A Morphometric Study of *Plagioporus sinitsini* Mueller (Digenea: Opecoelidae) from the Gallbladder of Three Cyprinid Hosts from the Blanco, San Marcos, and Comal Rivers in Central Texas

THESIS

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By

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CHAPTER I

INTRODUCTION

Several ecological surveys of fish helminths have been conducted that report the trematode *Plagioporus sinitsini* Mueller in the gallbladder of various cyprinid, catostomatid, and poeciliid hosts (Table 1).* Martin (1976) studied the helminths of the cyprinids *Cyprinella venusta* Girard and *Cyprinella lutrensis* (Baird and Girard) from Canyon Reservoir and the Blanco, Guadalupe, Little Blanco, and San Marcos Rivers and reported *Plagioporus* sp. in the gallbladder of both fish species from the San Marcos River. Davis and Huffman (1977) studied the helminths of the poeciliids *Gambusia affinis* (Baird and Girard) and *Gambusia geiseri* Hubbs and Hubbs from the San Marcos River and reported *P. sinitsini* in *G. affinis* only. Underwood and Dronen (1984) reported *P. sinitsini* in *Dionda episcopa* Girard and *Cyprinella venusta* from the San Marcos River in their ecological study of the helminths of San Marcos River fishes.

In September 1989, the gallbladders of *Cyprinella venusta*, *Notropis amabilis* Girard, and *Dionda episcopa* from the San Marcos River were all discovered to be parasitized by *Plagioporus sinitsini*. This condition provided an excellent opportunity to study host-induced morphological variation in *P. sinitsini*. This study was conducted from September 1989 to October 1990.

* Scientific names for piscine hosts in this study follow Robins et al. (1991).

Table 1. Previously reported hosts of *Plagioporus sinitsini*.

Taxonomy	Reference	Locality
Catostomatidae		
Catostomus commersoni	Mueller, 1934	Oneida Lake, New York
Catostomus commersoni	Dobrovolny, 1939	Huron River, Michigan
Catostomus commersoni	Bangham, 1955	South Bay, Ontario
Catostomus commersoni	Fischthal, 1956	New York
Catostomus commersoni	Williams & Ulmer, 1980	Wisconsin
Hypentelium nigricans	Dobrovolny, 1939	Huron River, Michigan
Hypentelium nigricans	Aliff, 1977	Kentucky
Hypentelium nigricans	Williams & Ulmer, 1980	Wisconsin
Moxostoma anisurum	Aliff, 1977	Kentucky
Moxostoma macrolepidotum	Williams & Ulmer, 1980	Wisconsin
Cyprinidae		
Campostoma anomalum	Dobrovolny, 1939	Huron River, Michigan
Campostoma anomalum	Aliff, 1977	Kentucky
Dionda episcopa	Underwood, 1981	San Marcos River, Texas
Hyborrhynchus notatus[1]	Dobrovolny, 1939	Huron River, Michigan
Nocomis biguttatus[2]	Dobrovolny, 1939	Huron River, Michigan
Nocomis biguttatus	Dobrovolny, 1939	Huron River, Michigan
Nocomis biguttatus	Bangham, 1955	South Bay, Ontario
Nocomis biguttatus	Muzzall, 1982	Red Cedar River, Michigan
Nocomis micropogan	Dobrovolny, 1939	Huron River, Michigan
Notropis ardens	Aliff, 1977	Kentucky
Notropis boops	Aliff, 1977	Kentucky
Notropis chrysocephalus[1]	Aliff, 1977	Kentucky
Notropis cornutus[1, 2]	Dobrovolny, 1939	Huron River, Michigan
Notropis cornutus[1]	Dobrovolny, 1939	Huron River, Michigan
Notropis cornutus[1]	Muzzall, 1982	Red Cedar River, Michigan
Notropis heterolepus	Dobrovolny, 1939	Huron River, Michigan
Notropis lutrensis[1]	Martin, 1976	San Marcos River, Texas
Notropis rubellus	Dobrovolny, 1939	Huron River, Michigan
Notropis rubellus	Aliff, 1977	Kentucky
Notropis stramineus	Muzzall, 1982	Red Cedar River, Michigan
Notropis venustus[1]	Martin, 1976	San Marcos River, Texas
Notropis venustus[1, 2]	Lindholm, 1979	San Marcos River, Texas
Notropis venustus[1]	Underwood, 1981	San Marcos River, Texas

Table 1. Continued.

Taxonomy	Reference	Locality
Notropis volucellus	Dobrovolny, 1939	Huron River, Michigan
Notropis whipplei[1]	Aliff, 1977	Kentucky
Pimephales notatus	Aliff, 1977	Kentucky
Rhinichthys atratulus	Aliff, 1977	Kentucky
Semotilus atromaculatus	Muzzall, 1982	Red Cedar River, Michigan
Semotilus atromaculatus	Fischthal, 1956	New York
Semotilus corporalis	Fischthal, 1956	New York
Poeciliidae		
Gambusia affinis	Davis & Huffman, 1977	San Marcos River, Texas
Gambusia affinis	Aliff, 1977	Kentucky
Lebistes reticulatus[2]	Dobrovolny, 1939	Huron River, Michigan

[1]After Robins et al. (1991)
Hyborrhynchus notatus = *Pimephales notatus*
Notropis chrysocephalus and *Notropis cornutus* = *Luxilis cornutus*
Notropis lutrensis = *Cyprinella lutrensis*
Notropis venustus = *Cyprinella venusta*
Notropis whipplei = *Cyprinella whipplei*

[2]Experimental infection; all other hosts listed harbor naturally occurring infections of *Plagioporus sinitsini.*

Choice of Host

Cyprinella venusta, *N. amabilis*, and *D. episcopa* were chosen as host species because of the presence of *P. sinitsini* in these fish and because of the abundance of these fish in the San Marcos, Comal, and Blanco Rivers. Additionally, these fish all belong to the same family, Cyprinidae, providing an excellent opportunity for investigating the effects of minute differences between the hosts upon various aspects of the parasite. Martin (1976) reported finding *Plagioporus* sp. in the gallbladder of *Cyprinella lutrensis*, but unfortunately this fish is no longer found in the San Marcos River (personal communication, B. J. Whiteside, Southwest Texas State University, San Marcos). Davis and Huffman (1977) reported finding *P. sinitsini* in the poeceliid *Gambusia affinis* from the San Marcos River, but this information was discovered after the present study was underway.

Host Distribution and Habitat in Texas

Dionda episcopa, the roundnose minnow, is distributed in the Colorado, San Antonio, Nueces, and Rio Grande drainages in west-central Texas. Primarily herbivorous, *Dionda episcopa* is abundant in shallow, vegetated pools of clear, low gradient creeks and rivers. Little has been reported about the biology of this species (Lee and Gilbert, 1980a).

Cyprinella venusta, the blacktail shiner, is abundant throughout Texas, inhabiting turbid waters from the Rio Grande Basin east to the Texas-Louisiana border. Until recently (Robins et al., 1991) this species was known under the binomen *Notropis venustus* (Girard). Its typical habitat is clear, large, sandy-bottomed streams, although some local populations occur over substrates with more gravel and rubble (Lee and Gilbert, 1980b).

Notropis amabilis, the Texas shiner, is found from the Colorado River to the Rio Grande drainages in Texas and is typically found in clear springs and headwater tributaries over substrates of sand, gravel, and rubble. Few studies have been published on the biology of *N. amabilis* (Lee and Gilbert, 1980c).

Taxonomic Description of the Trematode, *Plagioporus sinitsini*

Martin (1976) refers to *P. sinitsini* as *Plagioporus* "A" in her study of helminths from the San Marcos and Blanco Rivers because an authoritative specific determination could not be made. Davis and Huffman (1977) and Underwood (1981) all report this trematode as *Plagioporus sinitsini.* Specimens collected in the present study fit the type description given by Mueller (1934) and therefore the trematode in the present study is referred to as *Plagioporus sinitsini.*

The genus *Plagioporus* was erected by Stafford (1904) for *P. serotinus*, a new species from the intestine of the red horse sucker, *Moxostoma macrolepidotum* (LeSueur). Miller (1940) described the type species in more detail than Stafford and indicated that in *P. serotinus* and other species of this genus, the excretory vesicle is short, reaching forward at the most to the level of the posterior testis.

