**SUPPLEMENTARY FILE**

*De novo* transcriptome assembly and identification of GPCRs in two species of monogenean parasites of fish

**Authors**

Víctor Caña-Bozada, F. Neptalí Morales-Serna, Emma J. Fajer-Ávila, Raúl Llera-Herrera

**Affiliations**

Centro de Investigación en Alimentación y Desarrollo, A.C. Mazatlán, Sinaloa, Mexico

Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán, Sinaloa, Mexico

**Functional annotation**

Although the annotation was done for all the putative proteins, we present results of the longest, not redundant proteins to avoid overrepresentation of sequences. After removing redundant sequences (isoforms), the representative proteins were reduced from 47,187 to 23,857 in *R. viridisi*, and from 25,696 to 12,020 in *S. longicornis*. For *R. viridisi*, 3214, 2761, 2798, 2114, and 1205 protein sequences were aligned to the Swiss-Prot, Pfam, GO, KEGG, and COG databases, respectively. In addition, 6824 proteins were predicted with TM domain and 2823 with a signal peptide. For *S. longicornis*, 5849, 5422, 5647, 4132, and 2074 proteins were aligned to the Swiss-Prot, Pfam, GO, KEGG, and COG databases, respectively. In addition, 2579 proteins were predicted with a transmembrane region and 1098 with a signal peptide. Complete results are shown in Supplementary Table S1-S3.

According to the Swiss-Prot annotation innexin unc-9 (13 in *R. viridisi* and 11 in *S. longicornis*), FMRFamide receptor (6 and 6), innexin unc-7 (5 and 8), uncharacterized protein K02A2.6 (13 and 24), FMRFamide-activated amiloride-sensitive sodium channels (5 and 7), histone-lysine N-methyltransferase SETMAR (6 and 12), Tigger transposable element-derived protein 6 (7 and 7), potassium voltage-gated channel protein Shaw (4 and 7), cathepsin L/L1 (3 and 8) were among the most abundant proteins with annotation. The UniProt KeyWords transferase (36.7% in *R. viridisi* and 42.1% in *S. longicornis*), virulence (23.8% and 19.7%), DNA-binding (16.5% and 12.4%), and ANK repeat (8.8% and 10.2%) were the most abundant annotated (Supplementary Table S7).
The prediction indicated that the annotated proteins are vinculated, at level four, mainly with phenotypes such as cytokinesis variant emb (3.6% in \textit{R. viridisi} and 4.2% in \textit{S. longicornis}), muscle arm development defective (3.2% and 2.6%), sexually dimorphic development variant (2.7% and 2.5%), carbon dioxide response variant (2.8% and 2.9%), cell proliferation increased (2.2% and 2.5%), developmental morphology variant (2.1% and 1.9%), negative chemotaxis variant (2% and 2.3%), and drug-induced gene expression variant (2.1% and 1.7%). At level three, organelle organization biogenesis variant (5.5% and 6.3%), embryonic cell organization biogenesis variant (3.5% and 4.1%), egg-laying variant (3.3% and 2.9%), aldicarb response variant (3% and 2.4%), organism pathogen response variant (3.3% and 3.6%), cell fate specification variant (3.4% and 3.1%), chemotaxis variant (2.9% and 2.8%) and cell stress response variant (1.8% and 2%) were the most represented worm phenotypes (Supplementary Table S3).

Among the most represented GO terms appeared ATP binding (4.5% in \textit{R. viridisi} and 4.7% in \textit{S. longicornis}), metal ion binding (3.9% and 3.8%), protein binding (3.7% and 3.4%), DNA binding (2.8% and 2.7%), RNA binding (2.3% and 2.3%), nucleic acid binding (1.9% and 1.6%), and calcium ion binding (1.8% and 1.5%), protein kinase activity (1.2% and 1%), protein serine/threonine kinase activity (1% and 1%), and G protein-coupled receptor activity (1% and 0.6%) within the Molecular Function (Supplementary Figure S1); protein phosphorylation (0.6% and 0.6%), regulation of transcription, DNA-templated (0.8% and 0.6%), positive regulation of transcription by RNA polymerase II (0.6% and 0.7%), transmembrane transport (0.6% and 0.6%), and G protein-coupled receptor signaling pathway (0.6% and 0.4%) within the Biological Process (Supplementary Figure S2); and nucleus (7.2% and 7.1%), cytoplasm (6.1% and 6.9%), integral component of membrane (5.1% and 4.9%), cytosol (4.7% and 4.5%), plasma membrane (4.8% and 4.3%), and membrane (3.4% and 3%) within the Cellular Component (Supplementary Figure S3).

The KEGG annotations showed that membrane trafficking [BR:ko04131] (3.4% in \textit{R. viridisi} and 3.1% \textit{S. longicornis}), chromosome and associated proteins [BR:ko03036] (3.3% and 3.1%), transporters [BR:ko02000] (2.1% and 1.8%), exosome [BR:ko04147] (2% and 1.8%), protein kinases [BR:ko01001] (1.9% and 1.4%), messenger RNA biogenesis [BR:ko03019] (1.7% and 1.5%), ion channels [BR:ko04040] (1.7% and 1.4%) and peptidases and inhibitors [BR:ko01002] (1.1% and 1.1%) were among the main metabolic pathways in which the likely proteins are involved (Supplementary Figure S4).

