# Mitogenome Organization, Diversity, and Evolutionary Relationships of Proteocephalidean Tapeworms (Cestoda, Onchoproteocephalidea) Unveiled by a Genome Skimming Approach

PVA, RJdS, DJ, WT, AH, GN & DJM

São Paulo State University & UNC Charlotte

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**Philippe Vieira Alves**, Reinaldo J da Silva, Daniel Janies, Willian Taylor, April Harris, Gari New, Denis Jacob Machado

Background
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Material & Methods
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Results
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Conclusion
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Acknowledgments
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# What are proteocephalids?

**Onchoproteocephalidea** Caira, Jensen, Waeschenbach, Olson et Littlewood, 2014
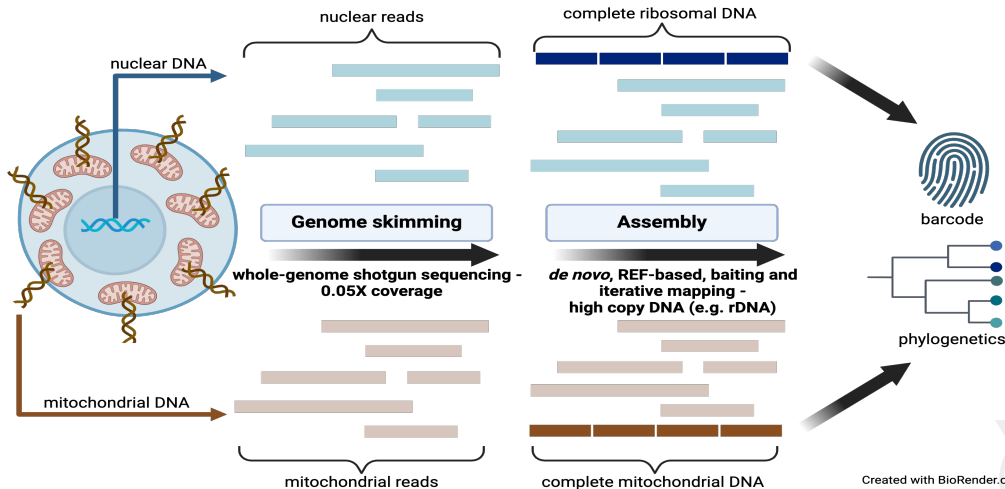
- Proteocephalidea + hook-bearing cestodes "Tetraphyllidea" (Onchobothriidae)
- Onchoproteocephalidea I and Onchoproteocephalidea II
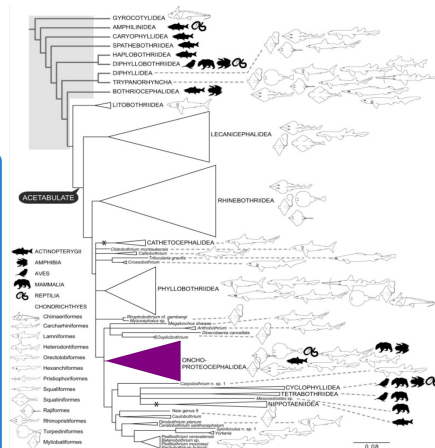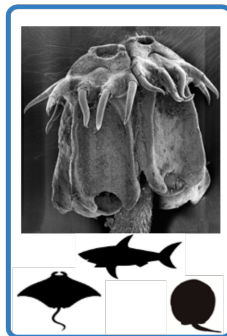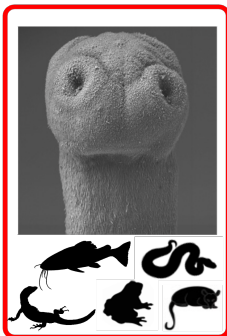


