A Novel Pegivirus in Indian River Bottlenose Dolphin

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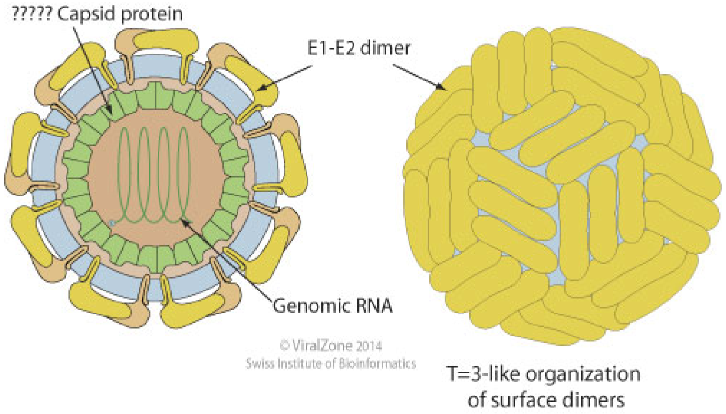
Talk Outline

- **Indian River Lagoon (IRL) – Overview**
- **Introduction to the HERA project**
- **Discovery of Novel *Pegivirus* in Bottlenose Dolphin**
- **Genetic Characterization of Bottlenose Dolphin Pegivirus**
- **Future Directions**



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The Indian River Lagoon (IRL)

- An estuary of National Significance
- 40% of Florida's East Coast
- Most Bio Diverse Estuary in U.S. (~ 4,000 spp.)
- Commercial & recreational activities support ~ 19,000 jobs & generate > $250 m in annual income

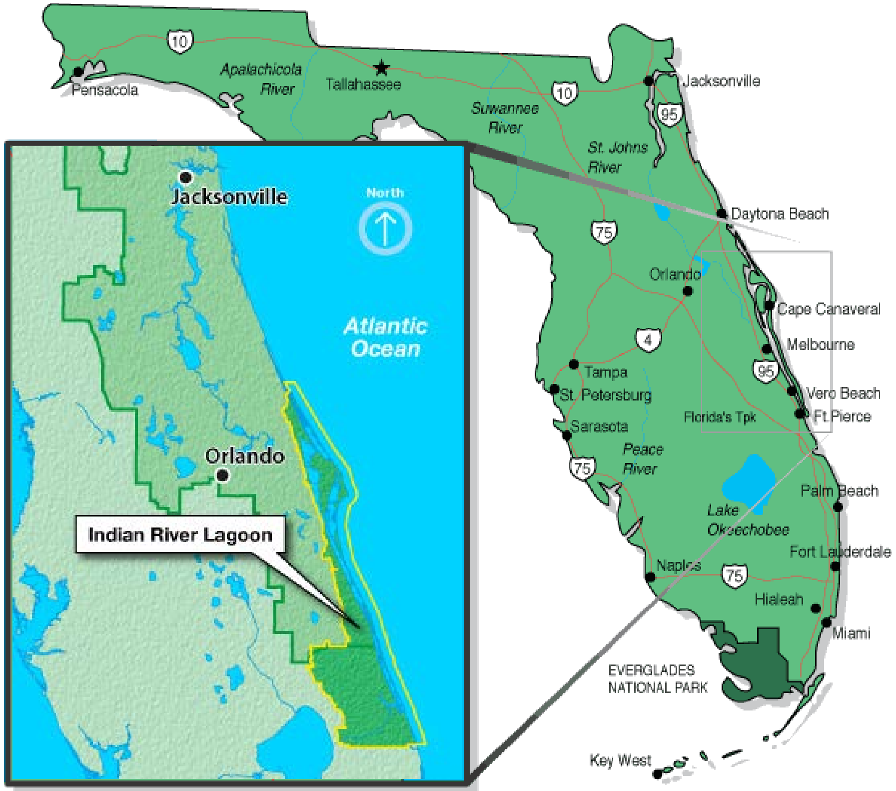


Image credit: St. Johns River Water Management District & UCF

IRL – Agriculture Based

- Rapid Development and Population Increase:
    - Influx of pollutants from water runoff
    - Increasing levels of copper and mercury
- Key Concerns - loss of seagrass meadows, oyster beds, mangrove habitat, toxic algal bloom, water quality, and fish kills

