A SURVEY OF MONOGENETIC TREMATODES FROM THE GILLS OF *SALMO GAIRDNERI*, *SALVELINUS FONTINALIS*, AND *SALMO TRUTTA* IN WATAUGA COUNTY, NORTH CAROLINA

A Thesis

by

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ABSTRACT

A Survey of Monogenetic Trematodes
from the Gills of Salmo gairdneri,
Salvelinus fontinalis, and Salmo trutta
in Watauga County, North Carolina

February 1974

Ernest James Arlart, B. S., Wake Forest University

Directed by: Dr. Richard N. Henson

Gyrodactylus salmonis Yin and Sproston, 1948, and Gyrodactylus colemanensis Mizelle and Kritsky, 1967, were found on the gills of Salmo gairdneri Richardson, and Salvelinus fontinalis Mitchell, 1815, in Watauga County, N. C.. This is the first report of these species of Gyrodactylus in North Carolina. Examination of Salmo trutta L., 1758, revealed no parasites on the gills. Host specificity was not observed.

ACKNOWLEDGEMENT

This author wishes to express his thanks and appreciation to Dr. Richard N. Henson, without whose unswerving aid and guidance, this paper would have been impossible. I would also like to thank the members of my thesis committee, Dr. F. Ray Derrick and Dr. William R. Hubbard, for their help and encouragement. I would like to express my appreciation to the Appalachian State University Library for its assistance and reliability in obtaining the literature for my research. Dr. Wilmer A. Rogers' aid in identification was most helpful. In addition, I want to thank those friends and students who aided in collecting host specimens.

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INTRODUCTION

This study is a survey of the monogenetic trematodes parasitizing the gills of Salmo gairdneri Richardson, Salmo trutta L., 1758, and Salvelinus fontinalis Mitchell, 1815, from stocked ponds and streams in Watauga County, N. C.. (See Map, p. 15.)

The scarcity of knowledge concerning monogenean parasites of trout provides an open area for investigation. At present, there is no publication on monogenetic trematodes of trout in N. C..

It is hoped this study will contribute to the knowledge of host specificity, speciation, and distribution of monogeneans in North America.

Members of the class Trematoda, order Monogenea, these microscopic parasites complete their life cycle on one host. The order Monogenea is characterized by the main organ of attachment, the Haptor, which, at some stage in the worms' development, has 12 to 16 marginal hooklets and 2 or 4 centrally located, large hooks or anchors (Hoffmann, 1967).

Most species live on fish, both fresh water and marine. Some live on the gills, the body and the fins, and some on both. The food of the monogenean is host mucus, epithelium, and sometimes blood (Hoffmann, 1967). Their feeding organ is a muscular pharynx.

The style and citations of this thesis follow the format of The Journal of Parasitology.

Trout in Watauga County carry two species of monogenea, both of the genus Gyrodactylus Nordmann, 1832. This genus comes under the suborder Monopisthocotylea Odhner, 1912, having anterior adhesive glands, and maintaining the larval haptor in the adult (Yamaguti, 1963); family Gyrodactylidae Cobbold, 1864, characterized by its viviparity; subfamily Gyrodactylinae Monticelli, 1892, with bilobed anterior, well developed haptor with one pair of large hooks (anchors), 16 marginal hooklets, and a cirrus, or copulatory organ, armed with spines (Hoffmann, 1967).

The only genus of this subfamily in North America is Gyrodactylus Nordmann, 1832. There are numerous species and subspecies or varities of this genus, causing confusion in the taxonomic status. The species G. salmonis Yin and Sproston, 1948, has the synonyms G. elegans salmonis Yin and Sproston, 1948, and G. elegans var. B. Mueller, 1936 (Malmberg, 1970). The species G. colemanensis Mizelle and Kritsky, 1967, still remains valid according to Malmberg (1970). These two species of Gyrodactylus are found on trout in Watauga County, N. C..

REVIEW OF LITERATURE

Thus far, no publication has dealt with monogenetic trematodes on trout in the waters of western North Carolina. These flukes are known to damage the gills of Salmo gairdneri as reported by Pratt (1919) at the New York State Fish Hatchery at Cold Spring Harbor, Long Island, N. Y.. In this case, practically all the trout a year or more old were infected. In many cases the infection was so severe that the gills were shriveled and functionless, resulting in death. Van Cleave (1921) draws the conclusion, from Pratt's paper, that this epidemic was caused by a representative of the genus Gyrodactylus, but no specific determination of the parasite was given.

