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Original Article

Identification of the Parasitoid Wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) in Pistachio Orchards Using DNA Barcoding

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ABSTRACT

Identification of *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae), a parasitoid of various species within the Coccinellidae family, is essential for the development of effective biological control strategies in pistachio orchards. This study aimed to accurately identify *D. coccinellae* utilizing DNA barcoding techniques. Research was conducted throughout 2023 on coccinellid specimens collected regularly from pistachio orchards in Siirt. Among the collected Coccinellidae species, *D. coccinellae* DNA was successfully identified in the *Hippodamia variegata* specimen among a total of 11 individuals. The mitochondrial COI gene region of *D. coccinellae* was analyzed by DNA barcoding analysis using Polymerase Chain Reaction (PCR) and sequencing methods. PCR results and DNA sequencing confirmed the accurate identification of *D. coccinellae*, demonstrating high similarity with reference sequences in the NCBI database. The sequencing results exhibited high accuracy and reliability, with a similarity rate of 97.13% and an E-value of 0.0. Phylogenetic relationships were assessed using the Maximum Likelihood (ML) method. Phylogenetic analyses clearly illustrated the relationships of D. coccinellae with other Hymenoptera species. The phylogenetic tree indicated that *D. coccinellae* is most closely related to *Meteorus obfuscatus*, with a genetic distance of 0.10, providing insights into interspecies relationships.

Key words: *Dinocampus coccinellae*, Coccinellidae, DNA barcoding, parasitoid, COI gene.

Siirt Fıstığı Bahçelerinde Coccinellidae Türlerinin Parazitoidi *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae)'nın DNA Barkodlama ile Tanımlanması

ÖZ

Siirt fıstığı bahçelerinde, Coccinellidae familyasından çeşitli türlerin parazitoidi olan *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae)'nın tanımlanması, etkili biyolojik kontrol stratejilerinin geliştirilmesi açısından kritik öneme sahiptir. Bu çalışma, *D. coccinellae*''nin DNA barkodlama yöntemi kullanılarak kesin tanımlanmasını amaçlamıştır. Araştırma, 2023 yılı boyunca Siirt fıstığı bahçelerinden düzenli olarak toplanan coccinelid örnekler üzerinde gerçekleştirilmiştir. Çalışma sonucunda, toplanan Coccinellidae türleri arasında, toplam 11 birey içinde *Hippodamia variegata* örneği üzerinde *D. coccinellae* DNA'sı doğru bir şekilde tespit edilmiştir. *Dinocampus coccinellae*'nin mitokondrial COI gen bölgesi, Polimeraz Zincir Reaksiyonu (PCR) ve dizileme yöntemleri kullanılarak DNA barkodlama analizi ile incelenmiştir. PCR sonuçları ve DNA dizileme analizi, *D. coccinellae*'nin doğru bir şekilde tanımlandığını ve yüksek bir benzerlik oranıyla NCBI veritabanındaki referans dizilerle uyumlu olduğunu ortaya koymuştur. Dizileme sonuçları, %97.13'lük bir benzerlik oranı ve 0.0 E-değeri ile yüksek doğruluk ve güvenilirlik sağlamıştır. Filogenetik ilişkilerin belirlenmesinde Maximum Likelihood (ML) yöntemi kullanılmıştır. Filogenetik analizler, *D. coccinellae*'nın diğer Hymenoptera türleriyle olan akrabalık ilişkilerini net bir şekilde göstermiştir. Filogenetik ağaç, *D. coccinellae*'nın en yakın akrabası olarak *Meteorus obfuscatus* ile 0.08 genetik uzaklığa sahip olduğunu ortaya koymuş, bu da türler arasındaki akrabalık ilişkileri hakkında bilgiler sunmuştur.

Anahtar kelimeler: *Dinocampus coccinellae,* Coccinellidae, DNA barkodlama, parazitoid, COI geni

INTRODUCTION

Pistachios are of significant importance to Türkiye's agricultural diversity and substantially contribute to the regional economy. Effective management of harmful organisms is essential for achieving high productivity in pistachio cultivation. While traditional chemical control methods are commonly employed for pest management, they have raised growing concerns due to their negative environmental and health impacts (Dhankhar and Kumar, 2023). As a result, there is an increasing interest in exploring more environmentally friendly and sustainable alternatives. Biological control, one such alternative, focuses on regulating pest populations through the utilization of their natural enemies (Nazir et al., 2019).

