

Characterization of Socotra (Yemen) honey bees (*Apis mellifera*) using morphometric and genetic markers

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Abstract

The honey bee (*Apis mellifera* L.) from Socotra archipelago (Yemen) has been characterized based on 32 morphometrical characters and on the sequence analysis of the tRNA^{Leu} - COII intergenic region of the mtDNA. Morphometric clustering by discriminant analysis grouped almost all honey bees from Socotra (93.8%) with *Apis mellifera litorea* Smith reference samples; however one of these samples was allocated with *Apis mellifera jemenitica* Ruttner (6.2%). Consistently, sequences of the tRNA^{Leu} - COII intergenic region of the Socotran samples (n = 2) were identical with Somalian honey bee (Acc: O1d FJ477995, *A. m. litorea*), and the sequences had a *P*_o element and one sequence repeat of the *Q* element. This indicates that the honey bee from Socotra belongs to the Z subgroup of the A lineage (formerly was part of the O lineage). Morphometric and sequence analyses clearly supports the statement that the native honey bee of Socotra is not the same as the bees called *A. m. jemenitica* from, Ethiopia, Yemen, Chad, Oman, Somalia, Saudi Arabia, Uganda, and Sudan, which were used as reference samples in this study.

Key words: Socotra, *Apis mellifera litorea*, morphometrics, mtDNA, Yemeni honey bee, Oriental lineage.

Introduction

Morphometric and molecular markers have been used successfully in the intra-specific characterization of the honey bee (*Apis mellifera* L.) worldwide. Five main lineages: the Oriental lineages (O), the African lineage (A), the north Mediterranean lineage (C), the western Mediterranean lineage (M) and (Y) lineage from Ethiopia (Ruttner, 1988; Engel, 1999; Franck *et al.*, 2001; Sheppard and Meixner 2003; Whitfield *et al.*, 2006, Alburaki *et al.*, 2011; Fontana *et al.*, 2018) are recognized based on molecular markers. Socotra (Yemen) is the largest, biologically most diverse archipelago in the Arabian region and is well-known for its exceptional biodiversity and endemism (Van Damme and Banfield, 2011). For example, 73% of the Isopod species and 95% of terrestrial mollusks in Socotra are endemic (Taiti and Ferrara, 2004; Neubert 2006; 2009). The Socotra honey bee *A. mellifera* is aggressive, and beekeeping is still traditional in the archipelago; only recently, beekeepers started swarm-trapping and used locally available materials to construct hives. Alkathiri and Khanbash (2009) compared several morphological traits of the indigenous honey bee of Socotra with honey bee samples collected from mainland Yemen; they found nonsignificant colour and size variations between both populations, thus they mentioned that Socotra honey bee is an aggressive phenotype of the Yemeni honey bee *Apis mellifera jemenitica* Ruttner (Khanbash, 2003). Though, at no time were the data of the Socotra bees compared with other honey bee subspecies from neighbouring countries such as the Litorean honey bee *Apis mellifera litorea* Smith from Ethiopia. Other studies (Alattal *et al.*, 2014a; 2014b; Alqarni, 2011), based on classical morphometric and molecular approaches, identified many honey bee samples from neighbouring countries such as: Yemen, Somalia, Oman and Saudi Arabia as *A. m. jemenitica*, but distinction were less in relation to *A. m. litorea*.

Alghamdi *et al.* (2015) reported significant colour and size variation between African and Asian populations of *A. m. jemenitica*. Recently, honey bee samples from Saudi Arabia and Yemen were assigned to Y lineage (Cridland *et al.*, 2017). In this study morphometric analysis and sequence analysis of the tRNA^{Leu} - COII region of the mtDNA (Estoup *et al.*, 1995; Franck *et al.*, 2001; Whitfield *et al.*, 2006) were used to characterize the honey bee of Socotra and to determine its lineage.

