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ORIGINAL ARTICLE

Genetic analysis of fish iridoviruses isolated in Taiwan during 2001–2009

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Abstract To investigate the genetic relationships between field strains of iridoviruses gathered from various fish species in Taiwan, viruses that were collected from 2001 to 2009 were analyzed. Open reading frames encoding the viral major capsid protein (MCP) and adenosine triphosphatase (ATPase) were sequenced for phylogenetic analysis. Our results indicated that iridoviruses from Taiwan aquaculture fishes could be classified into two groups: prior to 2005, the viruses were closely related to members of the genus *Ranavirus*; and after 2005, they were similar to members of the genus *Megalocytivirus*. Based on the analysis of MCP amino acid sequences, virus isolates were divided into 4 major genotypes that were related to ISKNV, RSIV, FLIV, and GIV, respectively. Pairwise comparisons of MCP genes showed that the ranavirus was an epidemic pathogen for economically important species in the major production regions and cultured marine fish, while the megalocytivirus isolates were sensitive to host range. In addition, the distribution of synonymous and non-synonymous changes in the MCP gene revealed that the iridoviruses were evolving slowly, and most of the variations were synonymous mutations. The *Ka*/*Ks* values were lower than one, and hence, the viruses were under negative selection.

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Introduction

Iridoviruses have been widely reported over the past 20 years in epizootics of finfish and amphibians in many countries. The virus has caused epidemics in Asia through international trade. Piscine iridoviruses that have been reported in Asian countries are classified into three genera: *Lymphocystivius, Ranavirus* and *Megalocytivirus* [6, 11, 26, 29, 33]. In Japan, red sea bream iridoviral disease (RSIVD) has been documented in 31 cultured seawater fishes [15]. In China, a molecular epidemiology study indicated that over 50 species of cultured and wild seawater fish were infected by infectious spleen and kidney necrosis virus–like (ISKNV-like) viruses [33]. In Korea, megalocytiviruses were reported in 10 freshwater ornamental fish species and 7 cultured fish species [6, 14].

Several iridovirus infections have been reported in Taiwan. The first one was reported in grouper, with diagnostic evidence provided by electron microscopy observations [1]. According to the histological, genetic, and microscopic characteristics, the epidemic strains were

similar to RSIV [3, 32]. In previous studies, the grouper iridovirus TGIV was isolated in Taiwan (TGIV) using the KRE cell line [5], while a GK cell line was suitable for culture of grouper iridovirus (GIV), which was determined by molecular characterization to belong to the genus *Ranavirus* [19, 30]. Previous studies have shown that Singapore grouper iridovirus (SGIV) was transferred from Taiwan through diseased grouper fry [20].Viruses from cultured seawater fish in Taiwan were recently determined to be more closely related to the RSIV genotype than to the ISKNV genotype [31]. However, these findings were limited to one fish species of grouper and a single-culture environment. The genetic relationships between epidemic strains, geographic distribution, and host susceptibility of seawater and freshwater fish in Taiwan are unclear. In addition, the degree of variation in such iridoviruses is also unclear between different hosts and environments.

The most important gene for the analysis of genetic relationships in members of the family *Iridoviridae* is the major capsid protein (MCP) gene because this gene product is a structural protein with highly conserved domains [6, 8, 9] that encapsulate the most detailed evolutionary information. In addition, other genes with highly conserved regions, such as those encoding adenosine triphosphatase (ATPase), DNA polymerase, and methyltransferase can provide complementing information [34]. In this study, the genetic diversity of the complete MCP and ATPase genes of iridovirus isolates isolated from seawater and freshwater fishes between 2001 and 2009 in Taiwan was investigated. Furthermore, we evaluate the genetic changes of these isolates and determine the relationships between the epidemic strains. These data could provide important epidemiological information about viral transmission in different fish species and be used to discriminate imported carry-in from endemic circulation.

Materials and methods

Viruses

Twenty-three iridovirus isolates from seawater and freshwater fish from farms distributed throughout all seven Taiwan prefectures (Pingtung, Kaohsiung, Penghu, Tainan, Chiayi, Nantou and Taipei) between 2001 and 2009 were collected. The virus samples were collected from eight species of cultured fish in Taiwan: 11 isolates from giant grouper (*Epinephelus lanceolatus*), five from orange-spotted grouper (*Epinephelus coioides*), three from giant seaperch (*Lates calcarifer*), one from crimson snapper (*Lutjanus erythropterus*), one from silver sea bream (*Rhabdosargus sarba*), one from largemouth bass (*Micropterus salmoides*), one from rock bream (*Oplegnathus fasciatus*), and one from marble goby (*Oxyeleotris marmoratus*). Sources of the fish iridoviruses used in this study are listed in Table 1. "GG" refers to fish species of giant grouper; "IV" represents iridovirus, followed by the geographic site of isolation, the isolate number, and the year of isolation.

DNA extraction and primer design for PCR

Viral DNA was extracted from pooled organ tissues using a QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The extracted DNA was eluted with 100 µl of elution buffer. Primers for the genes encoding MCP and ATPase were designed based on the nucleotide sequence of ISKNV (AF371960), RSIV (AY310918), Korean flounder iridovirus (FLIV) (AY633992) and GIV (AY666015). Primers RMCP-F and RMCP-R were derived from the full-length DNA sequences of the MCP gene and were used to amplify approximately 1392 base pairs of the genomes of the isolates belonging to the genus *Ranavirus*. Primers MMCP-F and MMCP-R were used to amplify the MCP gene of the isolates belonging to the genus *Megalocytivirus*, and the expected product size was 1362 base pairs. For amplification the ATPase gene, a similar strategy was used for primer design. Both sets of primer sequences were modified slightly, as shown in Table 2, to amplify the expected PCR products.

