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ORIGINAL PAPER

The complete mitochondrial genomes of *Gnathostoma doloresi* from China and Japan

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Abstract *Gnathostoma doloresi* is one of the neglected pathogens causing gnathostomiasis. Although this zoonotic parasite leads to significant socioeconomic concerns globally, little is known of its genetics and systematics. In the present study, we sequenced and characterized the complete mitochondrial (mt) genomes of *G. doloresi* isolates from China and Japan. The lengths of the mt genomes of the *G. doloresi* China and Japan isolates are 13,809 and 13,812 bp, respectively. Both mt genomes encode 36 genes, including 12 protein-coding genes (PCGs), 2 ribosomal RNA genes, and 22 transfer RNA genes. The gene order, transcription direction, and genome content are identical with its congener *G. spinigerum*. Phylogenetic analyses based on concatenated amino acid sequences of 12 PCGs by Bayesian inference (BI) indicated that *G. doloresi* are closely related to *G. spinigerum*. Our data provide an invaluable resource for studying the molecular epidemiology, phylogenetics, and population genetics of *Gnathostoma* spp. and should have implications for further studies of the diagnosis, prevention, and control of gnathostomiasis in humans and animals.

Keywords *Gnathostoma doloresi* · Mitochondrial genome · Mitochondrial DNA · Phylogenetic analyses

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Introduction

The *Gnathostoma* spp. are the etiological agents of the zoonotic gnathostomiasis with a worldwide distribution, and gnathostomiasis has received increasing attention because of its public health significance (Diaz 2015; Jeremiah et al. 2011; Liu et al. 2015a; Nawa and Nakamura-Uchiyama 2004; Vargas et al. 2012). Humans get infected with *Gnathostoma* by consuming raw or undercooked food (fish, frogs, poultries, and snakes). Most patients with gnathostomiasis show cutaneous lesions (Chai et al. 2003). The *Gnathostoma* invasion of visceral organs, such as the lung, ear, gastrointestinal tract, uterus, eye, and kidney, leads to mechanical injuries and function damage (Herman and Chiodini 2009). The larval *Gnathostoma* may also intrude brain and spinal cord, causing serious neurognathostomiasis (Intapan et al. 2010), eosinophilic meningitis, intracerebral hemorrhage (Intapan et al. 2010), subarachnoid hemorrhage, and lesion in spinal nerve root (Katchanov et al. 2011). Fortunately, gnathostomiasis can be treated by drugs of ivermectin and albendazole (Nontasut et al. 2000).

The metazoan mitochondrial (mt) genomes are typically circular, ranging from 14 to 18 kb in size. It usually encodes

36–37 genes, including 12–13 protein-coding genes (PCGs), 2 ribosomal RNA (*rrn*S and *rrn*L) genes, and 22 transfer RNA genes (*trn*s) (Wolstenholme 1992). No introns are within genes, and only very short spacer regions exist between the genes (Wolstenholme 1992). Mt genomes have been effectively used as genetic markers in phylogenetic studies of many taxa including parasites because of the properties of compactness, maternal inheritance, haploidy, relatively high mutation rates, and the lack of recombination (Chen et al. 2016; Tao et al. 2014; Yang et al. 2016).

Of the 12 species in the genus *Gnathostoma* (Liu et al., 2015a), *G. doloresi* is frequently reported for causing gnathostomiasis, especially in Asia (Chai et al. 2003; Liu et al. 2015a; Nawa and Nakamura-Uchiyama 2004; Xuan et al. 2002). Though the advanced DNA technologies are continuously improved, our knowledge of the genetics of *G. doloresi* is still scarce. The mt *cox*1 and the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) of *G. doloresi* is available (Li et al. 2015), and only one complete mt genome of *Gnathostoma* nematodes has been sequenced (Liu et al. 2015a). Therefore, the objectives of the present study were to determine the complete mt genomes of *G. doloresi* China and Japan isolates and to analyze the phylogenetic relationships of *G. doloresi* with 17 other representative nematodes using the concatenated mt amino acid sequences.

