BIOMINERALIZATION

Introduction

Frédéric MARIN
UMR CNRS 6282
Université de Bourgogne – Franche-Comté
Dijon

BIOGÉOSCIENCES

UMR 6282 Biogéosciences, DIJON

> 120 permanent & non-permanent employees

- **TEAM ECO/EVO**
- **TEAM BIOME**
- **TEAM SAMBA**
- **TEAM SEDS**
- **TEAM CRC**

SUB-TEAM Biomineralizatio n

- Exists since 2003
- 2 permanent members

cnrs dépasser les frontières

École Pratique des Hautes Études

uB UNIVERSITÉ DE BOURGOGNE

UBFC UNIVERSITÉ BOURGOGNE FRANCHE-COMTÉ

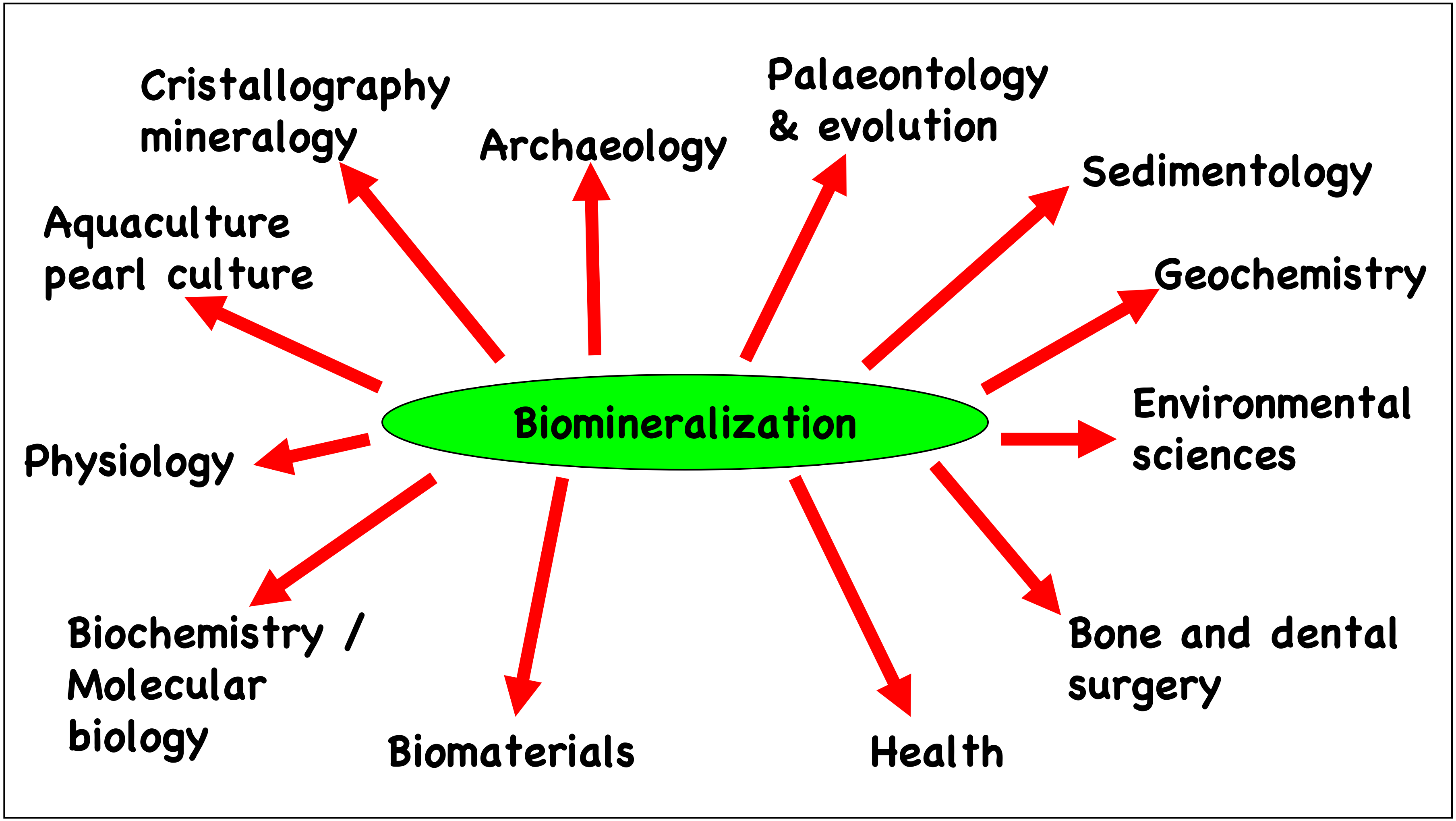
Biomineralization: different meanings…

Mineralized structures produced by living systems

Process by which living systems produce minerals

Scientific discipline in its own

Biomineralization: scientific discipline



A little pinch of history…

A brief history of biomineralization

Very first use of biominerals:
- Shells sewed on clothes, ornaments

Ex: Grimaldi children: 11000 years BP.

- Teeth as ornaments (necklaces)

- Currency: cowries (*Cypraeidae*)

- 1st glue from bone and skin collagen

A brief history of biomineralization

1st dental implant in Mayas: 7-8th century BP

3 implants made from bivalve nacre (Bobbio, 1972). Completely osteointegrated in the jaw.

A brief history of biomineralization

Middle Age:

- Religious symbols

From XVIIth century:

- Marquetery work

- Manufactured objects

A brief history of biomineralization

<u>From XVIIth century</u>:

- Jewels: pearls

- Industrial fabrication of glue from bone/skin collagen

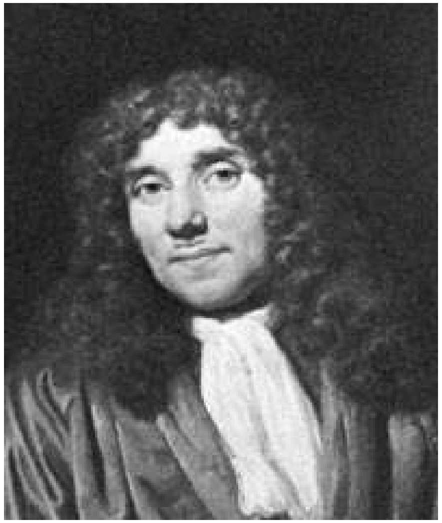
<u>From XIXth century</u>:

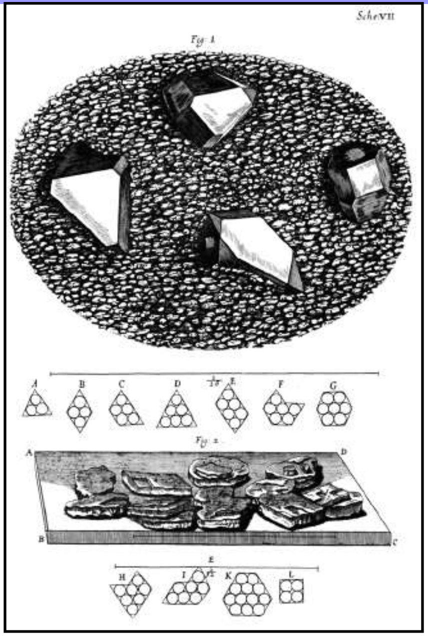
- Soil enrichment

A brief history of biomineralization

Andreas Vesalius: the Basel skeleton (1543): **anatomical Museum of the Basel Univ.**

Jan Swammerdam / Anton van Leeuwenhoek: 1st optical microscopes, circa 1660…





Robert Hooke: ***Micrographia*** **(1665): 1st observations of biominerals under microscope**
('of gravels in urine ')

A brief history of biomineralization

Clopton Havers (1657-1702):
1st descriptions of bone microstructure.
Osteologia nova, or some new Observations of the Bones, and the Parts belonging to them, with the manner of their Accretion and Nutrition (1691).

De Lasone (1751): 1st experiments of bone calcining.

1811-1823: Braconnot/Odier: chitin discovery

Frémy (1855): 1st chemical characterization of biominerals.

- Bone, teeth.
- Crustacean teguments.
- Gorgonian coral exoskeleton
- Mollusc shell (nacre) and cuttlefish bone.

A brief history of biomineralization

2nd half of XIXth century – 1st half of XXst century

- Several chemical analyses of biominerals.
- Several observation with optical microscope (Schmidt, Boggild)

After War period:

- Development of biochemistry and of several techniques for separating biomolecules:
    - *Amino acid analysis: bone, teeth, shells (1953-1955).*

- XRD of biomolecules: *3D structure of bone collagen (1954, G. N. Ramachandran).*

A brief history of biomineralization

The Sixties:

- Several biochemical characterizations of biominerals.

- Development of electron microscopy: TEM, SEM.

The Seventies:

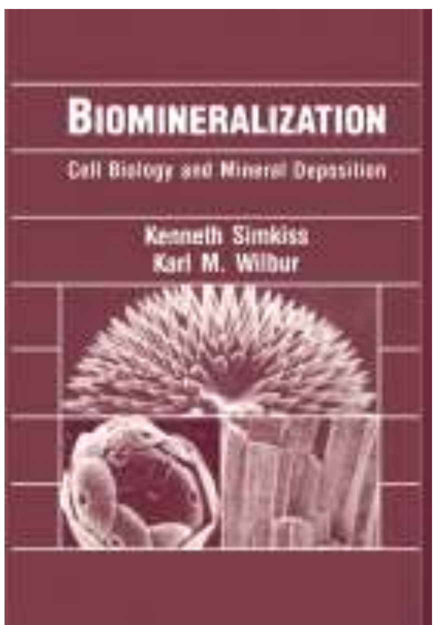
- 1^{st} International Symposium on Biomineralization: 1970.
  16th Symposium in China in 2021: BIOMIN XVI

- 1^{st} molecular models on biomineralization.

A brief history of biomineralization

H. Lowenstam, K. Simkiss, A. Veis, M. Glimcher, W. Traub, B. Landis, A. Salleudin, J. Oldak, K. Wilbur, S. Weiner, H. Mutvei, M. Crenshaw, P. Westbroek, L. Addadi...

Lowenstam & Weiner (1989)



Simkiss & Wilbur (1989)

A brief history of biomineralization

TODAY...

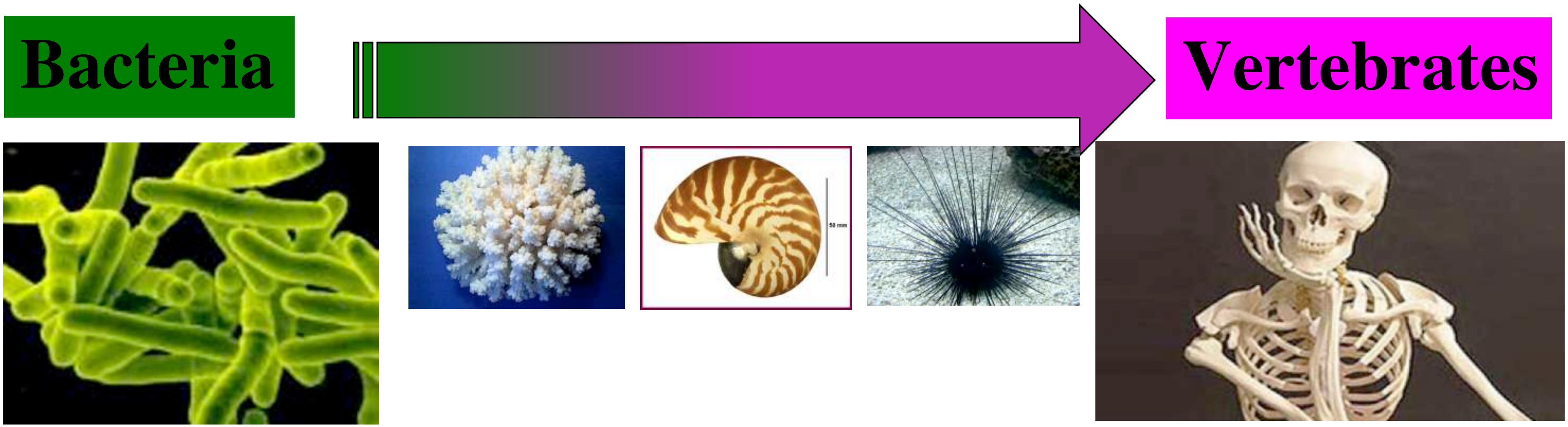
Beside the Int. Symp. on Biomineralization, several important scientific events:

- ***Gordon Research Conference (GRC) on Biomineralization***

- ***ICCBMT (International Conference on the Chemistry & Biology of Mineralized Tissues)***

Several international or national conferences have sessions dedicated to biomineralization: ***Goldschmidt Conference, Int. Marine Biotech Conf., Int. Sclerochronology Conf., ...***

Biomineralization, a widespread phenomenon



Biomineralization:

55 phylums

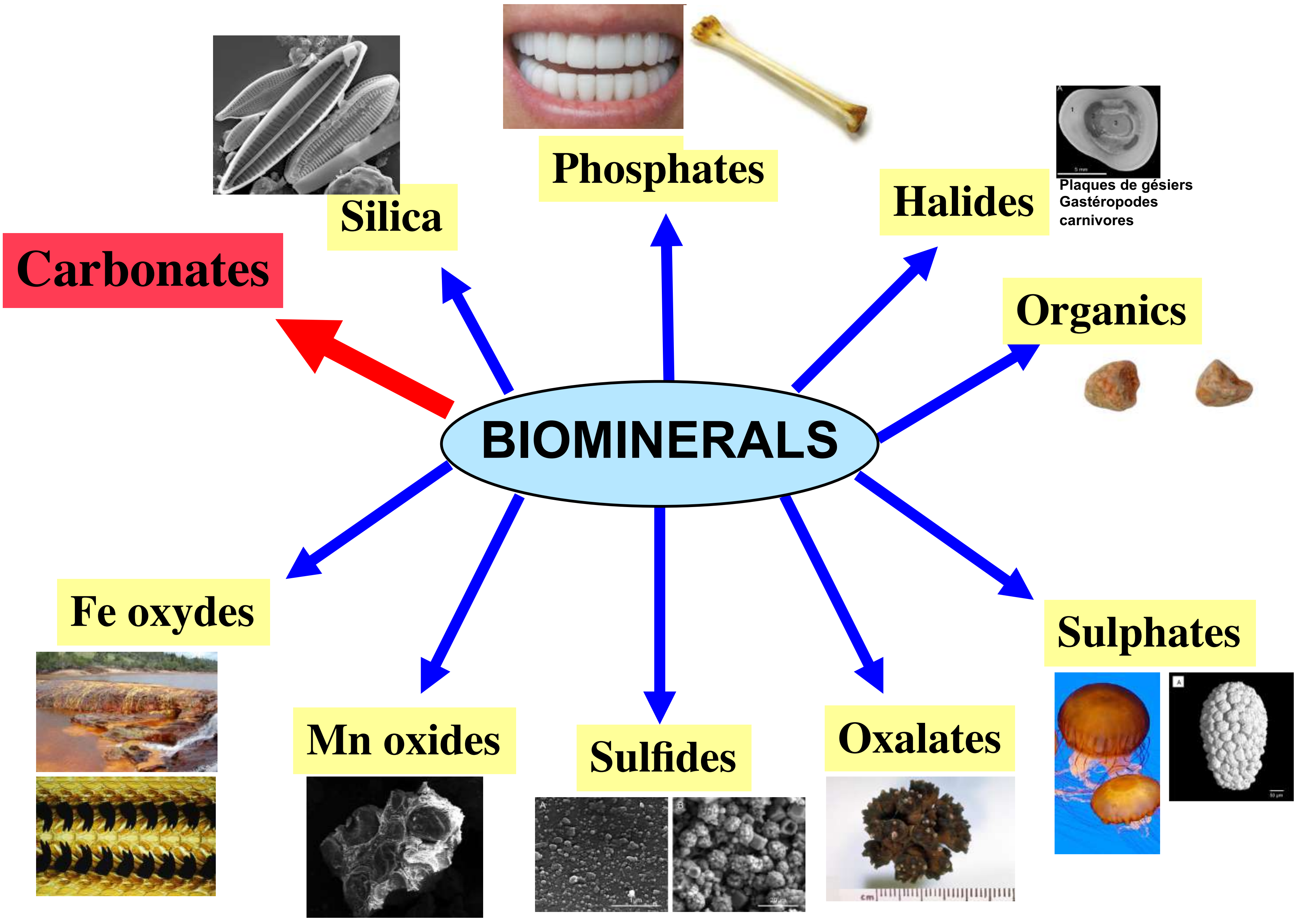
(living or fossils)

Bacteria

Archaea

Eucarya

About 70 different minerals!!



BIOMINERALS
Carbonates
Silica
Phosphates
Halides
Plaques de gésiers
Gastéropodes
carnivores
Organics
Sulphates
Oxalates
Sulfides
Mn oxides
Fe oxydes

2 types of biomineralizations

Biologically-induced

Biologically-controlled

Biologically-induced mineralizations

- No specific macromolecular machinery

- Formed crystals = look like chemically-precipitated crystals

- Depend on environmental conditions

- No control on the shape & layout of the crystals

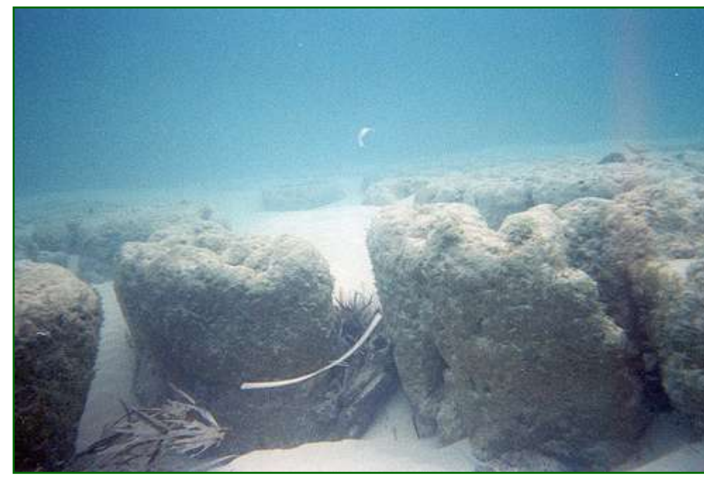
Who, what ? Bacteria, fungi, protists, algae, pathological mineralizations in metazoans

A well-known example: stromatolites

Photo Ch. Pomerol.

Stromatolites

Carbonates predominantly formed by bacterial communities

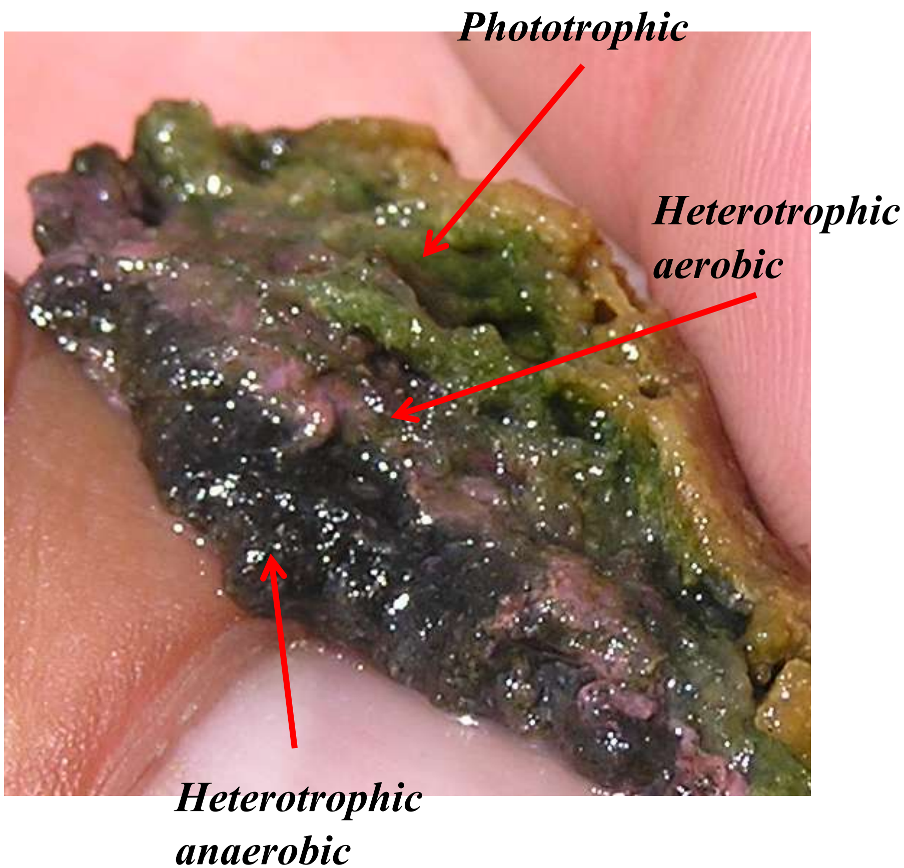


Bahamas

Salda Lake (Turkey)

Laminar structure of stromatolites

Microbial communities in biofilm forming different layers



www.mnhn.fr/mnhn/mineralogie/histoire/index/collections/sedimentaires.htm

Stromatolite fossile en dôme sur le site de forage de la formation de Tumbiana, photographiée par l'équipe. © Kevin Lepot

A very old origin...

Most ancient stromatolites: 3.5 billion years (Australia & South Africa)
Formed in anoxic terrestrial atmosphere.

Stromatolites from Australia (Shark Bay)

http://www.routard.com/images_contenu/communaute/photos.jpg

Slow growth - 0,4 mm per year

Modern stromatolites

Bahamas

Salda Lake (Turkey)

Salt Lake (EU)

Biologically-controlled mineralizations

1. *Very specific molecular & cellular machinery*
2. *Space delineation (where crystallization takes place)*
3. *Formed crystals = different from their chemical counterparts*
4. *Multi-scale organization*
5. *Far less dependent on environmental conditions*
6. *Mineral deposition = controlled by an organic matrix*

WHO ? Magnetotactic bacteria, «protists», «algae», metazoans

Biologically-controlled mineralizations

1. Very specific molecular & cellular machinery

- **Specialized cells. For bone: osteoblasts, osteocytes, osteoclasts**

- **Specialization of cells in organs: the mollusk mantle**

- **This kind of organ appears early during development**

- **Gene regulatory network (GRN) far upstream the formation of the specialized organ**

Biologically-controlled mineralizations

1. Very specific molecular & cellular machinery: the mollusk mantle

Edible pacific oyster *Crassostrea gigas / Magallana gigas*

Calcifying mantle

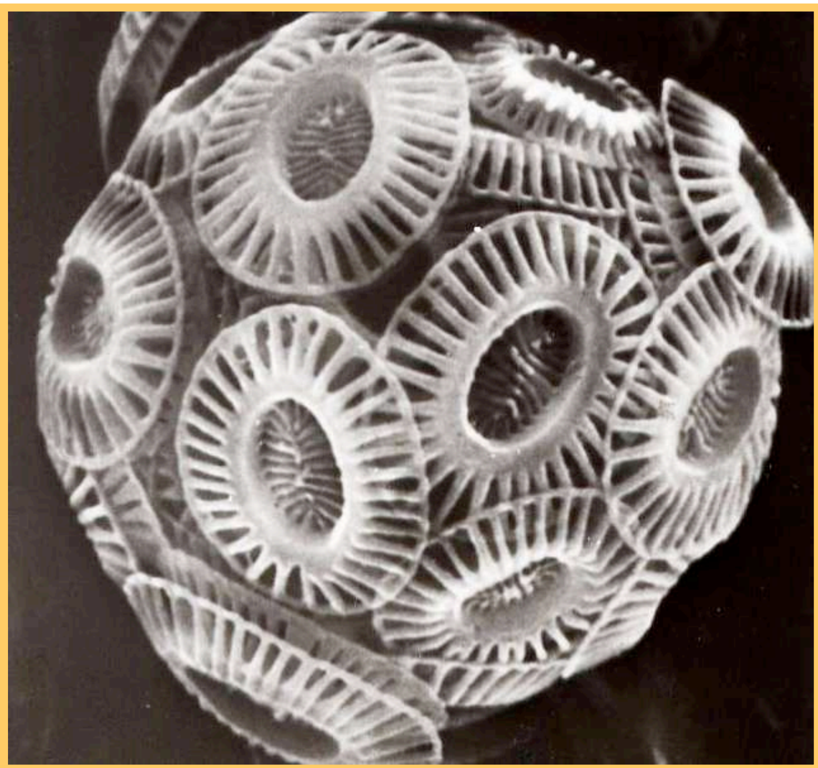
$$HCO_3^- + Ca^{2+} \longrightarrow CaCO_3 + H^+$$

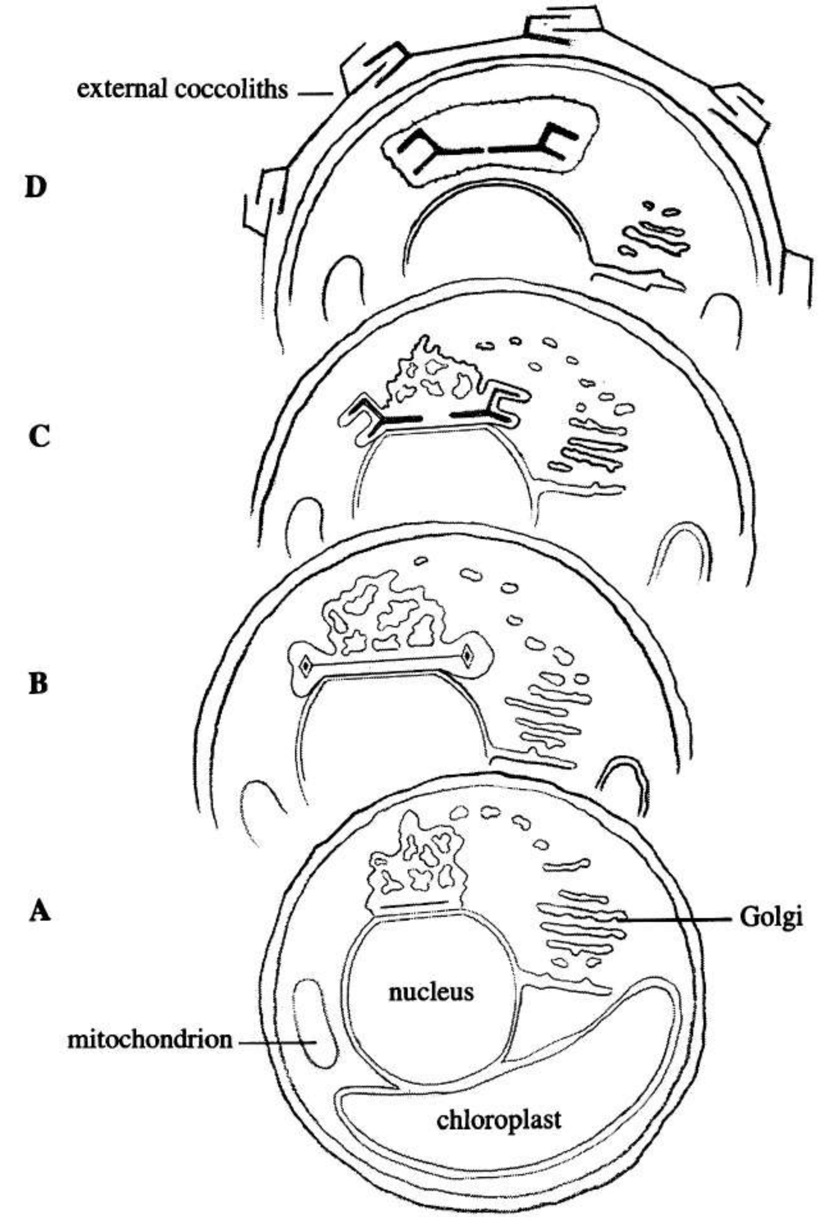
- **Translocation of precursor inorganic ions and secretion**
- **Secretion of amorphous granules**
- **Shell matrix secretion**
- **Proton reabsorption**

Biologically-controlled mineralizations

2. Space delineation

1st example: coccolithophore algae
Formation of coccoliths in a vesicle

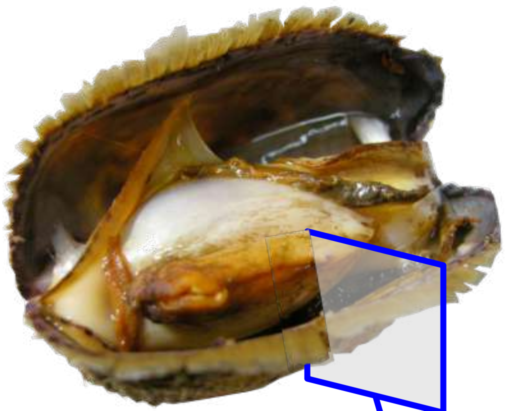


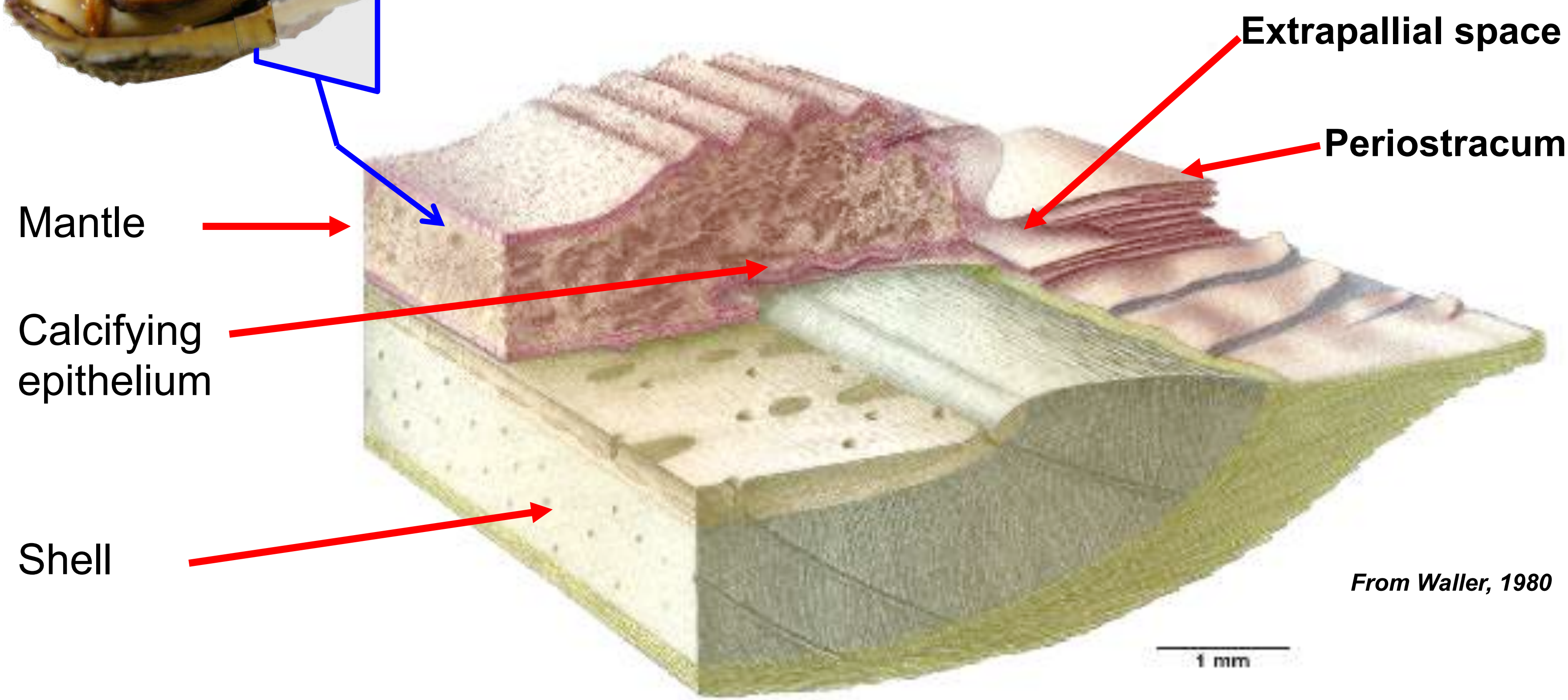


Biologically-controlled mineralizations

2. Space delineation

2nd example: the mollusk shell
Confinement by an organic membrane



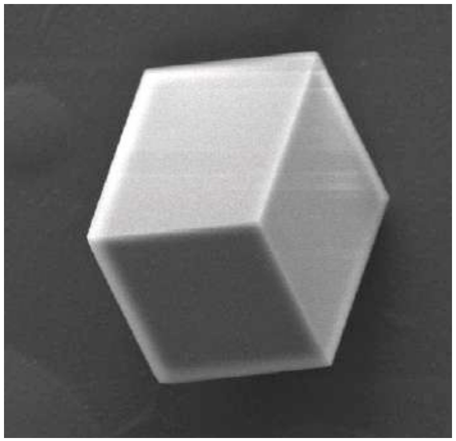


From Waller, 1980

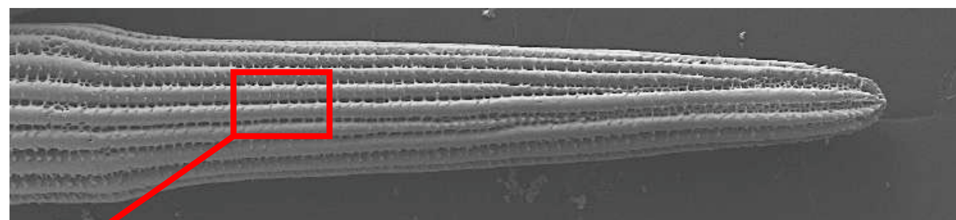
Biologically-controlled mineralizations

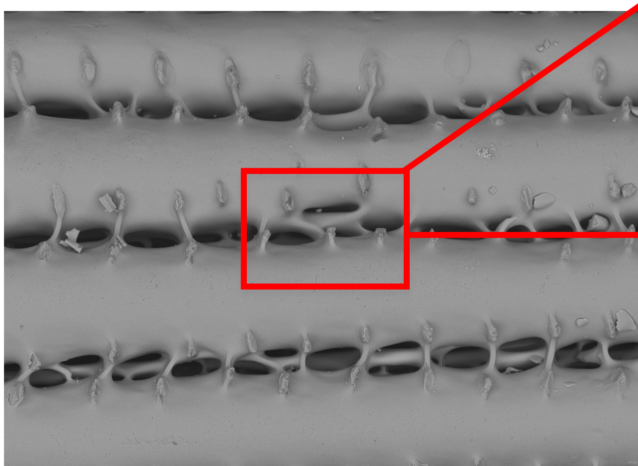
3. Formed crystals = different from their chemical counterparts

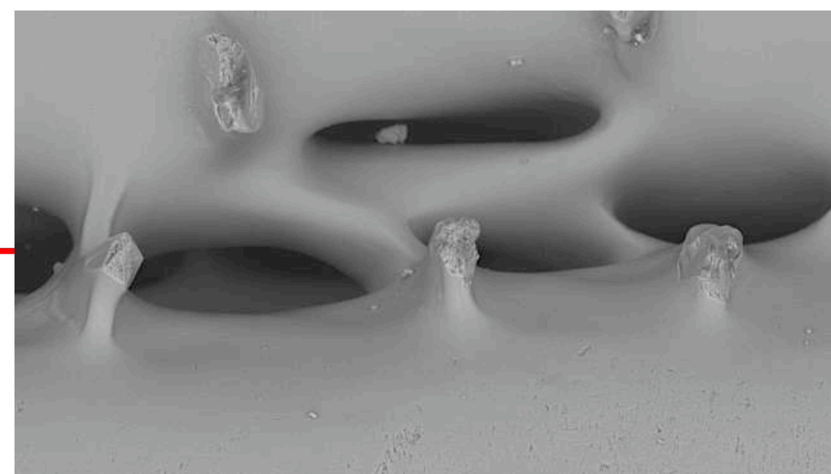
Abiotic calcite



Biological calcite





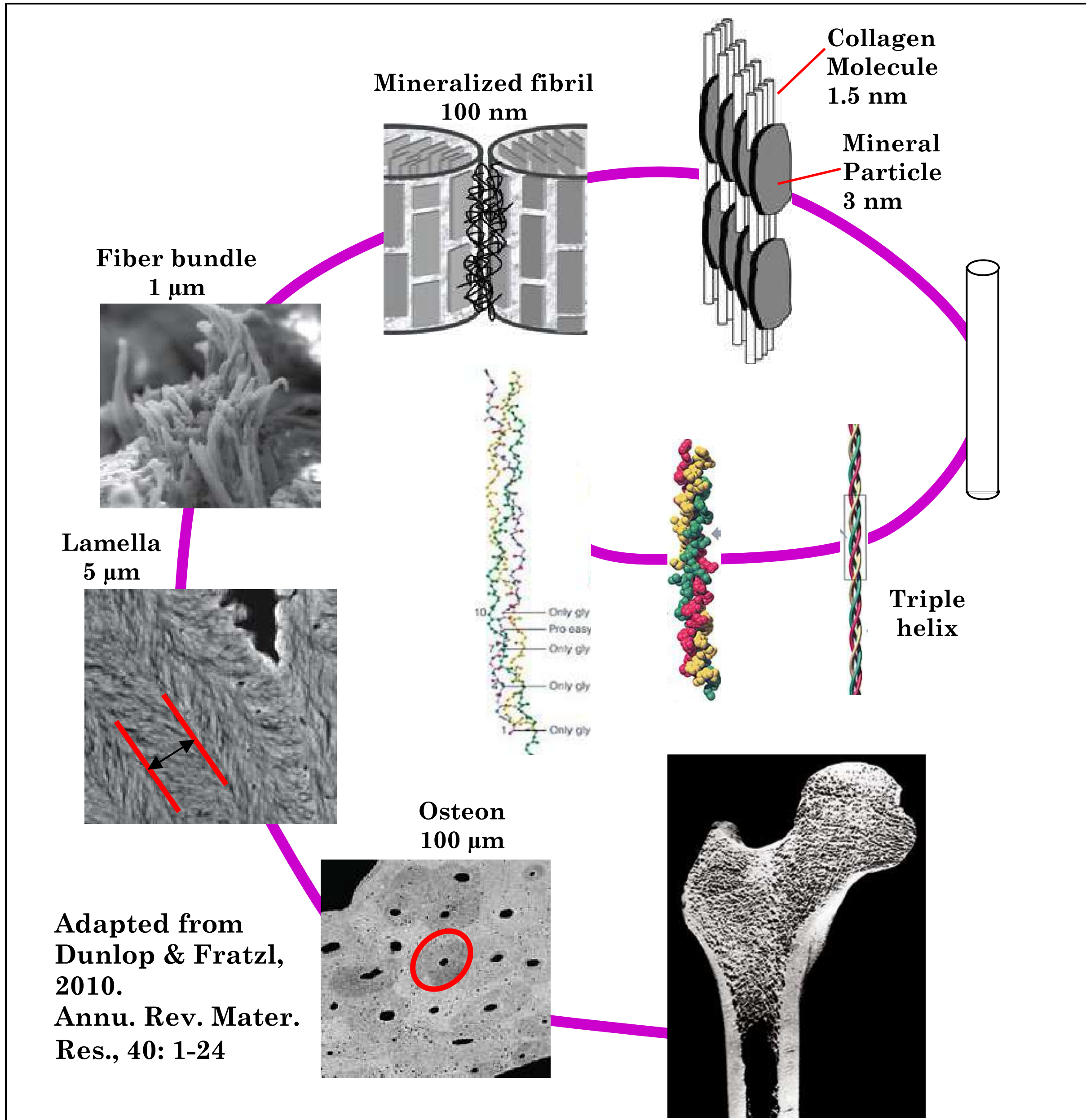


Biologically-controlled mineralizations

4. Multi-scale organization

Vertebrate bone:

At least 7 levels of hierarchy, from nm to cm



Adapted from Dunlop & Fratzl, 2010. Annu. Rev. Mater. Res., 40: 1-24

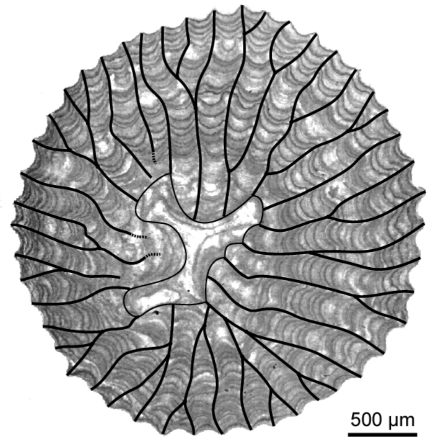
Biologically-controlled mineralizations

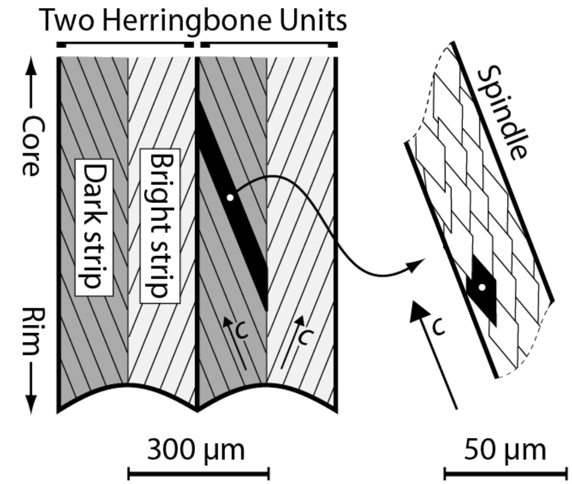
4. Multi-scale organization

Red coral

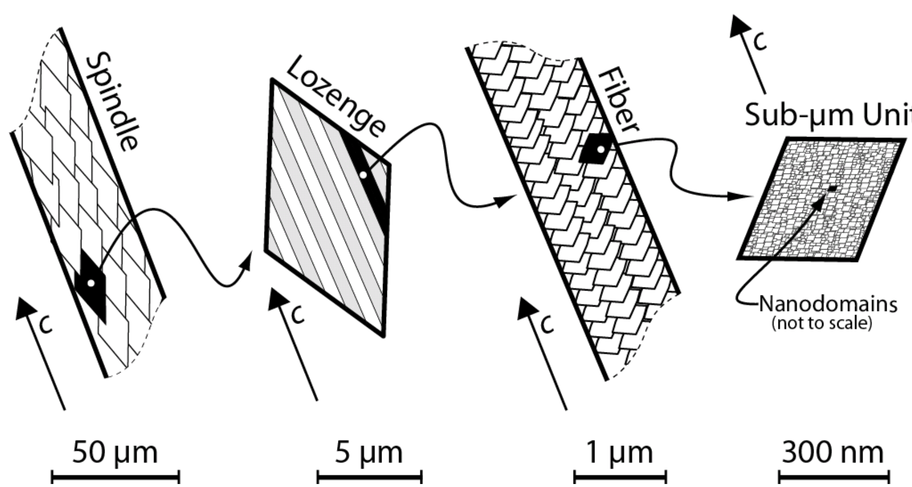
(Corallium rubrum) - Mg Calcite

http://www.uco.es/dptos/zoologia/zoolobiolo_archivos/practicas/practica_3/C_Corallium_rubrum.jpg





7 levels of hierarchy, from nm to cm



Vielzeuf et al., 2008. Am. Mineralogist, 93: 1799-1815.
Vielzeuf et al., 2010. Am. Mineralogist, 95: 242-248.

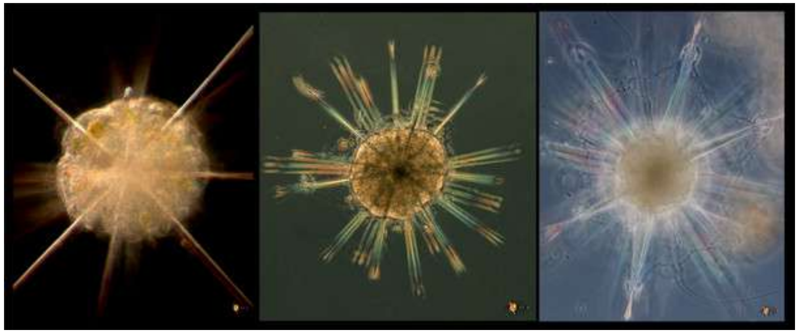
Biologically-controlled mineralizations

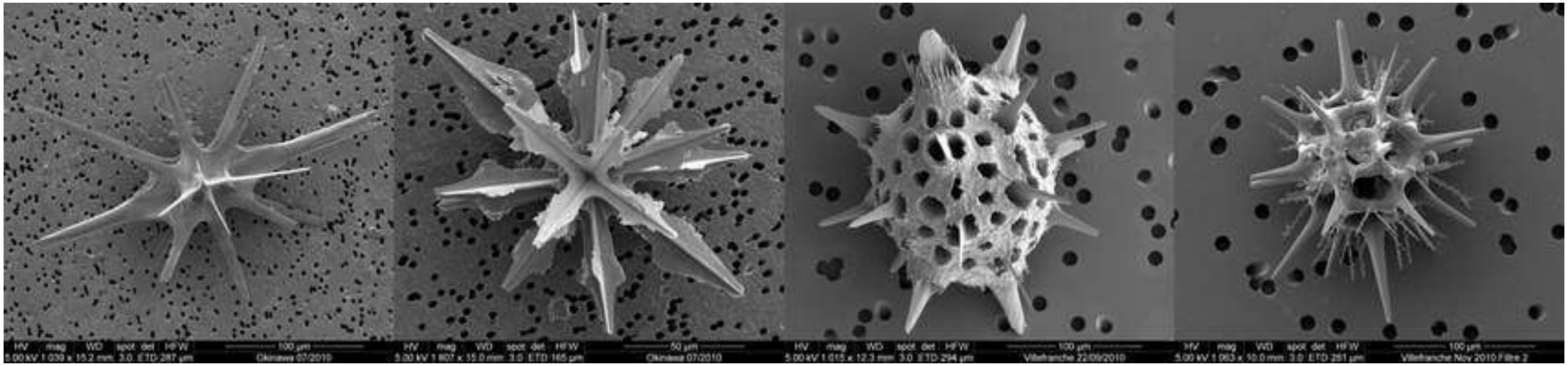
5. Far less dependent on environmental conditions

The example of Acantharians: planctonic marine protists

$SrSO_4$: celestite

Very undersaturated in marine environment = highly unstable





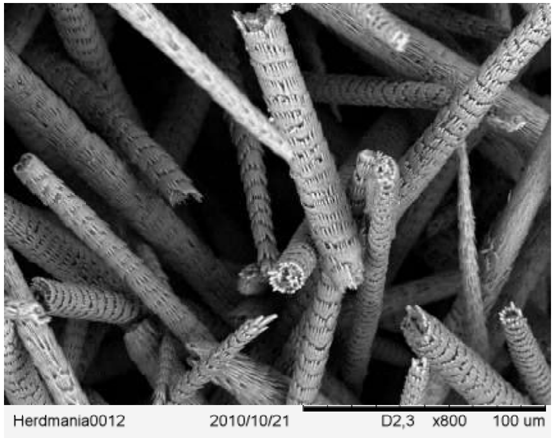
Biologically-controlled mineralizations

5. Far less dependent on environmental conditions

The example of freshwater mussel

Acidic water, under-saturated in $CaCO_3$

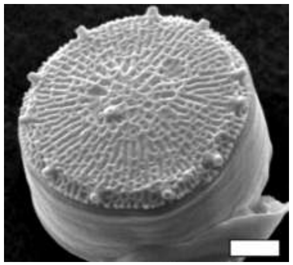
The example of the ascidian, *Herdmania momus*



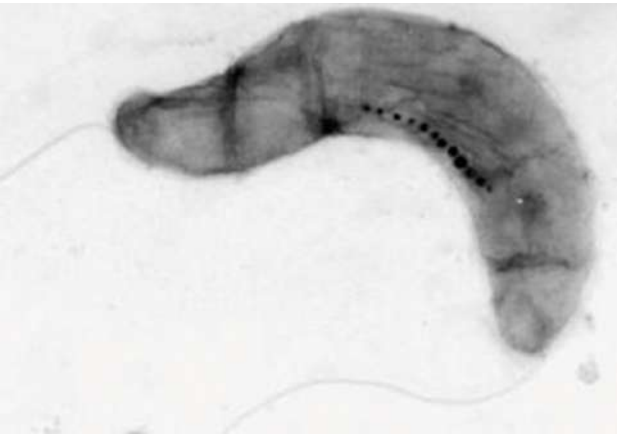
Spicules made of vaterite

Biologically-controlled mineralizations

6. Controlled by an organic matrix

	Silica		$CaCO_3$		Ca-P
	Diatoms	**Demosponge**	**Sea urchin**	**Mollusk**	**Chordate**
	 Photo M. Hildebrand	 Photo A. Frijsinger & M. Vestjens	 http://en.academic.ru/dic.nsf/enwiki/6124580		
	Thalassiosira pseudonana	***Suberites domuncula***	***Strongylocentrotus purpuratus***	***Pinctada sp.***	***H. sapiens sapiens***
PROTEINS	Silaffins, Frustulins SITs ($Si(OH)_4$ transporters)	Silicateins Galectins Collagens Selenoprot. M Silicase…	SpSM50, SpSM32, SpSM37, SpSM29, SPU_005989-91-92, SpPM27, SPU_027906, SpSM30-A to F SpC-lectin MSP130…	Aspein, MSI31, Prismalin-14, N19 Nacrein / N66, N14 / N16 / Pearlin, MSI60, MSI7, Pfty1-2, KRMP1-4, Shematrins 1-7 Prismin, Pif177, Prisilkin-39, mpn88 pfp-16, msi25 Several ESTs…	DSPP MEPE + ASARM pept. DMP1, DMP2 DPP Amelogenin Ameloblastin Enamelin Amelotin Biglycan Kallikrein-4 MMP20 Enamelysin Collagen…
OTHER COMPONENTS	LCPAs (long chain polyamines) PTMs	PTMs	PTMs	PTMs + Pol	PTMs + Pol

An example of biologically-controlled mineralization: magnetotactic bacteria

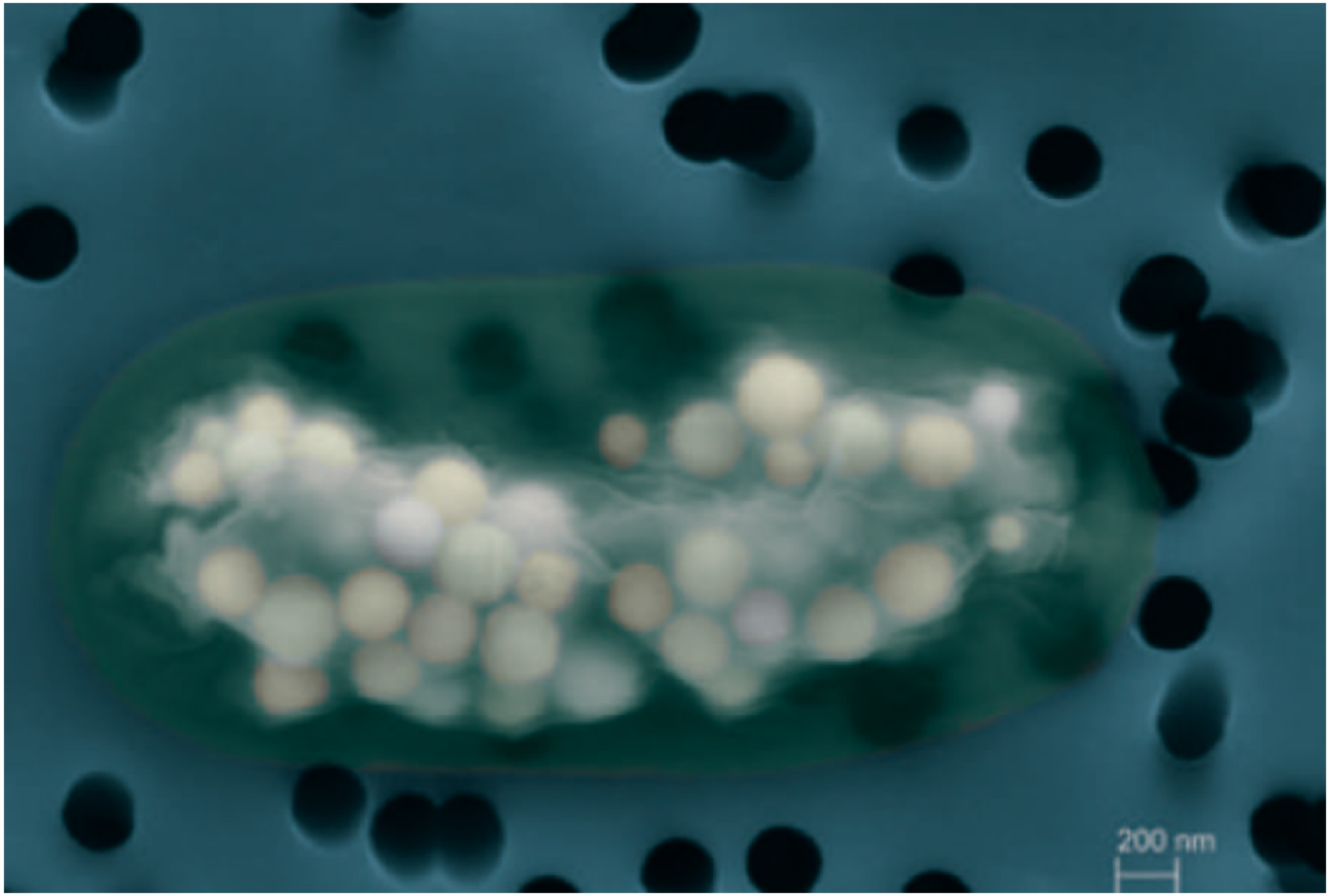


- Magnetite nanograins (25-100 nm)

synthesized in an organite (vésicule), the magnetosome

* Processus = controlled by about 20 different proteins,
  the «Mam family ».