Mueller (1934) described *P. sinitsini* from two specimens taken from the gallbladder of a fingerling *Catostomus commersoni* from Oneida Lake, Syracuse, New York (Fig. 1). Measurements of a flattened, mounted specimen included: body length: 1.22 mm, body width: 0.52 mm, oral sucker: 0.1173 x 0.065 mm, pharynx: 0.048 x 0.065 mm, acetabulum diameter: 0.255 mm, and eggs: 0.070 x 0.035 mm. Other characteristics described by

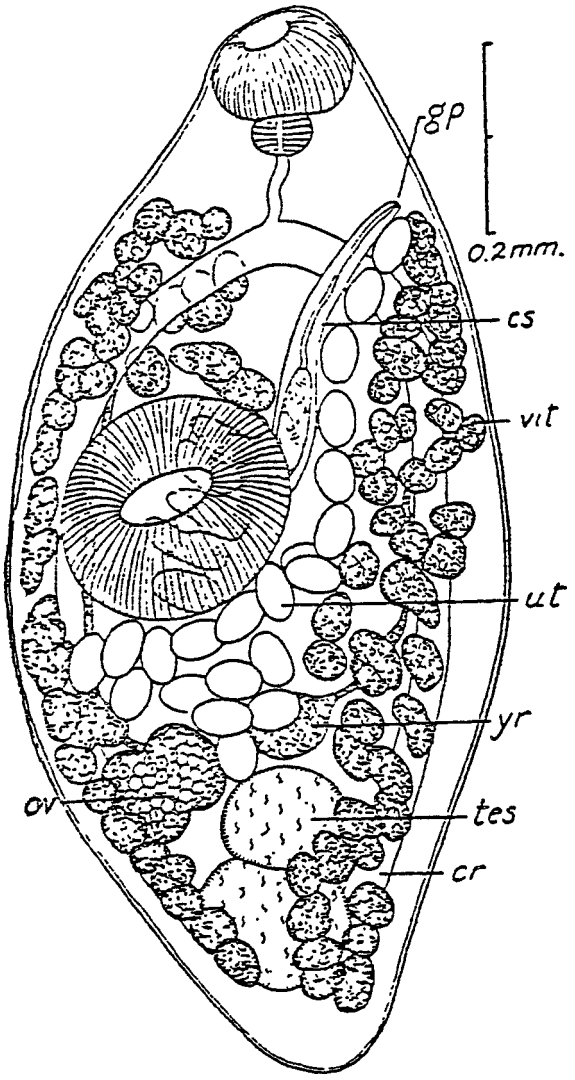

Figure 1. *Plagioporus sinitsini*, ventral view (from Mueller, 1934). (Legend: cr = intestinal crura, cs = cirrus sac, gp = genital pore, ov = ovary, tes = testis, ut = uterus, vit = vitellaria, yr = yolk reservoir)

Mueller (1934) included: smooth, thin cuticula; terminal oral sucker orifice; no prepharynx present; acetabulum located near the center of the body; wide intestinal crura extend to the middle of the second testis; vitellaria extend from the esophagus to the caudal extremity; ventral genital pore on the left margin at the pharyngeal level; slightly bilobed ovary located to the right; uterus consists of several small loops anterior to the ovary; elongate cirrus sac overlaps the anterior portion of the acetabulum on its dorsal side and it possesses a straight ejaculatory duct and a cylindrical vesicula seminalis; testes were serially oblique; ellipsoidal, operculate, straw-colored eggs, with about 22 present in mature worms.

Miller's (1940) key to species of *Plagioporus* included the following characteristics for *P. sinitsini*: smooth cuticula, spherical acetabulum not over twice the diameter of the oral sucker, testes near posterior tip of body, vitellarian follicles not numerous in post-testicular body region, and body not strongly fusiform.

The Life Cycle of *Plagioporus sinitsini*

Dobrovolny (1939) first described the sporocyst, its development, and completed the life cycle of *P. sinitsini*. *Goniobasis livescens* Menke, an operculate snail of the family Pleuroceratidae, serves as both the first and second intermediate host. Within the snail, sporocysts are long and saclike, with a short, obtuse, muscular anterior end and a rounded posterior end. The terminal birth port is surrounded by large cells, giving it the appearance of a pseudo-sucker. Cotylocercous cercariae develop in sporocysts and then develop into metacercariae *in situ* , meaning they never leave the sporocysts. Cercariae possess an oral sucker without stylets and a short, bell-shaped tail, which functions as a sucker. Judging from the large number of sporocysts bearing cercariae, there is thought to be two or more

generations of sporocysts. The sporocysts with mature metacercariae emerge from the rectum of the snail and are readily eaten by various fishes. Metacercariae excyst in the intestine of the fish and then migrate through the ductus choledochus and the cystic ducts into the gall bladder, where they develop into sexually mature worms in 2-4 weeks. Eggs from the worms are carried into the intestine with the bile and are discharged from the fish with the excrement. The long, pear-shaped, ciliated miricidium hatches in the water but its penetration and subsequent development in the snail was not observed by Dobrovolny, yet he did note the absence of a stylet and the poorly developed penetration glands in the miricidium.

Lindholm and Huffman (1979) completed the life cycle of *P. sinitsini* from the San Marcos River in *Cyprinella venusta*, using the intermediate host *Elimia (=Goniobasis) comalensis* Pilsbury. The pleuroceratid genus *Goniobasis* was separated into *Oxytrema* for the eastern species and *Elimia* for the southwestern species on the basis of anatomical differences found by Taylor (1966). In Lindholm and Huffman's study, *P. sinitsini* sporocysts were collected from *E. comalensis* from the San Marcos River and were fed to *C. venusta* from the Blanco River, where the trematode had not been found to occur. The parasite was experimentally shown to infect the gall bladder of this fish. The authors described the life cycle of this trematode just as Dobrovolny (1939) did, with the exception of the description of the cercariae. Lindholm and Huffman described the cercariae as being of the microcercous type with a stylet present. Dobrovolny described a cotylocercous cercaria with the stylet absent, which Olsen (1974) also noted in the description of the life cycle of *P. sinitsini*. Schell (1970) recorded a cotylomicrocercous cercaria in his description of the life cycle of *P. sinitsini* and in the key to the cercariae, he indicated that cotylocercous and cotylomicrocercous are equivalent terms.

Literature Review

Several studies concerning host-induced morphological variation among trematodes have been conducted. Beaver (1937) studied 300 mounted, unflattened specimens of *Echinostoma revolutum* (Froelich) and found substantial variation for all parts measured. He attributed some of the variation to the different species of hosts and he reported that in one case, the appearance of worms taken from a pig were so different that they could easily have been described as a different species had their origin not been known. In another early study, Rankin (1938) examined studies describing 20 different species of *Brachychoelium* Dujardin in North American salamanders. He believed that too many species had been described on too few specimens from a limited number of hosts and he indicated that characters considered specific in describing many species are probably nothing more than normal individual differences. Berrie (1960) experimentally infected ducks, chickens, pigeons, herring-gulls, and rats with *Diplostomum phoxini* (Faust) and reported significant differences between adult flukes recovered from different host species. Dogiel (1966) reports that the trematode *Prosotocus confusus* Looss, which usually lives in the anterior part of the frog's small intestine, sometimes inhabits the stomach; the stomach dwellers usually differ from their intestinal relatives in size, in the structure of their ovaries, the shape of the vitelline follicles, and the size of the oral and ventral suckers. Watertor (1967) experimentally infected amphibians and reptiles with *Telorchis bonnerensis* Waitz and reported interspecific and intraspecific variation among trematodes of same age; the trematodes varied considerably in overall size, sucker ratios, distribution of vitelline follicles, and position of the cirrus sac and ovary. Kinsella (1971) studied intraspecific variation in *Quinqueserialis quinqueserialis* (Barker and Laughlin) in a variety of rodent hosts and found that body length, body width, oral sucker width, uterine coil

width, testis lenth, ovary length, cirrus sac length, and metraterm length were all host-dependent; egg length was the only measurement for which there was no significant difference found. Blankespoor (1974) examined more than 1500 laboratory-reared adults of *Plagiorchis noblei* Park from 17 mammalian and avian hosts and concluded that body size, sucker size, oral sucker position, esophagus length, gonad size and position, extent of vitellaria, and cirrus sac size and position are not stable taxonomic characters; he found the only reliable characters to be egg size and ratios of oral sucker and acetabulum sizes. The most recent study reviewed was conducted by Le Brun et al. (1988) on *Diplozoon gracile* Reichenback-Klinke, a monogenetic trematode. They examined this parasite in 4 cyprinid species, compared them to already described *Diplozoon* species, and concluded that the parasites of these 4 cyprinids all belong to the same species. The researchers doubted the taxonomic value of morphological and biometrical characters because of their great variability, therefore they conducted a genetic study based on electrophoretic analysis and completed successful cross-infestations.

Objectives

The objectives of this study were 1) to examine any morphological variation of *P. sinitsini* and the prevalence and mean intensity of infection of this parasite with respect to host species, sex, size, and location of collection, 2) to examine seasonal effects upon prevalence, mean intensity, and stages of the parasite, and 3) to determine the geographical and ecological distribution of this parasite in the San Marcos, Comal, and Blanco rivers in Central Texas.

CHAPTER II

MATERIALS AND METHODS

Host Collection and Necropsy

Cyprinella venusta, *Notropis amabilis*, and *Dionda episcopa* were collected from various sites along the San Marcos River from October 1989 to September 1990. An attempt was made to make monthly collections of at least ten individuals of each host species from the San Marcos River during the study period. Only *N. venustus* could be collected from the Blanco River, and once it was discovered that this host did not harbor *P. sinitsini*, collections from this river were discontinued. Martin (1976) reported the same results for *P. sinitsini* in *C. venusta* and *C. lutrensis* from the Blanco River. The same situation existed for the host *D. episcopa* from the Comal River and collections were discontinued. Although the intermediate host *E. comalensis* is found in the Comal River, Rupp (1977, unpublished student paper written for D. G. Huffman, Southwest Texas State University, San Marcos) did not report any findings of *P. sinitsini* in his study of the larval trematodes of gastropods from the Comal River. His illustrations of the larval trematodes of *E. comalensis* did not reveal any cotylocercous or cotylomicrocercous cercariae that could possibly be from *P. sinitsini*.

