Limited Cross-Species Virus Transmission in a Spatially Restricted Coral Reef Fish Community

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Abstract

The Great Barrier Reef (GBR) – the largest coral reef ecosystem in the world – supports over 1200 fish species with some of the highest population densities and diversities seen in vertebrates, offering a high potential for virus transmission among species. As such, the GBR represents an exceptional natural ecosystem to determine the impact of host community diversity on virus evolution and emergence. In recent decades the GBR has also experienced significant threats of extinction, making it one of the most vulnerable ecosystems on the planet. However, our understanding of virus diversity and connectivity in tropical reef fishes remains poor. Here, we employed metatranscriptomic sequencing to reveal the viromes of 61 reef fish species. This identified a total of 132 viruses, 38 of which were vertebrate-associated and therefore likely infecting the fish, including a novel isolate of *Santee-cooper ranavirus* (*Iridoviridae*). Notably, we found little evidence for virus transmission between fish species living within a very restricted geographical space – a 100 m^2 coral reef ecosystem – suggesting that there might be important host genetic barriers to successful cross-species transmission despite regular exposure. We also identified differences in virome composition between reef fish families, such that cryptobenthic reef fishes – characterized by small body sizes and short life-spans – exhibited greater virome richness compared to large reef fishes. This study suggests that there are important barriers to cross-species transmission, and that successful emergence in a reef fish community likely requires active host adaptation, even among closely related host species.

Introduction

Despite their long evolutionary history, extensive diversity and complex ecological interactions, fish are severely under-sampled in studies of viral ecology and evolution. Economically, fish represent approximately 401 billion USD to the global economy and supply a yearly average of 20.5 kilograms per capita for consumption [1]. Yet fish face continual and potentially ruinous threats from emerging viral infections [2]. Climate-associated changes in species interactions are likely to have devastating ecological and economic consequences, particularly in the context of infectious disease emergence [3]. It was recently estimated that climate change may be responsible for the extinction of almost half of the economically important fish species in the tropical Pacific region by 2100 [4].

Tropical coral reefs are particularly vulnerable to biodiversity loss. In recent decades, global warming has caused numerous coral bleaching events worldwide, with detrimental cascading effects on reef ecosystem functioning and biodiversity [5, 6, 7]. While tropical coral reefs make up only a small fraction of the marine environment, they support enormous biodiversity, accounting for approximately one-third of all currently described marine fish species [8]. Many are ‘cryptobenthic reef fishes’, characterized by small body sizes (i.e. adult sizes of approximately five centimetres), short life-spans, cryptic behaviour and benthic positioning on coral reefs [9, 10]. These diverse reef fish assemblages are of significant economic and cultural value to humans through aquaculture, fisheries, tourism and the aquarium trade [10, 11, 12].

Despite their economic and socio-ecological importance, little is known about the natural diversity of viruses that infect reef fishes, and how ecological and phylogenetic variability within a reef fish community impacts cross-species virus transmission and disease emergence. Host community diversity likely plays a central role in virus emergence, as contact between donor and recipient hosts is a prerequisite for virus transmission [13]. Yet, revealing the exact nature of that role has proven challenging [14]. On one hand, a high diversity of hosts could provide more transmission opportunities for viruses, thereby elevating the risk of disease emergence. Conversely, increased host community diversity may reduce the probability of disease emergence through the ‘dilution effect’, in which species richness provides more possible hosts for pathogens, in turn reducing disease occurrence in some species [14, 15]. As tropical coral reefs are considered biodiversity hotspots, they serve as an ideal natural ecosystem for exploring the impact of host diversity on the extent, pattern and evolution of virus diversity. Indeed, reef fishes display some of the highest densities and highest diversities of potential vertebrate hosts on the planet. In our study location, a standard sampling area of 3.5 m^2 consistently supports between 50 to 150 fishes belonging to 15 to 25 species [74]. Furthermore, this density and richness is sustained year-round [75], offering a continual potential for cross-species transmission and high individual densities all in a readily transmitting aquatic medium.

Much of our knowledge on the tropical reef virosphere is skewed towards those viruses associated with coral species and their symbionts [16]. While epizootic infections have previously been reported in tropical reef fishes across the Western Atlantic and Gulf of Mexico [17], there is minimal data on viral diversity in fish from the Great Barrier Reef (GBR), Australia, although this is the largest and among the most threatened reef ecosystems in the world [5, 6, 18]. A recent metatranscriptomic analysis of the pygmy goby (*Eviota zebrina*) identified three viruses (families *Arenaviridae, Hantaviridae, Paramyxoviridae*) despite *Eviota* exhibiting maximum lifespans of between 60 and 100 days (including a 24 to 26 day pelagic larval phase) [19, 20].

The rate of infectious disease emergence is expected to increase in marine environments, particularly in the context of climate change [3]. As ocean temperatures continue to rise, many Eastern Australian tropical fishes have begun to shift their distribution poleward to temperate reefs situated at higher latitudes, through tropicalization [21, 22]. While the broad-scale ecological impacts of tropicalization are becoming increasingly apparent in native temperate fishes [22], it is unclear how this will impact virus ecology and infectious disease emergence.

Tropical ornamental fishes may also act as viral vectors of disease in economically important farmed species. For example, in Australia, dwarf gourami (*Trichipodus lalius*) can transmit infectious kidney and spleen necrosis virus (genus *Megalocytivirus, Iridoviridae*) to domestic fishes, often with detrimental impacts including disease outbreaks in iconic species such as Murray cod (*Maccullochella peelii*) [23, 24]. Moreover, tropical wrasses (Labridae) have been considered effective biological control agents in aquaculture for their natural ability to consume pests [25]. However, temperate cleaner wrasses have been reported to be important drivers of outbreaks of viral haemorrhagic septicaemia virus (*Rhabdoviridae*) in farmed salmonids [26]. As such, revealing viral diversity in ornamental tropical reef fishes is imperative to understanding the risk of disease emergence in both wild and domestic fish populations.

Revealing the nature of viral transmission within and among species in natural environments is central to our understanding of disease emergence. With exceptionally high species diversity and highly variable individual abundances and ecologies, coral reefs offer an exciting opportunity to explore cross-species virus transmission in a complex natural high-diversity ecosystem. Our goal, therefore, was to employ total RNA-sequencing (i.e. metatranscriptomics) to reveal the diversity, abundance and composition of viruses infecting reef fishes from the GBR, utilising a fish community from a 100 m^2 coral reef ecosystem. In particular, we aimed to: (i) reveal viral diversity and evolution in tropical reef fishes, (ii) identify how often viruses are exchanged in a spatially restricted reef fish community, (iii) determine whether there are differences in virome composition between reef fish families, as well as between cryptobenthic reef fishes and large reef fishes, that differ enormously in size, metabolic rate, lifespan and fecundity [10], and (iv) identify novel viruses that may pose an emerging threat to Australian fisheries, aquaculture and the aquarium trade.

Materials and Methods

Animal ethics

Fish were collected under a Great Barrier Reef Marine Park Authority permit (G16/37684.1) and James Cook University Animal Ethics permit A2752.

Tropical reef fish sample collection

Seemingly healthy fishes (n = 193) were collected in early April 2021 at Orpheus Island, GBR (18°36'44.3"S 146°28'59.4"E). These included 61 species across 16 reef fish families: Gobiidae (gobies) (n = 30 species), Labridae (wrasses) (n = 6), Pomacentridae (damselfishes) (n = 5), Blenniidae (blennies) (n = 5), Acanthuridae (surgeonfishes) (n = 3), Apogonidae (cardinalfishes) (n = 2), Monacanthidae (filefishes) (n = 2), Tetraodontidae (pufferfishes) (n = 1), Pseudochromidae (dottybacks) (n = 1), Chaetodontidae (butterflyfishes) (n = 1), Atherinidae (silversides, hardyheads) (n = 1), Serranidae (groupers) (n = 1), Tripterygiidae (triplefin blennies) (n = 1), Muraenidae (moray eels) (n = 1), Bythitidae (brotulas) (n = 1) and Ophichthidae (snake eels) (n = 1). (Supplementary Table 1). Importantly, of these species, 42 were collected from the reef fish community within a 100 m^2 sampling area (along the northern margin of Pioneer Bay) (Supplementary Table 1). All fish caught were euthanized using clove oil, transported to the lab on ice, then placed either dissected (liver and gills) or whole in RNAlater. Specimens were then stored at -80°C until RNA extraction.

RNA extraction, library preparation and metagenomic next-generation sequencing

Tissue specimens were combined based on species (e.g. liver and gills) and total RNA was extracted using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany) as previously described in [18, 26]. RNA was quantified using a UV-Vis cuvette spectrophotometer (DeNovix, Delaware, United States) and a parallel capillary electrophoresis instrument (Fragment Analyzer) (Agilent, California, United States). RNA from each individual fish was then pooled within each species (Supplementary Table 1), resulting in a total of 61 RNA sequencing libraries. These libraries were constructed using the Truseq Total RNA Library

Preparation Protocol (Illumina). Host ribosomal RNA (rRNA) was depleted with the Ribo-Zero Plus Kit (Illumina) and paired-end sequencing (150 bp) was carried out on the NovaSeq 500 platform (IIllumina). To reduce the impact of index hopping and false virus-host assignments, each library was sequenced on two different lanes. Library construction and metatranscriptomic sequencing were performed by the Australian Genome Research Facility (AGRF).

Virus discovery pipeline and genome annotation

Illumina sequencing reads were first quality trimmed using Trimmomatic v.0.38 [28] then *de novo* assembled into contigs using MEGAHIT v.1.2.9 [29]. The resultant contigs for each library were used as a query against the NCBI nucleotide (nt) and non-redundant protein (nr) databases using BLASTn and Diamond (BLASTX) with an e-value search threshold of 1 x 10^{-5} [30]. Contigs with positive matches to viral sequences were inspected and predicted into open reading frames (ORFs) using Geneious Prime (v.2022.0) [31] (www.geneious.com). We first assigned a virus to a fish library if it was identified on both sequencing lanes. Next, to distinguish between those viruses infecting fish or those associated with diet or gill contamination (e.g. invertebrates, fungi, algae), we used our predicted viral sequences as a single query against the NCBI nt and nr databases using BLAST implemented in Geneious Prime. Using these results, we then assigned a virus as ‘vertebrate-associated’ based on sequence similarity to established vertebrate viruses on the NCBI databases, as well as e-value. Final confirmation was based on phylogenetic analysis (see below) as vertebrate and non-vertebrate viruses often exhibit a large degree of genetic divergence and hence are phylogenetically distinct [47, 51]. Viruses were defined as novel based on the broad criteria by The International Committee on Taxonomy of Viruses (https://talk.ictvonline.org). Viral genomes were annotated using the NCBI conserved domain (CDD) search and ‘Live Annotate and Predict’ tool in Geneious Prime, using related sequences obtained from NCBI/GenBank with a gene similarity cut-off of 25%.

Phylogenetic analysis

To infer the evolutionary history of the viruses discovered here, we estimated family level phylogenetic trees using amino acid sequences of conserved genomic regions. These included RNA-dependent RNA polymerase (RdRp) for RNA viruses, and the DNA polymerase and major capsid protein for DNA viruses. We then aligned our novel viral sequences with those available on the NCBI/GenBank database (August 2021) using MAFFT v.7.450, employing the E-INS-i algorithm [32]. The amino acid sequence alignment was pruned using TrimAl v.1.2 to remove ambiguously aligned regions with a gap threshold of 0.9 and a variable conserve value [33]. The best-fit model of amino acid substitution was estimated with the ‘ModelFinder Plus’ (-m MFP) flag in IQ-TREE [34, 35]. Using these data, we then estimated phylogenetic trees using a maximum likelihood (ML) approach with 1,000 bootstrap replicates using IQ-TREE.

Abundance estimation and virome statistical analysis

We employed RNA-seq by Expectation-Maximization (RSEM) (v1.2.28) to quantify the relative abundance of transcripts within each fish species transcriptome [36]. These included both viral genes and the stably expressed host reference gene, ribosomal protein S13 (RPS13). Abundance measures were standardised by dividing values against the total reads for each library. We calculated both alpha and beta diversity to compare virome composition between reef fish families, as well as between cryptobenthic reef fishes and large reef fishes. We also compared non-fish virome composition (i.e. those viruses associated with diet, environment or microbiome) as a form of internal control as these viruses are not impacted by aspects of host biology. Accordingly, we used Rhea scripts to calculate alpha diversity, including viral abundance, observed virome richness and Shannon diversity [37]. Statistical comparisons of alpha diversity were modelled using generalized linear models (GLMs) and tested using a likelihood-ratio test (χ^2) and Tukey’s post hoc analysis with the *multcomp* package [38]. To compare viral communities between reef fish assemblages, we calculated beta diversity using a Bray–Curtis distance matrix with the *phyloseq* package [71]. These data were then tested using permutational multivariate analysis of variance (permanova) with the *vegan* package (adonis) [72]. All plots were constructed using *ggplot2* [39].