Image credit: Indian Riverkeeper (©Jacqui Thurlow-Lippisch)

Image credit: TCPALM

The Dolphin Health and Environmental Risk Assessment Program (HERA)

Collaborative, multi-disciplinary and integrated research program designed to access environmental and anthropogenic stressors, and determine the health and long-term viability of Atlantic Bottlenose dolphin populations

HERA Research Projects

- **Surveillance for Emerging Infectious Diseases**
- **Assess Immune Status of IRL Dolphins**
- **Antibiotic Resistance Patterns and Exposure Sources**
- **Molecular Genetic Studies / Disease**
- **Health Effects of Mercury, PCB, and Organochlorine Exposure**
- **2012 - 2013 UF WAVDL joins HERA!**

July 2012 IRL Sampling

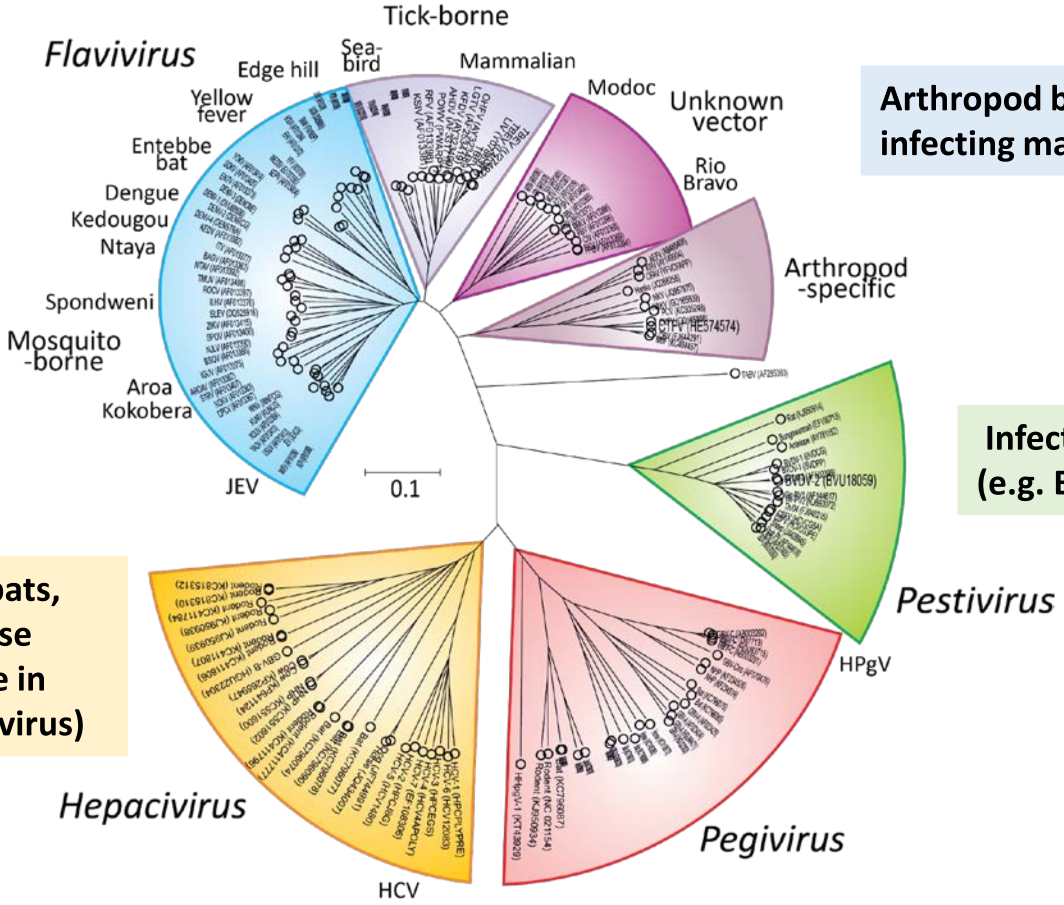
- 12 Dolphins Sampled
  - Blood (buffy)
  - Fecal
  - Blowhole
- UF WAVDL Virus Discovery
  - Samples processed for next generation sequencing (NGS)
  - Samples frozen back for virus isolation

