Supporting Information

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Specimen Collection

Three hundred thirty-five specimens representing 14 species of asteroids were collected on the North American Pacific Coast between 19 September 2013 and 2 April 2014 (Table S1). Of these specimens, 138 individuals had been maintained in public aquaria (Monterey Bay Aquarium, Vancouver Aquarium, Long Marine Laboratory, Seattle Aquarium, Port Townsend Marine Science Center, and California Science Center), and the remainder were collected by hand from intertidal or subtidal habitats. Animals were classified as symptomatic and asymptomatic by contributors based on the presence (symptomatic) or absence (asymptomatic) of sea-star wasting disease (SSWD) signs, which included (*i*) loss of body turgor (deflation) and weakness, (*ii*) foci of epidermal pallor and tissue loss, (*iii*) sloughing of rays and/or rupture of the body wall with evisceration, and (*iv*) death. A purely visual approach to symptomatic versus asymptomatic categorization was necessary. More symptomatic (n = 286), were collected than asymptomatic individuals (n = 49). Among the species studied, the most numerous were *Pisaster ochraceus* (n = 88) and *Pycnopodia helianthoides* (n = 78), with fewer *Pisaster brevispinus* (n = 63), *Pisaster giganteus* (n = 35), *Evasterias troschelii* (n = 33), and *Patiria miniata* (n = 11), and all other species represented by <10 individuals. After collection, animals were either frozen at −20 °C immediately (for field samples) or chemically anesthetized (at some aquariums, with MS-222 or $MgCl_2$) and then frozen. Subsequently, specimens were then either sectioned into rays or sent as intact whole animals to the Department of Microbiology at Cornell University for further analysis.

Sampling Procedure and DNA Extraction

Asteroid specimens were thawed at room temperature and then sampled by removing an ∼2-mm-wide strip running from the aboral surface to the ambulacral groove of the animal (including tube feet, ampullae, and axial canal) with sterilized scissors and forceps. Samples were taken from the widest part of the ray of most specimens. Samples were placed into sterile Bead Basher tubes (Zymo Research) and frozen before nucleic acid extraction. To assess within-animal microbial detection variability, two symptomatic individuals of *P. ochraceus* obtained from Cuyler Harbor, San Miguel Island (Channel Islands), CA, were partitioned into 23 samples each, representing the distal tip, two midpoint samples, and body wall of the central disk between each of the five rays, in addition to samples of gonad, pyloric caeca/digestive tract, and tube feet. Each sample was ∼0.2 g and was placed into a sterile Bead Basher tube and frozen before analysis. All samples were weighed before nucleic acid extraction. Holobiont DNA was extracted from samples using the ZR Tissue and Insect Mini kit (Zymo Research) following the manufacturer's protocols.

Viral Metagenomics

Viral metagenomes (i.e., metaviromes) were prepared following Gudenkauf et al. (1). Tissue samples (∼1-cm cross-section of a ray including body wall, epidermis, pyloric caeca, and gonad) were excised from specimens (Tables S2–S5) with sterilized dissecting scissors and placed into sterile 50-mL Falcon tubes. Samples were then amended with 50 mL of 0.02 μm-filtered PBS and homogenized in a NutriBullet for 1 min. Tissue homogenates were centrifuged at 3,000 × g for 5 min, after which supernatants (5–50 mL) were sequentially filtered through GF/A (Whatman), and then through 0.2-μm polyethersulfone (PES) (VWR) filters. Filtered homogenates were amended with 10% (wt/vol) PEG-8000 and precipitated overnight at 4 °C. Samples were then centrifuged at 12,000 × g for 1 h at 4 °C to pellet high molecular weight organic matter (including viruses). The supernatant was decanted, and pellets were resuspended in 1 mL of nuclease-free water (Promega). Samples were then syringe-filtered through 0.2-μm PES filters and then amended with 0.2 volumes of chloroform and mixed. The mixtures were centrifuged for 10 min at 12,000 × g, and the supernatant was transferred to a new microcentrifuge tube. The purified DNA was treated with TURBO DNase (14 U), RNase One (20 U), and Benzonase nuclease (1 μL) and incubated at 37 °C for 2 h. At the conclusion of nuclease treatment, samples were amended with 20 μM EDTA. Viral DNA was extracted from 500-μL subsamples of nuclease-treated purified viral preparations using the ZR Viral DNA kit (Zymo Research), following the manufacturer's recommendations. Viral RNA was extracted using the RNA Mini Isolation kit (Zymo Research), and coextracted DNA was removed by using the DNA-free RNA kit (Zymo Research). After extraction, DNA was amplified using the WGA2 kit (Sigma Aldrich), and RNA was amplified using the WTA kit (Sigma Aldrich) before being submitted to the Cornell Core Biotechnology Resource Center Genomics Facility for sequencing using Illumina 2 × 200-bp HiSeq chemistry. All sequences have been deposited in GenBank under BioProject accession no. PRJNA253121.

For each library, sequence data were transferred to the CLC Genomics Workbench 4.0 where they were trimmed for quality (n < 2 ambiguous bases, sequences >200 bp or <200 bp were discarded) and assembled into contiguous sequences (minimum overlap 0.2 and identity 0.95). ORFs on contigs were determined using the GetORF algorithm (EMBOSS) (2). ORF sequences were analyzed by BLASTx comparison against the National Center for Biotechnology Information (NCBI) nonredundant (nr) protein database via the Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA) (camera.calit2.net) (3). Contigs were phylogenetically annotated according to the top ORF hit (i.e., lowest E-value), where hits E > 10^{-3} were removed from further analysis. Recruitment information for each contig was then assigned to produce quantitative information on phylogenetic representation within each library.

