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BRIEF REPORT

First Report of *Proctoeces maculatus* (Digenea: Fellodistomidae) Infecting the Ribbed Mussel, *Geukensia demissa*: Detection of a Unique Haplotype in New England, USA

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Abstract
Parasites play critical roles in ecosystems, influencing host populations and community dynamics. Despite their ecological significance, the diversity and genetic structure of parasites in the ribbed mussel, *Geukensia demissa* (Dillwyn), a key species in salt marsh ecosystems, remain poorly understood. This study provides the first record of the trematode *Proctoeces maculatus* (Looss, 1901) infecting *G. demissa* in the Herring River estuary, Cape Cod, Massachusetts. Among 50 mussels examined, *P. maculatus* prevalence was 66%, with infection intensities ranging from light (less than 20 sporocysts per individual) to over 300 sporocysts per individual. Heavily infected mussels displayed mantle discoloration, suggesting potential pathological impacts. Molecular analysis of the 28 S ribosomal RNA gene identified a unique haplotype, GD1, restricted to *G. demissa* populations in Cape Cod. Haplotype network analysis revealed GD1's distinctiveness within *P. maculatus* populations, while genetic divergence (K2P: 0.001–0.003) indicated incipient host-associated differentiation rather than cryptic speciation. These findings highlight the role of ecological partitioning, such as the salt marsh-specific habitat of *G. demissa*, in shaping parasite genetic structure.

Keywords 28S · Haplotype · Complex · Parasite

Salt marshes are among the most productive ecosystems in the world, providing critical ecosystem services such as nutrient cycling, water filtration, and shoreline stabilization [1–3]. The Atlantic ribbed mussel (*Geukensia demissa*) plays an integral role in maintaining the health and stability of these habitats [4, 5]. Through the filtration of water and the recycling of nutrients through their feces and pseudofeces, ribbed mussels enhance the productivity of marsh vegetation, particularly *Spartina alterniflora*, a keystone species in the eastern United States [6, 7]. Additionally, their dense aggregations stabilize sediments and mitigate erosion, further supporting salt marsh resilience in the face of environmental stressors, including sea-level rise [5].

Geukensia demissa is native to the Atlantic coast of North America, ranging from the southern Gulf of St. Lawrence (Maritime Canada) to Palm Beach, Florida [8]. It has also been reported in other regions, including the west coast of North America and Venezuela; however, the latter record requires verification, as it may represent its congener, *Geukensia granosissima*, rather than *G. demissa*. These introductions were likely facilitated by unintentional transport through the aquaculture trade [9, 10]. Despite the ecological importance of *G. demissa* in its native range, the diversity and identity of its associated parasites remain understudied. One molecular study identified the protists *Cryptosporidium parvum* Tyzzer, 1912 and *Giardia lamblia* Stiles, 1902 infecting *G. demissa* in Orchard Beach, New York [11], and the only metazoan parasite known to infect the mussel on the East Coast is the trematode *Cercaria opaca* Holliman, 1961, based on historical morphological descriptions [12, 13].

As part of a routine parasite biomonitoring study in New England, sporocysts were found in massive densities (up to 300 sporocysts in a single mussel) within a population of ribbed mussels in the Herring River estuary– a tidally restricted estuary in Cape Cod, Massachusetts. In this study,

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we used DNA barcoding and haplotype analysis to report *Proctoeces maculatus* infection in the ribbed mussel for the first time, and provide details regarding its genetic interrelationships with conspecifics from blue mussels (*Mytilus edulis* Linnaeus). *Proctoeces maculatus* is a widely distributed marine trematode known for its complex life cycle involving multiple hosts [14, 15]. The species typically uses invertebrates such as molluscs and polychaetes as intermediate and paratenic hosts and often complete its life cycle within a fish vertebrate host, although on rare occasions both sporocysts and adults have been found in bivalve hosts (progenesis) [16].

Fifty specimens of *Geukensia demissa* were collected from the Herring River estuary in Wellfleet, Massachusetts, USA (41°55'54"N, 70°03'56"W) in August 2024. Mussels were transported live to Wheaton College, where they were dissected and examined under a stereomicroscope. Internal organs, including the mantle, gills, and gut, were thoroughly inspected using metal probes for the presence of parasites. Identified sporocysts were carefully removed using 1 mL plastic pipettes, transferred to circular glass microplates, and rinsed in deionized water. The sporocysts were dried using Kimwipe towelettes and frozen overnight at -30 °C.

The frozen sporocysts were digested in a proteinase K/lysis buffer solution, and genomic DNA (gDNA) was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A 900 bp fragment of the 28 S nuclear ribosomal gene region (D1–D3) was amplified via PCR using the forward and reverse primer pairs and cycling conditions outlined in Titus et al. [16]. Amplicons were sequenced in both directions by Azenta LLC (Plainfield, NJ). Sequences were screened for quality using BioEdit ver. 7.0 [17]. and initially identified using the NCBI BLASTn tool, which revealed 99.62% similarity to the trematode *Proctoeces maculatus* (GenBank accession: KU052939).

