IGC, TOKYO | OCTOBER, 2023

| DNA FINGERPRINTING AND AGE DATING OF HISTORIC NATURAL PEARLS: A COMBINED APPROACH

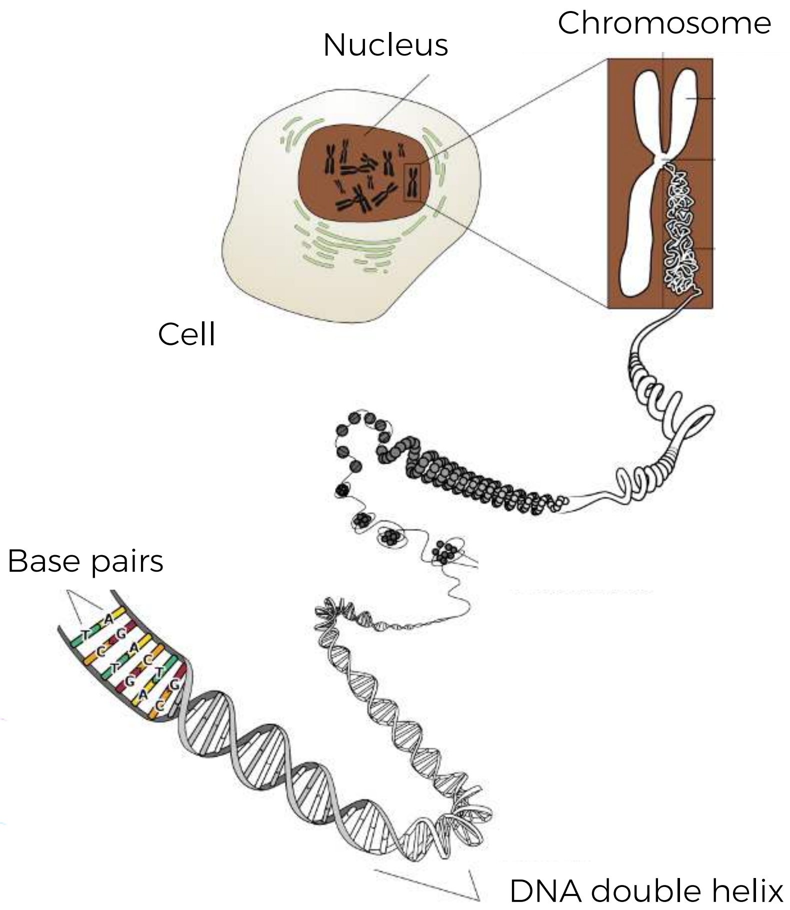
Presentation by Dr. Laurent E. Cartier FGA

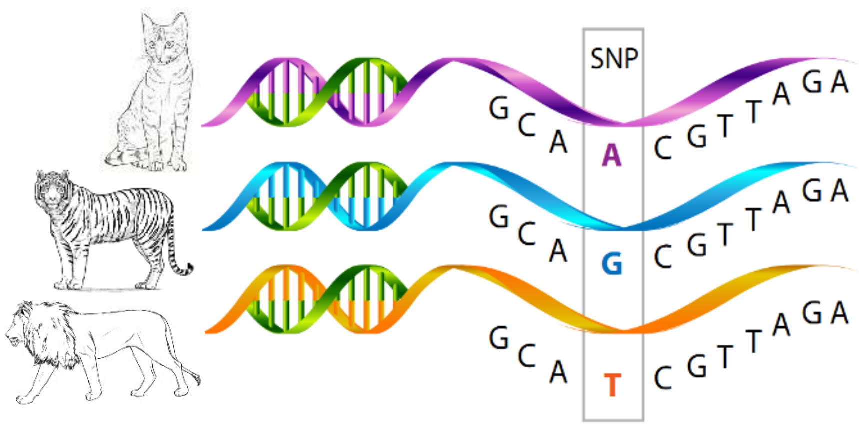
SSEF

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.





DNA FINGERPRINTING OF PEARLS

A pearl generally consists of 95% $CaCO_3$ and 5% H_2O & organic matter.

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DNA Fingerprinting of Pearls to Determine Their Origins

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Abstract

We report the first successful extraction of oyster DNA from a pearl and use it to identify the source oyster species for the three major pearl-producing oyster species *Pinctada margaritifera*, *P. maxima* and *P. radiata*. Both mitochondrial and nuclear gene fragments could be PCR-amplified and sequenced. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in the internal transcribed spacer (ITS) region was developed and used to identify 18 pearls of unknown origin. A micro-drilling technique was developed to obtain small amounts of DNA while maintaining the commercial value of the pearls. This DNA fingerprinting method could be used to document the source of historic pearls and will provide more transparency for traders and consumers within the pearl industry.

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Introduction

Pearls produced by oysters of the Pteriidae family are among the most valuable and oldest gems. Oyster shells and pearls have been used for human adornment since antiquity [1], [2], [3], [4], [5], [6]. Today pearls are cultured in domesticated saltwater oysters and freshwater mussels and have become a billion dollar industry [7]. Whereas a natural pearl forms without any human intervention in a wild oyster, a cultured pearl is the result of a human-induced injury. The value assigned to a pearl depends largely on its quality, rarity, and whether it originated naturally or through culture [8]. Thus there is significant interest in being able

are found in Australia, Burma, Indonesia and the Philippines [6], [7], [18]. Pearls from *P. margaritifera* are called black cultured pearls (or Tahitian cultured pearls) and are now produced mainly in French Polynesia, Fiji, Cook Islands and Micronesia [7], [19], [20], [21]. Akoya cultured pearls are produced mainly in China, Japan and Vietnam [6], [7]. Pearls from *P. radiata* are cultured exclusively in the Arabian/Persian Gulf. The majority of natural pearls come from *P. radiata* oysters, due to a long history of pearl fisheries in the Arabian/Persian Gulf [22]. Although they play a smaller role in the natural pearl trade, *P. maxima* and *P. margaritifera* oysters have produced many natural pearls of considerable size over the last centuries [4], [23], [24]. Natural pearls have a very

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

ABSTRACT: This article reviews the extraction of DNA (deoxyribonucleic acid) from biogenic gem materials (pearls, corals and ivory) for determining species identification and geographic/genetic origin. We describe recent developments in the methodology adapted for gem samples that is minimally destructive, as well as the successful DNA fingerprinting of cultured pearls from various *Pinctada* molluscs to identify their species. The DNA analysis methods presented here can also potentially be used for fingerprinting corals and ivory.

