Transcriptome and gene expression profiling in fish intestine and acanthocephalans

FINAL PROJECT MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 19th May 2023

Irena Vardić Smrzlić

Ciljevi projekta
O1. Procijeniti sezonske i dugoročne trendove koncentracija metala rijeke Krke (postaje: izvor (I), nizvodno od ulijevanja otpadnih voda (II), Brljansko jezero (III) te četiri pritoke: Krčić (IV), Kosovčica (V), Orašnica (VI), Butišnica (VII)) u: **O1.1.** vodi; **O1.2.** sedimentu.
O2. Odrediti biološke odgovore organizama na izloženost i/ili učinak metala u različitim okolišnim uvjetima praćenjem: **O2.1.** izravnih učinaka riječne i otpadne vode na laboratorijske organizme (alge i rakovi- testovi fito- i zoo-toksičnosti), koji pripadaju različitim trofičkim nivoima i različite su osjetljivosti na zagađenje od riba **O2.2.** biomarkerskih odgovora u probavilu nativnih riba te metalotioneina u kukašima, kao proteina odgovornih za homeostazu i detoksifikaciju metala, **O2.3.** histopatoloških promjena, posebno kvantitativnih i kvalitativnim promjena mukoznih stanica probavila riba **O2.4.** koncentracija metala u mekim tkivima (probavilo, mišić) i kalcificiranim strukturama riba (ljuske, otoliti) te u kukašima.
O3. Procijeniti bioraspoloživost i udio toksične frakcije metala unešenih hranom u ribe određivanjem: **O3.1.** udjela metabolički raspoložive frakcije (koja može biti i potencijalno toksična jer se ovi metali mogu vezati na biološki važne molekule); **O3.2.** detoksificirane frakcije metala (nemaju toksični učinak); **O3.3**. trofički raspoložive frakcije metala (raspoloživi za predatore); **O3.4.** koncentracija metala u sadržaju probavila riba (unos putem hrane).
O4. Odrediti aktivne stanične procese u kukaša i probavilu riba u okolišnim uvjetima različite izloženosti metalima profiliranjem: **O4.1.** raspodjele metala među citosolskim proteinima; **O4.2.** transkriptoma i ekspresije gena.

ACANTHOCEPHALA

- RNA extraction
- Commercial RNA sequencing (Novogene)

De novo transcriptome

Differential gene expression analysis

- DEG analyses by RNA-seq
- qPCR

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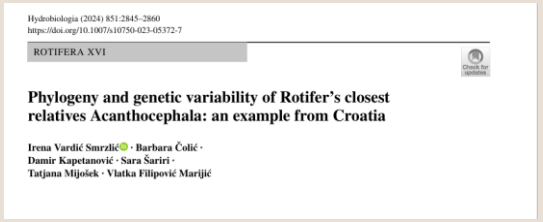
- Commercial sequencing (Novogene)
- DEG analyses

Transcriptome and DEG analysis under Cd2+ treatment


Hydrobiologia (2024) 851:2845–2860
https://doi.org/10.1007/s10750-023-05372-7

ROTIFERA XVI

Phylogeny and genetic variability of Rotifer's closest relatives Acanthocephala: an example from Croatia

Irena Vardić Smrzlić · Barbara Čolić · Damir Kapetanović · Sara Šariri · Tatjana Mijošek · Vlatka Filipović Marijić

Šariri S., Vardić Smrzlić I., Mijošek Pavun T, Filipović Marijić V. ***Scientific Reports*** *(under revision)*

- *Comparative Transcriptome Analysis of Gene Expression in Acanthocephala Parasites from Fish in Polluted and Reference Sites and under Cd2+ treatment* (in preparation)
- *Real-time PCR assays for quantification of up and down regulated genes from Acanthocephala* (in preparation)

SALMO TRUTTA

- RNA extraction
- Commercial RNA sequences (Novogene)

Transcriptome analysis

Gene expression analysis

- DEG analyses by RNA-seq
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Transcriptome analysis of Salmo trutta intestinal tissue infested by Acanthocephala (in preparation)

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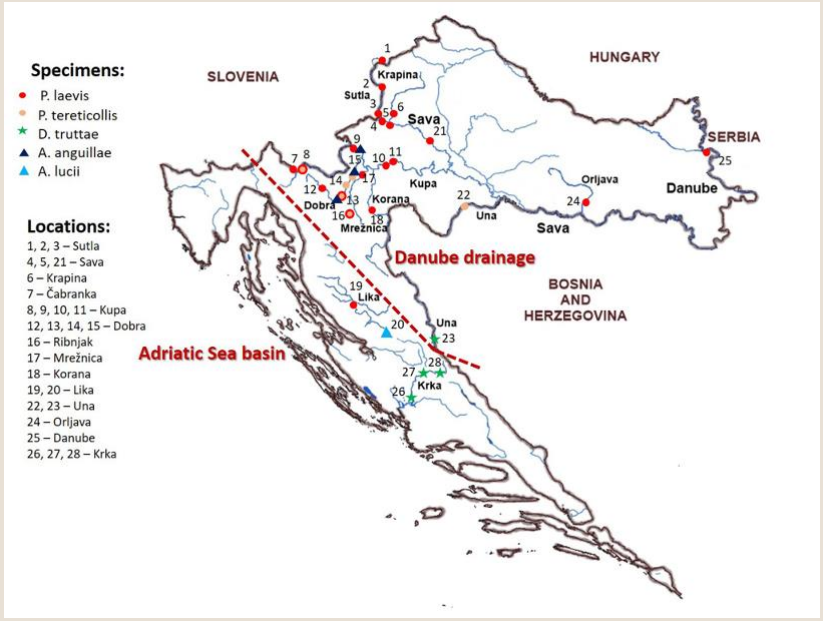


Fig. 1 Map of all sites with records of different Acanthocephala species in freshwaters of Croatia.

