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Original investigation

Genetic relationships of wild boars highlight the importance of Southern Iran in forming a comprehensive picture of the species' phylogeography

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ABSTRACT

Genetic relationships between wild boar populations in the southern regions of Iran still remain unclear. Therefore in the present study our aim was filling this gap and providing preliminary knowledge about the phylogenetic status of Iranian wild boar based on two datasets of the mtDNA control region (the 402-bp and 605-bp segments as the first and second datasets, respectively). Altogether 1063 and 490 sequences were used in the shorter and longer sequences, respectively (29 own samples and 1034 and 461 Genbank sequences from previous studies in the first and second datasets, respectively). Our findings revealed that the wild boars living in Southern Iran belong to the previously described three clades: Asian, Near Eastern 1 (NE1) and Near Eastern 2 (NE2) clades. The NE1 and NE2 clades sympatrically occupy a wide range across the southeast to southwest of Iran, whereas the Asian clade is restricted to a small area in the southeastern part of Iran. Our results propose the existence of a contact zone between the Near Eastern clades and the Asian clade in the studied area. The fixation index (F_{ST}) and the analysis of molecular variance (AMOVA) demonstrated significant genetic structure among the identified clades. Some identified haplotypes have a wide distribution, which suggests that they are probably ancestral haplotypes or are likely to be a result of recent population expansion. Overall, current research demonstrates that Iranian wild boars have an important phylogenetic position among the others in the world. In other words, Iran is a very important region for addressing some of the topical questions about phylogenetic relationships among the Eurasian wild boar clades.

Introduction

The Eurasian wild boar (*Sus scrofa* Linnaeus, 1758) has one of the widest geographic distributions of all terrestrial mammals (Oliver and Leus, 2008), inhabiting vast areas in Eurasia and Northern Africa (Herre and Rohrs, 1977; Clutton-Brock, 1987; Ruvinsky and Rothschild, 1998). Its success in wide distribution could be due to the remarkable adaptive plasticity of the species, its opportunistic omnivorous behavior (Massei et al., 1996; Fonseca, 2004), high reproduction rate (Fonseca, 2004) and human agency (Oliver and Leus, 2008). Wild boars have an important role in the formation of various ecosystems, but their relation with humans is particularly remarkable (Albarella et al., 2009).

In Iran, the wild boar is widely distributed except in the Central Desert and prefers areas with abundant water sources and vegetation (Ziaie, 2008). Iranian wild boars are found in a variety of habitats including forests, agricultural fields, rangelands, shrub lands and mangrove swamps. The main predator species that threaten wild boars in the country include leopard (*Panthera pardus*), brown bear (*Ursus arctos*), wolf (*Canis lupus*) and Eurasian lynx (*Lynx lynx*) (Ziaie, 2008). The decrease in population sizes of the nat-

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ural predators resulted in an increase in the population size of boars in some parts of its range, and led to extensive damage to the fields. On the other hand, droughts have led to the decline of local populations of wild boar in some areas of Iran, especially in the southern parts of the country. However, the droughts have increased the occurrence of crop damage in some areas by wild boars due to lack of food sources in natural habitats. Overall, both recent cases have caused damage to agriculture crops (Ashrafzadeh and Bordkhani, 2012) leading to conflicts within farming communities. Population sizes in many areas of the country are increasing (Karami et al., 2010), though populations are decreasing in regions where hunting intensity is high (for example in Minoo Island, Southwest Iran) or areas that are affected by severe drought. Moreover, mass mortality of wild boars mainly due to Fibromuscular dysplasia (FMD) and Ecthyma (Ziaie, 2008; Karami et al., 2010) has been observed in several areas. However, it is unclear how much these populations have been reduced by hunting and whether any subspecies and populations are presently threatened with extinction. The decrease in wild boar population size is a conservation concern in many regions of the world (Spencer et al., 2006).

Wild boars are very diverse in size and appearance across their large geographic range (Albarella et al., 2009) because of the high diversity in their ancestry (West et al., 2009). Different phenotypes can also indicate genetic differences among different populations (Amici et al., 2010; Gallo Orsi et al., 2014).

Furthermore, matrilineal groups are the common type of social organization among Suids (Podgórski et al., 2014). The social structure of wild boars is centered around philopatric adult females (Spitz and Janeau, 1990; Lemel et al., 2003; Iacolina et al., 2009) and involves high degrees of philopatry which can lead to remarkably increased levels of genetic differentiation (Peterson, 1992).

Phylogeny and genetic diversity of game species are one of the most important aspects of wildlife population management and conservation. Genetic relationships of wild boar are well studied in Europe and Asia (Huang et al., 1999; Kim et al., 2002; Larson et al., 2005; Fang et al., 2006; Larson et al., 2007; Scandura et al., 2008; Lattuada et al., 2009; Alves et al., 2010; Alexandri et al., 2012; Ottoni et al., 2012; Đan et al., 2013; Kusza et al., 2014; Vilaça et al., 2014; Veličković et al., 2015; Maselli et al., 2016; Veličković et al., 2016). However, there is little information on the phylogeny of the wild boars in the southern regions of Iran, and what there is, is mainly restricted to the studies of Larson et al. (2005, 2007) and Ottoni et al. (2012).

