





Sequencing Strepsiptera: NGS and museomics methods in a systematic study of the twisted-wing parasites

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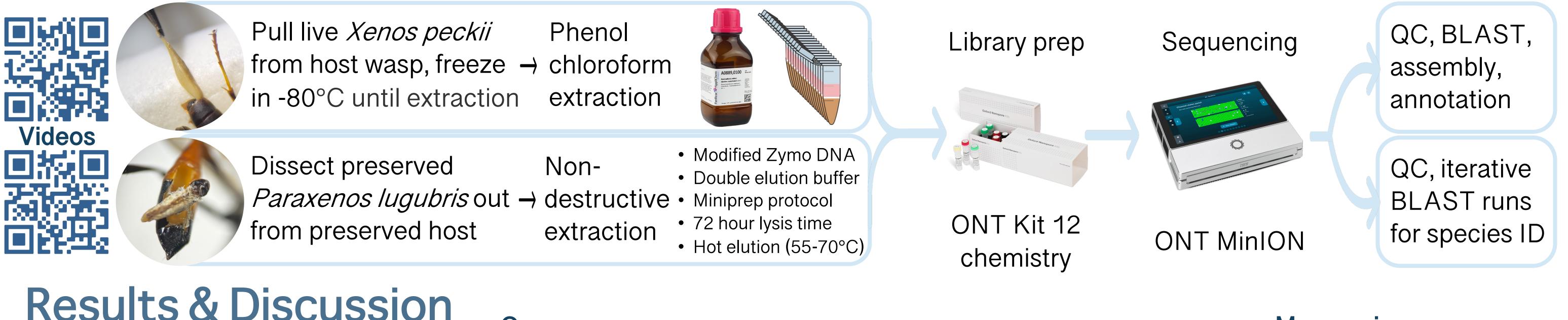
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Introduction

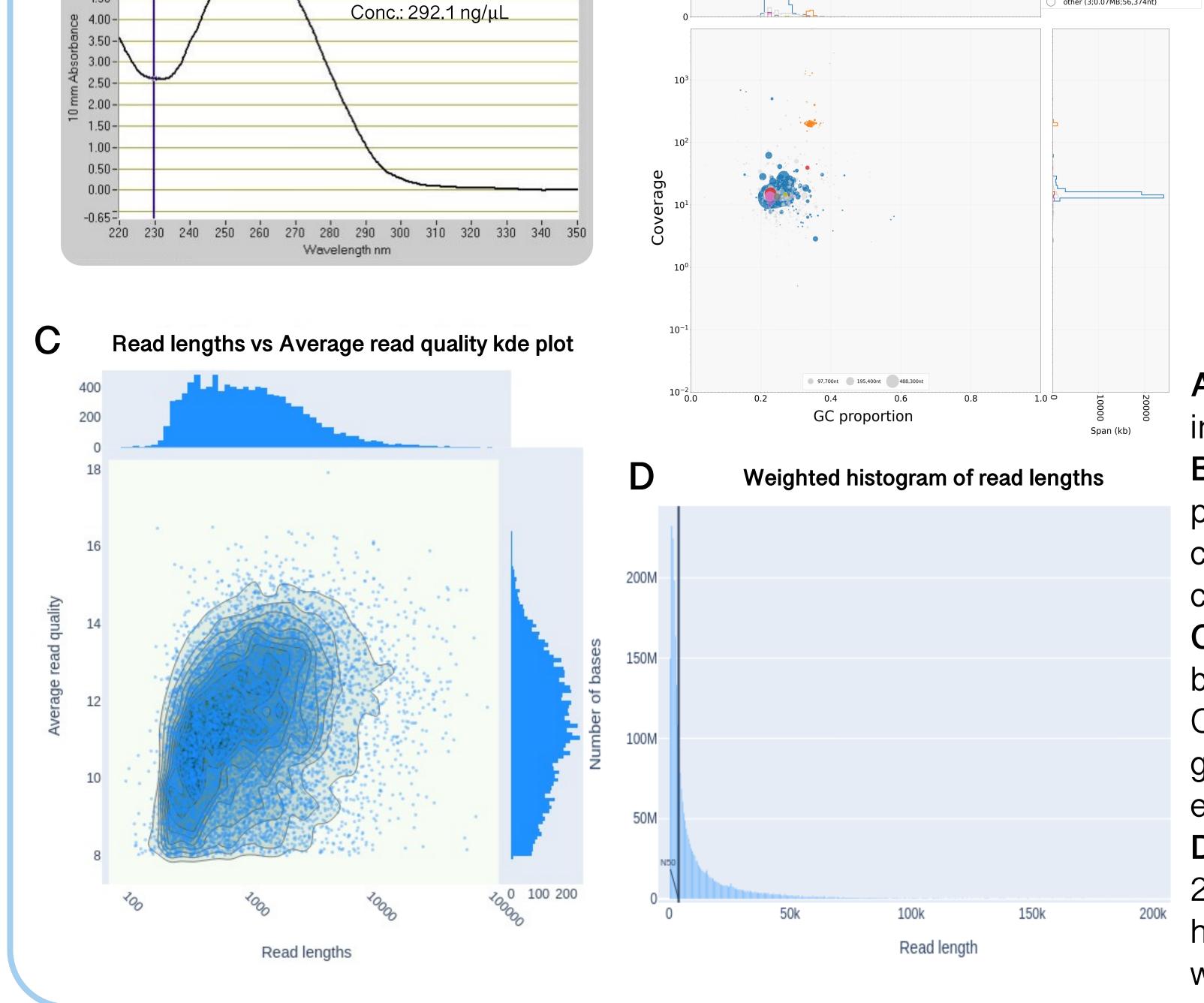
- Strepsiptera, or the twisted-wing parasites, are a small and enigmatic order of Insecta that parasitize several other major insect groups.¹ Rarely collected afield, only one whole genome and one molecular phylogeny are currently published for the order.²
- "Museomics" refers to the study of genomics using museum specimens as molecular material.³ The advent of improved extraction protocols and next generation sequencing allow for the inclusion of decade-old preserved specimens in molecular phylogenies.
- We present our methods and quality assessments in using the Oxford Nanopore Technologies MinION to sequence two

