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Original article

Genetic network analysis between *Apis mellifera* subspecies based on mtDNA argues the purity of specimens from North Africa, the Levant and Saudi Arabia

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ABSTRACT

Objectives: This study aimed to analyze the genetic relationships between honey bee subspecies using reference specimens and recently collected specimens from different parts of the world. The purity of these specimens was discussed in light of the obtained results.
Methods: The genetic networks were constructed between 21 subspecies of honey bees, *Apis mellifera* L.: 9 in Africa, 7 in Europe and 5 in Asia. The analysis was performed using the mtDNA of these subspecies and the Population Analysis with Reticulate Trees software. Some subspecies were represented by more than two specimens based on the available online sequences.
Results and conclusions: The subspecies *A. m. sahariensis* from Africa showed unique characteristics and is genetically isolated than all other studied bee subspecies. Specimens collected from Saudi Arabia showed genetic relatedness to *A. m. jemenitica*, *A. m. lamarckii*, and some European subspecies, suggesting high degree of hybridization. The close genetic relationship between the Egyptian bees, *A. m. lamarckii*, and the Syrian bees, *A. m. syriaca*, were emphasized. The overall genetic network showed the presence of three distinct branches in relation to geographical locations. The high accurateness of the used analysis was confirmed by previous phylogenetic studies as well as the genetic relationships between hybrid bees of *A. m. capensis* and *A. m. scutellata*. The genetic networks showed the presence of bee subspecies from Africa in all branches including Europe and Asia. The study suggests the impurity of some specimens mostly due to the hybridization between subspecies. Specific recommendations for future conservation efforts of bees were presented in light of this study.

1. Introduction

There is a number of honey bee, *Apis mellifera* L., subspecies from 20 up 33 (Ruttner, 1988; Engel, 1999; Sheppard and Meixner2003; Dogantzis and Zayed, 2019; Ilyasov et al., 2020). Morphometry and genetic methods are employed to discriminate between subspecies (Ruttner et al., 1978; Garnery et al., 1992; Estoup et al., 1995; Arias and Sheppard, 1996; Franck et al., 1998; Palmer et al., 2000; Meixner et al., 2013). However, the discrimination between some bee subspecies cannot be achieved by morphological characteristics (Garnery et al., 1995; Sheppard et al., 1996; Dukku, 2016). Typically, these subspecies can be divided into five lineages as A, Z, M, C, O, Y and C or Y (Ilyasov et al., 2020). Honey bee subspecies are assigned to these linages using morphometry and genetic methods; but variations of the results were observed between the two approaches. For Example, the Egyptian honey bees, *A. m. lamarckii*, in A lineage based on morphometry (Ruttner, 1988) but in O lineage based on mtDNA (Arias and Sheppard, 1996). Also, the Yemeni honey bees, *A. m. jemenitica*,

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in A lineage according to morphometry (Ruttner, 1988) and in Y lineage based on mtDNA (Coulibaly et al., 2019). Indeed, transition zones were detected between some subspecies (Kandemir et al., 2006a; Dukku, 2016; Alburaki et al., 2011).

In fact, these subspecies are geographically distributed and adapted to specific conditions (Ruttner, 1988). For example, subspecies of Europe are well adapted to temperate conditions while those of Gulf countries and North Africa can tolerate high temperature and arid conditions (Alqarni et al., 2011; Abou-Shaara et al., 2013; Al-Ghamdi et al., 2016). The higher number of subspecies occurs in Europe (13), then Africa (11), and finally the Western Asia and the Middle East (9) (Ilyasov et al., 2020). The productivity of these subspecies is varied and can be inferred from specific morphological characteristics such as body size, wing dimensions and corbicular area (Kolmes and Sam, 1991; Mostajeran et al. 2006). So, planned hybridization between them has been done in some countries including Egypt and Saudi Arabia (Sheppard et al., 2001; Abou-Shaara et al., 2013; Kamel et al., 2003). Such hybridizations can reduce the purity of bee subspecies. The overlap between bee subspecies especially those at boundaries between countries can also increase the hybridization degree between bee subspecies (Péntek-Zakar et al., 2015). Moreover, migratory beekeeping inside the same country can cause admixture between subspecies and bee hybrids (Marghitas et al., 2008).

Sequencing of mtDNA can be used in various purposes including detecting SNP markers in inbred female lines (Kim et al., 2019). Also, sequencing the mitogenome can contribute in the conservation genetic projects of honey bees (Haddad, 2016) as well as in understanding the hybridization between subspecies (Abou-Shaara and Bayoumi, 2019). The purity of honey bee subspecies has not been investigated in some countries including North Africa and Gulf countries. The available sequences from these areas is very limited and mostly from reference samples (Abou-Shaara, 2019; Abou-Shaara et al., 2020, 2021). Recently, sequences of some new specimens from various countries were available online. So, this study aimed to analyze these mtDNA sequences to understand the genetic relationships between honey bee subspecies and to evaluate the purity of the analyzed specimens. The results of this study are important in conservation programs aimed to keep the characteristics of the indigenous bee subspecies.

