Journal of Helminthology

cambridge.org/jhl

Mitochondrial genome of *Rhabdosynochus viridisi* (Monogenea: Diplectanidae), a parasite of Pacific white snook *Centropomus viridis*

V. Caña-Bozada[1], R. Llera-Herrera[2], E.J. Fajer-Ávila[1] and F.N. Morales-Serna[1,2]

[1]Centro de Investigación en Alimentación y Desarrollo, A.C., Mazatlán 82112, Sinaloa, Mexico and [2]Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán 82040, Sinaloa, Mexico

Short Communication

Cite this article: Caña-Bozada V, Llera-Herrera R, Fajer-Ávila EJ, Morales-Serna FN (2021). Mitochondrial genome of *Rhabdosynochus viridisi* (Monogenea: Diplectanidae), a parasite of Pacific white snook *Centropomus viridis*. *Journal of Helminthology* **95**, e21, 1–5. https://doi.org/10.1017/S0022149X21000146

Received: 7 February 2021
Accepted: 20 March 2021

Keywords:
Platyhelminthes; Monopisthocotylea; monogenean; mitogenome; fish parasite; marine water

Author for correspondence:
V. Caña-Bozada, E-mail: victorcana1991@hotmail.com

Abstract

We report the nearly complete mitochondrial genome of *Rhabdosynochus viridisi* – the first for this genus – achieved by combining shotgun sequencing of genomic and cDNA libraries prepared using low-input protocols. This integration of genomic information leads us to correct the annotation of the gene features. The mitochondrial genome consists of 13,863 bp. Annotation resulted in the identification of 12 protein-encoding genes, 22 tRNA genes and two rRNA genes. Three non-coding regions, delimited by three tRNAs, were found between the genes *nad*5 and *cox*3. A phylogenetic analysis grouped *R. viridisi* with three other species of diplectanid monogeneans for which mitochondrial genomes are available.

Introduction

Monogeneans are parasitic flatworms (Platyhelminthes) found mostly on freshwater and marine fish, although some species can infect aquatic or semi-aquatic sarcopterygians such as lungfish, and amphibians, freshwater turtles and hippopotamuses. This group of parasites is economically important as some species can cause disease and mortality in fish farmed around the world (Schelkle *et al.*, 2009; Ogawa, 2015). The short and direct life cycle of these parasites favours their proliferation within production tanks and cages. Monogeneans of the Diplectanidae family in particular pose a problem for the production of marine fish (Tokşen *et al.*, 2013; Andree *et al.*, 2015). Such is the case for *Rhabdosynochus viridisi* Montero-Rodríguez, Mendoza-Franco & López Téllez, 2020, a diplectanid species recently described as a threat to the pisciculture of Pacific white snook (*Centropomus viridis* Lockington, 1877) in Mexico (Montero-Rodríguez *et al.*, 2020; Morales-Serna *et al.*, 2020).

Although there are more than 250 species of diplectanids (Domingues & Boeger, 2008), mitochondrial genomes (mitogenomes) have been reported for only three species: *Pseudorhabdosynochus yangjiangensis* Wu & Li, 2005 [also known as *Laticola paralatesi* (Nagibina, 1976)] (Tingbao *et al.*, 2006), *Lepidotrema longipenis* (Yamaguti, 1934) Kritsky, Jiménez-Ruiz & Sey, 2000 and *Lamellodiscus spari* Zhukov, 1970 (Zhang *et al.*, 2018a). Mitogenomes are considered useful for reconstructing phylogenies and inferring population history due to haploid maternal inheritance (Boore *et al.*, 2005; Stewart & Larsson, 2014). However, some studies have questioned the basic assumptions about the evolutionary dynamics of mitochondrial DNA (mtDNA), that is clonality, near-neutrality and evolutionary rate constancy. They suggest that mtDNA is not a valid molecular marker of population genetics and phylogenetics, and that data on mtDNA might instead provide insight into many aspects of mitogenome evolution (Galtier *et al.*, 2009). Generating mitogenomes is important for supporting comparative mitogenomics studies at the boundary between evolutionary biology and medicine (Galtier *et al.*, 2009). In the present study, we assembled and annotated the mitogenome of *R. viridisi* using short-read sequencing, and inferred its phylogenetic relationship with other monogeneans for which mitogenomes are publicly available.

