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

Min-A Jeong, Ye-Jin Jeong and Kwang-Il Kim *

Department of Aquatic Life Medicine, Pukyong National University, Busan 48513, Korea; jm0613@pukyong.ac.kr (M.-A.J.); 201513346@pukyong.ac.kr (Y.-J.J.)
* Correspondence: kimki@pknu.ac.kr; Tel.: +82-51-629-5946; Fax: +82-51-629-5938

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

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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 cm^3), 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 cm^2 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^{TM} 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 cm^2 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 + G4 + 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 gsMapper (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.

gov/Blast.cgi, accessed on 10 May 2021). 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.

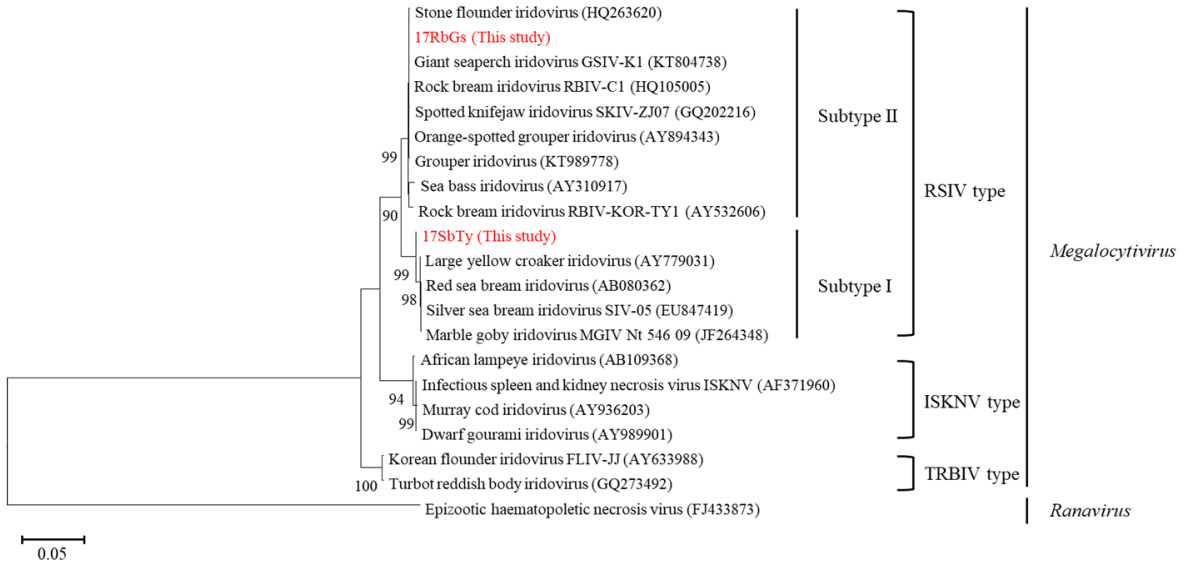
2.5. Pathogenicity of the Two RSIV Isolates in the Rock Bream

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; GensGen, 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).

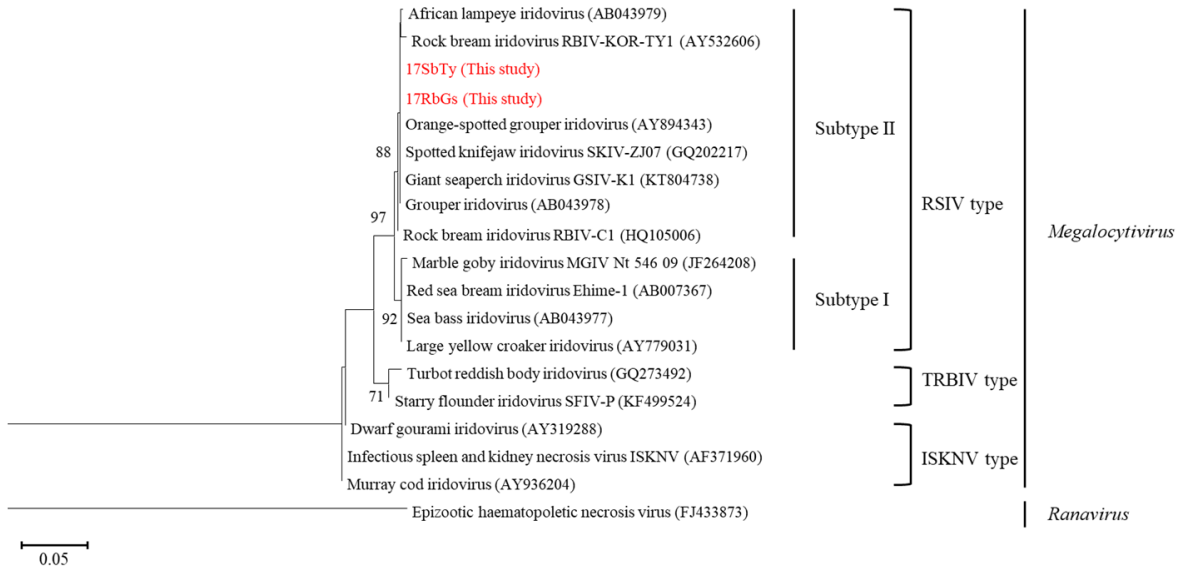
3. Results & Discussion

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).



(**a**)



(**b**)

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.

