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MOLECULAR CHARACTERIZATION OF *CONTRACAECUM RUDOLPHII* HARTWICH, 1964 (NEMATODA: ANISAKIDAE) FROM THE CORMORANT *PHALACROCORAX CARBO* IN IRAQ

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ABSTRACT

Contracaecum rudolphii Hartwich, 1964 is a nematode which causes major concerns to human and wildlife animal's health. However, the population genetics of *C. rudolphii* has been poorly studied in Iraq. In order to gain a deeper understanding in the outline of the genetic diversity of the nematode *C. rudolphii* that were isolated from its host cormorant *Phalacrocorax carbo* (Linnaeus, 1758), in the middle areas of Iraq, twenty specimens of *C. rudolphii* adults were isolated from nine individuals of *P. carbo*. The first (ITS-1) internal transcribed spacers (ITS) of ribosomal DNA (rDNA) of *C. rudolphii* were amplified using conventional polymerase chain reaction (PCR); then, the amplicons were subjected to sequencing. Concatenation of ITS-1 (rDNA) sequences resulted in four unique genotypes that have not been previously recorded in Iraq. The present study showed that the most common genotype occurred in 85% of *C. rudolphii*, and in 88.9% of cormorants. Furthermore, the infrapopulation difference in the genotypes was fairly high, with an average of 1.3 ± 0.48 genotypes per host of those with ≥two nematodes. All the sequences of the current study were distributed into two different populations. The sequences of ITS-1 for the first population had the highest similarity to ITS-1 sequence of *C. rudolphii* B, while the sequences of ITS-1 for the second population had the highest similarity to ITS-1 sequence of *C. rudolphii* A. This study provides an insight about the genetic divergence of *C. rudolphii* among *P. carbo* in Iraq. As well, the results likely support the hypothesis that *C. rudolphii* represents a complex of at least two sibling species.

Keywords: *Contracaecum rudolphii*, GenotypeITS-1, Iraq, *Phalacrocorax carbo*, Polymerase chain reaction (PCR).

INTRODUCTION

The roundworm *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae) is a parasitic intestinal nematode of the cormorants, pelicans, and ducks (Moravec, 2009). It is considered to have highly pathogenic effects on both wildlife and humans (Shamsi, 2009). In many regions, the species is progressively identified as an emerging concern for human health. The larvae of *Contracaecum* spp. are the causative agent of human Anisakidosis (Shamsi, 2014). Several reports have revealed that the larvae cause a severe and painful condition in humans following ingestion of under-cooked fish carrying third stage larvae of Contracaecum species including *C. rudolphii* (Takabayashi *et al*., 2014; Bookhout and Greene, 2019). *C. rudolphii* like to most other anisakid is transmitted through many hosts. The first intermediate hosts of *C. rudolphii* are crustaceans and larvae of aquatic insects; fish regards the second intermediate or paratenic hosts, while fishing birds are the final hosts (Bartlett, 1996; Dziekońska-Rynko and Rokicki, 2007). Anthropogenic shifts to natural landscapes have resulted in significant increases in population densities of wildlife species in some areas (Daszak *et al*., 2001; Barrueto *et al*., 2014). Such increases in the number of wildlife species are likely to increase the occurrence of *C. rudolphii* and promote extra transmission of *C. rudolphii* between birds and fish, also fish and humans (Kanarek, 2011).

The genetic variability of *C. rudolphii* is poorly investigated, regardless of the fact that discriminating the genetic population has significant consequences to considerate the evolutionary ecology of the species, and can give some important information for controlling the parasite in humans and wildlife animals (Shamsi *et al*., 2009; Cole and Viney, 2018). A previous study on *C. rudolphii* illustrated that the first (ITS1) and/or second (ITS-2) internal transcribed spacers (ITS) of ribosomal nuclear DNA (rDNA) provide genetic markers for the specific identification of number of ascaridoid species (Szostakowska and Fagerholm, 2012). In addition, some studies have shown that sibling species can be identified and distinguished based on of ITS-1 and/or ITS-2 (Zhu *et al*., 2000; Mattiucci *et al*., 2003; Li *et al*., 2005). Yet the broader of the molecular characterization and genetic polymorphism of *C. rudolphii* was not the topic of focused work in Iraq, although ITS-1 was used in one study from Thi-Qar southern of Iraq to identify the fourth-stage larvae of *Contracaecum microcephalum* (Rudolphi, 1809) and *C. septentrionale* (Kreis, 1955) which were isolated from proventriculus of *Nycticorax nycticorax* (Linnaeus, 1758) in Al-Sanaf marsh, southern of Iraq by Mohammad and Hbaiel (2019 a). Moreover, *C. rudolphii* has been morphologically identified and recorded recently by Al-Moussawi and Mohammad (2011); Al-Moussawi (2017) and Mohammad and Hbaiel (2019 b).

