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► **To cite this version:**

Raphaël Martin-Roy, Jakob Thyrring, Xavier Mata, Pernille Bangsgaard, Ole Bennike, et al.. Advancing Responsible Genomic Analyses of Ancient Mollusc Shells. Environmental and Agronomical Genomics Symposium, Feb 2024, Toulouse, France. hal-04790112

HAL Id: hal-04790112

<https://hal.science/hal-04790112v1>

Submitted on 19 Nov 2024

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Advancing Responsible Genomic Analyses of Ancient Mollusc Shells

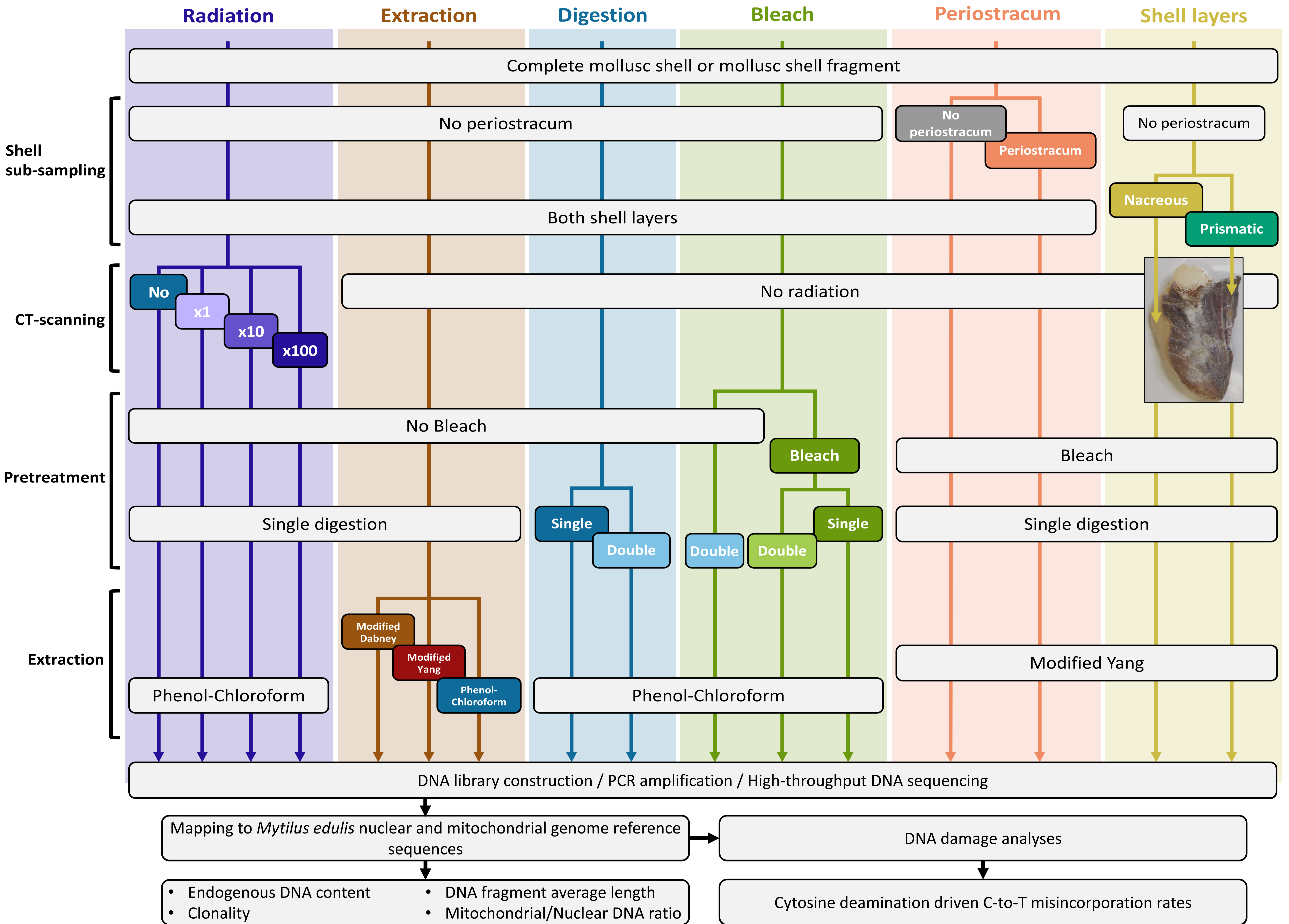


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The DNA entrapped in ancient mollusc shells has the potential to shed light on the evolution and ecology of this diverse phylum. It could help reconstruct responses to past climate, aquaculture practices, pollution and extinction events at unprecedented time resolution. Such studies are lacking partly due to our **limited knowledge of DNA preservation in calcium carbonate shells** and the **need for optimizing methods** for genomic data generation from ancient shells. Here, we applied **high-throughput DNA sequencing to 27 mollusc shells dated to 111-6,500 years Before Present** to investigate the impact of **micro computed tomography (micro-CT) scanning** on ancient shell DNA to preserve morphological information, the performance of commonly used **silica-based DNA extraction methods** and **predigestion treatments**, and DNA recovery from the **organic (periostracum)** and **carbonated (aragonite, calcite)** mollusc shell layers. We propose **recommendations for efficient and responsible genomic analyses** of ancient mollusc shells. **Micro-CT scans can be used** without harming their DNA. Ancient shells' DNA is protected in preservation niches and its recovery **was improved by double-digestion and predigestion bleach wash** of shell powder. The **periostracum, the calcite and the aragonite layers are good DNA reservoirs**, the latter appearing as the **best substrate** for genomic analyses. Our results also give insight on **long-term molecular preservation in biominerals**.

1. EXPERIMENTAL WORKFLOW

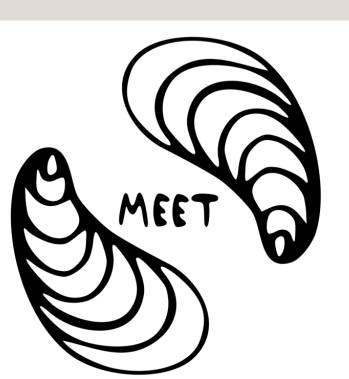
