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Evaluating DNA quality in Coleoptera and Lepidoptera: Impact of fixation and preservation in various trapping methods

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Abstract

Despite advancements in barcoding and metabarcoding, preserving high-quality DNA from field-collected arthropods remains challenging. Although various fixatives and preservatives are used for DNA recovery in Coleoptera (Carabidae) and Lepidoptera (Noctuidae, Nolidae, Geometridae, and Tortricidae), their effects on DNA quality across trapping methods are not fully understood. This study evaluates fixation and preservation strategies affecting DNA integrity, focusing on pH changes before and after tissue grinding to improve consistency. For Carabidae, *Calathus fuscipes* (L.) were collected with a Malaise trap, while *Platynus assimilis* (Paykull) were collected via emergence traps and pitfall traps (with and without roof), using propylene glycol as a fixative. Preservation methods included storage in propylene glycol, 96% ethanol, or drying, with samples kept at −20°C for 1 year. Propylene glycol samples were washed with distilled water prior to grinding. Additional fixatives in individual trapping included ethylene glycol, propylene glycol, ethanol, brine, ethyl acetate, vinegar, and drying (with and without silica gel), stored at −20°C for 3 months. For Lepidoptera, specimens were categorized by size: large—*Agrostis exclamationis* (L.) (Noctuidae), medium—*Meganola strigula* (Denis et Schiffermüller) (Nolidae), *Eupithecia insigniata* (Hübner) (Geometridae), and small—*Pelochrista caecimaculana* (Hübner) (Tortricidae). Specimens were treated with chloroform (vapor and soaked) or cyanide vapors and stored at room temperature for 3 months. DNA quality was assessed through fragmentation analysis and PCR amplification of COI fragments (658, 313, and 157 bp for Coleoptera and 658, 311, and 220 bp for Lepidoptera) with Sanger sequencing. Results showed reduced DNA integrity in diluted Malaise trap samples, while distilled water washing improved readability in emergence trap samples. Brine proved a cost-effective preservative. For Lepidoptera, DNA preservation depended on sample size and fixative, with small chloroform-soaked specimens yielding non-sequencable DNA, while vapor-treated samples remained sequencable. This study offers insights to optimize DNA yield and preservation for arthropod research.

KEYWORDS
arthropod barcoding, Carabidae, COI amplification, cyanide vapor, fragmentation analysis, molecular methods, PCR success, propylene glycol, UV LED light trap, DNA preservation

INTRODUCTION

Recent advancements in arthropod trapping methods have greatly improved our capacity to study diverse taxa. Techniques like Malaise traps, emergence traps, pitfall traps (both with and without roof), and UV LED light traps have been refined to capture a wide variety of arthropods efficiently (Devigne & Biseau, 2014; Infusino et al., 2017; Kopr et al., 2023; Marrec et al., 2015). As these methods evolve, the need for effective fixation and preservation protocols becomes increasingly crucial to maintain specimens for both morphometrical and molecular analyses (Beermann

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et al., 2021; Bisanti et al., 2009; Packard, 2021; Vogt, 2018). Stabilizing specimens directly in the field, followed by controlled storage, helps to safeguard DNA integrity for long-term analysis (Ballare et al., 2019; Camacho-Sanchez et al., 2013; Gotoh et al., 2014; Santos & Fernandes, 2021).

The integration of molecular tools, such as barcoding and metabarcoding, with traditional trapping techniques has enhanced our understanding of species interactions, community dynamics, and ecological responses to environmental changes (Bewick et al., 2016; Graux et al., 2024; Hao et al., 2020; Kumar et al., 2022; Šigut et al., 2017). High-quality genomic DNA (gDNA) is essential for downstream applications widely used in conservation and ecological research (Kress et al., 2015; Vargovčík et al., 2024) and increasingly within citizen science initiatives (Chiovitti et al., 2019; Svenningsen et al., 2021). In these contexts, effective fixation and preservation methods are critical for taxa like Coleoptera and Lepidoptera, which play key roles in biodiversity studies (Guo et al., 2022; Remmel et al., 2024; Santos & Fernandes, 2021; Shokralla et al., 2014). However, selecting an optimal preservative can be challenging, as methods that maintain specimen morphology may not always preserve DNA integrity—a critical factor for molecular analyses (Marquina et al., 2020; Moreau et al., 2013). Complementary laboratory experiments using freshly killed specimens could establish baseline DNA quality and better isolate preservation effects from environmental degradation, enhancing future protocols (Camacho-Sanchez et al., 2013; Dean & Ballard, 2001).

For Coleoptera, commonly used preservatives include 96% ethanol, 70% denatured ethanol, ethylene glycol, propylene glycol, saturated salt solution (brine), ethyl acetate, simple drying, and acetic acid (Graux et al., 2024; Jureková et al., 2019; Kwon et al., 2022; McCravy & Willand, 2007; Steininger et al., 2015). In contrast, preserving Lepidoptera often involves agents like chloroform or cyanide vapors, which help retain the morphological features essential for accurate species identification (Bibi et al., 2021; Iserhard et al., 2013; Porto et al., 2015; Willows-Munro & Schoeman, 2015). Each preservative presents trade-offs; while effective for morphological or morphometrical preservation, some agents may compromise DNA quality, limiting their suitability for molecular applications (Hajibabaei et al., 2005; Moreau et al., 2013; Schield et al., 2016). Furthermore, DNA quality can be influenced not only by the choice of fixatives but also by collection duration and transport conditions, emphasizing the need for comprehensive preservation strategies (Camacho-Sanchez et al., 2013; Pokluda et al., 2014).

To optimize DNA preservation, particularly for molecular applications, additional factors, such as fragmentation levels, must be considered (Gossner et al., 2016; Höfer et al., 2015; Ruppert et al., 2023; Zizka et al., 2022). Maintaining DNA quality involves more than just ensuring purity and quantity; it also requires minimizing fragmentation and optimizing gDNA accessibility during Proteinase K incubation, which is critical for efficient PCR amplification and sequencing (Frazer et al., 2020; Knebelsberger & Stöger, 2012).

Previous studies on Coleoptera have explored different fixatives for DNA preservation, yielding varying results (Bisanti et al., 2009; Ferro & Park, 2013; Reiss et al., 1995). Propylene glycol, ethanol, and ethylene glycol are frequently used for samples collected with pitfall traps (Höfer et al., 2015; Kwon et al., 2022; Nagy, 2010). However, while ethanol, acetone, and ethyl acetate are effective for preserving morphological features, they can disrupt protein structures and cause DNA fragmentation, which limits their use in protocols like ddRAD-seq (Feng et al., 2021; Griebenow & Klibanov, 1996; Lesch et al., 2015; Reiss et al., 1995; Tóth et al., 2014). Propylene glycol and ethylene glycol have shown greater promise for long-term DNA preservation due to their ability to reduce DNA fragmentation when compared with ethanol or Renner solution (Bhakuni, 1998; Höfer et al., 2015; Nakamura et al., 2020). Minimizing handling during post-fixation preservation is also essential for preventing DNA degradation (Beermann et al., 2021; Egonyu et al., 2021).

Research on the combination of propylene glycol as a fixative in traps with ethanol (70% or 96%) as a preservative is limited but promising (Höfer et al., 2015; Moreau et al., 2013; Rubink et al., 2003). For long-term preservation with pure propylene glycol, residuals can interfere with PCR enzymes, potentially impacting amplification and sequencing quality (Ferro & Park, 2013; Patrick et al., 2016). This issue may be mitigated by washing samples with distilled water prior to analysis (Celere & Gostoli, 2004; Dhale et al., 2004).

Preserving Lepidoptera poses unique challenges, requiring a balance between DNA and morphological quality. UV LED traps commonly employ chloroform or cyanide vapors as killing-fixation agents, which work effectively with dried specimens (Infusino et al., 2017; Landry & Landry, 1994; Stutt & Willmer, 1998). The efficacy of these agents depends largely on their ability to penetrate and interact with cells and peptide structures, which may vary based on specimen size and preservation conditions (Khavani et al., 2020; Suvarna et al., 2019). Electrochemical studies have noted impacts of these reagents on DNA quality and sequencing accuracy (Porfireva et al., 2021). Cyanide has been shown to yield cleaner, more amplifiable DNA compared with chloroform, making it a preferred option in certain cases (Demeke & Jenkins, 2010; Hoy, 2003; Psifidi et al., 2010).

External factors, including temperature, pressure, and ionic strength, are also essential for preserving high-molecular-weight gDNA across different taxa (Bhakuni, 1998; Nagy, 2010; Suvarna et al., 2019). Conditions like shaking and elevated temperatures (e.g., 37°C) may inhibit robust amplicon formation, impacting sequencing outcomes (Ruppert et al., 2023). Successful amplification and Sanger sequencing of full-length DNA fragments, especially the ~658 base pairs (bp) from the mitochondrial cytochrome c oxidase subunit 1 (COI) gene (Henter et al., 2016; Kress et al., 2015; Robinson et al., 2021), underscore the challenge of maximizing high-molecular-weight DNA recovery across trapping methods (Feng et al., 2021; Pokluda et al., 2014; Tóth et al., 2014). This study seeks to address current gaps in optimal fixation and preservation methods, with the following objectives:

1. Evaluate the impact of washing propylene glycol-fixed samples with distilled water, and monitor pH changes before and after sample grinding, using buffer T1 and tissue samples from Coleoptera (Carabidae) and Lepidoptera collected through conventional trapping methods.
2. Perform stepwise PCR amplification of COI barcodes targeting progressively shorter amplicon sizes, followed by agarose gel electrophoresis to assess amplification success. Validate the most successful full-length products by Sanger sequencing.
3. Analyze gDNA integrity through fragmentation analysis, informed by preliminary concentration measurements.

MATERIALS AND METHODS

Experimental design

This study systematically evaluates various trapping methods, fixation and preservation strategies for Coleoptera: Carabidae and Lepidoptera. The approach to sample handling was designed to fit the specific mode of investigation for each trapping method. Samples were collected, identified, measured, and subsequently maintained in a controlled environment to minimize unnecessary handling and preserve DNA integrity. Carabidae specimens were collected using Malaise traps, individual trapping, emergence traps, and pitfall traps (with and without roofs), while Lepidoptera specimens were trapped using UV LED light traps. The number of replicates was adjusted according to the specific trapping methods and standardized fixation–preservation combinations. Hence, this study presents four independent sub-experiments, as outlined below:

Malaise traps—propylene glycol was used as the fixation agent (without replacement, diluted by rainfall), and 70% ethanol was used for preservation. A total of eight replicates were employed, and collections were made on three sampling dates. Samples from this method were stored at −20°C for 1 year.

