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Morphological and molecular detection of tick Rhipicephalus (Boophilus) on ruminants in Mosul city

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Abstract

This study aimed to identify and characterize *Rhipicephalus* (*Boophilus*) *annulatus* and *Rhipicephalus sanguineus* ticks and infesting ruminants in Mosul, Iraq, using both morphological and molecular methods. Tick specimens were collected from multiple locations across Mosul (Thmaarat, Baadush, Hmaydat, Souq Al-Ghanam, Damerji, Damerji Sagheer, Ryhana, Al-mawali, Ihlayla, Al-uboor, Btyasha, Dijlah, Al-Tanak and Zanazel) from 27 buffalo, 39 cattle, 42 sheep, and 24 goats and identified based on standard morphological keys under a stereoscopic dissecting microscope. Key diagnostic features included the lateral angle of the basis capitulum, shape of palp pedicles, cervical field structure, scutum coloration, eye convexity, and genital cleft shape. Molecular identification was performed using PCR targeting the ITS (*5.8S rRNA* and *16S rRNA* gene regions. All tick samples tested positive, and representative amplicons were sequenced (GenBank accession numbers OR534233 for *R. annulatus* and OR759793 for *R. sanguineus*). Phylogenetic analysis revealed a 97.80%-100% identity with isolates from Egypt, Cameroon, South Africa, Pakistan, India, Thailand, China, and USA. Both Iraqi isolates clustered within clades of their respective species, confirming their identity and genetic similarity to international strain. These findings provide morphological and molecular confirmation of *Rhipicephalus* spp. in Mosul and contribute to regional tick surveillance data.

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Introduction

The most ectoparasites playing an important role as a vector of pathogen are ticks, influencing animal and human health status around the world. Logical assimilation of disparate control measurements, including acaricides, bacterial, herbal, phytogenic, vegetal, and floristic repellents, natural predator management, and vaccine, is demanded for inventive access to decrease the hazards associated with tick-borne diseases and ticks feeding. In feeding on their host, that within 10 to 15 minutes of finalize insertion of the tick's mouth parts into the skin, small momentary bloodbaths appeared at a 5-10 seconds intervals near to the hypostomal tip, every was noticeable for 1/10 seconds before being replaced by a little ejection of pure fluid from the tick, and the blood flow into this place increased about 10-30 minutes later and the interruption between The establishment of the baths extended, the drainage of every blood pools was accompanied by a regurgitation of saliva, mouth parts opening and become higher constant as continue of feeding (1,2). It is also worth noting a comprehensive world of tick infestations meta-analysis assigned with the international per annum trend in the world homelands, abstinent, tropical areas and hemispheres; attachment site, tick life instars, animal age grades and sexes and monthly and seasonally ticks infestations around the world especially in the meridians and equatorial areas in domesticated groups of ruminant had appropriate consequence (3). In a study of Makawi *et al.* (4) which was focused on some types of ticks dispersed in Iraq and on numerous types of domesticated as

well as wildlife animals, and the tick dispersal influenced by the surrounding like humidity and temperature by the *Rhipicephalus.* New insight into the definite genetic aggregation of the tick *Rhipicephalus microplus* in Brazil and admits that the Brazilian *Rhipicephalus* community consist of at least 2 different communities (5). The molecular phylogenetic investigation, based on *16SrRNA* and *COX1* genomes has been used to recognize the species and determine their close natural selection of them (6). Some modern works of the severity of tick-borne pathogenic agents in animals have been achieved using artificial culture to maintenance and growth of pathogens also line of cells or sequential passages of blood of animals or tissue accommodates with needle injection as the dominant approach of injection and in natural transmitting of tick-borne pathogens invade the animals with saliva of tick, which abetments improvement of infection by adaptation the host immunological and responses of cells at the attachment area of ticks (7). The hard ticks have the development and growth effect of small ruminants, especially goats and sheep, employing the bailiwick growth coefficient of carcass structures (skinny and fleshy) and the composition of non-carcass (edible and not-edible) organs (8). So, speaking of some treatment and control criteria, many farmers and animal husbandry have been recommended the use of oak-based products to establish the daily milk yield (9). Also, give the zinc supplement to local growing lambs have a role in reduction the impact of heat stress including exposure to ticks (10), so the recent studies have been focus the biological control against numbers of species belong to arthropods (i.e. ticks and mites) using (Vertimec pesticide+Metarism) in 1 ml/ 1 ml concentration which is that the supreme ratio for killing these creatures, it reached (96.77% and 92.33%) for plant also, the highest killer rate may reach (100%) of mites (11). In Iraq and Algeria, there were a lot of studies centered around the ticks and many morphometric and phylogenetic studies concerned with the wide ranges of tick species on numbers of ruminants (goats, sheep, cattle, buffalo and other animals) and laying a major part of these species in transfer a non-negligible number of pathogens (12).

