Studies on the Life History

of <u>Diplostomulum</u> <u>scheuringi</u>

(Trematoda: Strigeoidea)

THESIS

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Ву

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TABLE OF CONTENTS

LIST	OF	FIGUI	RES	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	•	٠	•	•	iii
LIST	OF	TABLI	ES	•	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	iv
ACKNO	DWLI	EDGMEN	NTS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	v
Chapt	er																							
I.	IN	ITRODU	JCTI	ON	[•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1
		Lite The	erat Gen	ur us	е <u>1</u>	Re y 1	evi .oc	lew le1	/ .ph	• • y s	•	•	•	•	•	•	•	•	•	•	•	•	•	1 5
II.	MI	ETHODS	5 AN	D	MA	TE	ERI	[A I	S	•	•	•	• `	•	•	•	•	•	•	•	•	•	•	11
		Feed	ling	E	lx p Pr	er	in	ien lur	its	; ЭП	·	• Pr	•	•	•	• • i c	•	• of	•	•	•	•	•	11
		Stud	Spe ly A	, ci re	me a	ns	f .	or	•	stu •	ıdy •	•	•	•	•	•	•	•	•	•	•	•	•	12 13
III.	RI	ESULTS	5.	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	15
		Deve Desc	elop ≿rip	me ti	nt on	al c	. S of	beq <u>Ty</u>	ue 1c	enc ode	e elp	• hy	• <u>'</u> S	sp	•	•	•	•	•	•		•	•	15 17
IV.	DI	cscuss	SION		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	35
ν.	St	JMMARY	Y.	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	•	•	•	•	•	41
LITER	RATU	JRE CI	TED		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	42

LIST OF FIGURES

Figure

1.	The developmental sequence depicting the transformation of <u>D</u> . <u>scheuringi</u> metacercaria into the adult	20
2.	Metacercaria of <u>D</u> . <u>scheuringi</u> obtained from the intestine of the Pied-billed Grebe after feeding experiments	22
3.	An early developmental stage in the maturation of <u>D</u> . <u>scheuringi</u> metacercaria into the adult	24
4.	Developmental stage in the maturation of \underline{D} . <u>scheuringi</u> metacercaria into the adult showing the formation of the concavity at the junction of the forebody and hindbody	26
5.	Developmental stage in the maturation of <u>D</u> . <u>scheuringi</u> metacercaria into the adult showing the differentiation of the gonads	28
6.	Pre-adult stage in the maturation of <u>D</u> . <u>scheuringi</u> metacercaria into the adult	30
7.	An early adult stage in the development of <u>D</u> . <u>scheuringi</u>	32
8.	A fully mature <u>Tylodelphys</u> adult (approximately 3 weeks old) obtained from the intestine of <u>Podilymbus</u> <u>podiceps</u> after feeding experiments	34

LIST OF TABLES

Table

- The species of <u>Tylodelphys</u> adults that are currently recognized, their hosts, and the locality from which each is reported. 6
- Minimum-maximum measurements of the two species of <u>Tylodelphys</u> described in North America and the specimens recovered in the present study. . 37

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CHAPTER I

INTRODUCTION

The metacercaria of the larval strigeiod trematode, <u>Diplostomulum scheuringi</u>, is a common parasite of fishes in the area of San Marcos, Texas. The trematode is found free in the coelom of the mosquitofish, <u>Gambusia</u> <u>affinis</u>, and in the vitreous humor of the eye of numerous other fishes. Although the metacercaria has been described for more than half a century and the sporocyst and cercaria have been found in the snail <u>Helisoma anceps</u>, adults have not been identified. The objective of this study was to procure adults through infection of the definitive hosts with metacercaria obtained from the mosquitofish, thereby completing the life cycle and determining the true identity of the parasite.

Literature Review

Hughes (1929) described the metacercaria of \underline{D} . <u>scheuringi</u> from the eyes of yellow perch, <u>Perca</u> <u>flavescens</u>, obtained from Douglas Lake, Michigan. Apparently the metacercaria is not extremely host specific as it has been reported from numerous fish hosts including

members of the families Centrarchidae, Cyprinidae, Esocidae, Gadidae, Ictaluridae, Percidae, Percopsidae, and Salmonidae (Bangham 1944) and Poeciliidae (Haderlie 1953). Although the parasite generally occurs in the vitreous humor of the eye, it has also been reported free in the coelom of the mosquitofish and the bluegill (<u>Lepomis</u> <u>macrochirus</u>) by Haderlie (1953).

The metacercaria has been reported from Michigan (Hughes 1929), New York (Mueller and Van Cleave 1931), Massachusetts (Palmer 1939), Wisconsin (Bangham 1944), Minnesota (Chandler 1951), California (Haderlie 1953), Virginia (Etges 1961), North Dakota (Voth and Larson 1968), Canada (Tedla and Fernando 1969; Cone and Anderson 1977), Arkansas (Cloutman 1975), West Virginia (Rubertone and Hall 1975), South Carolina (Aho et al. 1976), Texas (Davis and Huffman 1977a), Wyoming (Hendrickson 1978), and Tennessee (Stout 1980). It is probable that this is only a partial list because the parasite is believed to be more widely distributed.

The sporocyst and cercarial stages were first identified from the gastropod, <u>Helisoma anceps</u>, obtained from Mountain Lake, Virginia (Etges 1961). A study by Aho et al. (1982) indicated that <u>H</u>. <u>trivolvis</u> also serves as the first intermediate host.

