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Eski ve Yeni Böcek Örneklerinde Farklı DNA İzolasyon Protokollerinin Karşılaştırılması

Muhammed TATAR[1*], Aslıhan Esra BİLDİRİCİ[1], Fatih ÖLMEZ[1], Zemran MUSTAFA[1], Göksel TOZLU[2]

Öne Çıkanlar:

- Eski ve yeni böcek örneklerinden genetik materyal izole etmek için üç farklı DNA izolasyon yöntemi (fenol-kloroform, salting out ve DArT Seq) kullanılmıştır.
- İzolasyon yöntemleri zaman ve kalite açısından karşılaştırılmıştır.
- Örnek tipleri için en uygun yöntem olarak fenol-kloroform seçilmiştir.

Anahtar Kelimeler:

- Böcek
- Coleoptera
- Cerambycidae
- DNA İzolasyonu
- Genetik materyal

ÖZET:

Eklem bacaklılar şubesinde yer alan ve 1.000.000'dan fazla tür sayısı ile dünyada en kalabalık hayvan grubu, böceklerdir. Böcekler, bitkileri tozlaştırarak, organik maddeleri ayrıştırarak, toprak dokusunu iyileştirerek ve insanlara besin sağlayarak yeryüzündeki yaşamın sürdürülebilirliğini sağlar. DNA izolasyonu, Sanger dizileme, DNA parmak izi, gen-genom analizi gibi analizlerin yapılabilmesi için bir zorunluluktur. İzole edilmiş böcek DNA örnekleri; tür tanımlama, taksonomik ilişkiler, böcek-bitki ve -mikrofauna ilişkilerinin karşılaştırılması, genomik, popülasyon ve genetik çeşitliliğin ortaya çıkarılması, biyocoğrafya tespiti, tarımsal zararlılarla mücadele vb. çalışmaların yürütülmesinde kullanılabilir. Bu çalışmada, müze (eski) ve yeni böcek örneklerinden genetik materyal elde etmek için üç farklı izolasyon yönteminin zaman ve kalite açısından karşılaştırılması amaçlanmıştır. Çalışmada kullanılan örnekler, Cerambycidae familyasından (Coleoptera) aynı böcek türüne aittir. En fazla 9-12 ay önce toplanan numuneler "yeni örnek" olarak kabul edilirken, "eski örnekler" 7-28 yıllık numunelerden oluşmaktadır. Karşılaştırma için, Fenol-Kloroform, Salting Out ve DArT Seq olmak üzere üç farklı DNA izolasyon protokolü takip edilmiştir. Yeni toplanan örneklerde, kalite jeli görüntüleri ve A260/280 ve A260/230 değerleri dikkate alındığında, kalite açısından en iyi sonuçlar fenol-kloroform yöntemi ile elde edilmiştir. A260/280 değerleri tek başına incelendiğinde hem eski hem de yeni böcek örneklerinde saflık açısından DArT seq protokolü en uygun protokol olarak görünmektedir. Zaman açısından, fenol-kloroform yöntemi yaklaşık 2 saat süren en kısa protokoldür. Buna göre, fenol-kloroform yönteminin hem eski hem de yeni böcek örnekleri için daha iyi bir seçim olduğu sonucuna varılmıştır. Konsantrasyon ayarlanmasından sonra elde edilen DNA'lar, sonraki deney aşamaları için kullanılabilir veya -20°C'de stok materyal olarak saklanabilir. Bu bağlamda, kısa sürede yüksek-kalite DNA elde etmek için en uygun protokolün belirlenmesi, çalışmaların devamlılığı için önemlidir. Bulgularımız, teşhis ve sistematik durum belirleme çalışmalarına hız kazandırması ve özellikle böcek türlerinin taksonomik ilişkilerinin incelenmesi açısından önemlidir. Klasik taksonomiye ek olarak moleküler taksonominin kullanılması, böceklerin tür bazında tanımlanmasına olarak sağlar. Bu tür çalışmalar, böcek moleküler biyolojisi üzerine çalışan araştırmacılar için faydalı bilgiler üretecektir.

Comparison of Different DNA Isolation Protocols in Old and New Insect Samples

Highlights:

- Three different DNA isolation methods (phenol-chlorofom, salting out and DArT Seq) were used to isolate genetic material from old and new insect samples
- Isolation methods were compared in terms of time and quality
- Phenol-chloroform was chosen as the most appropriate method for the sample types

Keywords:

- Insect
- Coleoptera
- Cerambycidae
- DNA isolation
- Genetic material

ABSTRACT:

