Complete Genome Sequences and Pathogenicity Analysis of Two Red Sea Bream Iridoviruses Isolated from Cultured Fish in Korea

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Abstract: In Korea, red sea bream iridovirus (RSIV), especially subtype II, has been the main causative agent of red sea bream iridoviral disease since the 1990s. Herein, we report two Korean RSIV isolates with different subtypes based on the major capsid protein and adenosine triphosphatase genes: 17SbTy (RSIV mixed subtype I/II) from Japanese seabass (Lateolabrax japonicus) and 17RbGs (RSIV subtype II) from rock bream (Oplegnathus fasciatus). The complete genome sequences of 17SbTy and 17RbGs were 112,360 and 112,235 bp long, respectively (115 and 114 open reading frames [ORFs], respectively). Based on nucleotide sequence homology with sequences of representative RSIVs, 69 of 115 ORFs of 17SbTy were most closely related to subtype II (98.48–100% identity), and 46 were closely related to subtype I (98.77–100% identity). In comparison with RSIVs, 17SbTy and 17RbGs carried two insertion/deletion mutations (ORFs 014R and 102R on the basis of 17SbTy) in regions encoding functional proteins (a DNA-binding protein and a myristoylated membrane protein). Notably, survival rates differed significantly between 17SbTy-infected and 17RbGs-infected rock breams, indicating that the genomic characteristics and/or adaptations to their respective original hosts might influence pathogenicity. Thus, this study provides complete genome sequences and insights into the pathogenicity of two newly identified RSIV isolates classified as a mixed subtype I/II and subtype II.

Keywords: red sea bream iridoviral disease; red sea bream iridovirus; complete genome; insertion-deletion mutations; pathogenicity

1. Introduction

The virus species infectious spleen and kidney necrosis virus (ISKNV) (genus Megalocytivirus, family Iridoviridae) causes red sea bream iridoviral disease (RSIVD), which has a high mortality rate, in more than 30 susceptible freshwater and marine fish species [1]. According to the World Organization for Animal Health, it is a major fish disease [2]. Phylogenetic analyses based on major capsid protein (MCP) or adenosine triphosphatase (ATPase) genes have shown that the species can be classified into three major genotypes: red sea bream iridovirus (RSIV), ISKNV, and turbot reddish body iridovirus (TRBIV) [3]. The RSIV and ISKNV types can each be further categorized into two subtypes (I and II) [3]. Since the first outbreak of an RSIV-type infection among red sea breams (Pagrus major) in Japan in 1990 [4], RSIVs have been the predominant genotypes detected in marine fish in East Asian countries, including Korea [5–7]. In China, ISKNV and TRBIV types were first isolated from mandarin fish (Siniperca chuatsi) in 1998 [8,9] and from turbot (Scophthalmus maximus) in 2002, respectively [10]. In Korea, two genotypes of Megalocytivirus have been reported as endemic and have been taxonomically classified as RSIV [6,7,11] and TRBIV types [12]. Of note, RSIV subtype II has been identified as the major causative pathogen of endemic RSIVD in cultured marine fish in Korea [5].
Recently, an ISKNV/RSIV recombinant type was isolated from red sea bream (*Pagrus major*) in Taiwan, known as RSIV-Ku [13]. Its genome shares a high degree of homology with ISKNV-type viruses, except for specific nucleotide sequences that are closely related to RSIV-type viruses, implying that RSIV-Ku is a natural recombinant of ISKNV- and RSIV-type viruses [13]. Moreover, RSIV SB5-TY from a diseased Japanese seabass (*Lateolabrax japonicus*) in Korea is believed to be a genetic variant of RSIV-type viruses based on sequence difference in MCP and ankyrin repeat domains [5]. The emergence of genetic recombinants or variants of *Megalocytivirus* is a possibility, especially in RSIVD-endemic regions, such as Korea. Therefore, pathogenicity and complete genome sequence analyses of isolates in susceptible hosts are crucial for epidemiological studies, such as studies of source tracking and virus transmission.

In this study, we determined the complete genome sequences of two RSIVs identified in two cultured marine fish species (Japanese seabass and the rock bream (*Oplegnathus fasciatus*)) in Korea, and analyzed insertion/deletion mutations (InDels). In addition, to evaluate their pathogenicity, a challenge test was performed on rock breams, which are known to be highly susceptible to RSIV infection.

### 2. Materials and Methods

#### 2.1. Viral Culture

Primary cells derived from the fins of rock breams were grown in the L-15 medium supplemented with 10% fetal bovine serum (Performance Plus; Gibco, Grand Island, NY, USA) and 1% antibiotic-antimycotic solution (Gibco), as described by Lee et al. [14]. Briefly, caudal fin tissue was collected from juvenile rock bream (bodyweight, 5.4 ± 0.8 g), minced into small pieces (approximately 1 cm³), and then washed with phosphate-buffered saline (PBS). Cells treated with a 0.25% trypsin-EDTA solution (Gibco) at 20 °C for 1 h were filtered through a cell strainer (pore size: 70 µm; Falcon, NY, USA). Filtered cells were collected via centrifugation at 500 × g for 10 min at 4 °C and were then resuspended in the culture medium and seeded in 25 cm² tissue culture flasks. The primary cells were incubated at 25 °C, and the medium was replaced daily. The cells were subcultured (split ratio: 1:2) when monolayer cells reached >90% confluence.

Tissue samples (spleen and kidney, 50 mg) were collected from diseased Japanese seabass in Tongyeong and rock bream in Goseong in 2017. To identify RSIV infection, real-time polymerase chain reaction (PCR) [15] was carried out. Briefly, each 20 µL real-time PCR mixture contained 1 µL of DNA, which was extracted using the yesG™ Cell Tissue Mini Kit (GensGen, Busan, Korea), 200 nM each primer and probe (Table A1), 10 µL of the 2× HS Prime qPCR Premix (Genet Bio, Daejeon, Korea), 0.4 µL of the 50× ROX dye, and 5.6 µL of nuclease-free water. Amplification was performed using a StepOne Real-time PCR system (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95 °C for 10 min, followed by 40 cycles of 94 °C for 10 s (denaturation) and 60 °C for 35 s (annealing and extension). Tissue samples that were RSIV-positive, as determined by real-time PCR, were used as the viral inoculum.

Viral infection (each tissue homogenate, 10 mg/mL) was induced in 75 cm² tissue culture flasks (Greiner Bio-one, Frickenhausen, Germany) containing monolayers of primary cells at passage 15. RSIV-infected cells were propagated at 25 °C for 7 days in L-15 medium containing 5% fetal bovine serum and 1% antibiotic-antimycotic solution. After the appearance of the cytopathic effect (rounded cells; Figure A1), the infected cells were collected and subjected to three freeze-thaw cycles. After centrifugation at 500 × g for 10 min, the virus-containing supernatants were collected and stored at −80 °C until use. The cultured RSIVs were designated as 17SbTy and 17RbGs based on the sampling year, common name of the fish, and sampling site (i.e., 2017, Japanese seabass, Tongyeong and 2017, rock bream, Goseong).
2.2. Phylogenetic Analysis

For genotyping, genes encoding MCP and ATPase were amplified with the primers listed in Table A1 and sequenced using an ABI 3730XL DNA Analyzer (Applied Biosystems, CA, USA) by Bionics Co. (Seoul, Korea). Then, the MCP and ATPase gene sequences were quality-checked by base-calling using ChromasPro (ver. 1.7.5; Technelysium, Tewantin, Australia). Each sequence was identified using Nucleotide Basic Local Alignment Search Tool (BLASTn; https://blast.ncbi.nlm.nih.gov/Blast.cgi). Contigs were generated using the ChromasPro and aligned using the ClustalW algorithm in BioEdit (ver. 7.2.5). Phylogenetic trees were generated by the maximum likelihood method via the Kimura two-parameter (K2P) model with a gamma-distribution and invariant sites (K2P + G + I) using MEGA (ver. 11). The MCP and ATPase genes of epizootic haematopoietic necrosis virus (GenBank accession no. FJ433873) were used as outgroup in the phylogenetic analyses. Support for specific genotypes of the RSIVs were determined with 1000 bootstrap replicates (≥70%).

