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Original article

Characterization of the native honey bee (*Apis mellifera jemenitica*) in the south western region of Saudi Arabia using morphometric and genetic (mtDNA COI) characteristics

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ABSTRACT

Apis mellifera jemenitica incorporates a few perceived subspecies that vary in their natural properties and farming qualities. Mitochondrial COI gene sequence (mtCOI) has not been used before for bee identification in the southwestern region of Saudi Arabia. The aim of this work was to study the morphometry and analyzing the mtCOI of all collected bees. The nucleotide sequence of the mtCOI gene was analyzed. Similarity searches and distances between each obtained DNA and sequences available in GenBank were made. Morphometric analysis revealed close similarities among the studied bees, but these similarities are different from those previously indicated in earlier studies of the same region. Molecular studies revealed that the collected bees are similar to each other and some other sequences found in GenBank, but these bees are a new hybrid or subspecies that are different from those previously reported in the same region, indicating the emergence of a new hybrid.

1. Introduction

The Western honey bee (*Apis mellifera* L.) is a profoundly variable species, with around 31 perceived subspecies (Chen et al., 2016; Eimanifar et al., 2018; Engel, 1999; Hepburn and Radloff, 1998; Meixner et al., 2011; Ruttner, 1988; Sheppard and Meixner, 2003). Inside subspecies, there are additionally ecotypes and reproducing lines, which are significant regarding the conservation planning and sustainable practice of local strains (Dukku and Danailu, 2020; Ilyasov et al., 2020).

In the Kingdom of Saudi Arabia two races are mainly prevalent; the indigenous or native honey bees (*Apis mellifera jemenitica*) and the carniolan hybrid bees (*Apis mellifera carnica*, imported from Egypt) (Alqarni et al., 2013). AMJ is widespread in many parts of Saudi Arabia as well as in the northern parts of Africa (Al-Ghamdi and Nuru, 2013; Horth et al., 2017). In Saudi Arabia it is found in the south and southwestern areas while the carniolan race is prominent in almost all the regions of the kingdom. AMJ bears some of the unique traits, i.e., smaller size, more slender, shorter setae, and more yellow in coloration. Therefore, it was considered as a distinct ecotype of Saudi populations (Alqarni, 2011; Khan et al., 2017; Ruttner, 1988). Several workers studied key morphological traits while confirming the identity of native honey bees and concluded that the Saudi Arabian race of AMJ is the smallest honey bee with pale coloration compared with other reference honey bee races found in the Arabian region (Ahmed et al., 2012; Alattal et al., 2014; Al-Ghamdi and Nuru, 2013; Alattal et al., 2014a). Overall, Saudi AMJ seems well acclimatized to the unique climatic conditions of this region (Al-Ghamdi et al., 2017, 2020; 2021; Alattal et al., 2014; Alqarni et al., 2013; Taha and AL-Kahtani, 2019). However, this local strain is progressively subject to dissemination by human beekeeping endeavors at a disturbing rate. The local demand for high-yielding honey bee colonies has led to mass importations and colony movements, resulting in endangering the indigenous races and ecotypes by endorsing involuntary hybridization (Alattal et al., 2014; De La Rúa et al., 2009; Meixner et al., 2011). These unique beekeeping practices as well as economically driven processes are very popular in the southwest region in Saudi Arabia, which in the end are bringing about the loss of both genetic variety and specific adaptations to local conditions (Al-Ghamdi et al., 2017; Alqarni et al., 2013; Meixner et al., 2013).

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High genetic diversity among the worker class is important for honey bee survival. The loss of genetic diversity through the inbreeding process is of genuine concern and colonies with decreased hereditary variety are less competent at controlling hive environment (Jones et al., 2004; 2005;; Taha and Al-Kahtani, 2019) and also more prone to develop diseases (Bienefeld et al., 1989; Desai et al., 2015). This decrease in hereditary variety may likewise influence the failure of honey bee populations to adjust to new dangers, such as varroa. Thus, there it is a dire need to recognize and keep up local strains in detached conservation apiaries. Contrasted with other districts, Aseer is the calmest area with a mean greatest temperature of under 32 °C, a base temperature under 2 °C, and the most noteworthy yearly precipitation in Saudi Arabia of 456.6 mm. There are several factors which control the production of honey bee colonies and some of these are internal (e.g., fecundity of queens, race of bees, saved food, beekeeping experience and size of colony) and others are exterior (e.g., temperature, rainfall, humidity, nectar and pollen sources). The adaptation of the bees with their local environment is directly related to bee size, which corresponds to its daily practices such as foraging, manufacture of pollen, brood, and honey (Al-Ghamdi et al., 2017; Alattal and Alghamdi, 2015).

Considering its (AMJ) wide geographical range from Arabia to West Africa, homogeneity is being discussed (Al-Ghamdi et al., 2013; Alattal et al., 2014; Hepburn and Radloff, 1997). There is a pressing need to investigate the differences within the population of AMJ found in several geographical ranges. Subsequently, the point of this work is to assess the morphometric traits of AMJ bees in the southwestern region of Saudi Arabia.

