PARASITE COMMUNITY STRUCTURE IN 5 SPECIES OF DAMSELFLIES (ODONATA: ZYGOPTERA) FROM TEAL RIDGE, STILLWATER OKLAHOMA.

By

CRYSTAL MARIE WILES

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Thesis Approved:

Dr. Matthew Bolek

Thesis Adviser

Dr. Monica Papes

Dr. Mason Reichard

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Abstract: Few ecological studies exist on parasite community structure in insects and compared to other invertebrate and vertebrate groups, insects have been largely ignored in studies on parasite community structure. This is surprising because some insects, such as odonates, have become model systems for studies on host parasite interactions, and there is a desperate need for descriptive studies on their parasite community structure. In this study I examined 530 individual damselflies of five species (*Argia apicalis*, *Enallagma civile*, *Ischnura hastata*, *Ischnura verticalis*, and *Lestes disjunctus australis*) for their parasites and report parasite community structure parameters for these hosts. All damselflies were collected from Teal Ridge a semi-permanent wetland located in Stillwater, Oklahoma during the summer and fall of 2010–2012. I report the first record of juvenile *Serpinema* cf. *trispinosum* nematodes, along with new host records and geographical distribution information for gregarine parasites from Oklahoma damselflies. The parasite compound community of this odonate assemblage consisted of a total of 549 individual parasites, comprised of seven taxa including; five species of gregarines, two species of helminths, and one species of mite. None of the individual parasite species were host specific to a single damselfly species and all parasite species infected at least two species of damselflies. Average parasite species richness was low among the four species of damselflies ranging from a low of 0.2 $\pm$ 0.4 (0–2) for *I. hastata* to a high of 0.3 $\pm$ 0.6 (0–2) for *E. civile*. There was no relationship in damselfly size and parasite abundance, intensity, or species richness among any of the damselfly species examined. This study indicates that the parasite community structure of damselflies was most similar to Mariluan, 2012 study on the parasite communities of benthic aquatic insects and drastically differed in terms of standard measures of parasite community structure in vertebrate hosts which are much higher in terms of parasite prevalence, mean abundance, mean intensity and species richness.

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CHAPTER I

OVERVIEW

Ecological studies have concentrated on free-living animals with little attention placed on organisms such as parasites. As stated by Price (1980, 1984), "Small, highly specialized organisms are different in many aspects of their biology from larger, more generalized animals". Communities of specialists such as parasites may be organized in different ways from generalized animal communities. The complexity of parasitic life cycles and recruitment strategies make them very different from free-living organisms. Due to these differences, populations and communities of parasites can be studied at a number of hierarchical levels of organization (Bush et al., 2001). These have been defined as follows: 1) infrapopulation and infracommunity - a population or community of parasites in an individual host; 2) component population and component community - all the infrapopulations and infracommunities of parasites in a population of hosts in a given habitat; 3) suprapopulation and compound community - all the free living parasite stages of a given parasite species (population) and all the parasite species stages (community) in the environment, and all the parasite species in all stages of development, within all hosts in an ecosystem. Despite these differences in structure and complexity parasites have been shown to possess certain unique qualifications that permit them to contribute significantly to certain concepts in community ecology. First, communities

have unambiguous boundaries, the host. Second, since each host represents a separate and distinct habitat for a population or community of parasites, parasite communities may be replicated easily, resulting in the potential for a useful comparative approach (Aho, 1990). It is because of this that it can be argued that parasitologists deal with the most clearly structured habitats of any ecological system (Price, 1990).

Parasitic community structure can vary from isolationist to interactive and anywhere in between (Esch et al., 1990; Bush et al., 2001). Isolationist communities are depauperate and are structured by random events, while interactive communities are species rich and the role of interactions and competition are important in structuring these communities (Esch et al., 1990). Studies on freshwater and marine fish (Kennedy et al., 1986; and Muzzall and Bowen, 2002; Holmes, 1990), amphibians (Bolek and Coggins, 1998; 2000; 2001; 2003), reptiles (Aho, 1990; Pérez-Ponce De León et al., 2001; Jiménez-Ruiz et al., 2002), birds (Bush and Holmes, 1986; Bush, 1990; Glass et al., 2002), and mammals (Van den Bussche et al., 1987; Montgomery, 1988; and Bordes and Morand, 2008) have helped develop predictions which can be important in determining parasite community structure in vertebrate hosts. The following factors have been identified by these investigators as important in producing diverse parasite communities: 1) The complexity of the hosts alimentary canal and/or its physiology (ectothermy/endothermy); 2) Host vagility; 3) Host diet breadth; 4) Selective feeding by a host on prey which serve as intermediate hosts for a wide variety of parasites; 5) Exposure of a host to direct life-cycle parasites which enter by penetration; and 6) Host size and/or age.

Although descriptive analyses of parasite community structure are now available for a wide variety of vertebrate hosts, few such studies have been conducted on invertebrates and most of these are on molluscs and crustaceans (Kuris, 1990; Bush et al., 1993; Yoder and Coggins, 1998). However, important features distinguish invertebrates from vertebrates as hosts for parasites. First invertebrates are usually smaller than vertebrates, second the life span of most invertebrates is much shorter than vertebrate hosts, and third many invertebrates, particularly molluscs and arthropods, serve as intermediate hosts for parasites that complete their development in a vertebrate host (Kuris, 1990; Bush et al., 1993). Among the few studies on arthropod parasite community structure, most have concentrated on marine arthropods such as crustacean that predominantly serve as intermediate hosts for parasites of vertebrates and terrestrial insects that serve as hosts for parasitoids (Kuris, 1973; 1974; Price, 1973; Askew and Shaw, 1986; Bush et al., 1993). More recently, Mariluan et al. (2012) examined a community of benthic insects (plecoptera, ephemeroptera, trichoptera and diptera) from Patagonia and reported community parameters for trematode and nematode infections in these hosts. However, what is needed now are studies on a broader range of insects that serve as hosts for a wide variety of endo-parasites and ecto-parasites that utilize arthropods not only as intermediate hosts but also as definitive hosts. One such group of insects is the odonata (Percival et al., 1995; Clopton, 1995; Clopton, 2004; Clopton et al., 2010; Hays, et al., 2007; Lajeunesse, 2007; Bolek and Janovy, 2007a; 2007b; Bolek et al., 2009; 2010; Wiles and Reyda, 2011a; 2011b).

Within the order Odonata the suborder Zygoptera (damselflies) are carnivorous aquatic based insects that spend part of their life cycle in the water and the other part of

their life cycle in a terrestrial environment (Corbet, 1999). It is their carnivorous nature and distribution in aquatic and terrestrial habitats that allow damselflies to serve as hosts for a wide range of endo and ecto-parasites. Surveys of damselflies indicate that they serve as hosts for a wide variety of protozoan, platyhelminth, nematode, nematomorph, mite and dipteran parasites (Corbet, 1999). However, most surveys of damselfly parasites have concentrated on specific parasite species descriptions such as gregarine protozoa (Cielocha et al., 2011), or in life cycle studies of digentic trematodes of vertebrate hosts (Bolek et al., 2009; 2010). Few studies have examined multiple species of damselflies from a single location for their parasite assemblages (Lajeunesse, 2007; Bolek et al., 2010; Cielocha et al., 2011; Wiles and Reyda, 2011a; 2011b). Additionally, all of these studies have concentrated on a single group of parasites such as gregarines (Cielocha et al., 2011; Wiles and Reyda, 2011a; 2011b), digenean trematodes (Bolek and Janovy, 2007a; 2007b; Bolek et al., 2009; 2010) and mites (Lejeunesse, 2007). A recent review by Baker (2011) summarized all the recent publications on odonate parasite life cycles, and the ecology and behavior of odonate parasite interactions. However, to my knowledge no parasite community studies exist on damselfly parasites.

Oklahoma has a rich damselfly fauna including three families, 10 genera and 52 species of zygopterans, which have varied habitats, flying seasons and foraging strategies (Abbott, 2005; 2011; Westfall and May, 2006; May and Dunkle, 2007), making it an excellent location for comparative studies on parasite community structure of damselflies. Previous surveys of multiple species of damselflies for gregarines, digeneans and mites indicate that some parasite species appear to be specialists where as others are generalists (Lejeunesse, 2007; Bolek et al., 2009; 2010; Cielocha et al., 2011).

These studies suggest that distinct life histories of damselflies and their parasites probably play a major role in parasite distribution in their zygopteran hosts. By examining numerous species of damselfly across two common families in Oklahoma for their parasite communities, the present comparative study will address the following questions: 1) What species of parasites infect a community of damselflies? 2) Are individual damselfly species dominated by specialist, generalist, direct or indirect life cycle parasites; ecto or endo-parasites. 3) Does damselfly species, sex, size and age (teneral or adult) determine parasite species and/or community distribution at the infra and component community level?

The present thesis is divided into three chapters including:

(1) Damselflies as paratenic hosts for *Serpinema* cf. *trispinosum* and re-description of adult *Serpinema trispinosum* from turtle definitive hosts.

(2) Gregarine diversity from four species of damselflies form Oklahoma with descriptions of their oocysts of and re-description of oocysts of *Hoplorhynchus acanthatholius.*

(3) Parasite community structure in four species of damselflies from Oklahoma.

