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First record of Wax Scale Insect *Ceroplastic rusci fayhaa* (Linnaeus 1753) (Hemiptera: Coccoidea: Coccidae) in Baghdad, Iraq

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Abstract

The insect has been diagnosed and named by the National Center for Biotechnology Information (NCBI), USA as Wax Scale Insect *Ceroplastic rustic fayhaa* (Linnaeus 1753) (Insecta: Hemiptera: Coccoidea: Coccidae). The diagnosis was made based on DNA analysis with a sequence similarity of 97% for the species *Ceroplastic rusci* (Linnaeus1753), in this study. It was recorded as a new insect pest on *Myrtus communis* (L. 1753), (Myrtaceae). DNA analysis confirms that it was first recorded in Iraq. These insects were initially observed during April 2023 in the gardens of the College of Basic Education, Mustansiriyah University, in the Sabaa Abkar area in Baghdad / Iraq.

Keywords: Wax Scale Insect, *Ceroplastic rusci fayhaa,* Biotechnology, Linnaeus 1753, DNA analysis

Introduction

Fig trees are among the most widespread fruit crops in the world, and pests including the *Ceroplastic rusci* (L) are among the factors that affect the low production of this fruit due to the nature of its nutrition. And the damage it causes to the parts and fruits of the tree (1,2). It is one of the main pests on fig trees and other fruit trees. *Ceroplastic r.*(L). It spreads greatly in some areas of northern the world and the installation of other fruits. Like some citrus fruits, apples, some forest trees, ornamental shrubs, and shrubs (3,4). This is also why, just repentance of the fig trees, because they nourish prophecy and their secretion of the honest symposium, which fall on the

parts of the trees and planted prophets are carried out and bring about a collection of dust and development of posts upon them. It caused severe tree injury, fracture death, and tree weakening per year (5,6). Phase dispersal second instar is considered the factor that is to break the important issues to transfer the mouth from one orchard to another orchard between logic through the transfer of methods and their injuries the purpose of multiplication and the establishment of the new orchards (7,8). Many attempts have been made to use different control methods to control pests, but chemical pesticides remain the most widely used method against scale insects of all kinds., despite their uselessness in many cases and their role in affecting the environment and public health (9,10). Mineral oils were among the substances that gave positive results for controlling various types of insects on a large scale on various crops (11,12). Insecticides belonging to the group of neonicotinoids, gave satisfactory results in controlling scale insects and mealybugs (13,14). He also introduced the growth regulator Applaud, one of the newer substances with a specialized effect on Hemiptera insects (15,16). It affects entire roles that are prevented from developing and accessing themselves. It also influences languages and causes the creation of possessions, thus the abundance that makes them unfulfilled (17,18,19).

Material and Methods

DNA was extracted from the insect using a standard DNA extracted from Conda / USA, Intron / Korea, Intron / Korea, Intron / Korea, Intron / Korea, Conda / USA, Integrated DNA technologies /USA, intron biotechnology/Korea companies, as shown in Tables 1 and 2.

Table 1. The Materials of DNA Extraction from Insects

	Material	Cat #	company
1	Agarose	8100.11	Conda / USA
2	Red safe staining solution	21141	Intron / Korea
3	6X Loading dye	21161	Intron / Korea
4	Ladder 100 bp	24073	Intron / Korea
5	Premix pcr	25025	Intron / Korea
6	TBE buffer 10 X	IBS.BT004	Conda / USA
7	Primer	---	Integrated DNA technologies /USA
8	G- spin DNA extraction kit	17045	intron biotechnology/Korea

Table 2. List of apparatus used in DNA extraction from Insects.

Company	Origin	Apparatus	No
Euro Clone	Italy	AURA TM PCR Cabinet	1
Bio San	Germany	Micro spin 12, High-speed Mini-centrifuge	2
Dig system	Germany	V-1 plus, Personal Vortex for tubes	3
Bio San	Germany	Bio TDB-100, Dry block thermostat built	4
Lab net	Germany	Biopette Variable Volume 2-20 µl 0.5-10 µl ,20-200 µl ,10-100 µl ,100-1000 µl	5
Supplier	Chain	Mini-Power Supply 300V, 2200V	6
Lab net	USA	Multi Gene Opti Max Gradient Thermal Cycler	7

CBS, Scientific	USA	Electrophoreses	8
Lab net	USA	Document system	9
Vilber lourmat	France	UV. transmission	10
Bio san	Lativa	Micro spin	11
Bio san	Lative	Combi-spin	12
Kernpfb	Germany	Balance	13
Jrad	China	Incubation	14
Gosonic	China	Microwave	15
Rovsun	China	Water distilater	16
Nabi	Korea	Nanodrop	17

DNA extraction from insects

G-spin DNA extraction kit, intron biotechnology, cat no. 17045

Kit Contents

Protocol:

-Measure 25 mg of the ground tissue sample, then transfer it to a 1.5 mL tube using a spatula.