Discovery of a Novel *Pegivirus* in Bottlenose Dolphin

- **RNA from serum, fecal, and blowhole were extracted using Qiagen kit**
- **A cDNA library was generated & sequenced using a v3 chemistry 600-cycle kit on a MiSeq platform (Illumina)**
- ***De novo* assembly was performed in CLC Genomics, SPAdes, & Velvet**
- **Blastx searches of the assembled data retrieved near complete genome of a novel *Pegivirus* (Family *Flaviviridae*) from serum samples**

Near Complete Genome

Family *Flaviviridae*



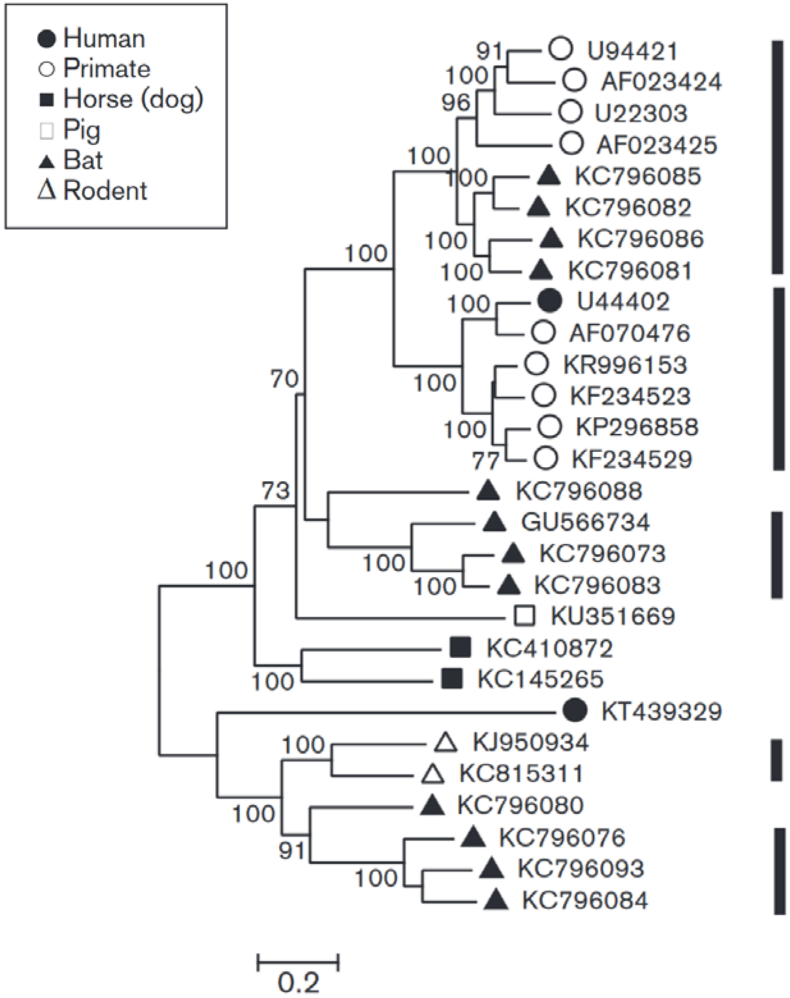
Arthropod borne viruses infecting mammals and birds

Infect pigs and ruminants (e.g. Bovine Viral Diarrhea)

Infect horses, rodents, bats, cows and primates. Cause progressive liver disease in human (e.g. hepatitis C virus)