2.3. Determination of Complete Genome Sequences by Next-Generation Sequencing

Viral nucleic acids were extracted from gradient-purified virions using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany). Next, 1 µg of the extracted DNA was employed to construct sequencing libraries using the QIAseq FX Single Cell DNA Library Kit (Qiagen). Sequencing libraries of 17SbTy and 17RbGs were constructed, with average lengths of 648 bp and 559 bp, respectively. The quality of the libraries was evaluated using the Agilent High Sensitivity D 5000 ScreenTape System (Agilent Scientific, CA, USA), and the quantity was determined using a Light Cycler Real-time PCR system (Roche, Mannheim, Germany). The high-quality libraries (300–600 bp) were sequenced (pair-end sequencing, 2 × 150 bp) by G&C Bio Co. (Daejeon, Korea) on the Illumina HiSeq platform (Illumina, San Diego, CA, USA). To assess the quality of the sequence data, FastQC [16] and MultiQC [17] were employed. Low-quality sequences (base quality <20) and the Illumina universal adapters were trimmed from the reads using Trim-Galore software (ver. 0.6.1; https://www.bioinformatics.babraham.ac.uk/projects/trim_galore, accessed on 21 June 2020). High-quality reads were mapped and assembled into contigs using gMapper (ver. 2.8). Nucleotide errors in the reads were corrected with the Illumina sequencing data using Proovread [18].

2.4. Complete Genome Sequence Analysis

2.4.1. Construction of a Circular Map

The composition, structure, and homologous regions of the genomic DNA were analyzed and circular map was generated using the cgview comparison tool [19]. Coding regions were classified according to a clusters of orthologous groups (COG) analysis. To determine COG categories, a comparative analysis was performed based on the proteins encoded in 43 complete genomes representing 30 major phylogenetic lineages described by Tatusov et al. (1997 and 2001) [20,21] using the COG program on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/COG, accessed on 12 April 2021). The genes were categorized in accordance with their functional annotations.

2.4.2. Gene Annotation and Open Reading Frame (ORF) Analysis

To identify putative ORFs, the full-length genome sequences of 17SbTy and 17RbGs were annotated using Prokka (ver. 2.1). ORFs were predicted using NCBI ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder, accessed on 15 April 2021), and then the amino acid sequences of the putative ORFs were checked by Protein BLAST (BLASTp; https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 16 April 2021). Nucleotide sequence homologies of the putative ORFs of 17SbTy with those of 17RbGs and representative megalocytiviruses, i.e., Ehime-1 (GenBank accession no. AB104413; RSIV subtype I and the ancestral strain of RSIVD) [22], ISKNV (GenBank accession no. AF371960) [8], and TRBIV (GenBank accession no. GQ273492) [23] were determined using BLAST (https://blast.ncbi.nlm.nih.
Furthermore, to analyze genetic relatedness among viruses in Iridoviridae, amino acid sequences of 26 conserved genes [24,25] were retrieved from NCBI GenBank. A phylogenetic tree based on the deduced amino acid sequences of 26 concatenated genes was constructed by the maximum likelihood method with the LG model and gamma-distributed rates with invariant sites (LG + G4 + I) [26] using MEGA (ver. 11.). Support for specific genera of iridoviruses was determined with 1000 bootstrap replicates (≥70%).

2.4.3. Analysis of InDels in RSIVs
To identify InDels in coding regions, the nucleotide sequences of 17SbTy and 17RbGs were compared with those of the ancestral RSIV (Ehime-1 isolated from a red sea bream in Japan in 1990; RSIV subtype I) [22] and an RSIV genome previously reported in Korea (RBIV-KOR-TY1 isolate found in a rock bream in 2000; RSIV subtype II; GenBank accession no. AY532606) [27]. Genomic sequences coding for functional proteins were aligned using the ClustalW algorithm in BioEdit (ver. 7.2.5), and InDels in the coding regions were detected.

2.5. Pathogenicity of the Two RSIV Isolates in the Rock Bream
Healthy rock bream (body length: 8.75 ± 1.95 [mean ± SD]; body weight: 6.79 ± 4.16 g) were obtained from an aquaculture farm in Geoje, Korea, after confirming that they were RSIV-free by PCR, as described in the Manual of Diagnostic Tests for Aquatic Animals for RSIVD [2,28], and by real-time PCR [15] (Table A1). The fish were acclimated in a 500 L aqua tank at 25.0 ± 0.5 °C for 2 weeks and were fed a commercial diet once daily. Each day, 50% of rearing water was replaced with temperature-adjusted (25 °C) fresh seawater. To prepare a viral inoculum, viral genome copy numbers of cultured 17SbTy and 17RbGs were determined by real-time PCR [15] with a standard curve constructed using the serial dilutions of a plasmid containing the MCP gene of 17RbGs. In a challenge test, each fish group was intraperitoneally injected with 0.1 mL of 17SbTy (n = 18; 10^4 viral genome copies per fish), 17RbGs (n = 18; 10^4 viral genome copies per fish), or PBS (n = 18; a negative control). After the viral challenge, the fish were maintained at 25.0 ± 0.5 °C in 30 L aqua tanks for 3 weeks, with 50% of water exchanged daily. DNA was extracted from the spleen tissue of dead fish, and RSIV infection was confirmed by real-time PCR. Survival rates were compared among the experimental groups by the log-rank test using GraphPad Prism (ver. 8.4.3.). Statistical significance was set at p-values < 0.05. Furthermore, the nucleotide sequences around four InDels in coding regions (ORFs 014R, 053R, 054R, and 102R on the basis of the 17SbTy isolate) were compared between cell-cultured isolates and viruses from RSIV-infected fish. DNA was extracted from three fish in each experimental group, and PCRs were carried out with each specific primer set (Table A1). Each 20 µL PCR mixture contained 1 µL of DNA (extracted using the yesG™ Cell Tissue Mini Kit;_colsGensGen, Korea), 500 nM each primer, 10 µL of the 2× ExPrime Taq Premix (Genet Bio, Daejeon, Korea), and 7 µL of nuclease-free water. Amplification was performed on an Alpha Cycler 1 machine (PCRmax, Staffordshire, UK) under the following conditions: 95 °C for 10 min, followed by 35 cycles at 94 °C for 30 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 60 s (extension). The amplicons were sequenced using the ABI 3730XL DNA Analyzer (Applied Biosystems) by Bionics Co. Contigs were assembled using ChromasPro (ver. 1.7.5) and aligned using the ClustalW algorithm in BioEdit (ver. 7.2.5).

3. Results & Discussion
The complete genome sequences of two RSIV isolates collected from representative fish susceptible to RSIVD (17SbTy from a Japanese seabass and 17RbGs from a rock bream) in Korea were investigated, and a comparative analysis of the pathogenicity of the isolates was performed. A phylogeny based on genes encoding MCP and ATPase revealed that 17RbGs belongs to RSIV subtype II, which has been the predominant genotype in marine fish in Korea since the 1990s [5]. Notably, 17SbTy grouped with subtype I or II of RSIV in
phylogenetic analyses based on MCP or ATPase, respectively (Figure 1). Comparisons of 17SbTy with Ehime-1 (ancestral RSIV subtype I) and 17RbGs (RSIV subtype II), showed 99.63% and 98.24% identity for the MCP gene and 99.03% and 100% identity for the ATPase gene, respectively. Golden mandarin fish iridovirus, an RSIV subtype I reported in Korea in 2016 [29], shares 99.9% sequence homology with Ehime-1 in both the MCP and ATPase genes. Unlike golden mandarin fish iridovirus, 17SbTy was classified as a mixed RSIV subtype (subtype I/II).

![Figure 1](image-url)

Figure 1. Phylogenetic trees based on the complete nucleotide sequences of the (a) major capsid protein gene (MCP; 1362 bp) and (b) adenosine triphosphatase gene (ATPase; 721 bp) of two red sea bream iridovirus (RSIV) isolates (17SbTy and 17RbGs) collected from cultured fish in Korea. The phylogenetic trees were constructed using the maximum-likelihood method in MEGA (ver. 11). Bootstrap values were obtained from 1000 replicates, and the scale bar represents 0.05 nucleotide substitutions per site. The two RSIV isolates (17SbTy and 17RbGs) from this study are highlighted in bold and red color.
The complete genomes of 17SbTy (122,360 bp, GenBank accession no. OK042108), and 17RbGs (122,235 bp, GenBank accession no. OK042109) were similar in size to the genomes of most representative megalocytiviruses, RSIV (Ehime-1; 112,415 bp), ISKNV (112,080 bp), and TRBIV (110,104 bp), except for scale drop disease virus (GF_MU1; GenBank accession no. MT521409; 131,129 bp). The sequences were circularly permuted and assembled into a circular form, similar to most *Megalocytivirus* genomes (Figure 2). In addition, the G+C contents of the 17SbTy and 17RbGs genomes were 53.28% and 53.13%, respectively.