Several attempts to obtain adults of <u>D</u>. <u>scheuringi</u> have been undertaken. Lautenschlager (1956) fed the metacercaria of an unknown <u>Diplostomulum</u> sp. obtained from

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the brain of the newt, Triturus viredescens, to day-old chicks. When he sacrificed the birds 36, 48, 72, and 96 hours after feeding, he found that no development had taken place. Etges (1961) collected fishes and newts from the same lake as Lautenschlager and found that the metacercariae in both host groups were morphologically identical. He considered them to be specimens of D. scheuringi. He injected some of these metacercaria down the esophagus of 22 day-old chicks and 31 white lab mice. One chick and one mouse were posted within 2 hours of feeding and the metacercariae were found dead in the upper small intestine of both animals. All his other test animals yielded negative results except one mouse in which the metacercaria had been inadvertently injected down the Three encysted forms were found in the trachial trachea. auxillary muscles of this animal. Etges postulated that this encysted form might develop in a cold-blooded host and that four obligatory hosts might be necessary in order for the life cycle to be completed.

Holliman and Whitlock (1975) published an abstract in which they indicated that the metacercaria of \underline{D} . <u>scheuringi</u> matured in the Snapping Turtle (<u>Chelydra</u> <u>serpentina</u>). No paper on the report was ever published and a later communication with Holliman indicated that the validity of the report was open to question.

Feeding experiments by Huffman (pers. comm.) in which infected mosquitofish were fed to chicks, domesticated

ducklings, Muscovy Ducks, and several species of turtles have all failed to produce adults.

Recent studies have concentrated on the biology and population dynamics of the metacercaria. Davis and Huffman (1977a, 1977b), S. Schneider (1978), and D. Schneider (1982) studied the helminth fauna of mosquitofish collected from various habitats in the area of San Marcos, Texas. Their studies showed that the prevalence of D. scheuringi was higher in lentic environments and that the population densities were extremely variable from year to year. They also found that larger mosquitofish generally had a higher prevalence and a higher mean intensity of D. scheuringi even though the parasite appears to have a one-year lifespan. Other studies by Aho et al. (1976) and Aho et al. (1982) have concentrated on the effects of thermally altered waters on the population biology of D. scheuringi in the mosquitofish.

In North America, the majority of species in the genus <u>Diplostomulum</u> which have known life cycles belong in the family Diplostomatidae (Hoffman 1960). The metacercaria of <u>D. scheuringi</u> most closely resembles those of species from the diplostomatid genus <u>Tylodelphys</u>. Sudarikov (1971), in a review of the order Strigeidida, characterized the metacercaria of <u>Tylodelphys</u> spp. as having a large, elongated forebody and a small, rounded hindbody. They do not form cysts and have a small ventral sucker, poorly developed lateral suckers, and highly developed digestive

and excretory systems. Living specimens are very motile and move by worm-like motions when removed from their hosts. The metacercariae usually occur in the vitreous humor of the eye or in the brain of numerous fishes. The metacercaria of <u>D</u>. <u>scheuringi</u> exhibits these characteristics suggesting that the species may belong in the genus Tylodelphys.

The Genus <u>Tylodelphys</u>

Adults of <u>Tylodelphys</u> spp. are characterized by having indistinct segmentation between the body regions, a conical hindbody with a prominent genital cone, and symmetrically developed testes with the anterior testis slightly wider than the posterior one (Dubois 1951).

Although Yamaguti (1971) lists twenty-two species (four of these are represented only by larval stages) in the genus <u>Tylodelphys</u>, there is a great deal of controversy about the validity of some species (Dubois 1961, 1964, 1970; Niewiadomska 1964). A review of the current literature indicates that sixteen species are widely accepted. Adults have been reported from seven orders of birds and from five continents (Table 1). Of the species reported, seven are found in Europe, four in India, three in North America, and two each in Africa and South America. Only two species, <u>T</u>. <u>conifera</u> and <u>T</u>. <u>podicipina</u>, are reported as occuring on more than one continent. Both of these species are found in Europe and

Table 1. The species of <u>Tylodelphys</u> adults that are currently recognized, their hosts, and the locality from which each is reported.

Species	Hosts	Locality
T. aegyptius El-Naffar et al. 1980	Ardea goliath	Egypt
<u>T. americana</u> (Dubois 1936)	Mycteria americana	Brazil
	Tantalus loculator	
<u>T. circibuteonis</u> Odening 1962	Buteo buteo	Germany
	<u>Circus</u> aeruginosus	
T. <u>clavata</u> (Nordmann 1832)	Ardea cinerea	Europe
	<u>Circus</u> <u>aeruginosus</u>	
<u>T</u> . <u>conifera</u> (Mehlis 1846)	Podiceps cristatus	Switzerland
	P. grisegena	Germany
	Lophodytes cucullatus	North America
<u>T. darteri</u> Mehra 1962	Anhinga melanogaster	India
<u>T. duboisilla</u> (Mehra 1962)	A. melanogaster	India
T. elongata (Lutz 1928)	Podiceps dominicus	Brazil

	Jabiru mycteria	Venezuela
	<u>Mycteria</u> <u>americana</u>	
<u>T. excavata</u> (Rudolphi 1803)	<u>Ciconia</u> <u>ciconia</u>	Europe
	<u>C. nigra</u>	
	Nycticorax nycticorax	
	Anas platyrhynchos	
	Podiceps cristatus	
,	Mergus merganser	
	Buteo buteo	
	<u>Circus</u> aeruginosus	
<u>T. glossoides</u> (Dubois 1928)	Colymbus asiaticus	Switzerland
<u>T. immer</u> Dubois 1961	Gavia immer	North America
	<u>Strix</u> <u>varia</u>	
T. mashonensis Beverley-Burton 1963	Ardea cinerea	Rhodesia
T. podicipina Kozicka and Niewiadomska 1960	Podiceps cristatus	Poland
	<u>P. grisegena</u>	
	P. nigricollis	

Table 1 cont.

