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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Detection and Identification of Wheat Pest *Chaetopteroplia segetum* (Coleoptera: Scarabaeidae)

Buğday Zararlısı *Chaetopteroplia segetum* (Coleoptera: Scarabaeidae)'un Tespiti ve Tanımlanması

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Abstract

Anisopliines are an important group of insects associated with both cultivated plants and wild weeds. *Chaetopteroplia segetum* (Herbst, 1783) (Coleoptera: Scarabaeidae), although preferring natural plants, can cause significant damage to agricultural crops such as wheat, rye, and corn, posing a major threat to agricultural fields. This species is widely distributed across Europe and Asia, and *C. segetum* has been reported in several provinces in Türkiye. However, despite its extensive geographical range, the phylogenetic relationships within the Scarabaeidae family remain poorly understood. This study aimed to detect, identify, and investigate the phylogenetic relationships of this wheat pest across various agricultural fields in the Çivril district of Denizli province, Türkiye. The surveys were systematically conducted between June and July 2023, with monthly sample collections carried out at predetermined locations within twelve different fields. The collected samples were morphologically characterized, and the morphological identification was further confirmed through molecular analysis of representative samples from each field. After DNA isolation, a partial fragment of the cytochrome oxidase I (COI) gene was amplified using universal barcode primers LCO1490F and HCO2198R. BLAST analysis revealed that all collected individuals exhibited 99%–100% similarity with previously reported *C. segetum* sequences. Furthermore, a phylogenetic tree was constructed using the neighbor-joining method with MEGA X software, based on COI gene region data, to elucidate the relationships among the individuals. Currently, there is limited information on the molecular classification of the Scarabaeidae family in Türkiye. The findings of this study contribute to bridging this gap by providing a foundation for future molecular research aimed at better understanding the genetic structure and phylogenetic relationships within this family.

Keywords: *Chaetopteroplia segetum*, Wheat, COI, DNA barcoding, Türkiye

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Öz

Anisopliines, hem kültür bitkileri hem de yabani otlarla ilişkili önemli bir böcek grubunu oluşturur. *Chaetopteroplia segetum* (Herbst, 1783) (Coleoptera: Scarabaeidae), doğal bitkileri tercih etmesine rağmen, buğday, çavdar ve mısır gibi tarım ürünlerine önemli zararlar verebilen bir türdür ve bu özelliğiyle tarım alanlarında büyük bir tehdit oluşturmaktadır. Bu tür, geniş coğrafi yayılımı ile Avrupa ve Asya'da yaygın olarak bulunmakta olup, Türkiye'de de birçok ilde *C. segetum* örneklerine rastlanmıştır. Ancak, bu türün Scarabaeidae ailesindeki filogenetik ilişkileri henüz yeterince incelenmemiştir. Bu çalışmanın amacı, Türkiye'nin Denizli ilinin Çivril ilçesinde yer alan farklı tarım alanlarında buğday zararlısı olarak görülen *C. segetum*'u tespit etmek, tanımlamak ve filogenetik ilişkilerini araştırmaktır. Haziran ve Temmuz 2023 tarihleri arasında yapılan sörveyler, on iki farklı alandan sistematik bir şekilde gerçekleştirilmiş olup, önceden belirlenen noktalardan aylık örneklem toplama işlemi yapılmıştır. Toplanan örnekler, morfolojik olarak detaylı bir şekilde karakterize edilmiş ve morfolojik tanımlamalar, her bir alandan seçilen temsilci örneklerin moleküler analizleriyle doğrulanmıştır. DNA izolasyonu sonrası, LCO1490F ve HCO2198R evrensel barkod primerleri kullanılarak mitokondriyal DNA'nın sitokrom oksidaz I (COI) gen bölgesinin bir kısmı çoğaltılmıştır. Elde edilen veriler, BLAST analizi ile toplanan tüm bireylerin, daha önce bildirilen *C. segetum* dizileriyle %99–100 benzerlik gösterdiğini ortaya koymuştur. Ayrıca, COI gen bölgesine dayalı olarak, bireyler arasındaki filogenetik ilişkileri göstermek amacıyla MEGA X yazılımı ile neighbor-joining yöntemiyle bir filogenetik ağaç oluşturulmuştur. Türkiye'de Scarabaeidae ailesinin moleküler sınıflandırmasına dair mevcut veriler sınırlı olduğundan, bu çalışma, bu aileye ait genetik yapı ve filogenetik ilişkilerin daha iyi anlaşılmasına yönelik gelecekteki moleküler araştırmalara önemli bir temel oluşturmaktadır.

