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The effect of environmental pollution on the American cockroach in Iraq for the genes COX1, COI LCO

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ABSTRACT

This research aims to determine the effect of environmental pollution on some genetic markers of the American cockroach by conducting molecular tests on the COX1 and COI LCO genes. The current study was conducted at Samarra University, College of Education/Laboratory of the Department of Life Sciences. Samples of adult American cockroaches were collected from six Iraqi cities: Duhok, Kirkuk, Samarra, Baghdad, Nasiriyah, and Basra, between September 2023 and September 2024. Three samples were taken from each city for molecular study. DNA was extracted, polymerase chain reaction (PCR) was performed, and the samples were sent for DNA sequencing analysis. The results showed 100% similarity between samples in the DNA analysis of the COX1 gene, indicating that this gene is suitable for studying convergence between species. However, the COI LCO gene showed interspecies differences and is suitable for studying environmental effects.

1. INTRODUCTION

Cockroaches are one of the most widespread insects in the world, comprising more than 5,000 species. They are considered urban pests that cause harm to humans, such as disease transmission and food spoilage. Cockroaches can live in all locations and environments. They can live in both hot and cold regions, but prefer warm environments. The American cockroach, one of the most widespread insects in the order of cockroaches, is known for its low mobility and is most active at night. In the dark, they cease movement and tend to remain dormant (Nguyen et al., 2020: Ademolu et al,2020). The American cockroach is one of the most widespread insects in Iraq. Originally from South America, it is 40 mm long, reddish-brown in color, and lives in warm areas such as kitchens, heating rooms, warehouses, and sewage systems. It emerges at night for feeding and other activities. Long-lived cockroaches live for more than a year and are among the most annoying household pests, causing damage to household materials and stored products. They are characterized by their mixed diet, feeding on plants, meat, and food scraps. The American cockroach has a very high capacity for reproduction and adapting to different environmental conditions (Zhao et al., 2022: Cerreta et al.,2022). The environment is constantly changing, and survival under these conditions is difficult, especially for organisms such as insects, which play a major role in the long-term dynamics of ecosystems. Therefore, their extinction or decline in numbers will impact the human food chain and the processes taking place in agricultural soil, affecting crop quality. There are also insect species, such as bees, whose life cycles are affected by temperature changes. Furthermore, human practices, such as the use of Excessive pesticides and chemicals affect insects, in addition to the effects of global warming (Barton et al., 2017: Dominic et al.,2023).

Genes provide the most important information about insect development, including mitochondrial DNA (mtDNA), which in the American cockroach consists of a double strand ranging in size from 14,903 to 19,513 base pairs. This gene has been classified as an important taxonomic tool. Among these genes is the COI gene, considered one of the most important genes used in insect classification. It has several types and is a common region across all organisms, but differs in the distribution of nitrogenous bases. It is widely used in insect classification to determine similarities between species (Shi et al., 2021; Ma et al., 2018).

The American cockroach feeds on many species and is widespread in all environments. Therefore, it contains proteins, heavy and light nutrients, and even heavy elements, including cobalt, zinc, and lead (Boate et al., 2021: Hamanaka et al.,2016). This study aims to know the effect of environmental pollution on the American cockroach by studying the genes COX1 and COI LCO.

DNA extraction

Measure 25 mg of ground tissue sample, and then transfer into 1.5 ml tube using a spatula,Add 200 µl Buffer CL, 20 µl Proteinase K and 5 µl RNase A Solution into sample tube and mix by vortexing vigorously ,Incubate the lysate at 56℃ (preheated heat block or water bath) for 10 ~ 30 min,After lysis completely, add 200 µl of Buffer BL into upper sample tube and mix thoroughly. Then incubate the mixture at 70℃ for 5min,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, and mix well by gently inverting 5 - 6 times or by pipetting. DO NOT vortex. After mixing, briefly centrifuge the 1.5 ml tube to remove drops from inside of the lid ,Carefully apply the mixture from step 6 to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuge at 13,000 rpm for 1 min. Discard the filtrate and place the Spin Column in a 2 ml Collection Tube (reuse) ,Add 700 µl of Buffer WA to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and reuse the Collection Tube ,Add 700 µl of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm. Discard the flow-through and place the Column into a 2.0 ml Collection Tube (reuse), Then again centrifuge for additionally 1 min to dry the membrane. Discard the flow-through and Collection Tube altogether,Place the Spin Column into a new 1.5 ml tube (not supplied), and 30 - 100 µl of Buffer CE directly onto the membrane. Incubate for 1 min at room temperature and then centrifuge for 1 min at 13,000 rpm to elute.

Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025)

iNtRON's *Maxime* PCR PreMix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. *Maxime* PCR Pre Mix Kit (*i*-Taq) is the product what is mixed every component: *i*-Taq DNA Polymerase, dNTP mixture, reaction buffer, and so on-in one tube for 1 rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has every components for PCR, so we can do PCR just add a template DNA, primer set, and D.W.. The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. It is suitable for various sample's experience by fast and simple using method.

Results and Discussion

Table (1)COX1 gene primers

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-GGAGCCCCAGATATAGCTTTCC - 3'	57	54.5	420 base pair
Reverse	5'-AAATTGGATCTCCCCCTCCTG - 3’	56.6	52.4	

Table (2)COI LCO gene primers

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-GGTCAACAAATCATAAAGATATTGG-3	55	54.5	658 base pair
Reverse	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	55	52.4	

Diagnosis of Gene

Figure (1) shows the results of the extracted DNA samples from the entire legs of the American cockroach, 17 samples from six different cities in Iraq. The first two samples were from the city of Samarra in central Iraq, three samples from the city of Baghdad in central Iraq, three samples from the city of Nasiriyah and Basra in southern Iraq, and three samples each from the cities of Kirkuk and Dohuk in northern Iraq. The samples showed the concentration of the extracted DNA (22.9, 6.8, 17.6, 7.9, 22.5, 11.4, 8.1, 19.5, 10.5, 13.6, 6.9, 11.8, 8.4, 7.3, 18.6, 6.0, 28.3). The samples were transferred on the electrophoresis device using agarose gel at a concentration of 1% and an electrical potential difference of 5 volts/cm2 for an hour. The gel was then photographed under ultraviolet rays (UV). The results showed that the bands resulting from the transfer process using a high amount of purity, if the purity ranged for all samples From 1.6 to 1.8, this is the acceptable value for performing a PCR for the COX, COI LCO genes. Only cockroach legs were used to obtain the best results and avoid the presence of proteins, fats, and sugars. Cockroach legs are characterized by low nutritional content, as well as hormones and digestive enzymes. They contain well-formed skeletal muscles, which facilitates the extraction process. The extracted samples were registered in the Global GenBank. The MTCOI gene is the most widely used gene in insect diagnosis because it has several different shapes and sizes. It can be used as a primary tool in differentiating between insect species, even within the same genus. If the difference between the American and German cockroaches is 25%, then the MTCOI gene is an important diagnostic tool in insects (Sharawis & Assagafa: 2021).

