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Research paper

# Characterization of the complete mitochondrial genome of *Cavisoma magnum* (Southwell, 1927) (Acanthocephala: Palaeacanthocephala), first representative of the family Cavisomidae, and its phylogenetic implications

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ABSTRACT

The phylum Acanthocephala is a small group of endoparasites occurring in the alimentary canal of all major lineages of vertebrates worldwide. In the present study, the complete mitochondrial (mt) genome of *Cavisoma magnum* (Southwell, 1927) (Palaeacanthocephala: Echinorhynchida) was determined and annotated, the representative of the family Cavisomidae with the characterization of the complete mt genome firstly decoded. The mt genome of this acanthocephalan is 13,594 bp in length, containing 36 genes plus two non-coding regions. The positions of *trn*V and SNCR (short non-coding region) in the mt genome of *C. magnum* are different comparing to those of the other acanthocephalan species available in GenBank. Phylogenetic analysis based on amino acid sequences of 12 protein-coding genes using Bayesian inference (BI) supported the class Palaeacanthocephala and its included order Polymorphida to be monophyletic, but rejected monophyly of the order Echinorhynchida. Our phylogenetic results also challenged the validity of the genus *Sphaerirostris* (Polymorphida: Centrorhynchidae). The novel mt genomic data of *C. magnum* are very useful for understanding the evolutionary history of this group of parasites and establishing a natural classification of Acanthocephala.

## 1. Introduction

The phylum Acanthocephala is a small group of endoparasites with approximately 1300 species reported from the alimentary tract of various vertebrates worldwide (Gazi et al., 2012; Amin, 2013; Gazi et al., 2015). According to the traditional classification, Acanthocephala was classified into four classes, including Eoacanthocephala, Palaeacanthocephala, Polyacanthocephala and Archiacanthocephala (Amin, 1987, 2013). This morphology-based classification was supported by some molecular phylogenetic analyses based on 18S/28S or 18S + 28S + *cox*1 genetic data, respectively (García-Varela et al., 2002; García-Varela and Nadler, 2006; Verweyen et al., 2011). However, recent mitogenomic phylogenetic results suggested that Polyacanthocephala should be considered as a member of Eoacanthocephala and represented a synonym of Eoacanthocephala (Gazi et al., 2016; Muhammad et al., 2019a,b). The interrelationships of these classes needs further studies.

Among the four classes, Palaeacanthocephala with over 840 species placed into three orders Echinorhynchida, Polymorphida and Heteramorphida, has the richest species diversity. In the class Palaeacanthocephala, the order Echinorhynchida includes more than 470 species, which are mainly parasitic in teleost fish, and represents the largest group (Amin, 1987; Pichelin and Cribb, 2001; Smales, 2012; Amin, 2013). The monophyly of Palaeacanthocephala and Polymorphida was strongly supported by some phylogenetic studies based on mitochondrial (mt) genomic data (Gazi et al., 2015; Gazi et al., 2016; Muhammad et al., 2019a,b; Song et al., 2019), but some regarded Palaeacanthocephala as paraphyly based on 18S genetic data (Herlyn

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et al., 2003). The non-monophyly of Echinorhynchida was reported by many previous phylogenetic studies based on morphological characters (Monks, 2001) and/or molecular data (Near et al., 1998; García-Varela et al., 2000, 2002; Near, 2002; García-Varela and Nadler, 2005; García-Varela and Nadler, 2006; Verweyen et al., 2011; Gazi et al., 2015; García-Varela et al., 2019).

The taxanomy and classification of *C. magnum* has gone through numerous revision for nearly a century. It was originally placed in family Echinorhynchidae, genus *Oligoterorhynchus* Monticelli, 1914 (Southwell, 1927). Then a new family Oligoterorhynchidae and genus *Cavisoma* was established containing *C. magnum* (Van Cleave, 1931). Subsequently, genus *Cavisoma* was elevated to a sub-family level as Cavisominae and moved into family Echinorhynchidae (Meyer, 1932). *Cavisoma*/Cavisominae was regarded as a separated family (Petrochenko, 1956). However, Golvan suggested *Cavisoma* should be placed in family Fessisentidae (Golvan, 1969). Later, genus *Cavisoma* was retained in family Cavisomidae within the order Echinorhynchida (Amin, 1985; Arthur et al., 1995; Amin, 2013; Amin et al., 2018).

The family Cavisomidae is a small group of acanthocephalans in order Echinorhynchida (Meyer, 1932) which mainly parasitizes in fishes, which currently includes ten genera (Amin, 2013). Recently, molecular phylogenetic results considered this family to be a paraphyletic group based on sequenced *cox*1 data (Lisitsyna et al., 2019). Among the 10 genera in Cavisomidae, genus *Cavisoma* contains only one species *C. magnum* (Southwell, 1927), commonly occurring in the marine fishes of the orders Perciformes, Mugiliformes and Gonorynchiformes in Pacific Ocean (Amin et al., 2018).

The mt genomic data are important and useful genetic markers for phylogenetic studies to determine the evolutionary relationships of high taxa in Acanthocephala (Gazi et al., 2012; Weber et al., 2013; Gazi et al., 2015, 2016; Muhammad et al., 2019a,b; Song et al., 2019). However, our present knowledge regarding mt genome sequences of acanthocephalans is still very limited. To date, only three species of Echinorhynchida have been reported, namely *Leptorhynchoides thecatus* Linton, 1891 (Rhadinorhynchidae), *Echinorhynchus truttae* Schrank, 1788 (Echinorhynchidae) and *Brentisentis yangtzensis* Yu and Wu, 1989 (Illiosentidae) (Steinauer et al., 2005; Weber et al., 2013; Song et al., 2019). The lack of mt genomic data of representatives belonging to different family-level taxa embarrassed to establish a natural classification of Acanthocephala based on mitogenomic phylogenetic analysis.