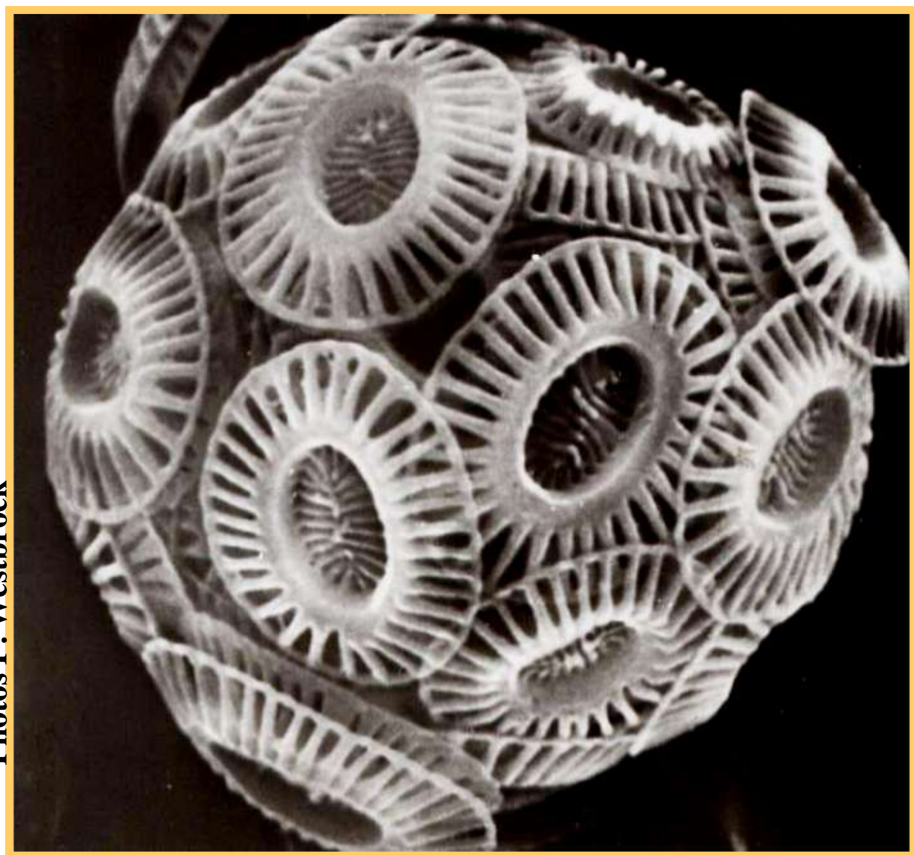
Another example of controlled mineralization:
Photosynthetic bacteria with Ca nodules



Couradeau et al., Science, 2012

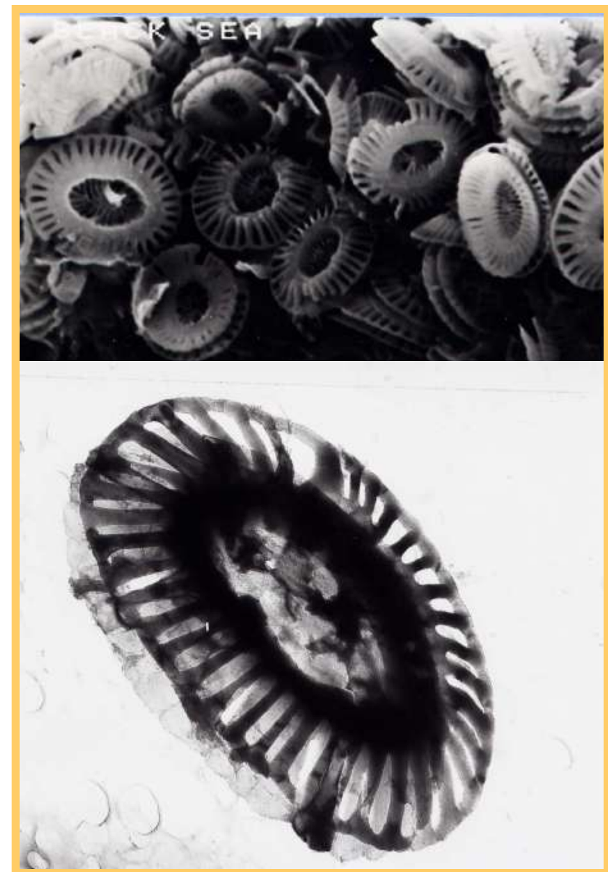
Another example of controlled mineralization: coccolithophore algae

Calcite plates secreted by unicellular algae, coccolithophorids (Haptophytes)



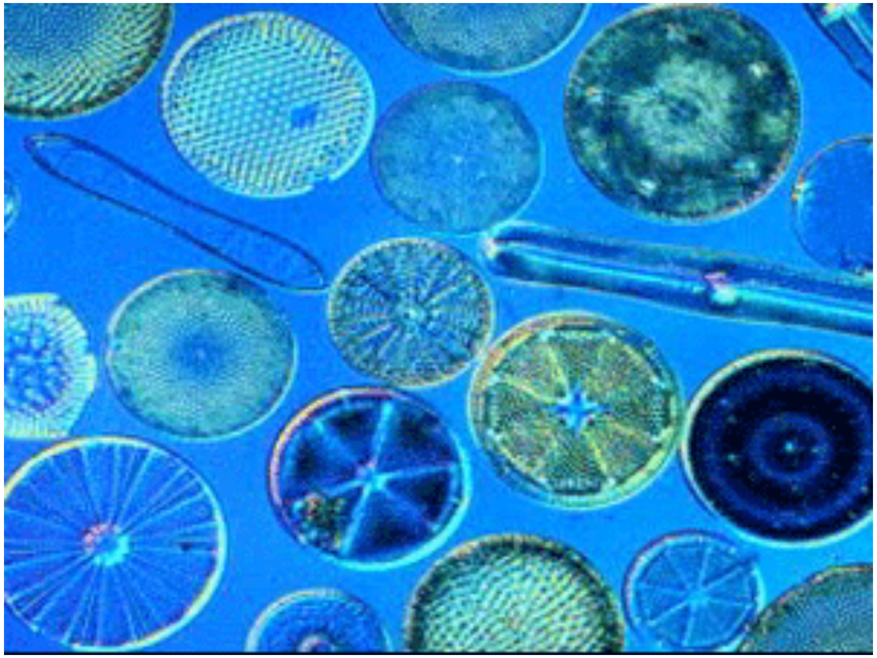
Photos P. Westbroek

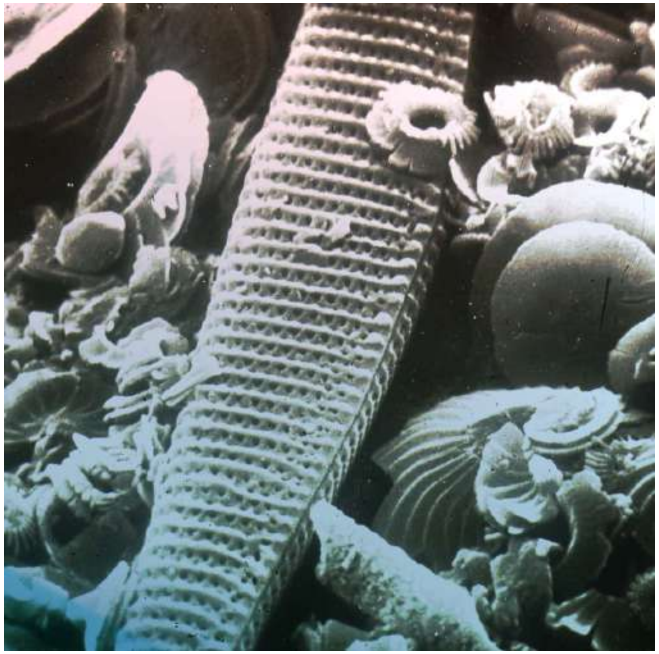
Photos P. Westbroek



Photos P. Westbroek

Another example:
diatoms (bacillariophyte algae): Trias to now

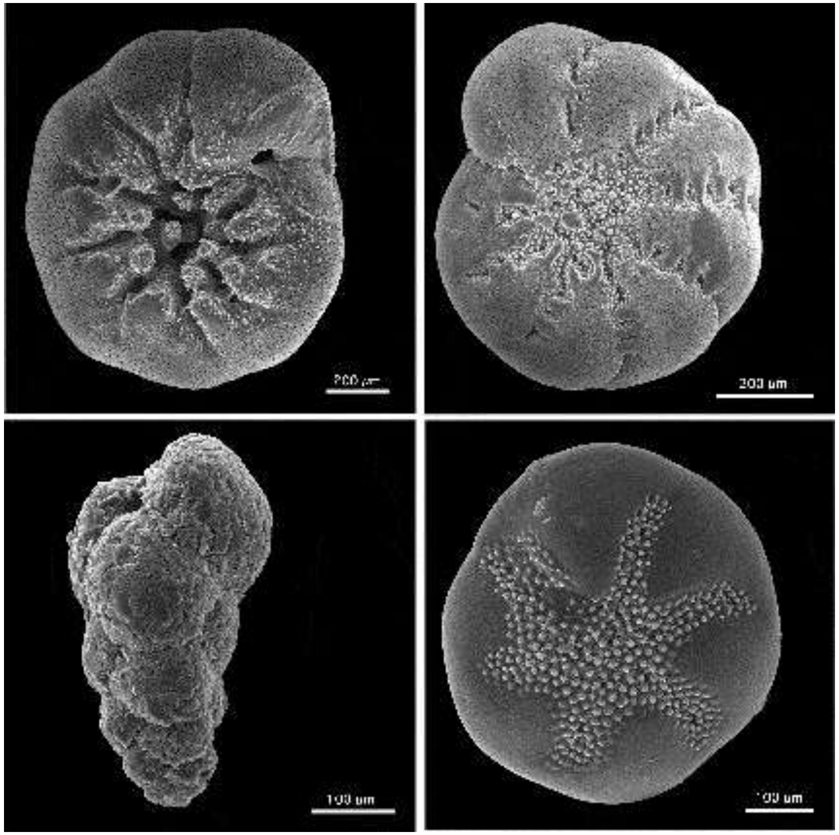




- Freshwater and marine water: diatomites

- Key-player in the regulation of silica at global scale

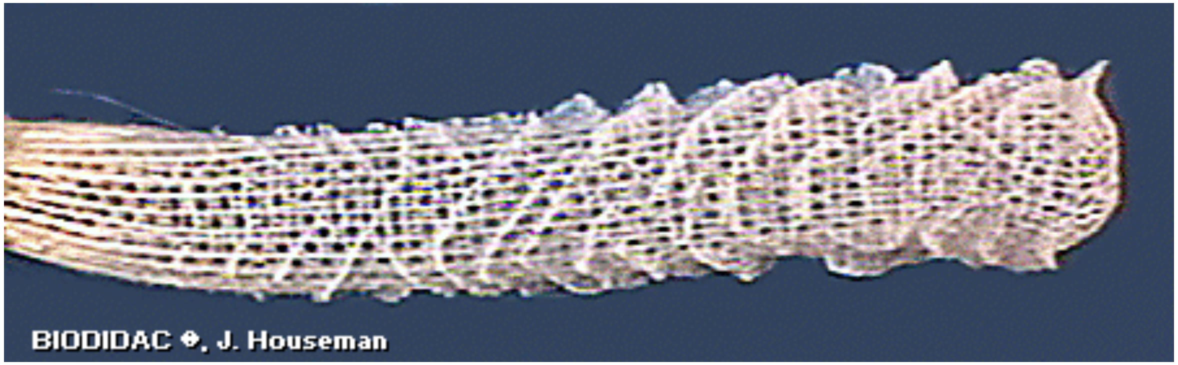
Another example: Foraminifera

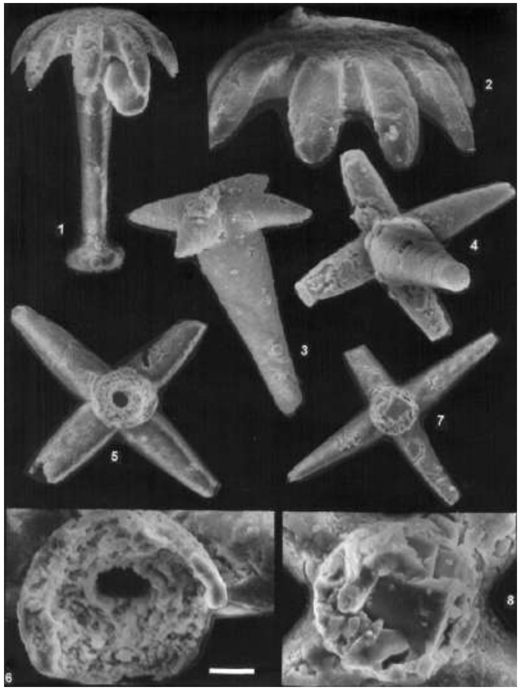


Nummulites

Calcite or aragonite

Another example: sponges (Porifera)





2 mineralogies:

$CaCO_3$
Calcarea)

SiO_2
(Hexactinelles
& Demosponges)

SiO_2 + $CaCO_3$
Few demosponges

Another example: cnidarians

In cnidarian, 2 polymorphs of $CaCO_3$

Octocorallia

Scleractinia

CALCITE

ARAGONITE

The example of brachiopods and bryozoans

Brachiopods

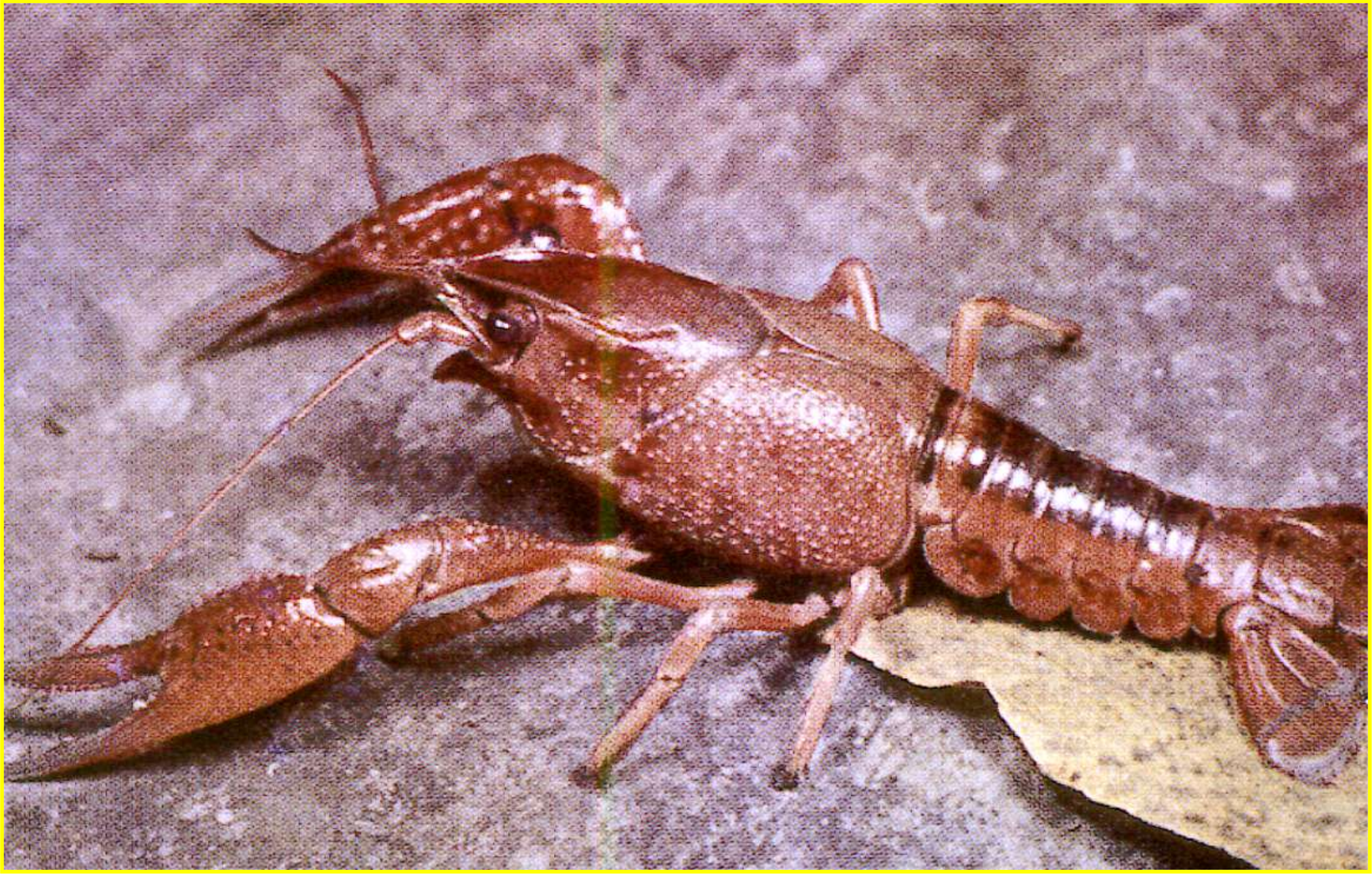
CALCITE

Exception: lingulid = Ca-phosphate !

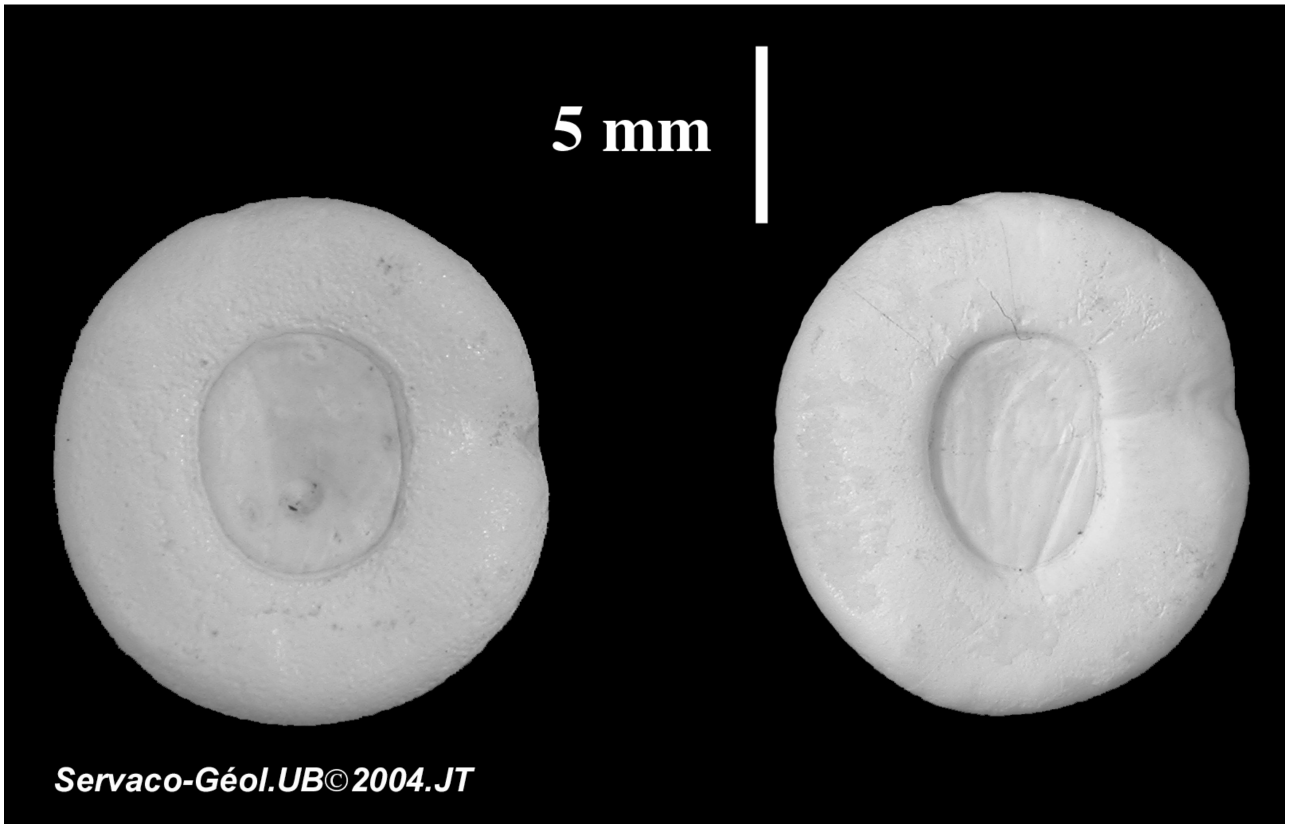
Bryozoans

CALCITE
(+ aragonite)

Example in arthropods

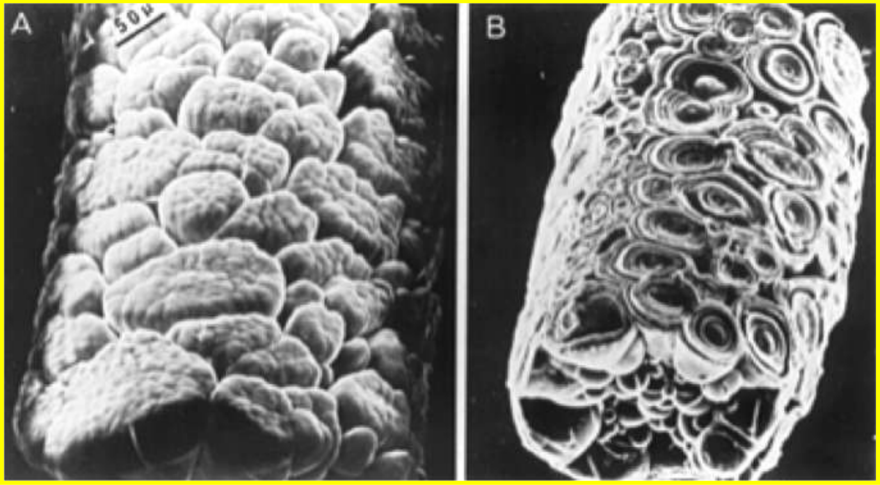


In arthropods: gastroliths



Biologically-controlled mineralization in arthropods: calcium storage structures

During the molting process (ecdysis)



10 hours after molting

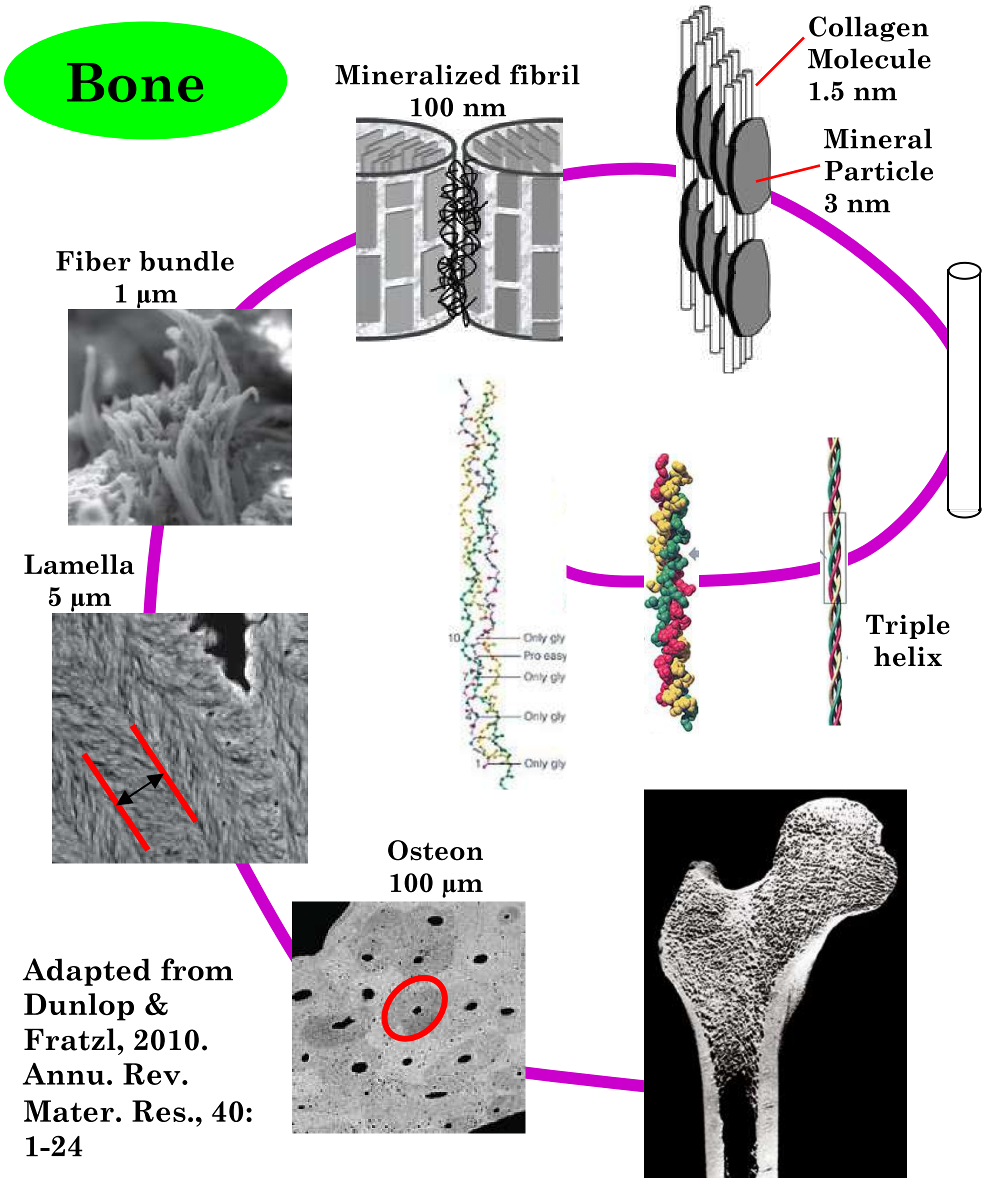
Biologically-controlled mineralization: the mollusk shell

Biologically-controlled mineralization: the mollusk shell

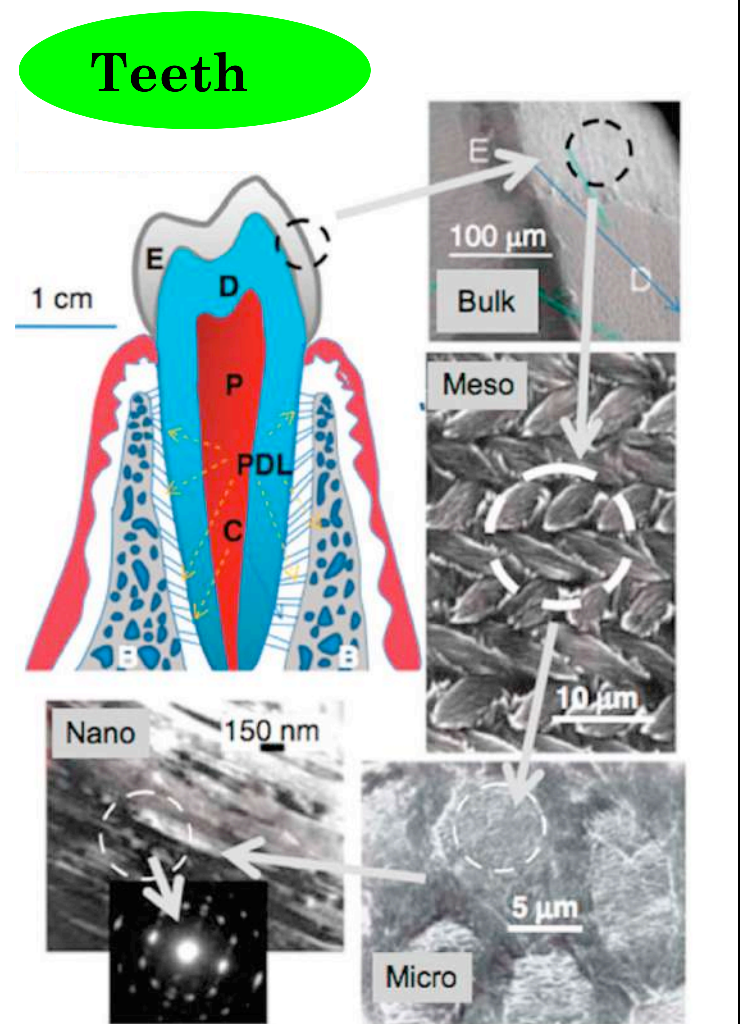
Thanatocenosis

Reef from Upper Cretaceous:
Rudist bivalves

The vertebrates…



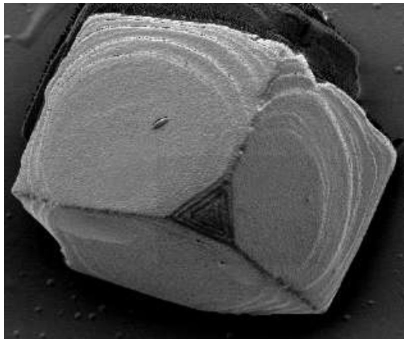
Adapted from Dunlop & Fratzl, 2010. Annu. Rev. Mater. Res., 40: 1-24

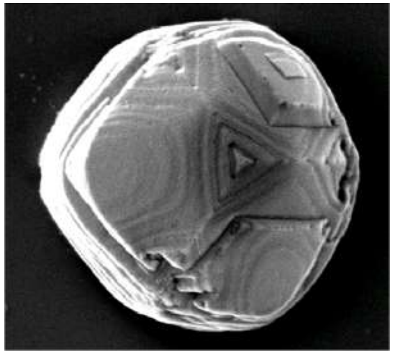


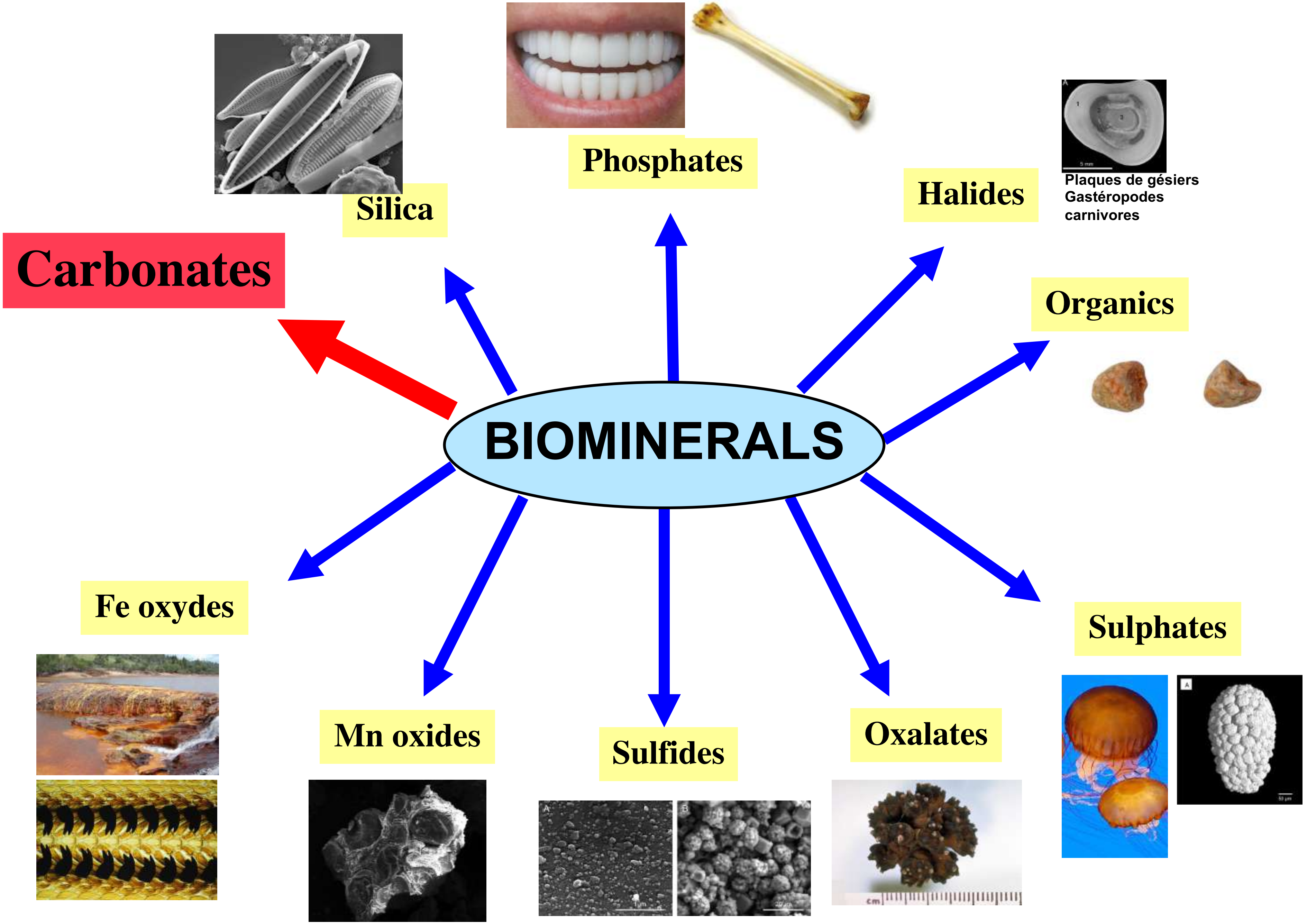
Adapted from Palmer et al., 2008. Chem. Rev. 108: 4754-4783 and from Tamerler & Sarikaya, 2008. MRS Bull., 33: 504.

Marine biocalcification:

Origin & evolution






Silica
Phosphates
Halides
Plaques de gésiers
Gastéropodes
carnivores
Carbonates
Organics
BIOMINERALS
Fe oxydes
Sulphates
Mn oxides
Sulfides
Oxalates

Biomineralization in $CaCO_3$ & carbonate cycle in the sea

Remark: surface sea water: highly supersaturated with respect to calcium carbonate

However, places where spontaneous calcium carbonate precipitation occurs are rare (Bahamas)

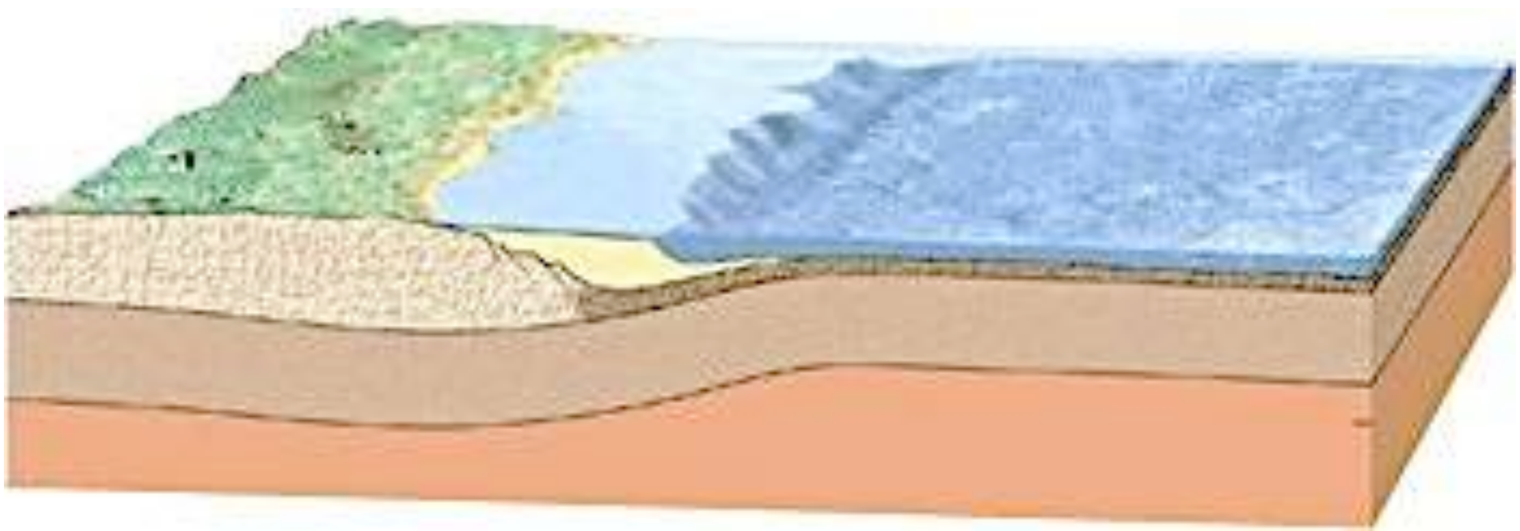
In sea water, several inhibitors of precipitation:
- Magnesium.
- Organic polymers: polysaccharides, proteins…
- Other: phosphates, citrates…

$CaCO_3$ biomineralization & the carbonate cycle

Production:
Marine $CaCO_3$
5 - 5.7.10^9 T/year
(Milliman, 1993)

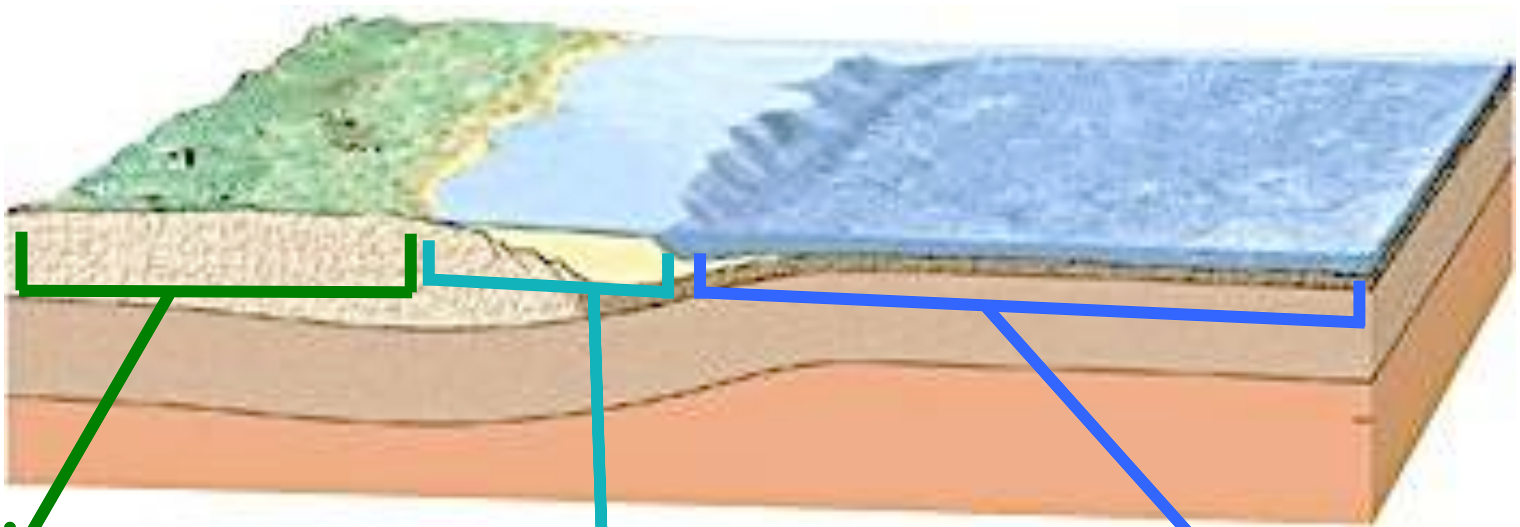
40% redissolved:
2 . 10^9 T/year (CCD)

60% accumulates:
3 . 10^9 T/year



Continental carbonates not taken into account

Machine EARTH: a factory for producing $CaCO_3$…



CONTINENTS
(soils, lakes, rivers…)

Unknown production?

- Bacteria, fungi, plants, mollusks (lacustrine domains)

EPICONTINENTAL PLATFORM

Estimated production: 2,5 $*10^9$ T/year
(corals: 0,9 . 10^9 T/year)

- Benthic
- Aragonite & (Mg) calcite
- Corals, foraminifera, mollusks, bryozoans, red algae, green algae

OPEN OCEAN

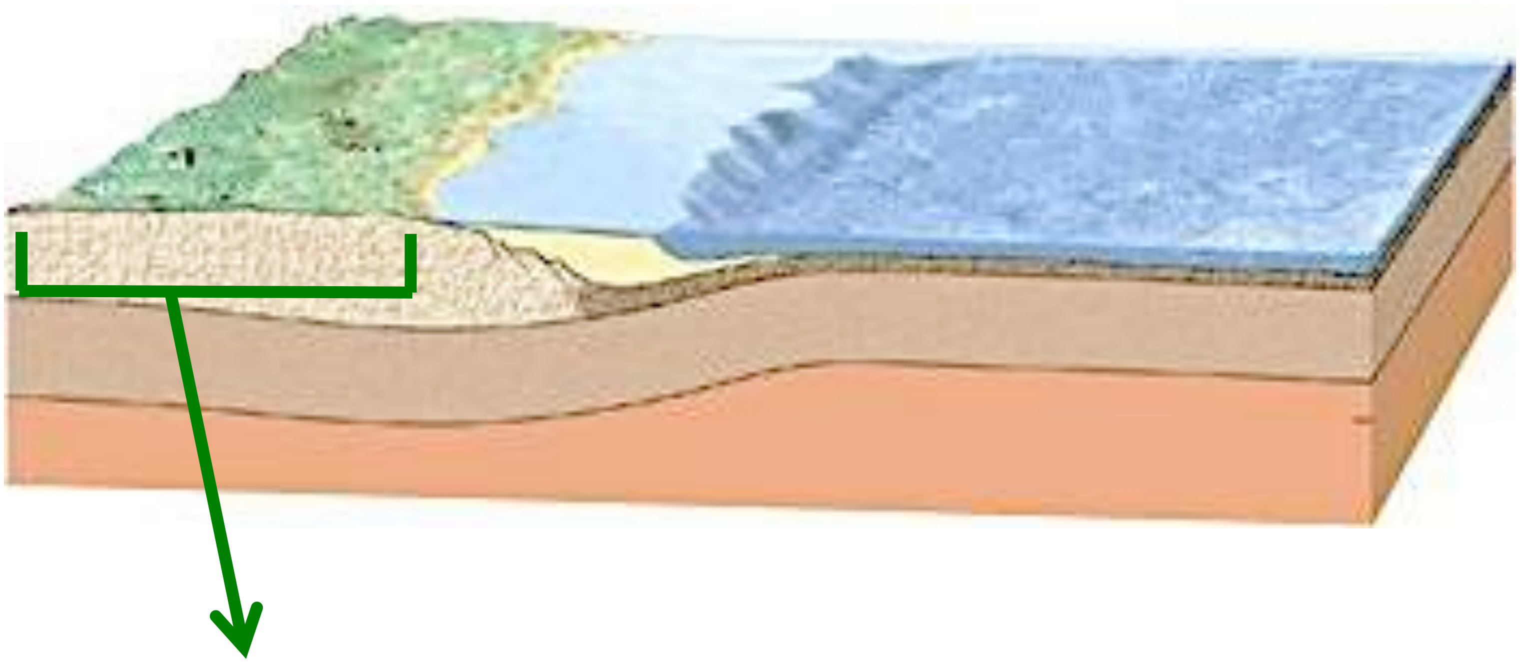
Estimated production: 2,4 $*10^9$ T/year ?

- Planktonic
- Calcite
- Coccolithophore algae, planktonic foraminifera, 'pteropods'

From Milliman, 1993; Wollast, 1993; Langer et al., 1997

MARINE PRODUCTION: 5 to 5.7.10^9 T/year

Continental domain…



<u>Highly variable production</u>:

- Important in lacustrine domain
- Almost 0 in hot deserts
- In temperate forrests: micro-organisms (bacteria, fungi): important but poorly quantified; calcite in needles...
- In tropical areas: locally, can be important; example of Iroko trees

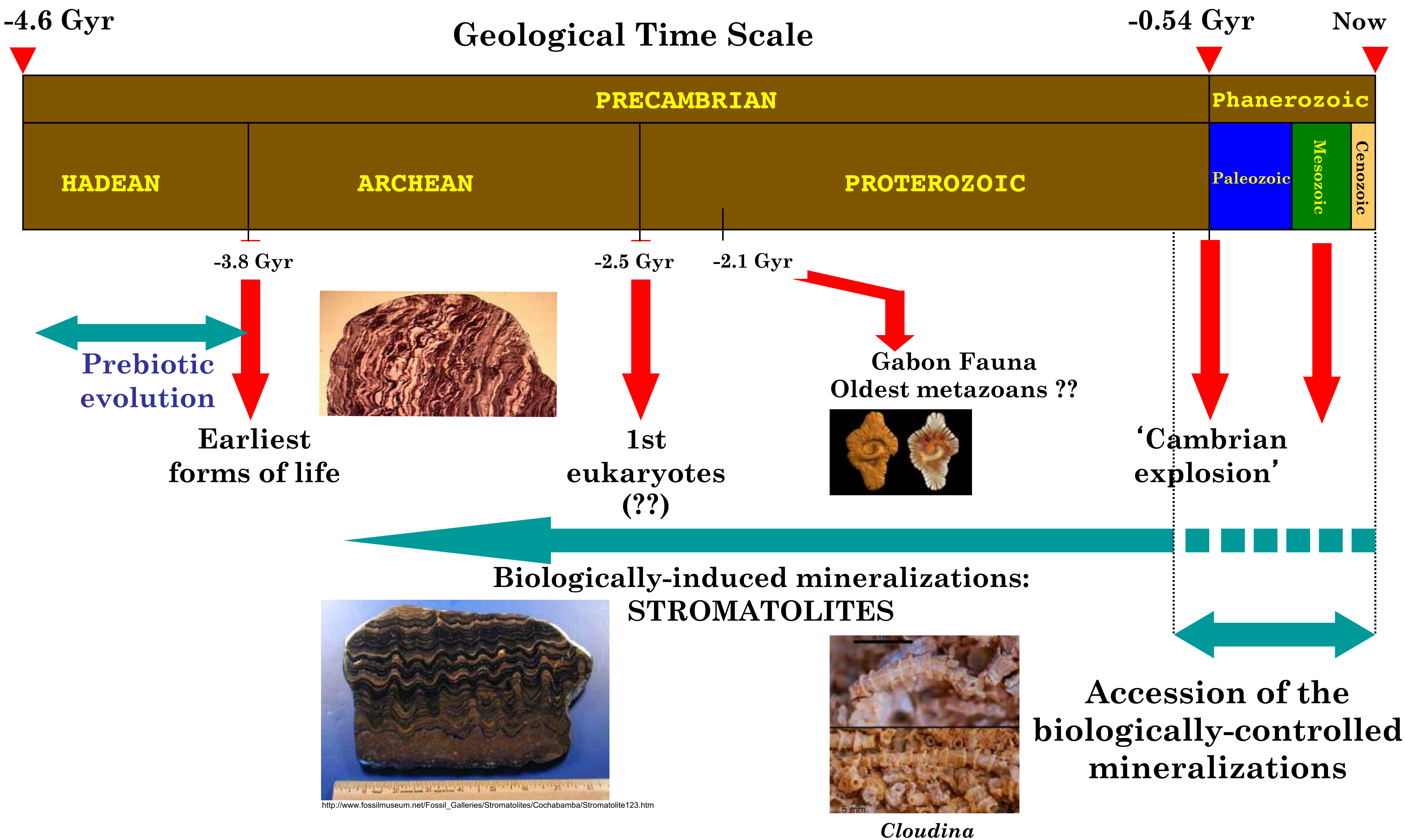
QUESTION:

Was this « calcifying regime » constant across the geological times?