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CHAPTER II

DAMSELFLIES AS PARATENIC HOSTS FOR *SERPINEMA* CF. *TRISPINOSUM* AND RE-DESCRIPTION OF JUVENILE AND ADULT *SERPINEMA TRISPINOSUM* FROM DAMSELFLY AND TURTLE HOSTS.

ABSTRACT: Third-stage juveniles of the nematode *Serpinema* cf. *trispinosum* (Leidy, 1852) were collected from the mid-gut of four species of adult damselflies from Teal Ridge, a non-irrigated restored semi-permanent wetland located in Stillwater, Oklahoma. This is the first record of juveniles of *Serpinema* cf. *trispinosum* from damselflies. *Serpinema trispinosum* adults have been reported from 18 species of North and Central American freshwater turtles, whereas microcrustaceans such as copepods serve as intermediate hosts in this nematode's life cycle. My review of the literature indicates that this nematode has also been reported from a single species of aquatic snail in Canada, a single species of cichlid fish from Mexico and five species of amphibians from various locations in North, Central and South America suggesting that a wide range of invertebrates and vertebrates may serve as paratenic hosts in the life cycle of this nematode. Dietary studies of the 18 species of freshwater turtles reported as definitive hosts for *S. trispinosum* indicate that aquatic insects including damselflies are more commonly reported in turtle diet

studies than are fish or amphibians. Since larval damselflies predominantly feed on microcrustaceans my discovery of *S.* cf. *trispinosum* in damselflies may reflect the importance of damselflies as paratenic hosts of turtle parasites in this genus. In this study, I provide new host and geographical distribution information for juvenile and adult *S. trispinosum* from damselfly and turtle hosts from Oklahoma along with new morphological measurements for juvenile and adult male and female *S. trispinosum*.

INTRODUCTION

Although damselflies are commonly surveyed for parasites few studies report nematodes from these hosts (Willis, 1971; Corbet, 1999; Baker, 2011). As a result, we know very little about the host specificity and distribution of nematodes in odonates (see Corbet, 1999; Baker, 2011). One such species is *Serpinema trispinosum* (Leidy, 1852) (Camallanidae) an intestinal nematode that infects New World turtles as definitive hosts (Baker, 1979; Moravec and Vargas-Vazques, 1998a). Although the entire life cycle of *S. trispinosum* has not been elucidated, evidence suggests that the life cycle of *S. trispinosum* resembles that of other Camallanid nematodes which infect microcrustacean first intermediate hosts, numerous invertebrate and vertebrate paratenic hosts and vertebrate definitive hosts (Baker, 1979; Hoffman, 1999; Moravec and Vargas-Vazques, 1998a). The only published information on a partial life cycle of *S. trispinosum* was provided by Moravec and Vargas-Vazquez (1998a). These investigators infected laboratory-reared copepods *Macrocyclops albidus* with first-stage juveniles of *S. trispinosum* recovered from red eared slider turtles *Trachemys scripta elegans* and found that the *S. trispinosum*

developed to second-stage juveniles in *M. albidus* (Moravec and Vargas-Vazquez, 1998a). Additionally, Moravec and Vargas-Vazques (1998b) and others (Bartlett and Anderson, 1985; Cabrera-Guzman et al., 2007; Cabrera-Guzman et al., 2010; Gonzalex and Hamann, 2007) recovered third stage juvenile nematodes that conformed to the description of *S. trispinosum* from a variety of naturally infected paratenic hosts. These paratenic hosts included the Mayan cichlid (*Cichlasoma urophthalmus*) in Mexico, five species of anurans (*Lysapsus limellum, Rana catesbeiana, R. clamitans, R. forreri, R. pipiens*) from various locations in North, Central and South America and the great pond snail (*Lymnaea stagnalis*) from Canada. These authors hypothesized that paratenic hosts became infected with this nematode when they ingested infected microcrustaceans, whereas turtle definitive hosts become infected with *S. trispinosum* when they ingest infected paratenic hosts with *S. trispinosum*.

While *S.* cf. *trispinosum* juveniles have been reported from various groups of invertebrates and vertebrate paratenic hosts, until now no reports existed of these nematodes from damselfly hosts (Moravec and Vargas-Vazquez, 1998b; Gonzalex and Hamann, 2007). This observation is an important one because both laboratory and field surveys indicate that microcrustaceans are an important component of larval damselfly diets (Corbet, 1999) more so than in the diets of aquatic snails, amphibians or some fish (Baker, 1928; Becker, 1983; Bolek et al., 2010). Therefore, my observation of *S.* cf. *trispinosum* in damselflies may reflect the importance of damselflies as paratenic hosts of turtle parasites in this genus. In this study, I provide new host and geographical distribution information for juvenile and adult *S.*

trispinosum from damselfly and turtle hosts from Oklahoma along with new morphological measurements for juvenile and adult male and female *S. trispinosum*. Additionally, I review the literature on the diet of the 18 species of turtles reported as definitive hosts of *S. trispinosum* (Baker, 1987) and I argue that damselflies may be a more important paratenic host than are freshwater gastropods, amphibians or fish in the life cycle of this nematode.

MATERIALS AND METHODS

Description of damselfly collection and necropsy

During September 2010–September 2012 a total of 530 teneral and adult damselflies of five species representing two families were collected from Teal Ridge Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405; Table I). All damselflies were collected between 9:00 AM and 6:00 PM with an aerial net, placed in a 1 liter plastic jar, and stored on ice until transport to the laboratory. In the laboratory all damselflies were identified to species, stage and sex based on descriptions and keys in Abbott (2005; 2011), Westfall and May (2006), and May and Dunkle (2007).

Total body length and head width were recorded for each individual damselfly to the nearest 1.0 mm and 0.1 mm, respectively. Each teneral or adult zygopteran was then killed by removing the head. At necropsy, individual damselflies were placed in odonate saline (Fielden, 1960) and the abdominal sterna was peeled back. The entire gut was removed and gently teased apart with forceps on a microscope slide, and examined for juvenile nematodes. Nematodes were fixed in 70% ethanol

cleared in glycerol, according to Pritchard and Kruse (1982). Pearson's correlations were calculated for damselfly total body length and head width, along with *S.* cf. *trispinosum* intensity and abundance for each infected damselfly species (Sokal and Rohlf, 1981).

Description of turtle collection and necropsy

Two red eared sliders *Trachemys scripta elegans* were collected on May 25, 2010 from Forty-one Cut-off Lake McCurtain, Co., Oklahoma (N 33° 45' 10.404" W 94° 45' 58.86"). Turtles were killed by overdose with an injection of sodium barbital. After death, the plastron was removed with a hand saw and all internal organs were remove;d and examined for all helminths. Each organ was placed individually in a petri dish and examined under a stereomicroscope. Sex of turtles was determined for all individuals by gonadal inspection during necropsy. Nematodes were fixed in 70% ethanol and cleared in glycerol, according to Pritchard and Kruse (1982).

Infection parameters and morphological characteristics for worm identification

Prevalence, mean intensity, and mean abundance are reported according to Bush et al. (1997). All values are reported as the mean $\pm$ 1 standard deviation (SD). Nematodes were identified based on descriptions in Baker (1979), Bartlett and Anderson (1985), and Moravec and Vargas-Vasquez (1998a; 1998b). Voucher and type specimens of parasites and damselfly hosts will be deposited in the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska and the K.C. Emerson Entomology Museum, Oklahoma State University-Stillwater, respectively.

Measurements of six juvenile, four male and eight female *Serpinema trispinosum* were taken using a calibrated micrometer on an Olympus BX-51 upright research microscope configured for bright-field and differential interference contrast microscopy with plain fluorite objectives and digital photographs were taken with an Olympus 5 megapixel digital camera. The following measurements were recorded for juvenile nematodes recovered from damselflies: total length; greatest body width; buccal cavity anterior length and width; buccal cavity anterior and posterior length and width; muscular esophagus length; glandular esophagus length; distance of excretory pore from anterior end; distance of nerve ring from anterior end; distance of genital primordium from anterior end; and tail end length.

The following measurements were recorded for male and female nematodes: total length; greatest width; buccal cavity anterior length and width; buccal cavity posterior length and width; muscular esophagus length; glandular esophagus length; distance of excretory pore from anterior end; distance of nerve ring from anterior end; distance of genital primordiud from anterior end; tail end length; spicule length and distance of vulva from posterior end. All measurements are reported as a range in microns unless otherwise noted.

RESULTS

Of the 5 damselfly species examined only the blue-fronted dancer, *Argia apicalis*, was not infected with *S.* cf. *trispinosum*. A total of 29 juvenile *S.* cf. *trispinosum* were recovered from teneral (N = 6) and adult (N = 14) life stages of the four other species of damselflies examined. The prevalence, mean abundance and mean intensity of *S.* cf. *trispinosum* in each species of infected damselfly was as

following: 2.5 % (7/278), 0.04 ± 0.3, 1.6 ± 1.5 for *E. civile*; 3 % (4/140), 0.04 ± 0.2, 5.0 ± 0.5 for *I. hastata*; 8 % (5/65), 0.09 ± 0.3, 0.3 ± 0.5 for *I. verticalis*; 10 % (4/42), 0.2 ± 0.8, 2.3 ± 1.5 for *L. disjunctus australis*. There were no significant correlations in damselfly total body length or head width and intensity or abundance of *S.* cf. *trispinosum* for any of the four infected damselfly species ($P > 0.05$).

