MOLECULAR DIVERSITY AND PHYLOGENETIC ANALYSIS OF SOME IRAQI HONEY BEE POPULATION INFERRED FROM MITOCHONDRIAL DNA GENE SEGMENTS (COI AND 16S rDNA)

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(Received 12th Jul 2022; accepted 14th Sep 2022)

Abstract. Honeybee is one of the most important insects considering its role in agriculture, ecology and economy as a whole. In the present study, honeybee subspecies discrimination, genetic diversity and phylogenetic relationships among honeybee populations of Iraq were investigated at two different mitochondrial regions. Two partial genes cytochrome oxidase subunit I (COI) and 16S rDNA regions of mtDNA of the honeybee population in Sulaimani, Iraq, have been studied. Portions of the mitochondrial 16S ribosomal DNA (16S rDNA) and cytochrome C oxidase I (COI) genes were amplified by PCR and then subjected sequencing analysis. All honeybee subspecies belong to the lineage, the honeybee populations in three locations at Baghdad belonged to the C mtDNA lineage. The estimated genetic diversity for the populations ranged from 0.39 in Kafri population to 0.47 in Erbil population, and total genetic diversity among loci was calculated as 0.47 while average within population genetic diversity was 0.44. GST value was 0.085. The Phylogenetic tree showed two main clusters; the first one comprised of three populations (Dohuk, Erbil, and Sulaymaniyah), and the second one included two communities (Kirkuk and Kafri).
Keywords: *Iraqian bee Apis mellifera, COI region, 16sRNA, genetic variation, phylogenetic analysis*

Introduction

The honeybee is an economically and ecologically important insect species in Iraq (Al-Ameri and Alhasan, 2021). The north and northeast cities, Dohuk, Erbil, Sulaimani, Kirkuk, and Kafri of Iraq comprise an essential center of honey production and provide a considerable amount of the country's honey needs. This region is a well-known modernized bee keeping area in Iraq. These cities have well-defined ecological characteristics, and because of favorable environmental conditions, they are major destinations for seasonal colony migrations from other regions of the country. Due to desirable and similar climates, there are fewer reciprocal colony migrations among these provinces, and it appears that these areas have their exclusive groups of honeybees (Said-Ahmad, 2018). Based on morphometric and genetic studies, the twenty-six of the honeybee (*Apis mellifera* L.) subspecies are identified and clustered into five evolutionary lineages: M from Northern and Western Europe and Northern Africa, A from Southern and Central Africa, C from the Northern Mediterranean region and Eastern Europe, O

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2101_229242
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from the Eastern Mediterranean and the Near and the Middle East regions and Y from the east African country of Ethiopia (Franck et al., 2001).

Based on morphometric studies, the most Middle East subspecies, Anatolian honey bee (*A. mellifera anatoliaca* Maa), Caucasian honeybee (*A. mellifera caucasica* Gorbachev), and Iranian honey bee (*A. mellifera meda* Skorikow) have been clustered within the O lineage (Farshineh et al., 2007). However, the genetic analysis found that the honeybee subspecies in the Middle East belonged to the C lineage, which is from the Northern Mediterranean region and Eastern Europe (Bouga et al., 2011). The morphometric traits are still considered very important in the classification of honeybees in the world, but this classic method is not fit to analyze the phylogenetic relationship and characterize the subspecies of the honeybee. Moreover, the morphological approach is more sensitive to environmental conditions (Rahimi et al., 2016). Studying population genetic structure and diversity is the basis of our understanding of biodiversity and the conservation of species (Allendorf et al., 2012).

Whole-genome and partial gene sequencing has become routine for population genetic studies. Due to the recent rapid developments in sequencing technology, it is now possible to gain insights into the genomic structure of populations with unprecedented power and accuracy (Luikart et al., 2018). The COI gene of the mitochondrion is the standard DNA barcode region utilized to identify the different species of insect (i.e. honeybee and wasp) because the COI part has great discrimination power for most insect groups and this gene is a region unique to the genus *Apis* (Özdil et al., 2009; Rahimi et al., 2016). The wide ranges of climates and habitats exist in Iraq; however, only one honeybee subspecies (*A. mellifera meda*) has been identified and described in the northeast of Iraq by Ruttner (1988) and Said-Ahmad (2018). Thus, the honeybee populations in different regions in Iraq can be identified by restriction and sequence analysis of the mitochondrial DNA (mtDNA) region. The main directions of mtDNA based discrimination of honey bees included: Restriction Fragment Length Polymorphisms by restriction enzymes (RFLP) (Garnery et al., 1992) and RFLP of fragments amplified by PCR (PCR-RFLP) (Ivanova et al., 2010); Polymorphism of PCR products with specific (Arias and Sheppard, 2005) or random primers – Random amplified polymorphic DNA (RAPD) amplification (Tunca and Kence, 2011); and Sequencing of the mtDNA with the quest of single nucleotide polymorphisms (SNPs) (Ilyasov et al., 2019). One of the important and essential aims in race breeding of the honeybee is determining the genetic variation amongst the populations of honeybee.

