Characterization of the Complete Mitochondrial Genome Sequence of *Contracaecum* sp. (Nematoda: Ascarididae) from China

Yuan-Ping Deng (ghost978106510@163.com)
Research Center for Parasites & Vectors, College of Veterinary Medicine,Hunan Agricultural University
Rong Li
Research Center for Parasites & Vectors, College of Veterinary Medicine,Hunan Agricultural University
Hui-Mei Wang
Research Center for Parasites & Vectors, College of Veterinary Medicine,Hunan Agricultural University
Guo-Hua Liu
Research Center for Parasites & Vectors, College of Veterinary Medicine,Hunan Agricultural University
Ya Tu
Beijing Wildlife Rescue and Rehabilitation Center, Beijing

Research Article

Keywords: Contracaecum sp., Contracaecum, mitochondrial genome

Posted Date: April 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1513649/v1

Abstract

Contracaecum spp. is a zooparasitic anisakid nematode and occurs in gastrointestinal tracts of vertebrate/invertebrate animals, humans included, causing gastrointestinal pain, diarrhea, and increasingly severe vomiting. To date, thousands of sequences have been published in GenBank, while complete mitochondrial genomes (mitogenome) of *Contracaecum* spp. are still limited. Although the complete mitogenome of *Contracaecum* species isolated from night herons in Beijing has been reported, the detailed information about it is still puzzling. In the present study, we describe the detailed characteristics within the complete mt DNA *Contracaecum* sp.. The AT-content in the complete mitogenome of *Contracaecum* sp. was 72.2%. The nucleotide diversity (Pi) among genus *Contracaecum* ranged from 0.124 (*cox*1) to 0.181 (*nad*4). Based on the computational algorithms Maximum Likelihood (ML) and Bayesian Inference (BI) within Ascaridoidea and Heterakoidea, the results supported *Contracacum* sp. Beijing isolate was a new species in the genus *Contracaecum*, and supported the family Ascaridiidae was paraphyletic.

Introduction

Contracaecum species were known as globally parasitizing nematodes causing severe pathogenic influences on vertebrate and invertebrate animals, including humans (Shamsi 2019). It has an indirect life cycle and piscivorous birds are its definitive hosts, leading to hemorrhages, necrosis, and severe ulcerative eosinophilic granulomas in intestinal tracts in birds (Zhang et al. 2021). In Australia, *Contracaecum* spp. was the first human anisakidosis detected in the human body with symptoms of gastrointestinal pain, diarrhea, and increasingly severe vomiting, though it was not identified at the species level (Shamsi and Butcher 2011; Shamsi et al. 2019).

In the past decades, over 100 species from the genus *Contracaecum* have been described the morphologies and partial mitochondrial (mt) sequences (Shamsi et al. 2019; Zhang et al. 2021). Some of them could be identified and distinguished by morphological features, while it is difficult to judge if there are cryptic species complexes within *Contracaecum* spp., such as *C. rudolphii*, *C. ogmorhini*, and *C. osculatum*. Studies show that even in cryptic species from the same species, considerable differences within hosts and biographylogy still exist (Shamsi et al. 2009; Timi et al. 2014; Mattiucci et al. 2014; Liu et al. 2016). Herein, it is better to understand more molecular information about *Contracaecum* spp. to proport a new insight to identify potential cryptic species complex, mitogenomes including. Recently, although the complete mitogenome of *Contracaecum* sp., which was collected from black night herons from Beijing, China, has been published (GenBank no. MN892395), the uploaded sequence was not annotated. Yet, the detailed information of the complete mt sequence was still in a puzzle that was inconvenient to cite the mt sequence of *Contracaecum* sp. for further studying.

Therefore, in the present study, we aim to (i) reassemble and annotate the complete mitogenome of *Contracaecum* sp., which was isolated from black herons in Beijing, China, and describe detailed information of the complete mt sequence of *Contracaecum* sp.; (ii) based on uploaded annotated

sequences of Ascaridoidea spp. and Heterakoidea spp., conducting phylogenetic analyses to support Zhang et al. (2021) hypothesis; and (iii) provide more detailed molecular features within genus *Contracaecum* for successive studies.

Method And Material

Parasites and molecular identification

Specimens' helminths were obtained from the digestive tracts of hosts grey and night herons in Beijing Zoo, China. The species were washed with ultrapure water and physiological saline solution, then fixed and collected into 75% ethanol (v/v) until further studying. The species were preliminarily identified as *Contracaecum* roundworms based on hosts and primary characteristic morphology (Zhang et al. 2021). For additional examination of molecules, the total genomic DNA of several species was extracted using QIAamp® DNA Micro Kit as the instruction recommended. The identity of these roundworms was confirmed based on polymerase chain reaction (PCR) amplification of partial *cox*1 (with primers JB3-JB4.5) (Bowles et al. 1992; Bowles and McManus 1994) and ITS (with primers NC5-NC2) (Newton et al. 1998; Chilton et al. 2001). The obtained ITS sequence totally matched with that of *Contracaecum* sp. (GenBank no. MW538933 ~ 36). And partial *cox*1 sequence showed 99.7% identity with *Porrocaecum reticulatum* (GenBank no. MF113244).

