DNA Fingerprinting as a Tool in Modern Gemmology
脫氧核醣核酸 (DNA) 指紋科技作為現代寶石學的一種工具



Laurent E. Cartier   Michael S. Krzemnicki   Bertalan Lendvay

Laurent E. Cartier[1], Michael S. Krzemnicki[1], Bertalan Lendvay[2]
[1] Swiss Gemmological Institute SSEF, Basel, Switzerland
[2] University of Zürich, Institute of Forensic Medicine, Zürich, Switzerland
Email: laurent.cartier@ssef.ch

脫氧核醣核酸(DNA)指紋作為現代寶石學工具進一步説明了多學科研究合作的重要性。將珍珠、珍貴珊瑚和象牙追溯到其物種相關和地理來源的能力可以提供更大的明晰度，並有助於遏制非法材料的貿易，從而限制偷獵和走私，並為記錄歷史物品提供更多信息。

Fig. 1 A selection of pearls from different species. DNA fingerprinting can help us in identifying which species a pearl came from. *Photo: Michael Krzemnicki, SSEF*
精選不同種類的珍珠。脫氧核醣核酸(DNA)指紋可以幫助我們識別珍珠來自哪個物種。

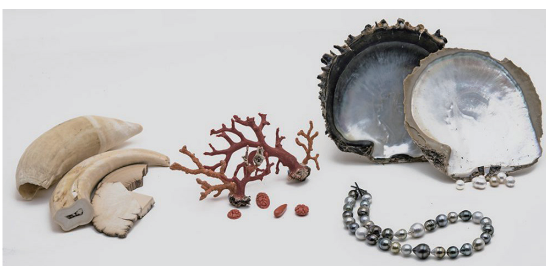

Fig. 2 Teeth, horn, ivory, corals and pearls are all organic gems that can contain DNA, allowing us to identify specific species conclusively. Narwhale, musk ox (Greenland), elephant and mammoth ivory, red coral (Spain), shell-Pinctada margaritifera (Tahiti), Pinctada maxima (Bali). *(Reference samples collected by H.A. Hänni)*
Photo by Vito Lanzafame, SSEF
牙齒、長角、象牙、珊瑚和珍珠都是含有DNA的有機寶石，讓我們能夠最終確定特定的物種。獨角鯨，麝牛（格陵蘭），大象和猛獁象牙，紅珊瑚（西班牙），貝殼 - 珠母貝（大溪地），大珠母貝（巴厘島）。

Pearl testing was long limited to determining whether a pearl is natural or cultured, and whether a pearl has been treated or not. Considerable advances in pearl research in recent years have achieved significant breakthroughs, namely DNA fingerprinting for species identification and radiocarbon age dating (Krzemnicki & Hajdas, 2011; Meyer et al., 2013).

SSEF pioneered DNA fingerprinting of pearls in 2013, in collaboration with scientists from ETH Zürich. This was the first published report (Meyer et al., 2013) of oyster DNA extraction from a pearl, allowing pearls of unknown origin to be traced and fingerprinted and matched to the specific oyster species in which they formed. In addition to refining methods for testing and fingerprinting pearls in recent years, these methods have been adapted and applied to precious coral and ivory samples too.

In partnership with the Institute of Legal Medicine at the University of Zürich since 2018, one of the leading forensic research facilities in Switzerland, quasi non-destructive genetic analysis methods have been developed with a focus on forensic validation that can accurately and sensitively determine the origin of pearls and precious corals processed for jewellery (Lendvay et al., 2022). These methods considerably enhance the scientific ability to document the provenance of historic and modern pearls and coral items. Furthermore, for corals, given that some species are listed by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), the developed methodology could contribute toward resource conservation efforts by offering full disclosure of species origin through scientific testing.

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. As pearls, corals and ivory are formed by living organisms and cells, they are in contact with host cells and thus DNA during their formation (Cartier et al., 2018). Fortunately, negatively charged DNA molecules are known to have a high affinity for the Ca^{2+} ions of $CaCO_3$, which enhances the conservation potential of DNA in pearls and precious corals as both consist of calcium carbonate (Barton et al., 2006). Variations in the genetic code of different pearl and coral producing species can allow them to be separated on the basis of the recovered DNA.