Insects belong to the Arthropoda phylum and are the most numerous animal group in the world, with more than 1.000.000 species. Insects ensure the sustainability of life on earth by pollinating plants, improving soil texture by decomposing organic materials, and providing nutrients to humans. DNA isolation is a necessity for performing analyses such as Sanger sequencing, DNA fingerprinting, and gene-genome analysis. Isolated insect DNA samples can be used to carry out studies such as species identification, taxonomic relations, comparison of insect-plant and –microfauna relations, revealing genomic, population, and genetic diversity, bio-geography determination, combating agricultural pests, etc. In this study, it is aimed to compare three different isolation methods in terms of time and quality for obtaining genetic material from museum samples (old) and new samples of insects. The samples used in this investigation belong to the same insect species of Cerambycidae family (Coleoptera). Samples collected at most 9-12 months ago are considered as "new specimen", whereas "old specimens" consist samples of 7-28 years old. Three DNA isolation protocols: Phenol-Chloroform, Salting Out and DArT seq, are compared in this study. Considering quality gel images and A260/280 and A260/230 values in newly collected samples, the best results in terms of quality were obtained with the phenol-chloroform method. When A260/280 values were examined alone, DArT seq protocol appears to be the most appropriate protocol in terms of purity in both old and new insect samples. In terms of time, the Phenol-chloroform method was found to be the shortest protocol lasting for about 2 h. Accordingly, the phenol-chloroform method was deduced to be the better choice for both old and new insect samples. After concentration adjustments, the obtained DNAs can be used for the next experimental stages or stored as stock material at -20 ºC. Determining the appropriate protocol to obtain high-quality DNA in a short time is important for the continuation of the studies. Our findings are important in terms of accelerating the studies for identification and determining systematic status and especially in examining the taxonomic relationships of the insect species. Using molecular taxonomy in addition to classical taxonomy allows the identification of insects on a species basis. These types of studies will generate useful information for researchers who study insect molecular biology.

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INTRODUCTION

Insects, belong in the phylum of Arthropoda, are the most numerous animal group, with more than one million described species that can be found almost everywhere in the world. They ensure the sustainability of life on earth by pollinating plants, decomposing organic matter, improving soil texture and providing nutrients to humans. Tukey boasts a diverse flora and fauna, with numerous endemic species, especially insect species, as it is partly located within the borders of three quarters of the Europe and Central Asia warm regions (Caucasus, Iran-Anatolia and Mediterranean Basin). Especially, the family of Cerambycidae (Insecta: Coleoptera) contains genera with a high number of endemic species. In addition to endemic species, there are harmful species among Cerambycidae species. Many species that cause economic damage to cultivated plants and forest trees, there are also species that feed on weeds and undertake important tasks in terms of biological control (Lodos, 1998).

It is a well-known fact that faunistic and systematic studies have a very important place in biological sciences and that most of the biological sciences have difficulty in interpreting their own findings without a systematic order (Lodos, 1998). Faunistic and systematic studies have been started many years ago in developed countries, and a great deal of progress has been made on the distribution areas and host determination of existing insect and other animal species (Tozlu, 1997).

The presence of a large number of subspecies in addition to the species in the existing catalogs indicates that the classical classification is insufficient for species identification. Therefore, in addition to making use of classification based on measuring body parts separately and proportioning to each other in some insects, electron microscopy techniques, which are based on examining the detailed structure of a certain organ or region of insects, were also used in insect systematic applications. However, electron microscope techniques are not preferred because of their cost. In recent years, molecular marker systems have been developed for the diagnosis of species that cannot be separated from each other, especially with classical systematic applications (Güz and Kılınçer, 2012). The use of molecular techniques in the classification of insects has added a new dimension to insect systematics. In addition to the definitive diagnosis of the species, existing in a habitat with molecular techniques, the proportional, morphological, physiological, nutrition, reproduction, population density, etc. changes of the species in the evolutionary process over time can be revealed. For this reason, the use of molecular taxonomy together with classical classification for the identification of individuals on the specie basis should be supported.

Based on this basic information, this study aimed to isolated DNA from Cerambycidae type insects from the Coleoptera family, which is likely to have a large number of endemics, harmful and sub-species content in Turkey. The insect was also preferred because it has an exoskeleton and a hard structure and contains plenty of chitin, in order to evaluate the difficulties in obtaining genetic material. Thus, three different isolation protocols were followed for isolation: Phenol-chloroform (Gemmel and Akiyama, 1996), DarT Seq and Salting Out (Aljanabi and Martinez, 1997), to be examined for their different advantages. These isolation protocols were preferred because they are the most frequently used methods in standard genetic material isolation experiments in molecular genetics laboratories and also because they are applied to different samples in our current laboratory due to their various advantages (cost, time, quality, ease of application, etc.).

MATERIALS AND METHODS

Collecting ınsects

The new samples collected from the provinces of Ağrı, Ardahan, Bayburt, Bingöl, Erzurum, Iğdır, Kars and Muş, which are a gateway region for Transcaucasia, Mesopotamia and Anatolia with the Eastern Anatolia Region (by Muhammed Tatar) in April-September 2021. The samples were generally

collected from gorges and forest areas, grass areas in rural areas, under stones, over bush or flowering plants. Trap, Japanese umbrella, sapirator and pit traps (for 1 liter mixture; 900ml of water, 100 ml of red wine, 25 ml of vinegar and 25 g of sugar) are the methods used in the material collection. The old samples previously collected and preserved in the Entomology Museum (Atatürk University, Erzurum, Türkiye) (by Göksel Tozlu). The old samples were also collected with the help of the same methods and kept under room conditions in the museum. The name and localization of the samples are represented in Table-1, and photographs of the species are given in Figure-1.