PCR amplification, TA cloning and sequencing

PCR was carried out in 50-µl reactions containing 1XPCR buffer for Blend Taq (TOYOBO), 0.2 mM dNTPs, 10 pmol of each primer, 1.25 units of Blend Taq DNA polymerase, and 100 ng of extracted genomic DNA. PCR was performed in a PTC-200 DNA thermal cycler (MJ Research Inc., USA) at 95°C for 5 min; 35 cycles of 94°C for 30 sec, 54-58°C for 50 sec, and 72°C for 1 min; and extension at 72°C for 7 min. The PCR products were cloned into the pGEM-T vector (Promega) and sequenced using an ABI PRISM 377 DNA sequencer with the BigDye Terminator Kit (Applied Biosystems, Inc.). Several independent PCR clones (6-8 clones) were analyzed to produce a consensus nucleotide sequence for each virus isolate.

Phylogenetic analysis

Nucleotide and amino acid sequences were analyzed using the BioEdit 7.0.4 software. Multiple sequences were aligned using the ClustalW program. Phylogenetic relationships among species were determined using the neighbor-joining (NJ) method [21], and the reliability of the NJ tree was inferred using the Felsenstein bootstrap method with 1000 replications. This study compared the

Table 1 Sources of iridoviruses isolated from cultured fish in Taiwan between 2001 and 2009

Virus name	Fish species	Size (g)	Year	Accession no.	
				MCP	ATPase
GGIV/Pt/1691/01	Giant grouper (*Epinephelus lanceolatus*)	9.4	2001	JF264367	JF264227
GGIV/Pt/1241/01	Giant grouper (*Epinephelus lanceolatus*)	2.2	2001	JF264359	JF264219
GGIV/Pt/1348/01	Giant grouper (*Epinephelus lanceolatus*)	8.2	2001	JF264363	JF264223
CSIV/Pt/2431/02	Crimson snapper (*Lutjanus erythropterus*)	3.3	2002	JF264357	JF264217
LMBIV/Kh/460/03	Largemouth bass (*Micropterus salmoides*)	5.3	2003	JF264364	JF264224
GGIV/Tn/337/04	Giant grouper (*Epinephelus lanceolatus*)	51.3	2004	JF264360	JF264220
GGIV/Cy/346/04	Giant grouper (*Epinephelus lanceolatus*)	7.4	2004	JF264366	JF264226
OSGIV/Pt/403/04	Orange-spotted grouper (*Epinephelus coioides*)	3.5	2004	JF264365	JF264225
OSGIV/Ph/617/04	Orange-spotted grouper (*Epinephelus coioides*)	11.6	2004	JF264361	JF264221
OSGIV/Tn/352/05	Orange-spotted grouper (*Epinephelus coioides*)	4.2	2005	JF264358	JF264218
SSBIV/Pt/703/05	Silver sea bream (*Rhabdosargus sarba*)	[a]	2005	JF264356	JF264216
GSIV/Pt/836/05	Giant seaperch (*Lates calcarifer*)	200	2005	JF264350	JF264210
GSIV/Pt/843/05	Giant seaperch (*Lates calcarifer*)	5	2005	JF264354	JF264214
GGIV/Pt/36/06	Giant grouper (*Epinephelus lanceolatus*)	10	2006	JF264347	JF264207
GGIV/Pt/48/06	Giant grouper (*Epinephelus lanceolatus*)	10.3	2006	JF264351	JF264211
GGIV/Pt/96/06	Giant grouper (*Epinephelus lanceolatus*)	[a]	2006	JF264355	JF264215
GSIV/Pt/113/06	Giant seaperch (*Lates calcarifer*)	2.2	2006	JF264353	JF264213
OSGIV/Pt/141/06	Orange-spotted grouper (*Epinephelus coioides*)	2.2	2006	JF264345	JF264205
RBIV/Tp/45/08	Rock beam (*Oplegnathus fasciatus*)	2.6	2008	JF264352	JF264212
OSGIV/Pt/308/08	Orange-spotted grouper (*Epinephelus coioides*)	4.7	2008	JF264349	JF264209
GSIV/Pt/327/08	Giant seaperch (*Lates calcarifer*)	6.8	2008	JF264346	JF264206
GSIV/Pt/610/08	Giant seaperch (*Lates calcarifer*)	52	2008	JF264362	JF264222
MGIV/Nt/546/09	Marble goby (*Oxyeleotris marmorata*)	24	2009	JF264348	JF264208

[a] Spleen

Table 2 PCR primers used for gene amplification

Virus genus	Primer	Sequence	Product (bp)
Ranavirus	RMCP-F	5′-ATGACTTGTACAACGGGT-3′	1392
	RMCP-R	5′-TTACAAGATAGGGAACCCCAT-3′	
	RATPase-F	5′-TGTGTTAAAATGTCTCAATA-3′	972
	RATPase-R	5′-CGAATCATGGAAAGCACCGCAACC-3′	
Megalocytivirus	MMCP-F[a]	5′-ATGTCTGC**R**ATCTCAGGTG-3′	1362
	MMCP-R[b]	5′-T**Y**ACAGGATAGGGAAGCCTG-3′	
	MATPase-F[c]	5′-ATGGAAATC**M**AAGAGTTGTCC**Y**TG-3′	720
	MATPase-R	5′-TTAC**R**CCACGCCAGCCTTGTA-3′	