The purpose of the current study is to quantify the genetic divergence of *C. rudolphii* Hartwich, 1964 from the *P. carbo* (Linnaeus, 1758) in Iraq.

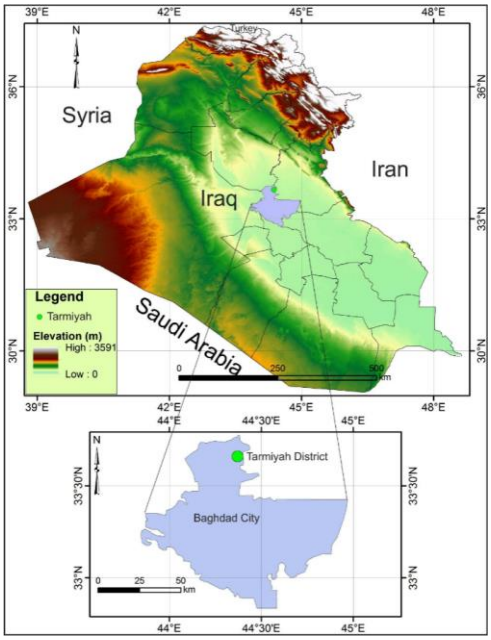
MATERIALS AND METHODS

Collection of parasites

Adult nematodes were collected from nine infected *P. carbo* obtained from trappers that were collected from Tarmiyah district (33.6732° N, 44.3615° E), Baghdad province, central Iraq (Map 1), for an unrelated project (Al-Moussawi, 2017). The cormorants were identified according to Allouse (1961) and Salim *et al*. (2009), the sex of each was recorded; they were dissected, and the digestive tracts were opened and searched for nematodes. Adult nematodes were collected, cleared with lactophenol and identified microscopically. The identification of

the adult nematode was based on the characteristic features collected from different works (York and Maplestone, 1962; Amato *et al*., 2006; D'Amelio *et al*., 2012; Moravec and Scholz, 2016; Al-Moussawi, 2017). The diagnostic features of the anterior end (cephalic extremity of the nematode) such as the presence of labia, interlabia and interlabial bifurcation in the base were considered for identification. The main identification features in males are the number of spicules, the shape of each spicules tip, the number and distribution of papillae on the tail, number of precloacal and postcloacal papillae. In females, the main identification features are the distance of vulva from the anterior and posterior ends, and the shape of the tail. The identification of the specimens was based on the previously mentioned criteria of *C. rudolphii*, regardless of the morphometric measurements. The identifying of all specimens were conducted by the third author. The morphometric measurements were not considered in this study.

All nematodes were counted, preserved in 70% ethanol (v/v) and kept at room temperature prior DNA extraction; the sex of each nematode was identified under a dissecting microscope. Representative voucher specimens of *C. rudolphii* were deposited in the Iraq Natural History Research Center and Museum, University of Baghdad. From the pool of the adult nematodes removed from the nine infected cormorant specimens (eight males and one female), 20 (eight males and 12 females) adult worms were selected for genetic analyses. The number of analyzed nematodes differed among cormorant individuals. For heavily parasitized hosts, a maximum of five nematodes were selected for genotyping, while all collected nematodes were genotyped for hosts with one, two or three parasites. This study protocol was approved by the local ethics committee (Ref.: BEC/1019/0015), in Department of Biology, College of Science, University of Baghdad.



Map (1): Map of study site in the central Iraq, Tarmiyah district, Baghdad province. (The map was processed using ArcGIS 10.3.1 software)

Table (1): Gene Bank accession numbers of the twenty *Contracaecum rudolphii* isolated from nine Cormorants collected in Tarmiyah district, Baghdad province (33.6732° N, 44.3615° E), central Iraq.