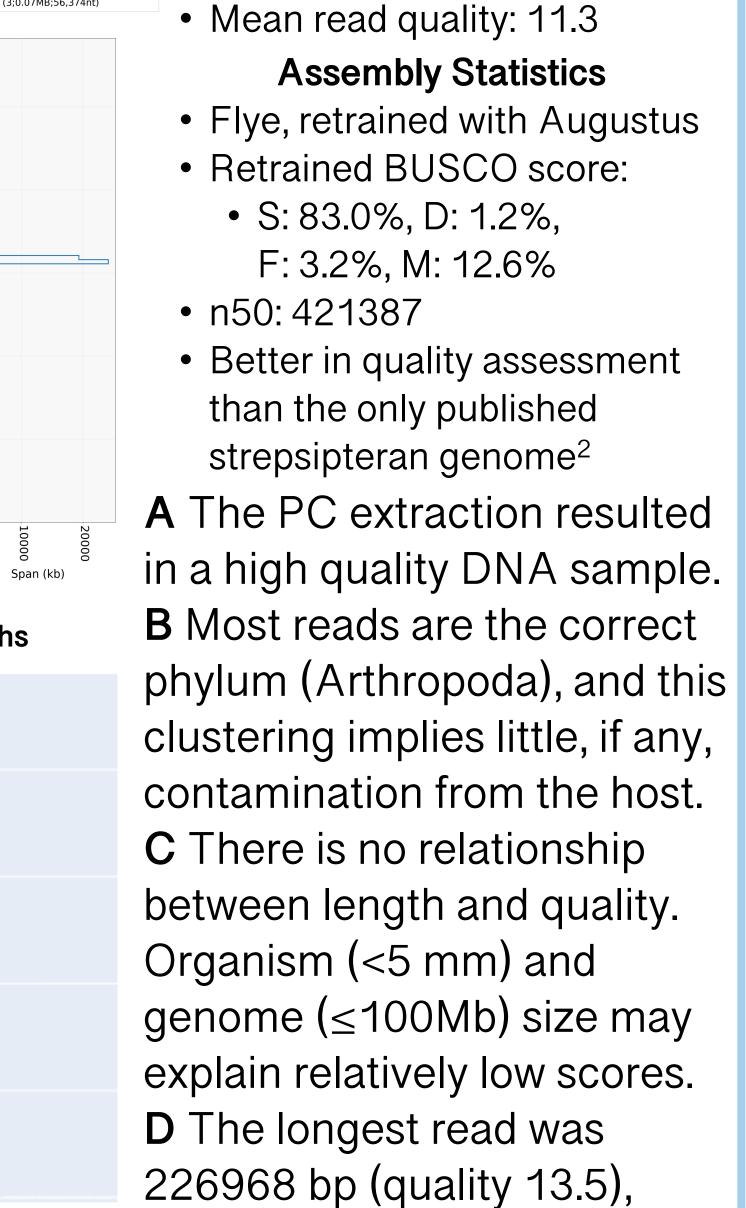
individuals: 1) the whole genome of a female Xenos peckii (Strepsiptera: Xenidae) and 2) a test extraction of a female Paraxenos Iugubris (Strepsiptera: Stylopidae) museum specimen, generated by our novel nondestructive DNA extraction protocol.

