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Short Communication

Molecular Characterization of the Native Honey-Bee Populations in Oman

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Abstract

This study was conducted to characterize the native honey bee populations in Oman based on the *mt*DNA COI–COII intergenic region. Fifty samples of honey bees were collected from 8 Governorates in Oman. Analysis of the samples using the COI–COII intergenic region revealed that all samples belong to the *Apis mellifera*. Polymerase chain reaction analysis of the samples produced the following amplicon sizes (612 bp PoQ, 812 bp PoQQ and 1000 bp PoQQQ). The majority of the samples belonged to the PoQ group (52%) and PoQQ group (42%). Only three samples (6%) belonged to the PoQQQ group. This is the first study that characterized phylogenetic relationship of *Apis mellifera* honey bees from Oman. It indicates the presence of diversity in the population of these honey bees in Oman. © 2020 Friends Science Publishers

Keywords: Bees; Honey; *A. mellifera*; *A. florea;* COI–COII intergenic region

Introduction

Honey is a natural product processed by honey bees when they ingest nectar from flowers (Sajwani *et al.* 2007). The honey bee is an important insect in Oman. Honey bees belong to the family Apidae (Hymenoptera) and they show complete metamorphosis (Yadav *et al.* 2017). Presently, there are ten species of honey bee recognised belonging to the genus *Apis*, including *A. mellifera, A. cerana, A. koschevnikovi, A. florea, A. andreniformis, A. nulensis, A. dorsata, A. binghami, A. nigrocincta* and *A. laboriosa*. Most of these species look alike, with some differences in their colours and sizes. Three species of honey bee are native to Asia and one species is native to the Euro-African region (Yadav *et al.* 2017). Based on sequence analysis, the genus *Apis* was found to cluster into three sections: giant bees, cavity-nesting bees, and dwarf bees (Gupta 2014). *Apis mellifera* has evolved into several subspecies, which are grouped into four evolutionary branches: the European North Mediterranean (C), the West-Mediterranean (M) lineages, the Oriental (O) lineage and the African (A) lineage (Ruttner 1988; Garnery *et al.* 1992; Wallberg *et al.* 2014).

Two species of honey bees are known from Oman; the dwarf honey bee (*A. florea*) which is locally known as "Abu Tuwaiq" and the cavity-nesting bee (*A. mellifera*) locally known as "domesticated Omani honey bee" (Al-Farsi *et al.* 2018). *Apis florea* mostly inhabits in mountains and trees. Bees of this species build a single comb, and their honey is expensive. The domesticated Omani honeybee (*A. mellifera*) was imported from Yemen to Oman before 400 years by the King Saif Bin Sultan Al-Yarubi. Omani beekeepers traditionally use palm trunks to keep the honey of Omani honey bees. In the 1970s, the Ministry of Agriculture and Fisheries of Oman started changing keeping honey bees from palm trunks to Langstroth Beehive. *A. mellifera* produce higher amount of honey compared with *A. florea*. In 2016, there were 100000 hives in Oman producing around 600 tons of honey (Al-Farsi *et al.* 2018).

Based on sequence data, the native honey bee in Saudi Arabia is divided into three clusters (Alattal *et al.* 2014). The Ministry of Agriculture and Fisheries of Oman did an experiment to compare the morphological differences between Omani bees and other bees in the region (Elbassiouny 2009). They found that the length of proboscis in Omani bees is different from Yamani, Carinolic and Italian Bees. However, there are no studies related to the honey bee population in Oman based on the sequence data.

Mitochondrial DNA (mtDNA) has been widely used as a valuable tool in phylogenetic studies of species and subspecies of honey bees (Garnery *et al.* 1992). Phylogenetic analysis of honey bee populations is important as it helps identify the relationship of honey bee populations

from this part of the world to honey populations in other countries. In addition, it helps identify the potential presence of new genetic resources of honey bees for future studies that target the improvement of honey quality and quantity.