Host		Nematodes			
ID	Sex	Number of specimens	GenBank accession number	Sex	Genotype
C1	Female	2	MT308989	Female	G4
			MT308991	Male	G4
C2	Male	1	MT012368	Female	G2
C3	Male	5	MT308990	Female	G4
			MT308999	Female	G4
			MT012369	Male	G3
			MT308994	Male	G4
			MT308998	Male	G4
C4	Male	3	MT308997	Female	G4
			MT309003	Female	G4
			MT308993	Male	G4
C5	Male	1	MT309002	Female	G4
C6	Male	2	MT308996	Female	G4
			MT308992	Male	G4
C7	Male	2	MT309000	Female	G4
			MT308995	Male	G4
C8	Male	2	MT308988	Female	G4
			MT309001	Male	G4
C9	Male	2	MT012366	Female	G4
			MT012367	Female	G1

DNA extraction and amplification

Total genomic DNA was extracted using ABIOpure TM Total DNA kit (ABIOpure, USA) following manufacturer's instructions. A thermal cycler was used to amplify segments of ITS-1 from each specimen by using the conventional PCR. The forward primer 5`-GGCTTATGGCTTGCTGTGTG-3` and reverse primer 5`-CGCCCGCATATCCAAGAATG-3`, were used. The primers were designed by using Pick primer tool found within NCBI GenBank. Many genes were used as reference genes for these primers, the NCBI GenBank accession numbers for these reference genes are AJ634783.1, MN557377.1, MN557376.1, MK424808.1, MK424807.1, MK424806.1, MK424801.1, MK424800.1, MK161411.1, MK161410.1, MK161409.1, MK161408.1, JQ071409.1, JQ071406.1, JQ071404.1, JQ071398.1, JQ071395.1, JQ071394.1, JQ071393.1, JF424597.1, FM210432.1, FM210251.1, FM177542.1, FJ467620.1, FJ467618.1, DQ316968.1, AY821753.1, AY821752.1 and AY821751.1. The primer melting temperature (Tm) for forward primer is 59.83^{o}C, while the Tm for reverse primer is 59.48^{o}C.

Thirty cycles of amplification in an Eppendorf thermocycler were following the program of denaturation at 95^{o}C for 30 second, annealing at 65^{o}C for 30 second, and extension at 72^{o}C for 30 second. An initial denaturation step consisting of incubation at 95^{o}C for 5 min and a final extension step consisting of incubation at 72^{o}C for 7 min were also added. After PCR

amplification, all the samples were separated on 1% agarose. Gels were run using OWL Electrophoresis System (thermos, USA), in 1X TBE buffer (Tris-acetate EDTA); samples of DNA were mixed with 1/10 volume of loading buffer and loaded into the wells on the gel. TBE was added to cover the gel and run for 75 min at 100 v/m Amp. The gel was stained with ethidium bromide 1 µl/ml. DNA bands were visualized using Gel imaging system (Major Science, Taiwan).

Sequencing and sequence analysis

The PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email and then analyzed using genious software. Nucleotide sequence data reported in this paper are available in the GenBank database under the accession numbers MT012366 to MT012369 and the accession numbers MT308988 to MT309003 (Tab. 1). For each sequence, the NCBI Blast program was used for homology search (http://www.ncbi.nlm.nih.gov/).

Phylogenetic analysis

Phylogenetic tree was constructed using the PhyML program which is hosted at the http://www.phylogeny.fr/simple_phylogeny.cgi website; gene sequences were aligned by MUSCLE alignment (Edgar, 2004) and the curation for the aligned sequences was achieved using Gblocks. Then, the tree was constructed using PhyML program with the application of specific parameters; the model used for constructing the tree was General time-reversible (GTR) and the starting tree was constructed using Bio neighbor-joining (NJ). The tree topology and branch length were adjusted simultaneously using a hill-climbing algorithm and a fast distance-based method was used to draw the trees with modification of the tree to improve its likelihood at each iteration (Guindon *et al*., 2010). A tree in the Newick format, the output of PhyML program, was submitted to FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) for the purpose of visualization and coloring of the strains and the branches. The tree was constructed to analyze the phylogenetic relationship among the twenty sequences of *C. rudolphii* in the present study (Tab. 1), as well as to analyze the phylogenetic relationship of these sequences with additional more sequences of related species with adequate geographic coverage (Tab. 2).