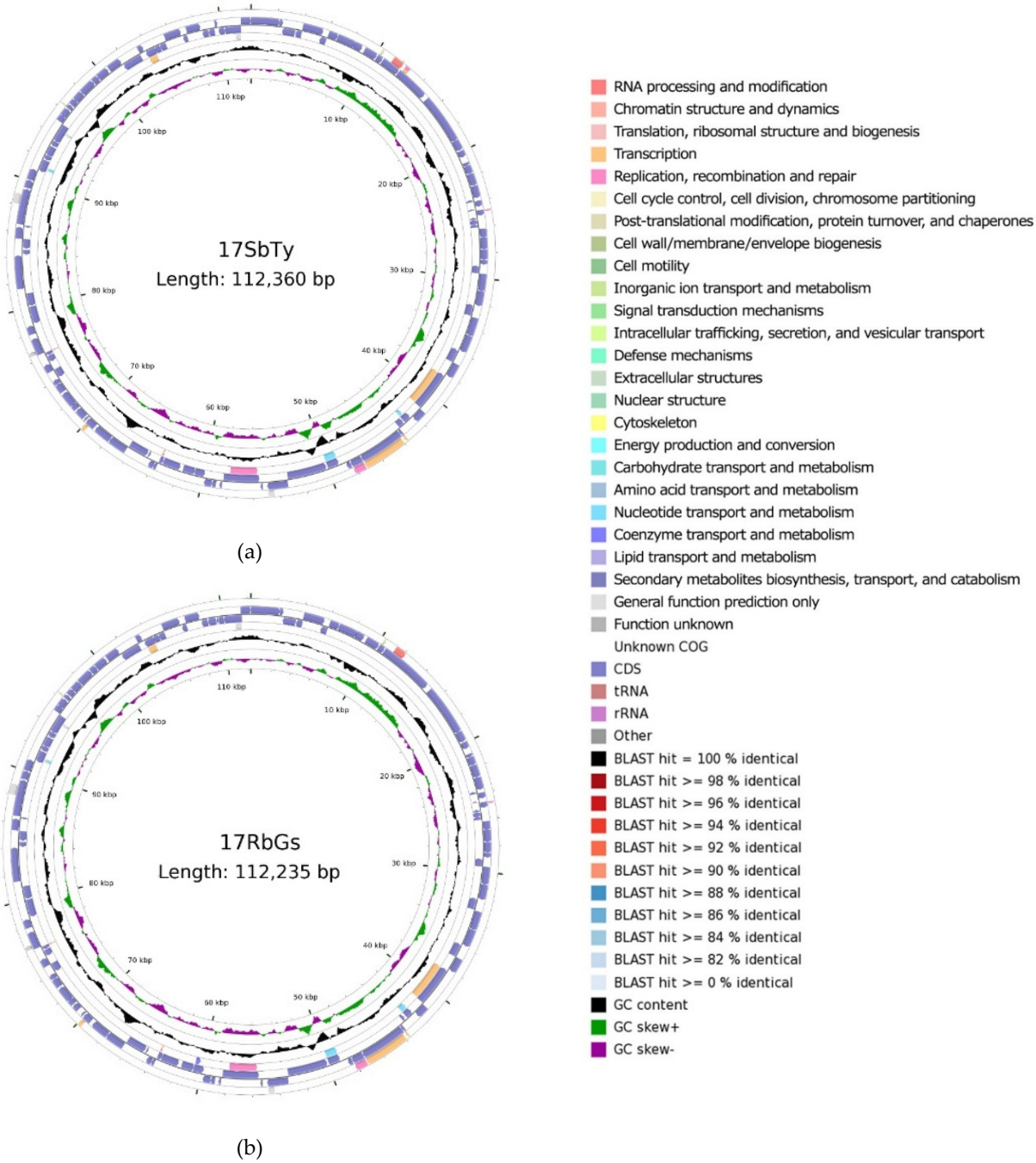


Figure 2. Circular genome maps of (**a**) 17SbTy (112,360 bp) and (**b**) 17RbGs (112,235 bp). From the inner ring to the outer ring, the first and eighth circles represented the genomic length (kbp) and nucleotide positions, respectively. The second and third circles show the G+C skew and G+C content, 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).

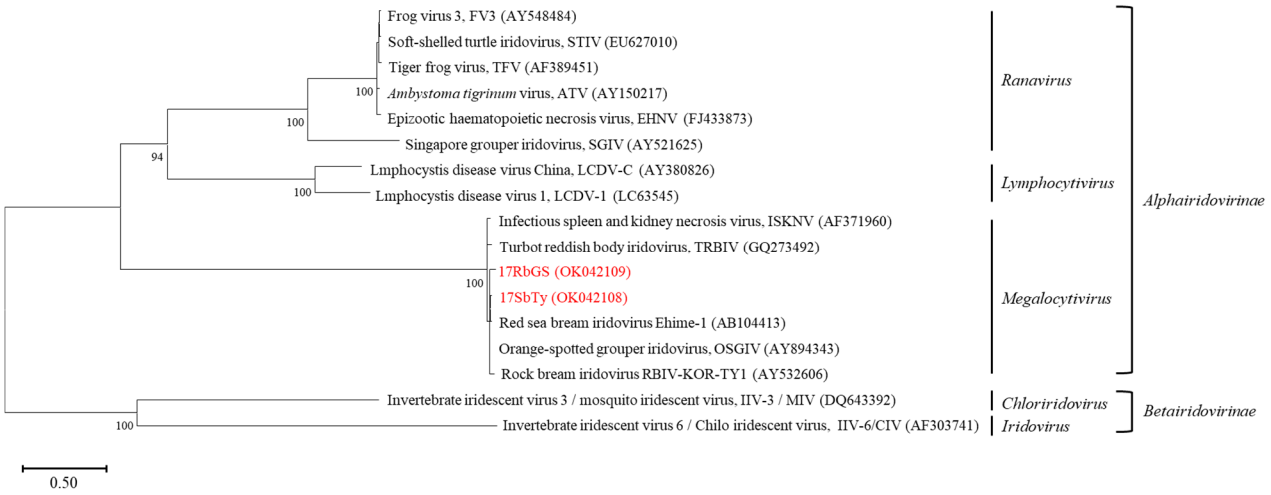


Figure 3. Phylogenetic trees based on the deduced amino acid sequences of the 26 concatenated genes conserved for members of the family *Iridoviridae*. The tree was constructed by the maximum-likelihood method under the LG model and gamma-distributed rates with invariant sites (LG + G4 + I) in MEGA (ver. 11). The two RSIV isolates (17SbTy and 17RbGs) from this study are highlighted in bold and red color.

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, Ehimel-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).

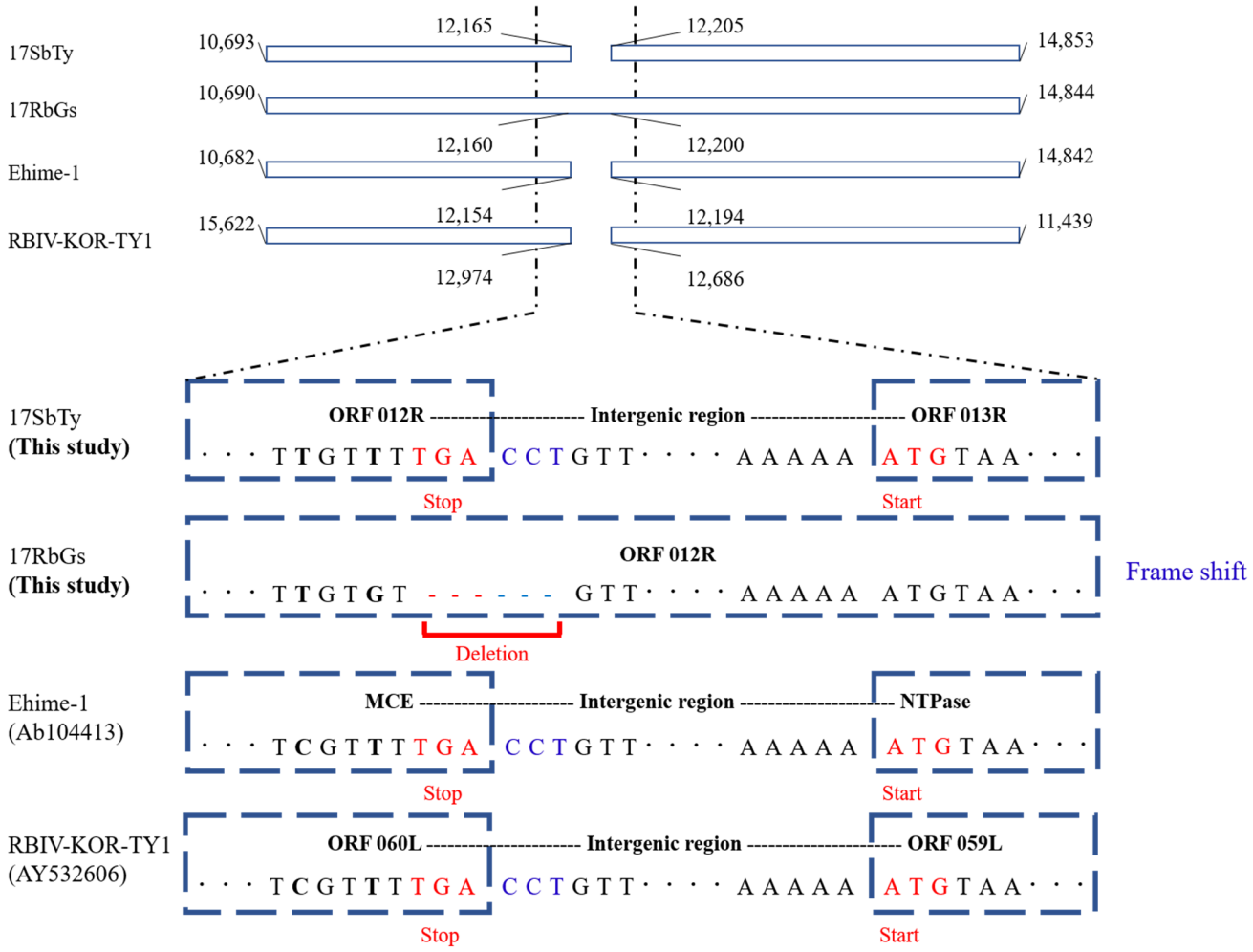
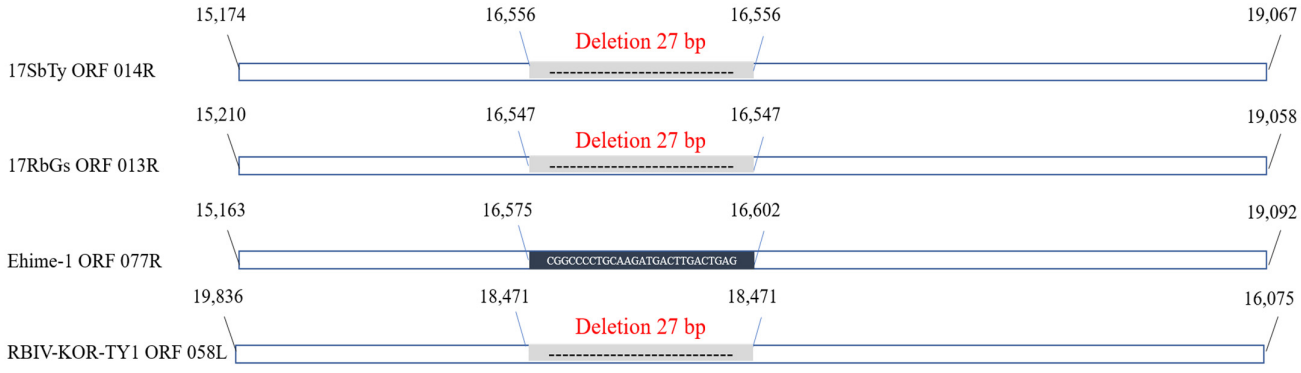