In the present study, the complete mt genome of *C. magnum* was sequenced for the first time based on specimens collected from the *Mugil cephalus* Linnaeus, 1758 (Mugiliformes: Mugilidae) in the Arabian Gulf. In order to test the monophyly of the order Ehinorhynchida, and to assess the evolutionary relationships of Cavisomidae with the other families in Ehinorhynchida, the phylogenetic analysis based on the amino acid sequences of 12 mt protein-coding genes (PCGs) was performed using Bayesian inference (BI).

## 2. Materials and methods

### 2.1. Parasites collection

Acanthocephalan specimens were collected from the intestine of the flathead grey mullet *Mugil cephalus* Linnaeus (Mugiliformes: Mugilidae) in the Arabian Gulf (29°58′33″N, 48°28′20″E) and then stored in 70% ethanol until study. The specimens were identified as *C. magnum* based on the morphology and size of trunk, proboscis, neck, lemnisci and proboscis receptacle, the armature of proboscis, the number and size of cement glands and testes and the morphology of eggs (Amin et al., 2018). Voucher specimens were deposited in College of Life Sciences, Hebei Normal University, Hebei province, China (HBNU–A-2019F020L).

**Table 1**
Primers used for amplification and sequencing of the complete mitochondrial genome of *Cavisoma magnum*.

| Primers | Sequence (5′ → 3′) | Gene/Region | Size (bp) |
|---|---|---|---|
| CMF1 | GACTGCGCTAAGGTAGCGTG | 16S | 328 |
| CMR1 | GGTCTAAACTCAGATCACGTAC | | |
| CMF2 | GAGTTACTTAAGGGTTAACAG | 16S - *nad*5 | 4583 |
| CMR2 | CTGCTAAATACACACCTCAC | | |
| CMF3 | CATGATGGTTTTGTTGTTATG | *nad*5 | 305 |
| CMR3 | GTGGGAGCAGCTATAGCAAG | | |
| CMF4 | AGAGTTTTGTTTAGCTTTAG | *nad*5 - *cyt*b | 1725 |
| CMR4 | AACCGACTTAAAGACCACAC | | |
| CMF5 | GGTTATGTATTGCCTTGAGG | *cyt*b | 426 |
| CMR5 | CTCTGGCTTAATGTGGATAG | | |
| CMF6 | GGTATTACATTTTTGTTCAACC | *cyt*b - 12S | 1643 |
| CMR6 | AACACTATTACACAGGTATC | | |
| CMF7 | CTCTATTTCAGATAAAGTCTG | 12S | 395 |
| CMR7 | GTGTTGACGGGCGATATGTAC | | |
| CMF8 | GTTTATCGTATGATTGTTAGTGC | 12S - 16S | 5336 |
| CMR8 | GTGTTAATACAACTAACACC | | |

### 2.2. Molecular procedures

For molecular studies, the genomic DNA was extracted from one specimen (preserved in ethanol) using small-scale sodium dodecyl-sulfate /proteinase K solution with column purification system (Wizard_SV Genomic DNA Purification Kit, Promega, Madison, USA) according to the operation manual (Gasser et al., 2006). Long PCR reactions were conducted in a total volume of 30 μl, containing 13.5 μl PrimeSTAR Max DNA polymerase (Takara, Dalian, China), 13.5 μl $ddH_2O$, 1.5 μl of total genomic DNA and 1.5 μl of each primer (25 pmol) (Table 1). Long PCR reactions were performed with an initial denaturation at 96 °C for 1 min, 12 cycles for 15 s at 96 °C, 25 s at 52–56 °C, 0.5–5 min at 60 °C. Then the denaturation were performed at 94 °C for 2 min, 22 cycles of 15 s at 96 °C, 25 s at 52–56 °C, 0.5–5 min at 66 °C. The 68 °C final-extension was conducted for 10 min.

The amplified fragments were cloned in pMD19-T vector, and positive clones were sequenced by Sangon Company (Shanghai, China) and Genewiz (Beijing, China) using primer walking strategy (Table 1). For checking the chromatograms the Chromas v.1.62. was used. The sequences were assembled using DNASTAR (https://www.dnastar.com). The codon usage and nucleotide composition were analyzed using MEGA7 (Kumar et al., 2016). The boundaries of PCGs, as well as *rrn*L and *rrn*S were identified by comparison with other echinorhynchid acanthocephalan. The alignment of sequences was done using MAFFT 7.130. Amino acids sequences from 12 PCGs were derived using MEGA7, with invertebrate mt code chosen. Secondary structures and boundaries (data not shown) of 22 transfer RNA genes (tRNAs) with specific anticodons were identified using ARWEN (Laslett and Canback, 2008) or by comparing with those of other echinorhynchids species. The location of two ribosomal RNA genes (rRNAs) were identified by comparison with those of echinorhynchid species. Codon usage and relative synonymous codon usage (RSCU) of PCGs were calculated using PhyloSuite v1.1.15 (Zhang et al., 2020), with the plugin ggplot2 (Wickham, 2016) used to draw the RSCU figure.

