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Molecular phylogenetic relationship among different species of the genus *Dactylopius* (Hemiptera, Dactylopiidae)

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Abstract

This research offers an extensive molecular characterization of *Dactylopius opuntiae* populations from Palestine, utilizing three genetic markers (*18S* rRNA, *COI*, and *12S* rRNA) in conjunction with RAPD-PCR analysis. The findings demonstrated pronounced genetic similarities across Palestinian isolates, corroborating the concept of a recent invasion or a shared ancestral origin. According to *18S* rRNA sequences, Palestinian isolates exhibited 100% similarity among themselves and with several GenBank isolates, while their identities with *D. confusus* ranged from 99.8% to 100%, therefore affirming a highly proximate evolutionary link. Mitochondrial markers demonstrated marked enhanced discriminating power. The *COI* sequence similarity among Palestinian *D. opuntiae* isolates varied from 99.6% to 100%, but diminished to 87.9% to 89.4% with *D. confusus*, 78.5% to 79.6% with *D. coccus*, and 79.1% to 80.7% with *D. tomentosus*. Likewise, *12S* rRNA study revealed identities ranging from 71.7% to 74.2% between *D. opuntiae* and *D. tomentosus*, indicating considerable evolutionary difference. Therefore, *12S* rRNA marker offered stronger selective strength for species delimitation. RAPD-PCR produced 14 bands, with 78.6% exhibiting polymorphism, and categorized Palestinian isolates into four principal genetic groupings. The data indicate that mitochondrial markers are particularly efficient for species delimitation and phylogenetic reconstruction within *Dactylopius* genus. Therefore, the findings obtained in this study provide valuable molecular data that can enhance biological control initiatives, refine pest identification accuracy, and aid in the monitoring of invasion routes and population dispersion of *D. opuntiae* in agricultural settings.

Key Words

12S gene, *18S* gene, *COI* gene, *Dactylopius opuntiae*, OPA 03 primer, Palestine, RAPD-PCR typing

Introduction

There are only eleven species in the scale-covered insect genus *Dactylopius* Costa (1829). The bodies of mature females of all *Dactylopius* species appear similar: dark red, oval in shape, and covered in white waxy filaments that act as a protective barrier against environmental conditions like heat, cold, and predators (Mazzeo et al. 2019). Aggregations of nymphs and adult females are typically found on cladodes, usually near the base of the spines.

Although the amount varies by species, adult females naturally produce a red glucosidal hydroxyanthrapurin, or carminic acid. For example, in *Dactylopius coccus* (Costa, 1829) it makes up 18–26% of body weight, while, in

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other species, it accounts for 6–8%. They feed on sap and eventually kill their hosts. In several countries in Europe, Africa, Asia, and Australia where other cacti can become invasive under favorable conditions, the false carmine cochineal scale, *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera, Dactylopiidae), was introduced to biologically manage these infestations (Mazzeo et al. 2019).

The cactus is grown extensively nowadays, mostly for its fruits, throughout the majority of Mediterranean Basin, including Palestine. In regions like Palestine, where cacti play a significant role in agricultural output and rural development as additional source of income for farmers, the cochineal *Dactylopius* has appeared as a major pest (Adwan et al. 2024). The genus *Dactylopius* has eleven species, including *D. opuntiae*, which infect *Opuntia ficus-indica* (L.) Mill. (Caryophyllales: Cactaceae) and other *Opuntia* species worldwide causing damage (Adwan et al. 2024; Rodríguez-Leyva et al. 2024).

According to Paterson et al. (2011), *D. opuntiae* is considered the most harmful species in the genus *Dactylopius*. After that it has spread throughout the Mediterranean region in countries like Israel (Spodek et al. 2013), Spain (Ben-Dov and Sanchez-Garcia 2015), Morocco (Bouharroud et al. 2016), Lebanon (Moussa et al. 2017), Cyprus (Ülgentürk and Hocaali 2019), Algeria (El Bouhissi et al. 2022), and Syria (Assad et al. 2024). It becomes the most aggressive insect against *O. ficus-indica*. In 2012, the first documented case of *D. opuntiae* insect on cacti was discovered in Lebanon (Moussa et al. 2017). In Israel, the first was in 2013 (Spodek et al. 2013). In Jordan the first case of *D. opuntiae* insect on cacti was observed in 2018 (Bader and Abu-Alloush 2019). In 2020, the first infestation of the *D. opuntiae* insect was recorded in Syria (Assad et al. 2024). *D. opuntiae* was first detected in Palestine in 2013, according to the Palestinian Ministry of Agriculture (Adwan et al. 2024).

The quantity, location, and kinds of pores and setae are the primary ways that the taxonomic classifications of the Dactylopiidae influence the physical traits of adult females. However, the accuracy of morphological investigations is limited by the intricacy of relationships between phytophagous insects and their hosts, adult stage requirements, and environmental conditions (Silva et al. 2013). Genetic variation assessments are increasingly dependent on DNA information obtained through molecular techniques such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD), simple sequence repeats (SSRs), and microsatellites (Kumari and Thakur 2014). In turn, molecular characterization provides access to a large genomic region and enables assessments of polymorphism both within and among *Dactylopius* species based on direct DNA molecule analysis. One of the most common methods used for genotyping is the RAPD technique. As the genetic diversity across species decreases, their identified pieces are more likely to be monomorphic. On the other hand, when substantial amounts of polymorphisms are found, the fragments' variability rises. Using this method also has an additional advantage of often aligning with morphological data to support the molecular descriptors (Silva et al. 2013).

By combining morphological and molecular approaches, this study not only enhances taxonomic resolution and pest management but also integrates evolutionary insights into sustainability science, contributing to long-term agricultural and ecological resilience. In Palestine, knowledge of *D. opuntiae* biology, genetic variation, and distribution remains limited (Adwan et al. 2024). This study was therefore conducted to analyze mitochondrial (*COI* and *12S* rRNA) and nuclear (*18S* rRNA) genes from *Dactylopius* spp. to assess the phylogenetic relationships among different species retrieved from GenBank. Moreover, it aimed to identify *D. opuntiae* using molecular techniques and to estimate the genetic variation of *D. opuntiae* populations using RAPD markers in isolates collected from different areas in Palestine.

