

**Unexpected cross reactivity of MAb IP5B11 used for  
VHSV diagnostics resolved.**

*Niels Lorenzen, Niels Jorgen Olesen, Tohru Mekata  
and Takafumi Ito*

- Background
- The paper
- Perspectives

# VHS & VHSV



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# **Production and characterization of monoclonal antibodies to four Egtved virus structural proteins**

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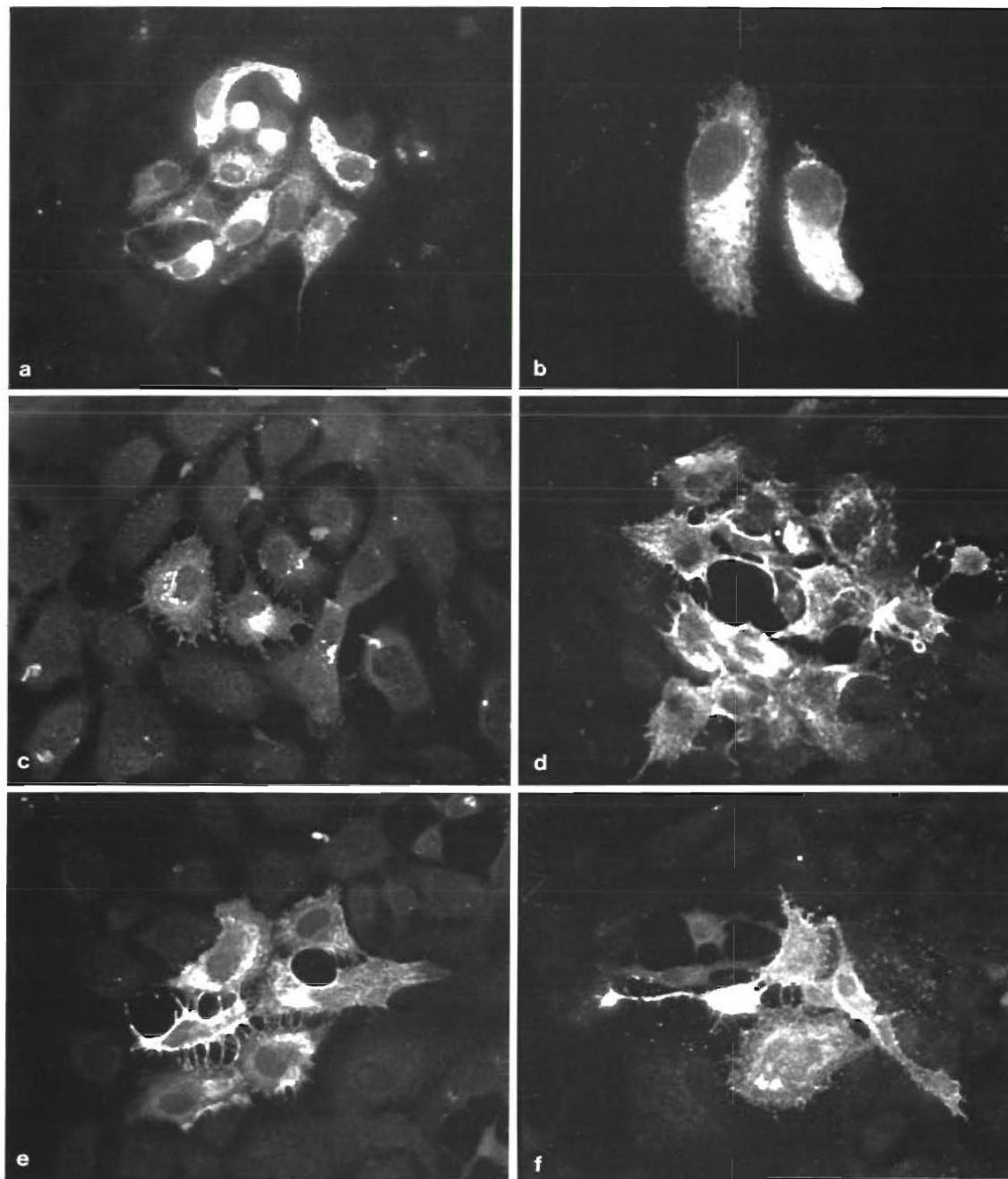
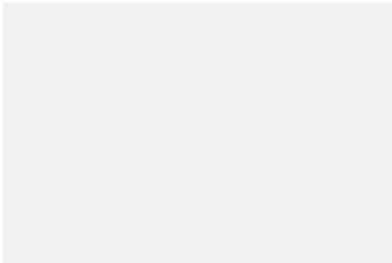


Fig. 3. Immunofluorescence micrographs illustrating the cellular staining patterns seen in Egtved virus-infected EPC cells fixed 24 p. i. and examined with protein-specific MAbs as primary antibodies. (a) G-specific MAb, reticular type,  $\times 370$ . (b) Same,  $\times 880$ . (c) G-specific MAb, Golgi type,  $\times 370$ . (d) N-specific MAb,  $\times 370$ . (e) M<sub>1</sub>-specific MAb,  $\times 370$ . (f) M<sub>2</sub>-specific MAb,  $\times 370$



## Typing of viral hemorrhagic septicemia virus by monoclonal antibodies

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Niels Lorenzen,<sup>3</sup> Katja Einer-Jensen<sup>3</sup> and Niels Jørgen Olesen<sup>3</sup>

**Table 2.** ELISA and IFAT results of mAbs against various genotypes of VHSV

Values are shown as ELISA/IFAT. ELISA: + + + +, absorbance value of sample was >150 % of the value with IP5B11 for each virus isolate; + + +, 150 %>absorbance value of sample ≥ 75 %; + +, 75 %>absorbance value of sample ≥ 35 %; +, 35 %>absorbance value of sample ≥ 15 %; -, negative, absorbance value of sample <15 %. IFAT: +, positive; -, negative.

