A STUDY ON THE PARASITE DIVERSITY AND COMMUNITY ECOLOGY OF THREE SPECIES OF TEXAS FRESHWATER TURTLES

A Thesis

by

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ABSTRACT

In this study, the metazoan parasites of three species of freshwater turtles (the spiny softshell, *Apalone spinifera*, the common snapping turtle, *Chelydra serpentina*, and the red-eared slider, *Trachemys scripta elegans*) were surveyed at 16 sites across the state of Texas. A total of 42 species of metazoan parasites were recovered from 15 *A. spinifera*, nine *C. serpentina*, and 55 *T. s. elegans*, representing 16 new host-parasite associations and 17 new locality records. The synonymy of *Acanthostomum nuevoleonensis* by Brooks (1980) is refuted and the species is redescribed. Two new species of monogenean worms in the genus *Neopolystoma* are reported, one from *C. serpentina* and *A. spinifera* and another from *T. s. elegans*. Through non-metric multidimensional scaling and analysis of similarities, *A. spinifera* was found to contain a significantly distinct parasite community from *C. serpentina* and *T. s. elegans*. A range of water parameters (ammonia, carbon dioxide, chloride, dissolved oxygen, hardness, nitrite, nitrate, pH, salinity, temperature, and turbidity) were recorded on each sampling trip and compared to parasite abundance and diversity. Ammonia levels were positively correlated with abundance of acanthocephalans. Carbon dioxide levels were negatively correlated with parasite diversity and monogenean abundance. Chloride levels were negatively correlated with parasite diversity. Dissolved oxygen levels were positively correlated with parasite diversity and monogenean abundance. Turbidity was positively correlated with parasite abundance, acanthocephalan abundance, and digenean abundance, and negatively correlated with parasite diversity. Parasite abundance was

significantly lower in west Texas and western river basins, and lower in rivers than ponds. Acanthocephalan abundance was significantly lower in rivers than ponds. Leech abundance was highest in the Trinity river basin. Turbidity had the strongest correlations in this study. As water clarity increased, diversity increased and abundance of certain taxa decreased, indicating clearer water may have greater food web diversity and healthier hosts. This study adds valuable data on host-parasite associations, parasite distributions, and parasite ecology of turtles in the state of Texas. Many of these findings are likely transferable to other host taxa and should be studied in greater depth. Parasite diversity is not well known, even in common species, highlighting the need for more diversity surveys.

DEDICATION

This thesis is dedicated to my educators and mentors, past and present. Thank you for believing in me and pushing me to greater things.

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This project would not have been possible without the contributions of a number of people. First and foremost I would like to thank my advisor, Dr. Norman Dronen, for teaching me so many valuable skills, always being available, offering direction and inspiration, and being my biggest advocate. I would also like to thank my committee members, Dr. Toby Hibbitts and Dr. Thomas Craig for their invaluable assistance with parasite and turtle identification, equipment acquisition, trapping methodology, and writing edits during the course of this research.

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This work was supervised by a thesis committee consisting of Dr. Norman O. Dronen (advisor) and Dr. Toby J. Hibbitts of the Department of Wildlife and Fisheries Sciences and Dr. Thomas M. Craig of the Department of Veterinary Medicine and Biomedical Sciences.

All work for the thesis was completed by the student, in collaboration with Charlayna Cammarata. Undergraduate assistants Kelsey Garner and Travis Doggett assisted with a large portion of field and lab work for this project.

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CHAPTER I

INTRODUCTION

Parasite diversity is a key component in understanding ecosystem complexity. With conservative estimates of around 40% of known species being parasitic, parasitism is the most common life strategy (Dobson et al., 2008). The high number of unsampled host species and amount of cryptic speciation potentially uncovered through genetic analysis indicates that this number is likely higher (Jousson et al., 2000; Steinauer et al., 2007). Helminths, or parasites in the phyla Acanthocephala, Nematoda, Platyhelminthes, and the subclass Pentastomida, often have complex life cycles, sometimes traveling through many hosts throughout development (e.g. Parker et al., 2003; Poulin, 2011). Endohelminths, or internal helminths, are associated with predator-prey relationships, as they are typically transmitted through consumption. Current estimates indicate that parasites are involved in nearly 75% of all trophic linkages in food webs due to their complex life cycles and dependence on hosts (Lafferty, 2008; Lafferty et al., 2006). Consequently, healthy ecosystems with greater numbers of trophic linkages are believed to be higher in parasite diversity. Parasite diversity is therefore a good indicator of ecosystem diversity and total ecosystem health (Hudson et al., 2006; Marcogliese, 2005). Despite these findings, parasite assemblages remain highly understudied, with many new species being described every year and with many life cycles completely unknown (Blasco-Costa and Poulin, 2017; Dobson et al, 2008; Poulin, 2014).

Previous research suggests that parasitic species may be trophic regulators in the same capacity as top predators (e.g. Dougherty et al., 2016; Lafferty et al. 2006); however, most conservation plans do not implement any efforts to preserve parasite diversity, and often work to eradicate parasites to alleviate stressors on threatened species. In order to conserve the total diversity in an ecosystem, parasite diversity must be taken into account when making conservation plans. For this to be possible knowledge of the parasites species present in a given ecosystem is vital. Studies on parasite diversity are difficult as they generally require collection and euthanasia of a large number of hosts. In spite of the challenges, studies on parasite communities are needed to understand the full breadth of diversity in ecosystems (Hudson et al., 2006; Dobson et al., 2008).

Environmental factors can alter parasite assemblages in significant ways. Some environmental factors associated with changes in parasite abundance and diversity are temperature, dissolved oxygen, turbidity, salinity, nutrient pollution, metal pollution, pesticide/herbicide pollution, and habitat alteration (e.g. Bourque and Esch, 1974; Lafferty and Kuris, 1999; Banu and Khan, 2004; Nachev and Sures, 2009; Shea et al., 2012; Chapman et al., 2015; Ahmad et al., 2016). Due to the complex and diverse nature of parasite life cycles, the effects of environmental factors are variable, and often contradictory. Bourque and Esch (1974) found that nematode abundance responded differently to thermal pollution between two wetlands. Goednkegt et al. (2015) reported trematode infectivity to increase with increasing temperatures; however, predation on cercaria also increased with increasing temperatures, which then decreased trematode

infectivity. In their review, Lafferty and Kuris (1999) found a variety of possible outcomes on parasite-host interactions impacted by environmental stressors. Pollutants can increase parasite infectivity by increasing host susceptibility or decrease parasite infectivity by decreasing host survival and therefore parasite transmission. Zargar et al. (2012) found varying intensities of monogeneans on fish across polluted lakes, with decreasing intensities in one polluted and eutrophied lake and increasing intensities at a different polluted and eutrophied lake. Current trends in environmental degradation and climate change point to a change in currently observed parasite diversity (e.g. Brooks and Hoberg, 2007; Strona, 2015; Cizauskas et al. 2017). With the convoluted nature of environmental effects on parasites, it is vital to continue research in smaller systems that can be used to clarify the bigger picture.

The state of Texas can be broken up into 12 major ecological regions and 15 major river basins. Ecological regions, or ecoregions, are large stretches of land that are grouped based on the native vegetation, hydrology, and geochemistry (Griffith et al., 2007). Aquatic communities can be characterized by the ecoregion in which they reside, as aquatic community assemblages tend to vary greatly among different ecoregions (Warry and Hanau, 1993; Stoddard, 2005). The ecoregions found within the state of Texas are the Arizona/New Mexico Mountains, Chihuahuan Desert, High Plains, Southwestern Tablelands, Central Great Plains, Cross Timbers, Edwards Plateau, Southern Texas Plains, Texas Blackland Prairies, East Central Texas Plains, Western Gulf Coastal Plains, and South Central Plains. These ecoregions have many characteristic features such as the vegetative communities (hardwood forest, prairie,

scrublands, etc.) and soil characteristics (sand or clay, acidic or basic, shallow or deep, etc.) (Griffith et al., 2007).

The river basins delimit the area drained by each major river and its tributaries and may cross multiple ecoregions (Bureau of Economic Geology, 1996). The separation between these basins can often be a determining factor in the range of aquatic species, as seen in freshwater mussel diversity (Burlakova et al., 2011). The river basins found in the state of Texas are the Brazos, Canadian, Colorado, Cypress, Guadalupe, Lavaca, Neches, Nueces, Red, Rio Grande, Sabine, San Antonio, San Jacinto, Sulphur, and Trinity Basins. The water chemistry and biotic communities may change across the river basin, since the common river basin is the only connecting factor (Ford et al. 2016). Texas can also be viewed in respects of latitudinal and longitudinal gradients. North and East Texas are typically wetter while South and West Texas are typically drier (Texas Commission on Environmental Quality, 2018), which could lead to shifts in parasite diversity and abundance due to changes in intermediate host abundance and larval dispersal (Janzen and Schoener, 1968; Froeschke et al. 2010).

In the state of Texas, only four metazoan parasite surveys have been conducted on freshwater turtles (Harwood, 1932; Everhart, 1957; Dinuzzo, 1981; McAllister et al., 2008). Harwood (1932) conducted a survey of the endohelminths of 50 species of amphibians and reptiles over the course of two and a half years in the vicinity of Houston, Texas. Over the course of this study, eight species of turtles were collected: 16 red-eared sliders, *Trachemys scripta elegans* (Wied, 1839), 16 Mississippi mud turtles, *Kinosternon subrubrum hippocrepis* Gray, 1856, 14 three-toed box turtles, *Terrapene*

carolina triunguis (Agassiz, 1857), nine common snapping turtles, *Chelydra serpentina* (Linnaeus, 1758), four spiny softshells, *Apalone spinifera* (LeSueur, 1827), two razor-backed musk turtles, *Sternotherus carinatus* (Gray, 1856), two ornate box turtles, *Terrapene ornata* (Agassiz, 1857), and one chicken turtle, *Deirochelys reticularia* (Latreille in Sonnini and Latreille, 1801). The four *A. spinifera* were reported as "*Amyda ferox*" but based on location are believed to be *A. spinifera*. Everhart (1957) conducted a survey of the endohelminths of *T. s. elegans* from two localities in Southern Texas and six localities near Stillwater, Oklahoma. A total of 79 turtles, 56 from Texas and 23 from Oklahoma, were collected during the course of this study. McAllister et al. (2008) surveyed endoparasites of 18 species of amphibians and reptiles from 11 counties in Arkansas and six counties in Texas (Bowie, Cass, Denton, Johnson, Somervell, and Webb). Of these 18 species, only two were turtles and one was a tortoise: five ornate box turtles, *Terrapene ornata ornata* (Agassiz, 1857), four yellow mud turtles, *Kinosternon flavescens* (Agassiz, 1857), and one Texas tortoise, *Gopherus berlandieri* (Agassiz, 1857). Dinuzzo (1981) collected 124 *T. s. elegans* over the course of a year from one location in Burleson County, Texas. This data was never formally published and specimens have not been located, so the host associations and locality records reported cannot be verified. Two of these studies were range restricted (Harwood, 1932; Dinuzzo, 1981), two did not sample many individuals (Harwood, 1932; McAllister et al., 2008), and two only sampled one species (Everhart, 1957; Dinuzzo, 1981). Parasite community structure varies greatly between geographical locations, parasite species, host species, individual hosts, different environments, and different seasons (Ernst and Ernst, 1977;

Esch and Gibbons, 1967; Poulin, 2006; Readel et al., 2008). For this reason, it is useful for studies to cover multiple host species across broader geographic and temporal ranges in order to reveal a clearer picture of the diversity in parasite communities in that area.

The three most common native species of freshwater turtles in Texas are the spiny soft shelled turtle, *Apalone spinifera* (Trionychidae) [syns. *Amyda*, *Aspidonectes*, *Platypeltis*, *Trionyx*], the common snapping turtle, *Chelydra serpentina* (Chelydridae) [syns. *Testudo*], and the red-eared slider, *Trachemys scripta elegans* (Schoepff, 1792) (Emydidae) [syns. *Chrysemys*, *Emys*, *Pseudemys*, *Testudo*]. Three subspecies of *A. spinifera*, *A. s. pallida* (Webb, 1962), *A. s. emoryi* (Agassiz, 1857), and *A. s. guadalupensis* (Webb, 1962) and one subspecies of *T. scripta*, *T. s. elegans*, are found in Texas. These three turtle species are evolutionarily distinct, belonging to three separate families. As adults, *A. spinifera* are primarily carnivorous, *C. serpentina* are scavenging omnivorous, and *T. s. elegans* are primarily herbivorous (Ernst and Lovich, 2009). *Apalone spinifera* and *T. s. elegans* are typically found swimming in the water column or basking while *C. serpentina* are more benthic dwelling and rarely bask (Ernst and Lovich, 2009). Thirty-two parasite species are known to infect *A. spinifera*, 67 species from *C. serpentina*, and 76 species from *T. scripta* (Appendix). These turtle species tend to be heavily parasitized, as their omnivorous food habits often bring them in contact with infected intermediate hosts (snails, ostracods, copepods, crayfish, amphibians, fish, etc.) or free floating parasite eggs and larvae while feeding (Everhart, 1958; Grosmaire, 1977).

The main objective of this study was to survey the metazoan parasites of *A. spinifera*, *C. serpentina*, and *T. s. elegans* from Texas, reporting the differences in species assemblages across the state and analyzing the host-parasite-environment relationships in these community assemblages. In addition, samples were collected from the same site as a previous study on parasites of *T. s. elegans* (Dinuzzo, 1981), and the parasite assemblages observed between these temporally distant surveys are compared. Through this project, the knowledge of the distributions and host associations of metazoan parasites in Texas freshwater turtles has been clarified, and the effects of environmental factors on parasite assemblages in aquatic ecosystems have been elucidated.

CHAPTER II

PARASITE DIVERISTY AND COMMUNITY STRUCTURE IN TEXAS FRESHWATER TURTLES

II.1 Introduction

Parasite diversity is a key component in understanding ecosystem complexity. With around 40% of known species being parasitic, parasitism is the most common life strategy (Dobson et al., 2008). The high number of unsampled host species and amount of cryptic speciation potentially uncovered through genetic analysis indicate that this number is likely higher (Jousson et al., 2000; Steinauer et al., 2007). Helminths, or parasites in the phyla Acanthocephala, Nematoda, Platyhelminthes, and the subclass Pentastomida, often have complex life cycles, sometimes traveling through many hosts throughout development (e.g. Parker et al., 2003; Poulin, 2011). Endohelminths, or internal helminths, are associated with predator-prey relationships, as they are typically transmitted through consumption. It is believed that parasites are involved in nearly 75% of all trophic linkages in food webs due to their complex life cycles and dependence on their hosts (Lafferty, 2008; Lafferty et al., 2006). Consequently, healthy ecosystems with greater numbers of trophic linkages are believed to be higher in parasite diversity. Parasite diversity is therefore a good indicator of ecosystem diversity and total ecosystem health (Hudson et al., 2006; Marcogliese, 2005). Despite these findings, parasite assemblages remain highly understudied, with many new species being

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Parasite diversity is particularly understudied in reptile, amphibian, and fish hosts (Dobson et al., 2008). In the state of Texas, only four metazoan parasite surveys have been conducted on freshwater turtles (Harwood, 1932; Everhart, 1957; Dinuzzo, 1981; McAllister et al., 2008). Harwood (1932) conducted a survey of the endohelminths of 50 species of amphibians and reptiles over the course of two and a half years in the vicinity of Houston, Texas. Over the course of this study, eight species of turtles were collected: 16 red-eared sliders, *Trachemys scripta elegans* (Wied, 1839), 16 Mississippi mud turtles, *Kinosternon subrubrum hippocrepis* Gray, 1856, 14 three-toed box turtles, *Terrapene carolina triunguis* (Agassiz, 1857), nine common snapping turtles, *Chelydra*

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The main objective of this study was to survey the metazoan parasites of three common species of native Texas freshwater turtles and observe the differences in species

assemblages across the state. In addition, samples were collected from the same site as a previous study on parasites of *T. s. elegans* (Dinuzzo, 1981), and the parasite assemblages observed between these temporally distant surveys are compared. This project is crucial to understanding the diversity present in aquatic ecosystems so that changes in these assemblages can be monitored in the future.

II.2 Materials and methods

II.2.1 Field materials and methods

Turtles of the species *A. spinifera* (*A. s. pallida* and *A. s. emoryi*), *C. serpentina*, and *T. s. elegans* were captured using baited hoop nets ranging in size from 1.5 m long by 0.75 m in diameter to 1.8 m long by 0.9 m in diameter. Nets were set in shallow areas along the banks of the bodies of water and anchored using 1.2 m metal rebar poles and baited with deer, chicken, or fish. Nets were left for around 24 hours to allow time for turtles to catch the scent of the bait and enter the trap. Bycatch, such as fish, alligators, or non-target turtle species, were immediately released when encountered. Target turtles were transported in plastic tubs with 15 cm diameter holes cut out for aeration and a damp sponge in the bottom to prevent desiccation to the Laboratory of Parasitology, Department of Wildlife and Fisheries Science at Texas A&M University in College Station, Texas for euthanasia and necropsy. Two sites in west Texas were over six hours from College Station, so on these trips turtles were processed in the field. Specific GPS locations were recorded for each collection location using the Garmin eTrex 30 GPS unit. Capture and euthanasia of turtles was approved by the Institutional Animal Care

and Use Committee of Texas A&M University, reference number 040564 and collections were carried out under Texas Parks and Wildlife Department, scientific research permit number SPR-0716-172.

A separate aspect of this project was to analyze environmental influences on parasite diversity. Each time turtles were collected, a range of environmental variables and water parameters were recorded and correlated with parasite abundance and diversity. These data are reported in chapter two.

II.2.2 Lab materials and methods

In the lab, turtles were weighed, measured (carapace length, carapace width, shell depth, circumference, and weight), and euthanized using an intracoelomic injection of 50% MS222 solution at a dosage of 1 mL/kg followed by an overdose of KCl injected into the brain, following the methods by Conroy et al. (2009). After the initial injection of MS222, turtles were monitored until the legs and neck were limp (usually around 30 minutes after injection) before KCl was administered. The spinal cord was severed before necropsy commenced. The combination of a bone saw and aviation wire cutters were used to cut between the carapace and plastron, and then a scalpel was used to separate the plastron from the skin and musculature. All external surfaces were checked for leeches and other metazoan ectoparasites, which were collected when found. All internal organs including the esophagus, stomach, small intestine, large intestine, heart, lungs, liver, gall bladder, gonads, kidneys, bladders, and spleen were removed and searched individually for metazoan parasites under a dissecting microscope. Spirorchiid

blood flukes were collected following a modification of the methods outlined by Snyder and Clopton (2005). After processing turtles, carcasses were donated to the Biodiversity Research and Teaching Collection at Texas A&M University where they are permanently housed.

II.2.3 Parasite processing

All metazoan parasites were relaxed in a Stentor dish with 7% saline solution. Soft-bodied helminths were heat-fixed under light coverslip pressure, placed in a petri dish with AFA (alcohol-formaldehyde-acetic acid) and left overnight, and stored in 70% ethanol until further processing. Hard-bodied parasites such as nematodes, pentastomids, and mites were moved from saline directly to 70% ethanol. Acanthocephalans were placed in tap water in the refrigerator overnight to relax the specimens and then placed directly into 70% ethanol. Moving female acanthocephalans into tap water frequently induced oviposition which facilitated egg measurements and offered a more unobstructed view of the internal structures. Eggs laid by gravid females were examined directly and measured to facilitate identification of species in multiple species infections. Leeches were removed and placed in tap water to which increasing concentrations of ethanol were added until the leeches were flat. They were then placed in 70% ethanol for permanent storage and identification.

Heat-fixed specimens were stained in Semichon's carmine, destained in acid alcohol, dehydrated through a graded ethanol series (70%, 80%, 95%, 100%, 100%), cleared in xylene, and mounted on a slide in Canada balsam. Nematodes and

acanthocephalans were moved from 70% ethanol to a mixture of equal amounts of 70% and glycerine for clearing, temporarily mounted on a slide in glycerine for identification, and subsequently stored in a vial in glycerine for future observations. Where sample size permitted, a small subset of specimens was placed directly in 95% ethanol for future molecular analysis.

Spirorchiid blood flukes were flat-fixed in 95% ethanol and will be analyzed morphologically and molecularly for a separate project. They were not included in any of the reported diversity in this paper.

Parasites were keyed out to genus using the available dichotomous keys (Khalil et al., 1994; Gibson et al., 2002; Jones et al., 2005; Bray et al., 2008; Anderson et al., 2009). For species level identification, body measurements were made and compared to original parasite descriptions. Leeches were keyed to species using the keys by Klemm (1985) and Moser et al. (2016).

II.2.4 Statistical analyses

Ecological terms follow Bush et al. (1997). Prevalence, mean intensity, median intensity, and 95% confidence intervals were calculated in the online application Quantitative Parasitology (Reiczigel et al., 2013). This software accounts for the non-normal distributions characteristic of parasite communities. Confidence intervals are only given for median intensity when the sample size was larger than five. Taxonomic diversity indices were calculated for each sample location using R version 3.1.4 (taxondive function in vegan package; R Core Team. 2017). Parasite communities of the

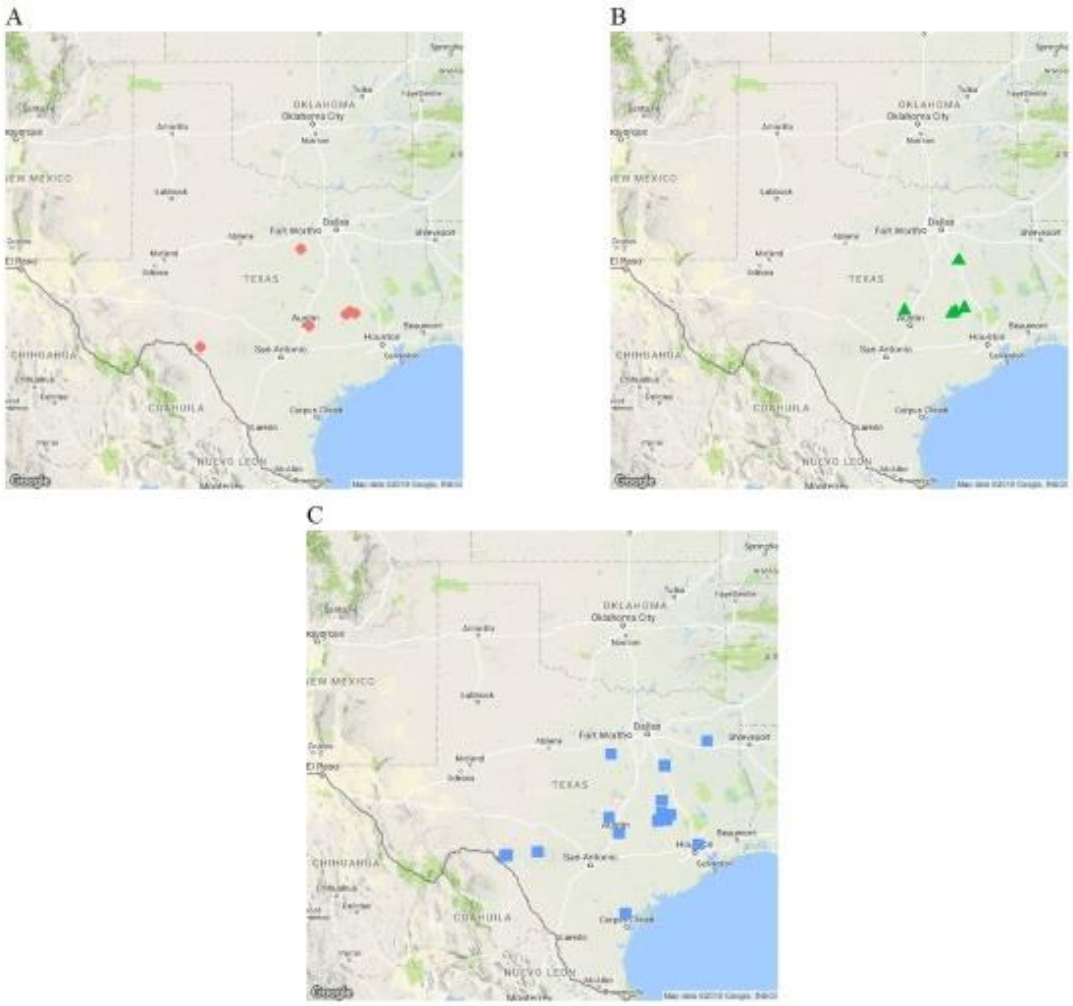
three host species were analyzed using an analysis of similarities [ANOSIM] (anosim function in vegan package) and non-metric multidimensional scaling [nMDS] (metaMDS function in vegan package). This analysis was also performed among the two subspecies of *A. spinifera* captured in this study, *A. s. pallida* and *A. s. emoryi*. The purpose of nMDS is to collapse the community data into two dimensions for visualization and interpretation (Kruskal, 1964). This method differs from other ordination methods, such as principal components analysis (PCA), in using rank orders instead of Euclidean distances. ANOSIM is a multivariate method of data analysis that can be used to compare variation in species abundance among a grouping variable, such as host species (Clarke, 1993). These two analyses give quantitative and visual representation of the differences in community data.

II.3 Results

A total of 15 *A. spinifera* (11 *A. s. pallida* and four *A. s. emoryi*), nine *C. serpentina*, and 55 *T. s. elegans* were collected and necropsied for this study. Turtles were collected from 16 properties in 13 different towns across Texas, USA: Barksdale, Bryan, College Station, Comstock, Del Valle, Franklin, Gladewater, Glen Rose, Humble, Iola, Leander, Sinton, and Streetman (Fig. 1). All turtles examined in this study were infected with at least two species of parasite except one *A. spinifera* which was only infected with *S. contorta*. As many as 10 species were recovered from a single individual host. Every organ system except the reproductive tract was found to be

infected with at least one species, with the small intestine being the most commonly infected site.