Anahtar Kelimeler: *Chaetopteroplia segetum*, buğday, COI, DNA barkodlama, Türkiye

1. Introduction

The subtribe Anisopliina (Scarabaeidae: Rutelinae: Anomalini) are distributed across the Palaearctic, Oriental, Ethiopian, Nearctic, and Neotropical biogeographical regions. Anisopliina species are associated with both cultivated and wild grasses. As adults, they feed on grass pollen or immature grass seeds, while the larvae feed on grass roots. Members of this subtribe are characterized by an elongated and recurved clypeal apex, a feature that allows adults to extract and consume pollen-loaded grass anthers (Mico et al., 2001; Jameson et al., 2007). According to current classifications (Machatschke, 1972; Potts, 1974; Baraud, 1992), the subtribe Anisopliina comprises nine genera and approximately 100 species distributed across the World: *Anisoplia* Schönherr, *Anthoplia* Medvedev, *Anomalacra* Casey, *Brancoplia* Baraud, *Callirhinus* Blanchard, *Chaetopteroplia* Medvedev, *Hemichaetoplia* Baraud, *Rhinyptia* Burmeister, and *Tropiorhynchus* Blanchard (Jameson et al., 2007).

The genus *Chaetopteroplia*, revised by Baraud (1986), encompasses twelve species (Jameson et al., 2007). While *Chaetopteroplia segetum* (Herbst, 1783) prefers non-cultivated plants (Hurpin, 1962), it poses a significant threat to various crops, including wheat, rye, and corn (Jameson et al., 2007). *C. segetum* has been reported in several provinces across Türkiye: Mardin and Malatya (Sert and Özdemir, 2019), Tekirdağ (Altın and Özder, 2020), and Van (Özgökçe et al., 2024). A study conducted in Van identified *C. segetum* as the most prevalent and densely populated species (Özgökçe et al., 2024). The subspecies *C. segetum velutina* exhibits a wide distribution throughout Türkiye and is commonly observed in provinces such as Afyon, Ağrı, Antalya, Balıkesir, Bursa, Çanakkale, Çorlu, Denizli, Edirne, Erzurum, Eskişehir, İzmir, Kars, Konya, Manisa, Muğla, Ordu, Sivas, Tekirdağ (Lodos et al., 1978; Rozner and Rozner, 2009), Eskişehir (Küçükkaykı et al., 2013), Adana, Afyon, Ankara, Antalya, Artvin, Bitlis, Erzincan, Erzurum, Hatay, Iğdır, Kars, Konya, Mersin, Rize, Trabzon (Polat et al., 2018). The global distribution of *C. segetum* extends across Europe (including Greece, Rhodes, and Ukraine) and Asia (Crimea, Türkiye) (Löbl and Smetana, 2006). Despite its extensive range, the phylogeny within the Scarabaeidae family remains poorly characterized. It is recognized that the Scarabaeid lineage is exclusively confined within the Scarabaeidae family (Bell et al., 2004; Philips et al., 2004).

