# SOLID Heritage Bone Diagenesis

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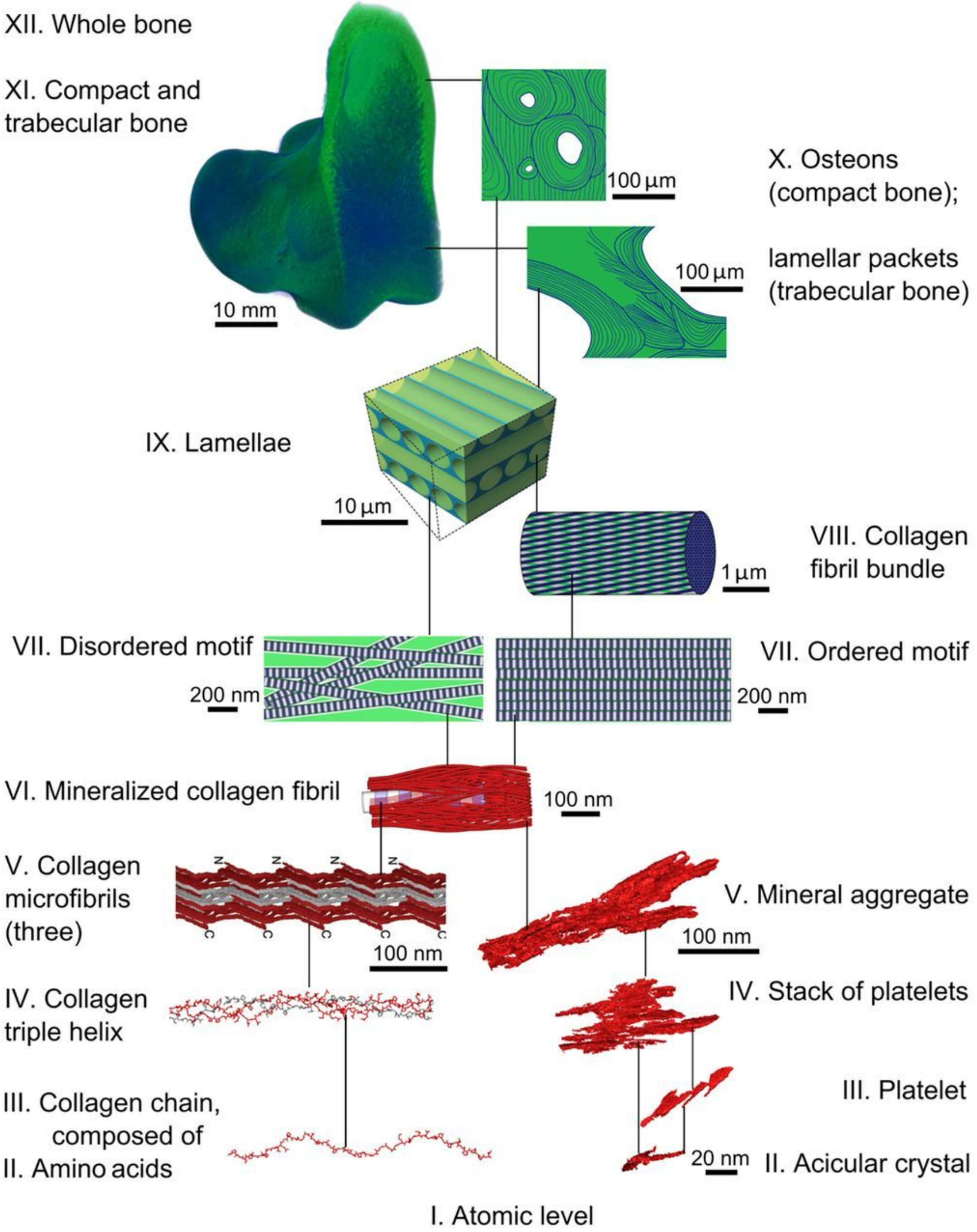



Image Reznikov, N., Bilton, M., Lari, L., Stevens, M. M., & Kröger, R. (2018). Fractal-like hierarchical organization of bone begins at the nanoscale. *Science*, **360**(6388). https://doi.org/10.1126/science.aao2189

# Taphonomy

Skeletal tissues may be either exposed on the ground surface (Biostratinomy) or buried in various burial environments (Diagenesis)

- Biostratinomy covers the pre-burial histories of bones.
- Diagenesis covers the post-burial histories of bones.

# What is Diagenesis?

- Post-mortem each object seeks to reach equilibrium with the conditions of its burial environment.
- A series of physical, chemical, and biological processes affect its preservation state. These are called diagenetic trajectories or diagenetic pathways.
- Three diagenetic pathways are identified for bone:

1. Biological deterioration of the composite (i.e. microbial attack).
2. Chemical deterioration of the inorganic component (i.e. dissolution).
3. Chemical deterioration of the organic component (i.e. hydrolysis).

Collins et al. (2002), *Archaeometry* **44**(3):383–395

Hedges, R. E. M. (2002), *Archaeometry* **44**(3):319-328

# Diagenetic Parameters

## State of preservation of bone characterized by 12 simple measurements

- Microstructure

  Histology: Oxford Histological Index; Collagen Birefringence Index; Cracking; Inclusions

- Physical

  Porosity: Mercury Porosimetry

  Biomechanical properties: density measurements (micro- and nano-indentation)

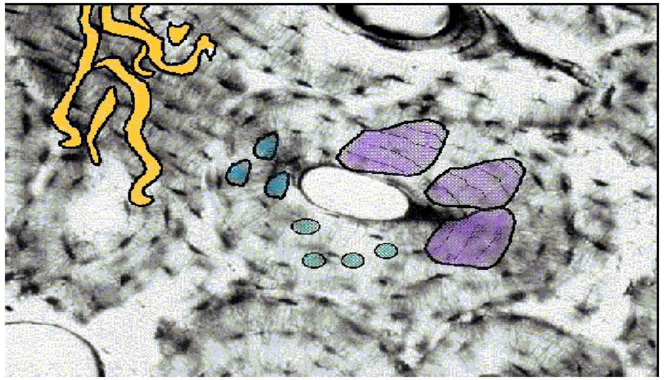
- Inorganic

  IRSF (crystallinity); C:P (Carbonate-to-Phosphate); Identification of other mineral phases

- Organic

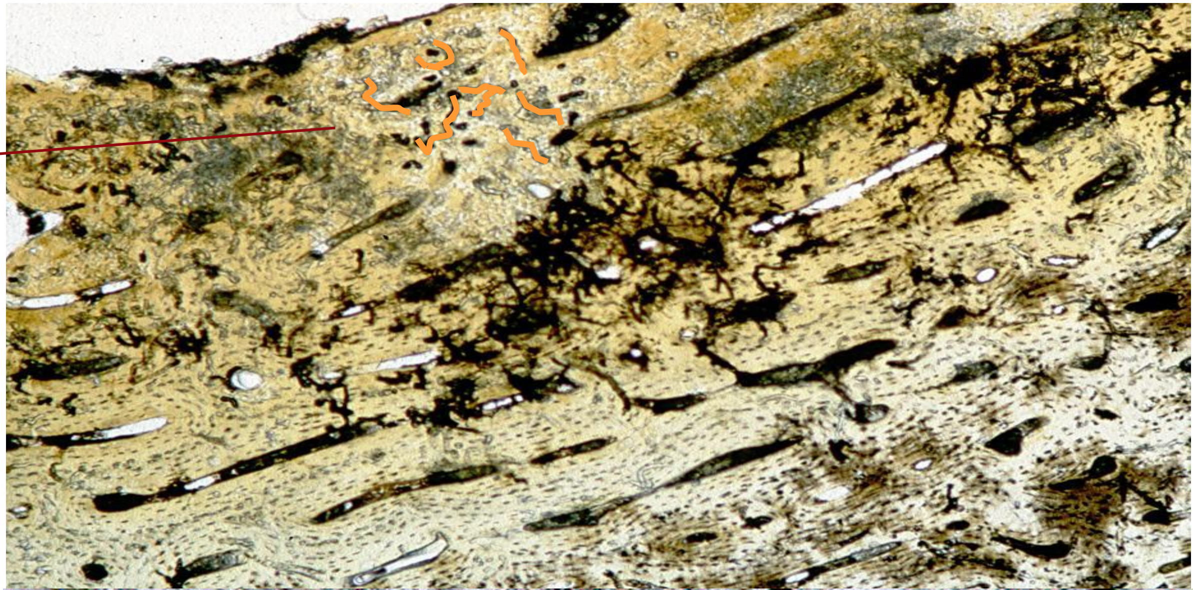
  %N of Whole bone; % 'Collagen'; C:N ratio of 'Collagen'

# Histology



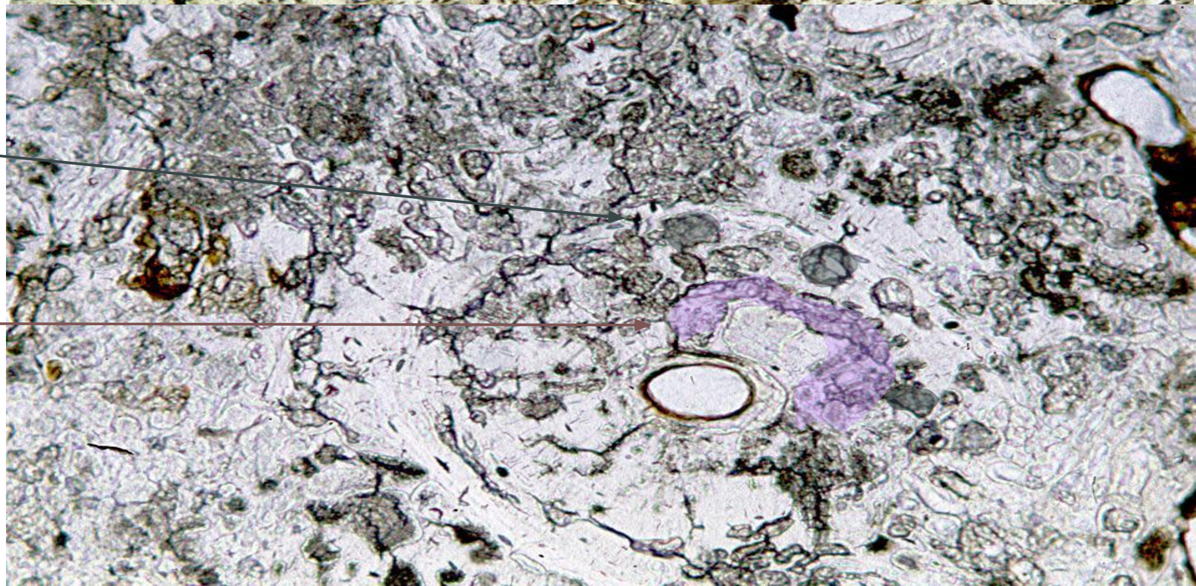
Wedl tunnelling

**Is this just expanded and stained canaliculi?**



Budded

lamellar



# Histology: Quantitative Assessment

*Oxford Histological Index*

| Index | Approximate % of intact bone | Description |
|---|---|---|
| 0 | <5 | No original features identifiable, except that Haversian canals may be present |
| 1 | <15 | Haversian canals present, small areas of well-preserved bone present, or lamellate structure is preserved by the pattern of destructive foci |
| 2 | <50 | Some lamellate structure is preserved between the destructive foci |
| 3 | >50 | Some osteocyte lacunae preserved |
| 4 | >85 | Bone is fairly well preserved, with minor amounts of destructive foci |
| 5 | >95 | Very well preserved, virtually indistinguishable from modern bone |

Millard (2001), The deterioration of bone. In *Handbook of Archaeological Sciences*, D. R. Brothwell, A. M. Pollard (eds.). Wiley: New York. 637–674.

*Birefringence Index*

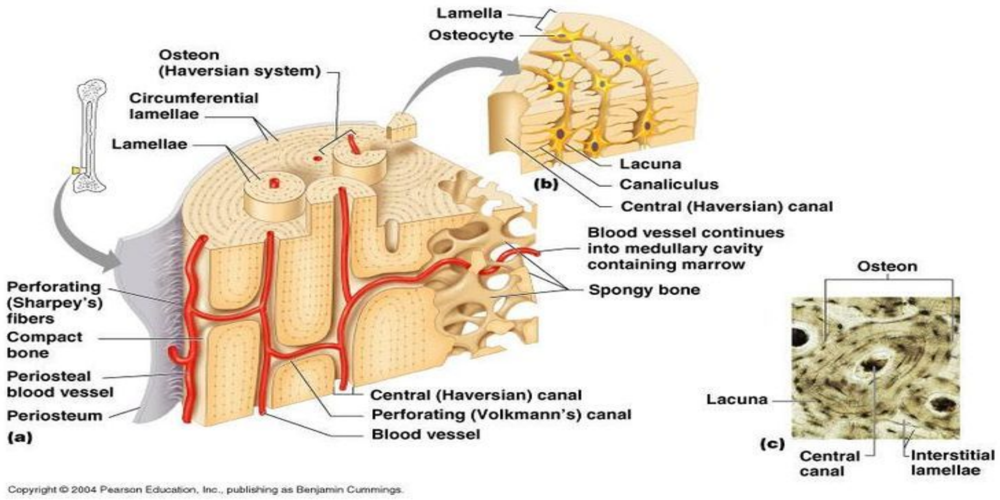
| Grade | Intensity of Birefringence |
|---|---|
| 0 | Absent |
| 0.5 | Reduced |
| 1 | Normal, comparable to fresh bone |

Jans et al. (2002), *Archaeometry* **44**(3):343–352

# Histology: Qualitative Assessment

- **Fungi**
  - Branches
- **Bacteria**
  - Linear-Longitudinal
  - Budded
  - Lamellate

- Fungi (and cyanobacteria in marine environments) create branched tunnels in the bone matrix called Wedl tunnels.
- Bacteria reorganize the mineral content of bone by creating microscopic focal destructions (MFD) called non-Wedl tunnels.




Hackett (1981), *Medicine, Science and the Law*, **21**:243–65

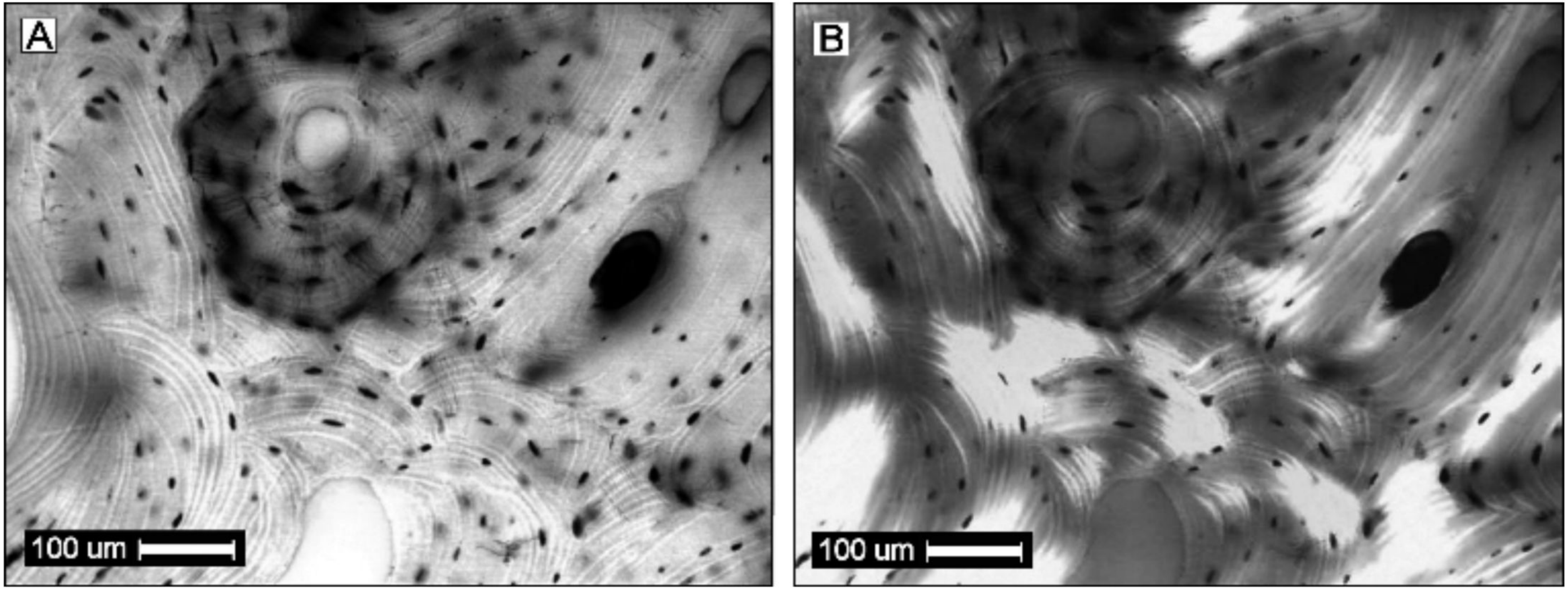
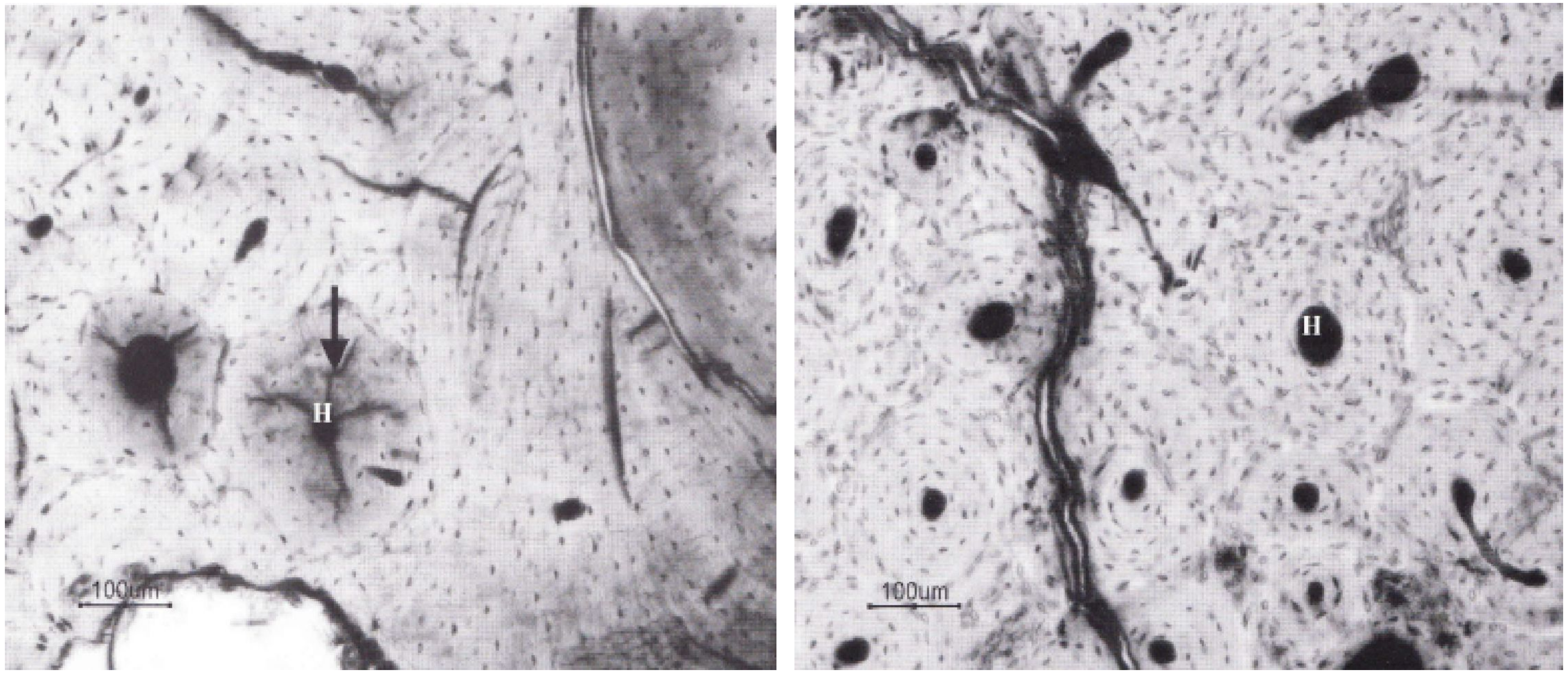