-Add 200 µL of CL buffer, 20 µL of Proteinase K, and 5 µL of RNase A solution into the sample tube and mix by rotating vigorously (Table 3).

- Incubate the lysate at 56 °C (heated thermal block or water bath) for 10

30 min.

-After completely lysing, add 200 µL of Buffer BL into the top sample tube and mix well. Then incubate the mixture at 70 for 5 minutes.

- Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles. Then, carefully transfer 350 ~ 400 µL of the supernatant into a new 1.5 mL tube (not provided).

- Add 200 µl of absolute ethanol into the lysate, mixing well by gently agitating 5 - 6 times or by pipetting. do not swirl. After mixing, briefly centrifuge the 1.5 mL tube to remove drops from the inside of the cap.

-Apply the mixture from step 6 to the spin column (in a 2 mL collection tube) without wetting the tip, close the cap, and centrifuge the concentrate at 13,000 rpm for 1 min. one. Discard the filter and place the spin column in a 2 mL collection tube (reuse).

- Add 700 µL of buffer into the spin column without wetting the tip, and centrifuge for 1 min at 13,000 rpm. Discard the flow and reuse the collection tube.

Add 700 µL of buffer WB to a spin column without wetting the tip, and centrifuge for 1 min at 13,000 rpm. Discard the flow and place the column in a 2.0 ml collection tube (reuse), then centrifuge again for one minute to dry the membrane. Discard the flow-through and collection tube completely.

- Place the spin column in a new 1.5 mL tube (not provided), and 30–100 µL of CE buffer directly on the membrane. Incubate for 1 minute at room temperature and then centrifuge for 1 minute at 13,000 rpm to elute.

Table 3. The types of Buffers

Label	Contents 50 Columns
Buffer CL	25 ml
Buffer BL	25 ml

Buffer WA	**40 ml**
Buffer WB	**10 ml**
Buffer CE	**20 ml**
Spin Column / Collection Tube	**50 ea**
RNase A (Lyophilized powder)	**3 mg x 1 vial**
Proteinase K (Lyophilized powder)	**g x 1 vial**

Agarose gel electrophoresis of DNA:

Electrophoresis was performed to identify DNA fragments after the extraction process or to detect the result of the PCR reaction while a DNA standard was present to discriminate the bundle size of the result of the PCR reaction on an agarose gel.

Agarose gel preparation:

According to (20), an agarose gel at a 1.5% condensation was made by dissolving 1.5 g of agarose in 100 mL of a pre-made TBE solution. The agarose is heated to a boil and then allowed to cool at (45-50 °C). The gel was poured into the casting plate where the agarose support plate was prepared after fixing the comb to make holes that would hold the samples. Pour the gel gently so that it does not form air bubbles and leave for 30 minutes to cool. The comb is gently removed from solid agarose. The plate was fixed to its bracket in the Electrophoresis horizontal unit represented by the tank used for Electrophoresis. The reservoir is filled with TBE buffer which covers the surface of the gel.

Sample preparation:

3 µl of processor loading buffer (intron/Korea) was mixed with 5 µl of DNA presumed to be electrophoresis Fig. (1) (loading dye), after mixing the loading process is now to the holes of the gel. An electric current of 7 V\C2 was exposed for 1–2 hours until the dye reached the other side of the gel. The gel was tested by a UV light source with 336 nm after the gel was placed in the pool on 30 µl safe nucleic acid staining solution and 500 ml of distilled water.

