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Comparison of Three Methods of DNA Extraction from *Parachipteria willmanni* (Acari: Oribatida) Collected in Turkey

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Abstract: We compared three DNA extraction methods; a Chelex resin (C100), Qiagen DNA extraction kit and Cethyl Trimethyl Ammonium Bromide (CTAB) protocol that obtained from Parachipteria willmanni van der Hammen, 1952 mite specimens preserved in ethanol. The aim of the study is to find the most efficient protocol for obtaining DNA from Parachipteria willmanni. All methods are successful in isolating DNA from P. willmanni, but among these three methods, maximum amount of DNA is obtained with Chelex-method. DNAs obtained by these three methods were successfully applied to RAPD-PCR.

Keywords: Chelex-method; DNA extraction; Oribatida; *Parachipteria willmanni*; RAPD-PCR.

1. Introduction

The ordo Oribatida (Acari) members are saprophagous microarthropods that generally lived in soil ecosystems worldwide [1]. They are the important part of the soil ecosystems and readily sampled in large numbers [2].

Characterization of Acari species is primarily based on morphological differences. Nevertheless, molecular analysis within and between mite species would better clarify their phylogenetic diversity because of their small size [3]. These studies require high quality DNA and so, choosing the most effective DNA isolation protocol is the foremost step before starting the study. Because the prime problem of DNA extraction from small specimen is its quality [4]. Besides, morphologically cryptic species often occur in mite groups [5].

Extraction and purification of DNA is an essential step before starting a study belongs to a molecular biology. In molecular studies, isolated DNA from target sample must be pure enough for PCR amplification and also yield of DNA must be sufficient. Several soil or sediment samples like Acari, contain extremely high amounts of polysaccharides, proteins, and tannins that would interfere with the DNA isolation protocols [6].

The main problem in the isolation and purification of DNA from samples contains degradation of DNA by endonucleases. Recent studies have proposed DNA isolation methods of different mite species [3, 6-9].

Although, there are many studies on identification of this group by classical methods [10-15], molecular-based studies have not found from Turkey.

The aim of this research contains improvement a method to isolate DNA from *Parachipteria willmanni*. Therefore, we compared three different DNA isolation procedures and we made RAPD-PCR with extracted DNA from these methods. This is the first report on the comparison of DNA isolation methods from *P. willmanni* in Turkey.

2. Materials and Methods

2.1. Sample Collection and Identification

Parachipteria willmanni belongs to the family Achipteriidae (Acari: Oribatida), was collected in moss and lichen from Erciyes Mountain, Turkey. Examination of *P. willmanni* was conducted with traditional methods [16]. Identification of samples was done by morphologically. Alcohol (75% ethanol) conserved individual specimens of *P. willmanni* were used for DNA extraction methods.

2.2. DNA Extraction Methods

The collected samples were killed in 95% ethanol. For each DNA extraction method, DNA was isolated from individual mites. The sampled DNA was quantified by taking the optical density (OD) measurements at 260 and 280 with a spectrophotometer (ACTGene Micro-Spectrophotometer) and the purity was evaluated by the ratio of OD_{260}/OD_{280}. The A_{260}/A_{280} ratio demonstrate the DNA purity, 1.8-2.0 values suggest “pure DNA” [17].

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2.3. Chelex-100 Protocol

The DNA was extracted according to the method of Edwards, *et al.* [18] modified. An individual *P. willmanni* mite was crushed in a microtube containing 40µl 5% Chelex® solution (Sigma) and 4 µl Proteinase K (10 mg/ml) and strenuously vortexed for 10 seconds. The samples were incubated at 56°C for 30 min, vortexed again for 10-15 seconds, then heated to 100°C for 4 minutes. Suspensions were vortexed and centrifuged at 14.000 rpm for 4 min to allow DNA solution from top of the tube. The DNA solution was transferred to the 0.5 ml. tube and stored at -20°C until used.

2.4. Qiagen DNA Extraction Kit

The genomic DNA was individually extracted from *P. willmanni* mite using Qiagen DNeasy tissue kit (Qiagen, Hilden, Germany), following the manufacturer's instruction manual.

2.5. Cethyl Trimethyl Ammonium Bromide (CTAB) Protocol

The procedure of DNA extraction was performed according to the method of Desloire, *et al.* [7] with modification. 200 µL of extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH, 8.0) and 4 µl Proteinase K (10 mg/ml) were added to the individual mite homogenate, then incubated at 65°C for 1 h. After incubation an equal volume of chloroform/isoamyl alcohol was added. DNA was then precipitated by adding one volume of 100% cold ethanol and spun at 14.000 rpm for 30 min. The DNA pellet was washed with 100 µl of 70% ethanol, dried for 10-15 min at 50°C. Finally, DNA was resuspended and dissolved in 20 µl 10 mM Tris-Cl 1mM EDTA (TE) buffer.

