Aspects of the life cycle of *Apharyngostrigea pipientis* in Central Florida wetlands

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Abstract

Apharyngostrigea pipientis (Trematoda: Strigeidae) is known to form metacercariae around the pericardium of anuran tadpoles in Michigan and other northern locations. Definitive hosts are thought to be wading birds, while the intermediate host is a freshwater snail. *Apharyngostrigea pipientis* is not commonly reported from Florida, yet we have found several populations of snails (*Biopholaria havaensis*) and tadpoles, primarily the Cuban treefrog (*Osteopilus septentrionalis*), to host this trematode. We used experimental infections to elucidate the transmission dynamics and development of *A. pipientis* inside the tadpole host. Surprisingly, we found two types (species?) of cercariae being shed from *B. havaensis* that enter Cuban treefrog tadpoles to form seemingly identical metacercariae. Further, both of these develop into metaceracariae inside the tadpoles over 5-7 days after wondering inside the host's body cavity as mesocercariae, and metacercariae are commonly concentrated around the pericardium cavity. However, they differ in entry mode, with one being ingested, whereas the other penetrates the skin. This project is ongoing.

1. Introduction

The study of parasitology plays a vital role in understanding the foundation of communities in different ecosystems. Parasites have the ability to exploit their hosts, directly affecting the health of the organism and the environment. By having these abilities, it could mean a change in the way the organism contributes to and balances the overall ecosystem (Poulin, 1999).

In Central Florida Wetlands there is a significant diversity within the parasite populations. Previously conducted local surveys of parasite populations in different amphibian and reptile populations in Central Florida Wetlands have been conducted at Florida Southern College (G. Langford, Pers. Comm.). Interestingly, in 2012, the parasite *Apharyngostrigea pipientis* (Phylum- Platyhelminthes; Class-Trematoda; Family-Strigeidae) was identified in tadpoles of *Osteopilus septentrionalis* (Cuban treefrog) in a collection site at a local park about four kilometers from the college. This was surprising because previously this parasite had only been identified in anuran hosts in Michigan (Oliver, 1940) and other northern locations such as Chicago (Huges, 1928). More current research is also finding that distributions of many parasites in the Strigeidae family are expanding (Locke et al., 2011).

A. pipientis is a member of the Strigeidae family. The distinguishing morphological characteristics of this family include their division into fore and hind bodies (Hernández-Mena et al., 2014). Within the Strigeidae family, Duboisiella is the only genus that is known to infect mammals. The other members of this family are known to infect the intestinal track of their final host, the bird (Shoop, 1989). The White Ibis (*Eudocimus albus*), specifically, is most likely to be the final host of *A. pipientis* in this experiment because of its presence in the wetland collection site. This bird has also been found to host other genuses in the Strigeidae family (Hernández-Mena et al., 2014).

Furthermore, *A. pipientis* has been known to form metaceracariae encysted around the pericardium of anuran tadpoles. When observed in a container in the lab, only a small few will swim at any given moment (Oliver, 1940). In the Central Florida collection site

where the research was conducted, planorbid snails (*Biopholaria havaensis*) were found to be intermediate hosts.

A previous study, conducted at Florida Southern College in Summer 2013, served to distinguish between two different parasites found in the collection site; one being *A. pipientis*, and the other, an unidentified species (G. Langford, Pers. Comm.). The ceracariae of *A. pipientis* and the other parasite found, differed morphologically in that the hind body of the unidentified parasite was much smaller and narrower. While both were fork tailed, the hind body of *A. pipientis* was significantly larger and was also characterized by the light yellow coloring of the fore body.

For the experimental infections, the two types of ceracariae were obtained from planorbid snails at the collection site. The Cuban treefrog tadpoles were exposed to the ceracariae in the lab. It was observed that *A. pipientis* entered into the tadpole by being digested, while the unidentified parasite's mode of entry was penetration through the tail of the tadpole. Once both ceracariaes had entered the body cavity of the tadpole, they were found to shed their tail within 24 hours of penetration. Over the next 5-7 days, both of the mesoceracariae from the two parasites moved around the body cavity of the tadpole and eventually developed into a metaceracariae encysted around the pericardium of the tadpole. The metaceracariae from both *A. pipientis* and the unidentified parasite were morphologically identical.