Mostly, clades of Eurasian wild boar were identified based on the sequences of the mitochondrial DNA (mtDNA) control region (e.g., Larson et al., 2005, 2007; Ottoni et al., 2012). Among genetic markers, mtDNA is highly polymorphic, almost exclusively maternally inherited and without genetic recombination (Alves et al., 2003), which makes it a powerful tool in evolutionary studies. Therefore, the mtDNA control region can be particularly useful in understanding phylogenetic relationships between wild boars of Southern Iran and those living in other parts of the world. In the present study, we investigated the genetic diversity and phylogenetic relationships of wild boar populations in a poorly studied region of the world, Southern Iran.

Material and methods

Samples

A total of 29 wild boar samples was collected from a non-studied region and another that had been sampled before, namely Minoo Island (MI: 14 individuals) and Khabr National Park (KNP: 15 individuals) located in Southwest and South Central Iran, respectively (Figs. 1 and S1). The tissue samples were obtained from wild boars freshly shot by legally licenced hunters. No animals were killed for the purposes of this study, and all procedures contributing to this work complied with the national regulations of the Iranian Department of Environment. Samples were preserved in 96% ethanol till further laboratory analysis.

DNA extraction, amplification and sequencing

Whole genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. All samples were amplified for the 605 nucleotides of the mtDNA control region fragment with L15387 (5′-CTCCGCCATCAGCACCCAAAG-3′) and H16108n (5′-GCACCTTGTTTGGATTRTCG-3′) primers described in a previous study (Luetkemeier et al., 2010). PCR amplification (AccuPower® PCR Premix Kit, Bioneer) was performed in 25 μl volume containing 1 U of *Top* DNA polymerase, 250 μM of each dNTP, 10 mM Tris–HCl, 30 mM KCl, 1.5 mM $MgCl_2$ and 2 pmol of each primer. PCR was performed under the following conditions: 94 °C for 3 min, 30 cycles of 45 s at 94 °C, 45 s at 60 °C, 50 s at 72 °C. After 30 cycles, a final extension was continued for 5 min at 72 °C. The PCR products were checked using electrophoresis on 1.5% agarose gels. The Big Dye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems, ABI) was used for double-strand cycle sequencing according to the manufacturer's protocols. Sequences were determined on an ABI PRISM 3730xl automatic DNA sequencer (Applied Biosystems).

Alignment and sequence analysis

Sequences were edited by SeqScape version 2.6 (Applied Biosystems). ClustalW in Mega version 6 (Tamura et al., 2013) was used for alignment of sequences. The aligned sequences generated in this study have been deposited in GenBank (accession numbers KF285540-KF285559 and KF955578- KF955586).

In this study, phylogenetic analyses carried out on two datasets comprising: (1) a shorter, 402-bp segment of the mtDNA control region encompassing a sequence commonly used in other studies on wild boar (e.g., Scandura et al., 2008; Veličković et al., 2015) (Table S1); and (2) a longer sequence including a 605-bp segment of the control region (Table S2). However, the shorter (402-bp) segment is included within the longer (605bp) segment. We calculated genetic diversity (haplotype and nucleotide diversity), parsimony informative sites and polymorphic sites using DnaSp version 5 (Librado and Rozas, 2009).

The sequences in the present study were combined with 1034 (the first data set, 402-bp segment) and 461 (the second data set, 605-bp segment) GenBank entries (Tables S1 and S2) to generate the data sets in order to estimate the phylogenetic relationships among wild boar populations across the entire species range in the world. The best-fit model of nucleotide substitution was determined using jModeltest 0.1.1 (Posada, 2008). The best model was HKY + I + G for both datasets, according to both the Corrected Akaike information criterion (AICc) and the Bayesian information criterion (BIC). The Bayesian phylogenetic tree was reconstructed using MrBayes 3.2.2 (Ronquist and Huelsenbeck, 2003) in two independent runs of four Monte Carlo Markov chains (MCMC) for 40,000,000 generations and sampling every 100 generations. Two sequences of the Common warthog (*Phacochoerus africanus*) (GenBank accession numbers: NC_008830 and DQ409327) (Wu et al., 2007) were used as outgroups. A median-joining network analysis was performed in Network 4.6.1.2 (Bandelt et al., 1999), for clarifying the relationships between the haplotypes inferred in the present study.

Analysis of molecular variance (AMOVA) and F_{ST} (Weir and Cockerham, 1984) statistics were estimated using Arlequin 3.5 (Excoffier and Lischer, 2010), with 10,000 permutations, aiming to determine levels of genetic structure. These statistics were only performed on the data of the wild boar clades from Southern Iran.

Results

Altogether 29 individuals from Southern Iran were successfully sequenced for the 605-bp segment of the mtDNA control region. Our datasets comprised the newly obtained sequences and previously published sequences (Tables S1 and S2) from wild boars. A total of 147 and 144 haplotypes was detected within the first (402-bp) and second (605-bp) datasets, respectively. Within the longer (605-bp) sequence, 63 parsimony informative sites were identified among 92 polymorphic sites. Based on the 605-bp sequence, the haplotype and nucleotide diversities were estimated as about 0.972 (±0.002) and 0.0143 (±0.00034), respectively. Using the 605-bp segment, seven haplotypes (H1: MI1, GL769, and GL752; H2: KNP1, and GL771; H3: KNP2; H5: GL783; H7: GL794; H8: GL793; H9: GL792) could be identified from wild boars of the south of Iran, including a novel haplotype (H3) that was reported for the first time. For detailed information on haplotypes see Fig. 1 and Tables S1 and S2.