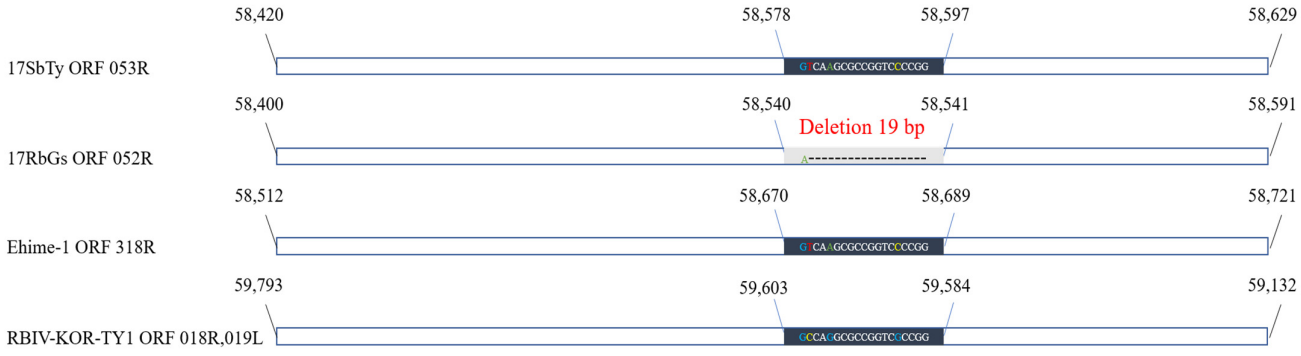
Figure 4. Schematic representation of a deletion of the termination codon in ORF 012R of 17RbGs causing a frameshift mutation. The aligned sequences are genomes of 17SbTy, 17RbGs, and two representative RSIVs (Ehime-1 [RSIV subtype I] and RBIV-KOR-TY1 [RSIV subtype II]). The nucleotide sequences surrounded 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.



(**a**)



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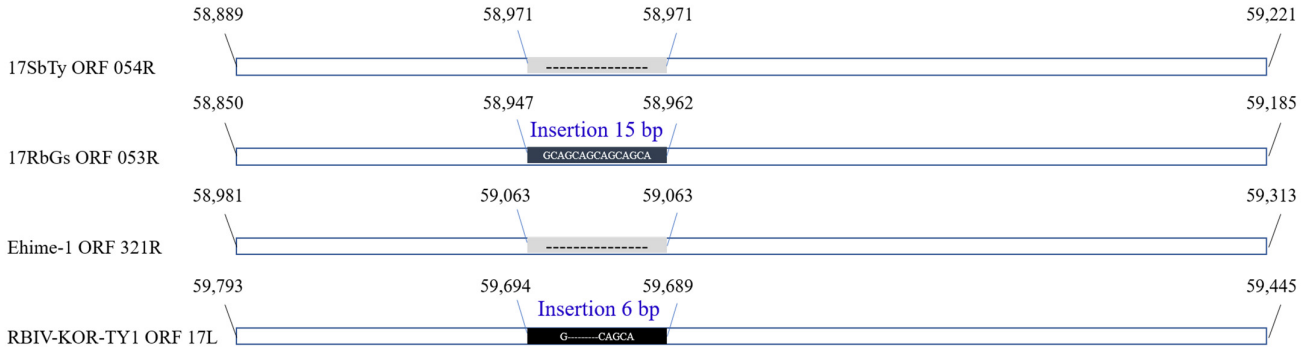

(**c**)

Figure 5. *Cont.*

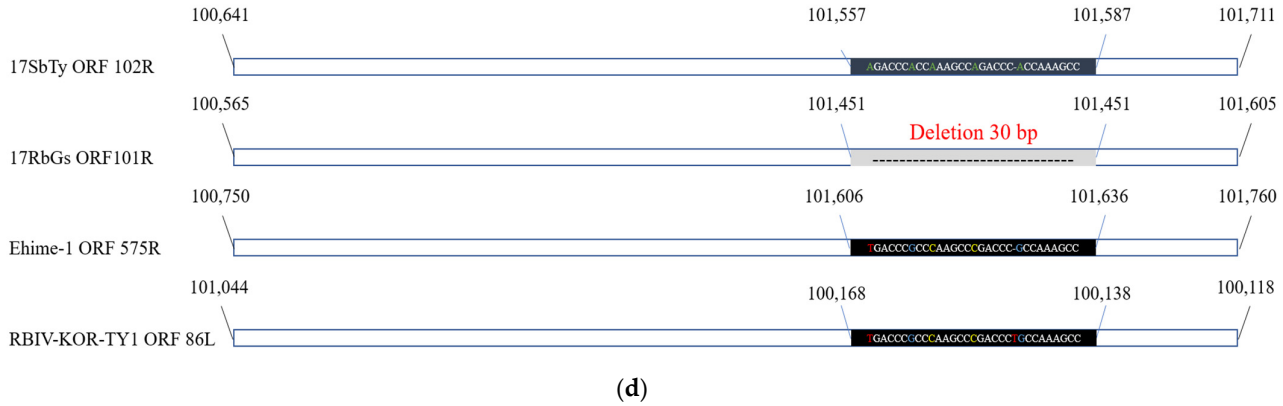

Figure 5. Schematic representation of insertion/deletion mutations (InDels) (>10 bp) in the coding regions as (**a**) ORF 014R, (**b**) ORF 053R, (**c**) ORF 054R and (**d**) ORF 102R based on the 17SbTy when compared with the genomes of 17RbGs and two representative RSIVs (Ehime-1 [RSIV subtype I] and 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.

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.

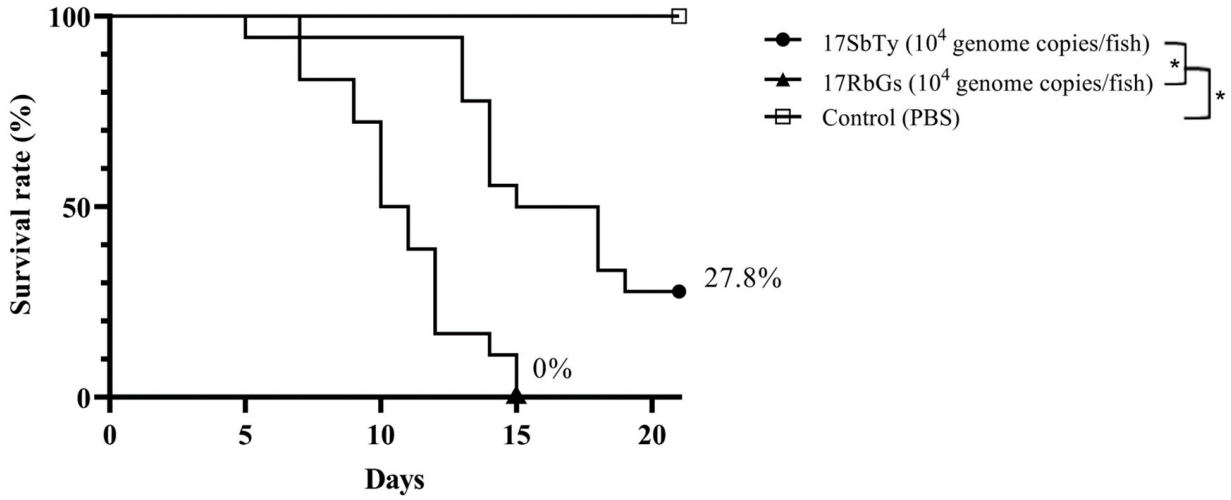

Figure 6. Survival rates (%) of rock breams after intraperitoneal injection with the two RSIV isolates (either 17SbTy or 17RbGs, 10^4 genome copies per fish). Statistical analysis was performed by the log-rank test (* $p < 0.05$).