Viral metagenomes contained between 5 × 10^5 and 4 × 10^6 reads, of which 41–80% assembled into contiguous sequences (Tables S2–S5). We focused analyses on contigs for annotation because the short length of sequence reads (200 bp) does not permit confidence in phylogenetic assignment. Between 2% and 60% of sequence reads assembling into contigs matched homologous proteins in the nonredundant database and could be assigned to a specific taxon based on their affiliation. Of these annotated contigs, the percentage of viral annotations was 0.02–25.45% of total sequence reads. The greatest proportion of viral reads was obtained in samples from *E. troschelii* from the Vancouver region (7.8–25.4% of reads) whereas the smallest was for *P. ochraceus* from Olympic National Park.

The majority of annotated reads within DNA viral metagenomic libraries were to Bacteria (45%) and Eukarya (39%) whereas viruses accounted for 0.1–68% (mean 15.3%). Among viral reads, most annotations were to bacteriophage (mean = 67%) and fewer to viruses of eukaryotic organisms (33%). In DNA metaviromic libraries, similarities to RNA viruses constituted only a small percentage (mean = 1.5%) of total viral reads

whereas, in RNA libraries, they constituted a much greater proportion (mean 43%).

Eukaryotic viral annotated reads in DNA libraries clustered primarily to phycodnaviruses and mimiviruses, both within the proposed family *Megaviridae*. Large representation of this viral family is likely a consequence of their extremely large size compared with other known viruses, or the presence of viruses that infect microscopic algae that may be present on animal surfaces or within digested food material or those present on the animal's exterior. In RNA libraries, Dicistroviruses constituted a large proportion of reads (41 ± 30% of symptomatic reads and 61 ± 9% of asymptomatic reads). Only two groups of viruses were disproportionately present in symptomatic compared with asymptomatic libraries: retroviruses and parvoviruses, the latter constituting a small fraction (2.23 ± 1.44% of total viral reads) (Fig. S1). Retroviral annotations are likely spurious because they were detected in DNA libraries (and have RNA as nucleic acids). With the exception of three samples (small *P. helianthoides* and *E. troschelii* from the Vancouver region and an asymptomatic *P. ochraceus* from Santa Cruz, CA), all hits to parvoviruses were from symptomatic asteroids ($n = 7$ libraries). Therefore, we targeted parvovirus-like viruses for further analyses.

Global assembly of all 28 metagenomes and screening for parvovirus-like fragments by BLASTn against a database of all known parvoviruses revealed the presence of a near-complete densovirus. The densovirus-like genome was not in our blank metavirome at any E-value by BLASTn analysis. We have previously observed densoviruses as a constituent of echinoid viral communities (1). The densovirus-like genome fragment was 5,050 nt and bore two ambisense ORFs that represent a well-characterized structural ORF (encoding VP4 and VP1) and nonstructural ORF (encoding NS1). We did not observe palindromic repeats at the distal ends of the genome fragment, suggesting that the contig does not represent a full viral genome. The VP1 protein (coat protein) was most similar to *Sibine fusca* densovirus (YP_006576514; E-value 2×10^{-17} by BLASTx) and the VP4 protein (major structural protein) was most similar to *Dysaphis plantaginea* densovirus (ACI01075; E-value 4×10^{-8} by BLASTx) whereas the NS1 protein was most homologous with *Periplaneta fuliginosa* densovirus (NP_051020; E-value 2×10^{-78} by BLASTx). Phylogenetic analysis based on the NS1 protein, which is a commonly used phylogenetic marker in studies of parvoviruses, indicated that the sea star-associated densovirus (SSaDV) is similar to a virus observed in the echinoid *Tripneustes gratilla*, but distinct from vertebrate parvoviruses and bocaviruses (Fig. 3).

Quantitative PCR of SSaDV

Quantitative PCR (TaqMan) primers were designed using Primer3 (4) specific to the nonstructural protein 1 (NS1; forward primer, 5′-ttaaggatcgggttcgtgtc-3′; reverse primer, 5′-tgcaagcggattaggtttct-3′; probe, 5′-tcaattggatgagtgcaccatttttga-3′; oligonucleotide standard, 5′-tttaaggatcgggttcgtgtcttcaattggatgagtgcaccatttttgaagaattatgataagaaacctaatccgcttgcag-3′) and viral gene product 4 (VP4; forward primer, 5′-ttgcattaattcctgctggt-3′; reverse primer, 5′-tgtaccaccagtgggatagc-3′; probe, 5′-tgatgtcatgcaaactgttgccaca-3′; oligonucleotide standard, 5-tttgcattaattcctgctggtagttacataaagtctgtatctattgatgtcatgcaaactgttgccacaactggctatcccactggtggtacaa-3′) of SSaDV. Primer specificity was checked by comparison of designed primers/probes and standards against the nonredundant database at NCBI by BLASTn. Quantitative PCR was conducted on asymptomatic and symptomatic asteroids in 25-μL reactions containing 1 X Universal Master Mix (Life Technologies), 200 pmol of each primer, and 1 μL of extracted DNA. Thermal cycling was performed in an ABI 7300 real-time thermal cycler. Thermal cycling comprised an initial denaturing step at 95 °C for 5 min, followed by 60 cycles of denaturing (95 °C for 30 s) and annealing (55.5 °C for 30 s). Each run of samples was accompanied by oligonucleotide standards over eight orders of magnitude and no-template controls. Detected gene-copy numbers were corrected for differences in extracted biomass by multiplying values by total extract volume and then dividing by the mass of asteroid tissue extracted. The practical detection limit of the VP4 primer set, based on the range of standards used in our study, was 2×10^2 copies per reaction, or 35 copies·mg^{-1} tissue. Quantitative PCR was performed on field-collected asteroids ($n = 339$) and for individual experiments as noted in subsequent sections. Viral loads were corrected for differences in the mass of holobiont extracted. Quantitative PCR inhibition was assessed in the first 80 samples analyzed by spiking samples with an internal oligonucleotide standard (10^4 copies per reaction) and comparing its cycle threshold value (Ct) to its respective standard run in a separate reaction. In these first 80 samples, no inhibition was observed.

