



IGC, NANTES | AUGUST, 2019

| DNA FINGERPRINTING OF PRECIOUS CORALS AND PEARLS

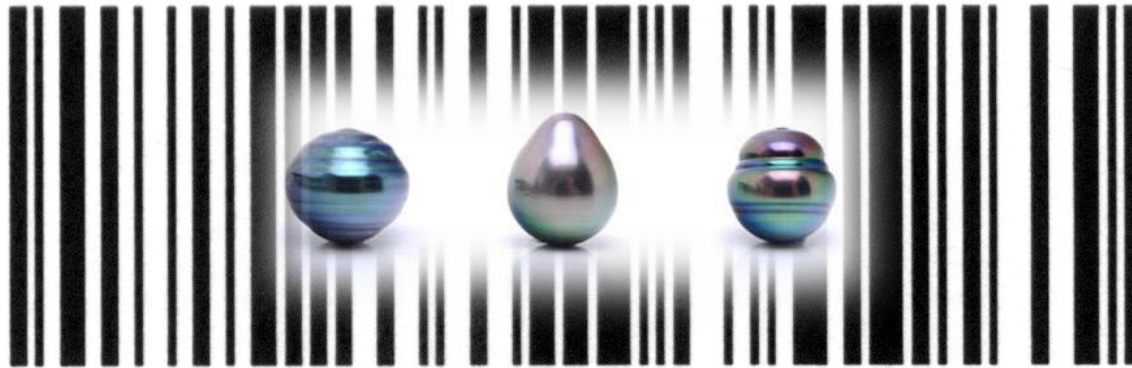
Presentation by Dr. Laurent E. Cartier FGA

with B. Lendvay, N. Morf, M.S. Krzemnicki, J.B. Meyer

SSEF 

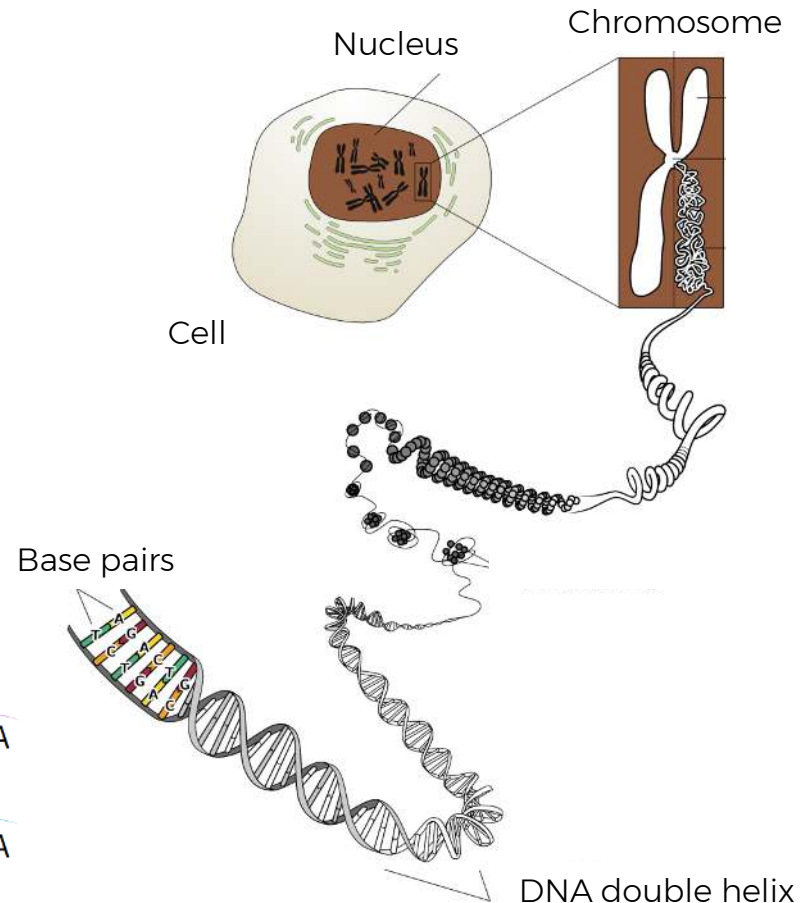
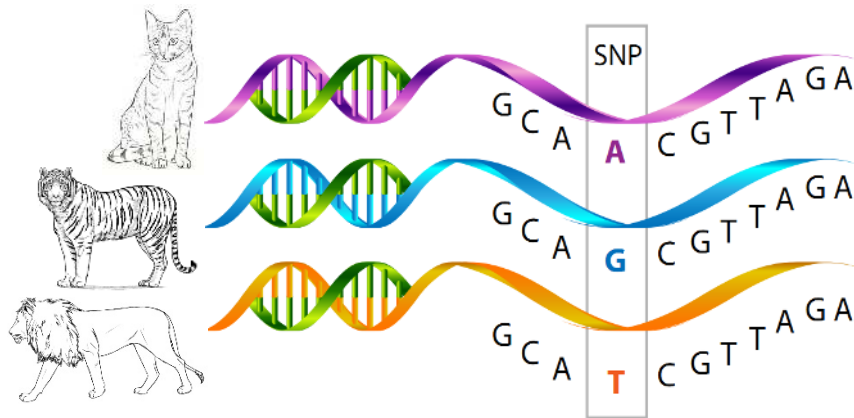
| RESEARCH QUESTIONS

- Is there oyster DNA in a pearl?
- Could this DNA be used to conclusively identify the species of a pearl?
- If it's possible for pearls, is it also possible to find DNA in precious coral and ivory for species identification?
- Can a non-destructive method be developed to test samples?
- Validation of methods



| WHAT IS DNA?

