New Trends and Methodologies in Cultural Heritage Studies: *Leonardos Codice del volo*

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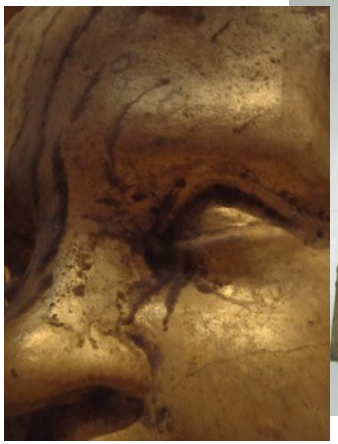
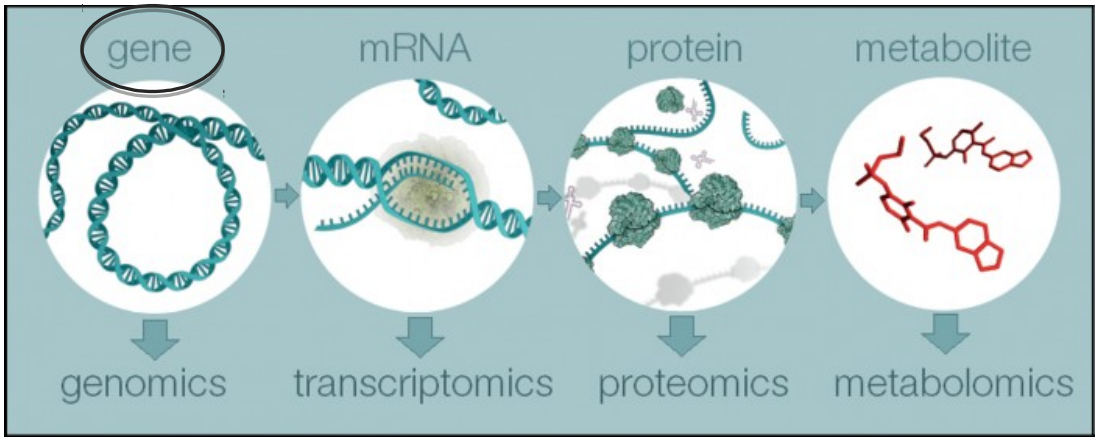
Deterioration of materials by fungi and bacteria

- Aesthetical spoiling
- Acid corrosion
- Material loss due to degradation of organic components (paper, leather, parchment, linen)
- Mechanical attack



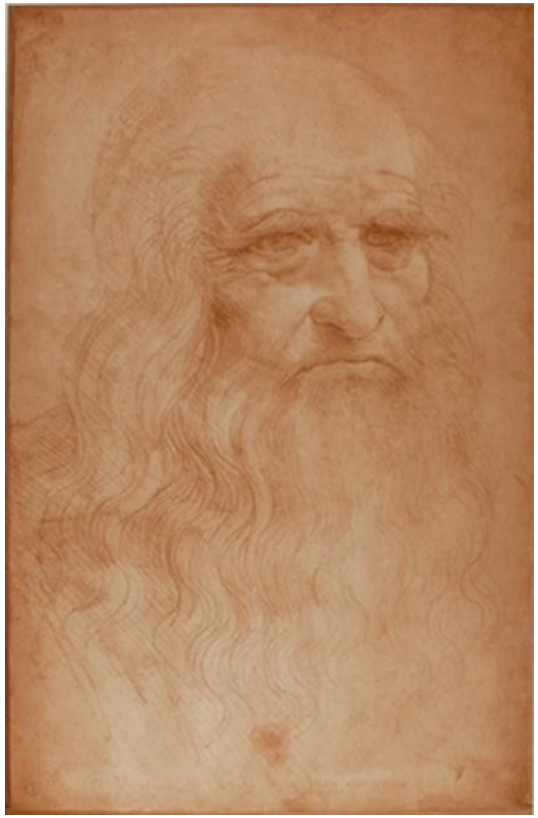
New Trends and Methodologies in Cultural Heritage Studies

New methods have evolved, enabling the study of microbial communities from their DNA, RNA, proteins and metabolites ("omics"-analyses).

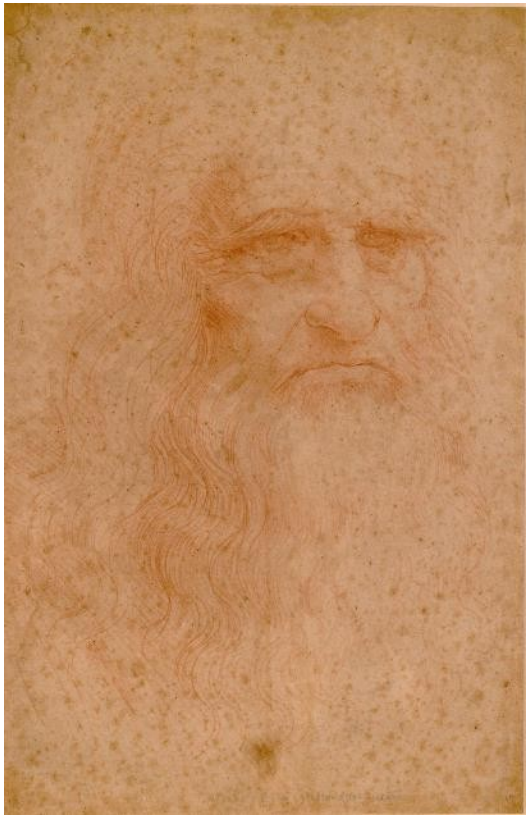


Molecular techniques have a big potential in the field of cultural heritage!

Self-portrait of Leonardo da Vinci_ red chalk Royal Library in Turin (since 1950)

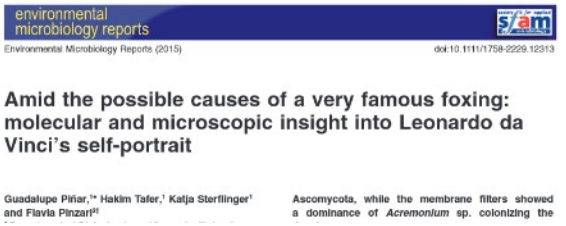


Alinari, 1898



ICPAL, now

- Damage occurred 1890-1950
- "Fox marks"_accumulation of iron salts and moisture.
- Biological analyses 1960-2000
- Current analyses:
  - SEM_spots eroded with loss of cellulose on the surface and *Aspergillus*-like conidia
  - Sampling: swabs, membranes for cultivation and molecular analyses



environmental microbiology reports

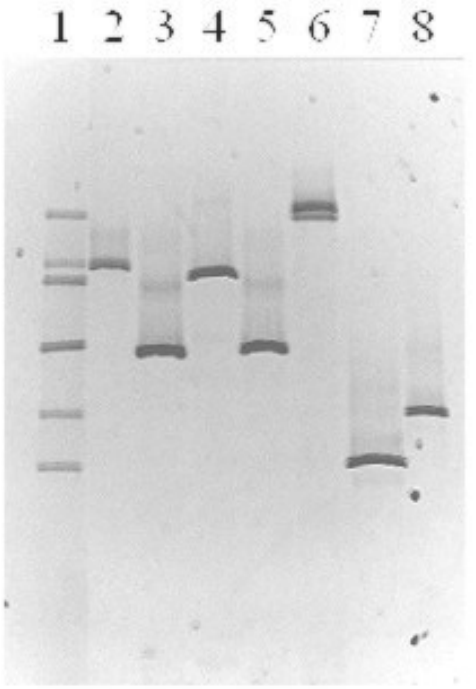
Environmental Microbiology Reports (2015) doi:10.1111/1758-2229.12313

