American Journal of Bioscience and Bioengineering
2024, Vol. 12, No. 3, pp. 48-55
https://doi.org/10.11648/j.bio.20241203.12

Research Article

A Fast, Simple and Low-cost DNA Extraction Protocol from Common Ants and Beetles for Multiple Molecular Applications

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Abstract

The rapid development of molecular biology tools in insect systematics, invasion research, evolutionary ecology and biodiversity analysis has led to faster and greater progress in understanding insect behavior and biology. Efficient DNA extraction is the foremost step and serves as the vital foundation. Several rapid DNA extraction methods have been established, which are often time-consuming and labour-intensive. Here, a simple, fast, low-cost DNA extraction protocol for common insect samples was developed basing on 28 specimens of 16 insect species (7 ants, 9 bark and ambrosia beetles). The new protocol was shown to be feasible and highly efficient by comparison with commercial kit in terms of DNA yield, purity and PCR sensitivity. The concentration of DNA through the new rapid method was higher than that through commercial kit, whether in ant or beetle samples. A better quality of DNA extracted via kit was indicated by A_{260}/A_{280} mostly ranging from 1.80 to 2.00. There was little difference between DNA extracted from adult and nymphal insects. PCR sensitivity of extracted DNA using both protocols was comparable. For nested PCR, amplification after two rounds yielded a bright signal using template DNA through both methods. But for PCR using primers of LCO1490 and HCO2198, the success ratio was lower (85.18%). Through BLAST, these amplicons were matched to related data with high identity. By combining this protocol with variable analysis platforms such as common PCR, loop-mediated isothermal amplification, and high throughput sequencing, it could assist insect diagnostics, biological surveys and invasion researches.

Keywords

DNA Extraction, Insects, Molecular Analysis, Rapid, Simple, Low-Cost

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Received: 14 April 2024; **Accepted:** 27 April 2024; **Published:** 17 May 2024

1. Introduction

The application of molecular biology tools in insect systematics, invasion research, evolutionary ecology and biodiversity analysis has accelerated in recent years, resulting in faster and greater progress in understanding insect behavior and biology [1-4]. Timely tracking methods are essential for monitoring insect population dynamics. Among current tracing approaches, molecular methods play an important role [5]. DNA barcoding system for taxonomic identification is based on the assumption that sequence conservation is normally much higher intraspecifically than interspecifically [6]. Plant-insect molecular interaction, including plant defense signal production, insect elicitor perception and effector secretion for efficient infection, remains a perennial issue [7]. Taking advantage of high-throughput sequencing platforms, many insect genome maps have been drawn [8, 9]. Expansion or contraction of specific gene family revealed by comparative analysis can be the underlying reason for changes in insect biology and social behavior [10]. Insecticide resistance caused by overuse of chemicals has weakened pest management programs and remains one of the most popular topics for researchers [11]. Above all, DNA sequences with the encoded genetic information are the central component of molecular biological techniques.

DNA extraction from insect materials is essential and fundamental for molecular biology tools, with the central focus on DNA recovery in high quantity and quality. Several commonly used protocols include Chelex extraction, conventional extraction using CTAB, and commercial kits based on solid phase extraction [12]. However, most of them are not cost effective, time consuming and labour intensive. Currently, the research purpose is to reduce operating time, labour and cost without compromising DNA quantity and quality too much [13]. A fast, simple and low cost extraction approach with sufficient yield and purity of DNA would greatly improve the overall efficiency of molecular analysis.

Insects pose a significant threat to agriculture, forestry and horticulture all over the world. The international transfer of non-native insects is a serious challenge to local ecosystems [14]. Non-native insects are considered invasive and are subject to strict embargoes as international trade increases [15]. Ants are typical members on invader list with strong destructive capabilities across the world. Invasive ants can alter the local ecosystem and greatly reduce native biodiversity [16]. It has been reported that the economic loss associated with ant invaders since 1930 amounts to US$51.93 billion, of which 92% are damages mainly to agriculture and social welfare [17]. Bark and ambrosia beetles, which spend most of their life cycle in wood, are abundant in forests. Some of them can kill millions of trees in Europe, North America and Asia, each year with a random breakout. Unfortunately, they are often transmitted in packaging and wood products [18]. Rapid screening and accurate diagnosis of these invaders is important for monitoring, decision making and supporting system.

In this study, a fast, simple and low-cost DNA extraction protocol was established with sufficient nucleic acid quantity and quality for further molecular analysis such as DNA barcoding etc. This protocol is believed to improve diagnostic efficiency and do favor to multiple molecular processes.