The purpose of this experiment was to further elucidate the life cycle of the smaller, unidentified ceracariae. Exposure studies were repeated with increased sample size and narrower sampling windows. This study was constrained by the seasonal lifecycle of the parasite, which only runs for a few summer months.

2. Methods

Collection of Planorbid snails

In order to conduct the experimental infections, *B. havaensis* were collected from the "Common Grounds" on Edgewood Drive, 3.38 kilometers from Florida Southern College. The collection site included standing water in the middle of a grass field. 3,980 snails were collected from August 25, 2016, to October 27, 2016. When collecting snails, researchers waded into the standing water and used a net to capture snails. The snails were stowed in a plastic 20 gallon bucket. For each day the snails were collected, field notes were taken that included: the number of snails collected, the temperature, and the date the collection took place. The amount of snails collected on each trip to the wetland ranged from 20-530.

After snails were obtained in the field, they were brought back to Florida Southern College to determine which snails were hosts of the parasite. Three to five snails were placed in a small glass jar filled with "snail water". The snails were allowed to sit in the jar for over twenty-four hours to allow time for them to shed the ceracariae. After twenty-four hours, the jars containing the snails and snail water were observed under a dissection microscope**.** The snail water was observed and the presence or absence of ceracariae was recorded for each snail. When a jar was found to contain the ceracariae, the snails inside were isolated from the others and placed into their own jar containing "snail water". These snails were allowed to sit out for 12-24 hours, and then they were observed to see which snail had shed the ceracariae. Only the smaller,

narrower ceracariae were found when this experiment was conducted. No *A. pipientis* were collected.

Once a snail was identified to host the ceracariae, the dimensions of the snail were recorded, and it was placed in a tank of "snail water" in order to preserve the snail to obtain ceracariae when needed. The snail was fed lettuce, and a bubbler was placed in the water to provide oxygen. The table below includes the described data collected.

Table 1: Table containing data describing collection of Planorbid snails and identification of snails hosting the parasite.

Date	Number collected	Time	Temp (F)	Parasite Present?	If yes, diameter of snail?	Notes	Allowed to sit in snail water for:
8/25/16	20	2:36 PM	91	No		All snails checked 8/26	23 hours
8/26/16	90	6:20 PM	90	No		All snails checked 8/29	26 hours
9/2/16	120	6:46 PM	82	No		All snails checked 9/3	29 hours
9/3/16	50	8:00 AM	79	No		All snails checked 9/6	22 hours
9/6/16	120	5:32 PM	85	No		All snails checked 9/11	27 hours
9/10/16	150	5:30 PM	87	No		All snails checked/9/12	13 hours
9/16/16	90	5:00 PM	88	Yes (1)	0.75 cm	All snails checked 9/17	12 hours
9/23/16	500	5:00 PM	89	Yes (1)	0.21 cm	All snails checked 9/24	12 hours
9/24/16	455	9:00 AM	81	No		All snails checked 9/26	14 hours
9/25/16	505	9:00 AM	83	Yes (1)	0.62 cm	All snails checked 9/27	23 hours
9/30/16	420	7:30 AM	80	Yes (1)	0.23 cm	All snails checked 10/1	24 hours
10/12/16	530	5:25 PM	81	No		All snails checked 10/13	17 hours
10/13/16	500	5:35	78	No		All snails checked	12 hours

		PM				10/18	
10/20/16	210	8:00 AM	77	No		Back from break, area was mowed down, almost no standing water left. Snails checked 10/21/16	15 hours
10/22/16	100	12:00 PM	66	No		All snails checked 10/23	16 hours
10/23/16	120	5:00 PM	67	No		All snails checked 10/24	19 hours
10/27/16	0	12:00 PM	82	No		No standing water present	22 hours

Experimental Infections

After snails that hosted the parasite were found, the ceracariae collected from the snails were used to experimentally infect the Cuban treefrog tadpoles. The tadpoles were collected from mesocosums on the Florida Southern College campus. The tadpole's collected were all identified and their growth stage was determined by using the Gosner Staging System for Anurans. The Gosner stage and length were recorded for each tadpole used in the infection.