In this study, we empirically assess the consistency two different mitochondrial regions, in estimating honeybee subspecies discrimination, genetic diversity and phylogenetic relationships among honeybee populations of Iraq. Nowadays, there is no information on the COI and 16s rDNA sequence variation of honeybee in Iraq, so the objective of this genetic study was to investigate the genetic diversity of honeybee populations of the nine locations in Sulaymaniyah province, Iraq via using the COI and 16s rDNA mitochondria genome and the PCR technique and gene sequencing.

The primary objective of this study was to determine the level of genetic differentiation among Iraqi honeybee populations, as determined by PCR pattern analysis of the COI and 16S rDNA gene regions of mtDNA. Additionally, the results of this study were compared with those of other earlier mitochondrial studies of honeybees, allowing for a much more accurate estimation of the genetic structure of Iranian honeybee populations than had been possible previously.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Materials and Methods

Honeybee samples

One hundred and eighty adult workers honeybee from various colonies of *A. mellifera* were collected from nine different localities distributed in (Kela spi (Apiary 1), Penjwen, Raparin, Qaradakh, Kela spi (Apiary 2), Bakrajo (Apiary 1), Bakrajo (Apiary 2), Awbara and Bazyan) Sulaymaniyah province- Kurdistan region – Iraq, during the spring season of 2021 (*Figure 1*). Honeybee sampling was collected from fifteen colonies of most active apiaries in each region. Two workers of honeybees were selected from each colony (15 workers honeybees per apiary). Samples were stored in 97% ethanol and kept at -20°C until DNA extraction (Al-Ameri and Alhasan, 2021).

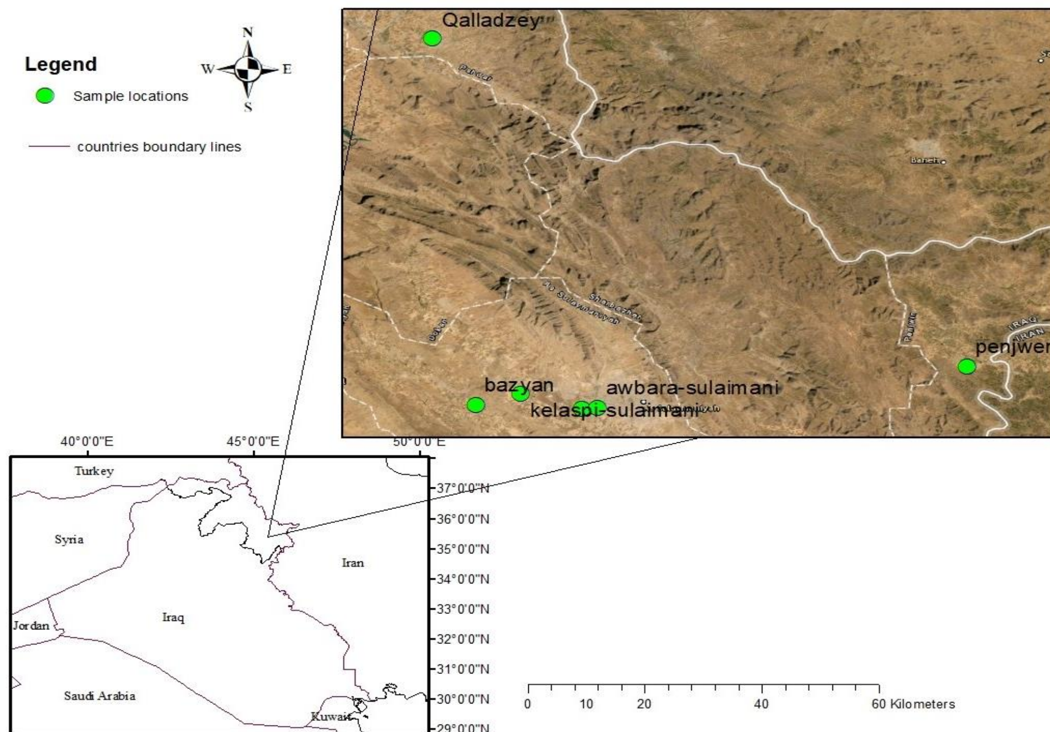

***Figure 1.** Map of the study site*

Molecular analysis

PCR technique

PCR technique was performed for detection, sequencing and genotyping of *A. mellifera* based on two different mitochondrial regions. This technique was carried out according to method described by Said-Ahmad (2018) with minor modification as following steps.

Genomic DNA extraction

Total genomic DNA was extracted from the head, thorax and leg sections of each worker honeybee, using the genomic DNA extraction kit (Fermentas, Germany) following the manufacturer's instructions; 50 µL elution buffer was used for extracting. Extracted genomics was stored at –20°C before running PCR.

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PCR amplification

The mtDNA variation was analyzed by performed on PCR amplified products. Mitochondrial regions were amplified according to Bouga et al. (2005) with slight modifications. Two sets of primers were used for amplifying: 16S rDNA and COI gene regions. These were 5’- CAACATCGAGGTCGCAAACATC-3’ and 5’-GTACCTTTT GTATCAGGGTTGA-3’ for 16S rDNA and 5’-GATTA CTTCCTCCCTCATTA-3’ and 5’-AATCTGGATAGTCTG AATAA-3’for COI segment (Rahimi et al., 2016).