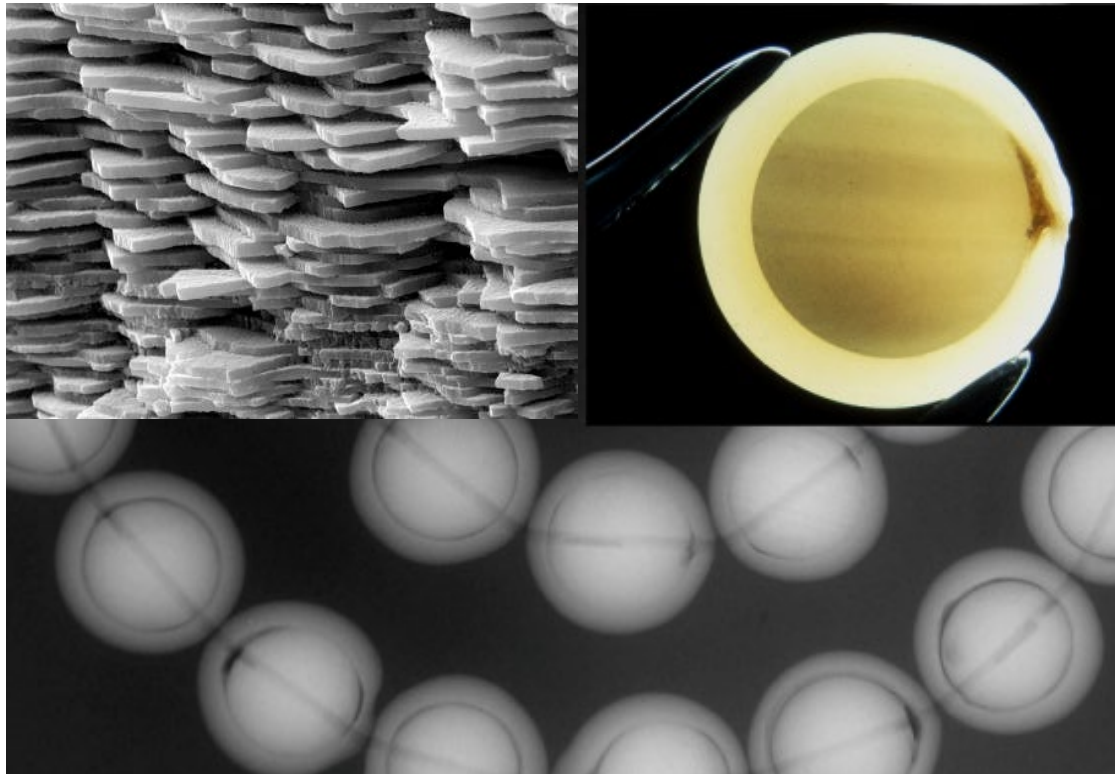
- Deoxyribonucleic acid (DNA): Contains all the information an organism needs to develop, live and reproduce. It is formed by the four nucleobases (or 'bases') adenine (A), cytosine (C), guanine (G) and thymidine (T). The order of the bases (e.g. ATCGGTT...) codifies the specific instructions for any living organism.
- Genome: An organism's full set of DNA, including all of its genes.



| OYSTER DNA IN A PEARL?

The nacreous part of a pearl consists of approximately 92% CaCO_3 , 4% organic matter (OM), 4% water and minute amounts of residual substances (Taylor & Strack, 2008).

The OM (consisting mostly of conchioline and porphyrines), which is also secreted by the pearl sac, serves as a framework for the CaCO_3 matrix during the biomineralization process. OM can also be found in concentrated pockets.



Photos: H.A. Hänni, SSEF

DNA EXTRACTION AND PREPARATION

2013

OPEN ACCESS Freely available online



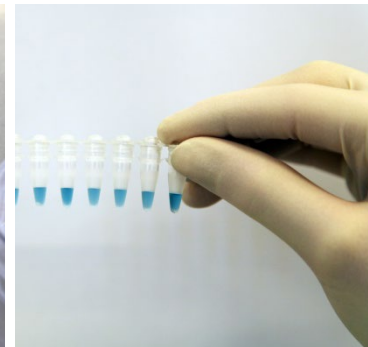
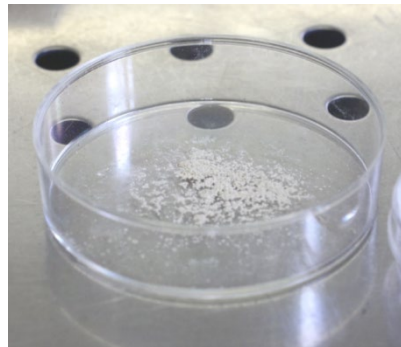
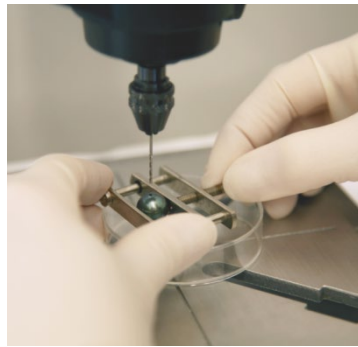
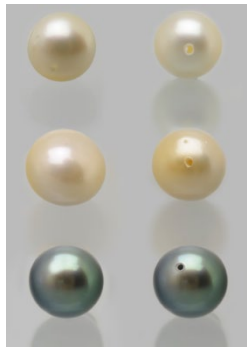
DNA Fingerprinting of Pearls to Determine Their Origins