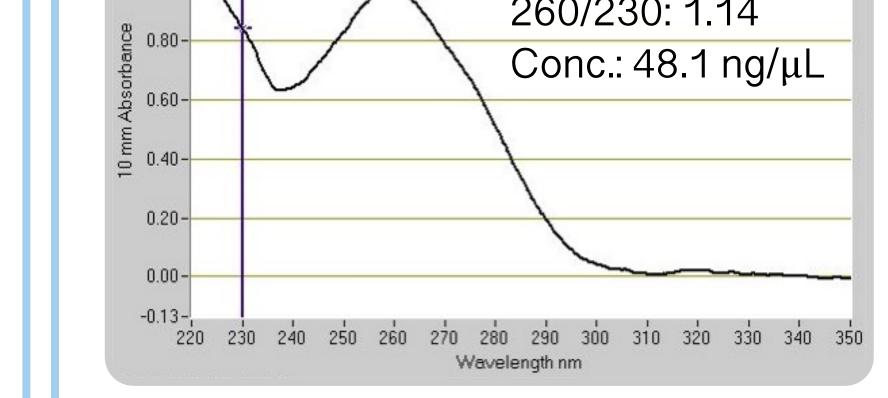
Material & Methods



		Genome			Museomics
Nanodrop Plot (PC Extraction QC)	R	Blobplot	Filtered Read Statistics:		Nanodrop Plot (QC)
6.47-	10000		• n50:3918.0	1.34-	
6.00- 5.50- 260/280: 2.14	(q) (q) 6000	Ani	hit (564;4.94MB;30,751nt) teobacteria (74;1.32MB;34,115nt) nelida (3;0.49MB;420,607nt) matoda (2;0.39MB;350,948nt)	1.20-	260/280: 1.91
5.00-260/230:224	ගි 4000	Pla Pla Asc	tyhelminthes (1;0.26MB;258,097nt) comycota (2;0.16MB;151,746nt) ardata (1:0.06MB;50,700nt) • Mean read length: 1935.4 bp	1.00	

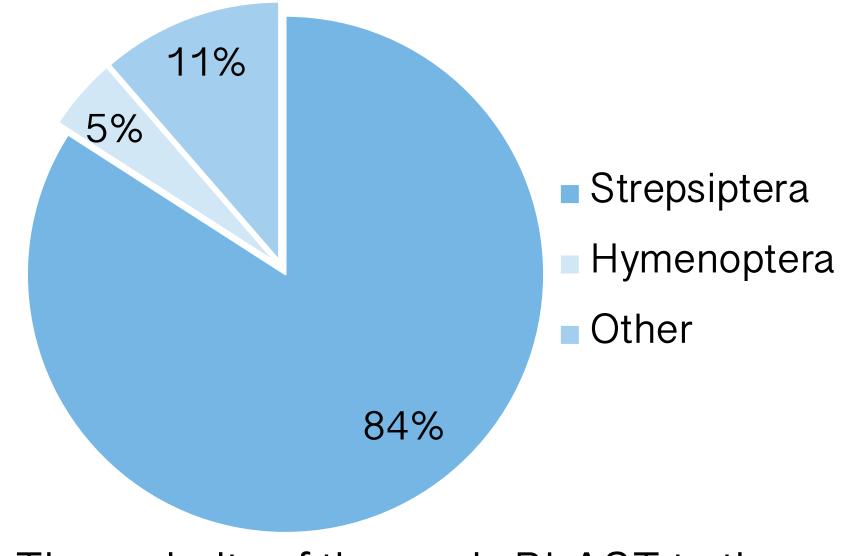






This 260/280 ratio is generally accepted as pure for DNA, but the 260/230 ratio indicates possible contaminants.

BLAST Result for 44 Nanopore Reads



^{200k} highest quality (Phred score) was 18.9 (length 1379). The majority of the reads BLAST to the correct order, verifying endogenous DNA.

Conclusions & Future Directions

- Phenol chloroform extractions combined with nanopore sequencing can yield genome sequences for singular insect specimens < 5 mm in size.
- Nondestructive sampling methods on museum specimens can extract enough DNA for anchored hybrid enrichment (AHE) analyses.⁴

We will take more steps to polish this genome. It will eventually serve as a reference genome in the construction of our molecular phylogeny of Strepsiptera. All extant taxa sampled will be sourced from museums and extracted with our final nondestructive protocol. Currently, we are acquiring taxa, optimizing our protocol, and designing baits for the AHE analyses.

References

- 1. Kathirithamby J (2018). Biodiversity of Strepsiptera. Insect biodiversity, 2, 673-703.
- 2. Niehuis O et al. (2012). Genomic and morphological evidence converge to resolve the enigma of Strepsiptera. *Current Biology, 22*(14), 1309-1313.
- 3. Raxworthy CJ & Smith BT. (2021). Mining museums for historical DNA: advances and challenges in museomics. *Trends in Ecology & Evolution, 36*(11), 1049-1060.
- 4. Goodman A et al. Assessment of AHE probes on locus capture of Odonate specimens across time and museums. *Insect Systematics and Diversity.* (Submitted 2022).