Individual trapping—eight replicates were used across nine different preservation agents: ethylene glycol, propylene glycol, drying with and without silica gel, 96% ethanol, 70% ethanol, brine, ethyl acetate, and vinegar (industrial acetic acid derived from potato starch). Samples were stored at −20°C for 3 months.

Propylene glycol washing—this was applied to emergence traps, pitfall traps (both with and without roofs), and individual trapping. Four replicates were used for the washing treatment, where distilled water was applied before the pre-lysis step for samples preserved in 100% propylene glycol. Control samples, either dried or preserved in 96% ethanol, were included, with eight replicates each, in total 96 samples. Samples were stored at −20°C for 1 year.

UV LED light trap (Lepidoptera)—four replicates were used for each of the three size categories. Specimens were trapped killed, and preserved for 6 h using chloroform vapor, chloroform-soaked, or cyanide vapor fixatives, resulting in a total of 36 samples categorized by size (small, medium, and large). Samples were stored dry at room temperature for 3 months. Cyanide vapor served as a benchmark for chloroform, as it has no effect on fragment size (Willows-Munro & Schoeman, 2015).

Sample set up and extraction

In this study, a total of 192 Coleoptera (Carabidae) specimens and 36 Lepidoptera specimens were used. Carabidae specimens were selected based on the most frequently sampled species for each trapping method, ensuring homogeneity within each sub-experiment. For Lepidoptera, efforts were made to standardize the samples by selecting individuals of comparable sizes across the three size categories.

In Malaise traps, 24 specimens of *Calathus fuscipes* (L.) (Coleoptera: Carabidae) were collected during a 14-day exposure period, as specified by Kopr et al. (2023), on the following dates: April 13–28 (14 days), April 28–May 12 (28 days), and May 12–26 (42 days). Initially, 100% propylene glycol was used as the fixation medium; however, it became diluted over the 42-day period due to precipitation. Cumulative dilution estimates indicate that the propylene glycol concentration decreased from 100% to approximately 74.2% after the first 14 days, then to 54.1% after 28 days, and finally to about 33.4% after 42 days without replenishment (provided in data repository). These estimates were based on daily precipitation data, an approximate evaporation rate of 0.5 mm/day, and the structural constraints of the trap (dimensions: 190 × 142 × 65 mm; total volume: 1200 mL; fixative volume: 800 mL). This modeling provided a stepwise estimation of concentration decline under the local weather conditions during the study. The trap was located in Cerová vrchovina, near the border of southern Slovakia and northern Hungary, close to the village of Drňa (48°15′35.35″ N, 20°07′15.76″ E) at an elevation of 148 m a.s.l.

In the individual trapping, 72 specimens of *Platynus assimilis* (Paykull, 1790) (Coleoptera: Carabidae) were collected on January 13, 2024, near the city of Ústí nad Orlicí (49°59′23.4″ N, 16°28′24.6″ E) at an elevation of 496 m a.s.l.

In the propylene glycol washing sub-experiment, 96 specimens of *P. assimilis* were collected using various trapping methods, including emergence traps, pitfall traps (with and without roofs), and individual trapping. During the 24-day exposure period, specimens from all methods, except individual trapping, were fixed in pure propylene glycol following the protocols outlined by Sapia et al. (2006) and Hohbein and Conway (2018). Specimens collected one time via individual trapping served as controls for trap type, as they were not exposed to field conditions. After field fixation, the specimens were sorted by trapping method and further preserved using one of three treatments divided into thirds: (i) one-third of the total specimens were stored in fresh propylene glycol, (ii) one-third were transferred to 96% ethanol, and (iii) one-third were left to dry without any additional preservative. The 96% ethanol and drying treatments served as positive controls for preservation type. All samples were subsequently stored at −20°C for 1 year to simulate long-term preservation. Prior to molecular analysis, a washing was performed on specimens preserved in fresh propylene glycol. These specimens were divided into two groups: (i) the washed group, comprising one-sixth of the total specimens, underwent a washing procedure with distilled water, and (ii) the unwashed group, comprising an additional one-sixth, which remained as a control. gDNA was extracted from all samples after the 1-year storage period to evaluate the impacts of trapping method, preservation type, and washing treatment on DNA quality. The sub-experiment began on July 15, 2023, in Podyjí National Park, located in Southern Moravia, Czech Republic, near the village of Lukov (48°52′26.9″ N, 15°53′34.2″ E) at an elevation of 385 m a.s.l.

For the UV LED light trap (Lepidoptera), a total of 36 specimens were categorized by size: large–*Agrostis exclamationis* (L.) (Noctuidae); medium–*Meganola strigula* (Denis & Schiffermüller, 1775) (Nolidae), *Eupithecia insigniata* (Hübner, 1790) (Geometridae); and small–*Pelochrista caecimaculana* (Hübner, 1799) (Tortricidae). Specimens were collected on April 6, 2024, at Cerová vrchovina, along the border between southern Slovakia and northern Hungary, near the village of Gamerský Jablonec—Vadokaš (48°11′6.4″ N, 19°59′15.4″ E) at an elevation of 225 m a.s.l.

The right hind femur of each specimen was carefully dissected for genetic analysis using sterilized forceps, which were cleaned between samples by washing in 96% ethanol and wiping with a disposable towel. Femurs preserved in the storage agent were air-dried on filter paper in a Petri dish for 48 h before gDNA extraction. gDNA was extracted using the NucleoSpin Tissue Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), following the manufacturer's protocol with adjustments to monitor propylene glycol washing sub-experiment and to measure pH before and after the pre-lysis step (Data S1). pH was measured using an Orion Star 3 meter with a semi-micro electrode (8103BN; Thermo Fisher Scientific, Waltham, MA, USA). The measurement was taken in a 1.5 mL Eppendorf tube containing 180 μL of buffer T1 and the femur sample ("before" measurement). The femur was then ground with a sterile pestle, and pH was measured again ("after" measurement). Proteinase K (25 μL) was added to aid digestion. Between measurements, the electrode was rinsed with distilled water and wiped dry.

Post-extraction, gDNA was eluted in 100 μL of BE buffer and stored at −20°C. DNA concentrations were measured using a NanoDrop Spectrophotometer (ND-2000c; Thermo Fisher Scientific), yielding an average concentration of 2 ng/μL of dsDNA, with BE buffer as a baseline. According to manufacturer specifications, pure DNA generally shows a 260/280 ratio around 1.8 and a 260/230 ratio between 2.0 and 2.2. Samples below these thresholds were further purified with the MinElute PCR Purification Kit (cat. no.: 28006; Qiagen, Hilden, Germany), using 10 μL of gDNA to enhance readability for fragmentation analysis (see Data S2).

PCR success and fragmentation analysis

Polymerase chain reaction (PCR) was performed using a total reaction volume of 20 μL, which included 10 μL of hot start Combi PPP Master Mix (C210; Top-Bio, s.r.o.), 3 μL ddH2O, 1 μL each of forward and reverse primers, and 5 μL of gDNA per sample. The same PCR protocol was applied for both Coleoptera: Carabidae and Lepidoptera (Table S1).

For Coleoptera, we initially targeted amplification of the full-length COI region (~658 bp) using primers dgLCO1490 (5′-GGT CAA CAA ATC ATA AAG AYA TYG G-3′; Meyer & Paulay, 2005) and dgHCO2198 (5′-TAA ACT TCA GGG TGA CCA AAR AAY CA-3′; Meyer, 2003), as described by Ruppert et al. (2023). When amplification was unsuccessful in a replicate, we tested the same biological sample again with a shorter 313-bp region, using the reverse primer dgHCO2198 and forward primer mlCOIintF (5′-GGW ACW GGW TGA ACW GTW TAY CCY CC-3′; Leray et al., 2013). For samples that still did not yield a product, a 157-bp minimalist region was targeted using the primer pair ZBJ-ArtF1c (5′-AGA TAT TGG AAC WTT ATA TTT TAT TTT TGG-3′) and ZBJ-ArtR2c (5′-WAC TAA TCA ATT WCC AAA TCC TCC-3′; Zeale et al., 2011).

This stepwise approach was similarly followed for Lepidoptera samples, starting with the full-length COI region (~658 bp) using dgLCO1490 and LepR (5′-TAA ACT TCT GGA TGT CCA AAA AAT CA-3′; Hajibabaei, Janzen, et al., 2006). If unsuccessful, we then targeted a 311-bp region using LepF (5′-ATT CAA CCA ATC ATA AAG ATA TTG G-3′; Hajibabaei, Janzen, et al., 2006) and MH-MR1 (5′-CCT GTT CCA GCT CCA TTT TC-3′; Hajibabaei, Janzen, et al., 2006). For samples failing both attempts, a 220-bp region was amplified with lib220-1 (5′-TGG TAT TCC AAT TAT AGG TG-3′; Hajibabaei, Smith, et al., 2006) and LepR (see Tables S2 and S3 for a primer overview).

Due to varying sample sizes across experiments, we adapted our PCR approach by testing biological replicates whenever an amplicon could not be obtained. PCR success rates of full-length COI were then converted to percentages, allowing us to account for amplification failures without introducing technical replicates. We defined a threshold for DNA degradation: if over 75% of replicates failed to amplify, we categorized the sample as degraded.

PCR success was evaluated on 3% agarose gels, with Combi PPP Master Mix used for gel-ready reactions.

Successful replicates were selected for cross-validation via Sanger sequencing to provide a comprehensive view of PCR outcomes. For Coleoptera, the best-looking positive product (full-length COI) from each preservative replicate was sequenced, while for Lepidoptera, all positive PCR products from each size and fixative replicate were sequenced. Sequencing was performed on an ABI 3500 Genetic Analyzer at the Faculty of Agri Sciences, Department of Animal Morphology, Physiology, and Genetics (Brno, Czech Republic).

Further analysis was conducted using the 5200 Fragment Analyzer System (Agilent Technologies) with the DNF-468-33-HS Genomic DNA 50-kb Kit (Agilent Technologies). Average fragment sizes were calculated with the PROSize v.5.0.1.6 software (Agilent Technologies). DNA fragments above the high-molecular-weight threshold (>50 000 bp) were not further subdivided. As with PCR, we did not include technical replicates in fragment analysis. For samples yielding no measurable results, we referenced NanoDrop data to confirm low or undetectable concentrations, where relevant. Fragment analysis was performed by the Institute of Molecular Genetics of the CAS, Laboratory of Genomics and Bioinformatics, Prague, Czech Republic.

Statistical analysis

All statistical analyses were performed in the R environment (v.4.3.2; R Development Core Team, 2023) with a significance threshold of $\alpha = 0.05$. These analyses evaluated the effects of different trapping methods, fixation, and preservation strategies on the average DNA fragment size (bp) in Coleoptera (Carabidae) and Lepidoptera samples. This multi-step approach ensured that the chosen model accurately reflected the data characteristics and supported meaningful analysis of the effects across all experimental factors.

