#### Characterization of a Novel Circovirus from a Stranded Longman's Beaked Whale (*Indopacetus pacificus*)

#### Nelmarie Landrau Giovannetti, Kuttichantran Subramaniam, Terry Fei Fan Ng, David S. Rotstein, Kristi West, and Thomas B. Waltzek









## **Talk Outline**

- Introduction to the family *Circoviridae* 
  - Biology
  - Clinical disease
  - Taxonomy
- Objective
- Stranded Hawaiian Longman's beaked whale
  - Clinical case history
  - Viral genomic characterization
    - Beaked whale circovirus (BWCV) genome annotation
    - Phylogenetic analysis
    - Sequence identity matrix
    - BWCV diagnostic PCR
- Conclusions/Future Directions





# **Biology of Circoviridae**

- Circular, ssDNA
  - 1.8-2 kbp
- Non-enveloped nucleocapsid with icosahedral symmetry
  - 17 nm diameter



Swiss Institute of Bloinformatics

T=1

- 2 genes: capsid and replicationassociated protein
  - Rolling cycle replication

#### **Clinical Disease of Porcine Circovirus 2 (PCV2)**

- Associated with the occurrence of postweaning multisystemic wasting syndrome (PMWS) in pigs between 6-18 weeks:
  - Gross pathology
    - Poor body condition
  - Microscopic pathology
    - Lymphoid depletion
    - Cytoplasmic botryoid inclusions
  - Disease outcome
    - Immunocompromised
    - Secondary infection lead to increased mortality







#### Niederwerder et al., 2015

# **Taxonomy of the family** *Circoviridae*

- Genus: Cyclovirus (43 species)
  - 16 bat cycloviruses
  - -9 invertebrate cycloviruses
  - 7 mammalian cycloviruses
  - 11 human cycloviruses









# **Taxonomy of the family** *Circoviridae*

- Genus: *Circovirus* (27 species)
  - 2 fish circoviruses (Barbel circovirus 1<sup>st</sup> circo reported in fish)
  - 8 bat circoviruses
  - 11 avian circoviruses (beaked and feather disease virus)
  - 5 mammalian circoviruses (porcine circovirus 1-3)







# Objective

• To characterize a novel circovirus from a stranded Longman's beaked whale



#### Stranded Hawaiian Longman's Beaked Whale



- Gross pathology
  - Broken jaw
  - Cookie cutter shark bites
- Microscopic pathology
  - Mild lymphocyte depletion
    - Immunocompromised
  - Cerebral encephalitis
  - Pulmonary edema
- Co-infection of an alphaherpesvirus & beaked whale morbillivirus (BWMV)

Jacob et al. 2016; West et al. 2013

## Viral Genomic Characterization

- Illumina MiSeq Next Generation Sequencer (NGS)
  Beaked whale circovirus (BWCV) was discovered
- Phylogenetic analysis IQ tree software
- Sequence identity matrix Sequence Demarcation Tool v1.2
- Development of specific BWCV conventional PCR



# **BWCV Genome Annotation**

- 2,021 bp, GC content of 46.1%
- Has the nine conserved nonanucleotide (5'- TAGTATTAC -3')



# CV Tree (replication gene)

- ML phylogenetic analysis
- Rep AA alignment (351 aligned characters)
- 1000 bootstraps



## **CV Tree** (replication gene)

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### **Circovirus Sequence Identity Matrix**

Pairwise identity (%)

Genus Circovirus – full genome nt alignment (2094 aligned characters)