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.

Table A1. PCR primers used in this study.

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	
ATPase 1F	ATPase	ATG GAA ATC MAA GAR TTG TCC YTG	This study
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	
RSIV 1094F	Major capsid protein	CCA GCA TGC CTG AGA TGG A	[15]
RSIV 1221R		GTC CGA CAC CTT ACA TGA CAG G	
RSIV 1177 probe		FAM-TAC GGC CGC CTG TCC AAC G-BHQ1	
1-F	*Pst* I fragment	CTC AAA CAC TCT GGC TCA TC	[28]
1-R		GCA CCA ACA CAT CTC CTA TC	
4-F	DNA polymerase gene	CGG GGG CAA TGA CGA CTA CA	
4-R		CCG CCT GTG CCT TTT CTG GA	
14R-1F	ORF 014R *	ATG AAG AAA TTT GAT TTT TGY RKA TGT C	This study
14R-260R		TCA TCC TCA GAG TCG CGG	
14R-430F		GCT CAG TTG TTC AAG ATG CC	
14R-999R		ATG CGT ATC ACA GTA CGC G	
14R-848F		CCA TAG AGG ATA ACA GCG C	
14R-1202F		ACA AGC GGG ACC TAT GCA A	
14R-1841R		TAC ATC GGC TCC TCA ACT G	
14R-1620F		AGA ACT GGA GGA CTC ACA	
14R-2011F		CAC CGT GAA CTG CGC ATC T	
14R-2630R		GTC 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 CCY ATT ATC TTC	
53R-RB-192R		CTA AGC GCG CCT GGC TGG	
53R-SB-210R		CTA AGC AGC CCT GGC GGG	
ORF54-1F	ORF 054R *	ATG CCG ACT ACC AAA CAC A	
ORF54-348R		TCA AAA CTC AAA GGC GCC G	
102R-1F	ORF 102R *	ATG AGT GCA ATA AAG GCA AAT G	
102R-222R		GTC CCG CAC GCC GTT GTT	
102R-424F		CGC GTG CAT GCA ATG TAT	
102R-797F		GCA ATG TCT GTC 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 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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 001R	111,584	2196	2973	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	99.70%	ORF 001R	99.70%	ORF 639R	98.18%	76L	93.44%	69L	92.91%
ORF 002R	2198	2467	270	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV RSIV Ehime-1	100.00%	ORF 002R	96.67%	ORF 010R	100.00%	75L	91.30%	68L	87.26%
ORF 003L	2476	3495	1020	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV RSIV Ehime-1	100.00%	ORF 003L	98.53%	ORF 016L	100.00%	74R	93.63%	67R	93.94%
ORF 004L	3544	4032	489	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 004L	95.09%	ORF 019L	100.00%	73R	90.24%	66R	84.72%
ORF 005R	4015	5625	1611	hypothetical protein	RSIV subtype I	PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 005R	98.08%	ORF 018R	100.00%	71L	93.61%	65L	93.42%
ORF 006L	5528	6043	516	hypothetical protein	RSIV subtype I	PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 006L	97.29%	ORF 026R	100.00%	70L	95.20%	-	-
ORF 007R	6065	6796	732	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 007R	96.86%	ORF 029R	100.00%	69L	86.07%	64L	-
ORF 008R	6808	8241	1434	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 008R	97.63%	ORF 033R	100.00%	68L	93.58%	63L	88.95%
ORF 009R	8192	8860	669	hypothetical protein	RSIV subtype I	LYCIV Zhoushan	100.00%	ORF 009R	98.06%	ORF 037R	98.80%	67L	90.69%	62L	91.68%
ORF 010R	9087	10,130	1044	hypothetical protein	RSIV subtype II / ISKNV subtype I	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RSIV-Ku LYCIV Zhoushan OSGIV	100.00%	ORF 010R	99.81%	ORF 042R	98.46%	66L	92.82%	61L	92.53%
ORF 011R	10,181	10,651	471	RING-finger-containing E3 ubiquitin ligase	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 RBIV-C1 LYCIV Zhoushan RSIV_121 17RbGs	100.00%	ORF 011R	100.00%	ORF 049R	98.51%	65L	91.30%	60L	89.17%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 012R	10,693	12,165	1473	mRNA capping enzyme	RSIV subtype II / ISKNV subtype I	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RSIV-Ku LYCIV Zhoushan OSGIV	100.00%	ORF 012R	99.93%	MCE	97.49%	64L	93.36%	59L	93.28%
ORF 013R	12,205	14,853	2649	putative NTPase I	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1	99.96%	-	-	NTPase	97.92%	63L	93.36%	58L	93.42%
ORF 014R	15,174	19,067	3849	DNA-binding protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1	100.00%	ORF 013R	99.48%	ORF 077R	96.78%	62L	91.81%	57L	93.08%
ORF 015R	19,064	19,870	807	putative replication factor and/or DNA binding-packing	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV	100.00%	ORF 014R	92.94%	ORF 092R	97.65%	61L	93.80%	56L	93.06%
ORF 016R	19,934	20,446	513	hypothetical protein	RSIV subtype II	RSIV KagYT-96 GSIV-K1 OSGIV	100.00%	ORF 015R	89.35%	ORF 097R	96.30%	59L	92.84%	55L	88.95%
ORF 017R	20,918	21,178	261	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 SKIV RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV	100.00%	ORF 016R	95.40%	ORF 099R	98.08%	57L	96.17%	54L	95.40%
ORF 018R	21,185	21,832	648	helicase family	RSIV subtype II	RSIV KagYT-96 GSIV-K1 OSGIV	100.00%	ORF 017R	99.23%	ORF 101R	99.23%	56L	97.22%	53L	97.38%
ORF 019R	21,843	22,784	942	Serine-threonine protein kina	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 SKIV RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 018R	100.00%	ORF 106R	96.92%	55L	90.98%	52L	89.81%
ORF 020R	22,807	23,751	945	hypothetical protein	RSIV subtype II	RSIV KagYT-96 GSIV-K1 SKIV RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 019R	100.00%	ORF 111R	97.67%	54L	90.08%	51L	90.48%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 021L	23,785	23,979	195	hypothetical protein	RSIV subtype II	RSIV KagYT-96RSIV RIE12-1GSIV-K1SKIVRBIV-C1RSIV_121OSGIV17RbGs	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	RSIV KagYT-96 RSIV 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	RSIV KagYT-96 RSIV 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	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-KOR-TY1 OSGIV	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	RSIV KagYT-96 RSIV 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	RSIV KagYT-96 RSIV 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	RSIV KagYT-96 RSIV 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%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 028R	26,167	26,850	684	cytosine DNA methyltrans-ferase	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	915	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	801	hypothetical protein	RSIV subtype I	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	363	Erv1/Alr family	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	600	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	1350	hypothetical protein	RSIV subtype I	LYCIV Zhoushan	100.00%	ORF 032R	97.04%	ORF 162R	99.56%	41L	88.96%	40L	90.53%
ORF 034R	30,981	32,129	1149	hypothetical protein	RSIV subtype I	LYCIV Zhoushan	100.00%	ORF 033R	91.91%	ORF 171R	91.22%	40L	89.65%	39L	98.43%
ORF 035L	32,122	33,000	879	hypothetical protein	RSIV subtype I	LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 034L	93.97%	ORF 179L	100.00%	39R	90.22%	38R	90.90%
ORF 036R	33,066	34,505	1440	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 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	1350	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	1056	hypothetical protein	RSIV subtype I	PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 037L	95.17%	ORF 197L	100.00%	36R	91.49%	35R	88.93%
ORF 039R	36,909	38,048	1140	hypothetical protein	RSIV subtype I	PIV2010 LYCIV Zhoushan	100.00%	ORF 038R	95.53%	ORF 198R	99.91%	35L	88.64%	34L	88.88%