**Figure 1.2** (a) Transmitted light image of medieval human bone from Trondheim, Norway. Histological preservation is excellent, but staining around the central osteon illustrates the fine canalicular network that connects the tissues with the soil environment. (b) The section viewed in polarized light with a quarter-lambda plate. The spectacular birefringence arises from the alignment of collagen fibrils and HAP in the bone lamellae

# Histology

## Cracking

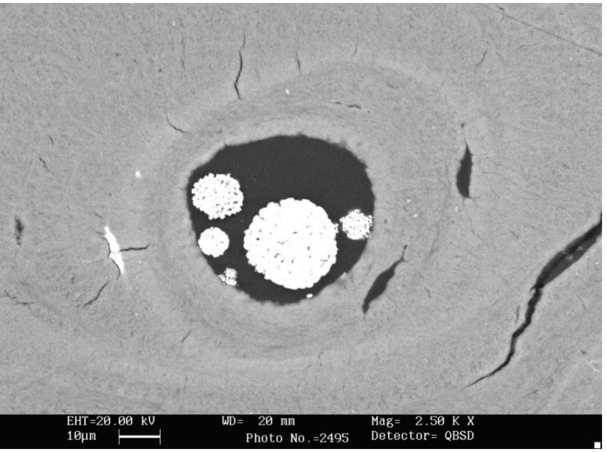
- Micro-fissures due to deterioration of the inorganic and/or organic components.
- Decoupling of mineral-collagen bond, demineralization or thermal stress.
- Large fissures maybe due to physical stress.

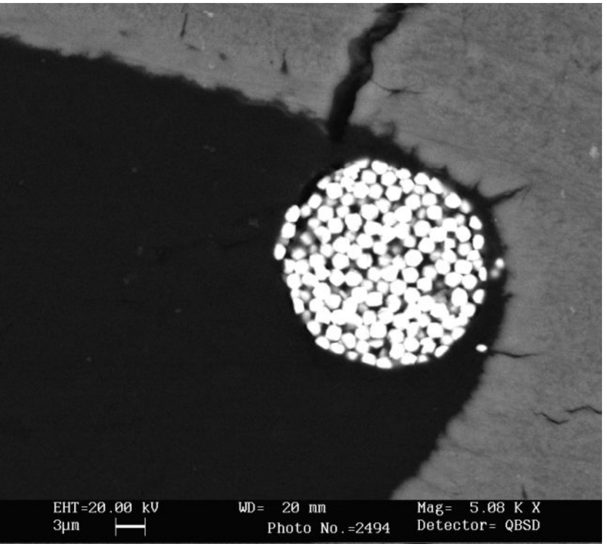


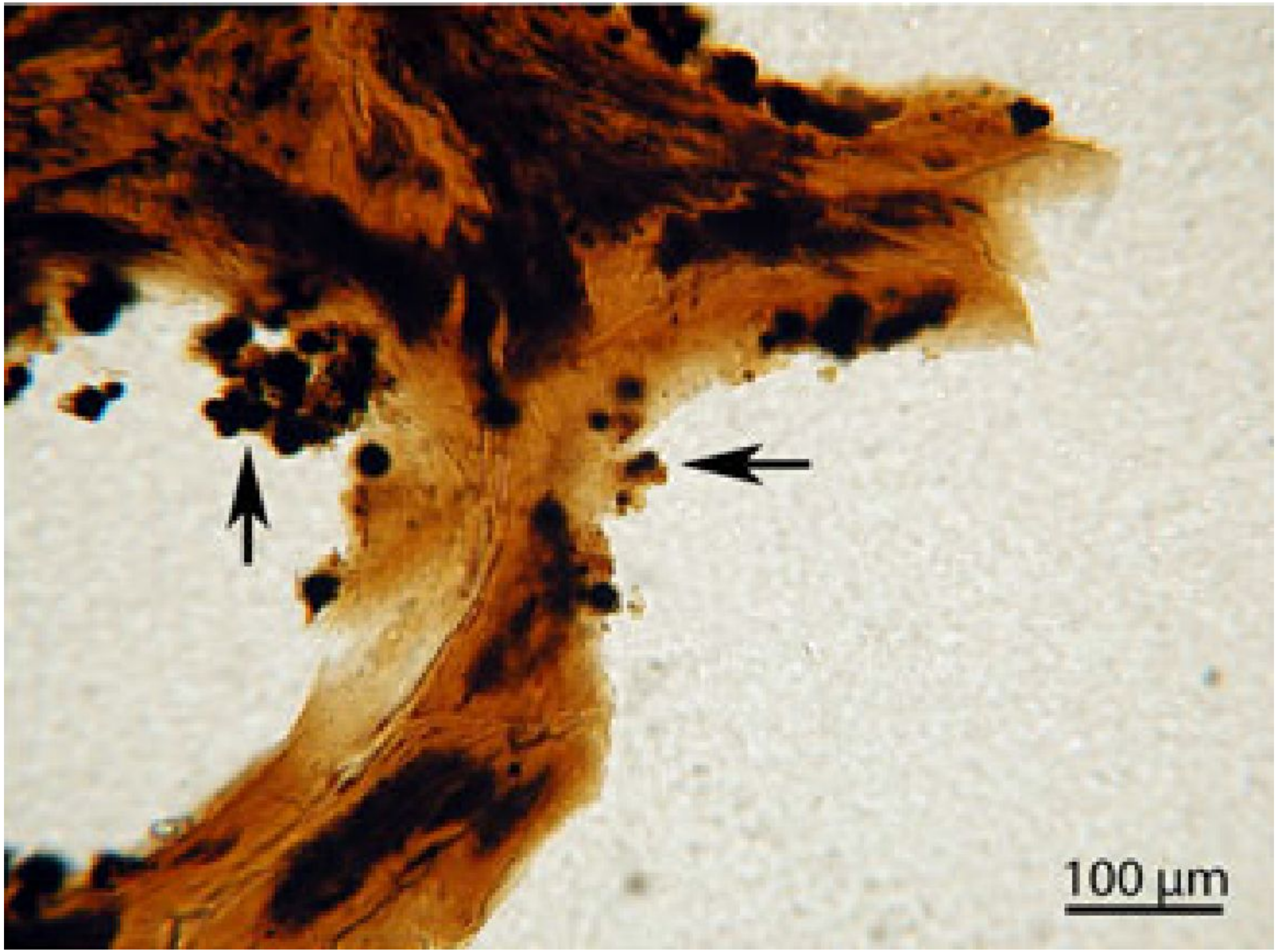

Jans (2005), *Histological Characterisation of Diagenetic Alteration of Archaeological Bone.* PhD Thesis, Vrije Universiteit: Amsterdam.

# Histology

## Inclusions - Pyrite framboids




Hollund et al. (2012), *Int. J. Osteoarchaeol*. **22**:537–548

# Diagenetic Parameters

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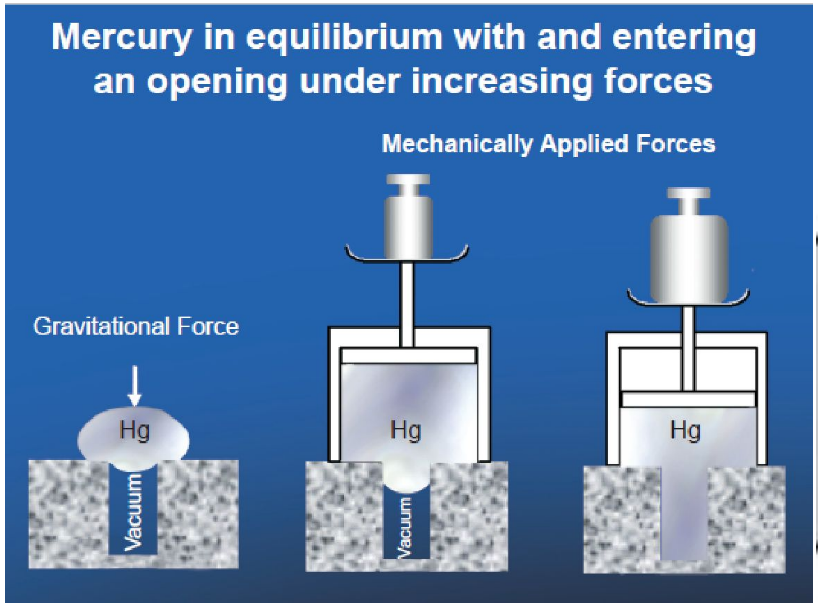
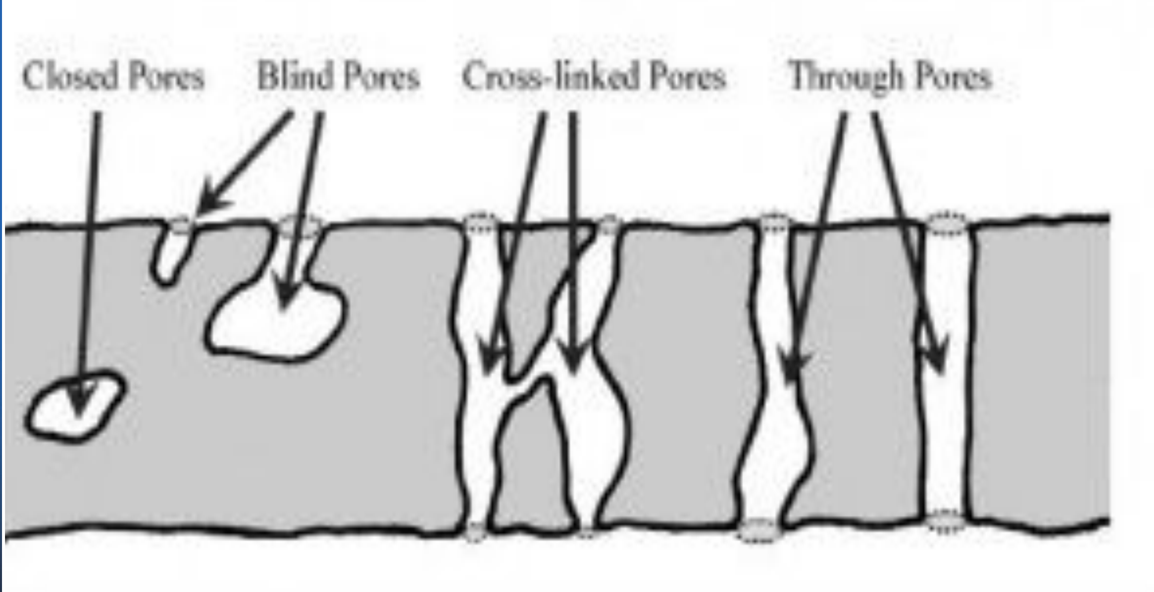
- Organic

%N of Whole bone; % 'Collagen'; C:N ratio of 'Collagen'

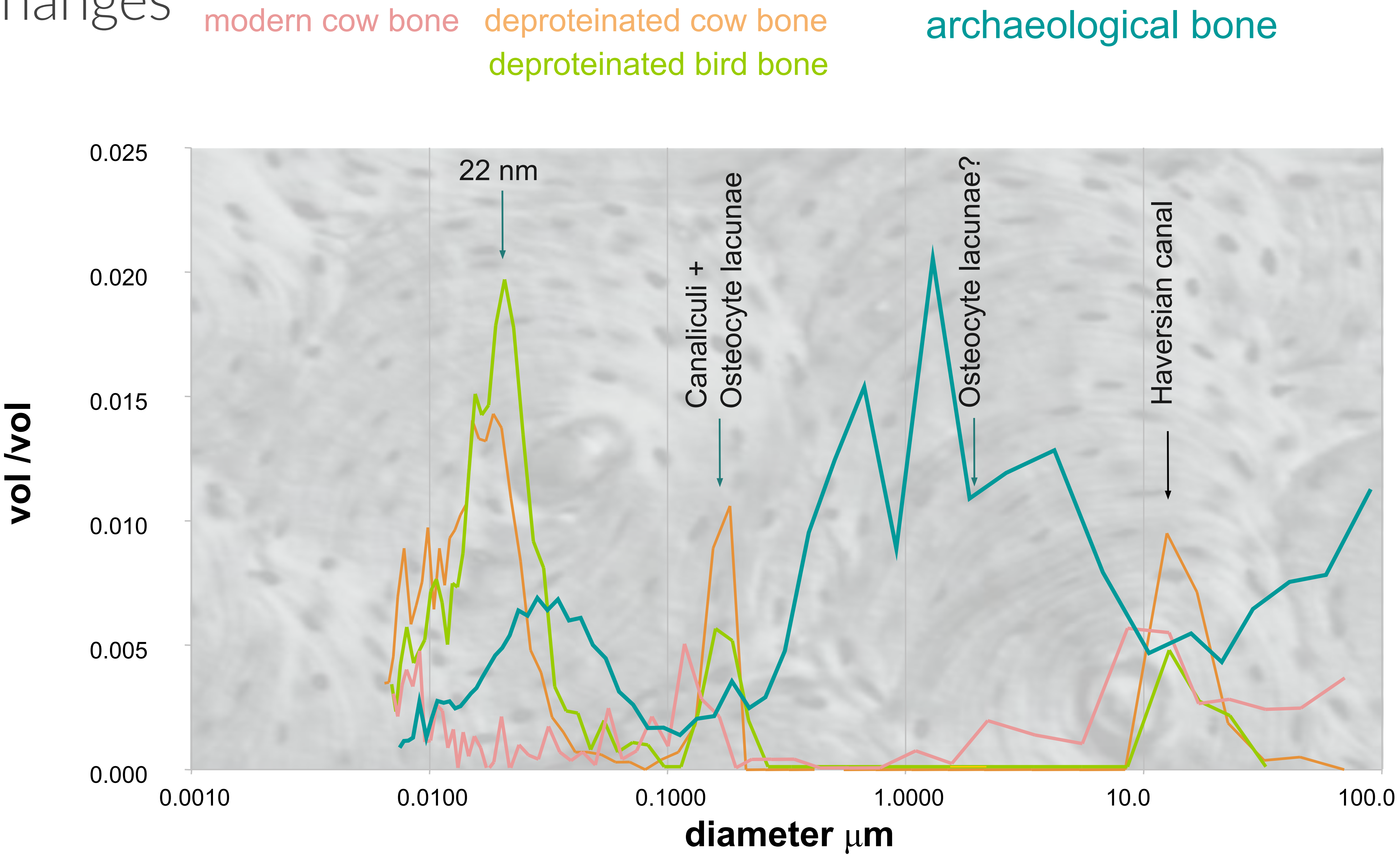
# Porosity

Mercury intrusion porosimetry: to measure intrusion of mercury into a porous structure under stringently controlled pressures (pore size distribution, volume, diameter, etc.).

