# Near chromosome-level genome assembly of *Hamiltosporidium tvaerminnensis*

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#### Microsporidia genome size variation

#### Genome reduction ...

and

Secondary genome expansion ...

... lead to high variation in genome size (< 3 Mb to > 50 Mb)



#### Microsporidia genome size variation



#### Hamiltosporidium tvaerminnensis

- Transmission: Vertical and horizontal
- Asexual
- Fat body, ovaries





infected D. magna Pictures: Ebert (2005) 6

spores

#### Materials and Methods

 Genome assembly: PacBio, Oxford Nanopore, and Illumina sequencing

• Annotation: PacBio Iso-Seq to sequence full-length transcripts, providing reliable isoform information without assembly steps

### Final assembly

- 21.64 Mb
  - N50 1.44 Mb
- BUSCO score 94 %
- 39.56% interspersed repeats
  - retroelements 7.07%
  - DNA transposons 7.04%
  - other 25.45%



#### Iso-Seq (full-length transcript data)

- Number of protein-coding genes in 21.64 Mb: 3,573 Encephalitozoonidae: genome size 2-3 Mb, ~2,000 genes
- We observed Alternative PolyAdenylation (APA), which could be a potential mechanism for gene regulation in microsporidia

The motif for cleavage of protein-coding mRNA is shared with other Metazoans:



#### Conclusion

• Genome size variation in microsporidia can potentially be explained by repetitive sequence content:

-> comparative genomic analyses aiming to understand the evolution of genome reduction and expansion should focus on understanding how and why repetitive sequence is purged or aggregated in the studied species.

## Thank you!

Ebert Group Dieter Ebert Peter Fields

and Jean-François Pombert





JOURNAL ARTICLE ACCEPTED MANUSCRIPT

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C2 Canad Canadian ikad 195 https://doi.org/10.1002/g2iournal/ikad 195

G3 Genes|Genomes|Genetics, jkad185, https://doi.org/10.1093/g3journal/jkad185 Published: 11 August 2023 Article history ▼





Assembly	
Total length (Mb)	21.64
GC content (%)	26.60
Contig N50 (Mb)	1.44
Contig number	17
BUSCO completeness score (%)	94
Annotation	
Total length of repeats (Mb)	8.56
Number of protein-coding genes	3,573
Mean gene length (bp)	1,391
Number of predicted introns	98
Number of Iso-Seq confirmed introns	17
Number of genes with confirmed introns	15
Mean intron length (bp)	28

#### 1) Ancestral lifecycle

(as in H. magnivora)





- *H. magnivora* depends on two hosts
- vertical transmission to offspring
- horizontal transmission to second host (and back)
- sexual reproduction in the second host

 New feature additonally allows to transmit horizontally among *D. magna* **3)** Absence of second host

(as in *H. tvaerminnensis*)



- *H. tvaerminnensis* can colonize areas were the second host is absent
- sexual reproduction is absent

FIGURE 4 Putative evolutionary change in the *Hamiltosporidium* life cycle. (1) The ancestral life cycle of *Hamiltosporidium* might have involved asexual vertical transmission to offspring and transmission to a second host where sexual reproduction could take place, as is the case for *Hamiltosporidium magnivora*. (2) However, a novel transmission strategy arose wherein horizontal transmission amongst the host *Daphnia magna* without sexual reproduction (3) allowing this derived *Hamiltosporidium* group to colonize geographical regions where the second host is absent, as is the case for *Hamiltosporidium tvaerminnensis*. Sexual reproduction would then only be possible in the presence of the now facultative second host. This figure illustrates our hypothesis and is a speculation based on our results *Angst et al. (2022)* 