2. Materials and Methods

2.1. Sampling

The area of study (Fig. 1) lies roughly inside 17°E and 42°N and includes southwestern areas of Saudi Arabia, which is partitioned by steep rough mountains into two primary subdivisions, a lowland beach front plain at the west, recognized as "Tihama"(HB-1), and a mountainous zone with a height of 3,005 m highlands at its top at the east, recognized as Aseer Mountains (HB-2,HB-3 and HB-4). The southwestern Aseer highlands constitute one of the most fascinating and unusual ecoregions in the country with its sparkling mountain streams, forests drenched in mist and incredible high-altitude agricultural terracing. These highlands receive variable rainfall particularly during the summer with most rain falling in April/May and July/August and ranges between 600 and 800 mm, rising to over 1,000 mm in the wettest areas. Rainfall in this region is adequate for most crop requirements, as well as for forest cover, estimated at about two million hectares. The natural forest of juniper in the highlands is probably the most extensive anywhere in Arabia. Also in the highlands, there are thickly wooded Acacia valleys of various species. Terraced agriculture growing cereals, several fruits growing orchards and variety of vegetables crops are also very prominent in this region throughout the year.

Despite the fact that the geographical area of the southwestern district of Saudi Arabia is debatable, numerous specialists have thought about it to be related to Afrotropical region, or rather considered as a transitional zone between Palearctic and Afrotropical region.

The present investigation was conducted at the Honey Bee Research Center at King Khalid University, Abha, Saudi Arabia during 2018. Samples of worker honeybees were collected from apiary colonies of four different locations in the southwestern region, Saudi Arabia, labeled as HB-1, HB-2, HB-3, and HB-4, with different altitudes, latitudes, and climatic conditions (Table1, Fig. 1). Free-flying worker bees were collected by using 38 cm diameter sweep net at chest height from 5 colony/apiary (5 workers/colony) and anesthetized with ethyl acetate and preserved in small plastic/killing bottles containing 70% ethyl alcohol and brought to the laboratory until morphometric and genetic analysis could be performed. All bees were dissected and their morphological parts were

Fig. 1. A map of Saudi Arabia showing the area of study (enclosed by the black Rectangle) and sample localities. It comprises southern western part of Saudi Arabia Inset.

Table 1
Geographical details of various places of *A. mellifera jemenitica* collected from southwest region of Saudi Arabia.

Location Code	District	Region	Latitude °N	Longitude °E	No of sample (5 colonies/apiary)
HB-1	Al Shuqaiq	Jezan	17°41'52.1"N	42°03'49.7"E	25
HB-2	Al namas	Aseer	19°07'05.8"N	42°07'33.1"E	25
HB-3	Manhal Jama (Gregar)	Aseer	18°14'46.5"N	42°33'25.4"E	25
HB-4	Sabt Beni Beshr	Aseer	17°56'44.5"N	43°08'17.4"E	25

Table 2
List of the selected morphometric characters measured in this analysis with their Ratner's numbers and abbreviations.

Morphometric traits (mm) except CI and HN	BODY	FLL	PROBL	FEML	TIBL	METL	METW	HLL	HN	T3L +T4L	WMW	MSLEN	FWL	FWW	CI	PMC
Ruttner No.	(9+6)	–	4	5	6	7	8	–	–	10 +11	13	15+16	17	18	29 +30	32+33 +34

Abbreviations: Body size = BODY; Flagellum length = FLL; Proboscis length = PROBL; Femur length = FEML; Tibia length = TIBL; Metatarsus length = METL; Metatarsus width = METW; Hind leg length = HLL; No. of hamuli = HN; Length of metasomal terga III & IV = T3L+T4L; Wax mirror width = WMW; Metasoma slenderness = MSLEN; Forewing length = FWL Forewing width = FWW; Cubital Index = CI; Metasoma Pigmentation (%) = PMC.

mounted on regular glass slides for morphometric analysis under Stereozoom microscope Zeiss apo SV11.

Approximately five bees were collected from four to six randomly chosen marked and unmarked hives within each large apiary (apiaries 1 through 5), and from all hives at apiaries 6 through 9 where every hive was fitted with a marking device. Free-flying bees were collected in the vicinity of each apiary by sweeping at chest height within the perimeter of each apiary for one min using a clean 38 cm diameter sweep net. The bees collected in the sweep nets were transferred into a plastic bag. The bag was sealed, rolled tightly to minimize bee movement within the bag, and immediately frozen on dry ice. All bee samples were placed into a –20° C freezer at the laboratory until analyzed for the presence of marks.