Statistical analyses

The obtained results were stated as percentage and mean ± standard deviation (SD). Data analysis was done by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The data were assessed by chi-square test. The genetic diversity between populations was assessed used ANOVA. P values < 0.05 were considered statistically significant.

RESULTS

Morphological identification

Morphological examinations revealed that all the examined nematodes in the present study had the most typical features of the adult *C. rudolphii*. There were no significant morphological differences of *C. rudolphii* among the individual worms; all were found to share most of the morphological characters with very minor differences among them. Body of the adults of *C. rudolphii* is yellowish, coiled, covered with a striated cuticle and tapering at its two ends. Head small, with three triangular interlabia, which are wider at their base; and three, one dorsal and two sub ventral, round, large and equal in size labia, each of them bear

two oval cephalic papillae on their dorsal sides. The excretory pore locates at the base of interlabium. Lips followed immediately by fine dense annulations of the collar area. Oesophagus is muscular, cylindrical, followed by short globular ventriculus connected to a posterior ventricular appendix. Tail conical and pointed at its end. The male has 2 equal spicules; the shape of the spicule, the groove along spicule length, and the shape of the tip are the characteristic features for this species. Females are larger than males, vulva in the anterior third of the body, tail longer than in males.

PCR ITS-1, Sequence and phylogenetic analyses

The ITS-1 (309 bp) amplicons from individual *C. rudolphii* (n=20) represented single bands on agarose gels. Examination of ITS-1 sequences (309 bp) revealed four unique Genotypes: G1, G2, G3 and G4 (Accession #MT012367, # MT012368, # MT012366 and # MT012367) respectively. The most common genotype is G4 occurred in 17 of the 20 (85%) *C. rudolphii*, and in eight of the nine (88.9%) of *P. carbo*, while the other genotypes (G1, G2 and G3) were found only once (Tab. 2). Out of the seven birds parasitized with mean intensity of more than one, only two (28.6%) were parasitized with individuals having different genotypes. Results also showed that there was an average (±SD) of 1.3 ± 0.48 genotypes per host for the seven cormorants with ≥two analyzed nematodes. The proportion of genotypes did not differ significantly by nematode sex (X^2=3.363, d.f.=3, p=0.33). Co-occurring males and females of *C. rudolphii* were identified in six specimens of *P. carbo*. In two of those cases, more than one genotypes were detected, indicating that at least one of the *C. rudolphii* in these individual hosts was maternally unrelated. Pairwise comparisons of nucleotide sequences among the four genotypes of *C. rudolphii* showed differences of 2.1-3.8% (ITS-1). Comparison among all sequences showed 26 variations in nucleotides. Of these, 17 (65.4%) were single-base substitutions, of which seven (41.2%) were intermediate changes between either the purines (A<->G; n=4) or the pyrimidines (C<->T; n=3) and 10 (58.2%) were transversions (substitutions between a purine and a pyrimidine), although 9 variations (34.6 %) related to the events of deletion / insertion.

The phylogenetic relationship among the 20 isolates of *C. rudolphii* in the present study and additional more sequences of related species (Tab. 3) with adequate geographic coverage were illustrated in Diagram (1) and Table (4). All the sequences of the current study (n=20; genotypes n=4) were distributed in two different populations the first one (Population I) is the cluster of G2 and G4 population (mean of genetic diversity =0.007 ± 0.0013) and the second (Population II) is the cluster of G1 and G3 population (mean of genetic diversity=0.018±0.0089) (Tab. 4). Significant difference was noticed between the means of genetic diversity of these two populations (F= 36.17, P=0). Interestingly, as shown in Diagram (1), the two isolates of the first population (Accession #MT012367 and #MT012369) are closely clustered to *C. septentrionale* (Accession #MK424799) that isolated from night heron *Nycticorax nycticorax* (Linnaeus, 1758) in Thi-Qar, Iraq by Mohammad and Hbaiel (2019 a), while the genotypes present in that population were dispersed, with *C. microcephalum* (Accession #MK424795) that was isolated from *N. nycticorax* in Thi-Qar, Iraq (Mohammad and Hbaiel, 2019 a). Furthermore, the sequences of *Contracaecum* sp. isolated from fish collected from Parishan Lake, Islamic republic of Iran (Shamsi and Aghazadeh-Meshgi, 2011) and Lake Nasser, Egypt (Younis *et al*., 2017) were highly dispersed, and no obvious correlations of close clusters were noticed with the two populations presented in the current study. The Blast analyses (Tab. 5) revealed that sequences of ITS-1