Image source: https://talk.ictvonline.org

Genus *Pegivirus*



A **New World monkeys & African bats**

C **Old World primates**

I **Old & New World bats**

B **Asian & African bats**

K **Pigs**

E **Horse**

D **Horse**

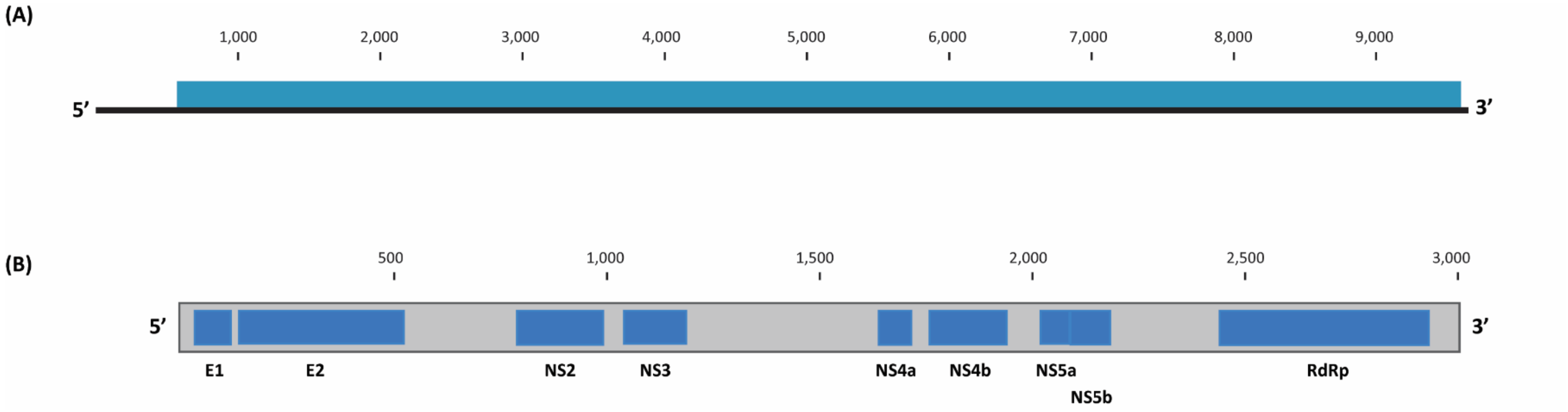
H **Primates**

J **Rodents**

F **Old & New World bats**

G **Old & New World bats**

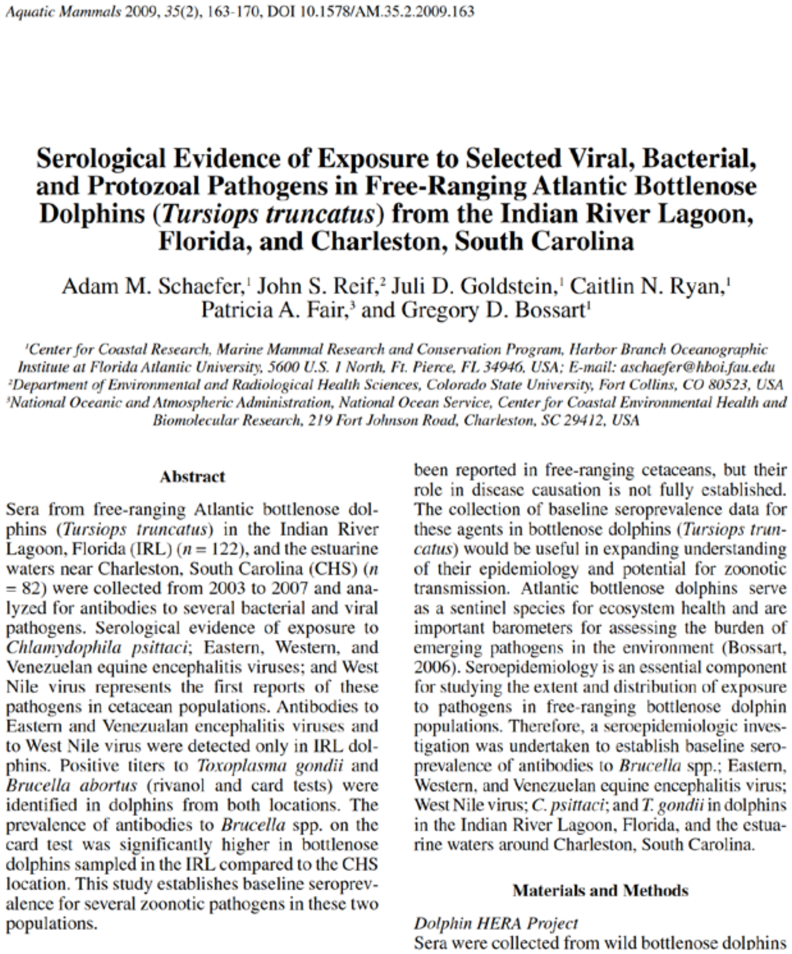
Dolphin pegivirus (DPgV)