Two different experimental infections were conducted. To experimentally infect the tadpoles, each tadpole was put in a small glass jar that contained the "snail water". Three ceracariae were obtained after being shed from the host snail and pipetted into the jar containing the tadpole. Each tadpole infected was dissected at a different twenty-four hour interval. The two tables below indicate the data recorded from each experimental infection.

Table 2: Table containing data recorded when setting up infections on September 9, 2016.

	Tadpole Infections Set Up 9/9	
Room Temp	10 Tadpoles collected at 3:47 on 9/27	
Gosner Stage 26-30	Tadpole Infected (Hours)	Tadpole Length
All Infections	24	0.71
Room Temperature	42	0.73
	72	0.73
	96	0.72
	120	0.8
	144	0.75
	168	0.71
	192	0.74
	216	0.74

Table 2: Table containing data recorded when setting up infections on November 21, 2016.

Tadpole Infections Set up 11/21	
7 Tadpoles Collected at 4:45 PM	
Tadpole Infected (Hours)	Length of Tadpole (cm)
192	0.74
216	0.72
240	0.72

Each tadpole was dissected at the indicated time interval and the mesoceracariae/metaceracariae was observed. Pictures were taken of the mesoceracariae/metaceracariae at each time interval and characteristics of the parasite were recorded.

A picture of the ceracariae prior to experimental infections was obtained on November 16, 2016. To take this picture, ceracariae were pipetted out of the jar

containing the shed ceracariae. Then they were pipetted onto a slide and observed under a microscope.

3. Results

Figure 1: Picture of mesoceracariae 24 hours after infection of host tadpole on 09/27/16.

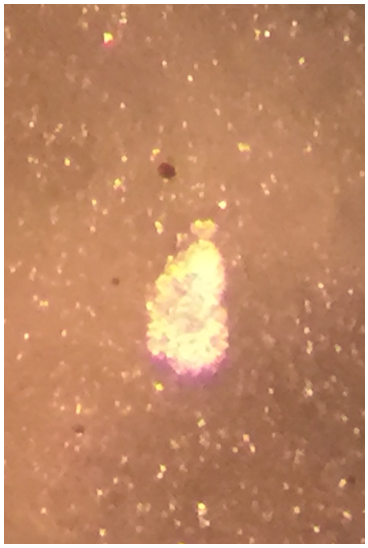


Figure 2: Picture of mesoceracariae 42 hours after infection of host tadpole on 09/27/16.

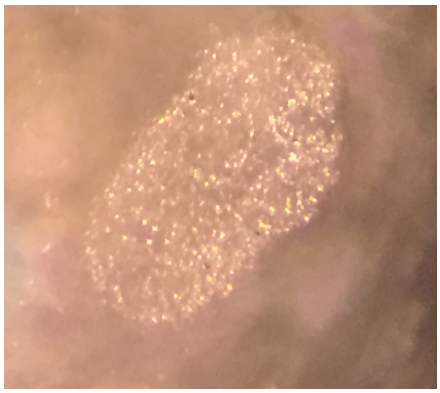


Figure 3: Picture of mesoceracariae 72 hours after infection of host tadpole on 09/27/16.

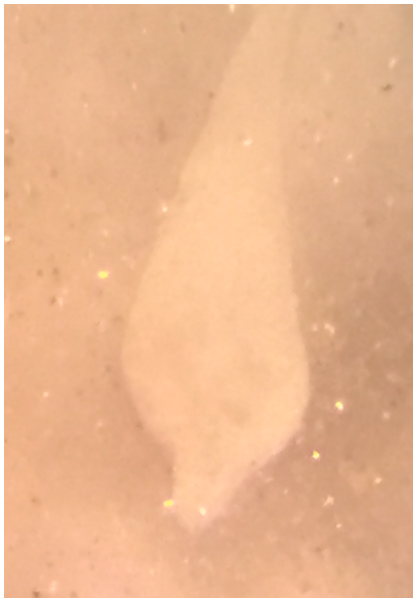


Figure 4: Picture of mesoceracariae 96 hours after infection of host tadpole on 09/27/16.