Materials and methods

Sample collection

Twenty-three isolates of *D. opuntiae* were obtained in 2023 from natural populations of *O. ficus-indica* found in several West Bank Palestinian districts. Nine, 7, 2, 2, 3 isolates were collected from Governorate of Nablus (2,860 ha, 32.221°N, 35.254°E), Governorate of Tulkarem (2,880 ha, 32.311°N, 35.028°E), Governorate of Jenin (3,730 ha, 32.462°N, 35.301°E), Governorates of Bethlehem (2,280 ha, 31.705°N, 35.204°E) and Governorates of Hebron (7,410 ha, 31.529°N, 35.094°E), respectively. According to the established taxonomic keys for *Dactylopius* species, these samples were morphologically diagnosed based on the physical features of the insect, such as its size, shape, and presence of waxy filaments (da Silva et al. 2022). Subsequently, the nymphs were transported for additional examination to the laboratories of the Faculty of Veterinary Medicine and Agricultural Engineering, An-Najah National University, Tulkarem, Palestine.

Genomic DNA isolation

Twenty-three fresh adult female *D. opuntiae* samples were obtained and treated with absolute ethanol to remove the white, waxy material before being washed with distilled water. Then, the processed isolates were kept at -20 °C until genomic DNA was extracted.

The DNeasy Blood and Tissue Kit (Qiagen Hilden, Germany) was used to extract the genomic DNA of a single *D. opuntiae* organism, following the manufacturer's instructions. Each sample was homogenized using a micropestle before extraction. The recovered insect genome, which contained the target DNA fragments for sequencing was amplified using specific gene primers. The amplified products were submitted to a commercial sequencing company (Smart Gene Labs, Nablus, Palestine) for sequencing with the Spectrum Compact CE System (Promega).

PCR amplification

DNA amplification was carried out using the specific primers mentioned (Table 1). Three genetic markers of this insect were targeted and amplified, one nuclear gene (*18S*) and two mitochondrial genes (*12S* and *COI*).

DNA sequencing and bioinformatics analyses

Fifteen insect samples were bidirectional sequenced with the primers listed in Table 1. Following the manufacturer's instructions, the resultant fragments of PCR products were cleaned up with the Wizard SV Gel and PCR Clean-Up System kit (Promega), and the purified PCR products were then sequenced using the dideoxy chain termination technique. The sequence reads were trimmed to eliminate poor-quality nucleotides at the two termini of each continuous strand. These sequences were uploaded into bioinformatics sequence databases to obtain an accession number.

Nucleotide sequences were compared to other closely matched DNA sequences of similar genes deposited in the GenBank database using a Basic Local Alignment Search Tool (BLAST) system for bioinformatics analysis. The *COI*, *12S*, and *18S* nucleotide sequences obtained from *D. opuntiae* insect populations collected in Palestine were multiple aligned with other sequences of the same genes that were downloaded from the National Center for Biotechnology Information database using the Clustal W tool in the software program MEGA Version 6 (Tamura et al. 2013). The maximum likelihood method (MLM), which is based on the Tamura–Nei model (Tamura and Nei 1993), was used to assess the evolutionary relationships between the continuous partial DNA sequences of the *12S*, *COI*, and *18S* genes determined in Palestine and those sequences previously published and already retrieved from the National Center for Biotechnology Information database. The topology with the highest log likelihood value was chosen to automatically generate the first tree for the heuristic search after 1000 bootstrap resampling. The neighbor-joining and BioNJ algorithms were subsequently employed on a matrix of pairwise distances calculated using the p-distance method; the stability of the groups in the MLM was evaluated. The phylogenetic tree was depicted to scale, with branch lengths measured in the number of substitutions per site.

Randomly amplified polymorphic DNA (RAPD)-PCR typing

The RAPD-PCR was conducted using the OPA 03 5′-AGT CAG CCA C-3′; as previously described (Silva et al. 2013), with minor modifications. The total volume of reaction was 25 µl, which had 12.5 µl of PCR premix (GoTaq® Green Master Mix, Promega), 1 µl of 20 µM of the primer, and 3 µl of the *D. opuntiae* genomic DNA template (50–70 ng). The reaction mix was optimized by adjusting the concentrations of dNTPs (0.4 mM), $MgCl_2$ (3 mM), and Taq DNA polymerase to 1.5 U per reaction. Amplification was carried out in thermal cycler (Mastercycler personal, Eppendorf, Germany) under the subsequent PCR conditions: 94 °C for 3 minutes (initial denaturation), followed by 34 cycles of 30 seconds at 94 °C (denaturation), 45 seconds at 32 °C (annealing), and 2 minutes at 72 °C (elongation), ending with a final extension of 5 minutes at 72 °C. The PCR fragments were separated by electrophoresis on a 2.0% agarose gel using 1.0X Tris-acetate-EDTA (TAE) buffer. A 100 bp DNA ladder (GeneDireX) was used as a molecular size marker. The gel was stained with a solution of ethidium bromide containing 0.5 µg/ml water. The image was obtained with the aid of a photodocumentation system. The binary scoring method was then used to score the DNA banding pattern in the gel image, recording 1 and 0 for the presence and absence of PCR bands, respectively. The unweighted pair group method for arithmetic averages (UPGMA) was used to analyze a binary matrix, the clustering process carried out using Ward linkage method and interval measurements based on Squared Euclidean distance to construct the dendrogram, using SPSS statistics software version 21 (IBM).

Results

PCR amplification

The *COI*, *12S* and *18S* genes of *D. opuntiae* were successfully amplified from 23 extracted DNA samples. Amplification of the *COI* gene consistently produced fragments of 710-bp, the *12S* gene yielded fragments of 460-bp, and the *18S* gene produced fragments of 620-bp. The results of PCR amplification are presented (Fig. 1).