18S rRNA + 28S rRNA
**Caira et al. 2014**

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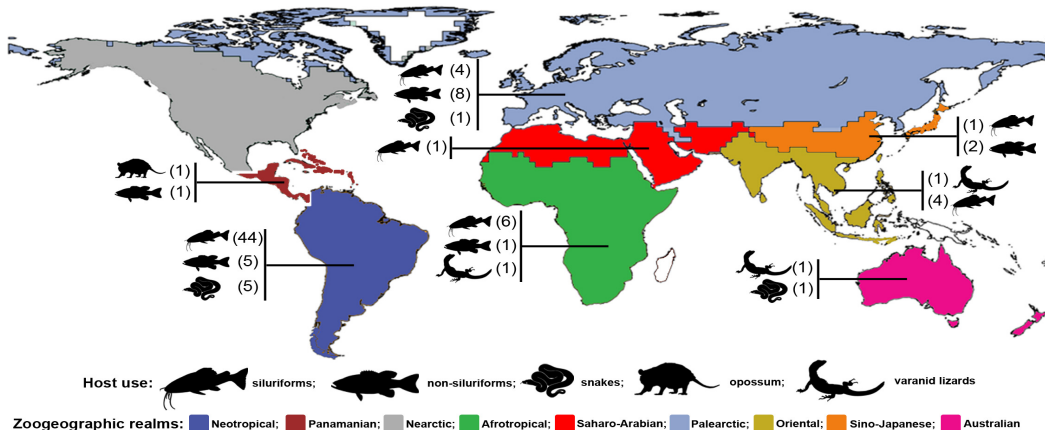# What is genome skimming?



Scalable and cost-effective strategy.

# Taxon sampling



Ethanol-preserved, vouchered proteocephalid specimens.

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# Molecular workflow



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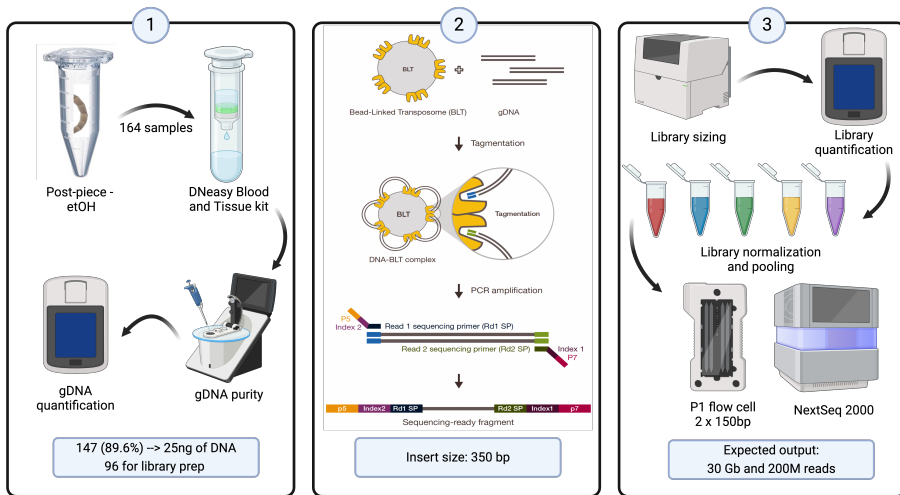
# Computational workflow
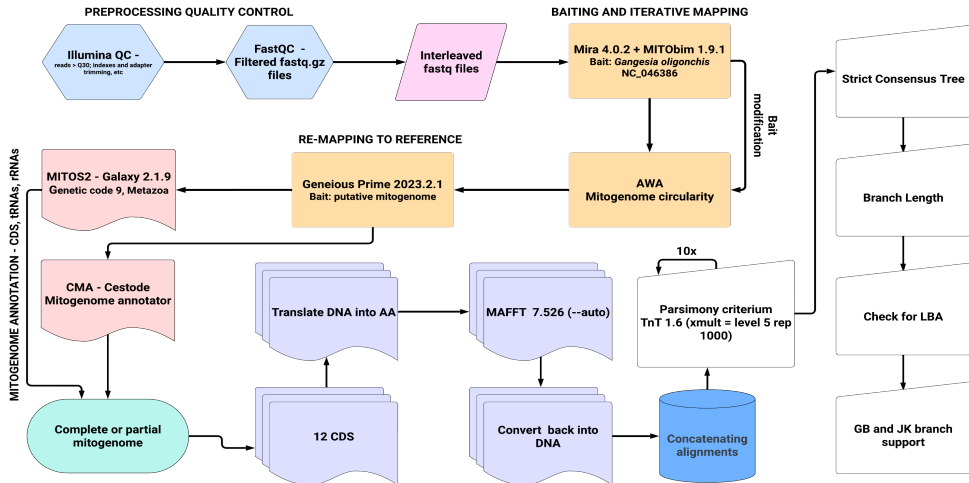


