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

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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 and decorative arts, the study of biogenic gem materials constitutes an important part of gemmological research.

Natural pearls form in wild molluscs without any assistance, whereas cultured pearls are the result of human intervention on cultivated pearl-producing molluscs (Strack, 2006; Hänni, 2012). Pearls and their shells consist of secretions of different polymorphs of calcium carbonate ($CaCO_3$) such as aragonite, calcite and vaterite. Pearls are sometimes coloured by organic pigments.

Precious corals have not been cultivated commercially so far, and those used in jewellery and *objets d'art* represent the coral skeleton (secreted by living polyps), 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 taken force (e.g. under the Convention on International Trade in Endangered Species of Wild Fauna and Flora, or CITES; www.cites.org/eng/niaps). CITES regulates the trade in biogenic gem materials that are produced by species included in its Appendices I, II or III. Among these are various species of precious coral, queen conch (pearls) and giant clam (pearls).

The ability to trace biogenic gem materials back to their species-related and geographic origins can provide greater transparency and help curb trade in illegal materials (and thus restrict poaching and smuggling). Furthermore, such research can yield important information

on the sources and trade routes of biogenic gems in historic items. The aim of this article is to provide an overview of DNA fingerprinting techniques to a gemmological audience, with particular emphasis on a previous detailed publication by some of the authors (Meyer et al., 2013) that focused on technical aspects concerning pearl genetics for species identification.

GEMMOLOGICAL RESEARCH

Traditional gemmological testing of pearls, corals and ivory in past decades was carried out mainly to separate these biogenic gem materials from imitations and, in the case of pearls, to separate natural from cultured (i.e. since the appearance of the latter on the market in the early 20th century: Anderson, 1932; Farn, 1986). This testing mostly has been visual, focusing both on the surface of examined materials and their internal structures. In addition, pigment analysis to help detect pearl treatments and gather more information for the possible determination of pearl species has also been carried out (Li and Chen, 2001; Elen, 2002; Karampelas et al., 2011). In recent years, research has focused on three-dimensional visualisation techniques of pearls and their internal structures (Krzemnicki et al., 2010; Revol et al., 2016; Mannes et al., 2017). Radiocarbon age dating of pearls has also been reported (Krzemnicki et al., 2017).

Research on corals in gemmology is much sparser than for pearls, and has focused on spectroscopic approaches (Rolandi et al., 2005; Henn, 2006; Smith et al., 2007; Karampelas et al., 2009). Elephant ivory has also been studied using techniques such as Raman and Fourier-transform infrared spectroscopy (Edwards and Farwell, 1995; Edwards et al., 2006), along with detailed visual analysis and preliminary trace-element studies (Yin et al., 2013). In addition, geochemical research on isotopes present in ivory has been conducted (van der Merwe et al., 1990; Ziegler et al., 2016).

BIOLOGICAL RESEARCH

A large amount of research has been carried out on the biological formation and characteristics of pearl-producing molluscs and also corals. This research is rarely linked to, and used in, gemmology. Much of this work focuses on detailed genetic aspects of pearl-producing molluscs, including various *Pinctada* species used for pearl farming. Among these are *P. maxima* (Kono et al., 2000; Lind et al., 2012), *P. margaritifera* (Arnaud-Haond et al., 2003a), *P. mazatlanica* (Arnaud-Haond et al., 2003b) and the *Pinctada* genealogy (Cunha et al., 2010), as well as the Akoya pearl oyster complex

Figure 1: Biogenic gem materials suitable for DNA testing include items such as these from the SSEF and H. A. Hänni collections: cultured pearls and associated shell material (*P. maxima* and *P. margaritifera*, ~15 cm tall), corals (including *Corallium rubrum* branches up to ~10 cm tall) and ivory (warthog and mammoth). Photo by Vito Lanzafame, SSEF.

(Wada and Tëmkin, 2008; Al-Saadi, 2013) and Pteriidae-family species such as *Pteria sterna* (Arnaud-Haond et al., 2005). The above-mentioned Akoya complex includes *Pinctada* species such as *P. fucata*, *P. imbricata*, *P. martensii* and *P. radiata*, which are closely related in genetic terms (Cunha et al., 2010). In addition, the genetics of freshwater mussel species used in cultured pearl production have been intensively studied (Peng et al., 2012; Shi et al., 2015). Most of this research was done with the goal of reducing mortality during pearl cultivation and improving the quality of cultured pearls.

Precious coral species such as *Corallium rubrum* (Mediterranean or Sardinian coral) have been studied extensively to understand their formation mechanisms (Grillo et al., 1993; Allemand and Bénazet-Tambutté, 1996), genetic diversity (Ledoux et al., 2010) and populations (Santangelo et al., 2003). Again, little of this biological research has crossed over to the gemmological community.