![Figure 2](image_url)

**Figure 2.** Circular genome maps of (a) 17SbTy (112,360 bp) and (b) 17RbGs (112,235 bp). From the inner ring to the outer ring, the first and eighth circles represented the genomic length (kbp) and nucleotide positions, respectively. The second and third circles show the G+C skew and G+C content, respectively. The fourth and fifth circles represent rRNA and tRNA genes on forward and reverse strands, respectively. The sixth and seventh circles indicate the functional categories of the protein-coding sequences in terms of clusters of orthologous groups (COG) on the forward and reverse strands, respectively.
In total, 115 and 114 putative ORFs were predicted in 17SbTy and 17RbGs, respectively (Table A2). The putative ORFs of 17SbTy (total length 104,868 bp, 93.3% of the genome) ranged in size from 111 to 3849 bp and encodes 36 to 1282 amino acid residues. Of the 115 ORFs, 70 were located on the sense (R) strand, and 45 were on the anti-sense (L) strand (Table A2). The putative ORFs of 17RbGs (total length 105,003 bp, 93.6% of genome) ranged in size from 111 to 4155 bp, encoding for 36 to 1384 amino acid residues. Of the 114 ORFs, 68 were located on the R strand and 46 were on the L strand. Of the annotated ORFs in 17SbTy (115 ORFs) and 17RbGs (114 ORFs), 43 (37.7%) and 42 (36.8%), respectively, could be assigned to a predicted structure and/or functional protein. The complete nucleotide sequences of 17SbTy and 17RbGs were closely related to rock bream iridovirus-C1 (RBIV-C1, GenBank accession no. KC244182) with identities of 99.56% and 99.69%, respectively. A comparison of the complete nucleotide sequences of 17SbTy and 17RbGs revealed 97.69% identity. In the ORFs of 17SbTy, nucleotide sequence identities were 87.99–100% with Ehime-1 (RSIV subtype I), 88.22–100% with 17RbGs (RSIV subtype II), 86.07–97.58% with ISKNV, and 80.25–99.66% with TRBIV (Table A2). Notably, the best matches for the nucleotide sequences of the 115 ORFs of 17SbTy were RSIV subtype II viruses (97.48–100% identity for 69 ORFs) and RSIV subtype I viruses (98.77–100% identity for 46 ORFs).

A total of 20 protein-coding genes in both 17SbTy (17.39%; 20/115 ORFs) and 17RbGs (17.54%; 20/114 ORFs) were annotated in the COG database, and these genes were assigned to nine functional groups (Table A3): (i) amino acid transport and metabolism; (ii) nucleotide transport and metabolism; (iii) translation, ribosomal structure, and biogenesis; (iv) transcription; (v) replication, recombination, and repair; (vi) signal transduction mechanisms; (vii) mobilome, prophages, transposons; (viii) general function prediction only; and (ix) function unknown. The nine functional groups identified in both 17SbTy and 17RbGs belonged to four major categories: metabolism, information storage and processing, cellular processes, and poorly characterized. Furthermore, both 17SbTy and 17RbGs harbored the 26 conserved genes that were shared by all members of the family Iridoviridae, including genes encoding enzymes and structural proteins involved in viral replication, transcriptional regulation, protein modification, and host-pathogen interactions [24,25]. The ORFs corresponding to these 26 core genes are listed in Table A4. A phylogenetic tree based on the concatenated amino acid sequences of the 26 conserved genes revealed that 17SbTy and 17RbGs can be assigned to the genus Megalocytivirus. Furthermore, 17SbTy was closely related to Ehime-1 (Figure 3).

![Figure 3. Phylogenetic trees based on the deduced amino acid sequences of the 26 concatenated genes conserved for members of the family Iridoviridae. The tree was constructed by the maximum-likelihood method under the LG model and gamma-distributed rates with invariant sites (LG + G4 + I) in MEGA (ver. 11). The two RSIV isolates (17SbTy and 17RbGs) from this study are highlighted in bold and red color.](image-url)
As described by Eaton et al. (2007) [24], several annotated genes within the family *Iridoviridae* contain frameshift mutations. InDels are a type of frameshift mutation that can affect the translation of a functional protein. The complete genome of 17SbTy showed 133 InDels when compared to the Ehime-1 and 17RbGs genomes (data not shown). Notably, although the genomes of several RSIVs, including 17SbTy, Ehime-1, and RBIV, encode two functional proteins—an mRNA-capping enzyme (ORF 012R, positions 10,693–12,165 in the 17SbTy genome) and a putative NTPase I (ORF 013R, positions 12,205–14,853 in the 17SbTy genome)—17RbGs possesses only a single functional protein (ORF 012R, positions 10,690–14,844 in the 17RbGs genome; Figure 4). A frameshift mutation caused by a short InDel [a 6 bp deletion, including a stop codon (TGA) and an intergenic codon (CCT)] explained the difference in the total number of ORFs between 17RbGs (n = 114) and 17SbTy (n = 115; Figure 4 and Table A2).

**Figure 4.** Schematic representation of a deletion of the termination codon in ORF 012R of 17RbGs causing a frameshift mutation. The aligned sequences are genomes of 17SbTy, 17RbGs, and two representative RSIVs (Ehime-1 [RSIV subtype I] and RBIV-KOR-TY1 [RSIV subtype II]). The nucleotide sequences surrounded by blue dashed lines are coding regions. The termination and start codons are shown in red, and the deleted sequences in the intergenic region are highlighted in blue.

Among the InDel regions in 17SbTy identified in comparisons with the Ehime-1 and 17RbGs genomes, 18 regions contained >10 bp mutations, and only four InDels were identified in coding regions (ORFs 014R, 053R, 054R, and 102R in 17SbTy). Although two ORFs encode known functional proteins (ORF 014R, which is involved in DNA binding, and ORF 102R, which is a myristoylated membrane protein; Figure 5a,d), two additional ORFs (ORF 053R and 054R) have not yet been functionally characterized (Figure 5b,c). Of
the InDels found in the ORFs known to encode functional proteins, a 27 bp deletion in a DNA-binding protein with an FtsK-like domain was identified in 17SbTy (ORF 014R), in 17RbGs (ORF 013R), and RBIV-KOR-TY 1 (ORF 058L), but not in Ehime-1 (ORF 077R; Figure 5a). The FtsK-like domain in spotted knifejaw iridovirus (an RSIV-type) [30] participates in host immune evasion by inhibiting transcriptional activities of NF-κB and INF-γ, indicating that the deleted sequences in the gene encoding a DNA-binding protein might affect viral replication and/or pathogenicity. Furthermore, ORF 102R of 17SbTy, located in the same region as ORF 575R in Ehime-1, encodes a myristoylated membrane protein, known as a viral envelope membrane protein of iridovirus, and its function may be conserved throughout the family Iridoviridae [31]. Thus, an InDel in the coding region of a viral membrane protein (a 30 bp deletion in ORF 101R of 17RbGs) may alter the regulation of viral entry into host cells at the onset of the infection cycle.

Figure 5. Cont.
No rock bream infected with 17RbGs survived 15 days post-injection, whereas 27.8% (5/18) of the 17SbTy-infected rock bream survived 21 days post-injection (Figure 6). The difference in survival rates between the 17SbTy- and 17RbGs-infected rock breams was significant (log-rank test, \( p < 0.001 \)). The nucleotide sequences of the four InDel regions (ORFs 014R, 053R, 054R and 102R on the basis of the 17SbTy isolate) were identical in the cell-cultured isolates and viruses from dead fish (Figure A2). These results suggest that several of the genetic factors identified in the genomic analysis, including the InDels in coding regions, may influence virulence. Another noteworthy observation is that the apparent difference in virulence between the RSIV isolates may be due to adaptations to their respective original hosts (Japanese seabass for 17SbTy and rock bream for 17RbGs). Further molecular epidemiological studies, including analyses of RSIV replication and pathogenic determinants, are needed to elucidate the transmission of RSIV.

**Figure 5.** Schematic representation of insertion/deletion mutations (InDels) (>10 bp) in the coding regions as (a) ORF 014R, (b) ORF 053R, (c) ORF 054R and (d) ORF 102R based on the 17SbTy when compared with the genomes of 17RbGs and two representative RSIVs (Ehime-1 [RSIV subtype I] and RBIV-KOR-TY1 [RSIV subtype II]). Numbers indicate the positions of the InDels in the genome; white bars represent genome fragments, black bars denote insertions, and gray bars represent deletions.

**Figure 6.** Survival rates (%) of rock breams after intraperitoneal injection with the two RSIV isolates (either 17SbTy or 17RbGs, \( 10^4 \) genome copies per fish). Statistical analysis was performed by the log-rank test (\( ^* p < 0.05 \)).