Results

Total diversity and abundance of viruses discovered in tropical reef fish

We sequenced 61 metatranscriptomes of tropical reef fish species for virus discovery, resulting in a median of 99,658,615 (range 45,583,181 – 121,905,422) reads and 371,944 (range 161,447 – 626,467) contigs per library. From this, we identified a total of 132 viruses infecting apparently healthy fish and their associated invertebrate and microbial species. This included 38 vertebrate-associated viruses, comprising 11 negative-sense single-stranded RNA viruses (-ssRNA) (*Hantaviridae, Chuviridae, Orthomyxoviridae, Rhabdoviridae, Paramyxoviridae*), 18 positive-sense single-stranded RNA viruses (+ssRNA) (*Astroviridae, Hepeviridae, Picornaviridae, Flaviviridae, Coronaviridae*), one double-stranded RNA virus (dsRNA) (*Reoviridae*), six single-stranded DNA viruses (ssDNA) (*Parvoviridae, Circoviridae*), and two double-stranded DNA viruses (dsDNA) (*Iridoviridae*) (Figure 1).

Our metatranscriptomic analysis also identified 94 viruses that shared sequence similarity and phylogenetically clustered with viruses associated with invertebrate (arthropods, crustaceans, molluscs, platyhelminths, myriapods, nematodes) and fungal hosts (Supplementary Figures 1-4). These viruses were classified within the *Flaviviridae* (27% of total non-vertebrate virome abundance), *Narnaviridae* (24%), *Picornaviridae* (20%), *Nodaviridae* (10%), *Hepeviridae* (7%), *Solemoviridae* (4%), *Totiviridae* (1%), *Partitiviridae, Iflaviridae*, *Picobirnaviridae*, *Phenuiviridae*, *Qinviridae, Rhabdoviridae*, *Tombusviridae*, *Mimiviridae*, *Natareviridae, Jingchuvirales* (all less than 1%) [40, 41]. We also identified viruses that were related to negeviruses (2% of non-vertebrate virus abundance) [42] and quenyaviruses (<1%) [43] (Supplementary Figure 1). While many of these families include viruses that are known to infect fishes (e.g. *Flaviviridae, Picornaviridae, Nodaviridae,* *Hepeviridae, Totiviridae, Rhabdoviridae*), the viruses identified here were highly divergent and clustered with those that infect a broad range of invertebrate and fungal species (Supplementary Figures 1-4). We therefore focused our analysis on vertebrate-associated viruses.

Genome organisation and phylogenetic relationships of vertebrate-associated viruses

-ssRNA viruses

Five actinoviruses were identified (subfamily *Actantavirinae*, *Hantaviridae*) (Figure 2). All of these were novel, with the exception for pygmy goby hantavirus, previously identified in *E. zebrina* [19]. We identified complete and partial actinovirus genomes in *Trimma fangi*, *Luposicya lupus*, *Cephalopholis boenak* and *Pleurosicya sp.* (Figure 2; Supplementary Table 2). The genome of *L. lupus* actinovirus contained all three expected actinovirus genomic regions (Figure 2). Notably, the S segment possessed an additional ORF upstream of the nucleoprotein in antisense orientation, also seen in the genome of perch actinovirus, but not in other actinoviruses [44].

Two partial genomes of paramyxoviruses were identified, one in *E. zebrina* (i.e., pygmy goby paramyxovirus) [19] and the other in the *Enneapterygius tutuilae*. This virus was highly divergent from other classified ray-finned fish paramyxoviruses, including the genus *Aquaparamyxovirus* (Figure 2). We also identified partial genomes (L protein and nucleocapsid sequences) of two novel rhabdoviruses in *Trimma okinawae* and *Trimma striatum*. *T. okinawae* rhabdovirus fell within the monophyletic *Tupavirus* group that infects birds and mammals [45, 46], while *T. striatum* rhabdovirus clustered with the dimarhabdoviruses [47] (Figure 2). Among other -ssRNA viruses, we identified a novel pischichuvirus in *Salarius guttatus* and orthomyxovirus in *Cheilodipterus quinquelineatus* that showed sequence similarity to quaranjaviruses (Figure 2; Supplementary Table 2).

+ssRNA viruses

Astroviruses were detected in seven fish species. All of these were members of the Gobiidae, except for *Blenniella sp.* (Blenniidae) which clustered with astroviruses that infect a broad range of ray-finned and jawless fish species (Figure 3). Similarly, we identified a divergent virus from this group in *L. lupus*, tentatively named goby astrovirus 3. This virus exhibited a genome of 7,355 nt with a predicted 5' untranslated region (UTR) of 347 nt, two main ORFs, and a 3' UTR of 347 nt. One ORF encoded the astrovirus ORF1a protein (protease) and the other, a polyprotein encoding RdRp and capsid protein. Notably, this arrangement is also seen in the genome of Wenling rattails astrovirus 5, but not in other currently described fish astroviruses that exhibit three main ORFs [47].

The complete genomes of three novel picornaviruses and partial sequences of five novel picornaviruses were identified (Figure 3). Although these fish species were members of the same community, all eight viruses were highly divergent. Phylogenetic analysis of the 3D polymerase gene reveals clustering with other fish picornaviruses, except for *E. tutuilae* picornavirus and *Exyrias* picornavirus that clustered with *Ampivirus*, identified in the common newt (*Lissotriton vulgaris*) [48] (Figure 3).

We also identified the full genome of a novel hepacivirus (*Flaviviridae*) in *Chaetodon aureofaciatus* that contained conserved genomic regions – Core C, Envelope E1, NS2, NS3, NS4, NS5B – and fell within a distinct group of aquatic hepaciviruses [47] (Figure 3).

dsRNA viruses

The near complete genome of a novel reovirus was identified in *Istigobius nigroocellatus*. This included six segments encoding the VP1 (guanyl transferase), VP2 (RdRp), VP3 (helicase), VP4 (NTPase), VP5 (outer capsid protein) and VP6 (core protein) proteins. Phylogenetic analysis of the VP2 gene revealed clustering within the *Aquareovirus* genus, as this virus shared 48-60% amino acid similarity with its closest relatives (Supplementary Table 2; Supplementary Figure 5).

ssDNA viruses

We discovered a basal group of four parvoviruses that fell within the subfamily *Parvovirinae* (*Parvoviridae*). The genome of *Ecsensius stictus* parvovirus was a single contig of 4286 nt, containing two main ORFs. The left ORF was 1374 nt and encoded the conserved non-structural protein NS1 that has DNA helicase and ATPase function [65]. The right ORF encoded the structural VP1 protein (2571 bp). *I. ornatus* parvovirus exhibited a genome of 4284 nt and contained three main ORFs, one encoding NS1 (1878 nt) and the other two, encoding putative structural proteins (Figure 4).

We also identified the partial genome of a novel icthamaparvovirus in *L. lupus* and a novel circovirus in *Abudefduf bengalensis* that clustered within a distinct clade of fish circoviruses. (Figure 4).

dsDNA viruses

Analysis of the *Halichoeres melanurus* meta-transcriptome identified nine conserved proteins sharing 99-100% similarity with all currently described *Santee-Cooper ranavirus* isolates such as largemouth bass virus, manadarin fish ranavirus, koi ranavirus, doctor fish virus, and guppy virus 6 (*Iridoviridae*; Supplementary Table 2). We used the highly conserved major capsid protein and DNA polymerase for phylogenetic analysis [50], which further confirmed a novel *Santee-cooper ranavirus* isolate, tentatively named *H. melanurus* ranavirus (Figure 5). Largemouth bass virus and mandarin fish ranavirus are highly lethal in farmed populations, causing 95-100% mortality [60, 61]. Our metatranscriptomic analysis also identified a novel iridovirus in *E. tutuilae* that fell sister to erythrocytic necrosis virus (ENV) [51] and clustered with other erythrocytic-like viruses identified in amphibians and reptiles [52, 53, 54, 55]. ENV was the closest relative across all proteins identified (Supplementary Table 2).

Cross-species virus transmission in a reef fish community

Despite the large number of viruses identified, we only found evidence for one cross-species transmission within our GBR ecosystem. This involved astroviruses found in five different fish species that exhibited high levels of amino acid sequence similarity and phylogenetic clustering (Figure 3). Specifically, phylogenetic comparisons of ORF1b (RdRp) revealed two viral species: goby astrovirus 1, identified in *I. goldmanni*, and goby astrovirus 2, identified in *I. nigroocellatus, I. decoratus, Asterropteryx semipunctatus,* and *Cabillus tongarevae* (Figure 4). These two viruses exhibit 82.5% amino acid sequence similarity across the virus genome, while the four sequences of goby astrovirus 2 had 96.8% amino acid similarity (Figure 3).

We also detected related viruses (i.e., those from the same virus family) in several different fish species, including the *Hantaviridae, Rhabdoviridae, Paramyxoviridae,* and *Picornaviridae* (Figure 6). However, most of these were highly divergent and likely reflect common ancestry rather than direct host-jumping in the reef ecosystem. For example, out of the eight picornaviruses identified in this study, the closest relatives were *Asterropteryx spinosa* picornavirus and *A. semipunctatus* picornavirus that shared only 44% amino acid similarity.

Also of note was the identification of viral species not directly infecting the fish themselves, but rather associated with the local environment, diet or microbiome (i.e., non-fish) that were transmitted between reef fish assemblages. These were quenyaviruses (95% amino acid similarity between *Pomacentrus moluccensis* and *A. bengalensis*), flavi-like viruses (91.2% between *I. goldmanni* and *I. ornatus*), narnaviruses (97.7% between *I. goldmanni, I. nigroocellatus* and *I. rigillus*) and totiviruses (93.5% between *Amblygobius buanesis* and *Amblygobius rainfordi*) (Supplementary figs 1, 2 and 4; Figure 6). That there were more instances of cross-species transmission of non-fish viruses compared to those viruses that actively replicate in fish suggests that the latter group are subject to strong host barriers, even among closely related species.

Comparisons of viral alpha and beta diversity between reef fish families

We next compared vertebrate virome composition between reef fish families, as well as between cryptobenthic reef fishes and large reef fishes (i.e. that differ in size). In our data set, cryptobenthic reef fish families included the Gobiidae, Apogonidae, Blenniidae, Tripterygiidae, Bythitidae and Pseudochromidae, while the large reef fish families included the Pomacentridae, Acanthuridae, Tetraodontidae, Atherinidae, Serranidae, Monacanthidae, Chaetodontidae, Labridae, Muraenidae, and Ophichthidae [10].

Three statistical measures were used to assess alpha diversity: viral abundance (i.e., standardised number of viral reads), observed viral richness (i.e., the number of viruses) and Shannon diversity. Notably, we found an association between fish size and observed viral richness, with cryptobenthic reef fishes harbouring more viruses than large reef fishes (χ^2 = 2.795, df = 1, P = 0.028). In particular, the Tripterygiidae exhibited greater observed viral richness than all other reef fish families (χ^2 = 16.678, df = 15, P = 0.007). However, we found no association between reef fish family and viral abundance (P = 0.153), Shannon diversity (p = 0.901) or beta diversity (R^2 = 0.334, P = 0.064). Likewise, we identified no significant differences in viral abundance (P = 0.271), Shannon diversity (P = 0.142) nor beta diversity (R^2 = 0.048, P = 0.121) between cryptobenthic reef fishes and large reef fishes.

As a form of internal control, we repeated our analyses of viral abundance and diversity on the non-fish viruses identified here. This analysis revealed no significant differences in viral abundance between reef fish families (P = 0.994) nor between cryptobenthic reef fishes and larger reef fishes (P = 0.355). Similarly, we found no significant difference in observed viral richness between fish families (P = 0.733), although cryptobenthic reef fishes exhibited higher observed non-vertebrate viral richness than large reef fish families (χ^2 = 10.805, df = 1, P = 0.016). We observed no difference in Shannon diversity between fish families (P = 0.453), as well as between cryptobenthic reef fishes and larger reef fishes (P = 0.070). Finally, we found no association between beta diversity and reef fish families (R^2 = 0.279, P = 0.126) nor between cryptobenthic reef fishes and large reef fishes (R^2 = 0.335, P = 0.058).

Discussion

The GBR supports over 1200 species of fish and is the largest coral reef ecosystem in the world, comprising 2500 reefs across approximately 344,400 km^2 [5]. Despite the global importance of the GBR, little is known about the natural diversity of viruses that infect tropical reef fishes, as well as the ecological and evolutionary processes that allow such viruses to spread within a reef fish community. To this end, we employed metatranscriptomic sequencing to characterize the viromes of 61 tropical reef fish species, including those occupying a 100 m^2 reef fish community. This identified a total of 132 viruses, including 38 vertebrate-associated viruses and 94 that infected a broad range of invertebrate and fungal hosts.