2.2. Morphometric analysis

Collected honey bees of the subspecies *A. mellifera jemenitica* were morphologically identified following Ruttner et al. (1978); (1988;)). Different morphological parts were dissected out, then seated on glass slides and covered with cover slips except tergites and sternites. The later were seated on glass bars so as to preserve their ordinary shape and also to facilitate the procedure of measurement. The slides were dried in the oven at 40 to 45 °C. All measurements were obtained with the help of a stereo binocular microscope supplied with an ocular scale calibrated against a stage micrometer. Measurements of different parameters including wings and every wing venation angle were gained with the help of a slide projector. The following morphometric characters were studied in the present investigation: body size, flagellum length, proboscis length, femur length, tibia length, metatarsus length, metatarsus width, hind leg length, number of hamuli, length of metasomal terga III and IV, wax mirror width, metasoma slenderness, forewing length, forewing width, cubital index, and metasoma pigmentation (%) (Table 2).

2.3. Molecular analysis

Total genomic DNA (gDNA) from all collected bees was extracted using DNeasy® Blood & Tissue extraction kit (QIAGEN) using spin-column protocol. Three legs of each bee were used to extract gDNA (Techer et al., 2017). Integrity of the purified gDNA was checked using 0.5% agarose gel electrophoresis, while its quantity was determined using UV-spectrophotometer (GENESYS™ 10S UV-Vis) at 260/280 nm wavelength according to Sambrook and Green (Dong et al., 2012). Samples were aliquoted and stored at -80 °C till use.

To amplify a part of mitochondrial cytochrome oxidase subunit 1 (mtCOI) gene, identical to "DNA Barcode" area which is used for universal animal identification (Hebert et al., 2003), one primer pair was used, namely LCO1490 (5'-GGTCAACAAATCATAAAGA TATTGG-3', forward primer) and HCO2198 (5'-TAAACTTCAGGGT GACCAAAAAATAC-3', reverse primer) (Folmer et al., 1994). The amplification reaction (25 μL) for each sample was composed 1 μL gDNA (20 ng); 1 μL forward primer (LCO1490, 25 μM), 1 μL reverse primer (HCO2198, 25 μM), 2 μL dNTPs mix (25 mM each, Promega) 2 μL $MgCl_2$ (25 mM, Promega) 2.5 μL 10X PCR-Buffer (without $MgCl_2$; Promega), 0.2 μL *Taq* DNA Polymerase (5 U/μL; Promega) and 15.3 μl purified water. The PCR amplification program was heating at 954 °C for 120 seconds, followed by 35 cycles of denaturation at 92 °C for 60 seconds, annealing at 50 °C for 59 seconds, extension at 72 °C for 60 seconds. The reaction was finalized by heating at 72°C for 10 min. Amplification products were checked using 1.5% agarose gel electrophoresis for size purity.

Products of PCR of each sample were purified using Wizard® SV Gel and PCR Clean-Up System kit (Promega) and then sent to Macrogen (Korea) for DNA sequence analysis using the above-mentioned primers (LCO1490/ HCO2198). Results of DNA sequence analysis were submitted to GenBank.

DNA sequences obtained were trimmed from 5' and 3' ends to remove primer sequences and bad sequence areas using the BioEdit Program. Consensus sequence of forward and reverse sequencing results for each sample were prepared. To search GenBank for similar sequence to the honey bee samples, the BLAST option for nucleotides (BLASTn) was used at https://blast.ncbi.nlm.nih.gov/Blast.cgi. Obtained similar sequences for each honey bee sample were aligned to the corresponding bee sample using MEGA-X program (Version 10.0.5) and results were saved as a MEGA file format. These saved files were used to calculate the distances (pairwise distances) and to make phylogeny (Maximum Likelihood) trees. In addition, distances between HB1-HB4 and phylogeny tree were calculated.

3. Statistical analysis

Statistical analysis of the data was performed through calculating the colony mean, standard deviation and covariance of the morphometric characters. In addition, ANOVA and t-test were applied for testing the significance of the results.

4. Results

4.1. Morphometric analysis

Collected honey bees were morphologically identified and results are shown in Table 3.

Table 3
Results of the selected morphometric characters measured according to Ratner's numbers.

