Uşak Üniversitesi Fen ve Doğa Bilimleri Dergisi
Usak University Journal of Science and Natural Sciences

http://dergipark.gov.tr/usufedbid
DOI: 10.47137/usufedbid.1148641

Araştırma Makalesi / Research Article

First DNA Barcode of *Bruchidius varius* (Coleoptera: Chrysomelidae: Bruchinae) from Turkey

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Geliş: 25 Temmuz 2022 Kabul: 02 Ağustos 2022 / Received: 25 July 2022 Accepted: 02 August 2022

Abstract

The DNA barcode of *Bruchidius varius* collected from Dilek Peninsula and Büyük Menderes Delta National Park (DPNP) in Aydın province of Turkey is given for the first time. As DNA barcode, mitochondrial cytochrome c oxidase subunit I (COI) with a length of approximately 628 bp is sequenced and an illustrative barcode is presented along with nucleotide composition of the target gene location. Nucleotide composition of the given sequence includes 38.63% T, 27.98% A, 17.01% C and 16.38% G and showing a strong AT bias (66.61%). GC percentage is 33.39%. BLAST tool in NCBI is used to check the taxonomic position of the sequenced sample. DNA barcoding result fully corresponds with the morphological identification of the samples.

Keywords: *Seed beetles, Bruchidius varius, COI sequence, DNA barcode, nucleotide composition.*

Özet

Dilek Yarımadası ve Büyük Menderes Deltası Milli Parkı'ndan (Aydın) toplanmış *Bruchidius varius* türüne ait DNA barkodu ülkemizden ilk kez verilmiştir. DNA barkodu olarak yaklaşık 628 bp uzunluğundaki mitokondriyal sitokrom c oksidaz altünite 1 (COI) geni kullanılmıştır. Ayrıca ilgili gen bölgesi sekansının nükleotid kompozisyonu verilmiştir. Nükleotid kompozisyonunda kuvvetli bir AT yönelimi (%66,61) görülmüş olup nükleotid oranları %38,63 T, %27,98 A, %17,01 C ve %16,38 G şeklindedir. GC oranı %33,39'dur. Dizilenen örneğin taksonomik durumu NCBI'da bulunan BLAST aracıyla kontrol edilmiştir. DNA barkodlama sonucu, örneklerin morfolojik teşhisi ile örtüşmektedir.

Anahtar Kelimeler: *Tohum böcekleri, Bruchidius varius, COI sekansı, DNA barkodu, nükleotid kompozisyonu.*

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1. Introduction

The Bruchinae, seed beetles, constitutes an economically important subfamily of Chrysomelidae, and many species are significant pests on stored products [1]. *Bruchidius varius* (Olivier, 1795) is widely distributed in Palaearctic region including Europe, Asia and North Africa [2]. This species was reported from several Fabaceae species (*Medicago* spp., *Vicia* spp., *Trifolium* spp., *Genista* spp.) [3].

Morphological species identification of seed beetles requires a hard taxonomic work, because the group is diverse and species look alike very much. DNA barcoding is a promising powerful instrument which facilitates the taxonomic work and reduces the need of taxonomic expertise [4]. The 5′-region of the mitochondrial cytochrome c oxidase subunit I (COI) with a length of approximately 658 bp is selected as a standard barcode for animals [5].

The main purpose of this paper is to provide first DNA barcode (COI sequence) of *B. varius* collected from Turkey and evaluate its importance and benefits for taxonomy of the species.

2. Material and Methods

Examined materials were collected from Dilek Peninsula and Büyük Menderes Delta National Park (DPNP), a protected area in Aydın province in Western Turkey. Specimens were collected with a sweeping net and preserved in ethanol. Prior to DNA extraction, specimens were identified morphologically using identification keys [6]. For DNA extraction and purification, EurX GeneMATRIX Tissue & Bacterial DNA purification kit (Poland) were used. Universal HCO/LCO primers were used to replicate the target region (Table 1). Sequences were obtained from Macrogen (Netherlands).

Table 1. Universal primers used in this study

LCO1490	GGTCAACAAATCATAAAGATATTGG
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA

3. Results and Discussion

Material examined: DPNP, Aydın; 18.06.2020, 37°36’44’’N, 27°12’58’’E, 1m, 3 specimens.

The COI sequence of *Bruchidius varius* was obtained successfully using LCO reverse primer. The electropherogram of the COI reverse sequence (Figure 1) shows that sequencing was clear and successful; however, some bases at the initial and end were ambiguous. Those parts were omitted from the sequence and a COI barcode consisting of 628 bp were obtained (Figure 2).

