

Research Article

The Mitochondrial Genome of the *Anisakis simplex* (Nematoda: Anisakidae) from Rockfish *Sebastes* sp.

Yujun Shuai and Jinhong Zhao*

Department of Medical Parasitology, Wannan Medical College, Wuhu 241002, China.

Abstract | *Anisakis simplex* is a zoonotic disease-causing parasitic nematode belonging to the Anisakidae family. In this study, the nematode samples of *A. simplex* were collected from rockfish *Sebastes* sp. From the comparison and analysis of the mitochondrial genome of *Anisakis simplex*, the results revealed a full-length genome with 13,903 bp, including 12 protein-coding genes, 2 rRNAs and 22 tRNAs. There was no encoding gene of *atp8*, which was consistent with the genome characteristics of *Anisakis* nematodes. Additionally, 25 related nematodes belonged to 5 different families served as study subjects for the construction of phylogenetic trees.

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***Correspondence** | Jinhong Zhao, Department of Medical Parasitology, Wannan Medical College, Wuhu 241002, China; **Email:** zhaojh@wnmc.edu.cn

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Keywords | *Anisakis simplex*, Nematoda, Mitochondrial genome, Phylogenetic analysis, Genome map, Anisakiasis



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Introduction

Anisakis simplex (Rudolphi, 1809) (Nematoda: Anisakidae) is a parasitic nematode that can cause zoonotic diseases (Lopienska *et al.*, 2019). Since the pathogenic role of *Anisakis* in humans was reported in 1960 (Van *et al.*, 1960), there has been increasing understanding of parasitic diseases transmitted by fish (Pravettoni *et al.*, 2012; Ramilo *et al.*, 2023). Anisakiasis is gradually becoming a growing issue in epidemiology as the number of globally identified cases increases (Audicana and Kennedy, 2008; Llorens *et al.*, 2018). The planktonic crustaceans, sea-fish, cephalopods, and marine mammal are all the part of the complicated life cycle of *A. simplex* (Fæste *et al.*, 2014). The larvae of *A. simplex* are frequently parasitic

in the fish's ventral muscle (Suzuki *et al.*, 2021). The tendency to enjoy raw or undercooked fish strongly enhances the risk of Anisakiasis due to the dietary habits, the most common nematode infection is the third-stage larvae (L3) of *A. simplex* (Cipriani *et al.*, 2021; Roca-Geronès *et al.*, 2020). *A. simplex* can cause gastrointestinal and allergic reactions to human (Audicana and Kennedy, 2008).

As we all know, for the maternal inheritance and relatively conserved genomic architecture of mitochondrial genomes, the information of mitochondrial genomes can provide useful molecular markers for studying the ecology, population genetic structure, and phylogeny of organisms (Li *et al.*, 2008a; Kim *et al.*, 2006).

Materials and Methods

Samples collecting and sequencing

The *A. simplex* samples were collected from rock-fish *Sebastes* sp. in Santa Barbara, California, USA (119°42' W, 34°25' N). The nematode samples were washed by sterile distilled water, then clipped off the head and tail and the remaining part of nematodes were ground and crumbled. The total genomic DNA was extracted from nematode samples using the TI-ANamp Genomic DNA Kit (TIANGEN, Beijing, China) and followed the manufacturer's directions. We used 9 pairs of PCR primers (Supplementary Table 1) to amplify the whole mitochondrial genome of the *A. simplex*, and sequenced the PCR products by GENERAL BIOL Co. (Chuzhou, China) using Sanger sequencing technology.

Sequence analysis

The resulting multiple sequences were manually corrected, edited and spliced using DNAMAN software (Version 8) and annotated after alignment with other existing sequences in the GenBank DNA database. The sequence of complete mitochondrial genome was uploaded to NCBI under the accession number OQ354213.