The most representative Pfam domains were protein kinase domain (2% and 1.8%), protein tyrosine and serine/threonine kinase (2% and 1.8%), leucine rich repeat (1.5% and 1.2%), 7 transmembrane receptor (rhodopsin family) (1.4% and 1.1%), immunoglobulin domain (1.2% and 1.1%), tetratricopeptide repeat (1.2% and 1.1%), WD domain, G-beta repeat (1.1% and 1.1%), RNA recognition motif. (a.k.a. RRM, RBD, or RNP domain) (1.1% and 1.1%), and ankyrin repeat (0.7% and 1.2%) (Supplementary Table S8).

Among the most representative COG terms were serine threonine protein kinase (5.6% and 4.6%), major facilitator Superfamily (4.2% and 3.1%), phosphatidylinositol kinase activity (3.6% and 2.5%), and calcium-binding protein (3.5% and 3.1%) (Supplementary Table S9).

**Lineage-specific GPCR**

To identify lineage-specific GPCR, the predicted GPCRs of \textit{R. viridisi} and \textit{S. longicornis} (Queries) were aligned against the NCBI non-redundant protein (nr) databases (Subject) limited by the option “Organism”, which included the sister classes, Cestoda (taxid:6199), Trematoda (taxid:6178); the basal class, Rhabditophora (taxid:147100) and the taxa
Lophotrochozoa (taxid:1206795) (exclude: Platyhelminthes), Spiralia (taxid:2697495) (exclude: Lophotrochozoa), Protostomia (taxid:33317) (exclude: Spiralia), Bilateria (taxid:33213) (exclude: Protostomia) and Vertebrata (taxid:7742). 16 (eighth of *R. viridisi* and 8 of *S. longicornis*) dataset of e-values were obtained. Those predicted GPCRs with e-value >1e-05, detected through heatmaps using the ggtree package in R-studio (R version 4.0.4) ggtree library (Yu et al., 2017), were considered to be specific for Monogenea. According to Kerfeld and Scott (2011) "sequences with a recent shared ancestry will have a high degree of similarity; their alignments will have many identical residues, few substitutions and gaps, and tiny e-values; conversely, sequences with an ancient common ancestor will be deeply divergent, with few shared sequence identities, many gaps, and larger e-values", therefore, considering that sequences with hits can be interpreted as sequences sharing evolutionary history, the e-values were correlated between different datasets through Spearman analysis. For instance, all e-values of the alignment of GPCRs of *R. viridisi* (Query) and Trematoda (Subject) were correlated with the e-values obtained by aligning GPCRs of *R. viridisi* (Query) against GPCRs of Cestoda (Subject) (Supplementary figure). Similar evolutionary patterns among taxa are expected to result in correlation values close to 1, whereas in those taxa where the selective pressure is different, for example due to adaptive issues, correlation values far away 1 may be expected.

We propose that the correlation values can be used to explore evolutionary patterns. Proteins with low mutational rate, usually essential proteins, show lower e-values even between distant taxa, whereas those with a higher mutation rate, usually proteins related to adaptive processes, tend to show higher e-values.
Code Availability

**Assembly:**
Trimmomatic (v0.35) R1_001.fastq.gz R2_001.fastq.gz Paired_R1.fastq.gz
Unpaired_R1.fastq.gz PairedR2.fastq.gz R2.fastq.gz ILLUMINACLIP:all_adapters.fa:2:30:10
SLIDINGWINDOW:4:15 LEADING:5 TRAILING:5 AVGQUAL:20 MINLEN:40
Trinity (v2.8.6) --seqType fq --max_memory 100G --left Paired_R1.fastq --right PairedR2.fastq --SS_lib_type RF --CPU 32 --normalize_by_read_set

**Filtered of contaminant sequences of the assembly**
makeblastdb (Blast v2.7.1) -in assembly_trinity.fasta -out assembly_trinity.fasta -parse_seqids -dbtype nucl
blastx (Blast v2.7.1) -query assembly_trinity.fasta -db uniprot_Bacter.fasta -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -out transcriptome_Bacter

fastagrep.pl (https://github.com/rec3141/rec-genome-tools) -X -f code_transcriptome_Bacter assembly_trinity.fasta > code_transcriptome_Bacter.fasta
makeblastdb (Blast v2.7.1) -in uniprot_Gyro_Hmicro_Hmicro_Sman_Smed_Bacter.fasta -out uniprot_Gyro_Hmicro_Hmicro_Sman_Smed_Bacter.fasta -parse_seqids -dbtype prot
blastx (Blast v2.7.1) -db uniprot_Gyro_Hmicro_Hmicro_Sman_Smed_Bacter -query code_transcriptome_Bacter.fasta -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -max_target_seqs 1 -out best_hit_bac
makeblastdb -in assembly_trinity_without_bacter.fasta -out assembly_trinity_without_bacter.fasta -parse_seqids -dbtype nucl
blastx -query assembly_trinity_without_bacter.fasta -db fish_host.pep -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.0000000000000001 -out transcriptome_Host

