

DNA analyses of ancient parchment manuscripts

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What is the DNA and its function?

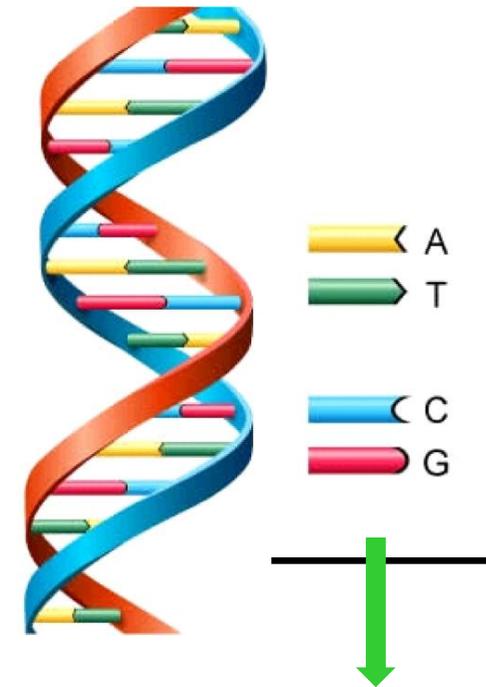


- **Deoxyribonucleic acid (DNA)** contains the genetic instructions for the development, survival, reproduction and function of living organisms. All known cellular life and some viruses contain DNA

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

What is DNA made of?

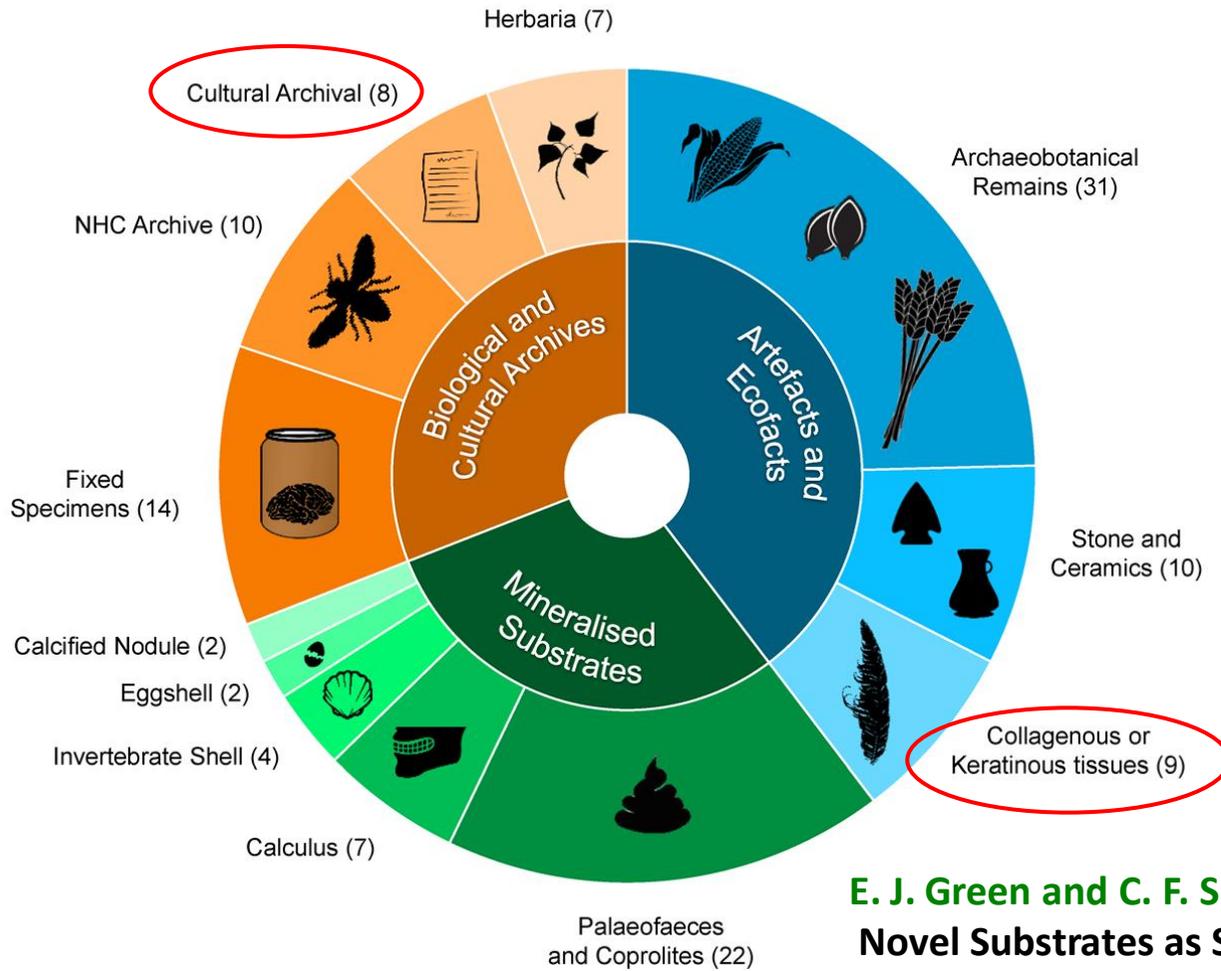
- DNA is made of chemical building blocks called **nucleotides**
- The nucleotides are made of three parts: a phosphate group, a sugar group and one of **four types of nitrogen bases**
- The four **nitrogen bases** are: adenine (**A**), thymine (**T**), cytosine (**C**) and guanine (**G**). The order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



Yes!