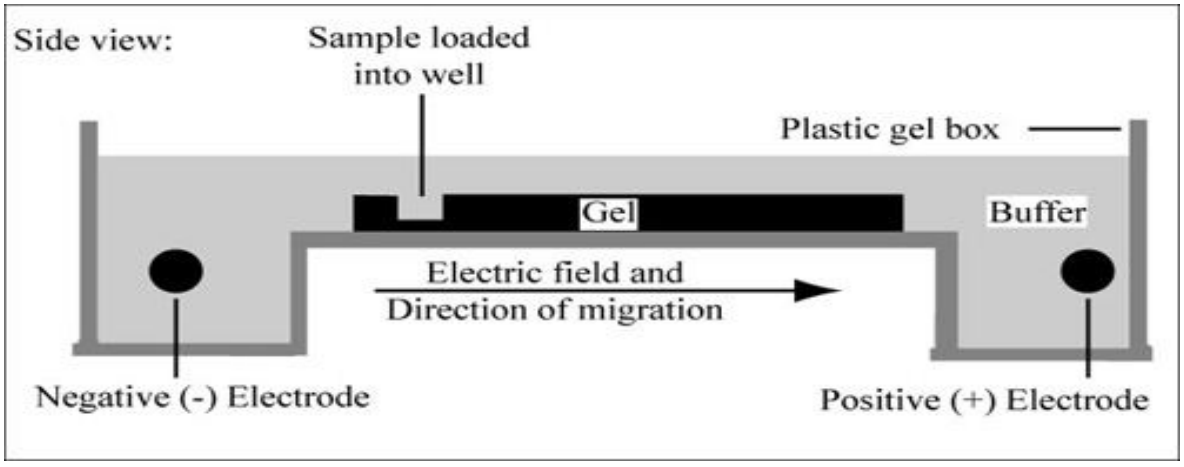

Figure .1 Working the electrophoresis system

Nucleic Acid Red Safe staining solution

Nucleic Acid Red Safe stain solution (20,000x) is a nucleic acid safe stain, an alternative to conventional ethidium bromide (ETBR) stains for the detection of nucleic acid in agarose gels. It emits a green fluorescence when binding to DNA or RNA. This new spot contains two fluorescence excitations when bound to nucleic acid, one positioned at 309nm and another at 419nm. In addition, it has a single visible excitation at 514 nm. The fluorescence emission from Red Safe BOON to DNA is centered at 537 nm. DNA staining solution red (20,000×) is as sensitive as ETBR. The protocol for Red Safe (20,000×) nucleic acid staining solution is similar to that for ETBR. Compared to ETBR, which is known to be a strong mutagenic, the Safe Red (20,000x) nucleic acid staining solution results in significantly fewer mutations in the AMES test. In addition, the DNA-safe staining solution (20,000×) had a negative result in the mouse medullary chromosome erythrocyte assay and the sperm chromosomal aberration test. It is therefore wise to choose Safe Red (20,000×) DNA staining solution instead of ETBR for the detection of DNA in agarose gels. (cat no. 21141).

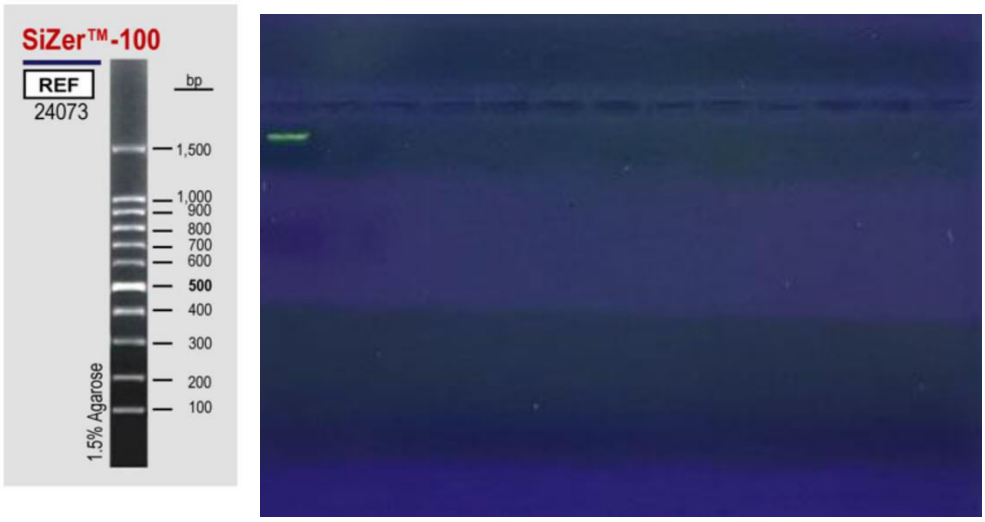

Figure |2: Gel electrophoresis of genomic DNA extraction from insect, 1% agarose gel at 5 vol /cm for 30 min. and in the left SiZer DNA Markers (intron)

DNA Nano Droplet

If you are using a Nano Dropper to measure your samples, place 1-2µL of DNA Nano-droplets on a pedestal 3. Close the cap and click Measure, make sure to record concentration and purity Fig 3.

Note: Purity is measured under a column 260/280 (good clarity ranges from 1.80-2.00). Repeat for each sample (Table 4).

Figure 3. Nano drop (Nabi / Korea)

Primers used in the reaction

The primers (Table 4) were lyophilized, and dissolved in free ddHO to give a final concentration of 100 p mol/µl as a stock solution and the stock was kept at -20. To prepare a 10 mol/µl concentration with a working suspension of the primer, 10 were screened µL of stock solution in 90 µL of free ddH2O water to reach a final volume of 100 µL, by IDT (Integrated DNA Technologies, Canada).