Figure 1: Map of the collection locations of turtles across Texas. A) *Apalone spinifera*, B) *Chelydra serpentina*, and C) *Trachemys scripta elegans*. Darker points indicate more captures in that location.



Cumulatively, five species of acanthocephalans, nine species of nematodes, 14 species of trematodes, two species of cestodes, five species of monogeneans, five species of leeches, one species of pentastomid, and one species of mite were recovered.

Acanthocephalans and nematodes were the most abundant parasites while trematodes were the most diverse. Nematodes of the genus *Spiroxys* were the most common parasite of *A. spinifera*, recovered from 100% of turtles. Nematodes of the genus *Falcaustra* were the most common parasite of *C. serpentina*, recovered from 100% of turtles. Acanthocephalans of the genus *Neoechinorhynchus* were the most common parasite of *T. s. elegans*, recovered from 91% of turtles. Sixteen new host records and 17 new locality records are recorded herein. Table 1 lists the parasites recovered in this study with the prevalence, intensity, site of infection, and locality. Spirorchiid blood flukes are being analyzed as part of a separate project and so are not included.

Occasionally, chironomid larvae were recovered from the intestines of turtles. These larvae were typically dead, and could usually be found in the debris on the carapace of the turtle as well, and were likely ingested during feeding. On one occasion, a large number of live chironomid larvae were found covering the carapace and throughout the digestive tract of two *T. s. elegans* collected in Humble, Texas. Tokeshi (1993) stated that these organisms have evolved commensal relationships with many slow-moving benthic organisms, which could explain this finding. These specimens could not be identified but were saved in 70% ethanol and will be deposited in a museum collection.

II.3.1 Host-community analysis

Analysis of the parasite communities between the three host species was performed to determine if parasite species assemblages were distinct among host

species. This analysis was also performed among the two subspecies of *A. spinifera*. The analysis of similarities revealed significant segregation between host species (ANOSIM statistic = 0.78; p = 0.001). When nMDS was performed (stress= 0.15), *A. spinifera* separated from the other two host species, as seen in Fig. 2. No segregation was found between the two subspecies of *A. spinifera*.

Figure 2: Plotted nMDS ordination showing parasite species grouping by host species. AS= *Apalone spinifera*, CS= *Chelydra serpentina*, and TSE= *Trachemys scripta elegans*.

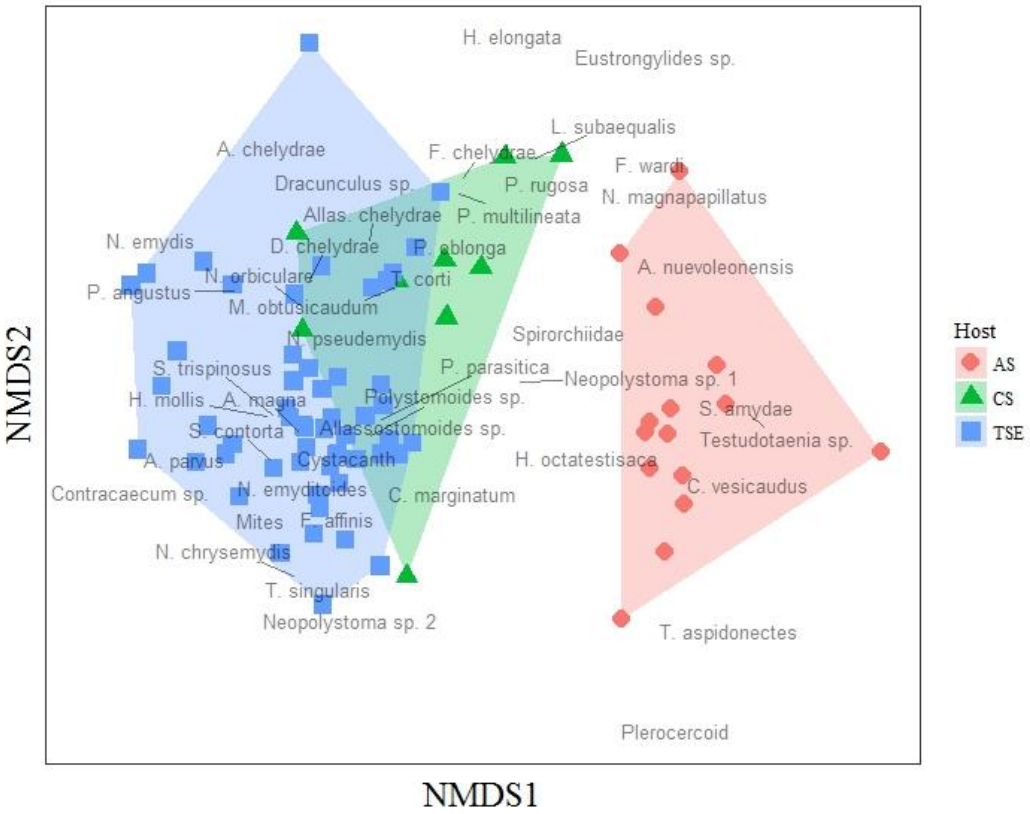


Table 1: Metazoan parasites recovered from 15 *Apalone spinifera*, nine *Chelydra serpentina*, and 55 *Trachemys scripta elegans*. All localities are towns in Texas, USA. For median intensity, confidence intervals could only be calculated for sample sizes greater than five. Bolded species name indicates a new host association and bolded locality indicates the first report in Texas.

Host	Species		Site of infection	Localities	Prevalence % [95% CI]	Mean intensity (range) [95% CI]	Median intensity [95% CI]
Apalone spinifera (n=15)	Acanthocephala	Cystacanth	Liver	Glen Rose	6.67	1	1
		Neoechinorhynchus magnapapillatus	Small intestine, Large intestine	**College Station**	6.67	11	11
		Neoechinorhynchus sp.	Small intestine	Glen Rose	6.67	1	1
	Nematoda	***Eustrongylides* sp.**	Mesentery	College Station	6.67	2	2
		Falcaustra wardi	Small intestine, Large intestine	**College Station**	6.67	22	22
		Spiroxys amydae	Esophagus, Stomach, Small intestine, Stomach cysts	College Station, Comstock, Del Valle, Glen Rose,	73.33 [44.9-92.2]	34.91 (3-120) [17-65.1]	14 [3-66]
		Spiroxys sp.	Stomach, Stomach cysts	Comstock, Del Valle	26.67 [7.8-55.1]	20.75 (1-71) [1-53.5]	5.5
	Trematoda	*Acanthostomum nuevoleonensis*	Small intestine	**Del Valle**	6.67	54	54
		***Allassostomoides* sp.**	Small intestine	Glen Rose	6.67	2	2
		Cephalogonimus vesicaudus	Stomach, Small intestine	College Station, Del Valle, Glen Rose	46.67 [21.3-73.4]	33.57 (1-82) [14.2-59.6]	26 [1-82]
		Teloporia aspidonectes	Small intestine	**College Station**	6.67	1	1
		Telorchis corti	Small intestine	College Station, Glen Rose	13.33 [1.7-40.5]	7 (1-13) [1-7]	7
	Cestoda	Plerocercoid	Liver	Comstock	6.67	6	6
		Testudotaenia testudo	Small intestine, Large intestine	**Comstock, Del Valle, Glen Rose**	53.33 [26.6-78.7]	7.13 (1-23) [3.38-14.3]	3 [1-13]
	Monogenea	***Neopolystoma* sp. 1**	Conjunctival sac of eye	**Comstock**	6.67	1	1
		Polystomoides coronatum	Mouth, Trachea	College Station, Comstock, Del Valle, Glen Rose,	53.33 [26.6-78.7]	1.25 (1-3) [1-1.75]	1 [1-1]
	Hirudinea	***Helobdella octatestisaca***	Carapace, Skin	Comstock	13.33 [1.7-40.5]	1.5 (1-2) [1-2]	1.5
		Placobdella parasitica	Carapace, Plastron, Skin	College Station, Del Valle, Glen Rose	26.67 [7.8-55.1]	3.25 (1-9) [1-7]	1.5
		Placobdella rugosa	Carapace, Plastron, Skin	College Station	13.33 [1.7-40.5]	5 (4-6) [4-5]	5
	Arthropoda	*Levisunguis subaequalis*	Lungs, Trachea	**College Station, Del Valle**	20 [4.3-48.1]	9 (5-16) [5-12.7]	6

Table 1: Continued

Host	Species		Site of infection	Localities	Prevalence % [95% CI]	Mean intensity (range) [95% CI]	Median intensity [95% CI]
Chelydra serpentina (n=9)	Acanthocephala	*Neoechinorhynchus* sp.	Small intestine, Large intestine	College Station, Iola, Streetman	33.33 [7.5-70.1]	4.67 (1-12) [1-8.33]	1
	Nematoda	*Eustrongylides* sp.	Body cavity, Liver	College Station	11.11	2	2
		Dracunculus globocephalus	Kidney, Bladder	**College Station, Streetman**	22.22 [2.8-60]	3 (2-4) [2-3]	3
		Dracunculus sp.	Kidney, Rectum epithelium	College Station, Iola	22.22 [2.8-60]	1	1
		Falcaustra chelydrae	Small intestine, Large intestine	College Station, Iola	66.67 [29.9-92.5]	235.5 (2-516) [91.7-385]	218 [2-516]
		Falcaustra affinis	Large intestine	Leander	11.11	14	14
		Falcaustra sp.	Small intestine, Large intestine	College Station, Streetman	22.22 [2.8-60]	234.5 (16-453) [16-453]	234.5
		Serpinema trispinosus	Esophagus, Small intestine	College Station, Iola, Leander, Streetman	77.78 [40-97.2]	10.14 (1-42) [3.86-27.1]	5 [1-42]
		Spiroxys contorta	Stomach	College Station	11.11	1	1
	Trematoda	*Allassostomoides chelydrae*	Large intestine	**College Station, Iola**	22.22 [2.8-60]	2 (1-3) [1-2]	2
		Auridistomum chelydrae	Small intestine	College Station	11.11	1	1
		Telorchis corti	Small intestine	College Station	11.11	4	4
	Monogenea	***Neopolystoma* sp. 1**	Conjunctival sac of eye	**Iola, Streetman**	22.22 [2.8-60]	1.5 (1-2) [1-2]	1.5
		Polystomoidella oblonga	Bladder	College Station, Iola	44.44 [13.7-78.8]	2.25 (1-6) [1-3.5]	1
	Hirudinea	***Helobdella elongata***	Skin	College Station	11.11	3	3
		Placobdella parasitica	Carapace, Plastron, Skin, Eye, Rectum	College Station, Iola, Streetman	44.44 [13.7-78.8]	17 (10-24) [11.5-20.5]	17
		Placobdella multilineata	Carapace	**Gladewater**	11.11	1	1
		Placobdella rugosa	Carapace, Plastron, Skin	College Station, Iola	44.44 [13.7-78.8]	108.75 (3-333) [3.5-274]	49.5
	Arthropoda	***Levisunguis subaequalis***	Lungs, Trachea, Bladder	**College Station**	33.33 [7.5-70.1]	19 (10-34) [10-27]	13
Trachemys scripta elegans (n=55)	Acanthocephala	Cystacanth	Mouth cyst, Small intestine cysts	College Station, Leander	5.45 [1.1-15.1]	3.33 (1-8) [1-5.67]	1
		Neoechinorhynchus chrysemydis	Small intestine, Large intestine	**Gladewater, Glen Rose, Leander**	14.55 [6.5-26.7]	65.4 (4-227) [20.5-148]	25.5 [4-181]
		Neoechinorhynchus emydis	Small intestine, Large intestine	College Station, Leander, Sinton	9.09 [3-20]	204.8 (12-740) [37.6-594]	48

Table 1: Continued

Host	Species		Site of infection	Localities	Prevalence % [95% CI]	Mean intensity (range) [95% CI]	Median intensity [95% CI]
Trachemys scripta elegans (n=55)	Acanthocephala	*Neoechinorhynchus emyditoides*	Small intestine, Large intestine	Barksdale, Bryan, College Station, Comstock, Gladewater, Glen Rose, Humble, Iola, Leander, Streetman	56.36 [42.3-69.7]	79.7 (3-336) [57.2-114]	50 [24-66]
		Neoechinorhynchus pseudemydis	Small intestine, Large intestine, Bladder	**Bryan, College Station, Del Valle, Franklin, Glen Rose, Leander, Streetman**	38.18 [25.4-52.3]	195.7 (4-1587) [102-499]	37 [10-203]
		Neoechinorhynchus sp.	Small intestine, Large intestine	Comstock, Gladewater, Streetman	5.45 [1.1-15.1]	36 (5-98) [5-67]	5
	Nematoda	***Contracaecum* sp.**	Stomach	Leander	1.82	1	1
		Falcaustra affinis	Small intestine, Large intestine	Bryan, College Station, Comstock, Gladewater, Glen Rose, Leander, Streetman	23.64 [13.2-37]	50 (5-236) [27-111]	21 [5-60]
		Falcaustra sp.	Large intestine	Barksdale, College Station, Gladewater, Glen Rose, Leander	16.36 [7.8-28.8]	12.89 (1-78) [3.67-39.8]	4 [1-14]
		Serpinema trispinosus	Stomach, Small intestine, Heart, Pancreatic cysts	Barksdale, Bryan, College Station, Comstock, Franklin, Gladewater, Glen Rose, Humble, Iola, Leander, Sinton, Streetman	89.09 [77.8-95.9]	30.33 (1-163) [21.2-44.1]	14 [9-23]
		Spiroxys amydae	Small intestine	College Station	1.82	2	2
		Spiroxys contorta	Stomach	Barksdale, College Station, Comstock, Gladewater, Glen Rose, Leander, Humble, Streetman	32.73 [20.7-46.7]	10 (1-35) [7.1-15.4]	9 [5-14]
		Spiroxys sp.	Stomach, Small intestine, Stomach cysts	Bryan, College Station, Franklin, Gladewater, Glen Rose, Streetman	16.36 [7.8-28.8]	9.44 (1-39) [3.56-19.7]	3 [1-20]
	Trematoda	*Allassostoma magnum*	Small intestine, Large intestine	**Barksdale, Bryan**	5.45 [1.1-15.1]	2.67 (1-6) [1-4.33]	1
		Allassostomoides chelydrae	Large intestine	**College Station**	1.82	1	1
		Allassostomoides parvus	Large intestine	**Glen Rose**	1.82	1	1
		Allassostomoides sp.	Small intestine, Large intestine	College Station	5.45 [1.1-15.1]	10.7 (1-19) [1-16.7]	12

Table 1: Continued

Host	Species		Site of infection	Localities	Prevalence % [95% CI]	Mean intensity (range) [95% CI]	Median intensity [95% CI]
Trachemys scripta elegans (n=55)	Trematoda	***Clinostomum marginatum***	Small intestine	Leander	1.82	1	1
		Dictyangium chelydrae	Large intestine	**College Station, Franklin, Sinton**	10.91 [4.1-22.2]	4.7 (1-11) [2.67-7.67]	4 [1-11]
		Heronimus mollis	Lungs	Bryan	1.82	2	2
		Macravestibulum obtusicaudum	Small intestine	**College Station, Sinton**	9.09 [3-20]	9.4 (1-41) [1.2-25.4]	2
		Protenes angustus	Small intestine	Bryan, Glen Rose, Leander, Sinton	7.27 [2-17.6]	2.25 (1-5) [1-4.25]	1.5
		Telorchis corti	Small intestine	College Station, Gladewater, Leander, Sinton, Streetman	14.55 [6.5-26.7]	47.43 (1-234) [9.38-132]	11.5 [1-48]
		Telorchis singularis	Small intestine	Bryan, Gladewater, Leander	7.27 [2-17.6]	8 (1-17) [2.68-14.2]	7
		Telorchis sp.	Small intestine	Comstock	1.82	17	17
	Monogenea	*Neopolystoma orbiculare*	Bladder, Rectum	Barksdale, Bryan, College Station, Comstock, Del Valle, Gladewater, Leander, Sinton	29.09 [17.6-42.9]	2.81 (1-8) [1.88-4]	2 [1-3]
		***Neopolystoma* sp. 2**	Conjunctival sac of eye	**Comstock**	1.82	3	3
		Polystomoides coronatum	Mouth	Barksdale, Bryan, College Station, Comstock, Franklin, Gladewater, Glen Rose, Leander, Sinton, Streetman	56.36 [42.3-69.7]	3.87 (1-25) [2.9-6.75]	3 [2-4]
	Hirudinea	*Helobdella octatestisaca*	Carapace, Skin	Barksdale, Comstock, Del Valle	5.45 [1.1-15.1]	2 (1-3) [1-2.67]	2
		Placobdella parasitica	Carapace, Plastron, Skin	Barksdale, Bryan, College Station, Comstock, Del Valle, Gladewater, Glen Rose, Humble, Leander, Streetman	40 [27-54.1]	15.78 (1-136) [5.95-39.3]	4 [1-8]
		Placobdella rugosa	Carapace, Plastron, Skin, Rectum	College Station	7.27 [2-17.6]	4.25 (1-7) [1.75-6]	4.5
		Placobdella sp.	Plastron, Skin	College Station, Gladewater, Glen Rose	5.45 [1.1-15.1]	1	1
	Arthropoda	Mite	Skin	Glen Rose	3.64 [0.4-12.5]	9 (3-15) [3-9]	9
		Levisunguis subaequalis	Lungs, Trachea, Dorsal muscle, Stomach	**College Station, Leander, Sinton**	9.09 [3-20]	5.6 (1-11) [2-9.2]	4

II.3.2 Host-parasite associations

The following is a list of the parasite species recovered in this study with prevalence and site of infection for each host species.

Acanthocephala

Encysted acanthocephalans (cystacanths) were recovered from the liver of 1 of 15 *A. spinifera* and the lining of the mouth and small intestine of 3 of 55 *T. s. elegans*. These could not be identified to species.

Eocanthocephala: Neoechinorhynchida: Neoechinorhynchidae

Neoechinorhynchus chrysemydis Cable and Hopp, 1954 were recovered from the small and large intestine of 8 of 55 *T. s. elegans*. This is the first report this parasite from Texas.

Neoechinorhynchus emydis (Leidy, 1850) were recovered from the small and large intestine of 5 of 55 *T. s. elegans*.

Neoechinorhynchus emyditoides Fisher, 1960 were recovered from the small and large intestine of 31 of 55 *T. s. elegans*. This was the most common acanthocephalan species recovered in this study.

Neoechinorhynchus magnapapillatus Johnson, 1969 were recovered from the small and large intestine of 1 of 15 *A. spinifera*. This is the first report of *N. magnapapillatus* from *A. spinifera* and the first report of this parasite from Texas.

Neoechinorhynchus pseudemydis Cable and Hopp, 1954 were recovered from the small and large intestine of 21 of 55 *T. s. elegans*. In one turtle, *N. pseudemydis* was also

recovered from the bladder. This turtle had the highest abundance of acanthocephalans (1,587) and it is likely that these worms were overflowing into the bladder from the large intestine. This is the first report this parasite from Texas.

Neoechinorhynchus sp. were recovered from the small and large intestine of 1 of 15 *A. spinifera*, 3 of 9 *C. serpentina*, and 3 of 55 *T. s. elegans*. These worms were either all larval or only males, precluding specific identification.

Nematoda: Enoplea: Dioctophymatoidea: Dioctophymidae

Eustrongylides sp. were recovered from the mesentery of 1 of 15 *A. spinifera* and the body cavity and liver of 1 of 9 *C. serpentina*. These nematodes were larval and were surrounded in thickened, cyst-like tissue along the length of their bodies. This is the first report of *Eustrongylides* sp. from *A. spinifera*. Molecular analysis is being conducted to determine the specific identity of these specimens.

Secernentea: Ascaridida: Kathlaniidae

Falcaustra affinis (Leidy, 1856) were recovered from the large intestine of 1 of 9 *C. serpentina* and the small and large intestine of 13 of 55 *T. s. elegans*.

Falcaustra chelydrae Harwood, 1932 were recovered from the small and large intestine of 6 of 9 *C. serpentina*.

Falcaustra wardi (Mackin, 1936) were recovered from the small and large intestine of 1 of 15 *A. spinifera*. This is the first report of *F. wardi* from *A. spinifera* and the first report of this parasite in Texas.

Falcaustra sp. were recovered from the small and large intestine of 2 of 9 *C. serpentina* and the large intestine of 9 of 55 *T. s. elegans*. These worms were either all larval or only females, precluding specific identification.

Anisakidae

Contracaecum sp. was recovered from the stomach of 1 of 55 *T. s. elegans*. This parasite, typically found in piscivorous birds such as herons, was larval, the turtle being a dead end host. This is the first report of *Contracaecum* sp. from *T. s. elegans*.

Spirurida: Camallanidae

Serpinema trispinosus (Leidy, 1851) were recovered from the esophagus and small intestine of 7 of 9 *C. serpentina* and the stomach and small intestine of 49 of 55 *T. s. elegans*. Additionally, larval worms were also recovered from the heart and pancreatic cysts of 2 of 55 *T. s. elegans*. These were likely intermediate stages in a migration through the host. These parasites were typically highly aggregated at the duodenum. This was the most common helminth species of *C. serpentina* and *T. s. elegans* in this study. *Serpinema trispinosus* and *S. microcephalus* have both been reported from all three turtle species in past studies, however, Baker (1979) clarified the distinction in morphology and locality between these two species, with *S. trispinosus* having different ridge patterns in the buccal cavity and being found in North America. It is likely that all *S. microcephalus* reported from North American turtles are actually specimens of *S. trispinosus*. A review of the catalogued specimens would be necessary to confirm this hypothesis.

Dracunculidae

Dracunculus globocephalus (Mackin, 1927) were recovered from the kidney and bladder of 2 of 9 *C. serpentina*. This is the first report of this parasite from Texas. *Dracunculus* sp. were recovered from the kidney and rectal epithelium of 2 of 9 *C. serpentina*. These worms were all female and therefore unidentifiable, but are likely *D. globocephalus* based on host species and location.

Gnathostomatidae

Spiroxys amydae Cobb, 1929 were recovered from the esophagus, stomach, stomach cysts, and small intestine of 11 of 15 *A. spinifera* and the small intestine of 1 of 55 *T. s. elegans*. Adults in the stomach were typically found in a single mass of individuals and always associated with ulcers in the stomach lining. The worms recovered from cysts were always larval and likely intermediate in a migration through the host. This was the most common helminth species of *A. spinifera*. This is the first report of this parasite from *T. s. elegans*.

Spiroxys contorta (Rudolphi, 1819) were recovered from the stomach of 1 of 9 *C. serpentina* and the stomach of 18 of 55 *T. s. elegans*. Adults in the stomach were typically found in a single mass of individuals and always associated with ulcers in the stomach lining.

Spiroxys sp. were recovered from the stomach and stomach cysts of 4 of 15 *A. spinifera* and the stomach, stomach cysts, and small intestine of 9 of 55 *T. s. elegans*. These worms were all larval and therefore unidentifiable, but are likely *S. contorta* in *T.*

s. elegans and *S. amydae* in *A. spinifera* based on typical host associations. The worms recovered from cysts were likely intermediate in a migration through the host.

Platyhelminthes: Trematoda: Diplostomida: Clinostomatidae

Clinostomum marginatum (Rudolphi, 1819) was recovered from the small intestine of 1 of 55 *T. s. elegans*. This parasite is typically found in herons and egrets, so was likely a dead-end infection in the turtle. This is the first report of this parasite in *T. s. elegans*.

Echinostomida: Heronimidae

Heronimus mollis (Leidy, 1856) were recovered from the lungs of 1 of 55 *T. s. elegans*. The genus *Heronimus* has undergone significant taxonomic debate, but is currently considered to be monotypic.

Microscaphidiidae

Dictyangium chelydrae Stunkard, 1943 were recovered from the large intestine of 5 of 55 *T. s. elegans*. This is the first report of this parasite from Texas.

Paramphistomatidae

Allassostoma magna Stunkard, 1916 were recovered from the small and large intestine of 3 of 55 *T. s. elegans*. Large red blisters were found on the intestinal lining where these trematodes were attached. This is the first report of this parasite from Texas.

Allassostomoides chelydrae (MacCallum, 1919) were recovered from the large intestine of 2 of 9 *C. serpentina* and 1 of 55 *T. s. elegans*. This is the first published report of this parasite from *T. s. elegans* and the first record in Texas. Dinuzzo (1981)

reported this trematode from *T. s. elegans* in his thesis, but as this data was never published and specimens cannot be located, his record is insufficient.

Allassostomoides parvus (Stunkard, 1916) was recovered from the large intestine of 1 of 55 *T. s. elegans*. This is the first report of this parasite from *T. s. elegans* and the first record in Texas.

Allassostomoides sp. were recovered from the large intestine of 1 of 15 *A. spinifera* and the small and large intestine of 3 of 55 *T. s. elegans*. These worms were all immatures, precluding specific identification. This is the first report of *Allassostomoides* sp. from *A. spinifera*.

Pronocephalidae

Macravestibulum obtusicaudum Mackin, 1930 were recovered from the small intestine of 5 of 55 *T. s. elegans*. This is the first report of this parasite in Texas.

Teloporia aspidonectes (MacCallum, 1917) was recovered from the small intestine of 1 of 15 *A. spinifera*. This is the first report of this parasite in Texas.

