

A novel Asfarvirus-like virus identified as a potential cause of mass mortality of abalone

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Supplemental data 1. Negative staining and TEM observation of the semi-purified fraction.

Semi-purified fractions were prepared from cryopreserved ($-80\text{ }^{\circ}\text{C}$) healthy and diseased black abalone collected from Owase and Omaezaki, respectively, in 2015. Approximately 0.1 g of soft-body tissues, excluding the midgut, were recovered from each abalone. Tissues were minced with a razor, combined with 10 volumes of autoclaved seawater, and crushed with a Potter-type glass homogenizer. The crushed samples were centrifuged at $15,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$, and the supernatants were passed through a 0.22- μm syringe filter (Millipore). The filtrates were diluted with autoclaved seawater and centrifuged at $150,000 \times g$ for 1 h at $4\text{ }^{\circ}\text{C}$ to sediment the particles. Supernatants were decanted and the resulting pellets were resuspended in a small amount of autoclaved seawater. Resuspended samples were divided for negative staining and TEM observation.

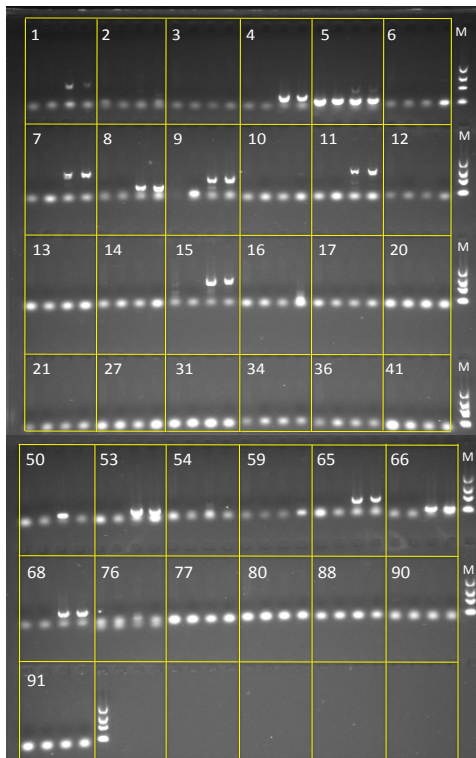
For negative staining observation, samples were absorbed to formvar film-coated grids and stained with 2% phosphor tungstic acid solution. The grids were observed using a transmission electron microscopy (JEOL1400 plus, Jeol).

For TEM observation, resuspended samples were sandwiched between copper disks and frozen in liquid propane at $-175\text{ }^{\circ}\text{C}$. Frozen samples were submerged in 2% glutaraldehyde, 1% tannic acid in ethanol, and 2% distilled water at $-80\text{ }^{\circ}\text{C}$ for 2 days. The samples were dehydrated through ethanol and infiltrated with propylene oxide (PO) and were put into a 70:30 mixture of PO and resin (Quetol-812, Nisshin EM Co.) for 1 h. The samples were transferred to fresh 100% resin and polymerized. Thin sections (70

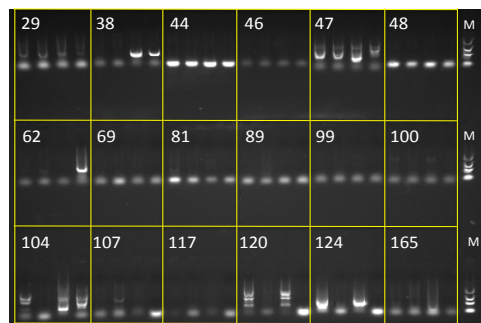
nm) were prepared and stained with 2% uranyl acetate and examined using the JEOL1400 plus transmission electron microscope.

Fig. S2 shows an example of electron microscopic (EM) observation. In images obtained from both analyses, spheroids of various sizes were found in the fractions prepared from both healthy and diseased abalone. AbALV was difficult to identify from observed particle morphology.

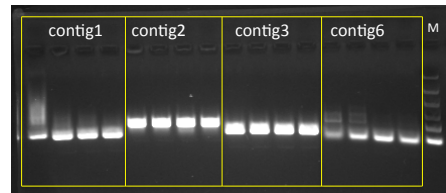
Electrophoresis images of PCR products generated by primers designed with primer3