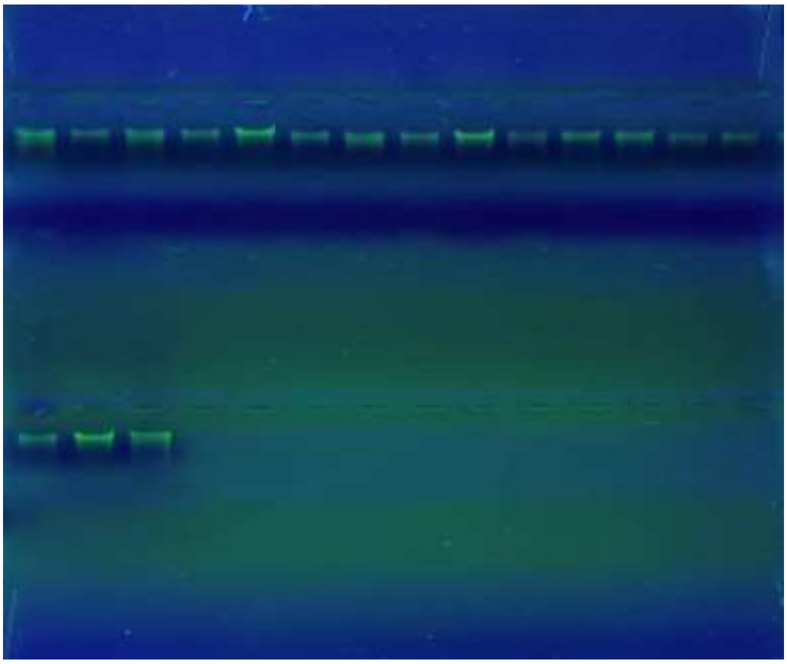

Figure (4) The result of DNA transfer

COX Gene Amplification

The results of the COX gene expression from mitochondrial DNA in the leg cells of the American cockroach were obtained from six samples collected from different cities in Iraq. PCR results were carried out using an electrophoresis device on a 1% agarose gel at a voltage of 5 V/cm² for an hour. The gel was then imaged under ultraviolet light. The results showed that the bands resulting from the PCR process, under the appropriate conditions for COX gene amplification, had a molecular weight of 420 base pairs, as shown in Figure (2). Gene 1. COX is the CYTCHROME C OXIDASE. Cytochrome C oxidase is the key enzyme in Complex IV of the electron transport chain (ETC) in mitochondria. It plays a crucial role in energy production within living cells(Yin et al,2022; Cox,2021; Wang et al,2023).

Especially in aerial organisms such as flying insects and humans, COX is a multi-component protein complex consisting of three subunits encoded by the nuclear genome of eukaryotes. It is the final enzyme for all aerobic metabolism. It plays an important role in energy balance and energy production in the form of ATP. It is found largely in muscles, particularly the pectoral muscles and leg muscles, which require high energy, particularly flight and jumping muscles. It is also abundant in insects with high resistance to pesticides, such as cockroaches and rice weevils. The biological function of COX is that this enzyme catalyzes the final reaction in the electron transport chain, catalyzing the transfer of electrons from the cytochrome C molecule to molecular oxygen (O_2), resulting in its reduction to water (H_2O)(Blomberg,2021; Noodleman et al,2023; Saura et al,2022)

It is one of the most widely used genes in in sect classification and determining the genetic similarities and divergences between different species. COX also represents a major site for regulating oxidoreductase phosphorylation (OXPHS). The function of COX is to transfer electrons from cytochrome c to molecular oxygen, which leads to the formation of water and the release of energy needed to produce ATP. The enzyme cytochrome c oxidase (COX) is one of the most important tools in the study of genetic convergence and molecular evolution, especially through the analysis of the genes of the first subunit (COX1 or MT-CO1) located in mitochondrial DNA (mtDNA) (Mesquita et al., 2021; Kadenbach,2018; Ramzan et al ,2021).