[a] Nucleic acid code R refers to A or G

[b] Nucleic acid code Y refers to T or C

[c] Nucleic acid code M refers A or C. Modified nucleotides are shown in bold

nucleotide and amino acid sequences of the MCP and ATPase genes with previously reported iridoviruses to determine the relationship between Taiwan iridovirus isolates. Sequences that were retrieved from the GenBank databases are shown in Table 3. Pairwise comparisons of the maximum nucleotide identities for MCP among different hosts using the MEGA 4.0.2 program [27] are shown in Table 4. Ratios of non-synonymous nucleotide substitutions per non-synonymous site (*Ka*) and synonymous nucleotide substitutions per synonymous site (*Ks*) were calculated using the MEGA 4.0.2 program. Sequences of the MCP and ATPase genes determined in this study were submitted to GenBank, and the accession numbers are listed in Table 1.

Table 3 Iridovirus reference strains selected from the GenBank database

Virus	Genus	Origin	MCP accession no.	ATPase accession no.	References
SGIV	*Ranavirus*	Singapore	AAS18087	AY521625	[25]
GIV	*Ranavirus*	Taiwan	AAV91066	AY666015	[30]
FV3	*Ranavirus*	USA	AAT09750	AY548484	[28]
TFV	*Ranavirus*	China	AAL77814	AF389451	[10]
ATV	*Ranavirus*	North America	AAP33191	AY150217	[13]
TRBIV	*Megalocytivirus*	China	AY590687	AY608684	[22]
DGIV	*Megalocytivirus*	Southeast Asia	AY285744	AY319288	[26]
ISKNV	*Megalocytivirus*	China	AAL98730	AF371960	[9]
OSGIV	*Megalocytivirus*	China	AAX82316	AY894343	[17]
RSIV	*Megalocytivirus*	Japan	AY310918	AB007367	[16]
FLIV	*Megalocytivirus*	Korea	AY633992	N/A	[6]

N/A not applicable, *SGIV* Singapore grouper iridovirus, *GIV* grouper iridovirus, *FV3* frog virus 3, *TFV* tiger frog virus, *ATV* Ambystoma tigrinum stebbensi virus, *TRBIV* turbot reddish body iridovirus, *DGIV* dwarf gourami iridovirus, *ISKNV* infectious spleen and kidney necrosis virus, *OSGIV* orange-spotted grouper iridovirus, *RSIV* red sea bream iridovirus, *FLIV* Korean flounder iridovirus

Table 4 Comparisons of maximum identities in the MCP genes of isolates in our study and the genotypes of reference viruses in GenBank

Genotype and host	Virus strain/nucleotide sequence maximum identity (%) (number of isolates)			Total number of fish species
	GIV[a]	ISKNV[b]	RSIV[c]	
GGIV (Giant grouper)	97.9–100 (n = 5)	94.9	99.6–99.9 (n = 3)	8
OSGIV (Orange-spotted grouper)	98.2–100 (n = 3)	99.9 (n = 2)	95.1	5
GSIV (Giant seaperch)	98.6[d] (n = 1)	99.5–99.8[d] (n = 3)	99.7 (n = 1)	5
CSIV (Crimson snapper)	97.9[d] (n = 1)	51.8	50.8	1
SSBIV (Silver sea bream)	51.8	99.9 (n = 1)	95.1	1
RBIV (Rock bream)	51.3	92.9	93.3 (n = 1)	1
MGIV (Marble goby)	51.7	94.2	97.9[d] (n = 1)	1
LMBIV (Largemouth bass)	97.9 (n = 1)	52	51.3	1
Total number of isolates	11	6	6	

[a] GenBank accession number AY666015

[b] GenBank accession number AF371960

[c] GenBank accession number AY310918

[d] First reported in this study

Results

Phylogenetic analysis of MCP

The MCP open reading frame (ORF) of was 1362 bp long and encoded 453 amino acids of the isolates belonging to the genus *Megalocytivirus*. In isolates belonging to the genus *Ranavirus*, the ORF was 1392 bp long and coded for a protein of 463 amino acids. In this study, we compared the full-length nucleotide and amino acid sequences of MCP with those previously reported from iridoviruses to determine the relationships among the 23 Taiwanese isolates. Based on the MCP nucleotide phylogenetic tree (Fig. 1), the 23 isolates were divided into 6 groups within the genera *Ranavirus* and *Megalocytivirus*. Isolates belonging to the genus *Megalocytivirus* were divided into 3 major groups: six isolates were closely related to the ISKNV genotype (group I), five isolates clustered with RSIV (group II), and 1 isolate was closely related to TRBIV and FLIV (group III). In the genus *Ranavirus*, the bootstrap values supported three major groups: three isolates were closely related to GIV (group V), eight isolates were related to SGIV (group VI) and FV3, and TFV was in group IV. In contrast, only five groups were supported in the phylogenetic tree of amino acid sequences (Fig. 2). In the *Megalocytivirus* cluster, the phylogenetic tree of amino acid sequences is similar to the one based on nucleotide sequences, consisting of three groups; however, all of the isolated strains that were related to ranaviruses from fish were clustered in groupV.

