Wednesday September 5th – Langevin / Cartier
Virology 1 & 2
Moderator - Tom Waltzek (University of Florida)

9:30 AM	Virology 1	Waltzek - Expansion of the Mimivirus Host Range From Microbes to Vertebrates
9:45 AM		**Koda - Repeated Detections of Red Seabream Iridovirus in Florida Pompano Maricultured in the Caribbean Sea**
10:00 AM		Watlzek - Phylogenomic Characterization of Carp Edema Virus
10:15 AM		Lovy - Carp Edema Virus Associated with Natural Mortality of Wild Carp in New Jersey
10:30 AM		**Refreshments**
10:45 AM	Virology 2	**Sriwanayos - Phylogenomic Characterization of Ranaviruses Detected in Fish and Amphibians in Thailand**
11:00 AM		**Walker - Phylogenomic Characterization of Acipenserid Herpesvirus 1 in Lake Sturgeon (*Acipenser fulvescens*)**
11:15 AM		Subramaniam - Phylogenomic Characterization of Squamate Erythrocytic Iridoviruses
11:30 AM		**Haggard - Genomic Characterization of Percid Herpesvirus 1 Associated with Epidermal Hyperplasia in Walleye *Sander vitreus***
11:45 AM		Waltzek - Genomic Characterization of the First Fish Bunyaviruses through Next-Generation Sequencing

Expansion of the Mimivirus Host Range from Microbes to Vertebrates

Thomas Waltzek[1*], Kuttichantran Subramaniam[1], Gavin Wilkie[2], and Andrew Davison[2]

[1] Department of Infectious Disease and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA kuttichantran@ufl.edu tbwaltzek@ufl.edu

[2] MRC – University of Glasgow Centre for Virus Research, United Kingdom gavin.wilkie@gmail.com andrew.davison@glasgow.ac.uk

Mimiviruses are ubiquitous among varied aquatic habitats where they infect unicellular eukaryotes including phagocytic flagellates and amoebae. Mounting evidence suggests that mimi-like viruses also infect diverse marine algae and perhaps even metazoans including reef-building corals and sponges. Here, for the first time, we characterize a new branch of mimiviruses responsible for lethal diseases in critically endangered sturgeon, expanding their host range from aquatic microbes to vertebrates, potentially spanning three eukaryotic supergroups. Purified viral DNA from the White Sturgeon Mimivirus (WSMV) was used to generate a DNA library for sequencing on an Illumina MiSeq sequencer. The resulting sequence reads were trimmed and assembled using multiple assembly softwares. The complete genome (427,714 bp) was recovered and is predicted to encode 365 open reading frames within the unique region and inverted terminal repeats. The success of mimiviruses, as evident by their abundance across varied hosts and environments, may be linked to their extraordinary genomic complexity and plasticity. We found that sturgeon mimiviruses, contrasted against mimiviruses that infect microbes lacking an acquired immunity, carry a repertoire of immune evasion genes likely pirated from their vertebrate hosts. Accumulating evidence would suggest that we are only now realizing the influence of mimiviruses and other giant viruses have on ocean biogeochemical cycling and eukaryotic biodiversity through a combination of bottom-up and top-down mechanisms.

Conference Session Designation: (Virology)
Presentation Format: **(** Oral)

Repeated Detections of Red Seabream Iridovirus in Florida Pompano Maricultured in the Caribbean Sea

Samantha A. Koda[1*], Kuttichantran Subramaniam[1], Deborah B. Pouder[2], Roy P. Yanong[2], Salvatore Frasca, Jr.[3] and Thomas B. Waltzek[1]

[1] Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida USA samanthakoda@ufl.edu kuttichantran@ufl.edu tbwaltzek@ufl.edu

[2] University of Florida/IFAS School of Forest Resources and Conservation, Program in Fisheries and Aquatic Sciences, Tropical Aquaculture Laboratory, Ruskin, Florida USA dbpouder@ufl.edu rpy@ufl.edu

[3] Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Florida USA sfrasca@ufl.edu

