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First detection of Megalocytivirus (*Iridoviridae*) in trash fish used for aquaculture feed in Sabah, Malaysia

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Abstract: Trash fish is a popular aquaculture feed in Sabah, Malaysia. Among the many fish species, Indian mackerel (*Rastrelliger kanagurta*), spotted sardine (*Sardinella* sp.), smoothbelly sardine (*Ambligaster leiogaster*), mackerel scad (*Decapterus macarelus*), round scad (*Decapterus* sp.) and big eyed scad (*Selar crumenopthalmus*) are the most commonly used trash fish. However, the increasing number of disease outbreaks occurring throughout aquaculture farms has prompted us to conduct this study with the aim to determine if Iridovirus presents in six different species of trash fish namely; Indian mackerel, spotted sardine, smoothbelly sardine, mackerel scad, round scad and big eyed scad collected throughout Sabah. In this study, DNA from 230 fish specimens was subjected to nested-PCR and DNA sequencing analyses. The results showed 15.22% of specimens were found to have been contaminated with Infectious Spleen and Kidney Necrosis Virus (Megalocytivirus, Iridoviridae). This indicates that trash fish can harbor viral pathogen and may likely to transmit it to culture fish during feeding. The reliance on trash fish for aquaculture feeding can jeopardize the sustainability of the aquaculture industry. Therefore, the use of pellet feeds made from certified virus-free ingredients may be the best choice to minimize viral outbreaks.

Key Words: *Iridoviridae,* trash fish, cage aquaculture

Introduction

Marine aquaculture has expanded over the year and is now becoming one of the most important socioeconomic activities in Sabah, Malaysia. It helps to fill in the gap between the fish supply and the increasing demand for fish protein as the number of Malaysian population increases and coupled with the declining fishery catches from natural marine environment.

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However, the aquaculture expansion is not without challenges. These challenges can include fish diseases, limited supply of high-quality seeds, inconsistent supply of aquaculture feeds and limited areas suitable for aquaculture operation. Nevertheless, diseases seem to be the main obstacle of sustainable aquaculture in Sabah. Although many of the disease outbreaks occurring in aquaculture farms are caused by bacteria (Ransangan and Mustafa, 2009), virus, particularly betanodavirus which cause viral nervous necrosis (VNN) has been reported as the main hindrance to seed production in hatcheries throughout the country (Ransangan and Manin, 2010).

In addition to disease, the aquaculture in Sabah also depends on trash fish for feeding. The commonly used trash fish can comprise of small fish species such as mackerels, sardines and scads. These fish species normally have low consumer preferences and likely to have little commercial value. Like in other places around Southeast Asia, trash fish are widely used for aquaculture feeding due to its comparatively low cost than the dry pellet feeds. However, the inconsistent supply and the question about its quality are now becoming one of the important issues being debated in aquaculture forums and meetings around the world. Trash fish have been shown to carry pathogenic bacteria and viruses which likely to be transmitted to culture fish during feeding (Gomez *et al.*, 2010). Earlier study by Kim *et al*. (2007) has shown that iridovirus can be transmitted from mixed trash fish (Pacific cutlass *Trichiurus lepturus,* Yellow croaker *Larimichthys polyactis,* Balloon fish *Diodon holocanthus Linnaeus*) to culture flounder in Korea. Gomez *et al*. (2010) also detected viral nervous necrosis (Nodaviridae) in trash fish (Japanase jack mackerel) that used to feed the culture fish in Japan. The reliance on trash fish and the many fish disease outbreaks occurring in aquaculture farms throughout Sabah have motivated us to conduct this study with the aim to determine the possibility of iridovirus contamination in trash fish.

Methods and Materials

Sample collection

Specimens of trash fish were collected from fishermen at five fishery landing stations (Kuala Penyu, Lahad Datu, Semporna, Kudat and Kota Kinabalu) in Sabah. Locations of these landing stations are indicated in Fig. 1. A total of 230 fish specimens comprising of six different species were collected and analyzed in this study (Tab. 1). During collection, specimens were chilled in ice. Tissues (spleen, kidney and liver) from individual fish specimens were removed aseptically in the laboratory and pooled in the 1.5 ml microcentrifuge tubes, and stored in the -20°C until DNA extraction.

