



Molecular Characterization of *Hyalomma dromedarii* from Bikaner, India

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Abstract. In this study, partial nucleotide sequences of cytochrome oxidase (COXI) gene and P-18 protein gene of *Hyalomma dromedarii* from one humped camels of National Research centre on Camel, Bikaner, India, were amplified by polymerase chain reaction from the salivary glands of the ticks. 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* isolates from Kenya and Ethiopia, respectively. With other species of *Hyalomma*, *H. dromedarii* from India exhibited 86.2–90.2 nucleotide identity. With other genera of the family Ixodidae, *Hv. dromedarii* from India showed 82.9–84.78 % nucleotide identity. But, salivary gland protein gene P-18 of *H. dromedarii* from Bikaner was having 90.4 % sequence identity with that of *H. asiaticum*.

Key words: *Hyalomma dromedarii*; Dromedary camel; National Research centre on Camel; Bikaner; India

Introduction

Hyalomma dromedarii can behave as a three- or two host species, with the two-host life cycle seemingly the most common.

Camels are the main hosts of the adults, which also parasitize other domestic animals.

Nymphs and larvae can parasitize the same hosts as adults, especially camels, but rodents, hedgehogs, and birds can also serve as hosts [ANDERSON, 2002].

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].

H. dromedarii is a vector of many disease agents such as protozoa [d'OLIVEIRA *et al.*, 1997], bacteria [MONTASSER, 2005], virus [GUMES, 2006] and rickettsia [KERNIF *et al.*, 2012].

Ticks species can be morphologically identified using

taxonomic keys for endemic species in several geographic regions [HUBALEK and RUDOLF, 2012].

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].

Molecular methods, such as the sequencing of the mitochondrial 12S [NORRIS *et al.*, 1996], 18S [MANGOLD *et al.*, 1998] and 16S rDNAs [NORRIS *et al.*, 1996]; mitochondrial cytochrome oxidase subunit 1 (COX1); and nuclear internal transcribed spacer 2 (ITS2), have been developed to identify arthropods, including ticks [SONG *et al.*, 2011].

The field of vector-host interaction has gained tremendously by high-throughput analysis of salivary gland transcripts and proteomes, collectively called the sialome [RIBEIRO and FRANCISCHETTI, 2003].



This approach allows for the description of secretory products involved in blood-feeding of hematophagous organisms.

The analysis of sialomes in terms of their protein domain compositions allows for a comparative analysis of sialome complexity and diversity that gives us insights into evolution of blood-feeding behavior in arthropods [MANSA *et al.*, 2008].

Research works have been carried out for the characterization of the salivary gland proteins of *H. dromedarii* at protein level [KANDIL and HABEEB, 2009].

Said and his team recently undertook the molecular characterization of Bm86 orthologue from *H. dromedarii* [SAID *et al.*, 2012].

National Research Centre on Camel (NRCC), Bikaner, Rajasthan state, India, is a premiere institute carrying out the excellent research activities exclusively on camels. At NRCC herd, inspite of the regular application of the acaricides, tick infestation is an unavoidable menace in the Dromedary camels.

In Indian Dromedary camels, the clinical cases of only species specific viral infections such as camelpox [BALAMURUGAN *et al.*, 2009] and contagious ecthyma [NAGARAJAN *et al.*, 2010] have been reported both in organized farms and field conditions.

Various studies have demonstrated that during the rainy seasons, the incidence of both camelpox [WERNERY *et al.*, 1997] and contagious ecthyma [NAGARAJAN *et al.*, 2010] outbreaks increases during rainy seasons with the appearance of more severe forms of the disease.

This may be due to the fact that besides the moisture affords the conducive environment for the virus stability and subsequent transmission to susceptible animal, the involvement of arthropods abundant during rainy seasons may serve as a mechanical vector of the virus.

This idea was evidenced by the isolation of camelpoxvirus (CMLV) from *H. dromedarii* [WERNERY *et al.*, 1997].

An outbreak of camelpox in September 2008 and contagious ecthyma in September 2010 occurred among the Dromedary camels maintained at NRCC

herd, Bikaner, Rajasthan state, India (unpublished data).

In Rajasthan state of India, September month is the monsoon period.

It was speculated that ticks infested the Dromedary camels of the NRCC herd could have served as the mechanical vectors in the above mentioned both camelpox and contagious ecthyma outbreaks.

Prior to the studies on the vector potentiality of the ticks in the transmission of the diseases, it is imperative to identify the species of the ticks infesting the camels of NRCC herd.

Till date, there is no published report on work related to the identification of tick species infesting the one humped camels (*Camelus dromedarius*) maintained at NRCC herd, India.

Further, no information is available about the salivary gland protein (P-18) gene of *H. dromedarii*.