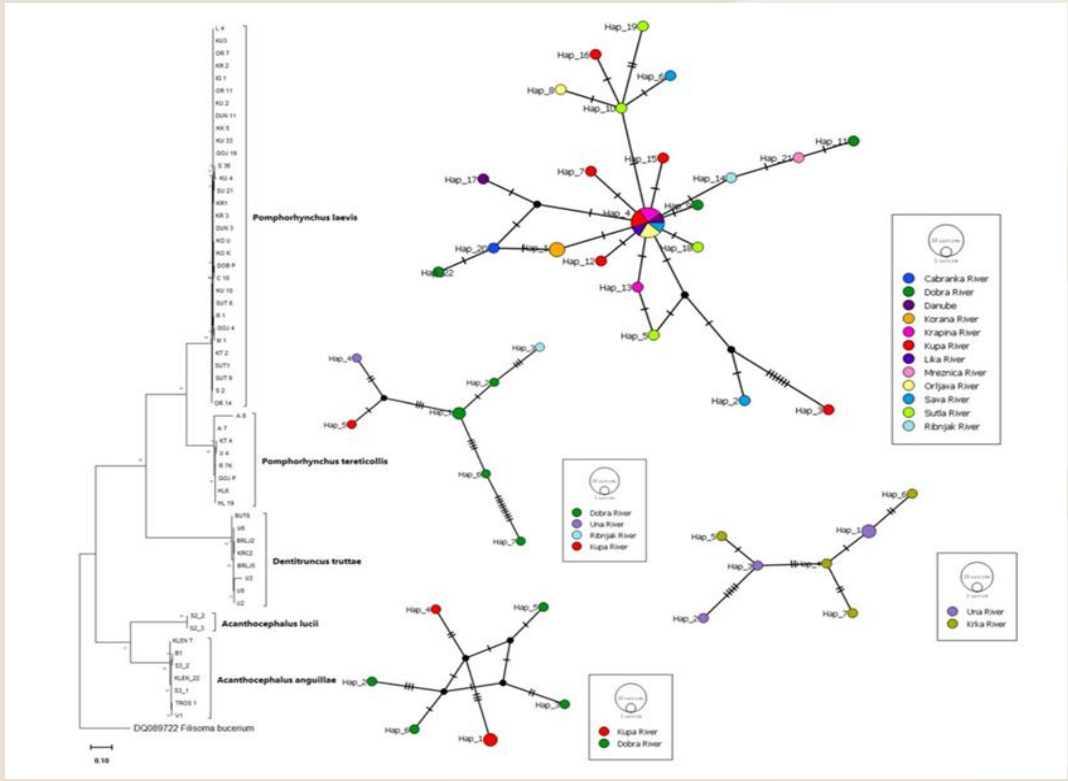


Fig. 2 Phylogenetic analyses of members of three Acanthocephala genera from Croatia based on partial COI marker sequence (565 bp).

The MJ network analysis of *D. truttae* based on COI sequence analyses do not reflect clear geographic structuring of Krka River and Una River specimens (Fig. 2). The large haplotype diversity (HD = 0.964) is due to the large number of haplotypes shared by only one or two individuals.

ACANTHOCEPHALA

De novo transcriptome analysis of _Dentitruncus truttae_ reveals metal-binding proteins in Acanthocephala

*Sara Šariri[1], Irena Vardić Smrzlić[1] *, Tatjana Mijošek Pavin[1], Vlatka Filipović Marijić[1] * *(Scientific Reports 2025)*