Sequencing, assembling, and annotation

The genomic DNA sample was fragmented to a size of 350 bp. The DNA libraries were sequenced for high throughput sequencing (HTS) on Illumina Hiseq 6000 platform (Novogene Co. Ltd. Tianjin, China) and 250 bp paired-end reads were generated. The raw data then were obtained and recorded in FASTQ format. Then, the reads with low-quality bases (Phred quality < 5) or uncertain reads with repetitive "N" bases were discarded to acquire clear data. The partial *cox*1 sequence was used as the initial reference to assemble complete sequences of *Contracaecum* sp. using Geneious Prime 2022.0.1 (Kearse et al. 2012). The assembly was operated with parameters as follows: (i) minimum overlap within the ranges of 150–200 bp; (ii) minimum overlap identity among 98% − 100%; and (iii) maximum gap of 5 bp. The completely circular mt genome of roundworm was then got verification by long PCR with primers as listed (**Table S1**, Fig. 1).

All 12 protein-coding regions were preliminarily identified the start and stop codons by ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) in NCBI. Later, two ribosomal RNAs (rRNAs, *rrn*L and *rrn*S) were framed using the Tandem repeats finder (Benson 1999). The 12 genes were then further confirmed by identifying similar sequences with former published Ascarididae (*Ascaris lumbricoides*, GenBank no. NC_016198). The tRNAscan-SE 2.0 (Chan et al. 2021) with cutoff score of 1.0, and MITOs (Bernt et al. 2013) were applied to search 22 potential transfer RNAs (tRNAs).

Nucleotide variation in mtDNA genomes among Contracaecum spp.

Based on available mitogenome sequences of genus *Contracaecum* in NCBI, pairwise alignment of 12 protein genes was conducted in Clustal X1.83 to compute nucleotide diversity. The complete protein alignments from the genus *Contracaecum*, *C. osculatum*, *C. rudolphii*, *C. ogmorhini*, and *Contracaecum* sp. in the present study, were analyzed by DnaSP v5 using sliding windows (Librado and Rozas 2009). The parameters of the sliding window were followed with 300 bp window length and a default 25 bp step site to calculate the nucleotide diversity (Pi or π). Each boundary of protein genes was identified due to mid-point position, and we then graphed nucleotide diversity for 12 protein genes from *Contracaecum*.

Phylogenetic analysis

Total of 41 mt sequences of nematodes, from families Ascaridoidea and Heterakoidea, were applied to analyze phylogeny with outgroups *Enterobius vermicularis* (GenBank accession no. EU281143) and *Wellcomia siamensis* (GenBank accession no. NC_016129) (**Table S2**). Each amino acid sequence was aligned using MAFFT computational algorithm (Katoh et al. 2019). The aligned sequences were then concatenated to obtain a single alignment dataset. The ambiguous gaps in the alignment were excluded by Gblocks 0.91b with default parameters “less stringent” (Dereeper et al. 2008). Computational algorithm Maximum likelihood (ML) (Guindon et al. 2010) was conducted to perform a phylogenetic tree with the best model “JTT + I + G + F” screened by ProtTest 3.4.2 (Darriba et al. 2011) and 1,000 replicates. Bayesian analysis was operated with MrBayes 3.2 (Ronquist et al. 2012) and “GTR + F + G” was selected as the best suitable model by ModelFinder in IQTree v.2.1.3 (Kalyaanamoorthy et al. 2017). Four Markov chains were progressed with 1,000,000 MCMC generations, with sampling analysis tree every 100 generations. The residual trees were calculated with Bayesian posterior probabilities (BPP), burning first 250 trees.

Results And Discussion

Mitogenome organization and composition

The filtered dataset of the sequenced mt genome of *Contracaecum* sp. is nearly 2Gb with a total of 8,677,194 x 2 clean reads for further assembling. The circular mt genome of *Contracaecum* sp. (GenBank accession: WM056322) assembled was 14,082 bp in size, shorter than Zhang et al. (2021) published, with 12 PCGs, 22 tRNAs, 2 rRNAs, and two non-coding regions (NCRs) (Table 1, Fig. 2). Total of 36 genes were transcribed in the forward direction and gene arrangement was recognized into the typical GA3 pattern which is mostly observed in roundworms (Liu et al. 2013a). Consistent with previous reports, there was a tendency of T base (48.7%) companying high A-T bases biased (71.2%). Total ten intergenic regions among the complete mt genome of *Contracaecum* sp. ranging from 1 bp to 16 bp (Table 1). One short (122 bp) was located between nad4 and *cox*1, and one long non-coding region (691 bp) was placed in tRNA-Ser_2 and tRNA-Asn. The values of AT-skew were negative from − 0.475 (*nad*6) to -0.111 (NCRs), inversely, the values of GC- skew were positive with scope 0.226 (*nad*4) – 0.674 (*nad*3), suggesting Ts and Gs were more frequently used in the genome.

Table 1

The organization of the complete mt genome of *Contracaecum* sp. from Beijing, China.

Gene/Region	Strand	Positions	Size (bp)	Number of aa[a]	Ini/Ter codons	Anticodons	In
tRNA-Asn (N)	H	1-60	60			GTT	0
tRNA-Tyr (Y)	H	61-116	56			GTA	0
*nad*1	H	117-989	873	290	TTG/TAG		0
*atp*6	H	993-1591	599	199	ATT/TA		+3
tRNA-Lys (K)	H	1592-1653	62			TTT	0
tRNA-Leu2 (L_2)	H	1654-1708	55			TAA	0
tRNA-Ser1 (S_1)	H	1709-1759	51			TCT	0
*nad*2	H	1760-2605	846	281	TTG/TAA		0
tRNA-Ile (I)	H	2619-2678	60			GAT	+13
tRNA-Arg (R)	H	2679-2732	54			GCG	0
tRNA-Gln (Q)	H	2733-2787	55			TTG	0
tRNA-Phe (F)	H	2788-2846	59			GAA	0
*cyt*b	H	2847-3953	1107	368	TTG/TAA		0
tRNA-Leu1 (L_1)	H	3961-4017	57			TAG	+7
*cox*3	H	4018-4782	766	255	TTG/T		0
tRNA-Thr (T)	H	4783-4843	60			TGT	0
*nad*4	H	4844-6073	1230	409	TTG/TAA		0
Intergenic region	H	6074-6195	122				0
*cox*1	H	6196-7771	1576	525	TTG/T		0
tRNA-Cys (C)	H	7772-7829	58			GCA	0
tRNA-Met (M)	H	7831-7890	60			CAT	+1
tRNA-Asp (D)	H	7907-7963	57			GTC	+16
tRNA-Gly (G)	H	7965-8021	57			TCC	+1
*cox*2	H	8022-8713	692	230	TTG/TA		0
tRNA-His (H)	H	8714-8778	65			GTG	0