Obstacle description

Tiny has conventionally been used to recognize ticks on their morphometrical features. Points such as host and body area predilection, morphometric attributes, geographical region, environmental needs are crucial in the detection and taxonomy of species of tick (13). Molecular techniques such as PCR and DNA barcoding, which detect species-specific DNA sequences, offer a more accurate and reliable method for tick identification and phylogenetic analysis (14). These tools help in understanding species distribution and evolutionary relationships, especially under changing environmental conditions.

Rationale for investigation

Although the diversity and distribution of ticks in South Africa are relatively well-documented (15), there is a significant lack of comprehensive taxonomic and molecular data in many regions, including parts of Iraq. The South African Biodiversity Act (NBI, 2008) emphasized that most invertebrate groups remain poorly studied. This study aimed to provide essential baseline data on tick distribution and taxonomy by achieving the following objectives. To identify the morphological and characteristics of ticks infesting ruminants. To preferentially molecular identification of ticks species using DNA barcoding techniques. To resolve the phylogenetic relationships of ticks species collected from animals.

Materials and methods

Ethical Approval

The Governmental Animal Carefulness Panel in the Veterinary Medicine College, Mosul University, has ethically allowable this study number UM.VET.2023.151 dated on 15/2/2023.

Study period and areas

Between May 2023 and October 2023, the study was carried out in a number of Mosul city regions, including Thmaarat (101 animals), Baadush (113 animals), Hmaydat (131 animals), Souq Al-Ghanam (110 animals), Damerji (112 animals), Damerji Sagheer (90 animals), Ryhana (66 animals), Al-mawali (111 animals), Ihlayla (120 animals), Al-uboor (8 animals), Btyasha (7 animals), Dijlah (7 animals), Al-Tanak (7 animals) and Zanazel (9 animals).

Hard tick collection

The number of hard ticks were collected was 2147 from buffalo (n=27), cows (n=39), sheep (n=42), and goats (n=24). Considering that the ticks were collected from various body parts of the animals and preserved in absolute ethanol until further examinations. The hard ticks were identified morphologically according to Estrada-Pena *et al.* (16). Under a stereoscope microscope with a 25x magnification based on the structural form developed by Walker (17) the tick samples were identified to genus level. Through the figures, the detection and arrangement of body regions of hard ticks were available. Taxonomic dichotomies of *Rhipicephalus (Boophilus) sanguineus* and *Rhipicephalus (Boophilus) annulatus* have been epitomized by consequent features of *R. sanguineus*: Shape of the lateral angle of the basis capitulum. Length of palp pedicle. Shape of cervical fields. Also, the spiracles and plates structures. Coloration of scutum. The forms of genital cleft. Adanal and accessory adanal plates constructions. Shape of the eyes. The shape of Coxa 1 and its structure. Hypostome and mouthparts components. At last, the existence or non-existence of a caudal appendage in males of ticks.

As well as the whole-body forms of the ticks were obtained applying a digicam with sensor-shift OIS and automatic focus for nearby in need of loss of perfection and examined under a stereoscope dissecting microscope with 25x magnification power based on morphological common traits.

DNA extraction

Following the manufacturer's instructions, the total DNA had been isolated from 32 ixodid ticks using AddPrep blood and tissue genomic extraction mini kit (Addbio, South Korea) (18). Briefly, twenty milligrams of each tick's crushed tissue was put into a 1.5 milliliter microcentrifuge tube. Next, 200 microliters of lysis buffer and 20 microliters of proteinase K solution (20 mg/ml) were added to the sample tube, mixed, and incubated at 56°C for 8 hours until the tissue was fully lysed. 200 µl of binding solution was added, pulse-vortexing for 15 seconds, and then incubated for 10 minutes at 56°C. Two hundred microliter of 100% ethanol was added and properly mixed, the lysate was placed on a spin column with a 2.0 ml collection tube and centrifuged at 13,000 rpm for one minute. Following two rounds of washing with 500 µl of each of the washing 1 and washing 2 solutions, the spin column was centrifuged for one minute at 13,000 rpm. Following a final centrifugation at 13,000 rpm for one minute to dry the spin column, 80 µl of elution solution was added and centrifuged for one minute at 13,000 rpm. For further tests, the eluted DNA was stored at -20 °C (18-21).

