Biocodicology:

A novel strategy for the biological analysis of parchment

GUADALUPE PIÑAR

A…KADEMIE DER BILDENDEN KÜNSTE WIEN

INSTITUT FÜR NATURWISSENSCHAFTEN UND TECHNOLOGIE IN DER KUNST

g.pinarlarrubia@akbild.ac.at

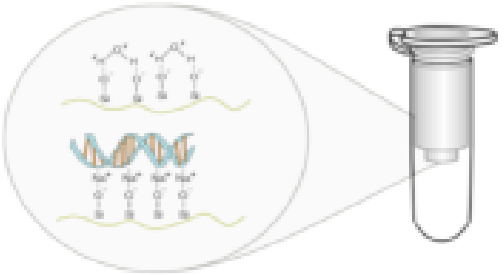
What is Codicology?

Codicology is the study of the physical structure of the book. It is often referred to as "book archaeology" and focuses on the materials (parchment, paper, pigments, inks, etc.) and techniques used to make books, including their binding.

What is biocodicology?

- **Biocodicology is the study of the biological information stored in manuscripts.** It aims to expand the field of codicology by including biomolecular techniques, in order to better understand how manuscripts were produced and used throughout history and how this can help focus our view of the past.

- This emerging field offers the possibility of interrogating manuscripts in novel ways.



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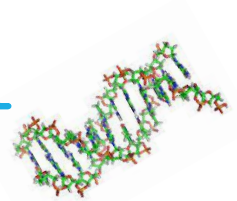
The evolution of codicological analysis:
from manuscript to molecules

1. Studies for the **animal identification**: The follicle patterns of animals used to produce parchment and leather have served as a reference for breed and species identification since the beginning of parchment manufacture.

2. Studies of the **biological microenvironment** of manuscripts, to identify potentially harmful micro-organisms that inhabit these documents and to assess the risk they pose (by cultivation of microorganisms / genetics).

3. Studies of **biomolecules** (proteins, genetic material): Identify all the biomolecules present, providing a '**bioarchive**' of the object.Tree types of biomolecular analyses can be undertaken:
    - proteomics (proteins)
    - genetics (aDNA) / microbiome (microbial genetics)



Biocodicology—a step by step guide

There are various aspects that can be investigated:

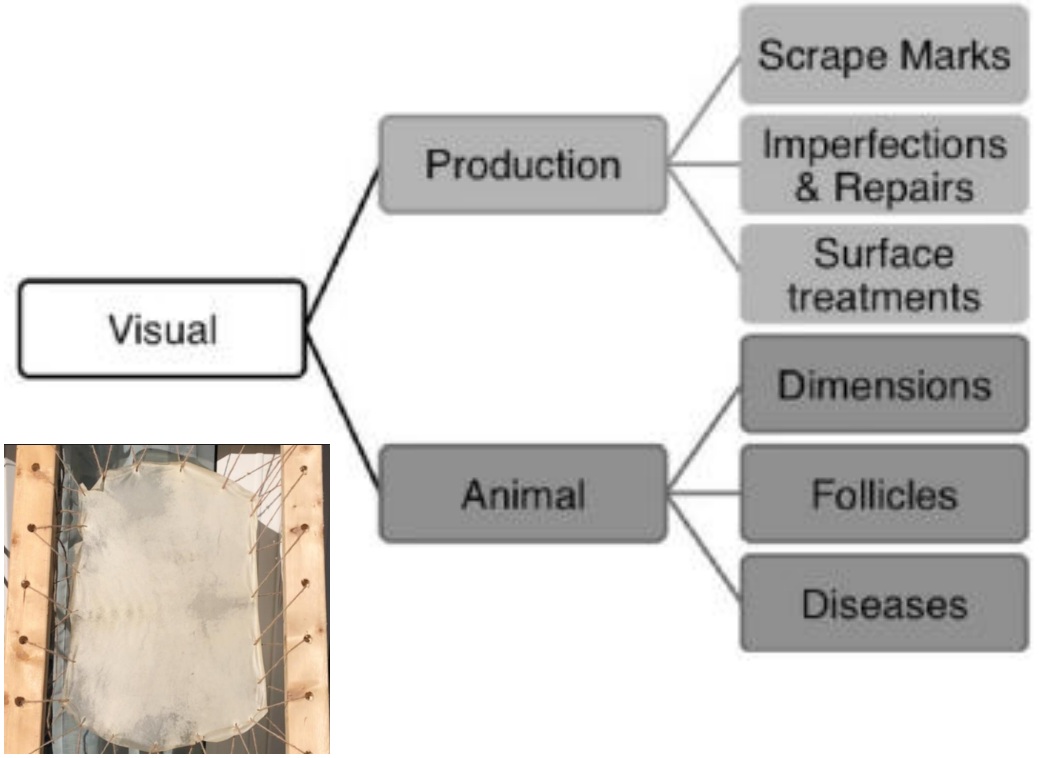
- **Visual analysis:** production and animal analysis

- **Biomolecular:** protein and genetic analysis

The selection of the techniques to be employed will depend on the question being posed!

Visual analysis:

Always the first step!



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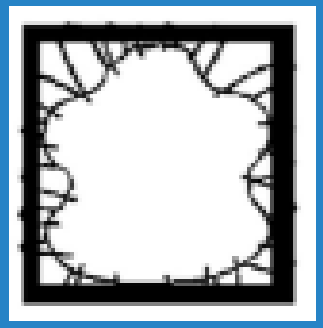
Materials selected for the manufacturing



Materials of non-animal origin:

- Wood used for boards
- vegetable fibres used for sewing thread,
- cloth linings and occasionally metal fastenings.

Materials of animal origin:

- alum-tawed skin
- tanned leather,
- parchment, fur, silk linings,
- fish glue, casein glue,
- egg white finish, beeswax,
- even tendons to make thread.

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Animal identification

Follicle patterns

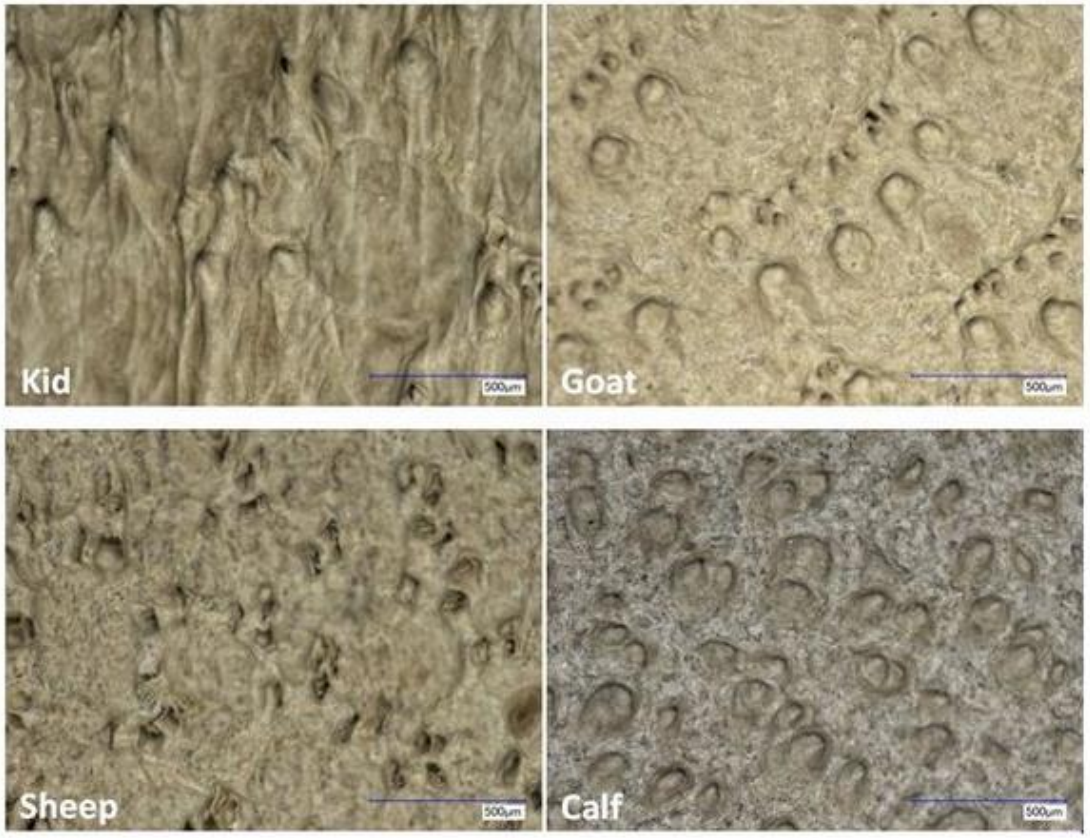
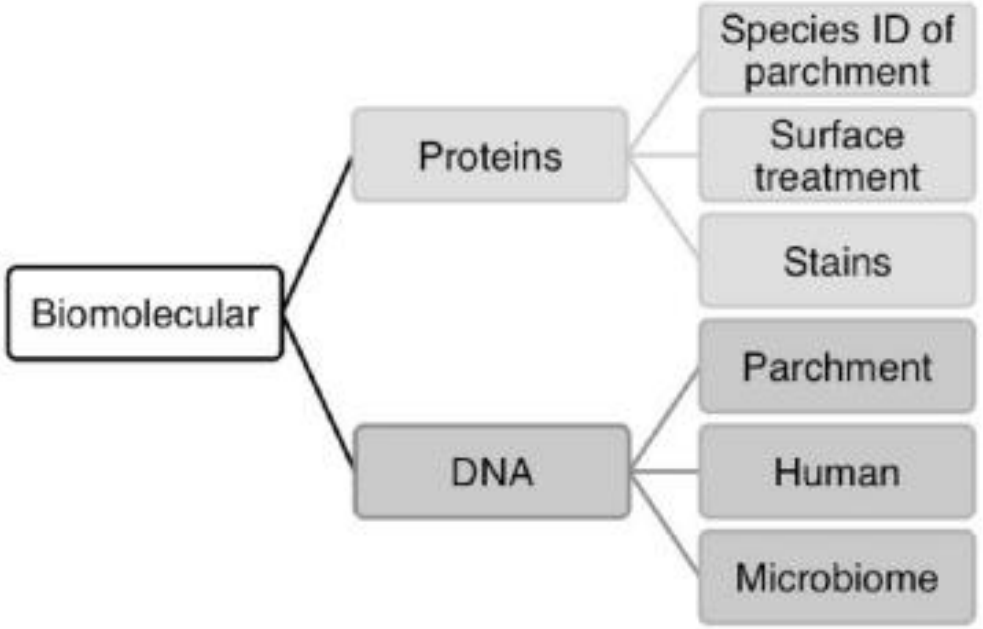


Figure 5: A comparison of parchment follicle patterns from kid, goat, sheep and calf at 200x magnification.

Pics. British Library: Collection Care Blog
https://www.bl.uk/blogs

Biomolecular analysis:

If visual analyses are not enough!



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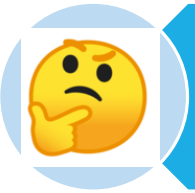
Biomolecular analysis: Sampling

The biggest challenge in biomolecular analysis of manuscripts and objects of cultural heritage has been the development of novel sampling techniques.