The mitochondrial genome map of *A. simplex* was visually analyzed via CGView Serve (Grant and Stothard, 2008) (<http://cgview.ca>), then manually corrected to ensure annotation accuracy. The colors of the circle represented different genetic traits. The phylogenetic sequences were constructed with 12 protein-coding genes (PCGs) of *A. simplex* and 24 other nematodes, of which *Wuchereria bancrofti* (GenBank accession No. NC016186) was selected as the outgroup. The PCGs of 25 nematodes species were compared using MEGA X software for sequencing results and manually trimmed for end-uniform sequences. Then, the topology structure of the obtained PCGs sequence was subsequently analyzed using the Maximum Likelihood (ML) method with 1,000 bootstrapping times.

Results and Discussion

The entire mitochondrial genome of *A. simplex* is a circular DNA molecule structure, lacking the *atp8* gene, and all genes are transcribed in the same direction, which is consistent with the general characteristics of most other nematodes (Kim et al., 2006). The entire length of *A. simplex* mt DNA is 13,903

bp (Table 1), with a base composition of A 22.8%, T 48.3%, C 9.8%, G 19.1%, and a high percentage of A + T (71.1%). There are a total of 36 genes (Figure 1), including 12 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and 2 ribosomal RNA genes (rRNAs), which are labeled on the entire mitochondrial genome in the same arrangement as the mitochondrial genomes of other *Anisakis* (Yamada et al., 2017). The start codons of 12 PCGs are TTG (*nad1*, *atp6*, *nad2*, *cox3*, *nad4*, *cox1*, *cox2*, *nad3*, *nad6*), ATT (*nad5*, *nad4L*) and ATA (*cytb*), respectively. Additionally, 7 PCGs are predicted to end in TAA (*nad1*, *atp6*, *nad2*, *nad4*, *nad6*) and TAG (*cox2*, *nad3*), while 5 PCGs are predicted to terminate in the incomplete stop codon T (*cytb*, *cox3*, *cox1*, *nad5*, *nad4L*). The 22 tRNAs, with a length range of 52bp (tRNA-Ser^(AGN)) to 62 bp (tRNA-Lys), and two rRNAs, small subunit rRNA (12S; 699 bp) and large subunit rRNA (16S; 957 bp) are each positioned between tRNA-His and *nad3* and between tRNA-Glu and tRNA-Ser^(UCN), respectively.

The 12 mitochondrial PCGs of *A. simplex* in this study were compared with those of other nematode species downloaded from GenBank (Table 2) through the systematic evolutionary tree with the outgroup of the *Wuchereria bancrofti*. The phylogenetic tree showed that *A. simplex* in this study belonged to the family Anisakidae, and it forms a clade with *Anisakis simplex* (NC007934), *Anisakis pegreffii* (NC034329) and *Anisakis simplex* (AP017678) as a monophyletic group with 100% boot strap, and then clustered with *Pseudoterranova azarasi* (NC027163). The other nematodes in the family Ascarididae, Toxocaridae, Heterocheilidae, Ascaridiidae and Heterakidae have stronger bootstrap value supporting (Figure 2).

Since the discovery of *Anisakis* in humans (Van et al., 1960), there has been a growing researching of fished vector-borne parasitic diseases and an increase in the study of *A. simplex* (Pravettoni et al., 2012; Ramilo et al., 2023). In this study, the mitogenome of *A. simplex* was sequenced, and the total length was 13,903 bp and the related phylogenetic tree was established. The most mitogenome characteristics of *A. simplex* in this study was consistent with other *A. simplex* reported before (Mohandas et al., 2014), while there just shared a 98.70% identity with *A. simplex* (GenBank accession No. NC007934) by comparing the GenBank DNA database in NCBI. We hope that the mitochondrial genome of *A. simplex* in the study would

Table 1: Organization of the *Anisakis simplex* mitochondrial genome.

