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Characterization of the complete mitochondrial genome of *Plagiorchis maculosus* (Digenea, Plagiorchiidae), Representative of a taxonomically complex digenean family

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ABSTRACT

Despite the highly divergent morphology, pathogenicity and worldwide distribution of digenean parasites belonging to one of the largest families, the Plagiorchiidae, there are no complete mitochondrial (mt) genomes published to date for plagiorchiids. In this study, we obtained nuclear ribosomal DNA (ITS region and 28S rDNA) sequences and the complete mt genome sequences of *Plagiorchis maculosus* (Rudolphi 1802) Braun, 1902, and assessed its phylogenetic relationship with other xiphidiates, based on the mtDNA sequences. The obtained ITS and 28S rDNA sequences were identical to the corresponding sequences of *P. maculosus* available in GenBank. The complete mitochondrial genome of *P. maculosus* (14,124 bp) contained 36 genes (*atp*8 is absent) and a long non-coding region (NCR) with two sets of repeated sequences of 283 nucleotides each. The phylogenetic tree resulting from Bayesian inference (BI) analyses based on concatenated nucleotide sequences of all 36 genes of *P. maculosus* and other xiphidiates mitochondrial genomes, indicated that *P. maculosus* (and the Plagiorchiidae) is phylogenetically closest to the Brachycladiidae and Paragonimidae. The present study describes the first mitochondrial genome from the type genus of the family Plagiorchiidae. The overall gene arrangement, nucleotide composition, A + T contents, AT and GC skew and codon usage with relative synonymous codon usage (RSCU) for 12 PCGs are described. Characterization of mitochondrial genomes from additional plagiorchiid taxa is necessary to make further progress in phylogenetic and epidemiological studies of these digeneans as well as accurate diagnostics of these parasites including those parasitic in humans.

1. Introduction

The Plagiorchiidae Lühe, 1901 is a very large, globally distributed family of digeneans parasitic in the digestive system of a variety of vertebrate hosts including humans [1,2]. Plagiorchiids parasitize virtually all parts of the digestive tract and other visceral organs of their definitive hosts including the bile ducts, gall bladder, liver, lungs, ureters and kidneys [1]. The Plagiorchiidae is the central family of the large superfamily Plagiorchioidea Lühe, 1901. Despite its obvious systematic, phylogenetic and practical importance, it remains one of the least understood and phylogenetically understudied groups of digeneans [3]. Despite extensive work done on the systematics and biology of the Plagiorchiidae, there are still numerous questions and controversies regarding the systematic position of a number of genera and evolutionary interrelationships among its members [1,4]. This situation stems from the extreme morphological diversity of the taxa currently placed in the clearly non-monophyletic Plagiorchiidae [1] and the lack of agreement regarding reliable diagnostic criteria. Moreover, poor original descriptions, unknown ranges of morphological variability and, in some cases, low host specificity among plagiorchiids have also added confusion to their systematics [6].

Plagiorchis Lühe, 1899 is the most species rich genus in the family containing more than 140 formally described species [7]. Species of *Plagiorchis* have a three-host life cycle that includes freshwater snails as

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first intermediate hosts [6,8,9], arthropods as second intermediate hosts and vertebrates, including humans, as definitive hosts [10–12]. Human plagiorchiasis likely results from ingestion of insect larvae or adults containing metacercariae of *Plagiorchis* [13]. To date, 12 cases of natural human infections by five different *Plagiorchis* species have been reported in Thailand, Indonesia, Japan, Korea and the Philippines [10,12]. *Plagiorchis maculosus* (Rudolphi, 1802) Braun, 1902 was originally described as *Fasciola maculosa* from the intestine of *Hirundo rustica* Linnaeus, 1758 and later transferred to *Plagiorchis* by Braun [14]. It is a common intestinal fluke of insectivorous birds and is one of the most widely spread species of this genus [15,16].