- **The genome is 9,649 bp and encode a polyprotein which is cleaved by viral and cellular proteases into structural and non structural proteins**

Serological Evidence of Flavivirus in IRL Dolphin

- **Sera from 122 IRL dolphins were collected from 2003-2007**
- **Analyzed for antibodies to several bacterial and viral pathogens**
- **Seropositive to West Nile virus (flavivirus)**



Aquatic Mammals 2009, *35*(2), 163-170, DOI 10.1578/AM.35.2.2009.163

Serological Evidence of Exposure to Selected Viral, Bacterial, and Protozoal Pathogens in Free-Ranging Atlantic Bottlenose Dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida, and Charleston, South Carolina

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Abstract

Sera from free-ranging Atlantic bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida (IRL) ($n = 122$), and the estuarine waters near Charleston, South Carolina (CHS) ($n = 82$) were collected from 2003 to 2007 and analyzed for antibodies to several bacterial and viral pathogens. Serological evidence of exposure to *Chlamydophila psittaci*; Eastern, Western, and Venezuelan equine encephalitis viruses; and West Nile virus represents the first reports of these pathogens in cetacean populations. Antibodies to Eastern and Venezualan encephalitis viruses and to West Nile virus were detected only in IRL dolphins. Positive titers to *Toxoplasma gondii* and *Brucella abortus* (rivanol and card tests) were identified in dolphins from both locations. The prevalence of antibodies to *Brucella* spp. on the card test was significantly higher in bottlenose dolphins sampled in the IRL compared to the CHS location. This study establishes baseline seroprevalence for several zoonotic pathogens in these two populations.

been reported in free-ranging cetaceans, but their role in disease causation is not fully established. The collection of baseline seroprevalence data for these agents in bottlenose dolphins (*Tursiops truncatus*) would be useful in expanding understanding of their epidemiology and potential for zoonotic transmission. Atlantic bottlenose dolphins serve as a sentinel species for ecosystem health and are important barometers for assessing the burden of emerging pathogens in the environment (Bossart, 2006). Seroepidemiology is an essential component for studying the extent and distribution of exposure to pathogens in free-ranging bottlenose dolphin populations. Therefore, a seroepidemiologic investigation was undertaken to establish baseline seroprevalence of antibodies to *Brucella* spp.; Eastern, Western, and Venezuelan equine encephalitis virus; West Nile virus; *C. psittaci*; and *T. gondii* in dolphins in the Indian River Lagoon, Florida, and the estuarine waters around Charleston, South Carolina.