ANSWER:

NO

Macro-evolution of $CaCO_3$-based biomineralization across geological times

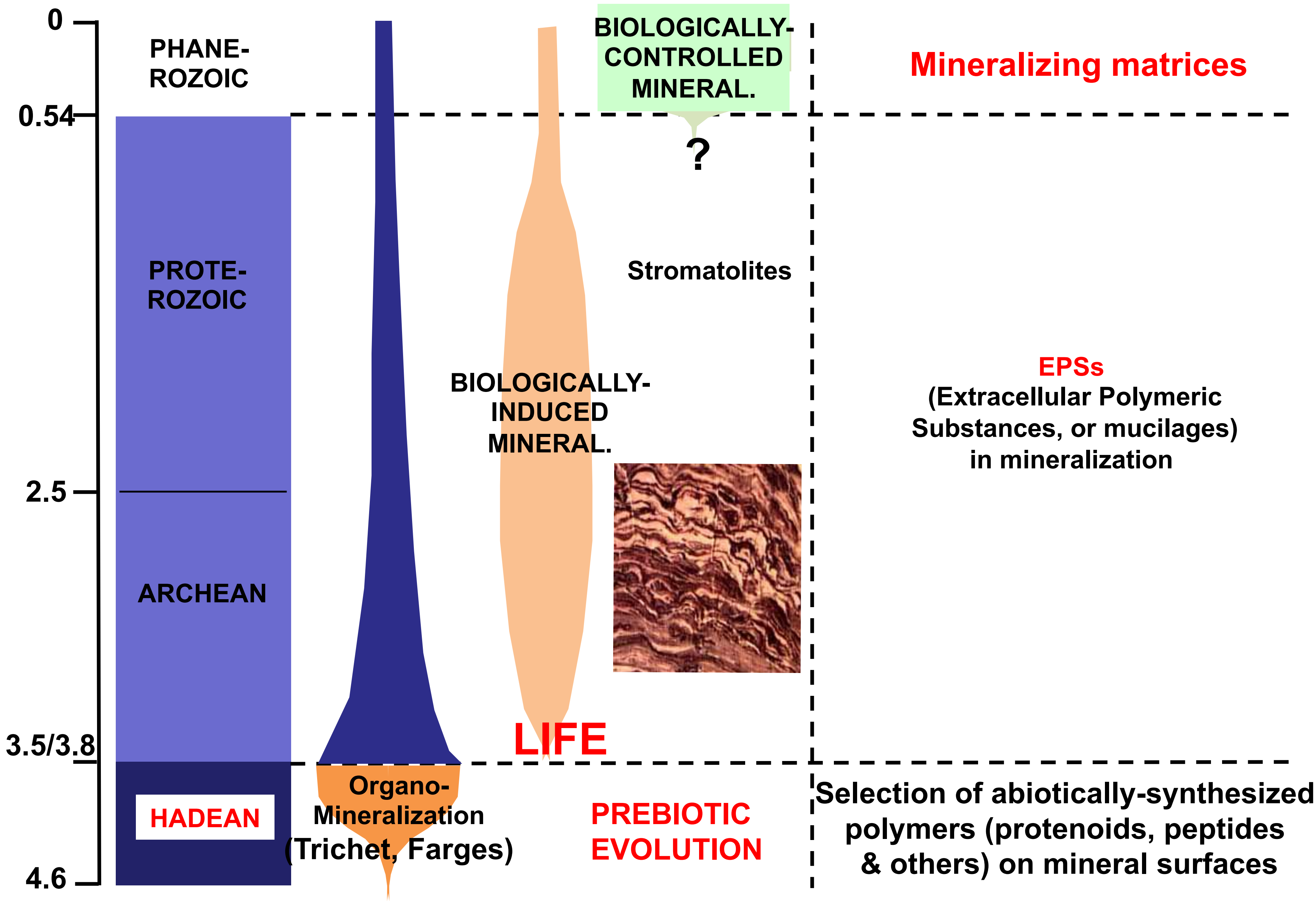


In the Hadean Times…

No life yet, no biomineralization...

But synthesis of organic macromolecules (« primitive soup »)

These components may have reacted with mineral surfaces (FeS, silicates): 1st « organo-mineralizations »: polymer selection by mineral surfaces


0
0.54
2.5
3.5/3.8
4.6
PHANE-ROZOIC
PROTE-ROZOIC
ARCHEAN
HADEAN
BIOLOGICALLY-CONTROLLED MINERAL.
?
Stromatolites
BIOLOGICALLY-INDUCED MINERAL.
LIFE
Organo-Mineralization (Trichet, Farges)
PREBIOTIC EVOLUTION
Mineralizing matrices
EPSs
(Extracellular Polymeric Substances, or mucilages) in mineralization
Selection of abiotically-synthesized polymers (protenoids, peptides & others) on mineral surfaces

Archean & Proterozoic Times…

- Biologically-induced mineralizations
- First biologically-controlled mineralizations ?

Stromatolites

Photo Ch. Pomerol.

Oldest mineralized construction:

-3,5 billion years (ARCHEAN)

Today's stromatolites

Shark Bay, Australia

Stromatolites

Coupling between photosynthesis & biomineralization

Photosynthesis
(cyanobacteria) :

Carbon dioxide (CO_2)

↓

Organic matter
+ oxygen (O_2)

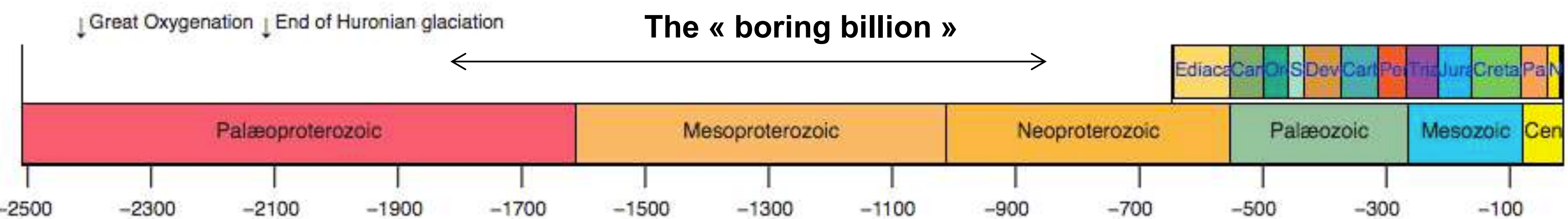
Stromatolites played a key-role in the formation of an oxidizing atmosphere

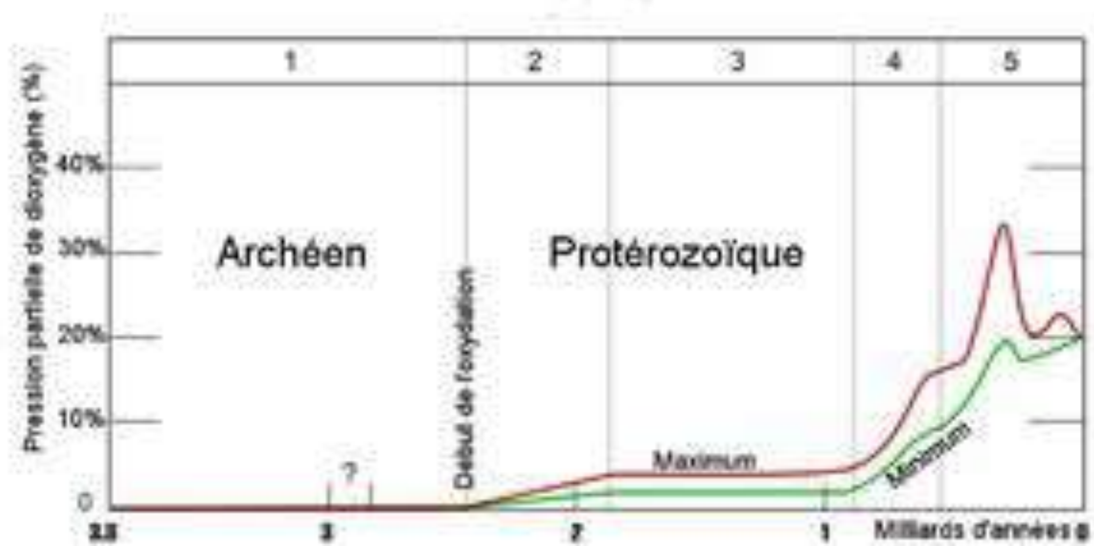
The ‘GOE’: Great Oxidation Event

* Increase of O2 level due to photosynthesis

* 2.4 à 2.1-2 billion year ago (Paleoproterozoic)

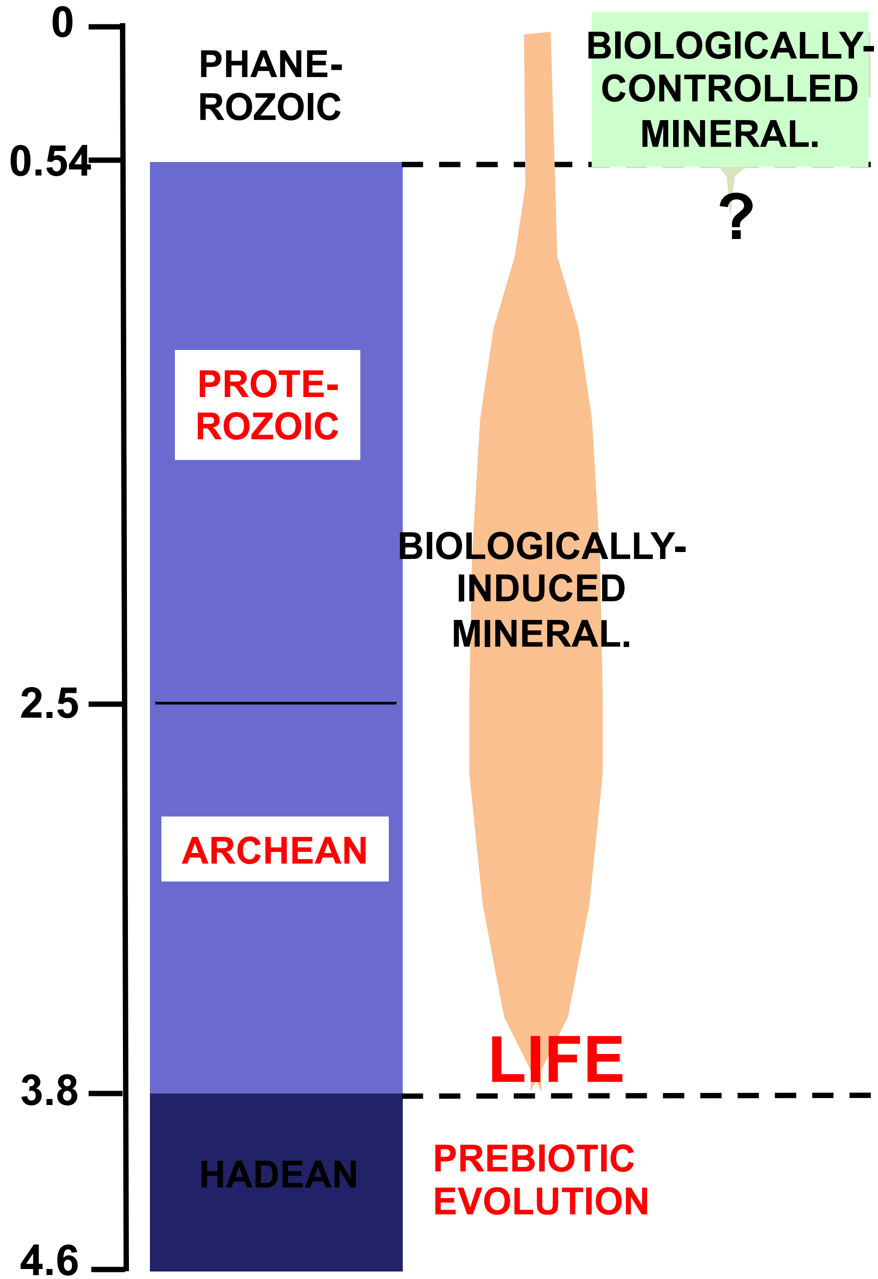
* Extinction of anaerobic life





Fonction(s) of stromatolite biomineralization in cyanobacteria

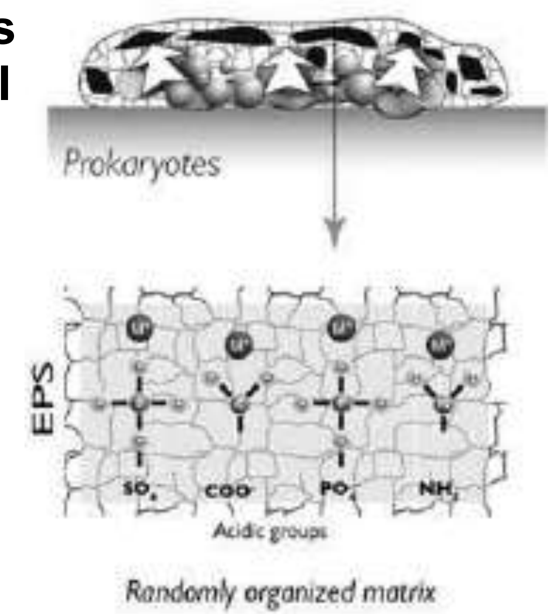
- Support
- Structuring of bacterial communities
- Protection against dessiccation
- Protection against UVs



Complex and specialized mineralizing matrices:
(fine-tuned at gene level)
Multiple molecular functions

Fundamental roles of EPS in bacterial biomineralization:

- Peculiar micro-environment
- Nucleation / Template
- Inhibition of mineralization
- 'Non specialized' ?



(From Dupraz et al., 2009)

***Prerequisites**: - Handling of intracellular Ca fluxes – Ca, 2nd messenger*
- Set up of a cytoplasmic regulatory machinery

Selection of abiotically-synthesized polymers (protenoids, peptides & others) on mineral surfaces

Multicellular eukaryotic life (fungi): 0.9 to 1 billion year old

(Loron et al., Nature, 570, 2019)

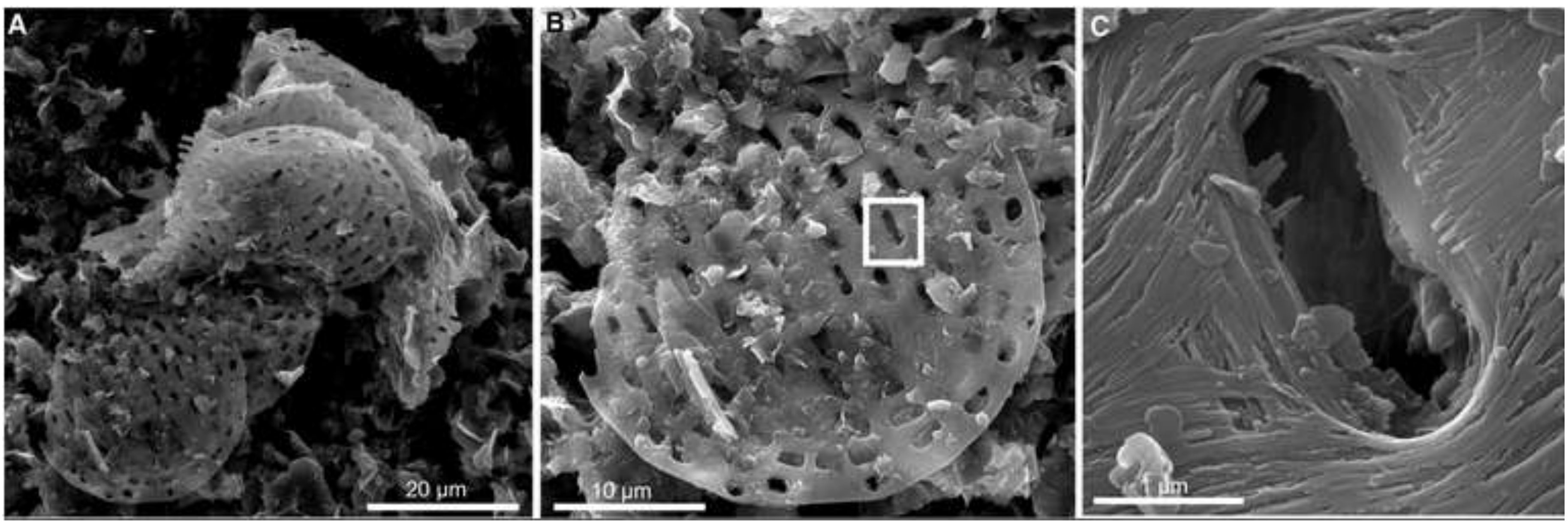
Outcrop from Canadian Arctic

Photographies prises au microscope optique des fossiles du champignon *Ourasphaira giraldae*, un organisme eucaryote multicellulaire à paroi organique (chitine) composé de filaments en forme de "T" et segmentés (hyphes) reliés à une vésicule sphérique (spore). Les spécimens mesurent entre 30 et 80 microns de diamètre (0.03 à 0.08 mm) et sont datés entre 0.9 et 1 milliard d'années. Documents C.

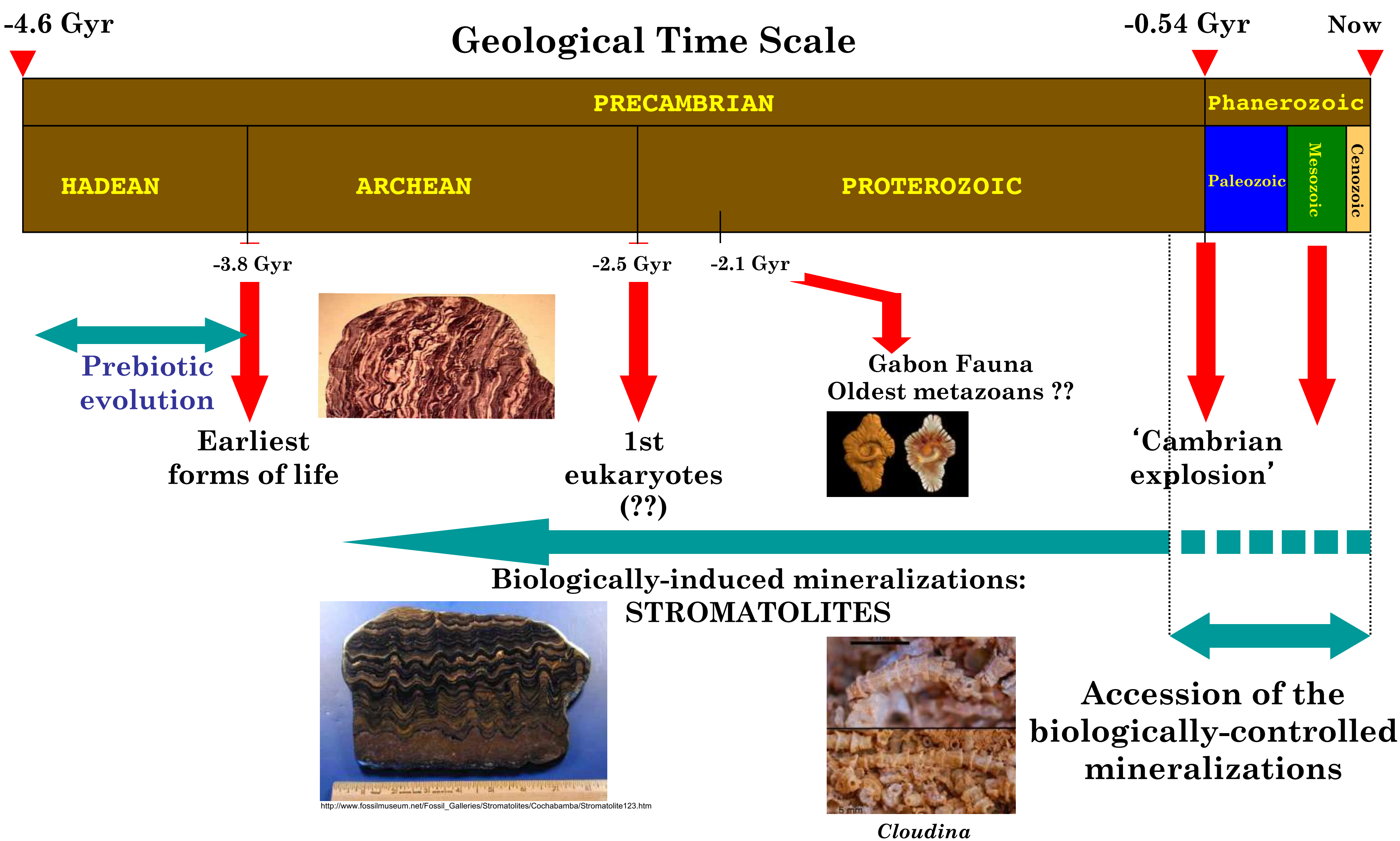
➔ **Chitin: one of the oldest polymers among Eukaryotes**

Very first complex biomineralized forms of life
(Cohen et al., 2017, Science Advances)

* **Eukaryotic cells**
* **Hydroxyapatite**
* **810 million year ago**
* **Yukon, Canada**
* **Dated by rhenium & osmium isotopes**



Macro-evolution of $CaCO_3$-based biomineralization across geological times



Around the Proterozoic / Cambrian transition

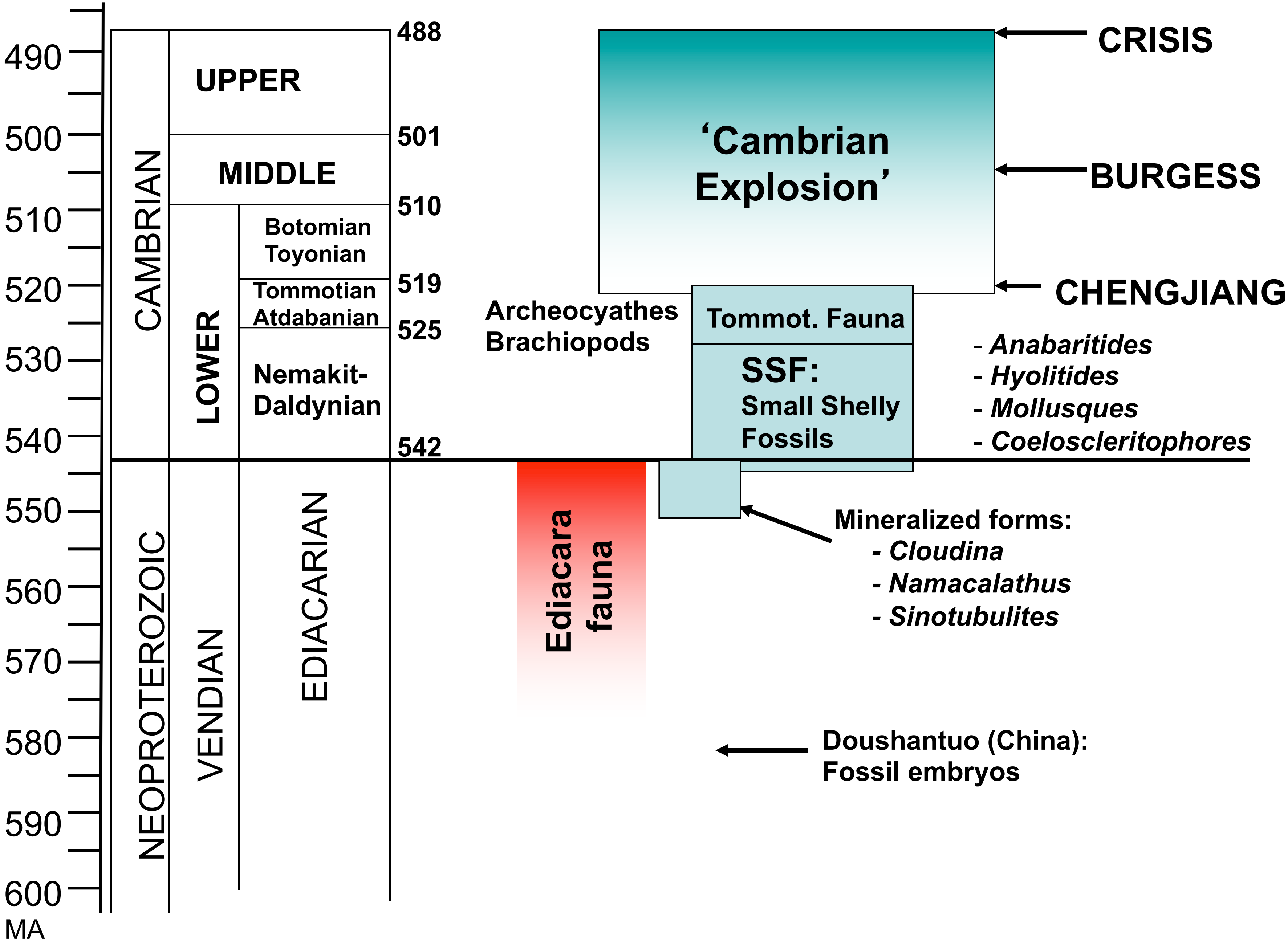
Biologically-controlled mineralization inherits the Earth

Appearance of biomineralization among metazoans =

One aspect of the « Cambrian Explosion »

What were the major steps at the Precambrian / Cambrian transition ?

P/C TRANSITION : SUCCESSION OF BIOLOGICAL EVENTS



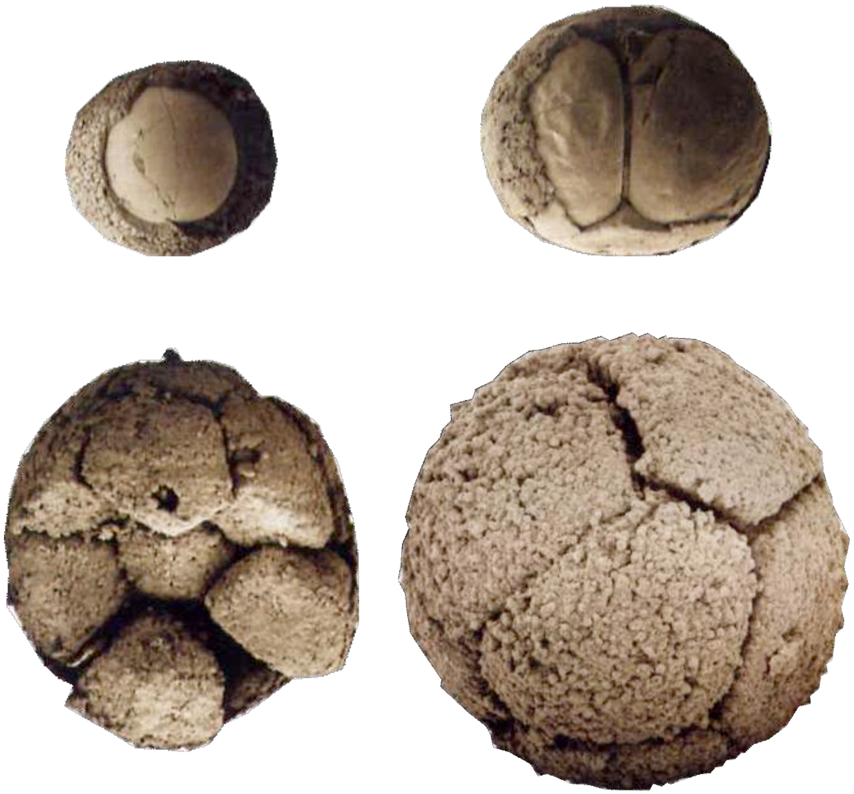
Doushantuo Formation (Chine)

1st 'fossil embryos' that are remarkably preserved:
Phosphatic deposits
Xiao, Zhang & Knoll, Nature, 391 (1998).

Age: 590 to 565 MY

Anterior or contemporary to Ediacara Fauna

- **Macro-algae**
- **Cnidarians ?**
- **Sponges ?**
- **Bilaterians ?**
- **Simple adult metazoans ?**

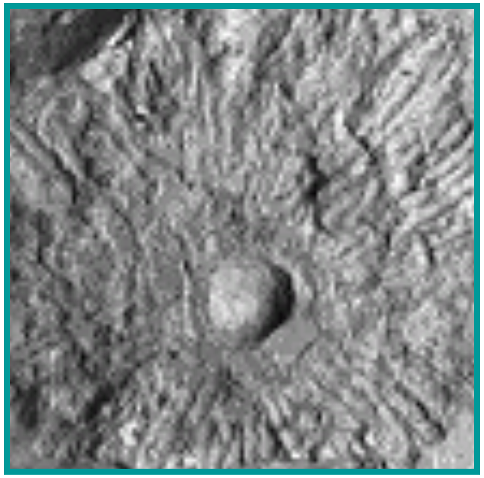


Ediacara fauna

P.A. Bourque, 1995

Ediacara fauna

Dickinsonia

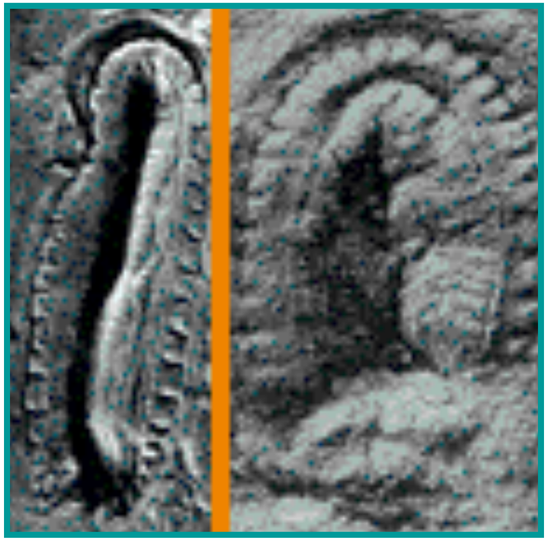


Eoporpita

Tribrachidium

Pteridinium

Spriggina



Kimberella

Photos extraites de de http://www.ucmp.berkeley.edu/vendian/critters.html

Ediacara fauna: some features

- **Worldwide distribution: > 30 sites on the 5 continents**
  (initial discovery in South Australie in 1946)

- **Soft-bodied fauna, unmineralized**

- **Microphage, short trophic chain**

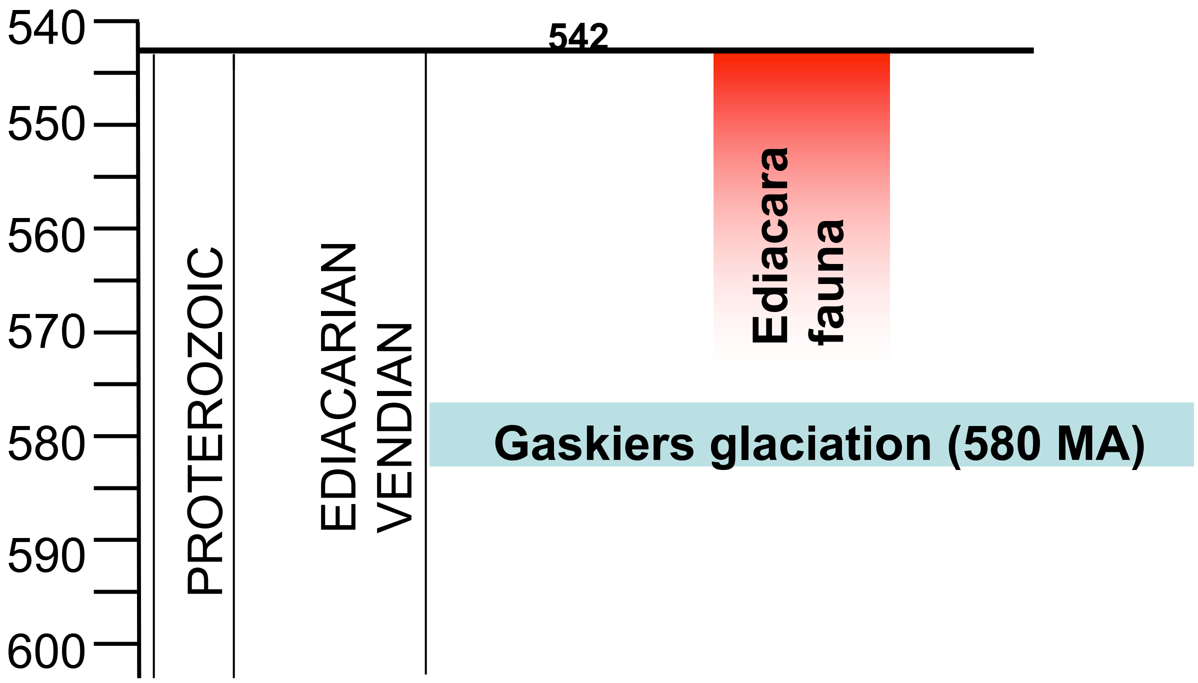
- **Adapted to a low level of O_2 : flat organisms, high surface exchange**

- **Increase of diversity from 570 à 545 MA**

- **Circa 70 'genera' in about 100 'species':**
  - ***70% cnidarian-like » = médusoid shape***
  - ***Flat worms***
  - ***Arthropods***
  - ***Unknown phylums***

Ediacara fauna & paleoenvironnemental context

***Canfield et al., Science, <u>315</u>, 92-95 (2007)* :**
Late Neo-Proterozoic deep-ocean oxygenation and the rise of animal life.



Ocean oxygenation due to deglaciation ?

Ediacara fauna & its relationship with metazoans: a debated question

Ediacara has nothing to do with metazoans that appeared later = VENDOBIONTS

Aborted experiment without offsprings

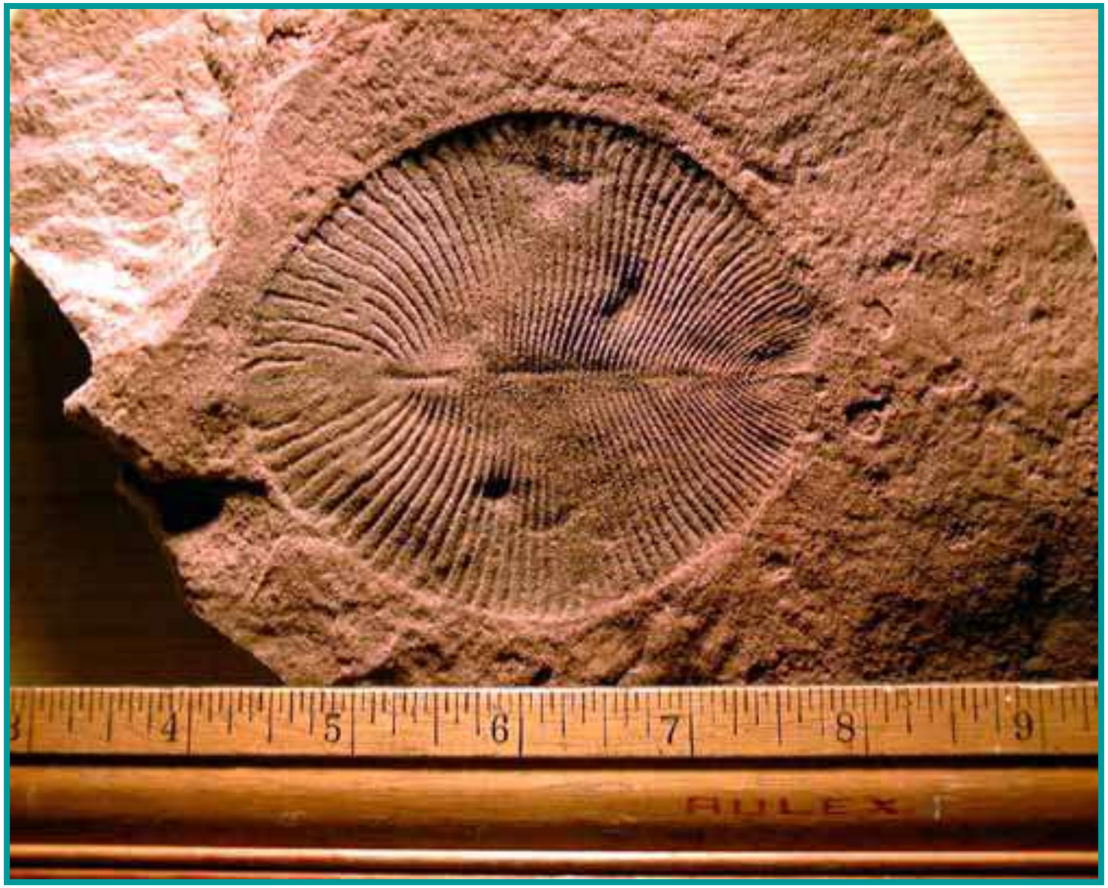
Buss & Seilacher, 1994: Vendobionts = sister-group of metazoans

Ediacara fauna is a mixture of representatives of disappeared and living phylums

- ***Sponges***
- ***Cnidarians***
- ***Flat worms***
- ***Arthropods ??***
- ***Echinoderms ??***

The *Dickinsonia* case

Bobrovskiy et al., Science, 361: Sept 2018.



LIPIDIC BIOMARKERS

Cholesteroids typical of metazoans

Early mineralized forms preceding the P/C transition

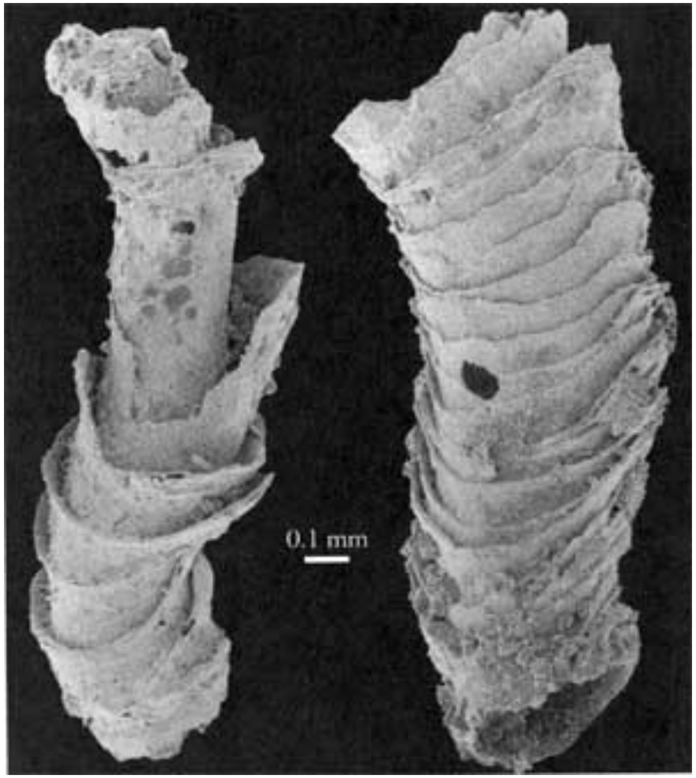
Cloudina:
- Germs. Am. J. Sci., 272 (1972)

- World distribution

- Calcified nested cones
- about the same age as EDIACARA

- Disappeared at the P/C transition

http://www.palaeos.com/Proterozoic/Neoproterozoic/Ediacaran/Ediacaran2.htm



Other mineralized forms:

- ***Namacalathus***

  Grotzinger et al. Paleobiology, 26 (2000)

- ***Namapoikia***

  Wood et al. Science, 296 (2002)

- ***Sinotubulites***

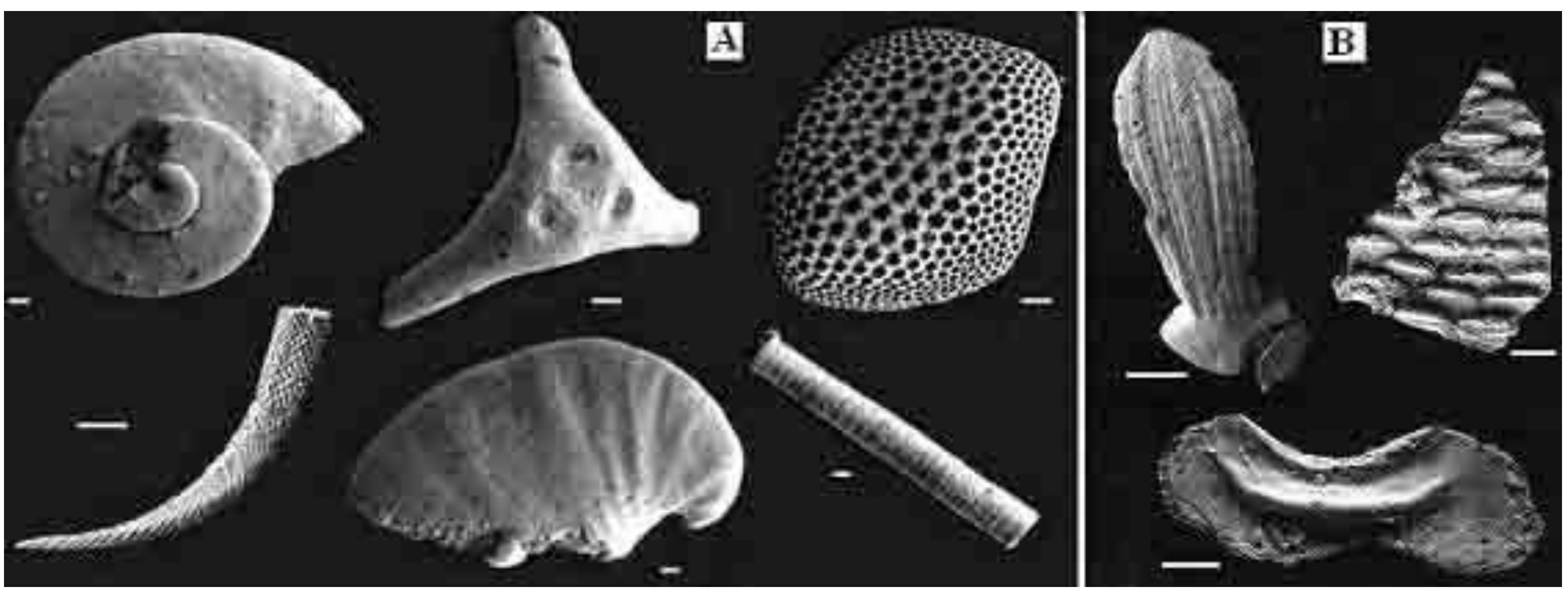
1st traces of predation on Cloudina

Bengtson & Zhao. Science, 257 (1992) **Hua et al. Palaios 18 (2003)**

Small Shelly Fossils of the Lower Cambrian

- World distribution
- Conical shapes, plates, sclérites and tubes
- 1st diversification of skeleton-bearing organisms: about 40 genera

- Uncertain affinities: sponges, cnidarians, mollusks…
- 1st attested mollusk: *Helcionella*

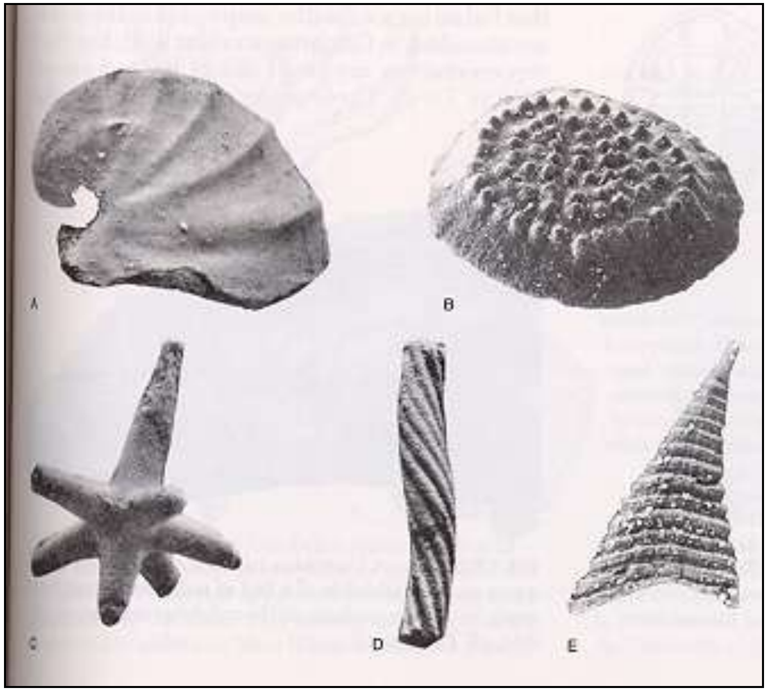


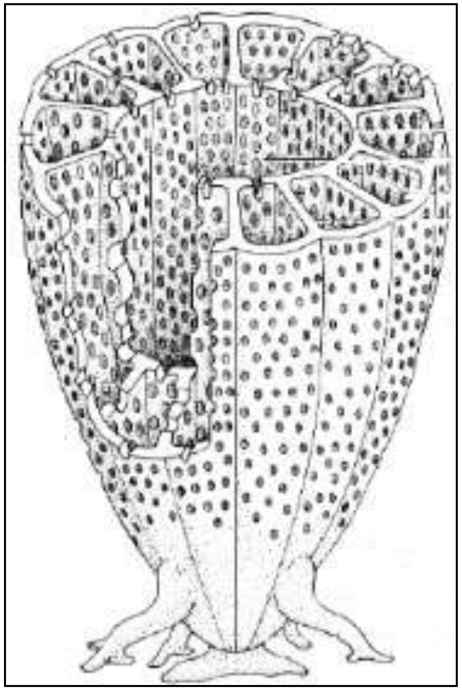
Li & Zhou, 2001 Peng *et al.*, 2001

Tommotian Fauna
(middle of Lower Cambrian)

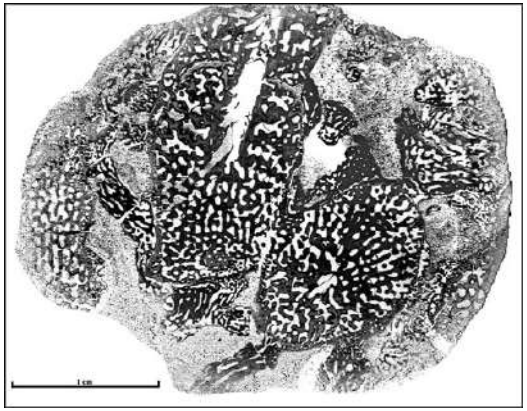
- World distribution
- Radiation of SSF

- Appearance of:
  - Archeocyatha
  - Brachiopods
  - Trilobites
  - Echinoderms



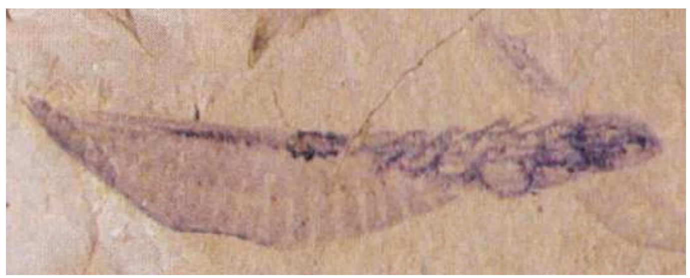


Archeocyathe



Archeocyathus atlanticus

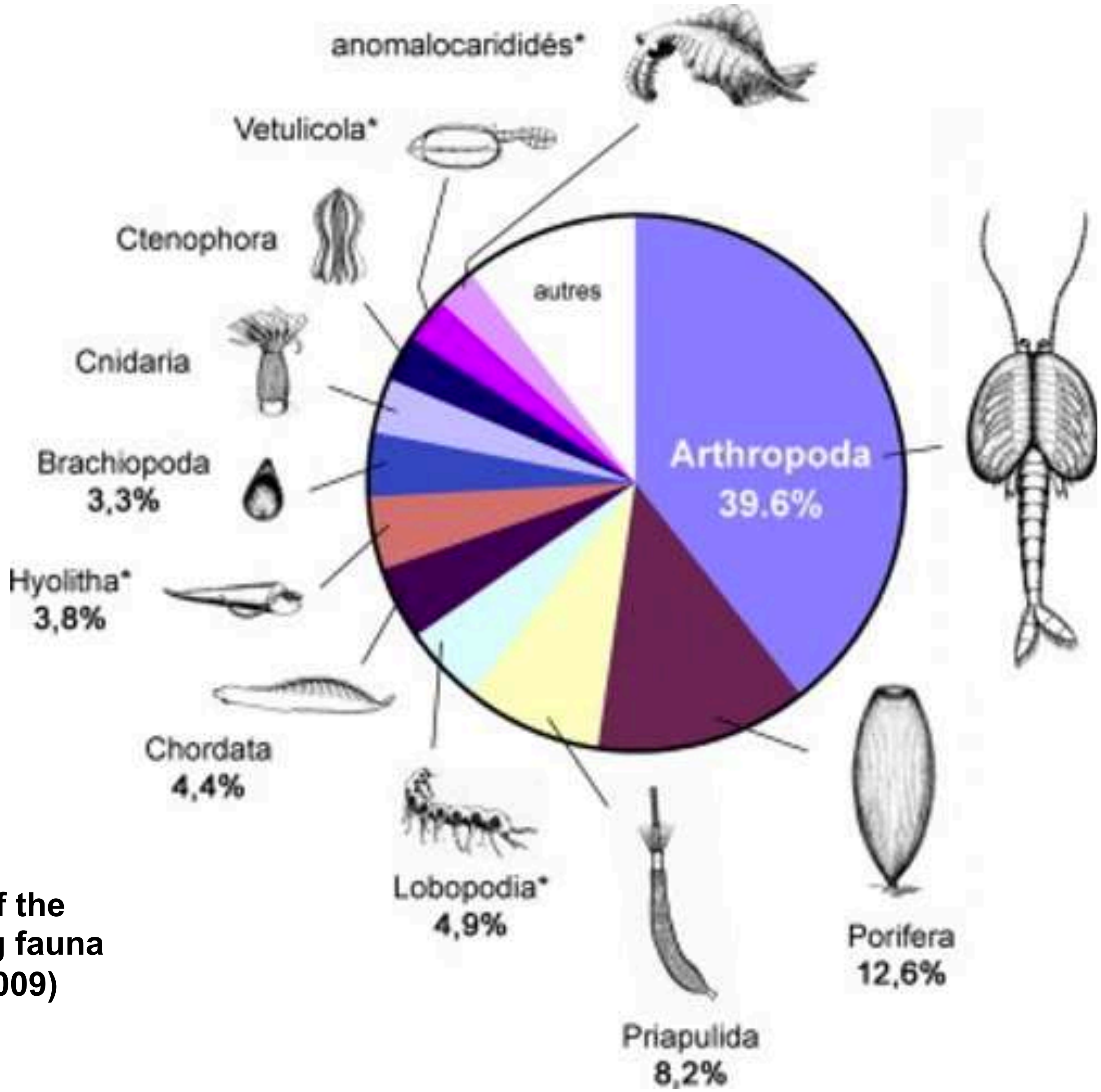
Chengjiang Fauna: the beginning of the Cambrian Explosion



- Age: end of Lower Cambrian (Atdabanien)
- < 150 species
- Circa 20 phylums
- One of the 1st modern ecosystem

Photos J. Vannier

Chengjiang fauna: beginning of the 'Cambrian Explosion'



Diversity of the Chengjiang fauna (Vannier, 2009)

The Burgess shales fauna

- Age : Middle Cambrian

- 37 phylums

- 70% metazoans with soft-bodies

- Sponges
- Cnidarians
- Annelids
- Priapulians
- Onychophores
- Mollusks
- Arthropods
- Echinoderms
- Chordates

Burgess Shales fauna

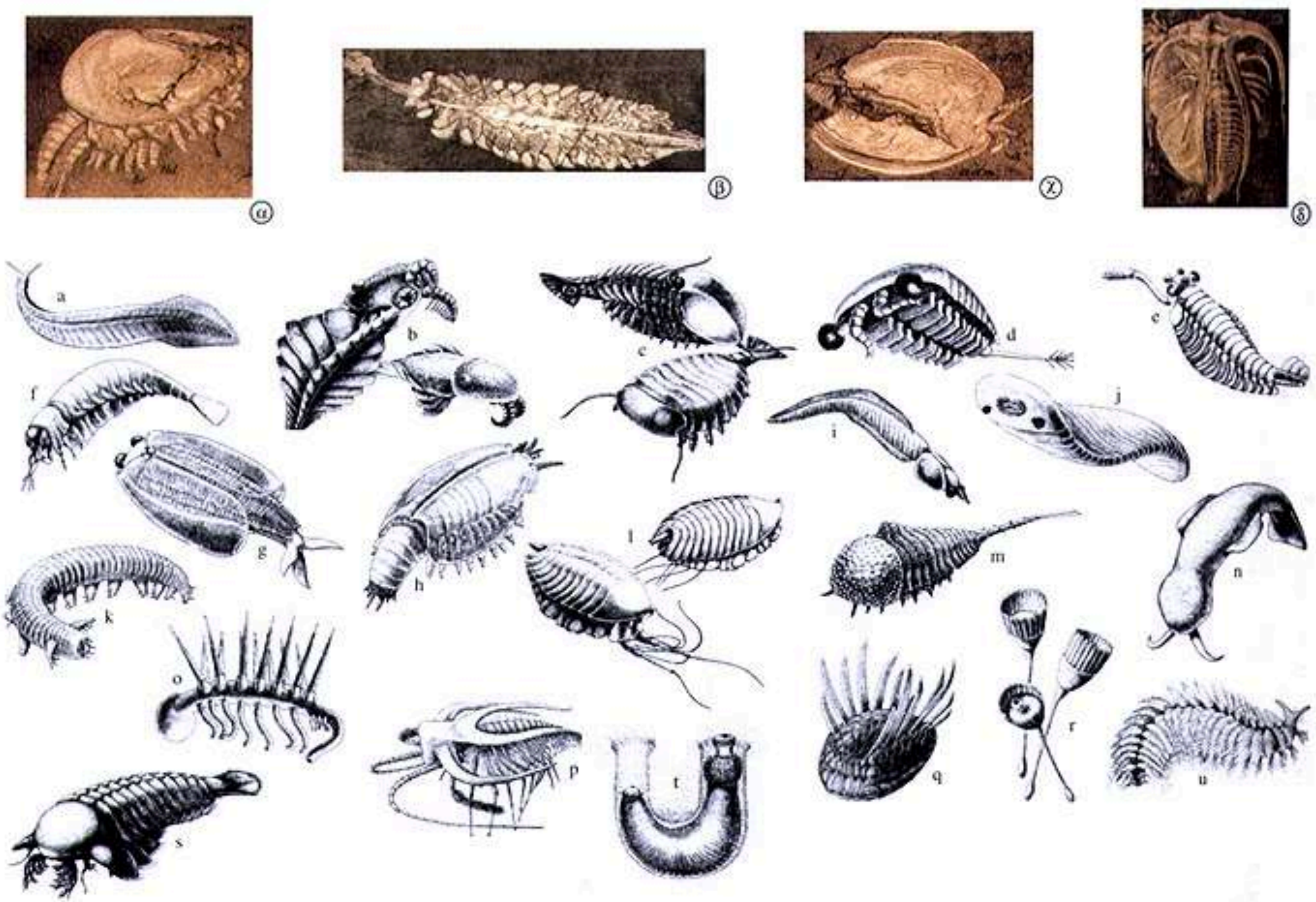


Fig. 235 La faune cambrienne de Burgess Pass (Colombie britannique).
α. *Canadaspis*. β. *Opabinia*. χ. *Leanchoilia*. δ. *Marella splendens*.

