Assessing ‘non-destructive’ DNA extraction method in small crustaceans kept in wet collections

Samuel C. Bernardes[‡§], Thomas von Rintelen[‡], Serena Alexander[|], Fiona Lorenz[¶], Kristina von Rintelen[‡]

‡ Museum für Naturkunde, Berlin, Germany
§ Faculty of Life Sciences, Humboldt Universität Berlin, Berlin, Germany
| School of Biological and Environmental Science, University College Dublin, Dublin, Ireland
¶ Institute of Biology, Freie Universität Berlin, Berlin, Germany

Corresponding author: Samuel C. Bernardes (samuel.geneticafarm@gmail.com)

Academic editor: Editorial Secretary

Abstract

Specimens in natural history museums are a valuable resource for biological research, such as taxonomic, biodiversity or evolutionary studies. However, the quality of DNA and even morphological characters can decrease over time, depending on previous fixation and long-term preservation methods. In recent years, advances in DNA extraction and sequencing techniques have allowed researchers to obtain DNA from museum specimens, even when the DNA was very fragmented. Extraction methods should ideally be morphologically non-destructive, leaving diagnostic characters intact for future taxonomic studies. Here, we assess whether the whole-body extraction widely used for several taxa would be destructive for small crustaceans kept in wet collections. We extracted the DNA from over 70 small (1-3 cm) and relatively fragile shrimps collected during the last 30 years by using: i) a piece of abdominal tissue and ii) from the entire remaining body of the animal. We photographed several samples before and after the lysis, focusing on taxonomically relevant characters. Although DNA concentration was higher in the whole-body extractions, the presence of intact DNA was not correlated to the amount of lysed tissue. The resulting genomic libraries had little to no difference in yield. The taxonomically relevant characters were primarily preserved in larger specimens, whereas smaller specimens (< 1.5 cm) became too fragile to handle or were damaged. We conclude that this method must be carried out carefully in smaller crustaceans, depending on size and taxon. We advise against using it with type specimens as the advantages do not outweigh the risks. Our experiment may provide future research with quantitative and qualitative evaluations to help scientists weigh their decisions when extracting DNA from wet collection material.

Keywords

Museum, ancient DNA, genomics, DNA preservation, molecular methods

Introduction

About two centuries' worth of biological information is currently contained in natural history collections (NHC) (Billerman and Walsh 2019). While these institutions play an invaluable role in taxonomic and evolution research, much of this evergrowing knowledge has historically been gained from morphological studies, with DNA often being poorly preserved and sampled. This is particularly true for samples collected before or during the growth of molecular biology in the past 40 years (Lindahl 1993, Dillon et al. 1996, Hahn et al. 2022). Overlapping with the onset of the molecular era, the urgency of the biodiversity crisis has twisted this trend by establishing a demand for defining (or challenging) the limitations in retrieving this previously ignored type of data (Alberch 1993, Krishtalka and Humphrey 2000, Billerman and Walsh 2019, Raxworthy and Smith 2021).

However, obtaining DNA from NHC is rarely straightforward. Different molecular biology methods vary in their demands in terms of DNA molecular integrity: although the advances in next-generation sequencing (NGS) have introduced us to a genomic era where retrieving vestigial DNA from virtually any type of sample is possible, a considerable proportion of the research projects in systematics and biodiversity require DNA fragments that are not too short to allow the amplification and sequencing of markers that vary from a few hundred up to a couple of thousand basepairs long. In general, an overall intact structure is largely preferred to avoid employing complex techniques to recover damaged DNA, such as an ancient DNA approach (Mandrioli 2008 , Zimmermann et al. 2008, Briggs and Heyn 2012, Gansauge and Meyer 2013, Linderholm 2016, Prosser et al. 2016). Even NGS techniques, which have shifted the demands from the extracted DNA from sequencing single markers to producing viable libraries, still exhibit a variety of protocols and kits that require different degrees of integrity – i.e. with or without fragmentation step, with or without PCR step or ability to skipping specific steps during library preparation (Van Dijk et al. 2014).