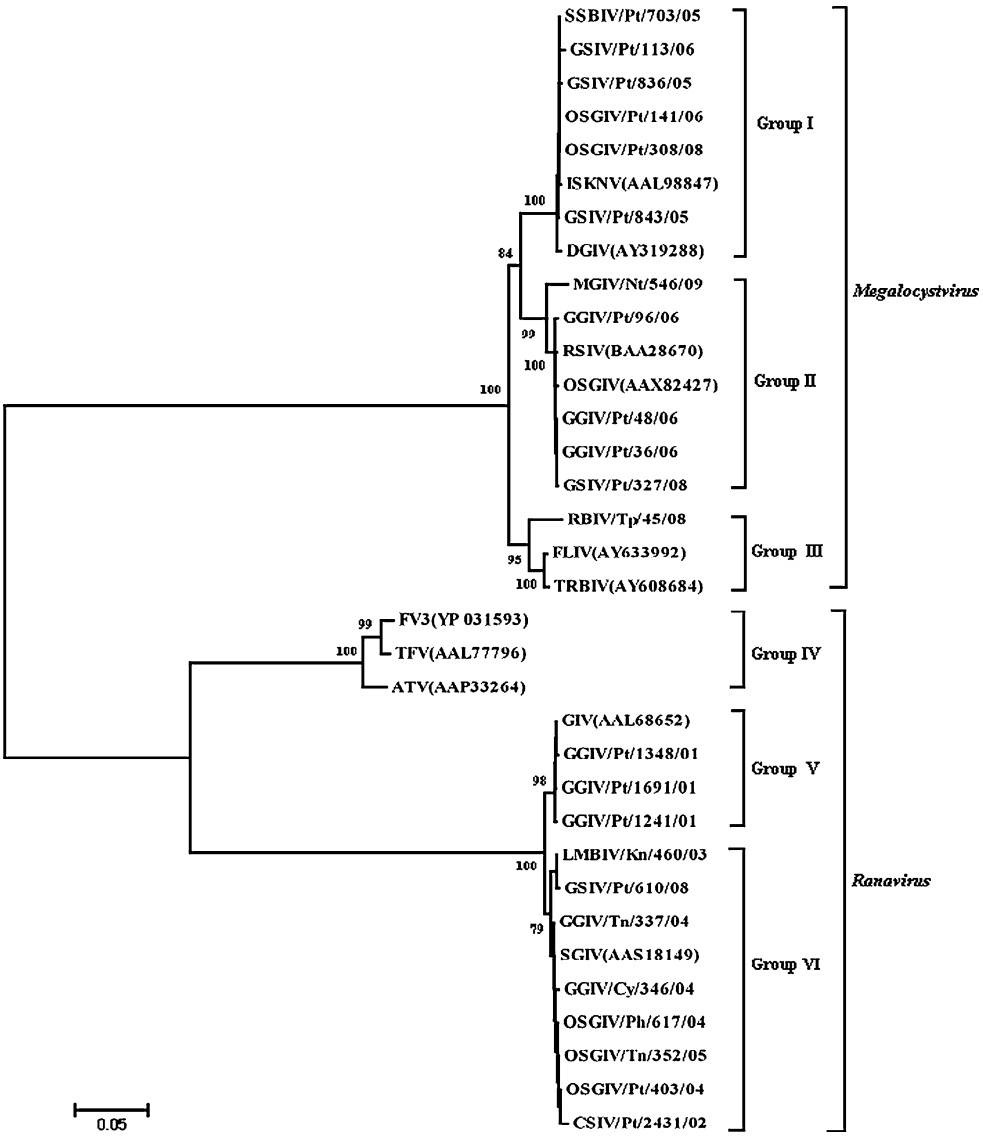

Fig. 1 Phylogenetic analysis of iridovirus MCP. The full-length nucleotide sequences of the MCP genes from 23 Taiwan isolates were compared with previously reported sequences. Full virus names and accession numbers of previously published sequences are described in the legends of Tables 1 and 3. Numbers at the tree nodes indicate bootstrap values of 1000 replicates; bootstrap values below 70 are not shown. The scale bar is a genetic distance marker

Phylogenetic analysis of ATPase

The full-length ATPase gene of the megalocytiviruses was 720 bp long, encoding a protein of 233 amino acids, whereas in the ranaviruses, it was 972 bp long, encoding a protein of 242 amino acids. In this study, we compared nucleotide and amino acid sequences of the ATPase genes with those previously reported from iridoviruses to determine the relationships among the 23 Taiwanese isolates. Based on the ATPase nucleotide phylogenetic tree (Fig. 3), the 23 isolates could be divided into 5 groups in the genera *Ranavirus* and *Megalocytivirus*. The bootstrap values supported three major groups belonging to the genus *Megalocytivirus*. In group I, six isolates were closely related to the ISKNV genotype, and five isolates were related to RSIV (group II). There was one isolate related to TRBIV (group III). In the genus *Ranavirus*, the bootstrap values supported two major groups: the previously reported iridoviruses that originated from frogs species were localized in group IV, and all of the isolates that were collected from fish species were localized to group V and group VI. Based on the amino acid sequences (Fig. 4), phylogenetic analysis indicated that the 23 isolates could only be divided into 3 groups. All of the isolated strains that were closely related to members of the genera *Ranavirus* and *Megalocytivirus* were in group I and III, respectively.