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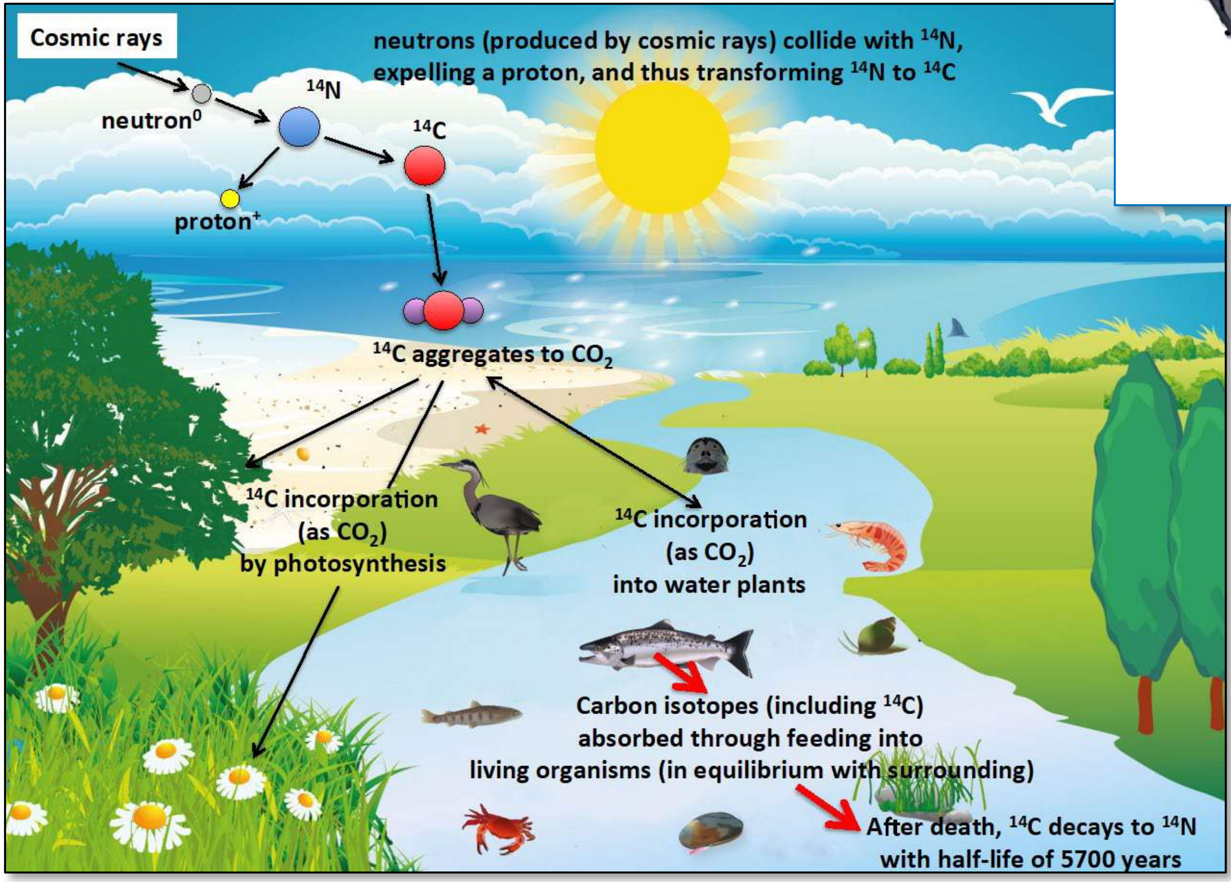
Biogenic gems—often called 'organic gems' (see Galopim de Carvalho, 2018, for a recent discussion of terminology)—are some of the oldest-used gem materials and have been cherished since pre-history (Hayward, 1990; Tsounis et al., 2010; Charpentier et al., 2012). Rather than having a geological origin, these gem materials—such as pearls, precious corals and ivory (e.g. Figure 1)—are products of biomineralisation processes in which living animals produce mineral substances (e.g. calcium carbonate or calcium phosphate) in terrestrial and marine environments (Mann, 2001). Due to their importance in jewellery

which consists of $CaCO_3$ as well as protein, glycosaminoglycans and proteoglycans (Debreuil et al., 2012). They can be coloured by carotenoids and other types of pigments.

Finally, elephant ivory from African (*Loxodonta spp.*) and Asian (*Elephas spp.*) elephant tusks is comprised of collagen and carbonate-rich hydroxyapatite (dahllite, $Ca_{10}[PO4]_6[CO_3] \cdot H_2O$; Edwards et al., 2006). Ivory can be found in a large number of animal species, of which elephant ivory is the most studied due to its value, recognition and cultural importance. In recent years, fossilised mammoth ivory has appeared more widely on the market, as elephant ivory trade restrictions have

RADIOCARBON CYCLE

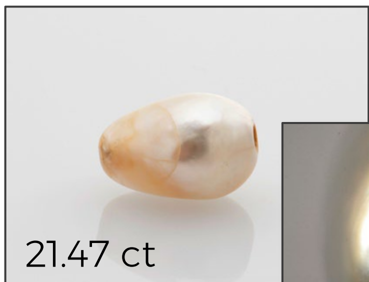


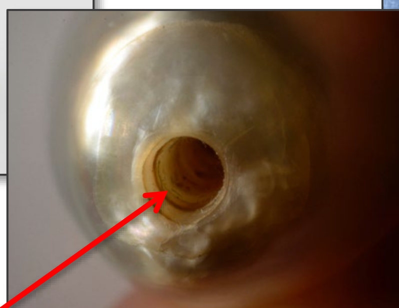
MICADAS Mini Carbon Dating System (Accelerator Mass Spectrometer).
Image ETH Zurich & ionplus AG

Figure: M.S. Krzemnicki, SSEF
Using background illustration
From www.fisheriesireland.ie

SAMPLING OF PEARLS

Sampling for age dating and DNA is carried out using similar method. Lowest sample weight used to data has been 2mg (0.001 carat, 0.004 grains).





A minute amount (less than 0.004 g) of calcium carbonate is taken from inside the drill-hole.

„quasi“ non-destructive testing even for objects of archaeological and cultural heritage.

0.02 ct powder of calcium carbonate for age dating.

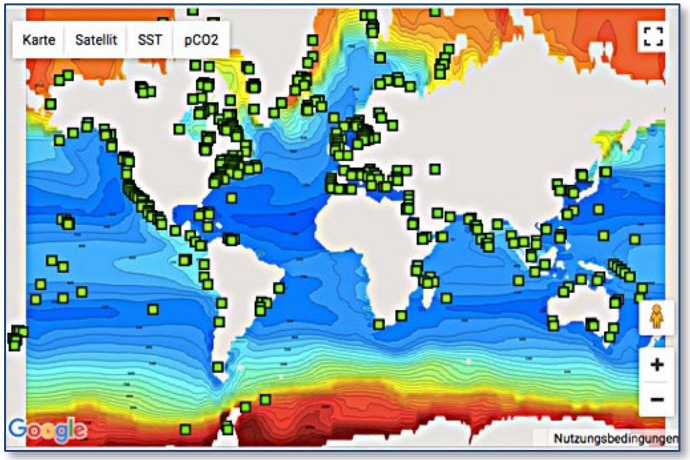
AGE DATING CHALLENGES

Water reservoir age (old age effect)

Analysed raw data has to be corrected for reservoir age. This means that the geographic location of the pearl should be known (at least approximately).

Contamination with geological calcium carbonate and diagenesis

Difference between suspension feeder (e.g. Pinctada molluscs) and
Soil grinding feeder (e.g. haliotis or other gastropods).
Difference between material from excavations and “fresh” shells/pearls in jewellery



Source:
http://radiocarbon.ldeo.columbia.edu/research/resage/res_ff.cgi

ONE OF WORLD'S OLDEST NATURAL PEARLS

This natural pearl was recovered (via shells found in the site) from Umm Al Quwain in U.A.E and was dated back to 5550 B.C.