																											- 100
>AF418552_GoCV	100.0																										- 95
>EU056309_SwCV	73.4	100.0																									
>AY228555_DuCV	69.1	67.5	100.0																							-	
>AF027217_PCV2	56.5	55.8	53.4	100.0																							-85
>AB277746_BFDV	55.7	56.9	53.8	77.8	100.0																						
>KC339249_BataCV2	55.2	55.1	54.5	63.5	65.1	100.0																				- H	
>JX863737_BatACV1	55.4	52.2	53.3	57.0	57.0	58.9	100.0																				- 76
>KJ020099_MiCV	54.5	55.9	53.2	58.1	57.1	56.5	66.5	100.0																			
>JQ814849_BatACV3	54.1	53.5	54.7	56.0	55.8	55.4	55.0	59.3	100.0																	-	- 71
>KT783484_BatACV4	54.8	52.0	55.6	54.6	56.5	53.6	56.2	57.0	74.7	100.0																	-66
>KJ641711_BatACV8	54.5	53.2	54.5	55.6	54.8	53.4	56.2	54.1	56.9	54.7	100.0																
>KJ641727_BatACV5	55.0	53.1	52.7	53.0	56.4	54.0	55.6	55.7	56.9	54.8	69.3	100.0															61
>KJ641723_BatACV7	53.3	51.6	51.3	52.4	53.1	53.0	56.0	53.8	55.0	54.5	60.8	61.3	100.0														- 56
>KJ641724_BataCV6	53.9	52.6	55.1	52.3	54.1	55.3	57.2	54.2	56.8	56.1	61.1	61.6	64.1	100.0													
>AF012107_PCV1	54.3	56.4	51.3	54.3	54.4	54.5	55.7	55.0	52.5	52.7	53.9	53.7	53.7	51.6	100.0												
>DQ172906_StCV	52.5	54.8	54.1	55.0	53.9	56.0	53.0	56.4	55.4	55.8	55.0	55.7	53.4	54.5	65.6	100.0											
>GQ404851_ChimpACV	54.8	54.2	52.4	53.5	54.2	51.8	53.1	51.6	53.8	53.9	54.4	56.7	54.4	54.3	66.2	68.0	100.0										
>AI301633_CaCV	53.1	52.5	51.1	54.1	53.6	53.5	54.5	52.8	54.2	54.7	53.4	54.3	53.0	54.7	59.9	64.7	62.6	100.0									
>DQ146997_RaCV	54.0	55.0	53.2	54.5	54.3	53.5	56.1	52.4	55.5	56.9	55.5	55.3	54.9	53.4	62.9	64.3	62.7	67.8	100.0								
>DQ845075_FICV	52.9	51.8	54.3	55.7	53.6	54.7	54.5	53.4	54.6	55.0	54.0	54.8	54.2	54.2	64.0	65.3	62.6	65.5	65.1	100.0							
>KP793918_ZfiCV	55.1	55.5	53.5	53.2	53.0	53.7	53.4	53.7	54.3	54.5	55.4	55.0	52.9	56.6	62.4	63.7	65.6	64.8	64.8	78.2	100.0						
>DQ845074_GuCV	52.3	53.2	52.5	53.6	54.7	54.8	51.8	55.1	55.7	55.5	54.6	53.5	53.1	52.6	60.3	60.2	58.4	62.1	59.8	61.9	60.5	100.0					
>AF252610_PICV	54.7	54.9	52.2	54.1	54.9	54.5	54.7	54.5	54.1	54.7	54.8	53.6	53.0	53.7	58.9	60.2	57.8	60.6	59.8	60.4	60.6	56.5	100.0				
>GQ404856_HuACV	51.7	52.4	53.0	52.3	53.8	53.6	56.2	54.7	54.2	55.0	53.7	55.5	52.7	53.6	55.0	52.7	51.9	54.4	52.6	54.6	52.3	54.4	54.8	100.0			
>GU799606_BarCV	55.6	53.7	52.1	51.7	52.8	54.0	53.0	54.1	53.5	54.0	53.5	53.7	52.6	52.4	52.8	54.9	53.0	52.1	52.3	54.1	52.8	54.4	52.6	58.2	100.0		1
>JQ011377_EcatfishCV	51.9	51.6	54.3	55.9	54.4	56.5	54.3	55.8	53.0	55.0	53.9	54.3	51.7	52.0	55.3	57.1	53.9	54.9	52.8	55.7	54.6	54.0	52.0	56.2	55.4	100.0	
>JO821392_CanineCV	54.5	55.2	51.9	52.9	53.8	54.6	56.7	55.0	54.5	53.2	53.2	54.4	54.2	54.8	55.5	52.9	53.7	54.6	52.7	54.6	53.0	53.7	54.0	54.1	535	54.7	100.0
>Beaked_whale	48.7	51.0	50.2	54.2	51.7	50.8	50.6	50.0	55.1	49.5	51.7	51.4	51.1	51.2	49.2	52.7	51.5	49.6	50.6	51.9	48.8	50.7	49.9	51.5	49.2	50.8	51.5 100.0

- X < 80% = new species
- Range of all CVs = 48.7-78.2%
- Range of all CVs to BWCV =  $48.7-54.2\% \rightarrow 1^{st}$  marine mammal CV!

# **BWCV Diagnostic PCR**

- 1. Lung
- 2. Cerebrum
- 3. Cerebellum
- 4. Scapular LN
- 5. Mediastinal LN



## **Conclusions/Future Directions**

- Our stranded Longman's beaked whale was co-infected with alphaherpesvirus, BWMV & BWCV
- This is the first description of a circovirus in a marine mammal
  BWCV can be considered a new species because X < 80%</li>
- The presence of the virus and its role in disease is yet to be proven
  - Positive PCR screening for BWCV on all tissues suggest a systemic infection and/or circulation in the blood
  - Future Direction:
    - *in situ* hybridization (ISH)



### References

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# Thank you! Questions?