While NGS techniques and the variety of approaches led to the development of alternative methods for dealing with damaged and ancient DNA, NHC specimens still present a further challenge: the preservation of the specimen to allow future examination (Dillon et al. 1996, Gilbert et al. 2007, Thomsen et al. 2009). It became common to extract DNA from some groups of small animals (especially arthropods) by submitting the entire body of the animal to the lysis solution. This so-called 'non-destructive' approach relies on maximising the number of lysed cells, while preserving the taxonomic characters in the exoskeleton that is left more or less intact. However, the assessment and comparison of extraction methods for museum specimens are mostly

focused on samples that were not only collected a long time ago but are also preserved in ways that neglect DNA preservation, such as dry collection or formalin (Dean and Ballard 2001, Dillon et al. 1996, Vink et al. 2005, Gilbert et al. 2007, Zimmermann et al. 2008, Thomsen et al. 2009, Chen et al. 2010, Jaksch et al. 2016, Wang et al. 2019, Patzold et al. 2020, Straube et al. 2021, Hahn et al. 2022).

The assessment of these approaches also suffers from a taxonomic bias: although this method has become commonplace for most small arthropods, such as mites (e.g. Klimov and OConnor (2008)), the evaluation of methods is still centred around insects since they are small, commonly studied and are typically preserved dry, pinned in collection boxes (e.g. Dillon et al. (1996), Dean and Ballard (2001), Gilbert et al. (2007), Zimmermann et al. (2008), Thomsen et al. (2009), Chen et al. (2010), Lang et al. (2015), Wang et al. (2019), Patzold et al. (2020)). With regards to wet collections, however, this sort of study focuses on trying to salvage DNA from samples preserved in formalin (Zimmermann et al. 2008, Jaksch et al. 2016, Straube et al. 2021, Hahn et al. 2022).

Small crustaceans, despite their hard exoskeleton, often have sensitive external structures like setae, teeth, spinules and other ornaments (Garm and Watling 2013) and are preferably kept in wet collections (e.g. alcohol) (Martin 2016, Uebeler et al. 2022). For atyids, which range from ~ 1 up to 12 cm in body length, traits of taxonomic importance often involve structures on the exoskeleton that may be small and/or delicate enough to be easily destroyed or deformed – particularly in the smaller species in the *Caridina* group, which rarely surpasses 30 mm in length (e.g. de Mazancourt et al. (2020)). They are usually preserved in ethanol 70-80% in long-term collections, but, in the field, they are often fixed and transported in ethanol 95% (Ng 2017, Uebeler et al. 2022). The higher alcohol concentration is suited to DNA preservation but has deleterious impacts on morphological studies. Thus, a trade-off between alcohol and specimen fixation must be considered by the researcher; samples that have been improperly fixed earlier in their collection may suffer with DNA degradation despite proper preservation in the long term.

Extraction methods and kits specifically focused on retrieving DNA from museum samples, either old or ill-preserved, have been explored for a couple of decades (Raxworthy and Smith 2021) and a number of methods and kits available to deal with low-quality or low-quantity DNA have been developed (Carøe et al. 2018, Ruiz-Gartzia et al. 2022, Settlecowski et al. 2023). However, even though extracting DNA from the animal's entire body might be an option to increase the yield for molecular studies, museomics studies either rely on proper taxonomy or lead to changes in it. Therefore, the specimens used in such works should be available for taxonomists later and the molecular age should not impede further morphological analyses and/or referencing. Since atyids are rather small and possess various very fine taxonomic characters, we deem them as a good proxy for testing whether the whole-body extraction approach often used for terrestrial arthropods amongst other groups is viable for smaller animals kept in the wet collection. Here, we evaluate the efficiency and viability of a supposedly non-destructive whole-body extraction for museum crustaceans by comparing the amount of extracted DNA and the effects of the lysis process on the body.

Materials and Methods

Sample selection

We selected 74 specimens, each belonging to a single lot of atyid shrimps (Crustacea, Decapoda) from the crustacean wet collection of the Museum fuer Naturkunde, Berlin (ZMB – former Zoologisches Museum Berlin). Each of the chosen specimens belongs to an individual species and their collection dates ranged from three up to 30 years prior to the DNA extractions (extractions were performed in 2022; sample collection years ranged between 1992 and 2019; Suppl. material 1). None of those samples was a type specimen of any kind. Since our main goal was to compare methods in terms of DNA yield and DNA integrity, while assessing specimen destruction, we decided that 30 years was a good age limit as older samples could be more vulnerable to contamination. Samples varied in size, from ~ 1 up to 3 cm. Whenever the collection ID referred to lots with several individuals, one specimen was chosen according to their state of conservation, including only those samples with a clear light colour and overall intact body integrity. However, some specimens that were already damaged (either by handling or by long-term preservation) were included (e.g. Fig. 3e) to test how it may affect the post-lysis integrity (see Discussion). A piece of muscular tissue was retrieved from a dorsal section of the abdomen that is not necessary for identification (Klotz et al. 2023), while the rest of the body was preserved for the alternative extraction method. Thus, two sets of extractions were prepared for each individual: one with the fragment of muscular tissue (hereafter TS) and one with the whole body (herafter WB). Twenty-six of the samples including species of various sizes were chosen as proxies to evaluate the preservation of taxonomic characters. These were photographed with a focus on said characters prior to the lysis and again after the DNA extraction. Photos were taken with a DMC6200 camera mounted on an M205 C stereomicroscope (both by Leica) by using the Leica Application Suite software (LAS) v. 4.13 following the protocol available in Uebeler et al. (2022).