Materials and methods

Sixteen honey bee samples of 20 workers each were taken from sixteen local colonies at 9 locations within Socotra Island (figure 1). To perform morphometric studies, 10 workers from each sample were preserved in 70% ethanol and were then dissected according to Ruttner *et al.* (1978). Body parts were mounted on slides, which were then scanned using a high resolution scanner (600 dpi) connected to desk-top computer system supported with image tool software (Image tool[®] 3.0). Classical morphological traits associated with the honey bee size, wing angles and cuticle pigmentations were used in this study (Goetze, 1964; Ruttner, 1988). In total, thirty two morphometric characteristics reported previously by Ruttner (1988) as highly discriminatory, were measured (table 1). Colony sample means were calculated for each character of each bee sample. Afterwards, reference data representing the measurements of the corresponding characters for seven other *A. mellifera* subspecies from nine countries (Somalia, Chad, Yemen, Kenya, South Africa, Tanzania, Mozambique, Saudi Arabia and Jordan) were obtained from Oberursel Bee Research Institute (Frankfurt, Germany) and were included in the data set (table 2). Subsequently, discriminant analysis using Wilk's lambda was used to verify reallocation probabilities and cluster distances. Analysis was performed using PASW 18 (2009).

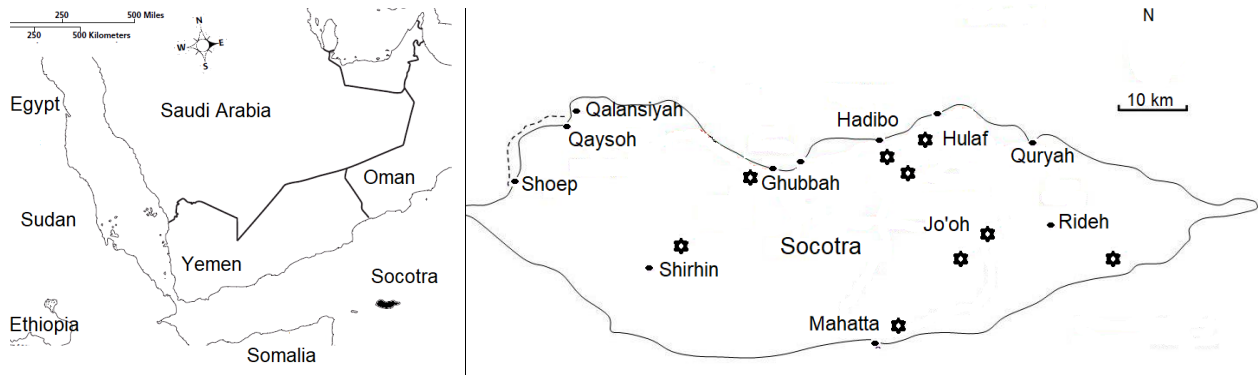


Figure 1. Map of the Arabian Peninsula (left) showing some neighbouring countries; (right) map of Socotra Island showing sampling locations (★), (Hadibo: n = 4: 12°38'N 54°24'E), (Ghubbah: n = 3: 12°35'N 53°46'E), (Mahatta: n = 1: 12°21'N 54°25'E), (Rideh: n = 3: 12°28'N 54°16'E), (Hulaf: n = 1: 12°39'N 54°75'E), (Shirhin: n = 1: 12°28'N 53°41'E), (Jo'oh: n = 3: 12°30'N 54°81'E).

Table 1. Means ($M \pm SD$) of 32 morphometric characteristics measurements used for the characterization of the Socotran honey bee (n = 16) according to Ruttner (1988). Length measurements were given in mm.

Characteristic	Mean	SD
Proboscis (prob)	559.63	13.286
Length femur (fem)	246.51	5.920
Length tibia (tib)	292.32	6.857
Length metatarsus (ltar)	185.50	5.571
Width metatarsus (wtar)	105.33	2.681
Pigment tergite 2 (pt2)	9.18	0.243
Pigment tergite 3 (pt3)	8.40	0.311
Pigment tergite 4 (pt4)	6.12	1.440
Length tergite 3 (lt3)	200.52	5.302
Length tergite 4 (lt4)	194.68	5.268
Length sternite 3 (lst3)	251.94	7.973
Length wax mirror (lwm)	129.65	6.166
Width wax mirror (wwm)	203.11	7.457
Length sternite 6 (lst6)	233.01	6.590
Width sternite 6 (wst6)	273.67	9.734
Length forewing (lfw)	847.83	15.510
Width forewing (wfw)	297.46	4.740
Cubital 1	48.99	2.315
Cubital 2	24.16	0.877
Wing angle (a4)	30.65	1.101
Wing angle (b4)	104.21	2.973
Wing angle (d7)	100.56	1.581
Wing angle (e9)	20.51	0.599
Wing angle (g18)	95.24	2.042
Wing angle (j10)	49.33	1.082
Wing angle (j16)	90.77	2.286
Wing angle (k19)	77.94	2.335
Wing angle (l13)	14.52	0.596
Wing angle (n23)	85.33	1.402
Wing angle (o26)	39.67	1.943
Complete leg	726.09	16.913
Body size (Lt3lt4)	391.44	10.070