The Coccinellidae family, which includes predatory insects, plays a crucial role in biological control applications. These beetles are particularly effective in managing populations of pest insects, such as aphids (Riddick, 2017). However, members of the Coccinellidae family can also be susceptible to their own natural enemies. In this context, the parasitoid wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) is recognized for parasitizing ladybugs within the Coccinellidae family (Koyama and Majerus, 2008). This cosmopolitan wasp has been documented parasitizing more than 50 species of ladybugs in both natural and infested environments (Ricupero et al., 2023). In Europe, *Coccinella septempunctata* L. serves as its most prevalent host (Berkvens et al., 2010). The taxonomic classification of *D. coccinellae* is as follows:

- Phylum Arthropoda (Arthropods)
  - Subphylum Hexapoda (Hexapods)
    - Class Insecta (Insects)
      - Order Hymenoptera (Sawflies, wasps, ants, bees)
        - Family Braconidae (Braconid wasps)
          - Genus (*Dinocampus*)
            - Species (*Dinocampus coccinellae*)

Coccinellidae, commonly known as ladybugs, play a crucial role in maintaining ecological balance and providing biological control against various pest species within agricultural ecosystems (Ranjbar et al., 2024). Numerous species of Coccinellidae have been in pistachio orchards globally, including in Türkiye (Souliotis et al., 2002; Bolu, 2004; Özgen and Karsavuran, 2005; Salehi et al., 2013; Dilmen and Özgökçe, 2020). Research has identified approximately 22 species of Coccinellidae in Turkey, 17 species in Iran, and 8 species in Greece. Notably, *Coccinella septempunctata*, *Hippodamia variegata*, *Adalia bipunctata*, and *Oenopia conglobata* are particularly prevalent. Therefore, the identification and monitoring of *D. coccinellae* can be considered a critical element in the biological control of pests. However, distinguishing between closely related species with similar morphological features requires the expertise of taxonomic specialists for precise identification. Accurate taxonomic classification and identification based on external morphology and genital organs demand significant expertise, time, and resources (Wang et al., 2024). Consequently, in recent years, DNA barcoding has increasingly been employed for the accurate and rapid identification of species. DNA barcoding is a molecular technique that allows for species-level identification using genetic material (Antil et al., 2023). This method is typically performed by sequencing the mitochondrial COI (cytochrome c oxidase I) gene region, which is sufficiently variable to distinguish between species in many groups (Hebert et al., 2003). DNA barcoding offers a significant advantage in differentiating morphologically similar species (Smith et al., 2006) and has thus been widely used in taxonomic studies and biodiversity monitoring (Poolprasert et al., 2019; Huang et al., 2020; Abdalla et al., 2022; Baena-Bejarano et al., 2023).

The aim of this study is to identify the parasitoid *D. coccinellae* associated with Coccinellidae species in pistachio orchards in Siirt DNA barcoding. This identification will facilitate the assessment of the impact of this parasitoid on beneficial ladybugs and contribute to the evaluation of biological control strategies, ultimately leading to the development of more effective pest management approaches.

MATERIALS AND METHODS

Sample Collection and Field Study

The study was conducted in 2023, during which coccinellid specimens were collected from pistachio orchards across various districts of Siirt, including Merkez, Tillo, Baykan, Kurtalan, and Şirvan (Figure 1).

