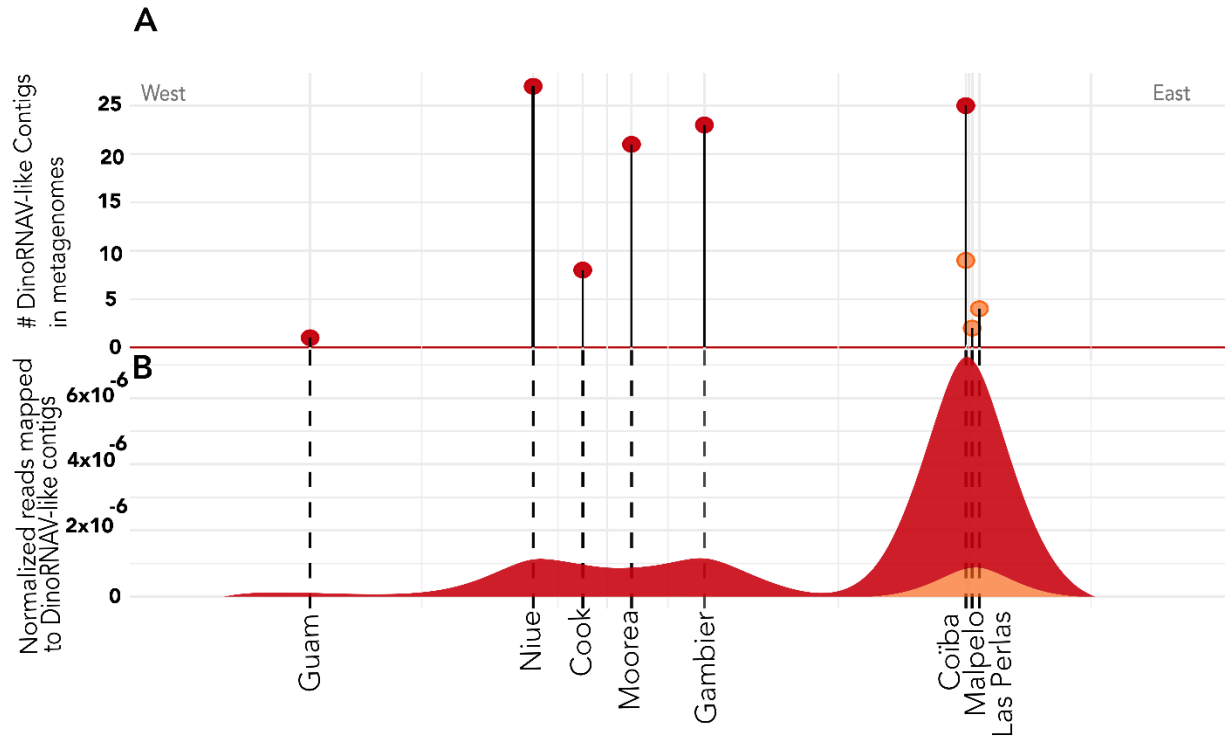
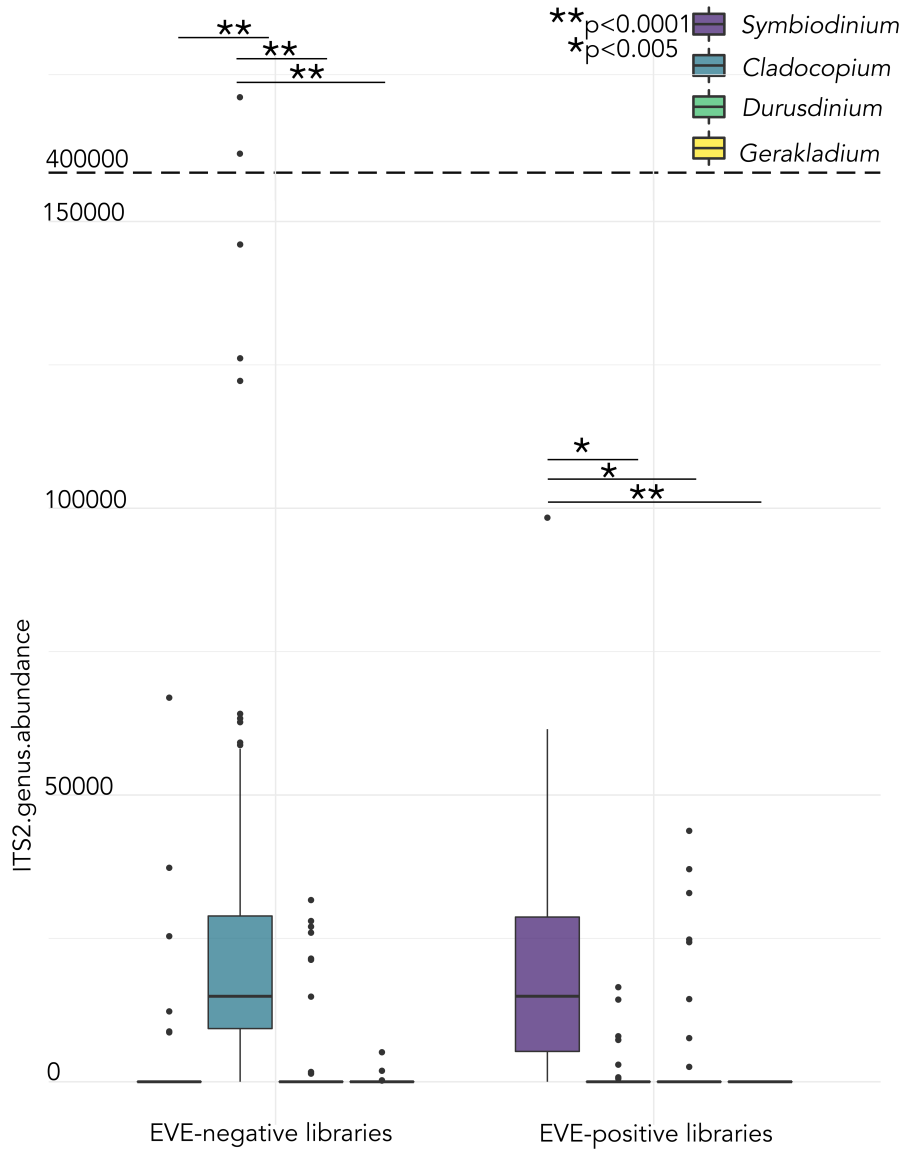


