Characterization of potentially oncogenic viruses in bottlenose dolphin (*Tursiops truncatus*) tumor tissues

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Talk outline

- Introduction
  - Health and Environmental Risk Assessment (HERA) Project
  - Orogenital papillomas in bottlenose dolphins
  - Papillomaviruses (PV)
  - Herpesviruses (HV)
- Molecular viral diagnostic methods
  - PCR and NGS
- Novel PV and HV discovered
- Conclusions and future directions

HERA project

US National Marine Fisheries Service Scientific Research Permit Nos. 998-1678 and 14352

- From 2003-2015: **Health assessments in 360 dolphins** (Bossart et al. 2017)

  - Charleston, South Carolina (CHS)
  - Indian River Lagoon, Florida (IRL)

IRL BD capture for health assessment
Photo credit Georgia Aquarium/Addison Hill www.georgiaaquarium.org

Physical examination on a IRL BD
Photo credit Georgia Aquarium/Addison Hill www.georgiaaquarium.org

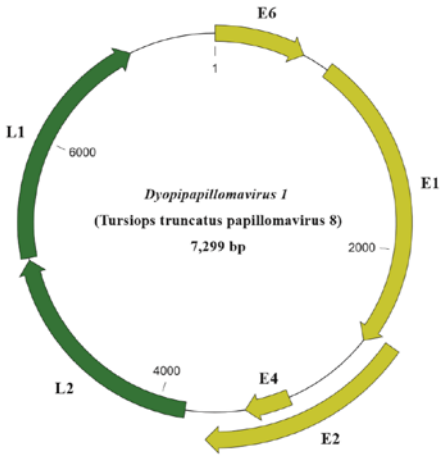
Orogenital papillomatosis

- Endemic disease in both populations (Bossart et al. 2017)
- Both sexes, self-limiting disease
- **Associated with papillomaviruses and herpesviruses**
- **Causal link remains to be demonstrated**

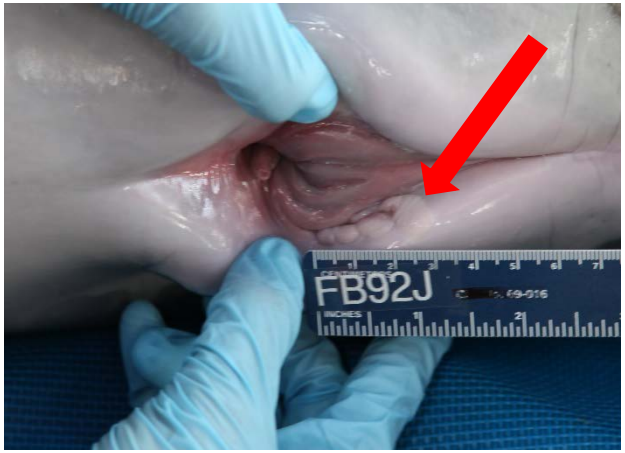
Multiple genital papillomas on the penis of an IRL BD (Bossart et al. Dis Aquatic Org 125: 141-153, 2017)

Family *Papillomaviridae*

- Circular double-stranded DNA
- Unenveloped spherical nucleocapsid
- 49 PV genera
  - *Omikronpapillomavirus*
  - *Upsilonpapillomavirus*
  - *Dyopipapillomavirus*
- Nine TtPV types
  - TtPV2 (first cetacean PV North America - Rehtanz et al. 2006)
  - TtPV8 (Cortés-Hinojosa et al. 2018 – in press)
  - TtPV9 (Rodrigues et al. 2018 – in press)