Isolate	Genotype	mAb								
		IP5B11	VHS-1.24	VHS-9.23	VHS-3.80	VHS-7.57	VHS-5.18	VHS-3.75	VHS-10	VHS-1.88
DK-F1	I	+ + + +	+ + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-Hededam	I	+ + + +	+ + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-3592B	Ia	+ + + +	+ + + / +	+ + + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-3971	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-3946	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-5151	Ia	+ + + +	+ + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-6137	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-7974	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-9695377	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-200149	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-200051	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
FR-07-71	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
FR-23-75	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
FR-02-84	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
CZ-R5	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
CZ-2077	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
DK-5927	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
AU-8/95	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
CH-F1 262 BFH	Ia	+ + + +	+ + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
PL-202473	Ia	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
M Rhabdo	Ib	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p8	Ib	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p40	Ib	+ + + +	+ + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p85	Ib	+ + + +	+ + / +	+ + + / +	+ + + + / +	- / -	+ + + + / +	- / -	- / -	- / -
1p86	Ib	+ + + +	+ + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p93	Ib	+ + + +	+ + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p116	Ib	+ + + +	+ + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p120	Ib	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
1p121	Ib	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
5p276	Ib	+ + + +	+ / +	+ + + / +	+ + + + / +	- / -	+ + + + / +	- / -	- / -	- / -
SE-SVA-14	Ib	+ + + +	+ / +	+ + + / +	- / -	- / -	+ + + / +	- / -	- / -	- / -
SE-SVA-1033	Ib	+ + + +	+ + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
UK-96-43	Ib	+ + + +	+ + / +	+ + + / +	+ + + / +	- / -	+ + + / +	- / -	- / -	- / -
4p37	Ib	+ + + +	+ / +	+ + + / +	+ + + + / +	- / -	+ + + + / +	- / -	- / -	- / -
DK-2835	Ic	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -
DK-5123	Ic	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -
DK-5131	Ic	+ + + +	+ + + / +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -
FIA01a.00	Id	+ + + +	+ / +	+ + + / +	+ + / +	- / -	- / -	- / -	- / -	- / -
FIP02b.00	Id	+ + + +	+ / +	+ + + / +	+ / +	- / -	- / -	- / -	- / -	- / -
NO-A163-68	Id	+ + + +	+ + + / +	+ + + / +	- / -	- / -	- / -	- / -	- / -	- / -
EG46										
GE-1.2	Ie	+ + + +	- / -	+ + / +	- / -	- / -	- / -	- / -	- / -	- / -
TR206239-1	Ie	+ + + +	- / -	+ + / +	- / -	- / -	- / -	- / -	- / -	- / -
1p49	II	+ + + +	+ + + / +	+ + + / +	+ + / +	+ / +	- / -	- / -	- / -	- / -
1p52	II	+ + + +	+ + + / +	+ + + / +	+ + / +	+ + + / +	- / -	- / -	- / -	- / -
1p53	II	+ + + +	+ + + / +	+ + + / +	+ + / +	+ + / +	- / -	- / -	- / -	- / -
1p54	II	+ + + +	+ + + / +	+ + + / +	+ + / +	+ / +	- / -	- / -	- / -	- / -
2p51	III	+ + + +	+ + + / +	- / -	- / -	- / -	- / -	+ + + / +	- / -	- / -
4p101	III	+ + + +	+ + + / +	- / -	- / -	- / -	- / -	+ + + / +	- / -	- / -
4p168	III	+ + + +	+ + + / +	- / -	- / -	- / -	- / -	+ + + / +	- / -	- / -

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## Characterization of a rhabdovirus isolated from carpione *Salmo trutta carpio* in Italy

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## INTRODUCTION

According to Melotto & Oppi (1987), carpione *Salmo trutta carpio* is a salmonid species with an uncertain taxonomic classification. Apparently, carpione are present only in Garda Lake, Italy, where they have become highly adapted, spawning twice a year in reproduction areas at depths of 70 to 300 m. The species is mainly planktrophagous. In spring 1988, attempts were initiated by the Ente Sviluppo Agricolo del Veneto (ESAV) to propagate carpione artificially

with the aim of increasing the population, which in recent years has suffered a significant reduction (Melotto & Oppi 1987). Fertilized eggs, obtained from captured wild spawners, were transferred to the ESAV experimental lake station in Bardolino, Italy, and hatched at 10 to 12°C. Fry were fed with phytoplankton. After 2 wk, the fry were transferred to a trout farm supplied with river water. At the end of June, 3 mo after hatching, high mortality occurred in the fry. This paper describes the isolation of a novel virus from the diseased fish.

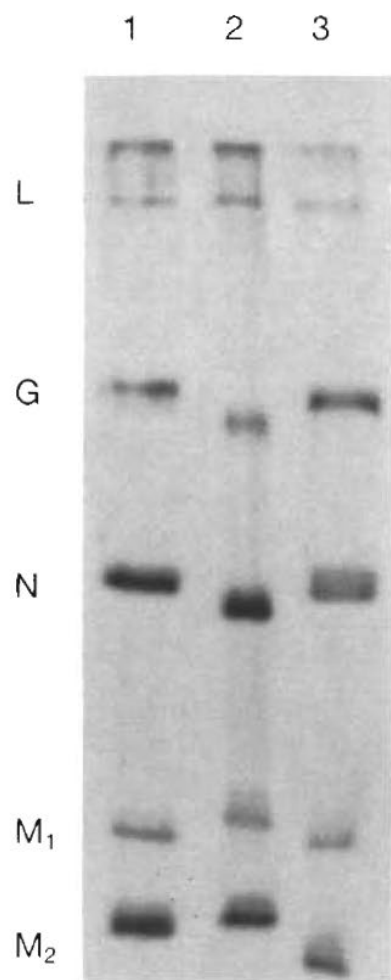


Fig. 3. SDS-PAGE gel stained with silver to reveal the 5 structural proteins (L, G, N, M<sub>1</sub>, and M<sub>2</sub>) of: (1) VHSV, (2) IHNV, and (3) carpione virus

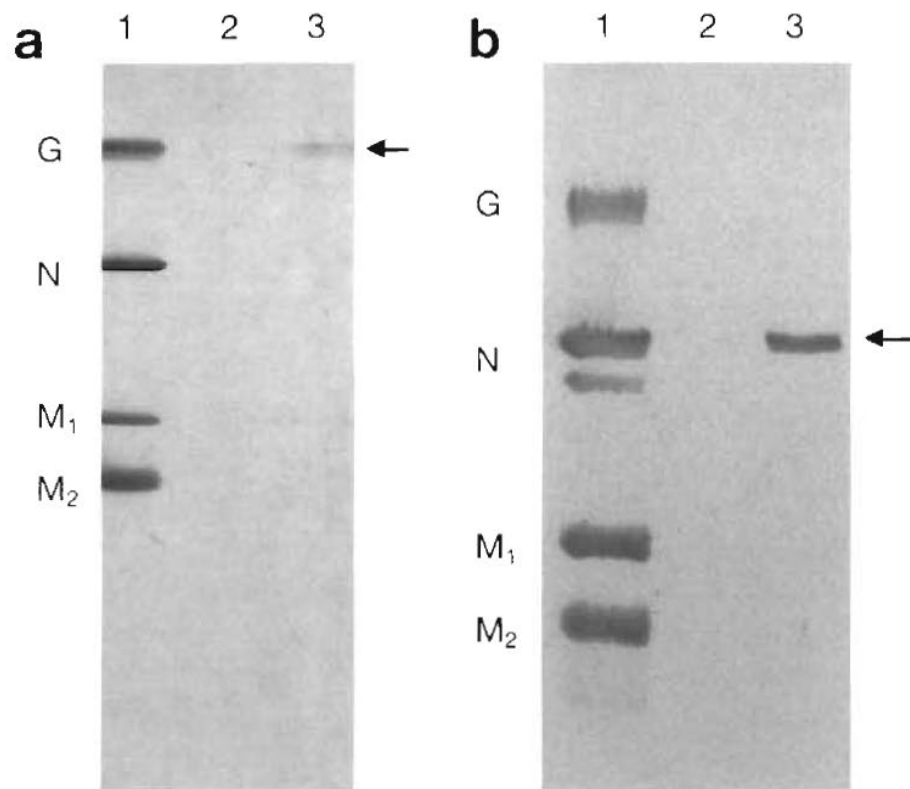


Fig. 4. Western blotting with (a) K59 (rabbit Ig to VHSV) and (b) a pool of MABs to VHSV, on the structural proteins of: (1) VHSV, (2) IHNV, and (3) carpione virus. Note reactions between K59 and carpione virus G protein (arrow, a) and between the MAB pool and carpione N protein (arrow, b)