Present study describe Omani honey bee populations using *mt*DNA COI–COII intergenic region by comparing them to related honey bees in the Middle Eastern region.

Materials and Methods

Fifty samples of honey bees were collected from 8 governorates in Oman (Al-Dhakhlia, Al-Batinah, Al-Dahira, Al-Sharqia, Al-Buraimi, Musandm, Al-Wusta and Dhofar) (Table 1). The samples were kept in 50 mL tubes with 70% methanol and stored at -20°C.

All the 50 samples were subjected to DNA extraction. However, each sample (one honey bee) was grinded separately and used for DNA extraction according to Al-Sadi *et al*. (2012) to avoid contamination or mixing two phylogenetic populations together. The *mt*DNA COI–COII intergenic region was amplified using the primer pairs E2 (5'-GGCAGAATAAGTGCATTGGGC-3') and H2 (5'-CAATATCATTGATGACCTTA-3') (Cornuet *et al.* 1991; Garnery *et al.* 1992). Polymerase chain reaction (PCR) was performed according to Garnery *et al.* (1992) in an Applied Biosystems Veriti™ 96-Well Thermal Cycler using an illustra PuReTaq Ready-To-Go PCR Beads. The denaturation was at 92°C for 3 min, followed by 30 cycles of 92°C for 30 s, 47°C for 90 s, and 72°C for 45 s. This was followed by a final elongation step of 72°C for 10 min (Garnery *et al.* 1992; Syromyatnikov *et al.* 2018).

Sequencing was conducted using the same primers used in PCR. The sequences from Oman were compared with representative sequences of honey bees in GenBank (National Centre for Biotechnology Information, NCBI). Sequences from this study and refence sequences of 105 *A. mellifera* from GenBank were aligned and optimized manually using MEGA v. 6 (Tamura *et al.* 2013). A maximum likelihood analysis was performed using raxmlGUI version 1.3 (Silvestro and Michalak 2012). The search for the optimal ML tree was conducted with 1,000 separate runs and the bootstrap support values above 50% were displayed on the tree. *Apis mellifera* ligustica (NC 001566) was used as an outgroup. Printing of the resulting trees was done using MEGA v. 6, while Adobe Illustrator CS v.6 was used to prepare the layout.

Results

PCR of the COI–COII intergenic region produced three amplicon sizes: 612 bp, 812 bp and 1000bp, designating for the populations PoQ, PoQQ and PoQQQ, respectively (Fig 1). The *mt*DNA COI–COII intergenic region of 50 honey-bee samples was sequenced. The sequences of 29 representative samples were deposited in GenBank under the accession numbers from MF326653 to MF326681. The majority of the samples in the present study belong to the PoQ sequences (52%) and PoQQ sequences (42%). Only three samples (6%) were found to belong to PoQQQ and these samples were collected from Adam, Al-Jable Al-Akhdar and Dank. Phylogenetic analysis was conducted based on the COI–COII sequence data of 50 individuals from the present study and a total of 105 members of *Apis mellifera*, with *Apis mellifera ligustica* (NC 001566) as the outgroup taxon. Phylogeny results indicated that a haplotype of a single lineage was found in the samples collected from Oman (Fig. 2). All these samples showed haplotypes known from the Oriental (O) evolutionary lineage.

Table 1: Sampling details and their GenBank accession numbers

Sample No.	Location
DH2	Dalkoot
S7	Al Kamel
DH6	Takah
DH3	Rkhioot
S9	Al Kamel
DH1	Dalkoot
DH4	Rkhioot
D5	Adm
H8	Yankul
H5	Dank
S3	Wadi Bani Khalid
H4	Ibri
DH5	Rkhioot
S8	Al Kamel
H6	Ibri
B10	Al-Auabi
S1	Wadi Bani Khalid
DH7	Takah
B6	Al-Rustaq
D6	Adm
S5	Al Mudibi
B3	Shinas
D2	Izki
D1	Izki
H3	Ibri
B4	Sohar
B11	Al-Auabi
B8	Al-Rustaq
S6	Sur
H7	Ibri
B12	Al-Auabi
H1	Ibri
S10	Al Mudibi
B7	Al-Rustaq
S2	Wadi Bani Khalid
BR1	Muhadah
B9	Al-Rustaq
B5	Al-Rustaq
DH8	Murbat
D3	Nizwa
S4	Ibra
B4	Sohar
W1	Mohut
D4	Al-Jable Al-Akhdar
M2	Khasab
M1	Khasab
H2	Ibri
B4	Sohar
D8	Al-Jable Al-Akh
DH10	Salalah