In this study, primary objective was to detect and identify the pests belonging to the *C. segetum* that have invaded wheat fields in the Denizli province of Türkiye. This purpose, we collected samples from 12 different wheat fields, focusing on specimens suspected of causing damage to wheat crops. The collected samples underwent comprehensive morphological characterization studies, and a representative sample from each field was subjected to detailed molecular characterization. In line with this study that molecular characterization studies on these specific species are scarce both globally and within Türkiye. Furthermore, our thorough literature review did not reveal any previous studies in Türkiye that molecularly characterized this particular insect. In light of this, our study not only contributes to the understanding of the molecular profile of *C. segetum* but also addresses a notable gap in existing research. By explaining the molecular characterization steps used in the study, it will be easier for researchers working in this field. The comprehensive data obtained from our research not only lays the groundwork for future investigations on *C. segetum* but also furnishes critical insights for the development of effective pest control strategies tailored to the unique characteristics of this species.

2. Materials and Methods

2.1. The Study Site and Sampling

Surveys were systematically conducted in June and July 2023, encompassing 12 distinct wheat fields situated in the Çivril district of Denizli province, southwestern Türkiye. (*Table 1*). Sampling was executed randomly, targeting various wheat plants within each site. A standard sweep net with a diameter of 35 cm was used for sample collection. A substantial number of collected samples were subsequently preserved in 99.5% alcohol for conservation until employed in molecular analyses. Samples of the pest species from every location were meticulously labeled and stored at –20°C, awaiting utilization in the experimental phases for molecular diagnosis. The geographical coordinates of all sampled fields were precisely determined using GPS technology and are presented in *Table 1*.

Table 1. Wheat fields where samples were collected for the study in the Denizli, Türkiye

Sampling area no	Wheat fields	Coordinates
1	Yakacık	N: 38º 28.42′3′′
		E: 29º 68.84′3′′
2	Ömerköy 1	N: 38º 27.39′0′′
		E: 29º 76.34′7′′
3	Emircik	N: 38º 29.11′3′′
		E: 29º 79.91′6′′
4	İnceköy	N: 38º24.74′8′′
		E: 29 º78.38′9′′
5	Karamanlı	N: 38º 25.38′2′′
		E: 29º 82.07′3′′
6	Yalınlı	N: 38º 24.17′2′′
		E: 29º 82.23′4′′
7	Menteş	N: 38º 27.50′7′′
		E: 29º 71.87′8′′
8	Balçıkhisar	N: 38º 29.82′3′′
		E: 29º 80.07′6′′
9	Seraserli	N: 38º 23.07′2′′
		E: 29º 80.95′6′′
10	Yakasomak	N: 38º 29.71′2′′
		E: 29º 72.75′4′′
11	Kızılcasöğüt	N: 38º 28.28′3′′
		E: 29º 74.01′9′′
12	Ömerköy 2	N: 38º 27.39′0′′
		E: 29º76.34′7′′

2.2. Identification of the Samples

2.2.1. Morphological Characterization

Chaetopteroplia segetum mature individuals are 9-13 mm in length, with a width of 6-9 mm, displaying a broad and oval-shaped body. The head and pronotum are densely covered with yellowish hairs, while the brownish elytra exhibit a sparser distribution of these hairs (*Figure 1*). Additionally, it has been reported that males have longer antennae and thickened front claws, whereas females have a paler coloration on their elytra (Machatschke, 1961).

Figure 1. Chaetopteroplia segetum adult form

2.2.2. Molecular Characterization

To confirm the morphological identification, COI gene region of rDNA was analyzed from 12 representative samples from each fields.

2.2.2.1. DNA Extraction

The total genomic DNA was extracted from the right hind legs of insects using the High Pure PCR Template Preparation Kit (Roche), following the manufacturer's instructions. The insect legs were washed with pure water and then left in the laminar box for 1 hour to dry. Subsequently, the dried insect legs were kept in the freezer at a temperature of -80°C for 15 minutes. Following these procedures, the washed and dried legs were transferred to an Eppendorf tube and thoroughly crushed with a sterile tube. All final DNA products were suspended in a 70 µL elution buffer and stored at -20°C until PCR amplification.