2. Materials & Methods

2.1. Insect Specimens

A total of 28 specimens of 16 insect species were collected and used in this study, including 7 species of ants and 9 species of bark and ambrosia beetles. Some of them were reserved samples, the others were donated by other labs. These samples were stored in pure ethanol/dry condition for different periods of time (Table 1). Six samples of *Hylurgus ligniperda* were collected from *Pinus* spp..

Table 1. *Sample list.*

No.	Species	Collection Time	Storage	Origin	Host
1	*Messor barbarus*	2023.10	Ethanol	reserved sample	/
2	*Pogonomyrmex barbatus*	2023.10	Ethanol	reserved sample	/
3	*Atta mexicana*	2023.10	Ethanol	reserved sample	/
4	*Ectatomma opaciventre*	2023.10	Ethanol	reserved sample	/
5	*Wasmannia auropunctata*	2023.1	Ethanol	Donated by Guangzhou Customs	/
6	*Solenopsis geminata*	2023.7	Dry condition	reserved sample	/
7	*S. geminata*	2014.5	Dry condition	reserved sample	/
8	*S. geminata*	2019.6	Dry condition	reserved sample	/

No.	Species	Collection Time	Storage	Origin	Host
9	*S. geminata*	2016.6	Dry condition	reserved sample	/
10	*S. invicta*	2021.7	Ethanol	reserved sample	/
11	*S. invicta*	2023.6	Ethanol	Donated by Guangzhou Customs	/
12	*Solenopsis* sp.	2019.4	Dry condition	reserved sample	/
13	*Solenopsis* sp.	2019.10	Dry condition	reserved sample	/
14	*Hylurgus ligniperda*	/	Ethanol	reserved sample	*Pinus radiata*
15	*H. ligniperda*	2011.5	Dry condition	reserved sample	/
16	*H. ligniperda*	2014.1	Dry condition	reserved sample	*P. radiata*
17	*H. ligniperda*	2014.10	Dry condition	reserved sample	*P. sylvestris*
18	*H. ligniperda*	2019	Ethanol	reserved sample	/
19	*H. ligniperda*	2018.10	Ethanol	reserved sample	*P. radiata*
20	*Xyleborus affinis*	2020.10	Ethanol	Donated by Guangzhou Customs	/
21	*X. perforans*	2021.12	Ethanol	Donated by Guangzhou Customs	/
22	*Ips. grandicollis*	/	Ethanol	Donated by Nanjing Customs	/
23	*I. sexdentatus*	/	Ethanol	Donated by Nanjing Customs	/
24	*Xylosandrus crassiusculus*	2022.7	Ethanol	Donated by Guangzhou Customs	/
25	*X. germanus*	2023.6	Ethanol	Donated by Guangzhou Customs	/
26	*X. germanus*	2021.1	Ethanol	reserved sample	*Pinus* sp.
27	*X. compactus*	2020.6	Ethanol	Donated by Guangzhou Customs	/
28	*Orthotomicus erosus*	2023.3	Ethanol	Donated by Guangzhou Customs	/

2.2. Rapid DNA Extraction Using a New Protocol

A new rapid protocol was developed for DNA extraction from insect samples.

Solutions:

Extraction Buffer A: 0.2% (g/mL) NaOH solution.

Extraction Buffer B: 4.5% (v/v) Tween 20 solution.

The entire process involves following steps:

Sample crushing: 0.1-0.5 mg of each sample (intact body or fragments) was placed into a PCR tube (0.2 mL). Use a pipette tip to crush the insect material.

Adding buffers: 10 uL Buffer A and 2 uL Buffer B were added, mixed and centrifuged briefly.

Extraction: Heat the mixture to 95 ℃ for 15 min. Add 10 μL ddH_2O to make the final DNA solution.

2.3. DNA Extraction Using Commercial Kit

Total DNA was extracted from these samples using UE small amount genomic DNA preparation kit (UElandy Biotechnology Co., LTD, China) according to the manufacturer's introduction. First, collect 0.5-1.0 mg of insect samples and place in a centrifuge tube (1.5 mL). Crush insect remains with a pipette tip. Second, add 350 uL PBS solution and 0.9 uL RNase A into the tube. Vortex for 15 s, and leave the mixture at room temperature for 1 min. Third, add 150 uL Buffer C-L and 8 uL Proteinase K. Vortex for 1 min, and centrifuge briefly. Heat the mixture to 56 ℃ for 10 min. Fourth, add 350 uL Buffer P-D. Vortex for 30 s, and centrifuge at 12,000 r/min for 10 min. Fifth, transfer the supernatant to a DNA preparation tube (with the DNA adsorbent film). Centrifuge at 12,000 r/min for 1 min. Sixth, discard the filtrate and add 500 uL Buffer W1 into the DNA preparation tube. Centrifuge at 12,000 r/min for 1 min. Repeat the last step twice with 700 uL Buffer W2. Dissolve DNA with 100 uL eluent at room temperature for 5 min and centrifuge at 12,000 r/min for 1 min.

