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Phylogenetic diversity and DNA barcoding of the camel tick *Hyalomma dromedarii* (Acari: Ixodidae) of the Eastern region of Saudi Arabia

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Abstract. Hard ticks are causative agents of physical illness and vectors of important diseases of human and livestock. The hard tick *Hyalomma dromedarii* Koch, 1844 is a major ectoparasite of livestock in the Kingdom of Saudi Arabia (KSA), of which, the one-humped dromedaries *Camelus dromedarius* is the most economically and culturally important and a potential reservoir of Middle East respiratory syndrome coronavirus (MERS-CoV) disease. Here we report on the molecular phylogenetic diversity of *H. dromedarii* collected from camels in the Eastern Province of KSA based on the mitochondrial *cytochrome oxidase I* (*COI*) gene. Maximum likelihood (ML) phylogenetic analysis of *COI* sequences of the studied ticks identified 11 haplotypes. All *H. dromedarii* ticks from KSA belonged to eight haplotypes diverged into two distinguished genetic clades (A-B). These results indicate that *H. dromedarii* ticks from KSA are monophyletic species with two distinguished lineages with low intra-specific genetic divergence and sharply structured isolated populations with high level of genetic differentiation. This is a first report of DNA barcode of *H. dromedarii* ticks from KSA and the Arabian Peninsula, which is an important step towards broader phylogenetic studies on larger tick samples from the region. The studies are important for better understanding its interactions with camels and other hosts and role in zoonotic disease transmission (e.g. MERS-CoV or Alkhurma virus) to pinpoint effective control strategies.

INTRODUCTION

The Arabian camel, the one-humped dromedaries *Camelus dromedarius* is one of the most popular livestock in the Kingdom of Saudi Arabia (KSA) due to its economic, veterinary and cultural importance. In their natural desert habitats, camels are usually raised under warm and dry climatic conditions and are therefore subjected to severe stress conditions, which render them susceptible to many diseases and ailments, mainly due to infestation by ectoparasites such as hard ticks (Al-Shammery *et al.*, 2011; Abdullah & Faye, 2012).

Due to their parasitic and blood-feeding habits, ticks directly impact their host(s) with severe body damage and illness and transmission of numerous pathogens (viral, bacterial, rickettsial and protozoan) of important infectious zoonotic diseases. These diseases cause chronic illnesses, mass mortality and severe economic losses in livestock due to physical damage and reduced productivity (Sonenshine & Mather, 1994; Rees *et al.*, 2003; Jongejan & Uilenberg, 2004; Randolph, 2008; Cangi *et al.*, 2013).

Hard ticks (family: Ixodidae) have a worldwide distribution with a higher

preference to temperate regions than soft ticks (family: Argasidae). *Hyalomma* ticks are widely distributed in the Middle East and the Arabian Peninsula and many Asian countries. These ticks are highly adapted to humans and their domesticated environments and animals, and therefore, became of significant economic and public health importance.

The hard tick of genus *Hyalomma* Koch, 1844 contains an estimated 30 recognized species and subspecies, which inhabit regions of long dry seasons in Asia (Oriental), Africa (Afrotropical) and Europe (Palaearctic). *Hyalomma* ticks infest mammals, birds and reptiles, and despite their medical and veterinary importance, the taxonomic position, population genetics and evolutionary history of many species are yet to be resolved (Rees *et al*., 2003; Randolph, 2008; Sands *et al*., 2017).

Of these ticks, *H.* (*Euhyalomma*) *dromedarii* Koch, 1844, *H.* (*Euhyalomma*) *schulzei* Olenev, 1931, as well as *H. asiaticum* Schulze et Schlottke, 1929, are of the species most closely-related and associated with camels and mainly exist in areas of their sympatry, including KSA, Egypt, Sudan and Iran. The wide distribution of *H. dromedraii* is largely due to its association and adaptation to the widely-distributed livestock hosts, such as the camel *C. dromedaries*. *Hyalomma dromedarii* is mainly a multi-host species with single host preferences (Hoogstraal & Aeschlimann, 1982; van Straten & Jongejan, 1993; Rees *et al.*, 2003; Apanaskevich *et al.*, 2008).