for each G2 and G4 (represented population I) had the highest similarity (97.83%) to ITS-1 sequence of *C. rudolphii* B (Accession# JQ071394 and #AJ634783), while the sequences of ITS-1 for each G1 and G3 (represented population II) had the highest similarity (98.55% and 98.20%) respectively to ITS-1 sequence of *C. rudolphii* A (Accession# JQ071414). Moreover, sequences of ITS-1 for each of G2 and G4 (represented population I) had the less similarity (96.74%) and (96.81%) to ITS-1 sequence of *C. rudolphii* F (Accession# JF424597), and the sequences of ITS-1 for each of G1 and G3 (represented Population II) had the less similarity (96.46% and 96.73%) to ITS-1 sequence of *C. rudolphii* F (Accession# JF424597). The phylogenetic relationship among the twenty isolates of *C. rudolphii* in the present study and additional more published sequences of *C. rudolphii* A, B and F were illustrated in Diagram (2). Results showed that population I (G2 and G4) are highly related to *C. rudolphii* B, while population II (G1 and G3) are highly related to *C. rudolphii* A. The identities and phylogenetic distances of the selected and most related ITS sequences showed that the isolates of *C. rudolphii* analyzed in this study are likely related to two species *C. rudolphii* A and B.

Table (2): Distribution of nuclear genotypes of *C. rudolphii* identified in nine Cormorants from Tarmiyah district, Baghdad province (33.6732° N, 44.3615° E), central Iraq.

Host		Nematodes		Genotypes			
ID	Sex	Analyzed	Sex (M:F)	G1	G2	G3	G4
C1	Female	2	(1:1)	0	0	0	2
C2	Male	1	(0:1)	0	1	0	0
C3	Male	5	(3:2)	0	0	1	4
C4	Male	3	(1:2)	0	0	0	3
C5	Male	1	(0:1)	0	0	0	1
C6	Male	2	(1:1)	0	0	0	2
C7	Male	2	(1:1)	0	0	0	2
C8	Male	2	(1:1)	0	0	0	2
C9	Male	2	(0:2)	1	0	0	1
Total		20	(8:12)	1	1	1	17

Table (3): Host, location and GenBank accession number of sequences of the related species with adequate geographic coverage.

Taxa	Host	Location	GenBank accession number	References
Contracaecum sp.	Barboid fishes	Parishan Lake, the largest freshwater lake in Iran.	FM210435 FM210434 FM210433	Shamsi and Aghazadeh-Meshgi (2011)
Contracaecum sp.	*Oreochromis niloticus* (Linnaeus, 1758); *Tilapia galilaea* (Linnaeus, 1758); *Lates niloticus* (Linnaeus,	Lake Nasser, Egypt	KX580602 KX580603 KX580604 KX580605 KX580606	Younis *et al*. (2017)

	1758); *Synodontis frontosus* Vaillant, 1895*; Mormyrus caschive* Linnaeus, 1758*; Chrysichthys Auratus* (Geoffroy Saint-Hilaire, 1809); *Hydrocynus forskahlii* (Cuvier, 1819); *Alestes Baremose* (Joannis, 1835); and *Labeo niloticus* (Linnaeus, 1758)		KX580607	
C. rocephalum	*Nycticorax nycticorax*	Thi-Qar, Iraq	MK424795	Mohammad and Hbaiel (2019 a)
C. septentrionale	*Nycticorax nycticorax*	Thi-Qar, Iraq	MK424799	Mohammad and Hbaiel (2019 a)

