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COX1 gene and ITS-2 region: A comparative study of molecular diagnosis of *Parabronema skrjabini* in camels (*Camelus dromedaries*), Al-Najaf Province, Iraq

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Abstract

The current study was contributed to the analysis of the nucleotide sequence pattern of the nucleotide sequence of the tissue DNA isolates based on the Internal Transcribed Spacer ribosomal DNA (ITS2rDNA) gene and Cytochrome c oxidase subunit 1 mitochondrial DNA (COX1 mtDNA) using the traditional polymerase chain reaction (150 samples of abomasum collected directly from camel carcasses after working in Al-Najaf slaughterhouse from 1/10/2019 to 1/2/2020). ITS2rDNA were well suited for the prepared primer with size783 bp and identical ratio ranged 81.17-99.73% of the same species, as indicated a high similarity of the isolates taken from two-humped camels in China or less related to *Parabronema skrjabini* in sheep and goat. In addition, the study identified the number of the mutations within the four COX1 gene and ITS-2 region, which were the most conservative region of the host's species, supporting the concept of host specificity with *Parabronema skrjabini*. The COX1 gene and ITS-2 region applied to confirm the diagnosis using a universal primer, as it included eight isolates with a size of 689 bp, identical values were ranged from 84.99-98.02% depending on the multiple sequence alignment and showed an increase in the substitution level among isolates at an upper taxonomic level. Studying of the COX1 gene and ITS-2 region in *Parabronema skrjabini* demonstrated a significant relation in the cluster and an early common ancestor with isolates of the two-humped camel (China). As for the COX1 gene and ITS-2 region, the phylogenetic relationship supported the ribosomal gene results, especially with *Habronema muscae* or related species such as *Habronema majus*, *Dirofilaria repens*, *Dipetalonema evansi*, *Setaria tundra*, *Cercopithifilaria sp* towards the root node. Therefore, considering COX1 gene and ITS-2 region as an ideal tool in determining the phylogenetic history of the sequence maps, but less conservative mode than the ITS2 ribosome gene based to a taxonomic species level.

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Introduction

Parabronema skrjabini: one of the spirurida nematodes that infect abomasum of the domesticated ruminants such as camel, sheep and goats (1). Sarwar (2) was identified a genus *Parabronema*, a first time was described from Russia (1924). Most abomasal nematodes are causes diarrhea and lack of absorption, weakness, low weight and histopathological changes in abomasa mucosa which lead to reduce in production or working efficiency of the infected animal (3). *P. skrjabini* is known with an indirect biological cycle, which require an intermediate host (flies) such as *Stomoxys bengalensis*, *paregle spp.* and *Lyperosia spp* (4), after 1-2 days fly's egg begin to hatch and feed on the

contaminated dung. Finally, Larva3 of the parasite penetrating the intestine wall of the flies by the anterior lancet (5). In small ruminants, *P. skrjabini* is isolated from sheep and recorded high prevalence rate of the gastrointestinal nematodes during December (100%), however less in March about 44.47% (6). Garedaghi *et al* (7) recorded infection rate 4.91% in sheep. Most researchers depended on microscopically examination of faeces and adult worms (3). Recent researches used a molecular technique to clarify the genotype of *P. skrjabini* in many host (8). Different factors effect on the prevalence rate of abomasal worms (age, sex, body condition and immunity status) Borji (9), and he observed high prevalence of gastrointestinal nematode in sheep's females than males, adult sheep with high ability to resistance nematode's invasion compared to the young ages due to development of immunity response, in other hand the seasonal fluctuation associated with the activation of the intermediate host (10). Epidemiological studies of abomasal nematodes are showed that annual temperature changing during year play an important role in larval development or arrested (7). Arabian camels (*Camelus dromedaries*) are important livestock animal live in harsh environments (11), the number of camels according to FAO, 2003 statistics is about 12.5 million camels about 70% of the world population. There are an increased the economic importance of camels has increased with utilization of their products such as milk, meat and skin (12), due to its geographical distribution, few studies were concentrated on gastrointestinal nematodes in camels and difficulties in taking samples due to the fact that the Bedouin majority is rising (13), Al-Taif (14) was the first study on one-humped camel's infection in Iraq.

Limited information has prompted us to identify this nematode by morphological and molecular distinguishing; and sequencing of ITS2r DNA and COX1mt DNA for determinate a diagnostic level.