The use of nucleotide sequences has fostered significant progress in our understanding of the plagiorchioid systematics and phylogeny [4,5,17] including species diversity, taxonomic status and identification of *Plagiorchis* spp. [4,6,9,18,19]. Mitochondrial DNA (mtDNA) genes have proved to be particularly useful for accurate species identification and delimitation as well as for studies of inter- and intra-specific variation in trematodes characterized by high levels of morphological uniformity [20,21]. However, sequences of complete mitochondrial genomes of any plagiorchiid are currently lacking. In this study, we provide a complete mt genome sequence and functional annotation of *P. maculosus*, report the composition of its mt genome and analyse its molecular phylogenetic relationships with other xiphidiatan taxa. In addition, we sequenced the ITS region and partial 28S gene of *P. maculosus* from Pakistan to compare with previously available sequences of the species from Europe.

2. Materials and methods

2.1. Trematode specimens and DNA extraction

A total of five adult specimens of *P. maculosus* were recovered from the intestine of their definitive hosts, barn swallow *H. rustica* (3 specimens) and cinereous tit *Parus cinereus* Vieillot, 1818 (2 specimens) collected in the Swabi district, Khyber Pakhtunkhwa province, Pakistan. Specimens were killed with hot water, fixed in 80% ethanol and stored in a freezer [22]. The permanent mounts of three voucher specimens were prepared according to the protocol recommended by Lutz et al. [22] and identified morphologically as *P. maculosus* based on existing keys and descriptions [1,23,24]. For molecular studies, the total genomic DNA was extracted from two individual *P. maculosus* (each from a different host), using small-scale sodium dodecyl-sulfate (SDS)/proteinase K treatment [25] and mini-column purification (Wizard® SV Genomic DNA Purification System, Promega, Madison, USA) according to the manufacturer's instructions.

2.2. Amplification of ITS and 28S rDNA

The ITS rDNA region was amplified with the primers BD1 (5′-GTC GTA ACA AGG TTT CCG TA-3′) and BD2 (5′-TAT GCT TAA ATT CAG CGG GT-3′) [26], and the partial 28S rDNA gene was amplified with LSU-5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) [27,28]. Both rDNA markers were amplified by PCR using Ex Taq™ Version 2.0 PCR mix (Takara, Dalian, China); annealing temperature was set at 55 °C for both markers. The positive amplicons were purified using EZNA Gel Extraction Kit (OMEGA Bio-tek Inc., Doraville, GA, USA) and sequenced directly by Genewiz sequencing company (Beijing, China).

2.3. PCR-based sequencing of complete mitogenome

Six pairs of platyhelminth-universal primers were used for amplification and sequencing of six genes (partial) by conventional PCRs. The sequence data obtained which was further used to design seven pairs of primers (Table 1) for amplification of seven medium to long fragments (1–3.5 kb) of overlapping regions. Long PCR reactions were conducted in total volume of 28 µl containing 12.5 µl PrimeStar Max DNA polymerase premix (Takara, Dalian, China), 1 µl of each primer (synthesized in Genewiz, Suzhou, China), 1 µl DNA templates and 12.5 µl dd H_2O. Long-PCRs were performed with an initial denaturation for 2 min at 98 °C, followed by 10 cycles, each including denaturation for 15 s at 92 °C, annealing for 30 s at 50–56 °C and extension for 1–3 min (depending on the fragment length) at 68 °C followed by denaturation for 2 min at 92 °C, and 22 more cycles each including steps of 10 s at 92 °C, 30 s at 50–56 °C and 1–3 min at 68 °C, and the final extension for 10 min at 68 °C. PCR products were either sequenced directly (size up to 2 kb) or cloned (size above 2 kb) in pMD19-T vector. PCR or bacterial products were sequenced in Genewiz (Beijing, China) and GenScript (Nanjing, China) using the primer-walking strategy.

Table 1
Primers used for amplification and sequencing of complete mitochondrial genome of *Plagiorchis maculosus*.