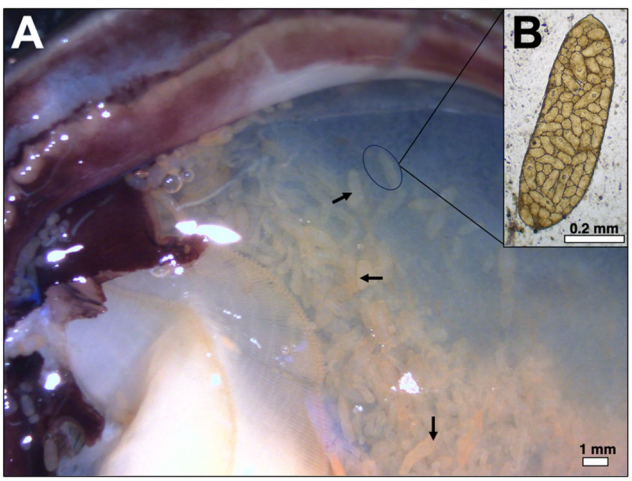

Fig. 1 **A**. Sporocysts (arrows) of *Proctoeces maculatus* infecting the mantle tissue of *Geukensia demissa*. **B**. Single sporocyst harboring numerous cercarial bodies

The sequences were integrated with all verified *Proctoeces* (including *P. maculatus*) 28 S sequences available in the GenBank database (table S1), aligned using the MUSCLE tool, and edited in Jalview ver. 2 [18]. Sequence AY222284 was omitted from the analyses since previous studies found that it was likely misassigned as *P. maculatus* [13, 19]. The final edited alignment was 788 bp in length. Genetic distances (Kimura-2-parameter) were calculated in MEGA 11 [20] to quantify levels of genetic divergence. To determine the evolutionary relationship among haplotypes of *P. maculatus* from *G. demissa* and other geographic localities and hosts, a haplotype network was constructed in PopART [21]. All sequences generated were submitted to the GenBank database (Accession nos. PQ885363– PQ885369).

The overall prevalence of *Proctoeces maculatus* in the sampled ribbed mussels (*Geukensia demissa*) was 66% (33 out of 50 individuals), with infection intensities ranging widely. Some mussels exhibited light infections (no more than 20 sporocysts identified per mussel), while others harbored over 300 sporocysts, primarily concentrated near the margins of the mantle tissue (Fig. 1). High infection intensities were associated with pale discoloration of mantle organs, suggesting potential pathological effects on heavily infected hosts.

The genetic diversity of *P. maculatus* in the northeastern United States reveals significant regional structuring, probably shaped by both historical and ecological processes. Three unique haplotypes were recovered (Fig. 2). The first haplotype, NE1, was previously identified by Titus et al. [16] in two hosts: the blue mussel (*M. edulis*) from southern New England and the polychaete *Sabella pavonina* Savigny. Notably, *S. pavonina* was reported as a host for a single *P. maculatus* specimen from Tunisia, demonstrating a geographically broad but sporadic host association. The second haplotype, ME1, was found exclusively in blue mussels sampled from Maine [16]. This study recovered a third, unique haplotype, herein designated as GD1, which exclusively infects *G. demissa* populations from Cape Cod (Fig. 2). While this haplotype appears host-specific to ribbed mussels, overall genetic divergence from other *P. maculatus* populations in New England was low (K2P distances: 0.001–0.003), suggesting that GD1 does not represent a cryptic lineage. Instead, the genetic data indicate that this haplotype may be in the incipient stages of host-associated differentiation or genetic isolation. This finding aligns with broader patterns of localized differentiation observed in parasites inhabiting hosts with restricted ranges or specialized ecological niches [22].

Titus et al. [16] demonstrated gene flow between *P. maculatus* populations in New England and global regions,

