


Isolation of Common Midwife Toad Virus and a Recombinant Frog Virus 3 in North American Bullfrogs (*Lithobates catesbeianus*)

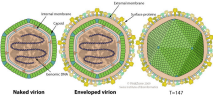



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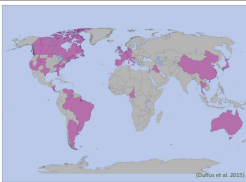


Iridovirus Taxonomy

- Diverse group of Nucleocytoplasmic Large dsDNA Viruses (NCLDV)
- Virion: enveloped icosahedral capsid (120-350 nm)
- Taxonomy
 - Genus *Iridovirus* (arthropod hosts)
 - Genus *Chloriridovirus* (arthropod hosts)
 - Genus *Lymphocystivirus* (fish hosts)
 - Genus *Megalocytivirus* (fish hosts)
 - Genus *Ranavirus* (fish, amphibian, & reptilian hosts)
 - FV3, BIV, EHNV, ATV, ECV, SCR
 - Unassigned members: CMTV, ADRV...

Ranavirus

- Ranaviruses are globally emerging pathogens
- Infect at least 175 species across 53 families of ectothermic vertebrates on all continents (except Antarctica)
- Six different species: three infect amphibians
 - *Ambystoma tigrinum virus* (ATV) (Jancovich et al. 1997)
 - North America
 - *Bahle Iridovirus* (BIV) (Speare and Smith 1992)
 - Australia, Europe*, North America*
 - *Frog Virus 3* (FV3) (Granoff et al. 1965)
 - North America, Europe, Asia
- Unassigned members: Emerging CMTV & CMTV-like clade
 - *Common Midwife Toad Virus* (CMTV) (Balseiro et al. 2009)
 - Europe
 - *Andrias davidianus* (ADRV) (Geng et al. 2011)
 - Asia



Ranavirus: Threats to Global Ranaculture/Aquaculture

- FV3-like virus epizootics in ranaculture/aquaculture:
 - Pig frog (*Rana gryllis*) used for food in China (Zhang et al. 2001)
 - Tiger frogs (*Hoplobatrachus tigerinus*) in China (He et al. 2002) and Thailand (Kanchanakhan et al. 2002)
 - North America **bullfrog** (*Lithobates catesbeianus*) in **Georgia, USA** (Majji et al. 2006, Miller et al. 2007), **Brazil** (Mazzoni et al. 2009), and **Korea** (Kim et al. 2011)
 - Chinese softshell turtle (*Pelodiscus sinensis*) (Huang et al. 2009)
 - Marbled sleeper goby (*Oxyeleotris marmoratus*) farm in Thailand (Prasankok et al. 2005)
- Facilities rearing sturgeon for food and restoration efforts (Waltzek et al. 2014)
- Lethal CMTV-like virus in most farms in China rearing endangered Chinese giant salamander (*Andrias davidianus*) for food and medicinal purposes (Geng et al. 2011, Chen et al. 2013)




Georgia FV3-like Ranaculture Isolates (RI)

- Compare the phylogenomic diversity of two lethal American bullfrog viral isolates from the same ranaculture facility to 15 other previously sequenced ranavirus isolates
- First Georgia (bullfrog) ranaculture epizootic
 - RC:RC_1998_IDGA_Chinchar in 1998 (Majji et al. 2006) = FV3 strain
 - > Virulence than FV3 isolate from leopard frog
- Second Georgia (bullfrog) ranaculture epizootic
 - RC:RC_2006_GA_Gray in 2006 (Miller et al. 2007) = FV3-like (new species?)
 - > Virulence than FV3 isolate from leopard frog (Hooverman et al. 2010)

Objectives

- Compare the phylogenomic diversity of two lethal American bullfrog isolates from the same ranaculture facility to 15 other previously sequenced ranavirus isolates



Common Name	Scientific Name	Country of Isolation	Year of Isolation	Reference
1 Alpine Newt	<i>Mesotriton alpestris cyreni</i>	Spain	2008	Mavian et al. (2012)
2 Bullfrog	<i>Lithobates catesbeianus</i>	USA	2006	Matthew Gray
3 Bullfrog	<i>Lithobates catesbeianus</i>	USA	1998	Gregory Chinchar
4 Brown-Spotted Grouper	<i>Epinephelus tauvina</i>	Singapore	1998	Song et al. (2004)
5 Chinese Giant Salamander	<i>Andrias davidianus</i>	China	2010	Jiang et al. (2011)
6 Edible Frog	<i>Pelophylax esculentus</i>	Netherlands	2013	van Beurden et al. (2014)
7 Egyptian Tortoise	<i>Testudo kleinmanni</i>	Germany*	1996	Stohr et al. (2015)
8 Gecko	<i>Uroplatus fimbriatus</i>	Germany*	2001	Stohr et al. (2015)
9 Leopard Frog	<i>Lithobates pipiens</i>	USA	1963	Tan et al. (2004)
10 Pig Frog	<i>Rana grylio</i>	China	1995	Lei et al. (2012)
11 Redfin Perch	<i>Perca fluviatilis</i>	Australia	1984	Jancovich et al. (2010)
12 Sheatfish	<i>Silurus glanis</i>	Germany	1989	Mavian et al. (2012)
13 Softshelled Turtle	<i>Trionyx sinensis</i>	China	1997	Huang et al. (2009)
14 Sonoran Tiger Salamander	<i>Ambystoma tigrinum stebbinsi</i>	USA	1995	Jancovich et al. (2003)
15 Spotted Salamander	<i>Ambystoma maculatum</i>	USA	1998	Morrison
16 Tiger Frog	<i>Rana tigrinum rugulosa</i>	China	1999	He et al. (2002)
17 Yellow Grouper	<i>Epinephelus awoara</i>	Taiwan	2000	Tsai et al. (2005)