Serious damage to fish by these ectoparasitic flukes, especially under crowded hatchery conditions, has been reported from several sources. Yin and Sproston (1948) attribute fatal epidemics in aquaria and among young fry in breeding tanks to Gyrodactylus elegans Nordmann, 1832. This species, having a wide distribution in Europe, North America, the U.S.S.R., and Eastern Asia, is common on pond fishes. Dr. Ron Clauda (personal communication, 1972) of the Armstrong Fish Hatchery, Marion, N. C., reveals the death of several hundred trout in a commercial pond in Watauga County, N. C.. This epidemic was believed to be caused by Gyrodactylus.

Viviparity, characteristic of Gyrodactylus is conducive to the production of epidemics among fishes (Mizelle, 1937). These parasites have one host and are spread via direct contact between hosts. By using their posterior hooks and anterior adhesive organ, they move along the surface of the host and may catch hold of a new host which comes in contact with the old host. According to Mizelle (1937), there are less numerous records of outbreaks caused by oviparous species.

There is some evidence that early infestation with monogeneans results in great structural modifications in young fishes. One example is the complete separation of the gill membranes from the isthmus (Hubbs, 1927).

Antagonism between different species of parasites to which fish are naturally exposed has been discussed by Bauer (1959). Paperna (1964) has found that the infestation of carp gills by Dactylogyrus vestator Nybelin, 1924, creates an environment unfavorable for the survival of other species. Histological changes, mainly hyperplasia of the epithelial lining and of the mucus goblet cells leave unsuitable sites for Dactylogyrus extensus Mueller and Van Cleave, 1932. Eventually, regeneration and healing of the gill tissue enable a renewed infestation by the excluded species, but Dactylogyrus vestator resumes dominance with the elimination of the other species (Paperna, 1964).

Atkins (1901) reports an infestation of young lake trout with Gyrodactylus elegans at the Craig Brook Station of the U. S. Fisheries Commission. Gyrodactylus brevis Crane and Mizelle, 1967, was found

on the external surface of Salmo gairdneri in the Navarro River, Mendocino County, Calif., by Crane and Mizelle (1967). Muller (1936) described G. elegans var. B from the cutthroat, brown, and brook trout in New York. Yin and Sproston (1948) later named this G. elegans salmonis. According to Yin and Sproston (1948) there are six subspecies of G. elegans. Rogers believes Muller's G. elegans var. B is definitely a new species as described by Yin and Sproston (Personal communication, 1973). Malmberg (1970) raises G. elegans salmonis to the species rank G. salmonis Yin and Sproston, 1948.

Mizelle and Kritsky (1967) described G. colemanensis, n. sp., from the steelhead, Salmo gairdneri Richardson, Coleman National Fish Hatachery, Shasta County, Calif.; and the Navarro River, Mendocino County, Calif.. Hathaway and Herlevich (1973) described G. colemanensis from Salvelinus fontinalis in Colorado.

According to Dr. Wilmer A. Rogers (Personal communication, 1973) of Auburn University, the "Gyros" are highly host specific and even subspecies or strain specific many times.

MATERIALS AND METHODS

A total of 89 trout were collected from various locations in Watauga County, N. C. from June 22, 1972 to May 16, 1973. Of this number: 40 were Salmo gairdneri, 40 were Salvelinus fontinalis, and 9 were Salmo trutta.

Host fish were collected by seining or by hook and line. Fish were transported alive in separate containers to the laboratory. This was done to prevent the unnatural mixing of parasite species between different host species.

Parasites were recovered from the gills of the host as suggested by Mizelle (1938). Fish were killed by severing the spinal cord at the base of the skull. The gills were removed and placed in 15 ml plastic, screw-top bottles and covered with tap water. A record of host species, length and sex of host, location of collection site, and date of collection was kept on each host collected. Parasites collected from a set of gills were given a reference number corresponding to this information. The gills were frozen for 12 to 36 hours in the covered bottles. This was an effective means of removing the parasites from the gills as well as killing them in a relaxed condition. The bottles were then removed from the freezer, allowed to thaw, shaken for 30 seconds to loosen any parasites remaining on the gills, and then emptied into separate petri dishes. Each dish was diluted with tap water to clear the remaining mucus and blood. They were examined under a dissecting

scope to locate any parasites present. All parasites were transferred with a capillary pipette to ½-dram vials of 70% ETOH. Each vial was labeled with the appropriate identification number and capped for storage until permanent slides could be made. These could be stored for six months or more with no apparent harm to the parasites if the vials were tightly capped.

