SESSION 3. MOLECULAR BIOLOGY STUDIES

IF STONES COULD SPEAK…
WHAT WOULD THEY TELL US ABOUT THEIR HISTORY?

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Session 3: Molecular biology studies

Development of high-throughput molecular techniques:

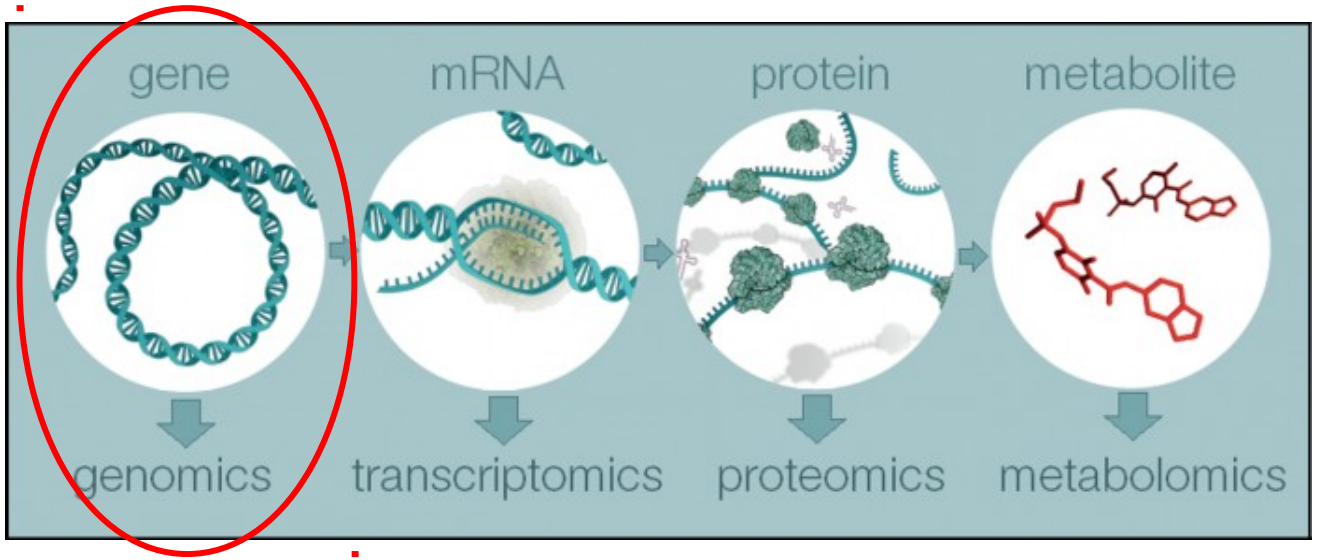
- High-throughput molecular methods are undergoing a real revolution. Nowadays, these methodologies enable to analyze even the smallest traces of DNA from ancient objects.

- The development of new working protocols and bioinformatics pipelines are making possible to assign single DNA molecules to a certain animal, plant, microorganisms or even to humans.

- The variety of strategies, methodologies and sequencing platforms available are reflecting the wide range of possibilities that scientists have today to choose the best technique to better suit the purpose of the study.

New Trends in molecular biology applied to Cultural Heritage Studies

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



What is the potential and what are the challenges of new molecular techniques in the field of cultural heritage?

Metagenomics studies performed in Cultural Heritage to date

Purpose:

- To asses the conservation state of an object.
- To estimate the biodeterioration risk of an object in the future.
- To identify the causative agents related with a specific biodeterioration phenomenon.
- For monitoring conservation/restoration treatments.

Are there any other interesting questions?

Potential of metagenomics analyses in Cultural Heritage studies

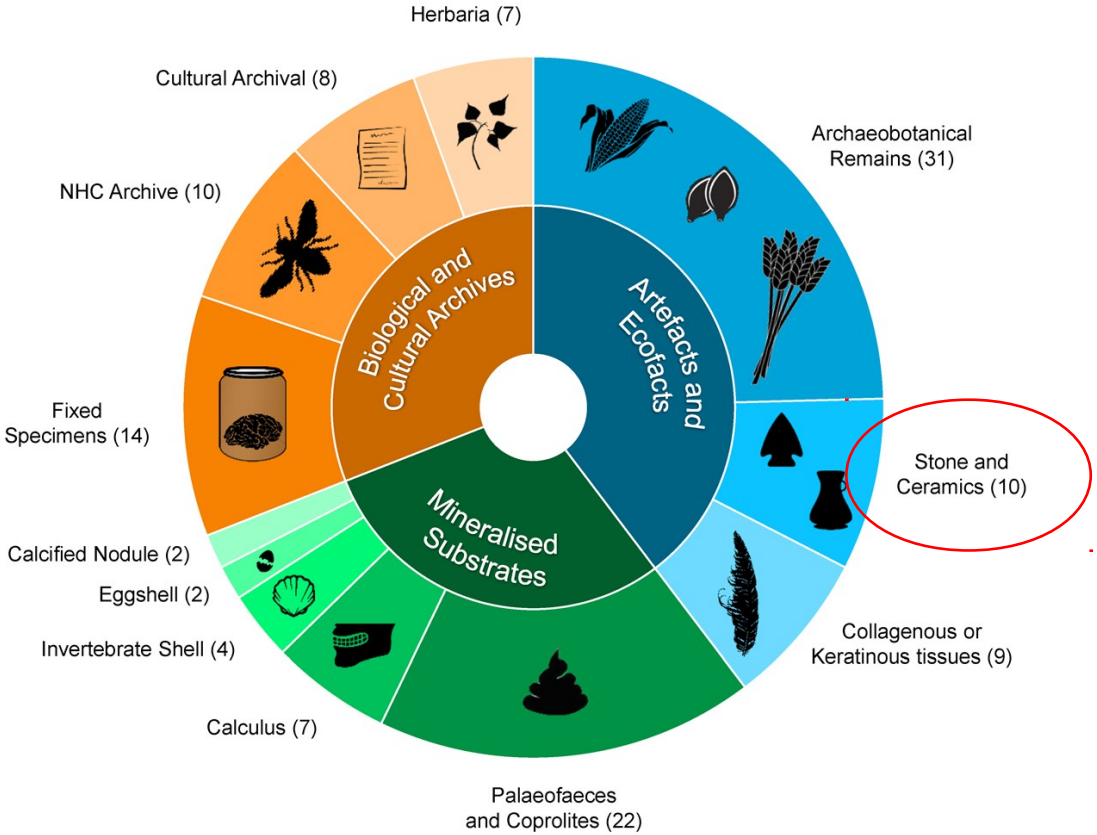
Data generated by Metagenomics studies may answer other interesting questions:

- The composition of materials (as animal skin in parchments…).
- The selection of materials at the time of manufacturing.
- The history of use of the objects.
- To provide amazing insights into the geographic origins and possible migrations of historical artefacts (geolocation).
- To elucidate the storage conditions…etc.

These answers can be useful for reconstructing the history of the investigated objects!

Important in other fields, as archaeology, history, philology, and last but not least, criminology

Can metagenomic studies help to reconstruct the history of the investigated objects?

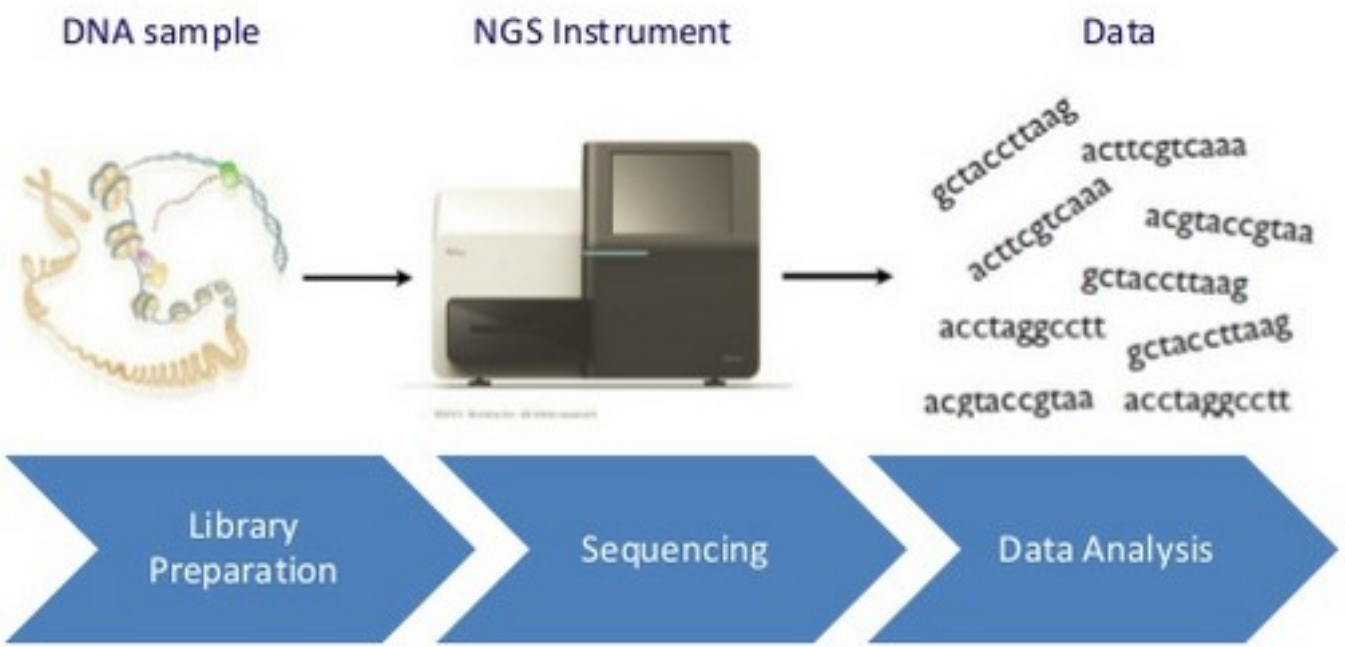


E. J. Green and C. F. Speller. 2017. Genes 8(7), 180;
Novel Substrates as Sources of Ancient DNA

Yes!

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.