Large-scale analyses of combined morphological characters and molecular markers of different tick species could reveal important information on their phylogeny (Hoogstraal *et al.*, 1969; Rees *et al.*, 2003; Cangi *et al*., 2013; Sands *et al.*, 2017). The most basal lineages in *Hyalomma* species are currently confined to Eurasia and molecular mitochondrial (mtDNA) clock estimates suggest that early diversification events of the genus member species occurred by large-scale tectonic events and global environmental changes, which significantly contributed to their current species richness and biodiversity (Sands *et al.*, 2017).

Different molecular markers used in DNA barcoding provide different genetic and chronological information on species based on their genomic loci (nuclear or mitochondrial) and function (metabolic enzymes), which are exposed to various evolutionary processes and mutation rates (Lv *et al*., 2014a, b; Abdullah *et al.*, 2018). Rees *et al*. (2003); analyzed mitochondrial and nuclear sequences of *H. dromedarii*, *H. truncatum* and *H. marginatum rufipes* collected from one-humped camels in Ethiopia. These species are well-differentiated morphologically and genetically; however, incongruence between morphology and mtDNA phylogeny was observed, with multiple individuals of *H. dromedarii* and *H. truncatum* present on the same mtDNA genetic lineage as *H. marginatum rufipes*. Thus, individuals with similar morphology of *H. dromedarii* and *H. truncatum* are indistinguishable from *H. marginatum rufipes* based on mtDNA alone. Subsequent analysis of ITS2 of designated 'normal' and 'putative hybrid' individuals from the above ticks showed inter-individual variation within 'putative hybrids', particularly in *H. dromedarii*, which strongly suggested that gene flow has occurred among these *Hyalomma* species, which was not reflected on morphology of their individuals (Rees *et al*., 2003).

The hard tick species commonly found in KSA are *H. dromedarii*, *H. impeltatum*, *H. schulzei*, *H. anatolicum*, *Boophilus kohlsi*, *Rhipicephalus turanicus* (Al-*Shammery et al.*, 2011). Despite the enormous economic and cultural importance of camels in the Arabian Peninsula including KSA, there have been few studies that focused on the ectoparasites infesting them; the most important of them are ticks (Al-Shammery *et al.*, 2011). These studies have been mere classical ecological and taxonomic surveys; however, no study addressed the species molecular structure composition and phylogenetics of camel ticks. These studies are very important to understand the evolutionary relationships

between different species and genera and their interactions with their hosts either as nuisance agents or vectors of diseases, and therefore put suitable plans of control.

Therefore, our main objective was to study the species composition of the ticks infesting the Arabian camel (*C. dromedarius*) in KSA, using both ecological and molecular approaches. This was to establish the phylogenetic relationships between species/genera that will be identified using mitochondrial gene markers. This study could lead to the development of molecular assays to differentiate between morphologically indistinguishable tick species or subspecies of groups or complexes. This information is lacking in KSA and will guide the control of these ticks and minimize the risk of diseases that might be transmitted to host camels and humans.

MATERIALS AND METHODS

Sample collection and identification of ticks

Samples of ticks were collected from on animal bodies from different camel breeding yards in Jubail city, the Eastern region of KSA (Fig. 1, Table 1). Ticks were collected while they were feeding on camels from different body parts (eyelids, ears, neck, hump, chest, legs or tail). The camels were of different age, sex and originated from different countries. Ticks were stored at room temperature then freezed overnight for species identification. Ticks were photographed and identified to the species level using the key of Soulsby (1982). Ticks were sorted to determine the site and density of infestation: according to body organs, sex, age and origin of camels (Table 2). After species identification, tick samples were preserved in 70% molecular grade ethyl alcohol and stored at -20°C for further processing for molecular and phylogenetic analysis.

Extraction of genomic DNA from ticks

Genomic DNA was extracted from the four pairs of legs of individual ticks using the Favorprep™ Tissue genomic DNA extraction Mini kit (Cat. No. FATGK001-1, Favorgen Biotech Corp.) according to manufacturer's instructions with minor modifications. The concentration of eluted DNA was measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific) and analyzed on 1% agarose gel. Agarose gel (1%) was prepared by mixing 1 gm of agarose (Bioline) in 100 ml of 0.5x TBE buffer (1x TBE; 89 mM Tris Base, 89 mM Boric acid, 2 mM EDTA, pH=8.0) (Sambrook *et al.*, 1989). Gel was stained with acridine orange (1 µg/ml) and visualized under the InGenius LHR gel imaging and analysis system (Model No. Lw135M-SYMB, Syngene, United Kingdom). The following DNA size ladders were used: Hyperladder II (Bioline) 50-2000 bp; Hyperladder III (Bioline) 500-5000 bp; Hyperladder V (Bioline) 25-500 bp and DNA Ladder (Solis Biodyne) 100-3000 bp.