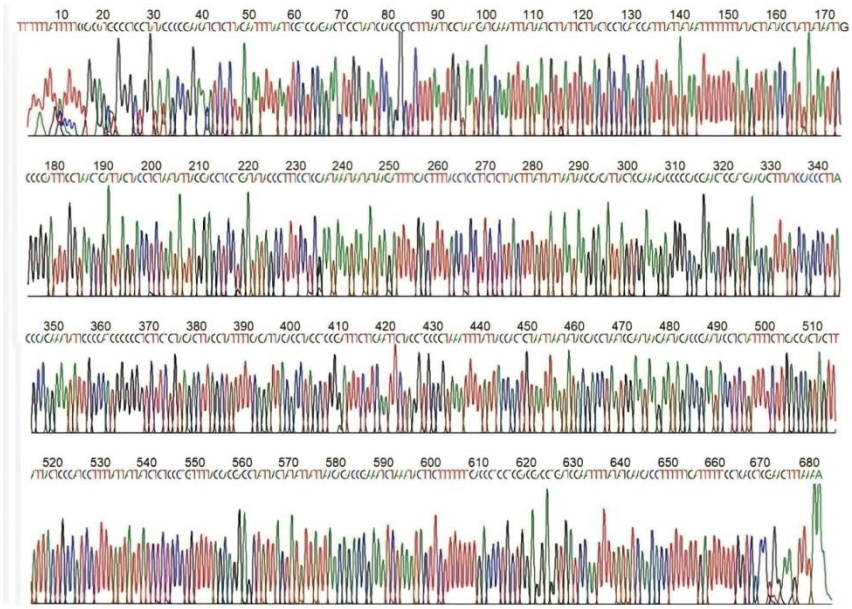

Figure 1. The electropherogram of COI sequence obtained from *B. varius*.

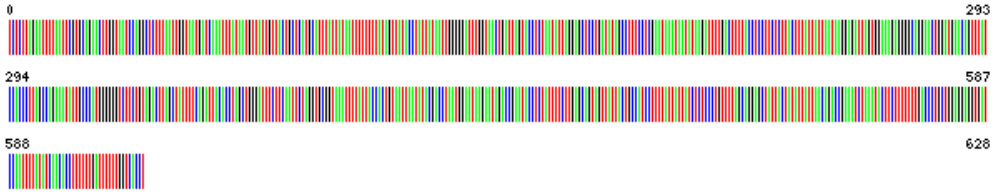

Figure 2. Illustrative COI barcode of *B. varius*.

Nucleotide composition of the given sequence is as in Table 2, including 38.63% T, 27.98% A, 17.01% C and 16.38% G and showing a strong AT bias (%66.61). GC percentage is 33.39%.

Table 2. Nucleotide composition of the COI sequence of *B. varius*.

Species	Primer	A	T	G	C	AT%	GC %	Total bp
B. varius	LCO	27.98	38.63	16.38	17.01	66.61	33.39	629

The BLAST tool provided by NCBI [7] is used to check the COI sequence obtained from our samples and a distance tree of the results is presented in Figure 3. Fast minimum evolution option is used to infer the phylogenetic tree. The figure shows 10 closest sequences in NCBI nucleotide database, and our sample sequence fits well in the distance tree. This result supports the morphological identification of the samples which is done prior to DNA barcoding.

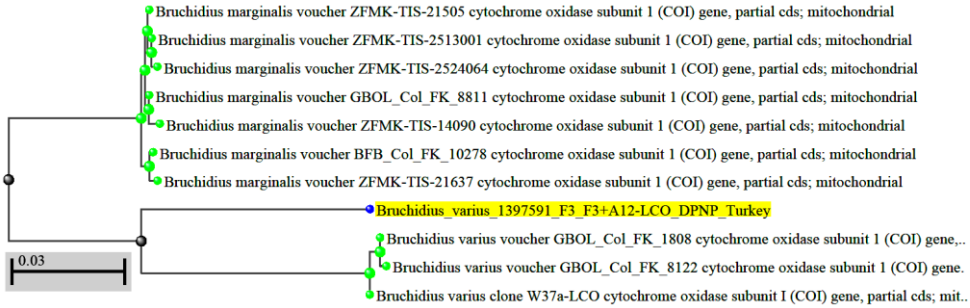

Figure 3. Distance tree inferred from BLAST tool in NCBI website using closest 10 COI sequences.

Although there are several faunistic papers dealing with Bruchinae of Turkey [i.e., 3; 8; 9], the seed beetles distributed in Turkey hasn't been fully documented yet. According to the Palaearctic catalogue of Bruchinae [2], approximately 120 species occur in Turkey. As mentioned previously, species identification of seed beetles can be challenging and needs taxonomic expertise. This may lead to misidentifications. These beetles are important agricultural pests and correct identification is essential to biological or agricultural control of these pests. Although DNA barcoding is an expensive and lengthy period for now, it can provide robust identification results for those lacking taxonomic expertise.

Acknowledgements

This study is a part of the master thesis of the first author. We sincerely thank to TÜBİTAK for supporting this study (Project number: 119Z544).

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