Table 1. The sequence of primers utilized in the current research for the PCR amplification of both nuclear (*18S*) and mitochondrial (*12S* and *COI*) marker genes in *D. opuntiae* insect.

Target gene	Primer sequence 5′→3′	Annealing temperature	Amplicon size	Reference
18S	18S-F CTGGTTGATCCTGCCAGTAG	58 °C	620-bp	(Ramírez-Puebla et al. 2010)
	18S-R CCGCGGCTGCTGGCACCAGA			
***12*S**	12S-F AAGAGTGACGGGCRATTTGTACATA	52 °C	460-bp	(Ramírez-Puebla et al. 2010)
	12S-R GTGCCAGCAGTWGCGGTT			
COI	PcoF1 CCTTCAACTAATCATAAAAATATYAG	45 °C	710-bp	(Wang et al. 2019)
	LepR1 TAAACTTCTGGATGTCCAAAAAATCA			

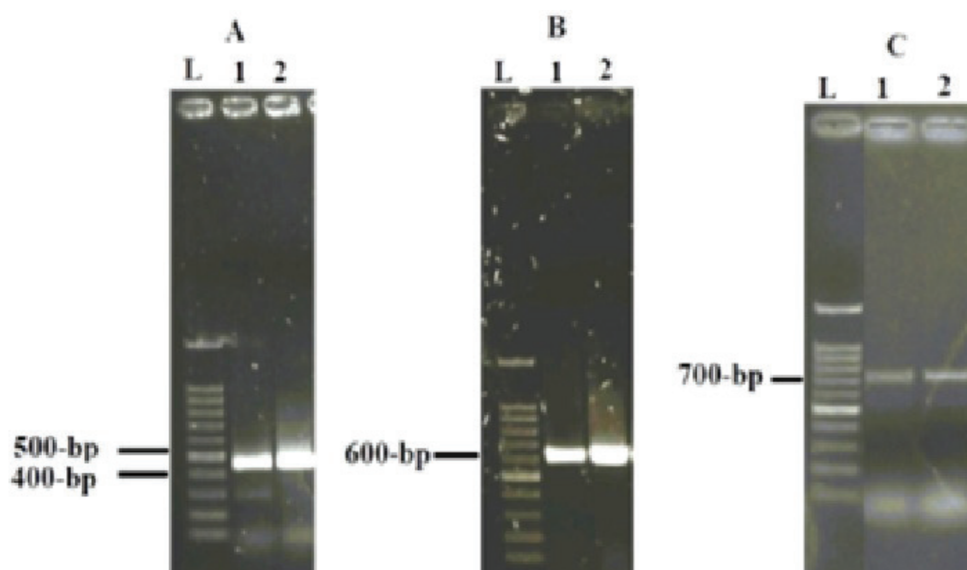

Figure 1. PCR products for *12S*, *COI* and *18S* gene sequences for *D. opuntiae* isolated in Palestine. Lane L for Ladder (100-bp); **A.** Lanes 1 and 2 for *12S* gene sequences (460-bp); **B.** Lanes 1 and 2 for *18S* gene sequences (620-bp) and **C.** Lanes 1 and 2 for *COI* gene sequences (710-bp).

Fifteen isolates were Sanger-sequenced and analyzed. The sequences of the mentioned genes showed high identity with the same genes belonged to the *D. opuntiae* using Basic Local Alignment Search Tool (BLAST). These sequences were previously deposited in GenBank database under the accession numbers PP939951, PP939953, PP939957, PP939959, PP939964, PP939966, PP939970, PP939972, PP939976, PP939979, PP939982, PP939986, PP939988, PP939997 and PP939992 for *18S* gene, accession numbers PP946184, PP946186, PP946190, PP946192, PP946197, PP946200, PP946203, PP946205, PP946208, PP946212, PP946214, PP946217, PP946219, PP946222 and PP946228 for *COI* gene and accession numbers PP958583, PP958586, PP958590, PP958592, PP958597, PP958600, PP958603, PP958606, PP958610, PP958612, PP958615, PP958619, PP958621, PP958624 and PP958627 for *12S* gene.

DNA sequencing and bioinformatics analyses

Phylogenetic analyses were performed using the sequences of Palestinian *D. opuntiae* isolates in comparison with reference sequences of other *Dactylopius* species retrieved from GenBank. The investigation of the nuclear gene (*18S*) and two mitochondrial genes (*12S* and *COI*) yielded the following phylogenetic trees as shown in (Figs 2–4).

The 18S gene phylogeny

Identity among *D. opuntiae* isolates collected from Palestine showed 100% based on *18S* gene sequences. The same 100% identity was recorded among our isolates and those downloaded from GenBank. According to the phylogenetic tree based on *18S* gene sequencing data, *D. ceylonicus* (Green, 1896), *D. confusus* (Cockerell, 1896), *D. austrinus* De Lotto, 1974 and *D. opuntiae* were all clustered together. In addition, *D. confertus* (De Lotto, 1967) was very close to these species. The *18S* rRNA gene sequences from *D. confusus*, *D. ceylonicus*, *D. austrinus* and *D. confertus* that retrieved from GenBank data base and the isolates of *D. opuntiae* that were collected from Palestine or retrieved from GenBank showed identities of 99.8%–100%, 99.1%–99.6%, 99.4% and 99.1%, respectively. However, the identity between *D. coccus* and *D. opuntiae* was 97.6% and between *D. tomentosus* (Lamarck, 1801) and *D. opuntiae* was 96.2%–96.4% (Fig. 2).