PCR cocktail was run in a total volume 25 µL reaction mixture containing 12.5 µL of 2× PCR master mix (AMPLIQON, Denmark), 1.0 µL of each primer (10 pmol), 1.5 µL of genomic DNA template. The volume was completed to 25 µL with free nuclease water. Each gene was amplified separately. The PCR was then continued with the following program: initial denaturation 94°C for 4 min, 35 cycles of 94°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 2 min, followed by a final extension step at 72°C for 10 min. The amplified products obtained were next electrophoresed on 1.5% agarose gel to verify the size of the fragment (Bakr et al., 2021).

DNA sequencing analysis

PCR products of DNA samples were prepared for sequencing by using forward primers of COI and 16S rDNA genes and sent to Macrogen Company (238, Teheran-ro, Gangnam-gu, Seoul, Republic of Korea). Sequence results were aligned using BioEdit Sequence Alignment Editor and compared with previously sequences were deposited in the National Center for Biotechnology Information (NCBI) GenBank. The sequences of *Apis* species in this study were submitted in NCBI-GenBank.

Phylogenetic tree analysis

The phylogenetic analysis was carried out via utilizing Molecular Genetic Evolutionary Analysis (MEGA) 11 software (French software company, France, 1991). Phylogenetic trees of the COI and 16S rDNA sequences were performed by utilizing the maximum likelihood (ML) method (Kishino and Hasegawa, 1989) and branching confidence was carried out by utilizing 1000 bootstrap replication. Analysis of strains identification by analysis of genetic trees between local *A. mellifera* isolates and known *A. mellifera* isolates at NCBI-Blast. Finally, the *A. mellifera* isolates identified in NCBI-GenBank were provided for the GenBank registration number.

Results and Discussion

Results of the PCR Technique, to confirm that the desired portion of 16S rDNA and COI genes have been amplified, gel electrophoresis was conducted. The gel documentation image obtained by BioDoc Analyzer shows that the results were positive for all the nine samples selected for gel electrophoresis. It reveals that desired regions of mtDNA were properly polymerized. The sizes of the PCR-amplified mtDNA regions for all populations studied were found to average 1080bp for 16S rDNA and 1000 bp for COI mtDNA gene region (*Figures 2 and 3*) and this is consistent with many studies such as Rahimi et al. (2018).

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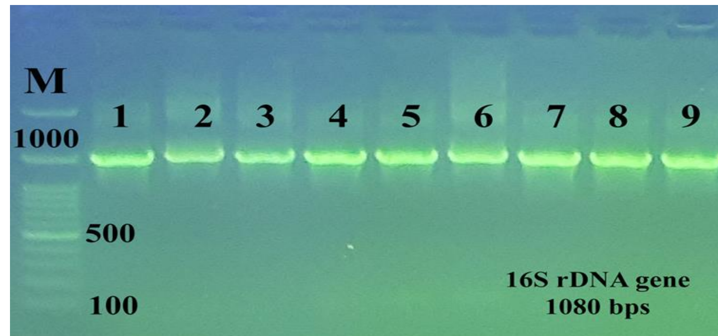

Figure 2. *Agarose gel electrophoresis 1.5% of PCR product of the 16S rDNA gene region of Apis mellifera, Lane M: DNA Marker of 100 bps, Lane 1 to 9 are samples of honeybee*

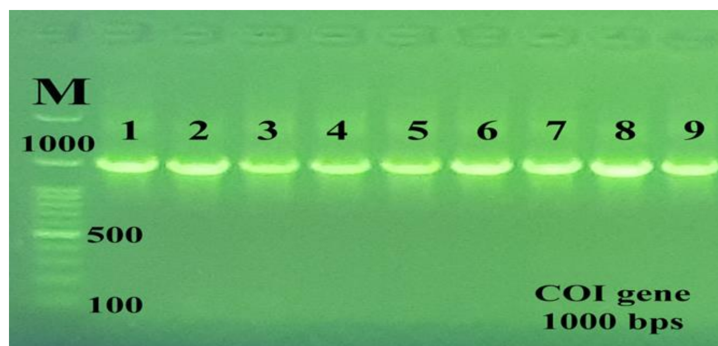

Figure 3. *Agarose gel electrophoresis 1.5% of PCR product of the COI gene region of A. mellifera, Lane M: DNA Marker of 100 bps, Lane 1 to 9 are samples of honeybee*

DNA sequencing technique and genetic tree analysis

In the present study, we have used different two mtDNA regions (COI and 16S rDNA) with different samples of the mitochondrial genome to discrimination the subspecies of honeybee; verify the nature and distribution of genetic variation within and between some Iraqi honeybee populations.

For genetic diversity of the COI and 16S rDNA region of mtDNA, random samples of honeybee were selected in nine different locations of Sulaimani province (Kela spi (Apiary 1), Penjwen, Raparin, Qaradakh, Kela spi (Apiary 2), Bakrajo (Apiary 1), Bakrajo (Apiary 2), Awbara and Bazyan). The selected samples were sent to Macrogen Company in South Korea for the purpose of discrimination *A. mellifera* subspecies; verify the nature and distribution of genetic variation within and between some Iraqi honeybee populations. Our results showed *ligustica* as subspecies of honeybee population in Iraq (*A. mellifera ligustica* Spinola 1806). This subspecies has also acquired a specific resistance against diseases and pests, which attack other honeybees. Our results revealed that the ability of honeybee to adapt to environmental changes depends significantly on the genetic diversity in the honeybee. Effective conservation of a vulnerable species depends mainly on the knowledge of patterns of genetic variation. *A. mellifera* L. includes several recognized subspecies that differ in their biological properties and agricultural characteristics. Distinguishing between honeybees subspecies is complicated (Syromyatnikov et al., 2017). However, Rahimi et al. (2016) and Rahimi et al. (2017) indicated that there is one local subspecies of honey bee in the north and northeast

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Kurdistan region of Iraq, *A. mellifera meda*, which during the past thousand's years of Iraqi civilization has adapted itself with different climatic conditions and plant flora varieties of the Middle East.