*rrn*L	H	8779-9737	959				0
*nad*3	H	9738-10073	336	111	TTG/TAG		0
*nad*5	H	10077-11659	1583	527	ATT/TA		+3
tRNA-Ala (A)	H	11660-11716	57			TGC	0
tRNA-Pro (P)	H	11724-11780	57			TGG	+7
tRNA-Val (V)	H	11781-11837	57			TAC	0
*nad*6	H	11838-12272	435	144	TTG/TAA		0
*nad*4L	H	12275-12505	231	76	ATT/TAA		+2
tRNA-Trp (W)	H	12506-12563	58			TCA	0
tRNA-Glu (E)	H	12565-12624	60			TTC	+1
*rrn*S	H	12625-13335	711				0
tRNA-Ser2 (S_2)	H	13336-13391	56			TGA	0
Non-coding region	H	13392-14082	691				0
In: Intergenic nucleotides.							
[a]The inferred length of amino acid (aa) sequence of 13 protein-coding genes; Ini/Ter codons: initiation and termination codons.							

Protein-coding genes

TTG was the most common initial codon in this study, followed by ATT. TTG was used as the start codon for nine genes (*cox*1-3, *cyt*b, *nad*1-4, and *nad*6), excluding genes *atp*6, *nad*4L, and *nad*5 (Table 1). The rest three PCGs used ATT as the initial codon. Generally, TAG and TAA were shared as common stop codons in metazoan (Hu et al. 2004). In this study, TAA was the most frequent termination among *nad*6, *nad*4L, *nad*4, *cyt*b, and *nad*2. the genes *nad*1 and *nad*3 used TAG as the stop codon. The rest genes respectively used incomplete stop codons T (*cox*1 and *cox*3) or TA (*atp*6, *cox*2, and *nad*5).

A sum of 3422 amino acids was translated by 12 PCGs. TTT (480) was the most common codon used in encoding with Phe. Followed by codons GTT (Val), TTG (Leu), and ATT (Ile), the amounts were 219, 216, and 214 (Table 2), respectively. Leu (519) and Phe (499) were the most frequently coded amino acids, while Arg (34) was the rarest. There was a tendency of Gs and Ts in the same amino acid by comparing the relative synonymous codon usage (RSCU) (Table 2). The AT content of 12 protein genes ranged from 66.7% (*cox*1) to 78.9% (*nad*6) (Table 3). There was an obvious use of Ts and Gs, all rates of AT-skews were negative ranging from − 0.475 to -0.111, and whole values for GC-skew were positive from 0.226 to 0.674. Ts were the most used bases in nad6 (-0.475), followed by nad3 (-0.464) and nad2 (-0.451). Similarly, Gs were the most used bases in nad3 (0.674), nad4L (0.569) and atp6 (0.526).

Table 2
Amino acids frequency of *Contracaecum* sp. mitochondrial protein-coding genes.

Amino acid	Codon	Number	RSCU (%)	Amino acid	Codon	Number	RSCU (%)
Phe	TTT	480	1.92	Tyr	TAT	154	1.84
Phe	TTC	19	0.08	Tyr	TAC	13	0.16
Leu	TTA	199	2.3	Stop	TAA	5	1.43
Leu	TTG	216	2.5	Stop	TAG	2	0.57
Leu	CTT	76	0.88	His	CAT	54	1.86
Leu	CTC	2	0.02	His	CAC	4	0.14
Leu	CTA	10	0.12	Gln	CAA	20	0.98
Leu	CTG	16	0.18	Gln	CAG	21	1.02
Ile	ATT	214	1.92	Asn	AAT	100	1.79
Ile	ATC	9	0.08	Asn	AAC	12	0.21
Met	ATA	76	0.86	Lys	AAA	35	0.71
Met	ATG	101	1.14	Lys	AAG	63	1.29
Val	GTT	219	2.61	Asp	GAT	62	1.65
Val	GTC	13	0.16	Asp	GAC	13	0.35
Val	GTA	49	0.59	Glu	GAA	32	0.84
Val	GTG	54	0.64	Glu	GAG	44	1.16
Ser	TCT	139	3.08	Cys	TGT	53	1.96
Ser	TCC	6	0.13	Cys	TGC	1	0.04
Ser	TCA	14	0.31	Trp	TGA	21	0.57
Ser	TCG	5	0.11	Trp	TGG	53	1.43
Pro	CCT	66	3.11	Arg	CGT	33	3.88
Pro	CCC	7	0.33	Arg	CGC	1	0.12
Pro	CCA	9	0.42	Arg	CGA	0	0
Pro	CCG	3	0.14	Arg	CGG	0	0
Thr	ACT	89	3.24	Ser	AGT	121	2.68
Excluding abbreviated stop codons (TA and T).							
Stop = Stop codon.							