Tick barcoding (Amplification of target *COI* gene)

The mitochondrial *cytochrome oxidase C* subunit I (*COI*) gene fragment was amplified by the universal primers LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAA AATCA) (Folmer *et al.*, 1994). The targeted *COI* gene fragment (~710 bp) was amplified from individual ticks by PCR. The reaction components were: 1 µl (10-20 ng) genomic DNA and 2 µl 10 pmole of each primer, 15 µl of 2X PCR master ready mix containing 2X KAPA *Taq* and nucleotides (Kapa Biosystems), and the reaction was completed by PCR grade water to a final volume of 30 µl. The thermocycling reaction conditions were: one cycle at 94°C initial denaturation for 30 sec.; 40 cycles of denaturation at 94°C for 30 sec., annealing (at 40°C for 30 sec., extension at 72°C for 60 sec.); and a final extension cycle at 72°C for 10 min. The PCR reaction products were analyzed on 1-1.5% agarose gel and stained with acridine orange using appropriate molecular size Hyperladder II (Bioline). The amplified PCR products (amplicons) were kept under -20°C until used in the sequencing.

Sequencing and bioinformatics analyses of *COI*

1. Direct Sequencing and editing

The purified PCR products of *COI* were sent for direct sequencing or after purification from gel using the kit MEGA-spin Agarose Gel Extraction (Favorgen Biotech Corp.). Sequencing reactions were carried out in both directions according to the Sanger dideoxy sequencing method (Sanger *et al.*, 1977) at the Advanced Genetic Technologies Center (AGTC) (University of Kentucky, Lexington, KY, USA). The obtained DNA sequence chromatograms were edited, assembled into single contig and aligned using the BioEdit software (BioEdit Sequence Alignment Editor, version 7.0.9.0, Hall, 1999; http://www.mbio.ncsu.edu/Bioedit/bioedit.html). Each single edited sequence was pairwise aligned against homologous sequences in the GenBank database using the NCBI BLASTn (BLASTN 2.7.1+) (Zhang *et al.*, 2000) against non-redundant genomic sequences (nt/nr) datasets of tick taxa for conferring sequence identity and further downstream bioinformatics analysis and phylogenetics. The multiple sequence alignments were done using Clustal W for DNA (Thompson *et al.*, 1994) incorporated in BioEdit software or NPS@ (Network Protein Sequence @nalysis) (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_server.html). The aligned BioEdit file was exported in PAUP/NEXUS format for further phylogenetic analysis as described below.

2. Multiple alignments of *COI* sequences

After PCR reaction purification, the amplicons of a representative of 26 tick samples were sequenced in both directions. The obtained sequences from these specimens as together with homologous sequences retrieved from the GenBank for various *H. dromedarii* (Hdro) from different geographical areas were initially multiple aligned using MUSCLE algorithm (Hdro-India-GQ483461.1, Hdro-Ethiopia-AJ437061.1, Hdro-Kenya-AJ437071.1, Hdro-Iran-KT920181.1, Hdro-UAE-MG188800.1, Hdro-Australia-AF132822.1), and afterwards they were online trimmed using http://users-birc.au.dk/biopv/php/fabox/alignment_trimmer.php. The trimmed aligned sequences were saved as fasta file, which have been used as a matrix to estimate the phylogeny of the entire alignments by constructing Maximum Likelihood (ML) tree (Tamura & Nei, 1993) in MEGA7 (Kumar *et al.*, 2016). All phylogenetic trees were constructed and performed with 1000 bootstrap replications to evaluate the reliability of the constructions. *Haylomma aegyptium* (Haeg) *(*AF132821.1) was used as an out-group in the phylogenetic tree.

3. Phylogenetic analysis of ticks *COI* by Maximum Likelihood

The phylogenetic relationships of the studied ticks were inferred by using ML tree method based on the Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (-645.2009) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

4. Inference of population genetic structure and genetic distances

To estimate the genetic variation between the resultant tick linages through descriptive values, including the number of haplotypes (H), haplotype diversity (Hd) (Nei, 1987), and nucleotide diversity (Nei, 1987) using the software package DnaSP v. 5.1 (Librado & Rozas, 2005).