Keeping these in view, in the present study, the cytochrome oxidase (EC 1.9.3.1) subunit I gene and P-18 protein gene of the ticks infesting the Dromedary camels (*Camelus dromedarius*) maintained at NRCC herd, India, were cloned and sequenced and its phylogenetic relationship with other tick species is described.

Material and methods

A field experiment was carried out in the period 2009–2011 with

Ticks

Six adult ticks were collected from the ground of camel pens of NRCC herd, India.

Morphological features were examined under stereo microscope and identified using the guide to identification of species [KANDIL and HABEEB, 2009] and morphological features of *H. dromedarii* was identified as per the keys described by Apanaskevich and co-workers [APANASKEVICH *et al.*, 2008].

Isolation of salivary glands, genomic DNA and cellular RNA

Salivary glands of six *H. dromedarii* were isolated as per the protocol



described by El-Kammah and his team [EL-KAMMAH *et al.*, 2005].

Genomic DNA and total cellular RNA were extracted from the tick salivary glands using respective Genei Ultrapure™ Mammalian Genomic DNA Purification Kit–Tissues and Total RNA isolation kit–Cells and Tissues (Bangalore GeNei Pvt. Ltd, India) according to the manufacturer's instructions.

Polymerase chain reaction

The Exon 1 region of the mitochondrial cytochrome oxidase gene of *H. dromedarii* was amplified from the total genomic DNA isolated from the salivary glands of female ticks using the forward primer of COI 5' TTA CCG CGA TGA TTA TAT TCA ACA AAT CAT 3' and reverse primer of COI 5' GTT CTT TTT TTC CAG TAT TAA AAC AAA TAA 3'.

The sequences of the primers were deduced from the GenBank accession No. AJ437083.

Using total cellular RNA extracted from the tick salivary glands as a template, cDNA was synthesized by Easyscript First Strand cDNA Synthesis Kit according to the manufacturer's instructions. From the resultant cDNA synthesized, the P–18 protein gene of *H. dromedarii* was amplified using the forward primer of

5' GAG CGG ATC CAT GAT TTT ATG
GGC GCT TTG 3'

and reverse primer of

5' CGC GCT CGA GTT ACC ACT CAA
TCT TGA CTG 3'.

The sequences of the primers were deduced from the GenBank accession No. EU000252.

PCR amplification was performed with the following thermal profiles: initial denaturation of 94°C for 3 min. followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 55°C for 1 min for COXI gene; 57°C for 1 min for P–18 gene and extension at 72°C for 1 min. and final extension at 72°C for 10 min.

The PCR amplified products were checked on 1% agarose gel.

Cloning and sequencing of PCR amplified DNA fragments

The PCR amplified products were checked in 1.2% agarose gel.

The purified amplicons corresponding to genes encoding COXI and P–18 of *H. dromedarii* were cloned into pGEM–T Easy vector (Promega Corp., Medison, USA).

The ligated mixtures for both genes were individually transformed into *Escherichia coli* DH 5α [SAMBROOK *et al.*, 1989].

The positive clones were confirmed by colony PCR using gene–specific primers and restriction analysis with EcoRI.

The positive clones were sequenced at the sequencing facility, Delhi University (South campus), Delhi.

Since pGEM–T easy vector was used for the cloning purpose, universal T7 and SP6 primers were used for the sequencing of recombinant clones.

The primer sequences used for the sequencing were based on respective promoter sequences.

The determined nucleotide sequences and the deduced amino acid sequences of the GIF and UDG were analyzed with the BLAST program (NCBI) search of GenBank.

Nucleotide identity and comparison of the sequences with published sequences of members of Ixodidae available in the GenBank database were carried out using the computer software BioEdit version 7.0.9.

These sequences were compared in Clustal X [THOMPSON *et al.*, 1997] and a phylogenetic tree was constructed based on the amino acid sequences by the neighbor–joining method using Mega 4 (Molecular Evolutionary genetics Analysis software with bootstrap values calculated for 1,000 replicates [TAMURA *et al.*, 2007]).

Results and discussion

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.

Table 1.

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

Sl.No.	Tick species	NCBI Accession No	Percent nucleotide identity
1	<i>Hyalomma dromedarii</i> –Bikaner	GQ483461	–
2	<i>H.dromedarii</i> –HY01–Ethiopia	AJ437061	99.2
3	<i>H.dromedarii</i> –HY02–Ethiopia	AJ437062	99.2
4	<i>H.dromedarii</i> –HY13–Kenya	AJ437071	99.1
5	<i>H.detrutum</i>	EU827694	90.2
6	<i>H.marginatum</i>	EU827692	88.3
7	<i>H.aegyptium</i>	AF132821	87.8
8	<i>H.lusitanicum</i>	EU827732	87.1
9	<i>H.truncatum</i> –HY86	AJ437087	86.2
10	<i>Rhipicephalus appendiculatus</i>	AF132833	84.7
11	<i>Rhipicephalus sanguineus</i>	AF132839	84.3
12	<i>Dermacentor variabilis</i>	AF132831	84.4
13	<i>Boophilus annulatus</i>	AF132825	82.9

The nucleotide sequences of COXI gene of *H. dromedarii* from Bikaner showed a higher homology to *H. dromedarii* isolates from Ethiopia.