Because of concerns that parvoviruses may be reagent contaminants, as observed in previous studies of parvovirus–circovirus hybrids (5), we rigorously interrogated a parallel "blank" viral metagenome but did not detect SSaDV. We also did not detect SSaDV in blank DNA extracts or in extracts of many asteroid specimens by PCR, strongly suggesting that SSaDV is not among recently described contaminants in molecular biology spin columns and reagents (5).

We sought to assess variability in viral load within individual animals and to determine whether nonlethal sampling of animals was feasible. Two *P. ochraceus* were sectioned into 22 tissue samples each, including body wall, arm tip, gonad, and digestive tract ($n = 20$), and nonlethal samples from tube feet ($n = 1$) and coelomic fluid ($n = 1$). We found considerable variability within and between both animals in SSaDV detectability (Fig. S2). SSaDVs were most consistently and with greatest abundance detected in tissues of samples of the interradial body wall of the central disk. Aboral and lateral body wall tissues were among the most variable tissues in which viral load was detected (SSaDV was detected in 11–38% of samples). Tube-feet samples demonstrated considerable variability in viral load, and variability was even greater in aboral and lateral body wall tissue of the arm. Overall, SSaDV was detected in 36–47% of tissue samples in symptomatic sea stars. These results suggest that single-location sampling of tissues describes potential viral load and prevalence, but SSaDV may in fact be present or more abundant elsewhere in animals. It is impractical to assay entire animals for every asteroid tested; therefore we feel that a combined sample of aboral and lateral body wall and tube feet (as used in this study for assessment of overall viral prevalence and load) adequately describes viral presence and abundance. Furthermore, both coelomic fluid and tube feet captured the presence of viruses and represent sublethal sampling that was used in later challenge experiments.

Quantitative Reverse Transcriptase PCR of SSaDV

To establish whether SSaDV was actively replicating, reverse-transcriptase quantitative PCR (qRT-PCR) was performed targeting the VP4 protein. RNA was extracted from *E. troschelii*, *P. ochraceus*, and *P. helianthoides* collected from several locations with the ZR RNA Mini Isolation Kit (Zymo Research). Extracted RNA was purified from coextracted DNA using the DNA-free RNA kit (Zymo Research). Total RNA was then converted to cDNA using SuperScript III (Life Technologies) before quantitative PCR analysis following the protocol above. For each sample, both cDNA and no-reverse transcriptase controls were quantified, and viral transcripts were corrected for no-RT controls and weight of sample extracted. Transcription of the structural gene was observed only in symptomatic *P. helianthoides* and *E. troschelii* compared with asymptomatic individuals. In *P. ochraceus,* transcription was significantly higher ($P < 0.05$, df = 10; *t* test) in asymptomatic than in symptomatic animals (Fig. 7).

Detection of SSaDV in Northwestern Atlantic Coast Samples

Similar SSWD-like symptoms were reported in Atlantic Coast asteroid populations between 2011 and 2013. Samples of apparently symptomatic *Asterias forbesii* from the Northwestern Atlantic Coast ($n = 15$) were obtained from the Mystic Aquarium. Samples were collected on 13 October 2012, 20 October 2012, 18 October 2013, 23 October 2013, 25 October 2013 ($n = 2$), 26 October 2013, and February 2014 ($n = 5$). Samples of asteroid tissues, similar to those from the Pacific Coast (described in *Sampling Procedure and DNA Extraction*), were collected, and DNA was extracted using the ZR Tissue and Insect kit (Zymo Research). Quantitative PCR targeting the NS1 and VP4 regions of SSaDV was performed on DNA extracts. SSaDV was detected in 9 of 15 samples, including all 3 samples from 2012, but only 2 of 5 samples from 2014. We also detected SSaDV by NS1 gene presence, which concurred with VP4 results in 3 asteroids (1 from 2012 and 2 from 2013). Therefore, the SSaDV detected in Pacific Coast asteroid samples was detected in asteroids from both the Atlantic Coast during the same time period of the Pacific Coast asteroid mortality event as well as in samples from 2012, which predates the disease event on the West Coast. The fact that some asteroids were positive only using the VP4 primers suggests that Atlantic asteroid tissues may have contained related, but not identical, densovirus genomes as well.

Longitudinal Study of SSaDV Dynamics

Eight asymptomatic small (~20-cm diameter) *P. helianthoides* were collected from Salsbury Point, Hood Canal in January 2014 and transported to a holding facility at USGS Marrowstone Laboratory before being shipped overnight to the Department of Microbiology at Cornell University. Three living animals were held overnight in a 20-L aquarium with juvenile (2-cm diameter) *P. helianthoides* from northern British Columbia (later found to have high loads of SSaDV) whereas the remainder were maintained in a second 20-L holding tank. After 1 d, the three animals that had cohabitated with juveniles were placed in the holding tank with the remaining five animals. Tube-foot clippings (~10 tube feet from each animal) were collected from each individual after 2 d, and load of SSaDV was determined by qPCR. At this time, the three asteroids that had cohabitated with juvenile asteroids were positive for SSaDV whereas the remaining five were negative. After 7 d, individual animals were placed in eight separate, 3.7-L aquaria in artificial seawater (Instant Ocean) at 8 °C on a 12-h light:dark cycle with continuous aeration and charcoal filtration. After the commencement of the experiment (where animals were housed individually), all seven asteroids had detectable abundances of SSaDV, suggesting that it had been transmitted from the infected asteroids. None of the animals fed on ~0.5 g of mussel tissue (obtained from a local supermarket) placed in each aquarium.