E. J. Green and C. F. Speller. 2017. Genes 8(7), 180; Novel Substrates as Sources of Ancient DNA

How can DNA analysis help the study of ancient manuscripts?



Medieval manuscripts, represent not only an irreplaceable documentary record but also a remarkable reservoir of **biological information**

1. 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. Objects carries a specific **microbiome** (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 **“biological pedigree”** for each single manuscript

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 are working 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
- Institute of Science and Technology in Art, 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, Vienna

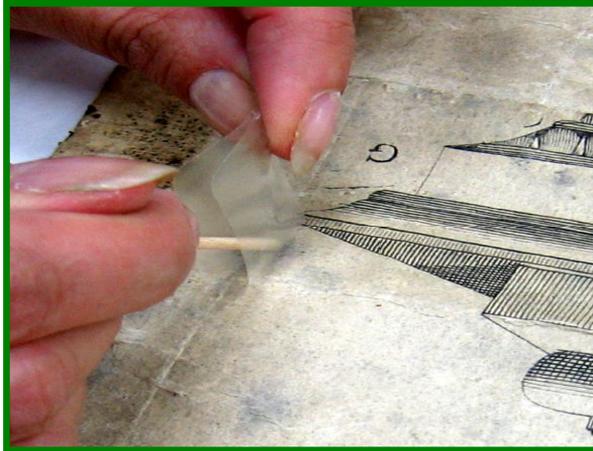
Questions posed in this project:

- 1. What animal species were used for the manufacturing 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 parchments?**
interesting for several practical conservation reasons

1. Sampling

Different sampling possibilities!

Non-invasive or minimal invasive sampling



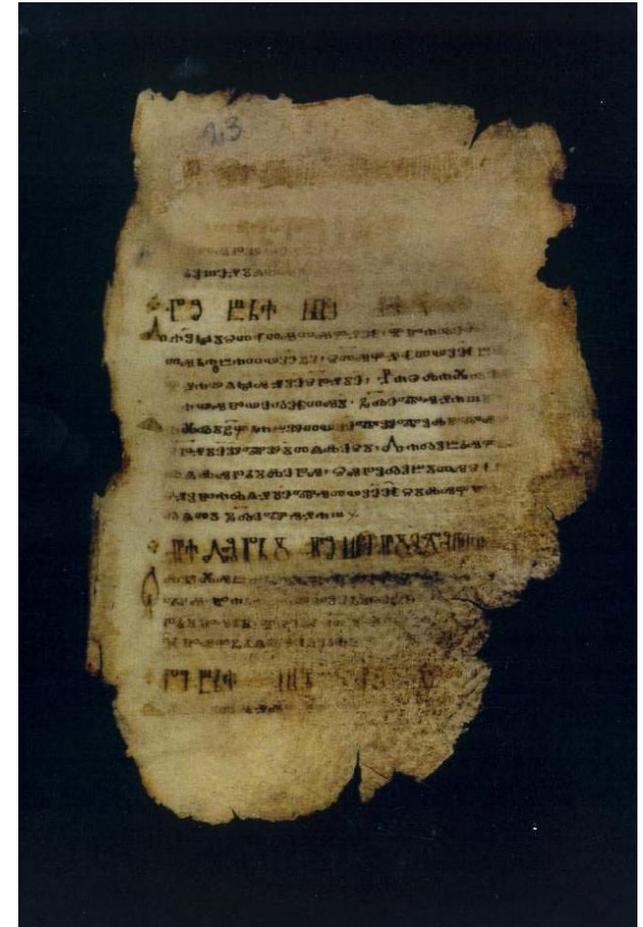
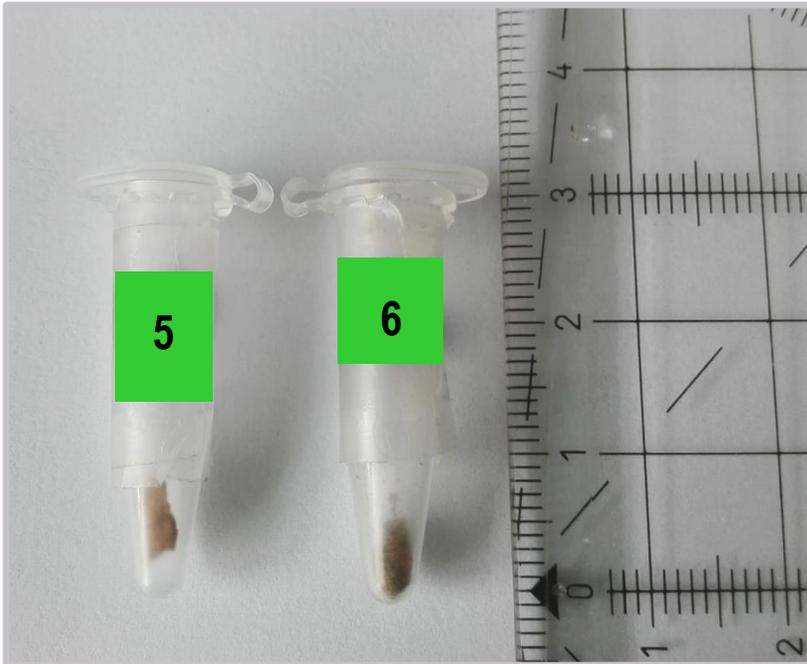
Samples investigated in this study:

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

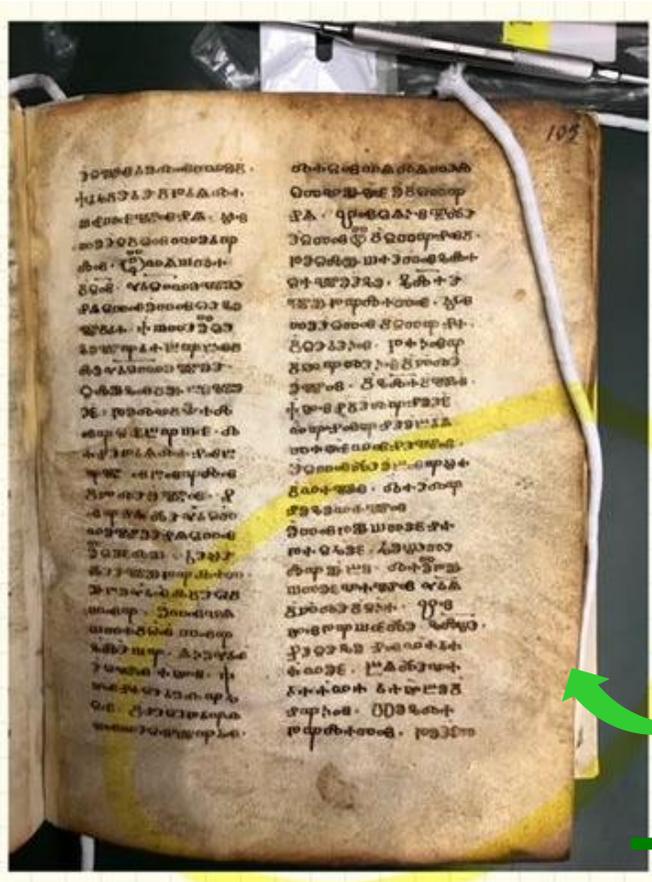


Samples investigated in this study:

Codex Assemanianus (Vat. Slav. 3)

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

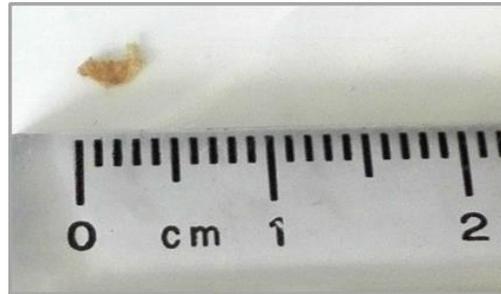
- Sample Vat-Slav-3P
- Sample Vat-Slav-3Rb



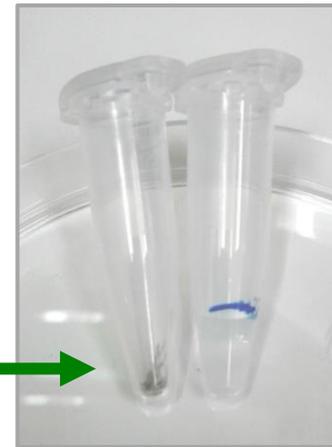
Folio 102r (pic. F. Cappa)



Eraser
rubbings



3P



3Rb

2. Molecular Methods

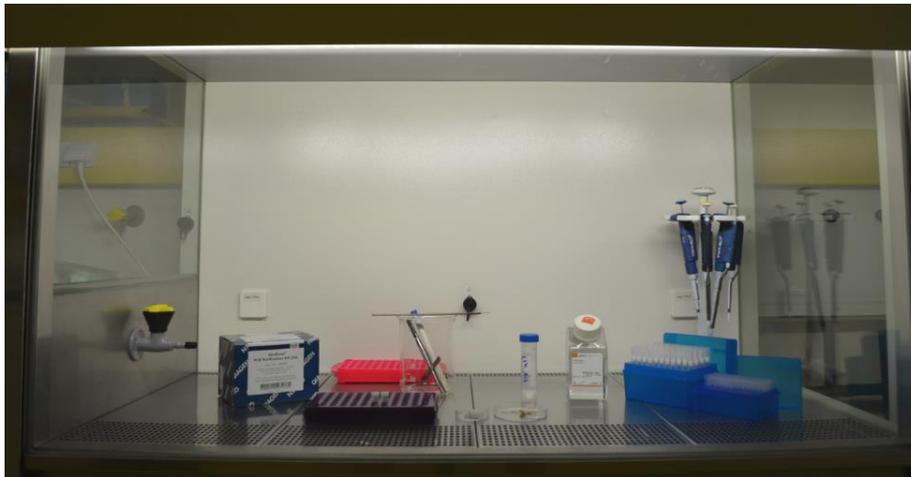
Next Generation Sequencing (NGS) technologies

(different sequencing platforms, bioinformatics pipelines, data analyses)