### 4. Conclusions

Phylogenetic trees based on genes encoding MCP and ATPase revealed that two RSIV isolates (17SbTy from a Japanese seabass and 17RbGs from a rock bream) can be
classified as RSIV mixed subtype I/II and subtype II, respectively. According to complete genome analysis, these isolates (17SbTy, 112,360 bp; 17RbGs, 112,360 bp) have the genomic organization, G+C content, coding capacity, and conserved core genes typical of the species ISKNV. Notably, the best matches for the nucleotide sequences in the 115 ORFs of 17SbTy were RSIV subtype II (69 matching ORFs; 97.48–100% identity) and RSIV subtype I (46 matching ORFs; 98.77–100% identity). In comparison with RSIVs, 17SbTy and 17RbGs had InDels in ORFs 014R and 102R (based on the 17SbTy genome), encoding a DNA-binding protein and myristoylated membrane protein, respectively. The survival rates of rock breams infected with these isolates differed significantly, suggesting that the genomic differences between these viruses and/or adaptations to their respective original hosts may have altered their pathogenicity. Thus, the complete genome sequences of these RSIV isolates provide basic information for molecular epidemiology and are expected to provide insight into viral replication in general and the pathogenicity of these viruses in susceptible hosts in particular.

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Institutional Review Board Statement: Animal experiment was performed with the approval of the Animal Ethics Committee of the Pukyong National University (Permission No. PKNUIACUC-2021-33).

Informed Consent Statement: Not applicable.

Data Availability Statement: Publicly available datasets were analyzed in this study. The full genome sequences generated in this study can be found in the National Center for Biotechnology Information (NCBI) GenBank (Accession No. OK042108 and OK042109).

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1, PCR primers used in this study; Table A2, Predicted ORFs based on a comparison of isolates 17SbTy to 17RbGs and representative ISKNVs; Table A3, The coding sequences (CDSs) determined via COG classification of 17SbTy and 17RbGs in four functional categories; Table A4, ORF locations of the 26 conserved core genes conserved in the family Iridoviridae. Figure A1, Cytopathic effects (CPEs) in rock bream fin cells under the influence of a tissue homogenate from (A) an RSIV (17SbTy)-infected Japanese seabass and (B) an RSIV (17RbGs)-infected rock bream; Figure A2, Comparison of nucleotide sequences covering the four InDels in coding regions (ORFs 014R, 053R, 054R and 102R on the basis of the 17SbTy isolate) between the cell-cultured isolates and viruses from RSIV-infected rock breams.
| Primer          | Target                  | Sequence (5'-3')                                                                                                                                                                                                 | Reference |
|----------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| MCP 1F         | Major capsid protein    | ATG TCT GCR ATC TCA GGT GC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------| [29]      |
| MCP 300R       |                         | CCA GCG RAT GTA GCT GTT CTC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| MCP 600F       |                         | CAA GCT GCG GCG CTG GGA GG                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| MCP 800R       |                         | GGC GCC ACC TGR CAC TGY TC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| MCP 1015F      |                         | CTC ATT TTA CGA GAA CAC CC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| MCP 1362R      |                         | TYA CAG GAT AGG GAA GCC TGC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| MCP 1F         | ATPase                  | ATG GAA ATC MAA GAR TTG TCC YTG                                                                                                                                                                                                                                     |           |
| ATPase 218R    |                         | CAG TTR GGC AAY AGC TTG CT                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| ATPase 529F    |                         | GGG GGY AAC ATA CCM AAG C                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| ATPase 721R    |                         | CTT GCT TAC RCC ACG CCA G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| RSV 1094F      | Major capsid protein    | CCA GCA TGC CTG AGA TGG A                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------| [15]      |
| RSV 1221R      |                         | GTC CGA CAC CTT ACA TGA CAG G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| RSV 1177 probe |                         | FAM-TAC GGC CGC CTG TCC AAC G-BHQ1                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 1-F            | Pst I fragment          | CTC AAG CAC TCT GCC TCA TC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------| [28]      |
| 1-R            |                         | GCA CCA ACA CAT CTC CTA TC                                                                                                                                                                                                                                           |           |
| 4-F            | DNA polymerase gene     | CGG GGGCAA TGA CGA CTA CA                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 4-R            |                         | CCC CTT GTG CCT TTG CTA GC                                                                                                                                                                                                                                           |           |
| 14R-1F         |                         | ATG AAG AAA TTT GAT TTT TGY RKA TGT C                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-260R       |                         | TCA TCC TCA GAG TCG CNG                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-430F       |                         | GCT CAG TGG TCC AAG ATG CC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-999R       |                         | ATG CGT ATC ACA GTA CGC G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-848F       |                         | CCA TAG AGG ATA ACA CGC C                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-1202F      |                         | ACG AGC GGG ACC TAT GCA A                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-1841R      |                         | TAC ATG GCC TCA ACG ACT G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-1620F      |                         | AGA ACT GGA GGA CTC ACA                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-2011F      |                         | CAC GTG GAA CTG CGC ATC T                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-2630F      |                         | GTG AGG TAT GTT TCC TGG TGT                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-2309F      |                         | GTA TGA TCG AGG AGA TCG CA                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-2740F      |                         | GAA CAC CGA GAG AGT GGA GAT G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-3241R      |                         | AGT AGT CTA CCA CAG TTG C                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-3190F      |                         | TGT CAG CTA AAG GTC AGT GAT G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-3494F      |                         | GTA TGT TGG ACT ACA TCG ACC C                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 14R-3849R      |                         | TCA TTG ATT TTC ATT YAC ACC MAG                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 53R-1F         | ORF 053R *              | ATG CCA CAG CGY ATT ATC TTC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 53R-192R       |                         | CTA AGC CGC CCT GCC TGG                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 53R-SB-210R    |                         | CTA AGC AGC CCT GCC GGG                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| ORF54-1F       | ORF 054R *              | ATG CCG ACT ACC AAA CAC A                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| ORF54-348R     |                         | TCA AAA CTC AAA GCC GCC G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 102R-1F        |                         | ATG AGT GCA ATA AAG GCA AAT G                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 102R-222R      |                         | GTC CCG CAC GCC GTT GTT                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 102R-424F      | ORF 102R *              | GCG GTG CAT GCA ATG TAT                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 102R-797F      |                         | GCA ATG TCT GTT AGG TGG C                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |
| 102R-1071R     |                         | CTA GGC AAA TGC AGC AAT AAC                                                                -----------------------------------------------------------------------------------------------------------------------------------------------------------------|           |

* Open reading frame on the basis of 17SbTy isolate.
Table A2. Predicted ORFs based on a comparison of isolates 17SbTy to 17RbGs and representative ISKNVs.