<u>T</u> .	<u>p</u> . <u>robrauschi</u> Dubois 1969	Podiceps grisegena	Alaska
$\underline{\mathrm{T}}$.	<u>rauschi</u> (Singh 1956)	Dissoura episcopus	India
<u>T</u> .	<u>spinata</u> Gupta 1962	Anastomus oscitans	India

in North America. Yamaguti (1971) also recognized four other species that are represented only by the metacercaria.

Little is known about the life cycle of members of the genus. Only four species, <u>T</u>. <u>clavata</u>, <u>T</u>. <u>excavata</u>, <u>T</u>. <u>conifera</u>, and <u>T</u>. <u>podicipina</u>, have had their complete life cycle determined. Adults of all four species are found in the intestine of birds (Table 1). Metacercariae of <u>T</u>. <u>excavata</u> are found in the spinal column and brain of seven species of frogs (Niewiadomska 1963). The metacercariae of the other three species occur in the vitreous humor or in the brain of numerous fishes (Wisniewski 1958; Dubois 1970; Sudarikov 1971; Yamaguti 1971). Earlier larval stages of these four species have been reported from various gastropods (Genetsinskaya 1959; Niewiadomska 1962; Dubois 1970; Sudarikov 1971).

Only three species have been reported from North America. Dubois (1961) described <u>T</u>. <u>immer</u> from the Common Loon (<u>Gavia immer</u>) collected in Oklahoma. This species was also reported from the Barred Owl (<u>Strix</u> <u>varia</u>) in Lousiana (Shoop and Corkum 1980). <u>T</u>. <u>conifer</u> was reported from a Hooded Merganser (<u>Lophodytes</u> <u>cucullatus</u>) in Canada by Bain and Threlfall (1977). The other species, <u>T</u>. <u>podicipina</u> <u>robrauschi</u>, was described by Dubois (1969) from the Red-necked Grebe (Podiceps <u>grisegena</u>) obtained in Alaska.

No larval stages of these three species have been

reported from North America (Shoop and Corkum 1980). In Europe, the metacercariae of <u>T</u>. <u>conifera</u> and <u>T</u>. <u>podicipina</u> are found in the vitreous humor of the eye of numerous fishes (Wisniewshi 1958; Kozicka and Niewiadomska 1960) and adults are reported from <u>Podiceps</u> spp. (Niewiadomska 1964).

CHAPTER II

METHODS AND MATERIALS

Feeding Experiments

The two hosts for these feeding experiments were a female Green-backed Heron, <u>Butorides striatus</u>, captured in San Marcos, Texas on September 11, 1983 and a female Pied-billed Grebe, <u>Podilymbus podiceps</u>, captured near Brownsville, Texas on October 14, 1983. The heron was started on feeding experiments shortly after capture. The grebe was isolated and fed mealworms for one week prior to feeding it infected mosquitofish. This isolation period should have allowed time for any parasites already infecting the bird to lose all larval characteristics.

Naturally-infected mosquitofish were collected from local ponds with handnets, held in facilities at the laboratory, and fed to the birds at a rate of 100 to 200 per day for at least 3 weeks. Additional food was provided in the form of small, cultured blue talapia, <u>Tilapia auria</u>. The tilapia were periodically examined and found to be free of trematode infections. After experimental feeding, the birds were sacrificed by exposure to chloroform and necropsied.

Necropsy Procedures and Preparation

of Specimens for Study

Each bird was necropsied in a systematic fashion with particular attention being given to the gastrointestinal tract. After the birds were sacrificed, the body cavity was opened and the entire visceral mass removed and placed in physiological saline solution. The viscera were seperated into organ-system components which were then placed in individual containers of saline. The liver, gall bladder, spleen, lungs, and ovary were teased apart or cut into thin slices and examined with the aid of a Ziess binocular dissecting microscope. The gastrointestinal tract was cut into short pieces. Each piece was then opened and the intestinal mucosa examined under the dissecting scope. All parasites were removed with fine forceps and placed in physiological saline until they could be properly fixed.

All specimens were fixed in hot AFA. The parasites were then stained in Semichon's acetic carmine, destained to the desired color in a 2% acid-alcohol solution, and dehydrated through an ethanol series. A small number of the specimens were counter-stained with fast green in 95% ethanol before they were further dehydrated. Complete dehydration was accomplished in absolute ethanol before the specimens were cleared in a xylene-absolute ethanol series and mounted in Permount.

Study Area

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Naturally-infected mosquitofish used in the feeding experiments were collected from two ponds near San Marcos, Texas. One pond was located on property owned by the McCoy Corporation. Fish were collected from this pond from September 5 to September 27, 1983. After this date, the number of infected fish collected per unit effort decreased and another pond had to be located. The second pond was located on the Southwest Texas State University agricultural farm and provided infected fish for the remainder of the study.