Table A2. *Cont.*

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.	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	RSIV subtype I	LYCIV Zhoushan	100.00%	ORF 040R	95.90%	ORF 226R	97.79%	33L	91.36%	32L	92.59%
ORF 042L	42,327	42,943	582	deoxyribo-nucleoside kinase	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	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	transcription elongation factor TFIIS	RSIV subtype I	PIV2016PIV2014aPIV 2010LYCIV ZhoushanRSIV Ehime-1	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	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	probable XPG/RAD2 type nuclease	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	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 046R	97.53%	ORF 261R	100.00%	26L	92.00%	26L	90.43%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 048L	49,064	50,002	939	ribonucleotide diphosphate reductase small subunit	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	laminin-type epidermal growth factor	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	LRP16 like protein macro domain-containing protein	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	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 050R	97.27%	ORF 300R	100.00%	20L	89.95%	21L	94.54%
ORF 052L	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	hypothetical protein	RSIV subtype I	PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 052R	92.55%	ORF 318R	100.00%	18.5L	89.89%	19L	91.76%
ORF 054R	58,889	59,221	333	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 053R	88.22%	ORF 321R	100.00%	17L	92.81%	17L	89.47%
ORF 055R	59,236	59,823	588	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	99.66%	ORF 054R	92.35%	ORF 324R	99.66%	16L	91.50%	16L	92.35%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 056L	59,881	60,672	792	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 LYCIV Zhoushan RBIV-KOR-TY1 OSGIV	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	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 LYCIV Zhoushan OSGIV	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	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 OSGIV	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	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 LYCIV Zhoushan RSIV_121 RBIV-KOR-TY1 OSGIV 17RbGs	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	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 LYCIV Zhoushan RSIV_121 OSGIV	100.00%	ORF 059R	98.04%	ORF 351R	96.55%	11L	95.02%	11L	94.90%
ORF 061R	63,919	64,311	393	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 RBIV-C1 RSIV_121 RBIV-KOR-TY1	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	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 LYCIV Zhoushan RSIV_121 RBIV-KOR-TY1 OSGIV 17RbGs	100.00%	ORF 061L	100.00%	ORF 360L	98.77%	9R	97.53%	9R	98.77%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 063L	64,727	66,274	1548	hypothetical protein	RSIV subtype II	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	66,345	67,802	1458	myristoylated membrane protein	RSIV subtype II	RSIV KagYT-96RSIV RIE12-1GSIV-K1LYCIV ZhoushanOSGIV	100.00%	ORF 063R	99.73%	ORF 374R	97.46%	7L	94.51%	7L	94.65%
ORF 065R	67,819	69,180	1362	major capsid protein	RSIV subtype I	LYCIV Zhoushan	100.00%	ORF 064R	98.24%	MCP	99.63%	6L	94.57%	6L	94.27%
ORF 066R	69,326	70,090	765	NIF-NLI interacting factor-like phosphatase	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 065R	98.35%	ORF 385R	100.00%	5L	95.17%	5L	92.82%
ORF 067R	70,164	70,340	177	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 066R	99.44%	ORF 388R	100.00%	4L	91.78%	4L	97.89%
ORF 068R	70,413	71,196	486	hypothetical protein	RSIV subtype I	LYCIV Zhoushan	100.00%	ORF 067R	96.30%	ORF 390R	99.79%	3L	90.00%		86.59%
ORF 069R	71,268	71,735	468	DNA dependent RNA polymerase subunit H like protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 068R	99.36%	RPOH	100.00%	2R	93.83%	2R	94.25%
ORF 070R	71,705	72,841	1137	probable trans-membrane amino acid transporter	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1 LYCIV	100.00%	ORF 069R	97.89%	ORF 396R	100.00%	1L	93.23%	1L	92.52%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 071R	72,956	73,672	717	hypothetical protein	RSIV subtype II	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	73,681	74,061	381	hypothetical protein	RSIV subtype II	RSIV KagYT-96 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	74,033	74,752	720	ATPase(adenosine triphos-phatase)	RSIV subtype II	RSIV KagYT-96 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	74,762	75,397	636	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV	97.48%	ORF 073R	97.48%	ORF 413R	97.16%	121L	86.09%	112L	84.54%
ORF 075L	75,418	75,924	507	hypothetical protein	RSIV subtype II	RSIV KagYT-96 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	75,955	76,242	288	probable tran-scriptional activator RING-finger domain-containing E3 protein	RSIV subtype II	RSIV KagYT-96 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	76,312	77,625	1314	ankyrin repeat-containing protein	RSIV subtype II	RSIV KagYT-96 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 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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 078R	77,958	78,632	675	FV3 early 31KDa protein homolog	RSIV subtype II	RSIV KagYT-96 GSIV-K1 RSIV_121 OSGIV	99.85%	ORF 077R	99.85%	ORF 436R	98.22%	117L	93.79%	108L	94.82%
ORF 079L	78,686	80,062	1377	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 17RbGs	100.00%	ORF 078L	100.00%	ORF 450L	96.27%	116R	86.68%	107R	85.92%
ORF 080L	80,123	81,133	1011	immediate-early protein ICP46	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 17RbGs	100.00%	ORF 079L	100.00%	ORF 458L	98.32%	115R	93.18%	106R	93.08%
ORF 081R	81,568	84,150	2583	putative tyrosine kinase	RSIV subtype II	GSIV-K1	100.00%	ORF 080R	99.96%	ORF 463R	97.99%	114L	93.69%	105L	93.26%
ORF 082L	84,194	84,574	381	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV	100.00%	ORF 081L	99.74%	ORF 483L	97.38%	113R	92.66%	104R	92.89%
ORF 083L	84,682	85,425	744	proliferating cell nuclear antigen	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 082L	100.00%	ORF 487L	98.39%	112R	94.35%	102R	96.01%
ORF 084R	85,445	86,341	897	tumor necrosis factor recepter - assosiated factor-like protein	RSIV subtype II	RSIV KagYT-96RSIV RIE12-1GSIV-K1RBIV-C1RSIV_12117RbGs	100.00%	ORF 083R	100.00%	ORF 488R	97.99%	111L	93.09%	101L	90.41%
ORF 085L	86,338	86,493	156	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV 17RbGs	100.00%	ORF 084L	100.00%	ORF 492L	96.79%	110R	90.38%	100R	91.03%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 086R	86,546	89,308	2763	D5 family NTPase	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	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 subtype II	RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV	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 subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 OSGIV 17RbGs	100.00%	ORF 087R	100.00%	ORF 506R	97.25%	-	-	97L	80.25%
ORF 089L	90,937	91,901	888	HIT-like protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	99.89%	ORF 088L	99.55%	ORF 515L	96.83%	-	-	-	-
ORF 090L	91,953	92,324	372	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV 17RbGs	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 subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV	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	RSIV subtype I	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	ankyrin repeat containing protein	RSIV subtype I	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 094R	95,098	95,613	516	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan RSIV Ehime-1	100.00%	ORF 093R	97.29%	ORF 535R	100.00%	101L	93.80%	92L	92.83%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 095R	95,588	96,229	642	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	99.07%	ORF 094R	98.75%	ORF 539R	98.91%	100L	86.49%	91L	86.67%
ORF 096R	96,283	96,606	324	RING-finger-containing E3 ubiquitin ligase	RSIV subtype II	RSIV KagYT-96 GSIV-K1 RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV 17RbGs	100.00%	ORF 095R	100.00%	ORF 543R	97.53%	99L	91.05%	90L	84.26%
ORF 097R	96,655	97,146	492	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 096R	100.00%	ORF 545R	97.36%	97.5L	94.51%	89L	92.48%
ORF 098R	97,137	97,888	738	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 097R	100.00%	ORF 550R	98.10%	96L	94.58%	88L	93.77%
ORF 099R	97,896	99,059	1164	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	100.00%	ORF 098R	99.91%	ORF 554R	96.91%	95L	91.21%	87L	91.02%
ORF 100R	99,084	99,584	501	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 099R	100.00%	ORF 562R	98.60%	94L	95.41%	86L	93.01%
ORF 101R	99,594	100,520	927	probable RNA binding protein	RSIV subtype II	RSIV KagYT-96RSIV RIE12-1GSIV-K1SKIVRBIV-C1RSIV_121OSGIV17RbGs	100.00%	ORF 100R	100.00%	ORF 569R	97.84%	93L	92.22%	85L	92.02%
ORF 102R	100,641	101,711	1071	myristoylated membrane protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	98.62%	ORF 101R	99.69%	ORF 575R	95.94%	-	-	83L	91.36%
ORF 103L	101,692	103,263	1572	hypothetical protein	RSIV subtype I	PIV2016 PIV2014a PIV2010 LYCIV Zhoushan	98.85%	ORF 102L	98.54%	ORF 586L	98.20%	88R	92.24%	82R	93.26%