Table 4. The specific primer 28S of gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	**5'- GAG AGT TMA ASA GTACGT GAA AC- 3'**	**52.3**	**44**	**700-860 base pair**
Reverse	**5'- TCG GAR GGAACC AGC TAC TA - 3'**	**56**	**42**	

The optimal condition (Initial denaturation and annealing) After doing several experiments to acquire this condition, the temperature was changed through the work of (gradient PCR) for all samples to determine the optimal condition, and the concentration also changed the DNA template between (1.5-2µL) where these two factors are considered among the factors Important factors in primer annealing with complement. (Fig. 4).

Score	Expect	Identities	Gaps	Strand
1070 bits(579)	**0.0**	**621/642(97%)**	**0/642 (0%)**	**Plus/ Plus**

Query 1
ATCCGGGCGCGCGGAATTCAGGACCGATCGGCGGACGTTCGCGTCTCGTCGGTCGAC
TCG 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 32
ATCCGGGCGCGCGGAATTCAGGACCGATCGGCGGACGTTCGCGTCTCGTCGGTCGAC
TCG 91

Query 61
ATATTTCCGTCGCCGACCCTCAACCGAGGGTGTCGGACGTCGCGACCCGTTCGCCATT
TT 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 92
ATATTTCCGTCGCCGACCCTCAACCGAGGGTGTCGGACGTCGCGACCCGTTCGCCATT
TT 151

Query 121
GTCGATGCTCAGGGACGCGTCGCCAGTTCGCCAGCGCCGTCGCCTCGGCGGCG**C**CGC
GA**T** 180
|||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct 152
GTCGATGCTCAGGGACGCGTCGCCAGTTCGCCAGCGCCGTCGCCTCGGCGGCG**G**CGC
GA**C** 211

Query 181
GATGGC**C**CGTTTCCGTTGTTTTCGACGCGGACGGTAGCTTTGCGCG**G**GGGTATTCGAC
GC 240
|||||| |||||||||||||||||||||||||||||||| ||||||||||||
Sbjct 212
GATGGC**G**CGTTTCCGTTGTTTTCGACGCGGACGGTAGCTTTGCGCG**C**GGGTATTCGAC
GC 271

Query 241
GGCCCACTCGTTCGGCGTCAC**G**TCGG**A**GGC**T**TCGTGC**TC**GCGCTCGCGTCGACGCCG
GTC 300
|||||||||||||||||| |||| ||| ||| || ||||||||||||||||||
Sbjct 272
GGCCCACTCGTTCGGCGTCAC**T**TCGG**T**GGC**G**TCGGGC**GG**GCGCTCGCGTCGACGCCG
GTC 331

Query 301
AGCGACGAATCTTCGGGCCTCTTTCCGACCCGTCTTGAAACACGGACCA**G**GGAGTCT
AGC 360

```
          |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
Sbjct                                                                 332
AGCGACGAATCTTCGGGCCTCTTTCCGACCCGTCTTGAAACACGGACCAAGGAGTCT
AGC  391

Query                                                                 361
GTGCGCGCGATTCGCGTGGTATCCGAGCCGTCGTAGTCGCGTTTCGGCGCGACAAAC
GCG  420
          ||||||||| ||||| |||||||||||||||||||||||||||| | ||||||
Sbjct                                                                 392
GTGCGCGCGAGTCGCGGGGTATCCGAGCCGTCGTAGTCGCGTTTCGGCGCGGCGAAC
GCG  451

Query                                                                 421
TCTCGACACGATACGAAACCCGTGTACGTACGTTGTGCGAAGGCGTAATGAAAGTGA
AAG  480
           |||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                 452
GCTCGTCACGATACGAAACCCGTGTACGTACGTTGTGCGAAGGCGTAATGAAAGTGA
AAG  511

Query                                                                 481
GCGACGCTTCGAGCGTCGCCGGGGAAAGATGGCGTTCCGAGTTCACGTATCGCGGTC
GCA  540
          ||| ||||||| |||||||||||| |||||||||||||||||||||||||||||||
Sbjct                                                                 512
GCGGCGCTTCGCGCGTCGCCGGGGGAAGATGGCGTTCCGAGTTCACGTATCGCGGTC
GCA  571

Query                                                                 541
CGTGTACCGCGACCGGACGCCCGCATTCCCAGGGCGTCTCGCTCGTACGGCCGTGGC
CGG  600
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct                                                                 572
CGTGTACCGCGACCGGACGCCCGCATTCCCAGGGCGTCTCGCTCGTACGGCCGTGGC
CGG  631

Query  601  GGTTCGCTTCGGTCGCGGACAAACTGAGGCGCACCTGCAGCG  642
             |||||||||||||||| ||||||||||||||||||
Sbjct  632  GGTTCGCTTCGGTCGCGGACGAACTGAGGCGCACCTGCAGCG  673
```

Figure 4. D1_DF 642 *Ceroplastes rusci* isolate S6A180a large subunit ribosomal RNA gene, partial Sequence ID: MT317013.1 Length: 760 Number of Matches: 1, Range 1: 32 to 673.