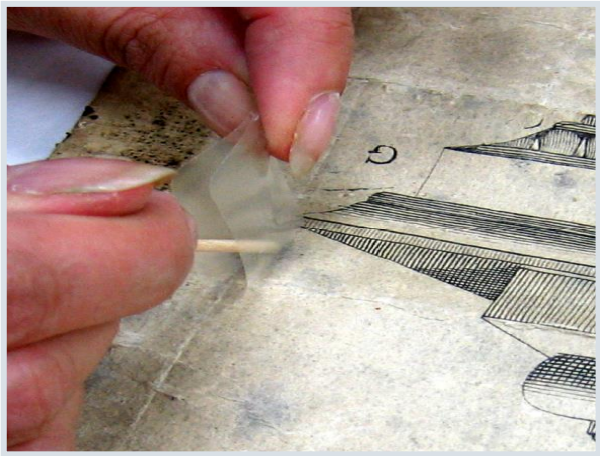
The development of non-invasive sampling techniques has allowed the access to thousands of previously unanalyzed documents all with the approval of conservators and curators.



The decision of where and whether to sample lies with the conservator who has the best understanding of the condition of the document.

The question posed must be taken as the starting point, because depending on the enquiry, a different methodological approach must be taken.

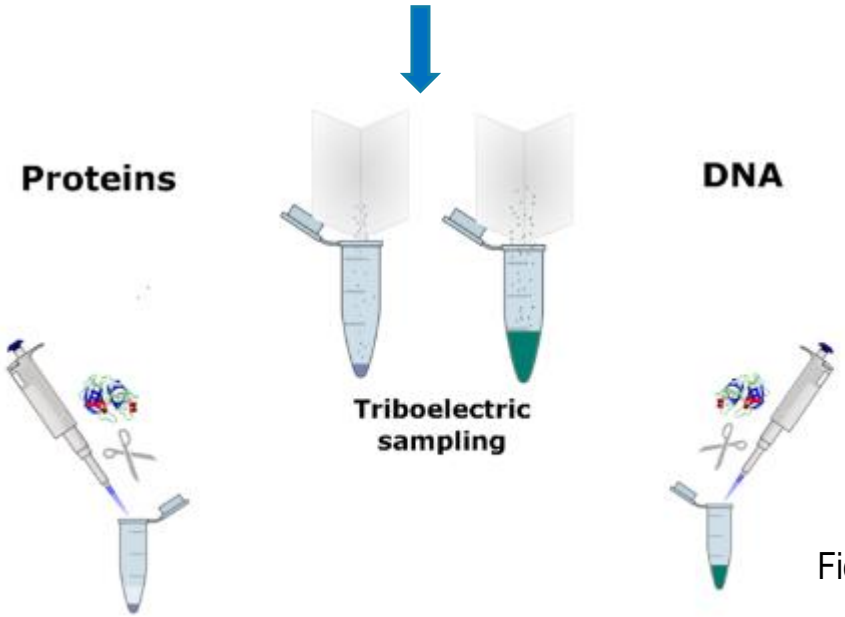
Non-invasive or minimal invasive sampling



Sampling: Triboelectric sampling

gentle rubbing of a PVC eraser on the parchment surface

Pics. University of York
https://www.york.ac.uk/news-and-events



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How much sample is needed?

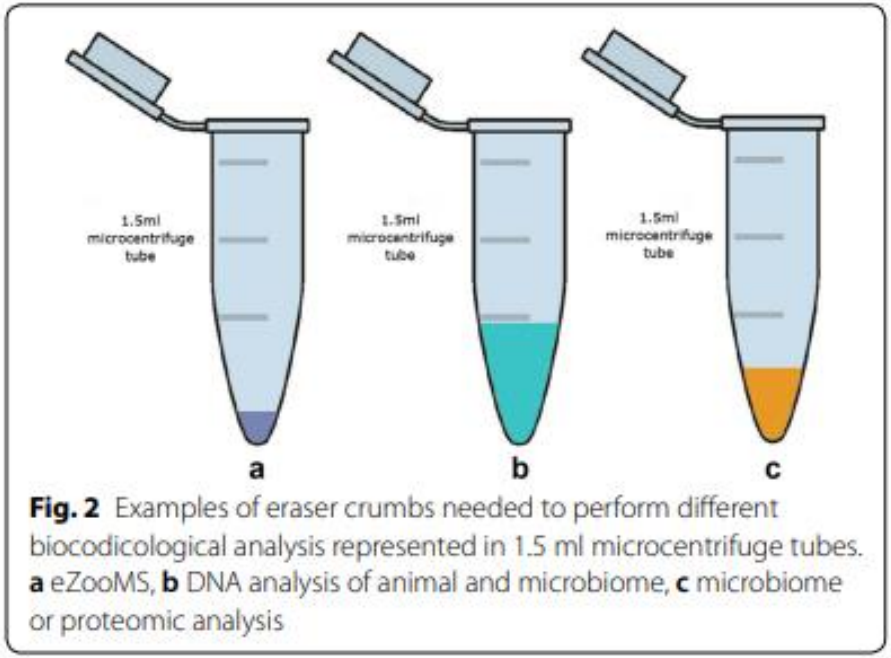

Fig. 2 Examples of eraser crumbs needed to perform different biocodicological analysis represented in 1.5 ml microcentrifuge tubes. **a** eZooMS, **b** DNA analysis of animal and microbiome, **c** microbiome or proteomic analysis

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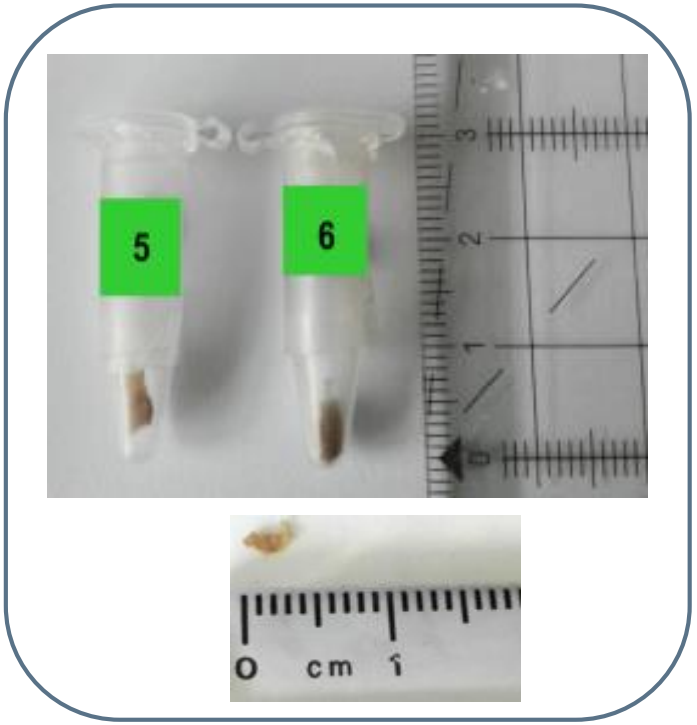
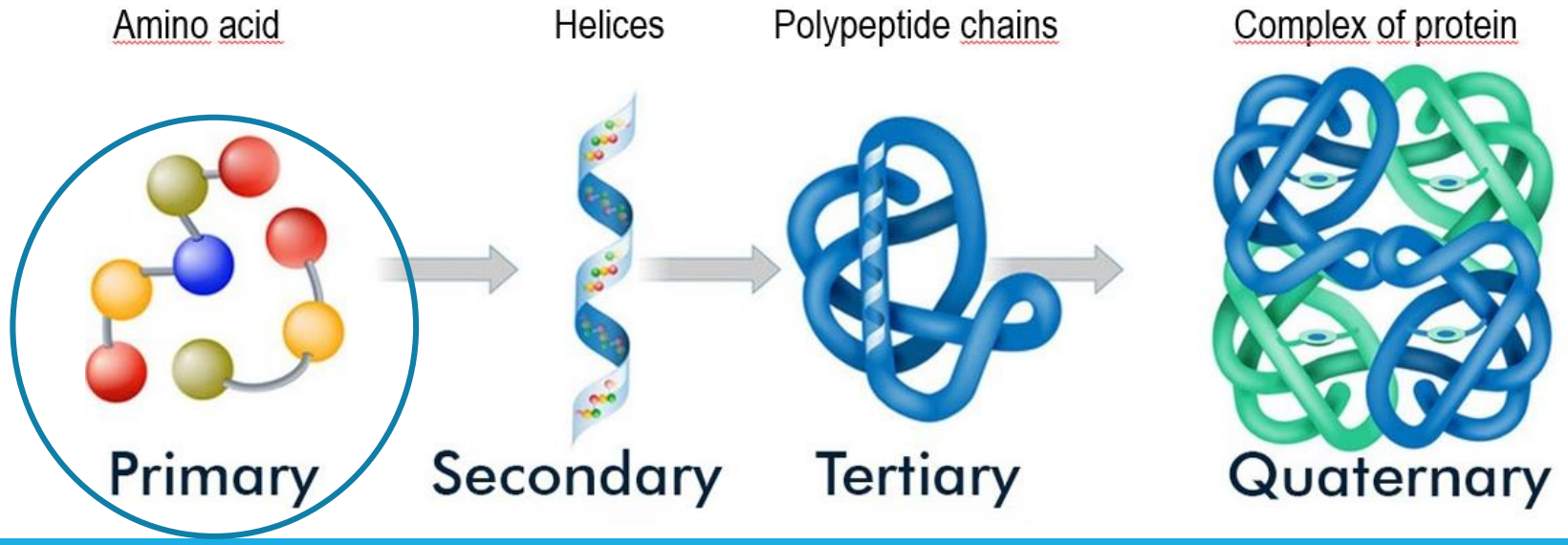


Piñar et al. 2020. Environ Microbiol 22: 3218–3233

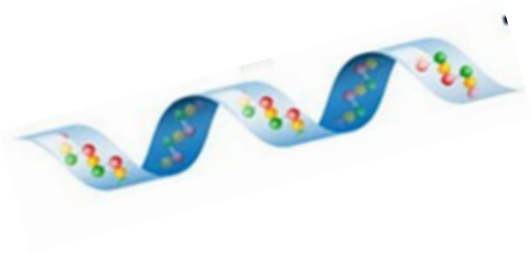
What is protein analysis?

- Proteins are composed of chains of molecules called amino acids. The sequence of these specific amino acids is what gives proteins their **primary structure**.
- Minor changes in primary structure can allow the discrimination between different species, allowing **species identification**.

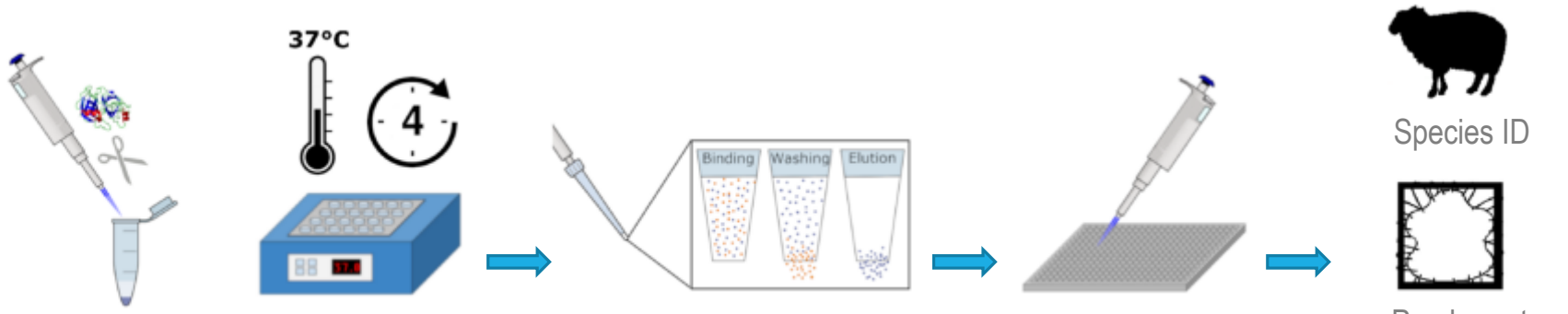


Protein analysis

- The basic form of protein analysis is called **peptide mass fingerprinting** (PMF).
- Proteins have **tissue specificity**, allowing the identification of not only the species but also of the biological tissue.
- Proteins seem to have much more **robust survival rates** than DNA (earliest proteins identified dated to over 3.8 million years!).
- The study of all the proteins present in a sample is known as **proteomics** and when applied to historic or ancient proteins it is called **palaeo-proteomics**.