Source: Charpentier et al., 2012

2000 YEAR OLD PEARL FROM AUSTRALIA

SHORT REPORTS

The Brremangurey pearl: A 2000 year old archaeological find from the coastal Kimberley, Western Australia

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Abstract

A small marine pearl was recovered at the Brremangurey rockshelter, on the Kimberley coast, from layers dating to approximately 2000 years ago. In an area famous for its pearls and history of cultured pearl production, public interest centred on whether the pearl was as old as the layer in which it was contained, or whether it was a recent cultured pearl that had infiltrated down from above. The near-spherical shape of the pearl hinted at a possible cultured origin. Owing to the uniqueness and historic cultural significance of this find, non-invasive analytical techniques were used to investigate whether the Brremangurey pearl was cultured or natural. Midden analysis was further used to assess the likely origin of the pearl within the stratified deposits. Analysis confirmed that the pearl is of natural origin and a dense midden lens of Pinctada albina *shells is its likely origin.*

Introduction

During excavations in 2011 at Brremangurey, a north Kimberley coastal rockshelter, a small nacreous marine pearl was recovered from within the site's shell midden. Although there is no record of pearls being of cultural importance to Australia's Indigenous peoples, the pearl generated much excitement and many questions from Kimberley locals, both around the site and further afield. Given the pearling heritage of the Kimberley, many of these questions related to the age and origin of the pearl. Although recovered from a layer which was radiocarbon dated to 1800–1906 cal. BP, local pearl experts raised the possibility that it could be an intrusive cultured pearl, based on its size, colour and spherical shape. We acknowledge that the pearl is most likely an incidental find in archaeological terms, but the public interest in its history, age and origin compelled us to develop tools to address these questions. As a unique object of historical value to many, a programme of non-invasive analyses was developed; we hope some of the techniques presented here will provide a constructive pathway to others working in these fields.

Background

Brremangurey is a quartzite rockshelter located 70 m inland from the current shoreline on the north Kimberley coast (Figure 1). The site deposits span periods of the late Pleistocene and Holocene, with a dense mid- to late Holocene shell midden dominating the upper portion of the sequence; the pearl was recovered whilst screening these midden deposits. Despite having the appearance of a cultured pearl, it was recovered from a depth of 70–77 cm below datum (Square K26, Spit 14). Marine shell from this level was AMS radiocarbon dated to 1800–1906 cal. BP (Table 1). A detailed excavation report is currently being prepared for publication, as are papers on the shell midden analysis.

Measuring 5.9 mm in maximum diameter and weighing 0.25 g (Figure 2), the Brremangurey pearl is the only pearl to have been recovered from a prehistoric archaeological site in Australia and one of only a small number found in archaeological contexts globally (e.g. Charpentier et al. 2012; Koerper and Desautels-Wiley 2007 from the Arabian Gulf and southern California, respectively). The Kimberley coast is a well-known centre for the production of South Sea pearls, farmed from the large pearl oyster species *Pinctada*

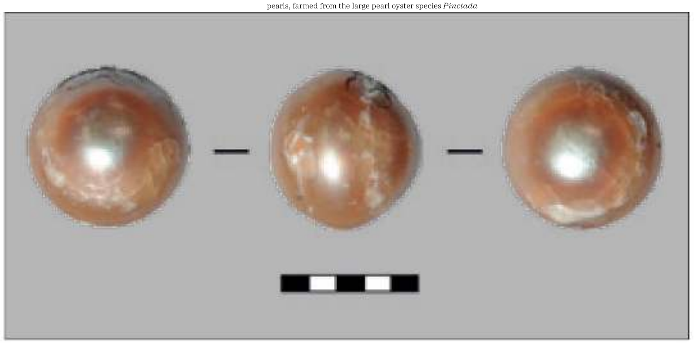


Figure 2 The Brremangurey pearl. Scale bar is in millimetres.

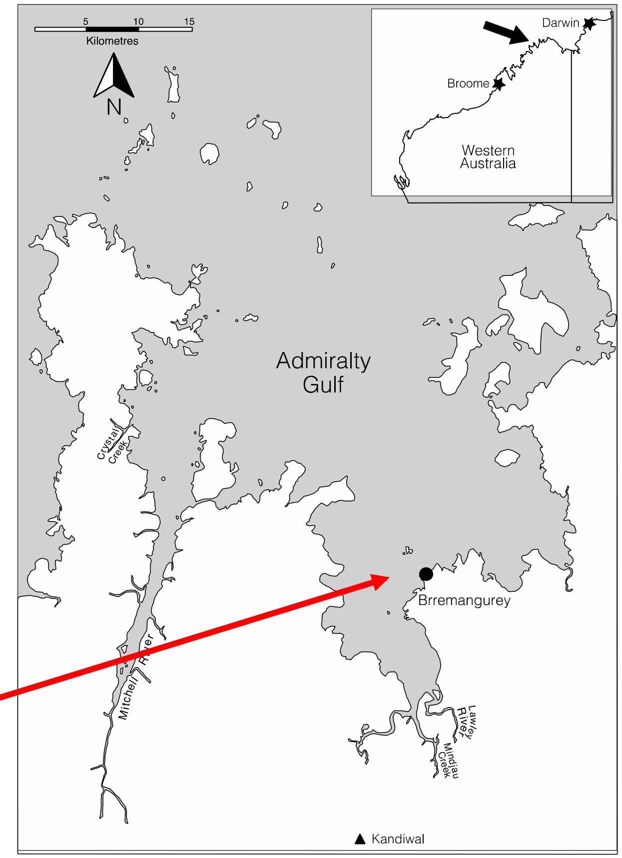


Figure 1 The location of the Brremangurey site on the shore of the Admiralty Gulf, northern Western Australia.

Source: Szabo et al. 2015

8500 YEAR OLD PEARLS FROM MEXICO

AMS radiocarbon dating of two modified pearls from the Covacha Babisuri site, Espíritu Santo Island, Baja California Sur, México, corroborates that traditional indigenous use and modification of pearls as items of adornment began at least 8,500 years ago. These are the oldest published modified pearls found in dated archaeological contexts anywhere in the world.

Source: Ainis et al. (2019)

THE QUEEN MARY PEARL

The 'Queen Mary Pearl' was once owned by Queen Mary (1867- 1953).

A drop-shaped natural pearl of 41.5 ct (166 grains) of finest quality and lustre, approximately 16.45 - 17.65 x 21.80 mm

For comparison, the Peregrina pearl: weighs approximately 202.24 grains or 50.56 carats, measuring approximately 17.35 - 17.90 x 25.50 mm