NGS-workflow for metagenomics



Sampling
DNA extraction
Down-steps:
Bias in target amplification

Sequencing-platform

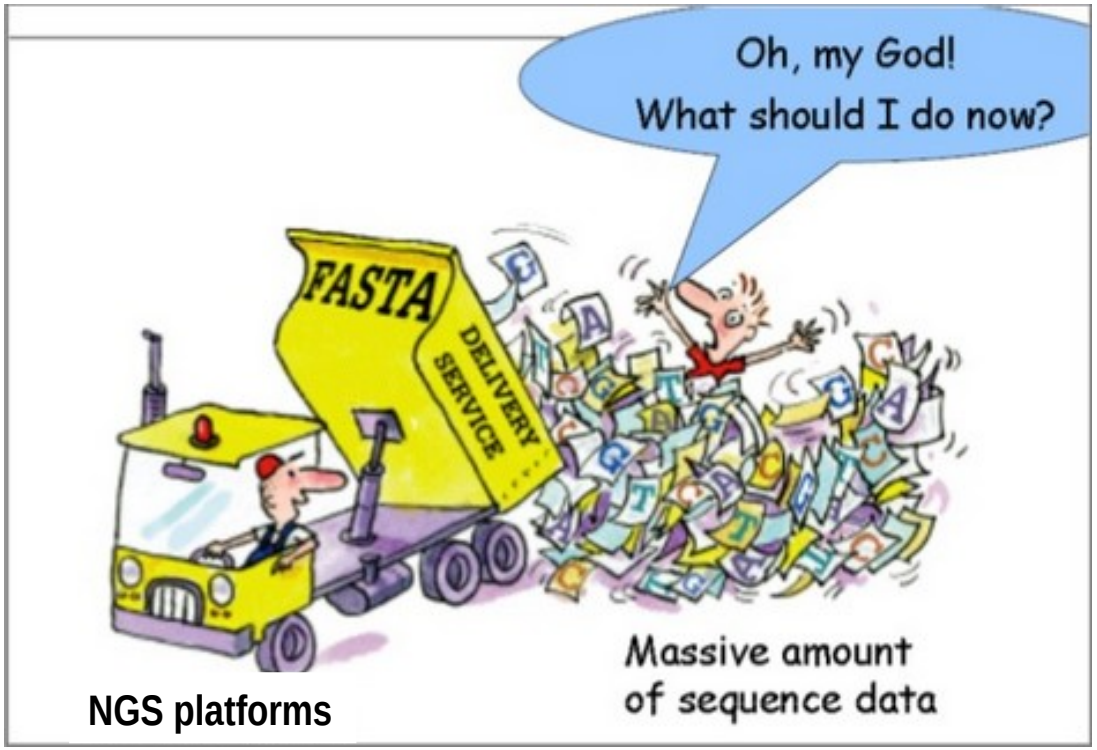
Big data

What are the challenges in this workflow ?

Challenges in data analysis: Big Data!

NGS challenges:

- Bioinformatics pipelines
- Available sequences in databases
- Storing of data
- Interpretation of results!



Case-study: “CSI Vienna“

- Custom officers found three possibly smuggled marble statues of an unknown origin, two of them representing human torsos, and one representing a small young girl head, at a flea market in Vienna. The seller said he had found them under the rubble of an old building.

- The statues were taken to the Museum of Art History in Vienna and analyzed by experts of the museum

- Sampling and molecular analyses were performed by our team (BOKU) in order to answer some intriguing questions…

Questions posed in this case-study:

1. Is it possible to reconstruct the history of the storage of each single object?

1. Were the statues stored individually or together?

1. Is it possible to elucidate the geographical shift of some of the objects?

Workflow to answer these questions:

Metagenomics

1. Sampling (non-invasive sampling)
2. Molecular methods (to analyze even smallest traces of DNA)

- DNA extraction (Quality/quantity of DNA)
- Target amplification
  - 16S rDNA: Ion 16S Metagenomic kit (Two primers sets: primer set V2-4-8 and primer set V3-6, 7-9)
  - 18S rDNA of Eukaryotes: V4 region (Primers 528f / 706r)
  - ITS regions of fungi: ITS1 region (Primers ITS1f /I TS2r)
- Down-steps:
  1. DNA library preparation (PGM-Ion Torrent)
  2. Next Generation Sequencing (NGS) technologies (Ion Torrent seq. platform)