Protein analysis



Extraction **Purification** **Analyses** **Results**

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What is genetic analysis?

DNA and its function

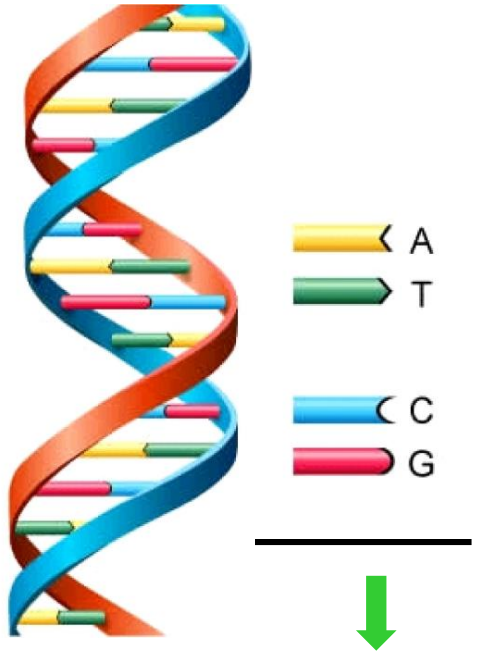
Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions for the development, survival, reproduction and function of living organisms. All known cellular life and some viruses contain DNA.

The main role of DNA in the cell is the long-term storage of biological information

By extracting and sequencing DNA we can detect the species and sex of the parchment animal. By analyzing the small differences in the genetic code we can determine possible breed variation (dependent on geography) and relatedness to other individuals.

What is DNA made of?

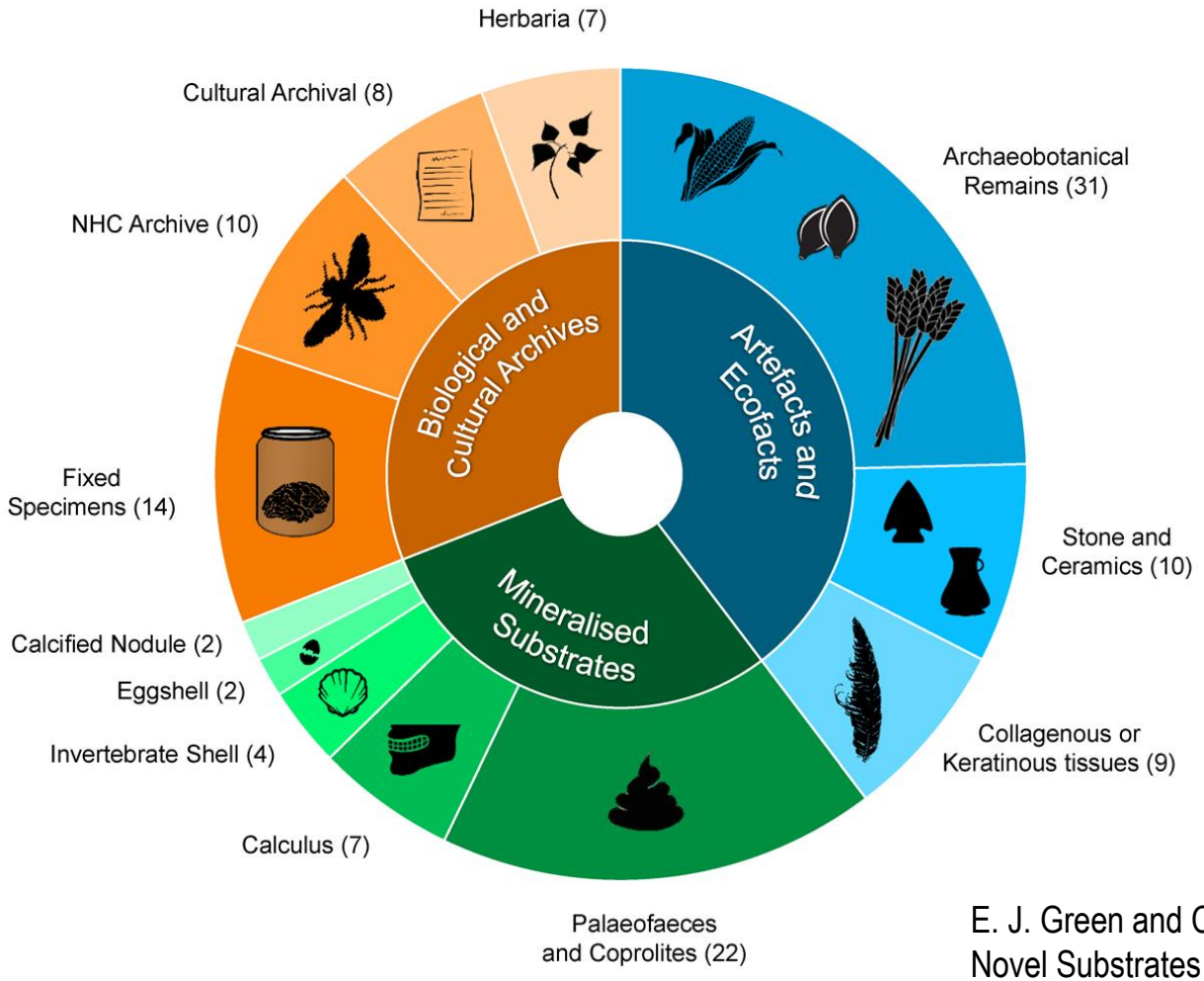
- DNA is made of chemical building blocks called **nucleotides**
- The nucleotides are made of three parts: a phosphate group, a sugar group and one of **four types of nitrogen bases**
- The four **nitrogen bases** are: adenine (**A**), thymine (**T**), cytosine (**C**) and guanine (**G**). The order (or sequence) of these bases determines what biological instructions are contained in a strand of DNA.
- In a strand of DNA, **nucleotides are linked into chains** binding the complementary nucleotides.



These 4 letters (nitrogen bases) represent a **code**.
We can decipher the "**genetic code**" contained in the DNA

Can DNA analyses help the study of ancient manuscripts?

A...



Yes!

E. J. Green and C. F. Speller. 2017. Genes 8: 180; Novel Substrates as Sources of Ancient DNA

How can DNA analysis help the study of ancient manuscripts?

1. Genetic analysis: The history of an object leaves its own genetic fingerprint represented by **aDNA**:

- the **animal species** from which a parchment was made
- the **plant** from which the **ink** was prepared
- the hands (**humans**) by which an object was manufactured and through which it was passed during its history

2. Microbiome: specific of each object (bacteria, fungi and viruses), as indicator for the conditions under which it was stored and its **state of conservation**

The compilation of these data results in an individual "bioarchive" for each single manuscript

what is the microbiome of an object?

The **microbiome** is the community of microorganisms (such as fungi, bacteria and viruses) that exists in a particular environment (in this case documents!)

provides information about the state of preservation and reflects past storage and environmental conditions.

Case studies in genetics

FWF Project P 29892-G29: "The Origin of the Old Church Slavonic-Glagolitic Manuscripts"

Interdisciplinary project:

❖Philologists, computer vision scientists, chemists and physicists, microbiologists and conservators worked together to investigate the history of some of the oldest Slavonic manuscripts and palimpsests.

- Department of Slavonic Studies,University of Vienna
- Computer Vision Lab, Institute of Computer Aided Automation, Vienna University of Technology (TU)
- Institute of Science and Technology in Art (INTK), Academy of Fine Arts, Vienna
- European Research Centre for Book and Paper Conservation-Restoration, Danube University, Krems
- Department of Biotechnology, University of Natural Resources and Life Sciences, (BOKU) Vienna

Questions posed in this project:

1. **What are the animal species that were used for the manufacture of the parchments?**

   some items can be grouped together as originating from the same or closely related parchments, and/or localities

2. **What is the colonizing microbiome on the surface of the parchment?**

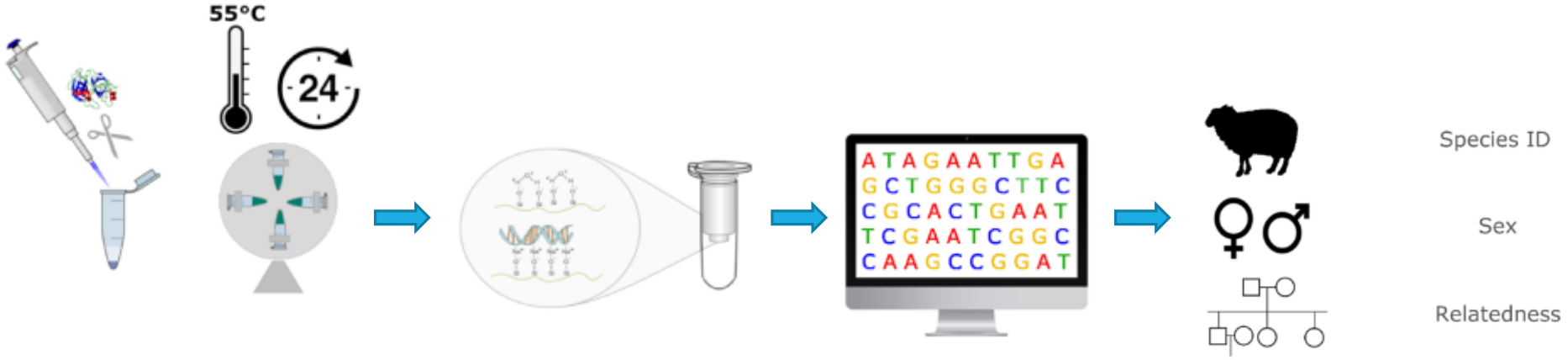
   interesting for several practical conservation reasons

Workflow to answer these questions

1. **Sampling** (piece of object, or non-invasive sampling with PVC erasers)
2. **Molecular methods** (DNA-based analyses to analyze even smallest traces of DNA)
   - **DNA extraction procedure**
     - Quality/quantity of DNA.
   - **Down-steps:**
     - DNA library preparation
     - Next Generation Sequencing **(NGS)** technologies
3. **Non-molecular methods**

   In situ observations (light microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM).