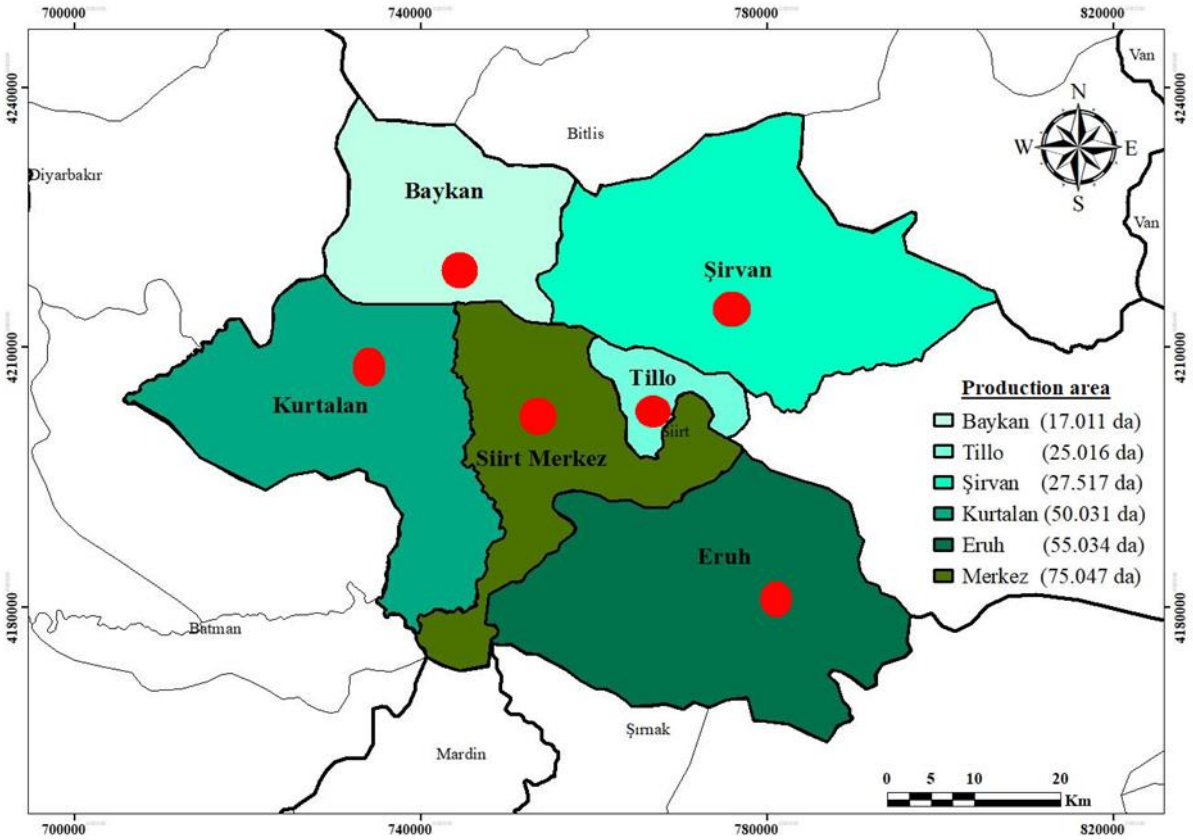

Figure 1. Sampling locations (H. Dilmen, Original, Esri ArcGIS 10.2).

Sampling occurred every 15 days from April to October. During this process, a minimum of 10 ladybug specimens from the Coccinellid species were collected from each orchard (Table 1). Specimens were gathered using Japanese umbrellas and sterile collection vials. Each specimen was separated and placed in individual labeled tubes. The collected specimens were separated and labeled with only one individual per tube. To prevent damage to the specimens, the tubes were transported to the laboratory in portable coolers containing refrigerants.

Table 1. Coccinellidae species collected in Siirt pistachio orchards

Orchard location	Coccinellidae Species
Merkez, Tillo, Baykan, Kurtalan, Şirvan, Eruh	*Hippodamia variegata* (Goeze), *Oenopia conglobata* (Linnaeus, 1758), *Coccinella septempunctata* (Linnaeus, 1758), *Adalia decempunctata* (Linnaeus, 1758).

Examination of Ladybug Specimens for Parasitoid

In the laboratory, ladybug specimens were meticulously examined using an Olympus SC61 stereo microscope, which was equipped with an Olympus SC50 camera and CellSens Entry software. The Coccinellidae individuals collected during the study exhibited noticeable changes in external appearance, such as paleness or darkening, and displayed signs of weakness and lethargy. Furthermore, some specimens presented swelling or deformities in specific body regions. These individuals were set aside for molecular analysis due to suspicions of parasitism. Suspected parasitized specimens were preserved by freezing at -20°C for subsequent DNA isolation. All individuals were utilized in the analysis.

DNA Isolation and PCR Amplification

DNA isolation from coccinellid specimens was conducted utilizing the Invitrogen PureLink Genomic DNA Mini Kit, which is specifically designed to isolate high-purity DNA from a range of biological samples. The isolation procedure was carried out in accordance with the manufacturer's guidelines. The isolated DNA was subsequently stored in 1.5 mL Eppendorf tubes at -20°C. DNA purity and quantity were measured using a NanoDrop spectrophotometer, confirming the acquisition of DNA at appropriate concentrations.

The isolated DNA was amplified for the mitochondrial COI (cytochrome c oxidase I) gene region using Polymerase Chain Reaction (PCR). The primers targeted a region of approximately 658 base pairs: LCO1490-F (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198-R (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). The PCR reaction mixture was prepared in a total volume of 50 µL, which included 5 µL of 10X PCR buffer, 2.5 mM MgCl2, 200 µM dNTP mix, 0.2 µM of each primer, 1.25 U of Taq DNA polymerase, and 100 ng of DNA template. The PCR program on the MiniAmp Plus Cycler was optimized as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 5 minutes.