Megalocytiviruses (MCVs) are finfish pathogens negatively impacting ornamental and food fish aquaculture around the world. Within the genus *Megalocytivirus*, *Infectious spleen and kidney necrosis virus* (ISKNV) is the only recognized species. ISKNV is subdivided into three genotypes: ISKNV, red seabream iridovirus (RSIV), and turbot reddish body iridovirus (TRBIV). Red seabream iridoviral disease (RSIVD), caused by genotypes ISKNV and RSIV, is listed as a disease reportable to the World Organization for Animal Health (OIE). RSIV was first reported in 1990 from Shikoku Island, Japan in cultured Red Seabream (*Pagrus major*). Since then, there have been repeated cases of RSIVD in Asian maricultured species. More recently, RSIVD has been reported multiple times in Florida Pompano (*Trachinotus carolinus*) reared in net pens in the Caribbean Sea. Histopathological examination of affected fish revealed microscopic lesions typical of MCV, including cytoplasmic basophilic inclusions in various internal organs. From outbreaks that occurred in 2010 and 2014, we sequenced the full MCV genomes from infected internal tissues using an Illumina MiSeq sequencer. Maximum Likelihood phylogenomic analyses based on full genomic alignments of the pompano and other previously sequenced MCVs revealed the pompano MCV is supported within the RSIV genotype. Partial amplification and sequencing of the MCV myristylated membrane protein gene from archived formalin-fixed paraffin-embedded tissue sections, displaying the aforementioned microscopic lesions, revealed previous outbreaks in Caribbean maricultured pompano were also due to the RSIV genotype. Although these cases are the first detections of RSIV in maricultured Florida Pompano, RSIV has previously been reported in Snubnose Pompano (*Trachinotus blochii*) maricultured in Japan. These cases extend the known geographic range of RSIV into the Caribbean and suggest further investigation is needed to determine the risk RSIV poses to the mariculture of Florida pompano.

Conference Session Designation: (Virology)
Presentation Format: (Oral)
Student Presentation: (Yes)

Phylogenomic Characterization of Carp Edema Virus

Thomas Waltzek[1*], Kuttichantran Subramaniam[1], Jan Lovy[2], Nissin Moussatche[3], Richard Condit[3], Gavin Wilkie[4], and Andrew Davison[4]

[1] Department of Infectious Disease and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA kuttichantran@ufl.edu tbwaltzek@ufl.edu
[2] Office of Fish & Wildlife Health & Forensics, New Jersey Division of Fish & Wildlife, Trenton, NJ 08625, USA Jan.Lovy@dep.nj.gov
[3] Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL 32610, USA nissin@ufl.edu, ilcondit@ufl.edu
[4] MRC – University of Glasgow Centre for Virus Research, United Kingdom gavin.wilkie@gmail.com, andrew.davison@glasgow.ac.uk

Double-stranded DNA viruses (dsDNA) infect a wide range of homeothermic and poikilothermic vertebrates. However, only the dsDNA families *Alloherpesviridae* and *Iridoviridae* are well studied among poikilothermic vertebrates (e.g. fish, amphibians, and reptiles). Herein, we report the phylogenomic characterization of a fish poxvirus, carp edema virus (CEV) that infects common carp (*Cyprinus carpio*) varieties including koi. CEV is a globally emerging virus that has negatively impacted facilities rearing common carp for food, sport, and recreation. In this study, we built a DNA library from CEV infected gill tissue DNA derived from an outbreak that occurred in wild common carp in New Jersey in 2017. Sequencing of the library was performed on an Illumina MiSeq sequencer and the resulting data trimmed and assembled using multiple assembly softwares. The nearly full genome (>450,000 bp) was recovered including the inverted terminal repeats. Ultrastructural examination revealed abundant large spheroid particles within the cytoplasm of gill epithelial cells consistent with previous studies. The mature CEV virion appears to possess a single lateral body, similar to previous reports of fish poxviruses in Atlantic salmon (*Salmo salar*) and ayu (*Plecoglossus altivelis*). Maximum Likelihood phylogenetic analysis based on the concatenated amino acid sequences of seven conserved poxvirus proteins revealed that CEV is the sister taxon to the salmon gill poxvirus and together they form the most basal branch of the *Chordopoxvirinae*. The genetic distinctness of the fish poxviruses argues that they represent a new genus within the *Chordopovirinae* that we suggest could be named Piscipoxvirus. However, completion of the CEV genome annotation is needed to determine whether the fish poxviruses share a suite of derived genomic features that support the creation of the proposed genus.