Gene	Position From To	Size (bp)	AA (bp)	Spacer(+)	Overlap(-)	Start codon	Stop codon	anticodon
nad1	1 873	873	290	13(+)		TTG	TAA	
atp6	887 1486	600	199	6(+)		TTG	TAA	
trnK	1493 1554	62		8(+)				TTT
trnL2UUR	1563 1617	55		0				TAA
trnS1AGN	1518 1669	52		0				TCT
nad2	1670 2515	846	281	7(+)		TTG	TAA	
trnI	2523 2583	61		0				GAT
trnR	2584 2642	59		1(-)				ACG
trnQ	2642 2696	55		0				TTG
trnF	2697 2756	60		0				GAA
cytb	2757 3855	1099	366	0		ATA	T	
trnL1CUN	3856 3910	55		0				TAG
cox3	3911 4676	766	255	0		TTG	T	
trnT	4677 4732	56		0				TGT
nad4	4733 5962	1230	409	0		TTG	TAA	
NCR	5963 6088	126		0				
cox1	6089 7664	1576	525	0		TTG	T	
trnC	7665 7720	56		0				GCA
trnM	7722 7782	61		1(+)				CAT
trnD	7791 7849	59		8(+)				GTC
trnG	7856 7911	56		6(+)				TCC
cox2	7912 8610	699	232	0		TTG	TAG	
trnH	8609 8665	57		2(-)				GTG
rrnL	8672 9628	957		6(+)				
nad3	9629 9964	336	111	0		TTG	TAG	
nad5	9965 11546	1582	527	0		ATT	T	
trnA	11548 11604	58		0				TGC
trnP	11621 11677	57		16(+)				TGG
trnV	11680 11735	56		2(+)				TAC
nad6	11736 12170	435	144	0		TTG	TAA	
nad4L	12171 12402	232	77	0		ATT	T	
trnW	12403 12460	58		0				TCA
trnE	12469 12528	60		8(+)				TTC
rrnS	12529 13227	699		0				
trnS2UCN	13231 13283	53		3(+)				TGA
AT	13284 13782	499		0				
trnN	13783 13839	57		5(+)				GTT
trnY	13845 13903	59		0				GTA

AA: Amino acid; **AT:** AT rich region; **NCR:** Non-coding region.

Table 2: *The species used to construct the phylogenetic tree and their GenBank accession numbers.*

Family	Genus	Species	GenBank accession number	Reference
Ascarididae	<i>Baylisascaris</i>	<i>Baylisascaris ailuri</i>	NC015925	Xie <i>et al.</i> , 2011b
		<i>Baylisascaris transfuga</i>	NC015924	Xie <i>et al.</i> , 2011b
		<i>Baylisascaris schroederi</i>	NC015927	Xie <i>et al.</i> , 2011b
		<i>Baylisascaris procyonis</i>	NC016200	Xie <i>et al.</i> , 2011a
	<i>Ascaris</i>	<i>Ascaris suum</i>	NC001327	Wolstenholme <i>et al.</i> 1994
		<i>Ascaris lumbricoides</i>	NC016198	Park <i>et al.</i> , 2011
		<i>Ascaris ovis</i>	KU522453	Unpublished
	<i>Toxascaris</i>	<i>Toxascaris leonine</i>	NC023504	Liu <i>et al.</i> , 2014
Toxocaridae	<i>Toxocara</i>	<i>Toxocara cati</i>	NC010773	Li <i>et al.</i> , 2008b
		<i>Toxocara canis</i>	AM411108	Li <i>et al.</i> , 2008b
		<i>Toxocara malaysiensis</i>	NC010527	Li <i>et al.</i> , 2008b
Heterocheilidae	<i>Ortleppascaris</i>	<i>Ortleppascaris sinensis</i>	KU950438	Zhao <i>et al.</i> , 2018
Anisakidae	<i>Pseudoterranova</i>	<i>Pseudoterranova azarasi</i>	NC027163	Liu <i>et al.</i> , 2015
	<i>Anisakis</i>	<i>Anisakis simplex</i>	AP017678	Unpublished
		<i>Anisakis simplex</i> *	OQ354213	this study
		<i>Anisakis simplex</i>	NC007934	Kim <i>et al.</i> , 2006
		<i>Anisakis pegreffii</i>	NC034329	Yamada <i>et al.</i> , 2017a
	<i>Contracaecum</i>	<i>Contracaecum osculatum</i>	NC024037	Mohandas <i>et al.</i> , 2014
		<i>Contracaecum rudolphii</i> B	NC014870	Unpublished
Ascaridiidae	<i>Ascaridia</i>	<i>Ascaridia galli</i>	NC021642	Liu <i>et al.</i> , 2013
		<i>Ascaridia galli</i>	OQ286042	Shuai <i>et al.</i> , 2023
		<i>Ascaridia columbae</i>	NC021643	Liu <i>et al.</i> , 2013
Heterakidae	<i>Heterakis</i>	<i>Heterakis beramporia</i>	NC029838	Wang <i>et al.</i> , 2016
	<i>Heterakis</i>	<i>Heterakis gallinarum</i>	NC029839	Wang <i>et al.</i> , 2016
Onchocercidae	<i>Wuchereria</i>	<i>Wuchereria bancrofti</i>	NC016186	McNulty <i>et al.</i> , 2012