The three smuggled statues

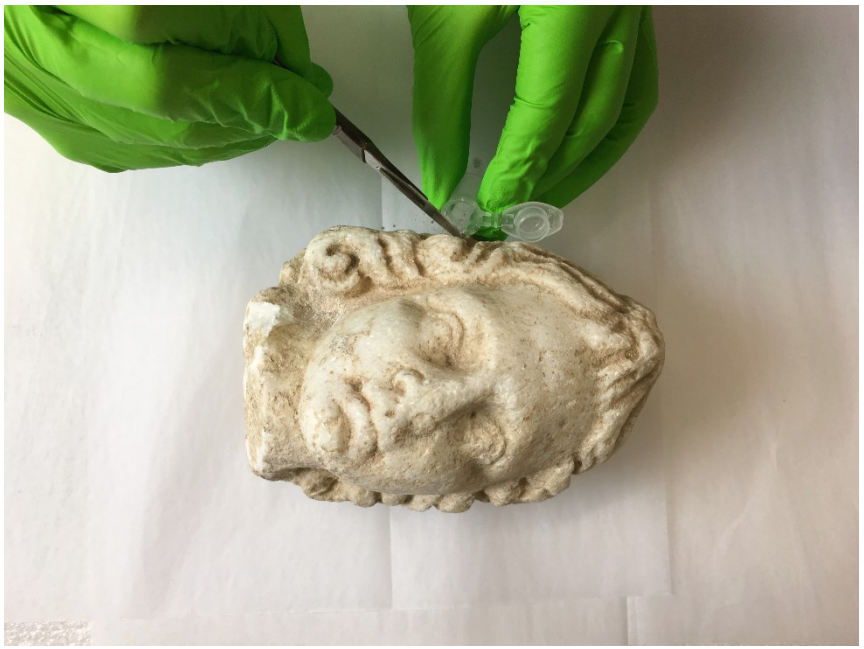
S1

S2

S3

Sampling procedure

Non-invasive sampling



Ion Torrent sequencing platform

Extremophile Center (BOKU):
Ion Torrent Sequencing Platform

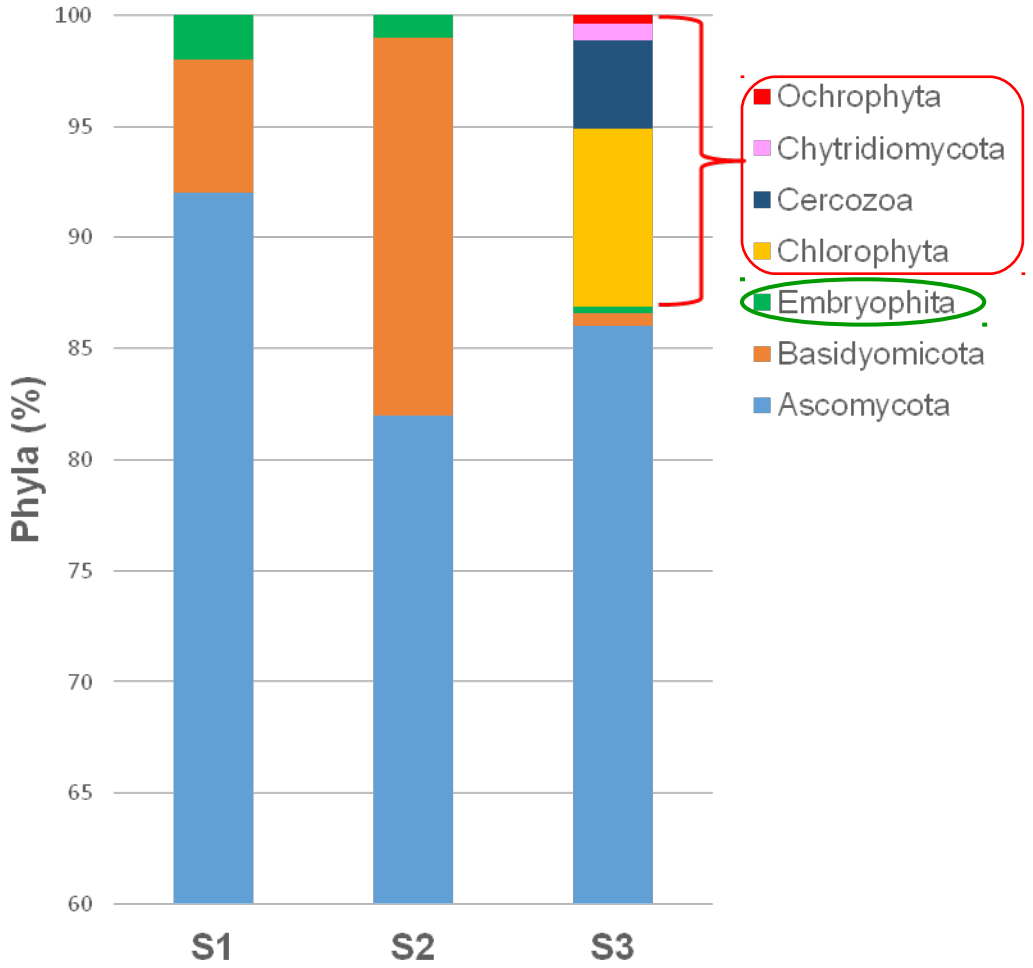
ION Chef™ System

ION Proton™ Bench Top

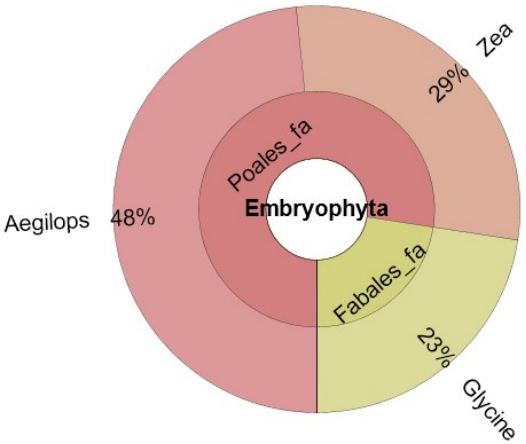
ION PGM™ System

Results

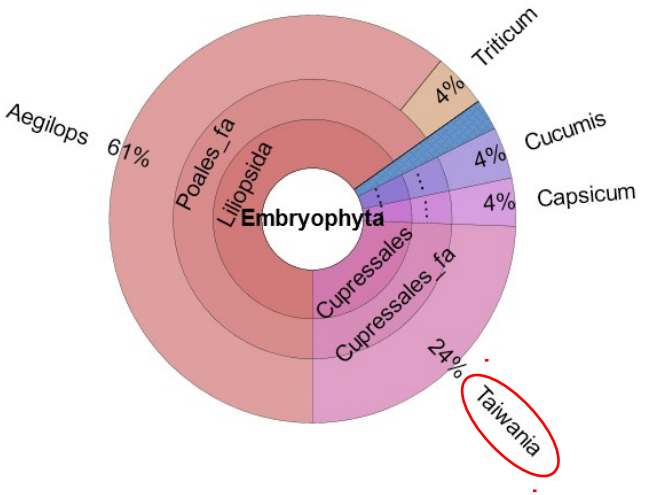
Microbiome: eukaryotes



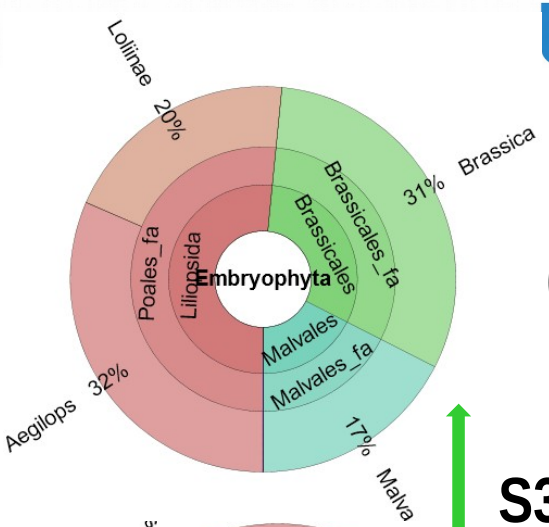
Biological markers: Embryophyta + Chlorophyta



