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Characterization and Phylogenetic Analysis of *Hyalomma dromedarii* (Acari, Ixodidae) in Hot Arid Region of Bikaner, India

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ABSTRACT

The main objective of this study was to investigate the Morphological and Molecular identification with phylogenetic analysis of camel ticks *Hyalomma dromedarii* found on one-humped camels (*Camelus dromedarius*) in hot arid region of Bikaner. For this purpose, a total of 205 ticks were collected from camels in various location of the Bikaner city and NRCC, Bikaner, during summer of 2009. In these location highest suspected species infesting the camels belongs to only *Hyalomma* genus in summer season of 2009 have been found and reported in the Ph.D. thesis of the first author. The large majority of them are *Hyalomma dromedaii* (90%) was identified by using DNA based techniques (Cytochrome oxidase subunit I (COXI) gene) and its specific morphologically features examined and dorsal and ventral view are showing in male and female and the ratio of male ticks (60%) was more than female ticks (40%). Phylogenetic analysis study demonstrated that *H. dromedarii* ticks in the hot region of Bikaner are very similar at the genetic level from hot region of Kenya and Ethiopia, because sequence analysis revealed that COXI gene of *H. dromedarii* from Bikaner shared 99.1–99.2 % sequence identity at the nucleotide level with *H .dromedarii* isolatesfrom Kenya and Ethiopia.

Key words: *Morphology character, Molecular identification, Phylogenetic analysis, H. dromedarii*

Introduction

The camel tick, *Hyalomma dromedarii* (Acari.-Ixodidae) is the most important tick infesting camels in India. The morphological and molecular identification with phylogenetic analysis of the camel tick has not been fully investigated in India. *H. dromedarii* is widely distributed in desert, semi desert and steppes wherever camels occur. *H. dromedarii* is widely distributed throughout north Africa, the northern regions of west, central, and East Africa,Arabia,Asia Minor, the middle East and central and South Asia.(Apanaskevich *et al.*, 2008)

Camel ticks can however be important vectors of viruses affecting man for example, *Hyalomma a. anatolicum* is a major vector of Crimea-Congo haemorrhagic fever (CCHF) virus in the USSR, Pakistan and Nigeria. CCHF has also been isolated from *H. dromedarii* and *H. impeltatum*, another *Hyalomma* commonly found on camels (Hoogstraal *et al.*, 1981) (Converse and Moussa, 1982) have recovered Quaranfil virus from *H. dromedarii* in Iraq, Kuwait

and Yemen. *H. dromedarii* is vector of many disease agents to farm animals such as protozoa (dOliveira *et al.*, 1997) bacteria (Montasser, 2005), Virus (Gunes, 2006) and rickettsia (Kernif *et al.*, 2012).

In the UAE, a spotted fever group *Rickettsia* sp. and *Theileria annulata* were recorded for the first time in *H. dromedarii* ticks in the country (Al-Deeb *et al.*, 2015). There are many ticks' species which transmit various haemo-protozoan (*Babesia caballi, Theileria equi, Theileria annulata*) diseases to camel (Rathore *et al.*, 2013), some time they also cause tick paralysis (Rathore *et al.*, 2014). However, morphological identification can be difficult because it requires some entomological expertise, and it is difficult to identify a specimen that is damaged or at an immature stage of its life cycle (Hubalek and Rudolf, 2012). The typical morphology characters of males *H. dromedarii* can be distinguished from other *Hyalomma* species by a narrow, sub-triangular Parma, usually very large subanal shields, and a long dorsal prolongation of the spiracular plates and females of *H. dromedarii* can be distinguished from other *Hyalomma* species by a narrow V-shaped genital aperture (Estrada-pena *et al.*, 2004; Apanaskevich *et al.*, 2008)

It was reported that DNA bar-coding was a very good tool that clinicians could utilize to get the correct identification of tick specimens. To date, the use of a COXI gene fragment is the standard marker for DNA bar-coding. Molecular methods, such as the sequencing of the mitochondrial 12S (Norris *et al*., 1996), and 16S rDNAs (Norris *et al*., 1996) mitochondrial cytochrome oxidase subunit 1 (COXI); and nuclear internal transcribed spacer 2 (ITS2), have been developed to identify arthropods, including ticks (Song *et al*., 2011).

This is the first report on the morphological character and molecular identification with phylogenetic analysis of camel tick species infesting the one humped camels maintained at hot arid region of Bikaner, India. Therefore, the aim of this study was to investigate the morphological and molecular identification with phylogenetic analysis of camel ticks *Hyalomma dromedarii* found on camels in hot arid region of Bikaner city of Rajasthan, India.