The COI gene phylogeny

According to *COI* gene sequences, the identity among Palestinian *D. opuntiae* isolates ranged from 99.6%–100%, while the identity between Palestinian *D. opuntiae* isolates and *D. opuntiae* isolates sequences from GenBank revealed slightly lower similarity values ranging from 97.3%–98.7%. However, the identity between *D. opuntiae* and *D. confusus* ranged from 87.9%–89.4%, between *D. opuntiae* and *D. tomentosus* ranged from 79.1%–80.7% and between *D. opuntiae* and *D. coccus* ranged from 78.5%–79.6%. These results indicate clear genetic differentiation between *D. opuntiae* and related species based on *COI* marker (Fig. 3).

The 12S gene phylogeny

Moreover, based on *12S* gene sequences, the identity among Palestinian *D. opuntiae* isolates ranged from 98.6%–100%, while the identity between Palestinian

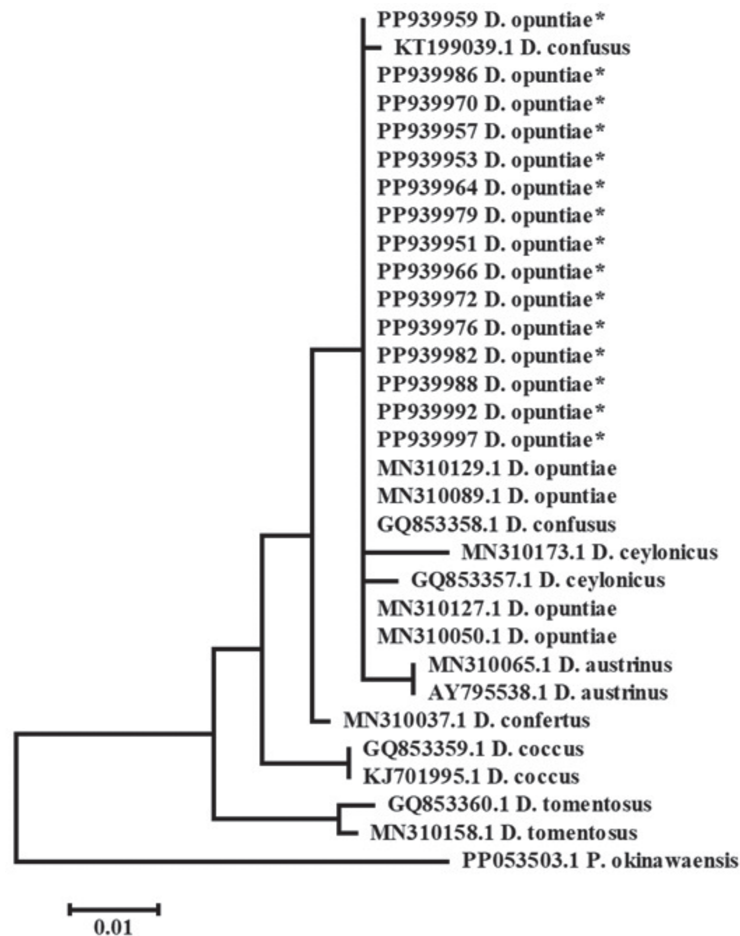

Figure 2. Phylogenetic tree of *18S* gene sequences (526-bp). Sequences from Palestine (noted by asterisk) and other sequences were obtained from GenBank. The sequence of *Pulleniatina okinawaensis* was used as an out-group to study phylogenetic relationship among different species. Phylogenetic analyses were conducted in MEGA6.

D. opuntiae isolates and those retrieved from GenBank is 98.3%–99.7%. However, the identity between *D. opuntiae* isolates and *D. confusus* ranged from 90.0% to 92.2%, *D. opuntiae* isolates and *D. ceylonicus* is 84.7%–86.7%, *D. opuntiae* isolates *D. austrinus* is 83.3%–85.0%. In addition, *D. coccus* and *D. tomentosus* showed when compared to *D. opuntiae* isolates, 81.7–82.8% and 71.7–74.2% identity, respectively (Fig. 4).

RAPD-PCR typing

In respect to RAPD-PCR results, the number of fragments produced by the primer OPA 03 was 14. The length of the fragments ranged from approximately 300 to 3000–bp. The number of polymorphic and monomorphic bands was 11 and 3, respectively, with a percentage of polymorphism 78.6%. Data are illustrated (Fig. 5).

The dendrogram obtained by UPGMA for the overall *D. opuntiae* isolates recovered from Palestine were clustered into 4 groups C1, C2, C3 and C4 at cut-off similarity of 80%. Nevertheless, isolates in clusters C1, C2, C3 and C4 were grouped at cut-off similarity 92%, 88%, 86% and 86%, respectively. Cluster C3 and C4 could be subdivided into 2 sub-clusters. Data are shown (Fig. 6).

Discussion

Three molecular markers—*18S* rRNA, *COI*, and *12S* rRNA—demonstrated considerable variance in their ability to discriminate species within the genus *Dactylopius*, according to the present study. All *D. opuntiae* isolates from Palestine showed 100% identity and shared full or near-complete similarity with other sequences found in GenBank, demonstrating exceptional sequence conservation in the *18S* rRNA gene. *18S* gene is more useful for revealing higher phylogenetic relationships than for distinguishing closely related species, as its high degree of conservation suggests a slow rate of evolution (Dong et al. 2021; Zhang and Bu 2022). *D. opuntiae*, *D. confusus*, *D. ceylonicus*, and *D. austrinus* show minimal sequence divergence and close clustering in recent phylogenetic analyses using conserved ribosomal markers (Ramírez-Puebla et al. 2010).