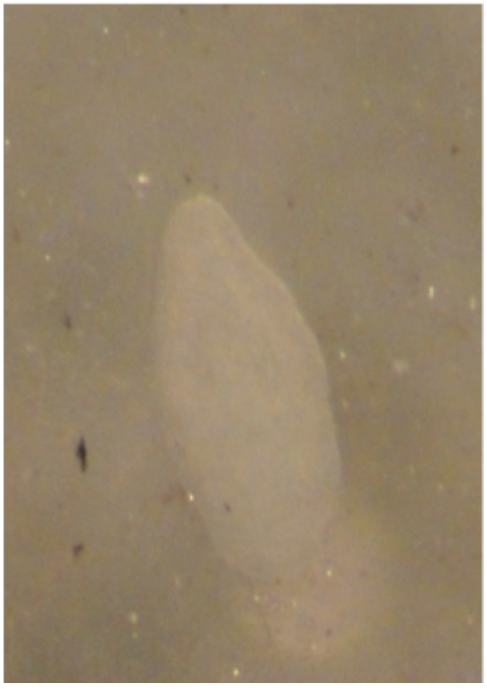


Figure 5: Picture of mesoceracariae 120 hours after infection of host tadpole on 09/27/16.

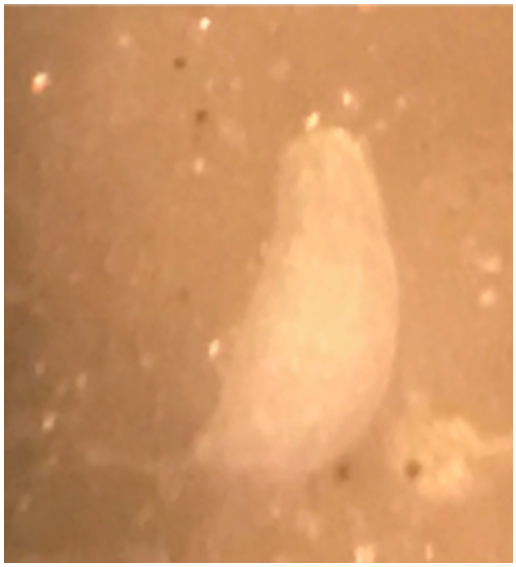


Figure 6: Picture of mesoceracariae 144 hours (6 days) after infection of host tadpole on 09/27/16.

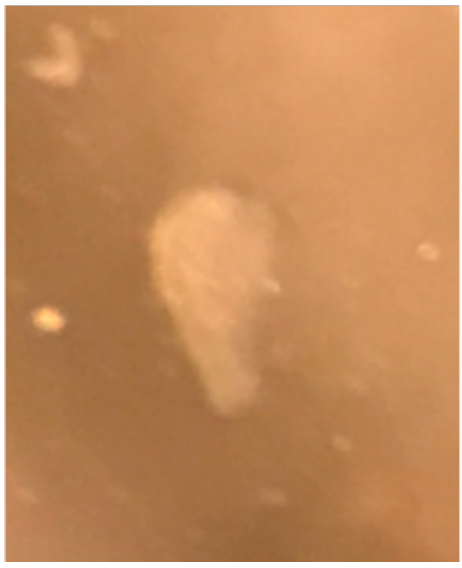


Figure 7: Picture of mesoceracariae 168 hours (7 days) after infection of host tadpole on 09/27/16.

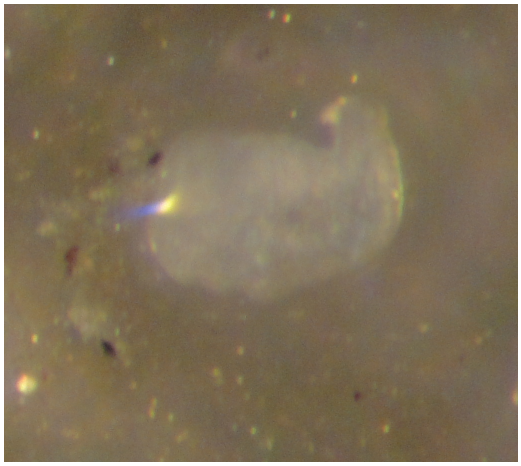


Figure 8: Picture of mesoceracariae moving around the body cavity of the tadpole 168 hours (7 days) after infection of host tadpole on 09/27/16.