Amid the possible causes of a very famous foxing: molecular and microscopic insight into Leonardo da Vinci's self-portrait

Guadalupe Piñar,[1*] Hakim Tafer,[1] Katja Sterflinger[1] and Flavia Pinzari[2]

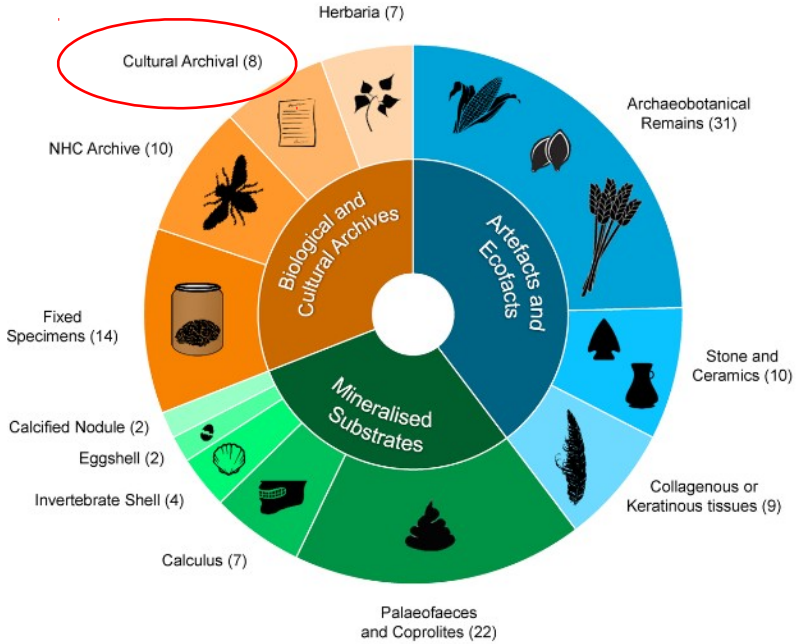
Ascomycota, while the membrane filters showed a dominance of *Acremonium* sp. colonizing the

Non-destructive sampling of cells and DNA



Lane 1: mixture of seven fungal strains; lane 2: *Chaetomium globo*sum; lane 3: *A. hollandic*us; lane 4: *Cladosporium cladosporioides*; lane 5: *E. chevalieri*; lane 6: *Alternata alterna*ria; lane 7: *A. versicolor*; lane 8: *A. terreus*.

Metagenomics can help to reconstruct the history of the investigated objects!



Every step in the history of an object leaves its own genetic fingerprint represented by DNA, a specific **"biological pedigree"** that allows us to get an idea of everything that it has been through during its history.

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

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A GENETIC TIME TRAVEL WITH YOUR ART

The biological information contained in our cultural heritage brings added value to a specific artefact and helps answering crucial questions that may arise when dealing with valuable objects. We offer a biological pedigree certificate for objects of art which enables:

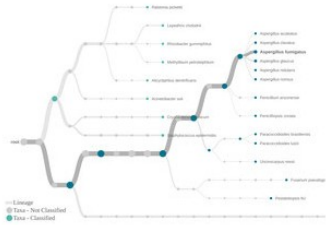
- **Certification of authenticity of valuable artefacts based on the microbial pedigree.**
- **Identification of the biological material and microbial species of artefacts**
- **Insights into the geographical origin of artefact.**
- **Inference of the travel history and the storage conditions.**
- **Grouping of objects of unknown origin based on their microbial pedigrees.**
- **Deciphering the usage history of the object.**

These state-of-the-art analyses provide new and valuable insights into the history of artifacts in the hands of art collectors, curators, auction houses, criminologists, archaeologists, art historians, restorers, codicologists and philologists.

ABOUT US

Science is team work...

more information



OUR METHODS

Molecular, non-destructive, state-of-the-art...

more information

REFERENCES

Highest standards, highest confidence...

more information

Methods

- Non-destructive sampling of dust and debris with swabs, membranes or needles.
- Nanopore identifies DNA bases by measuring the changes in electrical conductivity generated as DNA strands pass through a biological nanopore.
- Bioinformatic analysis and interpretation of data, report.

Feasibility study using the Nanopore sequencing technology

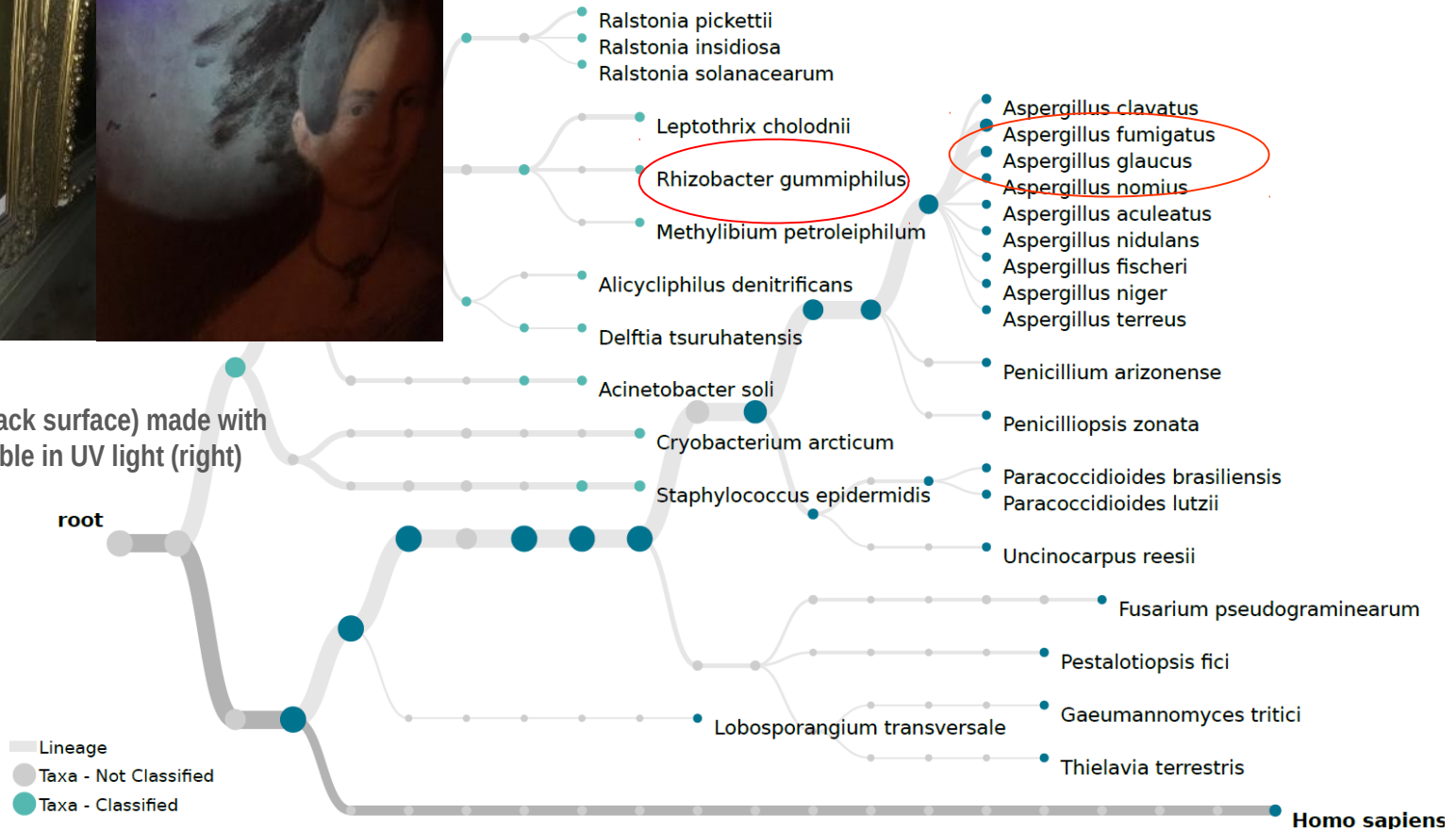
The main purpose was to develop a protocol **easy** for the operator to use, **fast** and **relatively low cost**. Following steps were evaluated independently:

- Feasibility of
  for further dire
- Suitability of
  genome amp
  libraries for use with Nanopore devices.
- Evaluation of the **complexity of the Nanopore sequencing** technology – by monitoring run parameters in real time
- Evaluation of **results** and creation of a "**microbial pedigree**" for each single sample, as a reference dataset for current and future comparisons.

The technology is feasible for the biological diagnosis of cultural heritage objects!

Test sample: “Microbial pedigree” of an oil painting with active fungal infection

Retouching areas (black surface) made with Arabic gum, only visible in UV light (right)



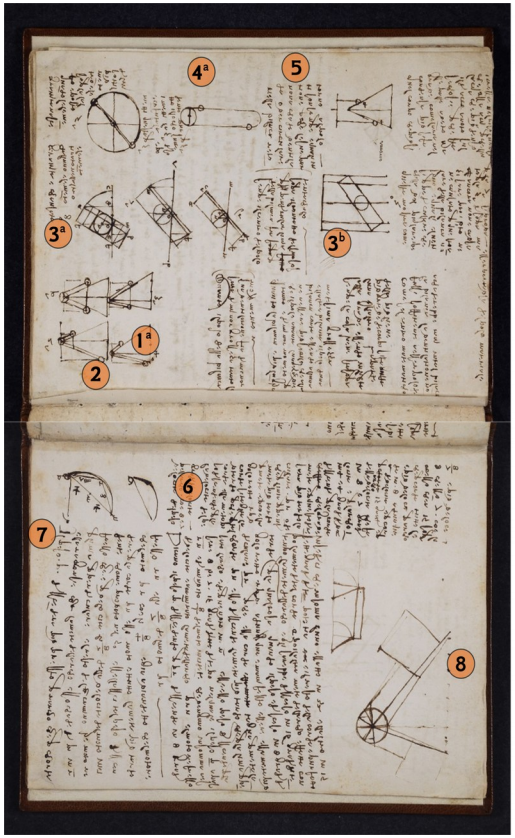
Codice del volo

Samples were taken from three pages of the Flight Code. Of these, two are part of those detached (pages 1 and 17) and reinserted at different times and one (page 13) that has always been part of the original manuscript.

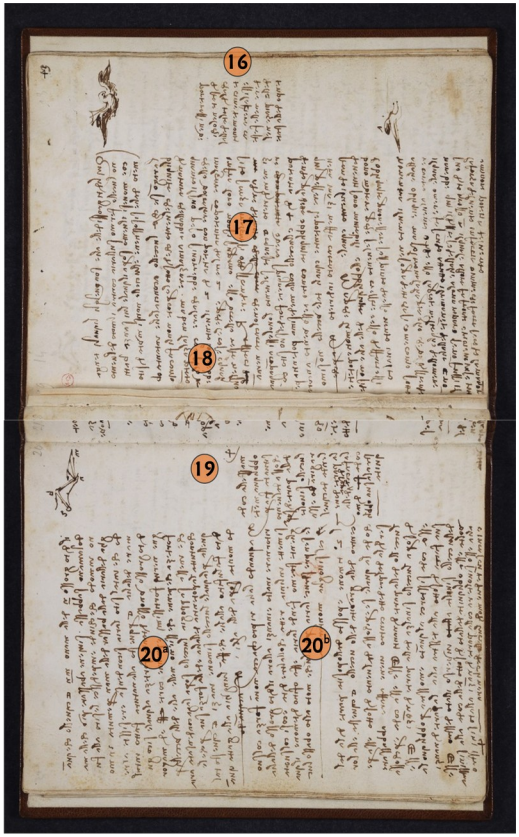
The sampling operations have been carried out both on the front and on the back of the sheets after observation of the areas under the stereomicroscope trying to understand altered and more intact areas according to the chosen numbering.

The samples were taken using sterile swabs of the MWE DRYSWAB type with different points.