Electrophoresis images of PCR products generated by manually designed primers

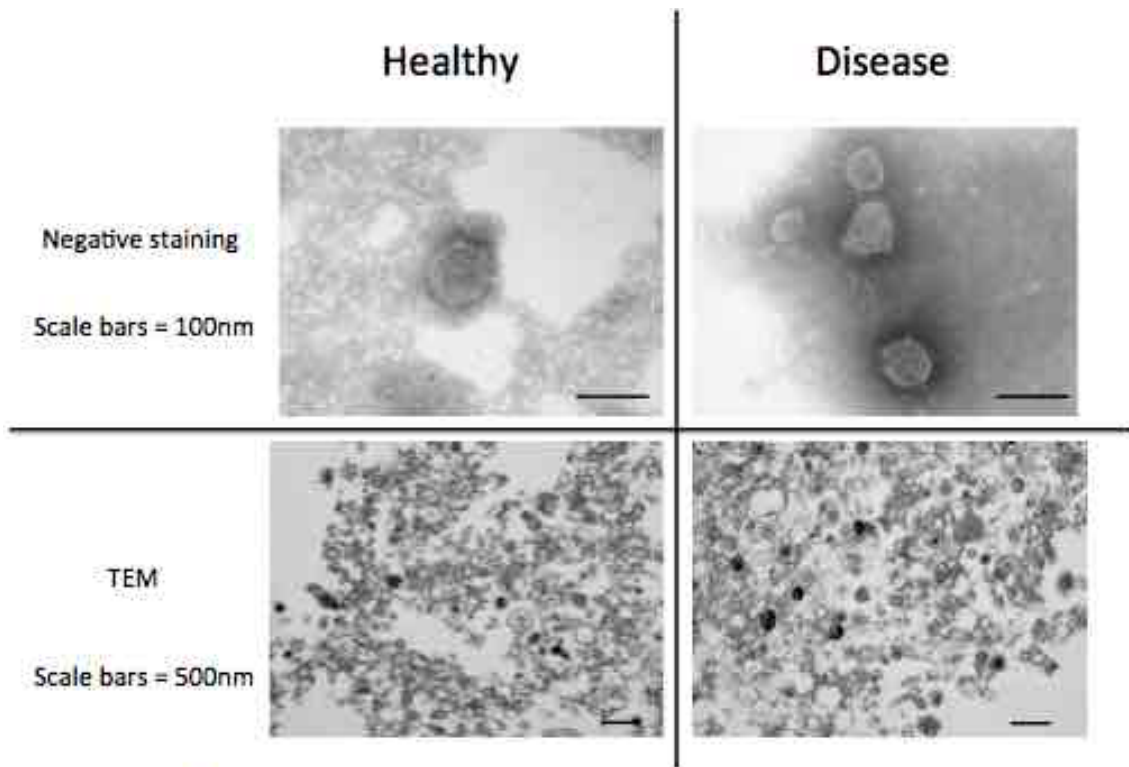


Electrophoresis images of RT-PCR products



Supplemental Fig 1

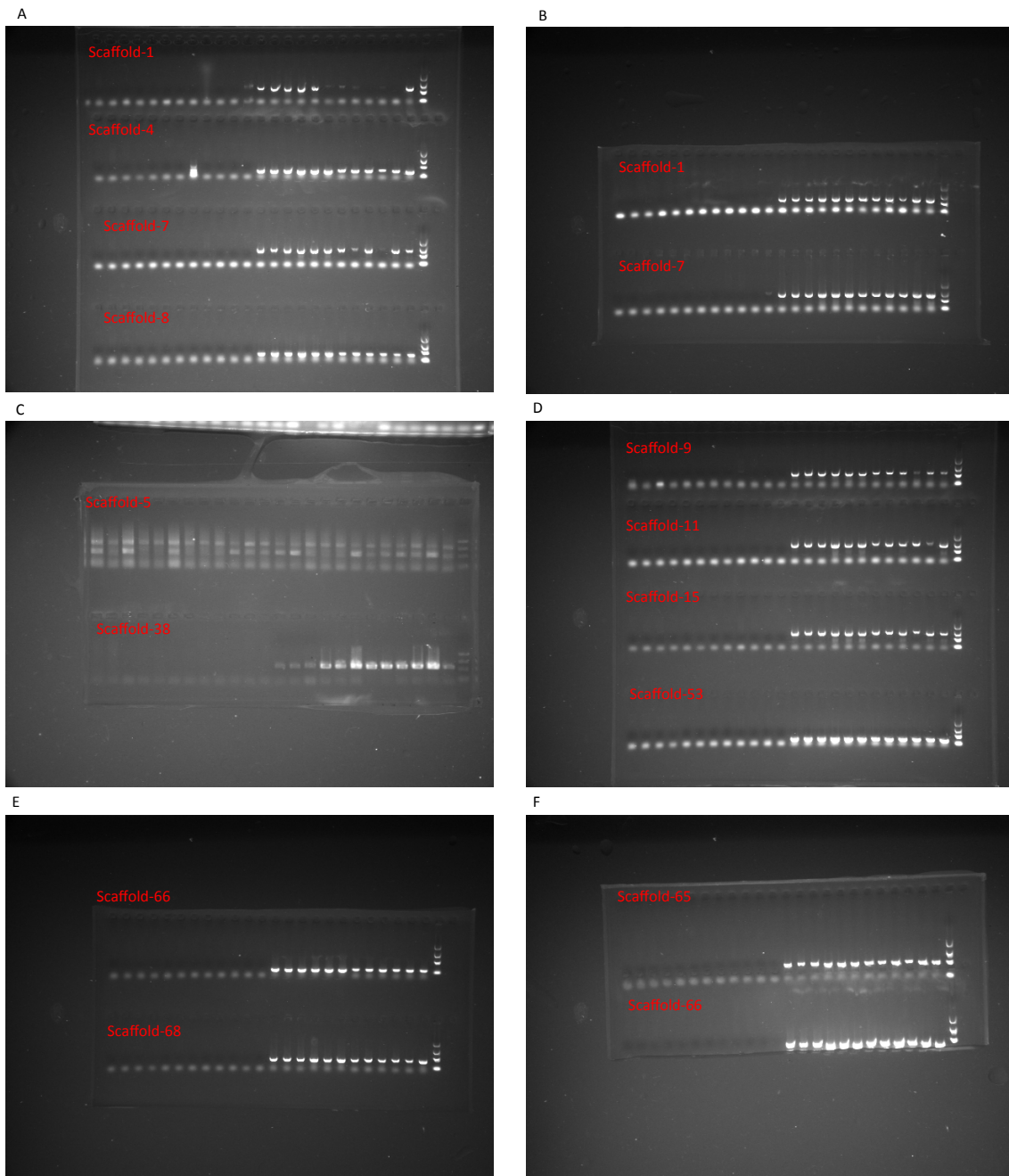
Supplemental Figure 1. Electrophoresis images of PCR and RT-PCR products for pooled DNA/RNA samples. The squares and numbers indicate electrophoresis images of target genes corresponding to each number. PCR products from healthy abalone isolated in 2015 and 2016, diseased black abalone (*Haliotis discus discus*), and diseased giant abalone (*Haliotis madaka*) (N=6 per group) are shown. M indicates the DNA molecular-weight size marker (2,000, 1,000, 500, and 100 bp for PCR; 600, 500, 400, 300, 200, and 100 bp for RT-PCR).



