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Trematode Gill Parasite Infection in Brachyrhaphis Episcopi

Kara Million

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+1 (256) 824-7339 (fax)
honors@uah.edu

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Student Name: Kara Million

Department: Biological Sciences

College: Science

Degree: Bachelor of Science

Advisor Name: Dr. Bruce Stallsmith

Full title of project as it should appear in Graduation Program and on transcript:

Trematode Gill Parasite Infection in *Brachyrhaphis episcopi*

Abstract (should be included at the beginning of your project as well):

The purpose of this study was to examine samples of *Brachyrhaphis episcopi,* a Panamanian live-bearing stream fish, for the presence of monogenean trematode gill parasites of the genus *Dactylogyrus.* Fish were collected from five stream sites in Panama during May. The gill arches of the fish were removed and stained in an acetocarmine solution, then examined under a dissecting microscope at 50x magnification. 109 fish were collected and examined, and 146 parasites were found. A correlation was found between host length and the number of parasites on the host. While very few parasites were found on fish collected from Rio Macho, Rio Guaybo, and Quebrada Sardinilla, many parasites were found on the fish collected from Rio Frijolito and Rio Mendoza. Rio Frijolito and Rio Mendoza also yielded a significantly higher prevalence of parasite infection than the other three streams. The number and prevalence of parasites on the fish may be influenced by the environmental factors of each site, as there were significant differences in characteristics from stream to stream. Samples of the parasites were mounted whole on slides and examined under a digital confocal microscope to begin work on describing new species. Because these fish have never been examined for parasites before, we are fairly certain we have found undescribed species of gill parasites.

Approved by:

Project Advisor: Bruce Stallsmith (Signature) Date: 11/30/11

Department Chair: Debra M. Moriarity (Signature) Date: 11/30/11

Honors Program Director: (Signature) Date: 12-2-11

Trematode Gill Parasite Infection in *Brachyrhaphis episcopi:*

Analysis of presence, prevalence, and characteristics of monogenean parasites on livebearing *B. episcopi* from five stream sites in Panama.

Senior Honors Thesis

Submitted in partial fulfillmentof the requirements for the Honor's Certificate upon Graduation

By

Kara Million

University of Alabama in Huntsville

Huntsville, Alabama 35899

Fall 2011

Abstract:

The purpose of this study was to examine samples of *Brachyrhaphis episcopi,* a Panamanian live-bearing stream fish, for the presence of monogenean trematode gill parasites of the genus *Dactylogyrus*. Fish were collected from five stream sites in Panama during May. The gill arches of the fish were removed and stained in an acetocarmine solution, then examined under a dissecting microscope at 50x magnification. 105 fish were collected and examined, and 146 parasites were found. A correlation was found between host length and the number of parasites on the host. While very few parasites were found on the fish collected from Rio Macho, Rio Guaybo, and Quebrada Sardinilla, many parasites were found on the fish collected from Rio Frijolito and Rio Mendoza. Rio Frijolito and Rio Mendoza also yielded a significantly higher prevalence of parasite infection than the other three streams. The number and prevalence of parasites on the fish may be influenced by the environmental factors of each site, as there were significant differences in characteristics from stream to stream. Samples of the parasites were mounted whole on slides and examined under a digital confocal microscope to begin work on describing new species. Because these fish have never been examined for parasites before, we are fairly certain we have found undescribed species of gill parasites.

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Introduction

This project began as an examination of *Brachyrhaphis episcopi* for the presence of *Dactylogyrus* parasites or parasites of a similar genus. The following sections introduce the *Dactylogyrus* parasite genus, the potential host fish (*B. episcopi*), and the Panama stream sites from which the fish were collected.