Materials and Methods

Collection of ticks from camels

The 205 engorged adult ticks were collected from camels in and around hot region of Bikaner city and NRCC herd during summer period from June to July 2009. All the camels were naturally infested with many engorged ticks, which predominantly attach itself in the Nostrils, Hump, and Tail and between the foot and attaches at the usual sites of the camels. Ticks were detached from camels using strong forceps into plastic tubes covered by a piece of cloth and secured by rubber band. The field data of each sample such as date, locality, and number of examined camels were recorded (El-Kammah *et al.*, 2001). The ticks were cleaned several times in sterile 1 x PBX (pH 7.2).

Morphological characters of camel ticks

Morphological features were examined under stereo microscope and identified using the guide to identification of species and morphological features of *H. dromedarii* was identified as per the key described by Apanaskevich and co-workers (2008). Then permanent mounting of adult *H. dromedarii* was prepared. Ticks internal organs were removed and washed with distilled water. The ticks specimen were softened by boiling it in 10 per cent KOH solution for 2-5 minutes and washed thoroughly in distilled water until KOH was removed. The specimen was transferred to glacial acetic acid for 15 minutes for dehydration, passed through carboxylol (1:3, Carboxylic acid: Xylene) and xylene for 5 minutes each for clearance. The specimen was mounted in DPX on slide as a permanent specimen and identified as male and female. Morphologically examined in details using LM, especially the dorsal and ventral surfaces of adult males and females. Male and female ticks were fixed on plastic disk one dorsally and another one ventrally. The adult ticks in these two positions were photographed by digital camera fixed on stereomicroscope (El-Kammah *et al.*, 2001).

Molecular Identification with phylogenetic analysis of camel ticks

To confirm the morphological identification proceeded on *H. dromedarii* from Bikaner. After this study we confirmed the suitability of mitochondrial genes as DNA markers for reliable molecular identification of above tick specimens. Keeping these in view, in the present study use of a COXI gene fragment is the standard marker for DNA marker.The cytochrome oxidase subunit I gene of the ticks infesting the camels maintained at hot region of Bikaner and NRCC herd, India, were cloned and

sequenced and its phylogenetic relationship with other tick nucleotide sequence identity of cytochrome oxidase gene from different species of *Hyalomma* and other *ixodid* ticks found at hot regions of other country. Genomic DNA isolation,PCR amplification,cloning and sequence analysis was followed as per Changal *et al.,* 2014.

Results

Among 205 engorged adult ticks which were collected from different parts of city around 123 (60%) were male ticks and 82 (40%) were female. both male and female *H. dromedarii* ticks have been identified based on their morphological features as given below and are depicted in figures 1 and 2. However, the camels tick *H. dromedarii* was the most abundant tick species found on camels recording 90%. The nucleotide sequences of COXI gene of *H. dromedarii* from Bikaner showed a higher homology (99.1 & 99.2%) to *H. dromedarii* isolates from Ethiopia and Kenya. The results of the present study indicate that the tick species infesting the one humped camels, maintained at hot region of Bikaner andNRCC herd, India is *Hyalomma dromedarii.*

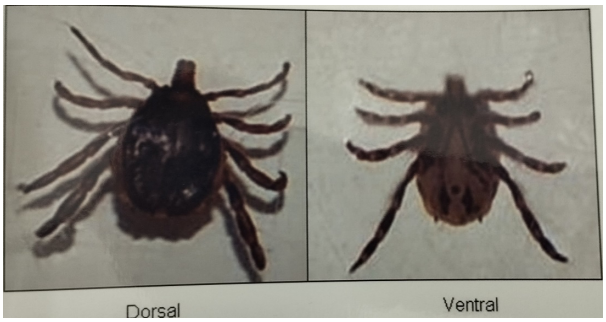

Fig. 1. Morphology character of dorsal and ventral view of male *H.dromedarii*

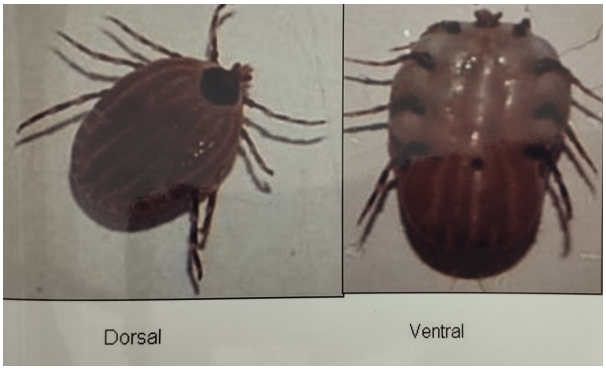

Fig. 2. Morphology character of dorsal and ventral view of Female *H.dromedarii*

Male *H. dromedarii* morphological showed following character

Genital structures: Adanal plates long, lateral margin markedly convex, anteriomedian margin concave, median projection prominent, posteriomedian margin deeply concave, posterior margin rounded; subanal plates vary in shape and size, usually very large and transversally aligned. Sclerotized plaques present ventrally on median (as tiny sclerite) and paramedian festoons.

