The Role of Phylogeny on the Taxonomy and Nomenclature of Ixodid Ticks

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ABSTRACT

The phylogenies based on nucleotide sequences using distance and neighbor-joining methods were congruent. The ITS-2 DNA region was amplified from *Boophilus annulatus* (Say, 1821) and *Rhipicephalus sanguineus* (Latreille, 1806) and was sequenced and analyzed to clarify the phylogenetic relationship of these *Boophilus* and *Rhipicephalus* species. The data suggested that the gene shared a similarity in sequence compared to ticks ITS-2 found in Gen Bank. The results indicated that the ITS-2 nucleotide sequence of *B. annulatus* strain Egypt and *R. sanguineus* strain Egypt reflected the phylogenetic relationship between the two species**.**

Key Words: Ticks; ITS-2; Phylogeny; *Boophilus annulatus*; *Rhipicephalus sanguineus*.

INTRODUCTION

Recent advances in phylogeny of ticks nomenclatures have been made or are likely to be made. Study of tick genomes has provided insight into the phylogeny and evolution of ticks. A number of changes to the taxonomy of ticks has occurred or has been proposed resulting to account for recent advances in our knowledge of tick phylogeny, Barker and Murrell (2004).

Murrell *et al*. (2001b) and Murrell and Barker (2003) proposed that the genus *Boophilus* Curtice, 1891 be relegated to a subgenus of the genus *Rhipicephalus* Koch, 1844 because the genus *Rhipicephalus* is paraphyletic without the inclusion of the species of *Boophilus*. Accordingly, some species or species groups of *Rhipicephalus* were elevated to the rank of genus, by placing the *Boophilus* species as a subgenus of *Rhipicephalus.* Since there is a lot of literatures on the *Boophilus* species and hundreds, perhaps thousands of people use these names regularly, the name *Boophilus* still be used (Murrell *et. al*., 2001b). However, Murrell and Barker (2003) moved all five species from the genus *Boophilus* to the genus *Rhipicephalus*.

The present study aims to study the evolutionary relationships of *Rhipicephalus* and *Boophilus* species using nucleotide sequence of rDNA (ITS-2).

MATERIALS AND METHODS

Tick species

Boophilus annulatus (Say, 1821) adults were collected from the experimental station, Faculty of Agriculture, Cairo University. *Rhipicephalus sanguineus* (Latreille, 1806) adults were obtained from laboratory colonies in Applied Parasitic Acarine Research Laboratories, Faculty of Agriculture, Cairo University. All engorged females were kept in an incubator regulated to 27-30^{o}C and 75% R.H. for oviposition. The eggs used were in the organogenetic stage (14-day-old) (El Kammah *et al*., 1982 and 1987).

PCR amplification of the ITS-2

Total genomic DNA from examined eggs was isolated and purified by using DNA isolation kits (Biospin Tissue Genomic DNA Extraction Kit (Bioflux) Bioer Technology Co., Ltd., China. Two degenerative primers were designed from half of the 5.8S to the end of the 28S rDNA genes (Poucher *et. al*., 1999). The ITS-2 DNA region was amplified using forward (5'-CTGCGAGACTTGGTGTGAAT-3') and reverse (5'-TATGCTTAAGTTCAGCGGGT-3') primers. The PCR program used was 95^{o}C for 5 min; 35 cycles at 95^{o}C for 1 min, 60^{o}C for 1 min, 72^{o}C for 2 min and one cycle at 72^{o}C for 5 min. The PCR amplification was performed in a total volume of 50 µl. The optimized conditions were 2 µl dNTPs (0.2 mM), 3 µl $MgCl_2$ (1.5 mM), 5 µl 10X reaction buffer, 2.5 µl of each primer (0.5 µM), 2 µl DNA (100 ng) and 1 µl Taq DNA polymerase (2 units) and sterile distilled water up to 50 µl (Williams *et. al*., 1990). PCR products were checked by electrophoresis using 1.5% agarose gel in 1× TAE buffer.