Materials and methods

Parasites and DNA extraction

The adult specimens of *Gnathostoma* were collected from naturally infected wild boars in Fujian province, China, and Mie Prefecture, Japan. They were detected by light microscopic examination and identified as *G. doloresi* according to morphological criteria (Camacho et al. 2002; Li et al. 2015) and multiple PCR assay (Li et al. 2014). The samples were fixed in 70 % alcohol and stored at −20 °C until further use. Total genomic DNA was isolated from individual adult *G. doloresi* using sodium dodecyl sulfate/proteinase K treatment, followed by spin-column purification (Wizard® SV Genomic DNA Purification System, Promega) (Liu et al. 2015a).

Long PCR amplification and sequencing

Nine pairs of PCR primers were used to amplify overlapping long fragments of the complete mt genome of *G. doloresi*, as shown in Table 1. The primers were designed according to relatively conserved regions of mtDNA sequences from *G. spinigerum*, *Cooperia oncophora* (NC004806), *Teladorsagia circumcincta* (NC013827) (Jex et al. 2009), *Haemonchus contortus* (NC010383) (Jex et al. 2008a), and *Cucullanus robustus* (GQ332426) (Park et al. 2011).

PCR reactions were conducted in a total volume of 50 μl, using 25-μl PrimeStar Max DNA polymerase premix (Takara, Dalian, China), 20 pmol of each primer (synthesized in Genewiz, Suzhou, China), and 0.3 μl DNA template in a thermocycler (Biometra, Göttingen, Germany). The PCR cycling conditions began with the initial denaturation at 98 °C for 1.5 min, then 22 cycles of denaturation at 98 °C for 18 s, annealing at 52–62 °C for 8 s, and extension at 60 °C for 0.5–5 min, followed by 98 °C denaturation for 1.5 min, plus 28 cycles of 98 °C for 18 s (denaturation), 52–62 °C for 8 s (annealing), and 60 °C for 0.5–5 min according to the product length, with 10 min of the final extension at 66 °C. A negative control (contains no DNA) was included in each amplification run. Every amplicon (3 μl) was evidenced by electrophoresis in a 3 % agarose gel and stained with ethidium bromide (Sangon Biotech, Shanghai, China) (not shown). The PCR products were then sequenced by Invitrogen Biotechnology Company (Shanghai, China) using a primer-walking strategy.

Sequence analyses

Sequences were assembled manually and aligned against sequences of the complete mt genome of *G. spinigerum* using the computer program MAFFT 7.122 (Katoh and Standley 2013) to identify gene boundaries. Each PCG was translated into amino acid sequence using the invertebrate mitochondrial genetic code in MEGA 6 (Tamura et al. 2011) and aligned based on the amino acid sequences using default settings. The translation start and stop codons were identified to avoid gene overlap and to optimize the similarity with the gene lengths of *G. spinigerum* mt genome (Liu et al. 2015a). tRNA genes were identified using the program tRNAscan-SE (Lowe and Eddy 1997) by recognizing potential secondary structures and anticodon sequences by eye. Two rRNA genes were predicted by comparison with that of *G. spinigerum* (Liu et al., 2015a).

Phylogenetic analyses

The concatenated amino acid sequences of *G. doloresi* mt genome, conceptually translated from individual genes of the mt genome, were aligned with those of published mt genomes of selected species, including *Baylisascaris ailuri* (NC_015925) (Xie et al. 2011), *Ascaris suum* (HQ704901) (Liu et al. 2012), and *Ascaris lumbricoides* (HQ704900) (Liu et al. 2012) [Ascarididae]; *Toxocara canis* (EU730761) (Jex et al. 2008b) and *Toxocara malaysiensis* (NC_010527) (Li et al. 2006) [Toxocaridae]; *Anisakis simplex* (KC965056) (Mohandas et al. 2014) and *Pseudoterranova azarasi* (NC_027163) (Liu et al. 2015b) [Anisakedae]; *Ascaridia galli* (NC_021642) (Liu et al. 2013) [Ascaridiidae]; *G. spinigerum*

Table 1 Sequences of primers used to amplify PCR fragments from *Gnathostoma doloresi*