2.2.2.2. PCR Amplification

The partial fragment of cytochrome oxidase I (COI) gene of mtDNA was amplified using the universal barcode primers LCO1490F, 5'GGTCAACAAATCATAAAGATATTGG3' and HCO2198R, 5' TAAACTTCAGGCTGACCAAAAAATCA 3'of (Folmer et al., 1994). PCR reactions were performed with Xpert Fast Hotstart Mastermix (Grisp, Portugal), according to the company's protocol instructions. Each reaction mixture for PCR with a volume of 25 µL contained 12.5 µL Master Mix (Xpert Fast Hotstart Mastermix, Grisp), 3 ml of DNA matrix, 1 µL for word primer, 1 µL of reverse primer and distilled water up to 25 µL. Negative controls were used in every PCR reaction by adding all reagents except genomic DNA. PCR was carried out using a Kyratec PCR Cycler with the following settings: 3 min at 95°C; followed by five cycles of 15 s at 94°C, 15 s at 50°C, and 1 min at 72°C; followed by a further 35 cycles a final extension of 10 min at 72°C. The quality of the PCR products was examined by electrophoresing the samples on 1.5% agarose gels and TBE buffer and staining with the Xpert Green DNA Stain (Grisp).

2.2.2.3. Sequence Analyses

The amplification products obtained through PCR were submitted for sequencing at BMLabosis in Ankara. The nucleotide sequences obtained from the PCR products were subjected to a meticulous search in the GenBank database at the National Biotechnology Information Center (NCBI). Alignment of the identified sequences, as well as selected outgroup sequences retrieved from the NCBI database, was accomplished using BioEdit version 7.2.6 software. A sequence similarity search was performed using NCBI-BLASTn (Basic Local Alignment Search Tool), and sequences exhibiting more than 98% homology were considered as belonging to the same species.

2.2.2.4. Phylogenetic Analyses

Phylogenetic analysis of the beetle sequences was conducted using MEGA X software. The Fasta files of the sequences were aligned, and pairwise estimation was carried out employing the ClustalW tool within the MEGA X software, following default settings. The aligned sequences were then exported in Mega format. The phylogenetic analysis was performed utilizing the Neighbor-Joining Method and Kimura-2-Parameter, with 1000 bootstrap supports. Both pairwise and multiple alignments were conducted with a gap opening penalty of 15.00 and a gap extension penalty of 6.66. The entire phylogenetic analysis process was repeated 1000 times with bootstrap support to ensure robustness in the results.

3. Results and Discussion

Chaetopteroplia segetum were collected from 12 distinct wheat fields. Macroscopic examinations were conducted, focusing on external traits such as general appearance, antennal structure, legs, wings, coloration, body segmentation, and other anatomical features for initial species identification (Machatschke, 1961). Following both macroscopic and microscopic assessments, it was determined that all samples belonged to the same genus (*Chaetopteroplia segetum*). within the order Coleoptera. One representative sample from each area was subsequently chosen for further molecular characterization studies. Upon successful DNA isolation, PCR was carried out for all selected samples, resulting in visible amplified products at 600-700 bp for each of the 12 samples and a 100-bp DNA ladder was used to determine and confirm the size of the PCR products (*Figure 2*).

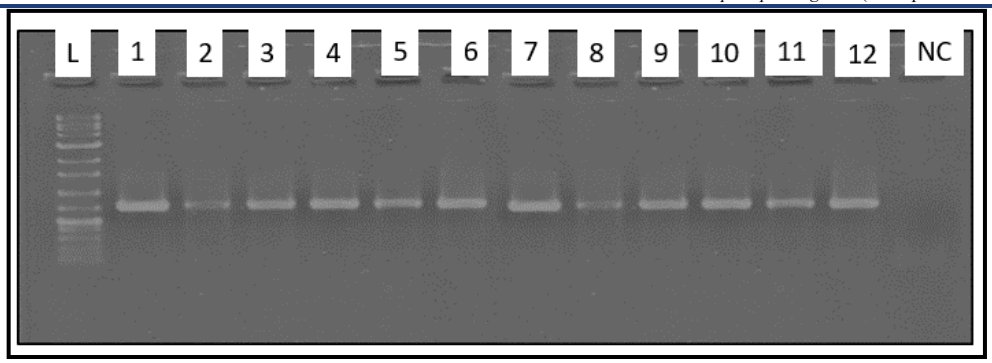

Figure 2. Polymerase Chain Reaction (PCR) bands of Chaetopteroplia segetum samples (L; DNA ladder-100 bp, NC; Negative Control)