SHORT REPORT

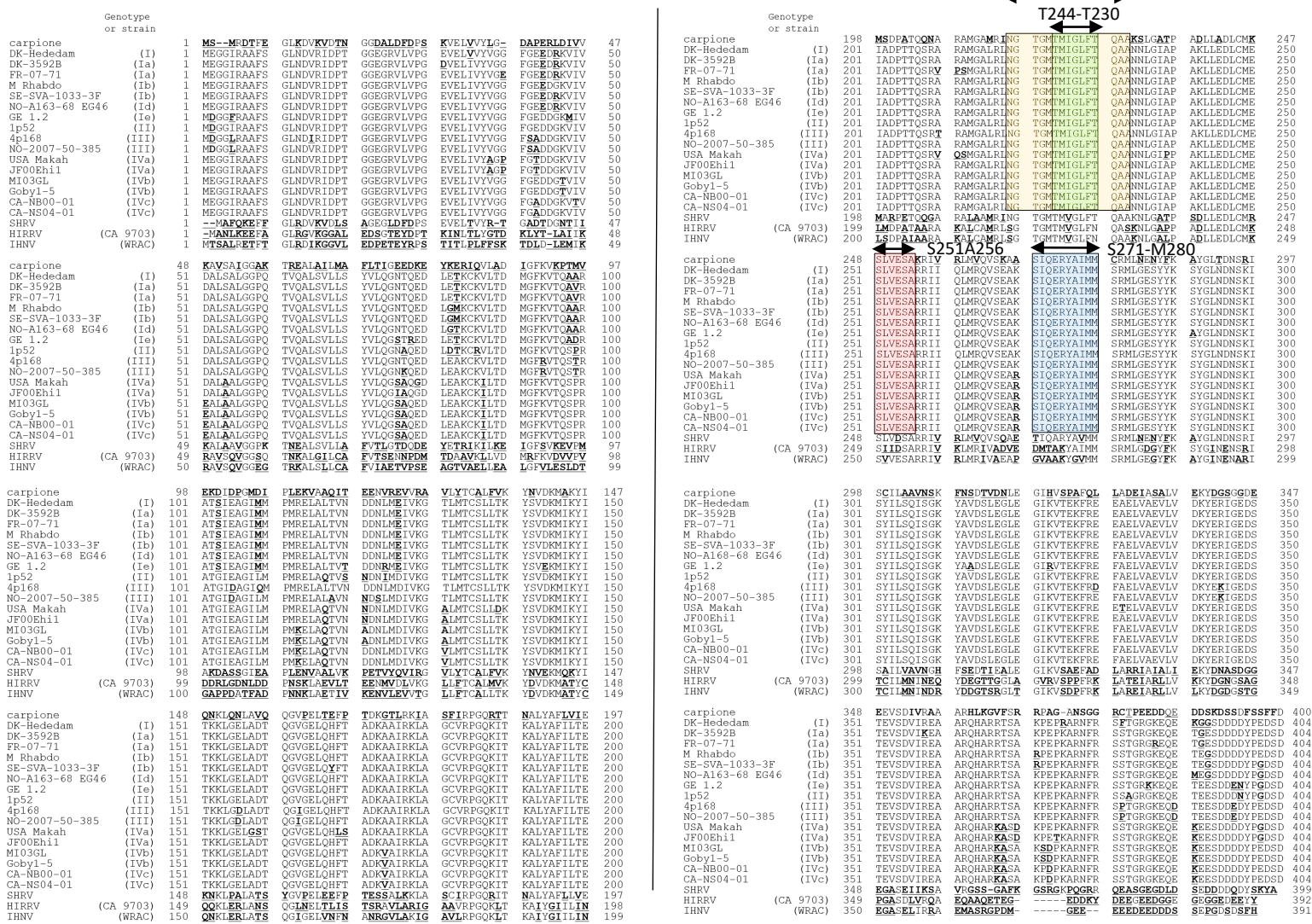
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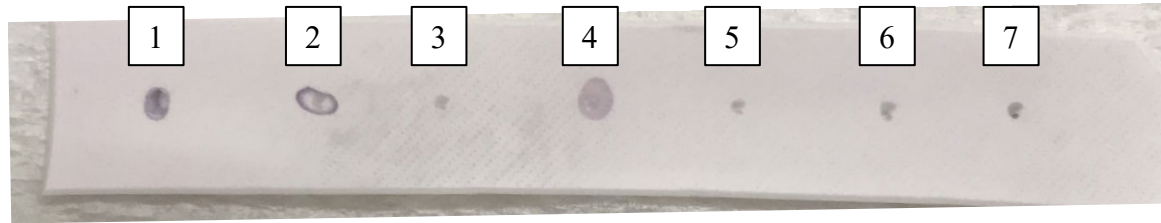
# Epitope mapping of the monoclonal antibody IP5B11 used for detection of viral haemorrhagic septicaemia virus facilitated by genome sequencing of carpione novirhabdovirus

Takafumi Ito<sup>1\*</sup> , Tohru Mekata<sup>1,3</sup>, Niels Jørgen Olesen<sup>2</sup> and Niels Lorenzen<sup>2</sup>

# Alignment of N-protein amino acid sequences



# Epitope identification using synthetic peptides



- 1: Purified VHSV isolate (JF00Ehi1)
- 2: Purified CARRV isolate
- 3: Purified HIRRV isolate (8401H)
- 4: N219 (NH<sub>2</sub>-NGTGMTMIGLFTQAA-COOH)
- 5: N224 (NH<sub>2</sub>-TMIGLFT-COOH)
- 6: N251 (NH<sub>2</sub>-SLVESA-COOH)
- 7: N271 (NH<sub>2</sub>-SIQERYAIMM-COOH)

The small black dots are the markings made with a pencil to show the dots.

