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DNA Barcoding of *Docotettix cornutus* Ribaut, 1948 (Hemiptera: Cicadellidae)

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Abstract*:* *Docotettix cornutus* Ribaut 1948 (Hemiptera: Cicadellidae) is a species that can cause economic damage to crops such as tomato, olive, pomegranate, and maize and is distributed worldwide. Since morphological identification is very difficult due to the small size of this pest there is a lack of experts and no record of molecular identification, DNA barcoding was aimed. In this study, *D. cornutus* was collected from pomegranate orchards in Aydın, İzmir and Manisa provinces (Türkiye) using Japanese umbrellas and suction tubes. After morphological identification of the species, HCO/ LCO universal primers were used to amplify the COI gene region of the specimen, and healthy reads were made between 499 bp (SE05) and 675 bp (SE07). Sequence analysis of the specimen was performed by Triogen Biotechnology (Istanbul-Türkiye). *D. cornutus* was registered in Genebank for the first time and a GeneBank accession number was obtained. When the specimens were compared with other registered specimens in the BLAST module at NCBI, it was seen that the closest species to D. cornutus was *Synophropsis lauri*.

Keywords: COI, DNA barcoding, Docotettix cornutus, Turkiye

Docotettix cornutus **Ribaut, 1948 (Hemiptera:Cicadellidae)'UN DNA BARKODLAMASI**

Öz: *Docotettix cornutus Ribaut 1948 (Hemiptera: Cicadellidae) domates, zeytin, nar ve mısır gibi bitkiler üzerinde ekonomik zarara neden olabilen ve tüm dünyada yayılış gösteren bir türdür. Morfolojik teşhisi zararlının küçük olması ve uzman eksikliği gibi nedenlerle oldukça zor yapılabilmekte olup, moleküler teşhisi ile ilgili herhangi bir kayıt da bulunmaması nedeniyle DNA barkodlamasının yapılması amaçlanmıştır. Çalışmada D. cornutus; Aydın, İzmir ve Manisa illerinde (Türkiye) nar bahçelerinden japon şemsiyesi ve emgi tüpü yardımıyla toplanmıştır. Morfolojik olarak tür teşhisi yapıldıktan sonra; örneklerin COI gen bölgesinin çoğaltılması için HCO/ LCO evrensel primerler kullanılmış ve 499bç (SE05) ile 675bç (SE07) arasında sağlıklı okuma yapılmıştır. Örneklerin sekans analizi Triogen Biyoteknoloji (İstanbul-Türkiye) tarafından yapılmıştır. D. cornutus ilk kez Genbank'a kaydedilmiş ve Gen Bankası erişim numarası alınmıştır. Örnekler; NCBI'da BLAST modülünde diğer kayıtlı örneklerle karşılaştırıldığında D. cornutus 'a en yakın türün Synophropsis lauri olduğu görülmüştür.*

Anahtar kelimeler: *COI, DNA barkodlama, Docotettix cornutus, Türkiye*

INTRODUCTION

Docotettix cornutus Ribaut, 1948 (Hemiptera: Cicadellidae) has been found on economically important plants such as tomato, olive, pomegranate, maize (Tedeschi and Alma 2006; Lodos and Kalkandelen 1984; Başpınar et al., 2013). Their body length usually varies between 2 mm and10 mm. In general, they have a narrow triangle-like body structure. They lay their eggs in plant tissue and have a stinging-sucking mouth structure. Cicadelliade species can cause damage by sucking the sap of leaves of plants, carrying viral disease agents and blocking the conduction tissues during feeding (Lodos, 1986).

Reliable identification of species is crucial for taxonomic classification. Traditional species identification methods are based only on morphological characteristics. Many criteria such as the appearance and behavioral characteristics of individuals belonging to the same species living in different ecosystems have proved that the use of advanced technologies instead of traditional methods based only on morphological characteristics is a more reliable method (Komazaki et al., 2011). The COI gene region can be used as an identification marker in insect studies as well as in most animal groups (Dayan and Yıldırım, 2023). Compared to other protein-coding genes, it has many important advantages such as having extensive phylogenetic data and ease of processing (Folmer et al., 1994; Polat et al., 2018). For these reasons, this study aimed to perform gene barcoding of *D. cornutus*.