### 2.3. Phylogenetic analysis

Phylogenetic analysis, using BI, was carried out based on concatenated amino acid sequences of 12 PCGs. The in-group samples contain 16 acanthocephalan species belonging to four classes, including Eoacanthocephala, Archiacanthocephala, Polyacanthocephala and Palaeacanthocephala. *Philodina citrina* Ehrenberg, 1832 (FR856884.1) and *Rotaria rotatoria* Pallas, 1766 (NC013568) (Rotifera: Bdelloidea) were selected as out-groups. Detailed information of mt sequences from

**Table 2**
The species, taxonomy and GenBank accession numbers for acanthocephalan phylogenetic analysis in this study. Newly sequenced species is shown in bold.

| Species | Systematic position | GenBank accession no. |
|---|---|---|
| ***Cavisoma magnum*** | **Palaeacanthocephala; Echinorhynchida** | **MN562586** |
| *Leptorhynchoides thecatus* | Palaeacanthocephala; Echinorhynchida | NC_006892 |
| *Brentisentis yangtzensis* | Palaeacanthocephala; Echinorhynchida | MK651258 |
| *Echinorhynchus truttae* | Palaeacanthocephala; Echinorhynchida | FR856883 |
| *Sphaerirostris picae* | Palaeacanthocephala; Polymorphida | MK471355 |
| *Centrorhynchus aluconis* | Palaeacanthocephala; Polymorphida | KT592357 |
| *Centrorhynchus milvus* | Palaeacanthocephala; Polymorphida | MK922344 |
| *Southwellina hispida* | Palaeacanthocephala; Polymorphida | KJ869251 |
| *Plagiorhynchus transversus* | Palaeacanthocephala; Polymorphida | KT447549 |
| *Oncicola lushes* | Archiacanthocephala; Oligacanthorhynchida | NC_016754 |
| *Macracanthorhynchus hirudinaceus* | Archiacanthocephala; Oligacanthorhynchida | FR856886 |
| *Hebesoma violentum* | Eoacanthocephala; Neoechinorhynchida | KC415004 |
| *Paratenuisentis ambiguus* | Eoacanthocephala; Neoechinorhynchida | FR856885 |
| *Acanthosentis cheni* | Eoacanthocephala; Gyracanthocephala | KX108947 |
| *Pallisentis celatus* | Eoacanthocephala; Gyracanthocephala | JQ943583 |
| *Polyacanthorhynchus caballeroi* | Polyacanthocephala; Polyacanthorhynchida | KT592358 |

**Table 3**
Organization of the mitochondrial genome of *Cavisoma magnum*.

| Gene/Region | Position 5′ to 3′ | Size (bp) | No. of aa | Ini/Ter codons | anti-codon | Int. seq. |
|---|---|---|---|---|---|---|
| *cox*1 | 1–1539 | 1539 | 512 | GTG/TAG | | –2 |
| tRNA-Gly (G) | 1538–1590 | 53 | | | TCC | 8 |
| tRNA-Gln (Q) | 1599–1666 | 68 | | | TTG | 0 |
| SNCR | 1667–1887 | 221 | | | | 0 |
| tRNA-Tyr (Y) | 1888–1940 | 53 | | | GTA | 0 |
| *rrn*L | 1941–2857 | 917 | | | | 0 |
| tRNA-Leu (L1) | 2858–2910 | 53 | | | TAG | 0 |
| *nad*6 | 2911–3322 | 412 | 137 | GTG/T | | –1 |
| tRNA-Asp (D) | 3322–3375 | 54 | | | GTC | 0 |
| tRNA-Ser (S2) | 3376–3438 | 63 | | | | 0 |
| *atp*6 | 3439–4017 | 579 | 192 | ATT/TAG | | –11 |
| *nad*3 | 4007–4352 | 346 | 115 | ATG/T | | 0 |
| tRNA-Trp (W) | 4353–4418 | 66 | | | TCA | 0 |
| LNCR | 4419–4891 | 473 | | | | 0 |
| tRNA-Lys (K) | 4892–4944 | 53 | | | CTT | −6 |
| tRNA-Glu (E) | 4939–4991 | 53 | | | TTC | 0 |
| tRNA-Thr (T) | 4992–5044 | 53 | | | TGT | −22 |
| *nad*4L | 5023–5277 | 255 | 84 | GTG/TAG | | −16 |
| *nad*4 | 5262–6465 | 1204 | 401 | ATT/T | | 0 |
| tRNA-Val (V) | 6466–6526 | 61 | | | TAC | 166 |
| tRNA-His (H) | 6693–6746 | 54 | | | GTG | −1 |
| *nad*5 | 6746–8356 | 1611 | 536 | ATG/TAG | | −1 |
| tRNA-Leu (L2) | 8356–8407 | 52 | | | TAA | 4 |
| tRNA-Pro (P) | 8412–8462 | 51 | | | TGG | 0 |
| *cyt*b | 8463–9593 | 1131 | 376 | GTG/TAG | | 5 |
| *nad*1 | 9599–10,486 | 888 | 295 | GTG/TAG | | −27 |
| tRNA-Ile (I) | 10,460–10,519 | 60 | | | GAT | −18 |
| tRNA-Met (M) | 10,502–10,562 | 61 | | | CAT | −4 |
| *rrn*S | 10,559–11,104 | 546 | | | | 0 |
| tRNA-Phe (F) | 11,105–11,158 | 54 | | | GAA | −6 |
| *cox*2 | 11,153–11,779 | 627 | 208 | ATG/TAG | | −10 |
| tRNA-Cys (C) | 11,770–11,825 | 56 | | | GCA | 20 |
| *cox*3 | 11,846–12,523 | 678 | 225 | ATG/TAA | | −2 |
| tRNA-Ala (A) | 12,522–12,576 | 55 | | | TGC | –12 |
| tRNA-Arg (R) | 12,565–12,624 | 60 | | | TCG | −5 |
| tRNA-Asn (N) | 12,620–12,673 | 54 | | | GTT | −3 |
| tRNA-Ser (S1) | 12,671–12,723 | 53 | | | GCT | −9 |
| *nad*2 | 12,715–13,593 | 879 | 292 | GTG/TAG | | 1 |