Dactylogyrus

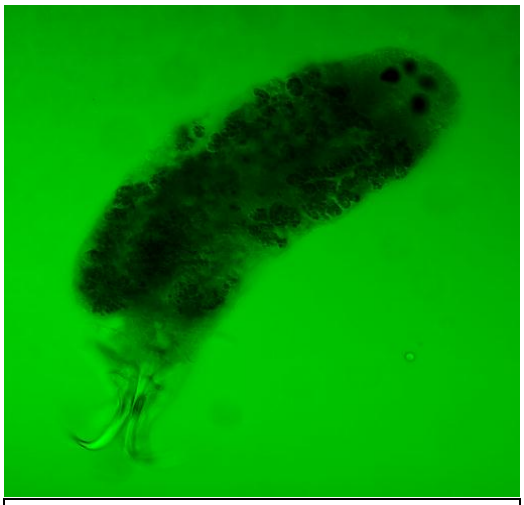


Figure 1. Confocal image of dactylogyrid. Host fish: *Etheostoma zonale* (banded darter). Haptor and eyespots obvious. Photo by Kara Million.

Dactylogyrus (family Dactylogyridae) are a monogenean class of parasitic trematodes (Hoffman 2006). Dactylogyrus are nearly transparent flatworms that have elongated bodies equipped with four eyespots on the head and a pair of dorsal anchors on a haptor apparatus, as well as 14 marginal hooks which they use to latch onto their host (See Figures 1 and 2). They are typically found on the gill arches of fish and complete their entire life cycle on a single host. The parasites appear to have a preference for attaching to the middle sections of the gill filaments (Turgut and others 2006). *Dactylogyrus* feed on the blood and mucus of the host, which can place significant stress on the fish's body and cause secondary infections. In some studies, *Dactylogyrus* infections have been shown to reach their peak during the fish's reproductive seasons, most likely because the stress of reproduction causes the fish to be more vulnerable to infection (Ozturk and others 2006).

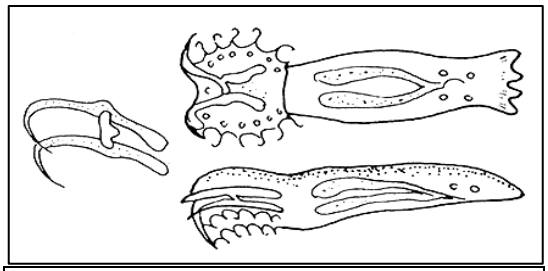


Figure 2. Illustration of *Dactylogyrus* parasite. Left: anchor hooks. Right: ventral and side views. FAO 2011.

Dactylogyrus are highly host-specific, with each species of dactylogyrid corresponding to a preferred species of fish (Simkova and others 2006). It is thought that mechanical or chemical stimuli on the surface of the host are responsible for the ability of the parasites to discern between species of fish and find

their preferred hosts (Buchmann and others 2001). One study demonstrated a competitive exclusion phenomenon between two *Dactylogyrus* species on the reared carp (Papernon 1964).

Figure 3. *Brachyrhaphis episcopi.* Andreas J 2005.

Brachyrhaphis episcopi

Brachyrhaphis episcopi (common name: Bishop) are livebearing poecilid fishes native to freshwater systems in South America (Parenti and others 1999). Males are between and mm in length, and females are between and mm. During breeding seasons, males will adapt one of their fins into an intromittent gonapodium used to inseminate the females. They prefer calm zones of flowing waters, standing waters, marshes and other shallow areas. They are often kept as aquarium fish. Brachyrhaphis have been shown to display more bold and aggressive behavior in habitats where there is heavier predation than in low-predation habitats, despite the common impression that low predation promotes aggressiveness in fish (Archard and others 2010).

Panama Stream Sites

Figure 4. Map of Soberania National Park, Panama. Stream sites highlighted in yellow. Top left: R. Macho. Bottom left: R. Mendoza. Middle: R. Frijolito. Bottom far right: Q.

Fish were collected from five stream sites in Panama, each with its own unique environment and geographic features. According to Dr. Stallsmith, who collected the fish from the sites, Rio Guaybo is a highland stream about 80 miles west of the Canal. The

other four sites- Rio Macho, Rio Mendoza, Rio Frijolito, and Quebrada Sardinilla (see figure 4)- are located in Soberania National Park in heavier, wetter rainforest east of the Canal. A collection permit and an export permit were obtained from the National Environmental Authority in Panama to authorize the collection and exportation of the fish from the stream sites.