Both male red eared sliders were infected with 19 male and 16 female and 28 male and 14 female *S. trispinosum* respectfully. Morphological measurements for juvenile, male and female worms are provided bellow.

***Serpinema trispinosum* (Leidy, 1852)**

(Figures 1–2)

General: Nematoda, Spirurida, Camallanoidea, Camallanidae, Camallaninae, *Serpinema*. Translucent orange to red in life for adults. Medium-sized fusiform worms. Cuticle smooth. Cephalic end with brown buccal capsule consisting of two lateral valves. Buccal capsule laterally compressed, composed of three parts (two valves and a basal ring), width and length subequal. Valves marked internally by longitudinal ridges, most numerous near anterior margin of buccal capsule. Anterior margin of valve bearing two small, elongate sclerotized plates. Buccal valves supported by dorsoventral tridents on each side, consisting of three posteriorly directed prongs extending beyond basal ring, prongs subequal. Tridents attached to buccal capsule by means of anteriorly directed, divided process supporting each valve. Mouth opening slit like surrounded by four subapical mouth papillae. Amphids not seen. Nerve ring near posterior end of tridents. Excretory pore slightly posterior to nerve ring. Glandular oesophagus. Oesophagus long, slender; anterior

muscular portion clearly divided from posterior glandular portion, glandular portion generally equal to muscular portion. Tail with three terminal cuticular spikes (mucrons) in juveniles and adult females. (Rigby *et al*., 1997)

Juveniles (six specimens): Body is colorless, 700 ± 325 (881–1009) long and 58 ± 4.6 (51–63) wide, with a smooth cuticle. Cephalic end bears 8 small papillae and lateral amphids. Buccal capsule is divided into anterior globular portion [length 29.3 ±2.6 (25.2–32.8), width 29.4 ± 2.6 (25.2–32.8) with inner ridges and narrower, smooth posterior portion (length 23.3 ± 3.2 (20.2–27.7), width 13.6 ± 1.2 (12.6–15.12)]. Each side of anterior portion of capsule bears more than 10 narrow, long longitudinal ridges extending approximately along anterior two thirds of this portion of capsule, and few (2–3) very short ridges. Posterior portion of capsule is simple, thick-walled. Buccal capsule opens into oesophagus through large esophageal funnel with sclerotized walls. Length of muscular oesophagus is 175 ± 19.3 (146.2–201.6), of glandular oesophagus 123.9 ± 10.6 (108.4–133.6); length ratio of the muscular oesophagus and glandular oesophagus is 1:0.7–0.8. Nerve ring and excretory pore are 85.1 ± 6.6 (73.1–90.7) and 94.7 ± 2.4, respectively, from anterior extremity. Oval genital primordium is 794.7 ± 152.7 (625–1046) from anterior end of body. Tail is conical, 54.9 ± 7.0 (42.8–63.0) long, with three terminal cuticular spikes; length of dorsal spike 10–13; of ventrolateral spikes 8–10.

Males (four specimens): Length of body 8636.6 ± 1277.9 (7418.2–9780.3), maximum width 247.0± 17.4 (230.2–267.0). Length of entire buccal capsule, including basal ring, 105.3 ± 10.7 (91.9–114.4), width 137.3 ± 5.3 (132.7–144.2); basal ring 91.3 ± 20.9 (61.3–105.4) long and 15.2 ± 1.7 (12.6–16.0) wide; length of

tridents 66.0 ± 25.7 (42.7–100.8). Muscular oesophagus measuring 340.8 ± 203.4 (39.0–470.0) in length, maximum width 106.6 ± 5.6 (101.5–112.1); glandular oesophagus 547.0 ± 78.5 (459.5–614.1) long, width 106.0 ± 24.5 (81.7–139.2). Deirids, nerve-ring and excretory pore 367.56–384.48, 100.46–112.14, and 459.45–614.1 respectively from anterior extremity. Posterior end of body with broad caudal alae supported by pedunculate papillae; alae opaque. Caudal papillae: seven pairs of preanal and six pairs of postanal thin, pedunculate papillae present; first three pairs of postanals close one to another, next two pairs forming group approximately at middle of tail; last pair of small postanal papillae situated laterally near caudal extremity. Cloacal opening surrounded by two transverse mounds, appearing in lateral view as two pairs of small sessile papillae. Large (right) spicule well sclerotized, 650–775 long; its posterior end slender, sharply pointed, bearing small dorsal, posteriorly oriented barb near tip. Small (left) spicule weakly sclerotized, hardly visible, 190-375 long, with simple, sharply pointed end. Tail conical, 115–130 long, its tip rounded.

Females (eight specimens): Body length of females 9886.8 ± 3527.5 (1262.5–11854.8), maximum width 386.0 ± 62.2 (320.4–520.0). Length of whole buccal capsule including basal ring 126.9 ± 23.1 (91.9–163.4), width 157.4 ± 24.2 (112.3–200.0); basal ring 107.2 ± 9.8 (95.6–126.0) long and 22.3 ± 4.2 (16.0–25.2) wide; length of tridents 90.6 ± 32.4 (40.8–125.0). Muscular oesophagus measuring 484.6 ± 31.0 (450.0–541.8) in length, maximum width 141.0 ± 12.1 (122.5–160.2); glandular oesophagus 508.5 ± 58.8 (403.2–612.6) long, width 139.9 ± 32.1 (100.0–201.6). Deirids, nerve-ring and excretory pore 408.4–453.9, 122.52–160.2, and 403.2–612.6,

respectively, from anterior extremity. Vulva equatorial or somewhat postequatorial, 5144–6623from posterior end of body. Vagina directed anteriorly. Uterus filled with larvae 186.9–315.06 long and 10.68–16.02 wide. Tail conical, 224.62–306.3 long, its tip bearing three cuticular processes 6 long.

DISCUSSION

Morphological comparisons of juvenile *S.* cf. *trispinosum* collected from damselfly hosts during this study and similar data reported for *S. trispinosum* from fish, snails and anurans indicates that specimens recovered from damselflies overlap in most of their morphological characteristic (8/12) with the descriptions of this nematode recovered from fish by Moravec and Vargas-Vazquez (1998b) (see Table II). In contrast, morphological characteristics of *S. trispinosum* recovered from aquatic snails and anurans by Bartlett and Anderson (1985) and González and Hamann (2007; Table II) only overlapped in 1 of 12 and 6 of 12 morphological characteristics reported for juvenile *S.* cf. *tripinosum* recovered from damselflies in this study. One reason that little overlap exists in the characteristics in specimens of *S. trispinosum* recovered from damselflies in this study and the nematodes recovered from aquatic snails and fish by Bartlett and Anderson (1985) and González and Hamann (2007) is that few individuals were measure. However, recent studies on nematodes of amphibians by Rhoden and Bolek (2011) and Vhora and Bolek (2013) indicate that host induced morphological variation is a common phenomenon in some nematodes and it may be that these nematodes experience similar morphological plasticity in there paratenic hosts. Although specimens of *S.* cf. *trispinosum* from damselfly hosts were morphologically distinct from the report of Bartlett and

Anderson (1985) and González and Hamann (2007) the most significant distinguishing characteristics for juvenile *S. trispinosum* and other camallanids identification is the morphology of the buccal capsule. Moravec and Vargas-Vazquez (1998a; 1998b) described third stage juveniles *S. trispinosum* recovered from the body cavity of fish and the intestines of a *Trachemys scripta* infected with adults of this species all collected from the same location in the Yucatan, Mexico. They indicated that the buccal capsule of juvenile *S. trispinosum* belongs to the *Paracamallanus*-type, which is divided into an anterior globular portion with inner ridges and narrower and a smooth posterior portion. The specimens recovered from damselfly hosts in this study agree with the morphological description of the buccal capsule reported for juvenile *S. trispinosum* by Moravec and Vargas-Vazques (1998a; 1998b; see Fig. 1C-E) but differ morphologically from description of the buccal capsule of juveniles of other camallanid genera such as *Camallanus* and *Spirocamallanus* (Stromberg and Crites, 1974; Santos et al., 1999). However, because few descriptions of other species and genera of juvenile camallanids exist in the literature (Stromberg and Crites, 1974; Santos et al., 1999) I adapted the conservative view and refer to these nematodes as *S.* cf. *trispinosum* until the life cycle is completed in the laboratory or molecular data is available to match juvenile and adult nematodes.

