

# Molecular insights of mitochondrial *16S rDNA* genes of the native honey bees subspecies *Apis mellifera carnica* and *Apis mellifera jementica* (Hymenoptera: Apidae) in Saudi Arabia

Reem Alajmi<sup>1</sup>, Rewaida Abdel-Gaber<sup>1,2\*</sup>, Loloa Alfozana<sup>1</sup>

<sup>1</sup>Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

<sup>2</sup>Faculty of Science, Department of Zoology, Cairo University, Cairo, Egypt

Corresponding author: Rewaida Abdel-Gaber

E-mail: [rewaida@sci.cu.edu.eg](mailto:rewaida@sci.cu.edu.eg)

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**ABSTRACT.** The honey bee *Apis mellifera* is of major importance for the world's agriculture and is also suitable for environmental monitoring. It includes several recognized subspecies distinguished by using morphological and morphometric variants. Here, 200 adult worker *Apis mellifera* honey bees were collected from Hail region, Saudi Arabia. Mitochondrial *16S rDNA* was conducted to detect molecular polymorphism among honey bee *A. mellifera* subspecies. The amplified and sequenced gene regions of mtDNA revealed the presence of two different subspecies of *Apis mellifera carnica* (gb| MH939276.1) and *Apis mellifera jementica* (gb| MH939277.1). The sequences were compared with each other and with others retrieved from the GenBank demonstrating a high degree of similarity (up to 72%). The NJ tree indicated that all *Apis* species are clustered together in one clade in addition to the genetically origin of *Apis* species within family Apidae as a paraphyletic group within the African lineage.

**Keywords:** *Apis mellifera*; Genetic characterization

## INTRODUCTION

Bees are the world's most important pollinators in natural and agricultural ecosystems. They are very diverse with over 20,000 species (Engel, 2005, 2011a). Honey bees belong to the family Apidae with other social bees. The subfamily Apidae is comprised of a single genus, *Apis*. The genus *Apis* comprises *Apis florae* (the little bee), *Apis dorsata* (the giant bee), *Apis cerana* (the eastern bee) and *Apis mellifera* (the western bee). The honey bee, *Apis mellifera* Linnaeus, 1758, is naturally found throughout Europe, Africa and Western Asia (Miguel et al., 2011). Traditionally, the intraspecific taxonomy of the honey bee *Apis mellifera* L. has been based on morphology. Twenty-nine subspecies of *A. mellifera* are currently recognized on the basis of morphometric characteristics (Sheppard and Meixner, 2003; Miguel et al., 2011). These subspecies are also described as 'geographic races' because their distributions correspond to distinct geographic areas (Ruttner, 1992). Five evolutionary lineages have been characterized based on morphometric, molecular, ecological, ethological, and physiological traits (De la Rúa et al., 2005). The four primary lineages are found in the Mediterranean Basin, including the African lineage (A), west and north European lineage (M), south-east European lineage (C), and Near and Middle Eastern lineage (O) (Franck et al., 2000a, 2001; Miguel et al., 2007; Cánovas et al., 2008). The fifth proposed lineage is north-east African (Y) (Franck et al., 2001). Although morphological characteristics are still considered very important in the classification of honey bees, this approach is not well suited to characterize honey bee subspecies and analyze phylogenetic relationships because they can be sensitive to environmental selection pressures (Franck et al., 2000b).

A number of molecular markers such as nuclear DNA (Hall, 1990; Tarès et al., 1993), mitochondrial DNA (mtDNA) (Moritz et al., 1986; Smith et al., 1989; Smith, 1991; Hunt and Page, 1992; Garnery et al., 1993; Oldroyd et al., 1995; Arias and Sheppard, 1996; Pedersen, 1996; De la Rúa et al., 2000), and microsatellites (Estoup et al., 1993; Garnery et al., 1998), are also used to study genetic variability in honey bees (Boore, 1999). The sequencing and characterization of the mtDNA genome have been very useful for analyzing the phylogeny and population genetic structure of the *Apis* species and of *A. mellifera* subspecies because it contains regions with variable evolutionary rates. In general, mtDNA contains genes for two ribosomal subunits (12S and 16S), 22 tRNA, and 13 proteins (three subunits of cytochrome c oxidase, cytochrome B, subunits 6 and 8 of ATP F0 synthase, and seven subunits of NADH dehydrogenase) (Shao et al., 2003; Silvestre and Arias, 2006). Genetic markers such as the mtDNA COXI–COXII intergenic region are unique to the genus *Apis* (Cornuet and Garnery 1991). Variations in the sequences of this region or the length of fragments produced using endonucleases are used extensively to differentiate the five honeybee lineages and to discriminate among *A. mellifera* subspecies (Garnery et al., 1992; Franck et al., 2000a; Sheppard and Smith, 2000). To date, no study has characterized Saudi honey bees. Therefore, the goal of this study was to determine the genetic diversity and phylogenetic relationships of *Apis mellifera* subspecies of Saudi Arabia via mitochondrial *16S rDNA* genes.

## MATERIALS AND METHODS

### Honey bee sample collection

A total of 100 samples were collected from adult worker bees of each breeder (200 samples) from honey bees in the Hail region of Saudi Arabia. Bee samples were collected during the month of September 2017 after approval from the beekeepers. Bees were collected inside the wooden cages containing food with passage of air and sunlight. The samples were transferred alive to an Entomology lab, frozen for 5 min to immobilize them, and then stored in 95% ethanol until processed. The two strains were identified according to the external characteristics with measurements of different body parts. Some samples were sent to a specialist for definition of the bee breeds with Dr. Yahya Al-Attal, Faculty of Agriculture, King Saud University, Saudi Arabia.