The Bayesian phylogenetic trees, based on both datasets, represent a previously reported topology (Larson et al., 2005) that included four clades (1–4) (Figs. 2 and 3). These clades are also well supported by posterior probabilities. In total, wild boars living in Southern Iran fell into the clades 3 and 4 (Figs. 1–3). The Iranian wild boars from Khuzestan (MI1 and GL783), Chaharmahal and Bakhtiari (GL769), Kohgilouye and Boyerahmad (GL752 and GL771), Kerman (KNP1 and KNP2) and Sistan and Baluchestan (GL793) provinces are clustered within the clade 4, whereas two haplotypes (GL792 and GL794) from Sistan and Baluchestan province, as most of the eastern population of wild boar in the area studied, are placed in the clade 3 (Figs. 1 and S1).

In addition, the topology observed by other authors (Larson et al., 2007; Scandura et al., 2008; Ottoni et al., 2012; Alexandri et al., 2012; Veličković et al., 2015; Vilaça et al., 2014; Khalilzadeh et al., 2016) is unambiguously supported by our phylogenetic analyses, which clearly separate five clades including Asian, Near Eastern 1 (NE1), Near Eastern 2 (NE2), European (E1) and Italian (E2) (Figs. 2 and 3). Wild boars from Southern Iran were nested in three clades, namely Asian, NE1 and NE2 (Figs. 1 and S1). It seems the Asian clade has a smaller geographic range within Southern Iran: it is identified in the sequences (GL792 and GL794) of Sistan and Baluchestan province, whereas the other two clades (NE1 and NE2) have relatively wide distributions in Southern Iran (Figs. 1 and S1). All in all, as it was expected, NE1 and NE2 were the most frequent clades in Southern Iran.

Wild boars from the Khabr national park (Kerman province, Iran) (15 individuals) represented two haplotypes (KNP1 and KNP2) within the NE1 clade, whereas wild boars from Minoo Island (Khuzestan province, Iran) (14 individuals) formed only one haplotype (MI1) within the NE2 clade (Figs. 1 and S1). Four other haplotypes were identified in Sistan and Baluchestan (GL792, GL793, and GL794) and Khuzestan (GL783) provinces.

Wild boars from Khuzestan (MI1), Chaharmahal and Bakhtiari (GL769: DQ872944) and Kohgilouye and Boyerahmad (GL752: DQ872938) situated in Southern Iran with wild boars from Kurdistan (KR075779), West Azerbaijan (KR075778) and Qazvin (KR075779) situated in the northwest of Iran, individuals from Tarsus Forest (GL754: DQ872940) and Izmir (GL940: DQ872966) located in Turkey and 12 individuals from Greece (JF774381–JF774392) share a common haplotype (H1 in Figs. 2 and 3). Fifteen individuals from Khabr national park (12 individuals: KNP1 in Fig. S1), Kohgilouye and Boyerahmad (one individual: GL771) and Kermanshah (one individual: GL781) were identified as sharing a common haplotype (H2 in Figs. 2 and 3). It is interesting that wild boars (105 sequences) from Southeast Iran (GL792), Northern Iran, Russia, Turkmenistan, China, Taiwan, Japan, Vietnam, Australia, New Zealand, Germany and United Kingdom share a common haplotype (H7 in Fig. 2 and Table S1; H9 in Fig. 3 and Table S2) within the clade 3 or Asian clade.

Median-joining networks have been constructed in order to further elucidate the differences between the wild boar clades (Figs. 4 and S2). The networks supported the identified clades and estimated relationships in the phylogenetic trees. The results obtained by AMOVA analysis, based on the 605-bp sequence, revealed a significant differentiation among the wild boar clades (NE1, NE2, and Asian) inhabiting Southern Iran ($F_{ST} = 0.947$; $P < 0.0001$) (Table 1).

Discussion

Iran has a special position in the northern hemisphere because of its rich biodiversity, in particular because of its role as a connecting bridge between Europe and Asia (Sagheb Talebi et al., 2013). In other words, unlike many other Asian countries, Iran is located at the meeting place of several zoogeographical regions including the Palearctic, Oriental and Ethiopian regions, and acts as a transition zone between a variety of humid to arid ecosystem types (Sagheb Talebi et al., 2013). Except for the interior deserts and the lowlands along the Caspian Sea, Persian Gulf and Makran Sea, ca. half of Iran is formed of high mountains (Noroozi et al., 2008).

On the other hand, recent studies have confirmed that most of the territory of Iran were important refugial areas for several animal and plant species during the Quaternary glaciations (Djamali et al., 2009; Rajaei et al., 2013; Naderi et al., 2014; Ashrafzadeh et al., 2016). These regions harbour a high proportion of unique haplotypes and geographically distinct clades for several species, including the fat dormouse (*Glis glis*) (Naderi et al., 2014) and the brown bear (*Ursus arctos*) (Calvignac et al., 2009; Ashrafzadeh et al., 2016).

The wild boar has a wide distribution in the areas studied in this work including Khabr national park and Minoo Island in Southern Iran, and occupies a variety of habitats, from semi-desert to mountains, woodlands, grasslands, riverbanks, streams, marshes and farms. Moreover, wild boars in these areas are regarded as an agricultural pest, and various methods are used to eliminate or limit their populations, including illegal and legal hunting, charging their farm fences with electricity, capturing boars with dogs and poisoned baits for decreasing the population sizes and reducing damage. The species from Minoo Island is currently faced with the decline of local populations due to several threatening factors including hunting, habitat destruction etc. At present there is no modern wildlife management of Iranian wild boars, and the populations are in dire need of the development of management action plans.