Electrophoresis and DNA Purification

PCR products were analyzed using 1.5% agarose gel electrophoresis, which was prepared by dissolving agarose in 1x TAE buffer. The electrophoresis was performed at 90 volts and 100 mA for approximately 45 minutes. A 100 bp DNA ladder (used as a molecular weight marker) was run alongside the samples to determine the size of the PCR products. After electrophoresis, the gel was stained with ethidium bromide for 20 minutes and visualized under UV light using a transilluminator to confirm the presence and size of the amplified DNA fragments. Once the correct size of the amplification products was confirmed, gel extraction and purification processes were carried out. The purified DNA was prepared for sequencing.

DNA Sequencing and Bioinformatics Analysis

PCR products were sent to MedSantek (Istanbul) for sequencing, which was performed using the Sanger sequencing method. The resulting sequences were compared with reference sequences in the NCBI GenBank database using the BLAST tool, enabling the accurate identification of Dinocampus coccinellae. Sequence alignment was carried out using Clustal W within the MEGA X software, an approach known for its reliability in aligning homologous sequences (Kumar et al., 2018). For phylogenetic analysis, the Maximum Likelihood (ML) method, as described by Felsenstein (1981), was employed. This method is well-suited for estimating evolutionary relationships due to its robustness in modeling nucleotide substitutions and its ability to handle varying rates of evolution across sites, making it a reliable choice for constructing accurate phylogenetic trees. This method was chosen as it provides high accuracy in generating phylogenetic trees, especially when dealing with large datasets and complex models of evolution, making it a widely accepted approach in molecular phylogenetics.

RESULTS AND DISCUSSION

This study identified the parasitoid *Dinocampus coccinellae* from Coccinellidae species in pistachio orchards in Siirt using DNA barcoding techniques.

PCR amplification targeting the mitochondrial COI gene region was successfully achieved, yielding the expected 658 base pair products in all samples. The PCR results for *D. coccinellae* are presented in the agarose gel electrophoresis image (Figure 2). The presence of a clear and single band in all samples indicates that the DNA fragments were of uniform size and that the target gene region was successfully amplified. The results of the study show that the amplification was successful and that *D. coccinellae* DNA was accurately detected in the samples (Figure 2). Therefore, this gel electrophoresis result supports the accurate identification of *D. coccinellae* and the reliability of the DNA barcoding method. As a result of the study, among the 11 different individuals collected from four distinct Coccinellid species, *D. coccinellae* DNA was accurately identified *in Hippodamia variegata* specimens collected from the central district of Siirt. This finding confirms that *H. variegata* in this region can host the parasitoid, highlighting the need for further research on this relationship.

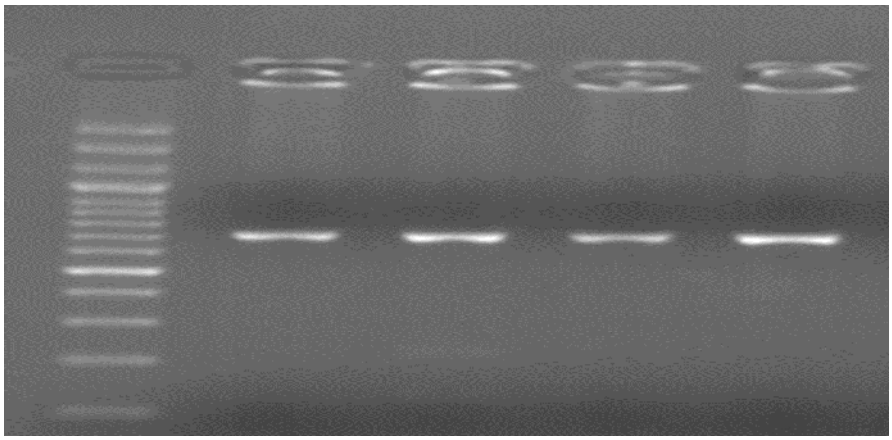

Figure 2. Polymerase chain reaction results of the mitochondrial COI gene region of *Dinocampus coccinellae*. *M* Marker (DNA leader; 100bp).

Additionally, the sequencing results were compared with the NCBI database. In the "Matched Sequences" column, the reference sequence for the analyzed sample, *D. coccinellae*, is shown. The "Score" column indicates a high similarity score of 819, suggesting a strong match between the sequences. The "E-value" is given as 0.0, indicating that the comparison is statistically significant and not due to random chance. The "Percentage Identity" column shows a value of 97.13%, meaning that the analyzed sequence is 97.13% identical to the reference sequence (Table 2). Furthermore, the comparison of the obtained sequences with the NCBI database confirms the accurate identification of *D. coccinellae* and shows a high level of agreement with other records in the NCBI database. These results confirm that the *D. coccinellae* specimen was correctly identified using DNA barcoding.