Genetic distances between the resultant tick lineages were estimated in MEGA7 using the Tamura-Nei model (Tamura & Nei, 1993). To examine the degree of genetic structure in the studied ticks, Arlequin (Excoffier *et al.*, 2005) was first used to generate between-lineages *Fst* values, with the significance of each value determined by a permutation test. To study genetic differentiation and whether there is a population structuring to infer the patterns of genetic relatedness between lineages, the total genetic diversity distributed among populations were estimated in population

samples using pairwise F-statistics (*Fst*) (Weir & Cockerham, 1984) and Arlequin was also used to estimate how genetic variation is partitioned in this system, by conducting several Analyses of Molecular Variance (AMOVA) (Excoffier *et al*., 1992), implemented in the Arlequin.

RESULTS

Tick collection and species identification
A total of 180 adult ticks were collected from on 21 camels (2 males and 19 females) from two camel stock yards in different locations in Jubail city (Fig. 1). Ticks were collected while they were feeding on camels from different body parts starting by the eyelid, ears, neck, hump, chest, legs, ending by the tail. Eighty ticks were collected from six camels in December 2012 and 100 ticks were collected from 15 camels in February 2013 (Table 1). Most of the camels (n=17, all females) were originated from Oman, while one camel was originated each from KSA (n=1 male), Sudan (n=1 male), Egypt (n=1 female) and Pakistan (n=1 female). The camel female age range was 4-8 years, while for males was 7-10 years. Twenty (11.1%) ticks were collected from males, 10 from the tail of a Sudanese male, and 10 from the leg of a Saudi male. A total of 160 (88.9%) ticks were collected from different body parts of female camels with variable infestation intensities (Table 1&2). The Pakistani female camel was the most infested with 30 ticks collected from the chest. The least infested were Omani female camels regardless of camel age, with four ticks collected from udder or tail. The overall tick infestation intensity was 8.57/camel (n= 21), with 8.42/camel (n=19) for females and 10/camel (n=2) for males (Table 2). Camel chest was the most infested organ with an average of 11.66 ticks (n=6) and the least infested organ was the tail with an average of 6 ticks (n=6). All information on the ticks collected