To determine the most appropriate family for the generalized linear model (GLM), we initially assessed the skewness and kurtosis of the raw average fragment size data using the *moments* package (Komsta, 2022). Following recommendations from Bullard et al. (2010), we aimed to capture the distribution's asymmetry in the model selection process. For symmetric data (skewness between −0.5 and +0.5), a Gaussian distribution with log-normal transformation was applied. For data with high right-skewness (skewness >+1), a Gamma distribution with a log link function was chosen, aligning with approaches outlined by Hooper et al. (2010), Monich et al. (2014) and Alfahad et al. (2023). If left-skewed data had been encountered (skewness <–1), we would have explored generalized additive models (GAMs) with log-normal transformation, as suggested by Wood (2020), to flexibly accommodate any non-linearity in the data distribution.

Outliers were then identified based on standardized residuals exceeding an absolute value of 2 and a Cook's distance threshold calculated as 4 divided by the number of observations. Observations meeting both criteria were excluded to refine the dataset, improve model accuracy, and enhance the fit.

In the next step, the finalized GLM model incorporated pH (calculated as $\mathrm{pH}_{\mathrm{before}}$—$\mathrm{pH}_{\mathrm{after}}$) as a covariate to control for its potential influence on the response variable. This model was fitted using the *"glm()"* function, allowing for robust interpretation of results. To confirm the relevance of including pH, we conducted an Analysis of Deviance with a Chi-square test to compare models with and without the pH covariate, which indicated whether the addition of pH significantly improved the model fit.

We further applied the Breusch–Pagan test using the *lmtest* package to assess heteroscedasticity (Zeileis & Hothorn, 2002). If heteroscedasticity was detected, we adjusted for it by calculating heteroscedasticity-consistent standard errors using the *"coeftest()"* function. Finally, model residuals were tested for normality with the Shapiro–Wilk test. If non-normality was found, bootstrapping was conducted with 1000 resamples using the *boot* package to provide bias-corrected estimates and strengthen the robustness of our inference (Canty & Ripley, 2024).

For the individual trapping method using nine preservative agents, as well as for Lepidoptera samples collected in UV LED light traps and fixed with chloroform or cyanide, we performed pairwise contrasts. These comparisons were conducted using the *emmeans* package with Dunnett's adjustment "dunnettx" for preservative agents and Tukey's adjustment "tukey" for Lepidoptera samples. All data and model result visualizations were generated using the *ggplot2* package (Wickham, 2016). In each graph, a dashed line represents the barcode library threshold, set at 10000 bp. A minimum average fragment size of 10 kb is generally considered sufficient to produce high-quality DNA barcodes for accurate species identification and subsequent genetic analyses (Johnson et al., 2023).

RESULTS

Mitochondrial cytochrome c oxidase subunit 1 amplification with different primer pairs

The success rate of amplifying the full-length COI region varied by exposure time, preservation agents, trapping methods, and fixatives. Amplification success was defined as the probability of obtaining a full-length amplicon across biological replicates. A success threshold of 75% was set for each sub-experiment, achieving success in three out of four or six out of eight replicates.

In the Malaise trap sub-experiment, the first exposure time, conducted after a 14 days, achieved a 100% amplification success rate for the 658-bp fragment (Figure S1). After 28 days without a change in the fixation agent, the success rate dropped to 63%, with amplicon sizes reduced to 313 bp in five out of eight samples (Figure S2). By the third exposure time, after 42 days, no samples produced successful results, and all fragment sizes had dropped to 313 bp (Figure S2). Sanger sequencing confirmed the expected sequences for each selected top-quality product based on gel results from the first and second intervals, despite the second falling below the 75% threshold (Figure S1).

In the individual trapping experiment, nine different preservation agents were tested, with seven achieving or exceeding the 75% amplification success threshold (Figure S3). Ethyl acetate and vinegar showed lower success rates, at 38% and 25%, respectively, and did not yield any sequenceable products. Of the samples preserved in ethyl acetate, five out of eight produced 313-bp fragments, while vinegar-preserved samples yielded 313-bp fragments in five out of eight samples and a 157-bp fragment in one sample (Figure S2).

In the propylene glycol washing sub-experiment, washing with distilled water enhanced amplification success for samples collected from emergence traps, individual traps, and pitfall trap without roof, compared with unwashed samples (Figure S4). Full-length COI from washed specimens collected in emergence and individual traps was successfully sequenced. However, amplification from pitfall trap samples, regardless of roof presence, remained inconsistent, with fragment sizes reduced to 313 bp (Figure S2).

In the Lepidoptera UV light-trapping sub-experiment, fixation using chloroform vapors, chloroform-soaked, and cyanide vapors achieved amplification success rates above the 75% threshold, though some limitations were noted (Figure S5). Specifically, in the small size category (*Plutella xylostella*), samples fixed by chloroform soaking were not sequenceable, despite achieving a 100% amplification success rate. In the medium size category (*Eupithecia insigniata*), samples fixed with cyanide failed to amplify, likely due to primer incompatibility. Multiple primer pair combinations were tested (Table S3), but none produced successful amplification, with readable amplicon size limited to 220 bp (Figure S6).

Average fragment size

To evaluate the effects of various factors on DNA integrity, measured as average fragment size, we conducted GLM analyses on the log-transformed fragment sizes using a Gaussian distribution for optimal fit.

For the fitted model, an analysis of deviance was conducted on the Malaise trap samples, comparing a baseline model with exposure time as the predictor to an extended model that included both exposure time and pH (Figure S7). The addition of pH reduced the residual deviance from 24.833 (20 degrees of freedom) to 16.703 (19 degrees of freedom), representing a statistically significant improvement to the model's explanatory power (χ^2 test = 30.175, df = 1, $p < 0.01$). Consequently, both exposure time and pH were retained in the final model, supporting precipitation-related dilution that influences average fragment size (Figure 1).

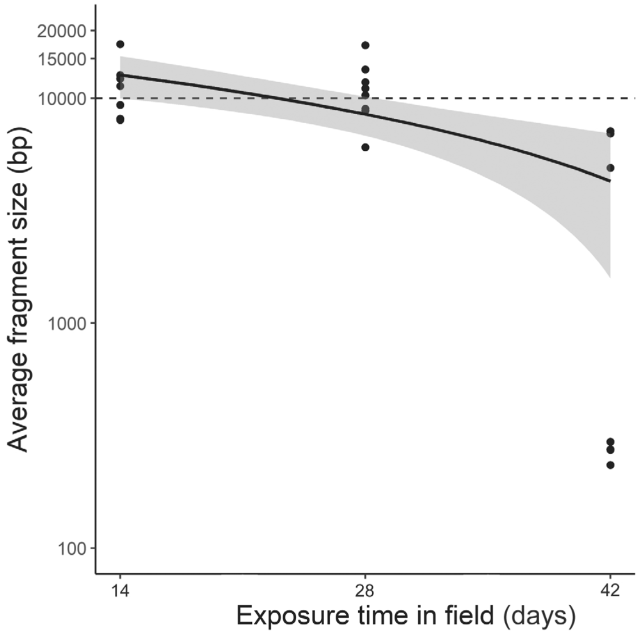

FIGURE 1 Average fragment size from the Malaise trap samples initially fixed with pure propylene glycol and progressively diluted by rainfall over the exposure time period. Samples were preserved in 70% ethanol for 1 year at −20°C. The dashed line represents the barcode library threshold, and the gray bands around the trend line denote the 95% confidence interval. A significant difference was detected based on model fit (χ^2 test: $P < 0.05$). The y-axis is shown on a logarithmic scale.

For individual trapping, to evaluate the fitted model, we conducted non-parametric bootstrapping and heteroscedasticity-consistent (HC1) standard errors to address residual non-normality and heteroscedasticity (Figure S8). The bootstrapped standard errors were consistent with the original estimates, indicating minimal bias and confirming the robustness of our model. An analysis of deviance comparing models with and without pH showed a modest improvement in fit with pH included ($p = 0.07$) (Figure S8). Pairwise comparisons of preservation efficacy across nine preservation agents were conducted using estimated marginal means (EMMs) from the GLM, adjusted with a Dunnett-type correction (Figure S9). The results indicated that 70% ethanol, ethyl acetate, and vinegar significantly reduced fragment preservation compared with ethylene glycol, with 70% ethanol showing a markedly lower DNA yield, while ethyl acetate and vinegar exhibited the greatest reductions. Other fixation methods, including 96% ethanol, brine, drying with silica gel, drying without silica gel, and propylene glycol, showed no significant differences from ethylene glycol, suggesting comparable effectiveness in preserving fragments (Figure 2).

For the propylene glycol washing sub-experiment, interactions among trapping method, preservation agents, and washing were evaluated as follows (Figure 3): (i) the interaction between trapping method and preservation agents showed that adding pH to the model slightly reduced residual deviance, though this reduction was not significant ($p = 0.77$), suggesting a limited role for pH in explaining

