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Evaluation of Extraction Methods of DNA from Dry Collection Material of *Urophora cuspidata* and *Urophora macrura* (Diptera: Tephritidae)

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Abstract: Different DNA extraction protocols are evaluated for DNA isolation from various samples. In our study we compared three DNA extraction methods; a Chelex resin (C100), Qiagen DNA extraction kit and Cethyl Trimethyl Ammonium Bromide (CTAB) protocols obtained from dry collected materials of *Urophora cuspidata* and *Urophora macrura* (Diptera: Tephritidae) samples. Although, the hihgest yield of DNA was obtained from C100 method, the purest DNA was obtained with Qiagen protocol. Using RAPD-PCR, we demonstrate the efficacy of Qiagen protocol on these samples collected up to 6 years ago.

Keywords: *Urophora cuspidata*; *Urophora macrura*; Tephritidae; DNA extraction; Chelex; RAPD-PCR.

1. Introduction

From the order Diptera, family Tephritidae or real name fruit flies have a worldwide distribution area with about 5000 species. Tephritidae is known with an economically important pest species in some fruits [1]. Generally, larvae of species belonging to this family feed on culture or wild plant fruits, so they renamed as “fruit flies”. Some species create a gal so, they called “gal flies”. *Urophora* larvae develop in the receptaculum of Asteraceae species and create various types of gals. Many species of this genus used as biological control agents of weed seeds [2]. Until now, 4.400 tephritid species belonging to 481 genera, and 900 species belonging to 137 genera are known in the World and Palearctic region, respectively [3-5]. In Turkey, totally, 133 species were recorded in the studies conducted till now [6].

The systematic describes the relationships between organisms. Correct identification of taxonomic groups allows organizing the information and correcting identification keys [7]. DNA analysis conducted in recent years has contributed to the elucidation of the evolutionary relationships between organisms. Nowadays, DNA differences between species can be measured reliable and thus the species can be detected by DNA sequencing.

The objective of molecular systematic studies is to introduce the structure of target population, and determine the intraspecific and interspecific phylogenetic relationships. For this purpose genetic marker systems are used effectively. The creation of molecular markers is based on naturally occurring polymorphisms [8]. Classical genetics has been used before for understanding of the diversity of insects. Morphological characters have been used as phenotypic markers [9]. In entomology, the use of DNA-based techniques are particularly important in determining the taxonomic and phylogenetic relationships in many areas of research [10].

The most important step of the molecular studies is to achieve the highest yield and pure DNA. Extraction and purification of DNA from small and dry specimens are main problems [11]. Dry insect specimens are commonly held in entomology collections. DNA is normally degraded as a function of heat and time and molecular-based techniques are generally limited to collected samples for molecular work [12].

The primary aim of this study was to obtain DNA isolation protocol from air-dried collection material. We wanted to develop a DNA extraction method from pinned collections of *Urophora cuspidata* and *Urophora macrura*. In this paper, C100, CTAB and Qiagen DNA extraction kit were used. This is the first report on the comparison of DNA extraction methods from single *Urophora cuspidata* and *Urophora macrura* collection materials for PCR analysis.

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2. Materials and Methods

2.1. Insect Materials

The samples were collected in Turkey in 2009 (Table 1). The adults of *Urophora* were collected by insect net. The net was swung at random around the host plants or adult specimens seen on the host plants were collected. The samples were killed in a killing jar containing ethyl acetate. Samples were prepared in the laboratory. Specimens were collected from different provinces in Turkey. Species were identified according to Hering [13], Korneyev and White [14], and Merz [15]. The terminology follows primarily White, *et al.* [16], and [15]. Extensive synonymy and bibliography are found in Thompson [17]. Air-dried collection materials of individual specimens of these species were used for DNA extraction methods.