Identity analysis using MCP

In order to clarify the relationships between fish hosts and virus strains, pairwise comparison methods were used to determine the maximum identity of the MCP gene from the 23 isolates. Representative strains of each group (GIV,

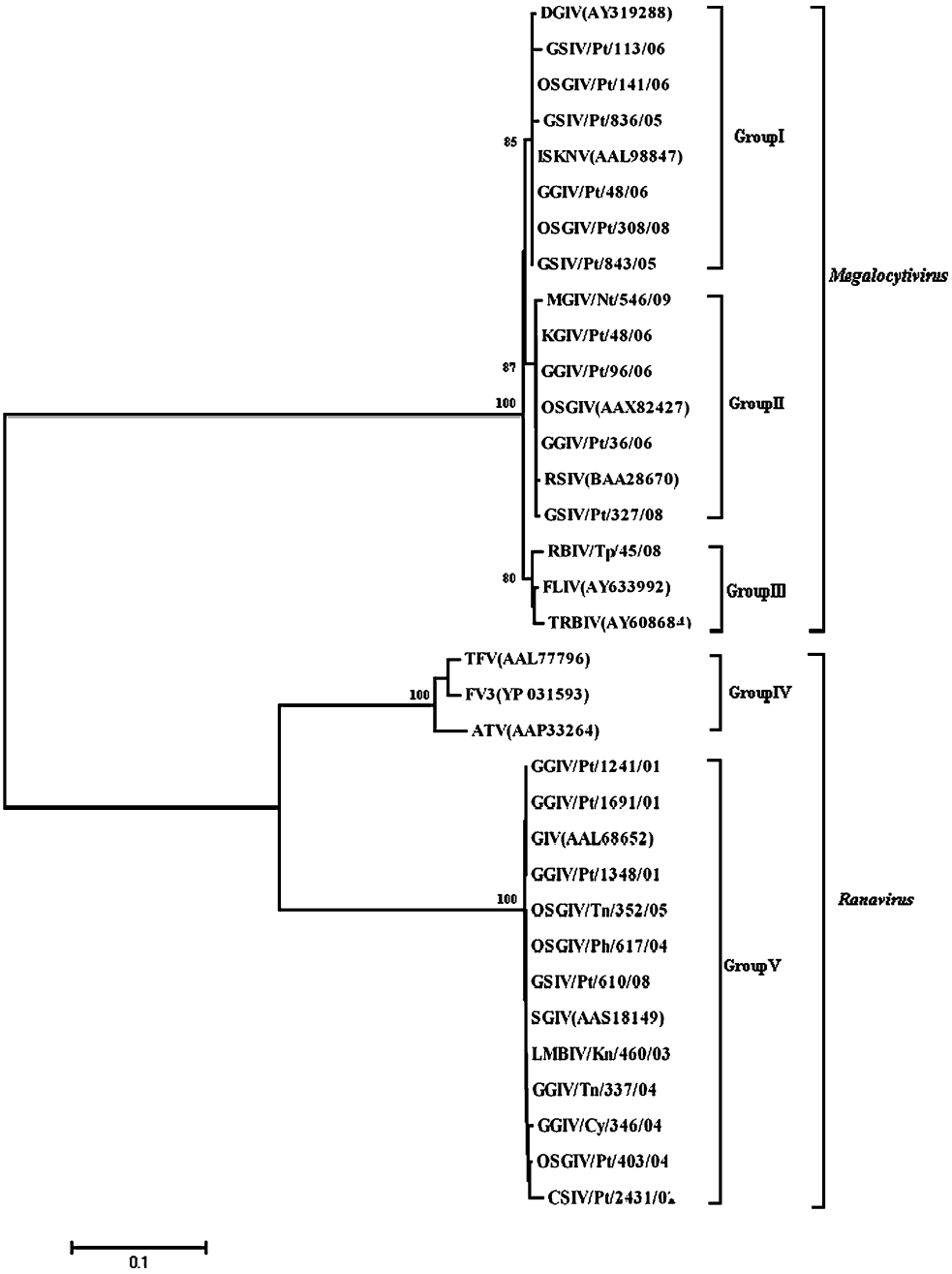

Fig. 2 Phylogenetic analysis of the MCP amino acid sequences. All of the specifications of the tree are similar to those in Fig. 1

ISKNV, and RSIV) were included in our analysis. Comparison of GIV-related isolates from five different fish species revealed that they had a high degree of sequence identity to each other (97.9-100%). ISKNV-related isolates from three different fish species were also found to be highly identical to each other, with a high degree of sequence identity (99.5-99.9%). Finally, RSIV-related isolates that were found in three different fish species had a high degree of sequence identity to each other (97.9-99.9%). Furthermore, RBIV/Tp/45/08 showed a high degree of identity to RSIV (93.32%) (Table 4). These results demonstrate that giant seaperch, giant grouper, and orange-spotted grouper are susceptible to ranaviruses and megalocytiviruses. In Taiwan, the ranavirus was responsible for the epidemic affecting mostly marine cultured fish of the Pingtung, Kaohsiung, Penghu, Tainan, and Chiayi regions. Megalocytivirus isolates were found in both seawater and freshwater fish. Additionally, infections with GIV identified in crimson snapper and RSIV in marble goby were reported for the first time. These results indicated that viral transmission is possible in both seawater and freshwater fish species.