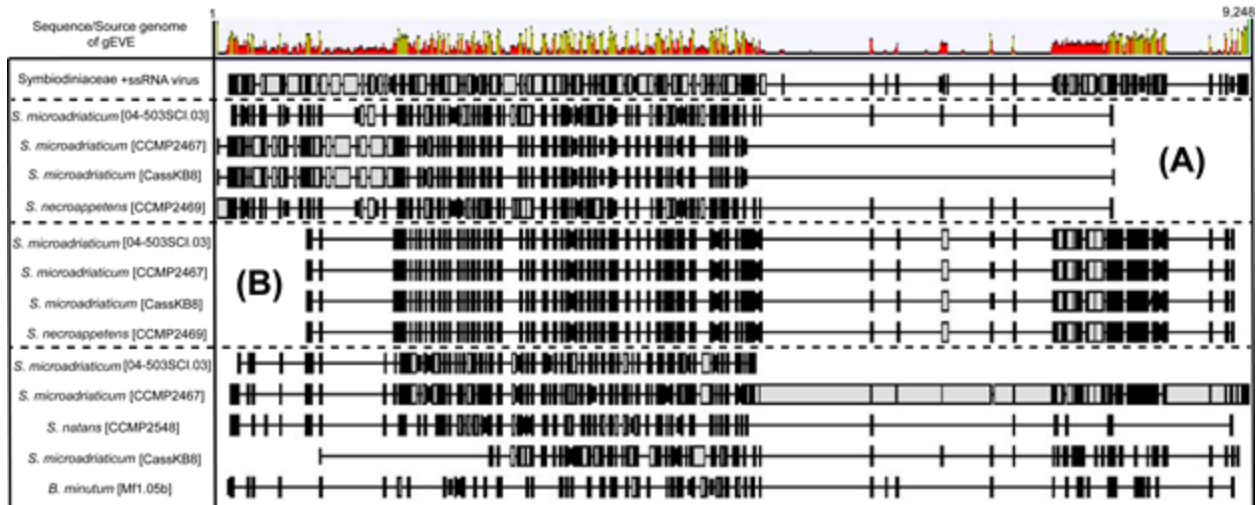
Supplementary Figures - *Endogenous viral elements reveal associations between a non-retroviral RNA virus and symbiotic dinoflagellate genomes*