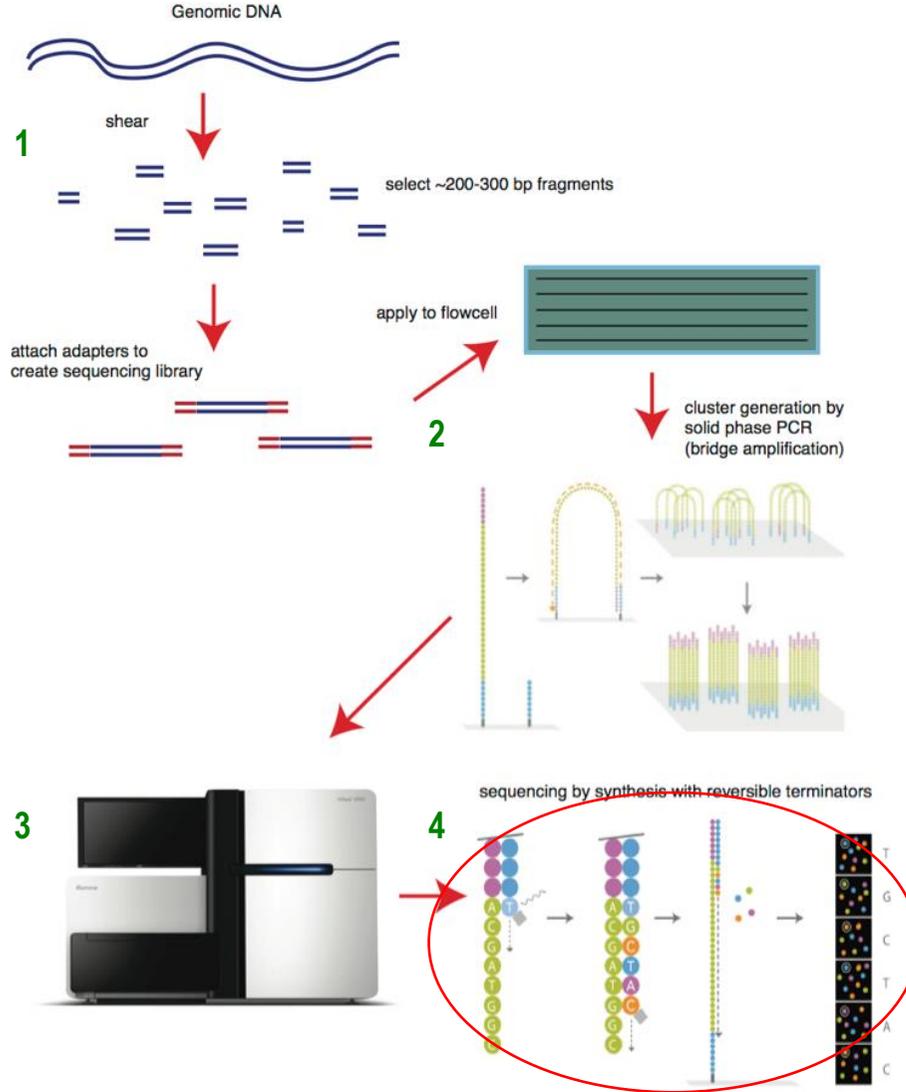
Extraction of ancient DNA (aDNA)



- 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**

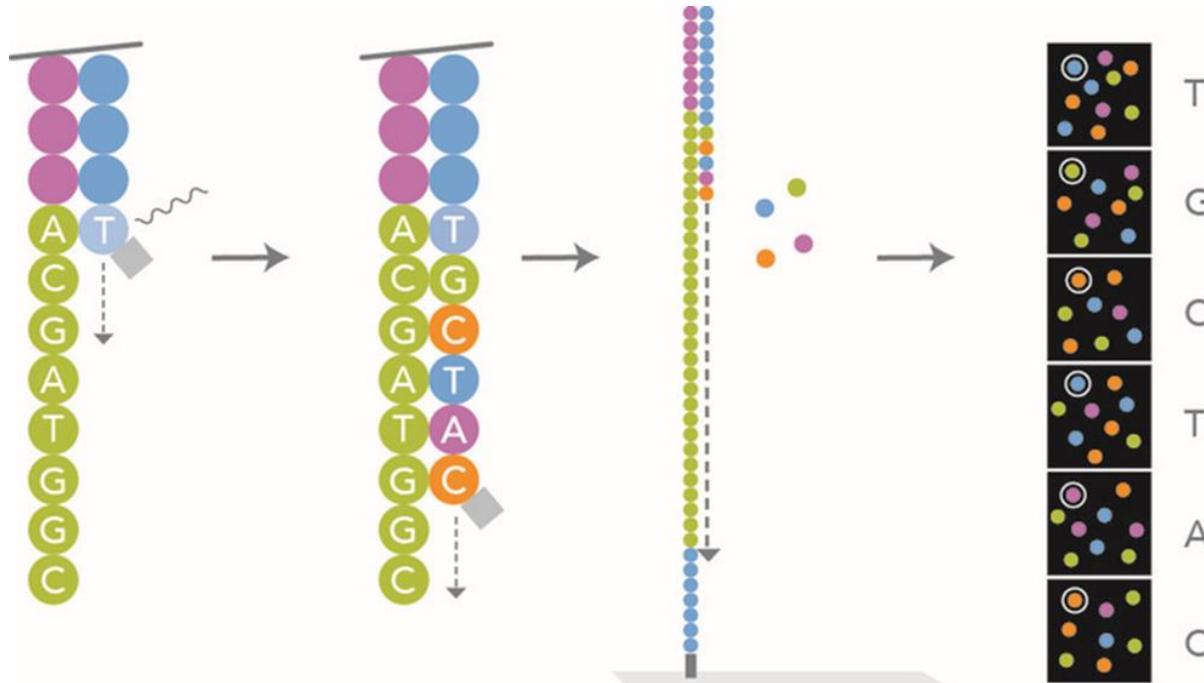
2. **Cluster generation** (add to flow cell and amplification)

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

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

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

Results

Results:

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

1. Animal species used for the manufacture of parchment:



Species	Reads sample 5	Reads sample 6
<i>Homo sapiens</i>	208416	64370
<i>Bos Taurus</i> (calf)		4630
<i>Ovis aries</i> (sheep)	49223	