## Molecular analysis

**DNA extraction, PCR amplification, and sequencing:** Mitochondrial genomic DNA was extracted from the thoracic legs of the ethanol-preserved samples. The tissue was homogenized using a glass pestle and a mortar. The genomic DNA in the homogenate was extracted using DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. The purity and concentration of extracted DNA was determined with a NanoDrop 2000 UV-VIS Spectrophotometer (Thermo Fisher Scientific Inc, USA) at 260/280 nm. The extracted DNA was stored at -20°C until use. Mitochondrial *16S rDNA* gene fragments were amplified using GeneJET™ PCR Purification kit [Thermo (Fermentas)] following the manufacturer's protocol in a total volume of 50 µl including 5 µl 10 × buffer, 5 µl of each dNTP (10 mM), 10 µl of each primer (1 pmol/ µl), 0.3 µl of Taq polymerase (5 U/ml), 2.5 µl MgCl<sub>2</sub> (50 mM), and 2 µl of total genomic DNA. PCR amplification and subsequent DNA sequencing was carried out using the following mitochondrial *16S rDNA* primers: 16SF (5'-CAC CTG TTT ATC AAA AAC AT-3') and 16SR (5'-CGT CGA TTT GAA CTC AAA TC-3'), designed by Crozir and Crozier (1993).

**The PCR cyclic conditions were processed under the following conditions:** 95°C for 15 min (initial denaturation), 35 cycles of 15 s at 95°C (denaturation), 30 s at 62°C (annealing), and 1 min at 72°C (extension), and finally post-PCR extension for 5 min at 72°C. Each amplicons was examined by (1%) agarose gel electrophoresis in 1× Tris–acetate–EDTA (TAE) buffered gel stained with 1% Ethidium bromide. This was then visualized with a UV transilluminator, and bands with the predicted size were purified using QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. Amplicons were sequenced (in both directions) using an ABI Prism Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) with an Applid Analyzer Biosystems (Thermo Fisher Scientific, USA) using the same primers for annealing.

## Sequence alignment and phylogenetic analysis

BLAST search was done to identify related sequences on NCBI database. Sequences were aligned directly using CLUSTAL-X multiple sequence alignment (Thompson et al., 1997) and others available from GenBank™ selected to represent all available tapeworm lineages with an emphasis on taxa presumed to be related to the analyzed groups. The alignment was manually corrected using the alignment editor of software BIOEDIT 4.8.9 (Hall, 1999). Phylogenetic calculations were performed with the UPGMA method. The data were analyzed with the Maximum Composite Likelihood (MCL) approach based on the Kimura 2-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Phylogenetic and evolutionary analyses were conducted using MEGA version 6 (Tamura et al., 2013). The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000).

## RESULTS

Molecular analyses based on the partial *16S rDNA* gene sequence were performed to investigate the taxonomy and classification of the two recovered *Apis mellifera* subspecies. The amplified and sequenced gene regions and GC content in the samples from *Apis mellifera carnica* and *Apis mellifera jementica* were 1157 bp/29% and 682 bp/25.7%, respectively. The sequences were deposited in GenBank under the accession numbers, MH939276.1 and MH939277.1, respectively. The sequences were compared with each other and with other sequences available on GenBank from varied geographical regions (Table 1). The calculation of the percentage of identity between these novel sequences and a range of sequences for other arthropods of different host species demonstrated a high degree of similarity (up to 72%) with intra-specific differences that varied from 0.1% to 23.8% (Figure 1).

**Table 1.** Arthropoda species used in the phylogenetic analysis of the present sub-species *Apis mellifera*

Parasite species	Class/Order/Family	Source	Accession no.	Sequence length (bp)	Percent identity (%)
<i>Apis mellifera scutellata</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	MG552702.1	16364	91
<i>Apis mellifera capensis</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	MG552697.1	16456	91
<i>Apis mellifera sahariensis</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	MF351881.1	16569	91
<i>Apis mellifera lamarckii</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KY464958.1	16589	91
<i>Apis mellifera syriaca</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KP162643.1	15428	91
<i>Apis mellifera intermissa</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KM458618.1	16343	91
<i>Apis mellifera monticola</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	MF67858.1	16343	91
<i>Apis mellifera syriaca</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KY926882.1	16343	91
<i>Apis mellifera carnica</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	JQ778284.1	1009	91
<i>Apis mellifera ligustica</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KX908209.1	16343	91
<i>Apis mellifera meda</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KY464957.1	16248	91
<i>Apis mellifera</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KX113622.1	515	91
<i>Apis florea</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KU571744.1	520	90
<i>Apis dorsata breviligula</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	FJ932648.1	468	90
<i>Apis laboriosa</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	JQ317319.1	469	90
<i>Apis mellifera mellifera</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	KJ396191.1	16051	91
<i>Trigona pallens</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	L22902.1	458	76
<i>Trigona hypoea</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	L22901.1	459	79
<i>Scaptotrigona luteipennis</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	L22900.1	461	78
<i>Acyrtosiphon pisum</i>	<i>Insecta/Homoptera/Aphididae</i>	GenBank	AK341909.1	661	74
<i>Melipona compressipes</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	L22899.1	459	76
<i>Pediculus humanus corporis</i>	<i>Insecta/Phthirapter/Pediculidae</i>	GenBank	KM579465.1	668	82
<i>Daphnia pulex</i>	<i>Crustacea/Branchiopoda/Daphniidae</i>	GenBank	DQ470571.1	442	72
<i>Nasonia vitripennis</i>	<i>Insecta/Hymenoptera/Pteromalidae</i>	GenBank	EU746617.1	2747	78
<i>Drosophila melanogaster</i>	<i>Insecta/Diptera/Drosophilidae</i>	GenBank	BT003592.1	3412	72
<i>Drosophila melanogaster</i>	<i>Insecta/Diptera/Drosophilidae</i>	GenBank	KP730804.1	342	73
<i>Bombyx mori</i>	<i>Insecta/Lepidoptera/Bombycidae</i>	GenBank	KP729110.1	15658	77
<i>Xylocopa virginica</i>	<i>Insecta/Hymenoptera/Apidae</i>	GenBank	L22905.1	463	82
<i>Anopheles gambiae</i>	<i>Insecta/Diptera/Culicidae</i>	GenBank	MG753749.1	14844	74
<i>Tribolium castaneum</i>	<i>Insecta/Coleoptera/Tenebrionidae</i>	GenBank	KJ003060.1	526	72
<i>Rhodinus prolixus</i>	<i>Insecta/Hemiptera/Reduviidae</i>	GenBank	EU822953.1	316	72
<i>Ixodes scapularis sterol</i>	<i>Chelicerata/Arachnida/Ixodidae</i>	GenBank	XM002408215.1	3339	72