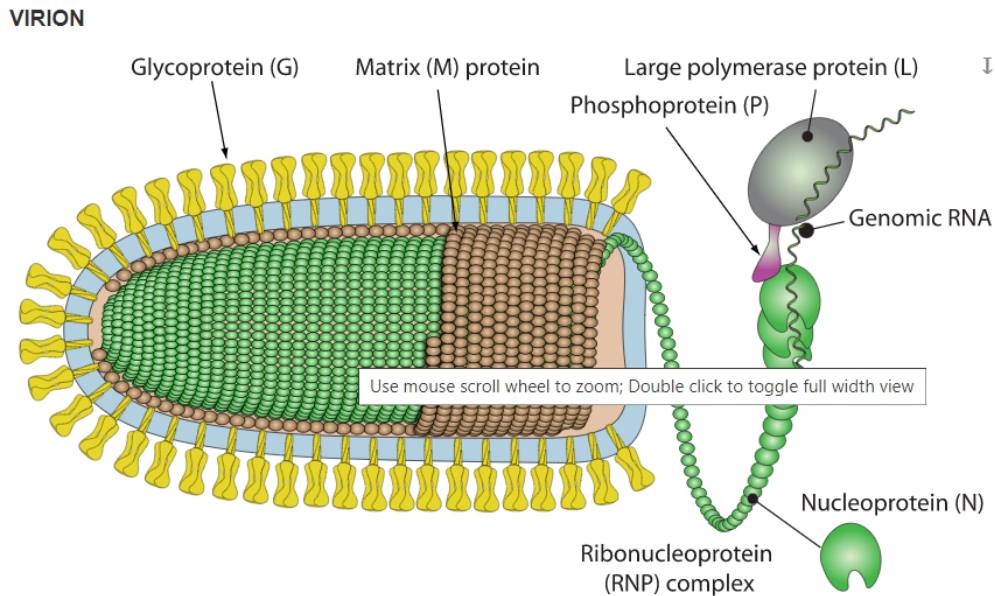
**Figure 3 Epitope mapping of IP5B11** using synthetic oligopeptides in dot-blot analysis. Purified VHSV isolate (JF00Ehi1) and the CARRV isolate were used as positive controls. Purified HIRRV isolate (8401H) was used as negative control. The purified viruses and synthetic oligopeptides were blotted onto a PVDF membrane. The membrane was incubated with mAb IP5B11 and subsequently immunostained with HRP conjugated secondary antibodies. Dot 1, JF00Ehi1; 2, CARRV; 3, HIRRV; 4, N219-A233 (NH<sub>2</sub>-NGTGMTMIGLFTQAA-COOH); 5, T224-T230 (NH<sub>2</sub>-TMIGLFT-COOH); 6, S251-A256 (NH<sub>2</sub>-SLVESA-COOH); 7, S271-M280 (NH<sub>2</sub>-SIQERYAIMM-COOH).

## Alignment of N-protein amino acid sequences

Genotype or strain					← T244-T230 →				
carpione		198	<b>MSDPATQONA</b>	RAMGAMRING	TGMTMIGLFT	QAA <b>KSLGATP</b>	ADLL <b>ADLCMK</b>		247
DK-Hededam	(I)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
DK-3592B	(Ia)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
FR-07-71	(Ia)	201	IADPTTQSRV	<b>PSMGALRING</b>	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
M Rhabdo	(Ib)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
SE-SVA-1033-3F	(Ib)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
NO-A163-68 EG46	(Id)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
GE 1.2	(Ie)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
1p52	(II)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
4p168	(III)	201	IADPTTQSRT	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
NO-2007-50-385	(III)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
USA Makah	(IVa)	201	IADPTTQSRV	<b>QSMGALRING</b>	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
JF00Ehi1	(IVa)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
MI03GL	(IVb)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
Goby1-5	(IVb)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
CA-NB00-01	(IVc)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
CA-NS04-01	(IVc)	201	IADPTTQSRA	RAMGALRING	TGMTMIGLFT	QAANNLGIAP	AKLLEDLCME		250
SHRV		198	<b>MARPETQOGA</b>	<b>RALAMRING</b>	TGMTMVGLFT	QAAK <b>NLGATP</b>	<b>SDLLEDLCMR</b>		247
HIRRV	(CA 9703)	199	<b>LMDPATAARA</b>	<b>KALCAMRLSG</b>	TGMTMVGLFN	QAA <b>SKNLGAP</b>	<b>ADLLEDLCMK</b>		248
IHNV	(WRAC)	200	<b>LSDPATAARA</b> <sup>256</sup>	<b>KALCAMRLSG</b>	TGMTMVGLFN	QAA <b>SKNLGALP</b>	<b>ADLLEDLCMK</b>		249
carpione		248	<b>SLVESAKRIV</b>	<b>RLMVQVSKAA</b>	<b>SIQERYAIMM</b>	<b>CRMLNENYFK</b>	<b>AYGLTDNSRI</b>		297
DK-Hededam	(I)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
DK-3592B	(Ia)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
FR-07-71	(Ia)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
M Rhabdo	(Ib)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
SE-SVA-1033-3F	(Ib)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
NO-A163-68 EG46	(Id)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
GE 1.2	(Ie)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	<b>AYGLNDNSKI</b>		300
1p52	(II)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
4p168	(III)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
NO-2007-50-385	(III)	251	SLVESARRII	QLMRQVSEAK	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
USA Makah	(IVa)	251	SLVESARRII	QLMRQVSEAR	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
JF00Ehi1	(IVa)	251	SLVESARRII	QLMRQVSEAR	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
MI03GL	(IVb)	251	SLVESARRII	QLMRQVSEAR	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
Goby1-5	(IVb)	251	SLVESARRII	QLMRQVSEAR	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
CA-NB00-01	(IVc)	251	SLVESARRII	QLMRQVSEAR	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
CA-NS04-01	(IVc)	251	SLVESARRII	QLMRQVSEAR	SIQERYAIMM	SRMLGESYYK	SYGLNDNSKI		300
SHRV		248	SLVDSARRIV	<b>RLMVQVSOAE</b>	<b>TIQARYAVMM</b>	SRML <b>NENYFK</b>	<b>AYGLNDNSRI</b>		297
HIRRV	(CA 9703)	249	<b>SIIDSARRIV</b>	<b>KLMRIVADVE</b>	<b>DMTAKYAIMM</b>	SRML <b>GDGYFK</b>	<b>SYGINENSRI</b>		298
IHNV	(WRAC)	250	<b>SVVESARRIV</b>	<b>RLMRIVAEAP</b>	<b>GVAAKYGVMM</b>	SRML <b>GEGYFK</b>	<b>AYGINENARI</b>		299
carpione		298	<b>SCILAAVNSK</b>	<b>FNSDVTVDNLE</b>	<b>GIHVSPAFQL</b>	<b>LADEIASALV</b>	<b>EKYDGSGGDE</b>		347
DK-Hededam	(I)	299	<b>SVYLGQISCK</b>	<b>YAVDLSLEGL</b>	<b>GIKVTKEKRE</b>	<b>FAPIVAEHLV</b>	<b>PKYMERISGDE</b>		350

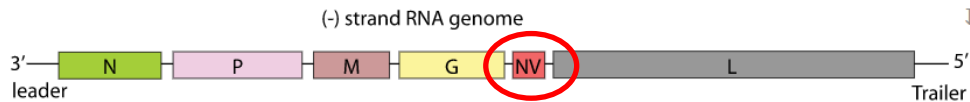
# Carpione rhabdovirus carries the NV-gene

## Novirhabdovirus



Enveloped, bullet shaped. 180 nm long and 75 nm wide.