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# Results summary



96 sequenced libraries

Rejected after
sequencing = **6**

Rejected after
QC = **3**

Fragmented - 3
or more parts = **8**

Fragmented - 2 parts
= **18**

Complete = **61**
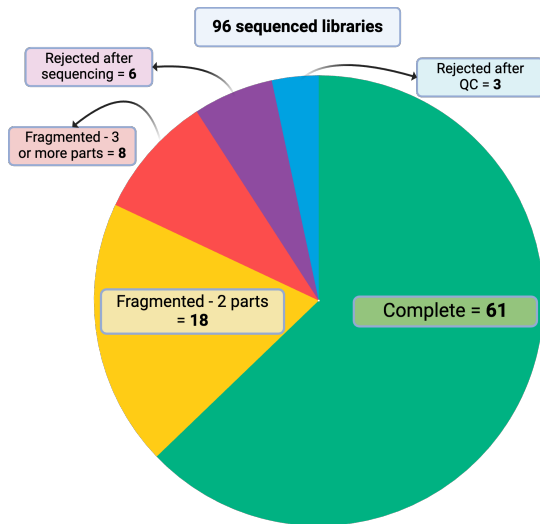
- **Observed output:**
  - 56% of the expect nº reads
  - 32% of expected nº base pairs

- **Samples age:**
  - from 35 y.o. to 11 y.o.

- **Mislabeled samples:**
  - Eight (8) names corrected, including a
    bothriocephalid tapeworm labeled as
    *Proteocephalus pentastoma*

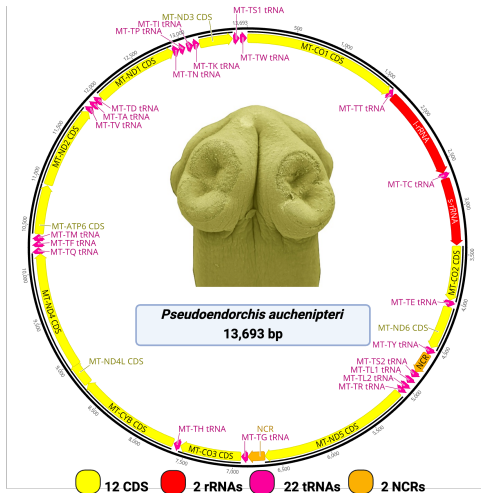- **Hardly assembling (repetitive regions):**
  - Between *trnaY* and *trnaS2*
  - Between the coding ND5 and *trnaG*

- **Material returned to collections:**
  - MHNG-PLAT: 65%
  - IPCAS: 77%

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# Mitogenome characterization



Mitochondrial genome map of *Pseudoendorchis auchenipteri* (13,693 bp)

- **Mitogenome size:**
  - 13,522 to 14,008 bp (mean 13,734 bp)

- **Number and order of genes:**
  - 36 (ATP8 is missing)
  - 2 non-coding regions
  - Same gene order and transcribed in the same direction