Fish were generally collected with a 20 ft bag seine; one collection of *D. episcopa* was made from Landa Lake (formed from the Comal Springs) by electrofishing. Live fish were transported back to the laboratory in polystyrene containers filled with river water. Upon arrival the containers were equipped with aerators to provide suitable conditions for the hosts prior to parasitological examination, which was within 36 hours of capture.

The spinal cord of each fish was severed at the base of the skull before length and weight measurements were taken. Following sex determination, the entire gallbladder was excised from each fish, placed in 0.75% saline solution, and examined under a dissecting microscope for the presence of the trematode . The number of adult and immature helminths was recorded.

Parasite Collection and Preservation

Trematodes were removed from the host by puncturing the gall bladder wall. Gravid trematodes were placed in distilled water to stimulate the release of their eggs. The trematodes were then flattened under coverslip pressure and fixed in AFA (alcohol-formalin-acetic acid) solution. The fixed trematodes were stained for 24 hours with acetic carmine and were then dehydrated in an 85%, 95%, 99%, and 100% ethanol series. Before being mounted in Canada balsam, the trematodes were cleared by transferring them through a series of alcohol-xylene solutions with increasing xylene concentrations. Measurements of body length, body width at acetabulum, oral sucker diameter, and acetabulum diameter were taken from mounted specimens using a calibrated ocular micrometer. Only sexually mature, egg-containing adults were measured. It is considered that the comparative measurements used in this study are satisfactory since all specimens were treated in the same manner.

Description of the Study Areas

This study was conducted in the Guadalupe and Blanco river basins on the Edwards Plateau in the vicinity of Hays County, Texas (Figure 2). Specifically, the study sites were located on the Blanco, San Marcos, and Comal Rivers. All three rivers experienced drought conditions during the study period.

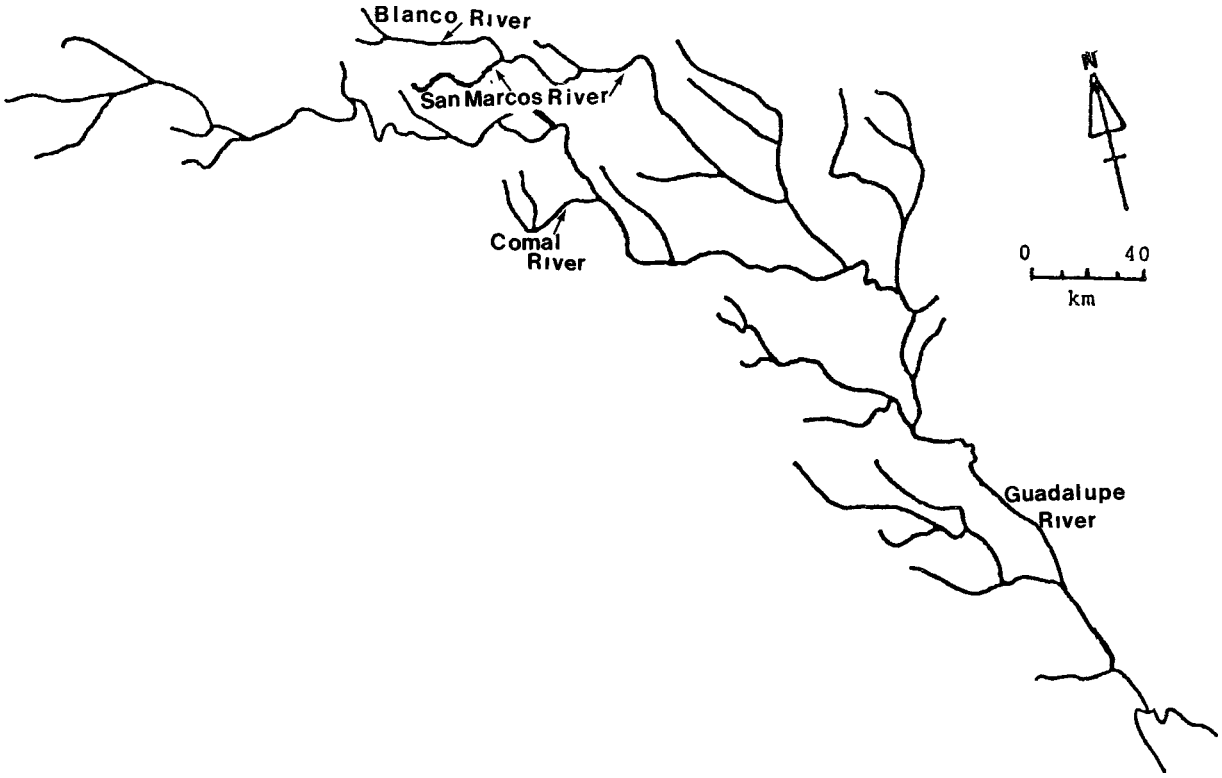

Figure 2. Map of the Blanco, San Marcos, and Comal Rivers in Central Texas.

Description of the San Marcos River

The primary source of water for the Upper San Marcos River, located in San Marcos, Hays County, Texas, is the Edwards Aquifer. The water emerges from fissures and many small openings in the Edwards limestone, creating Spring Lake. The river flows from Spring Lake and converges with the Blanco River before emptying into the Guadalupe River. Because the river is springfed, physico-chemical conditions are very stable, with temperatures ranging from 20-21°C.

Fish were collected from six different sites (sites A-F) in the San Marcos River. Site A was downstream from Spring Lake dam in a run between the falls at Pepper's Restaurant and Aquarena Springs Drive (Fig. 3). It was the primary collection site for *D. episcopa*, with 78 *D. episcopa* captured from this site. The water depth in the run ranged from 60 cm to 130 cm. A few *D. episcopa* were collected in faster-moving water along the left bank. Delta arrowhead (*Sagittaria platyphylla*) and filamentous algae were the predominant plant species in this area. Most *D. episcopa* were collected in slower-moving water in the center of the river where common hornwort (*Ceratophyllum demersum*) was abundant. The endangered Texas wild rice (*Zizania texana*) was present at the end of the run just before the bridge and elephant ear (*Colocasia antiquarum*) lined the banks of the river. The substrate at the site consisted of silt-covered cobble and gravel.

Five *D. episcopa* and 22 *N. amabilis* were collected from site B at Rio Vista Park on the San Marcos River (Fig. 3). *Dionda episcopa* were collected in shallow (approximately 20 cm deep), slow-moving water along the right bank below the dam. Eelgrass (*Vallisneria americana*) was the predominant aquatic plant species in this area and the substrate was silt-

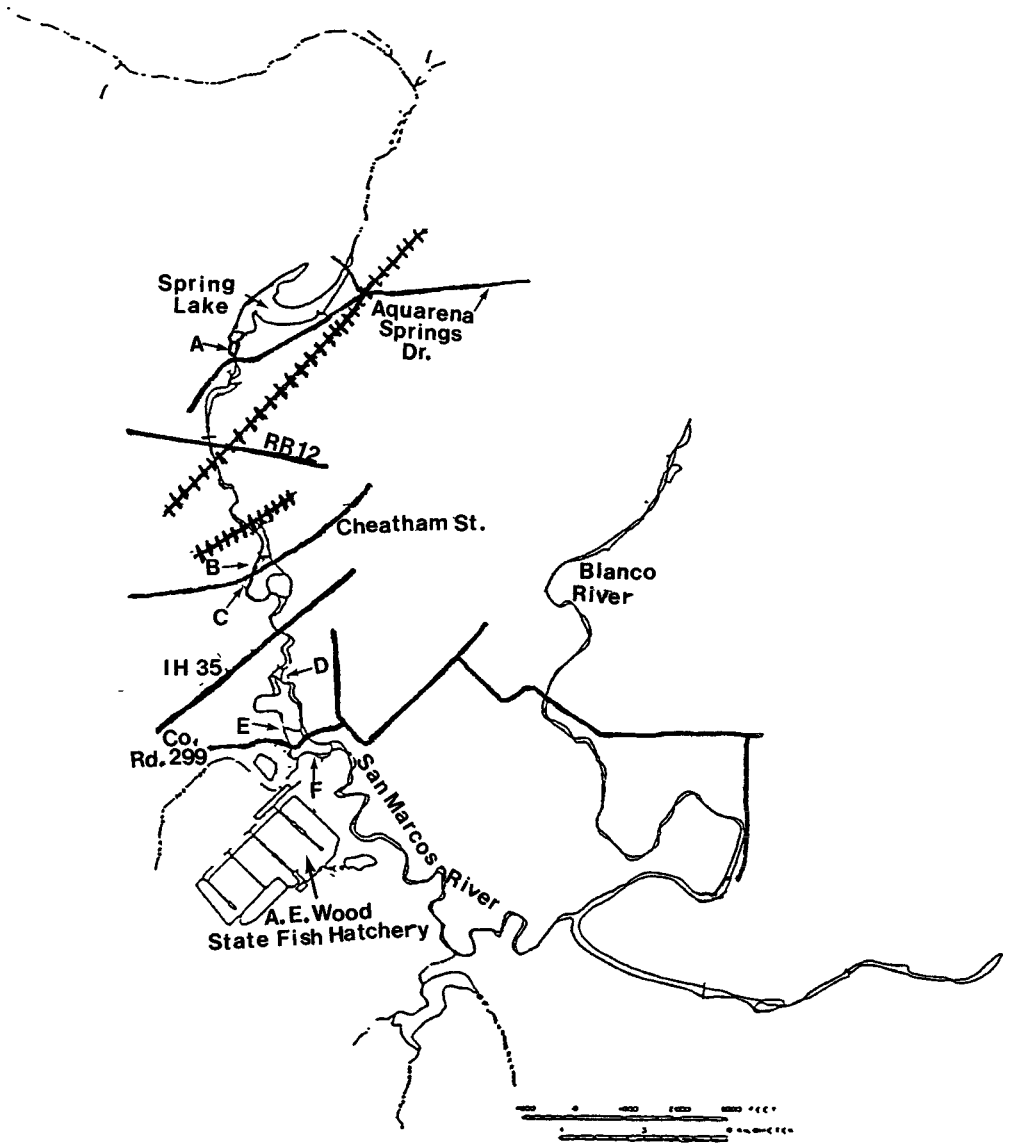

Figure 3. Map of the study sites for the San Marcos River.

