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MOLECULAR IDENTIFICATION OF THE RED PALM MITE *RAOIELLA INDICA* HIRST (ACARI: TENUIPALPIDAE) IN BASRAH PROVINCE, SOUTHERN IRAQ

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Abstract. *Raoiella indica* Hirst (Prostigmata: Tenuipalpidae) is a mite that feeds on a variety of plants and has been found in many countries around the world and the Middle East. In Iraq, it was found on the leaves of the date palm (*Phoenix dactylifera*) cultivars. In this study, mite specimens collected from Basrah orchards were examined microscopically and diagnosed morphologically to differentiate the *Raoiella* spp. This family Tetranychidae (Chelicerata: Acari) contains ~1200 species, many of which are of agricultural importance. Mitochondrial genomes of only a few Tetranychidae species are now been sequenced. The mitochondrial genomes of Tetranychidae are essential for phylogenetic analysis and population genetics. The mitochondrial genome of Raoiella indica (Family Tetranychidae), a widespread palm pest, was sequenced in this study. The central region of the mitochondrial COI region has been extensively studied for intra- and interspecific variation. All of these researches add up to a large database of sequencing information for the Tetranychidae family. This is the first research on red date palm mites on date palm trees in Iraq to our knowledge. Future research is needed, including surveys, biological studies, and host determination, as well as effect assessments and control measures.

Keywords: red palm mite, Raoiella indica, Hirst

1. Introduction

Iraq and Basrah Province are famous for palm cultivation and the production of dates, which are distinguished by their diversity, quality, and important agricultural export. Palm trees are affected by a variety of pests that cause them severe damage. Mite of all kinds is considered one of the most important of these pests, including the date palm dust mite and the Indian red palm mite [1, 2].

The palm is considered a suitable medium for the living of many types of arthropods such as insects, spiders, scorpions, mites, polypods, some crustaceans, and others where the palm is an integrated ecosystem consisting of living and non-living components suitable for the living of many organisms [3, 4].

Wakil *et al.* [5] mentioned that 51 insects are wandering in the date palm, in addition to the dust spider and false palm mites, and there are three types of mites that feed on palm sap, seven types of mites, four of which are predatory on Stem Borers and Dubas eggs, and some of them are saprotrophic. *Phoenix dactylifera* L., the date palm, is one of the most significant plants belonging to the palm family Arecaeae. The genus Phoenix is distinguished from the rest of the genera in the palm family by the palmate leaves folded in length and directed upwards, and the kernel of its fruits with a distinct groove [6].

Palm groves are spread over a large area of land in Basrah Province (Figure 1). It is also grown in urban areas and on roadsides for aesthetic purposes [2]. Palm trees have a wide geographical range and are widespread in the United States, the Arabian Peninsula, Pakistan, and North Africa. They live in a wide range of temperatures and different types of soil. It is believed that their original homeland is in southern Iraq and the Arabian Gulf region, which is important economically, culturally, and socially in many countries across the world, especially in the Middle East [1].

The date palm in Iraq is exposed to many agricultural pests and non-vital factors such as neglect, salinity, and excessive cutting, in addition to the wars and economic blockade that Iraq has gone through, which affected the deterioration of the date palm number. The most important pests of palm are the red palm weevil, a scale insect (armored, green, soft), palm Dubas bug, locusts, date minor worm, and date major worm [7, 8].

Figure 1: A view of date palm orchards in Basrah Province, Southern Iraq.

The types of mites that infect plants live in all environments from the soil, plants, water, food, dung, human, animal, and insects, their sizes range between (100-450) microns and some of them cannot be seen with the naked eye [9].

Palm and date pests include diseases, jungles, insects, mites, snake worms, and mice. Some of them only infect date palms, while others infect other species and types of ornamental palms, fruit trees, forests, ornamental plants, vegetables, and field crops.

An inventory and classification of the types of mites found in and on the date palm in the central region of Iraq were conducted by Al-Jubouri [10], the study showed the presence of 26 mite families comprising 34 genera, including *Raoiella indica*, distributed according to the nature of their nutrition and environment into three plant-fed families, twelve predatory families, three parasitic families, five fungi-feeding families, and three saprotrophic families.

Molecular studies have indicated the Middle East as the region of origin for this mite [11], which would be linked to the origins of the date palm, *Phoenix dactylifera*, which is believed to have originated from Southeast Asia (the Arabian Peninsula to southern Pakistan) [12, 13].

