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An overview of viral diseases Asian sea bass (*Lates calcarifer*) and the research highlights

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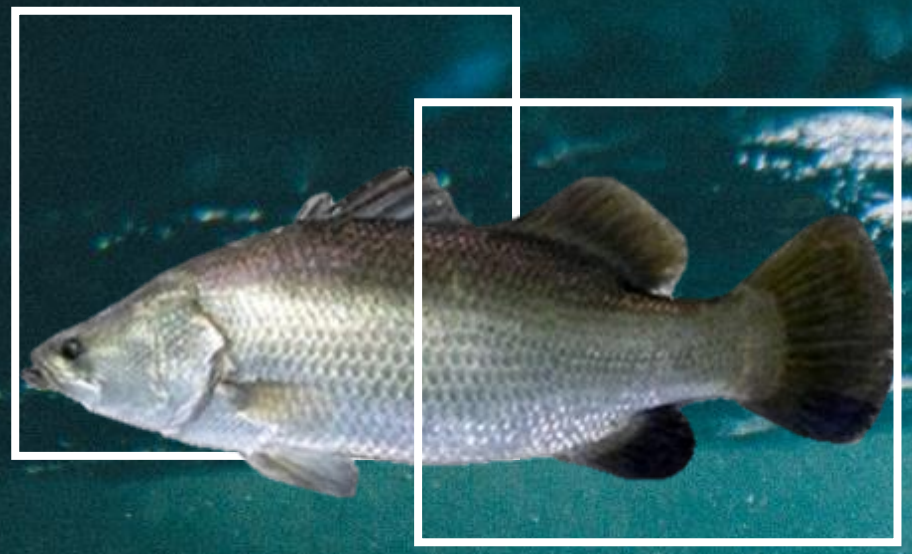
Fish Health Section

Asian Fisheries Society

BIOTEC
a member of NSTDA

MAHIDOL UNIVERSITY Centex Shrimp BIOTEC-NSTDA



Scientific name

Lates calcarifer (Bloch 1790)

Common names

- Asian sea bass
- Barramundi
- Barramundi perch
- Giant sea perch

Attractive species for aquaculture

- Tasty, nutritious meat
- Fast growth rate, large size, tolerance to a range of environmental conditions
- High demand in the domestic and export markets

Production

>110,000 MT in 2020

Asian sea bass culture

Asian sea bass can be cultured in

- *open*
- *semi-closed*
- *closed systems*

- *freshwater*
- *brackish*
- *marine settings*

each with specific disease challenges!!

List of viral pathogens in Asian sea bass

	Virus name	Family/Genus	First report in Asian sea bass	Subsequent reports
DNA virus	Infectious spleen and kidney necrosis virus (ISKNV)	Iridoviridae/ Megalocytivirus	Vietnam, 2017	Thailand, China
	Red sea bream iridovirus (RSIV)		India, 2020	-
	Turbot reddish body iridovirus (TRBIV)		Taiwan, 2020	-
	Scale drop disease virus (SDDV)		Singapore, 2012	Malaysia, Indonesia, Thailand
	Lymphocystivirus	Iridoviridae/Lymphocystivirus	Singapore, 1983	Thailand 1987, Australia 1990
	Lates calcarifer herpesvirus (LCHV)	Alloherpesviridae	Vietnam, Singapore, 2017	Thailand
RNA virus	Nervous necrosis virus (NNV)	Nodaviridae/Nodavirus	Malaysia, Australia, 1987	Indonesia, Singapore, Taiwan, China, Israel, Philippines, Tahiti, India, Iran
	Lates calcarifer Birnavirus (LCBV)	Birnaviridae/ Blosnavirus-like	Singapore, 2019	-

Iridoviridae family

bony fish, reptiles and amphibians

- Subfamily: *Alphairidovirinae*
  - Genus: *Lymphocystivirus*
  - Genus: *Megalocytivirus*
  - Genus: *Ranavirus*

insects and crustaceans

- Subfamily: *betairidovirinae*
  - Genus: *Chloriridivirus*
  - Genus: *Daphniairidovirus*
  - Genus: *Decapodiridovirus*
  - Genus: *Iridovirus*

All members in the *Iridoviridae* family are proposed to be called **"iridovirids"** (rather than iridoviruses) to avoid confusion to the genus *Iridovirus* (Chinchar et al, 2017)

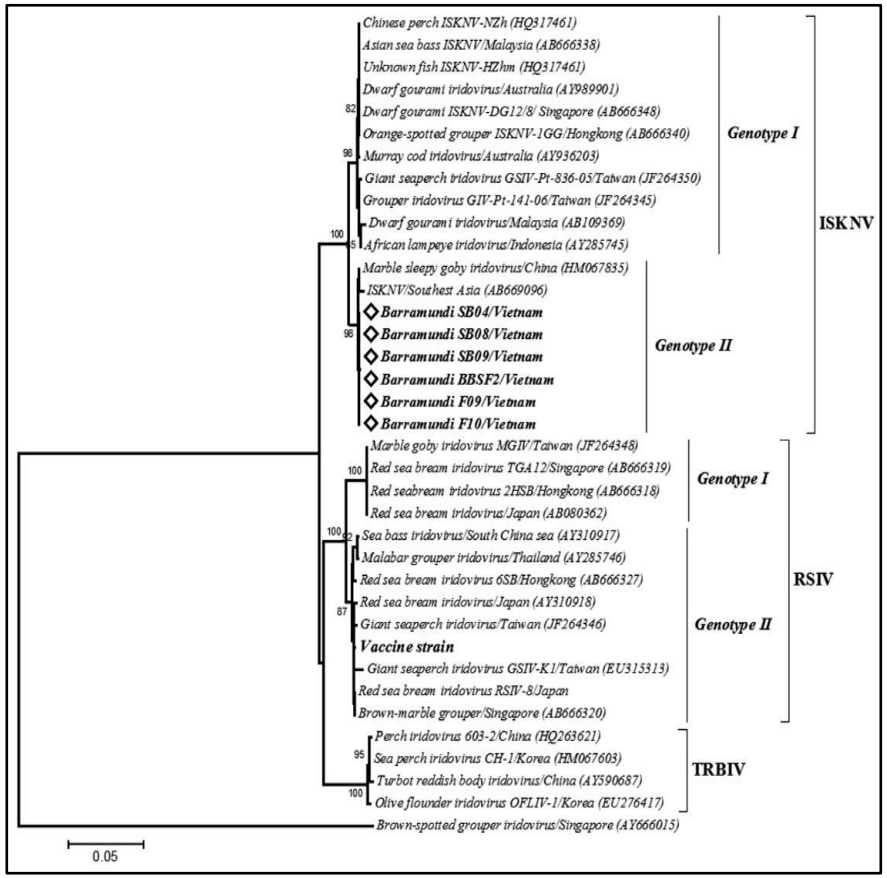
Infectious spleen and kidney necrosis disease (ISKND)

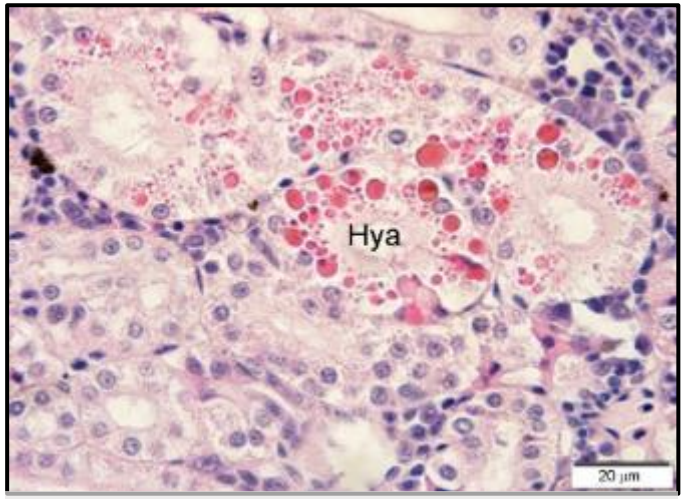
Causative agent	• Infectious spleen and kidney necrosis virus (ISKNV) or called *Megalocytivirus* ISKNV • Enveloped icosahedral viruses (130-150 nm in diameter) with a linear double-stranded DNA genome of 111 kb
Clinical sign	darkening, pale gills, ascites, enlarged spleen (splenomegaly)
Host	> 150 fish species of both marine and freshwater fish
Mortality	High mortality up to 85% of Asian sea bas
Susceptible stage	fingerling, juveniles, and grow-out of Asia sea bas
Transmission	horizontal & vertical
Distribution	• Widely distributed • Cases in Asian sea bass = Vietnam, Thailand, China
Histopathology	Basophilic hypertrophied cells (megalocytes) in spleen, kidney, liver, and gills
Diagnosis	PCRs, qPCR, isothermal amplification, immunological based techniques
Genotyping	MCP gene
Cell culture	Grunt fin-1 (GF-1) =Vacuolization Mandarin fish fry cells (MFF-1) => Rounding cells & detachment
Prevention	Biosecurity + general managements

First report of ISKNV in Asian sea bass

Dong et al. 2017 Fish Shellfish Immunol 68:65-73.

- *Affected sea bass farms in Vietnam in 2012–2014*
- ***Loss $435,000/ year***
- *A commercial oil-based vaccine (RSIV) showed inadequate protection*
- *Based on MCP gene, the virus was identified as Megalocytivirus ISKNV genotype II*

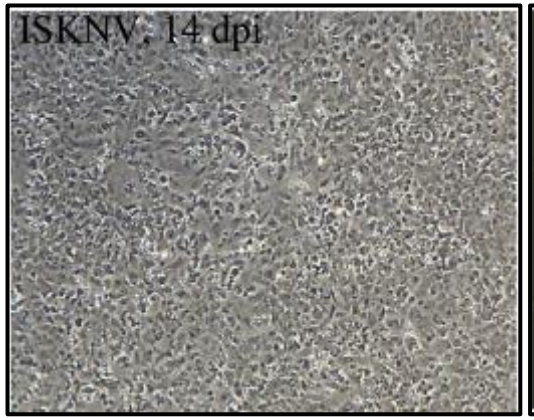




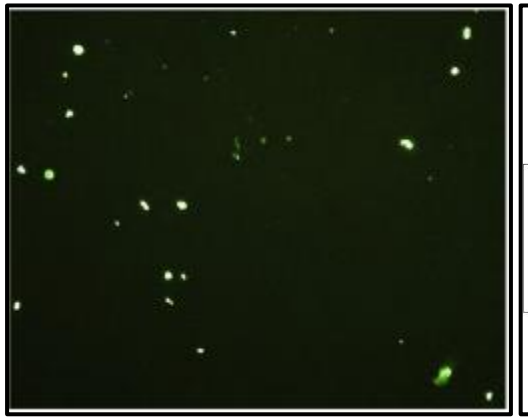
Hyaline degeneration in kidney tubules

First report of ISKNV in Thailand

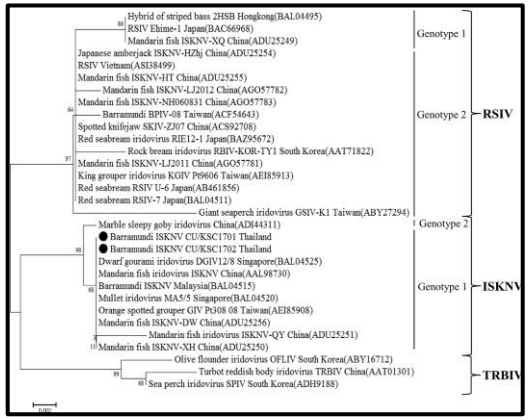
Thanasaksiri et al 2019 Aquaculture 500: 188-191