S1 (2%)

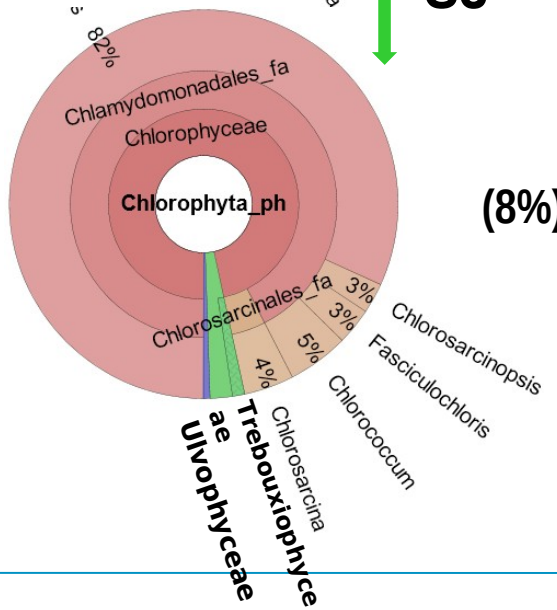


S2 (1%)



(0.3%)

S3



(8%)

S1 and S2: Biological markers for land plants, indicating that the statues were stored (or buried) most probably in agricultural soils!

S3: high presence (8% of eukaryotic sequences) of green algae of the phylum Chlorophyta

Geographical assignment

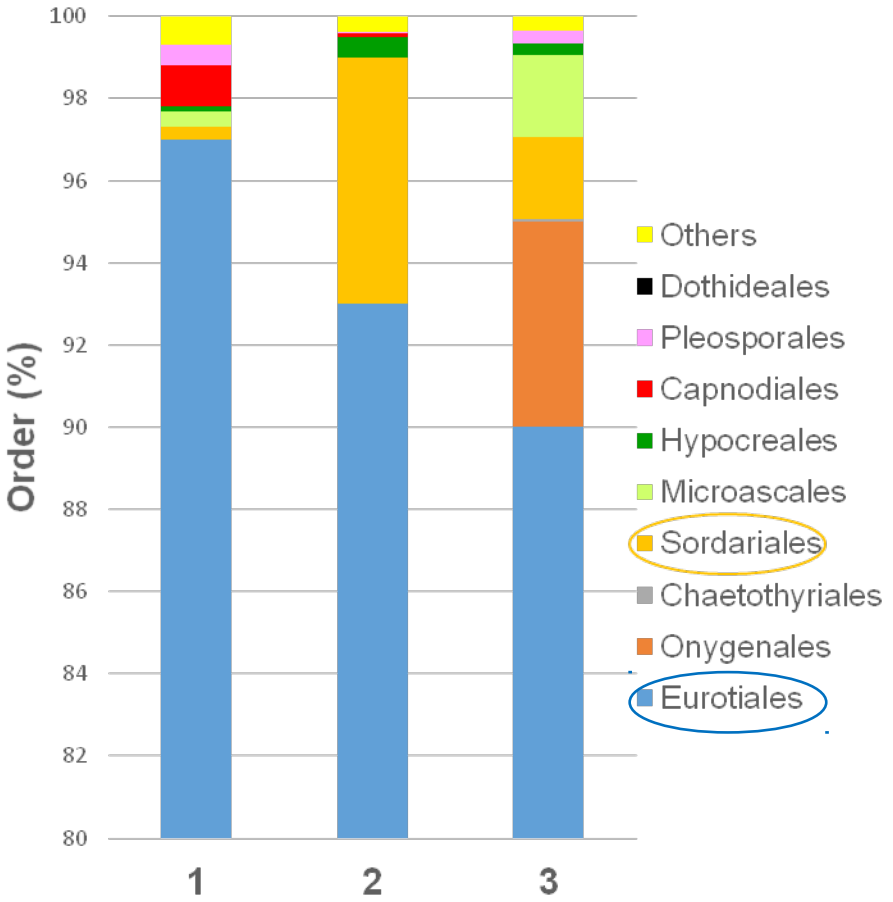
For each genus found in the samples, the geographic spread was plotted by fetching information from the Global Biodiversity Information Facility. The only genus that showed a strong geographical locality was *Taiwania*, a tree belonging to the Cypress family which is found only in Taiwan and in the south of the PRC

Geographical spread of *Taiwania Hyata*. The outliers outside Asia are located in botanical gardens

Biological markers: Fungi (Ascomycota)

Plant-associated or decaying plant - associated fungi:

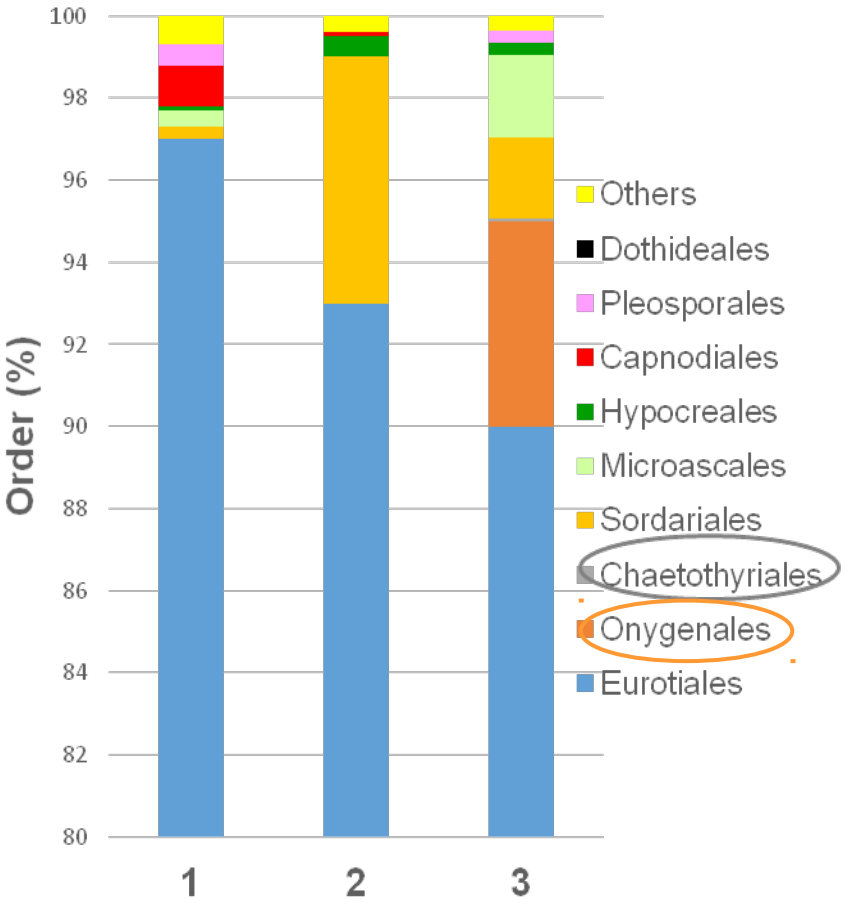
- **Eurotiales**: *A. sydowii* (S1/S2/S3) saprophytic fungus in soil and seawater. *P. glandicola* and *P. citrinus* (S1), plant-associated common in cereals
- **Sordariales**: *Chaetomium* (S1/S3), *Thielavia basicola* (S2) phytopathogenic fungi
- **Microascales**: *Microascus* (S1/S3) in soils and decaying plant material
- **Hypocreales**: *Gibberella intricans* (S1), *Fusarium* and *Acremonium* (S3) plant-associated fungi
- **Pleosporales**: *A. alternata* (plant pathogen) and *Phoma* (S1/S3) in association with land plants



Biological markers: Fungi (Ascomycota)

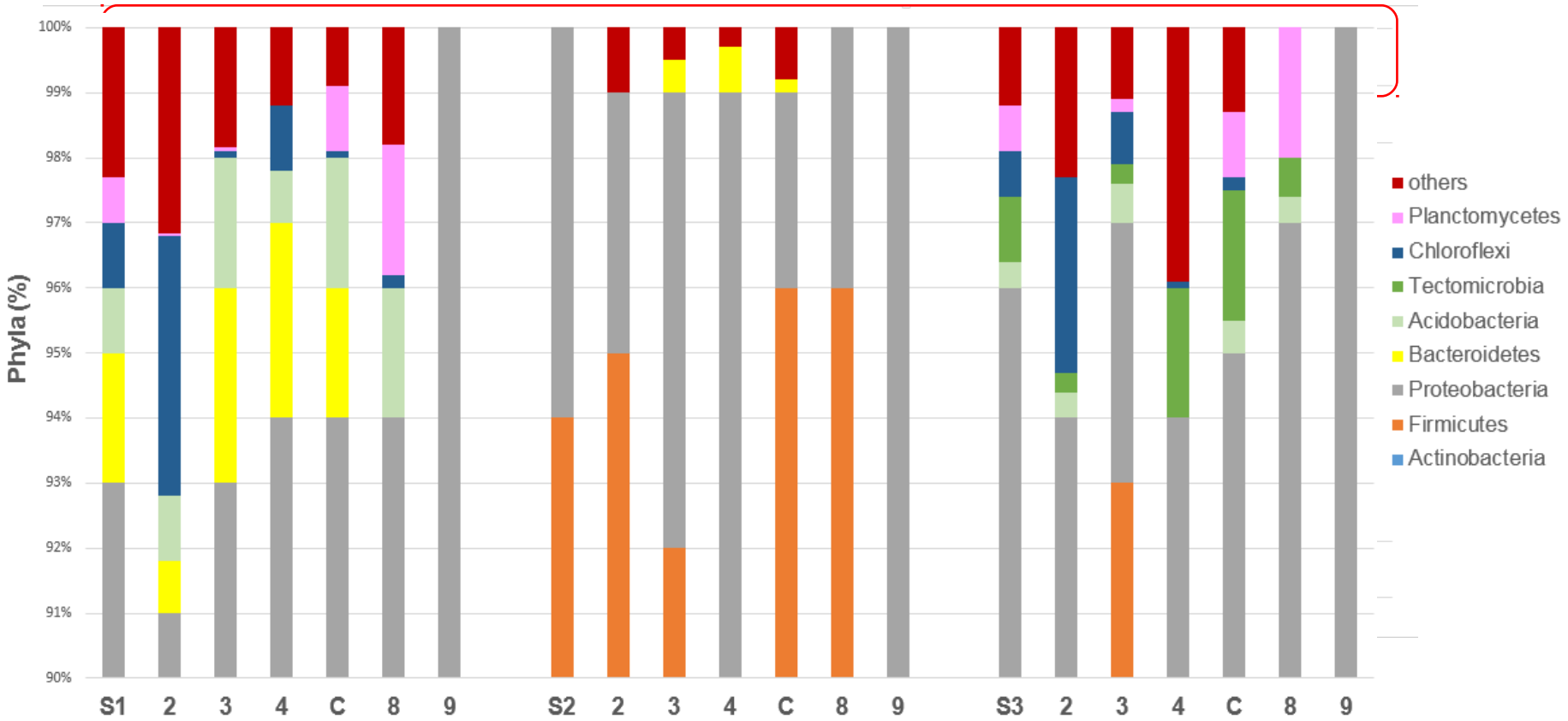
Hypersaline and / or extreme environment