DNA extraction

The DNA from the pooled tissues of individual fish specimens was extracted using

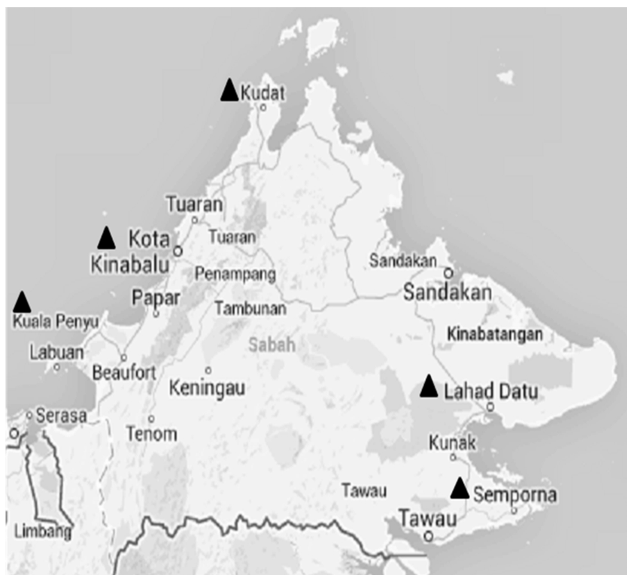

Fig. 1: Map indicates (black triangle) the location of the five fishery landing stations at which the trash fish specimens were collected.

the DTAB (dodecyltrimethylammonium bromide) – CTAB (hexadecyltrimethylammonium bromide) method according to Philips and Simon (1995). Pooled tissues were homogenized using sterile pestle and mortar in the presence of extraction buffer (DTAB, NaCl, 1M Tris 8.8, 0.5M EDTA) followed by incubating the tissue homogenate at 75˚C for 5 min. Then, 700µl chloroform was added into tissue homogenate before centrifuging at 15000 g for 5 min. Subsequently, 400 µl of the upper aqueous solution was transferred into a new tube. Then, 100 µl and 900 µl of CTAB lysis solution (CTAB, NaCl) and sterile distilled water respectively were added into the aqueous solution. The mixture was incubated at 75˚C for 5 min and allowed to cool down on ice bath for 5 min. The mixture was again centrifuged at 15000 g for 10 min. The supernatant was discarded, and the pellet was resuspended in 200 µl NaCl solution. The suspension was then incubated at 75˚C for 5 min and cooled down for 5 min before centrifugation at 15000 g for 5 min. Then, all the clear solution containing DNA was transferred into the new tube. The DNA was precipitated using 95% ethanol at room temperature for 5 min. Finally, the DNA pellet was collected by centrifugation at 15000 g for 5min. The DNA pellet was briefly air dried, dissolved in TE buffer and stored in -20^{0}C until used.

PCR amplification

Specific fragment of major capsid protein (MCP) gene was targeted in the PCR assay. First PCR assay was performed in 50 µl total volume; containing 5X GoTaq® Flexi PCR Buffer (Promega), 0.2mM of DNTPs (Promega), 1.7mM of $MgCl_2$ (Promega), 10 µM of IRF-1 and IRR-1 primers (Tab. 2), 0.3 units of Taq-polymerase (Promega) and 2 µl of DNA template. The PCR amplification was conducted as follow; pre denaturation at 95°C for 3 min, 30 cycles of denaturation at 94°C for 1 min; annealing at 55°C for 1 min; extension at 72°C for 1 min and final extension at 72°C for 5 min. Both positive and negative controls were included in every PCR assay. Nested PCR assay was carried out as the condition of first PCR assay except the primers used in the nested PCR assay are IRVF-2 and IRVR-2 (Razak *et*

Tab. 1: Number of fish specimens analyzed and results of the nested PCR assay.

Trash fish	Locality	No. of fish specimens	No. of positive specimens
Indian mackerel, *Rastrelliger kanagurta*	Kuala Penyu	10	0
	Semporna	25	0
Spotted sardine, *Sardinella* sp.	Kuala Penyu	3	0
	Kota Kinabalu	16	13
Round scad *Decapterus* sp.	Kuala Penyu	13	9
Smoothbelly sardine, *Ambligaster leiogaster*	Kuala Penyu	32	2
	LahadDatu	11	4
Mackerel scad, *Decapterus macarelus*	Kudat	30	1
	LahadDatu	30	6
	Kota Kinabalu	30	0
Big eyed scad, *Selar crumenopthalmus*	Kudat	30	0
Total		230	35

Tab. 2: PCR primers used in the amplification of major capsid protein gene of Iridoviridae.