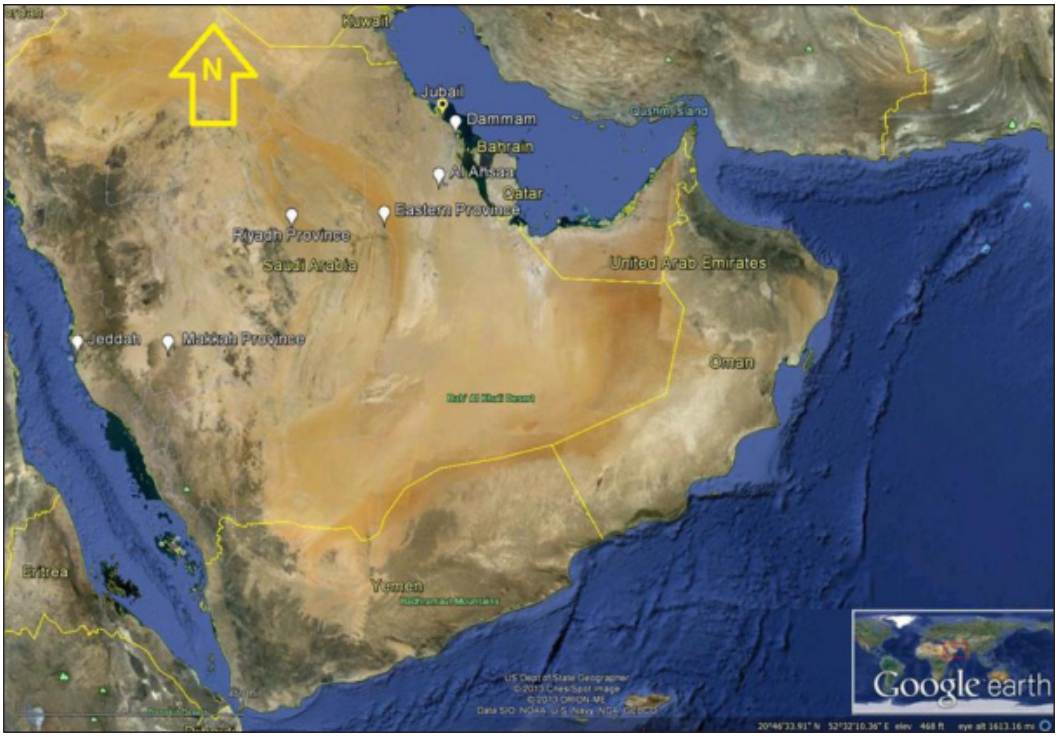

Figure 1. A map of Saudi Arabia showing Jubail region in the Eastern Province, where tick samples were collected from camel farms.

Table 1. Camels geographic origin and infestation intensity of ticks per camel organ, Jubail, Eastern Province, Saudi Arabia

Camel origin (no.)	Camel organ	Tick/organ (no.)	Infestation intensity (Tick/organ)
Egypt (1)	chest	70 (6)	11.66
Oman (17)	ear	10 (1)	10
Pakistan (1)	leg	10 (1)	10
Sudan (1)	neck	10 (1)	10
Saudi (1)	tail	38 (6)	6
	udder	42 (6)	7
Total		180 (21)	

Table 2. Infestation intensity of ticks per camel sex and age

Camel sex	Tick/camel (no.)	Camel age (years)
Female	8.42 (19)	4–8
Male	10 (2)	7–10
Overall	8.57 (21)	

from different body parts of camels and infestation rate are shown (Table 1&2).

Morphological identification of tick species showed that all were *H. dromedarii* (subfamily: Hyalomminae), the major tick species infesting the desert one-humped camel, *C. dromedarius*, in the Middle East including the Eastern region of Saudi Arabia, the largest and most biodiverse country in the Arabian Peninsula. This was subsequently shown by homologous sequence alignments in the GenBank.

PCR-amplification and bioinformatic analysis of *COI* from ticks

The amplified *COI* fragment from all *H. dromedarii* ticks was similar in size and around the expected fragment length of 700 bp flanked by the used primers, showing no apparent size polymorphisms between different tick individuals However, some samples had an extra band (>700 bp), but the reason for this is unknown. The *COI* sequences of the Saudi tick samples were deposited in the NCBI GenBank and were assigned temporary accession numbers: MK177457-MK177460.

Phylogenetic analysis of *H. dromedarii* ticks based on *COI* sequences

To investigate the phylogenetic relationships and genetic divergence among *H. dromedarii* ticks, ML tree was built from all tick *COI* sequences from KSA and those retrieved from the GenBank from various geographic regions from Asia (Hdro-UAE-MG188800.1, Hdro-Iran-KT920181.1, Hdro-India-GQ483461.1, Hdro-Australia-AF132822.1) and East Africa (Hdro-Ethiopia-AJ437061.1, Hdro-Kenya-AJ437071.1). The ML tree topology showed the presence of 11 haplotypes that belonged to two clades (lineages), A and B (Fig. 2). The Saudi *H. dromedarii* tick samples belonged to eight haplotypes (H1, 2, 6, 7, 8, 9, 10, 11) in two distinguished clades, A and B. Clade A included H1, which contained 12 Saudi sequences (Hdro-KSA1-6, 8, 10-13, 18) and H2, which included only one singleton, Hdro-KSA7. Clade B included H6, which contained single sequence (Hdro-KSA14), H7 (Hdro-KSA15), H8 (Hdro-KSA19), H9 (Hdro-KSA16), H10 (Hdro-KSA9) and H11 (Hdro-KSA17). The amplified *COI* fragment length range was 456-692 bp for clade A and 327-677 bp for clade B. Interestingly, there were a clear genetic diversity in *COI* sequences of *H. dromedarii* from KSA and not all shared the same genetic content compared to *H. dromedarii* sequences from other geographical regions (Fig. 2). Clade A included ticks collected in December 2012 and February 2013, while all ticks in clade B were collected in February 2013. In addition, the ticks