2. Methods

2.1. Software and resources

The sequences of mtDNA for all honey bee subspecies used in the study were obtained from the NCBI (ncbi.nlm.nih.gov/). Also, two genetic programs (software) were used in the analysis MEGA6 (Tamura et al., 2013) and Population Analysis with Reticulate Trees software (Leigh and Bryant, 2015).

2.2. Subspecies from Africa

Nine subspecies belong to African countries were analyzed (Table 1). Some subspecies were represented by two specimens or more including *A. m. capensis*, *A. m. intermissa*, *A. m. sahariensis* and *A. m. scutellata*. Also, hybrids between *A. m. scutellata* × *A. m. capensis* were included in the analysis.

2.3. Subspecies from Europe

Seven subspecies from European countries were incorporated into the analysis (Table 2). Five specimens of *A. m. ligustica*, and eleven specimens in total were considered during the construction of the genetic network.

2.4. Subspecies from Asia

Five subspecies from Asian countries were used in the analysis (Table 3). Fourteen specimens of hybrid bees from Saudi Arabia were used during the analysis and considered as *Apis mellifera* without specifying the subspecies name.

2.5. Genetic networks

The mtDNA sequences were aligned using Multiple Sequence Comparison by Log-Expectation (Edgar, 2004) prior to the establishment of the genetic networks. Then, the genetic networks were constructed using TCS networks (Clement et al., 2002) and number of mutations between common ancestors was presented in the final constructed networks. The analysis was performed for subspecies from each geographical location individually followed by an overall network for all subspecies.

3. Results

3.1. Africa

The genetic network showed the clear relationships between specimens of *A. m. scutellata* and *A. m. capensis* (Fig. 1). These two subspecies occur in South Africa (Eimanifar et al., 2018) and there is hybridization between them in some areas (Eimanifar et al., 2016; Eimanifar et al., 2018). Therefore, the hybrids between these two subspecies were placed very close to the maternal source as SCU X CAP close to *A. m. capensis* and CAP X SCU close to *A. m. scutellata*. This confirms the accurateness of the network analysis based on the mtDNA. The specimen of *A. m. scutellata* from Mexico (abbreviated as SCU 1) was close to *A. m. capensis*, *A. m. scutellata* and their hybrid bees. This bee from Mexico was a hybrid from drones of Italian bees and queen of *A. m. scutellata* (Gibson and Hunt, 2016). Therefore, its mtDNA was close to *A. m. scutellata*. The Egyptian bees, *A. m. lamarckii*, were close to a specimen of *A. m. intermissa* (specimen 1). The sample of the Egyptian bees was a reference sample from Egypt (Eimanifar et al., 2017a) while the *A. m. intermissa* sample was from Morocco. The second specimen was close to *A. m. scutellata* and away from the first specimen of *A. m. intermissa* and *A. m. lamarckii*. This specimen was collected from Algeria (Hu et al., 2016). The genetic variations between the two specimens of *A. m. intermissa* can be attributed to the hybridization between bee subspecies in North Africa. Indeed, no pure *A. m. lamarckii* bees occur in Egypt currently due to the long period of hybridization between Carniolan and Italian bees with Egyptian bees since 1930s (Page et al., 1981; Sheppard et al., 2001; Kamel et al., 2003; Abou-Shaara, 2019).

The two specimens of *A. m. sahariensis* were identical to each other and placed very away than all other subspecies with high number of mutations of 4424 than *A. m. monticola*, *A. m. intermissa* and *A. m. lamarckii*. This specific subspecies is geographically isolated than the rest of bee subspecies of Africa (Haddad et al., 2017). This can explain the occurrence of high number of mutations in the ancestors of these bees. Two subspecies *A. m. unicolor* and *A. m. simensis* were very close to *A. m. scutellata* (specimen 8) while *A. m. adansonii* was close to *A. m. scutellata* (specimen 4). In a similar way, a previous study showed the close relationship between the Malagasy honey bees, *A. m. unicolor*, and *A. m. scutellata* and *A. m. capensis* (Boardmanet al., 2019a). Also, The Ethiopian bees *A. m. simensis* were close to A. m. scutellata (Boardman et al., 2020a). Additionally, *A. m. adansonii* showed close relationships to *A. m. scutellata* and *A. m. capensis* (Boardman et al., 2020b). This study showed the distinct of *A. m. monticola* than the other subspecies with some relationship with *A. m. capensis*, this is in line

Table 1
Mitogenome data of *Apis mellifera* subspecies from Africa.