DNA extraction

In order to prepare the samples for extraction, tissue pieces had the preservation alcohol thoroughly dried, whereas the shrimp bodies were washed with distilled DNA-free water. Although we did not have a sensitive enough scale to weigh the small pieces of muscle tissue, we estimated the dry volume of samples using graph paper (~ 4 mm²). Following the instructions in the protocol 'Purification of Genomic DNA from Tissue Samples in QIAamp® 96 DNA QIAcube®,' reagents containing precipitate were incubated at 37°C until the solution was clear. Then, all reagents were equilibrated to room temperature before the procedure. Both tissue samples and the whole shrimp bodies were submitted to the same lysis solution: 180 µL ATL buffer + 20 µL proteinase K. The TS samples were then incubated for 6 hours at 55°C, whereas the WB samples were incubated for 30

minutes at 37°C. Following digestion, both sets of lysates were submitted to the automated extraction programme in QIAcube® HT.

All the precautions were taken during extraction to avoid contamination, both environmental and cross-contamination: all utensils and the counter were decontaminated with ultraviolet light and DNA AWAY (ThermoFisher Scientific) before and after use; all new tubes were kept in ultraviolet light for an hour before use; samples were washed from the preservation alcohol in a closed environment under negative air pressure before tissue pieces were taken and dried in the same enclosure.

Quality assessment

DNA extracts were quantified in FLUOstar Omega® and had the fragment sizes assessed through agarose gel electrophoresis. For the 26 samples that were photographed, the assessment was done in detail through Agilent TapeStation®. Differences in yield between the two methods were statistically compared through a paired t-test. We visually checked for DNA integrity in the electrophoresis quality control and classified for the presence and absence of fragments larger than 1500 bp. The difference between the methods was statistically compared through McNemar's test.

In order to test whether any of the extraction methods would provide better results for NGS methods, we chose five samples (ten total replicates: five TS and five WB) that had distinct collection dates across the tested range and presented rather contrasting results between the two methods and produced libraries from them. Libraries can be prepared with a very wide variety of DNA sources, but samples with very low quality or quantity are likely to require specific methods or some sort of special treatment to obtain successful preparations (Tyler et al. 2016, Mcdonough et al. 2019). Although further optimisation has been necessary for atyids according to our experience, we deemed that leaving the protocol as unaltered as possible would help to test the viability of the extracts and the overall success rate of the methods. In addition, since contamination cannot be ruled out without sequencing or, at the very least, with PCR using taxon-specific primers, we chose samples with similar TapeStation profiles, which may be seen as an indication that DNA from both samples had the same source.

Aliquots of 100 ng of DNA from the extracts were then used to prepare libraries with the NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina®, aiming for fragments 250-350 bp long. We followed the protocol made available by the manufacturer: samples were incubated for 6 min at 37°C for fragmentation (step 1.1.5), indices were diluted to 1.5 µM (10 times) (step 1.2.1) and we used 7 cycles in the PCR enrichment (step 1.4.3). The success of the procedure was measured by comparing the size and concentration of the fragments in Agilent TapeStation. Library concentrations were compared through a paired t-test.

Pictures of the samples from before and after the digestion were visually compared. The examination checked for the integrity and shape of the characters that would allow the identification of the species: rostrum length and indentation, carapace length, telson and

uropodia length and preservation of the setae, thoracic appendages, dactyli and exoskeleton adornments. Comparison was made subjectively as to perceive changes that would impede identification to species-level.

All the statistical analyses and plots were made in R (R Core Team 2021) with the packages: dplyr (Wickham et al. 2023), tidyverse (Wickham et al. 2019) and ggpubr (Kassambara 2021).