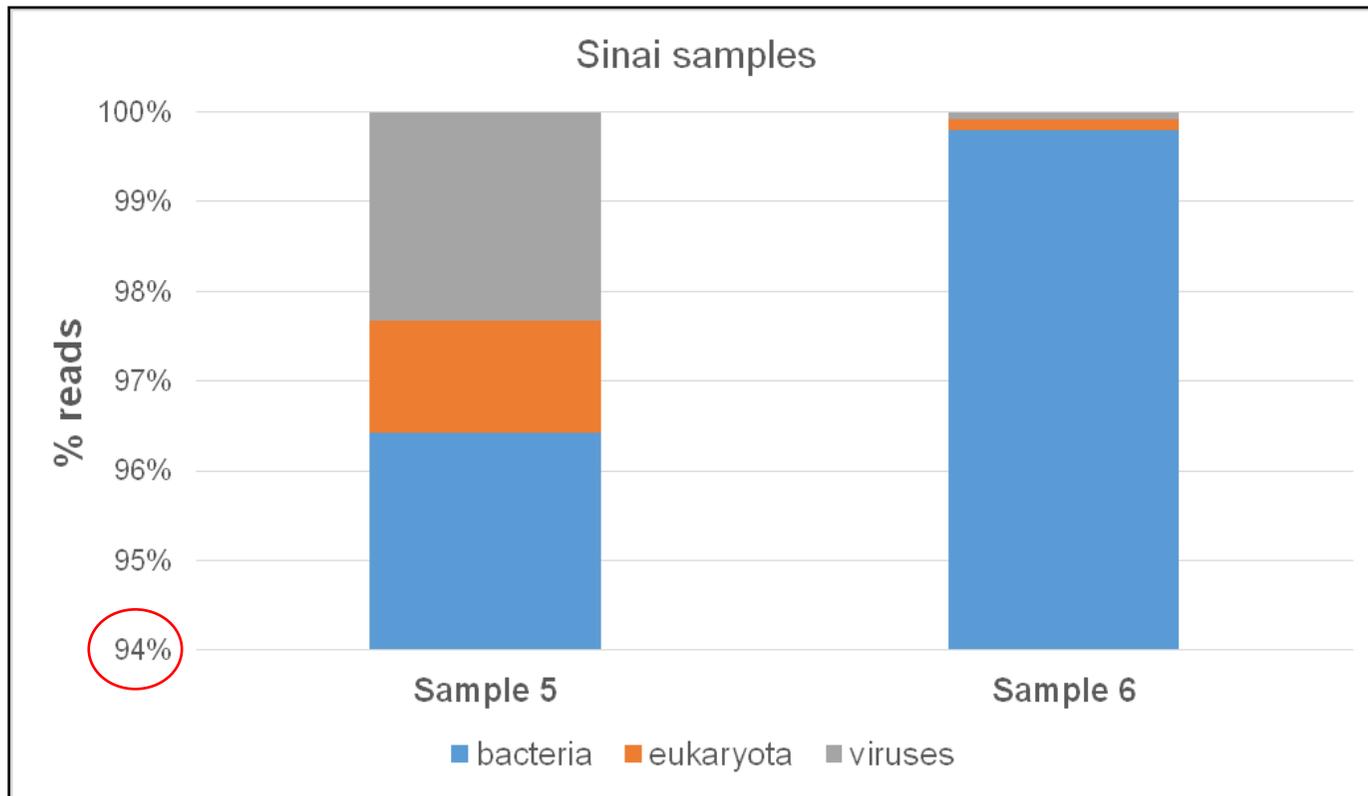
- Reads aligned against: *Homo sapiens* , *Bos taurus* , *Ovis aries* ,
Capra hircus , *Sus scrofa* 

Results:

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



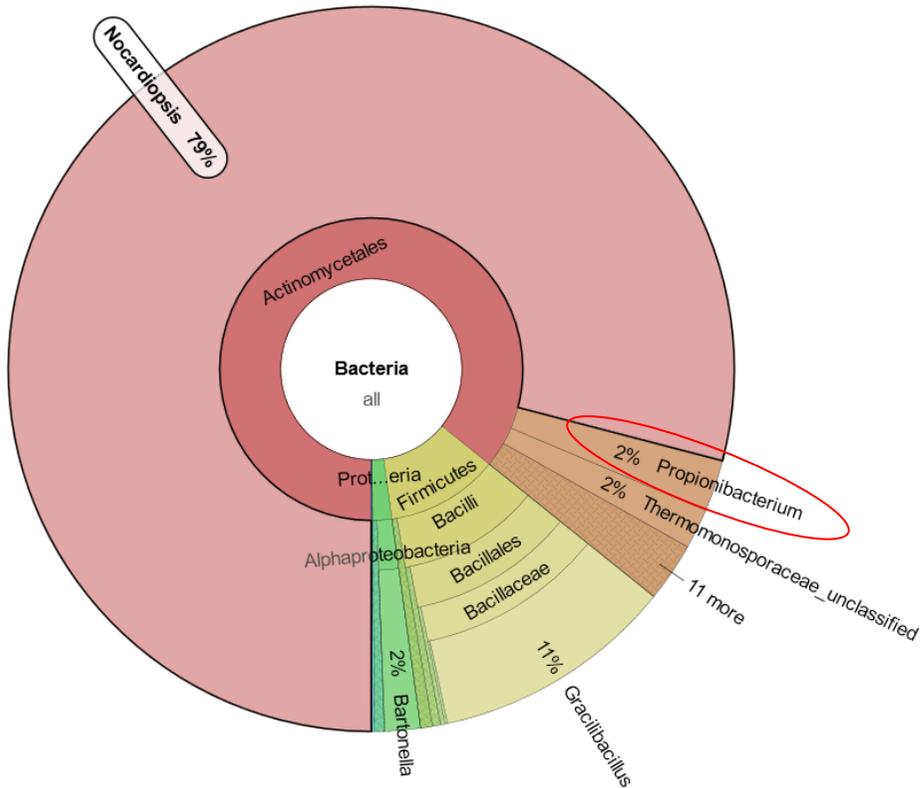
2. Microbiome colonizing the surface of the parchment