Polymerase chain reaction

The source of the primers used in this investigation was Macrogen Co., Korea (Table 1). The PCR master mix was set in a 25 µl volume containing 12.5 µl of Add Taq DNA Master (2x) (Addbio, Korea), 1 µl of each forward and reverse primer, 8.5 µl of PCR-grade water, and 2 µl of DNA were added as a template. The T100 BioRad Thermocycler, USA, was used to conduct the PCR. Initial denaturation was started up for 10 minutes at 95 °C, followed by 35 cycles of denaturing at 95°C for 45 seconds, annealing (as indicated for each primer in Table 1) for 45 seconds, and extension at 72°C for 1 minute. One cycle of final extension at 72°C for five minutes, followed by a holding step at 4°C. After amplification, the PCR product was subjected to gel electrophoresis using 1.5% agarose (Addbio, Korea) and 8 µl of the DNA ladder (AddBio, Korea) was used. After electrophoresis, the gel was documented, for the determination of expected bands.

Table 1: Primers with corresponding annealing temperatures and amplicon sizes used in molecular detection of *Rhipicephalus* spp.

No.	Primers (target gene)		Sequence (5^- to 3^-)	TmºC	Size (pb)	References
1	ITS (*5.8S rRNA*)	F	CGAGACTTGGTGTGAATTGCA	60	1500	(20,21)
		R	TCCCATACACCACATTTCCCG			
2	S1 (*16S rRNA*)	F	CCGGTCTGAACTCAGATCAAGT	56	460	(22,23)
		R	GCTCAATGATTTTTTAAATTG CTGT			

DNA sequencing and phylogenetic analysis

The two target gene positive samples (*5.8S rRNA* and *16S rRNA*) were sequenced with the forward primers (Macrogen, Korea). Following that, the samples were sent to GenBank (NCBI) for accession numbers. The acquired gene sequences were compared to those from other nations that already existed in the GenBank using BLAST. The MUSCLE program was used to perform multiple alignments using MEGA 11 software. The phylogenetic analysis of the *5.8S rRNA* and *16S rRNA* genes was performed using the Maximum Likelihood technique, which is based on the Tamura-Nei model in MEGA11 software. The strength of the groupings in the tree was assessed using 100 bootstrap resamples (24).

Statistical analysis

All data were analyzed using SPSS software (version 26). To compare means across groups, one-way ANOVA was used. Notable variation had been discovered ($P<0.05$), Duncan's multiple range test helped identify which specific groups differed. For comparisons within the same group over time or under different conditions, paired t-tests were applied. The expression of the results mean ± standard error (SE), and different letters were used to indicate statistically significant differences according to Duncan's test at the 0.05 level.

Results

Specimen Evidence and Morphological Analysis of Tick

A total of 132 animals competed in this study. The age of the animals ranged from 6 months to 5 years. 6 months – 2 years for each sheep and goats and (1 year- 5 years) for each cattle or cows and buffalo as shown in table 2, with the rate of tick infestation reached to 31.8% for sheep and goat (6 month to 2 years) and 78.8% infestation rate for cattle and buffalo (1 year- 5 years) and the total infestation rate of all ruminants was 55.30%.

Blending the sex of animal groups elevated infestation rates with ticks in females more than males, as shown in Table 3.

Table 2: Infestation rate of animals according to age

Animal groups	Age of animals	No. of examined animals	No. of infested animals	% of infested animals
Sheep and goats	6 months- 2 years	66	21	31.80%
Buffalo and cows	1 year- 5 years	66	52*	78.80%
Total ruminants	6 months- 5 years	132	73	55.30%

* Mean significant at P<0.05 with paired T-test.

Table 3: Infestation rate of animals according to sex

Animal groups	Sex of animals		No. of examined animals	No. of infested animals		% of infested animals	
	Males	Females		Males	Females	Males	Females
Sheep and goats	18	48	66	1	20	1.50%	30.30%*
Buffalo and cows	8	58	66	3	49	4.50%	74.20%*
Total ruminants	26	106	132	4	69	3.00%	52.30%*

* Mean significant at P<0.05 with paired T-test.

Ticks attachments on many parts of the bodies of ruminants like udder, thigh, around the anus, hindlimbs, ears, and abdomen. The most affected parts of the bodies are udder and thigh, as it represented in Table 4.

Table 4: The infestation rate of infested animals with ticks according to the site of infestation

Site of infestation	No. of infested animals	% infestation rates
Udder	65 a	49.20%
Thighs	52 b	39.40%
The anal region	38 c	28.80%
Hindlimbs	7 d	5.30%
Ears	5 d	3.80%
Abdomen	5 d	3.80%

Different letters mean significant at P<0.05 with one-way ANOVA Duncan's test.

In precisely which based on morphologically classified virtue, 30 larvae, 77 nymphs, 324 adults (217 females and 107 males) were recognized as belonging to the *Rhipicephalus (Boophilus)* spp.