Genetic analysis



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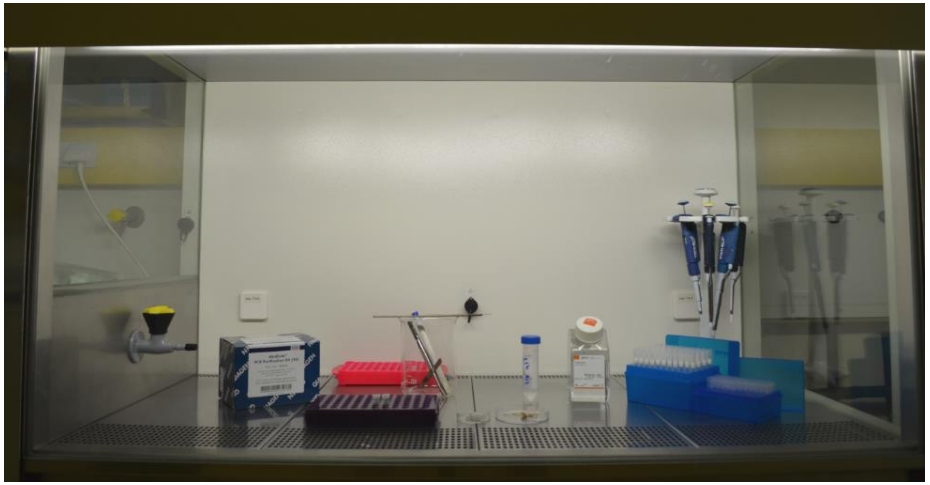
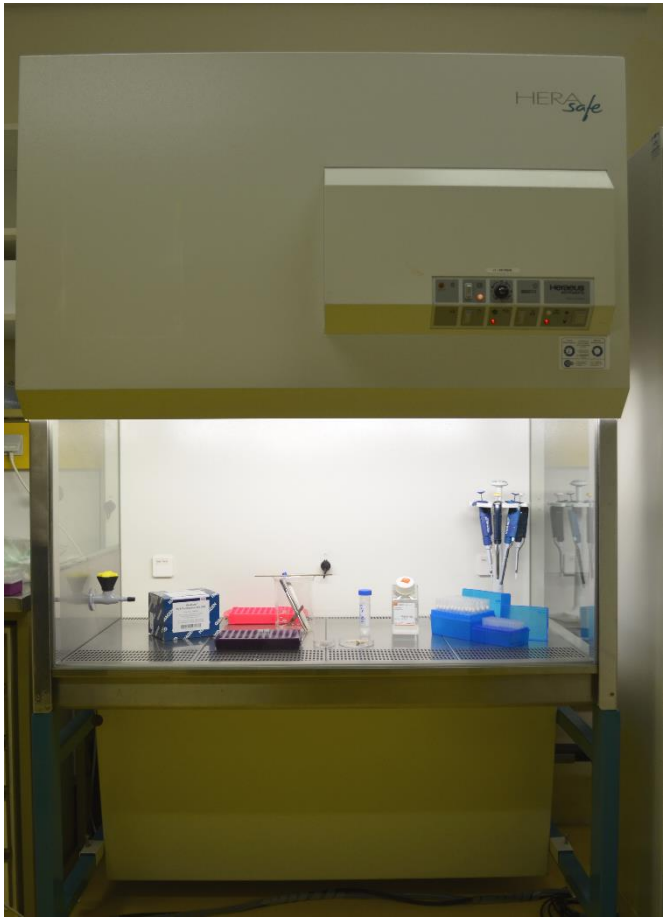
Extraction of ancient DNA (aDNA)

The extraction of **aDNA** is a delicate and difficult task that requires expertise and care.

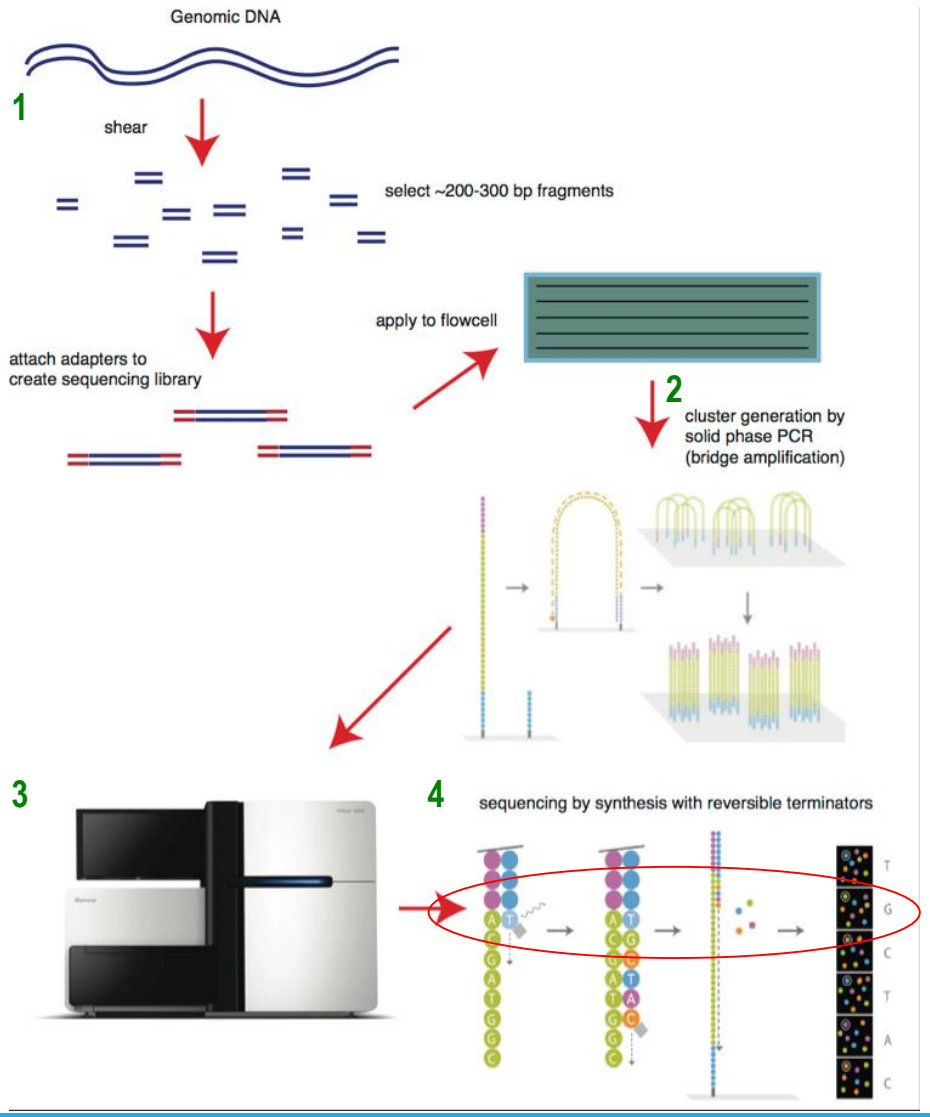
There are strict criteria to ensure the **reliability of aDNA** results.

Critical steps in DNA extraction procedures: **special rooms, devices and equipment .**





Sequencing



Illumina sequencing Platform steps:

1. **Library construction**

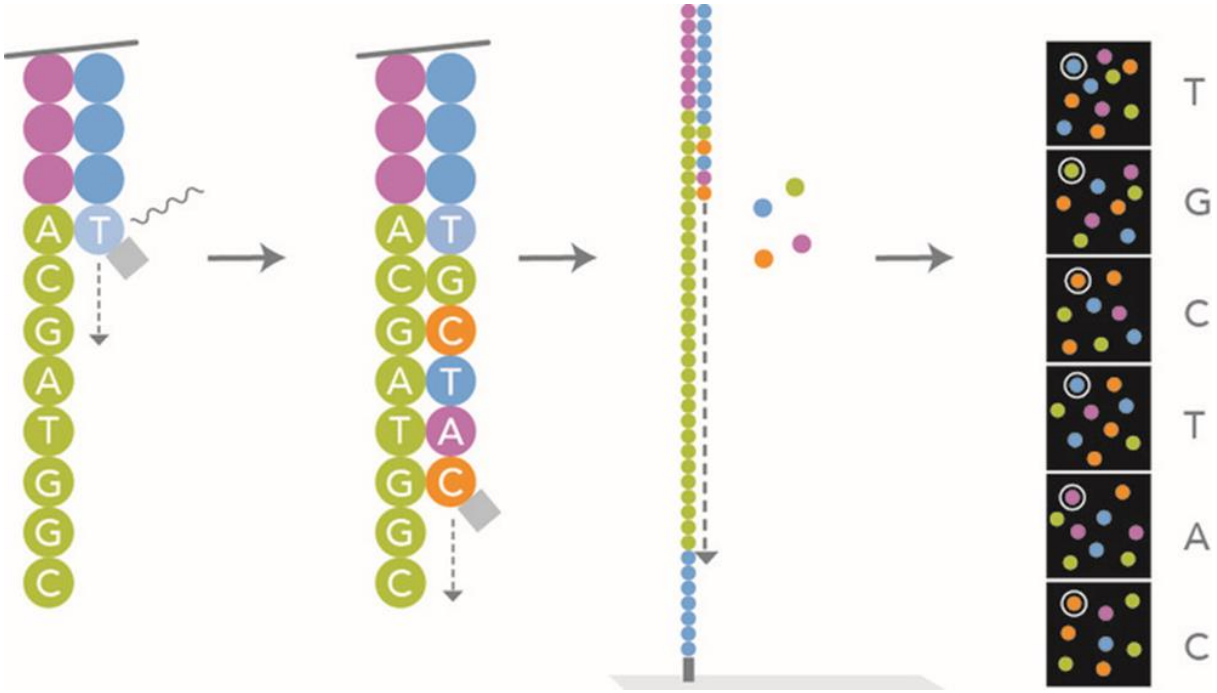
2. **Cluster generation** (add to flow cell and amplification)

3. **Sequencing** ("read" of single base at a time)