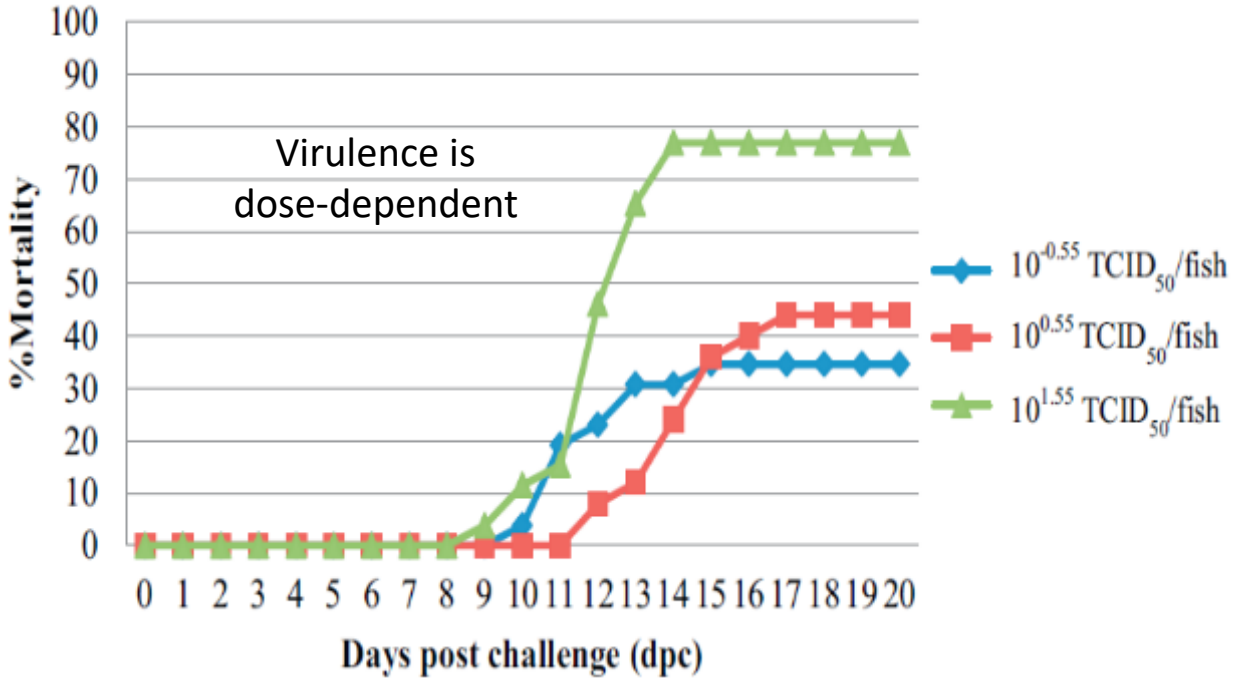
Inoculation in GF cells



Immunofluorescence using M10 mAb

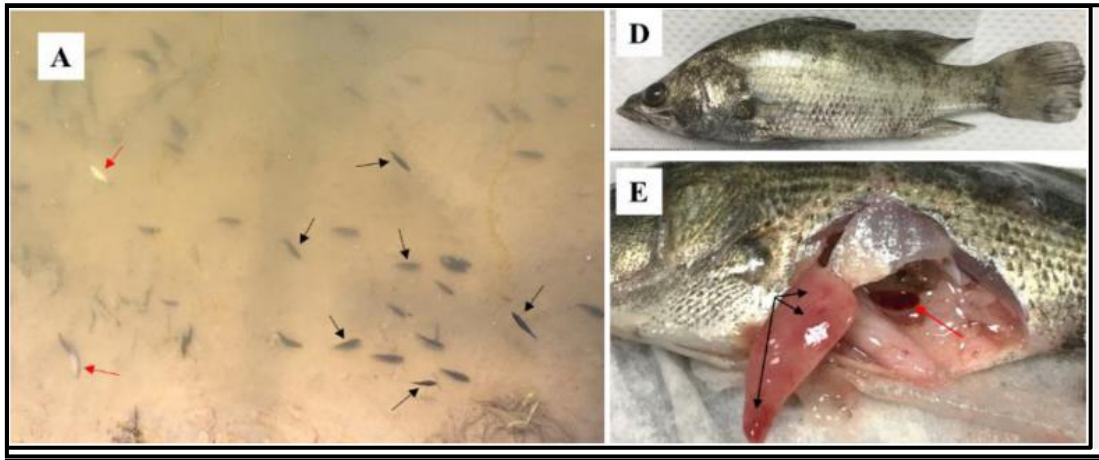


ISKNV genotype I based on MCP sequence

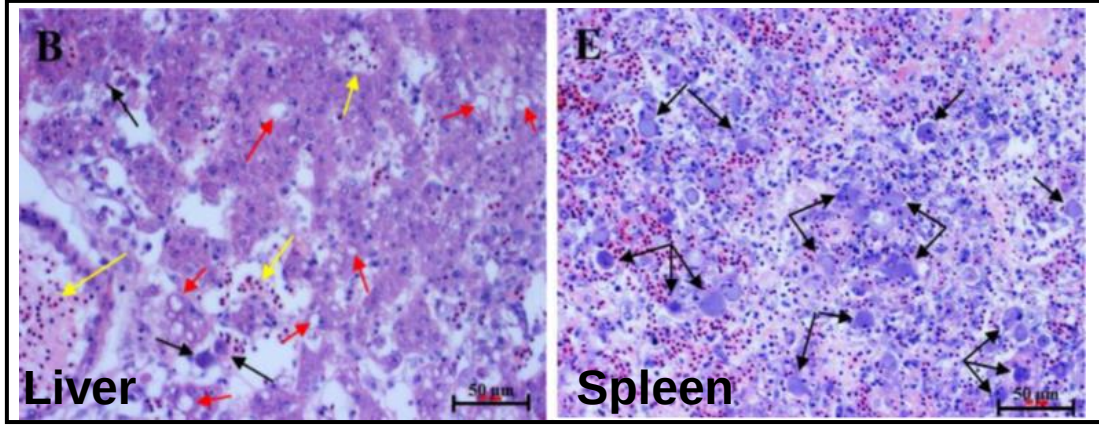


First report of ISKNV in China

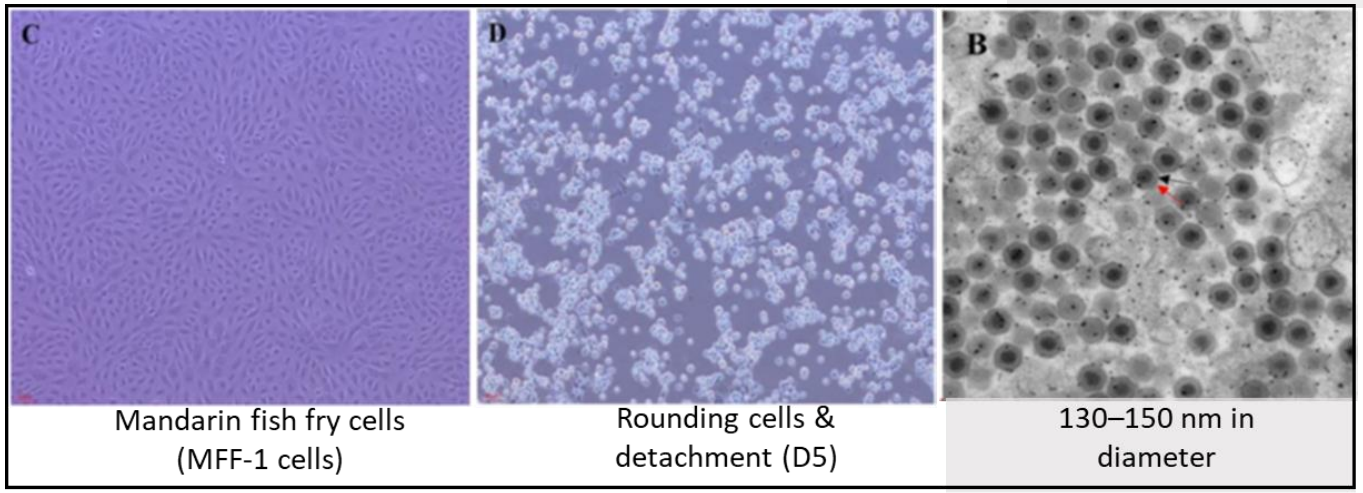
Zhu et al (2021) Aquaculture 534:736326



Body darkened, lethargic symptoms & mass mortalities (85.89%).
Liver hemorrhage and splenomegaly.



Enlargement cells observed in liver, kidney and particularly in spleen.



Mandarin fish fry cells (MFF-1 cells)

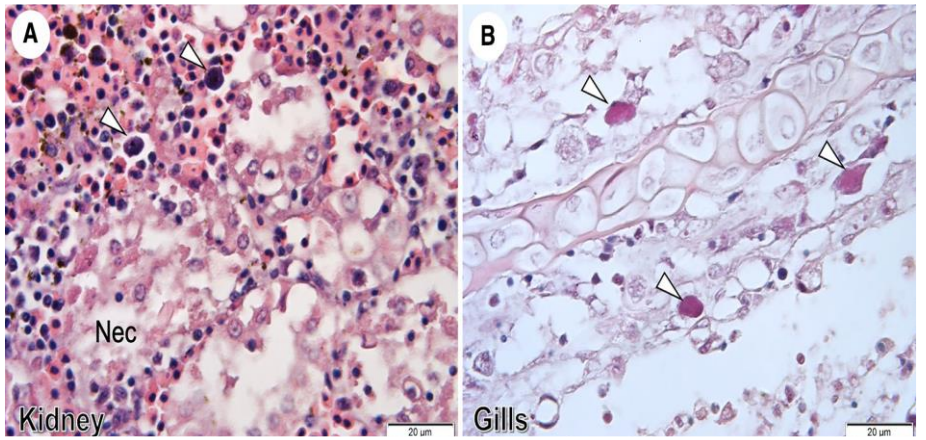
Rounding cells & detachment (D5)

130–150 nm in diameter

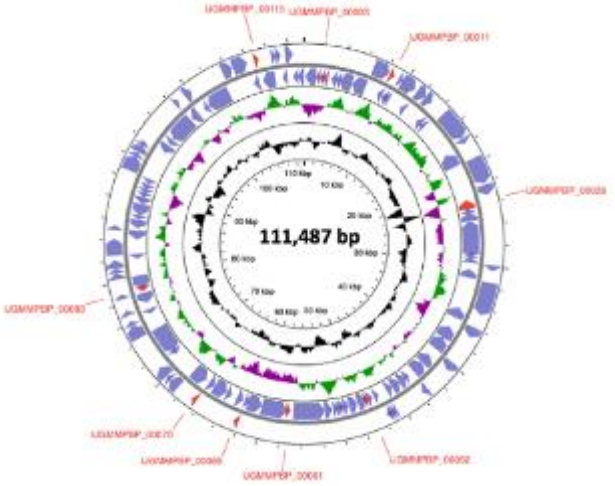
ISKNV in freshwater cultured Asian sea bass & their genome sequences

Kerddee et al 2021 Archives of Virology 166:3061–3074

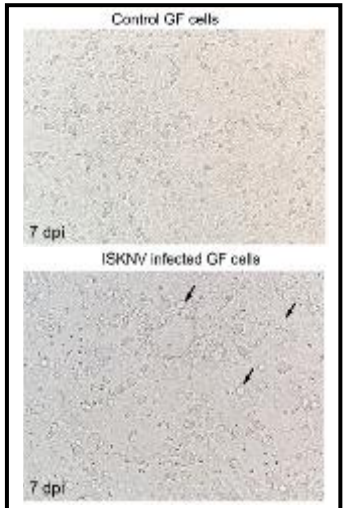
40-50% cumulative mortality of grown-out Asian sea bass in Thailand



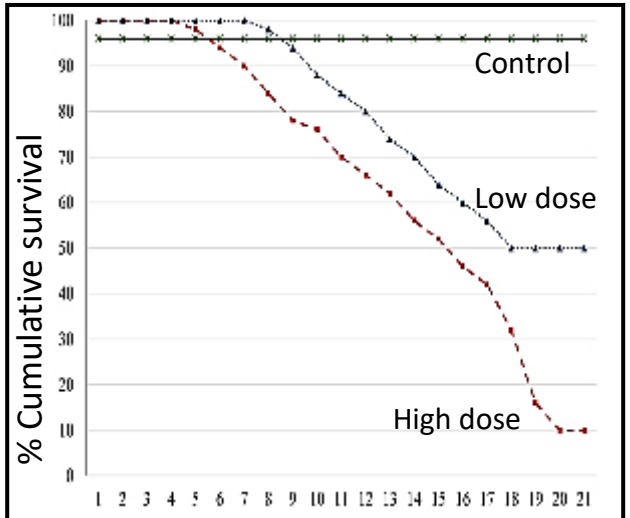
Basophilic inclusion bodies (megalocytosis) in kidney and gills



Genome of ISKNV genotype I



Vacuolization CPE



Day post challenge

Red sea bream iridovirus (RSIV)

First case report of RSIV in Asian sea bass

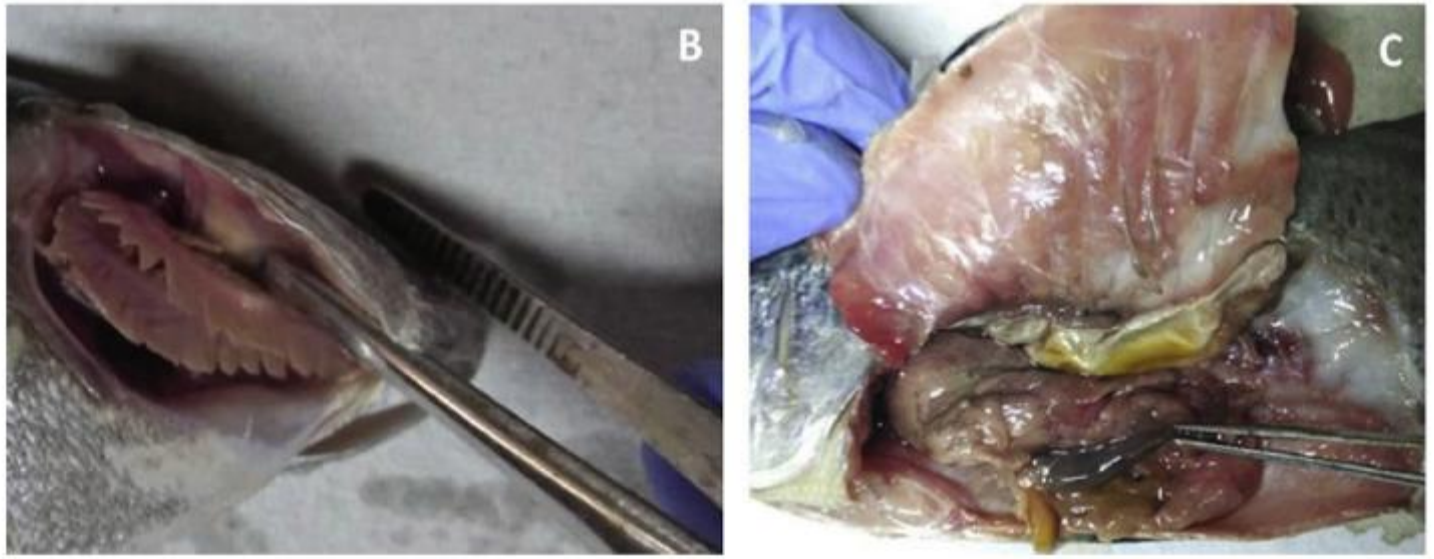
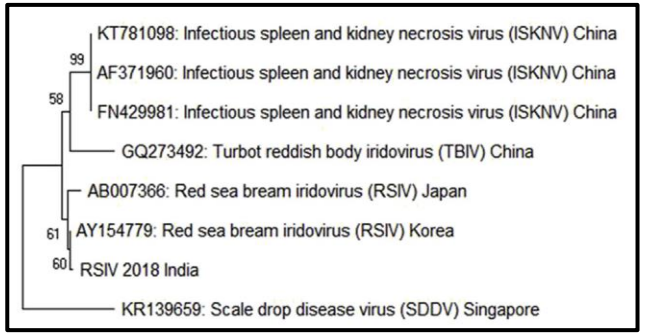
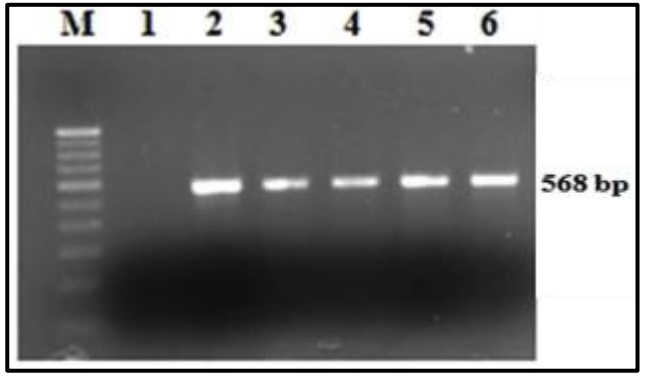
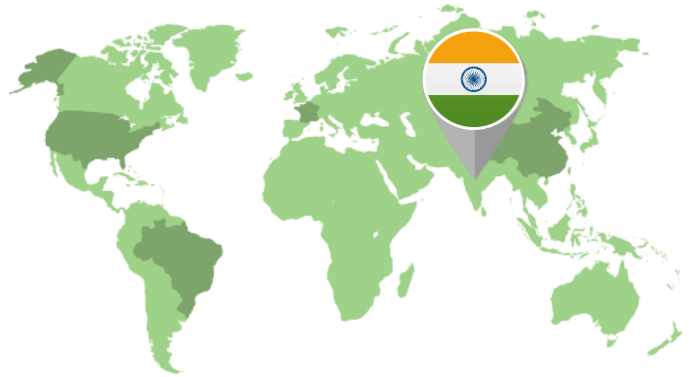