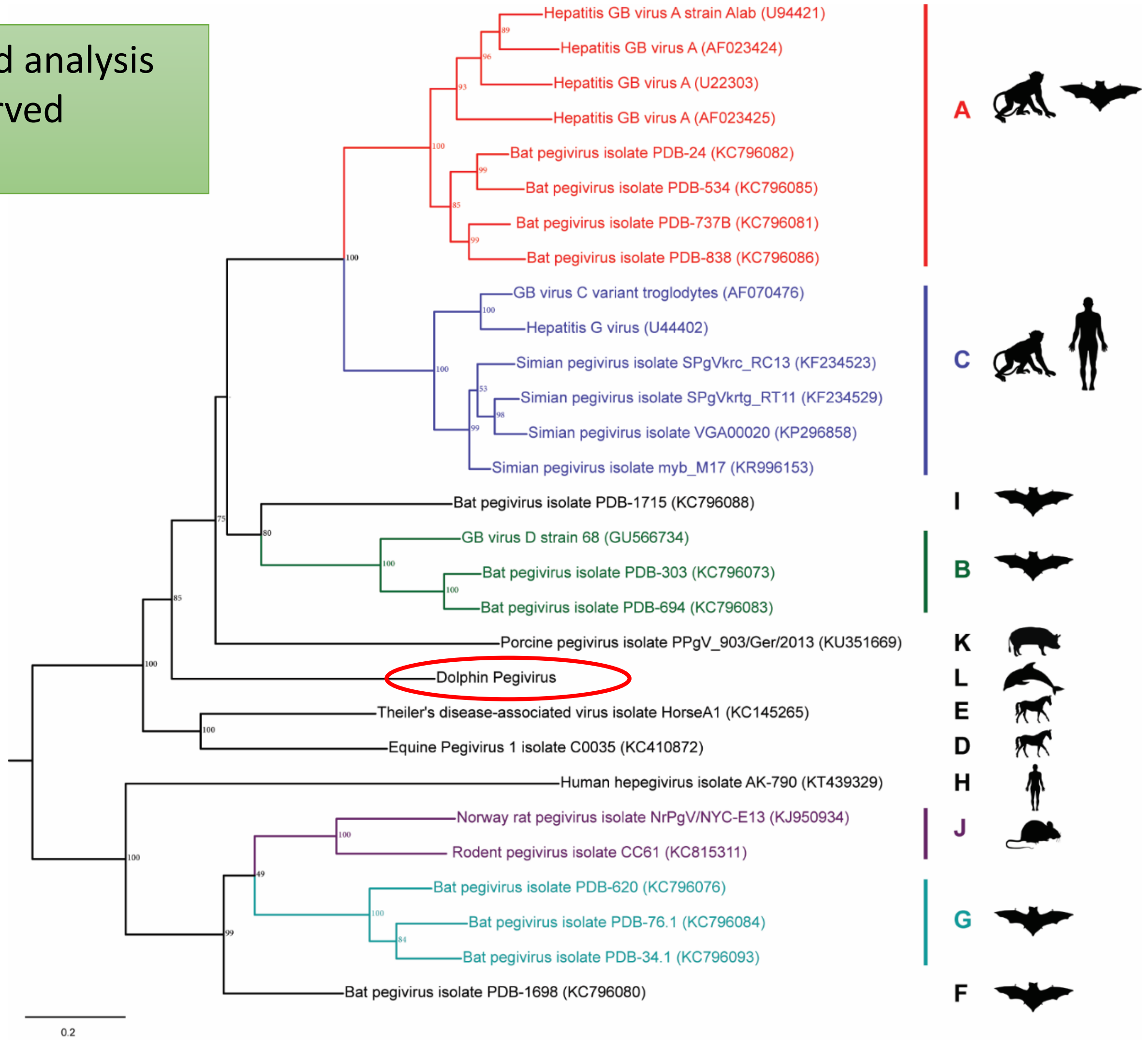
Materials and Methods

Dolphin HERA Project

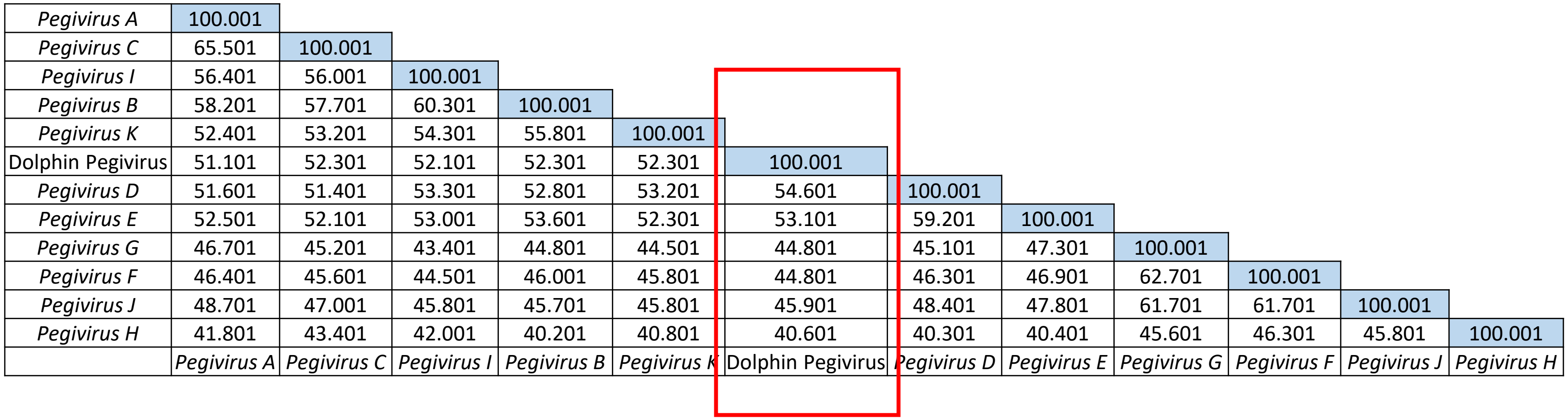
Sera were collected from wild bottlenose dolphins