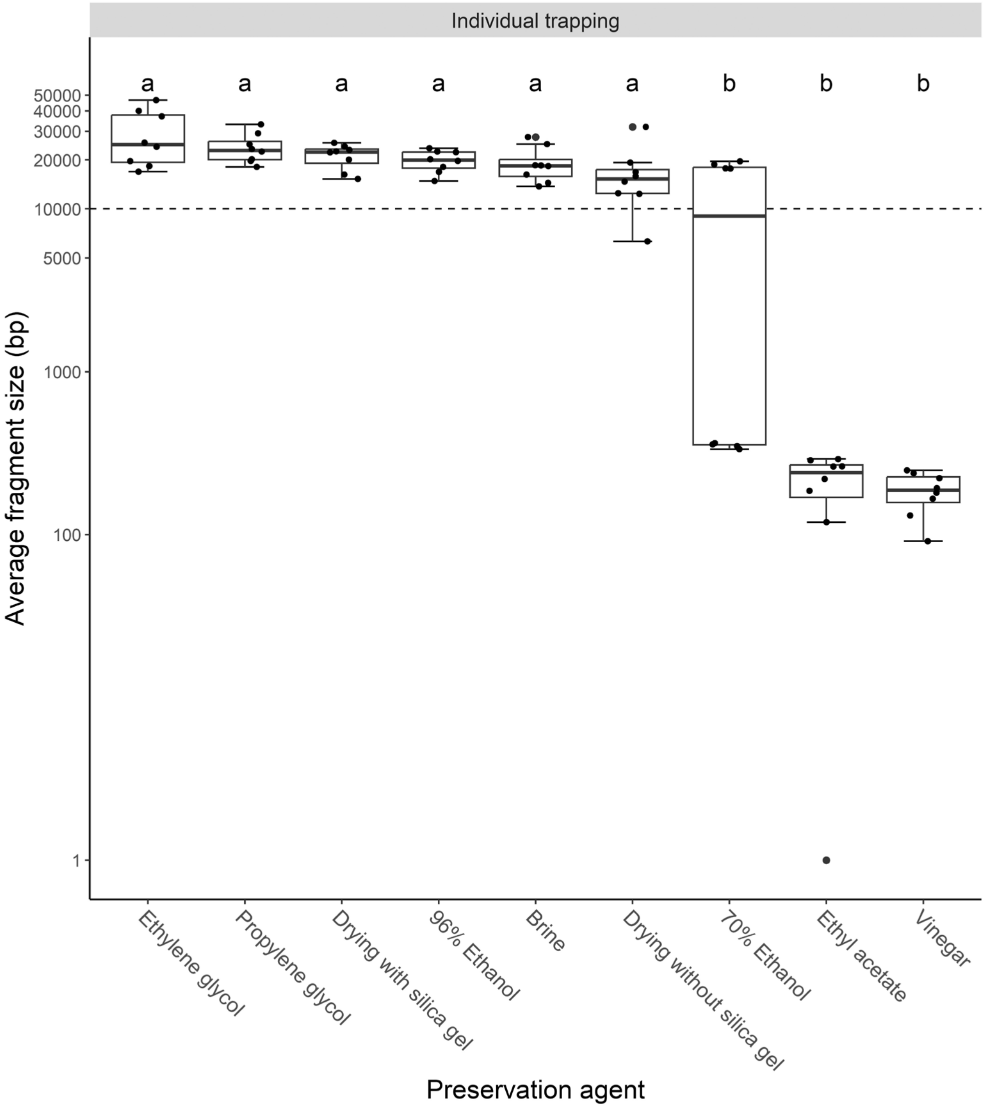

FIGURE 2 Average fragment size from the individual trapping sub-experiment using nine different preservation agents, arranged in descending order of effectiveness in maintaining larger fragments. Samples were stored for 3 months at −20°C. The dashed line represents the barcode library threshold. Boxplot details: The top and bottom of each box represent the third and first quartiles, the line within indicates the median, whiskers extend to 1.5× the interquartile range. The dots represent individual data points. Different letters indicate significant differences from the baseline treatment (ethylene glycol), based on pairwise comparisons ($p < 0.05$). The y-axis is shown on a logarithmic scale.

average fragment variability (Figure S10). Positive associations were noted for pitfall trap with roof combined with either dried or propylene glycol preservation, indicating potential improvement in preservation compared with the emergence trap baseline. Overall, however, the pitfall trap with and without roof had negative associations with fragment size, which showed worse integrity compared with the emergence trap. (ii) For the interaction of preservation agents and washing, HC1 standard errors confirmed a positive association for dried samples (Estimate = 2.022, SE = 0.682, $p < 0.01$), while pH showed a potential, though not significant, effect ($p = 0.073$). Washing alone showed no impact among four trapping methods. (iii) In the interaction of trapping method and washing, the pitfall trap with roof and dried preservation was negatively associated with fragment size (Estimate = −7.425, SE = 1.188, $p < 0.01$), indicating reduced effectiveness compared with the emergence trap washing (Figure S11). In contrast, individual collection without washing (Estimate = 3.300, SE = 1.336, $p < 0.01$) and pitfall trap without roof and without washing (Estimate = 4.109, SE = 1.464, $p < 0.01$) were positively associated with fragment size. The main effect of pH remained non-significant (Estimate = −1.154, SE = 2.067, $p = 0.58$), indicating limited impact.

In the Lepidoptera UV light-trapping sub-experiment, model inclusion of pH to some extent improved fit ($p = 0.045$), suggesting that pH may contribute to explaining variations in average fragment size across fixation agents and sizes (Figure S12). Significant interactions were found, particularly for smaller samples, where fixation method had a pronounced effect on DNA fixation. Pairwise contrasts indicated that cyanide was significantly

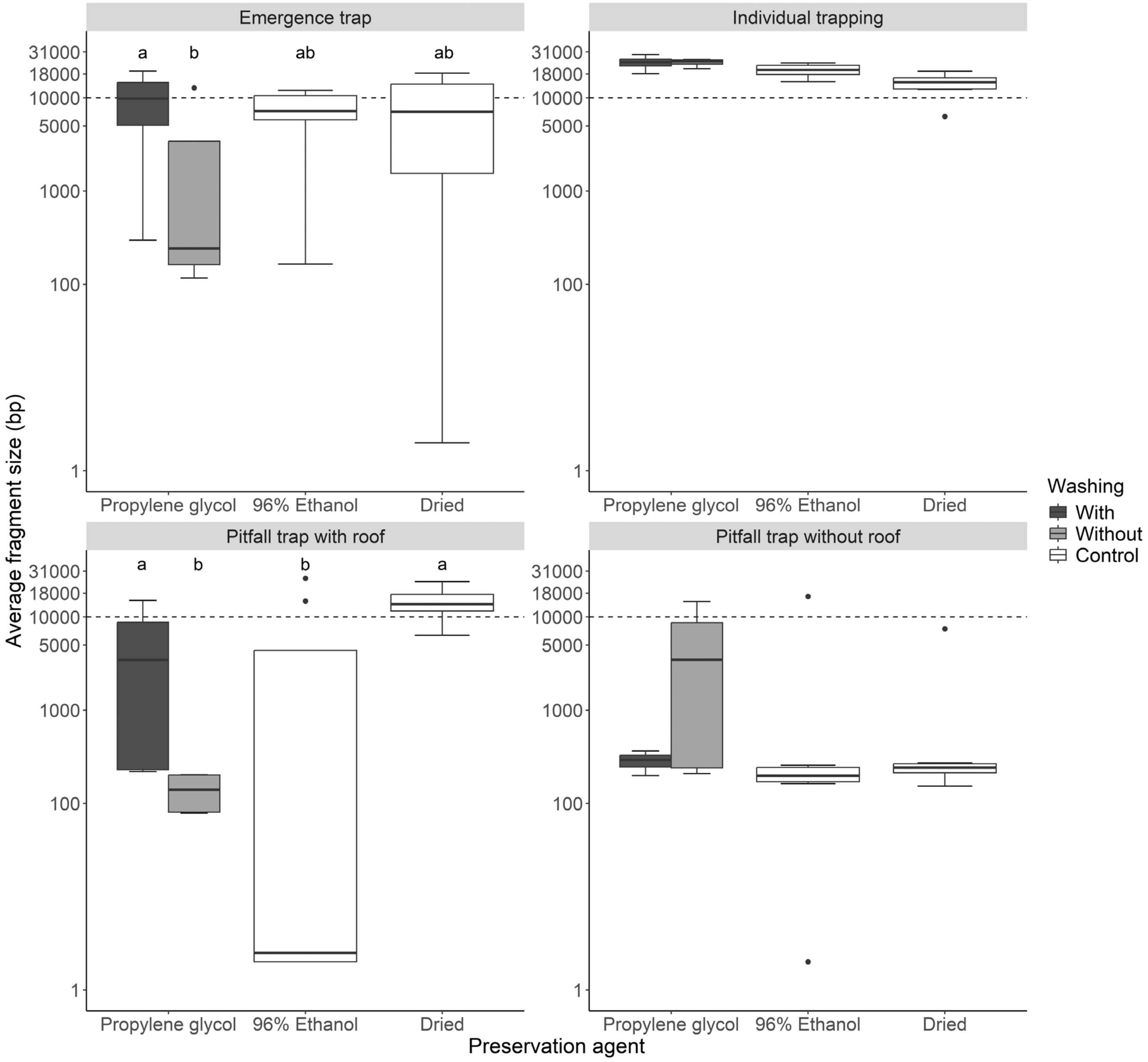

FIGURE 3 Average fragment size from four trapping methods comparing samples preserved in propylene glycol (with and without distilled water washing), alongside controls stored in 96% ethanol and dried without silica gel. Samples were stored at −20°C for 1 year. The dashed line represents the barcode library threshold. Boxplot details: The top and bottom of each box represent the third and first quartiles, the line within indicates the median, whiskers extend to 1.5× the interquartile range, and dots denote outliers. Significant differences between treatments are indicated by different letters (pairwise contrasts: $P < 0.05$). The y-axis is shown on a logarithmic scale.

more effective in preserving DNA fragments than both chloroform-soaked and chloroform vapors for the small category ($p < 0.01$ for both comparisons). No significant differences were detected between large and medium size categories, suggesting that the preservation effectiveness of cyanide diminishes with larger sizes (Figure 4).

DISCUSSION

Genetic tools, such as metabarcoding, are becoming increasingly valuable in ecological research, where they significantly expedite species identification (Kress et al., 2015; Remmel et al., 2024; Shokralla et al., 2014) and enable detailed investigations of population dynamics in tandem with morphometric analyses (Tatsuta et al., 2017) and citizen science initiatives (Chiovitti et al., 2019; Steininger et al., 2015; Svenningsen et al., 2021). Trapping methods, including Malaise traps, emergence traps, various forms of pitfall traps (both with and without roof), and UV LED light traps, have proven effective for collecting a broad spectrum of Coleoptera and Lepidoptera species (Devigne & Biseau, 2014; Infusino et al., 2017; Skvarla et al., 2021). Despite the varied methodologies employed by these

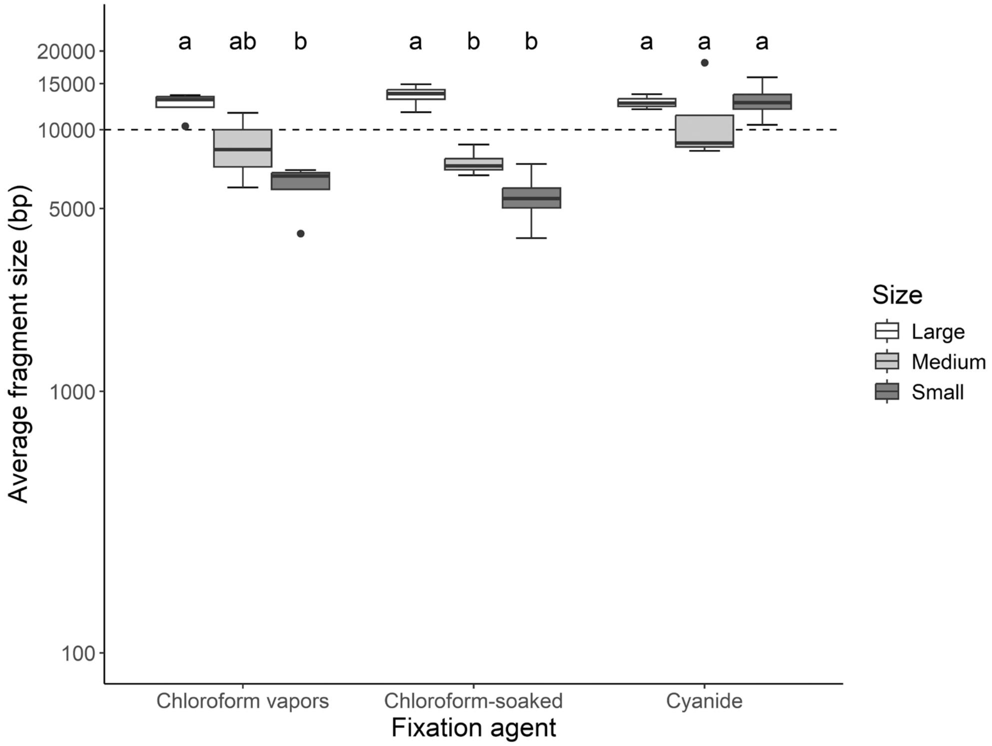

FIGURE 4 Average fragment size from Lepidoptera UV light-trapping samples, comparing three size categories (large, medium, and small) across three fixation agents (cyanide, chloroform-soaked, and chloroform vapors). Samples were stored at room temperature for 3 months. The dashed line represents the barcode library threshold. Boxplot details: The top and bottom of each box represent the third and first quartiles, the line within indicates the median, whiskers extend to 1.5× the interquartile range, and dots denote outliers. Significant differences between treatments are indicated by different letters (pairwise contrasts: $P < 0.05$). The y-axis is shown on a logarithmic scale.