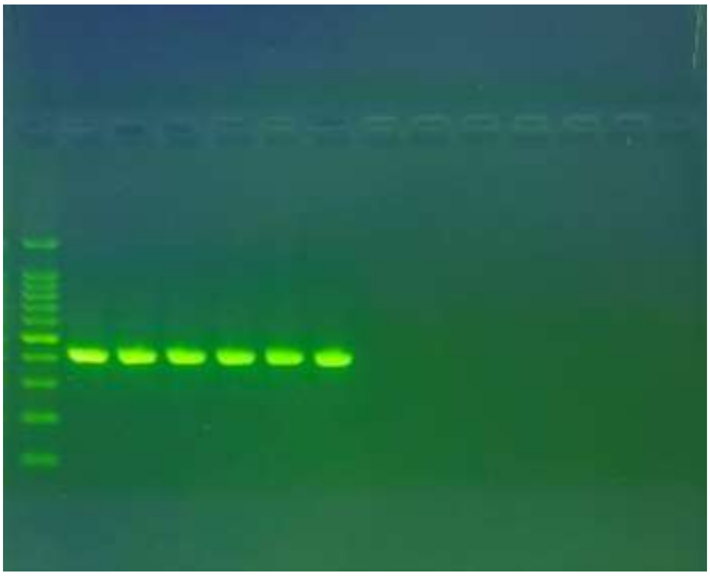

Figure (4) The result of gene transfer after performing PCR COX1.

Results of the COX1 gene sequencing

After amplifying the COX1 gene using the PCR technique, the sequences of the nitrogenous bases were determined for six samples from six different cities in Iraq. The PCR products were sent to a laboratory in South Korea.

BASRA 400

Query 1 ATATAAGATTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAG 60

||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

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Query 61 AAAGAGGTGCCGGAACAGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATG 120

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Query 121 CCGGAGCATCTGTTGATCTAGCAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTC 180

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Query 181 TAGGAGCTGTAAATTTTATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAG 240

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Query 241 AACGAATTCCCCTTTTCGTATGATCAGTAGCTATTACAGCattattatta ttattatCTC 300

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Sbjct 549 TACCAGTGCTTGCTGGAGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCT 608

Query 361 TTTTTGATCCAGCAGGAGGGG 381

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Sbjct 609 TTTTTGATCCAGCAGGAGGGG 629

Nasiriyah 400

Query 1 ATATAAGATTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAG 60

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Sbjct 249 ATATAAGATTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAG 308

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Sbjct  609  TTTTTGATCCAGCAGGAGGGGG  630
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Baghdad 379

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Samarra 400

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Kirkuk 397

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DHUK 405

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Sbjct  611  TTTGATCCAGCAGGAGGGGG  630
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2. THE AMERICAN COCKROACH PHYLOGENETIC TREE FOR THE COX GENE

Figure (3) The genetic tree of the American cockroach for the COX1 gene. Using the National Center for Biotechnology Information (NCBI) website, then accessing the BLAS NUCLEOTIDE subwindow and comparing them to the recorded species, the species were 100% similar to each other, 100% identical to the recorded species NC016956.1 from the United States, 100% identical to the recorded species OL589367 from Vietnam, 67% identical to the recorded species 220409.1 from Malaysia, and 93% identical to the recorded species LC619069.1 from Japan.

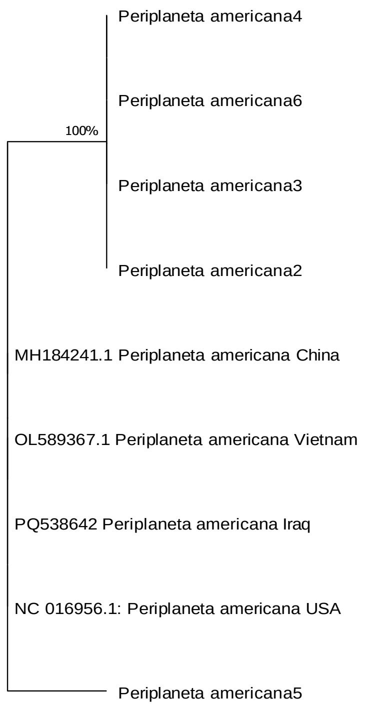

Figure (3) The genetic tree of the American cockroach for the COX1 gene

3. RESULTS OF THE COI LC GENE MIGRATION

The results of the COI LC gene migration from mitochondrial DNA in the leg cells of the American cockroach were shown for six samples collected from different cities in Iraq. PCR results were migration using an electrophoresis device on agarose gel at a concentration of 2% and an electrical potential difference of 5 V/cm² for an hour, then the gel was imaged under ultraviolet light.

Figure (4) The result of gene transfer after performing PCR COI LC.