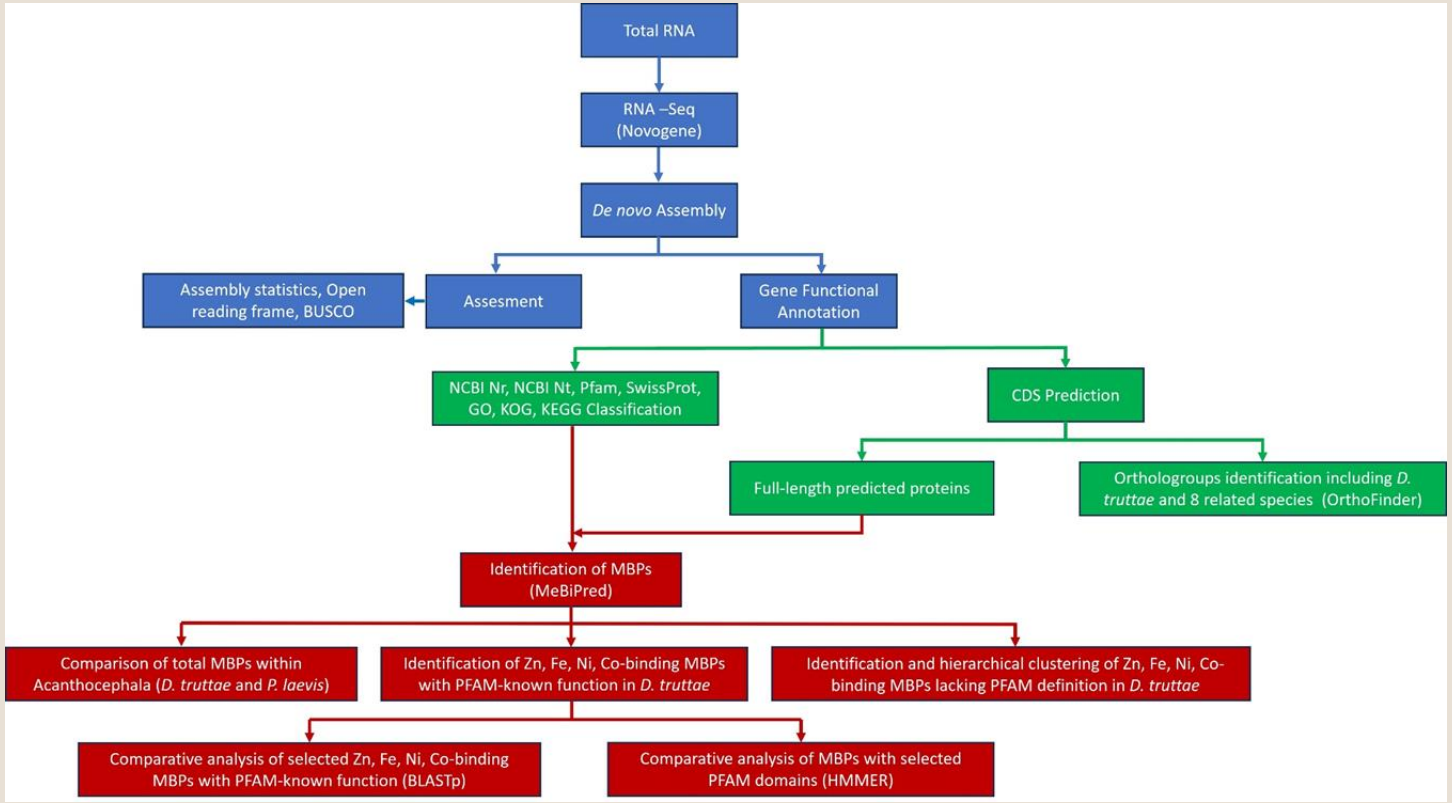


Figure 3. Workflow for RNA-Seq Analysis and Identification of Metal-Binding Proteins in *D. truttae*

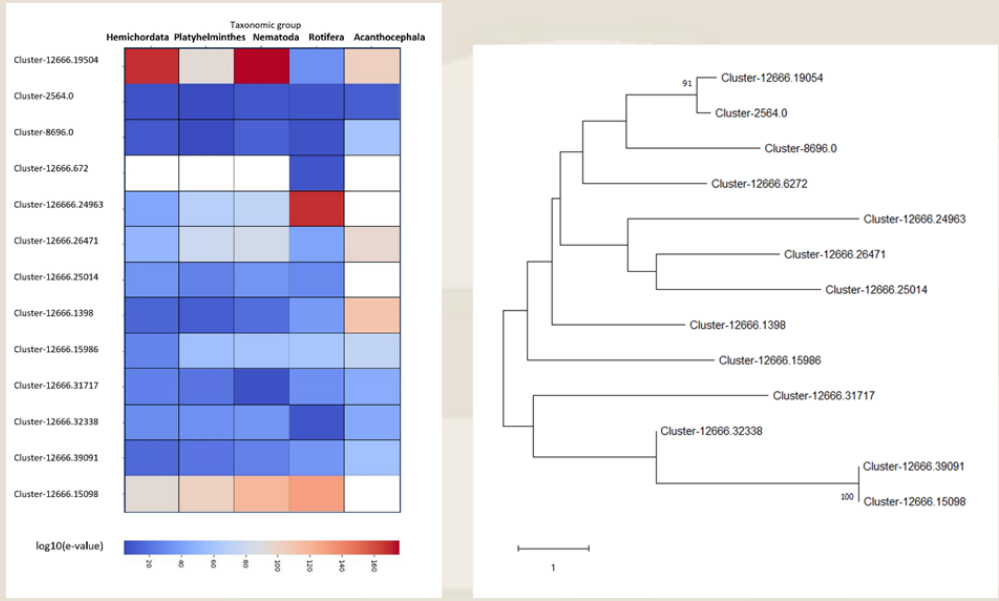


Figure 4. Phylogenetic analysis of *D. truttae* **zinc metalloproteases** using 1000 replicates and Whelan Goldman + Freq Model.