Table 2. BLAST analysis results from NCBI database.

Matched Sequences	Score	E-value	Percentage Identity	Accession length	Accession
Dinocampus coccinellae	819	0.0	%97.13	658	OR039339.1
Dinocampus coccinellae	819	0.0	%97.13	658	OR039335.1
Dinocampus coccinellae	819	0.0	%97.13	658	OR039333.1
Dinocampus coccinellae	819	0.0	%97.13	658	OR039332.1

The phylogenetic tree generated from the sequencing data in this study illustrates the genetic relationships among various Hymenoptera species, with a specific focus on *D. coccinellae*, a parasitoid of Coccinellidae species. Constructed using the Maximum Likelihood (ML) method and DNA barcoding, the tree provides insights into the genetic affinities and evolutionary relationships among these species (Figure 3).

This phylogenetic tree was created to show the evolutionary relationships of *D. coccinella* with other braconid and closely related species. *Hippodamia variegata*, used as an outgroup in the phylogenetic analysis, is evolutionarily distinct from the other species in the analysis and has been used as a reference point. Located at the bottom of the tree, *H. variegata* was selected as the outgroup in this study. The outgroup is distinctly separated from the other species. The distance value of 0.12 in the tree indicates how different the outgroup is from the ingroup. This shows that *H. variegata* is a species that diverged much earlier than all the other species in this tree. *Dinocampus coccinella* is at the top of the tree, and when examining its evolutionary relationships with other species, its closest relatives are *Meteorus obfuscatus* and *Perilitus* sp. The close evolutionary relationship of these three species is represented by short branches that indicate their genetic distances. For example, there is only a 0.08 short branch length between *D. coccinella* and *M. obfuscatus*, which indicates that these two species are genetically quite close to each other. It can be said that they diverged from a common ancestor a short time ago.

Additionally, the presence of some short branches (e.g., 0.02, 0.04) in the phylogenetic tree indicates that these species are genetically closer to each other. On the other hand, longer branches (e.g., 0.16) represent greater genetic differences and more ancient evolutionary separations. Notably, *D. coccinella* has a longer branch length compared to other braconid species, indicating a relatively early separation in the evolutionary process within this group.

The red bootstrap support values (e.g., 96%, 69%, 63%) presented on the tree represent the statistical reliability of the branches. High bootstrap values (90% and above) indicate that the corresponding branching is strong and reliable. For example, the 96% support value between *D. coccinella* and *M. obfuscatus* provides high

reliability for their relationship as relatives. However, some branches have low support values, such as the 53% support value between Braconidae sp. and *Cotesia glomerata*. This indicates that the reliability of this branching is weaker and that more data or analysis is needed regarding the relationships between these species.

In conclusion, this phylogenetic analysis has revealed the genetic relationships and evolutionary distinctions of *D. coccinella* with other species. The branch lengths in the tree indicate the evolutionary distances between species, while the bootstrap support values reflect the confidence in these relationships. *Dinocampus coccinella* has a close evolutionary relationship with its nearest relatives, *M. obfuscatus* and *Perilitus* sp., but this trio represents an earlier separation compared to other species. The low support values on the branches indicate uncertainties in some familial relationships and suggest that further investigation of these relationships is needed.