For measuring and identification, parasites were mounted both stained and unstained. The two stains used were Delafield's Hemotoxylin and Harris' Hemotoxylin. Best results were obtained using an iron alum mordant along with Harris' Hemotoxylin. Several methods of mounting were used: balsam, CMC 10, and Hoyer's medium. Since the alcohol series could be cut short by using Hoyer's medium, a water soluable, clear mounting medium, Hoyer's was used predominantly. Its use, however, requires drying slides four days at 45° C and ringing the coverslips with nail polish.

The Wild M 30 phase contrast microscope was used for further observations. All drawings were accomplished with the aid of the Wild camera lucida attachment at 1250x using the 100x phase contrast, oil immersion objective. Measurements were made using an eyepiece micrometer, following the method described by Malmberg (1970).

Identification was based primarily on the works of Mizelle and Kritsky (1967), Mueller (1936), Yin and Sproston (1948), and personal communication with W. A. Rogers of Auburn University.

EXPERIMENTAL RESULTS

Of 89 fish examined, only 10 Salvelinus fontinalis and 26 Salmo gairdneri were infected. None of the 9 Salmo trutta were parasitized by monogeneans on their gills.

Two species of Gyrodactylus were found. Salvelinus fontinalis and Salmo gairdneri were infected with both G. salmonis and G. colemanensis. Tables I through XI give the numbers of fish infected and species of parasite found, by location of collection.

Until the publication of Hathaway and Herlevich (1973) there had been no report of G. colemanensis on Salvelinus fontinalis.

The comparison of measurements in Tables XII and XIII confirms the identification of these two species of Gyrodactylus. The mean values and ranges for measurements, given in microns, are in Tables XIV and XV.

Distinctive characteristics of both species, as given by Mizelle and Kritsky (1967), are: anchor bars folded less than 90^{o}; peduncular bar absent; superficial bar (ventral bar) present; superficial bar with shield; peduncle without skirt-like structure; anchor folds present; deep bar (dorsal bar) without notch, not butterfly shaped; hooks shorter than anchors; deep bar without median posterior projection; superficial bar rami do not extend to tip of anchor base; marginal hook loop of one thread. Terms found in parentheses in the above description are suggested by Malmberg (1970).

Distinctive characteristics of G. salmonis (Figs. 1 - 5) are: superficial bar rami same length or shorter than bar width; basal border of hooklet (sickle) concave; cirral spinelets subequal; anchor knobs near middle of anchor base; hook shank without proximal enlargement; hooklet point does not project beyond vertical from tip of hooklet toe.

Distinctive characteristics of G. colemanensis (Figs. 6 - 10) are: superficial bar rami longer than bar width; superficial bar shield does not extend beyond anchor point arc; cirral spinelets subequal and not fused; hooklet shank with proximal enlargement; hooklet toe with shelf.

It was found that anchor bars vary considerably. The most reliable characteristics were the marginal hooklet sickle and hooklet shank (Figs. 4 and 9).

DISCUSSION

These *Gyrodactylus* species do not appear to be extremely host specific. They do seem to prefer *Salvelinus fontinalis* and *Salmo gairdneri* to *Salmo trutta*. However, sampling was random and due to the comparatively small number of *Salmo trutta* collected in Watauga County, this conclusion may be erroneous.

Both species were found on the gills of the host, but may also live on the body surface and fins. This was not observed.

The difference in numbers between the two species on one host may be attributed to chance in sampling or could be a case of competitive exclusion. Due to the small size of these parasites and the difficulty in handling or even detecting them, a quantitative study would be difficult if not impossible.

The state of North Carolina stocks those streams sampled, between March and August. It is not possible, by any reliable means, to determine the length of time the fish were in the stream before sampling. The Appalachian State University Research Pond was stocked in the spring of 1972.

This work presents a new locality record for both species of *Gyrodactylus* and supports the new host record for *G. colemanensis* on *Salvelinus fontinalis*, the Brook trout, reported by Hathaway and Herlevich (1973).