Fig. 1. A Diseased Asian seabass; B Infected Asian seabass showing pale gills C Enlarged spleen of diseased Asian seabass.



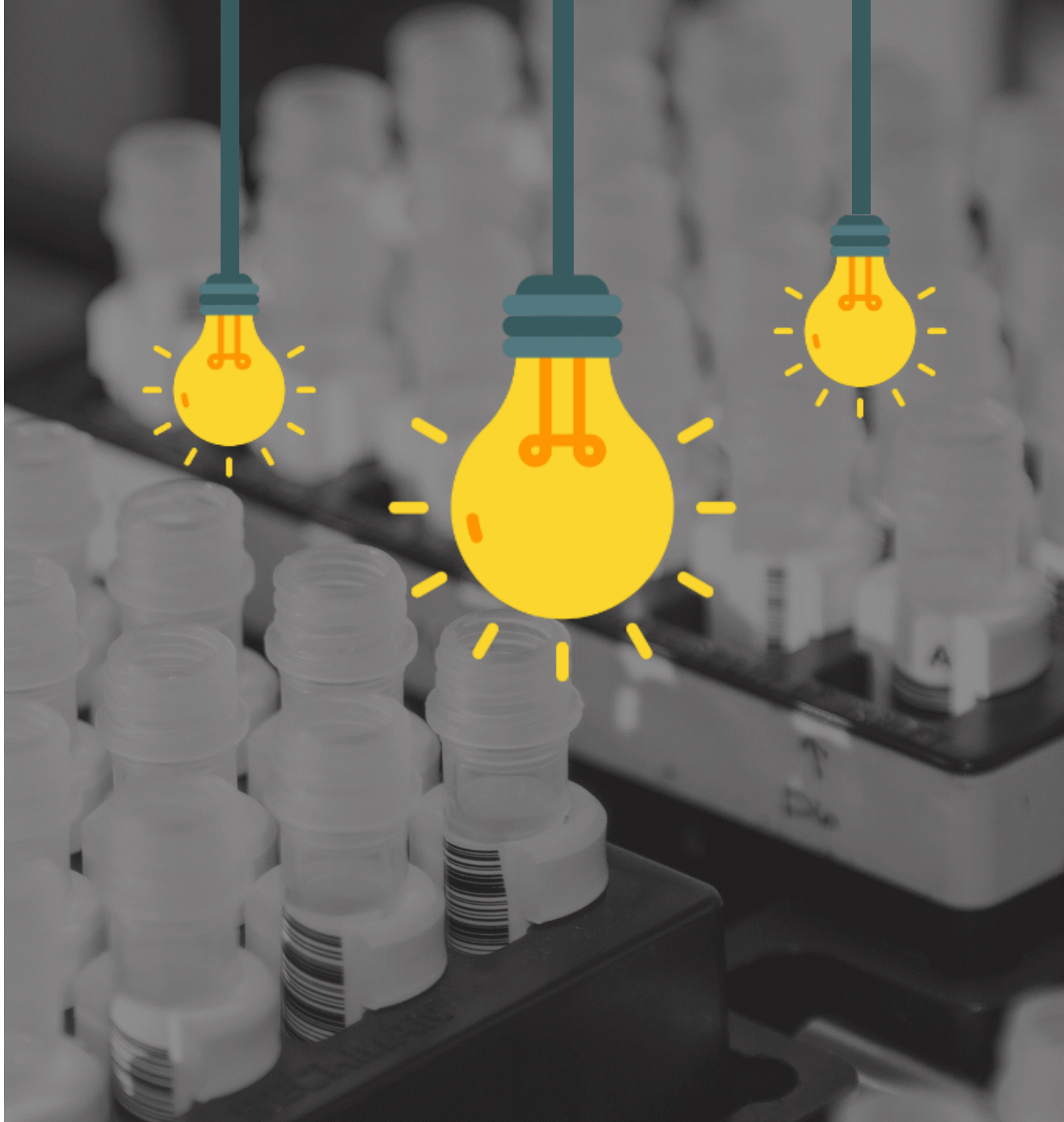


- **80-90% mortality of grow-out open-caged Asian sea bass in the brackish water environment in India.**
- **The affected fish were RSIV positive using DNA polymerase PCR and sequence analysis.**
- **The RSIV isolated from India is phylogenetically more closely related to Korean isolate of RSIV.**
- **Histopathogical examinations were investigated.**
- **The challenge test with tissue homogenate reproduced 100% mortality in the healthy sea bass.**
- **Report to OIE.**

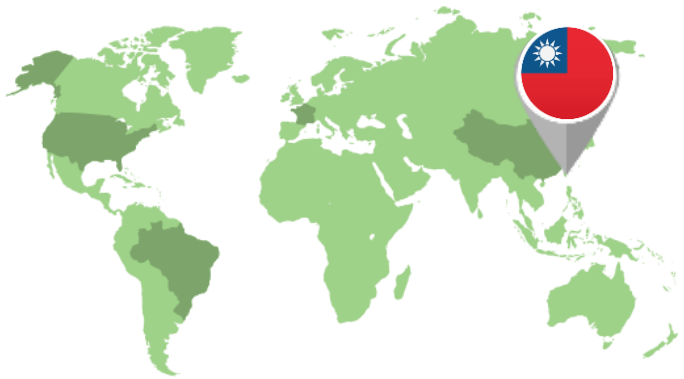


Girisha et al, 2020 Aquaculture 520: 734712

Turbot reddish body iridovirus (TRBIV)

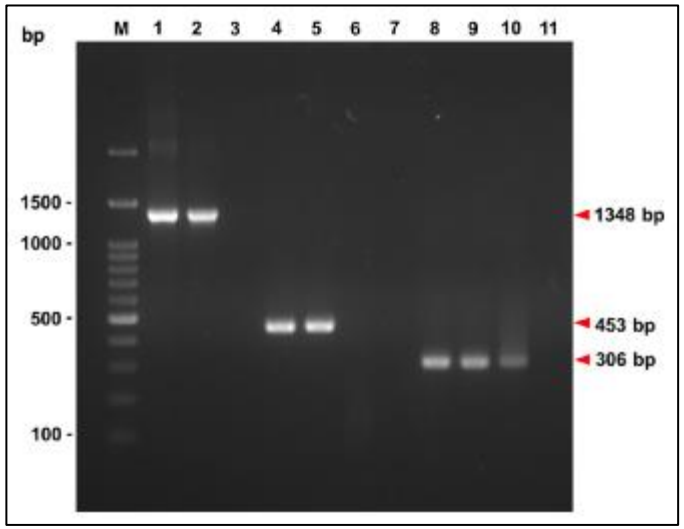


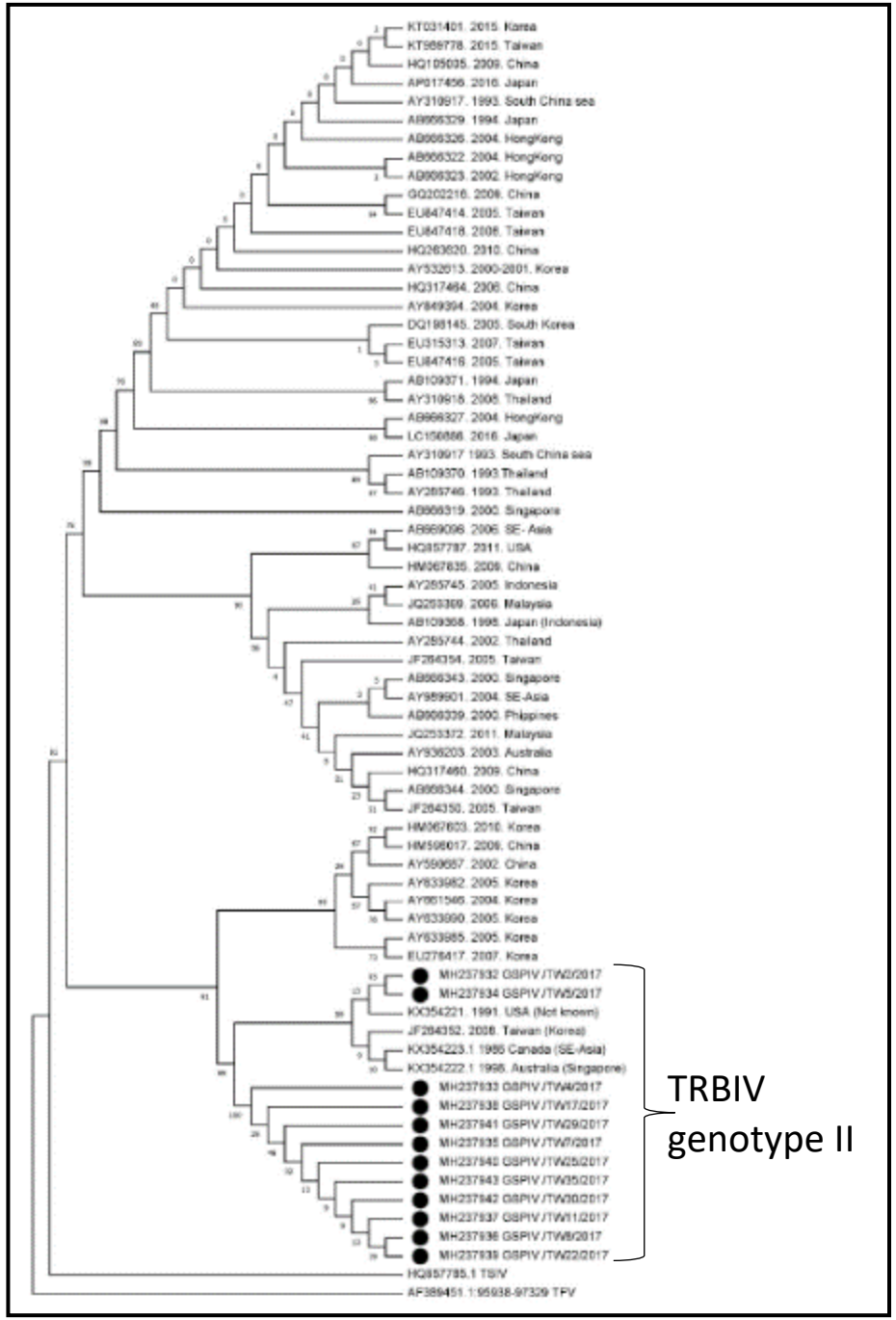
First case report of TRBIV in Asian sea bass



Tsai et al, 2020. Viruses 12: 681–699.