Morphological characteristics of adult male and female *S. trispinosum* recovered from Oklahoma red eared sliders overlapped in most characteristics with the re-description of these nematodes from Mexican red eared sliders by Moravec and Vargas-Vazquez (1998b; see Table III). Male and female nematodes in this study

differed in one of 15 morphological characteristics (the location of derides from the anterior end) and three of 14 morphological characteristics (total length, location of derides from the anterior end and location of vulva from posterior end) respectively from the re-description of this species by Moravec and Vargas-Vazquez (1998b; see Table III). More importantly, the buccal capsule morphology was consistent for the genus *Serpinema* as described by Yeh, 1960 and Moravec and Vargas-Vazquez, 1998a; large, orange-brown buccal capsule consisting of two lateral valves; inner surface of each valve supported by 16–21 narrow, longitudinal, sometimes incomplete ridges (see Fig. 2). Additionally, tail morphology of females (Fig. 2) and the posterior end of males and spicule length was consistent for the species *S. trispinosum* in the re-description by Moravec and Vargas-Vasquez (1998a).

Serpinema trispinosum has been reported from 18 species of turtles across four families (Baker, 1987) suggesting that *S. trispinosum* is a generalist nematode. Along with infecting a wide range of turtle definitive hosts that vary in their diet and habitat, this nematode also infects a wide range of paratenic hosts including fish, frogs, snails, and damselflies (Table II). Dietary studies of the 18 known turtle definitive hosts indicate that all four groups of paratenic hosts have been reported in the diet contents of most of these turtles (Table IV). However, although this is the first report of damselflies serving as paratenic hosts for *S.* cf. *trispinosum*, these observations are important for several reasons. First, among the 18 species of turtle definitive hosts for *S. trispinosum*, larval and adult odonates are the most commonly reported food item in turtle definitive hosts for this nematode (14/18 turtle species) and odonates can make up to 50% of the frequency of turtle diets (Table III). Second,

microcrustaceans including copepods which serve as first intermediate hosts for *S. trispinosum* are the predominant food items of larval damselflies (Corbet, 1999; Bolek *et al*., 2010) suggesting that these insects come in contact with microcrustacean infected with *S. trispinosum* commonly. Finally, because both teneral and adult damselflies were infected with this nematode in this study this observation indicates that these nematodes survive the molting process from larval damselfly to adult damselfly and provides a more plausible mechanism of how semi-terrestrial turtles such as *Terrapene carolina* become infected with this nematode.

Although fish, aquatic snails and amphibians are also commonly reported in the diet of turtle definitive hosts for this nematode, hundreds of field surveys on helminth parasites of fish, aquatic snails and amphibians indicate that these hosts are rarely infected with this nematode (see Baker, 1987; Hoffman 1999; Zimmermann et al., 2011). One possible reason that aquatic snails and anurans are not commonly reported as paratenic hosts for this nematode may be that both aquatic snails and anurans rarely ingest microcrustaceans in their diets. Aquatic snails predominantly feed on aquatic algae and detritus; whereas larval and adult anurans feed on algae or larger prey items respectively and both snails and anurans only accidentally ingest microcrustaceans (Thorp and Covich, 2001; Bolek et al., 2010). In contrast, it is less clear why other fish species have not been reported as paratenic hosts for *S. trispinosum* because microcrustaceans are a common component in the diet of fish. Clearly, laboratory life cycle studies will have to be conducted in order to test the host specificity of this nematode in fish paratenic hosts to address this issue. Finally,

further surveys of damselflies and other odonates are needed to define the role of odonates as paratenic hosts in the life cycle of *S. trispinosum*.

Although surveys of nematodes are now available for a wide variety of vertebrate and invertebrate hosts, few such studies have been conducted on damselflies (see Willis, 1971; Baker, 1987; Corbet, 1999; Baker, 2011; Hoffman 1999; Zimmermann et al., 2011). I hope that the present study provides an incentive for comprehensive studies on damselfly nematodes, which will aid in alleviating the current lack of knowledge on the occurrence, distribution and biogeography of nematodes infecting damselfly hosts.

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Figure 1. *Serpinema* cf. *trispinosum* collected from the mid-gut of the eastern forktail, *Ischnura verticalis* from Teal Ridge, Stillwater, Oklahoma. **(A)** Third Stage juvenile, general view; scale-bar = 50 μm. **(B)** Tail note the three terminal cuticular spikes; scale-bar = 10 μm. **(C-E)** Buccal capsule, lateral view. Note the ridges in buccal valve scale-bar = 15 μm.

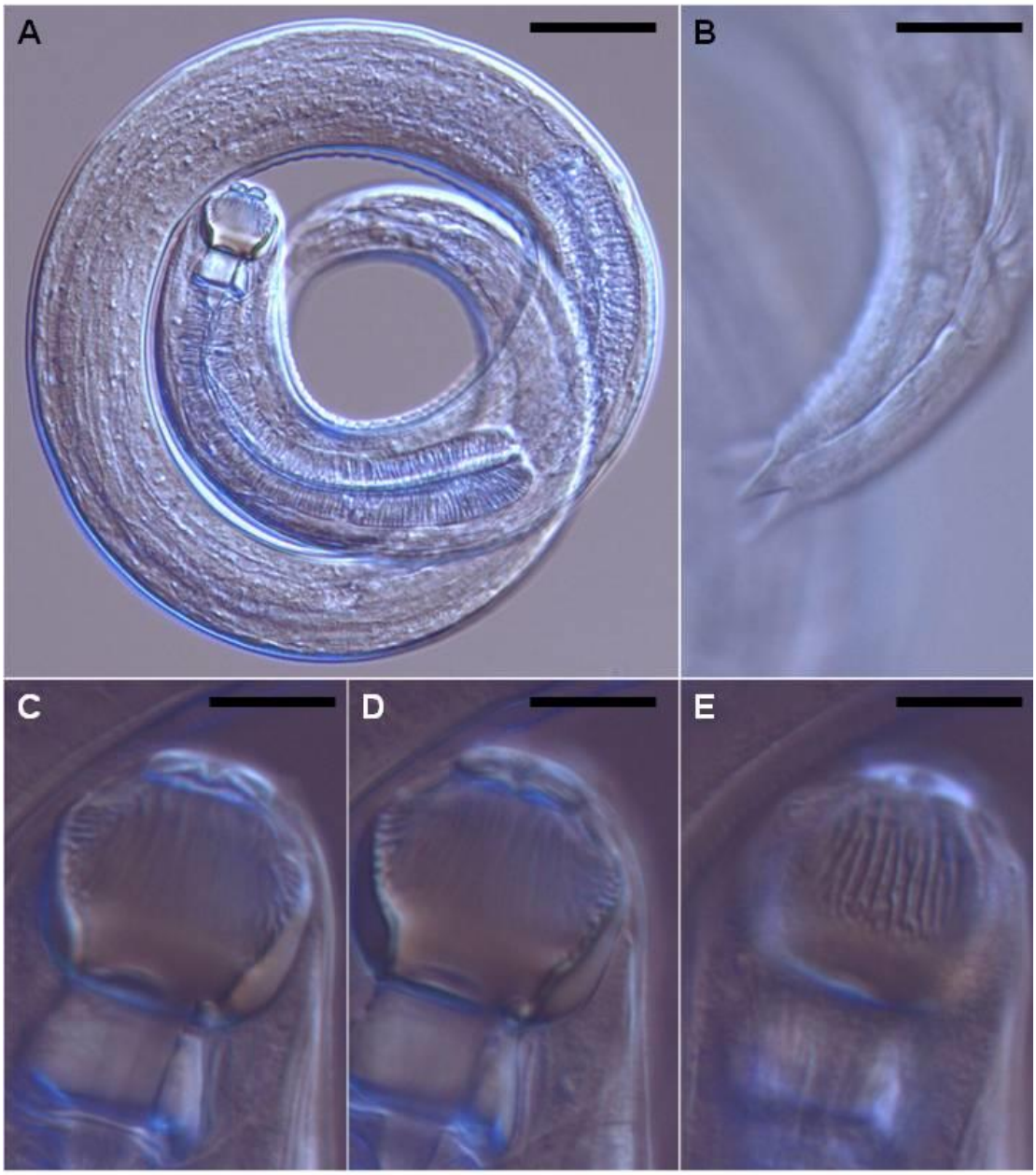
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Figure 2. *Serpinema trispinosum* collected from *Trachemys scripta elagans* from Oklahoma. **(A)** Anterior end of adult female, general view; scale-bar = 100 μm. **(B)** Buccal capsule of female, lateral view. Note the ridges in buccal valve scale-bar = 20 μm **(C)** Tail of female note the three terminal cuticular spikes; scale-bar = 10 μm. **(D)** Buccal capsule of male, lateral view. Note the ridges in buccal valve scale-bar = 15 μm.

