



Phylogenetic analysis of some hard ticks from India using mitochondrial 16s rDNA

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ABSTRACT

The present study was conducted to analyze inter-relationships between Indian hard tick using mitochondrial 16s rDNA. The sequence alignment consisted of 85 sequences, 32 sequences of 16s rDNA belonging to 7 species of two genera viz. *Rhipicephalus* and *Hyalomma* generated from PCR amplified products and 53 sequences of hard ticks from India available in genbank database. The NJ analyses conducted in MEGA6 revealed that Haemaphysalinae is basal to the clade of Rhipicephalinae + Hyalomminae in the metastriate lineage while Ixodinae was basal in Ixodidae. There were two large clades, one clade of *Hyalomma anatolicum*, *Hy. excavatum*, *Hy. hussaini* and *Hy. brevipunctata* and second clade of *Rhipicephalus (Rhipicephalus) + Rhipicephalus (Boophilus)*. The results provide evidence for the contention of polyphyly of *Rh. (Rh.) sanguineus* and species complex status of *Rh. (B.) microplus*. A further molecular study from wider distributional area using more genetic markers is needed to confirm these observations.

1. INTRODUCTION

India is predominantly an agricultural country with about 70% of its population dependent on income from agriculture [1]. The livestock sector especially the dairy sector comprising of approximately 199 million cattle and 105 million buffaloes in India is an important part of the rural agribusiness Indian economy [1]. As tick species parasitizing buffaloes and cattle are similar so the threat of tick borne diseases carried from cattle to buffalo and vice versa is possible and equal attention to the health care of cattle and buffalo should therefore be taken [2]. Ghosh and Nagar [1] have reviewed the various tick borne diseases threatening livestock in India and the recent report of spread of Kyasanur forest disease (KFD) from endemic regions in South India to other regions [3] is very alarming so it is all the more pertinent to have knowledge of tick distribution, speciation and evolution. Ticks are classified in the sub-order Ixodida of the order Parasitiformes, one of the two orders of mites (Acari) consisting of about 900 species divided into two major families: Argasidae Canestrini, 1890 of soft ticks and Ixodidae Murray, 1877 of hard ticks. The third family Nuttalliellidae Schulze, 1935 contains

only a single species *Nuttalliella namaqua* Bedford, 1931 in a single genus *Nuttalliella* Bedford, 1931. In the last two decades, molecular phylogenetics has revolutionized the phylogeny and systematics of Arthropods [4], especially Arachnids [5] and Insects [6]. The family Ixodidae of prostriate and metastriate hard ticks has 6 subfamilies Ixodinae, Bothriocrotoninae, Amblyomminae, Haemaphysalinae, Hyalomminae and Rhipicephalinae [7-11]. Recently, there have been reports of prevalence of sibling/cryptic species in different hard tick genera viz. *Rhipicephalus*, *Rhipicephalus (Boophilus)*, *Haemaphysalis* and *Hyalomma* Koch, 1844 [12, 13]. Thus there can be possible difficulties in the study of disease transmission and vector control as proper identification of the vector and understanding of the relationships between closely related species is a must for devising any effective control strategy [14-16]. The traditional morphology based identification is sometimes problematic due to variations caused by blood meal [17] and chances of geographical strains of tick species having different vectorial capacity [18-20], genetic introgression, fertile hybrids [21-22], and resistance to acaricides [23] are always there. During the past two decades several molecular markers have been used to resolve relationships and solve problems facing systematics of hard ticks in the family Ixodidae [24-40]. In this context, there is lack of any study on molecular analysis of hard ticks from the India [2, 41]. This crucial gap in information related to hard ticks prompted us to carry out molecular investigation on members of the family Ixodidae from India using mitochondrial 16s rDNA sequences.

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2. MATERIAL AND METHODS

2.1 Material

The hard ticks infesting cattle and buffalo hosts were field collected from different animal farms located in the state of Haryana (India) (Table 1). After initial separation of hard ticks identification up to species level was done by using standard identification keys available [42-46]. The ticks were photographed using a trinocular stereo-zoom microscope (Labomed™) and subsequently preserved in 100% ethanol in a -20°C deep freezer (Bluestar).

2.2 DNA extraction

DNA was extracted from individual hard ticks using DNeasy® DNA isolation kit (Qiagen). For this, individual ticks were crushed with sterile glass pestle in liquid nitrogen and subsequently DNA was extracted by following the protocol provided with the kit. The DNA was quantified using Tecan's Infinite® NanoQuant and stored at 4°C. Quality of DNA was checked by resolving on 0.8% Agarose gels using standard procedure.

2.3 PCR amplification and sequencing

PCR was performed to amplify 16s rDNA from individual hard tick DNA samples with the following primer pairs: S16S FP (5'-CTGCTCAATGAATATTTAAATTGC-3') and S16S

RP (5' -CGGTCTAAACTCAGATCATGTAGG-3') [47]. PCR reactions were performed in 25µl reaction mixture that had 100ng DNA template and 1.5U of Taq Polymerase (GeNei™) per reaction along with standard reaction ingredients. The PCR cycling conditions set in the program were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 sec (denaturation), 50°C for 40sec (annealing), 72°C for 40 sec (extension) and a final extension step of 72°C for 5 min. PCR products were resolved on 2% Agarose gels and compared with 100bp DNA standard ladder as the expected product size was in range of 420-440bp. PCR products were purified by using Geneipure™ Quick PCR Purification kit (GeNei™) and sent for commercial DNA sequencing to 1st base sequencing service (Malaysia).

2.4 Sequence details and analysis

A total of 32 sequences were generated for 16s rDNA of 7 species of two hard tick genera during the present study while 53 sequences of hard ticks of various species from India available in the genbank database were retrieved and used for deriving phylogenetic relationships (Table 2). Multiple sequence alignment of eighty five 16s rDNA sequences was generated with Muscle software tool executed in MEGA6 phylogenetic analysis software [48]. The alignment was manually edited to remove any alignment errors and exported as mega and fasta format files.