The yield and purity of final DNA solutions produced by two protocols above were tested using a Nanodrop 2000c spectrophotometer (Thermo Scientific, USA).

2.4. PCR Amplification of COI Loci

Two sets of primers were employed for amplification of

COI regions. One is LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'-TAAACTTCAGGG TGAC-CAAAAAATCA-3') [19] with an amplicon of ~710 bp. The other is nested primers, of which the external primers include CI-J-2183 (5'-CAACATTTATTTTGATTTTGG-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATAT-3') [20], and the internal primers consist of J2210 (5'-TCGCATATTATTAGGCAAGAAAGAG-3') and N2739 (5'-AGAAATGTTGTGGGAAGAG-3') [21]. The first round of PCR produced a fragment of ~1300 bp, and the second produced a fragment of ~500 bp. The reaction mixture was in 50 μl volume consisting of 2 ul DNA solution, 1 ul each primer (20 μM), 25 ul reaction buffer (2 ×Es Taq MasterMix, CWBIO, China), and 21 ul ddH_2O. Amplification was conducted on a GeneExplorer thermal cycler (BIOER Technology, China). Amplicons were sequenced by BGI Co., Ltd (Guangdong, China) and the result was analyzed using BLAST search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

3. Results

3.1. Yield and Purity of Extracted DNA

The yield and purity of extracted DNA were analyzed using a Nanodrop 2000c spectrophotometer, and results varied depending on the protocol used. In general the concentration of DNA through the new rapid method was higher than that through commercial kit, whether in ant or beetle samples. A better quality of DNA extracted via kit was indicated by A_{260}/A_{280} mostly ranging from 1.80 to 2.00. A low A_{260}/A_{280} (<1.80) suggests protein contamination, while a high value (>2.00) points to RNA contamination. The A_{260}/A_{280} of DNA extracted via the new rapid method was mostly below 1.80, suggesting protein contamination. There was little difference between DNA extracted from adult and nymphal insects.

In ants, the difference between DNA concentrations extracted through the new rapid method and the commercial kit could be as high as over 10 folds. The highest DNA concentration was observed in *S. geminata* using the new protocol. However, the purity of DNA was better using kit. For ants, only adult samples were tested.

Table 2. *DNA extraction and amplification results.*

No.	Species	Sample Condition	Protocol	Yield (ng/μL)	Purity A_{260}/A_{280}	Amplification	
						LCO_{1490}+HCO_{2198}	nested primers
1	*M. barbarus*	Fragments (adult)	UE	5.6	1.87	√	√
		Fragments (adult)	AB	24.7	1.57	√	√
2	*P. barbatus*	Fragments (adult)	UE	6.2	1.72	√	√
		Fragments (adult)	AB	34.7	1.61	√	√
3	*A. mexicana*	Fragments (adult)	UE	4.3	1.56	√	√
		Fragments (adult)	AB	51.3	1.43	√	√
4	*E. opaciventre*	Fragments (adult)	UE	7.1	2.03	√	√
		Fragments (adult)	AB	81.3	1.37	×	√
5	*W. auropunctata*	adult	UE	4.6	2.06	√	√
		adult	AB	32.6	1.55	√	√
6	*S. geminata*	adult	UE	3.8	1.92	√	√
		adult	AB	72.6	1.44	√	√
7	*S. geminata*	adult	UE	7.6	1.63	√	√
		adult	AB	56.8	1.73	√	√
8	*S. geminata*	adult	UE	4.7	1.74	√	√
		adult	AB	92.1	1.69	√	√
9	*S. geminata*	adult	UE	4.6	1.99	√	√
		adult	AB	47.9	2.32	√	√