Joana B. Meyer^{1,2*}, Laurent E. Cartier^{2,3*}, Eric A. Pinto-Figueroa⁴, Michael S. Krzemnicki², Henry A. Hänni⁵, Bruce A. McDonald¹

Destructive

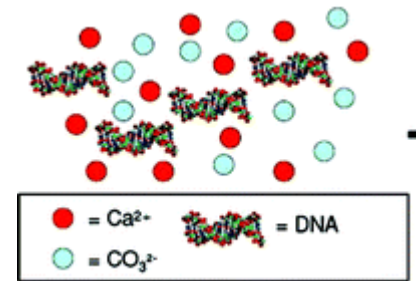
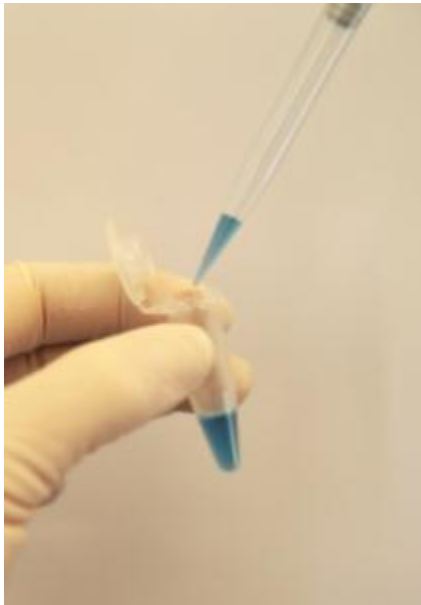


Quasi non-destructive



DNA EXTRACTION AND PREPARATION

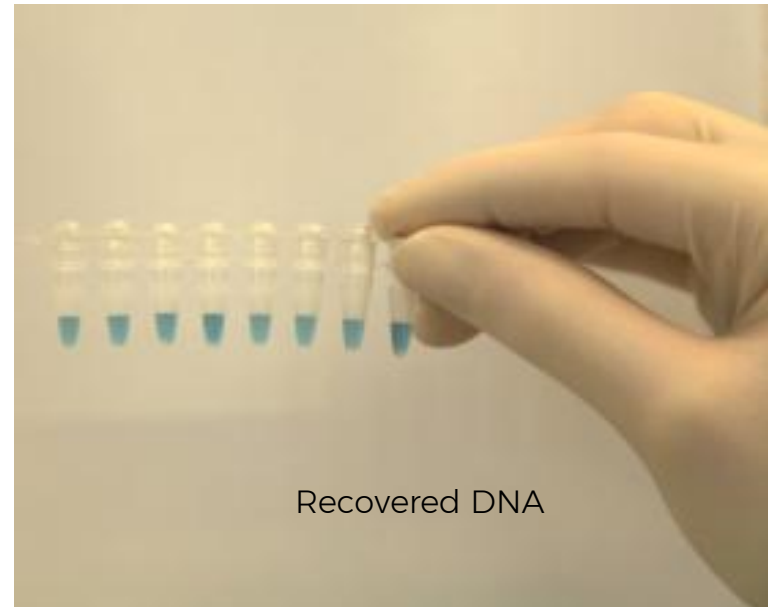
- The negatively charged DNA molecule is known to have a high affinity for the Ca^{2+} ion of CaCO_3 , which might enhance its conservation in pearls and corals.
- EDTA (0.5M pH=8, overnight 56°C) used to liberate DNA from CaCO_3 matrix.



Source: Sommerdijk et al. (2007)

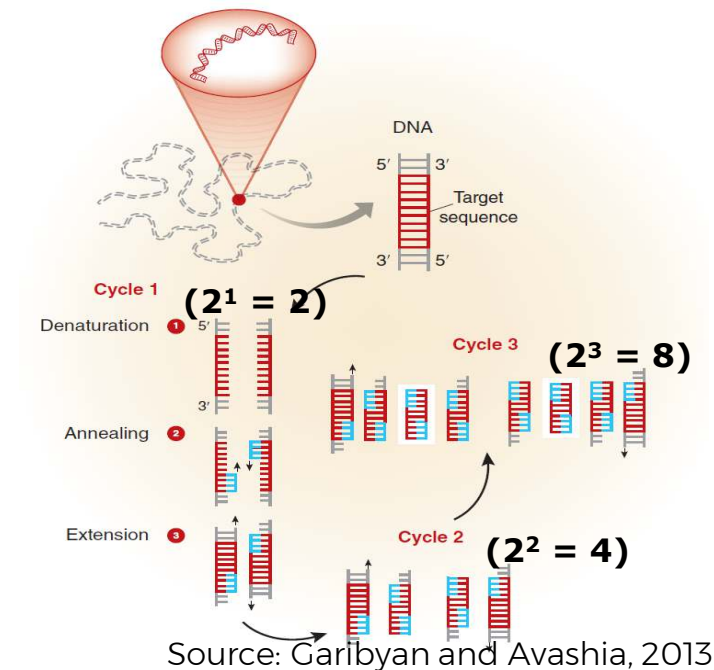
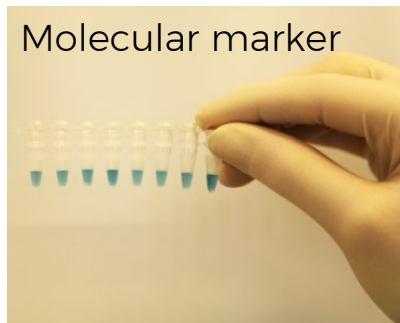
| DNA EXTRACTION AND PREPARATION

- Kit needed to concentrate DNA from recovered sample powder.
- Having positive and negative controls is critical.
- Depending on the species that needs to be identified different protocols are possible and different markers in different molecular markers in the genome are targeted (e.g. ITS2, 16S, Cox1, Cox2).