For *mtDNA* fingerprinting, samples were preserved in absolute ethanol. Then, total genomic DNA was extracted from thoraces of single adult bee worker per colony (n = 2) using DNeasy Blood & Tissue Kit (Qiagen, Valencia, California). The tRNA^{Leu} - COII intergenic region of the *mtDNA* was amplified using gene specific primers (E2) 5'-GGCAGAATAAGTGCATTG-3', (H2) 5'-CAATATCATTGATGACC-3' (Cornuet *et al.*, 1991; Garnery *et al.*, 1992) by GeneAmp 9700 thermocycler (Applied Biosystems) as described by Garnery *et al.* (1992), with minor modifications. PCR products were then sequenced in both directions using an automated 96 capillary ABI 3730XI DNA analyser (Applied Biosystem). After that, sequences were manually checked and assembled using Geneious® (Drummond *et al.*, 2011), and were aligned using CLUSTALW (Thompson *et al.*, 1994). To explore similarity matches, variable sequences were then analysed with Basic Local Alignment Search Tool (BLAST) search program (National Center for Biotechnology Information site - NCBI), and compared with other sequences available in GenBank (table 3), then the number of polymorphic sites were determined between different subspecies (<http://www.ncbi.nlm.nih.gov/>) using Geneious® 5.2.2.

Results and discussion

Discriminant analysis of reference groups confirmed their reallocation to their original subspecies (n = 73, 100%). However, in cross-validated grouping two sets of measurements of the reference *A. m. jemenitica* (n = 18, 11%) were allocated with *A. m. litorea* group. Fifteen samples (93.8%) of the Socotra honey bees analysed in this study grouped with *A. m. litorea* reference group. One of the samples was allocated with *A. m. jemenitica* (6.2%; figure 2a). Although, the analysis shows higher proximity between our samples and reference samples from neighbouring subspecies, Socotra honey bees are clearly not like *Apis mellifera syriaca* Skorikov and most of them are outside of the cloud

Table 2. Name, number (N) and origin of reference honey bee subspecies included in the morphometric analysis of Socotran honey bee, data were obtained from Oberursel Bee Research Institute, Frankfurt, Germany.

Subspecies	(N)	Bees/Colony	Country
<i>A. m. carnica</i> Pollmann	(20)	20	Hungary, Romania, Yugoslavia, Austria
<i>A. m. ligustica</i> Spinola	(11)	20	Italy
<i>A. m. meda</i> Skorikov	(8)	20	Turkey, Iran, Iraq
<i>A. m. syriaca</i> Skorikov	(9)	20	Jordan, Palestine, Lebanon
<i>A. m. lamarckii</i> Cockerell	(7)	20	Egypt
<i>A. m. jemenitica</i> Ruttner	(18)	20	Yemen, Chad, Oman, Somalia, Saudi Arabia, Uganda, Sudan
<i>A. m. litorea</i> Smith	(10)	20	Tanzania, Kenya, Mozambique, South Africa

Table 3. Comparison of sequence of the tRNA^{Leu}-COII region (P and Q fragments) among the Socotran honey bee and seven other honey bee subspecies. *The number of the nucleotide at the sequence where variation took place.