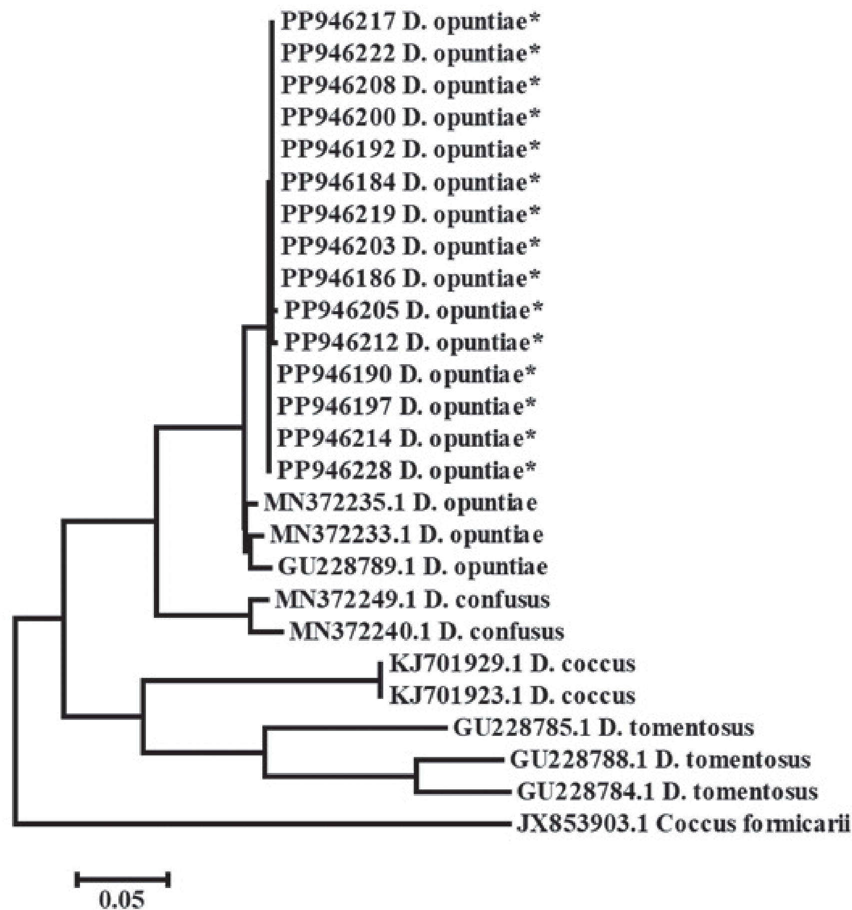

Figure 3. Phylogenetic tree of *COI* gene sequences (522-bp). Sequences from Palestine (noted by asterisk) and other sequences were obtained from GenBank. The sequence of *Coccus formicarii* was used as an out-group to study phylogenetic relationship among different species. Phylogenetic analyses were conducted in MEGA6.

This work's phylogenetic clustering strongly supports the hypothesis that *D. opuntiae*, *D. confusus*, *D. ceylonicus*, *D. austrinus*, and *D. confertus* are closely related. Due to either fast evolutionary divergence, incomplete lineage sorting, or insufficient selective capacity of the conserved *18S* marker (Wang et al. 2023), the sequence identity values between *D. opuntiae* and *D. confusus* are extraordinarily high, ranging from 99.8% to 100%. Similar results were found in recent studies on cochineal insects and other Hemipteran taxa, suggesting that nuclear ribosomal genes could only tell closely related species apart, even when there were obvious physical differences (van Steenderen et al. 2021). The tight link between these species could be a sign of cryptic species complexes, which are often found in insects when ribosomal markers that evolve slowly are analyzed (Zhang and Bu 2022).

In contrast, the mitochondrial *COI* marker provided substantially greater discriminatory power. Isolates of *D. opuntiae* from Palestine showed very high levels of intraspecific identity (99.6%–100%), but much lower sequence identities with allied species, particularly *D. confusus* (87.9%–89.4%), *D. tomentosus* (79.1%–80.7%), and *D. coccus* (78.5%–79.6%). These findings confirm that the *COI* region is suitable for DNA barcoding and species delineation within *Dactylopius*, and they show clear interspecific divergence. Due to the higher rate of mutation and change in mitochondrial genes compared to nuclear ribosomal genes, *COI* has been shown to be an effective barcode marker in insects (D'Ercole et al. 2023; Zhang and Bu 2022). According to recent reviews, *COI* is still a reliable signal for distinguishing closely related insect species and finding hidden genetic variation (Magoga et al. 2022).

At a high level of variation, the *12S* rRNA marker was considered the strongest discriminating molecular marker in comparison to the extremely conserved *18S* marker and the moderate variable *COI* marker in this study. Intraspecific identity values for Palestinian *D. opuntiae* isolates ranged from 98.6% to 100%, but similarities to *D. confusus*, *D. ceylonicus*, *D. austrinus*, *D. coccus*, and *D. tomentosus* were lower. *D. tomentosus* is well-supported in its distant phylogenetic position by the significantly low identity scores (71.7%–74.2%) between *D. opuntiae* and *D. tomentosus*, which indicate significant evolutionary divergence. In a similar vein, recent molecular studies on cochineal insects (van Steenderen et al. 2021) discovered that mitochondrial markers like *COI* and *12S* successfully separated *D. tomentosus* lineages from *D. opuntiae* and related species. The mitochondrial markers

