University of Wisconsin-Stevens Point

Utilizing Molecular Analyses to Identify Helminth Communities of Waterfowl

Michaela M. Meehl, Reece A. Mullen, Sarah A. Orlofske, Robert C. Jadin

Introduction

Parasite surveys are lacking across multiple host taxonomic groups, waterfowl included, leaving gaps in the understanding of parasite ecology. Furthermore, taxonomic resolution in parasite surveys has steadily decreased due to the loss of experts who can properly identify the parasites to the species level using only morphology.[1] One potential solution is to use molecular analyses to identify parasite species.[2,3]

Objectives

1. Analyze helminth parasite DNA collected from waterfowl in Green Bay, WI to confirm the identity of known and possible new species.
2. Provide a framework for continued monitoring of parasite species through environmental changes.

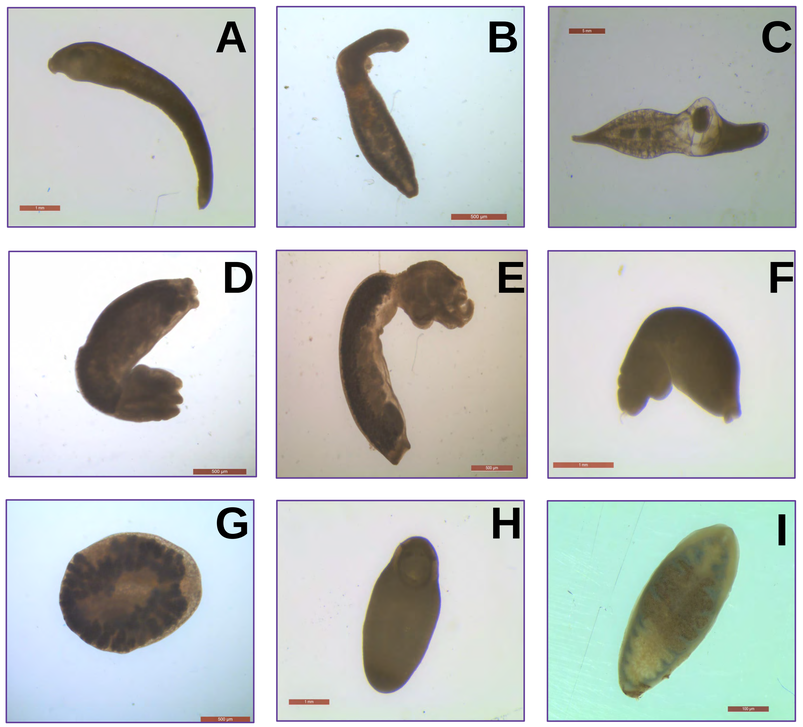

Figure 1. Photo vouchers of individual parasites collected and their identity based on molecular results.
A: *H. conoideum* **B:** *E. mordvilkowi* **C:** *Psilochasmus* sp.
D: Strigeidae sp. A **E:** Strigeidae sp. B **F:** *A. gracilis*
G: *C. prussica* **H:** *Z. lunata* **I:** Cyclocoelidae sp.

Methods

In 2019 and 2020, 18 waterfowl specimens of 5 species were donated by hunters from Green Bay, WI for parasite collection following standardized dissection methods. Parasite DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit with extended time in the 50ºC water bath. We amplified internal transcribed spacer regions [ITS-1] and [ITS-2] and cytochrome oxidase 1 (CO1) genes by polymerase chain reaction. We assessed PCR via electrophoresis, and successfully amplified samples were sequenced by GENEWIZ. Chromatographs were edited using Geneious R6 6.1.6, and sequences were analyzed using the NCBI Basic Local Alignment Search Tool (BLAST) to determine species identification based on comparison with GenBank records.