Morphometric traits (mm) except CI and HN	Ruttner No.	HB-1	HB-2	HB-3	HB-4	Average	Mean
BODY	(9+6)	3.91±0.03	3.92±0.03	3.93±0.02	3.94±0.03	3.91–3.95	3.92
FLL	–	2.31±0.01	2.33±0.00	2.29±0.01	2.27±0.00	2.29–2.33	2.30
PROBL	4	5.32 ± 0.01	5.30 ± 0.01	5.32 ± 0.02	5.31 ± 0.00	5.30–5.32	5.31
FEML	5	2.40±0.01	2.39±0.01	2.40±0.02	2.41±0.01	2.39–2.41	2.40
TIBL	6	2.69±0.04	3.00±0.04	2.81±0.02	2.91±0.03	2.69–2.91	2.85
METL	7	2.22±0.02	2.23±0.02	2.24±0.01	2.24±0.01	2.22–2.29	2.23
METW	8	1.01±0.01	1.02±0.01	1.02±0.01	1.01±0.00	1.01–1.02	1.01
HLL	–	7.06 ± 0.03	7.02 ± 0.01	7.01 ± 0.02	7.00 ± 0.03	7.00–7.06	7.02
HN	–	22.3±0.02	22.4±0.02	22.1±0.02	22.6±0.01	22.1–22.7	22.35
T3L+T4L	10+11	3.81 ± 0.05	3.84 ± 0.04	3.82 ± 0.04	3.83± 0.05	3.81–3.84	3.82
WMW	13	2.01±0.01	2.02±0.01	2.01±0.01	2.03±0.01	2.00–2.01	2.01
MSLEN	(15:16)	8.41±0.00	8.42±0.01	8.41±0.01	8.47±0.00	8.40–8.47	8.42
FWL	17	7.90 ± 0.05	7.52 ± 0.05	7.89 ± 0.04	7.66 ± 0.05	7.53–7.90	7.74
FWW	18	3.20±0.03	3.21±0.02	3.22±0.03	3.22±0.05	3.20–3.25	3.21
CI	(29:30)	2.10±0.01	2.19±0.01	2.18±0.01	2.14±0.00	2.10–2.20	2.15
PMC	32+33+34	5.30±0.03	5.29±0.02	5.28±0.05	5.30±0.03	5.29–5.33	5.29

All the character values are given in mm except CI (stated in degrees) and HN (provided

Table 4
GenBank accession numbers of the submitted consensus mtCOI of different worker honey bees.

Bee code	GenBank Accession number
HB1	MK510149
HB2	MK510150
HB3	MK510151
HB4	MK510152

4.2. mtDNA analysis

The submitted consensus COI-HB1-4 sequences got the GenBank Accession numbers as shown in Table 4.

BLAST search of HB-1 DNA sequence showed the presence of several similar sequences. Some of these sequences were aligned with HB-1 and both the distance study and phylogenetic tree (Fig. 2) were calculated.

Calculation of distances revealed that HB-1 and HB-2 are very distant from the sequences listed in Fig. 2. The phylogenetic tree (Fig. 2) revealed that all compared insects cluster in different clade while HB-1, HB-2 and *Apis mellifera sahariensis* are in a clade together. HB-1 and HB-2 show a common ancestor with *Apes mellifera sahariensis* rather than other species present in the tree, but both are still far from *A. mellifera sahariensis* as indicated by the distance study.

A BLAST search of HB-3 consensus sequence showed similar results to those found in GenBank. Distance study revealed that HB-3 is shown to be distant from most of the studied sequences. The phylogenetic tree (Fig. 2) revealed that HB-3 is in the same clade with KT276006.1 *Apis mellifera*, KT275960.1 *Apis mellifera*, MF543447.1 *Apis mellifera*, KP887097.1 *Apis mellifera* clone H091 and KP887077.1 *Apis mellifera* clone H066 and differs from all other sequences.

A BLAST search of HB-4 consensus sequence showed the presence of several similar sequences found in GenBank. Calculation of distance revealed that HB-4 is very close to a clone found in Abha, Saudi Arabia, the MK510153.1 *Apis mellifera* clone-Enas-Hamed-8-Abha clone. The phylogenetic tree (Fig. 2) revealed that HB-4 is related to many of the examined organisms. HB-4 was shown to be arising from different anscistors other than those of HB-1, HB-2 and HB-3.

Distance study (Table 5) among HB1-HB4 revealed that they are distant from each other and is confirmed by the pattern of the phylogenetic tree (Fig. 3).

5. Discussion

Studies on the morphometric of AMJ were carried out in four districts in Aseer region of Saudi Arabia during 2018. The mean estimations of thirteen morphological characters of honey bee workers from the data showed that there were contrasts in estimations of all morphological characters of honey bee workers, among areas as well as other previously studied regions. The acquired results of Aseer honey bees cleared that the gross mean of body length ranged from 3.80mm (Ahmed et al., 2012) to 3.82 (Ruttner, 1988) and 3.91 (present investigation). Probosis length was 5.10 (Ahmed et al., 2012) to 5.31 (Alqarni, 2011) and 5.32mm (Present investigation). The gross mean of FWL was 8.00 mm. The tallest forewing was 8.07 mm (Alqarni, 2011), while the shortest one was found to be 7.90 mm (present investigation). The upper value of hooks (hamuli) number was 22.07 (Alqarni, 2011), while the lower one was 22.3. The gross mean of FL was 2.24 mm. The longest femur was 2.29 mm, while the shortest one was found to be 2.21 mm. The gross mean of TL was 2.82 mm. The longest hind leg is 7.06 mm in current study, while the shortest one was 6.91 mm (Ahmed et al., 2012). The overall mean of Length of tergites 3 & 4 (mm) was found to be 3.81 mm while lowest was 3.75 (Alqarni et al 2011). The metasomal slenderness was found to be 8.41 mm, while the shortest one was found to be 8.37 mm (Ahmed et al., 2012; Alqarni, 2011). Yellow color (%) of the metasoma was highest 5.90, while lowest 5.30 in the present study.