Amino acid	Codon	Number	RSCU (%)	Amino acid	Codon	Number	RSCU (%)
Thr	ACC	6	0.22	Ser	AGC	2	0.04
Thr	ACA	9	0.33	Ser	AGA	36	0.8
Thr	ACG	6	0.22	Ser	AGG	38	0.84
Ala	GCT	72	2.5	Gly	GGT	112	2.22
Ala	GCC	24	0.83	Gly	GGC	21	0.42
Ala	GCA	11	0.38	Gly	GGA	23	0.46
Ala	GCG	8	0.28	Gly	GGG	46	0.91
Excluding abbreviated stop codons (TA and T).							
Stop = Stop codon.							

Table 3
Nucleotide composition and skews of *Contracaecum* sp. mitochondrial genome.

Gene	Nucleotide frequency				A + T (%)	AT-skew	GC-skew
	A (%)	G (%)	T (%)	C (%)			
*atp*6	22.0	22.0	49.1	6.9	71.1	-0.380	0.526
*cox*1	19.5	21.8	47.2	11.5	66.7	-0.416	0.307
*cox*2	21.2	22.1	46.5	10.1	67.7	-0.373	0.372
*cox*3	18.9	20.9	49.8	10.4	68.7	-0.449	0.333
*cyt*b	19.7	22.0	47.6	10.7	67.3	-0.415	0.343
*nad*1	19.5	20.5	50.5	9.5	70.0	-0.444	0.364
*nad*2	20.7	18.2	54.6	6.5	75.3	-0.451	0.474
*nad*3	20.0	21.4	54.4	4.2	74.4	-0.464	0.674
*nad*4	21.4	17.0	50.9	10.7	72.3	-0.408	0.226
*nad*4L	22.9	17.3	55.0	4.8	77.9	-0.411	0.569
*nad*5	21.2	18.8	51.9	8.1	73.1	-0.420	0.398
*nad*6	20.7	13.3	58.2	7.8	78.9	-0.475	0.261
*rrn*S	30.2	19.7	40.4	9.7	70.6	-0.143	0.340
*rrn*L	27.3	17.5	48.3	6.9	75.6	-0.277	0.436
22 tRNA	31.5	18.7	40.8	9.0	72.3	-0.129	0.352
NCR	37.4	10.3	46.7	5.6	84.1	-0.111	0.290
Total	23.5	19.0	48.7	8.9	72.2	-0.350	0.364

Transfer RNA genes, ribosomal RNA genes and non-coding region

The length of 22 tRNAs ranged from 51 bp (tRNA-Ser_1) to 65 bp (tRNA-His). As one of the most conserved and amplest RNA, the secondary structure of a typical cloverleaf consisted of one acceptor stem, a dihydrouridine loop (D-loop), an anticodon loop, a TΨC loop, and related arms fixing with them (Su et al. 2020). However, in nematodes, most tRNAs were different from other metazoan animals. In our study, 16 of 20 tRNAs (excluding tRNA-Ser_1 and tRNA-Ser_2) lacked a TΨC loop, replaced by several nucleotide residues which compromised the TV-replacement loop (Hu et al. 2004). The tRNAs tRNA-His, tRNA-Ile, and tRNA-Met were observed in a relatively standard cloverleaf structure with a TΨC loop, though the latter two (tRNA-Ile and tRNA-Met) lacked DHU-stem. The tRNA-Ser1 and tRNA-Ser2 were similar to previous

reports with one TΨC-loop but lacked D-loop (Su et al. 2020), while tRNA-Lys had a TΨC-arm with a short of TΨC-loop.

Ribosomal RNAs of *Contracaecum* sp. were fixed as GA3 pattern. The *rrn*L was located between tRNA-His and *nad*3 with a size of 959 bp, the rrnS gene was located between tRNA-Glu and tRNA-Ser_2 with a size of 711 bp (Table 1). The content of A + T for *rrn*L and *rrn*S were 75.6% and 70.6%, respectively. There were two NCRs among the mt genome of *Contracaecum* sp.. One short region was placed in *nad*4 and *cox*1 with a length of 122 bp, and the long region was situated between tRNA-Ser_2 and tRNA-Asn with a length of 691 bp.

Nucleotide variation of genus Contracaecum

Based on aligned nucleotide sequences among species *C. osculatum*, *C. rudolphii*, *C. ogmorhini*, and *Contracaecum* sp., nucleotide diversities (Pi) were calculated based on the sliding window. The values of Pi were ranged from 0.124 to 0.181 by analyzing window 300 bp and default step 25 bp (Fig. 3). The most variable genes were *cyt*b (0.178), *nad*2 (0.181), *nad*4 (0.179) and *nad*6 (0.172), and the most conserved genes were *cox*1 (0.124) and *cox*2 (0.130) in *Contracaecum* (Fig. 3). Protein genes *cox*1 and *cox*2 seemed to be the most stable genes in *Contracaecum* nematodes with the least variation, which could be used as molecular markers to identify species from *Contracaecum*. Results also supported that *nad*2 and *nad*4 could act as alternative markers among nematodes isolated from different environments.

Phylogenetic analyses

The present phylogenetic tree is based on the 12 PCGs of 41 available mt sequences from the superfamily Ascaridoidea and Heterakoidea (**Table S2**). Two phylogenetic trees, both BI and ML, had similar topologies, excluding species within the superfamily Heterakoidea. The topologies of ML and BI phylogenetic trees were highly similar to previous studies (Liu et al. 2016; Zhang et al. 2021; Zhao et al. 2021). The present sample formed a branch with *Contracaecum* nematodes, indicating a closer relationship within the genus *Contracaecum* with strong support (Fig. 4), but a distinct distance from species that had been reported. According to the structure of phylogenetic trees, results supported previous reports that superfamily Ascaridoidea and Heterakoidea were monophyly, and families (including Ascarididae, Anisakidae, Heterocheiidae, Toxocaridae, and Cucullanidae) within it were all monophyletic (Li et al. 2018; Zhao et al. 2021).