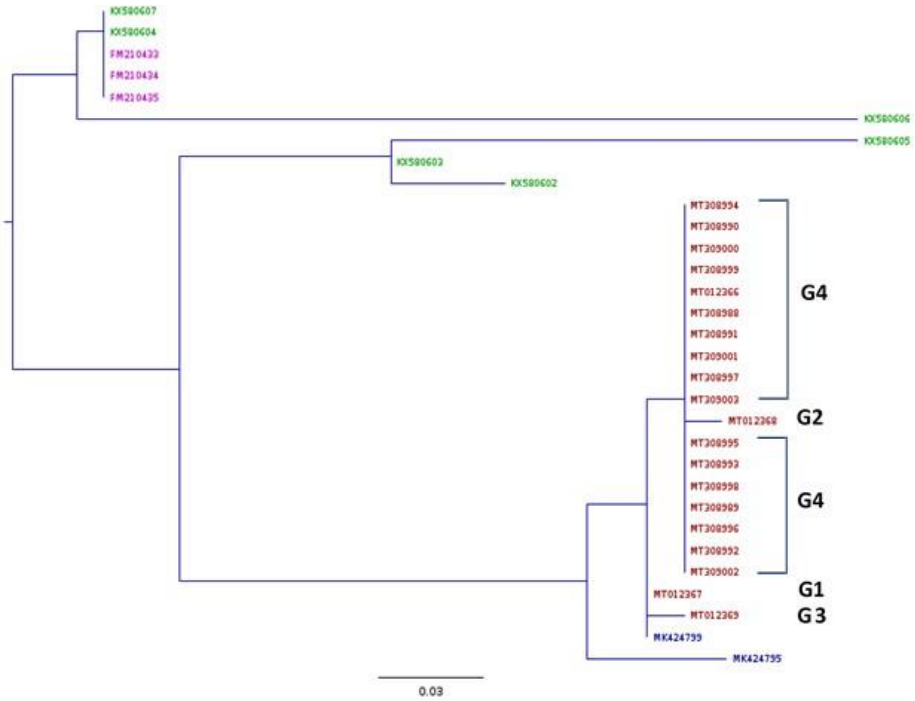


Diagram (1): Phylogenetic tree of the aligned 31 ITS-1 sequences from *Contracaecum* spp. including 20 ITS-1 sequences of *C. rudolphii*, identified in this study using the PhyML program which is hosted at the http://www.phylogeny.fr/simple_phylogeny.cgi website.

Table (4): The two populations of *C. rudolphii* identified in nine Cormorants from Tarmiyah district, Baghdad province (33.6732° N, 44.3615° E), central Iraq.

Population	Geneotypes	GenBank Accession number of the isolates	Mean of genetic diversity (SD)
Population I	G2	MT012368	0.007 (0.0013)
	G4	MT308994 MT308990 MT309000 MT308999 MT012366 MT308988 MT308991 MT309001 MT308997 MT309003 MT308995 MT308993 MT308998 MT308996 MT309002 MT309002 MT308994	
Population II	G1	MT012367	0.018 (0.0089)
	G3	MT012369	

Table (5): The two populations of *C. rudolphii* identified in nine Cormorants from Tarmiyah District (33.6732° N, 44.3615° E), central Iraq and their identity to some *C. rudolphii* isolates from NCBI GenBank.

Genotype (Population)	GenBank Accession Number	Identity
G2 (Population I)	MT012368	97.83% with *C. rudolphii* B (JQ071394
		97.83% with *C. rudolphii* B (AJ634783)
		96.38% with *C. rudolphii* A (JQ071415)
		96.74% with *C. rudolphii* F(JF424597)
G4 (population I)	MT308994	97.87% with *C. rudolphii* B(JQ071394)
		97.83% with *C. rudolphii* B (AJ634783)
		96.45% with *C. rudolphii* A (JQ071415)
		96.81% with *C. rudolphii* F(JF424597)
G1 (population II)	MT012367	98.55% with *C. rudolphii* A(JQ071414)
		97.83% with *C. rudolphii* B (AJ634783)
		96.46% with *C. rudolphii* F(JF424597)

G3 (population II)	MT012367	98.20% with *C. rudolphii* A(JQ071414)
		97.09% with *C. rudolphii* B(JQ071394)
		96.73% with *C. rudolphii* F(JF424597)

