

Isolation, characterization and molecular identification of a novel aquareovirus that infects the endangered fountain darter, *Etheostoma fonticola*

L. R. Iwanowicz*, D. D. Iwanowicz, C. R. Adams, T. D. Lewis, T. M. Brandt, L. R. Sanders, R. S. Cornman

*Corresponding author: liwanowicz@usgs.gov

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Table S1. Primers utilized for endpoint PCR and direct sequencing.

Genome segment	GenBank accession no.	Oligonucleotide ID	Oligonucleotide sequence	Target	Expected product size (bp)
Segment 6	NA	AQ1 AQ2	AWSCCKTAYATCTATGGCTT TTRGAGACGAAMAKNGASGC	~1331–1773	~443
Segment 1	KU194213	FDRS1.1F FDRS1.1R	TCCCCGTTTCTGGACTCAAC GTCAACTGGGCGGAGATCAT	1885–2465	600
Segment 2	KU194214	FDRS2.1F FDRS2.1R	AAACTCGCCTCCTCTCAAGC TGTCGAGGGCTTTTTCGTCA	430–1030	601
Segment 3	KU194215	FDRS3C1F FDRS3C1R	CGCTACATCCGGTTCTTTGA GACGATAAGCGACTTCCAGATAG	2621–3340	720
Segment 4	KU194216	FDRS4.1F FDRS4.1R	CCCACCACATCTCCCCTCTA GAGTCAGCGGAGTGGATAGC	1121–1947	827
		FDRS4C11gapF FDRS4C11gapR	TCGCATGGAGACGTCAACAA TAGAGGGGAGATGTGGTGGG	308–1140	833
Segment 5	KU194217	FDRS5C2F FDRS5C2R	ATGCCGATGTTCCAGTTCC TCCATGTGAGCGGTGATGAC	563–1295	733
Segment 6	KU194218	FDRS6C9F FDRS6C9R	ATCAGCTAGAGTCCGGCTCA ATGATGGCGGATGGATCGAC	171–978	808
Segment 7	KU194219	FDRS7C3F FDRS7C3R	CAGAGATCCCCGGTCAAC GGCGATGTTCAACAGGTCTA	232–1082	851
Segment 8	KU194220	FDRS8.1F FDRS8.1R	TATGAAATCCCTCGTCGGCG TAGACCAGGGCCTGAATCGA	546–1313	768
Segment 9	KU194221	FDRS9.1F FDRS9.1R	TCGTGGTGCAATCGAGTCAA GCGATGTCGTACTTTGCGTC	136–726	591
Segment 10	KU194222	FDRS10.1F FDRS10.1R	GTAACCGCCATGGAGACCAA GCGACCTTCCCTGATCTAGC	19–458	440
Segment 11	KU194223	FDRS11.1F FDRS11.1R	AGCAGGCATGGCTCAAGATT CACAGGCACGGTAGAACTGT	18–345	328

Table S2. Length of HSP|%id of HSP. NSH = no significant hit, PS = partial segment, SA = segment absent.

Species	A	A	A	B	B	C	G	Unassigned		
EFR _e V ORF	SMReV	MSReV	Chum salmon reovirus	Green river chinook virus	Fall chinook aquareovirus	Golden shiner reovirus	American grass carp reovirus	Grass carp reovirus GCRV918	Omega ω	Kappa κ
S1	1290 89	1290 88	1290 83	1288 62	1290 58	1290 45	1290 44	1242 32	0.010	1.20
S2	1274 95	1274 95	1234 93	PS (NSH)	1274 76	1274 60	1272 59	1274 44	0.009	1.21
S3	1209 95	1209 96	1209 86	1208 75	1165 74	1157 54	1170 53	1095 36	0.009	1.28
S4	819 69	604 74	389 68	SA	490 38	365 34	470 30	432 23	0.014	1.12
S5	722 87	719 86	722 80	719 59	722 58	721 37	720 35	521 28	0.012	1.14
S6	653 88	653 91	623 88	628 68	653 70	649 53	649 53	649 31	0.012	1.16
S7a	198 68	197 67	190 67	85 75	86 62	17 47	NSH	NSH	0.395	1.33
S7b	272 74	272 75	185 68	247 45	247 43	214 26	215 30	NSH	0.004	1.53
S8	417 90	416 89	416 87	398 61	398 59	398 44	416 41	290 26	0.045	1.39
S9	349 89	349 87	349 86	349 57	346 53	346 41	346 37	NSH	0.027	1.26
S10	298 80	298 84	298 77	297 39	297 37	NSH	NSH	NSH	0.033	1.42
S11	235 77	235 74	147 64	234 48	231 48	212 24	184 27	NSH	0.136	1.49

Table S3. Average coverage of each genomic segment of the *Etheostoma fonticola* aquareovirus using different sequencing platforms.