Discussion

Findings from this study show that the Omani populations of honey bees show genetic differences and belong to the Oriental (O) evolutionary lineage. Alattal *et al.* (2014) showed that most honey bee populations from Saudi Arabia

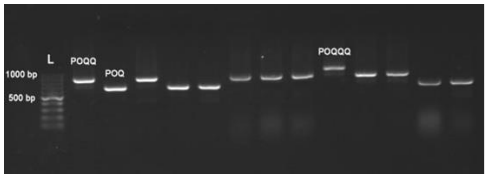

Fig. 1: Structural organization of the COI–COII intergenic region of mtDNA on 1.5% agarose gel. L is the 100 BP ladder; PoQ, PoQQ and PoQQQ sequences correspond to the O lineage

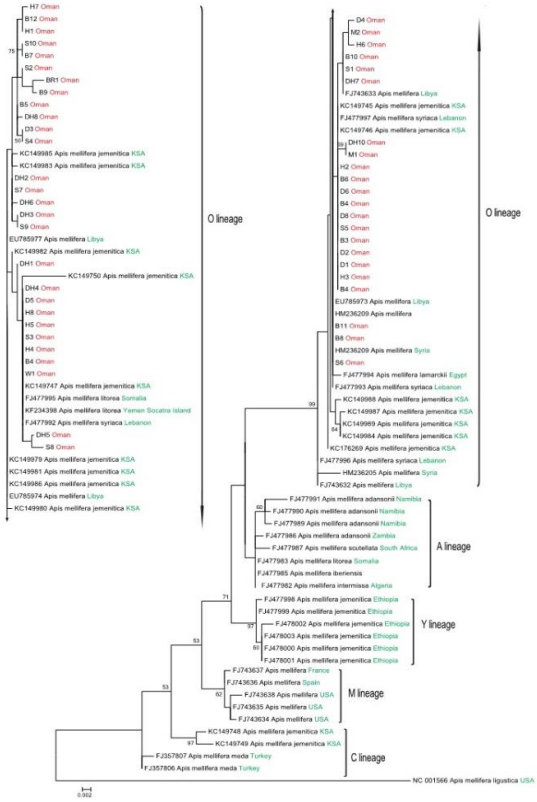

Fig. 2: Maximum likelihood tree revealed by RAxML from an analysis of COI-COII intergentic region sequence data for *Apis mellifera*. RAxML bootstrap supports (≥50%) are given at the nodes and the tree is rooted to *A. mellifera ligustica* (NC 001566)

belonged to the O lineage, with only few belongings to the A lineage. The individuals from Oman clustered populations from Saudi Arabia, Yemen, Lebanon and other countries, which is expected for *Apis mellifera* from this part of the world. However, the Omani populations showed subclustering within the O lineage, which may indicates the presence of genetic diversity among *A. mellifera* population in the country (Techer *et al.* 2015).

Conclusion

This is the first study that characterized phylogenetic relationship of *A. mellifera* honey bees from Oman. It indicates the presence of diversity in the population of these honey bees in Oman. Future studies should address morphological features of these honey bees and elucidate their genetic structure using population genetic analysis.

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Author Contributions

KH Al-Aghbari, HM Al-Sabbari and KS Al-Maani collected samples, extracted DNA and did PCR, SS Maharachchikumbura and AM Al-Sadi did phylogenetic analysis, all authors wrote and approved the manuscript

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