Materials and Methods

The methods used in this project mainly focused on examining the soft gill tissue of the fish for parasites and preparing the found parasites for confocal imaging.

Figure 5. Dr. Stallsmith and Gisela (Smithsonian Marine Lab) collecting fish from dipnet. Stallsmith B 2011.

Collection of fish

Brachyrhaphis episcopi were collected using a dip net from each of the five Panama stream sites in May. Fish were not selected based on size or sex. The fish were euthanized in a solution of clove oil dissolved in 10% ethanol and 90% water. The fish were then preserved in 10% phosphate buffered formalin. The fish were measured for standard length with a digital caliper (.01 mm) and weighed with a digital scale (.0001 g). The sex of each fish was determined based on the presence or absence of a fully-developed intromittent gonapodium.

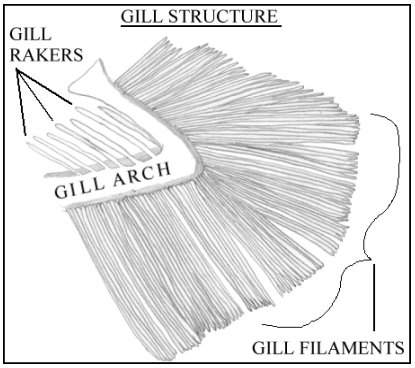


Figure 6. Structure of fish gill with rakers, arch, and filaments labeled. Fisheries and Oceans Canada 2005.

Examination of Gill Tissue

The gill arches of each fish were removed using fine forceps by forcing open the operculum and pulling out the gill

arches whole. The gills were stained in a 0.5% acetocarmine solution for at least 24 hours, then examined under a dissecting microscope at 50x magnification. Gill arches were separated from one another using the forceps and feathered through with a needle (See Figure 6). The arches were examined on one side, then flipped over on the other side to ensure that all the parasites were found and counted. The number of parasites found on each fish was recorded.

Preservation and Mounting of Parasites

Parasites found on the fish were fixed in a 10% solution of alcohol-formalin-acetic acid (AFA) for one hour, then stored in 70% ethanol until ready to be mounted onto slides. The parasites were mounted onto slides using double coverslips and Kleermount solution, a variant of damar mounting substance. Each sample was arranged ventral side down on a coverslip in a drop of Kleermount. Once the sample was in position, a second cover slide was placed on top and gently tapped into place. Once the air bubbles were removed from between the coverslips, the sample was mounted onto a slide using another drop of Kleermount. The slides were allowed to air dry for at least 24 hours before they were examined under a microscope (Upton 2005). A micropipette was used to transfer the parasites in and out of the solutions and onto the slides.

Confocal Images

To aid with description of new species, samples of the parasites were examined under a confocal microscope. The slides were examined at 63x magnification using an oil immersion lens. The images were processed and stored using Zen 2009 LE software.

Results and Discussion

A total of 105 fish were collected and a total of 146 parasites were found on the fish. While three of the stream sites yielded little to no parasites on the fish collected, two of the sites---Rio Frijolito and Rio Mendoza--- yielded an abundance of parasites. In the following sections the data and findings are

discussed in detail. Tables of the raw data from each site are located in the Appendix.

Number and Prevalence of Parasites Found

Very few parasites were found on the fish collected from Rio Macho, Rio Guaybo, and Quebrada Sardinilla, while a relative abundance of parasites were found on the fish collected from Rio Frijolito and Rio Mendoza. Not only did Frijolito and Mendoza have a higher number of total parasites found on the fish, but the prevalence of parasite infection among the hosts was also much higher for both sites than for Rio Macho, Rio Guaybo, and Quebrada Sardinilla (See Figure 7). Several factors may be responsible for the difference in parasite infection among the different populations.

Stream Site	# fish collected	# parasites found	Infection prevalence
R. Frijolito	22	27	45.5%
R. Guaybo	16	5	0.31%
R. Macho	23	3	0.13%
R. Mendoza	24	110	75.0%
Q. Sardinilla	20	1	0.05%

Figure 7 . Summary of data for all five sites: number of fish collected, number of parasites found at each site, and prevalence (percentage of infected fish/ total population). Bush and others 1997.