No.	Species	Sample Condition	Protocol	Yield (ng/μL)	Purity A_{260}/A_{280}	Amplification	
						LCO_{1490}+HCO_{2198}	nested primers
10	*S. invicta*	adult	UE	4.8	1.79	√	√
		adult	AB	36.4	1.46	√	√
11	*S. invicta*	adult	UE	4.5	2.3	√	√
		adult	AB	42.3	1.65	√	√
12	Solenopsis sp.	Fragments (adult)	UE	5.8	2.07	×	×
		Fragments (adult)	AB	81.3	1.54	×	×
13	Solenopsis sp.	Fragments (adult)	UE	6.3	1.41	√	√
		Fragments (adult)	AB	32.8	1.76	√	√
14	*H. ligniperda*	Fragments (adult)	UE	6	1.05	√	√
		Fragments (adult)	AB	48.6	1.87	√	√
15	*H. ligniperda*	Fragments (adult)	UE	4.3	1.15	√	×
		Fragments (adult)	AB	74.9	1.65	√	√
16	*H. ligniperda*	Fragments (adult)	UE	5.6	2.29	√	√
		Fragments (adult)	AB	28.9	1.78	×	√
17	*H. ligniperda*	Fragments (nymph)	UE	6.1	1.88	√	√
		Fragments (nymph)	AB	64.3	2.05	√	√
18	*H. ligniperda*	adult	UE	7.9	1.71	√	√
		adult	AB	52.8	1.45	√	√
19	*H. ligniperda*	adult	UE	7.5	1.62	√	√
		adult	AB	38.1	1.68	√	√
20	*X. affinis*	adult	UE	7.2	1.77	×	√
		adult	AB	41.2	1.74	×	√
21	*X. perforans*	Fragments (adult)	UE	5.1	1.69	×	√
		Fragments (adult)	AB	56.2	1.98	×	√
22	*I. grandicollis*	Fragments (adult)	UE	5.3	1.98	×	√
		Fragments (adult)	AB	80.8	1.5	×	√
23	*I. sexdentatus*	Fragments (adult)	UE	4.7	2.11	√	√
		Fragments (adult)	AB	91.5	1.36	√	√
24	*X. crassiusculus*	Fragments (adult)	UE	3.6	1.81	√	√
		Fragments (adult)	AB	184.5	1.9	√	√
25	*X. germanus*	Fragments (adult)	UE	4.4	2.49	√	√
		Fragments (adult)	AB	74	1.84	√	√
26	*X. germanus*	Fragments (adult)	UE	7.3	3.45	√	√
		Fragments (adult)	AB	56.6	1.76	√	√
27	*X. germanus*	Fragments (adult)	UE	5.3	1.96	√	√
		Fragments (adult)	AB	144.6	1.39	√	√
28	*O. erosus*	Fragments (adult)	UE	4.8	1.65	√	√

No.	Species	Sample Condition	Protocol	Yield (ng/μL)	Purity A_{260}/A_{280}	Amplification $LCO_{1490}+HCO_{2198}$	Amplification nested primers
		Fragments (adult)	AB	175.2	1.34	√	√

UE: UE small amount genomic DNA preparation kit;
AB: the new rapid protocol for DNA extraction.
×: amplification failed using specific primer set;
√: amplification successful using specific primer set.

For beetles, the concentration gap between two methods was similar to that for ants. The highest DNA concentration was recorded in *X. crassiusculus* using the new protocol. Little difference was observed between DNA concentrations from adult and nymphal insects.

3.2. Amplification of DNA Barcode

All the DNA solutions were further used as the templates to conform about corresponding quantity and quality, and to determine their suitability for routine molecular analysis. Results show that PCR sensitivity of extracted DNA using both protocols was comparable. For nested PCR, amplification after two rounds yielded a bright signal (amplicon near 500 bp) using template DNA through both methods. But for PCR using primers of LCO1490 and HCO2198, the success ratio was lower (85.18%). The first round of nested PCR could sometimes yield faint signals, mainly due to the sensitivity of electrophoresis. These PCR products were further sequenced to verify the accurate amplification. Through BLAST, these amplicons were matched to related data with high identity. Altogether, results above indicate that these two DNA extraction protocols were suitable for genetic analysis and species identification for insect samples.

4. Discussion

The molecular tools and corresponding working model have been deeply applied in researches on organisms, ecosystem and the big nature. First of all, DNA in good quantity and quality is the foundation. Several factors affect the efficiency of DNA extraction from different organisms, such as preservatives, temperature and humidity [22]. For insects, ethanol and isopropanol are widely used preservatives [23]. Ethanol solutions in the concentration ranging from 95% to 100% are the most effective for further molecular analysis, because ethanol could cross the cell membrane and inactivate enzymes including the DNase. It is also supposed to eliminate microorganisms present. Temperature is another vital factor influencing insect preservation. Ultralow temperature is often used for long-term preservation of insect specimens for genetic researches. Moisture can assist microbial growth and speed up specimen decay, so it needs to be get rid of so that microorganisms can be eliminated. In this study, 28 test specimens were used, which were stored either in pure ethanol or dry condition at room temperature. The oldest specimen was collected in 2018 and stored in pure ethanol. The DNA extracted through either protocols worked well in PCR amplification. Insect specimens dried at a high temperature and stored for a long time at room temperature could fail in DNA extraction due to DNA degradation.