Table A2. *Cont.*

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.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 104R	103,311	103,724	414	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV	99.52%	ORF 103R	99.52%	ORF 591R	99.28%		94.31%	81R	95.48%
ORF 105L	103,721	104,518	798	RNase III-like ribonuclease	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 RBIV-C1 RSIV_121 17RbGs	100.00%	ORF 104L	100.00%	RNC	97.99%	87R	94.16%	80R	94.86%
ORF 106L	104,484	104,951	468	Uvr/REP helicase	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	100.00%	ORF 105L	93.80%	ORF 600L	97.44%	86R	92.55%	79R	92.95%
ORF 107L	104,948	105,451	504	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 RBIV-KOR-TY1 OSGIV	100.00%	ORF 106L	92.86%	ORF 605L	97.83%	85R	92.74%	78R	90.73%
ORF 108R	105,565	106,869	1305	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	100.00%	ORF 107R	95.21%	ORF 606R	97.70%	84L	93.16%	77L	91.58%
ORF 109L	106,896	107,255	360	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV	100.00%	ORF 108L	98.89%	ORF 617L	98.33%	83R	93.46%	76R	92.48%
ORF 110R	107,319	10,8425	1107	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 OSGIV	100.00%	ORF 109R	99.82%	ORF 618R	97.92%	82L	93.59%	75L	93.22%
ORF 111L	108,474	108,971	498	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RSIV_121 OSGIV 17RbGs	100.00%	ORF 110L	100.00%	ORF 628L	97.99%	81R	95.78%	74R	94.32%
ORF 112L	108,984	109,457	474	hypothetical protein	RSIV subtype II	RSIV KagYT-96 RSIV RIE12-1 GSIV-K1 RBIV-C1 RBIV-KOR-TY1 OSGIV 17RbGs	100.00%	ORF 111L	100.00%	ORF 632L	95.81%	-	-	73R	85.56%

Table A2. *Cont.*

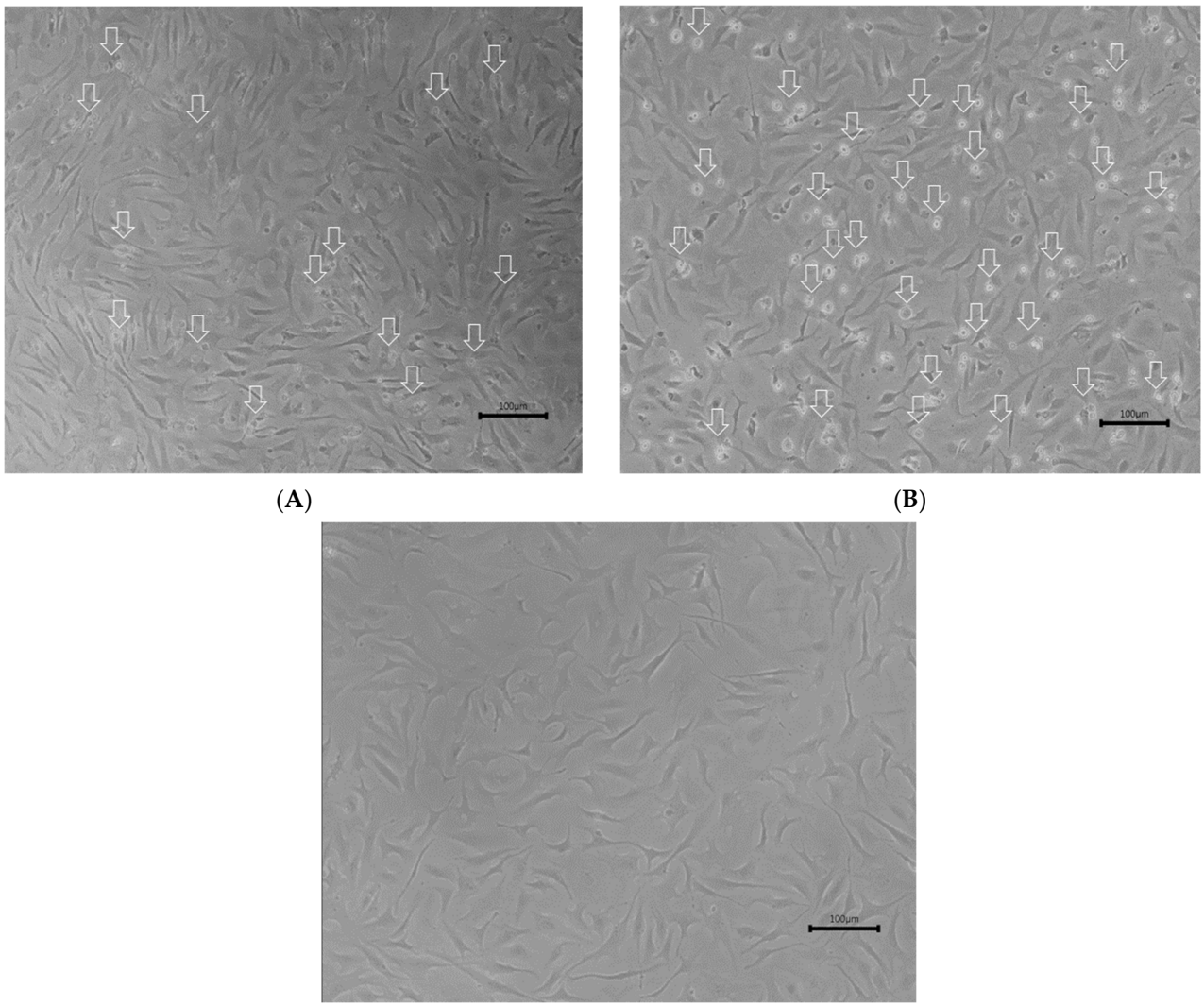
Gene ID 17SbTy	Position		CDS Size (NT)	Predicted Structure and/or Function	Best-Match Homolog			Homolog to 17RbGs		Homolog to Ehime_1 (AB104413.1)		Homolog to ISKNV (AF371960)		Homolog to TRBIV (GQ273492)	
	Start	End			Genotype	Isolates	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)	ORF no.	Identity (%)
ORF 113R	109,545	109,769	225	hypothetical protein	RSIV 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	RSIV 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	RSIV 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%

Table A3. The coding sequences (CDSs) determined via COG classification of 17SbTy and 17RbGs in four functional categories.