Then the results showed that the bands resulting from the migration process using the PCR technique and according to the appropriate conditions for the COI LCO gene assembly had a molecular weight of 580 base pairs as shown in Figure (4-5).

4. RESULTS OF THE COI LC GENE SEQUENCING

After amplifying the COI LC gene using the PCR technique, the sequences of the nitrogenous bases were determined for six samples from six different cities in Iraq. The PCR products were sent to an external laboratory, and the results were presented.

BASRA

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Query  15   ATTTTCGGTGCCTGATCAGGTATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAG  74
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Sbjct  134  CATGCCTTCATTATAATTTTCTTTATAGTAATACCAATCATAATTGGGGGATTTGGTAAT  193
Query  195  TGATTAGTACCACTAATATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATA  254
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  194  TGATTAGTACCACTAATATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATA  253
Query  255  AGATTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGA  314
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          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 254  AGATTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGA  313
Query 315  GGTGCCGGAACAGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGA  374
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 314  GGTGCCGGAACAGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGA  373
Query 375  GCATCTGTTGATCTAGCAATTTTTTCTTTACATCTAGCAGGTGTATCCTCAATTCTAGGA  434
          |||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct 374  GCATCTGTTGATCTAGCAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGA  433
Query 435  GCTGTAAATTTTATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGA  494
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 434  GCTGTAAATTTTATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGA  493
Query 495  ATTCCCCTTTTCGTATGATCAGTAGCTATTACAGCattattattattattatCTCTACCA  554
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 494  ATTCCCCTTTTCGTATGATCAGTAGCTATTACAGCATTATTATTATTATTATCTCTACCA  553
Query 555  GTGCTTGCTGGAGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTT  614
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 554  GTGCTTGCTGGAGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTT  613
Query 615  GATCCAGCAGGAGGGGGTGATCCAATTTTATATCAACACTTATTC  659
          |||||||||||||||||||| ||||||||||||||||||||||||
Sbjct 614  GATCCAGCAGGAGGGGGTGACCCAATTTTATATCAACACTTATTC  658
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Nasiriyah

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Query  34    AGCTATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTCTGGGCAACCAGGTT   93            ||
|||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct 31   AGGTATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTC-GGGCAACCAGGTT  89
Query 94   CATTAATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCATGCTTTCATTATAA  153
          || |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct 90   CACTAATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCATGCCTTCATTATAA  149
Query 154  TTTTCTTTATAGTTATACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACCACTAA  213
          ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 150  TTTTCTTTATAGTAATACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACCACTAA  209
Query 214  TATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAACATAAGATTCTGATTATTAC  273
          |||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct 210  TATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATAAGATTCTGATTATTAC  269
Query 274  CACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAACAGGAT  333
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 270  CACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAACAGGAT  329
Query 334  GAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGATCTAG  393
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 330  GAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGATCTAG  389
Query 394  CAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTTTATCT  453
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 390  CAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTTTATCT  449
Query 454  CCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTTCGTAT  513
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 450  CCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTTCGTAT  509
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Query 514  GATCAGTAGCTATTACAGCattattattattattatCTCTACCAGTGCTTGCTGGAGCAA  573
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 510  GATCAGTAGCTATTACAGCATTATTATTATTATTATCTCTACCAGTGCTTGCTGGAGCAA  569
Query 574  TTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGGAGGGG  633
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 570  TTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGGAGGGG  629
Query 634  GTGACCCAATTTTATATCAACACTTATTC  662
           |||||||||||||||||||||||||||||
Sbjct 630  GTGACCCAATTTTATATCAACACTTATTC  658
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Baghdad