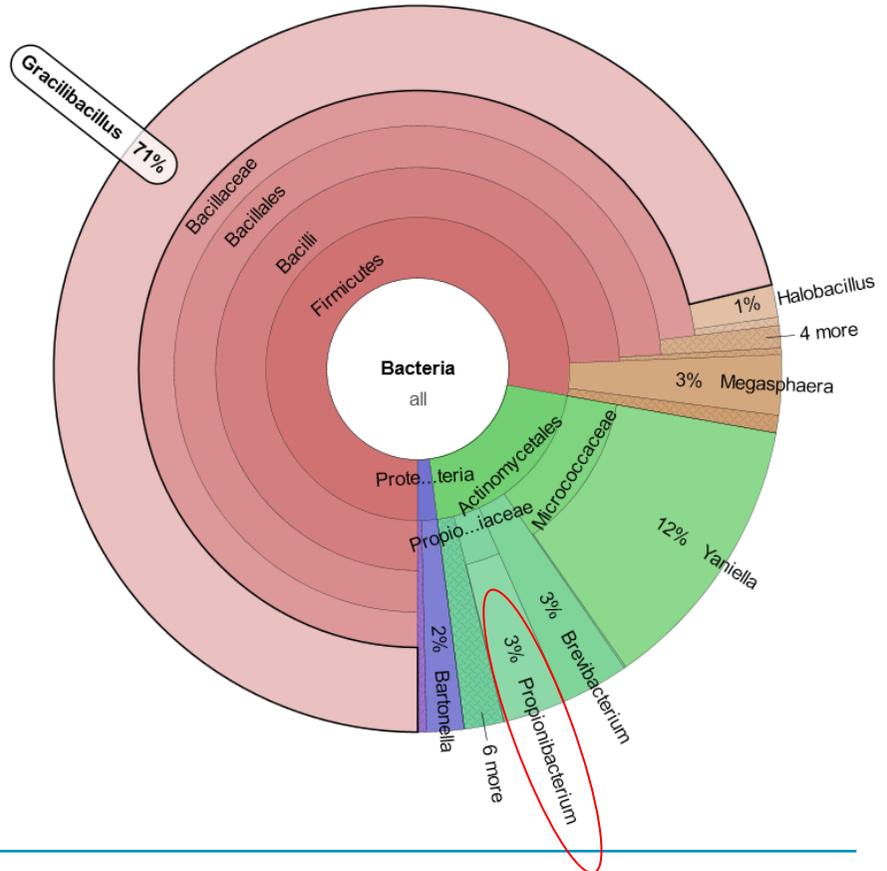
Results:

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

Sample 5



Sample 6



Results:

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

- 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)

Results:

Codex Assemanianus (Vat. Slav. 3)

1. Animal species used for the manufacture of parchment:

■ Vaticana.Slav.3P:



■ Vaticana.Slav.3Rb:



Species	Reads Vat.Slav.3P	Reads Vat.Slav.3Rb
<i>Homo sapiens</i>	7852951	143268
<i>Ovis aries</i> (sheep)	55146	24465

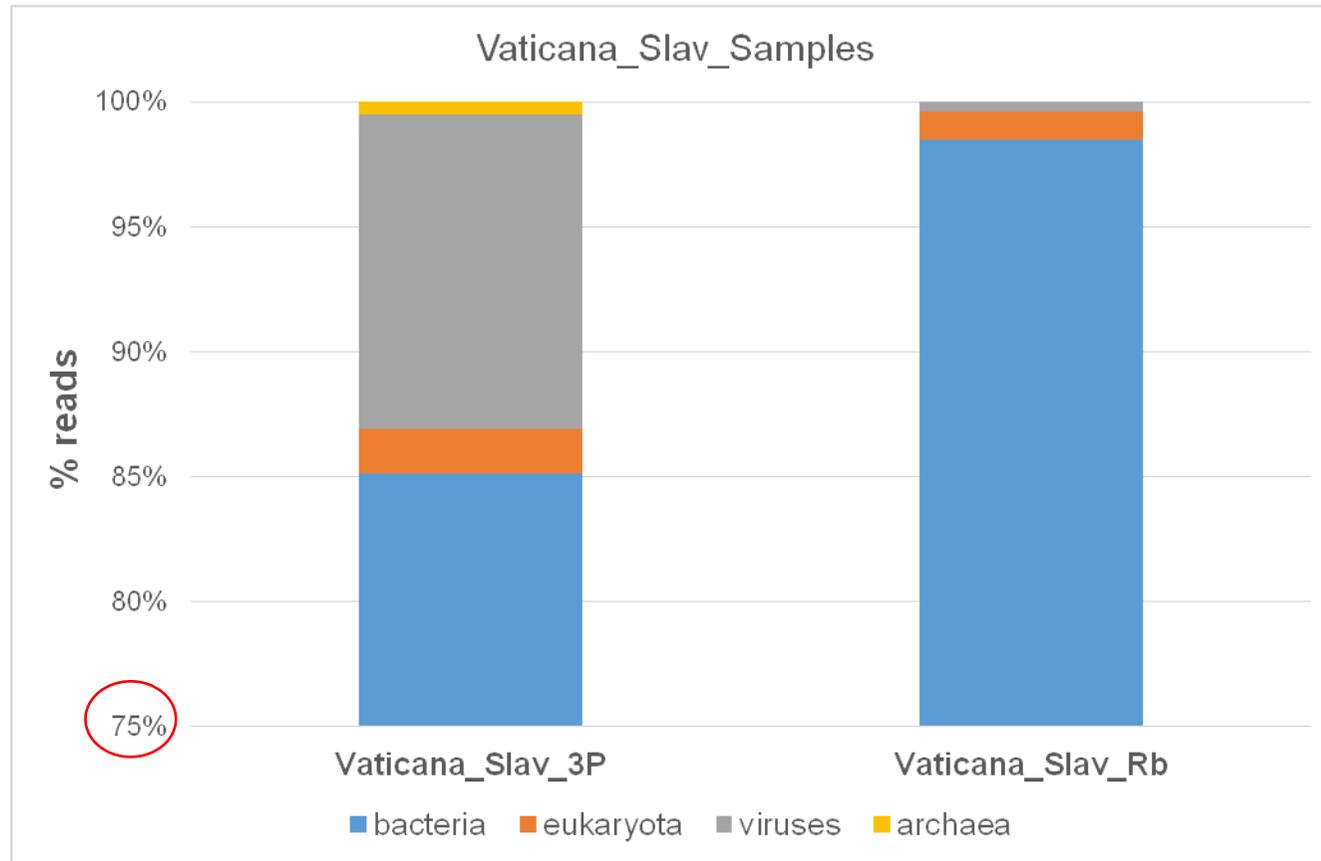
- Reads aligned against: *Homo sapiens* , *Bos taurus* , *Ovis aries* ,
Capra hircus , *Sus scrofa* 

