

The following supplement accompanies the article

Low intraspecific variation of *Frog virus 3* with evidence for novel FV3-like isolates in central and northwestern Canada

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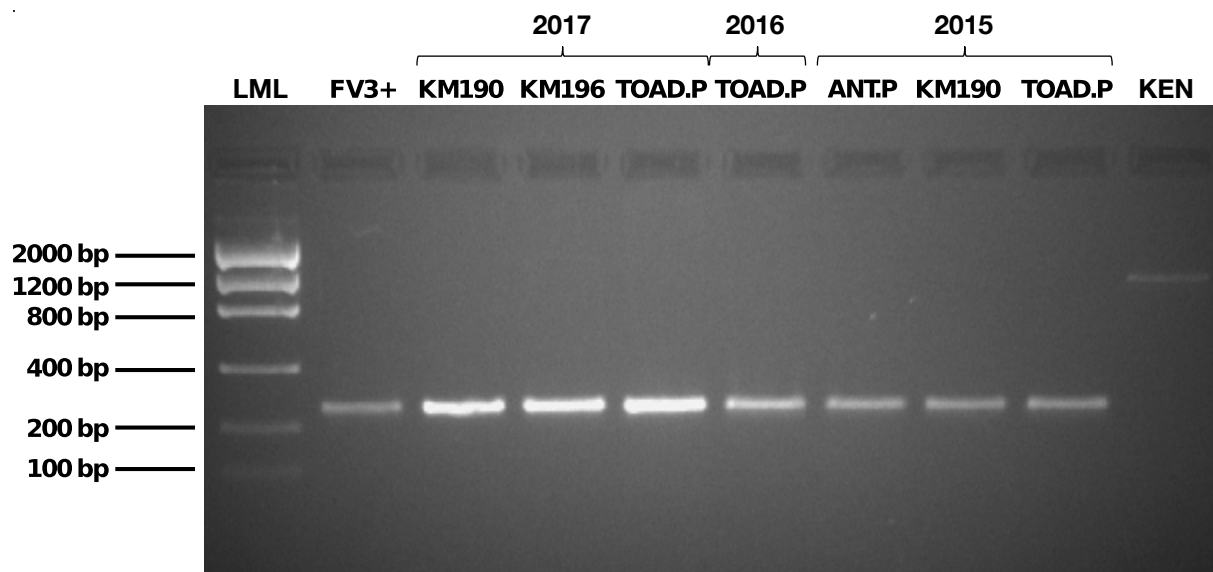


Figure S1: PCR amplification of the vIF-2 α region of eight FV3-like samples sampled across Canada. LML: Low mass ladder; FV3+: Cultured FV3 positive control. Site abbreviations as follows: TOAD.P: Toadlet pond; ANT.P: Antoinette's pond. See Table 2 and Figure 1 for site locations.