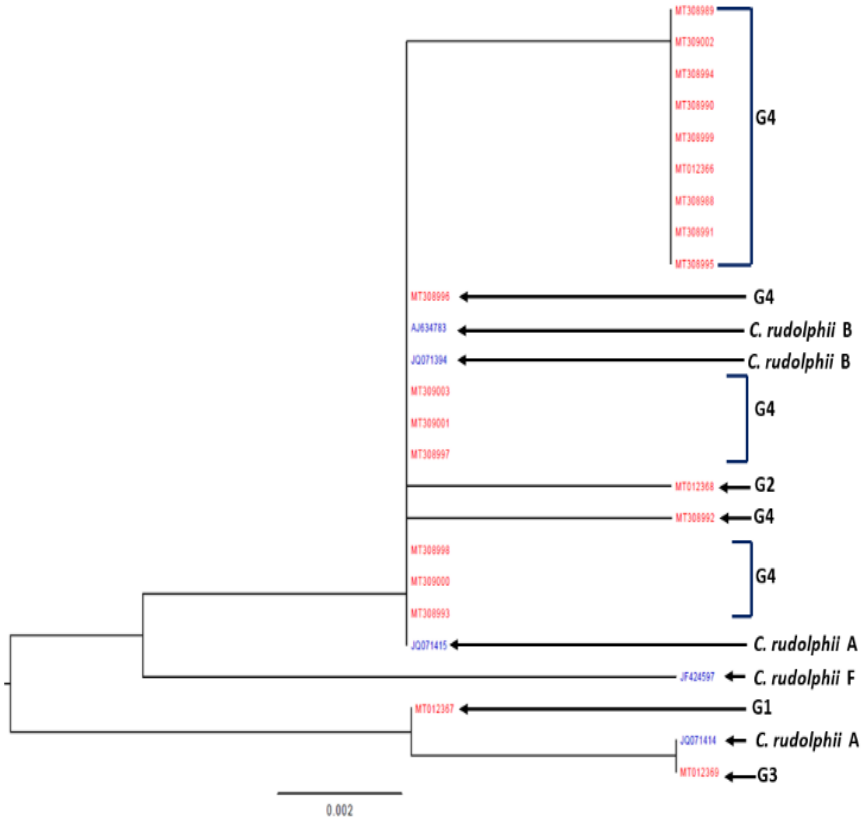


Diagram (2): Phylogenetic tree of the aligned 31 ITS-1 sequences from *Contracaecum* spp. including 20 ITS-1 sequences of *C. rudolphii*, identified in this study and its relation to published sequences of *C. rudolphii* A, B and F using the PhyML program which is hosted at the http://www.phylogeny.fr/simple_phylogeny.cgi website

DISCUSSION

The present investigation is the first study to use a genetic approach for the characterization of *C. rudolphii* from nine infected cormorants in central part of Iraq. Based on morphological characteristics, all nematodes collected from cormorants belong to *C. rudolphii*. The *C. rudolphii* showed morphological and morphometric similarities with the same species parasitizing cormorants in the world (Mattiucci *et al*., 2002; Amato *et al*., 2006; Moravec and Scholz, 2016); however, morphology and morphometric descriptions are not fully considered in this study because all data regarding microscopic identification has been published before (Al-Moussawi and Mohammad, 2011; Al-Moussawi, 2017).

The results obtained from the current study revealed four new different genotypes of *C. rudolphii* that were not previously recorded in Iraq. All recorded genotypes showed high percentages of sequence homology with some published ITS-1 genome of *C. rudolphii*, which means that there were few differences between nucleotide sequences of the isolates analyzed in this study and others. These minor differences may be due to the effects of the geographical location or the difference of the host that harbored the parasites (Cole and Viney, 2018). Assuming the enormously extensive range of hosts that are susceptible to *C. rudolphii* (Torres *et al*., 2000) and the capability of those hosts to spread broadly, the wide spreading of *C. rudolphii* genotypes and the related low population are likely to be probable. The existence of different genotypes of *C. rudolphii* agreed with the results of Shamsi *et al*. (2009) who showed that the *C. rudolphii* isolated from *P. carbo* in Australia revealed the existence of different genotypes. All the sequences of the current study are distributed into two different populations one of them is closely clustered to *C. septentrionale* isolated from *N. nycticorax* in Thi-Qar, Iraq (Mohammad and Hbaiel, 2019 a) while the genotypes presence in this population were dispersed, with *C. microcephalum* isolated from *N. nycticorax* in Thi-Qar, Iraq (Mohammad and Hbaiel, 2019 a).The sequences of *Contracaecum* sp. isolated from fish collected from Parishan Lake (Shamsi and Aghazadeh-Meshgi, 2011) and Lake Nasser (Younis *et al*., 2017) were highly dispersed, and no clear correlations of close clusters were observed with the two populations presented in this current study. The observed close clustered relation and dispersion between the isolates of this study and others are likely to be a function of host differences (bird versus fish) and geographical location.