DNA in natural and cultured pearls

Natural pearls form in wild molluscs without any assistance, whereas cultured pearls are the result of human intervention on cultivated pearl-producing molluscs. Pearls and their shells consist of secretions of different polymorphs of calcium carbonate ($CaCO_3$) such as aragonite, calcite and vaterite. The nacreous part of a pearl consists of approximately 92% $CaCO_3$, 4% organic matter, 4% water and minute amounts of residual substances (Taylor and Strack, 2008). The organic matter (consisting mostly of conchioline and porphyrines), which is also secreted by the pearl sac, serves as a framework for the $CaCO_3$ matrix (Fig. 3) during the biomineralisation process. Organic matter can also be found in concentrated pockets (e.g. cavities). Both are likely sources of minute amounts (nano-grams) of oyster DNA that can be recovered in natural and cultured pearls.

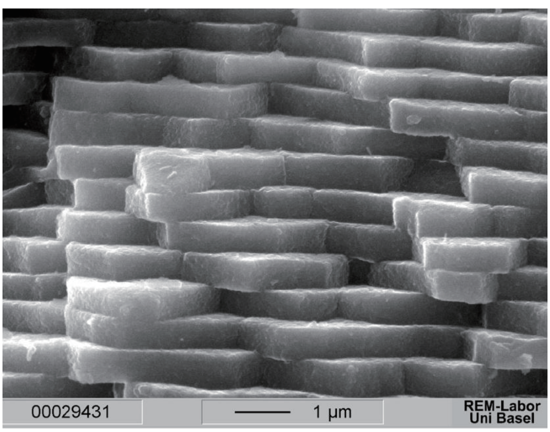

Fig. 3 Scanning electron microscopy reveals the individual aragonite tablets in a cross-section through pearl nacre. DNA is thought to be found in organic matter between the individual tablets. *Image by Henry A. Hänni and Marcel Düggelin, Zentrum für Mikroskopie, University of Basel, Switzerland*.
掃描電子顯微鏡顯示通過珍珠層的橫截面中的單個石片。DNA被認為存在於各個頁片之間的有機物中。

Although species identification may be possible for pearls of different colours (that have characteristic pigments that give the pearl its colour) using scientific instruments such as UV-visible photospectrometry and micro-Raman spectroscopy, this may not always be conclusive. Furthermore, it is rarely possible to determine the species of white to slightly cream coloured pearls (e.g. Fig. 4) conclusively using current methods.

The eight pearl species that can currently be distinguished conclusively using these DNA fingerprinting methods are:

- *Pinctada radiata* (Persian Gulf & Ceylon pearl oyster)
- *Pinctada imbricata* (Atlantic pearl oyster)
- *Pinctada fucata/martensii* (Akoya pearl oyster)
- *Pinctada maxima* (South Sea pearl oyster)
- *Pinctada margaritifera* (Tahitian black-lipped pearl oyster)
- *Pinctada mazatlanica* (Panama pearl oyster)
- *Pinctada maculata* (Pipi pearl oyster)
- *Pteria sterna* (Rainbow-lipped pearl oyster)

Fig. 4 A natural pearl necklace with pearls likely from Pinctada radiata of the Arabian/Persian Gulf. DNA fingerprinting can provide further documentation of the provenance for such exceptional pearls.
Photo by Luc Phan, SSEF
一串天然珍珠項鍊，珍珠可能來自阿拉伯/波斯灣的經輻射珠母貝。DNA指紋技術可以進一步證明這種特殊的珍珠之來源。

Coral species determination

Fig. 5 A selection of precious coral used in jewellery today. The ring in the middle of the photo consists of a dark Aka coral bead, the most expensive variety of precious coral in the world. *Samples courtesy of Enzo Liverino.*
Photo: Laurent E. Cartier
當今珠寶中的珍貴珊瑚。圖片中間的一顆深色阿卡紅珊瑚珠指環，是世界上最昂貴的珍貴珊瑚品種。

Precious corals used in jewellery and objets d'art represent the coral skeleton (secreted by living polyps), which consists of $CaCO_3$ as well as protein, glycosamino-glycans and proteoglycans (Debreuil et al., 2012). As in pearls, minute amounts of DNA have been found by our research team in precious corals. This DNA can be extracted using methods we have developed and used to determine which species or species group the precious coral is from. The DNA fingerprinting technology developed represents a game-changing way of assessing the species identity of precious corals found in the trade. Importantly, the technique is quasi non-destructive, requires considerably less sample material than other methods, with testable DNA being recovered from as little as 2.3 milligrams (0.0115 carats) of material (Lendvay et al., 2020).