Subspecies	GenBank	Base pairs	Specimen	Country	Abbreviation
A. m. adansonii	MN585109.1	16466	voucher="1284	Niger	ADA
A. m. capensis	KX870183.1	16470	–	South Africa	CAP 1
A. m. capensis	MG552694.1	16344	voucher="ST"	South Africa	CAP 2
A. m. capensis	MG552688.1	16453	voucher="MB"	South Africa	CAP 3
A. m. capensis	MG552696.1	16459	voucher="WD"	South Africa	CAP 4
A. m. capensis	MG552692.1	16435	voucher="RD"	South Africa	CAP 5
A. m. capensis	MG552691.1	16515	voucher="PE"	South Africa	CAP 6
A. m. capensis	MG552690.1	16434	voucher="PB"	South Africa	CAP 7
A. m. capensis	MG552687.1	16468	voucher="LB"	South Africa	CAP 8
A. m. capensis	MG552686.1	16473	voucher="LA"	South Africa	CAP 9
A. m. capensis	MG552685.1	16428	voucher="GT"	South Africa	CAP 10
A. m. capensis	MG552684.1	16380	voucher="GE"	South Africa	CAP 11
A. m. capensis	MG552683.1	16442	voucher="CT"	South Africa	CAP 12
A. m. capensis	MG552682.1	16447	voucher="CD"	South Africa	CAP 13
A. m. capensis	MG552681.1	16467	voucher="BD"	South Africa	CAP 14
A. m. capensis	MG552695.1	16439	voucher="SW"	South Africa	CAP 15
A. m. capensis	MG552693.1	16467	voucher="SF"	South Africa	CAP 16
A. m. capensis	MG552689.1	16463	voucher="MF"	South Africa	CAP 17
A. m. scutellata × *A. m. capensis*	KX943034.1	16340	–	South Africa	SCU X CAP
A. m. capensis × *A. m. scutellata*	MG552697.1	16456	voucher="KL"	South Africa	CAP X SCU
A. m. intermissa	KY926883.1	16343	voucher="1814″	Morocco	INT 1
A. m. intermissa	KM458618.1	16336	–	Algeria	INT 2
A. m. lamarckii	KY464958.1	16589	voucher="1842″	Egypt	LAM
A. m. monticola	MF678581.1	16343	voucher="1626″	Kenya	MONT
A. m. sahariensis	MF351881.1	16569	–	Algeria	SAH 1
A. m. sahariensis	NC_035883.1	16569	–	Algeria	SAH 2
A. m. simensis	MN585108.1	16523	voucher="2721	Ethiopia	SIM
A. m. unicolor	MN119925.1	16373	voucher="2520	Madagascar	UNI
A. m. scutellata	KJ601784.1	16411	Hybrid	Mexico	SCU 1
A. m. scutellata	KY614238.1	16288	voucher="1982″	South Africa	SCU 2
A. m. scutellata	MG552698.1	16479	voucher="BL"	South Africa	SCU 3
A. m. scutellata	MG552703.1	16339	voucher="VR"	South Africa	SCU 4
A. m. scutellata	MG552702.1	16364	voucher="UP"	South Africa	SCU 5
A. m. scutellata	MG552701.1	16450	voucher="SP"	South Africa	SCU 6
A. m. scutellata	MG552700.1	16462	voucher="PT"	South Africa	SCU 7
A. m. scutellata	MG552699.1	16454	voucher="KR"	South Africa	SCU 8

Table 2
Mitogenome data of *Apis mellifera* subspecies from Europe.

Subspecies	GenBank	Base pairs	Specimen	Country	Abbreviation
A. m. anatoliaca	MT188686.1	16256	voucher = 3377	Turkey	ANA
A. m. carnica	MN250878.1	16358	voucher = 1668	Austria	CAR
A. m. caucasica	MN714160.1	16274	–	–	CAU
A. m. iberiensis	MN585110.1	16560	voucher = 1964	Portugal	IBR
A. m. ruttneri	MN714162.1	16577	voucher = 2050	Malta	RUT
A. m. ligustica	NC_001566.1	16343	–	–	LIG 1
A. m. ligustica	MT859135.1	16467	–	–	LIC 2
A. m. ligustica	KX908209.1	16465	–	–	LIC 3
A. m. ligustica	MH341408.1	16426	voucher = CNU7294	–	LIC 4
A. m. ligustica	MH341407.1	16449	voucher = CNU7293	–	LIC 5
A. m. mellifera	KY926884.1	16343	voucher = 1410	Norway	MEL 1