The lateral angle of the basis capitulum is sharp, short of the palp pedicles as shown in Figure 1 (a,b). The shape of cervical fields is straight and large (Figure 2, a). The color of scutum is pale to dark (Figure 2, a,b). Coxae 1 have invisible anterior spurs from the dorsal side (Figure 2, c). Spiracles have sparse and the plates have narrow tails, about half the width of the nearest festoon (Figure 3, a). The genital cleft has a wide U shape, and the cornua are recognizable (Figure 3, b). Genital cleft has U or V shape (Figure 4). Accessory adanal plates are large and the adanal plates are trapezoidal and narrow as represented in (Figure 5). Eyes are slightly convex in both males and females (Figure 6). The hypostome has 4+4 columns of teeth, and Coxae 1 are indistinct (Figure 7). The 2 and 3 Coxae have no spurs (Figure 8, a). The spurs of Coxa 1 are short in length (Figure 8, b). The accessory adanal plate is unrecognizable as well as an unclear cut adanal plate (Figure 9). The second to last ventral plate spurs or (accessory adanal and adanal plate) are not detachable from the dorsal side in both male and female. Finally, the caudal appendage of the nourishing male is absent (Figure 10a and b).

Figure 1: *Rhipicephalus sanguineus* (a) Lateral angle of basis capitulum is sharp (red arrows, left, closeup image right). (b) Short of palp pedicle (red arrow).

Figure 2: (a) The shape of cervical fields is straight and large (red arrow), (b) The color of scutum is pale to dark to pale (yellow arrow). (c) Coxa 1 has invisible anterior spurs from the dorsal side (black arrow).

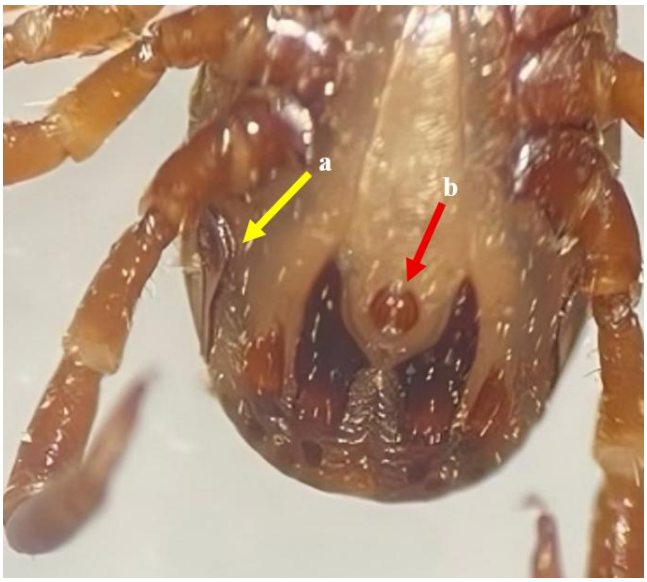

Figure 3: (a) Spiracles are sparse and the plates have narrow tail (yellow arrow). (b) The genital cleft has a wide U shape and the cornua is recognizable (red arrow).

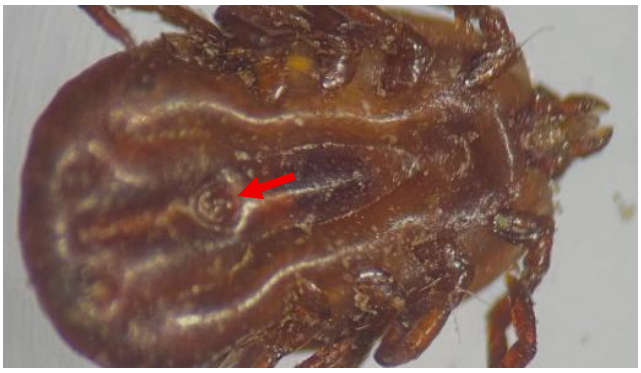

Figure 4: The genital cleft has a U or V shape (red arrow).

Figure 5: Accessory adanal plate is large (yellow arrow), and the adanal plate is trapezoidal and narrow (black arrow).

Figure 6: The eye is convex slightly (red arrow).

Figure 7: *Rhipicephalus annulatus* Hypostome has 4+4 columns of teeth (red arrow, left, and closeup image, right).