Raoiella indica Hirst (Figure 2) (Prostigmata: Tenuipalpidae), was described for the first time on coconut in India in 1924 [14]. It affects a variety of host plants, including coconut, date palms [15], bananas and other palms [16, 9], and ornamentals, such as Heliconiaceae, Strelitziaceae, and Zingiberaceae [17, 18].

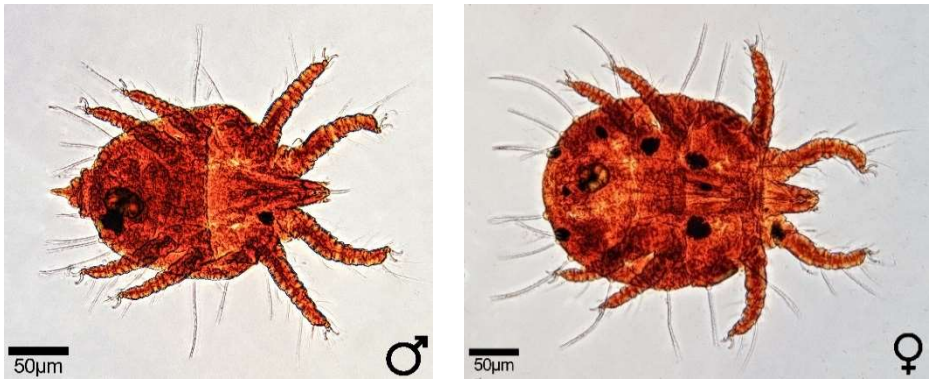

Figure 2: The adult male and female of *Raoiella indica* [19].

Understanding species diversity, phylogenetic trends, and evolutionary processes require species molecular identification. Only accurate identifications allow for cross-study comparisons and the replication or expansion of previous experiments. Species identification is also critical in the development of (biological) pest control techniques for pest species.

This study aims to diagnose the red palm mite pest molecularly because of its importance and the damage it causes to the date palm crop in Iraq, this will contribute to the preparation of integrated plans to combat it and limit its spread.

2. Materials and methods

A. DNA Extraction

The DNA extraction process was carried out in the Genetic Engineering Laboratory of the College of Agriculture, University of Basra, and the extraction was done using a special extraction kit (Geneaid Korea, GS100), according to the company's equipped instructions, which include the following steps [20]:

1. The sample (about 25 mg of mite) was subjected to flash freezing using liquid nitrogen, and then ground using a small pestle to a fine powder.
2. The powder was transferred to a 1.5 ml Eppendorf tube, 200 µl of GST buffer and 20 µl of Proteinase K enzyme were added to it, then the mixture was shaken well using a Vortex vibrator for 25 seconds, then the samples were incubated in a water bath at a temperature 60 °C for 1 hour, with the sample manually stirred five times every 15 minutes.

3. To increase the efficiency of DNA collection in the seventh step, the Elution Buffer was incubated at a rate of 200 µl/sample in the water bath at 60° C until it was used at the end of the extraction.
4. After incubation, the samples were centrifuged at 10,000 rpm for 2 minutes, then the filtrate was transferred to a new 1.5 mL Eppendorf tube, and a 200 µl/sample of GSB buffer was added and vigorously shaken with a vibrator for 10 seconds.
5. Add 200 µl of absolute ethyl alcohol to each sample and stir gently for 10 seconds until fine strands (representing precipitated DNA strands) appear. The sample was transferred to a spin column (consisting of a filter tube and a 2-ml collection tube) and centrifuged. For 1 minute at 10,000 rpm, centrifuged again to ensure proper disposal of the precipitate, after which the collection tube was discarded and replaced with a new tube.
6. 400 µl of W1 was added, then the centrifugation process was carried out at a speed of 10000 rpm for 1 min. minutes, then the filtrate was discarded and then the centrifugation process was repeated to dry the filter membrane, and the collection tube was replaced with a new Eppendorf tube.
7. A Hundred microliters of the Elution Buffer (which was previously incubated at 60°C in the third step above) was added to the sample, it was added to the center of the filter disc and left for a minute so that the filter disc saturated with the buffer to increase the extraction efficiency, then the sample was centrifuged at 10,000 rpm for 1 minute to collect the extracted DNA, the sample was kept at -20°C until use.

B. Estimation of DNA Quality and Quantity of Genomic DNA

The quantity and quality of mtDNA for all samples extracted by the Nanodrop device equipped with the American company Thermo-Scientific. It was estimated at wavelengths 260-280 nm, to identify the quantity and purity of the extracted DNA for use in subsequent experiments [20].

C. Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was conducted to amplify the COXI-COXII gene region in the mitochondrial genome. The pair of primers detailed in Table (1) was used because this region has high heterogeneity between species and high stability within one species in arthropods in general. The primers were prepared by the Korean company Macorogen and were prepared at a concentration of 10 picomoles/microliter, according to the manufacturer's instructions to be ready for use.
The PCR reaction was performed using a reaction mixture of 50 µl of the reaction components, 25 µl of the prepared reaction mixture (RED, Amplicon, Denmark, Taq DNA Polymerase Master Mix), 2.5 µl of each primer, and 100 ng of template DNA, then full size to 50 microliters using DD-Water and amplification using a thermal cycler (Techne TC-3000X, UK) according to the thermal cycling program mentioned in Table (2).

Table (1) Primers used in the PCR polymerase chain reaction.

Primer	Nitrogenous base sequence 5' to 3'	Annealing Temperature Tm	Expected piece size (bp)	Reference
LCO1490	GGTCAACAAATCATAAAGATATTGG	54.80	~710	[20]
HC02198	TAAACTTAGGGTGAAAAAAATCA	59.62		

Table (2): Thermal cycling program for PCR reactions for the CO1-CO11 genomic region.

Primer	Stages	Temperature C°	Time (minutes)	Number of cycles	References
LCO1490/ HC02198	Initial denaturation	94	4	1	[20]
	Denaturation	94	0.5	35	
	Annealing	57	1		
	Extension	72	1		
	Repeat	2-4			
	Terminal extension	72	10	1	
	Storage	4		∞	

PCR Technique Relay for the Product

To ensure the success of the amplification process for the targeted gene segments, electrophoresis of the PCR reaction product was carried out on a (2%) agarose gel at a voltage of 95 mV, and 1X TAE solution was used as an electrolyte buffer to conduct the migration process, which was carried out in a migration tank (Cleaver Scientific MultiSUB electrophoresis unit ev222, England). (Promega, USA) Diamond Dye at X1 concentration was used to stain the samples and the DNA molecular weight index (Ladder (100 bp), after migration, the samples were detected using Gel documentation (Cleaver "Clear view UVT254/312, England).

D. PCR product reading of the nitrogenous base sequences (DNA sequencing)

After confirming the success of the amplification of the targeted gene segments by electrophoresis, the samples (PCR product) were sent to the Korean company Macro gene to read the sequences of nitrogen bases for each sample.

E. Molecular identification of samples

The results of the readings of the nitrogenous base sequences of the target gene segments located within the COX1-COX11 genome in the mitochondrial genome of the red palm mite samples used

in this study were analyzed after they were processed using the Chromas Ver program. 2.6.6 (Technelysium Pty, Ltd). These sequences were matched with copies of sequences deposited at the US National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST), and the diagnosis was made. Depending on the Maximum score, the degree of coverage for the sequencing of the sample bases, the Query cover, and the identity percentage provided by the research tool, where the highest rates of the above-mentioned indicators were adopted to confirm the diagnosis of samples to the gender level [20].

After confirming the diagnosis, the samples were deposited and registered in the NCBI gene bank.

3. Results and discussion

The results of the electrophoresis (Figure 3) of the PCR product showed the appearance of three clear bands with a size of about 750 base pairs, confirming the amplification of the COX1-COX2 target region. The molecular diagnostics results also confirmed that the diagnosed samples (KLA1-KLA3) belong to the species *Raoiella indica* and were recorded in The US National Center for Biotechnology Information NCBI, according to the registration numbers (ON386172.1 - ON386174.1), respectively (Table 3). These results are compatible with the results of the phenotypic diagnosis of the same samples, and thus the diagnosis process for the mite under study was confirmed.

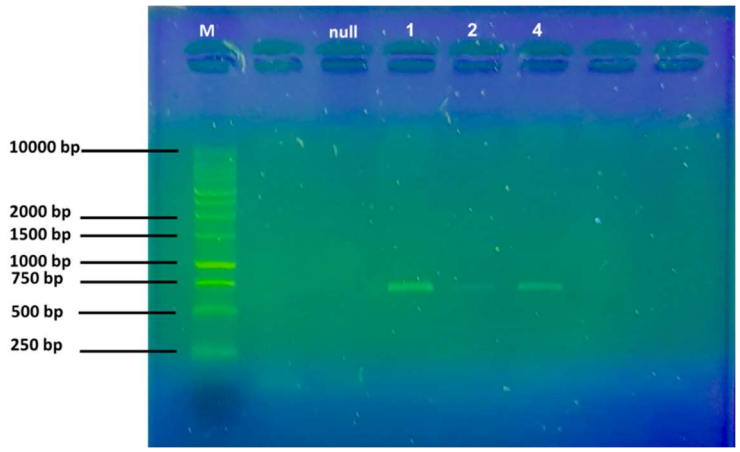

Figure 3: The PCR product electrophoresis results for *Raoiella indica* red palm mite samples.