Laboratory Studies

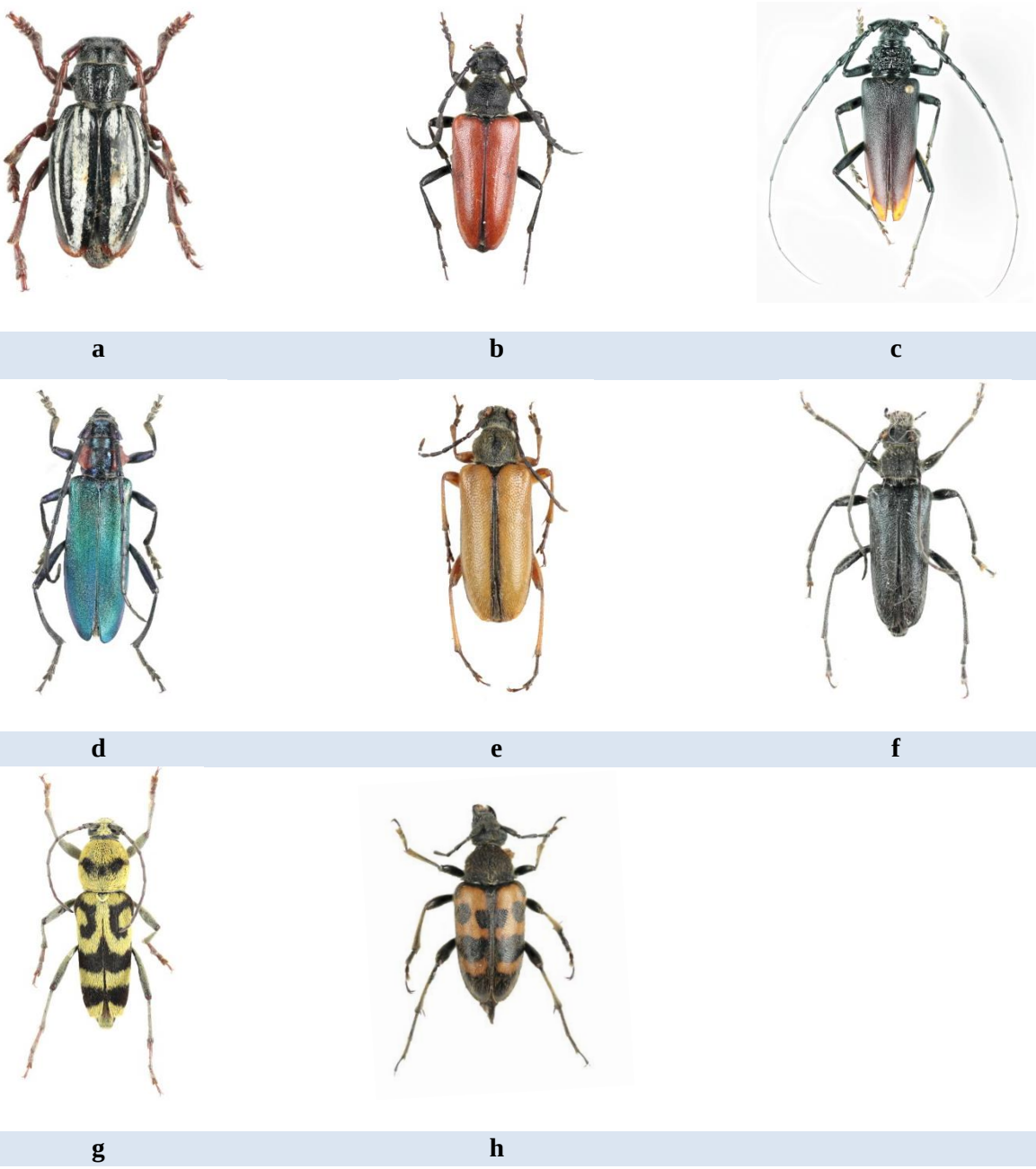
Taxonomic study on insect

The Cerambycidae samples, which were brought with proper preservation, were pinned in the laboratory with insect needles suitable for their size (approximately 1/3 of the needle remains on top) or affixed one by one on insect sticking cards of appropriate sizes.

Pictures of the dorsal, lateral, and ventral views of the specimens at Atatürk University, Biodiversity Application and Research Center were combined using Leica Macroscope, Canon 70 DSLR camera, Canon EOS utility program and Adobe Photoshop CS6 program. Some photos were taken with Canon EOS 1100D camera, Canon EF 100 mm, f/2.8L Macro lens, Kaiser digital shooting unit and combined using Helicon focus 6.7.1 program on Lenovo brand computer (Figure 2). The specimens prepared for collection were kept in Atatürk University, Faculty of Agriculture, Department of Plant Protection Entomology Museum (EMET) and in personal insect storage cabinets.