Supplemental Fig. 2

Supplemental Figure 2.

Example of electron microscopic (EM) observation. In images obtained from negative staining and TEM analyses, spheroids of various sizes were found in the fractions prepared from both healthy and diseased abalone.



Supplemental Figure 3. Full-length electrophoresis images for analyses described in Figure 2.

(A) Electrophoresis images for Scaffolds 1, 4, 7, and 8. Because Scaffold 1 had many false-negative results among diseased abalone, PCR and electrophoresis were repeated,

and the results from the repeated test (B) were described in the main text. (B) Electrophoresis images for Scaffolds 1 and 7. Scaffold 7 electrophoresis images show a weak positive signal from a healthy abalone sample in the 12th well. Thus, results from (A) were described in the main text. (C) Electrophoresis images for Scaffolds 5 and 38. (D) Electrophoresis images for Scaffolds 9, 11, 15, and 53. (E) Electrophoresis images for Scaffolds 66 and 68. (F) Electrophoresis images for Scaffolds 65 and 66. Because the Scaffold 66 PCR product migrated too far, the image shown in (E) was used in the main text. PCR products from healthy abalone in 2015 and 2016, diseased black abalone (*Haliotis discus discus*), and giant abalone (*Haliotis madaka*) (N=6 per group) were electrophoresed, stained with GelGreen, and visualized using an LED transilluminator. The rightmost lane is the DNA molecular-weight size marker (2,000, 1,000, 500, and 100 bp).

Supplemental Table 1. Primers for PCR- and RT-PCR-based screening of sequences possibly derived from putative causative agent, and results of PCR and RT-PCR for pooled DNA samples

