

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/302827544>

Characterization of the Socotran Honeybee (*Apis mellifera* Linnaeus, 1758) using Morphometric and Genetic Markers (Insecta: Apidae)

Conference Paper · November 2013

CITATIONS

0

READS

145

1 author:



Dr. Mohamad Alsharhi

Thamar university

19 PUBLICATIONS 58 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Morphometric and genetic characterization of the Socotran honeybee (Hymenoptera: Apidae) [View project](#)



توصيف المصادر النباتية والجغرافية للأعسالى اليمنية [View project](#)

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

Yehya ALATTAL¹, Mohamad ALSHARHI¹, Ahmed ALGHAMDI¹, Stephan FUCHS²

¹Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

²Institut für Bienenkunde, Polytechnische Gesellschaft, Goethe-University, Oberursel, Frankfurt, Germany

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).

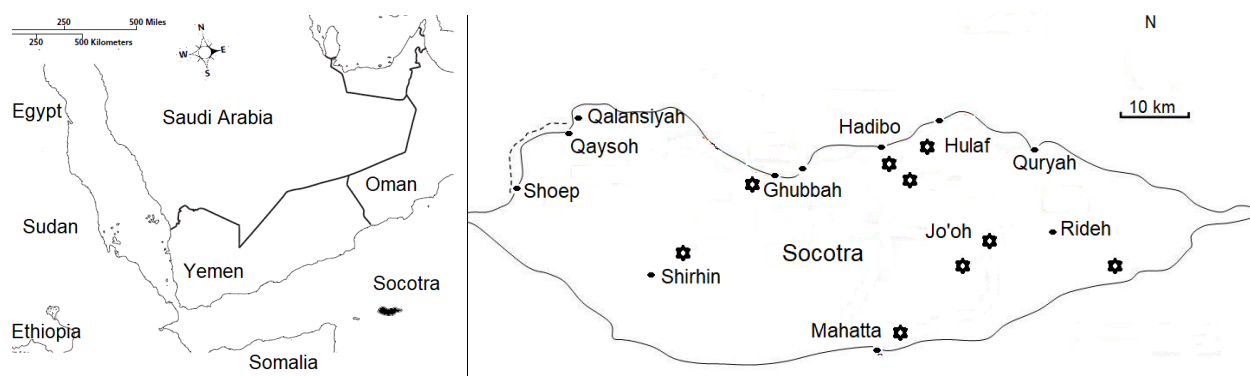


Figure 1. Map of the Arabian Peninsula (left) showing some neighbouring countries; (right) map of Socotra Island showing sampling locations (★), (Hadibo: n = 4: 12°38'N 54°24'E), (Ghubbah: n = 3: 12°35'N 53°46'E), (Mahatta: n = 1: 12°21'N 54°25'E), (Rideh: n = 3: 12°28'N 54°16'E), (Hulaf: n = 1: 12°39'N 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
Samples with related genotype (Gene bank No., Name, origin)	*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 plus deletion from nt 6-22	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

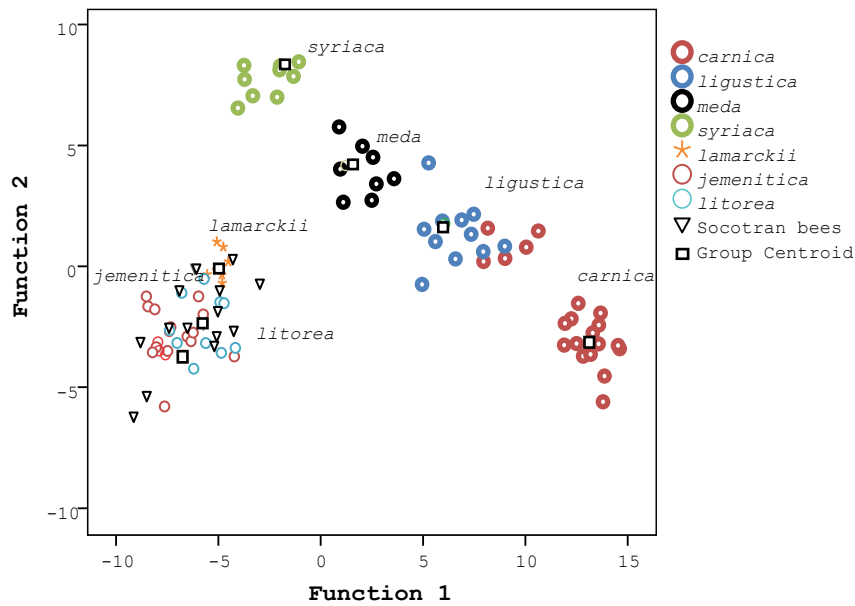
points for *Apis mellifera lamarckii* Cockerell, *A. m. jemenitica* and *A. m. litorea* (figure 2b). Table 4 shows the Euclidean distances values between the Socotran honey bee samples and the seven other reference groups used in this study.