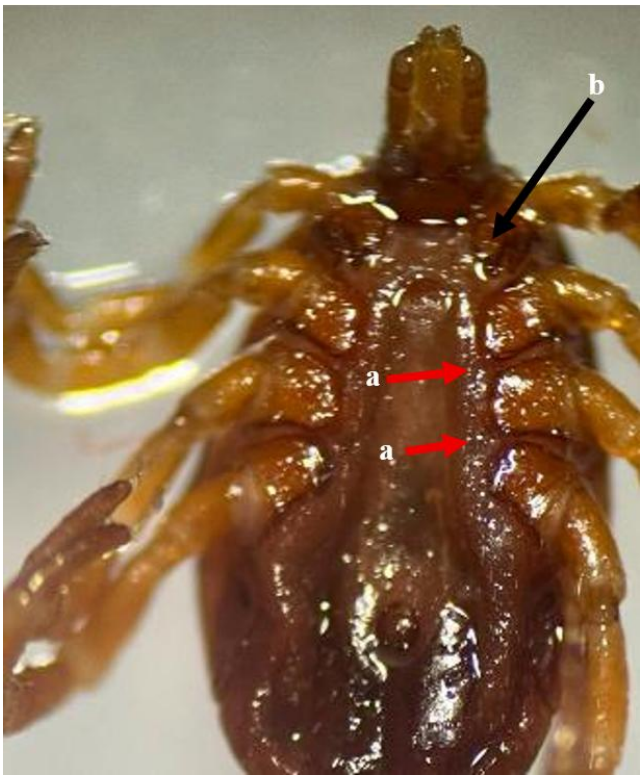

Figure 8: (a) The 2 and 3 coxae have no spurs (red arrows), (b) the spurs of Coxa 1 are short in length (black arrow).

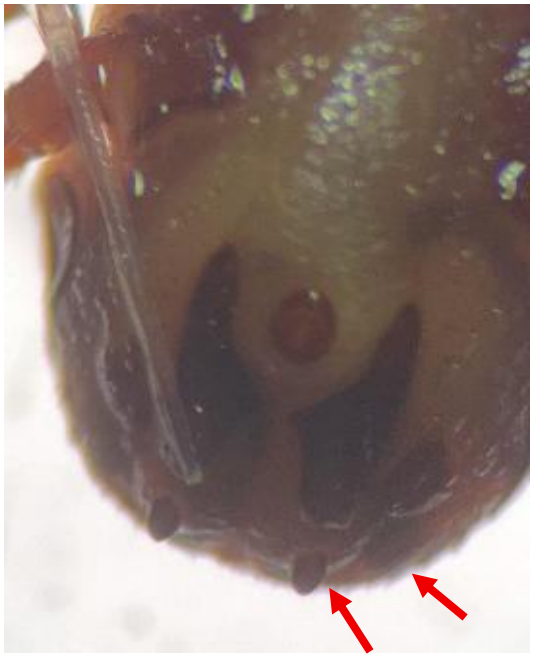

Figure 9: Accessory adanal and adanal plates are not detachable from the dorsal side in both male and female and they were within the body margins (red arrows).

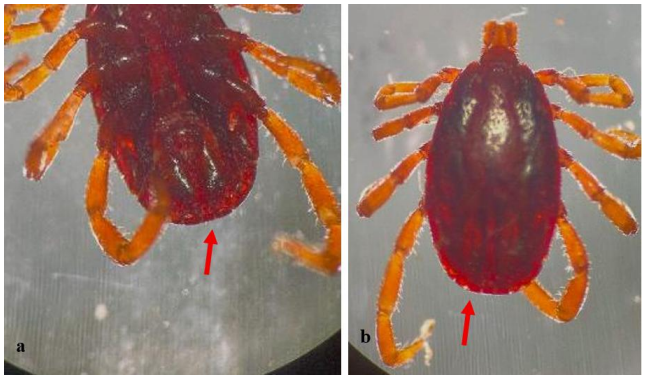

Figure 10: (a,b) Caudal appendage of nourishing male is absent from the ventral and dorsal aspect (red arrows)

Molecular results

PCR mixtures were prepared in 20 µl volumes, comprising a terminal concentration of 1X AddBio Master Mix.

The assortment of DNA density insights was 34.1 to 66.7 ng/µl, accompanied by the average value was 45.15 ng/µl, as well as the selected DNA virtue value, which ranged from 1.78-1.91, with an average of pureness value of about ~1.86.

Regarding the *5.8S rRNA* and *16S rRNA* specific primers, so the sequences of *5.8S rRNA* and *16S rRNA* genes approved that thirty-two samples were collected after extraction of DNA were from *R. sanguineus* and *R. annulatus*, intensification of the two target genes of *5.8S rRNA* and *16S rRNA* from exceptional DNA of *R. sanguineus* and *R. annulatus* isolated proceeded in amplicons of the predicted size, which were 1500 pb and 460 pb in length, respectively (Figures 11 and 12).