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	
MH939276.1 <i>Apis mellifera carnica</i>																																			
MH939277.1 <i>Apis mellifera jementica</i>	1.9																																		
MG552702.1 <i>Apis mellifera scutellata</i>	12.7	14.6																																	
MG552697.1 <i>Apis mellifera capensis</i>	12.7	14.6	0.0																																
MF351881.1 <i>Apis mellifera sahariensis</i>	2.1	0.0	14.6	14.6																															
KY464958.1 <i>Apis mellifera lamarchii</i>	12.8	14.6	0.0	0.0	14.7																														
KP162643.1 <i>Apis mellifera syriaca</i>	12.8	14.6	0.0	0.0	14.7	0.0																													
KM458618.1 <i>Apis mellifera intermissa</i>	12.7	14.6	0.0	0.0	14.6	0.0	0.0																												
MF67858.1 <i>Apis mellifera monticola</i>	12.8	14.6	0.0	0.0	14.7	0.0	0.0	0.0																											
KY926882.1 <i>Apis mellifera syriaca</i>	12.8	14.6	0.0	0.0	14.7	0.0	0.0	0.0	0.0																										
JQ778284.1 <i>Apis mellifera carnica</i>	2.1	0.0	14.6	14.6	0.0	14.6	14.6	14.6	14.6	14.6																									
KX908209.1 <i>Apis mellifera ligustica</i>	12.7	14.6	0.0	0.0	14.6	0.0	0.0	0.0	0.0	0.0	0.0	14.6																							
KY464957.1 <i>Apis mellifera meda</i>	12.7	14.6	0.0	0.0	14.6	0.0	0.0	0.0	0.0	0.0	0.0	14.6	0.0																						
KX113622.1 <i>Apis mellifera</i>	2.1	0.0	14.6	14.6	0.0	14.6	14.6	14.6	14.6	14.6	0.0	14.6	14.6																						
KU571744.1 <i>Apis florea</i>	1.9	0.1	14.0	14.0	0.1	14.0	14.0	14.0	14.0	14.0	0.1	14.0	14.0	0.1																					
FJ932648.1 <i>Apis dorsata breviligula</i>	2.1	0.1	14.1	14.1	0.1	14.1	14.1	14.1	14.1	14.1	0.1	14.1	14.1	0.1	0.1																				
JQ317319.1 <i>Apis laboriosa</i>	1.9	0.1	14.0	14.0	0.1	14.0	14.0	14.0	14.0	14.0	0.1	14.0	14.0	0.1	0.1	0.1																			
KJ396191.1 <i>Apis mellifera mellifera</i>	12.8	14.6	0.0	0.0	14.7	0.0	0.0	0.0	0.0	0.0	0.0	14.6	0.0	0.0	14.6	14.0	14.1	14.0																	
L22902.1 <i>Trigona pallens</i>	2.4	1.7	15.2	15.2	1.6	15.2	15.2	15.2	15.2	15.2	1.6	15.2	15.2	1.6	1.5	1.3	1.2	15.2																	
L22901.1 <i>Trigona hypogea</i>	2.6	1.8	16.4	16.4	1.7	16.4	16.4	16.4	16.4	16.4	1.7	16.4	16.4	1.7	1.3	1.5	1.4	16.4	0.1																
L22900.1 <i>Scaptotrigona luteipennis</i>	2.6	1.6	16.2	16.2	1.5	16.3	16.3	16.2	16.3	16.3	1.5	16.3	16.2	1.5	1.1	1.2	1.4	16.3	0.2	0.1															
AK341909.1 <i>Acyrtosiphon pisum</i>	2.9	2.6	14.1	14.1	2.5	14.1	14.1	14.1	14.1	14.1	2.5	14.1	14.1	2.5	2.5	3.0	2.4	14.1	2.1	2.2	3.0														
L22899.1 <i>Melipona compressipes</i>	2.8	2.0	16.3	16.3	1.9	16.4	16.4	16.3	16.4	16.4	1.9	16.4	16.3	1.9	1.7	1.7	1.5	16.4	0.2	0.1	0.1	3.3													
KM579465.1 <i>Pediculus humanus corporis</i>	10.5	12.6	10.3	10.3	13.4	10.3	10.3	10.2	10.3	10.3	13.4	10.3	10.3	13.4	12.1	12.2	11.3	10.3	12.2	13.1	12.2	11.5	13.0												
DQ470571.1 <i>Daphnia pulex</i>	5.2	4.3	16.4	16.4	4.2	16.4	16.4	16.4	16.4	16.4	4.2	16.4	16.4	4.2	4.1	3.7	3.8	16.4	3.4	3.7	3.8	1.9	4.4	13.8											
EU746617.1 <i>Nasonia vitripennis</i>	13.3	15.1	14.6	14.6	16.3	14.5	14.5	14.5	14.5	14.5	16.3	14.5	14.5	16.3	15.6	16.9	14.6	14.5	15.7	18.3	15.6	11.8	18.1	4.1	16.9										
BT003592.1 <i>Drosophila melanogaster</i>	5.1	2.9	13.5	13.5	2.9	13.5	13.5	13.5	13.5	13.5	2.9	13.5	13.5	2.9	2.7	2.3	2.4	13.5	1.9	2.4	2.4	2.0	2.7	13.2	2.0	15.0									
KP730804.1 <i>Drosophila melanogaster</i>	5.4	3.4	13.0	13.0	3.1	13.1	13.1	13.0	13.1	13.1	3.0	13.0	13.0	3.0	3.2	2.7	2.8	13.1	2.0	2.8	2.8	2.1	3.2	13.6	2.2	15.6	0.0								
KP729110.1 <i>Bombyx mori</i>	13.1	13.1	2.3	2.3	14.0	2.4	2.4	2.4	2.4	2.4	14.0	2.5	2.4	14.0	12.5	12.6	11.7	2.4	11.8	13.5	13.5	10.3	14.4	11.7	13.6	14.3	12.9	13.4							
L22905.1 <i>Xylocopa virginica</i>	3.0	1.4	17.6	17.6	1.4	17.6	17.6	17.6	17.6	17.6	1.4	17.6	17.6	1.4	1.0	1.0	1.5	17.6	1.3	1.0	0.9	2.9	1.1	14.9	4.0	18.2	3.0	3.6	14.5						
MG753749.1 <i>Anopheles gambiae</i>	13.4	13.5	5.0	5.0	14.5	5.0	5.0	5.0	5.0	5.0	14.5	5.0	5.0	14.5	14.0	14.0	14.0	5.0	15.1	16.2	16.1	12.2	17.5	11.9	13.4	11.8	15.5	16.0	2.0	16.1					
KJ003060.1 <i>Tribolium castaneum</i>	4.7	3.2	14.2	14.2	3.1	14.2	14.2	14.2	14.2	14.2	3.1	14.2	14.2	3.1	3.1	2.7	2.6	14.2	2.2	2.5	3.0	1.7	2.6	13.7	1.8	15.7	1.3	1.6	10.3	3.1	15.2				
EU822953.1 <i>Rhodnius prolixus</i>	7.1	2.8	14.2	14.2	2.7	14.2	14.2	14.2	14.2	14.2	2.7	14.2	14.2	2.7	3.2	3.2	3.2	14.2	3.5	3.9	4.2	2.4	4.3	18.2	3.4	15.8	1.9	2.1	12.7	3.6	13.2	2.4			
XM002408215.1 <i>Ixodes scapularis stercorator</i>	16.7	17.7	10.4	10.4	19.2	10.5	10.5	10.5	10.5	10.5	19.2	10.4	10.5	19.2	15.9	16.0	17.1	10.5	18.7	20.2	18.7	25.5	20.1	12.1	23.8	14.8	16.3	17.0	17.3	18.6	16.6	18.5	18.2		