Materials and methods

Monogenean *R. viridisi* were obtained from the gills of juvenile *C. viridis* maintained in the Laboratory of Parasitology at the Centro de Investigación en Alimentación y Desarrollo (CIAD), Mazatlán, northwestern Mexico (Morales-Serna *et al.*, 2020). Prior to the present study, 10 samples of monogeneans ($n = 10$) were randomly taken from ten individual fish contained in a single 400-l tank. These specimens were fixed with formaldehyde and stained with Gomori's trichrome or partially digested with proteinase K to observe their morphological characteristics under a microscope (BX53; Olympus). Monogeneans were identified as *R. viridisi* according to the method of Montero-Rodríguez *et al.* (2020). Voucher specimens were deposited in the Colección de Parásitos de Peces del Noroeste del Pacífico

CAMBRIDGE UNIVERSITY PRESS

(CPPNP-1379) at CIAD-Mazatlán. *Rhabdosynochus viridisi* morphologically resembles *Rhabdosynochus alterinstitus* Mendoza-Franco, Violante-Gonzalez & Vidal-Martinez, 2008; however, the former can be distinguished by the presence of tegumental scales and an accessory piece with a proximal bifurcation of the vagina. For the present study, 260 live specimens were isolated from a single fish host, washed in distilled water and then fixed in RNAlater (Thermo Fisher, Waltham, MA). Of these specimens, 60 were first observed for tegumental characteristics and then partially digested with proteinase K to observe the sclerotized structures. All the specimens examined were identified as *R. viridisi*.

The DNA and RNA were isolated from the remaining 200 individuals of *R. viridisi*. Reamplification, sequencing, assembling, annotation and phylogenetic analysis were performed as described by Caña-Bozada *et al.* (2021). However, in the present study, mitochondrial genes of the diplectanids *P. yangjiangensis*, *L. longipenis*, and *L. spari* (GenBank accession numbers JQ038231.1, NC_039617.1 and MH328204.1, respectively) were used as references to obtain DNA contigs and for annotation. The DNA and cDNA assembled using MEGA X software (Kumar *et al.*, 2018) and TRINITY v. 2.8.6 (Grabherr *et al.*, 2011), respectively, failed to find a region within the mitogenome. However, an assembly of the cDNA paired reads obtained with MEGAHIT (Li *et al.*, 2016) (parameter: –k-list 21, 29, 39, 59, 79, 99, 119, 141) and posteriorly reassembled with CAP3 (Huang & Madan, 1999), with an identity cut-off of 95% (setting: –p N > 95), enabled such a missing region to be found, and therefore a nearly complete mitogenome was obtained. The identity and boundaries of protein-coding genes (PCGs) and rRNA were determined using ORF Finder tools [available from the National Center for Biotechnology Information (NCBI)] with the genetic code 9, and a comparison with alignments of the mitochondrial genes of species closely related to *R. viridisi* (species of the family Diplectanidae; see the codes in supplementary table S1) in MEGA X. OGDRAW v. 1.1 (Greiner *et al.*, 2019) was used to visualize the resulting mitogenome. In addition, coverage of the mitochondrial genome was visualized using Integrative Genomics Viewer (Thorvaldsdóttir *et al.*, 2013) and BLAST Ring Image Generator (Alikhan *et al.*, 2011).

The PCGs and rRNA genes of the new mitogenome of *R. viridisi* and 36 monogenean mitogenomes available in GenBank (supplementary table S1) were used for a phylogenetic analysis. Two planarian species (*Crenobia alpina* and *Obama* sp.) were used as outgroups (Zhang *et al.*, 2018a). The best partition scheme and the optimal models of molecular evolution as determined by PartitionFinder 2 (Lanfear *et al.*, 2017) are presented in supplementary table S2. Based on the corrected Akaike information criterion, the optimal model of nucleotide evolution was GTR + G + I. A maximum likelihood (ML) tree was constructed in RAxML v. 8.1.12 (Stamatakis, 2014), with 1000 bootstrap replicates and joint branch length optimization. In addition, Bayesian inference (BI) was performed using MrBayes v. 3.2.2 (Ronquist *et al.*, 2012) over ten million generations, sampling the Markov chain at a frequency of 100 generations and using the default settings. The best partition scheme and the optimal models of molecular evolution for the analysis of BI are presented in supplementary table S3. The CREx program (Bernt *et al.*, 2007) was used to analyse rearrangement events in the mitogenomes and to make pairwise comparisons of gene orders of Diplectanidae utilizing the breakpoint dissimilarity measurement.