Table 1: Samples' names and localization

Samples Number and Name	Material Examined	
	New species (N)	Old species (O)
1) *Dorcadion* (*Cribridorcadion*) *wagneri karayaziense* Bernhauer and Peks, 2016	Türkiye, Erzurum Prov., Yakutiye Dist., Akdağ Village, 1915 m, 07.V.2021, M. Tatar.	Türkiye, Erzurum Prov., Atatürk Üniv., 1850 m, 39° 54′ 4.51"N - 41° 14′ 53.46"E, 30.IV.2019, M. Tatar.
2) *Vadonia bitlisiensis* Chevrolat, 1882	Türkiye, Erzurum Prov., Çat Dist., Çirişli Pass, 2200 m, 14.VII.2021, M. Tatar.	Türkiye, Erzurum Prov., Ilıca Dist., Ağzıaçık Pass, 2050 m, 19.VII.2005, G. Tozlu.
3) *Cerambyx* (*Cerambyx*) *cerdo cerdo* L., 1758	Türkiye, Bingöl Prov., Solhan Dist., Buğlan Pass, 1805 m, 23.VI.2021, M. Tatar.	Türkiye, Bingöl Prov., Solhan Dist., Buğlan Pass, 2120 m, 25.VI.2005, G. Tozlu.
4) *Aromia moschata ambrosiaca* Steven, 1809	Türkiye, Erzurum Prov., Karayazı Dist., Göksu, 2267 m, 03.VIII.2021, M. Tatar.	Türkiye, Kahramanmaraş Prov., Central Dist., 673 m, 13.VI.2000, G. Tozlu.
5) *Cortodera flavimana corallipes* Pesarini & Sabbadini, 2009	Türkiye, Bayburt Prov., Central Dist., Kop Pass, 2250 m, 15.VI.2021, M. Tatar.	Türkiye, Erzurum Prov., Ilıca Dist., Atlıkonak, 1900 m, 1.VI.2000, G. Tozlu.
6) *Cortodera alpina armeniaca* Pic, 1898	Türkiye, Muş Prov., Hasköy Dist., 1273 m, 20.V.2021, M. Tatar.	Türkiye, Erzurum Prov., Ilıca Dist., Atlıkonak, 1900 m, 1.VI.2000, G. Tozlu.
7) *Chlorophorus* (*Immaculatus*) *varius varius* O.F. Müller, 1766	Türkiye, Erzurum Prov., Olur Dist., Yeşilbağlar, 1200 m, 16.VI.2021, M. Tatar.	Türkiye, Erzincan Prov., Üzümlü Dist., 1279 m, 24.VI.1994, G. Tozlu.
8) *Pachytodes erraticus* Dalman, 1817	Türkiye, Erzurum Prov., İspir Dist., Madenköprübaşı, 1400m, 13.VII.2021, M. Tatar.	Türkiye, Kars Prov., Sarıkamış Dist., Akkurt, 1400m, 23.VI.2000, G. Tozlu.



a **b** **c**

d **e** **f**

g **h**

Figure 1: Samples. [**a)** *Dorcadion* (*Cribridorcadion*) *wagneri karayaziense* Bernhauer and Peks, 2016, **b)** *Vadonia bitlisiensis* Chevrolat, 1882, **c)** *Cerambyx* (*Cerambyx*) *cerdo cerdo* L., 1758, **d)** *Aromia moschata ambrosiaca* Steven, 1809, **e)** *Cortodera flavimana corallipes* Pesarini & Sabbadini, 2009, **f)** *Cortodera alpina armeniaca* Pic, 1898, **g)** *Chlorophorus* (*Immaculatus*) *varius varius* O.F. Müller, 1766, **h)** *Pachytodes erraticus* Dalman, 1817

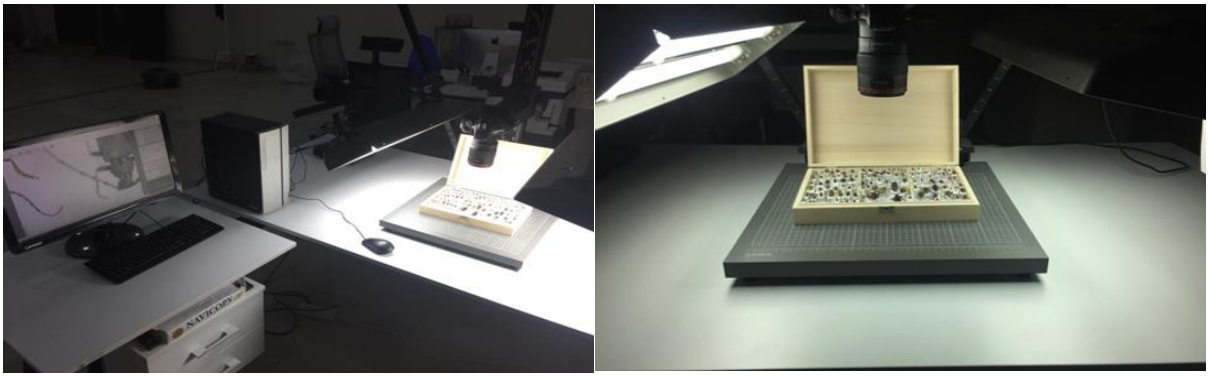


Figure 2: Imaging unit and insect containment box

The identification of the species was made by experts (Pierpaolo Rapuzzi [Prepotto, Italy], Mikhail Leontievitch Danilevsky [Moscow, Russia] and Maxim A. Lazarey [Moscow, Russia]).