Materials and methods

Collecting 150 samples of abomasum directly from camel carcasses after working in Al-Najaf slaughterhouse. Before that, we installed data of the camels for determining the sex, age and periods. Transferring the samples in a cold box to the parasitology lab of the College of Veterinary Medicine, Al-Qadisiyah University, gross examination of the samples grossly for collecting the adults, isolated worms were taken by forceps to diagnose species of parasites, depending on the diagnostic characteristics described by (1,15). Molecular identification of the nematodes uses extracted of 20 rDNA isolates and traditional PCR technique with higher purity of DNA (16-18), PCR master mix prepared by using AccuPower® PCR PreMix Kit and this master mix done according to company instructions included: DNA template 5µl, Forward primer 1.5µl, Reveres primer 1.5µl and PCR water 12µl. The PCR primers used in this analysis to the amplify ITS2rDNA gene of *P. skrjabini*, it is being designed according to (9), while COX1mtDNA gene was prepared as (non-degenerate primer) according to the molecular study of other Spirurida (19), these primers were produced from Bioneer company, Korea as following table 1, and reaction conditions of PCR for amplification showed as in the table 2.

Table 1: Sequences of primers used for identification of *Parabronema skrjabini* using traditional PCR

No	Gene name	Primer name	Primer Sequence	PCR Size
1	ITS2 rDNA gene	Para-Ir-F	5'-GTA GGT GAA CCT GCG GAAGG-3	783bp
		Para-Ir-R	5'-CTGAGC TGA GGT CAA CGA AT-3	
2	COX1 mt DNA gene	F	5'-TGATTGGTGGTTTTGGTAA-3	689bp
		R	5'-ATAAGTACGAGTATCAATATC-3	

Table 2: Cycling conditions of PCR for amplification of *Parabronema skrjabini*

No	PCR step	Temp.	Time	repeat
1	Initial Denaturation	94C	5min	1
2	Denaturation	94C	30sec.	
3	Annealing	58C	30sec	35 cycle
4.	Extension	72C	1 min	
5.	Final extension	72C	5min	1
6.	Colding	4C	Forever	-

Positive products of the PCR ribosomal genes were transferred in an ice bag by DHL to Macrogen Company in Korea for conducting DNA sequencing with DNA sequencing method then the sequencing data submitted into NCBI. The DNA sequencing study was carried out using the local alignment tool in NCBI website http://www.ncbi.nlm.nih.gov/blast/, either multiple sequence alignment of the partial small subunit rDNA gene or pairwise sequence model for determining the genetic variation and substitution among the local isolates and other species depending on the nucleotide sequences (20-22).

The phylogenetic tree is using the Maximum Composite Likelihood system. Phylogenetic analysis that uses the ITS2 rDNA gene according to Hasheminasab *et al.* (3) and COX1mtDNA sequences of *P. skrjabini*, that determined by UPGMA analysis.

Results

Electrophoresis of ITS2rDNA gene products

Results of electrophoresis showed that analysis ITS2rDNA for *P. skrjabini* was positive at 783bp product size (Figures 1,2).

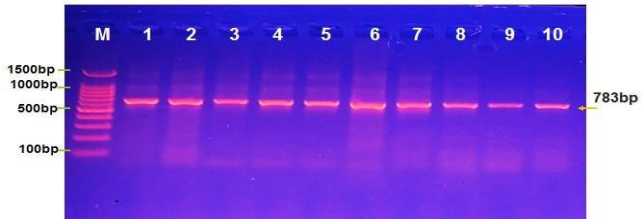

Figure 1: Agarose gel electrophoresis image that showed PCR product analysis ITS2-rDNA for *Parabronema Skrjabini*. M (Marker ladder 1500-100bp). Lane (1-10) positive *Parabronema skrjabini* isolates at 783bp product size.

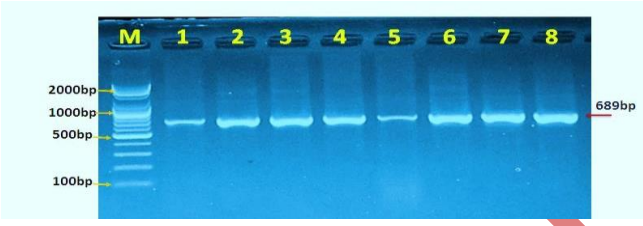

Figure 2: Agarose gel electrophoresis image that showed PCR product analysis COX1-mtDNA for *Parabronema skrjabini*. M (Marker ladder 2000-100bp). Lane (1-8) positive *Parabronema skrjabini* isolates at 689 bp product size.

Electrophoresis of the COX1mtDNA gene products

Results of electrophoresis showed that analysis COX1mtDNA for *P. skrjabini* was positive at 689bp pr

DNA sequencing analysis and phylogenetic tree of ITS2rDNA gene

PCR products are estimated at 783bp in the size and that submitted to the NCBI website. The current sequences were compared to the previous results documented on the NCBI website, ITS2-rDNA have a higher homogeneity range 98.01-99.73% with Bacterain camel isolates, but less similarity to other isolates from the small ruminant about 81.17-86.29% LC275906, LC275902, LC325445, LC275908, LC275903, LC325447, LC325450 and 84.04% of *P. smithi* MH445384. *Habronema muscae* or *Habronema majus* collected from the equines or donkey with a homogeneity ratio about 83.08-86.99% (Table 3). Phylogenetic tree of *Parabronema skrjabini* depending on ITS2 rDNA sequences uses the maximum log-likelihood method. The current study showed the overall nucleotide identity among isolates reported in Iraq and other sites of the world as indicated in the (Figure 3-6). The differences in the substitution ratio have a clear divergence among local isolates and Basra isolates from small ruminants, reaching 71-point mutation and a number of the gaps on NCBI website (Table 4).