Ruttner (1988) identified 5 populations of *Apis mellifera jemenitica* from Asian and African regions (Somalia, Yemen, Sudan, Chad, Oman and Saudi Arabia), and recognized morphometric variation among the populations of *A. m. jemenitica* of West and East Africa. Also, the morphometric estimates of AMJ of Ethiopia differ considerably from those reported for *A. m. jemenitica* from East and West Africa, especially in the length of hairs and pigmentation. Recently, significant morphometric variations revealed three and eighteen (Alattal et al., 2014a) well-defined clusters of the native honey bee in Saudi Arabia. The comparative analysis of all morphometric data showed a greater variation of Aseer population of AMJ than the AMJ presented in other regions of KSA as well as outside the kingdom. Generally, the obtained results of our study cleared that honey bee populations at southwestern region of Saudi are mixed, which may be due to importation of other strains and genetic introgression with introduced honey bee subspecies. The occurrence of three morphologically unmistakable ecotypes of the local honey

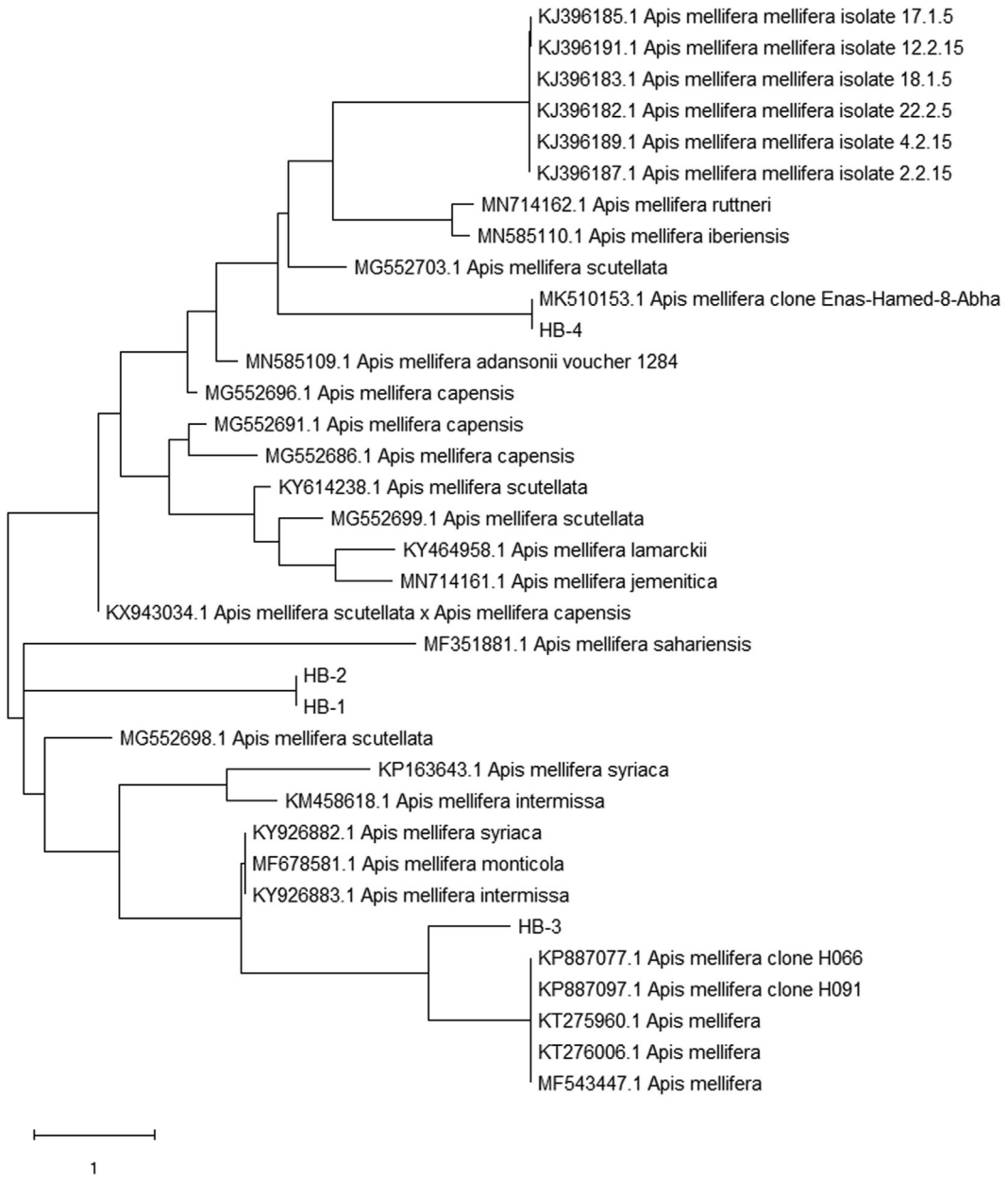

Fig. 2. Phylogenetic tree showing HB-1, HB-2, HB-3, HB-4 and other *Apis mellifera* clones recovered in GenBank.