Segment	Mean coverage (PGM)	Mean coverage (Miseq)	Composite coverage (PGM + MiSeq)	Normalized expression relative to S1
1	42x	164x	241x	1.0
2	216x	496x	722x	3.0
3	88x	434x	528x	2.3
4	118x	162x	290x	1.9
5	164x	470x	645x	4.7
6	639x	2412x	3097x	24.6
7	429x	2438x	2892x	33.8
8	339x	2573x	2933x	36.4
9	616x	3076x	3717x	54.4
10	898x	8191x	9133x	151.4
11	1161x	4175x	5391x	112.7

Figure S1. Alignment of the putative ‘universal’ aquareovirus primers AQ1 (A) and AQ2 (B) with a target sequence of a diversity of aquareoviruses that depicts mismatch precluding their use as universal. K = G/T, M = A/C, N = A/T/C/G, S = C/G, W = A/T, Y = C/T

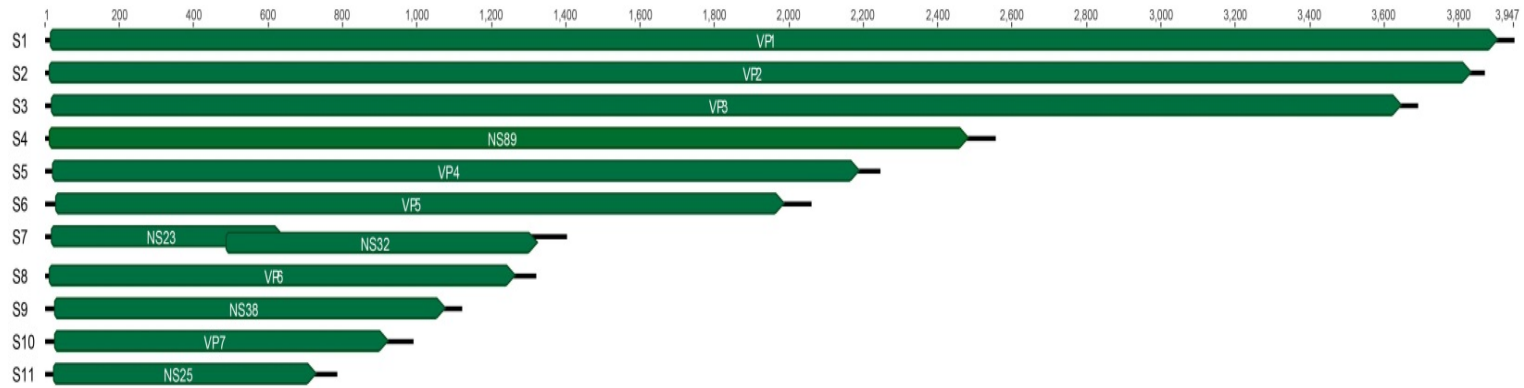


Figure S2. Graphical depiction of the EFRv genome. The genome consists of 10 monocistronic segments, and one partially overlapping, out-of-phase, polycistronic segment of dsRNA. Open reading frames are identified in green and the number of amino acid residues is indicated in parentheses. Specific ORF coordinates, estimated protein molecular weight and biochemical properties are listed in Table 2. Conserved terminal sequences characteristic of Aquareovirus A and G are indicated in black and blue, respectively.

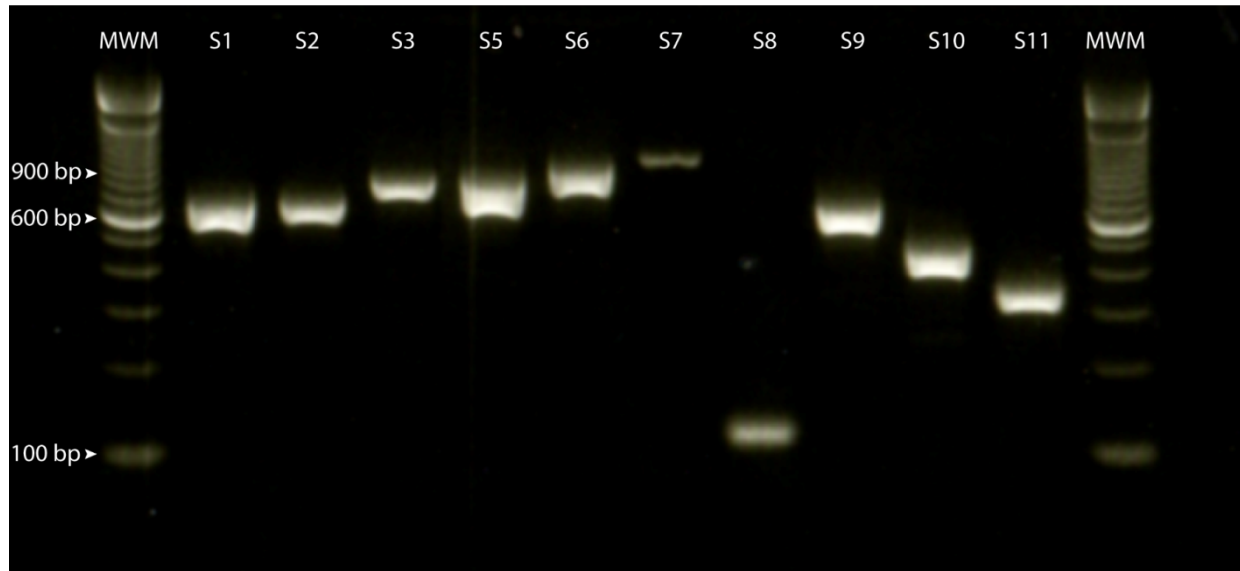


Figure S3. Agarose gel electrophoresis of PCR amplicons for 10 of the 11 segments. Amplicons were the result of PCR under identical conditions.