Plagiorchiida: Auridistomidae

Auridistomum chelydrae (Stafford, 1900) was recovered from the small intestine of 1 of 9 *C. serpentina*. *Auridistomum georgiense* Bogitsh, 1959 was described from *C. serpentina* in Georgia. This species differed from *A. chelydrae* based on a larger overall size, lobed testes, and the lack of a prominent Laurer's canal. Body size and testis shape are notoriously variable characteristics in many trematodes, and the Laurer's canal is often not visible, even in well-fixed specimens. Further morphological and molecular work is needed to determine if *A. georgiense* is truly a distinct species.

Cephalogonimidae

Cephalogonimus vesicaudus Nickerson, 1912 were recovered from the stomach and small intestine of 7 of 15 *A. spinifera*. These parasites were typically associated with the duodenum, but were found throughout the small intestine when infection intensity was high.

Cryptogonimidae

Acanthostomum nuevoleonensis Caballero and Caballero, 1964 were recovered from the small intestine of 1 of 15 *A. spinifera*. This is the first report of *A. nuevoleonensis* in the USA. This parasite was synonymized with *A. megacetabulum* Thatcher, 1963 by Brooks (1980), who stated that the size difference in oral spines and sucker sizes were likely due to host induced effects. Tkach and Snyder (2003) pointed out the tenuous nature of this synonymy, as chitonous elements are unlikely to vary greatly within a single species. The major differences between the two species are the size of oral sucker, pharynx, ventral sucker, and oral spines, with the oral spine length being much greater in *A. megacetabulum* (68 versus 16-32). For this reason, the synonymy (Brooks 1980) is rejected and *A. nuevoleonensis* is redescribed in the next section.

Telorchiidae

Protenes angustus (Stafford, 1900) were recovered from the small intestine of 4 of 55 *T. s. elegans*. The genera *Telorchis* and *Protenes* have been the subject of much taxonomic debate. MacDonald and Brooks (1989) placed *P. angustus* in the genus *Telorchis* based on a morphological character tree which placed this species in a clade

with *Telorchis corti*. The unusual location of the genital pore, different than any member of the genus *Telorchis*, would indicate that this is a generic level trait, and so *P. angustus* is left in the genus *Protenes* for this study.

Telorchis corti Stunkard, 1915 were recovered from the small intestine of 2 of 15 *A. spinifera*, 7 of 9 *C. serpentina*, and 8 of 55 *T. s. elegans*. This species of trematode is a generalist known to infect many species of reptiles and amphibians. A large number of species were synonymized with this species by Wharton (1940) and MacDonald and Brooks (1989); however, some of these synonymys were rejected after molecular analysis of specimens (Pulis et al., 2011). A full molecular revision of this genus is needed to clarify the taxonomy and reveal defining morphological traits.

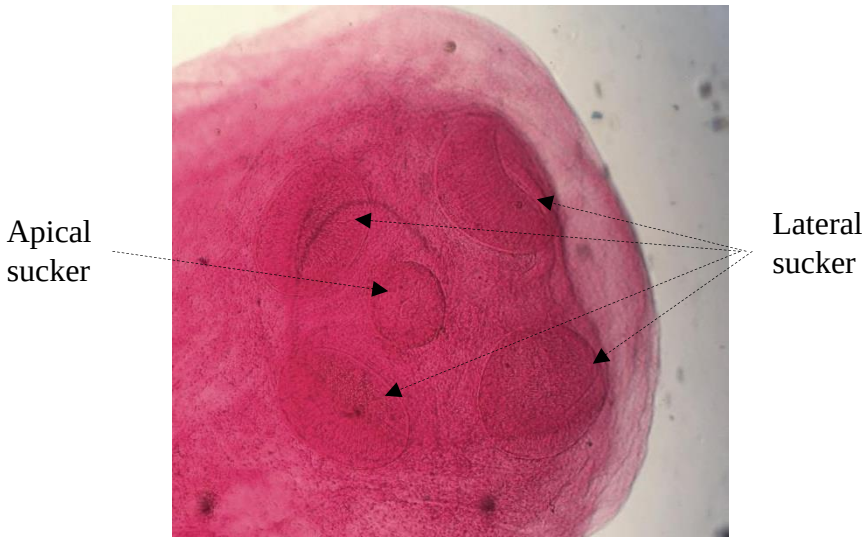
Telorchis singularis (Bennett, 1935) were recovered from the small intestine of 4 of 55 *T. s. elegans*. This was the largest trematode species recovered in this study, with individuals as large as 17 millimeters long.

Telorchis sp. were recovered from the small intestine of 1 of 55 *T. s. elegans*. These worms were all immatures with underdeveloped reproductive systems and no eggs, precluding specific identification.

Cestoda: Onchoproteocephalidea: Proteocephalidae

Encysted plerocercoids (Fig. 3) were recovered from the liver of 1 of 15 *A. spinifera*. The protoscoleces of these cestodes contained four lateral suckers and one apical sucker, placing them in the Family Proteocephalidae. Collaboration is ongoing to determine the specific identity of this parasite through molecular analysis, which will be detailed in a future report.

Figure 3: Plerocercoid protoscolex showing sucker arrangement. Recovered from the liver of *Apalone spinifera*.



Testuodotaenia testudo (Magath, 1924) were recovered from the small and large intestine of 8 of 15 *A. spinifera*. This is the first report of *T. testudo* in Texas. This parasite was recovered from three locations across Texas. Some general measurements are given for the worms collected from each location. Molecular analyses are being conducted to determine whether these samples are truly monotypic.

Central samples: Scolex width 400-1,180 (783). Sucker width 130-300 (230). Immature proglottids measured 140-370 (233) by 510-1,700 (1030). Mature proglottids measured 350-2,950 (1,519) by 1,200-1,600 (1,372). Cirrus sac length 350-650 (474). Gravid proglottids measured 1,550-4,300 (2,493) by 1,075-2,650 (1,870). Cirrus sac length 400-675 (513). Eggs measured 17.5-20 (18.1).

Northern samples: Scolex width 480-870 (683). Sucker width 200-270 (239). Immature proglottids measured 100-460 (221) by 410-1,150 (633). Mature proglottids measured 900-1,100 (993) by 600-1,600 (1,025). Cirrus sac length 200-490 (318). Gravid proglottids measured 1,350-4,175 (2,355) by 1,080-1,500 (1,327). Cirrus sac length 460-810 (593). Eggs measured 15-25 (20.8).

Western samples: Scolex width 510-700 (590). Sucker width 200-240 (218). Immature proglottids measured 100-400 (234) by 530-960 (665). Mature proglottids measured 680-1580 (1040) by 720-1,280 (952). Cirrus sac length 230-440 (375). Gravid proglottids measured 1,225-3,375 (2,405) by 900-1,600 (1,179). Cirrus sac length 380-590 (470). Eggs measured 17.5-25 (20.8).

Monogenea: Polyopisthocotylea: Polystomatidae

Neopolystoma orbiculare (Stunkard, 1916) were recovered from the bladder and rectum of 16 of 55 *T. s. elegans*. At the two sites where this parasite was found in the rectum, they were only recovered from this location within the host. This could be an insight into the life history of this parasite, possibly traveling to the rectum for release of eggs.

Neopolystoma n. sp. 1 were recovered from the conjunctival sac of the eye of 1 of 15 *A. spinifera* and 2 of 9 *C. serpentina*. This is the first report of *Neopolystoma* from the eye of *A. spinifera*, and the first report in *C. serpentina* in Texas. A description of this new species follows in the next section.

Neopolystoma n. sp. 2 were recovered from the conjunctival sac of the eye of 1 of 55 *T. s. elegans*. This is the first report of *Neopolystoma* from the eye of *T. s. elegans*.

Based on measurements these specimens represent a new species. A description of this new species follows in the next section.

Polystomoides coronatum (Leidy, 1888) were recovered from the mouth of 8 of 15 *A. spinifera* and the mouth of 31 of 55 *T. s. elegans*. In a single *A. spinifera*, one individual was found in the trachea near the connection to the lungs. This worm had likely moved to this location from the mouth, as it is not a typical location for monogenean infection in turtles. The original description of this species (Leidy, 1888) and later redescriptions (Stunkard, 1917; Price, 1939) are lacking in key morphological characters. While many synonyms have been accepted for this species, the ranges for some of the key morphological characters are too large to represent a monotypic group, particularly the number and length of the genital spines (Bychowsky, 1957; Timmers and Lewis, 1978; Lenis and Garcia-Prieto, 2009). A full revision comparing morphology and genetics is needed to clarify the taxonomy of this species. Collaboration is ongoing to determine whether the specimens in the current study are truly a single species through molecular analysis, which will be detailed in a future report. A redescription of this species based on morphology is located in the next section.

Polystomoidella oblonga (Wright, 1879) were recovered from the bladder of 4 of 9 *C. serpentina*. Although this parasite was recovered from *T. s. elegans* in a past study (Acholonu, 1969), it appears to be host specific to *C. serpentina* at the sites sampled in this study.

Annelida: Clitellata: Rhynchobdellida: Glossiphoniidae

Helobdella elongata (Castle, 1900) were recovered from the skin of 1 of 9 *C. serpentina*. This is the first report of this species on *C. serpentina*. This species was only recovered one time during this study and it is possible that it was using that turtle as a substrate and not a host. See notes on *H. octatestisaca* below.

Helobdella octatestisaca Lai and Chang, 2009 were recovered from the carapace and skin of 2 of 15 *A. spinifera* and 3 of 55 *T. s. elegans*. This is the first record of *H. octatestisaca* from *A. spinifera*. These leeches have typically been considered predators of small invertebrates. Those found on turtles were thought to be depredating leeches of the genus *Placobdella* (Richardson et al. 2017). However, Stark et al. (2017) found that *Helobdella stagnalis* were facultative parasites of four amphibian species in Europe. The fact that the specimens of *H. octatestisaca* in this study were recovered from individuals that had no other leech parasites and appeared to have blood-filled ceca indicates that this species may have a facultative relationship with turtles.

Placobdella multilineata Moore, 1953 was recovered from the carapace of 1 of 9 *C. serpentina*. This is the first record of this parasite from Texas. Only one individual of this species was recovered in this study.

Placobdella parasitica (Say, 1824) were recovered from the carapace, plastron, and skin of 4 of 15 *A. spinifera*, the carapace, plastron, skin, eye, and rectum of 4 of 9 *C. serpentina*, and the carapace, plastron, and skin of 22 of 55 *T. s. elegans*. This was the most common species of leech recovered in this study.

Placobdella rugosa (Verrill, 1874) were recovered from the carapace, plastron, and skin of 2 of 15 *A. spinifera*, the carapace, plastron, and skin of 4 of 9 *C. serpentina*, and the carapace, plastron, skin, and rectum of 4 of 55 *T. s. elegans*. This is the first report of this species on *A. spinifera*.

Placobdella sp. were recovered from the plastron and skin of 3 of 55 *T. s. elegans*. These were likely *P. parasitica* but due to poor fixation were unable to be identified.

Arthropoda: Arachnida (Acari)

Two *T. s. elegans*, collected on the same day in the same location, were found to be hosting a number of parasitic mites. These mites were located on the skin around the cloaca and axillae of the two turtles. These mites appear to represent a new species and a description of these specimens is currently in progress.

Maxillopoda (Pentastomida): Porocephalida: Sebekidae

Levisunguis subaequalis Curran et al., 2014 were recovered from the lungs and trachea of 3 of 15 *A. spinifera*, 3 of 9 *C. serpentina*, and 2 of 55 *T. s. elegans*. Additionally, larval specimens were recovered encysted in the dorsal muscle, lung, and stomach wall of three *T. s. elegans* from separate locations. In one *C. serpentina*, a single pentastomid was found in the bladder, likely the result of an aberrant migration to the lungs. The only location where this parasite was found as an adult in *C. serpentina* and *T. s. elegans* had an unusually high abundance of the mosquitofish, *Gambusia affinis* (Baird and Girard, 1853), the known intermediate host of *L. subaequalis* (Curran et al.,

2014). It is likely that opportunistic feeding on mosquitofish by *C. serpentina* and *T. s. elegans* resulted in infection at this site. These parasites were typically associated with excess mucus in the lungs of the host turtle. This is the first report of *L. subaequalis* from *T. s. elegans* and *C. serpentina*, the first record since its description, and the first record in Texas.

II.3.3 Parasite taxonomy

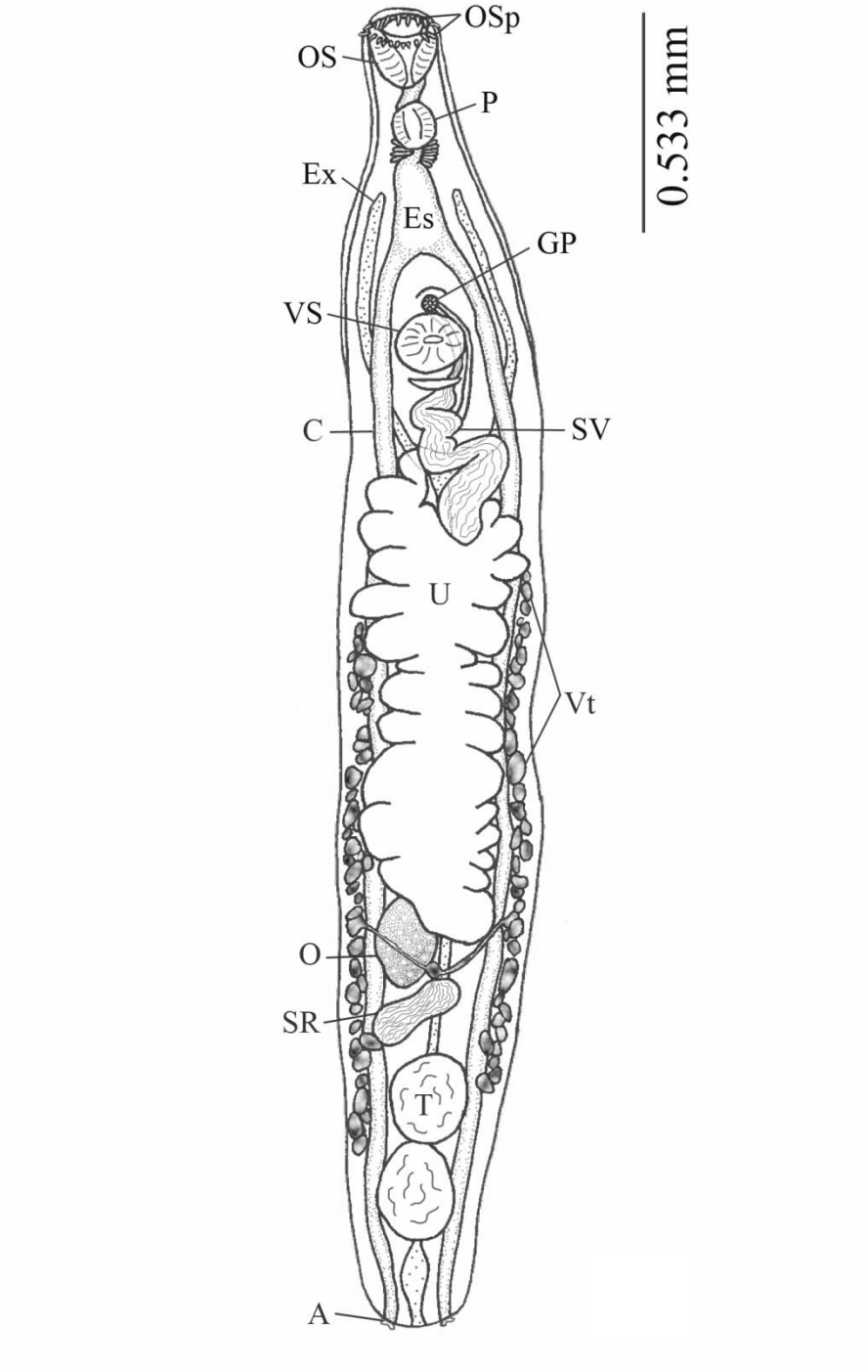
Redescription of *Acanthostomum nuevoleonensis* (Fig. 4)

Redescription [based on 20 gravid adults, measurements in micrometers with ranges followed by means]:

Body elongate, widest between ventral sucker and testes, 1,450-3,200 (2645) by 290-610 (461). Forebody 460-770 (604), comprising 19-32% (23%) of total body length. Tegument spinous, with regularly spaces spines diminishing in size and number toward posterior end. Oral sucker bell-shaped, 130-185 (159) by 120-180 (151). Spines triangular in shape with widest end embedded in cutaneous tissue, 18-22 (20) in number, 20-36.3 (26.2) by 7.5-12.5 (10.2). Spines regularly spaced around circumference of oral sucker, up to half often missing, likely lost during removal from host. Ventral sucker sub-spherical, 90-170 (135) by 115-190 (153). Oral sucker approximately the same size as ventral sucker, 0.87-1.13 (0.99) width ratio. Cutaneous invaginations present anterior and posterior to ventral sucker. Prepharynx short if present, 0-60 (14) long. Pharynx round, close to oral sucker, 80-110 (101) by 50-100 (81). A number of dark-staining cells located at posterior end of pharynx, possibly digestive glands. Oral sucker

approximately twice the size of pharynx, 1.5-2.4 (1.89) width ratio. Esophagus thick and sinuous, 80-210 (125) long. Bifurcation located just anterior to the ventral sucker.

Figure 4: Illustration of *Acanthostomum nuevoleonensis*. OS= Oral sucker, OSp= Oral spines, P= Pharynx, Ex= Exretory bladder, Es= Esophagus, GP= Genital pore, VS= Ventral sucker, C= Cecae, SV= Seminal vesicle, U= Uterus, Vt= Vitellaria, O= Ovary, SR= Seminal receptacle, T= Testis, and A= anus.



Ceca much narrower than esophagus, extending to posterior end of body, opening through two separate anal apertures. Testes in tandem with complete margins, 60-200 (108) from posterior end of body. Anterior testis 130-300 (221) by 140-250 (199) and posterior testis 150-320 (256) by 120-225 (182). Genital pore medial, located directly anterior to ventral sucker. Cirrus sac convoluted, widening at posterior extent, 55-100 (83) at widest. Seminal vesicle occupying posterior portion of cirrus sac, oblong in shape. Ovary located anterior to testes, slightly to left of midline, 540-1,450 (1,137) from ventral sucker, spherical in shape, 110-250 (192) by 100-180 (156). Ovary smaller than testes, 0.63-1 (0.82) width ratio. Seminal receptacle teardrop-shaped, located between ovary and anterior testis, tapering toward right side of the body, 80-250 (166) by 60-170 (89). Vitelline glands follicular, oblong in shape, confined to extracecal space, extending from posterior end of cirrus sac to middle of posterior testis. Ootype and Mehlis' gland situated to right of ovary, on the medial line of the body. Laurer's canal present. Uterus occupying intercecal space, winding between ovary and cirrus sac. Eggs numerous, developing protein-tanned color as they progress through uterus, do not develop in size, 20-35 (26.3) by 12.5-15 (13.1). Excretory bladder Y-shaped, branching at posterior testis and extending to middle of esophagus. Excretory pore located medially at posterior extremity.

Host: Spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae).

Locality: Del Valle, Travis County, Texas, USA.

Site of infection: Small intestine.

Representative DNA sequences: Specimens are currently being processed for genetics and voucher sequences will be uploaded to GenBank as soon as they are available.

Remarks:

Acanthostomum nuevoleonensis can be differentiated from *A. megacetabulum* mainly by the size of oral spines, the size of the oral sucker, and the length of the eggs (Table 2). This study is the first report of this species outside of Mexico. The recovery of this parasite from the type host in a geographically distant location indicates that the originally described specimens were not an accidental host switch, as inferred by Brooks (1980).

Table 2: A comparison of the body measurements of *Acanthostomum* species, including specimens collected in this study. All measurements are in micrometers.

Measurements	***A. megacetabulum* (Thatcher, 1963) Yamaguti 1971**	***A. nuevoleonensis* Caballero and Caballero, 1964**	***A. nuevoleonenesis* (this study)**
Body Length	1800-3900	1427-1647	1450-3200
Body Width	390-600	311-348	290-610
Forebody Length	–	–	460-770
Oral Spine Number	19-21	18-20	18-22
Oral Spine Length	69	16-25	20-36.3
Oral Spine Width	17	8	7.5-12.5
Oral Sucker Length	270-320	107-119	130-185
Oral Sucker Width	250-320	102-107	120-180
Prepharynx Length	0-130	37-39	0-60
Pharynx Length	140-160	74-82	80-110
Pharynx Width	130-140	74-78	50-100
Esophagus Length	–	41-82	80-210
Ventral Sucker Length	–	94-102	90-170
Ventral Sucker Width	190-200	94-115	115-190
Ovary Length	190-200	131-148	110-250
Ovary Width	140-180	94-98	100-180
Seminal Receptacle Length	220-280	49-82	80-250
Seminal Receptacle Width	140-180	29-86	60-170
Anterior Testis Length	190-230	86-111	130-300
Anterior Testis Width	140-220	111-123	140-250
Posterior Testis Length	140-250	107-123	150-320
Posterior Testis Width	190-230	102-115	120-225
Egg Length	31-33	25-29	24.2-29.2
Egg Width	14-15	14-16	12.5-14.2

Description of *Neopolystoma* n. sp. 1 (Fig. 5)

Type host: Common snapping turtle, *Chelydra serpentina* (Linnaeus, 1758) (Testudines: Chelydridae).

Type locality: Iola, Brazos County, Texas, USA.

Other host: Spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae).

Other localities: Streetman, Navarro County, Texas, USA; Comstock, Val Verde County, Texas, USA.

Site of infection: Conjunctival sac of the eye.

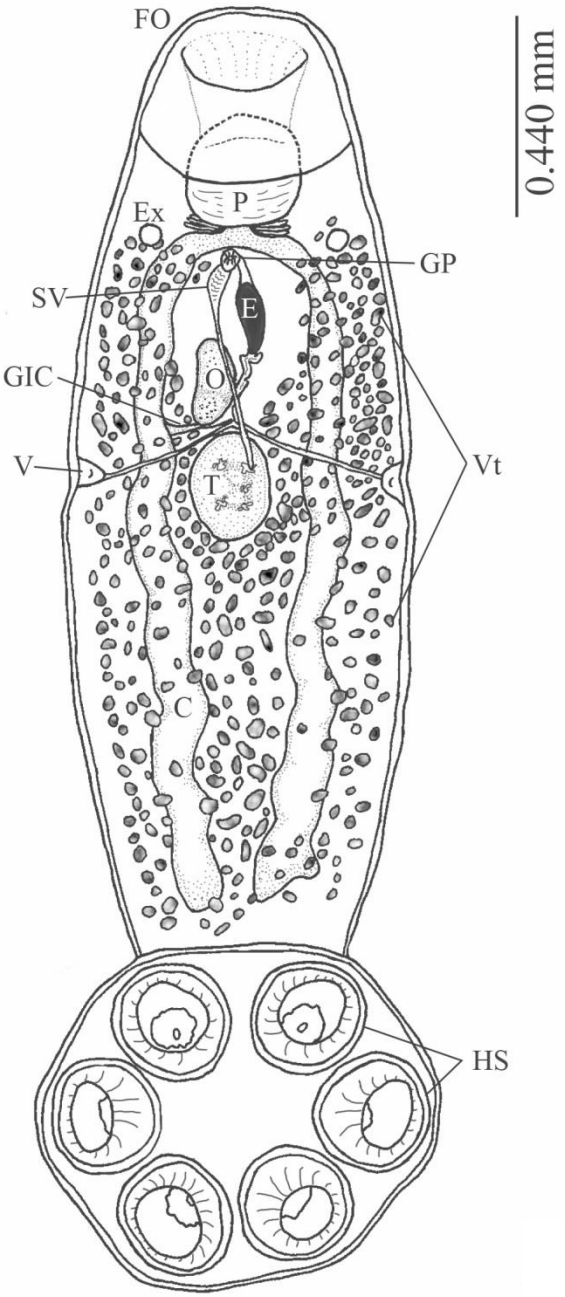
Type material: The holotype and two paratypes will be deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska, USA.

Prevalence and intensity: Across all sites, prevalence in *C. serpentina* was 22.22% (2 of 9) and *A. spinifera* was 6.67% (1 of 15). For sites where the parasites were recovered, prevalence in *C. serpentina* was 66.67% (2 of 3) and *A. spinifera* was 25.00% (1 of 4). Mean intensity for *C. serpentina* was 2 (1–2) and for *A. spinifera* was 1.

Description [based on 3 sexually mature worms, all measurements in micrometers unless otherwise stated]:

Delicate worms, able to extend considerably when alive, firmly attached to conjunctiva of host's eye. Body elongate, 2,525–2,825 (2,713.3) by 760–880 (813.3) at greatest width. Width at vagina 740–850 (796.7). Haptor compact, rounded, 650–710 (686.7) by 800–850 (830.0). Haptor length to body length ratio 0.23–0.28 (0.25).

Figure 5: Illustration of *Neopolystoma* n. sp. 1. FO= False oral sucker, P= Pharynx, Ex= Exretory bladder, GP= Genital pore, SV= Seminal vesicle, E= Egg, O= Ovary, GIC= Genito-intestinal canal, V= Vagina, T= Testis, Vt= Vitellaria, C= Cecae, and HS= Haptoral sucker.