Table 1: The details of hard tick populations analysed during the present study from Haryana (India).

S. No	Place of Collection	Species Identified	Species code used	Longitude and Latitude
1	NDRI Karnal	<i>Rhipicephalus (R) microplus</i> (Canestrini, 1888)	RBM	29.703504 76.983218
2	Chopra Colony, Rohtak Road, Gohana	<i>Rhipicephalus (B) microplus</i> (Canestrini, 1888)	RBM	29.127408 76.698571
3	Nuran Khera, Jind Road, Gohana	<i>Hyalomma brevipunctata</i> Sharif, 1928	HB	29.203916 76.581767
4	Gharaunda City	<i>Rhipicephalus (B) decoloratus</i> (Koch, 1844)	RBD	29.539130 76.967769
5	Hansi Road, Karnal	<i>Hyalomma excavatum</i> Koch, 1844	HE	29.687237 76.974474
6	Arainpura, Gharaunda, Karnal	<i>Rhipicephalus (B) microplus</i> (Canestrini, 1888)	RBM	29.548281 77.016225
7	Village Thuska Mahra, Rohtak Road, Gohana	<i>Rhipicephalus (R) sanguineus</i> (Latreille, 1806)	RRS	29.095420 76.688218
8	Ashok Vihar, Sonapat	<i>Hyalomma excavatum</i> Koch, 1844	HE	28.995288 77.006662
9	Garhi Brahmanan, Sonapat	<i>Hyalomma anatolicum</i> Koch, 1844	HA	28.994380 76.994348
10	Gangana, Jind Road, Gohana	<i>Hyalomma anatolicum</i> Koch, 1844	HA	29.236950 76.614468
11	Badthal, Nilokheri	<i>Rhipicephalus (B) microplus</i> (Canestrini, 1888)	RBM	29.867340 76.872346
12	Kaimla, Gharaunda	<i>Hyalomma hussaini</i> Sharif, 1928	HH	29.505624 76.997015
13	Kurana, Israna, Panipat	<i>Rhipicephalus (B) decoloratus</i> (Koch, 1844)	RBD	29.275754 76.718506
14	Namaste Chowk, Karnal	<i>Rhipicephalus (B) decoloratus</i> (Koch, 1844)	RBD	29.670994 76.990600

Table 2: Accession numbers of 16s rDNA sequences submitted to the GenBank nucleotide database in the study on hard ticks from Haryana (India).

S.NO	DETAILS OF SEQUENCE	POPULATION	SPECIES	Sequence ID
1.	lcl 1573839 T44 16SR	Badthal	RBM	KP210071
2.	lcl 1573838 E2 16SR	Namaste chowk	RBD	KP210070
3.	lcl 1573837 E1 16SR	Namaste chowk	RBD	KP210069
4.	lcl 1573828 A3 16SF	Gharonda city	RBD	KP210068
5.	lcl 1573827 A2 16SF	Gharonda city	RBD	KP210067
6.	lcl 1573826 A1 16SF	Gharonda city	RBD	KP210066
7.	lcl 1st BASE 1573836D3	Kaimla	HH	KP210065
8.	lcl 1st BASE 1573835D2 16SR	Kaimla	HH	KP210064
9.	lcl 1573834 D1 16SR	Kaimla	HH	KP210063
10.	lcl 1573833 C3 16SF	Kurana	RBD	KP210062
11.	lcl 1573832 C2 16SF	Kurana	RBD	KP210061
12.	lcl 1573831 C1 16SF	Kurana	RBD	KP210060
13.	lcl 1573830 B2 16SF	Arainpura	RBM	KP210059
14.	lcl 1573829 B1 16SF	Arainpura	RBM	KP210058
15.	lcl 1st BASE 1551253 K4 16SF	Nurankhera	HB	KP210057
16.	lcl 1st BASE 1547348 K1616SR	Badthal	RBM	KP210056
17.	lcl 1st BASE 1547347 K1516SR	Badthal	RBM	KP210055
18.	lcl 1st BASE 1547346 K1416SR	NDRI	RBM	KP210054
19.	lcl 1st BASE 1547345 K1316SR	NDRI	RBM	KP210053
20.	lcl 1st BASE 1547344 K1216SR	NDRI	RBM	KP210052
21.	lcl 1st BASE 1547343 K1116SR	NDRI	RBM	KP210051
22.	lcl 1st BASE 1547342 K1016SR	Thuska mahra	RBS	KP210050
23.	lcl 1st BASE 1547341 K9 16SR	Chopra colony	RBM	KP210049
24.	lcl 1st BASE 1547340 K8 16SF	Hansi road	HAE	KP210048
25.	lcl 1st BASE 1547339 K7 16SF	Hansi road	HAE	KP210047
26.	lcl 1st BASE 1547338 K6 16SF	Nurankhera	HB	KP210046
27.	lcl 1st BASE 1547337 K5 16SF	Nurankhera	HB	KP210045
28.	lcl 1st BASE 1547335 K3 16SF	Ashok Vihar	HAE	KP210044
29.	lcl 1st BASE 1547334 K2 16SF	Ashok Vihar	HAE	KP210043
30.	lcl 1st BASE 1547333 K1 16SF	Ashok Vihar	HAE	KP210042
31.	lcl 1st BASE 1364793 P4 16SF	Gangana	HA	KP210041
32.	lcl 1st BASE 1364792 P3 16SF	Garhi brahmanan	HA	KJ912623

2.5 Phylogenetic analysis

Phylogenetic analysis was performed in MEGA6 [48] using neighbor joining (NJ) method. The best fit model to derive relationships based on the current data set was estimated by using the model test tool in MEGA6 [48] which supported the - Tamura three parameter model + unequal frequency + gamma distribution (TPMuf+G). In all the analysis gaps and missing data were treated as partial deletion with 90% site coverage cut-off. The phylogenetic trees were constructed using the below mentioned parameters: nucleotide substitution model - Tamura 3-parameter model [49], test of phylogeny - bootstrap method, 1000 replications, substitutions to include - d: transitions + transversion, rates among sites - gamma distributed, pattern among lineages - different (heterogeneous) [50].