with Eimanifar et al. (2017b). This is on the contrary with Boardmanet al. (2019a,b), those authors found a close relationships between *A. m. simensis*, *A. m. adansonii* and *A. m. monticola*. The relationships between *A. m. adansonii*, *A. m. scutellata*, *A. m. monticola*, and *A. m. capensis* were highlighted (Arias and Sheppard, 1996; Franck et al., 2001). However, the present study supports the close relationship between *A. m. monticola*, and *A. m. capensis*, and between *A. m. adansonii* and *A. m. scutellata*. The number of mutations was extremely high in case of *A. m. sahariensis* only and not more than 20 in most subspecies while mutation numbers over 20 are shown in Fig. 1. The extreme variations between *A. m. sahariensis* and subspecies of Africa encourage the assessment of the sequences of this subspecies using modern sequencing techniques.

3.2. Europe

The genetic network clearly shows that *A. m. anatoliaca* and *A. m. caucasica* are placed in the same branch (Fig. 2). Accordingly, *A. m. anatoliaca* was close to *A. m. caucasica* found in Turkey (Boardman et al., 2020c). Also, *A. m. iberiensis* and *A. m. ruttneri* were highly close to each other. The close relationship between *A. m. carnica* and *A. m. ligustica* was previously highlighted (Boardman et al., 2019b), and is confirmed in this study. All specimens of the Italian honey bees, *A. m. ligustica*, showed close genetic relationships except specimen 4, suggesting the impurity of this specimen. The hybridization between specimen 4 and the Carniolan bees, *A. m. carnica*, is expected to be the reason behind the impurity of this specimen. The European bee, *A. m. mellifera*,

Table 3
Mitogenome data of *Apis mellifera* subspecies from Asia.

Subspecies	GenBank	Base pairs	Specimen	Country	Abbreviation
A. m. jemenitica	MN714161.1	16427	voucher = 154	Yemen	JEM
A. m. meda	KY464957.1	16248	voucher = 3284	–	MED
A. m. sinisxinyuan	MN733955.1	16886	–	China	SIN
A. m. syriaca	KY926882.1	16343	voucher = 1728	Syria	SYR 1
A. m. syriaca	KP163643.1	15428	Varroa resistant	–	SYR 2
Apis mellifera	MT745914.1	16386	Arabia-j-Z-61	Saudi Arabia	ARB 1
Apis mellifera	MT745913.1	16381	Arabia-n-Z-51	Saudi Arabia	ARB 2
Apis mellifera	MT745912.1	16352	Arabia-n-Z-53	Saudi Arabia	ARB 3
Apis mellifera	MT745911.1	16384	Arabia-md-Zc-71	Saudi Arabia	ARB 4
Apis mellifera	MT745910.1	16379	Arabia-t-Z-112	Saudi Arabia	ARB 5
Apis mellifera	MT745909.1	16390	Arabia-md-Z-61	Saudi Arabia	ARB 6
Apis mellifera	MT745908.1	16394	Arabia-t-Z-41	Saudi Arabia	ARB 7
Apis mellifera	MT745907.1	16390	Arabia-T-Z-61	Saudi Arabia	ARB 8
Apis mellifera	MT745906.1	16410	Arabia-A-Z-101	Saudi Arabia	ARB 9
Apis mellifera	MT745905.1	16419	Arabia-mK-Z-53	Saudi Arabia	ARB 10
Apis mellifera	MT745904.1	16425	Arabia-mK-Z-73	Saudi Arabia	ARB 11
Apis mellifera	MT745903.1	16428	Arabia-B-Z-34	Saudi Arabia	ARB 12
Apis mellifera	MT745902.1	16443	Arabia-A-Z-111	Saudi Arabia	ARB 13
Apis mellifera	MT745901.1	16445	Arabia-mK-Z-111	Saudi Arabia	ARB 14
Apis mellifera	MT745915.1	16400	Arabia-md-Zc-17	Saudi Arabia	ARB 15