Importantly, while we sampled coral reef fishes from a small spatial area, we identified a marked absence of cross-species virus transmission, with the only instance of host-jumping being the presence of a single viral species (*Astroviridae*) in four goby species (Figures 3 and 6). However, the variation across the four viral genomes is indicative of past host-switching throughout evolutionary history, which may span millions of years, rather than direct host-jumping within the ecosystem sampled here. Given the restricted spatial area considered, and the density of potential hosts, such a lack of cross-species transmission suggests that there may be important host genetic barriers to virus switching among the reef fishes sampled herein. This is supported by the observation that non-fish viruses – that are not impacted by aspects of host genetics – were characterized by higher levels of cross-species virus transmission. Although currently of unknown nature, these barriers are likely to be subtle and may reflect nuances in host cell receptor binding [56]. For instance, although the betacoronavirus RaTG13 sampled from *Rhinolophus affinis* bats is closely related (~96% sequence similarity) to SARS-CoV-2, it is unable to bind to the human ACE2 receptor [73].

Another notable result was that we detected differences in vertebrate virome composition between reef fish families, with cryptobenthic reef fishes harbouring more viruses than large reef fishes (Figure 7). Due to their small body size, cryptobenthic reef fishes exhibit significantly higher rates of metabolism compared to large reef fishes, resulting in high energy demands with a low tolerance for starvation [10]. Given their extremely short lifespans, a high degree of energy is used for their rapid growth as well as their complex reproductive strategies (e.g. sex change), such that there might be important energy trade-offs between growth, reproduction and immunity; particularly as immune systems require a substantial allocation of resources for recognizing and eliminating pathogens [10, 70]. While it is unclear if and how such energy demands impact immunity in cryptobenthic reef fishes, it is notable that this lifestyle is associated with a more diverse virome compared to large reef fishes, suggesting these species may be more susceptible to infection. Indeed, with a daily mortality rate of 8 to 9% [20, 76] investing in immunity may be a poor strategy, and it is possible that these energy-saving strategies are key to the spectacular success of these cryptobenthic fishes [77]. Whether as simple larvae in the pelagic realm, or on the reef, cryptobenthic fishes may be able to minimise energetically expensive activities such as immunity and swimming, freeing up energy to maintain adequate fecundity, despite exceptionally high mortality rates and infection risks.

Among reef fish families, the Tripterygiidae exhibited the greatest observed viral richness, with *E. tutuilae* harbouring four viral species. *E. tutuilae* is a generalist on coral reefs, utilising sand and rubble, soft coral, cave and open reef microhabitats [67]. It is therefore possible that habitat generalism may increase interactions with other fish species and hence increase the likelihood of being infected by a larger number of viruses. In contrast, *Gobiodon* species are extreme habitat specialists and may often only occupy a single coral species as habitat for its entire life with minimal interactions with other fish species [10, 68]. This lifestyle may explain why all five *Gobiodon* species examined here harboured no vertebrate-associated viruses, as well as few non-vertebrate associated viruses (Figure 1).

Given the phylogenetic distance between fish and other vertebrate classes, as well as the long-term associations through virus-host co-divergence [47, 57], it was not unexpected that 95% of the vertebrate-associated viruses discovered here clustered with other fish viruses. A case in point comes from the phylogeny of the *Hantaviridae* that clearly reflects the broad evolutionary history of vertebrates with the *Agantavirinae* (jawless fish) falling basal to the *Actantavirinae* (ray-finned fish)*, Reptantavirinae* (reptiles) and *Mammantavirinae* (mammals), respectively (Figure 2). Despite their likely long-term presence in ray-finned fish, actinoviruses have only recently been discovered, and have been associated with disease in farmed species [19, 27, 44, 47]. For instance, a novel actinovirus – perch actinovirus – was identified in diseased European perch (*Perca fluviatilis*) with high concentrations of viral RNA in gill endothelial cells and macrophages [44]. Our discovery of four actinoviruses in gobies suggests these viruses may be widespread in this fish family and hence should be monitored closely if interacting with farmed populations.

Another virus of concern identified in our study is a novel isolate of *Santee-cooper ranavirus* in *H. melanurus*. To the best of our knowledge, this is the first discovery of this virus in Australia. Our detection of this virus in seemingly healthy wrasses as well as its natural presence in cleaner wrasses (*Labroides dimidatus*), suggests this family of tropical reef fishes may be important reservoir hosts for the *Santee-cooper ranavirus* group [63]. For instance, disease outbreaks with high mortality rates have only been observed in farmed freshwater fish species including largemouth bass (*Micropterus salmoides*), mandarinfish (*Siniperca chuatsi*) and koi (*Cyprinus carpio*) [58, 59, 60, 61, 62].

Despite the long-known presence of viral erythrocytic necrosis in various fish species across the North Atlantic and Pacific Oceans, the genome of the causative virus – ENV – was only recently characterized [51]. We identified a closely related virus in *E. tutuilae*. Interestingly, viral erythrocytic necrosis in a juvenile triggerfish (*Rhinecanthus aculeatus*) has been reported nearby at Lizard Island [64], suggesting that this virus may be circulating between several reef locations along the GBR. Phylogenetic comparisons of DNA polymerase and major capsid protein revealed a distinct ‘ectothermic vertebrate’ clade that could be clearly classified as a novel genus within the *Betairidovirinae*, *Iridoviridae* (Figure 5).

While we observed significant differences in virome composition between coral reef fishes, there were necessary limitations in our sampling. For instance, cryptobenthic fishes made up

62% of the fish diversity in this study. In addition, 23 libraries contained only one individual. Such unequal sample sizes likely impacted the statistical power of our analyses. Accordingly, future work should balance the number of samples as well as increase the number of reef sampling sites. Comparing virome composition between entire reef fish communities is crucial to understanding how host community diversity affects viral dynamics and disease emergence, particularly in the context of biodiversity loss. As such, larger ecological studies are needed to better evaluate whether a dilution or amplification effect occurs in a reef fish community.

In sum, our study identified a large diversity and abundance of viruses in tropical reef fish assemblages. Notably, we identified a marked absence of virus exchange within a reef fish community, suggesting there may be important host genetic barriers for successful cross-species virus transmission. Importantly, our discovery of a novel *Santee-cooper ranavirus* isolate in seemingly healthy wrasses, highlights the importance of virological surveillance in marine wildlife, particularly as this virus can cause significantly high mortality in farmed fishes. As such, these species should be considered in biosecurity risk assessments and screened if utilized for aquaculture or aquarium operations [69]. Accordingly, future studies should also investigate its susceptibility in important Australian food fish to fully assess its emergence threat [63]. Overall, this study increases our knowledge on the severely understudied coral reef fish virome and provides the first data on virus-host interactions in a reef fish community.

Acknowledgements

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Data availability

All sequence reads are available on the NCBI Sequence Read Archive (SRA) under BioProject XXXX (submission: SUB11462050, awaiting ID) and all generated virus genetic sequences have been deposited in NCBI/GenBank under accessions XXXX - XXXX (submission: 2582698, awaiting accession IDs).

Competing Interests

The authors declare no competing interests.

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Tables

Table 1. Description of viruses identified in this study.

Figures

Figure 1. Standardized abundance of viral and host reads across reef fish libraries. Abundance (i.e., number of reads) of: (a) likely vertebrate-associated viruses; (b) non-vertebrate associated viruses (i.e., those from algae, fungi, coral, arthropods, crustaceans and protists); (c) host reference gene RPS13. Silhouettes represent the fish species and virus families are indicated by colour. (d) Total standardized abundance of vertebrate-associated viral families.

Figure 2. Genome structure and phylogenies of the RdRp gene of -ssRNA viruses identified in this study**.** Maximum likelihood phylogenies of novel and related virus species. Trees were midpoint rooted for clarity. Scale bar represents the number of amino acid substitutions per site. Tip labels represent virus name with NCBI/GenBank Accession. Tree branches are highlighted to broadly represent host taxonomy. Schematic genome diagrams illustrate genome orientation, length, predicted ORFs and gene products.

Figure 3. Genome organisation and phylogenies of the RdRp gene of +ssRNA viruses identified in this study. (a) Maximum likelihood phylogenies of the *Picornaviridae* and *Hepacivirus* (*Flaviviridae*), midpoint rooted for clarity. Scale bar represents the number of amino acid substitutions per site. Tip labels represent virus name with NCBI/Genbank Accession. Trees are highlighted to broadly illustrate host taxonomy. (b) Schematic genomes illustrate genome orientation, length and gene products. (c) Phylogeny of the *Astroviridae* and amino acid alignment of goby astrovirus 2 (GAst2) isolates against goby astrovirus 1 (GAst1). GAst2 are labelled to represent host species: GAst2 C.t = *C. tongaravae*, I.d = *I. decoratus*, A.s = *A. semipunctatus*, I.n = *I. nigroocellatus*.

Figure 4. Phylogenetic relationships of the ssDNA viruses identified in this study (a) Phylogenetic analysis of the NS1 gene of the *Parvoviridae*. Discovered viruses are represented as black circles. The tree is midpoint rooted for clarity. Scale bar represents the number of amino acid substitutions per site. (b) Schematic genomes (nt) of discovered parvoviruses. Coloured boxes represent both structural (NS1) and non-structural (VP) ORFs. (c) Unrooted phylogeny of the replication-associated protein of the genus *Circovirus* (*Circoviridae*). Discovered circovirus is represented as a fish symbol. Branches of both trees are highlighted to represent host class: blue, fish; red, invertebrates; green, birds; yellow, mammals.

Figure 5. (a) Phylogenetic analysis of the DNA polymerase gene among the *Iridoviridae.* Scale bar represents the number of amino acid substitutions per site. Tip labels represent virus name with NCBI/GenBank Accession. Viruses discovered here are represented as a black circle and fish symbol. Branches are highlighted to illustrate host class: blue, fish; purple, amphibians; green, reptiles; yellow, invertebrates. (b) Unrooted phylogeny of the MCP of the *Iridoviridae.* Branches are coloured to represent both subfamilies: orange, *Alphairidovirinae*; red, *Betairidovirinae*.

Figure 6. (a) Bipartite network illustrating viral families shared between fish taxa. Viral families are represented as a coloured box, with viruses connected between host species as a coloured line. (b) Network diagram illustrating vertebrate-associated viral species shared between fish. Coloured circle and lines illustrate viral species. (c) Network diagram revealing non-fish viral species shared between fish libraries. All fish silhouettes broadly represent host species at the family level.

Figure 7. (a) Vertebrate-associated viral richness observed in all tropical reef fish families (b) Comparison of observed vertebrate-associated viral richness between cryptobenthic reef fishes and large reef fishes.

Supplementary information

Supplementary Table 1. Host and meta-transcriptomic library information

Supplementary Table 2. Description of novel viruses identified in this study

Supplementary Figure 1. (a) Phylogenies of the RdRp gene of +ssRNA non-vertebrate associated viruses in fish metatranscriptomes. Discovered viruses in this study are represented as black circles. Maximum likelihood trees were midpoint rooted for clarity only. Scale bar represents amino acid substitutions per site. Tip labels represent virus name with NCBI/GenBank accession.

Supplementary Figure 2. (a) Phylogenies of the RdRp gene of +ssRNA non-vertebrate associated viruses in fish metatranscriptomes. Discovered viruses in this study are represented as black circles. Maximum likelihood trees were midpoint rooted for clarity only. Scale bar represents amino acid substitutions per site. Tip labels represent virus name with NCBI/GenBank accession.