Maxim PCR Pre Mix Kit (I-TAQ) 20µlrxn (Cat. No. 25025)

INTRON's *Maxim* PCR Pre Mix Kit contains not only different types of Research Pre Mix Kit but also the 2x Master Mix solution. Maxim PCR PRE MIX KIT (I-TAQ) is the completely mixed product: I-TAQ DNA mixture, DNTP mixture, buffer, and so on in one tube for 1 RXN PCR. This is the place to get the best result with the comfort system. The first reason is that it has an internal electric drive, so we can get the process done. The method is suitable for the sample from the quick methods and the simple method (Table. 5,6).

Table 5.: The Components of the Maxim PCR Pre Mix kit (i-Taq)

Material	**Volume**
i-Taq DNA Polymerase	**5U/µl**
DNTPs	**2.5mM**
Reaction buffer (10X)	**1X**
Gel loading buffer	**1X**
Components	**Concentration**
Taq PCR Pre Mix	**5µl**
Forward primer	**10 picomols/µl (1 µl)**
Reverse primer	**10 picomols/µl (1 µl)**
DNA	**2µl**
Distill water	**16 µl**
Final volume	**25µl**

Table 6.: Mixture of the specific interaction for diagnosis gene

Gene Diagnosis

Sequencing by ABI-310 Genetic Analyzer System

Gel Extraction Protocol (Sequencing)

Add absolute ethanol to the wash buffer before initial use. The DNA gel extraction step is as follows (21):

1- Gel dissociation

• Absorb a silica agarose gel containing relevant DNA fragments and remove any additional agarose to reduce the size of the silica gel.

• Transfer up to 300 mg of silica gel into a 1.5 mL micro centrifuge tube.

• Add 500µL of DF buffer to the sample and mix by Vortex.

- Incubate at 55-60°C for 10-15 minutes or until the silica gel is completely dissolved. During the incubation, turn the tube every 2-3 minutes.
- Cool the dissolved sample mixture to room temperature.
- 2- DNA binding
- Place the DF column in a 2 mL collection tube.
- Transfer 800µL of the sample mixture from (Step1) to the DF column.
- Centrifuge at 14-16,000 x g for 30 sec.
- Discard the flow and place the DF column back into the 2 mL collection tube.

3- Wash

- Add 600µl of Wash Buffer into the DF Column and let stand for 1 minute.
- Centrifuge at 14-16000×g for 30 seconds and then discard the flow-through.
- Place the DF Column back in the 2 ml Collection tube.
- Add 600µl of Wash buffer into the DF Column and let stand for 1 minute.
- Centrifuge at 14-16000×g for 30 seconds and then discard the flow-through.

- Place the DF Column back in the 2ml Collection tube.
- Centrifuge 14-16000×g again for 3 minutes to dry the column matrix.

4- DNA Elution

- Transfer the dried DF Column to a new 1.5 ml micro centrifuge tube.
- Add 20-50µl of Elution Buffer into the center of the column matrix.
- Let stand for 2 minutes the Elution Buffer is absorbed by the matrix.
- Centrifuge for 2 minutes at 14-16000×g to elute the purified DNA

Sequencing and Sequencing Alignment

PCR products were separated on a 2% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after ethidium bromide or blot red staining. Gene sequencing was performed by Microgen Korea, homology search was performed using the Basic Local Search Tool (Blast) software available at the National Center for Biotechnology (NCBI) online at (http://www.ncbi.nlm.nih.gov) and vital editing software. PCR products were purified and sequenced in both directions using the Big Dye Terminator V3.1 cycle sequencing kit on an ABI 3130 Genetic Analyzer (Applied Biosystem, Foster City, CA). The sequence is analyzed in the nucleotide databases using the NCBI's Basic Local Alignment Search Tool Bio ID program to search for local NCBI to determine the sample and present it to Gen Bank (ID). Related serials from the sample or S from the NCBI database (www.ncbi.nlm.gov/nucleotide) were obtained and included in multiple alignments using Bio ID program (22).

E-Value and Score

The expectation value is defined to give an estimate of how often you would expect to get the same correct similarity and the decrease in E-value. This indicates that the similarity score was high among the sequences which gives greater confidence. A value very close to zero means that these sequences are identical and the bit score: the statistical measure of moral similarity, a higher value indicates a high similarity score, and if dropped from the 50-point category no similarity is stated.

Phylogenetic tree

The evolutionary date was inferred using the way to join the neighborhood. Evolutionary distances have been calculated using the Jukes-Cantor model to determine the distance of the emergence and development designed in the Gene6 programmer.

NCBI Submission:

Submission of the local Iraq isolate or sample in NCBI.

