miRNA analysis in Common carp infected with KHV – SUPPLEMENTARY MATERIAL

Supplementary Table S1. Common carp and D. rerio reference sequences used for sRNA data processing - BlastN search.

<table>
<thead>
<tr>
<th>RNA Type</th>
<th>Number of Unique Reference Sequences</th>
<th>Reference Database</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA</td>
<td>420</td>
<td>miRBase v.21</td>
</tr>
<tr>
<td>pre-miRNA</td>
<td>480</td>
<td>miRBase v.21</td>
</tr>
<tr>
<td>snoRNA</td>
<td>244</td>
<td>Silva v.122; NCBI (November 2016); Rfam v.12.1</td>
</tr>
<tr>
<td>rRNA</td>
<td>4777</td>
<td>Silva v.122; NCBI (November 2016); Rfam v.12.1</td>
</tr>
<tr>
<td>tRNA</td>
<td>8135</td>
<td>Silva v.122; NCBI (November 2016); Rfam v.12.1</td>
</tr>
<tr>
<td>snRNA</td>
<td>392</td>
<td>Silva v.122; NCBI (November 2016); Rfam v.12.1</td>
</tr>
<tr>
<td>mRNA</td>
<td>68324</td>
<td>RefSeq (protein-coding, November 2016)</td>
</tr>
<tr>
<td>Repeat associated sequences</td>
<td>2313</td>
<td>Repbase (November 2016)</td>
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<tr>
<td>lncRNA</td>
<td>48</td>
<td>Silva v.122; NCBI (November 2016); Rfam v.12.1</td>
</tr>
<tr>
<td>Pseudogenes</td>
<td>104</td>
<td>NCBI (November 2016)</td>
</tr>
<tr>
<td>Introns</td>
<td>40375</td>
<td>UCSC (November 2016)</td>
</tr>
</tbody>
</table>

Supplementary Figure S1. Length distribution of clean sRNA reads from samples representing KHV infection phase P1 (A), phase P2 (B) and phase P3 (C).
Supplementary Figure S2. Distribution of total clean reads from samples (mean counts) representing 3 KHV infection phases among different types of RNA.
Supplementary Figure S3. Heatmap of sample-to-sample distance (similarity). The heatmap was built using DESeq2 on normalized (rlog) miRNA reads counts from all 9 samples (all 3 infection phases). The darker the color the smaller the distance and bigger similarity between samples.

Supplementary Figure S4. Results of the GO term annotation for targets of up-regulated miRNAs from P2 vs. P1 comparison. The GO terms annotation was performed by the Blast2GO software. Figure represents the pie chart graph of GO terms distribution from “Biological Process” (A), “Molecular Function” (B) and “Cellular Component” (C) category – numbers in brackets represent number of annotated carp sequences.
Supplementary Figure S4. Results of the GO term annotation for targets of down-regulated miRNAs from P2 vs. P1 comparison. The GO terms annotation was performed by the Blast2GO software. Figure represents the pie chart graph of GO terms distribution from “Biological Process” (A), “Molecular Function” (B) and “Cellular Component” (C) category – numbers in brackets represent number of annotated carp sequences.
Supplementary Figure S4. Results of the GO term annotation for targets of up-regulated and down-regulated miRNAs from P3 vs. P1 comparison. The GO terms annotation was performed by the Blast2GO software. Figure represents the pie chart graph of GO terms distribution from “Biological Process” (A), “Molecular Function” (B) and “Cellular Component” (C) category – numbers in brackets represent number of annotated carp sequences.
Supplementary Figure S5. Results of GO term enrichment analysis for targets of carp up-regulated miRNAs (P2 vs. P1 phase comparison). The GO terms enrichment analysis was performed by the Blast2GO software. The targets predicted for carp up-regulated miRNAs were used as a test set. All carp protein coding sequences were used as background. Figure represent enriched GO terms from the “Biological Process”, “Cellular Component” and “Molecular Function” categories. The size of dot denotes number of targets annotated by given GO term. The FDR ≤ 0.05 was set as the threshold for significant GO term enrichment.
Supplementary Figure S6. Results of GO term enrichment analysis for targets of carp down-regulated miRNAs (P2 vs. P1 phase comparison). The GO terms enrichment analysis was performed by the Blast2GO software. The targets predicted for carp down-regulated miRNAs were used as a test set. All carp protein coding sequences were used as background. Figure represent enriched GO terms from the “Biological Process”, “Cellular Component” and “Molecular Function” categories. The size of dot denotes number of targets annotated by given GO term. The FDR ≤ 0.05 was set as the threshold for significant GO term enrichment.
Supplementary Figure S7. The enriched KEGG pathway obtained for targets of down-regulated carp miRNAs (P2 vs. P1 phase comparison) – "Necroptosis" (dre04217). The KEGG pathway enrichment analysis was performed with the clusterProfiler R package. The zebrafish KEGG pathways served as reference. The targets predicted for down-regulated miRNAs which mapped on given pathway are marked as pink boxes.
Supplementary Figure S8. The enriched KEGG pathway obtained for targets of up-regulated carp miRNAs (P2 vs. P1 phase comparison) – “Fatty acid metabolism” (dre01212). The KEGG pathway enrichment analysis was performed with the clusterProfiler R package. The zebrafish KEGG pathways served as reference. The targets predicted for up-regulated miRNAs which mapped on given pathway are marked as pink arrows.
Supplementary Figure S9. Predicted hairpin structures of novel carp miRNA molecules identified in samples from phase P1. Dominant form of the mature miRNAs is indicated in blue. Opposite strand miRNAs (“miRNA*”) are indicated in red. The novel precursors were visualized using the UEA sRNA Workbench method.

A)

Novel-miRNA1
B) Novel-miRNA2
C)

Novel-miRNA3
D) Novel-miRNA4
E)

Novel-miRNA5
F)

Novel-miRNA6
G)
Novel-miRNA7
H)

Novel-miRNA8
Supplementary Figure S10. Predicted hairpin structures of novel carp miRNA molecules identified in samples from phase P2. Dominant form of the mature miRNAs is indicated in blue. Opposite strand miRNAs (“miRNA*”) are indicated in red. The novel precursors were visualized using the UEA sRNA Workbench method.

A) Novel-miRNA1
B)

Novel-miRNA2
C)

Novel-miRNA3
D)

Novel-miRNA4
E) Novel-miRNA5
F)

Novel-miRNA6
G)

Novel-miRNA7
H)

Novel-miRNA8
Novel-miRNA10
Supplementary Figure S11. Predicted hairpin structures of novel carp miRNA molecules identified in samples from phase P3. Dominant form of the mature miRNAs is indicated in blue. Opposite strand miRNAs (“miRNA*”) are indicated in red. The novel precursors were visualized using the UEA sRNA Workbench method.

A) Novel-miRNA1
B) Novel-miRNA2
C)

Novel-miRNA3
D)

Novel-miRNA4
E)

Novel-miRNA5