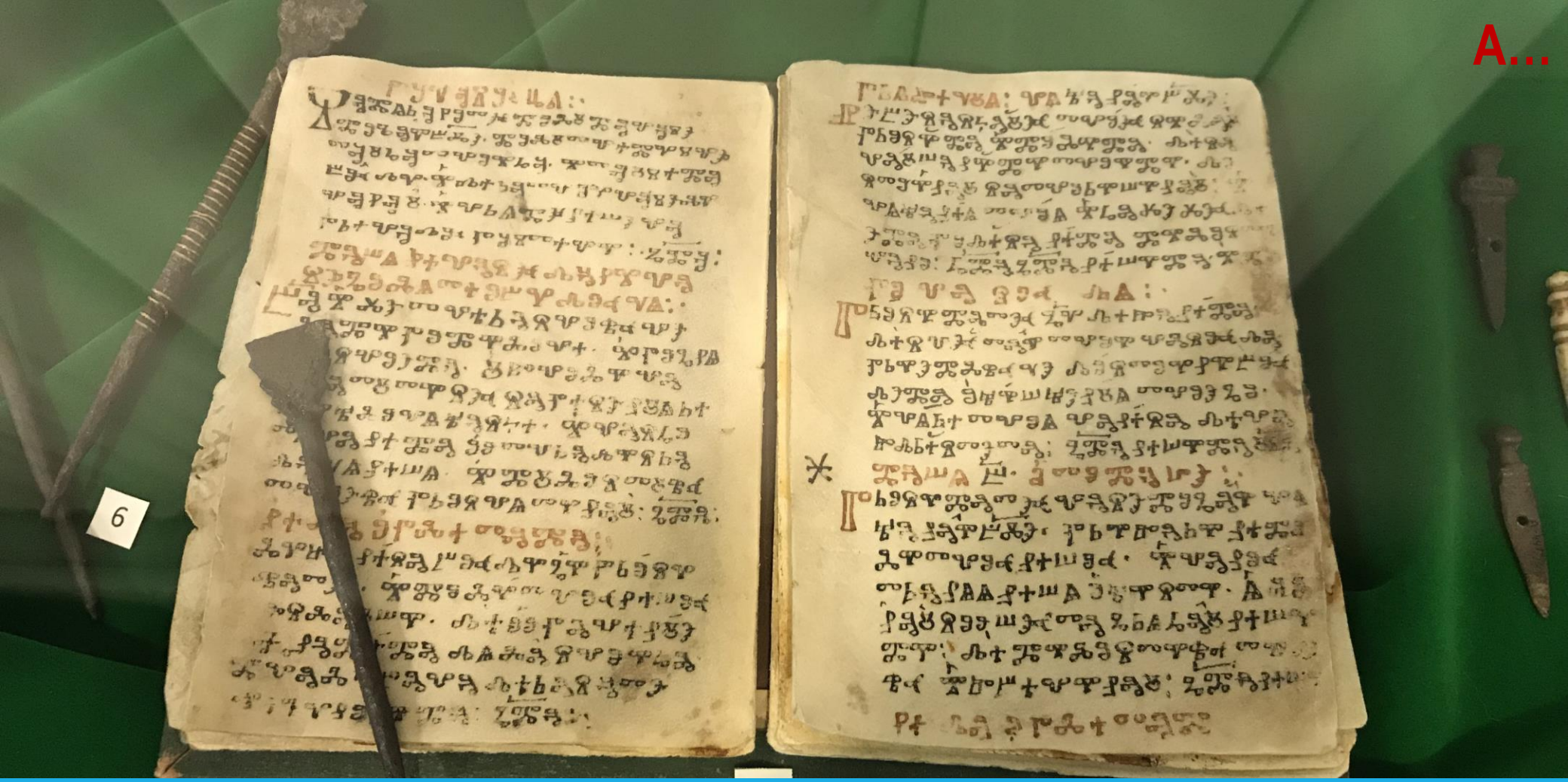
4. **Data analyses** (images transformed in basecalls and reads)

Data analyses

1. **Basecalling:**
   Images are translated in basecalls and "**reads**", like a kind of "**reading a code**"



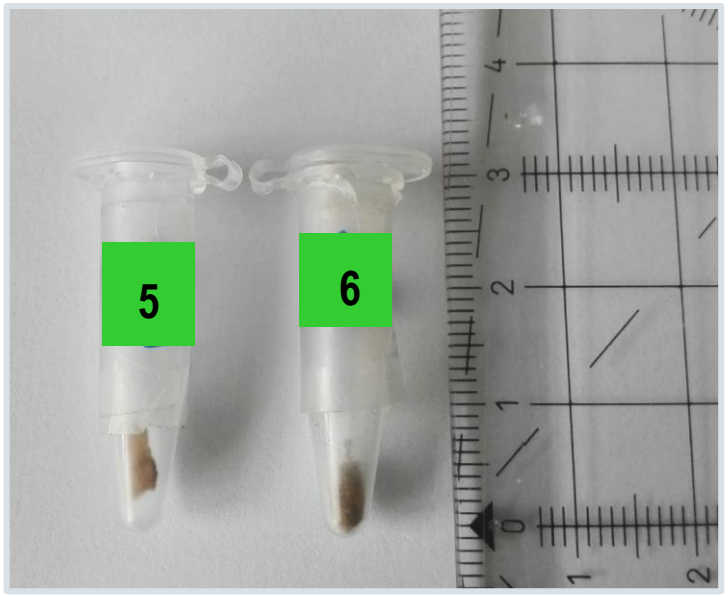
2. **Bioinformatic analyses:** Comparative analyses with DNA databases
   Enable the identification of sequences and the affiliation to animals, plants, bacteria, fungi, viruses and humans

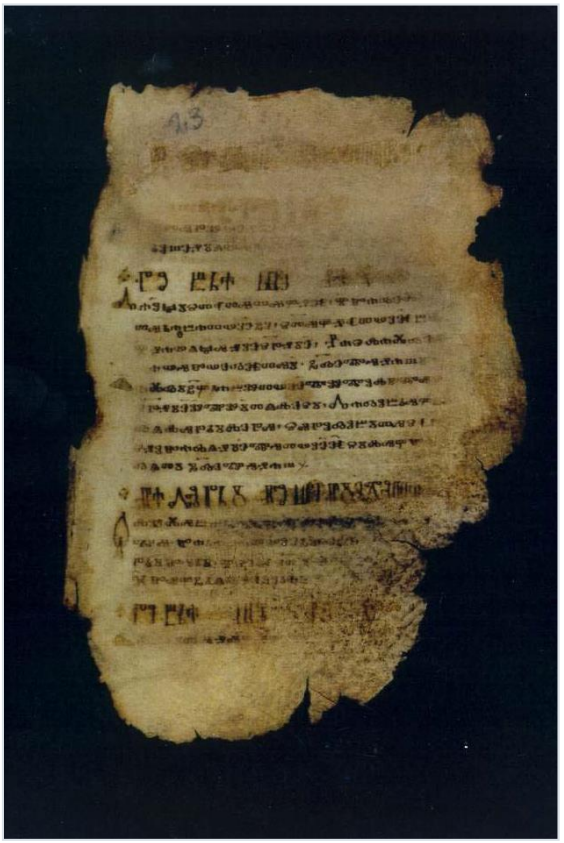


Some case studies

Missale Sinaiticum (Cod. Sin. Slav. 5/N)

Fragmentarily preserved and in a very bad state.
Written – probably in the second quarter of the 11th C.
Sample 5: Cod. Sin. Slav. 5N, Folio 3 or rather 4
Sample 6: Cod. Sin. Slav. 5N, Fragment EDV 68



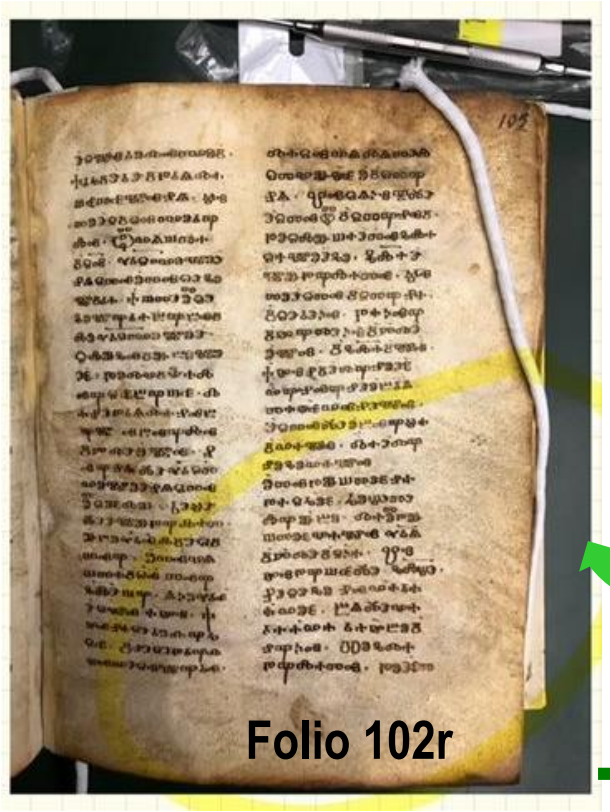


Piñar et al. 2020. Environ Microbiol 22: 3218–3233

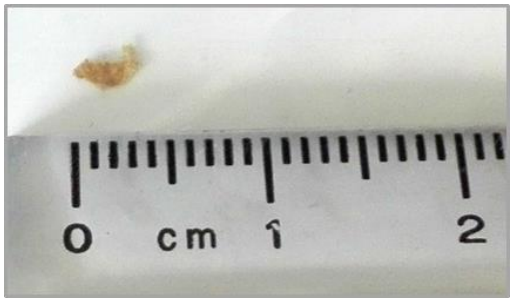
Codex Assemanianus (Vat. Slav. 3)

Found in Jerusalem in the 18th Century, and written between 1030-1050.