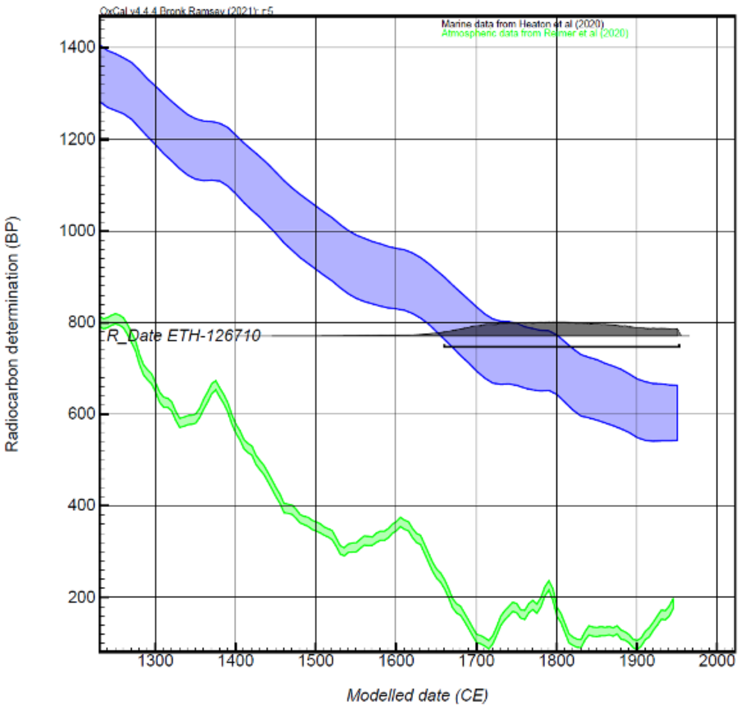
Queen Mary Pearl

La Peregrina

THE QUEEN MARY PEARL

THE QUEEN MARY PEARL

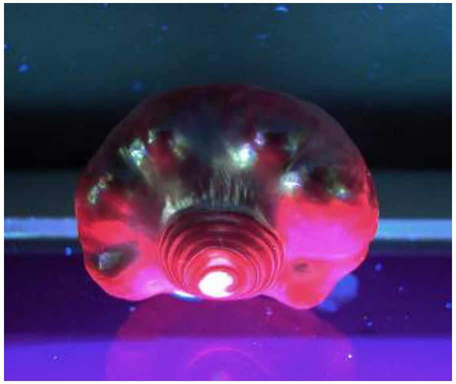
The purple band is the marine calibration curve (Heaton et al., 2020), and the green band is the atmospheric curve (Reimer et al., 2020).



GEOGRAPHIC ORIGIN OF PEARLS:

Historic pearl necklace from *Pteria sterna* analysed by DNA fingerprinting

Map showing habitat of *Pteria sterna* (name rainbow-lipped pearl oyster or Pacific wing oyster



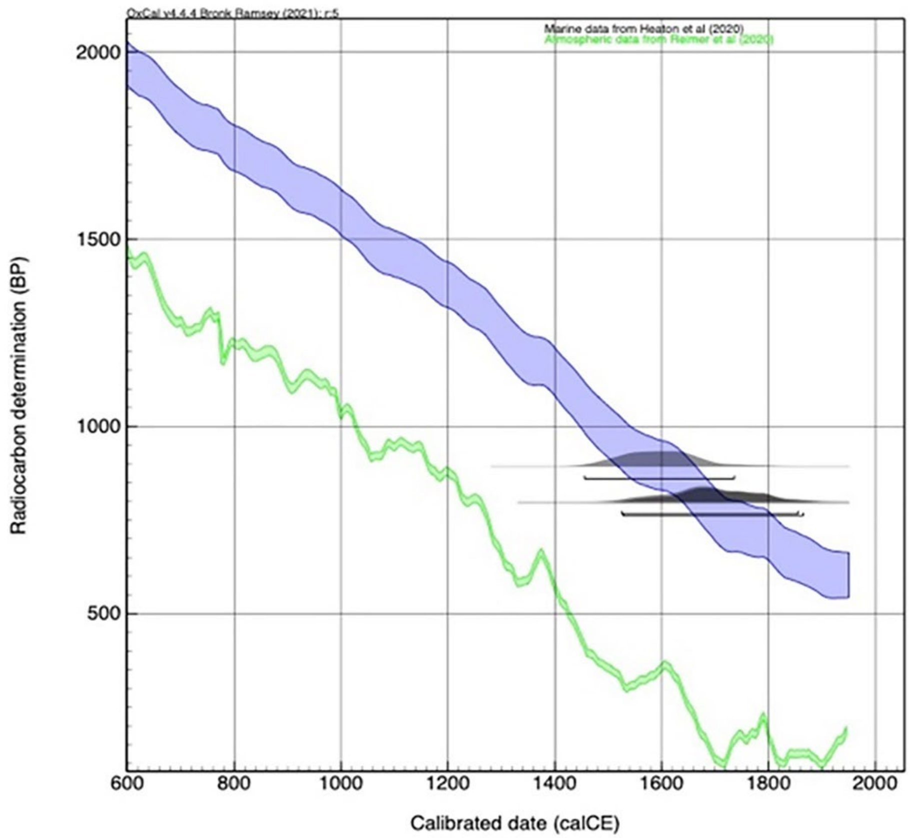
Characteristic reddish reaction under long-wave ultraviolet light.

HISTORIC NATURAL PEARL SET

This pearl jewellery set consisted of 63 natural pearls, with 61 of them being strung on a thread and two additional loose natural pearls. Three pearls were freshwater natural pearls, and it contained pearls from different species, including one not previously known to produce natural pearls (*Pinctada persica*).

HISTORIC NATURAL PEARL SET

As is often case with radiocarbon dating, the determined age indicates a period in history rather than a precise date. Based on our data, they probably formed between the 16th and 18th century AD with the highest probability of formation having been in the 17th century.



DISCOVERY OF NEW SPECIES

Pinctada persica was previously known as *Pinctada margaritifera var. persica* (Jameson, 1901; Lal et al., 2017), but recently has been recognized as a new species (Ranjbar et al., 2016).

Unlike its closely related species (*P. margaritifera*), *P. persica* has a distinct phenotypic color morph of mantle tissue, so that the orange mantle color of *P. persica* is the dominant morphotype compared to the black one.

Souce: Parvizi et al. (2017)