SNCR: short non-coding region, LNCR: long non-coding region, bp: base pair, aa: amino acid, Ini/Ter: initial/terminal codons, Int. seq.: intergenic sequences.

acanthocephalan species which were involved in the phylogenetic analysis were summarized (Table 2). Poorly aligned regions of the amino acids alignment were wiped off using Gblocks v.0.91b (Talavera and Castresana, 2007), with the resulting file converted into nexus format and then subjected to MrBayes v 3.1.1 (Ronquist and Huelsenbeck, 2003) using the mixed model (Castoe and Parkinson, 2006). BI analysis was run for 1,000,000 metropolis-coupled MCMC generations and sampling a tree with every 1000th generations with four independent Markov chain Monte Carlo (McMc) chains. The first 250 generations were treated as “burn-in”. The phylograms were viewed using FigTree v. 1.43 (Chen et al., 2014).

**Table 4**
Nucleotide composition of the mitochondrial genome of *Cavisoma magnum*.

| Nucleotide sequence | Size (bp) | A (%) | C (%) | T (%) | G (%) | A + T (%) | G + C (%) |
|---|---|---|---|---|---|---|---|
| Overall | 13,594 | 20.9 | 8.9 | 42.1 | 28.1 | 63.0 | 37.0 |
| Protein coding genes | 10,119 | 18.3 | 8.8 | 43.5 | 29.4 | 61.8 | 38.2 |
| Codon position[a] | | | | | | | |
| 1st | 3373 | 20.1 | 9.4 | 33.7 | 36.8 | 53.8 | 46.2 |
| 2nd | 3373 | 12.7 | 12.2 | 49.3 | 25.9 | 62.0 | 38.1 |
| 3rd | 3373 | 22.0 | 5.0 | 47.5 | 25.6 | 69.5 | 30.6 |
| Ribosomal RNA genes | 1463 | 31.0 | 9.6 | 35.9 | 23.6 | 66.9 | 33.2 |
| Transfer RNA genes | 1251 | 25.1 | 9.4 | 39.1 | 26.5 | 64.2 | 35.8 |
| Short non-coding region | 221 | 27.6 | 5.4 | 43.9 | 23.1 | 71.5 | 28.5 |
| Long non-coding region | 473 | 33.4 | 7.2 | 37.2 | 22.2 | 70.6 | 29.4 |

[a] Termination codons are excluded.

**Table 5**
Genetic code and codon usage for the 12 mitochondrial protein-coding genes of *Cavisoma magnum*.

| Codon | aa | No. | % | Codon | aa | No. | % |
|---|---|---|---|---|---|---|---|
| TTT | Phe | 250 | 7.41 | TAT | Tyr | 94 | 2.79 |
| TTC | Phe | 8 | 0.24 | TAC | Tyr | 16 | 0.47 |
| TTA | Leu | 230 | 6.82 | TAA | [a] | 0 | 0.00 |
| TTG | Leu | 201 | 5.96 | TAG | [a] | 0 | 0.00 |
| CTT | Leu | 84 | 2.49 | CAT | His | 34 | 1.00 |
| CTC | Leu | 5 | 0.15 | CAC | His | 12 | 0.36 |
| CTA | Leu | 28 | 0.83 | CAA | Gln | 14 | 0.42 |
| CTG | Leu | 17 | 0.50 | CAG | Gln | 19 | 0.56 |
| ATT | Ile | 153 | 4.54 | AAT | Asn | 32 | 0.95 |
| ATC | Ile | 11 | 0.33 | AAC | Asn | 1 | 0.03 |
| ATA | Met | 76 | 2.25 | AAA | Lys | 27 | 0.80 |
| ATG | Met | 70 | 2.08 | AAG | Lys | 31 | 0.92 |
| GTT | Val | 264 | 7.83 | GAT | Asp | 51 | 1.51 |
| GTC | Val | 22 | 0.65 | GAC | Asp | 14 | 0.42 |
| GTA | Val | 110 | 3.26 | GAA | Glu | 18 | 0.53 |
| GTG | Val | 134 | 3.97 | GAG | Glu | 65 | 1.93 |
| TCT | Ser | 100 | 2.96 | TGT | Cys | 73 | 2.16 |
| TCC | Ser | 4 | 0.12 | TGC | Cys | 9 | 0.27 |
| TCA | Ser | 18 | 0.53 | TGA | Trp | 51 | 1.51 |
| TCG | Ser | 8 | 0.24 | TGG | Trp | 74 | 2.19 |
| CCT | Pro | 28 | 0.83 | CGT | Arg | 16 | 0.47 |
| CCC | Pro | 10 | 0.30 | CGC | Arg | 1 | 0.03 |
| CCA | Pro | 20 | 0.59 | CGA | Arg | 12 | 0.36 |
| CCG | Pro | 7 | 0.21 | CGG | Arg | 10 | 0.30 |
| ACT | Thr | 37 | 1.10 | AGT | Ser | 87 | 2.58 |
| ACC | Thr | 4 | 0.12 | AGC | Ser | 13 | 0.39 |
| ACA | Thr | 22 | 0.65 | AGA | Ser | 44 | 1.30 |
| ACG | Thr | 5 | 0.15 | AGG | Ser | 65 | 1.93 |
| GCT | Ala | 85 | 2.52 | GGT | Gly | 214 | 6.34 |
| GCC | Ala | 9 | 0.27 | GGC | Gly | 29 | 0.86 |
| GCA | Ala | 36 | 1.07 | GGA | Gly | 35 | 1.04 |
| GCG | Ala | 17 | 0.50 | GGG | Gly | 139 | 4.12 |

[a] Stop (termination) codon excluded; no.: number of copies; aa: amino acid.