- *Fingerlings of L. calcarifer imported to Taiwan die up to 90%.*
- *Loss of appetite, lethargy, deep body color, petechiae of gills, severe anemia and an enlarged abdomen.*
- *Enlarged spleens and kidneys as well as reddish livers.*
- *Molecular detection revealed infection of TRBIV genotype II.*
- *Semi-nested PCR was developed to improve detection sensitivity.*





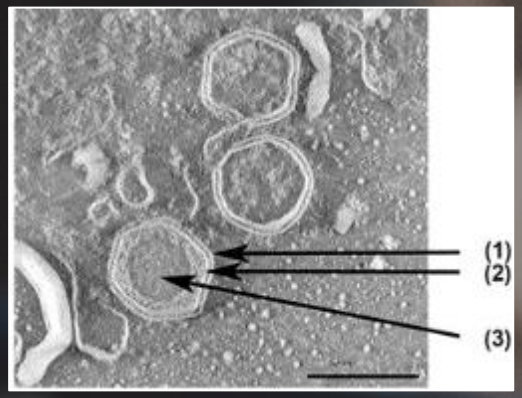
Scale drop syndrome

- SDDV (Scale drop disease virus)
  => Scale drop disease
- LCHV (Lates calcarifer herpesvirus)
  => SDD-like symptom
- A unique strain of *Vibrio harveyi*
  => scale drop and muscle necrosis

Scale drop disease (SDD)

Causative agent	• Scale drop disease virus (SDDV) • Enveloped hexagonal virions (100-180 nm) with double-stranded DNA genome of 131 kb (135 ORFs)
Clinical sign	Asian sea bass: Scale loss, some with “red belly”
Host	Asian sea bass, Yellowfin seabream (*Acanthopagrus latus*), European chub (*Squalius cephalus*), Tilapia (*Oreochromis* spp)
Mortality	Asian sea bass: 40-50% in marine, brackish, and freshwater culture
Susceptible stage	Juveniles, subadut, adult
Transmission	Horizontal-likely, Vertical??
Distribution	Singapore, Malaysia, Indonesia, Thailand, China, USA, UK (European chub iridovirus)
Histopathology	Basophilic hypertrophied cells (megalocytes) Multifocal necrosis, pyknosis and karyorrhexis Dermal inflammation and severe infiltration of lymphocytic inflammatory cells
Diagnosis	PCRs, qPCR, isothermal amplification
Genotyping	Major capsid protein (MCP) and adenosine triphosphatase (ATPase) genes
Cell culture	Seabass kidney (SK) SK21 cells Grunt fin (GF-1) cells MFF-1 cell
Prevention	Biosecurity + general managements

Scale drop disease (SDD)



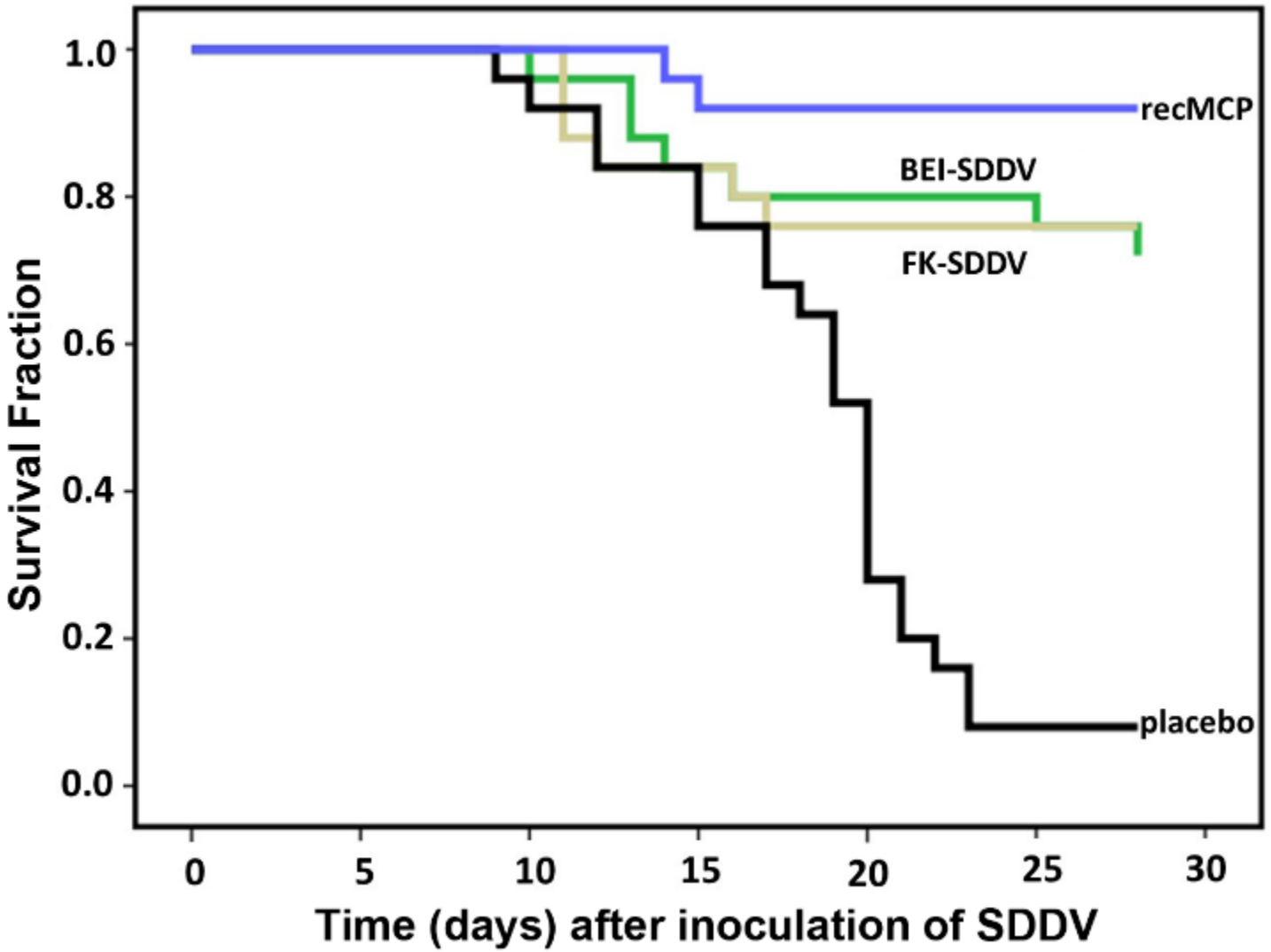
2012: Scale Drop Syndrome (SDS) – suspected viral etiology

2015: Scale Drop Disease (SDD)

- a novel Megalocytivirus (~68% genome identity to others)
- killed **40–50%** fish population (natural) **13–100%** (challenge test)
- confirmed in Singapore, Malaysia, and Indonesia
- may be circulated in SEA since 1992, misdiagnosed as Tenacibaculosis

Gibson-Kueh et al. 2012 J Fish Dis 35:19-27
de Groof et al. 2015 Plos Pathog 11(8): e1005074

Scale drop disease (SDD)

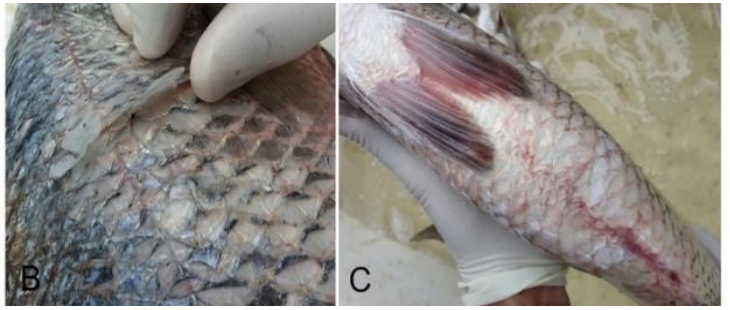


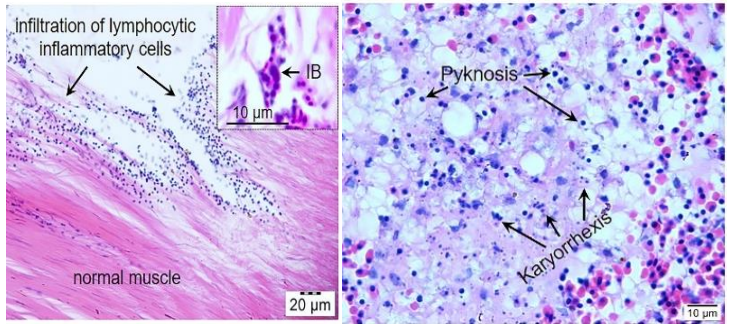
de Groof et al. 2015 Plos Pathog 11(8): e1005074

BEI, binary ethyleneimine

SDDV
in Thailand

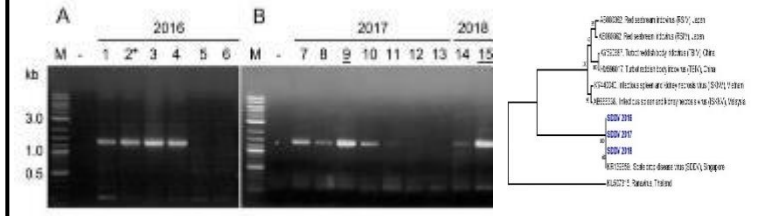
Senapin et al. 2019 J Fish Dis. 42, 119-127.
Kayansamruaj, et al 2020 J Fish Dis. 43,1287-1298

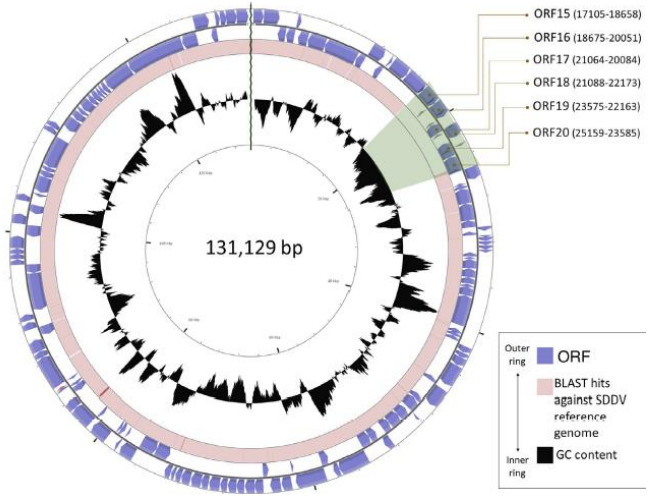




Gross signs and histopathology

- farmers call it “scale drop disease” or “red belly disease”
- observed since 2014
- affect adult and subadult fish
- mortality ~40%
- samples 2016-2018





Received: 10 February 2020 | Revised: 17 July 2020 | Accepted: 20 July 2020
DOI: 10.1111/jfd.13240

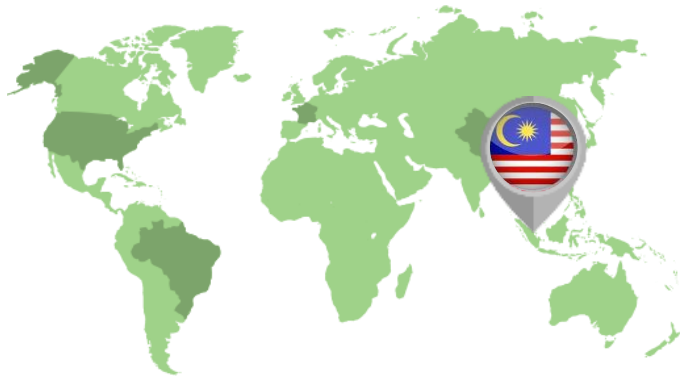
ORIGINAL ARTICLE

Journal of Fish Diseases WILEY

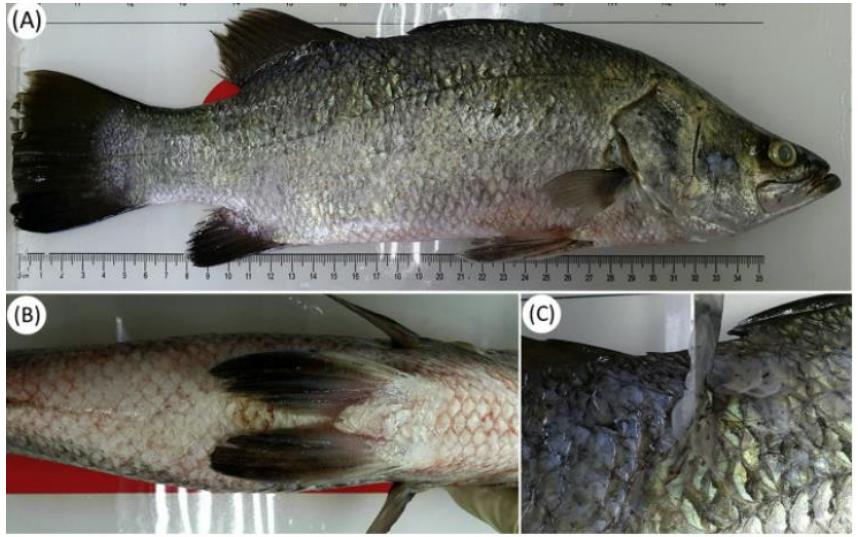
Draft genome sequence of *scale drop disease virus* (SDDV) retrieved from metagenomic investigation of infected barramundi, *Lates calcarifer* (Bloch, 1790)

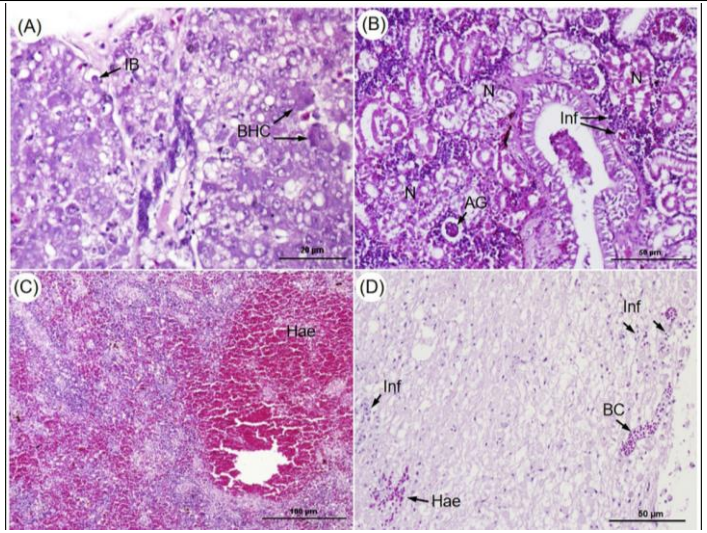
Pattanapon Kayansamruaj[1,2] | Chayanit Soontara[1] | Ha T. Dong[3,4] | Kornsunee Phiwsaiya[4,5] | Saengchan Senapin[4,5]