### GENOME



Negative-stranded RNA linear genome, about 11kb in size. Encodes for six proteins.

### DB LINKS

Nucleotide DB: [NCBI](#)

Protein DB: UniProtKB ★★



### TAXONOMY

Group V: [Negative sense ssRNA viruses](#)

Order: *Mononegavirales*

Family: *Rhabdoviridae*

Genus: *Novirhabdovirus*

### SPECIES

*Salmonid novirhabdovirus*

*Hirame novirhabdovirus*

*Piscine novirhabdovirus*

*Snakehead novirhabdovirus*

### REFERENCE STRAIN

Infectious hematopoietic necrosis virus

[Sequence](#) | [Genome](#) | [Proteome](#)

### Host

#### HOST

Fish.

#### CELL TROPISM

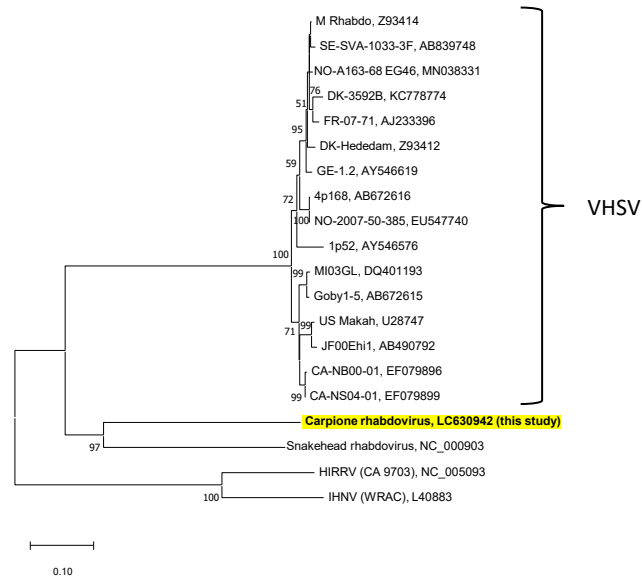
#### INTERACTIONS

[Host-virus interaction](#)

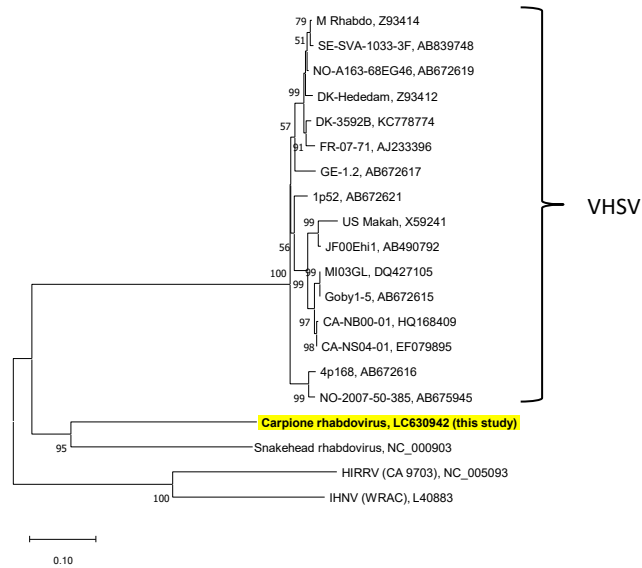
### Ecology and Disease

# Viral phylogeny based on amino acid sequences

(A) G protein



(B) N protein



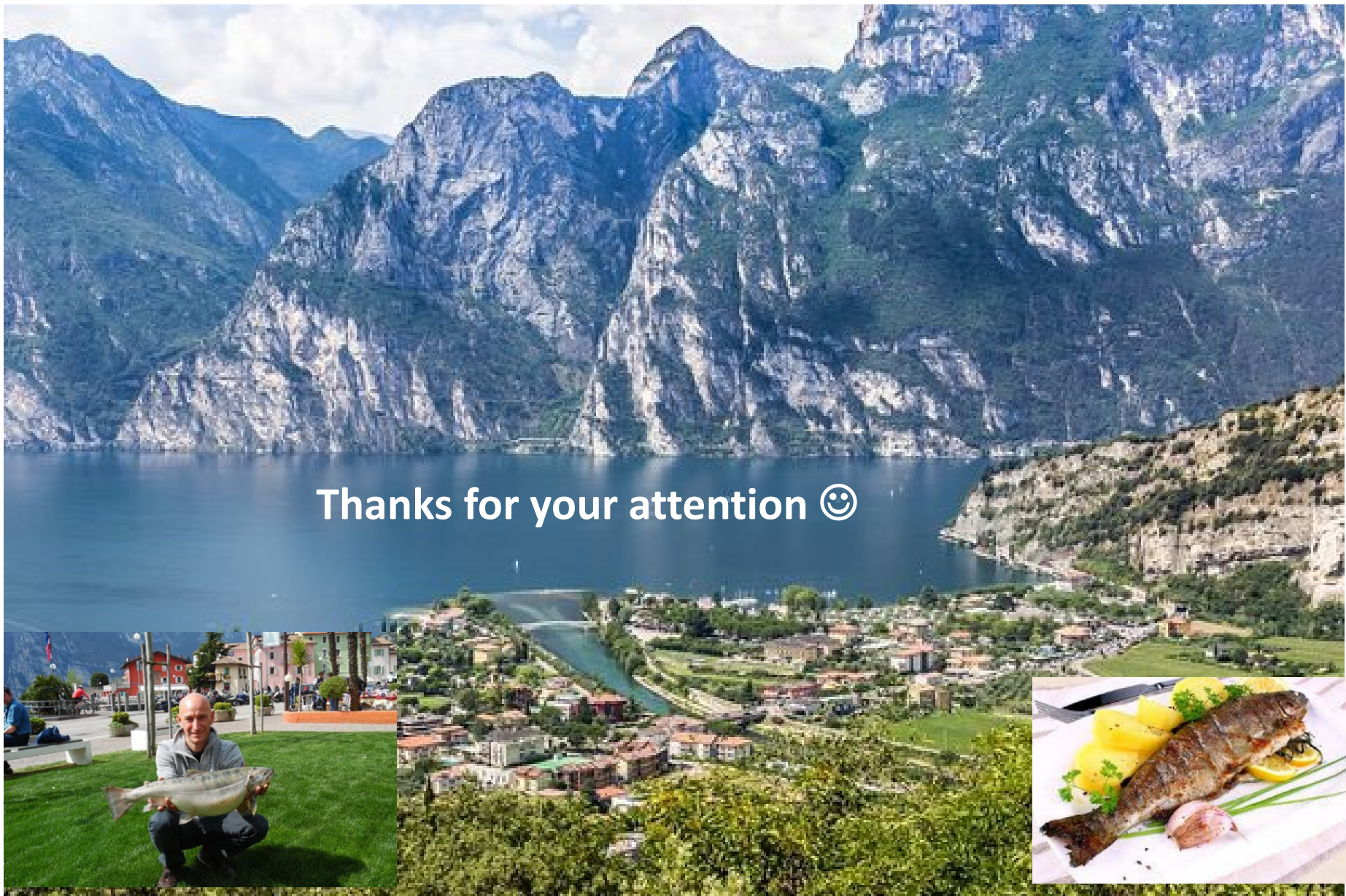
**Figure 1** Phylogenetic tree based on analysis of G and N protein amino acid sequences of piscine Novirhabdovirus by the neighbour joining method. Sixteen VHSV isolates, representatives of HIRRV, IHNV and SHRV isolates as well as the CarRV isolate 584 were included in the analysis. The accession numbers of the gene sequences used in the analysis are specified next to the names of the virus isolates. Bootstrap values above 50% are shown on the branches.