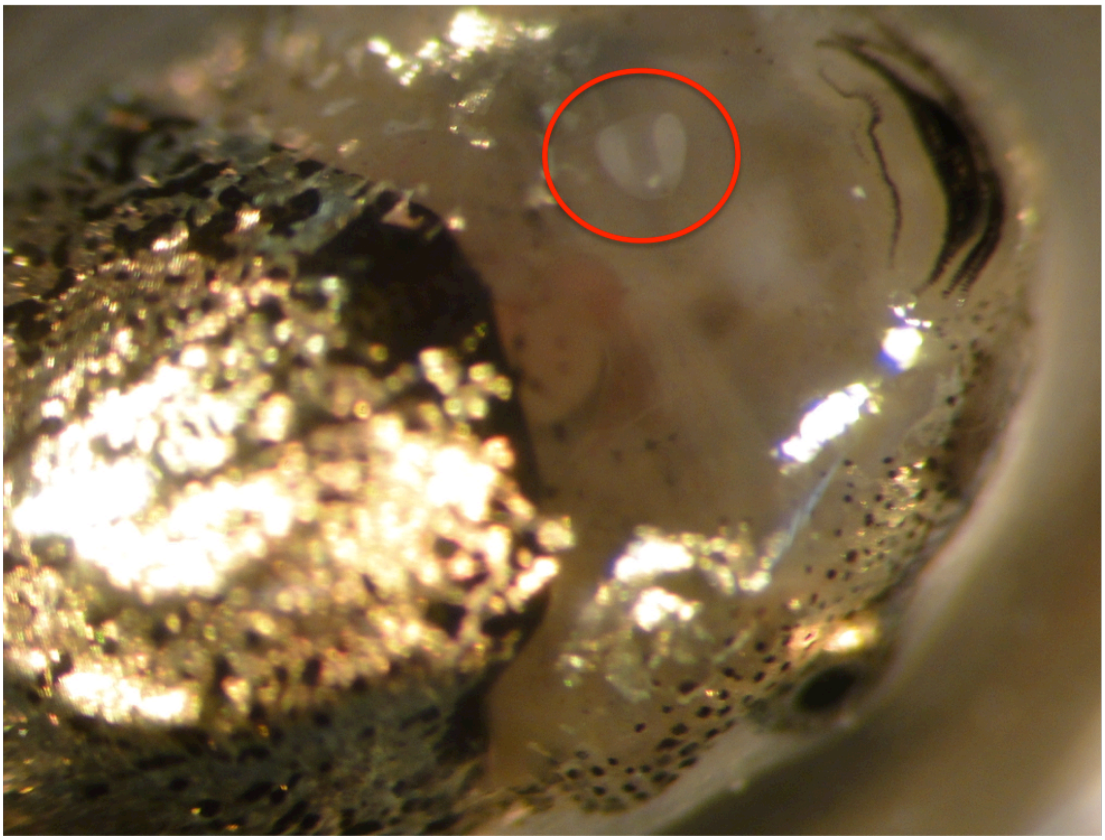


Figure 9: Picture of mesoceracariae 192 hours (8 days) after infection of host tadpole on 09/27/16.