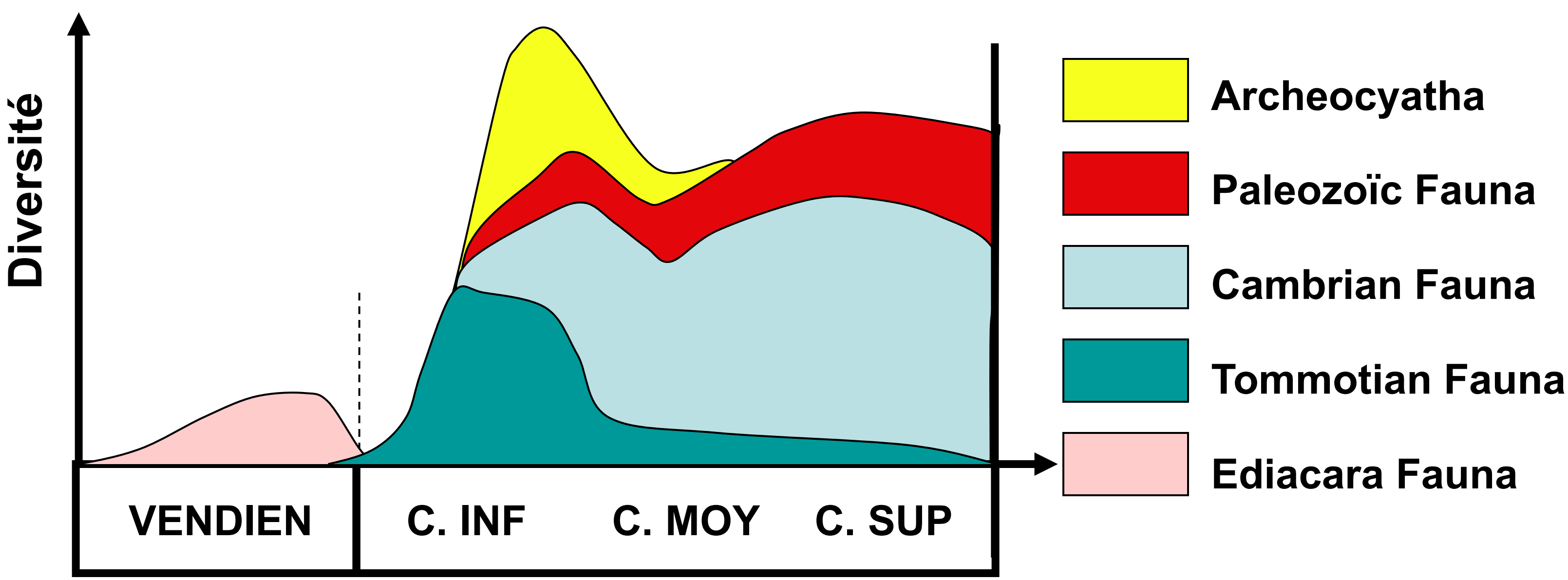
CARON J.-M. et coll., *Comprendre et enseigner la planète Terre*. Ophrys, 2003.

Représentations *(d'après Collins)* :
a. *Pikaia* (chordé). b. *Anomalocaris*. c. *Sidneyia*. d. *Sarotrocercus*. e. *Opabinia*. f. *Yohoia*. g. *Odaraia*. h. *Canadaspis* (malacostracé).
i. *Nectocaris*. j. *Odontogriphus*. k. *Aysheaia* (onychophore ?). l. *Leanchoilia*. m. *Habelia*. n. *Amiskwia*. o. *Hallucigenia*. p. *Marella*. q. *Wiwaxia*.
r. *Dinomischus*. s. *Sanctacaris* (arthropode chélicérate). t. *Ottoia*. u. *Canadia*.

In brief, the 'Cambrian Explosion' is:

- Novel 'bauplan' in metazoans
- Increase of diversity (at all levels, specific, generic...)
- Modern marine ecosystems
- Aborted experiments: Archeocyatha

- APPEARANCE OF BIOMINERALIZATION

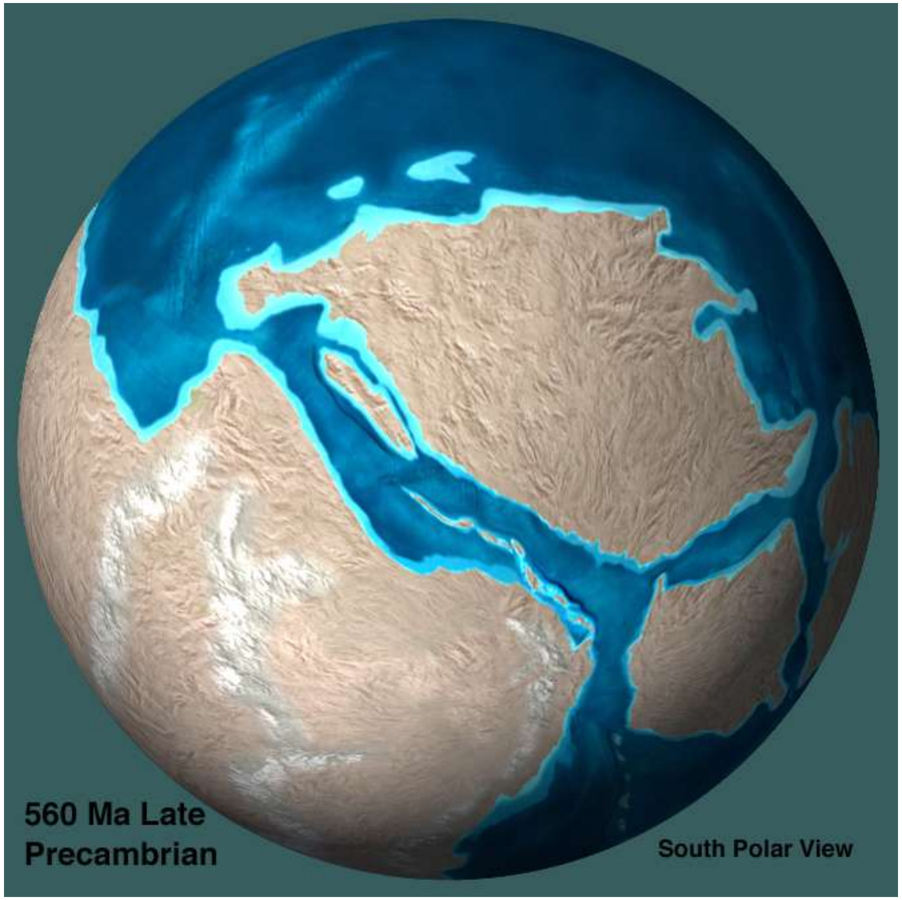


Adapted from Hallam & Wignall, 1997

Possible causes of the 'Cambrian Explosion'

EXTERNAL	**Tectonics** **Climat-related** **O_2** **Seawater chemistry: Ca**
INTERNAL	**Physiological** **Predation** **Genetic**

Causes related to tectonics



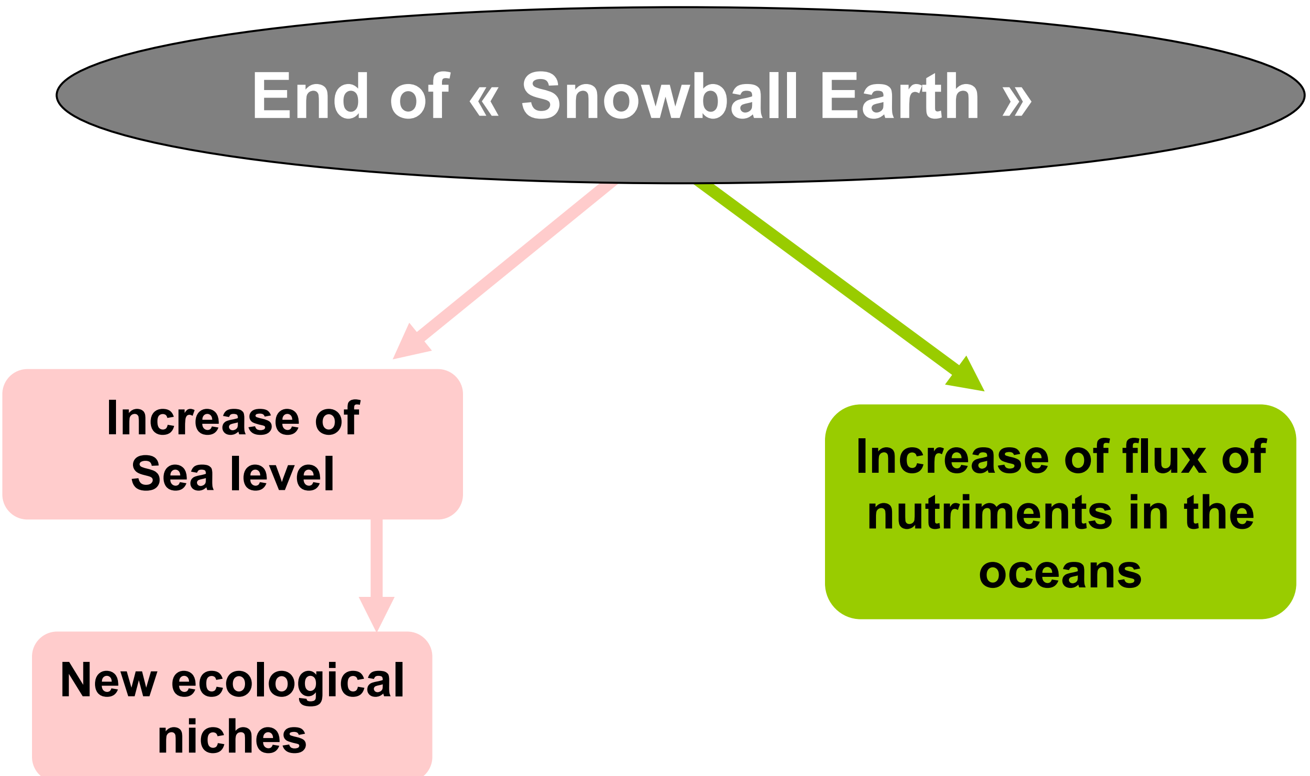
http://jan.ucc.nau.edu/~rcb7/560_LatePC_sp.jpg

Fragmentation of the supercontinent Rodinia

↓

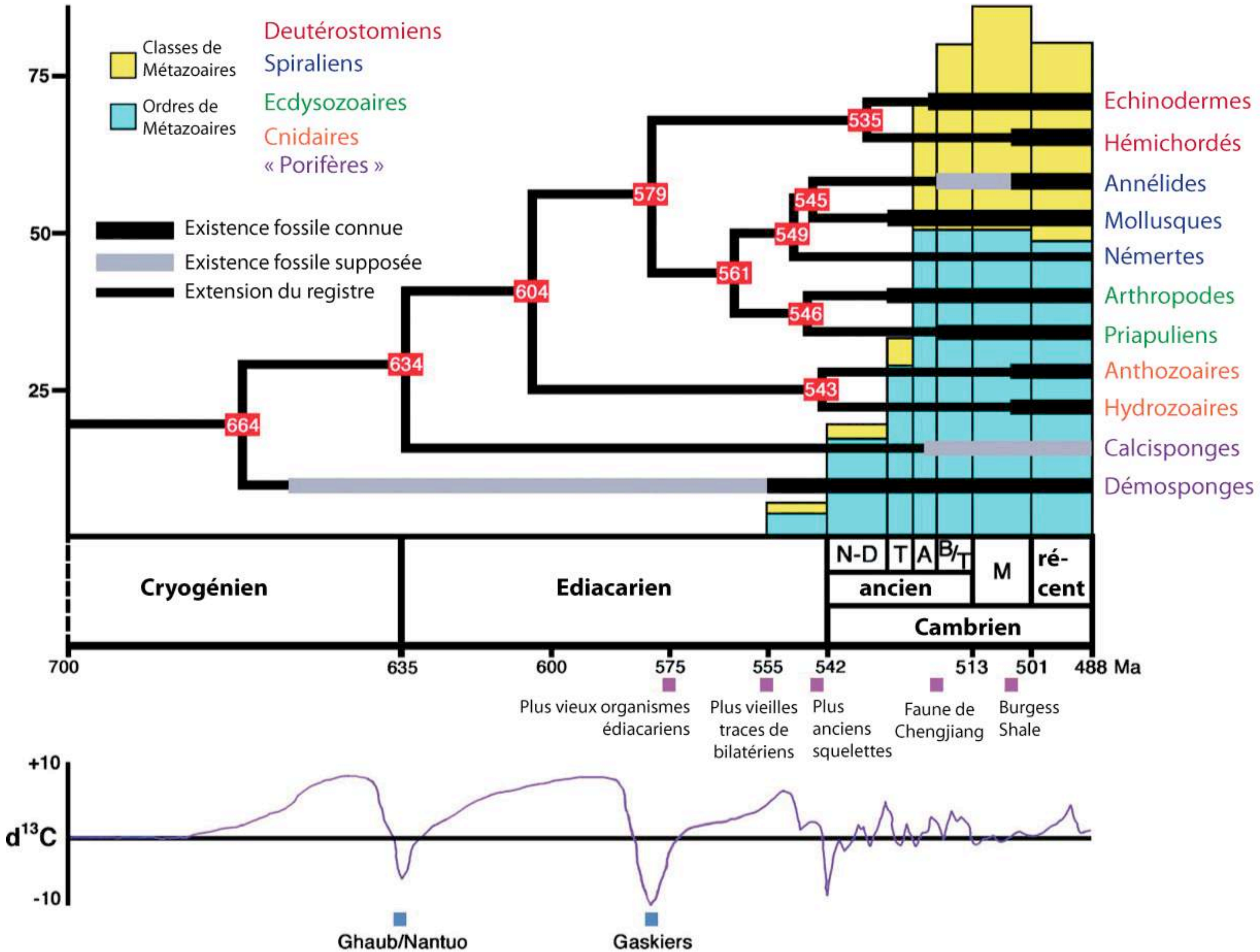
New epicontinental areas

Climatic causes



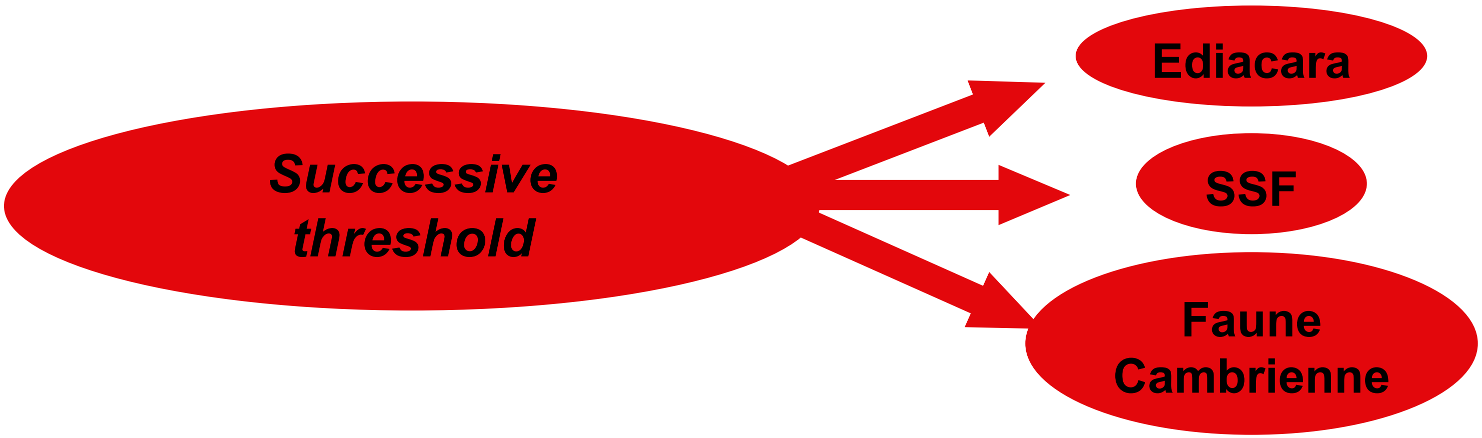
End of Snowball : due to a massive release of CO_2 ? (Pierrehumbert, Nature, 2004)

Climatic causes



Oxygen-related causes

Old hypothesis of Towe (1970) : O_2 & collagen



Raff & Raff. Nature, 228 (1970)

Kaufman & Knoll. Precamb. Res., 73 (1995)

Canfield. Nature, 396 (1998)

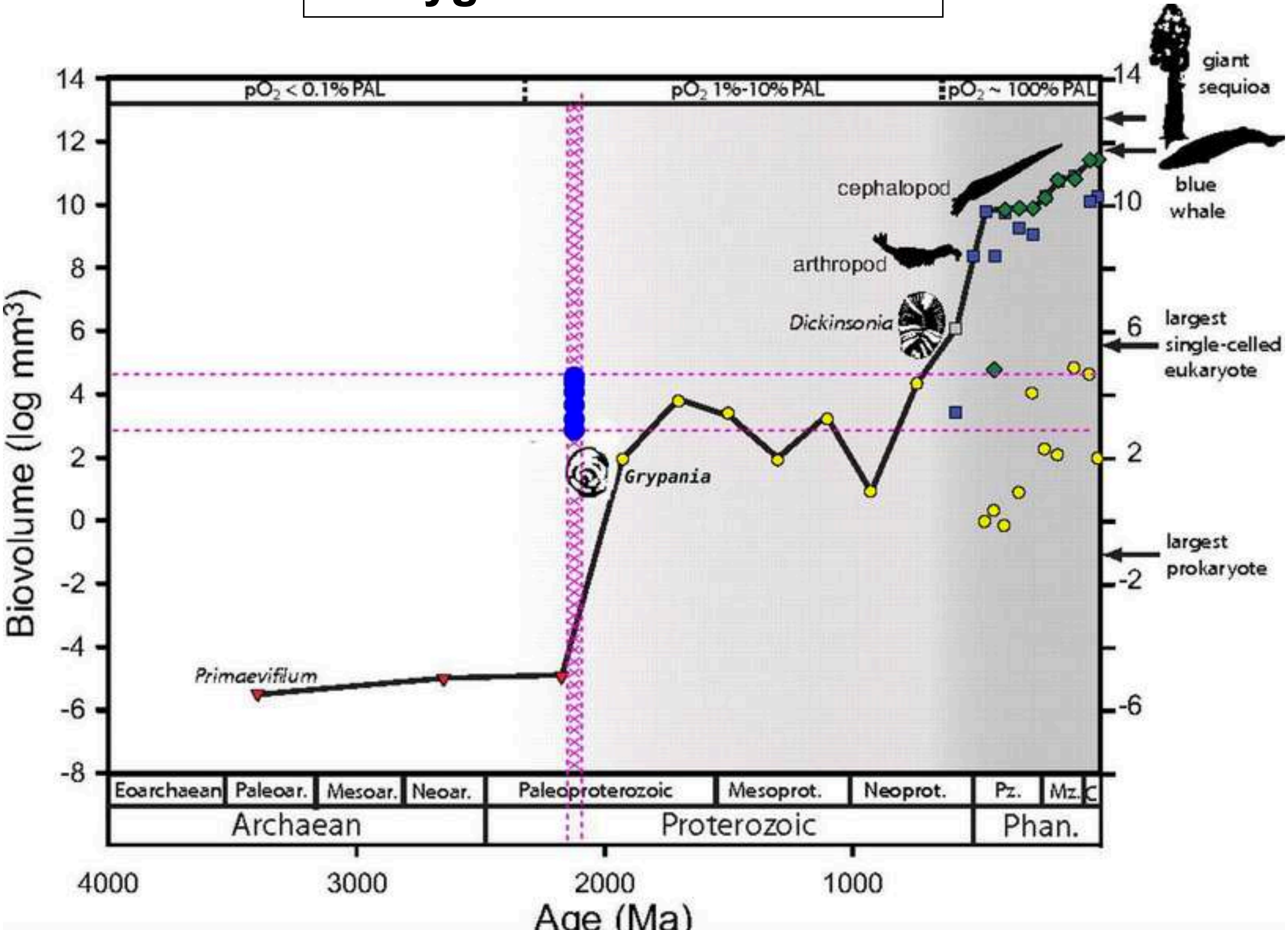
Shield. Terra Nova, 17 (2005)

Raymond & Segre. Science, 311 (2006)

Fike et al. Nature, 444 (2006)

Canfield et al. Science, 315 (2007)
Deep anoxic ocean until Gaskiers episode, then oxic ocean

Oxygen-related causes



Causes related to the chemistry of oceans

Hypercalcified bacteria of the Neoproterozoic, in Spitzberg:
Knoll et al. Palaios, 8 (1993)

NEOPROTEROZOIC OCEANS
SUPERSATUrATED WITH RESPECT TO $CaCO_3$

Without growth inhibitors, spontaneous precipitation

Fraiser et al. Palaios, 18 (2003)

Brennan et al. Geology, 32 (2004)

Corsetti & Grotzinger, Palaios, 20 (2005)

Kovalevych et al. Prec. Res., 144 (2005)

Decrease of the Mg^{2+}/Ca^{2+} ratio
Riding. Nature, 299 (1982)

Hardie, Geology, 31 (2003)

Stanley, P^3, 232 (2006)

Porter, Science, 316 (2007)

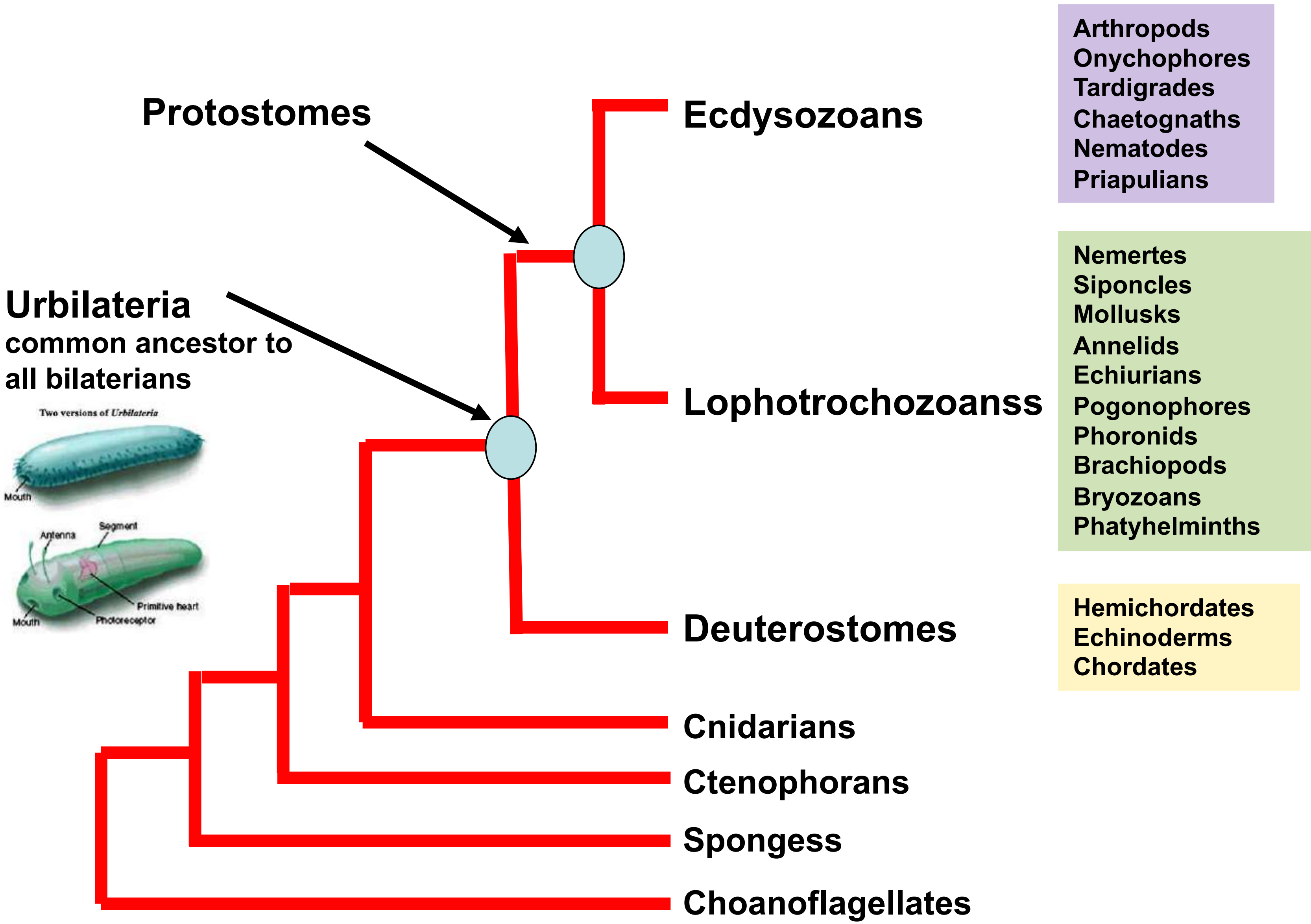
Ecological causes

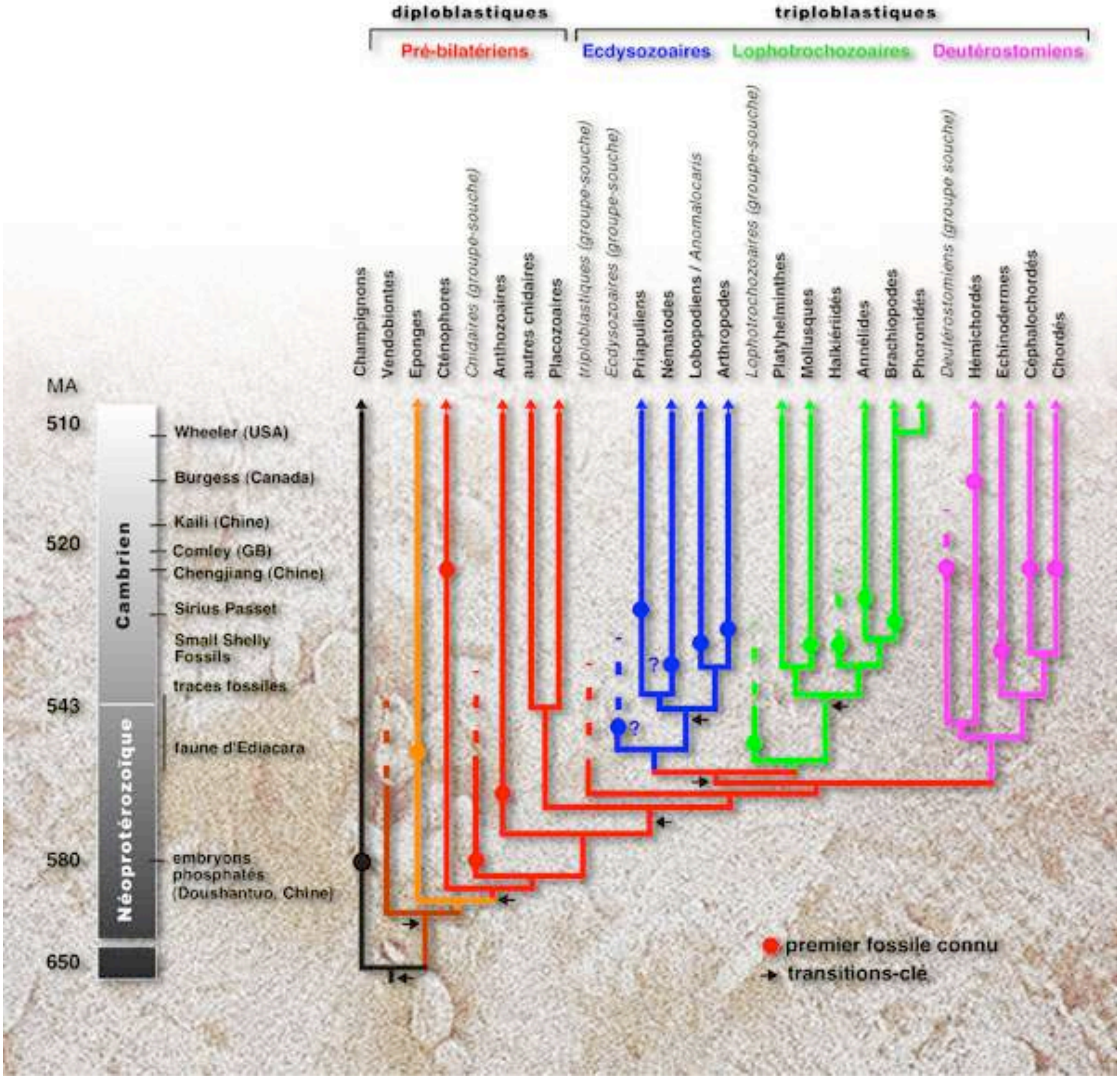
Appearance of predation

Traces of predation on trilobites: Nedin, Geology, 27 (1999)

***Anomalocaris*, the killer of Cambrian seas !!!**

Genetic causes: novel ‘bauplan’





diploblastiques
triploblastiques
Pré-bilatériens
Ecdysozoaires
Lophotrochozoaires
Deutérostomiens
Champignons
Vendobiontes
Eponges
Cténophores
Cnidaires (groupe-souche)
Anthozoaires
autres cnidaires
Placozoaires
triploblastiques (groupe-souche)
Ecdysozoaires (groupe-souche)
Priapuliens
Nématodes
Lobopodiens / Anomalocaris
Arthropodes
Lophotrochozoaires (groupe-souche)
Platyhelminthes
Mollusques
Halkieriidés
Annélides
Brachiopodes
Phoronidés
Deutérostomiens (groupe souche)
Hémichordés
Echinodermes
Céphalochordés
Chordés
MA
510
520
543
580
650
Cambrien
Néoprotérozoïque
Wheeler (USA)
Burgess (Canada)
Kaili (Chine)
Comley (GB)
Chengjiang (Chine)
Sirius Passet
Small Shelly Fossils
traces fossiles
faune d'Ediacara
embryons phosphatés (Doushantuo, Chine)
premier fossile connu
transitions-clé

Genetic causes: novel 'bauplan'

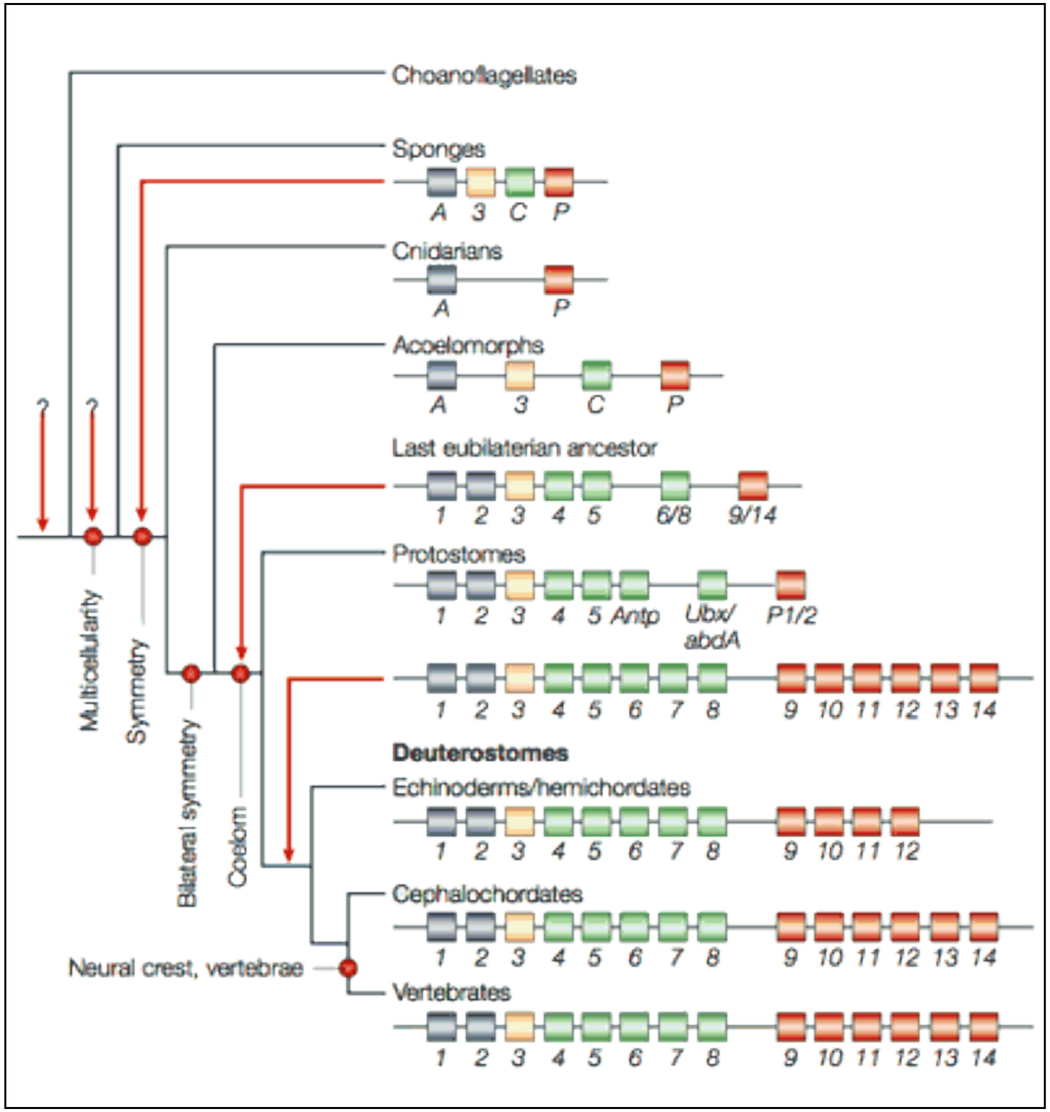
Role of Hox genes

- Hox genes code fortranscription factors

- They control the expression of other genes

- Development genes, structuring the antero-posterior axis

- Grouped in clusters (colinearity)
- Evolved by gene duplication



Garcia-Fernandez, Nature Reviews Genetics, *6*, 881-892 (2005)

Anti-calcification:

Marin et al. PNAS, 96 (1996)

Supersaturated ocean with respect to $CaCO_3$

↓

Requirement of inhibitors to avoid overgrowth of crystals

↓

Anticalcifying mucus may have played this role

↓

At P/C transition, mucus inhibitors would have played another function: to keep crystallization in check

Ediacara-type fauna

Appearance of biologically-controlled mineralization: what it implies

Proterozoic ecosystem

P.A. Bourque, 1995

Soft-bodied fauna

Cambrian 'modern' ecosystem

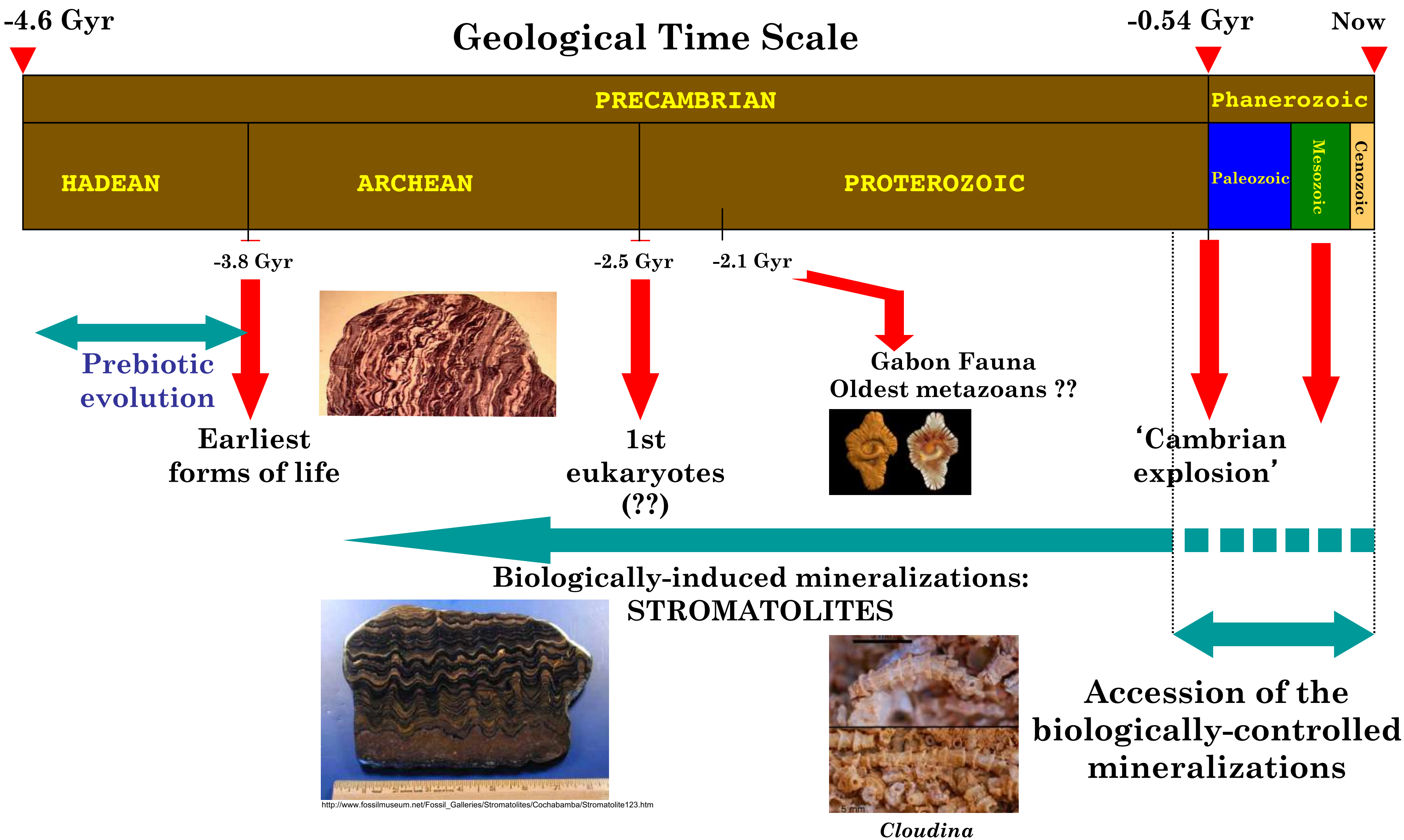
Mineralized fauna

Emergent properties

Controlled biomineralization → HIERARCHY

Controlled biomineralization → SPECIALIZED ORGANIC MATRIX

Macro-evolution of $CaCO_3$-based biomineralization across geological times

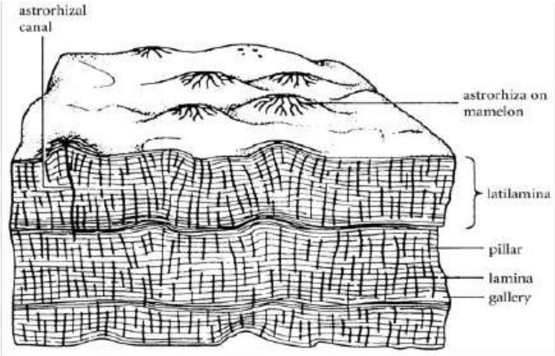


During the Paleozoic times

At world scale, biomineralizations dominated by reef systems in a ‘calcitic’ ocean: Paleozoic corals

-Tabulate corals
-Rugosans

-Stromatoporoids (sponge-like)

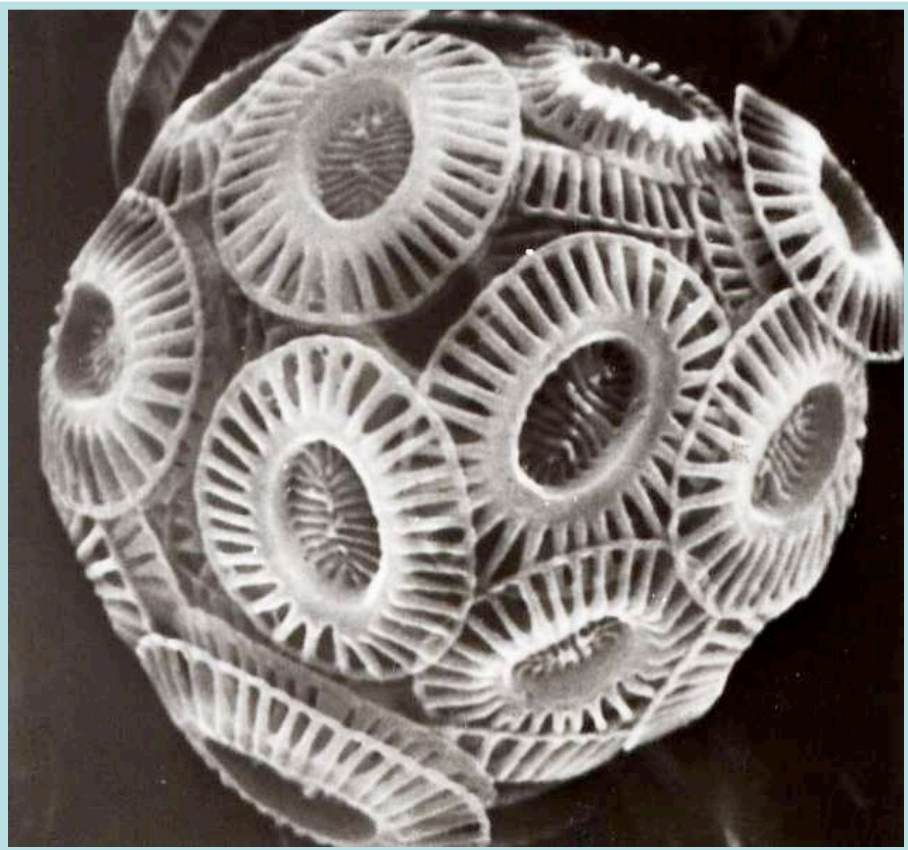


Extinction at the P/T crisis, 250 MY ago

During the Mesozoic times:

Emergence of planktonic organisms as important providers of biogenic calcium carbonate: the case of coccolithophore algae