Results and discussion

Mitogenome characterization

The sequencing runs yielded totals of 15.14 million and 12.10 million reads for the DNA and cDNA, respectively. Of these, 8.63 million and 3.81 million reads, respectively, were retained after quality trimming and filtering. The raw reads were deposited into the NCBI's SRA database under accession numbers SRR13359482 and SRR11563382 (BioProject PRJNA689569).

The DNA reads were assembled into 21 contigs with lengths varying from 234 to 5892 bp and an average coverage of 1128 X. The cDNA reads were assembled into 1 contig with an average coverage of 3153 X (supplementary fig. S1). The assemblies of DNA and cDNA were aligned to correct errors in the frame of translation of the DNA assembly. Specifically, the *nad*4, *nad*1 and *nad*6 genes each presented a region with 1 bp, which we considered false. The correction of errors in these three genes seemed to be related to the assembly obtained by MEGAHIT rather than to coverage (coverage >1000 times). Furthermore, alignment helped to predict the *trn*C gen and the first 123 bases in the *cox*3 gene. The use of MEGAHIT and CAP3 to assemble the cDNA enabled identification of the *trn*S1 gene and two non-coding regions (NCRs) of 585 and 239 bp located between the *trn*G and *trn*C genes. For *trn*S1, cDNA coverage was greater than 300 bp, whereas for DNA, coverage was less than 7 bp. The greater coverage of certain regions in the mitochondrial genome from the cDNA reads, together with the use of MEGAHIT and TRINITY for their assembly, allowed us to obtain all the genes in the mitogenome of *R. viridisi*. It is important to note that the sequences used from the pool of individuals enabled identification of 120 nucleotides (0.86%) in the mitogenome in which at least 20% of the nucleotide coverage differed from the consensus. Whereas the ribosomal genes presented the fewest nucleotide variations (*rrn*L 3 bp, 0.32%; *rrn*S 1 bp, 0.14%), the NCRs had the most variations (33 bp, 3.9%). These bases might represent the most common variations within *R. viridisi* (supplementary fig. S2).

The nearly complete mitogenome of *R. viridisi* is 13,863 bp in size and includes 22 tRNA genes (including two for the amino acids serine and leucine), 12 PCGs (*atp*8 gene is absent) and two rRNA genes (table 1 and supplementary fig. S3). The total base composition is 30% A, 46.9% T, 7.6% C and 15.5% G (supplementary table S4). The annotated sequence was deposited in the NCBI GenBank (accession number MW565922). The majority of PCGs use start codons ATG or GTG, which are canonical for genetic code 9, and either TAA or TAG as the stop codon, which has been also reported for other monogeneans (e.g. Vanhove *et al.*, 2018; Zhang *et al.*, 2019). In *L. longipenis* and *L. spari*, TTG and ATT have also been reported as start codons for the *nad*2 gene (Zhang *et al.*, 2018a). However, in *R. viridisi*, we only found ATG for *nad*2, and in *nad*4L, and TTG also appeared as start codon. The *cox*3 gene terminates with the abbreviated T codon, and thus was the only gene that does not use a canonical stop codon.

A peak was found in the DNA coverage on the first NCR, which had an AT content of 78.5%, but without repeating regions. This might indicate that the length of this NCR was underestimated; hence we refer to the *R. viridisi* mitogenome reported here as a nearly complete.

Three NCRs were found. The first consists of 585 bp located between the *trn*G and *trn*S1 genes and has an AT content of 78.5%. The second NCR is 239 bp in size and is located between

https://doi.org/10.1017/S0022149X21000146 Published online by Cambridge University Press

Table 1. Features of the mitochondrial genome of *Rhabdosynochus viridisi*.