Primer	Sequence (5′ → 3′)	Location/Region amplified
PMF1-F	ATAATTCAGGTTGTTTCAGCAGTG	Partial *cyt*b-partial *nad*4
PMF1-R	ACACACCAAGAATACCCAACTTC	
PMF2-F	TCTATTGTTGTTGCTTGTGTTAGAG	Partial *nad*4-partial *nad*1
PMF2-R	TTACACTCACATAAAACCCCTACG	
PMF3-F	CTCTTATGAGTTCTATTCGATCTGC	Partial *nad*1-partial *cox*1
PMF3-R	CTTAAGGTCTAAGCCAACCATAAAC	
PMF4-F	GCTATGGGAGCTATAGTTTGTTTG	Partial *cox*1-partial *rrn*L
PMF4-R	AATCGATATGAACTCTCCTCATCTC	
PMF5-F	TCCCTTCATGAGTTGAGTTAAGAC	Partial *rrn*S-partial *nad*5
PMF5-R	ATAACACTTACAAACCCCGTGTC	
PMF6-F	TTAGCTCTTTGGTGCACTCTTCTAC	Partial *nad*5-tRNA-H
PMF6-R	CTTTCAAAGAAACTTCGAACACCAC	

2.4. Mitogenome annotation and data analyses

The sequences obtained were quality-proofed and then assembled manually using DNAstar v.7.1 software [29]. At least four sequences (two from each strand) were aligned to ensure accuracy. *P. maculosus* mtDNA sequence was aligned against the complete mt genomes of other xiphidiatan trematodes using MAFFT 7.149 [30] in order to determine the gene boundaries and relative positions. Protein-coding genes (PCGs) were determined by ORFs tool available from the National Center for Biotechnology Information (NCBI), employing the relevant mitochondrial genetic code (Codon table 9). The tRNAs sequences and structures (structure data not shown) were identified using ARWEN [31] and MITOS [32] web servers. The two rRNA genes, 16S (*rrn*L) and 12S (*rrn*S) were identified by comparison with other trematode mt genomes in the alignment. The single non-coding region (NCR) was determined by recognition of boundaries between *trn*G and *cox*3. The manually annotated mitogenome was further used to generate the GenBank submission file (*.sqn), tables and comparative statistics using a GUI-based program, PhyloSuite-v.1.1.13 [33]. PhyloSuite was also used to calculate codon usage and relative synonymous codon usage (RSCU) in *P. maculosus* 12 mitochondrial PCGs. The obtained file was further sorted and used to draw RSCU figure using the ggplot2 [34], plugin of PhyloSuite. The nucleotide composition and AT/GC skew of *P. maculosus* mtDNA was calculated using PhyloSuite. Comparative line plots of Adenine + Thymine (A + T) content and AT/GC skew for all published and newly sequenced xiphidiatan mitogenomes were constructed in ggplot2 using the data generated by PhyloSuite. Tandem Repeats Finder [35] and mreps [36] were used to predict tandem repeats (TRs) in the NCR and their secondary structures were predicted by Mfold software [37]. The pairwise comparison of linear gene order and their rearrangement events in xiphidiates were assessed using the CREx program [38] employing the common interval measurement.

2.5. Phylogenetic analyses

To assess the phylogenetic interrelationship of *P. maculosus* and Plagiorchiidae within Xiphidiata, the newly obtained mt genome of *P. maculosus* was aligned with all xiphidiatan mt genomes published to date, namely *Brachycladium goliath* (KR703278), *Paragonimus westermani* (AF219379), *P. heterotremus* (NC_039430), *Eurytrema pancreaticum* (KP241855), *Dicrocoelium chinensis* (KF318786) and *D. dendriticum* (KF318787). A species of the order Diplostomida *Schistosoma japonicum* (AF215860) was used as outgroup. The nucleotide sequences of all 36 genes (12 PCGs, 2 rRNAs and 22 tRNAs) for each of the above species were extracted from GenBank files using PhyloSuite [33] and used for further analyses. The dataset was processed in PhyloSuite, adopting codon-based alignment of 12 PCGs and normal alignment of RNAs in batches using MAFFT, as integrated in PhyloSuite with the implementation of different modes of alignment. PhyloSuite was subsequently used for further analyses as follows: the ambiguous sites and gaps were deleted by GBlocks [39], the sequences were further concatenated into a single alignment and converted into nexus format files. ModelFinder [40] was used for the selection of the most appropriate evolutionary model. Based on the Akaike information criterion, GTR + F + I + G was chosen as the optimal model of nucleotide evolution. BI analysis was conducted in MrBayes 3.2.6 [41] with the following settings: 3,000,000 metropolis-coupled Markov chain Monte Carlo (MCMC) generations, sample frequency of 1000 and burnin parameter set at 750. Finally, phylogram and gene orders of each xiphidiate were visualized and annotated by iTOL [42] with the aid of several dataset files generated by PhyloSuite.