- **Onygenales (S3):** *Gymnoascus*, halophilic fungi
- **Capnodiales:** *Cladosporium halotolerans and C. sphaerospermum* (S1) in salty or hypersaline environments; *Vermiconia calcicola* (S3) and *Extremus antarcticus* (S3) both extremotolerant rock-inhabiting fungi isolated from marble monuments
- **Pleosporales:** *Alternaria chlamydospora* (S1) in salty soils



Microbiome: prokaryotes

Proportion of phyla vs. variable regions of 16S rDNA



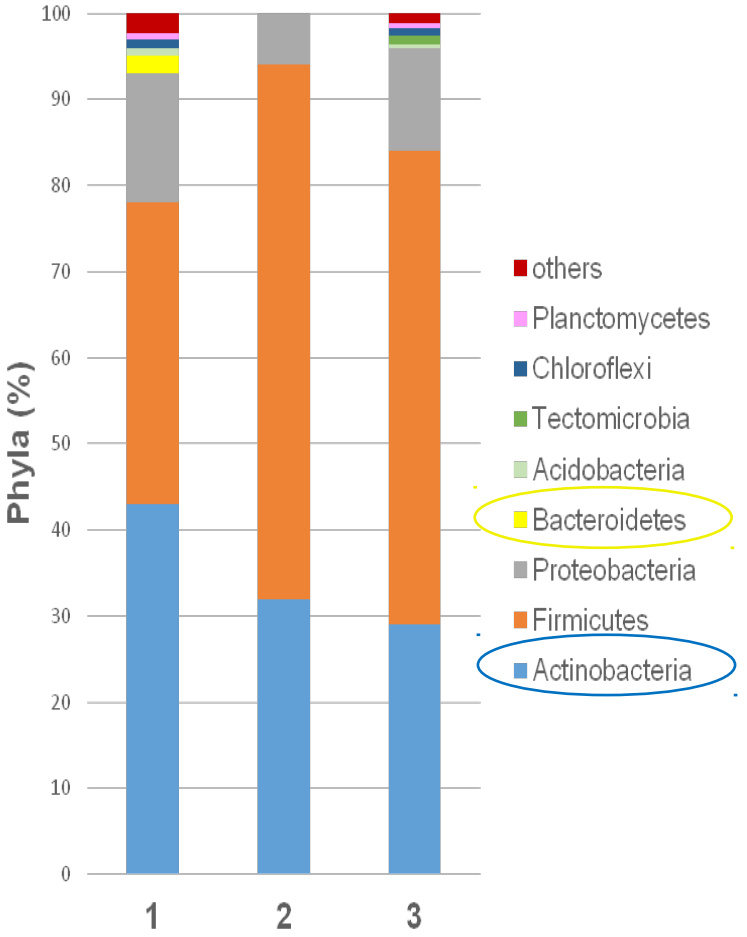
Biological markers: Bacteria

Soils/Agricultural soils:

- **Actinobacteria:** Agromyces (S1), Plantactinospora (S2), Solirubrobacter (S1/S3); **Firmicutes:** Paenibacillus (all), **Alpha-P:** Rhizobialles (all), **Beta-P:** Ralstonia (S2/S3), **Gamma-P:** Pseudomonas (S1) **Chloroflexi** (S1) (uncultured groups found in manure and fertilized soils)

Soil from animal's farm:

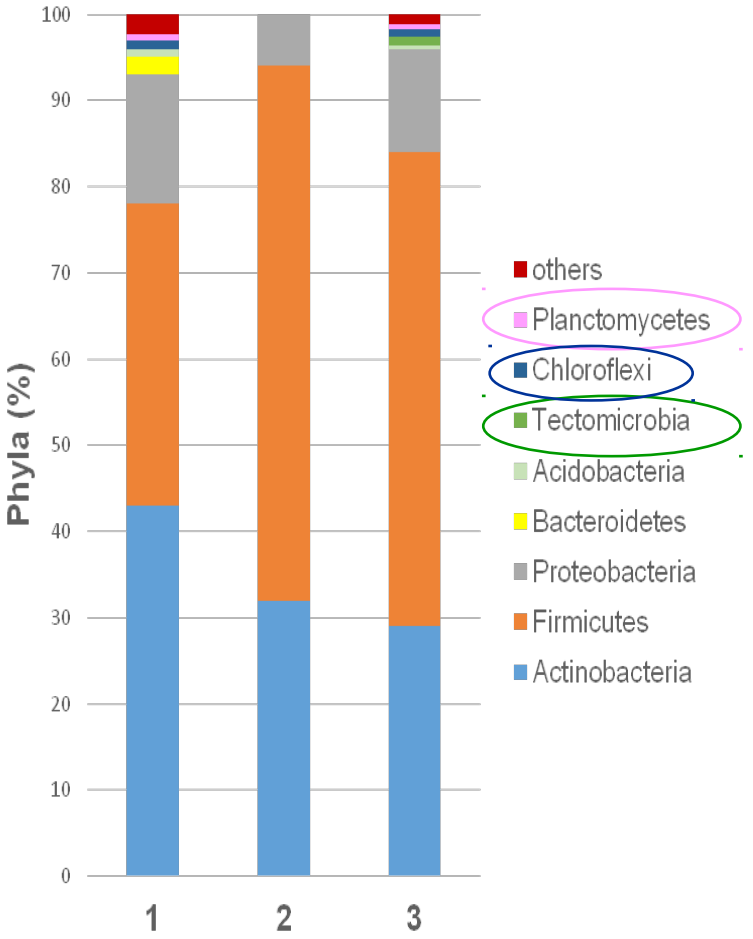
- **Actinobacteria:** Corynebacterium_1 (swine manure), (S1); **Bacteroidetes:** (gastrointestinal tract of animals); **Acidobacteria** subgroup-6 (pasture soils); **Firmicutes**, Intestinibacter, Christensenellaceae_R-7 group (uncultured rumen bacteria); Clostridium, Ruminococcaceae (gut microbiota of animals); Lactobacillus, Carnobacterium (S1) and B. timonensis (S2); **Gamma-P:** Enterobacterales (S1/S2)