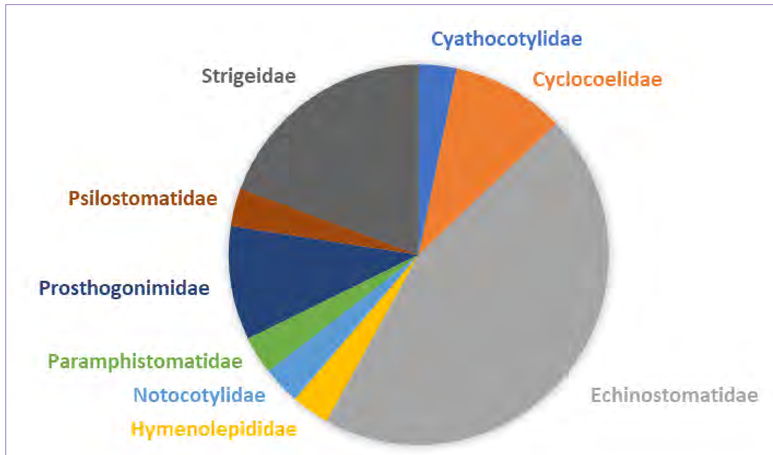

Figure 2. Proportion of parasites in each family.

Results

Based on morphology (see Fig. 1), 28 parasites were correctly identified to the family level. Fifty sequences from 30 individual parasites were obtained and analyzed. Species within the family Echinostomatidae predominated (47%) followed by Strigeidae (20%), Cyclocoelidae and Prosthogonimidae (10%), and all other families were represented by one parasite (3%) (Fig. 2). Based on molecular analysis, 15 species were identified (Fig. 3). There were 4 species of Echinostomatidae, 4 species of Strigeidae, and 1 species each from the remaining families. Using molecular analysis, 22 of the sequences matched existing GenBank records (78.68-100% identity).

Discussion

Based on morphology, we recognized 21 parasite morphotypes; however, our molecular analyses determined there were 15 species present. Molecular analysis provided higher resolution in identifications to the species level for Echinostomes and Strigeids but was inaccurate when analyzing rare or understudied families because of inadequate records in GenBank. We believe most discrepancies in morphological identification were caused by size differences, particularly in echinostomes. Echinostomes represented nearly half of the data set, primarily due to greater success in DNA extraction and PCR. In continuing this study, finding a successful DNA extraction and PCR process for all families will be crucial to enhancing this survey.

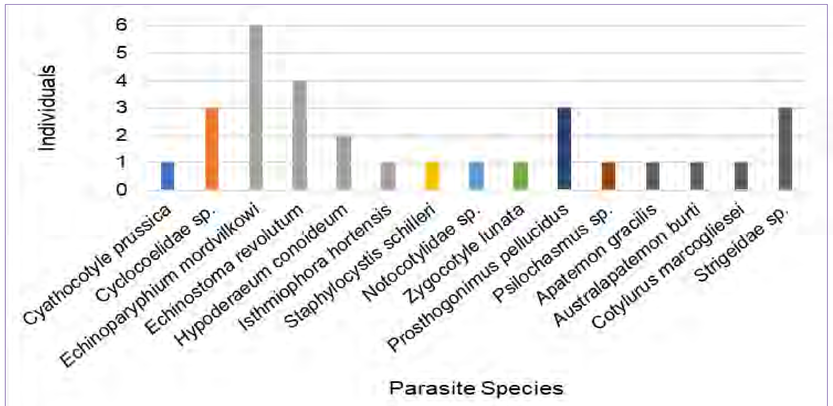

Figure 3. Number of parasites of each species, color coded by family.

Acknowledgements

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Citations

1. Poulin, R., & Leung, T. L. (2010). Taxonomic resolution in parasite community studies: are things getting worse?. *Parasitology*, 137(13), 1967–1973.
2. Bobrek, K., Hildebrand, J., Urbanowicz, J., and Gawel, A. (2019). Molecular Identification and Phylogenetic Analysis of *Heterakis dispar*. *Acta Parasitologica*, 64, 753–760,
3. Galazzo, D., Dayanandan, S., Marcogliese, D., McLaughlin J. D. (2003). Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA-sequence data and comparisons with European congeners. *Can. J. Zool.* 80, 2207–2217.