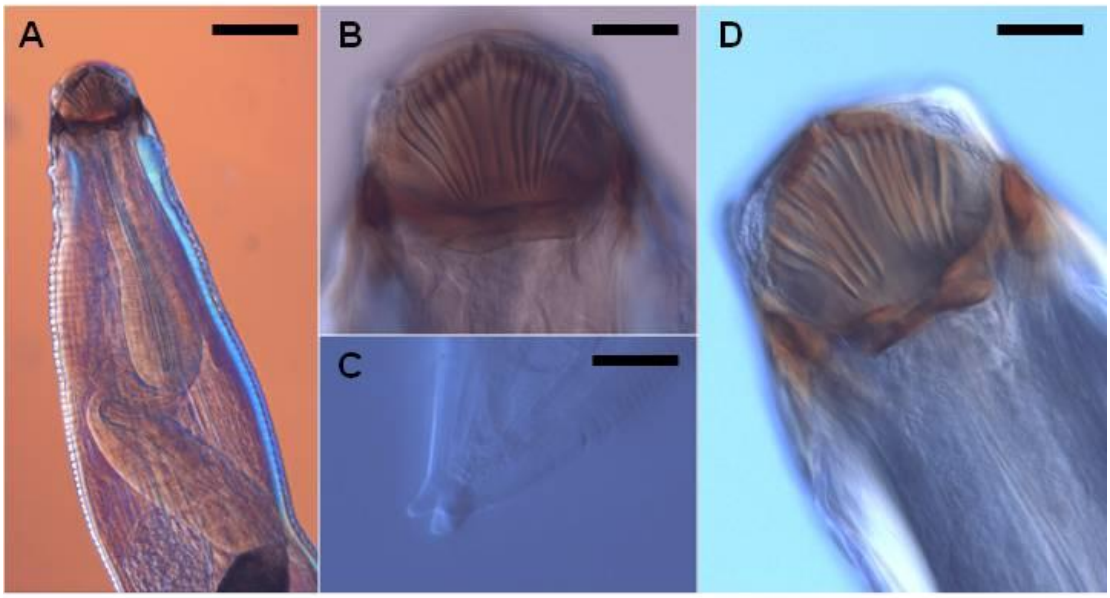
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TABLE I. Species and numbers of damselflies (separated by year) collected at Teal Ridge Stillwater, Payne County, Oklahoma and examined for *Serpinema trispinosum*.

Species	Number of damselflies in 2010	Number of damselflies in 2011	Number of damselflies in 2012	Total
Coenagrionidea				
Argia apicalis	0	4	1	5
Enallagma civile	43	190	45	278
Ischnura hastate	23	72	45	140
Ischnura verticalis	0	3	62	65
Lestidea				
Lestes disjunctus australis	8	28	6	42

Table II. Morphological characteristics of third stage juvenile *Serpinema* cf. *trispinosum* reported from various paratenic hosts.

	Paratenic Hosts Reported in Nature			
	This Study	Moravec and Vargas-Vazquez 1998b	Bartlett and Anderson, 1985	González and Hamann, 2007
Host Group	Damselflies	Fish	Snails	Anurans
# hosts collected	530	18	25	43
% (No. infected/no. examined)	4% (20/530)	17% (3/18)	8% (2/25)	16% (7/43)
# worms measured	6	5	2	11
Total Length (µm)	881–1009	980–1295	1200–1300	1170–1930
Greatest Width	51–63	50–75	68–76	46–80
Buccal Cavity Anterior Length	25–33	33–38	30–36	37–42.5
Buccal Cavity Anterior Width	25–33	33–40	N/G[†]	25–43
Buccal Cavity Posterior Length	20–28	18–20	16–20	16–22
Buccal Cavity Posterior Width	13–15	25–28	N/G[†]	14–29
Muscular Esophagus Length	146–202	175–225	216–220	198–305
Glandular Esophagus Length	108–134	130–175	174–190	184–275

Nerve Ring*	73–91	95–118	84–88	85–135
Excretory Pore*	92–98	120–145	140–144	127–159
Genital Primordiud*	625–1046	638–863	N/G†	N/G†
Tail End Length	43–63	60–70	64	53–115

*Distance from anterior end; †N/G = not given.

Table III. Morphological characteristics of adult *Serpinema trispinosum* reported from red-eared slider turtles.

	Male	Female

	This study	Moravec and Vargas-Vazquez 1998a	This study	Moravec and Vargas-Vazquez 1998a
	Oklahoma Sliders	Mexico Sliders	Oklahoma Sliders	Mexico Sliders
	Serpinema trispinosum	*Serpinema trispinosum*	*Serpinema trispinosum*	*Serpinema trispinosum*
No. worms measured	4	5	8	5
Total Length (µm)	7418–9780	4430–7530	10718–11855	7470–10350
Greatest Width	230–267	163–272	320–520	286–326
Buccal Cavity Anterior Length	92–114	105–132	92–163	114–150
Buccal Cavity Anterior Width	133–144	150–165	112–200	189–195
Basil Ring Length	12–16	12–15	16–25	15–21
Basal Ring Width	61–105	78–87	96–126	99–105
Muscular Esophagus Length	43–101	87–105	41–125	422–449
Glandular Esophagus Length	390–470	367–476	450–542	490–517

Nerve Ring*	100–112	81–129	123–160	108–114
Excretory Pore*	459–614	354–558	403–612	490–517
Genital Primordiud*	82–139	78–150	100–202	87–129
Deirids*	368–384	435–517	408–454	490–517
Large (right) Spicule Length	650–775	696–759	N/A†	N/A†
Small (left) Spicule Length	190–375	207–210	N/A†	N/A†
Vulva**	N/A†	N/A†	5144–6623	3060–5020
Tail End Length	115–130	195–231	225–306	218–299

*Distance from anterior end; **Distance from posterior end; †NA = not applicable.

Table IV. Four types of paratenic hosts for *Serpinema trispinosum* reported in the diet of 18 species of turtle definitive hosts.

	Odonates	Snails	Fish	Anurans	Reference
Chelydridae (Snapping Turtles)					

	Chelydra serpentine	Yes	Yes	Yes	Yes	Ernst et al., 1994
Emydidae (Pond Turtles)						
	Clemmys guttata	Yes	Yes	Yes	Yes	Ernst et al., 1994
	Clemmys insculpta	Yes	Yes	Yes	Yes	Ernst et al., 1994
	Chrysemys picta	Yes	Yes	No	No	Cooley et al., 2003
	Deirochelys reticularia	Yes*	No	No	Yes	Demuth and Buhlmann, 19
	Pseudemys concinna	Yes*	Yes	Yes (Carrion)	Yes	Bjorndal et al., 1997
	Pseudemys floridana	Yes*	No	No	No	Bjorndal et al., 1997
	Trachemys decussata	Yes	No	No	No	Seidel, 1990
	Trachemys scripta	Yes	Yes	Yes	Yes	Ernst et al., 1994
	Emydoidea blandingi	Yes*	Yes	Yes (Carrion)	Yes	Rowe, 1992
	Graptemys geographica	Yes	Yes	Yes (Carrion)	No	Ernst et al., 1994
	Graptemys pseudogeographica	Yes	Yes	Yes	No	Ernst et al., 1994
	Graptemys kohnii	Yes	Yes	Yes	Yes	Carr, 1952
	Terrapene carolina	Yes	Yes	Yes (Carrion)	Yes	Carr, 1952 Ernst et al., 1994, Platt et al., 2009

	Malaclemys terrapin	No	Yes	Yes (Carrion)	No	Ernst et al., 1994
Kinosternidae (Musk and Mud Turtles)						
	Kinosternon subrubrum	Yes	No	Yes (Carrion)	Yes	Ernst et al., 1994
	Sternotherus odoratus	Yes	Yes	No	Yes	Ernst et al., 1994
Trionychidae (Soft Shell Turtles)						
	Apalone spinifera	Yes	No	Yes	No	Martinho, 2008
Total		14	12	13	10	

* Indicates over 50% of turtle diet based on frequency of gut contents.

CHAPTER III

GREGARINE DIVERSITY FROM FOUR SPECIES OF DAMSELFLIES FROM OKLAHOMA WITH DESCRIPTIONS OF THEIR OOCYSTS AND RE-DESCRIPTION OF THE OOCYSTS OF *HOPLORHYNCHUS ACANTHATHOLIUS.*

ABSTRACT: As part of this study, I examined four species of damselflies (*Enallagma civile, Ishnura hastate, Ishnura verticalis* and *Lestes disjunctus australis*) from Teal Ridge Stillwater, Oklahoma for their gregarine parasites and I describe gregarine trophozoite, gametocyst and oocyst stages. Gregarines found included *Hoplorynchus acanthatholius, Nubenocephalous* sp., *Steganorynchus* sp, and one unknown gregarine. Both *E. civile* and *I. hastata* were infected with all four gregarines, *I. verticalis* was infected with *H. acanthatholius* and *Nubenocephalous* sp.; whereas *L. d. australis* was infected with *H. acanthatholius.* A second goal of this study was to isolate oocysts of *H. acanthatholius* from *E. civile* from the type locality, re-describe them and compare these oocysts to sporulated oocysts of this gregarine species isolated from Oklahoma damselflies. Morphological comparisons of oocysts of *H. acanthatholius* recovered from two damselfly species collected from Teal Ridge and oocyst recovered from *E. civile* from the type locality indicated that all possible host species pairs differed significantly for all oocyst morphological

characteristics except for the average total width of oocyst equator to terminal knob and the average length from the oocyst residdum to closet

terminal knob. These differences suggest that *H. acanthatholius* may represent multiple species of gregarines. However, another explanation for the observed oocyst size variation in different damselfly species and populations may be due to oocyst polymorphism. Similar polymorphism has been reported in oocysts of coccicidian species which are closely related to gregarines. I provide new host and geographical distribution information for gregarine parasites of damselflies from Oklahoma. My study puts into question previous studies on gregarine host specificity indicating that some gregarine species not only infect multiple species of damselflies in the same genus, but also different genera of damselflies and across different damselfly families.