Primer	Sequence (5′ to 3′)	Region	Amplicon size (kb)
GDC1F	GTTCCAGAATAATCGGCTATGCTT	*rrn*S–*cox*1	~940 bp
GDC1R	TAAAGAAAGAACATAATGAAAATG		
GDC2F	GAGTAAGTAGTAGTAAAGTAGGGTTAGGGAT	*cox*1–*rrn*L	~2684 bp
GDC2R	GTCAAATACAAACTTCTCTGACTCACAA		
GDC3F	AACCGTGATATTAGTTTAGCCCATTGT	*rrn*L–*cyt*b	~4242 bp
GDC3R	CCATTAGGATGAAACAGCCGAAA		
GDC4F	TGATGTATGAGGTTAATTTTGGTTGAG	*cyt*b–*cyt*b	~643 bp
GDC4R	GAAATATCATTCAGGCACAATATGAAC		
GDC5F	TTGTTGTGGTGACTATGATAAGGTTGG	*cyt*b–*cox*3	~984 bp
GDC5R	AATGGCATCAAGTTACTCTTACTCCCCT		
GDC6F	TTGTTGTATATTTTTAGAGAGTTTATGTT	*cox*3–*cox*3	~485 bp
GDC6R	ACATCCACAAAATGTCAATAAATAAT		
GDC7F	TGGGATTATTTATTGTCATTTTGTGGAT	*cox*3–*nad*1	~1702 bp
GDC7R	ATTCATAGAAATACGGCAATGTAAGTCA		
GDC8F	CACTTGATATGAGCGTCATTTGTTGG	*nad*1–*nad*1	~574 bp
GDC8R	CGAAAGCCACACTAGAATACTCCACATTA		
GDC9F	TGGGATTATTTATTGTCATTTTGTGGAT	*nad*1–*rrn*S	~2274 bp
GDC9R	ATTCATAGAAATACGGCAATGTAAGTCA		
GDJ1F	GTTCCAGAATAATCGGCTATGCTT	*rrn*S–*cox*1	~940 bp
GDJ1R	TAAAGAAAGAACATAATGAAAATG		
GDJ2F	CCTGCTTTTGGTATCGTGAGTCAGAGAA	*cox*1–*cox*1	~762 bp
GDJ2R	CAAACACATAACCCCTTACAACGACCTC		
GDJ3F	GAGTGTTTTTGCTTTGTTTGTTTTTGT	*cox*1–*rrn*L	~1325 bp
GDJ3R	TCCTTCACCCTTTTATTTCTATTTTTCA		
GDJ4F	GAGAAGATTGAGTATCAGTTGGGAGA	*rrn*L–*cyt*b	~5227 bp
GDJ4R	CAGATTAACAGGTACAAAAGGGAGTT		
GDJ5F	AACTCCCTCTTGTACCTGTTAATCTGA	*cyt*b–*cyt*b	~1136 bp
GDJ5R	AACCAACTCAACATCAAAACTGAAAAT		
GDJ6F	TTGTTGTGGTGACTATGATAAGGTTGG	*cyt*b–*cox*3	~984 bp
GDJ6R	AATGGCATCAAGTTACTCTTACTCCCCT		
GDJ7F	TTGGTTTATTGTCTTCTATTTTGATGTT	*cox*3–*nad*1	~2039 bp
GDJ7R	ATTCAGTGTAGTTAGCTGTTCTTTCTTC		
GDJ8F	CTGTGTATGAGCGACATTTACTAGGTAGG	*nad*1–*nad*1	~502 bp
GDJ8R	CCGATTCAGTTCCGCCAAGAT		
GDJ9F	GGATAGTAGGAGCATTGCGTGC	*nad*1–*rrn*S	~2585 bp
GDJ9R	TAGGGAATTGATGGATGATTTGTACC		

(NC_027726) (Liu et al. 2015a) [Gnathostomidae]; *Chabertia erschowi* (NC_023782) (Liu et al. 2014) and *Oesophagostomum quadrispinulatum* (NC_014181) (Lin et al. 2012) [Chabertidae]; *C. oncophora* (NC_004806) (Van der Veer and De Vries 2004), *Nematodirus oiratianus* (NC_024639) (Zhao et al. 2014), and *Trichostrongylus axei* (NC_013824) (Jex et al. 2009) [Trichostrongylidae]; *Dictyocaulus viviparus* (NC_019810) (Gasser et al. 2012) [Dityocanlidae]; and *Angiostrongylus cantonensis* (NC_013065) (Lv et al. 2012) [Metastrongylidae]. The sequence of *Trichuiris suis* Denmark isolate (KT449822) (Hawash et al. 2015) [Trichuridae] was used as an outgroup.

All inferred amino acid sequences were aligned using MAFFT 7.122. Divergent regions of the alignment were eliminated using Gblocks Server v. 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The default settings were used, selecting the option of less strict conservation of flanking positions. The alignment was then converted into the nexus format using Clustal X1.83 and subjected to phylogenetic analysis using Bayesian inference (BI). A mixed model (Castoe and Parkinson 2006) was used in the BI analysis using MrBayes 3.1.1. Four independent Markov chains were run for 1,000,000 metropolis-coupled MCMC generations and sampling trees every 1000 generations. The

first 250 trees (25 %) were discarded as burn-in, with the remaining trees being used for calculating Bayesian posterior probabilities (Bpp). The analysis was regarded as completed when the potential scale reduction factor was close to 1, and the average standard deviation of split frequencies was below 0.01. Phylograms were prepared using FigTree v. 1.42.

Results and discussion

General features of the mt genome of *G. doloresi*

The complete mt genomes of *G. doloresi* (Fig. 1) from China and Japan were 13,809 and 13,812 bp in length, respectively (GenBank accession numbers KU975390 and KX231806). The circular mt genomes, transcribed in the same directions, contain 12 PCGs (*cox*1-3, *nad*1-6, *nad*4L, *atp*6, and *cyt*b), 22 tRNA genes, 2 rRNA genes, and 2 non-coding regions (Table 2). The nucleotide composition of the *G. doloresi* China isolate mt genome is A = 25.02 %, T = 45.53 %, G = 21.70 %, and C = 7.75 %, with an obviously high A + T content of 70.55 %, and the nucleotide composition of the *G. doloresi* Japan isolate mt genome is A = 25.07 %, T = 45.68 %, G = 21.67 %, and C = 7.58 %, with an obviously high A + T content of 70.75 %, which is a little lower than that of *G. spinigerum* (71.13 %) (Liu et al. 2015a) and slightly higher than that of *T. canis* (NC_010690) (68.57 %) (Jex et al. 2008b).

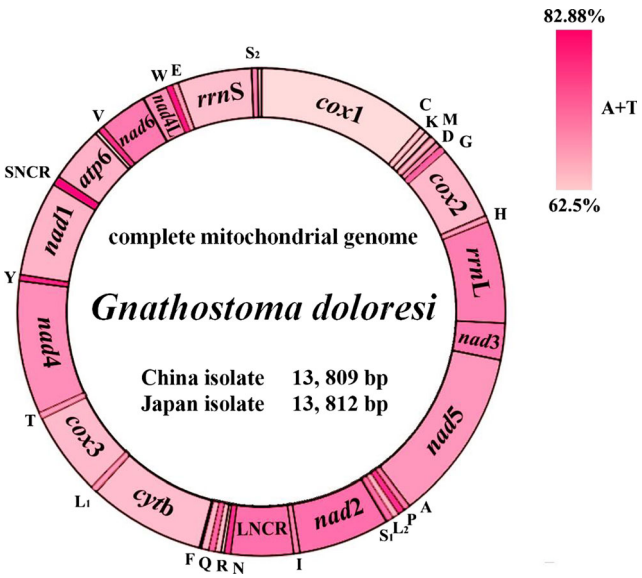

Fig. 1 Arrangement of the mitochondrial genomes of *Gnathostoma doloresi*. The scales are accurate. All genes are transcribed in the clockwise direction, using standard nomenclature. The 22 tRNA genes are represented by the *one-letter code* for the corresponding amino acid, with *numerals* differentiating each of the two leucine- and serine-specifying tRNAs (L_1 and L_2 for codon families CUN and UUR, respectively; S_1 and S_2 for codon families AGN and UCN, respectively). The A + T content for each gene or region is shown and represented by *color*