Genetic analyses on modern and ancient mitochondrial DNA of the wild boar have revealed several maternal clades worldwide. Giuffra et al. (2000) and Kijas and Andersson (2001) identified three divergent clades of *S. scrofa* mitochondrial sequences: the Asian clade, European clade I and European clade II, of which the European clade II consists solely of the present Italian wild boar. A strong phylogeographic structure of mtDNA sequences comprising four clades was revealed by Larson et al. (2005) among wild boars and domestic pigs. Furthermore, other studies have presented more details on Eurasian wild boar clades (e.g., Larson et al., 2007; Scandura et al., 2008; Ottoni et al., 2012; Alexandri et al., 2012; Kusza et al., 2014; Veličković et al., 2015; Vilaça et al., 2014).

Unfortunately, there is relatively little information on the phylogeny and genetic diversity of Iranian wild boars (Larson et al., 2005, 2007; Ottoni et al., 2012), particularly in Southern Iran. A

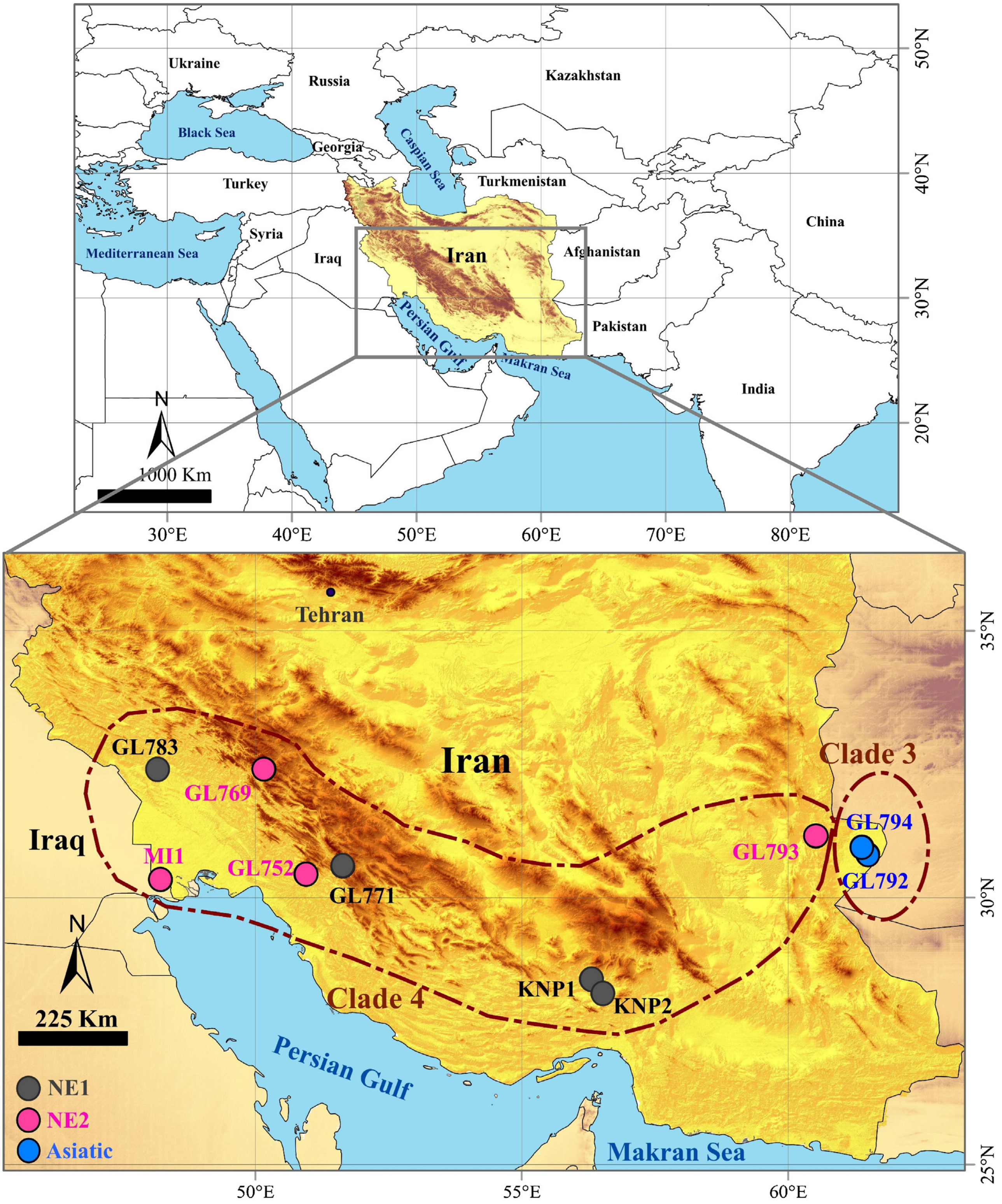

Fig. 1. Geographical distribution of wild boar clades in Southern Iran (see Fig. S1). Dashed lines represent the approximate range of the wild boar clades, based on Larson et al. (2005). Coloured circles refer to the clades based on other studies (e.g., Larson et al., 2007; Alves et al., 2010; Ottoni et al., 2012; Vilaça et al., 2014): black, pink, and blue circles indicate the Near Eastern 1, Near Eastern 2, and Asian clades, respectively. The following codes indicate samples from Larson et al. (2007): GL752, 769, 771, 783, 792–794 (see Tables S1 and S2) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