Nucleotide No. having variation	Sequence variation in P element										Sequence variation in Q element																					
	*25	29	31	35	37	49	54	55	3427	3428	3429	3430	3442	3443	3451	3455	3464	3475	3431	3493	3494	3495	3496	3498	3519	3571	3574	3575	3581	3593	3600	3608
O1d FJ477995 (<i>A. m. litorea</i>) (Somalia) (Socotran sampl. n = 2)	T	A	A	C	A	T	-	C	T	C	C	-	T	A	A	A	A	T	A	T	T	-	-	-	A	A	-	-	T	G	T	C
A27FJ477983 (<i>A. m. litorea</i>) (Somalia)	C	A	A	C	A	T	-	T	C	C	C	C	T	A	A	-	-	T	T	A	T	-	-	-	T	A	-	-	C	G	-	T
O1cFJ477992 (<i>A. m. syriaca</i>) (Syria)	T	A	A	C	A	T	-	C	T	C	C	-	T	A	A	A	-	T	A	T	T	A	A	-	A	A	-	-	T	G	T	C
O1cFJ477994 (<i>A. m. lamarckii</i>) (Egypt)	T	T	A	C	G	T	-	C	T	C	C	-	T	A	A	A	A	T	A	T	T	A	A	-	A	A	-	-	T	G	T	C
Y1FJ478000 (<i>A. m. jemenitica</i>) (Ethiopia)	T	A	T	T	A	A	A	T	T	C	A	-	T	A	T	A	A	T	T	A	T	-	-	-	T	A	-	-	C	G	T	T
C1NC001566 (<i>A. m. ligustica</i>) (USA)	complete deletion of the P								T	C	C	C	T	A	A	A	-	T	T	A	-	-	-	T	A	A	-	-	C	G	T	T
C2Gfj357807 (<i>A. m. meda</i>) (Turkey)	complete deletion of the P								T	C	C	-	-	A	A	A	-	T	T	A	T	-	-	-	A	T	-	-	C	G	T	T

points for *Apis mellifera lamarckii* Cockerell, *A. m. jemenitica* and *A. m. litorea* (figure 2b). Table 4 shows the Euclidean distances values between the Socotran honey bee samples and the seven other reference groups used in this study.

Sequence analysis of the PCR amplified tRNA^{Leu}-COII intergenic region of Socotra samples (n = 2, analysed) had a sequences of 598 bp length each, with one identical combination of the P₀ and Q sequences (tRNA^{Leu} = 62, P = 68 bp and Q = 198 bp, COII = 274), without any sequence variation between both samples. This Socotra haplotype sequence is exactly identical to a haplotype from Somalia (O1d: acc. FJ477995) (Franck *et al.*, 2000). This indicates that, the honey bee from Socotra belongs to the same lineage "Z lineage: formerly part of the O lineage". As well, sequence variation within the P and Q element was very minimal between the Socotra honey bee haplotype and one of the Syrian honey bee haplotypes, *A. m. syriaca*, and the Egyptian haplotype, *A. m. lamarckii*, (table 1). More sequence variation in the P and the Q element was found between Socotra honey bees haplotype and the other Somalian honey bee haplotype, *A. m. litorea*, a member of the A lineage, and the Yemeni haplotype from Ethiopia, a member of the Y lineage (Franck *et al.*, 2001) (table 1).

Table 4. The Euclidean distance between the Socotran honey bee samples and the seven other reference subspecies based on analysis of morphometric data of 32 characters.

Apis mellifera subspecies	Euclidean distance						
	<i>A. m. litorea</i>	<i>A. m. jemenitica</i>	<i>A. m. lamarckii</i>	<i>A. m. syriaca</i>	<i>A. m. meda</i>	<i>A. m. ligustica</i>	<i>A. m. carnica</i>
Socotra samples	9.7	10.8	14.7	15.3	11.8	14.5	21.0
<i>A. m. carnica</i>	17.5	19.7	19.8	19.1	15.0	10.7	0.0
<i>A. m. ligustica</i>	11.3	13.1	13.9	12.8	7.0	0.0	
<i>A. m. meda</i>	9.0	11.4	12.8	9.6	0.0		
<i>A. m. syriaca</i>	10.6	12.2	11.7	0.0			
<i>A. m. lamarckii</i>	9.5	7.3	0.0				
<i>A. m. jemenitica</i>	4.3	0.0					
<i>A. m. litorea</i>	0.0						