For ML and BI analyses, two trees had identical topologies within the superfamily Ascaridoidea. Among the family Ascarididae, the genera *Ascaris*, *Baylisascaris*, *Toxascaris*, and *Parascaris* had a closer relationship than *Ophidascaris* similarly to Zhou et al. (2021) reported. According to morphological descriptions of the genus *Ophidascaris*, *Ophidascaris* had been classified as a member genus of the superfamily Ascaridoidea (Pinto et al. 2010), and based on phylogenetic analyses, *Ophidascaris* more related to the family Ascaridae. While compared with other genera in Ascaridae, there was a relatively long evolutionary distance in Ascaridae. A previous study presented that the family Ascarididae was more related to Toxocaridae (Zhou et al. 2021). In this study, the family Ascarididae was closer taxa to

Anisakidae than Toxocaridae (Fig. 4). In addition, all five families and all eleven genera within the superfamily Ascaridoidea were monophyletic with strong support (Bpp = 1, Bf > 70, Fig. 4), verifying the correctness of former studies (Liu et al. 2016; Zhao et al. 2018).

In ML analysis, present findings showed strong statistical support (Bf = 100) that *Ascaridia galli* and *Heterakis* species were sister taxa, similar to former studies report (Liu et al. 2016). Similar to Liu et al. (2013b) reported *Ascaridia columbae* was more related to *Ascaridia* sp. than *A. galli*. However, especially in the BI analyzing tree, species *A. galli* formed a separate genus which created sister relationships with genera *Heterakis* and *Ascaridia* with strong support (Bpp = 1). The results proposed the hypothesis that Heterakidae had a closer relationship with Ascaridiidae, and the family Ascaridiidae was paraphyly within Heterakoidea. However, due to limited molecular data among Heterakoidea, it is better to catch more mt sequences to verify the relationship within this superfamily.

Conclusion

Based on analyses of molecular statistics for *Contracaecum* species, the present study showed detailed mitochondrial characteristics of the complete mitogenome of *Contracaecum* sp., and nucleotide diversity among the published *Contracaecum* species. Additionally, based on the nucleotide diversity, results emphasized that conservative *cox*1 and *cox*2 seem to be better markers in species identifications. In accordance with previous reports, *Contracaecum* sp. isolated from Beijing might be one new species of the genus *Contracaecum* based on the phylogenetic analysis, further verifying the hypotheses Zhang et al. (2021) supported before (Zhang et al. 2021). Additionally, consistent with Liu et al. (2016) supported that the families Heterakidae and Ascaridiidae were related, all genera and families cited in the present study were monophyly, excluding genus *Ascaridia* and family Ascaridiidae.

Declarations

Author's contributions

G-HL and Y-PD conceived and designed the study, and critically revised the manuscript. Y-T provided the sample worms and provided initial identification. Y-PD performed the experiments and analyzed the data. G-HL and Y-PD drafted the manuscript. R-L and H-MW helped in study design, study implementation, and manuscript preparation. All authors read and approved the final manuscript.

Funding

This study was supported by the Training Programme for Excellent Young Innovators of Changsha (Grant No. KQ2106044).

Declaration of Competing Interest

The authors declare that there are no competing interests.

Availability of data and material

The datasets generated and analyzed during the current study are available in GenBank.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580. https://doi.org/10.1093/nar/27.2.573
2. Bernt M, Donath A, Jühling F, et al (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol 69:313–319. https://doi.org/10.1016/j.ympev.2012.08.023
3. Bowles J, Blair D, McManus DP (1992) Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Mol Biochem Parasitol 54:165–173. https://doi.org/10.1016/0166-6851(92)90109-w
4. Bowles J, McManus DP (1994) Genetic characterization of the Asian Taenia, a newly described taeniid cestode of humans. Am J Trop Med Hyg 50:33–44
5. Chan PP, Lin BY, Mak AJ, Lowe TM (2021) tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. Nucleic Acids Res 49:9077–9096. https://doi.org/10.1093/nar/gkab688
6. Chilton NB, Newton LA, Beveridge I, Gasser RB (2001) Evolutionary relationships of trichostrongyloid nematodes (Strongylida) inferred from ribosomal DNA sequence data. Mol Phylogenet Evol 19:367–386. https://doi.org/10.1006/mpev.2001.0938
7. Darriba D, Taboada GL, Doallo R, Posada D (2011) ProtTest 3: fast selection of best-fit models of protein evolution. Bioinforma Oxf Engl 27:1164–1165. https://doi.org/10.1093/bioinformatics/btr088
8. Dereeper A, Guignon V, Blanc G, et al (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36:W465-469. https://doi.org/10.1093/nar/gkn180
9. Guindon S, Dufayard J-F, Lefort V, et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010
10. Hu M, Chilton NB, Gasser RB (2004) The mitochondrial genomics of parasitic nematodes of socio-economic importance: recent progress, and implications for population genetics and systematics. Adv Parasitol 56:133–212. https://doi.org/10.1016/s0065-308x(03)56003-1