recent study (Khalilzadeh et al., 2016) found that there were four mitochondrial clades including European (E1), Asian, NE1 (Near Eastern1) and NE2 (Near Eastern2) clades among wild boars in Northern Iran; this study also provided a striking result suggesting a contact zone between Asian wild boar (comprising the NE1, NE2, and Asian clades) and the European (E1) clade in Northwest Iran. However, no information was given in that study on the phylogeny and genetic diversity of wild boar populations in South Iran.

The findings of Bayesian phylogenetic and network analyses are in accordance with previous studies (Larson et al., 2005, 2007;

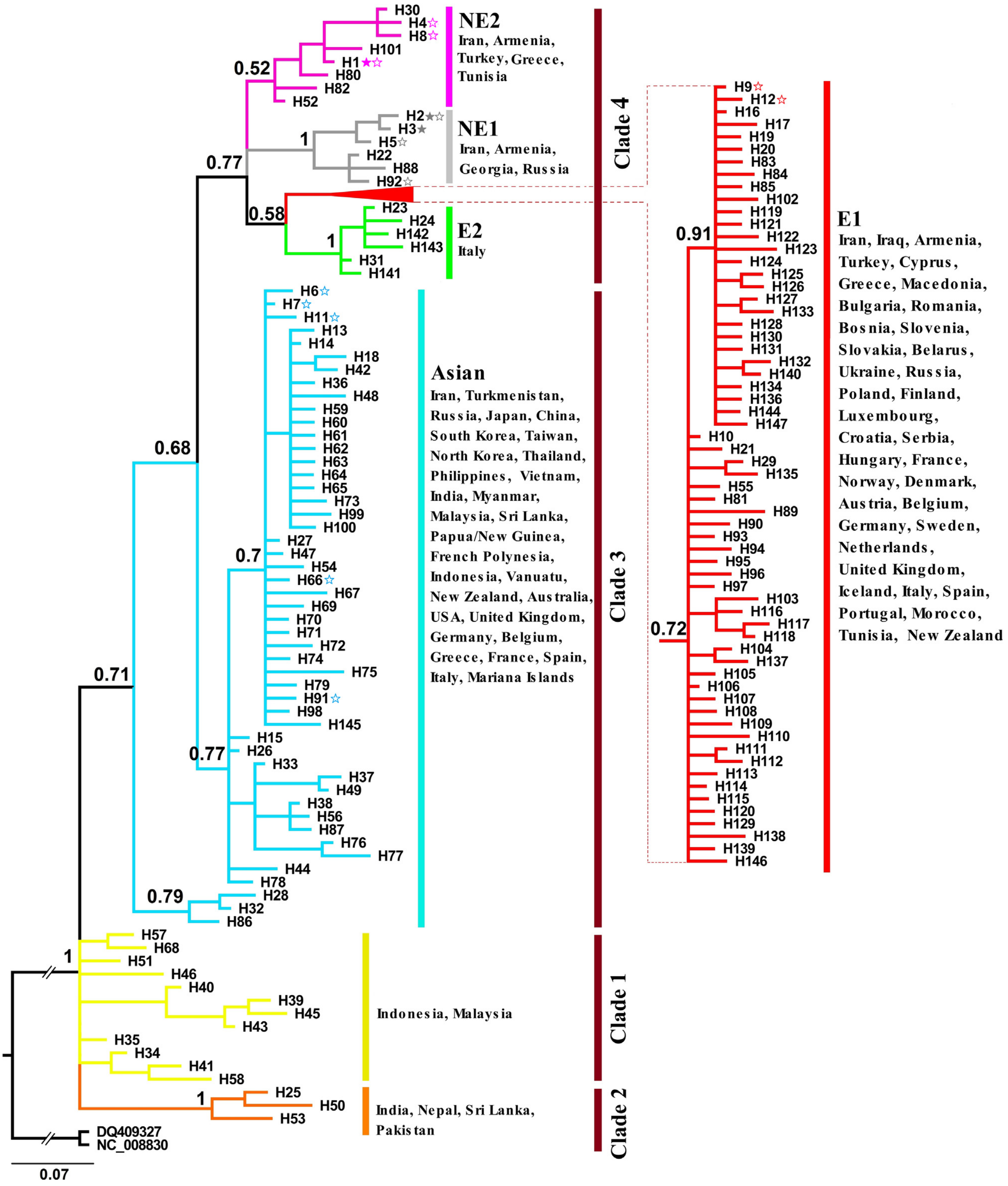

Fig. 2. Phylogenetic relationships of Iranian wild boars with other domestic and wild boars derived from an alignment of 402-bp mtDNA control region sequences and rooted with the common warthog (*Phacochoerus africanus:* DQ409327 and NC_008830). The numbers on the branches are posterior probabilities in the Bayesian inference. The main clades are displayed based on the topologies reported in other studies: (1) clades 1–4 are based on Larson et al. (2005), and (2) Near Eastern 1 (NE1), Near Eastern 2 (NE2), E1, E2 and Asian clades are based on others such as Larson et al. (2007) and Ottoni et al. (2012). Blanked and filled stars indicate newly identified and previously reported haplotypes from Iran, respectively.