Results and discussion

As expected, there was a significant difference in yield between the TS and WB assays (Fig. 1), as well as a clear correlation between age and yield. Our results also show that such correlation (curve inclination) is stronger for WB, but this is likely due to the difference in size of the individuals: since the size of the tissue fragments were similar, the maximum amount of DNA that could be yielded from them was also similar, whereas the outliers in body size increased the difference in yield between WB and TS. Meanwhile, there was little difference in the DNA preservation profile: where we managed to retrieve genomic DNA from samples, we did so in both sets (Fig. 2; Suppl. materials 2, 3). Obtaining DNA from museum samples is generally more challenging than from fresh samples and DNA quality is a main factor influencing the success of molecular methods (McGaughran 2020, Ruiz-Gartzia et al. 2022). Thus, whole-body extraction might be helpful to obtain more DNA to work with, which might be a necessity for very old samples and/or for projects that are in their first stages, when protocols are still being standardised.

A few of the samples had rather incongruent and unexpected profiles (intact DNA from the TS and degraded DNA from the WB; see Suppl. materials 2, 3). It is noteworthy that all these were large samples (larger than 2.5 cm in body length), which could suggest that it might have something to do with penetration; either of the lysis solution or the alcohol during fixation (Srinivasan et al. 2002). Although it cannot be ruled out, incomplete fixation is less likely since almost all specimens used in this study were fixed in 95-96% ethanol according to standard protocols for freshwater decapod crustaceans (particularly atyids) in the field and permanent collections (Ng 2017, Klotz et al. 2023). Nevertheless, not all large samples presented this problem and, without a proper test for contamination, we had to exclude these samples from further consideration and assumed that the similar profile was an indication that the DNA had the same source in both replicates.

A major problem we faced was due to the choice of extracting a piece of muscle from the anterior abdomen: we chose that region instead of an appendage to obtain tissue that was richer in DNA and, thus, have a fair comparison. However, several of our samples were already damaged, either by age (or shortcomings in preservation) or by handling (previous or our own) (e.g. Fig. 3e; see 'before' pictures in Suppl. material 2). Some of the smaller individuals also had their bodies split in half and/or their carapace broken, but the only taxonomic character that was visibly affected by this problem was carapace length. Since this problem did not seem to affect the finer characters, which were our main target

for assessment and since damage by handling is a problem that many, if not most, collections have to deal with, we decided to continue the procedure.

The lysis in and of itself did not seem to damage the taxonomic characters enough to hinder identification. However, while larger specimens presented no problems with preservation, smaller specimens were noticeably softened and cleared. In these individuals, the appendages became much more sensitive to breakage, detachment and loss or to being distorted or modified in a way that makes them difficult to study. In addition to this, some small individuals (< 1.5 cm) became so transparent that they became hard to find in the tube and so soft that they became very difficult to handle. Shorter lysis may diminish these effects, but the digestion period we employed was already very short and further reductions might be counterproductive in terms of yield. Different taxa have different demands in terms of morphological preservation, but we suggest that this method must be carried out carefully with smaller individuals because, although characters are not destroyed, the analysis might be hampered. Destruction is, thus, not null, relative to the nature of the characters required by taxonomic work. More importantly, we advise against using this method with type specimens as the advantages do not outweigh the risks.

Library results differences between TS and WB were not significant (Fig. 4; p-value = 0.096). All samples used in the preparation had rather large DNA fragments (> 1000 bp; Fig. 2), but all of them presented a wide range of fragment sizes. We predicted that WB samples would yield more concentrated libraries despite the similar profiles in fragment sizes because the initial proportion of larger fragments would be higher. However, our results show that even the most basic library preparation kits can produce viable libraries in rather shorn DNA samples. Samples from the WB group were just slightly higher than their TS counterparts in concentration and the library size between the two was similar.

Conclusions

Our experiment may provide future research with quantitative and qualitative evaluations to help scientists to weigh their decisions according to what they have available. Overall, the whole-body extraction significantly increases DNA yield while preserving morphologically relevant traits. We must underline, though, that additional taxonomic characters that are not limited to the exoskeleton might, in future, be employed and subsequently revealed to be more sensitive to digestion. Plus, there are different levels of alteration in the samples' bodies according to their size (and respective degree of penetration of the lysis solution). The larger quantity afforded by the lysis of the entire shrimp body may compensate for degradation (Mcdonough et al. 2019, McGaughran 2020, Settlecowski et al. 2023), but the demand for specific methods or procedures to treat degraded samples remains true, especially for older samples.