Coelomic fluid samples were obtained from each animal by drawing ~50 μL of fluid using a 3-mL syringe fitted with a 25 G needle from the perivisceral coelom. Coelomic fluid samples were obtained every 48 h until the animals died (after 11–14 d) or were euthanized by freezing. Aquarium water samples (50 mL syringe-filtered serially through 0.2- and 0.02-μm filters) were collected at 0 d, 4 d, and 10 d. Animals were killed by freezing at −80 °C after 14 d, and tissue samples were collected from arms per field samples. DNA was extracted from tissue, water column, and coelomic fluid samples. Over 14 d after isolation, coelomic fluid loads of SSaDV increased by over two orders of magnitude (Fig. S6). We also investigated the abundance of free-living viruses after 7 d and 14 d in aquaria. SSaDV abundance in the >0.02-μm-size fraction increased over time. Finally, we assessed viral load in euthanized animal tissues after 14 d. Tissue viral loads were similar to coelomic fluid loads in all animals. These results demonstrate that SSaDV abundance increases in animals over time during containment and that release/shedding of viruses occurs between 0 d and 14 d incubation. Further, these results show agreement between coelomic fluid viral loads and viral loads in tissues after 14 d.

Viral-Challenge Experiments

Asymptomatic adult *P. helianthoides* (size, 153 ± 35 cm average radius) were collected from Dabob Bay, Hood Canal, WA; Port Hadlock, Puget Sound, WA; San Juan Island, Straight of George, WA; and Langley, Puget Sound, WA, January–April 2014 before disease occurrence at each site. Animals were observed for disease signs until each experiment was started, and tissue samples (tube feet) were collected at the beginning of experiments to determine initial SSaDV load. Viral challenges were performed with homogenates prepared from symptomatic animals. Each asteroid was treated with either control or viral inocula (0.5 mL) by injection into the coelomic cavity.

Pairs of animals of similar size that were collected at the same time and location were haphazardly assigned to either the control or experimental treatment group. After injection, animals were observed every morning and evening. They were fed manila clams every 3 d. Tissue samples (tube feet) were collected for determination of SSaDV load after 8 d incubation and when the animals were killed. Early signs of disease (arm curling) were noted, and animals were killed when signs of tissue degradation were evident (lesions, arm amputation). Two experiments were conducted. In the first experiment, five control and five viral inocula (<0.02-μm size fraction) were performed. To further explore whether virus-sized particles might be the causative agent for disease, a second challenge was performed, where tissue from three animals that developed disease signs during the first experiment was used to make the control and viral inoculum for a second passage. The second experiment comprised 10 control and 10 viral inoculum-treated asteroids.

Survey of Museum Specimens

To investigate the historical presence of SSaDV in asteroids, we examined its occurrence in museum specimens collected from the Northeastern Pacific Coast between 1923 and 2010 (Table S6). Samples comprised tube feet excised from ethanol-preserved specimens in the echinoderm collection of the Natural History Museum of Los Angeles County. Specimens were selected to represent a range of species, dates, and geographic locations. Approximately 10 tube feet were removed from each specimen using sterile forceps and dissection scissors. The tube-foot samples were placed into sterile Bead Basher (Zymo) tubes and transported at room temperature to the laboratory at Cornell University. DNA was extracted from tube-foot samples using the Tissue and Insect Mini Kit (Zymo Research) according to the manufacturer's protocols. Viral presence and load were determined in samples by qPCR targeting the NS1 and VP4 regions of the SSaDV genome.

Survey of Nonasteroid Samples

The presence of SSaDV was examined in environmental reservoirs, including sediments, virioplankton (0.2 μm > virioplankton > 0.02 μm), bacterioplankton and larger particles (>0.2 μm), and sand filters in aquaria. DNA was extracted from subsamples (~200 mg) of sediment and sand-filter samples using the ZR Soil DNA kit (Zymo Research) according to the manufacturer's recommendations. DNA from water-column samples (which had been filtered onto 0.2-μm PES and 0.02-μm Anotop filters) was extracted using the ZR Viral DNA kit with modifications for filters (6).

SSaDV was detected in almost all sediments tested (13 of 15 samples) and was present in all sand-filter samples tested (Fig. 9). The abundance of SSaDV in sediments was generally lower than in sand filters; however, there was no difference between sediments at field sites and those from public aquaria. Interestingly, SSaDV was present in sediments at the bottom of

two aquaria that had previously housed symptomatic asteroids (samples were taken ~2 mo after asteroid death), but SSaDV was not detected in sediments at the bottom of the California Science Center kelp tank, which had never housed symptomatic asteroids. In plankton, SSaDV was found in the 0.02-μm-size fraction at abundances threefold higher than in the 0.2-μm-size fraction (Fig. 8). Mean SSaDV overall (2.5×10^6 copies·mL^{-1}) represents ~1–5% of virioplankton, which is higher than previous reports of metazoan viruses in plankton (6).

In addition to abiotic samples, the presence of SSaDV was assayed in specimens of ophiuroids and echinoids collected from public aquaria and in the field. DNA was extracted from tissue samples (for ophiuroids, a 0.5-cm section of arm; for echinoids, a 200-mg sample of gonad) using the ZR Tissue and Insect DNA kit (Zymo Research).

SSaDV was detected in 7 of 11 *Strongylocentrotus purpuratus*, in the single *Dendraster excentricus* from Bellingham, and in 2 of 6 ophiuroid specimens (Order Ophiurida) and not in the single specimen of *Gorgonocephalus eucnemis* (Order Euryalida) (Fig. S4). Although mortality of echinoids and ophiuroids has been reported in the field, we did not attempt to link disease in nonasteroids to microbiome composition.

1. Gudenkauf BM, Eaglesham JB, Aragundi WM, Hewson I (2014) Discovery of urchin-associated densoviruses (family Parvoviridae) in coastal waters of the Big Island, Hawaii. *J Gen Virol* 95(Pt 3):652–658.
2. Rice P, Longden I, Bleasby A (2000) EMBOSS: The European Molecular Biology Open Software Suite. *Trends Genet* 16(6):276–277.
3. Sun S, et al. (2011) Community cyberinfrastructure for Advanced Microbial Ecology Research and Analysis: The CAMERA resource. *Nucleic Acids Res* 39(Database issue): D546–D551.
4. Rozen S, Skaletsky H (2000) *Bioinformatics Methods and Protocols*, eds Krawertz S, Misener S (Humana, Totowa, NJ), pp 365–386.
5. Naccache SN, et al. (2013) The perils of pathogen discovery: Origin of a novel parvovirus-like hybrid genome traced to nucleic acid extraction spin columns. *J Virol* 87(22): 11966–11977.
6. Hewson I, et al. (2012) Temporal dynamics and decay of putatively allochthonous and autochthonous viral genotypes in contrasting freshwater lakes. *Appl Environ Microbiol* 78(18):6583–6591.