covered gravel. *Notropis amabilis* were collected in the shallow riffle area below the dam. The substrate in this area consisted of boulders, large cobble, and various-sized gravel. The riffle area was covered with filamentous algae and the banks were lined with elephant ear (*Colocasia antiquarum*).

Site C was just below the Cheatham Street crossing in a fairly deep pool of fast-moving water near the west bank (Fig. 3). Fifteen *N. amabilis* were collected at this site. Filamentous algae and *Hydrilla* sp. were the predominant aquatic plant species at this site. Elephant ear (*Colocasia antiquarum*) lined the west bank and the substrate was sand, gravel, and cobble.

Four *C. venusta*, 20 *N. amabilis*, and 5 *D. episcopa* were collected from site D just below Cape's Dam on the upstream end of Thompson's Island (Fig. 3). The river was approximately 90 cm to 125 cm deep and large boulders and cobble covered the riverbed. The water was very turbulent at this site. The predominant aquatic plant species was fanwort (*Cabomba caroliniana*) .

Site E was a shallow, fast-moving riffle area on the downstream end of Thompson's Island (Fig. 3) upstream from the County Road 299 bridge. Thirty-seven *N. amabilis* and 103 *C. venusta* were collected at this site. The substrate was silt-covered boulders, large cobble, and gravel. Filamentous algae and *Hydrilla* sp. were the predominant aquatic plant species. Elephant ear (*Colocasia antiquarum*) grew on the banks.

Fifteen *N. amabilis* were collected from site F, which was a fast-flowing riffle area close to the intake pumps for the A. E. Wood State Fish Hatchery (Fig. 3). The river was approximately 45 cm to 60 cm deep in this region and the substrate was mainly gravel with some silt. Filamentous algae and delta arrowhed (*Sagittaria platyphylla*) were the predominant plant species.

Description of the Blanco River

Station G was located in a backwater area on the east bank of the Blanco River just above Five Mile Dam. Physico-chemical conditions at this station were highly unstable, since the river fluctuated seasonally in depth and temperature. Fifteen *C. venusta* and 15 *N. amabilis* were captured at this site. The substrate consisted of cobble and gravel, covered with a thick layer of mud and silt. Waters were generally turbid. The predominant plant species was elephant ear (*Colocasia antiquarum*).

Description of the Comal River

The Comal River, located in New Braunfels, Comal County, Texas, originates from a series of springs in Landa Park (Fig. 4). Like the San Marcos River, these springs also arise from the Edwards Aquifer. The Comal River flows east for about 1.6 km, before converging with the Guadalupe River. The riverbed consists of a mixture of clay, silt, gravel, and sand. Water temperatures are stable, ranging from 20-21°C.

Forty-two *D. episcopa* were collected from the mouth of the spring run, site H (Fig. 4). The predominant aquatic plant species were eelgrass (*Vallisneria americana*), shining pondweed (*Potamogeton illinoensis*), and rush (*Juncus* sp.). The site had moderately fast-moving water, a water depth of approximately 110 cm, and a silty, cobble bottom.

Twenty-two *D. episcopa* were collected from site I at Landa Lake (Fig. 4). Elephant ear (*Colocasia antiquarum*) was the predominant plant species along the banks; the predominant aquatic plant species were eelgrass (*Vallisneria americana*), fanwort (*Cabomba caroliniana*), and floating primrose-willow (*Ludwigia repens*). The water depth ranged from 60 cm to 305 cm. Specimens of *D. episcopa* were collected by electrofishing.

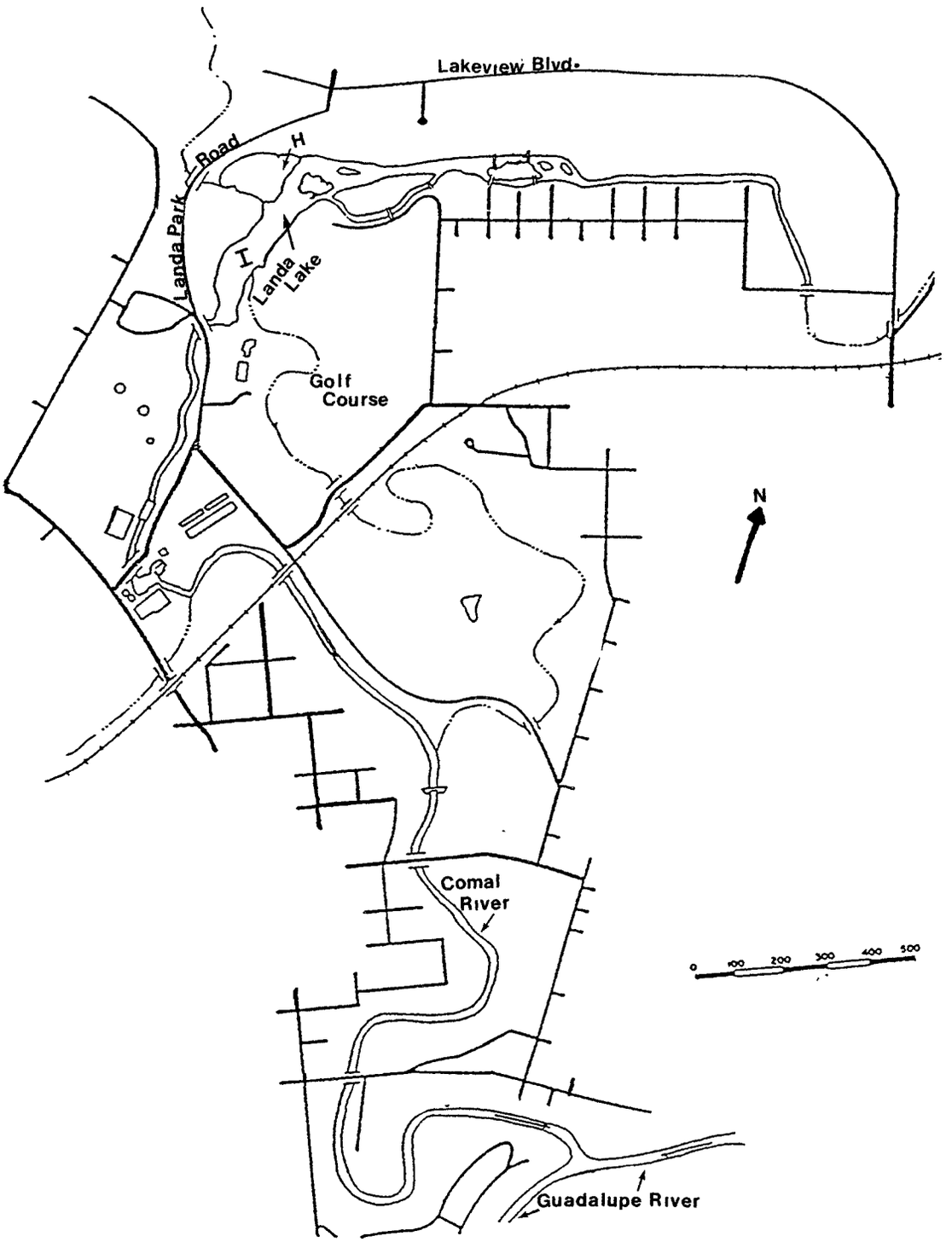

Figure 4. Map of the study sites for the Comal River.

Statistical Analysis

Statistical analysis of the morphometric data obtained from flattened, mounted specimens of *P. sinitsini* was performed with Statview 4.01 (Abacus Concepts) for the Macintosh computer. The computer program used analysis of variance and Fisher's PLSD, a pos hoc comparison, to determine any statistical differences in trematode morphological variation with respect to host species. Significance was recognized in all comparisons at $p \leq 0.002$. Additionally, prevalence and mean intensity of *P. sinitsini* with respect to host species were manually calculated. Statistical analysis of trematode morphological variation, prevalence, and mean intensity with respect to host sex, host size, season, and location of collection was not performed because unequal sample sizes, missing data for months when no hosts were collected, and an unequal distribution for size classses may contribute to a large source of bias from sampling artifacts.