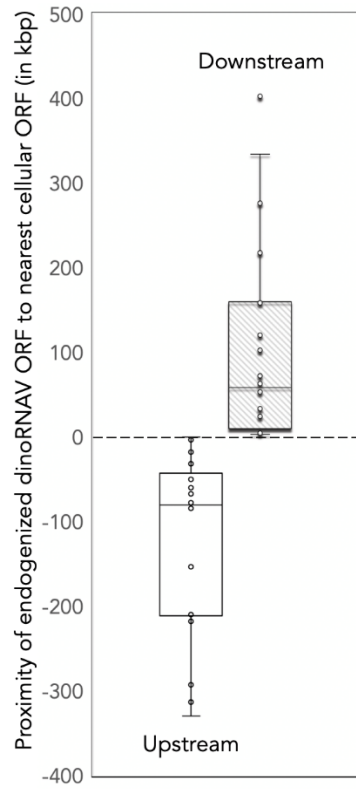
Supplementary Figure 1. Islands correlating with dinoRNAV EVE-like sequence detection among Tara Pacific metagenomes indicating reads mapped to dinoRNAV-like scaffolds within individual Tara Pacific metagenomic libraries, normalized by quality-controlled reads. See Supplementary Data 2 for supporting data (total n=120 contigs)



Supplementary Figure 2. DinoRNAV EVE detection - regardless of host species - largely correlated with *Symbiodinium* dominated communities. $F_{2,1044}=25.8$, $p<0.0001$, Type III ANOVA with Satterthwaite's. *Posthoc*: pairwise Tukey with Kenward-Roger approximation for linear mixed models (replicate libraries were randomized and nested within islands and examined if EVE-positive libraries (factor 1) are affiliated with genus (factor 2) with median and 95% CI error bars (n=263 unique metagenomes) . See Supplementary Data 5 for supporting data.