Insect DNA Isolation Studies

Phenol-chloroform method (Protocol-1)

Frozen in liquid nitrogen, front and hind legs or the whole body were grinded in the eppendorf tubes with a micropestle. The samples were homogenized by adding 100 µl 2% SDS buffer and then 700 µl more 2% SDS buffer and 15 µl proteinase-K (10 mg/ml) were added. The samples were rotated in a water bath at 60°C for 30 minutes for cell lysis. Then, one volume (~800 µl) phenol:chloroform:isoamyl alcohol (25:24:1) was added in the fume hood and shaken very well. The samples were centrifuged at 13000xg for 5 minutes at room temperature. The supernatant (upper phase, ~700 µl) was taken from a new eppendorf tube. To remove phenol residues, one volume of chloroform:isoamyl alcohol was added and shaken well and centrifuged at 10000xg for 5 minutes at room temperature. Approximately 600 µl of the supernatant was taken into a new eppendorf tube. To precipitate the total genomic DNA, 0.7 volume of 100% isopropanol was added and mixed well. The samples were incubated in the freezer for 30 minutes and then centrifuged at maximum speed for 30-45 minutes at 4°C to precipitate the DNA pellets. Isopropanol was removed and 70% ethanol (1 ml) was added to wash the pellet and centrifuged for 5 minutes at 1000xg at 4°C. The ethanol was removed, and the pellets were allowed dry and then dissolved in 20-50 µl Tris-Cl (pH=8.0) buffer.

Preparation of SDS buffer: (200 mM Tris-HCl (pH:8.0), 400 mM NaCl, 10 mM EDTA, %2 SDS at pH: 8.2) Dissolve 10 g SDS in 300 ml ddH_2O (if necessary, heat up to 40 °C), add 1,4612 g EDTA or 10 ml of 500 mM EDTA, add 11,684 g NaCl, add 12,114 g TRIS, adjust pH with NaOH or HCl, add ddH_2O up to 500ml.

Salting out method (Protocol-2)

Frozen in liquid nitrogen, front and hind legs or the whole body were grinded in the eppendorf tubes with a micropestle and then 400 µl TNE buffer was added and homogenized for 10-15 seconds. The lysate, which was mixed with 40 µl 20% SDS and 8 µl proteinase-K (20 mg/ml), was incubated overnight at 37°C by inverting at regular intervals. After incubation, the lysate was centrifuged at 10000xg for 30 minutes and the supernatant was taken into a new eppendorf tube, 0.1 volume 5M sodium acetate (pH=5.2) was added and vortexed for 10-15 seconds. The tubes were incubated at -20°C for 20 minutes and then centrifuged at 16000xg for 20 minutes at 4°C. The supernatant was taken into a new 1.5 ml eppendorf tube and two volumes of 98% ethanol were added. The gently inverted samples were incubated at -20°C for 15 minutes. By centrifugation at 16000xg at 4°C for 20 minutes, the DNA pellets were precipitated and after the ethanol was carefully removed, this time was washed with 70% cold ethanol. After centrifugation at 16000xg at 4°C for 6 minutes, the alcohol was removed, and the pellets were left to dry overnight at room temperature. The dried pellets were dissolved in 50 µl 1XTE (ingredients) buffer.

Preparation of TNE buffer (500 mL): 40 mL 5 M NaCl, 5 mL 1 M Tris-HCl (pH:8.0), 2 mL 0.5 M EDTA (pH:8.0) and add 453 mL ddH_2O on the mixture.

DArT seq method (Protocol-3)

Frozen in liquid nitrogen, front and hind legs or the whole body were grinded in the eppendorf tubes with a micropestle and 1 ml fresh buffer, preheated to 65°C, was added. The tubes were incubated at 65°C for 1 hour by inverting for 10 seconds at 10 minutes intervals. It was then centrifuged at 13500xg at room temperature for 15 minutes and the supernatant was taken into a new eppendorf tube. 1 ml chloroform:isoamyl alcohol (24:1) was added and inverted at room temperature for 30 minutes. After centrifugation at 13500xg at room temperature for 15 minutes, approximately 700 µl of the supernatant

was taken into a new eppendorf tube and 800 µl of cold isopropanol alcohol was added. The samples, which were slowly inverted ten times, were incubated for 10 minutes at room temperature and centrifuged at 13500xg for 30 minutes at room temperature to precipitate the DNA pellets. The supernatant was removed and for washing pellet, 1 ml of 70% ethanol was added and centrifuged at 13500xg for 10 minutes at room temperature. The supernatant was removed, and the pellets were allowed to dry overnight at room temperature. Dried pellets were dissolved in 100 µl ddH_2O.