**Figure 1.** Estimates of evolutionary divergence between sequences. The number of base substitutions per site from between sequences is shown. Analyses were conducted using the Maximum Composite Likelihood model. The analysis involved 34 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 168 positions in the final dataset. Evolutionary analyses were conducted in MEGA6

The comparison of nucleotide sequences and divergence showed that *16S rDNA* in the present species has the highest BLAST scores with lower divergence values than other apids previously sequenced; however, our sample was not identical to any prior specimen (Figure 2). A tree topology was automatically computed to estimate ML values (Figure 3). Two clades were clustered during the construction of the phylogenetic tree using the maximum likelihood and maximum parsimony of the present apids species. The major clade clustering all Pancrustacean species contained two classes: the first one was Crustacea represented here by *Daphnia pulex* (gb| DQ470571.1) within family Daphniidae; the second was Insecta with families Apidae (*A. mellifera scutellata* gb| MG552702.1, *A. mellifera capensis* gb| MG552697.1, *A. mellifera sahariensis* gb| MF351881.1, *A. mellifera lamarchii* gb| KY464958.1, *A. mellifera syriaca* gb| KP162643.1, *A. mellifera intermissa* gb| KM458618.1, *A. mellifera monticola* gb| MF67858.1, *A. mellifera syriaca* gb| KY926882.1, *A. mellifera carnica* gb| JQ778284.1, *A. mellifera ligustica* gb| KX908209.1, *A. mellifera meda* gb| KY464957.1, *A. mellifera* gb| KX113622.1, *A. florea* gb| KU571744.1, *A. dorsata breviligula* gb| FJ932648.1, *A. laboriosa* gb| JQ317319.1, *A. mellifera mellifera* gb| KJ396191.1, *Trigona pallens* gb| L22902.1, *T. hypogea* gb| L22901.1, *Scaptotrigona luteipennis* gb| L22900.1, *Melipona compressipes* gb| L22899.1, *Xylocopa virginica* gb| L22905.1), Aphididae (*Acyrtosiphon pisum* gb| AK341909.1), Pediculidae (*Pediculus humanus corporis* gb| KM579465.1), Pteromalidae (*Nasonia vitripennis* gb| EU746617.1), Drosophilidae (*Drosophila melanogaster* gb| BT003592.1,

gb| KP730804.1), Bombycidae (*Bombyx mori* gb| KP729110.1), Cuclicidae (*Anopheles gambiae* gb| MG753749.1), Tenebrionidae (*Tribolium castaneum* gb| KJ003060.1), and Reduviidae (*Rhodinus prolixus* gb| EU822953.1). The minor clade with the lowest blast scores and a high divergence value contained class Chelicerata represented by *Ixodes scapularis* sterol (gb| XM002408215.1). These results indicate that the recovered apid species are deeply embedded within the genus *Apis*.