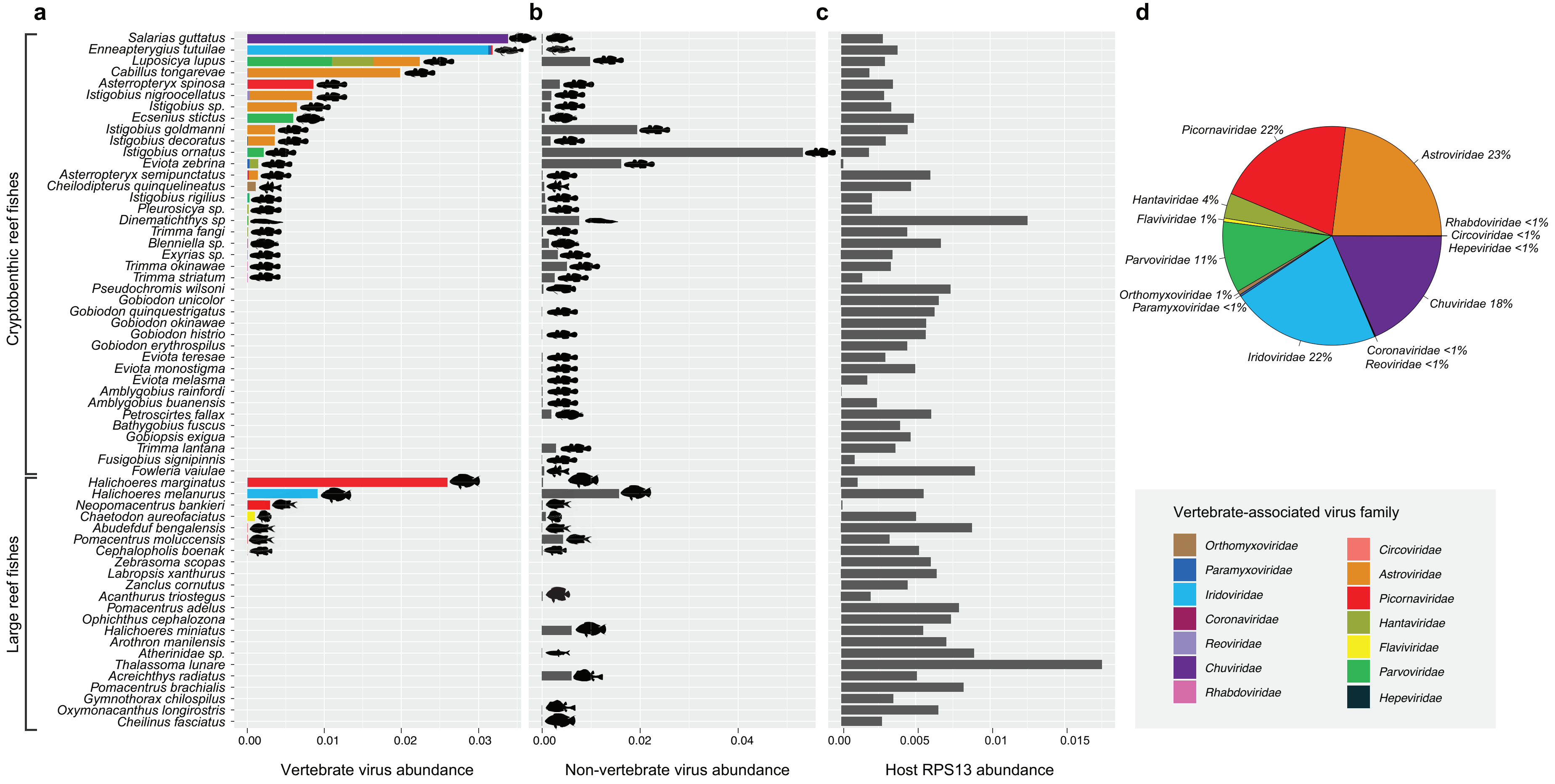
Supplementary Figure 3. (a) Phylogenies of the RdRp gene of -ssRNA non-vertebrate associated viruses in fish metatranscriptomes. Discovered viruses in this study are represented as black circles. Maximum likelihood trees were midpoint rooted for clarity only. Scale bar represents amino acid substitutions per site. Tip labels represent virus name with NCBI/GenBank accession.

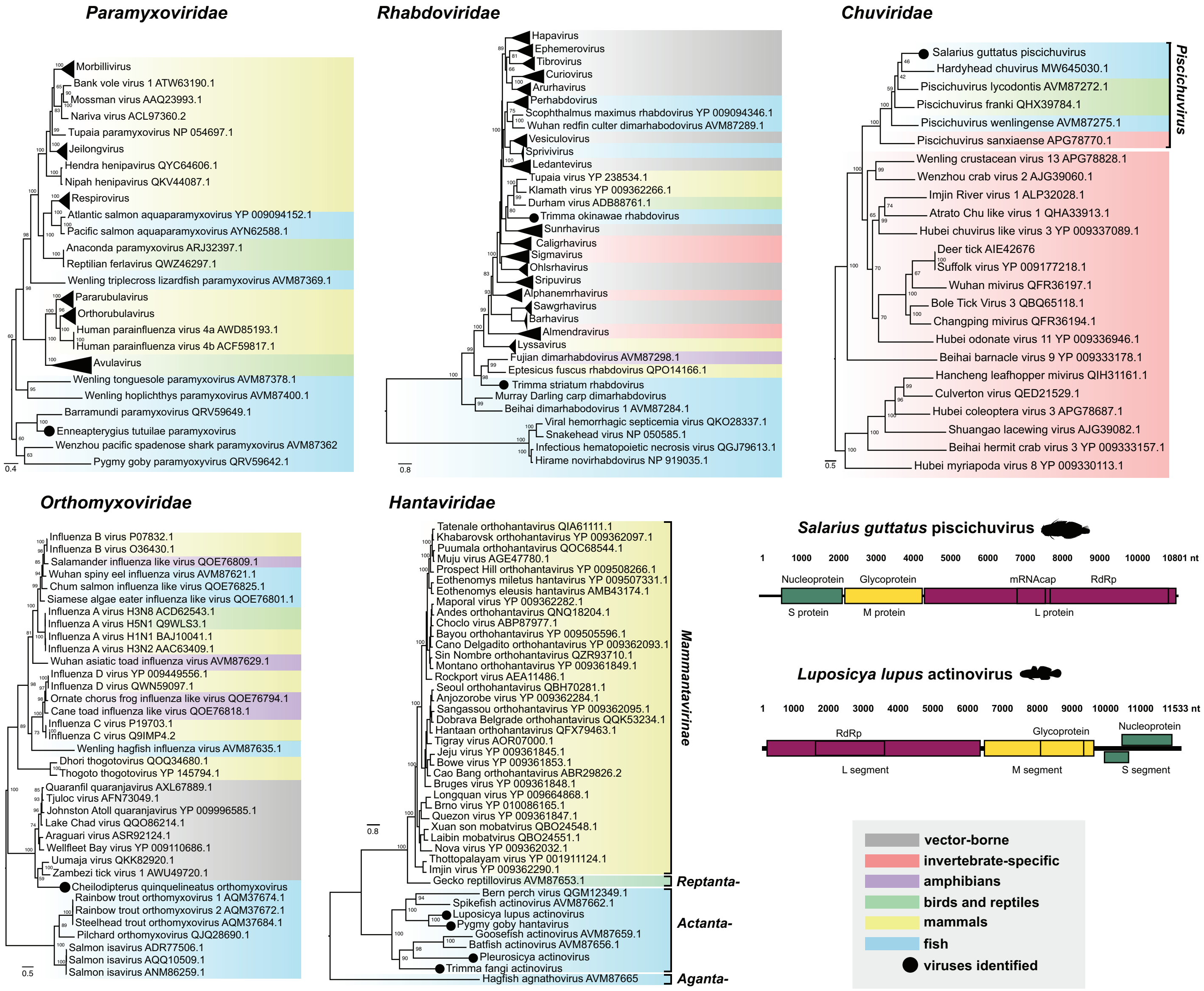
Supplementary Figure 4. (a) Phylogenies of the RdRp gene of dsRNA non-vertebrate associated viruses in fish metatranscriptomes. Discovered viruses in this study are represented as black circles. Maximum likelihood trees were midpoint rooted for clarity only. Scale bar represents amino acid substitutions per site. Tip labels represent virus name with NCBI/GenBank accession.

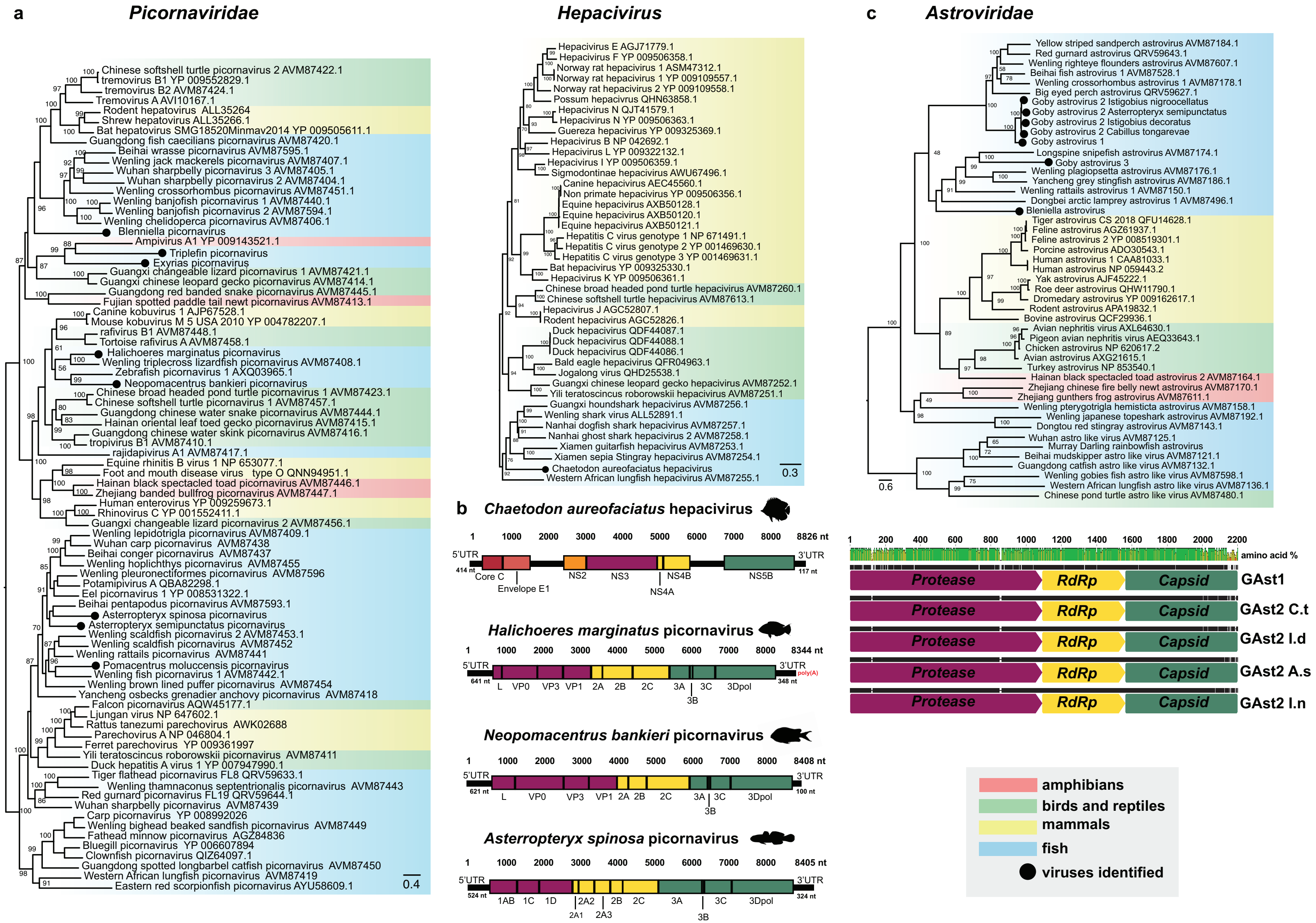
Supplementary Figure 5. (a) Phylogenetic tree of the VP2 (RdRp) gene of the subfamily *Spinareovirinae* (*Reoviridae*). Discovered virus represented as a black circle. The tree is midpoint rooted for clarity only. Scale bar represents amino acid substitutions per site and tip labels display virus name with NCBI/GenBank accession. Branches are highlighted to illustrate host class: blue, fish; green, birds; red, mammals.