Scaffolds	Scaffold length (bp)	Reads mapped	Mean coverage	F-primer	sequence(5'-3')	R-primer	Sequence (5'-3')	Expected product size (bp)	Results of PCR and RT-PCR of pooled DNA sample
Primers designed by Primer3									
scaffold_0001	49,616	715,710	1,983	F-seq_0001	GCGAATCCGATTTGGGCTGTGCGCCG	R-seq_0001	TCAAAATGAGGGCTCTCTGCCCATCG	795	D
scaffold_0002	39,570	1,726,916	6,135	F-seq_0002	AGGCCCGGATCCCGCGTGGT	R-seq_0002	CGCGCGCTTGGCCGCTTGGGG	1259	N
scaffold_0003	30,917	733,072	3,199	F-seq_0003	TGCCCTTATGGGGAGGATTCGACACT	R-seq_0003	TCCCGTTCAGGTTAGAGAGCGCTGG	323	N
scaffold_0004	19,721	349,693	2,412	F-seq_0004	CGGGCCCGCTACCGGAATCGGC	R-seq_0004	TCCCGTTCAGGTTAGAGAGCGCTGG	341	D
scaffold_0005	18,328	316,967	2,355	F-seq_0005	AGTGGGAAGGGGTGGGGCA	R-seq_0005	ACCAATGAGGTCAGGCGCCGCG	469	D
scaffold_0006	15,747	425,616	3,679	F-seq_0006	TGCTTATCCAAACCTGCCATGATCTCT	R-seq_0006	ACCCAGGAGCTTACGGTACAGGGGCA	538	N
scaffold_0007	14,418	288,679	2,748	F-seq_0007	ACGGCCCAACGATGGACGGCA	R-seq_0007	CGCGCGGGCGCCCAACT	990	D
scaffold_0008	9,744	109,705	1,541	F-seq_0008	TCTGTGCAATGAGGGGGCTGGAGGCA	R-seq_0008	ATTGGTGTATGGGGCGCCGACTCTTT	343	D
scaffold_0009	8,540	126,524	2,030	F-seq_0009	TCTTGGTGGGGTGGGGGCA	R-seq_0009	TGGGGGCGCCCTTGAATCCC	711	D
scaffold_0010	7,990	111,815	1,935	F-seq_0010	ACAGGGTGGAGGAACTGGTGCTGA	R-seq_0010	AGACCTGAAGGTTCTCCCAACAGT	836	N
scaffold_0011	7,378	148,766	2,768	F-seq_0011	CCCTTCCCTGGCTTCAGAAATGGGCA	R-seq_0011	TGCAGCCCGAAATGAGCGCGGT	1172	D
scaffold_0012	6,695	68,979	1,422	F-seq_0012	AGGAGCATGCACTTGTACAACAACTCC	R-seq_0012	ACTGCCCTTGGTGGAAATAAACCCGTTCA	900	N
scaffold_0013	6,183	61,861	1,384	F-seq_0013	AACACGACACAGCTGCAGCAACG	R-seq_0013	TGATGGCTTGTGGTAAGGGAGG	949	N
scaffold_0014	5,566	166,266	4,114	F-seq_0014	ACCACCACTATCGGTGGAGACGGA	R-seq_0014	ACACACCCGAGCTTGGAGAGGAA	607	N
scaffold_0015	5,385	71,380	1,810	F-seq_0015	CCCACTGTGCAAGTTTCTACACCA	R-seq_0015	TGGCTTTACAACTGTGGGGCGCAACA	1134	D
scaffold_0016	4,538	52,969	1,592	F-seq_0016	TAGCTGTGCTGGTGTGCACTTGTGAT	R-seq_0016	TAACTGGTGTGGCCCTTAACTGGTGT	1494	N
scaffold_0017	4,005	219,083	7,536	F-seq_0017	GGAAAGCCCAACCTCTCTCTGTC	R-seq_0017	TCTGATCTTAAAGTGGAGTTGTCCGTGGT	1190	N
scaffold_0020	3,586	140,370	5,376	F-seq_0020	AACCGGAGGCAAGGAGGAAACAGTAG	R-seq_0020	TTTCTCTTGGCACTTCACTCTTGG	474	N
scaffold_0021	2,853	57,456	2,662	F-seq_0021	TGTAATACACAGACTCAAGGCTACACAGGGT	R-seq_0021	ACGGTCAACAGTGAAGAGGAAAGGGATTC	1358	N
scaffold_0027	1,538	25,040	2,245	F-seq_0027	TGCTGTGGCACTCAGCAGCCCAATA	R-seq_0027	AGAGCCAGGAGGCAAGCACTGAC	488	N
scaffold_0031	1,444	26,554	2,537	F-seq_0031	AACACCGTGTCTGATGAAATGTCACAAAGGTA	R-seq_0031	TCCCGGCTAAAGCGGATTTGCTCAGA	589	N
scaffold_0034	1,382	47,949	4,824	F-seq_0034	CGCTTACGCGCAATAGCAAGCAAAACC	R-seq_0034	ATGGGCTTGGGTGTGTGCTGGTGA	747	N
scaffold_0036	1,241	13,963	1,566	F-seq_0036	GTCCCAAGGTTGATGAAACCAAGCCACT	R-seq_0036	GGGGGAGCTGGGTCTAACCAAGAAACAG	823	N
scaffold_0041	1,106	18,684	2,307	F-seq_0041	AGGAGGTGACAGACTAGGCTGGAACAACA	R-seq_0041	TCTGGTAAGCTTACTTGTGATGAAGAAACA	148	N
scaffold_0050	950	46,126	6,383	F-seq_0050	AGCGGAATGTTAAGCTGGGGACGGAT	R-seq_0050	TTCACCCCTCCGGGTGATCTCTCTCC	164	d
scaffold_0053	912	11,236	1,692	F-seq_0053	ACTGGGCAAGGCTTAAATAATGGCCGA	R-seq_0053	TCCGCACTTGTAAACTTCCGCATGGA	300	D
scaffold_0054	911	82,250	12,465	F-seq_0054	GGAGGGTGAAGGATTTGATGATGGT	R-seq_0054	CGGGTGAAGGATTTGATGGGGGGAAT	342	N
scaffold_0059	856	35,258	5,317	F-seq_0059	CACTTCCGCTGGTCTTAACTGATGTTA	R-seq_0059	TCTTAACAATACCGCTCACTTCCCACTCT	103	N
scaffold_0065	813	38,802	6,577	F-seq_0065	TGTCATGCAAGGAGCAAGTAGGGGGA	R-seq_0065	ACACCAACCGGTTAAACTCAACTAGT	553	D