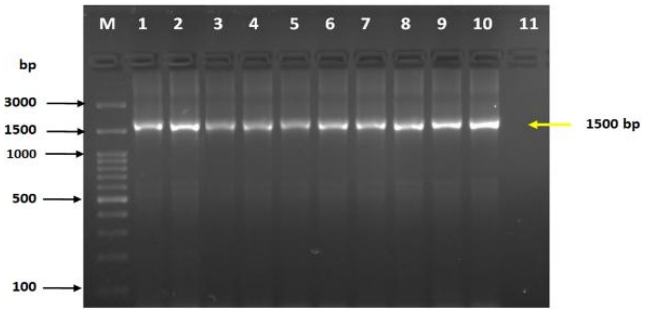

Figure 11: Polymerase chain reaction for the *5.8S rRNA* gene of *Rhipicephalus sanguineus.* Lane M: 100 bp DNA ladder. Lanes 1-10 are positive samples. Lane 11 negative control.

Phylogeny identification

Detonation similarities established the morphological detection ever since the *5.8S rRNA* gene showed (97.80%-100%) homogeneity with analogous sequences of the hard tick *R. sanguineus* from different nations. While the *16S rRNA* genes clarified the presence of (98.97%-100%)

identity with similar sequences of the hard tick *R. annulatus* from other countries (Table 5 and 6).

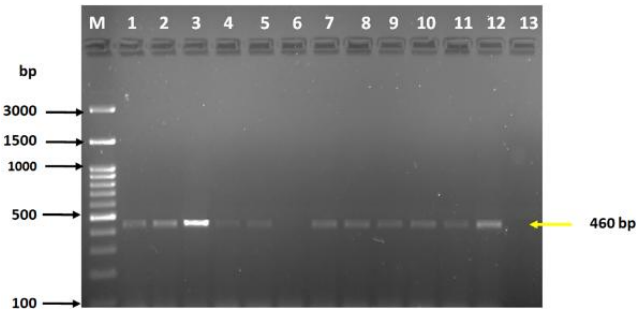

Figure 12: Polymerase chain reaction for the *16S rRNA* gene of *Rhipicephalus annulatus*. Lane M: 100 bp DNA ladder. Lanes 1-12 are positive samples. Lane 13 negative control.

Phylogenetic tree recognition explained the existence of genes sequencing for thirty-two specimens which had been shipped to the Microgen company in South Korea, and diagnosed in the city of Mosul for *R. sanguineus* and *R. annulatus*. They were registered in NCBI to have accession numbers for the sequencing of local genes, as mentioned in both Tables 5 and 6.

97% bootstrap support for *5.8S rRNA* and 51-95% bootstrap promote for *16S rRNA*, they recorded in NCBI to take the accession numbers as is aid before, the BLAST results of the captured 5.8S rRNA sequences of *R. sanguineus* had been showing (97.80%-100%) integrity with *R. sanguineus* from China OP419603, OP419604, OP419601, OP419596, China JQ737127, Egypt JQ412127, South Africa MK295618, MK295616. In the phylogenetic tree, the obtained sequences were grouped with disclosed sequences (Figure 13). The outcomes of the BLAST captured 16S rRNA sequences of *R. annulatus* had been demonstrated (98.97%-100%) rectitude with *R. annulatus* from India MG066700, India OM475710, Australia KC503256, Pakistan MN726556, Thailand MW541856, Egypt MF946466, Egypt KY945491, USA NC067926, Egypt MK737647, Cameron MW080159, so in the phylogenetic tree, the gained sequences were clustered with revealed sequences (Figure 14). The accord sequences of *R. sanguineus* and *R. annulatus* were uploaded to GenBank (OR759793.1) and (OR534233.1), respectively. There was an appearance of emblematic of *Rhipicephalus* (*Boophilus*), *sanguineus*, and *annulatus* grouping in one clade, which was supported with highly bootstrap evaluation.

Table 5: Sequence identity between local *Rhipicephalus sanguineus* strain L-RsM23 (OR759793) and other isolates has been recorded in the GeneBank

Sample Accession Number	Parasite Identified	Query Cover %	Identic Number %	GenBank Accession Number	Country Identification
OR759793	*Rhipicephalus sanguineus*	100	100	OP419603	China
		100	98.46	OP419604	China
		100	98.46	OP419601	China
		100	98.46	OP419596	China
		100	98.25	JQ737127	China
		100	98.24	MK295618	South Africa
		100	97.80	MK295616	South Africa
		100	98.03	JQ412127	Egypt

Table 6: Sequence identity between local *Rhipicephalus annulatus* strain L-RaM23 (OR534233) and other isolates has been recorded in the GeneBank