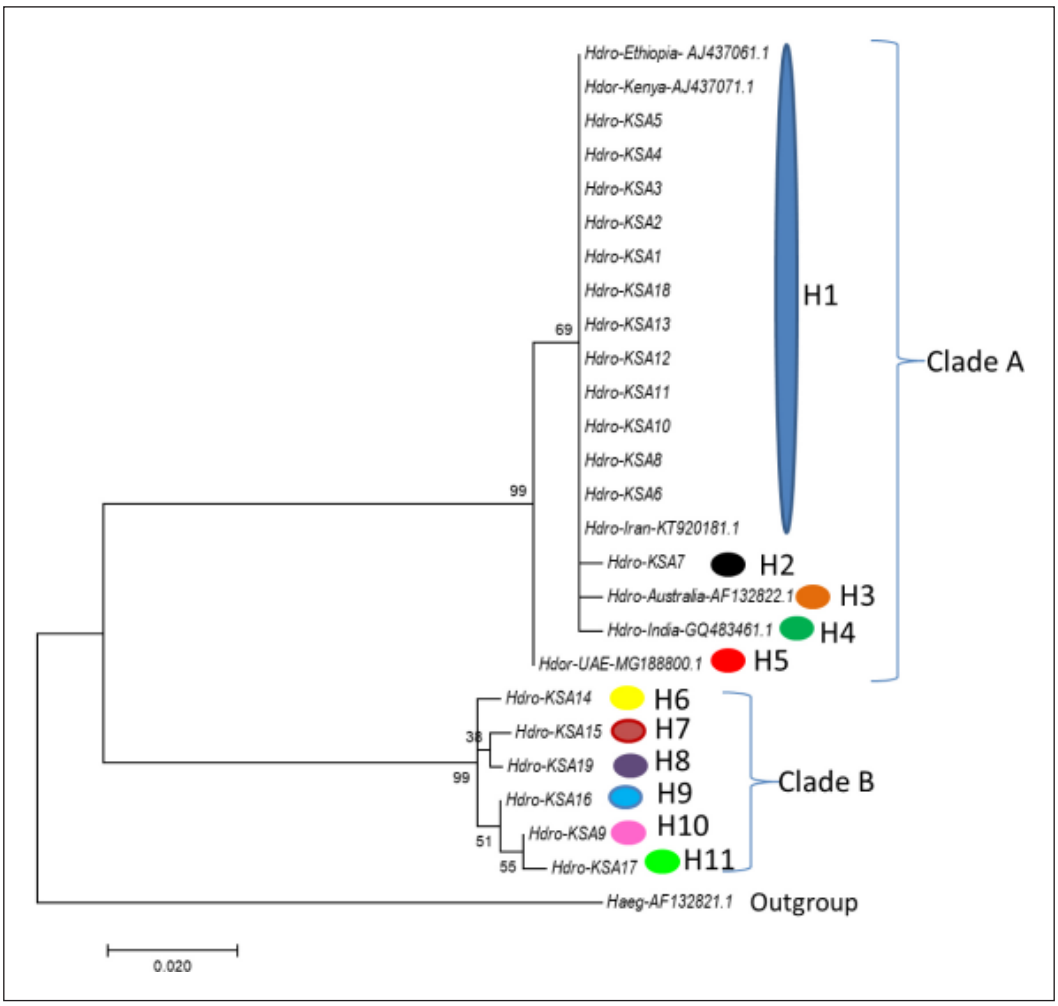

Figure 2. ML phylogenetic tree using *COI* sequences of *Hyalomma dromedarii* tick samples collected from different body parts of the one-humped camel *Camelus dromedarius* from Saudi Arabia (KSA) and *COI* sequences retrieved from the GenBank from different tick species from different geographic origin.

collected in December 2012 were from on camels originated from Oman (female) and Sudan (male), while all those collected in February 2013 were from on female camels originated from Oman. The camel movement, sex and body part of collection might also contribute to the genetic distance or level of variations among the studied ticks. These results show that *H. dromedarii* ticks from KSA are monophyletic species with two distinguished clades or lineages.