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Carp Edema Virus Associated with Natural Mortality of Wild Carp in New Jersey

Jan Lovy[1]*, Sarah E. Friend[1], Thomas B. Waltzek[2], and Lowia Al-Hussinee[2]

[1] Office of Fish and Wildlife Health and Forensics, N.J. Division of Fish and Wildlife, 605 Pequest Road, Oxford, NJ 07863 USA, Jan.Lovy@dep.nj.gov; Sarah.Friend@dep.nj.gov
[2] University of Florida, College of Veterinary Medicine, Aquatic Pathobiology Lab, Infectious Diseases and Immunology, 2173 Mowry Road, Gainesville, FL 32610 USA, Tbwaltzek@ufl.edu; lalhussinee@ufl.edu

In North America, carp edema virus (CEV), the cause of koi sleepy disease, has previously been limited to sporadic detections in koi imports as early as 1996, though not documented in wild carp populations. In May 2017, mass mortality of adult wild carp occurred in Mill Pond, Bergen County, NJ, USA. During the mortality fish showed signs of severe lethargy, often resting on the bottom of the pond. In a sampled moribund fish, histologic lesions were limited to the gill only. Microscopic lesions included diffuse lamellar fusion with extensive cell death suggestive of apoptosis seen as pyknosis, cytoplasmic condensation, and formation of apoptotic bodies. Transmission electron microscopy identified immature and mature pox-like virions consistent with CEV within gill epithelial cells. Amplification and sequencing of the CEV partial 4a gene sequence from the gill revealed this to belong to genogroup I, closely related to European viral strains associated with pond-farmed carp for food. Though this case marks the first documentation of CEV in a wild carp population in North America, it is possible that it was previously overlooked due to the lack of available diagnostic tests for this virus. The finding of CEV in wild carp emphasizes the need for strict biosecurity for hobby and commercial koi operators as transmission could be perpetuated through a "spillover and spillback" mechanism between wild carp and the commercial koi trade. Following the mortality in Mill Pond a follow-up survey was conducted in October 2017 to determine if the virus persisted in the surviving population. A total of 31 adult fish were captured by electrofishing and lethally sampled to screen their gills for CEV using a specific quantitative real-time PCR assay. The testing did not detect the virus in any of these collected samples, supporting that the virus is unlikely to persist in the gills following an epizootic, as has been previously reported.

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Phylogenomic Characterization of Ranaviruses Detected in Fish and Amphibians in Thailand

Preeyanan Sriwanayos[1,2*], Kuttichantran Subramaniam[1], Natalie K Stilwell[1], Kamonchai Imnoi[1], Jaree Polchana[2], Somkiat Kanchanakhan[3] and Thomas B Waltzek[1]

[1] Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32608 mameow@ufl.edu
[2] Aquatic Animal Health Research and Development Division, Department of Fisheries, Bangkok, Thailand 10900 jpolchana@gmail.com
[3] Chonburi Provincial Fishery Office, Department of Fisheries, Chonburi, Thailand 20000 kanchanakhan@yahoo.com