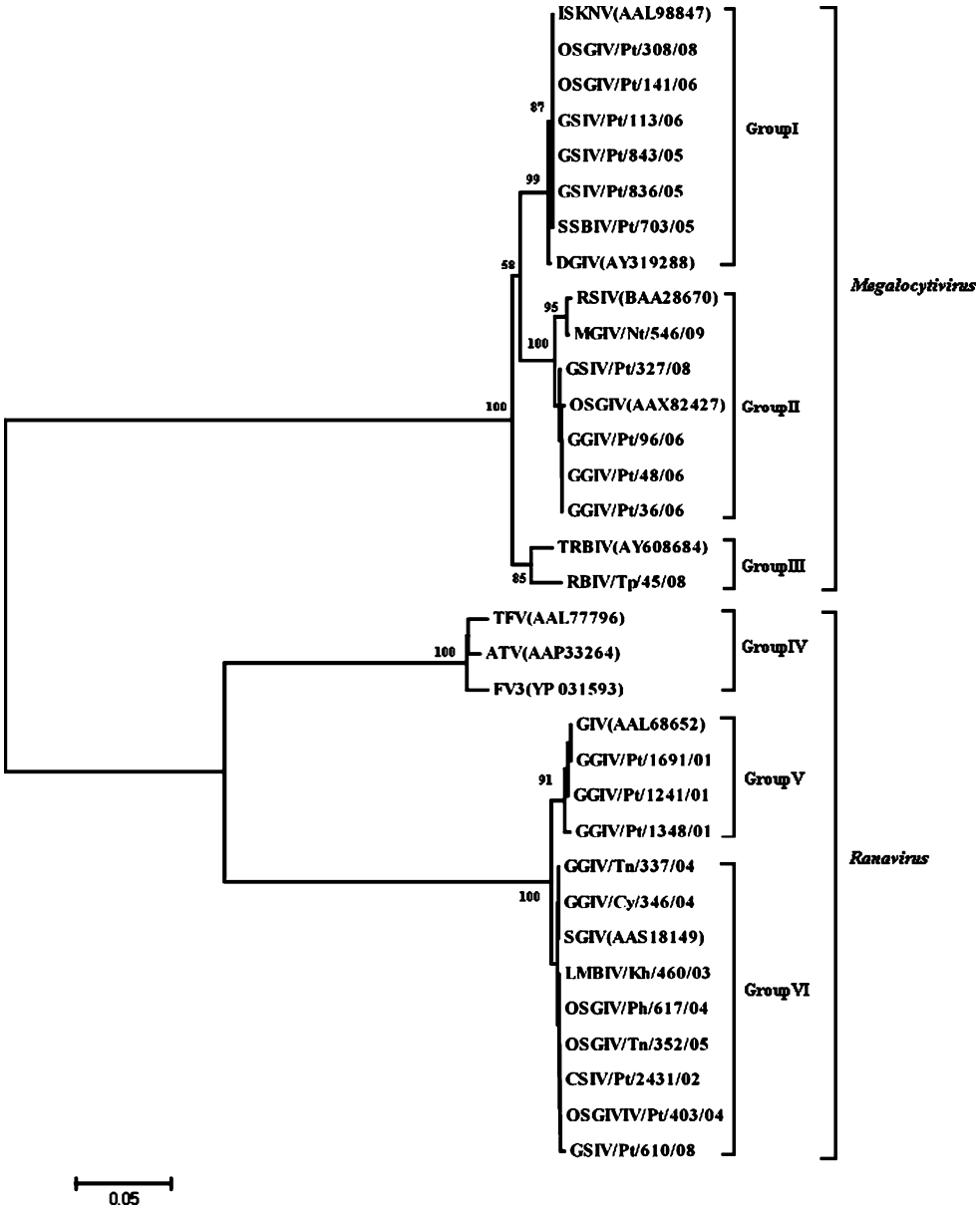

Fig. 3 Phylogenetic analysis of nucleotide sequences of the ATPase gene. All specifications of the tree are similar to those in Fig. 1

Analysis of genetic mutations in MCP

MCP is located on the surface of viral particles and is under selective pressure from host immune responses. To further investigate the extent of sequence variations in the MCP gene, we calculated the non-synonymous and synonymous substitution rates of 23 isolates and compared them to representative strains of GIV, ISKNV, and turbot reddish body iridovirus (TRBIV). The results are summarized in Table 5. Synonymous substitution rates ranged from 0 to 0.11%, whereas the non-synonymous rates were lower than 0.01%. Overall, synonymous substitutions were more frequent than non-synonymous substitutions in all isolates. The *Ka*/*Ks* values were lower than 1, indicating that the viruses are under negative selection. Furthermore, the analysis revealed that the nucleotide mutation sites were located in the first and second nucleotides but did not result in amino acid changes.