Table 2: Comparison of isolation methods

Phenol-chloroform method (Protocol 1)	Salting out method (Protocol 2)	DArT seq method (Protocol 3)
Homogenization with liquid nitrogen	Homogenization with liquid nitrogen	Homogenization with liquid nitrogen
Add 800 µl SDS buffer + 15 µl proteinase K (10 mg/mL)	Add 400 µl TNE buffer and homogenize for 10-15 seconds.	Add 1 mL extraction buffer
30 min. at 60 ºC in hot water bath with rotor	Add 40 µl % 20 SDS and 8 µl 20 mg/mL proteinase K and mix.	Invert tubes gently and vortex for 10 sec.
Add 1 volume phenol:chlorofom:isoamyl alcohol (25:24:1)	incubated at 37°C for overnight.	Incubate tubes at 65 °C for 1 h. Invert tubes every 10 min.
Centrifuge 5 min. at 13000g at room temperature	Centrifuge at 10000 g for 30 min.	Centrifuge tubes at 13 500g at room temperature for 15 min.
Take the upper phase bring to 2 mL eppendorf tube.	Take the supernatant to 2 mL eppendorf tube.	Transfer the supernatant to new 2 mL eppendorf tube and add 1 mL of chloroform:isoamyl alcohol (24:1).
Add 1 volume chloroform:isoamyl alcohol.	Add 0.1 volume of 5 M sodium acetate and mix by vortexing for 10-15 seconds.	Invert tubes for 30 min at RT slowly and centrifuge tubes at 13500g at room temperature for 15 min.
Centrifuge 5 min. at 10000g at RT	Hold the tubes at -20 ºC for 20 min.	Add 800 µl of cold isopropanol to new 1.5 mL eppendorf tubes.
Collect the upper phase bring to 1.5 mL eppendorf tube.	Centrifuged at 16000 g for 20 min. at 4 °C.	Take the upper phase and add to the new tube having isopropanol.
Add 0.7 volume of %100 isopropanol.	Collect the supernatant bring to 1.5 mL eppendorf tube and add 2 volumes of 98% cold ethanol to tube.	Invert tubes 10 times very slowly. Incubate at room temperature for 10 min. Centrifuge tubes at 13500g at room temperature for 30 min.
Put it 30 min in the freezer.	Hold it at -20 ºC for 15 min.	Discard the supernatant carefully and add 1 mL of %70 cold ethanol.
Centrifuge at 4 ºC for 30-45 min	Centrifuge at 16000g for 20 min at 4 ºC.	Centrifuge tubes at 13500g at room temperature for 10 min.
Wash the DNA pellet by adding 1 mL of %70 ethanol.	Remove the supernatant and add 70% cold ethanol.	Discard the supernatant carefully.
Centrifuge 5 min. at 4 ºC at 10 000g.	Centrifuge at 16000g for 6 min at 4 ºC and remove the alcohol.	Dry pellets at room temperature in tubes, with open caps overnight. The awaited samples are dissolved in 100 µl ddH_2O.
Samples are kept at room temperature for one night. The awaited samples are dissolved in in 20-50 µl Tris buffer (without EDTA).	Samples are kept at room temperature for one night. The awaited samples are dissolved in 50 µl 1 X TE buffer.	

Preparation of Fresh buffer (30mL): Add 0.15 g sodiumdisulfit and 0.6 g PVP-40 to 12.5 mL Extraction buffer and dissolve; add 12.5 mL Lysis buffer stock and 5 mL sarcosyl stock.

Preparation of Extraction buffer: 0.35 M sorbitol, 0.1 Tris-HCl (pH:8.0) and 5 mM EDTA (pH: 8.0).

Preparation of Lysis buffer: 0.2 M Tris-HCl (pH:8), 0.05 M EDTA (pH:8.0), 2 M NaCl and.- 2 % CTAB.

The protocols are summarized comparatively in Table-2.

Time required for homogenization was approximately 15 minutes. The pellet was incubated overnight at room temperature to remove alcohol from the pellet. These two steps are common to all three protocols.

Concentration and Purity Measurement

For determine the estimated concentrations of the isolated DNA samples, 1% agarose gel was used. Accordingly, 20 and 50 ng lambda DNAs were used as markers and concentrations were estimated based on the band brightness of the DNA samples loaded on the gel. Also, concentration measurements were supported by NanoDrop, a spectroscopic method. In addition, the purity levels of the isolated DNA were determined by measuring the absorbance values at 260, 280 and 230 nm. NanoDrop measurements were performed in triplicate using a blank.

RESULTS AND DISCUSSION

Protocol-1 was lasted 1 hour 45 minutes, Protocol-2 was lasted 13 hours 51 minutes, and Protocol-3 lasted 1 hour and 50 minutes. When the 1st and 3rd protocols were evaluated in terms of time, it was seen that they were the most efficient protocols.

For Protocol-1, it was observed that good results were obtained in the quality gel from six of the new samples and one of the old samples (Figure 3). The images were obtained from both old and new individuals of the first sample, *Dorcadion (Cribridorcadion) wagneri karayaziense* Bernhauer and Peks 2016. The reason for using the old specimen of *D. (C.) wagneri karayaziense* in all three protocols is that it is more recent than individuals of other species. According to the 1% agarose gel image, the obtained DNA concentration is in the range of 15-20 ng/µl compared to 20 ng lambda DNA used as a marker. According to the gel image, Protocol-1 appears to be suitable for new samples.