For molecular identification, 12 representative samples from diverse wheat fields underwent sequencing. The COI sequences were subjected to BLAST analysis, validating the insect identification, exhibiting a similarity range of 98%–100% with previously documented sequences from the National Biotechnology Information Center (NCBI) for C. segetum. The COI sequences of 12 samples were deposited in GenBank (Accession Numbers; OR805470-OR807318-OR807319-OR807320-OR807321-OR807326-OR813953-OR813954-OR809195-OR809196-OR809197- PP028382).

The phylogenetic tree construction employed the neighbor-joining method through MEGA X software, utilizing data derived from the COI gene region. This approach facilitated the elucidation of intricate molecular kinship relationships among individuals, providing detailed insights through the resulting phylogenetic tree (*Figure 3*) (*Table 2*). When the phylogenetic tree created in the study was examined, *C. segetum* from the *Anisoplia austriaca* species was evaluated as an external group.

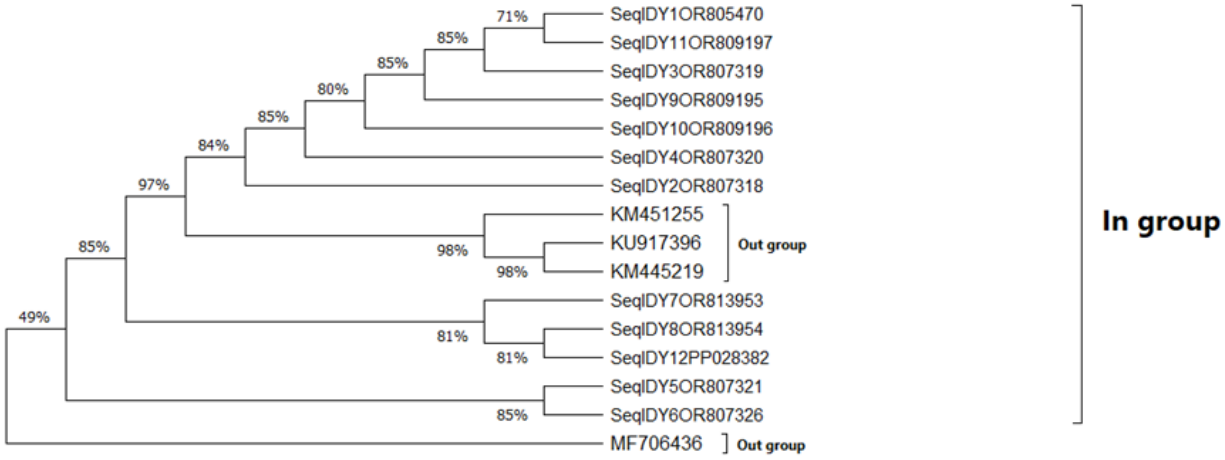

Figure 3. Neighbor-joining tree obtained as a result of phylogenetic analysis using the COI gene region

The nucleotide sequences were compared with those archived in the NCBI data bank to assess their similarities. Subsequently, a Neighbor Joining (NJ) molecular phylogenetic genealogy was constructed to analyze the sequence results of the obtained species. In the process of creating this phylogenetic tree, *C. segetum* KM451255 (Hendrich et al., 2015), KU917396 (Rulik et al., 2017), KM445219 (Hendrich et al., (2015) and *Anisoplia austriaca* MF706436 (Syromyatnikov et al., 2017), both species from the Coleoptera family, were utilized as an out group. The Bootstrap 1000 method was employed to enhance the robustness of the phylogenetic tree. The resulting optimal tree was then presented, with evolutionary distances computed using the Maximum Composite Likelihood method, expressed in units of the number of base substitutions per site. To provide additional insights, the proportion of sites where at least one unambiguous base is present in at least one sequence for each descendent clade was indicated next to each internal node in the tree. This comprehensive analysis involved a dataset comprising 16 nucleotide sequences. Ambiguities were systematically addressed by

removing all ambiguous positions for each sequence pair, utilizing the pairwise deletion option. The final dataset, containing a total of 673 positions, was subjected to evolutionary analyses, which were meticulously executed using the bioinformatics software MEGA 11.