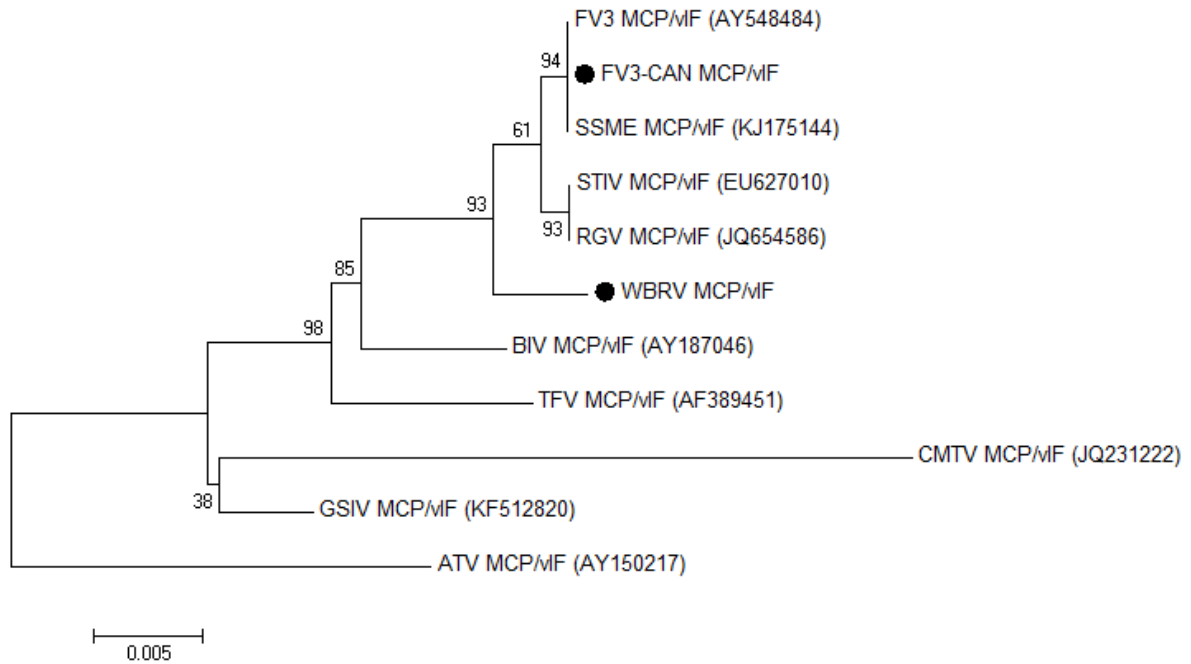


Figure S2: Maximum likelihood tree of the concatenated MCP and vIF-2 α of 10 ranaviruses, with *Ambystoma tigrinum* virus (ATV) as an outgroup. Two of the three samples found within this study are shown with a black circle. The recombinant samples sequenced in this study were not included due to the recombinant nature of the genes potentially coming from two different ranaviruses. Sequences were named based on given names from genbank sequences. FV3: *Frog virus 3*; SSME: Spotted salamander Maine; STIV: Soft-shelled turtle iridovirus; RGV: *Rana grylio* virus; BIV: *Bohle iridovirus*; TFV: Tiger frog virus; CMTV: *Common Midwife toad virus*; GSIV: Chinese giant salamander iridovirus. See Tables 4 and 5 for nucleotide polymorphisms.

Table S1: Primers used for DNA analysis. Primer positions were relative to the *Frog Virus 3* genome (AY548484).

Primer	Location	Nucleotide sequence
MCP forward	97310 – 97330	5' – TCC ACA GTC ACC GTG TAT CTT – 3'
MCP reverse	98061 – 98044	5' – TGC AGC AAA CGG ACA CTT – 3'
vIF-2 α forward	32947 – 32969	5' – AAC AAA TGC AAT GAC TGT AAA TG – 3'
vIF-2 α reverse	33195 – 33178	5' – ACA CAA AGG GGC ACA GTC – 3'

Table S2: Description of FV3-like samples taken across Canada between 2015-2017, including site codes, sampling location, host species, host life stage, and number of samples with given classifications. Homology percentage of genes were based on isolate of highest similarity.

Sample code	Location	Year	Species	Life stage	# samples	Gene	% Homology	Isolate of highest similarity
AMW	Antoinette's pond, NWT	2015	<i>Lithobates sylvaticus</i>	Metamorphic	6	MCP	100%	FV3
						vIF-2 α	100%	FV3
KAW	KM 190, NWT	2015	<i>Lithobates sylvaticus</i>	Adult	3	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
KTC	KM 190, NWT	2015	<i>Pseudacris maculata</i>	Tadpole	10	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
KTT	KM 190, NWT	2015	<i>Anaxyrus hemiophrys</i>	Tadpole	3	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
KTW	KM 190, NWT	2015	<i>Lithobates sylvaticus</i>	Tadpole	5	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
TAC	Toadlet pond, AB	2015	<i>Pseudacris maculata</i>	Adult	3	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
TAW	Toadlet pond, AB	2015	<i>Lithobates sylvaticus</i>	Adult	2	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
TMC	Toadlet pond, AB	2015	<i>Pseudacris maculata</i>	Metamorphic	2	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
TTW	Toadlet pond, AB	2015	<i>Lithobates sylvaticus</i>	Tadpole	6	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
KEN	Kennedy Drive, ON	2016	<i>Lithobates clamitans</i>	Tadpole	6	MCP	100%	FV3
						vIF-2 α	99.9%	CGSIV
MOA	Frink Marsh, ON	2016	<i>Lithobates clamitans</i>	Tadpole	1	MCP	100%	FV3
						vIF-2 α	100%	FV3
STL	Sturgeon road, ON	2016	<i>Lithobates clamitans</i>	Tadpole	2	MCP	100%	FV3
						vIF-2 α	100%	FV3
TIM	Maley, ON	2016	<i>Lithobates pipiens</i>	Adult	1	MCP	100%	FV3
						vIF-2 α	100%	FV3
TAW	Toadlet pond, AB	2016	<i>Lithobates sylvaticus</i>	Adult	2	MCP	100%	FV3
						vIF-2 α	100%	FV3
KAC	KM 190, NWT	2017	<i>Pseudacris maculata</i>	Adult	10	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
KTW	KM 190, NWT	2017	<i>Lithobates sylvaticus</i>	Tadpole	2	MCP	100%	FV3
						vIF-2 α	100%	FV3
K6AW	KM 196, NWT	2017	<i>Lithobates sylvaticus</i>	Adult	1	MCP	99.3%	WBRV (FV3)
						vIF-2 α	99.6%	WBRV (FV3)
K6TC	KM 196, NWT	2017	<i>Pseudacris maculata</i>	Tadpole	35	MCP	100%	FV3
						vIF-2 α	100%	FV3
K6TW	KM 196, NWT	2017	<i>Lithobates sylvaticus</i>	Tadpole	19	MCP	100%	FV3
						vIF-2 α	100%	FV3
TTW	Toadlet pond, AB	2017	<i>Lithobates sylvaticus</i>	Tadpole	7	MCP	100%	FV3
						vIF-2 α	100%	FV3
TTC	Toadlet pond, AB	2017	<i>Pseudacris maculata</i>	Tadpole	1	MCP	100%	FV3
						vIF-2 α	100%	FV3