Table-1. Origins of two *Urophora* species used in the study (Um: *U. macrura*, Uc: *U. cuspidata*)

Species	Locality	Province	Date collected
Um1 (♂)	N 40° 41′ 45′′ E 42° 09′ 96.6′′ Elevation: 1185 m.	Erzurum/Oltu/Tekeli village	09.06.2009
Um2 (♀)	N 40° 41′ 45′′ E 42° 09′ 96.6′′ Elevation: 1185 m.	Erzurum/Oltu/Tekeli village	09.06.2009
Um3 (♂)	N 40° 03′ 20.3′′ E 43° 39′ 22.8′′ Elevation: 1122 m.	Iğdır/Tuzluca	06.07.2009
Um4 (♀)	N 40° 03′ 20.3′′ E 43° 39′ 22.8′′ Elevation: 1122 m.	Iğdır/Tuzluca	06.07.2009
Um5 (♀)	N 40° 44′ 11.7′′ E 41° 39′ 52.4′′ Elevation: 613 m.	Erzurum/Uzundere/Kınalıçam village	20.07.2009
Um6 (♀)	N 40° 44′ 11.7′′ E 41° 39′ 52.4′′ Elevation: 613 m.	Erzurum/Uzundere/Kınalıçam village	20.07.2009
Uc1 (♂)	N 40° 24′ 17.9′′ E 41° 40′ 51.6′′ Elevation: 653 m.	Artvin/Yusufeli	20.07.2009
Uc2 (♂)	N 40° 18′ 40.4′′ E 42° 38′ 33.7′′ Elevation: 2089 m.	Kars/Sarıkamış	13.08.2009
Uc3 (♂)	N 39° 36′ 48′′ E 39° 49′ 30.9′′ Elevation: 1197 m.	Erzincan/Demirciler	13.07.2009
Uc4 (♀)	N 39° 36′ 48′′ E 39° 49′ 30.9′′ Elevation: 1197 m.	Erzincan/Demirciler	13.07.2009
Uc5 (♂)	N 39° 57′ 56′′ E 41° 03′ 48.9′′ Elevation: 1782 m.	Erzurum/Ilıca/Kayapa village	19.06.2009
Uc6 (♀)	N 39° 57′ 56′′ E 41° 03′ 48.9′′ Elevation: 1782 m.	Erzurum/Ilıca/Kayapa village	19.06.2009

2.2. DNA Extraction and Quantification

For DNA isolation three different methods were tested. Total genomic DNA was extracted from single *Urophora* specimens. The concentration and purity of DNA were determined by spectrophotometric method using the optical density (OD) measurements at 260 and 280 (ACTGene Micro-Spectrophotometer). The A260/A280 ratio demonstrate the DNA purity, 1.8-2.0 values suggest "clean DNA" Tixier, *et al.* [18].

2.2.1. Cethyl Trimethyl Ammonium Bromide (CTAB) protocol

DNA extraction procedure was performed according to the method of Desloire, *et al.* [19] modified. 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 homogenate, then incubated at 65°C for 1 h. An equal volume of chloroform/isoamyl alcohol was added after incubation, then DNA was 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.

2.2.2. Chelex-100 Protocol

The procedure of DNA extraction followed the method of [20] with modification. An individual *Urophora* specimens was ground in a microtube containing 40µl 5% Chelex® solution (Sigma) and 4 µl Proteinase K (10 mg/ml) and strenuously vortexed for 10 second. Then they were incubated at 56°C for 30 min, vortexed again for 10-15 seconds, then heated to 100°C for 4 minute. Suspensions were santrifuged at 14.000 rpm for 4 min to allow DNA solution from top of the tube. The supernatant, used as DNA template, was transfered to the 0.5 ml. tube and stored at -20°C until used.

2.2.3. Qiagen DNA Extraction Kit

The genomic DNA extraction was performed using Qiagen DNeasy tissue kit (Qiagen, Hilden, Germany), following the manufacturer's instruction manual from individual specimens.

Table-2. The quantity and purity of isolated DNAs showed by Nanodrop Spectrophotometer

Method	Concentration ng/ µl	Purity (A_{260}/A_{280} oran)
Chelex-100	1148,4333a	1,2408a
Qiagen kit	25,8417b	1,8383b
CTAB	127,4583c	1,4658c

*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

2.3. PCR Amplification

PCR amplification was carried in 15 µl reaction volumes containing 1.5 µl PCR buffer (10X buffer with (NH4)2 SO4, Fermentas), 0.5µl dNTPs (10mM stock solution), 2µl random primer (10µM, Opc7), 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 with 2 µl of DNA template. The template DNA was used from Qiagen kit that gave the purest DNA. PCR conditions and primer used are shown in Table 3. The PCR products were run in a Tris-Asedic Acid-EDTA buffer by 1% agarose gel for 1.5 h at 80V. The DNA photographed under UV light with ethidium bromide (Figure).