traps, practical guidelines for specimen preservation remain insufficient, particularly regarding protocols that ensure the reliability of both morphological and genetic assessments (Martoni et al., 2021; Nagy, 2010; Steininger et al., 2015; Vink et al., 2005). Although various fixatives and preservatives have been employed for the barcoding or metabarcoding of Coleoptera and Lepidoptera (Gossner et al., 2016; Pokluda et al., 2014; Steininger et al., 2015), a comprehensive comparison of these trapping methods, including the impact of preservation factors like pH, DNA fragmentation, and PCR success, has yet to be undertaken.

Impact of trapping, pH, and preservation on DNA integrity and PCR success

Each trapping method and fixation–preservation combination influences DNA quality, as indicated by PCR success and average fragment sizes. For Coleoptera (Carabidae), gradual dilution of fixative affected Malaise trap samples, which led to notable reductions in both PCR success and DNA fragmentation over exposure time, ultimately lowering DNA yield (Arbeli & Fuentes, 2007; Ballare et al., 2019). The degradation is further enhanced by acid hydrolysis, which increased the observed pH changes in the pre-lysis step (Gossner et al., 2016; Strickler et al., 2015; Tsuji et al., 2017). While this study focuses on the mitochondrial COI gene, we acknowledge that nuclear and ribosomal RNA genes, which degrade at different rates due to their distinct cellular structures, could provide valuable complementary insights (see Bloch et al., 2023). Future research adopting a multi-gene approach could further explore these differences (Foran, 2006).

In the individual trapping sub-experiment, preservation agents showed varying levels of success in maintaining DNA integrity. Ethylene glycol, propylene glycol, 96% ethanol, brine, and drying with and without silica gel effectively preserved high-quality DNA over a 3-month period at −20°C, making them suitable for molecular analysis (Dawson et al., 1998; Höfer et al., 2015; Martoni et al., 2021). Both ethylene glycol and propylene glycol are valuable for preserving DNA as well as specimen morphology (Kwon et al., 2022; Patrick et al., 2016; Steininger et al., 2015). However, ethylene glycol poses health risks, primarily renal toxicity and metabolic acidosis (Brent, 2012; Corley & McMartin, 2005), which restrict its use (Gossner et al., 2016). For the sole purpose of DNA preservation, 96% ethanol is an effective but costly option, making brine a more economical alternative (Marquina et al., 2020; McCravy & Willand, 2007; Seutin et al., 1991). The high salt concentration in brine can inhibit nuclease activity over short periods, helping to reduce DNA degradation (De Vos et al., 2021; Gajardo & Beardmore, 2012). Conversely, ethyl acetate and vinegar resulted in smaller, fragmented amplicons, likely due to lower pH caused by acid hydrolysis (An et al., 2014; Liu et al., 2014). The volatility of 70% ethanol on PCR success and fragment size may stem from evaporation and partial DNA dehydration, causing inconsistent preservation quality (Ladell et al., 2019; Nakahama et al., 2019; Nakamura et al., 2020; Robinson et al., 2021).

Propylene glycol combined with a distilled water wash in emergence and individual trapping enabled the extraction of large DNA fragments and improved PCR success compared with unwashed samples (Ascencio et al., 2016; Dhale et al., 2004; Ferro & Park, 2013). In contrast, while washing enhanced fragment readability in pitfall traps without roofs, it did not result in successful PCR amplification, indicating that the effectiveness of distilled water washing is dependent on the trapping method (Höfer et al., 2015; Nakamura et al., 2020; Pokluda et al., 2014). Undiluted propylene glycol was more effective at preserving large DNA fragments by encapsulating the tissue, which allowed effective removal of excess preservative during washing (Butterwort et al., 2022; Montgomery et al., 2021; Murthy et al., 2022). Additionally, pH did not appear to influence DNA integrity in propylene glycol-preserved samples, suggesting that acid hydrolysis was not a factor (Moreau et al., 2013; Nakahama et al., 2019; Vink et al., 2005); however, DNA degradation due to exonuclease activity caused by higher summer temperatures was observed (Bauer et al., 2012; Peng et al., 2018; Ruppert et al., 2023). Laboratory controls with freshly killed specimens could help isolate preservation effects from environmental degradation, providing baseline DNA quality and refining preservation protocols over longer storage periods (Camacho-Sanchez et al., 2013; Robinson et al., 2021; Ruppert et al., 2023).

For Lepidoptera, although amplification was successful in both chloroform vapor and chloroform-soaked samples, fragmentation analysis and pH stability indicated that cyanide vapors generally provided superior preservation, particularly for small-sized samples (Dean & Ballard, 2001; Willows-Munro & Schoeman, 2015; Xu et al., 2020). Although amplification was successful in chloroform-soaked samples, sequencing was inhibited, probably due to the interaction of chloroform with cells and peptide structures, which may hinder downstream processing (Almira et al., 2003; Khavani et al., 2020; Porfireva et al., 2021; Suvarna et al., 2019). However, our inability to amplify medium-sized cyanide-preserved samples requires careful primer selection (Willows-Munro & Schoeman, 2015).

Consequences for arthropod trapping methods in barcoding studies

Effective DNA preservation requires trapping and preservation strategies tailored to specific applications, as no single method universally meets all criteria (Kress et al., 2015; Moreau et al., 2013; Remmel et al., 2024). This study demonstrates that trapping methods and preservation agents influence DNA integrity, impacting PCR success and sequencing reliability in Coleoptera (Carabidae) and Lepidoptera (Graux et al., 2024; Shokralla et al., 2014; Vink et al., 2005).

Malaise trap: Diluted fixative led to DNA degradation in Coleoptera, highlighting the need for adequate fixative concentrations to maintain DNA integrity during prolonged field exposure (Camacho-Sanchez et al., 2013; Dean & Ballard, 2001; Martoni et al., 2021; Skvarla et al., 2021; Thomas, 2008). Regular fixative replenishment, such as using pure propylene glycol every 14 days, is recommended (Ballare et al., 2019; Nagy, 2010).

Emergence trap: Propylene glycol effectively preserved high-quality DNA in Coleoptera over 24 days (Höfer et al., 2015; Nakamura et al., 2020). Washing with distilled water further improved DNA extraction success, supporting its use in genetic and morphometric studies (Martoni et al., 2021).

Pitfall traps: Propylene glycol used in pitfall traps, both with and without roof, showed poor barcoding results after 24 days of field exposure, even when washed with distilled water. This outcome highlights challenges in preserving DNA quality during extended field exposure (Ballare et al., 2019; Jureková et al., 2019; Ruppert et al., 2023). As a result, these traps are better suited for biodiversity assessments than for molecular applications (Graux et al., 2024; Montgomery et al., 2021).

Individual trapping: Over a shorter 3-month period, pure propylene glycol effectively preserved DNA integrity, with no residual inhibition of enzymatic reactions observed, confirming its suitability (Ferro & Park, 2013; Patrick et al., 2016). Brine also emerged as a cost-effective alternative for scenarios where DNA quality is critical (McCravy & Willand, 2007; Seutin et al., 1991).

UV light-trapping for Lepidoptera: Fixation method and specimen size strongly influenced DNA quality. Cyanide vapor was more effective than chloroform soaking, particularly for smaller specimens (Khavani et al., 2020; Willows-Munro & Schoeman, 2015; Xu et al., 2020).

Ultimately, our findings underscore that effective DNA preservation for barcoding applications requires careful consideration of environmental conditions, limits of fixatives and preservatives, and arthropod size (Beermann et al., 2021; Gossner et al., 2016; Kumar et al., 2022; Peng et al., 2018; Strickler et al., 2015). Accounting for these factors can improve PCR success and sequencing reliability, advancing molecular study precision and biodiversity understanding for ecological efforts.

AUTHOR CONTRIBUTIONS

Dominik Stočes: Conceptualization; formal analysis; methodology; visualization; writing – original draft; writing – review and editing; funding acquisition; resources; project administration; data curation. **Jan Šipoš:** Conceptualization; formal analysis; visualization; methodology; supervision; writing – original draft; writing – review and editing. **Tamara Wijacki:** Conceptualization; methodology; writing – original draft; funding acquisition. **Aleš Knoll:** Supervision;

writing – original draft. **Tomáš Kopecký:** Conceptualization; visualization; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Datasets used in the present study can be found in the Zenodo.org data repository https://doi.org/10.5281/zenodo.14723906.

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REFERENCES

Alfahad, M.F., Ghitany, M.E., Alothman, A.N. & Nadarajah, S. (2023) A bimodal extension of the log-normal distribution on the real line with an application to DNA microarray data. *Mathematics*, *11*, 3360. Available from: https://doi.org/10.3390/math11153360

Almira, E.C., Panayotova, N. & Farmerie, W.G. (2003) Capillary DNA sequencing: maximizing the sequence output. *Journal of Biomolecular Techniques*, *14*, 270–277.