The greatest problem in the discrimination of subspecies was the presence of transition zones between ranges of subspecies, which led to the gradual changes in morphometric, and molecular features among neighbor subspecies. The honeybee subspecies can be variously assigned to the evolutionary lineages by different morphometry and mtDNA methods, the mitochondrial DNA based methods of identification the taxonomic affiliation of honeybees based on its nucleotide polymorphism.

At the moment, the most widely used genetic method for distinguishing between bee subspecies is the analysis of the mitochondrial DNA fragment located between the genes for cytochrome oxidase c subunits 1 and 2 (COI1 and COI2) (Abrahamovich et al., 2007; Alattal et al., 2014). This fragment is AT-enriched and significantly differs in its length and nucleotide composition between honeybee populations (Cornuet et al., 1991; Ostroverkhova et al., 2015). The COI1 gene is commonly used for species identification (including honeybees) (Sheffield et al., 2009; Packer and Ruz, 2017). Syromyatnikov et al. (2017) developed a PCR-RFLP procedure for the rapid identification of honeybee subspecies and they found that the COI1 gene could be used as a marker for distinguishing honeybee subspecies. In another study DNA, barcoding has been used for distinguishing honeybee subspecies in Turkey (Ozdil and Ilhan, 2012).

Our results for the discrimination subspecies in Iraqi population according to the previous two different partial genes of mtDNA region showed that they belong to the evolutionary Lineage C (The Italian honey bee), and the results of the present study coincides with the findings from the previous work of Rahimi et al. (2016), Tunca and Kence (2011). In a molecular study carried out by Özdil et al. (2009), similar results were reached on the honeybee samples collected from different 20 locations in Turkey. These samples were subgroups in the C lineage depending on the mtDNA regions. In addition, Al-Ameri and Alhasan (2021) indicated the honeybee populations in three locations (Abu Ghraib, Jadriya and Latifiya), at Baghdad – Iraq belonged to the C mtDNA lineage (this honeybee race group consists of the northern Mediterranean region and eastern European honeybee populations).

Based on morphometric studies, the most Middle East subspecies, Anatolian honeybee (*A. mellifera anatoliaca* Maa), Caucasian honeybee (*Apis mellifera caucasica* Gorbachev), and Iranian honeybee (*A. mellifera meda* Skorikov) have been clustered within the O lineage (Farshineh et al., 2007). In another study of Rahimi et al. (2018) on Genetic Variation in Iranian Honey bees, Inferred from PCR-RFLP Analysis of two mtDNA Gene Segments (COI and 16S rDNA) noted The Iranian subspecies honey bee composed of a shared clade with subspecies of Eastern Mediterranean, Near East and Eastern parts of Middle East (O branch). This result is very useful for the control of conservation of local honeybees, as the movement of colonies across the borderline of these neighboring countries, may affect the genetic structure of honeybee populations. The modern taxonomic pattern of honey bee is given according to the review done by Ilyasov et al. (2020), which included thirty-three distinct honey bee subspecies are distributed across all Africa (11 subspecies), Western Asia and the Middle East (9 subspecies), and Europe (13 subspecies). All honeybee subspecies are subdivided into five evolutionary lineages: lineage A (10 subspecies) and its sublineage Z (three subspecies), lineage M (three subspecies), lineage C (10 subspecies), lineage O (three subspecies), lineage Y (one subspecies), and lineage C or O (three subspecies).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Determining the DNA sequence for these two mtDNA regions, which was used to determine the nucleotide variation of *A. mellifera* (*Figures 4 and 5*).

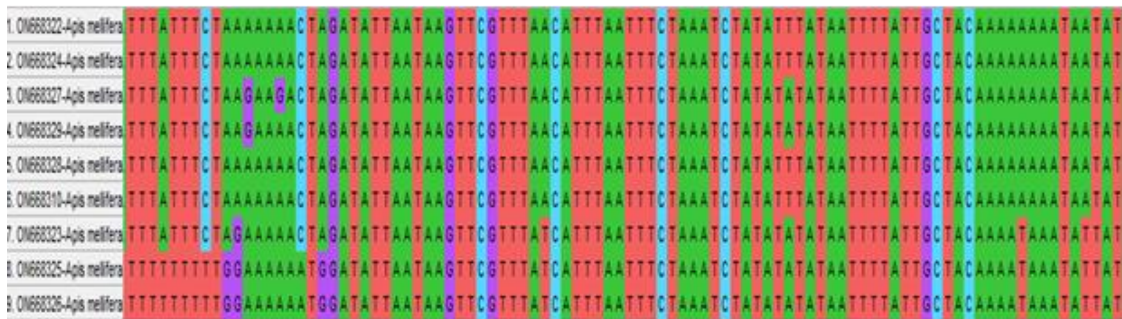

Figure 4. *Multiple sequence alignment analysis of partial mitochondrial 16S rDNA gene sequence in local A. mellifera*