## 3. Results and discussion

### 3.1. Gene content and organization

The mt genome of *C. magnum* was 13,594 bp long (GenBank accession no. MN562586) with 36 genes, including 12 PCGs (*nad*1–6, *nad*4L, *cox*1–3, *atp*6 and *cyt*b), 22 tRNAs and two rRNAs (*rrn*L and *rrn*S), with two non-coding regions (SNCR and LNCR). The lack of the gene *atp*8 in the mt genome of the other acanthocephalans has been reported (Gazi et al., 2016). All genes in *C. magnum* mt genome were encoded on the same strand and in the same direction (Fig. 1). In *C. magnum* mt genome, the gene contents of PCGs are identical with all the other mt genomes of acanthocephalans reported so far (Gazi et al., 2016; Muhammad et al., 2019a). Four translocations of tRNAs (*trn*S1, *trn*S2, *trn*V, and *trn*K) were observed. Genes were either positioned next to each other without intergenic spacers or separated by intergenic spacers of 1–166 nucleotides or even overlapped each other by 1–27 nucleotides (Table 3).

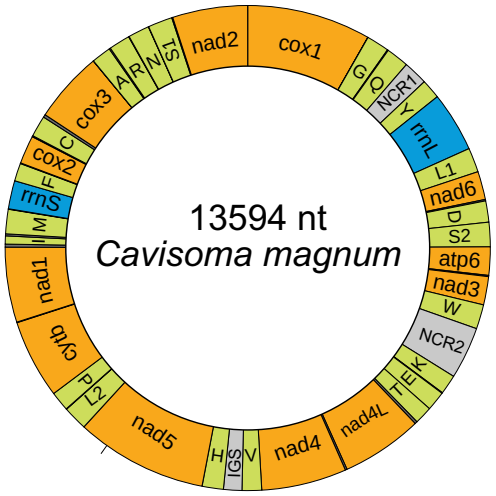


**Fig. 1.** Organization of the complete mitochondrial genome of *C. magnum*. All 22 tRNA genes are nominated by the one-letter code with numbers differentiating each of the two tRNAs serine and leucine. All genes are transcribed in the clockwise direction on the same strand. SNCR and LNCR represents short non-coding and long non-coding region, respectively.

In the mt genome of *C. magnum*, the nucleotide contents are 20.9% A (2847 bp), 28.1% G (3818 bp), 42.1% T (5718 bp) and 8.9% C (1211 bp) (Table 4). The overall A + T contents in the mt genome of *C. magnum* are 63.0%, which are less than those in *Acanthosentis cheni* Amin, 2005 (65.25%) and *Southwellina hispida* (Van Cleave, 1925) (63.9%), and more than the mt genomes of *Pallisentis celatus* (Van Cleave, 1928) (61.5%), *Plagiorhynchus transversus* (Rudolphi, 1819) (61.1%), *Oncicola luehei* (Travassos, 1917) (60.2%), *Hebesoma violentum* (Van Cleave, 1928) (59.4%), *Sphaerirostris picae* (Rudolphi, 1819) (58.1%) *Polyacanthorhynchus caballeroi* Diaz-Ungria & Rodrigo, 1960 (56.3%), *Centrorhynchus aluconis* (Müller, 1780) (55.6%) and *C. milvus* Ward, 1956 (54.5%) (Gazi et al., 2012; Pan and Nie, 2013; Song et al., 2016; Pan and Jiang, 2018; Muhammad et al., 2019a,b).

### 3.2. Protein-coding genes and codon usage

Twelve PCGs were 10,119 bp in length which encoded 3373 amino acids, and contained 3373 codons excluding termination codons. The 12 PCGs of *C. magnum* ranging in size from 255 bp (*nad*4L) to 1611 bp (*nad*5). Higher T content (43.5%) in 12 PCGs of *C. magnum* mtDNA, as also reported in other acanthocephalan species, correlates with relatively high frequency of T-rich codons (Gazi et al., 2016). The most frequently used codon is GTT for valine (7.83%), followed by TTT for phenylalanine (7.41%) and TTA for leucine (6.82%). The least commonly used codons are AAC, CGC, TCC and ACC which are used at

