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Comparison between two different DNA extraction methods to obtain high DNA quality from *Astacus leptodactylus*

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ABSTRACT

Most aquatic studies are performed at the molecular level, which needs reliable, quick DNA extraction protocols. Extraction of genomic DNA with high quality is one of the basic needs of molecular genetics. This investigation indicated two different methods for obtaining a sufficient quality of genomic DNA of crayfish (*Astacus leptodactylus)*. The DNA from *A. leptodactylus* was extracted using extraction methods Quick-DNA™ Tissue/Insect Miniprep Kit and Chelex method. We used eight samples of *A. leptodactylus* from the Anzali lagoon for this comparison. Total DNA was isolated from the abdominal tissues of all samples. Quantitative and qualitative parameters were measured using a spectrophotometer. The results showed that the highest quality of extracted DNA was in the Kit (1.82-1.9). The Chelex method showed the existence of proteins in the samples (1.48-1.73). In both methods, DNA was isolated, but the Kit method was more trustable and applicable for having good quality DNA.

INTRODUCTION

Most aquatic studies are performed at the molecular level, which needs reliable, quick, and cost-effective DNA extraction protocols. The various methods for genomic DNA created several DNA extraction methods. However, some methods, such as chloroform-based DNA extraction, are not safe for humans. Additionally, chloroform-based DNA extraction needs the use of toxic chemicals, magnetic separation, and silica-based DNA extraction inclined to be expensive (**Kumar *et al*., 2007**).

Overall, to obtain a sufficient quality of DNA, the A260 / 280 ratio should be between 1.8 and 2. besides, a lack of contaminations along with DNA, such as polysaccharides and phenols, is essential (**Abdel-Latif and Osman, 2017**). Generally, obtaining a good quality of DNA and purification is challenging due to proteins,

polysaccharides, and DNA polymerase inhibitors (**Shokoohi, 2021a)**. This material affects the quality and quantity of isolated DNA and interferes with PCR processing.

Pure and rapid DNA extraction is required for advanced genetic diversity and marker-assisted selection techniques. However, obtaining high-quality DNA is challenging and expensive because of several steps (**Shokoohi, 2021b)**.

According to studies, there are three crayfish families in the world. Astacidae is native to West Asia, and *A. leptodactylus* is native to Iran (**Chiesa *et al*., 2011**). Today, due to the high nutritional value of *A. leptodactylus*, their consumption has also increased. Having characteristics such as a cheap diet, high economic value, and good marketability globally, *A. leptodactylus* has particular economic and commercial importance (**Harlioğlu, 2008**). Therefore, this study aimed to compare DNA isolation quality using two different extraction methods from *A. leptodactylus.*

MATERIALS AND METHODS

Study area

Specimens of *A. Leptodactylus* were recovered from the Siah Darvishan River (GPS coordinates: 37° 25' 026.42" N and 49° 27' 307.12" E) (Figure 1). All samples were preserved in ethanol 96% then transported to the molecular laboratory for further analyses in 2017. The DNA was extracted from the abdominal tissues of all samples. The samples were divided into small pieces and placed in 1.5 microtubes. The number of microtubes was eight. Four times repeat for each extraction method DNA.

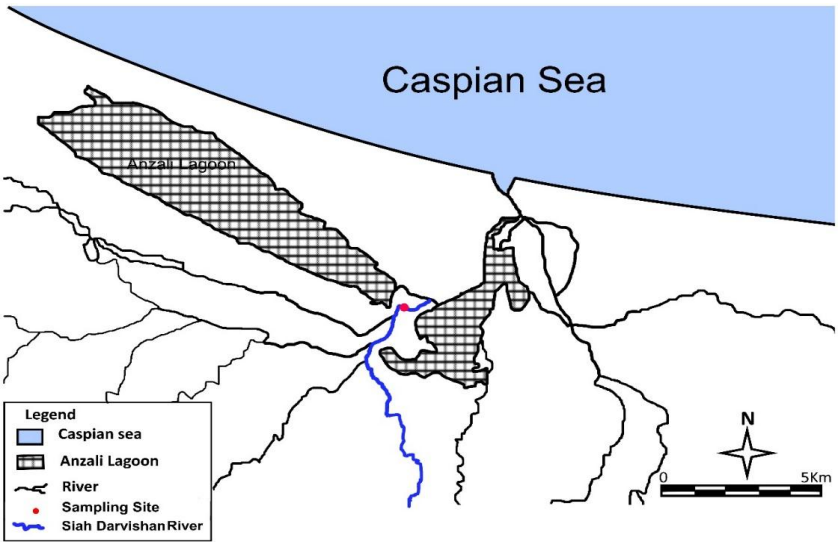

Figure 1. Location of the study area. The red circle is the sampling site

DNA extraction

Chelex method

In the Chelex method for extracting DNA (**Shokoohi, 2021c**), A piece of *A. leptodactylus* with a fine tip needle was hand-picked and transferred to a 1.5 ml Eppendorf tube containing 20 µL double distilled water. First, the crayfish in the tube was crushed and vortexed. Next, five µL of proteinase K and ten microliters of 5% Chelex® 50 were added to the microcentrifuge tubes that contained the crushed crayfish and mixed well. These tubes were incubated at 56 °C for two hours, then for 10 minutes at 95 °C to deactivate the proteinase K, and finally centrifuged for 2 min at 16000 x g (Sorvall™ Legend™ 14 Personal Microcentrifuge, USA) (**Shokoohi *et al.*, 2018**). The supernatant was stored at –20 °C.