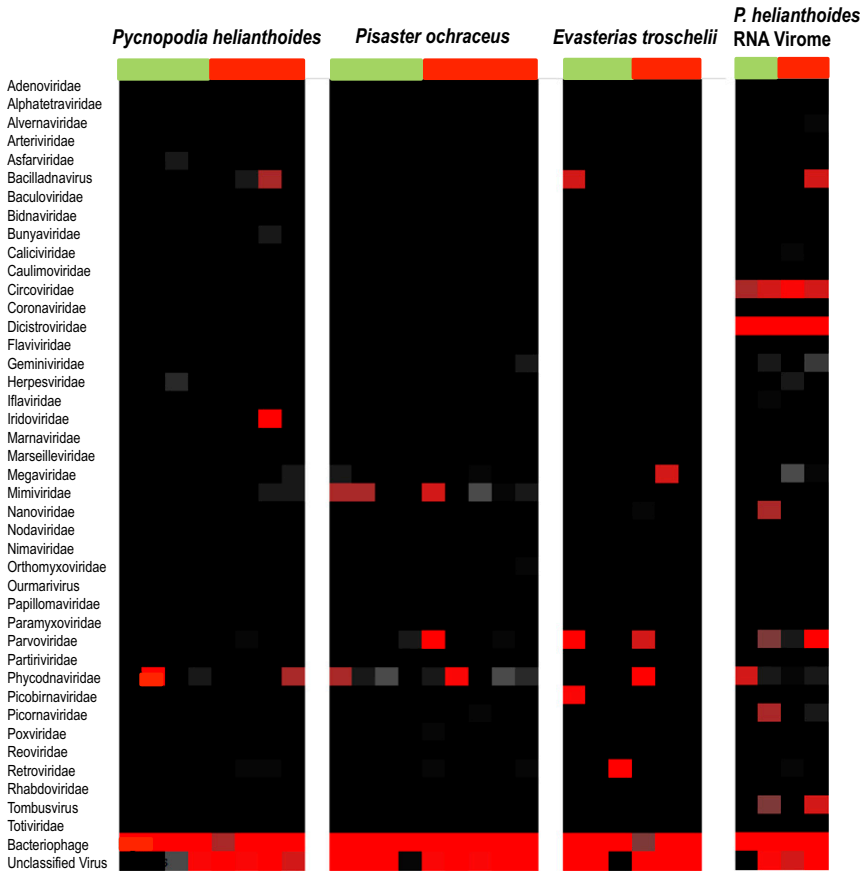

Fig. S1. Heat-map representation of viral families present in asymptomatic (green) and symptomatic (red) asteroid tissues. Annotation is based on the percentage of reads of total libraries and on BLASTx against the nonredundant database at NCBI. Each column represents a replicate metagenomic library.

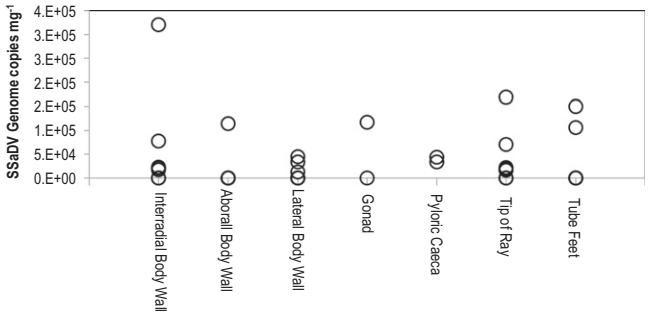

Fig. S2. Within-animal variability in SSaDV load as assessed by qPCR targeting the VP4 gene. Each circle indicates the load of SSaDV detected by qPCR in each tissue type. Analysis was performed on two symptomatic *P. ochraceus* obtained from Cuyler Harbor, San Miguel Island (Channel Islands), CA, on 18 January 2014. SSaDV was detected in 36% and 47% of subsamples from the symptomatic star, but detection varied from 11–77% of subsamples tested in individual tissues. Body-wall tissues, which were used to detect prevalence and load of SSaDV in field populations, yielded positive detection in only 11–38% of samples, suggesting the possibility of false negatives in the survey.

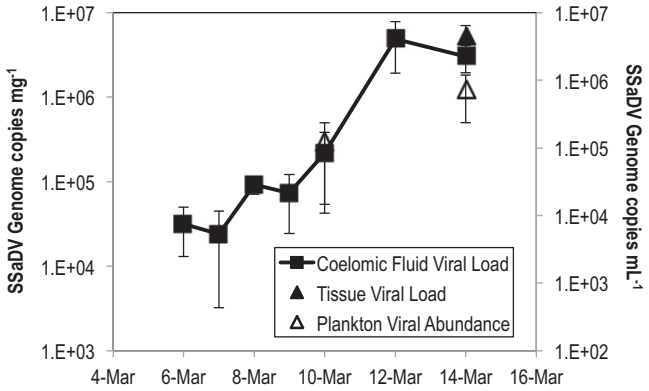

Fig. S3. Viral load in coelomic fluid and tissues and abundance in plankton during longitudinal study of SSaDV dynamics in aquaria. SSaDV load and abundance were determined by qPCR targeting the VP4 gene on the SSaDV genome. For coelomic-fluid load, viral abundance was normalized to total volume of coelomic fluid extracted.