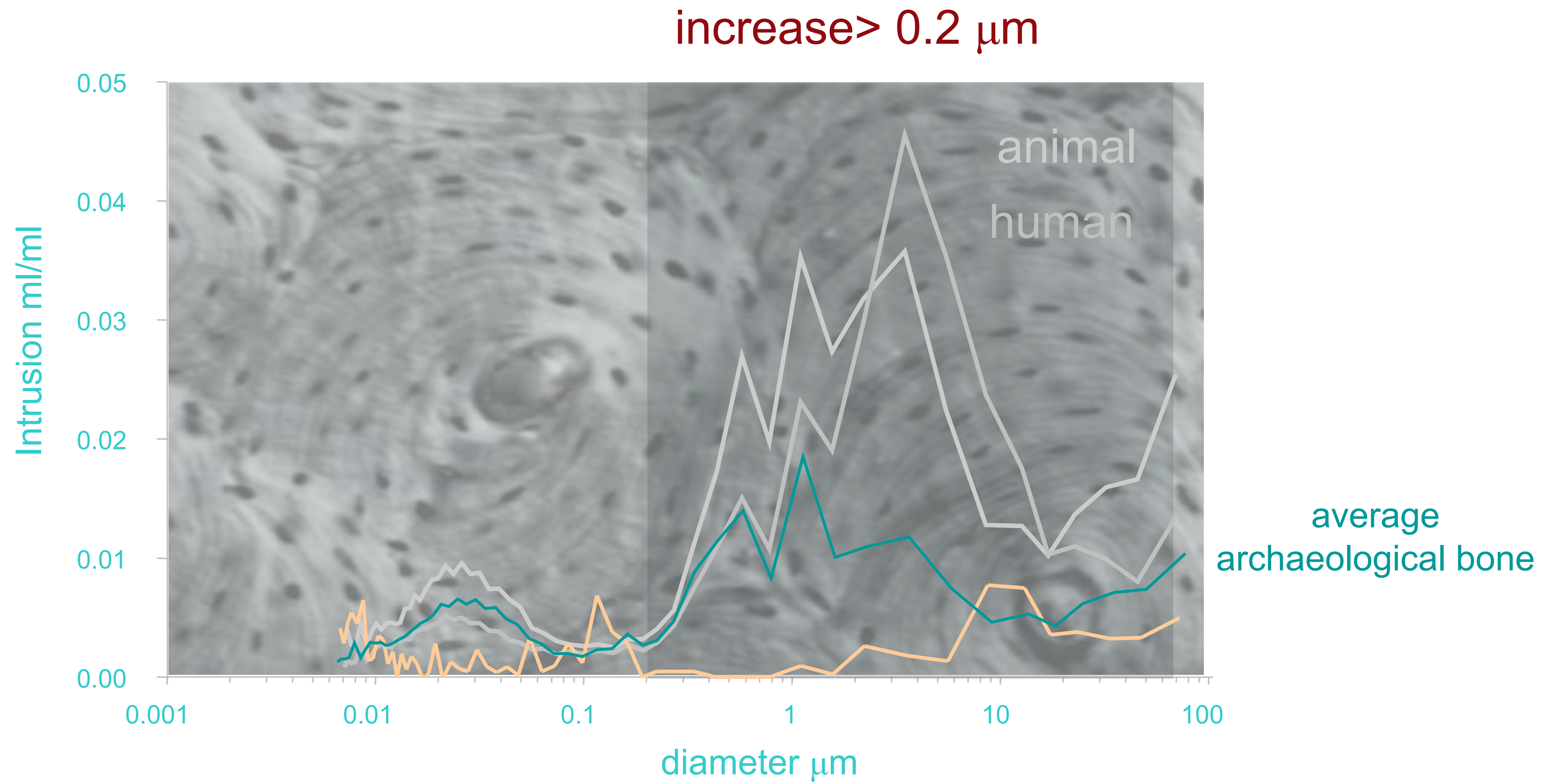



# Porosity changes

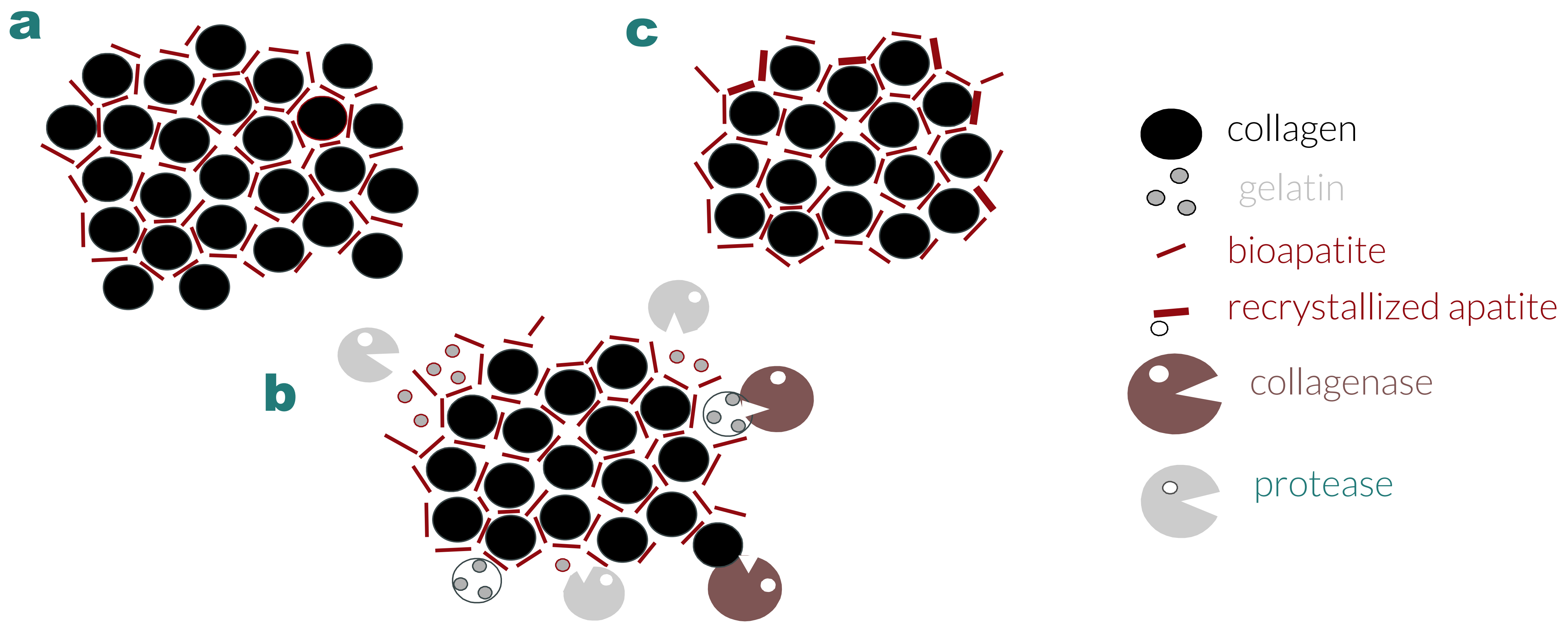


# Porosity: pH > 6.0

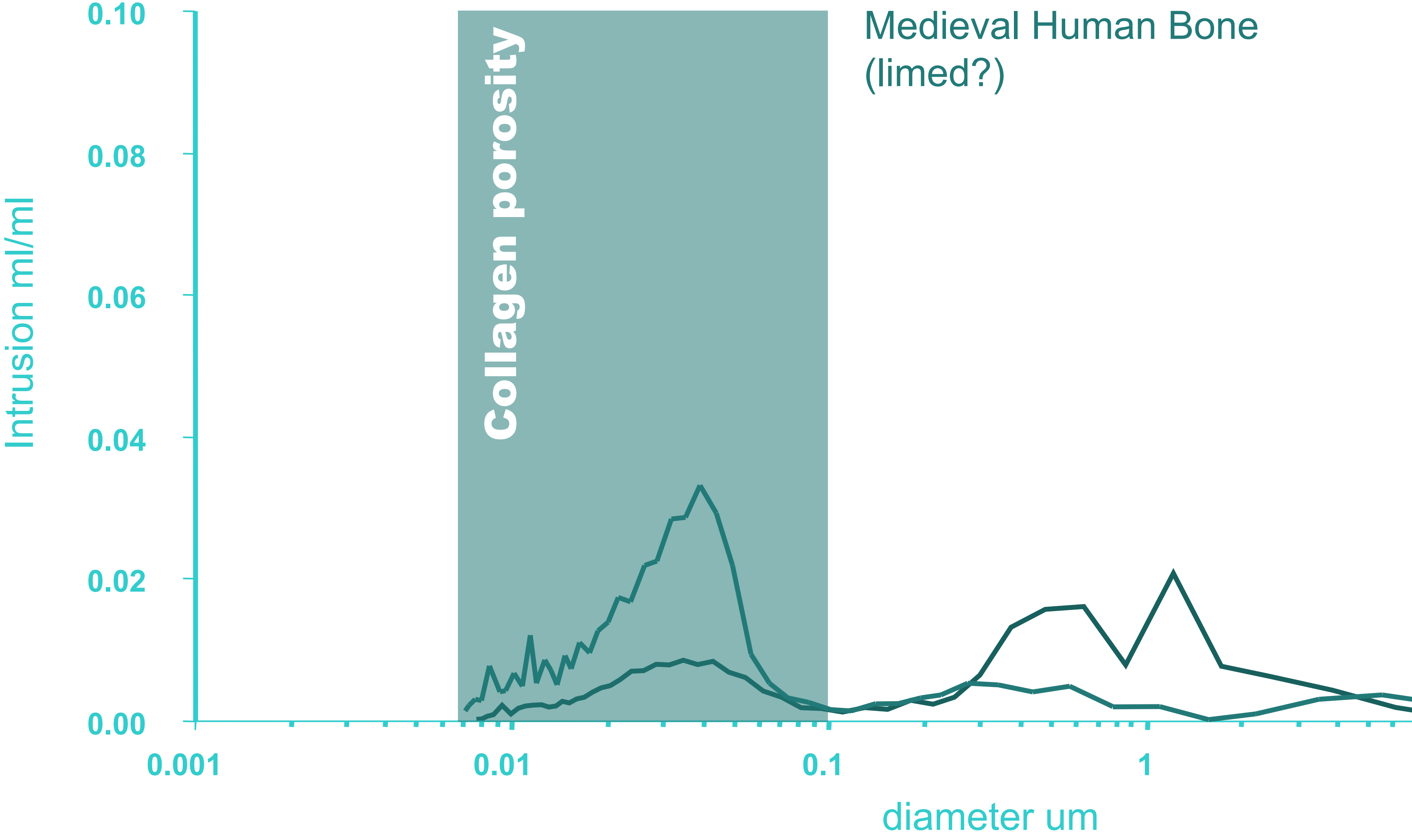


# Dissolution removes mineral

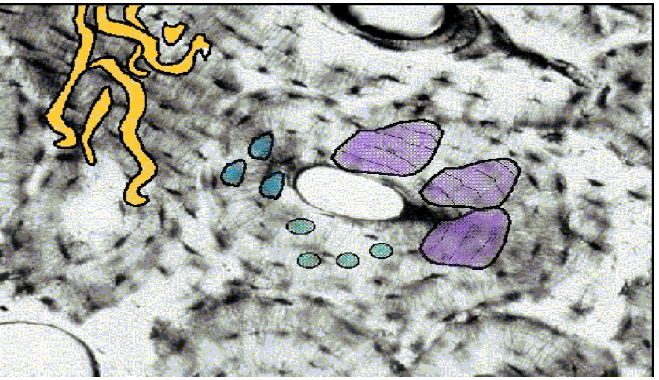
- Why no organic matter?
- Removal of mineral exposes collagen

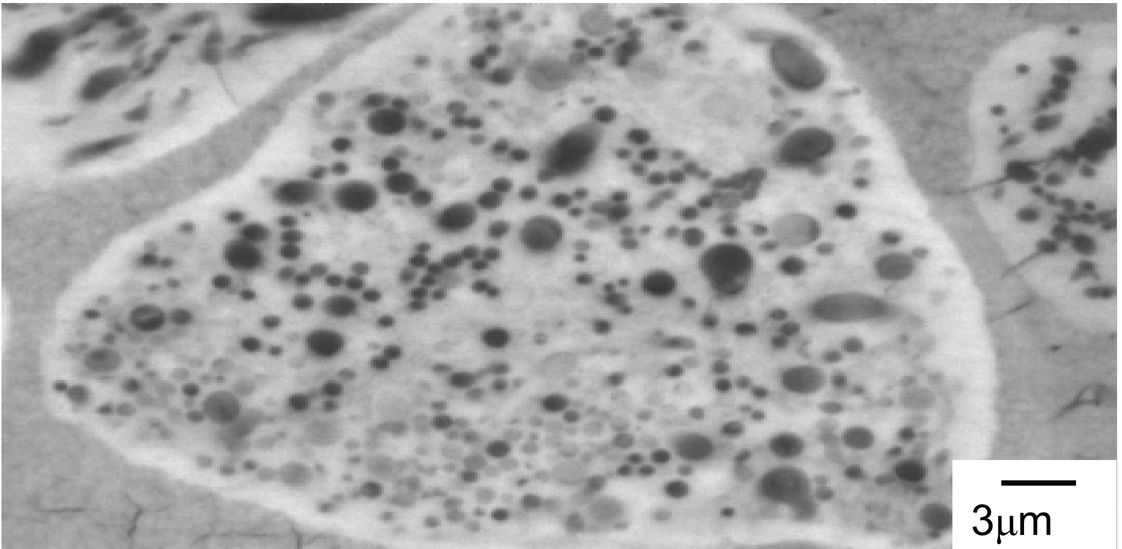


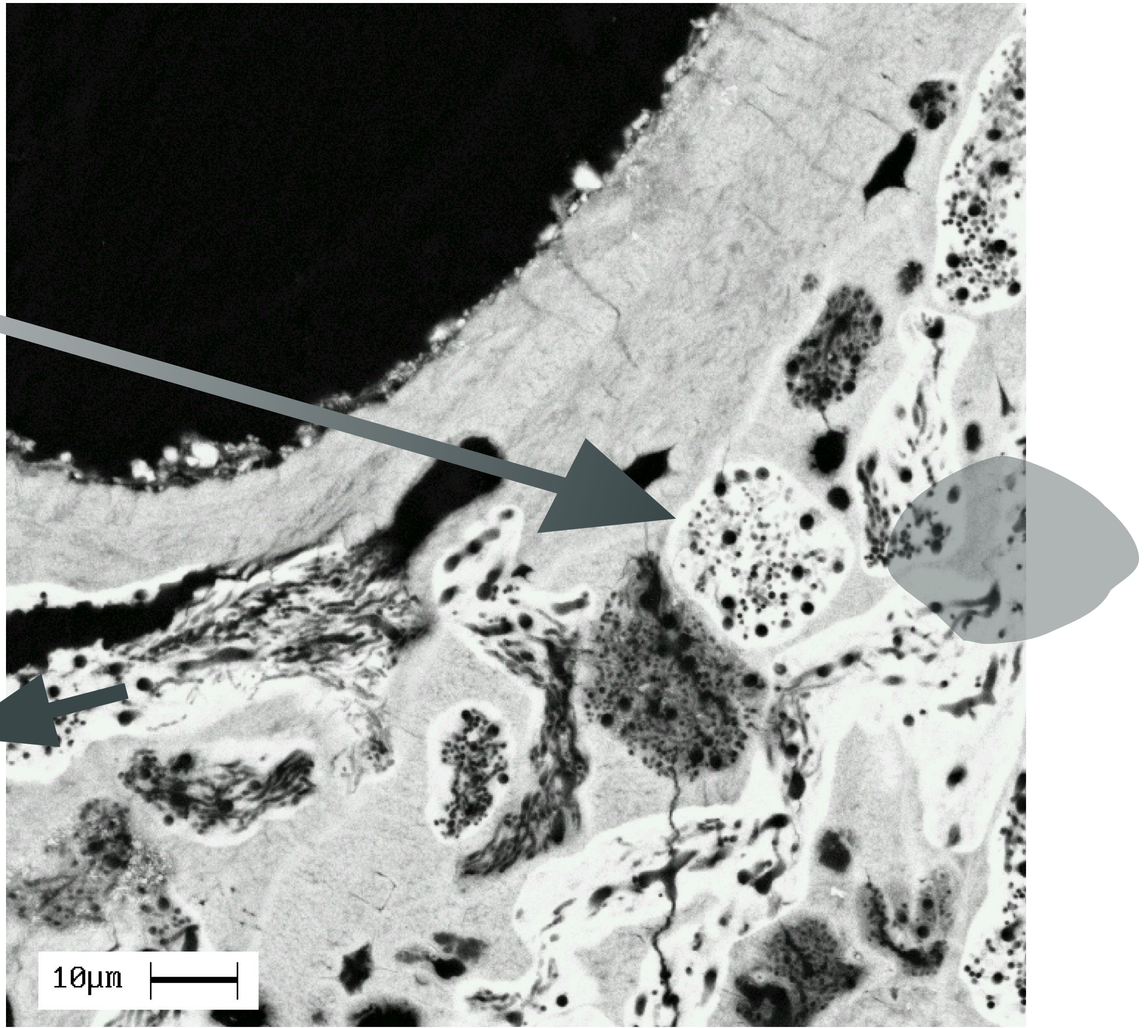
# Fossilization: Apigliano (& Çatalhöyük?)