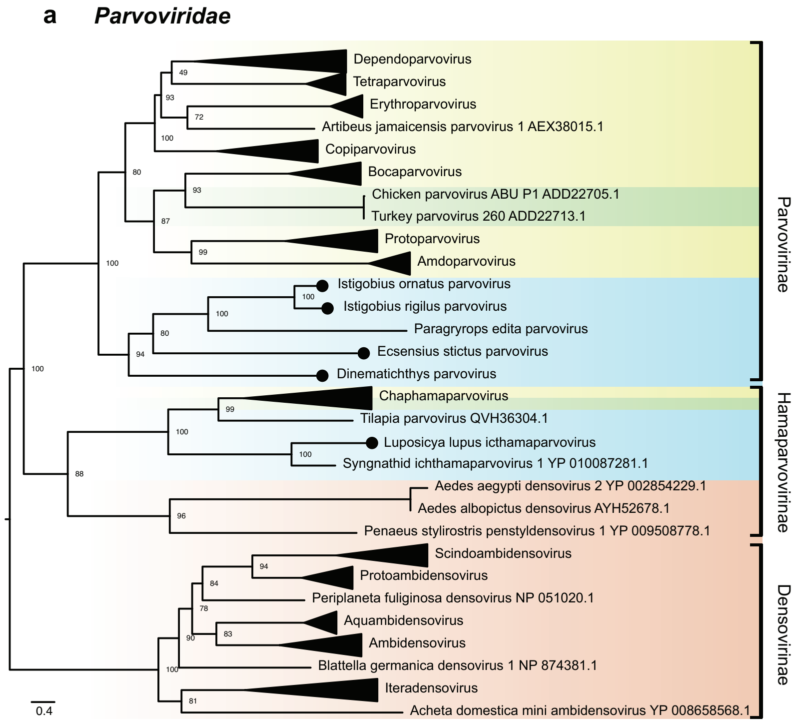
Virus name	No. of reads	Virus family	Host	No. of ORFs/Segments	Length (nt)	Complete genome
Goby astrovirus 2	24100	*Astroviridae*	*Cabillus tongarevae*	3	7226	Y
Goby astrovirus 2	2767	*Astroviridae*	*Istigobius decoratus*	3	7085	Y
Goby astrovirus 2	9023	*Astroviridae*	*Istigobius nigroocellatus*	3	7155	Y
Goby astrovirus 2	1184	*Astroviridae*	*Asterropteryx semipunctatus*	3	7045	Y
Goby astrovirus 1	2863	*Astroviridae*	*Istigobius goldmanni*	3	7087	Y
Goby astrovirus 3	6709	*Astroviridae*	*Luposicya lupus*	2	7355	Y
Blenniella astrovirus	59	*Astroviridae*	*Blenniella sp.*	1	744	N
Salarius guttatus piscichuvirus	40334	*Chuviridae*	*Salarias guttatus*	3	10801	Y
Abudefduf bengalensis circovirus	54	*Circoviridae*	*Abudefduf bengalensis*	1	873	N
Enneapterygius tutuilae letovirus	26	*Coronaviridae*	*Enneapterygius tutuilae*	1	856	N
Chaetodon aureofaciatus hepacivirus	1029	*Flaviviridae*	*Chaetodon aureofaciatus*	1	8826	Y
Luposicya lupus actinovirus	6144	*Hantaviridae*	*Luposicya lupus*	3	11533	Y
Pygmy goby hantavirus	972	*Hantaviridae*	*Eviota zebrina*	3	5894	N
Trimma fangi actinovirus	106	*Hantaviridae*	*Trimma fangi*	2	1319	N
Pleurosicya actinovirus	123	*Hantaviridae*	*Pleurosicya sp.*	2	2103	N
Cephalopholis boenak actinovirus	9	*Hantaviridae*	*Cephalopholis boenak*	1	1029	N
Istigobius decoratus hepevirus	16	*Hepeviridae*	*Istigobius decoratus*	1	393	N
Halichoeres melanurus ranavirus	9835	*Iridoviridae*	*Halichoeres melanurus*	9	10821	N
Enneapterygius tutuilae iridovirus	35545	*Iridoviridae*	*Enneapterygius tutuilae*	9	20970	N

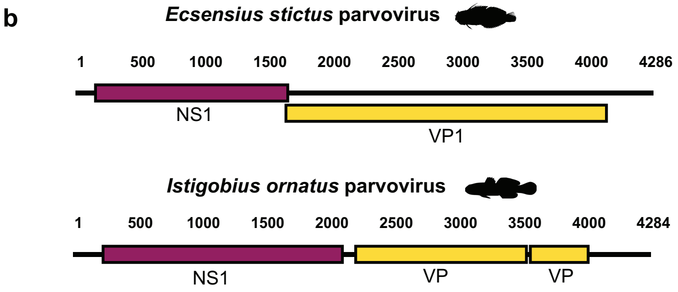
Cheilodipterus quinquelineatus orthomyxovirus	979	*Orthomyxoviridae*	*Cheilodipterus quinquelineatus*	5	9090	N
Pygmy goby paramyxovirus	238	*Paramyxoviridae*	*Eviota zebrina*	1	1314	N
Enneapterygius tutuilae paramyxovirus	322	*Paramyxoviridae*	*Enneapterygius tutuilae*	1	618	N
Istigobius ornatus parvovirus	1746	*Parvoviridae*	*Istigobius ornatus*	3	4284	Y
Istigobius rigillus parvovirus	266	*Parvoviridae*	*Istigobius rigillus*	1	1656	N
Ecsensius stictus parvovirus	2707	*Parvoviridae*	*Ecsensius stictus*	2	4286	Y
Dinematichthys parvovirus	122	*Parvoviridae*	*Dinematichthys sp.*	1	1236	N
Luposicya lupus icthamaparvovirus	12328	*Parvoviridae*	*Luposicya lupus*	1	1299	N
Blenniella picornavirus	2	*Picornaviridae*	*Blenniella sp.*	1	347	N
Enneapterygius tutuilae picornavirus	225	*Picornaviridae*	*Enneapterygius tutuilae*	1	3840	N
Exyrias picornavirus	44	*Picornaviridae*	*Exyrias sp.*	1	1373	N
Halichoeres marginatus picornavirus	27506	*Picornaviridae*	*Halichoeres marginatus*	1	8344	Y
Neopomacentrus bankieri picornavirus	2955	*Picornaviridae*	*Neopomacentrus bankieri*	1	8408	Y
Asterropteryx spinosa picornavirus	8307	*Picornaviridae*	*Asterropteryx spinosa*	1	8405	Y
Asterropteryx semipunctatus picornavirus	210	*Picornaviridae*	*Asterropteryx semipunctatus*	1	992	N
Pomacentrus moluccensis picornavirus	12	*Picornaviridae*	*Pomacentrus moluccensis*	1	321	N
Trimma okinawae rhabdovirus	13	*Rhabdoviridae*	*Trimma okinawae*	2	945	N
Trimma striatum rhabdovirus	2	*Rhabdoviridae*	*Trimma striatum*	1	488	N
Istigobius nigroocellatus reovirus	282	*Reoviridae*	*Istigobius nigroocellatus*	6	6966	N

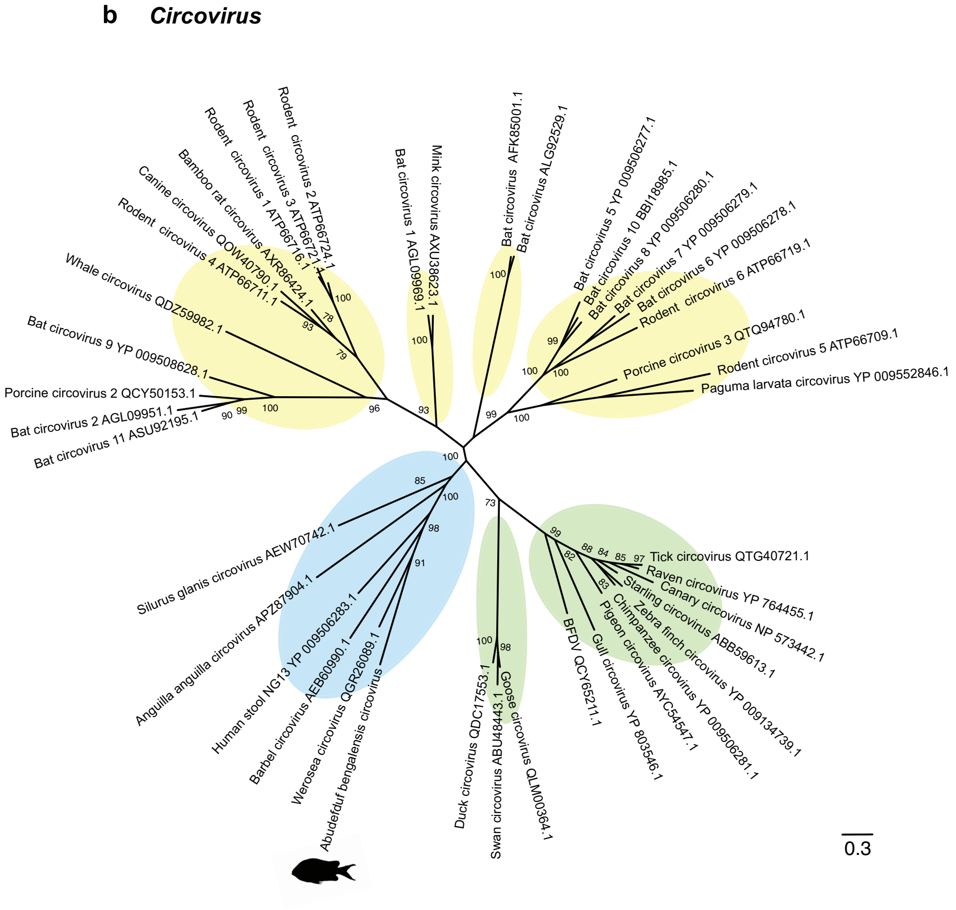

a
b
c
d
Cryptobenthic reef fishes
Large reef fishes
Salarias guttatus
Enneapterygius tutuilae
Luposicya lupus
Cabillus tongarevae
Asterropteryx spinosa
Istigobius nigroocellatus
Istigobius sp.
Ecsenius stictus
Istigobius goldmanni
Istigobius decoratus
Istigobius ornatus
Eviota zebrina
Asterropteryx semipunctatus
Cheilodipterus quinquelineatus
Istigobius rigilius
Pleurosicya sp.
Dinematichthys sp
Trimma fangi
Blenniella sp.
Exyrias sp.
Trimma okinawae
Trimma striatum
Pseudochromis wilsoni
Gobiodon unicolor
Gobiodon quinquestrigatus
Gobiodon okinawae
Gobiodon histrio
Gobiodon erythrospilus
Eviota teresae
Eviota monostigma
Eviota melasma
Amblygobius rainfordi
Amblygobius buanensis
Petroscirtes fallax
Bathygobius fuscus
Gobiopsis exigua
Trimma lantana
Fusigobius signipinnis
Fowleria vaiulae
Halichoeres marginatus
Halichoeres melanurus
Neopomacentrus bankieri
Chaetodon aureofaciatus
Abudefduf bengalensis
Pomacentrus moluccensis
Cephalopholis boenak
Zebrasoma scopas
Labropsis xanthurus
Zanclus cornutus
Acanthurus triostegus
Pomacentrus adelus
Ophichthus cephalozona
Halichoeres miniatus
Arothron manilensis
Atherinidae sp.
Thalassoma lunare
Acreichthys radiatus
Pomacentrus brachialis
Gymnothorax chilospilus
Oxymonacanthus longirostris
Cheilinus fasciatus
0.00
0.01
0.02
0.03
Vertebrate virus abundance
0.00
0.02
0.04
Non-vertebrate virus abundance
0.00
0.005
0.01
0.015
Host RPS13 abundance
Picornaviridae 22%
Astroviridae 23%
Hantaviridae 4%
Flaviviridae 1%
Rhabdoviridae <1%
Circoviridae <1%
Hepeviridae <1%
Parvoviridae 11%
Orthomyxoviridae 1%
Paramyxoviridae <1%
Chuviridae 18%
Coronaviridae <1%
Reoviridae <1%
Iridoviridae 22%
Vertebrate-associated virus family
Orthomyxoviridae
Paramyxoviridae
Iridoviridae
Coronaviridae
Reoviridae
Chuviridae
Rhabdoviridae
Circoviridae
Astroviridae
Picornaviridae
Hantaviridae
Flaviviridae
Parvoviridae
Hepeviridae