- Sample Vat-Slav-3P
- Sample Vat-Slav-3Rb



Eraser rubbings



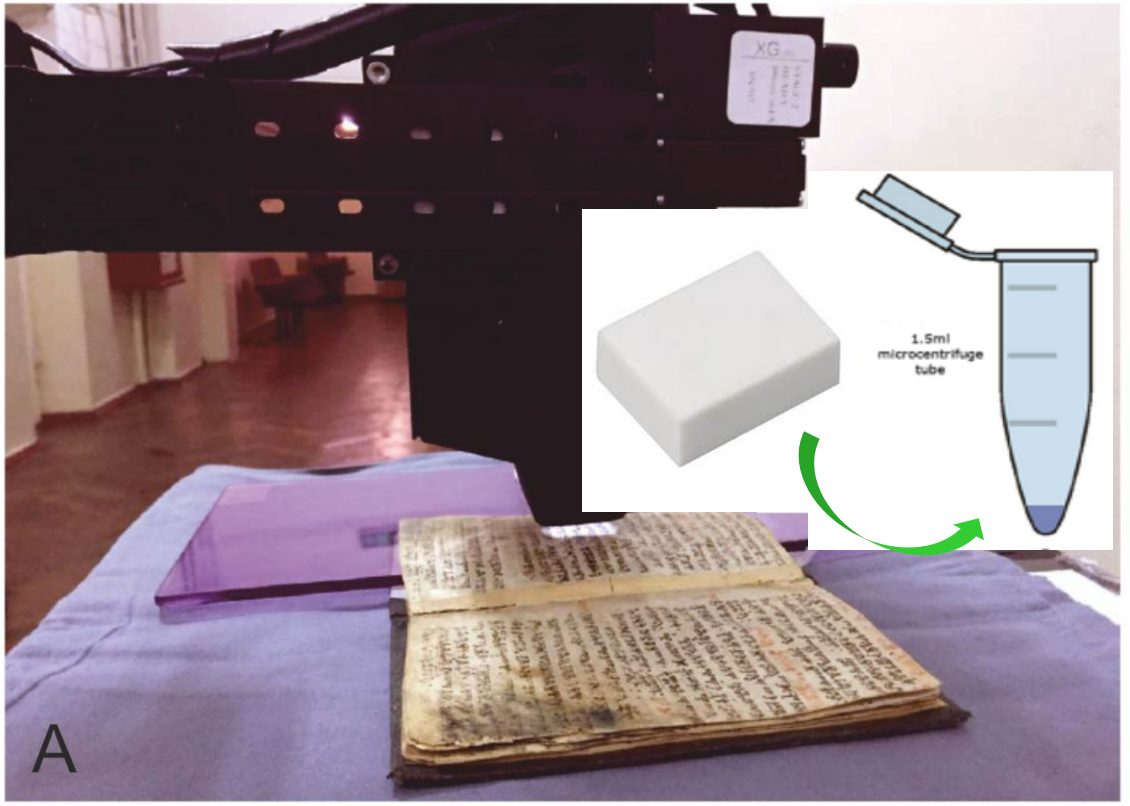
3P

3Rb

Piñar et al. 2020 Environ Microbiol 22: 3218–3233

The Kiev Folia

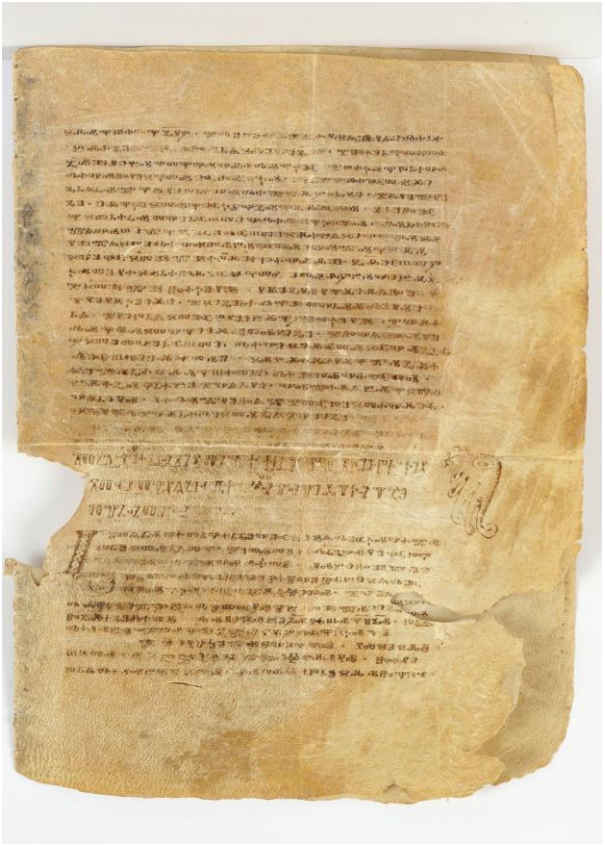
- The manuscript represents a Sacramentary and is the oldest surviving Slavonic manuscript found on Mt. Sinai/Egypt (dating most likely from the early 10th century, 925–935).
- It is written on seven parchment leaves which today are stored at the Vernadsky National Library of Ukraine in Kiev.

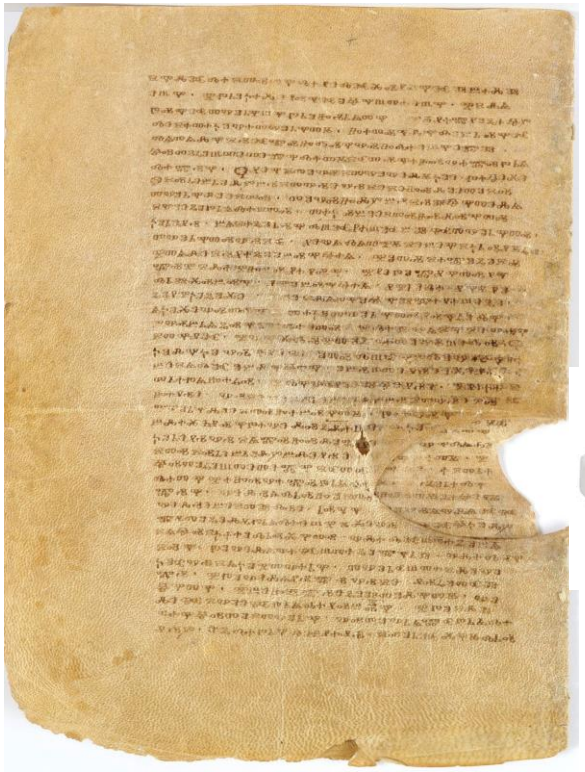


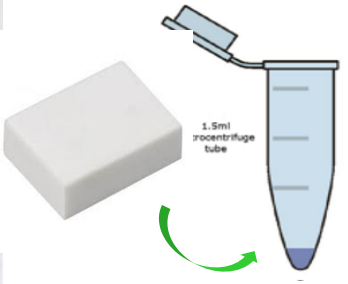
Cappa et al. 2022. Int Biodeterior Biodegrad 167: 105342

Glagolita Clozianus

Old Church Slavonic codex in Glagolitic script from the 11th century, consists of a total of 14 leaves. Two of these leaves are kept in the library of the Tyrolean Landesmuseum Ferdinandeum in Innsbruck.







https://www.uibk.ac.at/slawistik/institut/clozianus.html

Results

- Can we answer the questions posed?
- Can we estimate which animal was used for the manufacturing?
- What information does the microbiome provide?

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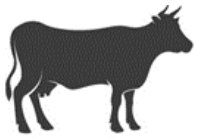
Animal origin of parchment

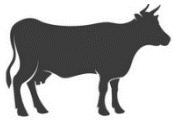



Missale (Sacramentarium) Sinaiticum

(Cod. Sin. Slav. 5/N)

1. Animal species used for the manufacture of parchment:

Sample 5: 

Sample 6: 

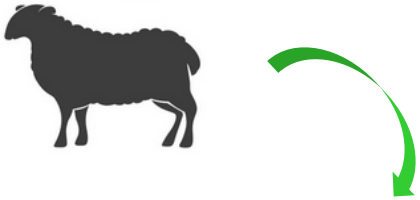
Species	Reads sample 5	Reads sample 6
Homo sapiens	208416	64370
Bos Taurus (**calf**)		**4630**
Ovis aries (**sheep**)	**49223**	

- **Reads aligned against:** *Homo sapiens*  *Bos taurus*  *Ovis aries* 
  Capra hircus  *Sus scrofa* 

Codex Assemanianus (Vat. Slav. 3)

1. Animal species used for the manufacture of parchment:

Vaticana.Slav.3P and Vaticana.Slav.3Rb:

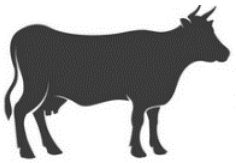


Species	Reads Vat.Slav.3P	Reads Vat.Slav.3Rb
Homo sapiens	7852951	143268
Ovis aries (**sheep**)	**55146**	**24465**

- **Reads aligned against:** *Homo sapiens*, *Bos taurus*, *Ovis aries*, *Capra hircus*, *Sus scrofa*

Kiev Folia

1. Animal species used for the manufacture of parchment:

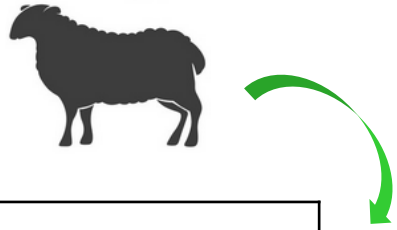

or


Species	Kiev Folia
Homo sapiens	5903087
Bos Taurus (**calf**)	**356967**
Capra hircus (**goat**)	347337

- **Reads aligned against:** *Homo sapiens*, *Bos taurus*, *Ovis aries*, *Capra hircus*, *Sus scrofa*

Glagolita Clozianus

1. Animal species used for the manufacture of parchment:

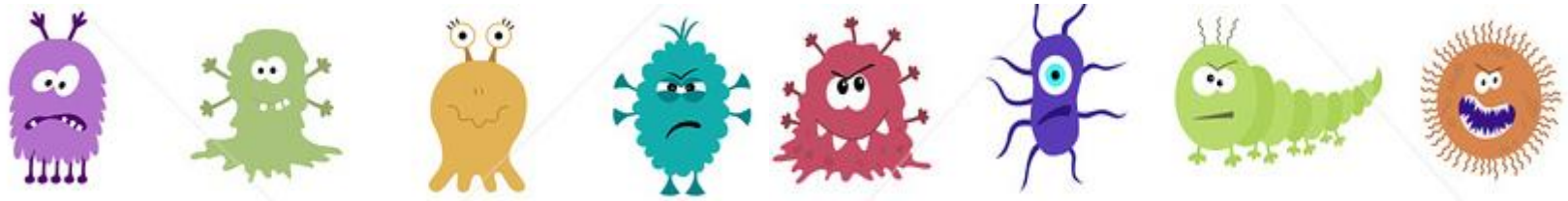


Species	Reads
Homo sapiens	207099
Ovis aries (**sheep**)	**150602**

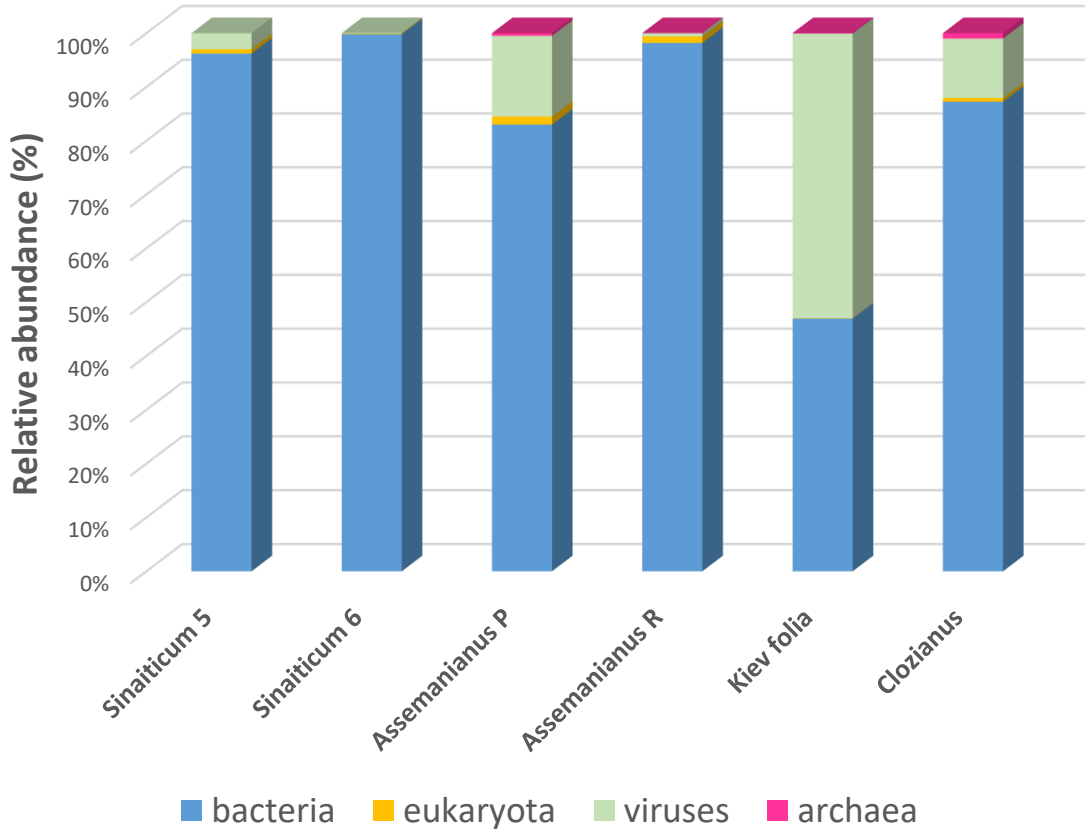
- **Reads aligned against:** *Homo sapiens*, *Bos taurus*, *Ovis aries*, *Capra hircus*, *Sus scrofa*

A...