Six haptoral suckers, muscular with a ring of plate-like skeletal structures, 240–250 (246.7) in diameter. Marginal hooklets not visible. Mouth subterminal, ventral. False oral sucker 230–330 (277) by 300–370 (346.7). Pharynx muscular, round, often overlapping with false oral sucker, 180–230 (213.3) by 220–270 (246.7). A mass of darkly stained cells are congregated at posterior edge of pharynx. Cecae bifurcating at posterior edge of pharynx and extending posteriorly, terminating short of anterior edge of the haptor, remaining unjoined, comprising 49.1–55.6% (53.3%) of total body length. Testis round to oblong, 160–240 (186.7) by 160–195 (171.7), located medially, posterior to ovary. Seminal vesicle located posterior to genital bulb, bulbous when filled with sperm, attached to testis by a long narrow canal. Genital bulb small, located posterior to bifurcation of intestinal caeca, 40–60 (51.7) wide. Genital spines 8 in number, 7.5–11.25 (9.5) in length, curved with crescent-shaped roots. Vaginae present, located approximately one third of body length from anterior end, lateral to testis, connecting anterior to testis by a narrow canal. Ovary longer than wide, comma shaped, 180–220 (196.7) by 80–110 (100.0). Uterus absent, ootype confined between testis and genital pore, containing a single spindle-shaped egg measuring 260–270 (265) by 120. Vitellaria in a continuous field stretching from posterior edge of pharynx to anterior edge of haptor, comprising 57.3–60.5% (59.1%) of total body length. Vitellaria absent in area taken up by ovary, testis, and uterus. Genito-intestinal canal wide, joining with cecum at level of the posterior edge of ovary. Ootype, genito-intestinal canal, and vaginae joining medially above testis. Excretory bladders circular, prominent, lateral to bifurcation of the cecae.

Remarks:

Neopolystoma n. sp. 1 differs from all described species in the genus *Neopolystoma* based on a number of characteristics (Table 1). *Neopolystoma* n. sp. 1 differs from *N. orbiculare, N. domitilae, N. rugosa, N. terrapenis, N. cayensis, N. cyclovitellum, N. exhamatum, N. kreffti, N. macleayi, N. novaeguineae, N. chelodinae, N. grossi, N. elizabethae, N. scorpioides, N. cribbi, N. liewi, N. palpebrae, N. queenslandensis, N. spratti*, and *N. tinsleyi* in at least six of the following measurements: body size, haptor size, false oral sucker width, pharynx size, ovary size, testis size, vitelline follicle extent, genital bulb width, number of genital spines, genital spine length, number of eggs, egg size, haptoral sucker width, and ratio of haptor to body length. *Neopolystoma* n. sp. 1 is most similar to *N. fentoni* and *N. guianensis*. *Neopolystoma* n. sp. 1 is larger in size than *N. fentoni*, has fewer eggs, and a smaller haptor length to body length ratio. *Neopolystoma* n. sp. 1 has a smaller testis size, smaller egg size, and vitelline follicles that do not reach the false oral sucker than *N. guianensis*.

In 2011, Platt et al. redescribed *N. liewi*, an eye monogenean from Asian turtles, and reported a tendency for wide variation in morphological characters due to coverslip fixation. The authors recommended that new species only be described based on specimens fixed in hot formalin without coverslip pressure. According to Platt et al. (2011), only false oral sucker width, genital bulb width, number of genital spines, genital spine length, number of eggs, egg size, haptoral sucker width, and ratio of haptor to body length are still acceptable measurements for comparison when specimens are fixed under

coverslip pressure. At least two of these measurements differ between *Neopolystoma* sp. 1 and all other described species.

Two specimens of *Polystomoides* sp. collected from a spiny softshell turtle were heat fixed without coverslip pressure to check if this method was viable. The two specimens curled and twisted, making them essentially useless for morphological measurement. For this reason, all of the specimens of *Neopolystoma* sp. 1 were fixed with light coverslip pressure.

Description of *Neopolystoma* n. sp. 2 (Fig. 6)

Type host: Red-eared slider turtle, *Trachemys scripta elegans* (Wied, 1839) (Testudines: Emydidae).

Type locality: Comstock, Val Verde County, Texas, USA.

Site of infection: Conjunctival sac of the eye.

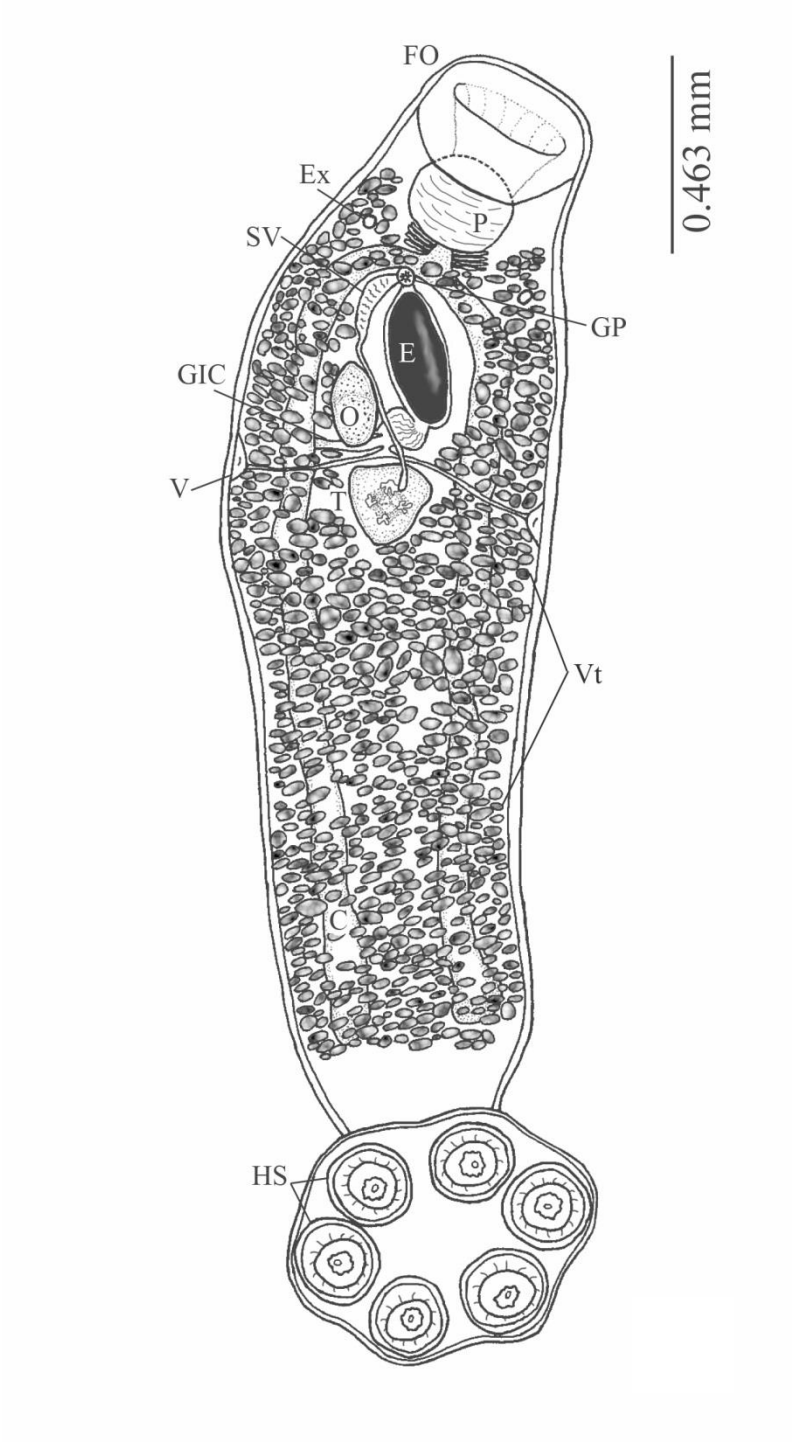
Type material: The holotype and two paratypes will be deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska, USA.

Prevalence and intensity: Across all sites, prevalence was 1.82% (1 of 56). For sites where the parasites were recovered, prevalence was 33.33% (1 of 3). Intensity was 3.

Description [based on 3 sexually mature worms, all measurements in micrometers unless otherwise stated]:

Delicate worms, more so than *Neopolystoma* n. sp. 1, able to extend considerably when alive, firmly attached to conjunctiva. Body elongate with tapered ends, 2,660–3,070 (2,800) long by 570–740 (657) at greatest width. Width at vagina 570–740 (647).

Figure 6: Illustration of *Neopolystoma* n. sp. 2. FO= False oral sucker, P= Pharynx, Ex= Exretory bladder, GP= Genital pore, SV= Seminal vesicle, E= Egg, O= Ovary, GIC= Genito-intestinal canal, V= Vagina, T= Testis, Vt= Vitellaria, C= Cecae, and HS= Haptoral sucker.



Haptor compact, wider than long, 540–650 (603) by 710–730 (720). Haptor length to body length ratio 0.19–0.26 (0.23). Six haptoral suckers, muscular with a ring of plate-like skeletal structures, 185–245 (225) in diameter. Marginal hooklets small, curved, 15 long. Mouth subterminal, ventral. False oral sucker 260–270 (263) by 320–400 (360). Pharynx muscular, round, often overlapping with false oral sucker, 170–200 (187) by 220–240 (233). A layer of darkly stained cells congregated at posterior edge of pharynx. Cecae bifurcating at posterior edge of pharynx and extending posteriorly, terminating short of anterior edge of haptor, remaining unjoined, comprising 52.9–58.9% (55.9%) of total body length. Testis round to lobate, 180–230 (197) by 110–160 (130), located medially, posterior to ovary. Seminal vesicle located posterior to genital bulb, bulbous when filled with sperm, attached to testis by a long narrow canal. Genital bulb small, located posterior to bifurcation of intestinal caeca, 20–25 (23.3) wide. Genital spines 8 in number, 11.25 in length, curved with crescent-shaped roots. Vaginae present, located approximately one third of body length from anterior end, lateral to testis, connecting anterior to testis by a narrow canal. Ovary longer than wide, 160–260 (197) by 70–100 (80). Uterus absent, ootype confined between testis and genital pore, containing a single spindle-shaped egg measuring 160–300 (225) by 70–125 (95). Vitellaria in a continuous field stretching from anterior edge of pharynx to before anterior edge of haptor, comprising 62.5–67.1% (61.5%) of total body length. Vitellaria absent in area taken up by ovary, testis, and uterus. Genito-intestinal canal wide, joining with cecum at level of posterior edge of the ovary. Ootype, genito-intestinal canal, and

vaginae joining medially above testis forming a bell-shaped atrium. Excretory bladders reduced, barely visible, lateral to bifurcation of cecae.

Remarks:

This description is based on three individuals taken from the conjunctival sac of a single *T. s. elegans*. These monogeneans were poorly fixed and no molecular specimen was saved, but key morphological characters allow for description of this species. The major difference between this species and *Neopolystoma* n. sp. 1 are the body width, haptor size, vitelline follicle extent, and genital bulb width.

Redescription of *Polystomoides coronatum* (Fig. 7)

Hosts: Red-eared slider, *Trachemys scripta elegans* (Wied, 1839) (Testudines: Emydidae); Spiny softshell turtle, *Apalone spinifera* (LeSueur, 1827) (Testudines: Trionychidae).

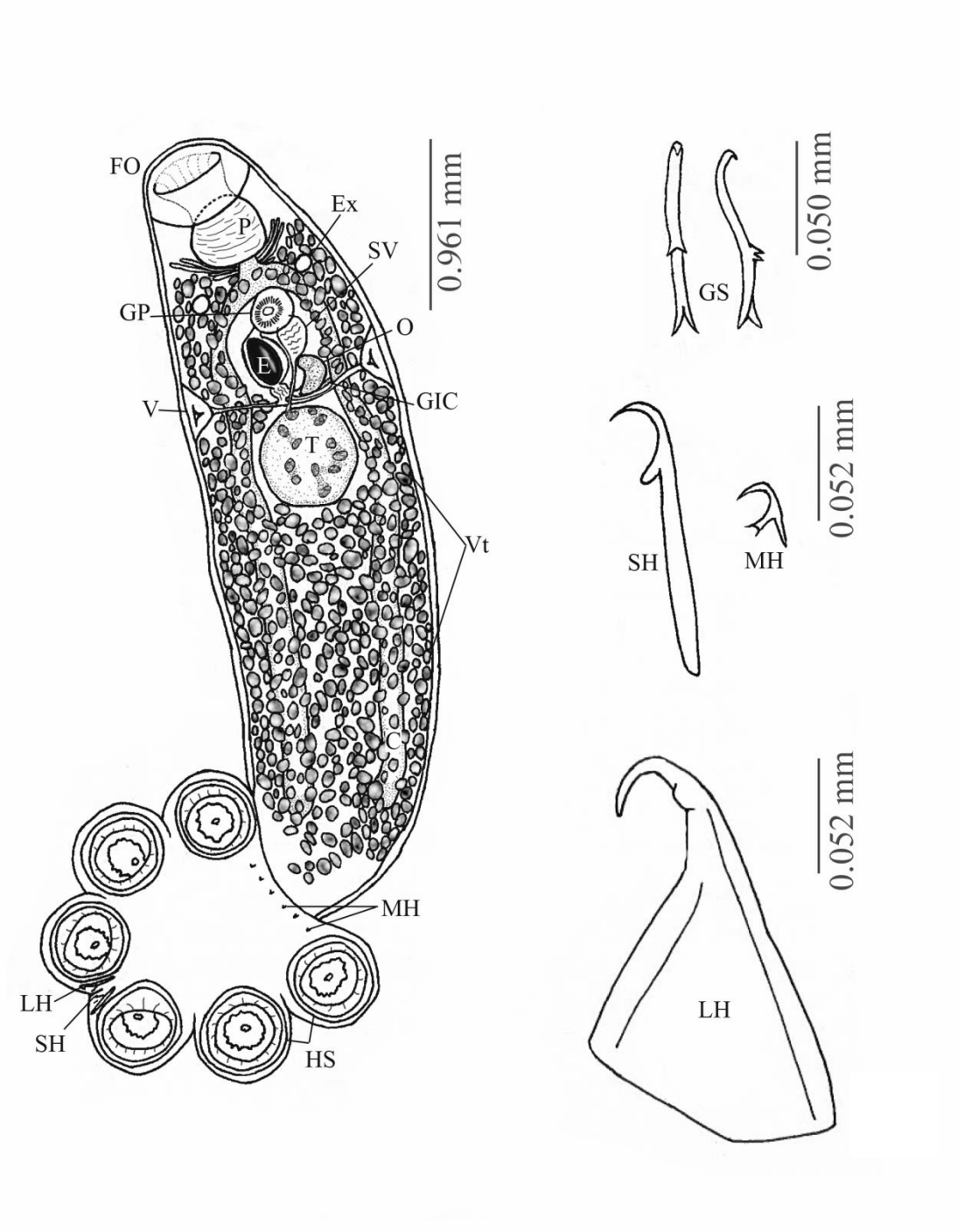
Localities: Barksdale, Edwards County; Bryan and College Station, Brazos County; Comstock, Val Verde County; Del Valle, Travis County; Gladewater, Upshur County; Glen Rose, Somervell County; Humble, Harris County; Leander, Williamson County; and Streetman, Navarro County, Texas, USA.

Site of infection: Mouth.

Redescription [based on 72 sexually mature worms, all measurements in micrometers unless otherwise stated]:

Hardy worms, light yellow when alive, egg large and orange if present, firmly attached to mucosa at back of host's mouth.

Figure 7: Illustration of *Polystomoides coronatum*. FO= False oral sucker, P= Pharynx, Ex= Exretory bladder, GP= Genital pore, SV= Seminal vesicle, E= Egg, O= Ovary, GIC= Genito-intestinal canal, V= Vagina, T= Testis, Vt= Vitellaria, C= Cecae, and HS= Haptoral sucker, LH= Large Hamulus, SH= Small Hamulus, MH= Marginal hooklets, and GS= Genital spines.



Body linguiform with tapered ends, 1,830–7,425 (3,894) by 530–1,550 (938), with greatest width at vagina. Haptor variable in shape, wider than long with suckers extending past the outer margins, 590–1,550 (1,056) by 790–2,050 (1,252). Haptor length to body length ratio 0.17–0.40 (0.28). Six haptoral suckers, muscular with a ring of plate-like skeletal structures, 200–550 (351) in diameter. Large Hamulus 95–233 (161) in length. Small Hamulus 31.3–161.5 (80.6) in length. Marginal hooklets small, curved, 12.5–25 (19.2). Mouth subterminal, ventral. False oral sucker 180–600 (311) by 260–760 (443). Pharynx muscular, round, often overlapping with false oral sucker, 140–610 (302) by 180–760 (365). A layer of darkly stained cells are congregated at the posterior edge of the pharynx. Cecae only visible in a few specimens, bifurcating at posterior edge of pharynx and extending posteriorly, terminating short of anterior edge of haptor, remaining unjoined. Testis round to oblong, 150–840 (448) by 120–760 (401), located medially, posterior to the ovary. Seminal vesicle located posterior to genital bulb, bulbous when filled with sperm, attached to testis by a long narrow canal. Genital bulb large, located posterior to bifurcation of cecae, 70–350 (173). Genital spines 28–40 (34) in number, 27–57 (41) in length, curved with branching roots. Vaginae well defined, located approximately one third of body length from anterior end, lateral to testis. Ovary longer than wide, 70–430 (187) by 70–240 (118). Uterus absent, ootype confined between the testis and genital pore, containing a single pear-shaped egg measuring 50–270 (179) by 35–200 (134). Vitellaria in a continuous field stretching from posterior edge of the pharynx to anterior edge of haptor. Vitellaria absent in area taken up by the ovary, testis, and uterus. Genito-intestinal canal joining with cecum at

posterior edge of ovary. Ootype, genito-intestinal canal, and vaginae joining medially above testis, forming a small round atrium. Excretory bladders round, prominent, lateral to the bifurcation of cecae.

Remarks:

Polystomoides coronatum is redescribed based on morphological data from the current study. Juveniles were determined to be individuals with no egg and under 2,000 µm in length. A large number of adults had no egg present (51%). It was noted that eggs were often released into the saline solution before specimens were heat fixed, which could result in this low number of gravid adults. The uterus when adults had recently oviposited was well developed but appeared collapsed, which was also used to differentiate juveniles from eggless adults.

The specimens recovered in this study match in measurements with the measurements of *P. coronatum* in the original description (Leidy, 1888) and both redescriptions (Stunkard, 1917; Price, 1939), with an expansion of the ranges (Table 3). The number of genital spines was found to be variable (28–40), but not as variable as reported by Price (1939) (14–40). Future molecular analyses will clarify the validity of the identifications in this chapter.

Table 3: A comparison of the body measurements of *Polystomoides coronatum* reported by Leidy (1888), Stunkard (1917), Price (1939), and the current study.

Characteristics	***P. coronatum*** **(Leidy, 1888)**	***P. coronatum*** **(Stunkard, 1917)**	***P. coronatum*** **(Price, 1939)**	***P. coronatum*** **this study**
Body length	4,000–6,000	3,150	3,000–6,400	1,830–7,425 (3,894)
Width		830	765–1,600	530–1,550 (938)
Haptor length				590–1,550 (1,056)
Haptor width		1,240	970–1,800	790–2,050 (1,252)
False oral sucker length		160	133–306	180–600 (311)
False oral sucker width		400	323–765	260–760 (443)
Pharynx length			274–460	140–610 (302)
Pharynx width		300	304–595	180–760 (365)
Ovary length			133–435	70–430 (187)
Ovary width		94	65–114	70–240 (118)
Testis length			285–680	150–840 (448)
Testis width		300	190–525	120–760 (401)
Genital bulb width			190	70–350 (173)
Number of genital spines	32		14–40	28–40 (34)
Genital spine length			20–26	26.5–57.3 (41.2)
Number of eggs			1	1
Egg length			228–250	50–270 (179)
Egg width				35–200 (134)
Haptoral sucker width		370	340–510	200–550 (351)
Large Hamulus length		132	95–197	95–233 (161)
Small Hamulus length		51	45–95	31.3–161.5 (80.6)
Marginal hooklet length		20	20–25	12.5–25 (19.2)
Haptor length:body length ratio				0.17–0.40 (0.28)

II.3.4 Regional associations

The following is a list of the locations sampled in this study with the number of each turtle species collected, diversity index for that location, and parasite species recovered. For each location, the GPS coordinates, ecoregion, and river basin are given.

Barksdale (29.73762, -100.0294)

This site was located in West Texas in the Edwards Plateau ecoregion and Nueces river basin. Water bodies on this property were lotic.

Three *T. s. elegans* were collected from this location. There were nine species of parasites recovered, with a taxonomic diversity index of 86.7. The parasite species recovered from this site were *N. emyditoides*, *F. affinis*, *S. trispinosus*, *S. contorta*, *A. magna*, *N. orbiculare*, *P. coronatum*, *H. octatestisaca* and *P. parasitica*.

Bryan (30.74547, -96.33327)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

Three *T. s. elegans* were collected from this location. There were 12 species of parasites recovered, with a taxonomic diversity index of 83.2. The parasite species recovered from this site were *N. emyditoides*, *N. pseudemydis*, *F. affinis*, *S. trispinosus*, *S. contorta*, *A. magna*, *H. mollis*, *P. angustus*, *T. corti*, *N. orbiculare*, *P. coronatum*, and *P. parasitica*.

TAMU Campus, College Station (30.61368, -96.331683)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

Three *A. spinifera* (*A. s. pallida*) were collected from this location. There were nine species of parasites recovered, with a taxonomic diversity index of 86.8. The parasite species recovered from this site were *N. magnapapillatus*, *Eustrongylides* sp., *F. wardi*, *S. amydae*, *T. corti*, *P. coronatum*, *P. parasitica*, *P. rugosa*, and *L. subaequalis*.

Three *C. serpentina* were collected from this location. There were 11 species of parasites recovered, with a taxonomic diversity index of 82.7. The parasite species recovered from this site were *Neoechinorhynchus* sp., *Eustrongylides* sp., *D. globocephalus*, *F. chelydrae*, *S. trispinosus*, *S. contorta*, *P. oblonga*, *H. elongata*, *P. parasitica*, *P. rugosa*, and *L. subaequalis*.

Four *T. s. elegans* were collected from this location. There were 11 species of parasites recovered, with a taxonomic diversity index of 81.3. The parasite species recovered from this site were *N. emyditoides*, *N. pseudemydis*, *F. affinis*, *S. trispinosus*, *S. amydae*, *T. corti*, *N. orbiculare*, *P. coronatum*, *P. parasitica*, *P. rugosa*, and *L. subaequalis*.

Aquaculture Facility, College Station (30.54398, -96.43777)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

One *A. spinifera* (*A. s. pallida*) was collected from this location. There were four species of parasites recovered, with a taxonomic diversity index of 66.5. The parasite species recovered from this site were *S. amydae*, *C. vesicaudus*, *T. aspidonectes*, and *P. coronatum*.

Two *C. serpentina* were collected from this location. There were seven species of parasites recovered, with a taxonomic diversity index of 75.7. The parasite species recovered from this site were *D. globocephalus*, *F. chelydrae*, *S. trispinosus*, *Al. chelydrae*, *Au. chelydrae*, *T. corti*, and *P. oblonga*.

Six *T. s. elegans* were collected from this location. There were 11 species of parasites recovered, with a taxonomic diversity index of 79.9. The parasite species recovered from this site were N. emydis, N. emyditoides, N. pseudemydis, F. affinis, S. trispinosus, *S. contorta*, *Al. chelydrae*, *D. chelydrae*, *M. obtusicaudum*, *T. corti*, and *N. orbiculare*.

Private Property 1, College Station (30.56647, -96.1665)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

One *A. spinifera* (*A. s. pallida*) was collected from this location. There were three species of parasites recovered, with a taxonomic diversity index of 81.1. The parasite species recovered from this site were *S. amydae C. vesicaudus*, and *P. parasitica*.

Two *T. s. elegans* were collected from this location. There were eight species of parasites recovered, with a taxonomic diversity index of 70.5. The parasite species

recovered from this site were *N. emyditoides*, *N. pseudemydis*, *S. trispinosus*, *S. contorta*, *D. chelydrae*, *M. obtusicaudum*, *P. coronatum*, and *P. parasitica*.

Private Property 2, College Station (30.55704, -96.20074)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

Three *T. s. elegans* were collected from this location. There were 11 species of parasites recovered, with a taxonomic diversity index of 83.1. The parasite species recovered from this site were *N. emyditoides*, *N. pseudemydis*, *F. affinis*, *S. trispinosus*, *S. contorta*, *Allassostomoides* sp., *D. chelydrae*, *M. obtusicaudum*, *N. orbiculare*, *P. coronatum*, and *P. parasitica*.

Comstock (29.65582, -100.92505)

This site was located in West Texas in the Chihuahuan Desert ecoregion and Rio Grande river basin. Water bodies on this property were both lentic and lotic.

Four *A. spinifera* (*A. s. emoryi*) were collected from this location. There were six species of parasites recovered, with a taxonomic diversity index of 79.5. The parasite species recovered from this site were *S. amydae*, *T. testudo*, Proteocephalan pleroceroids, *Neopolystoma* sp. 1, *P. coronatum*, and *H. octatestisaca*.

Three *T. s. elegans* were collected from this location. There were 10 species of parasites recovered, with a taxonomic diversity index of 84.1. The parasite species recovered from this site were *N. emyditoides*, *F. affinis*, *S. trispinosus*, *S. contorta*,

Telorchis sp., *N. orbiculare, Neopolystoma* sp. 2, *P. coronatum, H. octatestisaca*, and *P. parasitica*.

Del Valle (30.2213, -97.59953)

This site was located in Central Texas in the Texas Blackland Prairie ecoregion and Colorado river basin. Water bodies on this property were lentic.

Two *A. spinifera* (*A. s. pallida*) were collected from this location. There were six species of parasites recovered, with a taxonomic diversity index of 77.0. The parasite species recovered from this site were *S. amydae*, *A. nuevoleonensis*, *C. vesicaudus*, *T. testudo*, *P. coronatum*, and *L. subaequalis*.