Table 5
Distances between HB1, HB2, HB3 and HB4.

Bee code	1	2	3	4
1	HB-4			
2	HB-3	0.63766		
3	HB-2	0.71203	0.67692	
4	HB-1	0.71361	0.67580	0.00308

bee, *A.m. jemenitica* were reported in Saudi Arabia. This most likely reflects of the mixing of bees from different areas, as a result of the transport of colonies at the flowering periods of orchards. Most of the beekeepers shifted their colonies from Tihama to the mountainous region of Abha in the search for the flowering region each year, and back to Tihama from November and stayed there till March to avoid harsh winter session in Abha. In Tihama mainly *Acacia* spp. (locally known as sidr, dahiana, and samara) is their main source of their nectar apart from other wild but cultivated crop flowers. Due to these phenomena, the visual difference between the native ecotype and the imported honey bee is becoming obscured. In other words, transient beekeeping, rehearsed on a large scale, particularly in the southwestern region of Saudi Arabia, could make the gene pools of the southwestern region of Saudi bee populations homogenized, and genetic variations are certainly being lost.

Most studies (Al-Ghamdi and Alattal, 2020; Alattal et al., 2014b; Boardman et al., 2020) completed in Saudi Arabia on honey bees used the mtDNA COI–COII intergenic region (now called mtCOII) or HVSI and HVSII segments (Abu-Amero et al., 2008), but in our study we use COI region to compare all sequence with each other and other sequences found in GenBank. The distance studies and phylogenetic trees confirmed the morphometric results and showed that all bees are different from each other (except HB-1 and HB-2) but all are different from previously published species

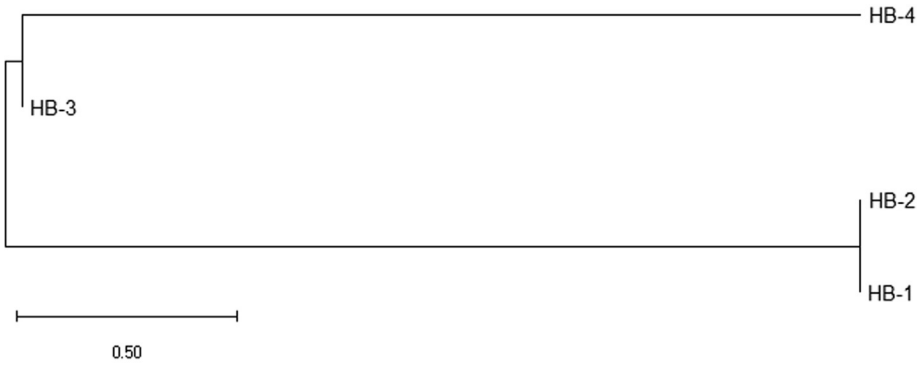

Fig. 3. Phylogenetic tree of HB1, HB2, HB3, and HB4.

and subspecies. In addition, molecular studies provided in our work showed little shared similarities (except HB-1 and HB-2) between the bees collected from different areas in the southwestern region of Saudi Arabia and other published species, indicating that the bees are hybrids of different other species. In contrary, the studied bees are not closely related to the previously studied bees in the same regions indicating the emergence of a new hybrid.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abu-Amero, K.K., Larruga, J.M., Cabrera, V.M., González, A.M., 2008. Mitochondrial DNA structure in the Arabian Peninsula. BMC Evol. Biol. 8, 45. https://doi.org/10.1186/1471-2148-8-45.

Al-Ghamdi, A., Alattal, Y., 2020. The complete mitochondrial genome and phylogenetic analysis of the Arabian Honeybee Apis mellifera jemenitica (Insecta: Hymenoptera: Apidae) from Saudi Arabia. Mitochondrial DNA Part B. 5 (3), 3673–3675.

Al-Ghamdi, A., Nuru, A., 2013. Beekeeping in the Kingdom of Saudi Arabia Past and Present Practices. Bee World 90, 26–29. https://doi.org/10.1080/0005772x.2013.11417527.

Al-Ghamdi, A.K., Khan, A., Al-Abbadi, A.A., Ghramh, H.A., Ahmed, A.M., 2020a. Mohammad Javed Ansari. *In vitro* antagonistic potential of gut bacteria isolated from indigenous honeybee race of Saudi Arabia against *Paenibacillus larvae*. J. Apic. Res. 59 (5), 825–833.