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Query 17   TTCGGTG-CTGATCAGGCATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTC  75
           ||||||| ||||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct 17   TTCGGTGCCTGATCAGGTATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTC  76
Query 76   GGGCAACCAGGTTCATTAATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCAT  135
           ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 77   GGGCAACCAGGTTCACTAATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCAT  136
Query 136  GCTTTCATTATAATTTTCTTTATAGTTATACCAATCATAATTGGGGGATTTGGTAATTGA  195
           || ||||||||||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct 137  GCCTTCATTATAATTTTCTTTATAGTAATACCAATCATAATTGGGGGATTTGGTAATTGA  196
Query 196  TTAGTACCACTAATATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAACATAAGA  255
           ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct 197  TTAGTACCACTAATATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATAAGA  256
Query 256  TTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGT  315
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 257  TTCTGATTATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGT  316
Query 316  GCCGGAACAGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCA  375
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 317  GCCGGAACAGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCA  376
Query 376  TCTGTTGATCTAGCAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCT  435
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 377  TCTGTTGATCTAGCAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCT  436
Query 436  GTAAATTTTATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATT  495
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 437  GTAAATTTTATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATT  496
Query 496  CCCCTTTTCGTATGATCAGTAGCTATTACAGCattattattattattatCTCTACCAGTG  555
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 497  CCCCTTTTCGTATGATCAGTAGCTATTACAGCATTATTATTATTATTATCTCTACCAGTG  556
Query 556  CTTGCTGGAGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGAT  615
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 557  CTTGCTGGAGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGAT  616
Query 616  CCAGCAGGAGGGGGTGACCCAATTTTATATCAACACTTATTC  657
           ||||||||||||||||||||||||||||||||||||||||||
Sbjct 617  CCAGCAGGAGGGGGTGACCCAATTTTATATCAACACTTATTC  658
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Samarra

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Query 1    ATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTCGGGCAACCAGGTTCATTA  60
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         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct 35   ATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTCGGGCAACCAGGTTCACTA  94
Query 61   ATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCATGCTTTCATTATAATTTTC  120
         ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct 95   ATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCATGCCTTCATTATAATTTTC  154
Query 121  TTTATAGTTATACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACCACTAATATTA  180
         |||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 155  TTTATAGTAATACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACCACTAATATTA  214
Query 181  GGAGCCCCAGATATAGCCTTCCCACGAATAAATAACATAAGATTCTGATTATTACCACCT  240
         ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct 215  GGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATAAGATTCTGATTATTACCACCT  274
Query 241  TCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAACAGGATGAACA  300
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 275  TCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAACAGGATGAACA  334
Query 301  GTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGATCTAGCAATT  360
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 335  GTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGATCTAGCAATT  394
Query 361  TTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTTTATCTCCACA  420
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 395  TTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTTTATCTCCACA  454
Query 421  ACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTTCGTATGATCA  480
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 455  ACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTTCGTATGATCA  514
Query 481  GTAGCTATTACAGCattattattattattatCTCTACCAGTGCTTGCTGGAGCAATTACT  540
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 515  GTAGCTATTACAGCATTATTATTATTATTATCTCTACCAGTGCTTGCTGGAGCAATTACT  574
Query 541  ATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGGAGGGGGTGAC  600
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 575  ATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGGAGGGGGTGAC  634
Query 601  CCAATTTTATATCAACACTTATTC  624
         ||||||||||||||||||||||||
Sbjct 635  CCAATTTTATATCAACACTTATTC  658
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KIRKUK

