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Research Article

Molecular Characterization of *Dactylopius opuntiae* (Cockerell, 1896) in Palestine

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Dactylopius opuntiae (Cockerell) (Hemiptera: Dactylopiidae) is a new invasive insect in our area, and the most destructive insect on cactus species. It is often referred to as prickly pear cochineal and produces large economic losses all over the globe. This research investigates the molecular genetics of *D. opuntiae*. Molecular studies exploring the evolutionary relationships of recently established and naturally occurring *D. opuntiae* populations are crucial in determining the routes of entrance. Here, the sequence variation in the *mtCOI*, *12S* and *18S* gene markers from specimens isolated from the Palestinian territories in 2023 were assessed to gain greater insight into the variations in DNA within and between Palestinian *D. opuntiae* populations. The results showed the presence of 12, 18, and 12 haplotypes for *COI*, *12S*, and *18S* gene markers, respectively. Furthermore, the results indicated low nucleotide diversity (π) (0.00133 for *COI*, 0.00258 for *12S*, and 0.00085 for *18S*) and high haplotype diversity (Hd) within haplotype phylogenetic trees (0.0.563 for *COI*, 0.628 for *12S*, and 0.425 for *18S*). Because the sequences deposited in GenBank are from few countries, it was difficult to track the origin of Palestinian sequences with *COI*, *12S*, and *18S* sequences deposited in GenBank from few areas of the world. In conclusion, the Palestinian *D. opuntiae* population exhibited the highest Hd and lowest π based on investigated gene markers. In addition, negative results for neutral tests indicated that a high number of uncommon mutations found in the populations, which may suggest new colonization events and increase in population number.

Keywords: *12S*; *18S*; *COI*; *Dactylopius opuntiae*; haplotype; haplotype diversity; nucleotide diversity

1. Introduction

The family Dactylopiidae (Signoret), which includes *Dactylopius* (Costa), is frequently referred to as cochineals. It infects plants belonging to the *Opuntia* and *Nopalea* genera of the Cactaceae family throughout Asia, Africa, Europe, and the Americas continents. One of the 11 species of the genus *Dactylopius* is *Dactylopius opuntiae* (Cockerell). It is abrasive and destructive, infecting *Opuntia ficus-indica* (*L.*) Mill. (Caryophyllales: Cactaceae) as well as other species of the genus *Opuntia* that inhabit various regions of the world [1, 2]. It is widely known that these insects synthesize a biological pigment called carminic acid, which plays a vital role in protecting them from predators [3]. In addition, this

insect is utilized in industries, including those that manufacture textiles, food coloring, medicine, and cosmetics.

In general, the morphological features of all *Dactylopius* species are similar: the body of an adult female is oval in shape, dark red, wingless and enveloped in a white waxy material. On cactus cladodes, adult females and nymphs live together in groups and consume the sap through beak-like mouthparts that eventually leads to kill their host. The nymph stage is the dispersal stage as it moves from one host to another through winds as waxy filaments. Furthermore, several species belonging to the *Dactylopius* genus have been used often as biological control agents in certain regions of the world to get rid of invading cacti [4]. In Palestinian territories, as well as other countries, cacti play a vital part in agriculture production and rural development, where they provide extra income for farmers.

According to Paterson et al. [2], *D. opuntiae* is considered the most hazardous species in the genus *Dactylopius*. It subsequently spreads over the Mediterranean area, becoming the most aggressive insect on *O. ficus-indica* in countries as Lebanon, Israel, Spain, Morocco, Cyprus, Algeria, and Syria [1, 4–9]. The first known incidence of *D. opuntiae* was verified on cactus plants in Lebanon in 2012 [4]. Later, in 2013, it was documented in Israel [1]. In Jordan, the *D. opuntiae* insect was first reported by Bader and Abu-Alloush [10]. In Syria, the insect was first reported in 2020 [9]. According to the Palestinian Ministry of Agriculture, the first detection of *D. opuntiae* in Palestinian territories was recorded in 2013 [1, 10, 11]. The rapid spread of this insect is largely driven by environmental factors, as these regions are typically arid and semiarid. With global climate change, these areas are expected to become even hotter and drier, further escalating the infestation and leading to greater economic losses [12]. The symptoms of this insect were verified on cladodes, as heavy, medium, and low infection. The cladodes seemed yellow, eventually became damaged, and collapsed. As it progressed to spread to other regions of Palestinian territories, the insect attacked more cacti and cause a serious loss of production. Farmers in several parts of the Palestinian territories have recently complained of economic loss due to the spread of this invasive insect in their cactus fields. In order to prevent the insect from spreading to other parts of the Palestinian territories and neighboring countries or regions, a national and regional strategy should be implemented to eradicate the insect and reduce the Palestinian and the regional cactus economic loss. The first step to achieve this goal is to identify and characterize the prevalent species.

Consequently, assessments of the polymorphism within and between *D. puntiae* populations are made possible by molecular characterization. Few molecular investigations on *D. puntiae* can be found in the literature; however, some correlated species of the genus *Dactylopius* can be highlighted [13–17]. In these studies, different gene markers were used such as ISSR and RAPD to assess genetic variability. According to the best of our knowledge, no prior research on this subject has been published. The primary focus of this work was to confirm the genetic diversity of *D. opuntiae* based on 2 mitochondrial gene markers which are cytochrome *C* oxidase subunit 1 (*COI*) and *12S* genes and 1 nuclear (nDNA) *18S* gene marker.