The pond owned by the McCoy Corporation was located at mile marker 206 along the north-bound access road to Interstate 35 in San Marcos, Texas. The pond is approximately 2 ha in surface area and roughly rectangular in shape. Unfortunately the pond is currently being filled with dirt. The most abundant aquatic plants were <u>Myriophyllum</u> sp. and filamentous green algae which grew primarily along the shore.

A majority of the infected fish were collected in the outlet situated at the south end of the pond. Heavy rains had caused the area around the outlet to be flooded, and large numbers of <u>H</u>. <u>anceps</u> and mosquitofish congregated in this area. The mosqitofish had a high prevalence of <u>D</u>.

<u>scheuringi</u>, possibly due to the presence of high numbers of the first intermediate host in the same area. Unfortunately, water levels in the outlet fell quickly, trapping the fish in the outlet which eventually went dry.

The second pond was located off of McCarty Lane just south of San Marcos. It is situated across Interstate 35 from the USFWS Fish Hatchery and Development Center, the effluent from which supplies the bulk of the water flowing into the pond. The pond is approximately 0.8 ha in area. The upper one-half of the pond was covered by a dense mat of <u>Chara</u> sp.. Other abundant aquatic plants included Potamogetan sp. and filamentous green algae.

This pond contained high densities of large mosquitofish which were infected with metacercaria. Large numbers of <u>H</u>. <u>anceps</u> and <u>H</u>. <u>trivolvis</u> were observed and might have been responsible for the heavy infection rate.

All avian scientific names in this paper correspond with those given in Howard and Moore (1980).

CHAPTER 111

RESULTS

No development of the metacercaria took place in the Green-backed Heron. However, 68 specimens of a trematode species were recovered from the small intestine of the grebe. These specimens represented various stages in the development of the metacercariae of <u>D</u>. <u>scheuringi</u> into egg-bearing adults. Figure 1 depicts this developmental sequence.

Developmental Sequence

Several specimens that were identical to the metacercaria of <u>D</u>. <u>scheuringi</u> were obtained from the intestine of the grebe (fig. 2). These specimens are probably metacercariae fed to the bird on the day it was sacrificed. As development begins, the length of the body decreases and the lateral suckers, tribocytic organ, and genital primordia become more distinct (Fig. 3). Overall morphology is still quite similar to that of the metacercaria. As development progresses, the organism continues to decrease in length and the posterior portion of the forebody begins to thicken (Fig. 4). An anteriorly opening concavity develops in this thickened region.

In more mature specimens (Fig. 5), the hindbody begins to enlarge and the genital primordia differentiate into the two bilobed testes and the single ovary. The first of the irregularly shaped vitelline glands appear along the anterior margin of the cavity which has now extended forward and obscures a portion of the tribocytic organ. At this stage of development, the length of the organism is the minimum value observed, being approximately one-half the length of the fully developed metacercaria.

The worm now begins to enlarge, with the hindbody growing much faster than the forebody (Fig. 6). The oral sucker is situated on a protrusion forming the most anterior point of the organism. The lateral suckers, which are large and well developed, are located on each side of and posterior to the oral sucker. The gonads increase in size and occupy the same positions as in the adult forms. The vitellaria are more numerous and widespread, becoming distributed throughout the hindbody and forward into the forebody to the ventral sucker. The copulatory bursa, with its distinctive genital cone, is now visible.

As development continues, the overall dimensions of the worm increase (Fig. 7). The vitellaria become more numerous and reach their furthest anterior position just forward of the ventral sucker. Eventually, ova develop and lie in the uterus situated between the lobes of the

testes. In fully mature individuals (approximately 3 weeks old) up to 30 eggs may be contained in the uterus at one time (Fig. 8).

Description of Tylodelphys sp.

The description is based on 19 sexually mature adults in whole mounts. All values given in the following text and tables are in micrometers unless otherwise noted and represent (min-max, mean).

Specimens (total length 833-1300, 1050) have a spoon-shaped forebody (473-789, 590 long by 386-605, 479 maximum width) that is slightly demarcated from the conical hindbody (359-576, 461 long by 368-579, 472 maximum width); ratio of hindbody length to forebody length 0.62-1.06 (mean 0.79). Oral sucker (49-96, 72 in diameter) unarmed, flanked on each side by well developed lateral suckers (89-148, 107 long) which vary in shape. Ratio of total body length to lateral sucker length 7.8-12.4 (mean 10.1). Prepharynx short; pharynx (51-82, 66 long by 31-60, 47 wide) bulbous, muscular; ratio of oral sucker length to pharynx length 0.71-1.36 (mean 1.10). Esophagus (0-11 long) short or absent; ceca extending to posterior of body. Ventral sucker (62-96, 75 long by 95-118, 109 wide) is a short distance (0-22, 8)anterior of the edge of the tribocytic organ (everted 167-271, 216 long by 156-215, 186 wide; withdrawn 156-233,