The number of haplotypes (H) in lineage 1 (Clade A) is five, while in linage 2 (Clade B) is six (Table 3). The estimated values of haplotype diversity (Hd) in Clade A is 0.376 and Clade B is 0.909 (Table 3). The nucleotide diversity (Pi=π) in Clade A is 0.00180 and Clade B is 0.00640 (Table 3). Genetic distance value measured in MEGA7 between the two lineages was high (13.8%). The *Fst* value provides an indication of the genetic distance between populations by measuring the difference in allele frequency between two populations. The highly significant results as shown in table (4) indicating an isolated population with a higher level of differentiation (Hartl & Clark, 1997). AMOVA was performed using all

Table 3. statistics of number of haplotypes (h), haplotype diversity (Hd) and nucleotide diversity (pi) of *COI* of *H. dromedarii*

Clade	Number of haplotypes (hap)		Haplotype diversity (Hd)	Nucleotide diversity (pi)
Clade A	5	Hap_1:Hdro-KSA6, Hdro-KSA8, Hdro-KSA10, Hdro-KSA11, Hdro-KSA12, Hdro-KSA13, Hdro-KSA18, Hdro-KSA1, Hdro-KSA2, Hdro-KSA3, Hdro-KSA4, Hdro-KSA5, Hdro-Ethiopia-AJ437061.1, Hdor-Kenya-AJ437071.1, Hdro-Iran-KT920181.1 Hap_2: Hdro-KSA7 Hap_3: Hdro-India-GQ483461.1 Hap_4: Hdor-UAE-MG188800.1 Hap_5: Hdro-Australia-AF132822.1	0.376	0.00180
Clade B	6	Hap_6: Hdro-KSA14 Hap_7: Hdro-KSA15 Hap_8: Hdro-KSA19 Hap_9: Hdro-KSA16 Hap_10: Hdro-KSA9 Hap_11: Hdro-KSA17	0.909	0.00640

Table 4. Pairwise *Fst* values of *COI* of the two lineages of *H. dromedarii*

	Lineage 1 (Clade A)	Lineage 2 (Clade B)
Lineage 1 (Clade A)	0.97559 +	0.000
Lineage 2 (Clade B)	0.95920 +	0.98839 +

+ refers to significance level (P=0.0500)
Number of permutations: 110

Table 5. Analysis of molecular variance (AMOVA) for the two distinguished lineages of *H. dromedarii*

Source of variation	Variance components	Percentage of variation
Among populations	16.2	97.56%
Within populations	0.4	2.44%

the different geographically populations (Table 5), it revealed a far higher genetic differentiation among the populations (97.56%) compared with that within populations (2.44%), again indicating a sharply structured population.

DISCUSSION

In this study, we provide a first report on ecology and molecular structure and phylogenetics of the tick *H. dromedarii*, a major ectoparasite of the one-humped camel *C. dromedarius* in the Arabian Peninsula, including the Eastern Province of KSA. The studied tick samples were collected from different body parts of female and male camels originated from five countries from Africa and Asia including KSA. The tick infestation intensities differed with camel sex, body organs and geographic origin, which might indicate diverse tick physiological needs as the richness and

accessibility for blood vessels or protective sheltering during animal movements for hiding from predators or unfavorable climatic conditions. There are complex tick-host-environment-human interactions that affect the tick temporal and spatial distribution, life cycle structure and longevity, blood-feeding physiology (period, frequency, length, size and number of blood-meals) and host-selection (movements and adaptation to humoral and cellular immune responses). These biotic and abiotic factors, mainly ambient temperature and humidity and the surrounding vegetation play important roles in tick vectorial capacity for transmission of various disease pathogens (Randolph, 2008). For individual ixodid ticks, long and slow blood-meals enhance the tick vectorial capacity, however, it could negatively impair tick population overall vectorial capacity due to the consumption of few meals in its life time (Randolph, 2008).

Currently, the molecular approach has become a significant trend in taxonomic and phylogenetic studies of tick species to resolve the difficulty of identification problems especially of tick related species or cryptic species of species complexes. One of the most important DNA markers used for molecular characterization of ticks is the mitochondrial *COI*. The 5'-end *COI* is the standard marker for DNA barcoding in various taxa (Estrada-Pena *et al*., 2107). Although *COI* has proved to be of limited use in identifying some species, and for some taxa, *COI* was ideal and proved more reliable in identifying and discriminating many species of ticks (Lv *et al*., 2014a, b; Zhang & Zhang, 2014). Abdullah *et al*. (2018) molecularly characterized two tick species (*H. dromedarii* and *H. excavatum)* based on *COI* and *16S* and suggested that both *H. dromedarii* ticks and camels could be the sources of Q-fever infection to human and other animals. Al-Deeb & Enan (2018) demonstrated that *H. dromedarii* ticks in the United Arab Emirates (UAE) are genetically very similar based on *COI*.