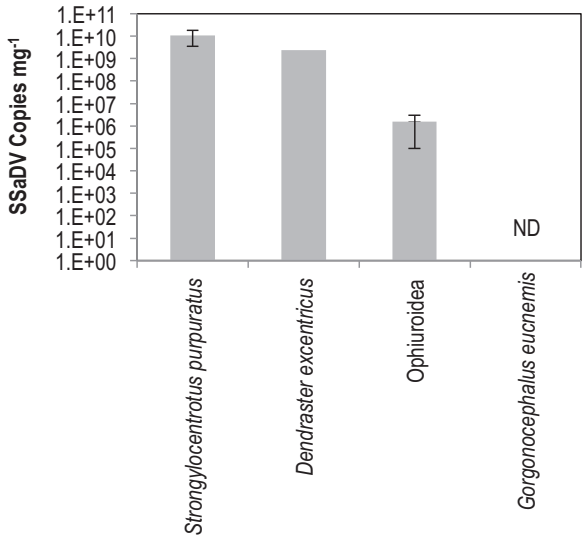

Fig. S4. Viral load in non-Asteroidea echinoderms as detected by qPCR targeting the VP4 gene on the SSaDV genome.

Table S1. Numbers of individual asteroids analyzed

Species	Location	Asymptomatic	Symptomatic
Patiria miniata	California Science Center (A)	0	4
	Monterey Bay Aquarium (A)	0	5
	Long Marine Laboratory/UCSC (A)	0	1
	Vancouver Aquarium (A)	0	1
Astrometis sertulifera	California Science Center (A)	0	4
Astropecten polyacanthus	California Science Center (A)	1	0
Dermasterias imbricata	Friday Harbor Laboratories (F)	1	0
	Santa Barbara, CA (F)	3	0
	Seattle Aquarium (A)	1	0
Evasterias troschelii	Port Hardy, British Columbia (F)	0	3
	Vancouver, British Columbia (F)	2	3
	Friday Harbor Laboratories (F)	3	2
	Olympic National Park (F)	0	2
	Dabob Bay, Hood Canal (F)	0	4
	Port Townsend, Puget Sound (A)	0	1
	Mukilteo, Puget Sound (F)	0	5
	Seattle Aquarium (A)	1	6
	Vancouver Aquarium (A)	0	1
Luidia foliolata	Vancouver Aquarium (A)	0	2
	Seattle Aquarium (A)	0	4
Mediaster aequalis	Vancouver Aquarium (A)	0	2
	Monterey Bay Aquarium (A)	0	1
Orthasterias sp.	Seattle Aquarium (A)	0	1
Pisaster brevispinus	Vancouver, British Columbia (F)	0	1
	Bainbridge Island, Puget Sound (F)	0	2
	Dabob Bay, Hood Canal (F)	0	1
	Carpinteria, CA (F)	0	28
	Seattle Aquarium (A)	0	4
	Vancouver Aquarium (A)	0	27
Pisaster giganteus	Santa Cruz, CA (F)	0	9
	Carpinteria, CA (F)	0	5
	Santa Barbara, CA (F)	0	4
	California Science Center (A)	0	6
	Monterey Bay Aquarium (A)	0	2
	Seattle Aquarium (A)	1	8
Pisaster ochraceus	Vancouver, British Columbia (F)	0	1
	Olympic National Park (F)	15	19
	Friday Harbor Laboratories (F)	0	5
	Dabob Bay, Puget Sound (F)	0	8
	Alki, WA (F)	0	4
	Mukilteo, WA (F)	0	4
	Santa Cruz, CA (F)	10	15
	San Miguel Island, Channel Islands (F)	0	2
	California Science Center (A)	0	5
Pycnopodia helianthoides	Vancouver, British Columbia (F)	5	2
	Friday Harbor Laboratories (F)	0	18
	Olympic National Park (F)	0	2
	Whidbey Island, WA (F)	0	4
	Dabob Bay, Hood Canal (F)	0	1
	Santa Barbara, CA (F)	0	2
	Vancouver Aquarium (A)	1	20
	Seattle Aquarium (A)	5	16
	Monterey Bay Aquarium (A)	0	2
Solaster stimpsoni	Friday Harbor Laboratories (F)	0	5
Stylasterias forreri	Vancouver Aquarium (A)	0	2
Total Individuals		49	286

A, aquarium; F, field site.

Table S2. Characteristics of viral metagenomes sequenced as part of this study: V1–V8

Library name	V1	V2	V3	V4	V5	V6	V7	V8
Species	*Dermasterias imbricata*	*Solaster stimpsoni*	*Solaster stimpsoni*	*Evasterias troschelii*	*Pycnopodia helianthoides*	*Evasterias troschelii*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*
S/A	S	S	S	S	S	A	A	A
Location	Friday Harbor Lab	Friday Harbor Lab	Friday Harbor Lab	Friday Harbor Lab	Friday Harbor Lab	Friday Harbor Lab	Friday Harbor Lab	Croker Island, Indian Arm, Burrard Inlet
Date	13 Oct 2013	10 Oct 2013	11 Oct 2013	7 Oct 2013	11 Oct 2013	8 Oct 2013	8 Oct 2013	29 Oct 2013
RNA/DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
No. of total reads	1,007,652	3,500,172	1,455,342	1,951,510	3,233,230	1,432,820	678,554	1,924,530
No. of reads assembling into contigs	649,193	2,816,306	1,093,359	1,143,245	1,706,209	949,592	395,484	1,316,347
No. of contigs	4,867	3,162	1,846	2,429	1,697	3,416	1,435	9,739
Avg contig length, bp	457	334	335	390	458	368	475	403
No. of annotated reads	80,229	184,712	105,821	307,056	265,216	540,673	152,439	285,639
No. of viral reads	29,025	5,244	33,776	19,204	18,931	973	374	5,293

S, symptomatic; A, asymptomatic.