One *T. s. elegans* was collected from this location. There were three species of parasites recovered, with a taxonomic diversity index of 57.8. The parasite species recovered from this site were *N. pseudemydis*, *N. orbiculare*, *H. octatestisaca*, and *P. parasitica*.

Franklin (31.05375, -96.32432)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

One *T. s. elegans* was collected from this location. There were seven species of parasites recovered, with a taxonomic diversity index of 73.7. The parasite species recovered from this site were *N. pseudemydis*, *S. trispinosus*, *S. contorta*, *Al. parvus*, *P. coronatum H. octatestisaca,* and *P. parasitica*.

Gladewater (32.57858, -94.9633)

This site was located in East Texas in the South Central Plains ecoregion and Sabine river basin. Water bodies on this property were lentic.

Seven *T. s. elegans* were collected from this location. There were 11 species of parasites recovered, with a taxonomic diversity index of 82.8. The parasite species recovered from this site were *N. chrysemydis*, *N. emyditoides*, *F. affinis*, *S. trispinosus*, *S. amydae*, *S. contorta*, *T. corti*, *T. singularis*, *N. orbiculare*, *P. coronatum*, and *P. parasitica*.

Glen Rose (32.24048, -97.83173)

This site was located in Central Texas in the Cross Timbers ecoregion and Brazos river basin. Water bodies on this property were lotic.

Four *A. spinifera* (*A. s. pallida*) were collected from this location. There were eight species of parasites recovered, with a taxonomic diversity index of 79.2. The parasite species recovered from this site were *Neoechinorhychus* sp., *S. amydae*, *Allassostomoides* sp., *C. vesicaudus*, *T. corti*, *T. testudo*, *P. coronatum*, and *P. parasitica*.

Five *T. s. elegans* were collected from this location. There were 11 species of parasites recovered, with a taxonomic diversity index of 79.2. The parasite species recovered from this site were *N. chrysemydis*, *N. emyditoides*, *N. pseudemydis*, *F. affinis*,

S. trispinosus, *S. contorta*, *Al. parvus*, *P. angustus*, *P. coronatum*, *P. parasitica*, and mites.

Humble (29.92578, -95.23422)

This site was located in East Texas in the South Central Plains ecoregion and San Jacinto river basin. Water bodies on this property were lentic.

Two *T. s. elegans* were collected from this location. There were four species of parasites recovered, with a taxonomic diversity index of 74.5. The parasite species recovered from this site were *N. emyditoides*, *S. trispinosus*, *S. contorta*, and *P. parasitica*.

Iola (30.69922, -96.05138)

This site was located in East Texas in the East Central Texas Plains ecoregion and Brazos river basin. Water bodies on this property were lentic.

Two *C. serpentina* were collected from this location. There were nine species of parasites recovered, with a taxonomic diversity index of 80.9. The parasite species recovered from this site were *Neoechinorhynchus* sp., *D. globocephalus*, *F. chelydrae*, *S. trispinosus*, *Al. chelydrae*, *Neopolystoma* sp. 1, *P. oblonga*, *P. parasitica*, and *P. rugosa*.

One *T. s. elegans* was collected from this location. There were three species of parasites recovered, with a taxonomic diversity index of 70.5. The parasite species recovered from this site were *N. emyditoides*, *S. trispinosus*, and *P. parasitica*.

Leander (30.63028, -97.89038)

This site was located in Central Texas in the Edwards Plateau ecoregion and Brazos river basin. Water bodies on this property were both lentic and lotic.

One *C. serpentina* was collected from this location. There were two species of parasites recovered, with a taxonomic diversity index of 58.7. The parasite species recovered from this site were *F. affinis* and *S. trispinosus*.

Six *T. s. elegans* were collected from this location. There were 16 species of parasites recovered, with a taxonomic diversity index of 74.6. The parasite species recovered from this site were *N. chrysemydis*, *N. emydis*, *N. emyditoides*, *N. pseudemydis*, *Contracaecum* sp., *F. affinis*, *S. trispinosus*, *S. contorta*, *C. marginatum*, *P. angustus*, *T. corti*, *T. singularis*, *N. orbiculare*, *P. coronatum*, *P. parasitica*, and *L. subaequalis*.

Sinton (28.12983, -97.39733)

This site was located in Central Texas in the Western Gulf Coastal Plains ecoregion and San Antonio Nueces river basin. Water bodies on this property were both lentic and lotic.

Three *T. s. elegans* were collected from this location. There were nine species of parasites recovered, with a taxonomic diversity index of 81.5. The parasite species recovered from this site were *N. emydis*, *S. trispinosus*, *D. chelydrae*, *M. obtusicaudum*, *P. angustus*, *T. corti*, *N. orbiculare*, *P. coronatum*, and *L. subaequalis*.

Streetman (31.94903, -96.236817)

This site was located in East Texas in the East Central Texas Plains ecoregion and Trinity river basin. Water bodies on this property were lentic.

One *C. serpentina* was collected from this location. There were seven species of parasites recovered, with a taxonomic diversity index of 72.8. The parasite species recovered from this site were *Neoechinorhynchus* sp., *D. globocephalus*, *F. chelydrae*, *S. trispinosus*, *Neopolystoma* sp. 1, *P. oblonga*, *P. parasitica*, and *P. multilineata*.

Four *T. s. elegans* were collected from this location. There were eight species of parasites recovered, with a taxonomic diversity index of 80.4. The parasite species recovered from this site were *N. emyditoides*, *N. pseudemydis*, *F. affinis*, *S. trispinosus*, *S. contorta*, *T. corti*, *P. coronatum*, and *P. parasitica*.

II.4 Discussion

Through this survey, the distributions and host associations of a number of metazoan parasite species have been elucidated at sites that had never been sampled. The fact that 17 new locality records and 16 new host associations are reported underscores our lack of understanding of parasite diversity and the clear need for more surveys. While some taxa may appear to be well sampled for parasites, parasite assemblages seem to be changing (Brooks and Hoberg, 2007; Parmesan, 2006) and need to be continually monitored. In the same manner that this paper can be compared to the study by Dinuzzo (1981), future studies will be able to compare to these results to track community changes. In this study many parasites were found in unusual sites within

hosts, emphasizing the need for thorough necropsy of hosts. Even sites that are typically uninfected (trachea, eyes, etc.) should be searched, as they may reveal dead-end infections, parasites mid-migration, or new sites of infection.

Parasite diversity was variable across sample sites and largely dependent on sample size. Taxonomic diversity was a more consistent measure of diversity across sample sites than species richness, as it is not influenced as heavily by species rarity. This measure of diversity is a useful comparative measure for studies where samples sizes are limited (Reiczigel et al, 2013). Data on environmental variables, climatic regions, and host body measurements were also collected during this study, and will be compared to parasite abundance and diversity in the next chapter.

Through nMDS and ANOSIM, *A. spinifera* was found to contain a significantly different parasite assemblage than *C. serpentina* and *T. s. elegans*. Interestingly, *A. spinifera* and *C. serpentina* have more similar feeding habits (as determined by gut content analysis in this study), and *A. spinifera* and *T. s. elegans* have more similar habitat use, being aerial baskers (Ernst and Lovich, 2009). This suggests that the parasite diversity observed in this study may be primarily driven by the evolutionary history between host and parasite, as *C. serpentina* and *T. s. elegans* are more closely related (Shaffer et al., 1997). The two subspecies of *A. spinfera*, *A. s. pallida* and *A. s. emoryi*, sampled in this study were analyzed but no differences were seen in the parasite communities. This could possibly be due to sample size since only four *A. s. emoryi* were collected. Research has shown distinct molecular differentiation between the

allopatric *Apalone* subspecies, and molecular analyses might reveal cryptic parasite diversity between these hosts (e.g. Weisrock and Janzen, 2000; McGaugh et al., 2008).

The last survey of freshwater turtle parasites in Texas was conducted by McAllister et al. (2008). This study only sampled four individual *K. flavescens,* a species not sampled in the current study. Dinuzzo (1981) is the most recent survey of *T. s. elegans,* and was conducted at a sample site used in the current study. Over the three decades since that study, there have been many global environmental changes in the form of urbanization, pollution, and increased annual temperatures due to global warming (Burrows et al., 2011; Grimmond, 2007). These changes will likely have impacts on parasite diversity and distributions, which will go unnoticed without regular surveys (Bellard et al., 2012; Bourque and Esch, 1974; Carlson et al., 2017; Cizauskas et al., 2016; Hautier et al., 2015; King et al., 2010).

In his thesis, Dinuzzo (1981) recovered 11 helminth species (excluding the Spirorchiidae) from 124 *T. s. elegans* at the TAMU aquaculture facility in College Station, Texas. Six *T. s. elegans* were collected from the same location in this study, and 11 helminth species (excluding the Spirorchiidae) were recovered. Dinuzzo reported one species of acanthocephalan (*N. emyditoides*), three species of nematodes (*S. microcephalus, S. contortus,* and *F. affinis*), five species of trematodes (*Al. chelydrae*, *H. mollis*, *T. corti*, *T. robustus*, and *P. angustus*), and two species of monogeneans (*P. coronatum* and *N. orbiculare*). In this study, three species of acanthocephalans (*N. emydis*, *N. emyditoides*, and *N. pseudemydis*), three species of nematodes (*S. trispinosus*, *S. contortus*, and *F. affinis*), four species of trematodes (*Al. chelydrae*, *D. chelydrae*, *M.*

obtusicaudum, and *T. corti*), and one species of monogenean (*N. orbiculare*) are reported. The record of *S. microcephalus* was likely a misidentification of *S. trispinosus*, since Baker (1979) clarified that *S. microcephalus* is found in the old world and *S. trispinosus* is found in the new world. The intensities of parasite species are similar between these two studies, with lower intensities of trematodes and monogeneans and higher intensities of nematodes and acanthocephalans. It would appear that a community shift has occurred in this system, with two added species of acanthocephalans and two added species of trematodes. Species losses cannot be confirmed due to the low sample size in the current survey. This community shift could be the result of turtle immigration from nearby water bodies, or a change in prevalence of species already present. Dinuzzo (1981) sampled 124 individuals from a relatively small area, so it is unlikely that parasite species were missed, and plausible that turtles have immigrated to the system since 1981, carrying new parasite species with them. Six species of spirorchiid blood flukes were recovered by Dinuzzo (1981), with prevalences up to 67% and intensities ranging from 1–22 individuals. This is contrary to the results of the current study, with a prevalence of 17% (only recovered from one turtle) and an intensity of three individuals. Spirorchiid blood flukes were found to be extremely delicate in the current study and began to die immediately after being removed from the host. The free living stages may have similar environmental sensitivities, and changes in the environment at the aquaculture facility could have reduced the abundance and diversity of this group. Turtles are still being collected from this site and blood flukes will be described with species identifications in a later report.

As the current study had low numbers of turtles collected per location, number of species sampled, and locations sampled, more surveys are still necessary to uncover the distributions of parasites of more host species across a broader area in Texas. It would be beneficial for all collecting ventures, for museum specimens and other projects, to save internal organs for parasites recovery. In addition to this, more data should be collected and reported when sampling for parasites. Host-parasite associations are still largely understudied and some factors not included in the study may be elucidated in future studies.

When sample size permitted, a small subset of specimens was saved in 95% ethanol for molecular analysis. These analyses are currently being conducted on the acanthocephalans, trematodes, cestodes, and monogeneans. This may lead to revision of this data as these identifications are based solely on morphological characters, which can fail to detect cryptic speciation. Nematode and pentastomid specimens are available for any interested parties who would like to add valuable genetic information on understudied species.

CHAPTER III

ENVIRONMENTAL AND REGIONAL INFLUENCES ON PARASITE ASSEMBLAGES IN TEXAS FRESHWATER TURTLES

III.1 Introduction

Parasitism is the most common life strategy, with an estimated 40% of known species being parasitic, and many taxa consisting mostly or entirely of parasitic species (e.g. Acanthocephala, Annelida, Arthropoda, Nematoda, Pentastomida, Platyhelminthes, Protozoa) (Dobson et al., 2008). With the high amount of cryptic speciation being uncovered through genetic analysis in parasites, the actual number of parasitic species is likely much higher than the estimates (Jousson et al., 2000; Steinauer et al., 2007). Helminths, the most common metazoan parasites recovered in the current study, have complex life cycles, sometimes traveling through many hosts throughout development from larvae to adults (Parker et al., 2003; Poulin, 2011). Helminths rely on predator prey relationships, as they are typically transmitted through consumption of infected hosts (Dronen, 1994). It is believed that overall parasites are involved in 75% of all trophic linkages in food webs due to their complex life cycles and dependence on their hosts (Lafferty et al., 2006; Lafferty, 2008). Consequently, healthy ecosystems with greater numbers of trophic linkages are higher in parasite diversity than those where the numbers of hosts in food chains have been reduced through pollution, disease, or natural causes (Johnson and Thieltges, 2010). Parasite diversity can therefore be a good indicator of ecosystem diversity and total ecosystem health (Marcogliese, 2005; Hudson

et al., 2006; Madanire-Moyo et al., 2012). Despite these findings, parasite diversity remains highly understudied, with new species being described every year, many life cycles completely unknown, and many host taxa, such as Neotropical groupers, completely unstudied (Dobson et al, 2008). Research suggests that parasitic species may be trophic regulators in the same capacity as top carnivores (e.g. Lafferty et al. 2006; Dougherty et al., 2016); however, most conservation plans do not implement any efforts to preserve parasite diversity, and often work to eradicate parasites to alleviate stressors on threatened species (Gomez and Nichols, 2013).

Environmental factors can alter parasite assemblages in significant ways. Some environmental factors associated with changes in parasite abundance and diversity are season, temperature, dissolved oxygen, turbidity, salinity, nutrient pollution, metal pollution, pesticide/herbicide pollution, and habitat alteration (e.g. Bourque and Esch, 1974; Dronen et al., 1982; Lafferty and Kuris, 1999; Banu and Khan, 2004; Nachev and Sures, 2009; Shea et al., 2012; Chapman et al., 2015; Ahmad et al., 2016). Due to the complex and diverse nature of parasite life cycles, the effects of environmental factors are variable, and often contradictory. Bourque and Esch (1974) found that nematode abundance responded differently to thermal pollution between two wetlands. Goednkegt et al. (2015) reported trematode infectivity to increase with increasing temperatures; however, predation on cercaria also increased with increasing temperatures, which then decreased trematode infectivity. In their review, Lafferty and Kuris (1999) found a variety of possible outcomes on parasite-host interactions impacted by environmental stressors. Pollutants can increase parasite infectivity by increasing host susceptibility or

decrease parasite infectivity by decreasing host survival and therefore parasite transmission. Zargar et al. (2012) found varying intensities of monogeneans on fish across polluted lakes, with decreasing intensities in one polluted and eutrophied lake and increasing intensities at a different polluted and eutrophied lake. Current trends in environmental degradation and climate change point to a change in currently observed parasite diversity (e.g. Brooks and Hoberg, 2007; Strona, 2015; Cizauskas et al. 2017). With the convoluted nature of environmental effects on parasites, it is vital to continue research in smaller systems that can be used to clarify the bigger picture.

The state of Texas can be broken up into 12 major ecological regions and 15 major river basins. Ecological regions, or ecoregions, are large stretches of land that are grouped based on the native vegetation, hydrology, and geochemistry (Griffith et al., 2007). Aquatic communities can be characterized by the ecoregion in which they reside, as aquatic community assemblages tend to vary greatly among different ecoregions (Warry and Hanau, 1993; Stoddard, 2005). The ecoregions found within the state of Texas are the Arizona/New Mexico Mountains, Chihuahuan Desert, High Plains, Southwestern Tablelands, Central Great Plains, Cross Timbers, Edwards Plateau, Southern Texas Plains, Texas Blackland Prairies, East Central Texas Plains, Western Gulf Coastal Plains, and South Central Plains. These ecoregions have many characteristic features such as the vegetative communities (hardwood forest, prairie, scrublands, etc.) and soil characteristics (sand or clay, acidic or basic, shallow or deep, etc) (Griffith et al., 2007).

The river basins delimit the area drained by each major river and its tributaries and may cross multiple ecoregions (Bureau of Economic Geology, 1996). The separation between these basins can often be a determining factor in the range of aquatic species, as seen in freshwater mussel diversity (Burlakova et al., 2011). The river basins found in the state of Texas are the Brazos, Canadian, Colorado, Cypress, Guadalupe, Lavaca, Neches, Nueces, Red, Rio Grande, Sabine, San Antonio, San Jacinto, Sulphur, and Trinity Basins. The water chemistry and biotic communities may change across the river basin, since the common river basin is the only connecting factor (Ford et al. 2016). Texas can also be viewed in respects of latitudinal and longitudinal gradients. North and east Texas are typically wetter while south and west Texas are typically drier (Texas Commission on Environmental Quality, 2018), which could lead to shifts in parasite diversity and abundance due to changes in intermediate host abundance and larval dispersal (Janzen and Schoener, 1968; Froeschke et al. 2010).

The objective of this study was to analyze the host-parasite-environment relationships in the parasites of three common species of native Texas freshwater turtles: the spiny soft shelled turtle, *Apalone spinifera* (Trionychidae) [syns. *Amyda*, *Aspidonectes*, *Platypeltis*, *Trionyx*], the common snapping turtle, *Chelydra serpentina* (Chelydridae) [syns. *Testudo*], and the red-eared slider, *Trachemys scripta elegans* (Schoepff, 1792) (Emydidae) [syns. *Chrysemys*, *Emys*, *Pseudemys*, *Testudo*]. Three subspecies of *A. spinifera*, *A. spinifera pallida* (Webb, 1962), *A. s. emoryi* (Agassiz, 1857), and *A. s. guadalupensis* (Webb, 1962) and one subspecies of *T. scripta*, *T. scripta elegans*, are found in Texas. These three turtle species are evolutionarily distinct,

belonging to three separate families. These turtle species tend to be heavily parasitized, as their omnivorous food habits often bring them in contact with infected intermediate hosts (snails, ostracods, copepods, crayfish, amphibians, fish, etc.) or free floating parasite eggs and larvae while feeding (Everhart, 1958; Grosmaire, 1977). Through this project, the effects of environmental factors on parasite assemblages in aquatic ecosystems have been elucidated for the parasite taxa of these three turtle species.

III.2 Methods

III.2.1 Field materials and methods

Turtles of the species *A. spinifera* (*A. s. pallida* and *A. s. emoryi*), *C. serpentina*, and *T. s. elegans* were captured using baited hoop nets ranging in size from 1.5 m long by 0.75 m in diameter to 1.8 m long by 0.9 m in diameter. Nets were set in shallow areas along the banks of the bodies of water and anchored using 1.2 m metal rebar poles and baited with deer, chicken, or fish. Nets were left for around 24 hours to allow time for turtles to catch the scent of the bait and enter the trap. Bycatch, such as fish, alligators, or non-target turtle species, were immediately released when encountered. Target turtles were transported in plastic tubs with 15 cm diameter holes cut out for aeration and a damp sponge in the bottom to prevent desiccation to the Laboratory of Parasitology, Department of Wildlife and Fisheries Science at Texas A&M University in College Station, Texas for euthanasia and necropsy. Two sites in west Texas were over six hours from College Station, so on these trips turtles were processed in the field. Specific GPS locations were recorded for each collection location using the Garmin eTrex 30 GPS

unit. Capture and euthanasia of turtles was approved by the Institutional Animal Care and Use Committee of Texas A&M University, reference number 040564 and collections were carried out under Texas Parks and Wildlife Department, scientific research permit number SPR-0716-172.

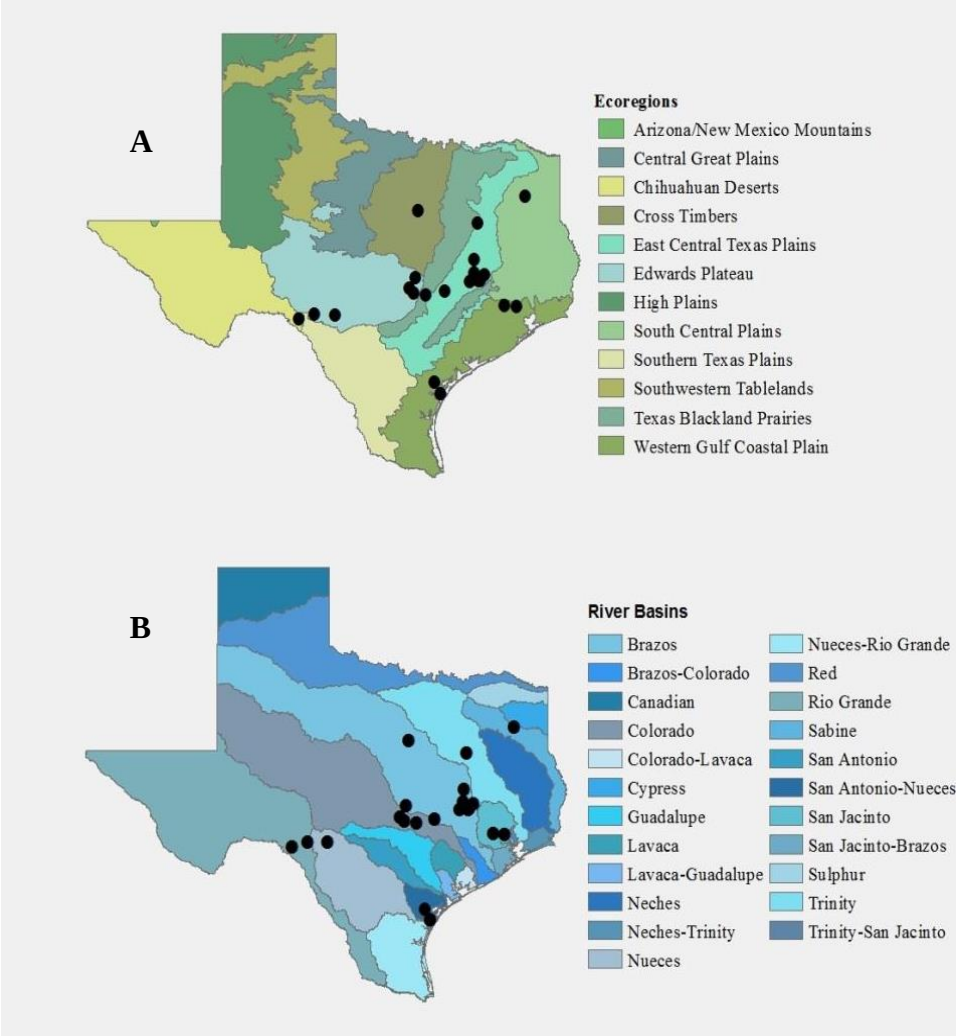
Whenever turtles were captured, a set of water parameters (i.e. temperature, turbidity, ammonia, carbon dioxide, chloride, dissolved oxygen, hardness, nitrite, nitrate, pH, and salinity) were taken from the location of capture. Ammonia, carbon dioxide, chloride, dissolved oxygen, hardness, nitrite, nitrate, and pH were analyzed using a colorimetric test kit (HACH: Ten-Parameter Test Kit, Model FF-2). Salinity was analyzed using a salinity refractometer (HACH: Refractometer, Salinity, FG100sa). A Secchi disk was used to measure turbidity as water clarity. In addition, aquatic vegetation, sediment composition, and water body type (lentic or lotic) were noted.

Capture sites were located across seven ecoregions and eight river basins in Texas (Fig. 8). One site was located in the Chihuahuan Desert and was characterized by arid shrubland with sand and gravel soils and both lentic and lotic water bodies. One site was located in the Cross Timbers and was characterized by open prairie with clay and gravel soils and lotic a lotic water body. Two geographically distant sites were located in the Edwards Plateau and were characterized by juniper and oak wooded grassland with clay and gravel soils and both lentic and lotic water bodies. One site was located in the Texas Blackland Prairies and was characterized by wooded grassland with clay and sand soils. Eight sites were located in the East Central Texas Plains and were characterized by oak woodlands with sand and clay soils and lentic water bodies. One

site was located in the Western Gulf Coastal Plains and was characterized by shrub and grasslands with sand and clay soils and both lentic and lotic water bodies. Two sites were located in the South Central Plains and were characterized by mixed pine forests with sand and clay soils and lentic water bodies.

Nine sites were located in the Brazos Basin, one site in the Colorado Basin, one site in the Nueces Basin, one site in the Rio Grande Basin, one site in the Sabine Basin, one site in the Nueces Basin, one site in the confluence of the San Antonio and Nueces Basins, one site in the San Jacinto Basin, and one site in the Trinity Basin. Lotic sites located in the upper Nueces and Brazos basins were markedly clearer and colder than other lotic and lentic sites.

Figure 8: Map of the study sites across Texas A) within ecoregions and B) within river basins.



III.2.2 Lab materials and methods

In the lab, turtles were weighed, measured (carapace length, carapace width, shell depth, circumference, and weight), and euthanized using an intracoelomic injection of 50% MS222 solution at a dosage of 1 mL/kg followed by an overdose of KCl injected into the brain. After the initial injection of MS222, the turtle was monitored until the legs

and neck were limp (usually around 30 minutes) before KCl was administered. The combination of a bone saw and aviation wire cutters were used to cut between the carapace and plastron, and then a scalpel was used to separate the plastron from the skin and musculature. All external surfaces were checked for leeches and other metazoan ectoparasites, which were removed when found. All internal organs including the esophagus, stomach, small intestine, large intestine, heart, lungs, liver, gall bladder, gonads, kidneys, bladders, and spleen were removed and searched individually for metazoan parasites under a dissecting microscope.

III.2.3 Parasite processing

Any metazoan parasites found were relaxed in a Stentor dish with 7% saline solution.