3. RESULTS

3.1 Tick diversity

In this context, the culmination of this study from the state of Haryana (India) resulted in identification of seven Ixodid tick species belonging to two genera viz. *Hyalomma* Koch, 1844 and *Rhipicephalus* Koch, 1844. Seven tick species that were identified belonging to these two genera are- *Hyalomma anatolicum* Koch, 1844, *Hyalomma excavatum* Koch, 1844, *Hyalomma hussaini* Sharif, 1928, *Hyalomma brevipunctata* Sharif, 1928, *Rhipicephalus (Rhipicephalus) sanguineus* (Latreille, 1806), *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888), and

Rhipicephalus (Boophilus) decoloratus (Koch, 1844). The study revealed that *Hyalomma anatolicum* and *Rhipicephalus (B.) microplus* are the most common vector species that infest the buffalo and cattle in Haryana. *Hyalomma excavatum* Koch, 1844, *Hyalomma brevipunctata* Sharif, 1928, and *Rhipicephalus (Boophilus) decoloratus* (Koch, 1844) have been reported during the present study for the first time from the state of Haryana, India.

3.2 Molecular Phylogeny

The molecular methods were employed to perform phylogenetic analysis of ticks infesting cattle from Haryana using mitochondrial 16S ribosomal DNA. The sequences obtained during the present study were compared with the sequences from India which resulted in some interesting inferences having consequent implications on the tick molecular evolution and systematics. When the dataset of eighty-five 16s rDNA sequences belonging to members of family Ixodidae from India was analysed it revealed 180 variable sites, 304 conserved sites, 117 parsimony informative sites and 57 singleton sites. When the evolutionary history was inferred for 16s rDNA sequences of the members of the family Ixodidae from India by the Neighbor-Joining method it resulted in a bootstrap consensus tree inferred from 1000 replicates where branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed (Figure 1a, b, c). The evolutionary distances were computed using the Tamura 3-parameter method in which rate variation among sites was modelled with a gamma distribution.

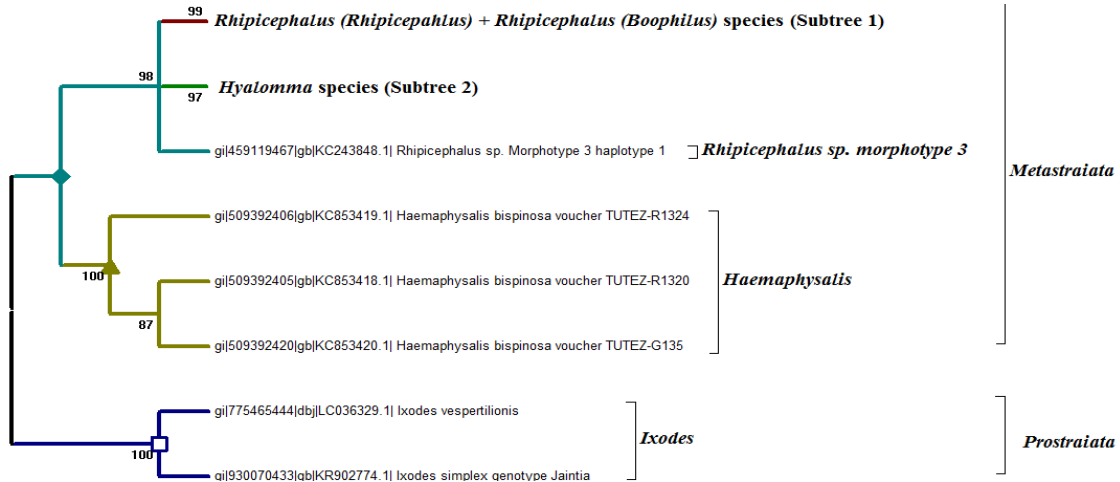


Fig. 1: A-Phylogenetic tree (50% consensus) generated by Neighbor-Joining method based on T3PM+G model with 1000 bootstrap replicates (values on branches are bootstrap support) for 16s rDNA sequences of hard tick species from India.