	Position				Codon	
Gene	From	To	Length (bp)	Intergenic nucleotides	Start	Stop
*cox*3	1	649	649		ATG	T
*trn*H	649	715	67	−1		
cytb	715	1803	1089	−1	ATG	TAG
*nad*4L	1805	2050	246	1	TTG	TAA
*nad*4	2020	3222	1203	−31	ATG	TAA
*trn*Q	3227	3290	64	4		
*trn*F	3290	3354	65	−1		
*trn*M	3345	3409	65	−10		
*atp*6	3409	3918	510	−1	ATG	TAA
*nad*2	3921	4793	873	2	ATG	TAA
*trn*A	4791	4856	66	−3		
*trn*D	4855	4916	62	−2		
*trn*V	4919	4983	65	2		
*nad*1	4984	5886	903		GTG	TAG
*trn*N	5889	5953	65	2		
*trn*P	5962	6025	64	8		
*trn*I	6024	6090	67	−2		
*trn*K	6092	6157	66	1		
*nad*3	6160	6513	354	2	ATG	TAA
*trn*W	6514	6575	62			
*cox*1	6576	8132	1557		ATG	TAA
*trn*T	8135	8197	63	2		
16S rRNA	8198	9131	934			
12S rRNA	9132	9868	737			
*cox*2	9869	10,441	573		ATG	TAG
*trn*L1	10,442	10,504	63			
*trn*S2	10,503	10,571	69	−2		
*trn*E	10,570	10,635	66	−2		
*nad*6	10,635	11,084	450	−1	GTG	TAA
*trn*Y	11,086	11,146	61	1		
*trn*L2	11,147	11,213	67			
*trn*R	11,219	11,285	67	5		
*nad*5	11,287	12,828	1542	1	ATG	TAG
*trn*G	12,831	12,895	65	2		
NCR	12,896	13,480	585			
*trn*S1	13,481	13,536	56			
NCR_copy2	13,537	13,775	239			
*trn*C	13,776	13,841	66			
NCR_copy3	13,842	13,863	22			

the *trn*S1 and *trn*C genes. It has an AT content of 91.2% and has a 182-bp region that consists of four tandem repeats (TRs) of between 39 and 48 bp each. The third NCR consists of 22 bp located between the *trn*C and *cox*3 genes, and has an AT content of 90.9%. The AT content of the second and third NCRs is much higher than in other parts of the mitogenome (supplementary

https://doi.org/10.1017/S0022149X21000146 Published online by Cambridge University Press

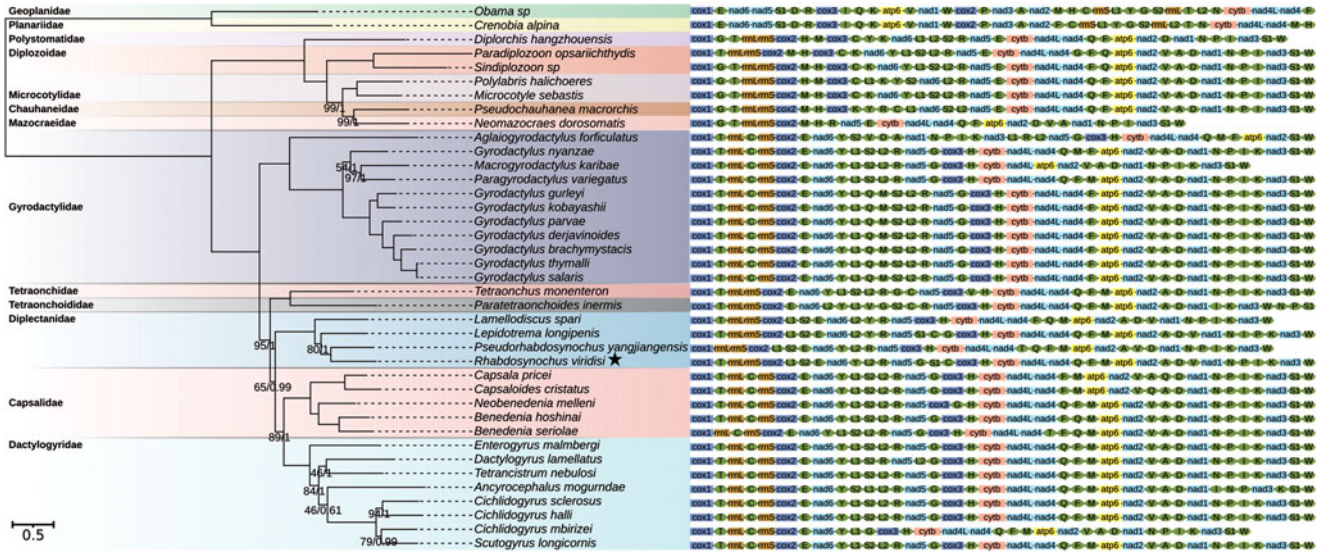

Fig. 1. Phylogenetic analysis of the mitochondrial genomes of seven species from five families (Polystomatidae, Diplozoidae, Chauhaneidae, Mazocraeidae and Microcotylidae) of the Polyopisthocotylea and 30 species from six families (Gyrodactylidae, Tetraonchidae, Tetraonchoididae, Diplectanidae, Capsalidae and Dactylogyridae) of the Monopisthocotylea. Statistical support values of maximum likelihood and Bayesian analyses are shown below the nodes (ML/BI), except for nodes with maximum support. The scale bar corresponds to the estimated number of substitutions per site. Monogenean families are shown in different colours. Gene order is displayed to the right of the tree.