MATERIAL and METHODS

Collection of samples

In this study*,* *D. cornutus* samples (20 adults) were collected from pomegranate orchards in Aydın, İzmir and Manisa provinces (Türkiye) using Japanese umbrellas and suction tubes in the summer of 2019 (July-August) and the coordinates are given in Table 3.

Laboratory studies

Specimen identification was carried out by Prof. Dr. Hüseyin BAŞPINAR (Aydın Adnan Menderes University, Faculty of

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Agriculture, Department of Plant Protection, Aydın, Türkiye). DNA isolation of 5 different insect samples that were morphologically identified was performed using the QIAGEN DNA isolation kit as described in the protocol.

The universal HCO/LCO primers used for amplification of the COI region are given in Table 1 (Amin et al., 2014; Polat et al., 2018).

Table 1. Universal primers used in the study

LCO1490	GGTCAACAAATCATAAAGATATTGG
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA

The PCR cycle was performed as indicated in Table 2. Sequencing of the samples was performed at Triogen Biotechnology using an ABI 3730XL DNA sequencer (Applied Biosystems). The primer pair used in PCR was also used in sequencing. The sequences obtained with both primers were aligned using Bioedit, Clustal X software (Hall, 1999) and a common sequence was obtained.

Table 2. The PCR cycles used in the study to clone COI gene regions

Stages of PCR		Temperature/ Time	Cycle number
Initial denaturation		94 °C / 4 min	1
Steps of cycles	Denaturation	94 °C / 45 sec	36
	Primer annealing	55 °C / 1 min	36
	Extension	72 °C / 1 min	36
Final extension		72 °C / 10 min	1

RESULTS and DISCUSSION

Adult (A) and aedagus (B) of *D. cornutus* are shown in Figure 1. The pest is 2-10 mm long and its body structure resembles a narrow triangle.

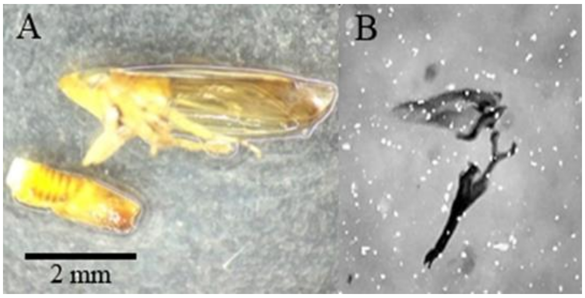


Figure 1. *Docotettix cornutus* (A) adult and its aedagus (B)

The band images of the samples after PCR are given in Figure 2.

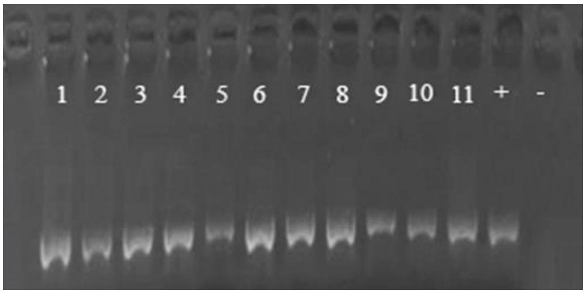


Figure 2. Gel image of eleven samples of *Docotettix cornutus*, positive control (+), and negative control (-).

NCBI accession numbers and locality information of the samples are given in Table 3

Table 3. NCBI accession numbers and locality information of the samples

Sample number	NCBI Gene Bank Accession Number	Locality
SE01	BQ0191204	39^0 4′ 8″ N- 26^O 53′ 28’’E
SE05	BQ0191205	37^{o}52′ 23″ N- 28^o 31′ 42″ E
SE07	BQ0191206	37^0 53′ 2″ N- 28^0 12′ 2″ E
SE14	BQ0191207	39^0 5′ 10’’ N – 26^0 54′ 9″ E
SE15	BQ0191208	37^0 47′ 58″ N – 27^0 55′ 40″ E

After morphological identification of the species, HCO / LCO universal primers were used to amplify the COI gene region of the specimens and healthy reads were made between 499 bp (SE05) and 675 bp (SE07).Thymine 33.33% - 33.89%, Cytosine 14.19% - 15.03%, Adenine 31.86% - 32.94% and Guanine 18.81% - 20.24% were observed in our samples.