Soft-bodied helminths were heat-fixed in 7% under light coverslip pressure, placed in a petri dish with AFA (alcohol-formaldehyde-acetic acid) and left overnight, and stored in 70% ethanol until further processing. Hard bodied parasites such as nematodes, pentastomids, and mites were moved from saline directly to 70% ethanol. Acanthocephalans were placed in tap water in the refrigerator overnight to relax the specimens and then placed directly into 70% ethanol. Moving female acanthocephalans into tap water frequently induced oviposition which facilitated egg measurements and offered a more unobstructed view of the internal structures. Eggs laid by gravid females were examined directly and measured to facilitate identification of species in multiple species infections. Leeches were removed and placed in tap water to which increasing

concentrations of ethanol were added until the leeches were flat. They were then placed in 70% ethanol for permanent storage and identification.

Heat-fixed specimens were stained in Semichon's carmine, destained in acid alcohol, dehydrated through a graded ethanol series (70%, 80%, 95%, 100%, 100%), cleared in xylene, and mounted on a slide in Canada balsam. Nematodes and acanthocephalans were moved from 70% ethanol to a mixture of equal amounts of 70% and glycerine for clearing, temporarily mounted on a slide in glycerine for identification, and subsequently stored in a vial in glycerine for future observations. Where sample size permitted, a small subset of specimens was placed directly in 95% ethanol for future molecular analysis.

Parasites were keyed out to genus using the available dichotomous keys (Khalil et al. 1994, Gibson et al. 2002, Jones et al. 2005, Bray et al. 2008, Anderson et al. 2009). For species level identification, body measurements were made and compared to original parasite descriptions. Leeches were keyed to species using the keys by Klemm (1985) and Moser et al. (2016).

III.2.4 Statistical analysis

All statistical analyses were performed in R version 3.4.1 (R Core Team, 2017). For water parameters, simple linear regressions (lm function) were performed on parasite abundance, species richness, and taxonomic diversity. Significant relationships were then plotted for visualization of relationships (ggplot function in the package ggplot2). Significance was analyzed at the 95% level. Abundances were log-transformed

to correct for normality. Tukey's Ladder of Powers transformation (transformTukey function in the package rcompanion) did not greatly alter the results of transformation, so the simpler log method was used. Diversity was analyzed as both species richness and taxonomic diversity (taxondive function in the package vegan). Species richness is simply the number of species in a given community while taxonomic diversity takes species relatedness into account, with communities with larger diversity indices having more unrelated parasite communities. Taxonomic diversity reduces error introduced from sample size and species rarity, and is more robust to minor changes in community composition (Warwick and Clarke, 1995; Clarke and Warwick, 1998; Luque and Poulin, 2008). The data set was split by host species in order to remove conflicting responses due to differing host responses and differing parasite communities. Data was also split and analyzed by abundances of different parasite taxa (acanthocephalans, digeneans, hirudineans, monogenean, and nematodes). Pentastomids and mites did not have large enough sample sizes for correlations to be visible. As *T. s. elegans* had the largest sample size (n=51), only this species was used to analyze responses of individual parasite taxa.

Season, latitudinal gradient, longitudinal gradient, ecoregion, river basin, sediment composition, water body type, turtle sex, and aquatic vegetation presence were separately compared to parasite abundance and diversity using one-way ANOVAs (aov function), and p-value correction was performed following the Bonferroni method ($p < 0.0056$). All of these characteristics change by location and host individuals. Whenever an ANOVA showed significance, Tukey's post-hoc test was used (TukeyHSD function)

to determine the relationship between variables. Tukey's test compares the differences between group's means to determine which groups from an ANOVA differ significantly. No difference was found in the parasite diversity among the two subspecies of *A. spinifera* captured in this study, *A. s. pallida* and *A. s. emoryi*, and so they were analyzed together.

III.3 Results

III.3.1 Sample sites

Turtles were collected from 16 properties across seven ecoregions and eight river basins. The properties were located in 13 towns across Texas: Barksdale, Bryan, College Station (Aquaculture Facility, Private Property 1, Private Property 2, and TAMU Campus), Comstock, Del Valle, Franklin, Gladewater, Glen Rose, Humble, Iola, Leander, Sinton, and Streetman. Sites varied in the type of water bodies present. The Barksdale site, located in the Edwards Plateau ecoregion and Nueces river basin, had a single vegetated river with gravel sediment.

The Bryan site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a single vegetated pond with primarily clay sediment.

The Aquaculture Facility site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a vegetated oxbow pond with primarily silt sediment and fish ponds with rubber liner substrate and no vegetation.

The Private Property 1 site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a single vegetated pond with primarily silt sediment.

The Private Property 2 site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a single vegetated pond with primarily clay sediment.

The TAMU Campus site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a single vegetated pond with primarily silt sediment.

The Comstock site, located in the Chihuahuan Desert ecoregion and Rio Grande river basin, had a vegetated pond with primarily clay sediment and an unvegetated river with primarily loam sediment.

The Del Valle site, located in the Texas Blackland Prairies ecoregion and Colorado river basin, had a vegetated pond with primarily silt sediment and a vegetated lake with primarily sand sediment.

The Franklin site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a vegetated lake with primarily sand sediment.

The Gladewater site, located in the South Central Plains ecoregion and Sabine river basin, had a vegetated pond with primarily silt sediment and a vegetated pond with primarily sand sediment.

The Glen Rose site, located in the Cross Timbers ecoregion Brazos river basin, had a single vegetated river with primarily rock and gravel sediment.

The Humble site, located in the South Central Plains ecoregion and San Jacinto river basin, had a single unvegetated pond with primarily clay sediment.

The Iola site, located in the East Central Texas Plains ecoregion and Brazos river basin, had a single vegetated pond with primarily silt sediment.

The Leander site, located in the Edwards Plateau ecoregion and Brazos river basin, had two vegetated ponds, both with primarily gravel sediments.

The Sinton site, located in the Western Gulf Coastal Plains ecoregion and San Antonio-Nueces river basin, had a vegetated pond and an unvegetated river, both with primarily clay sediment.

The Streetman site, located in the East Central Texas Plains ecoregion and Trinity river basin, had a single vegetated pond with primarily sand sediment.

III.3.2 Capture data

A total of 15 *A spinifera* (11 *A. s. pallida* and four *A. s. emoryi*), nine *C. serpentina*, and 55 *T. s. elegans* were collected and necropsied for this study. Four *T. s. elegans* were collected without water parameter data, so only 51 individuals of this species are used for water parameter analyses. A total of 18,853 individual parasites were recovered in this study: 8,271 acanthocephalans, 8,408 nematodes, 947 trematodes, 191 monogeneans, 906 leeches, 112 pentastomids, and 18 mites. A total of 42 different parasitic species were recovered in this study: five species of acanthocephalans, nine species of nematodes, 14 species of trematodes, two species of cestodes, five species of monogeneans, five species of leeches, one species of mite, and one species of pentastomid. The detailed localities, taxonomy, and host associations are listed out in the previous chapter (Table 1).

III.3.3 Regional and water quality analyses

Environmental parameters were highly variable across sites. Ammonia levels were highest (> 2 mg/L) at the Streetman and TAMU campus sites. Chloride levels were highest (> 200 mg/L Cl^-) at the Aquaculture Facility and Sinton sites. Carbon dioxide levels were highest (> 200 mg/L) at the Aquaculture Facility. Dissolved Oxygen levels were lowest (< 4 mg/L) at the TAMU campus, Aquaculture Facility, and Leander sites. Hardness levels were highest (> 500 mg/L CaCO3) at the TAMU Campus, Glen Rose, and Comstock, with Glen Rose and Comstock having levels over 1850 mg/L CaCO3 on one occasion. Nitrites were only detected on one occasion at three properties, Bryan, Del Valle, and TAMU campus, with levels over 2.0 mg/L NO_2^- at the Bryan site. Nitrate levels were highest (>4 mg/L NO_3^-) at the Del Valle site. Salinity was only detected (25 ppt) in the river at the Sinton site. Turbidity measured as water clarity (cm) was highest (< 20 cm visibility) at the Aquaculture Facility, Humble, Private Property 1, Private Property 2, and TAMU Campus sites. The Barksdale and Glen Rose sites and one pond at the Comstock site had the highest visibility, with water being clear to the bottom. The pH levels varied across sites from 6.5 to 9.0. The Barksdale, Bryan, Aquaculture Facility, Private Property 1, Private Property 2, TAMU campus, Comstock, Del Valle, Glen Rose, Humble, Iola, Leander, and Streetman sites were all basic (>7). The Gladewater site was measured as acidic (6.5) in the small pond and basic (7.5) in the lake. The Sinton site was measured as acidic (6.75) in the small pond and basic (7.25) in the river. The Franklin site was only sampled once and was neutral (7). Temperature

(°C) varied by season, averaging 21°C in the spring, 28°C in the summer, 24°C in the fall, and 17°C in the winter.

For water parameters, simple linear regressions were performed on parasite abundance, species richness, and taxonomic diversity. The data set was split by host species in order to remove conflicting responses due to different host responses and parasite communities. Parasite abundance and diversity were not significantly correlated to any water parameters for *A. spinifera* and *C. serpentina*, which could be a result of the small sample sizes in both groups (N=15 and N=9). Turbidity was significantly positively correlated with abundance (Fig. 9A, $p=7.47e^{-6}$, $R^2=0.34$) and significantly negatively correlated with taxonomic diversity (Fig. 9B, $p= 0.022$, $R^2= 0.1$) for parasites of *T. s. elegans* (n=51). Parasite species richness and taxonomic diversity were significantly negatively correlated with carbon dioxide (Fig. 10A, $p=0.018$, $R^2= 0.11$; Fig. 10B, $p=0.0024$, $R^2= 0.17$). Taxonomic diversity was significantly negatively correlated with chloride (Fig. 12, $p= 0.0075$, $R^2= 0.14$) and significantly positively correlated with dissolved oxygen (Fig. 11A, $p= 0.029$, $R^2= 0.094$). When data was analyzed by parasitic taxa abundances, only acanthocephalans, digeneans, and monogeneans showed significant relationships. Acanthocephalan abundance was significantly positively correlated with ammonia (Fig. 13, $p= 0.042$, $R^2= 0.082$) and turbidity (Fig. 9C, $p= 0.0024$, $R^2= 0.17$). Digenean abundance was significantly positively correlated with turbidity (Fig. 9D, $p=0.049$, $R^2= 0.077$). Monogenean abundance was significantly positively correlated with dissolved oxygen (Fig. 11B, p=

0.039, R^2= 0.084) and significantly negatively correlated with carbon dioxide (Fig. 10C, p= 0.024, R^2= 0.10).

Figure 9: Linear regressions for turbidity (water clarity), trend lines plotted with 95% confidence intervals. Significance coefficients are included on each plot. A) Correlation between turbidity and parasite abundance, B) Correlation between turbidity and taxonomic diversity, C) Correlation between turbidity and acanthocephalan abundance, and D) Correlation between turbidity and digenean abundance.

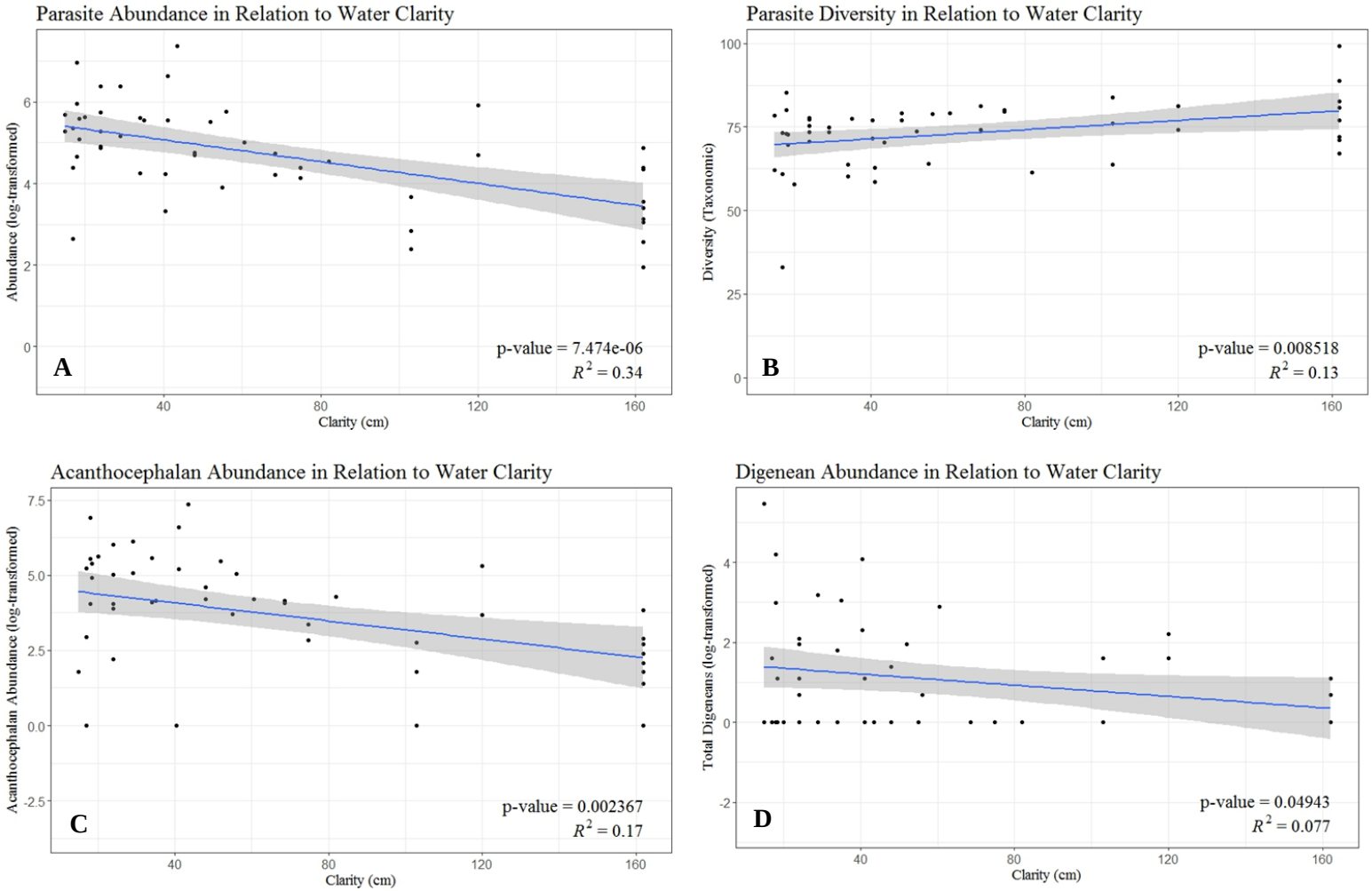


Figure 10: Linear regressions for carbon dioxide, trend lines plotted with 95% confidence intervals. Significance coefficients are included on each plot. A) Correlation between carbon dioxide levels and species richness, B) Correlation between carbon dioxide levels and taxonomic diversity, and C) Correlation between carbon dioxide levels and monogenean abundance.

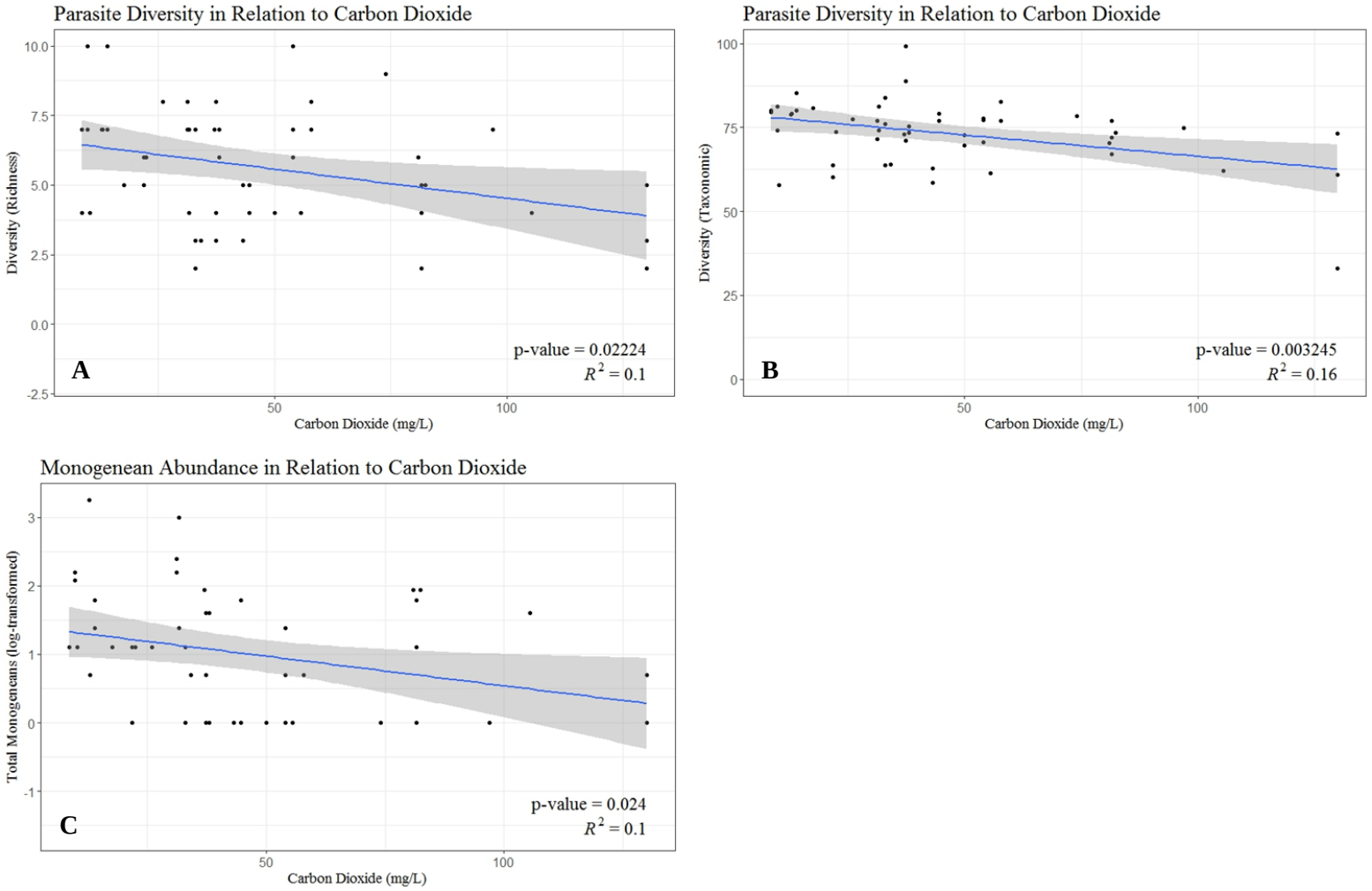


Figure 11: Linear regressions for dissolved oxygen, trend lines plotted with 95% confidence intervals. Significance coefficients are included on each plot. A) Correlation between dissolved oxygen levels and taxonomic diversity and B) Correlation between dissolved oxygen levels and monogenean abundance.

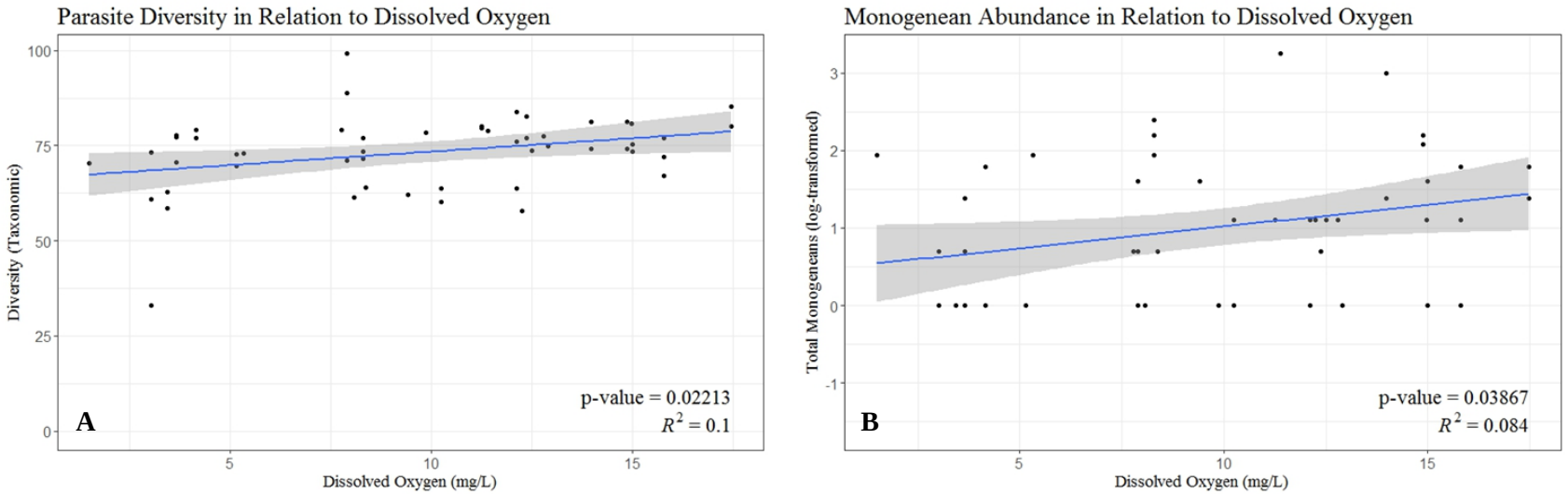


Figure 12: Linear regression for chloride levels and taxonomic diversity, trend line plotted with 95% confidence interval. Significance coefficients are included on the plot.

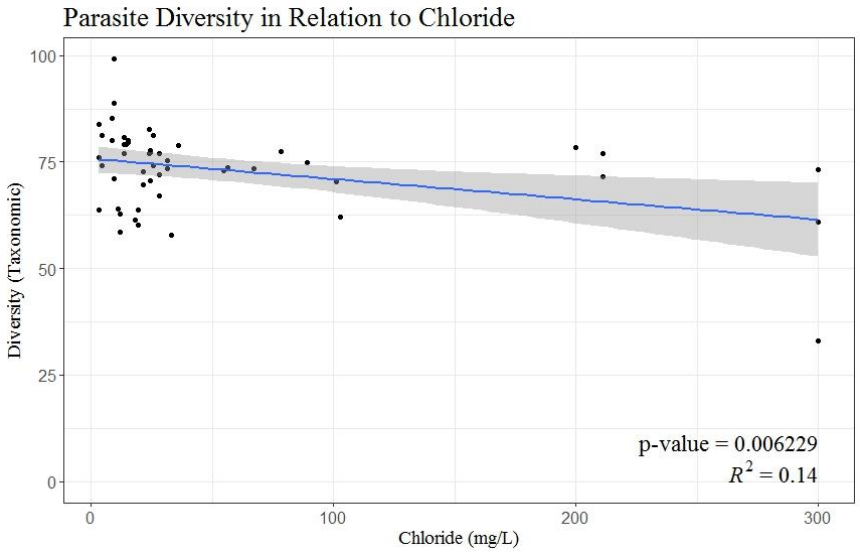
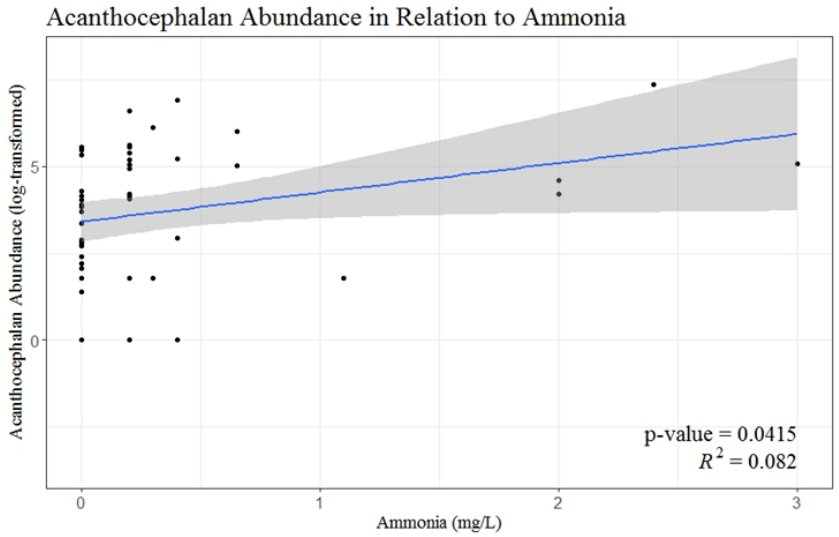


Figure 13: Linear regression for ammonia levels and acanthocephalan abundance, trend line plotted with 95% confidence interval. Significance coefficients are included on the plot.



Season, latitudinal gradient, longitudinal gradient, ecoregion, river basin, water body type, sediment type, and aquatic vegetation presence were all compared to parasite abundance and diversity using one-way ANOVAs, with Bonferroni p-value correction ($p< 0.0056$).

Total parasite abundance among all three host species, scaled logarithmically for normality, was significantly different among longitudinal gradient ($p=0.00028$) and river basins ($p=0.00055$). For longitudinal gradient, the West had significantly lower abundance than Central ($p=0.0056$) and the East ($p=0.00016$). For river basins, post-hoc tests showed no significant differences in abundance between basins. Total parasite abundance was significantly lower in lotic versus lentic systems ($p=0.00046$).

Total species richness and taxonomic diversity were not significantly correlated with any environmental variables.

Parasite abundance in *T. s. elegans*, scaled logarithmically for normality, was significantly different among river basins ($p=0.0013$) and water body type ($p=0.00039$). For river basins, post-hoc tests showed no significant differences in abundance between basins. Parasite abundance was significantly lower in lotic versus lentic systems ($p=0.00039$).