Discussion

In phylogenetic analysis, iridoviruses from Taiwanese fish clustered into two different genera. Prior to 2005, the epidemic strains were genetically similar to members of the genus *Ranavirus*; however, after 2005, they were closely related to members of the genus *Megalocytivirus* (Fig. 1). In previous studies, classification of the ranaviruses was based on host species and geographical range, as they are mostly detected in distinctive hosts and geographical

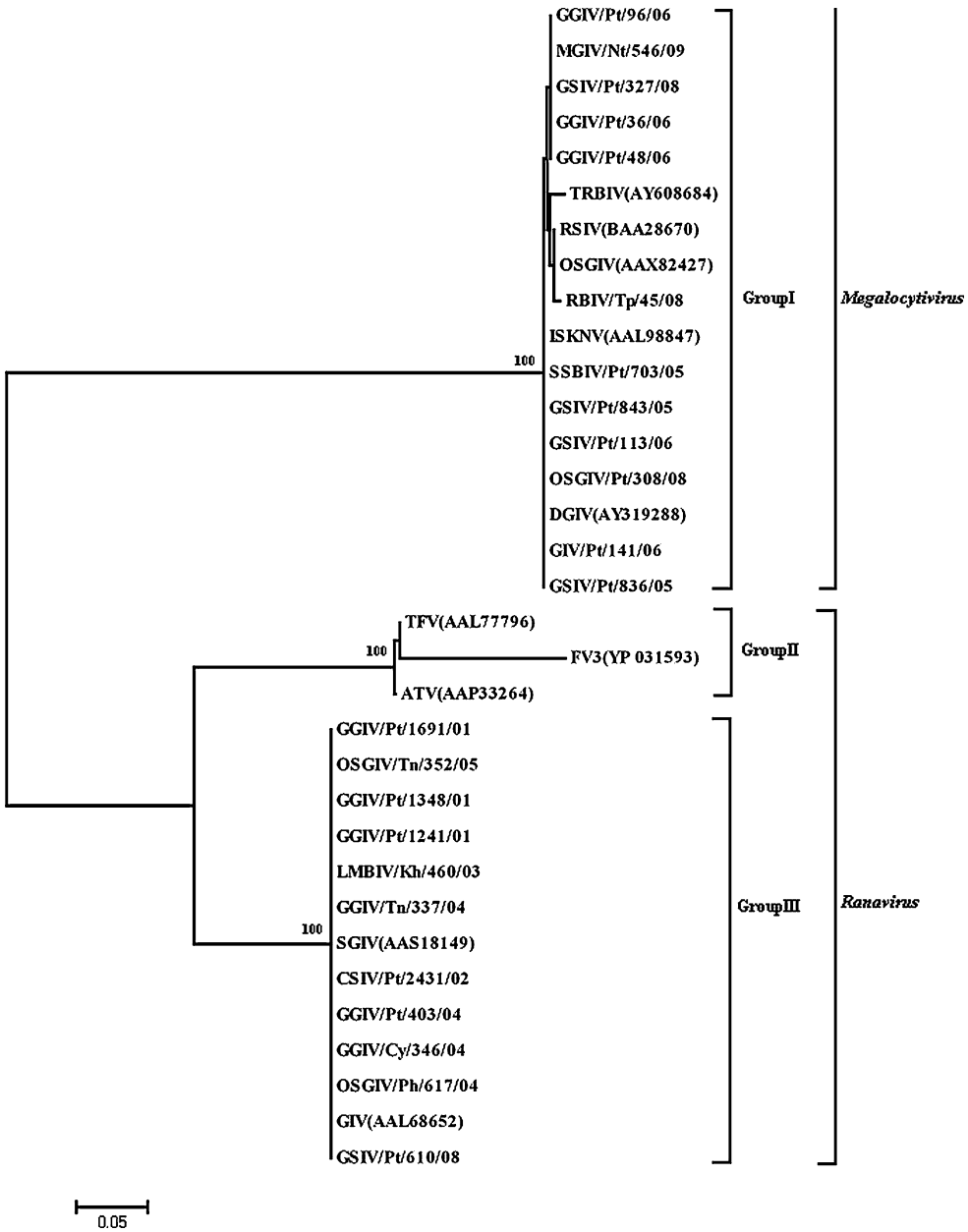

Fig. 4 Phylogenetic analysis of ATPase amino acid sequences. The specifications of the tree are similar to those in Fig. 1

regions [4, 12, 13, 18, 35]. Our results support this, since 11 strains of viruses in our study, collected from different fish species in different years, belonged to the same group and were similar to ranaviruses (Figs. 1, 2). According to the phylogenetic tree profiles of viral genomic DNA in the MCP genes, the genotypes of these isolates are closely related to SGIV and GIV. It appears that the viruses gradually adapted and evolved into the current local strains. Based on the phylogenetic tree profiles of amino acid sequences and nucleotide sequences of MCP, Taiwanese isolates belong to three genotypes in the genus *Megalocytivirus*, which include ISKNV, RSIV, and FLIV. An emerging strain (RBIV/Tp/45/08) invaded Taiwanese fish farms, and its genotype was found to be closely related to that of FILV and TRBIV (Figs. 1, 2). This strain originated from diseased rock bream that was imported as larvae from Korea, causing an epidemic point source in the farm. The cumulative mortality rate was 100% at 7 days after infection. Fortunately, there were no other farms near the outbreak.