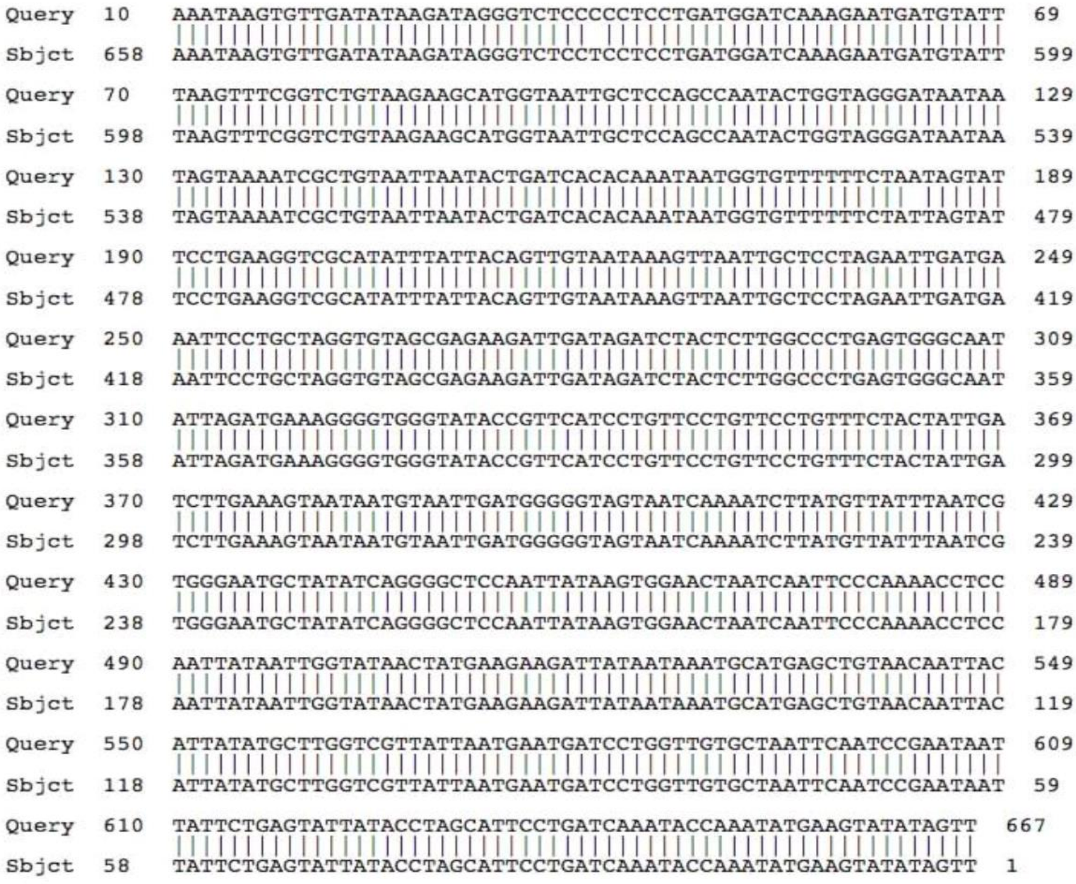
When the distance between within-group samples was calculated, it was observed that SE05 and SE07 carried the same haplotype, while SE15 and SE5 were the most distant haplotypes (1. 83%) (Table 4).

Table 4. Table of genetic distance between sequences within a group*

	SE14	SE07	SE01	SE05
SE14				
SE07	0,00770			
SE01	0,00770	0		
SE05	0,01012	0		
SE15	0,01010	0,01522	0,01522	0,01832

*Each number indicates the genetic distance between sequences.

The result of BLAST analysis with the DNA nucleotide sequence of the mtDNA COI gene region of D. cornutus is given in Figure 2..