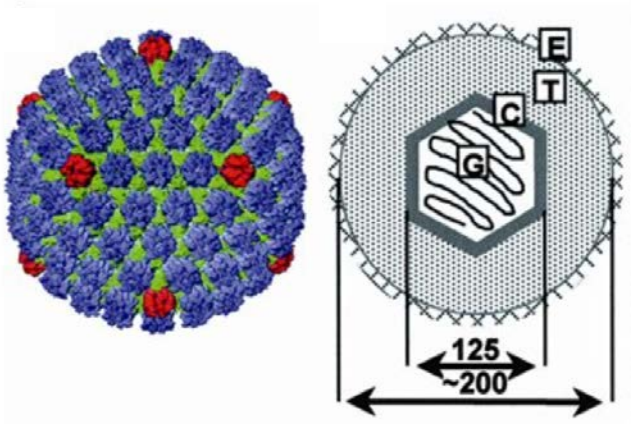
- **Proliferative lesions of the skin and mucosal membranes**



NOAA Fisheries Permit No 998-1678 Georgia Aquarium and Harbor Branch Oceanographic Institute

Family *Herpesviridae*

- Linear, double-stranded DNA
- Enveloped, icosahedral nucleocapsid
- Subfamilies *Alphaherpesvirinae* and *Gammaherpesvirinae*



*W. Chiu and H. Zhou; Zhou, Z.H., Dougherty, M., Jakana, J., He, J., Rixon, F.J. and Chiu, W. (2000). Seeing the herpesvirus capsid at 8.5 Å. Science, **288**, 877–880.*

- **Localized mucosal and cutaneous infections** (Limpscomb et al. 1996, Smolarek-Benson et al. 2006)
- **Fatal systemic infections** (Kennedy et al. 1992, Blanchard et al. 2001)

Genital lesions of two captive Atlantic bottlenose dolphins. Smolarek-Benson. Thesis, Master of Science, University of FLorida, 2005.

Genital tumors - herpesvirus

- Transmission electron microscopy: herpes-like particles (Bossart et al. 2005, Rehtanz et al. 2012)
- PCR and sequencing: partial sequences of DNA polymerase gene of a gammaherpesvirus provisionally named DeHV4 (Maness et al. 2011, Rehtanz et al. 2012)
- Partial sequences of DeHV4 reported in genital tumors in a managed and a Florida Keys stranded bottlenose dolphin (Smoralek-Benson et al. 2006)

Genital tumors of Atlantic bottlenose dolphins inhabiting the IRL, FL, USA associated with HV-infection
Rehtanz et al. Veterinary Microbiology 160, 297–304, 2012.

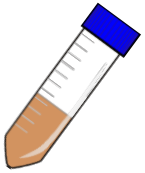
Objective

- Improve our understanding of potentially oncogenic viruses associated with tumor tissues in IRL bottlenose dolphins

- Tumor samples were screened for viral molecular approaches
  - PCR / Sanger sequencing (VanDevanter et al., 1996)
  - Next generation sequencing

Material and methods

- 5 genital tumor biopsies taken from IRL bottlenose dolphins
  - 4 ♂ and 1 ♀ captured between 2007 - 2012



DNA extraction (Qiagen)

Nested PCR herpesvirus (VanDevanter et al., 1996)

Sanger sequencing

NGS Nextera XT DNA Library Prep Kit

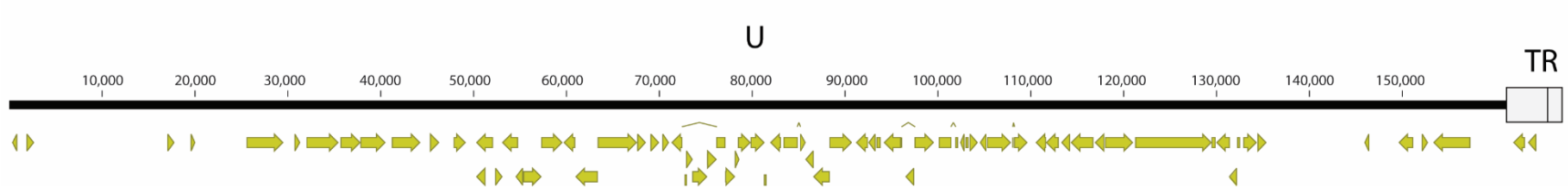
Illumina MiSeq Next Generation Sequencer