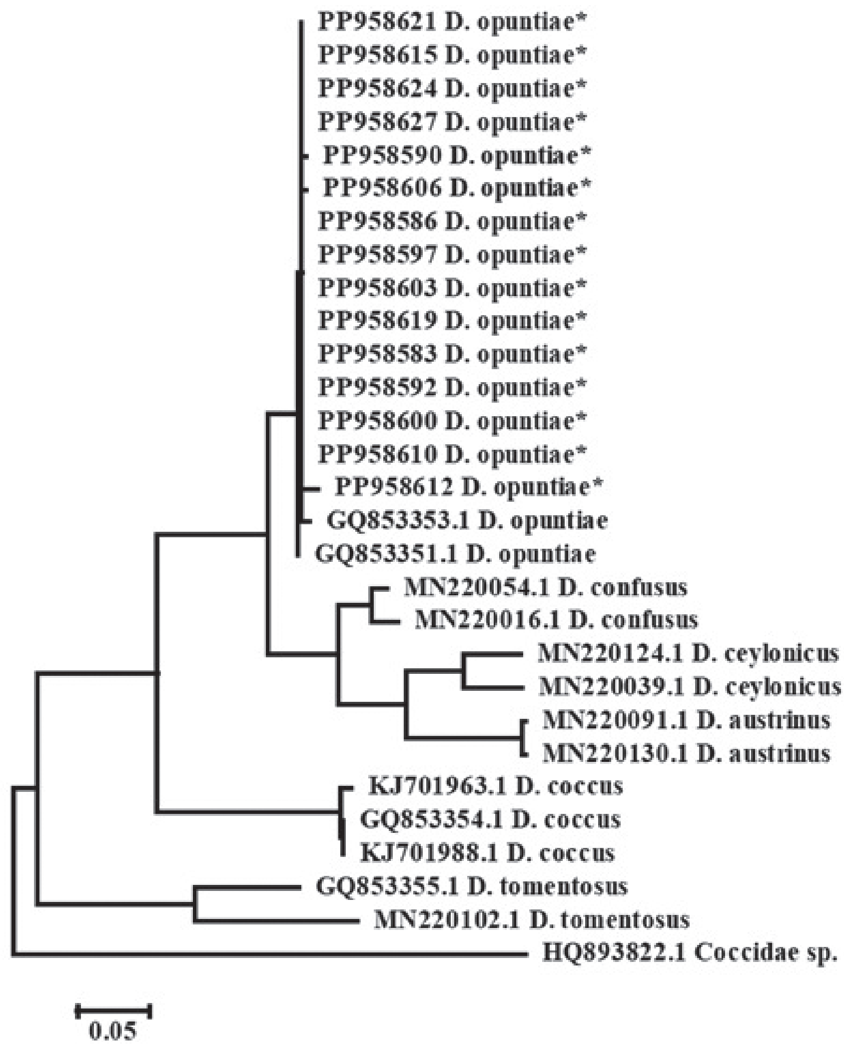

Figure 4. Phylogenetic tree of *12S* gene sequences (357-bp). Sequences from Palestine (noted by asterisk) and other sequences were obtained from GenBank. The sequence of *Coccidae* sp. was used as an out-group to study phylogenetic relationship among different species. Phylogenetic analyses were conducted in MEGA6.

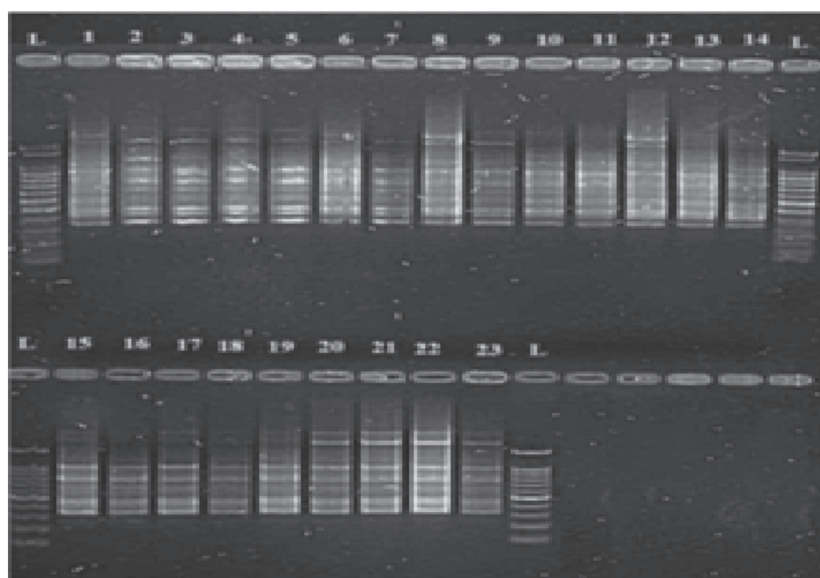

Figure 5. RAPD-PCR product profiles using the primer OPA 03. Lane *L* = ladder 100-bp. Lanes 1 to 23 = RAPD-PCR product of DNA isolated from *D. opuntiae* that recovered in Palestine.

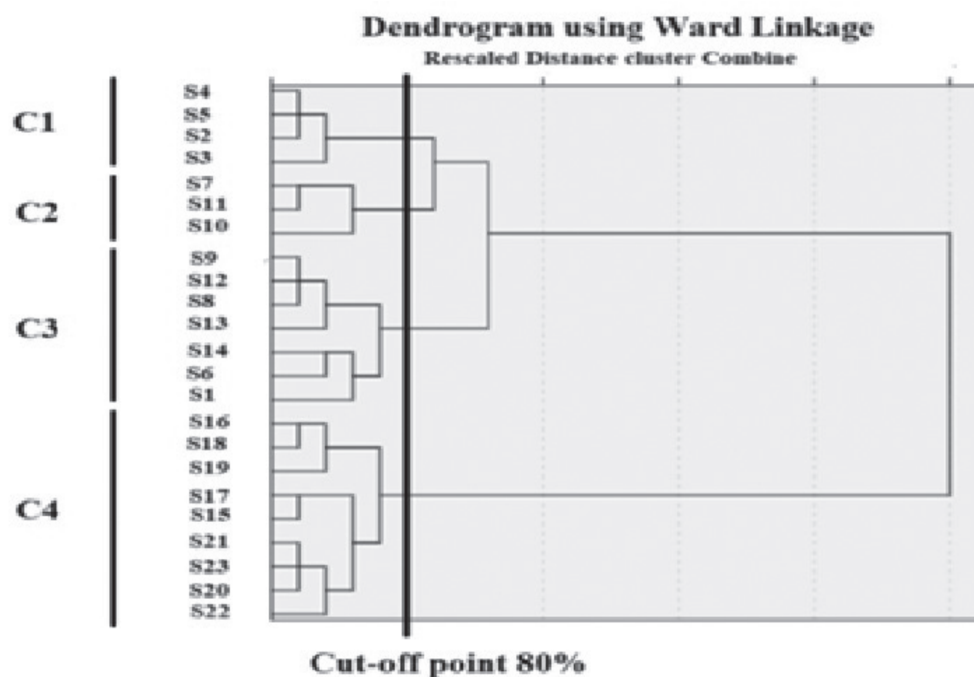

Figure 6. Dendrogram of Palestinian *D. opuntiae* isolates depending on the UPGMA method derived from analysis of the RAPD-PCR product profiles at similarity level 80%. A binary matrix was analyzed using the UPGMA, the clustering process carried out by Ward linkage method and interval measurements using Squared Euclidean distance to construct the dendrogram, using SPSS statistics software version 21 (IBM). C: Cluster.

are more diverse than the ribosomal genes, according to many insect phylogenetic studies (Dong et al. 2021).