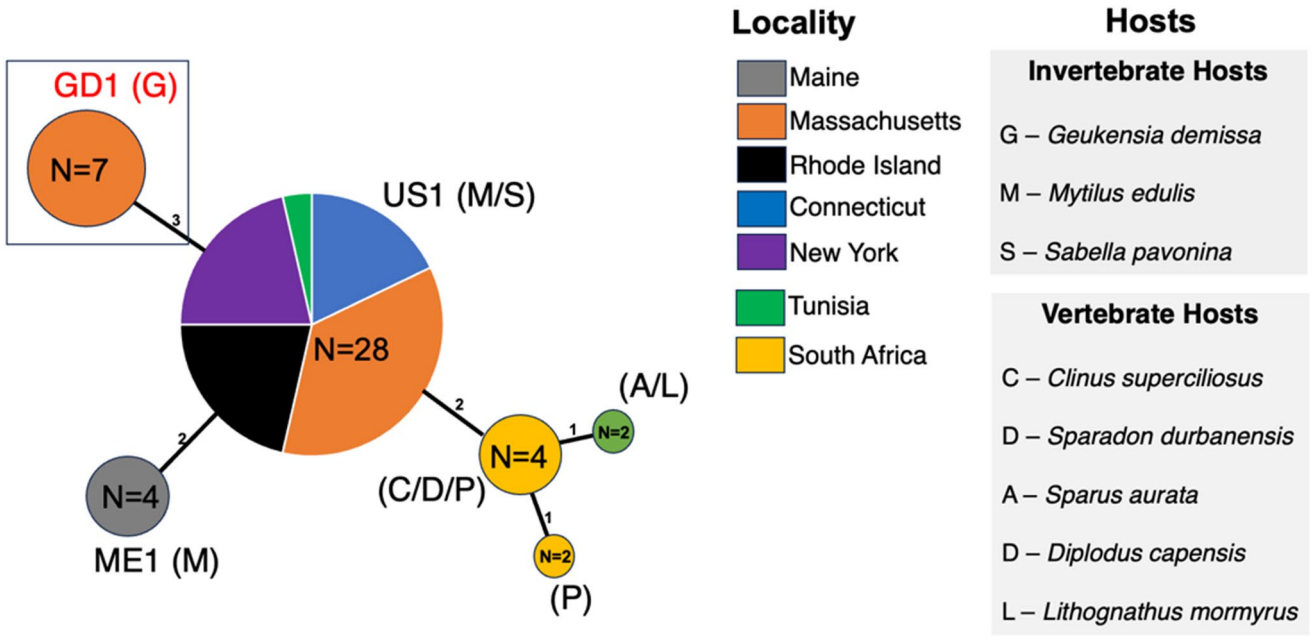

Fig. 2 Haplotype network of *Proctoeces maculatus* based on 28 S rRNA sequence data. Each circle represents a haplotype and size of circles is indicative of the number of individuals with that haplotype (N). Each connecting line between haplotypes represent a single mutational step and numbers above lines represent additional mutational steps. The recovered GD1 haplotype is highlighted in the black box along with two other North American haplotypes (US1 and ME1). Letters in parentheses next to haplotypes represent host(s) harboring specific trematode haplotypes

such as Tunisia, via haplotype sharing, suggesting anthropogenic and host-mediated dispersal. However, the presence of the new GD1 haplotype in *G. demissa* may reflect a shift in host-parasite dynamics driven by local ecological conditions. Ribbed mussels occupy distinct habitats within salt marshes, often isolated from intertidal zones and maritime structures (e.g. floating docks) where *M. edulis* predominates [23]. This ecological partitioning likely limits cross-infection opportunities, reinforcing host-associated genetic differentiation. Moreover, these mussels were collected from a tidally restricted region of the Herring River, which may contribute to the emergence of unique haplotypes by creating ecological conditions conducive to genetic isolation.

The emergence of the GD1 haplotype highlights the dynamic interplay between host specificity and environmental pressures in shaping parasite genetic diversity. While GD1 does not yet meet the criteria for a cryptic lineage, its potential for divergence underscores the importance of monitoring parasite populations for early signs of speciation. Our study also lends support to Vermaak's hypothesis that globally, *P. maculatus* likely represents a species complex [17]. Given the well-documented fitness impacts of *P. maculatus* on *M. edulis*, its presence in *G. demissa* raises concerns about broader ecological consequences, particularly if host-switching exacerbates disease burdens in salt marsh ecosystems. Additionally, the low genetic divergence between GD1 and other haplotypes in the region raises questions about the potential for gene flow across host species. Continued sampling of *P. maculatus* from underrepresented hosts and regions is critical for determining the full extent of its genetic diversity and connectivity. This avenue of research is likely to be challenging considering that *P. maculatus* has one of the broadest host ranges of any marine trematode [14, 17]. In addition, employing higher-resolution markers, such as microsatellites or SNP-based approaches, could provide more detailed insights into population structure and dynamics of this parasite.

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Author Contributions AAD designed the study, analyzed the data and wrote the manuscript. ER collected and analyzed the data, IV collected and analyzed the data, CG collected and analyzed the data.

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Data Availability All sequences were deposited into the GenBank database (Accession nos. PQ885363– PQ885369).