Table 3: Molecular diagnosis of red palm mite samples (*Raoiella indica*).

The isolate name	The scientific name	NCBI Registration Number
KLA1	*Raoiella indica*	ON386172.1
KLA2	*Raoiella indica*	ON386173.1
KLA3	*Raoiella indica*	ON386174.1

Several of the COI sequences discovered in this investigation were identical to sequences previously in the GenBank collection.

The discovery of natural groupings in phylogenetic trees that represent biological species is one use of phylogenetic analysis (DNA taxonomy). Our most inclusive assemblage of data shows that the samples that were examined belong to the genus and species of the red palm mite *Raoiella indica* (Figure 4).

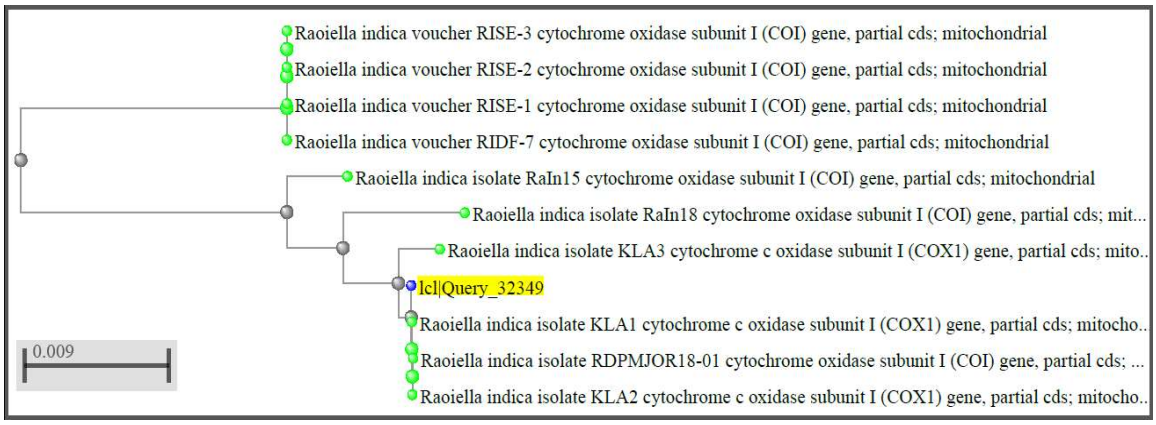

Figure 4: Maximum likelihood tree of the genus *Raoiella indica* based upon COI sequences. GenBank accession numbers and associated species name are given (see text) and the details are found here https://www.ncbi.nlm.nih.gov/nuccore/ON386174

The ability to comprehend and interpret evolutionary processes and ecological variety in mites depends on accurate species delineation and identification.

Mites are also a difficult group to identify morphologically, as many key traits exhibit high phenotypic plasticity and lack suitable identification characters.

Many ecological, behavioral, genetic, and pest-control research on diverse tetranychid species or strains have been carried out without the storage of voucher specimens, confusing the identity of the specimens under investigation.

In many circumstances, DNA barcoding can be a useful and effective method for species identification [21].

The use of a single (mitochondrial) gene for DNA barcoding or DNA taxonomy, on the other hand, appears to be inappropriate.

An integrative method incorporating nuclear and mitochondrial genes, morphological features, and ecological data (including, if possible, crossing studies) is required.

To circumvent the problem that gene trees are not always coherent with species trees and to detect hybridization, a combined analysis of mitochondrial and nuclear markers is widely used.

Finding adequate nuclear markers and strong geographic sample strategies that allow for intra- and interspecific variation evaluation is the problem.

The usefulness of several molecular markers has been studied by [22] and [23], although nuclear markers suitable for discriminating closely related species are still needed.
The D1-D2 region of the nuclear 28S rDNA gene has recently been proposed as a taxonomic identifier by [24]. It could be used to supplement mitochondrial DNA sequence-based DNA barcoding research.
In addition, to distinguish biological species, genetic testing for reproductive parasites and crossing tests utilizing iso-female lines should be normal procedures.

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