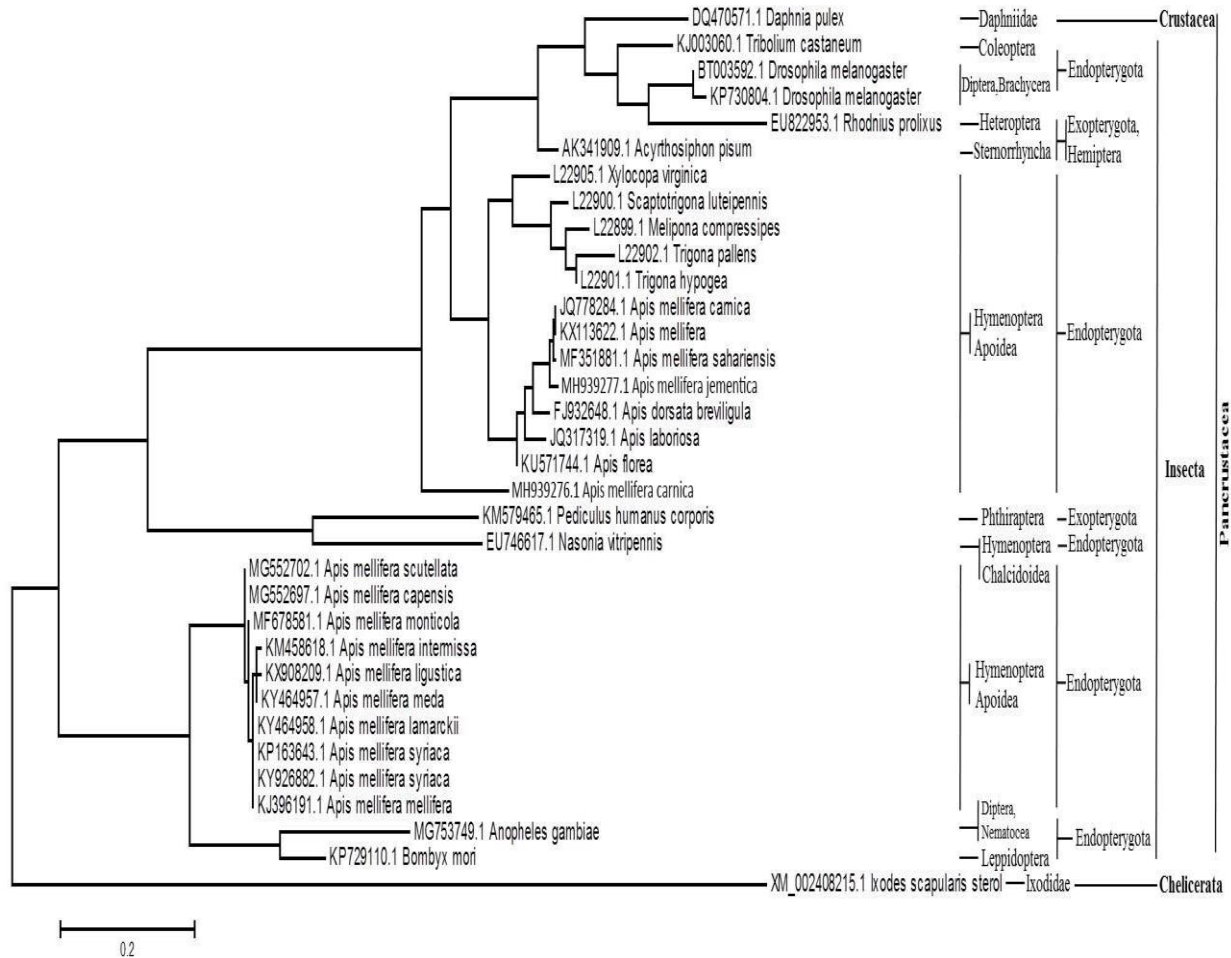
		10	20	30	40	50	60	70	80	90	100
MH939276.1	A. m. carnica	GGTTTAAATT	AGGCGACTGC	TCATGA-ATT	ATTTTAAATG	CTGCAGTATG	T---TGACT	GTACAAAGGT	AGCATAATAA	ATGTCCTTTT	TAA---TTGA
MH939277.1	A. m. jementica	-----	---CT---	GA..T.T---	---A.---	-----	-----	-----	-----	-----	-----
MG552702.1	A. m. scutellata	ATA..AT.AA	TTTTA..CA	GA.ATTATCC	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AC	T.G---GATC	C.ATAT..GA	.G.ACA.AAT
MG552697.1	A. m. capensis	ATA..AT.AA	TTTTA..CA	GA.ATTATCC	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AC	T.G---GATC	C.ATAT..GA	.G.ACA.AAT
MF351881.1	A. m. sahariensis	TAA..T.GG	TC.ATCTGCT	CA...AT.A	T...A...	-----	-----	-----	-----	-----	-----
KY464958.1	A. m. lamarkii	ATA..AT.AA	TTTTA..CA	GA.ATTAT.C	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAC
KF163643.1	A. m. syriaca	ATA..AT.AA	TTTTA..CA	GA.ATTAT.C	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAC
KM458618.1	A. m. intemissa	ATA..AT.AA	TTTTA..CA	GA.ATTATCC	CAA.....A	TATA.CCTCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAT
MF678581.1	A. m. monticola	ATA..AT.AA	TTTTA..CA	GA.ATTATCC	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAT
KY926882.1	A. m. syriaca	ATA..AT.AA	TTTTA..CA	GA.ATTAT.C	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAC
QJ778284.1	A. m. carnica	TAA..T.GG	TC.ATCTGCT	CA...AT.A	T...A...	-----	-----	-----	-----	-----	-----
KX908209.1	A. m. ligustica	ATA..AT.AA	TTTTA..CA	GA.ATTATCC	CAA.....A	TATA.CCTC.	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAC
KY464957.1	A. m. meda	ATA..AT.AA	TTTTA..CA	GA.ATTATCC	CAA.....A	TATA.CCTCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAC
KX113622.1	A. mellifera	----GTGCA	TCGTCT.AAT	GA..T.T---	---A.---	-----	-----	-----	-----	-----	-----
KU571744.1	A. florea	----AGTCGA	TCGTCT.CAT	GA..A.A---	---A.---	-----	-----	-----	-----	-----	-----
FJ932648.1	A. dorsata breviligula	----GTCGA	TCGTCT.AAT	GA.AT.T---	---A.---	-----	-----	-----	-----	-----	-----
QJ317319.1	A. laboriosa	----GTGCA	TCGTCT.CAT	GA..TTT---	---A.---	-----	-----	-----	-----	-----	-----
KJ396191.1	A. m. mellifera	ATA..AT.AA	TTTTA..CA	GA.ATTAT.C	CAA.....A	TATA.C.TCA	CTAAT.A.A.	T..A.G..AAC	T.G---GATC	C.ATAT..GA	.G.ACA.AAC
		110	120	130	140	150	160	170	180	190	200
MH939276.1	A. m. carnica	AGA-ATTGTA	TGAAAGAGAT	TA-----ATG	GAAATAATAA	ACTGTTCTCC	TAAATTTATT	TAATGGAAAT	TTAAATTTTT	TAGTTTAAAA	ATTCTTAAAA
MH939277.1	A. m. jementica	-----	-----	-----	A..T---AT.	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
MG552702.1	A. m. scutellata	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
MG552697.1	A. m. capensis	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
MF351881.1	A. m. sahariensis	-----	-----	-----	A..T---AT.	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
KY464958.1	A. m. lamarkii	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
KF163643.1	A. m. syriaca	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
KM458618.1	A. m. intemissa	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
MF678581.1	A. m. monticola	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
KY926882.1	A. m. syriaca	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.Y.	ATAAAA.T.C.	T.AA.....
QJ778284.1	A. m. carnica	-----	-----	-----	A..T---AT.	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
KX908209.1	A. m. ligustica	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
KY464957.1	A. m. meda	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
KX113622.1	A. mellifera	-----	-----	-----	A..T---AT.	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
KU571744.1	A. florea	-----	-----	-----	A..T---ATT	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