PAGE 1



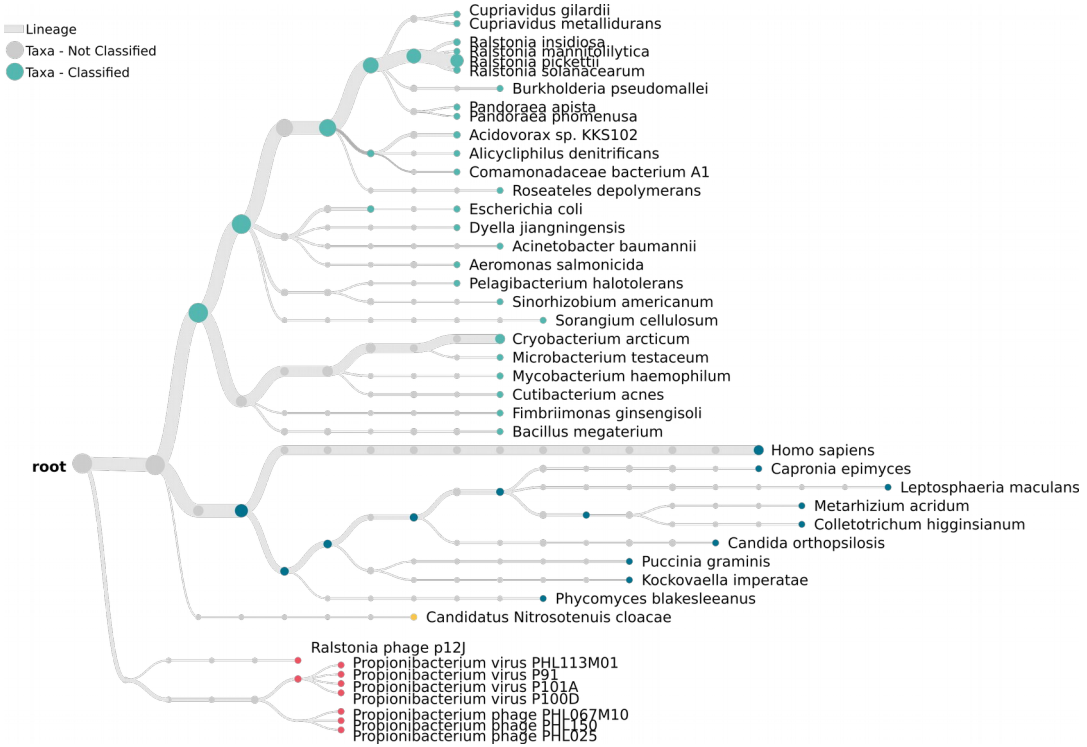
PAGE 13

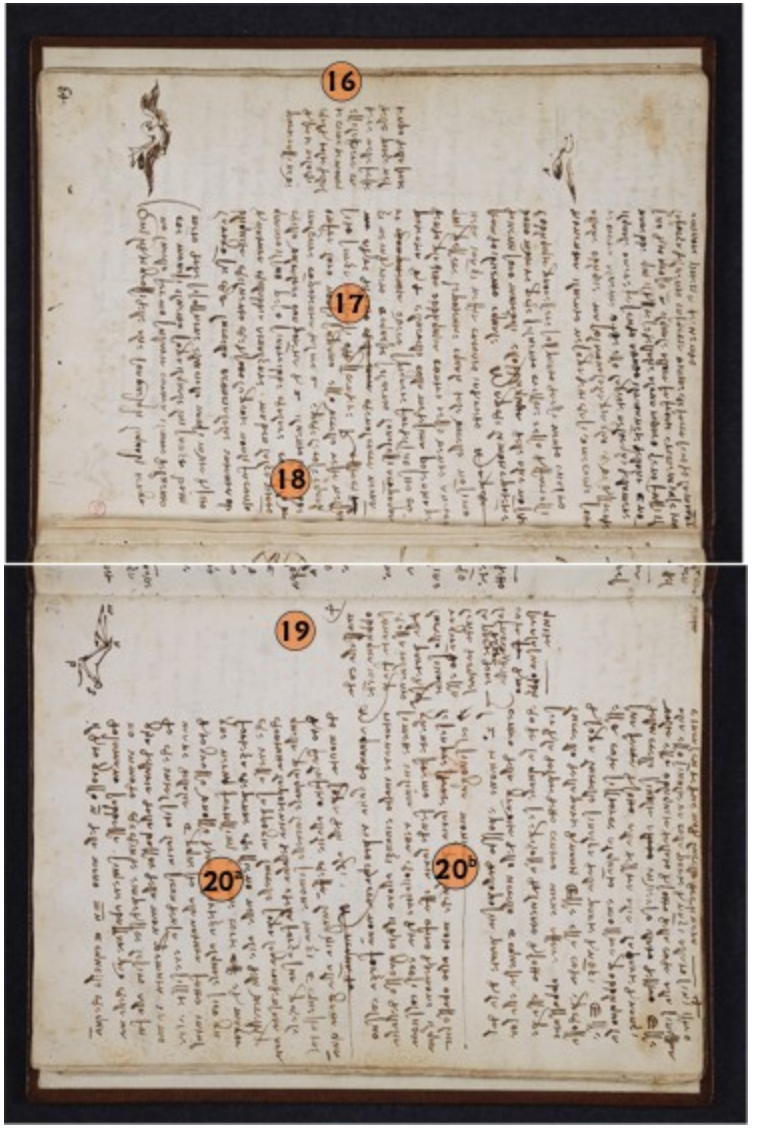


Codice del volo:page 13

Page 13 has always been part of the original manuscript.

16	pag 13 recto taglio esterno
17	pag 13 recto zona centro
18	pag 13 recto particelle
19	pag 13 verso interno pagina
20a	pag 13 verso centro
20b	pag 13 verso centro





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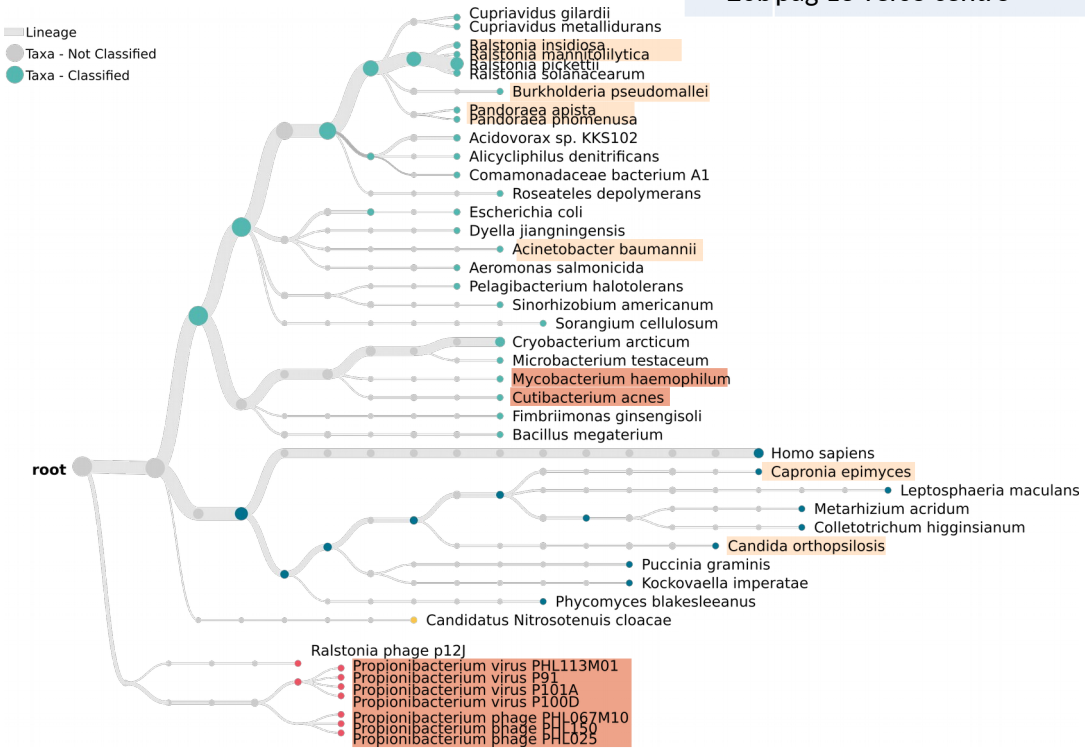

