Poster # 1367

asm2015
115th General Meeting | American Society for Microbiology
May 30–June 2, 2015 | New Orleans, Louisiana

Genome Sequence Analysis of Largemouth Bass Virus Isolates from Smallmouth Bass

Shannon Pipes and Jeff Newman
Department of Biology, Lycoming College, Williamsport, PA.

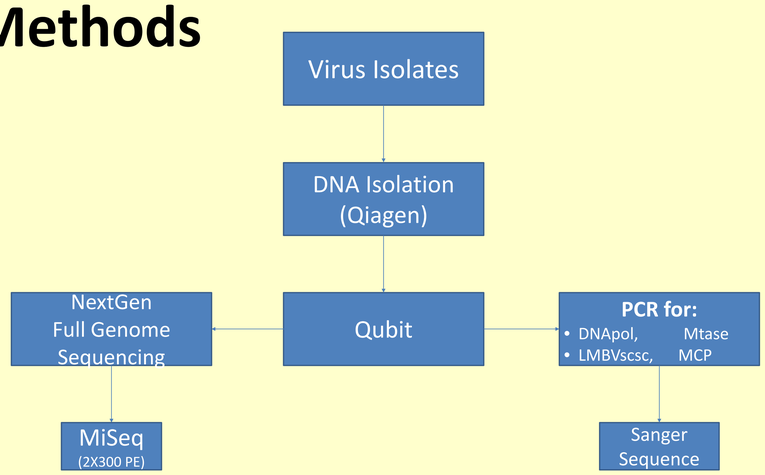
Abstract

The viral pathogen, Largemouth Bass Virus (LMBV), is a member of the family Iridoviridae and has been known to cause large fish kills among Largemouth Bass, Micropterus salmoides. The viral pathogen is also consistently isolated from other species where diseased fish are found. Throughout the Susquehanna River Basin since 2005, Smallmouth Bass, Micropterus dolomieu, have been suffering from wide-spread disease related deaths. Even though LMBV is not clinically known to affect Smallmouth Bass, this virus is prevalent among diseased fish infected with other pathogens such as Pseudomonas aeruginosa. The question we seek to address is whether there are significant genetic differences between LMBV isolates from smallmouth bass and largemouth bass. Total DNA was isolated from preparations of LMBV derived from smallmouth bass and largemouth bass isolates. Five LMBV-specific primer sets were used to amplify fragments from each preparation for conventional Sanger sequencing and the total DNA preparations are also being analyzed using MiSeq NextGen sequence analysis. After analysis of the sequences, there does not appear to be any significant difference between the DNA sequences of the virus when it is isolated from either the largemouth bass or smallmouth bass. Further analysis using BLAST and other comparison tools will be studied to determine any differences between the sequences.

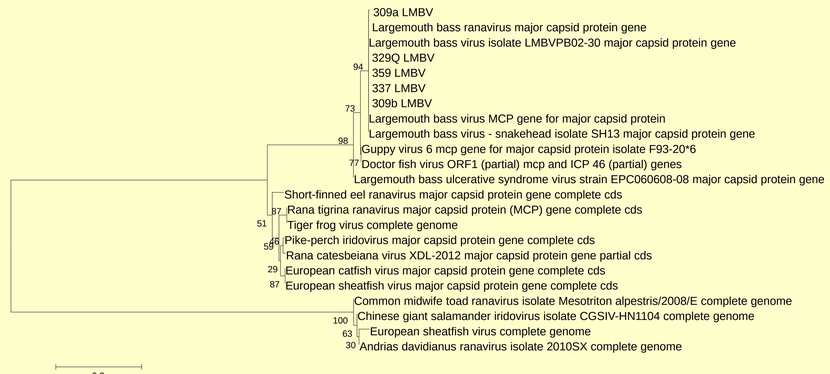
Background

- Isolates of LMBV were collected from smallmouth bass in the Susquehanna Watershed
- Is LMBV causing disease in the smallmouth bass?
- Is the smallmouth bass within the virus's normal host range?
- Are the SMB isolates different from the LMB isolates?

Methods



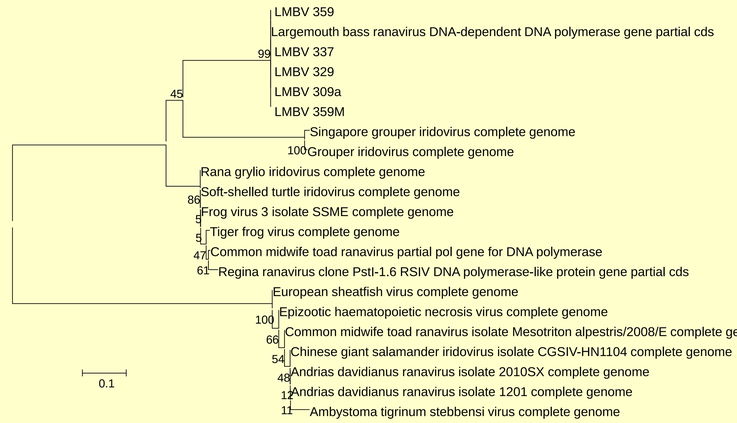
Analysis of MCP PCR Product:



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
309a LMBV	1																						
309b LMBV	2	0.002																					
329Q LMBV	3	0.002	0.000																				
337 LMBV	4	0.002	0.000	0.000																			
359 LMBV	5	0.002	0.000	0.000	0.000																		
Largemouth bass virus MCP gene	6	0.002	0.000	0.000	0.000	0.000																	
Largemouth bass virus snakehead isolate SH13- MCP	7	0.002	0.000	0.000	0.000	0.000	0.000																
Largemouth bass virus isolate LMBVPB02-30- MCP	8	0.002	0.000	0.000	0.000	0.000	0.000	0.000															
Largemouth bass ranavirus MCP	9	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000														
Guppy virus 6 mcp gene	10	0.023	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020													
Doctor_fish_virus	11	0.023	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.000												
Largemouth_bass_ulcerative_syndrome_virus_strain	12	0.025	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.023	0.011	0.011											
Pike-perch_iridovirus_major_capsid_protein	13	0.225	0.223	0.223	0.223	0.223	0.223	0.223	0.223	0.223	0.220	0.220	0.211										
Short-finned_eel_ranavirus_major_capsid_protein	14	0.227	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.227	0.227	0.218	0.055									
European_catfish_virus_major_capsid_protein	15	0.227	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.227	0.227	0.218	0.023	0.045								
European_sheatfish_virus_major_capsid_protein	16	0.227	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.227	0.227	0.218	0.023	0.045	0.000							
Rana_tigrina_ranavirus_major_capsid_protein_(MCP	17	0.230	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.229	0.229	0.223	0.029	0.064	0.039	0.039						
Tiger_frog_virus_complete_genome	18	0.230	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.223	0.025	0.064	0.039	0.039	0.000					
Rana_catesbeiana_virus_XDL-2012_major_capsid_protein	19	0.230	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.227	0.225	0.225	0.216	0.009	0.052	0.018	0.018	0.028	0.025				
Common_midwife_toad_ranavirus	20	0.639	0.636	0.636	0.636	0.636	0.636	0.636	0.636	0.634	0.634	0.634	0.630	0.623	0.618	0.618	0.618	0.618	0.620				
Chinese_giant_salamander_iridovirus	21	0.639	0.636	0.636	0.636	0.636	0.636	0.636	0.636	0.636	0.634	0.634	0.630	0.625	0.620	0.620	0.620	0.620	0.620	0.623	0.002		
Andrias_davidianus_ranavirus_isolate_2010SX	22	0.639	0.636	0.636	0.636	0.636	0.636	0.636	0.636	0.636	0.634	0.634	0.630	0.625	0.620	0.620	0.620	0.620	0.620	0.623	0.002	0.000	
European_sheatfish_virus_complete_genome	23	0.643	0.641	0.641	0.641	0.641	0.641	0.641	0.641	0.639	0.639	0.634	0.623	0.618	0.618	0.618	0.618	0.618	0.620	0.618	0.016	0.016	

Figure 1. Neighbor joining tree and distance matrix showing relationships among iridovirus Major Coat Protein Genes

Analysis of DNApol PCR Product:



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
LMBV 359M	1																				
LMBV 309a	2	0.00																			
LMBV 329	3	0.00	0.00																		
LMBV 337	4	0.00	0.00	0.00																	
LMBV 359M	5	0.00	0.00	0.00	0.00																
Largemouth_bass_ranavirus_DNA-dependent_DNA_polymerase_gene	6	0.00	0.00	0.00	0.00	0.00															
Rana_grylio_iridovirus_complete_genome	7	0.27	0.27	0.27	0.27	0.27	0.27														
Soft-shelled_turtle_iridovirus_complete_genome	8	0.27	0.27	0.27	0.27	0.27	0.27	0.00													
Common_midwife_toad_ranavirus_partial_pol_gene_for_DNA_polymerase	9	0.27	0.27	0.27	0.27	0.27	0.27	0.02	0.02												
Tiger_frog_virus_complete_genome	10	0.26	0.26	0.26	0.26	0.26	0.26	0.02	0.02	0.02											
Frog_virus_3_isolate_SSME_complete_genome	11	0.27	0.27	0.27	0.27	0.27	0.27	0.00	0.00	0.02	0.02										
Regina_ranavirus_clone_PstI-1.6_RSIV_DNA_polymerase-like_protein_gene	12	0.28	0.28	0.28	0.28	0.28	0.28	0.04	0.04	0.02	0.03	0.04									
Singapore_grouper_iridovirus_complete_genome	13	0.36	0.36	0.36	0.36	0.36	0.36	0.32	0.32	0.31	0.31	0.32	0.32								
Grouper_iridovirus_complete_genome	14	0.36	0.36	0.36	0.36	0.36	0.36	0.31	0.31	0.31	0.30	0.31	0.31	0.02							
Chinese_giant_salamander_iridovirus_isolate_CGSIV-HN1104_complete_genome	15	0.58	0.58	0.58	0.58	0.58	0.58	0.55	0.55	0.56	0.56	0.55	0.56	0.62	0.61						
Andrias_davidianus_ranavirus_isolate_2010SX_complete_genome	16	0.58	0.58	0.58	0.58	0.58	0.58	0.55	0.55	0.56	0.56	0.55	0.56	0.62	0.61	0.00					
Andrias_davidianus_ranavirus_isolate_1201_complete_genome	17	0.58	0.58	0.58	0.58	0.58	0.58	0.55	0.55	0.56	0.56	0.55	0.56	0.62	0.61	0.00	0.00				
European_sheatfish_virus_complete_genome	18	0.57	0.57	0.57	0.57	0.57	0.57	0.54	0.54	0.55	0.55	0.54	0.56	0.61	0.60	0.02	0.02	0.02			
Epizootic_haematopoietic_necrosis_virus_complete_genome	19	0.58	0.58	0.58	0.58	0.58	0.58	0.54	0.54	0.56	0.56	0.54	0.56	0.61	0.61	0.02	0.02	0.02	0.00		
Ambystoma_tigrinum_stebbensi_virus_complete_genome	20	0.58	0.58	0.58	0.58	0.58	0.58	0.57	0.57	0.58	0.58	0.57	0.59	0.62	0.61	0.04	0.04	0.04	0.04	0.03	
Common_midwife_toad_ranavirus	21	0.57	0.57	0.57	0.57	0.57	0.57	0.54	0.54	0.56	0.56	0.54	0.56	0.62	0.61	0.01	0.01	0.01	0.02	0.02	0.04