An, R., Jia, Y., Wan, B., Zhang, Y., Dong, P., Li, J. et al. (2014) Non-enzymatic depurination of nucleic acids: factors and mechanisms. *PLoS One*, *9*, e115950. Available from: https://doi.org/10.1371/journal.pone.0115950

Arbeli, Z. & Fuentes, C.L. (2007) Improved purification and PCR amplification of DNA from environmental samples. *FEMS Microbiology Letters*, *272*, 269–275. Available from: https://doi.org/10.1111/j.1574-6968.2007.00764.x

Ascencio, S., Choe, C., Meinke, M., Müller, R., Maksimov, G., Wigger-Alberti, W. et al. (2016) Confocal Raman microscopy and multivariate statistical analysis for determination of different penetration abilities of caffeine and propylene glycol applied simultaneously in a mixture on porcine skin ex vivo. *European Journal of Pharmaceutics and Biopharmaceutics*, *104*, 51–58. Available from: https://doi.org/10.1016/j.ejpb.2016.04.018

Ballare, K.M., Pope, N.S., Castilla, A.R., Cusser, S., Metz, R.P. & Jha, S. (2019) Utilizing field-collected insects for next-generation sequencing: effects of sampling, storage, and DNA extraction methods. *Ecology and Evolution*, *9*, 13690–13705. Available from: https://doi.org/10.1002/ece3.5756

Bauer, R., Begley, M. & Trakselis, M. (2012) Kinetics and fidelity of polymerization by DNA polymerase III from Sulfolobus solfataricus. *Biochemistry*, *51*, 1996–2007. Available from: https://doi.org/10.1021/bi201799a

Beermann, A.J., Werner, M.T., Elbrecht, V., Zizka, V.M.A. & Leese, F. (2021) DNA metabarcoding improves the detection of multiple stressor responses of stream invertebrates to increased salinity, fine sediment deposition, and reduced flow velocity. *Science of the Total Environment*, *750*, 141969. Available from: https://doi.org/10.1016/j.scitotenv.2020.141969

Bewick, A.J., Vogel, K.J., Moore, A.J. & Schmitz, R.J. (2016) Evolution of DNA methylation across insects. *Molecular Biology and Evolution*, *34*, 654–665. Available from: https://doi.org/10.1093/molbev/msw264

Bhakuni, V. (1998) Alcohol-induced molten globule intermediates of proteins: are they real folding intermediates or off pathway products? *Archives of Biochemistry and Biophysics*, *357*, 274–284.

Bibi, M., Bibi, S., Akhtar, N., Ullah, Z., Khan, M.F. & Qureshi, I.Z. (2021) Butterfly (order: Lepidoptera) species richness, diversity, and distribution in different localities of Battagram, Pakistan. *Saudi Journal of Biological Sciences*, *29*, 1853–1857. Available from: https://doi.org/10.1016/j.sjbs.2021.10.039

Bisanti, M., Ganassi, S. & Mandrioli, M. (2009) Comparative analysis of various fixative solutions on insect preservation for molecular studies. *Entomologia Experimentalis et Applicata*, *130*, 290–296. Available from: https://doi.org/10.1111/j.1570-7458.2008.00821.x

Bloch, M., Mou, D.-F., Helmick, E.E., Pelz-Stelinski, K. & Bahder, B.W. (2023) DNA degradation in Haplaxius crudus (Hemiptera: Cixiidae) and Diaphorina citri (Hemiptera: Liviidae) on yellow sticky traps in Florida. *Journal of Insect Science*, *23*, 14. Available from: https://doi.org/10.1093/jisesa/iead083

Brent, J. (2012) Current management of ethylene glycol poisoning. *Drugs*, *61*, 979–988. Available from: https://doi.org/10.2165/00003495-200161070-00006

Bullard, J.H., Purdom, E., Hansen, K.D. & Dudoit, S. (2010) Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. *BMC Bioinformatics*, *11*, 94. Available from: https://doi.org/10.1186/1471-2105-11-94

Butterwort, V., Dansby, H., Zink, F.A., Tembrock, L.R., Gilligan, T.M., Godoy, A. et al. (2022) A DNA extraction method for insects from sticky traps: targeting a low abundance pest, Phthorimaea absoluta (Lepidoptera: Gelechiidae), in mixed species communities. *Journal of Economic Entomology*, *115*, 844–851. Available from: https://doi.org/10.1093/jee/toac046

Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I. & Leonard, J. (2013) Preservation of RNA and DNA from mammal samples under field conditions. *Molecular Ecology Resources*, *13*(4), 663–673. Available from: https://doi.org/10.1111/1755-0998.12108

Canty, A. & Ripley, B.D. (2024) Boot: bootstrap R (S-plus) functions. R Package Version 1.3–31.

Celere, M. & Gostoli, C. (2004) Osmotic distillation with propylene glycol, glycerol and glycerol-salt mixtures. *Journal of Membrane Science*, *229*, 159–170. Available from: https://doi.org/10.1016/J.MEMSCI.2003.10.025

Chiovitti, A., Thorpe, F., Gorman, C., Cuxson, J.L., Robevska, G., Szwed, C. et al. (2019) A citizen science model for implementing statewide educational DNA barcoding. *PLoS One*, *14*, e0208604. Available from: https://doi.org/10.1371/journal.pone.0208604

Corley, R. & McMartin, K. (2005) Incorporation of therapeutic interventions in physiologically based pharmacokinetic modeling of human clinical case reports of accidental or intentional overdosing with ethylene glycol. *Toxicological Sciences*, *85*, 491–501. Available from: https://doi.org/10.1093/TOXSCI/KFI120

Dawson, M.N., Raskoff, K.A. & Jacobs, D.K. (1998) Field preservation of marine invertebrate tissue for DNA analyses. *Molecular Marine Biology and Biotechnology*, *7*, 145–152 PMID: 11541322.

De Vos, S., Rombauts, S., Coussement, L. et al. (2021) The genome of the extremophile Artemia provides insight into strategies to cope with extreme environments. *BMC Genomics*, *22*, 635. Available from: https://doi.org/10.1186/s12864-021-07937-z

Dean, M.D. & Ballard, J.W.O. (2001) Factors affecting mitochondrial DNA quality from museum-preserved Drosophila simulans. *Entomologia Experimentalis et Applicata*, *98*, 279–283. Available from: https://doi.org/10.1046/j.1570-7458.2001.00784.x

Demeke, T. & Jenkins, G.R. (2010) Influence of DNA extraction methods, PCR inhibitors, and quantification methods on real-time PCR assay of biotechnology-derived traits. *Analytical and Bioanalytical Chemistry*, *396*, 1977–1990. Available from: https://doi.org/10.1007/s00216-009-3150-9

Devigne, C. & Biseau, J. (2014) Urban ecology: comparison of the effectiveness of five traps commonly used to study the biodiversity of flying insects. *Biodiversity*, *5*, 165–174.

Dhale, A., Myrant, L., Chopade, S., Jackson, J. & Miller, D. (2004) Propylene glycol and ethylene glycol recovery from aqueous solution via reactive distillation. *Chemical Engineering Science*, *59*, 2881–2890. Available from: https://doi.org/10.1016/J.CES.2004.02.018

Egonyu, J.P., Kinyuru, J., Fombong, F., Ng'ang'a, J., Ahmed, Y.A. & Niassy, S. (2021) Advances in insects for food and feed. *International Journal of Tropical Insect Science*, *41*, 1903–1911. Available from: https://doi.org/10.1007/s42690-021-00610-8

Feng, Y., Ma, X., Kong, B., Chen, Q. & Liu, Q. (2021) Ethanol induced changes in structural, morphological, and functional properties of whey proteins isolates: Influence of ethanol concentration. *Food Hydrocolloids*, *111*, 106379. Available from: https://doi.org/10.1016/j.foodhyd.2020.106379

Ferro, M.L. & Park, J.-S. (2013) Effect of propylene glycol concentration on mid-term DNA preservation of Coleoptera. *The Coleopterists Bulletin*, *67*, 581–586. Available from: https://doi.org/10.1649/0010-065X-67.4.581

Foran, D.R. (2006) Relative degradation of nuclear and mitochondrial DNA: An experimental approach. *Journal of Forensic Sciences*, *51*, 766–770. Available from: https://doi.org/10.1111/j.1556-4029.2006.00176.x

Frazer, Z., Yoo, C., Sroya, M., Chokshi, C., Crossley, R. & Maronpot, R. (2020) Effect of different proteinase K digest protocols and deparaffinization methods on yield and integrity of DNA extracted from formalin-fixed, paraffin-embedded tissue. *The Journal of Histochemistry and Cytochemistry*, *68*, 171–184. Available from: https://doi.org/10.1369/0022155420906234

Gajardo, G.M. & Beardmore, J.A. (2012) The brine shrimp Artemia: adapted to critical life conditions. *Frontiers in Physiology*, *3*, 185. Available from: https://doi.org/10.3389/fphys.2012.00185

Gossner, M.M., Struwe, J.-F., Sturm, S., Max, S., McCutcheon, M., Weisser, W.W. et al. (2016) Searching for the optimal sampling solution: variation in invertebrate communities, sample condition, and DNA quality. *PLoS One*, *11*, e0148247. Available from: https://doi.org/10.1371/journal.pone.0148247

Gotoh, H., Miyakawa, H., Ishikawa, A., Ishikawa, Y., Sugime, Y., Emlen, D.J. et al. (2014) Developmental link between sex and nutrition: doublesex regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles. *Proceedings of the National Academy of Sciences*, *111*, 917–922. Available from: https://doi.org/10.1073/pnas.1320868111

Graux, Y., Querejeta, M., Gaba, S., Bretagnolle, V. & Boyer, S. (2024) A comparison of live versus kill pitfall traps to assess the diet of carabids through a metabarcoding approach. *Entomologia Experimentalis et Applicata*, *172*, 249–260. Available from: https://doi.org/10.1111/eea.13396

Griebenow, K. & Klibanov, A.M. (1996) On protein denaturation in aqueous–organic mixtures but not in pure organic solvents. *Journal of the American Chemical Society*, *118*, 11695–11700. Available from: https://doi.org/10.1021/ja9615127

Guo, M., Yuan, C., Tao, L., Cai, Y. & Zhang, W. (2022) Life barcoded by DNA barcodes. *Conservation Genetics Resources*, *14*, 351–365. Available from: https://doi.org/10.1007/s12686-022-01291-2

Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L. et al. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *360*, 1959–1967. Available from: https://doi.org/10.1098/rstb.2005.1727

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences*, *103*, 968–971. Available from: https://doi.org/10.1073/pnas.0510466103

Hajibabaei, M., Smith, M.A., Janzen, D.H., Rodriguez, J.J., Whitfield, J.B. & Hebert, P.D.N. (2006) A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes*, *6*, 959–964. Available from: https://doi.org/10.1111/j.1471-8286.2006.01470.x

Hao, M., Jin, Q., Meng, G., Yang, C., Yang, S., Shi, Z. et al. (2020) Using full-length metabarcoding and DNA barcoding to infer community assembly for speciose taxonomic groups: a case study. *Evolutionary Ecology*, *34*, 1063–1088. Available from: https://doi.org/10.1007/s10682-020-10072-y

Henter, H.J., Imondi, R., James, K., Spencer, D. & Steinke, D. (2016) DNA barcoding in diverse educational settings: five case studies. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *371*, 20150340. Available from: https://doi.org/10.1098/rstb.2015.0340

Höfer, H., Astrin, J., Holstein, J., Spelda, J., Meyer, F. & Zarte, N. (2015) Propylene glycol—a useful capture preservative for spiders for DNA barcoding. *Arachnologische Mitteilungen*, *50*, 30–36. Available from: https://doi.org/10.5431/aramit5005

Hohbein, R. & Conway, C. (2018) Pitfall traps: a review of methods for estimating arthropod abundance. *Wildlife Society Bulletin*, *42*, 597–606. Available from: https://doi.org/10.1002/wsb.928

Hooper, S.D., Dalevi, D., Pati, A., Mavromatis, K., Ivanova, N.N. & Kyrpides, N.C. (2010) Estimating DNA coverage and abundance in metagenomes using a gamma approximation. *Bioinformatics*, *26*, 295–301. Available from: https://doi.org/10.1093/bioinformatics/btp687

Hoy, M.A. (2003) *Insect molecular genetics: An introduction to principles and applications*, 2nd edition. San Diego: Academic Press.