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.

	Euclidean distance						
<i>Apis mellifera</i> subspecies	<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

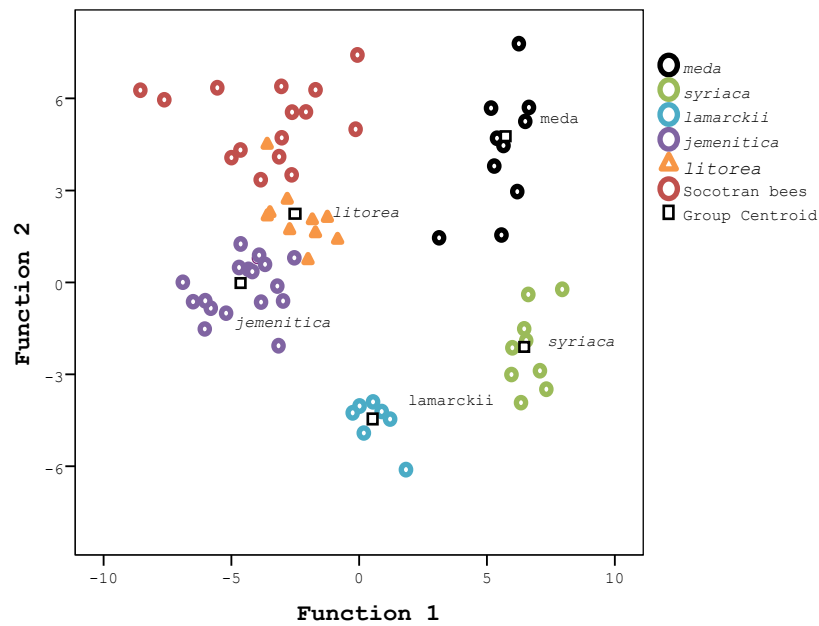


Figure 2. A): Discriminant analysis of morphological data. Samples include the Socotran honey bee data (n = 16) and the data for the seven reference subspecies obtained from the state institute for beekeeping in Frankfurt. **B):** Only subspecies with high similarity with the Socotran honey bees were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

Acknowledgements

We wish to thank Oberursel Bee Research Institute (Frankfurt, Germany) for providing us with reference data for the several bee subspecies used for comparison in this article. This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award No. (11-BIO 1743-2).