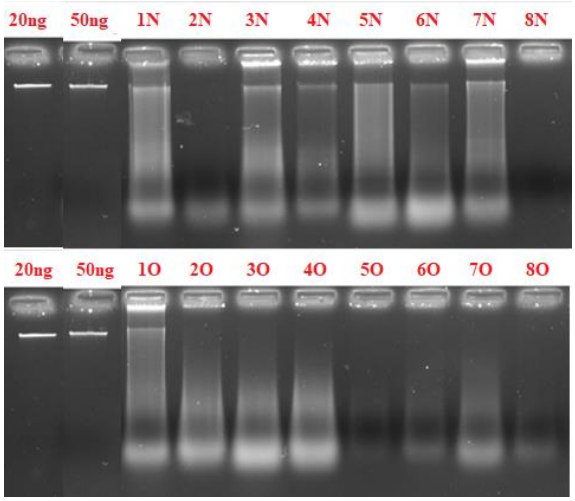


Figure 3: 1% agarose gel images for Protocol-1 (Phenol-chloroform method). [N: new sample, O: old sample]

Protocol-2: 1% agarose gel images were obtained from five of eight new specimens and two of old museum specimens (Figure-4). The DNA concentration obtained according to the 1% agarose gel image is in the range of 10-15 ng/µl, compared to 20 ng lambda DNA used as a marker.

For Protocol-3, it was observed that good results were obtained in the quality gel from three of the new samples and two of the old samples (Figure 5). The DNA concentration obtained according to the 1% agarose gel image is in the range of 10-15 ng/µl, compared to the 20 ng lambda DNA, used as a marker.

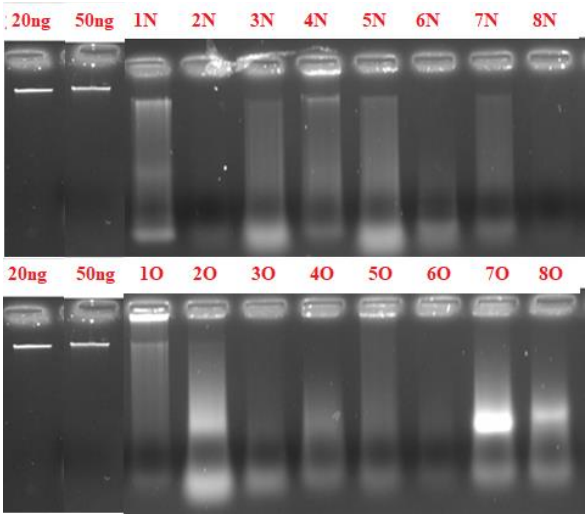


Figure 4: 1% agarose gel images for Protocol-2 (Salting out method). [N: new sample, O: old sample]

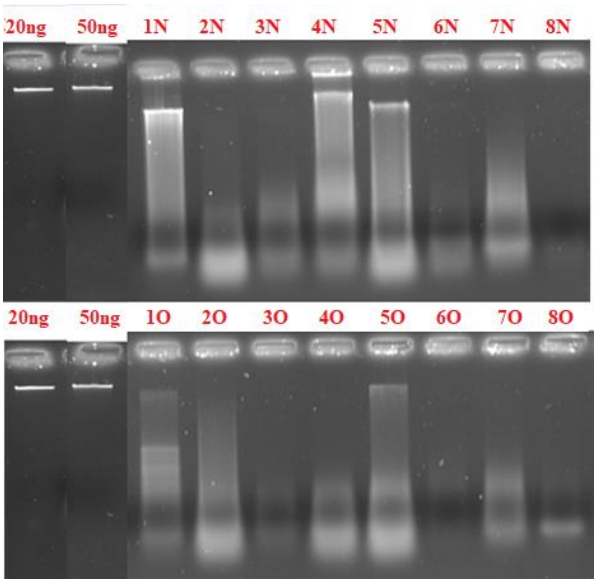


Figure 5: 1% agarose gel images for Protocol-3 (DArT seq method). [N: new sample, O: old sample]

Concentration of the isolated DNA with different protocols was determined qualitatively by 1% agarose gel electrophoresis method. According to the agarose gel images, the isolation method used in Protocol-1 was found to be successful in new individual samples.

In addition to quality gel images, concentration and purity measurements of the isolated DNA samples were confirmed using NanoDrop, a spectroscopic method (Table 3).