scaffold_0066	806	25,947	4,388	F-seq_0066	ACCAACAGTGTGTAAGCACTAAGCAAGGGA	R-seq_0066	ACGAAGTGAAGGAGGGGAAAGTCTAGT	185	D
scaffold_0068	800	27,063	4,579	F-seq_0068	GAACAGTGTCTTGGCCGCTTCTCTTCTA	R-seq_0068	CCCAACCGTTCGGCAACAGTAGGAC	460	D
scaffold_0076	770	35,673	6,469	F-seq_0076	GGCGTAGGAGGATTCCTATGAGGCT	R-seq_0076	TCTTATCACTCTCAACCAAGTGGGGC	123	N
scaffold_0077	768	87,025	15,462	F-seq_0077	TGATGTCGAATGAGCTTGGTGTGATGCA	R-seq_0077	AGGAGGATCCCAAGTAAATGGAATGGGGA	195	N
scaffold_0080	750	67,682	12,486	F-seq_0080	TGTGTTCTTAACTTGGGCTGGCGAGCT	R-seq_0080	ACCAACAACAATACCGCACTGCGCAAA	250	N
scaffold_0088	723	21,957	4,212	F-seq_0088	GGTGCACATGGCAAGGCTGGTGTGTT	R-seq_0088	AAACCAACCCCAAGCAGACTTGGGA	167	N
scaffold_0090	710	103,387	20,038	F-seq_0090	TCTAAGGTTCTCGCTCCGCTCGTCAA	R-seq_0090	CAAGGAGAGGGGTCTAGCCCAAGCAGA	404	N
scaffold_0091	701	22,077	4,369	F-seq_0091	ACGGTAAAGTCAAGGACCAAAACCCAGA	R-seq_0091	ACATACGCTGTGGTGTGGTGGGACAA	515	N
Manually designed primers									
scaffold_0029	1,496	25,766	2,221	F-seq_0029	CTGAAGTAAGTCACTCAAGCTCACATAG	R-seq_0029	CCAAAGTCTCAAACTAGGTGATGTCAGG	347	B
scaffold_0038	1,217	17,112	1,911	F-seq_0038	GATATGATAAATTTGGCAACAGTAATCTACCTGAAAGTACC	R-seq_0038	CAAAAGATGTTGTCAAAATTTTCACTATACTCCGCTACC	755	D
scaffold_0044	938	11,146	1,654	F-seq_0044	CGGATTAATGAAAGTCTAATACGGATTAATGAAAGGAAAGG	R-seq_0044	CAATTAATCCGCTTCAAGCTTCAATATCCCGC	552	B
scaffold_0046	1,025	15,142	2,037	F-seq_0046	GATATGCTCTTCTATGACAGATAGATGATATAAACC	R-seq_0046	ACTCATCTTCCCAAAACTTAAACTTTTGGCTTGGC	375	N
scaffold_0047	993	32,733	4,551	F-seq_0047	GTCTTTCAGGTTATACATAAGCTTAAATACC	R-seq_0047	AGTGTGAGTGGCGCAACATGCAAGGTG	333	B
scaffold_0048	972	53,853	6,909	F-seq_0048	CCCAAGGATGACCACTGCAATACC	R-seq_0048	CATCAAGCAATCAGATATGCGTGTAGCTACC	318	N
scaffold_0062	832	11,790	1,952	F-seq_0062	CATATATCACACAACAACCAACAGAAAGCC	R-seq_0062	TCTGGCAAGACTCTTCCCGGATGA	224	N
scaffold_0069	797	104,706	17,621	F-seq_0069	GATTAAGATTTCACTCACTCTTAAAGG	R-seq_0069	GCTAAAAGATATGAAAGAACTAATATCATGG	317	N
scaffold_0081	746	37,439	6,724	F-seq_0081	CCTAATCTGCTCAAACTCTTAAAGAAATAGG	R-seq_0081	TCTAATGAATFAGTAAATGAAAGGTGGTGAATCC	382	N
scaffold_0089	715	9,986	1,934	F-seq_0089	CAATCTATGTTTATACAACTGTATGTTG	R-seq_0089	AGGAAGTATAGAGGGTCTGTAGGCTAGGG	331	N
scaffold_0099	643	81,487	16,917	F-seq_0099	CTACATTAICAGAAGCTTCACTGATACATC	R-seq_0099	CTAGTACTCAAAAATGCTAAAATAAAGAAATATGG	275	N
scaffold_0100	640	5,705	1,255	F-seq_0100	GAGATGATATGAAGCAACGAAACG	R-seq_0100	CCGGAGGTTGAGGGTAACTATATCC	258	N
scaffold_0104	492	9,504	2,619	F-seq_0104	CATGAGTTTGTATGCTGGTGAAGTGTATGTTCCG	R-seq_0104	TGTATGCGGTGAGTGTGCAATGTCGG	398	B
scaffold_0107	612	95,237	21,033	F-seq_0107	AGCTTTCTTAAACTGCTTACGGAGCTAGT	R-seq_0107	GGTAAGAACCTCAAAAAGTATGAGGCTATC	273	N
scaffold_0117	574	2,913	675	F-seq_0117	GCAAGAAATGAATAGAGTAGAAATGG	R-seq_0117	GAACCTTAGGAGTTACGAAATGG	272	N
scaffold_0120	566	26,316	6,091	F-seq_0120	GTGACCAAGATATGAAAGATACAG	R-seq_0120	CTTTCTTTGGTTTGGAGGAGCGG	205	B
scaffold_0124	425	24,817	8,205	F-seq_0124	ACTTCCAGCAAGGCAATACCGCAC	R-seq_0124	TCTTGTGCTGGGATGCTTACTGCTTTGG	351	B
scaffold_0165	442	6,727	1,727	F-seq_0165	GTGTTTCCGTGATGTAACAGTGTGTTCACTATG	R-seq_0165	GTCAACTAGGATGAAATGATCATTTGATGTTG	298	N
Primers for RT-PCR									
contig_01	507	1,169,291	272,143	F-RT-contig_01	AGCTTGGTCCAGCAGCCGCGGTAA	R-RT-contig_01	TGGCAAGGGGAGCAGCCCGCA	151	B
contig_02	1,078	1,716,902	187,935	F-RT-contig_02	TGGCAAGGGGCTTCCCGCTGGCT	R-RT-contig_02	TCCCGCCCGGAGGAGCA	269	B
contig_03	591	2,463,373	531,773	F-RT-contig_03	TGGAGGGCTAGAGCGTGGGCT	R-RT-contig_03	TGCACCGGGGGCTTCACTAGGCT	147	B
contig_06	937	1,318,363	166,027	F-RT-contig_06	CCGCGCGGCACTTGGTGGCA	R-RT-contig_06	ACGCGCGGCGGCGCTGGA	116	N