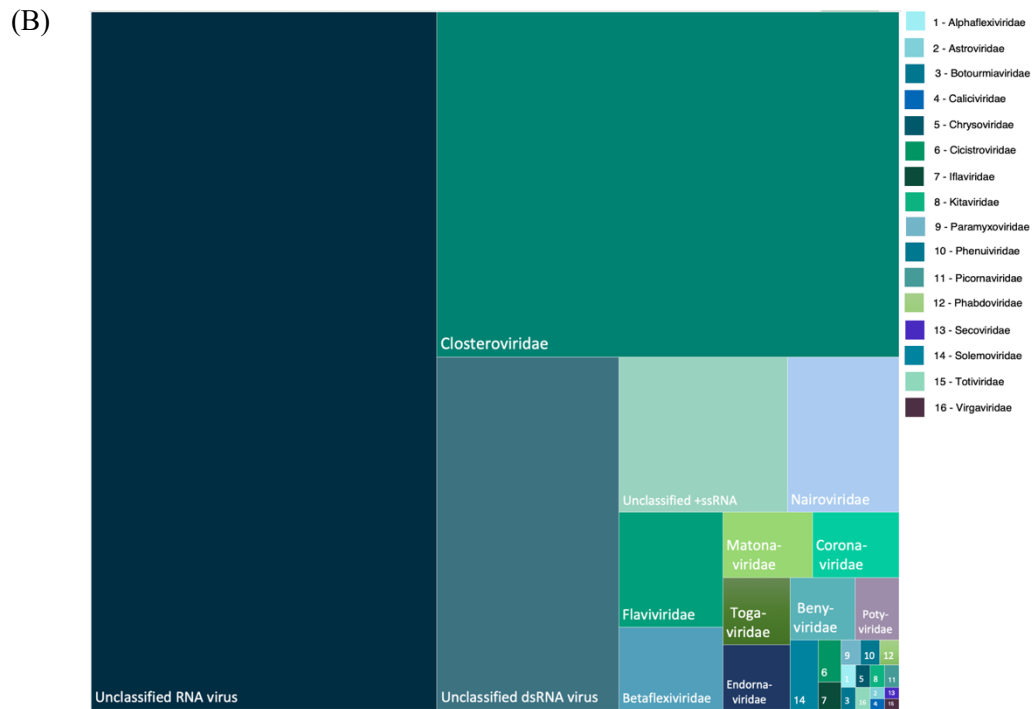
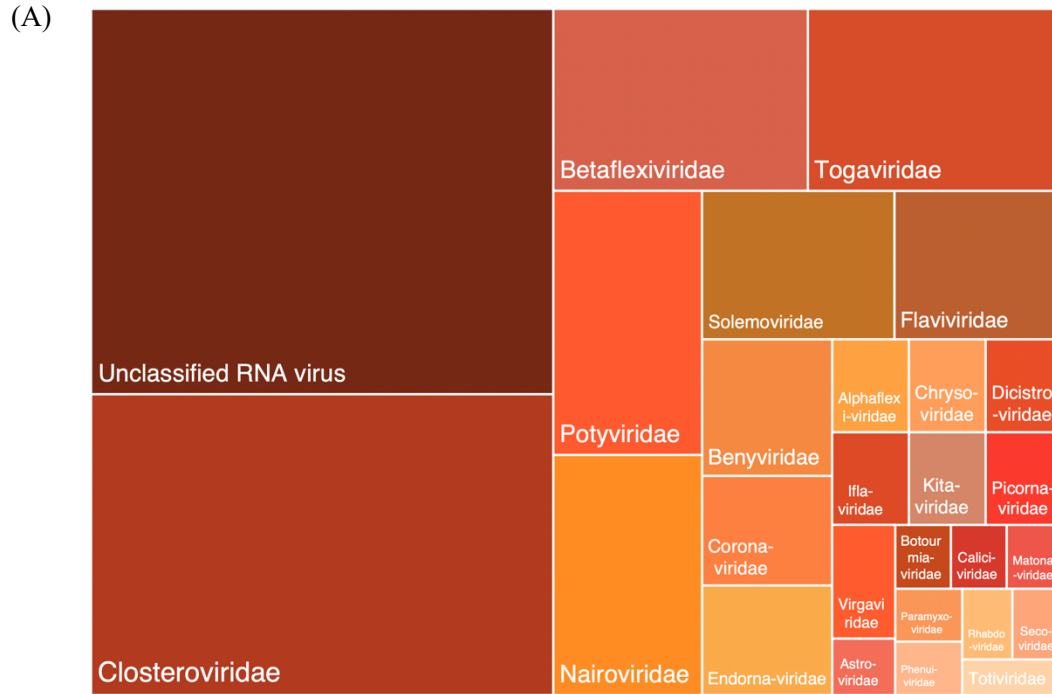
Supplementary Figure 3. Alignment of putative endogenous genomic dinorNAV EVEs to the Symbiodiniaceae-infecting +ssRNA virus genome assembled by Levin et al (2017)⁵⁷ from a *Symbiodinium* spp. transcriptome. Repeated “whole” genome EVEs (A & B) showing similarity across *Symbiodinium* genome are marked with dashed boxes. Putative whole genome EVEs were extracted from their respective scaffolds and aligned to the dinorNAV reference genomes using MAFFT (v7.464, Katoh and Standley 2013) and visualized in Geneious Prime (v.2021.0.1, restricted).