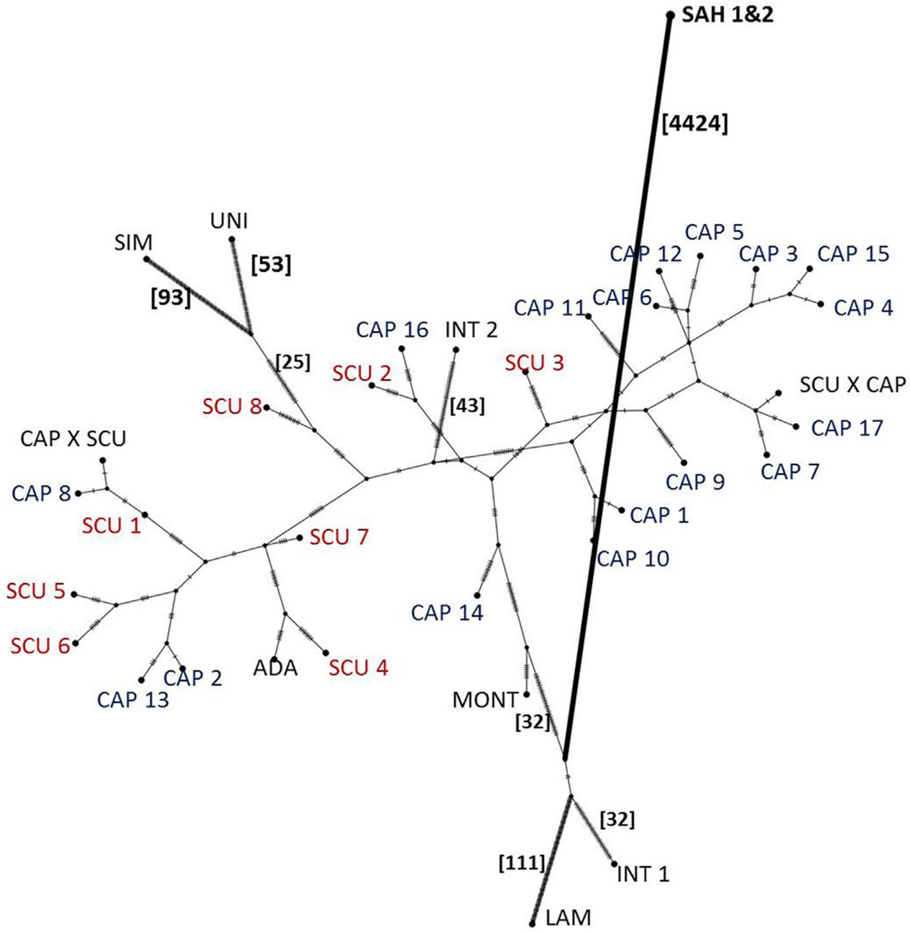

Fig. 1. The genetic network between *Apis mellifera* subspecies from Africa. ADA: *A. m. adansonii*, CAP: *A. m. capensis* (specimens 1–17), INT: *A. m. intermissa* (specimens 1–2), LAM: *A. m. lamarckii*, MONT: *A. m. monticola*, SAH: *A. m. sahariensis* (specimens 1–2), SIM: *A. m. simensis*, UNI: *A. m. unicolor*, and SCU: *A. m. scutellata* (specimens 1–8). The number of mutations over 20 is placed between brackets.

showed high variation (122 mutations) than the other subspecies. In fact, the available number of specimens for some European subspecies is low and further investigations are recommended especially at Central and Eastern parts of Europe.

3.3. Asia

The specimens collected from Saudi Arabia showed close relationships with *A. m. jemenitica* except specimens 1 and 2 (Fig. 3).