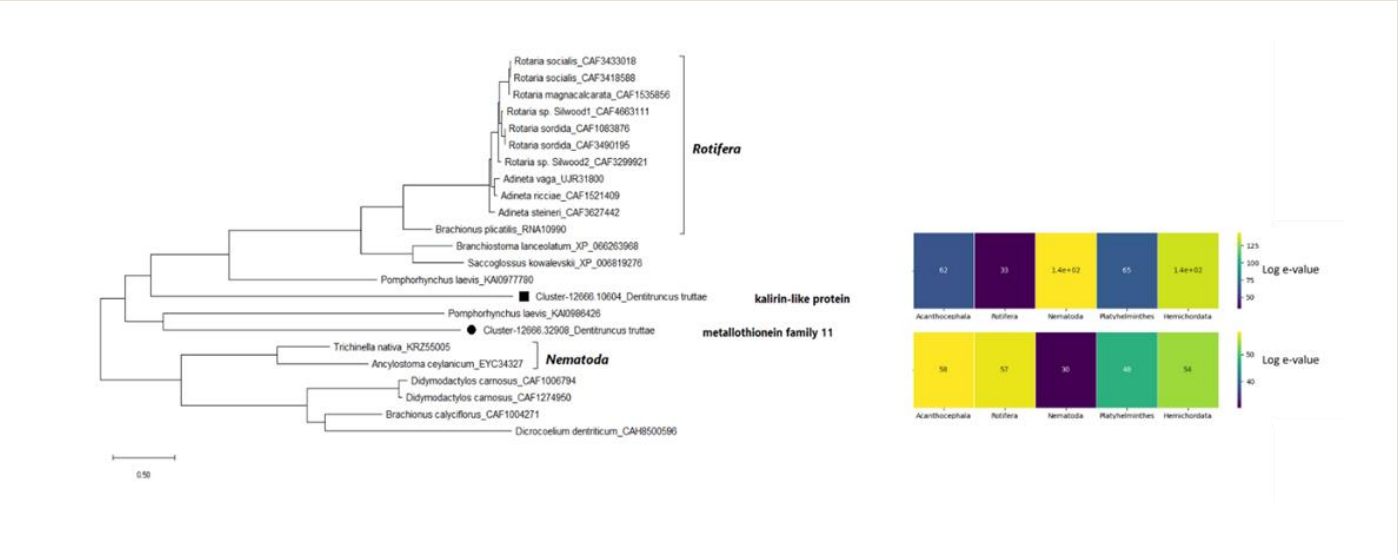
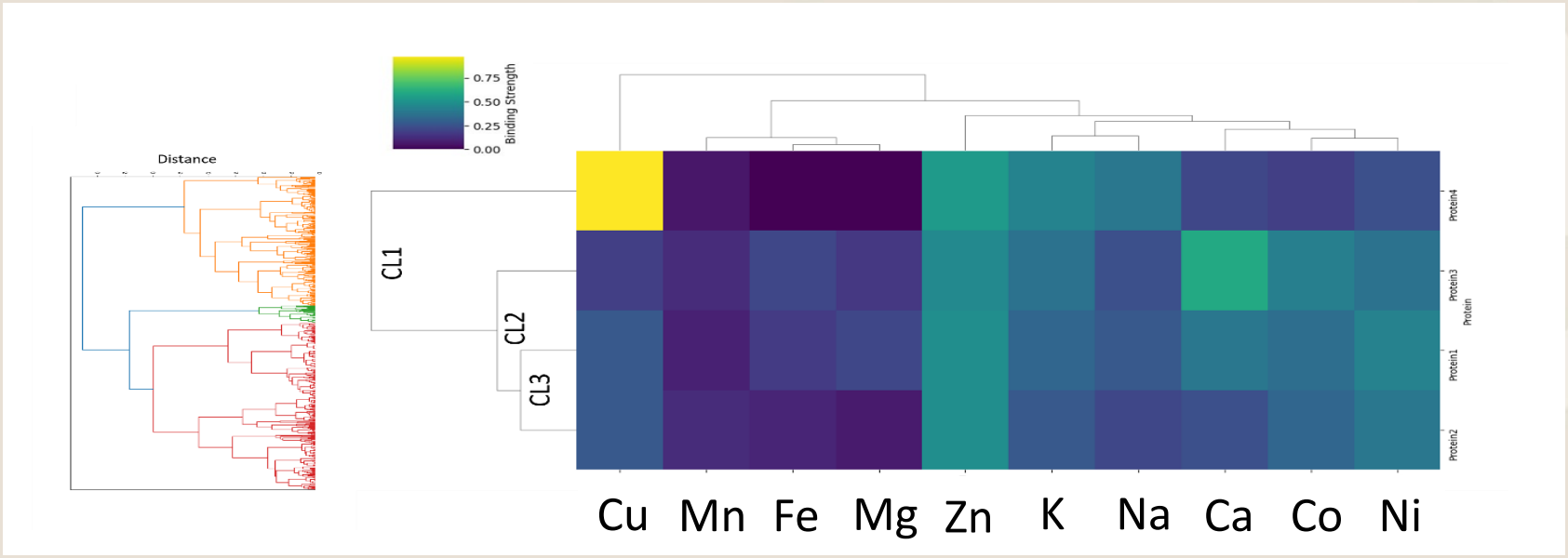


Figure 5. Phylogenetic analysis of two **metallothioneins** of *D. truttae* using 1000 replicates and JTT matrix-based Model

Figure 6. Hierarchical clustering heatmap of *D. truttae* **metal-binding proteins lacking PFAM definition** into three clusters (CL1, CL2, and CL3) based on metal binding preferences



The results presented provide a valuable basis for further investigations of metal homeostasis in these parasites and for solving many questions about the phylogeny, taxonomy, diversity and evolution of Acanthocephala.

ACANTHOCEPHALA

Comparative Transcriptome Analysis of Gene Expression in Acanthocephala Parasites from Fish in Polluted and Reference Sites and under Cd2+ treatment (in preparation)