Table S3. Characteristics of viral metagenomes sequenced as part of this study: V9–V16

Library name	V9	V10	V11	V12	V13	V14	V15	V16
Species	*Pisaster ochraceus*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*	*Pisaster ochraceus*	*Pisaster ochraceus*	*Pisaster ochraceus*
S/A	A	A	A	S	S	A	A	S
Location	Cates Park Reef, Burrard Inlet	Seattle Aquarium	Seattle Aquarium	Seattle Aquarium	Seattle Aquarium	Olympic National Park	Olympic National Park	Olympic National Park
Date	29 Oct 2013	26 Oct 2013	26 Oct 2013	26 Oct 2013	26 Oct 2013	19 Sep 2013	19 Sep 2013	19 Sep 2013
RNA/DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
No. of total reads	1,186,824	2,783,328	1,468,056	3,284,842	1,626,722	1,263,860	1,231,402	799,760
No. of reads assembling into contigs	753,998	1,379,247	830,906	2,234,614	1,045,925	829,203	746,472	464,081
No. of contigs	3,913	2,175	2,404	8,923	3,977	9,578	5,932	2,427
Avg contig length, bp	405	442	444	391	423	412	424	384
No. of annotated reads	154,622	517,622	160,642	486,945	488,751	89,716	98,735	46,038
No. of viral reads	10,062	10,686	19,715	6,544	38,382	3,752	12,078	71

S, symptomatic; A, asymptomatic.

Table S4. Characteristics of viral metagenomes sequenced as part of this study: V17–V24

Library name	V17	V18	V19	V20	V21	V22	V23	V24
Species	*Pisaster ochraceus*	*Pisaster ochraceus*	*Pisaster ochraceus*	*Pisaster ochraceus*	*Pisaster ochraceus*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*	*Evasterias troschelii*
S/A	S	S	S	A	A	S	A	S
Location	Olympic National Park	Santa Cruz	Santa Cruz	Santa Cruz	Santa Cruz	Gower Point	Gower Point	Cape Roger Curtis
Date	19 Sep 2013	8 Oct 2013	8 Oct 2013	8 Oct 2013	8 Oct 2013	17 Oct 2013	17 Oct 2013	17 Oct 2013
RNA/DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA
No. of total reads	1,255,558	1,235,128	1,503,372	1,928,276	1,136,824	1,407,560	1,806,572	2,615,580
No. of reads assembling into contigs	717,005	628,937	968,409	1,266,256	700,500	889,083	1,003,950	1,635,326
No. of contigs	4,238	4,248	5,195	8,241	2,615	3,264	1,365	1,234
Avg contig length, bp	390	432	433	421	383	399	458	593
No. of annotated reads	55,121	59,786	282,022	27,979	36,010	200,118	350,457	588,337
No. of viral reads	1,795	6,583	58,070	3,629	3,728	4,321	434	127,407

S, symptomatic; A, asymptomatic.

Table S5. Characteristics of viral metagenomes sequenced as part of this study: V25–V33

Library name	V25	V26	V27	V28	V30	V31	V32	V33
Species	*Evasterias troscheliii*	*Evasterias troschelii*	*Evasterias troscheliii*	*Asterina miniata*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*	*Pycnopodia helianthoides*
S/A	S	A	A	S	A	A	S	S
Location	Cape Roger Curtis	Cape Roger Curtis	Cape Roger Curtis	Vancouver Aquarium	Seattle Aquarium	Seattle Aquarium	Seattle Aquarium	Seattle Aquarium
Date	17 Oct 2013	17 Oct 2013	17 Oct 2013	17 Oct 2013	26 Oct 2013	26 Oct 2013	26 Oct 2013	26 Oct 2013
RNA/DNA	DNA	DNA	DNA	DNA	RNA	RNA	RNA	RNA
No. of total reads	1,069,772	645,446	461,852	1,047,486	2,098,860	1,590,274	634,154	1,449,257
No. of reads assembling into contigs	547,055	297,307	191,102	477,486	1,014,703	724,150	283,171	850,072
No. of contigs	2,231	564	337	1,082	25,017	11,066	9,809	10,431
Avg contig length, bp	665	631	615	685	718	687	702	834
No. of annotated reads	253,085	41,691	115,094	83,349	317,421	229,370	125,939	427,794
No. of viral reads	139,216	28,465	46,917	25,284	5,359	99,312	20,436	102,749

S, symptomatic; A, asymptomatic.

Table S6. **Natural History Museum of Los Angeles County specimens analyzed for detection of SSaDV**