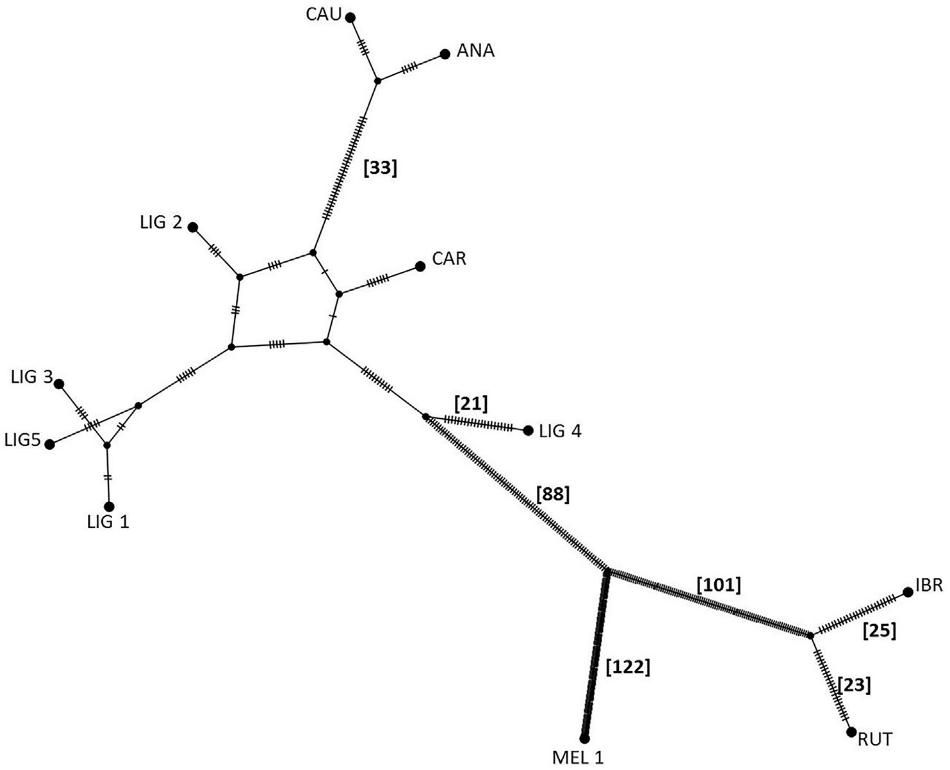

Fig. 2. The genetic network between *Apis mellifera* subspecies from Europe. ANA: *A. m. anatoliaca*, CAR: *A. m. carnica*, CAU: *A. m. caucasica*, IBR: *A. m. iberiensis*, RUT: *A. m. ruttneri*, LIG: *A. m. ligustica*, and MEL: *A. m. mellifera*. The number of mutations over 20 is placed between brackets.

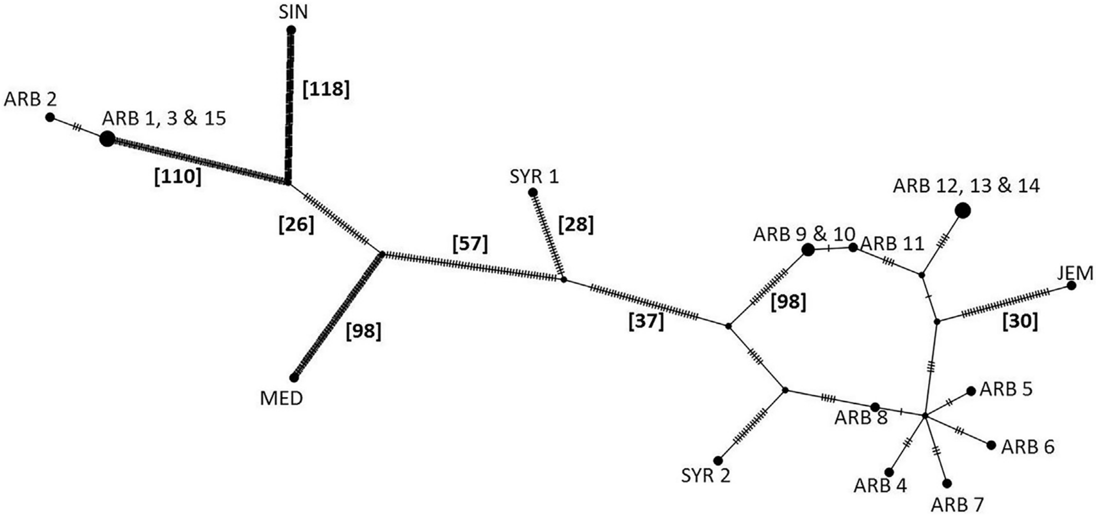

Fig. 3. The genetic network between *Apis mellifera* subspecies from Asia. JEM: *A. m. jemenitica*, MED: *A. m. meda*, SIN: *A. m. sinisxinyuan*, SYR: *A. m. syriaca*, and ARB: *Apis mellifera* (specimens from Saudi Arabia). The number of mutations over 20 is placed between brackets.

Indeed, none of the analyzed specimens from Saudi Arabia showed identical sequences with *A. m. jemenitica*, suggesting the high hybridization degree in these specimens. The importation of bee stocks from some countries including Egypt to Saudi Arabia has been done over a long period of time (Alqarni et al., 2011; Al-Ghamdi and Nuru, 2013; Al-Ghamdi et al., 2016); therefore, the potential hybridization between the native bees of Saudi Arabia, *A. m. jemenitica*, and the imported bees is highly expected. A close relationship between *A. m. jemenitica* and the Syrian honey bees, *A. m. syriaca*, was found (Boardman et al., 2020d), which is supported in this study mainly by specimen 2 of the Syrian bees. The highest number of mutations was found in *A. m. sinisxinyuan* (118) and *A. m. meda* (98). These two subspecies were separated than the other subspecies. The two specimens of the Syrian honey bees were not identical.