table S4). The high number of TRs and the high AT content in these three NCRs, which are delimited by three tRNAs, posed a challenge to completing the mitogenome of *R. viridisi*. To overcome this problem, we combined the results obtained using three assemblers. It seems that a long NCR between *nad*5 and *cox*3 is common in diplectanids (Zhang *et al.*, 2018a). The mitogenome of *R. viridisi* contains fewer TRs than those of *L. longipenis* and *L. spari*. Such differences in the number of TRs have also been observed among representatives of the Dactylogyridae family (Zhang *et al.*, 2018a, b).

Phylogenetic and gene order analyses

The phylogenetic analysis included seven species from five families of the Polyopisthocotylea and 30 species from six families of the Monopisthocotylea. The topologies of the ML and BI trees were identical; nonetheless, the BI tree was better supported statistically (fig. 1). Each subclass formed a monophyletic group. Within the Monopisthocotylea, *R. viridisi* appeared clustered with diplectanids, as a sister group of *P. yangjiangensis*, with strong bootstrap support. The diplectanids formed a sister group with the clade formed by the Dactylogyridae and Capsalidae, which is consistent with mitogenome trees previously reported for monogeneans (Zhang *et al.*, 2019).

The gene order in the mitogenome of *R. viridisi* was different from the other members of the Diplectanidae family. Three transposition events of tRNA genes were found between *R. viridisi* and *L. longipeni*, and two events were found between *R. viridisi* and both *P. yangjiangensis* and *L. spari* (supplementary fig. S4). Gene order rearrangement has been previously observed in monogeneans. For instance, Ye *et al.* (2014) reported different arrangements in tRNA genes in monopisthocotylids, which was explained by the duplication–random loss model and recombination model together with a parsimonious scenario. In another example, Zhang *et al.* (2019) reported that the order of the two rRNAs in the mitogenomes of two species of *Thaparocleidus* was different with respect to all Neodermata. They suggested that evolution of mitogenomic gene order arrangements is discontinuous in monogeneans, as gene orders in a proportion of monogenean taxa are highly variable, whereas the remaining are conserved. The present study revealed that, although in a different order, there are three tRNA genes (*trn*G, *trn*S1 and *trn*C) located between *nad*5 and *cox*3 in the diplectanids *L. longipenis* and *R. viridisi*. This is in contrast with the gene order in the other monogenean families, in which only *trn*G is typically located between *nad*5 and *cox*3 (fig. 1). It is possible that this gene order is conserved among diplectanids.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X21000146.

Financial support. This research was funded by the National Council of Science and Technology of Mexico (CONACYT) through the grant 'Ciencia de Frontera', FORDECYT–PRONACES No. 1715616. V.C.-B. thanks CONACYT for his graduate studentship.

Conflict of interest. The authors declare none.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Alikhan NF, Petty NK, Zakour NLB and Beatson SA (2011) BLAST ring image generator (BRIG): Simple prokaryote genome comparisons. *BMC Genomics* **12**, 1–10.

Andree KB, Roque A, Duncan N, Gisbert E, Estevez A, Tsertou MI, *et al.* (2015) *Diplectanum sciaenae* (Van Beneden & Hesse, 1863) (Monogenea) infecting meagre, *Argyrosomus regius* (Asso, 1801) broodstock in Catalonia, Spain. A case report. *Veterinary Parasitology: Regional Studies and Reports* **1**, 75–79.

Bernt M, Merkle D, Ramsch K, Fritzsch G, Perseke M, Bernhard D, Schlegel M, Stadler PF and Middendorf M (2007) CREx: Inferring genomic rearrangements based on common intervals. *Bioinformatics* **23**, 2957–2958.

Boore JL, Macey JR and Medina M (2005) Sequencing and comparing whole mitochondrial genomes of animals. *Methods in Enzymology* **395**, 311–348.

Caña-Bozada V, Llera-Herrera R, Fajer-Ávila EJ and Morales-Serna FN (2021) Mitochondrial genome of *Scutogyrus longicornis* (Monogenea: Dactylogyridea), a parasite of Nile tilapia *Oreochromis niloticus*. *Parasitology International* **81**, 102281.

Domingues MV and Boeger WA (2008) Phylogeny and revision of Diplectanidae Monticelli, 1903 (Platyhelminthes: Monogenoidea). *Zootaxa* **1903**, 1–40.