Coccolithophore algae



Photos P. Westbroek

Photos P. Westbroek

Photos P. Westbroek

Accumulation of coccolithes: chalk cliffs

Etretat cliffs

Cretaceous period

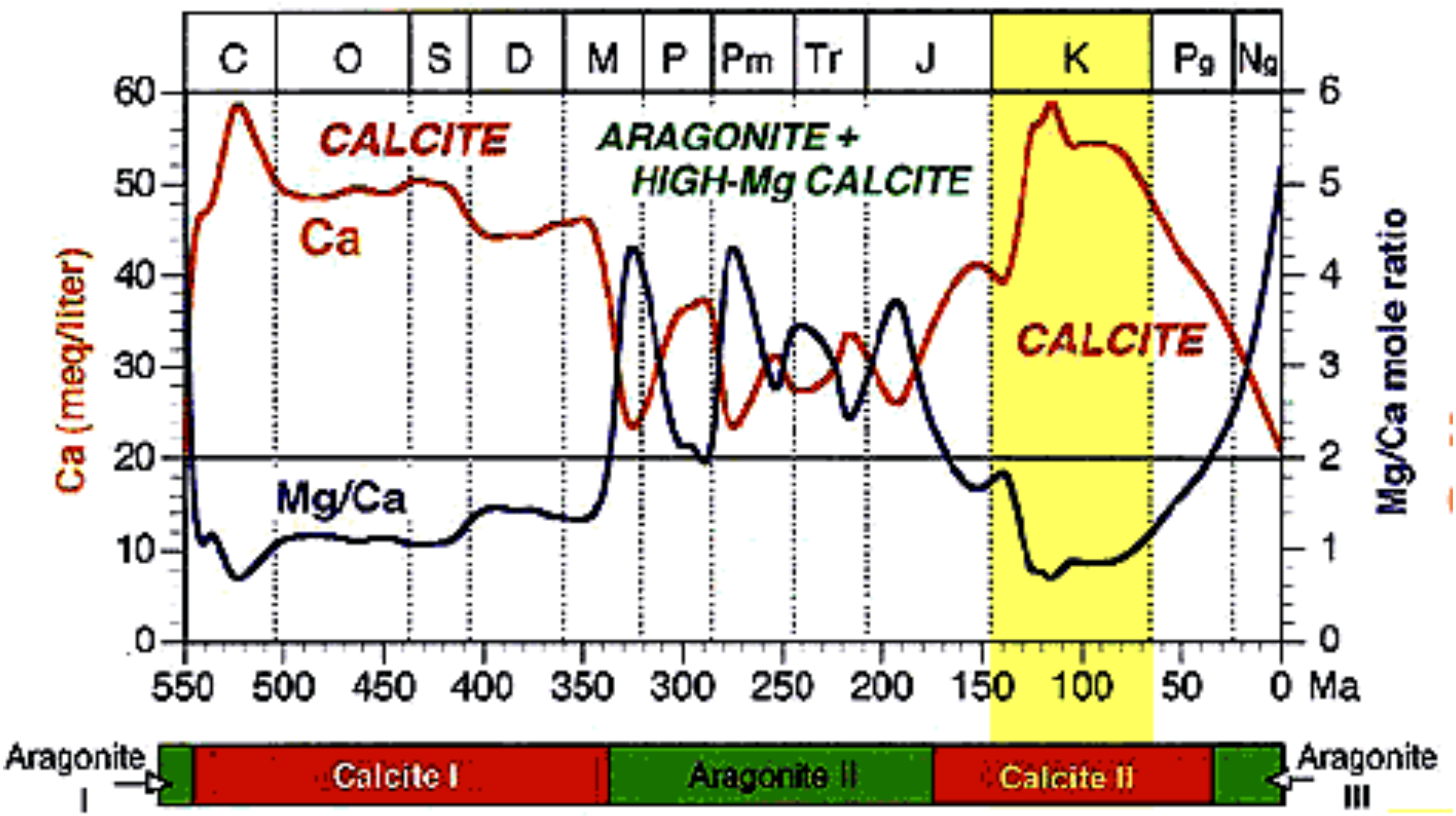
Accumulation of coccolithes: chalk cliffs

Seven Sisters, Sussex
(P. Standing, Geograph.org)

Today's bloom of coccolithophore algae

During the Mesozoic times:

Fluctuation of Mg/Ca ratio: High => Aragonite
Low => Calcite



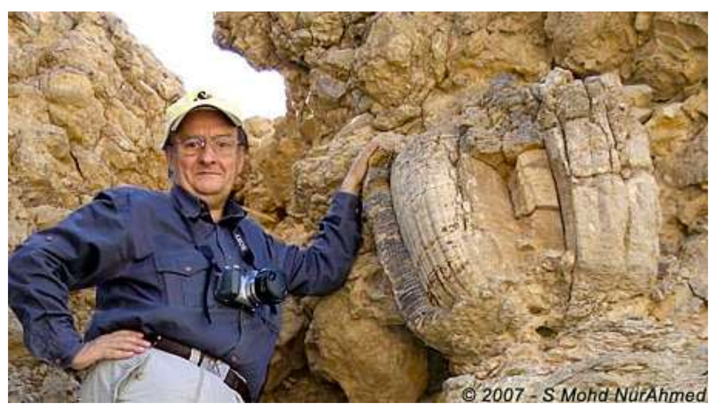
During the Mesozoic times:

While calcifying planktonic organisms emerged, reef systems continued to develop during the Trias, the Jurassic and the Lower Cretaceous: from 250 to 120 MY. They were dominated by Scleractinian corals (like today's corals)

Yonne Valley, Upper Jurassic

In the Upper Cretaceous, reef systems were dominated by rudists, a clade of highly specialized bivalves


Photo Schumann & Steuber



The take-away message

In brief:

Biologically-induced calcifications:
STROMATOLITES: Archean to Proterozoic

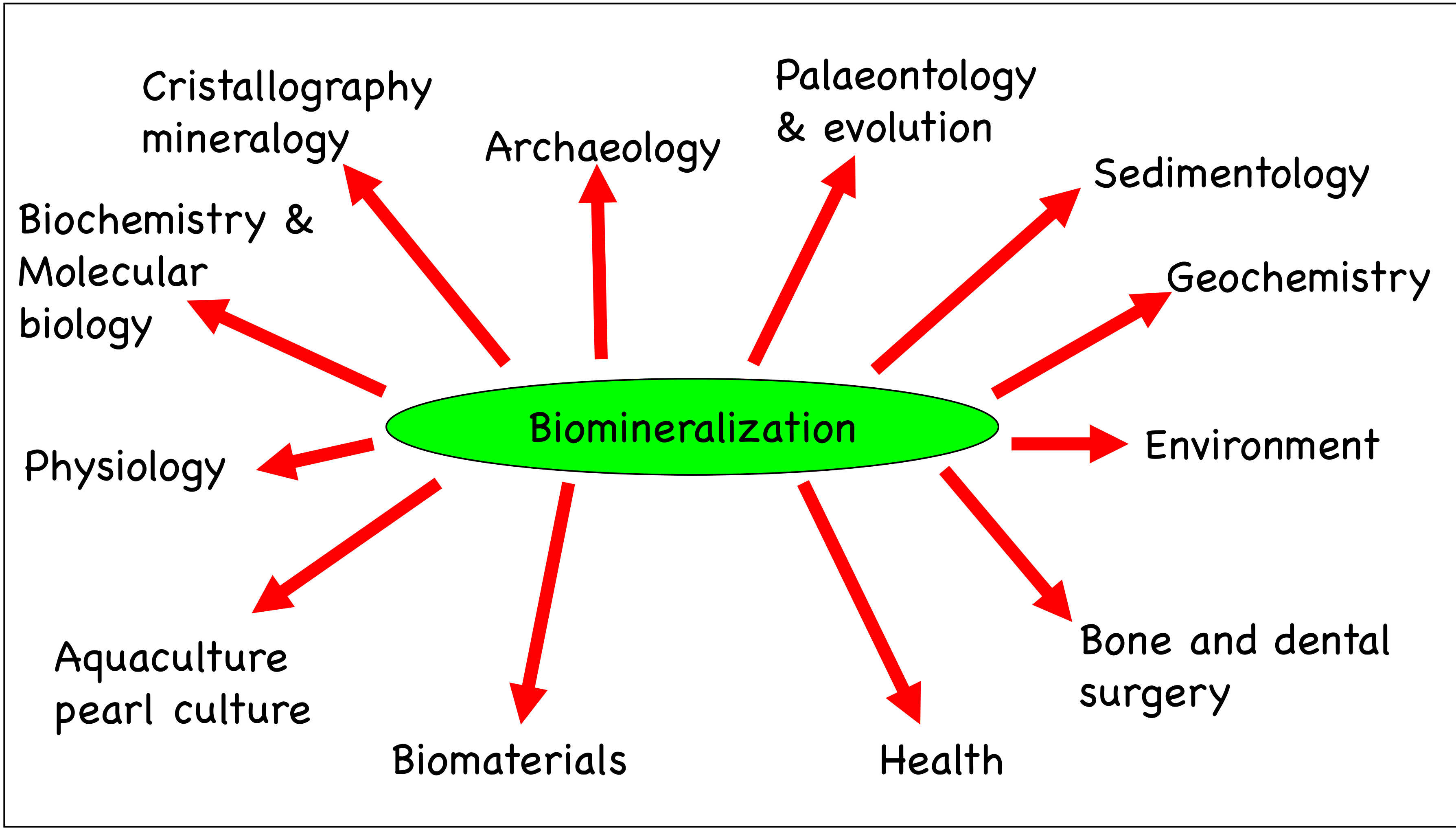
I. Biologically-controlled mineralization

- ***Cambrian Explosion: 544 My***
  - ***- Benthic fauna***
  - ***- Neritic domain***

- ***Mesozoic revolution (Jurassic)***
  - ***- Takeover by planktonic protists***
  - ***- Open oceanic domain***

Biomineralizations

Applications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Palaeontology
& evolution
Sedimentology
Biochemistry &
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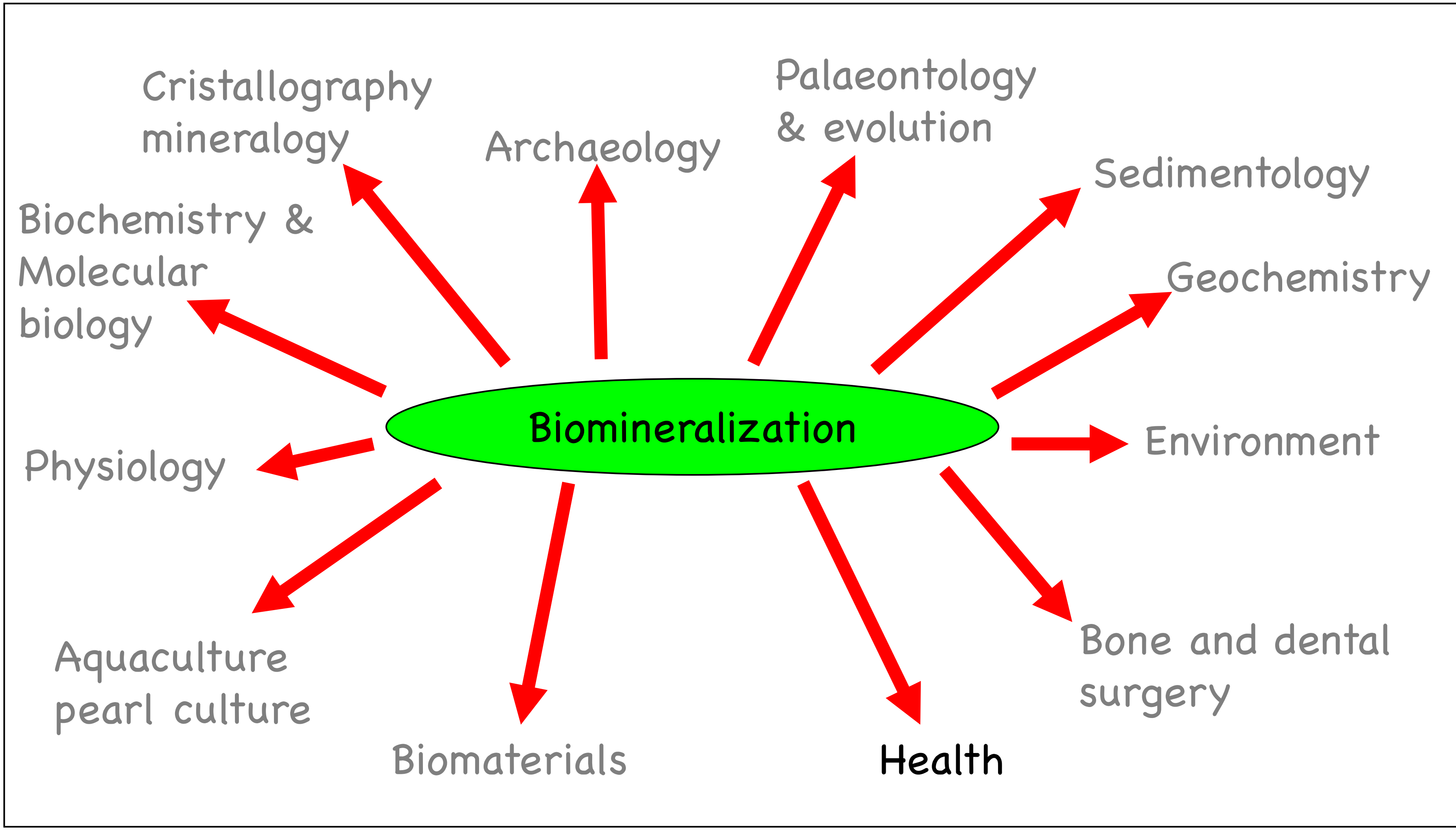
Environment
Aquaculture
pearl culture
Biomaterials
Health
Bone and dental
surgery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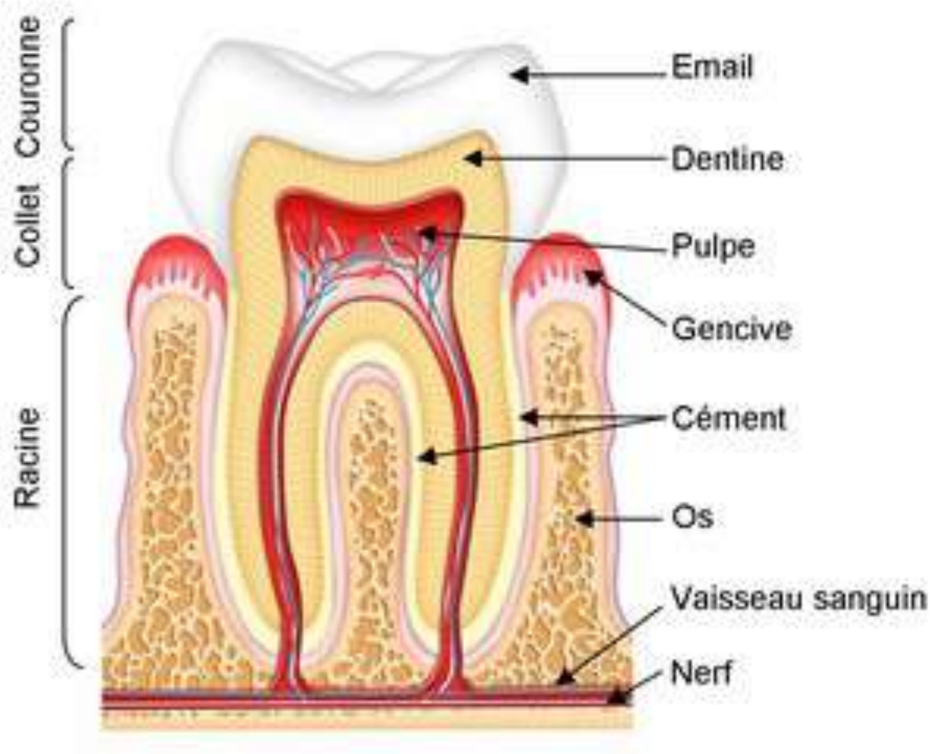
Dental surgery & implantology

- **Implants, a huge market: 3.5 billion Euros en 2014.**
- **High added value.**



Bone surgery

- **Population ageing**
- **Osteoporosis**
- **Hip / knee implants / prosthesis**

  *** In France, per year:**

  Knee: 30 000 prostheses

  Hip: 100000 prostheses

  *** In the USA: 285000 hip prostheses**

Bone surgery

Coral implants:
Porites sp.

- Decontaminated
- Thermic conversion in Ca-P

- Good porosity facilitating the migration of osteoblasts

- Relatively good mechanical properties but brittle
- Resorption capacity

Nephrology, urology

Kidney stones

Circa 5% of European population (1 à 10%), 5 to 15% in the USA. In France, 1 man on 10 and 1 woman on 20had, has or will have kidney stone problems

Calcium-based: 80% of the cases.

- Calcium oxalate (whewellite, weddellite)

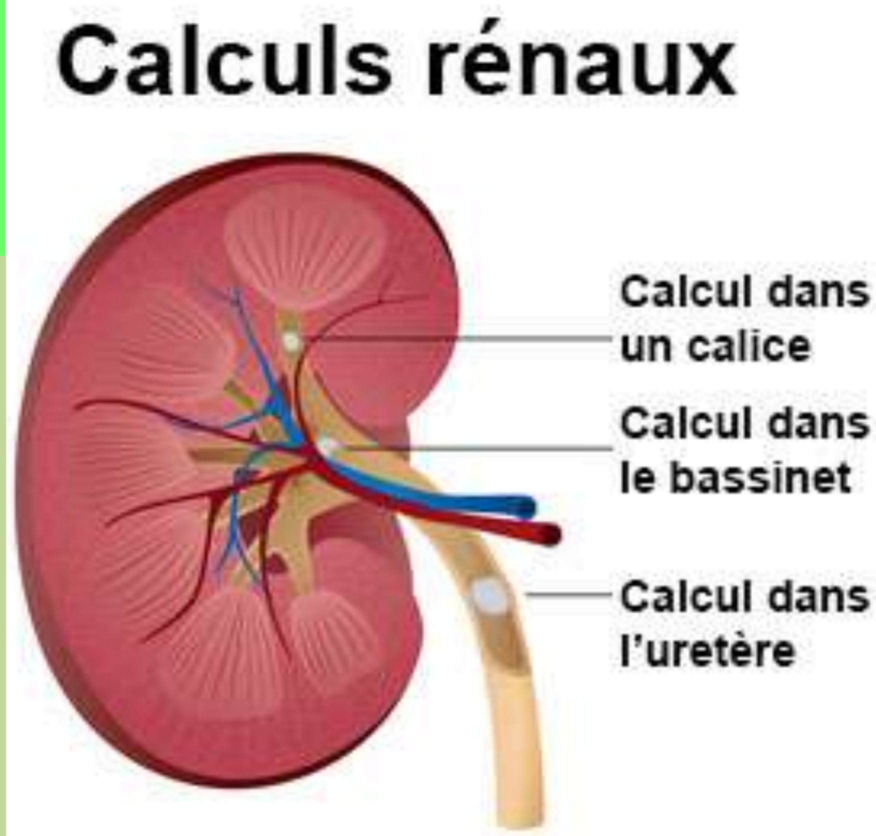
- Calcium phosphate (carbonate-apatite, brushite)

Non calcium-based: 20% of the cases

- Uric acid : 10% → gout

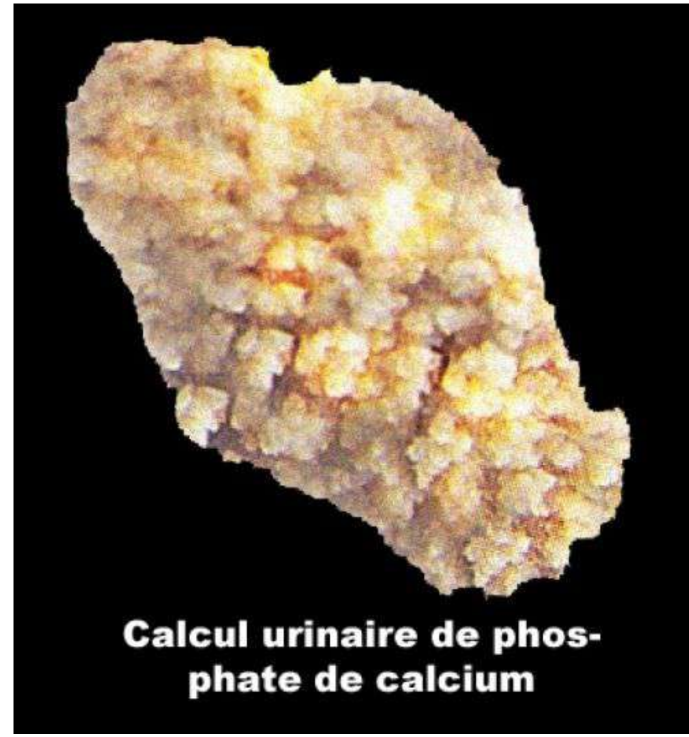
- struvite (Mg-phosphate): 10%

- cystine/xanthine: 1%.



Les calculs calciques

'Coralliform' kidney stone in calcium oxalate

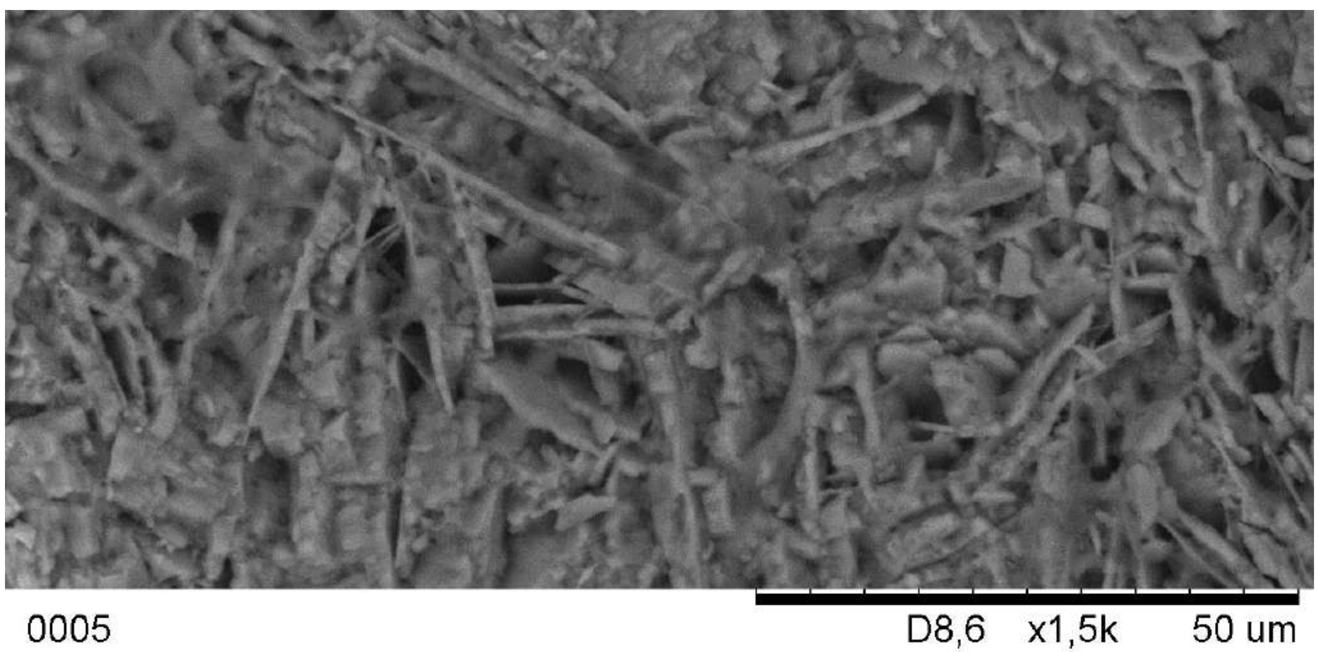


Crystallization inhibiteurs:

Non specific:
- Citric acid.
- Magnesium.
- Oligo-elements

Specific:
- GAGs
  - Nephrocalcin
  - Uropontin
  - Pyrophosphates

Uric acid kidney stones



Gastroenterology

PÄNCREATIC STONES

Up to 10% of the European population

More or less mineralized

Inhibitor of pancreatic stones: lithostatin

Pharmacology

Discovery of natural biactive substances, beneficial for health, extracted from biominerals

<u>The nacre example</u>:

- Bone surgery:

*** Osteoinductive and osteogenic properties of nacre matrix.**

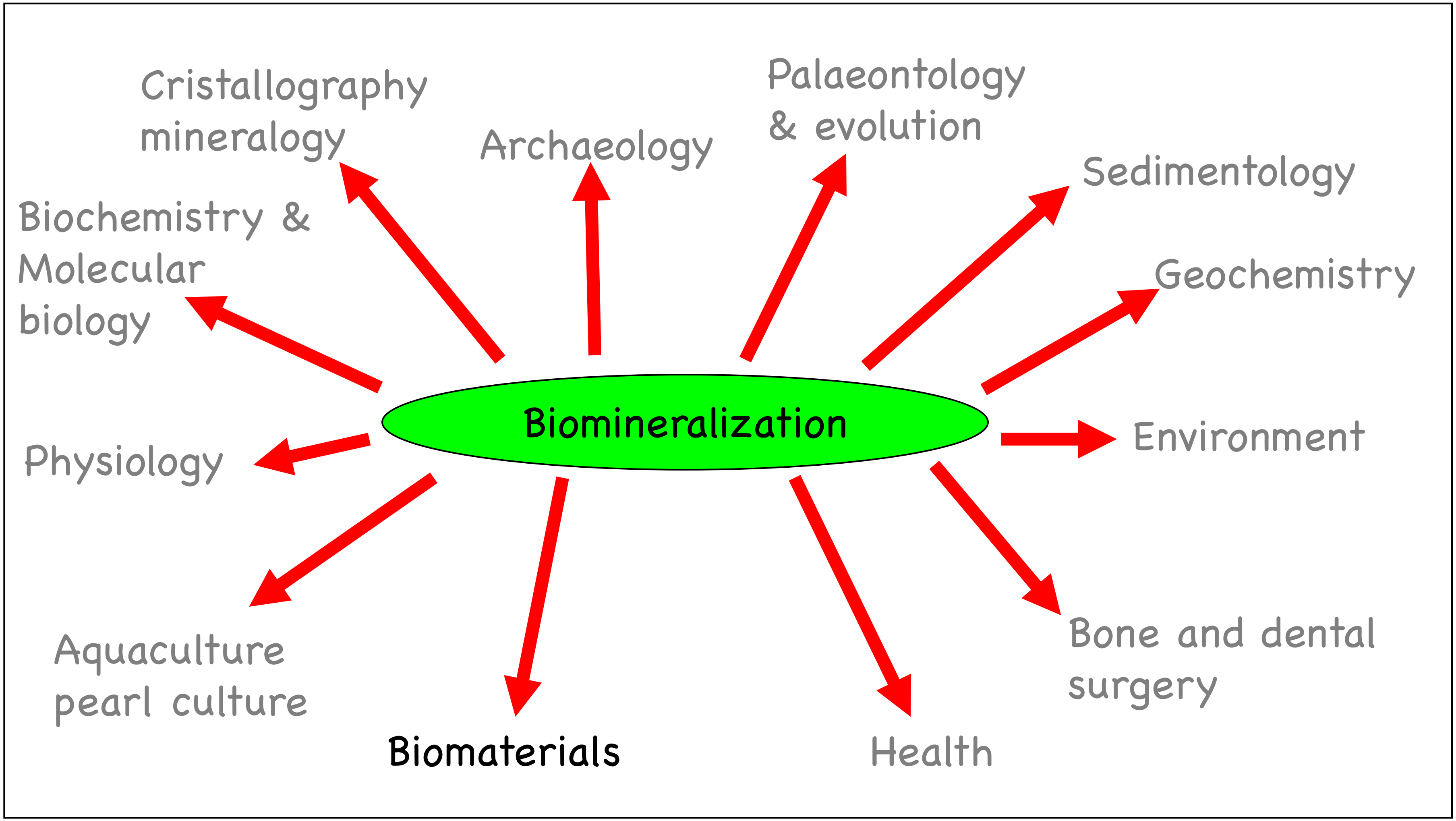
- Cosmetology / dermatology:

*** Nacre extracts in dermal applications.**

Pharmacology

Food supplement:

- Maerl (red algae)
- Nacre powder
- Coccoliths extracts
- 'Organic silica' (in reality, diatoms)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Construction materials

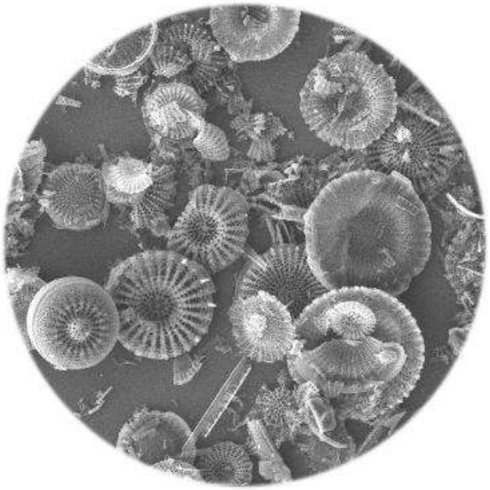
Limestone

Chalk

Lime

Other materials

Diatomites



- Filters (wines)

- Abrasives

- Depolluting

- Mineral filler for

Painting

- Some natural deposits:

California (Lompoc,the biggest in the world), Turkey, China, Island.

World prod.: 1.8 millions tons/yr, 50% from USA.

- Coastal marine deposits
- Lacustrine deposits in relation to volcanism

Biomimetic materials
Bioinspired materials

*Most of the processes used in industry for making materials are **costly**: sintering of ceramics made at high temperature*
*And / or **polluting** (polymers chemistry).*

➔ Development of a **Green Chemistry**, basedon synthesis processes made at room temperature, with a minimum of energy.

⇒ Nature fabricates biominerals under these conditions.

⇒ Observe Nature & try to mimic what it does: « bottom-up » approach

Biomimetic materials
Bioinspired materials

- **Nanocages (& nanotubes) made of silica or other**
- **Inspired from diatoms:**
  - **Applications: delivery of active substances in the body**

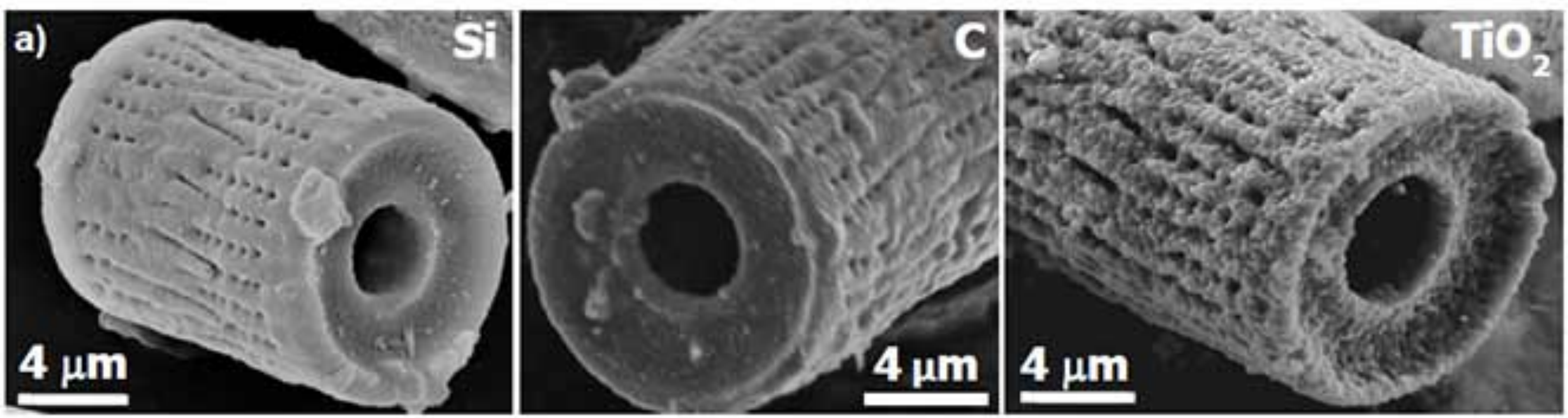
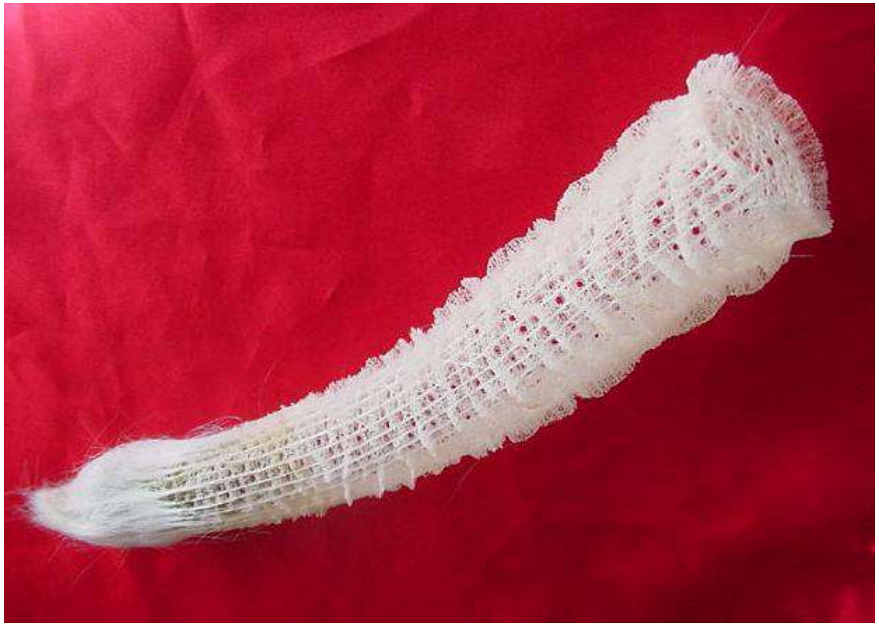


Figure 10. Secondary electron images of: a) porous Si, b) porous C, and c) TiO_2 replicas of *Aulacoseira* diatom frustules generated via shape-preserving gas/solid reactions.

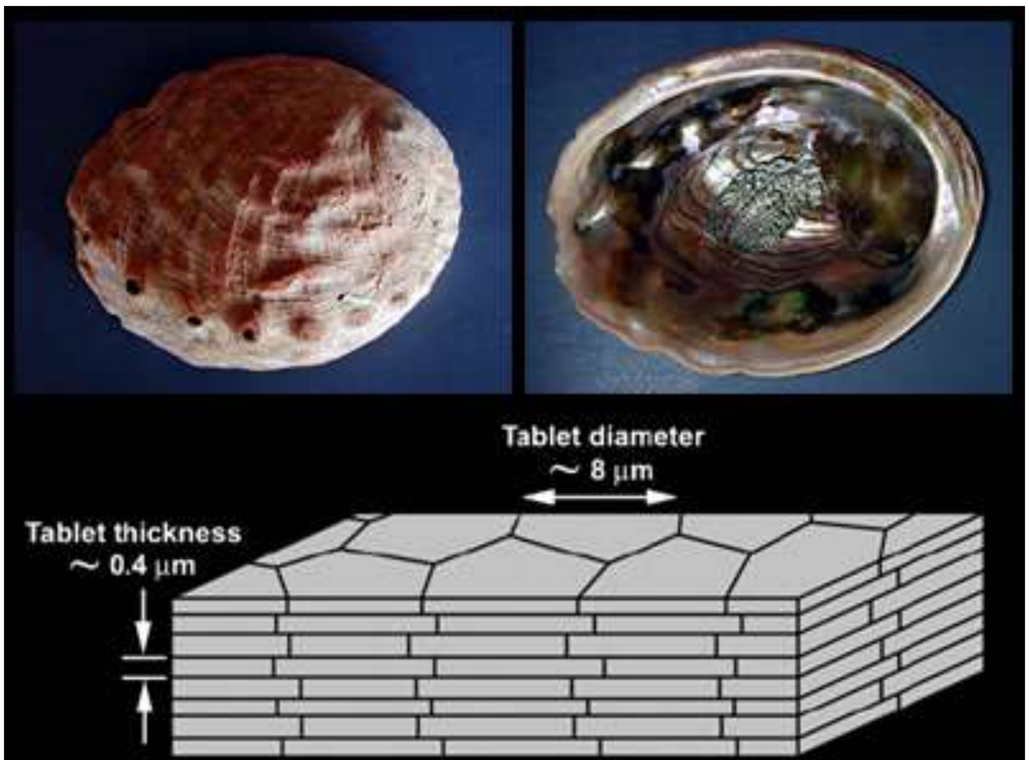
Biomimetic materials
Bioinspired materials

- Siliceous sponge spicules behaving like optical fibers



Biomimetic materials
Bioinspired materials

- Materials in ‘tablets’ that mimic nacre in its microstructure

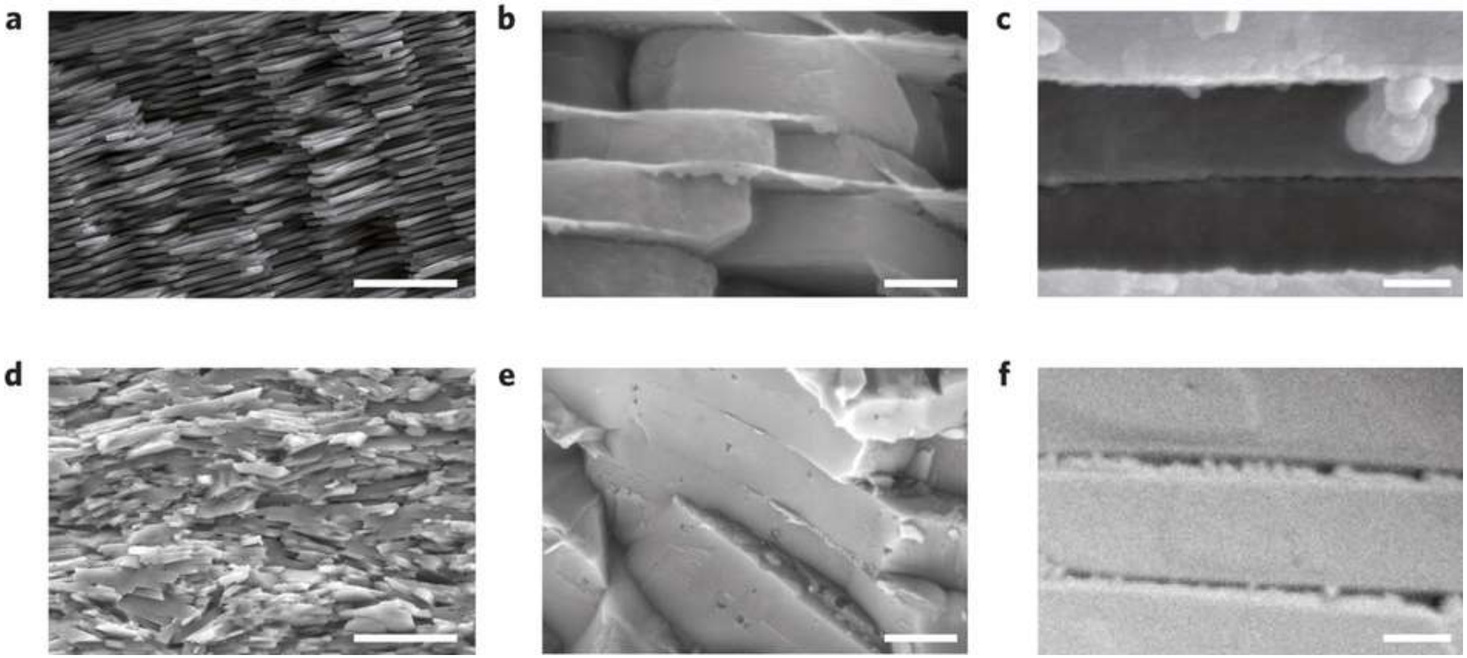


Resistance to fracture 1000 higher than that of chemical aragonite

$CaCO_3$: cheap !

Biomimetic materials
Bioinspired materials

- Materials in 'tablets' that mimic nacre in its microstructure



True nacre

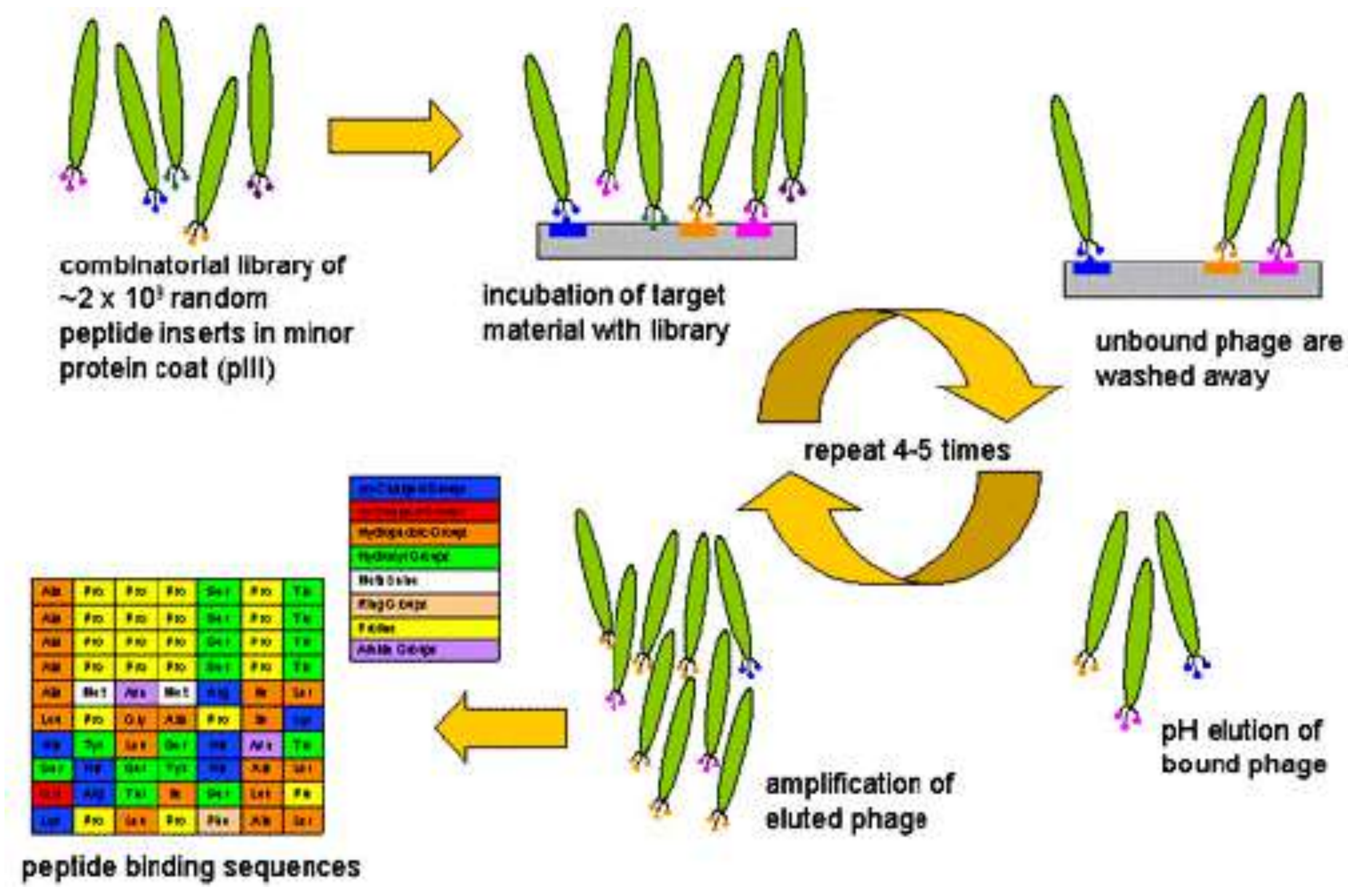
« Nacre » in aluminium oxide

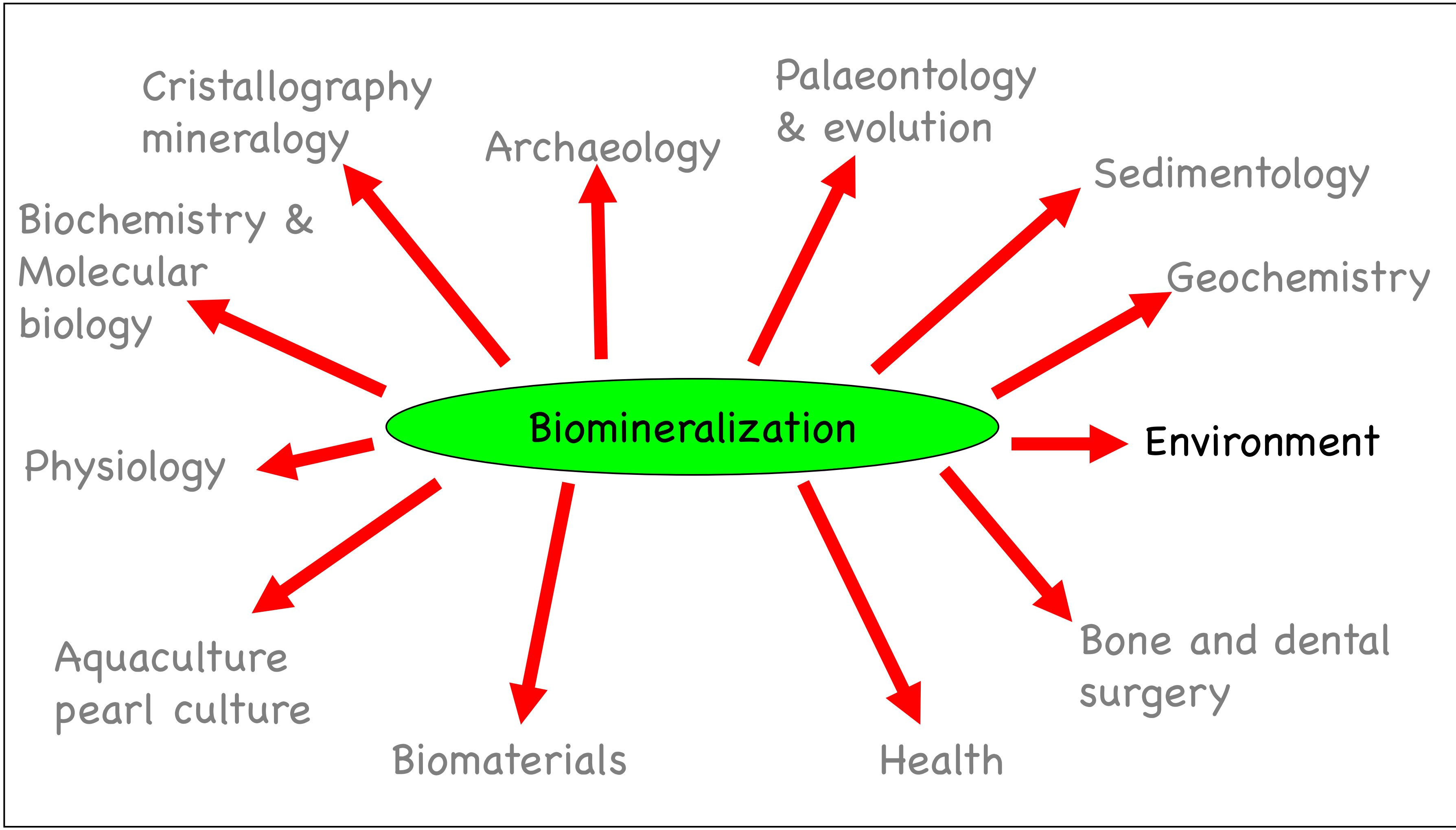
Bouville et al., 2014

Materials of the Future

Organo-mineral hybrid materials: nano-electronics, semi-conductors...

Phage display peptide library

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Environment: biofouling

Biofouling
(colonization of surfaces by living organisms that make 'crusts')

- Threat: endolithic microorganisms
- Use of calcifying bacteria to remineralize construction materials degraded by pollution

Environment: anti-fouling

Anti-fouling substances on boat hulls

- Super-tankers, gas (LNG) carrier without antifouling = up to 40% increase of fuel consumption

- > 25000 marine species able to colonize a submerged surface
- 1 to 2 mm of encrusting algae = 15% loss of speed.
- 1 m^2 of boat hull: can welcome up to 150 kg of organisms.