3. Results and discussion

3.1. Molecular identification of specimens

In addition to morphological examination, we confirmed the identity of our specimens using sequence data. The ITS rDNA region was 1250 bp long (GenBank accession no. MK641808) and the sequenced fragment of the 28S rDNA was 1382 bp long (GenBank accession no. MK641807). Sequences of both nuclear ribosomal DNA regions were identical to the corresponding sequences of *P. maculosus* (ITS rDNA, GenBank accession nos. AF316152, KJ533390 and KJ533391; 28S rDNA, KJ533395) [6,43]. Moreover, the partial *cox*1 sequences of our isolate have 99.05% similarity with the published *cox*1 sequences of *P. maculosus* (GenBank accession no. KJ533424, 423 bp) [6]. Thus, molecular data supported the morphological identification of our specimens as *P. maculosus*.

3.2. Mitochondrial genome arrangement and nucleotide usage

Mitochondrial genome of *P. maculosus*, was 14,124 bp long (GenBank accession no. MK641809). The circular mt genome comprised 36 genes: 12 protein-coding genes (*nad*1–6, *nad*4L, *cox*1–3, *cyt*b and *atp*6), 22 transfer RNA genes, two ribosomal RNA genes (*rrn*L and *rrn*S) with a single long non-coding region (Fig. 1). Genes were either separated by short intergenic spacers of 1–27 nucleotides or were positioned next to each other without intergenic spacers or even overlapped each other by 1–40 nucleotides (Table 2). Here, we visualized the arrangement of all 36 mt genes and NCRs of xiphidiates (Fig. 4 and Supplementary file 1: Fig. S1) however, the existence of two copies for a specific tRNA (other than *trn*L1, *trn*L2, *trn*S1 and *trn*S2), presence of pseudo tRNA genes, lack of a specific tRNA and discontinuous evolution of mitogenomic architecture in a specific group of animals, including helminths [44,45], creates some problems for the use of mitochondrial gene order as a tool for inferring phylogenetic relationships. The gene order in *P. maculosus* was similar to that in the Paragonimidae and Dicrocoeliidae except for the altered position of *trn*E and *trn*G and single NCR in *P. maculosus*. It should be noted, however, that *trn*Y and

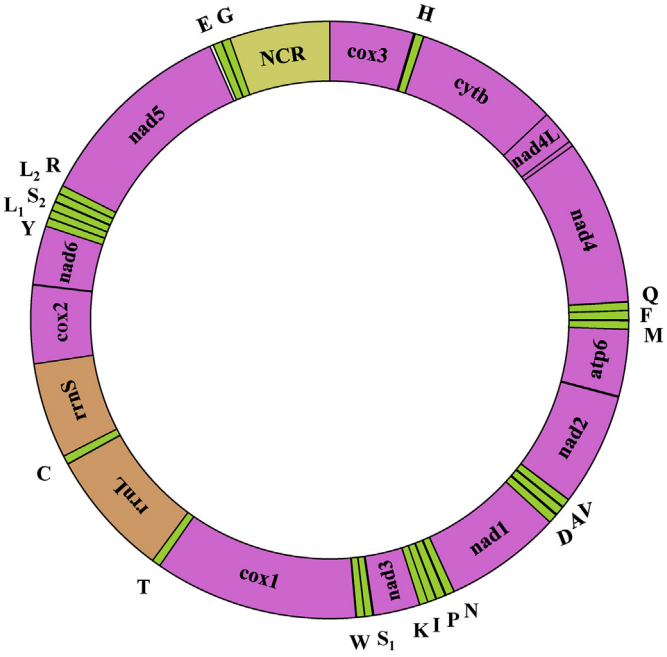

Fig. 1. Organization of the complete mitochondrial genome of *Plagiorchis maculosus*. All 22 tRNA genes are designated by the one-letter code with numbers differentiating each of the two tRNAs leucine and serine. All genes are transcribed on the same strand in the clockwise direction. NCR: non-coding region.