Acknowledgements

We thank the Museum für Naturkunde (MfN) Berlin, Center for Integrative Biodiversity Discovery (CIBD), for the financial and tangible support. Oliver Coleman and Antje Schwiering (MfN) provided the loan of all ZMB samples. Parts of this study were financed by the DAAD RISE programme (Research Internships in Science and Engineering). This grant given to SCB as a PhD mentor allowed SA to complete a summer research internship at the MfN and support SCB's lab work. Additionally, we would like to especially thank the MfN technicians Isabelle Waurick and Robert Schreiber as well as Berlin Center for Genomics in Biodiversity Research (BeGenDiv) together with their staff, Sarah Sparmann and Susan Mbedi. Finally, we would like to thank Prof. Dr. Rudolf Meier (MfN) for adding his expertise and knowledge to the project.

Author contributions

Samuel C. Bernardes: Conceptualisation (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead), validation (lead); visualisation (equal); writing – original draft (lead); writing – review and editing (lead). **Thomas von Rintelen**; Conceptualisation (lead); supervision (equal); methodology (equal), validation (equal); writing – review and editing (equal). **Serena Alexander**: Investigation (equal); methodology (equal), writing – review and editing (equal). **Fiona Lorenz**: Investigation (equal); methodology (equal), visualisation (equal); writing – review and editing (equal). **Kristina von Rintelen**: Conceptualisation (lead); supervision (lead); methodology (equal), validation (equal); visualisation (equal); writing – original draft (equal); writing – review and editing (equal).

Conflicts of interest

The authors have declared that no competing interests exist.

References

- Alberch P (1993) Museums, collections and biodiversity inventories. Trends in Ecology and Evolution 8 (10): 372-375. https://doi.org/10.1016/0169-5347(93)90222-B
- Billerman S, Walsh J (2019) Historical DNA as a tool to address key questions in avian biology and evolution: A review of methods, challenges, applications, and future directions. Molecular Ecology Resources 19 (5): 1115-1130. https://doi.org/10.1111/1755-0998.13066
- Briggs A, Heyn P (2012) Preparation of next-generation sequencing libraries from damaged DNA. Methods in Molecular Biology 840: 143-154. https://doi.org/10.1007/978-1-61779-516-9_18

- Carøe C, Gopalakrishnan S, Vinner L, Mak ST, Sinding M, Samaniego J, Wales N, Sicheritz-Pontén T, Gilbert MTP (2018) Single-tube library preparation for degraded DNA. Methods in Ecology and Evolution 9 (2): 410-419. https://doi.org/10.1111/2041-210X.12871
- Chen H, Rangasamy M, Tan SY, Wang H, Siegfried B (2010) Evaluation of five methods for total DNA extraction from western corn rootworm beetles. PLoS ONE 5 (8). https://doi.org/10.1371/journal.pone.0011963
- Dean M, Ballard JWO (2001) Factors affecting mitochondrial DNA quality from museum preserved *Drosophila simulans*. Entomologia Experimentalis et Applicata 98 (3): 279-283. https://doi.org/10.1046/j.1570-7458.2001.00784.x
- de Mazancourt V, Boseto D, Marquet G, Keith P (2020) Solomon's gold mine: Description or redescription of 24 species of *Caridina* (crustacea: Decapoda: Atyidae) freshwater shrimps from the solomon islands, including 11 new species. European Journal of Taxonomy 2020 (696): 1-86. https://doi.org/10.5852/ejt.2020.696
- Dillon N, Austin AD, Bartowsky E (1996) Comparison of preservation techniques for DNA extraction from hymenopterous insects. Insect Molecular Biology 5 (1): 21-24. https://doi.org/10.1111/j.1365-2583.1996.tb00036.x
- Gansauge MT, Meyer M (2013) Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. Nature Protocols 8 (4): 737-748. https://doi.org/10.1038/nprot.2013.038
- Garm A, Watling L (2013) The Crustacean Integument: Setae, Setules, and Other Ornamentation. In: Watling L, Thiel M (Eds) Functional Morphology and Diversity. Oxford University Press, New York, 31 pp. [ISBN 9780190267780]. https://doi.org/10.1093/ACPROF:OSOBL/9780195398038.003.0006
- Gilbert MTP, Moore W, Melchior L, Worebey M (2007) DNA extraction from dry museum beetles without conferring external morphological damage. PLoS ONE 2 (3). https://doi.org/10.1371/journal.pone.0000272
- Hahn E, Alexander M, Grealy A, Stiller J, Gardiner D, Holleley C (2022) Unlocking inaccessible historical genomes preserved in formalin. Molecular Ecology Resources 22 (6): 2130-2147. https://doi.org/10.1111/1755-0998.13505
- Jaksch K, Eschner A, Rintelen T, Haring E (2016) DNA analysis of molluscs from a museum wet collection: A comparison of different extraction methods. BMC Research Notes 9 (1): 1-12. https://doi.org/10.1186/s13104-016-2147-7
- Kassambara A (2021) ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.6.0. URL: https://rpkgs.datanovia.com/ggpubr/.
- Klimov P, OConnor B (2008) Origin and higher-level relationships of psoroptidian mites (Acari: Astigmata: Psoroptidia): Evidence from three nuclear genes. Molecular Phylogenetics and Evolution 47 (3): 1135-1156. https://doi.org/10.1016/j.ympev.2007.12.025
- Klotz W, Rintelen Tv, Annawaty A, Wowor D, Rintelen Kv (2023) *Caridina clandestina*, new species, an unusual new freshwater shrimp (Crustacea: Decapoda: Atyidae) from the remote high elevation Napu Valley of Sulawesi, Indonesia. *The Raffles Bulletin of Zoology* 71: 12-25. https://doi.org/10.26107/RBZ-2023-0002
- Krishtalka L, Humphrey PS (2000) Can Natural History Museums Capture the Future? BioScience 50 (7): 611-617. https://doi.org/10.1641/0006-3568(2000)050[0611:CNHMCT]2.0.CO;2