INTRODUCTION

Gregarines are single cell apicomplexan parasites which infect multiple groups of invertebrates such as insects and annelids. One group of arthropods that has been relatively well studied for gregarines in North America is the Odonata, the dragonflies and damselflies (Clopton, 1995; Percival et al., 1995; Clopton et al., 2007; Hays et al, 2007; Clopton et al., 2010; Cielocha et al., 2011). Gregarines in the order Eugregarinorida and the family Actinocephalidae parasitizing damselflies and most species have been assumed to be host specific at the species, genus and/or family level (Percival et al. 1995). Most surveys of damselflies for gregarine parasites have concentrated on species descriptions from a single odonate host from a single location (Percival et al., 1995; Richardson and Janovy, 1990; Cook et al., 2011), or new host records across multiple locations from a single species or genus of odonates (Cielocha et al., 2011; Wiles and Reyda, 2011a; 2011b). Currently there are

no studies comparing gregarine infections from multiple species, genera and/or families of damselflies.

Although no life cycles of damselfly gregarines have been elucidated it is hypothesized that damselflies become infected with these parasites when they either drink contaminated water with oocysts or when they ingest invertebrates which have gregarine oocysts attached to their bodies. The oocyst is the stage in the gregarines life cycle that contains the infective sporozoite stage. After being ingested by the odonate, the sporozoites escape from the oocyst in the gut of the odonate and enter gut epithelial cells where intracellular develop occurs. Trophozoites develop and emerge from the gut epithelial cells and attach to the epithelial tissue with a specialized structure known as an epimerite. Trophozoites are the main feeding stages of gregarines where they increase in size and develop into the sporont stage characterized by a lack of an epimerite. In most gregarine life cycles that have been elucidated two sporonts pair to form a gametocysts and perform syzygy. However, pairs of sporonts have never been reported in odonate hosts. Infected damselflies release the gametocyst stage in their feces. If the gametocyst lands in water, the gametocyst sporulates into hundreds of oocysts, dehisses and oocysts are released into the water. Once sporulated oocysts are ingested by damselflies the life cycle continues (Abro, 1976, Roberts and Janovy, 2005, Vivier and Desportes, 1990).

Although gregarines are considered the most diverse eukaryotic taxon because their members parasitize most invertebrate groups, descriptive work on gregarines has been hindered by a number of factors, such as the paucity of structural features in most gregarine life stages. However, recently Clopton and coworkers have

established criteria by which new taxa should be described. These studies have standardized terminology for shapes and proportions of different gregarine life stages (Clopton, 2004a; Clopton et al., 2004; Janovy et al., 2007a; 2007b). This body of published work indicates that sporulated oocyst shape and size are highly stable characters of major taxonomic importance. However, to date no studies have examined oocysts of gregarines of the same species that infect multiple families, genera and species of damselflies or species of damselflies infected with the same species of gergarines from different populations.

As part of this study, I surveyed four damselfly species (*Enallagma civile*, *Ishnura hastate*, *I. verticalis* and *Lestes disjunctus australis*) from Teal Ridge Stillwater, Oklahoma for their gregarine parasites and describe their trophozoite, gametocyst and oocyst stages. During my survey, I discovered a gregarine, *Hoplorhynchus acanthatholius* which infected multiple species, genera and families of damselflies. This observation is important because previous field studies suggest that *H. acanthatholius* is family specific and only infects damselflies in the family Coenagrionidae (Percival, et al., 1995). As part of this study I compared the oocysts of *H. acanthatholius* from multiple species, genera and families of damselflies collected from a single location in Oklahoma. Additionally, because it was unclear from the original description of *H. acanthatholius* by Percival, Clopton and Janovy, 1995 that these authors described sporulated oocysts of *H. acanthatholius* a second goal of this study was to isolate oocysts of *H. acanthatholius* from *E. civile* from the type locality. Once oocysts are isolcated they can be re-described and compared to sporulated oocysts of the gregarine isolated from Oklahoma damselflies.

MATERIALS AND METHODS

Description of damselfly collection and necropsy

During September 2010–September 2012 525 teneral and adult damselflies of four species representing two families were collected from Teal Ridge Stillwater, Payne County, Oklahoma (N 36° 6' 1.44", W 97° 4' 51.405; Table I). All damselflies were collected between 9:00 AM and 6:00 PM with an aerial net and placed in a one liter plastic jar and stored on ice until brought back to the laboratory within an hour of capture. In the laboratory all damselflies were identified to species, stage and sex based on descriptions and keys in Abbott (2005; 2011), Westfall and May (2006), and May and Dunkle (2007). For oocyst comparisons of *H. acanthatholius* from the type locality and type hosth, an aerial net was used to collect 100 adults of *E. civile* during June 2011 from Keystone Lake, Keith Co., Nebraska (41.21527° N, 101.5784°, and 41.20710° N, 101.40850° W). After identification, each live damselfly was placed individually, abdomen down, in a glass vial filled with two ml of aged tap water in order to collect damselfly fecal matter for gregarine gametocysts. After 36–48 hrs, damselfly fecal content was searched for gametocysts and any gametocysts found were removed and measured with a ruler to the nearest mm and photographed. All gametocysts were then placed in 1.5 ml plastic wells filled with aged tap water and watched daily for oocyst dehiscence. Any oocysts that were collected from the dehised gameotcysts were placed onto a wet mount slide and photographed with an Olympus BX-51 upright research microscope at 1000x total magnification. Digital photographs were taken with an Olympus 5 megapixel digital camera. All teneral and adult zygopterans were removed from the vile and killed by removing the head. At

necropsy, individual damselflies were placed in odonate saline and the abdominal sterna were peeled back. The entire gut was removed and gently teased apart with forceps on a microscope slide, and examined for gregarines.

Gregarine infection parameters and trophozoite preparation and identification

Prevalence, mean intensity, and mean abundance are reported according to Bush et al. (1997). All values are reported as the mean $\pm$ 1 standard deviation (SD). All gregarine trophozoite were examined as both fresh preparations and stained smears on microscope slides. Air-dried smears were fixed in alcohol–formalin–acetic acid (AFA), washed in 70% ethyl alcohol, stained in Semichon's acetocarmine, dehydrated in an ethanol series (70–100%), cleared in xylene, and mounted in Canada balsam (Pritchard and Kruse, 1982; Kula and Clopton, 1999). Fresh preparations of trophozoites were prepared as wet mounts and both fresh preparations and stained smears were examined using an Olympus BX-51 upright research microscope configured for brightfield and differential interference contrast microscopy with plain fluorite objectives. Digital photographs were taken with an Olympus 5 megapixel digital camera. Trophozoites were identified to genus based on the structure of the epimerite from the digitized images using Q Capture image analysis software and compared to trophozoite descriptions from previous work in Clopton (2012) and Percival, et al. (1995). Voucher specimens of parasites and damselfly hosts will be deposited in the H. W. Manter Parasitology Collection, University of Nebraska, Lincoln, Nebraska and the K.C. Emerson Entomology Museum, Oklahoma State University-Stillwater, respectively.

Morphological characteristics of oocysts

All measurements are reported as an average ± 1 SD followed by the range in microns unless otherwise noted. The following measurements were recorded for 30 oocysts recovered from each damselfly species and included the length between oocyst knobs; length from oocyst equator to oocyst knob; total width of oocyst equator edge to terminal knob; oocyst width at equator, width of oocyst midway between equator and terminal knob; oocyst equator width to terminal knob; terminal knob length; terminal knob width; diameter of oocyst residuum; length from oocyst residdum to inner curve; length from oocyst residdum to closet terminal knob; and average sporozite length and width (see Fig. 1). I follow the recommendations of Clopton (2004) for oocyst shapes. Because variances were heteroscedastic the Kruskal–Wallis test and the Kolmogorov–Smirnov 2-sample test were used to compare differences among all morphological characteristics of oocyts of *H. acanthatholius* and the unidentified gregarine recovered from different species and populations of damselflies (Sokal and Rohlf, 1981).

RESULTS

A total of 312 gregarine trophozoites were recovered from 68 of 525 (12.9%) damselflies collected from Teal Ridge, Stillwater Oklahoma. At least four species of gregarines including *H. acanthatholius, Nubenocephalus* sp., *Steganorhynchus* sp., and an unidentified gregarine (Fig. 1) infected the four species of damselflies sampled. All gregarine parasites from Teal Ridge infected two or more damselfly species, with *H. acanthatholius* infecting all four species of damselfly (Table I). Additionally, a total of 425 *H. acanthatholius* were recovered from 9 of 100 (9%) *E.*

civile collected from Keystone Lake, Keith Co., Nebraska with a mean abundance of 47 ± 43 and a mean intensity of 47 ± 43.5.

Only eight *E. civile* and one *L. d. australis* collected from Teal Ridge, Oklahoma shed gametocysts; whereas eight *E. civile* collected Keystone Lake, Nebraska shed gametocysts of *H. acanthatholius*. Of the 17 gametocysts recovered, only four gametocysts of *H. acanthatholius* and one gametocyst of the unknown gregarine dehisced and produced sporulated oocysts. Gametocysts stored in aged tap water dehisced by simple rupture within 6–11 days after being defecated by damselflies. The following are descriptions of trophozoites, gametocysts and oocysts if available of the four gregarine genera and/or species found.