De novo assembly in SPAdes

Blastx against the GenBank non-redundant database

Discovery of a novel gammaherpesvirus

- **PCR and Sanger sequencing: 5/5 samples positive**
  - 100% identical to DeHV4 (Smoralek-Benson et al. 2006, Rehtanz et al. 2011)
- **NGS approach: 4/5 samples presented sequences of a novel gammaherpesvirus**
  - 166,210 bp genome, 72 ORFs
  - 99% identical to common bottlenose dolphin gammaherpesvirus 1 (strain Sarasota) (Davidson et al. 2017)



Common bottlenose dolphin gammaherpesvirus 1 (strain IRL)

Commom bottlenose dolphin gammaherpesvirus 1 (strain Sarasota)

- Proliferative rectal lesion, female bottlenose dolphin
- First full genome of herpesvirus from a marine mammal

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Genome Sequence of a Gammaherpesvirus from a Common Bottlenose Dolphin (*Tursiops truncatus*)

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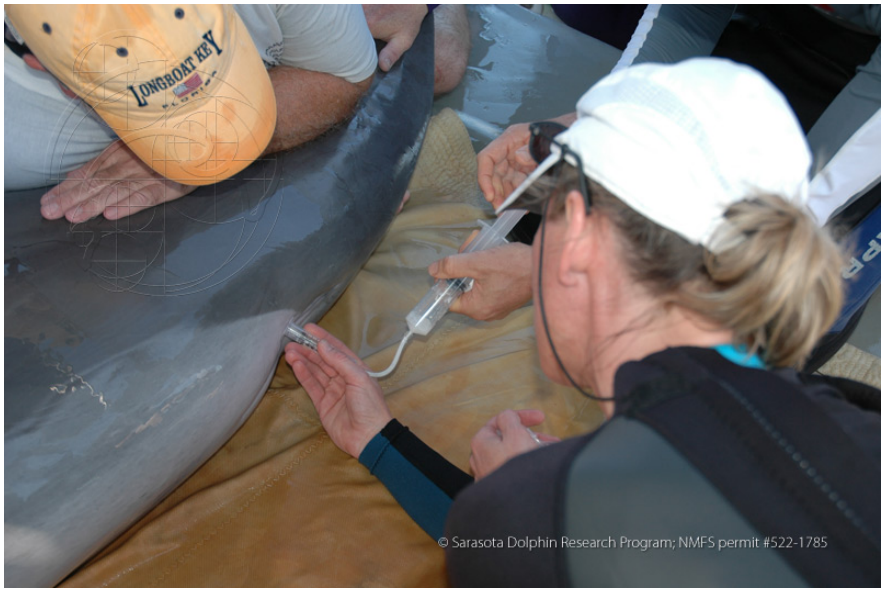
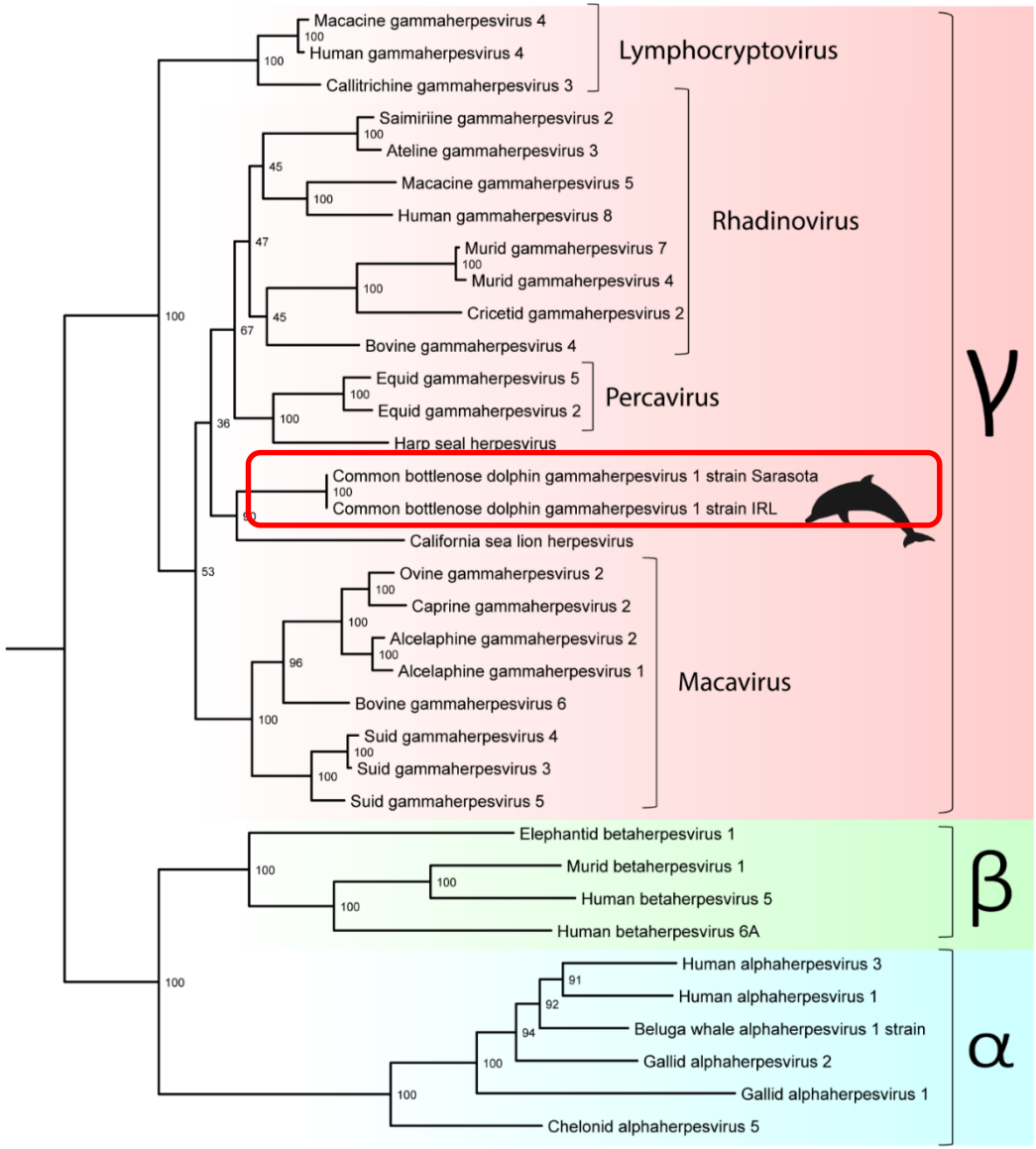