2. Materials and Methods

2.1. Insect Sampling. In 2023, *D. opuntiae* isolates were collected from wild *Opuntia ficus-indica* populations located in several Palestinian regions in the West Bank (Governorates of Nablus, Tulkarem, Jenin, Jordan Valley, Bethlehem, and Hebron). These samples were morphologically identified based on the insect physical traits such as its body shape, size, and the presence of waxy filaments, following the standard taxonomic keys for Dactylopius species [18]. The identification process was carried out by the research team and then transported to the laboratories of the Faculty of agriculture and Veterinary Medicine, An-Najah National University, Tulkarem, Palestine, for further analysis.

2.2. Genomic DNA Extraction. Fifty one freshly adult female *D. opuntiae* specimens that had just been collected were treated with 100% ethanol to get rid of the white, waxy substance prior being cleaned with distilled water. Following that, the treated samples were frozen at −20°C till the extraction of DNA.

According to the manufacturer's instructions, the DNA genome of a single *D. opuntiae* organism was extracted using the DNeasy Blood and Tissue Kit (Qiagen Hilden, Germany). A micropestle was used to homogenize the materials. The extracted genomic DNA molecules containing the target fragments for sequencing were sent to a commercial sequencing company (Smart gene labs, Nablus, Palestine) for bidirectional sequencing by the Spectrum Compact CE System (Promega).

2.3. DNA Amplification. Using the primers given in Table 1, three genes were amplified, two mitochondrial genes (COI, 12S) and one nuclear gene (18S).

2.4. PCR Product Clean up and DNA Sequencing. Following the manufacturer's instructions, the PCR reaction products were cleaned-up using the Wizard SV Gel and PCR Clean-Up System kit (Promega) and then the dideoxy chain termination technique was used to sequence the purified products, and subsequently, the sequence reads were trimmed to remove low-quality bases at both ends each continuous partial strand. The resulting sequences were submitted to primary bioinformatics sequence databases to obtain the accession number.

2.5. Bioinformatics Analyses. A Basic Local Alignment Search Tool (BLAST) system was used to compare the nucleotide sequences to other closely related DNA sequences of the same genes that were deposited in the GenBank database. The Clustal W tool in the computer software program MEGA Version 6 was used to perform a multiple alignment of the *COI*, *12S*, and *18S* nucleotide sequences that were obtained from *D. opuntiae* populations

Table 1: Primers sequences used in this research for the PCR amplification of *COI*, *12S*, and *18S* marker genes in *Dactylopius opuntiae*.

Gene	Primer	Sequence (5′ ⟶ 3′)	Annealing temperature	Amplicon size	Reference
18S	*18S-F*	CTGGTTGATCCTGCCAGTAG	58°C	570-bp	[19]
	18S-R	CCGCGGCTGCTGGCACCAGA			
12S	*12S-F*	AAGAGTGACGGGCRATTTGTACATA	52°C	413-bp	[19]
	12S-R	GTGCCAGCAGTWGCGGTTA			
COI	PcoF1	CCTTCAACTAATCATAAAAATATYAG	43°C	603-bp	[20]
	LepR1	TAAACTTCTGGATGTCCAAAAAATCA			

invading the Palestinian territories with other sequences of the same gene that retrieved from the GenBank database [21].

The origin of *D. opuntiae* individuals that invaded the Palestinian territories was conducted using the sequences obtained from nuclear and mitochondrial DNA. The phylogenetic relationships between the continuous partial sequences of the *COI*, *12S*, and *18S* genes identified in the Palestinian territories and those previously published sequences obtained from the GenBank database were ascertained using the maximum likelihood method (MLM), which is based on the Tamura–Nei model [22]. The neighbor-join and BioNJ algorithms were applied to a matrix of pairwise distances computed using the maximum composite likelihood (MCL) approach, and the topology with the highest log likelihood value was chosen to create the initial tree for the heuristic search automatically after 1000 bootstrap resampling; the robustness of the groups in the MLM was assessed. The estimated values of population genetic diversity indices including haplotype numbers (h), haplotype diversity (Hd), and nucleotide diversity (π) as well as neutrality indices (Tajima's D, Fu's, and Li's F test statistic, Fu and Li's D test statistic, and Fu's Fs statistic) were computed using computer software program DnaSP 5.1001 [23]. The median-joining (MJ) network of the haplotypes of the *COI*, *12S*, and *18S* sequences of *D. opuntiae* was examined using the computer software program Network 4.6.1.6 [24].

3. Results

3.1. Phylogenetic Analyses. The *COI*, *12S*, and *18S* gene markers of *D. opuntiae* genome were PCR amplified in all isolated DNA samples. Only 47, 49, and 50 out of 51 amplified PCR products were successfully sequenced using the Sanger technique for *COI*, *12S*, and *18S* genes, respectively. Using the BLAST system, the continuous partial DNA sequences of these genes exhibited a high degree of homology with the same genes corresponding to *D. opuntiae* available in GenBank database. The accession numbers PP946183–PP946229 for *COI* gene sequences, PP958583–PP958631 for *12S* gene sequences, and PP939950–PP939999 for *18S* gene sequences were deposited in GenBank database.