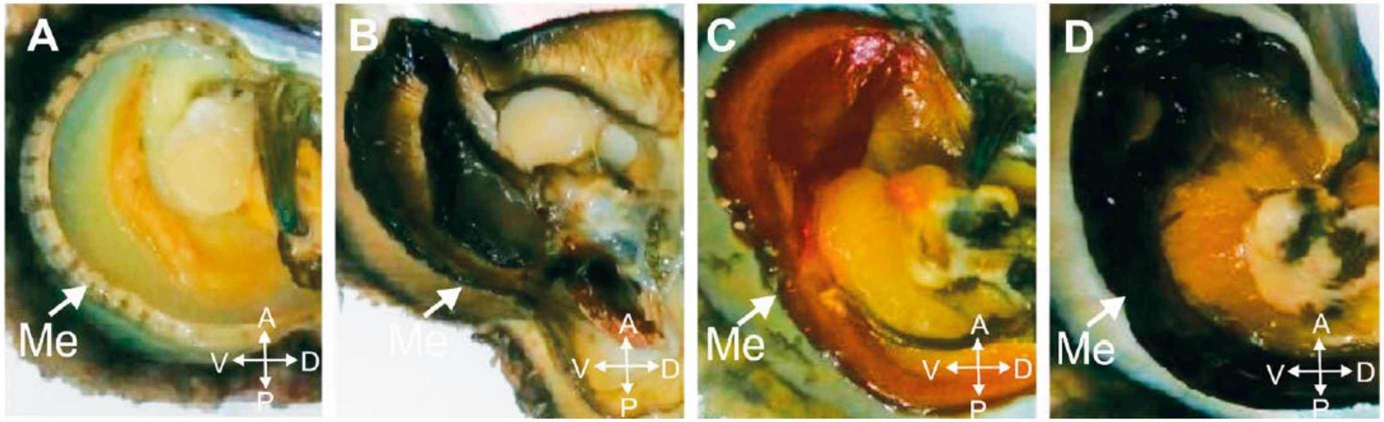
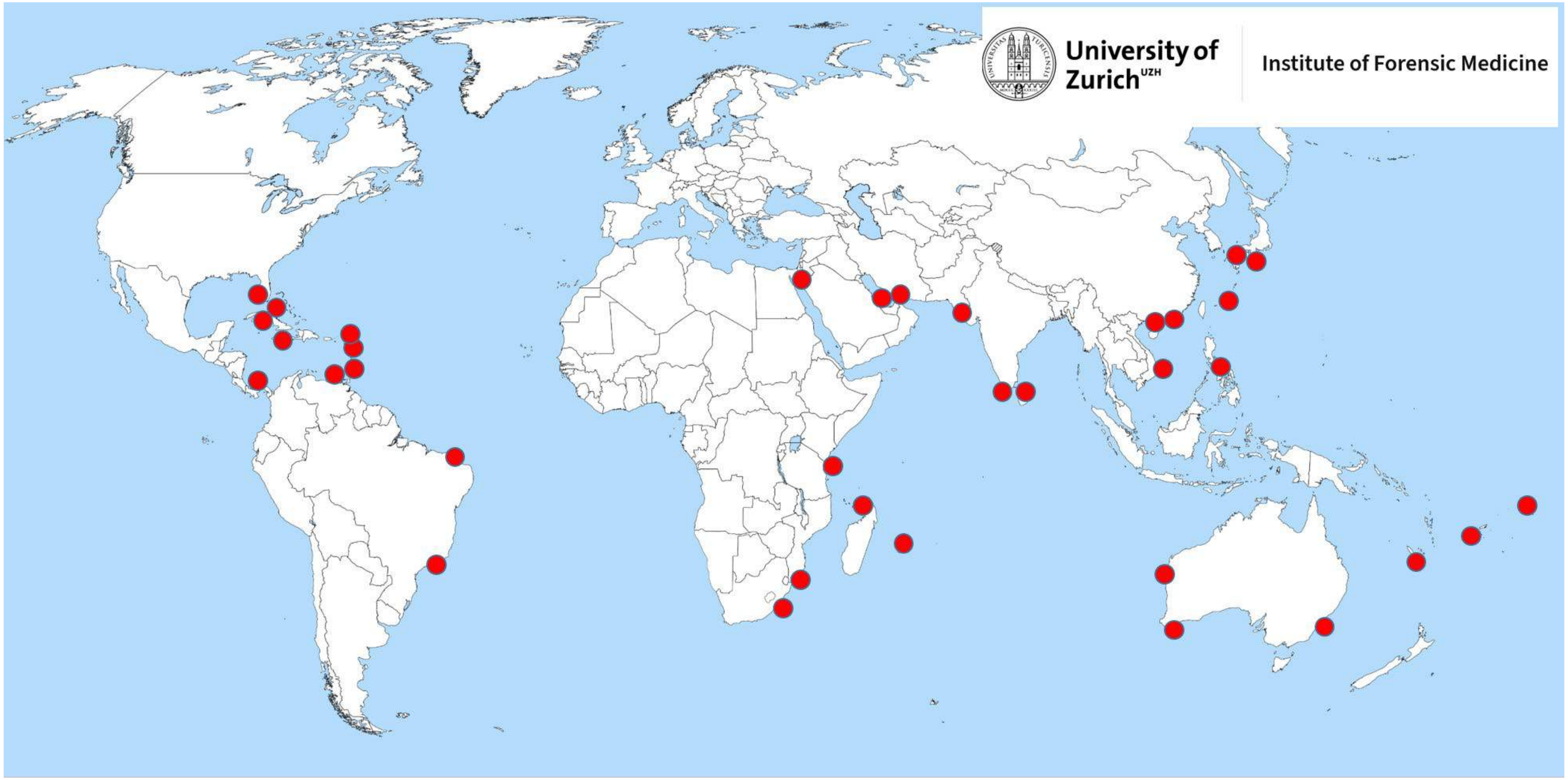


Figure 2. Mantle edge of the three study species. **A,** *Pinctada radiata*; **B,** *Pteria penguin*; **C–D,** *Pinctada persica*. Me—mantle edge; A—anterior; P—posterior; V—ventral; D—dorsal.

AKOYA COMPLEX SPECIES

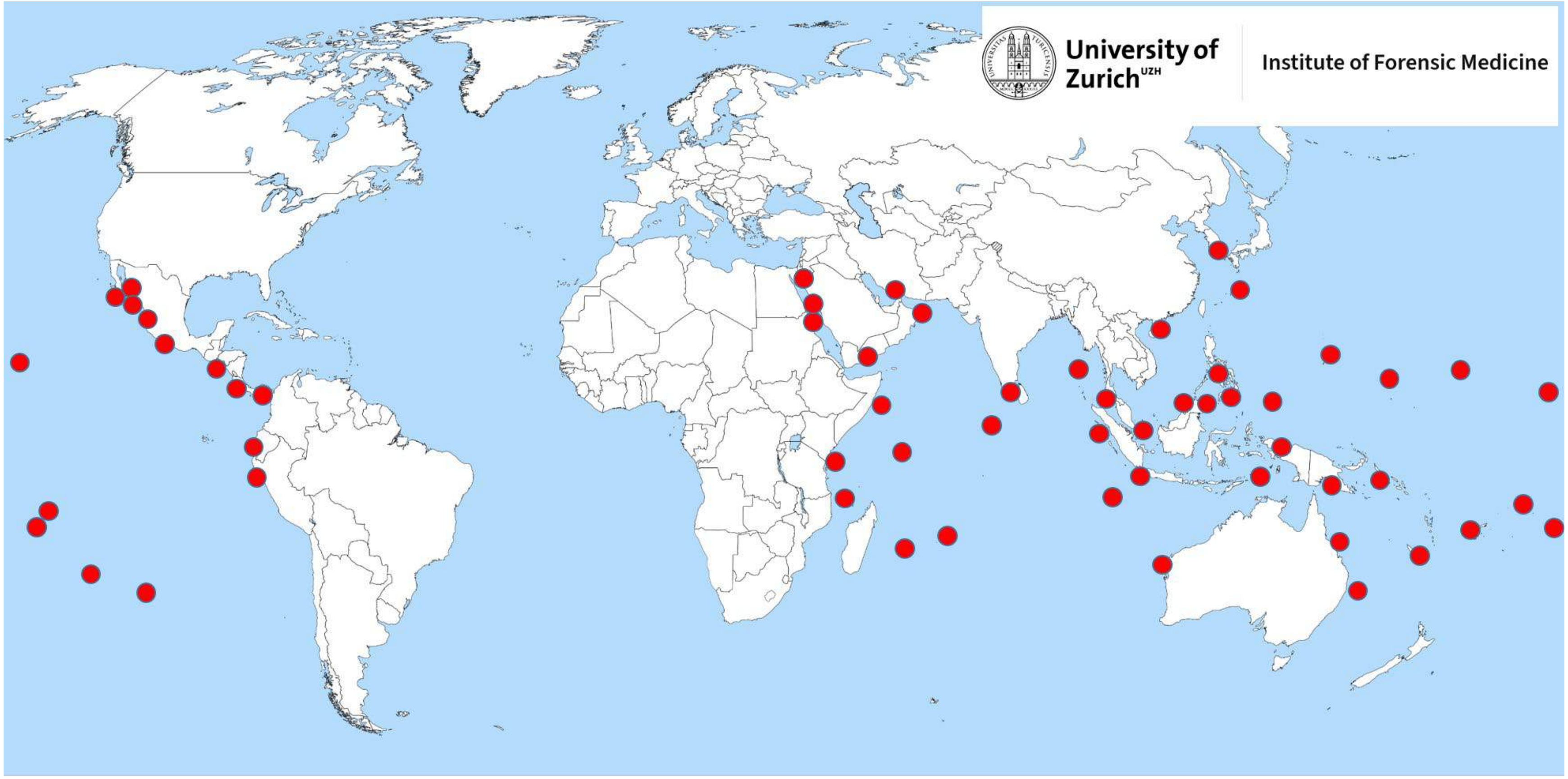
Akoya complex includes *Pinctada fucata-imbricata-martensii-radiata* species.