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APPENDIX

COLLECTION SITES

I. Goschen Creek.

II. Dutch Creek - Above the Falls.

III. Dutch Creek - Below the Falls.

IV. Flannery Fork.

V. Howard's Creek.

VI. Winkler's Creek.

VII. Lance Creek - A.S.U. Biological Research Pond.

VIII. Beaverdam Creek.

IX. Payne Branch.

X. Thunder Hole Creek.

XI. Boone Fork.

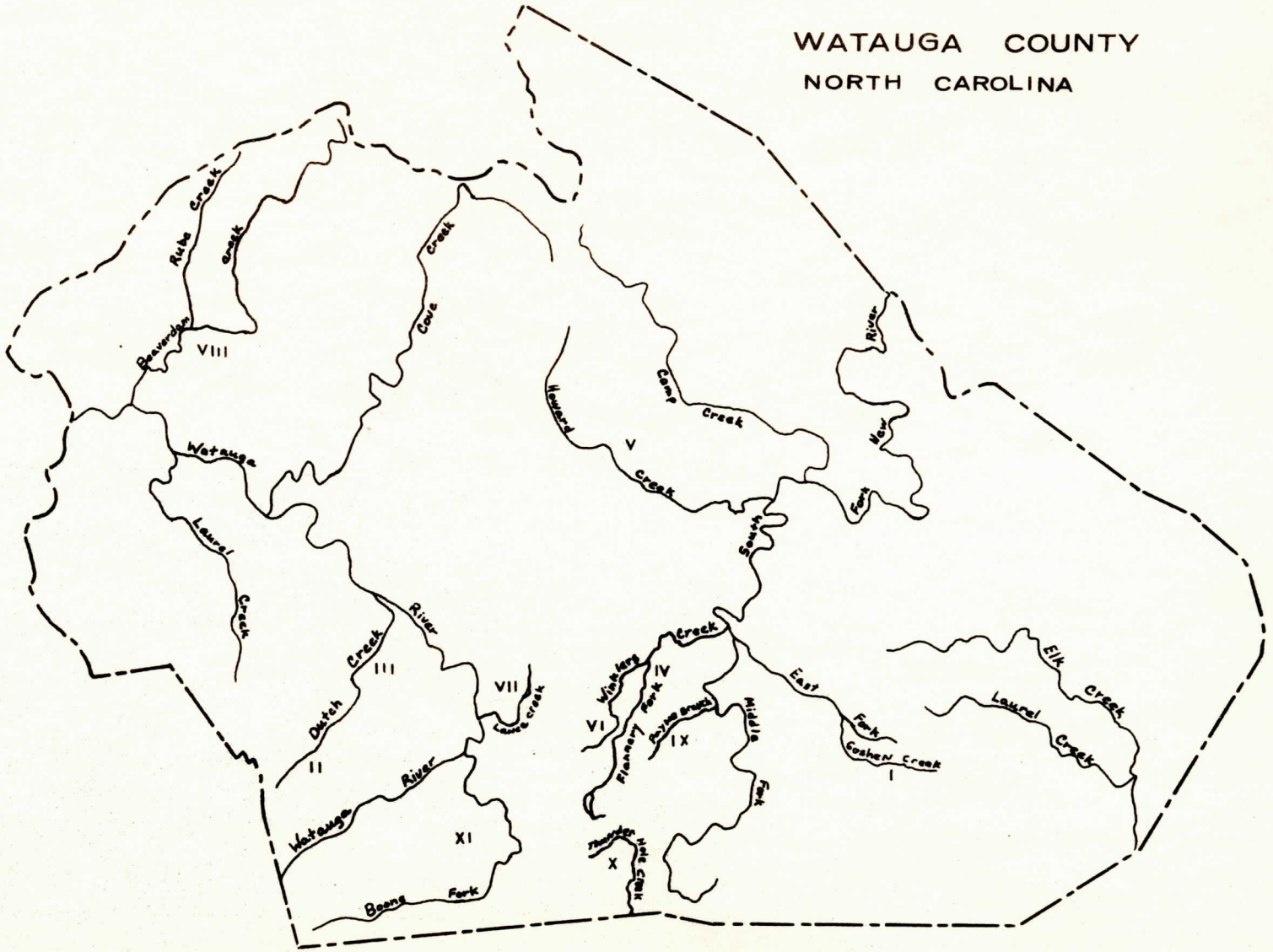

WATAUGA COUNTY
NORTH CAROLINA
Rube Creek
Creek
Beaverdam
VIII
Cove Creek
Howard
Camp Creek
River
Watauga
V
Creek
Laurel
Creek
South Fork
River
Creek
III
Dutch Creek
VII
Cove Creek
Winkler
IV
Creek
Elk creek
East Fork
Laurel Creek
Flannery Fork
Payne Branch
IX
VI
Middle Fork
II
Watauga River
Goshen Creek
I
XI
Beans Fork
Rocky Hole Creek
X

Table I. Host/Parasite Data-Goshen Creek

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
7/18/72	S. fontinalis	1	male	0	none	
9/10/72	" "	3	female	1	G. colemanensis	(100%)
9/10/72	" "	1	male	0	none	
10/25/72	S. gairdneri	1	male	1	G. salmonis	(100%)
10/25/72	S. fontinalis	5	3 male 2 female	2 male 1 female	G. colemanensis G. salmonis	(90%) (10%)
5/14/73	" "	3	female	1	G. colemanensis	(100%)

Table II. Host/Parasite Data-Dutch Creek, above falls

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
6/22/72	S. gairdneri	2	1 male 1 female	1 male	G. salmonis	(100%)
7/31/72	" "	1	female	0	none	
8/3/72	" "	1	male	0	none	
9/21/72	" "	8	7 male 1 female	7 male 1 female	G. colemanensis G. salmonis	(73%) (27%)

Table III. Host/Parasite Data-Dutch Creek, below falls

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
7/24/72	S. trutta	1	male	0	none	
10/26/72	S. gairdneri	4	2 male 2 female	4	G. salmonis	(100%)
11/1/72	" "	4	male	0	none	
4/2/73	" "	2	1 male 1 female	1 female	G. salmonis	(100%)