201 long by 93-167, 130 wide). Tandem testes are bilobed, bent into a "U" shape that opens ventrally; anterior testis (96 by 158, 118 long by 307-570, 380 maximum width) slightly wider than posterior testis (96-171, 127 long by 27-518, 327 wide). Ovary (56-84, 73 long by 86-140, 100 wide) pyriform, situated submedial, dorsal and anterior to testes. Uterus winding between lobes of testes before opening into the copulatory bursa; up to 30 ova (56-100, 84 long by 47-69, 58 wide) contained in uterus at one time. Copulatory bursa (110-271, 185 in diameter) round to ellipsoidal, terminal, containing prominant genital cone. Vitellaria irregular in shape, concentrated in hindbody at junction with forebody and in area of copulatory bursa; forming a narrow band between the lobes of the testes with most anterior extent just beyond the ventral sucker. Position in the forebody (percentile of forebody length) from most anterior point to posterior of lateral suckers 22.0-32.7% (mean 25.9%), anterior extent of vitellaria 41.7-66.3% (mean 54.1%), anterior edge of ventral sucker 44.7-67.7% (55.3%), anterior edge of tribocytic organ 50.7-77.2% (mean 67.0%).

Figure 1. The developmental sequence depicting the transformation of <u>D</u>. <u>scheuringi</u> metacercaria into the adult. The oldest possible infection is 21 days.

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Figure 2. Metacercaria of <u>D</u>. <u>scheuringi</u> obtained from the intestine of the Pied-billed Grebe after feeding experiments.

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Figure 3. An early developmental stage in the maturation of <u>D</u>. <u>scheuringi</u> metacercaria into the adult. Specimen obtained from the intestine of the Pied-billed Grebe after feeding experiments.



Figure 4. Developmental stage in the maturation of \underline{D} . <u>scheuringi</u> metacercaria into the adult showing the formation of the concavity at the junction of the forebody and hindbody.



Figure 5. Developmental stage in the maturation of \underline{D} . <u>scheuringi</u> metacercaria into the adult showing the differentiation of the gonads.



Figure 6. Pre-adult stage in the maturation of \underline{D} . scheuringi metacercaria into the adult.



0.25 mm

Figure 7. An early adult in the development of \underline{D} . <u>scheuringi</u>. The worm now exhibits characteristics of the genus <u>Tylodelphys</u>.



0.25 mm

Figure 8. A fully mature <u>Tylodelphys</u> adult (approximately 3 weeks old) obtained from the intestine of <u>Podilymbus</u> <u>podiceps</u> after feeding experiments.



CHAPTER IV

DISCUSSION

The fully mature specimens obtained from the Pied-billed Grebe in this study (Fig. 8) exhibit all the characteristics used by Dubois (1951) to define the genus <u>Tylodelphys</u>. This fact, together with the developmental sequence showing the transformation of <u>D</u>. <u>scheuringi</u> metacercaria into these adults, and the similarities existing between the metacercariae of <u>D</u>. <u>scheuringi</u> and those of known species of the genus <u>Tylodelphys</u>, leads to the conclusion that the larval trematode known as <u>Diplostomulum scheuringi</u> actually belongs in the genus Tylodelphys.

The host specificity of members of the genus <u>Tylodelphys</u> has only begun to be investigated. Initial work by Dubois (1955) indicated that adults of <u>T</u>. <u>conifera</u>, <u>T</u>. <u>excavata</u>, and <u>T</u>. <u>americana</u> have a narrow host specificity, and that <u>T</u>. <u>clavata</u> was euryxenic being reported from two systematically distant orders of birds. Later work by Niewiadomska (1964) found that <u>T</u>. <u>excavata</u> would mature in birds in three different orders and therefore should also be considered euryxenic. She also found that T. podicipina appears to

be stenoxenic since it has matured only in feeding experiments involving species in the order Podicipediformes. In this study, metacercaria from <u>G</u>. <u>affinis</u> fed to the Green-backed Heron did not develop while those fed to the grebe did. Since several species of the genus <u>Tylodelphys</u> are reported from the order Ciconiiformes, to which the herons belong, these results suggest that the adults of <u>D</u>. <u>scheuringi</u> are somewhat host specific. This conclusion is substantiated by the numerous feeding experiments by Huffman (pers. comm.) using <u>D</u>. <u>scheuringi</u> metacercaria from the mosquitofish. All of his studies have failed to produce adults.

The adults obtained in this study exhibit differences in morphology from the three species reported from North America. The hindbody of my specimens is much longer in relation to the forebody than the hindbody of these three species. According to measurements in Dubois (1964), the specimens from this study can be differentiated from <u>T</u>. <u>conifera</u> by their larger ventral sucker and the shorter distance between the posterior margin of the ventral sucker and the anterior margin of the tribocytic organ (0 to 22 compared to 45 to 71).

Table 2 list the measurements from the two species described from North America and the specimens recovered in this study. My specimens are shorter and more robust than \underline{T} . <u>immer</u> (Dubois 1961). In the majority of specimens obtained in this study the vitellaria extend to

Table 2. Minimum-maximum measurements of the two species of <u>Tylodelphys</u> described in North America and the specimens recovered in the present study.