Genomic analysis using all three markers shows that *D. opuntiae* populations in Palestine are rather variable within themselves, but very different from other *Dactylopius* species, particularly when it comes to mitochondrial markers. This tendency suggests that *D. opuntiae* populations may have been introduced relatively recently and have since spread rapidly from a small initial population. Adwan et al. (2024) found that a molecular investigation of Palestinian *D. opuntiae* populations supported the idea of recent invasion and population expansion episodes, since they found low nucleotide diversity and relatively high haplotype diversity.

Results of this study based on RAPD-PCR product demonstrated that the number of fragments produced by RAPD-PCR primer OPA 03 was 14. The length of the fragments ranged from approximately 300 to 3000-bp. The number of polymorphic and monomorphic bands was 11 and 3, respectively, with a percentage of polymorphism of 78.6%. These results were in contrast with those published previously (Silva et al. 2013), which showed that the number of fragments produced by RAPD-PCR primer OPA 03 was 12 fragments**,** size of the fragments ranged from 200 to 2000-bp, and number of polymorphic and monomorphic was 9 and 3, respectively, with a percentage of polymorphism of 75%. Results of RAPD-PCR typing method showed that the clustering was not associated with the isolate location. This result is in agreement with findings previously reported (Silva et al. 2013). In addition, results showed that isolates in clusters C1, C2, C3 and C4 were grouped at cut-off similarity 92%, 88%, 86% and 86%, respectively. Cluster C3 and C4 could be subdivided into 2 sub-clusters. These results indicate that the isolates of Palestinian *D. opuntiae* exhibited low genetic variation with a high level of relatedness. Our results were consistent with a study published previously (Silva et al. 2013), which showed that *D. opuntiae* isolates had low genetic variation with a high degree of relatedness.

Genetic structure of Palestinian *D. opuntiae* populations affects pest monitoring and management. The strong genetic similarity of isolates implies a recent introduction and rapid dissemination from a small founder population, which may help build molecular diagnostic tools for early diagnosis and invasion tracking. However, mitochondrial markers (*COI* and *12S*) may accurately distinguish closely related *Dactylopius* species, increasing quarantine surveillance and minimizing misidentification. RAPD-PCR polymorphism and clustering patterns show intraspecific variation that may affect adaptability, virulence, and biological control agent response. Therefore, constant molecular monitoring is needed to discover new genotypes and enhance integrated pest control. Multilocus molecular methods improve biological control, invasion monitoring, and *Dactylopius* species and lineage identification, according to similar research (van Steenderen et al. 2021).

Although this study did provide some useful insights, it does have several drawbacks that need to be noted. The genetic variety of *D. opuntiae* populations in Palestine may not have been completely captured by the small sample size of populations and isolates that were examined. More sophisticated genomic methods, including micro-

satellite or whole-genome sequencing, might offer finer resolution of population structure and invasion paths than the study's reliance on three molecular markers and RAPD-PCR analysis. That is why we need more molecular technologies and more extensive geographical sampling in future large-scale research.

Conclusion

In conclusion, genetic analysis of the *18S* rRNA, *COI*, and *12S* rRNA genes showed that Palestinian *D. opuntiae* isolates were quite similar to one another, suggesting that they may have descended from a common ancestor or experienced a single invasion. Although they distinguished *D. opuntiae* from less closely related taxa, the three molecular markers all pointed to a tight evolutionary link among *Dactylopiu*s species. While the mitochondrial markers *COI* and particularly *12S* offered stronger selective strength for species delimitation, the highly conserved *18S* rRNA gene was better suited for determining larger evolutionary connections within the genus. Among closely related *Dactylopius* species, the *12S* marker had the best resolution according to sequence divergence values. Overall, the findings highlight the importance of integrating multiple molecular markers with traditional taxonomy to better understand the evolutionary diversity and species boundaries within the genus *Dactylopius*.

Data availability statement

The data that support the results of this study are openly available in GenBank database at https://www.ncbi.nlm.nih.gov/genbank/.

Author contributions

All authors contributed equally to this work, participated in experimental design, sample collection, DNA extraction, PCR and DNA analyses. All authors interpreted the data, participated in writing the draft version of the manuscript, critically revised the manuscript for important intellectual contents, and approved the final version.

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References

Adwan G, Abuseir S, Omar G, Al-Fares H, Altamimi M, Dalab A, Khraim N, Abed Al-daym M (2024) Molecular Characterization of *Dactylopius opuntiae* (Cockerell, 1896) in Palestine. Psyche: A Journal of Entomology 2024: 1–11. https://doi.org/10.1155/psyc/9986708

Assad F, Chikh-Khamis Z, Bufaur M (2024) First Record of the Predators *Scymnus saturalis* and *Hyperaspis trifurcata* on *Dactylopius opuntiae* in Cactus Fields in Syria. Arab Journal of Plant Protection 42(1): 102–107. https://doi.org/10.22268/AJPP-001211

Bader AMK, Abu-Alloush AH (2019) First Record of the Cochineal Scale Insect, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae), in Jordan. Jordan Journal of Biological Sciences 12(2): 155–159.

Ben-Dov Y, Sanchez-Garcia I (2015) New Data on Several Species of Scale Insects (Hemiptera: Coccoidea) From Southern Spain. Boletin De La Sociedad Entomologica Aragonesa 56: 313–317.