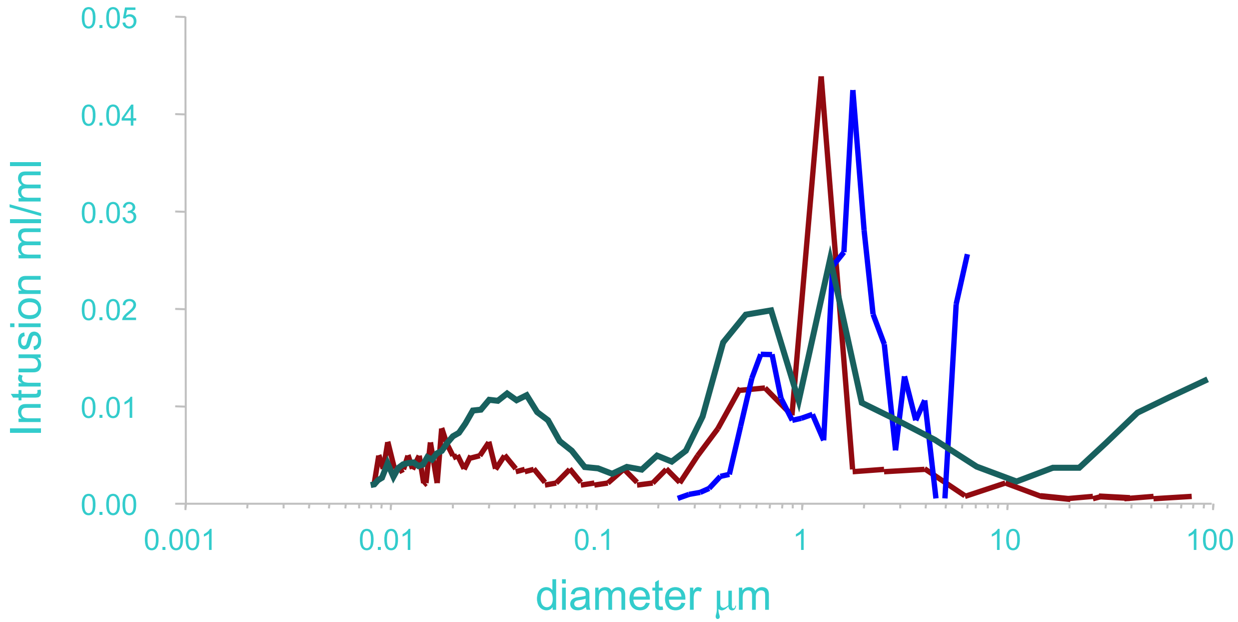

# Patterns of microbial attack





# Porosimetry trace



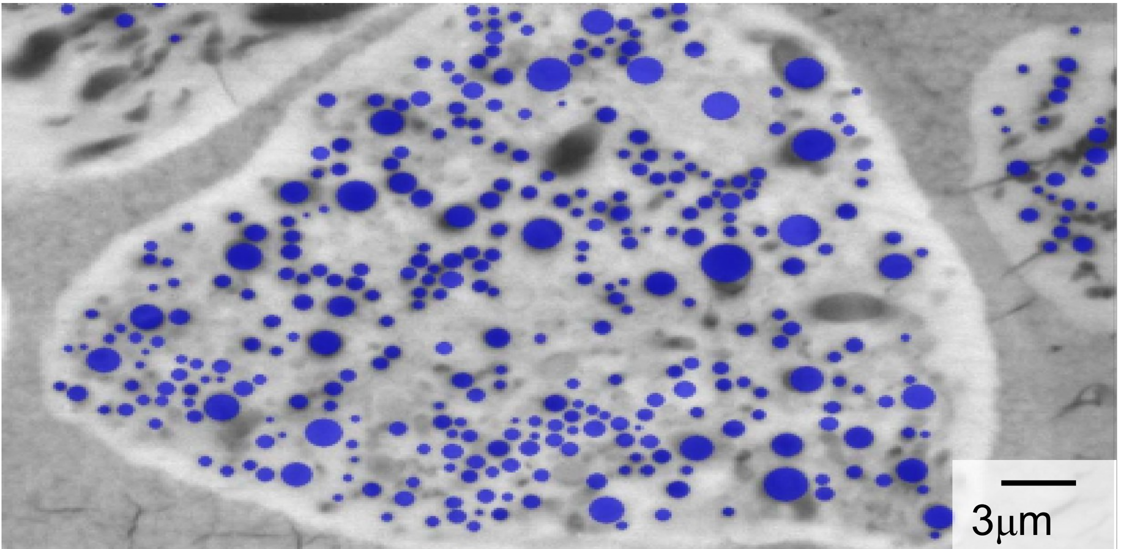
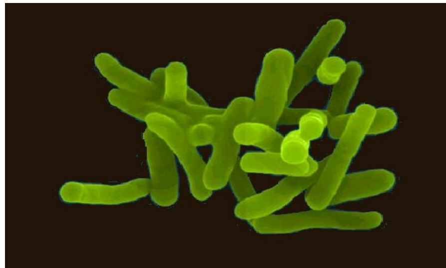
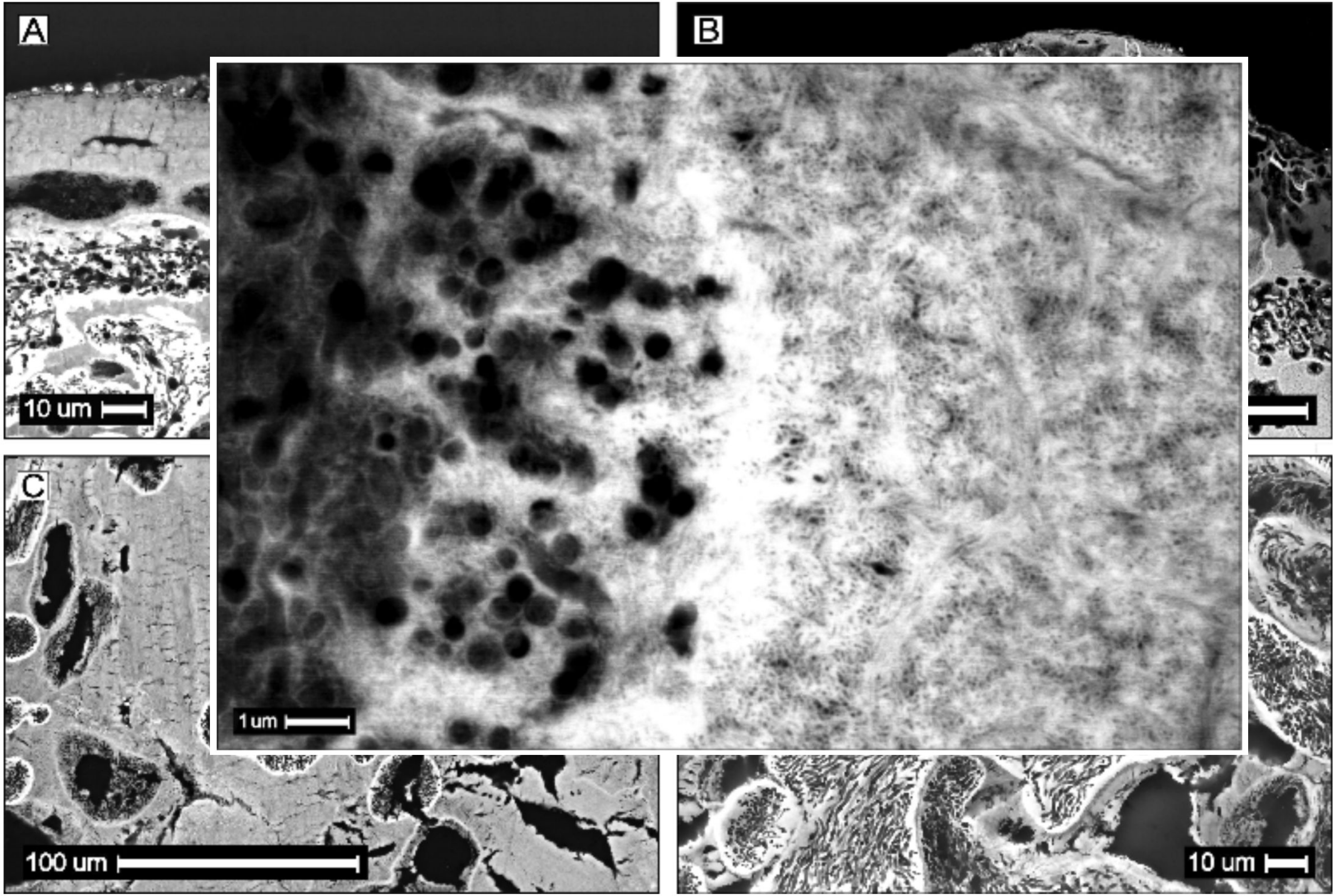
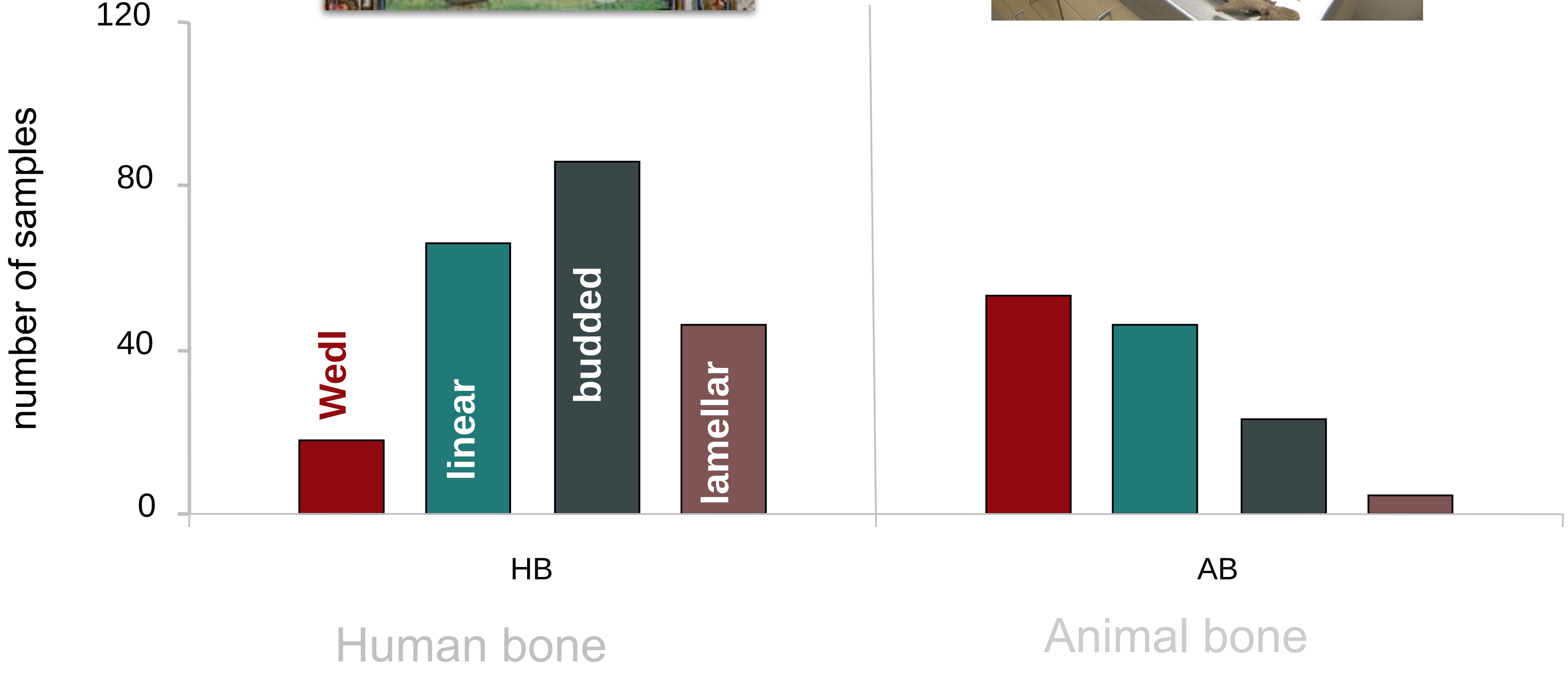