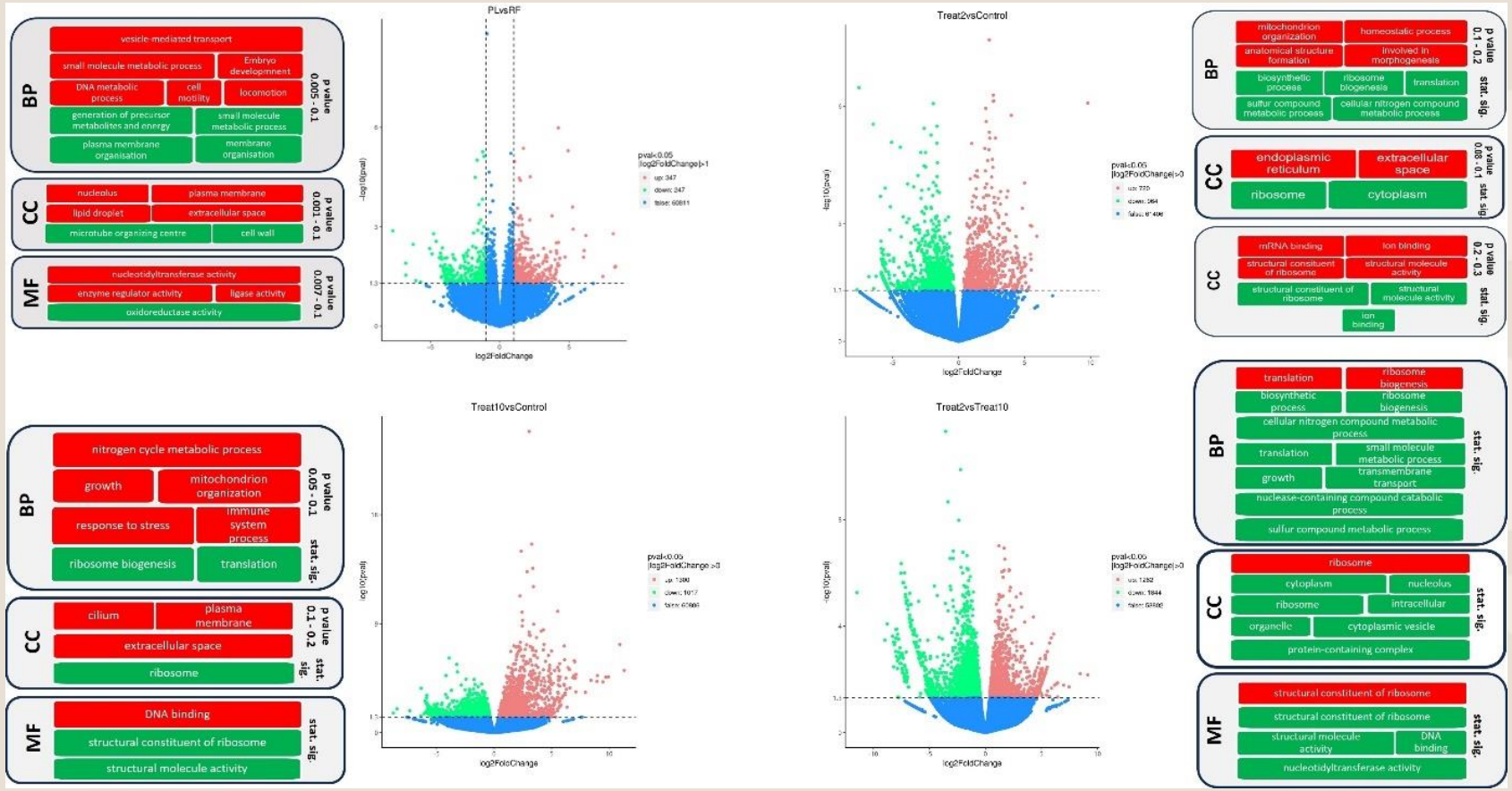


Figure 7. Gene Ontology Enrichment and Differential Expression Analysis

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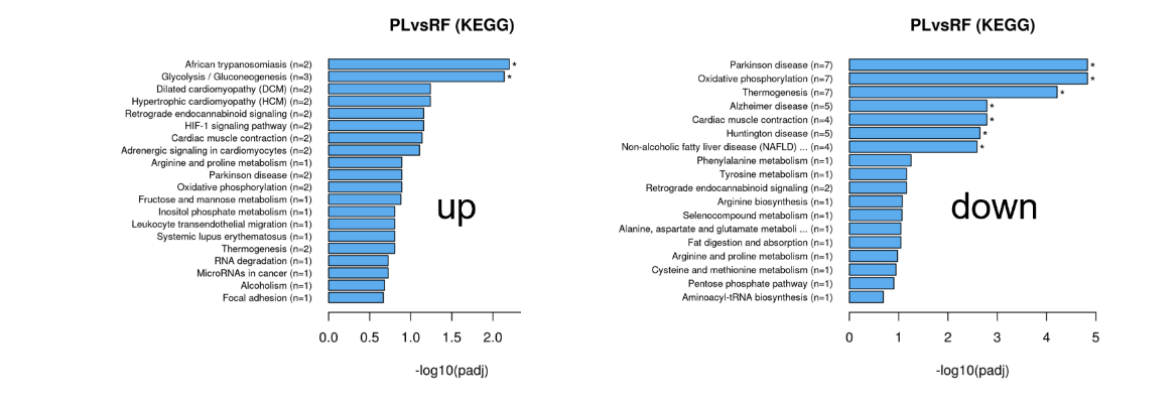
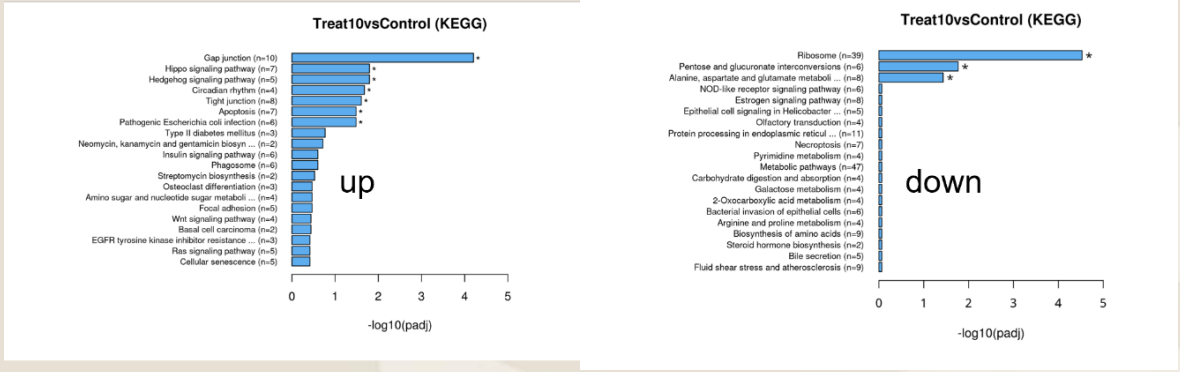