Al-Ghamdi, A. Mariam S. Al-Ghamdi, Ashraf M. Ahmed, Abdel Salam A. Mohamed, Ghada H. Shaker, Mohammad Javed Ansari, Moataza A. Dorrah, Khalid Ali Khan, Tahany H. Ayaad. (2021). Immune investigation of the honeybee Apis mellifera jemenitica broods: a step toward production of a bee-derived antibiotic against the american foulbrood. Saudi J. Biol. Sci. 28, 1528–1538. https://doi.org/10.1016/j.sjbs.2020.12.026.

Al-Ghamdi, A.A., Adgaba, N., Tadesse, Y., Getachew, A., Al-Maktary, A.A., 2017. Comparative study on the dynamics and performances of Apis mellifera jemenitica and imported hybrid honeybee colonies in southwestern Saudi Arabia. Saudi J. Biol. Sci. 24, 1086–1093. https://doi.org/10.1016/j.sjbs.2017.01.008.

Al-Ghamdi, A.A., Nuru, A., Khanbash, M.S., Smith, D.R., 2013. Geographical distribution and population variation of Apis mellifera jemenitica Ruttner. J. Apic. Res. 52, 124–133. https://doi.org/10.3896/ibra.1.52.3.03.

Ahmed, A., Mohammad, A., Yehya, A., Nuru, A., 2012. Morphometric diversity of indigenous honeybees, apis mellifera (linnaeus, 1758), in saudi arabia: (Insecta: Apidae). Zool. Middle East 57, 97–103. https://doi.org/10.1080/09397140.2012.10648968.

Alattal, Y., Aighamdi, A., Alsharhi, M., 2014. Population structure of the Yemeni honey bee (apis mellifera jemenitica) entails an urgent conservation strategy in Saudi Arabia. J. Entomol. 11, 163–169. https://doi.org/10.3923/je.2014.163.169.

Alattal, Yehya, Al Ghamdi, A., Al Sharhi, M., Fuchs, S., 2014a. Morphometric characterisation of the native Honeybee, Apis mellifera Linnaeus, 1758, of Saudi Arabia. Zool. Middle East 60, 226–235. https://doi.org/10.1080/09397140.2014.944431.

Alattal, Y., Alghamdi, A., 2015. Impact of temperature extremes on survival of indigenous and exotic honey bee subspecies, Apis mellifera, under desert and semiarid climates. Bull. Insectology 68, 219–222.

Alattal, Yehya, Alsharhi, M., AlGhamdi, A., Alfaify, S., Migdadi, H., Ansari, M., 2014b. Characterization of the native honey bee subspecies in Saudi Arabia using the mtDNA COI-COII intergenic region and morphometric characteristics. Bull. Insectology 67, 31–38.

Alqarni, A.S., 2011. Beekeeping in Saudi Arabia: current and future. Saudi Society for Agricultural Sciences Riyadh King Saud Univ. 21, 40 (in Arabic).

Alqarni, A.S., Balhareth, H.M., Owayss, A.A., 2013. Queen morphometric and reproductive characters of Apis mellifera jemenitica, a native honey bee to Saudi Arabia. Bull. Insectology 66, 239–244.

Bienefeld, K., Reinhardt, F., Pirchner, F., 1989. Inbreeding effects of queen and workers on colony traits in the honey bee. Apidologie 20, 439–450. https://doi.org/10.1051/apido:19890509.

Boardman, L., Eimanifar, A., Kimball, R.T., Braun, E.L., Fuchs, S., Grünewald, B., Ellis, J. D., 2020. The complete mitochondrial genome of Apis mellifera jemenitica (Insecta: Hymenoptera: Apidae), the Arabian honey bee. Mitochondrial DNA Part B. 5 (1), 875–876.

Chen, C., Liu, Z., Pan, Q., Chen, X., Wang, H., Guo, H., Liu, S., Lu, H., Tian, S., Li, R., Shi, W., 2016. Genomic Analyses Reveal Demographic History and Temperate Adaptation of the Newly Discovered Honey Bee Subspecies Apis mellifera sinisxinyuan n. ssp. Mol. Biol. Evol. 33, 1337–1348. https://doi.org/10.1093/molbev/msw017.

De La Rúa, P., Jaffé, R., Dall'Olio, R., Muñoz, I., Serrano, J., 2009. Biodiversity, conservation and current threats to European honeybees. Apidologie 40, 263–284. https://doi.org/10.1051/apido/2009027.

Desai, S.D., Kumar, S., Currie, R.W., 2015. Occurrence, detection, and quantification of economically important viruses in healthy and unhealthy honey bee (Hymenoptera: Apidae) colonies in Canada. Can. Entomol. 148, 22–35. https://doi.org/10.4039/tce.2015.23.

Dong, L., Lv, L.B., Lai, R., 2012. [Molecular cloning of Tupaia belangeri chinensis neuropeptide Y and homology comparison with other analogues from primates]., Dong wu xue yan jiu = Zoological research / "Dong wu xue yan jiu" bian ji wei yuan hui bian ji. https://doi.org/10.3724/sp.j.1141.2012.01075.