DNA sequencing and phylogenetic tree

The present study provides the first phylogenetic description of the mitochondrial COX1region in *P. skrjabini* and its role in the phylogenetic rate with other nematodes, then comparing of the substitution level among this nematode and some available sequences for other spirurida by BLAST software on the NCBI website. The mtDNA sequences have an identic level about 84.99-98.02% in eight local isolates of *P.skrjabini*. The COX1mtDNA gene were 689 bp in length after electrophoresis processing. we were able for comparing the identical ratio in the local sequences of the COX1mtDNA gene and determined 97.39-98.02% for *H. muscae* isolate KX868085 and *Habronema majus* 87.82-89.62% KX868083, the current sequences reported on the NCBI website.

Finally, the less identical species were estimated *Dirofilaria repens, Dipetalonema evansi, Setaria tundra, Cercopithifilaria sp.* the genetic variation ranged 84.99-86.34% as shown in the (Table 5). The phylogenetic tree constructed using the data of strong bootstrap value among the Parabronema isolates and some spirurida, in the (Figure 4) the monophyletic clade involved *Habronema sp.* and the current isolates at most common ancestor. The related species of China, Poland, Austria and Italy have inserted to the second cluster within same common ancestor by the bootstrap value (polyphyletic group). The number of mutations/ substitutions per isolate and the genetic variation 1.98-2.6% with *Habronema muscae* KX868085 (Table 6). Knowledge of the evolutionary principles as the substitution patterns and variability rate are a necessary step of the phylogenetic studies. We have identified the substitution model of the mitochondrial COX1mtDNA gene with related species. The percentage of transition bias was 79.7% T.i. and 11.9% T.v. of the total number of the substitutions, the most genotype is T/C, then A/G (Table 8). The results of local isolates with a number of species revealed that spirurida possesses an advanced level of the mutation, the common genotype of each nematode, we can be reflecting the percentage of the substitution about 15.26% and a number of mutations within the eight local isolates 47-71 mutation shown in the (Table 7). The percentage of nucleotides from the COX1 mtDNA gene within the current isolates and other isolates showed the rich A + T content as indicated in the (Figure 5), The ratio of higher A + T content observed within local isolates in three positions.

Table 3: The NCBI-BLAST homology sequence identity

Identical NCBI BLAST of *P. skrjabini*	Genbank Accession number	Host	NCBI-BLAST Homology Sequence identity (%)			
			P. skrjabini IQ. Camel isolate No. / Genbank Accession number			
			MT509436	MT509437	MT509438	MT509439
P. skrjabini	EU375510.1	Camel	99.18	99.73	99.51	99.45
P. skrjabini	EU420132.1	Camel	98.01	98.52	98.51	98.27
P. skrjabini	LC325447.1	sheep	86.29	86.29	86.29	86.29
P. skrjabini	LC275906.1	Goat	85.94	85.94	85.94	85.94
P.skrjabini	LC325450.1	Sheep	85.77	85.77	85.77	85.77
P. skrjabini	LC275903.1	Goat	85.28	85.94	85.28	85.28
P. skrjabini	LC275908.1	Goat	83.22	84.34	83.72	83.72
P. skrjabini	LC275902.1	Goat	83.17	82.61	82.61	82.61
P. skrjabini	LC325445.1	Sheep	81.17	81.17	81.17	81.17
P. smithi	MH445384.1	Elephant	84.04	84.04	84.04	84.04
Habronema muscaae	KX868082.1	Donkey	86.99	86.99	86.99	86.99
Habronema majus	KX868081.1	Donkey	83.08	83.08	83.08	83.08

Table 4: The number of the substitution of the camel, sheep and related species by Multiple sequence alignment tool

Parasite	host	Accession no	*P. skrjabini* IQ. Camel isolate No. / Gen Bank Accessing number			
			MT509436	MT509437	MT509438	MT509439
Parabronema skrjabini	Sheep	LC325447.1	71	71	71	71
Substitution %			13.1%	13.2%	13.1%	13.4%
Parabronema skrjabini	Camel	EU375510.1	6	2	2	4
Substitution %			0.8%	0.2%	0.2%	0.5%
Parabronema skrjabini	Camel	EU420132.1	5	3	3	4
Substitution %			0.6%	0.4%	0.4%	0.5%
Habroenma muscae	Donkey	KX868082.1	38	38	38	38
Substitution %			7.8%	7.9%	7.9%	8%
Parabronema smithi	Elephant	MH445384.1	34	34	34	34
Substitution %			5.7%	5.8%	5.8%	5.9%