- **Mean depth of coverage:**
  - from 14X to 227.4X

- **Nucleotide frequency (%):**
  - A = 24.9; T = 43.9; C = 12.1; G = 19.1

- **Start and stop codons of CDS:**
  - ATG/GTG and TAG/TAA, respectively
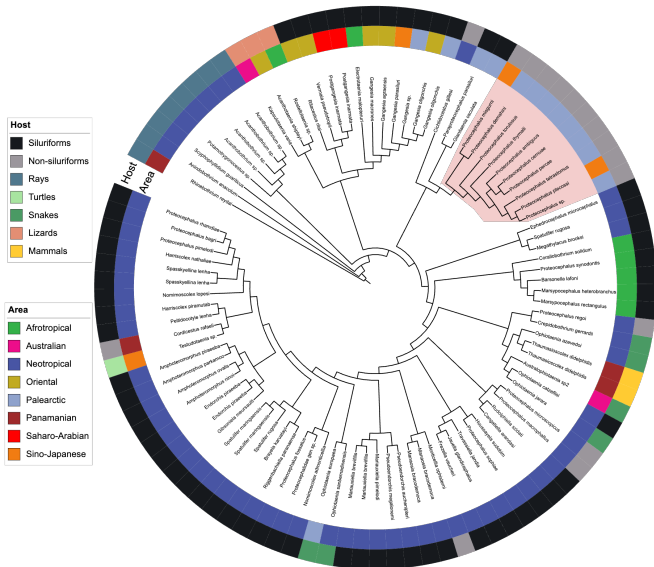  - CO3 CDS has only GTG as start codon

Created with BioRender.com
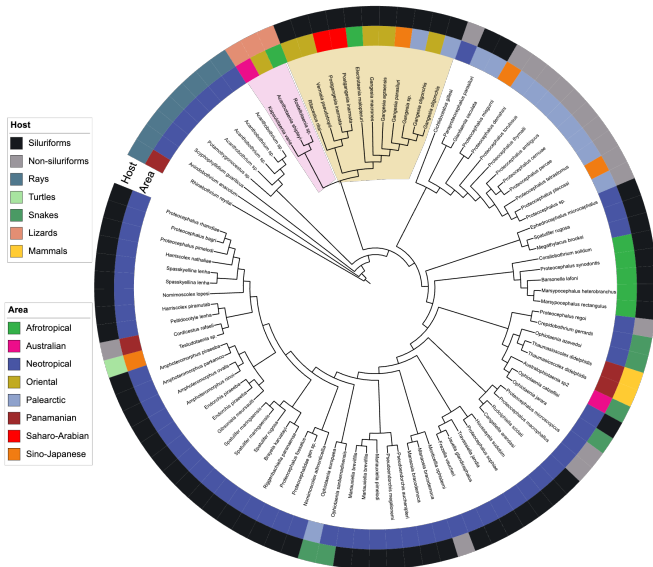
- Reciprocal monophyletism of Acanthotaeniinae in terrestrial hosts (pink clade) and Gangesiinae in siluriforms (yellow clade)

Tree created with itol.embl.del

Created with BioRender.com

- Reciprocal monophyletism of Acanthotaeniinae in terrestrial hosts (pink clade) and Gangesiinae in siluriforms (yellow clade)

- *Proteocephalus*-aggregate clade (Holarctic in non-siluriforms), including the type species *P. ambiguus*

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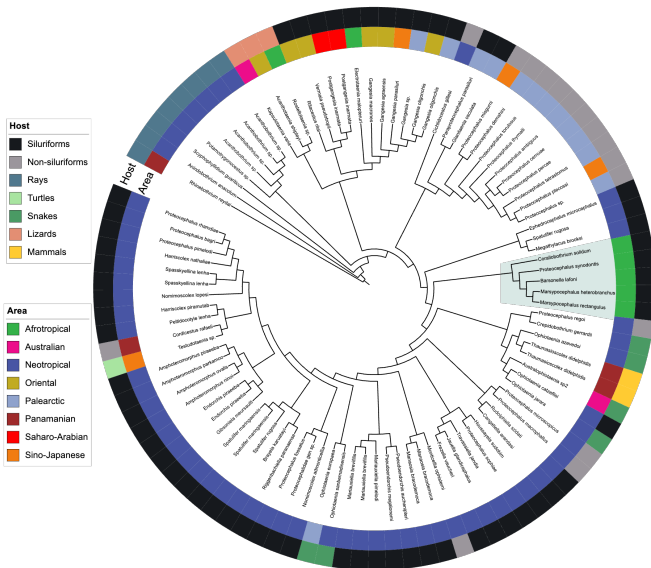- Morphologically unrelated, African clade in siluriforms