*trn*E have a unique position in the mt genome of *B. goliath* (Fig. 4). For the pairwise comparison of gene order among xiphidiatans, we used CREx program to calculate the pairwise similarity between all published xiphidiatan mitogenomes. The transformational pathway from *P. maculosus* to the very similar gene order observed in the Paragonimidae and Dicrocoeliidae (pairwise similarity value: 1186 over 1254) required a single transposition (Supplementary file 1: Fig. S1).

The nucleotide contents in the complete mt genome of *P. maculosus* are: 18.8% A (2,655), 22.8% G (3,218), 46.4% T (6,559) and 12% C (1,692) with total A + T percentage of 65.24% (9214). The entire mt genome, individual gene, and codon (1st, 2nd and 3rd codon position) exhibited a strong A + T bias (Supplementary file 2: Table S1). The overall A + T content in protein-coding genes is 65.27% which is very close to that of whole mt genome; the highest A + T value was observed in *nad*3 (69.2%) and the lowest in *cox*2 (62.8%). Both *rrn*L and *rrn*S consist of 1716 nucleotides and their A + T contents are 66.5% and 64.6% respectively. Noteworthy, the A + T content in the entire mt genome of *P. maculosus* is the highest among all xiphidiatan mt genomes sequenced so far (Fig. 2). Nevertheless, it is within the range observed in other trematode mt genomes, e. g., 51.7% in the Japan isolate of *P. westermani* (AF219379), 54.6% in the Indian isolate of *P. westermani* [46] and 73.3% in *Schistosoma spindale* [47].

3.3. Protein-coding genes and codon usage

Concatenated PCGs were 10,117 bp in length. The protein-coding genes were initiated with ATG or GTG and terminated with stop codons TAA or TAG or with an incomplete stop codon T (*cox*1 only) (Table 2). The overall codon usage, RSCU, and codon family proportion (corresponding to the amino acid usage) used in all 12 PCGs are presented in Fig. 3. The most common amino acids were leucine (L1, 5.53% and L2, 10.95%), serine (S1, 4.14% and S2, 6.28%) and Phenylalanine (12.67%). The frequently used codons were, UUU (396 instances), UUG-Leu and GUU-Val (221 instances each), while the least common codons were CCA-Pro (4 instances) and CGC-Arg (2 instances). The use of amino acid Phenylalanine and codon UUU (396 instances) in 12 PCGs of *P. maculosus* mitogenome were highest among all published

Table 2
The annotated mitochondrial genome of *Plagiorchis maculosus*.