At present, there is no other conclusive way of determining the genetic species which a precious coral piece in jewellery is from, unless clear visual characteristics are present. Given that a number of precious coral species are listed on the Convention on International Trade in Endangered Species (CITES) Appendix III, correct identification and declaration is vital in order for samples to be traded legally.

Using DNA fingerprinting, it is possible to identify a coral item genetically as one of the following:

- *Corallium rubrum* (Mediterranean red coral)
- *Corallium japonicum* complex (includes aka, moro, oxblood varieties)
- *Hemicorallium sp*. (includes deep sea Midway coral, garnet coral and Miss coral)
- *Pleurocorallium secundum* (Midway and Rosato coral)
- *Pleurocorallium elatius* complex (includes angel skin, boké, magai and momo varieties)
- *Pleurocorallium sp.* (*Pleurocorallium* which does not belong to *Pleurocorallium secundum* or the *Pleurocorallium elatius* complex)

DNA analysis is complemented by morphological and gemmological analyses for species identification.

Elephant Ivory or Mammoth Ivory?

DNA fingerprinting, together with a morphological analysis of an ivory sample, helps ascertain whether an item of ivory originated from a historic or modern source. This is particularly helpful in identifying cases of fraud where, for example, CITES-regulated elephant ivory is misrepresented and sold as mammoth ivory. To identify the species of ivory conclusively using commonly available gemmological lab techniques can be challenging, especially if Schreger lines are not evidently visible. Mammoth ivory is used in carvings and jewellery, and is mainly sourced from the remains of mammoths preserved in the permafrost of current-day Siberia. The mammoths, which were common to the region, became extinct about 10,000 years ago.

Fig. 6 A sample of ivory, about 5 centimetres in length, which was conclusively identified as being from extinct mammoth ivory (Mammuthus sp.), following DNA fingerprinting analysis. *Photo: SSEF*
一個長約5厘米的象牙樣本，經過DNA指紋分析，最終確定為來自已滅絕的猛獁象牙。

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. The method described in Cartier et al. (2020) requires much less material (ca. 100 mg for ivory) for testing of such samples. Although ivory is no longer widely used in jewellery, the ability to trace ivory back to its species-related and geographic origins can provide greater transparency and help curb trade in illegal materials (and thus restrict poaching and smuggling). It can also help in the documentation of historic samples.

Discovery of New Species and Examples of Tested Items

Our work on DNA in pearls and precious corals has uncovered new species used in jewellery, which were previously unreported in the trade. A number of submitted precious coral samples turned out to be *Pleurocorallium niveum* (found in the Pacific), which can be considered a new species for the jewellery world. A natural pearl jewellery set (thought to be from *Pinctada radiata*) we tested contained two pearls samples for DNA which were attributed to another species: *Pinctada persica* or *Pinctada margaritifera persica*, which is a rare member of the *Pinctada margaritifera* species complex. To our knowledge, this is the first time that pearls from **Pinctada persica** have been reported. To date this species has only been found exclusively in the Persian Gulf (Ranjbar et al. 2016). This shows the potential that DNA fingerprinting has to uncover previously unreported species of both pearls (and precious corals) used in jewellery and also to document species and geographic origins for pearls. In continuing this research, we are convinced that we will discover many new secrets about pearls in future.

A notable item that was submitted for DNA fingerprinting testing was a coral sautoir (Fig. 7) designed by Suzanne Belperron, one of the most influential jewellery designers of the 20th century. The item consisted of a sautoir with 123 plain, polished coral beads and a carved coral, and a pendant with 7 partly carved corals (up to 23.00 mm diameter). The corals were accentuated by black intersections of polished onyx and slightly frosted colourless quartz.

On this item both a carved bead and plain, polished bead were sampled. In both cases less than 10 mg of material was taken for DNA analysis and in both cases, the sequenced samples were attributed to the *Pleurocorallium elatius* complex. Three taxa belong to this complex: *Pleurocorallium elatius*, *Pleurocorallium konojoi* and *Pleurocorallium carusrubrum* (red skeletal axis) (sensu Tu et al. 2015). Identification of a specific species within this complex by SSEF is thus based on a combination of DNA, morphological and other analyses. The sampled beads were identified as being from *Pleurocorallium elatius*. This species is by far the most widely used of these three species in the jewellery trade, and includes the desirable angel skin, boké, magai and momo varieties.