Ranaviruses are emerging pathogens associated with epizootics in farmed and wild poikilothermic vertebrates (e.g. fish, amphibians, and reptiles) worldwide over the past two decades. In this study, we describe the full genomes of seven ranaviruses, each isolated from one of the following species: marbled sleeper goby (*Oxyeleotris marmorata*); goldfish (*Carassius auratus*); guppy (*Poecilia reticulata*); tiger frog (*Hoplobatrachus tigerinus*); Asian grass frog (*Fejervarya limnocharis*); and two from East Asian bullfrog (*H. rugulosus*) in Thailand. The full genomes of the fish and amphibian isolates were sequenced using an Illumina MiSeq sequencer. The nucleotide (nt) sequences of the major capsid protein (MCP) from the Thai isolates compared to a Chinese isolate from tiger frog were highly similar (99.8-100% nt identity). Comparison of the MCP sequences from the seven Thai isolates to 22 other fully sequenced ranaviruses, recovered from Genbank, displayed a lower nt sequence identity ranging from 93.1-98.9%. Phylogenomic analysis based on the concatenated locally collinear blocks alignment, generated using Mauve 2.4, for 29 fully sequenced ranaviruses revealed that these eight Asian isolates, including the Chinese isolate, formed a well-supported monophyletic group referred to as tiger frog virus (TFV) clade. Our findings confirm the international movement of TFVs among Asian cultured fish and amphibians. Biosecurity measures are needed to ensure TFV does not continue to spread throughout Southeast Asia and between this region and other parts of the world.

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Phylogenomic Characterization of Acipenserid Herpesvirus 1 in Lake Sturgeon (*Acipenser fulvescens*)

Logan Walker[1]*, Kuttichantran Subramaniam[2], Susan Marcquenski[3], Danielle Godard[3], Ryan Koenigs[4], Elizabeth Kieran[5], Salvatore Frasca[5], Jr., Karen Kerr[6], Andrew J. Davison[6], and Thomas B. Waltzek[2]

[1] Fisheries and Aquatic Sciences, College of Agricultural and Life Sciences, University of Florida, Gainesville, FL 32611, USA walkerlogan@ufl.edu
[2] Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA kuttichantran@ufl.edu, tbwaltzek@ufl.edu
[3] Bureau of Fisheries Management, Wisconsin Department of Natural Resources, Madison, WI 53707, USA smarcquenski@yahoo.com Danielle.Godard@wisconsin.gov
[4] Bureau of Fisheries Management, Wisconsin Department of Natural Resources, Oshkosh, WI 54901, USA Ryen.Kleiser@wisconsin.gov
[5] Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611 USA ekieran@ufl.edu, sfrasca@ufl.edu
[6] MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom karen.kerr@glasgow.ac.uk, andrew.davison@glasgow.ac.uk

Acipenserid herpesvirus 1 (AciHV1) was first isolated from moribund farmed juvenile white sturgeon (ws; *Acipenser transmontanus*) in California and later in Europe on an Italian farm rearing ws. Fish infected with this virus (AciHV1-ws) presented with focal white cutaneous plaques that upon histopathological examination revealed keratinocyte swelling and hyperplasia. In spring 2017, two wild, adult lake sturgeon (ls; *A. fulvescens*) captured from the Wolf River, WI, presented with cutaneous lesions similar to those previously reported in farmed ws in California and Europe. Biopsies were obtained for histopathologic evaluation and molecular diagnostic testing. Microscopic examination of the cutaneous lesions in these two ls revealed hyperplasia and hydropic change of keratinocytes consistent with previous cases of AciHV1-ws disease. A degenerate PCR targeting the DNA-dependent DNA polymerase (pol) of large DNA viruses generated the expected 500 bp amplicons from both skin samples. Sanger sequencing of the purified PCR products followed by BLAST analyses using the National Center for Biotechnology Information non-redundant nucleotide and protein databases confirmed the presence of an alloherpesvirus closely related to AciHV1-ws in both ls samples (AciHV1-ls). A DNA library was prepared from the DNA extracted from biopsied skin lesions and sequenced using a v3 chemistry 600 cycle kit on an Illumina MiSeq sequencer. The *de novo* assembly of 6,477,748 paired-end reads using the SPAdes genome assembler recovered a large Alloherpesvirus contig that was extended and joined to other contigs manually by PCR and Sanger sequencing, resulting in the complete AciHV1-ls genome sequence (201,788 bp). Maximum Likelihood phylogenetic analysis based on the concatenated amino acid alignments of the partial pol and exon two of the terminase (term) genes revealed that AciHV1-ls branches as the sister group to AciHV1-ws. The AciHV1-ls and AciHV1-ws amino acid sequences of the partial pol and term amino acid sequences were 93.1 and 100% identical, respectively. This study provides the first complete AciHV1 genome sequence and expands the host range of this virus to include lake sturgeon.