Genes	Position 5′ to 3	Length		Ini/Ter codons	tRNA anti-codon	Int. Seq. length (bp)
		bp	aa			
*cox*3	1–645	645	214	ATG/TAG		+11
tRNA-His (H)	657–723	67			GTG	+2
*cyt*b	726–1838	1113	370	ATG/TAG		0
*nad*4L	1839–2105	267	88	GTG/TAG		−40
*nad*4	2066–3334	1269	422	ATG/TAG		+3
tRNA-Gln (Q)	3338–3402	65			TTG	+1
tRNA-Phe (F)	3404–3473	70			GAA	+7
tRNA-Met (M)	3481–3545	65			CAT	0
*atp*6	3546–4058	513	170	ATG/TAG		+8
*nad*2	4067–4933	867	288	ATG/TAA		+2
tRNA-Val (V)	4936–5002	67			TAC	+7
tRNA-Ala (A)	5010–5076	67			TGC	+9
tRNA-Asp (D)	5086–5155	70			GTC	0
*nad*1	5156–6055	900	299	ATG/TAG		−1
tRNA-Asn (N)	6055–6120	66			GTT	+8
tRNA-Pro (P)	6129–6198	70			TGG	+5
tRNA-Ile (I)	6204–6268	65			GAT	0
tRNA-Lys (K)	6269–6332	64			CTT	+1
*nad*3	6334–6690	357	118	ATG/TAA		+8
tRNA-SerAGN (S1)	6699–6758	60			GCT	+2
tRNA-Trp (W)	6761–6821	61			TCA	+5
*cox*1	6827–8393	1567	522	ATG/T		0
tRNA-Thr (T)	8394–8460	67			TGT	0
*rrn*L	8461–9439	979				−2
tRNA-Cys (C)	9439–9503	66			GCA	0
rrnS	9504–10,240	737				0
*cox*2	10,241–10,840	600	199	ATG/TAA		+5
*nad*6	10,846–11,298	453	150	GTG/TAA		0
tRNA-Tyr (Y)	11,299–11,362	64			GTA	+3
tRNA-LeuCUN (L1)	11,366–11,426	61			TAG	−2
tRNA-SerUCN (S2)	11,425–11,489	65			TGA	+6
tRNA-LeuUUR (L2)	11,496–11,559	64			TAA	+3
tRNA-Arg (R)	11,563–11,625	63			TCG	0
*nad*5	11,626–13,191	1566	521	ATG/TAG		+27
tRNA-Glu (E)	13,219–13,285	67			TTC	+3
tRNA-Gly (G)	13,289–13,354	66			TCC	
NCR	13,355–14,124	770				

bp: base pairs; aa: amino acids; Ini/Ter: initiation/termination; Int. Seq.: intergenic sequences; NCR: non-coding region.

xiphidiate species (Supplementary file 3: Table S2).

3.4. Transfer and ribosomal RNA genes

A total of 22 tRNA genes identified in the mt genome of *P. maculosus*, varying in length from 60 bp to 70 bp (Table 2) accounted for 1506 bp in total concatenated length. Twenty of the tRNAs can be folded into the conventional cloverleaf secondary structure while the remaining two, tRNAs for serine (S1 and S2), are lacking the dihydrouridine (DHU) arm which is replaced by a loop of 6–12 nucleotides. The lack of D-arms in these two tRNAs in the *P. maculosus* mt genome is consistent with the previous reports on mt genomes of other digeneans where D-arms are replaced by 6–10 nt in *F. hepatica* [48], 6–9 nt in *O. viverrini* and *C. sinensis* [49], 8–11 nt in *F. gigantica* [50], 7–15 nt *E. hortense* [51] and 7–15 nt in *E. pancreaticum* [52].

In the mt genome of *P. maculosus*, the large ribosomal RNA gene and the small ribosomal RNA gene are located between *trn*T and *cox*2, separated by *trn*C. The length of *rrn*L (16S) and *rrn*S (12S) are 979 bp and 737 bp, respectively. The position of *rrn*L and *rrn*S in the mt genome of *P. maculosus* is also similar to that in mt genomes of other digenean trematodes with the exception of *S. japonicum* and *S. mansoni* in which two *trn*C (C1 and C2) are situated between *rrn*L and *rrn*S [53], and *Orientobilharzia turkestanicum* in which the *trn*C is not located between *rrn*L and *rrn*S [54]. The *rrn*S gene in *P. maculosus* is shorter than in all other digenean mt genomes published to date, except for *D. spathaceum* and *D. pseudospathaceum* (*rrn*S, 725 bp) [55].

3.5. Non-coding region

Apart from short inter-genic sequences (1–27), the single non-coding region (770 bp) found in the mt genome of *P. maculosus*, is located between *trn*G and *cox*3. The A + T content of this NCR is 65.6% which is quite similar to that of coding regions. The NCR region contains two sets of repeated sequences, 283 nucleotides each, which are located at positions 13,370–13,652 and 13,839–14,121. Since these repeated sets of sequences were not located next to each other, they were not detected by Tandem Repeats Finder [35] and mreps [36] web tools. Both sets of repeated nucleotides were found manually and were 100% identical; therefore, we provide the secondary structure as predicted by Mfold software, for only one of them (Supplementary file 4: Fig. S2). The repeats are separated from each other by a stretch of 186 nucleotides.