Scandura et al., 2008; Alves et al., 2010; Ottoni et al., 2012; Alexandri et al., 2012; Veličković et al., 2015; Vilaça et al., 2014; Khalilzadeh et al., 2016). The analyses revealed that wild boars from the southern part of Iran belong to the clades 3 and 4 (Larson et al., 2005). The findings show that clade 4 encompasses a relatively wide distribution in the southern regions of Iran from the east (Sistan and Baluchestan province) to the west (Khuzestan province), whereas clade 3 is restricted to a small area in Sistan and Baluchestan province (Figs. 1 and S1). Furthermore, our results suggest the existence of a contact zone between clade 4 (NE1 and NE2) and

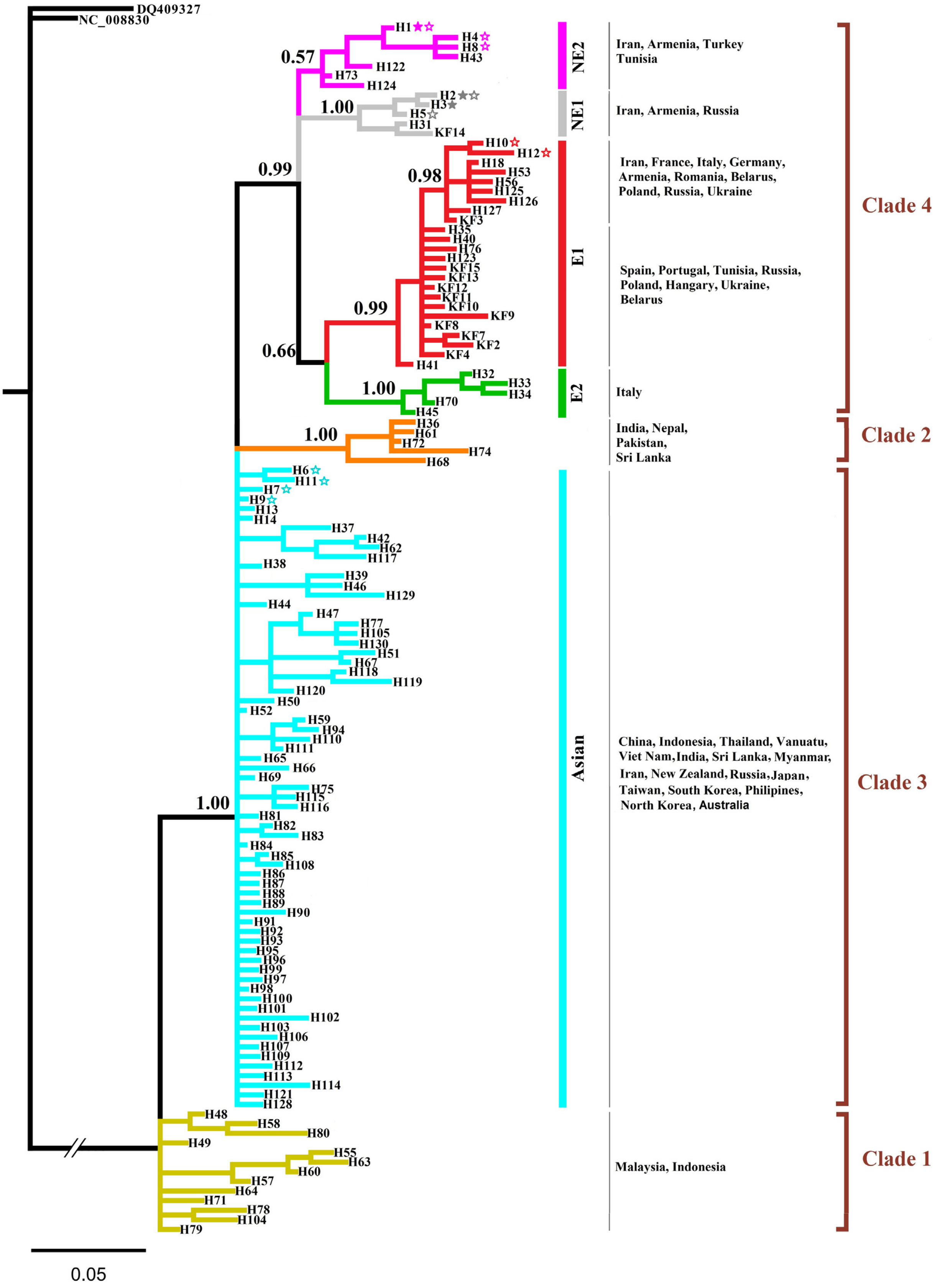

Fig. 3. Phylogenetic relationships of Iranian wild boars with other domestic and wild boars, based on the 605-bp mtDNA control region sequences and rooted with the common warthog (*Phacochoerus africanus:* DQ409327 and NC_008830). The numbers on the branches are posterior probabilities in the Bayesian inference. The main clades are displayed based on the topologies reported in other studies: (1) clades 1–4 are based on Larson et al. (2005), and (2) Near Eastern 1 (NE1), Near Eastern 2 (NE2), E1, E2 and Asian clades are based on others such as Larson et al. (2007) and Ottoni et al. (2012). Blanked and filled stars indicate newly identified and previously reported haplotypes from Iran, respectively.