Primers	DNA sequence (5'-3')	Target gene	Nucleotide position (nt)	Reference sequence
IRVF-2	GCTGCGTGTTAAGATCCC	MCP	244-262	AB669096
IRVR-2	CATGACAGGGTGACGTTGG	MCP	1189-1208	HQ317462
IRF-1	CATCATGTCTGCGATCTCAG	MCP	1-17	JQ253368
IRR-1	CACAGGATAGGGAAGCCTGC	MCP	1342-1360	JQ253368

al., 2014), and the DNA template are from the amplicons generated during the first PCR. The PCR products were analyzed on 1.5% EtBr-agarose gel electrophoresis and visualized under UV Gel documentation system (Alpha Innotech Corporation).

DNA Cloning

The PCR amplicon was first purified using MEGAquick-spin™PCR & Agarose Gel DNA Extraction System (iNtRON Biotechnology, Inc). Then, it was ligated into pGEM-TEasy vector (Promega) using T4 DNA ligase at 16°C for 24h. Subsequently, the ligation mixture was transformed into competent *E.coli* JM109 cells using the heat shock method described by Sambrook and Russell (2001). Then, the *E. coli* JM109 was spread on Luria-Bertani (LB) agar containing ampicillin (25mg/ml), IPTG (20 mg/ml) and X-gal (20 mg/ml), and incubated at

37˚C overnight. Next day, white colonies *E. coli* JM109 were aseptically picked, inoculated in LB broth containing ampicillin (25 mg/ml) and incubated at 37˚C overnight with moderate shaking of 150 rpm. Then, recombinant plasmid was extracted using the alkaline lysis method (Sambrook and Russell, 2001) and purified using the DNA-spin™ Plasmid DNA Purification Kit (iNtRON Biotechnology, Inc). The DNA insert was verified by restriction enzyme EcoR1 analysis following manufacturer's instruction (New England Biolabs). The plasmids with correct DNA insert were sent to AITBiotech Pte Ltd. Singapore for sequencing.

DNA sequence analyses

DNA contigs were assembled using the SeqMan Pro tool (DNASTAR Lasergene) and analyzed using BLASTN analysis (http://www.ncbi.nih.gov). Multiple alignments of the DNA sequences was done against selected sequences of Major Capsid Protein (MCP) gene downloaded from National Center for Biotechnology Information (NCBI) using the Clustal W method (Thompson, 1994). The lists of sequences analyzed in the study are as in Tab. 3 and Tab. 4. Phylogenetic tree against DNA sequences of major capsid protein gene from other iridovirus genera (see Tab. 3) was constructed using MegAlign program (DNASTAR, V 7.1) software that has the neighbor-joining algorithm. Similarly, second phylogenetic tree for the clustering of the viral strain was also constructed using the same program.

Tab. 3: List of nucleotide sequences of the major capsid protein gene used in the study for viral classification.

Source of Isolate	Virus	Origin	Accession Number
Aedes, *Ochlerotatustaeniorhynchus*	Chloriridovirus	USA	DQ643392
Aedes, *O. taeniorhynchus*	Chloriridovirus	USA	NC008187
Brown beetle, *Costelytrazealandica*	Iridovirus	New Zealand	AF025775
Crane fly, *Tipulapaludosa*	Iridovirus	UK	M33542
Blackfly larvae, *Simulium* sp.	Iridovirus	UK	NC021901
Olive flounder, *Paralichthysolivaceus*	Lymphocytivirus	Korea	AY297741
Seargent fish, *Rachycentroncanadum*	Lymphocytivirus	China	EF103188
Olive flounder, *P. olivaceus*	Lymphocytivirus	Korea	AY303804
Humpback grouper, *Cromileptesaltivelis*	Megalocytivirus	Malaysia	JQ253368
Humpback grouper, *C. altivelis*	Megalocytivirus	Malaysia	JQ253371
Orange spotted grouper, *E. coioides*	Megalocytivirus	Malaysia	JQ253372
Round scad, *Decapterus* sp.	Megalocytivirus	Malaysia	KF753318