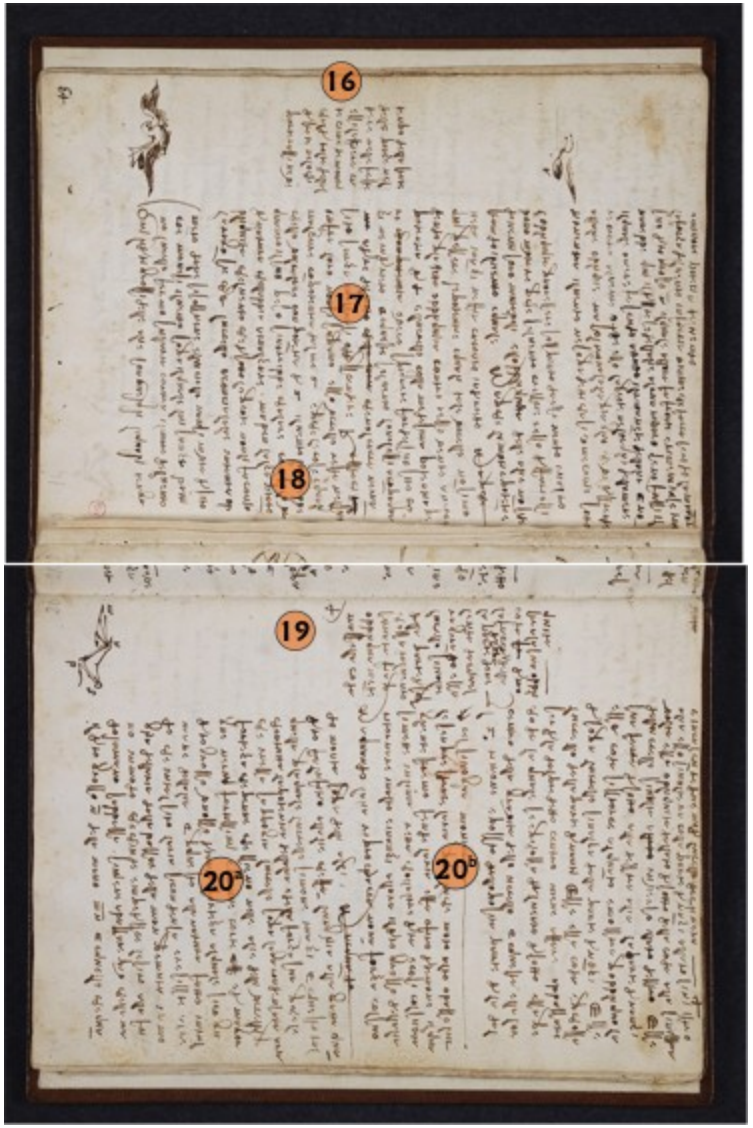

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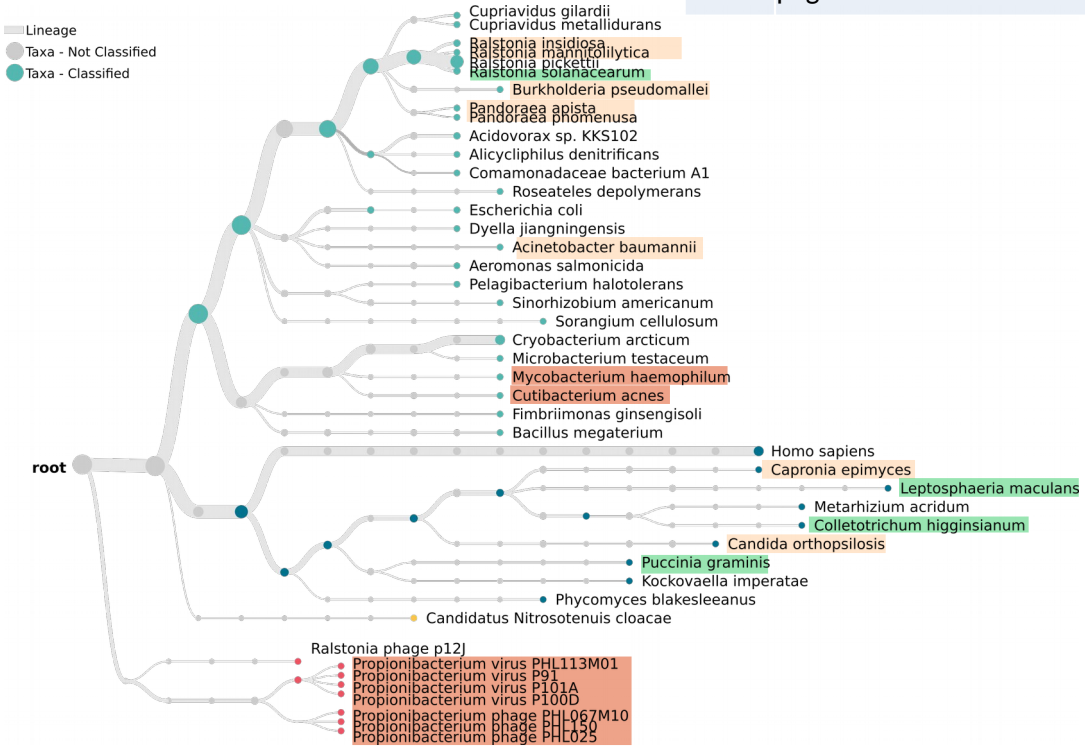