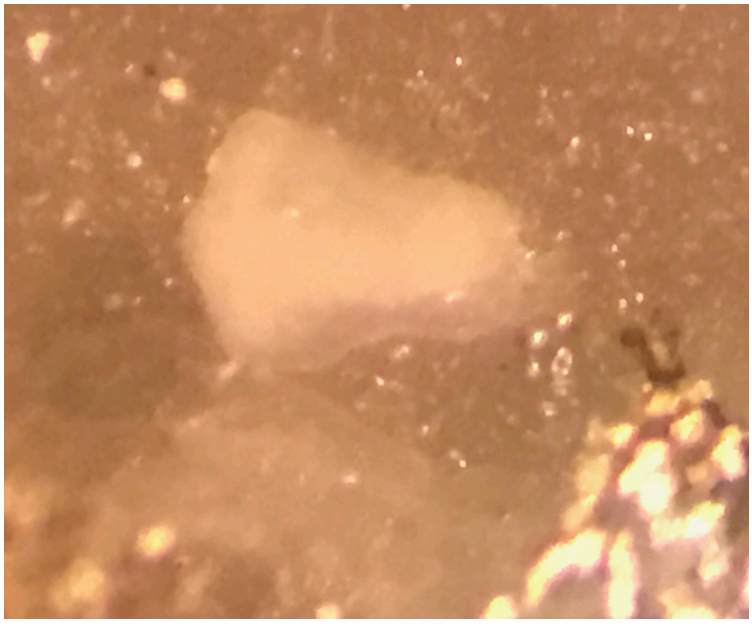


Figure 10: Picture of mesoceracariae 216 hours (9 days) after infection of host tadpole on 11/21/16.

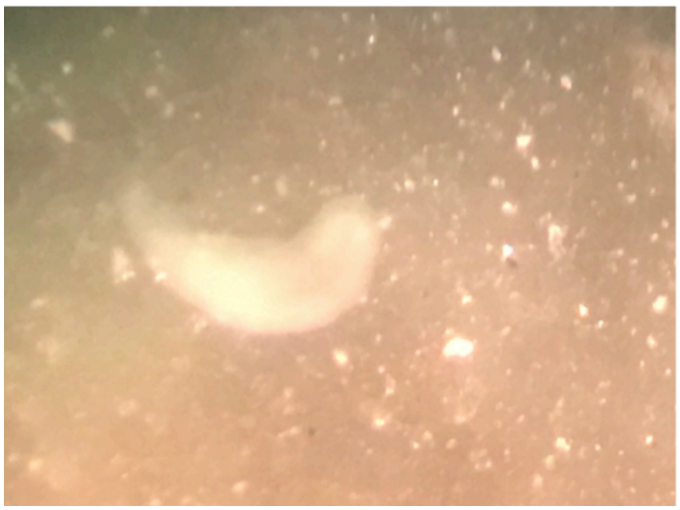


Figure 11: Picture of metaceracariae 240 hours (10 days) after infection of host tadpole on 11/21/16.

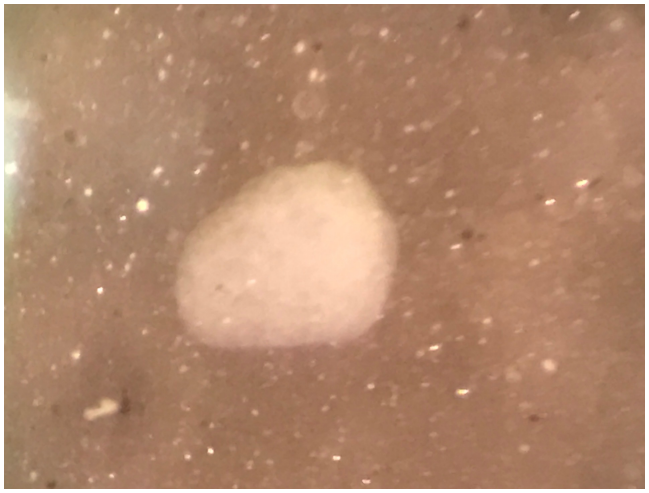


Figure 12: Picture of the metaceracariae 240 hours (10 days) after infection of host tadpole on 11/21/16.