Mean coverage=Number of reads/Average read length/Scaffold length
D: Amplification specific to diseased abalone, N: No amplification, B: Amplification in both abalone species, d: Amplification in only one diseased abalone species
F: forward, R: reverse

Supplemental Table 2. Primers used for gap closing

Scaffold	F-primer	R-primer
Scaffold-1	GTGGTCTTGATTACATGACACAGTAAAAATAGGGCATG	CGGGTCAATCCGTTGGTATCTTTGATGCGGTGTTCTAC
Scaffold-4	GCGGTGTTTTAGTATTTTCAATTAAGTCCAATTTGGTGATACC	CTCGAAAATGTTTTATGTCGATTCACCGAAAATACACATTACC
Scaffold-5	CAGTGAAAAATCCATCAACCGATCACTTGTGGACC	CCATACGAACAACCCATTTAGTGAACGCCCATACAATCTGG
Scaffold-7	CCGTGCAAAATACAAAACCTATCAGTCTCGGC	GTTTTCTTAAACCATCACCAATTAATCACTGCTCTGAAGGC
Scaffold-8	CCTAGACCGATTAGTGATACATCCATTCACGGC	GTCCGACGGAATATCTTCTGGAAGTCGGAG
Scaffold-9	CAATTATGGATTACAAAACATGCACTGGCGGGACACC	GTCGGAGGATCAAGTGTGTTGAAACAAAAGATGGCTC
Scaffold-11	GTGGATTAATATTTGGCCCAATCCCTAATTTACCAAGTGCCG	CATAGCAAAAAGGACAGTTATAAAAATCGGAAAATGTGCGG
Scaffold-15	CTTAAACACATTTATATCAGTGTGGCGGTGATTTGGCC	CCAAAAGATACAGTCTCAATATATATGCTTATCCCG
Scaffold-38	GGTTCACATGCTAAACATTTTCTAGCAACACTTGATGG	GGATACGGTAACTTACAGTATGATCTTGTGCC
Scaffold-53	CACCGTGATATCGCTATTGATATGACTGCAGCAG	CGAGAATAATAGCATAAAGATTGATGATCGGTGAGTC
Scaffold-65	GACATGGGGCAAAAAGAATTATAAATATAGCGGAGCC	CTCTTTCGCGCTCATATCAATTTACCAGTCAATTTGCC
Scaffold-66	CATGTAATTTATATCAAAAAACTAGACTTTGCCCGCC	CCTAATCTGGTGAACATTTGCGAAGTGGTTG
Scaffold-68	TTGCGGAAAAGCGGTGGGCAATAACTGG	GGAAAAGTAAAAATATAAATCGATCGCAACTTAGTATTGG

F: forward, R: reverse

Supplemental Table 3. Characterization of potential open reading frames of AbAV virus genome