Chance

Chance may play a role in the variation of infection between the populations. Monogeneans hatch in the water and the larvae swim in search of their hosts of preference. As the prevailing theory on host specificity is that each species of gill parasite has its own unique host, the parasites must be able to find that host and successfully attach to the gill tissue. More fish from each site must be collected to determine how large a role chance may play in the infection of the fish by parasites.

Variations in Sites

Another factor that may influence the parasite infection load on the hosts is the variations in the sites from which the fish were collected. Rio Guaybo is a highland stream about 80 miles to the west of the Canal. It runs over sand and rocks down the side of a dormant volcano. Rio Macho, Rio Frijolito, Rio Mendoza, and Quebrada Sardinilla are located in rainforest east of the Canal, where the highland streams level out before they flow into the Canal. Rio Mendoza is an especially deep and wide stream compared to the other sites. The fish from the other sites were collected in very shallow rivulets. The depth and flow rate of the water may have influenced the parasites' ability to find and attach to their hosts. More samples would have to be taken from all the sites to establish a more firm correlation between site location and parasite infection load.

Correlation Between Fish Length and Parasite Load

A strong correlation was found between the standard length of the host fish and the number of parasites found on the fish. The data was run through a PASW Statistics program, release 18 (see Figures 9-14). A one-tailed ANOVA regression yielded an F value of 25.98 with a significance of $p < 0.000$. The R coefficient was 0.45, and the r-squared was 0.2, establishing a statistically significant relationship between fish length and number of parasites.

The higher gill surface area on the larger fish could facilitate parasite infection more readily. Furthermore, the older age of the larger fish may have allowed them more time to accumulate parasites. This correlation is consistent with data for other fish populations and their dactylogyrid parasites (Adrian 2009).