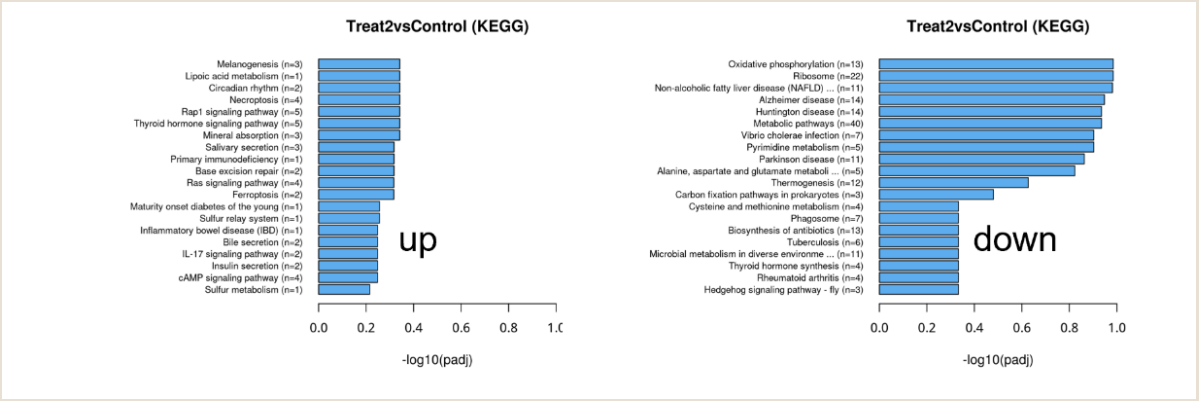
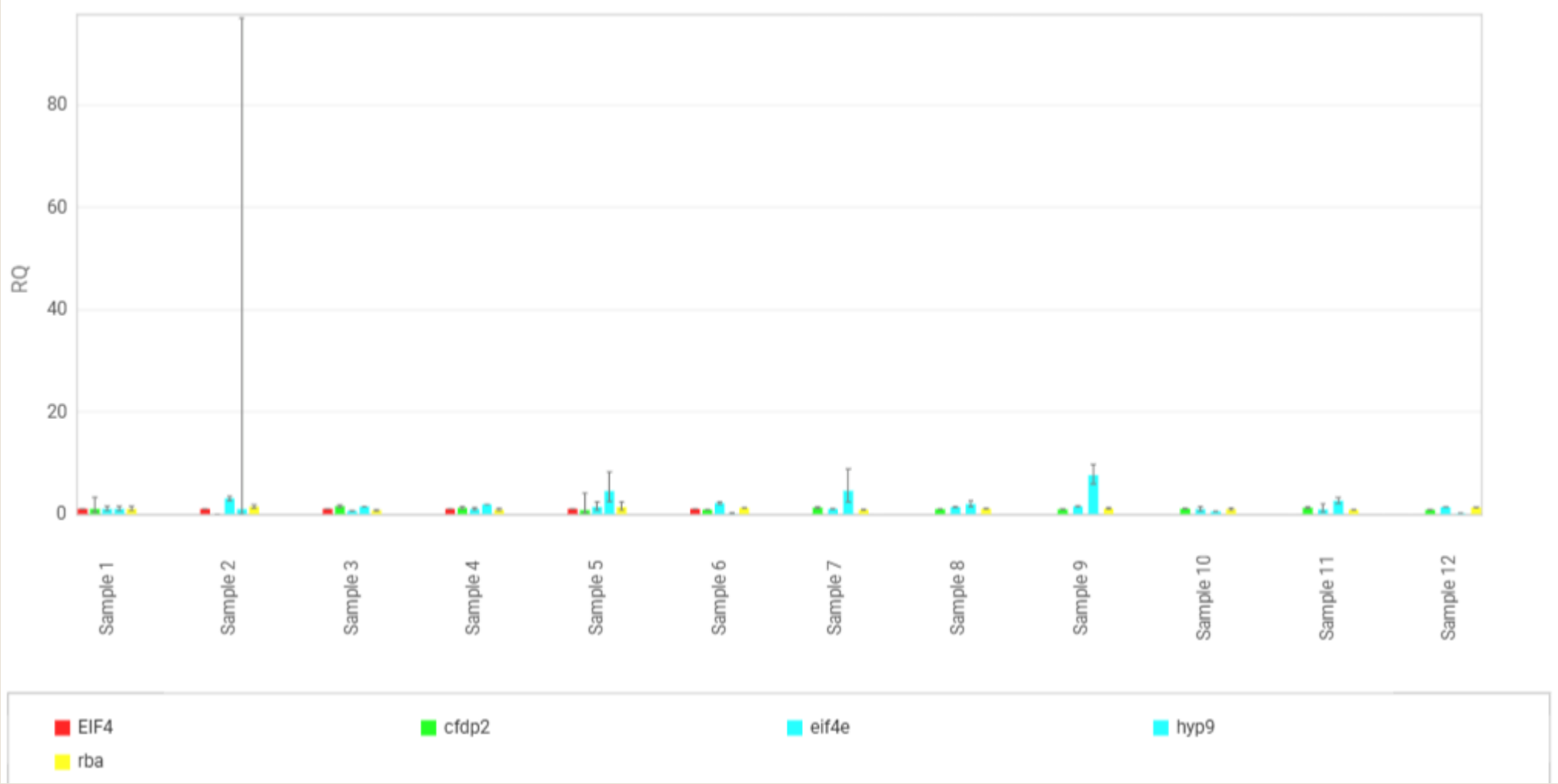


Figure 9. KEGG pathway enrichment analysis

ACANTHOCEPHALA

Real-time PCR assays for quantification of up and down regulated genes from Acanthocephala (*in preparation*)



SALMO TRUTTA INTESTINE

Transcriptome analysis of Salmo trutta intestinal tissue infested by Acanthocephala (*in preparation*)