SDDV
in Malaysia

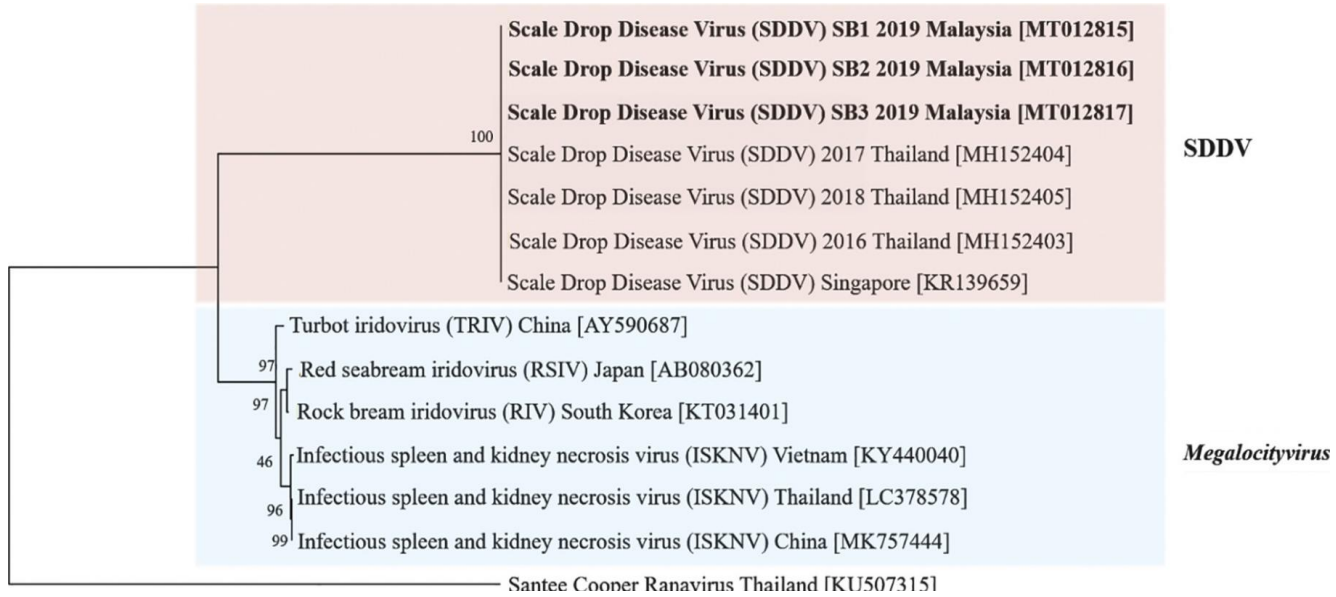


Nurliyana et al 2020 Aquaculture 528, 735600





Gross signs and histopathology



Phylogenetic tree based on MCP sequences

Fig. 1. External pathological changes of diseased barramundi. Affected fish presented with (A) wounded skin and fin fraying, as well as (B) hemorrhage at the base of fins. Additionally, there was (C) cutaneous discoloration and scale loss around the dorsal fin ('saddle back lesion') and (D) segmental gill necrosis

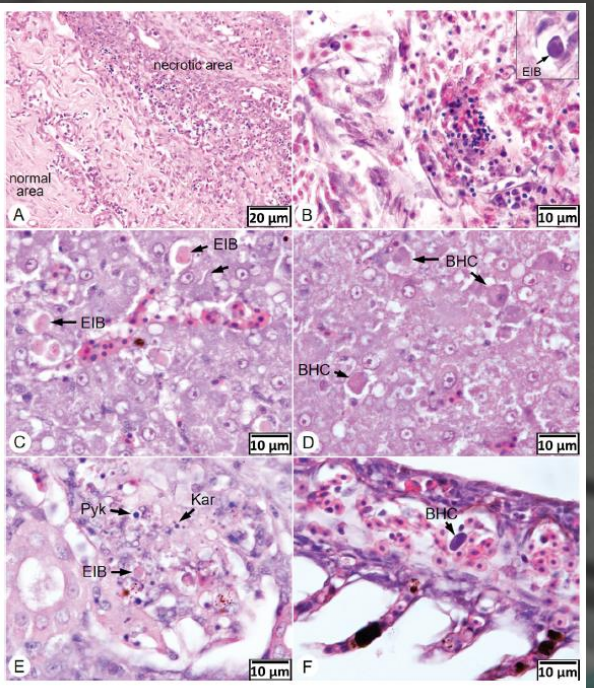


Fig. 2. Histopathological lesions in (A,B) skeletal muscle, (C,D) liver, (E) kidney, and (F) gill of diseased barramundi. BHC: basophilic hypertrophied cell (megalocytosis); EIB: eosinophilic inclusion body; Pyk: pyknosis; Kar: karyorrhexis

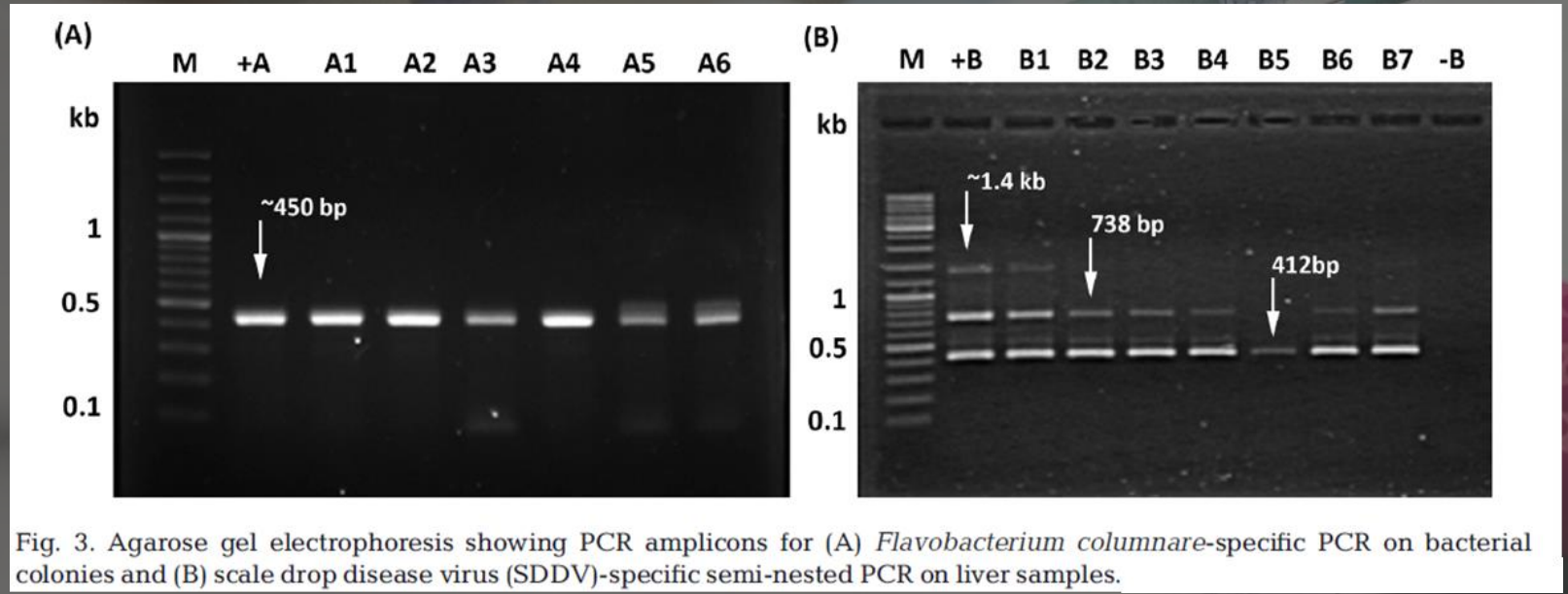
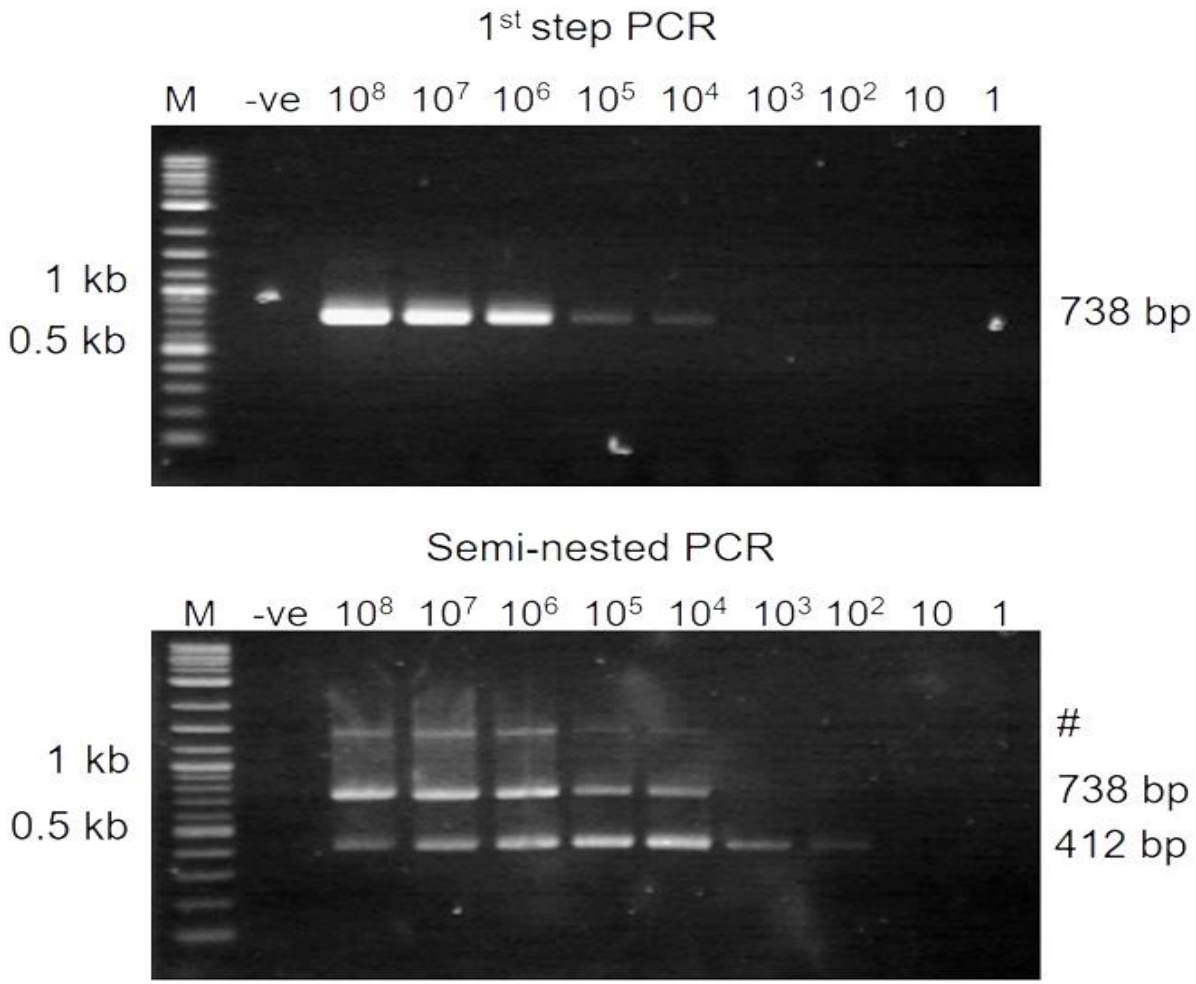


Fig. 3. Agarose gel electrophoresis showing PCR amplicons for (A) *Flavobacterium columnare*-specific PCR on bacterial colonies and (B) scale drop disease virus (SDDV)-specific semi-nested PCR on liver samples.