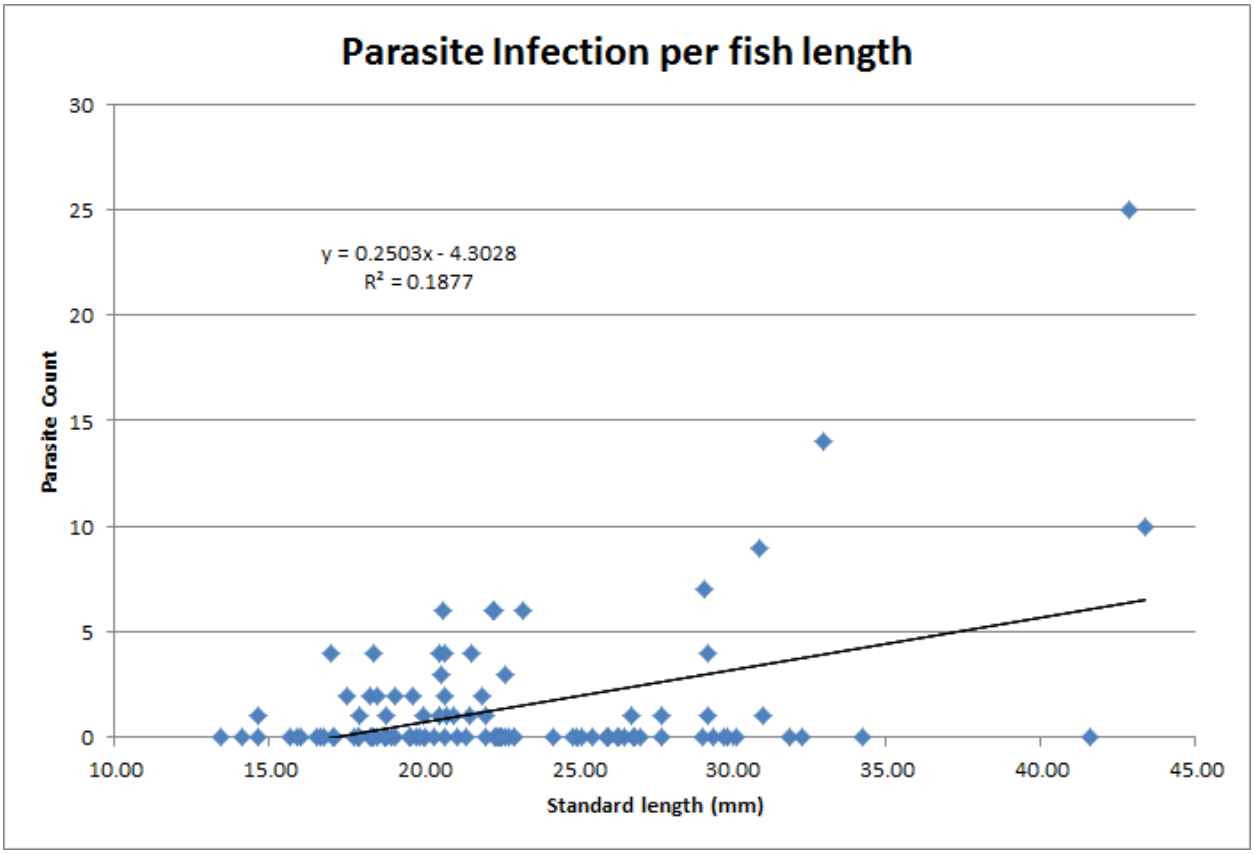


Figure 8. Scatter plot of parasite infection per fish length.

Descriptive Statistics			
	Mean	Std. Deviation	N
VAR00002	1.4038	3.33420	104
VAR00001	22.6353	5.66201	104

Figure 9. Descriptive statistics.

Correlations			
		VAR00002	VAR00001
Pearson Correlation	VAR00002	1.000	.451
	VAR00001	.451	1.000
Sig. (1-tailed)	VAR00002	.	.000
	VAR00001	.000	.
N	VAR00002	104	104
	VAR00001	104	104

Figure 10. Pearson correlation.

Variables Entered/Removed[a]

Model	Variables Entered	Variables Removed	Method
1	VAR00001	.	Stepwise (Criteria: Probability-of-F-to-enter <= .050, Probability-of-F-to-remove >= .100).

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics		
					R Square Change	F Change	df1
1	.451[a]	.203	.195	2.99116	.203	25.980	1

Figure 11. Variables entered/removed and model summary.

Model Summary

Model	Change Statistics	
	df2	Sig. F Change
1	102	.000

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	232.443	1	232.443	25.980	.000[a]
	Residual	912.595	102	8.947		
	Total	1145.038	103			

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-4.602	1.214		-3.790	.000
	VAR00001	.265	.052	.451	5.097	.000

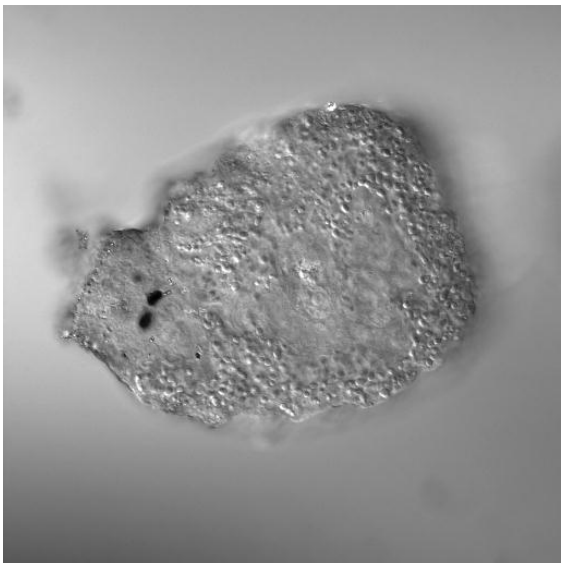
Figure 12. Model summaries.