# Conclusions

- Amino acid sequence alignment of carpione rhabdovirus N-protein with other fish rhabdovirus N-proteins allowed identification MAb IP5B11 epitope candidates.
- Dot blotting with synthetic peptides determined the exact epitope.
- The high specificity of IP5B11 for VHSV depends on very few conserved amino acids.
- Unexpected identification/lack of identification of VHSV using MAb IP5B11 should be followed up by gene sequencing or other confirmatory examination.
- The carpione rhabdovirus belongs to "*novirhabdoviridae*" with SHRV and VHSV as the closest relatives.

Thanks for your attention 😊



**Table 1 Identity ratio of amino acid of glycol(G)- and nucleocapsid(N)-proteins of CarRV and sixteen VHSV isolates, SHRV, HIRRV and IHNV isolates.**

Virus isolates, genotype (accession number)	Identity ratio between CarRV 583 (LC630942) (	
	G-protein	N-protein
VHSV DK-Hededam, I (Z93412)	45.6	47.8
VHSV DK-3592B, Ia (KC778774)	46.2	47.3
VHSV FR-07-7, Ia (AJ233396)	46.2	46.8
VHSV M rhabdo, Ib (Z93414)	46.2	47.8
VHSV SE-SVA-1033-3F, Ib (AB839748)	46.0	47.8
VHSV NO-A163-68 EG46, Ic (MN038331)	46.2	
(AB672619)		47.5
VHSV GE-1.2, Ie (AY546619)	45.6	
(AB672617)		47.3
VHSV 1p52, II(AY546576)	45.4	
(AB672621)		46.8
VHSV 4p168, III (AB672616)	46.4	46.0
VHSV NO-2007-50-385, III (EU547740)	46.4	
(AB675945)		46.3
VHSV US Makah, IVa (U28747)	47.2	
(X59241)		44.8
VHSV JF00Ehi1, IVa (AB490792)	46.8	46.5
VHSV MI03GL, IVb (DQ401193)	46.8	
(DQ427105)		46.3
VHSV Goby 1-5 (AB672615)	46.6	46.3
VHSV CA-NB00-01, IVc (EF079896)	46.2	
(HQ168409)		46.8
VHSV CA-NS04-01, IVc (EF079899)	46.4	
(EF079895)		46.8
SHRV (NC_000903)	55.0	57.4
HIRRV CA 9703 (NC_005093)	39.9	42.6
IHNV WRAC, M(L40883)	39.3	44.0

<sup>a</sup> Gap-excluded identity

# Lake Garda's Carpione (*Salmo carpio*)

*Salmo carpio* is an endemic trout of Northern Italy, inhabiting exclusively Lake Garda as the primary lacustrine watershed in Italy (surface area = 368 km<sup>2</sup>). The history of Lake Garda is relatively recent, dating back to the retreat of Southern Alpine glaciers at the end of the Pleistocene some 15,000 to 18,000 years BP. The carpione is thought to have originated within Lake Garda from an ancestral 'peninsular' brown trout and rap-



Figure 3.4 Map of sampling locations for individuals included in the Bayesian clustering analysis (see Figure 3.5: Lake Garda's carpione (WSC, *S. carpio* from the western lake bank and ESC, *S. carpio* from the eastern bank, Gratton *et al.*, 2014), stream resident brown trout (ALB, Albola River, Meraner *et al.*, 2013a), lake dwelling trout (GAL, lacustrine trout from Lake Garda and SAL, lacustrine trout from River Sarca, Gratton *et al.*, 2014), and reference populations (MAR, marble trout, SVE, Tyrrhenian brown trout and HAT, hatchery brown trout of Atlantic origin; Meraner *et al.*, 2013a).

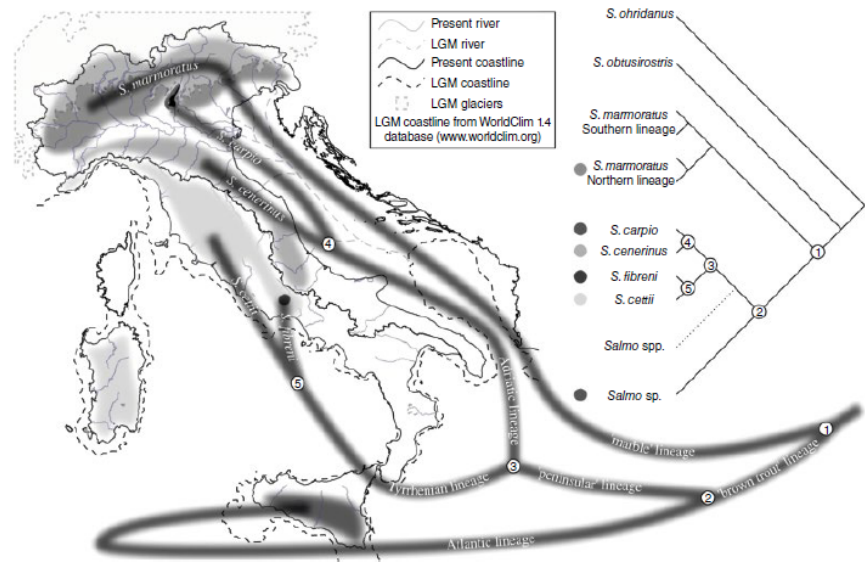


Figure 3.1 A plausible overview upon colonisation scenarios of major evolutionary lineages of the genus *Salmo* into the Italian peninsula, by taking into account inferred phylogenetic relationships and relative time of separation between them: ① split between 'marble' and 'brown trout' lineages; ② split between 'peninsular' and Atlantic lineages; ③ split between Adriatic and Tyrrhenian lineages; ④ split between *S. cenerinus* and *S. carpio*; ⑤ split between *S. cettii* and *S. fibreni*. Albeit the general pattern of east to west dispersal from the supposed origin of the genus in Ponto-Caspian refuge, Sicilian brown trout populations are likely to originate from Pleistocene west to east colonisation events from the North African Atlantic brown trout lineage, expanding back into the Central-Eastern Mediterranean.