ORF #	Nucleotide position	Orient ation	Length (codon)	Predicted similarity and/or function	Best BLASTP hits of AbAV ORF against virus database										
					Species	Family	Accession no. *	Total score	Query cover	E-value	Identity				
ORF-1	2	970	322												
ORF-2	1005	2846	+ 613												
ORF-3	2864	4534	+ 556												
ORF-4	4562	6229	+ 555												
ORF-5	6221	6709	+ 162	YbIA	Stenotrophomonas phage DLP4	Siphoviridae	AT992227.1	122	88%	4.00E-34	42.36%				
ORF-6	6772	7254	+ 160												
ORF-7	7297	7791	+ 164												
ORF-8	7842	8258	+ 138												
ORF-9	8308	8745	+ 142												
ORF-10	9256	9588	+ 110												
ORF-11	9663	10109	+ 148												
ORF-12	10162	10566	+ 134												
ORF-13	10601	11005	+ 134												
ORF-14	11042	11524	+ 160												
ORF-15	11576	12088	+ 170												
ORF-16	12085	13371	+ 428	inhibition of apoptosis protein (IAP-3)	Spodoptera frugiperda multiple nucleopolyhedrovirus	Baculoviridae	ACA02668.1	55.8	20%	9.00E-07	34.09%				
ORF-17	13438	13731	+ 97	Viral histone-like protein, A104R	African swine fever virus	Asfarviridae (NCLDV)	POC9E4.1	75.1	98%	3.00E-17	41.67%				
ORF-18	13755	14435	+ 226												
ORF-19	14415	15434	+ 339	Uncharacterized protein F317L	African swine fever virus	Asfarviridae (NCLDV)	POCAE0.1	87	71%	2.00E-17	24.00%				
ORF-20	15522	16454	+ 330	Ribonucleotide reductase small subunit	African swine fever virus	Asfarviridae (NCLDV)	AIT22235.1	269	98%	4.00E-87	45.90%				
ORF-21	16595	18979	+ 794	F778R	African swine fever virus	Asfarviridae (NCLDV)	AZX95821.1	559	93%	0	41.10%				
ORF-22	18971	22860	+ 1029	Putative helicase/primase complex protein:pF1055L	African swine fever virus	Asfarviridae (NCLDV)	POCA11.1	525	97%	1.00E-167	34.70%				
ORF-23	22117	22389	+ 90												
ORF-24	22416	22589	+ 57												
ORF-25	22622	22921	+ 99												
ORF-26	22928	23071	+ 47												
ORF-27	23074	23229	+ 51												
ORF-28	23268	23540	+ 90												
ORF-29	23601	23729	+ 42												
ORF-30	23937	24119	+ 60												
ORF-31	24148	24351	+ 67												
ORF-32	24373	24516	+ 47												
ORF-33	24545	24709	+ 54												
ORF-34	24742	25788	+ 348												
ORF-35	25820	26330	+ 236												
ORF-36	26272	27795	+ 407	Probable methyltransferase, EP424R	African swine fever virus	Asfarviridae (NCLDV)	AJL34069.1	213	89%	6.00E-63	38.36%				
ORF-37	27834	28658	+ 274												
ORF-38	28705	28971	+ 88												
ORF-39	29004	29579	+ 191	thymidine kinase	NY_014 poxvirus	poxvirus (NCLDV)	YP_009408474.1	123	96%	9.00E-34	34.24%				
ORF-40	29949	31238	+ 429	K421R	African swine fever virus	Asfarviridae (NCLDV)	AZX95828.1	186	96%	2.00E-52	30.57%				
ORF-41	31189	34002	+ 1121	RNA polymerase subunit 2	African swine fever virus	Asfarviridae (NCLDV)	CAN10153.1	1673	98%	0	64.88%				
ORF-42	34990	35913	+ 307												
ORF-43	35935	36381	+ 148												
ORF-44	36404	37339	+ 311	pP364R	African swine fever virus	Asfarviridae (NCLDV)	AXB49288.1	97.8	96%	3.00E-21	27.08%				
ORF-45	37336	41244	+ 1302	BA71V/M1249L	African swine fever virus	Asfarviridae (NCLDV)	POCAB5.1	442	78%	2.00E-132	29.82%				
ORF-46	41286	42533	+ 415	pM448R	African swine fever virus	Asfarviridae (NCLDV)	AAX3490.1	102	71%	1.00E-21	26.54%				
ORF-47	42522	42851	+ 99												
ORF-48	42848	43192	+ 114	Uncharacterized protein C129R	African swine fever virus	Asfarviridae (NCLDV)	POCA38.1	93.6	94%	5.00E-24	41.96%				
ORF-49	43218	43403	+ 61												
ORF-50	43450	45501	+ 683	Uncharacterized protein C717R	African swine fever virus	Asfarviridae (NCLDV)	POCAJ6.1	307	96%	1.00E-92	31.61%				
ORF-51	45524	45841	+ 105	Uncharacterized protein C122R	African swine fever virus	Asfarviridae (NCLDV)	POCA22.1	94	95%	1.00E-24	44.00%				
ORF-52	45843	46349	+ 168												
ORF-53	46401	47789	+ 462	Putative poly(A) polymerase catalytic subunit	African swine fever virus	Asfarviridae (NCLDV)	POC9D2.1	258	100%	6.00E-79	34.16%				
ORF-54	47822	48841	+ 330	Uncharacterized protein C315R	African swine fever virus	Asfarviridae (NCLDV)	POCAD6.1	149	93%	2.00E-40	31.88%				
ORF-55	48856	49255	+ 139												
ORF-56	49369	49596	+ 75												