Sample Accession Number	Identified	Query Cover %	Identic Number %	Genbank Accession Number	Country Identification
OR534233	*Rhipicephalus annulatus*	100	100	MG066700	India
		100	100	OM475710	India
		100	100	KC503256	Australia
		100	99.23	MN726556	Pakistan
		100	99.23	MW541856	Thailand
		100	99.23	MF946466	Egypt
		100	99.23	KY945491	Egypt
		100	98.97	NC_067926	USA
		100	98.97	MK737647	Egypt
		100	98.97	MW080159	Cameroon

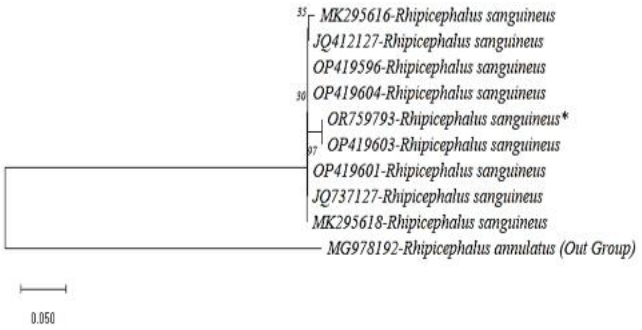

Figure 13: Phylogenic tree was constructed using the Maximum Likelihood method, depending on the Tamura-Nei model in MEGA11 with 1000 replicates. Fractional DNA sequences of integrated fragmentary *5.8S rRNA* had been used as feedback information, showing 97.8%-100% identity.

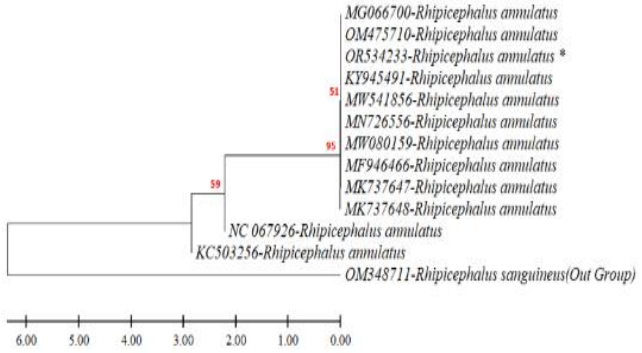

Figure 14: Phylogenic tree was constructed using the Maximum Likelihood method, depending on the Tamura-Nei model in MEGA11 with 1000 replicates. Fractional DNA sequences of integrated fragmentary *16S rRNA* had been used as feedback information, showing 98.97%-100% identity.

Discussion

The microscopic recognition of the hard ticks makes known the genus *Rhipicephalus* that was confined to infected ruminants. This study listed the infection rate with *Rhipicephalus* spp. 31.8% in sheep and goats, and 78.8% in cattle and buffaloes, and total infection rate was 55.3% in ruminants, and these results were matched with Sado Yousseu *et al.* (25) outcome, which they pointed that the highest ratio of infection with *Rhipicephalus* was in cattle and buffalo, while the lowest one seemed in sheep and goats, also agreed with the result of HEKİMOĞLU (26) regarding the infection rate in small ruminants 31.7%, while they did not agree with the infection rate with the tick in large ruminants in Babylon Province, Iraq, 21.3%. The reason is attributed to the variance in geological locales, altitude conditions, rational methodologies, election conscience for animal's samples, and the specific strain of the animals under examination (27). The results of this study showed rising rates of infestation with ticks in females than males for all ruminants under the inquiry, and this result was due to stress factors which the female animals were exposed to them more than males, as pregnancy, suckling, and newborn monitoring lead to a significantly greater burden in females than in males (28). Tick were predominantly found attached to specific anatomical sites of the ruminants, with the udder being the most affected area followed by the thighs and the anal region. The highest rate of tick infestation was on the udder of the females ruminants; tick infestation was necessarily associated with flawed udder health, but not with the milk income and quality (29). In the second place, the thick infestation of thighs region 39.40%, it goes back to its proximity of thighs from udder region, the same applies to the anal region 28.8% tick infestation rate, with noticeable differences between the three regions (udder, thighs, and anal region one), these result did not agree with what was indicated by Gopalakrishnan *et al*. (30), who found that the most predilection sites were on the ears, followed by anus and the external genitalia and around the eyes in goats, this is due to the nature of goats herding and wall and trees climbing behavior of these animals, another cause dependent on the thickness and temperature of the animal skin (31), our result was very close of the result of Fanos Tadesse *et al.* (32) that the udder and adjacent areas (thighs and anal region), while the ears and abdomen were less affected regarding the morphological aspect of *Rhipicephalus annulatus* and *Rhipicephalus sanguineus*. The visual specifications were much like to the studies of Amrutha *et al.* (33) and Do *et al.* (34). The distinguished hard ticks *R. annulatus* and *R. sanguineus* at the molecular level in this research and compare it with the works of other researchers, highlight the presence of close and different phylogenetic findings from dissimilar sectors of the world and systemic improvements on *R. sanguineous* complex has been crucial to renew the knowledge on the existence and dissemination of the ticks (35). Hard ticks, the family Ixodidae, are significant vectors of contagious infections internationally due to their geographical location (36). Phylogenetic recognition of the mitochondrial chromosomes containing *16S rRNA* and ITS genetic marker improved the morphological features regarding *Rhipicephalus sanguineus* and *Rhipicephalus annulatus*, ramified 23 nucleotide sequences: China OP419603, South Africa MK295616, and Egypt JQ412127, as a result, as recorded (37-39), who resolved with our fallout in query cover 100% and only the Chinese isolate had 100% query cover and 100% identic number (37) while 98.24% and 98.03% identic numbers for South Africa and Egyptian isolates. The phylogenetic analysis of *R. annulatus* confirmed high sequence identity with isolates from India, Australia, and other regions, indicating close genetic relatedness and possible global spread of these tick populations (40-42).