In this study, phylogenetic analysis using the MLM based on *COI*, *12S*, and *18S* gene sequences extracted from *D. opuntiae* specimens isolated from the Palestinian territories and previously samples sequenced for the same genes from other countries was performed in order to establish the geographic origin of the population of *D. opuntiae* attacking the Palestinian territories. Only the GenBank has reference strains for *COI*, *12S*, and *18S* genes from the United States, Australia, Mexico, South Africa, Namibia, and Saudi Arabia, which were used for the building of the phylogenetic tree. Figures 1, 2, and 3 show the molecular phylogenetic trees for *COI*, *12S*, and *18S* genes.

3.2. Mitochondrial DNA Analysis and Haplotypes. The understanding of the genetic diversity of the *D. opuntiae* population invading the Palestinian territories was enhanced by analyzing the mtDNA nucleotide sequences of 47 *COI* and 49 *12S* specimens. Twelve and 18 haplotypes were identified by the *COI* and *12S* sequencing data, respectively. The most prevalent haplotype for *COI* was Hap-1 (31 out of 47 specimens, 66%) and for 12S, Hap-1 (30 out of 49 specimens, 61.2%). Data are presented in Table 2 and Figures 4 and 5. Table 3 shows that π was 0.00133 and 0.00258 for the *COI* and *12S* sequences, respectively, while the Hd was 0.563 and 0.628. With a significant core Hap-1 haplotype, the MJ networks built with *COI* and *12S* sequence haplotypes did not demonstrate "star-like" phylogeny expansion, with the vast majority of the distinct haplotypes being closely associated to the founder or dominant haplotype (Hap-1) particularly in *12S* sequences; data are shown in Figure 5. The hypothesized (often ancestral) sequence denoted to as a median vector (red circle) is needed to link the network's present sequences as parsimoniously as possible. In the absence of the median vector, there would be no shortest junction between the dataset's sequences. The *COI* gene in the isolates from the Palestinian territories revealed a variety of mutations ranging from one mutation (e.g., acc. no. PP946224) to three mutations (e.g., acc. no. PP946203) located between the founder or dominant haplotype and the remaining haplotypes (Figure 4); similarly, the *12S* gene showed a range of one mutation (e.g., acc. no. PP958597) to three mutations (acc. no. PP958612). Data are shown in Figure 5. The levels of variation in *COI* genes were lower than those in *12S* sequences. Among 10 variable polymorphic sites observed in the *COI* sequences, there were 5 singleton variable sites (single nucleotide polymorphisms or SNPs) and five parsimony informative sites (PISs) separated the fundamental core haplotype of the *COI* gene from the other haplotypes (Table 3). The five PISs (two variants) were found on sites 32, 108, 121, 197, and 659, while the five SNPs (two variants) were found on sites 46, 47, 109, 432, and