Tab. 3: Continued

Source of Isolate	Virus	Origin	Accession Number
Round scad, *Decapterus* sp.	Megalocytivirus	Malaysia	KF753319
Round scad, *Decapterus* sp.	Megalocytivirus	Malaysia	KF753320
Smoothbelly sardinella, *Amblygater Leiogaster*	Megalocytivirus	Malaysia	KF753321
Smoothbelly sardinella, *Amblygater Leiogaster*	Megalocytivirus	Malaysia	KF753322
Smoothbelly sardinella, *Amblygater Leiogaster*	Megalocytivirus	Malaysia	KF753323
Smoothbelly sardinella, *Amblygater Leiogaster*	Megalocytivirus	Malaysia	KF753324
Mackerel scad, *Decapterus macarelus*	Megalocytivirus	Malaysia	KF753325
Mackerel scad, *Decapterus macarelus*	Megalocytivirus	Malaysia	KF753326
Sardinella sp.	Megalocytivirus	Malaysia	KF753327
Sardinella sp.	Megalocytivirus	Malaysia	KF753328
Sardinella sp.	Megalocytivirus	Malaysia	KF753329
Sardinella sp.	Megalocytivirus	Malaysia	KF753330
Sardinella sp.	Megalocytivirus	Malaysia	KF753331
Sardinella sp.	Megalocytivirus	Malaysia	KF753332
Sardinella sp.	Megalocytivirus	Malaysia	KF753333
Sardinella sp.	Megalocytivirus	Malaysia	KF753334
Sardinella sp.	Megalocytivirus	Malaysia	KF753335
Sardinella sp.	Megalocytivirus	Malaysia	KF753336
Sardinella sp.	Megalocytivirus	Malaysia	KF753337
Sardinella sp.	Megalocytivirus	Malaysia	KF753338
Rock bream iridovirus, *O. fasciatus*	Megalocytivirus	Korea	AY533035
Rock bream iridovirus, *O. fasciatus*	Megalocytivirus	China	HQ105005
Red sea bream, *Chrysophrys major*	Megalocytivirus	Japan	AB109371
Turbot, *Scophthalmusmaximus*	Megalocytivirus	China	AY590687
Mandarin fish, *Synchiropussplendidus*	Megalocytivirus	China	HQ317461
Mandarin fish, *S. splendidus*	Megalocytivirus	China	HQ317462
Frog virus, *Ranacatesbeiana*	Ranavirus	Brazil	DQ897669
Large mouthbass, *Micropterussalmoides*	Ranavirus	Germany	FR682503
Frog, *Ranaplancyichosenica*	Ranavirus	Korea	HM133594
Warthog, *Phacochoerusafricanus*	Outgroup (*Asfaviridae*)	USA	AY578706

Tab. 4: List of DNA sequences used in the strain divergence analysis.

Accession Number	Source of Isolate	Genus	Year	Country
HQ317459	Mandarin fish, *Synchiropussplendidus*	ISKNV	2002	China
HQ317460	Mandarin fish, *Synchiropussplendidus*	ISKNV	2009	China
HQ317461	Mandarin fish, *Synchiropussplendidus*	ISKNV	2006	China
HQ317462	Mandarin fish, *Synchiropussplendidus*	ISKNV	2007	China
HQ317465	Mandarin fish, *Synchiropussplendidus*	ISKNV	2006	China
JF264345	Orange spotted grouper, *E.coioides*	ISKNV	2006	Taiwan
JF264349	Orange spotted grouper, *E.coioides*	ISKNV	2008	Taiwan
JQ253368	Humpback grouper, *Cromileptesaltivelis*	ISKNV	2006	Malaysia
JQ253371	Humpback grouper, *Cromileptesaltivelis*	ISKNV	2006	Malaysia
JQ253372	Orange spotted grouper, *E. coioides*	ISKNV	2004	Malaysia
KF753318	Round scad, *Decapterus* sp.	ISKNV	2012	Malaysia
KF753319	Round scad, *Decapterus* sp.	ISKNV	2012	Malaysia
KF753320	Round scad, *Decapterus* sp.	ISKNV	2012	Malaysia
KF753321	Smoothbelly sardinella, *Amblygater Leiogaster*	ISKNV	2012	Malaysia
KF753322	Smoothbelly sardinella, *Amblygater Leiogaster*	ISKNV	2012	Malaysia
KF753323	Smoothbelly sardinella, *Amblygater Leiogaster*	ISKNV	2012	Malaysia
KF753324	Smoothbelly sardinella, *Amblygater Leiogaster*	ISKNV	2012	Malaysia
KF753325	Mackerel scad, *Decapterus macarelus*	ISKNV	2012	Malaysia
KF753326	Mackerel scad, *Decapterus macarelus*	ISKNV	2012	Malaysia
KF753327	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753328	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753329	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753330	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753331	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753332	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753333	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753334	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753335	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753336	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753337	*Sardinella* sp.	ISKNV	2013	Malaysia
KF753338	*Sardinella* sp.	ISKNV	2013	Malaysia
AB080362	Red sea bream, *Pagrus major*	RSIV	1992	Japan
AB109370	Malabar grouper, *E. malabricus*	RSIV	1993	Thailand
AB461855	Japanase amberjack, *Seriolaquingueradiata*	RSIV	2004	Japan
AB461856	Red sea bream, *Pagrus major*	RSIV	2005	Japan
AB666318	*Moronesaxatilis x micropterussalmoides*	RSIV	2004	Hong Kong
AB666319	Brown marbled grouper, *E. fuscoguttattus*	RSIV	2002	Singapore