- Lang M, Nagy O, Lang C, Orgogozo V (2015) High throughput preparation of fly genomic DNA in 96-well format using a paint-shaker. Fly 9 (3): 138-144. https://doi.org/10.1080/19336934.2015.1137401
- Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362 (6422): 709-715. https://doi.org/10.1038/362709a0
- Linderholm A (2016) Ancient DNA: The next generation - chapter and verse. Biological Journal of the Linnean Society 117 (1): 150-160. https://doi.org/10.1111/bij.12616
- Mandrioli M (2008) Insect collections and DNA analyses: How to manage collections? Museum Management and Curatorship 23 (2): 193-199. https://doi.org/10.1080/09647770802012375
- Martin J (2016) Collecting and processing crustaceans: An introduction. Journal of Crustacean Biology 36 (3): 393-395. https://doi.org/10.1163/1937240X-00002436
- Mcdonough S, Bhagwate A, Sun Z, Wang C, Zschunke M, Gorman J, Kopp K, Cunninghamid J (2019) Use of FFPE-derived DNA in next generation sequencing: DNA extraction methods. PLOS ONE 14 (4). https://doi.org/10.1371/JOURNAL.PONE.0211400
- McGaughran A (2020) Effects of sample age on data quality from targeted sequencing of museum specimens: What are we capturing in time? BMC Genomics 21 (1): 1-10. https://doi.org/10.1186/S12864-020-6594-0/TABLES/2
- Ng PL (2017) Collecting and processing freshwater shrimps and crabs. Journal of Crustacean Biology 37 (1): 115-122. https://doi.org/10.1093/jcbiol/ruw004
- Patzold F, Zilli A, Hundsdoerfer A (2020) Advantages of an easy-to-use DNA extraction method for minimal-destructive analysis of collection specimens. PLOS ONE 15 (7). https://doi.org/10.1371/JOURNAL.PONE.0235222
- Prosser SJ, Dewaard J, Miller S, Hebert PN (2016) DNA barcodes from century-old type specimens using next-generation sequencing. Molecular Ecology Resources 16 (2): 487-497. https://doi.org/10.1111/1755-0998.12474
- Raxworthy C, Smith BT (2021) Mining museums for historical DNA: advances and challenges in museomics. Trends in Ecology and Evolution 36 (11): 1049-1060. https://doi.org/10.1016/j.tree.2021.07.009
- R Core Team (2021) R: A language and environment for statistical computing. 4.1.2. R Foundation for Statistical Computing. Release date: 2021-11-01. URL: https://www.R-project.org
- Ruiz-Gartzia I, Lizano E, Marques-Bonet T, Kelley J (2022) Recovering the genomes hidden in museum wet collections. Molecular Ecology Resources 22 (6): 2127-2129. https://doi.org/10.1111/1755-0998.13631
- Settlecowski AE, Org A, Marks BD, Manthey JD (2023) Library preparation method and DNA source influence endogenous DNA recovery from 100-year-old avian museum specimens. bioRxiv https://doi.org/10.1101/2023.02.06.527363
- Srinivasan M, Sedmak D, Jewell S (2002) Effect of Fixatives and Tissue Processing on the Content and Integrity of Nucleic Acids. The American Journal of Pathology 161 (6): 1961-1971. https://doi.org/10.1016/S0002-9440(10)64472-0
- Straube N, Lyra M, Paijmans JA, Preick M, Basler N, Penner J, Rödel MO, Westbury M, Haddad CB, Barlow A, Hofreiter M (2021) Successful application of ancient DNA extraction and library construction protocols to museum wet collection specimens. Molecular Ecology Resources 21 (7): 2299-2315. https://doi.org/10.1111/1755-0998.13433