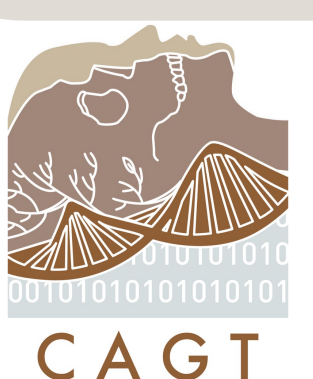


2. RESULTS

- NO** impact on endogenous DNA content.
- NO** additional DNA damage (fragmentation and cytosine deamination).
- NO** benefits from Phenol:Chloroform.
- Other methods are equivalent.
- Dabney € < Yang €€.
- Double-digestion increases endogenous DNA content.
- Double-digestion can recover less degraded DNA.
- BEST** performances for Bleach wash + Single-digestion.
- NO** additional DNA damage when using bleach (fragmentation and cytosine deamination).
- Periostracum increases endogenous DNA content.
- BUT** also increases clonality.
- Nacre: reduced cytosine deamination.
- Nacre: higher nuclear DNA content.

3. CONCLUSIONS and RECOMMENDATIONS

1. Acquire high-resolution 3D images of ancient mollusc shells prior to destructive DNA analyses. Mollusc shells can be scanned without significant impact on DNA molecules. Document scanning history/parameters to avoid unnecessary scanning. Micro-CT scans are better suited than photogrammetry/surface scans for the preservation of the 3D morphological information of shells. **2. Use silica-based methods for ancient mollusc shell DNA extraction.** No differences in performance were observed between protocols. However, 'modified Dabney' protocol is cheaper per sample. **3. Consider pre-digestion treatments for ancient mollusc shell DNA extraction.** All pretreatments allowed for increased endogenous DNA recovery from preservation niches within ancient molluscs' shell biominerals. Bleach wash followed by a single digestion step showed the best performance and is a cost effective way to reduce contamination. **4. Use the periostracum as an indicator of good DNA preservation in ancient mollusc shells.** Mollusc shell DNA extraction keeping the periostracum layer yielded higher endogenous DNA content. **5. Prefer well-preserved aragonite rather than calcite shell layers for ancient mollusc genomics.** The nacreous layer offers better protection to nuclear DNA, likely due to its microstructure organisation (densely packed aragonite crystals) compared to the prismatic layer (elongated calcite crystals).



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