Tab. 4: Continued

Accession Number	Source of Isolate	Genus	Year	Country
AB666320	Brown marbled grouper, *E. fuscoguttattus*	RSIV	2002	Singapore
AB666326	Orange spotted grouper, *E. Coioides*	RSIV	2004	Hong Kong
AB666321	Red sea bream, *Pagrus major*	RSIV	2004	Hong Kong
AB666335	Red sea bream, *Pagrus major*	RSIV	2004	Japan
AY532608	Barred knifejaw, *Oplegnathusfasciatus*	RSIV	2001	Korea
AY532611	Rock bream, *O. fasciatus*	TBRIV	2001	Korea
AY590687	Turbot, *Scophthalmusmaximus*	TBRIV	2005	China
EU276417	Olive flounder, *Paralichthysolivaceus*	TBRIV	2007	Korea
HM067603	Sea perch, *Lateolabrax* sp.	TRBIV	2010	Korea
FR682503	Large mouthbass, *Micropterussalmoides*	Ranavirus	2010	Germany

Results

Out of the 230 fish specimens analyzed, 35 specimens were found to have contaminated with Iridovirus (Tab. 1). These positive specimens were collected from four fishery landing stations (Kota Kinabalu, Kuala Penyu, Lahad Datu and Kudat). No iridovirus detected in fish specimens collected from Semporna. Representatives of the PCR amplification are shown in Figure 2. From six species of trash fish collected, four species were detected positive Iridovirus; there are two species of trash fish detected positive with iridovirus from Kuala Penyu namely the round scad, *Decapterus* sp. and smoothbelly sardine, *Ambligaster leiogaster*. Smoothbelly sardine and mackerel scad, *Decapterus macarelus* collected from Lahad Datu were also found to have contaminated with the virus. Meanwhile, only one species of trash fish collected from Kudat (mackerel scad, *Decapterus macarelus*) and Kota Kinabalu (*Sardinella* sp.) was found positive, respectively.

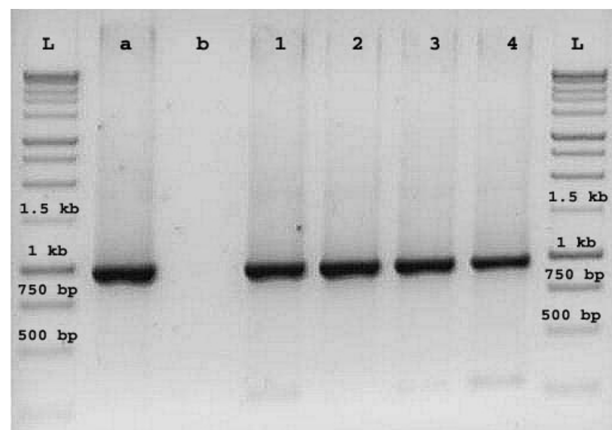

Fig. 2: PCR amplification of iridoviridae major capsid protein gene. Lane L= 1 kb DNA Ladder (Promega); Lane a = positive control (DNA of iridovirus); Lane b= negative control (nuclease free dH_2O); Lanes 1-2= Smoothbelly sardine (*A. leiogaster*); lane 3= Mackerel scad (*D. macarelus*) and lane 4: Round scad (*Decapterus* sp.).

From the 35 positive samples, 21 of the samples were successfully cloned and sequenced. The DNA sequences derived from this study have been deposited into GenBank with the following accession numbers; KF753318-38.

The BLAST analysis of the major capsid protein gene sequences of the virus amplified from the trash fish specimens in this study showed that the virus was highly similar to Megalocytivirus with nucleotide sequence similarity recorded at 96.53%. The virus exhibited low nucleotide sequence similarity to Ranavirus (54.30%), Chloriridovirus (49.70%), Lymphocytivirus (48.63%) and Iridovirus (45.56%). The phylogenetic tree analysis of the virus also showed that it was clustered within the Megalocytivirus group (Fig. 3). Strain divergence analysis of the phylogenetic tree (Fig. 4) further showed that the virus was highly similar to the Infectious Spleen and Kidney Necrosis Virus (ISKNV) with nucleotide sequence similarity of 99.40%. Nonetheless, the nucleotide sequence similarities of the virus with other strains of Megalocytivirus were low at 93.30% (RSIV) and 92.50% (TBRIV), respectively.