Results:

Codex Assemanianus (Vat. Slav. 3)

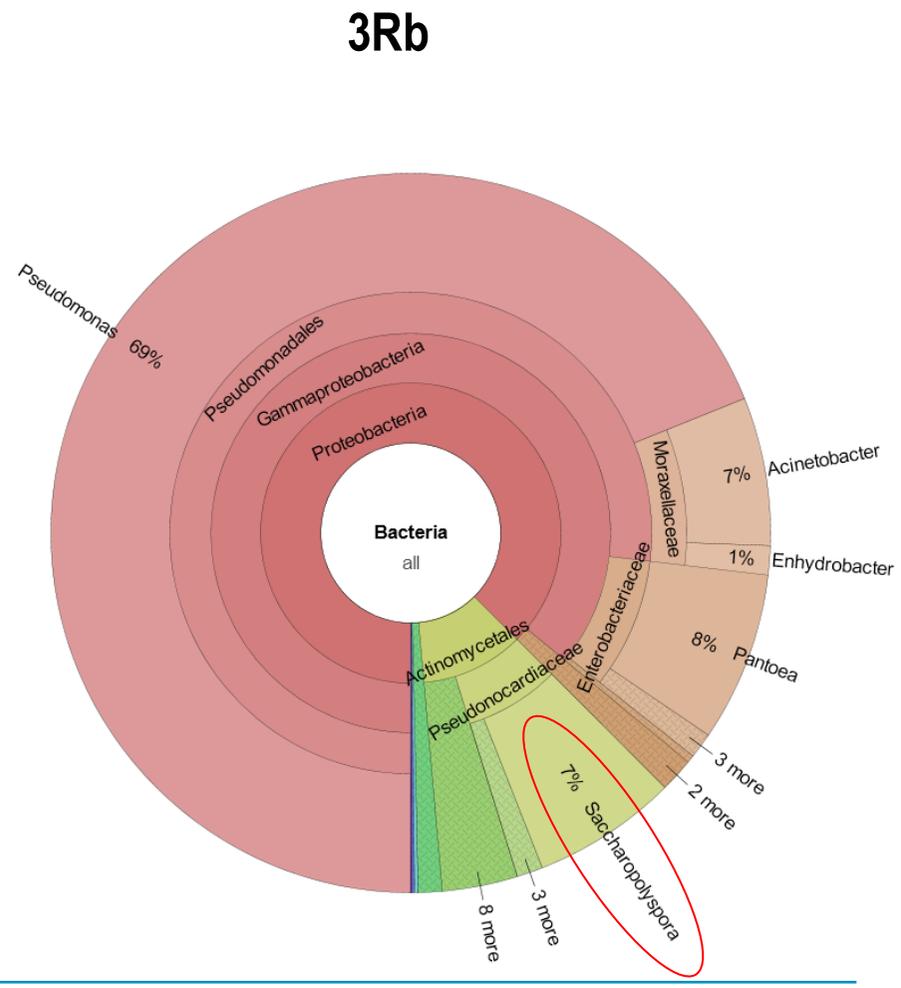
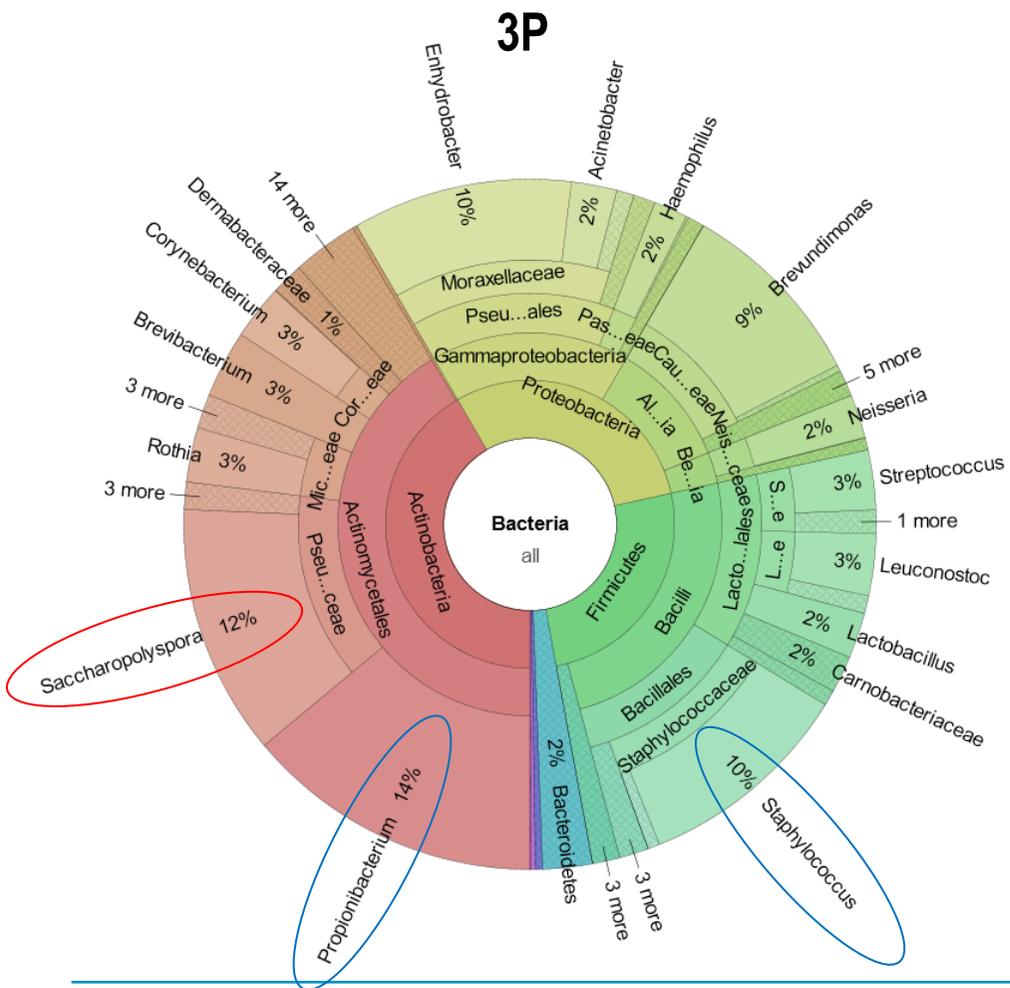