Table 5: The NCBI-BLAST homology sequence identity

P. skrjabini identical NCBI BLAST	Accession no.	Host	NCBI-BLAST Homology Sequence identity (%)			
			MT664736	MT664737	MT664738	MT664739
Habronema muscae	KX868085.1	donkey	97.60%	98.02%	97.85%	97.62%
Habronema majus	KX868083.1	donkey	89.11%	89.62%	89.46%	89.18%
Dirofilaria repens	AM749230.1	dog	86.06%	85.9%	86.02%	86.02%
Dipetalonema evansi	KR184805.1	camel	86.27%	86.34%	86.24%	86.15%
Setaria tundra	MK360915.1	deer	85.62%	86.09%	86.02%	85.71%
Cercopithifilaria sp.	KX898457.1	dog	85.37%	85.65%	85.13%	85.47%
P. skrjabini identical NCBI BLAST	Accession no.	Host	NCBI-BLAST Homology Sequence identity (%)			
			MT664740	MT664741	MT664742	MT664743
Habronema muscae	KX868085.1	donkey	97.59%	97.39%	97.63%	97.72%
Habronema majus	KX868083.1	donkey	89.04%	88.89%	89.25%	87.82%
Dirofilaria repens	AM749230.1	dog	85.56%	85.62%	86.02%	85.5%
Dipetalonema evansi	KR184805.1	camel	85.96%	86.06%	86.24%	84.99%
Setaria tundra	MK360915.1	deer	85.53%	85.4%	86.02%	84.99%
Cercopithifilaria sp.	KX898457.1	dog	85.27%	85.15%	85.34%	84.99%

Table 6: The mutation analysis of COX1mtDNA gene in *Parabronema skrjabini* and the substitutions among local isolates with *Habronema muscae* KX868085, the ratio of mutation range (1.98%-2.6%) by pairwise sequence alignment tool

Isolates of *P. skrjabini*	Accession number	NCBI-BLAST Homology nucleotides alignment / (COX1) gene			
		Identities	Sequence/n	Ratio	Genotype
No.1	MT664736	98%	459/ 11	2.39%	T/C,T/C,A/T,T/G,A/G,T/C,A/G,T/C,C/T,T/C,G/A
No.2	MT664737	99%	454/ 9	1.98%	T/C,T/G,A/G,T/C,A/G,T/C,C/T,T/C,G/A
No.3	MT664738	98%	475/ 10	2.15%	T/C,T/A,T/G,A/G,T/C,A/G,T/C,C/T,T/C,G/A
No.4	MT664739	98%	460/ 11	2.38%	T/C,T/C,A/T,T/G,A/G,T/C,A/G,T/C,C/T,T/C,G/A
No.5	MT664740	98%	459/ 11	2.41%	T/C,G/A,T/G,A/G,T/C,T/A,A/G,T/C,C/T,T/C,G/A
No.6	MT664741	97%	461/ 12	2.6%	T/C,T/C,T/A,T/G,A/G,T/C,T/A,A/G,T/C,C/T,T/C,G/A
No.7	MT664742	98%	465/ 11	2.36%	T/A,T/C,T/A,T/G,A/G,T/C,A/G,T/C,C/T,T/C,G/A
No.8	MT664743	98%	394/ 9	2.28%	T/A,T/C,G/A,T/G,A/G,T/C,A/G,T/C,C/T

Table 7: The mutation number of the nucleotides database from *COX1mtDNA* gene in *Parabronema skrjabini* and identify the substitutions with other spiurida by multiple sequence alignment tool:

NCBI isolates	Accession n	Host	N	Substitution	Genotype of all isolates NO.1-NO8
H. majus	KX868083	Donkey	47-51	10.35-11.06	T/C,T/G,T/A,A/G,C/T,A/T,G/A,T/A,G/T
D. repens	AM749230	Dog	58-66	13.47-14.75	G/A,T/C,T/G,T/A,A/G,C/T,A/T,T/A,G/T,G/C,C/A
D. evansi	KR184805	Camel	60-70	13.65-15.26	G/A,T/C,T/G,T/A,A/G,C/T,A/T,T/A,G/T,G/C,C/A
S. tundra	MK360915	Deer	60-71	13.78-15.26	G/AT/C,T/G,T/A,A/G,C/T,A/T,T/A,G/T,G/C,C/A
Cercopithifilaria	KX898457	Dog	66-70	14.43-15.21	G/A,T/C,T/G,T/A,A/G,C/T,A/T,T/A,G/T,G/C,C/A

Underline indicated to the most frequency genotype of NO.1-NO.8 isolates

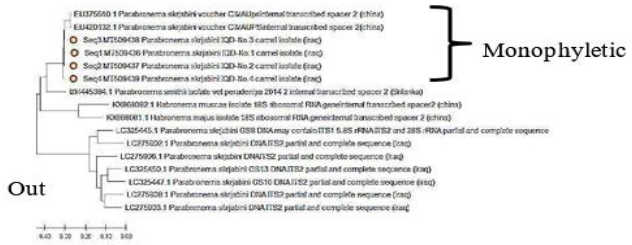

Figure 3: The phylogenetic tree analysis based on ITS2rDNA in local camel's isolates that used for confirmative genetic identification.