DNA sequence and phylogenetic analyses

The PCR products were then purified using QIAQuick Gel Extraction Kit #28706 (QIAGEN, www. quiagen. com) following manufacturer instructions and sequenced by automated DNA sequencing reactions, which were performed using a sequencing ready reaction kit (Life Technologies, www.invitrogen.com) in conjunction with ABI-PRISM and ABI-PRISM big dye terminator cycler. The sequences obtained in this study were submitted to the GenBank nucleotide

sequence databases (Accession numbers: JQ412126 and JQ412127). These sequences were subjected to alignment with ITS-2 sequences of the Gen Bank, EMBL, DDBJ, and PDB sequence database using the program BioEdit version 7.0.0 (Hall 1999). Evolutionary analyses were conducted in MEGA software version 4 (Tamura *et. al*., 2007) to generate a phylogenetic tree using the neighbor–joining methods based on Saitou and Nei (1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et. al*., 2004) and were in the units of the number of base substitutions per site. The nucleotide composition, nucleotide pair frequencies (16 pairs) and the overall transition/ transversion bias were accordingly estimated. Substitution pattern and rates were estimated under 2-parameter model (Kimura, 1980).

RESULTS AND DISCUSSION

Phylogenetic analysis

The topology of neighbor-joining tree of the Egyptian tick strains (accession numbers: JQ412126 and JQ412127) with 16 accession of *Rhipicephalus* in the GenBank database represented a monophyletic group (Fig. 1). The *B. annulatus* strain Egypt (JQ412126) was clustered with *R. (Boophilus) annulatus* and *R. (Boophilus) microplus* in the first cluster. It showed a close relationship to *B. annulatus* strains from Texas and Israel (accession numbers: AF271270 and AF271272, respectively); while *R. sanguineus* strain Egypt (JQ412127) was clustered with *R. sanguineus* and *R. turanicus* in the second cluster. Four *R. turanicus* accessions were closely related with each other and formed a monophyletic lineage.

ITS-2 sequence analyses

The ITS-2 nucleotide sequence of *B. annulatus* strain Egypt (JQ412126) showed a point mutation, namely base substitution when compared with *B. annulatus* strains from Texas and Israel (accession numbers: AF271270 and AF271272, respectively) (Table 1). This table showed that there are 1114 identical pairs (96.7%), 14 transitional pairs (1.2%) and 23 transversional pairs (2.1%). The nucleotide frequencies are 19.26% (A), 17.49% (T), 29.19% (C) and 34.06% (G). The estimated Transition/Transversion bias (R) is 0.57. On the other hand, comparing the ITS-2 nucleotide sequence of *R. sanguineus* strain Egypt (JQ412127) to *R. sanguineus* (JF758643 and AF271283) showed a point mutation (Table 2). There are 1100 identical pairs (99.5%), 5 transitional pairs (0.45%) and one-transversional pairs (0.09%). The nucleotide frequencies are 19.00% A, 16.20% T, 29.77% C and 35.02% G. The overall transition/ transversion bias (R) is 3.5.