| Gene ID 17SbTy | Position CDS (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------------|-------------------|------------------------------------|--------------------|-------------------|-------------------------------|-------------------------------|-----------------------------|
| ORF 001R      | 111,584 2196 2973 | hypothetical protein               | RSIV                 RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV PIV2016 PIV2014a PIV2010 LYCIV RSIV Ehime-1 | 99.70% Identity (%) ORF 001R 99.70% Identity (%) ORF 001R 98.18% Identity (%) 76L 93.44% Identity (%) 69L 92.91% |
| ORF 002R      | 2198 2467 270    | hypothetical protein               | RSIV                 PIV2016 PIV2014a PIV2010 LYCIV RSIV Ehime-1 | 100.00% Identity (%) ORF 002R 96.67% Identity (%) ORF 002R 100.00% Identity (%) 75L 91.30% Identity (%) 68L 87.26% |
| ORF 003L      | 2476 3495 1020   | hypothetical protein               | RSIV                 PIV2016 PIV2014a PIV2010 LYCIV RSIV Ehime-1 | 100.00% Identity (%) ORF 003L 98.53% Identity (%) ORF 003L 100.00% Identity (%) 74R 93.63% Identity (%) 67R 93.94% |
| ORF 004L      | 3544 4032 489    | hypothetical protein               | RSIV                 PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV | 100.00% Identity (%) ORF 004L 95.09% Identity (%) ORF 004L 100.00% Identity (%) 73R 90.24% Identity (%) 66R 84.72% |
| ORF 005R      | 4015 5625 1611   | hypothetical protein               | RSIV                 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% Identity (%) ORF 005R 98.08% Identity (%) ORF 005R 100.00% Identity (%) 71L 93.61% Identity (%) 65L 93.42% |
| ORF 006L      | 5528 6043 516    | hypothetical protein               | RSIV                 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% Identity (%) ORF 006L 97.29% Identity (%) ORF 006L 100.00% Identity (%) 70L 95.20% - - |
| ORF 007R      | 6065 6796 732    | hypothetical protein               | RSIV                 PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% Identity (%) ORF 007R 96.86% Identity (%) ORF 007R 100.00% Identity (%) 69L 86.07% Identity (%) 64L - |
| ORF 008R      | 6808 8241 1434   | hypothetical protein               | RSIV                 PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% Identity (%) ORF 008R 97.63% Identity (%) ORF 008R 100.00% Identity (%) 68L 93.58% Identity (%) 63L 88.95% |
| ORF 009R      | 8192 8860 669    | hypothetical protein               | RSIV                 PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV Zhoushan | 100.00% Identity (%) ORF 009R 98.06% Identity (%) ORF 009R 98.80% Identity (%) 67L 90.69% Identity (%) 62L 91.68% |
| ORF 010R      | 9087 10,130 1044 | hypothetical protein               | RSIV                 PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% Identity (%) ORF 010R 99.81% Identity (%) ORF 010R 99.46% Identity (%) 66L 92.82% Identity (%) 61L 92.53% |
| ORF 011R      | 10,181 10,651 471 | hypothetical protein, RING-finger-containing E3 ubiquitin ligase | RSIV                 PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% Identity (%) ORF 011R 100.00% Identity (%) ORF 011R 98.51% Identity (%) 65L 91.30% Identity (%) 60L 89.17% |
| Gene ID 175bTy | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|----------------|----------|---------------|-------------------------------------|---------------------|-------------------|--------------------------------|----------------------------|----------------------------|
| ORF 012R       | 10,693   | 12,165        | 1473 mRNA capping enzyme            | RSIV                 | 100.00% ORF 012R  | ORF 012R 99.93% MCE 97.49%     | ORF 012R 93.36% 59L 93.28%   |                          |
| ORF 013R       | 12,205   | 14,853        | 2649 putative NTPase I DNA-binding protein putative replication factor and/or DNA binding-packing | RSIV                 | 99.96% - - NTPase 97.92% 63L 93.36% 58L 93.42% |                          |                          |                          |
| ORF 014R       | 15,174   | 19,067        | 3849 DNA-binding protein            | RSIV                 | 100.00% ORF 013R  | ORF 013R 99.48% ORF 077R 96.78% | ORF 013R 93.36% 62L 91.81% 57L 93.08% |                          |
| ORF 015R       | 19,064   | 19,870        | 807 mRNA capping enzyme            | RSIV                 | 100.00% ORF 014R  | ORF 014R 92.94% ORF 092R 97.65% | ORF 014R 93.80% 61L 93.06% 56L 93.06% |                          |
| ORF 016R       | 19,934   | 20,446        | 513 putative NTPase I DNA-binding protein putative replication factor and/or DNA binding-packing | RSIV                 | 100.00% ORF 015R  | ORF 015R 89.35% ORF 097R 96.30% | ORF 015R 92.84% 59L 92.84% 55L 88.95% |                          |
| ORF 017R       | 20,918   | 21,178        | 261 putative NTPase I DNA-binding protein putative replication factor and/or DNA binding-packing | RSIV                 | 100.00% ORF 016R  | ORF 016R 95.40% ORF 099R 98.08% | ORF 016R 96.17% 57L 95.40% |                          |
| ORF 018R       | 21,185   | 21,832        | 648 putative NTPase I DNA-binding protein putative replication factor and/or DNA binding-packing | RSIV                 | 100.00% ORF 017R  | ORF 017R 99.23% ORF 101R 99.23% | ORF 017R 97.22% 56L 97.38% |                          |
| ORF 019R       | 21,843   | 22,784        | 942 putative NTPase I DNA-binding protein putative replication factor and/or DNA binding-packing | RSIV                 | 100.00% ORF 018R  | ORF 018R 100.00% ORF 106R 96.92% | ORF 018R 90.98% 55L 90.81% 52L 89.81% |                          |
| ORF 020R       | 22,807   | 23,751        | 945 putative NTPase I DNA-binding protein putative replication factor and/or DNA binding-packing | RSIV                 | 100.00% ORF 019R  | ORF 019R 100.00% ORF 111R 97.67% | ORF 019R 90.08% 54L 90.48% |                          |
| Gene ID 175bTye | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime _1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|----------------|----------|---------------|-------------------------------------|--------------------|------------------|---------------------------------|-------------------------------|-----------------------------|
| ORF 021L | 23,785-23,979 | 195 | hypothetical protein | RSIV subtype II | RSV KagYT-96RSIV RIE12-1GSIV-K1SKIVRBIV-C1 | 100.00% ORF 020L 100.00% | ORF 121L 96.91% 53R 91.24% | 50R - | |
| ORF 022R | 23,981-24,433 | 453 | hypothetical protein | RSIV subtype II | RSV KagYT-96 RSV RIE12-1 GSIV-K1 SKIV RBIV-C1 RSIV 121 OSGIV 17RbGs | 100.00% ORF 021R 100.00% | ORF 122R 96.47% 52L 88.91% | 49L 88.21% | |
| ORF 023L | 24,522-24,657 | 111 | hypothetical protein | RSIV subtype II | RSV KagYT-96 RSV RIE12-1 GSIV-K1 SKIV RBIV-C1 RSIV 121 RBIV-KOR-TY1 OSGIV 17RbGs | 100.00% ORF 022L 100.00% | ORF 127L 93.86% 51R 91.46% | - - | |
| ORF 024R | 24,712-25,140 | 429 | hypothetical protein | RSIV subtype II | RSV KagYT-96 RSV RIE12-1 GSIV-K1 RBIV-KOR-TY1 OSGIV 17RbGs | 100.00% ORF 023R 99.77% | ORF 128R 98.37% 50L 93.24% | 48L 91.61% | |
| ORF 025L | 25,208-25,378 | 171 | hypothetical protein | RSIV subtype II | RSV KagYT-96 RSV RIE12-1 GSIV-K1 SKIV RBIV-C1 RSIV 121 RBIV-KOR-TY1 OSGIV 17RbGs | 100.00% ORF 024L 100.00% | ORF 134L 97.66% 49R 94.74% | - - | |
| ORF 026L | 25,394-25,747 | 354 | PDGF/VEGF-like protein ORF 135L | RSIV subtype II | RSV KagYT-96 RSV RIE12-1 GSIV-K1 OSGIV | 100.00% ORF 025L 99.72% | ORF 135L 97.74% 48R 86.16% | 47R 87.39% | |
| ORF 027L | 25,744-26,007 | 264 | hypothetical protein | RSIV subtype II | RSV KagYT-96 RSV RIE12-1 GSIV-K1 SKIV RBIV-C1 RSIV 121 RBIV-KOR-TY1 OSGIV 17RbGs | 100.00% ORF 026L 100.00% | ORF 138L 97.35% 47R 93.18% | 46R 93.56% | |
| Gene ID 17SbTy | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|--------------|----------|--------------|------------------------------------|-------------------|------------------|-------------------------------|-----------------------------|---------------------------|
| ORF 028R     | 26,167   | 26,850       | cytosine DNA methyltransferase     | RSIV subtype I    | PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 99.85% ORF 027R | 97.95% ORF 140R | 99.85% 46L 94.74% 45L 94.88% |
| ORF 029R     | 26,844   | 27,758       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 028R | 96.17% ORF 145R | 100.00% 45L 88.74% 44L 89.84% |
| ORF 030R     | 27,763   | 28,563       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 029R | 97.50% ORF 151R | 100.00% 44L 90.02% 43L 89.51% |
| ORF 031R     | 28,570   | 28,932       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 030R | 97.80% ORF 156R | 100.00% 43L 94.21% 42L 95.04% |
| ORF 032L     | 29,016   | 29,615       | hypothetical protein               | RSIV subtype I    | PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 031L | 96.83% ORF 161L | 100.00% 42R 89.33% 41R 91.01% |
| ORF 033R     | 29,630   | 30,979       | hypothetical protein               | RSIV subtype I    | PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 032R | 97.04% ORF 162R | 100.00% 41L 88.96% 40L 90.53% |
| ORF 034R     | 30,981   | 32,129       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 033R | 91.91% ORF 171R | 91.22% 40L 89.65% 39L 98.43% |
| ORF 035L     | 32,122   | 33,000       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 034L | 93.97% ORF 179L | 100.00% 39R 90.22% 38R 90.90% |
| ORF 036R     | 33,066   | 34,505       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 035R | 93.75% ORF 180R | 100.00% 38L 90.71% 37L 90.90% |
| ORF 037R     | 34,514   | 35,863       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 99.93% ORF 036R | 93.85% ORF 186R | 99.93% 37L 90.11% 36L 90.96% |
| ORF 038L     | 35,860   | 36,915       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 037L | 95.17% ORF 197L | 100.00% 36R 91.49% 35R 88.93% |
| ORF 039R     | 36,909   | 38,048       | hypothetical protein               | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 038R | 95.53% ORF 198R | 99.91% 35L 88.64% 34L 88.88% |
| Gene ID 17SbTy | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Genotype | Isolates | ORF no. | Identity (%) | ORF no. | Identity (%) | ORF no. | Identity (%) | ORF no. | Identity (%) |
|---------------|----------|---------------|-------------------------------------|-------------------|-----------|----------|---------|--------------|---------|--------------|---------|--------------|---------|--------------|
| ORF 040L      | 38,121   | 41,279        | 3159                  | DNA dependent RNA polymerase second largest subunit | RSIV subtype I    | LYCIV Zhoushan | 100.00% | ORF 039L | 96.52% | RPO-2       | 98.54% | 34R         | 93.78% | 33R         | 94.98% |
| ORF 041R      | 41,362   | 42,264        | 903                   | hypothetical protein deoxyribo-nucleoside kinase | RSIV subtype I    | LYCIV Zhoushan | 100.00% | ORF 040R | 95.90% | RPO-2       | 97.79% | 33L         | 91.36% | 32L         | 92.59% |
| ORF 042L      | 42,327   | 42,943        | 582                   | RSIV subtype I    | LYCIV Zhoushan    | 100.00% | ORF 041L | 88.87% | TK          | 87.99% | 32R         | 92.16% | 31R         | 99.66% |
| ORF 043L      | 43,008   | 43,535        | 243                   | hypothetical protein transcription elongation factor TFIIS DNA dependent | RSIV subtype I | PIV2016 PIV2014a PIV2010 RSIV Ehime-1 | 98.77% | ORF 042L | 95.47% | ORF 237L | 98.77% | 31.5L | 88.89% | 30R | 93.42% |
| ORF 044R      | 43,603   | 43,824        | 222                   | RSIV subtype I    | LYCIV Zhoushan    | 100.00% | ORF 043R | 98.20% | ORF 238R | 100.00% | 29L         | 96.40% | 29L | 97.06% |
| ORF 045R      | 43,831   | 47,337        | 3507                  | DNA dependent RNA polymerase largest subunit probable | RSIV subtype I | LYCIV Zhoushan PIV2016 PIV2014a PIV2010 | 99.94% | ORF 044R | 97.69% | RPO-1       | 99.37% | 28L | 94.66% | 28L | 95.30% |
| ORF 046R      | 47,354   | 48,250        | 897                   | RSIV subtype I    | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% | ORF 045R | 98.33% | ORF 256R | 100.00% | 27L | 96.10% | 27L | 95.21% |
| ORF 047R      | 48,272   | 48,595        | 324                   | hypothetical protein | RSIV subtype I | LYCIV Zhoushan RSIV Ehime-1 | 100.00% | ORF 046R | 97.53% | ORF 261R | 100.00% | 26L | 92.00% | 26L | 90.43% |
| Gene ID | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------|-----------|--------------|-------------------------------------|--------------------|------------------|-------------------------------|----------------------------|----------------------------|
| ORF 048L | 49,064-50,002 | 939 | ribonucleotide diphosphate reductase small subunit laminin-type epidermal growth factor LRP16 like protein macro domain-containing protein | RSIV subtype I | PIV2016 PIV2014a PIV2010 RSIV Ehime-1 | 100.00% ORF 047L 98.08% | RR-2 100.00% 24R 94.68% 25R 95.21% |
| ORF 049L | 50,114-53,266 | 3153 | | RSIV subtype I | PIV2010 RSIV Ehime-1 | 100.00% ORF 048L 93.77% | ORF 291L 100.00% 23R 87.35% 24R 88.96% |
| ORF 050R | 53,339-54,934 | 1596 | | RSIV subtype I | PIV2016 PIV2014a PIV2010 RSIV Ehime-1 | 100.00% ORF 049R 95.60% | ORF 292R 100.00% 22L 93.41% 23L 93.52% |
| ORF 051R | 55,282-55,464 | 183 | | | | | |
| ORF 052R | 55,511-58,354 | 2844 | DNA polymerase family B exonuclease | RSIV subtype I | PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% ORF 051L 97.23% | DPO 100.00% 19R 95.11% 20R 93.15% |
| ORF 053R | 58,420-58,629 | 210 | | | | | |
| ORF 054R | 58,889-59,221 | 333 | | | | | |
| ORF 055R | 59,236-59,823 | 588 | | | | | |