Table IV. Host/Parasite Data-Flannery Fork

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
7/19/72	S. trutta	1	female	0	none	
9/16/72	" "	2	male	0	none	
10/5/72	S. fontinalis	3	2 male 1 female	1 male 1 female	G. colemanensis	(100%)

Table V. Host/Parasite Data-Howard's Creek

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite (%)
8/13/72	S. fontinalis	1	?	0	none
10/11/72	" "	2	male	0	none
5/16/73	" "	1	?	0	none

Table VI. Host/Parasite Data-Winkler's Creek

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite (%)
10/5/72	S. trutta	1	male	0	none
3/15/73	S. fontinalis	8	male	0	none*

*Collected on the day after the stream was stocked.

Table VII. Host/Parasite Data-Appalachian State U./Biological Research Pond (Lance Creek)

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
10/17/72	S. trutta	2	1 male 1 female	0	none	
10/17/72	S. gairdneri	1	male	0	none	
10/17/72	S. fontinalis	2	male	1	G. colemanensis	(100%)
5/2/73	S. gairdneri	4	male	4	G. salmonis	(100%)
5/2/73	S. trutta	2	1 male 1 female	0	none	
5/5/73	S. gairdneri	1	male	1	G. salmonis G. colemanensis	(66%) (34%)

Table VIII. Host/Parasite Data-Beaver Dam Creek

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
3/31/73	S. fontinalis	3	2 male	0	none	
			1 female	0		

Table IX. Host/Parasite Data-Payne Branch

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
4/20/73	S. gairdneri	4	3 female 1 male	4	G. salmonis	(100%)

Table X. Host/Parasite Data-Thunder Hole Creek

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
5/3/73	S. gairdneri	7	1 male 6 female	1 male	G. colemanensis	(100%)

Table XI. Host/Parasite Data-Boone Fork

Date of Collection	Host	No.	Sex	No. Inf.	Species of Parasite	(%)
5/7/73	S. fontinalis	6	5 male 1 female	2 male	G. colemanensis	(100%)