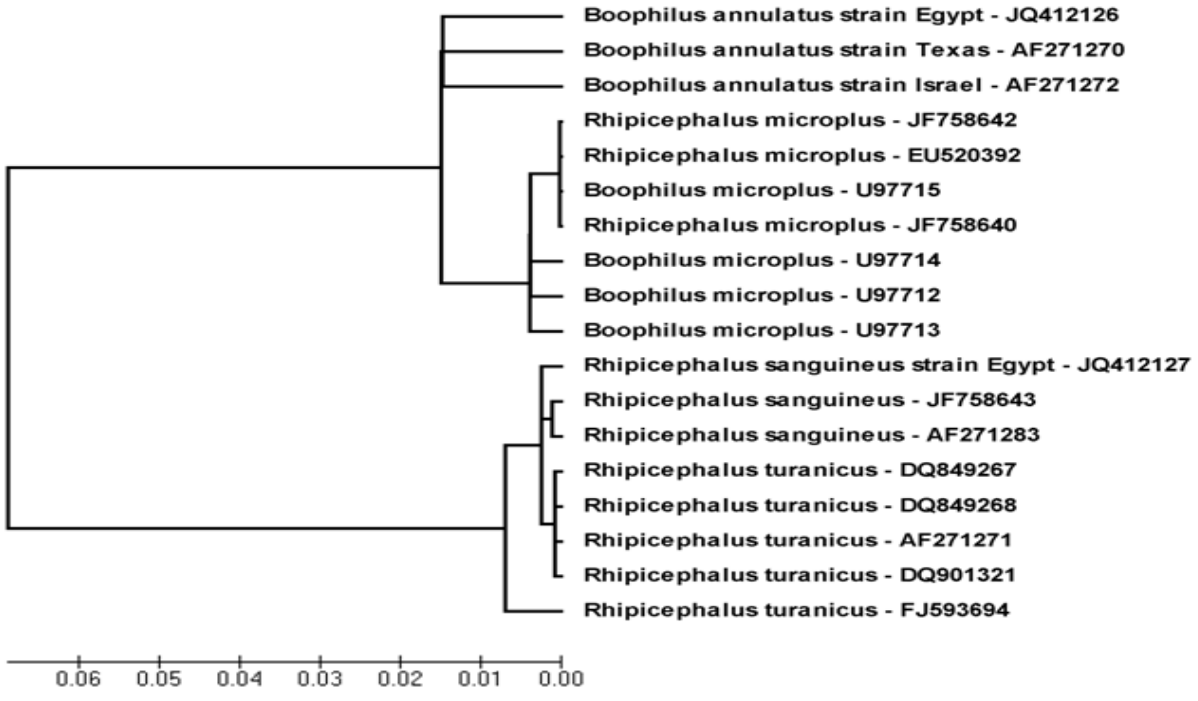

Fig. (1): Neighbor–joining dendrogram of 4 *Rhipicephalus* species (18 accessions) generated based on Saitou and Nei distances.

Table (1): ITS-2 nucleotide sequences (1151b) of *B. annulatus* strains, Egypt, Israel and Texas

Base Pairs	Nucleotide	No. of Base	Frequency
Identical	TT	193	0.167
	CC	328	0.285
	AA	215	0.187
	GG	378	0.328
Transitional (si)	T↔C	4	0.003
	A↔G	10	0.009
Transversional (sv)	T↔A	2	0.002
	T↔G	11	0.009
	C↔A	4	0.003
	C↔G	8	0.007

Table (2): ITS-2 nucleotide sequences (1105b) of three *R. sanguineus* accessions (JQ412127, JF758643 and AF271283)

Base Pairs	Nucleotide	No. of Base	Frequency
Identical	TT	177	0.160
	CC	328	0.297
	AA	209	0.18
	GG	386	9
Transitional (si)	T↔C	3	0.349
	A↔G	2	0.0027
Transversional (sv)	T↔A	1	0.0018
	T↔G	0	0.0009
	C↔A	0	0.000
	C↔G	0	0.000

Several changes to the nomenclature of ticks are imminent or to have been made. The morphology of any *Rhipicephalus* spp. (2- 3 host- tick) or *Boophilus* spp. (one host- tick) is quite significant and can easily be recognized by the naked eyes. They are also different biologically and physiologically. The phylogeny of the hard ticks is quite different to the working hypothesis of 40 years ago for taxonomists. The internal transcribed spacer (ITS) regions are very useful for distinguishing between closely related taxa, Hillis and Dixon (1991). Once an ITS region has been successfully amplified, it can be analyzed by additional techniques. Poucher *et. al*., (1999) distinguished 17 Ixodes tick species based on restriction enzyme analysis of the (ITS-2). In addition, El Kammah and El Fiky (2005) identified 3 tick species. ITS-2 rDNA has been sequenced extensively in ticks to study closely related species (McLain *et. al*., 1995; Zahler *et. al*., 1995; Zahler & Gothe 1997; Baker 1998; Fukunaga *et. al*., 2000 and Murrell et. *al*., 2001a).

Interaspecific variation in the ITS-2 of *Rhipicephalus* and *Boophilus* spp. was synonymised by Murrell and Barker (2003) and Barker (1998). In addition, some species of *Rhipicephalus* (74 species), should be put in *Boophilus* (5 species), or *Boophilus* should be synonymized with *Rhipicephalus* (Walker *et. al*., 2000). The results obtained herein reflect the phylogenetic relationship between one species of *Boophilus* and one species of *Rhipicephalus*. However, we propose that the name *Boophilus* is well known and has to be retained. This proposal disagrees with that suggested by Murrell and Brker (2003), that *Boophilus* is a subgenus of *Rhipicephalus*.

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