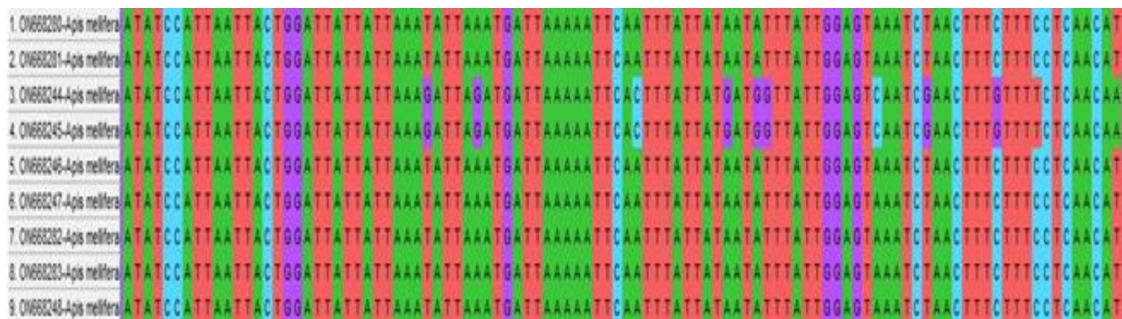

Figure 5. *Multiple sequence alignment analysis of partial mitochondrial COI gene sequence in local A. mellifera*

The phylogenetic tree was written using the evolution distance, which was measured using the Composite Likelihood Maximum method (UPGMA tree) in MEGA 11.0 version. The phylogenetic tree of the Iraqi population (*A. mellifera* L.) was drawn to illustrate the relationship within and between some China species that deposited on the GenBank database based 16S rDNA gene region by using Mega 11 program. *Figures 6 and 7* illustrated relationship of nine local Iraqi samples *A. mellifera ligustica* with each other according 16S rDNA gene region and with four china sequences of global isolates in GenBank, were the result showed that similarity between (ON668325 *A. mellifera ligustica* Kela spi and ON668326 *A. mellifera ligustica* Bakrajo), (ON668327 *A. mellifera ligustica* Bakrajo and ON668329 *A. mellifera ligustica* Bazyan) and (ON668323 *A. mellifera ligustica* Raparin and OM203348 *A. mellifera ligustica* China) reached 100, 92 and 74% respectively. While both figures (*Figs. 8 and 9*) showed relationship of nine local Iraqi samples *A. mellifera ligustica* with each other according COI gene region and with four China sequences of global isolates in GenBank, were the result indicated that similarity reached 100% between ON668244 *A. mellifera ligustica* Raparin and ON668245 *A. mellifera ligustica* Qaradakh and they have showed 62% similarity with OM203347 *A. mellifera ligustica* China.

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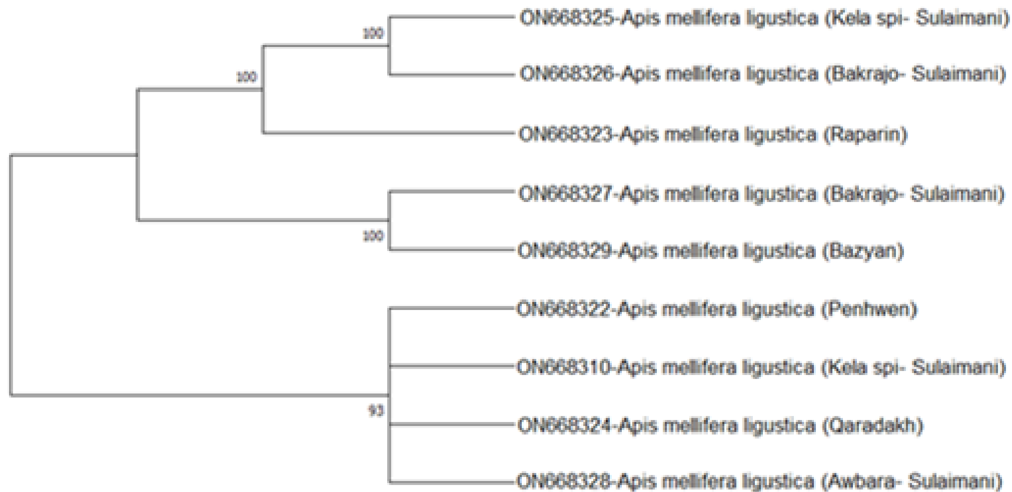

Figure 6. *Phylogenic tree of Apis mellifera haplotypes samples from Iraq: Kurdistan region: Sulaymaniyah province. The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model (Tamura et al., 2004) in MEGA11 software and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated partial mitochondrial 16S rDNA gene sequence was used as input data Apis mellifera typing detection*