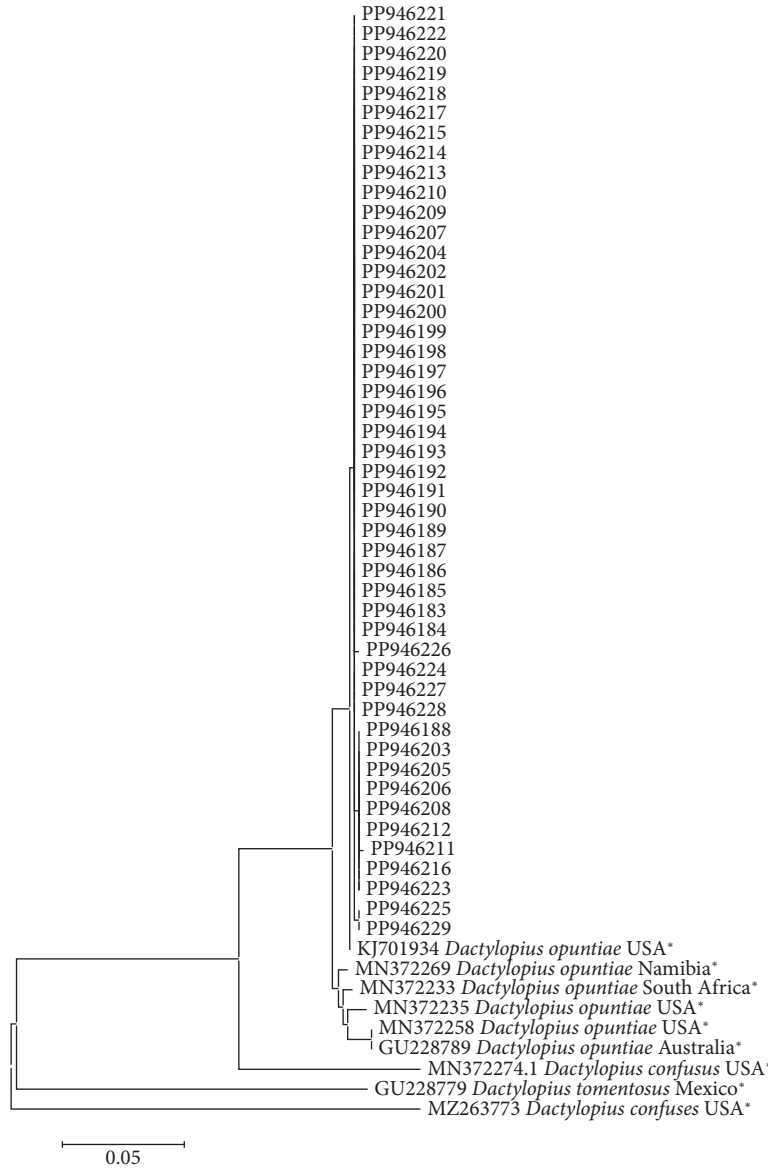

Figure 1: Phylogenetic tree based on the *COI* gene sequencing data using MLM. Reference sequences for the *COI* gene of *Dactylopius opuntiae* (denoted by asterisk) were retrieved from GenBank. Phylogenetic analysis was conducted using sequencing data (PP946183–PP946229) from Palestine. There were 57 nucleotide sequences that were studied, and the ultimate dataset had 519 positions. In MEGA6, evolutionary analyses were performed.

651. Compared with *COI* sequences, the polymorphism level in the *12S* sequences was greater. Among 23 variable polymorphic sites observed in the *12S* sequences, there were 5 PISs and 18 SNPs (Table 3) separating the fundamental core haplotype of the *12S* gene from the other haplotypes. At the following positions in *12S* sequences, 17 SNPs (two variants) were identified: 30, 47, 126, 130, 144, 188, 197, 199, 231, 324, 345, 394, 417, 440, 442, 451, and 466. On the other hand, one SNP (three variants) was determined on site position 464. Furthermore, 5 PISs (two variants) were detected on sites 93, 215, 443, 448, and 456. Tajima's $D = -1.74112$ ($p > 0.05$), Fu and Li's F test $= -1.95869$ ($0.10 > p > 0.05$), Fu and Li's D test $= -1.62541$ ($p > 0.05$), and $Fs = -9.413$ were the results of the *COI* gene neutrality test. Tajima's $D = -2.48637$ ($p < 0.01$), Fu and Li's F test $= -4.35766$ ($p < 0.02$), Fu and Li's D test $= -4.30463$ ($p < 0.02$), and $Fs = -16.976$ were the results of the neutrality test for the *12S* gene. Table 3 shows the data.

3.3. Nuclear DNA Analysis and Haplotypes. In addition to mtDNA analysis, nDNA nucleotide sequence analysis of fifty *18S* specimens was done in order to enhance the understanding of the genetic diversity of the *D. opuntiae* population invading the Palestinian territories. The *18S* gene sequencing demonstrated existence of 12 haplotypes. As shown in Table 2 and Figure 6, the most common haplotype