Quick-DNA™ Tissue/Insect Miniprep Kit

DNA extraction was done using the Kit method according to protocol. First, a piece of *A. leptodactylus* was hand-picked with a fine tip needle and transferred to a ZR BashingBead™ Lysis Tube (2.0 mm). DNA extraction was done using the Kit method according to protocol. First, a piece of *A. leptodactylus* was hand-picked with a fine tip needle and transferred to a ZR BashingBead™ Lysis Tube (2.0 mm). Then 750 µl, BashingBead™ Buffer was added to the tube and capped tightly. Then vortexed it at maximum speed for 10 minutes and centrifuged it in a microcentrifuge at ≥10,000 x g for 1 minute. Next, up to 400 µl supernatant was transferred to a Zymo-Spin™ III-F Filter in a Collection Tube and then centrifuged at 8,000 x g for 1 minute. Next, 1,200 µl of Genomic Lysis Buffer was added to the filtrate in the Collection Tube and mixed it well. Next, 800 µl of the mixture to a Zymo-Spin™ IICR Column1 was transferred in a Collection Tube and centrifuged at 10,000 x g for 1 minute. Then, the flow-through from the Collection Tube was discarded and repeated. Next, 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute. Next, 500 µl gDNA Wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuged at 10,000 x g for 1 minute. Next, the Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and added 100 µl DNA Elution Buffer directly to the column matrix. Then, centrifuged it at 10,000 x g for 30 seconds to elute the DNA. The supernatant was stored at –20 °C.

Spectrophotometric analyses of DNA

The absorbance ratio at 260–280 nm (A260/A230 ratio) was measured by Thermo Scientific NanoDrop™ 1000 Spectrophotometer (Thermo Scientific, Germany). We used one µL for measurement in a spectrophotometer for each sample and repeated it three times.

RESULTS

The quality of extracted DNA sample was evaluated by NanoDrop spectrophotometer. High-quality DNA with the absence of proteins and phenols, the 260/280 nm ratio must be 1.8. Since the matrix effect was reduced using the same samples, extraction methods can be attributed to the variations in the data. In this study, the 260/280 ratio was in the range of 1.51–1.9.

DNA quality can be affected by several ingredients of samples, such as lipids, polysaccharides, and polyphenols. The Chelex method created pure DNA samples with ratios in a range of 1.51-1.79 (table 1), whereas the purity ratio samples of extract DNA with the Kit were between 1.82–1.9 (table 2).

Table 1. The amplitude of DNA function was obtained using the Chelex extraction method for all sample extracts

Sample	DNA concentration	Unit	260/280	260/230	Sample type
A1	247±23	µg/mL	1.62±0.01	1.43±0.01	DNA
A2	256±29	µg/mL	1.51±0.02	1.23±0.02	DNA
A3	219±21	µg/mL	1.68±0.01	1.32±0.02	DNA
A4	248±20	µg/mL	1.79±0.03	1.62±0.01	DNA

When the DNA purity ratio is larger than 1.9, it indicates the presence of RNA in the sample. However, we did not have more than 1.9. When the DNA purity ratio is less than 1.7, it indicates protein in the samples. However, some samples of DNA extracted by the Chelex method suggested the presence of protein in the samples. The reason could be explained by the ability of some of the procedures to eliminate contaminating molecules.

Table 2. The amplitude of DNA function was obtained using the Kit extraction method for all sample extracts

Sample	DNA concentration	Unit	260/280	260/230	Sample type
A1	475±65	µg/mL	1.85±0.02	2.07±0.02	DNA
A2	429±45	µg/mL	1.82±0.01	1.92±0.01	DNA
A3	406±57	µg/mL	1.9±0.01	2±0.02	DNA
A4	606±72	µg/mL	1.89±0.02	2.08±0.01	DNA

DISCUSSION

This study had two methods for extracting DNA from *A. leptodactylus.* Both methods worked, but Miniprep Kit's quality was much better than the Chelex method. High-quality DNA is required for PCR and sequencing (**Abdel-Latif and Osman, 2017**). Therefore, it must be free of RNA, polysaccharides, or protein contamination. On the other hand, the Chelex method was cheaper than the Miniprep Kit method. Thus, in this study, between two methods in terms of saving time for eight samples preparation: the Chelex method takes time from 3 to 4 hours, and for all samples in the Kit, the method takes time from 1 to 2 hours.

Abdel-Latif and Osman (2017) studied obtaining high DNA quality from maize. They reported that the modified Mericon extraction method was the most efficient DNA extraction method, capable of providing high DNA yields with better quality, affordable cost, and less time than the Kit method. Their study did not match the present study, but the reason could be the study sample. The extraction of DNA from plants and animals is different. **Silva *et al.*** (2019) used two kinds of commercial Kit for extracting DNA from fish. They reported that both kits gave them high-quality DNA for the PCR target. **Hellberg *et al*.** (2014) compared three different DNA extraction kits for fish focused on minimizing time, costs, and labor. They reported that all Kits gave them high-quality DNA.

CONCLUSION

In conclusion, several factors are essential for having the highest quality of extracted DNA. First, the researchers need to know what the target of the research is. Second, time and cost affect the researcher's decision to choose the best way of extracting DNA. Third, quality is the most critical factor in molecular studies; therefore, further studies are required to improve the quality of the Chelex method.

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