The absorbance at 260nm and 280nm is used to evaluate the purity of DNA and RNA. A ratio of ~1.8 is generally considered "pure" for DNA. In either case, at 280nm, if the ratio is significantly low or close, it may indicate the presence of strongly absorbing protein, phenol or other contaminants. And again, the A_{260}/A_{230} ratio gives information about nucleic acid contamination. The A_{260}/A_{280} value obtained in the new samples in the protocol shows that the DNA is of pure quality. When the A_{260}/A_{280} values are examined, that the Phenol-chloroform and the DArT seq protocols are the most appropriate protocols in terms of purity for old and new insect samples. The A_{260}/A_{230} absorbance value of the DNA obtained by using Protocol-1 in new individual samples was in the normal range. According to the A_{260}/A_{280} value obtained for the museum samples, it was determined that the DNA was of good quality for some individuals and poor for others. While Protocol-2 gave acceptable results in terms of purity for new samples, A_{260}/A_{280} values for the old samples ranged from 1.1 to 2 and the value could not be read for some of the samples.

Table 3: NanoDrop concentration and absorbance values of samples

Sample	Cons. (ng/µl)			A260/A280			A260/A230		
	1. Prot.	2. Prot.	3. Prot.	1. Prot.	2. Prot.	3. Prot.	1. Prot.	2. Prot.	3. Prot.
1 N	-	-	952.2	-	-	1.4	-	-	1.3
2 N	329.38	655.3	985.6	1.7	0.9	1.9	1.9	1.1	1.4
3 N	2020	758.5	436.3	1	1	1.6	2.5	1.2	1.4
4 N	490.39	873.4	996.2	1.9	1.8	1.7	1.9	1.5	1.3
5 N	1202.9	1062.4	830.4	2.1	1	2	2.1	1.1	1.6
6 N	1575.9	1402.6	231.4	2	1.5	1.7	1.9	1.6	1.6
7 N	1428.6	862.87	943.8	1.9	1.2	2	1.9	1.3	1.8
8 N	55.52	360.8	78.3	1.8	1.5	1.8	2.1	1.5	2
1 O	-	1015	567	-	1.2	1.2	-	1.2	1.1
2 O	2145.48	1092.8	1171.6	1.6	2	2	2.9	1.7	1.8
3 O	-	-	403.2	-	-	1.3	-	-	1.3
4 O	-	1873	1159	-	1.1	1.9	-	2.1	1.7
5 O	115.9	1239.6	118	2.9	1.15	2.4	4.2	1.26	1.9
6 O	263.7	239.3	61.8	2.7	1.3	1.9	2.3	1.3	2.1
7 O	776	-	379.5	1.9	-	2	2	-	1.7
8 O	184.2	-	134.2	2.9	-	1.8	2.2	-	1.5

[N: new sample, O: old sample]

A study with a similar concept to our study was conducted by Güler et al. (2018), and nine different DNA isolation methods were compared in terms of PCR success using insect samples. According to the study results, while all methods were successful in the mitochondrial genome, inconsistent results were observed between the methods in the nuclear genome. In PCR amplification of both mitochondrial and nuclear genome regions, phenol-chloroform, salt precipitation and column-based commercial kit methods have been reported to give the best results for PCR. In addition, considering the cost and toxicity of the chemicals used, it has been determined that the salt precipitation method is the most suitable method for DNA isolation from insect tissues.

In another study conducted by Gutierrez-Lopez et al. (2015), manual and semi-automatic DNA isolation methods were compared in louse flies. According to the study results, the phenol-chloroform method was determined as the method with the lowest efficiency. The phenol-chloroform method, which is used as a routine DNA isolation method in many laboratories, is a disadvantageous method due to the extreme toxicity of the chemicals used (Chen et al, 2010). However, when evaluated in terms of the parameters taken into account in the study, it is considered one of the advantageous methods for new insect samples. Salting out and DArT seq protocols have an advantage over the phenol-chloroform method in terms of the toxicity of the chemicals used, however, although the DArT seq protocol is the most successful method for both old and new samples, it is the longest method in terms of time.

Other studies have also been carried out aiming to obtain the maximum amount of DNA from insect samples duet to their small size, that is, the lack of starting material (Dittrich-Schröder et al, 2012).

CONCLUSION

The species diversity and population densities of insects are quite high. This can be explained by the fact that insects can adapt to any habitat due to their high adaptability. Because of their beneficial and harmful roles in the ecosystem, these livings are included in research topics not only in basic sciences such as biology, but also in sciences such as biotechnology, agriculture, veterinary medicine, and forensic medicine.

Species identification is extremely important for all fields of biology, from population genetics to environmental sciences studies. Today, DNA barcoding helps determine the definitive diagnosis or species, the changes in the evolutionary process of species, the determination of the genes encoding the enzymes involved in silent processes, and the changes in the defense system they have shown against unsuitable environmental conditions. In addition, thanks to barcoding, the genetic variability and

populations composition, the observation of disease agents, and the geological processes depending on the presence of species in a habit can be explained (Güler et al, 2018). For this reason, in DNA isolation, which is the first step to reveal such data, it is very important to choose an efficient and useful method, as well as an economical method. In this study, the quality of DNA material isolated with different protocols was examined by looking at 1% DNA quality gel images and absorbance values of A_{260}/A_{280} and A_{260}/A_{230}. According to our results, the best DNA material for new samples was isolated by phenol-chloroform method. In addition, according to the absorbance value of A_{260}/A_{280}, Protocol-3 for new and old samples seems to be the appropriate method for DNA isolation.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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