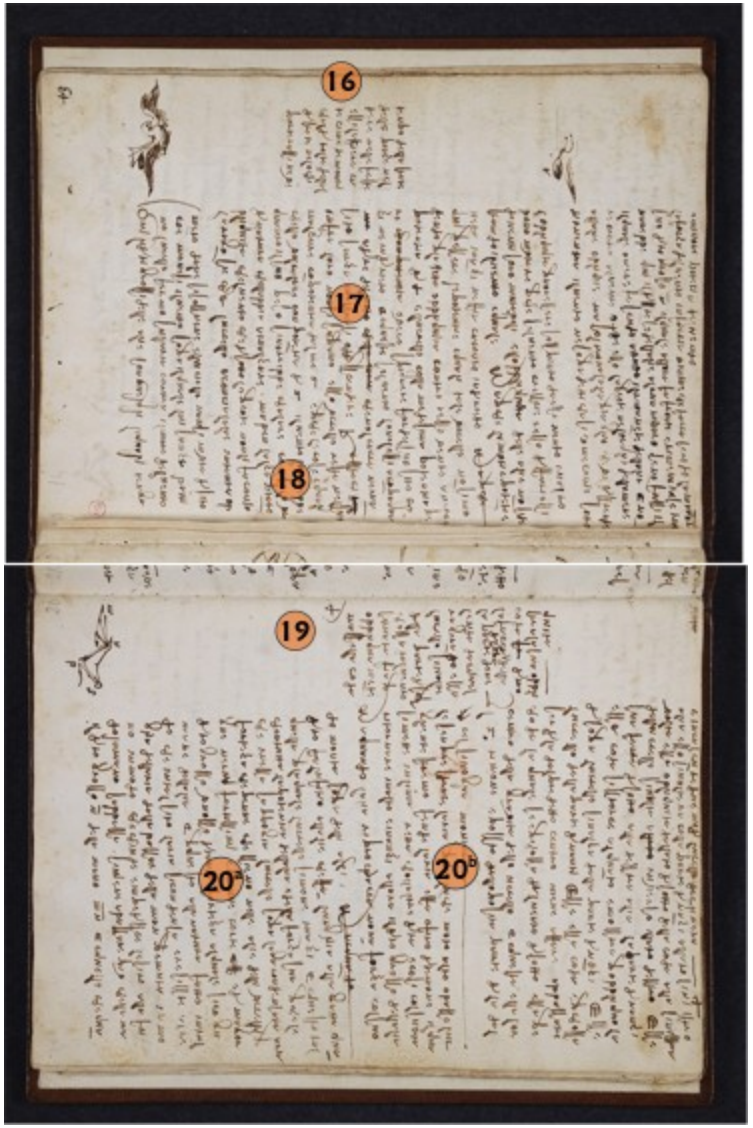

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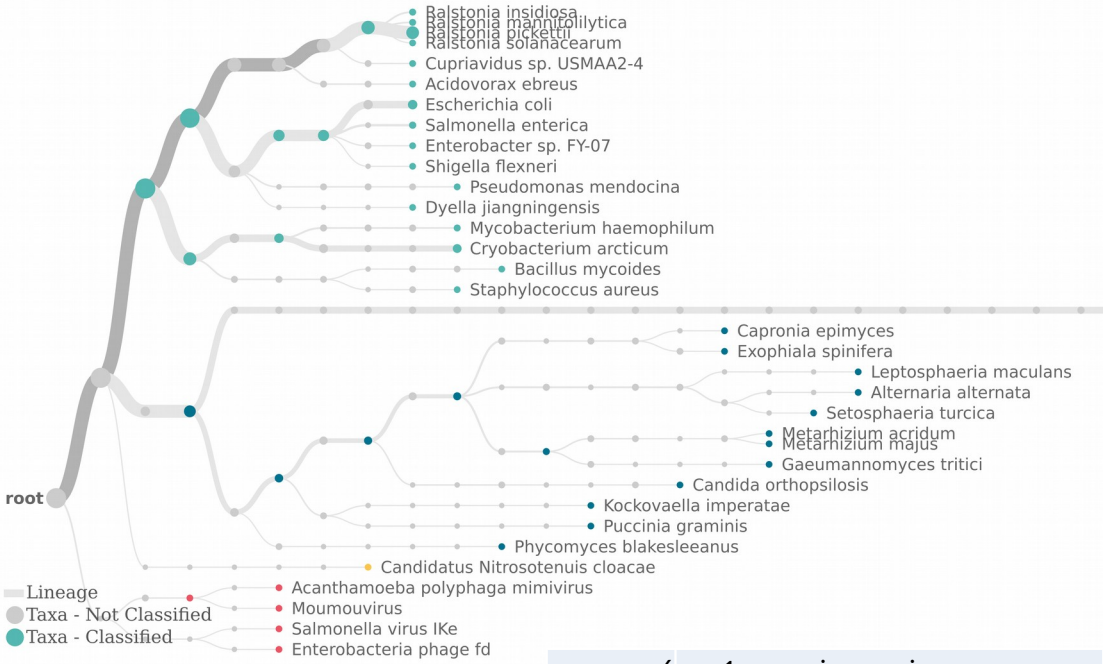
3a/b	pag 1 recto superficie
4a	pag 1 recto macchia centrale
5	pag 1 recto escremento



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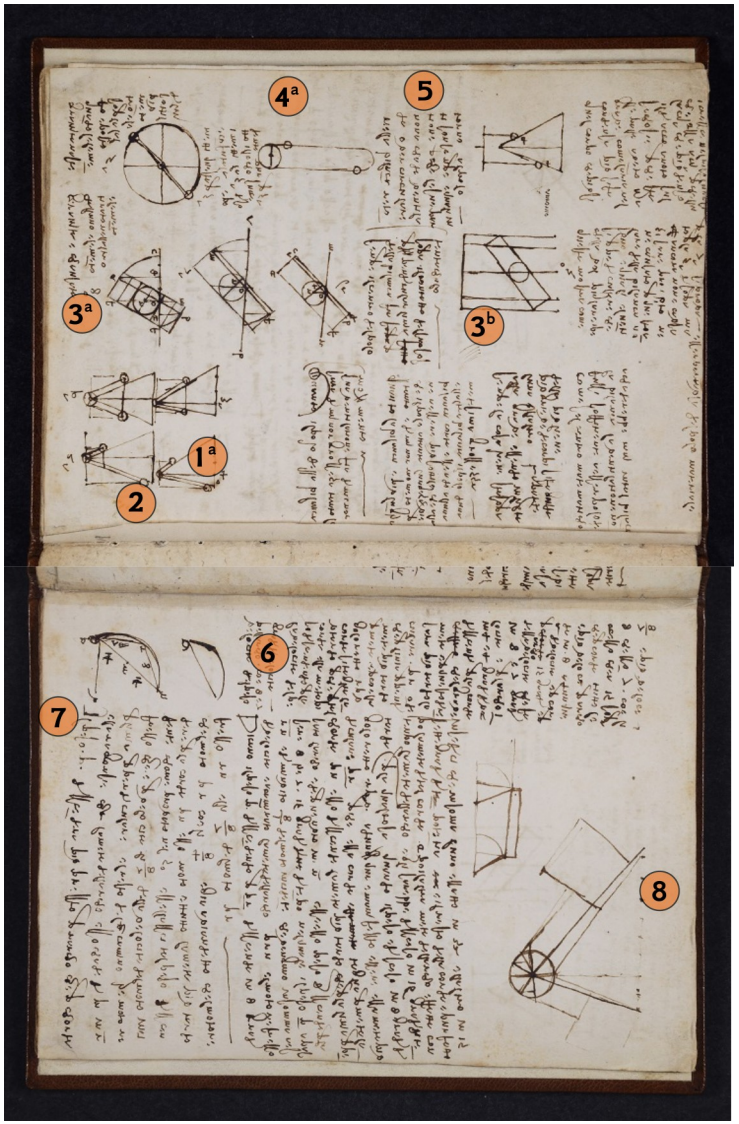