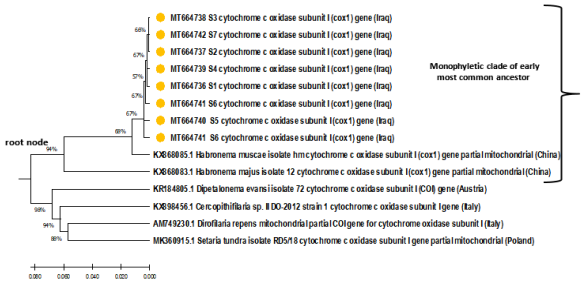

Figure 4: The phylogenetic tree analysis based on COX1mtDNA gene partial sequence in local *Parabronema skrjabini* isolate Iraqi Camel isolates that used for the genetic variation identification.

Table 8: The total number of T.i.: T.v. substitutions among *Habroenema muscae* KX868085 and NO.1-NO.8 local IQD-Camel isolates of cox1mtDNA by Pairwise sequence alignment in NCBI Website

Model	Genotype	No. Substitution %	Total (%)
T.i.	T/C	34	67(79.7)
	A/G	16	
	C/T	8	
	G/A	9	
T.v.	A/T	2	10(11.9)
	T/G	8	

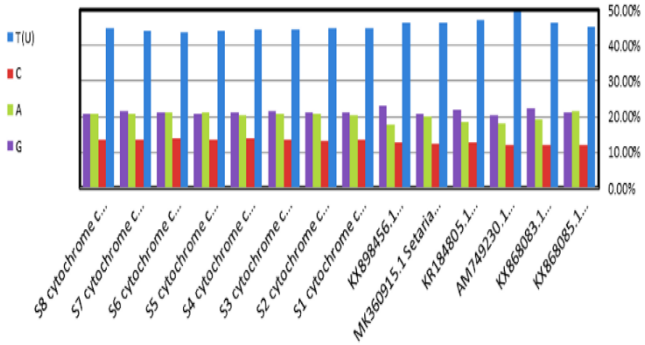

Figure 5: The nucleotide composition of the COX1mtDNA gene in the local isolates (No1-No8) and other isolates inserted to NCBI website by Mega 6.0 software.

Discussion

Phylogenetic tree and sequencing analysis of DNA gene ITS2 rDNA region

ITS2-rDNA sequences prepared in four isolates for answering the question about the identity of *P. skrjabini* from one-humped camel. The current findings of the molecular methods have confirmed this spirurida, PCR products being 783bp in size, that very linked to the Chinese isolates obtianed from the Bactrian camel, but relatively less in the identical values with the ITS2rDNA gene isolates of the small ruminants at Basra /Iraq and related species of the same family (*Habronema sp.* and *Parabronema smithi*), thus supporting the diagnostic standards of ITS2rDNAgene as one of the most specific markers among isolates of the same species. The local isolates estimated 98.01-99.73% shared on the same cluster with Chinese isolates (Accession number: EU375510, EU420132), while the identical level reported in the Basra isolates (small ruminant) about 81.17-86.29% LC275906, LC275902, LC275908, LC275903, LC325447, LC325450, LC325445). Some nematodes were determined in the family-Habronematidae as called *H.muscae* KX868082 and *H.majus* KX868081 obtained from equines or *Parabronema smithi* in the elephant MH445384, the homogeneity ratio ranged 83.08-86.29% according to the Blast analysis, where the phylogenetic tree created a paraphyletic group with the local isolates, comparing of *P.skrjabini* in the small ruminants combined to a separate common ancestor(outgroup). The current findings are greatly consistent with certain findings of the previous studies as Traversa (23) or the evolutionary analysis of the Iranian study (9).

Phylogenetic tree and sequencing analysis of COX1mt DNA region:

In the second identification, the mitochondrial COX1DNA gene in *P. skrjabini* is a first reported in Iraq. No published study of this nematode has previously reported with the COX1mtDNA gene, so the rate of divergence analysis can be discussed depending on the related spirurida. The COX1mtDNAgene considered a suitable tool in the phylogenetic studies because of the rapid variation rate in the several categorical levels (24). The COX1mtDNA sequences of *P. skrjabini* estimated 689bp in size. The current results are agreement with other studies in determining the evolutionary history such as a family Habronematidae (25).