Bacterial biofilm→ Foraminifera, algae
→ barnacles, mollusks, calcifying worms

Environment

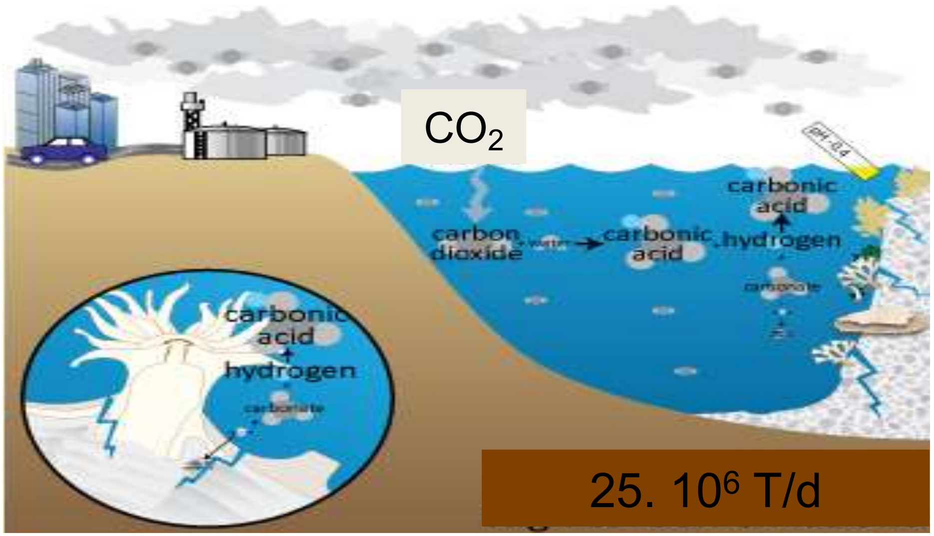
Ecological monitoring:
Biomineralizations = 'ecological sentinel' of the environment

Biomineralizations = archives of environmental parameters:

- **Temperature, salinity,**
- **Pollutions: heavy metals, organic pollutants, pesticides...**

Environment: CO_2 storage

CO_2 storage



Oceanic acidification

Moment	pH
Pre-industrial period	8.2
Today	8.1
2100	7.8

- Perennial storage (in comparison to vegetal storage)
- Trap CO_2 with calcifying organisms:
  - Calcifying bacteria

Environment: water dépollution

Biominerals, when reduced in powder have interesting surface charge properties, due to the presence of organic polyanionic macromolecules

- Flocculation of clay minerals that are in suspension in meteoric waters, during flood
- Trapping of metallic cations
- Example of maerl: alkalinisation of acidic waters (drinking water treatment)

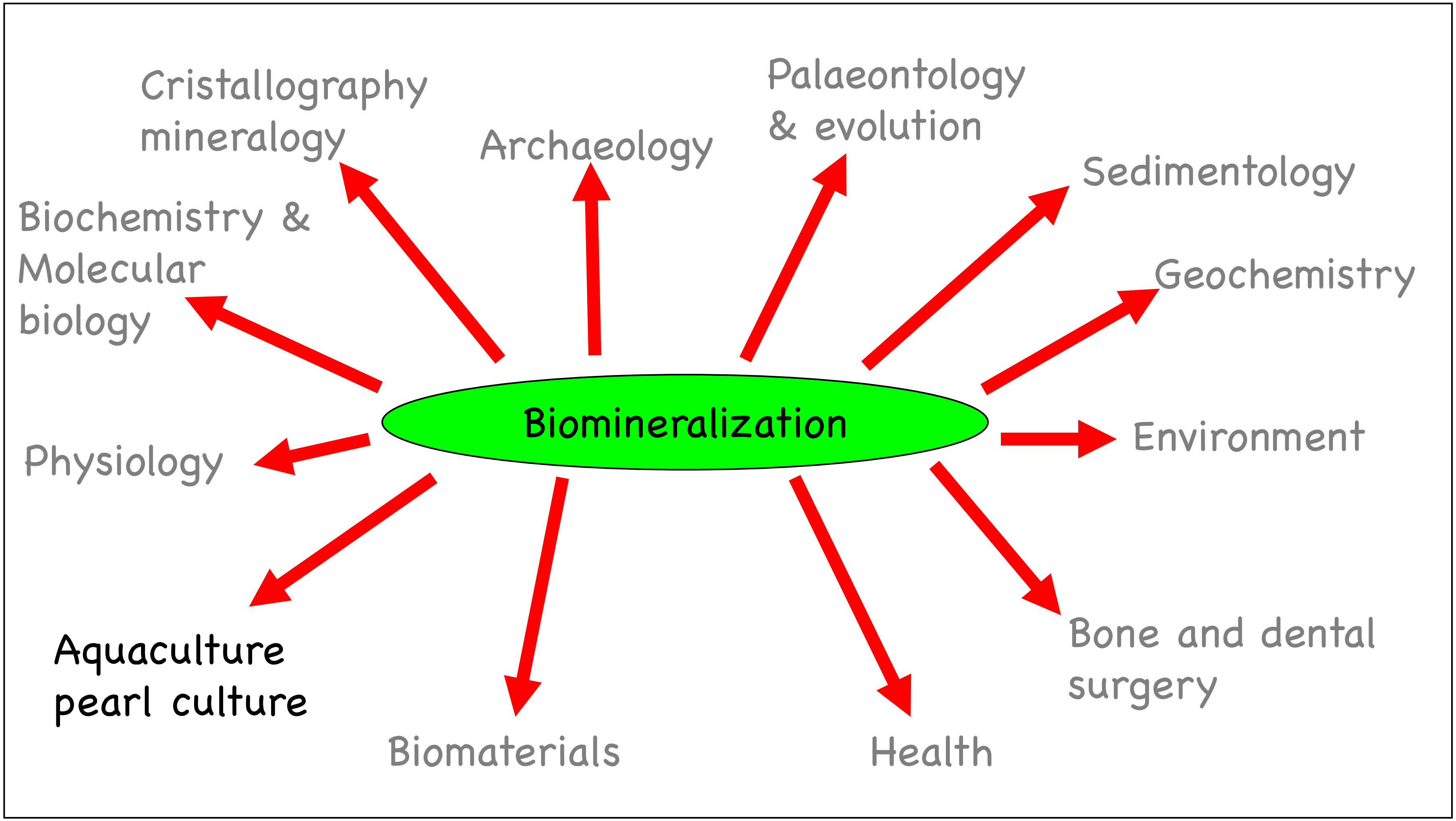
Environment: soils amendment

Soils poor in Ca

Soils poor in Mg

Acidic soils

- Crushed mollusk shells
- Chalk
- Maerl (rich in Mg)



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Zootechnics: aquaculture

Shellfish farming
(pathology affecting the shell of edible species)

Examples:

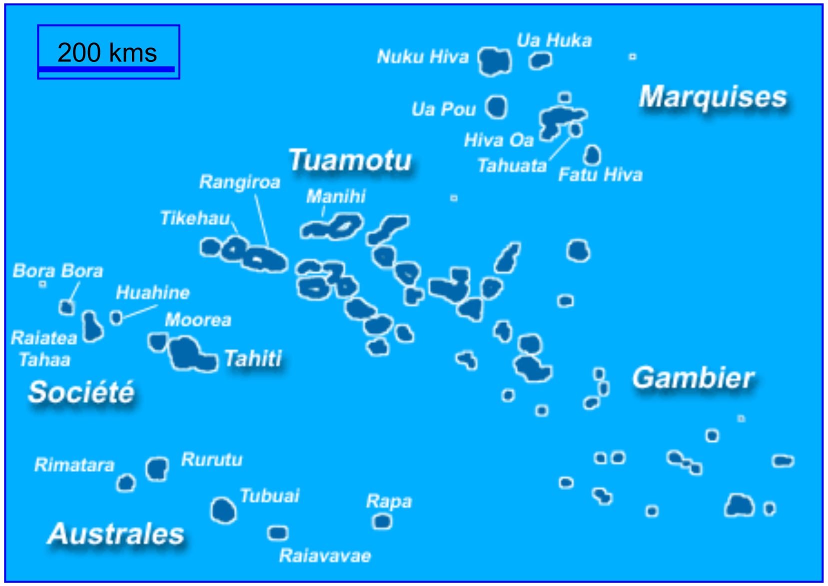
- Chambers in oyster shells in the 80 – TBT
- Scallop shells
- Manila clam (*Venerupis philippinarum*) brown ring disease

Zootechnics: pearl culture

PEARL CULTURE / AQUACULTURE

The French Polynesia example

Black pearl of French Polynesia

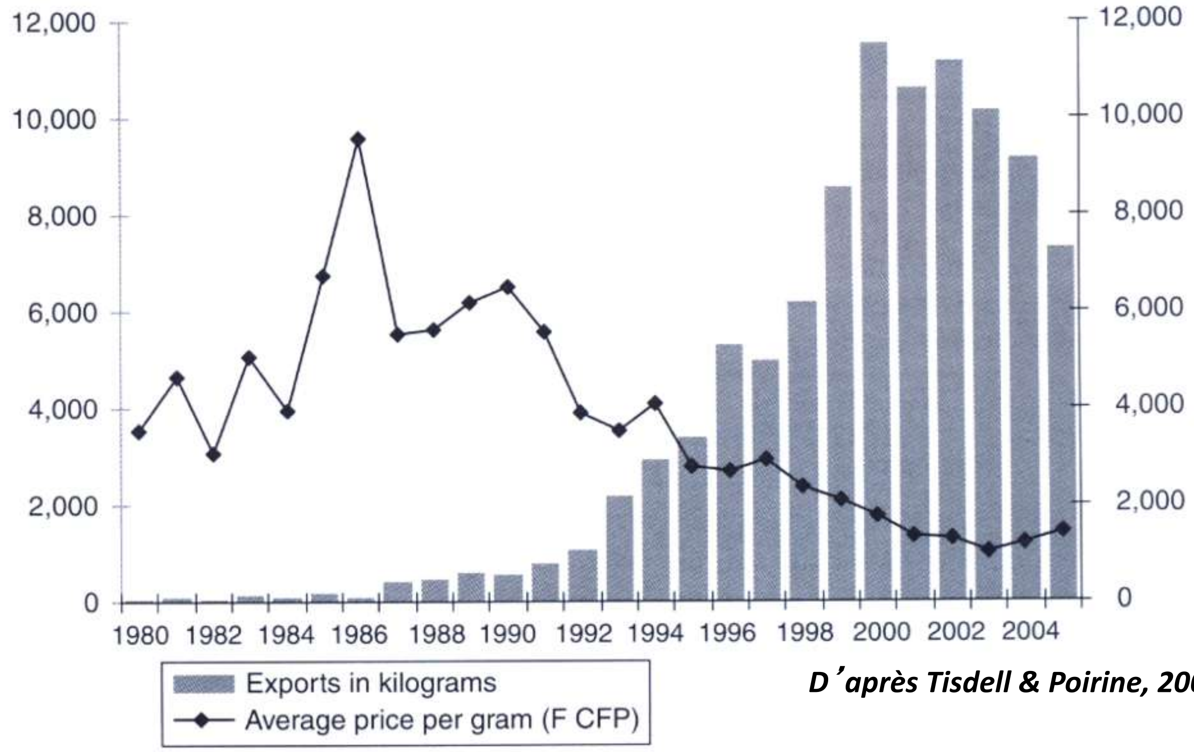


Population: circa 280 000
Pearl activity: 4000/5000
800 pearl farms
Polynesia : 90% of black pearl of southern seas

58% of exportation

120 millions US $ per year

Economic situation in French Polynesia



D'après Tisdell & Poirine, 2008

A domain in crisis between late 90 until 2015

Decrease of pearl quality

Decrease of demand

New competitors

Solution => Produce again pearls of quality

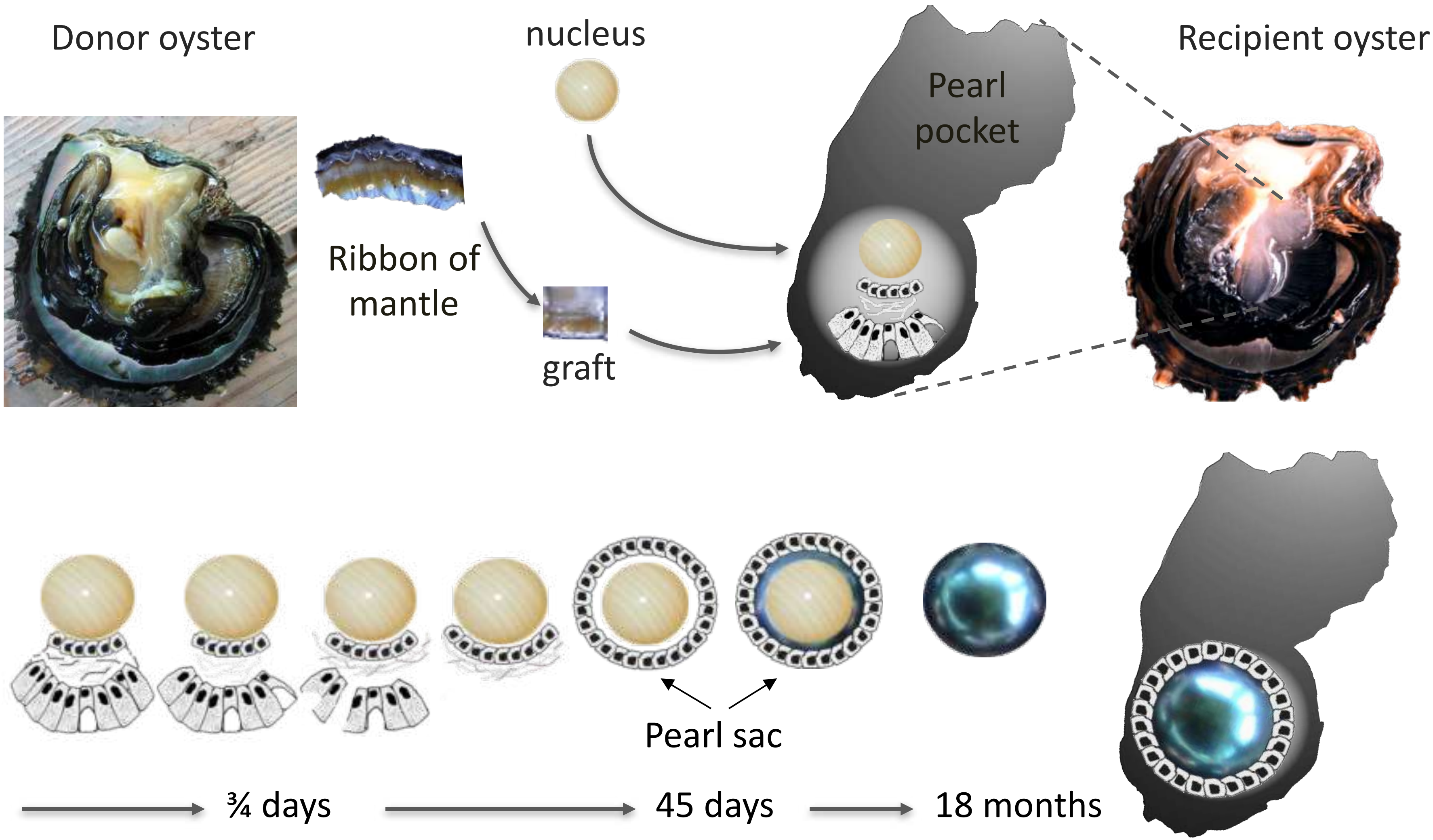
Service de la Perliculture

=> GDR ADEQUA (Ifremer 2008-2012), 10 partners

Film MNHN Paris (BOME, C. Milet)

Pearl culture in Polynesia

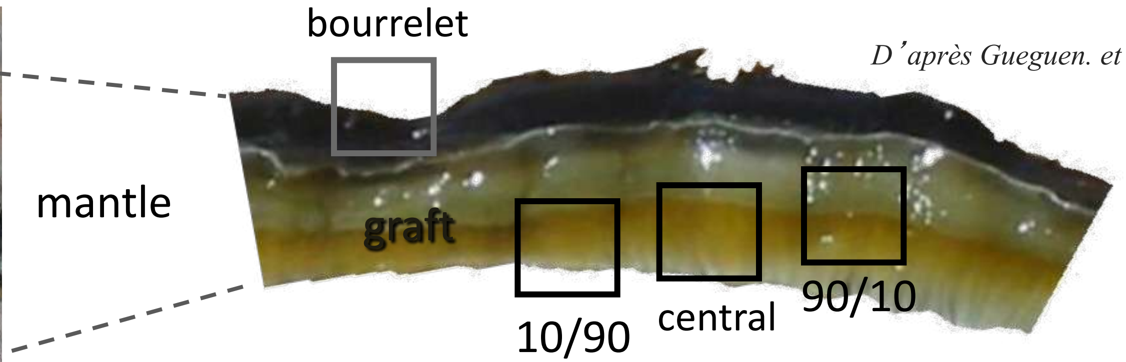
How to fabricate a pearl



Drawing C. Montagnani

Role of the graft in the quality of the pearl

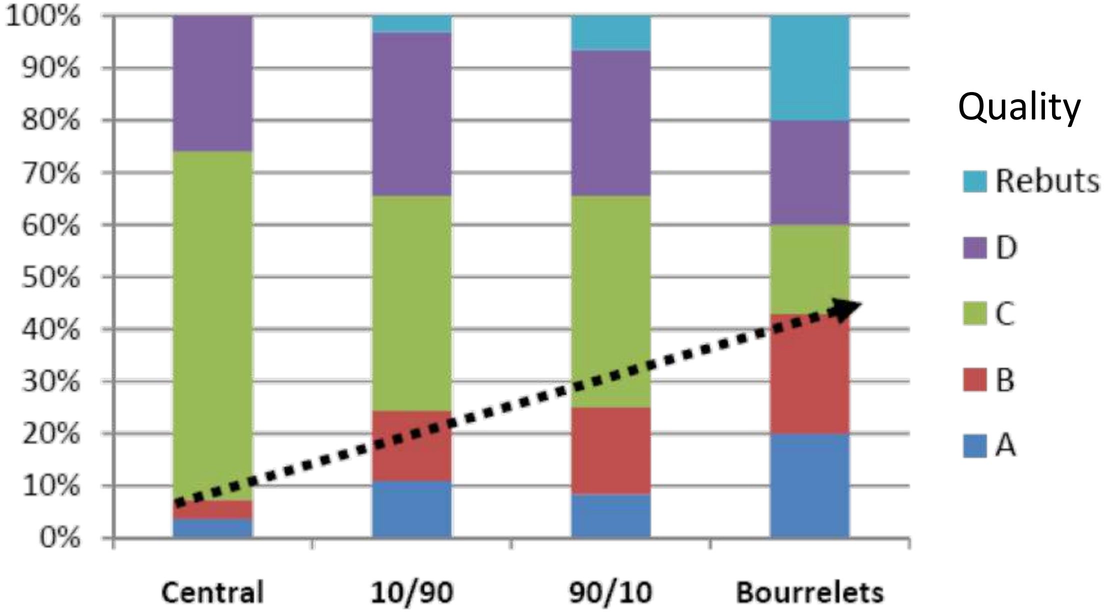
Huître donor



D'après Gueguen. et al, 2009

Where the graft is taken is important for the quality and color of the future pearl

Need to understand the mechanisms of mineral deposition regulation in the mantle



Mollusks and their shell biomineralization

Mollusks (120 000 living species)

Solenogasters
(350 species)

Caudofoveates
(250 species)

Polyplacophores
(900 species)

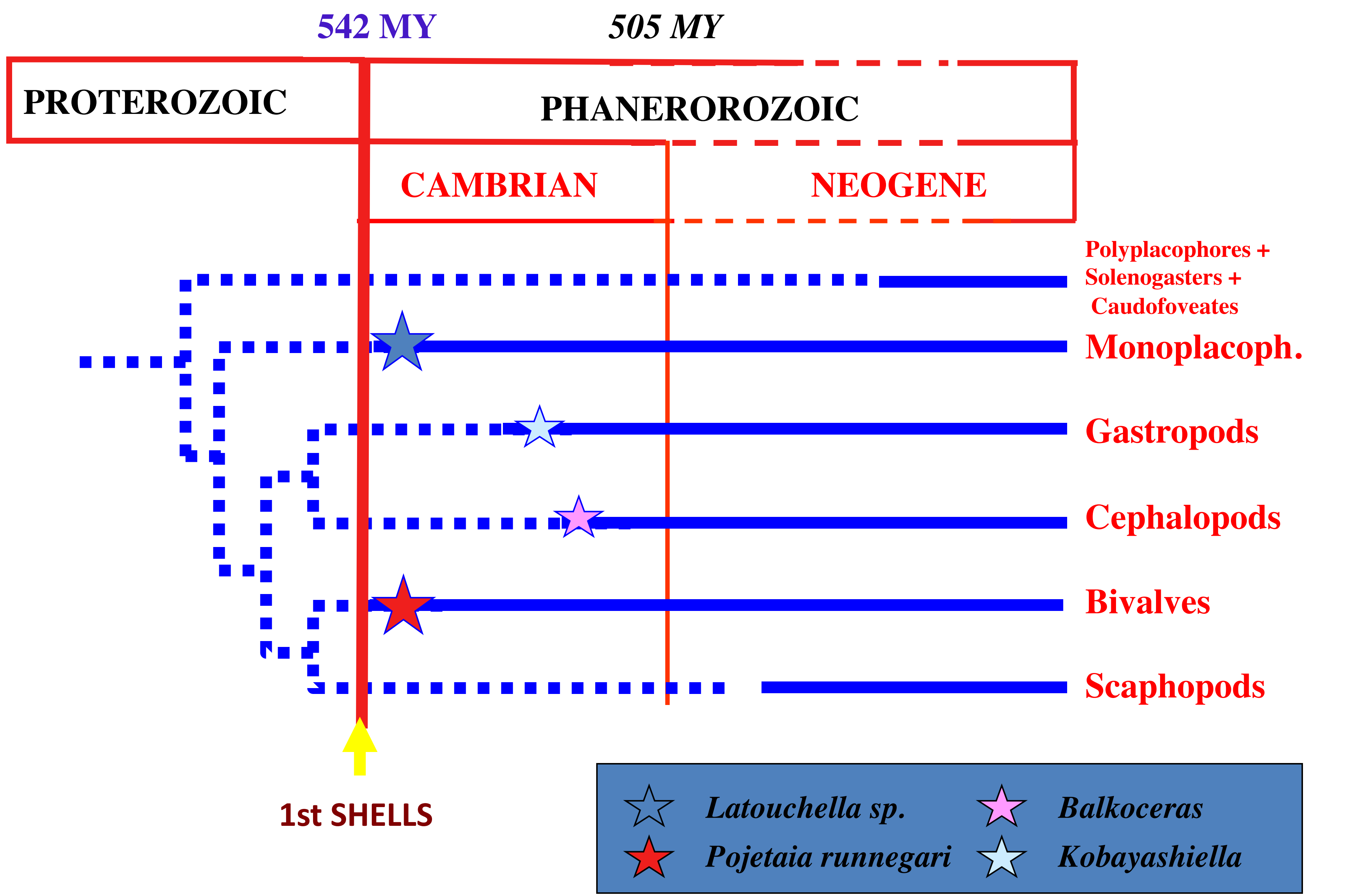
Monoplacophorans
(15 species)

Bivalves
(12 000 species)

Scaphopods
(400 species)

Gastropods
(103 000 species)

Cephalopods
(700 species)


542 MY
505 MY
PROTEROZOIC
PHANERIROZOIC
CAMBRIAN
NEOGENE
Polyplacophores +
Solenogasters +
Caudofoveates
Monoplacoph.
Gastropods
Cephalopods
Bivalves
Scaphopods
1st SHELLS
Latouchella sp.
Pojetaia runnegari
Balkoceras
Kobayashiella

- ***Since the Cambrian, continuous fossil record***

- ***Great adaptability***

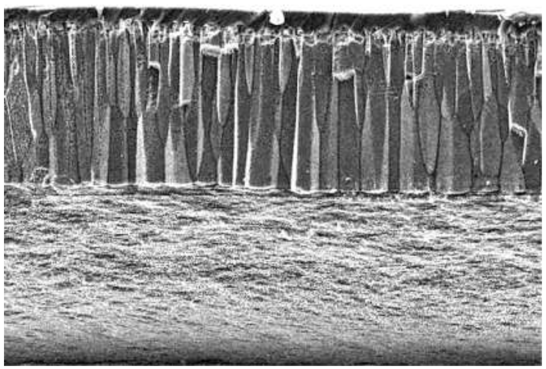
- ***Evolutionary success: efficient shelter against predation and dessiccation***

One of the keys of the

evolutionary success:

THE SHELL

The shell, a multi-layered material



Organic layer = périostracum

External layer

Internal layer

$CaCO_3$

	SHELL
Calcite	YES
Aragonite	YES
Vatérite	Traces
Monohydrocalcite	0
Protodolomite	0
Amorphe (ACC)	Traces

Physiology of calcification

Crassostrea gigas

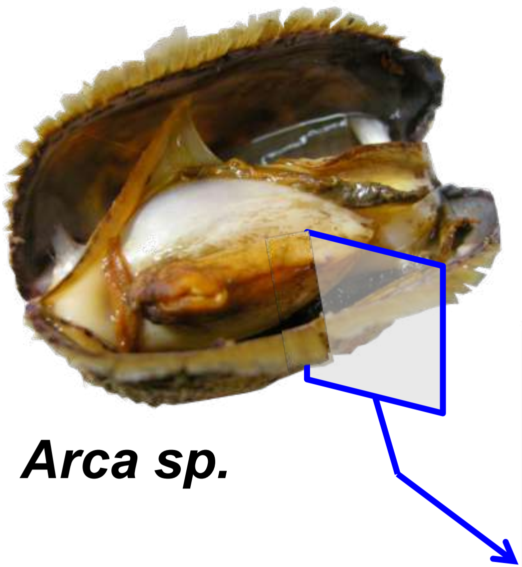
Calcifying mantle

$$HCO_3^- + Ca^{2+} \longrightarrow CaCO_3 + H^+$$

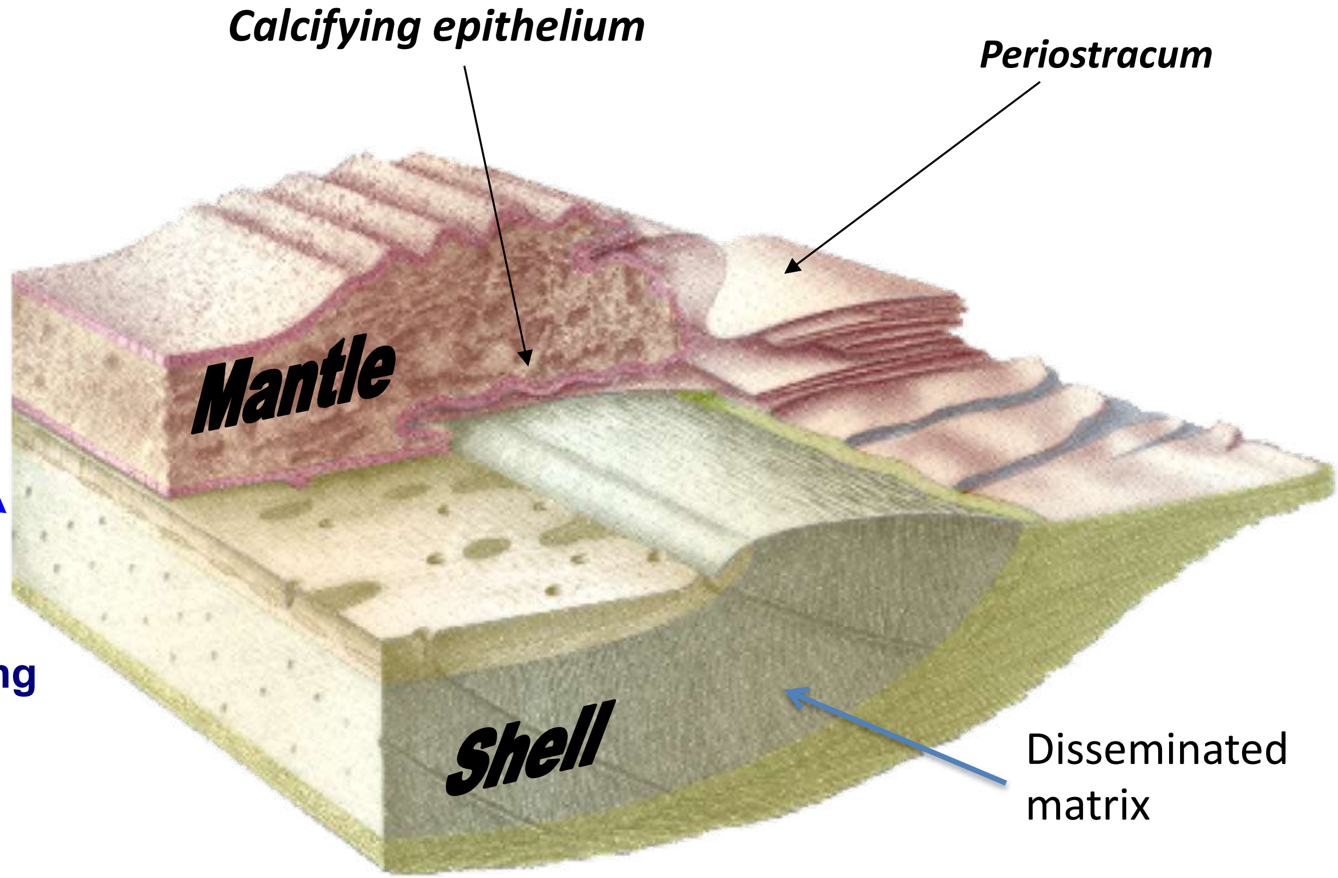
- Translocation of precursor ions & extrusion
- Secretion of amorphous granules
- Secretion of skeletal matrix
- Proton reabsorption

The mantle-shell interface in mollusks

http://tiritinyam.blogspot.com/2007/12/les-caixetes-de-vinars.html



Arca sp.

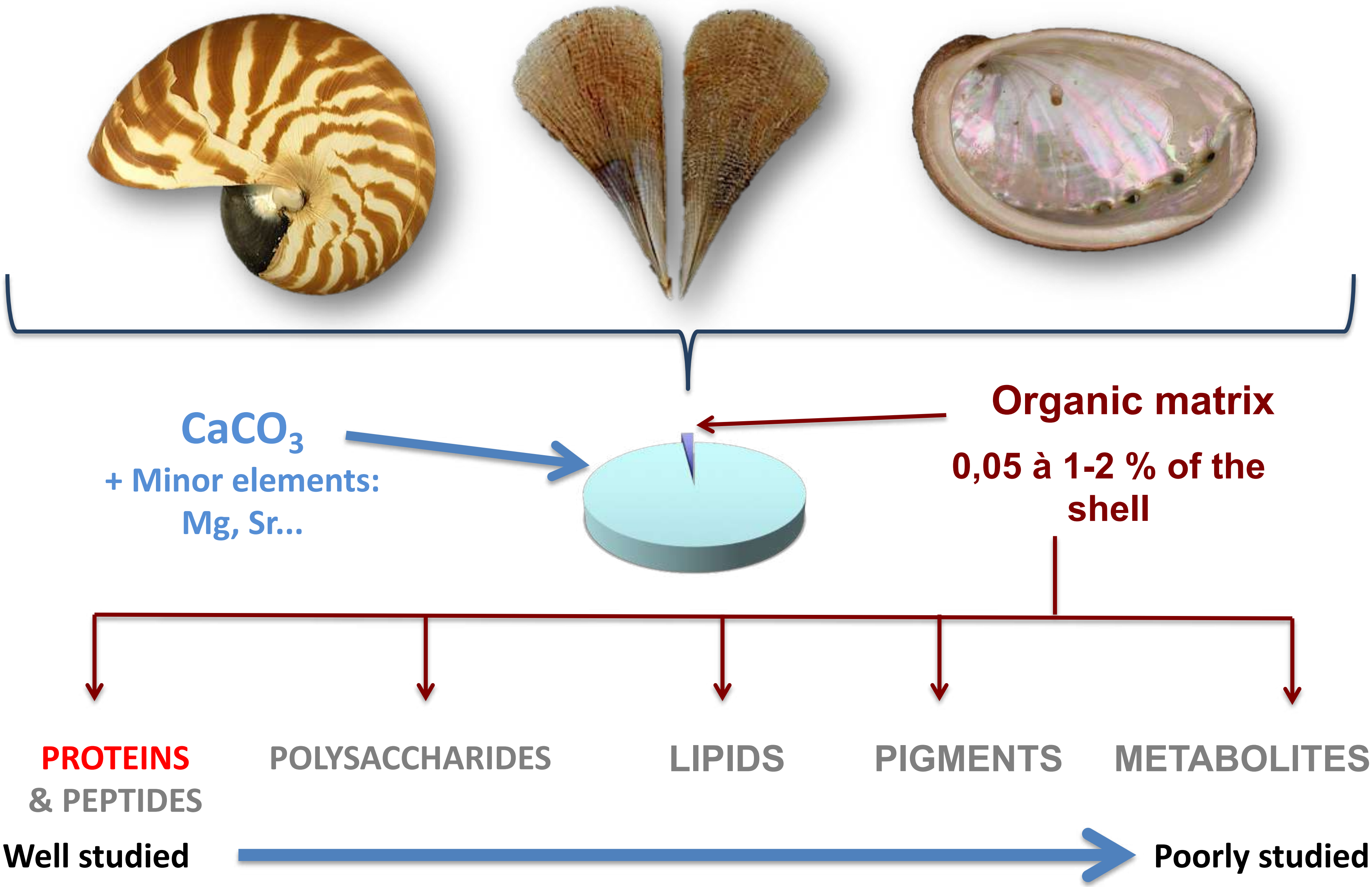


From Marin et al., 2012, Adapted from Waller, 1980

Interface mantle / growing shell: the 'extrapallial space':

- **Inorganic precursors**
- **Matrix**
- **'Self-assembling process'**

The shell, an organo-mineral material…



Mollusk shell matrix

- **Displays major roles in biomineralization**

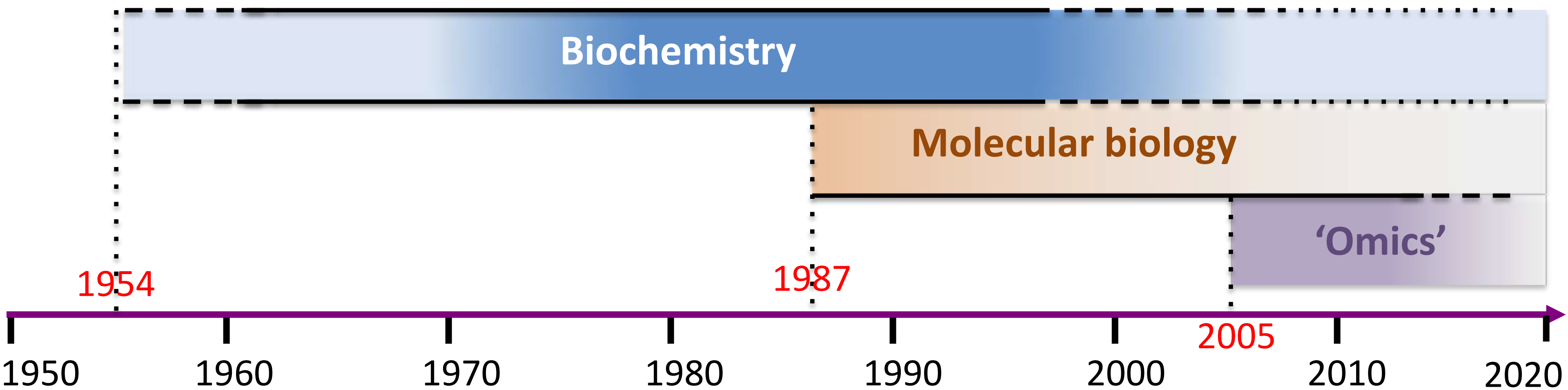
« MOLECULAR TOOLBOX ! »

& the main regulator of mineral deposition

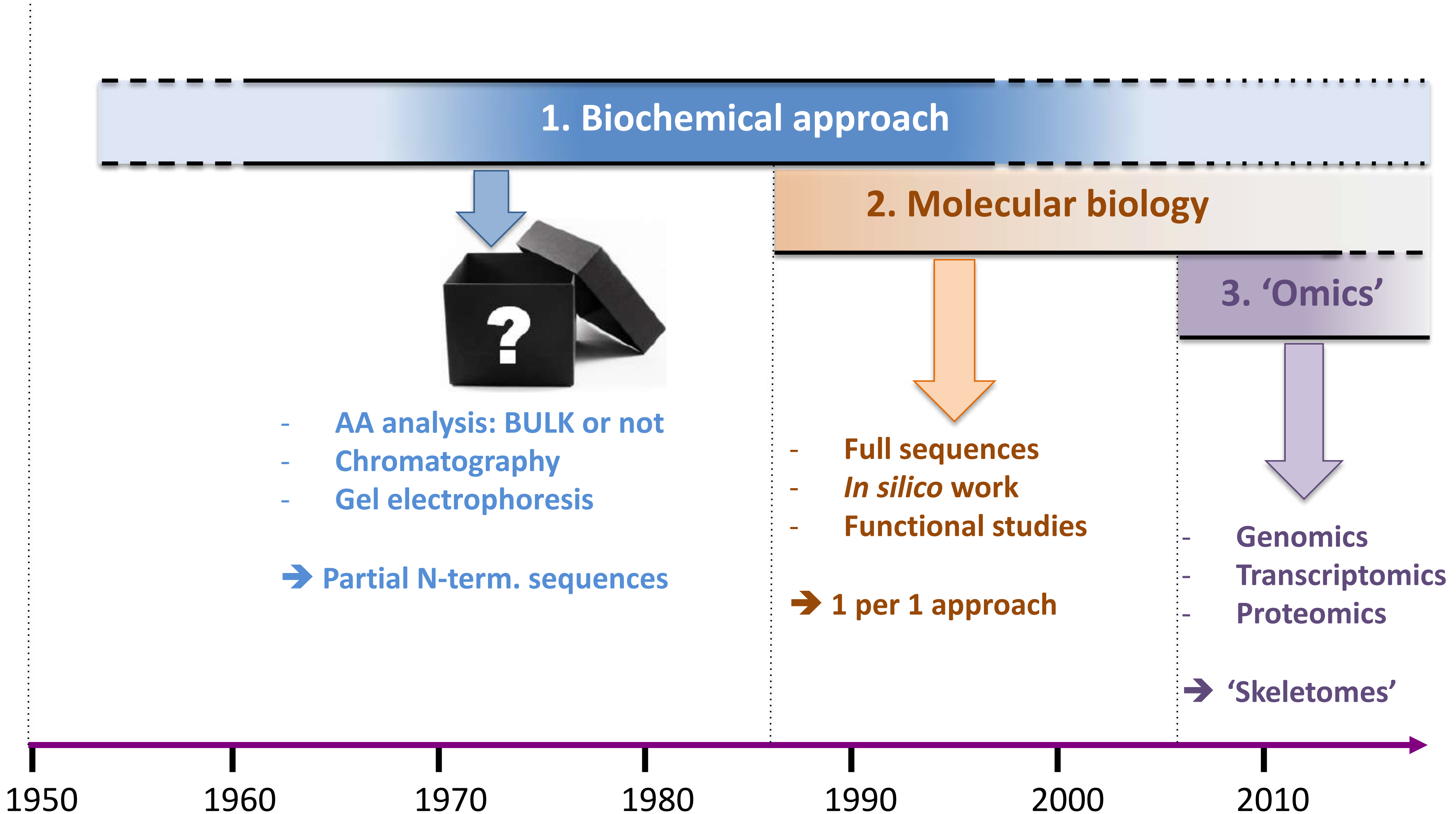
Drastic evolution of the concept of
« skeletal matrix » in the last decades...

The manner we perceive the shell matrix and its putative functions

has a lot to do with the techniques we use to analyze it !!



Mollusk shell matrix



(From Marin *et al.*, 2016)

Mollusk shell matrix

1. Biochemical approach

- AA analysis: BULK or not
- Chromatography
- Gel electrophoresis

➔ Partial N-term. sequences

- Proteins = molds around crystals or act as physical templates (hydrophobic)
- Proteins that bind calcium ions (polyanionic, Asp-rich)

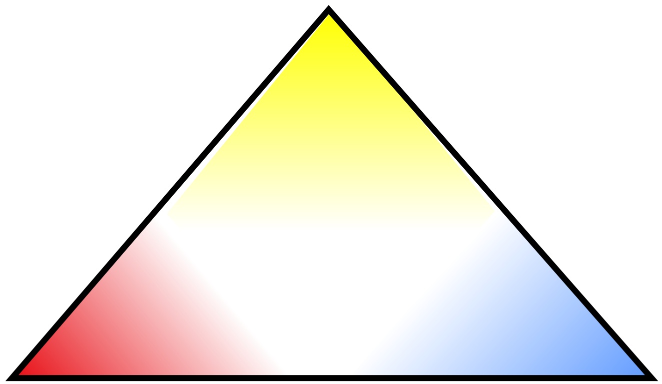
SIMPLE MOLECULAR MODELS

1950 1960 1970 1980 1990 2000 2010

IN THE MID-EIGHTIES: aa analysis, chromatography… No sequence data !!

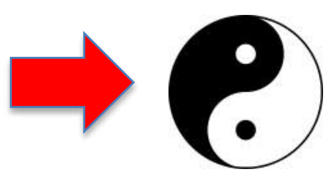
The ternary « sandwich model » for mollusks (nacre)

Chitin fibers
STRUCTURAL SUPPORT

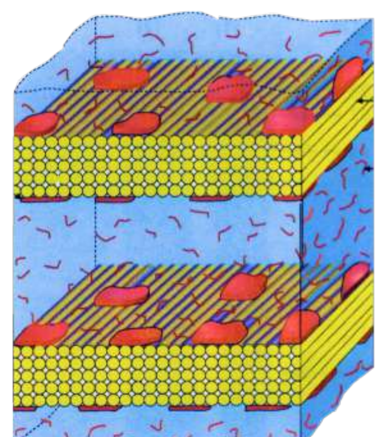


Soluble acidic proteins
NUCLEATION & INHIBITION

Insoluble hydrophobic proteins
MOLD, TEMPLATE then HYDROGEL

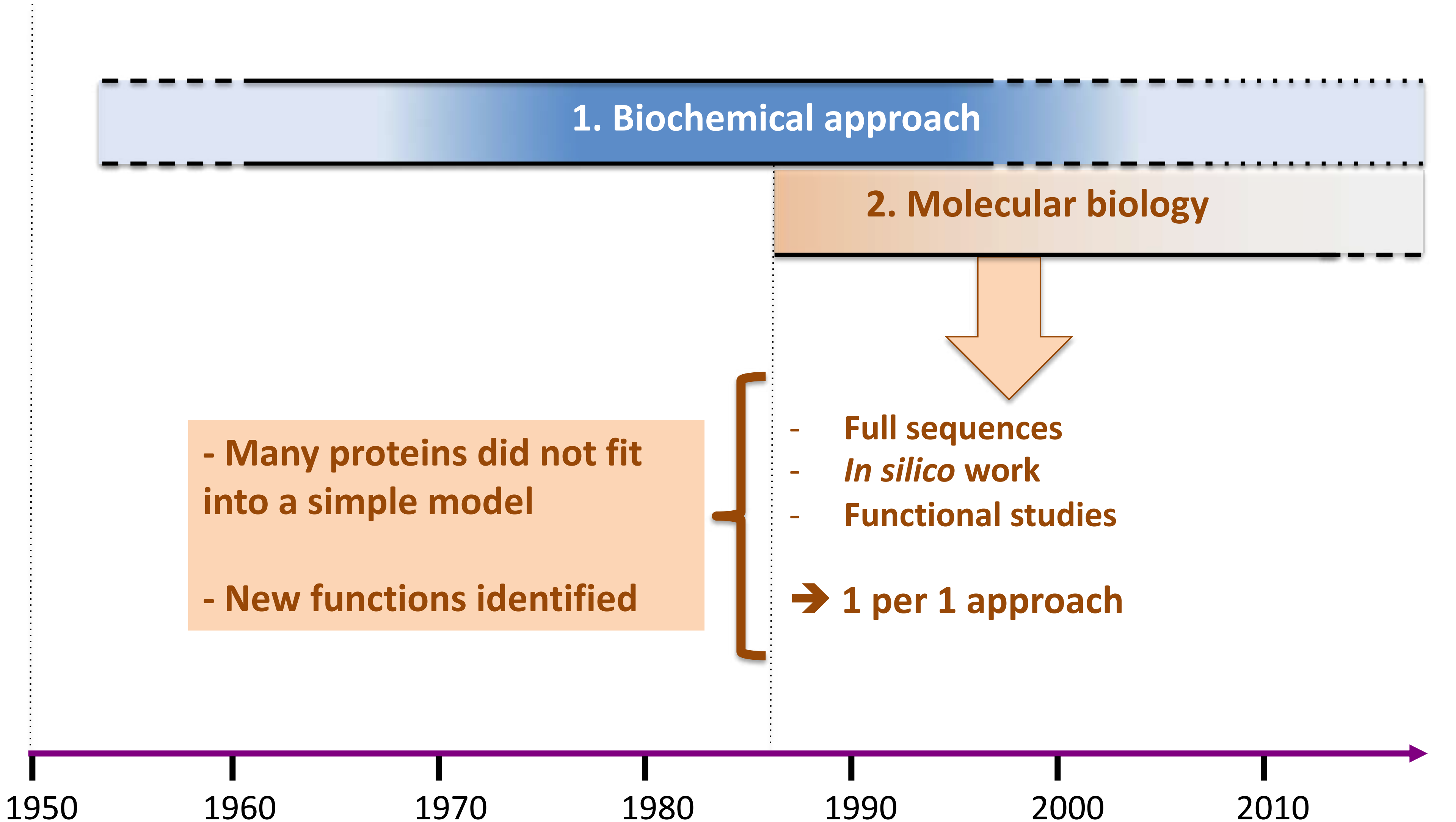


Yin & Yang

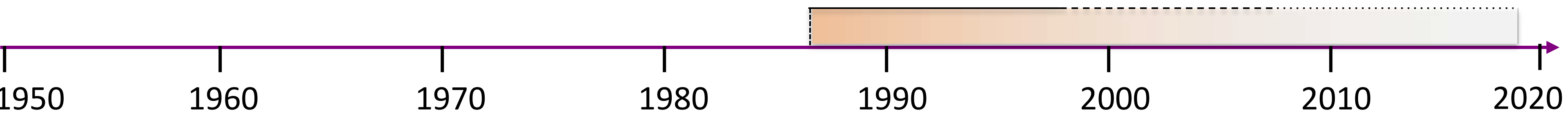


Levi-Kalisman *et al.*, 2001

Mollusk shell matrix



Analysis of the shell proteins: the molecular biology approach

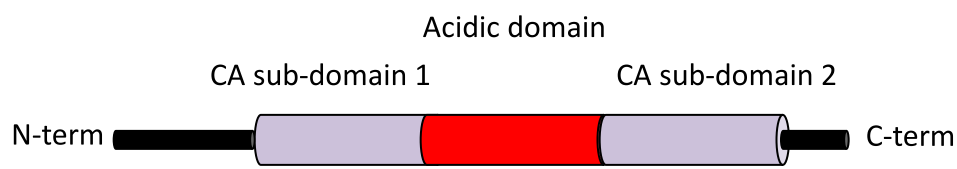


- Full sequences of shell matrix proteins
- Between 1996 and 2008, > 40 proteins for mollusks
- Overexpression and functional assays.
- **1 per 1 approach**.