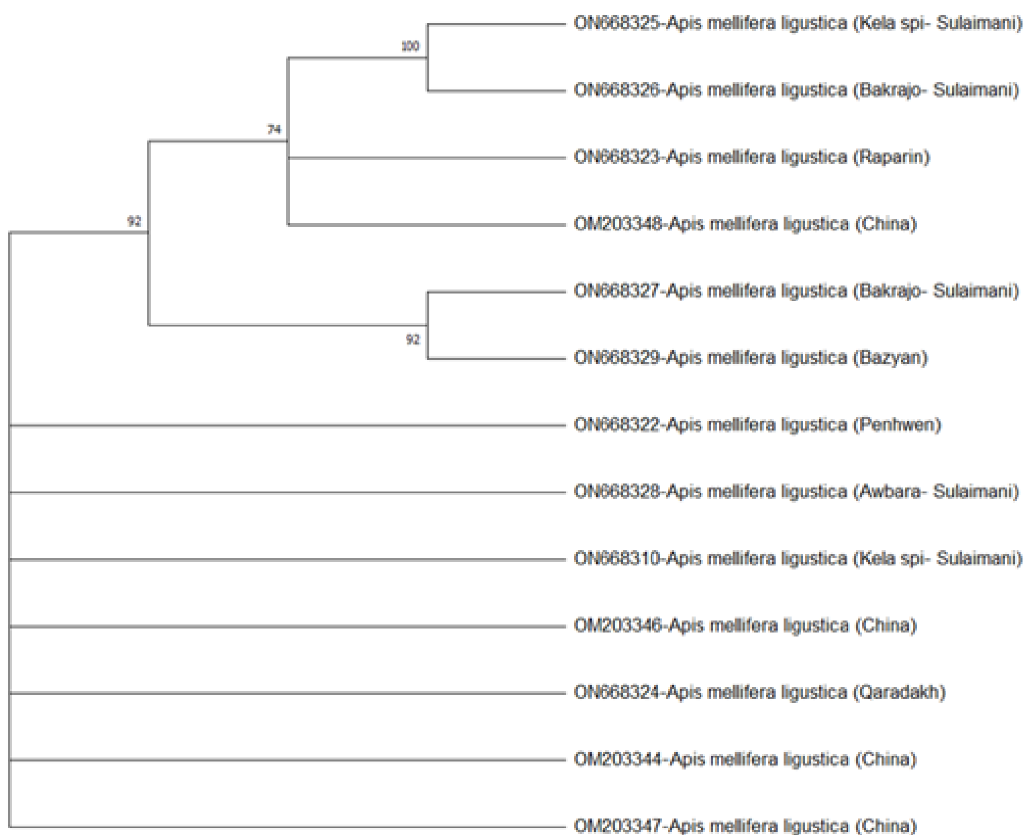

Figure 7. *Phylogenic tree of Apis mellifera haplotypes samples from Iraq: Kurdistan region: Sulaymaniyah province with some China species. The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated partial mitochondrial 16S rDNA gene sequence was used as input data Apis mellifera typing detection*

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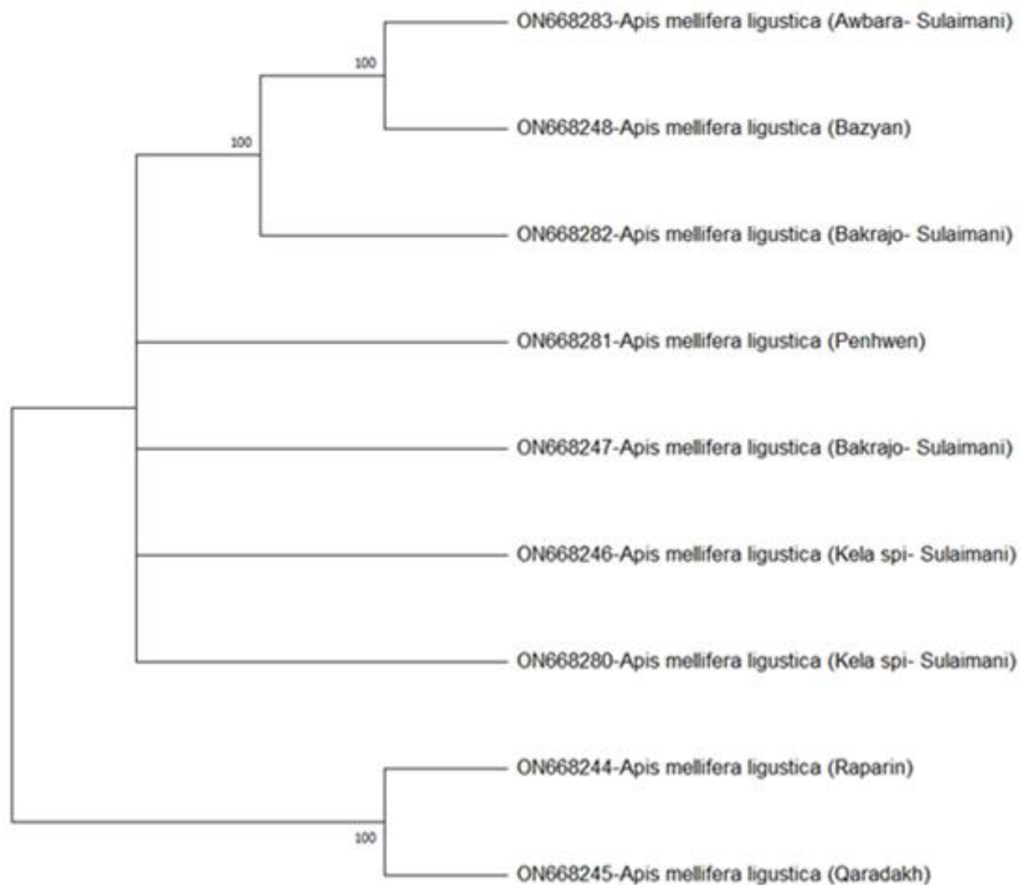