Table XII. Comparative Measurements of Gyrodactylus Species

Species	G. elegans*	G. elegans*	G. elegans**	G. salmonis
Subspecies	elegans	salmonis	var. B	
Author	Nordman, 1832	Yin & Sproston, 1948	Mueller, 1936	Yin & Sproston, 1948
Data	Byckowsky, 1933	Mueller, 1936	Mueller, 1936	Arlart, 1974
Mean Dia. Cirrus	15.3	13-17	13-17	15.2
Anchors				
length (Avg.)	82.0	60-70	65-70	62.1
point (Avg.)	35.0	32	32	33.9
Ventral Bar	20.0	25	25	30.1
Marginal Hooks				
length	32-35	48	48	44.9
sickle	4.5-5.0	8.0	8.0	8.0

*Yin & Sproston, 1948
**Mueller, 1936

Table XIII. Comparative Measurements of Gyrodactylus colemanensis

Species	G. colemanensis*	G. colemanensis
Author	Mizelle & Kritsky, 1967	Mizelle & Kritsky, 1967
Data	Mizelle & Kritsky, 1967	Arlart, 1974
Anchor length	49 (46-51)	50.6 (45.5-53.3)
Ventral Bar length	31 (27-33)	31.3 (20.7-42)
Marginal Hook		
length	29	29.8 (22.2-34.0)
sickle	6	5.8 (3.0-6.7)

*Mizelle & Kritsky, 1967

Tavle XIV. Measurements of G. salmonis Yin and Sproston, 1948/
Data: Arlart, 1974

	# measured	Mean*	Range*
Anchor length	138	62.1	51.8-68.1
Anchor Point length	131	33.9	25.2-38.5
Marginal Hook length	125	44.8	35.0-49.0
Sickle length	135	8.0	7.0-10.5
Sickle Filament length	120	15.0	10.1-19.3
Ventral Bar length	97	39.0	24.5-59.5
Ventral Bar width	78	30.8	21.0-49.0
Cirrus Dia.	66	15.2	7.0-24.5

*All measurements in microns

Table XV. Measurements of G. colemanensis Mizelle & Kritsky, 1967/
Data: Arlart, 1974

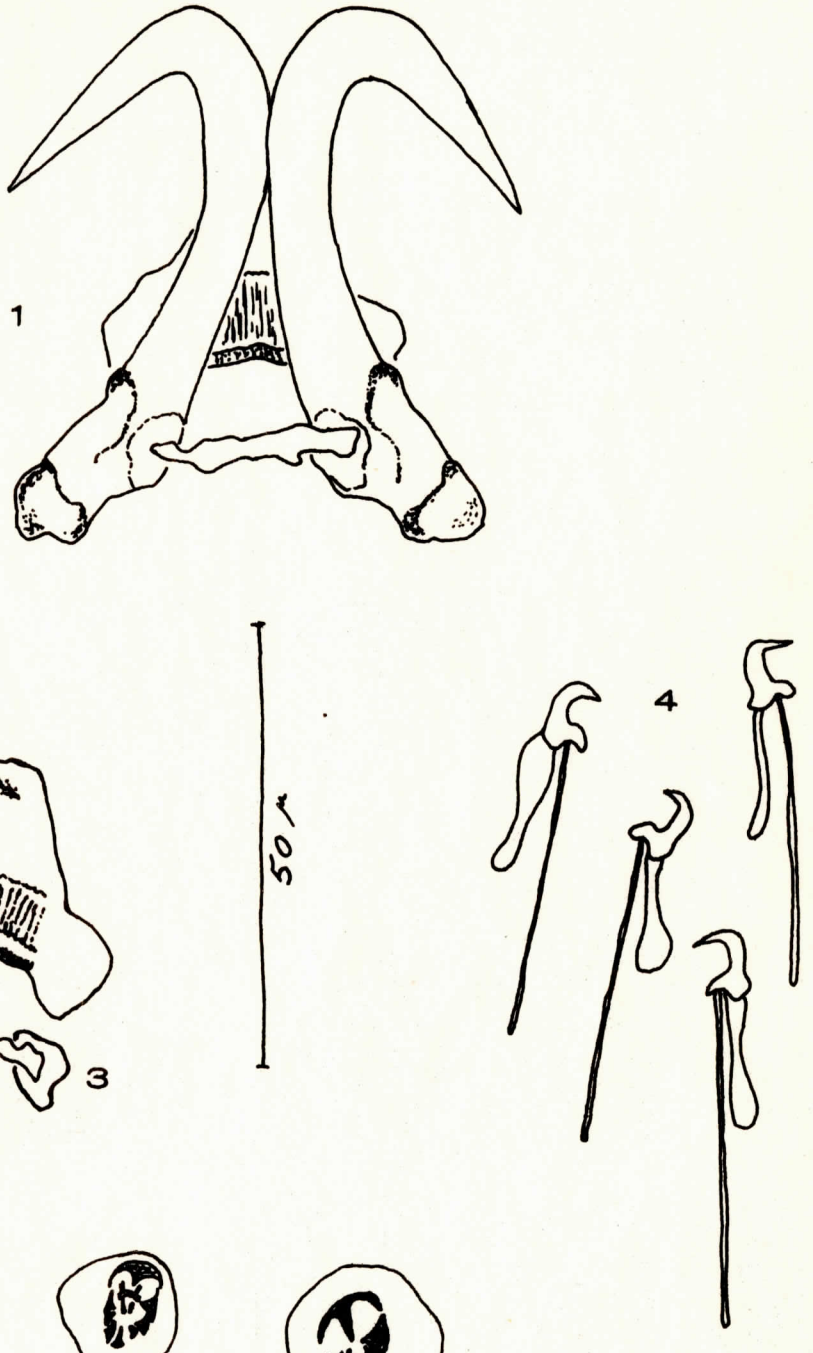
	# measured	Mean*	Range*
Anchor length	48	50.6	45.5-53.3
Anchor point length	47	22.5	16.3-26.3
Marginal Hook length	46	29.8	22.2-34.0
Sickle length	48	5.8	3.0-6.7
Sickle Filament length	18	9.8	7.0-16.3
Ventral Bar length	10	36.2	24.5-52.5
Ventral Bar width	10	31.3	20.7-42.0
Cirrus Dia.	16	12.5	7.4-17.5