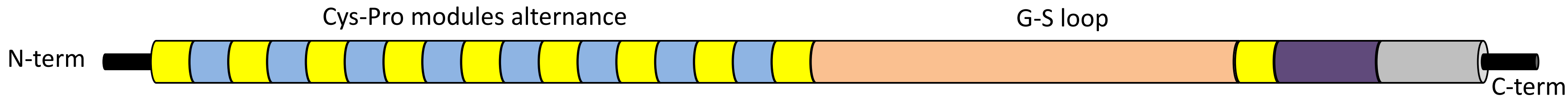
Most of the identified proteins did not fit into a ternary model

Examples:

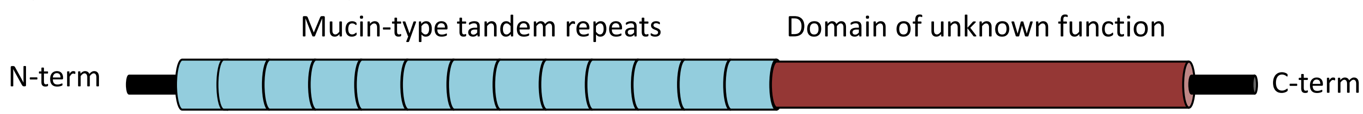
Nacrein *(Miyamoto et al., 1996)*



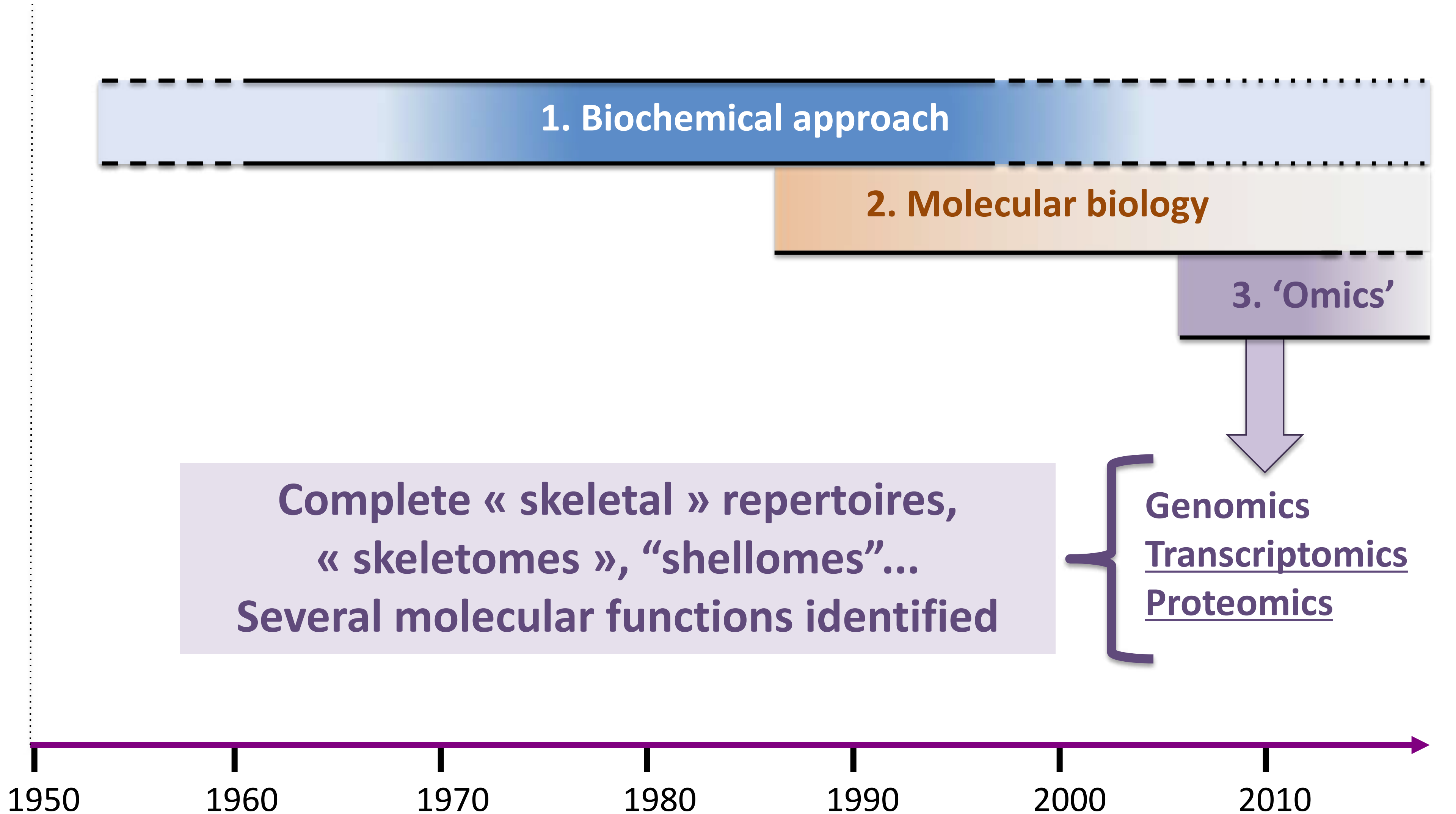
Lustrin A *(Shen et al., 1997)*



Mucoperlin *(Marin et al., 2000)*



Mollusk shell matrix



Analysis of the shell proteins: the 'omics Era

1950 | 1960 | 1970 | 1980 | 1990 | 2000 | 2010 | 2020

Proteomics ('skeletomics ')

Calified tissue

Skeletal matrix extraction

Enzymatic digestion

MS-MS analysis: collection of peptides

Transcriptomics

Living calcifying tissue

mRNA extraction
& conversion to cDNA

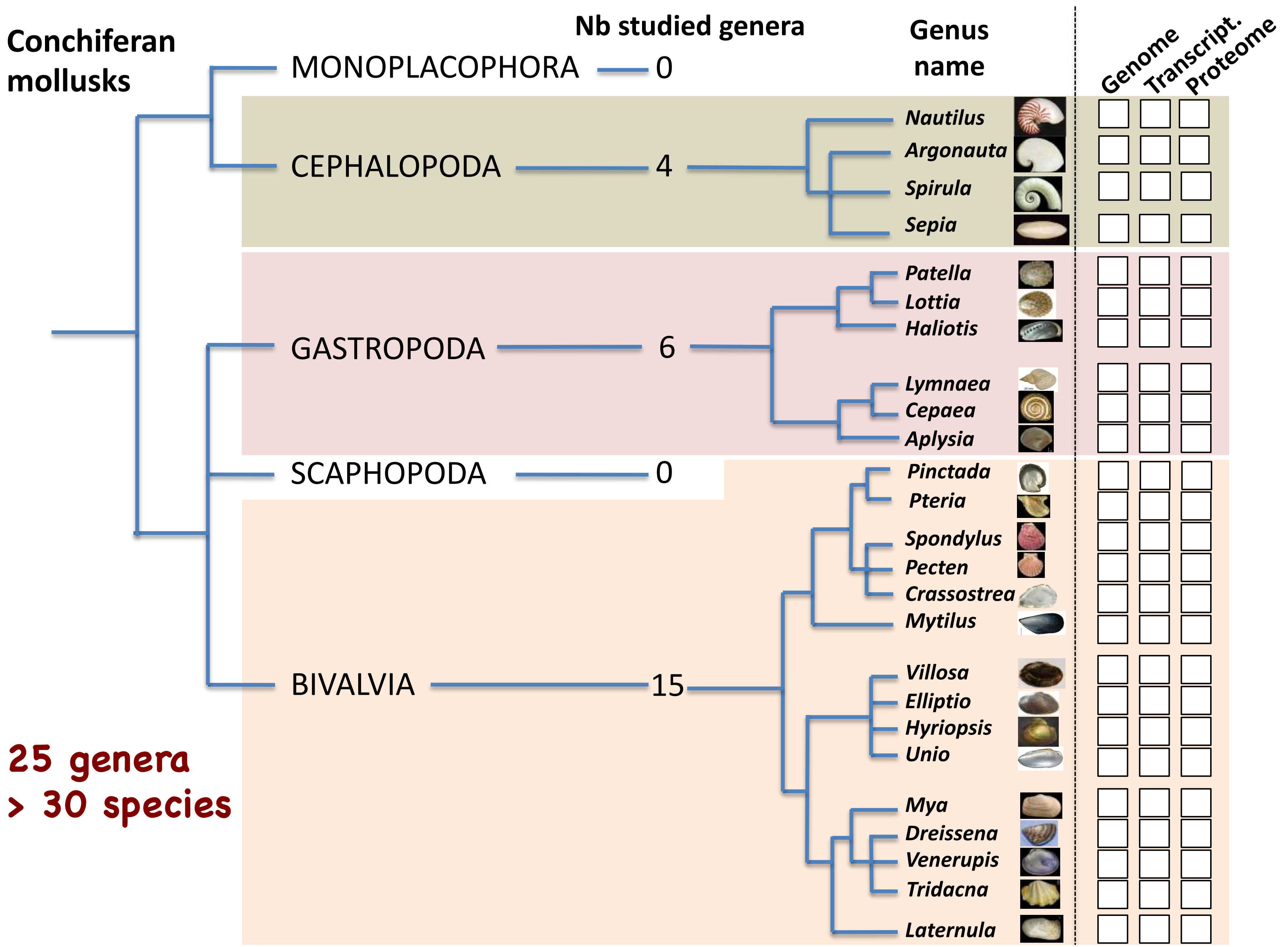
Next generation sequencing

Transcriptome / Secretome

In silico digestion &
comparison with MS-MS data

Full-length protein sequences

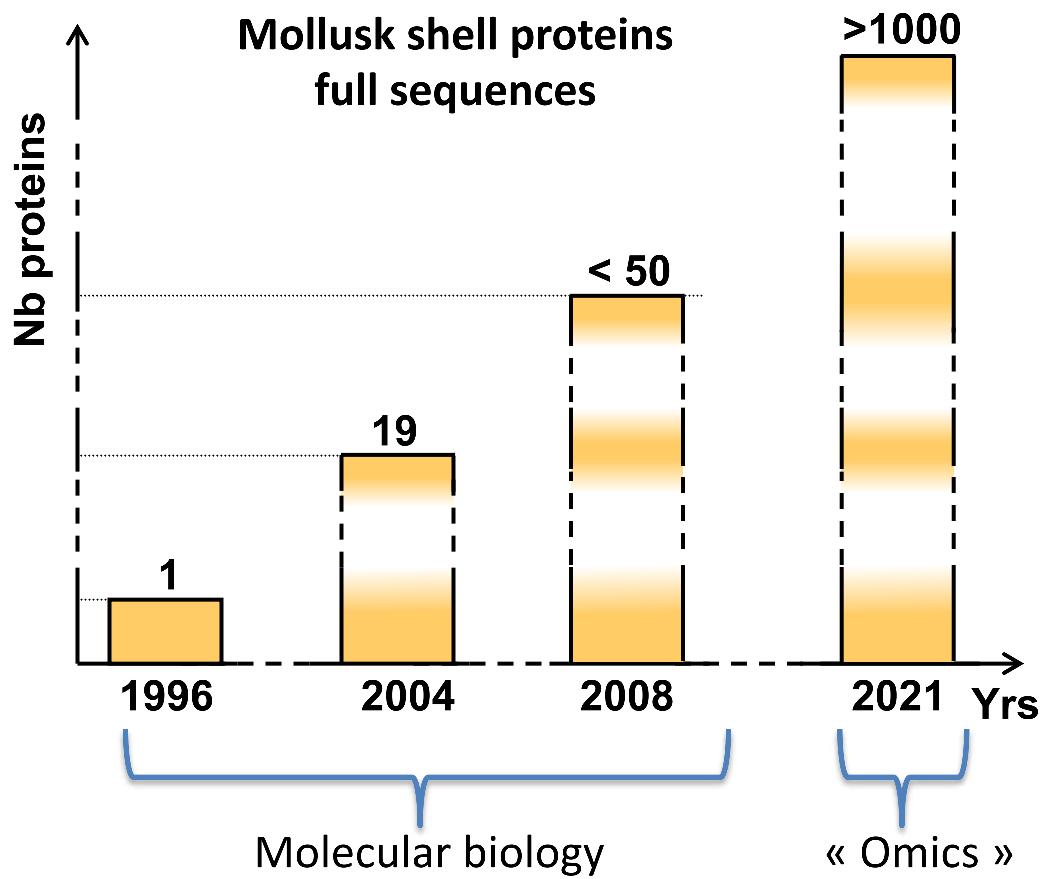
« Skeletal repertoires », « skeletomes »...

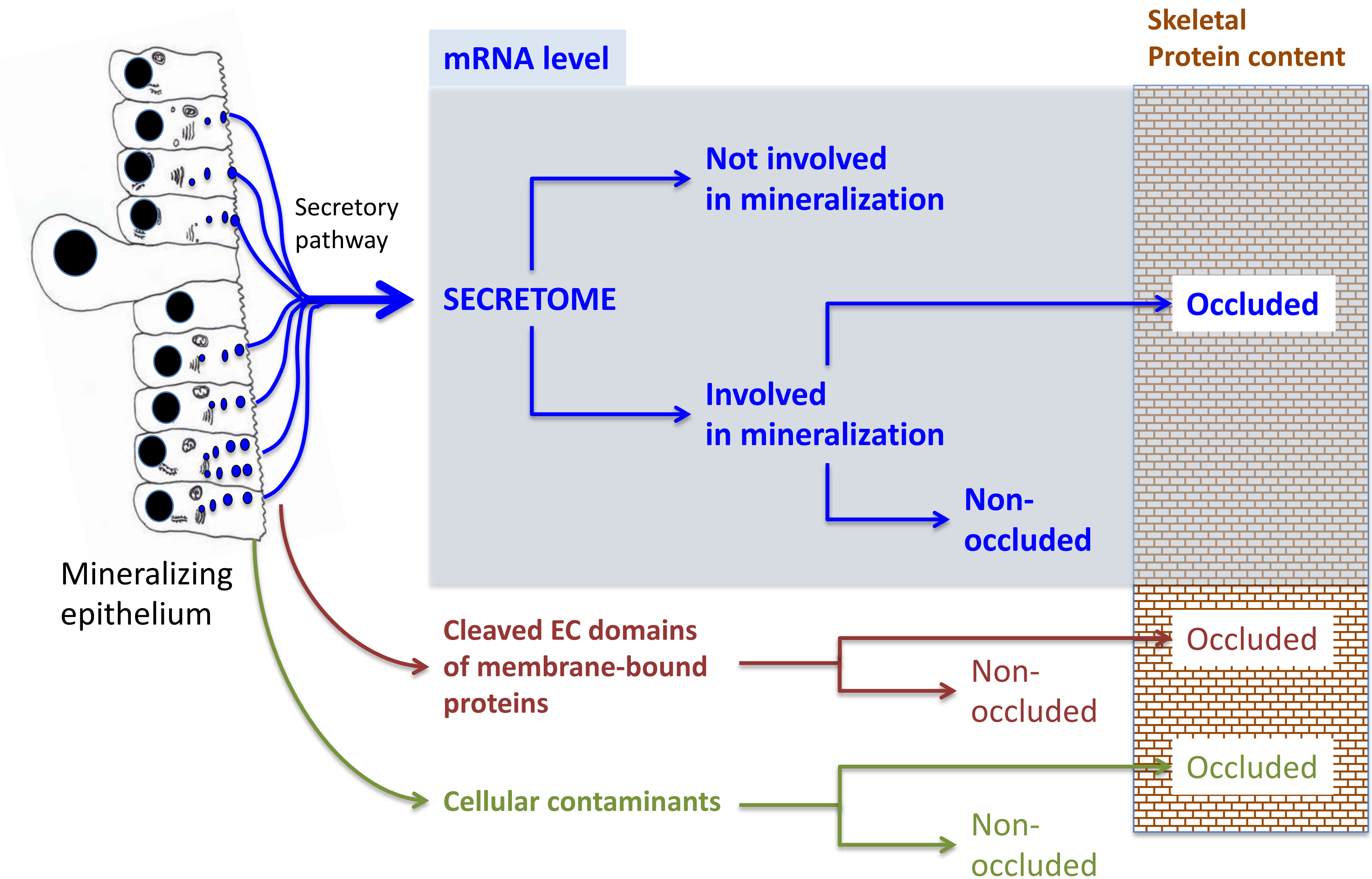


Conchiferan mollusks
Nb studied genera
Genus name
Genome
Transcript.
Proteome
MONOPLACOPHORA
0
CEPHALOPODA
4
Nautilus
Argonauta
Spirula
Sepia
GASTROPODA
6
Patella
Lottia
Haliotis
Lymnaea
Cepaea
Aplysia
SCAPHOPODA
0
BIVALVIA
15
Pinctada
Pteria
Spondylus
Pecten
Crassostrea
Mytilus
Villosa
Elliptio
Hyriopsis
Unio
Mya
Dreissena
Venerupis
Tridacna
Laternula
25 genera
> 30 species

Contribution of the ‘omics’ techniques

- Massive increase of the number of identified proteins
- Concept of skeletal matrix: blurred
- Several new functions identified
- Majority of novel unknown functions
- Are skeletal proteins all involved in mineralization ?
  ➔ cytoplasmic contaminants
  Necessity to clean properly skeletal tissues





Skeletal
Protein content
mRNA level
Secretory
pathway
SECRETOME
Not involved
in mineralization
Occluded
Involved
in mineralization
Non-
occluded
Mineralizing
epithelium
Cleaved EC domains
of membrane-bound
proteins
Occluded
Non-
occluded
Cellular contaminants
Occluded
Non-
occluded

The facts

- >> 1000 proteins
- Several protein families
- Several functional domains
- Low Complexity Domains

THE PROBLEMS

- Concept / outlines of skeletal matrix: blurred
- How to relate protein sequences and functions ?
- How do all these proteins work together ?

The possibilities

- Comparison between calcifying repertoires
- Sketch macroevolutionary trends
- Diagenesis of skeletal matrix

Example 1: *nacre in molluscs, single or multiple inventions ?*

**** One of the several shell microstructures***

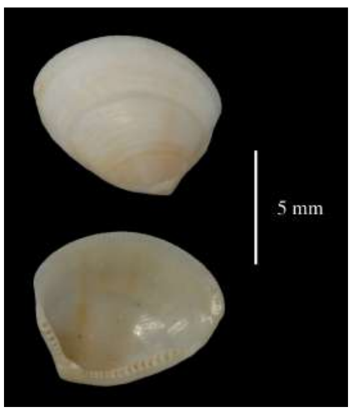
**** Always internal layer***

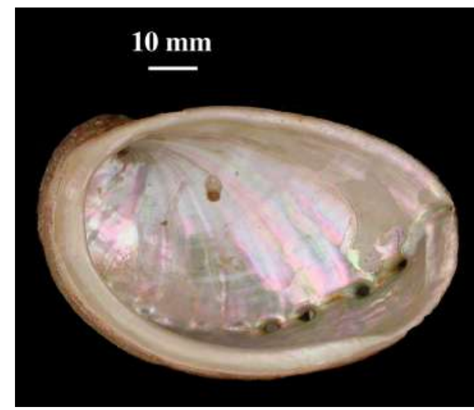
**** Laminar microstructure***

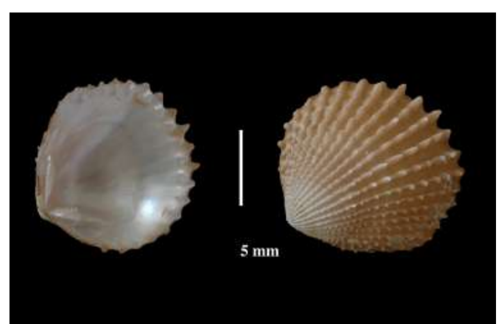
**** Nacre = typical of molluscs***

**** Always aragonitic***

**** Cambrian origin***



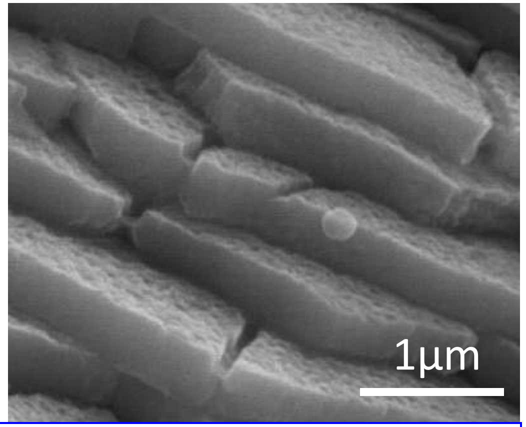


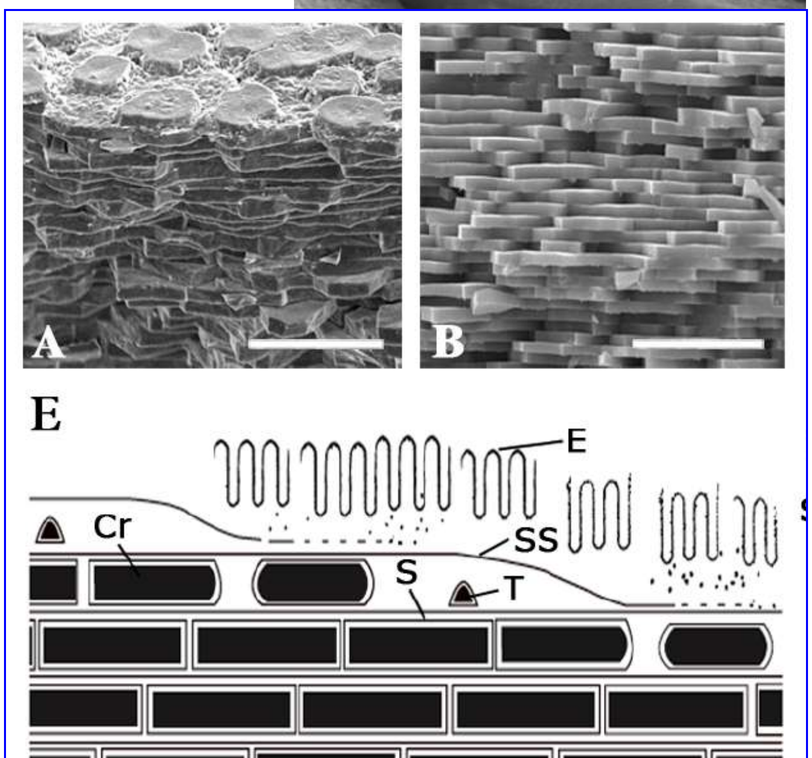


Nacre in mollusks

Brickwall nacre

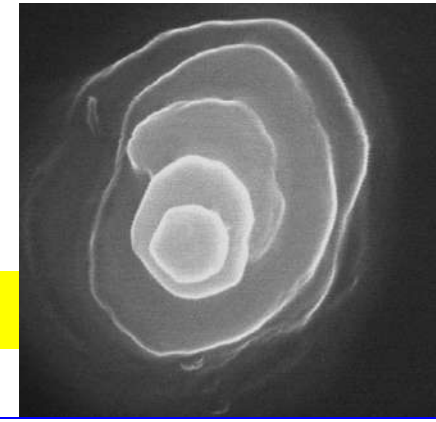
BIVALVES

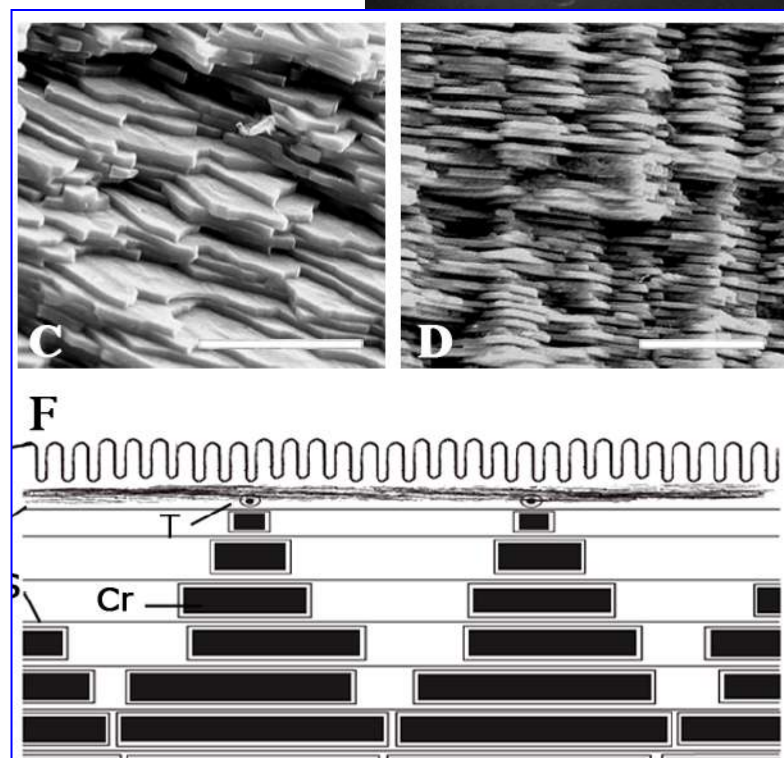




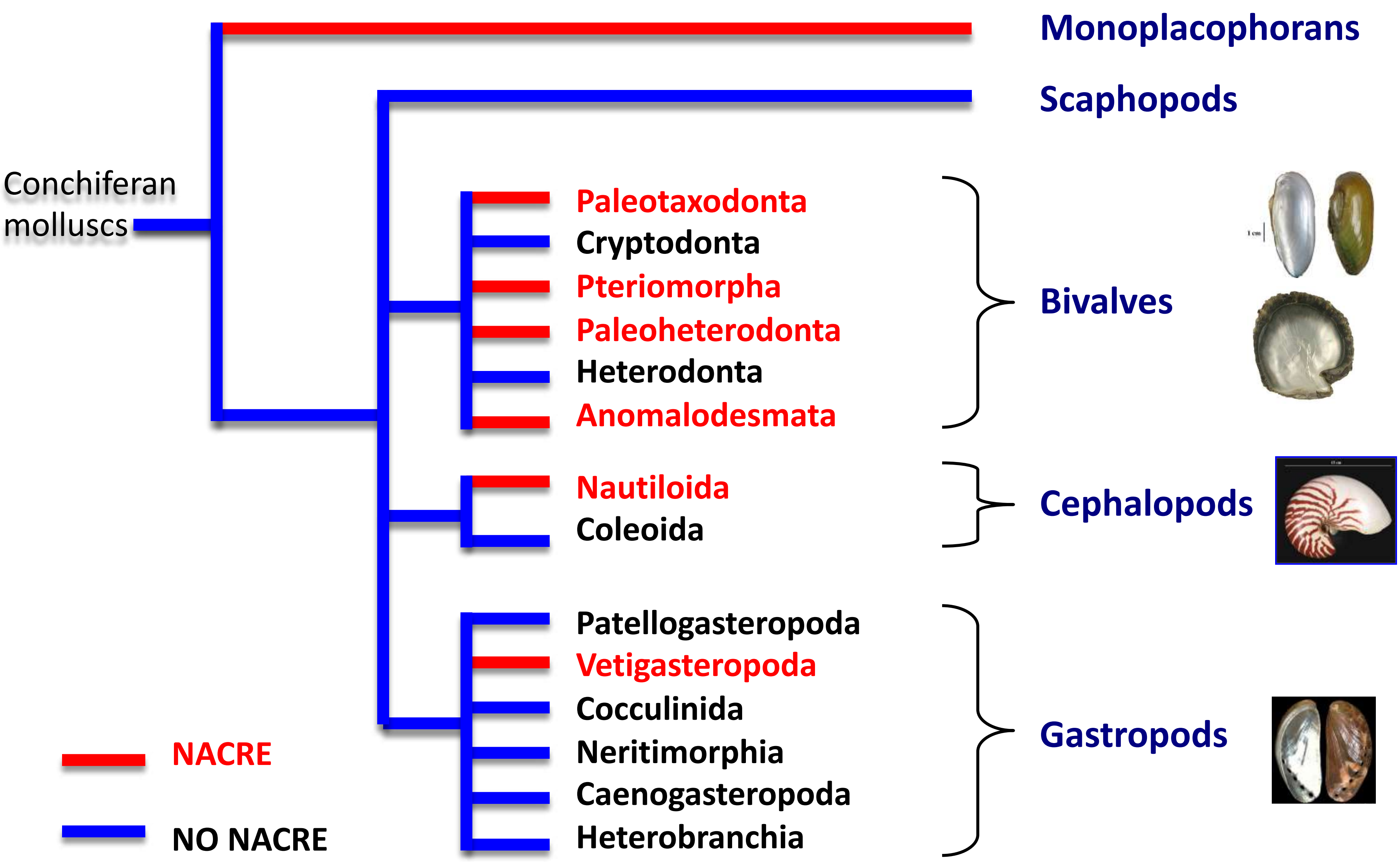
Columnar nacre

GASTROPODS





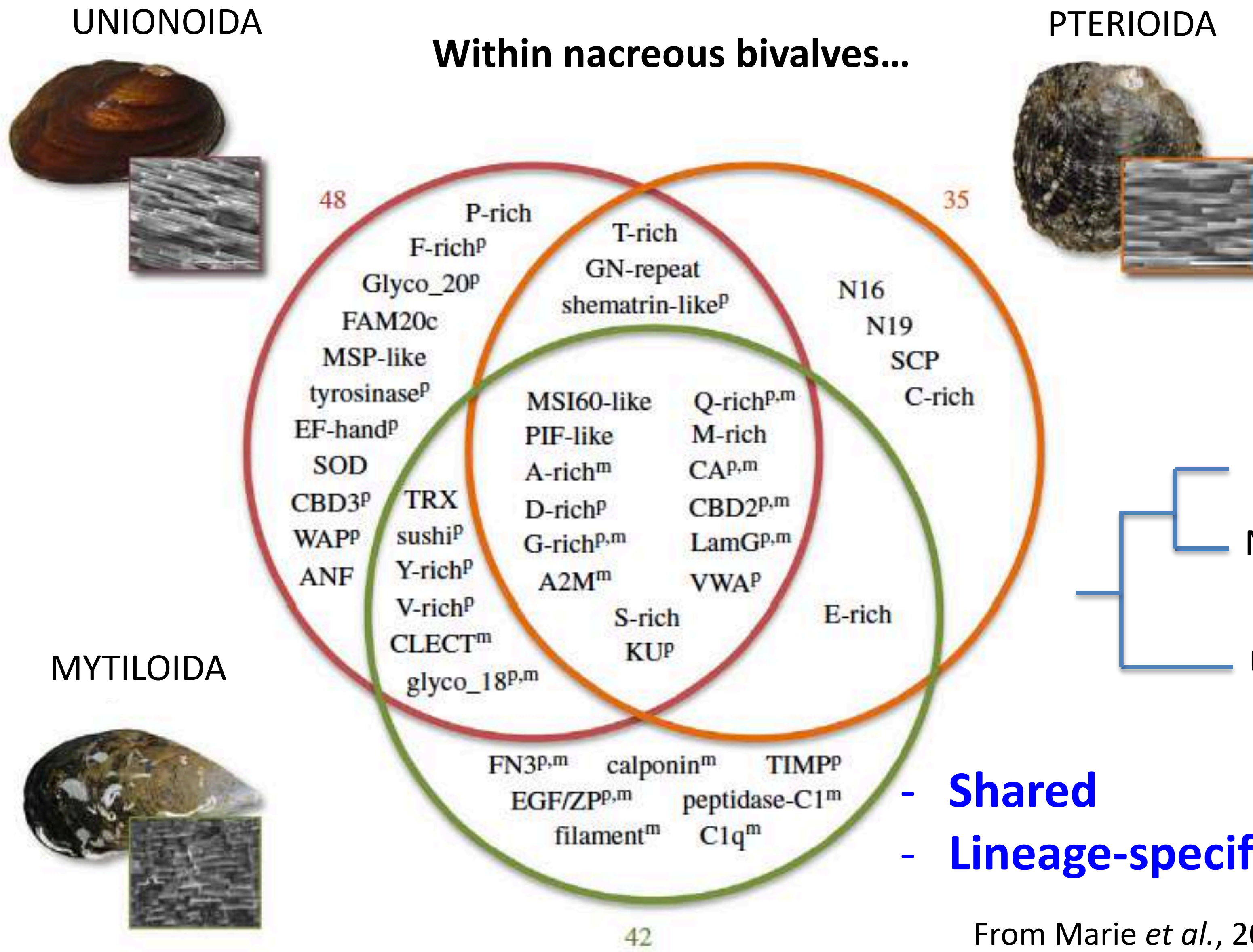
Example 1



Monoplacophorans
Scaphopods
Conchiferan molluscs
Paleotaxodonta
Cryptodonta
Pteriomorpha
Paleoheterodonta
Heterodonta
Anomalodesmata
Bivalves
Nautiloida
Coleoida
Cephalopods
Patellogasteropoda
Vetigasteropoda
Cocculinida
Neritimorphia
Caenogasteropoda
Heterobranchia
Gastropods
NACRE
NO NACRE

Example 1

Within nacreous bivalves...



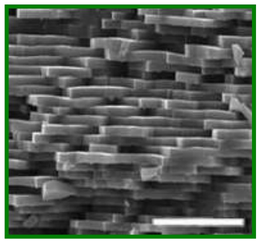
- **Shared**
- **Lineage-specific**

From Marie *et al.*, 2017

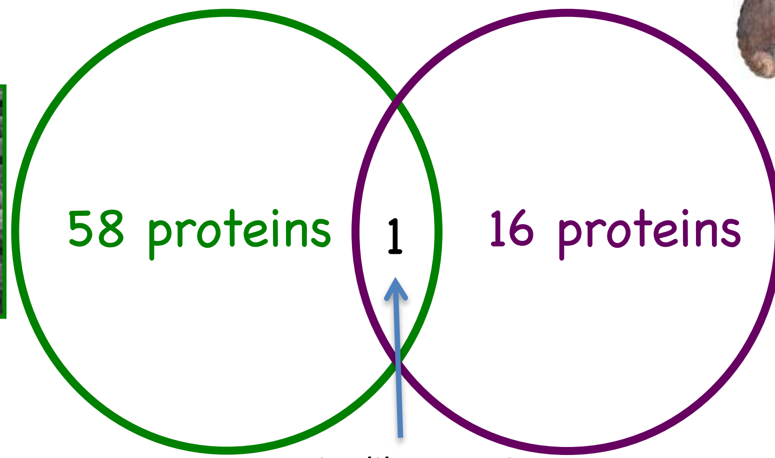
Example 1

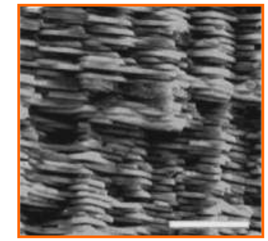
Nacreous bivalve *vs.* nacreous gastropod

Pearl oyster
Pinctada sp.



SHEET NACRE





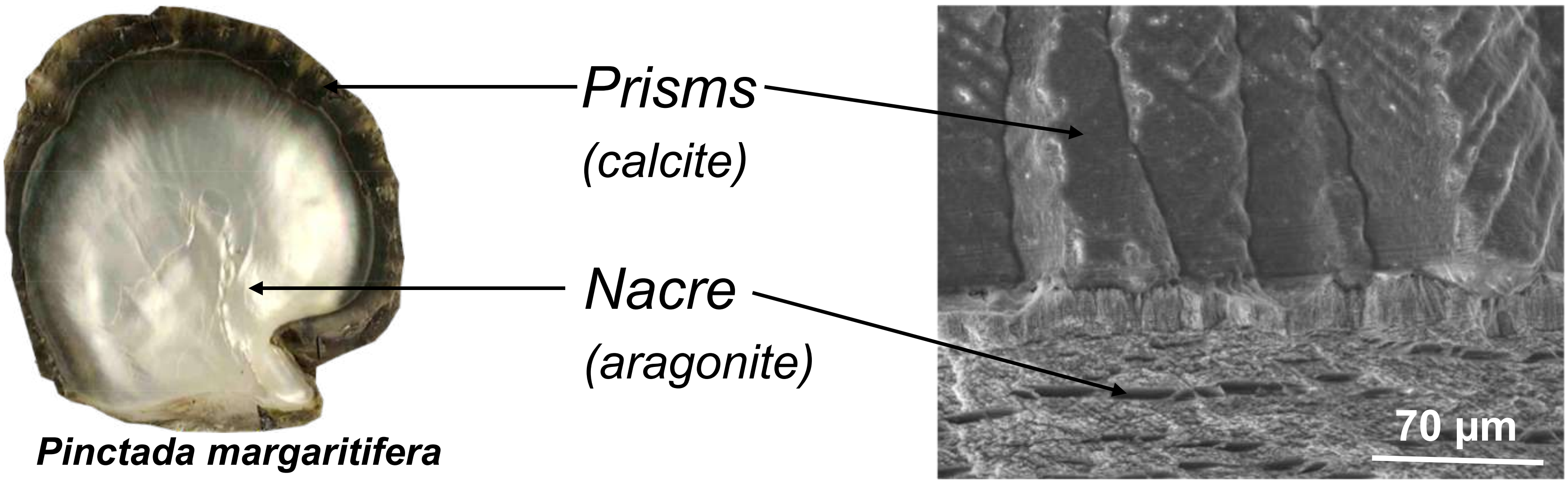
Abalone
H. asinina

COLUMNAR NACRE

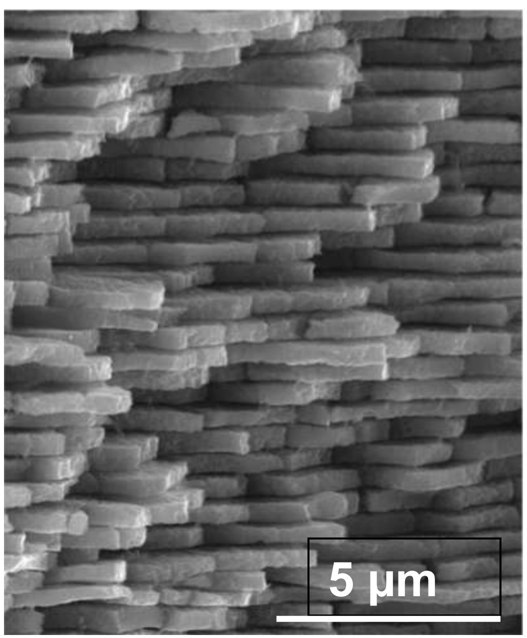
Adapted from Marie *et al.*, 2012

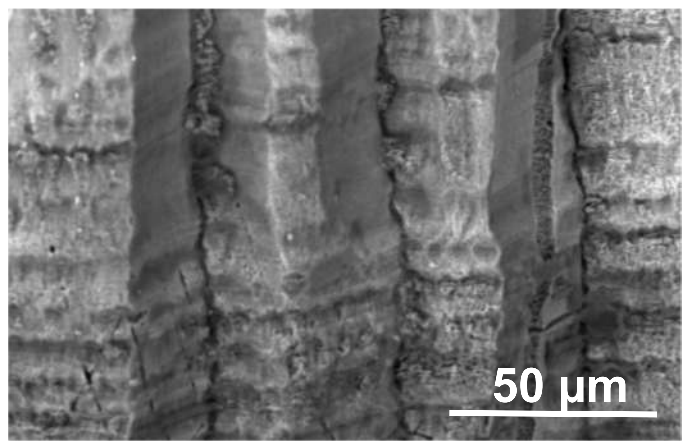
- **Within bivalves, <u>several similarities</u>, but also <u>taxon-specific nacre proteins</u>**
- **Between bivalves & gastropods, very different nacre proteins assemblages**
- **=> Independent inventions…, or fast evolution of nacre proteins (“drift” from a common nacre ancestor) ?**
- **Different assemblages can build similar microstructures: plasticity of the system => which ‘driving force’ constrains the system to produce nacre?**

Example 2: *prisms and nacre, same matrices ?*



Pinctada margaritifera





Are prisms and nacre made from the same shell matrix repertoire ?

ADEQUA Consortium

Example 2

A fundamental question that was left open for decades...

Amino Acids in the Proteins from Aragonite and Calcite in the Shells of Mytilus californianus

Abstract. *Hydroxylysine and hydroxyproline are absent in the calcified proteins of* Mytilus. *The organic matrices from the calcite layers have a consistently higher ratio of acidic to basic amino acids than the aragonitic shell units. The uncalcified shell units periostracum and outer ligament, have very few acidic residues, which may in part account for the lack of mineralization.*

Hare, PE, *Science*, 1963

Biol. Rev. (1967), 42, pp. 653–687

SUR LA STRUCTURE DES MATRICES ORGANIQUES DES COQUILLES DE MOLLUSQUES

PAR CH. GRÉGOIRE
Laboratoire de Biochimie, Université de Liège, Belgique

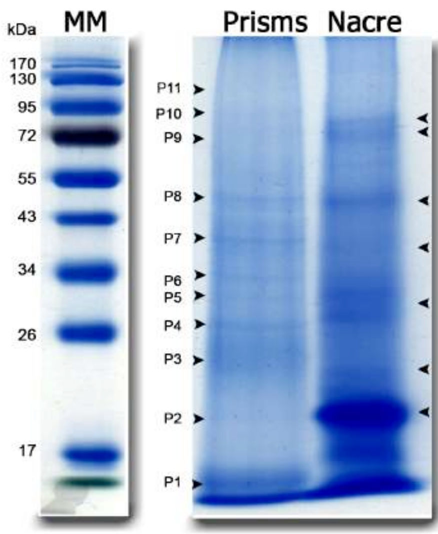
Grégoire, Ch, *Biol. Rev.,* 1967

Mollusk Shell Formation: Isolation of Two Organic Matrix Proteins Associated with Calcite Deposition in the Bivalve *Mytilus californianus*†

Stephen Weiner

Weiner, S, *Biochemistry*, 1983

Example 2



Prisms

Protein	Homology / domain	*Pmarg*	*Pmax*
Alveolin-like[a]	- / VP-rich RLCDs	P	P
MP10[a]	MP10 *Pfu* / VP-rich RLCDs	P	P
Shematrin8	Shematrin2 *Pfu* / GY-rich RLCDs	P>N	P>N
Shematrin9	Shematrin1 *Pfu* / GY-rich RLCDs	P	P
Shematrin3[a]	Shematrin3 *Pfu* / GY-rich RLCDs	P	P
Shematrin5[a]	Shematrin5 *Pfu* / GY-rich RLCDs	P	-
Shematrin6[a]	Shematrin6 *Pfu* / GY-rich RLCDs	P	-
Tyrosinase1[a]	Tyrosinase *Pfu* / Tyrosinase domain	P	P
Tyrosinase2[a]	Tyrosinase *Pfu* / Tyrosinase domain	P	P
Clp-1[a]	Clp1 *Cgig* / Glyco_18 domain	P	P
Clp-3[a]	Clp3 *Cgig* / Glyco_18 domain	P	P
Chitobiase[a]	- / Hex + Glyco_20 domains	P	P
EGF-like1[a]	EGF-like *Cgig* / 2 EGF + ZP domains	P	P
EGF-like2[a]	EGF-like *Cgig* / 2 EGF + ZP domains	P	P
Fibronectin1[a]	- / 5 FN3 domains	P	P
Fibronectin2[a]	- / 4 FN3 domains	P	P
Fibronectin3[a]	- / 5 FN3 domains	P	P
PUSP1[a]	- / 2 chitin-binding + LCT domains	P	P
PUSP15[a]	- / 2 chitin-binding + LCT domains	P	n.e.
PUSP16[a]	- / 2 chitin-binding domains	P	n.e.
Cement-like[a]	- / Poly-G RLCDs	P	P
KRMP7	KRMP2 *Pfu* / GY-rich RLCDs	P	P
Prismalin14[a]	Prismalin14 *Pfu* / GY-rich RLCDs	P	n.e.
PUSP2[a]	- / D-rich domain	P	P
Calmodulin[a]	- / Ef-hand domain	P	-
PUSP3[a]	- / -	P	P
PUSP4[a]	- / Q-rich RLCDs	P	P
PUSP5[a]	- / Q-rich RLCDs	P	P
PUSP7[a]	- / Poly-G RLCDs	P	P
PSPI1[a]	- / 2 kunitz-like domains	P	P
PSPI2[a]	- / 2 kunitz-like domains	P	-
CopAmOx[a]	- / Copper amine oxidase domain	P	-
Peroxidase[a]	Peroxidase / Peroxidase domain	P	-
MPN88	MPN88 *Pfu* / Q-rich RLCDs	P	-
PUSP9[a]	- / S-rich RLCDs	P	n.e.
PTIMP1[a]	- / TIMP domain	P	-
PTIMP2[a]	- / TIMP domain	P	-
PTIMP3[a]	- / TIMP domain	P	-
PUSP11[a]	- / 2 sushi domains	P	n.e.
PUSP12[a]	- / 3 sushi domains	P	n.e.

Nacre

Proteins	Homology / domain	*Pmarg*	*Pmax*
Pif-177	Pif-177 *Pfu* / VWA + 2 chitin-bd domains	N	N
MSI60	MSI60 *Pfu* / Poly-A + G-rich RLCDs	N	N
Nacrein	Nacrein / Carbonic anhydrase domain	N>P	N>P
Pearlin	N14 *Pfu* / C-rich + GN repeat domains	N	N
Linkine	- / C-rich domain	N	N
MRNP34	- / MG-rich RLCDs	N	N
NUSP1[a]	- / 2 chitin-binding + LCT domains	N	N
NUSP2[a]	- / GA-rich RLCDs	N	N
NUSP3[a]	- / -	N	N
NUSP4[a]	- / GAK-rich RLCDs	N	N
NUSP5[a]	- / -	N	N
NUSP6[a]	- / -	N	N
NUSP7[a]	- / G-rich RLCDs	N	N
NUSP8[a]	- / Chitin-binding domain	N	-
NUSP9[a]	- / Poly-A + poly-D RLCDs	N	N
NUSP10[a]	- / C-rich domain	N	-
NUSP11[a]	- / SCP domain	N	n.e.
NUSP12[a]	- / -	N	-
NUSP15[a]	- / -	N	-
NUSP17[a]	- / C-rich + GN repeat domains	N	-
NUSP18[a]	- / -	N>P	n.e.
NUSP19[a]	- / -	N	-
NUSP20[a]	- / -	N	-
NSPI1[a]	- / 2 kunitz-like + G-rich domains	N	N
NSPI2[a]	- / 2 kunitz-like domains	N	N
NSPI3[a]	- / 2 kunitz-like domains	N	-
NSPI4[a]	- / 2 kunitz-like domains	N	-
NSPI5[a]	- / 2 kunitz-like domains	N	-

Protein compositions = VERY différent !!!

Identification *of potential biomarkers of shell calcification* **=>**

48 prisms proteins (40 new, 28 complete)

32 nacre proteins (26 new, 10 complete)

Example 2

PRISMS

Hydrophobic: VP-rich, Poly-G
GY-rich

Hydrophilic polar: Q-rich

Acidic: D-rich

Basic: K-rich

Fibronectin

EGF-like

Sushi (adhesion/binding)

Chitin-binding

Peroxidase

Tyrosinase(phenol ox.)

Cu-amine oxidase

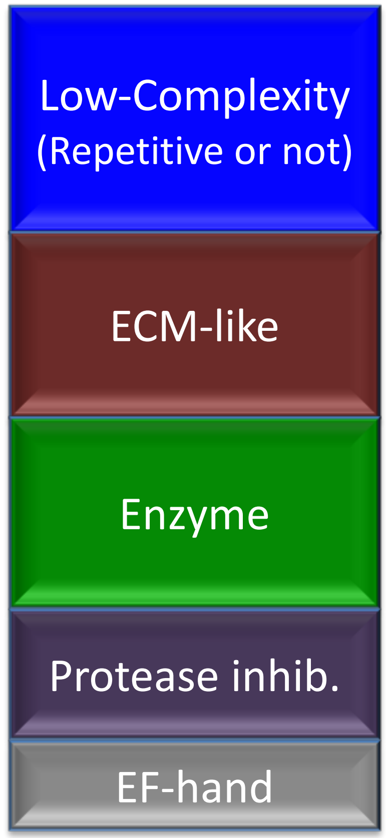
Clp (chitinase)

TIMP (MMP inhibitor)

Kunitz-like

Putative Ca-binding

Protein domains



NACRE

Hydrophobic: G-rich, Poly-G, Poly-A,
GN-rich, GA-rich, GAK-rich

Sulfur-rich: C-rich, M-rich,

Hydrophilic polar: N-rich

Acidic: D-rich, E-rich, poly-D

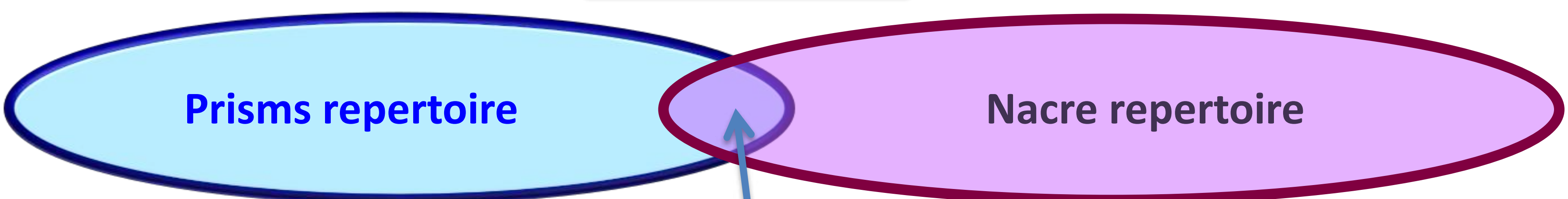
SCP-like

vWF

Chitin-binding

CA

Kunitz-like



Nacrein
NUSP18
Shematrin8

Marie et al., PNAS, 2012

Example 3: mosaic composition of shell matrix proteins

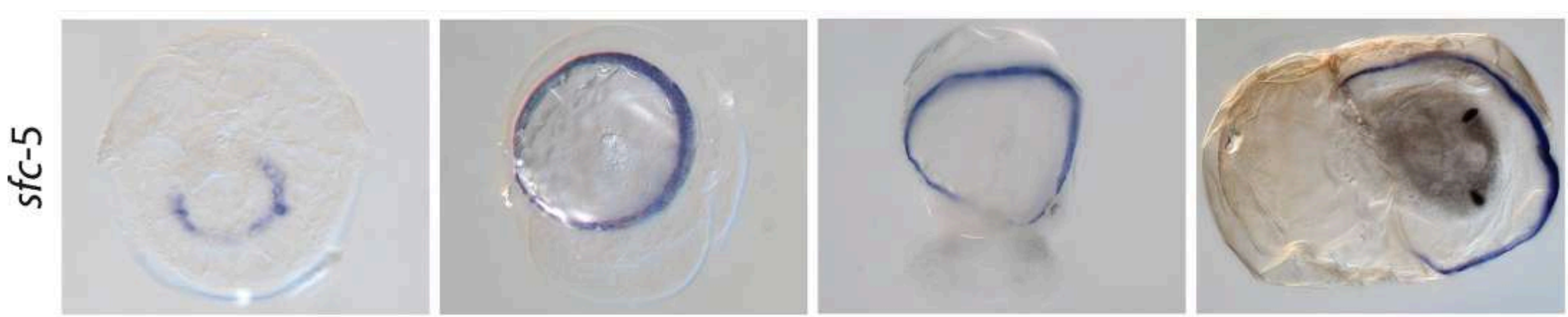
- Freshwater
- Pulmonate
- Belongs to a superfamily (Lymnaeoidea) that emerged in the Lower Jurassic (Dayrat et al., 2011)
- **Non nacreous: crossed-lamellar**

Lymnaea stagnalis

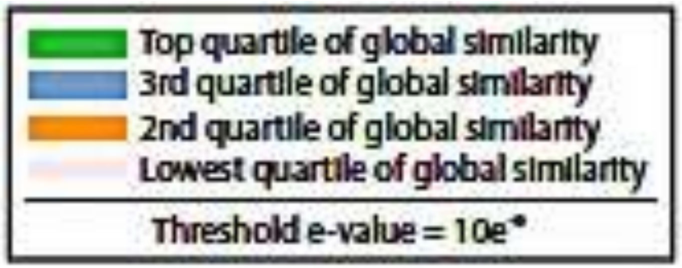
Herlitze et al., Gigascience, 2018
(Dan Jackson's group, Göttingen)

Proteomics of the shell matrix ➔ 40 proteins

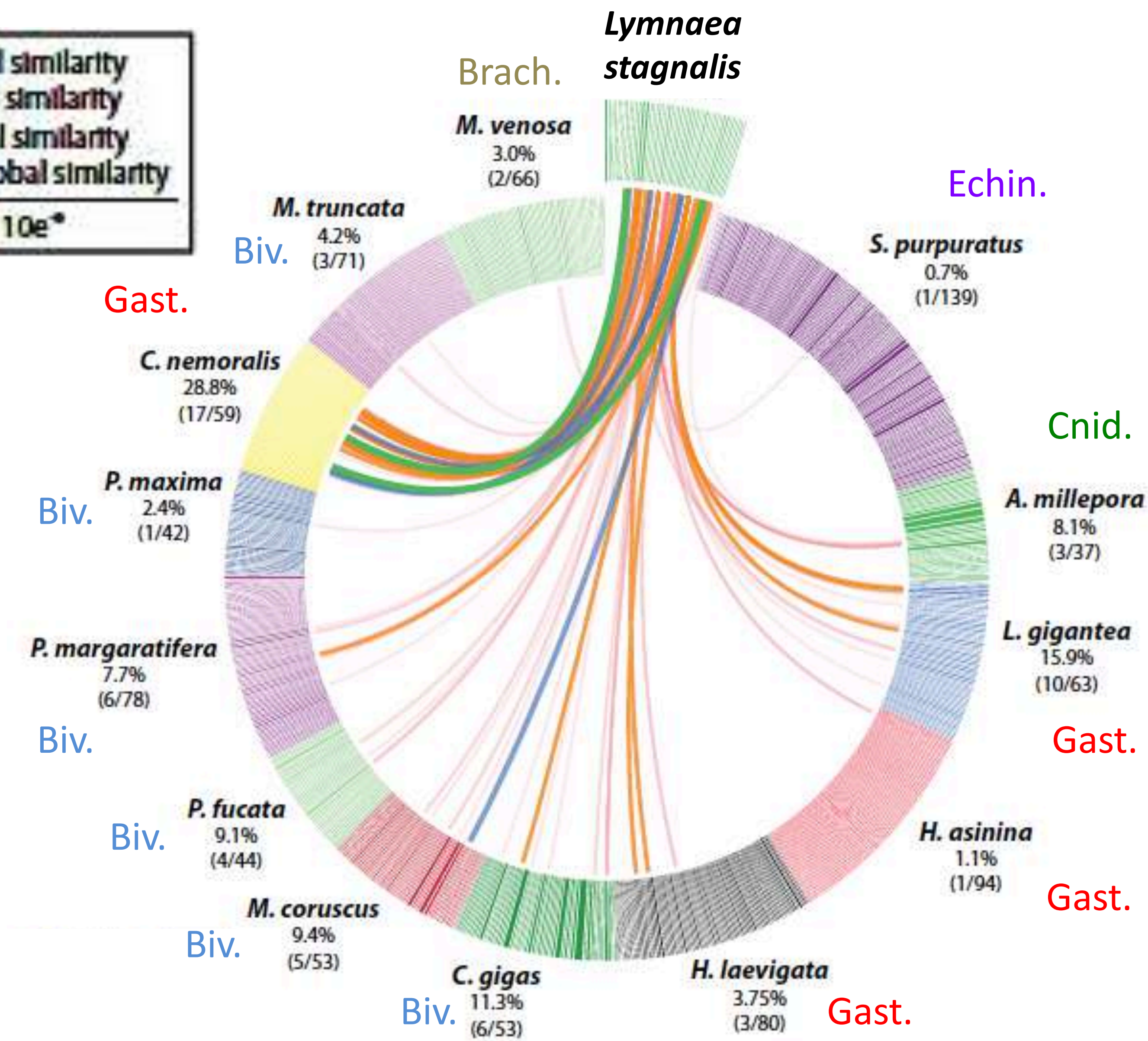
For all of them, verification of the expression of the corresponding gene by *ISH* at different developmental stages



Example 3



Similarity of shell proteins of *L. stagnalis* to skeletal proteins of other invertebrates:
A mosaic picture



Herlitze et al., Gigascience, 2018

Skeletal (CaCO3) proteins, some characteristics…

* Two dozens to >> 100 per model **(depending on cleaning, intracrystalline *vs.* intercrystalline)**

* Mostly <u>modular</u> (multidomains), 'chimeric proteins' ➔ Multifunctional

* Functions deduced from sequence analysis and comparison with known proteins of clearly identified functions: domain sequences similar to those found in vertebrates (in particular in ECM)

* Functional assays / gene knock-down : on very few proteins

Skeletal proteins

→ **Domains of clearly identified function**

- Enzymatic
- Sugar-binding
- Protease inhibitor
- Ca-binding
- Immunity
- ECM-like

→ **Domains of unknown / putative function (no homologues)**

- All LCD or RLCDs
- Other non-LCDs

A summary…

FEATURES

- Skeletal matrix proteins => Diversity => Plasticity
- Modular architecture of sequences
- Abundance of LCDs and RLCDs

MACROEVOLUTIONARY TRENDS

- Skeletal matrix proteins = complex evolutionary histories
  - Early recruitment (Cambrian or before) + 2^{ary} evolutions in the different lineages
  - Recent recruitment (lineage-dependent) and / or fast evolution: RLCDs

  - No phylogenetic signal (at high taxonomic level) !!!

