Role of structural variations in the genetic diversity and evolution of the large DNA *Cyprinid herpesvirus* 3

Nurul Novelia Fuandila

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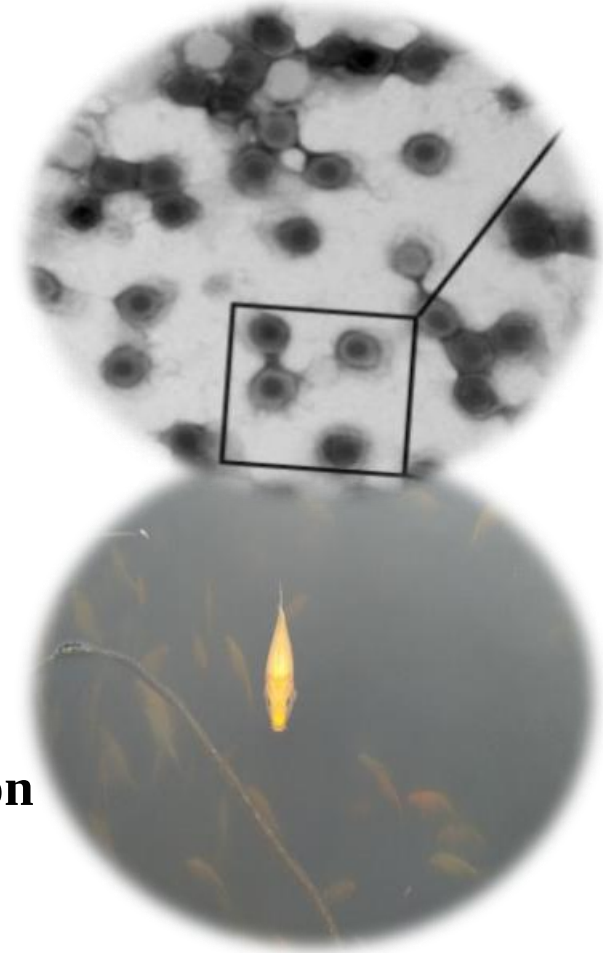
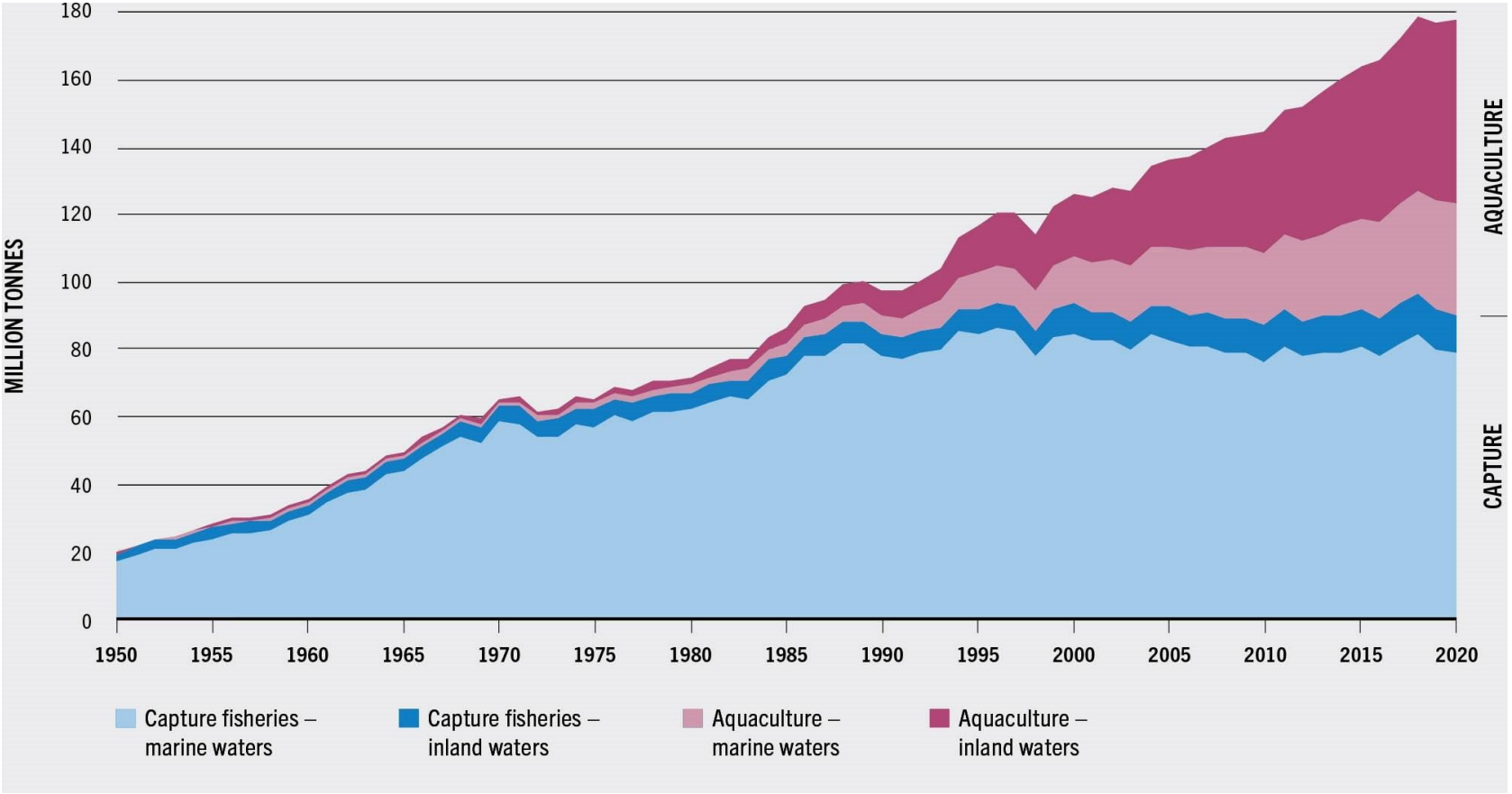
Presentation outline

- General introduction
- Research objectives
- Structural variations of CyHV-3 after *in vitro* passages
- Structural variations of CyHV-3 in carp populations of Indonesia
- Effect of *in vitro* serial passages and thermal stress on CyHV-3 evolution
- Conclusions
- Perspectives and recommendations



Current aquaculture

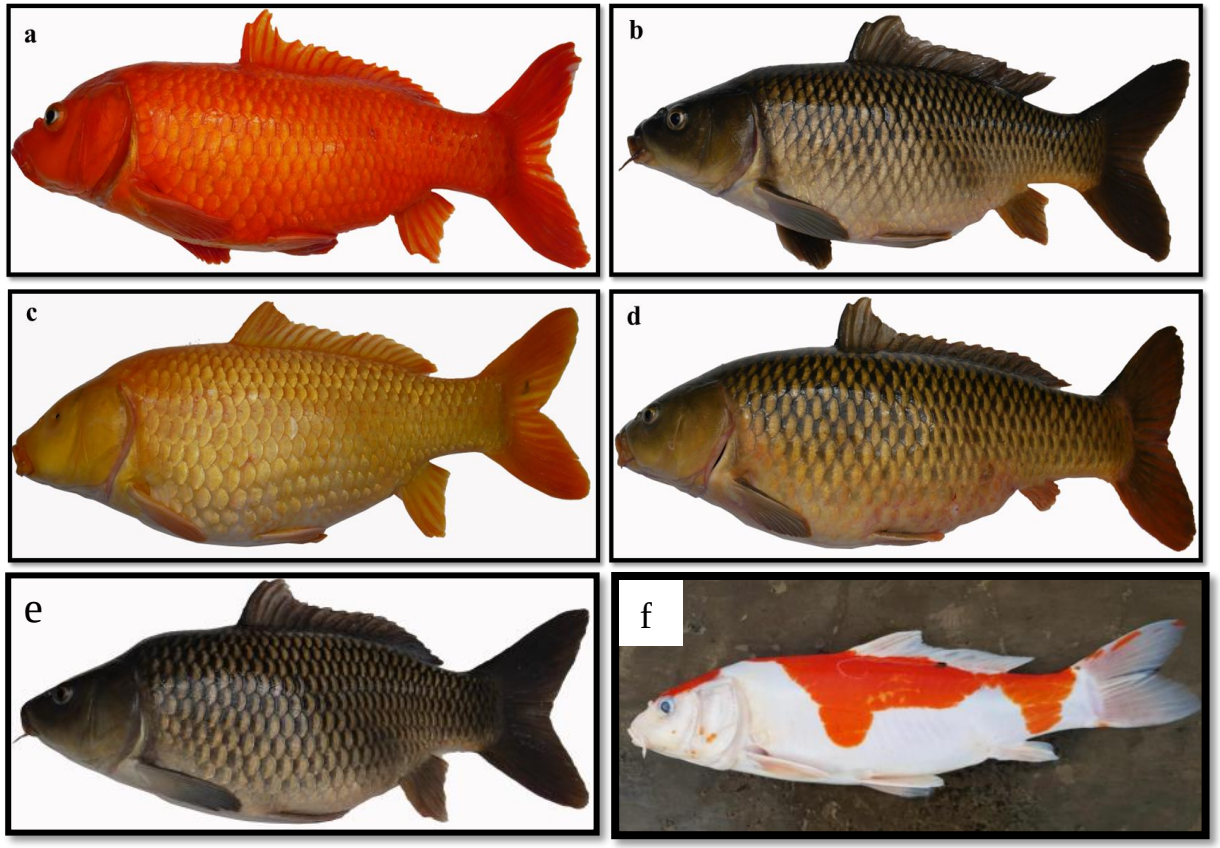
A major industry to contribute to food security



FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO.

Common carp

- Freshwater fish cultivated for human consumption and recreation (koi)
- Common carp listed as the top five species contributing to the aquaculture global production (8.6% in 2020) (FAO 2022)
- Common carp reproduce prolifically and thrive in various aquatic environments



Several strains of Common carp from Indonesia. a) Cangkringan; b) Mustika; c) Sinyonya; d) Punten; e) Rajadanu; f) Koi

FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO.

Carp viral diseases

Intensification

⇩

Increasing number of disease outbreaks

Main failure in common carp production

Cyprinid viral diseases. (FAO 2022)

Disease	Pathogen	Genome
Koi herpesvirus disease	Koi herpesvirus (KHV); Cyprinid herpesvirus-3 (CyHV-3)	dsDNA
Spring virenis of carp	Spring virenis of carp virus (SVCV)	(−) ssRNA
Grass carp hemorrhagic disease	Grass carp reovirus (GCRV)	dsRNA
Koi sleepy disease	Carp edema virus (CEV)	dsDNA
Carp pox disease	Carp pox herpesvirus (CPHV); Cyprinid herpesvirus-1 (CyHV-1)	dsDNA
Herpesviral haematopoietic necrosis	Herpesviral haematopoietic necrosis virus (HVHNV); Cyprinid herpesvirus-2 (CyHV-2)	dsDNA

FAO. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO.

Koi Herpesvirus Disease (KHVD)

Michel *et al.*, 2010

KHVD often leads to up to 95% mortality

KHVD is a seasonal disease, occurring when water temperature is between 18 °C and 28 °C

Rakus *et al.*, 2013

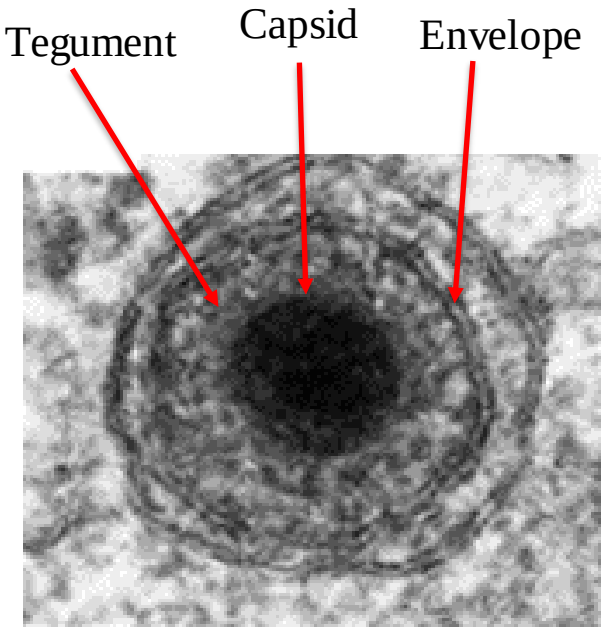
To date, there is no effective treatment against KHVD

Michel *et al.*, 2010. Cyprinid herpesvirus 3. Emerg Infect Dis. 2010
Rakus *et al.*, (2013). Cyprinid herpesvirus 3: An interesting virus for applied and fundamental research. Veterinary Research, 44(1), 85.

Cyprinid herpesvirus 3 (CyHV-3)

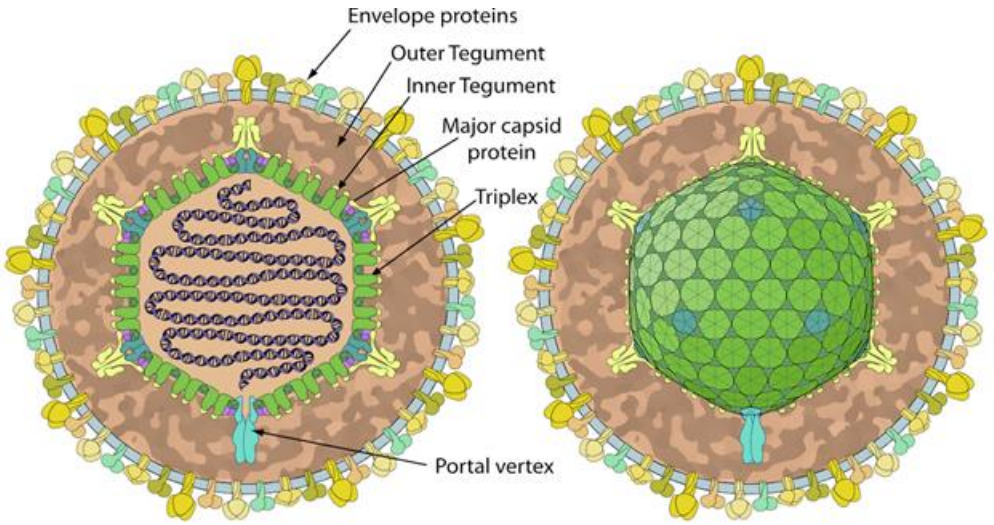
Order : *Herpesvirales*
Family : *Alloherpesviridae*
Species : *Cyprinid herpesvirus 3*

- *Koi herpesvirus* (KHV)
- *Cyvirus cyprinidallo3* (2022)



Mettenleiter *et al.*, 2009

Alloherpesvirus morphology and structure

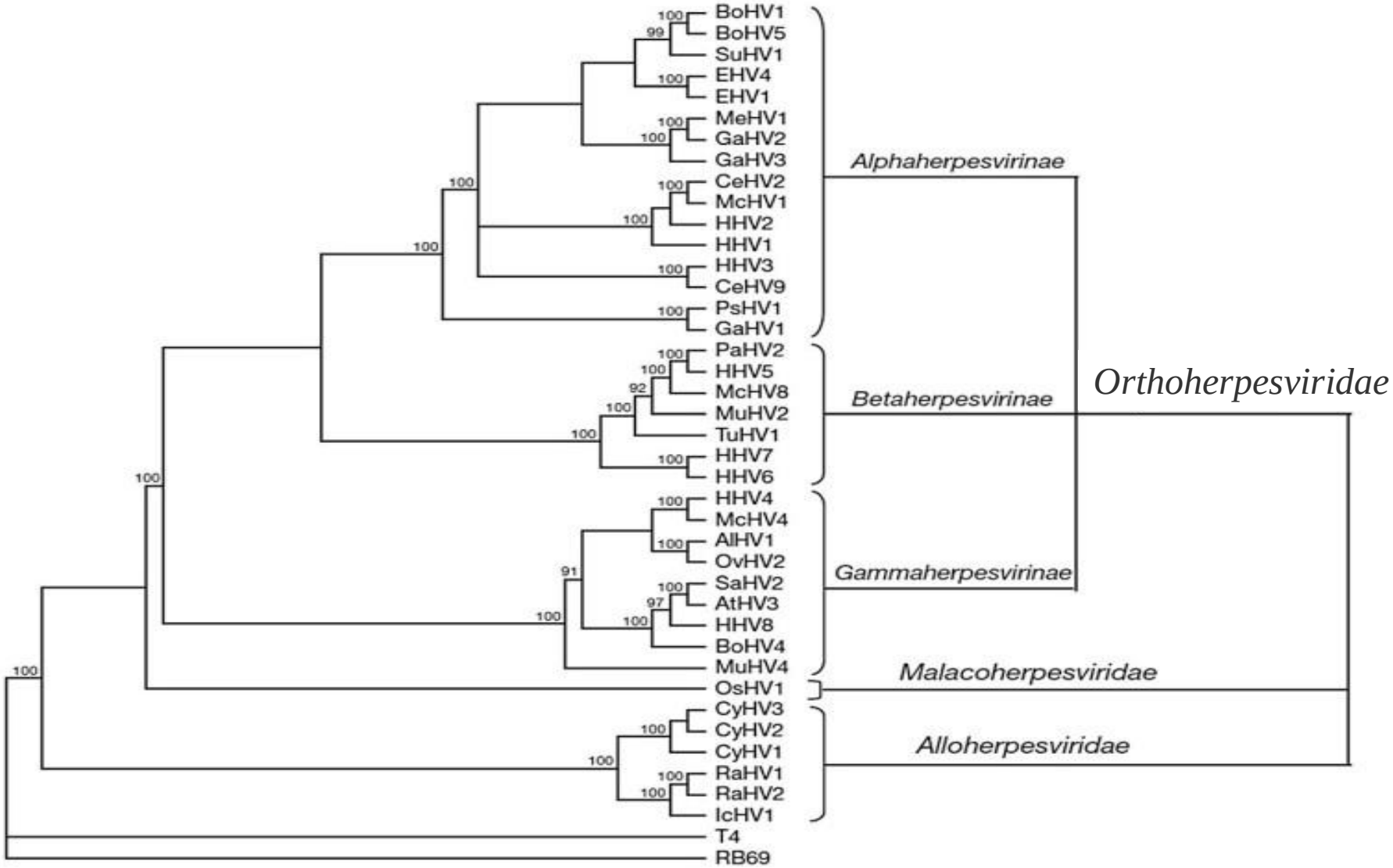


Source: Viral zone, Swiss Institute Bioinformatic

- CyHV-3 has a dsDNA genome of 295 kbp, the largest genome among herpesviruses

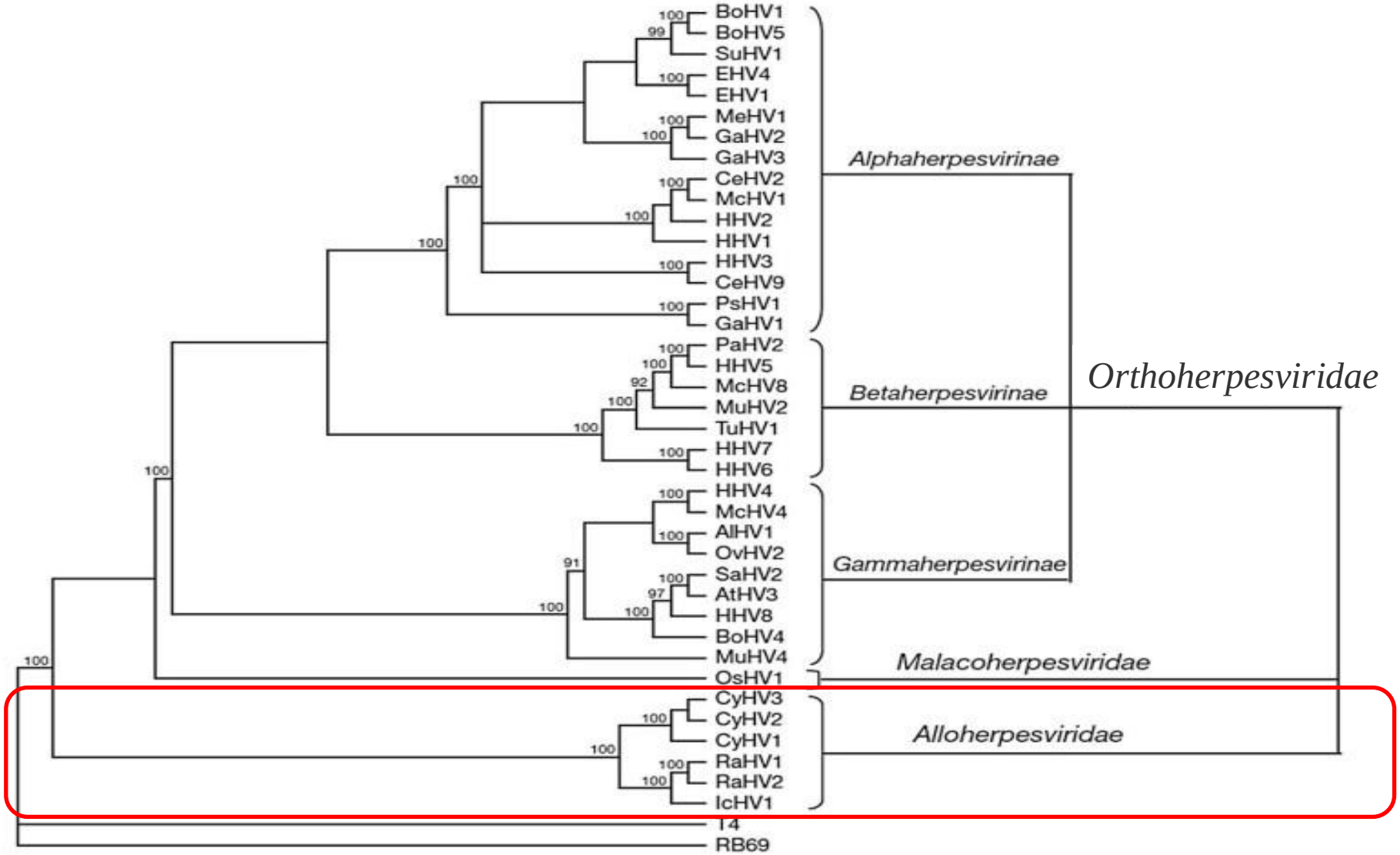
Mettenleiter *et al.*, 2009. Herpesvirus assembly: an update. Virus Res. 143: 222-234.

Phylogeny of herpesviruses



Waltzek *et al*,. (2009). Phylogenetic relationships in the family Alloherpesviridae. Dis Aquat Organ 84, 179–194.

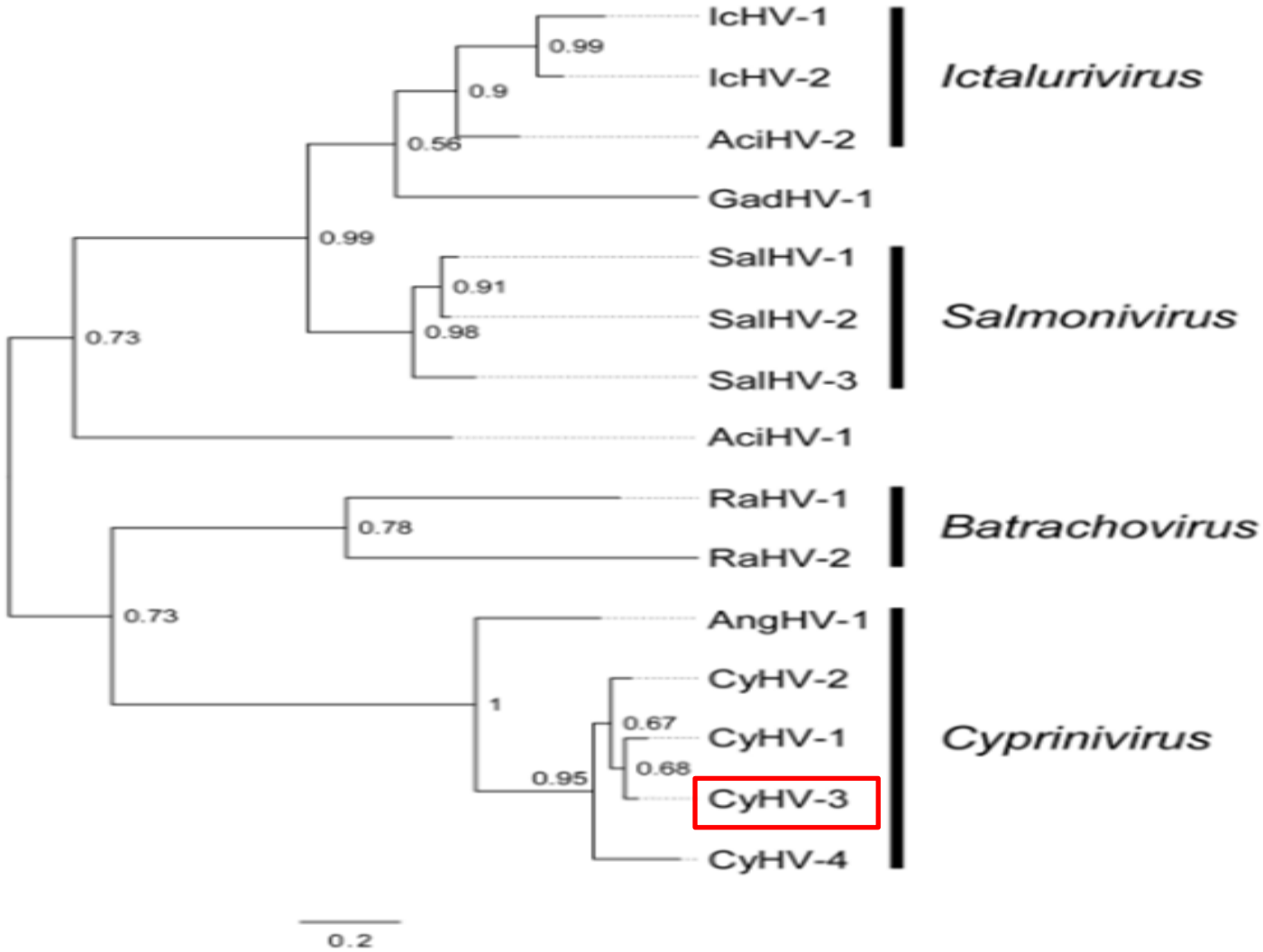
Phylogeny of herpesviruses



Waltzek *et al*,. (2009). Phylogenetic relationships in the family Alloherpesviridae. Dis Aquat Organ 84, 179–194.

Phylogeny of alloherpesviruses

Partial DNA polymerases of *Alloherpesviridae*



Waltzek *et al*,. (2009). Phylogenetic relationships in the family Alloherpesviridae. Dis Aquat Organ 84, 179–194.

Emergence of CyHV-3

KHVD outbreaks were first observed in 1996 and CyHV-3 first describe as etiological agent in 1998

1996-2002 → 2002-2005 →

Israel
United States
Germany, UK

Southeast Asia (Indonesia, Thailand)
China, Taiwan

(Hedrick, *et al*. *2000)*

Hedrick *et al*,. (2000). A Herpesvirus Associated with Mass Mortality of Juvenile and Adult Koi, a Strain of Common Carp. J Aquat Anim Health 12, 44–57

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Israel
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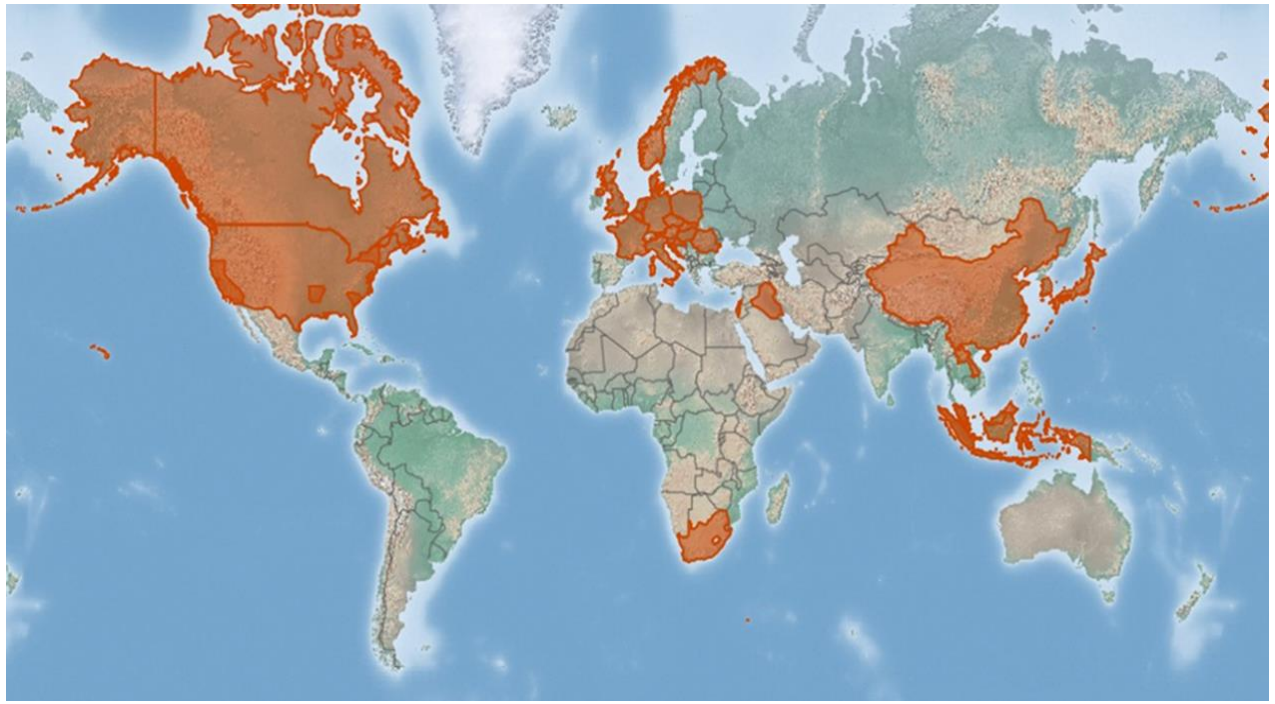
China, Taiwan

(Hedrick, *et al. 2000)*

This lethal virus has spread rapidly around the world



Positive CyHV-3

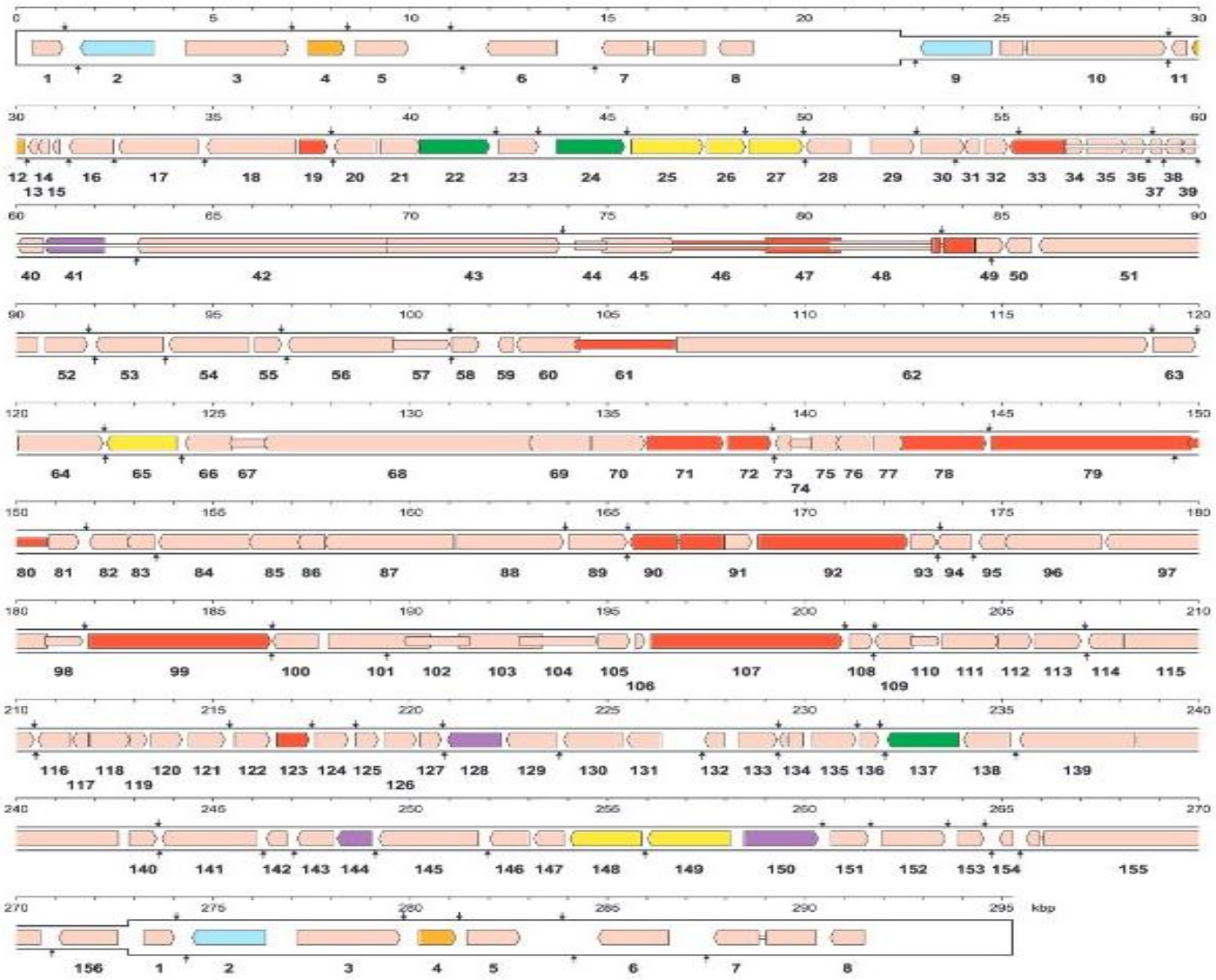
Hedrick *et al*,. (2000). A Herpesvirus Associated with Mass Mortality of Juvenile and Adult Koi, a Strain of Common Carp. J Aquat Anim Health 12, 44–57

Susceptible hosts for CyHV-3

S. No.	Host fish species	Nature of Infection
1	Common carp (*Cyprinus carpio carpio*)	Symptomatic
2	Koi carp (*Cyprinus carpio koi*)	Symptomatic
4	Goldfish (*Carassius auratus*)	Asymptomatic/carrier
5	Goldfish × koi carp hybrid	Symptomatic
6	Crucian carp × koi carp hybrid	Symptomatic
7	Goldfish × common carp	Symptomatic
8	Grass carp (*Ctenopharyngodon idella*)	Asymptomatic/carrier
9	Ide (*Leuciscus idus*)	Asymptomatic/carrier
10	Ornamental catfish (*Ancistrus* sp.)	Asymptomatic/carrier
11	Russian sturgeon (*Acipenser gueldenstaedtii*)	Asymptomatic/carrier
12	Atlantic sturgeon (*Acipenser oxyrinchus*)	Asymptomatic/carrier

Rathore *et al.*, (2012). Koi herpes virus: a review and risk assessment of Indian aquaculture. Indian J Virol 23, 124–133

CyHV-3 genome map

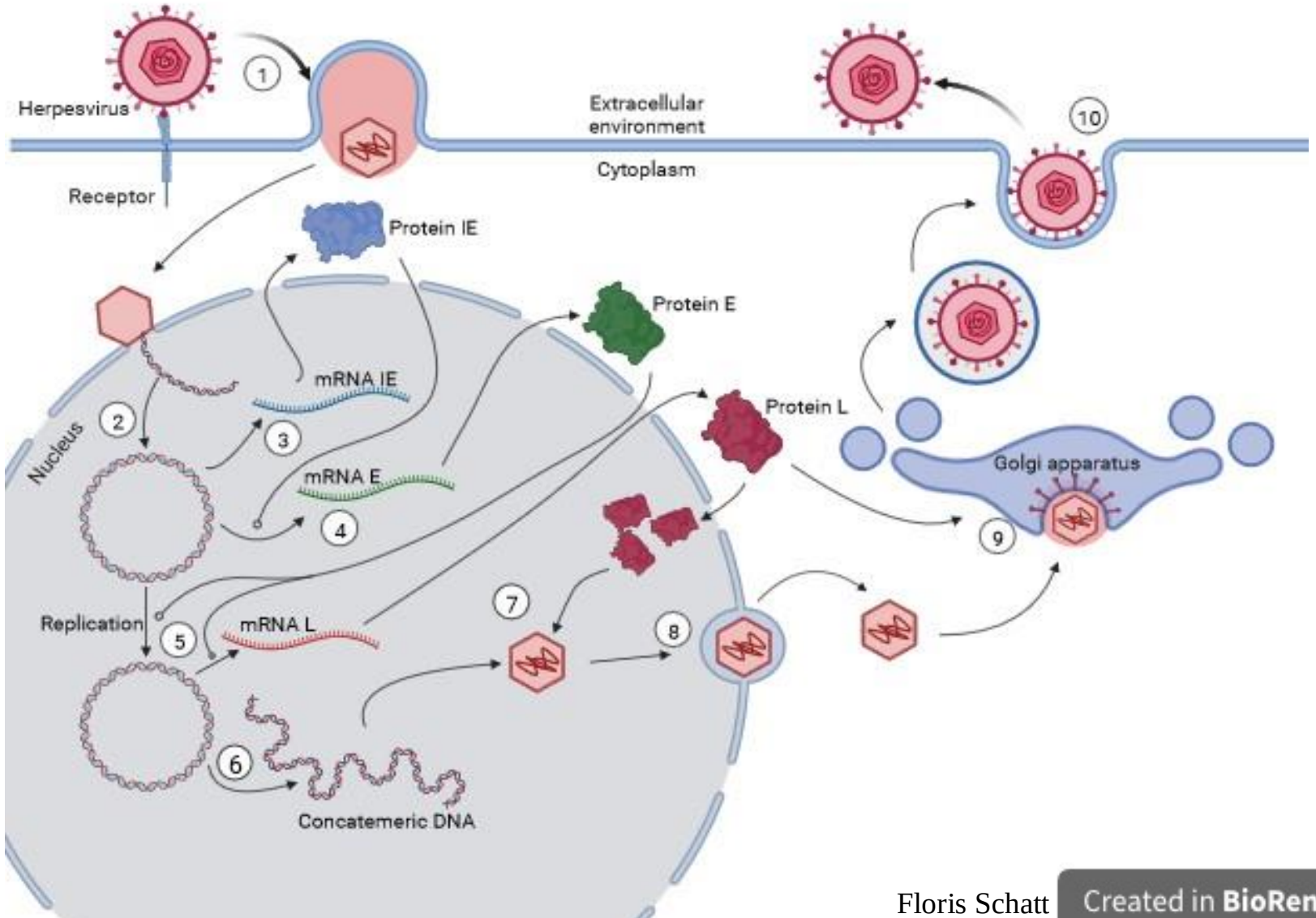


- CyHV-3 contains 156 open reading frames (ORFs) predicted to encode proteins
- Genome consists of a unique sequence flanked by a direct repeat (22kb) (Aoki *et al.*, 2007)

The vast majority of ORF has no available functional annotation

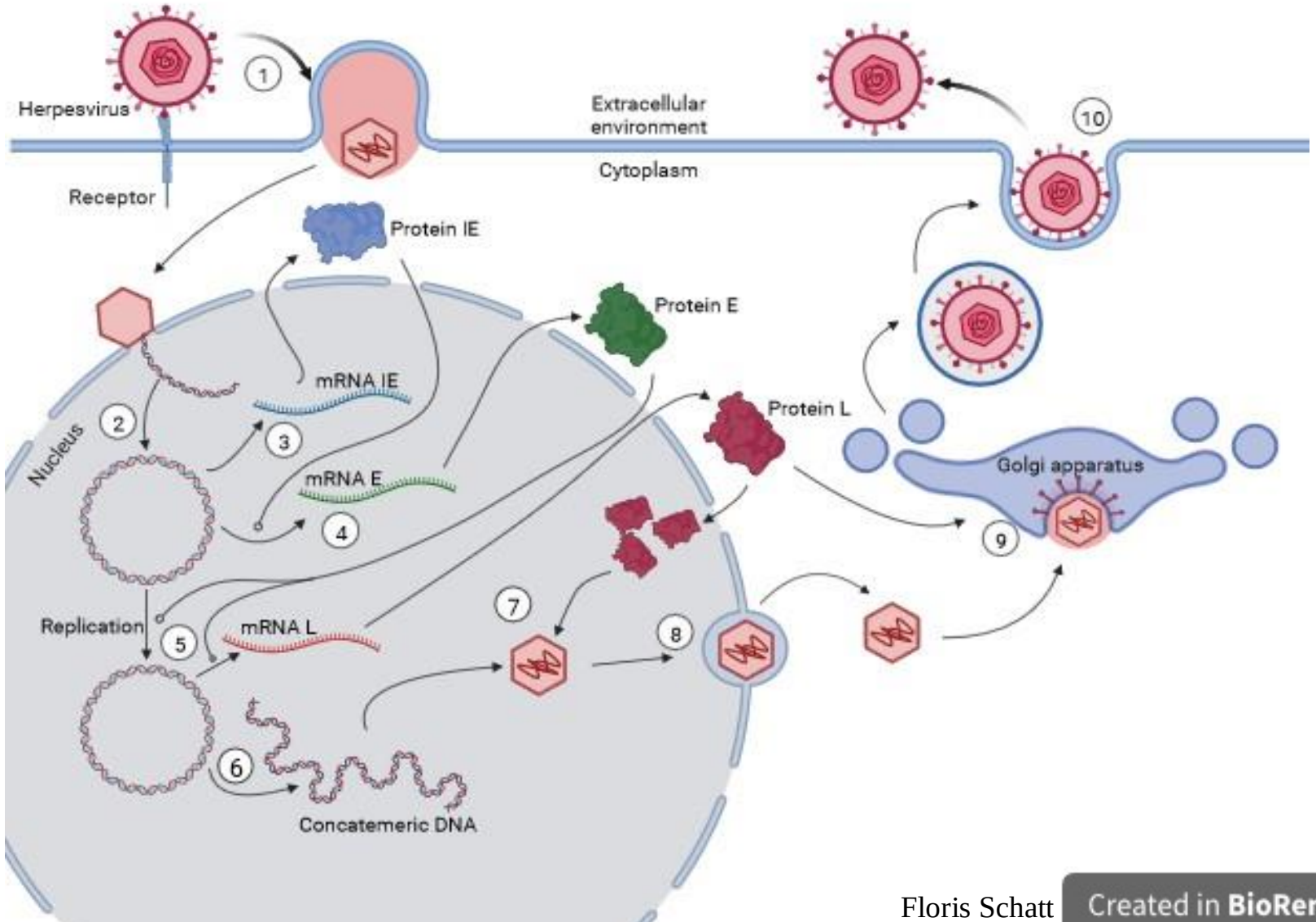
Aoki *et al*,. (2007). Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. J Virol 81

Lytic cycle of CyHV-3



1: Attachment and penetration
2: Decapsidation
3: Transcription of IE genes,
4: Transcription of E gene,
5: Transcription of late (L) viral gene
6: Viral replication in a rolling circle mode
7: Assembly of capsid proteins and encapsidation
8: Budding into the nuclear envelope,
9: Acquisition of the integument and budding into the Golgi apparatus
10: Release of virions by exocytosis

Lytic cycle of CyHV-3

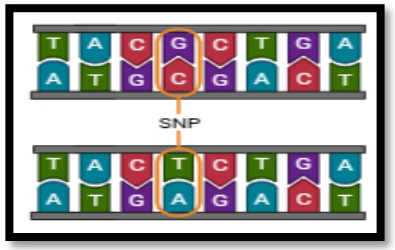


- **CyHV-3 regularly create new variants during replication :**
  - **Mutation**
  - **Recombination events**

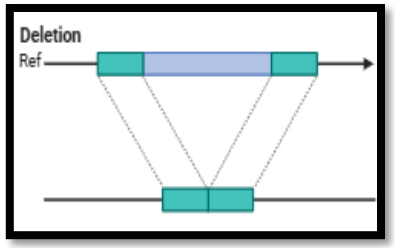
Floris Schatt

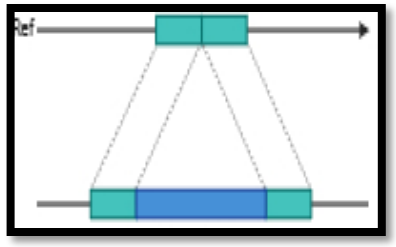
Created in BioRender.com

The molecular mechanisms of viral evolution

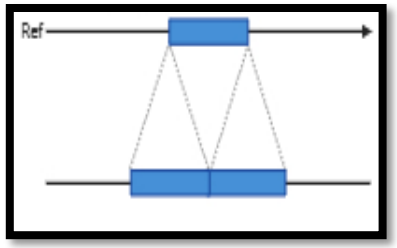


Single nucleotide polymorphism (**SNP**)

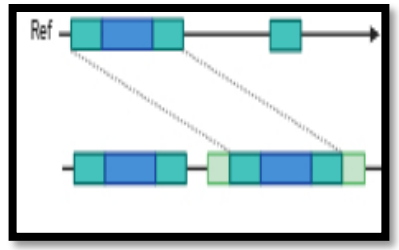




Sequence gain or loss through insertions, deletions and duplications



Fluctuations in tandem repeats



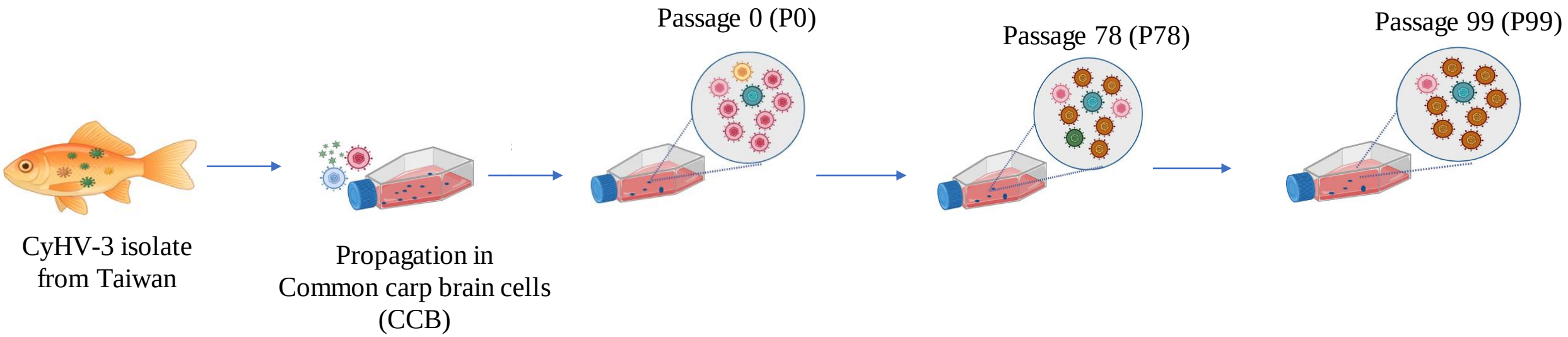
Recombination

- Genomic rearrangements, or structural variations (**SV**s)
- SVs play a crucial role in the evolution of DNA viruses, allowing them to adapt to different environments

- **CyHV-3 infections are the result of haplotype assemblies, both *in vivo* and *in vitro*** (Hammoumi *et al.*, 2016; Klafack *et al.*, 2019**)**

A haplotype is defined as a set of DNA variations that are located on the same chromosome

➢ **CyHV-3 infections are the result of haplotype assemblies, both *in vivo* and *in vitro*** (Hammoumi *et al.*, 2016; Klafack *et al.*, 2019)



Hammoumi *et al*,. (2016). Targeted genomic enrichment and sequencing of CyHV-3 from carp tissues confirms low nucleotide diversity and mixed genotype infections. PeerJ, e2516.

Klafack *et al.*, 2019. Cyprinid herpesvirus 3 evolves in vitro through an assemblage of haplotypes that alternatively become dominant or under-represented. Viruses 11, 0–12.

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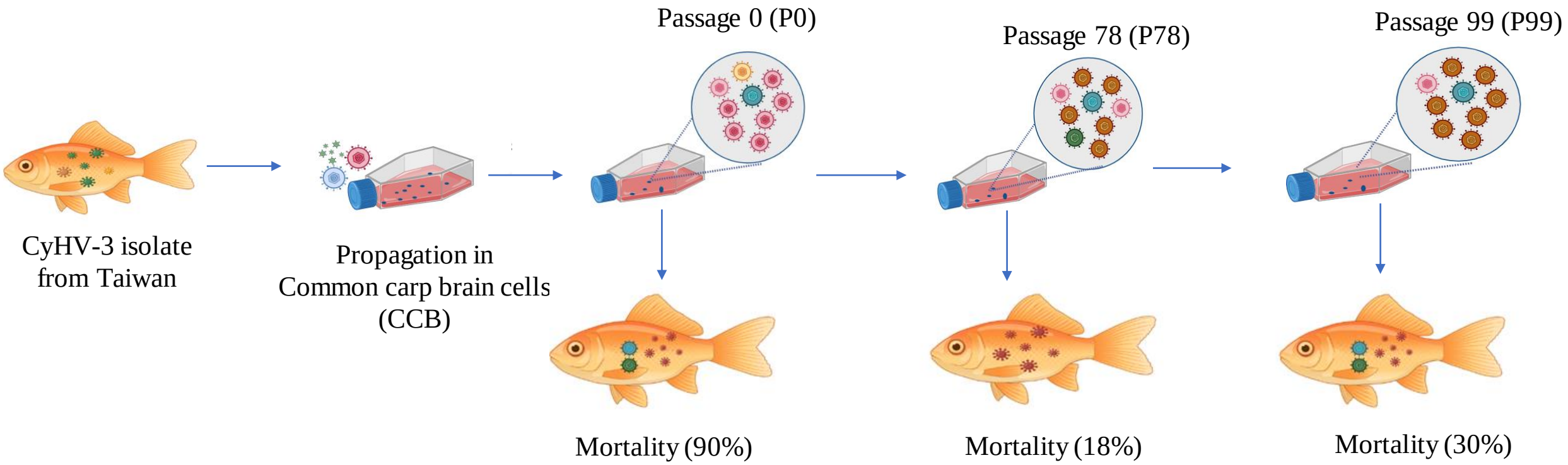
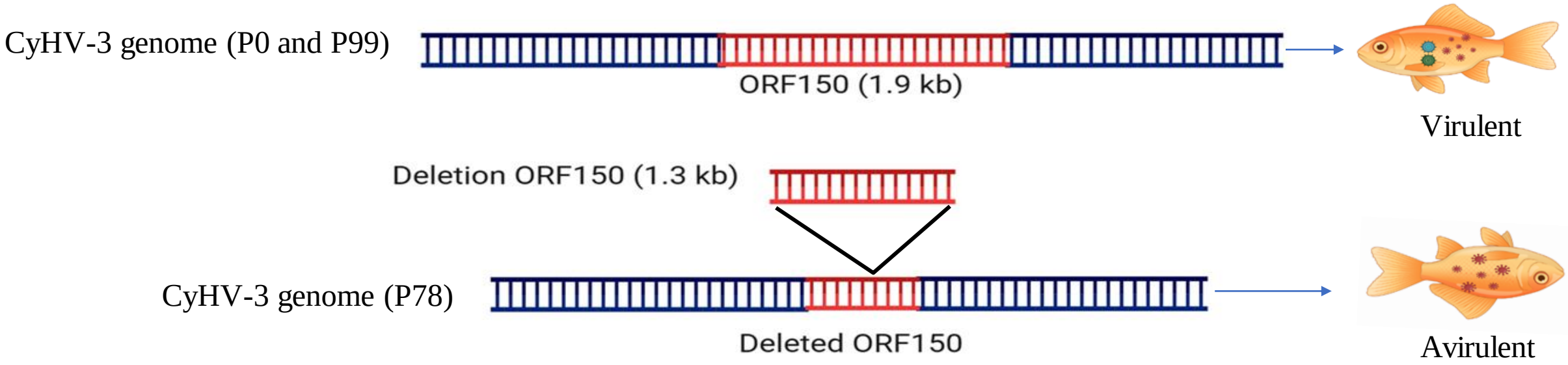


The molecular basis of this reduced virulence is unclear → basis of my study

Hammoumi *et al*,. (2016). Targeted genomic enrichment and sequencing of CyHV-3 from carp tissues confirms low nucleotide diversity and mixed genotype infections. PeerJ, e2516.

Klafack *et al.*, 2019. Cyprinid herpesvirus 3 evolves in vitro through an assemblage of haplotypes that alternatively become dominant or under-represented. Viruses 11, 0–12.

Short-read sequencing



ORF150 contains a RING (Really Interesting New Gene) domain that might play a role in interactions between the host and the virus, virus latency, replication, and breaking down host proteins.

Klafack *et al.*, 2019. Cyprinid herpesvirus 3 evolves in vitro through an assemblage of haplotypes that alternatively become dominant or under-represented. Viruses 11, 0–12.

- **The primary goal of my doctoral research was to understand the molecular mechanisms and factors that influence the evolution of haplotype assemblies in CyHV-3 throughout its replication cycles in cell culture and natural populations**

Understanding the evolutionary mechanisms of CyHV-3 may help for designing effective prevention strategies against infections, including advancing vaccine development efforts

1. What are the evolutionary mechanisms underlying haplotype assemblies in cell culture ?

2. What is the prevalence of the CyHV-3 and structural variations in carp populations in Indonesia ?

3. What are the accumulation patterns of SVs and SNPs in CyHV-3 genome under replicate cell cultures and after application of a thermal stress ?

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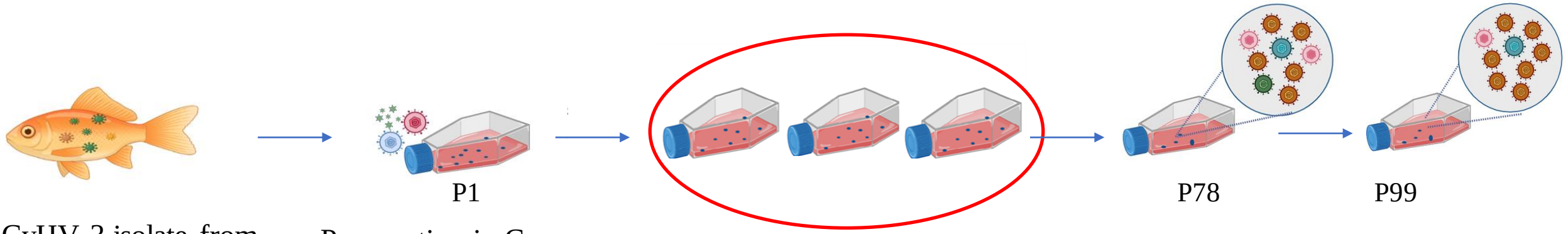
Characterization of haplotype assemblies and changes during serial cell culture passages

This first chapter is a follow-up to a previous study (Klafack *et al.*, 2019).



CyHV-3 isolate from Taiwan

Propagation in Common carp brain cells (CCB)

Haplotypes in intermediates passage ?

Klafack *et al.*, 2019. Cyprinid herpesvirus 3 evolves in vitro through an assemblage of haplotypes that alternatively become dominant or under-represented. Viruses 11, 0–12.

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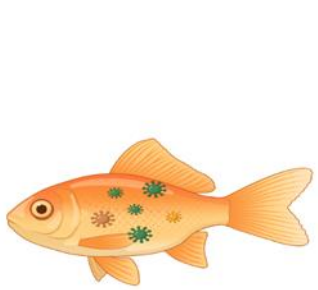
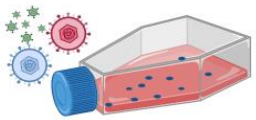
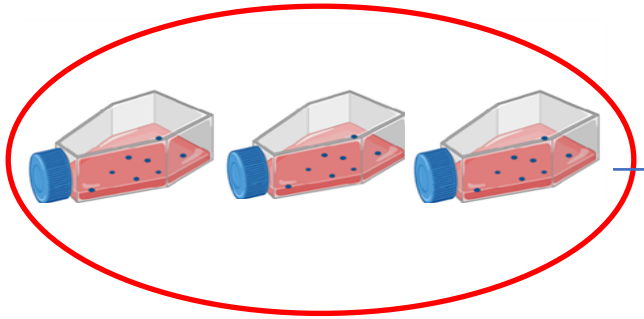


CyHV-3 isolate from Taiwan



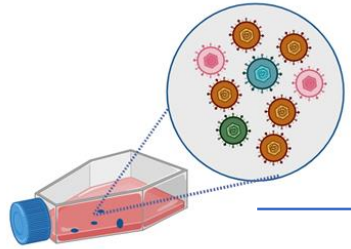
P1

Propagation in Common carp brain cells (CCB)



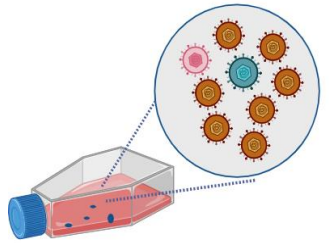
P10, P20, P30, P40, P50, P70

Intermediates passages

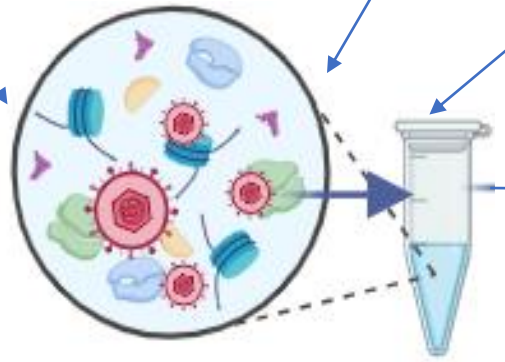


P78

P80



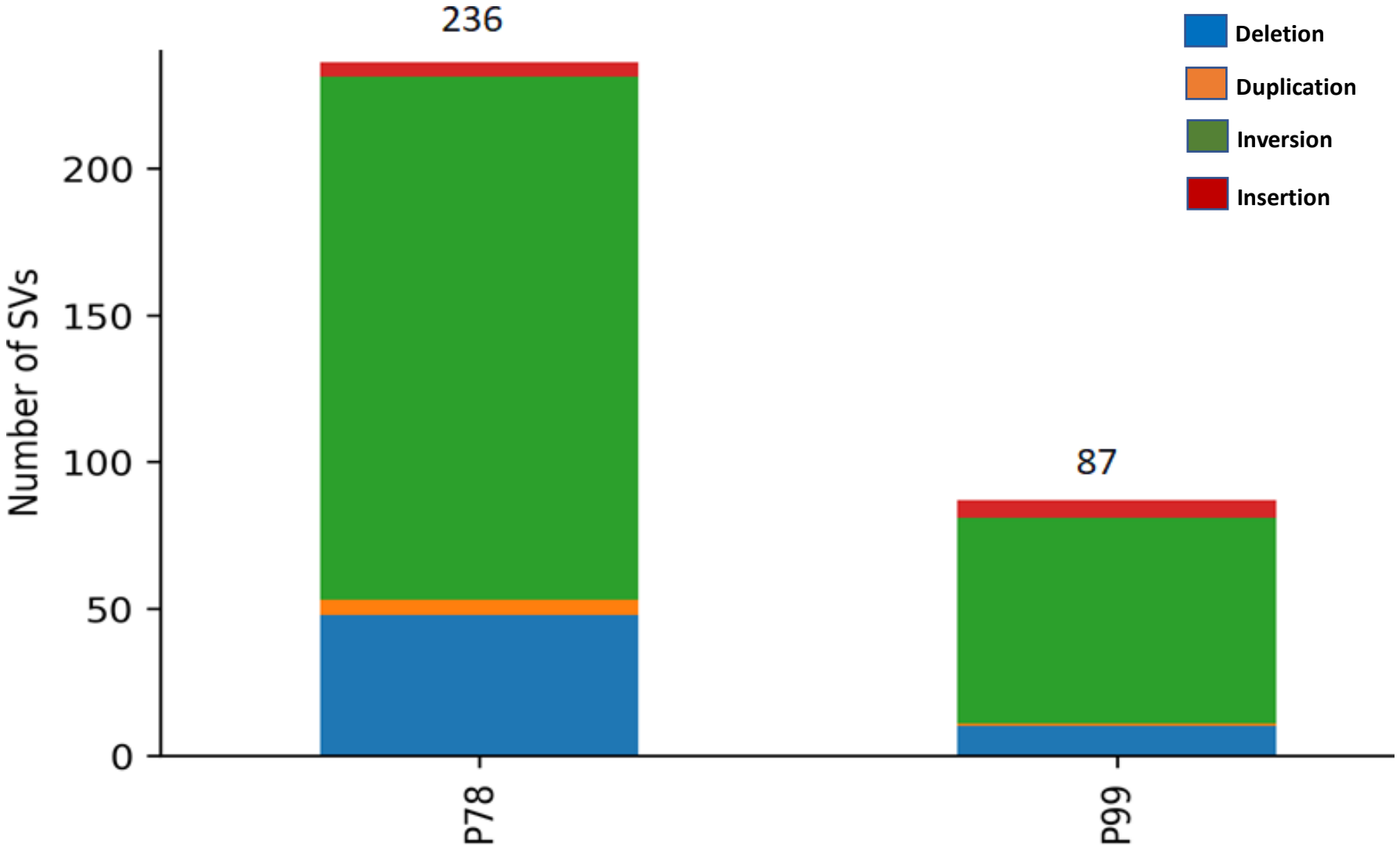
P99



Collect supernatant by centrifugation

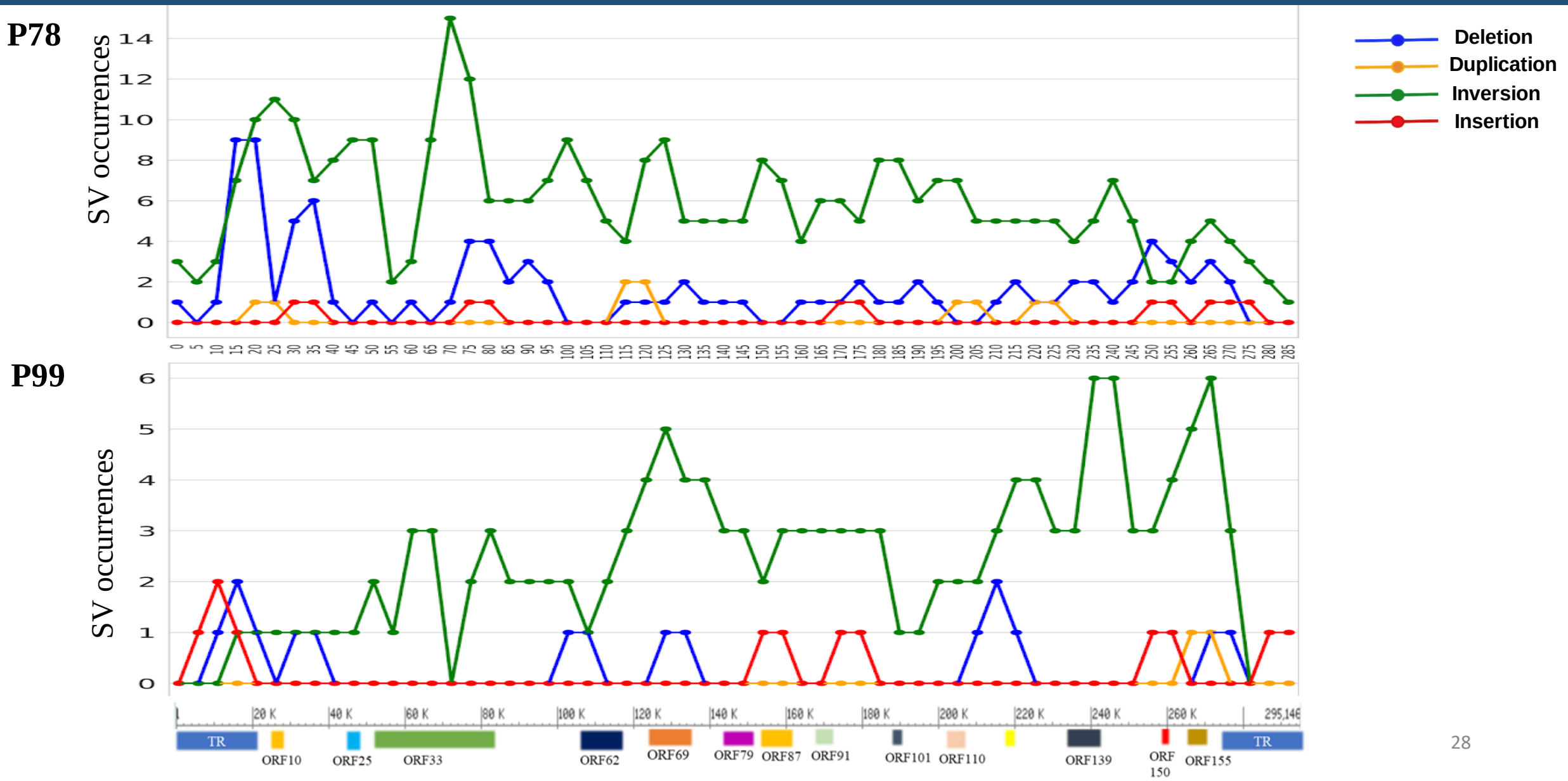
Long read sequencing

Detection of structural variations

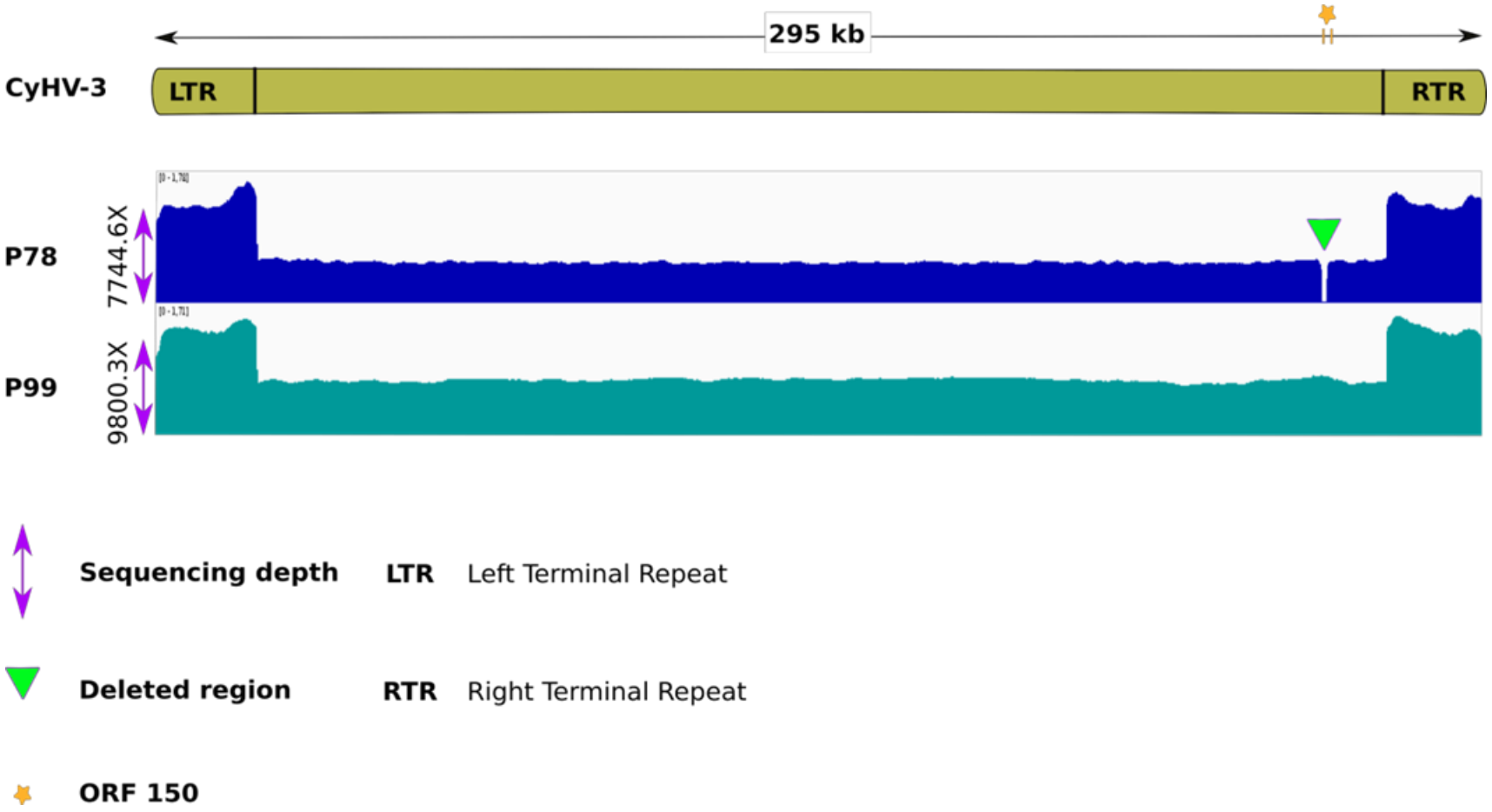


Fuandila, et al. 2022. Structural Variation Turnovers and Defective Genomes: Key Drivers for the in Vitro Evolution of the Large Double-Stranded DNA Koi Herpesvirus (KHV). Peer Community Journal :44.

The distribution of SVs detected in P78 and P99 genomes

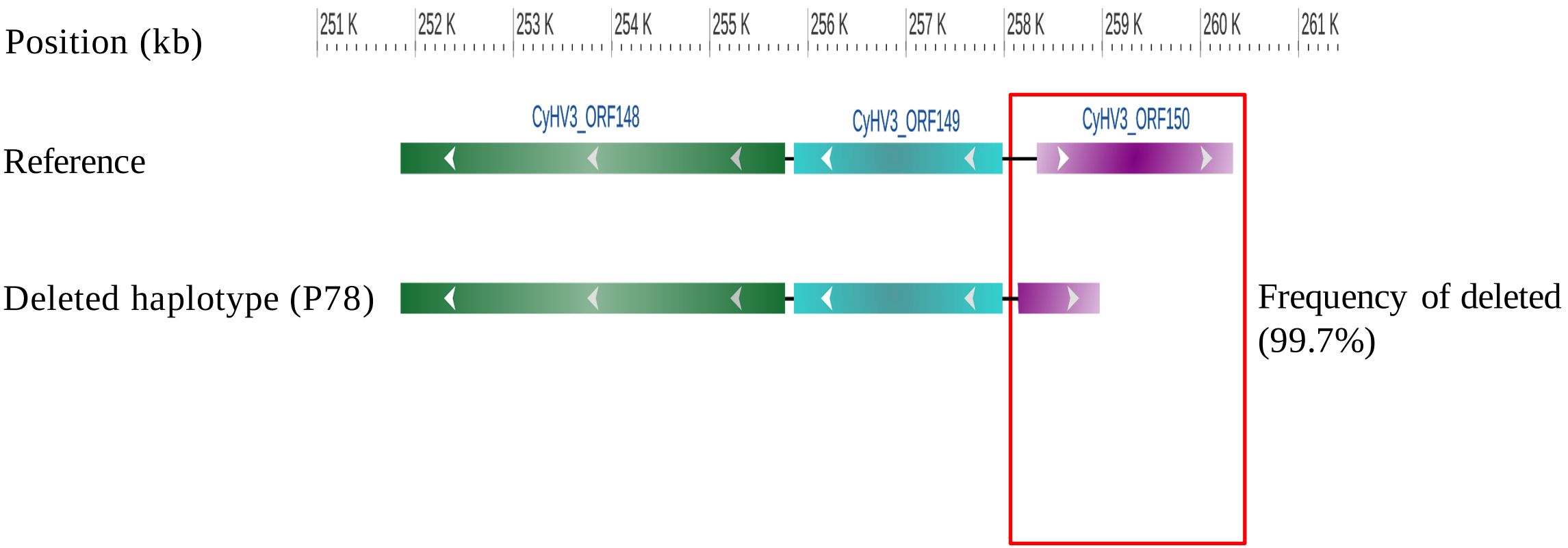


Sequencing coverage for P78 and P99 genomes



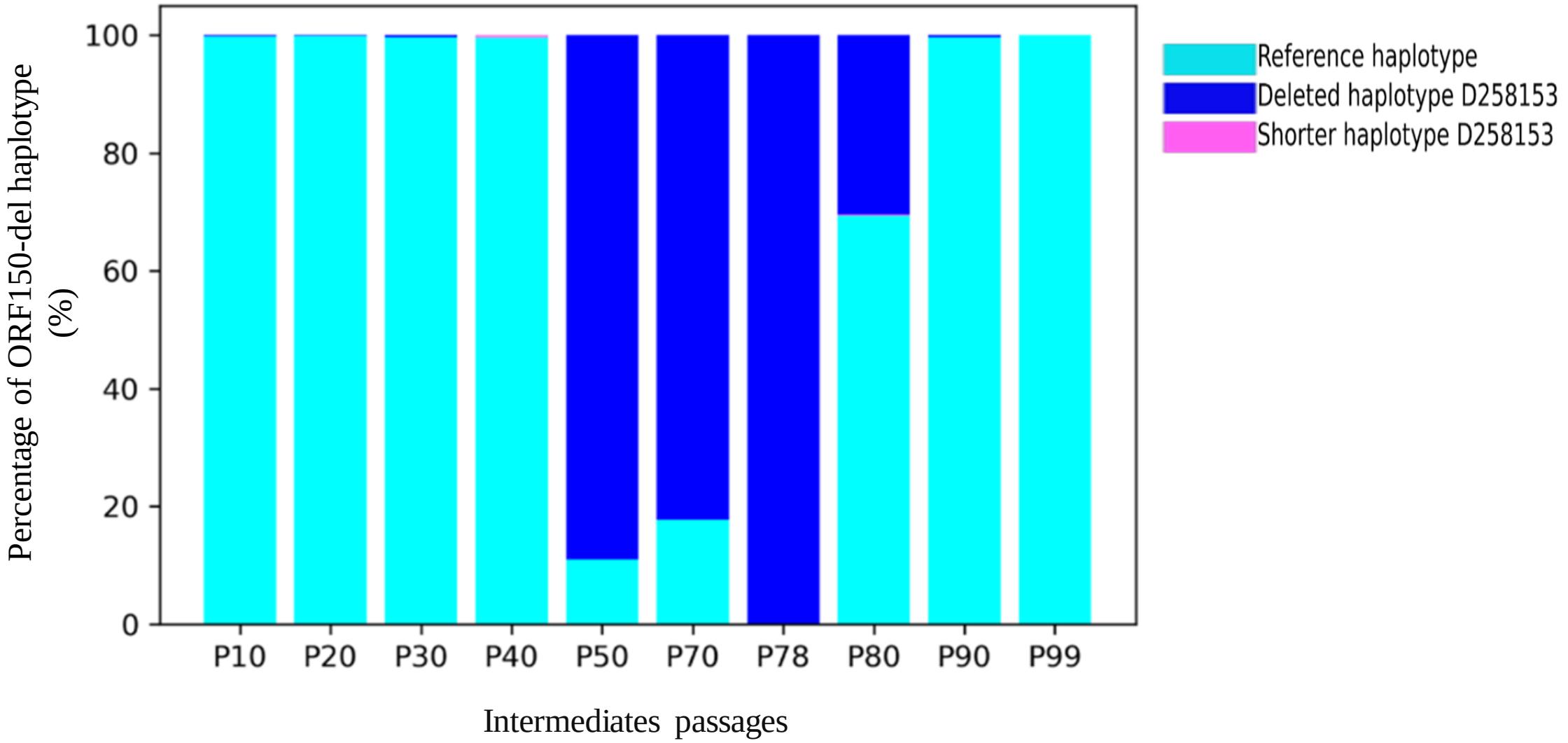
- A deletion in P78 is evident within the region spanning positions 258154 to 259617, corresponding to ORF150

Impact of structural variations on the ORF150 in P78

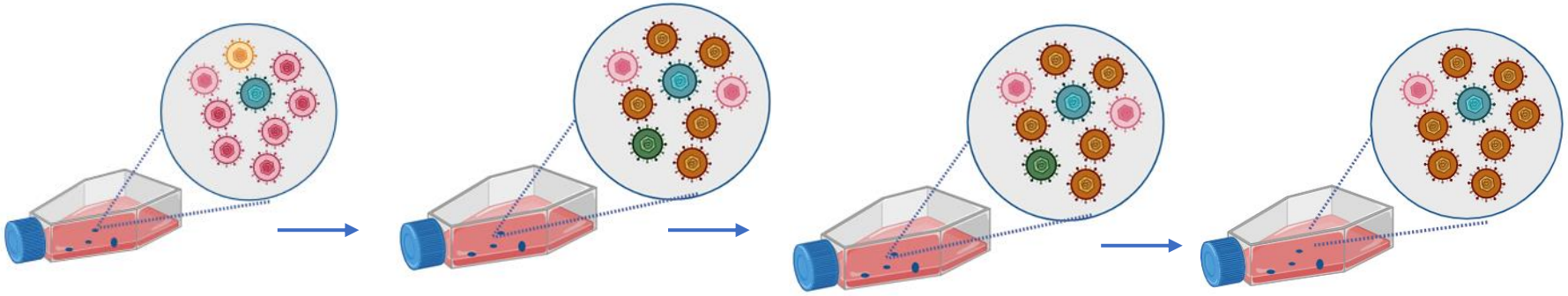


Impact of structural variations on the ORF150 in P78

Percentage of ORF150-del haplotype in ten intermediate passages



- The proportions of specific haplotypes in cell culture can rapidly change from one infection cycle to the next



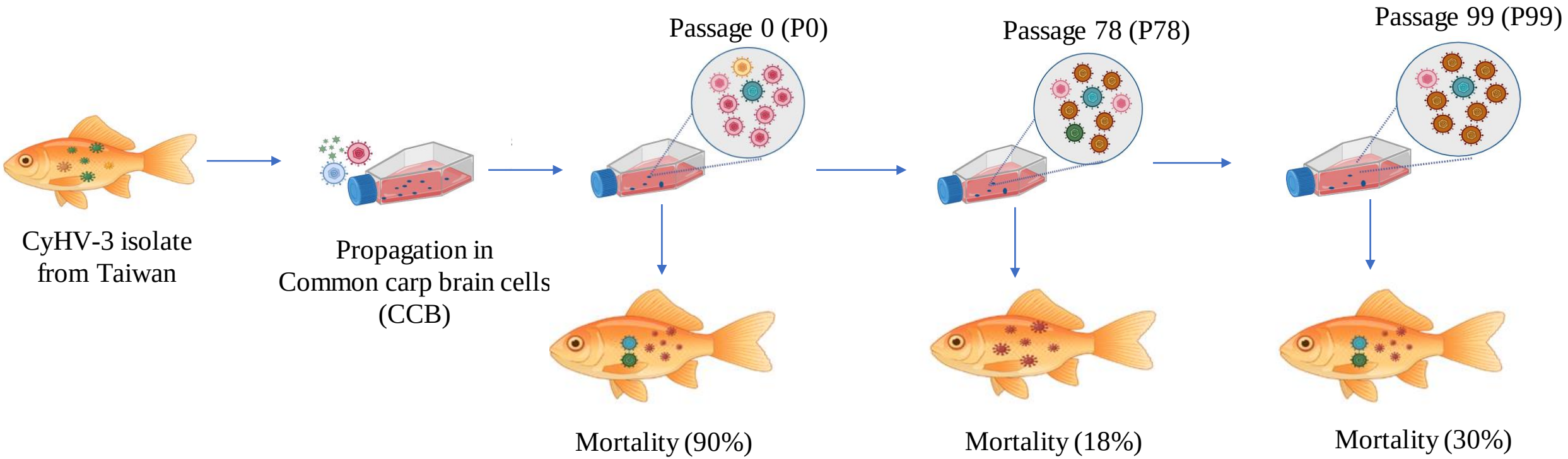
- After extensive in vitro serial passages, herpesviruses undergo genome alterations that might modify their phenotype (Kuny *et al.*, 2020)

- Accumulation of structural variations in CyHV-3 provide advantages in *in vitro* environments and represents an underestimated source of diversification (Gao *et al.*, 2018).

Gao *et al.*, (2018). Genomic and biologic comparisons of cyprinid herpesvirus 3 strains. Veterinary research
Kuny *et al.*, (2020). In vitro evolution of herpes simplex virus 1 (HSV-1) reveals selection for syncytia and other minor variants in cell culture. Virus Evol,

- **Our findings confirm that CyHV-3 can evolve rapidly during infectious cycles in cell culture**

- The existence of mixed haplotypes in the population of CyHV-3 could be a way to regulate virulence



Peer Community Journal

Section: Infections

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Structural variation turnovers and defective genomes: key drivers for the in vitro evolution of the large double-stranded DNA koi herpesvirus (KHV)

Nurul Novelia Fuandila[1], Anne-Sophie Gosselin-Grenet[1,2], Marie-Ka Tilak[1,1], Sven M Bergmann[1,3], Jean-Michel Escoubas[1,4], Sandro Klafack[1,5], Angela Mariana Lusiastuti[1,6], Munti Yuhana[1,7], Anna-Sophie Fiston-Lavier[1,8], Jean-Christophe Avarre[1,1], and Emira Cherif[1,1]

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Abstract

Structural variations (SVs) constitute a significant source of genetic variability in virus genomes. Yet knowledge about SV variability and contribution to the evolutionary process in large double-stranded (ds)DNA viruses is limited. Cyprinid herpesvirus 3 (CyHV-3), also commonly known as koi herpesvirus (KHV), has the largest dsDNA genome within herpesviruses. This virus has become one of the biggest threats to common carp and koi farming, resulting in high morbidity and mortalities of fishes, serious environmental damage, and severe economic losses. A previous study analyzing CyHV-3 virulence evolution during serial passages onto carp cell cultures suggested that CyHV-3 evolves, at least in vitro, through an assembly of haplotypes that alternatively become dominant or under-represented. The present study investigates the SV diversity and dynamics in CyHV-3 genome during 99 serial passages in cell culture using, for the first time, ultra-deep whole-genome and amplicon-based sequencing. The results indicate that KHV polymorphism mostly involves SVs. These SVs display a wide distribution along the genome and exhibit high turnover dynamics with a clear bias towards inversion and deletion events. Analysis of the pathogenesis-associated ORF150 region in ten intermediate cell passages highlighted mainly deletion, inversion and insertion variations that deeply altered the structure of ORF150. Our findings indicate that SV turnovers and defective genomes represent key drivers in the viral population dynamics and in vitro evolution of KHV. Thus, the present study can contribute to the basic research needed to design safe live-attenuated vaccines, classically obtained by viral attenuation after serial passages in cell culture.

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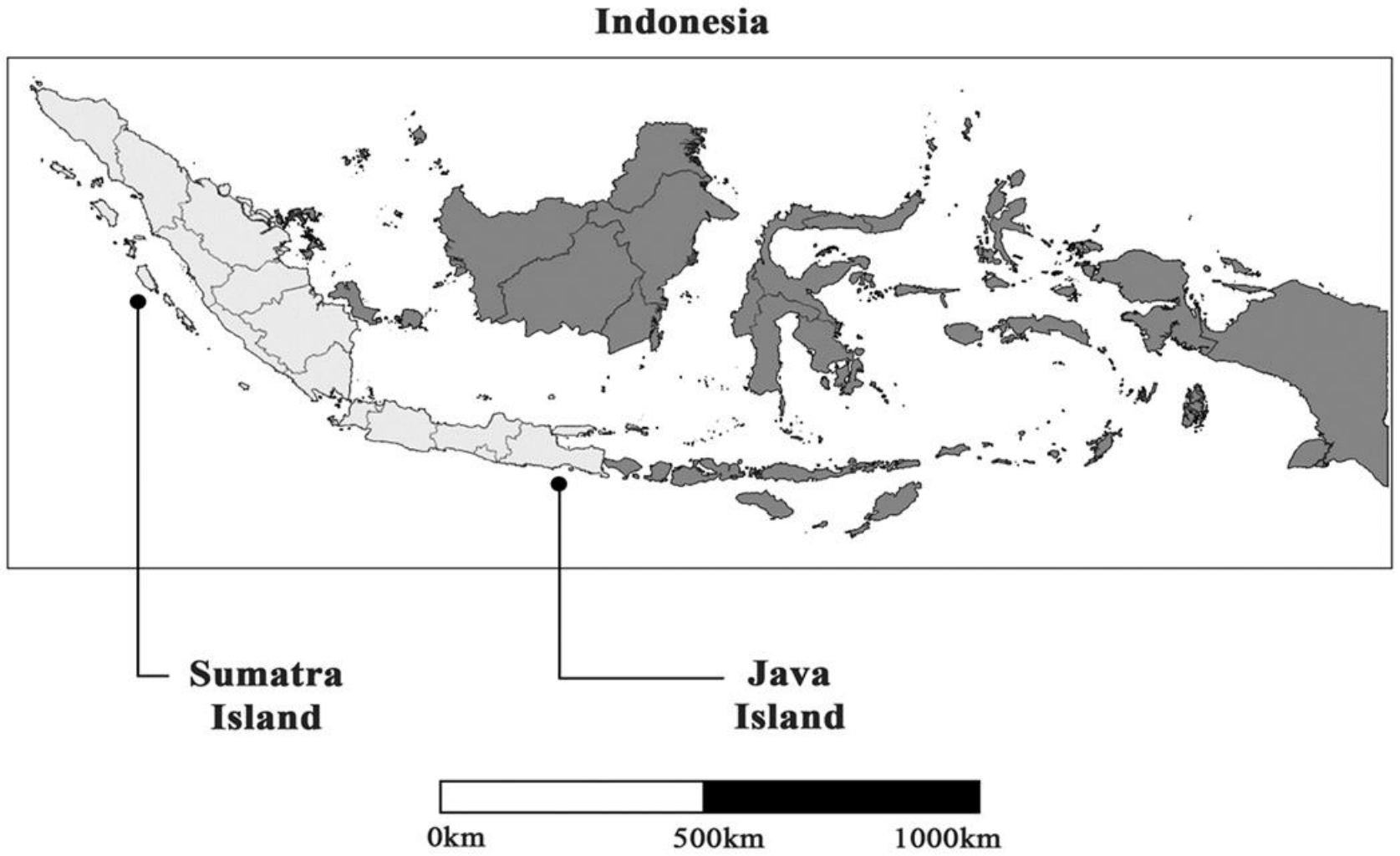
1. What are the evolutionary mechanisms underlying haplotype assemblies in cell culture ?

2. What is the prevalence of the CyHV-3 and structural variations in carp populations in Indonesia ?

3. What are the accumulation patterns of SVs and SNPs in CyHV-3 genome under replicate cell cultures and after application of a thermal stress ?

Sampling sites of common carp in Indonesia

- Sampling farms were located in enzootic and non-enzootic areas of Indonesia (January-May 2022)
- Sampling occurred during both epidemic and non-epidemic periods



Farming systems of common carp in Indonesia

Earthen pond farming

Floating net cage

Running water pond

- Frequent water temperatures in common carp farming exceeded the permissive temperature range, around 30°C

Clinical signs

- Collection of both symptomatic and asymptomatic common carp
- Fish were classified into three groups based on the level of observed clinical signs (Monaghan *et al.*, 2015):

Severe clinical signs

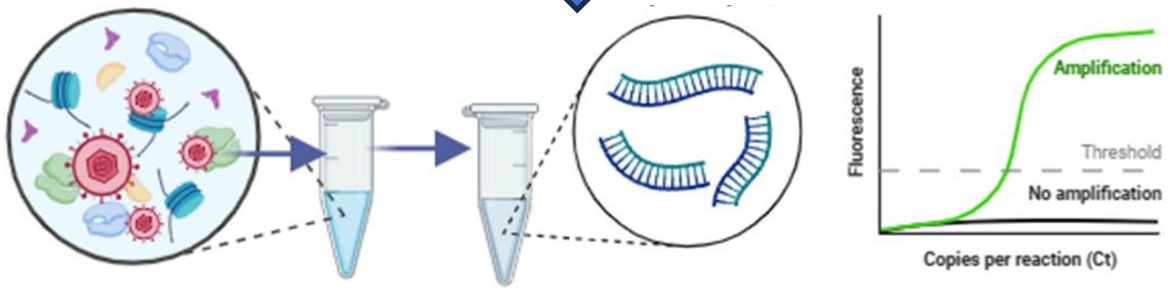
Intermediate clinical signs

Absence of clinical signs

Monaghan *et al.* (2015). Sensitivity of seven PCRs for early detection of koi herpesvirus in experimentally infected carp, Cyprinus carpio L., by lethal and non-lethal sampling methods. J Fish Dis 38, 303–319

Laboratory diagnostic

DNA extracted from the gills

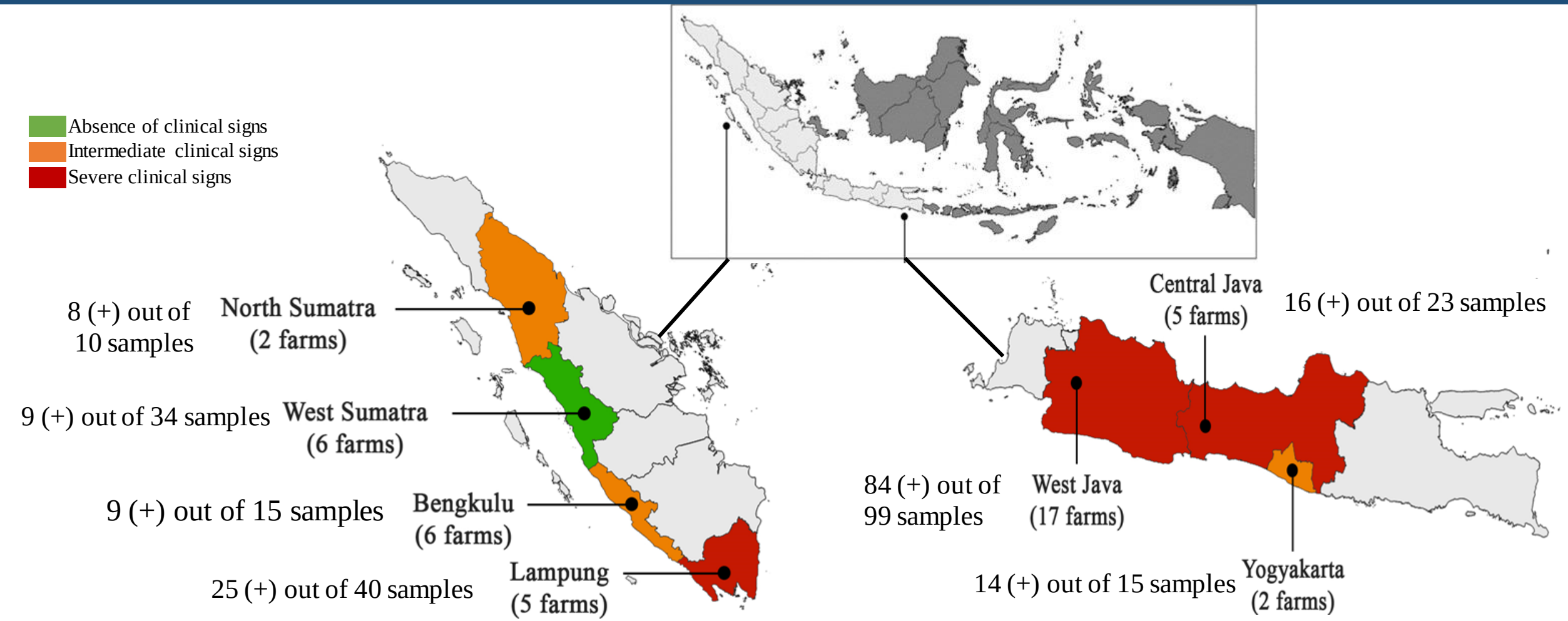


All samples were analyzed by qPCR to detect the presence of CyHV-3 and ORF150-del

5 samples were sequenced to detect other structural variations in ORF150

Amplicon-based sequencing (Oxford Nanopore)

Prevalence of CyHV-3 in one of the largest carp-breeding countries, Indonesia



Fuandila *et al.*, 2023.

- Of the 236 fish samples examined, 165 (70%) were infected by CyHV-3
- Only 66 of these infected fish (40%) displayed apparent and severe symptoms

Variations in ORF150

Analysis of a 3.3 kb region around ORF150 revealed:

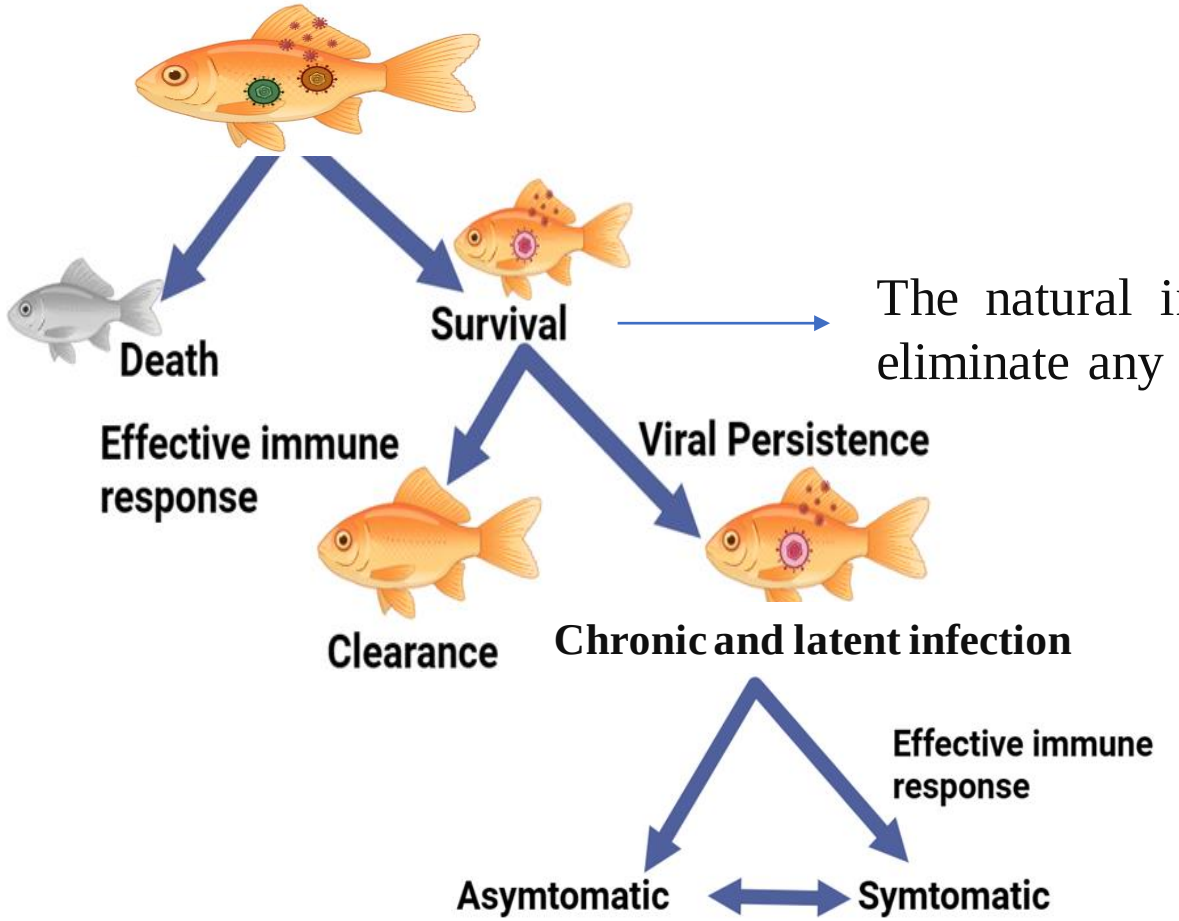
- no ORF150-del
- no SV
- a limited number of SNPs with very low allelic frequencies

- Many CyHV-3-positive fish appeared to be in good health
  - Fish surviving an initial virus exposure may harbor CyHV-3 in a latent state (Uchii *et al.*, 2009)

- ORF150 is not prone to frequent structural variations in natural populations
  - The evolution of CyHV-3 in its host is very slow (Hammoumi *et al.*, 2016)

Uchii *et al.*, (2014). Seasonal reactivation enables Cyprinid herpesvirus 3 to persist in a wild host population. FEMS Microbiol Ecol.
Hammoumi *et al*,. (2016). Targeted genomic enrichment and sequencing of CyHV-3 from carp tissues confirms low nucleotide diversity and mixed genotype infections. PeerJ.

Dynamics and evolution of viral infections *in vivo*



The natural immune response acts as a selective pressure to eliminate any SV that could be harmful or reduce fitness in vivo

Many fish that tested positive for CyHV-3 from semi-intensive systems appeared to be in good health, unlike those from intensive farms

The finding showed a connection between fish density and the symptoms

Fuandila, N.N., A.M. Lusiastuti, M. Yuhana, A. Santika, A.-S. Gosselin-Grenet, E. Cherif, and J.-C. Avarre. 2023. "Prevalence of Cyprinid Herpesvirus 3 and ORF150 Genomic Variations in Carp Populations of Indonesia." *Bulletin of the European Association of Fish Pathologists*, August, 101–12. https://doi.org/10.48045/001c.84009.

RESEARCH ARTICLE

Prevalence of Cyprinid herpesvirus 3 and ORF150 genomic variations in carp populations of Indonesia

N.N. Fuandila[1], A.M. Lusiastuti[2], M. Yuhana[3], A. Santika[4], A.-S. Gosselin-Grenet[5], E. Cherif[1], J.-C. Avarre[1]

[1] ISEM, Univ Montpellier, CNRS, IRD, Montpellier, France, [2] Research Center for Veterinary Science, National Research and Innovation Agency, Indonesia, [3] Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Indonesia, [4] Main Center for Freshwater Aquaculture Development, Sukabumi, Indonesia, [5] DGIMI, Univ Montpellier, INRAE, Montpellier, France

Keywords: CyHV-3, structural variations, carp farming

https://doi.org/10.48045/001c.84009

Bulletin of the European Association of Fish Pathologists

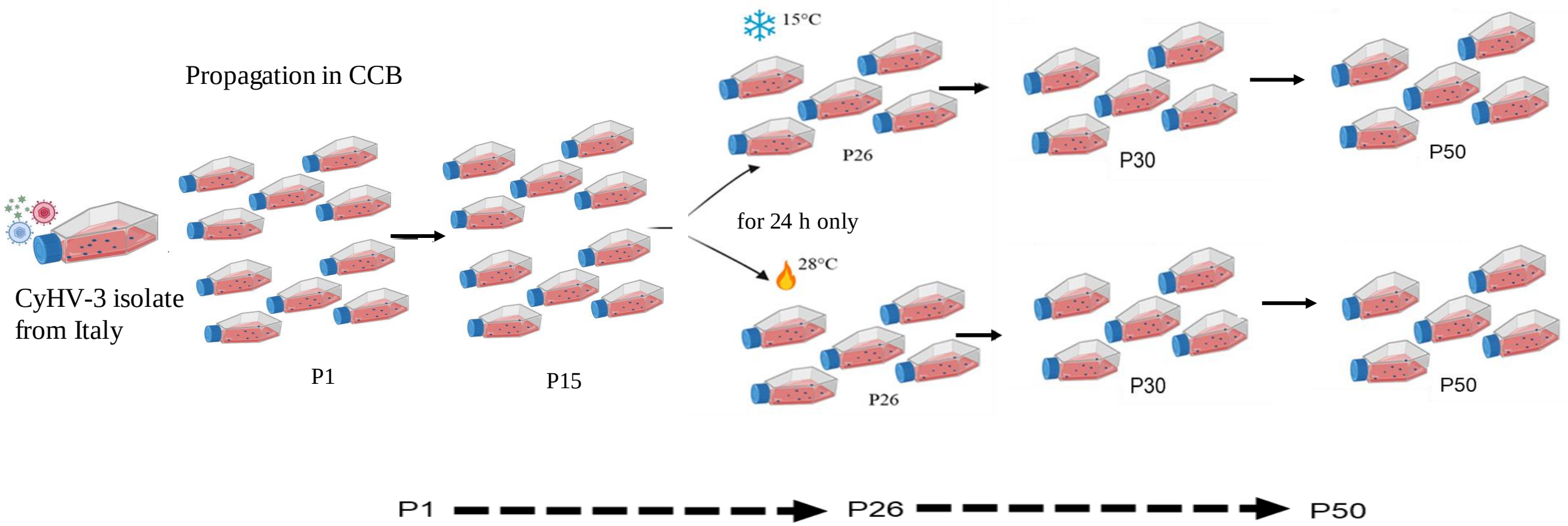
Cyprinid herpesvirus-3 (CyHV-3) is the etiological agent of koi herpesvirus disease (KHVD) in common and koi carp farming. This highly contagious pathogen has a 295-kb genome that harbours 156 open reading frames. Recent in vitro experimental evolution studies highlighted strong dynamics of genomic structural variations (SVs), in particular in the region of ORF150, an ORF potentially involved in virus multiplication and host inflammatory response. Among these SVs, a 1363-bp deletion could be associated with a loss of virulence. The present study aimed at investigating the genomic variations in the ORF150 region, and especially the deletion, in viruses isolated from carp populations of Indonesia. A screening of 236 fish from 43 different farms revealed a high prevalence of CyHV-3 (nearly 70%), both in symptomatic and asymptomatic common carp. However, in contrast with the results obtained in vitro, long read sequencing of the ORF150 region revealed a low level of genetic variations and the absence of the 1363-pb deletion. The complex interactions between the virus, the environment and the host, particularly the immune system, probably play an important role in this reduced variability.

1. What are the evolutionary mechanisms underlying haplotype assemblies in cell culture, particular ?

2. What is the prevalence of the CyHV-3 and structural variations in carp populations in Indonesia ?

3. What are the accumulation patterns of SVs and SNPs in CyHV-3 genome under replicate cell cultures and after application of a thermal stress ?

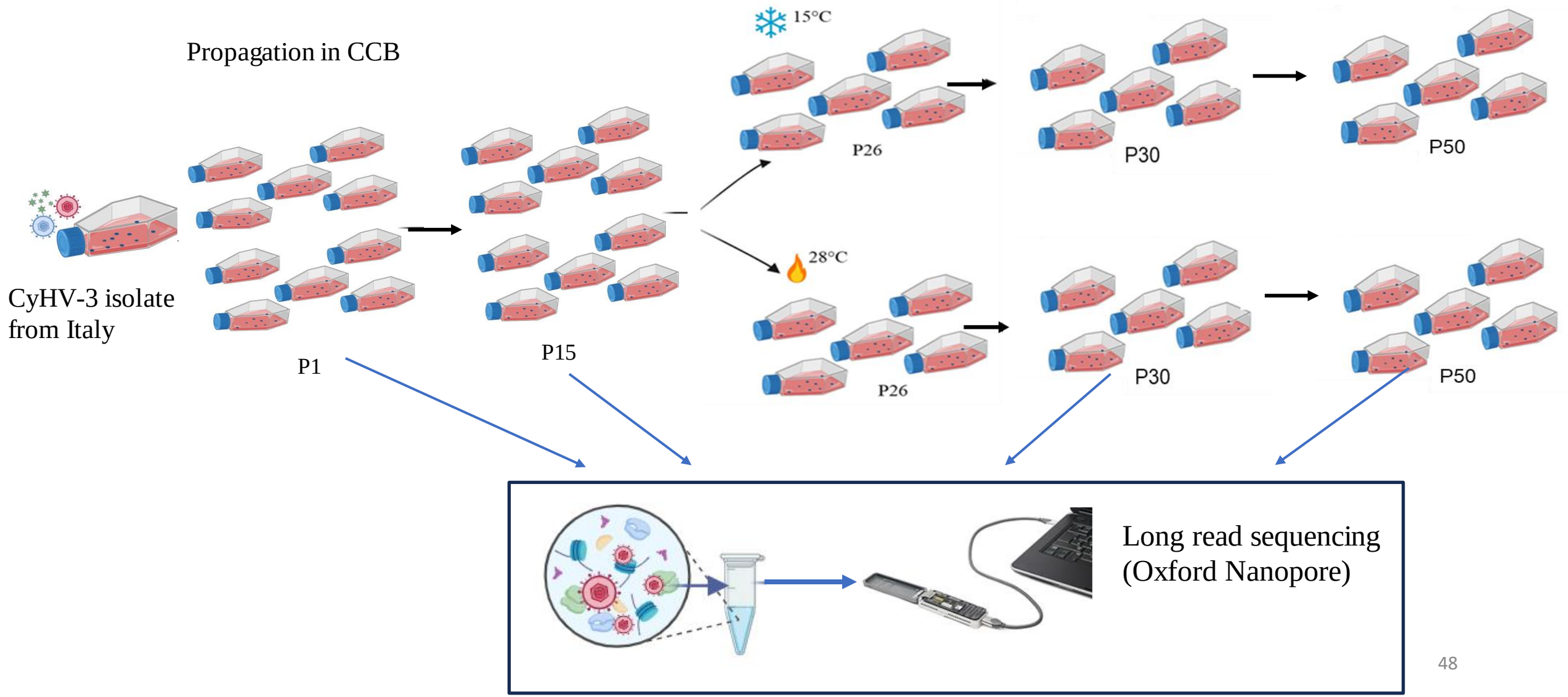
CyHV-3 propagation onto common carp brain (CCB) cells



A thermal stress was applied at passage 26 (P26):

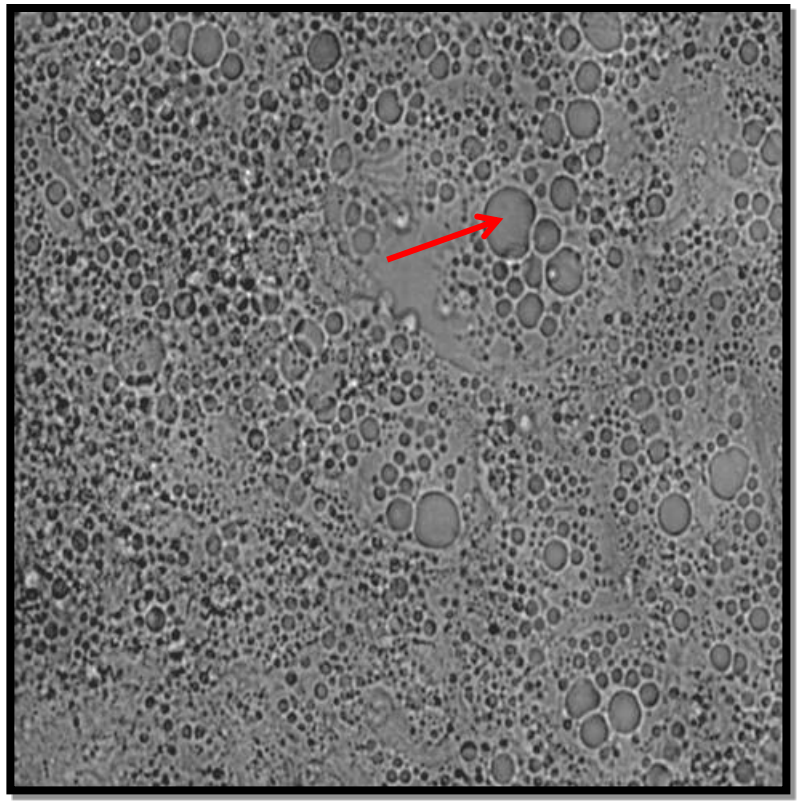
- five subcultures were exposed to 15°C (1 to 5) for 24 h
- five others were exposed to 28°C (6 to 10) for 24 h

CyHV-3 propagation onto common carp brain (CCB) cells

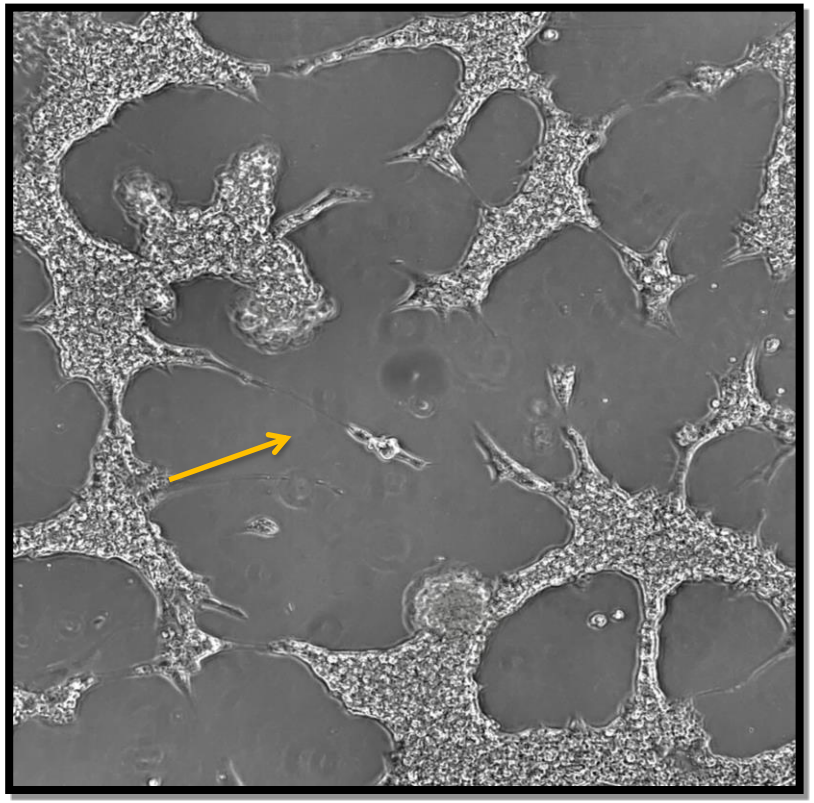


Cytopathic effects (CPE)

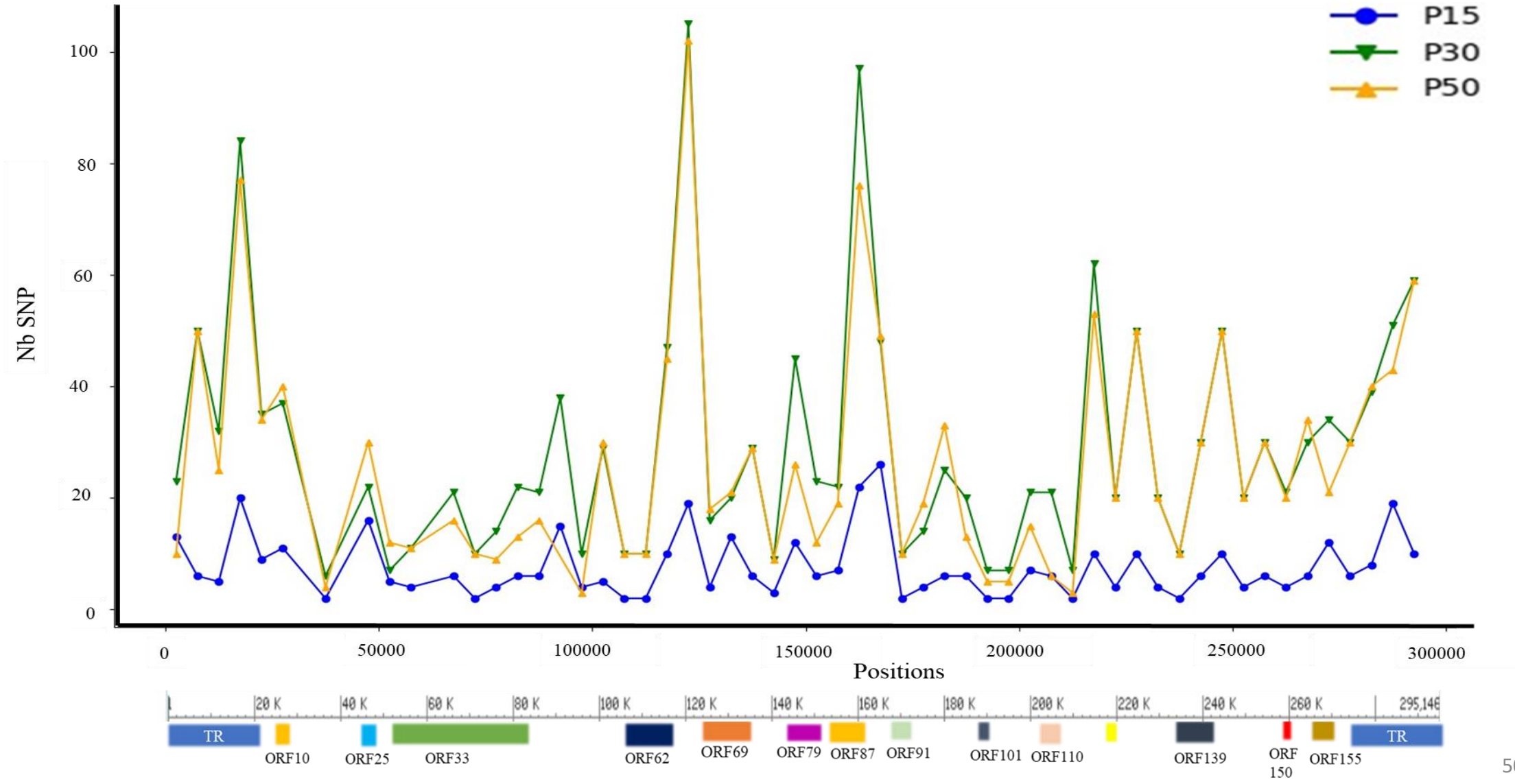
CPE observed at P8 (5 dpi)



CPE observed at P38 (5 dpi)

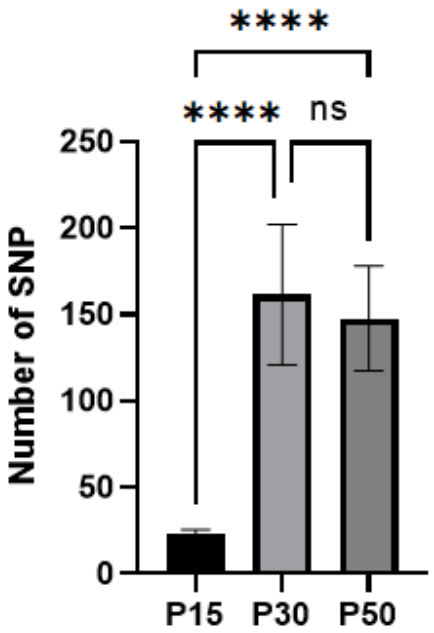


Number and position of Single nucleotide polymorphisms (SNPs)



Accumulation of SNP along serial passages and effect of thermal stress

Average number of SNPs between the three passages



Statistical test :
one way ANOVA
Tukey's post-hoc test

**** : p < 0,0001

Accumulation of SNP along serial passages and effect of thermal stress

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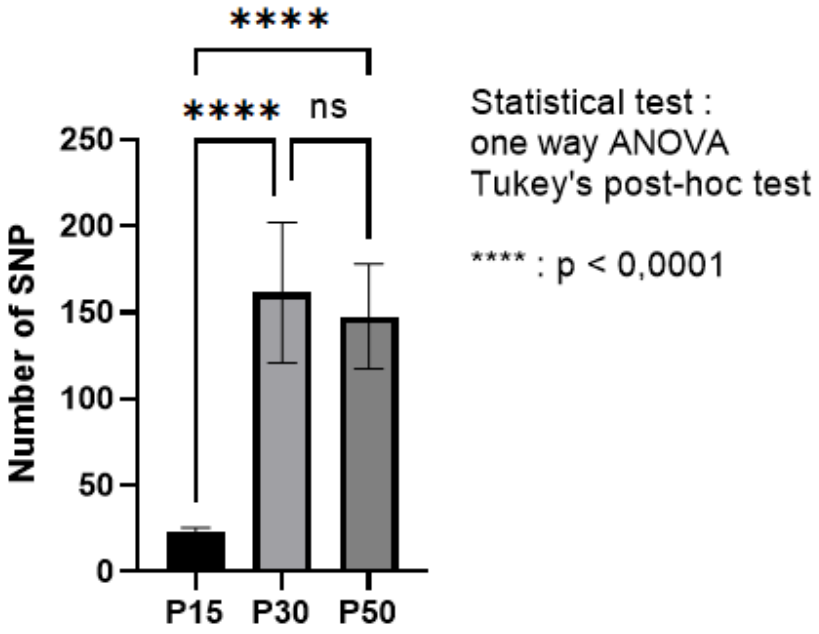
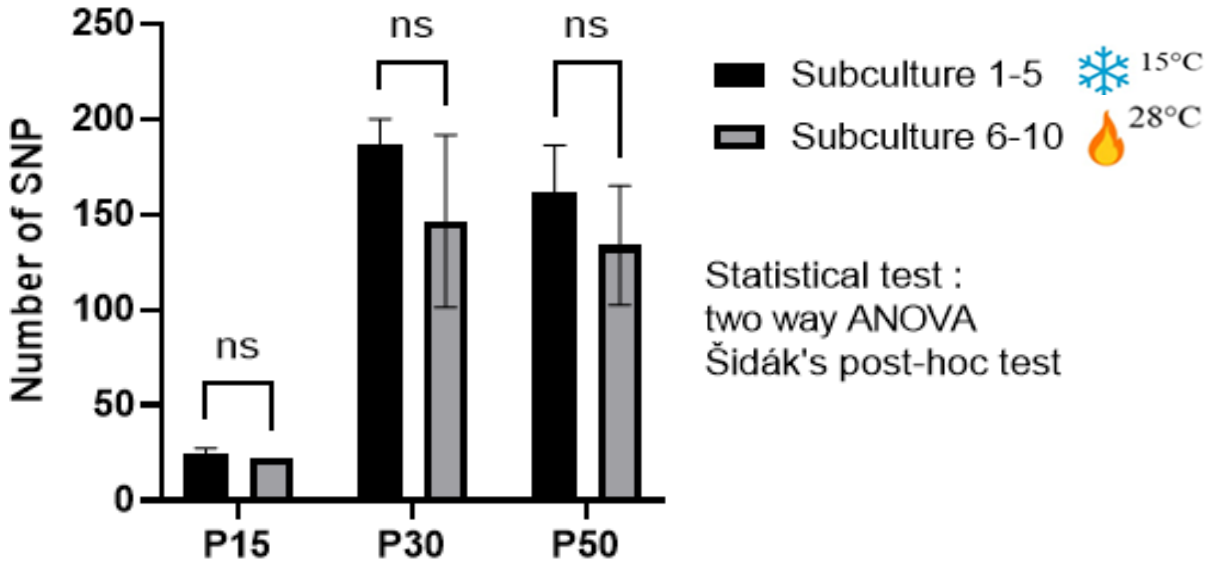


Statistical test :
one way ANOVA
Tukey's post-hoc test

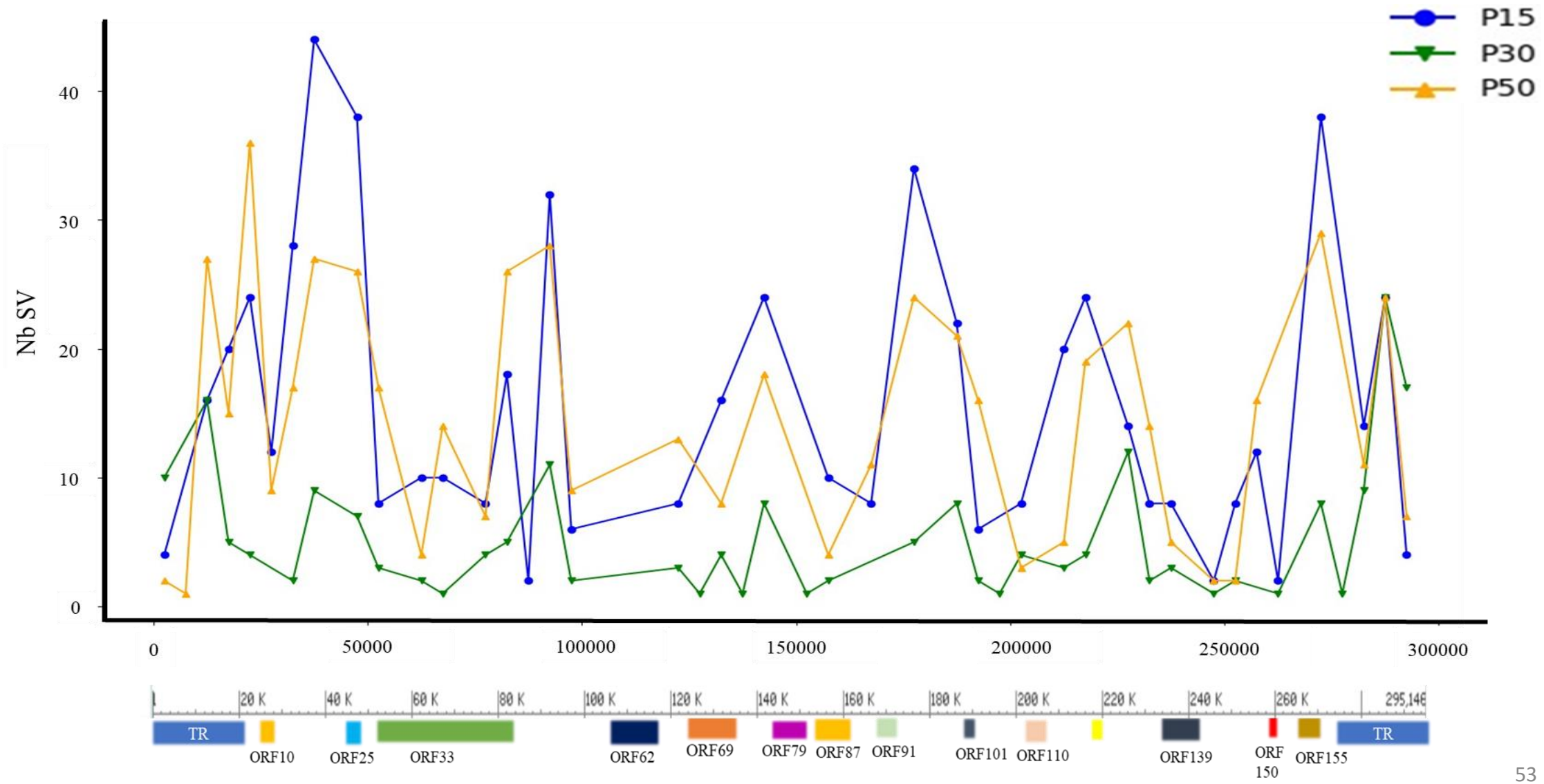
**** : p < 0,0001

Average number of SNPs the two series of subcultures (1-5 and 6-10)



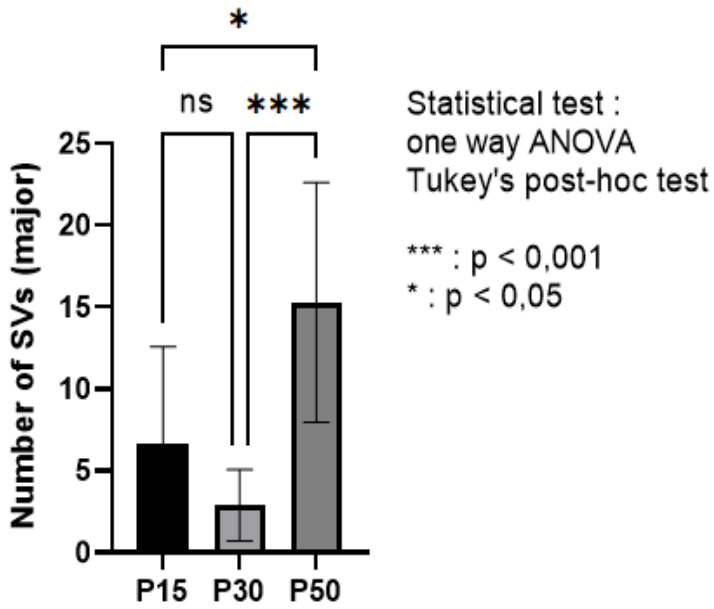
Statistical test :
two way ANOVA
Šidák's post-hoc test

Number and position of structural variations (SVs)



Accumulation of structural variations (SVs) along serial passages and effect of thermal stress

Average number of SVs between the three passages



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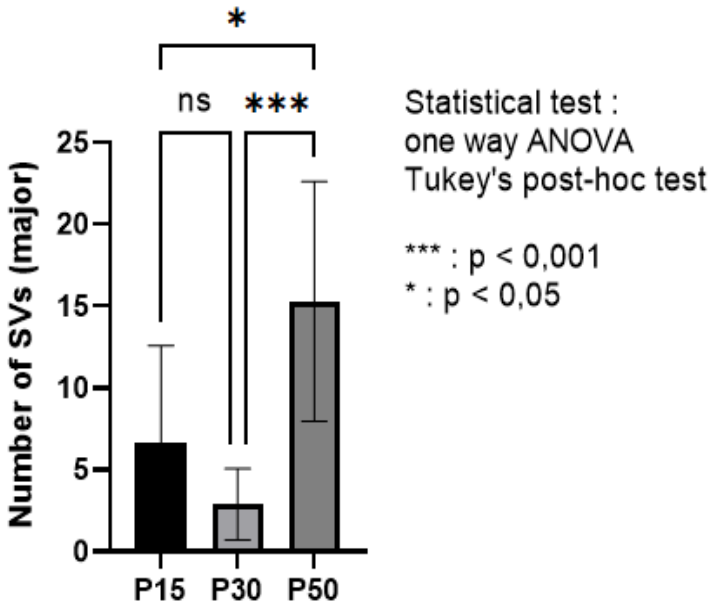
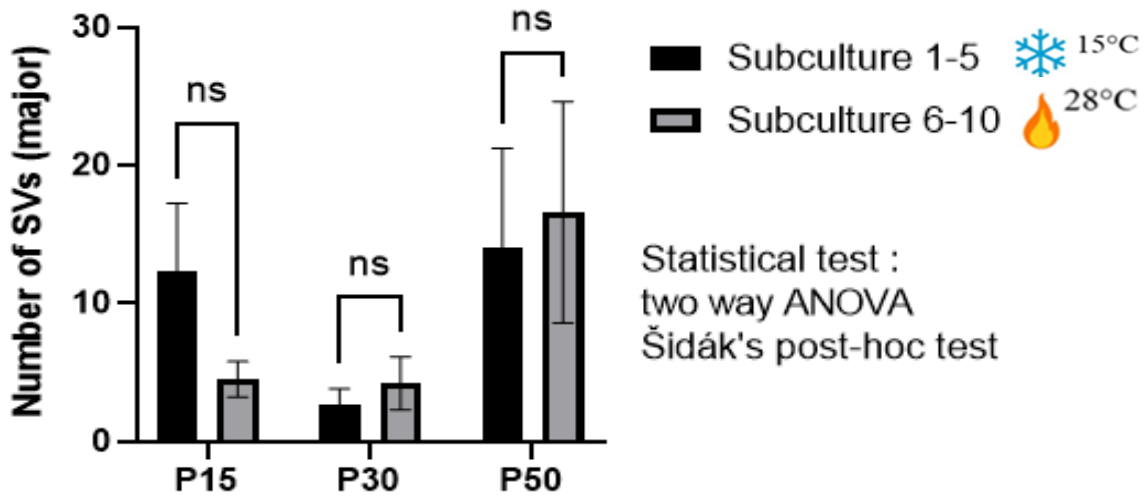


Average number of SVs the two series of subcultures (1-5 and 6-10)



- The lack of distinction between the two sets of subcultures suggests that both thermal stresses had a comparable reducing impact on the accumulation of SVs

Structural variations in open reading frames

- The majority of SVs: insertions and deletions, with a small proportion of duplications
- Small insertions or deletions located within variable numbers of tandem repeats (VNTR)
- The inserted sequences varied:
  - some closely resembled *Cyprinus carpio* DNA, others were duplications of different parts of CyHV-3 genomes
- Presence of very large insertions, up to 16 kb, which correspondence to unknown sequence
- SNP in the ORF131, leading to 183A or 183T

There is no observed deletions in ORF150, which contradicts with the previous findings

- Thermal stress seems to reduce the number of SVs but not the number of SNPs.
- The lower number of SVs observed at P30 implies that the thermal stress interrupted the accumulation dynamics of SV

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Dynamics and evolution of viral infections in heterogeneous environments

Factors influencing virus evolution:

- Environment
- Cellular conditions
- Viral genome stability

Implication of genetic changes

- Manipulating host immunity
- Pathogenesis
- Drug resistance

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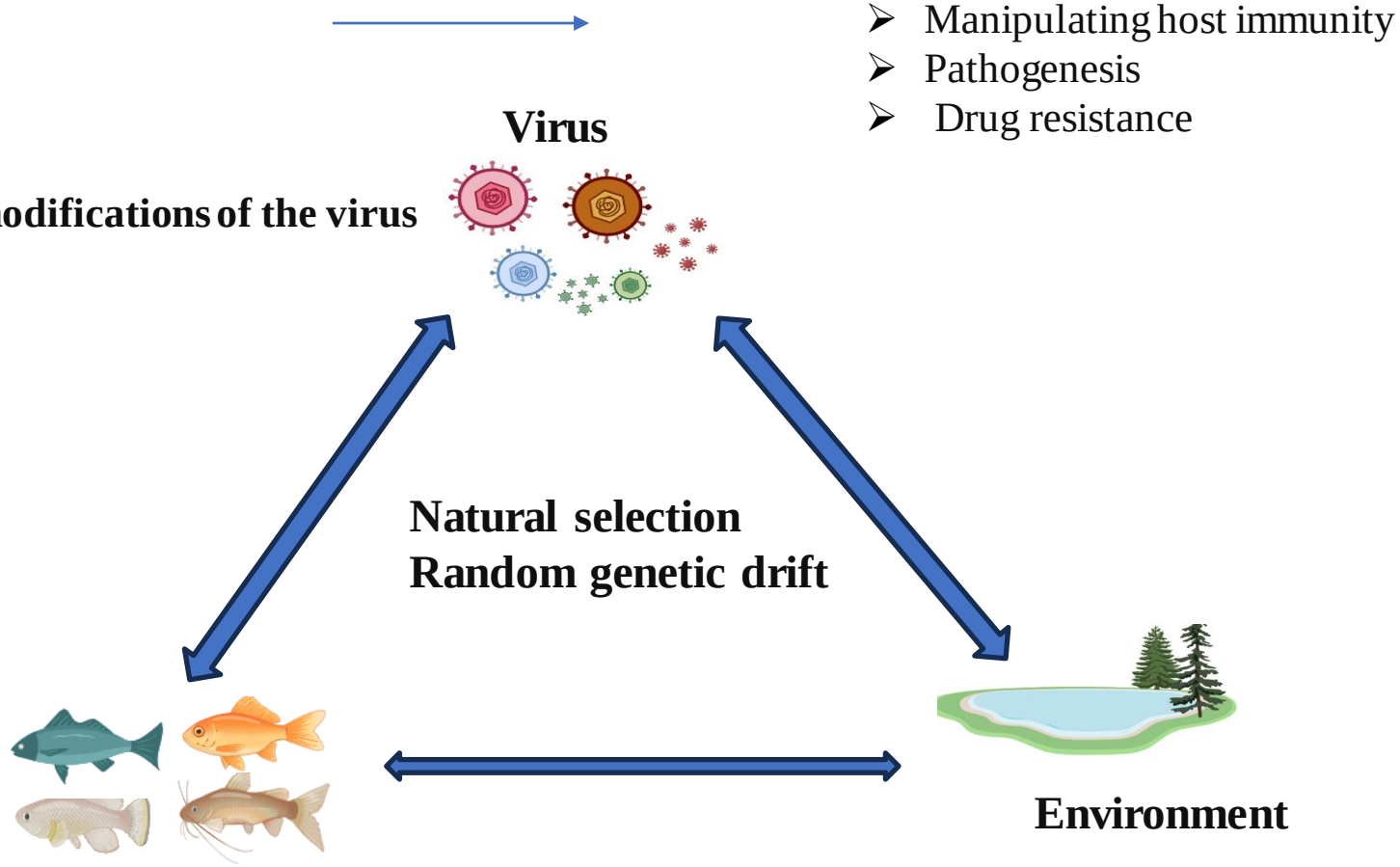


The instability in the ORF150 region after *in vitro* successive serial passages in KHV-T might be unique to this isolate

- Asian and European lineages exhibit genetic differences (Li *et al.*, 2015)
- Some mutations originated *in vivo* rather than in cell culture propagation (Davison *et al.*, 2013)

A SNP at position 226228 (on the reference KHV-U genome) was found in all P30 and P50 subcultures as a homozygous variant

- This SNP contributes to the *in vitro* fitness of CyHV-3 (Gao *et al.*, 2023)

The evolution of CyHV-3 *in vitro* does not reflect its evolution *in vivo*

Davison *et al*,. 2013. "Comparative Genomics of Carp Herpesviruses. Journal of Virology. 87 (5): 2908–22

Gao *et al.*, (2023). Virus-induced interference as a means for accelerating fitness-based selection of cyprinid herpesvirus 3 single-nucleotide variants in vitro and in vivo. Virus Evol 9

Li *et al.*, (2015). Whole-genome sequence of a novel Chinese cyprinid herpesvirus 3 isolate reveals the existence of a distinct European genotype in East Asia. Vet Microbiol

- This study is the first one to apply long read-sequencing to study the diversity of CyHV-3

- Structural variations play a substantial role in the rapid evolution of CyHV-3 *in vitro* and represent a probably underestimated source of diversification

- Structural variations need now to be studied *in vivo*, in order to understand their role in evolution

- The absence of ORF150-del both *in vivo* and in another KHV isolate *in vitro* indicates that this deletion is not sufficient to regulate CyHV-3 virulence

- A bigger sampling effort should be made to definitively confirm the absence of ORF150-del

Short term:

Continue passaging CyHV-3 from Italy is necessary to observe the accumulation dynamics of SV the genome in a high number of passages

Mid term:

It is important to note that this study solely explored variations in the ORF150 region and may not provide insights into the broader genomic diversity of CyHV-3. Therefore, additional research is necessary to assess CyHV-3 evolution through extensive field sampling

Long term:

The absence of the ORF150-deleted haplotype in carp populations will help the vaccination strategy

- KHVD outbreaks in Indonesia are no longer limited by temperature, and the severity of the disease is strongly related to density. Hence, optimal density and good aquaculture practices have to be considered to control disease outbreaks

- There is no CyHV-3-free area at the sampling sites, indicating a high prevalence of the disease in persistence condition. Enhancing the health of the host can help to reduce the losses caused by the disease

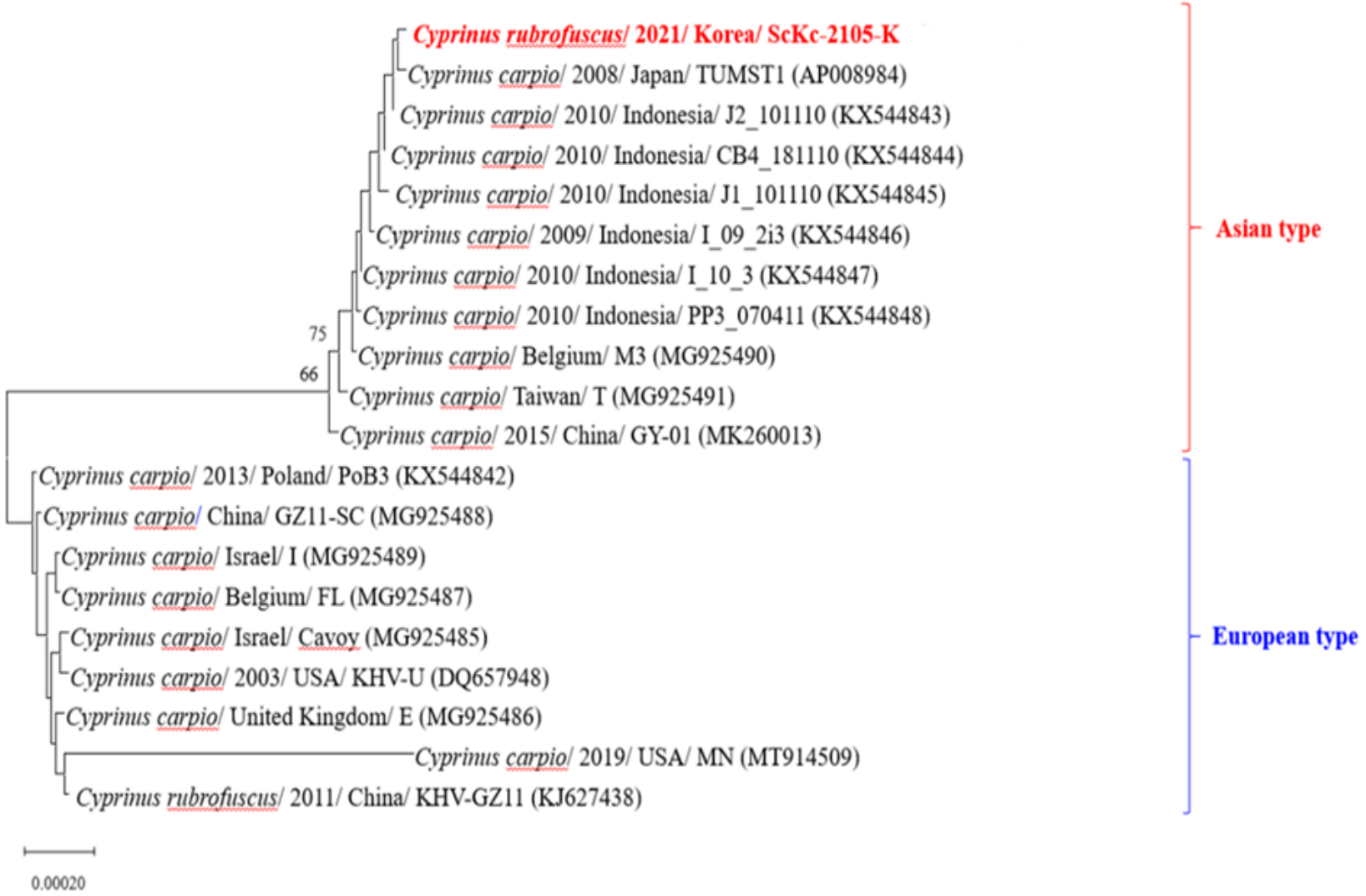
- Affordable prophylactic effort is urgently required to control disease by improving the host's immune response and also inhibiting viral replication in the host

Acknowledgements

- I would like to thank:
- the Embassy of France in Indonesia for granting CampusFrance fellowship during the study
- IRD, for providing additional scholarship through its ARTS programmes and research funding assistance
- all the members of ISEM (Univ Montpellier, IRD), DGIMI (Univ Montpellier, INRAE), France, for welcoming me well and for the prosperous academic environment
- KKP Indonesia for providing facility during *in vivo* study in Indonesia

THANK YOU

Molecular phylogeny based on the partial nucleotide sequence of *thymidine kinase* gene



Markers that focus on specific variable regions might not provide insights into the comprehensive diversity of the entire genome.

Jeong, Y et al. (2023). Genetic characterization of Alloherpesvirus (*Cyprinid herpesvirus-2* and *Koi herpesvirus*) and poxvirus (carp edema virus) identified from domestic and imported cyprinids in Korea. Fish Aquat Sci 26, 437–446.

Characterization of haplotype assemblies and changes during serial cell culture

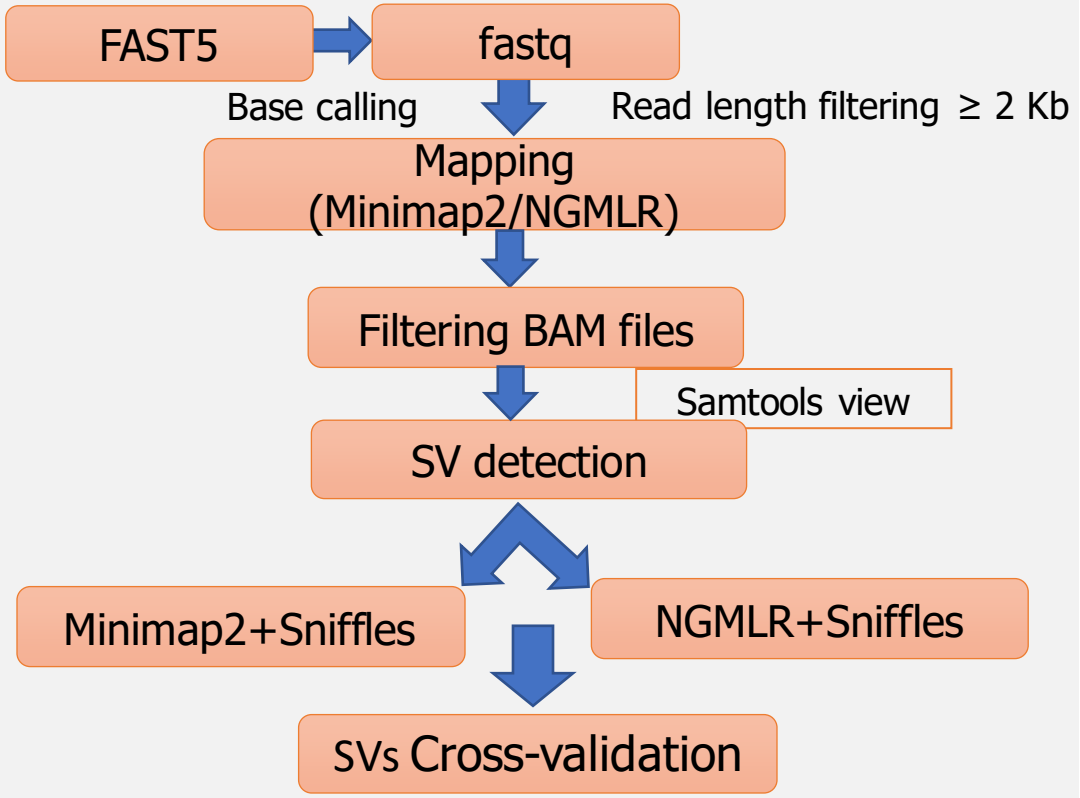
Long read sequencing

Sample and Library preparation

Long read sequencing

1. High molecular weight DNA extraction (P78 and P99)
2. Quality check
3. DNA library preparation
4. Long read sequencing using Oxford nanopore Minlon
5. Bioinformatic analysis

Bioinformatic analysis



2002

The first documented case of KHVD

→

2015

97% of regions of Indonesia were infected by KHVD

→

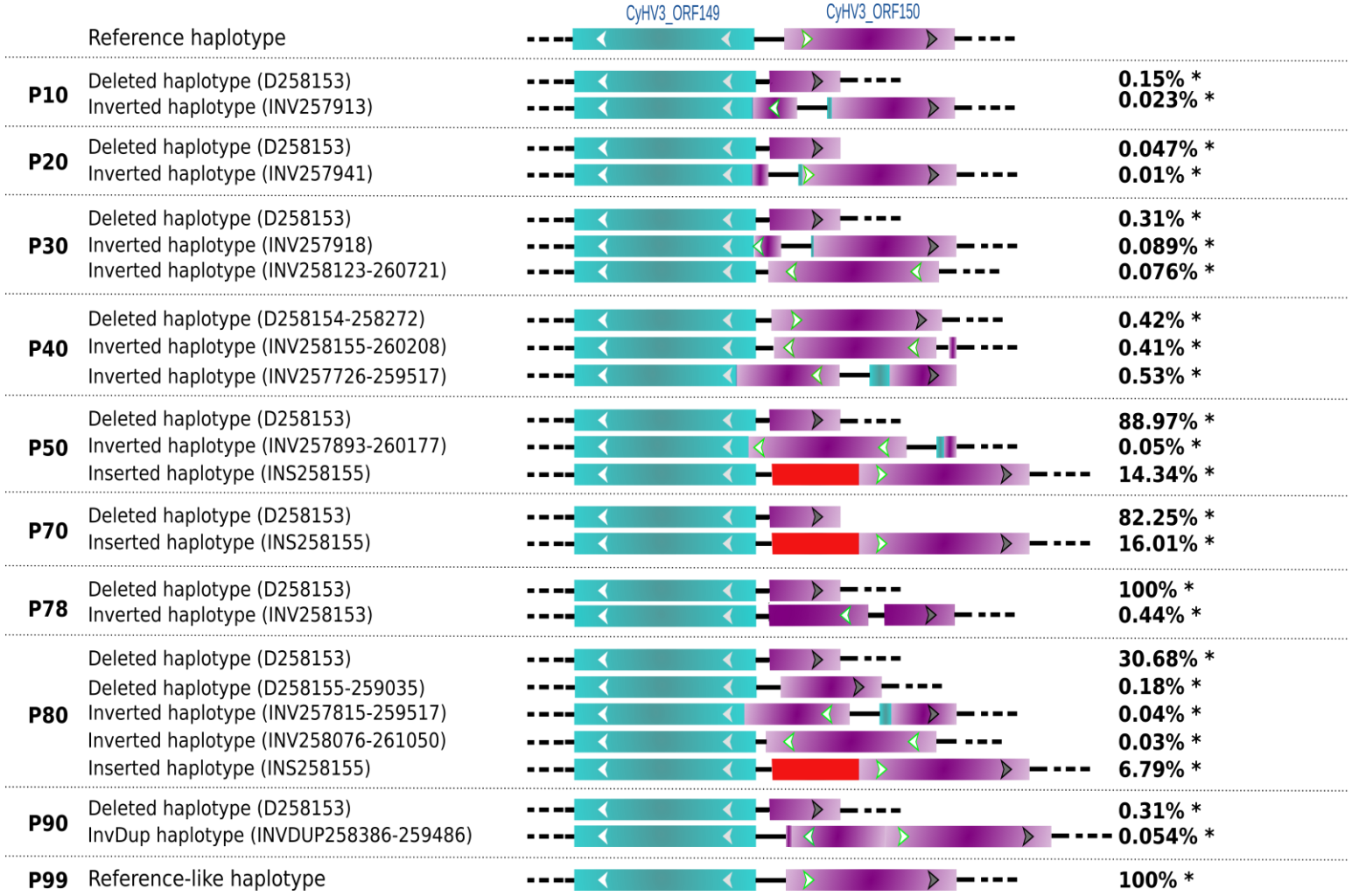
2023

All provinces were reported with KHVD (WAHIS 2023)

Sunarto A, Rukyani A, Itami T. Indonesian experience on the outbreak of koi herpesvirus in koi and carp (*Cyprinus carpio*) *Bull Fish Res Agen.* 2005;2(395):15–21

RESULTS

Impact of SV dynamics on the ORF149-ORF150 structure in the successive passages P10, P20, P30, P40, P50, P70, P78, P80, P90 and P99. The inversion is highlighted by an inverted arrow compared to the reference haplotype



Participation in the conferences

- **Journée des doctorants Indonesie, Montpellier 2021. Oral presentation**
- **18th ecology & evolution of infectious diseases meeting, Montpellier 2021. Poster presentation**
- **21st Journée des doctorants Indonesie, Paris 2023. Oral presentation**
- **EAFP International Conference on Diseases of Fish and Shellfish, Aberdeen 2023. Oral presentation**

PUBLICATION

- Fuandila, Nurul Novelia, Anne-Sophie Gosselin-Grenet, Marie-Ka Tilak, Sven M Bergmann, JeanMichel Escoubas, Sandro Klafack, Angela Mariana Lusiastuti, et al. 2022. "Structural Variation Turnovers and Defective Genomes: Key Drivers for the in Vitro Evolution of the LargeDoubleStranded DNA Koi Herpesvirus (KHV)." Peer Community Journal 2 (July): 44. https://doi.org/ 10.24072/pcjournal.154

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