Many studies have evaluated the genetic relationships between the epidemic strains, geographic distribution, and host susceptibility of members of the genus *Megalocytivirus* [23, 24]. In this study, we found six viruses of the genotype I group that were isolated from three different host fish species in both freshwater and marine environments between 2005 and 2008. The genotype II group consisted of five viruses isolated from three different host

Table 5 Numbers of nucleotide and amino acid substitutions and *Ka/Ks* ratios of iridovirus isolates

Isolate name	No. of nucleotide substitutions	No. of amino acid substitutions	Non-synonymous substitution rate (*Ka*)	Synonymous substitution rate (*Ks*)	Omega value (*Ka/Ks*)
OSGIV/Ph/617/04	3	0	0	0.009	0
OSGIV/Pt/403/04	4	1	0.001	0.009	0.111
OSGIV/Tn/352/05	3	0	0	0.009	0
OSGIV/Pt/141/06	2	0	0	0.003	0
OSGIV/Pt/308/08	2	0	0	0.003	0
GGIV/Pt/1348/01	1	0	0	0.003	0
GGIV/Pt/1691/01	1	0	0	0.003	0
GGIV/Pt/1241/01	2	0	0	0.006	0
GGIV/Tn/337/04	0	0	0	0	0
GGIV/Cy/346/04	2	2	0.002	0	0.002
GGIV/Pt/36/06	3	1	0.001	0.006	0.166
GGIV/Pt/48/06	3	1	0.001	0.006	0.166
GGIV/Pt/96/06	5	1	0.001	0.012	0.083
GSIV/Pt/843/05	3	0	0	0.006	0
GSIV/Pt/836/05	4	2	0.002	0.003	0.666
GSIV/Pt/113/06	7	2	0.003	0.006	0.5
GSIV/Pt/327/08	4	2	0.002	0.006	0.333
GSIV/Pt/610/08	11	1	0.001	0.030	0.033
CSIV/Pt/2431/02	8	4	0.004	0.012	0.333
LMBIV/Kn/460/03	9	1	0.001	0.024	0.041
SSBIV/Pt/703/05	2	0	0	0.003	0
RBIV/Tp/45/08	45	8	0.010	0.106	0.094
MGIV/Nt/546/09	29	2	0.006	0.062	0.096

[a] The sequences of SGIV (AAS18087), GIV (AAV91066), ISKNV (AAL98730), RSIV (AY310918) and TRBIV (AY590687) were used as references

fish species isolated between 2006 and 2009. Interestingly, a new strain of MGIV/Nt/546/09 was detected from the marble goby in 2009, and this virus was genetically distinct from other viruses belonging to genotype II. Our findings confirm previous results [7, 33] that suggested freshwater and marine fish species are susceptible to ISKNV. The results also demonstrate that giant seaperch, giant grouper, and orange-spotted grouper are more sensitive to members of the genera *Ranavirus* and *Megalocytivirus*. In Taiwan, ranaviruses are epidemic in Pingtung, Kaohsiung, Penghu, Tainan, and Chiayi, mostly in marine fish. In this study, the megalocytivirus isolates from both seawater and freshwater fish species were mostly collected from Pingtung County. When comparing affected locations identified in previous studies in Taiwan [2, 33], the endemic areas are the same for both the megalocytiviruses and the ranaviruses. We have demonstrated that the main production areas are centralized in Taiwan. Moreover, the production chain is divided into several segments in the larvae and juvenile stages, resulting in the fry being constantly subjected to stress due to movement of the fish in commercial transactions, forcing the fry to adapt to different rearing environments in a short period of time. Consequently, multiple strains of GIV, RSIV, and ISKNV exited simultaneously from the same geographic locations, and the strain variations of these isolates may not be related to geography in Taiwan.

Comparison of nucleotide and amino acid sequences of iridovirus strains from 23 isolates shows high sequence identity in GIV, RSIV, ISKNV, and FLIV. Most of the variations observed were synonymous mutations, and the nucleotide substitutions in these isolates occurred at the first and second nucleotide positions and did not cause amino acid changes. Mutations in some isolates caused amino acid changes, particularly in the first-found strains in this study. Moreover, genomic variations occurred more often in freshwater fish species like giant seaperch, large-mouth bass, and marble goby. We propose that the more frequent occurrence of non-synonymous mutations in freshwater fish species may be related to the fact that the references strains are most often detected in the seawater fish species or that these strains are involved in cross-species infections. However, this phenomenon requires more research. The *Ka*/*Ks* ratio of MCP segments from

iridoviruses were lower than one, indicating that the viruses are under negative selection. These results suggest that the viruses are stable in the environment and could infect in both seawater and freshwater fish hosts.

The sequence identities between Taiwanese isolates in the MCP and ATPase genes demonstrate that they belong to different species in the genera *Ranavirus* and *Megalocytivirus*, according to the phylogenetic tree profiles of viral amino acids in the MCP proteins, which clustered into distinct genotypes (I, II, III, andV). The MCP is located on the viral surface and is exposed to environmental pressures, and it therefore can be used as an indicator of relationships within the family *Iridoviridae*. Our results show that the amino acid sequence of the ATPase is more conserved than that of the MCP. Presently, the function of the ATPase is poorly understood. Therefore, the high variability of the MCP gene may be a good tool to study virus strain evolution, classification, and taxonomic differentiation. Continuous monitoring of the iridovirus genotypes with a worldwide database may improve the understanding of local viral genotype shifts and their relationship to worldwide epidemiology. This information will be useful for disease diagnosis and control, including vaccine development and future cures.

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