*All measurements in microns

FIGURES 1-5

Gyrodactylus salmonis

1. Anchors and bar complex.
2. Ventral bar.
3. Dorsal bar.
4. Marginal hooks.
5. Cirrus. (variations in cirral spinelet arrangement)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FIGURES 6-10

Gyrodactylus colemanensis

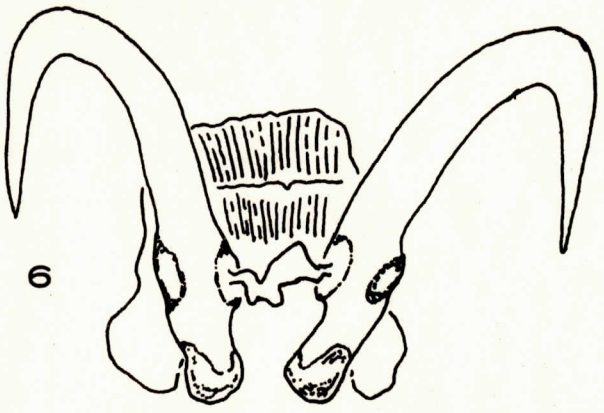
6. Anchors and bar complex.

7. Ventral bar.

8. Dorsal bar.

9. Marginal hooks.

10. Cirrus. (variations in cirral spinelet arrangement)



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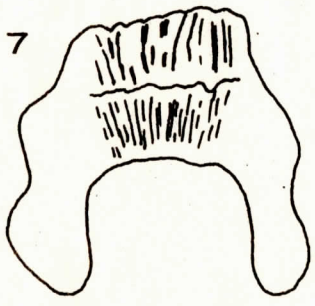

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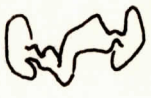

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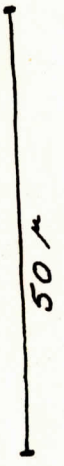

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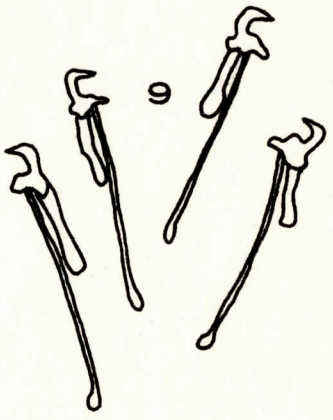

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VITA

The author was born in Chicago, Illinois in 1949. He attended primary and secondary school in Riverside, Illinois. He attended Wake Forest University in Winston Salem, N. C. from 1967 to 1971, receiving his Bachelor of Science degree in Biology. He is married and is presently living in Charlotte, N. C..

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