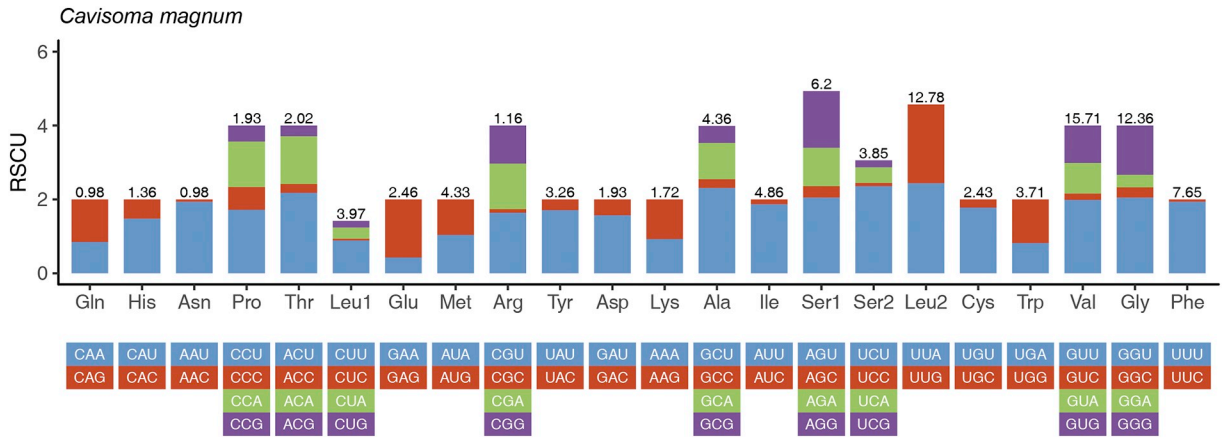


**Fig. 2.** Relative Synonymous Codon Usage (RSCU) of 12 PCGs of *Cavisoma magnum*, the codon families are labelled on the x-axis. Values on the top of each bar represent amino acid usage in percentage.

0.03%, 0.03%, 0.12% and 0.12%, respectively (Table 5). In 12 PCGs of *C. magnum* mt genome, leucine (16.7%) is the most frequently used amino acid, followed by valine (15.7%) and glycine (12.4%). The RSCU and overall codon usage for the construction of all 12 PCGs are shown (Fig. 2). The high frequency of these two amino acids are also reported in *S. picae* [leu: 14.0%, val: 11.0%] (Muhammad et al., 2019a) and *P. transversus* [leu: 15.0%, val: 14.4%] (Gazi et al., 2016).

The initiation and termination codons usages of 12 PCGs of mt genome of *C. magnum* show common features with the mt genomes of other acanthocephalan. GTG is the most frequently start codon which was used for six PCGs (*cox*1, *nad*6, *nad4*L, *cyt*b, *nad*1 and *nad*2). Four genes (*nad*3, *nad*5, *cox*2 and *cox*3) were inferred to use ATG as the start codon. Two genes (*atp*6 and *nad*4) were inferred to use ATT as the start codon. Among 12 PCGs, eight genes (*cox*1, *atp*6, *nad*4L, *nad*5, *cyt*b, *nad*1, *cox*2 and *nad*2) were terminated with complete stop codon TAG, while *cox*3 was inferred to terminate with complete stop codon TAA. The incomplete stop codon T, was used for the remaining three genes (*nad*6, *nad*3 and *nad*4) (Table 3). The presence of incomplete stop codon is also common in other acanthocephalan reported so far (Steinauer et al., 2005; Gazi et al., 2012; Pan and Nie, 2013; Gazi et al., 2015, 2016; Pan and Jiang, 2018; Muhammad et al., 2019a,b; Song et al., 2019). The codons information of 12 PCGs in *C. magnum* mt genome is provided (Table 3).

### 3.3. Ribosomal and transfer RNA genes

*rrn*L and *rrn*S were 917 bp and 546 bp in length, respectively (Table 3). The A + T contents of *rrn*L and *rrn*S were 67.6% and 65.6%, respectively. The *rrn*L in *C. magnum* is located between *trn*Y and *trn*L1, which has the same position as in other acanthocephalan species reported so far, except for *Macracanthorhynchus hirudinaceus* (Pallas, 1781), where *rrn*L lies between *trn*Y and *trn*L2 (Weber et al., 2013). The *rrn*S is located between *trn*M and *trn*F, which has the same position as in other acanthocephalan species sequenced so far except for *L. thecatus* and *P. celatus*, where it lies between *trn*S1 and *trn*F (Steinauer et al., 2005; Pan and Nie, 2013). In the complete mt genome sequences of *C. magnum*, the 22 tRNAs were identified ranging from 51 bp (*trn*P) to 68 bp (*trn*Q) in size. The anticodons (Table 3) and secondary structures (data not shown) of tRNAs were also identified. Twenty tRNAs genes contain TV-replacement loop and lack TΨC arm similar to tRNAs of other acanthocephalans. Two tRNAs *trn*S1 and *trn*S2 lacked dihydrouridine (DHU) arm, while the remaining tRNAs were predicted to be folded into 'cloverleaf' secondary structure (Gazi et al., 2016; Muhammad et al., 2019b).

### 3.4. Non-coding regions

In the mt genome of *C. magnum*, SNCR, 221 bp in length, is located between *trn*Q and *trn*Y. LNCR, which is 473 bp in length, is located between *trn*W and *trn*K. Their A + T contents are 71.5% and 70.6%, respectively (Table 4).

### 3.5. Gene order

The gene order of the mt genome provides useful information for inferring the metazoans phylogenetic relationships (Littlewood et al., 2006; Luo et al., 2008; Wang et al., 2017; Zhang et al., 2018; Song et al., 2019). In the mt genomes of *C. magnum* (Fig. 3), gene arrangement of PCGs and rRNAs is in the following order: *cox*1, *rrn*L, *nad*6, *atp*6, *nad*3, *nad*4L, *nad*4, *nad*5, *cyt*b, *nad*1, *rrn*S, *cox*2, *cox*3 and *nad*2, which is relatively conserved as all the other acanthocephalans (Wey-Fabrizius et al., 2013; Gazi et al., 2016; Muhammad et al., 2019b).