Comparing the genetic markers in identifying the nematode *P. skrjabini*

As a result of the sequence and phylogenetic data for each of the genetic markers, the ITS2rDNA gene has a fixed pattern of Indels (a common feature of ITS2rDNA region) and the similar model of the sequence differences presented the high conservative pattern of the same species, if the divergence level 1% among the individuals (26,27), thus able to identifying at the lower categorical levels as called the deep levels e.g. species and populations (28). The current results are agreement for the previous studies that indicated to the ribosomal DNA genes in identifying the nematode species, reporting the constant identical and substitution values in the individual identity of *P. skrjabini* (29). The inter-species identity of the ITS2rDNA gene comparing to Basra isolates of the sheep and goat have relatively different (23), as well with *P. smithi* habited in the elephant (25). The Parabronema isolates of the small ruminants were more divergence ratio than 1%and this unlike what the researchers suggested at the phylogenetic relationship (26).

The ITS2rDNA results are supporting the concept of the host specification; what raising a question about the *P. skrjabini* in the one-humped camel as a separate species of the genus Parabronema, that may need the compared studies with more isolates and diagnostic regions. The study results don't agree in confirming the unreliability of the nuclear rDNA gene according to the phylogeny analysis or decreased resolution at the species level (30). The multiple sequence alignment showed the a clear variation of ITS2rDNA region in *P.skrjabini* according to the sequences published in NCBI website, the mutation ratio reached 13.4% with Basra isolates taken from the small ruminants and the relatively less to the substitutions level has 8% of *H. muscae* (donkey) or *P.smithi* about 5.9% of early common ancestor, consequently, it is confirming the idea of *P. skrjabini* carries a unique pattern of the ITS2rDNA gene sequence. In the previous studies, the mitochondrial DNA variation within members of the same species was indicated 2-6% such as *Ostertagia ostertagi* (27). The mitochondrial DNA sequence of the related species range 10-20%; therefore, every two members may be a difference within 10% or more (27). The mitochondrial COX1gene has introduced a clear picture in recognizing the genetic individual variation mapping lead to determining for the cryptic species within known nematode, which not confirmed by the results of the current study (27), the sequences are appeared for all local isolate early related to a most common ancestor with Chinese isolates *Habronema muscae and, H. majus*.

The phylogenetic tree showed the relationship with species *Habronema sp.* at an early evolutionary period, this is also similar to the another spirurida, confirming the results of the previous studies as" a typical marker for determining evolutionary history" (27), analysis of the phylogenetic among other species in relation to multiple branches within a monophyletic group. We determined the few substitutions of the related species in a family Habeonematidae depend on COX1 mtDNA gene, that related to a separate branch during an early evolutionary time; if reported the substitution number of 9-12/per local isolate (the substitution ratio about 1.98-2.6%)with *H.*

muscae KX868085, then an increase in the mutations are reached 15.26% towards the early position according to the (Table,7),when comparing the substitution and identical ratios indicated for them in the results chapter between the local isolates both genes.

The study found out a mode of the most conservatism within the ITS2rDNA gene is relatively better at species level than COX1 mtDNA gene. The high A+T content of mitochondrial DNA, many copies and small size sees a reason for the rapid rate of the evolutionary pattern, which can be adapted to the current findings, where the increased rates of the A+T composition are higher than C+G bases (30).

Conclusion

COX1 gene and ITS-2 region as an ideal tool in determining the phylogenetic history of the sequence maps, but less conservative mode than the ITS2 ribosome gene based to a taxonomic species level.