Image analysis: Gordon Turner-Walker



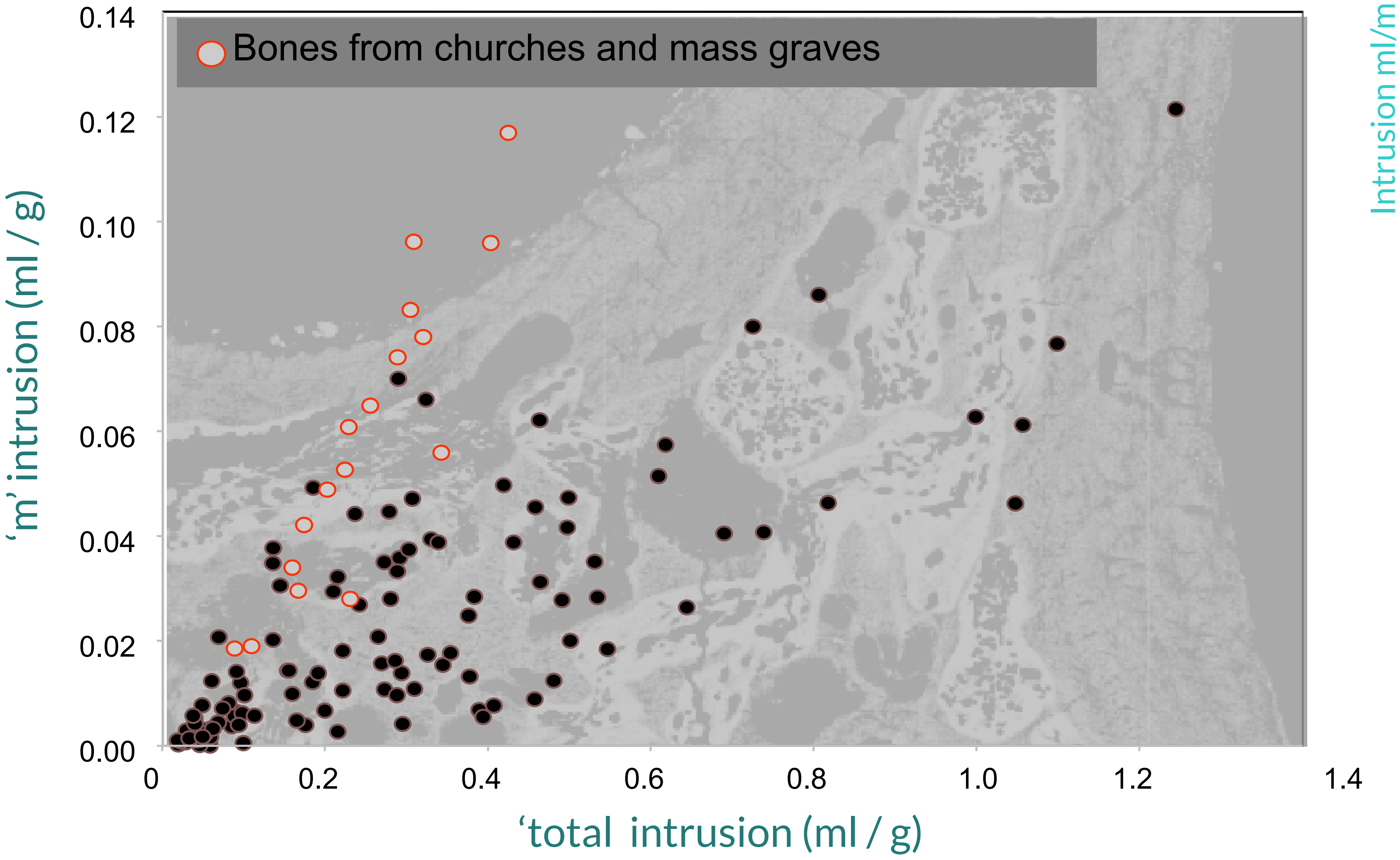

A
B
C
10 um
1 um
100 um
10 um

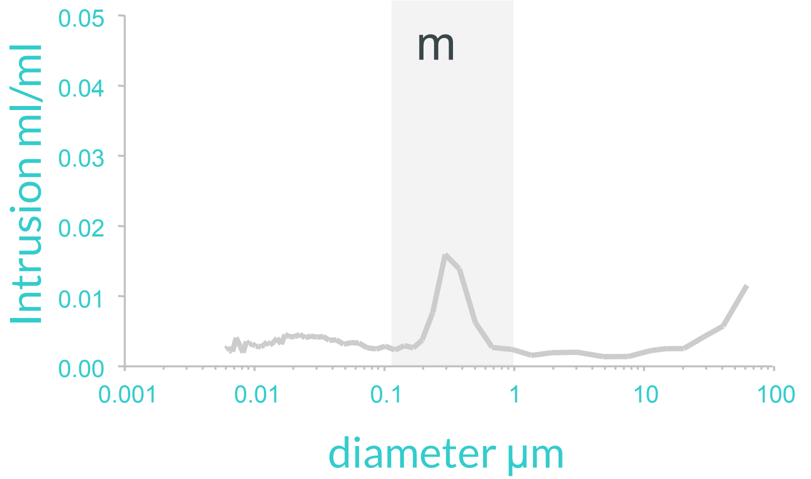
# Human vs. animal bone

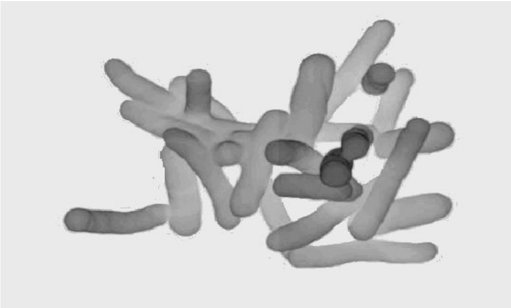



Miranda Jans

# Churchyards



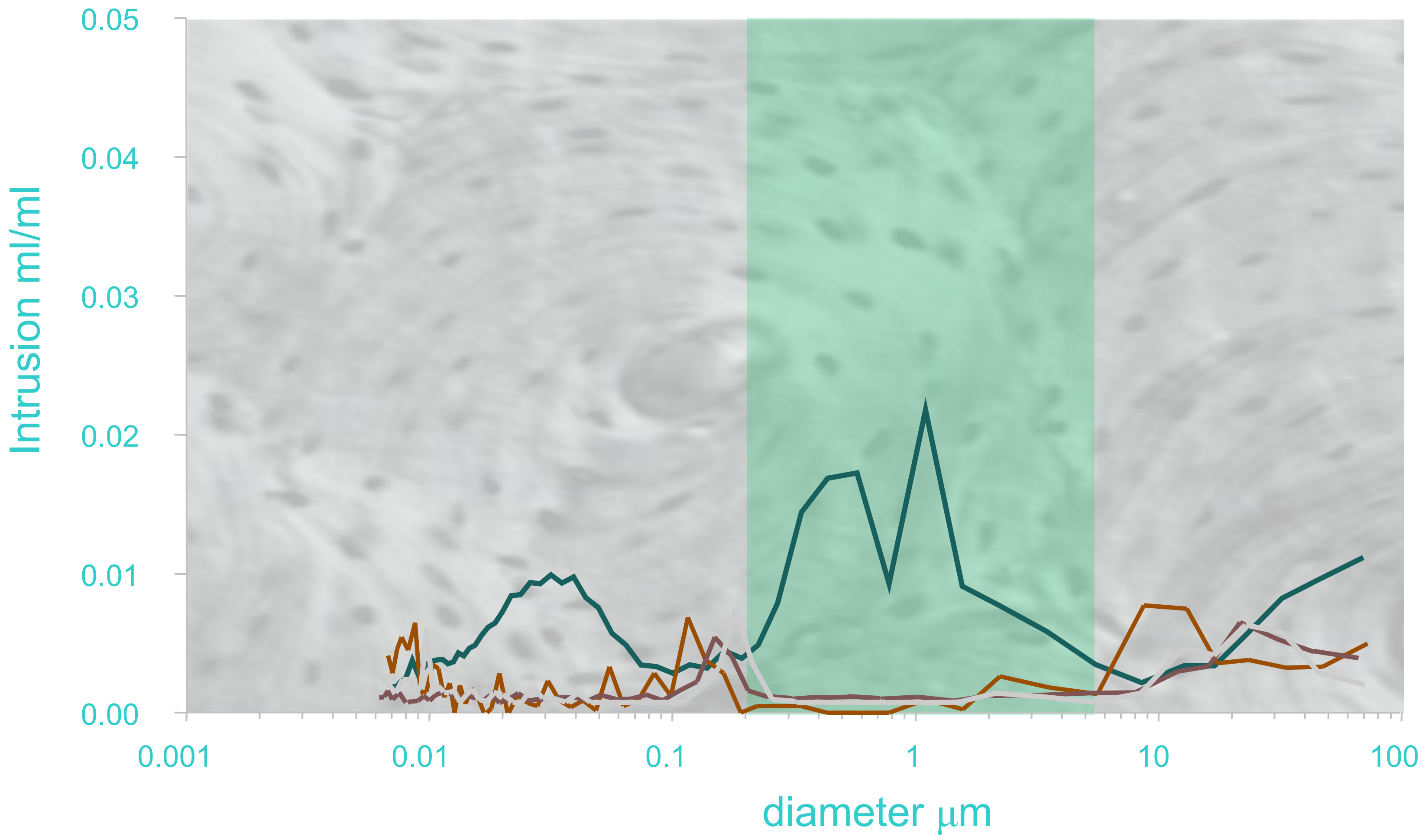



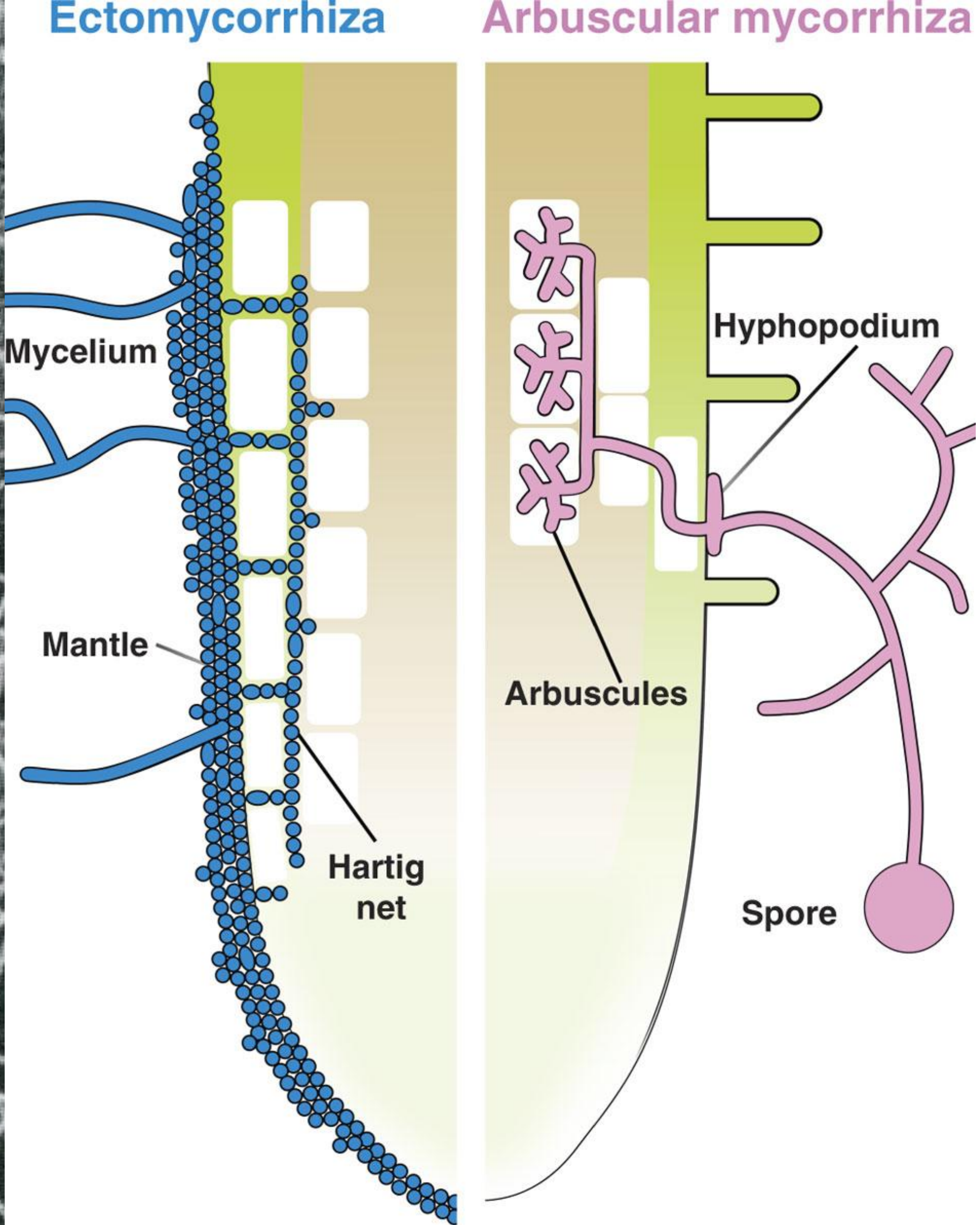
# Animal vs. human bone

- Human bone
  - Predominately microbial attack
  - Burial with intact blood supply
  - Putrefaction of intestine
  - Migration of gut bacteria *Clostrida* sp. into blood then bone
- Animal bone
  - Fungal attack (Wedl)
  - No putrefaction
  - Processed bone
  - [100% of interred animals (9) have microbial attack]

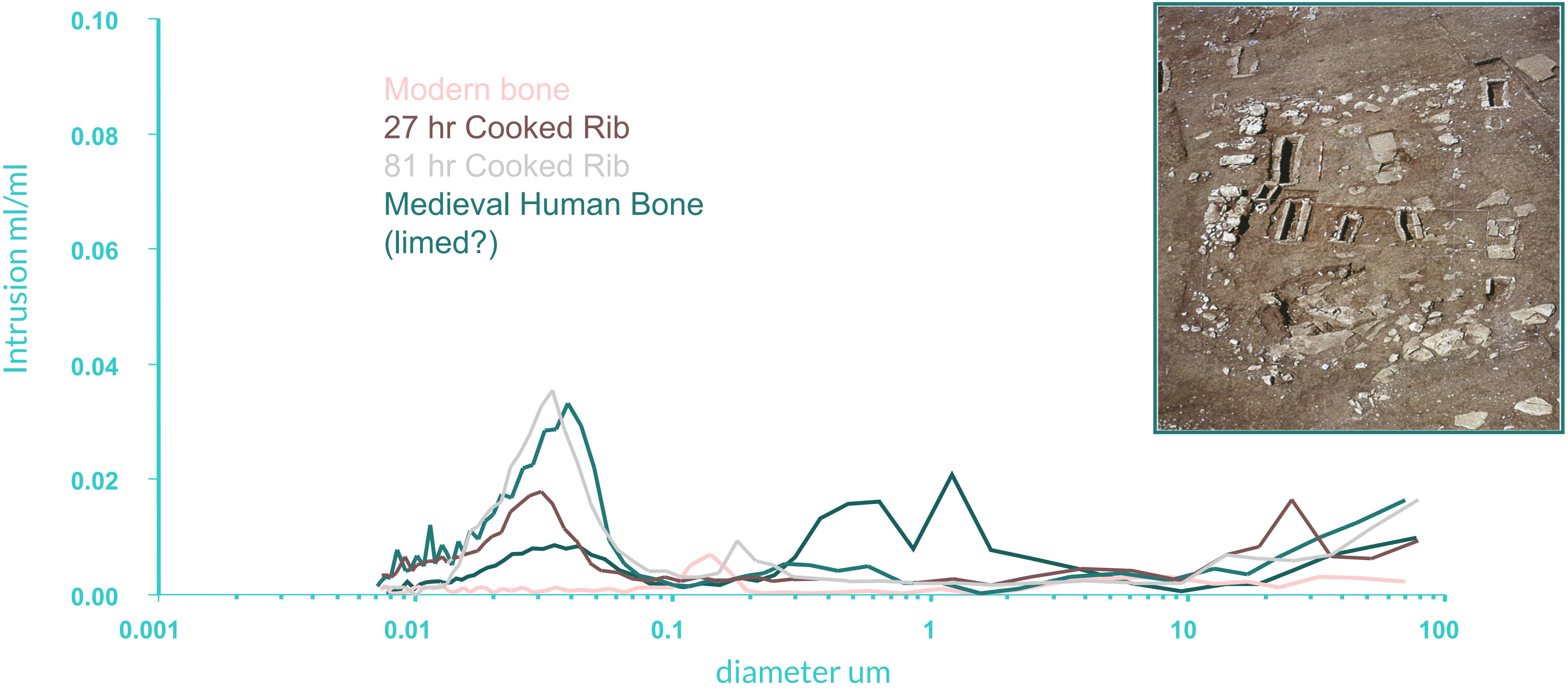
# Porosity: well preserved bone



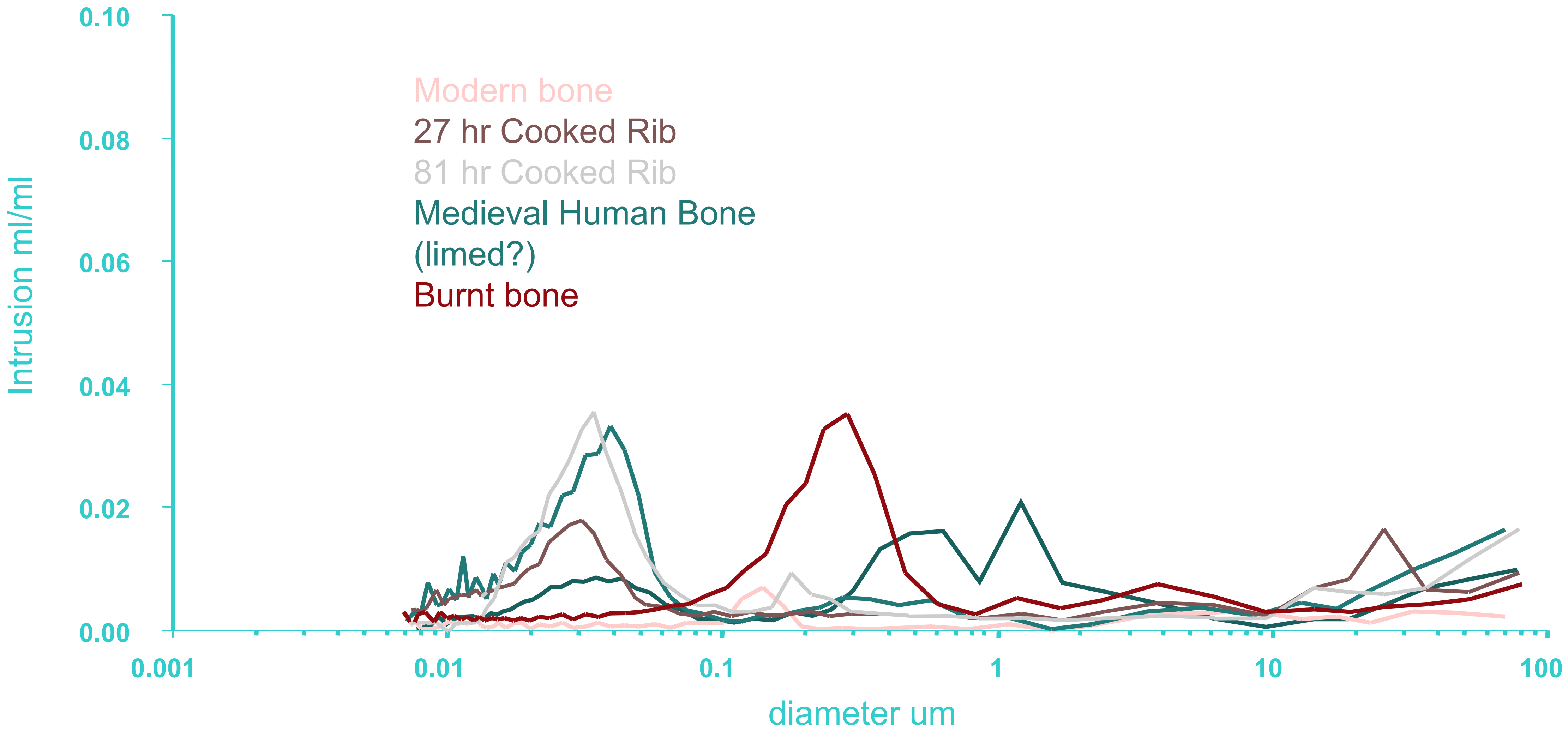
# Mycorrhizal fungi



# Boiling bone?

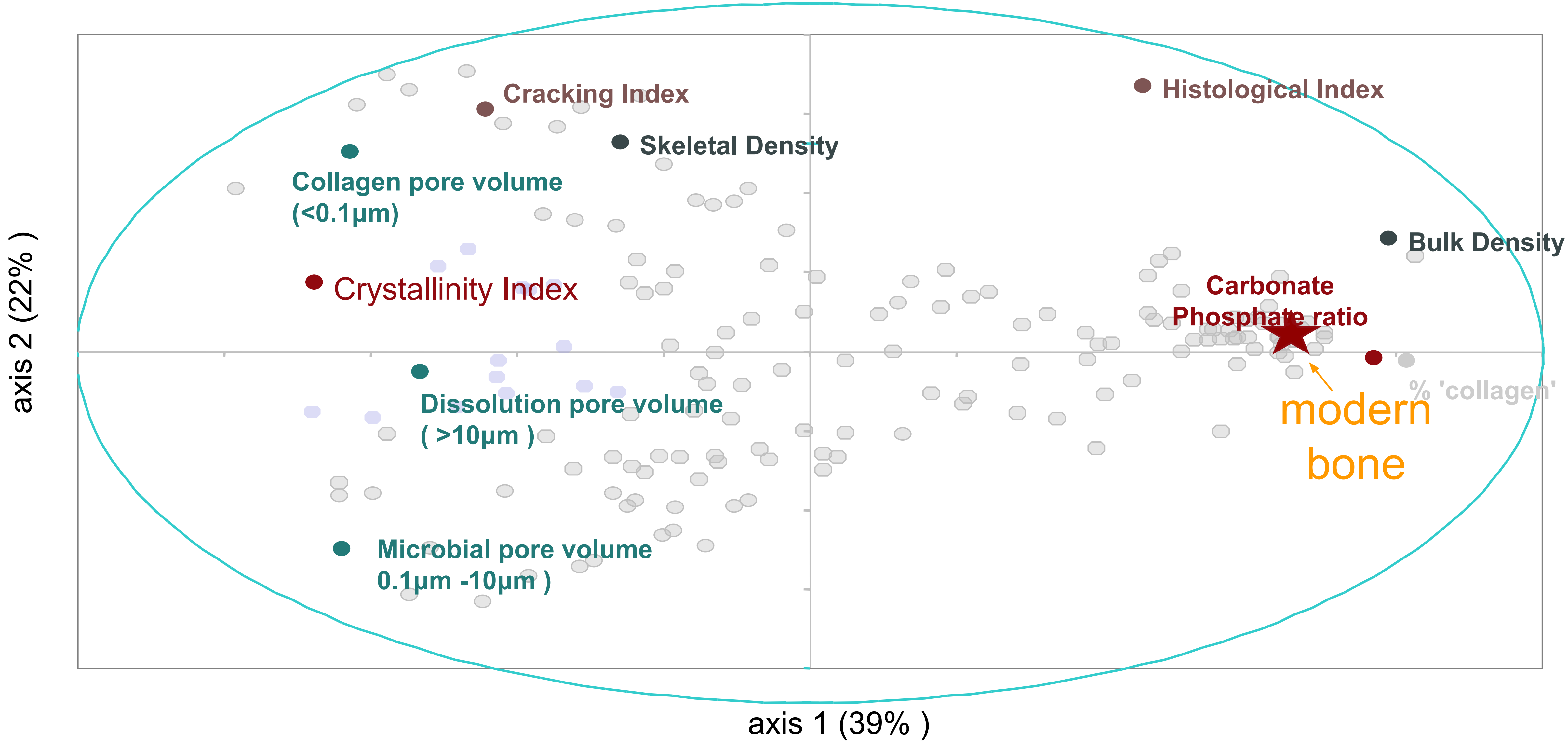


# Burnt bone?

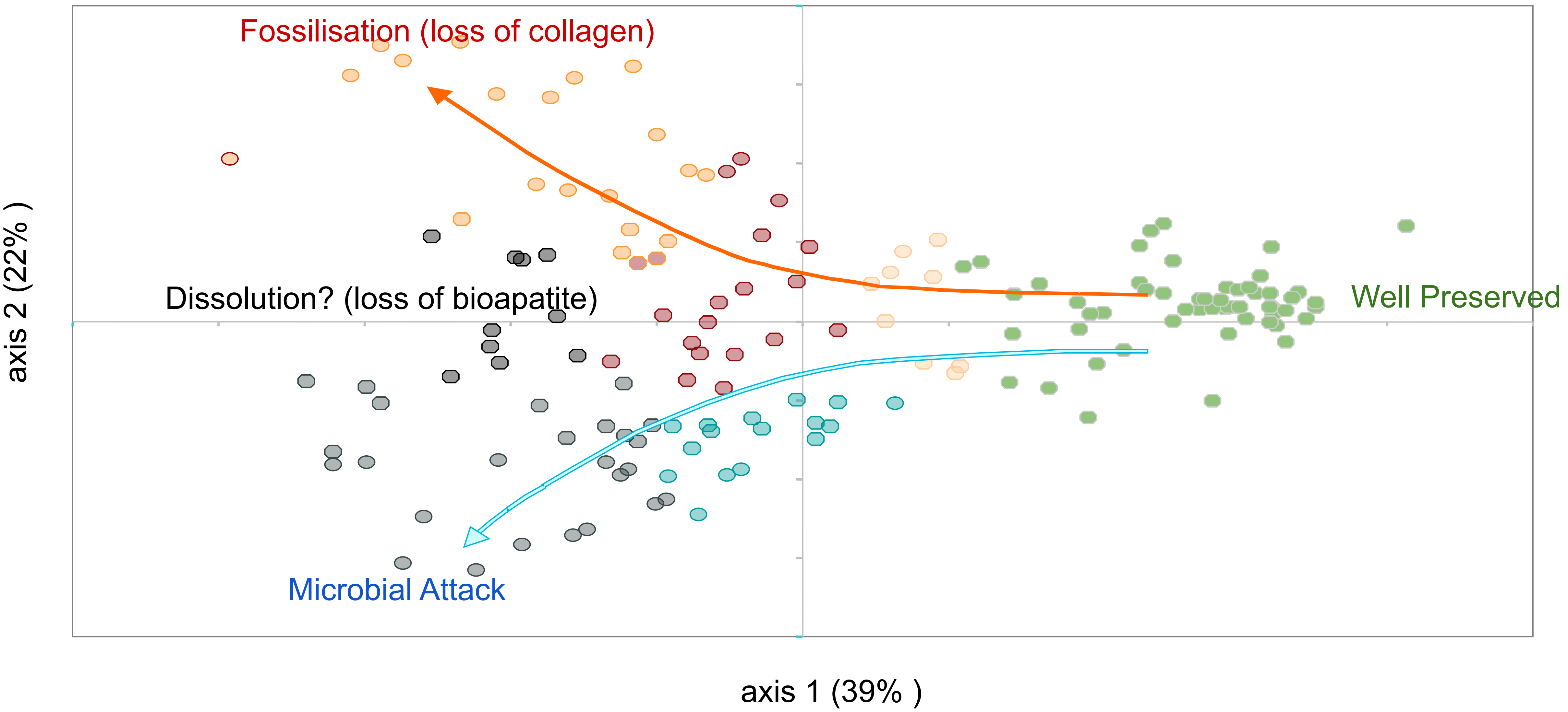


Modern bone
27 hr Cooked Rib
81 hr Cooked Rib
Medieval Human Bone
(limed?)
Burnt bone
Intrusion ml/ml
0.10
0.08
0.06
0.04
0.02
0.00
0.001
0.01
0.1
1
10
100
diameter um

# Principal Components Analysis

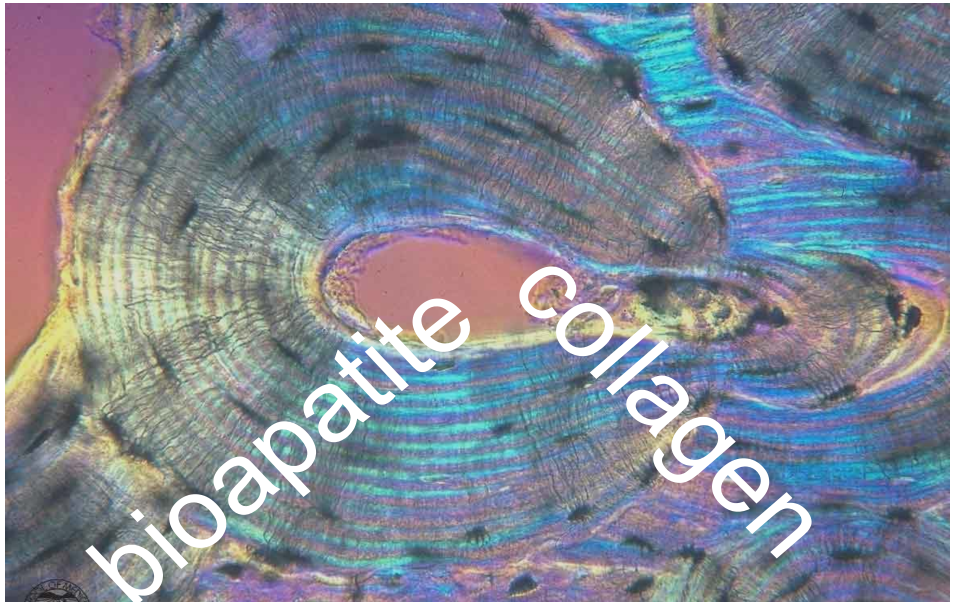


# PCA



Fossilisation (loss of collagen)
Dissolution? (loss of bioapatite)
Well Preserved
Microbial Attack
axis 2 (22% )
axis 1 (39% )

Bone corrosion




pH
saturation

1

3

2

time
temperature

**dissolution**

**microbial**

**fossilization**

# Legacy

# Bronze Age Britain?


Booth, T. J., Chamberlain, A. T., & Pearson, M. P. (2015). Mummification in Bronze Age Britain. *Antiquity*, 89(347), 1155–1173.