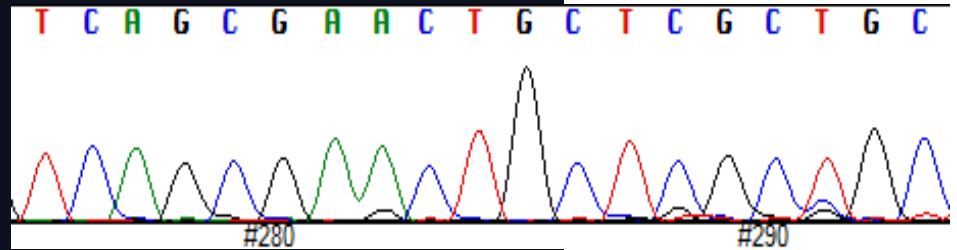
DNA AMPLIFICATION

- DNA amplification involves using short DNA sequences (**primers**) to select the portion of a genome for amplification. In PCR, sample temperature is repeatedly increased and decreased to help a DNA replication enzyme synthesise the target DNA sequence.
- PCR can produce thousands to millions of copies of the target sequence in several hours, which can then be analysed using visual inspection (e.g. gel electrophoresis) or they may be read through sequencing.



| DNA SEQUENCING

TCAGCGAACTGC...

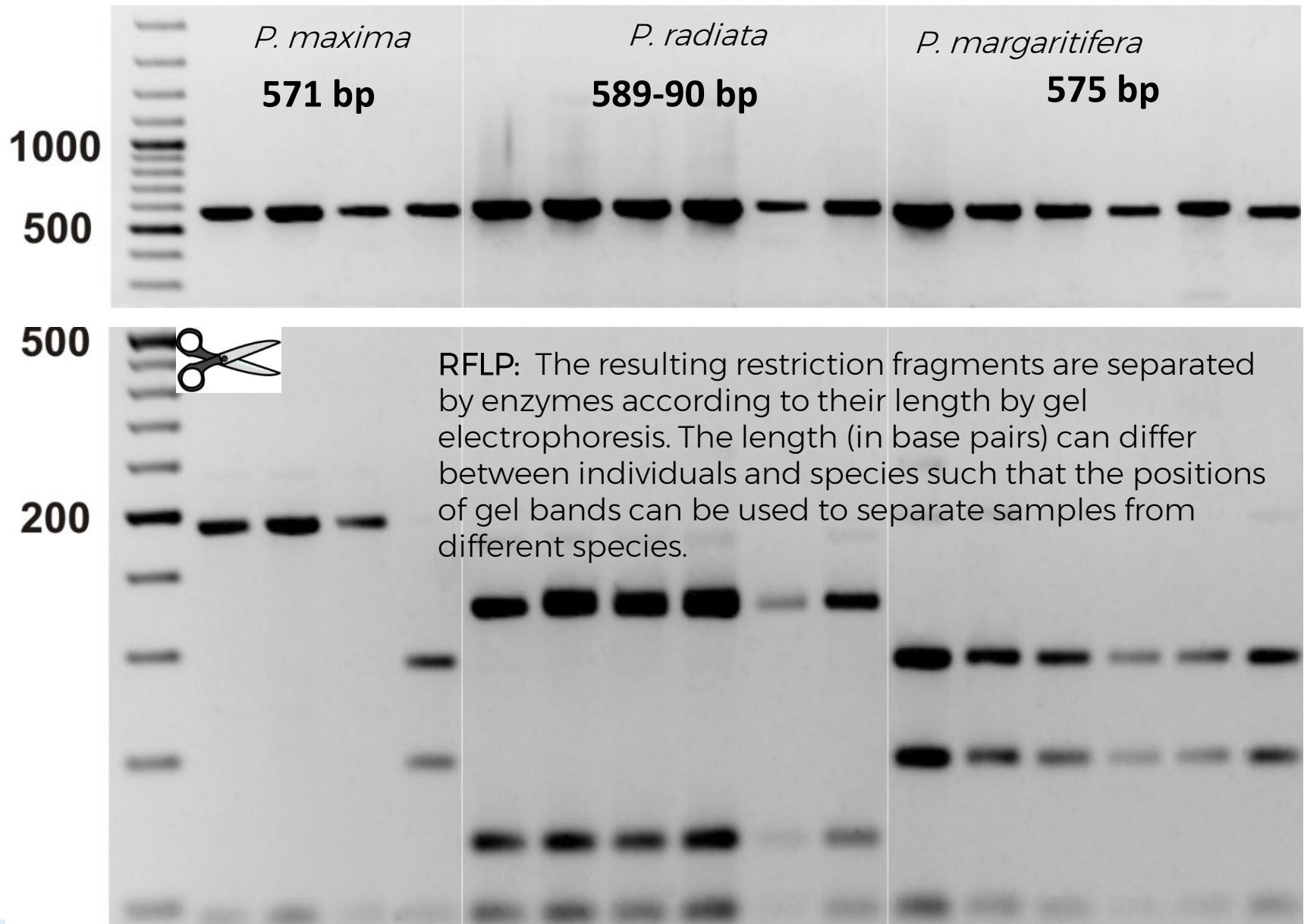


<http://blast.ncbi.nlm.nih.gov/Blast.cgi>

TCAGCGAACTGC... = *P. radiata* ITS2

Source: National Institute of health Web site

DNA SEQUENCING: ITS2-PCR & ITS2-RFLP



| PEARLS

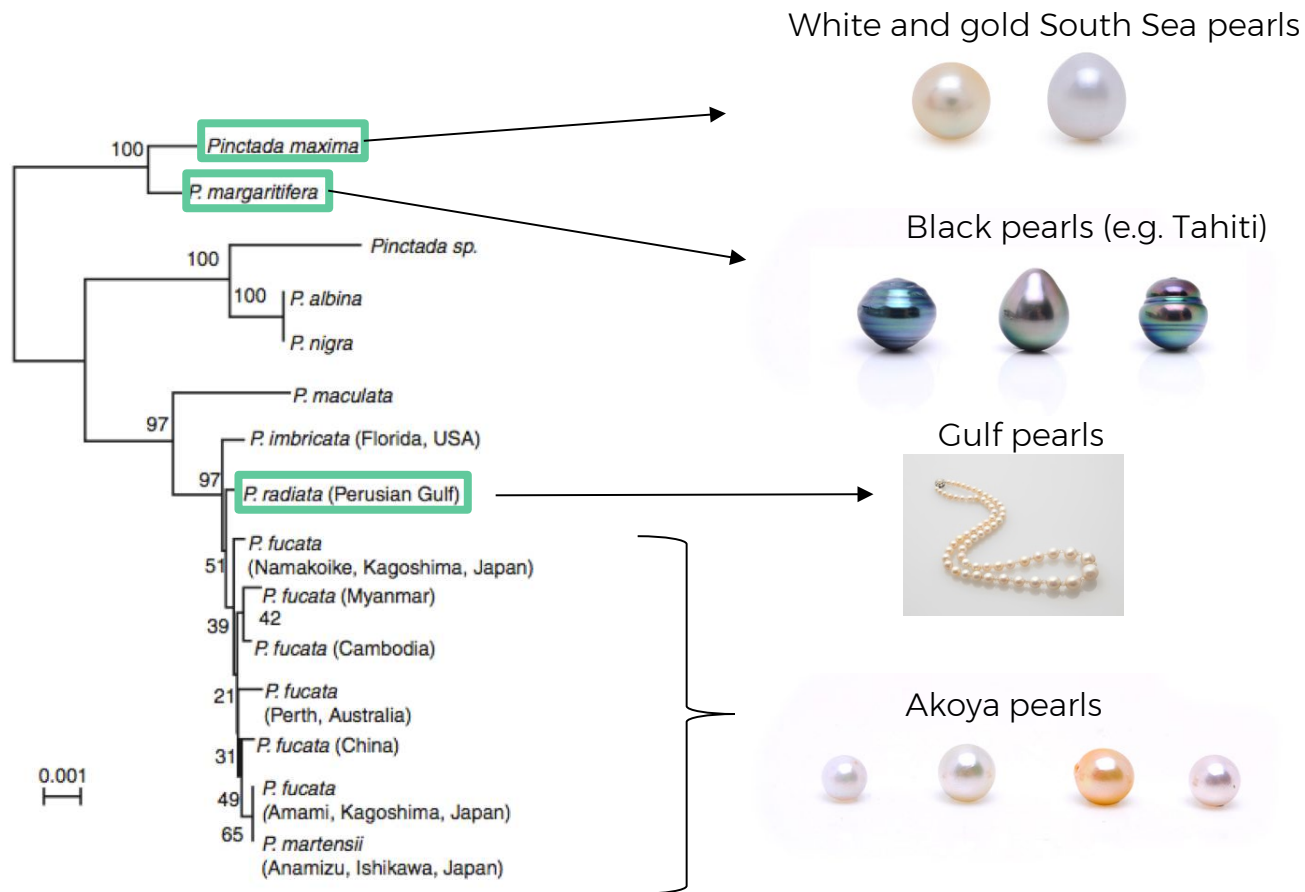
- Example of one cultured pearls that were submitted as *Pinctada maxima* (South Sea) samples and turned out to be *Pinctada margaritifera* after DNA analysis.