SDDV in freshwater cultured Asian sea bass co-infected with *Flavobacterium*

Kerddee et al 2020 DAO 140:119-128

Semi-nested PCR: 100 copies/µL



Molecular diagnosis of SDDV

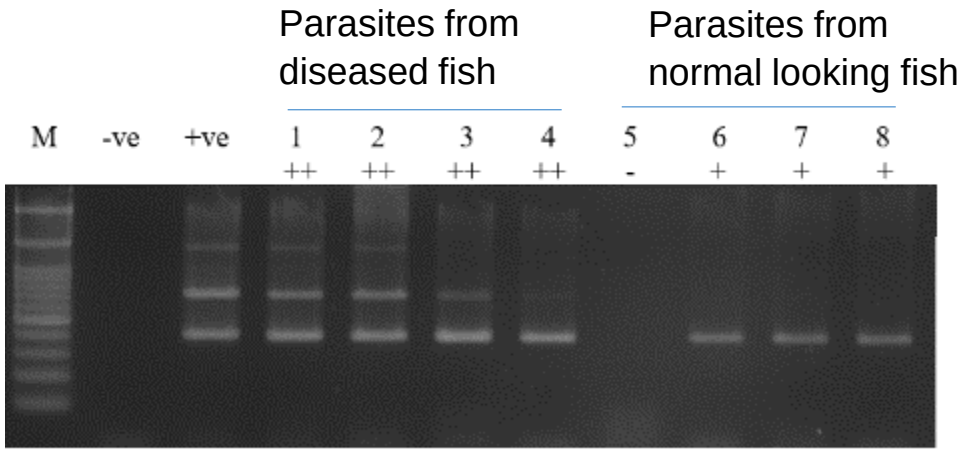
Charoenwai et al (2019)
J Virol Methods. 268: 37-41

Molecular diagnosis of SDDV

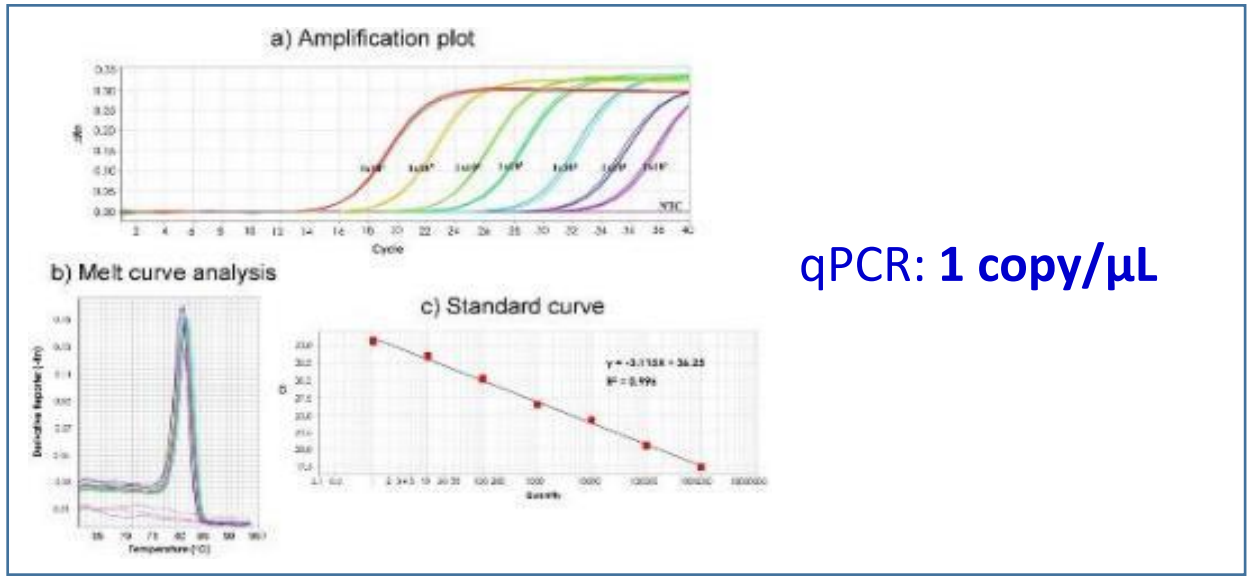
Non-lethal detection of SDDV from Asian sea bass and its potential carriers

Fish status	Samples	+ve/ total
Clinically sick fish	Blood	12/12
	Mucus	7/7
	Fin	11/15
Clinically healthy	Blood	4/4
	Mucus	4/4
	Fin	4/5

Lernanthopus sp. & *Diplectanum* sp.



Charoenwai et al (2021)
J Fish Dis. 44: 461– 467

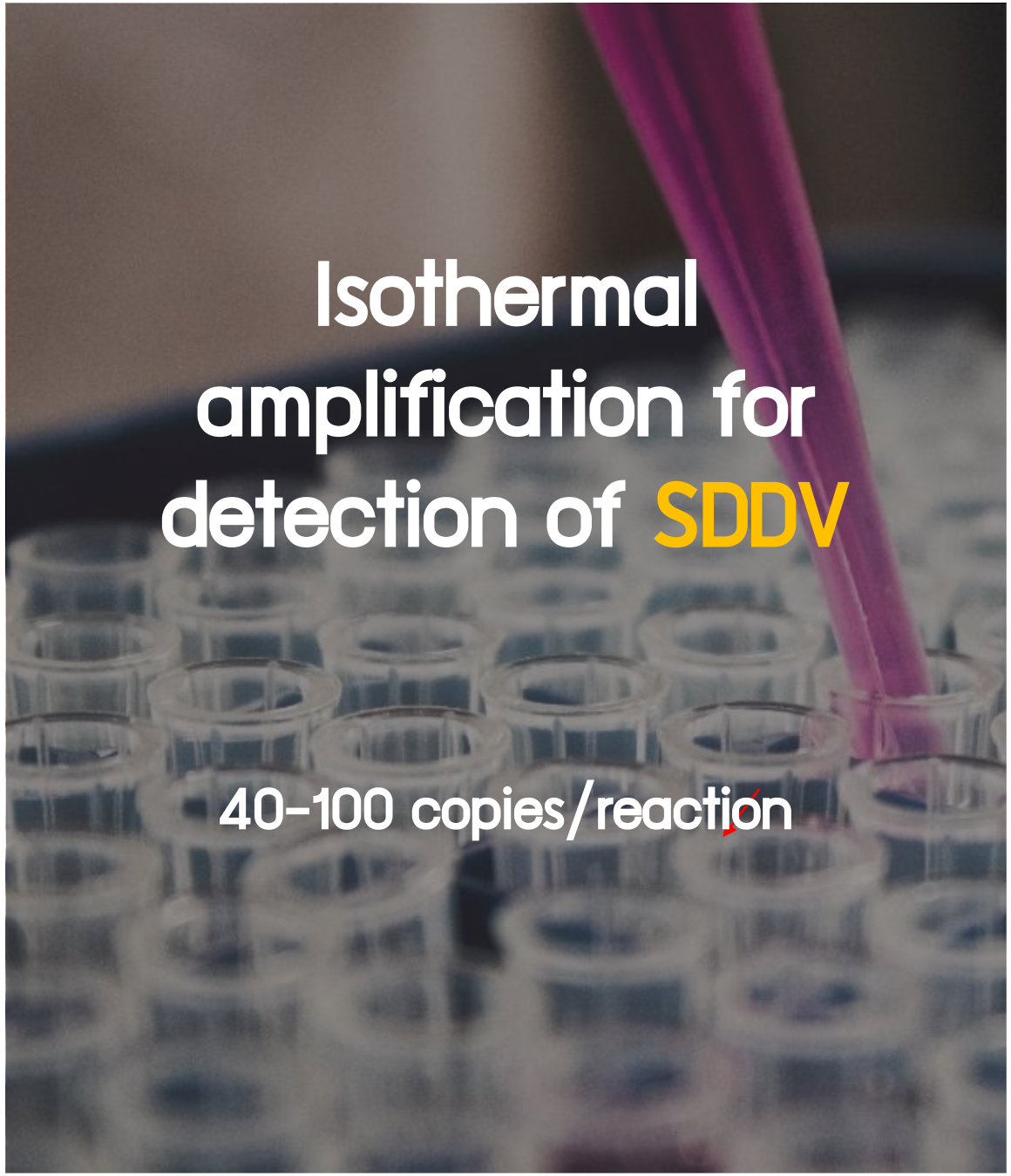


qPCR: **1 copy/µL**

Year	Farm	Fish health status	Positive sample/Number of tested samples	
			Semi-nested PCR*	qPCR
2016	Farm 1	Sick fish with scale drop symptoms	18/18	18/18[a]
	Farm 2	Unknown diseased fish	6/6	6/6
2017	Farm 3	Sick fish with scale drop symptoms	5/5	5/5
	Farm 4	Clinically healthy fish	0/12	8/12
	Farm 5	Clinically healthy fish	0/80	1/5
	Farm 6	Clinically healthy fish	0/10	0/5
	Farm 6	Clinically healthy fish	0/10	5/5
	Farm 8	Clinically healthy fish	0/10	1/5[a]
	Farm 9	Clinically healthy fish	0/10	5/5[a]
	Farm 10	Clinically healthy fish	0/5	5/5[a]
	Farm 11	Clinically healthy fish	0/10	2/5[a]
	Farm 12	Clinically healthy fish	0/13	3/5
2018	Farm 13	Sick fish with scale drop symptoms	2/2	2/2
2019	Farm 14	Sick fish with scale drop symptoms	Not done	3/3
		Total	**31/191 (16.2%)**	**64/86 (74.4%)**

Molecular diagnosis of SDDV

Sriisan et al, 2020
Dis. Aquat. Org. 139:131-137



Aquaculture 510 (2019) 386–391

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Colorimetric detection of scale drop disease virus in Asian sea bass using loop-mediated isothermal amplification with xylenol orange

Sirintip Dangtip[a], Jantana Kampeera[a], Rapheephat Suvannakad[a], Pakapreud Khumwan[a], Wansadaj Jaroenram[a], Molruedee Sonthi[b], Saengchan Senapin[c,d], Wansika Kiatpathomchai[a,*]

Received: 7 January 2021 | Revised: 30 April 2021 | Accepted: 4 May 2021

DOI: 10.1111/jfd.13448

RESEARCH ARTICLE

Journal of Fish Diseases WILEY

Development of cross-priming amplification (CPA) combined with colorimetric and lateral flow dipstick visualization for scale drop disease virus (SDDV) detection

Terawut Prasitporn[1,2] | Saengchan Senapin[3] | Akapon Vaniksampanna[4] | Siwaporn Longyant[1,2] | Parin Chaivisuthangkura[1,2]

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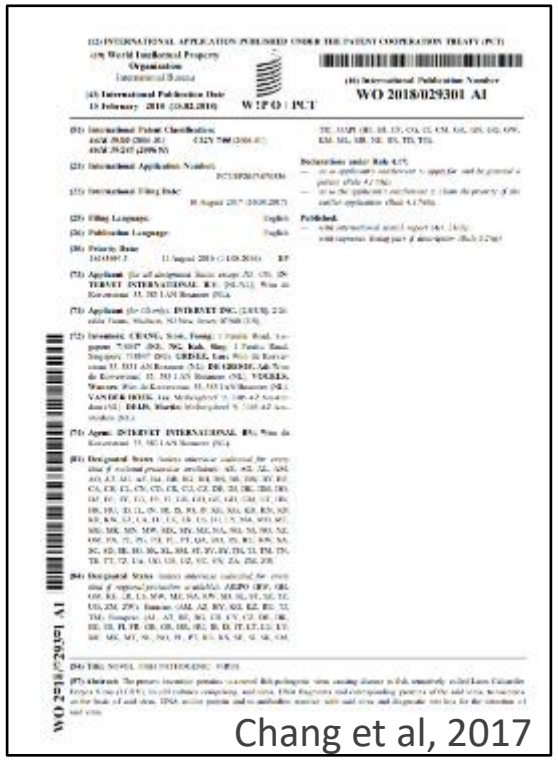
DOI: 10.1111/jfd.13541

RESEARCH ARTICLE

Journal of Fish Diseases WILEY

CRISPR-based platform for rapid, sensitive and field-deployable detection of scale drop disease virus in Asian sea bass (*Lates calcarifer*)

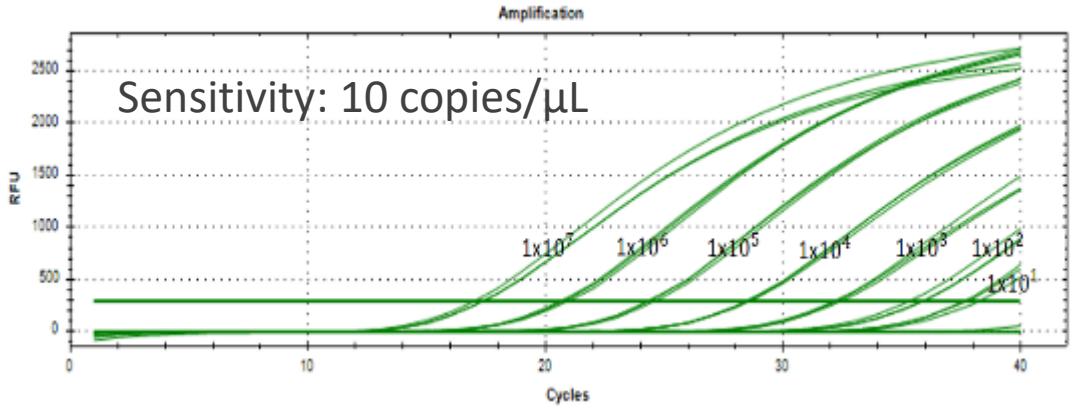
Thanwarat Sukonta[1] | Saengchan Senapin[1,2,3] | Watcharachai Meemetta[3] | Thawatchai Chaijarasphong[1,3]