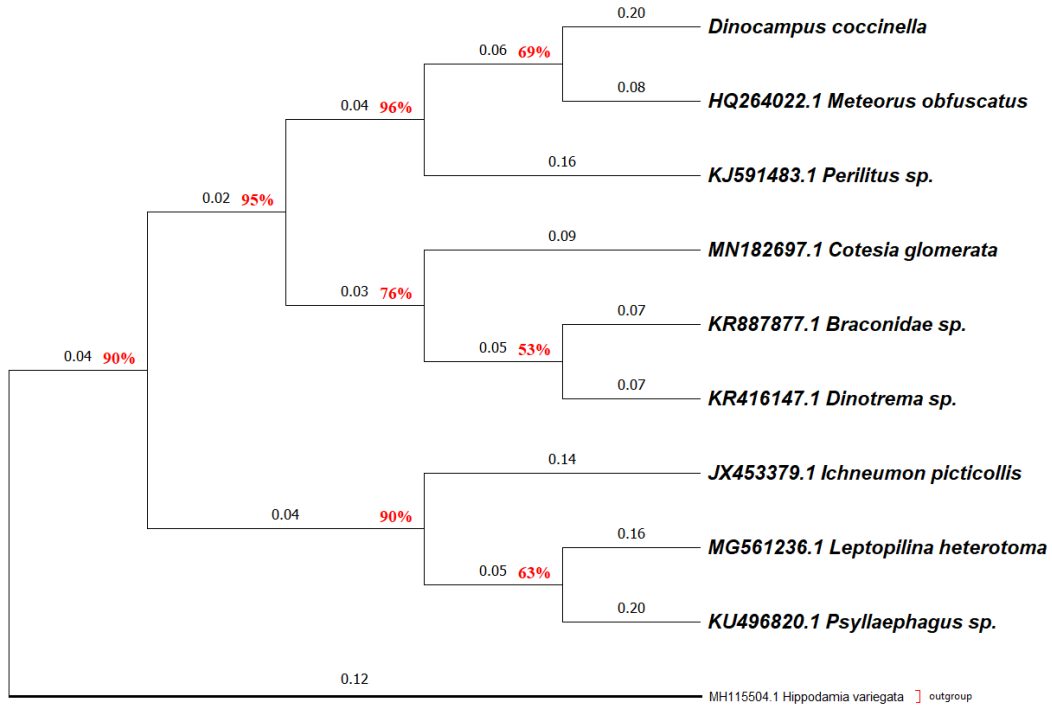

Figure 3. Phylogenetic relationships among *Dinocampus coccinellae* and its closely related species (Maximum Likelihood analysis).

The findings of this study indicate that *D. coccinellae* is present in Siirt pistachio orchards and can be effectively identified using DNA barcoding. The presence of *D. coccinellae* is a significant finding for pest management in pistachio orchards, highlighting the importance of understanding this species' role in biological control strategies. The DNA barcoding method has provided high accuracy and reliability in identifying *D. coccinellae*. Specifically, the use of the COI gene region has facilitated the differentiation of *D. coccinellae* from other similar parasitoid species. This confirms that DNA barcoding is an effective tool in the taxonomic studies of parasitoids and other insect species.

In recent years, mitochondrial gene regions, particularly the COI gene, have been widely used for species identification and diagnostics. Mitochondrial DNA, with its high evolutionary rate and nucleotide diversity, is recognized as an effective biomarker for distinguishing closely related species and populations. This method offers significant reliability for assessing genetic diversity and accurately identifying species (Hebert et al., 2003; Boehme et al., 2010).

The use of the mitochondrial COI gene region for diagnostic purposes has been successfully validated in many studies. For instance, Hebert et al. (2003) demonstrated the effectiveness of the COI gene region in species identification. It has been proven effective in distinguishing known species and discovering previously unknown ones (Cheng et al., 2023). In recent years, many parasitoid species have been identified using the COI gene region (Ceccarelli et al., 2012; Franjević et al., 2015; Chen et al., 2021; Al-Jalely et al., 2022; Tiring et al., 2023). Ricupero et al. (2023) sequenced and characterized the mtDNA of D. coccinellae using COI and 16S rRNA gene markers in ladybug species collected from various geographic regions, including China, the USA, Canada, Chile, and Italy.

In this study, the identification of D. coccinellae using DNA barcoding has once again validated the effectiveness of mitochondrial gene regions in species diagnostics, and the results are consistent with other studies. The

97.13% similarity rate obtained indicates that the DNA barcoding method is reliable for the accurate identification of D. coccinellae. This result shows that mitochondrial gene regions provide valuable information for biological control strategies and pest management, offering an important tool for ecosystem management. The findings emphasize the reliability and effectiveness of mitochondrial gene regions in species identification and suggest that future studies using this method could be highly beneficial for identifying insect species. Such analyses represent a crucial step in developing biological control strategies for pests and other insects.

CONCLUSION

This study has successfully identified *Dinocampus coccinellae* using DNA barcoding and has confirmed that this species acts as a parasitoid of coccinellid beetles in Siirt pistachio orchards. The 97.13% similarity rate obtained validates the accurate identification of *D. coccinellae* and confirms its parasitism of coccinellids. Furthermore, the phylogenetic analysis conducted in this study highlights the close genetic relationship of *D. coccinellae* with *Meteorus obfuscatus* and *Perilitus* sp., while also revealing the genetic distances between *D. coccinellae* and other Hymenoptera species, thus demonstrating the effectiveness of DNA barcoding in elucidating genetic similarities and their phylogenetic relationships.