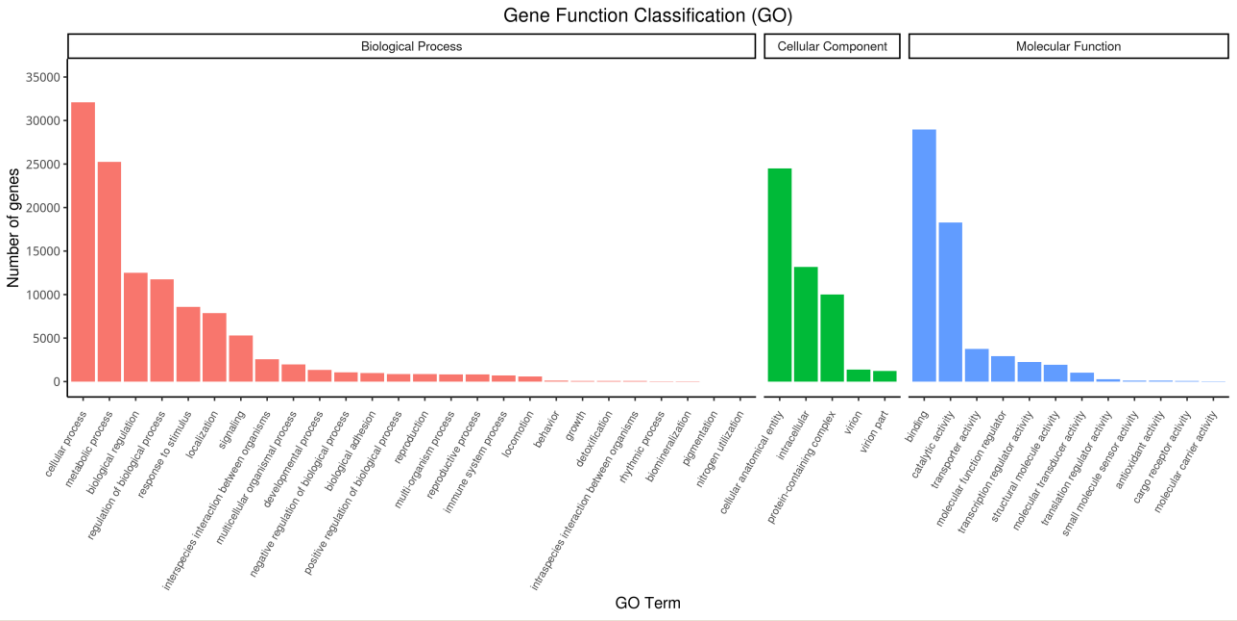


Figure 10. GO clasification

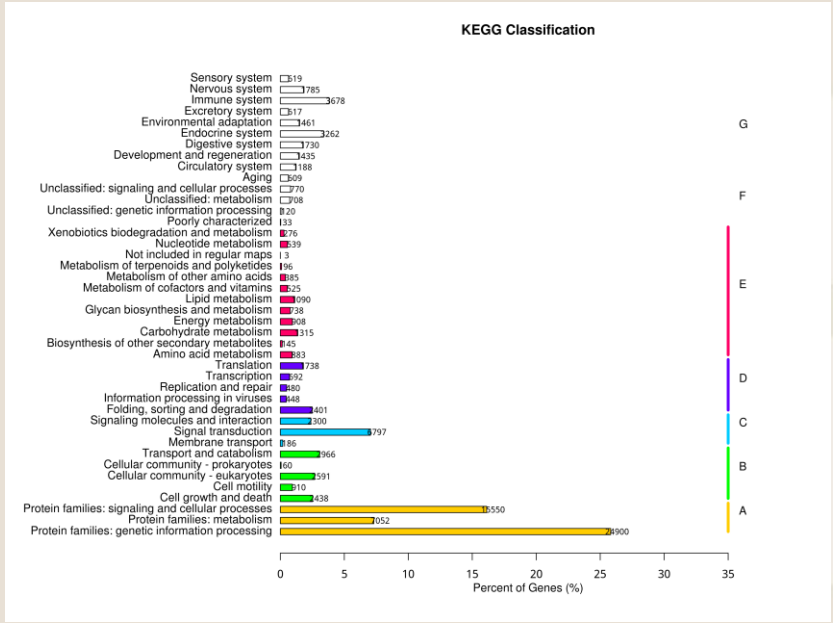


Figure 11. KEGG clasification

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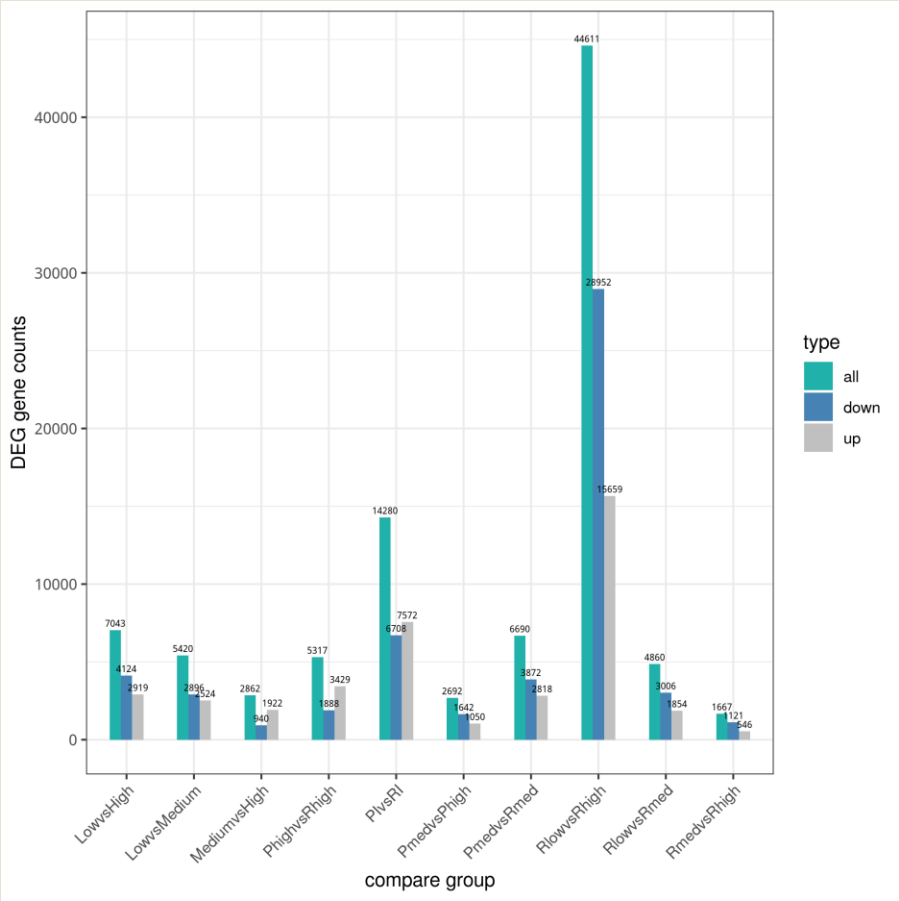