Akoya complex (*Pinctada imbricata / radiata / fucata*)

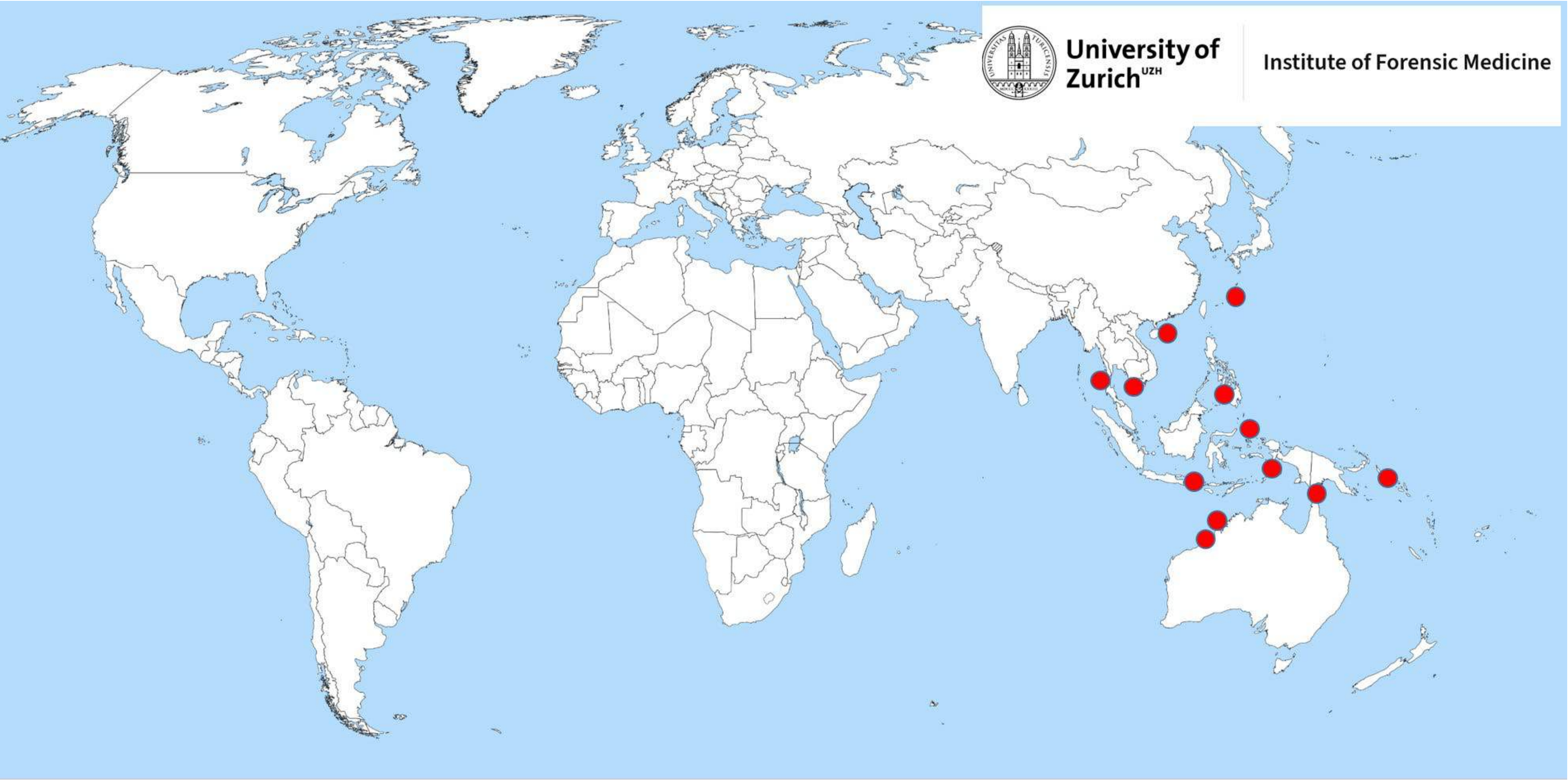
PINCTADA MARGARITIFERA COMPLEX

Pinctada margaritifera complex includes: var. *cumingii*, var. *typica*, var. *zanzibariensis*, var. *galtsoffi*.