Species richness and taxonomic diversity in *T. s. elegans* were not significantly different among any environmental variables.

When data was analyzed by parasitic taxa abundances, only acanthocephalans, leeches, and nematodes showed significant relationships. Acanthocephalan abundance

was significantly different among water body type (p=0.00065). For water body type, lentic systems had significantly higher acanthocephalan abundance than lotic systems.

Leech abundance was significantly different among river basins (p=0.0045). For river basins, leech abundance was significantly higher in the Trinity basin than the Brazos basin (p=0.0012) and the San Antonio Nueces basin (p=0.0052).

All significance coefficients from the linear regressions and ANOVAs are reported in tables 4 through 6.

Table 4: Significance coefficients (p-values and R^2 values) from simple linear regressions on parasite abundance and diversity. Significant values are bolded.

	Parasite Abundance				Parasite Diversity (Richness)				Parasite Diversity (Taxonomic)			
	Total		TS only		Total		TS only		Total		TS only	
	p-value	R2	p-value	R2	p-value	R2	p-value	R2	p-value	R2	p-value	R2
Ammonia	0.3	0.015	0.038	0.085	0.719	0.0018	0.45	0.012	0.86	0.00041	0.67	0.0038
CO2	0.43	0.0085	0.93	0.00017	0.16	0.026	**0.018**	**0.11**	0.19	0.023	**0.0025**	**0.17**
Chloride	0.71	0.0019	0.99	2.28E-06	0.55	0.0049	0.74	0.0024	0.08	0.041	**0.0075**	**0.14**
DO	0.6	0.004	0.28	0.024	0.14	0.029	0.14	0.044	0.66	0.0027	**0.029**	**0.094**
Hardness	0.071	0.044	0.13	0.046	0.095	0.038	0.11	0.051	0.77	0.0012	0.87	0.00053
pH	0.5	0.0064	0.63	0.0048	0.47	0.007	0.95	7.11E-05	0.77	0.0011	0.68	0.0034
Temperature	0.88	0.00032	0.66	0.0041	0.42	0.009	0.12	0.048	0.75	0.0014	0.57	0.0065
Turbidity	**0.0094**	**0.089**	**7.47E-06**	**0.34**	0.67	0.0026	0.3	0.022	0.13	0.031	**0.022**	**0.1**

Table 5: Significance coefficients (p-values and R^2 values) from simple linear regressions on parasite taxa. Significant values are bolded.

	Parasite Abundance (TS only)									
	Acanthocephalans		Digeneans		Leeches		Monogeneans		Nematodes	
	p-value	R2	p-value	R2	p-value	R2	p-value	R2	p-value	R2
Ammonia	**0.042**	**0.082**	0.26	0.026	0.055	0.073	0.28	0.024	0.056	0.072
CO2	0.47	0.011	0.33	0.02	0.072	0.065	**0.024**	**0.1**	0.58	0.0064
Chloride	0.083	0.06	0.11	0.052	0.06	0.07	0.74	0.0022	0.82	0.001
DO	0.66	0.004	0.15	0.041	0.28	0.024	**0.039**	**0.084**	0.92	0.00022
Hardness	0.64	0.0046	0.31	0.021	0.28	0.024	0.85	0.00079	0.28	0.024
pH	0.38	0.016	0.5	0.0093	0.15	0.041	0.66	0.0041	0.3	0.022
Temperature	0.54	0.0078	0.17	0.039	0.16	0.04	0.92	0.00021	0.41	0.014
Turbidity	**0.0024**	**0.17**	**0.049**	**0.077**	0.19	0.035	0.74	0.0023	0.41	0.014

Table 6: Significance coefficients (p-values) from one-way ANOVAs. Significant values are bolded. See Appendix for full table of significance coefficients from Tukey's post-hoc tests.

	Parasite Abundance		Parasite Diversity (Richness)		Parasite Diversity (Taxonomic)		Parasite Abundance (TS only)				
	Total	TS only	Total	TS only	Total	TS only	Acanthocephalans	Digeneans	Leeches	Monogeneans	Nematodes
Lentic/Lotic	**0.00046**	**0.00039**	0.32	0.72	0.88	0.092	**0.00065**	0.41	0.27	0.19	0.084
Sex	0.11	0.072	0.11	0.29	0.13	0.61	0.26	0.19	0.91	0.58	0.092
Aquatic Veg	0.13	0.41	0.05	0.16	0.016	0.057	0.1	0.6	0.028	0.91	0.6
Season	0.53	0.61	0.062	0.057	0.403	0.061	0.83	0.065	0.089	0.18	0.03
Latitude	0.83	0.14	0.95	0.41	0.54	0.53	0.12	0.12	0.41	0.29	0.25
Longitude	**0.00028**	0.0087	0.037	0.32	0.035	0.029	0.28	0.49	0.14	0.76	0.39
Ecoregion	0.009	0.022	0.27	0.62	0.88	0.63	0.03	0.19	0.4	0.097	0.08
River Basin	**0.00055**	**0.0013**	0.46	0.84	0.48	0.077	0.0059	0.57	**0.0045**	0.32	0.021
Sediment	0.039	0.13	0.38	0.78	0.33	0.2	0.55	0.12	0.034	0.04	0.67

III.3.4 Host measurement analyses

Simple linear regressions were also performed between parasite abundance, species richness, and taxonomic diversity and turtle weight, straight carapace length, straight carapace width, shell depth, curved carapace length, curved carapace width, and circumference. For *A. spinifera*, parasite abundance was significantly positively correlated with turtle weight (Fig. 14E, p=0.035, R2= 0.30), straight carapace length (Fig. 14A, p=0.017, R2= 0.36), straight carapace width (Fig. 14C, p=0.016, R2= 0.37), curved carapace length (Fig. 14B, p=0.016, R2= 0.37), curved carapace width (Fig. 14D, p=0.016, R2= 0.37). No correlation was seen in *C. serpentina* or *T. s. elegans*. ANOVAs were performed between host sex and melanism, and parasite abundance, species richness, and taxonomic diversity, but no significant relationships were found.

Figure 14: Linear regressions for body size of *A. spinifera* in relation to parasite abundance, trend line plotted with 95% confidence interval. Significance coefficients are included on the plot. A) Correlation between straight carapace length and parasite abundance, B) Correlation between curved carapace length and parasite abundance, C) Correlation between straight carapace width and parasite abundance, D) Correlation between curved carapace width and parasite abundance, and E) Correlation between weight and parasite abundance.

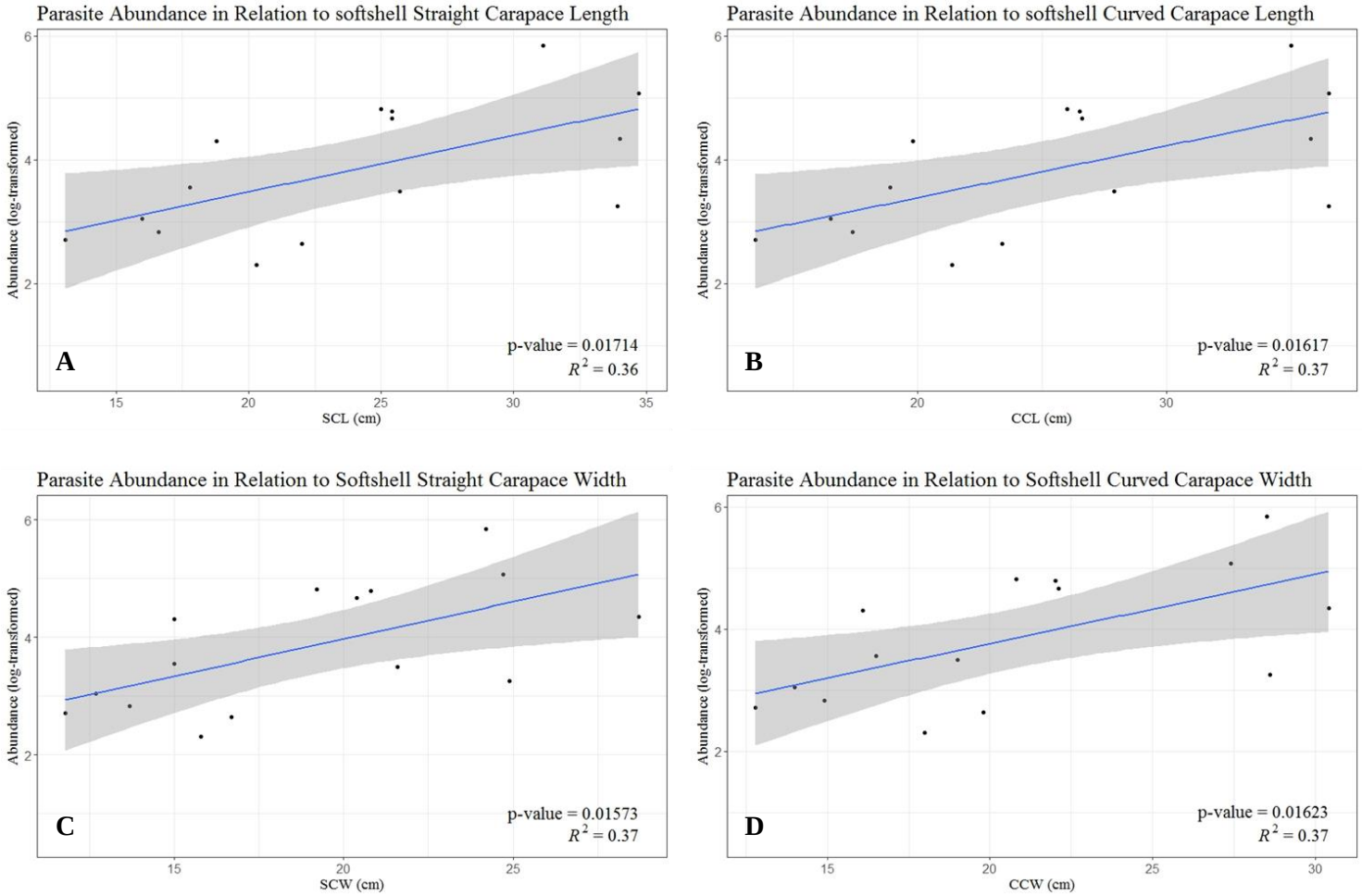
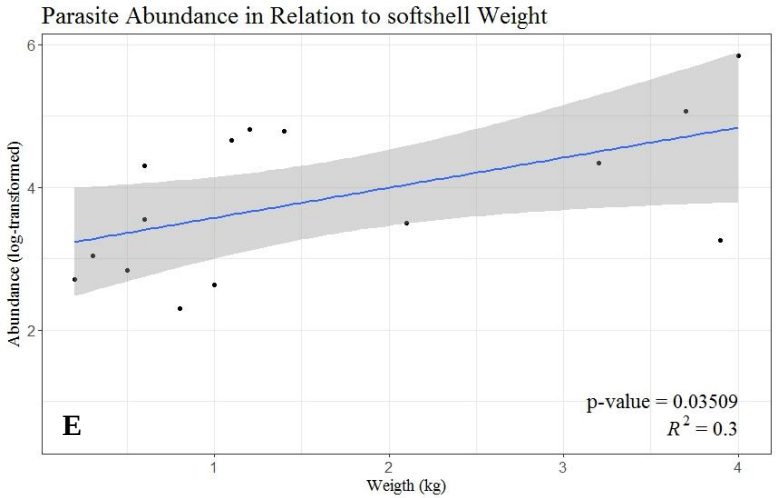


Figure 14: Continued.



III.4 Discussion

Many studies have shown through both surveys and meta-analysis that environmental changes are impacting parasite diversity and distributions (e.g. Bourque and Esch, 1974; Grimmond, 2007; King et al., 2010; Burrows et al., 2011; Bellard et al., 2012; Hautier et al., 2015; Cizauskas et al., 2016; Carlson et al., 2017). Of the three major diversity surveys of parasites of Texas freshwater turtles (Harwood, 1932; Everhart, 1957; Dinuzzo, 1981), only one unpublished thesis reported any ecological data (Dinuzzo, 1981). Season and water temperature as well as host sex and age class were compared to the abundances of different parasitic taxa in *T. s. elegans*. Dinuzzo found that female turtles had higher parasite abundances than male turtles, which was attributed to their larger size. Adult turtles had more parasites than juveniles, which was also attributed to body size. In the current study, no differences were found between host sex or body size (age class) on parasite abundance or diversity in *T. s. elegans*. A significant increase in parasite abundance was found in *A. spinifera* with increasing body size. Dinuzzo also found that parasites had lower intensities in the winter compared to the summer for all taxa but the trematodes, which had higher abundances in the winter. In the current study, trematodes and nematodes were found to have higher abundances in the spring, with no seasonality in acanthocephalans, monogeneans, and leeches. In addition, taxonomic diversity was higher in the summer than the fall. The difference observed in seasonality between the study by Dinuzzo (1981) and the current study could be attributed to a number of factors including confounding factors from multiple

highly variable study sites, different species assemblages responses, or climatic differences between the two temporally distant studies.

Trachemys scripta elegans was the most common host species captured during this study. This turtle was in high abundance at most sample sites, often seen basking or swimming when setting and collecting traps. *Apalone spinifera* was the second most common species, and was seen swimming in the river sites more often than *T. s. elegans*. *Chelydra serpentina* were only seen when captured in this study, and more than two were never captured in a single trap night. Apart from the three target species of turtles, only one Texas cooter, *Pseudemys texana*, and one razor-backed musk turtle, *Sternotherus carinatus*, were captured during the course of this study. As a result of the larger sample size, *T. s. elegans* revealed the clearest picture of the effects of environmental parameters and regionality on parasite diversity and abundance. Parasite abundance and species richness were variable across samples sites, and were largely dependent on sample size. Taxonomic diversity was a more consistent measure of diversity across sample sites than species richness, as it is not influenced as heavily by species rarity. This measure of diversity is a useful comparative measure for studies where samples size is limited (Reiczigel et al., 2013).

Many environmental and host physiological factors contribute to the abundance and diversity of parasites in a given ecosystem. For this reason it is doubtful that any single factor has any major significance in the determination of species assemblage dynamics. Many of the statistically significant correlations presented in this study are likely due to covariation of parameters at sites, and many of the important variables were

likely not measured in this study. Most correlation coefficients (R^2) presented with the linear models were low, indicating very little of the observed variation is explained by the model. The possible contributions of individual factors are discussed here, although the contribution may be insignificant in reality, and these hypotheses should be tested in future lab and fieldwork. Multivariate methods are currently being used to analyze which factors are contributing the most to the overall variance between sites, and will be reported in a future paper.

Turbidity, measured as water clarity, had the strongest correlations of any environmental parameters in this study, and is likely the strongest predictor of parasite abundance and diversity. Turbidity was negatively correlated with taxonomic diversity, while it was positively correlated with total parasite abundance, abundance of acanthocephalans, and abundance of digeneans. This increase in diversity in clearer water could be a result of reduced diversity of intermediate host species, increased predation on free living larval stages, reduced motility of free living larval stages, direct mortality of free living larval stages due to toxins often associate with turbid water, or cryptic interactions between host health and water quality. The increase in abundance in more turbid waters could be a result of increased abundance of specific intermediate host species or a greater availability of niche space within the host due to decreases in diversity. Abundance of acanthocephalans and digeneans was higher in more turbid waters, following the trend seen in total parasite abundance. Izyumova (1979) found that turbidity can negatively affect crustaceans through direct mechanical damage by suspended particles to the feeding appendages of larval stages. Conversely, Yilman and

Kulkoyluoglu (2006) found that certain species of ostracod crustaceans, the known intermediate host for acanthocephalans, increased in abundance with increasing turbidity. An increase in the intermediate host abundance of acanthocephalans would increase the abundance in turtle hosts, explaining the positive correlation between turbidity and abundance seen in the current study. Turbidity decreases direct mortality of cercaria from UV radiation and predation, and increases the abundance of snail intermediate hosts by reducing predation and increasing food availability (Zbikowska et al., 2006; Shah et al., 2013). An increase in cercaria survival and snail intermediate host abundance could explain an increase in overall trematode abundance in more turbid environments.

Carbon dioxide levels were negatively correlated with species richness, taxonomic diversity, and monogenean abundance. Parasitic nematode larvae respond to carbon dioxide as a host seeking stimulus (Sciacca et al., 2002). *Schistosoma* larvae, both miracidia and cercaria, exhibit host seeking responses to chemical stimuli including amino acids, fatty acids, ammonia, and several different glycoproteins (Sukhdeo and Sukhdeo, 2004; Haeberlein and Haas, 2008). If aquatic parasites which have larvae that must seek hosts, like monogeneans, rely on the same stimulus, high ambient carbon dioxide levels could interfere with this life cycle and reduce the abundance of specific parasites, therefore reducing the overall diversity.

Dissolved oxygen levels were positively correlated with taxonomic diversity and monogenean abundance. Monni and Cognetti-Varriale (2002) found that eels have a stronger immune response against monogeneans in oxygen rich water, leading to lower

levels of infection. Chapman et al. (2000) found higher prevalence of monogeneans in fish inhabiting oxygen deficient environments, which could be due to a high tolerance on the part of the parasite or a reduced immune response in the fish. The data in the current study contrast these findings. Some larval helminths have inhibited development in oxygen deficient environments (Thorson, 1969). It is likely that turtle monogeneans respond differently to oxygen levels than fish monogeneans. It may be that turtle monogenean adults and larvae require higher oxygen levels to persist. Laboratory studies observing survival and infectivity of monogeneans in water with different levels of carbon dioxide and oxygen could clarify the mechanisms behind this relationship.

Chloride levels were negatively correlated with taxonomic diversity. High levels of chlorides are known to interfere with osmoregulation in aquatic organisms (Karraker and Gibbs, 2011). This reduction in taxonomic diversity indicates a decrease in the number of parasite taxa, and could be due to toxic effects of chloride on certain free swimming larval stages, certain intermediate hosts, or adults within the host as water is ingested.

Ammonia levels were positively correlated with acanthocephalan abundance. At high levels, ammonia can be toxic to aquatic organisms by halting their ability to properly excrete waste (U.S. Environmental Protection Agency, 2013). The increase in acanthocephalan levels could be due to a decrease in host immunity, allowing higher numbers of acanthocephalans to invade. This correlation is confounding, as higher ammonia levels would reduce the survival of intermediate host species and free swimming larvae. Na et al. (2009) showed that certain ostracods have a high tolerance to

increased aquatic ammonia levels. If ammonia is reducing the abundances of other aquatic zooplankton, ostracods may be in high abundance in environments with raised ammonia levels, increasing the abundance of acanthocephalan parasites. More in depth field studies would be necessary to discover if this correlation observed is simply coincidental and that other factors are not causing the increased abundance. Laboratory experiments testing varying ammonia levels on the acanthocephalan life cycle could clarify the mechanisms behind this correlation.

Longitudinal gradient, river basin, and water body type were all significantly correlated with parasite abundance, while no variables were significantly correlated with diversity. Differences between parasite abundance across a longitudinal gradient and river basins were in an east to west distribution, with higher parasite abundance in the East. It is likely that the difference is due to the change in climate from east to west Texas, with lower precipitation in the west reducing the transmission of parasites. Lotic systems (rivers) had significantly lower parasite abundance than lentic systems (ponds and lakes). Higher flow in lotic systems could possibly inhibit transmission of free-swimming larval stages and therefore reduce the abundance of parasites within the hosts in those systems.

Host measurement analyses revealed that parasite abundance was higher in larger *A. spinifera*. Interestingly, this trend was not observed in the other two host species. There could be acquired immune responses to parasitism with age in *C. serpentina* and *T. s. elegans* that are not present in *A. spinifera*.

As this study was restricted in the number of turtles collected, number of species sampled, and locations sampled, more examination is still necessary to uncover the distributions of parasites of more host species across a broader area in Texas. The titration water parameter tests used in this study relied on rough visual estimations of color change for quantification of water parameters, only offering an estimate of the actual parameter value. Similar studies using more accurate data collection methods would be valuable, as they may reveal a more accurate representation of species responses to water parameters. It would be beneficial for all collecting ventures, for museum specimens and other projects, to save internal organs for examination for parasites. In addition to this, more data should be collected and reported when sampling for parasites. Associations between parasites and the environment are largely understudied and some factors not visible in the current study may be elucidated in a future meta-analysis.

CHAPTER IV

SUMMARY AND FUTURE DIRECTIONS

In this study, a total of 78 turtles of three different species were collected across the state of Texas and examined for parasites. All turtles were infected with at least one species of parasite, with an average of four species per turtle. A total of 42 species of metazoan parasites were recovered, with acanthocephalans and nematodes typically more abundant and trematodes typically more diverse. Sixteen new host records and 17 new locality records are reported. Two new species of *Neopolystoma* are reported and *Polystomoides coronatum* and *Acanthostomum nuevoleonensis* are redescribed. When parasite communities were analyzed between host species, *A. spinifera* was found to contain a significantly different parasite assemblage than *C. serpentina* and *T. s. elegans*. Parasite abundance was higher in more turbid waters and diversity was higher in less turbid waters with lower carbon dioxide and chloride levels and higher dissolved oxygen levels. Acanthocephalans had higher abundance in more turbid waters and water with higher ammonia levels, digeneans had higher abundance in more turbid waters, and monogeneans had higher abundances in water with higher dissolved oxygen and lower carbon dioxide levels. Parasite abundance was higher in eastern parts of Texas and lentic water bodies. Acanthocephalans were primarily driving the abundance differences observed. Parasite abundance was significantly higher in large *A. spinifera*, but no correlation was seen with body size in *C. serpentina* or *T. s. elegans*. Many of the correlations discovered in this study relate to changes in the quality of freshwater

ecosystems. In water of higher quality (lower turbidity, ammonia, carbon dioxide, chloride, higher dissolved oxygen), parasite diversity will be higher, since the food web will be more dynamic, maintaining a greater diversity of parasite life cycles. In lower water quality, more resilient species become hyper-abundant due to less competition for resources within the host and possible reduced host immunity to infection.

This project began as a simple community diversity survey. Due to the paucity of molecular data on many parasites, when sample size permitted specimens were saved in 95% ethanol for analysis. Future directions of this project include analyzing molecular specimens of specific taxa (acanthocephalans, trematodes, cestodes, and monogeneans) to uncover any cryptic species and reveal gene flow between populations. Collaboration is currently ongoing with Dr. Vasyl Tkach to study the genetics of many of the platyhelminth species reported in this survey, which will likely lead to revision of some identifications. Acanthocephalans will be genetically analyzed in collaboration with Dr. Florian Reyda and Dr. Anna Phillips to reveal details in morphological differences across sexes and between populations. A thorough molecular and morphological analysis of the mites recovered in this study is being conducted by Dr. Ray Fisher in order to describe this new species. All spirorchiid blood flukes collected in this study will be analyzed both morphologically and molecularly by Charlayna Cammarata for her dissertation. Nematode, leech, and pentastomid specimens are available for any interested parties who would like to add valuable genetic information on some understudied species.

This study highlights the need for further parasite surveys, particularly in undersampled locations and understudied host taxa. Even in locations and hosts that have been well studied, regular surveys are vital to reveal changes in parasite community assemblages.

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APPENDIX

Previously reported metazoan parasites of *Apalone spinifera* (32 species), *Chelydra serpentina* (67 species), and *Trachemys scripta* (76 species). Site of infection and locality is given for each species when given in the literature.

Host	Species		Site of Infection	Localities
Apalone spinifera	Acanthocephala	*Neoechinorhynchus chrysemydis*	Small intestine	Louisiana
		Neoechinorhynchus emyditoides	Small intestine	Louisiana
		Neoechinorhynhus sp.	Not given	Louisiana
	Cestoda	*Cylindrotaenia americana*	Intestine	Oklahoma
		Testudotaenia testudo	Intestine	Louisiana, Indiana, Illinois, Minnesota, Nebraska
		Proteocephalus trionyechinum	Mouth	Oklahoma
	Monogenea	*Polystomoides coronatum*	Intestine	Louisiana, Texas, Massachusetts
	Nematoda	*Cosmocercoides dukae*	Intestine	Oklahoma
		Cucullanus emydis	Intestine, Rectum	Oklahoma
		Falcaustra chelydrae	Stomach, Intestine	Texas
		Oswaldocruzia leidyi	Stomach, Small intestine	Oklahoma
		Serpinema microcephalus	Stomach, Small intestine	Louisiana, Oklahoma, Texas
		Serpinema trispinosus	Stomach	Oklahoma, Tennessee
		Spiroxys amydae	Stomach	Mississippi, Texas
		Spiroxys constricta	Stomach, Stomach cyst	Louisiana
		Spiroxys contorta	Small intestine	Michigan, Ohio, Oklahoma, Tennessee, Texas
	Trematoda	*Acanthostomum nuevoleonenis*	Bile ducts	Mexico
		Amphimerus ovalis	Intestine	Iowa, Minnesota
		Cephalogonimus vesicaudus	Intestine	Nebraska, Oklahoma, Mississippi, Texas
		Cotylaspis cokeri	Mesentery	Oklahoma
		Haplorhynchus evaginatus	Oviduct, Intestine	Tennessee
		Teloporia aspidonectes	Stomach	Illinois, Michigan, Massachusetts, NY

Continued.