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Conflict of interests

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References

1. Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals. London: Bailliere Tindall; 1982. 809 p. DOI: doi.org/10.1016/0035-9203(84)90110-X.
2. Sarwar MM. On the Transference of *Parabronema skrjabini* Rassowska, 1924 to Squamanema Thiel, 1925 and its Occurrence in Sheep and Goats in Indo-Pakistan. J Helminthol. 1954;28(3-4):143-150. DOI: doi.org/10.1017/S0022149X0003279X.
3. Hasheminasab SS. Molecular characterization of the first internal transcribed spacer of rDNA of *Parabronema skrjabini* for the first time in sheep. Ann Parasitol. 2015;61(4):241-246. DOI: 10.17420/ap6104.13
4. Liu Y, Zhao Z, Yang X, Yang L, Yang B, Zheng W, Li W, Luo X, Wang R, Gu W, Wang P. *Haematobium irritans* and *Haematobium titillans* as potential vectors of *Parabronema skrjabini* in camels (*Camelus bactrianus*) in Inner Mongolia, China. Parasitol, 2020;147(13):1509-1514. DOI: 10.1017/S0031182020001328
5. Sazmand A, Joachim A. Parasitic diseases of camels in Iran (1931-2017)-a literature review. Parasite. 2017;24(21):1-15. DOI: 10.1051/parasite/2017024
6. AL-Dahar AH, Al-Amery A. A prevalence of abomasal worms in sheep in Al-Kut province abattoir. Iraqi J Vet Med. 2015;39(2):38-41. DOI: 10.30539/iraqijvm.v39i2.175
7. Garedaghi Y, Hossein H, Asso F. Prevalence of abomasal nematodes in sheep slaughtered at Baneh Town. Am J Anim Vet Sci. 2013;8(3):142-145. DOI: 10.3844/ajavssp.2013.142.145
8. Gari Z, Melako M, Bekele T. Prevalence of abomasal nematodes in sheep slaughtered at Jimma Town Municipality Abattoir, South West Ethiopia. Inter J Adv Res Biol Sci. 2017;4(12):1-7. DOI: 10.22192/ijarbs.2017.04.12.001
9. Borji H. A study on gasterointetinal helminthes of camels in Mashhad abattoir, Iran. Iranian J of Vet res, 2010;11(2):174-179. DOI: 10.22099/IJVR.2010.162
10. Kuchboev A, Sobirova K, Karimova R, Amirov O, vonSamsonHimmelstjerna G, Krucken J. Molecular analysis of polymorphic species of the genus *Marshallagia* (Nematoda: Ostertagiinae). Parasit Vect. 2020;13(1):1-12. DOI: 10.1186/s13071-020-04265-1
11. Traversa D, Kuzmina T, Kharchenko VA, Iorio R, Klei TR, Otranto D. Haplotypic variability within the mitochondrial gene encoding for the cytochrome c oxidase 1 (COX1) of *Cylicocyclus nassatus* (Nematoda, Strongylida): Evidence for an affiliation between parasitic populations and domestic and wild equid hosts. Vet Parasitol. 2008;156(3-4):241-247. DOI: 10.1016/j.vetpar.2008.05.031
12. Radfar MH, Gowhari MA. Common gastrointestinal parasites of indigenous camels (*dromedarius*) with traditional husbandry managements (free-ranging system) in central deserts of Iran. J. Parasitol Dis. 2013;37:225-230. DOI: 10.1007/s12639-012-0170-8
13. Al-Taif KI. Helminths in camels in Iraq. Trop Anim Heal Product. 1974;6(1):55-57. DOI: 10.1007/BF02380750
14. Casiraghi M, Anderson TJC, Bandi C, Bazzocchi C, Genchi C. A phylogenetic analysis of filarial nematodes: Comparison with the phylogeny of Wolbachia endosymbionts. Parasitol. 2001;122:93-103. DOI: 10.1017/s0031182000007149
15. Mohammed NH. Prevalence, morphological and biochemical study of larval stage *Coenurus cerebralis* of *Taenia multiceps* in sheep. Iraqi J Vet Sci. 2020;34(1):159-163. DOI: 10.33899/ijvs.2019.125660.1124
16. Hajeel MS, Alfatlawi MAA. Sequencing-based phylogenetic-study of *Babesia* spp detected in tick tissues in Al-Diwaniyah province, Iraq. Iraqi J Vet Sci. 2019;33(1):9-12. DOI: 10.33899/ijvs.2019.125512.1034
17. Al-Fatlawi MAA, Ali M, Naser H. Molecular identification of allelic genotypes of pyrethroid-insecticide resistance in housefly, Iraq. Iraqi J Vet Sci. 2019;33(2):209-212. DOI: 10.33899/ijvs.2019.125538.1060
18. Alfatlawi MA, Jasim AA, Jarad NE, Khlaif SF. Clinical and molecular identification of ruling *Theileria annulata* strains in cattle calves in Al-Diwaniyah province, Iraq. Iraqi J Vet Sci. 2021;35(1):115-119. DOI: 10.33899/ijvs.2020.126429.1319
19. Blouin MS. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. Inter J Parasitol. 2002;32:527-531. DOI: 10.1016/s0020-7519(01)00357-5
20. Traversa D, Costanzo F, Iori OR, Aroch I, Lavy E. Mitochondrial cytochrome c oxidase subunit 1 (cox1) gene sequence of *Spirocerca lupi* (Nematoda, Spirurida) from different geographical areas: avenues for potential implications. Vet Parasitol. 2007;146:263-270. DOI: 10.1016/j.vetpar.2007.03.015
21. Ali MJ, Karawan AC, Al-Fetly DR, ALfatlawi MAA. Synergizing the deltamethrin larvicidal activity against *Aedes albopictus* larvae using cinnamaldehyde in Diwaniyah, Iraq. Iraqi J Vet Sci. 2020;34(2):317-320. DOI: 10.33899/ijvs.2019.126026.1212
22. Alfatlawi MA, Ismail YK, Ali MJ, Karawan AC, Ibadi IN. Molecular differentiation of *Thysaniezia* (*Helictometra*) *giardi* and *Moniezia* species based on 18s rRNA gene in small ruminants. Iraqi J Vet Sci. 2021;35(1):105-108. DOI: 10.33899/ijvs.2020.126407.1313
23. Galtier N, Nabholz B, Glemin S, Hurst GDD. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. Molecul Ecol. 2009;18(22):4541-4550. DOI: 10.1111/j.1365-294X.2009.04380.x
24. Thewarage LD, Dissanayake DS, Perera US, Bandara AT, Perera BVP, Wickramasinghe S, Rajapakse RPVJ. Morphology and molecular characterization of *Parabronema smithii* (Cobbold, 1882) (Nematoda: abronematidae) from wild asian elephant (*Elephas maximus maximus*) of Sri Lanka. Acta Parasitol. 2020;65(2):504-517. DOI: 10.2478/s11686-020-00193-3
25. Dallas JF, Irvine RJ, Halvorsen O, Albon SD. Identification by polymerase chain reaction (PCR) of *Marshallagia marshalli* and