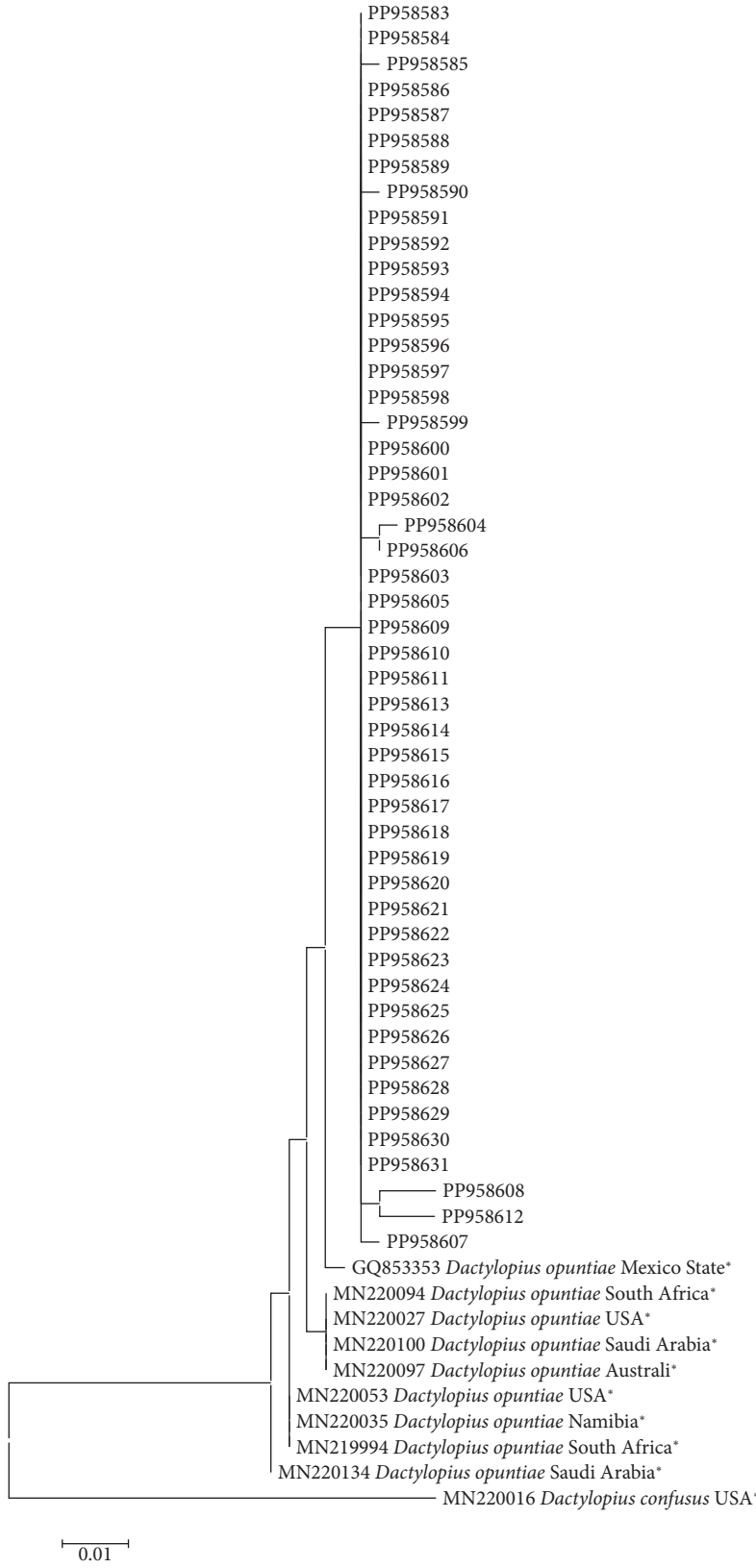

Figure 2: Phylogenetic tree based on the *12S* gene sequencing data using MLM. Reference sequences for the *12S* gene of *Dactylopius opuntiae* (denoted by asterisk) were retrieved from GenBank. Phylogenetic analysis was conducted using sequencing data (PP958583–PP958631) from Palestine. There were 59 nucleotide sequences that were studied, and the final dataset had 362 positions. Evolutionary analyses were carried out in MEGA6.

Figure 3: Phylogenetic tree based on the *18S* gene sequencing data using MLM. Reference sequences for the *18S* gene of *Dactylopius opuntiae* (denoted by asterisk) were retrieved from GenBank. Phylogenetic analysis was conducted using sequencing data (PP939950–PP939999) from Palestine. There were 64 nucleotide sequences that were studied, and the final dataset had 492 positions. Evolutionary analyses were carried out in MEGA6.

Table 2: *Dactylopius opuntiae* haplotype distribution for *COI*, *12S*, and *18S* sequences.

Haplotype	*COI* gene No. of sequences	*12S* rRNA region No of sequences	*18S* rRNA region No of sequences
Hap-1	31	30	38
Hap-2	3	1	1
Hap-3	1	2	2
Hap-4	1	1	1
Hap-5	2	1	1
Hap-6	1	1	1
Hap-7	1	2	1
Hap-8	1	1	1
Hap-9	1	1	1
Hap-10	1	1	1
Hap-11	3	1	1
Hap-12	1	1	1
Hap-13		1	
Hap-14		1	
Hap-15		1	
Hap-16		1	
Hap-17		1	
Hap-18		1	
Total	47	49	50

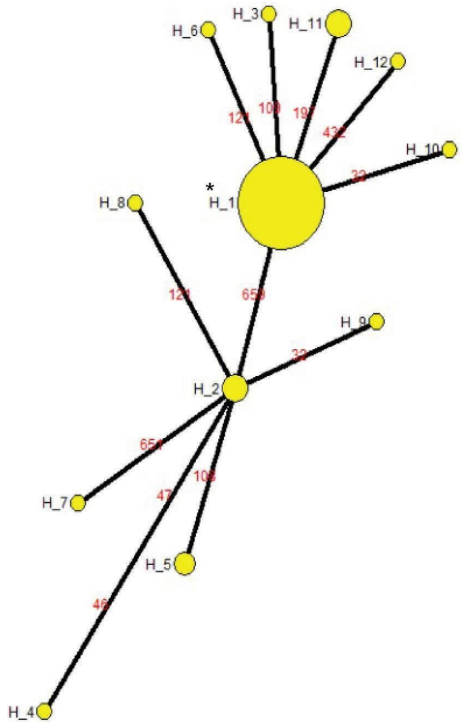

Figure 4: The *COI* gene's median-joining (MJ) network of the isolates of *Dactylopius opuntiae* haplotypes. Every haplotype is denoted by a yellow circle. The founder or dominant haplotype is indicated by the asterisk. The circle's size correlates with the frequency of haplotypes. Bars joined the haplotypes indicate the number of nucleotide substitutions which separate the *COI* founder haplotype from other haplotypes. The circles' measurements reflect the number of sequences that belong to each distinct haplotype.