11. Kalyaanamoorthy S, Minh BQ, Wong TKF, et al (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285
12. Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166. https://doi.org/10.1093/bib/bbx108
13. Kearse M, Moir R, Wilson A, et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinforma Oxf Engl 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199
14. Li L, Lü L, Nadler SA, et al (2018) Molecular Phylogeny and Dating Reveal a Terrestrial Origin in the Early Carboniferous for Ascaridoid Nematodes. Syst Biol 67:888–900. https://doi.org/10.1093/sysbio/syy018
15. Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinforma Oxf Engl 25:1451–1452. https://doi.org/10.1093/bioinformatics/btp187
16. Liu G-H, Nadler SA, Liu S-S, et al (2016) Mitochondrial Phylogenomics yields Strongly Supported Hypotheses for Ascaridomorph Nematodes. Sci Rep 6:39248. https://doi.org/10.1038/srep39248
17. Liu G-H, Shao R, Li J-Y, et al (2013a) The complete mitochondrial genomes of three parasitic nematodes of birds: a unique gene order and insights into nematode phylogeny. BMC Genomics 14:414. https://doi.org/10.1186/1471-2164-14-414
18. Liu G-H, Shao R, Li J-Y, et al (2013b) The complete mitochondrial genomes of three parasitic nematodes of birds: a unique gene order and insights into nematode phylogeny. BMC Genomics 14:414. https://doi.org/10.1186/1471-2164-14-414
19. Mattiucci S, Cipriani P, Webb SC, et al (2014) Genetic and morphological approaches distinguish the three sibling species of the Anisakis simplex species complex, with a species designation as Anisakis berlandi n. sp. for A. simplex sp. C (Nematoda: Anisakidae). J Parasitol 100:199–214. https://doi.org/10.1645/12-120.1
20. Newton LA, Chilton NB, Beveridge I, et al (1998) Genetic markers for strongylid nematodes of livestock defined by PCR-based restriction analysis of spacer rDNA. Acta Trop 69:1–15. https://doi.org/10.1016/s0001-706x(97)00105-8
21. Pinto RM, Pereira LC, Panizzutti M, Noronha D (2010) Nematode parasites of the Neotropical Rattlesnake, Crotalus Durissus Linnaeus, 1758 (Ophidia, Viperidae) from Brazil: new records and general aspects. undefined
22. Ronquist F, Teslenko M, van der Mark P, et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029
23. Shamsi S (2019) Parasite loss or parasite gain? Story of *Contracaecum* nematodes in antipodean waters. Parasite Epidemiol Control 4:e00087. https://doi.org/10.1016/j.parepi.2019.e00087
24. Shamsi S, Butcher AR (2011) First report of human anisakidosis in Australia. Med J Aust 194:199–200. https://doi.org/10.5694/j.1326-5377.2011.tb03772.x

25. Shamsi S, Norman R, Gasser R, Beveridge I (2009) Genetic and morphological evidences for the existence of sibling species within *Contracaecum* rudolphii (Hartwich, 1964) (Nematoda: Anisakidae) in Australia. Parasitol Res 105:529–538. https://doi.org/10.1007/s00436-009-1424-y
26. Shamsi S, Stoddart A, Smales L, Wassens S (2019) Occurrence of *Contracaecum* bancrofti larvae in fish in the Murray-Darling Basin. J Helminthol 93:574–579. https://doi.org/10.1017/S0022149X1800055X
27. Su Z, Wilson B, Kumar P, Dutta A (2020) Noncanonical Roles of tRNAs: tRNA Fragments and Beyond. Annu Rev Genet 54:47–69. https://doi.org/10.1146/annurev-genet-022620-101840
28. Timi JT, Paoletti M, Cimmaruta R, et al (2014) Molecular identification, morphological characterization and new insights into the ecology of larval Pseudoterranova cattani in fishes from the Argentine coast with its differentiation from the Antarctic species, P. decipiens sp. E (Nematoda: Anisakidae). Vet Parasitol 199:59–72. https://doi.org/10.1016/j.vetpar.2013.09.033
29. Zhang Q, Meng M, Huang C, et al (2021) Identification and genetic characterization of *Contracaecum* sp. (Nematoda: Anisakidae) from China. Integr Zool 16:929–938. https://doi.org/10.1111/1749-4877.12465
30. Zhao JH, Tu GJ, Wu XB, Li CP (2018) Characterization of the complete mitochondrial genome of Ortleppascaris sinensis (Nematoda: Heterocheilidae) and comparative mitogenomic analysis of eighteen Ascaridida nematodes. J Helminthol 92:369–378. https://doi.org/10.1017/S0022149X17000542
31. Zhao Q, Abuzeid AMI, He L, et al (2021) The mitochondrial genome sequence analysis of Ophidascaris baylisi from the Burmese python (Python molurus bivittatus). Parasitol Int 85:102434. https://doi.org/10.1016/j.parint.2021.102434
32. Zhou C-Y, Ma J, Tang Q-W, et al (2021) The mitogenome of Ophidascaris wangi isolated from snakes in China. Parasitol Res 120:1677–1686. https://doi.org/10.1007/s00436-021-07069-z