All samples Where possible near the full length of a 16S rRNA gene, ITS4, or other gene sequence, we selected one sample to be obtained (??? BP) favorably as entries sequencing. NCBI houses a series of biotechnology and bio field-related databases and is an important resource for bioinformatics tools and services. Major databases include the Gen Bank for DNA sequences and PubMed, a bibliographic database of biomedical literature. Other databases include the NCBI Epigenomics database. All these databases are available online through the Entrez search engine. NCBI was directed by David Lipman, [2] one of the original authors of the Blast sequence program [3] and a widely respected figure in bioinformatics. The ongoing work will add to this group where more species are published and are available for download in NCBI: https://www.ncbi.nlm.nih

Basic Local Search Tool (Blast)

The National Center for Biotechnology Information (NCBI) [1][2] is part of the United States National Library of Medicine (NLM), a division of the National Institutes of Health (NIH). It is approved and funded by the US government. NCBI is located in Bethesda, Maryland, and was founded in 1988 through legislation sponsored by Senator Claude Pepper.

Results and Discussion:

Sequencing and Sequence Alignment

Coccidae (wax scale insect) is one of the largest families of scale. Wax scale, as the common name suggests, is phytophagous and feeds on leaves and other parts of plants. Adults of most species lack masters and usually use terrestrial species (23). These results indicate large regularities in the pattern of aggregation variation across all pedigree ages that are best explained by the permanent action of mutation and dispersal processes. Thus, multi-hierarchical analysis embodies the predictions of the neutral theory of molecular evolution and the neutral theory of biodiversity. Neutral processes thus emerge as a unifying principle of ecology and evolution, which has profound implications for the assessment and conservation of biodiversity. The DNA extraction kit (DNA Biotechnology/Korea) was chosen for the DNA extraction. The method is because manual protocols are very slow. The other reason is that conventional methods such as phenol-chloroform may cause contamination of the obtained DNA. On the other hand, the automated method is quite expensive and requires special tools. In this study, isolation of the required region of the 28s gene from C. rusci mitochondrion DNA was carried out. (L. 1753), (Myrtaceae). These insects were initially observed in April 2023 in the gardens of the College of Basic Education, Mustansiriyah University, in the Sabaa Abkar district of Baghdad / Iraq. The results shown in Figures 4, 5, and 6 are indicated together with Tables 7 and 8 indicated that a yield of one band of the desired product with a molecular weight of 800 bp was obtained for the C. rusci fayhaa 28s gene from adult C. rusci fayhaa. Sequencing results are similar to those of C. rusci fayhaa S6A180A cytochrome oxidase subunit 1 28S coupon, partial CDs; Mitochondrial Sequence ID: MT317013.1 Length: 760 Number of Matches: 1 Range 1: 760 Number of Matches: 1, Range 1: 32 to 673. (24), These findings are consistent with those of a previous study (25) on mitochondrial DNA heteroplasmy in adult *C. rusci*. Sequencing of the amplified product of the *C. rusci* 28S gene. Table 7 shows that the results from 10 adult C. rusci samples showed a 97% concordance with that of the standard 28S gene from C. rusci that was recorded. Multiple hierarchical levels representing haplotype lineages of different ages showed a similar rate of distance decay for clustering similarity. In addition, we found strong logarithmic significant correlations between the hierarchical level (age of the lineage), the number of lineages, the size of the pedigree range, and grouping similarity. Similarity at the species level was closely related to similarity at the haplotype level for the entire assemblage.