Table A2. Cont.
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| Gene ID 17SbTy | Position | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------------|----------|------------------------------------|--------------------|-------------------|-------------------------------|-------------------------------|-------------------------------|
|               | Start    | End      | Size (NT) |                   | Identity (%) | ORF no. | Identity (%) | ORF no. | Identity (%) | ORF no. | Identity (%) | ORF no. | Identity (%) |
| ORF 056L      | 59,881   | 60,672   | 792       | hypothetical protein | 92.12% | ORF 055L | 95.58% | ORF 333L | 98.86% | 15R | 94.44% | 15R | 93.43% |
| ORF 057L      | 60,678   | 61,652   | 975       | hypothetical protein | 100.00% | ORF 056L | 99.90% | ORF 342L | 97.03% | 14R | 92.31% | 14R | 92.23% |
| ORF 058L      | 61,907   | 63,304   | 1398      | serine/threonine protein kinase | 100.00% | ORF 057L | 99.93% | ORF 349L | 97.49% | 13R | 90.19% | 13R | 91.91% |
| ORF 059L      | 63,311   | 63,643   | 333       | RING-finger-containing ubiquitin ligase | 100.00% | ORF 058L | 100.00% | ORF 350L | 98.50% | 12R | 96.36% | 12R | 95.80% |
| ORF 060R      | 63,662   | 63,922   | 261       | hypothetical protein | 100.00% | ORF 059R | 98.04% | ORF 351L | 96.55% | 11L | 95.02% | 11L | 94.90% |
| ORF 061L      | 63,919   | 64,311   | 393       | hypothetical protein | 100.00% | ORF 060R | 92.11% | ORF 353R | 97.96% | 10L | 92.62% | 10L | 92.11% |
| ORF 062L      | 64,470   | 64,631   | 162       | hypothetical protein | 100.00% | ORF 061L | 100.00% | ORF 360L | 98.77% | 9R | 97.53% | 9R | 98.77% |
| Gene ID 17SbTy | Position   | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------------|------------|---------------|-------------------------------------|---------------------|--------------------|-------------------------------|----------------------------|-----------------------------|
| ORF 063L      | Start 64,727, End 66,274 | 1548 | hypothetical protein                | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, RBIV-C1, LYCIV Zhoushan, RSIV 121 OSGIV, 17RbGs | 100.00% ORF 062L 100.00% ORF 373L 96.13% 8R 91.88% 8R 91.68% |
| ORF 064R      | Start 66,345, End 67,802 | 1458 | myristoylated membrane protein      | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, LYCIV Zhoushan OSGIV | 100.00% ORF 063R 99.73% ORF 374R 97.46% 7L 94.51% 7L 94.65% |
| ORF 065R      | Start 67,819, End 69,180 | 1362 | major capsid protein NIF-NLI factor-like phosphatase | LYCIV Zhoushan PIV2016 PIV2014a | 100.00% ORF 064R 98.24% MCP 99.63% 6L 94.57% 6L 94.27% |
| ORF 066R      | Start 69,326, End 70,090 | 765  | hypothetical protein               | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, EBIV-C1, LYCIV Zhoushan OSGIV, 17RbGs | 100.00% ORF 065R 98.35% ORF 385R 100.00% 5L 95.17% 5L 92.82% |
| ORF 067R      | Start 70,164, End 71,735 | 177  | hypothetical protein               | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, EBIV-C1, LYCIV Zhoushan OSGIV, 17RbGs | 100.00% ORF 066R 99.44% ORF 388R 100.00% 4L 91.78% 4L 97.89% |
| ORF 068R      | Start 70,413, End 71,196 | 486  | hypothetical protein               | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, EBIV-C1, LYCIV Zhoushan OSGIV, 17RbGs | 100.00% ORF 067R 96.30% ORF 390R 99.79% 3L 90.00% 86.59% |
| ORF 069R      | Start 71,268, End 71,735 | 468  | hypothetical protein               | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, EBIV-C1, LYCIV Zhoushan OSGIV, 17RbGs | 100.00% ORF 068R 99.36% RPOH 100.00% 2R 93.83% 2R 94.25% |
| ORF 070R      | Start 71,705, End 72,841 | 1137 | hypothetical protein               | RSIV KagYT-96, RSIV RIE12-1, GSIV-K1, EBIV-C1, LYCIV Zhoushan OSGIV, 17RbGs | 100.00% ORF 069R 97.89% ORF 396R 100.00% 1L 93.23% 1L 92.52% |
| Gene ID 17SbTy | Position CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------------|------------------------|-----------------------------------|--------------------|------------------|-------------------------------|-----------------------------|----------------------------|
| ORF 071R      | Start: 72,956 End: 73,672 Size: 717 | hypothetical protein               | RSIV RIE12-1 RSIV KagYT-96 GSIV-K1 OSGIV | 100.00% ORF 070R 99.86% ORF 401R 98.61% | 124L 93.01% | 115L 92.39% |
| ORF 072R      | Start: 73,681 End: 74,061 Size: 381 | hypothetical protein               | RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% ORF 071R 100.00% ORF 407R 98.69% | 123R 97.58% | 114R 95.90% |
| ORF 073L      | Start: 74,033 End: 74,752 Size: 720 | ATPase(adenosine triphosphatase)   | RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% ORF 072L 100.00% ORF 412L 99.03% | 122R 95.97% | 113R 95.97% |
| ORF 074R      | Start: 74,762 End: 75,397 Size: 636 | hypothetical protein               | RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 97.48% ORF 073R 97.48% ORF 413R 97.16% | 121L 86.09% | 112L 84.54% |
| ORF 075L      | Start: 75,418 End: 75,924 Size: 507 | hypothetical protein               | RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% ORF 074L 100.00% ORF 420L 97.24% | 120R 93.53% | 111R 92.28% |
| ORF 076L      | Start: 75,955 End: 76,242 Size: 288 | probable transcriptional activator RING-finger domain-containing E3 protein ankyrin repeat-containing protein | RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% ORF 075L 100.00% ORF 423L 98.96% | 119R 93.71% | 110R 92.01% |
| ORF 077R      | Start: 76,312 End: 77,625 Size: 1314 | hypothetical protein               | RSIV RIE12-1 GSIV-K1 | 100.00% ORF 076R 99.77% ORF 424R 96.88% | 118L 93.03% | 109L 92.03% |
### Table A2. Cont.