FJ932648.1	A. dorsata breviligula	-----	-----	-----	A..T---ATG	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
QJ317319.1	A. laboriosa	-----	-----	-----	A..T---AT.	-----	T	CT..A.TA.	-----G.A.	AG---T---	-----TA---
KJ396191.1	A. m. mellifera	.T.A..CA..	AT..TGAT..	.GAAGGT..A	A..T---T.	TACC..TATT	A.T.AATA.	A.TC.TCTA.	.AGTT..G.C.	ATAAAA.T.C.	T.AA.....
		210	220	230	240	250	260	270	280	290	300
MH939276.1	A. m. carnica	ATTTTAAATT	ATGGGGACGG	ATAAAGACCC	CTATAAGAAA	TTTAA---A	TTTTAAATTT	TTTAAATTTT	TATTTTAAATA	AAITTTTAAA	TAAAAATATT
MH939277.1	A. m. jementica	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
MG552702.1	A. m. scutellata	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.C....A.	.AG..ATT.	.TCTT..A.
MG552697.1	A. m. capensis	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A....A.	.AG..ATT.	.TCTT..A.
MF351881.1	A. m. sahariensis	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KY464958.1	A. m. lamarkii	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
KF163643.1	A. m. syriaca	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
KM458618.1	A. m. intemissa	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
MF678581.1	A. m. monticola	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
KY926882.1	A. m. syriaca	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
QJ778284.1	A. m. carnica	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KX908209.1	A. m. ligustica	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
KY464957.1	A. m. meda	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
KX113622.1	A. mellifera	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KU571744.1	A. florea	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
FJ932648.1	A. dorsata breviligula	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
QJ317319.1	A. laboriosa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KJ396191.1	A. m. mellifera	.AAA...ACC	TGAA..A.T-	-----C---	A.GAG..A.TC	A..T---T.	AA.ATCC.CC	-AATT..C.	A.....A.	.AG..ATT.	.TCTT..A.
		310	320	330	340	350	360	370	380	390	400
MH939276.1	A. m. carnica	ATTTTAAAG	ATTTAAAAAT	TTTGAATTGG	GGGAGGGAAT	T---GGGGT	AAAAA-TTTT	AAATTAATAA	CTTTTAAATT	TTAAAAAAT	TTTAAAAATT
MH939277.1	A. m. jementica	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
MG552702.1	A. m. scutellata	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
MG552697.1	A. m. capensis	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
MF351881.1	A. m. sahariensis	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KY464958.1	A. m. lamarkii	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
KF163643.1	A. m. syriaca	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
KM458618.1	A. m. intemissa	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
MF678581.1	A. m. monticola	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
KY926882.1	A. m. syriaca	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
QJ778284.1	A. m. carnica	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KX908209.1	A. m. ligustica	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
KY464957.1	A. m. meda	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA
KX113622.1	A. mellifera	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KU571744.1	A. florea	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
FJ932648.1	A. dorsata breviligula	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
QJ317319.1	A. laboriosa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KJ396191.1	A. m. mellifera	TA..AATCCT	.A.G..CAA	.AGATGAAT	T.C.ATA.T	G---ATTTT.	.T.TCAAA.	GTGA....C	TA..AATC.	AA..TTT..A	C.CC...AGA