3.4. All subspecies

The overall genetic network classified the subspecies into three clear branches (Fig. 4). Branch A contained subspecies from Africa plus two subspecies from outside it *A. m. ruttneri* and *A. m. iberiensis*. In a previous study, *A. m. ruttneri* from Malta showed a close

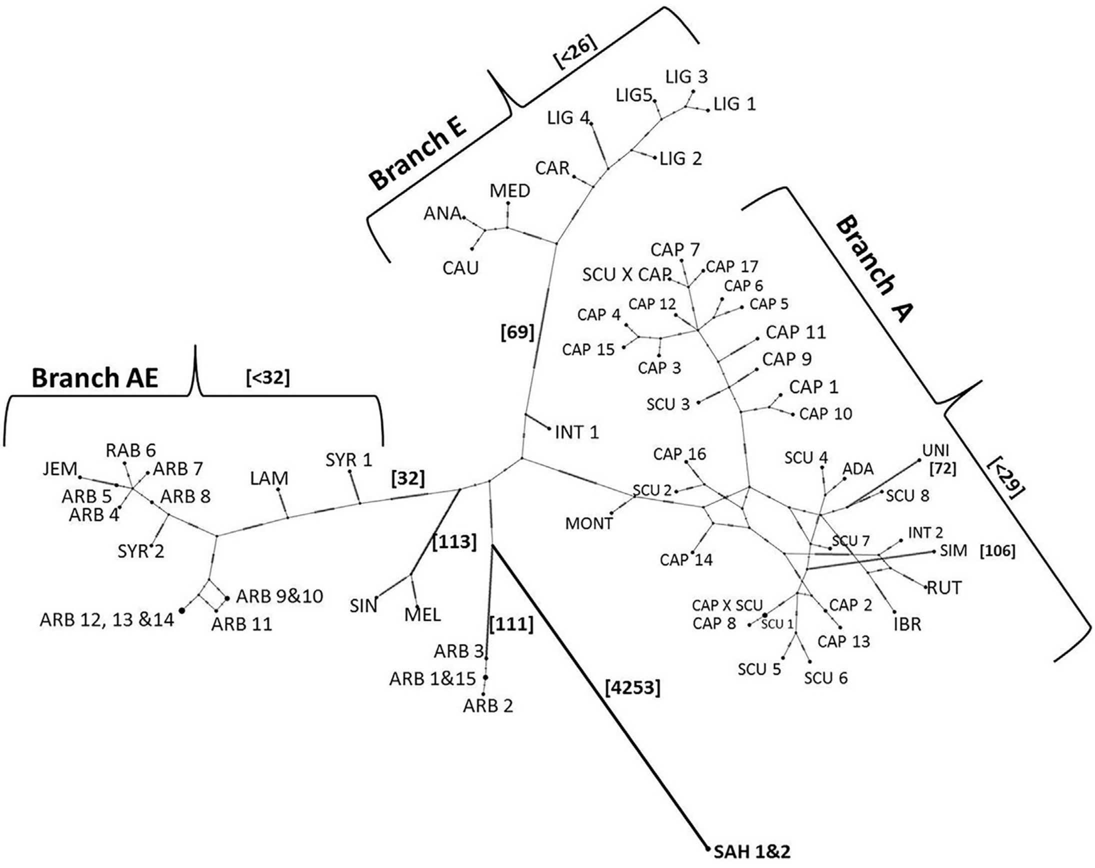

Fig. 4. The genetic network between *Apis mellifera* subspecies from Africa, Europe, and Asia. ADA: *A. m. adansonii*, CAP: *A. m. capensis* (specimens 1–17), INT: *A. m. intermissa* (specimens 1–2), LAM: *A. m. lamarckii*, MONT: *A. m. monticola*, SAH: A. *m. sahariensis* (specimens 1–2), SIM: *A. m. simensis*, UNI: *A. m. unicolor*, and SCU: *A. m. scutellata* (specimens 1–8). ANA: *A. m. anatoliaca*, CAR: *A. m. carnica*, CAU: *A. m. caucasica*, IBR: *A. m. iberiensis*, RUT: *A. m. ruttneri*, LIG: *A. m. ligustica*, and MEL: *A. m. mellifera*. JEM: *A. m. jemenitica*, MED: *A. m. meda*, SIN: *A. m. sinisxinyuan*, SYR: *A. m. syriaca*, and ARB: *Apis mellifera* (specimens from Saudi Arabia). The number of mutations is placed between brackets.

genetic relationship to *A. m. intermissa* from North Africa (Sheppard et al., 1997). Also, in a previous phylogenetic analysis *A. m. ruttneri* and *A. m. iberiensis* showed a close relationship to *A. m. sahariensis* and *A. m. intermissa* (Boardman et al., 2020d, e). These findings are supported partially by the results of this study. In fact, *A. m. intermissa* has a pivotal role in the transition zones between some subspecies of Africa such *as A. m. sahariensis* and *A. m. scutellata* (Dukku, 2016; Ilyasov et al., 2020) and in Europe such as *A. m. iberiensis* (Ruttner, 1988). This supports the idea of being *A. m. intermissa* the genetic root of bee subspecies in South Europe. The Egyptian bees, *A. m. lamarckii*, were not included in this branch. The number of mutations between subspecies of this branch was less than 29, suggesting their close genetic relatedness. The accuracy of the analysis is highlighted by the location of *A. m. scutellata* (specimens 1) in the African branch and not in the European branch. In fact, this specimen is a hybrid between drone of *A. m. ligustica* and queen of *A. m. scutellata* (Gibson and Hunt, 2016); therefore, the maternal source (mtDNA) is from Africa.