## Genetics of the Genus *Salmo* in Italy: Evolutionary History, Population Structure, Molecular Ecology and Conservation

Andreas Meraner<sup>1</sup> and Andrea Gandolfi<sup>2</sup>

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## Essential Role of the NV Protein of *Novirhabdovirus* for Pathogenicity in Rainbow Trout

Maria-Isabel Thoulouze,\* Edwige Bouguyon, Catherine Carpentier, and Michel Brémont

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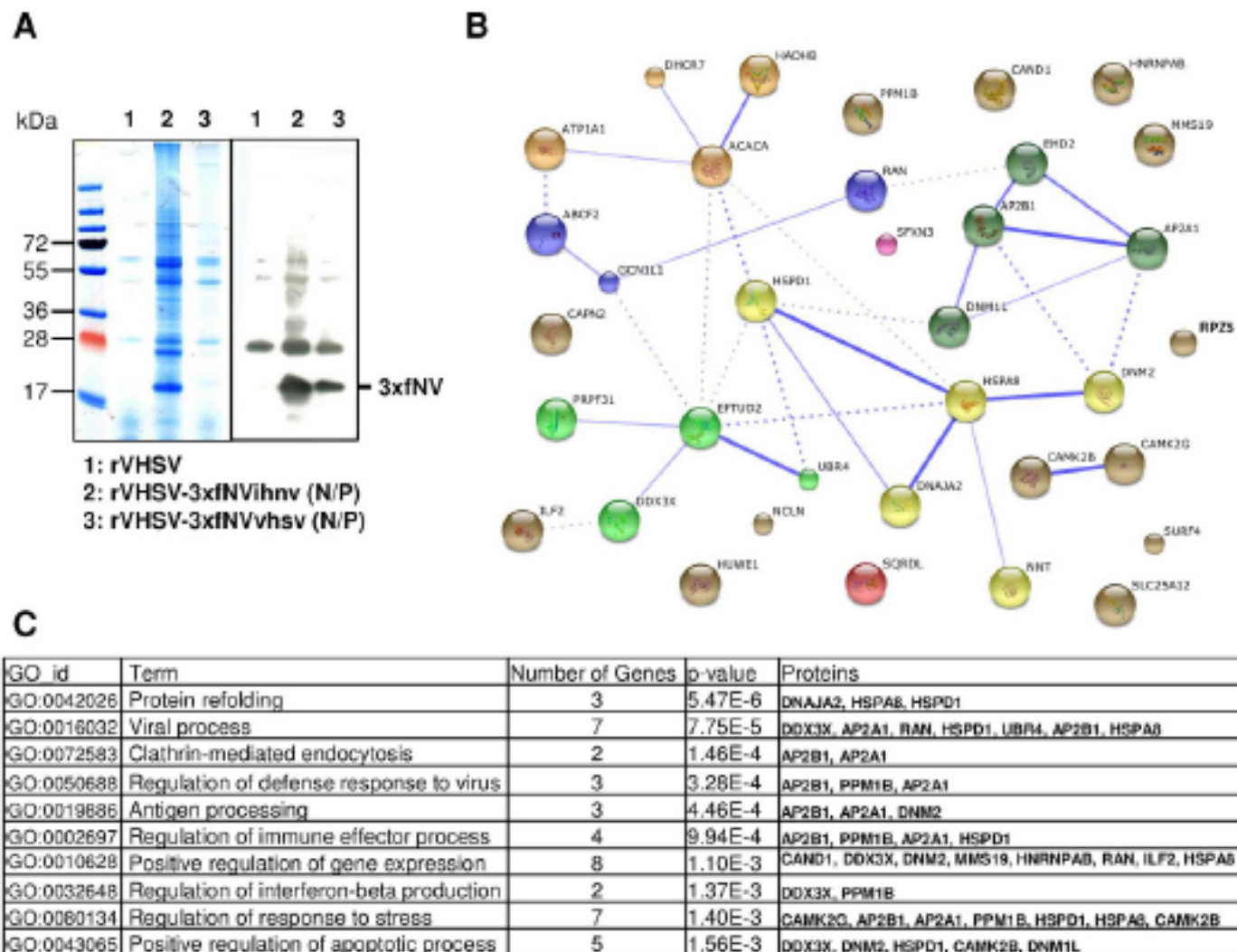
## NV Proteins of Fish Novirhabdovirus Recruit Cellular PPM1Bb Protein Phosphatase and Antagonize RIG-I-Mediated IFN Induction

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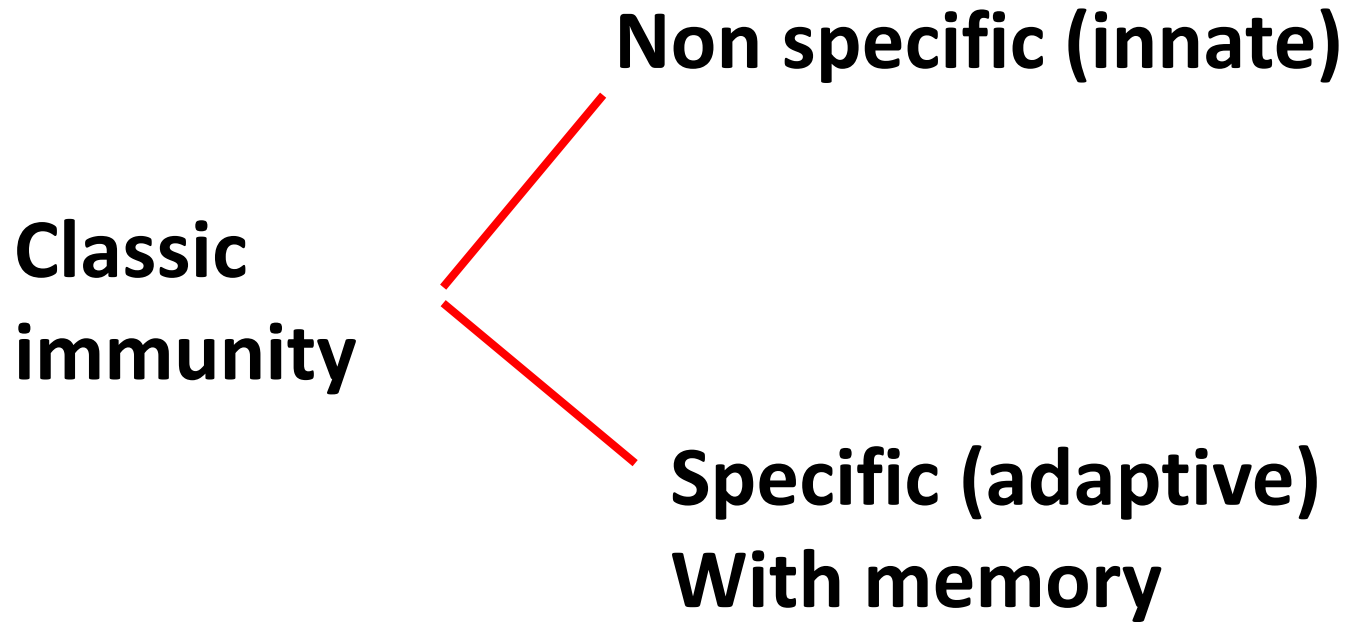
Stéphane Biacchesi<sup>1</sup>, Emilie Mérour<sup>1</sup>, Didier Chevret<sup>2</sup>, Annie Lamoureux<sup>1</sup>, Julie Bernard<sup>1</sup> & Michel Brémont<sup>1</sup>

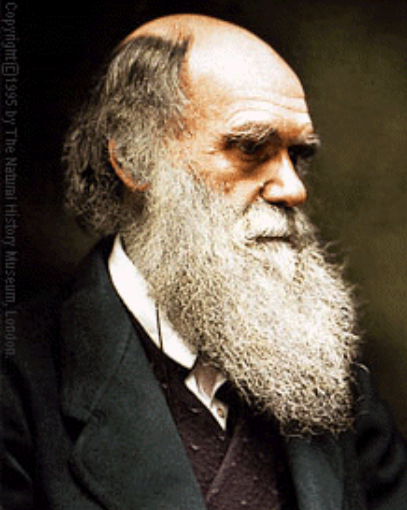


**Figure 3. Identification of host proteins that co-precipitated with VHSV and IHNV NV proteins.**

(A) Co-immunoprecipitation of cellular proteins associated with NV proteins. Lysates from rVHSV (background control; lane 1), rVHSV-3xFlagNVhsv (N/P) (lane 2) and rVHSV-3xFlagNVvhsv (N/P) (lane 3) infected cells were immunoprecipitated using anti-Flag mAb. Aliquot of each eluate was analyzed by electrophoresis on a polyacrylamide gel followed by Coomassie blue staining (left panel) or western-blotting using the anti-Flag mAb (right panel). (B) NV-associated host protein network. The protein-protein interaction network involving the 35 host proteins associated with NV were shown in the confidence view produced by STRING v10 analysis. Stronger associations are represented by thicker lines. (C) Gene ontology (GO biological processes) enrichment analysis of host proteins co-immunoprecipitating with NV proteins.

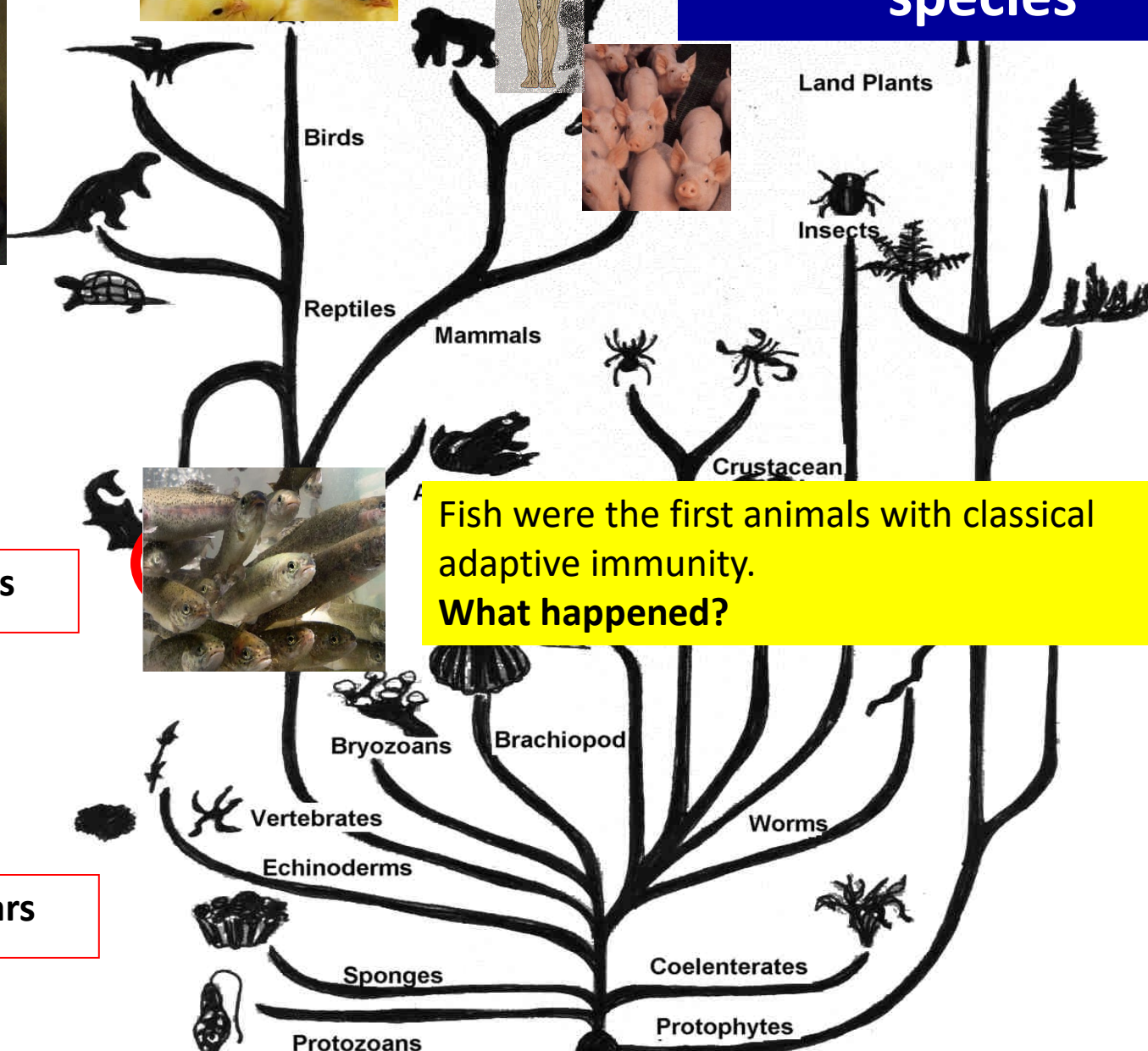
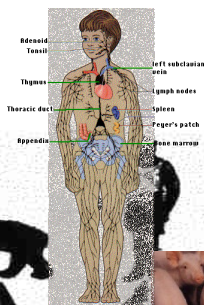
# Immune mechanisms





Charles Darwin

# Evolution of the species



500 mill. years

Fish were the first animals with classical adaptive immunity.  
**What happened?**

600 mill. years