***Hoplorynchus acanthatholius* (Percival, Clopton and Janovy 1995)**

(**Trophozoite Figure 1A; Oocysts Figure 2A-B)**

General: Apicomplexa, Eugregarinorida, Stenophoricae, Actinocephalidae, Menosporinae, *Hoplorynchus*. –

Trophozoite: Broadly ovoid protomerites, posterior margins are depressed, anterior margin nearly apiculoid and joining stalk of epimerite; epimerite is long, ending in a flattened blub boardered by anterior hooks (Percival et al., 1995).

Gametocyst: White in color, orbicular with persistent gelatinous epicyst; 0.9–1.1 mm in diameter.

Oocyst: Axially asymentic, biconical, crescentic, compressed in the plane perpendicular to the major axis, smooth (without spines), uniform in size and shape, and containing four sporozoites. Average sporozoite length (ASL) 7.22 ± 0.72 (5.76–

8.84); average sporozoite width (ASW) 1.66 ± 0.19 (1.18–2.13). Length between oocyst knobs (OKL) 13.07 ± 0.84 (11.68–14.71). Oocyst length from equator to terminal knob (OEWK) 5.99 ± 0.85 (4.55–8.82). Length from oocyst equator to oocyst knob (OLA) 6.5 ± 0.46 (5.53–7.6). Width of oocyst midway between equator and terminal knob (OMW) 4.29 ± 0.21 (3.81–4.73). Diameter of oocyst residdum (OrD) 2.33 ± 0.36 (1.82–3.16). Length of oocyst residdum to inner curve (OrICL) 1.28 ± 0.41 (0.57–2.1). Length from oocyst residdum to closet terminal knob (OrKL) 9.61 ± 0.5 (8.68–10.73). Oocyst width at equator (OW) 4.99 ± 0.59 (4.13–7.79). Total width of oocyst equator to terminal knob (OWEK) 10.87 ± 0.57 (9.78–11.99). Terminal knob length (TKL) 0.97 ± 0.18 (0.63–1.32). Terminal knob width (TKW) 1.48 ± 0.19 (1.1–1.87).

Type host: Enallgma civile

Other hosts: Ischnura verticalis, *Ischnura hastata*, and *Lestes disjunctus australis*

Type locality: Keystone Lake, Keith Co., Nebraska (41.21527° N, 101.5784° W and 41.20710° N, 101.40850° W).

Other localities: Teal Ridge Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405).

***Nubenocephalus* sp. (Clopton, Percival, and Janovy, 1993)**

(Figure 1B)

General: Apicomplexa, Eugregarinorida, Stenophoricae, Actinocephalidae, Acanthosporinae, *Nubenocephalus*.

Trophozoite: Epimerite broadly ovid, truncated posteriad, with broad, flexible equatorial tumidi that do not form hooks, spines, or digitiform processes, borne on a long, slender stalk (Clopton et al., 1993).

Type host: Argia bipunctulata

Other hosts: Argia apicalis, A. bipunctulata, Argia chelata A. fumipennis, A. moesta, A. nahuana, A. sedula, A. translate, A. vivida (Cielocha et al., 2011), *Enallagma civile, Hetaerina americana,* and *H. titia* (Clopton et al., 2010); *Ischnura hastata,* and *I. verticalis* (Wiles and Reyda, 2011a)

Type locality: Bowling Lake (Section 2, Township 10 North, Range 6 East), Lancaster County, Nebraska.

Other localities: Clear Creek, Atchison Co., Kansas (39°39’01”N; 95°24’53°W, Cielocha et al., 2011); Delaware River, Brown Co., Kansas (39°40’03”N; 95°39’35”W, Cielocha et al., 2011); Deroin Creek, Nemaha Co., Nebraska (40°15’38”N; 95°38’07”W, Cielocha et al., 2011); Easly Creek, Richardson Co., Nebraska (40°03’30”N; 95°52’37”W, Cielocha et al., 2011); Big Pond at Thayer Farm, Otsego Co., New York (42.80° N 74.91° W, Wiles and Reyda, 2011); Chain Ponds of the Thayer Farm, Otsego Co., New York (42.79° N 74.91° W, Wiles and Reyda, 2011); SUNY Oneonta College Camp station, Otsego Co., New York (42.50° N 75.06° W, Wiles and Reyda, 2011); Teal Ridge, Stillwater, Payne Co., Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405); Coal Creek, Rogers Co., Oklahoma (36°12’26”N; 95°54’50”W, Cielocha et al., 2011); Harmon Creek, Walker Co., Texas (30°44’42”N; 95°28’17”W, Cielocha et al., 2011); Lake Mineral Wells, Parker Co., Texas (32°48’59”N; 98°02’31”W, Cielocha et al., 2011); Russell Creek, Collin Co., Texas

(33°05’47”N; 96°45’04”W, Cielocha et al., 2011); Sam Houston State University Center for Biological Field Studies, Harmon Creek Walker Co., Texas, (N 30°40’53.54”,W 95°38’44.86”, Cielocha et al., 2011); Solomon Springs, Reeves Co., Texas (30°56’41”N; 103°47’01”W, Cielocha et al., 2011); Hammock Bridge on the Mopan River at Branch Mouth, Cayo District,, Belize (17°10’65.6”N; 89°4’81.3”W, Clopton et al., 2010); Barton’s Creek at Caesar’s Place, Imperial Mile Marker 60, Western Highway near Unitedville, Cayo District, Belize (17°12’33.5”N; 88°57’20.5”W, Clopton et al., 2010).

***Steganorhynchus* sp. (Percival, Clopton, and Janovy 1995)**

(Figure 1C)

General: Apicomplexa, Eugregarinorida, Stenophoricae, Actinocephalidae, Menosporinae, *Steganorhynchus.*

Trophozoite: Epimerite set on a long vermicular stalk, an ovid papilla enclosed in a retractile, globular sheath (Percival et al., 1995).

Type host: Enallgma civile

Other hosts: Ischnura verticalis, Ischnura hastate.

Type locality: Keystone Lake, Keith Co., Nebraska (41.21527° N, 101.5784° W and 41.20710° N, 101.40850° W).

Other localities: Teal Ridge Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405).

Unknown gregarine

(**Figure 1D; Figure 2C-D)**

General: Apicomplexa, Eugregarinorida, Stenophoricae, Actinocephalidae, Menosporinae.

Trophozoite: Broadly ovoid protomerites, posterior margins depressed, and deutromerites are deeply obdeltoid in shape.

Gametocysts: White in color, orbicular with persistent gelatinous epicyst; 0.8–1.0 mm in diameter.

Oocyst: An overall obese luniform shape, compressed perpendicular to the major axis, smooth (without spines), and containing four sporozoites. Average sporozoite length (ASL) 5.59 $\pm$ 1.04 (3.19–7.08) and average sporozoite width (ASW) 1.9 $\pm$ 0.35 (1.28–2.44). Length between oocyst knobs (OKL) 13.59 $\pm$ 1.36 (11.44–16.38). Oocyst length from equator to terminal knob (OEWK) 7.64 $\pm$ 0.94 (5.67–9.7). Length from oocyst equator to oocyst knob (OLA) 6.41 $\pm$ 0.86 (4.95–7.92). Width of oocyst midway between equator and terminal knob (OMW) 5.49 $\pm$ 0.71 (3.93–6.51). Diameter of oocyst residdum (OrD) 2.13 $\pm$ 0.76 (1.01–3.25). Length of oocyst residdum to inner curve (OrICL) 3.56 $\pm$ 0.65 (2.41–4.63). Length from oocyst residdum to closet terminal knob (OrKL) 8.2 $\pm$ 1.19 (6.23–10.1). Oocyst width at equator (OW) 7.39 $\pm$ 0.63 (6.41–8.77). Total width of oocyst equator to terminal knob (OWEK) 7.64 $\pm$ 0.94 (5.67–9.7). Terminal knob length (TKL) 1.51 $\pm$ 0.6 (0.82–2.53). Terminal knob width (TKW) 1.61 $\pm$ 0.61 (1.08–2.81).

Type host: Enellagma civile.

Other hosts: Ischnura hastata.

Type locality: Teal Ridge Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405).

Other localities: none known.

Statistical comparisons of oocyst morphology

The Kruskal–Wallis 1-way analysis of variance revealed significant differences between all morphological characteristics of oocysts of the unknown gregarine from *E. civile* and oocysts of *H. acanthatholius* from *E. civile* collected from Nebraska and Oklahoma and *L. d. australis* from Oklahoma (Table II). The Kolmogorov–Smirnov 2-sample tests showed that all possible host species pairs differed significantly ($P < 0.05$) for all oocyst morphological characteristics except for the average total width of oocyst equator to terminal knob and the average length from the oocyst residdum to closet terminal knob. (Table II).