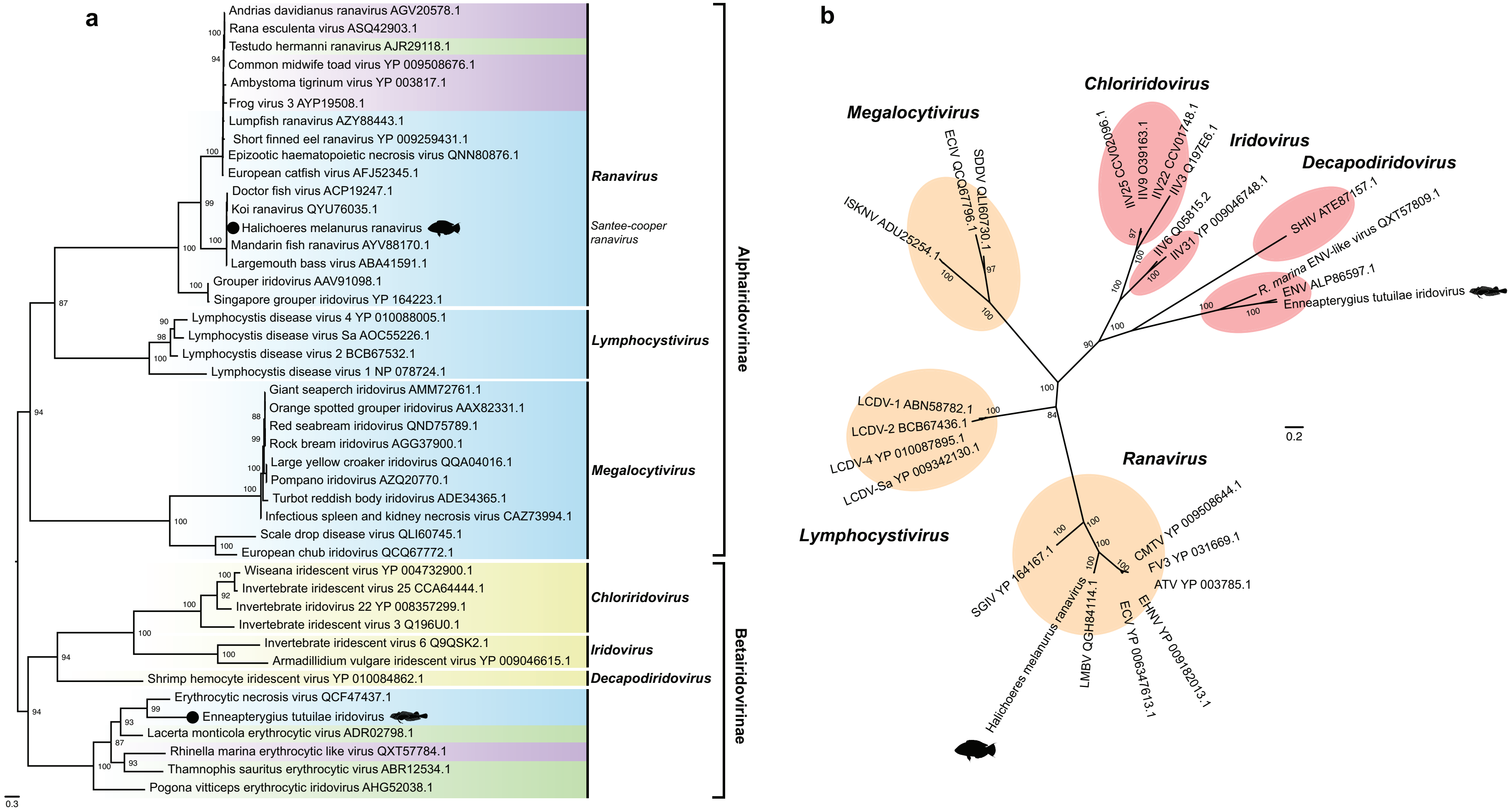

Paramyxoviridae
Morbillivirus
Bank vole virus 1 ATW63190.1
Mossman virus AAQ23993.1
Nariva virus ACL97360.2
Tupaia paramyxovirus NP 054697.1
Jeilongvirus
Hendra henipavirus QYC64606.1
Nipah henipavirus QKV44087.1
Respirovirus
Atlantic salmon aquaparamyxovirus YP 009094152.1
Pacific salmon aquaparamyxovirus AYN62588.1
Anaconda paramyxovirus ARJ32397.1
Reptilian ferlavirus QWZ46297.1
Wenling triplecross lizardfish paramyxovirus AVM87369.1
Pararubulavirus
Orthorubulavirus
Human parainfluenza virus 4a AWD85193.1
Human parainfluenza virus 4b ACF59817.1
Avulavirus
Wenling tonguesole paramyxovirus AVM87378.1
Wenling hoplichthys paramyxovirus AVM87400.1
Barramundi paramyxovirus QRV59649.1
Enneapterygius tutuilae paramyxovirus
Wenzhou pacific spadenose shark paramyxovirus AVM87362
Pygmy goby paramyoxyvirus QRV59642.1
0.4
Rhabdoviridae
Hapavirus
Ephemerovirus
Tibrovirus
Curiovirus
Arurhavirus
Perhabdovirus
Scophthalmus maximus rhabdovirus YP 009094346.1
Wuhan redfin culter dimarhabdovirus AVM87289.1
Vesiculovirus
Sprivivirus
Ledantevirus
Tupaia virus YP 238534.1
Klamath virus YP 009362266.1
Durham virus ADB88761.1
Trimma okinawae rhabdovirus
Sunrhavirus
Caligrhavirus
Sigmavirus
Ohlsrhavirus
Sripuvirus
Alphanemrhavirus
Sawgrhavirus
Barhavirus
Almendravirus
Lyssavirus
Fujian dimarhabdovirus AVM87298.1
Eptesicus fuscus rhabdovirus QPO14166.1
Trimma striatum rhabdovirus
Murray Darling carp dimarhabdovirus
Beihai dimarhabdovirus 1 AVM87284.1
Viral hemorrhagic septicemia virus QKO28337.1
Snakehead virus NP 050585.1
Infectious hematopoietic necrosis virus QGJ79613.1
Hirame novirhabdovirus NP 919035.1
0.8
Chuviridae
Salarius guttatus piscichuvirus
Hardyhead chuvirus MW645030.1
Piscichuvirus lycodontis AVM87272.1
Piscichuvirus franki QHX39784.1
Piscichuvirus wenlingense AVM87275.1
Piscichuvirus sanxiaense APG78770.1
Piscichuvirus
Wenling crustacean virus 13 APG78828.1
Wenzhou crab virus 2 AJG39060.1
Imjin River virus 1 ALP32028.1
Atrato Chu like virus 1 QHA33913.1
Hubei chuvirus like virus 3 YP 009337089.1
Deer tick AIE42676
Suffolk virus YP 009177218.1
Wuhan mivirus QFR36197.1
Bole Tick Virus 3 QBQ65118.1
Changping mivirus QFR36194.1
Hubei odonate virus 11 YP 009336946.1
Beihai barnacle virus 9 YP 009333178.1
Hancheng leafhopper mivirus QIH31161.1
Culverton virus QED21529.1
Hubei coleoptera virus 3 APG78687.1
Shuangao lacewing virus AJG39082.1
Beihai hermit crab virus 3 YP 009333157.1
Hubei myriapoda virus 8 YP 009330113.1
0.5
Orthomyxoviridae
Influenza B virus P07832.1
Influenza B virus O36430.1
Salamander influenza like virus QOE76809.1
Wuhan spiny eel influenza virus AVM87621.1
Chum salmon influenza like virus QOE76825.1
Siamese algae eater influenza like virus QOE76801.1
Influenza A virus H3N8 ACD62543.1
Influenza A virus H5N1 Q9WLS3.1
Influenza A virus H1N1 BAJ10041.1
Influenza A virus H3N2 AAC63409.1
Wuhan asiatic toad influenza virus AVM87629.1
Influenza D virus YP 009449556.1
Influenza D virus QWN59097.1
Ornate chorus frog influenza like virus QOE76794.1
Cane toad influenza like virus QOE76818.1
Influenza C virus P19703.1
Influenza C virus Q9IMP4.2
Wenling hagfish influenza virus AVM87635.1
Dhori thogotovirus QOQ34680.1
Thogoto thogotovirus YP 145794.1
Quaranfil quaranjavirus AXL67889.1
Tjuloc virus AFN73049.1
Johnston Atoll quaranjavirus YP 009996585.1
Lake Chad virus QQO86214.1
Araguari virus ASR92124.1
Wellfleet Bay virus YP 009110686.1
Uumaja virus QKK82920.1
Zambezi tick virus 1 AWU49720.1
Cheilodipterus quinquelineatus orthomyxovirus
Rainbow trout orthomyxovirus 1 AQM37674.1
Rainbow trout orthomyxovirus 2 AQM37672.1
Steelhead trout orthomyxovirus AQM37684.1
Pilchard orthomyxovirus QJQ28690.1
Salmon isavirus ADR77506.1
Salmon isavirus AQQ10509.1
Salmon isavirus ANM86259.1
0.5
Hantaviridae
Tatenale orthohantavirus QIA61111.1
Khabarovsk orthohantavirus YP 009362097.1
Puumala orthohantavirus QOC68544.1
Muju virus AGE47780.1
Prospect Hill orthohantavirus YP 009508266.1
Eothenomys miletus hantavirus YP 009507331.1
Eothenomys eleusis hantavirus AMB43174.1
Maporal virus YP 009362282.1
Andes orthohantavirus QNQ18204.1
Choclo virus ABP87977.1
Bayou orthohantavirus YP 009505596.1
Cano Delgadito orthohantavirus YP 009362093.1
Sin Nombre orthohantavirus QZR93710.1
Montano orthohantavirus YP 009361849.1
Rockport virus AEA11486.1
Seoul orthohantavirus QBH70281.1
Anjozorobe virus YP 009362284.1
Sangassou orthohantavirus YP 009362095.1
Dobrava Belgrade orthohantavirus QQK53234.1
Hantaan orthohantavirus QFX79463.1
Tigray virus AOR07000.1
Jeju virus YP 009361845.1
Bowe virus YP 009361853.1
Cao Bang orthohantavirus ABR29826.2
Bruges virus YP 009361848.1
Longquan virus YP 009664868.1
Brno virus YP 010086165.1
Quezon virus YP 009361847.1
Xuan son mobatvirus QBO24548.1
Laibin mobatvirus QBO24551.1
Nova virus YP 009362032.1
Thottopalayam virus YP 001911124.1
Imjin virus YP 009362290.1
Gecko reptillovirus AVM87653.1
Bern perch virus QGM12349.1
Spikefish actinovirus AVM87662.1
Luposicya lupus actinovirus
Pygmy goby hantavirus
Goosefish actinovirus AVM87659.1
Batfish actinovirus AVM87656.1
Pleurosicya actinovirus
Trimma fangi actinovirus
Hagfish agnathovirus AVM87665
Mammantavirinae
Reptanta-
Actanta-
Aganta-
0.8
Salarius guttatus piscichuvirus
1 1000 2000 3000 4000 5000 6000 7000 8000 9000 10000 10801 nt
Nucleoprotein
Glycoprotein
mRNAcap
RdRp
S protein
M protein
L protein
Luposicya lupus actinovirus
1 1000 2000 3000 4000 5000 6000 7000 8000 9000 10000 11000 11533 nt
RdRp
Glycoprotein
Nucleoprotein
L segment
M segment
S segment
vector-borne
invertebrate-specific
amphibians
birds and reptiles
mammals
fish
● viruses identified