Figure 2. Neighbor joining tree and distance matrix showing relationships among iridovirus DNA Polymerase Genes

Conclusion: PCR products including MCP, DNApol, Mtase, and LMBVscsc of new isolates cluster closely with previously identified LMBV isolates.

Table 1. Whole Genome Sequencing Stats

	329 (LMB)	309 (SMB)	337 (SMB)	359 (SMB)
reads	2,009,727	1,384,503	1,376,857	3,551,237
coverage	1350x	1040x	1650x	3025x
contigs	6	6	4	12
genome size	98,613	98,032	98,625	101,410
annotated genes (RAST)	105	107	107	108

Figure 3. Comparison of LMBV to closest Iridovirus genome (from redfin perch Accession FJ433873)

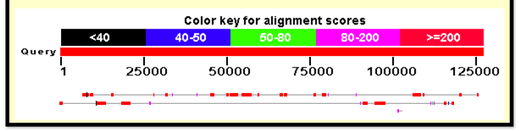


Figure 4. LMBV gene comparison

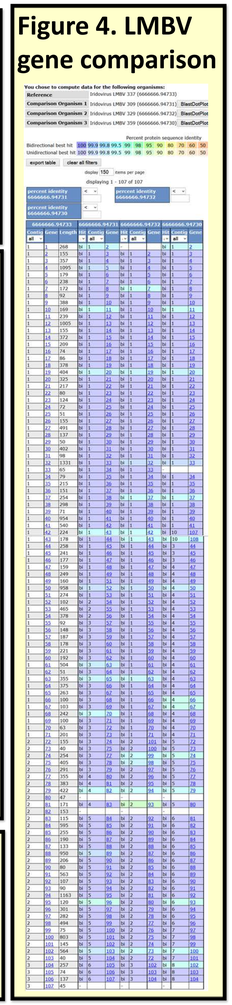
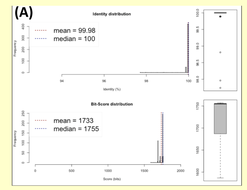
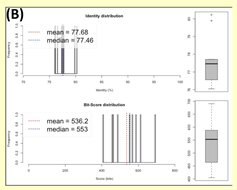


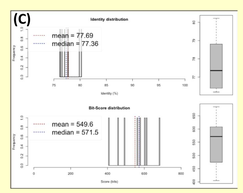
Figure 5. ANI Comparison:



(A) Comparison of LMBV329 isolated from Largemouth Bass and LMBV337 isolated from Smallmouth Bass



(B) Comparison of closest Iridovirus genome (Accession FJ433873) from Redfin Perch and LMBV329 isolated from Largemouth Bass



(C) Comparison of closest Iridovirus genome (Accession FJ433873) from Redfin Perch and LMBV337 isolated from Smallmouth Bass

Conclusions

- 1st genome sequences of LMBV
- No obvious differences observed in LMBV from SMB

Acknowledgements:

Funding provided by PA Fish and Boat Commission and CWI NextGen Access through GCAT-SEEK

References

L. Iwanowicz , C. Densmore , C. Hahn , P. McAllister & J. Odenkirk (2013) Identification of Largemouth Bass Virus in the Introduced Northern Snakehead Inhabiting the Chesapeake Bay Watershed, Journal of Aquatic Animal Health, 25:3, 191-196.