Sarasota Dolphin Research Program; NMFS permit #522-1785

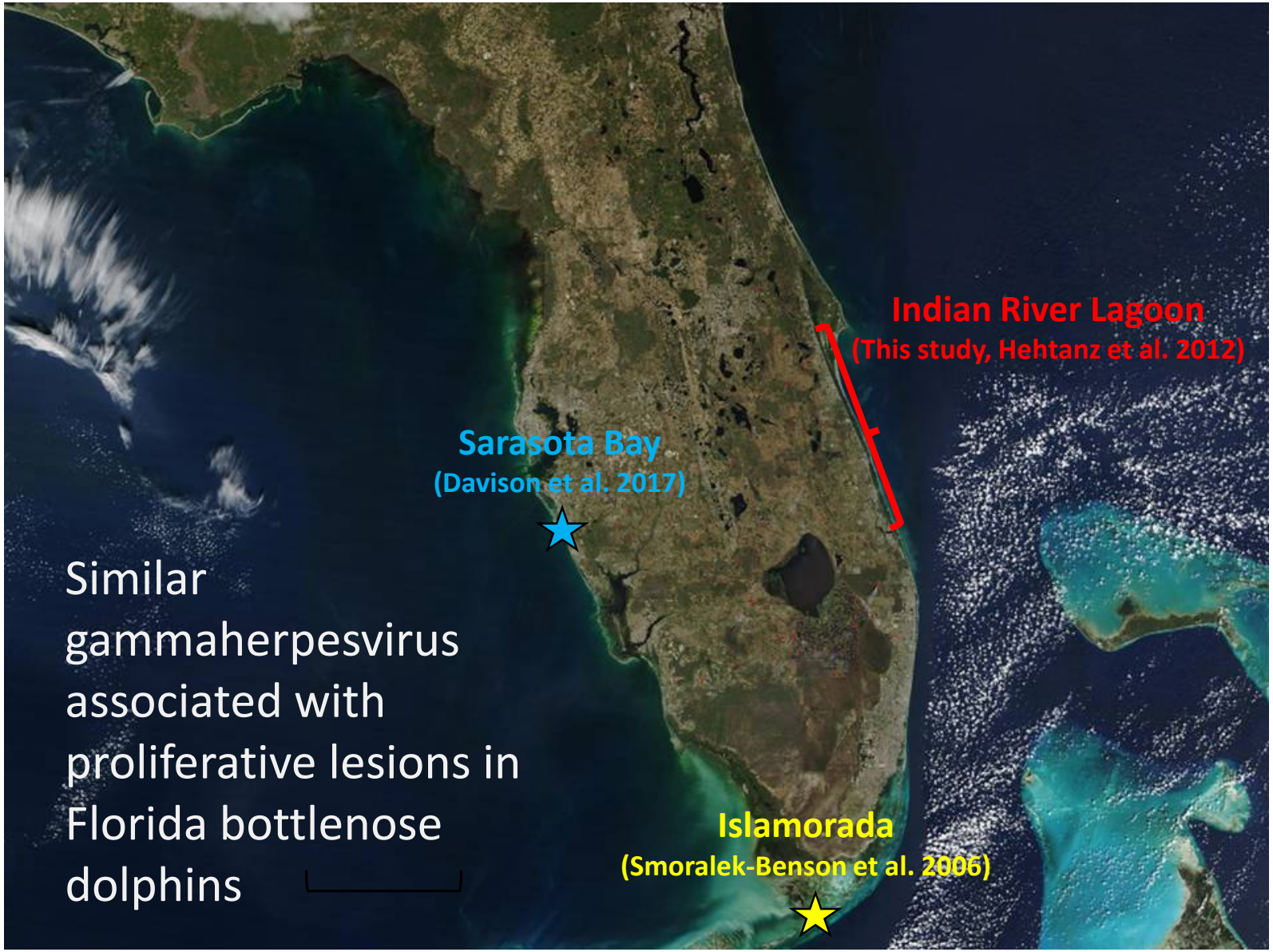
Delphind gammaherpesvirus 1

Phylogenetic analysis

- Maximum Likelihood
  - DNA polymerase gene
  - Amino acid alignment
  - 1000 bootstraps
- Clusters with common bottlenose dolphin gammaherpesvirus 1 (strain Sarasota)