Figure 1b: Subtree 1

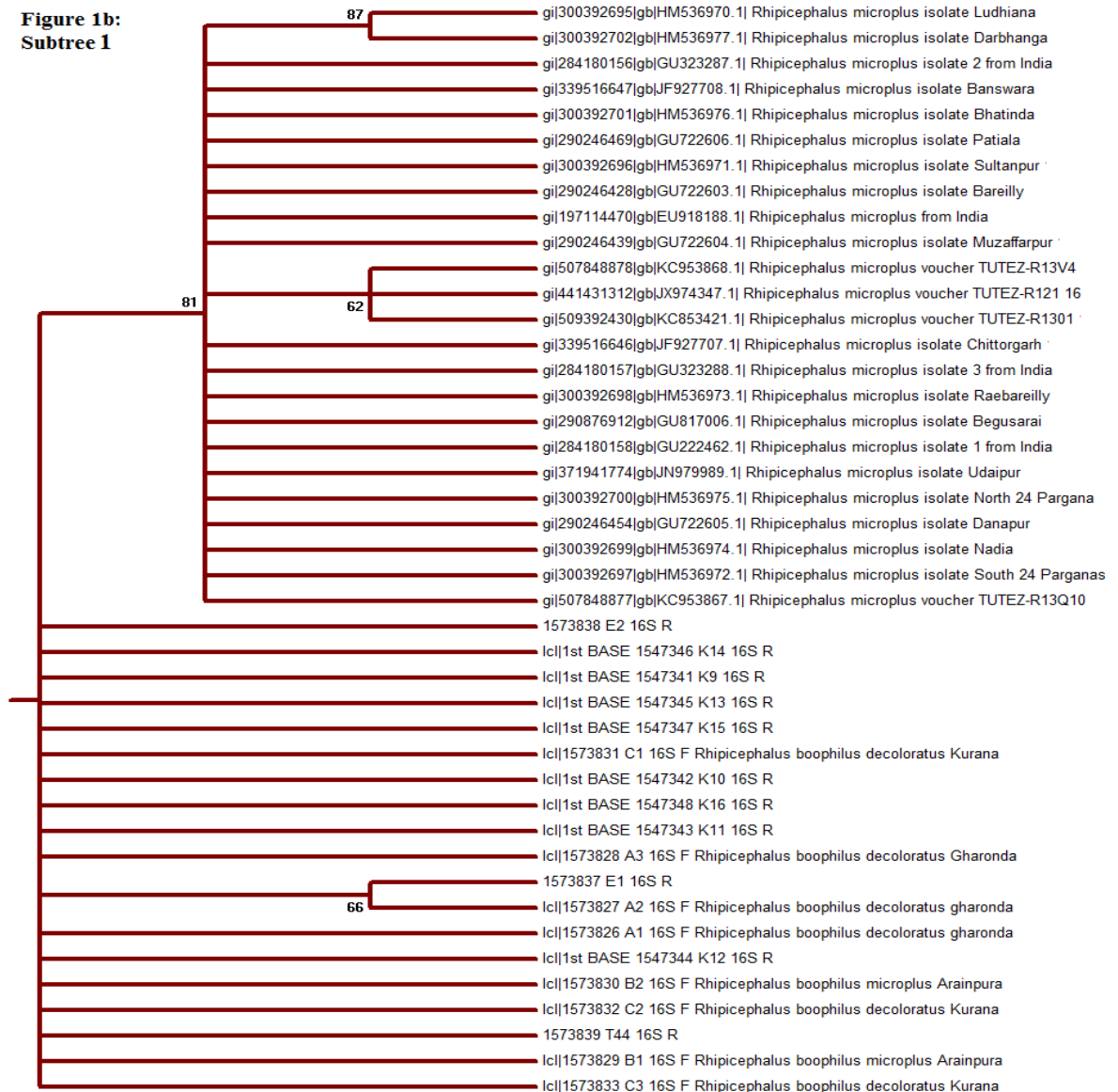


Fig. 1: B-Subtree 1 having *Rhipicephalus (Rhipicephalus) + Rhipicephalus (Boophilus)* species sequences.

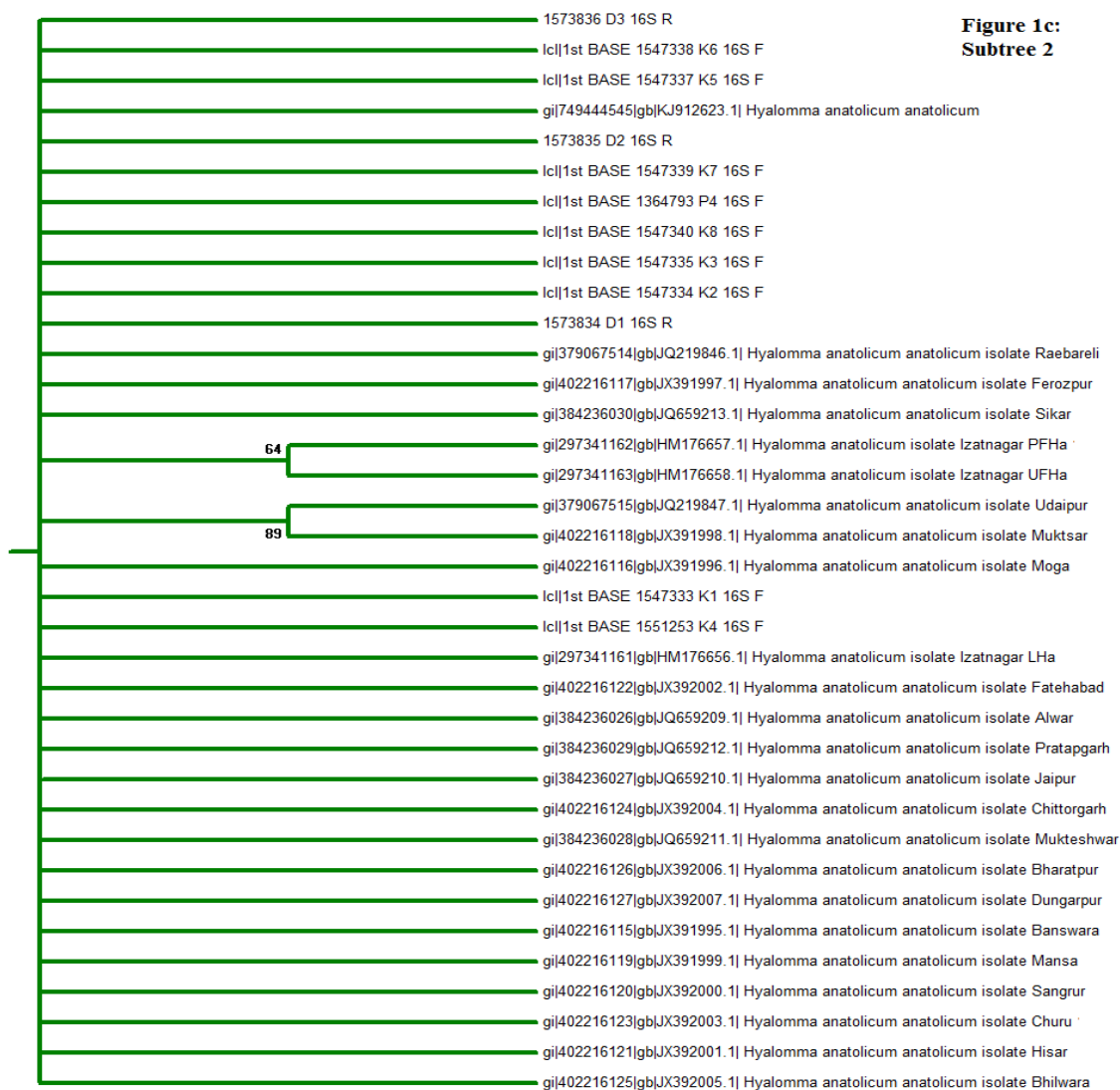


Fig. 1: C-Subtree 2 having *Hyalomma* species sequences

In the final analysis all positions with less than 90% site coverage were eliminated resulting in a total of 501 positions in the final dataset. The results revealed that genus *Ixodes* as the out-group was most basal. *Rhipicephalus (Boophilus) microplus* formed a clade having 98% BT support with *R. (B.) decoloratus*. Species of genus *Hyalomma* formed a separate clade with 97% BT support. Species *R. morphotype* was paraphyletic to the clade of *Hyalomma* and *Rhipicephalus*. *Haemaphysalis bispinosa* was paraphyletic to all the above with 100% BT support.