Black-lip pearl oyster *Pinctada margaritifera*

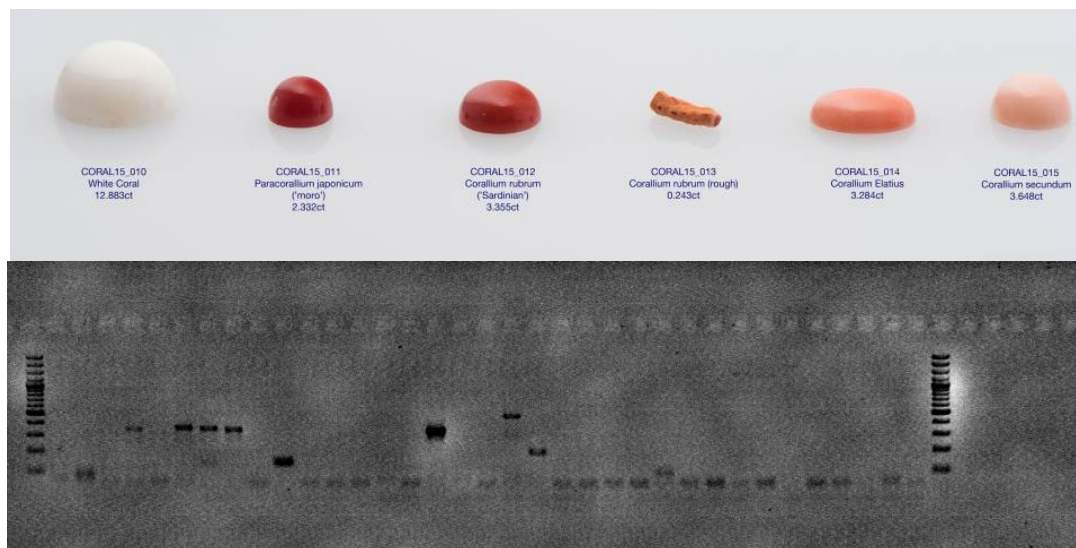
PEARLS

- 2013 study (Meyer et al. published in PlosOne) by SSEF and ETHZ showed that DNA could be extracted from pearls from the following species:



| DNA IN OTHER MATERIALS?

- If DNA can be found in pearls, can we also find it in corals?



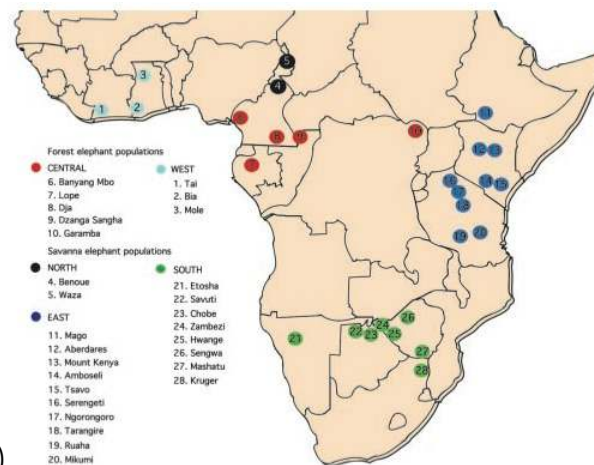
2016

2018

FEATURE ARTICLE

DNA Fingerprinting of Pearls, Corals and Ivory: A Brief Review of Applications in Gemmology

Laurent E. Cartier, Michael S. Krzemnicki, Bertalan Lendvay and Joana B. Meyer



Source: Wasser et al. (2004)

| MAIN VARIETIES OF PRECIOUS CORALS



Rubrum (Mediterranean coral)



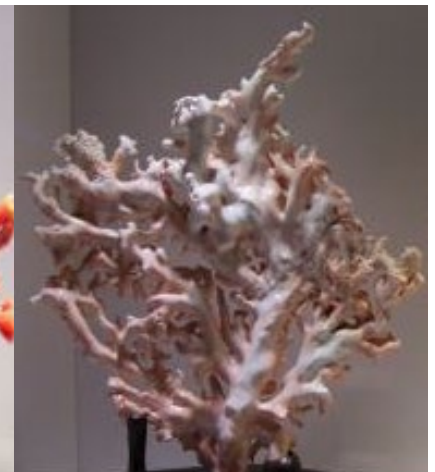
Elatius (Boké-Momo-Angel skin)



Konojoi (White coral)



Japonicum (Red coral, ox-blood)



Secundum (Midway)

| PRECIOUS CORAL REGIONS









Brought during Edo Period (1603-1868) to Japan from Italy. Until 1812 only source of precious coral worldwide

Hasso Nehanzu, 1772



| PRECIOUS CORALS

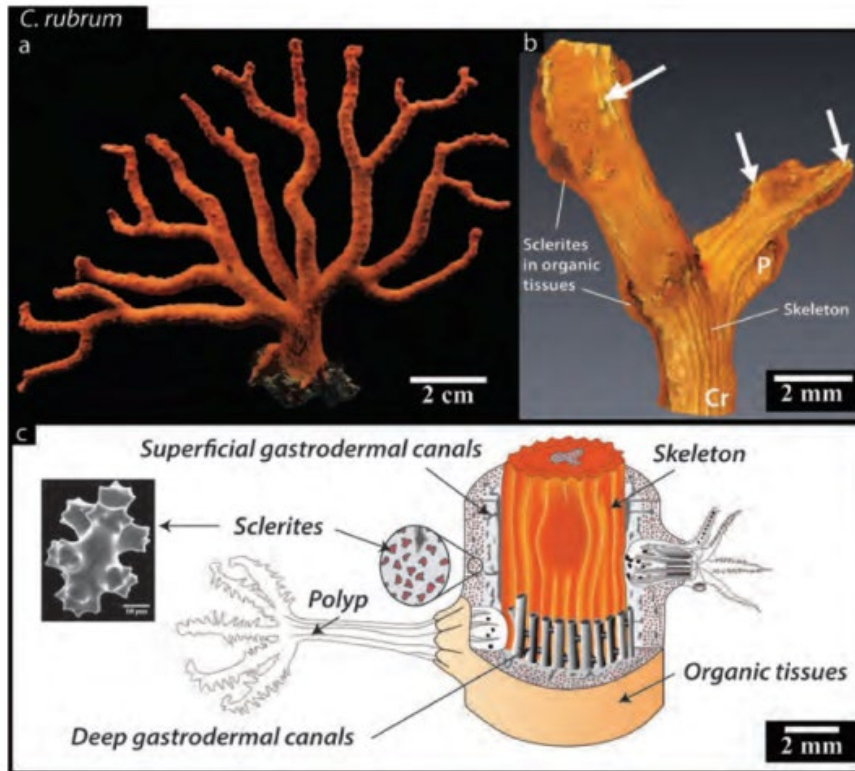
Precious corals consists of 8 main species used in the jewellery industry:

- *Corallium rubrum* (Mediterranean/ Sardinian coral) 
- *Corallium japonicum* (including oxblood coral) 
- *Pleurocorallium elatius* (including angel skin coral) 
- *Pleurocorallium konojoi* (white coral) 
- *Pleurocorallium secundum* (including Midway coral) 
- *Hemicorallium regale* 
- *Hemicorallium laauense* 
- *Hemicorallium sulcatum* 

! : CITES Appendix III listing (species included at the request of a country which then needs the cooperation of other countries to help prevent illegal exploitation).

PRECIOUS CORALS

Eugenius Johann Christoph Esper, published *Pflanzenthiere* in Nuremberg in 1791.

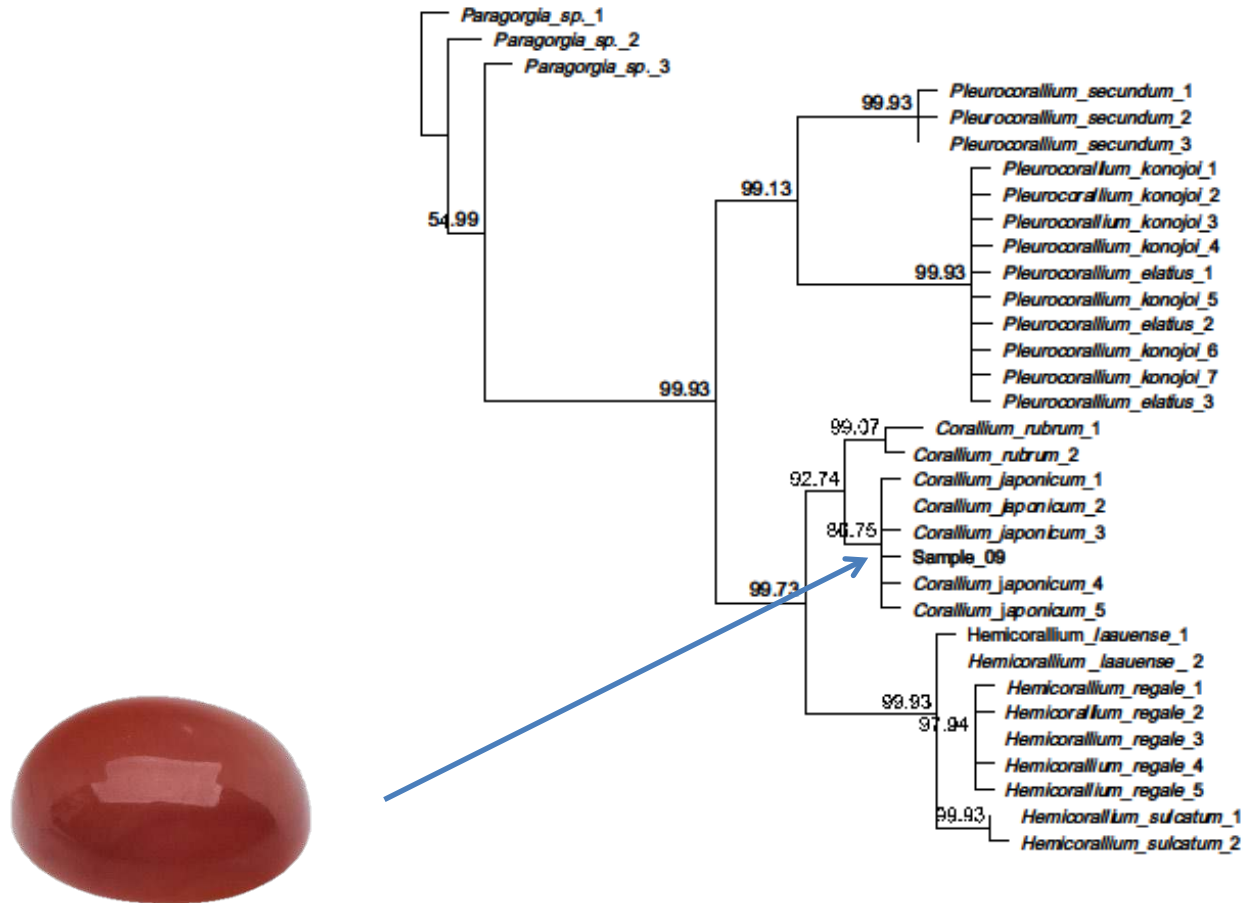


Source: Perrin et al., 2015

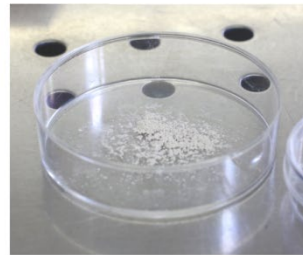
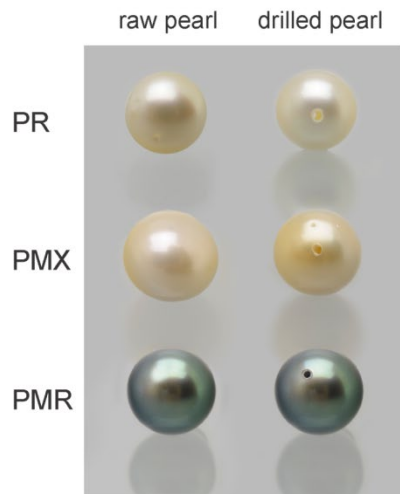