Figures

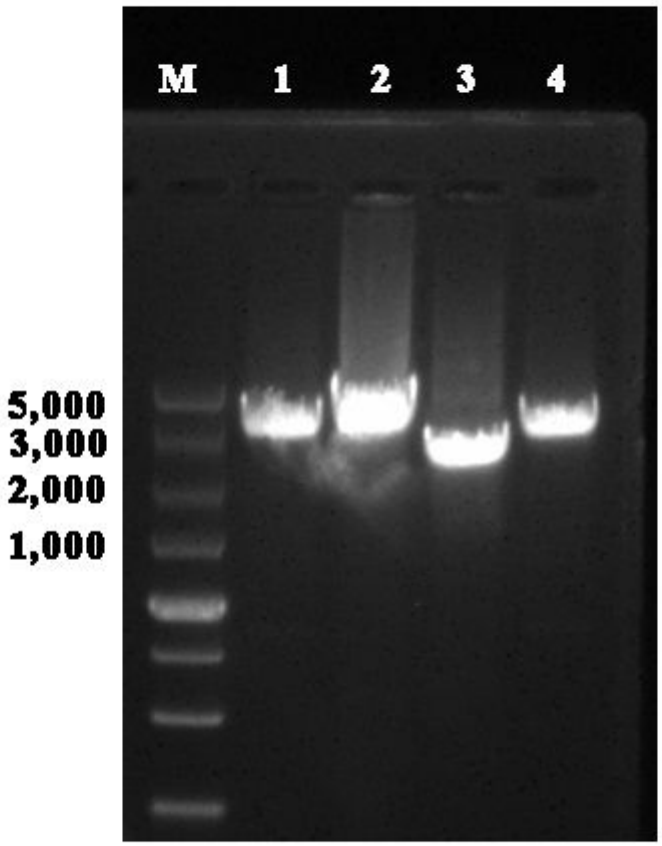

Figure 1

PCR amplicons from the mitochondrial genome of *Contracaecum* sp.. M: DL5,000 DNA marker; 1: Validation_01; 2: Validation_02; 3: Validation_03; 4:Validation_04.

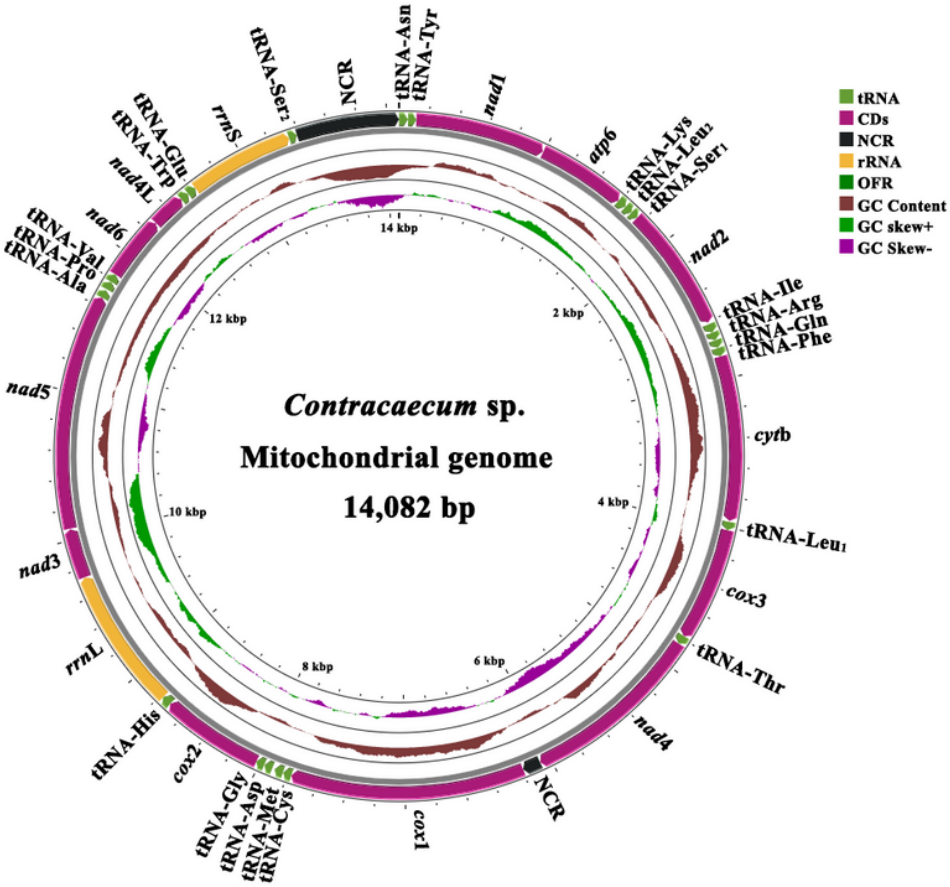

Figure 2

The organization of the complete mitochondrial genome sequence of *Contracaecum* sp.