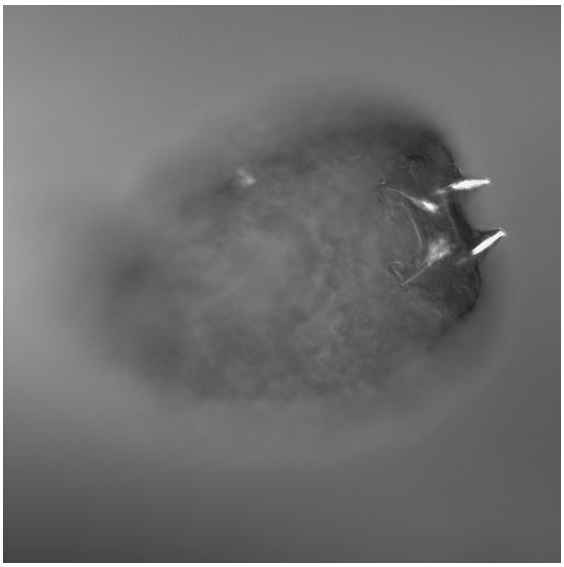
Coefficients[a]						
Model		Correlations			Collinearity Statistics	
		Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)					
	VAR00001	.451	.451	.451	1.000	1.000

Figure 13. Coefficients.

Collinearity Diagnostics[a]							
Model		Dimension		Eigenvalue	Condition Index	Variance Proportions	
						(Constant)	VAR00001
1		1		1.970	1.000	.01	.01
			2	.030	8.157	.99	.99

Figure 14. Colinearity diagnostics.





Figures 15 and 16. Confocal images of parasite found on fish from Rio Frijolito stream site. Taken with 63x oil immersion lens. Left: body, eyespots visible. Right: haptor bearing hooks. Photos by Kara Million.

Possible New Species of Parasites

Confocal microscope images were obtained of the parasites from the Rio Frijolito and Rio Mendoza populations. The observed physical characteristics for the parasites differed significantly from those of known *Dactylogyrus* species in both body shape and haptor arrangement (See Figures 15-16). The bodies of the Rio Frijolito parasites were more rounded in shape and the hooks were more narrow and curved in a talon-like manner. The parasites from the Rio Mendoza hosts appeared very diverse in shape and size. Because *B. episcopi* have previously never been examined for gill parasite infections, and because of the proposed host-specificity of monogeneans, these parasites are most likely undescribed species. More images of the parasites must be obtained to aid with species description.

Conclusion

This project has revealed an abundant amount of gill parasite infection on *Brachyrhaphis episcopi* collected from the Panama streams Rio Frijolito and Rio Mendoza. There appears to be very little gill parasite infection on *Brachyrhaphis* collected from Rio Guaybo, Rio Macho, and Quebrada Sardinilla. Chance and differences in environment from stream to stream are

possible reasons for the variation in parasite load from one fish population to another, as parasites must find and successfully infect their specific host, and some environments may facilitate infection more readily than others.

A strong correlation was established between fish length and number of parasites on the host, most likely due to the age of the fish. Older, larger fish have a larger gill tissue area and more time to accumulate parasite infection.

Images of the found parasites show significant differences in physical characteristics from previously described dactylogyrid species, suggesting the possibility of undescribed parasite species. This would be consistent with the host specificity of dactylogyrids. More images need to be obtained to enable the description of any new genera or species present on the host fish.

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Appendix: Raw Data

Brachyrhaphis episcopi	Rio Macho, Panama		May 21, 2011	
Sample #	Length (mm)	Mass (g)	gender	# parasites found
1	34.25	0.9875	F	0
2	22.95	0.2876	M	0
3	30.12	0.5260	F	0
4	26.53	0.3951	M	0
5	26.80	0.4153	F	0
6	26.71	0.3464	M	1
7	25.97	0.3477	M	0
8	27.01	0.4108	F	0
9	18.27	0.1183	F	0
10	15.68	0.0808	F	0
11	25.13	0.2777	F	0
12	19.62	0.1161	M	0
13	29.36	0.5716	F	0
14	25.93	0.3615	M	0
15	20.77	0.1458	M	1
16	25.46	0.3496	M	0
17	22.29	0.2038	M	0
18	22.50	0.2315	F	0
19	22.38	0.2128	M	0
20	21.00	0.1945	M	1
21	22.74	0.2046	F	0
22	21.11	0.2669	F	0
23	26.85	0.3745	F	0

