁵

Analysis of museum specimens of supposedly extinct stick insects from the Lord Howe Island and extant populations on a nearby island revealed them to be the same species. This indicates individuals from the nearby island are suitable for recolonizing the Lord Howe Island.

Figure 1. Examples of studies where ancient and/or historical DNA has provided important conservation insights.

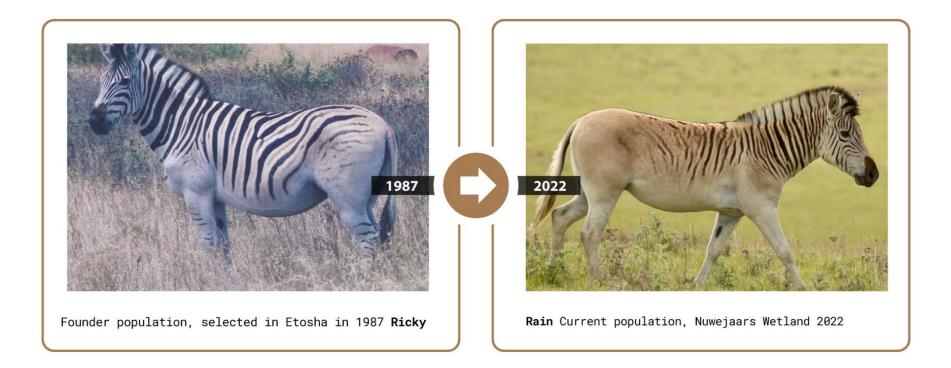
Colours around the icons indicate the type of provided conservation insight or informed policy action: blue, determining the historical distribution of species; purple, guiding conservation breeding; red, informing translocations/reintroductions; teal, assessing anthropogenic impacts on genetic diversity; yellow, resolving taxonomic issues.

Bringing extinct species back to life ٠



Jurassic Park (1993), Universal Pictures

- Bringing extinct species back to life
- Similar phenotype by cross-breading



- Bringing extinct species back to life
- Similar phenotype by cross-breading
- Genome editing of close related species





MAY PREHISTORY THUNDER FORWARD.

WE HAVE THE DNA, THE TECHNOLOGY AND THE LEADING EXPERTS IN THE FIELD. NEXT, WE WILL HAVE THE WOOLLY MAMMOTH. ALIVE AGAIN.

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COLOSSAL PROJECT 001

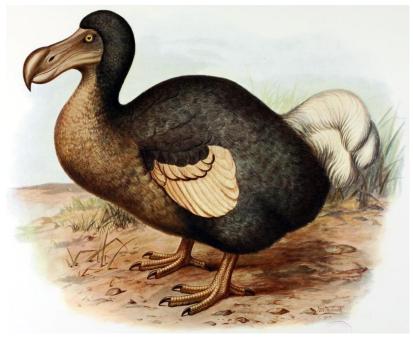


- Goals more general, e.g.:
 - Elephant genome
 - Elephant tracking technologies
 - Artificial womb
 - Ecological restoration
 - Synthetic biology applications
 - Vaccine
 - Sustainable agriculture
 - ...

- Bringing extinct species back to life
- Similar phenotype by cross-breading
- Genome editing of close related species
- Cloning

- Bringing extinct species back to life
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- Cloning
- Thylacine, Dodo, Quagga, Mammoth, Passenger pidgeon

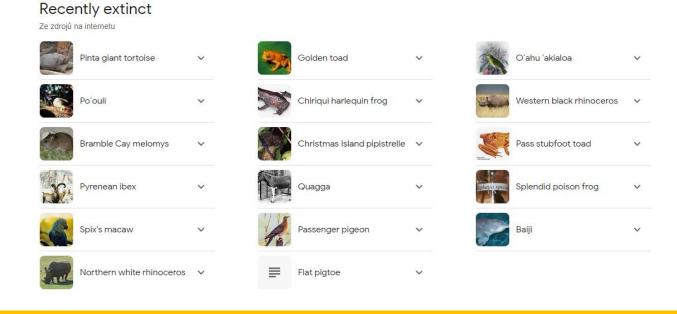




Extinct birds (1907), Frederick William Frohawk

- Bringing extinct species back to life
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- Thylacine, Dodo, Quagga, Mammoth, Passenger pidgeon
- Ecosystem, habitat do extinct species have a place?
- Efficiency of funding

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- Recently extinct species!



- Bringing extinct species back to life
- Similar phenotype by cross-breading
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- Ecosystem, habitat do extinct species have a place?
- Efficiency of funding
- Recently extinct species!
- At least about 200–2000 species every year

- Rhino and recently extinct species for deextinction
- Deextiction types
- sedaDNA subsampling (on boats in marine sedaDNA)
- Plant domestication
- Extraterrestrial sediment
- Climate change and sedaDNA
- Sampling in winter (lake sedaDNA)
- Domestication types
- Reintroduction of genetic diversity from past populations to current ones