No.	Category	COG Function	COG Descrption	17SbTy	17RbGS
1	Metabolism	Amino acid transport metabolism	quinoprotein dehydrogenase-associated putative ABC transporter substrate-binding protein	ORF 093L	ORF 092L
2		Nucleotide transport and metabolism	deoxynucleoside kinase	ORF 042L	ORF 041L
3			ribonucleoside-diphosphate reductase	ORF 048L	ORF 047L
4			HIT domain-containing protein	ORF 089L	ORF 088L
5	Information storage and processing	Translation, ribosomal structure and biogenesis	O-acetyl-ADP-ribose deacetylase	ORF 050R	ORF 049R
6		Transcription	DNA-directed RNA polymerase subunit B	ORF 040L	ORF 039L
7			transcription factor S	ORF 044R	ORF 043R
8			DNA-directed RNA polymerase subunit A&apos	ORF 045R	ORF 044R
9			phosphoprotein phosphatase	ORF 066R	ORF 065R
10			ribonuclease III	ORF 105L	ORF 104L
11		Replication, recombination and repair	DNA cytosine methyltransferase	ORF 028R	ORF 027R
12			flap endonuclease-1	ORF 046R	ORF 045R
13			DNA polymerase elongation subunit	ORF 052L	ORF 051L
14	Cellular process	Signal transduction mechanisms	protein-tyrosine-phosphatase	ORF 12R	ORF 012R
15			ankyrin repeat-containing protein	ORF 077R	ORF 076R
16			quinoprotein dehydrogenase-associated putative ABC transporter substrate-binding protein	ORF 093L	ORF 092L
17			ankyrin repeat-containing protein	ORF 115L	ORF 114L
18		Mobilome; prophages, transposons	hypothetical protein	ORF 086R	ORF 085R
19	Poorly characterized	General function prediction only	HIT domain-containing protein	ORF 089L	ORF 088L
20		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	015R	014R	092R	61L	57L	56L
4	Helicase family	018R	017R	101R	56L	54L	53L
5	Serine-threonine protein kinase	019R	018R	106R	55L	53L	52L
6	Erv1/Alr family	031R	030R	156R	43L	43.5L	42L
7	DNA dependent RNA polymerase second largest subunit	040L	039L	RPO-2	34R	33R	33R
8	Deoxynucleoside kinase	042L	041L	TK	32R	31R	31R
9	Transcription elongation factor TFIIS	044R	043R	238R	29L	29.5Lb	29L
10	DNA dependent RNA polymerase largest subunit	045R	044R	RPO-1	28L	29L	26L
11	Putative XPPG-RAD2-type nuclease	046R	045R	256R	27L	28L	27L
12	Ribonucleotide reductase small subunit	048L	047L	RR-2	24R	26R	25R
13	DNA pol Family B exonuclease	052L	051L	DPO	19R	20R	20R
14	Serine-threonine protein kinase	058L	057L	349L	13R	13R	13R
15	Myristoylated membrane protein	064R	063R	374R	7L	8L	7L
16	Major capsid protein	065R	064R	MCP	6L	7L	6L
17	NIF-NLI interacting factor	066R	065R	385R	5L	6L	5L
18	ATPase(adenosine triphosphatase)	073L	072L	412L	122R	116R	113R
19	Immediate early protein ICP-46	080L	079L	458L	115R	108.5R	106R
20	Putative tyrosin kinase/lipopolysaccharide modifying enzyme	081R	080R	463R	61L, 114L	57L, 106Lb	105L
21	Proliferating cell nuclear antigen	083L	082L	487L	112R	103Rb	102R
22	D5 family NTPase involved in DNA replication	086R	085R	493R	109L	101L	99L
23	hypothetical protein	098R	097R	550R	96L	89.5Lb	88L
24	Myristoylated membrane protein	102R	101R	575R	90.5L	85L	83R
25	RNase III-like ribonuclease	105L	104L	RNC	87R	83R	80R
26	Uvr/REP helicase	106L	105L	600L	86R	82.5R	79R



(**A**) (**B**)

(**C**)

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.

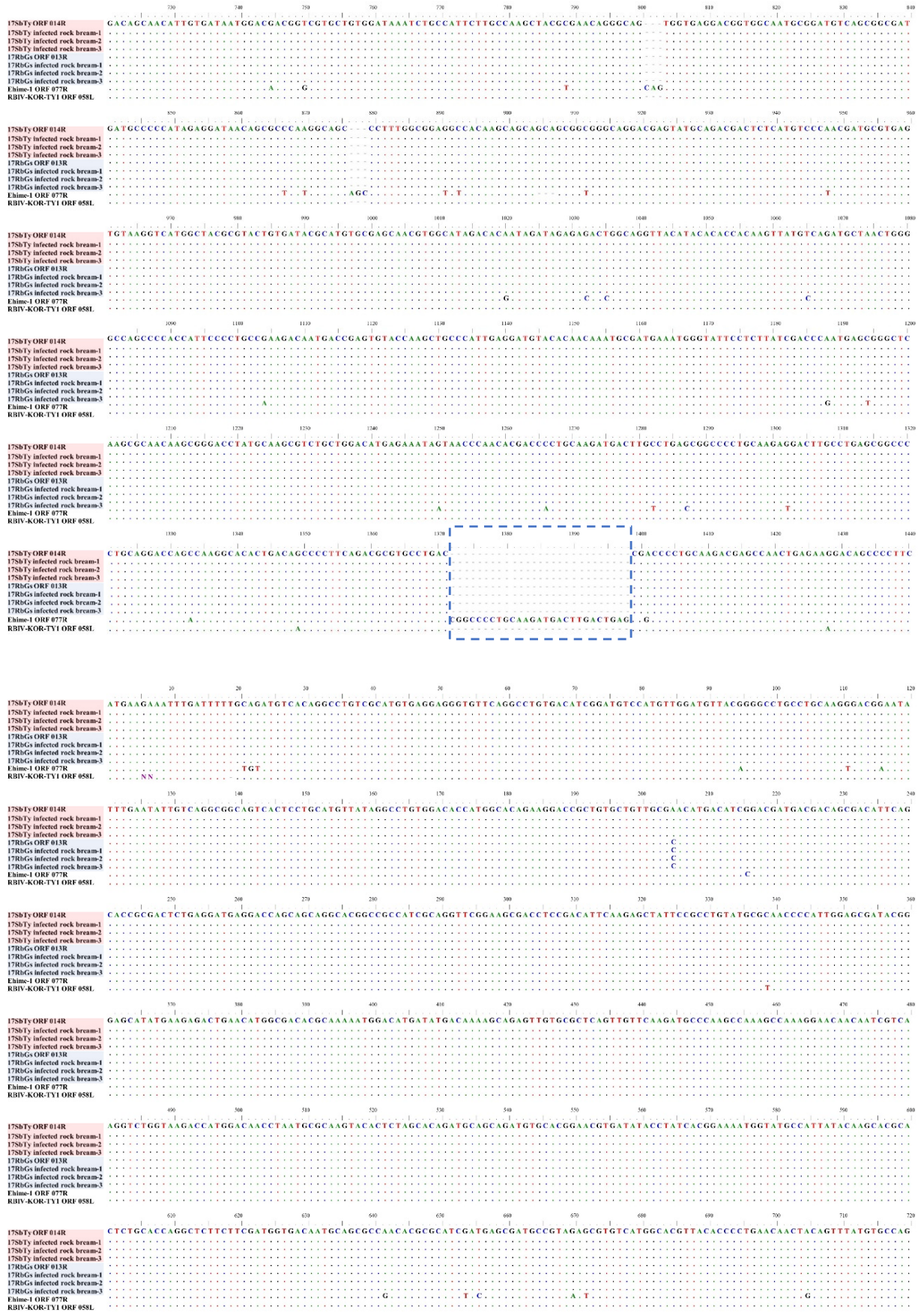
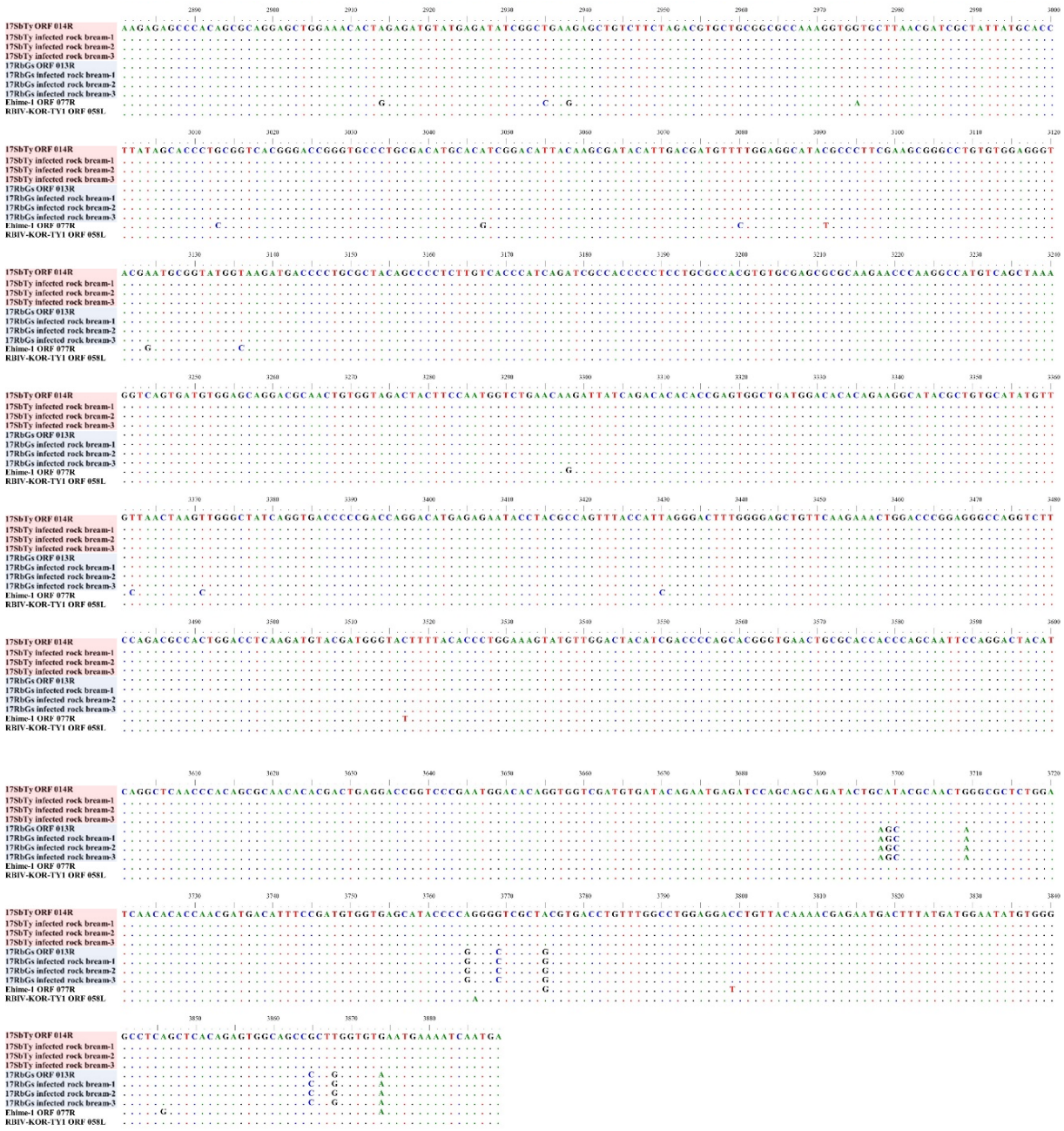