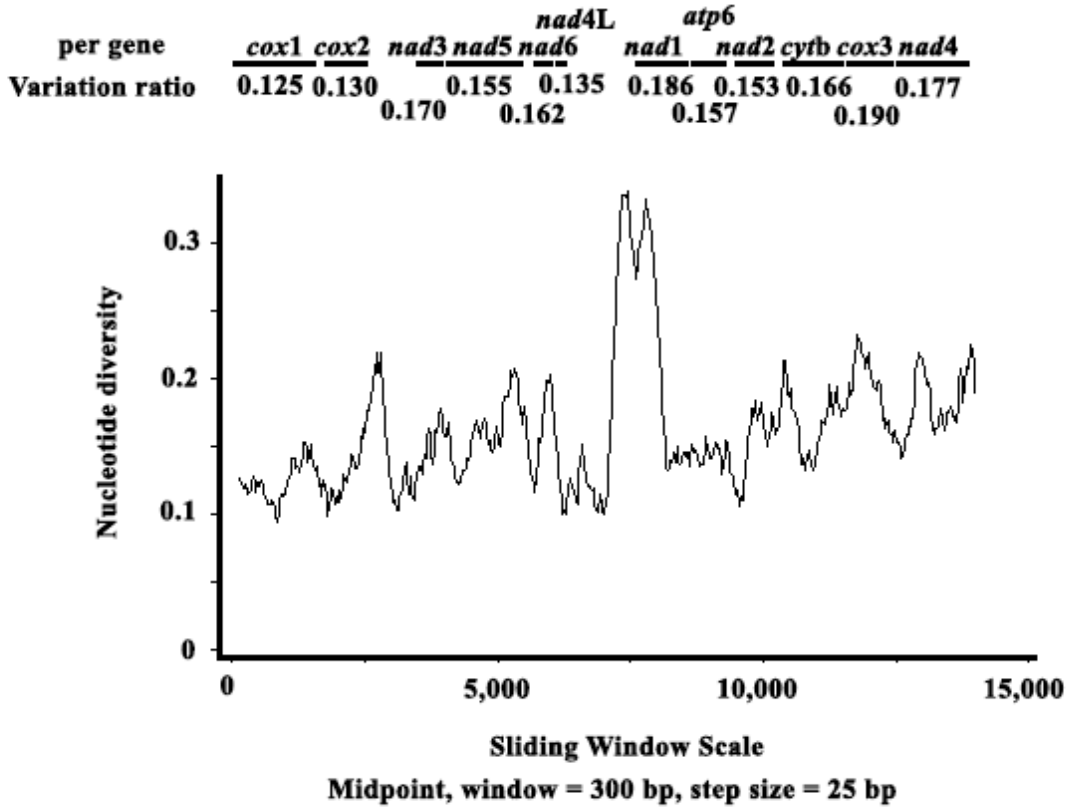

Figure 3

Sliding window analysis of the alignment of complete mtDNAs of available *Contracaecum* spp.. The black line shows the value of nucleotide diversity Pi (π) in a sliding window analysis of window size 300 bp with step size 25 bp, and the value is inserted at its mid-point. Gene boundaries are indicated with a variation ratio per gene.

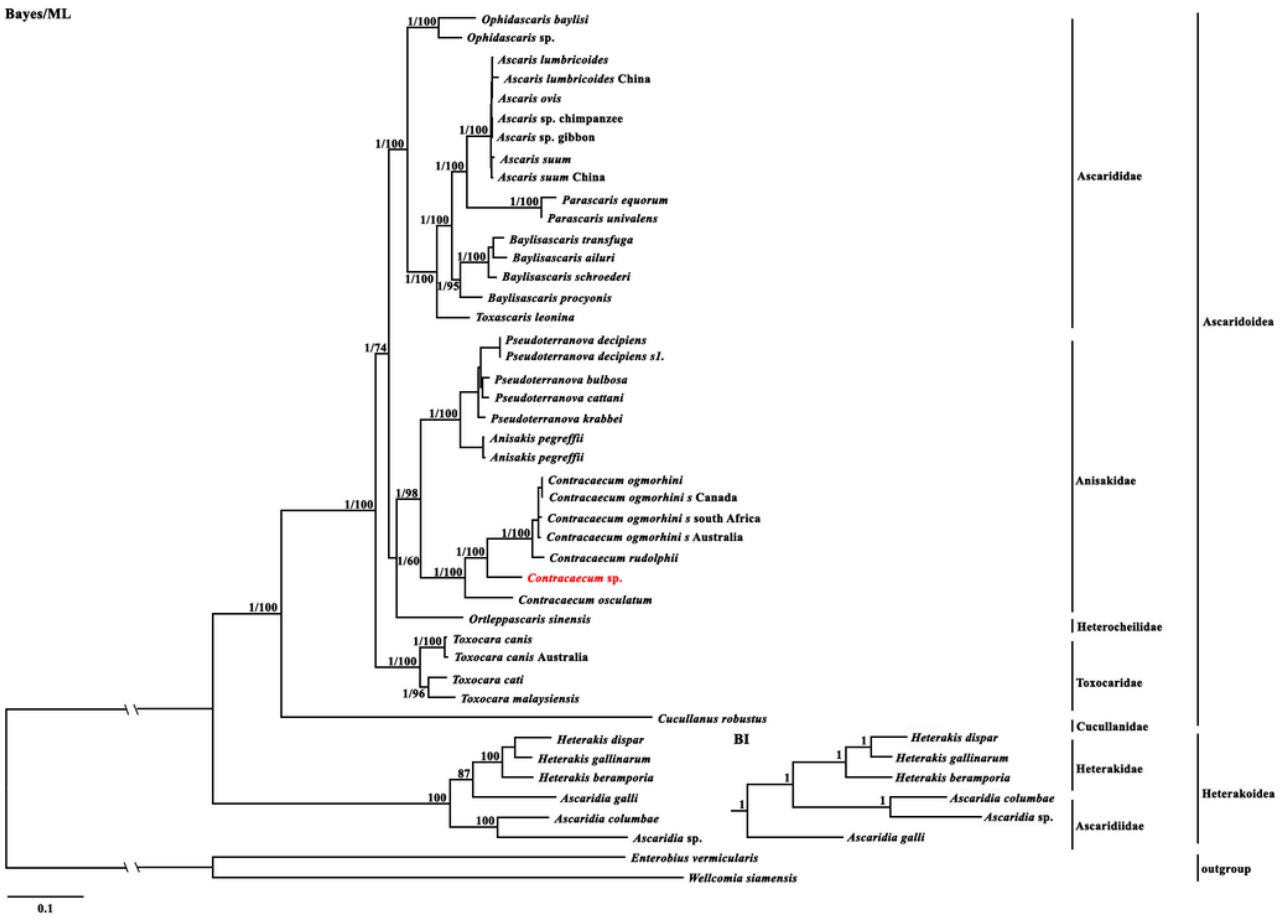

Figure 4

Phylogenetic relationships of *Contracaecum* spp. with species from Ascaridoidea and Heterakoidea. Analyses trees based on amino acid sequences of 12 protein genes by complete mitochondrial genome using Bayesian Inference (BI) and Maximum Likelihood (ML) with *Enterobius vermicularis* and *Wellcomia siamensis* as outgroups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx