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ORIGINAL PAPER

Characterization of the complete mitochondrial genome sequence of *Homalogaster paloniae* (Gastrodiscidae, Trematoda) and comparative analyses with selected digeneans

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Abstract Gastrodiscidae species are neglected but significant paramphistomes in small ruminants, which can lead to considerable economic losses to the breeding industry of livestock. However, knowledge about molecular ecology, population genetics, and phylogenetic analysis is still limited. In the present study, we firstly sequenced and analyzed the full mitochondrial (mt) genome of *Homalogaster paloniae* (14,490 bp). The gene contents and organization of the *H. paloniae* mt genome is the same as that of other digeneans, such as *Fasciola hepatica* and *Paramphistomum cervi*. It is interesting that unlike other paramphistomes, *H. paloniae* is flat in shape which is similar with Fasciola, such as *F. hepatica*. Phylogenetic analysis of *H. paloniae* and other 17 selected digeneans using concatenated amino acid sequences of the 12 protein-coding genes showed that Gastrodiscidae is closely related to Paramphistomidae and Gastrothylacidae. The availability of the mt genome sequence of *H. paloniae* should provide an important foundation for further molecular study of Gastrodiscidae and other digeneans.

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Introduction

Gastrodiscidae species are important paramphistomes parasiting in the large intestine of small ruminants such as goats, sheep and cattles, and *Homalogaster paloniae* (Poirier, 1883) is one of the most common species (Li 2011; Taylor et al. 2007). As a neglected pathogen, *H. paloniae* have been reported in Burma, China, Formosa, India, Indonesia, Japan, Philippines, and Thailand (Yamaguti 1971). *H. paloniae* (Poirier, 1883) usually inhabits in the caecum of animals. Mature eggs are expelled with feces from the intestine into the environment and will develop into miracidium after several days under favor conditions. Subsequently, miracidium will invade into freshwater snails (intermediate host). Miracidium will develop into cercaria, and escape from the freshwater snails, then develop into metacercaria on the water plants. Ruminants infect *H. paloniae* by intaking water plants polluted by metacercaria. Although animals infected with *H. paloniae* usually do not show obvious symptoms, it can cause considerable losses to the breeding of sheep and cattle under heavy burden (Guoqing 2006).

Since no effective vaccine is available, application of chemical drugs is the main methods for the prevention and control of *H. paloniae* and other paramphistomes. Accurate diagnosis of *H. paloniae* infection is essential for the prevention and control of this species. Traditional morphological methods have been widely used for a long time; however, these methods are time-consuming and inaccurate (Bott et al. 2009). Based on these restricted factors, molecular methods based on PCR were developed for species identification (Itagaki et al. 2003; Morgan and Blair 1995). Recently, the mitochondrial genome has been used for species identification, population diversity, phylogenetic

analysis, and so on (Cheng et al. 2016; Choi et al. 2012; Liu et al. 2012, 2015). Besides, advances in PCR-coupled sequencing together with bioinformatics analysis have been proved to be useful for the molecular study of species (Jex et al. 2010). In the present study, we aimed (i) to characterize the *H. paloniae* mt genome, and this is the first report about full mt genome of Gastrodiscidae; (ii) to assess the phylogenetic relationship between Gastrodiscidae and other family; and (iii) to provide useful information for further study of species identification, biology, population genetics and phylogenetic analysis.

Materials and methods

Parasites and DNA extraction

Adult flukes were collected from the caecum of a naturally infected black goat post-mortem in Macheng, Hubei province, PR China. After sufficiently washed with physiological saline, the specimens were fixed in 75 % (*v*/*v*) ethanol and preserved at −20 °C until use. These flukes were identified to be *H. paloniae* based on key morphological characteristics and parasitic positions (Guoqing 2006; Li 2011; Taylor et al. 2007).

Subsequently, total genomic DNA of *H. paloniae* was isolated from single worm by a kit (E.Z.N.A.® Tissue DNA Kit, D3396-01). The ITS-2 (the second internal transcribed spacer) region of *H. paloniae* was amplified and sequenced for further molecular identification (Itagaki et al. 2003); the sequence was 100 % similarity with a sequence available for *H. paloniae* (GenBank accession no. KM281535.1).

PCR-coupled sequencing of *H. paloniae* mitochondrial genome

Firstly, seven pairs of primers (Table 1) were designed and synthesized to amplify short fragments from *nad*1, *nad*4,

Table 1 Primers used for amplifying the mitochondrial genome of *Homalogaster paloniae*

Primers	Sequences (5′–3′)	Target gene
PFXCND1F	CGKAAGGGNCCNAAHAAGGTKGG	*nad*1
PFXCND1R1	CGVAHHCGHGGHARHGTDGCACG	*nad*1
PFXCND4F	GADTCBCCDTATTCDGARCG	*nad*4
PFXCND4R	GCHARCCADCGCTTVCCNTC	*nad*4
PFXCCOX1F	GAYCCDTTRGGWGGWGGDGATCC	*cox*1
PFXCCOX1R	ACAMACWCGACGWGGYAAHCC	*cox*1
PFXCCOX2F	AAGRTDRTDGGNCRBCARTGRTAYTG	*cox*2
PFXCCOX2R	CGWCCHGGDATWGCATCYATCTT	*cox*2
PFXCND5F	ATGCGNGCYCCNACNCCNGTDAG	*nad*5
PFXCND5R1	TGCTTVSWAAAAAANACHCC	*nad*5
PFXC12SF	CAGKGCCAGCAWYCKCGGTTA	*rrn*S
PFXC12SR	DDTGACGGGCGRTRTGTAC	*rrn*S
PFXCCYTBF	TDCCHTGRCAYCARATGTC	*cyt*b
PFXCCYTBR	AARAARTAYCAYTCHGGCTT	*cyt*b
PFXCF9	TTGTTTATGGCTGTGGGAGACT	*nad*5-*cyt*b
PFXCR1	ACAAAGGATTTTTAGAACCCCC	*nad*5-*cyt*b
PFXCF2	TGTTCTTTGGTTACCGACAG	*cyt*b-*nad*4
PFXCR2	AATGGGGGTAACGGTATCTTTG	*cyt*b-*nad*4
PFXCF3	GCCTTTGGGGCTGTTTTGTG	*nad*4-*nad*1
PFXCR3	ATTGAACCTAACAACCTAG	*nad*4-*nad*1
PFXCF4	TGGGGTTATTAGTCACATTTGTG	*nad*1-*cox*1
PFXCR4	CTTAATACCTGTTGGTATACC	*nad*1-*cox*1
PFXCF5	ATTCGATGGTACATGATACGTG	*cox*1-*rrn*S
PFXCR5	GGACGAACTTCATCGGCTGC	*cox*1-*rrn*S
PFXCF6	CCAGGTCTTTGTGCTGCTGA	*rrn*S-*cox*2
PFXCR6	CATCCGCAGACGTCACCA	*rrn*S-*cox*2
PFXCFF7	GTGATTTTGTGGGTGGTGTG	*cox*2-*nad*5
PFXCFR7	CTACTTTATTACACGTTGATAACG	*cox*2-*nad*5

*cox*1, *cox*2, *nad*5, *rrn*S, and *cyt*b based on the conserved regions of the mt genomes of *Fasciola hepatica* (Le et al. 2001a) and *Paramphistomum cervi* (Yan et al. 2013). PCR reactions (25 μl) were as follow: 1 × Taq polymerase buffer, 0.2 mM each of dNTP, 0.5 μM of each primer, 2 U Taq polymerase (Takara), and 2.5 μl genomic DNA in a thermocycler (Biometra) under the following conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C/30 s, 50 °C/30 s, 72 °C/1 min, and a final extension of 72 °C/7 min. Subsequently, PCR products were identified by agarose gel electrophoresis, and positive amplicons were sent for sequencing in both directions. Later, seven pairs of primers were designed based on the obtained seven short fragments to amplify the remaining sequences of the complete mt genome in seven long-PCR reactions (Table 1). The long-PCR reactions (25 μl) were performed in 0.8 mM of each dNTPs, 2.5 μl 10× LA Taq buffer, 0.5 μM of each primer, 2.5 U LA Taq polymerase (Takara), and 2.5 μl genomic DNA sample. PCR reactions were carried out under the following condition: 94 °C for 5 min, then 35 cycles at 94 °C/30 s, and annealed at 50 °C/30 s, followed by extension at 72 °C/8 min, and a final extension of 72 °C/7 min. Then, positive PCR amplicons were purified and cloned into pGEM-T vector (Promega, USA) for sequencing using a primer-walking strategy.

Assembly, annotation, and bioinformatics analyses

The complete *H. paloniae* mt sequences were assembled by bioinformatics analysis and manually adjustment. Gene boundaries of the mt genome sequence of *H. paloniae* were predicted by aligning against that of *F. hepatica* (Le et al. 2001a) and *P. cervi* (Yan et al. 2013) using the software Clustal X 1.83 (Thompson et al. 1997). The protein-coding genes, transfer RNA, ribosomal RNA, and non-coding regions were annotated as reported (Yan et al. 2013).

Nucleotide comparison of the complete mt genome of *H. paloniae* and other 17 selected digeneans was conducted. Meanwhile, the AT-skew and GC-skew were calculated as previous study (Baek et al. 2014; Yan et al. 2014).

Sliding window analysis of nucleotide variation

Pairwise alignment of the complete mt genome of *H. paloniae*, *Fischoederius elongates*, *P. cervi*, *Gastrothylax crumenifer*, and *Ogmocotyle sikae* was accomplished by MEGA v6.0 to predict variable nucleotide sites (Tamura et al. 2013). Subsequently, a sliding window analysis of *H. paloniae*, *F. elongates*, *P. cervi*,

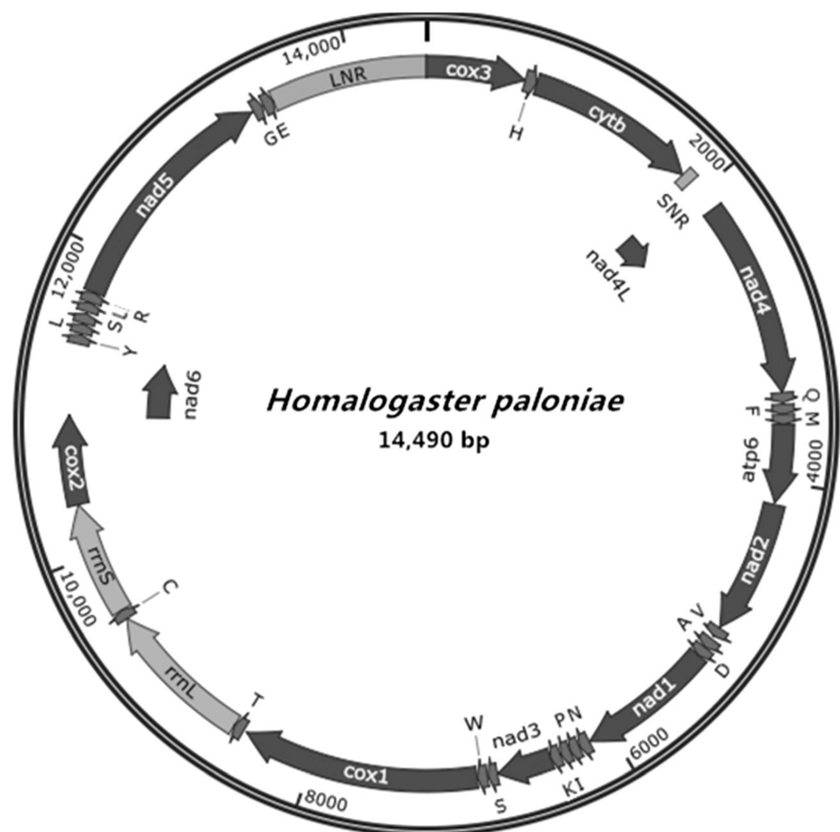

Fig. 1 Arrangement of the mitochondrial genome of *Homalogaster paloniae*. Genes organization of 12 protein-coding genes, 22 tRNA gene, two RNA genes, and two non-coding regions in the mitochondrial genome of *Homalogaster paloniae*

G. crumenifer, and *O. sikae* was accomplished using DnaSP v.5.0 to assess the nucleotide variation diversity for the 12 protein-coding genes among these five paramphistomes (Librado and Rozas 2009).

Phylogenetic analysis

All 12 protein-coding genes of *H. paloniae* mt genome and other 17 selected digeneans were translated, concatenated, and

Table 2 Content of the *Homalogaster paloniae* mitochondrial genome

Gene/region	Positions	Size (bp)	Number of aa[a]	Ini/Ter codons[b]	Anticodons	In[c]
*cox*3	1–645	645	215	ATG/TAG		0
*trn*H	647–721	75			GTG	+1
*cyt*b	724–1845	1122	374	ATG/TAG		+2
SNR	1846–1916	71				0
*nad*4L	1917–2180	264	88	ATG/TAG		0
*nad*4	2141–3421	1281	427	ATG/TAG		−40
*trn*Q	3423–3486	64			TTG	+1
*trn*F	3499–3563	65			GAA	+12
*trn*M	3564–3627	64			CAT	0
*atp*6	3628–4137	510	170	ATG/TAG		0
*nad*2	4149–5021	873	291	ATG/TAG		+11
*trn*V	5028–5091	64			TAC	+6
*trn*A	5113–5179	67			TGC	+21
*trn*D	5183–5248	66			GTC	+3
*nad*1	5249–6151	903	301	GTG/TAG		0
*trn*N	6154–6223	70			GTT	+2
*trn*P	6227–6291	65			TGG	+3
*trn*I	6292–6354	63			GAT	0
*trn*K	6360–6425	66			CTT	+5
*nad*3	6426–6782	357	119	ATG/TAG		0
*trn*S1	6785–6844	60			GCT	+2
*trn*W	6851–6915	65			TCA	+6
*cox*1	6919–8460	1542	514	GTG/TAG		+3
*trn*T	8484–8552	69			TGT	+23
rrnL[d]	8553–9536	984				0
*trn*C	9537–9601	65			GCA	0
rrnS[d]	9602–10,342	741				0
*cox*2	10,343–10,924	582	194	ATG/TAG		0
*nad*6	10,918–11,370	453	151	GTG/TAG		−7
*trn*Y	11,386–11,448	63			GTA	+15
*trn*L1	11,456–11,519	64			TAG	+7
*trn*S2	11,524–11,589	66			TGA	+4
*trn*L2	11,601–11,663	63			TAA	+11
*trn*R	11,666–11,733	68			TCG	+2
*nad*5	11,734–13,314	1581	527	ATG/TAA		0
*trn*G	13,325–13,390	66			TCC	+10
*trn*E	13,400–13,468	69			TTC	+9
LNR	13,469–14,490	1022				0

The inferred length of amino acid sequence of 12 protein-coding genes.

[a] Amino acid

[b] Initiation and termination codons

[c] Intergenic nucleotides

[d] Initiation or termination positions of ribosomal RNAs defined by adjacent gene boundaries

aligned for phylogenetic analysis, including *Clonorchis sinensis* (NC_012147) (Shekhovtsov et al. 2010), *Fasciola gigantica* (NC_024025) (Liu et al. 2014), *F. hepatica* (NC_002546) (Le et al. 2001a), *Fischoederius elongatus* (KM_397348) (Yang et al. 2015b), *G. crumenifer* (KM_400624), *Haplorchis taichui* (NC_022433.1) (Lee et al. 2013), *Hypoderaeum conoideum* (KM111525) (Yang et al. 2015a), *Metagonimus yokogawai* (KC330755.1), *O. sikae* (KR006934) (Ma et al. 2015), *Opisthorchis felineus* (EU_921260) (Shekhovtsov et al. 2010), *Opisthorchis viverrini* (JF729304.1) (Cai et al. 2012), *P. cervi* (NC_023095.1) (Yan et al. 2013), *Schistosoma haematobium* (NC_008074) (Littlewood et al. 2006), *Schistosoma japonicum* (AF215860) (Le et al. 2001b), *Schistosoma mekongi* (NC_002529) (Le et al. 2000), *Schistosoma spindale* (NC_008067) (Littlewood et al. 2006), and *Trichobilharzia regent* (NC_010976) (Webster et al. 2007). And *Taenia solium* (NC_004022.1) (Nakao and Sako 2003) was included as an outgroup control.

The amino acid sequences were aligned and subjected to phylogenetic analysis by maximum likelihood methods using MEGA v.6.0 with default settings (Tamura et al. 2013).

Results

Genome content and organization

The complete mitochondrial (mt) genome of *H. paloniae* (GenBank accession no. KT266674) is 14,490 bp in length (Fig. 1) and contains 12 protein-coding genes, 22 transfer ribonucleic acid (tRNA) genes, two ribosomal ribonucleic acid (rRNA) genes (*rrnS* and *rrnL*), and two non-coding regions (Table 2). All the genes are transcribed in the same direction, which is in accordance with other digeneans (Le et al. 2001a; Yan et al. 2013). The gene arrangement is similar with other digeneans except for *S. haematobium* and *S. spindale* (Littlewood et al. 2006).

As for the nucleotide composition, *H. paloniae* mt genome is obviously favor in T (Table 3). The nucleotide contents in the complete mt genome are 21.92 % (A), 9.28 % (C), 43.21 % (T), and 25.6 % (G). And the A + T content of mt genes range from 63.23 to 71.04 %, total A + T content is 65.12 %.

Annotation of *H. paloniae* mt genome

The *H. paloniae* mt genome has 12 protein-coding genes (Fig. 1). For these genes, the most commonly used start codon is ATG (nine of 12 protein genes), and GTG is used by the remaining genes (three of 12 protein genes) (Table 2), which is in agreement with other digeneans (Cai et al. 2012; Le et al. 2001a; Littlewood et al. 2006; Yan et al. 2013; Yang et al. 2015a). The termination codon is TAA for *nad*5, and TAG for the rest genes. No incomplete codons are used in the mt genome of *H. paloniae*.

Table 3 Nucleotide composition of the mitochondrial genome of *Homalogaster paloniae*

Gene	A (%)	C (%)	G (%)	T (%)	A + T (%)
*cox*3	22.02	8.37	22.48	47.13	69.15
*cyt*b	19.61	9.98	25.13	45.28	64.88
SNR	29.58	5.63	30.99	33.80	63.38
*nad*4L	23.48	8.71	23.86	43.94	67.42
*nad*4	18.35	9.45	24.82	47.38	65.73
*atp*6	17.25	10.00	25.69	47.06	64.31
*nad*2	17.41	8.48	24.05	50.06	67.47
*nad*1	19.38	7.75	28.13	44.74	64.12
*nad*3	18.21	6.72	25.21	49.86	68.07
*cox*1	19.97	10.89	25.81	43.32	63.29
*rrn*L	28.66	10.57	24.29	36.48	65.14
*rrn*S	26.86	11.20	25.10	36.84	63.70
*cox*2	23.02	9.62	27.15	40.21	63.23
*nad*6	16.34	7.73	26.71	49.23	65.56
*nad*5	18.28	8.67	27.64	45.41	63.69
LNR	31.90	6.46	22.50	39.14	71.04
Total	21.91	9.28	25.60	43.21	65.12

The 12 protein-coding genes encode 3359 amino acids excluding the termination codons (Table 4). Among all the amino acids, Phe (TTT 10.00 %) is the most used, followed by Leu (TTG 7.09 %), and Leu (TTA 6.91 %). The least used codon is Arg (CGC 0.12 %), followed by Leu (CTC 0.15 %), and Arg (CGG 0.15 %).

As for the tRNA genes and rRNA genes, the length of the 22 tRNA genes ranged from 59 to 72 bp (Table 2). The size of *rrnS* and *rrnL* were 741 and 984 bp, respectively (Table 3). The location of *rrnS* is between *trn*C and *cox*2 and *rrnL* is between *trn*T and *trn*C, and their A + T content was 63.70 and 65.14 %, respectively (Table 3).

In the *H. paloniae* mt genome, two non-coding regions were recognized based on their AT-rich features and locations (Yan et al. 2013), one short non-coding region (SNR 71 bp) and one long non-coding region (LNR 1022 bp) (Table 2). The location of SNR is between *cyt*b and *nad*4L, and LNR is located between *trn*E and *cox*3.

Comparative analyses of the mt genomes of *H. paloniae* and other digeneans

Nucleotide composition, AT skews and GC skews of the mt genome of *H. paloniae* and other digeneans were presented in Table 5. All the 18 digeneans mt genomes are rich in A + T. The nucleotide composition of *H. paloniae* is biased to T compared with A (AT skew = −0.327), and biased to G

Table 4 Codon usage for 12 protein-coding genes in *Homalogaster paloniae* mitochondrial genome

Amino acid	Codon	Number	Frequency (%)	Amino acid	Codon	Number	Frequency (%)
Phe	TTT	337	10.00	Ile	ATT	99	2.94
Phe	TTC	16	0.47	Ile	ATC	5	0.15
Leu	TTA	233	6.91	Ile	ATA	73	2.17
Leu	TTG	239	7.09	Met	ATG	106	3.14
Ser	TCT	106	3.14	Met	GTG	153	4.54
Ser	TCC	8	0.24	Thr	ACT	30	0.89
Ser	TCA	37	1.10	Thr	ACC	7	0.21
Ser	TCG	33	0.98	Thr	ACA	24	0.71
Tyr	TAT	159	4.72	Thr	ACG	23	0.68
Tyr	TAC	15	0.44	Asn	AAT	44	1.31
Stop	TAA	1	0.03	Asn	AAC	6	0.18
Stop	TAG	11	0.33	Asn	AAA	26	0.77
Cys	TGT	106	3.14	Lys	AAG	52	1.54
Cys	TGC	14	0.42	Ser	AGT	97	2.88
Trp	TGA	55	1.63	Ser	AGC	6	0.18
Trp	TGG	64	1.90	Ser	AGA	36	1.07
Leu	CTT	33	0.98	Ser	AGG	44	1.31
Leu	CTC	5	0.15	Val	GTT	152	4.51
Leu	CTA	15	0.44	Val	GTC	12	0.36
Leu	CTG	29	0.86	Val	GTA	99	2.94
Pro	CCT	56	1.66	Ala	GCT	86	2.55
Pro	CCC	2	0.06	Ala	GCC	7	0.21
Pro	CCA	13	0.39	Ala	GCA	22	0.65
Pro	CCG	13	0.39	Ala	GCG	23	0.68
His	CAT	42	1.25	Asp	GAT	58	1.72
His	CAC	8	0.24	Asp	GAC	3	0.09
Gln	CAA	14	0.42	Glu	GAA	32	0.95
Gln	CAG	15	0.44	Glu	GAG	55	1.63
Arg	CGT	42	1.25	Gly	GGT	150	4.45
Arg	CGC	4	0.12	Gly	GGC	13	0.39
Arg	CGA	8	0.24	Gly	GGA	37	1.10
Arg	CGG	5	0.15	Gly	GGG	53	1.57

compared with C (GC skew = 0.468), which is in accordance with that of other digeneans.

Nucleotide variability

The sliding window analysis was showed in Fig. 2; the highest level of nucleotide variability was within *cox*1, and the lowest was within *cox*3. In our study, *cox*1 and *nad*6 are the most conserved genes, and *cox*3 and *cyt*b are the least conserved.

Phylogenetic analyses

Waeschenbach and colleagues reported that the complete mt sequences are more reliable for phylogenetic analyses (Waeschenbach et al. 2012). Based on previous study, the concatenated amino acid sequence data of the 12 protein-coding genes of *H. paloniae* and other 17 digeneans (*C. sinensis*, *F. gigantica*, *F. hepatica*, *F. elongatus*, *G. crumenifer*, *H. taichui*, *H. paloniae*, *H. conoideum*, *M. yokogawai*, *O. sikae*, *O. felineus*, *O. viverrini*, *P. cervi*, *S. haematobium*, *S. japonicum*, *S. mekongi*, *S. spindale*, and *T. regent*) and one tapeworm (*T. solium*, as an outgroup) were used for the phylogenetic study. The relationship of *H. paloniae* with selected digeneans was showed in Fig. 3. The phylogenetic tree contains two clades with significantly strong support (100 %), one contains 13 members from eight families (Opisthorchiidae, Heterophyidae, Echinostomatidae, Fasciolidae, Notocotylidae, Gastrodiscidae, Paramphistomidae, and Gastrothylacidae), and the other

Table 5 Comparisons of nucleotide composition of the full genome of selected digeneans, including *Homalogaster paloniae*

Species	Nucleotide frequency (%)				Complete genome sequence			
	A	T	C	G	A + T %	AT skew	G + C %	GC skew
Clonorchis sinensis	17.25	42.90	12.37	27.47	60.15	−0.426	39.85	0.379
Opisthorchis viverrini	16.92	42.46	12.97	27.65	59.38	−0.430	40.62	0.361
Opisthorchis felineus	17.20	42.65	12.38	27.76	59.85	−0.425	40.14	0.383
Dicrocoelium chinensis	18.09	44.01	9.98	27.91	62.10	−0.417	37.9	0.473
Fasciola gigantica	15.26	47.40	9.38	27.97	62.66	−0.513	37.34	0.498
Fasciola hepatica	16.07	46.11	9.94	27.89	62.18	−0.483	37.82	0.474
Hypoderaeum conoideum	18.92	42.46	11.71	26.91	61.38	−0.384	38.62	0.394
Haplorchis taichui	19.56	39.67	12.41	28.32	59.23	−0.340	40.77	0.391
Metagonimus yokogawai	17.79	37.89	14.21	30.11	55.68	−0.361	44.32	0.359
Fischoederius elongatus	19.78	44.10	9.62	26.50	63.88	−0.381	36.12	0.467
Homalogaster paloniae	*21.91*	*43.21*	*9.28*	*25.60*	*65.12*	*−0.327*	*34.88*	*0.468*
Paramphistomum cervi	18.45	44.95	9.10	27.50	63.40	−0.418	36.60	0.503
Gastrothylax crumenifer	19.82	43.69	9.86	26.63	63.51	−0.376	36.49	0.460
Ogmocotyle sikae	22.46	44.03	8.74	24.76	66.49	−0.324	33.51	0.478
Trichobilharzia regenti	22.00	46.54	7.68	23.78	68.55	−0.358	31.45	0.512
Schistosoma haematobium	29.06	43.28	7.97	19.66	72.34	−0.197	27.66	0.423
Schistosoma japonicum	24.89	46.15	8.37	20.59	71.04	−0.299	28.96	0.422
Schistosoma mekongi	25.97	46.23	7.18	20.62	72.20	−0.281	27.8	0.483
Schistosoma spindale	30.57	42.13	7.06	20.22	72.70	−0.159	27.3	0.482

contains five members from the Schistosomatidae family. The tree indicated that *H. paloniae* was together with other paramphistomes including *F. elongatus*, *G. crumenifer*, *O. sikae*, and *P. cervi* in one sub-clade, but separated from *F. gigantica* and *F. hepatica* from Fasciolidae, and *H. paloniae* has the closest relationship with members from Paramphistomidae and Gastrothylacidae that inhabiting in small ruminants. Nevertheless, more mt genomes from digeneans are needed for further phylogenetic analyses in the future.

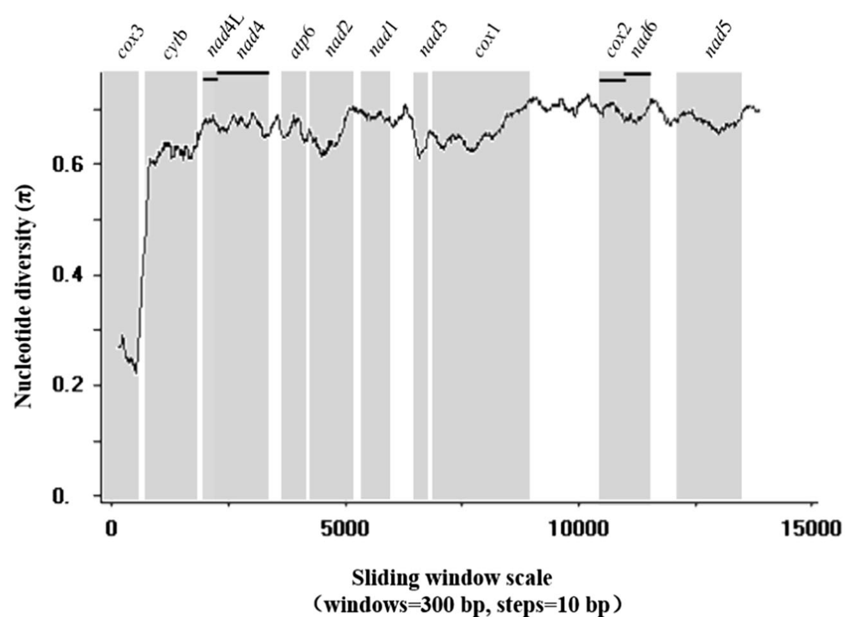

Fig. 2 A sliding window analysis of complete mt genome sequences of *Homalogaster paloniae*, *Fischoederius elongates*, *Paramphistomum cervi*, *Gastrothylax crumenifer*, and *Ogmocotyle sikae.* The *black line* in the picture showed nucleotide diversity in the sliding window analysis (windows = 300 bp; steps = 10 bp). There are two overlapping genes in the protein-coding genes, one is between *Nad*4L and *nad*4, and the other is between *cox*2 and *nad*6. All the 12 protein-coding genes are indicated using grey boxes

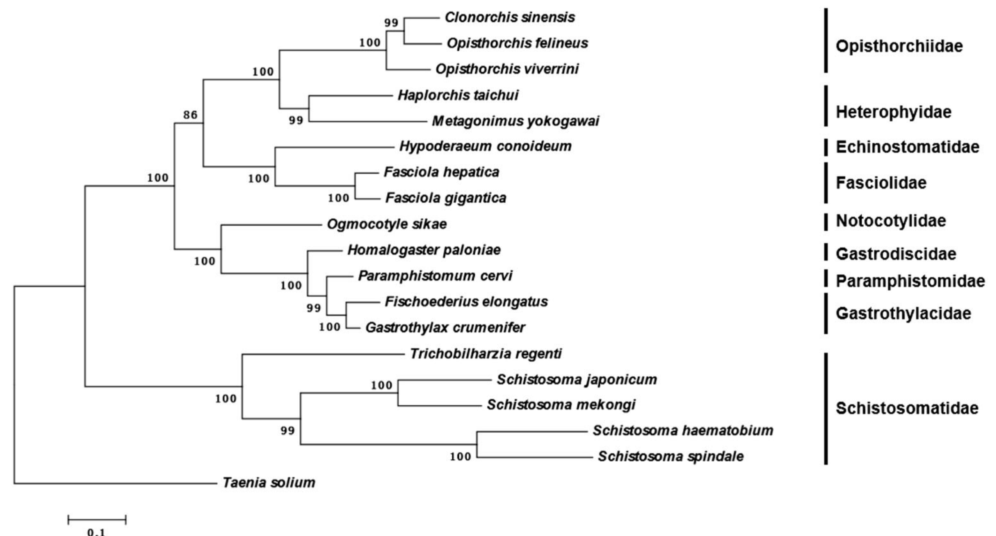

Fig. 3 The phylogenetic relationships of *Homalogaster paloniae* and other digeneans based on concatenated amino acid sequence data representing 12 protein-coding genes using *Taenia solium* as an outgroup

Discussion

As an important paramphistome, *H. paloniae* can lead to considerable economic losses to the breeding industry of small ruminants under heavy burden. Although the development of advances in technology, knowledge about epidemiology, biology, and genetics is still limited.

The present study firstly characterized the mt genome of *H. paloniae*. The gene content and organization are the same as other digeneans. Knowledge of the *H. paloniae* mt genome should provide useful for comparative study of this species and other digeneans.

As for the complete mt of *H. paloniae*, the gene arrangement is the same as other digeneans except for *S. haematobium* and *S. spindale* (Littlewood et al. 2006). All the protein-coding genes use complete codons, which is in accordance with other selected digeneans. Among all the 18 digeneans, all species show strand asymmetry (AT skew = −0.513 ~ −0.159; GC skew = 0.359 ~ 0.512). With the accomplishment of sliding window analysis, *cox*1 gene is the most conserved region among these four paramphistomes; this is in accordance with previous studies, which indicated the conserved characteristics of *cox*1 gene (Chibwana et al. 2013; Pérez-del-Olmo et al. 2014; Rollinson et al. 2009). Phylogenetic analyses can provide a basic understanding of the relationship of *H. paloniae* with other digeneans.

Although *H. paloniae* is closer to Fasciolidae in shape, phylogenetic analysis based on the complete mt genome of *H. paloniae* and other digeneans indicated that *H. paloniae* is closely related to paramphistomes, this is in accordance with their relationship in taxonomy.

Now, the *H. paloniae* mt genome is available; this should provide useful information for the study of epidemiology, biology, species identification, population genetic, and phylogenetic analyses.

Conclusions

In conclusion, our study firstly reported the complete mt genome sequence of *H. paloniae* and compared the mt genome of *H. paloniae* with other selected digeneans. The *H. paloniae* mt genome is the first mt genome available for Gastrodiscidae. Knowledge of mt genome of *H. paloniae* should enrich the mt genome databases of digeneans and also provide useful information for the study of epidemiology, biology, population genetics, as well as phylogenetic analyses.

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