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 (Figure 1).

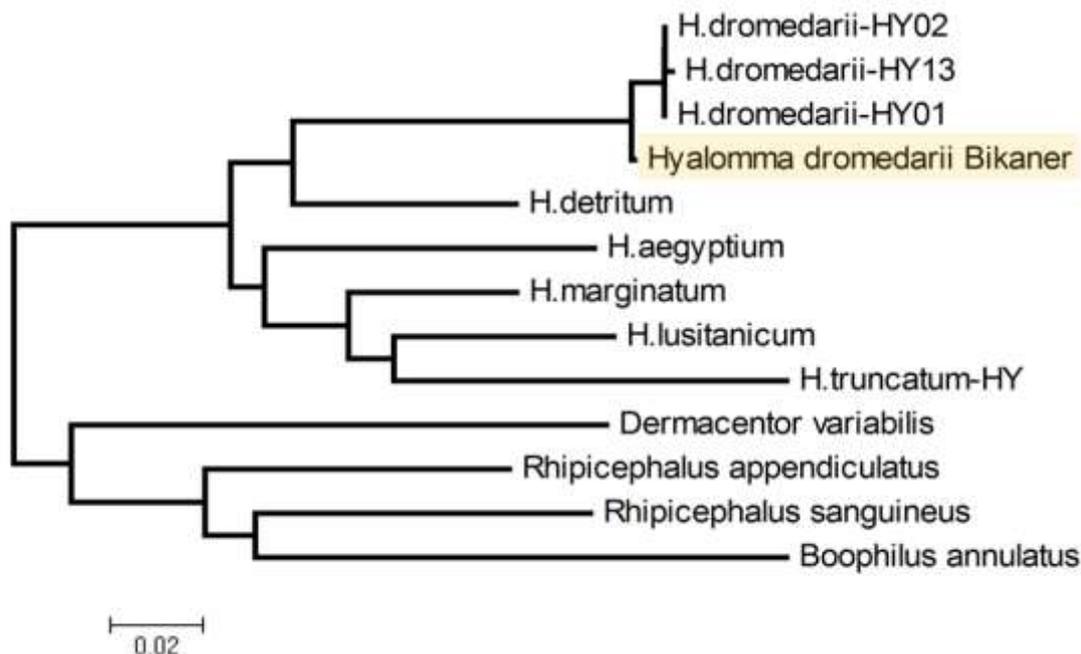


Figure 1. Phylogenetic tree based on nucleotide sequences of cytochrome oxidase gene from different *Hyalomma* species and ixodids, constructed by the neighbor-joining method using MEGA 4 (Molecular Evolutionary genetics Analysis software with bootstrap values calculated for 1,000 replicates). Horizontal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



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 & 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).

Although a low level of intraspecific variation in COX1 and ITS2 was observed among individuals of *Ixodes holocyclus* in Clade II, Song and his team [SONG *et al.*, 2011] noted that the ticks from different geographic ranges could be genetically distinguished.

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 [LV *et al.*, 2014].

The length of the P–18 gene sequenced was 461 bp. BLAST search analysis in NCBI database showed that it has closely matched with P–18 gene of *H. asiaticum* (Accession No. EU000252), which is the only one available sequence in NCBI database.

Pair-wise comparison of these two sequences showed that 461 bp of *H. dromedarii* from India was matching with the nucleotide base, 26 to 486 of P–18 gene of *H. asiaticum* from China (data not shown).

These partial 461 bp gene sequences compared with the corresponding nucleotide sequences of P–18 gene of *H. asiaticum* (GenBank

accession No. EU000252) and it was found that they had 90.4 % sequence identity with *H. asiaticum*.

The nucleotide sequences of the salivary gland protein (P–18) gene of *H. dromedarii* were submitted to GenBank, NCBI database and assigned accession number HM051110.

The results of the present study indicate that the tick species infesting the one humped camels, maintained at NRCC herd, India is *Hyalomma dromedarii*.

Further the baseline information about the salivary gland protein P–18 gene of the present study affords the avenues for the exploration of sialomics of *H. dromedarii* and other ixodid ticks from different geographical areas of India and thereby the development of a new generation vaccine for the control of ticks would be feasible in India.

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.

Conclusions

From the major findings of the present study, it is concluded that the tick species infesting the one humped camels, maintained at National Research Centre on Camel, India is *Hyalomma dromedarii*.

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