# Documenting Trematode Larvae in Marine Sponges

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## Background

Trematodes have never been documented in association with sponges in previous literature. This is an explorative study of the larval trematode stages in the sponge microenvironment.

**Study Organism: Digenean Trematodes**

**Phylum:** Platyhelminthes
**Subphylum:** Neodermata
**Class:** Trematoda
**Subclass:** Digenea

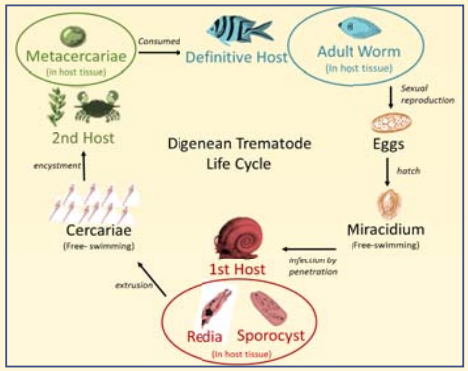


**Figure 1:** Typical Digenean Trematode Life Cycle. Personal diagram based on [1], [2]

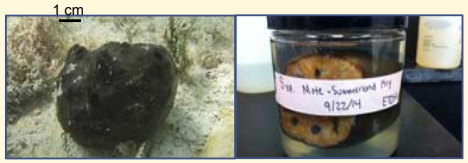


**Figure 2:** *Spongia* sp., Spongiidae, Greek bathing sponge from Summerland Key, FL

**Figure 3**: Location of Summerland Key Bay in the lower FL Keys (star); GPS location of Mote Marine Laboratory (orange: 24˚39'41.4"N 81˚27'16.5"W) and outline of approximate study area for sponge collections (yellow)

## Objectives

The goal of this research is to determine the role of sponges in the life cycle of these trematodes and elucidate whether any adaptive traits have evolved within this trematode population as a result of their unique sponge association.

This study focuses on **(1)** observing morphological adaptations of the trematode population through light microscopy **(2)** determining the prevalence of trematode cercariae's affinity towards sponges using experimental flow tanks **(3)** documenting the host-parasite histology using scanning electron microscopy (SEM) and **(4)** identifying the trematode taxa present in the sponge microenvironment using 18S rDNA molecular markers.

## Methods

**(1) Morphology**

- Whole mounted specimens are preserved and viewed unstained or stained with toluidine blue
- Histological sections processed according to *figure 4*

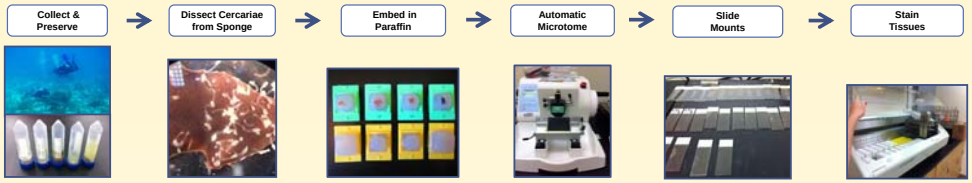


**Figure 4**: Histological procedures for observing cercariae morphology

**(2) Prevalence Studies**

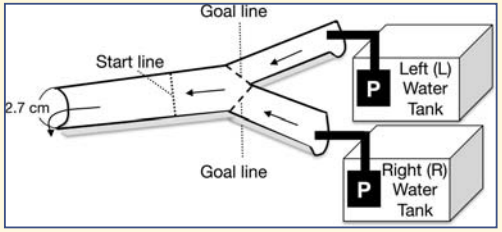


**Figure 5:** Top view of Y-shaped tank with one-way flow from two separate containers. Image source: [3]

**(3) Scanning Electron Microscopy (SEM)**

Micrographs generated through scanning electron microscopy will reveal the ultrastructure of cercarial 'attachment' to the sponge tissue. Attachment structures or marine biological adhesives can be observed. SEM will be performed in collaboration with Dr. Patricia Blackwelder at University of Miami.

**(4) Identification of Trematode Taxa**

Genomic DNA from cercariae will be extracted and a partial sequence of the 18S rRNA gene (rDNA) will be PCR amplified using universal 18S trematode primers that allow amplification across a wide range of taxa [4]. This type of preliminary screening is a useful tool for potentially mixed samples of uncertain taxonomic identity [5]. Amplicons will be sequenced at Auburn University's Aquatic Parasitology Lab, and compared with registered sequences of 18S digenean rDNA in GenBank using BLAST (NCBI) and ClustalW (DDBJ).

## Preliminary Results

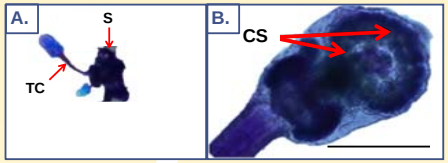


**Figure 6:** Whole mounted specimens stained with toluidine blue. ***A.*** Trematode cercariae (TC) attached to sponge (S); stereomicroscope magnification = 40x. ***B.*** Cercariae body region. Arrows show location of collar spines (CS) which completely encircle the oral sucker. Magnification = 100x; scale bar = 500µm

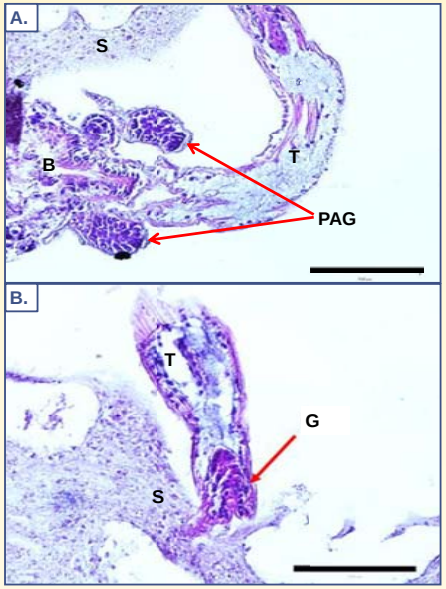


**Figure 7:** Hematoxylin and eosin stained sections (7µm thickness). ***A.*** Posterior adhesive glands (PAG) located on the posterior region of the body where the tail and body meet. Magnification = 200x, size bar = 200µm. ***B.*** Association of sponge tissue (S) and trematode cercariae tail (T). Arrows indicate location of a cluster of gland pores (G), that may secrete a marine biological adhesive similar to the PAG. Magnification = 400x, size bar = 100µm.

## References

[1] Smyth, J.D. The Physiology of Trematodes. W.H Freeman and Company: 1966.
[2] Cribb, T.H., Bray, R.A., Olson, P.D., Littlewood, D.T.J. 2006. Life Cycle Evolution in the Digenea: a New Perspective from Phylogeny. Advances in Parasitology, 54: 197-254.
[3] Katano, I., & Doi, H. 2014. Stream grazers determine their crawling direction on the basis of chemical and particulate microalgal cues. PeerJ 2:e503
[4] Waikagul, J. & Thaenkham, U. Approaches to Research on the Systematics of Fish- Borne Trematodes. Academic Press: 2014.
[5] Moszczynska, A., Locke, S., McLaughlin, D., Marcogliese§, D., & T.J. Crease. 2009. Development of primers for the mitochondrial COI gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminthes. *Molecular Ecology Resources*, 9: 75-82.