	<u>T. p. robrauschi</u>	T. immer	Tylodelphys sp.
Total Length (mm)	0.85-1.62	1.74-1.80	0.83-1.30
Forebody (mm)	0.55-0.99/0.40-0.80	1.04-1.14/0.53-0.58	0.47-0.79/0.39-0.79
Hindbody (mm)	0.26-0.63/0.31-0.68	0.60-0.79/0.41-0.47	0.36-0.58/0.37-0.58
Ratio of Hindbody Length			
to Forebody Length	0.41-0.66	0.53-0.76	0.62-1.06
Oral Sucker	70-135/57-120	115-120/105-115	49-91/51-96
Lateral Sucker Length	150-245	180-280	89-148
Pharynx	52-122/52-102	87-89/68-70	51-82/31-60
Ventral Sucker	80-120/100-155	84-100/110-122	62-96/93-118
Tribocytic Organ	120-320/110-265	260/210	160-270/93-220
Ratio of Oral Sucker Length			
to Pharynx Length	1.03-1.54 (1.21)	1.20-1.57 (1.36)	0.71-1.36 (1.10)
Esophagus	0-50	5-52	0-11
Anterior Testis	80-210/270-610	120-150/345-380	100-160/310-570

Table 2 cont.

Posterior Testis	100-240/250-530	150-170/320-350	100-170/270-520
Ovary	85-117/110-256	100-105/125-145	56-84/86-140
Eggs	85-98/54-63	94-104/57-68	56-100/47-69
Position in Forebody (in percent from Most Anterior Point to:	c of forebody length)		
Anterior Extent of Vitellaria	42-61/100	28-44/100	42-66/100
Anterior Edge of Ventral Sucker	52-72/100	56-60/100	45-68/100
Anterior Edge of Tribocytic Orga	an 60-80/100	61-70/100	51-77/100

just beyond the anterior margin of the ventral sucker. One specimen had vitelline follicles which extended anteriorly to a point one-third the distance between the ventral sucker and the bifurcation of the caeca. Dubois (1961) reports a measurement of greater than one-half this distance for the anterior extent of the vitellaria of \underline{T} . <u>immer</u>. My specimens also differ from \underline{T} . <u>immer</u> in that the testes are wider, the concavity at the junction of the body regions is well developed, and the lateral suckers are shorter in relation to the total body length.

The specimens obtained in this study are most closely resembled by T. p. robrauschi. Measurements from Dubois (1969, 1970) and my study of T. p. robrauschi paratypes do reveal some differences. The most noticeable is the size of the hindbody noted earlier. Another outstanding difference is the size of the lateral suckers. The maximum lateral sucker length obtained for specimens in this study was 148 and the ratio of total body length to lateral sucker length is 7.8-12.4 (mean 10.1). Dubois (1970) obtained lengths of 150-245 for the lateral suckers of T. p. robrauschi and established a ratio of 5.0-9.0 (mean 6.7). This is an extremely important difference since Dubois (1970) uses this ratio to separate T. podicipina, <u>T</u>. <u>immer</u>, and <u>T</u>. <u>glossoides</u> from all other species of Tylodelphys. He stated that a ratio of 5.0-8.5 is exhibited by these three species while values of 9.0-22.0 are found in the other species of Tylodelphys.

Other differences include a smaller ovary, shorter pharynx, and shorter oral sucker in specimens obtained in this study. My specimens also differ in that the vitellaria are not distributed as far anteriorly into the forebody as the vitellaria of <u>T</u>. <u>p</u>. <u>robrauschi</u> (Dubois 1969). Also, the vitellaria tend to be less concentrated at the junction of the forebody and hindbody and are more numerous in the region around the copulatory bursa in my specimens than in <u>T</u>. <u>p</u>. <u>robrauschi</u>.

The specimens obtained in this study can be easily distinguished from <u>T</u>. <u>immer</u> and <u>T</u>. <u>podicipina</u> <u>robrauschi</u> by their relatively longer hindbody and smaller lateral suckers. My specimens also differ from <u>T</u>. <u>immer</u> in that they are shorter and more robust and have a well developed concavity at the junction of the hinbbody and forebody. They differ from specimens of <u>T</u>. <u>p</u>. <u>robrauschi</u> in that the vitellaria do not extend into the forebody nearly as far as those of that species. On the basis of these differences in morphology, it is my opinion that the specimens recovered in this study represent a new species of the genus Tylodelphys.

CHAPTER V

SUMMARY

The life cycle of the larval, strigeiod trematode, <u>Diplostomulum</u> <u>scheuringi</u>, was investigated.

Metacercariae obtained from the mosquitofish were fed to a Green-backed Heron and a Pied-billed Grebe. No development took place in the heron, however, in the grebe the metacercariae matured into adults belonging to the genus <u>Tylodelphys</u>. These specimens exhibit distinct morphological differences from the known species of the genus and represent a new species. This is the first completion of the life cycle of a member of the genus <u>Tylodelphys</u> in North America and is the first report of the genus occuring in the Pied-billed Grebe.

Since the metacercariae matured in the Pied-billed Grebe and no development took place in the Green-backed Heron or in the numerous hosts used in feeding experiments by Huffman (pers. comm.), it appears that the parasite is somewhat host specific in regards to the definitive host.