Infusino, M., Brehm, G., Di Marco, C. & Scalercio, S. (2017) Assessing the efficiency of UV LEDs as light sources for sampling the diversity of macro-moths (Lepidoptera). *European Journal of Entomology*, *114*, 25–33. Available from: https://doi.org/10.14411/eje.2017.004

Iserhard, C.A., Brown, K.S., Jr. & Freitas, A.V.L. (2013) Maximized sampling of butterflies to detect temporal changes in tropical communities. *Journal of Insect Conservation*, *17*, 615–622. Available from: https://doi.org/10.1007/s10841-013-9546-z

Johnson, M.S., Venkataram, S. & Kryazhimskiy, S. (2023) Best practices in designing, sequencing, and identifying random DNA barcodes. *Journal of Molecular Evolution*, *91*, 263–280. Available from: https://doi.org/10.1007/s00239-022-10083-z

Jureková, N., Raschmanová, N., Kováč, Ľ., Miklisová, D. & Kováčik, Ľ. (2019) Type of fixative solution in pitfall traps as a decisive factor affecting community parameters of Collembola (Hexapoda) inhabiting superficial subterranean habitats. *The Science of Nature*, *106*, 1–10. Available from: https://doi.org/10.1007/s00114-019-1611-3

Khavani, M., Izadyar, M. & Housaindokht, M. (2020) The effects of amino acid sequence and solvent polarity on the self-assembling of cyclic peptide nanotubes and molecular channel formation inside the lipid bilayer. *Journal of Molecular Liquids*, *314*, 113660. Available from: https://doi.org/10.1016/j.molliq.2020.113660

Knebelsberger, T. & Stöger, I. (2012) DNA extraction, preservation, and amplification. In: Kress, W. & Erickson, D. (Eds.) *DNA barcodes. Methods in molecular biology*, Vol. *858*. Totowa, NJ: Humana Press. Available from: https://doi.org/10.1007/978-1-61779-591-6_14

Komsta, L. (2022) Moments, cumulants, skewness, kurtosis and related tests. http://www.komsta.net/

Kopr, D., Šipoš, J. & Schlaghamerský, J. (2023) Importance of stochastic assembly processes influencing beetle communities increases after logging. *Forest Ecology and Management*, *545*, 121296. Available from: https://doi.org/10.1016/j.foreco.2023.121296

Kress, W.J., García-Robledo, C., Uriarte, M. & Erickson, D.L. (2015) DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology & Evolution*, *30*, 25–35. Available from: https://doi.org/10.1016/j.tree.2014.10.008

Kumar, A., Choudhury, B., Dayanandan, S. & Khan, M.L. (Eds.). (2022) *Molecular genetics and genomics tools in biodiversity conservation*. Springer Singapore. Available from: https://doi.org/10.1007/978-3-030-88839-0

Kwon, T.S., Park, Y.K., Jung, J.K., Lee, Y.G., Park, C.W. & Park, Y.S. (2022) Effects of preservatives in pitfall traps for collecting arthropods: a comparison of ethylene glycol and five alternative preservatives. *Journal of Asia-Pacific Biodiversity*, *15*, 541–546. Available from: https://doi.org/10.1016/j.japb.2022.07.001

Ladell, B.A., Walleser, L.R., McCalla, S.G. et al. (2019) Ethanol and sodium acetate as a preservation method to delay degradation of environmental DNA. *Conservation Genetics Resources*, *11*, 83–88. Available from: https://doi.org/10.1007/s12686-017-0955-2

Landry, J.-F. & Landry, B. (1994) A technique for setting and mounting microlepidoptera. *Journal of the Lepidopterists' Society*, *1994*, 205–227.

Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V. et al. (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*, 34. Available from: https://doi.org/10.1186/1742-9994-10-34

Lesch, V., Heuer, A., Tatsis, V.A., Holm, C. & Smiatek, J. (2015) Peptides in the presence of aqueous ionic liquids: tunable co-solutes as denaturants or protectants? *Physical Chemistry Chemical Physics*, *17*, 26049–26053. Available from: https://doi.org/10.1039/C5CP03838C

Liu, D., Zou, X., Zhong, L., Lou, Y., Yang, B. & Yin, Y. (2014) New features of DNA damage by acid hydrolysis in MALDI-TOF mass spectrum. *International Journal of Mass Spectrometry*, *374*, 20–25. Available from: https://doi.org/10.1016/j.ijms.2014.10.001

Marquina, D., Buczek, M., Ronquist, F. & Łukasik, P. (2020) The effect of ethanol concentration on the morphological and molecular preservation of insects for biodiversity studies. *PeerJ*, *9*, e10799. Available from: https://doi.org/10.7717/peerj.10799

Marrec, R., Badenhausser, I., Bretagnolle, V., Börger, L., Roncoroni, M., Guillon, N. et al. (2015) Crop succession and habitat preferences drive the distribution and abundance of carabid beetles in an agricultural landscape. *Agriculture, Ecosystems & Environment*, *199*, 282–289. Available from: https://doi.org/10.1016/j.agee.2014.10.005

Martoni, F., Nogarotto, E., Piper, A.M., Mann, R., Valenzuela, I., Eow, L. et al. (2021) Propylene glycol and non-destructive DNA extractions enable preservation and isolation of insect and hosted bacterial DNA. *Agriculture*, *11*, 77. Available from: https://doi.org/10.3390/agriculture11010077

McCravy, K.W. & Willand, J.E. (2007) Effects of pitfall trap preservative on collections of carabid beetles (Coleoptera: Carabidae). *The Great Lakes Entomologist*, *40*, 154–165.

Meyer, C.P. (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society*, *79*, 401–459. Available from: https://doi.org/10.1046/j.1095-8312.2003.00197.x

Meyer, C.P. & Paulay, G. (2005) DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology*, *3*, e422. Available from: https://doi.org/10.1371/journal.pbio.0030422

Monich, U.J., Grgicak, C., Cadambe, V., Wu, J.Y., Wellner, G., Duffy, K. et al. (2014) A signal model for forensic DNA mixtures. Proceedings of the 2014 48th Asilomar conference on signals, Systems and Computers 978–1479982974. http://hdl.handle.net/1721.1/100950

Montgomery, G.A., Belitz, M.W., Guralnick, R.P. & Tingley, M.W. (2021) Standards and best practices for monitoring and benchmarking insects. *Frontiers in Ecology and Evolution*, *8*, 579193. Available from: https://doi.org/10.3389/fevo.2020.579193

Moreau, C.S., Wray, B.D., Czekanski-Moir, J.E. & Rubin, B.E.R. (2013) DNA preservation: a test of commonly used preservatives for insects. *Invertebrate Systematics*, *27*, 81–86. Available from: https://doi.org/10.1071/IS12067

Murthy, M.K., Khandayataray, P., Tara, M., Buragohain, P., Giri, A. & Gurusubramanian, G. (2022) Optimisation of DNA isolation and PCR techniques for beetle (order: Coleoptera) specimens. *International Journal of Tropical Insect Science*, *42*, 2761–2771. Available from: https://doi.org/10.1007/s42690-022-00736-3

Nagy, Z.T. (2010) A hands-on overview of tissue preservation methods for molecular genetic analyses. *Organisms Diversity & Evolution*, *10*, 91–105.

Nakahama, N., Isagi, Y. & Ito, M. (2019) Methods for retaining well-preserved DNA with dried specimens of insects. *European Journal of Entomology*, *116*, 486–491. Available from: https://doi.org/10.14411/eje.2019.050

Nakamura, S., Tamura, S., Taki, H. & Shoda-Kagaya, E. (2020) Propylene glycol: a promising preservative for insects, comparable to ethanol, from trapping to DNA analysis. *Entomologia Experimentalis et Applicata*, *168*, 89–96. Available from: https://doi.org/10.1111/eea.12876

Packard, G. (2021) When perception isn't reality: allometric variation in the exaggerated mandibles of male stag beetles (Coleoptera: Lucanidae). *Biological Journal of the Linnean Society*, *134*, 760–772. Available from: https://doi.org/10.1093/biolinnean/blab100

Patrick, H.J.H., Chomič, A. & Armstrong, K.F. (2016) Cooled propylene glycol as a pragmatic choice for preservation of DNA from remote field-collected Diptera for next-generation sequence analysis. *Journal of Economic Entomology*, *109*, 1469–1473. Available from: https://doi.org/10.1093/jee/tow047

Peng, Y., Wang, K., Fu, W., Sheng, C. & Han, Z. (2018) Biochemical comparison of dsRNA degrading nucleases in four different insects. *Frontiers in Physiology*, *9*, 624. Available from: https://doi.org/10.3389/fphys.2018.00624

Pokluda, P., Čížek, L., Stříbrná, E., Drag, L., Lukeš, J. & Novotný, V. (2014) A goodbye letter to alcohol: An alternative method for field preservation of arthropod specimens and DNA suitable for mass collecting methods. *European Journal of Entomology*, *111*, 175–179. Available from: https://doi.org/10.14411/eje.2014.024

Porfireva, A., Plastinina, K., Evtugyn, V., Kuzin, Y. & Evtugyn, G. (2021) Electrochemical DNA sensor based on poly(azure a) obtained from the buffer saturated with chloroform. *Sensors*, *21*, 2949. Available from: https://doi.org/10.3390/s21092949

Porto, D.S., Melo, G.A.R. & Almeida, E.A.B. (2015) Clearing and dissecting insects for internal skeletal morphological research with particular reference to bees. *Revista Brasileira de Entomologia*, *59*, 273–279. Available from: https://doi.org/10.1016/j.rbe.2015.11.007

Psifidi, A., Dovas, C.I. & Banos, G. (2010) A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. *Molecular and Cellular Probes*, *24*, 93–98. Available from: https://doi.org/10.1016/j.mcp.2009.11.001

R Development Core Team. (2023) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Reiss, R.A., Schwert, D.P. & Ashworth, A.C. (1995) Field preservation of Coleoptera for molecular genetic analyses. *Environmental Entomology*, *24*, 716–719. Available from: https://doi.org/10.1093/ee/24.3.716

Remmel, N., Buchner, D., Enss, J., Hartung, V., Leese, F., Welti, E.A.R. et al. (2024) DNA metabarcoding and morphological identification reveal similar richness, taxonomic composition and body size patterns among flying insect communities. *Insect Conservation and Diversity*, *17*(3), 449–463. Available from: https://doi.org/10.1111/icad.12710

Robinson, C.V., Porter, T.M., Wright, M.T.G. & Hajibabaei, M. (2021) Propylene glycol-based antifreeze is an effective preservative for DNA metabarcoding of benthic arthropods. *Freshwater Science*, *40*, 77–87. Available from: https://doi.org/10.1086/712232

Rubink, W., Murray, K., Baum, K. & Pinto, M. (2003) Long-term preservation of DNA from honey bees (Apis mellifera) collected in aerial pitfall traps. *Texas Journal of Science*, *55*, 159–168.