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Query  10    AAATAAGTGTTGATATAAGATAGGGTCTCCCCCTCCTGATGGATCAAAGAATGATGTATT  69
             |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  658   AAATAAGTGTTGATATAAGATAGGGTCTCCTCCTCCTGATGGATCAAAGAATGATGTATT  599

Query  70    TAAGTTTCGGTCTGTAAGAAGCATGGTAATTGCTCCAGCCAATACTGGTAGGGATAATAA  129
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  598   TAAGTTTCGGTCTGTAAGAAGCATGGTAATTGCTCCAGCCAATACTGGTAGGGATAATAA  539

Query  130   TAGTAAAATCGCTGTAATTAATACTGATCACACAAATAATGGTGTTTTTCTAATAGTAT  189
             |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  538   TAGTAAAATCGCTGTAATTAATACTGATCACACAAATAATGGTGTTTTTCTATTAGTAT  479

Query  190   TCCTGAAGGTCGCATATTTATTACAGTTGTAATAAAGTTAATTGCTCCTAGAATTGATGA  249
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  478   TCCTGAAGGTCGCATATTTATTACAGTTGTAATAAAGTTAATTGCTCCTAGAATTGATGA  419

Query  250   AATTCCTGCTAGGTGTAGCGAGAAGATTGATAGATCTACTCTTGGCCCTGAGTGGCAAT  309
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  418   AATTCCTGCTAGGTGTAGCGAGAAGATTGATAGATCTACTCTTGGCCCTGAGTGGCAAT  359

Query  310   ATTAGATGAAAGGGGTGGGTATACCGTTCATCCTGTTCCTGTTCCTGTTTCTACTATTGA  369
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  358   ATTAGATGAAAGGGGTGGGTATACCGTTCATCCTGTTCCTGTTCCTGTTTCTACTATTGA  299

Query  370   TCTTGAAAGTAATAATGTAATTGATGGGGGTAGTAATCAAAATCTTATGTTATTTAATCG  429
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  298   TCTTGAAAGTAATAATGTAATTGATGGGGGTAGTAATCAAAATCTTATGTTATTTAATCG  239

Query  430   TGGGAATGCTATATCAGGGGCTCCAATTATAAGTGGAACTAATCAATTCCCAAAACCTCC  489
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  238   TGGGAATGCTATATCAGGGGCTCCAATTATAAGTGGAACTAATCAATTCCCAAAACCTCC  179

Query  490   AATTATAATTGGTATAACTATGAAGAAGATTATAATAAATGCATGAGCTGTAACAATTAC  549
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  178   AATTATAATTGGTATAACTATGAAGAAGATTATAATAAATGCATGAGCTGTAACAATTAC  119

Query  550   ATTATATGCTTGGTCGTTATTAATGAATGATCCTGGTTGTGCTAATTCAATCCGAATAAT  609
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  118   ATTATATGCTTGGTCGTTATTAATGAATGATCCTGGTTGTGCTAATTCAATCCGAATAAT  59

Query  610   TATTCTGAGTATTATACCTAGCATTCCTGATCAAATACCAAATATGAAGTATATAGTT  667
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  58    TATTCTGAGTATTATACCTAGCATTCCTGATCAAATACCAAATATGAAGTATATAGTT  1
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Figure 2. BLAST analysis results with DNA nucleotide sequence of the mtDNA COI gene region of *Docotettix cornutus*

As a result of BLASTn, the species with the closest similar sequence was identified as Synophropsis lauri (Hemiptera: Cicadellidae). GenBank accession number of the identified sequence (Sequence ID): MK188542.1 Length: 658 bp, Similarity: 656/658 (%99).

The use of COI region in the identification of Cicadellidae species continues to develop as a common method (Demichelis et al., 2010; Lin etal.,2010; Sottile 2010; Kosovac et al., 2020). In our study, the COI gene sequence was examined, and it was determined that the similarity rate with the close species Synophropsis lauri was 99%. Foottit et al. (2014) determined 99% similarity in their study to determine the limits of genetic diversity of Cicadellidae species with nearby species and to determine the personal reflections of each species. Sreejith and Sebastian (2014) reported that the spread rate of Aleyrodidae is high, and the genetic similarity is high in their study with samples taken from nearby countries. In our study, samples taken from Manisa, Aydın and İzmir provinces showed the same similarity in the NCBI database, indicating that the transmission is proportional. With this study, DNA barcoding of *Docotettix cornutus* was performed for the first time and recorded in Genbank.

CONCLUSION

The fact that Cicadellids are small insects and the insufficient number of experts working on this subject makes morphological diagnosis difficult. However, it is clear that in cases where the number of specimens is small or the number of male individuals is low, identification can be made easily and reliable results can be obtained by using the COI gene region. Within the framework of our study, DNA barcoding of Docotettix cornutus was performed for the first time and recorded in NCBI Genebank.

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