The length of PCGs of *G. doloresi* China and Japan isolates were in the following order: *nad*5 > *cox*1 > *nad*4 > *cyt*b > *nad*1 > *nad*2 > *cox*3 > *cox*2 > *atp*6 > *nad*6 > *nad*3 > *nad*4L (Table 2). A total of 3360 amino acids are encoded in the mt genome of *G. doloresi* China and Japan isolates. In these mt genomes, six start codons (TTG, ATG, ATA, GTT, ATT, and TTT) and four stop codons (TAA, TAG, TA, and T) are used (Table 2). GTT is used as start codon in *cox*2 and *nad*4 genes, and ATT is used in *nad*5 and *cyt*b genes in the *G. doloresi* China isolate. Additionally, in the mt genome of the *G. doloresi* Japan isolate, GTT is used as start codon in *cox*2 and *nad*5 genes, and ATT is used only in the *cyt*b gene. In both mt genomes, *cox*1 is initiated with TTT. Furthermore, incomplete termination codon TA is used in *cox*1 and *cyt*b genes, and T is used in *cox*2, *nad*5, *nad*2, and *nad*4 (Table 2) in the mt genomes of the *G. doloresi* China and Japan isolates. The uncommon codon GTT was also used in *Ascaris suum* (Wolstenholme, 1992); ATT was used in *Caenorhabditis elegans* (Okimoto et al., 1992), *A. suum* (Okimoto et al., 1992), and many other species (Wolstenholme 1992).

A total of 22 tRNA sequences (ranging from 53 to 67 nucleotides in length) were identified in *G. doloresi* mt genomes, and their predicted secondary structures (not shown) are similar to that of *G. spinigerum* (Liu et al., 2015a). The *rrn*S gene of *G. doloresi* from the China and Japan isolates are 679 and 680 bp in length, and they are located between *trn*E and *trn*S2 genes. The *rrn*L gene is 939 and 938 bp in length, respectively, which lies between the *trn*H and *nad*3 genes. The A + T contents of the *rrn*L of the two *G. doloresi* isolates are 74.12 and 74.09 %, and the A + T contents of *rrn*S are 67.89 and 67.65 %, respectively.

The longer non-coding region (LNCR) of the *G. doloresi* China and Japan isolates is located between the *nad*2 and *cyt*b genes, and the shorter one (SNCR) is located between the genes *nad*1 and *atp*6. Their sizes are 568 and 573 bp (LNCR) and 86 and 89 bp (SNCR) (Table 2). The A + T contents of the NC2 are 75.88 and 75.22 %, and the NC1 are 84.88 and 83.72 %, respectively (Table 2).

Comparative analyses between *G. doloresi* and *G. spinigerum* mt genomes

The complete mt genome sequence of the *G. doloresi* China isolate was 13,809 bp in length, 4 bp shorter than that of *G. doloresi* Japan. A comparison of the nucleotide sequences of each mt gene, as well as the amino acid sequences, conceptually translated from all protein genes of the two *G. doloresi* isolates, is given in Table 3. Sequence difference across the complete mt genome between *G. doloresi* China and Japan

Table 2 Organization of *Gnathostoma doloresi* from China (GDC) and *Gnathostoma doloresi* from Japan (GDJ) and *Gnathostoma spinigerum* from China (GSC) mitochondrial genomes