Discussion

Feed can contribute up to 55% of farming costs in aquaculture (Chong *et al.,* 2011) and trash fish has been one of the major feeds of culture marine fish species globally (FAO, 2005). In Malaysia, trash fish is preferable due two main reasons: low-cost and the availability of supply. Moreover, farmers are also believed that trash fish can improve the marketability (quality and texture) of the culture fish (Othman, 2006). However, in the recent years, the use of trash fish is less recommended because of its inconsistent nutrient content, contributes to water pollution and may carry pathogens. Kim *et al.* (2007) reported that mixed trash fish used to feed flounder in Korea has shown to carry Iridovirus.

Results of the present study revealed that 35 individuals of the trash fish specimens were found to have contaminated with Iridovirus. Interestingly, the majority of the trash fish species examined in this study were plankton feeders (Gambang *et al.,* 2003). However, it was not known to us, at this stage, if the iridovirus detected in the trash fish could have originated from planktons. The study also suggests that the feed management in aquaculture in Sabah has to be properly done to ensure that all the trash fish use to feed culture fish are in good condition and free from pathogens. Freezing trash fish may kill most parasites, but some bacteria and viruses can tolerate to freezing condition, and may transmit the pathogens to culture fish during feeding (Sim *et al.,* 2005; Kim *et al.,* 2007). Dry pellet feeds can provide a better feeding option to aquaculture and may completely replace the