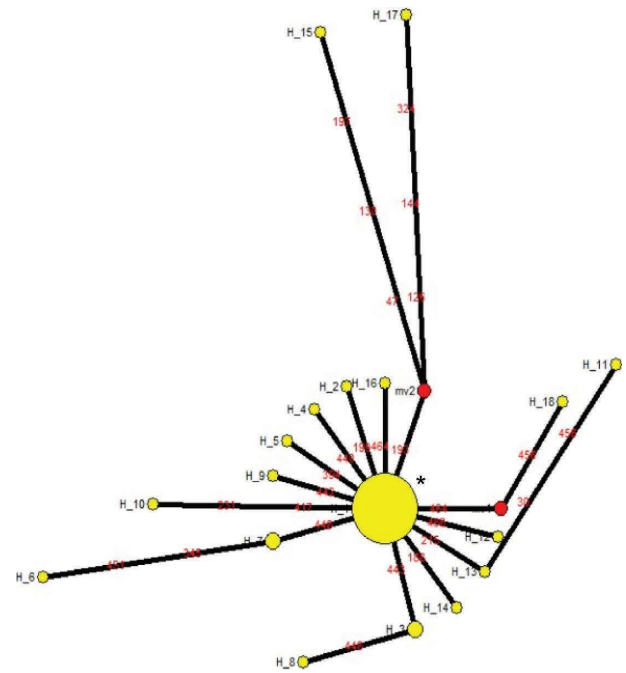

Figure 5: The *12S* gene's median-joining (MJ) network of the isolates of *Dactylopius opuntiae* haplotypes. Every haplotype is denoted by a yellow circle. The founder or dominant haplotype is indicated by the asterisk. The circle's size correlates with the frequency of haplotypes. Bars joined the haplotypes indicate the number of nucleotide substitutions which separate the *12S* founder haplotype from other haplotypes. The circles' measurements reflect the number of sequences that belong to each distinct haplotype. A hypothetical (usually ancestral) sequence known as a median vector (red circle) is needed to link current sequences within the network with maximum parsimony. There wouldn't be a shortest route joining the sequences in the data set without the median vector.

was Hap-1 (38 out of 50 specimens, 76%). According to Table 3, the Hd and π for the *18S* sequences were 0.425 and 0.00085, respectively. With a significant core Hap-1 haplotype, the MJ networks built using *18S* sequence haplotypes as well did not show "star-like" phylogenetic expansion; the majority of the distinct haplotypes were connected to the founder haplotype (Hap-1). The data are displayed in Figure 6. In Palestinian isolates, the *18S* gene showed mutational steps between the founder haplotype and others ranging from one mutation (e.g., acc. no. PP939985) to three mutations (acc. no. PP939959), as shown in Figure 6.

The *18S* sequences exhibited low levels of polymorphism. Among 12 variable polymorphic sites observed in the 18*S* sequences, there were 11 SNPs, and 1 was PIS separating the *18S* gene's primary core haplotype from the other haplotypes (Table 3). Eleven SNPs (two variants) were detected in *18S* sequences on positions 19, 29, 31, 35, 608, 615, 625, 626, 627, 629, and 636. Furthermore, one PIS (two variants) was detected on site 635. Tajima's D = −2.34485 ($p < 0.01$), Fu and Li's F test = −4.43979 ($p < 0.02$), Fu and Li's D test = −4.46711 ($p < 0.02$), and Fs = −13.311 were the results of the *18S* gene neutrality test. Table 3 demonstrates the data.

Table 3: Summary statistics for *COI*, *12S*, and *18S* genes polymorphism in *Dactylopius opuntiae* isolated from Palestine. Statistical significant differences are marked with asterisks.

Nucleotide diversity indices	Gene or region		
	COI	***12S* rRNA**	***18S* rRNA**
Number of nucleotide sites	683	500	657
Total number of sites (excluding sites with gaps/missing data)	680	498	654
Number of sequence	47	49	50
Invariable (monomorphic) sites	670	475	642
Variable (polymorphic) sites	10	23	12
Singleton variable sites	5	18	11
Parsimony informative sites	5	5	1
Haplotypes number	12	18	12
Haplotype (gene) diversity (Hd)	0.563	0.628	0.425
Variance of haplotype diversity	0.00742	0.00675	0.00796
Standard deviation of haplotype diversity	0.086	0.082	0.089
Nucleotide diversity (per site) (π)	0.00133	0.00258	0.00085
Sampling variance of π	0.0000001	0.0000003	0.0000001
Standard deviation of π	0.00027	0.00056	0.00023
Average number of nucleotide differences (k)	0.90102	1.285	0.555
Fu and Li's D test statistic	−1.62541	−4.30463*	−4.46711*
Fu and Li's F test statistic	−1.95869	−4.35766*	−4.43979*
Tajima's D	−1.74112	−2.48637**	−2.34485**
Fu's Fs statistic	−9.413	−16.976	−13.311

* $p < 0.02$.
** $p < 0.01$.