Pinctada margaritifera **species complex**

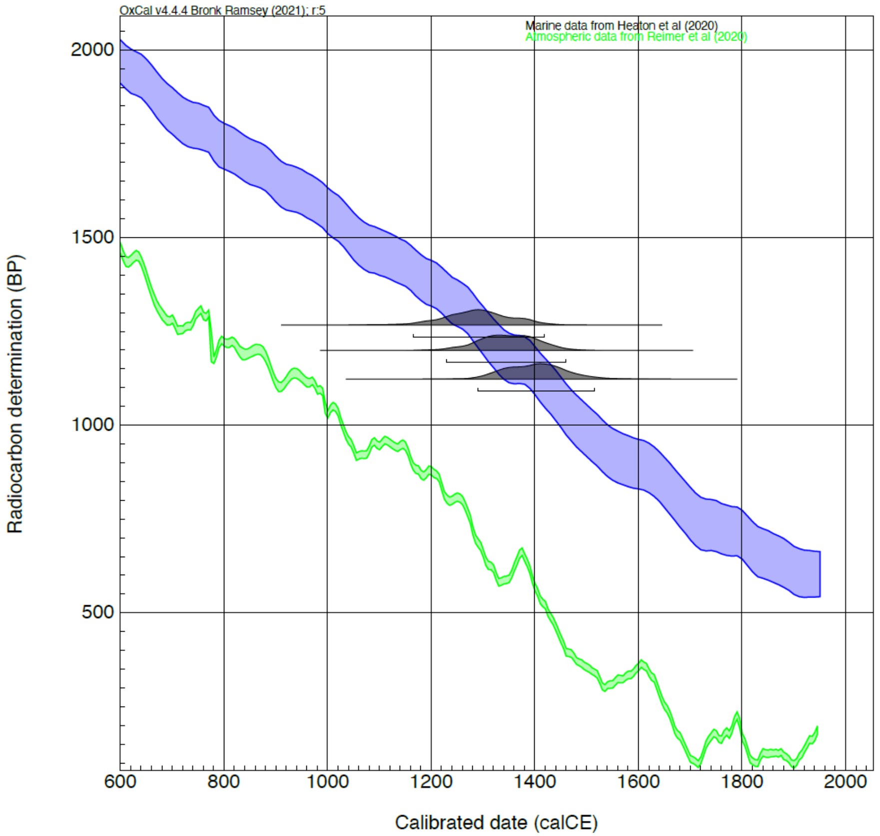
PINCTADA MAXIMA REFERENCES



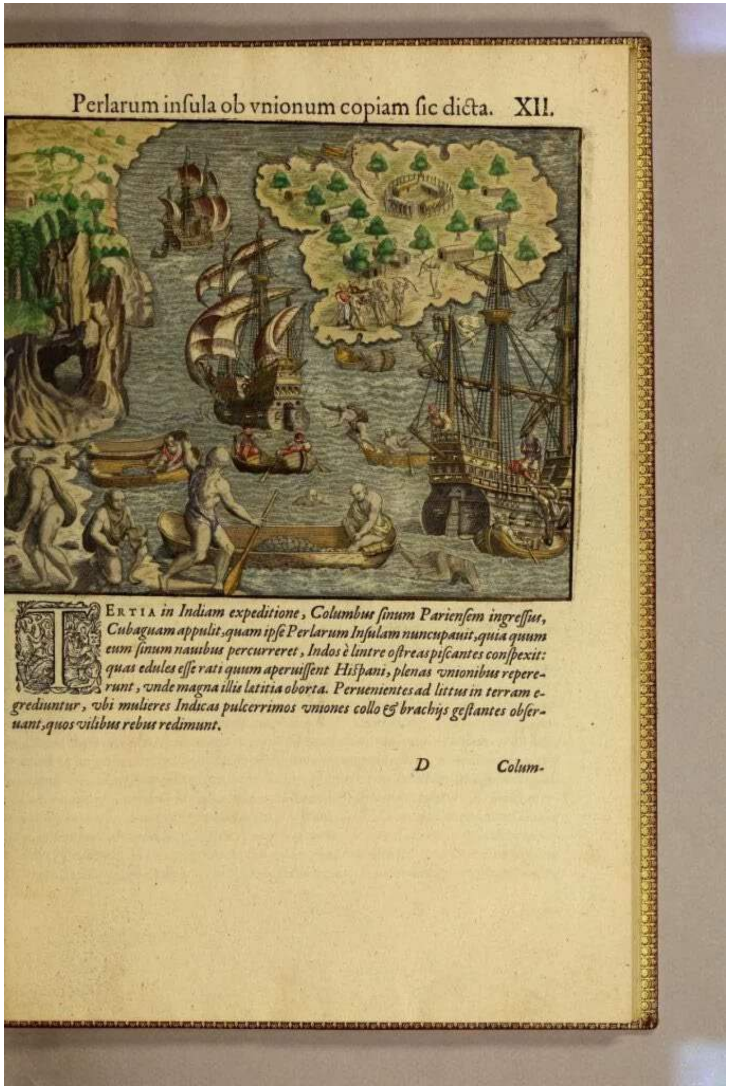
Pinctada maxima

DNA AND AGE?

Mexico had a plentiful supply of mother-of-pearl from oyster-beds off the coast of Baja California, as Hernán Cortés and the so-called conquistadores discovered (1522)when they encountered Pericú Indians wearing necklaces strung with red berries, shells and blackened pearls.



HISTORIC PEARLS



Perlarum inſula ob vnionum copiam ſic dicta. XII.

TERTIA in Indiam expeditione, Columbus ſinum Parienſem ingreſſus, Cubaguam appulit, quam ipſe Perlarum Inſulam nuncupauit, quia quum eum ſinum nauibus percurreret, Indos è lintre oſtreas piſcantes conſpexit: quas edules eſſe rati quum aperuiſſent Hiſpani, plenas vnionibus repererunt, vnde magna illis lætitia oborta. Peruenientes ad littus in terram egrediuntur, vbi mulieres Indicas pulcerrimos vniones collo & brachijs geſtantes obſeruant, quos vilibus rebus redimunt.

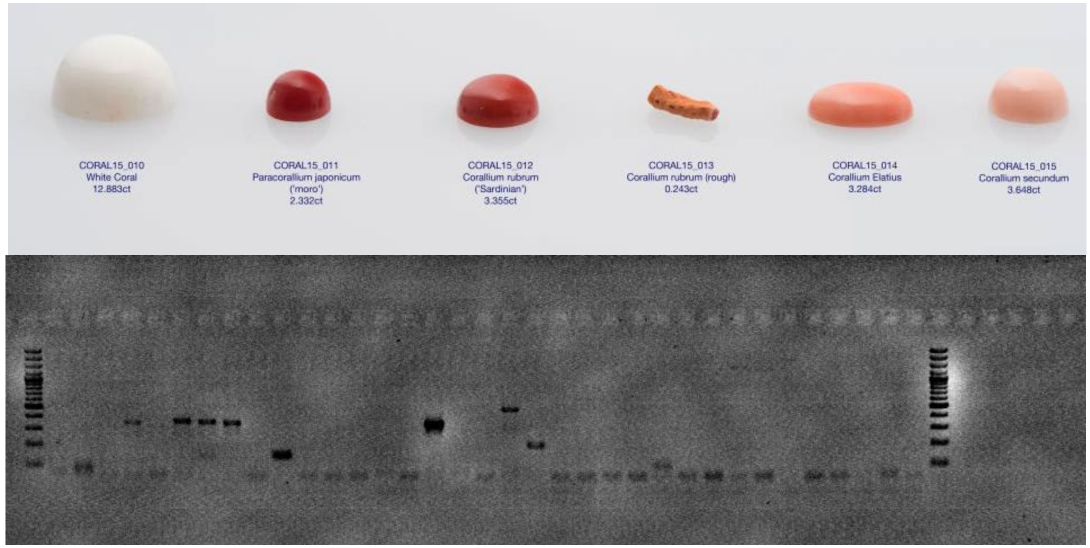
D Colum-

From Girolamo Benzoni's Americae pars quarta (Frankfurt am Main: Ioannis Feyrabend, 1594)

Jacopo Zucchi, The Coral Fishers, 1585. Oil on copper, 55 x 45cm. Galleria Borghese, Rome.

DNA IN PRECIOUS CORALS

Developments in precious coral DNA fingerprinting



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

2020

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DNA fingerprinting: an effective tool for taxonomic identification of precious corals in jewelry

Bertalan Lendvay[1,2] ✉, Laurent E. Cartier[2,3], Mario Gysi[1], Joana B. Meyer[4], Michael S. Krzemnicki[2], Adelgunde Kratzer[1] & Nadja V. Morf[1]

Precious coral species have been used to produce jewelry and ornaments since antiquity. Due to the high value and demand for corals, some coral beds have been heavily fished over past centuries. Fishing and international trade regulations were put in place to regulate fishing practices in recent decades. To this date, the control of precious coral exploitation and enforcement of trade rules have been somewhat impaired by the fact that different species of worked coral samples can be extremely difficult to distinguish, even for trained experts. Here, we developed methods to use DNA recovered from precious coral samples worked for jewelry to identify their species. We evaluated purity and quantity of DNA extracted using five different techniques. Then, a minimally invasive sampling protocol was tested, which allowed genetic analysis without compromising the value of the worked coral objects.The best performing DNA extraction technique applies decalcification of the skeletal material with EDTA in the presence of laurylsarcosyl and proteinase, and purification of the DNA with a commercial silica membrane. This method yielded pure DNA in all cases using 100 mg coral material and in over half of the cases when using "quasi non-destructive" sampling with sampled material amounts as low as 2.3 mg. Sequence data of the recovered DNA gave an indication that the range of precious coral species present in the trade is broader than previously anticipated.