2.6. RAPD-PCR

Amplification of both nuclear DNA that extracted by all methods was performed using 2 µl of DNA template in a 15 µl reaction containing 1.5µl PCR buffer (10X buffer with (NH4)2 SO4, Fermentas), 0.5µl dNTPs (10mM stock solution), 2µl random primer (10µM, Opc2), 0.25µl Taq Polymerase (5 u/µl, Fermentas), 1.5µl MgCl2 (25mM stock solution, Fermentas), 1.2µl BSA (10mg/ml) and 6.05µl of sterile distilled water. The temperature profile for the RAPD-PCR was a pre-denaturing step of 2.5 min at 94 °C, followed by 38 cycles of 45 s at 94°C, 45 s at 35°C and 45 s at 72°C, with a final extension step of 5 min at 72°C. The PCR products were electrophoresed in a Tris-Asedic Acid-EDTA buffer by 1% agarose gel for 1.5 h at 80V. The DNA was stained with ethidium bromide and the bands were photographed under UV light (Figure 2).

2.7. Statistical Analysis

The data were analysed by ANOVA and Tukey tests for comparison of isolation methods using SPSS program for Windows. Differences were regarded significant at $P<0.05$. The values were stated as mean±SD.

3. Results and Discussion

In the study, *Parachipteria willmanni* which is a new record for the Turkish fauna is identified by classical methods [19-21]. General characters of *P. willmanni*; length 620-660 (640) µm, width 410-430 (420) µm (n=6); body structure dotted and colored dark brown; porose area large, pedotectum I with strong distal tooth, tutorium with narrow (Figure 1A-C).

Although classical systematic is essential in identification, molecular identification methods are becoming important in systematic acarology [22]. In molecular-based studies, extraction of DNA is the critical step. The previous DNA extraction methods from arthropods contain toxic or corrosive chemicals, complex steps or require expensive materials [23]. We compared three different isolation protocols. These all protocols are practical, inexpensive, not time consuming and comparatively low toxic.

The present study shows that it is possible to extract DNA from *P. willmanni* by using all tested procedures. The yield and purity of DNA were determined spectrophotometrically. The statistical analyses results of differences between three isolation methods are shown in Table 1. The differences between isolation methods were statistically significant ($P<0.05$) and the difference was determined using the Tukey test (Table 1, letters).

During the DNA isolation procedures, remaining proteins are often present. These proteins tightly bound to DNA and may create some problems in subsequent studies like PCR. For determining the concentration and purity of DNA solution, the optical density (OD) at 260 and 280nm is measured. If the absorbance ratio (A_{260}/A_{280}) is between 1.8-2.0, it means that DNA is quite free of protein [17]. The final target of DNA extraction is to obtain DNA with the highest concentration and purity.

In our study, we have achieved DNA with highest yield by Chelex-100 (C100) method, while with highest purity with Qiagen DNA extraction kit. Although, with CTAB method we isolated much more DNA than Qiagen kit, with Qiagen kit method isolated DNA was purer than CTAB method (Table 1). The DNA extracted by kit exhibiting highest purity, but amount of DNA was negligible for other two methods.

In the study, we used samples stored in 75% ethanol. It was demonstrated that in DNA isolation protocols, usage of the samples stored in 75% ethanol has been resulted low DNA yield after isolation [24]. Using the best DNA isolation method with the best stored samples has been helpful in molecular phylogeny research of small insects and mites.

Cellular and nuclear membranes are the first barriers to obtain isolated DNA. Detergents, like SDS and CTAB used for disruption these barriers. Although, CTAB is generally used for plant and fungal DNA extraction [25, 26], now this procedure is effectively used for insects [27] and mites [7]. In this paper, CTAB method provided higher purity of DNA than C-100 method, while C-100 method was rapid and easy to perform and provided higher amount of DNA than CTAB. However OD_{260}nm/OD_{280}nm ratio indicated a protein contamination in C-100 protocol. Qiagen kit was as rapid as C-100, but quantity of isolated DNA was significantly less ($P<0.05$).