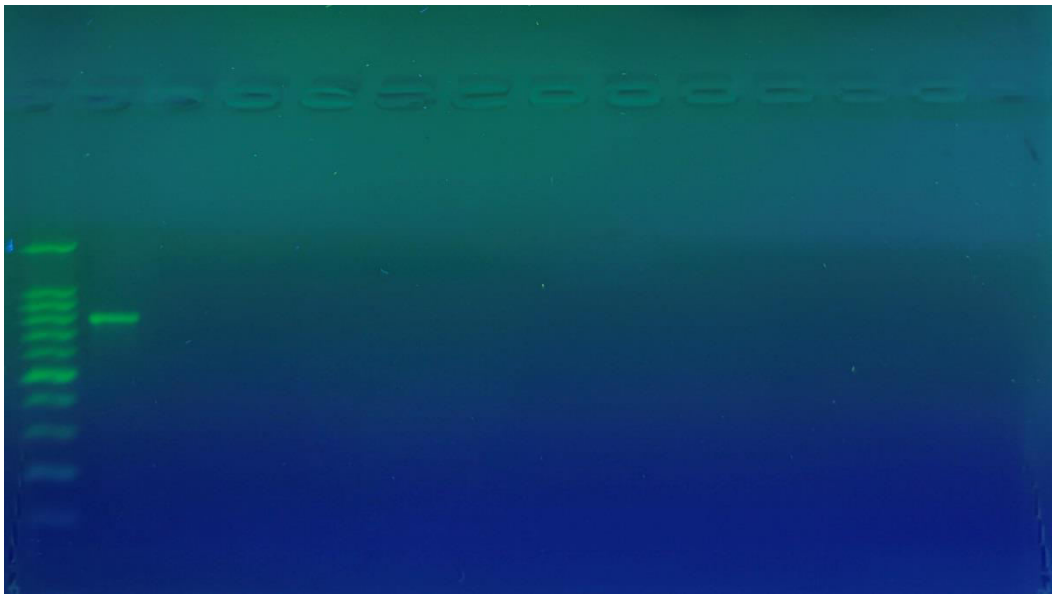

Figure (5) PCR product the band size 800 bp. The product was electrophoresis on 2 % agarose at 5 volt/cm^2. 1x TBE buffer for 1 hour. N: DNA ladder (100).

LOCUS MT317013 738 bp DNA linear INV 14-APR-2020
DEFINITION Ceroplastes rustic isolate S6A180a large subunit ribosomal RNA gene, partial sequence.
ACCESSION MT317013 REGION: 23..760
VERSION MT317013.1
KEYWORDS .
SOURCE Ceroplastes rusci
ORGANISM Ceroplastes rusci
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Paraneoptera; Hemiptera; Sternorrhyncha; Coccoidea; Coccidae; Ceroplastes.
REFERENCE 1 (bases 1 to 738)
AUTHORS Li,Y.
TITLE Molecular Identification and Phylogenetic Studies of Coccidae
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 738)
AUTHORS Li,Y.
TITLE Direct Submission
JOURNAL Submitted (05-APR-2020) The College of Forestry, Beijing Forestry University, No. 35 Qinghua East Road, Haidian District., beijing 100083, China
COMMENT ##Assembly-Data-START##
Sequencing Technology: Sanger dideoxy sequencing
##Assembly-Data-END##
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/isolate="S6A180a"
/db_xref="taxon:1285730"
rRNA <1..>738
/product="large subunit ribosomal RNA"
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61 gtcgactcga tatttccgtc gccgaccctc aaccgagggt gtcggacgtc gcgacccgtt
121 cgccattttg tcgatgctca gggacgcgtc gccagttcgc cagcgccgtc gcctcggcgg
181 cggcgcgacg atggcgcgtt tccgttgttt tcgacgcgga cggtagcttt gcgcgcgggt
241 attcgacgcg gcccactcgt tcggcgtcac ttcggtggcg tcgggcgggc gctcgcgtcg
301 acgccggtca gcgacgaatc ttcgggcctc tttccgaccc gtcttgaaac acggaccaag
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481 aagtgaaagg cggcgcttcg cgcgtcgccg ggggaagatg gcgttccgag ttcacgtatc
541 gcggtcgcac gtgtaccgcg accggacgcc cgcattccca gggcgtctcg ctcgtacggc
601 cgtggccggg gttcgcttcg gtcgcggacg aactgaggcg cacctgcagc gtgcacgctg
661 gtacccgaaa gatggtgaac tatgcccggc caggatgaag tcaggggaaa ccctgatgga
721 ggtccgcagc gattctga

Figure 6. Ceroplastes rusci isolate S6A180a large subunit ribosomal RNA gene, partial sequence
Gen Bank: MT317013.1

Table 7: The optimum condition of detection

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94°C	5 min.	1 cycle
2-	Denaturation -2	94°C	45sec	35 cycle
3-	Annealing	58°C	45sec	
4-	Extension-1	72°C	45sec	
5-	Extension -2	72°C	7 min.	1 cycle
Lab number	Student number	DNA Result	28S/ 700-860bp	
1	1	+	+	

Table 8. Sequencing ID in *Ceroplastic rusci fayhaa* (Linnaeus 1753) gene, score, expects and compatibility of DNA sequences obtained

Homo sapiens						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transversion Transition Transversion	205 211 252	C/G T/C G/C	ID: MT317013.1	*Ceroplastes rusci* isolate S6A180a large subunit ribosomal RNA gene,	97%

	Transversion	293	G/T
	Transversion	298	A/T
	Transversion	302	T/G
	Transversion	307	T/G
	Transversion	310	T/G
	Transversion	311	C/G
	Transition	381	A/G
	Transversion	402	T/G
	Transversion	408	T/G
	Transition	443	A/G
	Transition	445	A/G
	Transversion	452	T/G
	Transversion	457	A/T
	Transition	515	A/G
	Transition	523	A/G
	Transition	536	A/G
	Transition	652	A/G