CHAPTER III

RESULTS AND DISCUSSION

Prevalence and Mean Intensity of *Plagioporus sinitsini* with Respect to Host Species

Dionda episcopa

The prevalence and mean intensity of *Plagioporus sinitsini* was the highest in the gallbladders of *D. episcopa* from the San Marcos River (Table 2). Eighty-one *D. episcopa* (92.0%) were infected with a total of 3643 *P. sinitsini*; the mean intensity was 45.0. Underwood and Dronen (1984) achieved similar results and reported a prevalence of 95% and a mean intenstiy of 49.3 of *P. sinitsini* in *D. episcopa*. At site A (Pepper's), 77 fish (92.8%) were infected with a total of 3217 *P. sinitsini*. The prevalence of this trematode in *D. episcopa* was the highest at this site. The mean intensity was 41.8; the maximum number of trematodes recovered from a single fish was 214. At site B (Rio Vista Park), four fish (80.0%) were infected with a total of 426 *P. sinitsini*. The mean intensity was 106.5; the maximum number of trematodes recovered from a single fish was 204. The mean intenstiy of this parasite in *D. episcopa* was the highest at this site.

Sixty-two *D. episcopa* were collected from the Comal River during the months of February, July, and August, 1990. *Plagioporus sinitsini* was not found in any of these fish. This is surprising, as the intermediate host, *Elimia comalensis*, is present in large numbers in

Table 2. Prevalence and mean intensity of *Plagioporus sinitsini* in *Dionda episcopa*, *Cyprinella venusta*, and *Notropis amabilis* for all sites and for each site on the San Marcos River.

Host Species and Site	Prevalence[1] (Percent)	Mean Intensity[2]	Number of Parasites (min - max)
All San Marcos River Sites			
D. episcopa	92.0 (81/88)	45.0 (3643/81)	1-214
C. venusta	68.2 (73/107)	21.0 (1530/73)	1-56
N. amabilis	53.2 (67/126)	11.0 (738/67)	1-124
Site A - Peppers			
D. episcopa	92.8 (77/83)	41.8 (3217/77)	1-214
N. amabilis	52.9 (9/17)	7.8 (70/9)	1-29
Site B - Rio Vista Park			
D. episcopa	80.0 (4/5)	106.5 (426/4)	5-204
N. amabilis	63.6 (14/22)	7.4 (103/14)	1-44
Site C - Cheatham St.			
N. amabilis	46.7 (7/15)	10.3 (72/7)	2-28
Site D - Cape's Dam			
C. venusta	75.0 (3/4)	18.7 (56/3)	2-48
N. amabilis	50.0 (10/20)	16.3 (163/10)	7-42
Site E - Thompson's Isle			
C. venusta	68.0 (70/103)	21.1 (1474/70)	1-56
N. amabilis	59.5 (22/37)	14.2 (312/22)	1-124
Site F - Fish Hatchery			
N. amabilis	33.3 (5/15)	3.6 (18/5)	1-7

[1](Number of fish infected) x 100 / (number of fish examined). Numbers noted parenthetically.

[2](Total number of *P. sinitsini*) / (number of fish infected). Numbers noted parenthetically.

this river. Additionally, because the San Marcos and Comal Rivers are very similar in physicochemical characteristics and are in close proximity, the parasite was expected to occur here. The life cycle may have never been established here. Klaus (1991) suggests that the trematode may have been eradicated when the Comal Springs went dry during the drought of 1955-56. Neither *D. episcopa* nor *E. comalensis* were found in the Blanco River.

Cyprinella venusta

Plagioporus sinitsini was found in smaller numbers in *C. venusta* from the San Marcos River (Table 2). One hundred and seven *C. venusta* (68.2%) were infected with a total of 1530 *P. sinitsini*; the mean intensity was 21.0. Underwood and Dronen (1984) reported a prevalence of 20% and a mean intensity of 29.3 for *P. sinitsini* in *C. venusta*, but only 15 hosts were examined. At site D (Cape's Dam), 3 fish (75.0%) were infected with a total of 56 *P. sinitsini*. The prevalence of the parasite in *C. venusta* was the highest at this site. The mean intensity was 18.7; the maximum number of trematodes recovered from a single fish was 48. At site E (Thompson's Isle), 70 fish (68.0%) were infected with a total of 1474 *P. sinitsini*. The mean intensity was 21.1; the maximum number of trematodes recovered from a single fish was 56. The mean intensity for this parasite in *C. venusta* was the highest at this site.

Fifteen *C. venusta* were collected from the Blanco River during the month of June, 1990. *Plagioporus sinitsini* was not found in any of these fish. This is not surprising, as the intermediate host, *Elimia comalensis*, was not present in this river. *Cyprinella venusta* was not found in the Comal River.

Notropis amabilis

Plagioporus sinitsini was found in the smallest numbers in *N. amabilis* from the San Marcos River (Table 2). A total of 126 *N. amabilis* were collected from the San Marcos River of which 67 (53.2%) were infected with a total of 738 *P. sinitsini*; the mean intensity was 11.0. At site A (Pepper's), 9 fish (52.9%) were infected with a total of 70 *P. sinitsini.* The mean intensity was 7.8; the maximum number of trematodes recovered from a single fish was 29. At site B (Rio Vista Park), 14 fish (63.6%) were infected with a total of 103 *P. sinitsini.* The prevalence of the parasite in *N. amabilis* was the highest at this site. The mean intensity was 7.4; the maximum number of trematodes recovered from a single fish was 44. At site C (Cheatham Street Crossing), 7 fish (46.7%) were infected with a total of 72 *P. sinitsini.* The mean intensity was 10.3; the maximum number of trematodes recovered from a single fish was 28. At site D (Cape's Dam), 10 fish (50.0%) were infected with a total of 163 *P. sinitsini.* The mean intensity was 16.3; the maximum number of trematodes recovered from a single fish was 42. The mean intensity of the parasite in *N. amabilis* was the highest at this site. At Site E (Thompson's isle), 22 fish (59.5%) were infected with a total of 312 *P. sinitsini.* The mean intensity was 14.2; the maximum number of trematodes recovered from a single host was 124. At site F (State Fish Hatchery), 5 fish (33.3%) were infected with a total of 18 *P. sinitsini.* The mean intensity was 3.6; the maximum number of trematodes recovered from a single host was 7. The prevalence and mean intensity of this trematode was the lowest at this site.

Only 5 *N. amabilis* were collected from the Comal River during the month of February, 1990. *Plagioporus sinitsini* was not found in any of these fish. Again, *P. sinitsini* was expected

to occur in this host in the Comal River, as it did in the San Marcos River. Fifteen *N. amabilis* were collected from the Blanco River and were not found to be infected by this parasite.

Effect of Season upon the Prevalence and Mean Intenstiy of *Plagioporus sinitsini* Infection

Dionda episcopa

The prevalence of *P. sinitsini* in *D. episcopa* from the San Marcos River ranged from 33.3% in September, 1989 to 100% in the 7 of the other 8 months sampled (Table 3). Mean intensity of *P. sinitsini* ranged from a low of 1 in September, 1989 to a high of 84.9 in June, 1990. Small sample sizes during some months prevent analysis or speculation as to seasonal patterns of prevalence or mean intensity for *P. sinitsini* in *D. episcopa*. Underwood (1981) also found no evident seasonal patterns of *P. sinitsini* in *D. episcopa* from the San Marcos River.

Cyprinella venusta

The prevalence of *P. sinitsini* in *C. venusta* from the San Marcos River ranged from a low of 40.0% in February, 1990 to a high of 83.3% in June, 1990 (Table 4). Mean intensity of *P. sinitsini* ranged from a low of 3.8 in February, 1990 to a high of 18.2 in April, 1990. Again, small sample sizes and the absence of collections for certain months prevent speculation as to seasonal patterns of prevalence or mean intensity for *P. sinitsini* in *C. venusta*. Underwood (1981) did not report any data for *C. venusta* concerning prevalence or mean intensity with respect to season. Martin (1976) reported that *P. sinitsini* prevalence peaked in late winter and early spring for *C. venusta* and *C. lutrensis* and was the lowest in late summer. The author also reported that *P. sinitsini* mean intensity peaked in March for both

Table 3. Prevalence and mean intensity of *Plagioporus sinitsini* in *Dionda episcopa* during the months sampled from the San Marcos River.

Month	Prevalence[1] (Percent)	Mean Intensity[2]	Number of Parasites (min - max)
September 1989	33.3 (1/3)	1.0 (1/1)	1
November	76.9 (10/13)	13.9 (139/10)	1-45
December	100.0 (5/5)	18.2 (91/5)	5-59
January 1990	100.0 (9/9)	68.4 (616/9)	3-214
February	100.0 (10/10)	28.7 (287/10)	6-46
March	100.0 (11/11)	48.8 (537/11)	1-144
April	100.0 (8/8)	28.8 (230/8)	3-61
June	100.0 (18/18)	84.9 (1529/18)	7-204
July	100.0 (2/2)	28.5 (57/2)	5-52
August	77.8 (7/9)	17.3 (156/9)	1-57

[1] (Number of fish infected) x 100 / (number of fish examined). Numbers noted parenthetically.