Ostertagia gruehneri from Svalbard reindeer. Int J Parasitol. 2000;30(7):863-6. DOI: 10.1016/s0020-7519(00)00063-1
26. Blouin MS. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. Inter J Parasitol. 2002;32(5):527-531. DOI: 10.1016/s0020-7519(01)00357-5
27. Li F, Hu T, Duan NC, Li WY, Teng Q, Li H, Cheng TY. Sequence variation in two mitochondrial DNA regions and internal transcribed spacer among isolates of the nematode *Oesophagostomum asperum* originating from goats in Hunan Province, China. J Helminthol. 2016;90(1):1-6. DOI: 10.1017/S0022149X14000650
28. Gasser RB. Molecular tools-advances, opportunities and prospects. Vet Parasitol. 2006;136(2):69-89. DOI: 10.1016/j.vetpar.2005.12.002
29. Derycke S, Vanaverbeke J, Rigaux A, Backeljau T, Moens T. Exploring the use of cytochrome oxidase c subunit 1 (COXI) for DNA barcoding of free-living marine nematodes. PLoS One. 2010;5(10):e13716. DOI: 10.1371/journal.pone.0013716
30. Workentine ML, Chen R, Zhu S, Gavriliuc S, Shaw N, de Rijke J, Gilleard JS. A database for ITS2 sequences from nematodes. BMC Genet. 2020;21(1):1-4. DOI: 10.1101/689695

جين COX1 و منطقة ITS-2: دراسة مقارنة للتشخيص الجزيئي لطفيلي *Parabronema skrjabini* في الجمال العربية في محافظة النجف، العراق

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الخلاصة

ساهمت الدراسة الحالية في تحليل نمط تسلسل النوكليوتيدات لعزلات الحمض النووي للأنسجة استنادا إلى جين الدنا الريبوزومي المنقسم الداخلي والجين الفرعي ١ للميتوكوندريا باستخدام تفاعل متسلسل للبوليميراز التقليدي (١٥٠ عينة من الانفحة تم جمعها مباشرة من جثث الإبل بعد الذبح في مسلخ النجف من ٢٠١٩/١٠/١ إلى ٢٠٢٠/٢/١. كان جين الدنا الريبوزومي المنقسم الداخلي مناسب تماما للطبقة الأولية المحضرة بحجم ٧٨٣ نقطة أساس ونسبة مماثلة تراوحت ٨١,١٧-٩٩,٧٣٪ من نفس النوع، كما يتضح من التشابه الكبير للعزلات المأخوذة من الإبل ذات السنامين في الصين أو أقل ارتباطًا بـ *Parabronema skrjabini* في الأغنام والماعز. بالإضافة إلى ذلك، حددت الدراسة عدد الطفرات داخل الجينات الأربعة للجين الفرعي ١ للميتوكوندريا ومنطقة ITS-2، والتي كانت المنطقة الأكثر تحفظًا لأنواع المضيف، ودعم مفهوم خصوصية المضيف مع *Parabronema skrjabini*. تم تطبيق الجين الفرعي ١ للميتوكوندريا ومنطقة ITS-2 لتأكيد التشخيص باستخدام أساس عالمي، حيث اشتمل على ثماني عزلات بحجم ٦٨٩ نقطة أساس، وتراوحت القيم المتطابقة من ٨٤,٩٩-٩٨,٠٢٪ اعتمادًا على محاذاة التسلسل المتعدد وأظهرت زيادة في مستوى الإحلال بين العزلات عند مستوى تصنيفي أعلى. أظهرت دراسة الجين الفرعي ١ للميتوكوندريا ومنطقة ITS-2 في *Parabronema skrjabini* وجود علاقة مهمة في التركيب الجيني مع عزلات من الجمل ذي السنامين (الصين). أما بالنسبة للجين الفرعي ١ للميتوكوندريا ومنطقة ITS-2، فإن علاقة النشوء والتطور تدعم نتائج الجينات الريبوزومية، خاصة مع *Habronema muscae* أو الأنواع ذات الصلة مثل *Habronema majus* و *Dirofilaria repens* و *Dipetalonema evansi* و *Setaria tundra* و *Cercopithifilaria* sp تجاه عقدة الجذر. لذلك، بالنظر إلى الجين الفرعي ١ للميتوكوندريا ومنطقة ITS-2 كأداة مثالية في تحديد تاريخ تطور خرائط التسلسل، ولكن وضع أقل تحفظًا من جين الريبوسوم ITS-2 على أساس مستوى الأنواع التصنيفية.