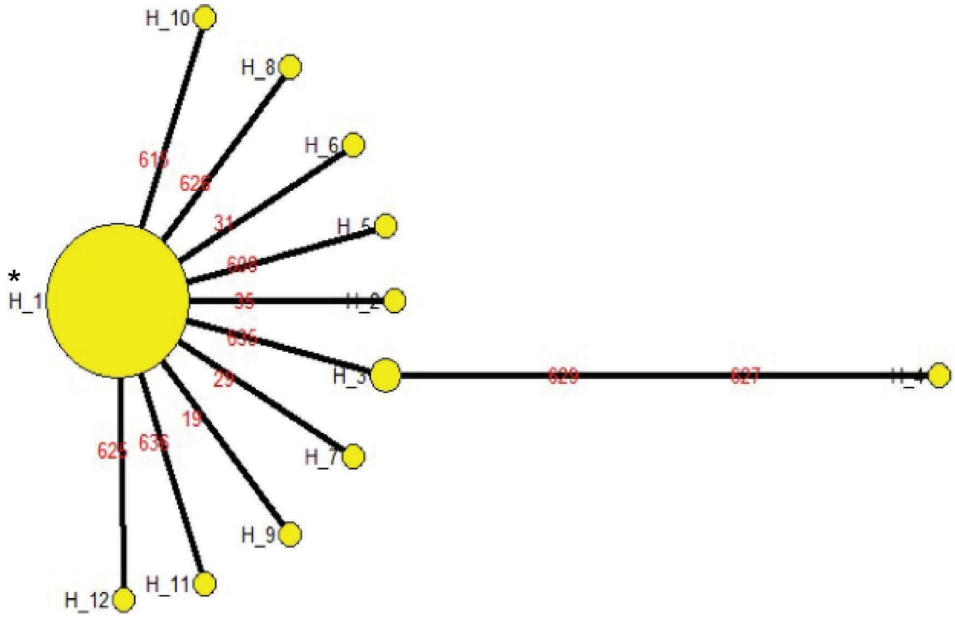

Figure 6: The *18S* gene's median-joining (MJ) network of the isolates of *Dactylopius opuntiae* haplotypes. Every haplotype is denoted by a yellow circle. The founder or dominant haplotype is indicated by the asterisk. The circle's size correlates with the frequency of haplotypes. Bars joined the haplotypes indicate the number of nucleotide substitutions which separate the *18S* founder haplotype from other haplotypes. The circles' measurements reflect the number of sequences that belong to each distinct haplotype.

4. Discussion

The data of the current study are the first to investigate genetic variation and differentiation of *D. opuntiae* populations invading the Palestinian territories based on *COI*, *12S*, and *18S* gene markers. As a molecular marker, mtDNA has been utilized extensively. In addition to being simple to use, it possesses beneficial biological features including near-neutrality, no recombination, and a clock-like evolutionary rate. However, employing mtDNA data for species delimitation, population genetics, and calculating the evolutionary history of populations and species is involved with some challenges. Other restrictions and exceptions to the simplicity of mitochondrial inheritance include complicated

processes of evolution, low effective population sizes, and maternal inheritance. The ability of molecular data to evaluate phylogenetic and phylogeographic hypotheses can be boosted by combining mtDNA and nuclear DNA markers [25]. The *mtCOI* marker is considered very helpful since it shows insignificant rates of insertions and deletions and may be employed to differentiate across species, especially in insects, even though it is a reasonably conserved area [26, 27]. The mitochondrial *12S* encoding gene can be advantageous for distantly related taxa, for example, genus [25]. One of the nuclear sequences with the slowest rate of evolution is the *18S* gene, which may be used for determining the ancestry of different species [28]. The *18S* marker genes were excluded from analysis since it is not easy to identify any variation between them through *Dactylopius coccus* lineages [19].

Using maximum likelihood cluster analysis, this study was unable to identify any obvious relationship between genetic and geographic distances. This is because the available sequences for marker genes (*COI*, *12S*, and *18S*) into GenBank with a dispersed geographic distribution are very limited. On the other hand, sequences from the USA and Mexico were grouped with the Palestinian sequences for the *12S* and *COI* genes, respectively.

The level of polymorphism detected in *D. opuntiae* isolates from Palestinian territories in *COI* and *18S* sequences was intermediate, with 12 haplotypes each for the *COI* and *18S* genes, while the level was high in Case *12S* sequences with 18 haplotypes. On PubMed and other websites, we did not find published papers related to this subject. Population samples from GenBank were retrieved and analyzed using DnaSP 5.1001 from invaded countries. Results of this study are in disagreement with information for *COI* haplotypes from South Africa and Namibia which were 6 and 4, respectively. However, the result from USA is consistent with information for *COI* haplotype number which was 10. The number of haplotypes for the *12S* and *18S* genes is in disagreement with information from USA, Australia, Saudi Arabia, South Africa, and Namibia, which showed the number of haplotypes had a range 1–6 and 1-2 for *12S* and *18S* gene, respectively.