Species	Date	Location	Depth, m	Museum catalog no.
Evasterias troschelii	28 Jun 1942	S side of Sunset Bay, Coos Co, OR	Inter.	LACM 1942-22.18
Evasterias troschelii	28 Jun 1942	S side of Sunset Bay, Coos Co, OR	Inter.	LACM 1942-22.18
Evasterias troschelii	29 Jul 1942	Reef and bight at Cape Arago Lighthouse, Coos Co, OR	Inter.	LACM 1942-48.18
Evasterias troschelii	29 Jul 1942	Reef and bight at Cape Arago Lighthouse, Coos Co, OR	Inter.	LACM 1942-48.18
Evasterias troschelii	29 Jul 1942	Reef and bight at Cape Arago Lighthouse, Coos Co, OR	Inter.	LACM 1942-48.18
Evasterias troschelii	29 Jul 1942	Reef and bight at Cape Arago Lighthouse, Coos Co, OR	Inter.	LACM 1942-48.18
Evasterias troschelii	29 Jul 1942	Reef and bight at Cape Arago Lighthouse, Coos Co, OR	Inter.	LACM 1942-48.18
Evasterias troschelii	26 Jul 1971	Arena Cove, Mendocino Co, CA	12	LACM-1971-103.4
Pisaster brevispinus	7–10 Aug 1942	Depoe Bay, OR	110–135	LACM 1942-55.6
Pisaster brevispinus	7–10 Aug 1942	Depoe Bay, OR	110–135	LACM 1942-55.6
Pisaster ochraceus	30 Oct 1947	Big Rock Beach, CA	Inter.	LACM 1947-25.9
Pisaster brevispinus	30 Jul 1971	Humboldt Co, CA	8–15	LACM 1971-109.8
Pisaster brevispinus	24–26 Oct 1980	Morro Bay, CA	Inter.	LACM 80-174.1
Pisaster brevispinus	6 Sep 1987	Wickaninish Bay, Vancouver Island, British Columbia	45	LACM 87-114.9
Pisaster brevispinus	28 Feb 2009	Palos Verdes, CA	15–18	LACM 2009-12.1
Pisaster brevispinus	2 Jul 1942	Douglas Co., OR	48–106	LACM 1942-30.5
Pisaster giganteus capitatus	31 Jul 1923	Point Fermin, CA	n/a	LACM 1923-6.2
Pisaster giganteus capitatus	13 Feb 1942	Palos Verdes, CA	Inter.	LACM 1942-4.17
Pisaster giganteus capitatus	13 Feb 1942	Palos Verdes, CA	Inter.	LACM 1942-4.17
Pisaster giganteus	17 Feb 1996	Corona del Mar, CA	Inter.	LACM 96-1.7
Pisaster giganteus	19 Feb 1996	False Point, La Jolla, CA	Inter.	LACM 96-2.3
Pisaster giganteus	18 Dec 1998	Cayucos, Morro Bay, CA	Inter.	LACM 98-89.4
Pisaster giganteus	22 Sep 2001	San Clemente Island, CA	17	LACM 2001-58.1
Pisaster giganteus	22 Sep 2001	San Clemente Island, CA	15	LACM 2001-59.1
Pisaster giganteus	22 Sep 2001	San Clemente Island, CA	15	LACM 2001-59.1
Pisaster giganteus	2 Sep 2007	San Clemente Island, CA	14	LACM 2007-126.1
Pisaster giganteus	29 Oct 2010	Santa Rosa Island, CA	17–21	LACM 2010-81.2
Pycnopodia helianthoides	30 Jun 1942	Coos Co, OR	Inter.	LACM 1942-25.16
Pycnopodia helianthoides	30 Jun 1942	Coos Co, OR	Inter.	LACM 1942-25.16
Pycnopodia helianthoides	13 Dec 1948	Bodega Head, Sonoma Co, CA	Inter.	LACM 1948-45.22
Pycnopodia helianthoides	7 Aug 1971	Ft. Bragg, Mendocino Co, CA	55	LACM 1971-133.4
Pycnopodia helianthoides	23 Oct 1976	Bear Harbor Ranch, Mendocino Co, CA	Inter.	LACM 1976-9.22
Pycnopodia helianthoides	24–26 Oct 1980	Morro Bay, CA	Inter.	LACM 1980-174.2
Pycnopodia helianthoides	6 Sep 1987	Wickaninish Bay, Vancouver Island, British Columbia	45	LACM 87-114.10
Pycnopodia helianthoides	6 Sep 1987	Wickaninish Bay, Vancouver Island, British Columbia	45	LACM 87-114.10
Pycnopodia helianthoides	15 Oct 1991	San Miguel Island, CA	16	LACM 91-135.2
Pycnopodia helianthoides	15 Oct 1991	San Miguel Island, CA	16	LACM 91-135.2
Pycnopodia helianthoides	22 Jun 1997	Diablo Canyon, San Luis Obispo Co, CA	Inter.	LACM 97-42.7
Pycnopodia helianthoides	2 Jul 2001	Astoria Canyon, Clatsop Co, OR	154	LACM 2001-61.1
Pycnopodia helianthoides	8 Jul 2001	Heceta Bank, Lane Co, OR	110	LACM 2001-60.1
Pycnopodia helianthoides	17 Jun 2006	Santa Barbara Island, CA	17	LACM 2006-66.1
Pycnopodia helianthoides	2 May 2008	San Miguel Island, CA	21	LACM 2008-52.1
Pycnopodia helianthoides	24 Oct 2010	Santa Rosa Island, CA	17–21	LACM 2010-81.1
Pisaster ochraceus	29 Jan 1939	White Cove, Santa Catalina Island, CA	73-146	LACM 1939-5.10
Pisaster ochraceus	1 Jun 1941	Tomales Point, CA	Inter.	LACM 1941-373.1
Pisaster ochraceus	1 Jun 1941	Tomales Point, CA	Inter.	LACM 1941-373.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Jul 1942	Coast Guard Station, Coos Bay, OR	Inter.	LACM 1942-44.1
Pisaster ochraceus	25 Oct 1976	Half Moon Bay, CA	Inter.	LACM 1976-10.26
Pisaster ochraceus	22 Nov 1988	SE of Point Conception, CA	Inter.	LACM 88-131.45
Pisaster ochraceus	1 Jul 1991	Curry Co, OR	Inter.	LACM 91.37.1
Pisaster ochraceus	1 Jul 1991	Curry Co, OR	Inter.	LACM 91.37.1
Pisaster ochraceus	26 Jun 1994	Sonoma Co, CA	Inter.	LACM 94-73.1
Pisaster ochraceus	22 Jun 1997	Diablo Canyon, San Luis Obispo Co, CA	Inter.	LACM 97-42.8
Pisaster ochraceus	8 Sep 2006	San Miguel Island, CA	14–21	LACM 2006-75.1
Pisaster ochraceus	2 Jul 1991	Cape Ferrelo, Curry Co, OR	Inter.	LACM-91-38.1
Pisaster ochraceus	2 Jul 1991	Cape Ferrelo, Curry Co, OR	Inter.	LACM-91-38.1
Pisaster ochraceus	26 Jun 1994	Sonoma Co, CA	Inter.	LACM 94-73.1
Pisaster ochraceus	22 Jun 1997	Diablo Canyon, San Luis Obispo Co, CA	Inter.	LACM 97-41.4

Co, County; Inter., intertidal; n/a, not available; NE, northeast; S, south; SE, southeast.