Created with BioRender.com

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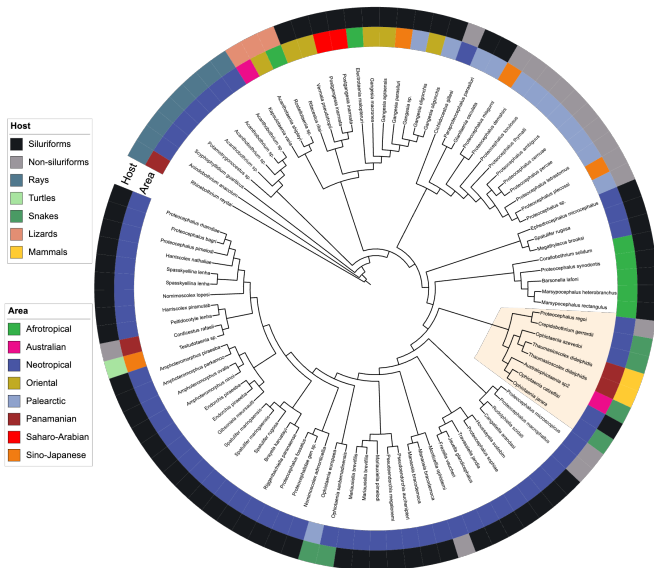
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- Eclectic clade, including fish, snake, and mammal proteocephalids from unrelated regions

Tree created with itol.embl.del

Created with BioRender.com
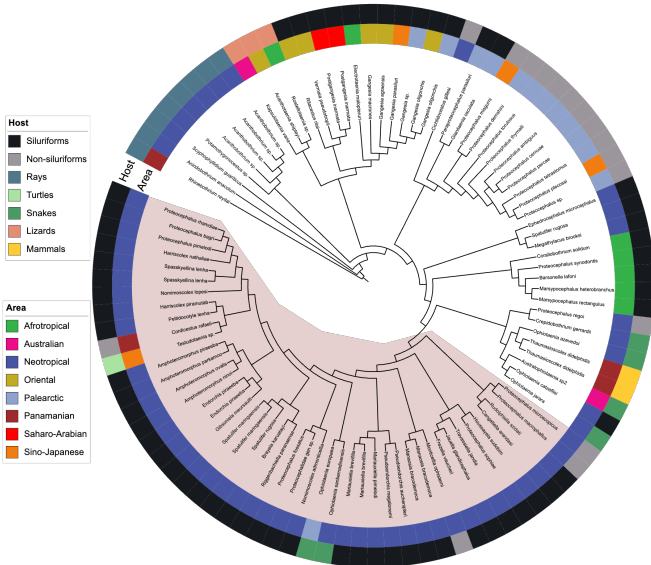
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- Eclectic clade, including fish, snake, and mammal proteocephalids from unrelated regions

- "Neotropical fish superclade", with turtle and snake proteocephalids from unrelated regions nested within it

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## Conclusion

▶ Genome skimming is a cost-efficient technique to sample decades-old museum samples even when the sequencing yield is smaller than expected

▶ Phylogenies produced using nuclear ribosomal DNA vs. mitochondrial DNA are largely concordant, but mtDNA trees are better resolved (especially for early diverging lineages)

▶ The mitogenomes generated here will set the stage for future phylogenetic studies on the evolutionary history of proteocephalid cestodes.

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