Code Availability Not applicable.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

References

1. Balke T, Stock M, Jensen K, Bouma TJ, Kleyer M (2016) A global analysis of seaward salt marsh extent: the importance of tidal range. Wat Res Res; 3775–3786
2. Gonneea ME, Maio CV, Kroeger KD, Hawkes AD, Mora J, Sullivan R, Madsen S, Buzard RM, Cahill N, Donnelly JP (2019) Salt marsh ecosystem restructuring enhances elevation resilience and carbon storage during accelerating relative sea-level rise. Estuar Coast Shelf Sci 217:56–68
3. Vernberg FJ (1993) Salt-marsh processes: A review. Environ Toxicol Chem 12:2167–2195
4. Jordan TE, Valiela I (1982) A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in nitrogen flow in a new England salt marsh. Limnol Oceanogr 27:75–90
5. Isdell RE, Bilkovic DM, Hershner C (2020) Large projected population loss of a salt marsh bivalve (*Geukensia* demissa) from sea level rise. Wetlands 40:1729–1738
6. Kemp PF, Newell SY, Krambeck C (1980) Effects of filter-feeding by the ribbed mussel *Geukensia Demissa* on the water-column microbiota of a *Spartina alterniflora* saltmarsh. Mar Ecol Prog Ser 59:119–131
7. Bertness MD (1984) Ribbed mussels and *Spartina alterniflora* production in a new England salt marsh. Ecology 65:1794–1807
8. Virgin SDS, Sorochan KA, Metaxas A, Barbeau MA (2019) Effect of temperature on the larval biology of ribbed mussels (*Geukensia demissa*) and insights on their Northern range limit. J Exp Mar Biol Ecol 512:31–41
9. Torchin ME, Hechinger RF, Huspeni TC, Whitney KL, Lafferty KD (2005) The introduced ribbed mussel (*Geukensia demissa*) in Estero de Punta Banda, Mexico: interactions with the native cord grass, *Spartina foliosa*. Biol Inv 7:607–614
10. Carlton JT (1999) Molluscan invasions in marine and estuarine communities. Malacologia 41:439–454
11. Tei FF, Kowalyk S, Reid JA, Presta MA, Yesudas R, Ghislaine Mayer DC Assessment and molecular characterization of human intestinal parasites in bivalves from Orchard Beach, NY, USA. Diversity; 13: 381
12. Epstein R Larval trematodes of marine gastorpods of Galveston Island, Texas. Master's thesis. Texas A&M University. https://hdl.handle.net/1969.1/ETD-TAMU-1972-THESIS-E64
13. Holliman RB (1961) Larval trematodes from the apalachee Bay area, Florida, with a checklist of known marine cercariae arranged in a key to their superfamilies. Tulane stud. Zool 9:1–76
14. Stunkard HW, Uzmann J.R. (1959) The life-cycle of the digenetic trematode, proctoeces maculatus (Looss, 1901) Odhner, 1911 [syn. P. subtenuis (Linton, 1907) Hanson, 1950], and description of cercaria Andranocercan N. sp. Biol Bull 116:184–193
15. Antar R, Gargouri L (2016) Morphology and molecular analysis of life-cycle stages of *Proctoeces maculatus* (Looss, 1901) (Digenea: Fellodistomidae) in the Bizerte. Lagoon Tunisia J Helminthol 90:726–736
16. Titus M, Varetto I, Grosser C, Russo E, Davinack AA (2025) First molecular characterization of *Proctoeces maculatus* (Looss, 1901) (Digenea: Fellodistomidae) infecting blue mussels (*Mytilus edulis*) from the Northeastern USA. J Helminthol 99:e23
17. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser.; 41: 95–98
18. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ (2009) Jalview version 2– a multiple sequence alignment editor and analysis workbench. Bioinformatics 25:1189–1191
19. Vermaak A, Kudlai O, Yong RQ-Y, Smit NJ (2023) Novel insights into the genetics, morphology, distribution and hosts of the global fish parasitic Digenean *Proctoeces maculatus* (Looss, 1901) (Digenea: Fellodistomidae). Parasitology 150:1242–1253
20. Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics, analysis version 11. Mol Biol Evol 38:3022–3027
21. Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116
22. Blasco-Costa I, Cutmore SC, Miller TL, Nolan MJ (2016) Molecular approaches to trematode systematics: 'best practice' and implications for future study. Syst Parasitol 93:295–306
23. Bertness BD, Grosholz E (1985) Population dynamics of the ribbed mussel, *Geukensia demissa*: the costs and benefits of an aggregated distribution. Oecologia 67:192–204
24. Oliva ME, Valdivia IM, Cardenas L, Munoz G, Escribano R, George-Nascimento M (2018) A new species of *Proctoeces* and reinstatement of *Proctoeces humboldti* George-Nascimento and Quiroga 1983 (Digenea: Fellodistomidae) based on molecular and morphological evidence. Parasitol Int 67:159–169
25. Wee NQ-X, Cribb TH, Bray RA, Cutmore SC (2017) Two known and one new species of *Proctoeces* from Australian teleosts: variable host-specificity for closely related species identified through multi-locus molecular data. Parasitol Int 66:16–26