# Leads to generalised ideas...

*Oxford Histological Index*

| Index | Approximate % of intact bone | Description |
|---|---|---|
| 0 | <5 | No original features identifiable, except that Haversian canals may be present |
| 1 | <15 | Haversian canals present, small areas of well-preserved bone present, or lamellate structure is preserved by the pattern of destructive foci |
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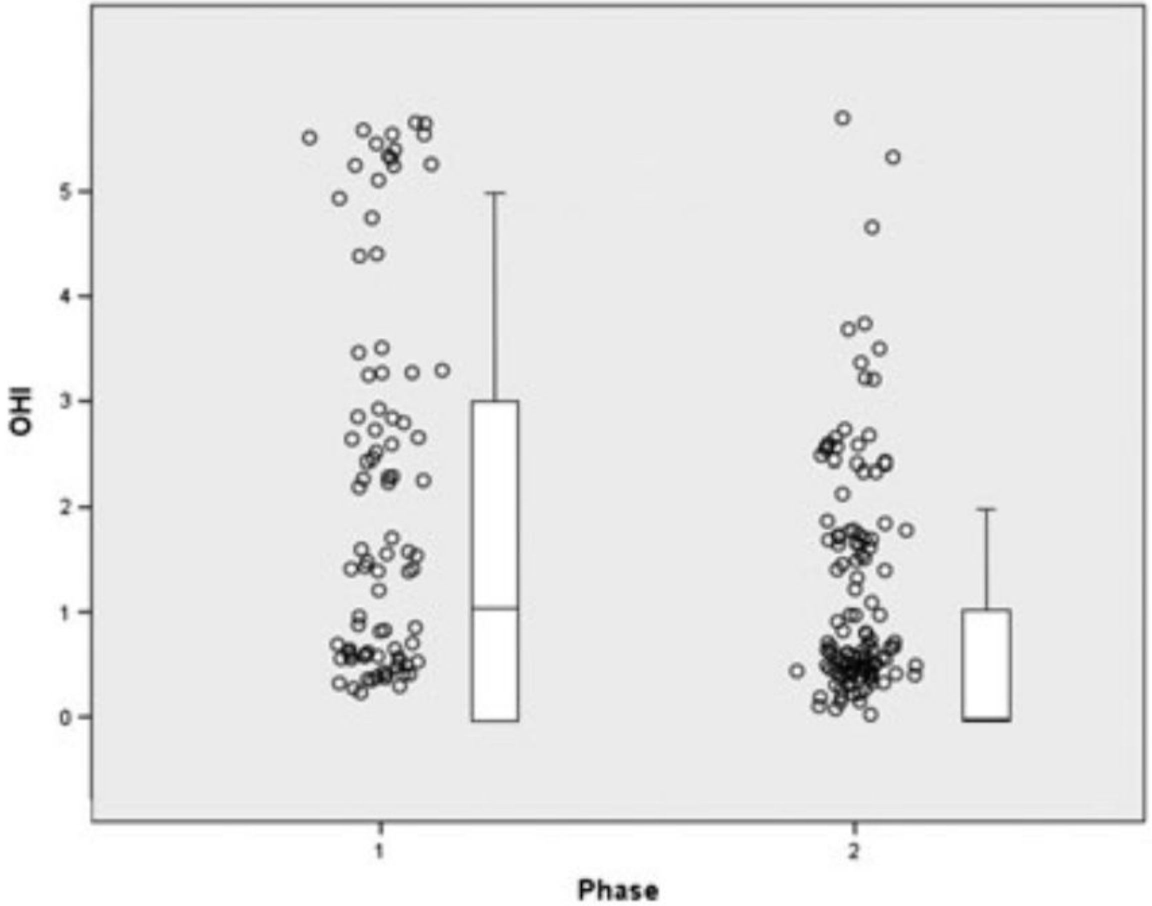
| 5 | >95 | Very well preserved, virtually indistinguishable from modern bone |

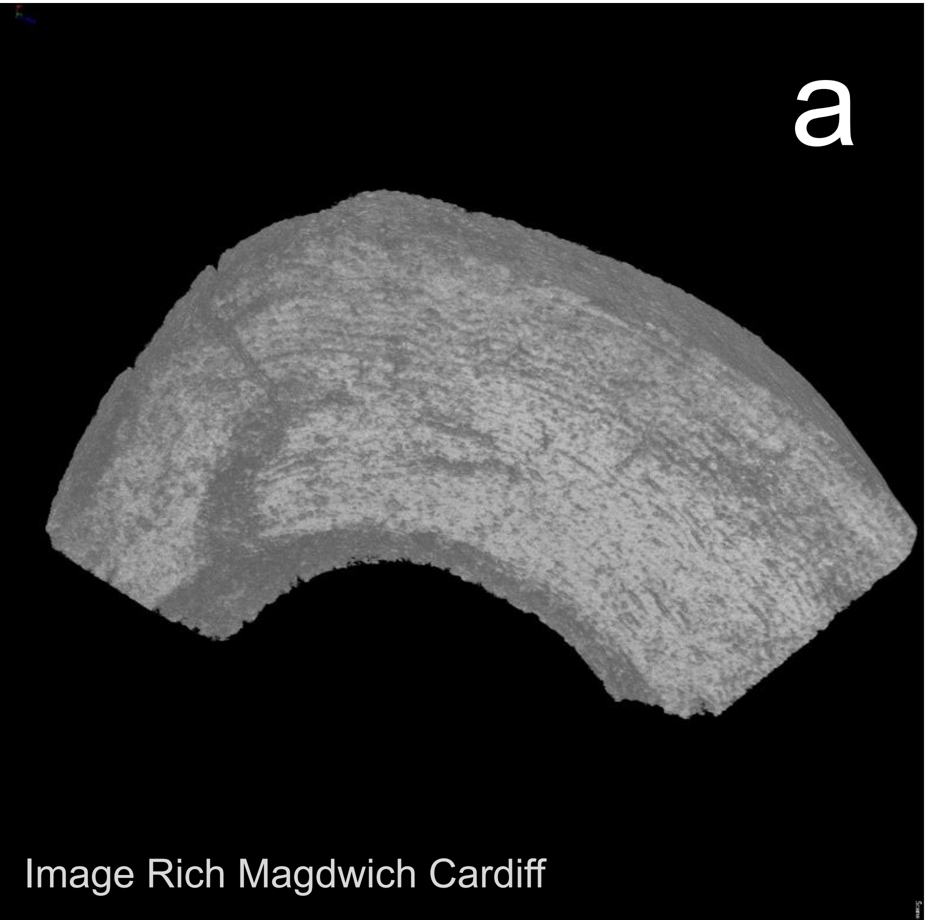


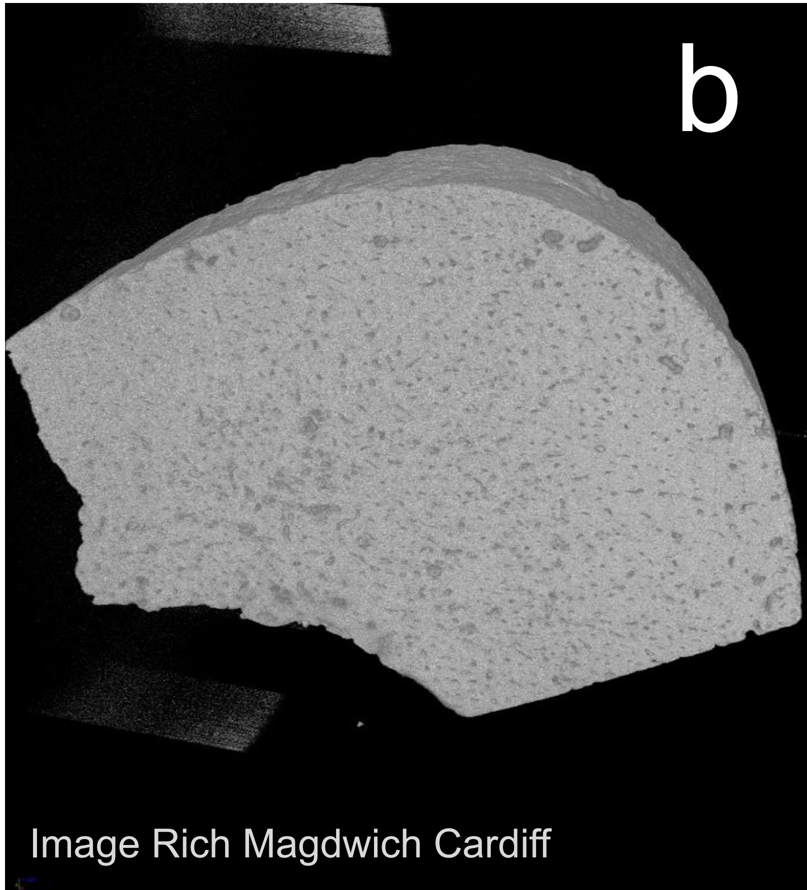

Well preserved histology

Poor histology

# What can you see here?

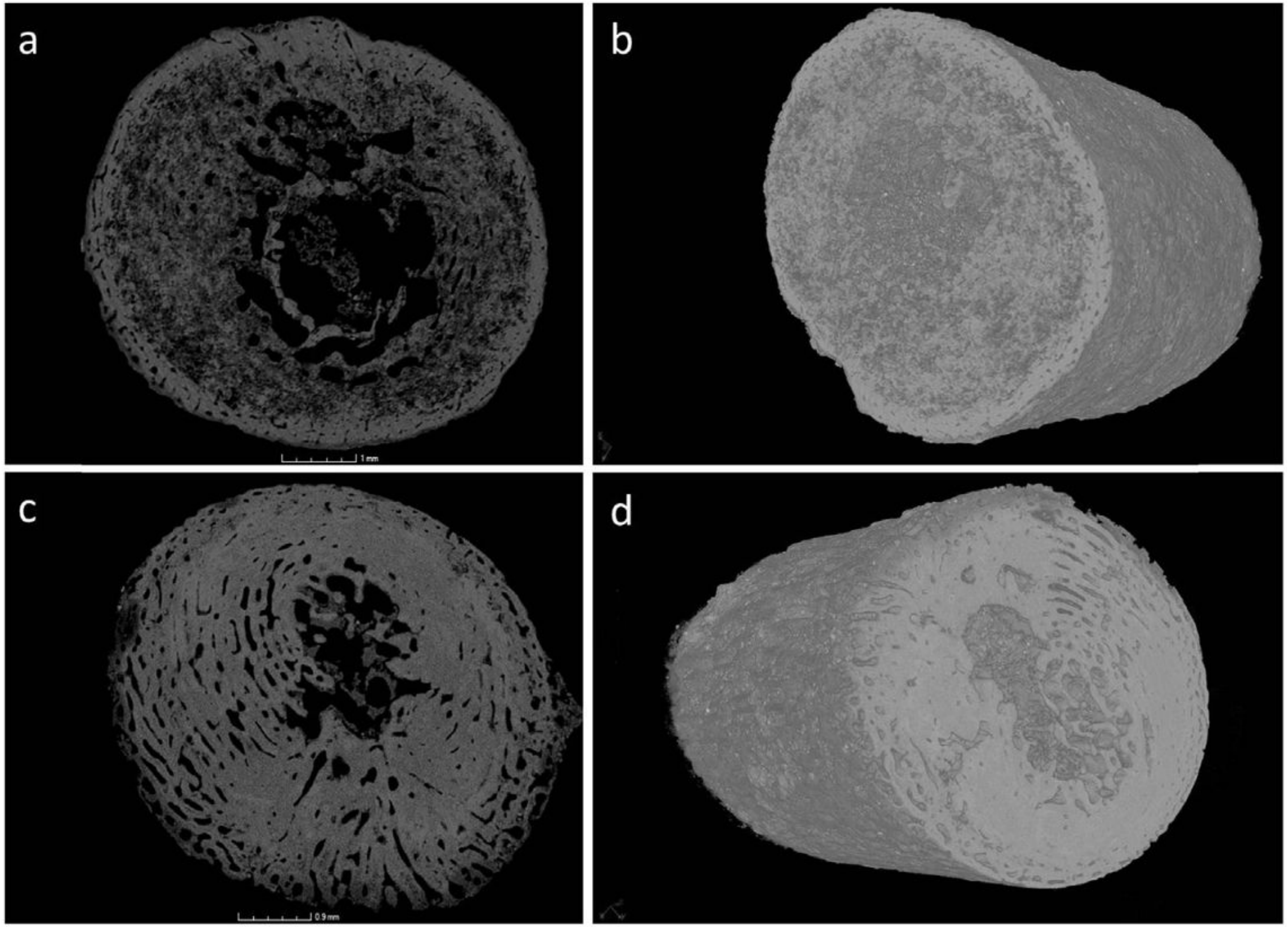
Computer Tomography (CT)



# CT imaging: Infant bone

Half of the infant samples studied here were free from bacterial bioerosion, further suggesting that histological analysis can be used to identify archaeological remains of stillborn and short-lived infants.




**The samples that were not tunnelled had “bright inclusions” in them. Probably  pyrite framboids. Thus, probably in anoxic conditions.**

Booth, T. J., Redfern, R. C., & Gowland, R. L. (2016). Immaculate conceptions: Micro-CT analysis of diagenesis in Romano-British infant skeletons. *Journal of Archaeological Science*, *74*, 124–134.

# Experimental burials

Anne-Marie Høier Eriksen,

# Experimental

(a) Raw bone fragments

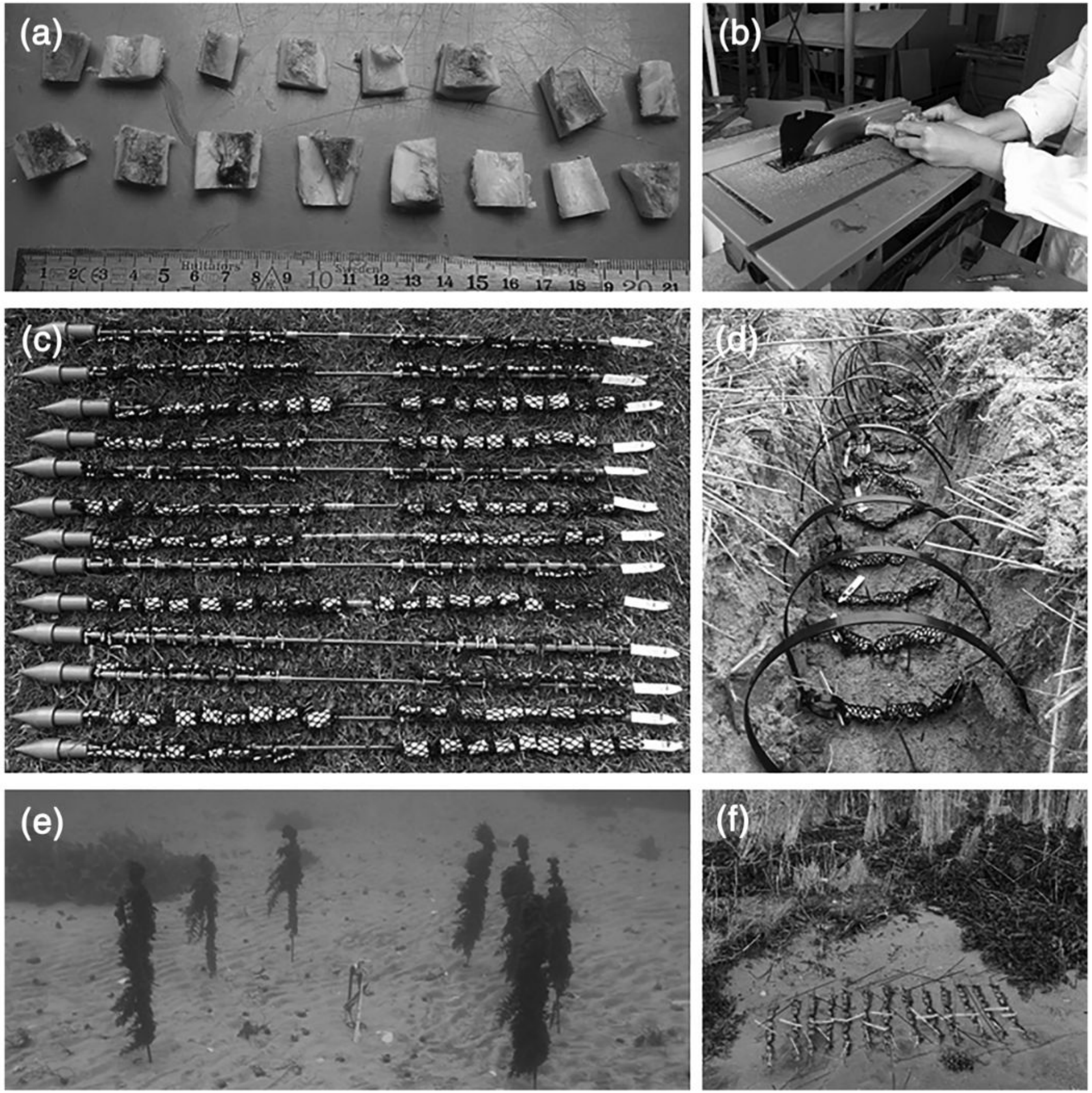
(b) cutting  fragments

(c) spears with raw,boiled and baked bone fragments were embedded at the two submerged study environments; half the fragments were embedded into the sediment and the other half were suspended in the water column

(d) terrestrial  environment the fragments were placed at the bottom of a 30 cm-deep trench;

(e) sea-bed spears overgrown with algae after 28 weeks

(f) bones at the tidal zone were left on the shore and subjected to wet–dry cycles.