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Phylogenomic Characterization of Squamate Erythrocytic Iridoviruses

Kuttichantran Subramaniam[1*], Thomas Waltzek[1], Nicole Stacy[2], Claire Grosset[3], and James Wellehan[2]

[1] Department of Infectious Disease and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA kuttichantran@ufl.edu, tbwaltzek@ufl.edu

[2] Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA stacyn@ufl.edu, wellehanj@ufl.edu

[3] Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, and the California Animal Health and Food Safety Laboratory, Davis, University of California, Davis, CA 95616, USA, (present address: Zoological Medicine Service, Faculté de medicine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC J2S 2M2, Canada claire.grosset@umontreal.ca

Erythrocytic iridoviruses (EIV) have been documented in squamates within the families Gekkonidae, Phyllodactylidae, Scincidae, Cordylidae, Lacertidae, Pythonidae, Colubridae, Viperidae, Varanidae, Iguanidae, Phrynosomatidae, Agamidae, and Chamaeleonidae. Interestingly, similar viral agents have also been reported in more than 20 species of anadromous and marine fishes throughout the Atlantic and Pacific Oceans, as well as amphibians. However, the phylogenetic relationship of these viruses to other iridoviruses remains unclear to date. In this study, we compared the light microscopic abnormalities of infected cells, the ultrastructural morphology and phylogenetic relationship of EIVs to other iridoviruses. Recently, EIVs were partially characterized in a wild Peninsula ribbon snake (*Thamnophis sauritus sackenii*) and captive bred inland bearded dragons (*Pogona vitticeps*). The Peninsula ribbon snake displayed two types of cytoplasmic inclusions in erythrocytes, polychromasia, anisocytosis, and hypochromasia, while the erythrocytes of the bearded dragon exhibited prominent blue-staining inclusions within normal appearing erythrocytes. Cytoplasmic inclusion bodies within erythrocytes of the Peninsula ribbon snake and bearded dragons examined by transmission electron microscopy revealed cytoplasmic icosahedral particles morphologically consistent with iridoviruses. The complete genome of the EIV from Peninsula ribbon snake (*Thamnophis sauritus sackenii*; TsEIV) comprises 111,413 bp nucleotides which encodes 115 potential open reading frames. Maximum Likelihood phylogenetic analysis based on 19 conserved genes revealed the squamate EIVs form a well-supported clade distinct from other established iridovirus genera, and likely represent founding members of a novel genus. We propose the genus Hemocytivirus for this new clade of iridoviruses to reflect their predilection for red blood cells.

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Genomic Characterization of Percid Herpesvirus 1 Associated with Epidermal Hyperplasia in Walleye (*Sander vitreus*)

Jaime A. Haggard[1]*, Kuttichantran Subramaniam[2], Vsevolod L. Popov[3], Thomas B. Waltzek[2]

[1] College of Agricultural and Life Sciences, University of Florida, Gainesville, FL 320611, USA j.haggard@ufl.edu
[2] Department of Infectious Disease and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611, USA kuttichantran@ufl.edu tbwaltzek@ufl.edu
[3] Department of Pathology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555 USA vpopov@UTMB.edu