- Thomsen PF, Elias S, Gilbert MTP, Haile J, Munch K, Kuzmina S, Froese D, Sher A, Holdaway R, Willerslev E (2009) Non-destructive sampling of ancient insect DNA. PLoS ONE 4 (4). https://doi.org/10.1371/journal.pone.0005048
- Tyler A, Christianson S, Knox N, Mabon P, Wolfe J, Van Domselaar G, Graham M, Sharma M (2016) Comparison of Sample Preparation Methods Used for the Next-Generation Sequencing of *Mycobacterium tuberculosis*. PLOS ONE 11 (2). https://doi.org/10.1371/JOURNAL.PONE.0148676
- Uebeler JL, Rumpf CMA, de Mazancourt V, Von Rintelen K (2022) Workflow protocol for the digitization of ethanol-preserved isopods to accelerate the process of species description. Museum für Naturkunde Berlin (MfN) - Leibniz Institute for Evolution and Biodiversity Science https://doi.org/10.7479/padn-cx88
- Van Dijk E, Jaszczyszyn Y, Thermes C (2014) Library preparation methods for next-generation sequencing: Tone down the bias. Experimental Cell Research 322 (1): 12-20. https://doi.org/10.1016/j.yexcr.2014.01.008
- Vink C, Thomas S, Paquin P, Hayashi C, Hedin M (2005) The effects of preservatives and temperatures on arachnid DNA. Invertebrate Systematics 19 (2): 99-104. https://doi.org/10.1071/IS04039
- Wang YS, Dai TM, Tian H, Wan FH, Zhang GF (2019) Comparative analysis of eight DNA extraction methods for molecular research in mealybugs. PLoS ONE 14 (12). https://doi.org/10.1371/journal.pone.0226818
- Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the Tidyverse. Journal of Open Source Software 4 (43). https://doi.org/10.21105/joss.01686
- Wickham H, François R, Henry L, Müller K, Vaughan D (2023) dplyr: A Grammar of Data Manipulation. URL: https://dplyr.tidyverse.org, https://github.com/tidyverse/dplyr
- Zimmermann J, Hajibabaei M, Blackburn D, Hanken J, Cantin E, Posfai J, Evans T (2008) DNA damage in preserved specimens and tissue samples: A molecular assessment. Frontiers in Zoology 5 (1): 1-13. https://doi.org/10.1186/1742-9994-5-18

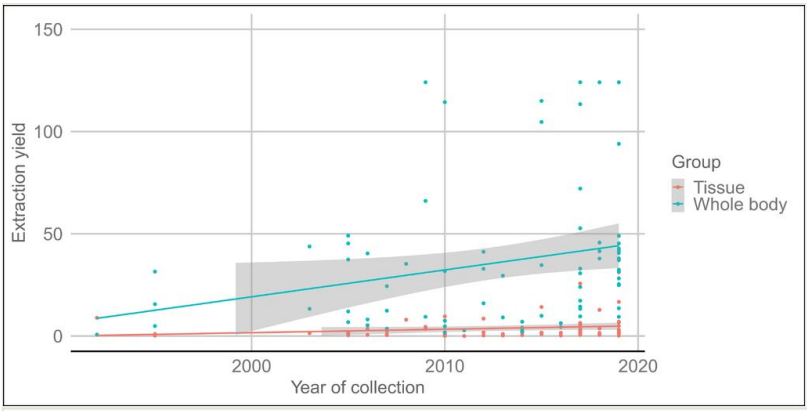

Figure 1.