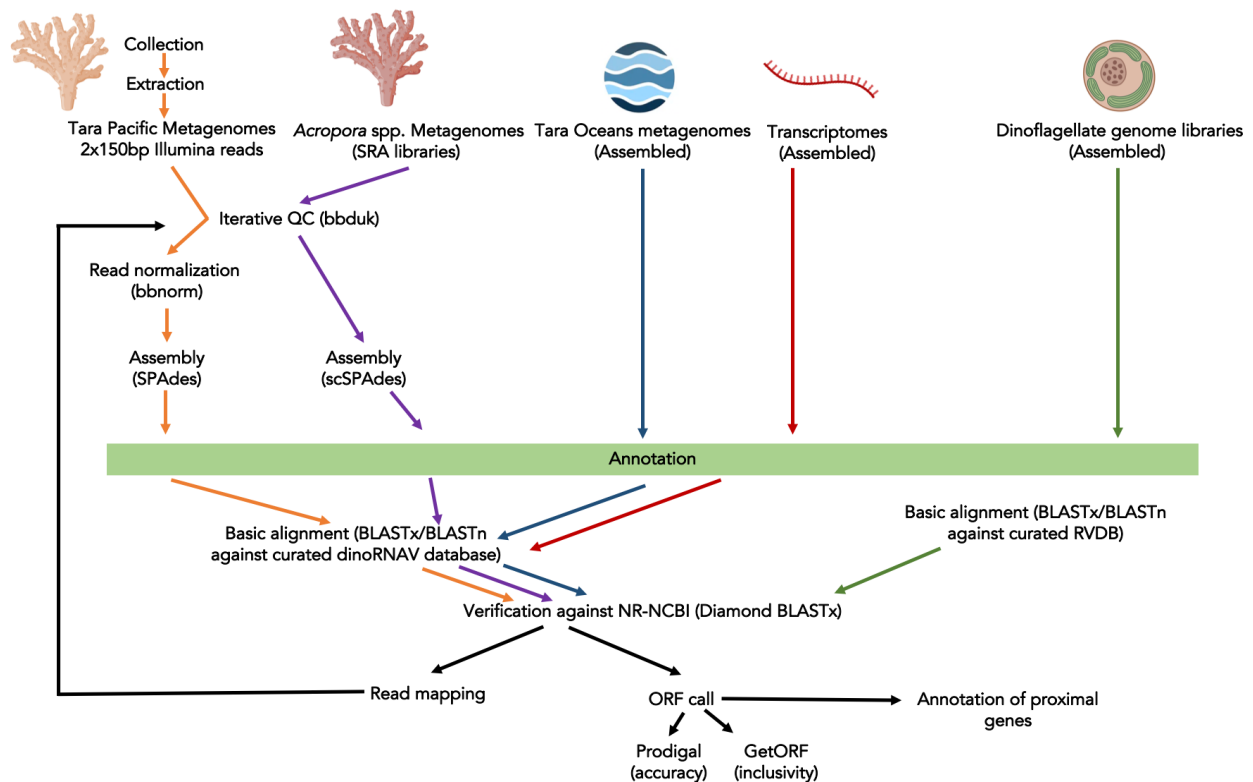
Supplementary Figure 4. Box and whisker plot indicative of median and quartiles of nearest 3' or 5' proximal genes (in bp; whiskers are 95% CI; n=22 integrations on n=18 chromosomes) to dinoRNAV EVEs on the chromosome level *Symbiodinium microadriaticum* assembly (Smic1.1N, GSE152150).



Supplementary Figure 5. Example secondary structural elements of *Symbiodinium* sp. (A1) transcript Locus_83084 (accn: GAKY01194223.1) including hairpin folds. *Symbiodinium* genome contains all core RNAi protein machinery, including Argonaute and Dicer and GAKY01194223.1 folds into several hairpins ($\Delta G = -142.5\text{kcal/mol}$; mfold v. 3.5)



Supplemental Figure 6. Composition of RNA virus families detected in Tara Pacific metagenomes where DinoRNAV EVE was also detected per identification and characterization of *RdRp* gene via Diamond BLASTx. (A) Map describes the composition of *unique* contigs affiliated with each RNA viral family (per *RdRp* detection) across 42 dinoRNAV EVE-positive metagenomes (representing n=26 families and 50 RNA viral contigs containing *RdRps* with unknown viral families; total of n=187 *unique* contigs); (B) Map describes the *total* number of contigs identified within each RNA viral family across 42 dinoRNAV EVE-positive metagenomes (per *RdRp* detection; n=3,5069 *total* contigs identified; average 834.97 per library).