fastagrep.pl -X -f code_transcriptome_Host assembly_trinity.fasta > code_transcriptome_Host.fasta
makeblastdb (Blast v2.7.1) -in Gyro_Hmicro_Hmicro_Sman_Smed_Fish.fasta -out Gyro_Hmicro_Hmicro_Sman_Smed_Fish.fasta -parse_seqids -dbtype prot
blastx -db Gyro_Hmicro_Hmicro_Sman_Smed_Fish.fasta -query code_transcriptome_Host.fasta -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -max_target_seqs 1 -out best_hit_fish
makeblastdb -in assembly_trinity_without_bacter_fish.fasta -out assembly_trinity_without_bacter_fish.fasta -parse_seqids -dbtype nucl
blastx -query assembly_trinity_without_bacter_fish.fasta -db uniprot_virus_fungi.fa -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -out transcriptome_virus_fungi

fastagrep.pl -X -f code_transcriptome_virus_fungi assembly_trinity.fasta > code_transcriptome_virus_fungi.fasta
makeblastdb (Blast v2.7.1) -in uniprot_Gyro_Hmicro_Hmicro_Sman_Smed_virus_fungi.fasta -out uniprot_Gyro_Hmicro_Hmicro_Sman_Smed_virus_fungi.fasta -parse_seqids -dbtype prot
blastx -db uniprot_Gyro_Hmicro_Hmicro_Sman_Smed_virus_fungi.fasta -query code_transcriptome_virus_fungi.fasta -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -max_target_seqs 1 -out best_hit_virus_fungi

**Prediction of ORFs**
TransDecoder.LongOrfs (v5.5) -t assembly_trinity_without_bacter_fish_virus_fungi.fasta
blastp (Blast v2.7.1) -query longest_orfs.pep -db uniprot_sprot.pep -max_target_seqs 1 -outfmt 6 > blastp.outfmt6
hmmscan (v3.2.1) --cpu 10 --domtblout TrinotatePFAM.out Pfam-A.hmm longest_orfs.pep > pfam.log
TransDecoder.Predict (v5.5) -t assembly_trinity_without_bacter_fish_virus_fungi.fasta --retain_pfam_hits TrinotatePFAM.out --retain_blastp_hits blastp.outfmt6

**Cluster of ORFs**
cd-hit (v4.6) -i assembly_trinity_without_bacter_fish_transdecoder.pep -o unigene.pep -c 1.00

Filtered of contaminant sequences of the Rabdo ORF
makeblastdb -in vibrio_spp_platyhelminthes.fasta -out vibrio_spp_platyhelminthes.fasta -parse_seqids -dbtype prot
blastp -db vibrio_spp_platyhelminthes.fasta -query rhabdo_unigene.pep -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -max_target_seqs 1 -out best_hit_vibrio
makeblastdb -in robalo_lates_tilapia_platyhelminthes.fasta -out robalo_lates_tilapia_platyhelminthes.fasta -parse_seqids -dbtype prot
blastp -db robalo_lates_tilapia_platyhelminthes.fasta -query rhabdo_unigene.pep -outfmt "6 qacc sacc qcovs qcovhsp pident evalue bitscore length" -evalue 0.00001 -max_target_seqs 1 -out best_hit_vibrio

Annotation
blastx -query assembly_trinity_without_bacter_fish_virus_fungi.fasta -db uniprot_sprot.pep -max_target_seqs 1 -value 0.00001 -outfmt 6 > blastx.outfmt6
RnammerTranscriptome.pl (v3.1.1) --transcriptome assembly_trinity_without_bacter_fish_virus_fungi.fasta --path_to_rnammer ~/bin/RNAMMER/rnammer
signalp (v4.1) -f short -n signalp.out
<assembly_trinity_without_bacter_fish_virus_fungi.fasta.transdecoder.pep> tmhmm (v2.0) --short
get_Trinity_gene_to_trans_map.pl (v3.1.1) assembly_trinity_without_bacter_fish_virus_fungi.fasta > assembly_trinity_without_bacter_fish_virus_fungi.fasta.gene_trans_map
Trinotate (v3.1.1) Trinotate.sqlite init --gene_trans_map
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Mapped
bowtie2-build (v2.3.4) assembly_trinity.fasta assembly_mapping
bowtie2 (v2.3.4) --no-unal -x assembly_mapping -1 Paired_R1.fastq.gz -2 Paired_R2.fastq.gz -S assembly_mapping.sam.gz
samtools (v1.7) view -bSo assembly_mapping.bam.gz assembly_mapping.sam.gz
samtools sort assembly_mapping.bam.gz -o assembly_mapping_sorted.bam
samtools index assembly_mapping_sorted.bam
samtools depth Rhabdo_contig.sorted.bam | awk '{sum+=$3} END { print "Average = ",sum/NR }' > OutFile.coverage

Evaluation of the transcriptome
python run_BUSCO.py (v.3.0.2) -i unigene.pep -o unigene -l metazoa_odb9 -m prot