Spiracular plate-dorsal prolongation long, narrow, and clearly distinct from body of plate; perforated portion of prolongation gently curved throughout its length.

Conscutum: dark brown to reddish brown in color; pale marbling absent; broadly oval in shape; widest near mid-length; slight narrowing in region of spiracular plates; cervical and lateral grooves very deep, up to one half to two thirds of length of conscutum; marginal grooves short, furrow-like, extending anteriorly for posterior 1/4th of conscutum; posteromedian.groove reaches parma; paramedian grooves well defined; caudal field well defined, laterally demarcated by moderate ridges; large punctations sparse, mainly on caudal and lateral fields, small punctations vary in density- usually very sparse, mainly on caudal and lateral fields; parma generally present, narrow, sub-triangular; four distinct festoons.

Capitulum: *Basis capituli,* without lateral projections; dorsal posterior margin angular, deeply concave; cornua modest.

Hypostome: Club-shaped; denticulate portion slightly longer than denticle-free portion.

Legs: *Coxae*: posteromedian and posterolateral spurs of coxa I long, subequal in length or posterolateral spur longer than posteromedian spur, juxtaposed, tapering to apices; coxae IIIV each with distinct, broadly arcuate posterolateral spur; coxae II and III each with modest, very broadly arcuate, posteromedian spur; posteromedian spur on coax IV distinct, triangular. Ivory-colored enamel band encircles distal portion of each segment of legs; incomplete or complete ivory-colored stripe on dorsal aspects of leg segments.

Female *H. dromedarii* morphological showed following character:

Genital structures: genital aperture narrow, triangular in shape (V-shaped); vestibular portion of va-

gina strongly bulging; preatrial fold of genital aperture flat.

Conscutum: yellow to light brown in color; pale marbling absent; nearly as broad as long; posterolateral angles distinct; cervical and lateral grooves deep, extending to posterior margin of scutum; large, deep punctuations sparse, evenly distributed over scutum.

Capitulum: Basis capituli dorsally lateral projections broad andshort, absent ventrally; dorsal posterior margin slightly concave; dorsal cornua inconspicuous.

Hypostome: clubshaped; denticulate portion slightly longer than denticle-free portion.

Legs: Coxae: posteromedian and posterolateral spurs of coxa I long, subequal in length or posterolateral spurlonger than posteromedian spur, tapering to apices, juxtaposed, posteromedian spur broad with bluntapex; coxae II-IV each with distinct, broadly triangular posterolateral spur, with rounded apex; coxaeII-IV each with modest, broadly arcuate, postero median spur. Coloration of legs was similar to that of male.

Molecular identification with phylogenetic analysis of camel ticks

As the standard DNA barcode, COI is the first choice for species identification of ticks, while 16S rDNA, ITS2 and 12S rDNA could be used as complementary to COXI, thereby circumventing situations where COI fails to produce reliable results. The size of COXI gene of *H. dromedarii* from Bikaner is 793 bp in length, which is only partial gene sequence. The resultant gene sequences were submitted to GenBank, NCBI database for which the assigned Accession No. is GQ483461. The percent nucleotide identity of COXI gene of *H. dromedarii* Bikaner isolate with different ixodid tick species from various parts of the world are shown in (Table 1).

The nucleotide sequences of COXI gene of *H. dromedarii* from Bikaner showed a higher homology to *H. dromedarii* isolates from Ethiopia and Kenya. A phylogenetic tree constructed using the partial nucleotide sequences of COXI gene of different ixodid tick species revealed that the *H. dromedarii* from Bikaner clustered with *H. dromedarii* isolates from Kenya and Ethiopia. *Boophilus annulatus* was considered as the out group in the phylogenetic tree (Fig.3). Phylogenetic tree based on nucleotide sequences of cytochrome oxidase gene from different *Hyalomma*

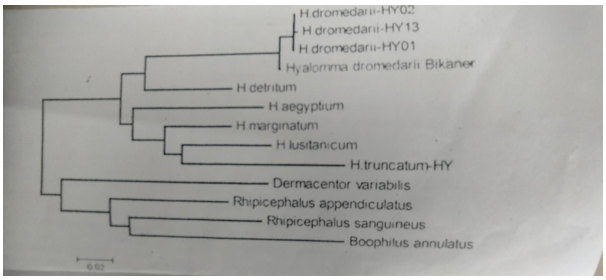

Fig. 3. Phylogenetic tree based on nucleotide sequences of cytochrome oxidase gene from different *Hyalomma* species and *ixodids*, constructed by the neighbour-joining method using MEGA 4 (Molecular evolutionary genetics analysis software with bootstrap values calculated for 1,000 replicates. Horizontal distances are proportional to the genetic distances. vertical distances are arbitrary. The numbers at each branch represent bootstrap values (1000 replicates).