In elephant research, the link between declining populations and demand for ivory has been widely researched (Maisels et al., 2013, Wittemyer et al., 2014). Much of the scholarly work has focused on declining elephant populations and how to address poaching.

Figure 3: Vigorous vortexing of the nacre sample material in EDTA solution is necessary to detach the DNA molecules from the $CaCO_3$ framework. Photo by L. Cartier, SSEF.

DNA FINGERPRINTING FOR SPECIES (AND ORIGIN) DETERMINATION

Pearls

Pearls and pearl oyster shells contain small amounts of organic matter interspersed within a nacreous mineral matrix (Cuif and Dauphin, 1996; Comps et al., 2000; see Figure 2). In particular, they consist of approximately 92% $CaCO_3$, 4% organic matter (mainly conchiolin and porphyrins), 4% water and minute amounts of other substances (Taylor and Strack, 2008). The organic material has been studied in detail and contains different types of proteins, but previously it had not been reported to contain DNA (Levi-Kalisman et al., 2001; Nudelman et al., 2006; Dauphin et al., 2009). Nevertheless, negatively charged DNA molecules are known to have a high affinity for the Ca^{2+} ions of $CaCO_3$ (Barton et al., 2006), which might enhance the conservation of DNA in biogenic gems such as pearls.

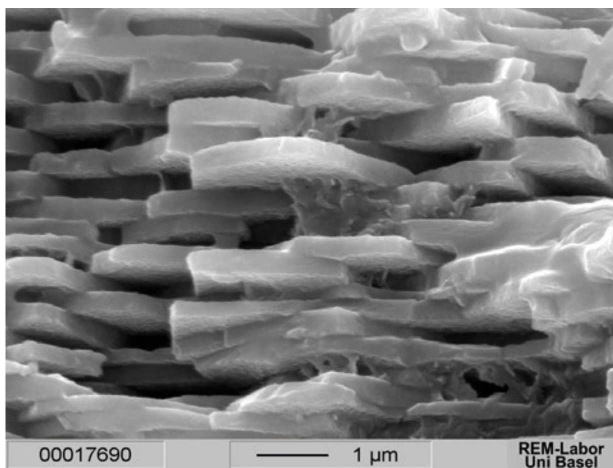

Figure 2: Scanning electron microscopy reveals the individual aragonite tablets in a cross-section through pearl nacre. DNA is thought to be found in organic matter between the individual tablets. Image by Henry A. Hänni and Marcel Düggelin, Zentrum für Mikroscopie, University of Basel, Switzerland.

Research by some of the present authors (Meyer et al., 2013) found DNA in organic matter within nacre from *P. margaritifera* (Tahitian black-lip pearl oyster), *P. maxima* (South Sea pearl oyster) and *P. radiata* (from the Arabian/Persian Gulf, part of the Akoya complex), thus allowing the separation of pearls (and mother-of-pearl) from different *Pinctada* species. A destructive technique for DNA identification was recently published by Saruwatari et al. (2018) focusing on *P. fucata* cultured pearls from Japan. For minimally destructive DNA extraction, the present authors developed a method that uses only a minute sample quantity and thus is applicable to jewellery-quality pearls (Meyer et al., 2013, and subsequent unpublished research by the authors).

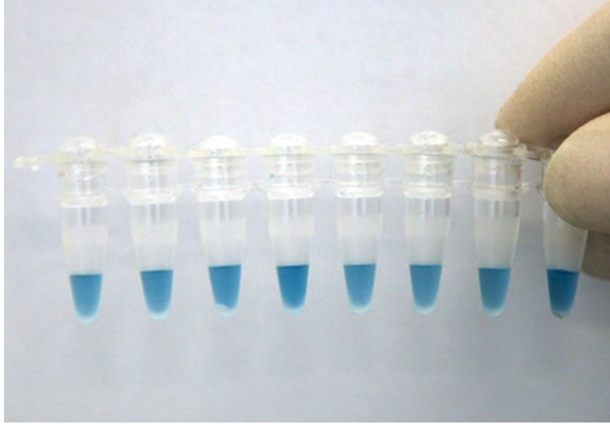

Figure 4: These tubes contain the amplified PCR product (internal transcribed spacer, ITS2) obtained for eight cultured pearls. Photo by L. Cartier, SSEF.