a
Picornaviridae
Chinese softshell turtle picornavirus 2 AVM87422.1
tremovirus B1 YP 009552829.1
tremovirus B2 AVM87424.1
Tremovirus A AVI10167.1
Rodent hepatovirus ALL35264
Shrew hepatovirus ALL35266.1
Bat hepatovirus SMG18520Minmav2014 YP 009505611.1
Guangdong fish caecilians picornavirus AVM87420.1
Beihai wrasse picornavirus AVM87595.1
Wenling jack mackerels picornavirus AVM87407.1
Wuhan sharpbelly picornavirus 3 AVM87405.1
Wuhan sharpbelly picornavirus 2 AVM87404.1
Wenling crossorhombus picornavirus AVM87451.1
Wenling banjofish picornavirus 1 AVM87440.1
Wenling banjofish picornavirus 2 AVM87594.1
Wenling chelidoperca picornavirus AVM87406.1
Blenniella picornavirus
Ampivirus A1 YP 009143521.1
Triplefin picornavirus
Exyrias picornavirus
Guangxi changeable lizard picornavirus 1 AVM87421.1
Guangxi chinese leopard gecko picornavirus AVM87414.1
Guangdong red banded snake picornavirus AVM87445.1
Fujian spotted paddle tail newt picornavirus AVM87413.1
Canine kobuvirus 1 AJP67528.1
Mouse kobuvirus M 5 USA 2010 YP 004782207.1
rafivirus B1 AVM87448.1
Tortoise rafivirus A AVM87458.1
Halichoeres marginatus picornavirus
Wenling triplecross lizardfish picornavirus AVM87408.1
Zebrafish picornavirus 1 AXQ03965.1
Neopomacentrus bankieri picornavirus
Chinese broad headed pond turtle picornavirus 1 AVM87423.1
Chinese softshell turtle picornavirus 1 AVM87457.1
Guangdong chinese water snake picornavirus AVM87444.1
Hainan oriental leaf toed gecko picornavirus AVM87415.1
Guangdong chinese water skink picornavirus AVM87416.1
tropivirus B1 AVM87410.1
rajidapivirus A1 AVM87417.1
Equine rhinitis B virus 1 NP 653077.1
Foot and mouth disease virus type O QNN94951.1
Hainan black spectacled toad picornavirus AVM87446.1
Zhejiang banded bullfrog picornavirus AVM87447.1
Human enterovirus YP 009259673.1
Rhinovirus C YP 001552411.1
Guangxi changeable lizard picornavirus 2 AVM87456.1
Wenling lepidotrigla picornavirus AVM87409.1
Wuhan carp picornavirus AVM87438
Beihai conger picornavirus AVM87437
Wenling hoplichthys picornavirus AVM87455
Wenling pleuronectiformes picornavirus AVM87596
Potamipivirus A QBA82298.1
Eel picornavirus 1 YP 008531322.1
Beihai pentapodus picornavirus AVM87593.1
Asterropteryx spinosa picornavirus
Asterropteryx semipunctatus picornavirus
Wenling scaldfish picornavirus 2 AVM87453.1
Wenling scaldfish picornavirus AVM87452
Wenling rattails picornavirus AVM87441
Pomacentrus moluccensis picornavirus
Wenling fish picornavirus 1 AVM87442.1
Wenling brown lined puffer picornavirus AVM87454
Yancheng osbecks grenadier anchovy picornavirus AVM87418
Falcon picornavirus AQW45177.1
Ljungan virus NP 647602.1
Rattus tanezumi parechovirus AWK02688
Parechovirus A NP 046804.1
Ferret parechovirus YP 009361997
Yili teratoscincus roborowskii picornavirus AVM87411
Duck hepatitis A virus 1 YP 007947990.1
Tiger flathead picornavirus FL8 QRV59633.1
Wenling thamnaconus septentrionalis picornavirus AVM87443
Red gurnard picornavirus FL19 QRV59644.1
Wuhan sharpbelly picornavirus AVM87439
Carp picornavirus YP 008992026
Wenling bighead beaked sandfish picornavirus AVM87449
Fathead minnow picornavirus AGZ84836
Bluegill picornavirus YP 006607894
Clownfish picornavirus QIZ64097.1
Guangdong spotted longbarbel catfish picornavirus AVM87450
Western African lungfish picornavirus AVM87419
Eastern red scorpionfish picornavirus AYU58609.1
0.4
Hepacivirus
Hepacivirus E AGJ71779.1
Hepacivirus F YP 009506358.1
Norway rat hepacivirus 1 ASM47312.1
Norway rat hepacivirus 1 YP 009109557.1
Norway rat hepacivirus 2 YP 009109558.1
Possum hepacivrus QHN63858.1
Hepacivirus N QJT41579.1
Hepacivirus N YP 009506363.1
Guereza hepacivirus YP 009325369.1
Hepacivirus B NP 042692.1
Hepacivirus L YP 009322132.1
Hepacivirus I YP 009506359.1
Sigmodontinae hepacivirus AWU67496.1
Canine hepacivirus AEC45560.1
Non primate hepacivirus YP 009506356.1
Equine hepacivirus AXB50128.1
Equine hepacivirus AXB50120.1
Equine hepacivirus AXB50121.1
Hepatitis C virus genotype 1 NP 671491.1
Hepatitis C virus genotype 2 YP 001469630.1
Hepatitis C virus genotype 3 YP 001469631.1
Bat hepacivirus YP 009325330.1
Hepacivirus K YP 009506361.1
Chinese broad headed pond turtle hepacivirus AVM87260.1
Chinese softshell turtle hepacivirus AVM87613.1
Hepacivirus J AGC52807.1
Rodent hepacivirus AGC52826.1
Duck hepacivirus QDF44087.1
Duck hepacivirus QDF44088.1
Duck hepacivirus QDF44086.1
Bald eagle hepacivirus QFR04963.1
Jogalong virus QHD25538.1
Guangxi chinese leopard gecko hepacivirus AVM87252.1
Yili teratoscincus roborowskii hepacivirus AVM87251.1
Guangxi houndshark hepacivirus AVM87256.1
Wenling shark virus ALL52891.1
Nanhai dogfish shark hepacivirus AVM87257.1
Nanhai ghost shark hepacivirus 2 AVM87258.1
Xiamen guitarfish hepacivirus AVM87253.1
Xiamen sepia Stingray hepacivirus AVM87254.1
Chaetodon aureofaciatus hepacivirus
Western African lungfish hepacivirus AVM87255.1
0.3
b
Chaetodon aureofaciatus hepacivirus
1 1000 2000 3000 4000 5000 6000 7000 8000 8826 nt
5'UTR 414 nt
Core C
Envelope E1
NS2
NS3
NS4B
NS4A
NS5B
3'UTR 117 nt
Halichoeres marginatus picornavirus
1 1000 2000 3000 4000 5000 6000 7000 8000 8344 nt
5'UTR 641 nt
L VP0 VP3 VP1 2A 2B 2C 3A 3C 3Dpol
3B
3'UTR 348 nt
poly(A)
Neopomacentrus bankieri picornavirus
1 1000 2000 3000 4000 5000 6000 7000 8000 8408 nt
5'UTR 621 nt
L VP0 VP3 VP1 2A 2B 2C 3A 3C 3Dpol
3B
3'UTR 100 nt
Asterropteryx spinosa picornavirus
1 1000 2000 3000 4000 5000 6000 7000 8000 8405 nt
5'UTR 524 nt
1AB 1C 1D 2A2 2B 2C 3A 3C 3Dpol
2A1 2A3
3B
3'UTR 324 nt
c
Astroviridae
Yellow striped sandperch astrovirus AVM87184.1
Red gurnard astrovirus QRV59643.1
Wenling righteye flounders astrovirus AVM87607.1
Beihai fish astrovirus 1 AVM87528.1
Wenling crossorhombus astrovirus 1 AVM87178.1
Big eyed perch astrovirus QRV59627.1
Goby astrovirus 2 Istigobius nigroocellatus
Goby astrovirus 2 Asterropteryx semipunctatus
Goby astrovirus 2 Istigobius decoratus
Goby astrovirus 2 Cabillus tongarevae
Goby astrovirus 1
Longspine snipefish astrovirus AVM87174.1
Goby astrovirus 3
Wenling plagiopsetta astrovirus AVM87176.1
Yancheng grey stingfish astrovirus AVM87186.1
Wenling rattails astrovirus 1 AVM87150.1
Dongbei arctic lamprey astrovirus 1 AVM87496.1
Bleniella astrovirus
Tiger astrovirus CS 2018 QFU14628.1
Feline astrovirus AGZ61937.1
Feline astrovirus 2 YP 008519301.1
Porcine astrovirus ADO30543.1
Human astrovirus 1 CAA81033.1
Human astrovirus NP 059443.2
Yak astrovirus AJF45222.1
Roe deer astrovirus QHW11790.1
Dromedary astrovirus YP 009162617.1
Rodent astrovirus APA19832.1
Bovine astrovirus QCF29936.1
Avian nephritis virus AXL64630.1
Pigeon avian nephritis virus AEQ33643.1
Chicken astrovirus NP 620617.2
Avian astrovirus AXG21615.1
Turkey astrovirus NP 853540.1
Hainan black spectacled toad astrovirus 2 AVM87164.1
Zhejiang chinese fire belly newt astrovirus AVM87170.1
Zhejiang gunthers frog astrovirus AVM87611.1
Wenling pterygotrigla hemisticta astrovirus AVM87158.1
Wenling japanese topeshark astrovirus AVM87192.1
Dongtou red stingray astrovirus AVM87143.1
Wuhan astro like virus AVM87125.1
Murray Darling rainbowfish astrovirus
Beihai mudskipper astro like virus AVM87121.1
Guangdong catfish astro like virus AVM87132.1
Wenling gobies fish astro like virus AVM87598.1
Western African lungfish astro like virus AVM87136.1
Chinese pond turtle astro like virus AVM87480.1
0.6
1 200 400 600 800 1000 1200 1400 1600 1800 2000 2200
amino acid %
Protease RdRp Capsid GAst1
Protease RdRp Capsid GAst2 C.t
Protease RdRp Capsid GAst2 I.d
Protease RdRp Capsid GAst2 A.s
Protease RdRp Capsid GAst2 I.n
🟥 amphibians
🟩 birds and reptiles
🟨 mammals
🟦 fish
● viruses identified


a
Parvoviridae
Dependoparvovirus
Tetraparvovirus
Erythroparvovirus
Artibeus jamaicensis parvovirus 1 AEX38015.1
Copiparvovirus
Bocaparvovirus
Chicken parvovirus ABU P1 ADD22705.1
Turkey parvovirus 260 ADD22713.1
Protoparvovirus
Amdoparvovirus
Istigobius ornatus parvovirus
Istigobius rigilus parvovirus
Paragryrops edita parvovirus
Ecsensius stictus parvovirus
Dinematichthys parvovirus
Chaphamaparvovirus
Tilapia parvovirus QVH36304.1
Luposicya lupus icthamaparvovirus
Syngnathid ichthamaparvovirus 1 YP 010087281.1
Aedes aegypti densovirus 2 YP 002854229.1
Aedes albopictus densovirus AYH52678.1
Penaeus stylirostris penstyldensovirus 1 YP 009508778.1
Scindoambidensovirus
Protoambidensovirus
Periplaneta fuliginosa densovirus NP 051020.1
Aquambidensovirus
Ambidensovirus
Blattella germanica densovirus 1 NP 874381.1
Iteradensovirus
Acheta domestica mini ambidensovirus YP 008658568.1
Parvovirinae
Hamaparvovirinae
Densovirinae
0.4


b
Ecsensius stictus parvovirus
1
500
1000
1500
2000
2500
3000
3500
4000
4286
NS1
VP1
Istigobius ornatus parvovirus
1
500
1000
1500
2000
2500
3000
3500
4000
4284
NS1
VP
VP


b
Circovirus
Rodent circovirus 2 ATP66724.1
Rodent circovirus 3 ATP66721.1
Rodent circovirus 1 ATP66716.1
Bamboo rat circovirus AXR86424.1
Canine circovirus QOW40790.1
Rodent circovirus 4 ATP66711.1
Whale circovirus QDZ59982.1
Bat circovirus 9 YP 009508628.1
Porcine circovirus 2 QCY50153.1
Bat circovirus 2 AGL09951.1
Bat circovirus 11 ASU92195.1
Bat circovirus 1 AGL09969.1
Mink circovirus AXU38623.1
Bat circovirus AFK85001.1
Bat circovirus ALG92529.1
Bat circovirus 5 YP 009506277.1
Bat circovirus 10 BBI18985.1
Bat circovirus 8 YP 009506280.1
Bat circovirus 7 YP 009506279.1
Bat circovirus 6 YP 009506278.1
Rodent circovirus 6 ATP66719.1
Porcine circovirus 3 QTQ94780.1
Rodent circovirus 5 ATP66709.1
Paguma larvata circovirus YP 009552846.1
Silurus glanis circovirus AEW70742.1
Anguilla anguilla circovirus APZ87904.1
Human stool NG13 YP 009506283.1
Barbel circovirus AEB60990.1
Werosea circovirus QGR26089.1
Abudefduf bengalensis circovirus
Duck circovirus QDC17553.1
Swan circovirus ABU48443.1
Goose circovirus QLM00364.1
BFDV QCY65211.1
Gull circovirus YP 803546.1
Pigeon circovirus AYC54547.1
Chimpanzee circovirus YP 009506281.1
Zebra finch circovirus YP 009134739.1
Starling circovirus ABB59613.1
Canary circovirus NP 573442.1
Raven circovirus YP 764455.1
Tick circovirus QTG40721.1
0.3