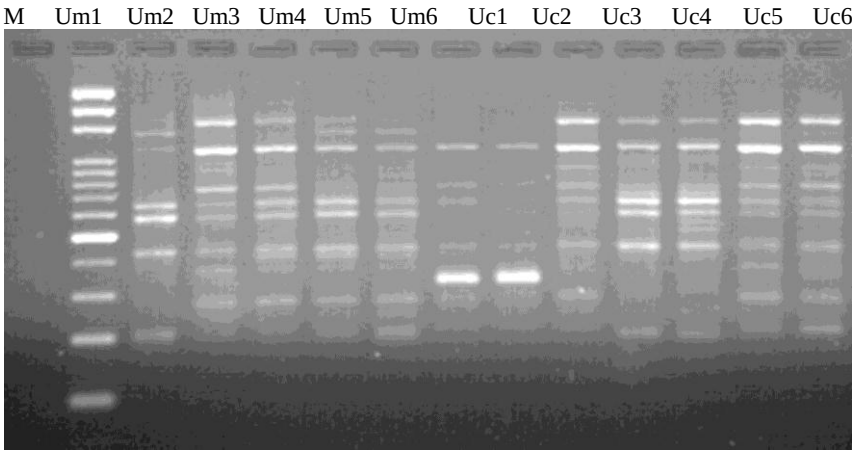
Table-3. PCR conditions and primer used in the study

Primer	Sequence (5'→3')	Denaturation	Annealing	Extension	Reference
Opc7	TTA GTG AGT A	94°C	35°C	72°C	Ercan et al. [20]

2.4. 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.

Figure. PCR amplification products from Opc7 random primer. Lane 1: 100 bp ladder; Lane 2 to 7: DNA extracted using Qiagen kit protocol with *U. macrura* specimens; Lane 8 to 13: DNA extracted using Qiagen kit protocol with *U. cuspidata* specimens. (Um: *U. macrura*, Uc: *U. cuspidata*)



3. Results and Discussion

We compared three conventional methods for extracting DNA from air-dried collection materials of *U. macrura* and *U. cuspidata* specimens; a CTAB method [21], a Chelex resin Huang, *et al.* [22], and Qiagen DNA extraction kit [23]. The ultimate aim of DNA extraction is to gain the highest molecular weight DNA without impurities. CTAB is a surfactant like sodium dodecyl sulfate (SDS) was always used in plant and fungal DNA extraction in past [24, 25]. Nowadays, this protocol is often used for DNA extraction in insects and crustaceans [26, 27]. In our study, we have obtained DNA with all tested procedures. The yield and purity of DNA were determined spectrophotometrically. The differences between isolation methods were statistically significant ($P<0,05$) and the difference was determined using the Tukey test (Table 2). We have achieved DNA with highest molecular weight by Chelex-100 (C100)

method, while with highest purity with Qiagen DNA extraction kit. In CTAB method, isolated DNA was more than Qiagen kit, but less pure.

The concentration and purity of DNA solution were determined by the measurement of the optical density (OD) at 260 and 280nm. If the ratio of absorbance (A260/A280) is between 1.8-2.0, it represents that DNA is fairly free of protein [18]. In present study, the purest DNA was achieved from Qiagen kit, but the amount of DNA was quite few and significantly less than C100 method ($P<0.05$). Although C100 method was comparatively easy and rapid, A260/A280 ratio have indicated a protein contamination in DNA solution obtained from this method (Table 2).

In order to determine whether the DNA extracted by the Qiagen kit that gave the purest DNA, could be used other molecular analyses, a RAPD primer (Opc7) was tried. Using the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) protocol and Opc7 primer, distinctive bands from *U. macrura* and *U. cuspidata* specimens were produced (Figure). RAPD-PCR is an important and sensitive method to approve the concentration and purity of the template DNA by producing consistent banding patterns [28].

In conclusion, choosing the best DNA isolation method have been a critical step in molecular phylogeny research of small insects. In the study, all tested protocols were found to be suitable for the DNA isolation from *U. macrura* and *U. cuspidata* specimens. The goal of the study is to gain DNA with high concentration and quality. We obtained high concentration of DNA with C100 method. Optimization of this method will provide high quality DNA for further studies. Additionally, this is the first study, that determine definitely the yield of DNA extracted from archived samples of *U. macrura* and *U. cuspidata* found in Turkey.

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