Biological markers: Bacteria

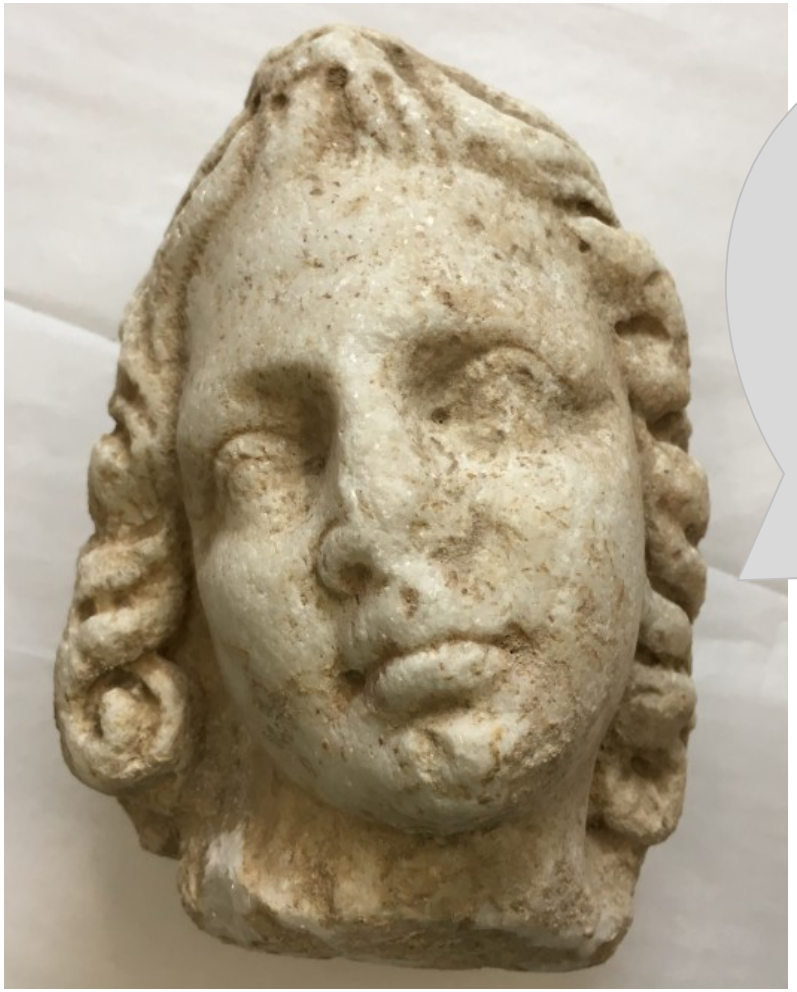
Saline environments and / or aquatic environment

- **Actinobacteria:** Rubrobacter (S3), **Firmicutes:** Salinicoccus, Jeotgalicoccus, Oceanobacillus, Virgibacillus, Marinococcus (S1).
- **Alpha-proteo:** Rhodospirillales MSB-1E8 group (seabirds and corals).
- **Gamma-P:** Salinisphaeraceae (S3).
- **Delta-P:** Desulfurellaceae H16 (microbial mats) (S3).
- **Tectomicrobia:** (marine sponges and seawater) (S3).
- **Chloroflexi** (sponges) (S3).
- **Planctomycetes** (aquatic bacteria) (S1/S3).



Summary and Conclusions:

- Metagenomics help to reconstruct the history of the three investigated statues by adding an "individual biological pedigree".
- No strong indication that all three statues could have been stored together for a longer period of time under house dust or building rubble.
- All three samples showed biological markers that indicate a contact with agricultural soil, being S1 and probably also S2 in contact with soil from animal's farm.
- A common storage in the recent past can be accepted cautiously only for the two torsi (S1 and S2), most probably in a farm.
- In S2, the detection of Taiwania sequences allowed a real geolocation of this sample, because this plant is native and endemic to Asia.
- The girl's head (S3) differs considerably from the other two objects in terms of mold and eukaryotic flora. This sample harbors inhabitants of aquatic (or even marine) environments as well as halophilic microorganisms, which are markers for saline environments (or marine).



Thank you for your attention!

European Geosciences Union

EGU General Assembly 2019

Vienna (Austria) on 7–12 April 2019

- **Session: BG1.68/ERE7.3/NH2.4 "Novel approaches on stone heritage conservation: Biomaterials, biotechnology and bioremediation"**

- **Convener: Patricia Sanmartín;**
- **Co-Conveners: Ana Z. Miller, Domenico Pangallo & Guadalupe Piñar**
- **https://meetingorganizer.copernicus.org/EGU2019/session/32679**

The deadline for abstracts submission is 10 Jan 2019, and for travel grants (Roland Schlich travel support) is 1 Dec 2018!