LITERATURE CITED

- Aho, J. M., J. W. Camp and G. W. Esch. 1982. Long-term studies on the population biology of <u>Diplostomulum</u> <u>scheuringi</u> in a thermally altered reservoir. J. Parasitol. 68: 659-708.
- Aho, J. M., J. W. Gibbons and G. W. Esch. 1976. Relationship between thermal loading and parasitism in the mosquitofish. <u>In</u> G. W. Esch and R. W. McFarlane (eds.), Thermal Ecology-II, ERDA Symposium Series (CONF-750425), p. 213-218.
- Bain, G. A. and W. Threlfall. 1977. Helminth parasites of Hooded Mergansers, <u>Lophodytes</u> <u>cucullatus</u> (L.) from Ontario. Proc. Helminthol. Soc. Wash. 44: 219-221.
- Bangham, R. V. 1944. Parasites of northern Wisconsin fish. Trans. Wis. Acad. Sci., Arts, and Lett. 36: 391-325.
- Beverly-Burton, M. 1963. A new strigeid, <u>Diplostomum</u> (<u>Tylodelphys</u>) <u>mashonense</u> n. sp., (Trematoda : Diplostomatidae), from the Grey Heron, <u>Ardea</u> <u>cinerea</u> L. in Southern Rhodesia, with an experimental demonstration of part of the life cycle. Rev. Zool. Bot. Afr. 68: 291-308.
- Chandler, A. C. 1951. Studies on the metacercariae of <u>Perca flavescens</u> in Lake Itasca, Minnesota. Am. <u>Midl. Nat. 45: 711-721.</u>
- Cloutman, D. G. 1975. Parasite community structure of largemouth bass, warmouth, and bluegill in Lake Fort Smith, Arkansas. Trans. Am. Fish. Soc. 104: 277-283.
- Cone, D. K. and R. C. Anderson. 1977. Parasites of pumpkinseed (<u>Lepomis gibbosus</u> L.) from Ryan Lake, Algonguin Park, Ontario. Can. J. Zool. 55: 1410-1423.
- Davis, J. R. and D. G. Huffman. 1977a. A comparison of the helminth parasites of <u>Gambusia affinis</u> and <u>Gambusia geiseri</u> (Osteichthyes : Poeciliidae) from the upper San Marcos River. Southwest. Nat. 22: 359-366.

- Davis, J. R. and D. G. Huffman. 1977b. Some factors associated with the distribution of heminths among individual mosquitofish, <u>Gambusia affinis</u>. Tex. J. Sci. 30: 43-53.
- Dubois, G. 1928. Descriptions de nouveaux trematodes d'oiseaux du genre <u>Hemistomum</u>. Bull. Soc. Neuchatel. Sci. Nat. 52: 33-44.

. 1936. Nouveaux principes de classification des trematodes du groupe des Strigeida. Note preliminaire. Rev. Suisse Zool. 43: 507-515.

. 1951. Nouvelle cle de determination des groupes systematiques et des genres de Strigeida Poche (Trematoda). Rev. Suisse Zool. 58: 639-691.

. 1955. Nature de specificite chez les Strigeides (Trematoda). Rev. Iber. Parasitol. Tomo Extraord. p. 133-144.

. 1961. Le genere <u>Diplostomum</u> Von Nordmann 1832 (Trematoda : Strigeida). Bull. Soc. Neuchatel. Sci. Nat. 85: 109-120.

. 1964. Du statut de quelques Strigeata La Rue, 1926 (Trematoda). I. Bull. Soc. Neuchatel. Sci. Nat. 87: 27-71.

_____. 1969. Notes Helminthologiques II : Diplostomatidae Poirier et Cyathocotylidae Poche (Trematoda). Rev. Suisse Zool. 76: 3-21.

_____. 1970. Synopsis des Strigeidae et des Diplostomatidae (Trematoda). Mem. Soc. Neuchatel. Sci. Nat. 10: 259-727.

- El-Naffar, M. K., R. M. Khalifa and A. A. Sakla. 1980. Parasitofauna of the Egyptian aquatic birds. II. Trematode parasites of the Giant Heron (<u>Ardea</u> <u>goliath</u>) in Assiut Governorate. J. Egypt. Soc. Parasitol. 10: 107-116.
- Etges, F. J. 1961. Contributions to the life history of the brain fluke of newts and fish, <u>Diplostomulum</u> <u>scheuringi</u> Hughes, 1926 (Trematoda : <u>Diplostomatidae</u>). J. Parasitol. 47: 453-458.
- Genetisinskaya, T. A. 1959. K faune cerkarij Moljuskov Rybinskogo Vodochranilisca. Ekol. Parasitol. p. 96-146.

- Gupta, R. 1962. Two new species of the genus <u>Tylodelphys</u> Diesing, 1850 (Trematoda : Diplostomatidae) from Indian birds. Proc. Nat. Acad. Sci. India Sect.B (Biol. Sci.) 32: 417-420.
- Haderlie, E. C. 1953. Parasites of fresh water fishes of northern California. Calif. Pub. in Zool. 57: 303-440.
- Hendrickson, G. L. 1978. Observations on strigeoid trematodes from the eyes of southeastern Wyomong fish. II. <u>Diplostomulum scheuringi</u> Hughes, 1929; <u>Neascus ptychocheilus</u> (Faust, 1917); and other types. Proc. Helminthol. Soc. Wash. 45: 64-68.
- Hoffman, G. L. 1960. Synopsis of Stigeoidea (Trematoda) of Fishes and Their Life Cycles. Fish. Bull. 175 Vol. 60.
- Holliman, R. B. and S. Whitlock. 1975. Adult stages of <u>Diplostomulum trituri</u> (Digenea : Diplostomatidae) in the snapping turtle, <u>Chelydra serpentina</u>. Program and Abstracts, 50th Annual Meeting, Am. Ass. Parasitol. Abstract 98: 68.
- Howard, R. and A. Moore. 1980. A Complete Checklist of the Birds of the World. Oxford University Press, Oxford, 712pp.
- Hughes, R. C. 1929. Studies on the trematode family Strigeidae (Holostomidae) No. XIX. <u>Diplostomulum</u> <u>scheuringi</u> sp. nov. and <u>D. vegrandis</u> (La Rue). J. Parasitol. 15: 267-271.
- Kozicka, J. and K. Niewiadomska. 1960. Studies on the biology and taxonomy of trematodes of the genus <u>Tylodelphys</u> Diesing, 1850 (Diplostomatidae). Acta Parasitol. Pol. 8: 379-401.
- Lautenschlager, E. W. 1956. A Study of <u>Diplostomulum</u> sp. (Trematoda : Diplostomatidae) in the Brain and Cranial Case of <u>Triturus viridescens</u> <u>viridescens</u>. M. S. Thesis. Univ. of Vir., Dept. of Biology. 27pp.
- Lutz, A. 1928. Estudios de Zoologia y Parasitologia Venezolanas. 133pp.