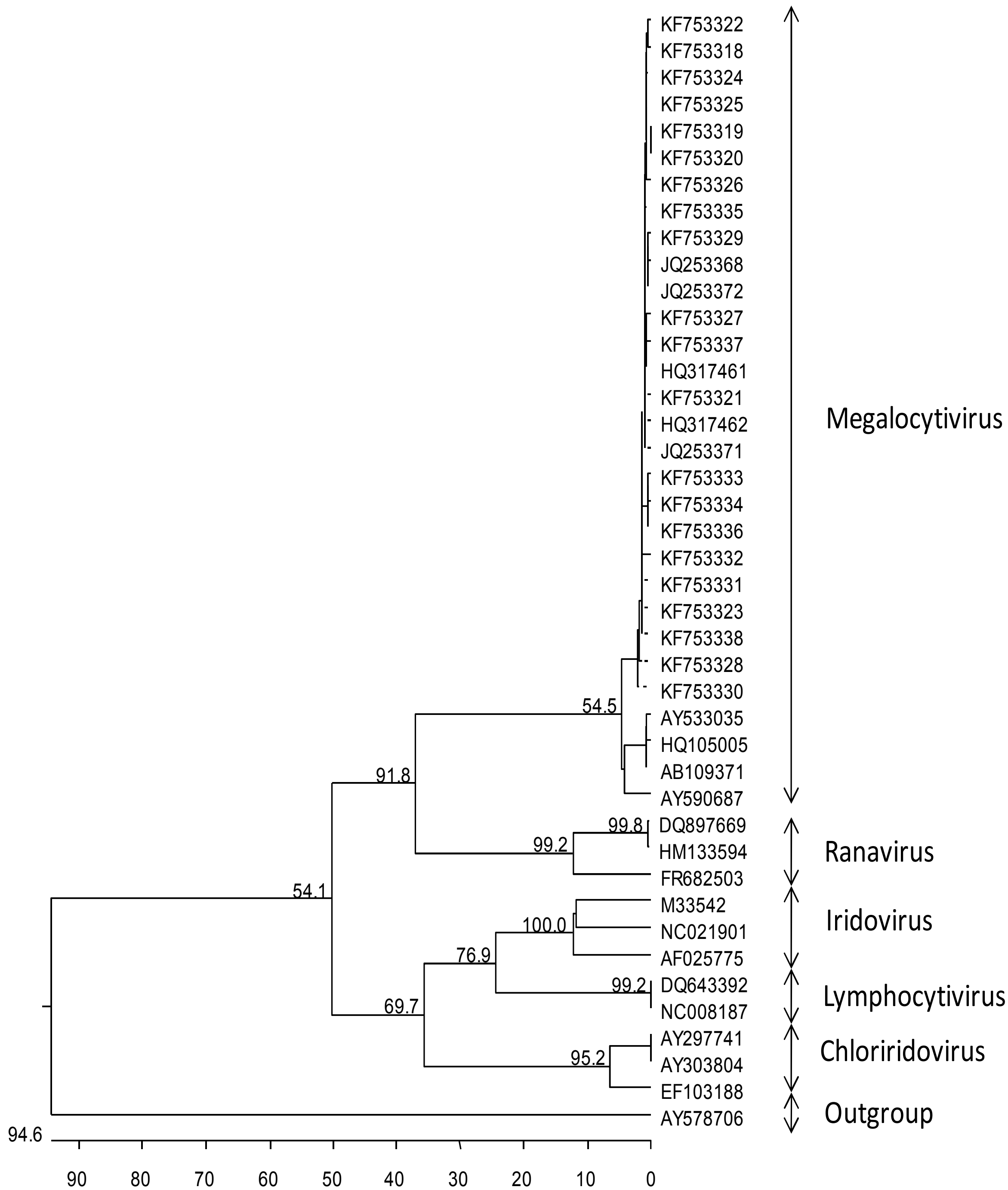

Fig. 3: Phylogenetic tree constructed based on the most variable region of the 42 major capsid protein gene sequences of Iridoviridae against the gene in the Asfaviridae (outgroup). Number at the tree nodes indicates bootstrap values of 1000 replicates. The distance scale is represented by the horizontal branches.

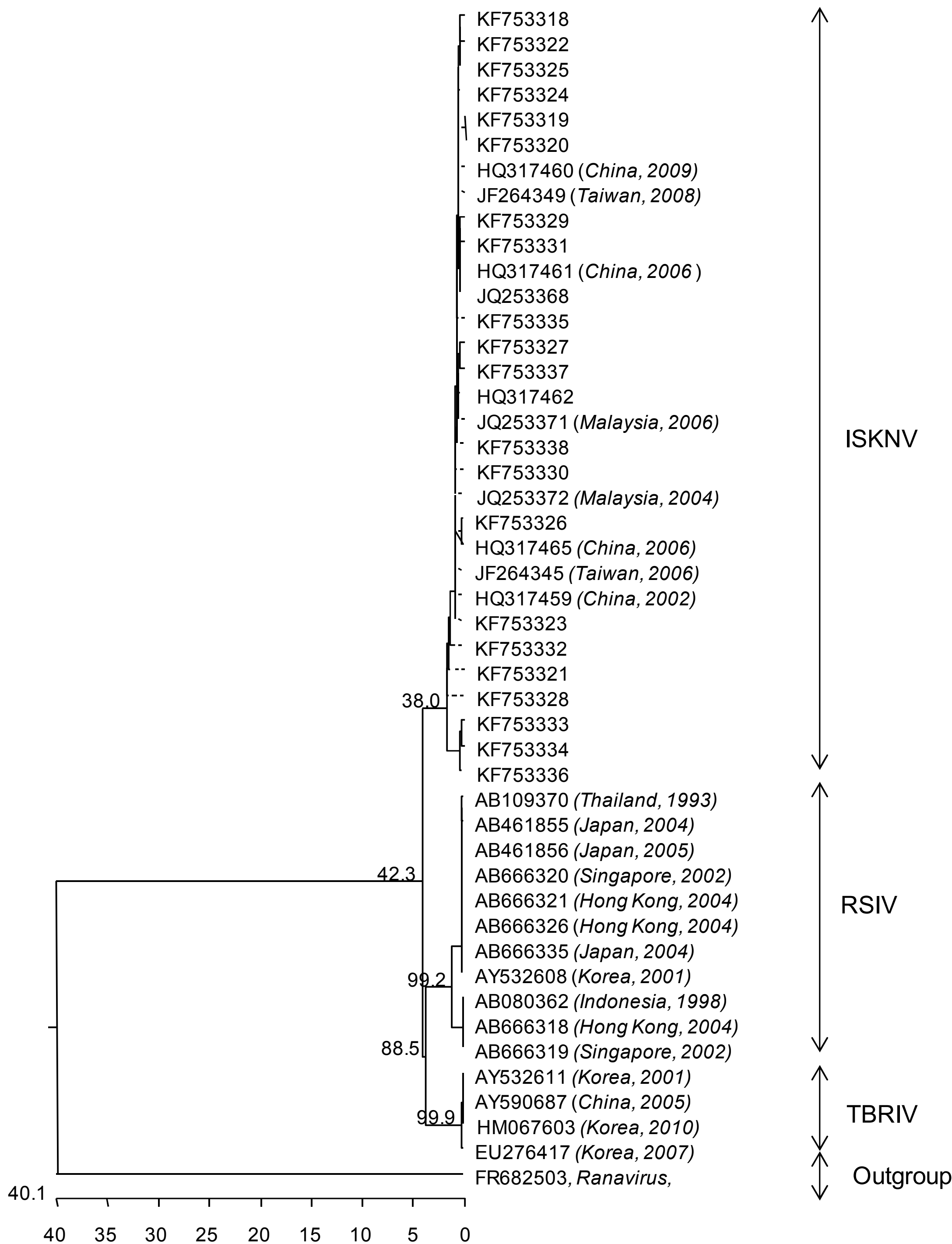

Fig. 4: Phylogenetic tree analysis of strain divergence based on 47 major capsid protein gene sequences of viral strains within the genus Megalocytivirus. Number at the tree nodes indicates bootstrap values of 1000 replicates. The distance scale is represented by the horizontal branches.

use of trash fish in the near future. By comparison, pellet feeds are more hygiene, improved nutrition quality tailored to the requirement of the intended culture fish. Furthermore, the use of pellet feeds has also been shown to significantly reduce environmental pollution (Chong *et al*., 2011).