Identifying *D. coccinellae* provides significant advantages for plant protection and integrated pest management (IPM) programs. Coccinellid species, which are beneficial insects providing biological control against pests, may have their effectiveness impacted by parasitism by *D. coccinellae*. Thus, determining *D. coccinellae* as a parasitoid is a critical step in understanding the populations of coccinellids and their impact on pest management. Additionally, identifying *D. coccinellae* in this manner allows for monitoring of the parasitoid's population dynamics and effectiveness, helping assess whether coccinellids are affected by its parasitism and the implications for pest control strategies. This identification also enables more targeted and effective implementation of biological control strategies. Determining the extent to which coccinellids are affected by *D. coccinellae* can assist in developing strategies to enhance the effectiveness of these species and reduce pest impacts. In conclusion, this study highlights the importance of DNA barcoding technology in biological control strategies and its contribution to accurately assessing the effects of parasitoids. Accurate identification using this method will provide valuable insights for future research on *D. coccinellae*.

Declaration of Interests

The Author declare that there is no conflict of interest.

Author Contributions

1st Halil DİLMEN: Conceptualization; supervision; data curation; molecular analysis; investigation; methodology; software; writing— original draft; writing—review and editing.

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REFERENCES

Abdalla, H. A., Gamal Fiteha, Y., Abdel-Salam Rashed, M., Mohamed Ali, R., Mohamed Abdel-Salam Hammad, A., Saber Mohamed Bream, A., & Magdy, M. (2022). Molecular Identification of Ladybird Beetles (Coccinella: Coccinellidae) Using DNA Barcodes. *Journal of Scientific Research in Science*, *39*(2), 179-193. https://doi.org/10.21608/jsrs.2022.275796.

Al-Jalely, B. H., Wang, P., Liao, Y., & Xu, W. (2022). Identification and characterization of olfactory genes in the parasitoid wasp *Diadegma semiclausum* (Hellén)(Hymenoptera: Ichneumonidae). *Bulletin of Entomological Research*, *112*(2), 187-196. https://doi.org/10.1017/S0007485321000675.

Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., ... & Toteja, R. (2023). DNA barcoding, an effective tool for species identification: a review. *Molecular biology reports*, *50*(1), 761-775. https://doi.org/10.1007/s11033-022-08015-7

Baena-Bejarano, N., Reina, C., Martínez-Revelo, D. E., Medina, C. A., Tovar, E., Uribe-Soto, S., ... & Gonzalez, M. A. (2023). Taxonomic identification accuracy from BOLD and GenBank databases using over a thousand insect DNA barcodes from Colombia. *Plos one*, *18*(4), e0277379. https://doi.org/10.1371/journal.pone.0277379

Berkvens, N., Moens, J., Berkvens, D., Samih, M. A., Tirry, L., & De Clercq, P. (2010). *Dinocampus coccinellae* as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe. *Biological control*, *53*(1), 92-99. https://doi.org/10.1016/j.biocontrol.2009.11.001.

Boehme, P., Amendt, J., Disney, R. H. L., & Zehner, R. (2010). Molecular identification of carrion-breeding scuttle flies (Diptera: Phoridae) using COI barcodes. *International Journal of Legal Medicine*, *124*, 577-581.

Bolu, H. (2004). Güneydoğu Anadolu Bölgesi antepfıstığı alanlarında bulunan avcı Coccinellidae türleri, yayılış alanları ve zararlı *Agonoscena pistaciae*'nın populasyon değişimi üzerine etkileri. *Bitki Koruma Bülteni*, *44*(1-4), 69-77.

Ceccarelli, F. S., Sharkey, M. J., & Zaldívar-Riverón, A. (2012). Species identification in the taxonomically neglected, highly diverse, neotropical parasitoid wasp genus *Notiospathius* (Braconidae: Doryctinae) based on an integrative molecular and morphological approach. *Molecular Phylogenetics and Evolution*, *62*(1), 485-495. https://doi.org/10.1016/j.ympev.2011.10.018

Chen, H. Y., Li, H. L., Pang, H., Zhu, C. D., & Zhang, Y. Z. (2021). Investigating the Parasitoid community associated with the invasive mealybug *Phenacoccus solenopsis* in Southern China. *Insects*, *12*(4), 290. https://doi.org/10.3390/insects12040290

Cheng, Z., Li, Q., Deng, J., Liu, Q., & Huang, X. (2023). The devil is in the details: Problems in DNA barcoding practices indicated by systematic evaluation of insect barcodes. *Frontiers in Ecology and Evolution*, *11*, 1149839. https://doi.org/10.3389/fevo.2023.1149839

Dhankhar, N., & Kumar, J. (2023). Impact of increasing pesticides and fertilizers on human health: A review. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2023.03.766

Dilmen, H., & Özgökçe, M. S. (2020). Siirt ili Antep fıstığı (Siirt çeşidi) bahçelerinde *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae)'nın popülasyon gelişmesi. *Plant Protection Bulletin*, *60*(3), 47-56.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of molecular evolution*, *17*, 368-376.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294–299. https://doi.org/10.16955/bitkorb.675288