A Dremel workstation with a fixed 1 mm drill head was used to make a small hole in the nacre, and then a second non-fixed 0.9 mm drill bit was used to slightly enlarge the interior part of the hole without damaging the surface around it. The sample material was collected in a Petri dish. Given that most pearls are already drilled for jewellery use, the extraction of 10–20 mg (0.05–0.10 ct) of nacre material from within a pre-existing drill hole is considered quasi-non-destructive. However, this is not the case for pearls that cannot be drilled. The drill powder was then suspended in 1,000–2,000 µl of ethylenediaminetetraacetic acid solution (0.5 M EDTA solution at pH 8.0), vigorously vortexed for two minutes (Figure 3) and incubated overnight at 56°C in a water bath. EDTA dissolves the calcium carbonate structure of the mother-of-pearl. Oyster DNA was extracted from the sample material using a DNA extraction kit according to specific protocols (see Meyer et al., 2013). To genetically discriminate between *Pinctada* species, PCR-RFLP analysis (see Glossary) was performed on a PCR-amplified DNA fragment (internal transcribed spacer, ITS2; see Figure 4), and compared to equivalent RFLP profiles obtained from reference-positive controls (i.e. fresh mollusc tissue from these species; see Figure 5). Alternatively, PCR amplification only of specific ITS2 regions that discriminate between the oyster species was performed.

The research by Meyer et al. (2013) showed that in most cases it was possible to separate pearl oyster species based on their DNA profile extracted from only a minute amount of nacre material. Interestingly, amplification was also successful from samples composed of white nacre powder (i.e. no organic matter evidently visible), indicating that DNA can be obtained through demineralisation from the $CaCO_3$ structure of the nacre and/or from small samples (e.g. 10–20 mg). As technology costs come down and these methods are further refined, the authors foresee DNA fingerprinting being carried out on even smaller amounts of nacre material.

Current research by the authors shows that DNA fingerprinting can be adapted to other species of pearl-bearing molluscs and their pearls. This includes a range of freshwater mussels such as Chinese *Hyriopsis schlegelii* and *Hyriopsis cumingii schlegelii* mussel hybrids or the American washboard mussel (*Megalonaias nervosa*) that is frequently used as bead nucleus material in Akoya,

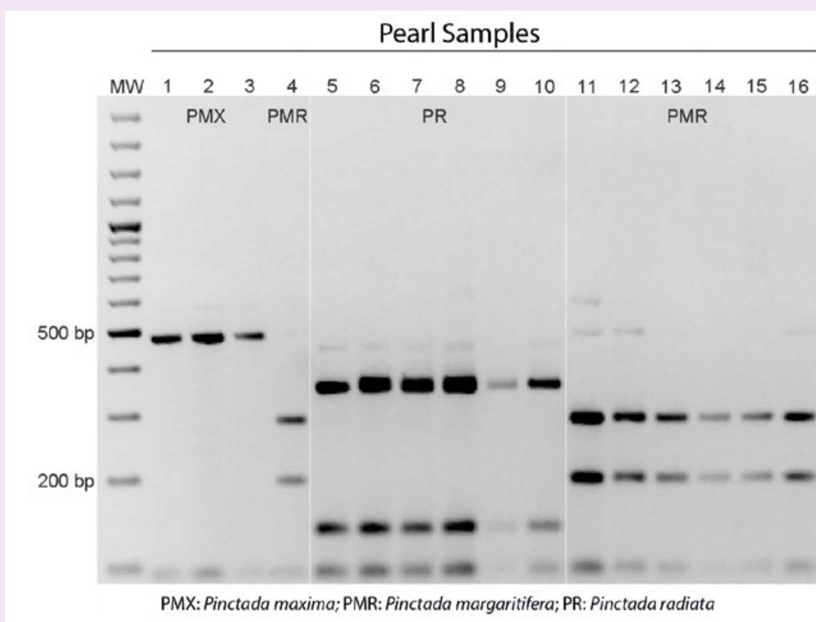

Figure 5: A PCR-RFLP assay of the ITS2 DNA fragment was used to differentiate the species associated with 16 cultured pearls. Lanes 1–3 correspond to *Pinctada maxima,* lanes 4 and 11–16 are for *P. margaritifera* and lanes 5–10 are for *P. radiata.* 'MW' corresponds to molecular weight and 'bp' to base pairs. Image by J. B. Meyer; modified from Meyer et al. (2013).

Figure 6: This five-row necklace contains 377 natural pearls ranging from 3.90 to 9.45 mm, likely from *P. radiata* of the Arabian Gulf. DNA fingerprinting could provide further documentation of the provenance for such exceptional pearls. Photo by Luc Phan, SSEF.