4. Discussion:

Branch AE contained *A. m. lamarckii*, *A. m. jemenitica*, *A. m. syriaca*, and all specimens from Saudi Arabia except 1, 2, 3 and 15. The number of mutations in this cluster was less than 32, suggesting the high genetic similarities between them. The close genetic relationship between the reference sample of *A. m. lamarckii* (the Egyptian bees) and *A. m. syriaca* (the Syrian bees) were confirmed in previous studies (Eimanifar et al., 2017a; Abou-Shaara, 2019; Abou-Shaara et al., 2020, 2021) in accordance to the present result. Also, a close relationship between *A. m. jemenitica*, *A. m. lamarckii* and *A. m. syriaca* were suggested (Boardman et al., 2020f). In fact, transition zones were found between some subspecies in the near East such as *A. m. meda* and *A. m. anatoliaca*, and also *A. m. syriaca* and *A. m. lamarckii* (Franck et al., 2000; Kandemir et al., 2006a; Alburaki et al., 2011). Such transitions are highly supported by the constructed network. High similarities between *A. m. lamarckii* and honey bees from Syria and Turkey based on the COI-COII intergenic region were found (Kandemir et al., 2006b) as well as between *A. m. lamarckii* and *A. m. syriaca* from Lebanon (Franck et al., 2000). This supports that *A. m. lamarckii* may represent the genetic root of bee subspecies of the Levant. It is clear that some samples from Saudi Arabia were genetically close to *A. m. jemenitica* especially specimen 5, and such specimen can be further used in conservation programs of honey bees in Saudi Arabia.

Branch E contained European honey bee subspecies with mutations less than 26, suggesting the close genetic relationships between these reference samples. The subspecies *A. m. intermissa* (specimen 1) showed genetic relationships with European subspecies with 69 mutations in the common ancestor. In other words, *A. m. intermissa* (specimen 1) and other European subspecies shared the same common ancestor. The highest number of mutations were recorded to specimens 1, 2, 3, and 15 from Saudi Arabia (111 mutations), *A. m. mellifera* and *A. m. sinisxinyuan* from China (113 mutations), and the two specimens of *A. m. sahariensis* (4253 mutations). The close relationship between *A. m. mellifera* and *A. m. sinisxinyuan* is highly supported by the phylogenetic study based on 13 protein coding genes (Yang et al., 2020). In fact, the Xinyuan honey bees, *A. m. sinisxinyuan*, occurs in limited areas in China (Zhao et al.2018), and have ability to adapt with temperate climates and to tolerate winter conditions (Chen et al.2016). Also, *A. m. mellifera* is well adapted to European conditions and can tolerate cold winter and temperate climates. This shows the similarities between these two subspecies in their behaviors. The mutations in specimens 1, 2, 3, and 15 from Saudi Arabia can be explained by their exposure to hybridization with other bee subspecies over long period. The isolation of *A. m. sahariensis* than all subspecies suggests its unique characteristics; however, re-assessment of its mtDNA sequences is required.

5. Conclusion

The genetic network showed that two subspecies from Europe (*A. m. ruttneri* and *A. m. iberiensis*) were close to subspecies from Africa than those from Europe. Also, *A. m. mellifera* from Europe and *A. m. sinisxinyuan* from China were close to each other although the geographical isolation between them. The low purity of some specimens was highlighted including samples collected from Saudi Arabia and specimen of *A. m. intermissa* and *A. m. Syriaca*. The highest number of mutations was recoded to *A. m. sahariensis*, suggesting its unique genetic characteristics. *A. m. intermissa* from Morocco, Algeria, and Tunisia should be reassessed as the genetic root for bee subspecies from South Europe, which can help in conservation programs. Specific specimens collected from Saudi Arabia showed high genetic relationships with the endemic bees, *A. m. jemenitica*, and should be considered in future conservation programs. A very close genetic relationship was detected between a specimen of *A. m. Syriaca* and the Egyptian bees, *A. m. lamarckii*. The assessment of new specimens from Egypt and the Levant is highly recommended to update the genetic status of bee subspecies from these regions. In general, collecting new samples of bees and sequencing of mtDNA is highly recommended to update the current genetic relationships between subspecies as a result of hybridization.

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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