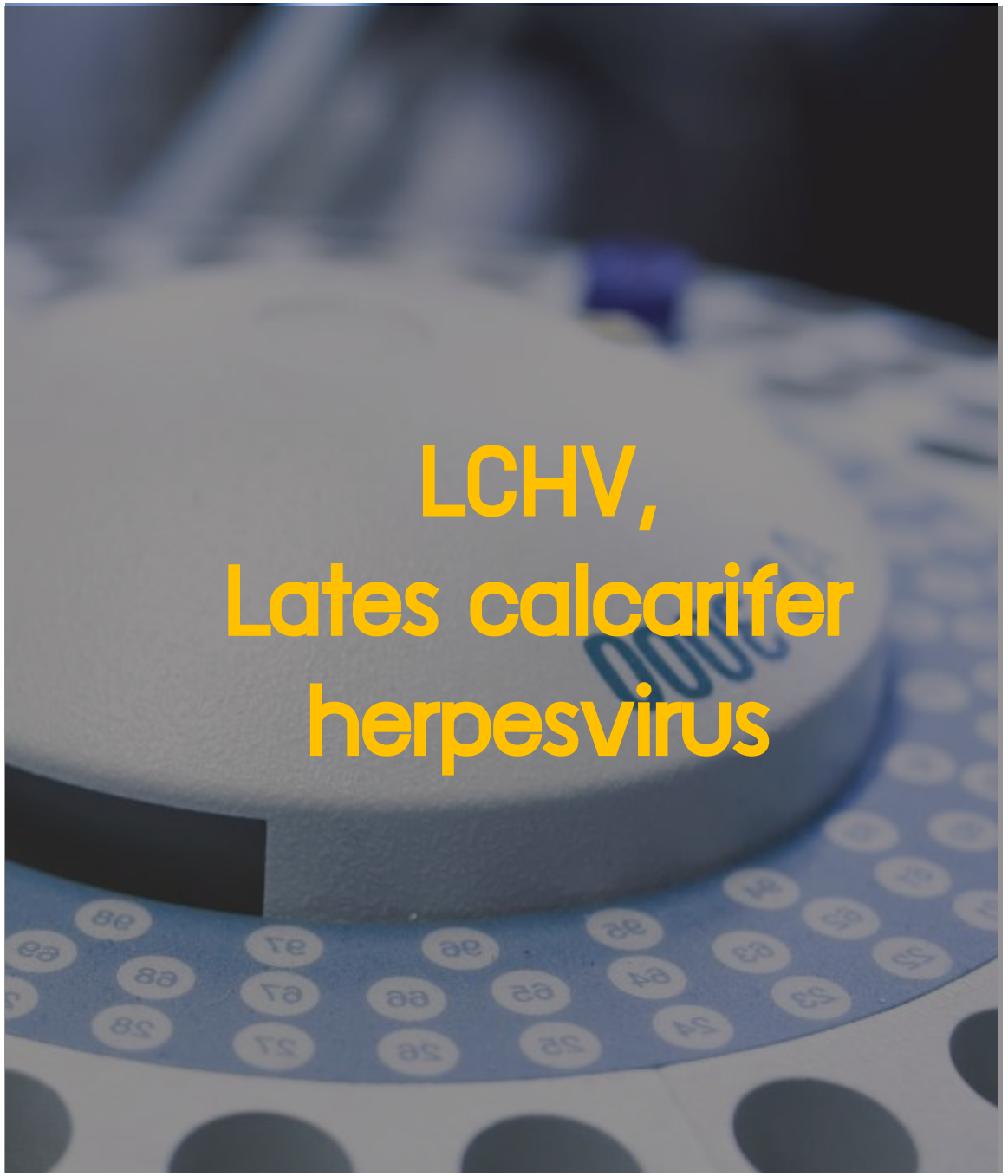
Chang et al, 2017

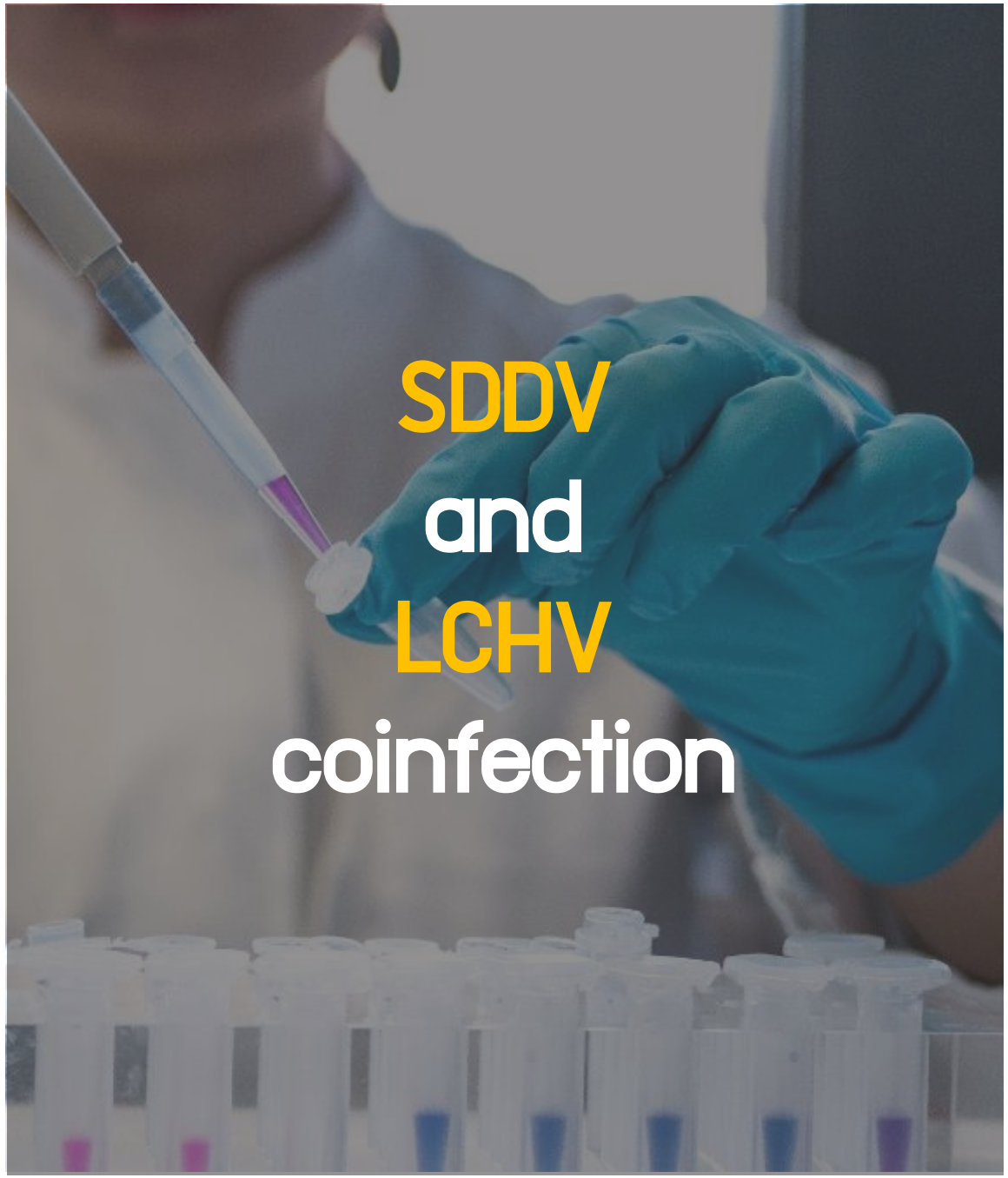
- Diseased fish with SDD-like symptom
- Singapore and Vietnam, 2015
- Mortality 30-70% (natural)
- 77% mortality in co-habitation or i.p. challenges
- Genome 130 kb
- Called Lates calcarifer herpes virus (LCHV)

Development of qPCR detection method for LCHV



Meemetta et al, 2020 J Virol Methods. 285: 113920





frontiers in Genetics

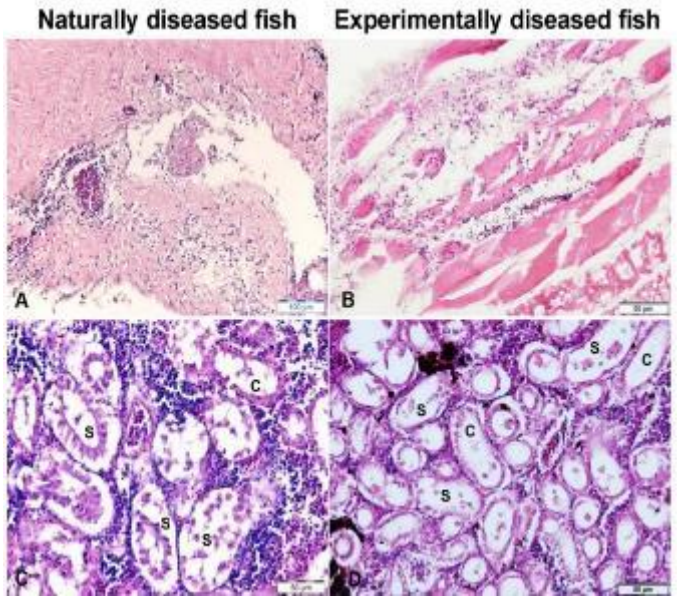
ORIGINAL RESEARCH
published: 18 June 2021
doi: 10.3389/fgene.2021.666897

Scale Drop Disease Virus (SDDV) and *Lates calcarifer* Herpes Virus (LCHV) Coinfection Downregulate Immune-Relevant Pathways and Cause Splenic and Kidney Necrosis in Barramundi Under Commercial Farming Conditions

Jose A. Domingos[1,2], Xueyan Shen[1], Celestine Terence[1], Saengchan Senapin[3,4], Ha Thanh Dong[3,5], Marie R. Tan[6], Susan Gibson-Kueh[1] and Dean R. Jerry[1,2]*

Scale Drop and Muscle Necrosis Disease (SDMND)

Caused by a pathogenic *V. harveyi*



Is toxin involved ?

Dong et al. Aquaculture (2017) 473:89-96

Viral Nervous Necrosis (VNN)

or Viral encephalopathy and retinopathy (VER) disease

Viral Nervous Necrosis (VNN) or Viral encephalopathy and retinopathy (VER) disease

Causative agent	• Betanodavirus, Nervous necrosis virus (NNV) • Non-enveloped icosahedral virus (appx 25 nm in diameter) with two positive-strand RNA segments of 3.1 and 1.4 kb
Clinical sign	Darkening of the skin & erratic swimming. Some with pop-eye
Host	> 60 fish species, mainly marine fish
Mortality	High mortality up to 100%
Susceptible stage	All stages, mainly larvae and juveniles
Transmission	Horizontal & vertical
Distribution	• Widely distributed • Cases in Asia sea bass = Malaysia, Australia, Indonesia, Singapore, Taiwan, China, Israel, Philippines, Tahiti, India, Iran
Histopathology	Vacuolation in brain and retina
Diagnosis	RT-PCRs, qPCR, isothermal amplification, Immunological based techniques
Genotyping	RNA 2 segment encoding a viral capsid protein
Cell culture	Striped snakehead cell line, SSN-1 E-11, a clone of SSN-1 & others
Prevention	Biosecurity + improvement of host immunity + water management + reduce stress

Viral Nervous Necrosis (VNN) disease

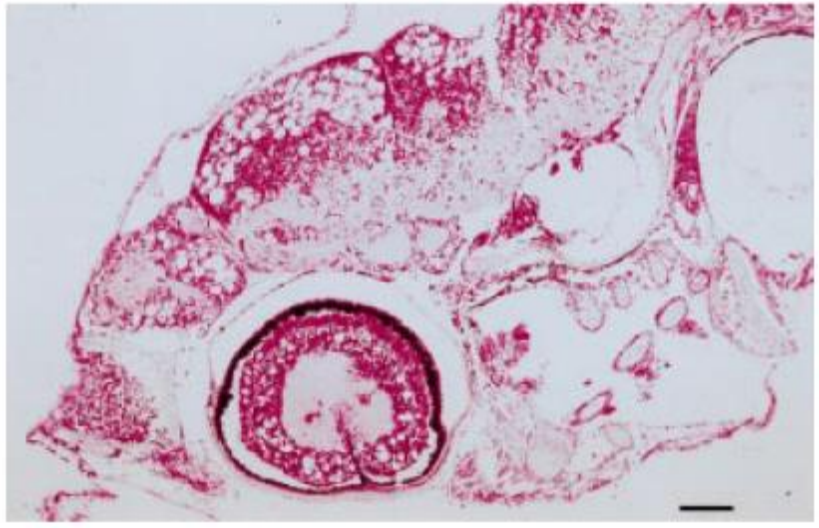


Figure 2 Barramundi larva with betanodavirus infection. Note severe vacuolation of the brain and retina (H & E, bar = 100 µm).

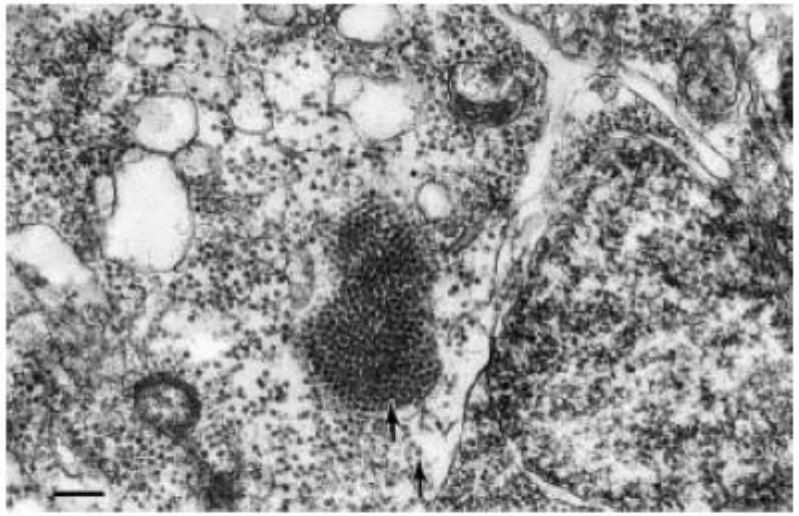


Figure 3 Transmission electronmicrograph of betanodavirus particles in the brain of a barramundi larva (bar = 250 nm).