Note: * means the species of *Anisakis simple* in this study.

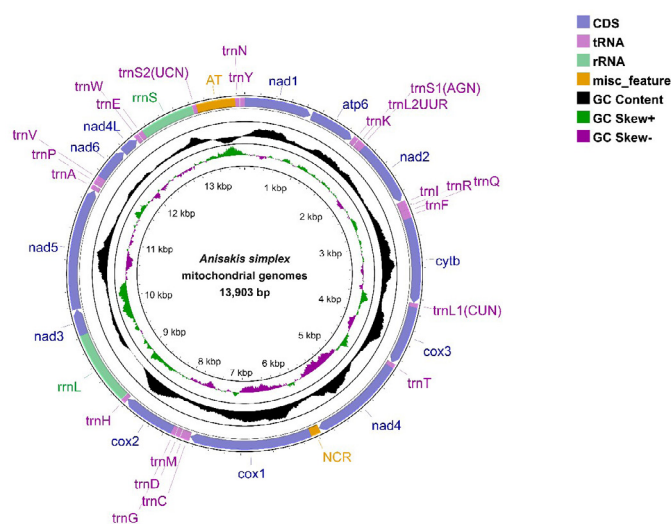


Figure 1: Mitochondrial genome map of *Anisakis simplex* in this study.

gives some more valuable genetic information for phylogenetic analysis, molecular epidemiology, and the biological evolution of related species.

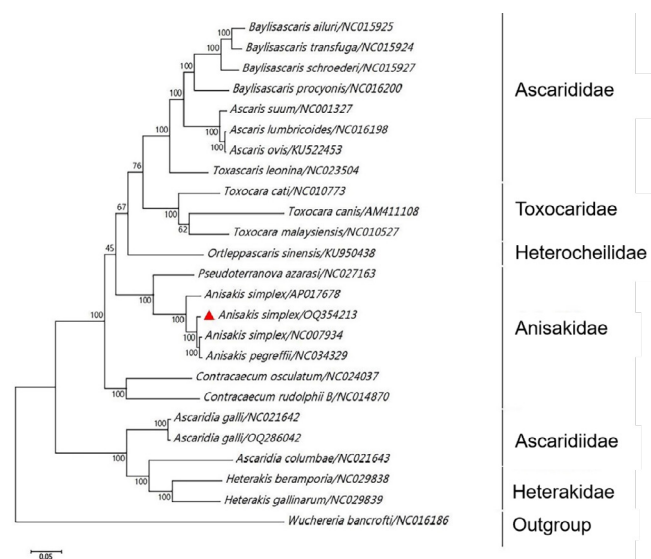


Figure 2: *Maximum Likelihood (ML) trees were composed of nucleotide sequences from 12 mitochondrial PCGs of 25 nematode species and were replicated 1,000 bootstrapping times using MEGA X software.*

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Novelty Statement

The mitochondrial genome of *A. simplex* in the study would give some more valuable genetic information for phylogenetic analysis and the biological evolution of related species.

Author's Contributions

YJS: Data Processing and Analysis, Methodology, Software, Visualization, Writing - original draft; JH.Z: Conceptualization, Data Processing and Analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing - review and editing.

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Data Availability Statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/nucleotide/OQ354213>.

Supplementary Material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjn/2024/42.1.81.87>

Conflict of interest

The authors have declared no conflict of interest.

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