Percid herpesvirus 1 (PeHV1), known informally as walleye herpesvirus, was first reported in walleye (*Sander vitreus*) in 1971 during a spawning event in the Bad Carrot River, Canada and subsequently, in the Northern United States. Infected adults displayed cutaneous whitish plaques during the spring spawning season. Genetic data confirming PeHV1 as a member of the family *Alloherpesviridae* (i.e. fish and amphibian herpesviruses) is lacking. In this study, a Canadian PeHV1 isolate was propagated on the walleye ovary (WO) cell line and infected WO cells were examined by transmission electron microscopy. As expected for a herpesvirus, enveloped virus particles with hexagonal nucleocapsids were observed within the cytoplasm of infected WO cells. DNA was extracted from infected WO cell culture supernatant and used to build a DNA library for sequencing on an Illumina MiSeq sequencer. The 13,099,218 paired-end reads were assembled *de novo* in SPAdes resulting in two herpesviral contigs that were joined manually by PCR and Sanger sequencing. The complete PeHV1 genome was determined to be 127,290 bp encoding 86 putative proteins including those conserved in all fish herpesviruses. Maximum Likelihood phylogenetic analysis based on the concatenated partial DNA-dependent DNA polymerase (pol) and second exon of the terminase (term) gene sequences (249 amino acid characters including gaps) revealed PeHV1 forms a novel branch between the alloherpesvirus genera *Ictalurivirus* and *Salmonivirus*. The genetic analysis of the partial PeHV1 pol (151 amino acid characters including gaps) and term (98 amino acid characters including gaps) sequences ranged from 34.6-72% and 35.9-77.2% identities to other alloherpesviruses, respectively. Our study provides the first sequence data supporting PeHV1 as a novel species in the family *Alloherpesviridae*. Challenge studies are planned to confirm PeHV1 is the causative agent of the observed cutaneous disease in adult walleye.

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Genomic Characterization of the First Fish Bunyaviruses through Next-Generation Sequencing

Thomas B. Waltzek[1,*], Kuttichantran Subramaniam[1], William N. Batts[2], James R. Winton[2], Nicholas B.D. Phelps[3], Sunil K. Mor[3], Sagar M. Goyal[3], Eric Leis[4], Corey Puzach[4], Rebecca McCann[4], Terry F.F. Ng[5,6], Eric Delwart[5,6], Susan V. Marcquenski[7], Marisa Barbknecht[8], Kelly Rock[8], Tony Goldberg[9], Michael A. Hoffman[8]

[1] Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA tbwaltzek@ufl.edu, kuttichantran@ufl.edu
[2] US Geological Survey, Western Fisheries Research Center, Seattle, WA 98115 bbatts@usgs.gov, jwinton@usgs.gov,
[3] Minnesota Veterinary Diagnostic Laboratory, St. Paul, MN 55108, USA phelp083@umn.edu, kumars@umn.edu, goyal001@umn.edu
[4] US Fish and Wildlife Service, La Crosse Fish Health Center, Onalaska, WI 54650 eric_leis@fws.gov, corey_puzach@fws.gov, Rebekah_McCann@fws.gov
[5] Blood Systems Research Institute, San Francisco, CA 94118, USA ylz9@cdc.gov, Eric.Delwart@ucsf.edu
[6] Department of Laboratory Medicine, University of California, San Francisco, CA 94143
[7] Wisconsin Department of Natural Resources, Madison, WI 53707, USA smarcquenski@yahoo.com
[8] Department of Microbiology, University of Wisconsin–La Crosse, La Crosse, WI 54601, USA mbarbknecht@uwlax.edu, mhoffman@uwlax.edu
[9] Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA tony.goldberg@wisc.edu

Members of the family *Bunyaviridae* are a diverse assemblage of negative-sense single-stranded RNA viruses. Bunyaviruses (BVs) typically replicate alternatively in arthropods and vertebrates with disease most often observed only in the vertebrate host. The family includes five genera: orthobunyaviruses vectored through mosquitoes, ticks, and flies; nairoviruses and phleboviruses vectored through ticks; tospoviruses vectored through thrips; and hantaviruses vectored through rodents. Here we report the first genomic characterization of piscine BVs isolated from goldfish, largemouth bass, and white sucker. Phylogenetic analyses revealed piscine BVs represent a new branch within the family. Future studies are planned to understand the clinical significance of these piscine BVs.

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