3.6. Molecular phylogeny

In our analysis we used the nucleotide sequences of all 36 mitochondrial genes from seven digeneans belonging to the Xiphidiata [27,56] using *S. japonicum* as the outgroup. The obtained phylogenetic tree is characterized by a strongly supported branch topology (all BI posterior probabilities values were 1.0) (Fig. 4). The phylogenetic tree indicates two major clades, one containing *P. maculosus* clustered together with members of the Paragonimidae and the Brachycladiidae and the other clade containing only three dicrocoeliids.

Recently, Kostadinova and Pérez-del-Olmo [56] emphasized the

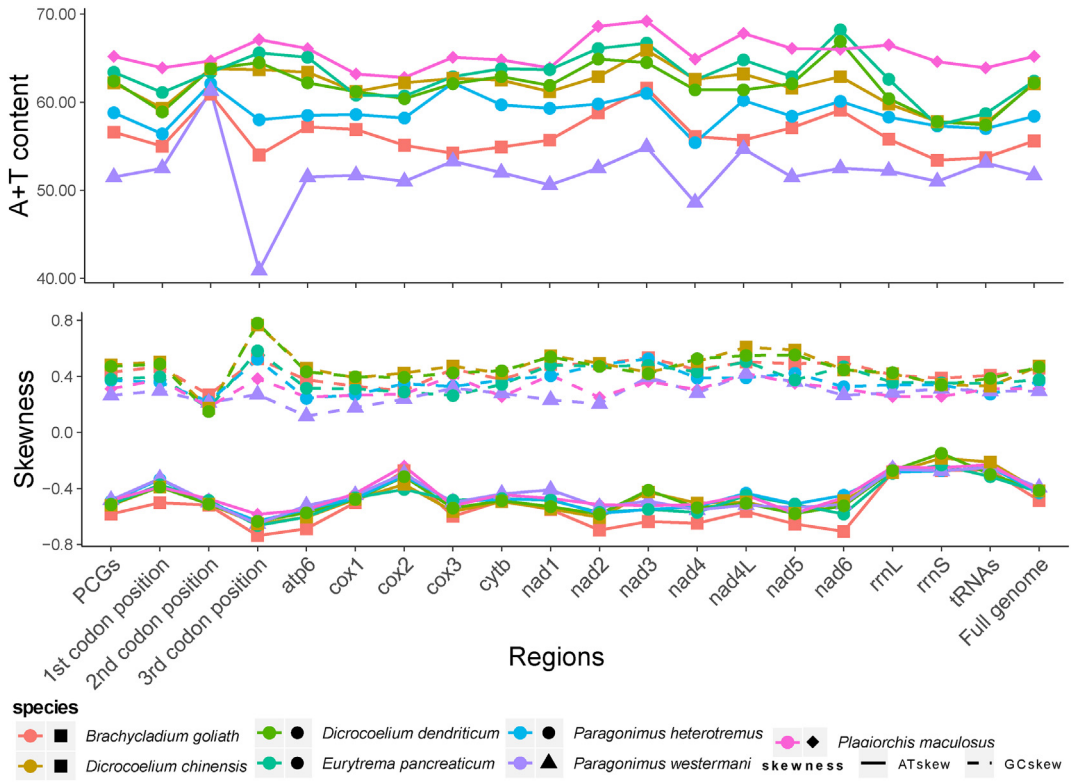

Fig. 2. A + T content and nucleotide skewness of genes, individual elements and the complete mitogenome of seven xiphidiates. Lines colour and point represent a specific xiphidiate species.

deficiency of molecular data on the central genus of the Plagiorchiidae and suggested the need of clarification of the content and position of the superfamily Plagiorchioidea based on molecular data on *Plagiorchis* as an important reference point. Interrelationships of a large number of taxa belonging to the Plagiorchiidae as well as the intra- and inter-specific variability within *Plagiorchis* have been previously studied based on 1–2 DNA regions only, such as nuclear rRNA (ITS, 28S) and partial mt *cox*1 gene [4,6,18]. However, the use of the complete mt genomes provides a more extensive set of characters for molecular phylogenetic analyses among Platyhelminthes than a few relatively short DNA targets [57].