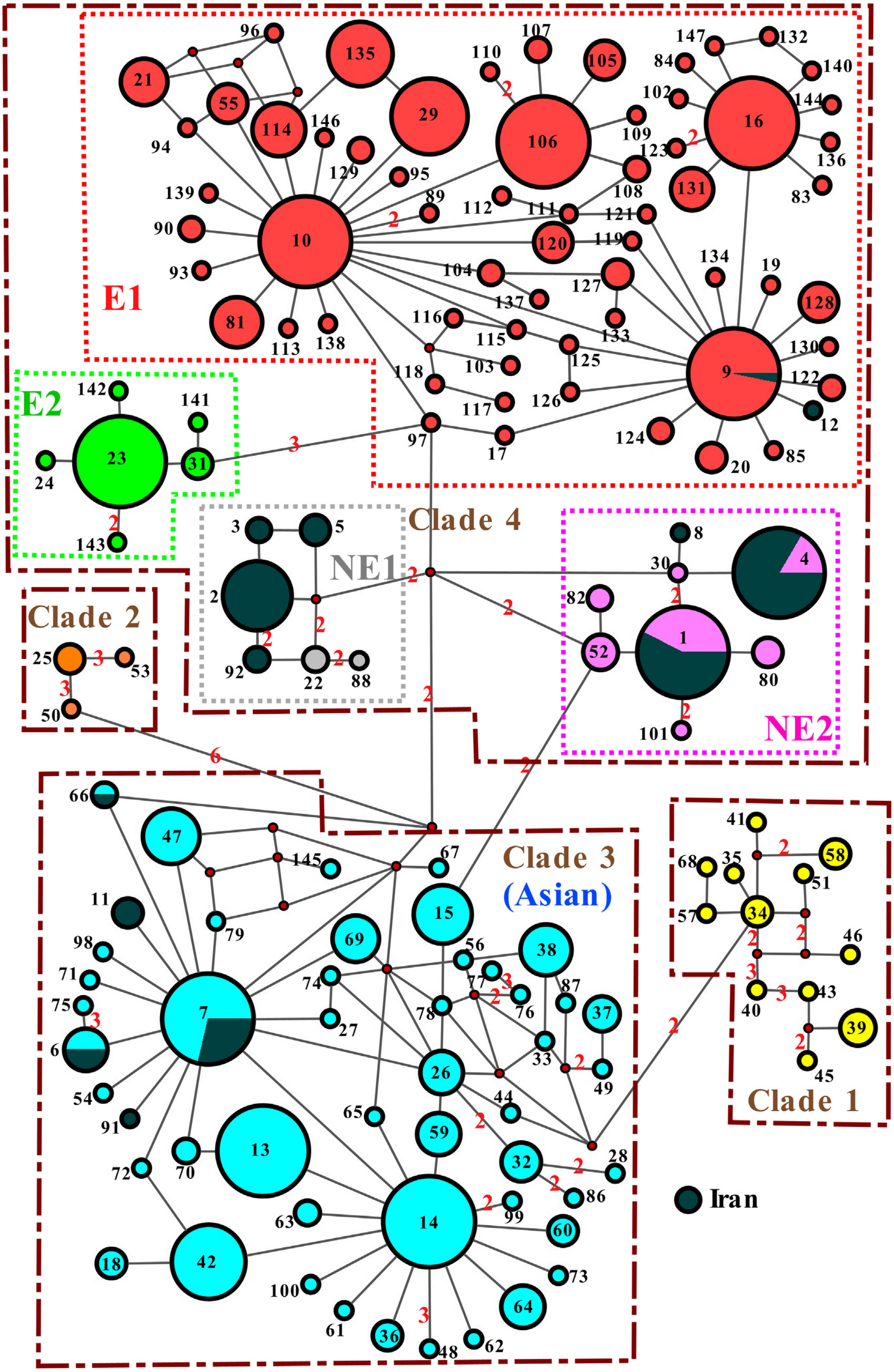

Fig. 4. Median-joining network of Iranian wild boars and other domestic and wild boars depicting the relationships between the main clades in a 402-bp segment of the mtDNA control region. The main clades are displayed based on the topologies reported in other studies: (1) clades 1–4 are based on Larson et al. (2005), and (2) Near Eastern 1 (NE1), Near Eastern 2 (NE2), E1, E2 and Asian clades are based on others such as Larson et al. (2007) and Ottoni et al. (2012). For detailed information on haplotypes (H1–H147) see Table S1.

Table 1
AMOVA results for wild boars from Southern Iran (NE1, NE2, and Asian clades) based on the 605-bp mtDNA control region.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation	*P*
Among clades	2	88.407	4.432	94.71	$P < 0.0001$
Within clades	33	8.176	0.248	5.29	
Total	35	96.58	4.680		
Fixation Index F_{ST}	0.947				

clade 3 (Asian) in Southeast Iran. Some wild boar specimens belonging to clades 3 and 4 have been reported by Larson et al. (2005) from Iran: (1) Clade 3, including a single individual (AY884638) from Golestan province in North Iran; (2) clade 4, which includes one specimen (AY884622) from Hamedan province in West Iran and another specimen (AY884725) from Iran. The geographical location of the last sample was uncertain.