Dukku, U.H., Danailu, G., 2020. An appraisal of subspecific classification of Apis mellifera L. in parts of West and Central Africa through landmark-based geometric morphometric analysis of forewings. J. Apic. Res. 59 (4), 722–729.

Eimanifar, A., Brooks, S.A., Bustamante, T., Ellis, J.D., 2018. Population genomics and morphometric assignment of western honey bees (Apis mellifera L.) in the Republic of South Africa. BMC Genomics 19, 615–641. https://doi.org/10.1186/s12864-018-4998-x.

Engel, M.M.S., 1999. The taxonomy of recent and fossil honey bees (Hymenoptera Apidae *Apis*). J. Hymenopt. Res. 8, 165–196. https://doi.org/http://www.pensoft.net/journals/jhr/.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Hebert, P.D., Cywinska, A., Ball, S.L., DeWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B Biol. Sci. 270, 313–321. https://doi.org/10.1098/rspb.2002.2218.

Hepburn, H.R., Radloff, S.E., 1998. Honeybees of Africa. Honeybees of Africa. https://doi.org/10.1007/978-3-662-03604-4.

Hepburn, H.R., Radloff, S.E., 1997. Biogeographical correlates of population variance in the honeybees (Apis mellifera L) of Africa. Apidologie 28, 243–258. https://doi.org/10.1051/apido:19970501.

Horth, L., Haddad, N., Nasher, A., 2017. Apis mellifera jemenitica in Yemen. Bee World 94, 66–68. https://doi.org/10.1080/0005772x.2017.1345225.

Ilyasov, R.A., Lee, M.L., Takahashi, J.I., Kwon, H.W., Nikolenko, A.G., 2020. A revision of subspecies structure of western honey bee Apis mellifera. Saudi Journal of Biological Sciences 27 (12), 3615.

Jones, J.C., Helliwell, P., Beekman, M., Maleszka, R., Oldroyd, B.P., 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, Apis mellifera. J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol. 191, 1121–1129. https://doi.org/10.1007/s00359-005-0035-z.

Jones, J.C., Myerscough, M.R., Graham, S., Oldroyd, B.P., 2004. Honey bee nest thermoregulation: diversity promotes stability. Science (80-.) 305, 402–404. https://doi.org/10.1126/science.1096340.

Khan, K.A., Ansari, Mohammad Javed, Al-Ghamdi, Ahmad, Nuru, Adgaba, Harakeh, Steve, Iqbal, Javaid, 2017. Investigation of gut microbial communities associated with indigenous honey bee (*Apis mellifera jemenitica*) from two different eco-regions of Saudi Arabia. Saudi J. Biol. Sci. 24 (5), 1061–1068.

Meixner, M.D., Leta, M.A., Koeniger, N., Fuchs, S., 2011. The honey bees of Ethiopia represent a new subspecies of Apis mellifera-Apis mellifera simensis n. ssp. Apidologie 42, 425–437. https://doi.org/10.1007/s13592-011-0007-y.

Meixner, M.D., Pinto, M.A., Bouga, M., Kryger, P., Ivanova, E., Fuchs, S., 2013. Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. J. Apic. Res. 52, 1–28. https://doi.org/10.3896/IBRA.1.52.4.05.

Ruttner, F., 1988. Evolution, in: Biogeography and Taxonomy of Honeybees. Springer Berlin Heidelberg, pp. 20–36. https://doi.org/10.1007/978-3-642-72649-1_3.

Ruttner, F., Tassencourt, L., Louveaux, J., 1978. Biometrical-statistical analysis of the geographic variability of *Apis mellifer* A L I. Material and Methods. Apidologie 9, 363–381. https://doi.org/10.1051/apido:19780408.

Sheppard, W.S., Meixner, M.D., 2003. Apis mellifera pomonella, a new honey bee subspecies from Central Asia. Apidologie 34, 367–375. https://doi.org/10.1051/apido:2003037.

Taha, E.K.A., AL-Kahtani, S.N., 2019. Comparison of the activity and productivity of Carniolan (Apis mellifera carnica Pollmann) and Yemeni (Apis mellifera jemenitica Ruttner) subspecies under environmental conditions of the Al-Ahsa oasis of eastern Saudi Arabia. Saudi J. Biol. Sci. 26, 681–687. https://doi.org/10.1016/j.sjbs.2017.10.009.

Techer, M.A., Clémencet, J., Simiand, C., Preeaduth, S., Azali, H.A., Reynaud, B., Hélène, D., 2017. Large-scale mitochondrial DNA analysis of native honey bee Apis mellifera populations reveals a new African subgroup private to the South West Indian Ocean islands. BMC Genet. 18, 53–74. https://doi.org/10.1186/s12863-017-0520-8.