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Query 1    CATCACTAAGAATATTAATTCGTGCTGAGCTCGGGCAACCAGGTTCATTAATTGGAGATG  60
         ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct 45   CATCACTAAGAATATTAATTCGTGCTGAGCTCGGGCAACCAGGTTCACTAATTGGAGATG  104
Query 61   ATCAAATTTATAATGTAATCGTTACTGCCCATGCTTTCATTATAATTTTCTTTATAGTTA  120
         |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||| |
Sbjct 105  ATCAAATTTATAATGTAATCGTTACTGCCCATGCCTTCATTATAATTTTCTTTATAGTAA  164
Query 121  TACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACCACTAATATTAGGAGCCCCAG  180
         ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 165  TACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACCACTAATATTAGGAGCCCCAG  224
Query 181  ATATAGCCTTCCCACGAATAAATAACATAAGATTCTGATTATTACCACCTTCATTAACTT  240
         ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
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Sbjct  225  ATATAGCCTTCCCACGAATAAATAATATAAGATTCTGATTATTACCACCTTCATTAACTT  284
Query  241  TATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAACAGGATGAACAGTATACCCAC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  285  TATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAACAGGATGAACAGTATACCCAC  344
Query  301  CACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGATCTAGCAATTTTTTCATTAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  345  CACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGATCTAGCAATTTTTTCATTAC  404
Query  361  ATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTTTATCTCCACAACAATTAATA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  405  ATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTTTATCTCCACAACAATTAATA  464
Query  421  TAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTTCGTATGATCAGTAGCTATTA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  465  TAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTTCGTATGATCAGTAGCTATTA  524
Query  481  CAGCattattattattattatCTCTACCAGTGCTTGCTGGAGCAATTACTATATTATTAA  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  525  CAGCATTATTATTATTATTATCTCTACCAGTGCTTGCTGGAGCAATTACTATATTATTAA  584
Query  541  CTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGGAGGGGGTGACCCAATTTTAT  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  585  CTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGGAGGGGGTGACCCAATTTTAT  644
Query  601  ATCAACACTTATTC  614
            ||||||||||||||
Sbjct  645  ATCAACACTTATTC  658
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DHUK