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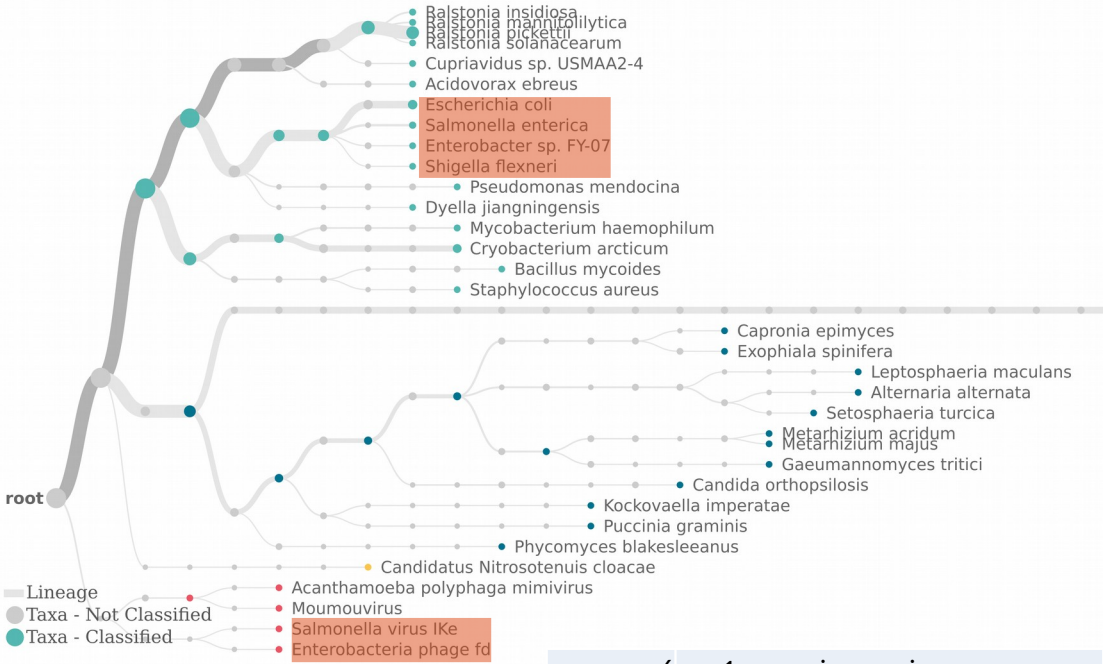

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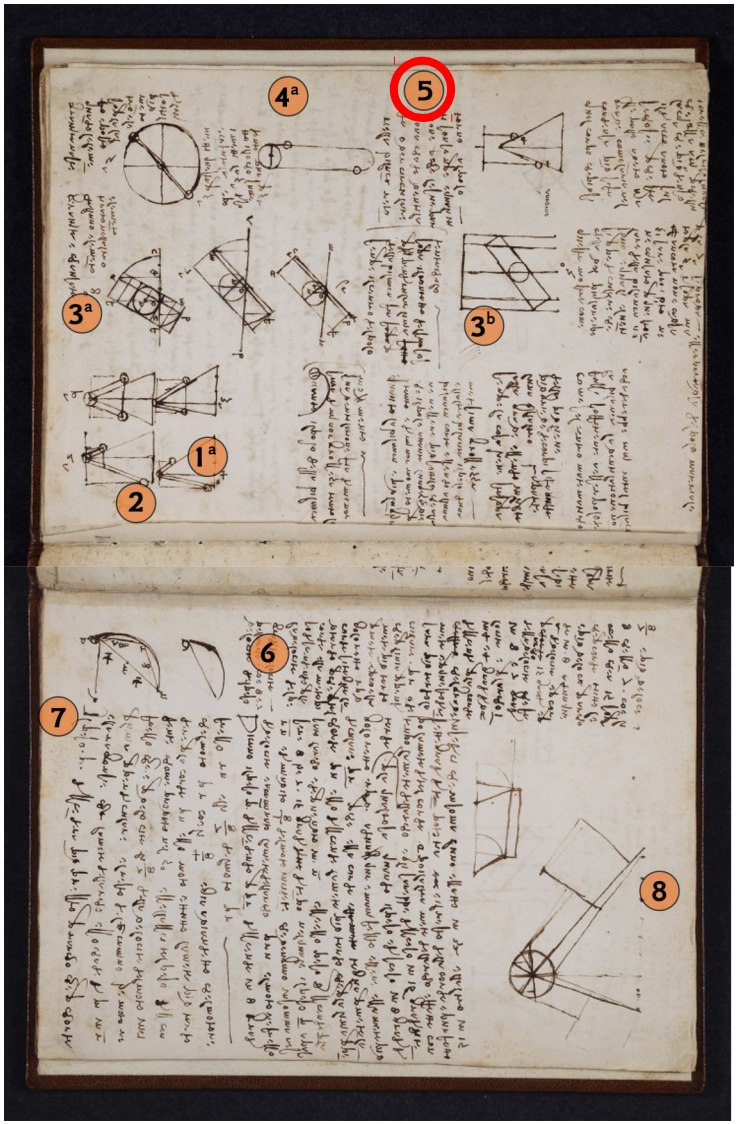

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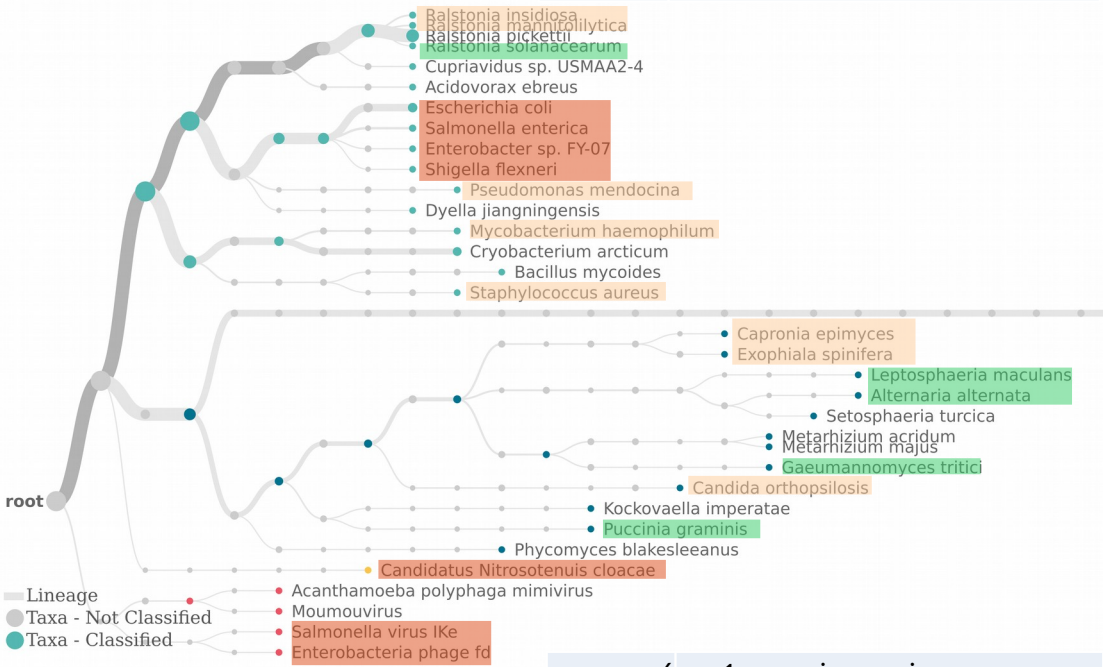