Figure 8. *Phylogenic tree of haplotypes of Apis mellifera samples from Iraq: Kurdistan region, Sulaymaniyah province. The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated of partial COX c subunit I gene sequence were used as input data Apis mellifera typing detection*

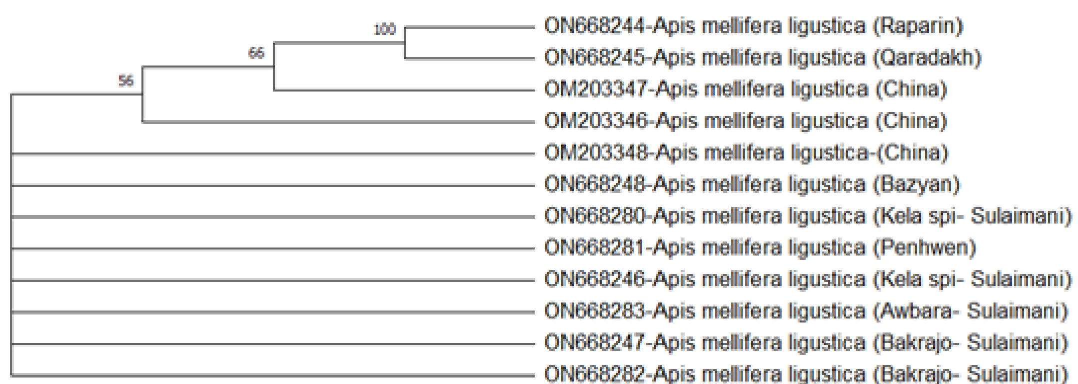

Figure 9. *Phylogenic tree of haplotypes of Apis mellifera samples from Iraq: Kurdistan region, Sulaimani province with some China species. The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software (Tamura et al., 2021) and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated of partial COX c subunit I gene sequence were used as input data Apis mellifera typing detection*

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Phylogenetic relationships among honey bee populations of Iraq based on ISSR markers showed two main clusters; the first one comprised of three populations (Dohuk, Erbil, and Sulaymaniyah), and the second one included two communities (Kirkuk and Kafri) (Said-Ahmad, 2018). In another molecular study neighbor, joining analysis showed that the three Russian breeds of *A. mellifera* subspecies: a separate cluster represents *A. mellifera caucasica, A. mellifera carnica*, and *A. mellifera carpathica*, as well as one of the haplotypes of *A. mellifera mellifera*. However, the second haplotype of *A. mellifera mellifera* is represented as a separate cluster, together with other *A. mellifera mellifera* subspecies from GenBank. In general, there is a low level of bootstrap value, but it should be taken into account that *A. mellifera* breeds are not species, but subspecies. Additionally, it should be noted that the identified SNP are stable, and this makes it possible to develop a method for differentiating subspecies of *A. mellifera* (Syromyatnikov et al., 2017). Generally, using neighbor-joining analysis of COI sequences is limited to the identification of honeybee subspecies. The COI gene is commonly used for species identification (including bees) (Sheffield et al., 2009; Packer and Ruz, 2017). However, Syromyatnikov et al. (2017) found that COI sequences also differ in honeybee subspecies and exhibit stable SNPs typical for individual subspecies.

A dendrogram based on the Unweighted Pair Group Method with Arithmetic mean (UPGMA) generated two sub-clusters. Honeybee populations of Golestan, Mazandaran, Guilan provinces were located in the first group. The second group included honeybee populations of Ardebil, West Azerbaijan, East Azerbaijan provinces, but this group showed a close relationship with other populations. The results showed obviously the ability of the ISSR marker technique to detect the genetic diversity among the honeybee populations (Rahimi et al., 2016).

The phylogenetic tree based on Neighbor-joining method divided 29 subspecies of Iranian honeybee to 5 distinct clusters. The Iranian subspecies honey bee composed of a shared clade with subspecies of Eastern Mediterranean, Near East and Eastern parts of Middle East (O branch) (Rahimi et al., 2018).

Honeybee populations genotype

The analysis of genetic diversity between Iraqi honeybee populations according to the 16S rDNA gene as shown from the *Table 1*, revealed that the consequences of the DNA sequence of *A. mellifera* indicate that responsible for Kela spi, Penjwen, Qaradakh and Awbara Sulaimani governorates, where the strain (G1) was found in 44.5% of samples and identical rates 100%. With the comparable sample in the gene bank under the symbol OM203348 from China, the strain (G2) relates to Raparin in 11.1% of the samples with an identical rate ranging from 99.10% when comparable sample in the gene bank with accession number OM203346 from China. *A. mellifera* indicate that (G3) responsible for Bakrajo and Bazyan, where the strain (G3) was found in 22.2% of samples and identical rates 99.55% when comparable sample in the gene bank with accession number OM203344 from China. the strain (G4) relates to Kela spi and Bakrajo in 22.2% of the samples with an identical rate ranging from 94.96% when comparable sample in the gene bank with accession number OM203347 from China. The isolates were recorded in GenBank with the following accession number: ON668310, ON668322, ON668323, ON668324, ON668325, ON668326, ON668327, ON668328 and ON668329.