In this study, it was evident that some species of trash fish used in Sabah have shown to carry Megalocytivirus. These virus-carrier trash fish may have the tendency to transmit the pathogen to susceptible culture fish as well as to wild fish, especially those that preyed on them. According to Fauquet *et al.,* (2005) iridoviruses can be transmitted naturally by cohabitation and feeding of the infected fish. The DNA analysis also revealed that the Megalocytivirus detected in the trash fish was highly similar to the ISKNV strain. Currently, Megalocytivirus has been reported to infect more than 50 fish species and blamed for the great loss to culture and wild fish populations (Yanong and Waltzek, 2010; Razak *et al.,* 2014). Obviously, this can become one of the greatest challenges to sustainable aquaculture in Malaysia. Since treatment options for virus are limited and not always successful, stopping its transmission route is seen to be the only practical solution for eradicating the virus in aquaculture. As such, the use of trash fish should be stopped, and the pellet feeds originated from virus-free certified ingredients should be promoted in order to sustain the aquaculture production in the country.

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References

- ✓ Chong R., Bousfield B. and Brown R. (2011) Fish Disease Management. Veterinary Bulletin, 1(8): 1-12.
- ✓ FAO (2005) Low value and trash fish in the Asia pacific region. Food and Agriculture Organization of the United Nations Regional Office for Asia and The Pacific, Bangkok.
- ✓ Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U. and Ball L.A. (2005) Virus Taxonomy. Eight Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, USA, pp 145-161.
- ✓ Gambang A.C., Rajali H.B. and Awang D. (2003) Overview of biology and exploitation of the small pelagic resources of the EEZ of Sarawak, Malaysia. Fisheries Research Institute Malaysia Sarawak.
- ✓ Gomez D.K., Mori K., Okiyana Y., Nakai T. and Park S.C. (2010) Trash Fish can be a source of betanodavirus for cultured marine fish. Aquaculture, 302: 158-163.
- ✓ Kim T.H., Gomez D.K., Choresa C.H. and Park S.C. (2007) Detection of major bacterial and viral pathogen in trash fish used to feed cultured flounder in Korea. Aquaculture, 272: 105-110.
- ✓ Othman M.F. (2008) Malaysia. FAO/NACA regional workshop on the future of mariculture: a regional approach for responsible development in the Asia-Pacific region. Guangzhao, China, 7-11 March 2006. FAO fisheries proceeding, No. 11. FAO, pp. 207-224.
- ✓ Philip A.J. and Simon C. (1995) Simple, efficient and nondestructive DNA extraction protocol for arthropods. Annals of the Entomological Society of America, 88(3): 281-283.

- ✓ Ransangan J. and Mustafa S. (2009) Identification of *Vibrio harveyi* isolated from diseased asianseabass *Lates calcarifer* by use of 16S ribosomal DNA sequencing. Journal of Aquatic Animal Health, 21: 150-155.
- ✓ Ransangan J. and Manin B.O. (2010) Mass Mortality of hatchery-produced larvae of Asian seabass, *Lates calcarifer* (Bloch), associated with viral nervous necrosis in Sabah, Malaysia. Veterinary microbiology, 4828: 1-5.
- ✓ Razak A.A., Ransangan J. and Sade A. (2014) First report of Megalocytivirus *(Iridoviridae)*in grouper culture in Sabah, Malaysia. International Journal of Current Microbiology and Applied Sciences, 3(3): 896-909.
- ✓ Sambrook J. and Russell D.W. (2001) Molecular cloning: a laboratory manual. CSHL Press. New York.
- ✓ Sim S.Y., Rimmer M.A., William K., Toledo J.D., Sugama K., Rumengan I. and Philips M.J. (2005) A practical guide to feeds and feed management for cultured groupers. NACA, Bangkok, Thailand. pp18.
- ✓ Yanong R.P.E and Waltzek T.B. (2010) Megalocytivirus infection in fish, with emphasis on ornamental species. Program in fisheries and aquatic sciences (FA182), University of Florida.

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