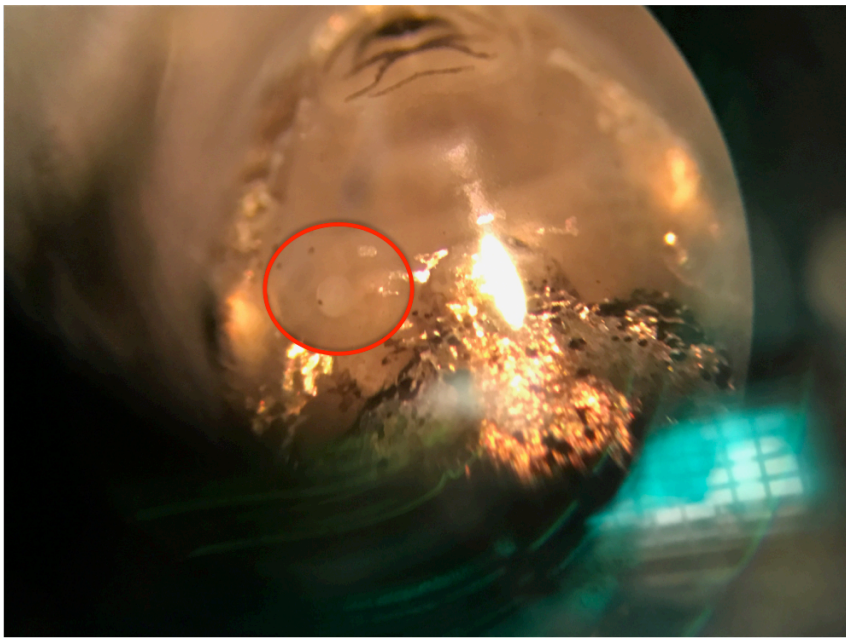
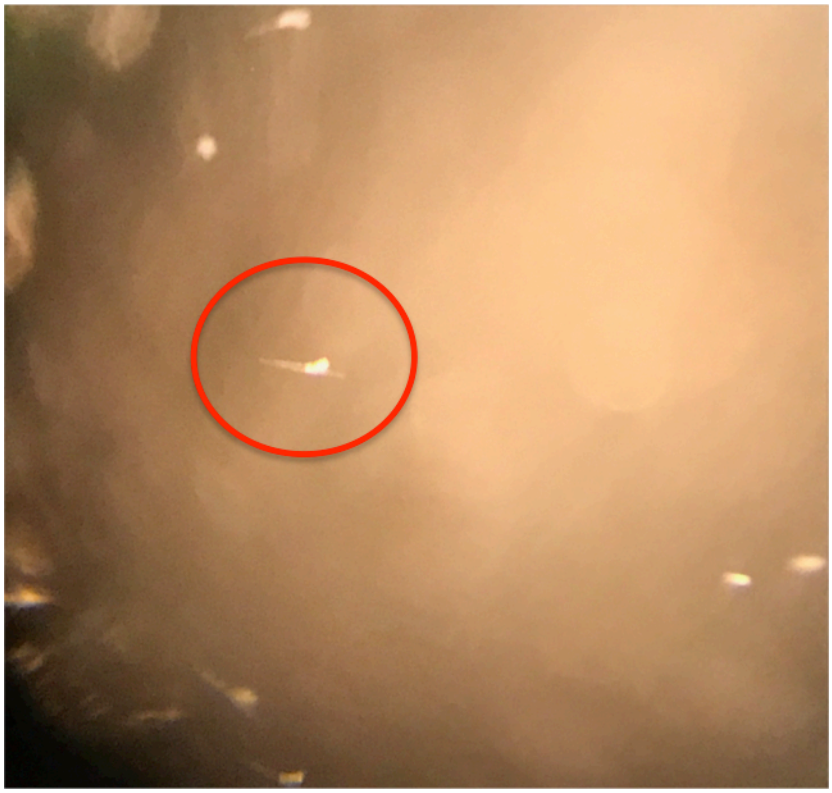


Figure 13: Picture of ceracariae taken on November 16, 2016.

Figure 14: Picture of ceracariae swimming in container taken on 11/30/16.



4. Discussion

Previous research conducted at Florida Southern College found that this smaller, narrower ceracariae, did cause metaceracariae around the pericardium of anuran tadpoles (Prev. Comm., G. Langford). Therefore, the results of this experiment confirmed this observation, because Figures 1-12 show the development of the cercariae in the Cuban Tree Frog Tadpole. Figure 1 shows a distinctive metacercariae that moved around the body cavity of the tadpole. It no longer has its tail, because it was shed almost immediately after penetration. Figures 2-10 show the mesoceracariae that were found moving around the body cavity of the tadpole. There was no extreme change observed until Day 10 (Figure 11 and 12), where it is obvious that the mesoceracariae had begun to

fold in on itself and turn into the metaceracariae. The metaceracariae observed on Day 10 was no longer moving around the body cavity. Rather, it was unmoving, located near the pericardium (Figure 12).