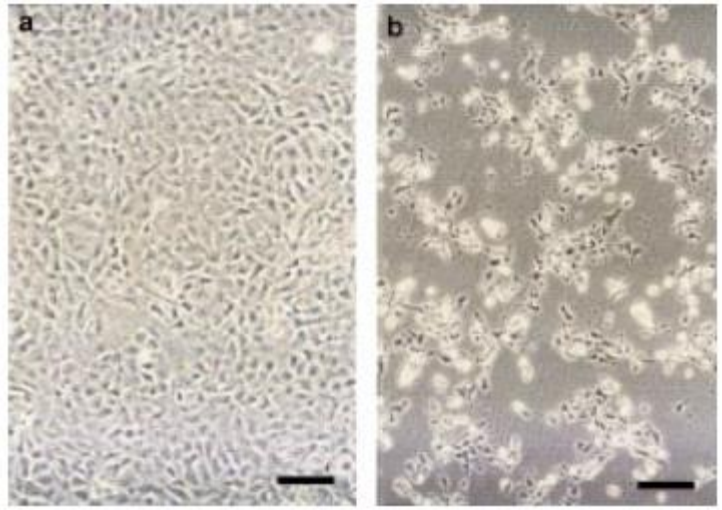
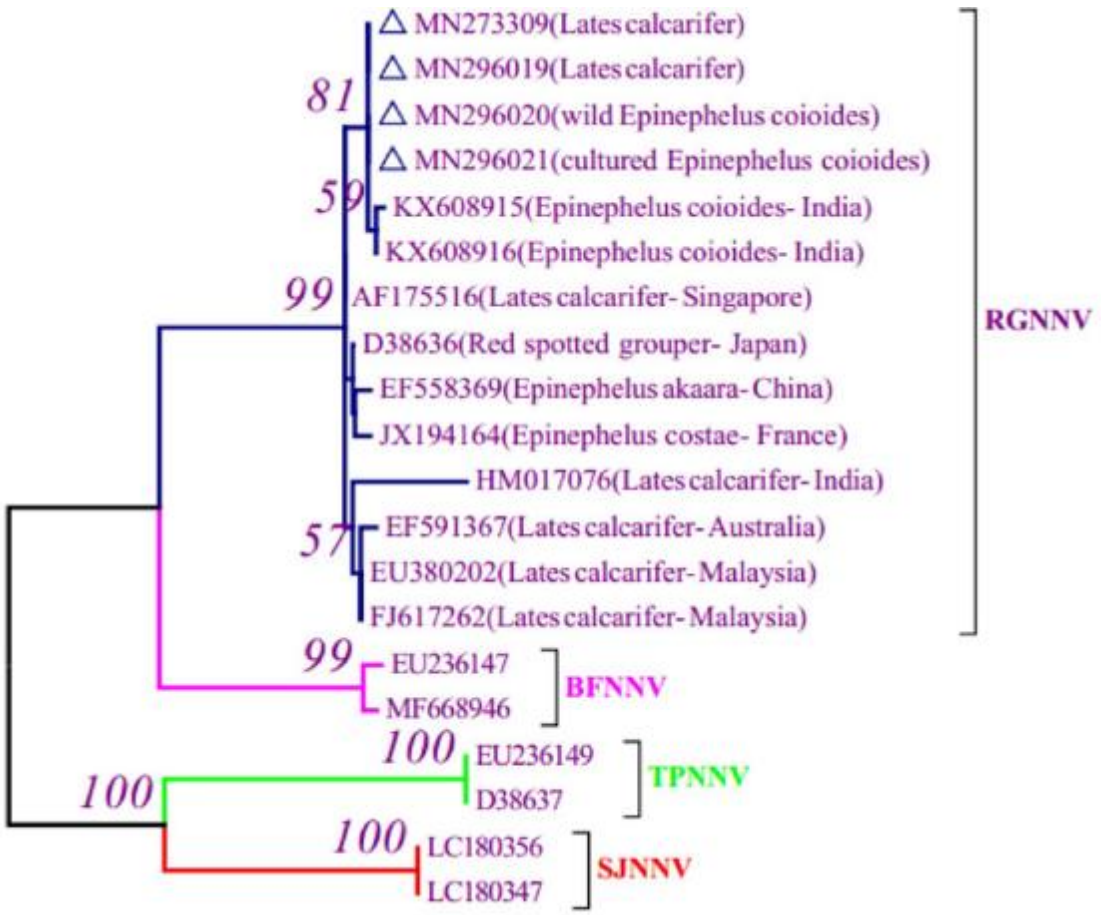
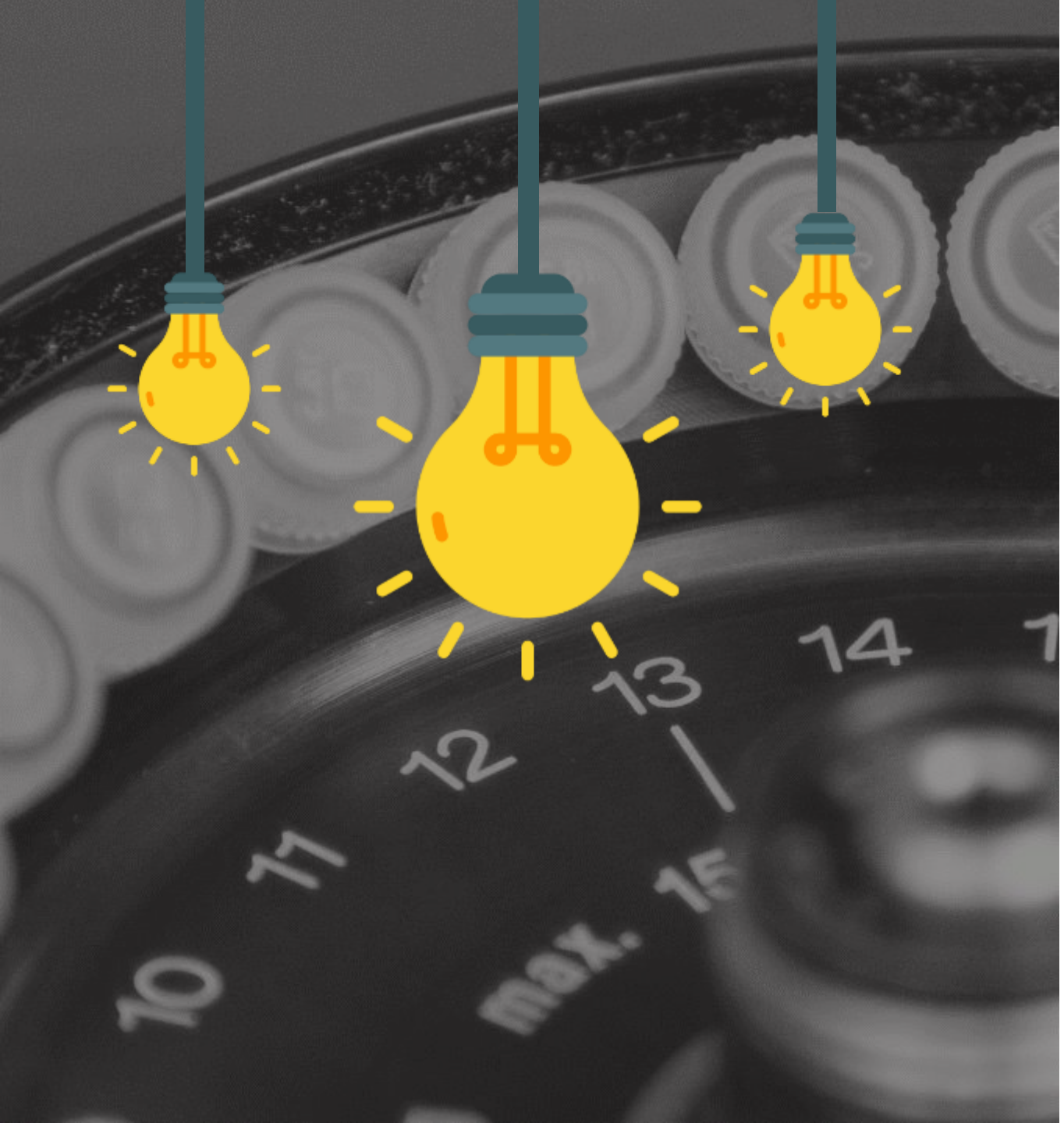


Figure 6 Fish cell line, barramundi/sea bass: (a) normal, non-infected cells (bar = 100 µm); (b) cells showing CPE caused by greasy grouper nervous necrosis virus (bar = 100 µm).

Munday et al Journal of Fish Diseases 2002, 25, 127-142



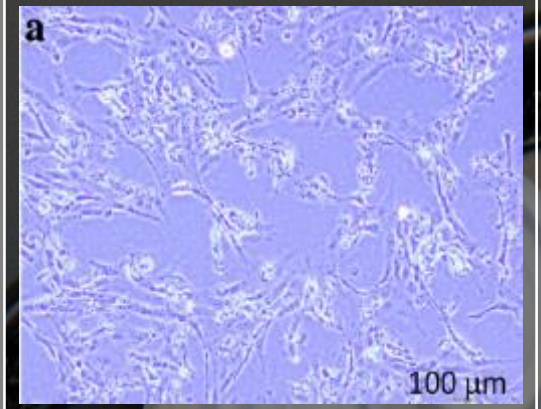
Ziarati et al. Current Microbiology (2020) 77:3919–3926



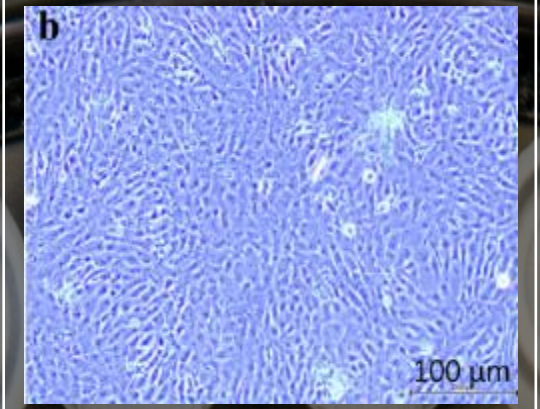
Lates calcarifer Birnavirus (LCBV)

LCBV, a novel RNA virus in Asian sea bass

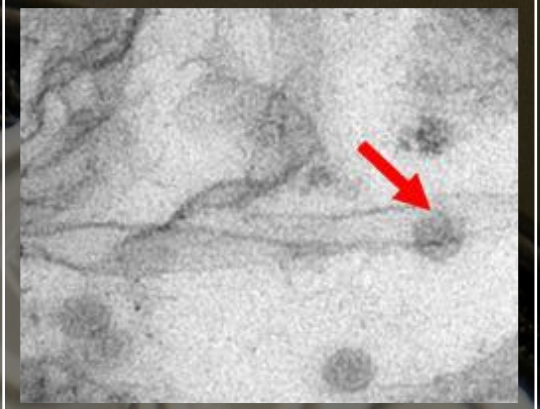
Chen et al. Virology Journal (2019) 16:71



Cell rounding and detachment

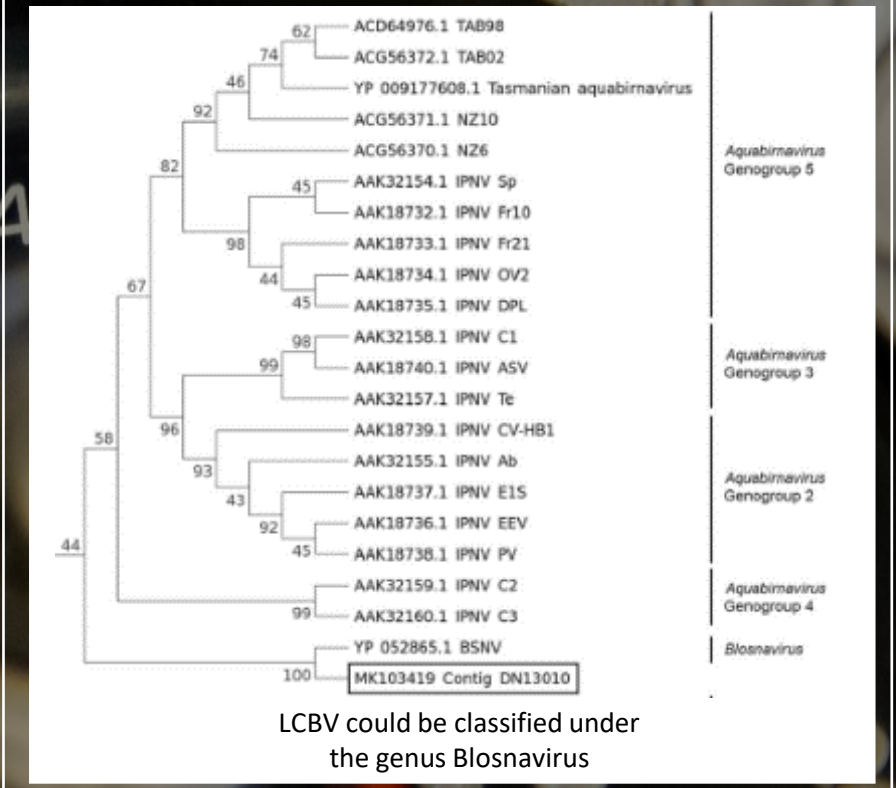


Control Bluegill Fry (BF-2) cells



Icosahedral virions with 50 nm in diameter

Experimental challenge of naïve fish with LCBV showing “white patch” lesions but mortality was not different with the control group.



LCBV could be classified under the genus Blosnavirus

Comments

- There are a growing number of viruses (and other pathogens) affecting Asian sea bass (and other species).
- No treatment for viral diseases in aquaculture, so 'Prevention is better than cure'.
- Vaccination and selective breeding are envisioned as future disease management strategies for aquacultured Asian sea bass.
- Promote systematic and regular surveillance for known and undiagnosed pathogens.
- Utilize regionally available Internet resources for actively sharing and updating information.
- Basic sciences should be continuously and financially supported.
- Promote aquaculture as a career option for the younger generation.

DAA 11 2022
KUCHING, SARAWAK MALAYSIA

11th Symposium on Diseases in Asian Aquaculture

23-26 August 2022
Kuching, Sarawak, Malaysia

Thank you
for your attention

Fish Health Section

Asian Fisheries Society