Microbiome



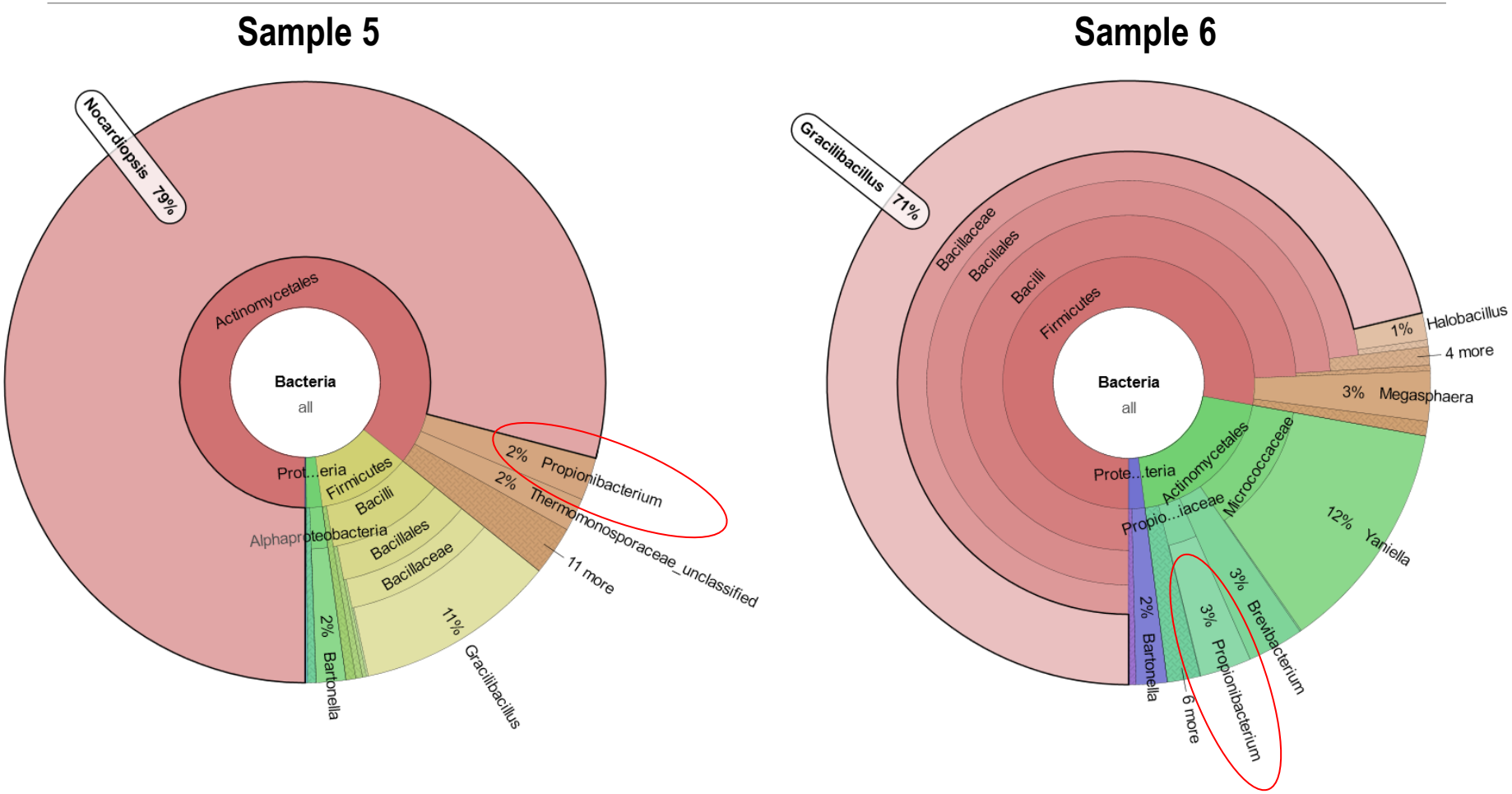
Microbiome



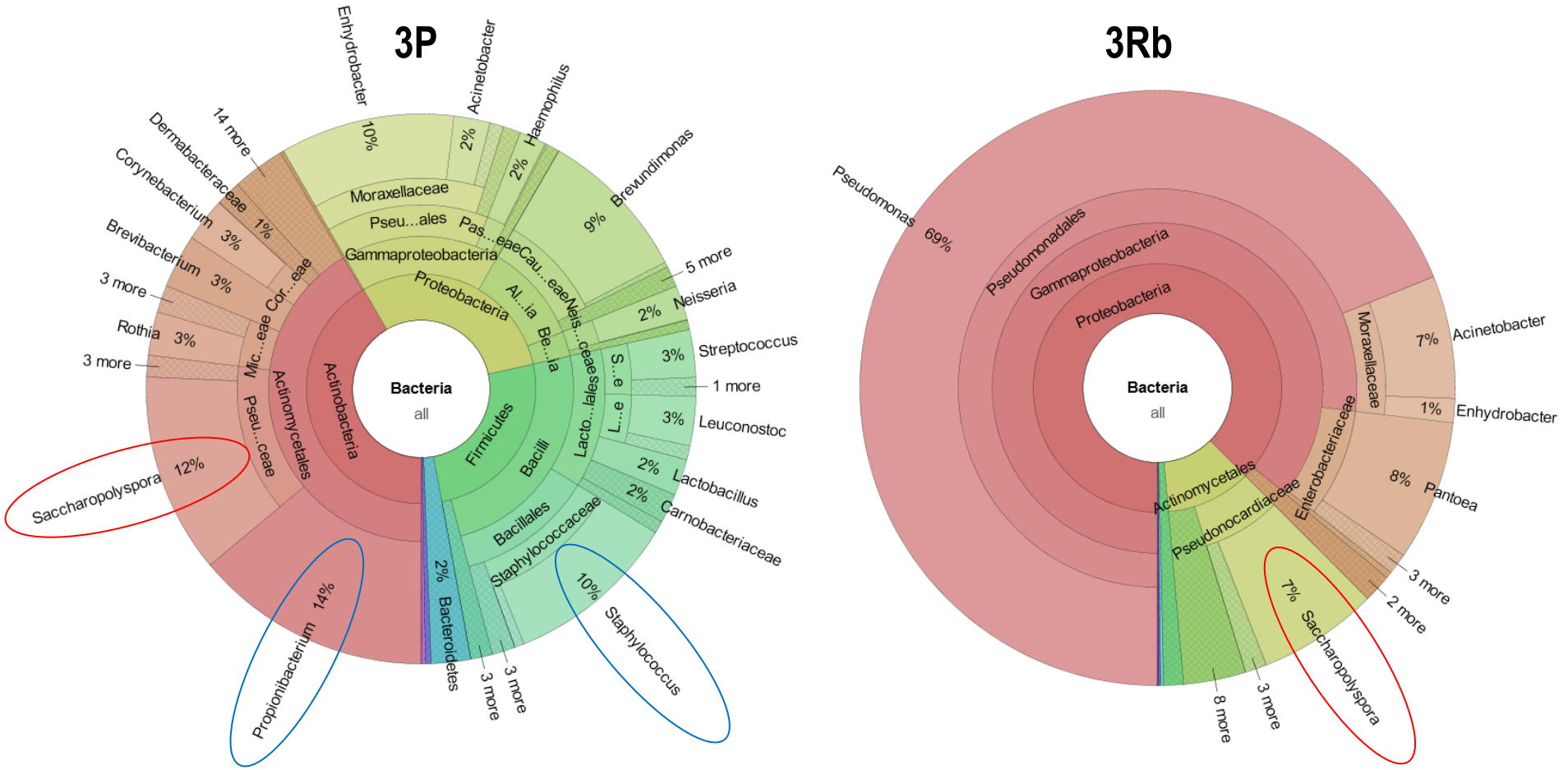
Piñar et al. 2020. Environ Microbiol 22: 3218–3233