a
Andrias davidianus ranavirus AGV20578.1
Rana esculenta virus ASQ42903.1
Testudo hermanni ranavirus AJR29118.1
Common midwife toad virus YP 009508676.1
Ambystoma tigrinum virus YP 003817.1
Frog virus 3 AYP19508.1
Lumpfish ranavirus AZY88443.1
Short finned eel ranavirus YP 009259431.1
Epizootic haematopoietic necrosis virus QNN80876.1
European catfish virus AFJ52345.1
Doctor fish virus ACP19247.1
Koi ranavirus QYU76035.1
Halichoeres melanurus ranavirus
Mandarin fish ranavirus AYV88170.1
Largemouth bass virus ABA41591.1
Grouper iridovirus AAV91098.1
Singapore grouper iridovirus YP 164223.1
Lymphocystis disease virus 4 YP 010088005.1
Lymphocystis disease virus Sa AOC55226.1
Lymphocystis disease virus 2 BCB67532.1
Lymphocystis disease virus 1 NP 078724.1
Giant seaperch iridovirus AMM72761.1
Orange spotted grouper iridovirus AAX82331.1
Red seabream iridovirus QND75789.1
Rock bream iridovirus AGG37900.1
Large yellow croaker iridovirus QQA04016.1
Pompano iridovirus AZQ20770.1
Turbot reddish body iridovirus ADE34365.1
Infectious spleen and kidney necrosis virus CAZ73994.1
Scale drop disease virus QLI60745.1
European chub iridovirus QCQ67772.1
Wiseana iridescent virus YP 004732900.1
Invertebrate iridescent virus 25 CCA64444.1
Invertebrate iridovirus 22 YP 008357299.1
Invertebrate iridescent virus 3 Q196U0.1
Invertebrate iridescent virus 6 Q9QSK2.1
Armadillidium vulgare iridescent virus YP 009046615.1
Shrimp hemocyte iridescent virus YP 010084862.1
Erythrocytic necrosis virus QCF47437.1
Enneapterygius tutuilae iridovirus
Lacerta monticola erythrocytic virus ADR02798.1
Rhinella marina erythrocytic like virus QXT57784.1
Thamnophis saurītus erythrocytic virus ABR12534.1
Pogona vitticeps erythrocytic iridovirus AHG52038.1
100
94
100
99
100
100
100
87
90
98
100
94
88
99
100
100
100
100
100
92
100
100
100
94
99
93
87
100
93
94
0.3
Ranavirus
Santee-cooper ranavirus
Lymphocystivirus
Megalocytivirus
Chloriridovirus
Iridovirus
Decapodiridovirus
Alphairidovirinae
Betairidovirinae
b
Megalocytivirus
Chloriridovirus
Iridovirus
Decapodiridovirus
Lymphocystivirus
Ranavirus
ISKNV ADU25254.1
ECIV QCQ67796.1
SDDV QLI60730.1
IIV25 CCV02096.1
IIV9 O39163.1
IIV22 CCV01748.1
IIV3 Q197E6.1
IIV6 O55815.2
IIV31 YP 009046748.1
SHIV ATE87157.1
R. marina ENV-like virus QXT57809.1
ENV ALP86597.1
Enneapterygius tutuilae iridovirus
LCDV-1 ABN58782.1
LCDV-2 BCB67436.1
LCDV-4 YP 010087895.1
LCDV-Sa YP 009342130.1
SGIV YP 164167.1
Halichoeres melanurus ranavirus
LMBV QGH84114.1
ECV YP 006347613.1
EHNV YP 009182013.1
ATV YP 003785.1
FV3 YP 031669.1
CMTV YP 009508644.1
100
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100
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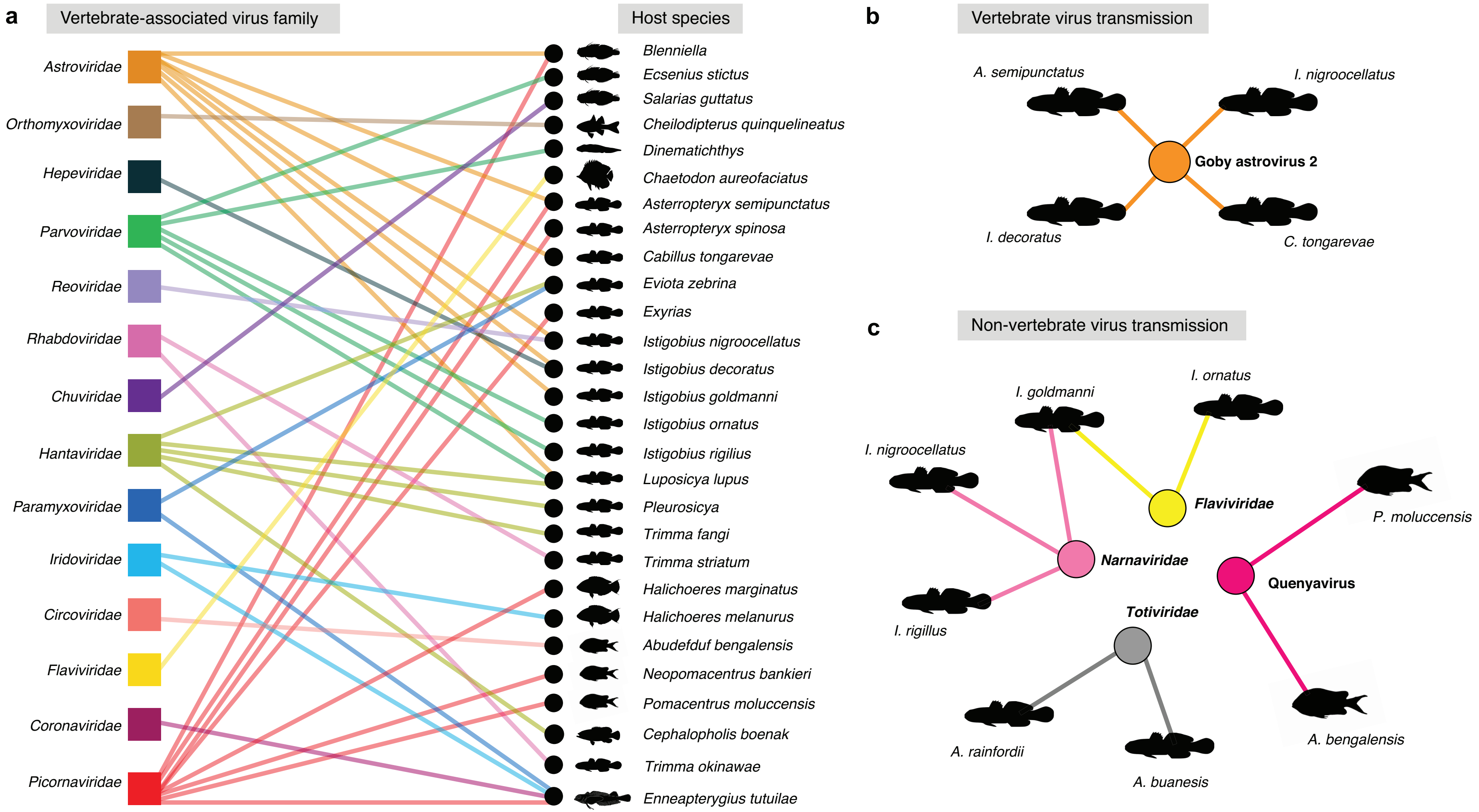
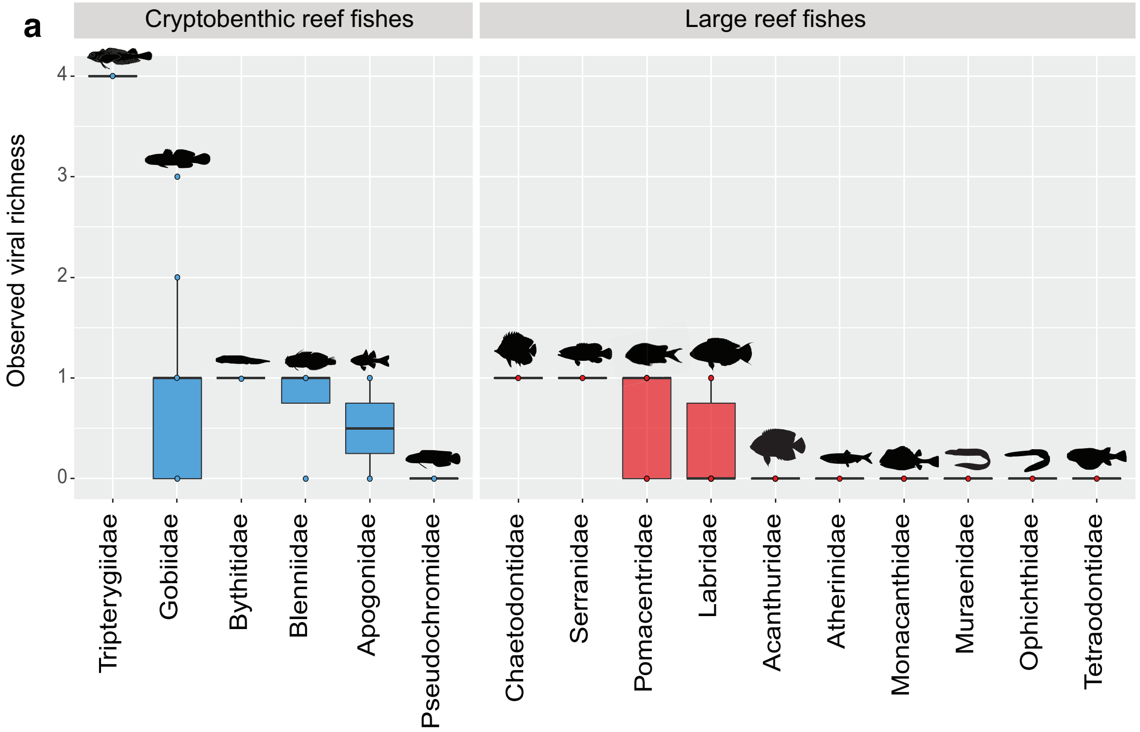
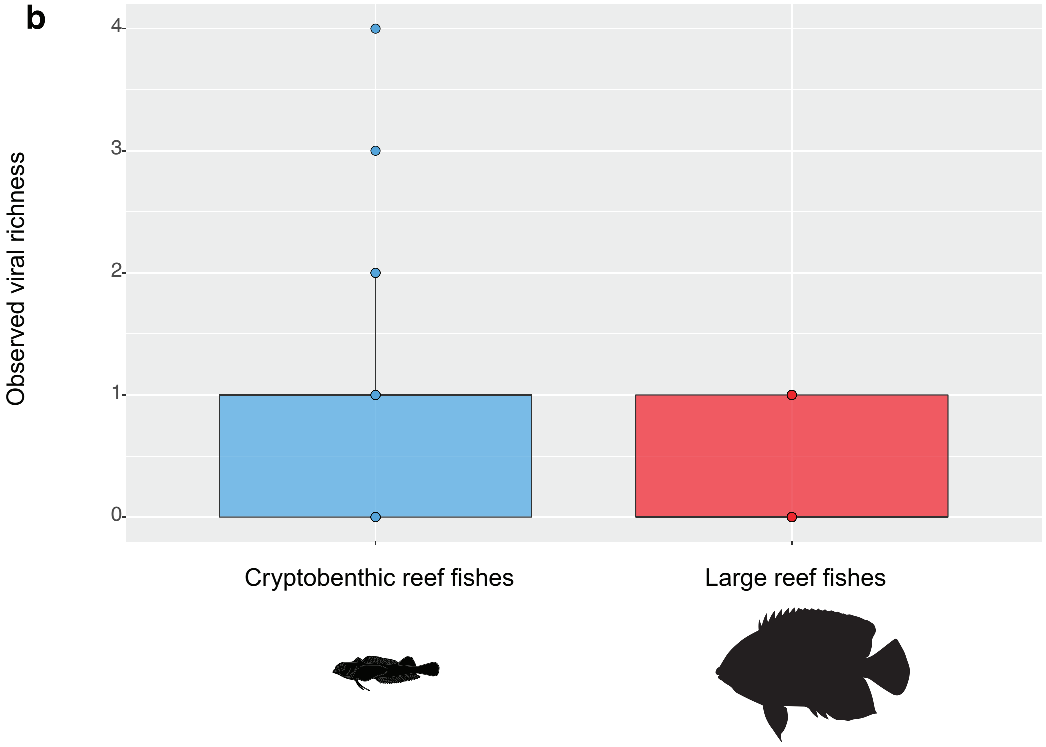
0.2


a
Vertebrate-associated virus family
Host species
Astroviridae
Orthomyxoviridae
Hepeviridae
Parvoviridae
Reoviridae
Rhabdoviridae
Chuviridae
Hantaviridae
Paramyxoviridae
Iridoviridae
Circoviridae
Flaviviridae
Coronaviridae
Picornaviridae
Blenniella
Ecsenius stictus
Salarias guttatus
Cheilodipterus quinquelineatus
Dinematichthys
Chaetodon aureofaciatus
Asterropteryx semipunctatus
Asterropteryx spinosa
Cabillus tongarevae
Eviota zebrina
Exyrias
Istigobius nigroocellatus
Istigobius decoratus
Istigobius goldmanni
Istigobius ornatus
Istigobius rigilius
Luposicya lupus
Pleurosicya
Trimma fangi
Trimma striatum
Halichoeres marginatus
Halichoeres melanurus
Abudefduf bengalensis
Neopomacentrus bankieri
Pomacentrus moluccensis
Cephalopholis boenak
Trimma okinawae
Enneapterygius tutuilae
b
Vertebrate virus transmission
A. semipunctatus
I. nigroocellatus
Goby astrovirus 2
I. decoratus
C. tongarevae
c
Non-vertebrate virus transmission
I. goldmanni
I. ornatus
I. nigroocellatus
Flaviviridae
P. moluccensis
Narnaviridae
Quenyavirus
I. rigillus
Totiviridae
A. rainfordii
A. buanesis
A. bengalensis

761

762


a
Cryptobenthic reef fishes
Large reef fishes
Observed viral richness
0
1
2
3
4
Tripterygiidae
Gobiidae
Bythitidae
Blenniidae
Apogonidae
Pseudochromidae
Chaetodontidae
Serranidae
Pomacentridae
Labridae
Acanthuridae
Atherinidae
Monacanthidae
Muraenidae
Ophichthidae
Tetraodontidae


b
Observed viral richness
0
1
2
3
4
Cryptobenthic reef fishes
Large reef fishes

763