[2] (Total number of *P. sinitsini*) / (number of fish infected). Numbers noted parenthetically.

Table 4. Prevalence and mean intensity of *Plagioporus sinitsini* in *Cyprinella venusta* during the months sampled from the San Marcos River.

Month	Prevalence[1] (Percent)	Mean Intensity[2]	Number of Parasites (min - max)
November 1989	66.7 (2/3)	16.5 (33/2)	15-18
January 1990	60.0 (9/15)	7.7 (69/9)	1-19
February	40.0 (4/10)	3.8 (15/4)	1-6
April	75.0 (6/8)	18.2 (109/6)	2-48
June	83.3 (20/24)	13.1 (261/20)	1-56
July	71.4 (5/7)	15.2 (76/5)	2-36
August	71.4 (15/21)	13.5 (202/15)	2-38
September	63.2 (12/19)	6.3 (75/12)	1-16

[1](Number of fish infected) x 100 / (number of fish examined). Numbers noted parenthetically.

[2](Total number of *P. sinitsini*) / (number of fish infected). Numbers noted parenthetically.

host species. Martin's explaination that these results may be due to the inability of the fry to swallow the large gastropod intermediate host is invalid as the hosts directly ingest the parasite sporocysts, not the intermediate host, as shown by Dobrovolny (1939).

Notropis amabilis

The prevalence of *P. sinitsini* in *N. amabilis* from the San Marcos River ranged from 12.5% in February, 1990 to 100% in September, 1990 (Table 5). Mean intensity of *P. sinitsini* ranged from a low of one in February, 1990 to a high of 16.1 in April, 1990. As in *D. episcopa* and *C. venusta*, small sample sizes and the absence of collections for certain months prevent speculation as to seasonal patterns of prevalence or mean intensity for *P. sinitsini* in *N. amabilis*.

Morphological Variation

Statistical analysis was performed on the following *P. sinitsini* measurements with respect to host species: body length, body width at acetabulum, width to length ratio, oral sucker diameter, acetabulum diameter, and oral sucker to acetabulum ratio. A comparison of means of measurements of adult *P. sinitsini* collected from *D. episcopa*, *C. venusta*, and *N. amabilis* from the San Marcos River was conducted using Fisher's PLSD test. Significant differences were found in body length, body width at acetabulum, oral sucker diameter, and acetabulum diameter of trematodes from different hosts (Table 6). Statistical analysis of *P. sinitsini* measurements with respect to host size, host sex, season, and location was not performed due to unequal sample sizes, missing data for months when no hosts were collected, and unequal distribution of size classes.

Table 5. Prevalence and mean intensity of *Plagioporus sinitsini* in *Notropis amabilis* during the months sampled from the San Marcos River.

Month	Prevalence[1] (Percent)	Mean Intensity[2]	Number of Parasites (min - max)
September 1989	100.0 (2/2)	2.0 (4/2)	1 - 3
November	33.3 (1/3)	2.0 (2/1)	2
January 1990	33.3 (5/15)	3.6 (18/5)	1 - 7
February	12.5 (1/8)	1.0 (1/1)	1
April	50.0 (11/22)	16.1 (177/11)	7 - 42
June	56.0 (14/25)	6.1 (1529/18)	7 - 204
July	33.3 (1/3)	2.0 (2/1)	2
August	65.8 (25/38)	13.7 (343/25)	1 - 124
September	46.7 (7/15)	10.3 (72/7)	2 - 28

[1](Number of fish infected) x 100 / (number of fish examined). Numbers noted parenthetically.

[2](Total number of *P. sinitsini*) / (number of fish infected). Numbers noted parenthetically.

Table 6. Comparison of means of measurements (in millimeters) of adult *Plagioporus sinitsini* collected from *Dionda episcopa*, *Cyprinella venusta*, and *Notropis amabilis* from the San Marcos River. (Standard deviations are noted parenthetically.)

Body Part	*D. episcopa*	*C. venusta*	*N. amabilis*	S. D.*
Total length	0.905 (0.228)	0.940 (282)	0.734 (229)	DE, NA; NA, CV
Width at acetabulum	0.342 (0.077)	0.354 (0.870)	0.299 (71)	DE, NA; NA, CV
Width/length ratio (%)	38.569 (5.837)	41.967 (7.382)	38.635 (6.359)	DE, NA; NA, CV
Oral sucker diameter	0.134 (0.028)	0.121 (0.020)	0.113 (0.015)	DE, NA; DE, CV
Acetabulum diameter	0.221 (0.045)	0.192 (0.030)	0.183 (0.030)	DE, NA; DE, CV
Oral sucker/acetabulum ratio (%)	61.366 (9.384)	63.542 (6.164)	63.075 (8.090)	None

* S. D. = significant difference; DE = *D. episcopa*; CV = *Cyprinella venusta*; NA = *N. amabilis*

Body Length, Body Width, Width/Length Ratio

Trematodes from *D. episcopa* and *C. venusta* were significantly greater in body length, body width, and width/length ratio than trematodes from *N. amabilis* (Table 6). Interaction bar graphs illustrate these differences (Fig. 5, 6, 7). No significant differences in body measurements were found in trematodes from *D. episcopa* and *C. venusta*. Larger trematodes were expected to be found in the larger hosts, which were *D. episcopa* and *C. venusta*. The *F* test results on fish length data revealed significant differences between these two hosts and *N. amabilis*, but no significant difference was found between *D. episcopa* and *C. venusta*. Martin (1976) reported mean measurements of morphometric data for *P. sinitsini* from both *C. venusta* and *C. lutrensis*, but did not separate the data according to host.

Oral Sucker, Acetabulum, Oral Sucker/Acetabulum Ratio

A different pattern was seen for sucker morphology. Trematodes from *D. episcopa* were significantly greater in oral sucker and acetabulum diameter than worms in *C. venusta* and *N. amabilis* (Table 6). No significant difference in oral sucker diameter or acetabulum diameter was found in trematodes from *C. venusta* and *N. amabilis*. No significant differences were found in oral sucker to acetabulum ratios in the three host species. Interaction bar graphs illustrate these findings (Fig. 8, 9, 10). While oral sucker and acetabulum diameter was significantly greater in *D. episcopa*, the ratio of the two suckers remained relatively constant in all of the host species. When an increase in the size of the oral sucker was observed, a corresponding increase in the size of the acetabulum was also observed. These results indicate that while the

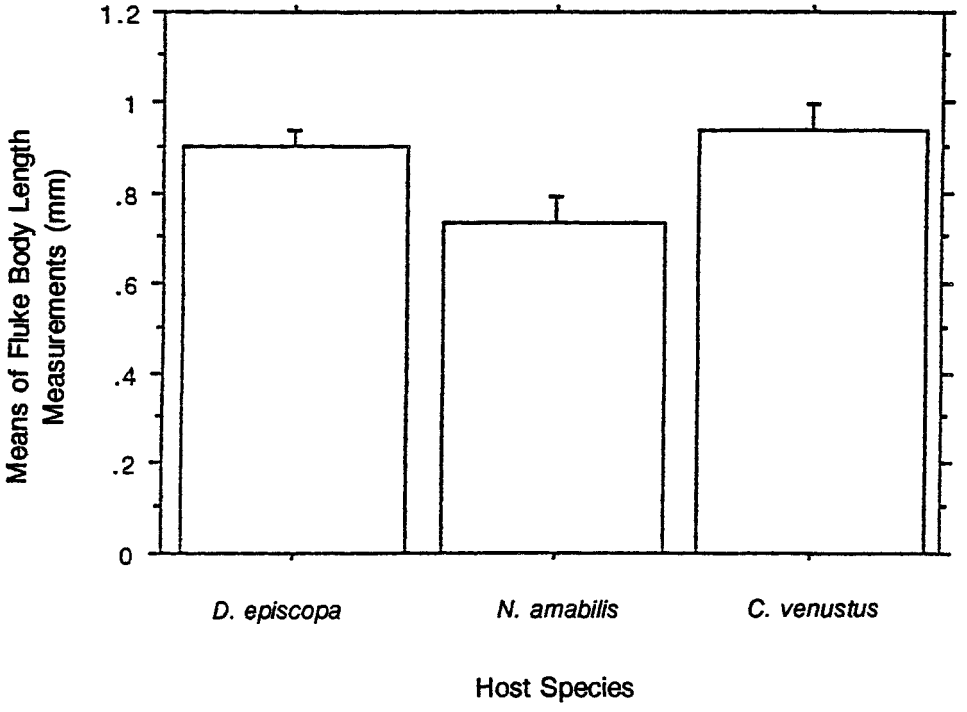

Figure 5. Interaction bar plot for body length of *Plagioporus sinitsini* collected from *Dionda episcopa*, *Notropis amabilis*, and *Cyprinella venusta* from the San Marcos River.