Brachyrhaphis episcopi	Rio Guaybo, Panama		May 21, 2011	
Sample #	Length (mm)	Mass (g)	gender	# parasites found
1	22.43	0.2314	F	0
2	30.98	0.4830	F	1
3	21.37	0.1794	F	0
4	13.45	0.0472	Juvenile	0
5	14.63	0.0676	Juvenile	1
6	16.54	0.1145	F	0
7	16.04	0.1005	F	0
8	19.09	0.1265	F	0
9	17.14	0.1175	F	0
10	18.82	0.1534	F	0
11	20.01	0.1564	F	1
12	20.03	0.1723	F	0
13	27.73	0.3265	F	1
14	31.85	0.6161	F	0
15	29.23	0.5146	F	1
16	32.25	0.6930	M	0

Brachyrhaphis episcopi	Rio Frijolito, Panama		May 21, 2011	
Sample #	Length (mm)	Mass (g)	Gender	# parasites found
1	22.66	0.2127	M	3
2	20.60	0.1331	M	3
3	20.50	0.1357	M	4
4	22.92	0.1932	F	0
5	21.55	0.1879	F	4
6	16.64	0.0746	F	0
7	18.74	0.1049	M	0
8	17.05	0.0816	F	0
9	17.89	0.0805	M	0
10	18.50	0.1081	F	0
11	18.79	0.0969	M	0
12	18.37	0.0961	F	4
13	19.77	0.1127	M	0
14	18.78	0.1116	M	1
15	18.30	0.1192	F	2
16	18.97	0.1615	F	0
17	22.02	0.1606	F	1
18	20.54	0.1390	F	1
19	26.34	0.3004	F	0
20	24.21	0.2552	F	0
21	29.86	0.4820	F	0
22	29.21	0.4794	F	4

Brachyrhaphis episcopi Quebrada Sardinilla, Panama May 20, 2011				
Sample #	Length (mm)	Mass (g)	Gender	# parasites found
1	41.59	1.8667	F	0
2	22.00	0.2035	F	0
3	14.15	0.0710	Juvenile	0
4	24.96	0.2832	M	0
5	15.90	0.0703	F	0
6	14.63	0.0611	Juvenile	0
7	16.77	0.0927	M	0
8	21.52	0.1705	M	1
9	17.78	0.1041	M	0
10	18.32	0.1145	M	0
11	19.56	0.1607	F	0
12	19.89	0.1500	F	0
13	20.69	0.1750	F	0
14	22.54	0.2100	F	0
15	22.66	0.2784	F	0
16	26.01	0.3365	F	0
17	24.86	0.3016	F	0
18	27.71	0.4760	F	0
19	29.04	0.4503	F	0
20	29.71	0.4359	F	0

Brachyrhaphis episcopi	Rio Mendoza, Panama		May 18, 2011	
Sample #	Length (mm)	Mass (g)	Gender	# parasites found
1	43.37	2.0365	F	10
2	42.89	2.1696	F	25
3	32.93	0.9134	F	14
4	30.87	0.7038	F	9
5	29.08	0.7166	F	7
6	26.28	0.3707	F	0
7	19.10	0.1561	M	2
8	20.67	0.2089	M	4
9	17.51	0.1132	F	2
10	17.00	0.1145	F	4
11	17.94	0.1273	F	1
12	19.67	0.1618	F	2
13	18.41	0.1480	F	0
14	17.91	0.1336	F	0
15	18.81	0.1602	F	0
16	20.72	0.1780	M	2
17	22.33	0.2077	M	6
18	20.34	0.1800	F	0
19	18.49	0.1302	M	2
20	22.52	0.2000	F	0
21	20.61	0.2018	F	6
22	22.22	0.2330	F	6
23	21.92	0.2225	M	2
24	23.21	0.3142	F	6