Bouharroud R, Amarraque A, Qessaoui R (2016) First Report of the Opuntia Cochineal Scale *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) in Morocco. EPPO Bulletin 46(2): 308–310. https://doi.org/10.1111/epp.12298

da Silva VP, Suma P, Claps L, Kondo T (2022) *Dactylopius* spp. (Hemiptera: Coccomorpha: Dactylopiidae). In: Kondo T, Watson GW (Eds) Encyclopedia of Scale Insect Pests. CABI Press, Wallingford, Oxfordshire, 390–394. https://doi.org/10.1079/9781800620643.0002

D'Ercole J, Vila R, Dapporto L, Pentinsaari M, Talavera G, Dincă V, Hebert PDN (2023) Molecular evolution in introduced insect species—a mitochondrial perspective. Frontiers in Ecology and Evolution 11: 1218690. https://doi.org/10.3389/fevo.2023.1218690.

Dong Z, Wang Y, Li C, Li L, Men X (2021) Mitochondrial DNA as a Molecular Marker in Insect Ecology: Current Status and Future Prospects. Annals of the Entomological Society of America 114(4): 470–476. https://doi.org/10.1093/aesa/saab020

El Bouhissi M, Ghefar M, Sadine SE, Gachi M (2022) Note on the Presence of *Dactylopius opuntiae* (Cockerell, 1896) on Prickly Pear in Algeria (Hemiptera: Dactylopiidae). Annales De La Recherche Forestière En Algérie 12(1): 01–06. https://asjp.cerist.dz/en/article/182825

Kumari A, Thakur SK (2014) Randomly amplified polymorphic DNA-A brief review. American Journal of Animal and Veterinary Sciences 9(1): 6–13. https://doi.org/10.3844/ajavsp.2014.6.13

Magoga G, Forni G, Brunetti M, Meral A, Spada A, De Biase A, Montagna M (2022) Curation of a reference database of *COI* sequences for insect identification through DNA metabarcoding: COins. Database (Oxford) 6: baac055. https://doi.org/10.1093/database/baac055

Mazzeo G, Nucifora S, Russo A, Suma P (2019) *Dactylopius opuntiae*, a New Prickly Pear Cactus Pest in the Mediterranean: An Overview. Entomologia Experimentalis et Applicata 167(1): 59–72. https://doi.org/10.1111/eea.12756

Moussa Z, Yammouni D, Azar D (2017) *Dactylopius opuntiae* (Cockerell, 1896), a New Invasive Pest of the Cactus Plants *Opuntia Ficus-Indica* in the South of Lebanon (Hemiptera, Coccoidea, Dactylopiidae). Bulletin de la Societe Entomologique De France 122(2): 173–178. https://doi.org/10.3406/bsef.2017.3194

Paterson ID, HoHmann JH, Klein H, Mathenge CW, Neser S, Zimmermann HG (2011) Biological Control of Cactaceae in South Africa. African Entomology 19(2): 230–246. https://doi.org/10.4001/003.019.0221

Ramírez-Puebla ST, Rosenblueth M, Chávez-Moreno CK, de Lyra MC, Tecante A, Martínez-Romero E (2010) Molecular Phylogeny of the Genus *Dactylopius* (Hemiptera: Dactylopiidae) and Identification of

the Symbiotic Bacteria. Environmental entomology 39(4): 1178–1183. https://doi.org/10.1603/EN10037

Rodríguez-Leyva E, García-Pascual E, González-Chávez MM, Méndez-Gallegos SdJ, Morales-Rueda JA, Posadas-Hurtado JC, Bravo-Vinaja Á, Franco-Vega A (2024) Interactions of *Opuntia ficus-indica* with *Dactylopius coccus* and *D. opuntiae* (Hemiptera: Dactylopiidae) through the Study of Their Volatile Compounds. Plants 13(7): 963. https://doi.org/10.3390/plants13070963

Silva DM, do ES Mergulhão AC, de Medeiros LV, Figueiredo MV, Burity HA (2013) Genetic Variability of *Dactylopius opuntiae* (Hemiptera, Dactylopiidae) on Forage Cactus in Northeast Brazil. Genetics and Molecular Research 12(4): 5236–5246. https://doi.org/10.4238/2013.October.30.8

Spodek M, Ben-Dov Y, Protasov A, Carvalho CJ, Mendel Z (2013) First Record of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Coccoidea: Dactylopiidae) from Israel. Phytoparasitica 42(3): 377–379. https://doi.org/10.1007/s12600-013-0373-2

Tamura K, Nei M (1993) Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and Chimpanzees. Molecular Biology and Evolution 10(3): 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 2013 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Ülgentürk S, Hocaali SS (2019) Pest Status of *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) and New Records of Scale Insects from Northern Turkish Republic of Cyprus. Munis Entomology & Zoology 14(1): 294–300.

van Steenderen CJM, Paterson ID, Edwards S, Day MD (2021) Addressing the red flags in cochineal identification: The use of molecular techniques to identify cochineal insects that are used as biological control agents for invasive alien cacti. Biological Control 152: 104426. https://doi.org/10.1016/j.biocontrol.2020.104426

Wang W, Zhang X, Garcia S, Leitch AR, Kovařík A (2023) Intragenomic rDNA variation – the product of concerted evolution, mutation, or something in between? Heredity 131(3): 179–188. https://doi.org/10.1038/s41437-023-00634-5

Wang YS, Dai TM, Tian H, Wan FH, Zhang GF (2019) Comparative Analysis of Eight DNA Extraction Methods for Molecular Research in Mealybugs. PLoS One 14(12): e0226818. https://doi.org/10.1371/journal.pone.0226818

Zhang H, Bu W (2022) Exploring Large-Scale Patterns of Genetic Variation in the COI Gene among Insecta: Implications for DNA Barcoding and Threshold-Based Species Delimitation Studies. Insects 13(5): 425. https://doi.org/10.3390/insects13050425