| ICORAL GENETICS

- Sample CB009. From *Corallium japonicum*



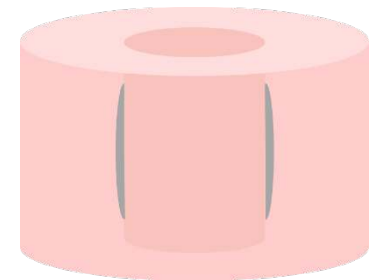
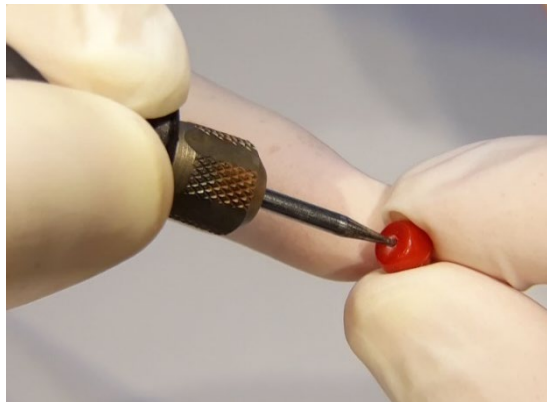
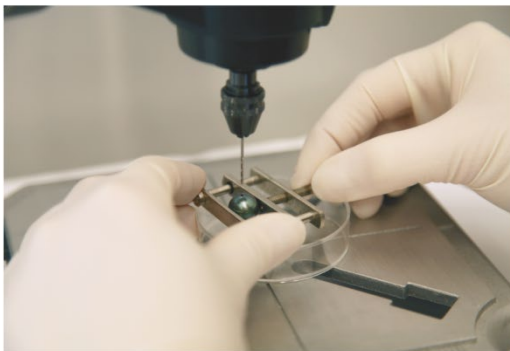
REFINING SAMPLING TECHNIQUES



- For recent coral DNA fingerprinting work we were able to extract DNA successfully with as low as 2mg sample material.



■ Coral material removed from backside of cabochon.



■ Drill hole enlarged on coral bead for sample extraction.

| IVORY

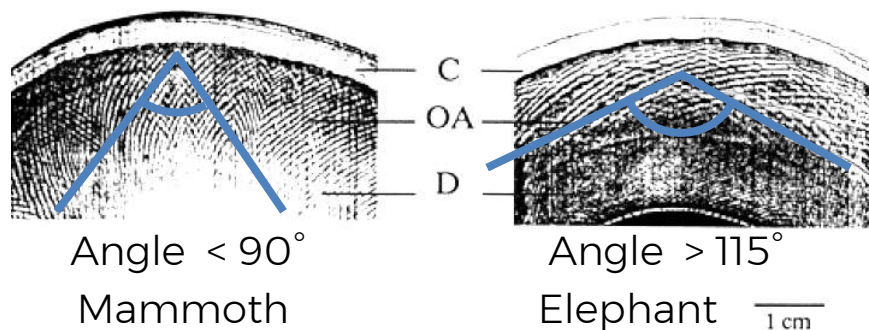
- Ivory is any mammalian tooth or tusk of commercial interest which is large enough to be carved.
- African (*Loxodonta africana*) and Asian elephants (*Elephas maximus*), along with their fossil relatives (e.g. *Mammuthus sp.*) belong to the mammalian order Proboscidea.
- How to distinguish between these species?



Photo: Evgenia Arbugaeva, NatGeo

IVORY

- If Schreger angles cannot be correctly oriented (i.e. Relative to cementum) or cannot be seen then species identification is challenging.
- DNA fingerprinting of samples is possible using cytochrome b region.



Source: Espinosa & Mann (2000)

Loxodonta africana	TATACCATTAAGGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Loxodonta africana	TATACCATTAAGGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTGTCTCCTGACATA
Loxodonta africana	TATACCATTAAGGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTGTCTCCTGACATA
Loxodonta africana	TATACCATTAAGGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Loxodonta cyclotis	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCCGACATA
Elephas maximus	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCAGACATA
Elephas maximus	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCAGACATA
Elephas maximus	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCAGACATA
Elephas maximus	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCAGACATA
Elephas maximus	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCAGACATA
Elephas maximus	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCAGACATA
Mammuthus primigenius	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Mammuthus primigenius	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Mammuthus primigenius	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Mammuthus primigenius	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Mammuthus primigenius	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA
Mammuthus primigenius	TATACCATCAAAGACTTCCTAGGATTACTTATCCTAATTTTACTTCTTCTACTCCTAGCCCTACTATCTCCTGACATA



| OUTLOOK

- Origin determination and documentation of natural & cultured pearls using sequencing, microsatellites and NGS.
- Understanding donor-host interaction in cultured pearl production, and bacterial influence.
- Species identification of ivory and corals to support CITES work and gemmological documentation. .



Photo: Andy Bardon

| THANK YOU