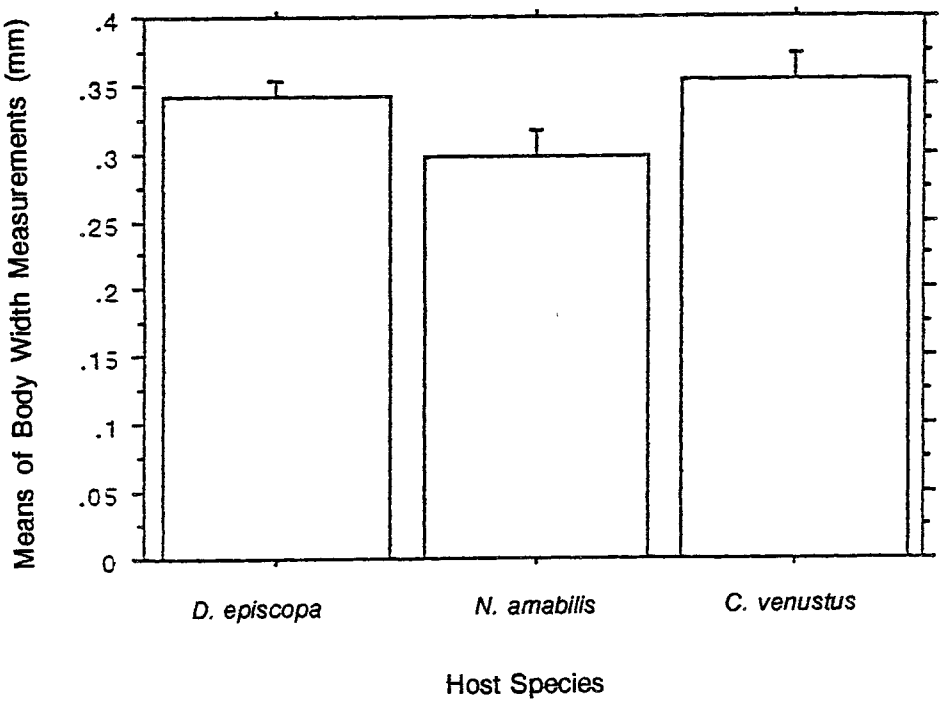

Figure 6. Interaction bar plot for body width (at acetabulum) of *Plagioporus sinitsini* collected from *Dionda episcopa*, *Notropis amabilis*, and *Cyprinella venusta* from the San Marcos River.

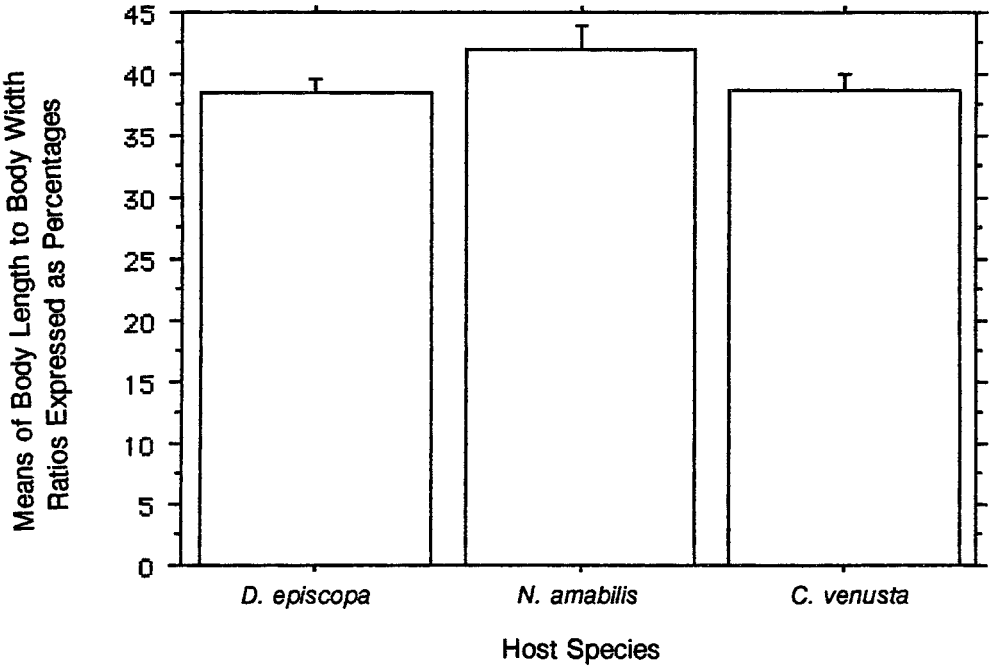

Figure 7. Interaction bar plot for body width/length ratio of *Plagioporus sinitsini* collected from *Dionda episcopa*, *Notropis amabilis*, and *Cyprinella venusta* from the San Marcos River.

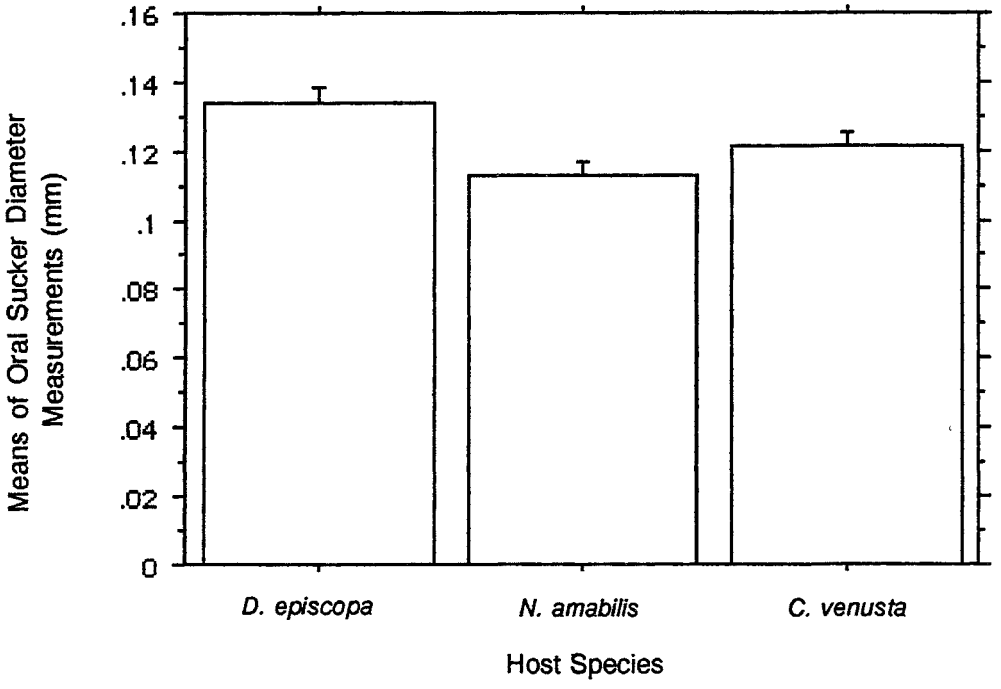

Figure 8. Interaction bar plot for oral sucker diameter of *Plagioporus sinitsini* collected from *Dionda episcopa*, *Notropis amabilis*, and *Cyprinella venusta* from the San Marcos River.

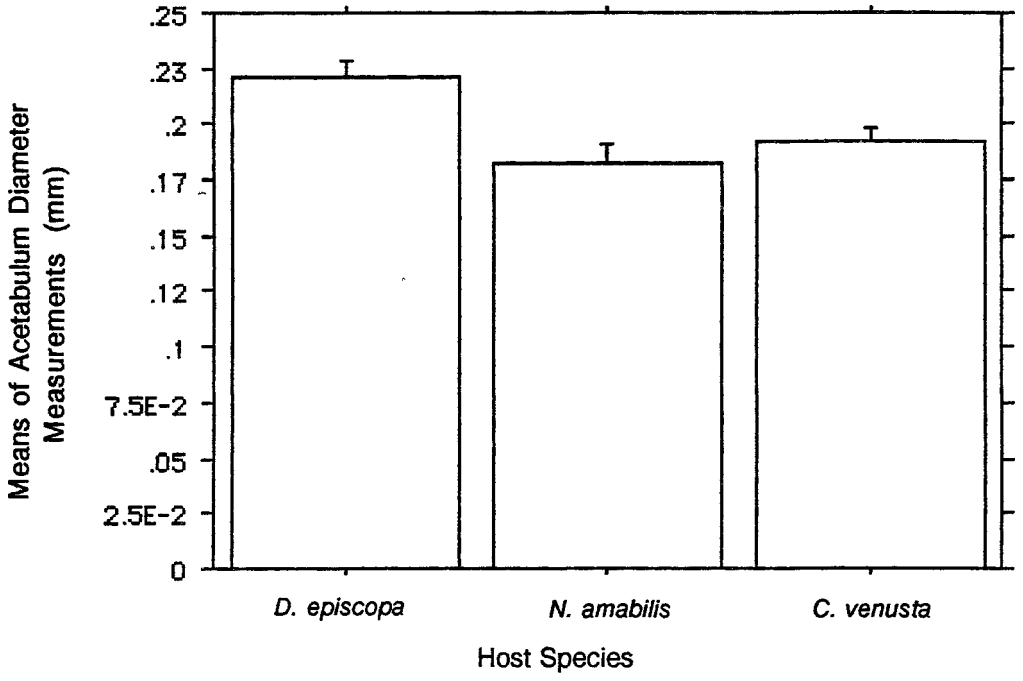

Figure 9. Interaction bar plot for acetabulum diameter of *Plagioporus sinitsini* collected from *Dionda episcopa*, *Notropis amabilis*, and *Cyprinella venusta* from the San Marcos River.