Hd and π were determined to quantify the diversity of genes within or across populations [29]. Based on *COI*, *12S*, and *18S* gene markers, the overall population showed high Hd and low π. All of the haplotypes in this analysis differed by just one, two, or three nucleotides, suggesting that the haplotypes based on various markers were closely associated and not highly diverse, as demonstrated by the low nucleotide-diversity values. The analysis of the median-joining network of the *COI*, *12S*, and *18S* gene markers, which did not exhibit "star-like" expansion networks, confirms this hypothesis by pointing to population expansion haplotypes that evolved from a central founder haplotype with few mutational steps. This suggests the rapid demographic expansion of *D. opuntiae* population size from small effective population size [30]. Both Tajima's D test and the Fu test also supported these results. Using DnaSP software, neutral tests such as Tajima's D [31], Fu and Li's D, and Fu and Li's F [32] tests were used to analyze the historical demographic expansions. The allele frequency distribution of segregating nucleotide sites serves as the basis for Tajima's D test. A positive value denotes a preference toward intermediate frequency alleles, whereas a negative value suggests a preference toward uncommon alleles, which is indicative of a recent increase in population. The neutral test for the Palestinian sample population revealed negative values for Tajima's D, Fu and Li's D, and Fu and Li's F in case *COI* gene sequences; it also obtained a significant value in Case *12S* and *18S* genes. This indicates a shift away from neutral mutation and these sequences have a recent population expansion. Factors that may have an impact on the population expansion of this insect may include the increasing area of cactus planting, where the insect can grow and feed, the absence of natural enemies, the tolerance to changing environmental factors, and other biotic and abiotic factors [4, 33]. Tajima's D test results that are negative indicate a bias toward rare alleles, which are suggestive of recent colonization events and population expansion in particular regions. The total negative results generated by neutral tests in this study suggest that there is an excess of uncommon mutations in the populations, which may be evidence of recent population growth. Fu's FS test may have provided more evidence for increasing the growth of this population [32], which is based on the distribution of alleles or haplotypes; negative values in this study (−9.413 for *COI* gene, −16.976 for *12S* gene, and −13.311 for *18S* gene) are before indicative of an excess of new mutations concomitant with population growth.

Hd and π obtained in the current study correlate well with information analyzed from invaded areas such as USA, Australia, Saudi Arabia, South Africa, and Namibia. Hd ranges from 0.656 to 0.846, 0.0 to 0.748, and 0.0 to 0.333 for *COI*, *12S*, and *18S* gene, respectively. In addition, π ranges from 0.00382 to 0.01204, 0.0 to 0.00472, and 0.0 to 0.00060, for *COI*, *12S*, and *18S* genes, respectively. The current study's findings showed that the *D. opuntiae* population had a low degree of nucleotide diversity. This model may be a consequence of a severe, recurrently or prolonged population bottleneck. In addition, human-initiated events or natural factors might contribute to the reduction of the effective population size [34].

Future research focusing on the assessment of the risk of disease transmission to the *Opuntia ficus-indica* plant, the development of pesticide resistance, and population spread might discover the data in this report to be beneficial for creating appropriate control programs in the Palestinian territories and other different countries. To understand more about this insect in the Palestinian territories and other countries in the region, additional molecular studies are required.

4.1. Conclusions. In this study, high Hd and low π were observed within the Palestinian *D. opuntiae* population based on *COI*, *12S* rRNA, and *18S* rRNA gene markers. This funding suggests that the population of *D. opuntiae* in the Palestinian territories has undergone recent expansion from a relatively small effective population size. The negative values observed in the neutral tests further support this

hypothesis, indicating an excess of rare alleles, which are characteristic of populations that have recently expanded.

From an invasion ecology perspective, this pattern of genetic diversity implies that *D. opuntiae* has likely undergone a demographic expansion following its colonization in the region. Such patterns are typical of invasive species that rapidly establish and spread in new environments. The phylogenetic analysis suggests that the spread of this insect might have occurred from a few introduction events, followed by rapid growth and developments.

The expansion of *D. opuntiae* in the Palestinian territories could be due to several factors including the availability of suitable host plants (such as *Opuntia ficus-indica*), the absence of natural enemies, and suitability of local environmental conditions. The low genetic variation between *D. opuntiae* populations in Palestinian territories and those in other regions suggests that the *D. opuntiae* in Palestinian could originate from human movement and activities.

In addition, the outcomes of this study highlight the importance of monitoring genetic diversity in invasive species. Understanding the genetic diversity and structure of *D. opuntiae* can be used to improve strategies for controlling its spread, particularly by identifying potential weaknesses related to its low genetic variation. Moreover, the possibility of population growth necessitates the preventive measures to prevent the spreading of invasive species to new areas or amplifying their impact on local ecosystems. Future studies must evaluate the insect's resistance to control methods and its potential to spreading to new regions, which could further escalate the ecological and agricultural challenges posed by this invasive species.

Data Availability Statement

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

Consent

This manuscript does not contain any person's data, and further consent for publication is not required.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

All authors interpreted the data, critically revised the manuscript for important intellectual contents, and approved the final version. Ghaleb Adwan and Sameh Abuseir conceived the experimental design, contributed in sequencing and phylogenetic analysis of PCR final products in this experiment and preparation and reviewing of the research paper with statistical analysis and also contributed in sample collection and DNA extraction and purification. Ghadeer Omar and Heba Al-Fares shared their experience in genetic analysis, review of gene primer design and linking it to genetic markers, drafted the manuscript, and participated actively in optimization of the PCR and sequencing process. Mohammad Altamimi and Abdelhafeed Dalab participated in DNA isolation and purification, helped in the genetic analysis and drafted the manuscript. Nimer Khraim and Mohammad Abed Al-Daym participated in sample collection, labeling, and processing in the lab, and in addition, they helped in the review of the scientific paper after writing it. Ghaleb Adwan and Sameh Abuseir contributed equally to this work.

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