Till now, there have been several methods for rapid extraction of DNA from insect samples [24-26]. Many of them are time-consuming and labour-intensive because they involve steps for washing out impurities and contaminants. A simple and non-destructive DNA extraction procedure was reported earlier [27], and tested feasible for three arthropod orders: Coleoptera, Diptera and Hemiptera. Researchers also used Flinders Technology Associates Plant Saver cards which were originally designed for plant DNA extraction, in rapid DNA extraction from insect materials in non-lab situations and coupled with the loop-mediated isothermal amplification (LAMP) method to realize fast diagnosis [28]. Sumit Jangra and colleagues evaluated and established a fast and zero-cost protocol for DNA extraction from small and soft-bodied insects for molecular applications [29]. Commercial kits are always the most expensive among existing extraction procedures. Time, cost and labour involved in DNA extraction from insects need to be reduced on condition that the quantity and quality of DNA are sufficient for further molecular analysis. Low biomass and small size of insect specimens add more difficulties to DNA extraction.

Here, a simple, fast, and cost-effective insect DNA extraction protocol has been developed, which is sufficient for routine PCR based and other diagnostic applications. Through analysis, this protocol was shown to be suitable for ants and common beetles. The low yield of DNA through commercial kit may be led by the fact that DNA was either not absorbed onto the column or irreversibly bound. Repeated washing steps could also contribute to DNA loss. Similarly, the high amount of DNA through the rapid procedure could be facilitated by little DNA loss during the process. Besides, it has been observed that the quantity of DNA gradually declined over time regardless of extraction methods. The decrease in both quantity and quality was enhanced for DNA through the rapid protocol. The reason can be related to the presence of

nucleases and ROS as impurities. Other impurities such as alkaloid and phenolics, also interfere with PCR amplification.

5. Conclusion

The rapid development of molecular biology tools in insect systematics, invasion research, evolutionary ecology and biodiversity analysis has led to faster and greater progress in understanding insect behavior and biology. Efficient DNA extraction is the foremost step and serves as the vital foundation. Several rapid DNA extraction methods have been established, which are often time-consuming and labour-intensive. Here, a simple, fast, low-cost DNA extraction protocol was established for common insect samples basing on 28 specimens of 16 insect species (7 ants, 9 bark and ambrosia beetles). The new protocol was shown to be feasible and highly efficient by comparison with commercial kit in terms of DNA yield, purity and PCR sensitivity. The concentration of DNA through the new rapid method was higher than that through commercial kit, whether in ant or beetle samples. A better quality of DNA extracted via kit was indicated by A_{260}/A_{280} mostly ranging from 1.80 to 2.00. There was little difference between DNA extracted from adult and nymphal insects. PCR sensitivity of extracted DNA using both protocols was comparable. Through BLAST, these amplicons were matched to related data with high identity. By combining this protocol with variable analysis platforms such as common PCR, loop-mediated isothermal amplification, and high throughput sequencing, the efficiency of insect diagnostics, biological surveys and invasion researches is supposed to be largely improved.

6. Recommendations

In this study, a simple, fast, and cost-effective insect DNA extraction protocol has been developed, which is sufficient for routine PCR based and other diagnostic applications, such as DNA barcoding. By combining this protocol with variable analysis platforms such as various PCR, loop-mediated isothermal amplification, and high throughput sequencing, the efficiency of insect diagnostics, biological surveys, insect systematics, invasion research, evolutionary ecology and biodiversity analysis is supposed to be largely improved. This DNA extraction protocol can also assist timely tracking of pests to choose appropriate control measures.

Abbreviations

DNA: Deoxyribonucleic Acid
PCR: Polymerase Chain Reaction
CTAB: Cetyltrimethyl Ammonium Bromide
NaOH: Sodium Hydroxide
PBS: Polybutadiene-Styrene
COI: Cytochrome Oxidase I
BLAST: Basic Local Alignment Search Tool
LAMP: Loop-Mediated Isothermal Amplification
ROS: Reactive Oxygen Species

Author Contributions

Wang Jiaying: Funding acquisition, Investigation, Writing – original draft
Cui Junxia: Project administration
Liu Li: Data curation, Investigation, Resources
Wang Yuanjing: Validation, Writing – review & editing
Yan Shuyi: experiment conduct
Chen Xianfeng: Conceptualization, Supervision

Funding

This study received support from the Ningbo Public Interest Plan (2023S182) and the Research Foundation of the General Administration of Customs of the People's Republic of China (2023HK048).

Conflicts of Interest

The authors declare no conflicts of interest.

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