Molecular insights of mitochondrial 16S rDNA genes of the native honey bees subspecies *Apis mellifera carnica* and *Apis mellifera jementica* (Hymenoptera: Apidae) in Saudi Arabia

	410	420	430	440	450	460	470	480	490	500
MH939276.1 A. m. carnica	TTTGGGATA	ATTATAAAG	---G---AAT	TA-----AA	TTTTTT-TTT	TG---GAAA	ATTATA-ACC	AAA-----AAA	TTTGTTTAA	AT-----TTT
MH939277.1 A. m. jementica	..GAATT..C.	..A.TGT..T	---TTTA..A	G-----	..AAA..TACC	TTA---GGG	..AA.CAGG	GT.AATA.TC	..T-.GG.T	..AGATCCA.A
MG552702.1 A. m. scutellata	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
MG552697.1 A. m. capensis	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
MF351881.1 A. m. sahariensis	..GAATT..C.	..A.T-GT..T	---T---A	AG-----	..AAA..-ACC	T---AGGG	..AAC--.G	GT-----T	..C.T-.G.T	..G-----A.C
KY464958.1 A. m. lamarkii	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
KP163643.1 A. m. syriaca	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
KM458618.1 A. m. intemissa	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
MF678581.1 A. m. monticola	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
KY926882.1 A. m. syriaca	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
JQ778284.1 A. m. carnica	..GAATT...A	..A.T-GT..T	---T---A	AG-----	..AAA..-ACC	T---AGGG	..AAC--.G	GT-----T	..C.T-.G.T	..G-----ACC
KX908209.1 A. m. ligustica	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
KY464957.1 A. m. meda	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
KX113622.1 A. mellifera	..GAATT...A	..A.T-GT..T	---T---A	AG-----	..AAA..-ACC	T---AGGG	..AAC--.G	GT-----T	..C.T-.G.T	..G-----ACC
KU571744.1 A. florea	..GATTT...A	..T-GT..T	---T---TA	AG-----	..AAA..-ACC	T---AGGG	..AAC--.G	GT-----T	..C.T-.G.T	..G-----G.C
FJ932648.1 A. dorsata breviligula	..GAATT...A	..T-T..T	---T---A	AG-----	..AAA..-ACC	T---AGGG	..AAC--.G	GT-----T	..C.T-.G.T	..G-----ACC
JQ317319.1 A. laboriosa	..GATTT...A	..T-T..T	---T---TA	AG-----	..AAA..-ACC	T---AGGG	..AAC--.G	GT-----T	..C.T-.G.T	..G-----ACC
KJ396191.1 A. m. mellifera	A..AATT..T	..CC.A..TTT	---TTTGA.T	A-----	..AAA..CA..	TTATAA.T	..ATTA.T	GT.ATATTC	A.ATCC.CCT	..ATTTTAA.A
	510	520	530	540	550	560	570	580	590	600
MH939276.1 A. m. carnica	AAAAAAGGA	A--AATTAA	A-AATTTT--	---AACCC	TTTAAAGGG	GGAATTA	ACCAAGGCG	GGTAAAT--	TAATCCCTT	TTTTTGGGA
MH939277.1 A. m. jementica	..T..G..TA.	..AGG..GGTT	TTGGCGAA--	---CC.T..	GAA..G.TTT	.....T..	..GG..T--	AAA...TT.A	..GGG..GCC	AAG-----
MG552702.1 A. m. scutellata	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
MG552697.1 A. m. capensis	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
MF351881.1 A. m. sahariensis	..T.T.G.TA.	---G..GTTT	GCG.CC.C--	---G.TGTT	GAA.T.A.AT	..A.....GG	CG..GTA--	TT...GTT.T	..G..TG..	CGAC..TTA.
KY464958.1 A. m. lamarkii	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
KP163643.1 A. m. syriaca	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
KM458618.1 A. m. intemissa	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
MF678581.1 A. m. monticola	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
KY926882.1 A. m. syriaca	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
JQ778284.1 A. m. carnica	..T.T.G.TA.	---G..GTTT	GCG.CC.C--	---G.TGTT	GAAC..A.AC	A.CGGCA..	..GA.TTA--	CCG..G..AA	.....	.....
KX908209.1 A. m. ligustica	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.CC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
KY464957.1 A. m. meda	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.CC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
KX113622.1 A. mellifera	..T.T.G.TA.	---G..GTTT	GCG.CC.C--	---G.TGTT	GAA.T.A.AT	..A.....GG	CG..GTA--	TT...GTT.T	..G..TG..	CGAC..---
KU571744.1 A. florea	..T.TTG.TA.	---G..G.TT	GCG.CC.C--	---G.TGTT	GAA.T.A.AT	AA.....	TG..G.A--	TT...T..T	..G..TG..	CGAC..---
FJ932648.1 A. dorsata breviligula	..T.T.G.TA.	---G..G.TT	GCG.CC.C--	---G.TGTT	GAA.T.A.AT	TA.....G	CG..GAA--	TT...TT.T	..G..TG--	.....
JQ317319.1 A. laboriosa	..T.T.G.TA.	---G..G.TT	GCG.CC.C--	---G.TGTT	GAA.T.A.AT	TA.....G	CG..GAA--	TT...TT.T	..G..TG--	.....
KJ396191.1 A. m. mellifera	T..TTG.A.C	TAA.....TT	GATCCA.AAT	AAGG.G.TT	AACATGA.CT	TTT...A.TC	---CCATGA	AA...T.AA	..GGAATT..	AACCAAAAT
	610	620	630	640	650	660	670	680	690	700
MH939276.1 A. m. carnica	---AATTAAAGG	AAATTCCAAT	TAAATTTAAG	GGAAATTTAA	AAAAAGGGAA	ATTGGGTTTT	TTTTGGGCGC	GGAAACCC	CTTTCCGGGA	AAATTTGGGT
MH939277.1 A. m. jementica	---.....T	TT..AAAGG.	TTT...T.A	A.GTTCCG	GTITTCG..	..CCTTT...	..AAAAAAAT	TCCTTTTAA.	..AA.TG..A.	TTT..T..AA
MG552702.1 A. m. scutellata	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
MG552697.1 A. m. capensis	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
MF351881.1 A. m. sahariensis	---CTTACA	TGA..TG.G.	..C..A.CG.C	..T..G.CAG	TTGGTTTCT	TC.ATAA.AA	AA.ATTAAAT	AATTTGA.GA	AAGGA..TTT	..TT..TAA.
KY464958.1 A. m. lamarkii	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
KP163643.1 A. m. syriaca	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
KM458618.1 A. m. intemissa	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
MF678581.1 A. m. monticola	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
KY926882.1 A. m. syriaca	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
JQ778284.1 A. m. carnica	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KX908209.1 A. m. ligustica	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
KY464957.1 A. m. meda	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
KX113622.1 A. mellifera	---...AAT	CT.ACACG..	..TG.G..C.A	ATCG.CG..	GT-----	-----	-----	-----	-----	-----
KU571744.1 A. florea	---...AAT	CT.ACACG..	..TG.G..C.A	ATCG.CG..	GT-----	-----	-----	-----	-----	-----
FJ932648.1 A. dorsata breviligula	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JQ317319.1 A. laboriosa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KJ396191.1 A. m. mellifera	G.TA.T.ATA	..AAAT..A	..TAC..TA	TTT..A..T.	..TTTAAT.T	C.CTA--AA	A.AAAATTT	AATCT.T--A	A.CAATTAA.	T..A.ATAA.
	710	720	730	740	750	760	770	780	790	
MH939276.1 A. m. carnica	TTTTTGGGAA	AAATTTTTTT	AAAAAGGGG	AAAAATTTGG	AAAAAATA	AAAAATTTTT	AAAAAGGGGG	GGCCCGGGG	CCCCAGAGG	TTTTTAA
MH939277.1 A. m. jementica	G.CCCAC...	..AAAA.C	CCCCCG..AA	CGGGGGG.AA	.....T	TTTT.AAGGA	GGTGA.ATTA	T.AATAT..G	GAA.GA..A	..GG..TG.G
MG552702.1 A. m. scutellata	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT...	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
MG552697.1 A. m. capensis	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT...	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
MF351881.1 A. m. sahariensis	..A.TAT...	TT.A.AA.AA	..T.T.TTATA	GTT...TA	C.T..T.GTG	C.TTAA...	..GG.TTTAAT	TATATTAAAT	..TTT..A.TT	AA.A..T..
KY464958.1 A. m. lamarkii	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT.G.	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
KP163643.1 A. m. syriaca	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT.G.	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
KM458618.1 A. m. intemissa	AGA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT.G.	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
MF678581.1 A. m. monticola	AAA.AAT.T	T...A..WGA.	...G..AAAA	TT.T.G.ATA	...T.TT...	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
KY926882.1 A. m. syriaca	AAA.AAT.T	T...A..WGA.	...G..AAAA	TT.T.G.ATA	...T.TT...	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
JQ778284.1 A. m. carnica	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KX908209.1 A. m. ligustica	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT...	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
KY464957.1 A. m. meda	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT...	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.
KX113622.1 A. mellifera	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KU571744.1 A. florea	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
FJ932648.1 A. dorsata breviligula	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
JQ317319.1 A. laboriosa	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
KJ396191.1 A. m. mellifera	AAA.AAT.T	T...A..GG.	...G..AAAA	TT.T.G.ATA	...T.TT.G.	T...A.CCAG	T..TCATC	ATTTT.ACTA	TAA.CTC.TT	..CAC..CT.