South Sea, Tahitian and Fijian pearl cultivation. We also are adapting this analytical approach to conch pearls (*Lobatus gigas*, formerly commonly known as *Strombus gigas*) and pearls from the giant clam (*Tridacna gigas*), in anticipation that DNA fingerprinting could contribute to more transparency in these CITES-regulated pearls.

Other Biogenic Gem Materials

The methodology used by Meyer et al. (2013) has been recently piloted by the authors on samples from various precious coral species commonly used in jewellery (including those from the Mediterranean, Asian and Midway Islands regions). This ongoing research should allow the separation of different species of precious corals and conclusively identify and distinguish non-CITES-regulated species (e.g. *Corallium rubrum*, or Mediterranean coral) from CITES-regulated species (e.g. *Corallium elatius*, known in the trade as Momo, Cerasuolo or Satsuma coral).

There have also been developments in techniques to determine geographic/genetic origin and species identification of seized elephant ivory using DNA and microsatellite methods (i.e. analysis of repeated DNA sequences in the genome that enable the distinction between different elephant population groups). However, so far this has been performed in a destructive way, requiring relatively large amounts of material (Comstock et al., 2003; Wasser et al., 2004, 2015). A less destructive method, like that used by Meyer et al. (2013), might be adapted to ivory too, thereby requiring much smaller amounts of material for testing such samples.

RESEARCH OUTLOOK AND CONCLUSIONS

DNA fingerprinting offers various advantages for the research and trade in biogenic gem materials. It provides a new option to increase transparency (through origin and species determination) and to help address fraud or illegal trading by separating protected from non-protected species.

The state-of-the-art minimally destructive extraction methodology outlined in this article can offer conclusive identification of the mollusc species to which a pearl corresponds, unlike other methods currently available in gemmology today. Furthermore, DNA analysis has the potential to reveal the geographic origin of cultured or natural pearls (Figure 6) based on more specific fingerprinting. For corals, species determination may considerably contribute toward resource conservation efforts and also provide more information on the provenance of historic items. As such, this research is relevant to the work of international customs officials within the context of biogenic gem materials protected by CITES. With ivory, origin determination based on DNA analysis has already been proven possible (Wasser et al., 2004). However, the available methodology requires large amounts of sample material and is thus not appropriate for jewellery or other items that cannot be destructively tested. Ongoing research and specifically next-generation sequencing (see Glossary) enables the screening of a large number of DNA sequences from smaller samples

at lower costs, subsequently reducing the amount of sample material required. DNA fingerprinting is, therefore, becoming less destructive and more useful for biogenic gem materials.

DNA fingerprinting as a tool in gemmology further illustrates the importance of multidisciplinary research collaborations (in this case, with marine biology and genetics scientists) to develop new gem-testing techniques for the 21st century.

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Glossary*

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.

DNA sequencing: The reading of nucleobases (A, C, G and T) in DNA. One can choose to sequence an entire genome (whole/full genome sequencing) of a tested sample or just sequence a few targeted nucleobases that are useful for distinguishing different species (DNA fingerprinting).

Genome: An organism's full set of DNA, including all of its genes.

Microsatellite: Repetitive DNA sequences that can be used as genetic markers to measure levels of relatedness between species or individuals. They can be used for genetic population studies and thus may offer more information on the geographic origin of individuals from a species.

Next-generation sequencing (NGS): Allows massive parallel sequencing of DNA, enabling a rapid and cost-effective way to sequence large amounts of genetic regions and whole genomes of organisms. With its ultra-high throughput, NGS has revolutionised genomic research.

DNA amplification and polymerase chain reaction (PCR): A method for amplifying DNA sequences. The technique involves using short DNA sequences called primers (see below) 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. As such, PCR can produce thousands to millions of copies of the target sequence in several hours, which can then be analysed. For example, it allows the identification of specific DNA sequences using visual inspection (e.g. gel electrophoresis) or they may be read through sequencing.

Primer: A primer is a short DNA sequence that serves as a starting point for DNA synthesis by PCR. Primers are selected according to the sequence region targeted for DNA amplification. These are, for example, regions in the genome of various oyster species (specific genetic markers) for which differences allow species determination.

Restriction fragment length polymorphism (RFLP): A technique based on variations in the DNA sequence (e.g. from different species) recognised by restriction enzymes. The resulting restriction fragments are separated according to their length by gel electrophoresis. The length (in base pairs) can differ between individuals and species such that the positions of gel bands can be used to separate samples from different species.

* Sources: National Human Genome Research Institute glossary (www.genome.gov/glossary), National Center for Biotechnology Information Probe glossary (www.ncbi.nlm.nih.gov/probe/docs/glossary) and Wikipedia.

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