Cappa et al. 2022. Int Biodeterior Biodegrad 167: 105342

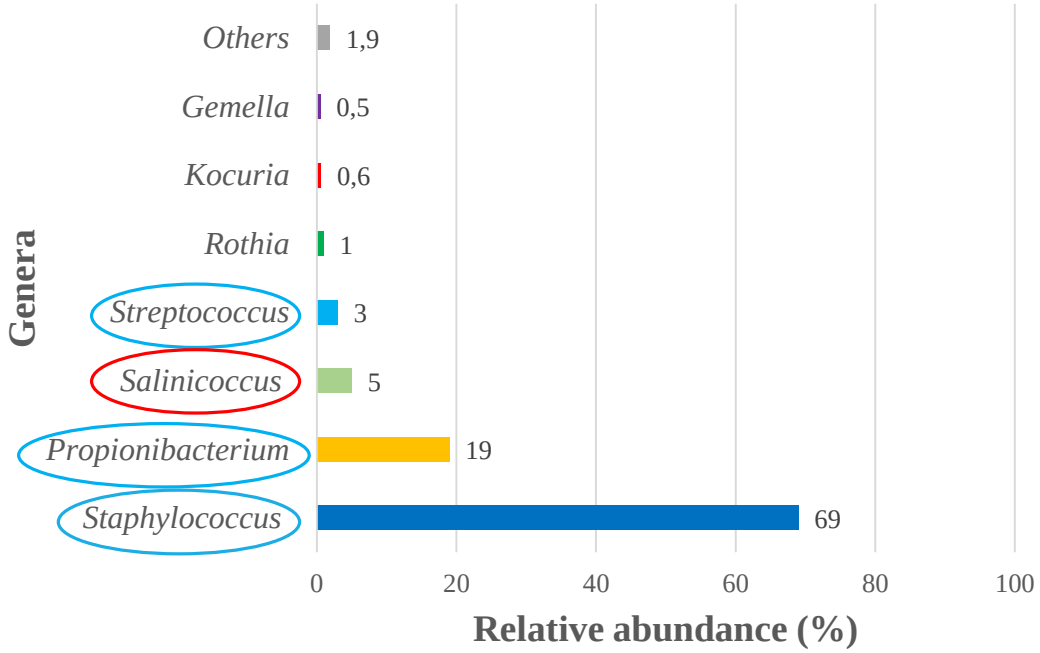
Missale (Sacramentarium) Sinaiticum



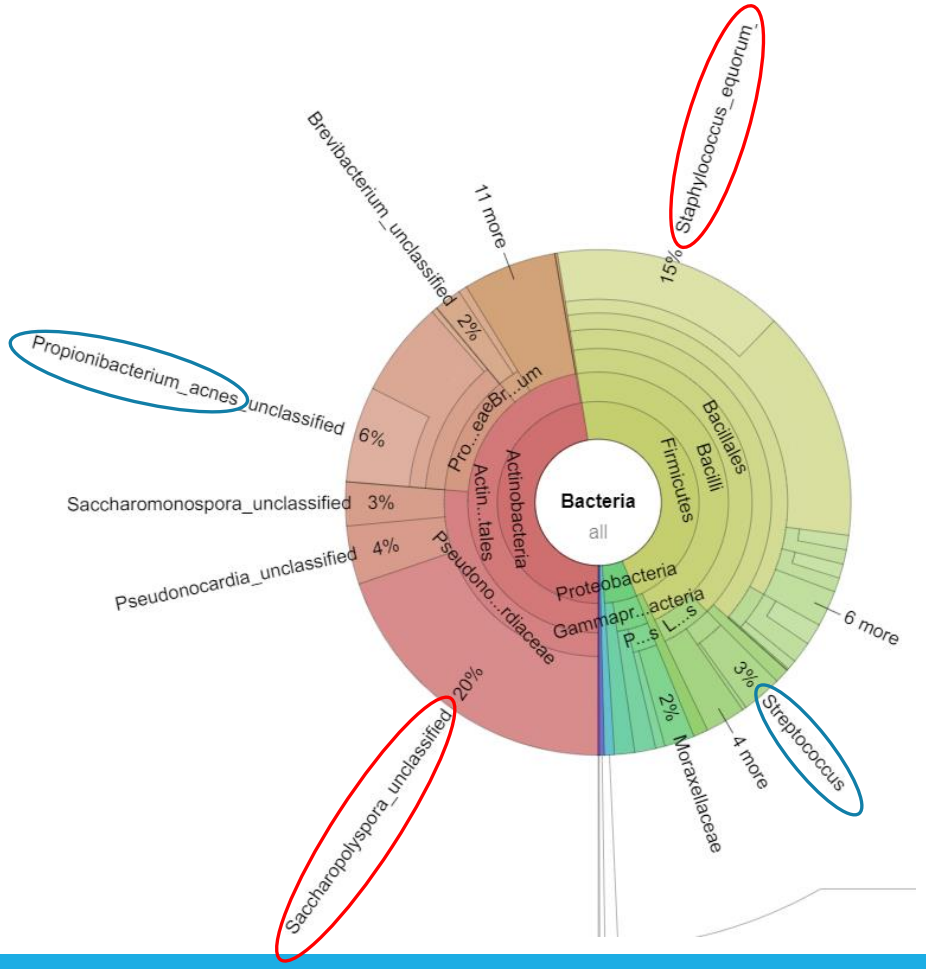
Codex Assemanianus



The Kiev Folia

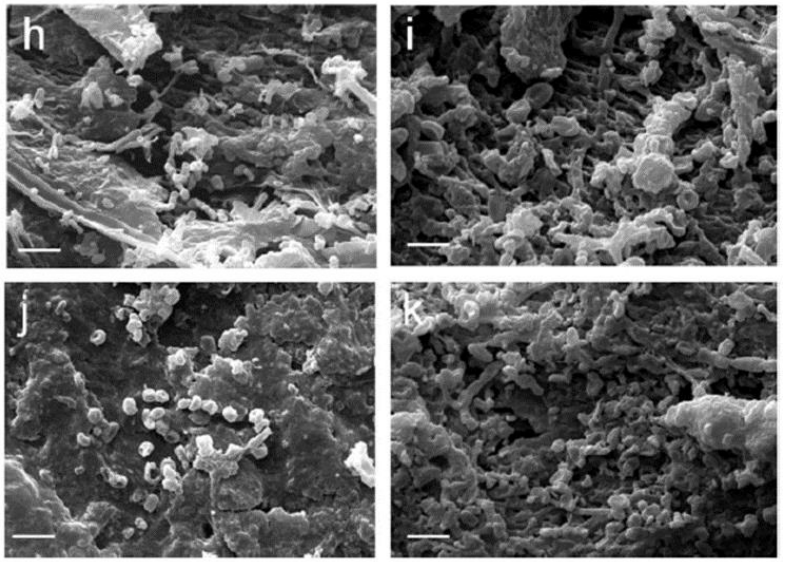


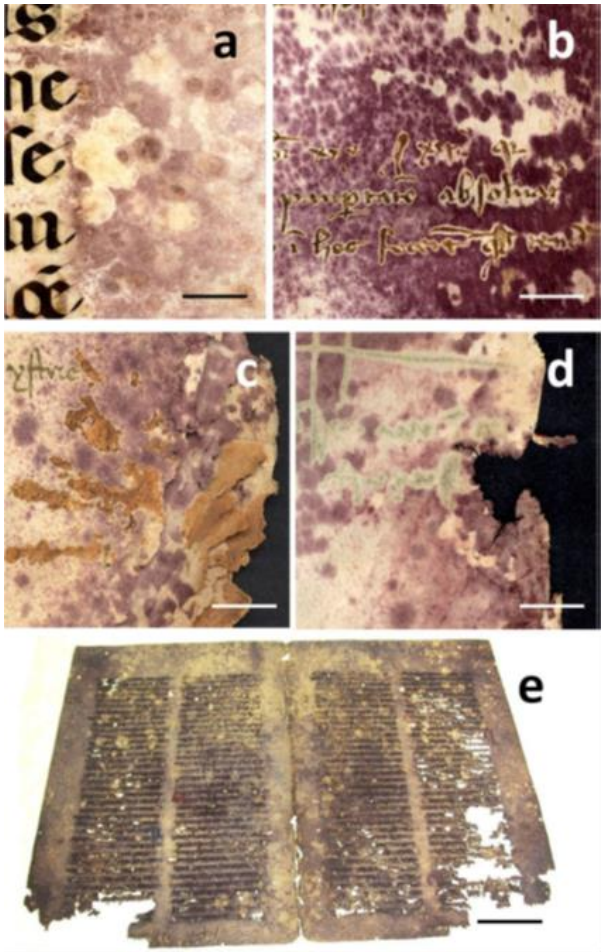
Glagolita Clozianus



Saccharopolyspora and the measles-like parchment discoloration

There is a common denominator in the measles-like parchment discoloration, namely ***Saccharopolyspora* species,** and allied species related to the presence of salt (NaCl) in the material.



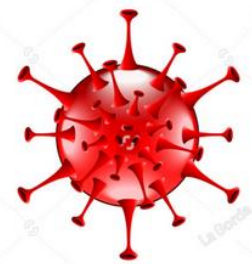


Piñar et al., 2015. Environmental Microbiology 17(2), 427–443
Teasdale et al., 2017. R. Soc. open sci. 4: 170988.

Viruses







Dasheen-mosaic virus

In *Missale (Sacramentarium) Sinaiticum*

Some viruses matched the Dasheen-mosaic virus, which infects the plant Taro (*Colocasia esculenta*)

Taro was a common plant in Egypt

Its primary use is the consumption of its edible corm and leaves, but it is also known for their high **anthocyanine** concentration

In ancient times some inks used for parchments were composed of **anthocyanines** extracted from plants (bark, leaves, fruits, seeds)

Were some inks composed of anthocyanine gained from Taro?

Bicchieri, 2014 (evidence of anthocyanine inks in the purple Codex Rossanensis)

Jaagsiekte sheep retrovirus (JSRV)

- Detected in **Missale Sinaiticum nr 5**, **Codex Assemnianus** and **Glagolita Clozianus** (**all made of sheep!**)
- This is a retrovirus, which is the causative agent of a contagious lung cancer in sheep, called ovine pulmonary adenocarcinoma (OPA).
- The exogenous infectious form of JSRV has an endogenous counterpart, which is present in the genomes of all sheep. The sheep genome has around 27 copies of endogenous retroviruses (enJSRVs).
- Endogenous JSRV has several roles in the evolution of the domestic sheep, as they are able to block the JSRV replication cycle and play a critical role in sheep embryo development.

The detection of this virus in these scrolls confirms the genetic analyses that identify the origin of the used sheepskin!

Last but not least:

When working on biodeteriorated parchment remember to protect yourself with gloves, glasses and mask.

BECAUSE….

Some of the microorganisms that colonize parchment are also human parasites and pathogens, or can produce toxic substances that causes severe allergies

Fungi can attack nails causing mycoses, and, together with bacteria, can enter lungs causing infections…

Never like this!

Conclusions:

- **Biocodicology** can help the study of ancient manuscripts by adding valuable biological information: an "**individual bioarchive**".
- **Protein and DNA** analyses enable to infer the **animal species** used for the manufacture of parchment.
- The **microbiome** colonizing the surface of the parchments correlates with the salty environment provided by the parchment samples, selecting **halophilic (salt-loving)** microorganisms, as ***Saccharopolyspora*** (common denominator) on most of parchment samples examined.
- Microorganisms from the **human skin microbiome**, most probably related with the intensive handling of the manuscripts that may be **potential pathogens**.
- Viruses can also provide interesting information, such as confirming the **animal origin of the skins** or the **vegetable origin of the dyes** used.

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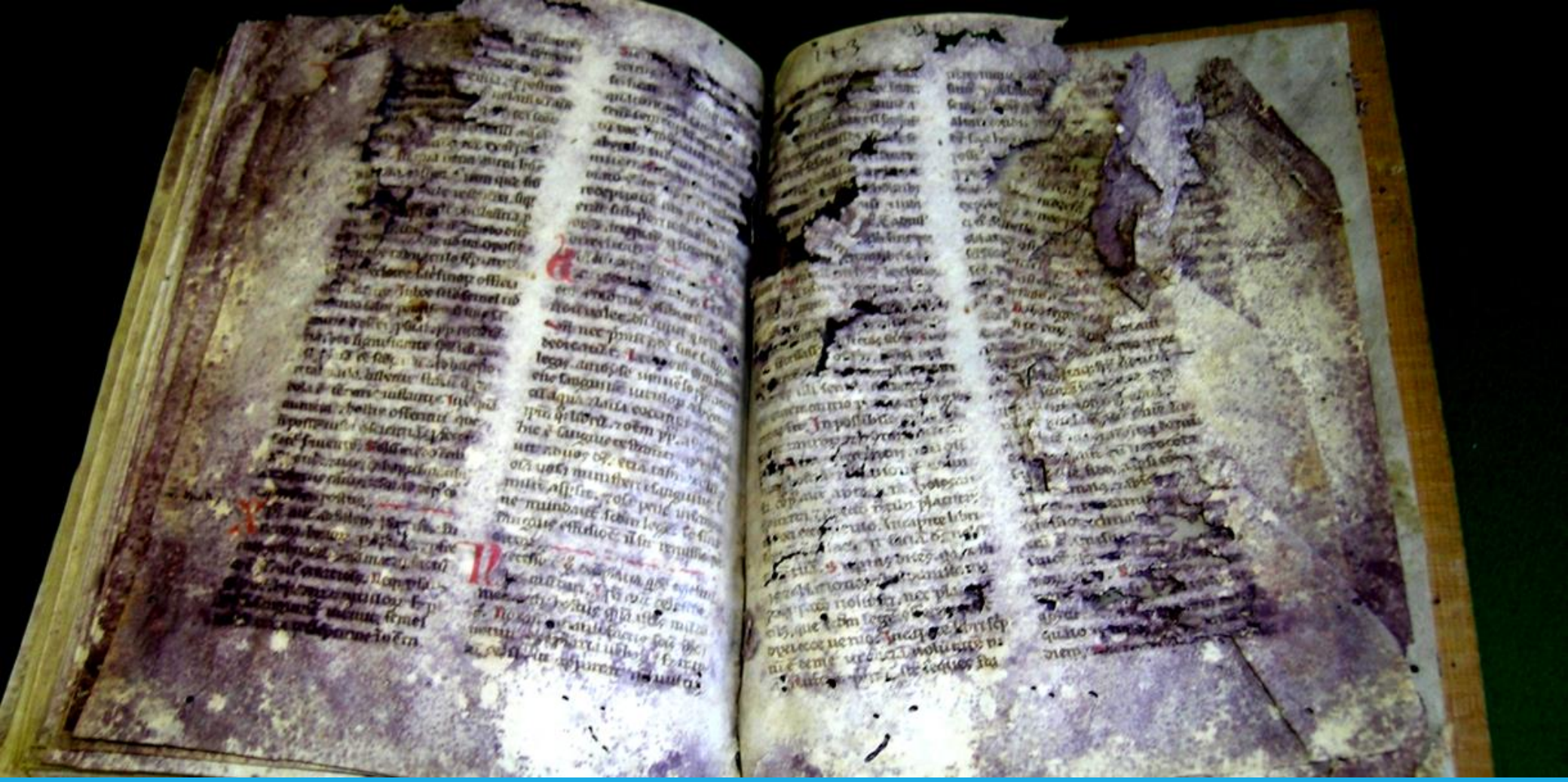

Thank you for your attention!