Table 1. Percent nucleotide identity of cytochrome oxidase gene of *Hyalomma dromedarii* from Bikaner with different species of *Hyalomma* and other ixodid ticks

S.N.	Tick species	NCBI Accession No	Percent nucleotide identity
1	*Hyalommadromedarii–Bikaner*	GQ483461	-
2	*H.dromedarii–HY01–Ethiopia*	AJ437061	99.2
3	*H.dromedarii–HY02–Ethiopia*	AJ437062	99.2
4	*H.dromedarii–HY13–Kenya*	AJ437071	99.1
5	*H.detritum*	EU827694	90.2
6	*H.marginatum*	EU827692	88.3
7	*H.aegyptium*	AF132821	87.8
8	*H.lusitanicum*	EU827732	87.1
9	*H.truncatum–HY86*	AJ437087	86.2
10	*Rhipicephalusappendiculatus*	AF132833	84.7
11	*Rhipicephalussanguineus*	AF132839	84.3
12	*Dermacentorvariabilis*	AF132831	84.4
13	*Boophilusannulatus*	AF132825	82.9

species and *ixodids*, constructed by the neighbour-joining method using MEGA 4(Molecular evolutionary genetics analysis software with bootstrap values calculated for 1,000 replicates. Horizontal distances are proportional to the genetic distances. vertical distances are arbitrary. The numbers at each branch represent bootstrap values (1000 replicates).

Discussion

This is the first report for characterization of morphology and molecular identification with phylogenetic analysis of ticks collected from camel at hot arid region of Bikaner city of Rajasthan, India. The camel tick, *Hyalomma dromedarii* is a very characteristic tick closely associated with camels and widely distributed in desert and steppes wherever camels occur. It may be involved in the transmission of various disease agents in India. It's a vector of many disease agents such as Protozoa, bacteria, virus and ricketesia (d'Oliveira *et al.,* 1997; Montasser, 2005; Gunes, 2006 and Loffis *et al.,* 2006). In the present study all ticks which were collected was *Hyalomma* genus based on the morphological character of *H.dromedarii* from other *Hyalomma* species were identified as described by (Estrada-pena *et al*., 2004; Apanaskevich *et al.,* 2008). The observed morphological characters are in concordat with our study. This hard ticks were more common found during summer and hot arid region respectively.

Based on our findings, *H. dromedarii* was the most abundant tick species found on camels recording 90%. Others studies were in agreement with our results as the most dominant tick species on camels are *H. dromedarii* (Hoogstraal and Kaiser, 1958; El-Kammah *et al.,* 2001 and Fard *et al.,* 2012). In addition to, the reference database of DNA sequencing of the COI gene is a very reliable identification tool in animal species. High levels of genetic diversity did not show among the *H. dromedarii* ticks collected from the four locations in the UAE (Hend *et al.,* 2016).

As the standard DNA barcode, COI is the first choice for species identification of ticks, while 16S rDNA, ITS2 and 12S rDNA could be used as complementary to COI, thereby circumventing situations where COI fails to produce reliable results. Moreover, either NN (Nearest Neighbour) or BLASTn could be used for tick species identification because both methods outperformed tree–based methods. We compared the sequences of COXI gene of *H. dromedarii* from Bikaner with the corresponding sequences of *H. dromedarii* from various parts of the world and other ixodid tick species available in the database. Sequence analysis revealed that COXI gene of *H. dromedarii* from Bikaner shared 99.1 and 99.2% sequence identity at the nucleotide level with *H. dromedarii* isolates from Kenya and Ethiopia, respectively. With other species of *Hyalomma, H. dromedarii* from Bikaner exhibited 86.2– 90.2 nucleotide identity. With other genera of the family Ixodidae, *H. dromedarii* from Bikaner showed 82.9–84.78% nucleotide identity (Table 1). Song and his team (Song *et al.,* 2011) noted that the ticks from different geographic ranges could be genetically distinguished.

This suggests that, to be effective, tick control measures for camels should concentrate on the dominant tick species and their hot season of abundance. Planned application of acaricide especially at the beginning of wet months might minimize the burden of ticks on the camels.

Therefore, it is recommended that extensive research work on the analysis of the genes involved in the phylogenetic analysis as well as various salivary gland protein genes of different ixodid tick species infesting the camels of different geographical areas of India needs to be carried out for the elucidation of evolution of hard ticks and the development of a common vaccine candidate gene for their control. Further, it is proposed that the vector potentiality of *H. dromedarii* in the transmission of the viral diseases among the Dromedary camels of the NRCC herd.In conclusion further studies on the effect of these species of ticks on the productivity of camels and the natural immune response of camels against *H. dromedarii* ticks is so far unknown

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Conflict of Interest

The authors declare that they have no conflict of interest.

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