Table 2. Sequences used in phylogenetic analyzes

No	Accession no	Species	Sites
1	OR805470	*C. segetum*	Yakacık
2	OR807318	*C. segetum*	Ömerköy[1]
3	OR807319	*C. segetum*	Emircik
4	OR807320	*C. segetum*	İnciköy
5	OR807321	*C. segetum*	Karamalı
6	OR807326	*C. segetum*	Yalınlı
7	OR813953	*C. segetum*	Balçıkhisar
8	OR813954	*C. segetum*	Seraserli
9	OR809195	*C. segetum*	Yakasomak
10	OR809196	*C. segetum*	Kızılcasöğüt
11	OR809197	*C. segetum*	Menteş
12	PP028382	*C. segetum*	Ömerköy[2]
13	KM451255	*C. segetum*	Germany: Brandenburg
14	KU917396	*C. segetum*	Germany: Mecklenburg
15	KM445219	*C. segetum*	Germany: Fuerstenwalde
16	MF706436	*A. austriaca*	Russia

The results of the study highlight the efficacy of COI-based pest identification, particularly concerning beetles, as evidenced by the COI marker profile. DNA sequence data have proven instrumental in elucidating the relationships among various groups of insect species at the generic level (Murthy, 2020). Molecular sequence information obtained from NCBI has accurately portrayed the relatedness among all collected scarabaeids, consistent with their morphological characters. Our observations align with the findings reported by Blaxter (2004). Qiu et al. (2009) propose that, where sequence information is available in GenBank for morphologically defined species and can be matched with certain DNA-based clusters, close relationships can be readily identified through sequence variation in field-collected samples. These clusters are likely to correspond to previously undescribed species. Mgocheki et al. (2012) reported the utility of sequence information based on mitochondrial markers for species delineation in both adults and grubs of scarabaeids, providing insights into larval taxonomy. Our studies underscore the significance of DNA sequencing in aligning various forms of scarabs, and minimizing the risk of misdiagnosis.

To date, only limited information exists regarding a formal cladistic classification of the Scarabaeidae superfamily (Browne and Scholtz, 1995). Consequently, there is an urgent need to investigate the genetic diversity within the Scarabaeidae family (Zahoor et al., 2013). It is essential to focus on sequencing partially genomic conserved regions, such as the mitochondrial COI gene (Foottit et al., 2008; Jalalizand et al., 2012; Tayat and Özder, 2023). DNA sequencing, particularly of the mitochondrial cytochrome-c oxidase subunit 1 gene (CO1), provides a powerful tool for accurately identifying various creatures, including certain insect species, especially those with similar morphologies. The exceptional resolution of the CO1 gene makes it a valuable resource for species differentiation and exploring hidden variations among closely related species (Sharif et al., 2023).

4. Conclusions

The fact that current information on the scarabaeoid fauna in Türkiye is limited underscores the importance of research efforts in this field. In particular, the insufficient documentation of the molecular classification of the Scarabaeidae superfamily emphasizes the need for further research. Such studies focusing on genetic diversity of harmful species like *C. segetum* throughout Türkiye could significantly contribute to control strategies for these species. Furthermore, it is believed that these investigations will provide critical insights for future research and species identification. Ultimately, detailed studies utilizing methods such as molecular characterization and phylogenetic analysis could establish a crucial foundation for the conservation and management of scarabaeoid species in Türkiye and globally.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

The collection of samples: Bayındır Erol, A.; Conducting experiments: Beram, R. C. and Tashıgul, N.; Creation of the manuscript: Beram, R. C. and Tashıgul, N. and Bayındır Erol, A.

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