Gene/region	Positions and nt sequence sizes (bp)			Start and stop codons			Anticodons
	GDC (5′–3′)	GDJ (5′–3′)	GSC (5′–3′)	GDC	GDJ	GSC (5′–3′)	GD/GS
*cox*1	7–1571 (1565)	7–1571 (1565)	1–1572 (1572)	TTT/TA	TTT/TA	ATA/TAG	
*trn*C	1572–1627 (56)	1572–1627 (56)	1573–1629 (57)				GCA
*trn*K	1627–1689 (63)	1627–1688 (62)	1629–1691 (63)				TTT
*trn*M	1698–1754 (57)	1697–1753 (57)	1699–1752 (54)				CAT
*trn*D	1760–1818 (59)	1759–1817 (59)	1753–1808 (56)				GTC
*trn*G	1820–1875 (56)	1819–1873 (55)	1813–1868 (56)				TCC
*cox*2	1876–2566 (691)	1874–2564 (691)	1869–2556 (688)	GTT/T	GTT/T	TTG/T	
*trn*H	2567–2621 (55)	2565–2619 (55)	2557–2612 (56)				GTG
*rrn*L	2622–3560 (939)	2620–3557 (938)	2613–3554 (942)				
*nad*3	3561–3896 (336)	3558–3893 (336)	3555–3890 (336)	TTG/TAA	TTG/TAA	TTG/TAA	
*nad*5	3900–5480 (1585)	3897–5481 (1585)	3894–5478 (1585)	ATT/T	GTT/T	TTG/T	
*trn*A	5485–5538 (54)	5482–5535 (54)	5479–5533 (55)				TGC
*trn*P	5542–5603 (62)	5539–5599 (61)	5535–5592 (58)				TGG
*trn*L2	5599–5653 (55)	5594–5649 (56)	5598–5652 (55)				TAA
*trn*S1	5652–5712 (61)	5648–5708 (61)	5651–5710 (60)				TCT
*nad*2	5723–6554 (832)	5719–6550 (832)	5721–6553 (833)	ATG/T	ATG/T	ATA/TA	
*trn*I	6555–6611 (57)	6551–6607 (57)	6554–6609 (56)				GAT
LNCR	6612–7179 (568)	6608–7180 (573)	6610–7359 (750)				
*trn*N	7180–7235 (56)	7181–7236 (56)	7360–7416 (57)				GTT
*trn*R	7269–7325 (57)	7270–7326 (57)	7448–7504 (57)				TCG
*trn*Q	7325–7378 (54)	7326–7379 (54)	7504–7557 (54)				TTG
*trn*F	7378–7444 (67)	7379–7445 (67)	7557–7624 (68)				GAA
*cyt*b	7468–8537 (1070)	7469–8538 (1070)	7648–8721 (1074)	ATT/TA	ATT/TA	ATA/TAG	
*trn*L1	8538–8593 (56)	8539–8593 (55)	8722–8776 (55)				TAG
*cox*3	8594–9358 (765)	8594–9361 (768)	8777–9544 (768)	TTG/TAG	TTG/TAG	TTG/TAG	
*trn*T	9360–9415 (56)	9363–9418 (56)	9547–9601 (55)				TGT
*nad*4	9419–10,640 (1222)	9422–10,643 (1222)	9602–10,826 (1225)	GTT/T	TTG/T	TTG/T	
*trn*Y	10,641–10,694 (54)	10,644–10,696 (53)	10,827–10,880 (54)				GTA
*nad*1	10,695–11,567 (873)	10,697–11,569 (873)	10,881–11,753 (873)	TTG/TAA	TTG/TAA	TTG/TAA	
SNCR	11,568–11,653 (86)	11,570–11,658 (89)	11,754–11,830 (77)				
*atp*6	11,654–12,166 (513)	11,659–12,168 (510)	11,831–12,409 (579)	ATA/TAG	TTG/TAG	ATT/TAG	
*trn*V	12,205–12,260 (56)	12,208–12,263 (56)	12,463–12,517 (55)				TAC
*nad*6	12,261–12,698 (438)	12,264–12,701 (438)	12,518–12,955 (438)	TTG/TAA	TTG/TAA	TTG/TAG	
*nad*4L	12,706–12,933 (228)	12,709–12,936 (228)	12,963–13,190 (228)	TTG/TAG	TTG/TAG	TTG/TAG	
*trn*W	12,934–12,990 (57)	12,937–12,992 (56)	13,191–13,247 (57)				TCA
*trn*E	12,991–13,045 (55)	12,992–13,048 (57)	13,255–13,311 (57)				TTC
*rrn*S	13,045–13,723 (679)	13,047–13,726 (680)	13,312–13,985 (674)				
*trn*S2	13,723–13,775 (53)	13,726–13,778 (53)	13,986–14,039 (54)				TGA

isolates was 3 %. The difference in amino acid sequences of the *G. doloresi* China and Japan isolate mt genomes was 2.3 %.