Ruppert, L., Segelbacher, G., Staab, M. & Winiger, N. (2023) Gauging DNA degradation among common insect trap preservatives. *Entomologia Experimentalis et Applicata*, *171*, 218–226. Available from: https://doi.org/10.1111/eea.13266

Santos, J.C. & Fernandes, G.W. (Eds.). (2021) *Measuring arthropod biodiversity: a handbook of sampling methods*. Switzerland AG: Springer Nature. Available from: https://doi.org/10.1007/978-3

Sapia, M., Lövei, G. & Elek, Z. (2006) Effects of varying sampling effort on the observed diversity of carabid (Coleoptera: Carabidae) assemblages in the Danglobe project, Denmark. *Entomologica Fennica*, *17*, 345–350. Available from: https://doi.org/10.33338/ef.84356

Schield, D.R., Walsh, M.R., Card, D.C., Andrew, A.L., Adams, R.H. & Castoe, T.A. (2016) EpiRADseq: scalable analysis of genome-wide patterns of methylation using next-generation sequencing. *Methods in Ecology and Evolution*, *7*, 60–69. Available from: https://doi.org/10.1111/2041-210X.12435

Seutin, G., White, B. & Boag, P. (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, *69*, 82–90. Available from: https://doi.org/10.1139/Z91-013

Shokralla, S., Gibson, J.F., Nikbakht, H., Janzen, D.H., Hallwachs, W. & Hajibabaei, M. (2014) Next-generation DNA barcoding: using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. *Molecular Ecology Resources*, *14*, 892–900. Available from: https://doi.org/10.1111/1755-0998.12236

Šigut, M., Kostovčík, M., Šigutová, H., Hulcr, J., Drozd, P. & Hrček, J. (2017) Performance of DNA metabarcoding, standard barcoding, and morphological approach in the identification of host–parasitoid interactions. *PLoS One*, *12*, e0187803. Available from: https://doi.org/10.1371/journal.pone.0187803

Skvarla, M.J., Larson, J.L., Fisher, J.R. & Dowling, P.G. (2021) A review of terrestrial and canopy malaise traps. *Annals of the Entomological Society of America*, *114*, 27–47. Available from: https://doi.org/10.1093/aesa/saaa044

Steininger, S., Storer, C., Hulcr, J. & Lucky, A. (2015) Alternative preservatives of insect DNA for citizen science and other low-cost applications. *Invertebrate Systematics*, *29*, 468–472. Available from: https://doi.org/10.1071/IS15003

Strickler, K.M., Fremier, A.K. & Goldberg, C.S. (2015) Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92. Available from: https://doi.org/10.1016/j.biocon.2014.11.038

Stutt, A.D. & Willmer, P. (1998) Territorial defence in speckled wood butterflies: do the hottest males always win? *Animal Behaviour*, *55*, 1341–1347. Available from: https://doi.org/10.1006/anbe.1998.0728

Suvarna, K., Layton, C. & Bancroft, J.D. (2019) *Bancroft's theory and practice of histological techniques*, 8th edition. Churchill: Elsevier Health Sciences. Available from: https://doi.org/10.1016/C2015-0-00143-5

Svenningsen, C.S., Frøslev, T.G., Bladt, J., Pedersen, L.B., Larsen, J.C., Ejrnæs, R. et al. (2021) Detecting flying insects using car nets and DNA metabarcoding. *Biology Letters*, *17*, 20200833. Available from: https://doi.org/10.1098/rsbl.2020.0833

Tatsuta, H., Takahashi, K.H. & Sakamaki, Y. (2017) Geometric morphometrics in entomology: basics and applications. *Entomological Science*, *21*, 164–184. Available from: https://doi.org/10.1111/ens.12293

Thomas, D.B. (2008) Nontoxic antifreeze for insect traps. *Entomological News*, *119*, 361–365. Available from: https://doi.org/10.3157/0013-872X-119.4.361

Tóth, M.E., Vígh, L. & Sántha, M. (2014) Alcohol stress, membranes, and chaperones. *Cell Stress and Chaperones*, *19*, 299–309. Available from: https://doi.org/10.1007/s12192-013-0472-5

Tsuji, S., Yamanaka, H. & Minamoto, T. (2017) Effects of water pH and proteinase K treatment on the yield of environmental DNA from water samples. *Limnology*, *18*, 1–7. Available from: https://doi.org/10.1007/s10201-016-0483-x

Vargovčík, O., Čiamporová-Zaťovičová, Z., Beracko, P. & Čiampor, F. (2024) Environmental gradients and optimal fixation time revealed with DNA metabarcoding of benthic sample fixative. *Scientific Reports*, *14*(1), 18396. Available from: https://doi.org/10.1038/s41598-024-68939-x

Vink, C.J., Thomas, S.M., Paquin, P., Hayashi, C.Y. & Hedin, M. (2005) The effects of preservatives and temperatures on arachnid DNA. *Invertebrate Systematics*, *19*, 99–104. Available from: https://doi.org/10.1071/IS04039

Vogt, G. (2018) Investigating the genetic and epigenetic basis of big biological questions with the parthenogenetic marbled crayfish: a review and perspectives. *Journal of Biosciences*, *43*, 189–223. Available from: https://doi.org/10.1007/s12038-018-9741-x

Wickham, H. (2016) *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.

Willows-Munro, S. & Schoeman, M.C. (2015) Influence of killing method on Lepidoptera DNA barcode recovery. *Molecular Ecology Resources*, *15*, 613–618. Available from: https://doi.org/10.1111/1755-0998.12331

Wood, S.N. (2020) Inference and computation with generalized additive models and their extensions. *Test*, *29*, 307–339. Available from: https://doi.org/10.1007/s11749-020-00711-5

Xu, Y., Ren, X., Wang, H., Wang, M. & Li, G. (2020) Evaluation of DNA degradation and establishment of a degradation analysis model for Lepidoptera specimens. *BioTechniques*, *68*, 138–147. Available from: https://doi.org/10.2144/btn-2019-0166

Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C. & Jones, G. (2011) Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, *11*, 236–244. Available from: https://doi.org/10.1111/j.1755-0998.2010.02920.x

Zeileis, A. & Hothorn, T. (2002) Diagnostic checking in regression relationships. *R News*, *2*, 7–10.

Zizka, V.M.A., Koschorreck, J., Khan, C.C. & Astrin, J.J. (2022) Long-term archival of environmental samples empowers biodiversity monitoring and ecological research. *Environmental Sciences Europe*, *34*, 10. Available from: https://doi.org/10.1186/s12302-022-00618-y

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. DNA extraction protocol for Macherey-Nagel NucleoSpin Tissue Kit—adjustments for propylene glycol washing and pH monitoring.

Data S2. Revised protocol for purification of 10 µL of gDNA using the Quick-Start Protocol of the MinElute PCR Purification Kit.

Table S1. PCR protocol for mitochondrial cytochrome c oxidase subunit 1 (COI) amplification with Coleoptera and Lepidoptera DNA.

Table S2. List of primers used for PCR amplification of a 658 bp, 313 bp and 157 bp mitochondrial cytochrome c oxidase subunit 1 (COI) amplicon of Coleoptera: Carabidae.

Table S3. List of primers used for PCR amplification of a 658 bp, 311 bp and 220 bp mitochondrial cytochrome c oxidase subunit 1 (COI) amplicon of Lepidoptera.

Figure S1. PCR success rate of full-length mitochondrial cytochrome c oxidase subunit 1 (COI) amplicon (658 bp) in the Malaise trap sub-experiment over three 14-day intervals (14, 28, and 42 days), using pure propylene glycol as the initial fixative.

Figure S2. Step-wise PCR success rates for mitochondrial cytochrome c oxidase subunit 1 (COI) in Coleoptera: Carabidae across four sub-experiments, using various preservation agents covered as biological replicates.

Figure S3. PCR success rate of full-length mitochondrial cytochrome c oxidase subunit 1 (COI) amplicon (658 bp) in the individual trapping sub-experiment across nine different preservation agents.

Figure S4. PCR success rate of full-length mitochondrial cytochrome c oxidase subunit 1 (COI) amplicon (658 bp) in the propylene glycol sub-experiment across four different trapping methods.

Figure S5. PCR success rate of the full-length mitochondrial cytochrome c oxidase subunit 1 (COI) amplicon (~658 bp) in the UV light-trapping sub-experiment, using chloroform vapor, chloroform soaking, and cyanide vapor as preservation agents.

Figure S6. Step-wise PCR success rates for mitochondrial cytochrome c oxidase subunit 1 (COI) amplification in Lepidoptera across the UV light-trapping sub-experiment, using chloroform vapor, chloroform-soaked, and cyanide vapor as fixation agents.

Figure S7. Model fit summary for the Malaise trap sub-experiment, showing the effects of exposure time and pH on average fragment size in Coleoptera: Carabidae. Model quality of the Generalized Linear Model (GLM) is assessed by the Akaike Information Criterion (AIC) and dispersion parameter.

Figure S8. Model fit summary for the individual trapping sub-experiment, showing the effects of nine preservation agents and pH on the average fragment size in Coleoptera: Carabidae.

Figure S9. Pairwise contrasts of DNA preservation efficacy across nine preservation agents, using ethylene glycol as the control.

Figure S10. Model fit summary for the propylene glycol washing sub-experiment, evaluating the interaction between trapping method and preservation agents in Coleoptera: Carabidae.

Figure S11. Model fit summary for the propylene glycol washing sub-experiment, evaluating the interaction between trapping method and washing in Coleoptera: Carabidae.

Figure S12. Model fit summary for Lepidoptera UV-light trapping, evaluating the interaction between fixation and size.

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