According to the proposed topology by other studies (e.g., Larson et al., 2007; Ottoni et al., 2012; Veličković et al., 2015; Vilaça et al., 2014), Southern Iran is populated by wild boar from three clades: Asian, NE1 and NE2 (Fig. 1). The NE1 and NE2 clades have a wide geographic distribution and are dominant in Southern Iran. However, the Asian clade has been identified in most of the eastern part of the area studied (Sistan and Baluchestan province), only in a small distribution range. However, further investigations and more intensive sampling will present broader facts regarding the geographic distribution of the wild boar clades in the southern part of Iran. The Asian clade has been reported by previous studies (Larson et al., 2007; Ottoni et al., 2012; Khalilzadeh et al., 2016) across the northeast to the northwest of Iran and has also been identified in specimens from Sistan and Baluchestan province situated in the southeast of Iran. The NE1 clade was already reported from the western and northwestern parts of Iran, and sequences belonging to the NE2 clade were previously obtained across the northeast to the northwest of Iran, southwest and southeast of the country (Larson et al., 2007; Ottoni et al., 2012; Khalilzadeh et al., 2016). Our findings indicate that the European clade (E1) is not distributed in Southern Iran. Khalilzadeh et al. (2016) obtained sequences (KR075769 and KR075770) of clade E1 from the northwest of Iran (Ardabil province), characterized as the easternmost distribution of the clade, whereas Larson et al. (2007) reported clade E1 sequences from Kermanshah province in West Iran (DQ872939) and Golestan province in the Southeast Caspian Sea (DQ872954).

This study revealed only one haplotype (MI1 in Figs. 1 and S1; also, H1 in Figs. 4 and S2) among wild boars from Minoo Island (14 individuals) that was placed within the NE2 clade. Wild boars on Minoo Island are probably good representatives of peripheral populations inhabiting their respective mainlands, because wild boars are good swimmers (Nowak and Paradiso, 1999; Allen et al., 2001) and their split probability can only increase on islands that are a very great distance from the mainland (Albarella et al., 2009). Among the 15 mtDNA control region sequences from Khabr national park, a total of two haplotypes (KNP1 and KNP2 in Figs. 1 and S1; also, H2 and H3 in Figs. 4 and S2) were identified that were placed within the NE1 clade.

The distribution ranges of the NE1 and NE2 clades overlap in the southern regions of Iran, and this result (sympatric distribution) is consistent with previous studies (Ottoni et al., 2012; Khalilzadeh et al., 2016). Inference of phylogenetic trees may be affected by contact in the regions of overlap. In other words, recent genetic hybridization or incomplete lineage sorting of ancestral variation potentially can be resulted in the admixture of individual genotypes among populations. In total, the overlapping distribution among different clades can be resulted in low posterior probability of the clades on phylogenetic tree. Other studies have also reported the sympatric distribution of different clades of wild boar (Watanobe et al., 2003). In addition, the geographic distribution of *S. scrofa* has been reported to overlap with those of other *Sus* species (*S. salvanius*, *S. barbatus* and *S. verrucosus*) in South-East Asia (Albarella et al., 2009; Groves, 1983). The AMOVA results based on wild boar sequences from Southern Iran show that 94.71% of the overall variation is observed among the identified clades (NE1, NE2, and Asian) in this area.

The recent mtDNA control region investigation revealed a strong genetic structuring within the northern populations of Iranian wild boar (Khalilzadeh et al., 2016). On the other hand, haplotypes such as H1, H7, H9, H10 and H14 (Fig. 4, Table S1) have a wide distribution, which suggests that they are probably ancestral haplotypes or, alternatively, these haplotypes may result from recent population expansions (Alves et al., 2010). Haplotypes H7, H9, H10 and H14 in Fig. 4 with more than 15 connections to other haplotypes are relatively ancestral compared to the remaining haplotypes.

Overall, our findings and other authors' results (Djamali et al., 2009; Rajaei et al., 2013; Naderi et al., 2014; Ashrafzadeh et al., 2016; Khalilzadeh et al., 2016) may reflect the existence of several probable glacial refugia during the Last Glacial Maximum in (or near) Iran. Recent investigations (Rajaei et al., 2013) based on the genetic data suggested the presence of a wide glacial refugium in the southeastern Zagros Mountains (with the center of Dena, situated in Kohgilouye and Boyerahmad province) and another one on the eastern coast of the Persian Gulf.

The MJ networks (Figs. 4 and S2) clearly show that the southern populations of Iranian wild boar belong to the NE1, NE2 and Asian clades, which are sharply differentiated from other clades. This result is in accordance with other studies (Larson et al., 2005, 2007; Ottoni et al., 2012; Khalilzadeh et al., 2016) indicating that wild boars inhabiting Iran do not belong to a unique clade.

Our research and previous phylogenetic studies (Larson et al., 2005, 2007; Ottoni et al., 2012; Khalilzadeh et al., 2016) demonstrate that Iranian wild boars have an important phylogenetic position among the wild boars of the world. In other words, the Middle East and particularly Iran is a very important region for addressing some of the questions about phylogenetic relationships between the Asian and European wild boar clades (Khalilzadeh et al., 2016). Nevertheless, since mtDNA markers only provide information on female-mediated gene flow (Waits et al., 2000), for a detailed assessment of the genetic differentiation and geographic structure of Iranian wild boar nuclear markers should also be used.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mambio.2018.04.001.

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