References

- ALATTAL Y., ALGHAMDI A., ALSHARHI M., FUCHS S., 2014a.- Morphometric characterisation of the native honeybee, *Apis mellifera* Linnaeus, 1758, of Saudi Arabia.- *Zoology in the Middle East*, 60 (3): 226-235.
- ALATTAL Y., 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.- *Bulletin of Insectology*, 67 (1): 31-37.
- ALBURAKI M., MOULIN S., LEGOUT H., ALBURAKI A., GARNERY L., 2011.- Mitochondrial structure of Eastern honeybee populations from Syria, Lebanon and Iraq.- *Apidologie*, 42: 628-641.
- AL-GHAMDI A., ADGABA N., KHANBASH M. S., SMITH D. R., 2013.- Geographical distribution and population variation of *Apis mellifera jemenitica* Ruttner.- *Journal of Apicultural Research*, 52: 124-133.
- ALKATHIRI H., KHANBASH M., 2009.- Studies on the Socotran honeybees *Apis mellifera jemenitica* in wadi Hadhramout, Yemen.- *The Sixth International Arab Beekeepers Union Conference*, 17-19 March 2009, Abha, Saudi Arabia.
- ALQARNI A., HANNAN M., OWAYSS A., ENGEL M., 2011.- The indigenous honey bees of Saudi Arabia (Hymenoptera, Apidae, *Apis mellifera jemenitica* Ruttner): their natural history and role in beekeeping.- *ZooKeys*, 134: 83-98.
- CORNUET J. M., GARNERY L., SOLIGNAC M., 1991.- Putative origin and function of the intergenic region between COI and COII of *Apis mellifera* L. mitochondrial DNA.- *Genetics*, 128 (2): 393-403.
- CRIDLAND J. M., TSUTSUI N. D., RAMÍREZ S. R., 2017.- The complex demographic history and evolutionary origin of the Western honey bee, *Apis mellifera*.- *Genome Biology and Evolution*, 9 (2): 457-472.
- DRUMMOND A. J., ASHTON B., BUXTON S., CHEUNG M., COOPER A., DURAN C., FIELD M., HELED J., KEARSE M., MARKOWITZ S., MOIR R., STONES-HAVAS S., STURROCK S., THIERER T., WILSON A., 2011.- *Geneious v5.4*.- [online] URL: <http://www.geneious.com/>
- ENGEL M., 1999.- The taxonomy of recent and fossil honey bees.- *Journal of Hymenoptera Research*, 8: 165-196.
- ESTOUP A., GARNERY L., SOLIGNAC M., CORNUET J., 1995.- Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models.- *Genetics*, 140 (2): 679-695.
- FONTANA P., COSTA C., DI PRISCO G., RUZZIER E., ANNOSCIA D., BATTISTI A., CAODURO G., CARPANA E., CONTESSI A., DAL LAGO A., DALL'OLIO R., DE CRISTOFARO A., FELICOLI A., FLORIS I., FONTANESI L., GARDI T., LODESANI M., MALAGNINI V., MANIAS L., MANINO A., MARZI G., MASSA B., MUTINELLI F., NAZZI F., PENNACCHIO F., PORPORATO M., STOPPA G., TORMEN N., VALENTINI M., SEGRÈ A., 2018.- Appeal for biodiversity protection of native honey bee subspecies of *Apis mellifera* in Italy (San Michele all'Adige declaration).- *Bulletin of Insectology*, 71 (2): 257-271.
- FRANCK P., GARNERY L., SOLIGNAC M., CORNUET J. M., 2000.- Molecular confirmation of a fourth lineage in honeybees from the Near East.- *Apidologie*, 31 (2): 167-181.
- FRANCK P., GARNERY L., LOISEAU A., OLDROYD B. P., HEBURN H. R., SOLIGNAC M., CORNUET J. M., 2001.- Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data.- *Heredity*, 86 (4): 420-430.
- GARNERY L., CORNUET J. M., SOLIGNAC M., 1992.- Evolutionary history of the honey bee *Apis mellifera* L. inferred from mitochondrial DNA analysis.- *Molecular Ecology*, 1 (3): 145-154.
- GOETZE G. K. L., 1964.- *Die Honigbiene in natürlicher und künstlicher Zuchtauslese. Teil I, II*.- Parey, Hamburg, Germany.
- KHANBASH M. S., 2003.- Studies on Socotran honeybee *Apis mellifera jemenitica* in wadi Hadhramout, Yemen, pp. 101-110. In: *The second international symposium on strategic development of Socotra and other Yemeni Islands*.
- NEUBERT E., 2006.- Land snails of the Socotra Archipelago, pp. 125-127. In: *Socotra - a natural history of the islands and their people* (CHEUNG C., VAN DAMME K., DEVANTIER L., Eds).- Odyssey Books and Guides, Hong Kong.
- NEUBERT E., 2009.- The continental malacofauna of Arabia and adjacent areas: VI. Pomatiidae of Arabia, Socotra and Northeast Africa, with descriptions of new genera and species (Gastropoda: Caenogastropoda: Littorinoidea).- *Fauna of Arabia*, 24: 47-127.
- PASW, 2009.- *SPSS for Window release 118.0.0*.- SPSS Inc., Chicago, USA.
- RUTTNER F., 1988.- *Biogeography and taxonomy of honeybees*.- Springer-Verlag, Berlin, Germany.
- RUTTNER F., TASSENCOURT L., LOUVEAUX J., 1978.- Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. I. Material and methods.- *Apidologie*, 9 (4): 363-381.
- SHEPPARD W. S., MEIXNER M. D., 2003.- *Apis mellifera pomonella*, a new honey bee subspecies from Central Asia.- *Apidologie*, 34 (4): 367-376.
- TAITI S., FERRARA F., 2004.- The terrestrial Isopoda (Crustacea: Oniscidea) of the Socotra Archipelago.- *Fauna of Arabia*, 20: 211-325.
- THOMPSON J. D., HIGGINS D. G., GIBSON T. J., 1994.- CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice.- *Nucleic Acids Research*, 22: 4673-4680.
- VAN DAMME K., BANFIELD L., 2011.- Past and present human impacts on the biodiversity of Socotra Island (Yemen): implications for future conservation.- *Zoology in the Middle East*, 3: 31-88.
- WHITFIELD C., BEHURA S., BERLOCHER S., CLARK A., JOHNSTON J., SHEPPARD W., SMITH D., SUAREZ A., WEAVER D., TSUTSUI N., 2006.- Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*.- *Science*, 314 (5799): 642.

Authors' addresses: Yehya ALATTAL (corresponding author: yalattal@ksu.edu.sa), Mohamad ALSHARHI, Ahmed ALGHAMDI, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; Stephan FUCHS, Institut für Bienenkunde, Polytechnische Gesellschaft, Goethe-University, Karl-vonFrisch-Weg 2, 61440 Oberursel, Frankfurt, Germany.

Received March 26, 2019. Accepted September 27, 2019.