In addition, the analysis of genetic diversity between Iraqi honeybee populations according to the COI gene are obtained in *Table 2*. The results of the DNA sequence of *A. mellifera* indicate that (G1) responsible for Kela spi, Penjwen, Bakrajo, Awbara and

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Bazyan from Sulaimani governorates, where the strain (G1) was found in 55.56% of samples and identical rates 100%. With the comparable sample in the GenBank under the symbol OM203348 from China, the strain (G2) relates to Raparin and Qaradakh in 22.22% of the samples with an identical rate ranging from 98.82% when comparable sample in the GenBank with accession number OM203346 from China. *A. mellifera* indicate that (G3) responsible for Bakrajo and Kela spi, where the strain (G3) was found in 22.22% of samples and identical rates 99.55% when comparable sample in the GenBank with accession number OM203344 from China. The strain (G4) relates to Kela spi and Bakrajo in 22.2% of the samples with an identical rate ranging from 99.89% when comparable sample in the GenBank with accession number OM203347 from China. The isolates were recorded in GenBank with the following accession number: ON668280, ON668281, ON668244, ON668245, ON668246, ON668247, ON668282, ON668283 and ON668248.

Table 1. *NCBI-BLAST Homology sequence identity based on 16S rDNA partial gene sequence between local A. mellifera of Sulaimani governorates isolates with NCBI-BLAST Apis mellifera related Genotypes isolates*

Local Isolation	GenBank submission accession number	NCBI -BLAST Homology sequence identity				
		NCBI-BLAST identity isolate	Genotype	Accession Number	Country	Identity (%)
Kela spi (Apiary 1)	ON668310	*A. mellifera*	G1	OM203348	China	100
Penjwen	ON668322	*A. mellifera*	G1	OM203348	China	100
Raparin	ON668323	*A. mellifera*	G2	OM203346	China	99.10
Qaradakh	ON668324	*A. mellifera*	G1	OM203348	China	100
Kela spi (Apiary 2)	ON668325	*A. mellifera*	G4	OM203347	China	94.96
Bakrajo (Apiary 1)	ON668326	*A. mellifera*	G4	OM203347	China	94.96
Bakrajo (Apiary 2)	ON668327	*A. mellifera*	G3	OM203344	China	99.55
Awbara	ON668328	*A. mellifera*	G1	OM203348	China	100
Bazyan	ON668329	*A. mellifera*	G3	OM203344	China	99.55

Table 2. *NCBI -BLAST Homology sequences identity based on the mitochondrial COI gene partial sequence between local A. mellifera of Sulaimani governorates isolates with NCBI-BLAST Apis mellifera related Genotypes isolates*

Local Isolation	Gene bank submission accession number	NCBI -BLAST Homology sequence identity				
		NCBI-BLAST identity isolate	Genotype	Accession Number	Country	Identity (%)
Kela spi (Apiary 1)	ON668280	*A. mellifera*	G1	OM203348	China	100
Penjwen	ON668281	*A. mellifera*	G1	OM203348	China	100
Raparin	ON668244	*A. mellifera*	G2	OM203346	China	98.82
Qaradakh	ON668245	*A. mellifera*	G2	OM203346	China	98.82
Kela spi (Apiary 2)	ON668246	*A. mellifera*	G3	OM203347	China	99.89
Bakrajo (Apiary 1)	ON668247	*A. mellifera*	G3	OM203347	China	99.89
Bakrajo (Apiary 2)	ON668282	*A. mellifera*	G1	OM203348	China	100
Awbara	ON668283	*A. mellifera*	G1	OM203348	China	100
Bazyan	ON668248	*A. mellifera*	G1	OM203348	China	100

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DOI: http://dx.doi.org/10.15666/aeer/2101_229242
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In another Genetic diversity study among honeybee populations of Iraq according to ISSR markers ranged from 0.39 in Kafri population to 0.47 in Arbil population, and total genetic diversity among loci was calculated as 0.47 while average within population genetic diversity was 0.44. Analysis of molecular variance (AMOVA) showed that genetic variation within populations was higher than among populations. Genetic diversity within populations is highly essential for the adaptation to changing environments and, as a consequence, for long-term survival of a species (Said-Ahmad, 2018).

In Iranian honey bee populations same mitochondrial region genes studied by Rahimi et al. (2018), they were amplified by PCR and then subjected to RFLP pattern analysis using 8 restriction enzymes, they found 3 novel composite genotypes (haplotypes). The average haplotype diversity (h) within populations was 0.0405.

Conclusions

The phylogenetic analysis of molecular data derived from sequencing two separate areas (COI and 16S rDNA) produced concordant results. In addition, our findings supported previous reports in the scientific literature that used DNA data and morphometric analysis to demonstrate the evolutionary relationships among *Apis* species. This study includes all described species and provides a broader perspective on the links between older and newly described species groups. As revealed for *A. mellifera* subspecies, the COI region has the potential to clarify the relationships between taxa within the genus Apis. The addition of 16S rDNA data strengthened the phylogeny established by mtDNA analysis.

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