Because of the small size, tRNAs in the mt genomes of acanthocephalan species are tend to have more variability in translocation (Kilpert and Podsiadlowski, 2006; Song et al., 2019). There are up to four translocations in tRNAs (*trn*S1, *trn*S2, *trn*V, and *trn*K) observed in the mt genomes of all acanthocephalan species sequenced so far. In *C. magnum* mt genome, *trn*V, *trn*S2 and SNCR have different positions, especially *trn*V and SNCR, which show complete new locations comparing to all the other acanthocephalan species reported so far. *trn*S2 situated between *trn*D and *atp*6, while *trn*V lied between *nad*4 and *trn*H. SNCR located between *trn*Q and *trn*Y.

### 3.6. Phylogenetic analysis

The phylogenetic analysis in the present study indicated that the selected representatives of the phylum Acanthocephala were divided into three different large branches with high nodal support (BPP = 1) (Fig. 4). The species *O. luehei* and *M. hirudinaceus* grouped together representing the monophyletic class Archiacanthocephala as basal to the remaining three classes of Acanthocephala. Apart from the phylogenetic status of Polyacanthocephala, this result agreed well with the previous molecular studies (Near et al., 1998; García-Varela et al., 2000; Near, 2002; García-Varela and Nadler, 2005; Verweyen et al., 2011; Weber et al., 2013; Gazi et al., 2015). The present phylogenetic relationships between four classes of Acanthocephala is also supported by the previous complete mitogenomics phylogenetic studies (Gazi et al., 2016; Muhammad et al., 2019a,b; Song et al., 2019). The species *C. magnum*, *L. thecatus*, *S. hispida*, *P. transversus*, *S. picae*, *Echinorhynchus truttae*, *B. yangtzensis*, *C. aluconis* and *C. milvus* formed the largest clade in the BI tree, that represents the class Palaeacanthocephalas. Within

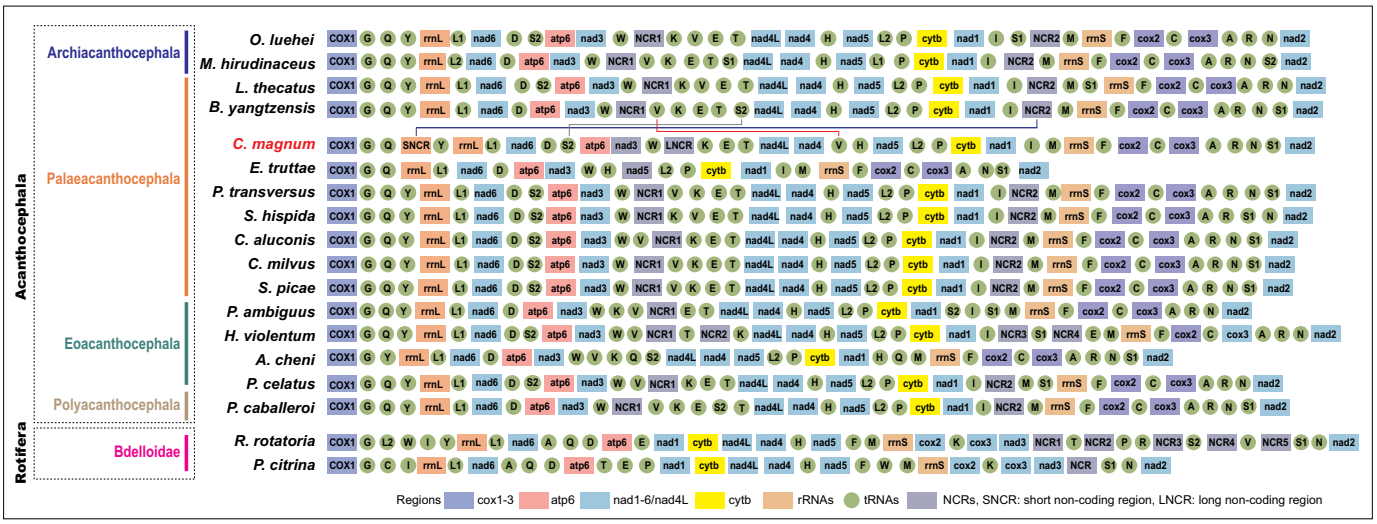


**Fig. 3.** Linearized comparison of the mitochondrial gene arrangement for 16 acanthocephalan species and two rotifer species. Gene and genome size are not to the scale. All genes are encoded in the same direction from left to right. The tRNAs are labelled by single-letter code for the corresponding amino acid. The red colour indicates the newly complete mtDNA sequenced species in this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the clade Palaeacanthocephala, the representatives of Polymorphida (including *C. aluconis*, *C. milvus*, *S. picae*, *P. transversus* and *S. hispida*) formed a monophyletic group, and the Polymorphidae (*S. hispida*) and Plagiorhynchidae (*P. transversus*) displayed more close relationship than the family Centrorhynchidae (*C. aluconis*, *S. picae* and *C. milvus*). The results are consistent with previous phylogenetic analyses based on the mitogenomic data (Gazi et al., 2016; Song et al., 2016; Muhammad et al., 2019a,b) or rDNA data (García-Varela et al., 2019). But the monophyly of Polymorphida is not supported by several previous molecular studies based on rDNA data (García-Varela and Nadler, 2005; Verweyen et al., 2011; Radwan, 2012).