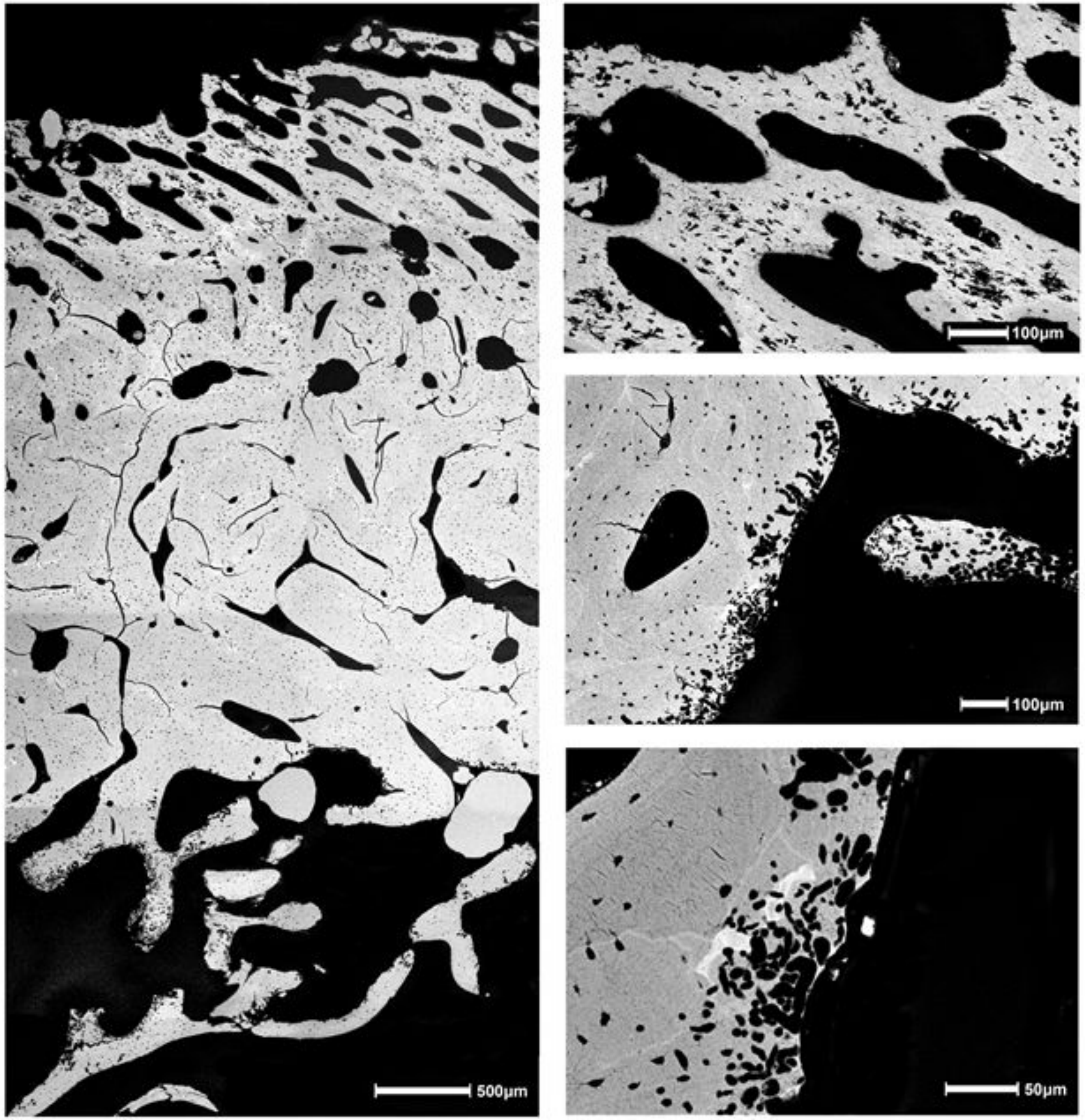



Eriksen, A. M. H., Matthiesen, H., Kontopoulos, I., Gregory, D., Snoeck, C., Zhang, G., Collins, M. J., & Gilbert, M. T. P. (2020). Rapid loss of endogenous DNA in pig bone buried in five different environments. *Archaeometry*, **62**(4), 827–846.

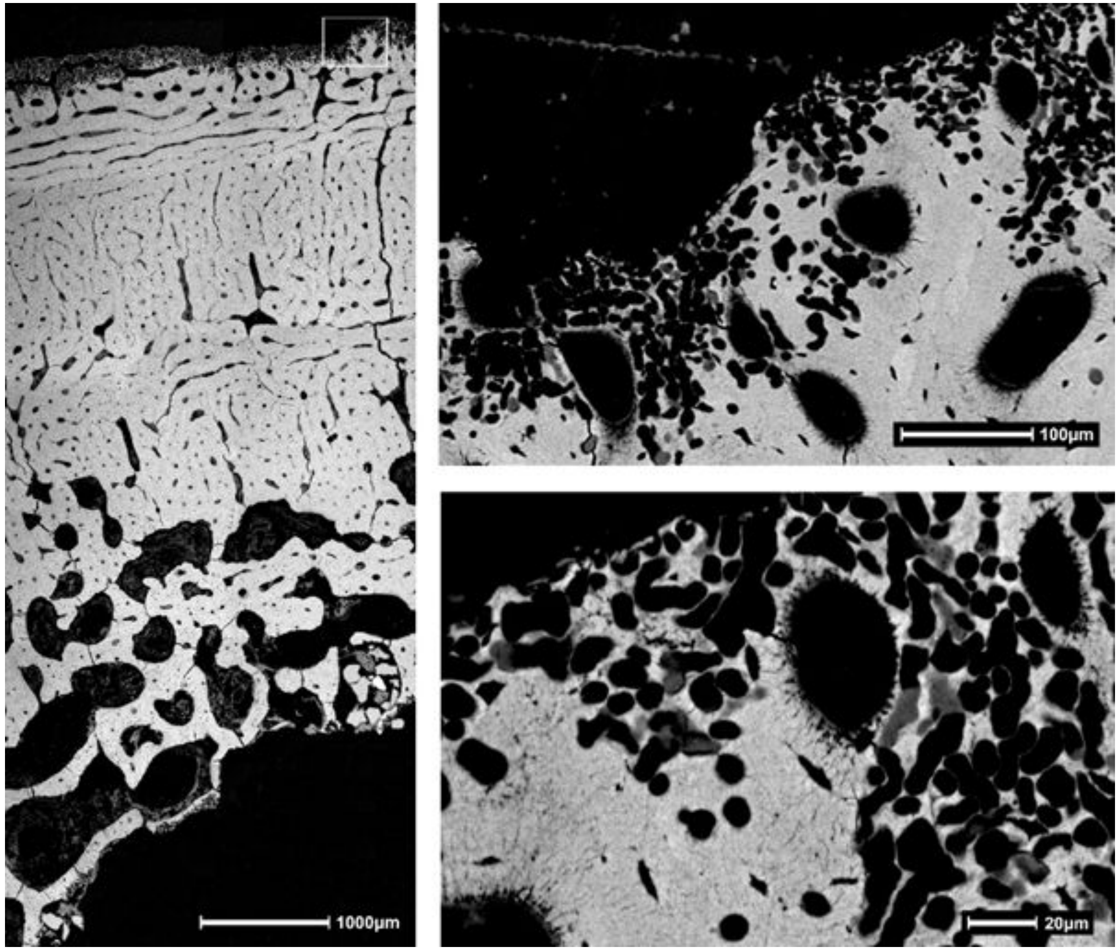
# Submerged gyttja

Baked bone, Submerged gyttja,
52 weeks.

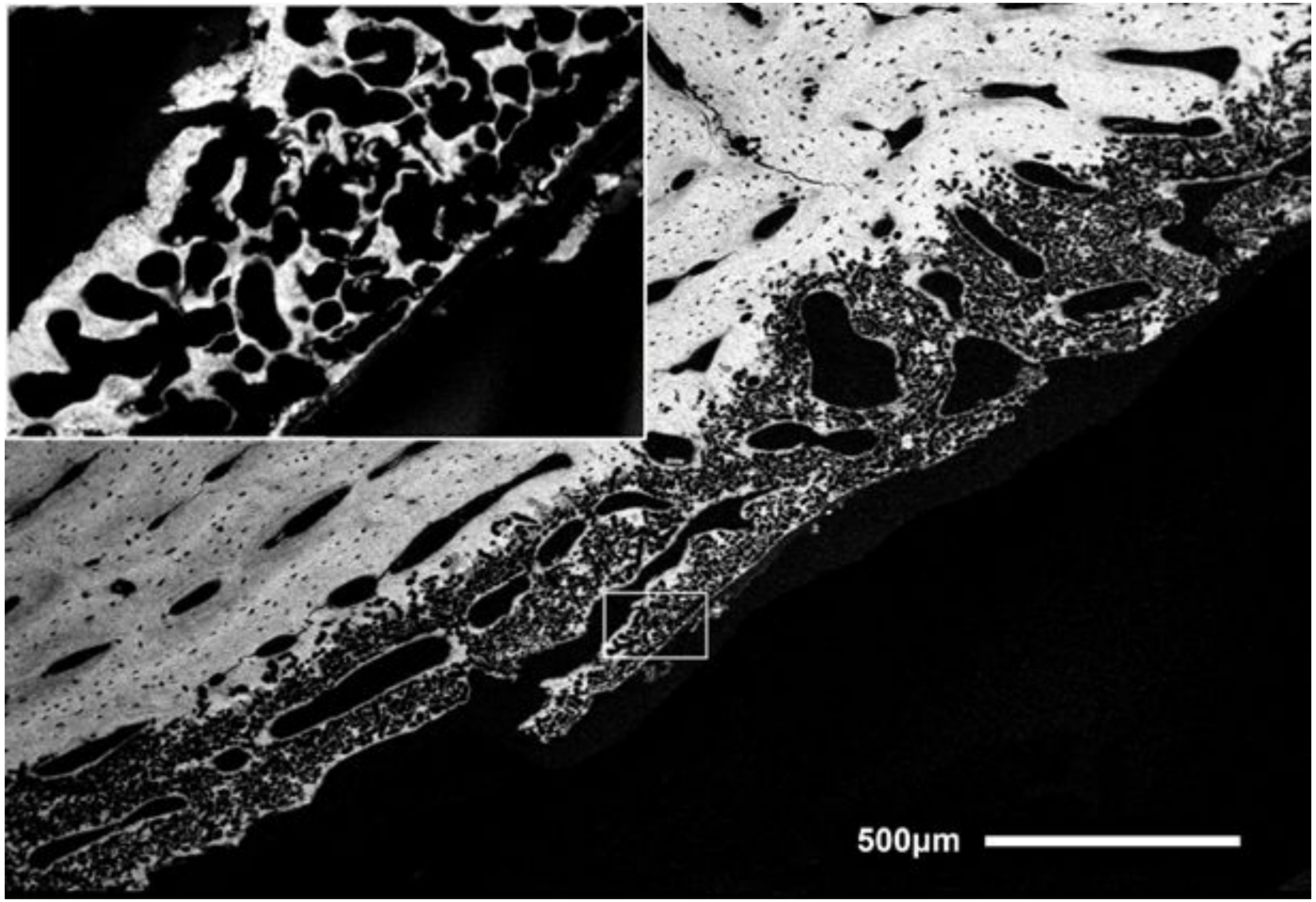
- chemical demineralisation on the periosteal surface.
- cyanobacterial attack.
- enlarged and ragged osteocyte lacunae and enlarged canaliculi.



# Tidal Zone: Cyanobacteria



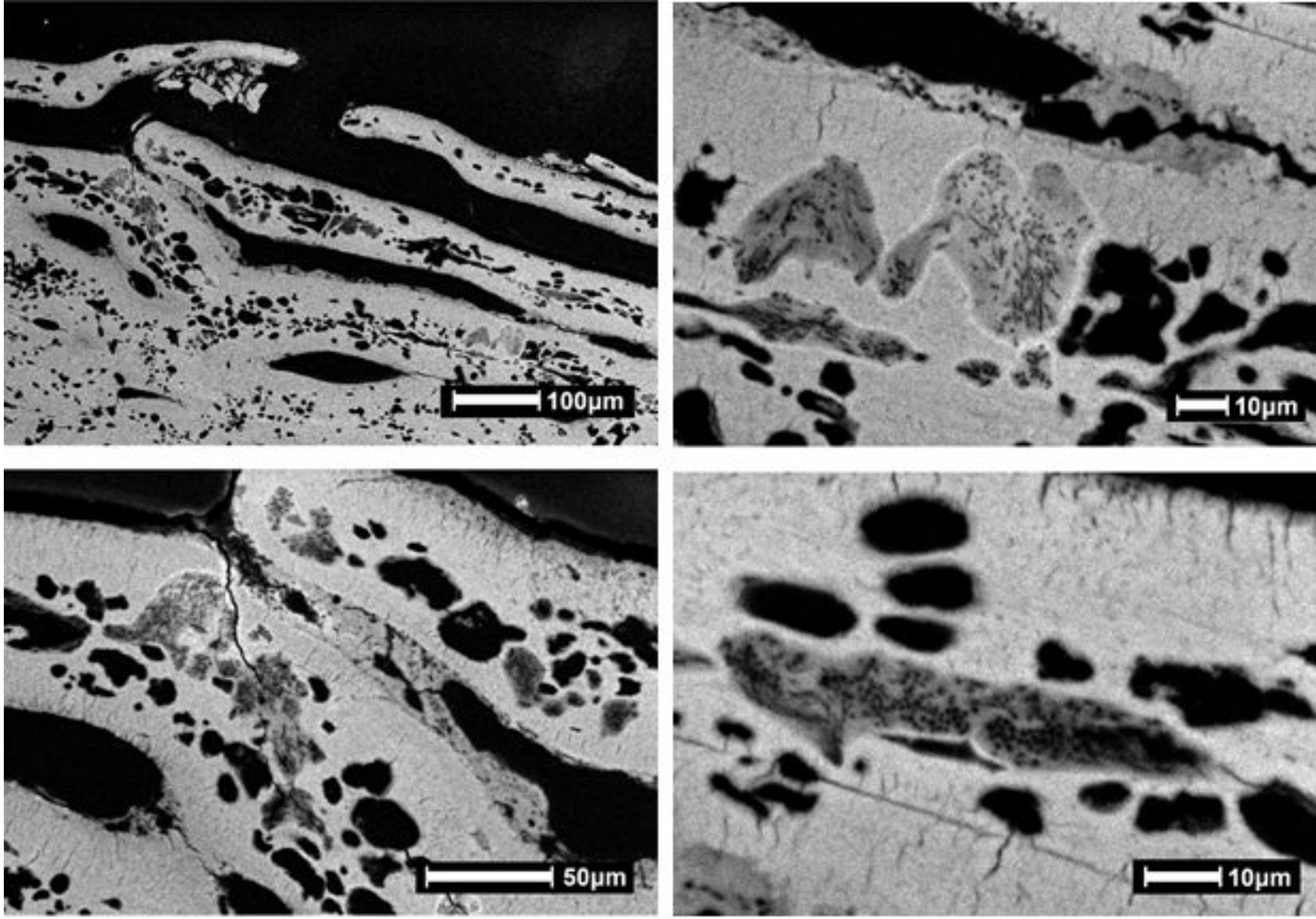

Tidal zone, buried for 52 weeks.
Enlarged area showing extensive cyanobacterial attack on the periosteal side. All samples at the tidal zone at 52 weeks showed this kind of damage.




Tidal zone, buried for 52 weeks.
Enlarged area showing extensive cyanobacterial attack on the periosteal side. All samples at the tidal zone at 52 weeks showed this kind of damage.