Distribution of the DNA yield per year of collection per extraction method: tissue extraction (TS) and whole-body extraction (WB). A trend line with the respective standard deviation was added for each extraction group. The grey shade representing the standard deviation range was removed where it included negative values. The difference between methods is significant ($t = -8.49$, $df = 71$, $p\text{-value} < 0.01$).

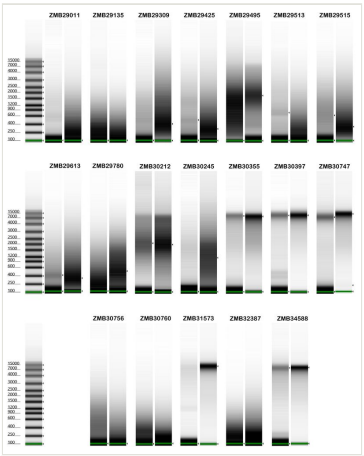

Figure 2.

Comparison of the Agilent TapeStation® profiles of photographed samples between tissue (TS, left column) and whole-body (WB, right column) extractions. Scales on the left represent fragment sizes in base pairs (bp). Samples are identified with their ZMB collection number. (For detailed profiles including samples that were excluded due to suspicions of contamination and the agarose gel images for the samples that were not photographed, see Suppl. material 2.)

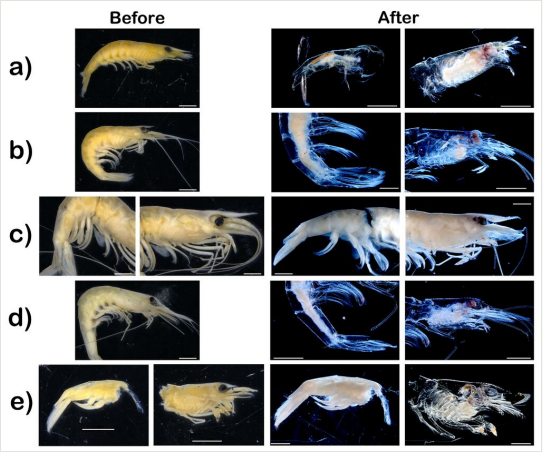

Figure 3.

Selected photographed samples before (left) and after (right) lysis, with emphasis on characters used for taxonomic identification. Specimens shown in the arrangement are ZMB29135-2 (a), ZMB29425-3 (b), ZMB30245-1 (c), ZMB31573-3 (d) and ZMB32141-2 (e). (For detailed pictures of all photographed samples, see Suppl. material 2.)

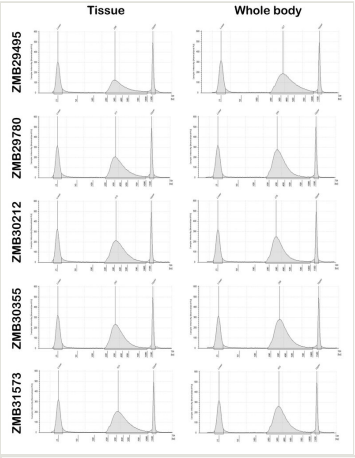

Figure 4.

Agilent TapeStation® graphs of prepared libraries per extraction method. Concentration is represented on the y-axis, whereas fragment sizes are represented on the x-axis.

Supplementary materials

Suppl. material 1: Supplementary Table S1

Authors: Samuel Chagas Bernardes, Thomas von Rintelen, Serena Alexander, Fiona Lorenz, Kristina von Rintelen
Data type: Collection data
Brief description: Table S1: Samples used in this study.
Download file (14.19 kb)

Suppl. material 2: Supplementary Figure S1

Authors: Samuel Chagas Bernardes, Thomas von Rintelen, Serena Alexander, Fiona Lorenz, Kristina von Rintelen
Data type: Images
Brief description: Figure S1: Agilent TapeStation profiles of all the photographed samples and detailed photos before and after lysis. The scale on the left represents size of the fragments in bp. Numbers above the pictures represent the sample ID. ID numbers in red represent samples that were removed from DNA yield and quality analyses due to the possibility of contamination (see text).
Download file (56.15 MB)

Suppl. material 3: Supplementary Figure S2

Authors: Samuel Chagas Bernardes, Thomas von Rintelen, Serena Alexander, Fiona Lorenz, Kristina von Rintelen
Data type: Electrophoresis image
Brief description: Figure S2: Agarose gel profile for the non-photographed samples. Paired tissue (TS) and whole-body replicates, as well as collection ZMB ID numbers are specified on the right.
Download file (2.46 MB)