The mt genome sequence of the *G. doloresi* China and Japan isolates are 270 and 267 bp shorter than that of *G. spinigerum* (Liu et al., 2015a). The arrangement of the mt genes (12 protein genes, 2 rRNA genes, and 22 tRNA genes) and 2 NCRs is the same (Liu et al. 2015a). A pairwise comparison of the nucleotide and the amino acid sequences of each mt gene was performed between the two *Gnathostoma* species (Table 3). The sequence lengths of the individual genes between the three taxa

Table 3 Comparison of nucleotide (nt) and/or predicted amino acid (aa) sequence differences between mitochondrial genomes of Chinese *Gnathostoma doloresi* (GDC) and Japanese *Gnathostoma doloresi* (GDJ) and Chinese *Gnathostoma spinigerum* (GSC)

Gene/region	Nt length (bp)			Nt diversity (%)			Amino acid no.			Amino acid diversity (%)		
	GDC	GDJ	GSC	GDC/GSC	GDJ/GSC	GDC/GDJ	GDC	GDJ	GSC	GDC/GSC	GDJ/GSC	GDC/GDJ
*cox*1	1565	1565	1572	13.0	13.2	2.9	521	521	523	6.7	5.2	1.5
*cox*2	691	691	688	16.1	15.9	2.7	230	230	229	14.3	12.6	1.8
*nad*3	336	336	336	19.3	19.3	2.7	111	111	111	18.8	18.8	1.8
*nad*5	1585	1585	1585	19.8	20.1	2.1	528	527	528	19.3	18.4	1.3
*nad*2	832	832	833	22.3	21.5	3	277	277	277	20.1	18	1.8
*cyt*b	1070	1070	1074	15.8	15.7	3.2	356	356	357	13.4	12	1.1
*cox*3	765	768	768	14.5	13.8	4.3	254	254	255	9.4	10.2	1.2
*nad*4	1222	1213	1225	17.5	16.0	7.2	403	405	408	14.9	14.8	3
*nad*1	873	873	873	19.8	19.8	2.5	290	289	290	15.5	15.2	0
*atp*6	513	510	579	18.1	17.8	2.7	170	170	192	14.6	11.7	1.8
*nad*6	438	438	438	20.0	19.8	1.6	145	145	145	19.2	19.2	0.7
*nad*4L	228	228	228	14.0	13.5	2.6	75	75	75	11.8	10.5	2.6
*rrn*L	939	938	942	17.4	17.2	1.1						
*rrn*S	679	679	674	11.1	11.4	0.9						
Overall	13,809	13,812	14,039	19.1	17.8	3	3360	3360	3390	14.6	14.3	2.3

were very similar (Table 3). The magnitude of sequence difference in each gene between the two *Gnathostoma* species ranged from 11.1 to 22.3 % for nucleotide sequences and 5.2 to 20.1 % for amino acid sequences (Table 3). The sequence difference across the entire mt genome between *G. doloresi* (China and Japan isolates) and *G. spinigerum* was 19.10 and 17.80 % at the nucleotide level and 14.60 and 14.30 % at the amino acid level.

The greatest difference of nucleotide sequences between the two *Gnathostoma* species was in the *nad*2 (22.3 and 21.5 %), whereas the least difference (11.1 %) was detected in the *rrn*S gene (Table 3). At the amino acid level, the PCGs that varied the most were *nad*2 (20.1 %), *nad*5 (19.3 %), and *nad*6 (19.2 %), whereas *cox*1 (6.7 %) and *cox*3 (9.4 %) are the most conserved genes between China *G. doloresi* and *G. spinigerum*. On the other hand, the major difference of PCGs between Japan *G. doloresi* and *G. spinigerum* were *nad*6 (19.2 %), *nad*3 (18.8 %), and *nad*5 (18.4 %), while *cox*1 (5.2 %) and *cox*3 (10.2 %) are the most conserved genes.