Conclusion

The hard tick's phylogenetic tree is scrutinized on the ruminant *Rhipicephalus annulatus* and *R. sanguineus* from a number of Mosul city regions. There is 98.97%-100% and 97.80%-100% for *R. annulatus* and *R. sanguineus* respectively. between episodes of *R. annulatus*, *R. sanguineus* and 9 countries included Egypt, Cameroon, South Africa, Pakistan, India, Thailand, China, and the USA. All-inclusive sweeping research is desirable in the coming generation for a higher clarity of *R. annulatus* and *R. sanguineus* ecology, also the disposal of these ticks in the different meteorological places in Mosul city as well as its brunt on the animal yield and the function of the morphological studies of particular communities of hard tick in ratifies different relationships which signify the differences between species.

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Conflict of interest

The author advocate that there is no conflict of interest around the publication of this study.

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الكشف المظهري والجزيئي لقراد *Rhipicephalus (Boophilus)* في المجترات في مدينة الموصل

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فرع الاحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

بيّنت الدراسة الحالية أن القراد الصلب يمكن تصنيفه بالإعتماد على مفتاح التصنيف ثنائي التفرع والمؤلف من عدة معايير أُخذت بنظر الاعتبار في هذا البحث للتعرف على القراد (مروحية الرأس) أو قراد المواشي، حيث ظهر جسم القراد بقاعدة رؤيس ذات زاوية جانبية حادة مع عنيقات لوامس قصيرة، مع مسافة عنقية كبيرة ومستقيمة في الجانب الظهري للقرادة ويتراوح لون الدرع من الشاحب إلى اللون الداكن والعيون محدبة قليلا في كلا الجنسين وأجزاء الفم صغيرة في الذكور مقارنة بالإناث، كما أن الشق التناسلي يشبه حرف U أو V باللغة الإنجليزية مع معايير تشخيصية أخرى الخ.. إن عينات القراد الصلب قد تم التعرف عليها على مستوى النوع باستخدام المجهر التشريحي المجسم طبقاً للمظهر الشكلي. جمعت عينات قراد المواشي من أعداد مختلفة من المجترات مأخوذة من مناطق متعددة من مدينة الموصل، ثمارات، بادوش، حميدات، سوق الغنم، دامرجي، دامرجي الصغير، ريحانة، الموالي، إحليلة، العبور، بطياشا، دجلة، حي التنك وأخيراً زنازين. إن التحليل الجزيئي باستخدام تقنية تفاعل السلسلة المتبلمر لكلا الجينين ITS (*16S rRNA*) and S1 (*5.8S rRNA*) كان إيجابياً لكل العينات المفحوصة وإن الأجزاء الناتجة من مضاعفة الحامض النووي تم نقلها لغرض التعرف على التسلسل الجيني. أكدت الدراسة على الخصائص الشكلية لقراد الماشية وذلك لإثبات دليل الشاهد مدعوماً بالصور التي تم أخذها لقراد الماشية تحت المجهر التشريحي المجسم والذي تم إثباته من خلال دراسة النشوء والتطور لعزلتين من عزلات جنس قراد الماشية *R. annulatus* و*R. sanguineus*، OR53423، OR759793 على التوالي، إن نسبة التطابق بين عزلتي وعزلات الدول الاخرى مصر، الكاميرون، جنوب أفريقيا، باكستان، الهند، تايلاند، الصين وأمريكا كانت ٩٧,٨٠%-١٠٠% وأظهرت دراسة شجرة النشوء وتطور بأن عزلتي جنس قراد المواشي واقعة مع عزلات الدول آنفة الذكر ضمن فرع حيوي واحد.