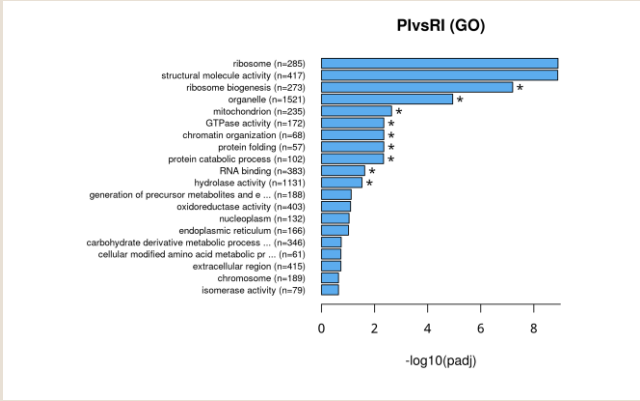

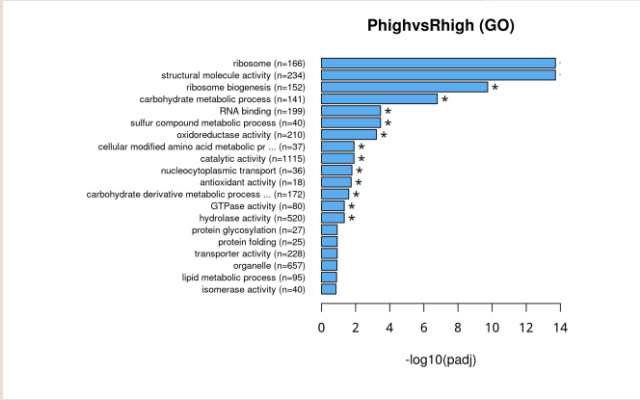

Figure 12. KO pathway enrichment analysis

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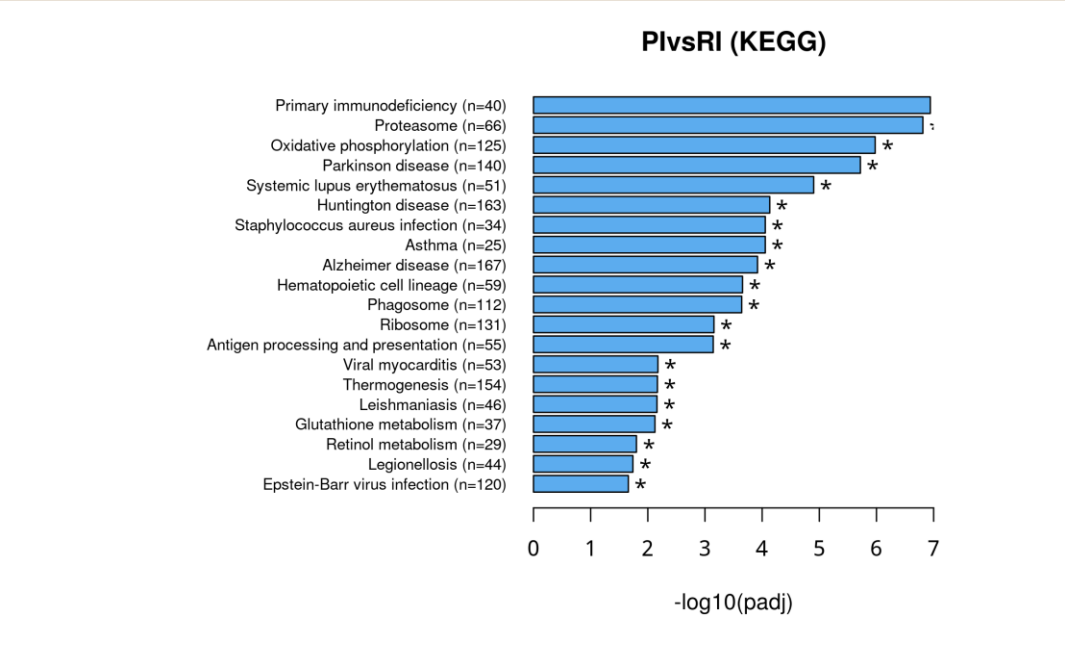

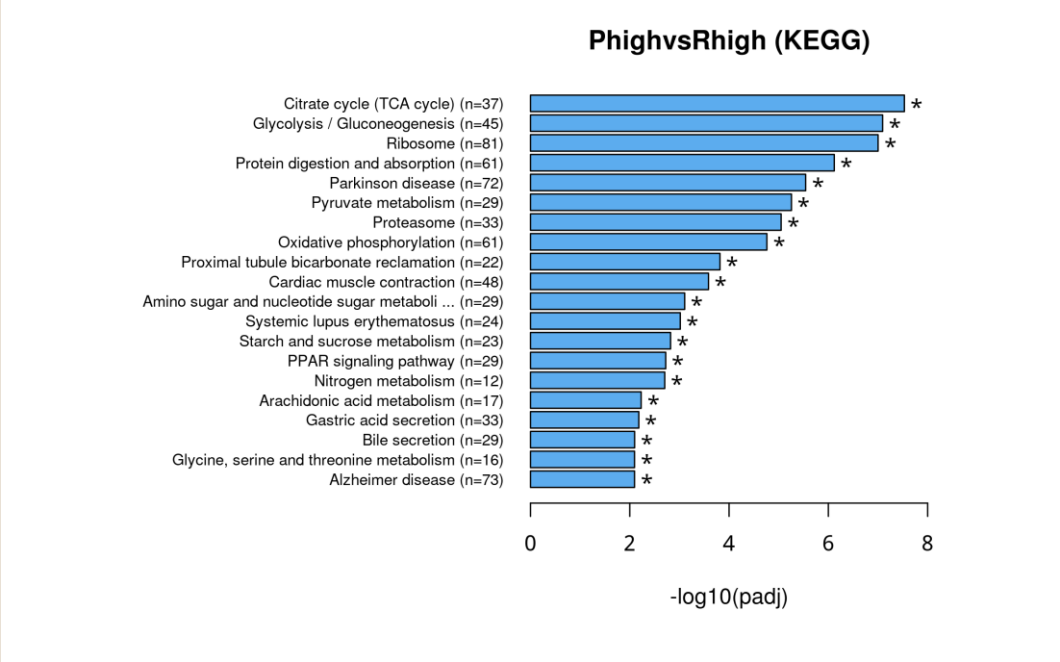

Figure 13. KEGG pathway enrichment analysis

THANK YOU!