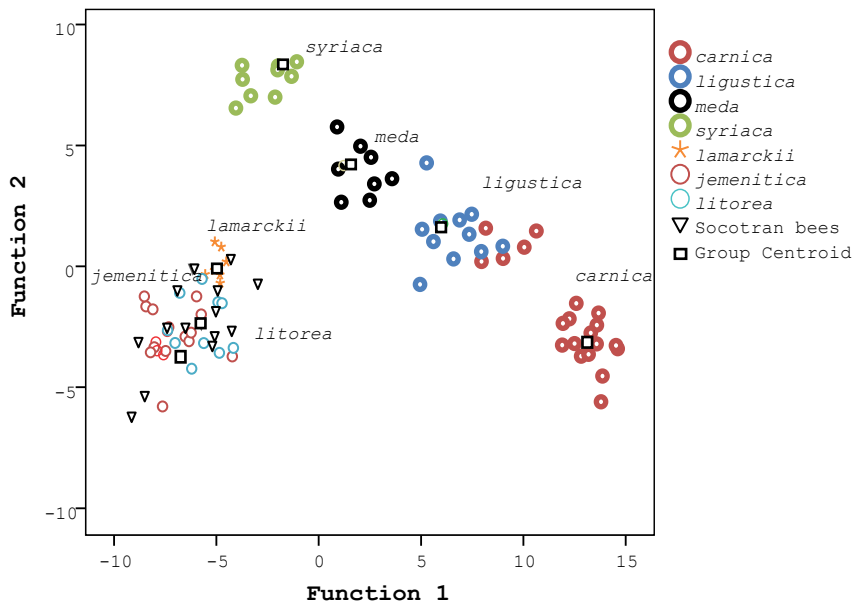
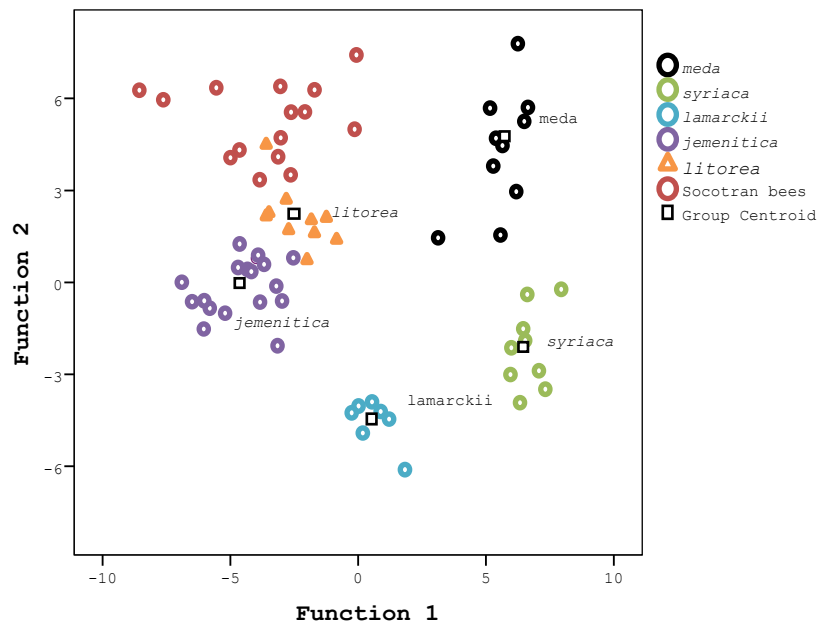
A**B**

Figure 2. A): Discriminant analysis of morphological data. Samples include the Socotran honey bee data ($n = 16$) and the data for the seven reference subspecies obtained from the state institute for beekeeping in Frankfurt. **B):** Only subspecies with high similarity with the Socotran honey bees were included in the analysis.

However, the morphometric reference data for the Litorean honey bees were collected from countries other than Somalia, while the genetic data in the NCBI were obtained from samples collected from Somalia. This may explore the need for further analysis of the Litorean honey bee from Somalia. Yet, we have no reports if Litorean honey bees are found in the Arabian Peninsula. However, morphometric data based on big honey

bee sample sizes described the local honey bee of Saudi Arabia as *A. m. jemenitica* (Alattal *et al.*, 2014a). Our results supports the statement that the native honey bee of Socotra is not the same as the bees called *A. m. jemenitica* from, Ethiopia, Yemen, Chad, Oman, Somalia, Saudi Arabia, Uganda, and Sudan, which were used as reference samples in this study.

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