To see whether this DNA extraction procedures could be used on PCR, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) was carried out with Opc2 random primer. As can be seen in Figure 2, DNA extracted by all three methods could be amplified with RAPD-PCR. Very clear bands were seen in Chelex extracted DNA. Banding patterns that obtained from other two methods were comparatively faint.

Consequently

These three protocols were all suitable for the DNA isolation from *P. willmanni* mite. The aim of this study is to obtain pure and high yield DNA with a rapid and easy method. Particularly, optimization of C100 method for further studies will allow to obtaining purer DNA with high concentration. In the study, we described for the first time a rapid and easy method for the extraction of DNA from *P. willmanni* found in Turkey. Furthermore, this is the first report that defined DNA isolation from *P. willmanni*.

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Figure-1. *Parachipteria willmanni* Hammen, 1952. A- Dorsal view, B- Ventral view, C- Prodorsal view

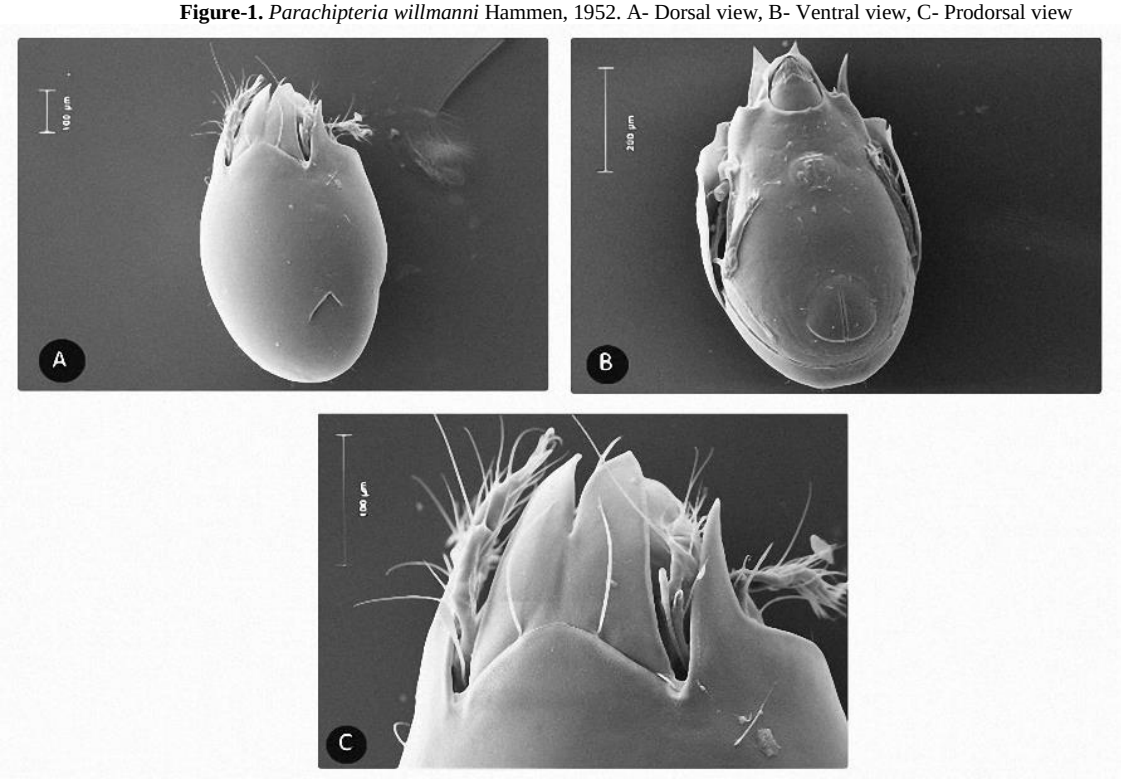

Table-1. The quantity and purity of isolated DNAs showed by micro-volume UV spectrophotometer

Method	Concentration ng/µl	Purity (A_{260}/A_{280} ratio)
Chelex-100	130,83[a]	1,19[a]
Qiagen kit	26,37[b]	1,81[b]
CTAB	64,98[c]	1,42[c]

+Six repetitions for each method were done

+Means followed by different letters are significantly different at $P < 0.05$, according to Tukey's comparison test

Figure-2. PCR amplification products from Opc2 random primer. Lane 1: 100 bp ladder; Lane 2 to 5: DNA extracted using C100 protocol; Lane 6 to 9: DNA extracted using CTAB protocol; Lane 10: Empty; Lane 11 to 13: DNA extracted using Qiagen DNA extraction kit. (All samples used for DNA extraction were *P. willmanni* specimens)