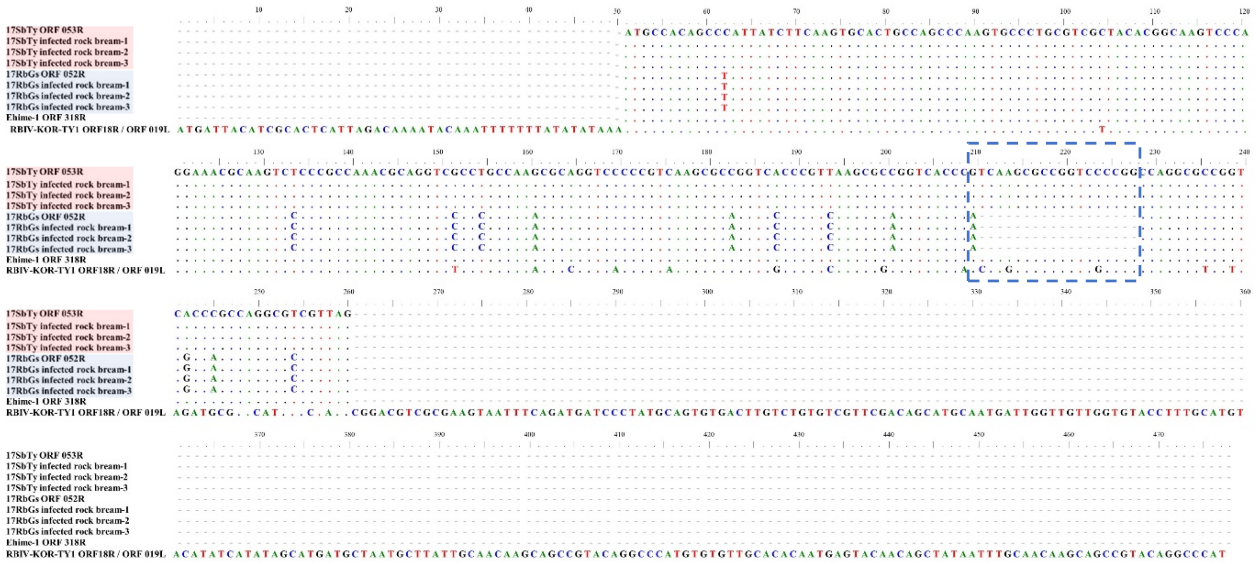
Figure A2. *Cont.*

Figure A2. *Cont.*

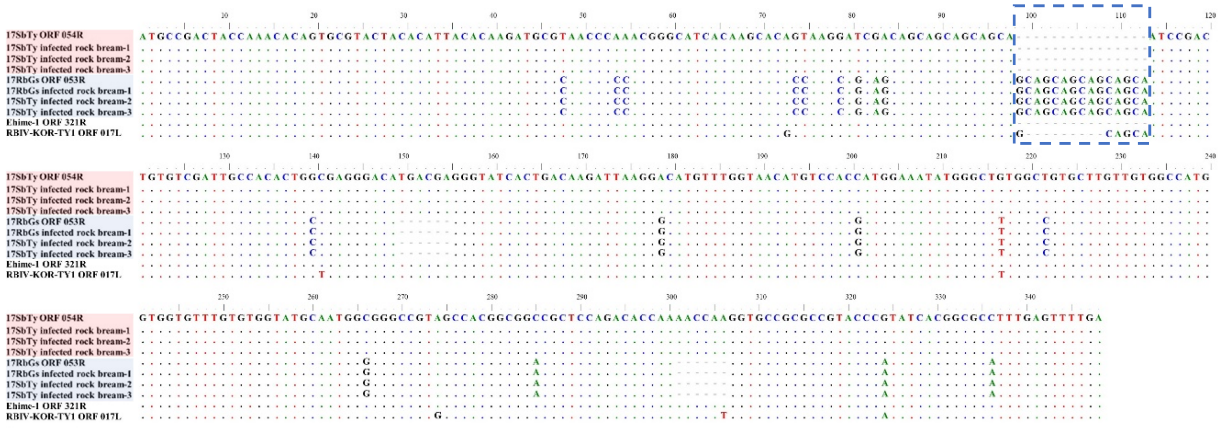


(**a**)

Figure A2. *Cont.*



(**b**)



(**c**)

Figure A2. *Cont.*

(**d**)

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 17SbTy isolate) between the cell-cultured isolates and viruses from RSIV-infected rock breams. The 17SbTy 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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