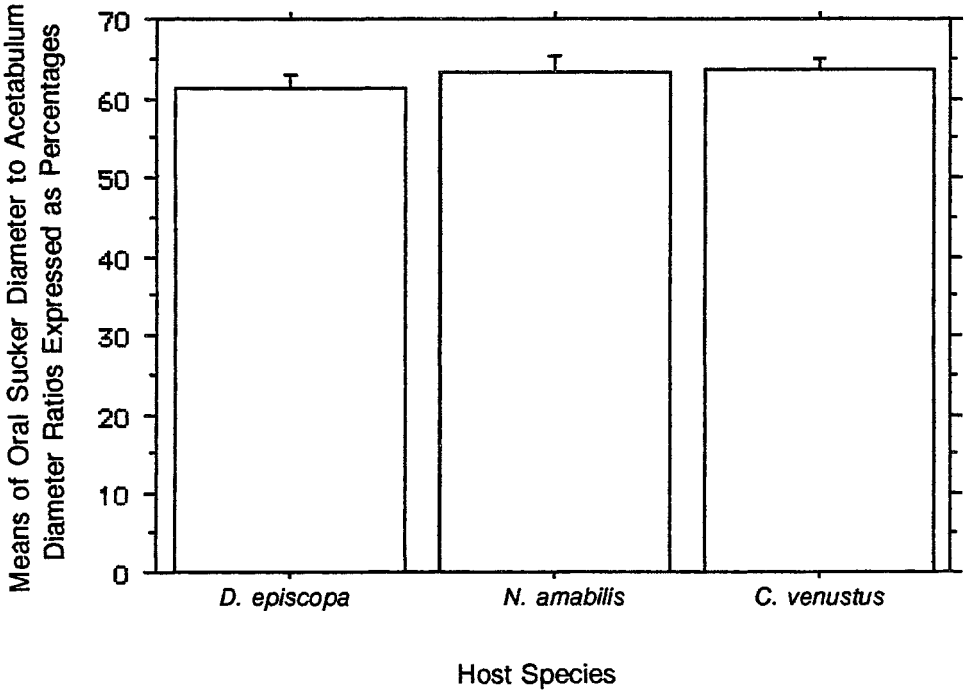

Figure 10. Interaction bar plot for oral sucker/acetabulum ratios of *Plagioporus sinitsini* collected from *Dionda episcopa*, *Notropis amabilis*, and *Cyprinella venusta* from the San Marcos River.

size of the oral sucker and acetabulum may be host-induced, ratios of sucker measurements remain constant and may be valid criteria used in describing trematode species. Blankespoor (1974) reported similar results in his study of the trematode *Plagiorchis noblei*.

Discussion of Morphological Variation

As long ago as 1923, parasitologists were aware that when a parasite develops in a different host, the parasite may undergo certain morphological changes which are fairly consistant as long as the parasite remains in or on that particular host (Chandler, 1923). At one time, parasitologists considered similar parasite species in two different hosts different, until they were definitely shown to be identical (Chandler, 1923). Growing evidence of host-induced morphological variation slowly changed opinions to consider these parasites identical until definitely shown to be distinct (Chandler, 1923). Scientists now realize that the phenotype of an individual is the resultant of interaction between its genotype and its environment (Haley, 1962). Changes in the host's external environment (macroenvironment), changes in the physiological condition of the host itself (microenvironment), and development of a parasite in a wide range of hosts may have a pronounced effect on parasite morphology and physiology (Dogiel, 1961). Several studies have demonstrated that pronounced variation in morphological characters of several families of trematodes may be host-induced (Beaver, 1937; Rankin, 1938; Berrie, 1960; Watertor, 1967; Kinsella, 1971; and Blankespoor, 1974). However, many host-induced morphological characters continued to be used in new species descriptions, causing invalid determinations to be made and making taxonomy more and more difficult. An early solution to the taxonomy problem was the erection of "hostal races" and trinomial nomenclature to indicate species in different hosts (Chandler, 1923).

Data obtained from natural infections of *P. sinitsini* in *D. episcopa*, *C venusta*, and *N. amabilis* in the present study indicate statistically significant variation in the following morphological characters: body length, body width at acetabulum, oral sucker diameter, and acetabulum diameter. There are some indications that the differences observed may be host-induced. Variation in body size was consistent among the hosts, with larger hosts (*D. episcopa* and *C. venusta*) harboring larger trematodes. Variation in sucker size was also consistent among the hosts (trematodes from *D. episcopa* had significantly larger suckers than trematodes from *C. venusta* and *N. amabilis*).

In addition to host-induced morphological variation, other sources of variation must be considered when describing trematodes. Trematodes have no skeletal structure and their overall shape and the shape and location of their organs is influenced by the extension and contraction of different muscles, by the accumulation of genital products in the seminal vesicle or uterus, and by accumulation of fluid in the excretory vesicle (Stunkard, 1957). *Plagioporus sinitsini* in the present study were often very gravid and expulsion of all of the eggs did not always occur. Because of contortions undergone by individuals in killing fluids, the specimens generally show little resemblance to their normal structure. All *P. sinitsini* specimens in the present study were mounted under coverslip pressure to avoid such contractions, but measurements of flattened specimens can also be misleading. According to Stunkard (1957), measurements of a flattened trematode can sometimes be 100% greater than those made on the same specimen in a contracted condition. However, because all of the specimens in the present study were treated in the same manner, some valid comparisons can be made. It was assumed in the present study that *P. sinitsini* reached their maximum size at sexual maturity, therefore all measurements were

made of adult specimens. Stunkard (1957) and Kinsella (1971) demonstrated that growth occurs past the time of sexual maturity in trematodes, so this may have also contributed to the variation that was seen. Conclusions of host-induced morphological variation would be better supported if experimental infections had been conducted so that the age of the flukes was known.

Because decisions for classifying helminth parasites are generally based on comparisons with previously described worms, more studies are needed on host-induced variation to better assess the stability of taxonomic characters used in classification. The literature indicates that two of the more stable characters are egg size (Kinsella, 1971 and Blankespoor, 1974) and oral sucker to acetabulum ratio (Blankespoor, 1974). Le Brun, et al. (1988) may have provided some solutions to the problem when they reported using electrophoretic analysis and cross-infestation experiments to determine if four trematodes belonged to the same species.

CHAPTER IV

SUMMARY

A morphological study of the trematode *P. sinitsini* from *D. episcopa*, *C. venusta*, and *N. amabilis* was conducted from September 1989 to October 1990. *Plagioporus sinitsini* was only recovered from fish from the San Marcos River in Central Texas. The intermediate host (*Elimia comalensis*) for this trematode species was abundant in the Comal River, but the trematode was absent from the Comal River fishes. This may be because the life cycle was never established there or because of eradication due to the drought the Comal Springs experienced in 1955 and 1956. The intermediate host was not found in the Blanco River.

A total of 88 *D. episcopa* were collected from two sites on the San Marcos River; the prevalence in these hosts was 92.0% and the mean intensity was 45.0 trematodes per host. A total of 107 *C. venusta* were collected from three sites on the San Marcos River; the prevalence in these hosts was 68.2% and the mean intenstiy was 21.0 trematodes per host. A total of 126 *N. amabilis* were collected from seven sites on the San Marcos River; the prevalence in these hosts was 53.2% and the mean intensity was 11.0 trematodes per host.

The season of collection of hosts had no apparent effect upon the prevalence or mean intensity of *P. sinitsini* in any of the three hosts. Small sample sizes and the absence of collections for certain months, prevented speculation as to seasonal patterns of prevalence or mean intensity.

Statistical analysis was performed on the following *P. sinitsini* measurements with respect to host species: body length, body width at acetabulum, width to length ratio, oral sucker diameter, acetabulum diameter, and oral sucker to acetabulum ratio. Statistical analysis of *P. sinitsini* measurements with respect to host size, host sex, season, and location was not performed due to unequal sample sizes, missing data for months when no hosts were collected, and unequal distribution of size classes. *Plagioporus sinitsini* from *D. episcopa* and *C. venusta* were significantly greater in body length, body width, and width/length ratio than trematodes from *N. amabilis*. These results indicate that these characters may be host-induced and were expected as *D. episcopa* and *C. venusta* were the larger hosts. While oral sucker and acetabulum diameter was significantly greater in *P. sinitsini* from *D. episcopa*, the ratio of the two suckers remained relatively constant in all of the host species. When an increase in the size of the oral sucker was observed, a corresponding increase in the size of the acetabulum was also observed. These results indicate that although the size of the oral sucker and acetabulum may be host-induced, ratios of sucker measurements remain constant and may be valid criteria used in describing trematode species.

Host-induced morphological variation may not have been the only source of variation in the morphometric data from *P. sinitsini*. Variation may also be due to the accumulation of eggs in the uterus, contortions undergone by specimens in killing fluids, and mounting the trematodes under coverslip pressure. Additionally, it has been demonstrated that growth occurs past the time of sexual maturity in trematodes, therefore the age of the trematodes may have contributed to variation.

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