2022

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Coral-ID: A forensically validated genetic test to identify precious coral material and its application to objects seized from illegal traffic

Bertalan Lendvay[a,b,*], Laurent E. Cartier[b], Federica Costantini[c], Nozomu Iwasaki[d], Meredith V. Everett[e], Michael S. Krzemnicki[b], Adelgunde Kratzer[a], Nadja V. Morf[a]

PRECIOUS CORALS

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

- *Corallium rubrum* (Mediterranean 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).

DISCOVERY OF NEW SPECIES

Discovery of a 'new' species (*Pleurocorallium niveum*, from the Hawaiian archipelago) that has never before been reported in the jewellery industry, but was identified in several submitted coral cabochons tested in different studies. Results of our ongoing research show that *Pleurocorallium niveum* (non-CITES-listed) corals were often mistakenly identified as *Pleurocorallium secundum* (CITES-listed).

Left: *Pleurocorallium niveum* from Hawaii at the Smithsonian Institution's National Museum of Natural History (Washington D.C.).

Right: A precious coral cabochon submitted as that was submitted as Pleurocorallium secundum but was identified as *Pleurocorallium niveum* using DNA fingerprinting. Photos: Bertalan Lendvay and SSEF.

PRECIOUS CORAL DNA

The six taxonomic groups distinguishable by the Coral-ID assay. Note that species in the species complexes cannot be differentiated based on mitochondrial markers. From Lendvay et al. (2022). For precious coral taxonomy, see Tu et al. (2015).

Taxonomic group	Species within group	CITES-listed	Primary color of skeletal axis	Distribution area
Corallium rubrum	*C. rubrum* *	No	uniform red to deep orange	Mediterranean Sea, North-East Atlantic [5]
***Corallium japonicum* species complex**	*C. japonicum* *	Yes	dark red with white center [29]	Japan, Taiwan [30]
	C. nix	No	dark red or pink, white center, white tip [31], [32]	New Caledonia [31]
	C. tortuosum	No	pale pink [33], white-transparent [26]	Hawaiian Islands [33], New Caledonia [26], Taiwan [26]
Hemicorallium	*H. abyssale*	No	pale pink, darker	Hawaiian Islands [33]
	H. aurantiacum	No	center [33]	New Caledonia [26]
	H. bathyrubrum	No	pale pinkish –	North-West Atlantic [34]
	H. bayeri	No	orange [26]	North-West Atlantic [34]
	H. ducale	No	deep pink to red [34]	East-Pacific [35]
	H. guttatum	No	white [34]	Hawaiian Islands [26]
	H. imperiale	No	dark pink [35]	East-Pacific [35]
	H. laauense *	No	milk white [26]	Hawaiian Islands [33],
	H. niobe	No	rich pink [35]	Emperor Seamount [4]
	H. regale *	No	white [33]	Western Atlantic [36]
	H. sulcatum *	No	white [36]	Hawaiian Islands [33]
			pale pink [33]	Taiwan, Japan [5],
			pink [26]	Philippines [4]
***Pleurocorallium elatius* species complex**	*P. elatius* *	Yes	red to pink with white center [29], orange [37]	Taiwan, Japan, Vietnam [5]
	P. konojoi *	Yes	milky white, pinkish center [37]	Japan, Taiwan, Vietnam [5]
	P. carusrubrum *	No	crimson, orange [26], [37]	Taiwan [37]
Pleurocorallium secundum	*P. secundum* *	Yes	pale pink, often almost white [33]	Hawaiian Islands [33], Taiwan [38]
other *Pleurocorallium*	*P.*	No	pure white -	New Caledonia [26]
	bonsaiarborum	No	transparent [26]	Malaysia [39]
	P. borneense	No	white with pink	New Caledonia [26]
	P. clavatum	No	center [39]	Japan [29]
	P. inutile	No	white [26]	Hawaiian Islands [29]
	P. porcellanum	No	white [29]	Hawaiian Islands [33]
	P. niveum *	No	white [29]	New Caledonia [26]
	P. norfolkicum	No	white [33]	New Caledonia [31]
	P. thrinax		white [26]	
			white [31]	

THANK YOU

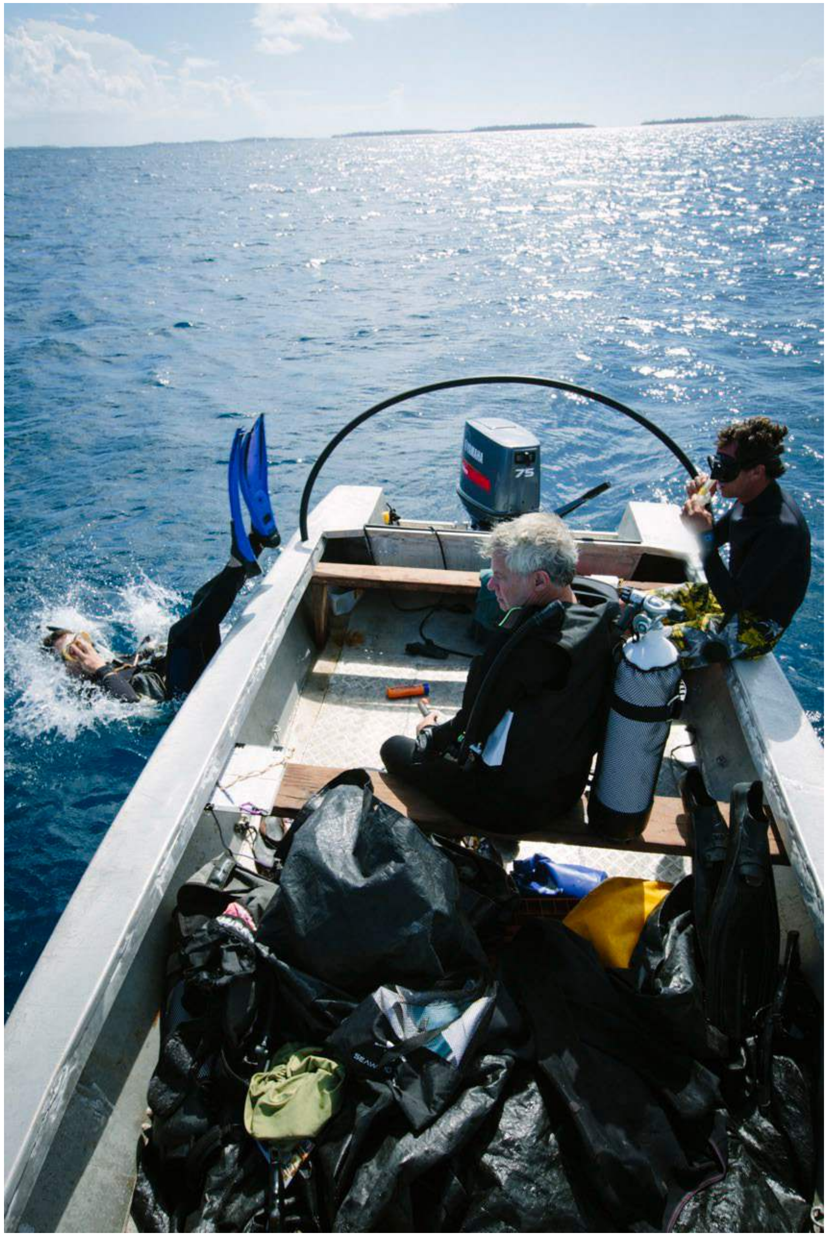

Photo: Andy Bardon