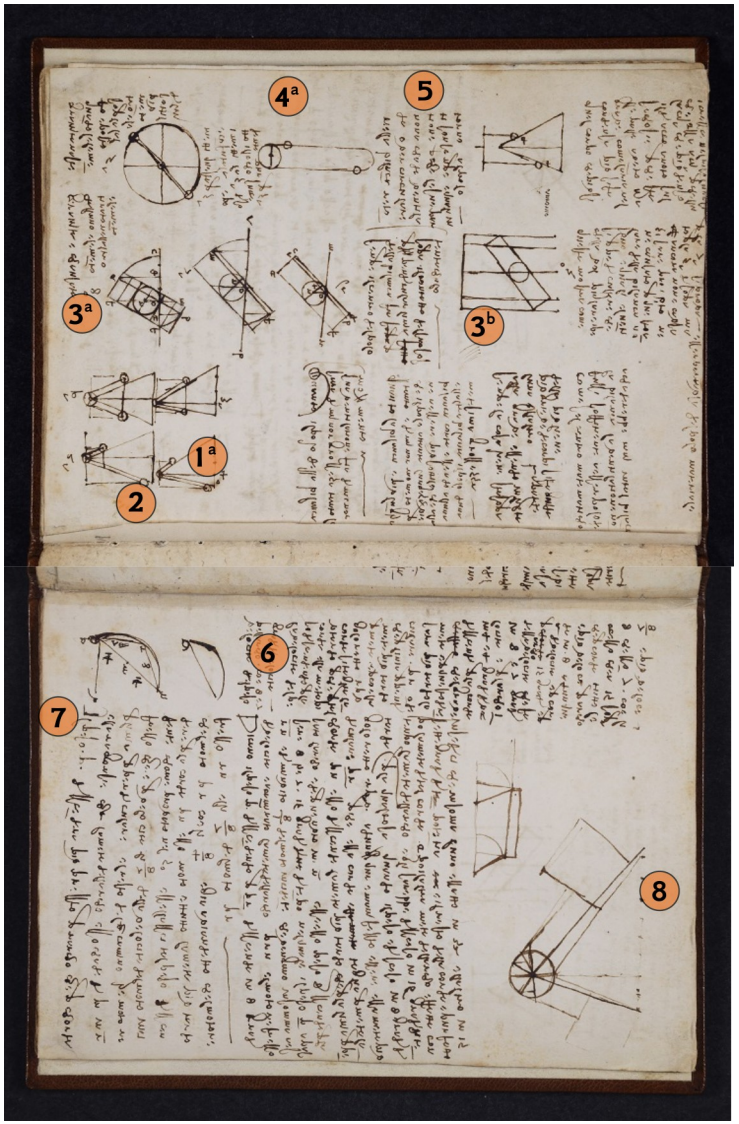

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ABOUT US

SCIENTIFIC EXPERIENCE IS OUR STRENGTH

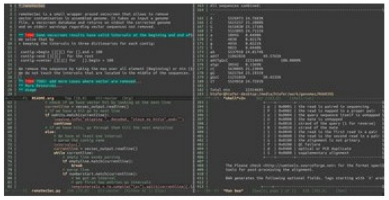
Univ. Prof. Dr. Katja Sterflinger

I have been working as geomicrobiologist in the field of cultural heritage since 1994 when I started to work on biodeterioration of antique monuments in the Sanctuary of Delos (Greece). Since that time I am absolutely facinated by both: art and biology! Today, I am a university Professor at the University of Natural Resources and Life Sciences Vienna and a consultant for the Federal Monuments Office in Austria and the Federal Office of Culture in Switzerland. I am teaching microbiology for restorers at the University of Fine Arts and the University of Applied Arts in Vienna.

You may also visit me at www.sterflinger.at

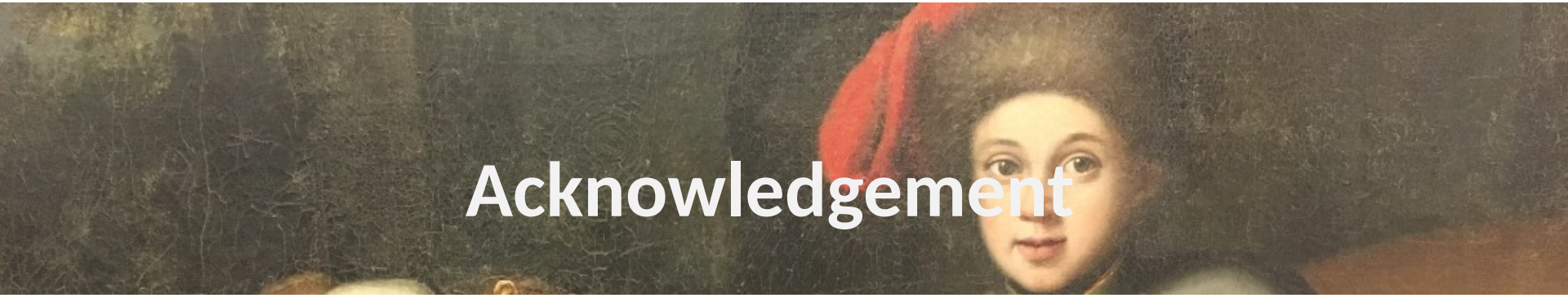
Dr. Guadalupe Pinar

After completing my PhD in Biology in Granada (Spain), I have been working as a senior scientist and teaching assistant at the University of Vienna and the University of Natural Resources and Life Sciences in Vienna. For almost 20 years, I have had the pleasure of working with art-historically valuable materials. My main activities include the development and optimization of molecular analyses to be applied on material samples from art and Cultural Heritage. I have extensive knowledge on Next Generation Sequencing technologies, including different protocols and sequencing platforms. The use of cutting-edge techniques in the field of molecular biology has enabled me to decipher the valuable information contained in the microbiomes of many incomparable objects of art.



Dr. Hakim Tafer

After my physics studies at the ETH Zurich, I started working in the field of bioinformatics in academia and in the industry (Siemens/Lyndbeck). I have more than 14 years of experience in bioinformatics and worked in fields as diverse as functional annotation of RNA molecules, comparative genomics, next generation sequencing analyses and proteomics. I am currently working as a senior scientist in the University group of Prof. Sterflinger where I am involved in genomics, transcriptomics, proteomics and metagenomics analyses of microorganisms. Together with Katja Sterflinger and Guadalupe Pinar, we are actively developing new methods to study the history of artifacts.



Acknowledgement

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