Our phylogenetic analysis also showed the species *S. picae* nested in the representatives of the genus *Centrorhynchus* Lühe, 1911. It is easy to understand that when we considered the taxonomic history of *Sphaerirostris*. Golvan erected the subgenus *Sphaerirostris* in the genus *Centrorhynchus* based on morphological characters (Golvan, 1956). Later, this subgenus *Sphaerirostris* was raised to a full generic level (Amin and Canaris, 1997). In fact, *Sphaerirostris* was established mainly based on the size and shape of trunk and testes and the morphology and armature of the proboscis. However, there are considerable variations in these characters among different species of *Centrorhynchus*. It seems to be unreasonable and unjustified to treat *Sphaerirostris* as a separated

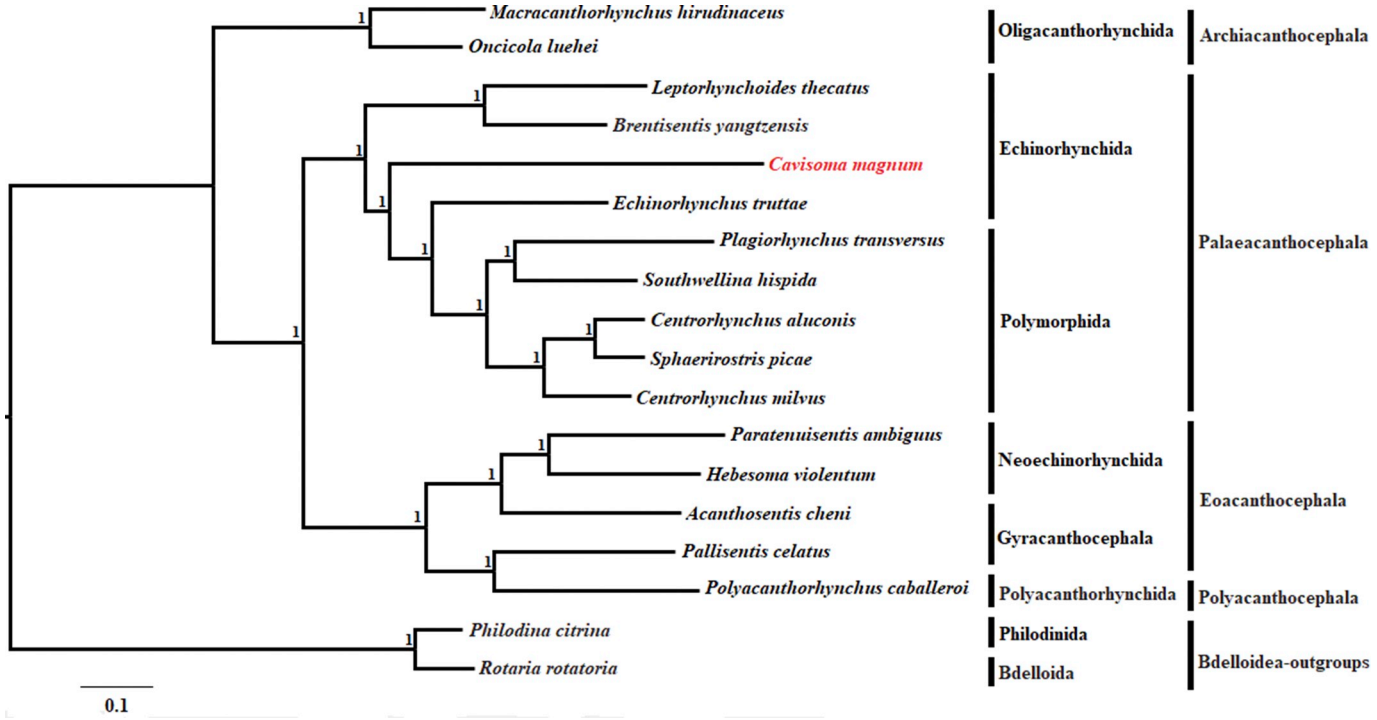


**Fig. 4.** Phylogenetic tree of acanthocephalans constructed using Bayesian inference (BI) based on the concatenated amino acid sequences of 12 PCGs. *Philodina citrina* (FR856884.1) and *Rotaria rotatoria* (GQ304898.1) (Bdelloidea: Rotifera) used as outgroup.

genus.

It is surprised that *C. magnum* did not cluster together with the other representatives of Echinorhynchida (*L. thecatus*, *E. truttae* and *B. yangtzensis*). The present phylogenetic analysis indicated that the order Echinorhynchida is not a monophyletic group, which is consistent with some previous studies based on morphological (Monks, 2001) or partial nuclear and/or mt genes (Near et al., 1998; García-Varela et al., 2000, 2002; Near, 2002; Herlyn et al., 2003; García-Varela and Nadler, 2005, 2006; Verweyen et al., 2011; Gazi et al., 2015, 2016; García-Varela et al., 2019). The species *P. caballeroi*, only representative of the class Polyacanthocephala formed a clade with *P. celatus*, one of the representatives of the class Eoacanthocephala. The relationship of Eoacanthocephala and Polyacanthocephala need further study. It is very useful to solve these above-mentioned systematic problems if more species of acanthocephalans representing more high-level taxa with their mt genomic data available.

## 4. Conclusion

The present study sequenced the complete mt genome sequences of *C. magnum* for the first time, which represents the first species of the family Cavisomidae with the characterization of the complete mt genome reported. Phylogenetic analyses of amino acid sequences based on amino acids of 12 PCGs using BI supported the class Palaeacanthocephala and its included order Polymorphida to be monophyletic, but rejected the monophyly of the order Echinorhynchida. Our phylogenetic results also challenged the validity of the genus *Sphaerirostris* (Polymorphida: Centrorhynchidae). The relationship between Polyacanthocephala and Eoacanthocephala remains unclear. The novel mt genomic data of *C. magnum* are very useful for understanding the evolutionary history of this group of parasites and establishing a natural classification of Acanthocephala.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

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