The complete mitogenome was not previously available for any member of the family Plagiorchiidae. Our analysis using all 36 genes of *P. maculosus* and other xiphidiatan digeneans (Published to date), represents the first attempt to infer the phylogenetic position of the Plagiorchiidae based on complete mt genome data. In some disagreement to the previously published data [27], the Paragonimidae (a member of the Gorgoderoidea according to Olson et al. [27]) in our analysis appears to be more closely related to the Plagiorchiidae (Plagiorchioidea) and Brachycladiidae (Allocreadioidea) than to the Dicrocoeliidae which belongs to the Gorgoderoidea. More detailed analyses incorporating complete mitochondrial genomes of a broader variety of taxa should shed more light on the interrelationships of this highly diverse major digenean lineage. Meanwhile, the availability of the complete mitogenome of *P. maculosus* will provide basis for development of new markers useful for plagiorchiid identification, phylogenetic and epidemiological studies.

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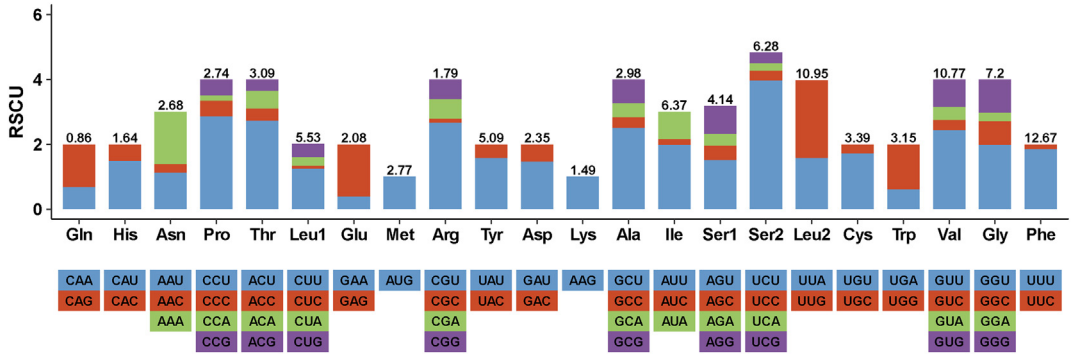

Fig. 3. Relative synonymous codon usage (RSCU) of *Plagiorchis maculosus* mitogenome. Codon families are labelled on the x-axis. Values on the top of the bars indicate percentage of each amino acid used for the construction of 12 protein-coding genes.

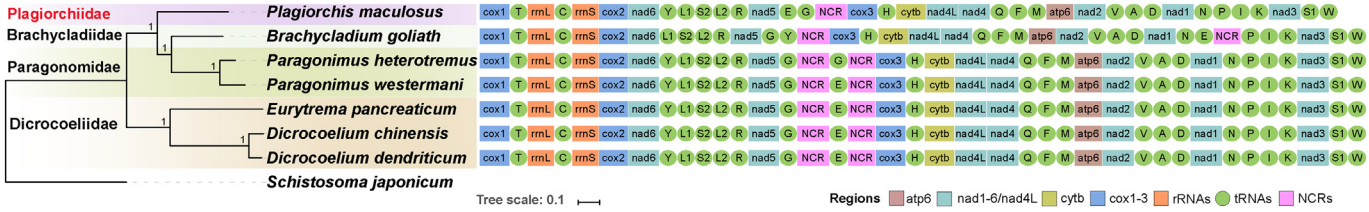

Fig. 4. Phylogeny based on Bayesian inference (BI) with mitogenomic architecture of four families of the suborder Xiphidiata, using almost complete mitogenomic datasets (excluding non-coding regions). *Schistosoma japonicum* is the outgroup. Families are shown in different colour with the gene order displayed to the right of the tree. Numbers above branches indicate posterior probabilities.

Conflict of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2019.04.001.

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