Shell matrix diagenesis

Frédéric MARIN

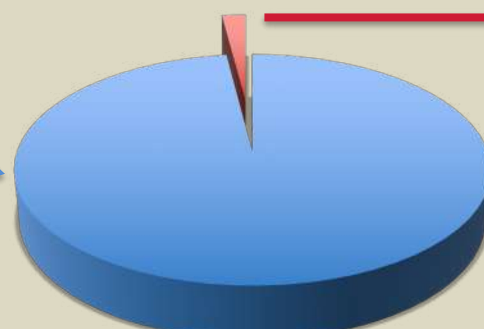
UMR 6282 CNRS BIOGEOSCIENCES
Université de Bourgogne – Franche Comté -
Dijon, FRANCE

Calcifying matrix in metazoans (non vertebrates)

Cnidaria | Bryozoa | Brachiopoda | Mollusca | Echinodermata

Decalcification

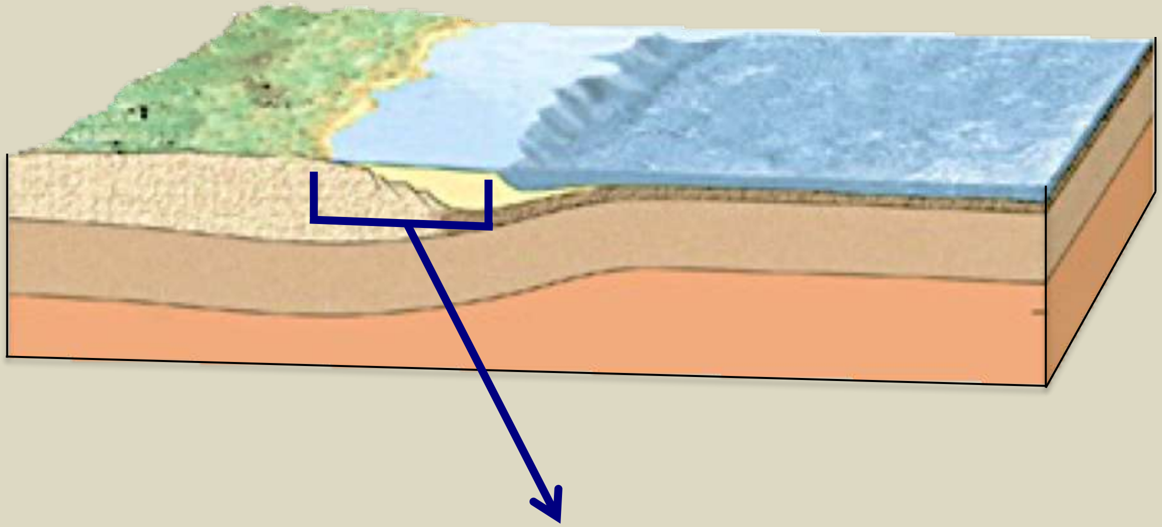
$CaCO_3$
+ Minor & Trace Elements
Mg, Sr...



ORGANICS
0.05 to 1-2 wt-% of the skeletal tissue

CaCO3 biomineralization & global carbonate cycle

Biogenic $CaCO_3$ in neritic environments: about $2.5.10^9$ T/yr



From Milliman, 1993;
Wollast, 1993;
Langer et al., 1997

Neritic environments

- **Benthic**
- **Aragonite & (Mg) calcite**
- **Corals, foraminif., molluscs, algae**

Estimated prod.: about 2,5 . 10^9 T/yr (corals: 0,9 . 10^9 T/yr)

Cnidaria Bryozoa Brachiopoda Mollusca Echinodermata

0.05 to 1-2 wt-% of the skeletal tissue

$1.2 \cdot 10^6$ T/yr < calcifying matrix < $50 \cdot 10^6$ T/yr

in neritic environments

Important source of biomolecules in the fossil record

How do these molecules fossilize ?

- Abelson, 1954. Amino acids in fossils. Carn. Inst. Washington Yearb., 53, 97-108.

SOME ASPECTS OF PALEOBIOCHEMISTRY

By Philip H. Abelson

Geophysical Laboratory, Carnegie Institution of Washington, Washington, D. C.

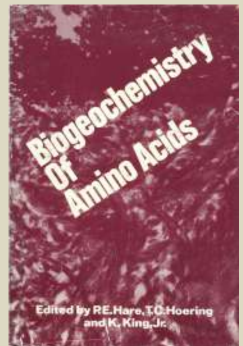
Organic chemicals of biological origin are being found in an increasing number of fossils, sediments, and sedimentary rocks. The existence of these

Paleobiochemistry / Molecular Paleontology

2 approaches

- **Work on fossils**
  - → Matrix extraction and analysis
- **Artificial diagenesis experiments**
  - → Heated samples, matrix extraction & analysis

The ‘Golden Age’ of fossil proteins analysis



Abelson

Vallentyne
Grégoire

Mitterer
Weiner
Westbroek

Collins
Muyzer

1950 1960 1970 1980 1990 2000 2010

PCR

The ‘Golden Age’ of fossil DNA analysis

Research on fossil proteins in biominerals overshadowed by that on fossil DNA

Degradation pathway of skeletal matrix proteins

Hydrolysis

Proteins → Peptides → Free amino acids

Racemization

L-Amino acids ⇄ D-Amino acids

Defunctionalization

Amino acids —*Decarboxylation / Desamination*→ **Amines / Organic acids / Hydrocarbons**

Condensation

Amino acids ⇄ Humic substances

Adapted from Mitterer 1985; Hare & Hoering, 1977

Hydrolysis of skeletal matrix proteins

1. Stability of peptidic bonds

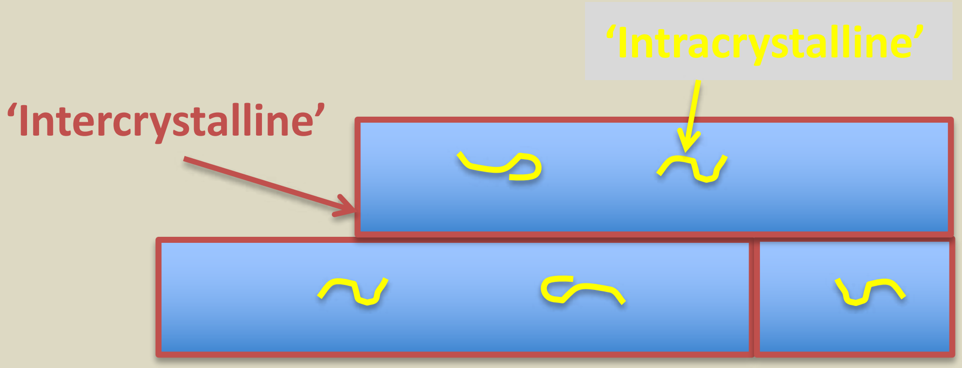
1. 3D structure of the protein

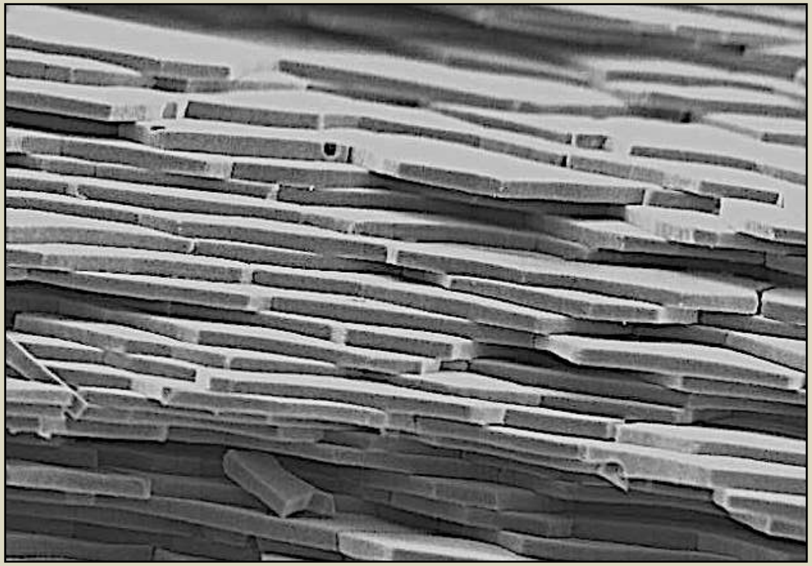
2. PTMs *(glycos. phosphor.)*

1. Localization in the biomineral

1. Water: interstitial/linked

2. CaCO3 polymorph: calcite vs. aragonite





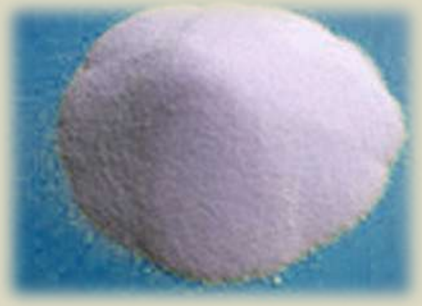
Nacre *Nucula* sp., Lutetian

Example 1: thermal stability of nacre proteins
Artificial diagenesis experiments

P. margaritifera

Nacre only !!
1st & 2^{nd} Bleaching treatments

3rd bleach



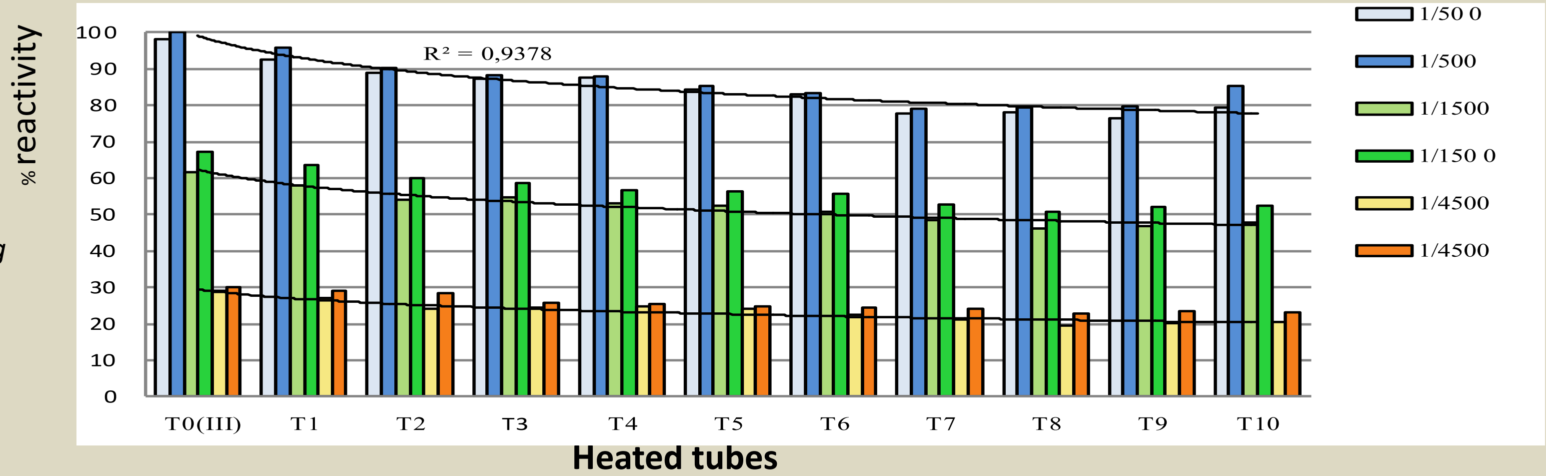
Heated at 100° C during 10 days (dry conditions)

- Daily sampling: T1, T2... T5... T10
- Matrix extraction
- Matrix quantification, ELISA, proteomics

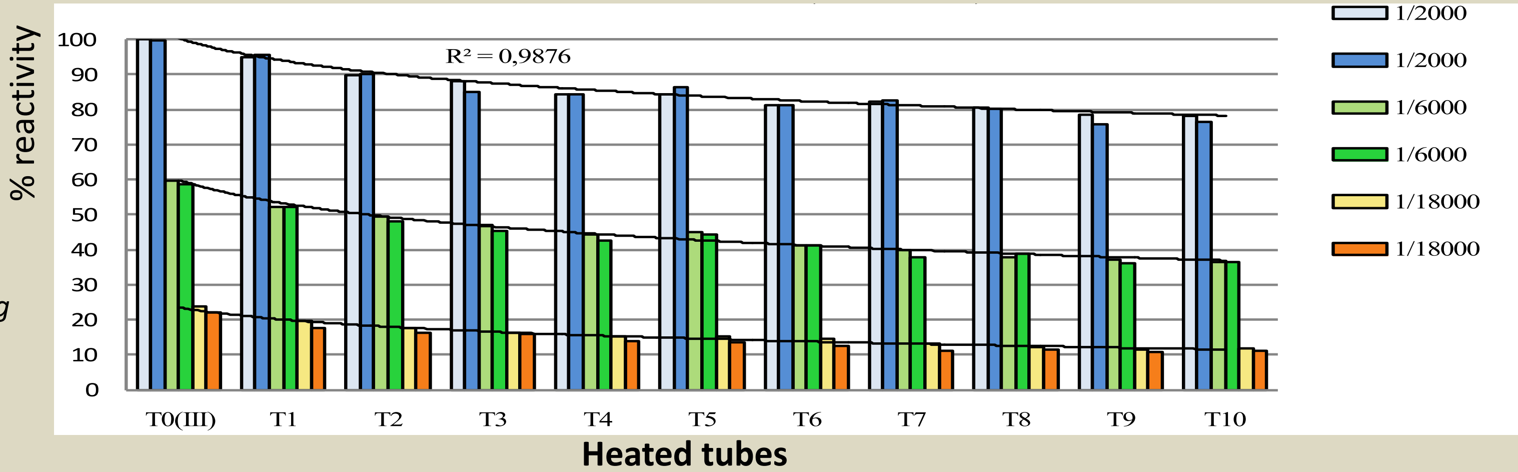
Example 1

Immunological tests on the 'intracrystalline' matrix

Antibody A: α–ASM $_{Pmarg}$



Antibody B: α–LS-AIM $_{Pmarg}$



After 10 days at 100°C, loss of 20% reactivity

Example 1

T0	T5 (120 hours)	T10 (240 hours)
Nacrein A1 (13) →	Nacrein A1 (7) →	Nacrein A1 (4)
Nacrein B3 (11) →	Nacrein B3 (6) →	Nacrein B3 (6)
Nacrein B4 (11) →	Nacrein B4 (5) →	Nacrein B4 (5)
Nacrein C5 (12)	-	-
PIF (11) →	PIF (3) →	PIF (3)
Gly-Rich protein 2 (3)	-	-
Shematrin 8 (2) →	Shematrin 8 (2)	Shematrin-like (1)
Perlin (3)	-	-
Asn-rich protein (1)	-	-
USP 7 (1) →	USP 7 (1) →	USP 7 (1)
USP 11 (1)	-	-
USP 12 (1)	-	-

(Parker et al., 2015)

*** Persistence of some shell proteins**

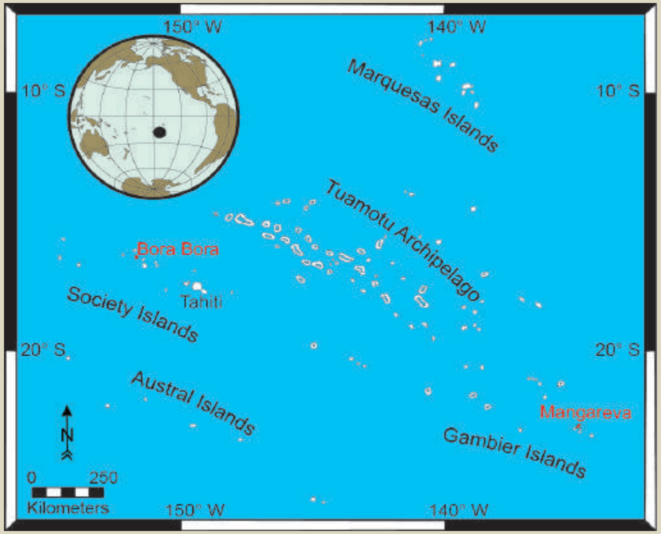
*** Apparent degradation of others: differential degradation**

*** Diagenesis: -> Disappearance of some proteins**

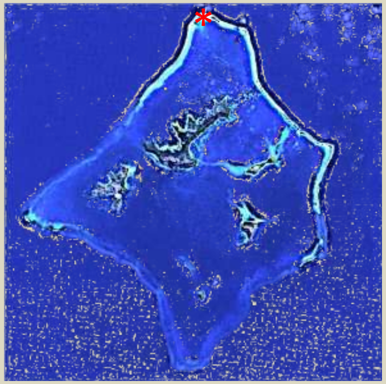
-> Decrease of the number of identified peptides

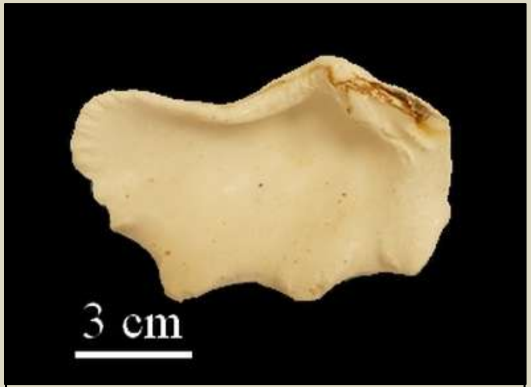
<u>Example 2</u>: sub-fossil *Tridacna of French Polynesia*

(Collaboration: Takeshi Takeuchi, OIST + the Univ. Geneva, Master work of A. Chmiel)

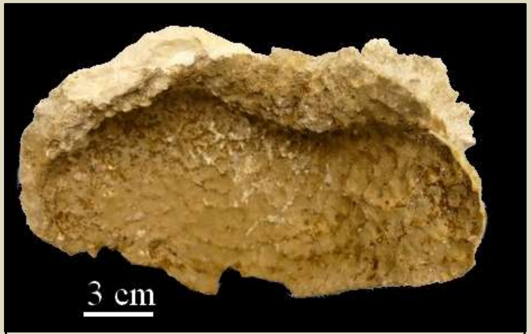


Mangareva





GAM-48: Collected alive



GAM-14: 3320 +/- 30 BP

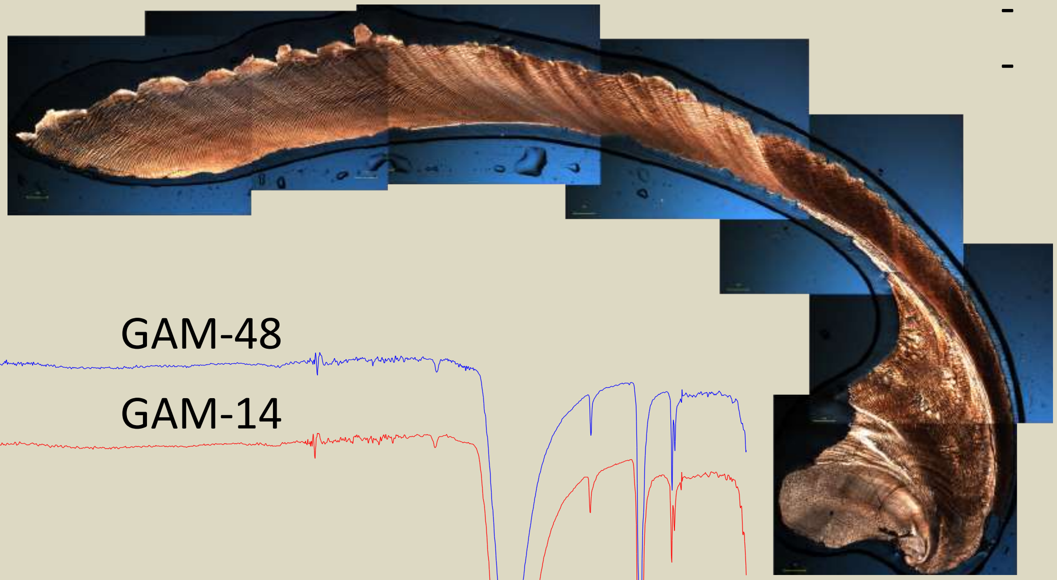
<u>Diagenetic context</u>:

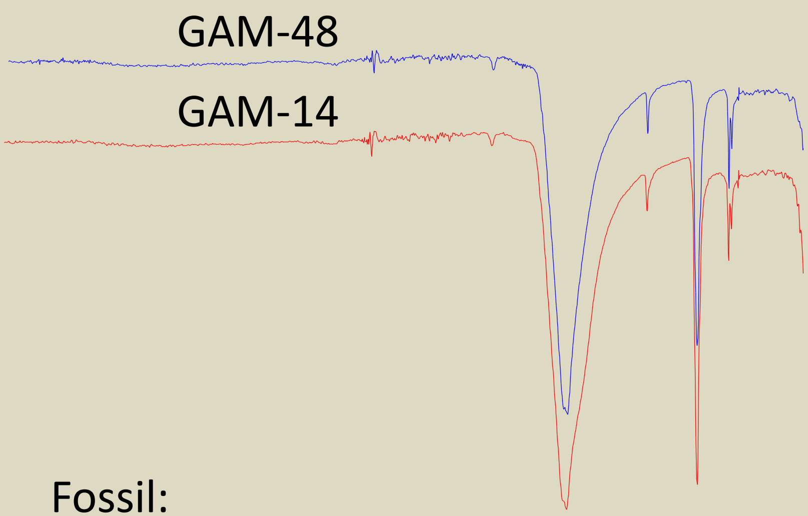
- **Reef flat**
- **Rich in percolating H_2O**
- **Not favourable for good preservation of aragonite**

Is there still a biochemical signal ?

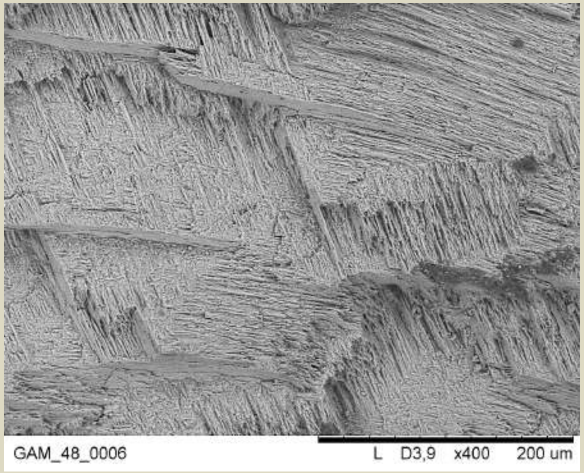
<u>Control on the mineral phase</u>:

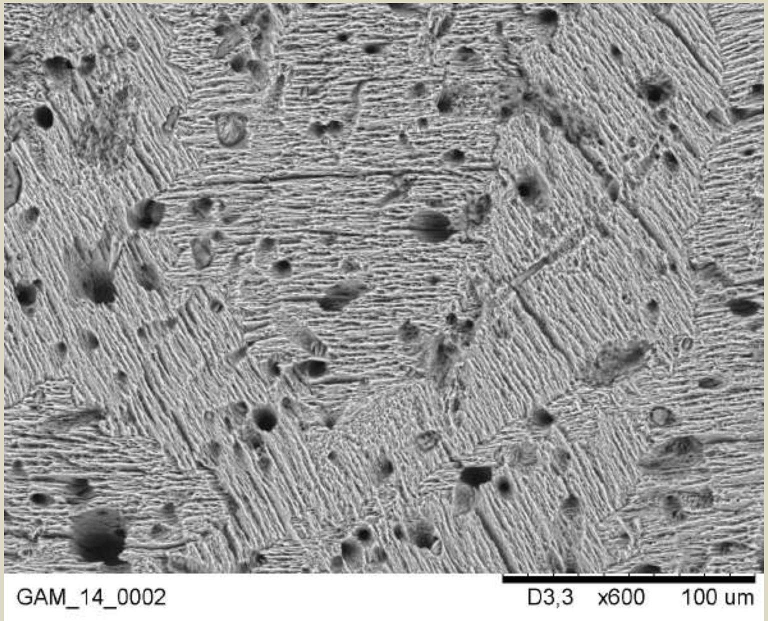
- Thin sections
- SEM
- Cathodoluminescence
- Epifluorescence
- XRF
- FT-IR





- Fully aragonitic
- Crossed-lamellar & complex crossed lamellar microstructures



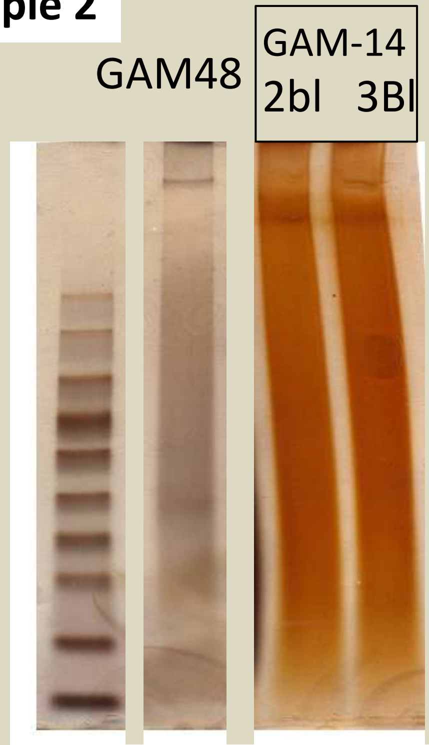


<u>Fossil</u>:

- No recrystallization into calcite.

- Important alterations & perforations in the outemost and innermost layers of the shell

Example 2



- 'Fossil' sample: 2 & 3 thorough bleaching steps: 2bl, 3bl

➔ get the most protected proteins

- Extraction and analysis on 1D gels
- Proteomics

Reference: transcriptome from *Tridacna crocea* *(Dr. Takeshi TAKEUCHI, Pr. Nori SATOH, OIST)*

	GAM-48	GAM-14, 2 bl	GAM-14, 3bl
Nb protein hits	134	40	32
Nb proteins hits identified by more than 2 peptides	46	4	7
Nb proteins hits identified by 1 peptides	88	36	25
	FRESH	SUB-FOSSIL	

Example 2

LIVING: GAM-48 | SUB-FOSSIL: GAM-14-2 & GAM-14-3

TRINITY_DN232224_c0_g1_i1|m.393634

Living: GAM-48

MRGIAVFAVLLLAVAANAQGPPTRTKKGKRPGDNPDIGLPSGKDIPDGRR
FAPPAAPRAAPVPPARAPAAAAPAPAPAGYRKALPPAAARPPVLPPAAGR
PAGGIPPTRTRKGKRPAGRMGPAYRVAPPAPLAPKNPPMFGNPWAFAPAP
AAPVAPAVSCEASCGYFWAPVCSVYGNTYDNDCRLGCSGEPYACEGQCPC
EEPKPAPAAASPMLGLFGSSSCGCGYHYDPVCTDDGDEVMNECLALCDGK
TIACSSHCPC

Protein sequence coverage: 25%

Sub-fossil: GAM-14-2 & GAM-14-3

MRGIAVFAVLLLAVAANAQGPPTRTKKGKRPGDNPDIGLPSGKDIPDGRR
FAPPAAPRAAPVPPARAPAAAAPAPAPAGYRKALPPAAARPPVLPPAAGR
PAGGIPPTRTRKGKRPAGRMGPAYRVAPPAPLAPKNPPMFGNPWAFAPAP
AAPVAPAVSCEASCGYFWAPVCSVYGNTYDNDCRLGCSGEPYACEGQCPC
EEPKPAPAAASPMLGLFGSSSCGCGYHYDPVCTDDGDEVMNECLALCDGK
TIACSSHCPC

Protein sequence coverage: 15%

Pro: 19.5; Ala: 17.4; Gly: 10.4 pI: 8.78

TRINITY_DN230191_c0_g1_i1|m.402396

Living: GAM-48

GQWEFDGSGAGGFGGQFPLG GLGLANWKYNAKTGQWEFDGFGAGGA
GSQFTGSGNWKWNATSGHWQFVGAGGQGKGQSWWTSNMKGAALLK
KLKALLQAQAMARWNEWQKTTKSGFVGRQRPSHIVQTIRGKMGRNEIGG
AKLAILGGKNRAKPMVQPKTRSPPNSNAN

Protein sequence coverage: 30%

Sub-fossil: GAM-14-2 & GAM-14-3

GQWEFDGSGAGGFGGQFPLGGLGLANWKYNAKTGQWEFDGFGAGGA
GSQFTGSGNWKWNATSGHWQFVGAGGQGKGQSWWTSNMKGAALLK
KLKALLQAQAMARWNEWQKTTKSGFVGRQRPSHIVQTIRGKMGRNEIGG
AKLAILGGKNRAKPMVQPKTRSPPNSNAN

Protein sequence coverage: 11%

Gly: 18.8; Ala: 10.6; Lys: 9.4 pI: 11.45

Example 2

LIVING: GAM-48

ETMNKVILIVFSGLLAVQLVSAQSHTTWAAAQVPGLGRMTPPTTDYPEYMLHMAVG
EIMRAPTENKAAYAAAKVYNPVMDMSDKVQQALEDRVLQLRHPPGTPYYRRKLDFD
VMQLVIGAYYKTLNISAPQQLGSFYGPPPANHWAGASQPVGPPARQPGPLPPAGPPA
GPAMGPPTSIRRGFRPRAQGIYSPFEPTPWELDRAVQDIHMARTEKQAVKAAAGVHR
IGLDLADIVVNALEEKIARLRRPNWTGFRPPPIPRGLNVHGLVRHAFYEIQRIAQAKAAA
DAAAAAAAAAKAKKTPPPPTPRAGSKIPTTLPPTPPPKPYKKPRQPSKPNPPPSPKKTK
PPKRDFMTDFIQNRRKQRQPPPAPKLFKQAQITRPPFVQPVRRQTLNPFPTQPSVPPY
FEPTKITRPPYVQPQRRDPVDPFFSQPSYRPKPQVEPFRPPPNVPRPPKINWKKKAAAK
PVPTSVSLTEKGPTSISAASSNSNKSPVSYETIPSQNSAKPAFMKIPKPNVPPAPFRSEPP
KPKSLFPKGNSGRPSDIPKAVLSSNKGKGKRPSSKTVEPLFQSTETTPSPEEQQLFNRYP
GLFENKARMRVANLAQHRLETVGPSAEISKPPLKGPNPQPPKVSNKKMPVSKPPQQA
AVKVAPKIIKPSKVWSPLGNLGSNINEILKFSIDGPSESVPIPTAAPLTTKAPTTTTKKPTT
TTPRKQEKVKPIRKTKVKRRRKVVSKAKSKSFAIKLAKKKPEKPKKPQGADKLTQLHKLL
EGISPSQLQTLVDLIKAKANEGKPKPLPKPEPLPKPKPIFAPPPPPGSEHKGPPREFRSQ
MQSSRSGPYRPNSDYGPPPDNRGPPPDWARGPGGRPRGPPGPPGPGGPGGPGMR
GGPDLSNPQIARLIRVMKQGGHPKNNFLSGRTPGSSAAAAGGEAPEAGEGPTGLLGN
PLMMSMLMNRGGQGGGGGIASLLGGGAAGGANPLAALMPGGAGGAGGGEGGM
NPAMLAAIMGGGQGGGGGGMGALGGGYGGMLAGLGL

Protein sequence coverage: 38%

SUB-FOSSIL: GAM-14-2 & GAM-14-3

ETMNKVILIVFSGLLAVQLVSAQSHTTWAAAQVPGLGRMTPPTTDYPEYMLHMAVG
EIMRAPTENKAAYAAAKVYNPVMDMSDKVQQALEDRVLQLRHPPGTPYYRRKLDFD
VMQLVIGAYYKTLNISAPQQLGSFYGPPPANHWAGASQPVGPPARQPGPLPPAGPPA
GPAMGPPTSIRRGFRPRAQGIYSPFEPTPWELDRAVQDIHMARTEKQAVKAAAGVHR
IGLDLADIVVNALEEKIARLRRPNWTGFRPPPIPRGLNVHGLVRHAFYEIQRIAQAKAAA
DAAAAAAAAAKAKKTPPPPTPRAGSKIPTTLPPTPPPKPYKKPRQPSKPNPPPSPKKTK
PPKRDFMTDFIQNRRKQRQPPPAPKLFKQAQITRPPFVQPVRRQTLNPFPTQPSVPPY
FEPTKITRPPYVQPQRRDPVDPFFSQPSYRPKPQVEPFRPPPNVPRPPKINWKKKAAAK
PVPTSVSLTEKGPTSISAASSNSNKSPVSYETIPSQNSAKPAFMKIPKPNVPPAPFRSEPP
KPKSLFPKGNSGRPSDIPKAVLSSNKGKGKRPSSKTVEPLFQSTETTPSPEEQQLFNRYP
GLFENKARMRVANLAQHRLETVGPSAEISKPPLKGPNPQPPKVSNKKMPVSKPPQQA
AVKVAPKIIKPSKVWSPLGNLGSNINEILKFSIDGPSESVPIPTAAPLTTKAPTTTTKKPTT
TTPRKQEKVKPIRKTKVKRRRKVVSKAKSKSFAIKLAKKKPEKPKKPQGADKLTQLHKLL
EGISPSQLQTLVDLIKAKANEGKPKPLPKPEPLPKPKPIFAPPPPPGSEHKGPPREFRSQ
MQSSRSGPYRPNSDYGPPPDNRGPPPDWARGPGGRPRGPPGPPGPGGPGGPGMR
GGPDLSNPQIARLIRVMKQGGHPKNNFLSGRTPGSSAAAAGGEAPEAGEGPTGLLGN
PLMMSMLMNRGGQGGGGGIASLLGGGAAGGANPLAALMPGGAGGAGGGEGGM
NPAMLAAIMGGGQGGGGGGMGALGGGYGGMLAGLGL

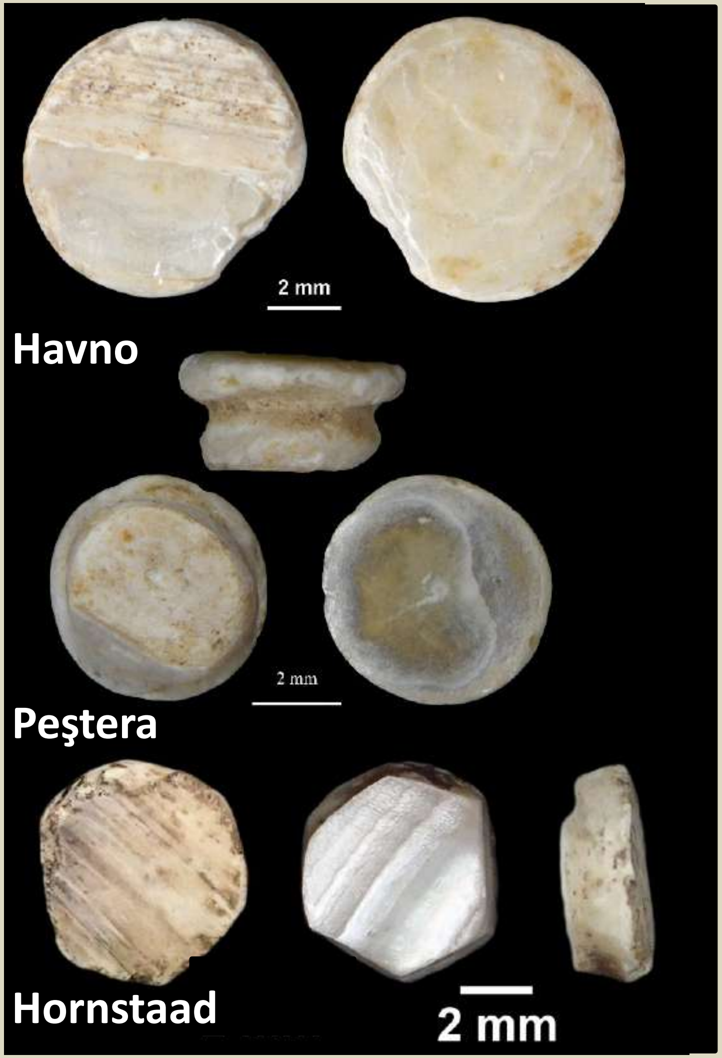
Protein sequence coverage: 4%

TRINITY_DN253411_c2_g2_i3|m.459507

Pro: 17.2; Gly: 10.1; Ala: 9.6 pI: 10.63

➔ **Follow the diagenetic behavior of one protein species**

<u>Example 3</u>: archaeological samples



2 mm

Groove

Double button pearl: « Doppelknöpfe »

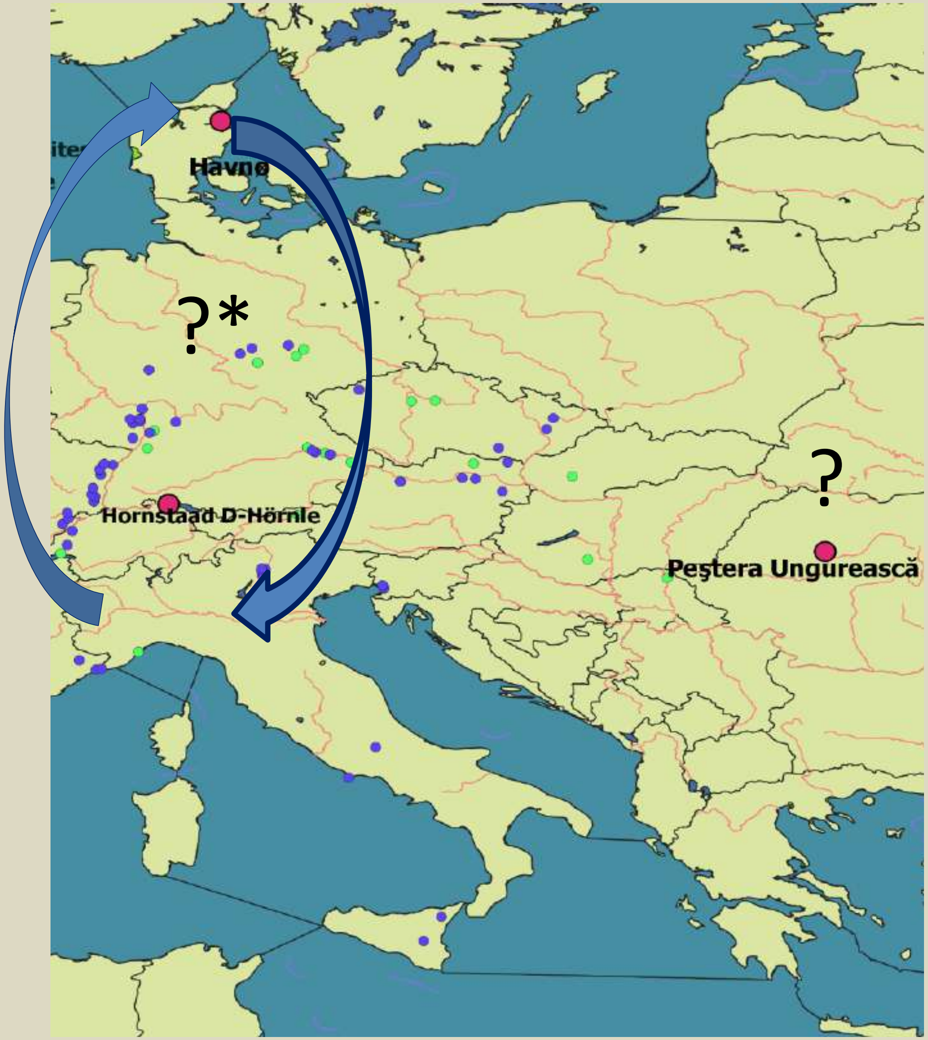
- Complex process of fabrication
- Inserted in pierced leather clothes

« Pearlies »

Example 3: archaeological samples

Archaeological sites

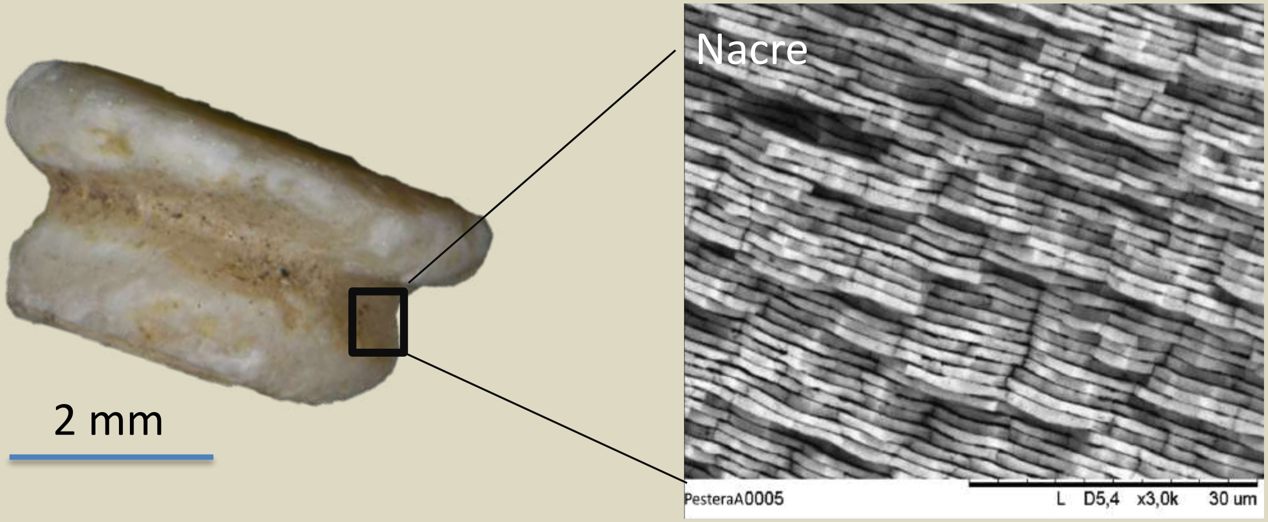
- **Havnø (Dk)**: Ertebølle Hunter-gatherers-fishers (*4420 - 3590 BC*)
- **Hornstaad (Sw)**: Neolithic (*3918 - 3902 BC*)
- **Peştera Ungurească (Ro)**: Late Neolithic / beginning Bronze Age



Marine origin? Long-distance exchange of raw materials ?

Example 3: archaeological samples

Scanning Electron Microscopy



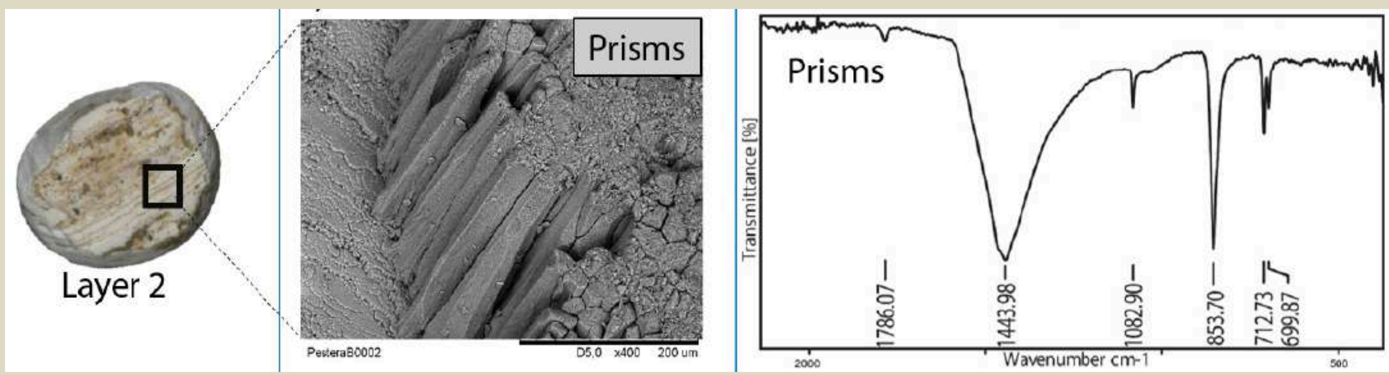
Nacre in bivalves

Paleotaxodonta: marine
Cryptodonta
Pteriomorpha: marine
Paleoheterodonta: fw
Heterodonta
Anomalodesmata: marine

Typical « brickwall » bivalve nacre

Clues:

1



2 Stable isotope geochemistry: $\delta^{18}O$ and $\delta^{13}C$: negative (suggests a freshwater / estuarine origin)

<u>Example 3</u>: archaeological samples

Micro-extraction & proteomics

Proteins in Unionoid shells & beads	Hyriopsis cumingii (Triangle sail mussel)
• Hic74	Unknown function
• Hic52	Unknown function
• Silkmapin	Unknown function

Hic74: Ala/Gly–rich
pI: 4.8; A: 30.8%; G: 25.6%; S: 10.6%

Hic52: Gly/Gln-rich
pI: 10.2; G: 28%; Q: 12%.

Silkmapin: Gly-rich
pI: 6.9; G: 33%.

Freshwater mussel !!
Unionidae

<u>Example 3</u>: archaeological samples

* Exploitation of local freshwater shell resources for making button-pearls.

* No use of marine nacreous shells.

* Ornaments = prestige, concept <u>not associated</u> to the rarity of the resource; what made the button-pearls rare was the effort made to fabricate them.

* No necessity of long-distance trading exchange of raw material but…

* ... Propagation of the know-how to craft double button pearls.

* Powerful use of proteomics in archeology...

In summary…

- Persistence of some shell proteins after long heating (10d, 100°C)
- Differential degradation pattern
- Diagenesis seen by proteomics:
  - Disappearance of some proteins
  - Decrease of the number of identified peptides

Time to revisit diagenesis of Skeletal Matrix Proteins by proteomics

Possibility to track the diagenetic behavior of one given protein

Well-suited for archaeological samples