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TGATCAGAGTATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTCGGGCAACC  84
            ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26   TGATCAG-GTATAGTGGGAACATCACTAAGAATATTAATTCGTGCTGAGCTCGGGCAACC  84
Query  85   AGGTTCACTAATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCATGCCTTCAT  144
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   AGGTTCACTAATTGGAGATGATCAAATTTATAATGTAATCGTTACTGCCCATGCCTTCAT  144
Query  145  TATAATTTTCTTTATAGTAATACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACC  204
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  TATAATTTTCTTTATAGTAATACCAATCATAATTGGGGGATTTGGTAATTGATTAGTACC  204
Query  205  ACTAATATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATAAGATTCTGATT  264
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  ACTAATATTAGGAGCCCCAGATATAGCCTTCCCACGAATAAATAATATAAGATTCTGATT  264
Query  265  ATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAAC  324
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  ATTACCACCTTCATTAACTTTATTACTAGCTAGTAGTATAGTAGAAAGAGGTGCCGGAAC  324
Query  325  AGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGA  384
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  325  AGGATGAACAGTATACCCACCACTAGCAAGAGGCATTGCTCATGCCGGAGCATCTGTTGA  384
Query  385  TCTAGCAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTT  444
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  TCTAGCAATTTTTTCATTACATCTAGCAGGTGTATCCTCAATTCTAGGAGCTGTAAATTT  444
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Query  445  TATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTT  504
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  445  TATCTCCACAACAATTAATATAAAACCTATTAATATAAAACCAGAACGAATTCCCCTTTT  504
Query  505  CGTATGATCAGTAGCTATTACAGCattattattattattatCTCTACCAGTGCTTGCTGG  564
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  505  CGTATGATCAGTAGCTATTACAGCATTATTATTATTATTATCTCTACCAGTGCTTGCTGG  564
Query  565  AGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGG  624
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  565  AGCAATTACTATATTATTAACTGACCGAAATCTAAATACATCCTTTTTTGATCCAGCAGG  624
Query  625  AGGGGGTGACCCAATTTTATATCAACACTTATTC  658
            ||||||||||||||||||||||||||||||||||
Sbjct  625  AGGGGGTGACCCAATTTTATATCAACACTTATTC  658
```

Analysis of the Scansink results for the COI LC gene

Tebil(1) shows the occurrence of many changes in the DNA strand through the presence of gaps, substitutions, inversions, and deletions in different ways for each sample.

Periplaneta Americana						
No.	**Type of substitution**	**Location**	**Nucleotide**	**Sequence ID with compare**	**Source**	**Identities**
1	**GAP**	**33**	**A\-**	.1	**Periplaneta americana isolate NH20 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial**	**99%**
2	**Transversion**	**33**	**C\G**	: .1	Periplaneta americana isolate NH20 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	**99%**
	GAP	**76**	**T\-**			
	Transition	**93**	**T\C**			
	Transition	**139**	**T\C**			
	Transversion	**164**	**T\A**			
	Transversion	**250**	**C\T**			
3	**GAP**	**24**	**-\C**	.1	Periplaneta americana isolate NH20 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	**99%**
	Transition	**34**	**C\T**			
	Transition	**93**	**T\C**			
	Transition	**139**	**T\C**			
	Transversion	**163**	**T\A**			
	Transition	**250**	**C\T**			
4	**Transition**	**92**	**T\C**	.1	Periplaneta americana isolate NH20 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	**99%**
	Transition	**140**	**T\C**			
	Transversion	**163**	**T\A**			
	Transition	**250**	**C\T**			
5	**Transition**	**82**	**G\A**	.1	Periplaneta americana isolate NH20 cytochrome oxidase subunit I (COI)	**99%**
	Transition	**126**	**T\C**			

	Transition	**139**	**T\C**		gene, partial cds; mitochondrial	
	Transversion	**400**	**T\A**			
	Transition	**635**	**T\C**			
6	**Transition**	**92**	**T\C**	.1	Periplaneta americana isolate NH20 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	99%
	Transition	**139**	**T\C**			
	Transversion	**163**	**T\A**			
	Transition	**250**	**C\T**			

Table (1) shows the differences between the studied samples.

The American cockroach phylogenetic tree for the COI LC gene

After accessing the GenBank website, going to the BLAST page and searching for the sequence for each sample in the search, the search results showed:

The results showed that the sample isolated from Basra matched the sample bearing the global number MH184229.1 with a 97% match rate, which is from China

The results showed that the sample isolated from Nasiriyah matched the sample bearing the global number MF149291.1 with a 97% match rate, which is from China.

The results showed that the sample isolated from Baghdad matched the sample bearing the global number MH1840721.1 with a 99% match rate, which is from Thailand.

The results showed that the sample isolated from Samarra matched the sample bearing the global number MH184075.1 with a 95% match rate, which is from China. The results showed that the sample isolated from Kirkuk city matched the sample bearing the global number MH184072.1 with a 97% match rate, which is from China.

The results showed that the sample isolated from Dohuk city matched the sample bearing the global number MF149272.1 with a 97% match rate, which is from China.

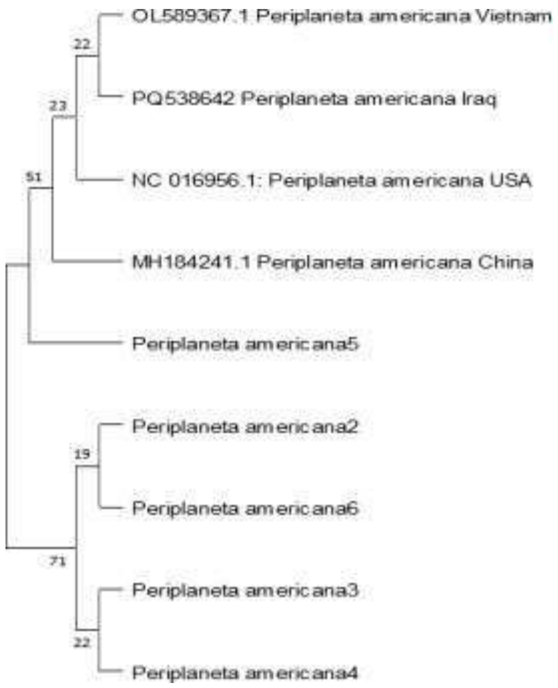

Figure (5) The genetic tree of the American cockroach for the COI LC gene.

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