Fig. 7 A coral sautoir designed by Suzanne Belperron. DNA fingerprinting of two samples showed they could be attributed to *Pleucorallium elatius*, a precious coral species found in Asian waters. *Photo: A. Chalain, SSEF*
由Suzanne Belperron設計的珊瑚項鍊。對兩個樣本的DNA指紋分析表明，它們可歸因於毛孔菌埃拉蒂烏斯鈀(Pleucorallium elatius)，這是一種在亞洲水域發現的珍貴珊瑚物種。

Outlook

The ability to trace pearls, precious corals and ivory 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) and provide further information to document historic items. 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 (Cartier et al., 2018; Saruwatari et al., 2018).

In addition, this technique can be combined with age dating of pearl, precious coral or ivory samples. The ability to date and trace pearls and precious corals back to their species-related and geographic origins can provide greater transparency, as well as supply important and fascinating information about modern and historic items. We look forward to combining and expanding these techniques further in future.

References

Barton H.A., Taylor N.M., Lubbers B.R., Pemberton A.C., 2006. DNA extraction from low-biomass carbonate rock: An improved method with reduced contamination and the low-biomass contaminant database. *Journal of Microbiological Methods*, **66**(1), 21–31

Cartier, L.E., Krzemnicki, M.S., Lendvay, B., Meyer, J.B., 2018. DNA Fingerprinting of Pearls, Corals and Ivory: A Brief Review of Applications in Gemmology. *Journal of Gemmology*, **36**(2), 152–160

Cartier, L.E., Krzemnicki, M.S., Gysi, M., Lendvay, B., Morf, N.V., 2020. A Case Study of Ivory Species Identification Using a Combination of Morphological, Gemmological and Genetic Methods. *Journal of Gemmology*, *37*(3), 282–297

Debreuil, J., Tambutté, É., Zoccola, D., Deleury, E., Guigonis, J.M., Samson, M., Allemand, D. and Tambutté, S., 2012 Molecular cloning and characterization of first organic matrix protein from sclerites of red coral, Corallium rubrum. Journal of Biological Chemistry, 287(23), 19367-19376

Krzemnicki, M.S., Hajdas, I., 2013. Age determination of pearls: A new approach for pearl testing and identification. *Radiocarbon*, 55(3), 1801-1809

Lendvay, B., Cartier, L.E., Gysi, M., Meyer, J.B., Krzemnicki, M.S., Kratzer, A., Morf, N.V., 2020. DNA fingerprinting: an effective tool for taxonomic identification of precious corals in jewelry. *Scientific reports*, *10*(1), 1-12

Lendvay, B., Cartier, L.E., Costantini, F., Iwasaki, N., Everett, M.V., Krzemnicki, M.S., Kratzer, A., Morf, N.V., 2022. Coral-ID: A forensically validated genetic test to identify precious coral material and its application to objects seized from illegal traffic. *Forensic Science International: Genetics*, *58*, p.102663

Meyer J.B., Cartier L.E., Pinto-Figueroa E.A., Krzemnicki M.S., Hänni H.A. and McDonald B.A., 2013. DNA fingerprinting of pearls to determine their origins. *PLoS ONE*, **8**(10), article e75606, 11 pp

Ranjbar, M. S., Zolgharnien, H., Yavari, V., Archangi, B., Ali Salari, M., Arnaud-Haond, S., Cunha, R.L, 2016. Rising the Persian Gulf Black-Lip Pearl Oyster to the Species Level: Fragmented Habitat and Chaotic Genetic Patchiness in *Pinctada persica. Evolutionary Biology* **43**, 131–143

Saruwatari, K., Suzuki, M., Zhou, C., Kessrapong, P. and Sturman, N., 2018. DNA techniques applied to the identification of Pinctada fucata pearls from Uwajima, Ehime Prefecture, Japan. *Gems & Gemology*, *54*(1), 40-50

Taylor J. and Strack E., 2008. Pearl production. In P.C. Southgate and J.S. Lucas, Eds., *The Pearl Oyster*, Elsevier, Oxford, 273–302

Tu, T.H., Dai, C.F. and Jeng, M.S., 2015. Phylogeny and systematics of deep-sea precious corals (Anthozoa: Octocorallia: Coralliidae). *Molecular Phylogenetics and Evolution*, 84, 173-184