- Mehra, R. K. 1962. Two new species of the genus <u>Tylodelphys</u> Diesing, 1850 (Trematoda : Diplostomatidae Poirier, 1886) from the Indian Snake Bird <u>Anhinga melanogaster</u> Pennant. Proc. Nat. Acad. Sci. India Sect. B (Biol. Sci.) 32: 50-56.
- Mueller, J. F. and H. J. Van Cleave. 1931. What is <u>Diplostomulum</u> <u>scheuringi</u>? J. Parasitol. 18: 126-127.
- Niewiadomska, K. 1962. Identity of <u>Tylodelphys</u> <u>clavata</u> (Ciurea, 1928) nec Nordmann, 1832, with <u>Tylodelphys</u> <u>excavata</u> (Rudolphi, 1803) (Trematoda : Diplostomatidae). Bull. Acad. Pol. Sci. Ser. Sci. Biol. 10: 431-433.
- . 1963. Further studies on the biology and taxonomy of trematodes of the genus <u>Tylodelphys</u> Diesing, 1850 (Diplostomatidae). Acta Parasitol. Pol. 11: 283-306.
- ______. 1964. Observations on the specificity in the genus <u>Tylodelphys</u> (Diesing 1850) (Trematoda, Diplostomatidae). Proc. Symp. Parasit. Worms and Aquat. Cond. pp. 159-165.
- Nordmann, A. von. 1832. Mikroskopische Beitrage zur Naturgeschichte der Wirdellosen Tiere. 118pp.
- Odening, K. 1962. Trematoden aus indischen Vogeln des Berliner Tierparks. Parasitenk D. 21: 381-425.
- Palmer, E. D. 1939. Diplostomiasis, a hatchery disease of freshwater fishes new to North America. Prog. Fish-Cult. Memo. 1-131. 45: 41-47.
- Rubertone, J. A. and J. E. Hall. 1975. A survey of fish parasites from the Greenbrier River. Proc. Helminthol. Soc. Wash. 42: 58-59.
- Rudolphi, C. A. 1803. Neue Beobachtugen uber die Eingeweidewurmer. Arch. Zool. U. Zoot. 3: 1-32.
- Schneider, D. 1982. A Survey of the Helminth Parasites of <u>Gambusia</u> <u>affinis</u> (Osteichthyes : Poediliidae) from the Blanco River in Hays County, Texas. M. S. Thesis. Southwest Tex. St. Univ., San Marcos. 101 pp.

- Schneider, S. S. 1978. An Ecological Survey of the Helminths of <u>Gambusia affinis</u> (Osteichthyes : Poeciliidae) from a Pond Eniviroment in San Marcos, Texas. M. S. Thesis. Southwest Tex. St. Univ., San Marcos. 129 pp.
- Shoop, W. L. and K. C. Corkum. 1980. <u>Tylodelphys</u> <u>immer</u> Dubois 1961 (Trematoda : Diplostomatidae) from the Barred Owl, <u>Strix</u> varia in Louisiana. J. Parasitol. 66: 701-702.
- Stout, R. B. 1980. Comparative Study on the Parasites of the Bluegill (<u>Lepomis macrochirus</u>, Rafinesque) from Cookeville City Lake and Center Hill Reservoir, Tennessee. M. S. Thesis. Tenn. Tech. Univ. 121 pp.
- Sudarikav, V. Y. 1971. The Order Strigeidida (La Rue, 1926) Sudarikov, 1959. <u>In</u> K. M. Skryabin (ed.). Trematodes of Animals and Man. Vol. 24. Nauka Press, Moscow. p. 71-272.
- Tedla, S. and C. H. Fernando. 1969. Observations on the seasonal changes of the parasite fauna of yellow perch (<u>Perca flavescens</u>) from the Bay of Quinte, Lake Ontario. J. Fish. Res. Board Can. 26: 833-843.
- Voth, D. R. and O. R. Larson. 1968. Metazoan parasites of some fishes from Goose River, North Dakota. Am. Midl. Nat. 79: 216-224.
- Wisniewski, W. L. 1958. Characterization of the parasitofauna of an eutrophic lake (Parasitofauna of the biocoenosis of Druzno Lake - Part I). Acta Parasitol. Pol. 6: 1-64.
- Yamaguti, S. 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vol. I. Keigaku Publ. Co., Tokyo. 1424pp.