Host	Species	Site of Infection	Localities	Host
Apalone spinifera	Trematoda	*Telorchis attenuatus*	Intestine	Oklahoma, Mexico
		Telorchis corti	Intestine	Nebraska, Oklahoma
		Telorchis erectus	Vasculature	Oklahoma
		Vasotrema amydae	Vasculature	Indiana, Michigan, Nebraska
		Vasotrema attenuatum	Vasculature	Nebraska
		Vasotrema brevitestis	Arteries	Nebraska
		Vasotrema longitestis	Arteries	Oklahoma, Tennessee
		Vasotrema robustum	Small intestine	Indiana, Michigan, Nebraska, Tennessee
	Hirudinea	*Placobdella ornata*	Not given	Unknown
		Placobdella parasitica	Not given	Alabama
		Placobdella sp.	Not given	Illinois
	Arthropoda	*Levisunguis subaequalis*	Lungs	Florida, Louisiana
Chelydra serpentina	Acanthocephala	*Acanthocephalus* sp.	Not reported	Illinois
		Neoechinorhynchus chrysemydis	Small intestine	Indiana
		Neoechinorhynchus emydis	Small intestine	Oklahoma
		Neoechinorhynchus pseudemydis	Small intestine	Illinois, Tennessee
		Neoechinorhynchus sp.	Not given	Louisiana
	Monogenea	*Neopolystoma domitilae*	Bladder, Cloaca	Mexico
		Neopolystoma orbiculare	Bladder	Louisiana, Oklahoma
		Polystomoidella oblonga	Bladder	Louisiana, Maryland, Nebraska, Oklahoma, Canada, Florida, Iowa
		Polystomoidella whartoni	Bladder	Texas
		Polystomoides coronatum	Mouth	Illinois, Louisiana, Oklahoma
	Nematoda	*Aplectana* sp.	Lower intestine	Louisiana, Ohio
		Atractis carolinae	Rectum	Texas
		Capillaria serpentina	Intestine, Rectum	Oklahoma, Texas

Continued.

Host	Species	Site of Infection	Localities	Host
Chelydra serpentina	Nematoda	*Cruzia testudinis*	Rectum	Texas
		Dracunculus globocephalus	Body cavity, Mesentery, Pelvic fasciae	Costa Rica, Illinois, Ohio, Oklahoma, Tennessee
		Eustrongylides sp.	Rectum epithelium	Ohio
		Falcaustra affinis	Intestine, Rectum	Texas, Wisconsin
		Falcaustra chelydrae	Intestine, Rectum	Costa Rica, Illinois, NY, Oklahoma, Tennessee, Texas
		Falcaustra sp.	Not given	Illinois
		Falcaustra wardi	Intestine	Oklahoma
		Foleyella sp.	Peritoneum	Ohio
		Icosiella quadrituberculata	Stomach cysts	Georgia
		Klossinemella caballeroi	Large intestine	Costa Rica
		Serpinema microcephalus	Stomach, Small intestine	Illinois, Iowa, Louisiana, NC, NY, Ohio, Oklahoma, Tennessee, Texas, Wisconsin
		Serpinema trispinosus	Stomach, Small intestine	Texas, Oklahoma, Illinois, Tennessee
		Spiroxys constricta	Stomach	Wisconsin
		Spiroxys contorta	Stomach	Illinois, Ohio, Oklahoma
		Spiroxys sp.	Stomach	Iowa
	Trematoda	*Allassostoma magnum*	Intestine, Cloaca	USA
		Allassostomoides chelydrae	Rectum	Louisiana, Nebraska
		Allassostomoides parvus	Intestine, Cloaca	Florida, Illinois, Maryland, Nebraska, Ohio, Oklahoma, Wisconsin, Canada
		Amphimerus ovalis	Bile ducts	North America
		Amphimerus sp.	Intestine, Bladder	Kentucky
		Auridistomum chelydrae	Intestine	Florida, Nebraska, Ohio, Oklahoma, Wisconsin, Canada
		Auridistomum georgiense	Esophagus	Georgia
		Cercaria ramonae	Intestine	Experimental

Continued.

Host	Species	Site of Infection	Localities	Host
Chelydra serpentina	Trematoda	*Cotylaspis stunkardi*	Digestive tract	NC
		Crepidostomum cooperi	Large intestine, Cloaca	Oklahoma
		Dictyangium chelydrae	Small intestine	Louisiana, Oklahoma
		Diplostomulum scheuringi	Small intestine	Virginia
		Eustomus chelydrae	Circulatory system	Michigan, NY, Wisconsin
		Haplorhynchus foliorchis	Arteries	Nebraska
		Haplorhynchus gracilis	Vasculature of lungs	Indiana, Wisconsin
		Haplorhynchus stunkardi	Lungs	Nebraska
		Heronimus mollis	Intestine	Illinois, Indiana, Iowa, Louisiana, Minnesota, Nebraska, NC, Ohio, Oklahoma, Texas, Canada
		Herpetodiplostomum delillei	Not given	Mexico
		Learedius sp.	Intestine	Tennessee
		Macravestibulum eversum	Intestine	Experimental (did not reach maturity)
		Microphallus opacus	Intestine	Ohio
		Microphallus ovatus	Heart	Ohio
		Neascus sp.	Heart	Oklahoma
		Spirorchis haematobium	Mesentery	Mississippi, Nebraska, Indiana, Iowa, Louisiana, Maryland, NC, NJ, NY, Ohio, Oklahoma, Tennessee, Texas, Wisconsin, Mississippi
		Spirorchis magnitestis	Stomach, Intestine	Illinois, Tennessee
		Spirorchis minutum	Stomach	Tennessee
		Telorchis aculeatus	Not given	Oklahoma
		Telorchis attenuatus	Stomach	Ohio, Oklahoma, Illinois, Mexico
		Telorchisbonnerensis	Intestine	Experimental
		Telorchis caudatus	Intestine	NC
		Telorchis clava	Small intestine	Oklahoma

Continued.

Host	Species	Site of Infection	Localities	Host
Chelydra serpentina	Trematoda	*Telorchis corti*	Small intestine	Illinois, Iowa, Louisiana, Nebraska, Ohio, Oklahoma, Wisconsin, Mexico
		Telorchis singularis	Small intestine	Louisiana
		Telorchis sp.	Not given	Iowa, Wisconsin
	Hirudinea	*Actinobdella annectens*	Not given	Ontario
		Desserobdella picta	Not given	Wisconsin
		Placobdella ali	Not given	Connecticut
		Placobdella hollensis	Not given	Minnesota
		Placobdella parasitica	Not given	Alabama, Illinois, North Carolina, Ohio, Ontario
		Placobdella multilineata	Not given	Illinois, Arkansas, Oklahoma
		Placobdella ornata	Not given	Illinois, Ontario
		Placobdella papillifera	Not given	Illinois
		Placobdella rugosa	Not given	Unknown
	Arthropoda	*Cloacarus faini*	Cloaca	Kansas
Trachemys scripta	Acanthocephala	*Leptorhynchoides* sp.	Not given	Iowa
		Neoechinorhynchus chelonos	Small intestine	SC
		Neoechinorhynchus chrysemydis	Small intestine	Alabama, Arkansas, Louisiana, NC, SC, Tennessee, Louisiana, Texas
		Neoechinorhynchus emydis	Intestine	Illinois, Oklahoma, Texas
		Neoechinorhynchus emyditoides	Small intestine	Alabama, Arkansas, Illinois, Louisiana, Mississippi, NC, SC, Texas, Virginia, Mexico
		Neoechinorhynchus magnapapillatus	Intestine	Alabama, NC
		Neoechinorhynchus moleri	Small intestine	Florida
		Neoechinorhynchus pseudemydis	Small intestine	Alabama, Arkansas, Illinois, Indiana, Louisiana, Mississippi, Missouri, NC, SC, Tennessee, Texas, Virginia
		Neoechinorhynchus schmidti	Small intestine	Mexico

Continued.

Host	Species	Site of Infection	Localities	Host
Trachemys scripta	Acanthocephala	*Neoechinorhynchus stunkardi*	Intestine	Arkansas, Illinois
		Neoechinorhynchus sp.	Not given	Alabama, Illinois, Louisiana, SC
	Cestoda	Cyclophyllidean cysticerci	Not given	NC
		Proteocephalan plerocercoid	Not given	Louisiana
		Testudotaenia testudo	Intestine	Oklahoma
	Monogenea	*Neopolystoma domitilae*	Bladder, Cloaca	Mexico
		Neopolystoma orbiculare	Bladder	Arkansas, Illinois, Louisiana, NC, Oklahoma, Tennessee, Texas, Mexico
		Polystomoidella oblonga	Bladder	Louisiana
		Polystomoidella sp.	Bladder	Louisiana
		Polystomoides coronatum	Mouth	Illinois, Louisiana, NC, Oklahoma, Texas, Mexico
		Polystomoides scriptanus	Mouth	Florida, North Carolina
		Polystomoides soredensis	Mouth	Indiana, Maine, North Carolina
		Polystomoides sp.	Not given	Iowa
	Nematoda	*Aplectana* sp.	Intestine	Louisiana
		Cissophyllus penitus	Intestine	North America
		Cucullanus cirratus	Intestine	Oklahoma
		Dracunculus globocephalus	Mesentery	USA
		Dracunculus sp.	Mesentery	Louisiana
		Falcaustra affinis	Intestine, Rectum	Illinois, Mexico, Arkansas, Texas
		Falcaustra chelydrae	Intestine, Rectum	SC, Tennessee
		Falcaustra concinnae	Intestine, Rectum	Texas
		Falcaustra gracile	Stomach	North America
		Falcaustra procera	Intestine, Rectum	Oklahoma, Texas
		Falcaustra sp.	Not given	Illinois
		Falcaustra tricirratus	Not given	Arkansas, Oklahoma, Texas

Continued.

Host	Species	Site of Infection	Localities	Host
Trachemys scripta	Nematoda	*Gnathostoma procyonis*	Muscle cyst	Louisiana
		Icosiella quadrituberculatus	Stomach cyst	Georgia
		Oxyuroidea sp.	Not given	Alabama
		Serpinema microcephalus	Stomach, Small intestine	Florida, Illinois, Louisiana, Oklahoma, SC, Tennessee, Texas
		Serpinema sp.	Not given	Alabama, Iowa, SC
		Serpinema trispinosus	Stomach, Small intestine	Arkansas, Mexico, Oklahoma, Tennessee, Texas, Wisconsin
		Spiroxys constricta	Stomach	Louisiana
		Spiroxys contorta	Stomach	Arkansas, Illinois, Louisiana, Oklahoma, Tennessee, Texas, Mexico
		Spiroxys sp.	Not given	Alabama, Iowa, SC
	Trematoda	*Allassostoma magnum*	Intestine, Cloaca	Illinois, Louisiana, Oklahoma
		Caballerodiscus resupinatus	Large intestine	Mexico
		Caballerodiscus tabascensis	Large intestine, Cloaca	Mexico, Panama
		Cephalogonimus vesicaudus	Intestine	Oklahoma
		Cotylaspis sp.	Small intestine	Louisiana
		Dictyangium chelydrae	Large intestine, Cloaca	Mexico, Arkansas
		Henotosoma haematobium	Heart	Tennessee
		Heronimus mollis	Lungs	Arkansas, Texas, Canada, Tennessee, Illinois, Indiana, Louisiana
		Macravestibulum eversum	Intestine	Experimental
		Macravestibulum kepneri	Small intestine	Texas
		Macravestibulum obstusicaudatum	Intestine	Oklahoma, SC, Tennessee
		Macravestibulum sp.	Intestine	SC
		Pneumatophilus variabilis	Lungs	Louisiana
		Protenes angustus	Intestine	Louisiana, Texas
		Spirorchis artericola	Heart	Illinois, Louisiana, NC, Oklahoma, Tennessee, Texas
		Spirorchis blandingioides	Mesentery	Tennessee

Continued.

Host	Species	Site of Infection	Localities	Host
Trachemys scripta	Trematoda	*Spirorchis elegans*	Submucosa of esophagus	Illinois, NC, Oklahoma, Mississippi
		Spirorchis parvum	Heart	Arkansas
		Spirorchis pseudemydae	Mesentery	Tennessee
		Spirorchis scripta	Heart, Arteries	NC, Tennessee
		Spirorchis sp.	Submucosa of esophagus	Illinois
		Telorchis attenuatus	Stomach	Arkansas, Oklahoma, Louisiana, Texas, Mexico
		Telorchis bonnerensis	Intestine	Experimental
		Telorchis chelopi	Not given	New York Aquarium
		Telorchis corti	Small intestine	Arkansas, Illinois, Iowa, Louisiana, Mexico, Oklahoma, Panama, SC, Texas
		Telorchis dissimilis	Stomach, Intestine	Arkansas, Mexico
		Telorchis membranaceus	Intestine	Mexico
		Telorchis nematoides	Intestine	Wisconsin
		Telorchis robustus	Small intestine	Louisiana, Texas, Tennessee
		Telorchis scabrae	Not given	New York Aquarium
		Telorchis singularis	Small intestine	Arkansas, Louisiana, Oklahoma, Texas
		Telorchis sp.	Not given	Iowa, SC
		Unicaecum dissimile	Blood vessels	Tennessee
		Unicaecum ruszkowskii	Mesentery, Small intestine	Mississippi, NC, Tennessee
	Hirudinea	*Helobdella octatestisaca*	Not given	Texas
		Placobdella parasitica	Not given	Texas, Alabama
		Placobdella ali	Not given	Connecticut
		Placobdella ornata	Not given	Unknown
		Placobdella rugosa	Not given	Texas
		Placobdella multilineata	Not given	Illinois

Continued.

Host	Species	Site of Infection	Localities	Host
Trachemys scripta	Arthropoda	*Amblyomma dissimile*	Not given	Panama
		Amblyomma sabanerae	Not given	Panama
		Caminacarus chrysemys	Cloaca	Louisiana
		Cistudinomyia cistudinis	**--**	**Chrysemys picta in Knipling 1937 cited as Chrysemys scripta in Mitchell, 2007**

Significance coefficients for the results of one-way ANOVAS and Tukey's post-hoc test. Significance was considered at the 5% level, with Bonferroni's correction ($p < 0.0056$). Significant correlations are bolded.

	Parasite Abundance		Parasite Diversity (Richness)		Parasite Diversity (Taxonomic)		Parasite Abundance (TSE only)				
	Total	TSE only	Total	TSE only	Total	TSE only	Acanthocephalans	Digeneans	Leeches	Monogeneans	Nematodes
Lentic/Lotic	**0.00046**	**0.00039**	0.32	0.72	0.88	0.092	**0.00065**	0.41	0.27	0.19	0.084
Sex	0.11	0.072	0.11	0.29	0.13	0.61	0.26	0.19	0.91	0.58	0.092
Aquatic Veg	0.13	0.41	0.05	0.16	0.016	0.057	0.1	0.6	0.028	0.91	0.6
Season	0.53	0.61	0.062	0.057	0.403	0.061	0.83	0.065	0.089	0.18	0.03
Spring-Fall	0.51	0.57	0.05	0.11	0.38	0.33	1	0.04	0.98	0.47	0.098
Summer-Fall	0.99	0.99	0.14	0.079	0.51	0.047	1	0.47	0.18	0.24	1
Winter-Fall	0.98	1	0.42	0.54	0.73	0.79	0.83	0.79	0.98	0.25	0.73
Summer-Spring	0.59	0.65	0.81	0.92	0.96	1	1	0.27	0.7	1	0.058
Winter-Spring	0.8	0.68	0.8	0.71	0.97	0.84	0.87	0.28	0.89	1	0.02
Winter-Summer	1	1	1	0.9	1	0.56	0.87	0.99	0.14	0.98	0.74
Latitude	0.83	0.14	0.95	0.41	0.54	0.53	0.12	0.12	0.41	0.29	0.25
North-Central	0.87	0.14	0.98	0.42	0.51	0.56	0.64	0.36	0.41	0.39	0.87
South-Central	0.92	0.58	0.95	0.75	0.99	0.97	0.34	0.61	1	0.4	0.4
South-North	0.99	0.97	0.98	1	0.78	0.68	0.11	0.15	0.73	0.88	0.22
Longitude	**0.00028**	0.0087	0.037	0.32	0.035	0.029	0.28	0.49	0.14	0.76	0.39
East-Central	0.44	0.55	0.14	0.34	0.61	0.76	0.48	0.65	0.16	0.75	0.9
West-Central	**0.0056**	0.045	0.7	1	0.91	0.023	0.38	0.82	0.95	1	0.36
West-East	**0.00016**	0.006	0.069	0.62	0.97	0.057	0.84	0.51	0.35	0.91	0.56
Ecoregion	0.009	0.022	0.27	0.62	0.88	0.63	0.03	0.19	0.4	0.097	0.08
CT-CD	0.77	1	0.94	1	0.92	0.98	1	1	1	0.68	1
ECTP-CD	0.016	0.51	0.3	0.99	0.88	0.91	0.92	0.72	0.99	0.84	0.99
EP-CD	0.76	1	0.91	1	0.96	0.99	1	1	1	0.21	0.98
SCP-CD	0.93	1	1	1	0.82	1	1	1	0.99	0.35	1

Continued.

	Parasite Abundance		Parasite Diversity (Richness)		Parasite Diversity (Taxonomic)		Parasite Abundance (TSE only)				
	Total	TSE only	Total	TSE only	Total	TSE only	Acanthocephalans	Digeneans	Leeches	Monogeneans	Nematodes
TBP-CD	0.86	0.89	1	1	1	0.55	0.91	1	1	0.98	0.47
WGCP-CD	1	1	0.83	1	1	0.96	0.75	0.56	0.98	1	0.92
ECTP-CT	0.52	0.14	0.94	0.84	1	1	0.32	0.61	0.92	0.99	1
EP-CT	1	0.97	1	0.99	1	1	0.99	1	1	0.98	1
SCP-CT	1	1	1	1	1	1	1	1	0.92	1	1
TBP-CT	1	0.79	0.99	1	1	0.83	0.71	1	0.99	1	0.21
WGCP-CT	0.99	1	1	0.99	1	1	0.92	0.51	0.99	0.74	0.53
EP-ECTP	0.45	0.39	0.96	0.99	1	1	0.61	0.65	0.67	0.39	1
SCP-ECTP	0.31	0.11	0.69	0.65	1	0.97	0.47	0.79	1	0.72	1
TBP-ECTP	0.97	1	0.81	0.95	1	0.82	1	0.92	1	1	0.14
WGCP-ECTP	0.49	0.26	1	1	1	1	0.048	0.98	0.61	0.9	0.31
SCP-EP	1	0.99	1	0.98	1	1	1	1	0.75	1	1
TBP-EP	1	0.95	0.99	0.99	1	0.72	0.88	1	0.98	0.99	0.14
WGCP-EP	0.99	0.97	1	1	1	1	0.52	0.59	1	0.26	0.33
TBP-SCP	1	0.85	1	1	0.99	0.64	0.83	1	1	1	0.2
WGCP-SCP	1	1	0.97	0.97	1	0.99	0.66	0.67	0.64	0.42	0.49
WGCP-TBP	0.99	0.78	0.95	0.98	1	0.92	0.32	0.79	0.91	0.99	0.91
River Basin	**0.00055**	**0.0013**	0.46	0.84	0.48	0.077	0.0059	0.57	**0.0045**	0.32	0.021
CB-BB	1	1	0.94	0.98	1	0.87	0.98	0.99	0.99	1	0.09
NB-BB	0.021	**0.0053**	1	1	0.53	0.12	0.13	0.93	1	1	0.32
RGB-BB	0.034	0.7	0.52	1	0.96	0.83	0.99	0.93	1	0.74	0.96
SANB-BB	0.68	0.39	1	1	1	1	0.095	0.95	0.95	0.81	0.17
SB-BB	0.2	0.058	0.99	0.97	0.98	0.77	0.36	0.99	0.9	1	1

Continued.

	Parasite Abundance		Parasite Diversity (Richness)		Parasite Diversity (Taxonomic)		Parasite Abundance (TSE only)				
	Total	TSE only	Total	TSE only	Total	TSE only	Acanthocephalans	Digeneans	Leeches	Monogeneans	Nematodes
SJB-BB	1	1	0.93	0.91	1	1	0.99	1	0.95	0.77	1
TB-BB	0.96	1	1	1	1	0.94	0.98	1	0.0012	1	1
NB-CB	0.47	0.21	1	1	0.67	0.16	0.34	1	1	1	0.86
RGB-CB	0.87	0.89	1	1	1	0.52	0.93	1	1	1	0.48
SANB-CB	0.99	0.77	0.97	0.99	1	0.92	0.31	0.87	0.89	1	0.93
SB-CB	0.97	0.65	1	1	0.98	0.54	0.64	1	1	1	0.24
SJB-CB	0.99	1	1	1	1	0.93	1	1	1	0.96	0.25
TB-CB	0.93	1	0.95	1	1	0.63	1	1	0.86	1	0.26
RGB-NB	0.96	0.67	1	1	0.31	0.97	0.81	1	1	0.8	0.98
SANB-NB	0.91	0.87	1	1	0.78	0.45	1	0.68	0.98	0.84	1
SB-NB	0.88	0.83	1	1	0.96	0.84	0.98	1	1	1	0.81
SJB-NB	0.15	0.1	1	1	0.88	0.62	0.21	1	0.99	0.98	0.79
TB-NB	0.017	0.032	1	1	0.93	0.83	0.1	1	0.073	1	0.83
SANB-RGB	1	1	0.89	1	1	0.96	0.76	0.68	0.98	1	0.94
SB-RGB	1	1	0.99	1	0.8	1	0.99	1	1	0.74	1
SJB-RGB	0.41	0.88	1	1	1	0.99	0.92	1	0.99	0.3	1
TB-RGB	0.052	0.8	0.78	1	0.92	1	0.9	1	0.072	0.81	1
SB-SANB	1	1	1	1	1	0.98	0.96	0.84	0.65	0.8	0.63
SJB-SANB	0.79	0.71	0.96	0.97	1	1	0.18	0.9	0.73	0.35	0.65
TB-SANB	0.44	0.56	1	1	1	0.99	0.083	0.92	**0.0051**	0.86	0.67
SJB-SB	0.61	0.52	1	1	1	0.99	0.5	1	1	0.92	1
TB-SB	0.16	0.28	0.99	1	1	1	0.3	1	0.11	1	1
TB-SJB	1	1	0.94	0.99	1	1	1	1	0.6	0.94	1

Continued.

	Parasite Abundance		Parasite Diversity (Richness)		Parasite Diversity (Taxonomic)		Parasite Abundance (TSE only)				
	Total	TSE only	Total	TSE only	Total	TSE only	Acanthocephalans	Digeneans	Leeches	Monogeneans	Nematodes
Sediment	0.039	0.13	0.38	0.78	0.33	0.2	0.55	0.12	0.034	0.04	0.67
Gravel-Clay	0.99	0.91	0.95	0.92	0.98	0.99	0.98	0.27	0.61	0.1	0.81
Loam-Clay	0.45	1	0.41	1	0.36	1	1	0.5	1	0.79	1
Sand-Clay	1	0.98	0.98	0.88	1	1	1	0.53	0.61	0.5	1
Silt-Clay	0.71	0.62	1	1	0.81	0.36	0.86	1	0.69	0.54	0.82
Loam-Gravel	0.58	1	0.68	1	0.54	0.98	0.96	0.98	0.94	0.098	0.93
Sand-Gravel	0.99	1	1	1	0.98	0.97	0.97	1	0.034	0.93	0.89
Silt-Gravel	0.25	0.1	0.92	0.89	0.98	0.53	0.44	0.31	1	0.79	1
Sand-Loam	0.41	1	0.69	1	0.34	1	1	0.96	0.91	0.26	1
Silt-Loam	0.038	0.84	0.31	1	0.75	0.67	1	0.57	0.96	0.28	0.94
Silt-Sand	0.71	0.25	0.97	0.83	0.79	0.25	0.89	0.62	0.046	1	0.9

Full table of significance coefficients for host measurement analyses in relation to parasite abundance. Significant correlations are bolded.

	Parasite Abundance					
	AS only		CS only		TSE only	
	p-value	R2	p-value	R2	p-value	R2
Weight	**0.0351**	**0.3**	0.81	0.0087	0.45	0.012
SCL	**0.017**	**0.36**	0.76	0.014	0.5	0.0093
SCW	**0.016**	**0.37**	0.82	0.0082	0.2	0.034
Depth	0.39	0.084	0.38	0.16	0.1	0.057
CCL	**0.016**	**0.37**	0.64	0.034	0.18	0.037
CCW	**0.016**	**0.37**	0.77	0.013	0.091	0.057
Circumference	0.089	0.29	0.87	0.0049	0.17	0.039

Full table of significance coefficients for host measurement analyses in relation to parasite species richness. Significant correlations are bolded.

	Parasite Diversity (Richness)					
	AS only		CS only		TSE only	
	p-value	R2	p-value	R2	p-value	R2
Weight	0.33	0.074	0.6	0.042	0.92	0.00021
SCL	0.22	0.11	0.82	0.0076	0.66	0.0041
SCW	0.15	0.15	0.94	0.00086	0.54	0.0076
Depth	0.95	0.00043	0.78	0.018	0.47	0.011
CCL	0.21	0.12	0.75	0.015	0.51	0.0091
CCW	0.17	0.14	0.2	0.22	0.5	0.0091
Circumference	0.45	0.066	0.39	0.13	0.5	0.0096

Full table of significance coefficients for host measurement analyses in relation to parasite taxonomic diversity. Significant correlations are bolded.

	Parasite Diversity (Taxonomic)					
	AS only		CS only		TSE only	
	p-value	R2	p-value	R2	p-value	R2
Weight	0.55	0.028	0.83	0.007	0.97	3.40E-05
SCL	0.74	0.009	0.85	0.0056	0.81	0.0012
SCW	0.62	0.019	0.62	0.037	0.78	0.0016
Depth	0.93	0.00099	0.46	11	0.99	6.97E-06
CCL	0.73	0.0092	0.99	5.19E-05	0.89	3.70E-04
CCW	0.72	0.01	0.44	0.088	0.78	1.60E-03
Circumference	0.8	0.0077	0.49	0.081	0.81	1.20E-03