Franjević, M., Glavaš, M., Hrašovec, B., Koletić, N., & Franjević, D. (2015). Genetic identification of new alien pest species Illinoia liriodendri and its parasitoid *Areopraon silvestre* in Croatia. *Periodicum biologorum*, *117*(4), 513-517. https://doi.org/10.18054/pb.2015.117.4.3713

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). "Biological identification through DNA barcodes." Proceedings of the Royal Society B: *Biological Sciences*, *270*(1512), 313-321. https://doi.org/10.1098/rspb.2002.2218

Huang, W., Xie, X., Huo, L., Liang, X., Wang, X., & Chen, X. (2020). An integrative DNA barcoding framework of ladybird beetles (Coleoptera: Coccinellidae). *Scientific Reports*, *10*(1), 10063. https://doi.org/10.1038/s41598-020-66874-1

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, *35*(6), 1547-1549.

Koyama, S., & Majerus, M. E. (2008). Interactions between the parasitoid wasp Dinocampus coccinellae and two species of coccinellid from Japan and Britain. From Biological Control to Invasion: The Ladybird *Harmonia Axyridis* as a Model Species, 253-264. https://doi.org/10.1007/978-1-4020-6939-0_17

Nazir, T., Khan, S., & Qiu, D. (2019). Biological control of insect pest. Pests Control and Acarology, IntechOpen. 21. https://doi.org/10.5772/intechopen.81431

Poolprasert, P., Senarat, S., Nak-eiam, S., & Likhitrakarn, N. (2019). Molecular taxonomic identification of predatory ladybird beetles inferred from COI Sequences (Coleoptera: Coccinellidae). *Malaysian Journal of Applied Sciences, 4*(2), 10-18.

Ranjbar, F., Jalali, M. A., Ahmadi, Z., Pons, X., Levi-Mourao, A., & Ugine, T. (2024). Comparison of the predatory impacts of indigenous and adventive ladybeetle species (Coleoptera: Coccinellidae) using a functional response approach. *Journal of Plant Diseases and Protection*, *131*(5), 1515-1523.

Ricupero, M., Zepeda-Paulo, F., Cabrera, N., Biondi, A., Dai, C., Zappalà, L., ... & Lavandero, B. (2023). The geographic scope of host use by the ladybeetle parasitoid *Dinocampus coccinellae*. *Biological Invasions*, *25*(9), 3009-3024. https://doi.org/10.1007/s10530-023-03090-8

Riddick, E. W. (2017). Identification of conditions for successful aphid control by ladybirds in greenhouses. *Insects*, *8*(2), 38. https://doi.org/10.3390/insects8020038

Salehi, T., Mehrnejad, M. R., & Pashaei Rad, S. (2013). Diversity pattern of adult ladybird (Coleoptera: Coccinellidae) communities on pistachio trees in southern parts of Iran in different months. *Zoology and Ecology*, *23*(4), 286-292. https://doi.org/10.1080/21658005.2013.838071

Souliotis C., Markoyiannaki-Printziou D., Lefkaditis F., (2002). The problems and prospects of ıntegrated control of *Agonoscena pistaciae* Burck. and Laut. (Hom.: Sternorrhyncha) in Greece. *Journal of Applied Entomology*. *126*(7-8), 384-388.

Smith, M. A., Woodley, N. E., Janzen, D. H., Hallwachs, W., & Hebert, P. D. (2006). DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences*, *103*(10), 3657-3662. https://doi.org/10.1073/pnas.051131810

Tiring, G., Tusun, A., Kalkan, Ç., & Satar, S. (2023). The first native pupae parasitoid of *Ceratitis capitata* in Türkiye: The molecular identification of Pachycrepoideus vindemmiae. *International Journal of Tropical Insect Science*, *43*(4), 1381-1385. https://doi.org/10.1007/s42690-023-01033-3

Özgen, İ., & Karsavuran, Y. (2005). Antepfıstığı ağaçlarında zararlı *Lepidosaphes pistaciae* (Archangelskaya) (Homoptera: Diaspididae)'nin doğal düşmanlarının saptanması üzerinde araştırmalar. *Turkish Journal of Entomology*, *29*(4), 309-316.

Wang, L., Wu, H., He, W., Lai, G., Li, J., Liu, S., & Zhou, Q. (2024). Diversity of Parasitoid Wasps and Comparison of Sampling Strategies in Rice Fields Using Metabarcoding. *Insects*, *15*(4), 228. https://doi.org/10.3390/insects15040228