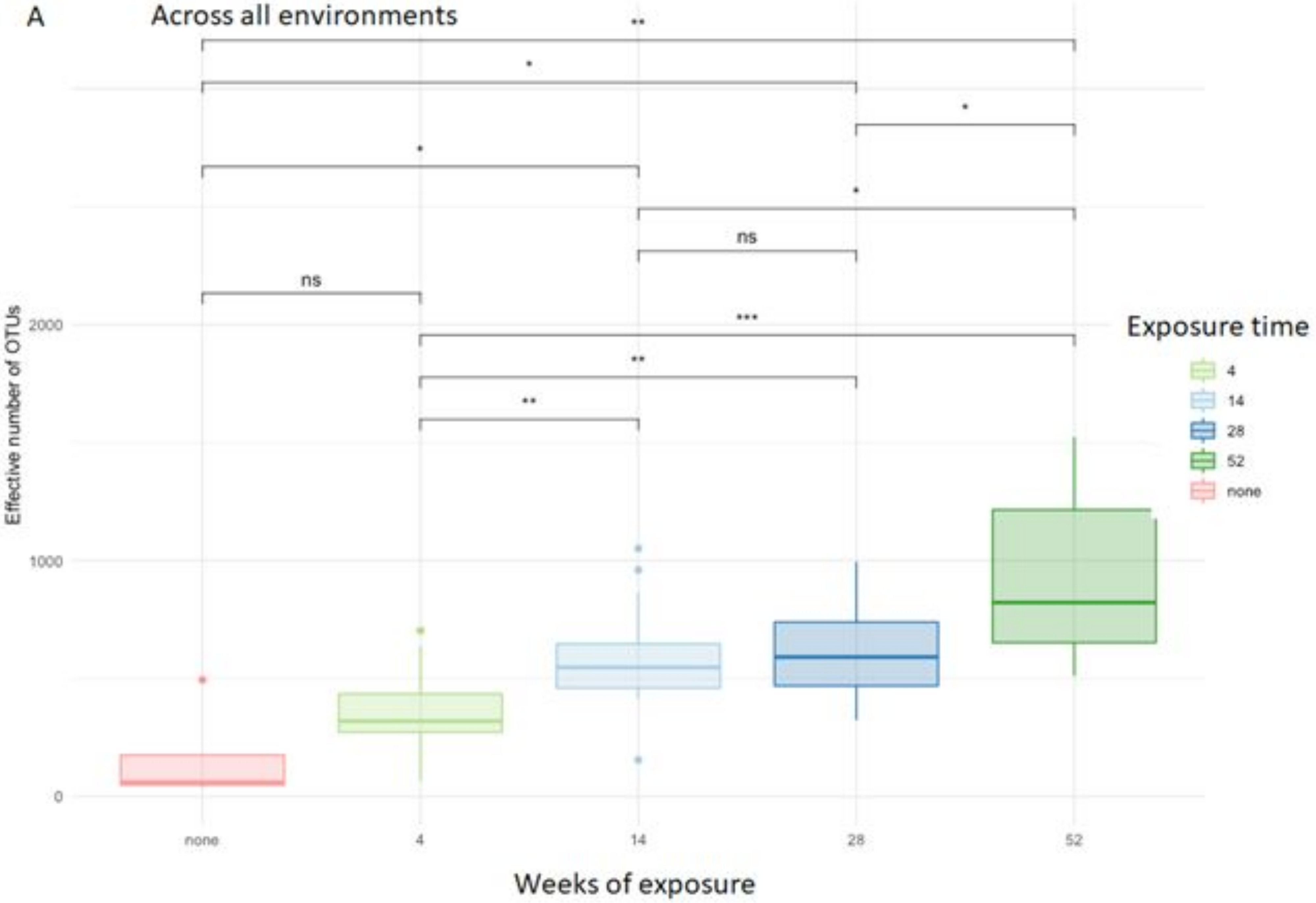
# Terrestrial

Terrestrial sand, 52 weeks. Microbial tunnelling is observed from the periosteal border and to 200 µm depth.



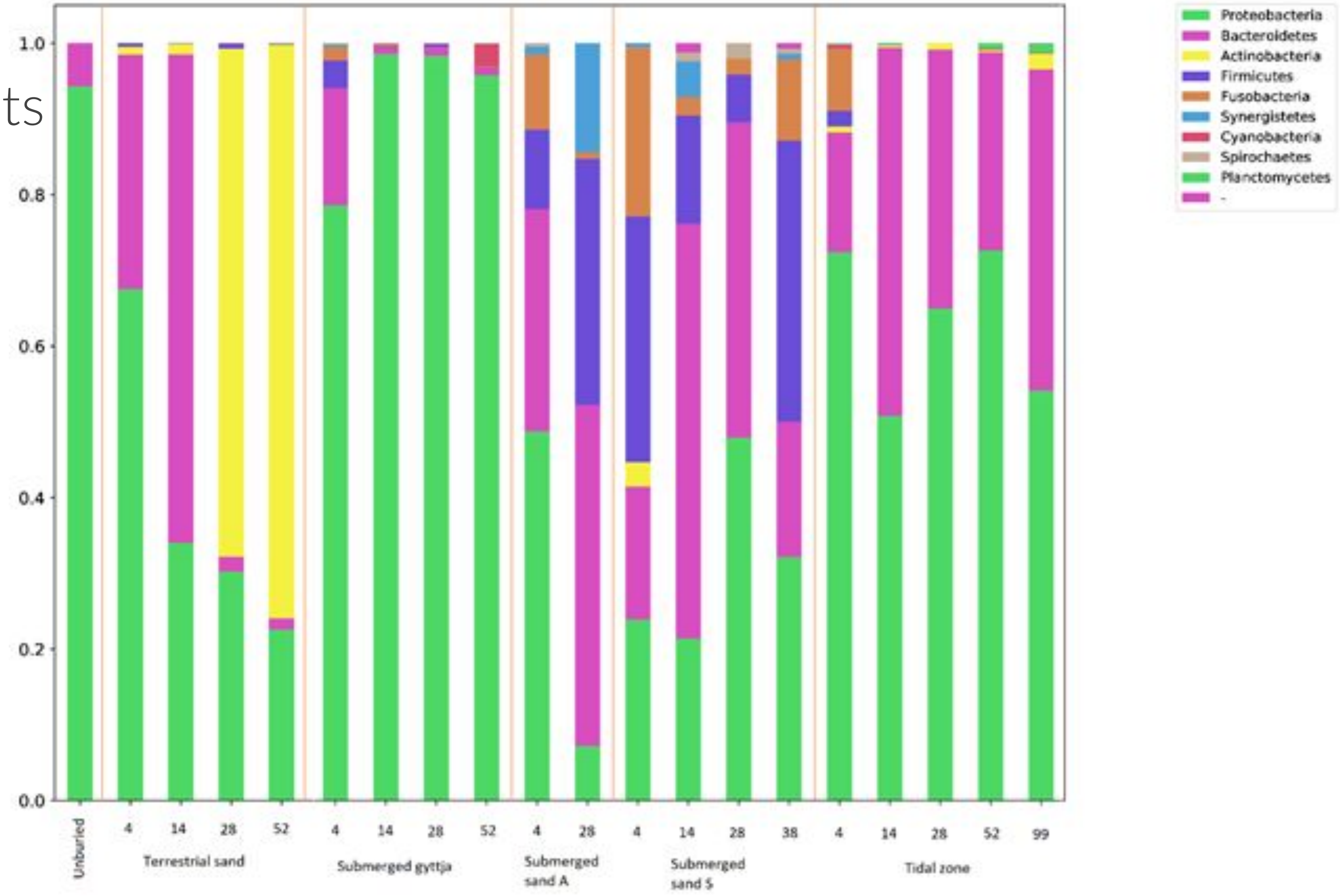
# DNA analysis of the microbial community

# Increase in microbial diversity




Eriksen, A. M. H., Nielsen, T. K., Matthiesen, H., Carøe, C., Hansen, L. H., Gregory, D. J., Turner-Walker, G., Collins, M. J., & Gilbert, M. T. P. (2020). Bone biodeterioration-The effect of marine and terrestrial depositional environments on early diagenesis and bone bacterial community. *PloS One*, **15**(10), e0240512.

# Burial Environments



# Actinobacteria: *Streptosporangium*

- Clostridiacea and Fusobacteriaceae in the two submerged sites,
  - both of which are known to be have members linked to collagenolytic enzymatic activity
- Xanthomonadaceae (a Proteobacteria) found in the Terrestrial site,
  - has previously been found in degrading bone from individuals were placed outside on the ground surface to decompose naturally at the Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville
  - Most common organism Actinobacteria *Streptosporangium*

# Neanderthal bone

3.95 Gb of Neanderthal DNA isolated from the Vindija Neanderthal Vi33.16 fossil showed that 90% of about 50,000 rRNA gene sequence reads were of bacterial origin, of which Actinobacteria accounted for more than 75%.

Actinobacteria also represented more than 80% of the PCR-amplified 16S rRNA gene sequences from a cave sediment sample taken from the same G layer as the Neanderthal bone.

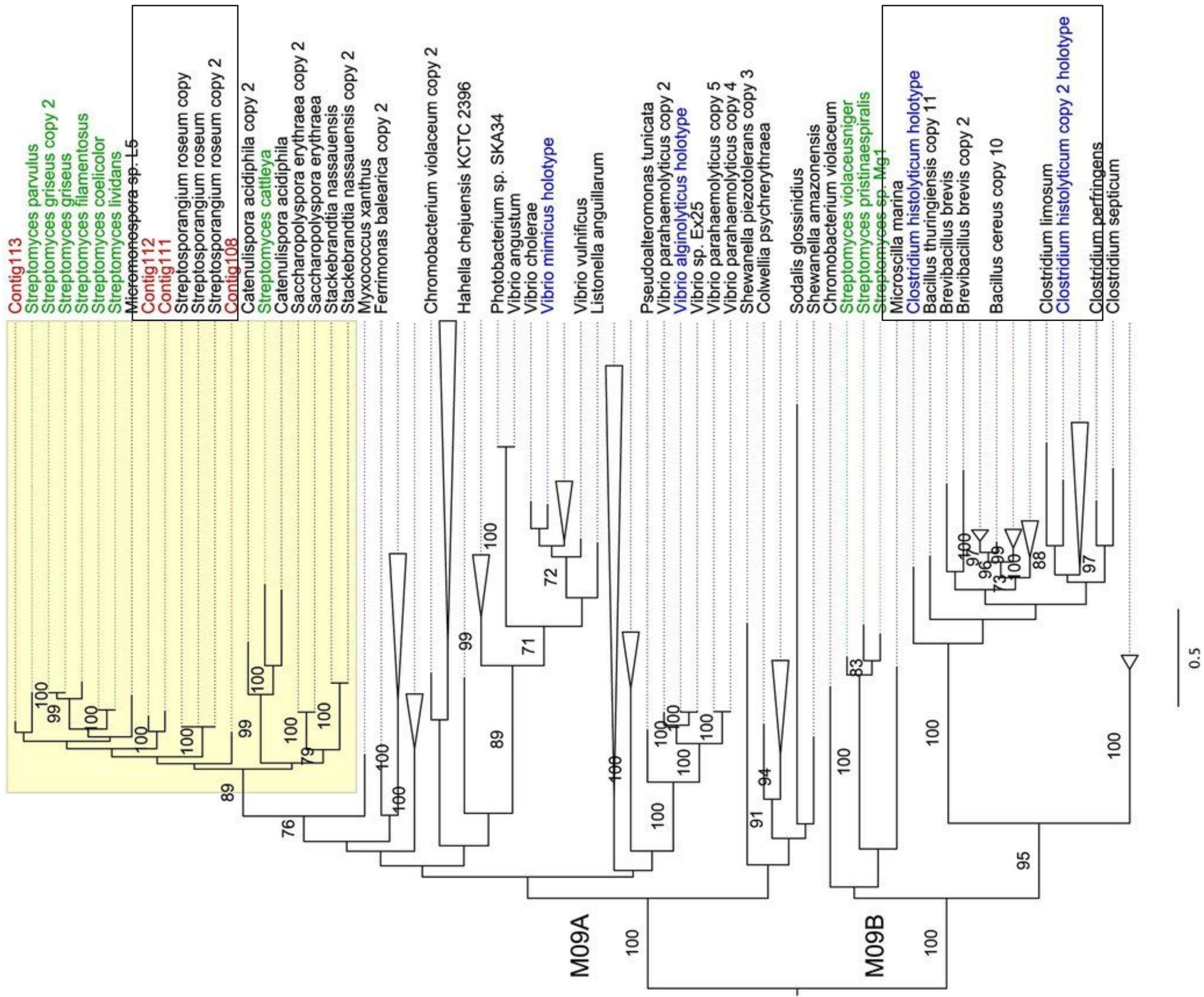
The bacterial DNA showed no signs of damage, and we hypothesize that it was derived from bacteria that have been enriched inside the bone.

Zaremba-Niedźwiedzka, K., & Andersson, S. G. E. (2013). No ancient DNA damage in Actinobacteria from the Neanderthal bone. PloS One, 8(5), e62799.

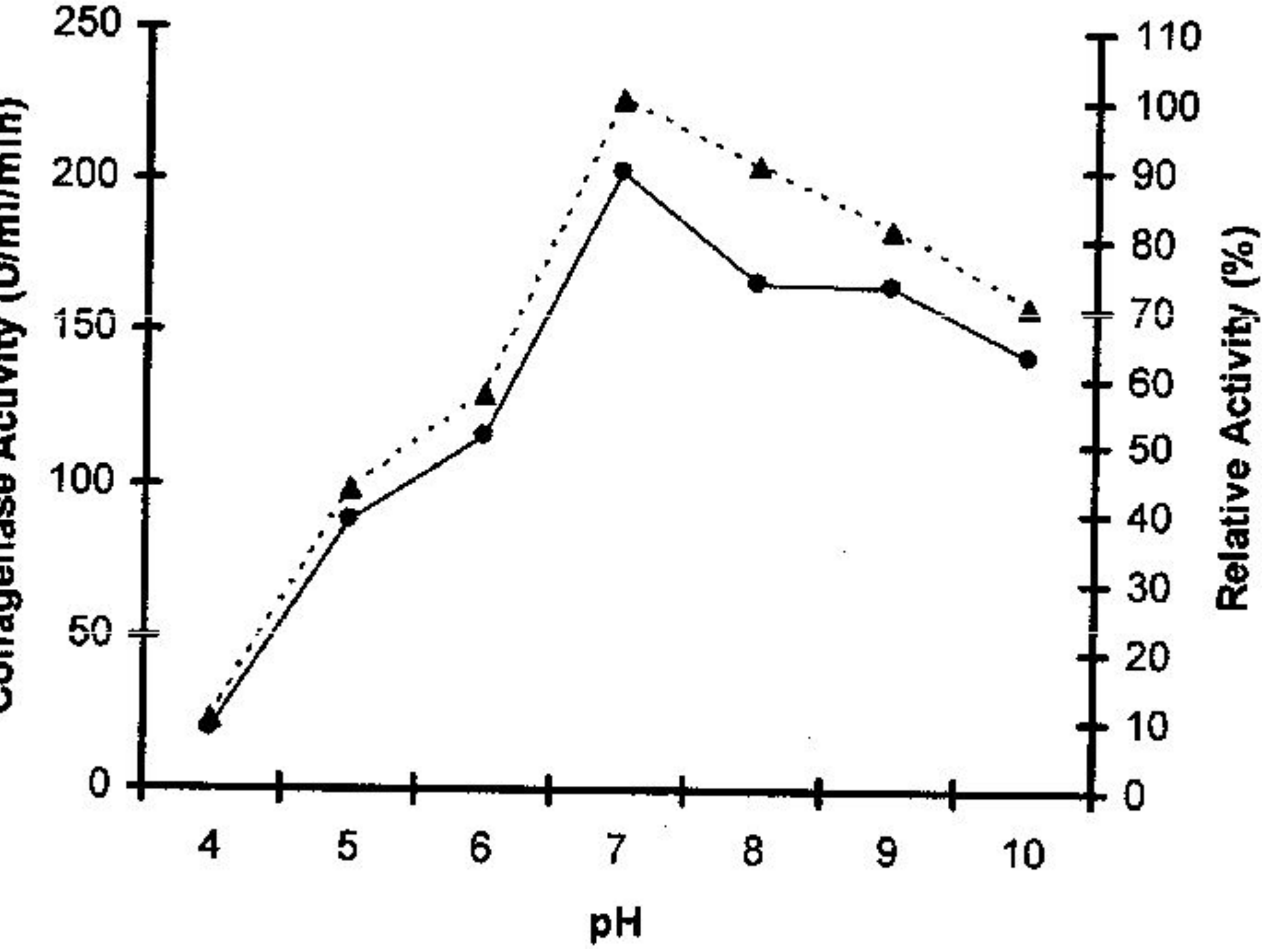
# Phylogeny of microbial collagenases.

Collagenase consensus sequences are coloured in red The actinobacterial clade is highlighted in yellow

doi:10.1371/journal.pone.0062799.g005

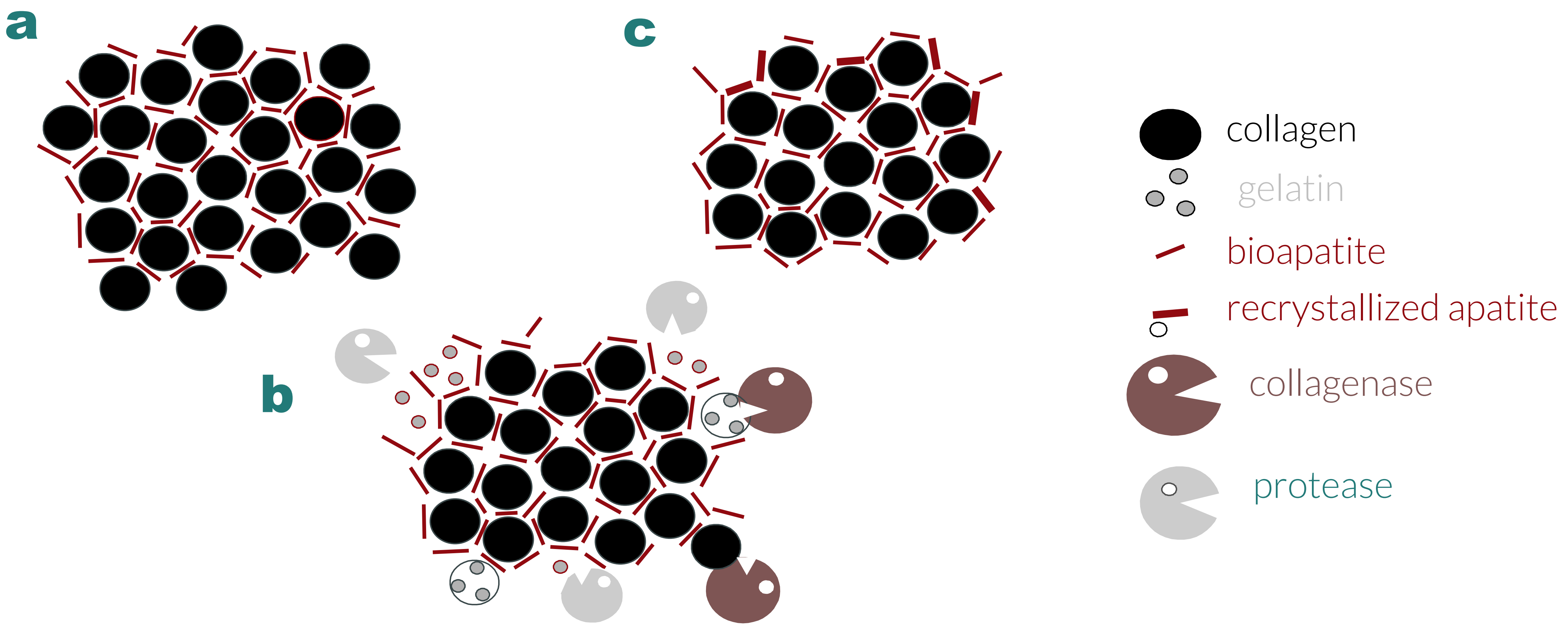


# Collagenase



# Dissolution removes mineral

- Why no organic matter?
- Removal of mineral exposes collagen



# SOLID

Henning and Scott