Supplementary Fig 7. Acquisition and bioinformatic analysis pipeline utilized for dinoRNAV EVE identification and verification. Five sources of data (newly assembled metagenomes from Tara Pacific, newly assembled metagenomes from publicly available *Acropora* holobionts, pre-assembled metagenomes from Tara Ocean databases, pre-assembled transcriptomes from Baumgarten et al, 2020¹⁰¹, and publicly available genomes from dinoflagellates) were quality controlled/assembled per a variety of software in the bbttools suite and SPAdes prior to alignment-based annotation against several databases (NR-NCBI, UniProt, RVDB, or manually curated databases of dinoRNAV genomes. Details not available in “Methods” can be found below:

Collection and sequencing of Tara Pacific coral metagenomes (see full methods in doi:10.5281/zenodo.4068293): Briefly, coral nubbins were collected from replicate colonies of a fire coral (genus: *Millepora*, n=60), and two stony corals (genera: *Porites*, n=108; and *Pocillopora*, n=101), as well as from immediately surrounding water. DNA was extracted via Quick-DNA/RNA Kit with supplemental enzymatic lysis (Zymo Research, Irvine, CA, USA). mechanically fragmented (300bp; Covaris E210, Covaris, Inc., USA), end-repaired and polyadenylated (NEBNext DNA Modules; New England Biolabs, MA, USA) before being ligated to adapters (NextFlex DNA barcodes; Bio Scientific Co) for clean-up and amplification. DNA was subjected to 2x150bp paired-end Illumina sequencing (Genoscope; Évry, France). Metagenomic libraries were trimmed for length, quality, and adapters (bbduk v.38.06), normalized (bbnorm v. 38.06; target depth 40x coverage) and assembled *de novo* (SPAdes v.3.12).

Publicly available metagenomes were quality controlled as detailed above, non-normalized, and assembled via SPAdes utilizing the single cell modifier (v.3.12).

Collection and sequencing of Tara Pacific coral amplicon libraries (see full methods in doi:10.5281/zenodo.4061797 and Belser et al, *in prep*): DNA was extracted using the Quick-DNA/RNA kit with supplemental enzymatic lysis (Zymo Research, Irvine, CA, USA), PCR amplified in triplicate using SYM-VAR-5.8S2 / SYM-VAR-REV (Hume et al. 2019), and cleaned (AMPure XP; Beckman Coulter, Brea, CA) prior to pooling and library construction (NEBNext DNA Modules; New England

Biolabs, MA, USA). Amplicons were sequenced on the Illumina platform. “Defining intragenomic variants” were utilized to differentiate taxonomic profiles within the Symportal analytical framework post-minimum entropy decomposition (Hume et al, 2019)¹¹¹.

Transcriptomes: To assess (1) if dinoRNAV EVEs were present in poly(A)-selected dinoflagellate transcriptomes, and (2) if these transcripts resembled endogenized dinoRNAVs in proximal gene composition, presence of a characteristic pre-mRNA spliced leader (SL) sequence (SL; as in Levin et al, 2017), or were differentially expressed under variable environmental conditions. Publicly accessible transcriptomes from the genus *Symbiodinium* (n=11 smRNA, n=11 mRNA; Supplemental Table ST.1B) were queried for dinoRNAV-like sequences. Reads were trimmed and quality controlled per the same description as the original study (cutadapt v. 3.1; Baumgarten et al, 2020). Pre-assembled RNA sequences from nine publicly available transcriptomes (Supplemental Data 1) were congruently queried. Transcripts sharing sequence similarity with dinoRNAVs, or containing whole dinoRNAV-like ORFs, and simultaneously annotating as dinoflagellate transcripts (i.e. with cellular ORFs or sequence similarity) were manually annotated. Transcripts were annotated for dinoRNAVs in congruent manner as metagenomes (described in Methods), reads were mapped via bmap (v.38.84), and RNA secondary structure was predicted via mfold (v.3.5).

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