| Gene ID | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------|----------|---------------|------------------------------------|--------------------|-------------------|---------------------------------|----------------------------|----------------------------|
| ORF 078R | 77,958   | 78,632        | Start End | FV3 early 31KDa protein homolog | RSIV KagYT-96 GSIV-K1 RSIV_121 OSGIV | 99.85% | 99.85% | ORF 077R | 98.22% | 117L | 93.79% | 108L | 94.82% |
| ORF 079L | 78,686   | 80,062        | Start End | hypothetical protein | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 17RbGs | 100.00% | 100.00% | ORF 078L | 96.27% | 116R | 86.68% | 107R | 85.92% |
| ORF 080L | 80,123   | 81,133        | Start End | immediate-early protein | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 17RbGs | 100.00% | 100.00% | ORF 079L | 98.32% | 115R | 93.18% | 106R | 93.08% |
| ORF 081R | 81,568   | 84,150        | Start End | putative tyrosine kinase | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 17RbGs | 100.00% | 100.00% | ORF 080L | 97.99% | 114L | 93.69% | 105L | 93.26% |
| ORF 082L | 84,194   | 84,574        | Start End | hypothetical protein | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV | 100.00% | 99.74% | ORF 081L | 97.38% | 113R | 92.66% | 104R | 92.89% |
| ORF 083L | 84,682   | 85,425        | Start End | proliferating cell nuclear antigen | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% | 100.00% | ORF 082L | 98.39% | 112R | 94.35% | 102R | 96.01% |
| ORF 084L | 85,445   | 86,341        | Start End | tumor necrosis factor receptor - associated factor-like protein | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% | 100.00% | ORF 083R | 97.99% | 111L | 93.09% | 101L | 90.41% |
| ORF 085L | 86,338   | 86,493        | Start End | hypothetical protein | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV 17RbGs | 100.00% | 100.00% | ORF 084L | 96.79% | 110R | 90.38% | 100R | 91.03% |
| Gene ID | Position CDS (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------|------------------|-----------------------------------|-------------------|-----------------|-------------------------------|----------------------------|----------------------------|
| ORF 086R | 86,546 89,308 2763 | D5 family NTPase | RSIV | 100.00% | ORF 085R 99.96% | ORF 493R 97.79% | 109L 94.29% | 99L 94.53% |
| ORF 087R | 89,389 90,018 630 | hypothetical protein | RSIV | 99.84% | ORF 086R 99.84% | ORF 502R 95.67% | 108.5L 91.61% | 98L 94.91% |
| ORF 088R | 90,058 90,930 873 | hypothetical protein | RSIV | 100.00% | ORF 087R 100.00% | ORF 506R 97.25% | - - | - 97L 80.25% |
| ORF 089L | 90,937 91,901 888 | HIT-like protein | RSIV | 99.89% | ORF 088L 99.55% | ORF 515L 96.83% | - - | - - |
| ORF 090L | 91,953 92,324 372 | hypothetical protein | RSIV | 100.00% | ORF 089L 100.00% | ORF 518L 98.66% | 105R 95.99% | 96R 94.62% |
| ORF 091L | 92,326 93,102 777 | hypothetical protein | RSIV | 98.71% | ORF 090L 98.71% | ORF 522L 98.20% | 104R 94.21% | 95R 90.09% |
| ORF 092L | 93,164 93,577 414 | suppressor of cytokine signalling 1 homolog ankyrin repeat containing protein | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% | ORF 091L 95.17% | ORF 524L 100.00% | 103R 88.38% | 94R 88.22% |
| ORF 093L | 93,584 95,029 1446 | hypothetical protein | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% | ORF 092L 97.99% | ORF 534L 100.00% | 102R 91.46% | 93R 92.39% |
| ORF 094L | 95,098 95,613 516 | hypothetical protein | PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 | 100.00% | ORF 093R 97.29% | ORF 535R 100.00% | 101L 93.80% | 92L 92.83% |
| Gene ID 175b1y | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------------|----------|---------------|-------------------------------------|-------------------|-----------------|-------------------------------|-----------------------------|-----------------------------|
| ORF 095R      | 95,588   | 96,229        | 642 hypothetical protein              | RSIV subype II    | 99.07%          | 98.75%                       | 98.91%                      | 100L 86.49%                 | 91L 86.67%                  |
| ORF 096R      | 96,283   | 96,606        | 324 RING-finger-containing E3 ubiquitin ligase | RSIV subype II    | 100.00%         | 100.00%                      | 97.53%                      | 99L 91.05%                 | 90L 84.26%                  |
| ORF 097R      | 96,655   | 97,146        | 492 hypothetical protein              | RSIV subype II    | 100.00%         | 100.00%                      | 97.36%                      | 97.5L 94.51%               | 89L 92.48%                  |
| ORF 098R      | 97,137   | 97,888        | 738 hypothetical protein              | RSIV subype II    | 100.00%         | 100.00%                      | 98.10%                      | 96L 94.58%                 | 88L 93.77%                  |
| ORF 099R      | 97,896   | 99,059        | 1164 hypothetical protein            | RSIV subype II    | 100.00%         | 99.91%                       | 96.91%                      | 95L 91.21%                 | 87L 91.02%                  |
| ORF 100R      | 99,084   | 99,584        | 501 hypothetical protein             | RSIV subype II    | 100.00%         | 100.00%                      | 98.60%                      | 94L 95.41%                 | 86L 93.01%                  |
| ORF 101R      | 99,594   | 100,520       | 927 probable RNA binding protein     | RSIV subype II    | 100.00%         | 100.00%                      | 97.84%                      | 93L 92.22%                 | 85L 92.02%                  |
| ORF 102R      | 100,641  | 101,711       | 1071 myristoylated membrane protein  | RSIV subype II    | 98.62%          | 99.69%                       | 95.94%                      | -                         | -                          | 83L 91.36%                  |
| ORF 103L      | 101,692  | 103,263       | 1572 hypothetical protein            | RSIV subype I     | 98.85%          | 98.54%                       | 98.20%                      | 88R 92.24%                 | 82R 93.26%                  |
| Gene ID 17SbTy | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------------|----------|---------------|-----------------------------------|-------------------|-------------------|---------------------------------|-----------------------------|-----------------------------|
|               | Start    | End           | Genotype                          | Isolates          | Identity (%)      | ORF no. (%)                     | Identity (%)                | ORF no. (%)                  |
| ORF 104R      | 103,311  | 103,724       | 414 hypothetical protein          | RSIV              | 99.52%            | ORF 103R                        | 99.52%                      | ORF 591R                    | 99.28%                      |
| ORF 105L      | 103,721  | 104,518       | 798 RNase III-like ribonuclease    | RSIV              | 100.00%           | RNC                            | 97.99%                      | 87R                         | 94.16%                      |
| ORF 106L      | 104,484  | 104,951       | 468 Uvr/REP helicase              | RSIV              | 100.00%           | ORF 105L                        | 93.80%                      | ORF 600L                    | 97.44%                      |
| ORF 107L      | 104,948  | 105,451       | 504 hypothetical protein          | RSIV              | 100.00%           | ORF 106L                        | 92.86%                      | ORF 605L                    | 97.83%                      |
| ORF 108L      | 105,565  | 106,869       | 1305 hypothetical protein         | RSIV              | 100.00%           | ORF 107L                        | 95.21%                      | ORF 606R                    | 97.70%                      |
| ORF 109L      | 106,896  | 107,255       | 360 hypothetical protein          | RSIV              | 100.00%           | ORF 108L                        | 98.89%                      | ORF 617L                    | 98.33%                      |
| ORF 110R      | 107,319  | 10,8425       | 1107 hypothetical protein         | RSIV              | 100.00%           | ORF 109R                        | 98.89%                      | ORF 618R                    | 97.92%                      |
| ORF 111L      | 108,474  | 108,971       | 498 hypothetical protein          | RSIV              | 100.00%           | ORF 110L                        | 100.00%                     | ORF 628L                    | 97.99%                      |
| ORF 112L      | 108,984  | 109,457       | 474 hypothetical protein          | RSIV              | 100.00%           | ORF 111L                        | 100.00%                     | ORF 632L                    | 95.81%                      |
| Gene ID | Position | CDS Size (NT) | Predicted Structure and/or Function | Best-Match Homolog | Homolog to 17RbGs | Homolog to Ehime_1 (AB104413.1) | Homolog to ISKNV (AF371960) | Homolog to TRBIV (GQ273492) |
|---------|-----------|---------------|-------------------------------------|-------------------|-----------------|--------------------------------|-----------------------------|-----------------------------|
| 17SbTy  |           |               |                                     |                   |                 |                                |                             |                             |
| ORF 113R | 109,545   | 109,769       | 225 hypothetical protein subtype II | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% ORF 112L 100.00% ORF 634L 92.06% 79L 93.78% 72L 92.27% |
| ORF 114L | 109,771   | 110,235       | 465 hypothetical protein subtype II | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs | 100.00% ORF 113L 100.00% ORF 635L 97.42% 78R 96.34% 71R 93.76% |
| ORF 115L | 110,232   | 111,566       | 1335 hypothetical protein subtype II | RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 OSGIV | 99.93% ORF 114L 99.93% ORF 641L 96.55% 77R 90.95% 70R 90.42% |
| No. | Category                          | COG Function                          | COG Description                                                                 | 17SbTy  | 17RbGS |
|-----|----------------------------------|---------------------------------------|---------------------------------------------------------------------------------|---------|--------|
| 1   | Metabolism                       | Amino acid transport and metabolism   | quinoprotein dehydrogenase-associated putative ABC transporter substrate-binding protein | ORF 093L | ORF 092L |
| 2   | Nucleotide transport and metabolism |                                       | deoxynucleoside kinase ribonucleoside-diphosphate reductase                      | ORF 042L | ORF 041L |
| 3   |                                  | HIT domain-containing protein          |                                                                                 | ORF 084L | ORF 087L |
| 4   |                                  |                                       |                                                                                 | ORF 088L |         |
| 5   | Translation, ribosomal structure and biogenesis | O-acetyl-ADP-ribose deacetylase | DNA-directed RNA polymerase subunit B                                            | ORF 050R | ORF 049R |
| 6   | Information storage and processing | DNA-directed RNA polymerase subunit A| DNA-directed RNA polymerase subunit A                                          | ORF 040L | ORF 039L |
| 7   | Transcription                     | DNA-directed RNA polymerase subunit B | DNA-directed RNA polymerase subunit A                                          | ORF 040L | ORF 039L |
| 8   | Replication, recombination and repair | phosphoprotein phosphatase ribonuclease III | DNA cytosine methyltransferase flap endonuclease-1                            | ORF 036R | ORF 035R |
| 9   |                                  | DNA polymerase elongation subunit      |                                                                                  | ORF 052L | ORF 051L |
| 10  |                                  |                                       |                                                                                  | ORF 052L | ORF 051L |
| 11  |                                  |                                       |                                                                                  | ORF 052L | ORF 051L |
| 12  |                                  |                                       |                                                                                  | ORF 052L | ORF 051L |
| 13  |                                  |                                       |                                                                                  | ORF 052L | ORF 051L |
| 14  | Signal transduction mechanisms    | protein-tyrosine-phosphatase ankyrin repeat-containing protein quinoprotein dehydrogenase-associated putative ABC transporter substrate-binding protein ankyrin repeat-containing protein | ORF 12R | ORF 012R |
| 15  | Cellular process                  | Dehydrogenase-associated putative ABC transporter substrate-binding protein ankyrin repeat-containing protein | ORF 093L | ORF 092L |
| 16  | Mobilome; prophages, transposons  | Hypothetical protein                   |                                                                                  | ORF 086R | ORF 085R |
| 17  | Poorly characterized              | General function prediction only       | HIT domain-containing protein                                                  | ORF 089L | ORF 088L |
| 18  | Function unknown                  | Hypothetical protein                   |                                                                                  | ORF 013R | ORF 012R |
Table A4. ORF locations of the 26 conserved core genes conserved in the family *Iridoviridae*.