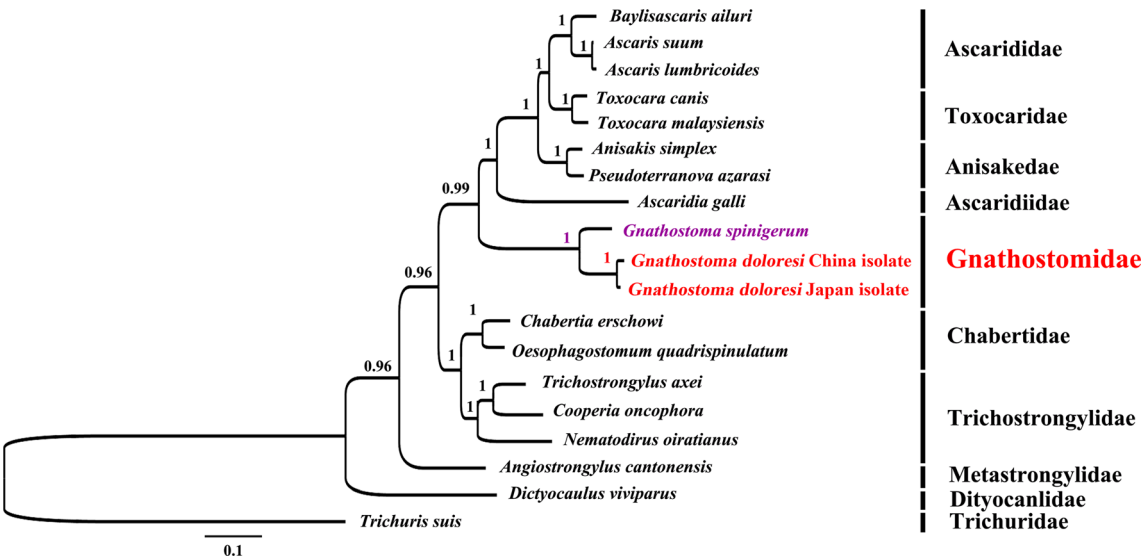

Fig. 2 Phylogenetic relationships of *Gnathostoma doloresi* and other species. Tree inferred from the concatenated amino acid sequence dataset of 12 protein-coding genes from 20 nematodes was performed by Bayesian inference (BI). *Trichuris suis* (KT449822) was chosen as an outgroup

Phylogenetic analyses

The phylogenetic tree inferred from the concatenated amino acid sequences of 15 nematode species (Fig. 2). The monophyly of the families Ascarididae, Toxocaridae, Anisakidae, Gnathostomidae, and Chabertidae were strongly supported in the phylogenetic analyses in the present study (Bpp = 1). Our data confirm that *G. spinigerum* is a member of the Gnathostomidae with strong support (Bpp = 1).

Significance and implications

In the present study, we determined the complete mt genome of *G. doloresi* from China and Japan isolates, which are neglected zoonotic nematode. The characterization of the *G. doloresi* mt genomes will provide a novel resource for the improved diagnosis of human gnathostomiasis using the molecular approach. Molecular tools using mt gene sequences as genetic markers have been proven effective in assisting clinical diagnosis and conducting molecular epidemiological investigations of parasites (Ma et al. 2015a; Ma et al. 2015b). In addition, there is great potential to employing mt genome markers to investigate genetic variation of *G. doloresi* from different epidemic locations worldwide. The availability of the complete mt genomes of *G. doloresi* will also enable the identification and differentiation of potential cryptic/sibling species of *Gnathostoma*.

Mt genome sequences have been proven useful in verifying the phylogenetic position of helminths, particularly when using sequences of 12 PCGs as markers in comparative analyses (Liu et al. 2015b; Zhao et al. 2014). In the present study, the *G. doloresi* China and Japan isolates were clustered with *G. spinigerum* in the same branch, showing that the *G. spinigerum* and *G. doloresi* China and Japan isolates are closely related *Gnathostoma* species with high support in the BI analysis (Bpp > 0.92, Fig. 2). The phylogenetic relationships of the selected nematodes are consistent with those of previous studies (Liu et al. 2015a; Liu et al. 2013; Zhao et al. 2014). To date, mt genomes of many species of the family Gnathostomatidae are still underrepresented or not represented. So, expanding taxa sampling is necessary for future phylogenetic studies of Gnathostomatidae species using mt genomic dataset.

Conclusion

The present study determined the entire mt genomes of the *G. doloresi* China and Japan isolates of human health significance. The *G. doloresi* mt genomes will provide a useful resource for studying the molecular epidemiology and phylogenetics of the *Gnathostoma* spp. and should have implications for further studies of the diagnosis, prevention, and control of gnathostomiasis in humans and animals.

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