Delphind gammaherpesvirus 1



Discovery of a novel papillomavirus

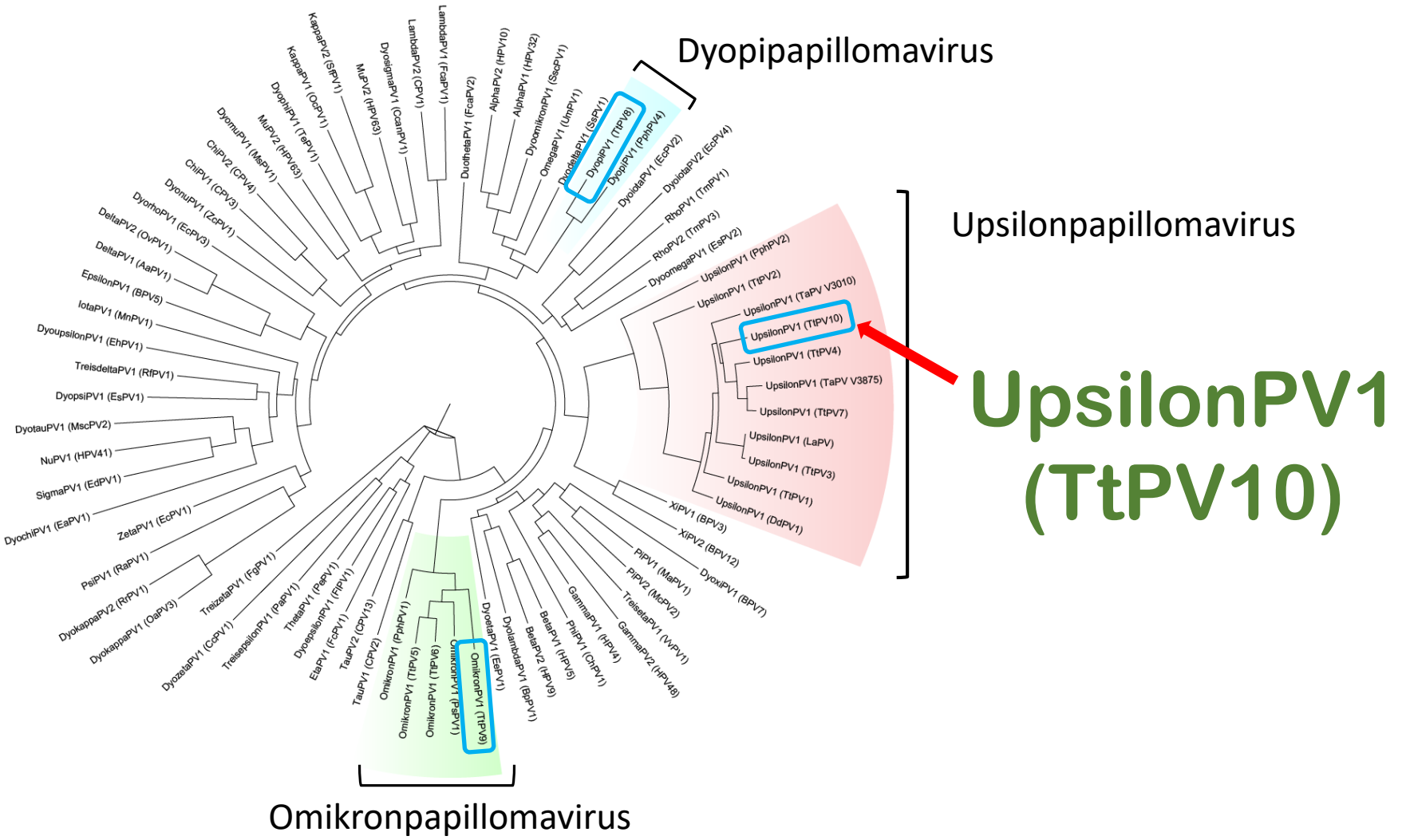
- 1/5 samples: 659 bp **partial genome of a novel UpsilonPV** by NGS approach
  - **Most closely related to UpsilonPV1 (TtPV4)**
    - Sequenced from a penile lesion in a stranded bottlenose dolphin (Robles-Sikisaka et al. 2012)
    - 84% nt identity of L1 gene

- PV Taxonomy:
  - Taxonomy based on genetic distance of L1
  - Pairwise nt identities 71-89%: different types

UpsilonPV1 (TtPV10)

Phylogenetic analysis

Maximum Likelihood - L1 gene - amino acid alignment - 1000 bootstraps



Conclusions and future directions

- Expansion of the number of potentially oncogenic viruses associated with genital tumors in bottlenose dolphins
- High prevalence in some free-ranging bottlenose dolphin populations
- Further research needed to confirm casual link between these viruses and the tumor tissues they were recovered from
- Potential health impacts in free-ranging and managed bottlenose dolphin populations

Acknowledgements

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THANK YOU!
ANY QUESTIONS?