4. DISCUSSION

4.1 Inter-relationships within Ixodidae

A review of literature reveals that there is no explicit study to infer relationships within hard ticks from India but some of the investigations had a few members from India using various molecular markers. The result of this first molecular study from

India have thrown open some important points for discussion as detailed hereunder that will be helpful for future studies on tick distribution, epidemiology, control, and in understanding the tick evolution and systematics. Based on the present 16S based phylogeny following points of importance could be inferred viz. subfamily Haemaphysalinae is basal in Metastriate while *Rhipicephalus* is paraphyletic to *Hyalomma* and *Rhipicephalus (Rhipicephalus)* form a clade with *Rhipicephalus (Boophilus)*.

In this context, Hoogstraal and Aehlimann [18] grouped Haemaphysalinae + Hyalomminae + Rhipicephalinae based on shared presence of hard hooking devices (Spines and Hooks on legs and mouthparts) and the results of the present study are in lines with their hypothesis. Further, the first molecular study based on 16s rDNA conducted to infer phylogenetic relationships of hard and soft ticks by Black and Piesmann [24] revealed that the members of subfamily Hyalomminae claded with members of

subfamily Rhipicephalinae and over the years different studies have supported monophyly of Metastriata including the present study [7, 25-28, 30, 51].

Similar to present study, Barker and Murrell [10] while reviewing phylogeny of Ixodidae presented a working hypothesis in which the subfamily Haemaphysalinae claded with (Rhipicephalinae + Hyalomminae). Recently, Burger *et al.*, [37] based on their mitochondrial genome analysis also reported paraphyly of genera *Rhipicephalus* + *Hyalomma*. In this context, Mans *et al.*, [52] based on their 18s Bayesian analysis also contended that there is very strong support for Metastriata and the clade of Haemaphysalinae + (Rhipicephalinae + Hyalomminae).

4.2 Status of different species groups:

When the individual species groups were considered for the sequences from India the majority of sequences belonged to three species groups' viz. Sanguineus group, Microplus group and Anatolicum group. These are individually discussed hereunder:

4.2.1 Sanguineus group

Recently, there has been considerable debate about the status of *Rhipicephalus sanguineus* sensu stricto as it has been considered as a species complex of 17 closely related species [53, 54, 55], however, there is no consensus [56] and the morphological similarities among ticks belonging to the *Rh. (Rh.) sanguineus* group make their identification a difficult task.

Based on the analysis of present data *Rh. (Rh.) morphotype 3* is paraphyletic with *Rh. (Rh.) sanguineus* from Mahra (Haryana) which is suggesting that the former might be a different cryptic/sibling species and that *Rh. (Rh.) sanguineus* species complex might be polyphyletic.

4.2.2 Microplus group

Rhipicephalus (Boophilus) microplus is a cattle parasite with a one host life cycle. It is thought to have originated in the Indian sub-continent but has now been introduced into many parts of the world including South East Asia with its Host [57-58]. *Rhipicephalus (Boophilus) annulatus* (Say, 1821) and *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) have long been thought to be sister species on the basis of morphology and molecular markers [10]. Labruna *et al.* [22] analyzed *Rh. (B.) microplus* from different geographical regions using 12s and 16s sequences and according to their hypothesis at least two different species share the name of *Rh. (B.) microplus*. According to them *Rh. (B.) microplus* from India and Nepal have been shown to be highly divergent from *Rh. (B.) microplus* from the Americas and Africa [22]. However, the phylogenetic placement of *Rh. (B.) microplus* from India and Nepal was not strongly resolved, though *Rh. (B.) microplus* from India clustered with *Rh. (B.) annulatus* in the 16S rRNA analysis of Labruna *et al.*, [22].

In this context, Estrada-Peña *et al.*, [59] reinstated as a separate species the cattle ticks from Australia previously known as *Rh. (B.) microplus* as *Rhipicephalus (Boophilus) australis* (Fuller, 1899). Interestingly, *Rh. (B.) decoloratus* during the

present study claded with from *Rh. (B.) microplus* suggesting that *Rh. (B.) microplus* is a species complex of at least four species viz. *Rh. (B.) microplus*, *Rh. (B.) annulatus*, *Rh. (B.) australis*, and *Rh. (B.) decoloratus* and the strains from India and Nepal need to be studied using several molecular markers to discern the sibling/cryptic species and inter-relationships within this important species complex.

4.2.3 Anatolicum group

H. anatolicum anatolicum and *H. anatolicum excavatum* were subspecies until Apanaskevich and Horak [60] raised those to the rank of species namely *Hyalomma anatolicum* Koch 1844 and *Hyalomma excavatum* Koch 1844 based on morphological characters. In this context, a recent study of Hosseini *et al.*, [61] using morphometric methods on male specimens of *Hyalomma anatolicum* have shown polymorphism in the important taxonomic characters and have recommended more studies on related species for proper identification of species. According to the present analysis there is very strong support for Anatolicum clade of *Hy. anatolicum* + *excavatum* + *hussaini* + *brevipunctata* with 97%BT support supporting the contention of Kaur *et al.*, [62] that it might be a group of species.