Phylogenetic Analysis

Maximum Likelihood analysis based on the conserved domains in IQ-TREE



Phenetic Analysis



Pegivirus A	100.001											
Pegivirus C	65.501	100.001										
Pegivirus I	56.401	56.001	100.001									
Pegivirus B	58.201	57.701	60.301	100.001								
Pegivirus K	52.401	53.201	54.301	55.801	100.001							
Dolphin Pegivirus	51.101	52.301	52.101	52.301	52.301	100.001						
Pegivirus D	51.601	51.401	53.301	52.801	53.201	54.601	100.001					
Pegivirus E	52.501	52.101	53.001	53.601	52.301	53.101	59.201	100.001				
Pegivirus G	46.701	45.201	43.401	44.801	44.501	44.801	45.101	47.301	100.001			
Pegivirus F	46.401	45.601	44.501	46.001	45.801	44.801	46.301	46.901	62.701	100.001		
Pegivirus J	48.701	47.001	45.801	45.701	45.801	45.901	48.401	47.801	61.701	61.701	100.001	
Pegivirus H	41.801	43.401	42.001	40.201	40.801	40.601	40.301	40.401	45.601	46.301	45.801	100.001
	Pegivirus A	*Pegivirus C*	*Pegivirus I*	*Pegivirus B*	*Pegivirus K*	Dolphin Pegivirus	*Pegivirus D*	*Pegivirus E*	*Pegivirus G*	*Pegivirus F*	*Pegivirus J*	*Pegivirus H*

- **Comparison of conserved domains of DPgV to other pegiviruses showed sequence identity ranged from 40.6 – 54.6%**

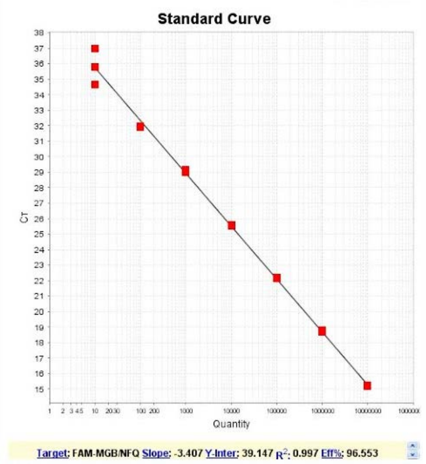
- **The ICTV suggests a species demarcation threshold of <69%**

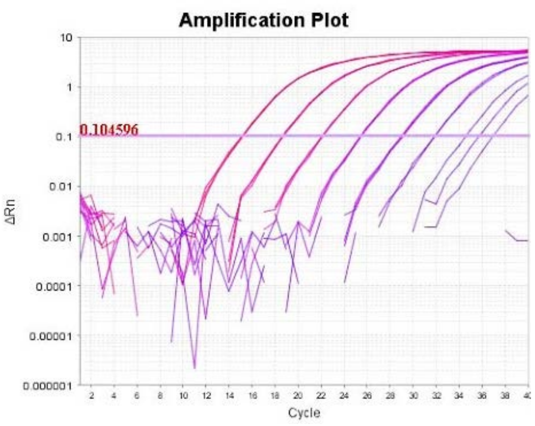
- **DPgV is the first pegivirus described from marine mammal & propose to be accepted as Pegivirus L within the genus *Pegivirus***

Future Directions

- **Complete the genome sequence by RACE**

- **Development & validation of molecular tools**
  - **RT-qPCR**
  - ***In situ* hybridization**





Acknowledgements

GEORGIA AQUARIUM

HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY

Colorado State University
COLLEGE OF VETERINARY MEDICINE
AND BIOMEDICAL SCIENCES

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THANK YOU FOR THE ATTENTION!

ANY QUESTIONS?