Genome Annotation, BLASTp Analysis and Alignment

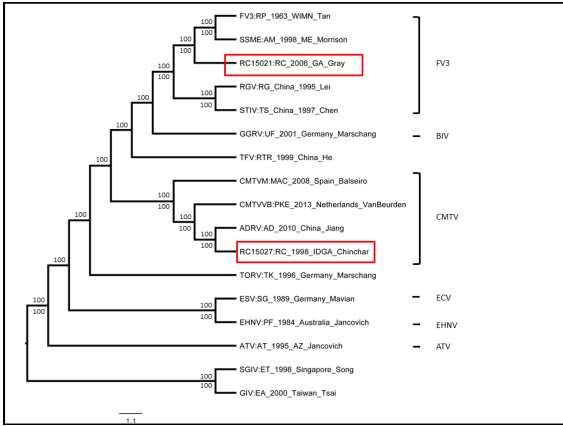
- RC15021:RC_2006_GA_Gray
 - De novo assembly
 - 4,330,026 paired end reads
 - 104,968 bps
 - 110 putative ORFs, 17-1,234 aa
 - G+C content = 56.08%
 - Mean coverage = 7,495x per nucleotide
 - BLASTp showed majority of ORFs had highest identity to FV3 or FV3-like viruses
 - 12 recombination events
 - Best hits from 18 ORFs displayed greatest identity with members of CMTV or CMTV-like viruses (ADRV)

- RC15027:RC_1998_IDGA_Chinchar
 - De novo assembly
 - 4,388,046 paired end reads
 - 106,890 bps
 - 108 putative ORFs, 16-1,294 aa
 - G+C content = 55.5%
 - Mean coverage = 5,884x per nucleotide
 - The BLASTp searches revealed the majority of ORFs with highest identity to CMTV or CMTV-like viruses (ADRV)
 - 2 recombination events
 - Best hits from 4 ORFs displayed greatest identity with FV3 and 1 ORF with TRV

• The final aligned concatenated dataset (53 genes for 17 taxa) was 68,136 bps in length including gaps

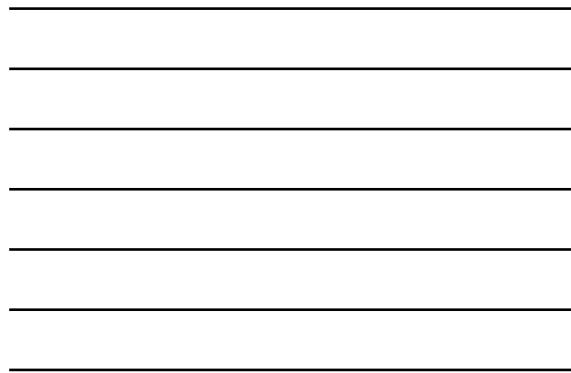
• All recombination events removed prior to phylogenomic analyses

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Discussion

- Two bullfrog viral isolates from the same ranaculture facility sequenced
 - First (RC15027:RC_1998_IDGA_Chinchar)
 - Majji et al. (2006) showed that the bullfrog viral isolate > virulence than FV3 from leopard frogs and predicted it was a novel species
 - This isolate is a member of the CMTV clade
 - Expands the known geographic range of CMTV clade into NA
 - 1998 becomes the earliest known CMTV epizootic (origins?)
 - Previously, earliest CMTV isolate was in 2007 in Spain
 - Second (RC15021:RC_2006_GA_Gray)
 - Hoverman et al. (2010) showed that this bullfrog viral isolate > virulence than FV3 from leopard frogs
 - First reported case of recombination in a FV3
 - Previously detected *in vitro* (Chinchar and Granoff 1986)
 - Recombination recently reported in ATV (Epstein and Storf 2016)



Discussion

RC15021:RC_2006_GA_Gray

RC15027:RC_1998_IDGA_Chinchar



Conclusions

- The first bullfrog epizootic in 1998 was found to be the earliest known detection of CMTV, expanding the geographic range into North America (origins/index case?)
- The second bullfrog epizootic in 2006 was found to be a chimeric FV3 having recombined with CMTV (first bullfrog epizootic?)
- Given bullfrogs are susceptible to multiple strains of ranaviruses they may serve as a mixing vessel for the evolution of virulent recombinant FV3 strains
- This bullfrog ranaculture facility may have led to the evolution of virulent recombinant FV3 strains (density dependent factors, e.g. host immunosuppression and ease of viral transmission)
- The American bullfrog was disseminated globally at the beginning of the 19th century and now is traded internationally as the most important amphibian species for human consumption
- The international trade in bullfrogs has undoubtedly contributed to the global spread of important amphibian diseases including Bd and ranaviruses (Garner et al. 2006; Schlogel et al. 2009)

Future Directions

- Selection Analyses (non-synonymous vs synonymous substitution rate d_n/d_s)
 - Detected positive selection in 12 genes (Abrams et al. 2013)
- Continue our preliminary recombination analyses
 - Hot spots within ranavirus genomes (paired repeated elements to facilitate homologous recombination)

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