| No. | Gene (GenBank Access. No.) | 17SbTy (OK042108) | 17RbGs (OK042109) | Ehime-1 (AB104413) | ISKNV (AF371960) | RBIV (AY532606) | TRBIV (GQ273492) |
|-----|----------------------------|-------------------|-------------------|-------------------|-----------------|----------------|-----------------|
| 1   | hypothetical protein        | 001R              | 001R              | 639R              | 76L             | 72L            | 69L             |
| 2   | Putative NTPase I           | 013R              | 012R              | NTPase            | 63L             | 59L            | 58L             |
| 3   | Putative replication factor and/or DNA binding-packing Helicase family | 015R | 014R | 092R | 61L | 57L | 56L |
| 4   | Serine-threonine protein kinase | 018R | 017R | 101R | 56L | 54L | 53L |
| 5   | Erv1/Alr family DNA dependent RNA polymerase second largest subunit | 019R | 018R | 106R | 55L | 53L | 52L |
| 6   | Putative replication factor TFIS DNA dependent RNA polymerase largest subunit | 031R | 030R | 156R | 43L | 43.5L | 42L |
| 7   | XPPG-RAD2-type nuclease      | 040L              | 039L              | RPO-2             | 34R             | 33R            | 33R             |
| 8   | Deoxynucleoside kinase       | 042L              | 041L              | TK                | 32R             | 31R            | 31R             |
| 9   | Transcription elongation factor TFIS DNA dependent RNA polymerase largest subunit Putative | 044R | 043R | 238R | 29L | 29.5Lb | 29L |
| 10  | DNA pol Family B exonuclease | 048L | 047L | RR-2 | 24R | 26R | 25R |
| 11  | Myristoylated membrane protein | 052L | 051L | DNA pol | 19R | 20R | 20R |
| 12  | Serine-threonine protein kinase | 058L | 057L | 349L | 13R | 13R | 13R |
| 13  | Myristoylated membrane protein | 064R | 063R | 374R | 7L | 8L | 7L |
| 14  | Major capsid protein         | 065R              | 064R              | MCP               | 6L              | 7L             | 6L              |
| 15  | NIF-NLI interacting factor  | 066R              | 065R              | ATPase (adenosine triphosphatase) | 385R | 5L | 6L |
| 16  | Immediate early protein ICP-46 | 073L | 072L | DNA pol | 412L | 122R | 116R | 113R |
| 17  | Putative tyrosin kinase/lipopolysaccharide modifying enzyme | 080L | 079L | 458L | 115R | 108.5R | 106R |
| 18  | Proliferating cell nuclear antigen | 081R | 080R | 463R | 61L, 114L | 57L, 106Lb | 105L |
| 19  | D5 family NTPase involved in DNA replication | 083L | 082L | 487L | 112R | 103Rb | 102R |
| 20  | Hypothetical protein         | 086R              | 085R              | DNA pol | 493R | 109L | 101L | 99L |
| 21  | Myristoylated membrane protein | 098R | 097R | 550R | 96L | 89.5Lb | 88L |
| 22  | RNase III-like ribonuclease  | 102R              | 101R              | 575R              | 90.5L           | 85L            | 83R             |
| 23  | Immediate early protein ICP-46 | 105L | 104L | RNC | 87R | 83R | 80R |
| 24  | Uvr/REP helicase             | 106L              | 105L              | 600L              | 86R             | 82.5R          | 79R             |
Figure A1. Cytopathic effects (CPEs) in rock bream fin cells under the influence of a tissue homogenate from (A) an RSIV (17SbTy)-infected Japanese seabass and (B) an RSIV (17RbGs)-infected rock bream. CPE of the rounding cells (arrows) in rock bream fin cells (A) after 3 days of inoculation with 17SbTy, and (B) 9 days of inoculation with 17RbGs, and (C) negative control (mock cells at passage 15). Scale bar = 100 μm.
Figure A2. Cont.
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Figure A2. Cont.
Figure A2. Comparison of nucleotide sequences covering the four insertion and deletions (InDels) in coding regions (ORFs (a) 014R, (b) 053R, (c) 054R and (d) 102R on the basis of 175bTy isolate) between the cell-cultured isolates and viruses from RSIV-infected rock breams. The 175bTy and 17RbGs from either cell-isolates or viruses from RSIV-infected rock bream are highlighted in red and blue boxes, respectively. The boxes consisting of blue dashed lines represent the InDel regions.

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