ORF-57	49571	51829	+ 752	BA71V-B962L	African swine fever virus	Asfarviridae (NCLDV)	AJL34086.1	624	98%	0	45.21%				
ORF-58	51838	52203	+ 121	FAD-linked sulphydryl oxidase (p14)	African swine fever virus	Asfarviridae (NCLDV)	Q9JFM9.1	86.7	79%	3.00E-21	43.75%				
ORF-59	52208	53632	+ 474												
ORF-60	53673	54137	+ 154												
ORF-61	54145	54903	+ 252	hypothetical protein D5b_00348	Faustovirus	Asfarviridae ? (NCLDV)	AMN83769.1	111	88%	1.00E-26	31.14%				
ORF-62	54905	56056	+ 383	B354L	African swine fever virus	Asfarviridae (NCLDV)	AZX95853.1	271	98%	1.00E-86	40.00%				
ORF-63	56071	56556	+ 161												
ORF-64	56587	57114	+ 175												
ORF-65	57107	57646	+ 179												
ORF-66	57715	59334	+ 539	BA71V-B602L (9RL)	African swine fever virus	Asfarviridae (NCLDV)	AJL34093.1	240	97%	9.00E-71	29.53%				
ORF-67	59371	60480	+ 369	Zinc finger protein B355R	African swine fever virus	Asfarviridae (NCLDV)	Q8V957.1	244	93%	6.00E-76	39.89%				
ORF-68	60482	62425	+ 647	major capsid protein p72	African swine fever virus	Asfarviridae (NCLDV)	AAX34439.1	583	99%	0	44.99%				
ORF-69	62439	62888	+ 149												
ORF-70	62906	64078	+ 390	Hypothetical protein PACV_238	Pacmavirus A23	Asfarviridae ? (NCLDV)	YP_009361587.1	137	91%	4.00E-35	28.49%				
ORF-71	64121	64741	+ 206	BA71V-B175L	African swine fever virus	Asfarviridae (NCLDV)	AJL34264.1	97.1	83%	1.00E-23	31.40%				
ORF-72	64824	65612	+ 262	B263R	African swine fever virus	Asfarviridae (NCLDV)	AZX95861.1	171	98%	1.00E-50	40.91%				
ORF-73	65597	65941	+ 114												
ORF-74	65964	70244	+ 1426	BA71V-B175L	African swine fever virus	Asfarviridae (NCLDV)	AJL34102.1	770	99%	0	34.52%				
ORF-75	70289	70861	+ 190	Hypothetical protein D6_00329	Faustovirus	Asfarviridae ? (NCLDV)	AMN84731.1	120	91%	2.00E-32	37.56%				
ORF-76	70882	74226	+ 1114	DNA polymerase beta	African swine fever virus	Asfarviridae (NCLDV)	POC972.1	748	96%	0	39.60%				
ORF-77	74257	74790	+ 177												
ORF-78	74785	75475	+ 296	putative ubiquitin carboxyl-terminal hydrolase 2-like	Harvovirus sp.	Mimiviridae (NCLDV)	AYV80395.1	55.1	86%	8.00E-07	23.88%				
ORF-79	75656	75904	+ 82												
ORF-80	75907	83604	+ 2565	CP2475L (p20)	African swine fever virus	Asfarviridae (NCLDV)	AZX95866.1	981	99%	0	29.29%				
ORF-81	83681	85270	+ 529	60 kDa polymerase	African swine fever virus	Asfarviridae (NCLDV)	AYJ22285.1	325	85%	1.00E-103	39.85%				
ORF-82	85284	85535	+ 83	Putative DNA-directed RNA polymerase subunit 10 homolog	African swine fever virus	Asfarviridae (NCLDV)	POC979.1	69.7	100%	1.00E-15	42.86%				
ORF-83	85547	86647	+ 366	CP312R	African swine fever virus	Asfarviridae (NCLDV)	AYW34807.1	135	83%	4.00E-35	31.68%				
ORF-84	86667	91013	+ 1448	Probable DNA-directed RNA polymerase subunit 1 homolog	African swine fever virus	Asfarviridae (NCLDV)	POC983.1	1637	99%	0	53.96%				
ORF-85	91071	92453	+ 460	Chan A, DNA ligase	African swine fever virus	Asfarviridae (NCLDV)	AZX95874.1	256	92%	4.00E-79	37.76%				
ORF-86	92512	93390	+ 292												
ORF-87	93442	94488	+ 348												
ORF-88	94588	95010	+ 140												
ORF-89	95041	95601	+ 186												
ORF-90	95651	96196	+ 181												
ORF-91	96247	96498	+ 83												
ORF-92	96528	96686	+ 52												
ORF-93	96727	97017	+ 96												
ORF-94	97055	97543	+ 162												
ORF-95	97575	98027	+ 150												
ORF-96	98082	98441	+ 119												
ORF-97	98470	98766	+ 98												
ORF-98	98761	99768	+ 335												
ORF-99	99831	100247	+ 138												
ORF-100	100251	100697	+ 148												
ORF-101	100798	102825	+ 675												
ORF-102	103488	103751	+ 87												
ORF-103	103786	104781	+ 331												
ORF-104	104796	105665	+ 289	Transcription factor S-II-related protein	African swine fever virus	Asfarviridae (NCLDV)	POCF8.1	123	67%	7.00E-32	39.30%				
ORF-105	105662	105841	+ 59												
ORF-106	105893	106333	+ 146												
ORF-107	106395	106610	+ 71												
ORF-108	106638	108146	+ 502	Hypothetical protein BpV2_110c	Bathyococcus sp. RCC1105 virus BpV2	Phycodnaviridae (NCLDV)	ADQ91277.1	137	92%	1.00E-33	24.03%				
ORF-109	108259	109359	+ 426	Hypothetical protein Hokovirus_3_43	Hokovirus HKV1	Mimiviridae (NCLDV)									