5. CONCLUSIONS

The results of the present study have confirmed some of the existing morphological and molecular hypotheses about hard tick phylogeny. A condensed phylogenetic tree is provided as a reference standard for any future studies from India (Figure 2). Furthermore, information about the inter-relationships of taxa not previously included in any phylogenetic study has been provided. In conclusion, the molecular evidence presented here suggests that *H. anatolicum*, *H. excavatum*, *H. hussaini* and *H. brevipunctata* represent closely related but rapidly diverging taxa, confirms that *Rh. (B.) microplus* is a species complex of at least four species, and asserts that *Rh. (Rh.) sanguineus* species complex might be polyphyletic.

Although the main aim of our study was to provide a phylogenetic tree as the basis for further comparative studies of inter-relationships within the hard ticks from India rather than a detailed analysis of phylogenetic relationships with other genera within family Ixodidae, still our preliminary results does not support merging the subfamily Hyalomminae within subfamily Rhipicephalinae. This hypothesis needs to be tested using more sequences from other genes covering whole of the metastriate lineage.

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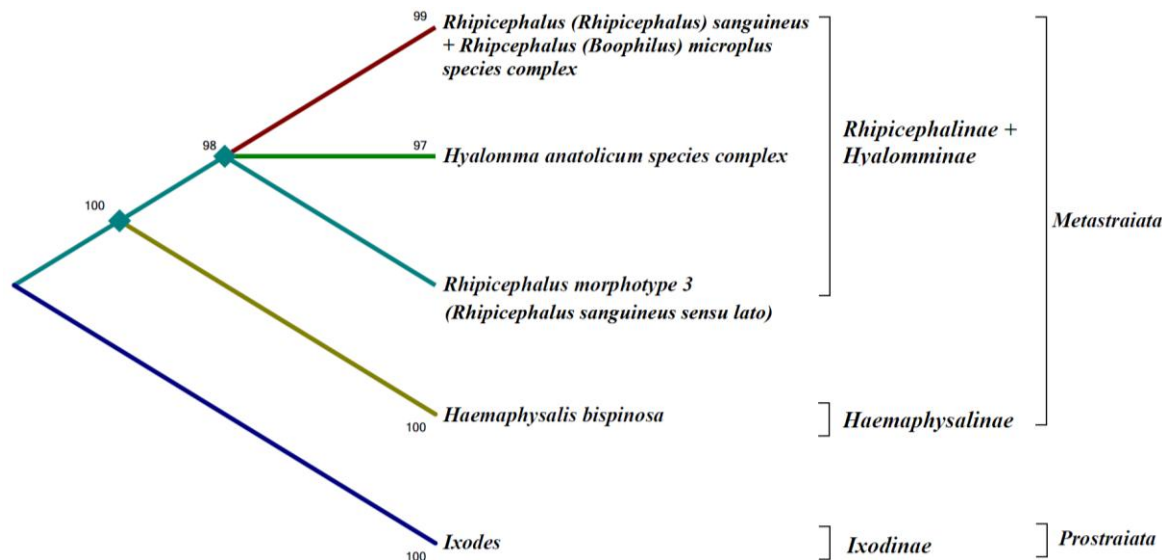


Fig. 2: Condensed Phylogenetic tree summarising the results of the present phylogenetic study on hard tick species from India recommended as a Key for future works

7. CONFLICT OF INTEREST

There exists no conflict of interest.

8. REFERENCES

- Ghosh S, Nagar G. Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: A review. *Journal of Vector Borne Diseases*. 2014; 51: 259–270
- Miranpuri GS. Ticks parasitizing the Indian buffalo (*Bubalus bubalis*) and their possible role in disease transmission. *Veterinary Parasitology*. 1988; 27: 357-362.
- Murhekar MV, Kasabi GS, Mehendale SM, Mourya DT, Yadav PD, Tandale BV. On the transmission pattern of Kyasanur Forest disease (KFD) in India. *Infectious Diseases of Poverty*. 2015; 4:37. doi:10.1186/s40249-015-0066-9.
- Hassanin A. Phylogeny of arthropoda inferred from mitochondrial sequences: strategies for limiting the misleading effects of multiple changes in pattern and rates of substitution. *Molecular Phylogenetics and Evolution*. 2006; 38: 100-116. Doi: 10.1016/j.ympev.2005.09.012.
- Masta SE, Longhorn SJ, Boore JL. Arachnid relationships based on mitochondrial genomes: asymmetric nucleotide and amino acid bias affects phylogenetic analyses. *Molecular Phylogenetics and Evolution*. 2009; 50(1):117-128. doi: 10.1016/j.ympev.2008.10.010.
- Cruickshank RH. Molecular markers for the phylogenetics of mites and ticks. *Systematic and Applied Acarology*. 2002; 7: 3-14.
- Klompen JSH, Oliver-Jr JH, Keirans JE, Homsler PJ. A re-evaluation of relationships in the metastraiata (Acari: Parasitiformes: Ixodidae). *Systematic Parasitology*. 1997; 38: 1-24.
- Klompen H, Dobson SJ, Barker SC. A new subfamily, Bothriocrotoninae n. subfam., for the genus *Bothriocroton* Keirans, King & Sharrad, 1994 status amend. (Ixodida: Ixodidae), and the synonymy of *Aponomma* Neumann, 1899 with *Amblyomma* Koch, 1844. *Systematic Parasitology*. 2002; 53(2):101-7.
- Horak IG, Camicas JL, Kierans JE. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names. *Experimental and Applied Acarology*. 2002; 28: 27–54
- Barker SC, Murrell A. Systematics and evolution of ticks with a list of valid genus and species names. *Parasitology*. 2004; 129: S15-S36.
- Barker SC, Murrell A. Phylogeny, evolution and historical zoogeography of ticks: a review of recent progress. *Experimental and Applied Acarology*. 2007; 28: 55-68.
- Liu GH, Chen F, Chen YZ, Song HQ, Lin RQ, Zhou DH. Complete mitochondrial genome sequence data provides genetic evidence that the brown dog tick *Rhipicephalus sanguineus* (Acari: Ixodidae) represents a species complex. *International Journal of Biological Sciences*. 2013; 9(4): 361-369.
- Burger TD, Shao R, Barker SC. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus (Boophilus) microplus*, contains a cryptic species. *Molecular Phylogenetics and Evolution*. 2014a; 76: 241-253.
- Klompen, JSH, Black WCIV, Keirans JE, Oliver JHJr. Evolution of ticks. *Annual Reviews of Entomology*. 1996; 41: 141–161.
- Jongejan F, Uilenberg G. The global importance of ticks. *Parasitology*. 2004; 129: S3–S14.
- Ghosh S, Azhahianambi P, Yadav MP. Upcoming and future strategies of tick control: a review. *Journal of Vector Borne Diseases*. 2007; 44: 79-89.
- Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C, Wang Q, Mei L, Lin X. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasites and Vectors*. 2014; 7 (1): 93.
- Hoogstraal H, Aeschlimann A. Tick–Host Specificity. *Bulletin de la Societe' Entomologique Suisse*. 1982; 55: 5–32.
- Gou H, Guan G, Liu A, Ma M, Chen Z, Liu Z, Ren Q, Li Y, Yang J, Yin H, Luo J. Coevolutionary analysis of the relationships between piroplasmids and their hard tick hosts. *Ecology and Evolution*. 2013; 3(9): 2985-2993.
- Lu X, Lin XD, Wang JB, Qin XC, Tian JH, Guo WP, Fan FN, Shao R, Xu J, Zhang YZ. Molecular survey of hard ticks in endemic areas of tick borne diseases in China. *Ticks and Tick Borne Diseases*. 2013; 4(4): 288-296.
- Rees DJ, Dioli M, Kirkendall LR. Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae). *Molecular Phylogenetics and Evolution*. 2003; 27: 131-142.
- Labruna MB, Naranjo V, Mangold AJ, Thompson C, Estrada-Pena A, Guglielmone AA, Jongejan F, de la Fuente J. Allopatric speciation in ticks: genetic and reproductive divergence between geographic

- strains of *Rhipicephalus (Boophilus) microplus*. BMC Evolutionary Biology. 2009; 9: 46.
23. Shyma KP, Kumar S, Sharma AK, Ray DD, Ghosh S. Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*. Experimental and Applied Acarology. 2012; 58 (4): 471-481.
 24. Black WCIV, Piesman J. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proceedings of the National Academy of Sciences. U.S.A. 1994; 91 (21): 10034-10038.
 25. Black WCIV, Klompen JSH, Keirans JE. Phylogenetic relationships among tick subfamilies (Ixodida: Ixodidae: Argasidae) based on the 18s nuclear rDNA gene. Molecular Phylogenetics and Evolution. 1997; 7(1): 129-144.
 26. Dobson SJ, Barker SC. Phylogeny of the hard ticks (Ixodidae) inferred from 18s rDNA indicates that the genus *Aponomma* is Paraphyletic. Molecular Phylogenetics and Evolution. 1999; 11(2): 288-295.
 27. Murrell A, Campbell NJH, Barker SC. A total evidence phylogeny of ticks provides insights into the evolution of life cycles and biogeography. Molecular Phylogenetics and Evolution. 2001; 21(2): 244-258.
 28. Norris DE, Klompen JSH, Black WCIV. Comparison of the mitochondrial 12S and 16S Ribosomal DNA genes in resolving phylogenetic relationships among hard ticks (Acari: Ixodidae). Annals of Entomological Society of America. 1999; 92: 117-129.
 29. Beati L, Keirans JE. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12s ribosomal DNA gene sequences and morphological characters. Journal of Parasitology. 2001; 87(1): 32-48.
 30. Guglielmo AA, Estrada-Pena A, Mangold AJ, Barros-Battesti DM, Labruna MB, Martins JR, Venzal JM, Arzua M, Keirans JE. *Amblyomma aureolatum* (Pallas, 1772) and *Amblyomma ovale* Koch, 1844 (Acari: Ixodidae): hosts, distribution and 16S rDNA sequences. Veterinary Parasitology. 2003; 113 (3-4): 273-288.
 31. Chitimia L, Lin RQ, Cosoroaba I, Wu XY, Song HQ, Yuan ZG, Zhu XQ. Genetic characterization of ticks from southwestern Romania by sequences of mitochondrial *cox1* and *nad5* genes. Experimental and Applied Acarology. 2010; 152: 305-311.
 32. Abdigoudarzi M, Nouredine R, Seitzer U, Ahmed J. rDNA-ITS2 identification of *Hyalomma*, *Rhipicephalus*, *Dermacentor* and *Boophilus* spp. (Acari: Ixodidae) collected from different geographical regions of Iran. Advanced Studies in Biology. 2011; 3(5): 221-238.
 33. Song S, Shao R, Atwell R, Barker S, Vankan D. Phylogenetic and phylogeographic relationships in *Ixodes holocyclus* and *Ixodes cornuatus* (Acari: Ixodidae) inferred from COX1 and ITS2 sequences. International Journal of Parasitology. 2011; 41: 871-880.
 34. Burger TD, Shao R, Beati L, Miller H, Barker SC. Phylogenetic analysis of ticks (Acari: Ixodida) using mitochondrial genomes and nuclear rDNA genes indicates that the genus *Amblyomma* is polyphyletic. Molecular Phylogenetics and Evolution. 2012; 64: 45-55.
 35. Burger TD, Shao R, Labruna MB, Barker SC. Molecular phylogeny of soft ticks (Ixodida: Argasidae) inferred from mitochondrial genome and nuclear rDNA sequences. Ticks and Tick Borne Diseases. 2014; 5: 195-207.
 36. Kulakova NV, Khasnatinov MA, Sidorova EA, Adel'shin RV, Belikov SI. Molecular identification and phylogeny of *Dermacentor nuttalli* (Acari: Ixodidae). Parasitology Research. 2014; 113 (5): 1787-1793.
 37. Lv J, Wu S, Zhang Y, Zhang T, Feng C, Jia G, Lin X. Development of a DNA barcoding system for the Ixodida (Acari: Ixodida). Mitochondrial DNA. 2014; 25 (2): 142-149.
 38. Ghosh S, Bansal GC, Gupta SC, Ray D, Khan MQ, Irshad H, Shahiduzzaman Md, Seitzer U, Ahmed JS. Status of tick distribution in Bangladesh, India and Pakistan. Parasitology Research. 2007; 101 (Suppl 2): s207-s216.
 39. Murrell A, Campbell NJ, Barker SC. Mitochondrial 12S rDNA indicates that the Rhipicephalinae (Acari: Ixodida) is paraphyletic. Molecular Phylogenetics and Evolution. 1999; 12: 83-86.
 40. Kaiser MN, Hoogstraal H. The *Hyalomma* ticks (Ixodoidea, Ixodidae) of Pakistan, India and Ceylon, with keys to subgenera and species. Acarologia. 1964; 6 (2): 257-286.
 41. Geevarghese G, Dhanda V. The Indian *Hyalomma* ticks (Ixodoidea: Ixodidae). Indian Council of Agricultural Research, New Delhi; 1987.
 42. Mangold JJ, Bargues MD, Mas-coma S. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). Parasitology Research. 1998; 84: 478-484.
 43. Keirans JE, Litwak TR. Pictorial Key to the Adults of Hard Ticks, Family Ixodidae (Ixodida: Ixodoidea), East of the Mississippi River. Journal of Medical Entomology. 1989; 26(5): 435-448.
 44. Mangold AJ, Bargues MD, Mas-coma S. 18S rDNA gene sequences and phylogenetic relationships of European hard-tick species (Acari: Ixodidae). Parasitology Research. 1998; 60: 31-37.
 45. Petney TN, Keirans JE. Ticks of the genera *Amblyomma* and *Hyalomma* from South-east Asia. Tropical Biomedicine. 1995; 12: 45-56.
 46. Walker AR, Bouattour A, Camicas JL, Estrada-Pena A, Horak IG, Latif AA, Pegram RG, Preston PM. Ticks of domestic animals in Africa: a guide to identification of species, Bioscience Reports, Edinburgh, Scotland, U.K; 2003.
 47. Tian Z, Xie J, Yin H, Luo J, Zhang L, Zhang P, Luo J. Discrimination between *Haemaphysalis longicornis* and *H. qinghaiensis* based on the partial 16S rDNA and the second internal transcribed spacer (ITS-2). Experimental and Applied Acarology. 2011; 54: 165-172.
 48. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013; 30: 2725-2729.
 49. Tamura K. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. Molecular Biology and Evolution. 1992; 9: 678-687.
 50. Tamura K, Kumar S. Evolutionary distance estimation under heterogeneous substitution pattern among lineages. Molecular Biology Evolution. 2002; 19: 1727-1736.
 51. Crampton A, McKay I, Barker SC. Phylogeny of ticks (Ixodida) inferred from nuclear ribosomal DNA. International Journal for Parasitology. 1996; 26: 511-517.
 52. Mans BJ, de Klerk D, Pienaar R, de Castro MH, Latif AA. The Mitochondrial Genomes of *Nuttalliella namaqua* (Ixodoidea: Nuttalliellidae) and *Argas africanus* (Ixodoidea: Argasidae): Estimation of Divergence Dates for the Major Tick Lineages and Reconstruction of Ancestral Blood-Feeding Characters. Public Library of Science One. 2012; 7(11): e49461. doi:10.1371/journal.pone.0049461
 53. Dantas-Torres F, Latrofa MS, Annoscia G, Giannelli A, Parisi A, Otranto D. Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. Parasites and Vectors. 2013; 6: 213 LID- 10.1186/1756-3305-6.
 54. Pegram RG, Clifford CM, Walker JB, Keirans JE. Clarification of the *Rhipicephalus sanguineus* group (Acari, Ixodoidea, Ixodidae). II. *R. sanguineus* (Latreille, 1806) and related species. Systematic Parasitology. 1987; 10:27-44.
 55. Burlini L, Teixeira KR, Szabo MP, Farnas KM. Molecular dissimilarities of *Rhipicephalus sanguineus* (Acari: Ixodidae) in Brazil and its relation with samples throughout the world: is there a geographical pattern? Experimental and Applied Acarology. 2010; 50: 361-74 LID - 10.1007/s10.
 56. Gray J, Dantas-Torres F, Estrada-Peña A, Levin M. Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Ticks and Tick Borne Diseases. 2013; 4:171-180.
 57. Hoogstraal H. Argasid and nuttalliellid ticks as parasites and vectors. Advances in Parasitology, 1985; 24: 135-238.

58. Petney, TN and Keirans, JE. Ticks of the genera *Boophilus*, *Dermacentor*, *Nosomma* and *Rhipicephalus* (Acari: Ixodidae) in Southeast Asia. *Tropical Biomedicine*. 1996; 13: 73-84
59. Estrada-Pena A, Venzal JM, Nava S, Mangold A, Guglielmo AA, Labruna MB. Reinstatement of *Rhipicephalus (Boophilus) australis* (Acari: Ixodidae) with redescription of the adult and larval stages. *Journal of Medical Entomology*. 2012; 49: 794-802.
60. Hosseini A, Dalimi A, Abdigoudarzi M. Morphometric Study on Male Specimens of *Hyalomma anatolicum* (Acari: Ixodidae) in West of Iran. *Iran Journal of Arthropod Borne Diseases*. 2011; 5(2): 23–31
61. Kaur H, Chhillar JS, Chhillar S. Mitochondrial 16s rDNA based analysis of some hard ticks belonging to genus *Hyalomma* Koch, 1844 (Acari:Ixodidae). *The Journal of Advances in Parasitology*. 2016; 3(2): 32-48.
62. Apanaskevich DA, Horak IG. The genus *Hyalomma* Koch, 1844. II. Taxonomic status of *H. (Euhyalomma) anatolicum* Koch, 1844 and *H. (E.) excavatum* Koch, 1844 (Acari, Ixodidae) with redescription of all stages. *Acarina*. 2005; 13(2): 181-197.

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