**Figure 2.** Sequence alignment of mitochondrial 16S rDNA gene of the honey bee subspecies *Apis mellifera jementica* and *Apis mellifera carnica* with the most closely related Holometabola species within family Apidae. (Only variable sites are shown. Dots represent bases identical to those of the first sequences, and dashes indicate gaps).



**Figure 3.** Molecular phylogenetic analysis by maximum likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-2409.1376) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 34 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 168 positions in the final dataset. Evolutionary analyses were conducted in MEGA6

## DISCUSSION

Apidae is the largest family of bees, with over 5,700 described species (Ascher and Pickering, 2012). There are four different species of honey bees in the world: little honey bee (*Apis florea*) that is native to southeast Asia, the Eastern honey bee (*Apis cerana*) of eastern Asia including Korea and Japan; the Giant honey bee (*Apis dorsata*) native to southeast Asia; and the Western honey bee (*Apis mellifera*) native to Europe, Africa, and western Asia (Daly et al., 1982; Oldroyd and Wongsiri, 2006; Rattanawanee et al., 2010). *Apis mellifera* is commonly known as the European, western, or common honeybee in different parts of the world and is extensively domesticated for honey, pollen, beeswax, propolis, royal jelly, and bee venom (Ratcliffe et al., 2011). It is a major agent of global ecology via pollination (Williams, 2002; Kajobe, 2006; Klein et al., 2007; Allsopp et al., 2008). It is a common model in scientific social behavioral studies (Breed et al., 2004; Solignac et al., 2004; Beye et al., 2006; The Honey Bee Genome Sequencing Consortium, 2006). The current study aimed to determine the genetic diversity and phylogenetic relationships of the *A. mellifera* subspecies of



Saudi Arabia. The *Apis* species are often identified via molecular markers due to the lack of taxonomists skilled in apiculture in Saudi Arabia.

DNA sequence-based identification techniques have revealed the morphological and ecological traits of many species during larval stages (Foltan et al., 2005; Smith et al., 2006; Hayashi and Sota, 2010). Mitochondrial DNA (mtDNA) markers have been widely used to address population and evolutionary questions from the *A. mellifera* honey bee. The sequencing and characterization of the mitochondrial DNA genome has been very useful for analyzing the phylogeny and population genetic structure of the *Apis* species and of the *A. mellifera* subspecies. It contains regions with variable evolutionary rates. In principle, the general pattern of subspecies distribution has been supported by various genetic studies using molecular tools (Garnery et al., 1993; Franck et al., 2000a; 2001; Whitfield et al., 2006; Cánovas et al., 2008). Mitochondrial introgressions can be assessed using the “DraI” test—a molecular test that highlights the sequence variability between subunits I and II of the cytochrome oxidase gene (Garnery et al., 1993). This test has been widely used to analyze the biogeography of *A. mellifera* L. subspecies and races (Rortais et al., 2011) as well as for other *Apis* species (Smith and Hagen, 1997).

We used this method to distinguish different haplotypes and group them into one of the four primary lineages (Ruttner, 1988; De la Rúa et al., 2009). The mitochondrial *16S rDNA* was studied here for the first time to detect the molecular polymorphism between two different subspecies of *Apis mellifera* worker honey bee. Previously, the mitochondrial CoxI–CoxII intergenic region was utilized to distinguish honey bee lineages. Schiff and Sheppard (1995) used mitochondrial DNA and allozyme variations to characterize 142 breeder queen colonies from 22 apiaries in the southeastern United States. In addition, Franck et al. (2000a) described the different haplotypes of the CoxI–CoxII intergenic region found in major honey bee lineages. Kozmus et al. (2007) analyzed the mtDNA of honeybee ecotypes as the initial step of molecular characterization of the indigenous honeybee populations from Serbia. Papachristoforou et al. (2013) combined analysis of mtDNA and microsatellite data from *A. m. cypria* to describe the genetic structure of the Cyprian honey bee population. Techer et al. (2015) analyzed the genetic diversity of the honeybee population in Rodrigues using the mitochondrial CoxI–CoxII intergenic region. Božić et al. (2016) analyzed mtDNA for the characterization of carniolan honey bee *A. M. carnica*.

This study indicated that closely related *Apis* species have 90% similarity in the standardized DNA sequence and distantly related species have less than 90% similarity in the same genes sequence. This agrees with Gurney et al. (2000). The NJ tree was constructed here based on the multiple aligned sequence data for different *Apis* species and other arthropods. The tree separates the genomes into two main clades. All *A. mellifera* species were included in one clade; Chelicerata represented by *Ixodes scapularis* sterol were in another clade. Our results verify the conclusions of Prentice (1998), Melo (1999), Michener (2007), Cardinal et al. (2010), and Haddad et al. (2017) who found that all *Apis* species are clustered together in one clade in addition to the genetic origin of *Apis* species within family Apidae as a paraphyletic group.

Franck et al. (2001) and Cánovas et al. (2008) demonstrated that there are five honey bee lineages found in the Mediterranean Basin. These results indicated that the recovered apid species are deeply embedded within the genus *Apis* within the African lineage. A recent similar study by Haddad et al. (2017) analyzed the mt genome haplotypes and indicated that the north African Sahara Honey bee, *A. m. sahariensis* in the African lineage was genetically distinct from the northern African lineage exemplified by *A. m. intermissa*. The branch length of *A. m. carnica* (gb| MH939276.1) was lower than *A. m. jementica* (gb| MH939277.1) indicating less divergence from its ancestor. This result was confirmed by data from Rukhsana et al. (2014) who indicated that the branch length of the NJ tree is a degree to indicate the divergence from the common ancestor.

## CONCLUSION

A recent field study provides tools for the rapid identification and phylogenetic analysis of honey bees inhabiting Saudi Arabia via mitochondrial *16S rDNA* gene from the recovered *Apis mellifera* subspecies. This yielded a unique genetic sequence that confirms their taxonomic position within the family Apidae.

## COMPETING INTERESTS

The authors have declared that they have no conflict of interest regarding the content of this article.

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## COMPLIANCE WITH ETHICAL STANDARDS

All procedures contributing to this work comply with the ethical standards of the relevant national guides on the are and use of laboratory animals and have been approved and authorized by the Institutional Animal Care and Use Committee (IACUC) at King Saud University, Riyadh, Saudi Arabia.

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