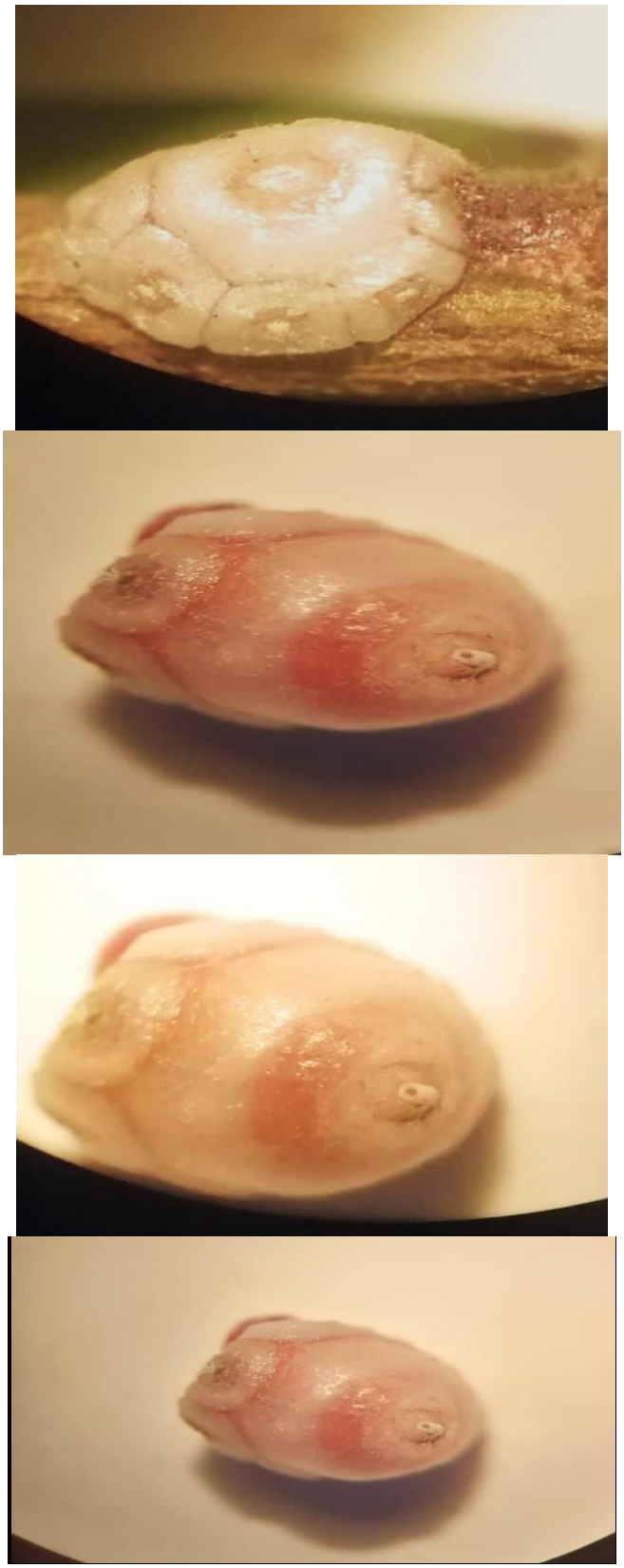

Figure 6. Adult and larvae of *Ceroplastic rusci fayhaa* (Linnaeus 1753) (Hemiptera: Coccidae) (125X).

Among the species belonging to this genus, *Ceroplastic Rusci* (Linnaeus, 1758), a wax scale, is of enormous economic importance with a wide range of host species around the world (26). *Ceroplastes rusci* is one of the first species reported from the Mediterranean region, and it has been known since the OPHRSTUS, i.e. 370-285 BC (27). In the first place, it is its home in the African region (28) and it has also been reported from the air -speaking, paper, eastern, Ethiopian, and Australian regions (29,30). *Ceroplastes rusci* was reported for the first time in Britain from the fruits of Ficus Carica imported from Italy (31), and it has a wide range of host plants in Algeria, Cyprus, Egypt, Greece, Israel, Italy, Lebanon, Morocco, Spain, Tunisia, Turkey, and India (32). Aside from the shape, Nerium Oleander, Pistacia Lentscus and P. Terebinthus are their common host plants (33). About 100 percent of the plant injury by *C. rusci. In Annona muricata* 100 percent infestation with lead has been recorded (34). *C. rusci* infection of figs was recorded with about 500 nymphs per twig (35). In India, C. rusci has been reported to infect trees *Mangifera indica*, *Dalbergia sissoo*, and *Syzygium sp*. and *Ziziphus mauritania* and *Citrus sp*. and Ficus benghalensis, F. 36). *C. rusci* has also been reported as a vector of plant viruses (37,38) which reported 39 plant species hosting *C. rusci*. The findings of the workers demonstrate that *C. rusci* has a wide range of host plants worldwide. The current survey of host plant species and infestation intensity was carried out in Uttaranchal, India, after which a checklist of host plants in the world was prepared.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process
During the preparation of this work NO AI tools were used by the authors.

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