- Microbiome colonizing the surface of the parchment



Results:

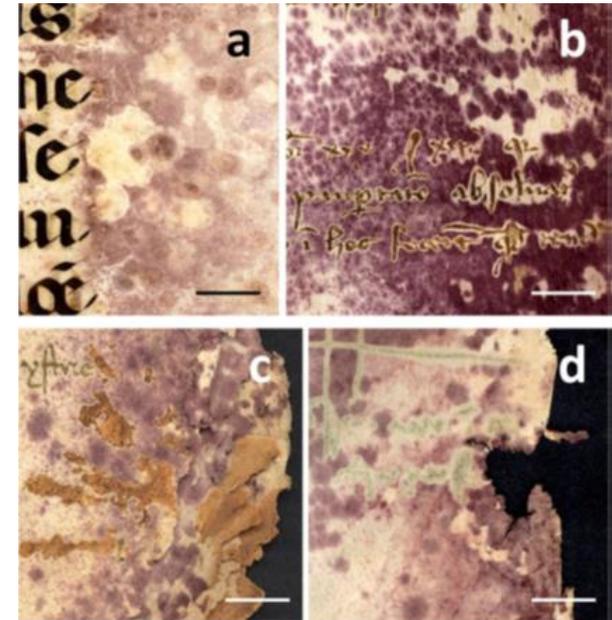
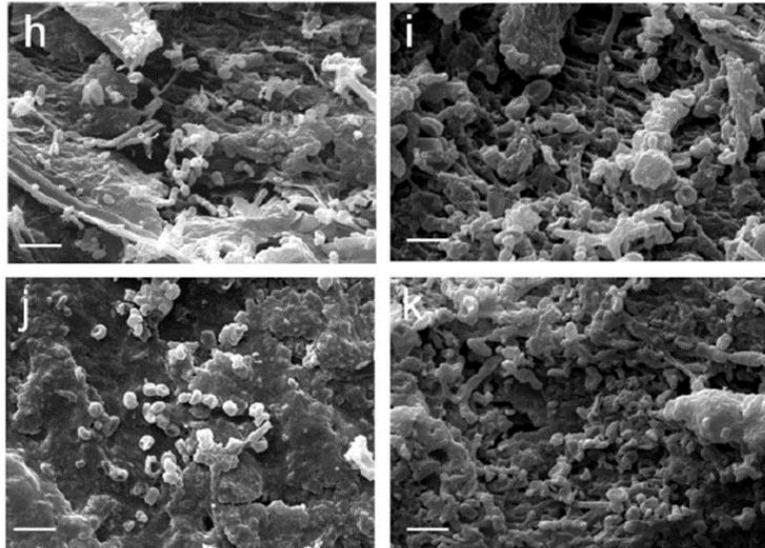
Codex Assemanianus (Vat. Slav. 3)



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.



- DNA analyses can help the study of ancient manuscripts by adding valuable biological information: an “**individual biological pedigree**”
- DNA analyses enable to infer the **animal species** used for the manufacture of parchment and, in some cases, the vegetable **origin of some inks**
- The **microbiome** colonizing the surface of the parchments correlates with the salty environment provided by the parchment samples, which selects **halophilic (salt-loving)** microorganisms.
- In particular, ***Saccharopolyspora*** species has been shown to be common denominator on most of parchment samples examined by molecular methods to date.
- It is worth noting the presence of microorganisms from the **human skin microbiome**, most probably related with the intensive handling of the manuscripts that may be **potential pathogens**.

Thank you for your attention!