Figure 13 and 14 show an image of the ceracariae just after being shed from the planorbid snail. Figure 13 shows the cercariae isolated on a slide while Figure 14 shows what the ceracaria looked like in the glass container. It was found that their behavior when contained was very similar to the behavior of *A. pipientis*, which has been characterized as intermittently sinking and rising, while swimming every so often, with only a small few swimming at any given moment (Oliver, 1940).

The identity of the parasite was analyzed upon examining the development of the trematode in the tadpole. For identification of this ceracariae, genuses were identified that were known to infect anuran tadpoles. Most ceracariae in the Strigeidae family that are known to form mesoceracariae in tadpoles infect the host by penetration of the tadpole's tail prior to metamorphosis. This is because the ceracaria are more adapted to penetrating in the strong muscled regions of the tail. After penetration, these mesocercariae will migrate from the tail to other parts of the tadpole so that they are able to survive metamorphosis when the frog no longer has the long, muscular tail (Shoop 1989). These characteristics of the family support the conclusion that this narrower cercariae is a part of the Strigeidae family.

When trying to narrow the identification of the parasite down to the genus, the closer relatives of *Apharyngostrigea* were explored. *Apharyngostrigea* is similar to *Cardiocephalus* and *Parastrigeidae* in that they have the tetracotyle type metacercaria stage (Shoop, 1989). However, the conclusion was drawn that this parasite was not a part

of the *Cardiocephalus* genus, because this genus is known to infect fish (Sukhdeo, 1994).

Consequently, the genus, *Parastrigeidae,* was researched further. This genus contains three species: *P. plataleae, P. diovadena, and P. cincta* (Hernadez-Mena et al., 2014). *Parastrigeidae* is similar to *Apharyngostrigea* in that they have uniformly distributed vitelline follicles in the hind body (Schell, 1970). However, *Parastrigeidae* have vitelline follicles in two symmetrical masses that are localized in lateral expansions of the dorsal lobe of the holdfast organ (Gibson et al., 2002). Additionally, *Parastrigeidae* is described as having a fore body pyriform that has lateral expansions (Schell, 1970). This characteristic confirms that *Parastrigeidae* have a wider fore body than hind body. This is different from *Apharyngostrigea* who have a larger and wider hind body (G. Langford, Prev. Comm.). Furthermore, *Parastrigea* is known to be found in the white ibis (Hernandez-Mena et al., 2014), which also supports the conclusion that the cercariae is a member of the *Parastrigea* genus.

One surprising obstacle to this experiment was the fact that when collecting snails from 8/25/16 to 10/27/16, no *A. pipientis* were found. Only the smaller, narrower ceracaria were found. Furthermore, a total of 3,980 snails where surveyed, however, only four snails were found to host the tremadode. Possible reasons for lack of parasite population could have been due to variability in the City of Lakeland's fertilizing methods, or an extremely large population of snails that led to a dilution of the parasite population. The limiting factor was that the life cycle of the parasite is seasonal and that research could no longer be conducted after the wetland had dried at the end of October.

Future research that should be conducted on this topic include using Polymerase Chain Reaction. Sequences from the barcode region of cytochrome oxidase I (CO1) should be obtained in order to compare its genome to determine its identity (Locke et al., 2011) and determine the species of *Parastrigea*. Morphologically, the *Parastrigea* species is known to have a pharynx and a fore body with two symmetrical masses of vitelline follicles (Gibson et. al., 2002). Therefore, using a microtome to section the mesocercariae would prove helpful to identifying the organism. Staining the mesoceracariae would also serve to further elucidate the internal structures of the trematode. Finally, because the Strigidae Family is known to have wading birds as their final host (Shoop,1989), experimental infection of a bird would also be vital in completion of the lifecycle.

5. Acknowledgements

I would like to thank Dr. Gabriel Langford for his extreme patience and guidance throughout the research process. Additionally, I would like to thank the Florida Southern College Biology Department for providing the recourses necessary for success.

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