Optimizing molecular identification techniques for *Stegastes adustus* erythrocytes infected with Haemohormidium-like apicomplexan parasites

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Abstract

Blood cells of Caribbean damselfish, *Stegastes adustus*, are infected with *Haemohormidium*-like apicomplexan parasites related to causative agents of malaria and toxoplasmosis in humans. However, host-to-host transmission of fish protozoan is poorly understood. We aim to establish the complete life cycle of these parasites. First, infected fish must be identified and protozoa isolated. We hypothesize that PCR primers targeting the fish apicomplexan 18S rDNA gene will complement microscopy techniques currently used to identify infected erythrocytes. Fish blood samples were analyzed through microscopy for parasitism. Next, whole blood samples were fractionated using a Percoll gradient, and DNA extracts from fractions were tested for infection using PCR. Preliminary results indicate infected cells are not separated adequately by the density gradient because protozoan DNA was present in all fractions. The PCR assay rapidly screens for infection compared to microscopy. Detection of infected fractions by PCR will allow us to determine if there is a pattern in fractions from numerous fish. Isolated infected cells could be used for further study including transmission experiments with live fish, as well as ultrastructure analysis.

Background

Apicomplexan parasites:
- Constitute the largest and best-known taxon of parasitic protozoa.
- Infect invertebrate and vertebrate species (Renoux, 2016).
- Share common structural features, in particular an apical complex of microtubules within the cell (Figure 1).

*Haemohormidium* is a genus of parasitic alveolates in the phylum Apicomplexa. Its primary hosts are fish.Leeches have been postulated as probable invertebrate vectors. (Renoux, 2016)

Problem: Host-to-host transmission of *Haemohormidium*-like apicomplexan fish protozoa (Figure 2) and whether or not the infection causes symptomatic disease is unknown.

Current Objective: To use DNA barcoding to target the evolutionarily conserved eukaryotic 18S small subunit (SSU) rDNA gene sequence to complement microscopic identification of *Haemohormidium*-like apicomplexans in infected *Stegastes adustus*.

Methods

I. Collection of Fish blood and Fecal matter

*Day 1: Live damselfish were collected from the reef and kept in individual buckets overnight.*

*Day 2: Blood samples were collected from clove oil-anesthetized fish and fecal matter recovered by filtration through a plankton mesh. Whole blood samples were examined for the presence of infection by Giemsa staining of a thin smear. Recovered fish were returned to the reef.*

II. Isolation of protozoa through Percoll gradient

*Figure 3: Processing of fish fecal matter (Robinson, 2016)*

III. Detection of apicomplexan DNA using microscopy and specific PCR primers

- DNA was extracted from recovered blood fractions using DNA extraction and purification QIAQuick kits.
- Novel primers, 226F (forward) and 685R (reverse), were used to selectively detect and amplify protozoan DNA found in low numbers in host fish blood through Polymerase Chain Reaction (PCR).
- Gel electrophoresis was used to verify PCR products.

- A variety of microscopic techniques were employed to capture images of infected cells or fecal oocysts from corresponding fish.

Results

Blood Fractionation on Percoll Gradients

*Figure 5: (Left to Right) Gradual improvement of Percoll gradients for blood samples*

Fecal Sample Large and Small Fractionation on Percoll Gradients

*Figure 6: Small (left) and Large (right) Percoll gradients of fecal samples. A total of three fractions were obtained for microscopy.*

Gel Electrophoresis for PCR products

*Figure 7: Pictures from the three fractions recovered from fecal matter Percoll gradient above. No oocysts were detected.*

III. Detection of apicomplexan DNA in fish whole blood.

*Figure 8: PCR detection of apicomplexan DNA in fish whole blood.*

Conclusions

- *Percoll gradients successfully separated blood and fecal matter components into density-dependent categories that may allow the isolation of infected red blood cells (RBCs).*
- *Haemohormidium*-like apicomplexan parasites were detected in fractionated feces and blood samples.
- 18S rRNA region of the blood parasites was successfully amplified via PCR
- PCR products confirmed fish classified as infected through microscopy and appears more sensitive than microscopy for detection of parasites in whole blood

Future Direction

- Repetition of experiments to further optimize the protocol
- Performance ultrastructure analysis of isolated infected cells to determine the actual structure of these novel *Haemohormidium*-like apicomplexan parasites
- Transmission experiments to further test our hypothesis that uninfected fish can become infected via contact with fish feral matter
- Western blots to probe whole or fractionated blood and fecal matter with antibodies that are specific to known apicomplexan proteins conserved in other well-studied species

References


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