Galtier N, Nabholz B, Glémin S and Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Molecular Ecology* **18**, 4541–4550.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, *et al.* (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**, 644–652.

Greiner S, Lehwark P and Bock R (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3.1: Expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Research* **47**, W59–W64.

Huang X and Madan A (1999) CAP3: A DNA sequence assembly program. *Genome Research* **9**, 868–877.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA x: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.

Lanfear R, Frandsen PB, Wright AM, Senfeld T and Calcott B (2017) Partitionfinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* **34**, 772–773.

Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, *et al.* (2016) MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. *Methods* **102**, 3–11.

Montero-Rodríguez M, Mendoza-Franco EF and Téllez NAL (2020) New species of *Rhabdosynochus* Mizelle and Blatz 1941 (Monogenoidea: Diplectanidae) from the gills of the cultured *Centropomus viridis* (Perciformes) in the Mexican Eastern Tropical Pacific. *Acta Parasitologica*. In press. doi:10.1007/s11686-020-00303-1

Morales-Serna FN, López-Moreno DG, Medina-Guerrero RM, Abad-Rosales SM, Martínez-Brown JM, Ibarra-Castro L and Fajer-Ávila EF (2020) Toxicity of formalin for juvenile *Centropomus viridis* and in vitro efficacy against the parasite *Rhabdosynochus* sp. (Monogenea: Diplectanidae). *Journal of Applied Ichthyology* **36**, 740–744.

Ogawa K (2015) Diseases of cultures marine fishes caused by Platyhelminthes (Monogenea, Digenea. *Cestoda*). *Parasitology* **142**, 178–195.

Ronquist F, Teslenko M, Van Der Mark P, *et al.* (2012) Mrbayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**(3), 539–542.

Schelkle B, Shinn AP, Peeler E and Cable J (2009) Treatment of gyrodactylid infections in fish. *Diseases of Aquatic Organisms* **86**, 65–75.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Stewart JB and Larsson N-G (2014) Keeping mtDNA in shape between generations. *PLoS Genetics* **10**, e1004670.

Thorvaldsdóttir H, Robinson JT and Mesirov JP (2013) Integrative genomics viewer (IGV): High-performance genomics data visualization and exploration. *Briefings in Bioinformatics* **14**(2), 178–192.

Tingbao Y, Kritsky DC, Yuan S, Jianying Z, Suhua S and Agrawal N (2006) Diplectanids infesting the gills of the barramundi *Lates calcarifer* (Bloch) (Perciformes: Centropomidae), with the proposal of *Laticola* n. g. (Monogenoidea: Diplectanidae). *Systematic Parasitology* **63**, 125–139.

Tokşen E, Nemli E, Değirmenci U and Karacalar U (2013) The effects of azamethiphos and trichlorfon on the control of *Diplectanum aequans* (Monogenea: Diplectanidae) infestations in cultured broodstock sea bass, *Dicentrarchus labrax*. *Bulletin of the European Association of Fish Pathologists* **33**, 144–149.

Vanhove MPM, Briscoe AG, Jorissen MWP, Littlewood DTJ and Huyse T (2018) The first next-generation sequencing approach to the mitochondrial phylogeny of African monogenean parasites (Platyhelminthes: Gyrodactylidae and Dactylogyridae). *BMC Genomics* **19**, 520.

Ye F, King SD, Cone DK and You P (2014) The mitochondrial genome of Paragyrodactylus variegatus (Platyhelminthes: Monogenea): differences in major non-coding region and gene order compared to Gyrodactylus. *Parasites & Vectors* **7**, 377.

Zhang D, Li WX, Zou H, Wu SG, Li M, Jakovlić I, *et al.* (2018a) Mitochondrial genomes of two diplectanids (Platyhelminthes: Monogenea) expose paraphyly of the order Dactylogyridea and extensive tRNA gene rearrangements. *Parasites & Vectors* **11**, 601.

Zhang D, Zou H, Wu SG, Li M, Jakovlić I, Zhang J, *et al.* (2018b) Sequencing, characterization and phylogenomics of the complete mitochondrial genome of *Dactylogyrus lamellatus* (Monogenea: Dactylogyridae). *Journal of Helminthology* **92**, 455–466.

Zhang D, Zou H, Jakovlić I, Wu SG, Li M, Zhang J, *et al.* (2019) Mitochondrial genomes of two *Thaparocleidus* species (Platyhelminthes: Monogenea) reveal the first rRNA Gene rearrangement among the Neodermata. *International Journal of Molecular Sciences* **20**, 4214.