In this study, some insights have appeared regarding sibling species for the studied population of *C. rudolphii*; the sequences of ITS-1 for the first population had the highest similarity to ITS-1 sequence of *C. rudolphii* B, while the sequences of ITS-1 for the second population had the highest similarity to ITS-1 sequence of *C. rudolphii* A. These results agreed with the results of some previous studies (Mattiucci *et al.*, 2002; Li *et al*., 2005), who represented that *C. rudolphii* isolated from *P. carbo* included two sibling species nominated *C. rudolphii* A and *C. rudolphii* B.

The current study focused on *P. carbo* and nematodes collected from one area (central part of Iraq). Considering that *C. rudolphii* has been identified at both higher and lower prevalence in other parts of the world (Torres *et al*., 2000; Dziekońska-Rynko and Rokicki, 2008, Kanarek, 2011), other population genetic structuring from different hosts or in different locations could be expected. This approach however, has significant limitations because it is time consuming and only few numbers of *C. rudolphii* can be collected for microscopic examination. The ITS-1 study therefore will provide an insight regarding genetic divergence of *C. rudolphii* in Iraq. Moreover, the results are likely to support the hypothesis that *C. rudolphii* represents a complex species at least two sibling species.

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التوصيف الجزيئي للدودة الخيطية *Contracaecum rudolphii* Hartwich, 1964 المعزول من غراب البحر *Phalacrocorax carbo* في العراق

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الخلاصة

تعد الدودة *Contracaecum rudolphii* Hartwich, 1964 واحدة من الديدان الخيطية التي تسبب مخاوف كبيرة على صحة الإنسان والحيوانات البريه. هناك القليل من الدراسات التي تخص هذا الطفيلي من الناحية الوراثية. أجريت الدراسة الحالية من اجل الحصول على فهم أعمق في التباين الوراثي للدودة اعلاه، التي عزلت من غراب البحر *Phalacrocorax carbo*، في المناطق الوسطى من العراق ، حيث تم عزل عشرين عينة من *C. rudolphii*من تسعة أفراد من *P. carbo*.

تم التحري عن الجين (ITS-1) من الدنا الريبوسومي (rDNA) المعزول من *C. rudolphii* بأستخدام تفاعل البلمرة المتسلسل التقليدي (PCR) ثم تم دراسة التسلسل الجيني Gene sequence.اثبتت دراسةالتسلسل الجيني-ITS 1 (rDNA)ظهور أربعة أنماط وراثية فريدة لم يتم تسجيلها سابقًا في العراق. أظهرت الدراسة الحالية أن النمط الجيني الأكثر شيوعًا وقع في 85٪ من *C. rudolphii*، وفي 88.9٪ من طائر الغراب. اضافةً الى ذلك ، كان الاختلاف في الأنماط الجينية مرتفعًا إلى حد ما، بمتوسط 1.3 ± 0.48 من الأنماط الجينية لكل مجموعة من المضائف المصابة باكثر من دودتين.

اظهرت نتائج جميع التسلسلات الجينية في هذه الدراسة ظهور مجتمعين مختلفين؛ اذ اظهر تسلسل ITS-1 للسكان الأول أعلى تشابه مع تسلسل ITS-1 لـ *C. rudolphii* B، بينما اظهرت تسلسلات ITS-1 للسكان الثاني أعلى تشابه مع تسلسل ITS-1 لـ *C. rudolphii* A.

قدمت هذه الدراسة حقائق معمقة حول الاختلاف الوراثي لـ *C. rudolphii* بين عينات النوع *P. carbo* في العراق. كما عززت هذه الدراسة فرضية كون الديدان الخيطية *C. rudolphii* تمثل معقدًا من نوعين منفصلين على الأقل.