Yet much research is needed to understand their complex population structure and resolve systematic relationships and ambiguities between many of the species studied so far (Lv *et al.*, 2014a). This is particularly important for closely-related and multi-host tick species, to accurately determine their species structure, host presence, ecological adaptations, genetics of vectorial capacity, immune responses and susceptibility to control measures including acaricides. Such information is valuable for the development of species and disease diagnostic assays, monitoring acaricides resistance and the development of pharmacological and vaccine candidates for effective control intervention strategies against tick infestations and the disease they transmit. The results of *COI* sequence phylogenetic analysis revealed that *H. dromedarii* ticks from KSA are monophyletic species with two distinguished lineages with low intra-specific genetic divergence. The genetic distance between the distinguished lineages indicated sharply structured isolated populations with high genetic differentiation. Moreover, the *H. dromedarii COI* sequences from KSA were highly similar enough to be shared among other geographic regions in East Africa (Ethiopia and Kenya) and Asia (Iran, India and Australia) (Fig. 2). This pattern of intra- and inter-species genetic variation in KSA *H. dromedarii* might be due to a number of interacting factors of tick, host and environmental variables, like homoplasy, ancestral polymorphisms, incipient speciation, tick-host specificity and interactions, inter-specific competitions for the available hosts or environmental conditions and host movement (Klompen *et al*., 1996; Rees *et al*., 2003; Randolph, 2008; Cangi *et al.*, 2013). Rees *et al.* (2003) studied the role of natural hybridization and introgression in the evolutionary process of ticks, especially when inter-species gene exchange events occurred. The authors suggested that biotic and abiotic climatic factors might be responsible for the disruption in maternal mitochondrial gene flow and limit gene exchange and dispersal between distinguished assemblages. These included the presence of juvenile hosts (young camels) in the environment, the ability of adult hosts to disperse across

the landscape and inter-specific competition between the immature stages of *H. rufipes* and *H. truncatum*. Investigation of larger number of morphology-based taxonomic hypotheses or characters and the mechanisms involved in the diversification of the genus *Hyalomma* using multiple mitochondrial and nuclear DNA markers provided maximum resolution and strongly supported the monophyly of *Hyalomma* (Sands *et al.*, 2017). There was congruence between morphological similarities and evolutionary associations such as that between *H. dromedarii*, *H. somalicum*, *H. impeltatum* and *H. punt*. However, wide-scale continental sampling revealed cryptic divergences within the African *H. truncatum* and *H. rufipes*, which suggested the need for more approaches to determine their true taxonomic lineages (Sands *et al.*, 2017).

These differential species- or genus-specific patterns of genetic polymorphism and phylogeographic structure and plasticity, gene flow and speciation and taxonomic complexes and ambiguities are due to various interacting factors: the type and number of molecular markers analyzed, type and stringency of genetic analysis, tick sample type, size, temporal and spatial characteristics, tick ability to survive in a variety of habitats and feed on multiple hosts. The complex evolutionary biology and community ecology of hard ticks resulted in complex vectorial capacities for disease transmission. Therefore, for effective and sustainable control interventions against tick infestations, physical damages and disorders and the disease they transmit to animals and humans, their phylogenetics needs complete and better understanding (Klompen *et al.*, 1996; Barker & Murrell, 2004; Ribeiro *et al.*, 2006; Jeyaprakash & Hoy, 2008; Esteve-Gassent *et al.*, 2016). Intensive comparative phylogeographic studies that include different molecular markers (mitochondrial and nuclear) will give deeper insights on the taxonomic status and genetic structure of different tick populations and species and help understand their vectorial capacities for disease transmission and therefore pinpoint the appropriate control strategies (Lv *et al.*, 2014a; Abdullah *et al.*, 2018; Al-Deeb & Enan, 2018).

In summary this study provides a first-hand report on ecology and molecular phylogenetics of the tick *H. dromedarii*, a major ectoparasite of livestock, especially the one-humped camel *C. dromedarius* from the Eastern Province of KSA, representing the most eastern part of the Arabian Peninsula. A larger-scale mutli-marker study using larger tick sample size covering different camel and other livestock farms and populations across KSA in different seasons, will provide better and broader understanding of the population structure and dynamics, host-range and blood-feeding patterns, physical environment and living habitats, not only of *H. dromedarii* but also for other co-existing tick or ectoparasite species infesting livestock and of public health and economic importance in KSA and the whole of Arabian Peninsula.

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