DISCUSSION

The individual gregarine genera and species and overall gregarine prevalence, mean abundance, and mean intensity for the four damselfly species examined in this study were very low. These observations are in contrast to previous studies on gregarines of damselflies which reported much higher prevalence, mean intensities and mean abundances than in the current study (Locklin and Vodopich, 2010, Cielocha *et al*., 2011). More importantly, my study indicates the lack of host specificity exhibited by gregarine parasites infecting damselfly hosts. All of the gregarine genera and all identified species of gregarines from this studied infected at

least two damselfly species, with *H. acanthatholius* infecting all four damselfly species. For most of the past century gregarines have been hypothesized as being host specific, with the possibility that every insect species could harbor their own unique host specific gregarines, thereby potentially making gregarines the most specious parasite (Levine, 1979). While laboratory cross-infections of these parasites in some insect groups such as beetles and cockroaches have yield results to show strict host specificity (Clopton et al., 1992; Clopton and Janovy, 1993) there have been arguments against the gregarines host specific traits in some field studies. Recent field studies on damselfly gregarines indicate that some species of gregarine can infect multiple species of damselflies in a single genus (Cielocha et al., 2011; Clopton, 2009). My study adds to this observation and indicates that some gregarine species can infect multiple genera and families of damselflies.

Morphological comparisons of oocysts of *H. acanthatholius* recovered from two damselfly species collected from Teal Ridge and oocyst recovered from *E. civile* from the type locality indicated that all possible host species pairs differed significantly for all oocyst morphological characteristics except for the average total width of oocyst equator to terminal knob and the average length from the oocyst residdum to closet terminal knob. These differences suggest that *H. acanthatholius* may represent multiple species of gregarines. However, another explanation for the observed oocyst size variation in different damselfly species and populations may be due to oocyst polymorphism. Similar polymorphism has been reported in oocysts of coccicidian protozoans which infect vertebrates and are closely related to gregarines (Gardner and Duszynski, 1990; Hill et al., 2012). Gardner and Duszynski (1990)

reported polymorphism in oocysts of a coccidian species (*Eimreia opimi*) from seven species of tuco-tucos (*Ctenomys* spp.) collected from 16 localities representing four distinct ecological habitats in Bolivia. Gardner and Duszynski (1990) used discriminant analysis on 256 individual oocysts selected randomly from each *Ctenomys* sp. and indicated that oocyst and sporocyst lengths and widths could not be used to separate morphotypes of *E. opimi* from different *Ctenomys* spp. from different geographic locations. More recently Hill et al. (2012) used morphologic and molecular traits to identify polymorphic oocysts of *Eimeria macropodis* in a captive Tammar wallaby (*Macropus eugenii*) population from Australia. Their study using molecular characterization of oocysts highlighted the need to use multiple genetic markers to accurately identify polymorphic oocyts of *E. macropodis*. My morphological analysis of oocysts of *H. acanthatholius* from different species and populations of damselflies suggests that a similar approach along with life cycle studies may be necessary for species identification of damselfly gregarines.

Finally, the unknown gregarine, recovered from *E. civile* from Teal Ridge is most likely a new species of gregarine. This is due to the unique obese luniform shape of the oocyst. When reviewing the literature of all known Menosporinae gregarine species it was determined that shape of this oocyst was unique and unlike any other described gregarine oocyst. Although the unique oocyst stage of this gregarine indicate that it belongs to a new species, unfortunately I did not find enough other gregarine life stages during this study for a formal description of this new species. Further work on collecting the rest of these stages needs to be done in order to describe this new species.

In conclusion this study has revealed many new questions about the host specificity and systematics of gregarines in damselfly hosts. This survey has expanded the range for gregarine parasites in Oklahoma damselflies and has expanded the known host range for many of these parasites including *L. d. australis* as a new host for the gregarine *H. acanthatholius.* My study puts into question previous studies on gregarine host specificity indicating that gregarine species not only infect multiple species of damselflies in the same genus, but also different genera of damselflies and across different damselfly families. Additionally, my work indicates that the gregarine oocyst may be polymorphic, similar to that of their coccidian relatives. It is my hope that the new questions that have arisen from this study will stimulates further studies on the distribution and identification of damselfly gregarines. One major problem in our understanding on the distribution of gregarine parasites of damselflies is the lack of knowledge on damselfly gregarine life cycles. For example, all of the gregarine species discovered during this study have yet to have their life cycles completed in the laboratory. Such life cycle studies would allow us to test hypothesis on host specificity of gregarines of damselflies and on the polymorphic nature of gregarine oocysts in damselflies observed in the present study.

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Figure 1. Morphological characteristics reported for oocysts of *H. acanthatholius* and the unknown gregarine. Sporozoite length (ASL); Sporozoite width (ASW); length between oocyst knobs (OKL); oocyst length from equator to terminal knob (OEWK); length from oocyst equator to oocyst knob (OLA); width of oocyst midway between equator and terminal knob (OMW); diameter of oocyst residdum (OrD); length of oocyst residdum to inner curve (OrICL); length from oocyst residdum to closet terminal knob (OrKL); oocyst width at equator (OW); total width of oocyst equator to terminal knob (OWEK); terminal knob length (TKL); terminal knob width (TKW).

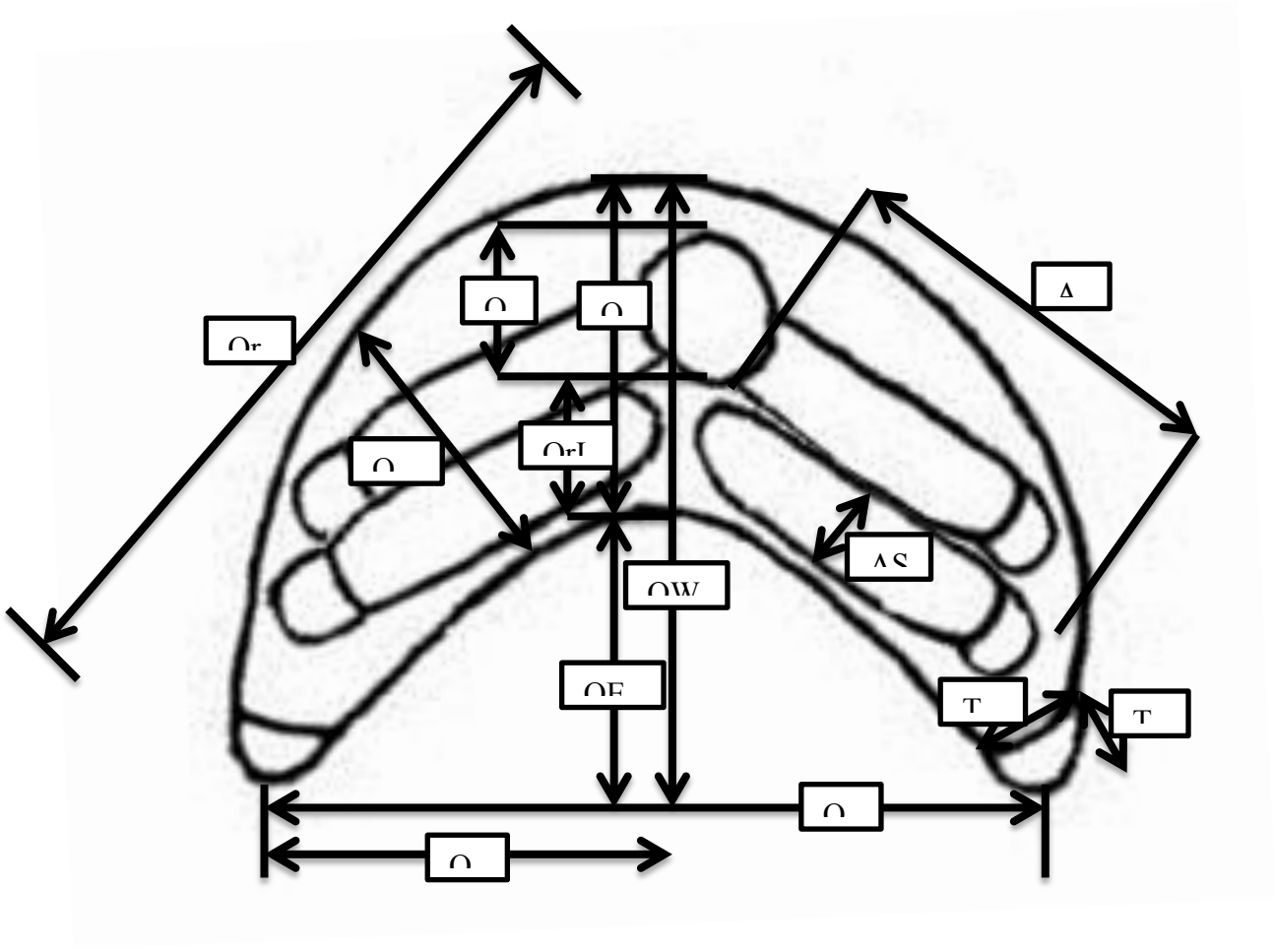

Figure 2. Brightfield and differential interference contrast photomicrographs of trophozoites of (A) *H. acanthatholius,* (B) *Steganorynchus* sp., (C) *Nubenocephalus* sp., and (D) unknown gregarine. Lower case symbols (a and b) higher magnification of epimerites of *H. acanthatholius* and *Steganorynchus* sp. Scale bar = 30 µm.

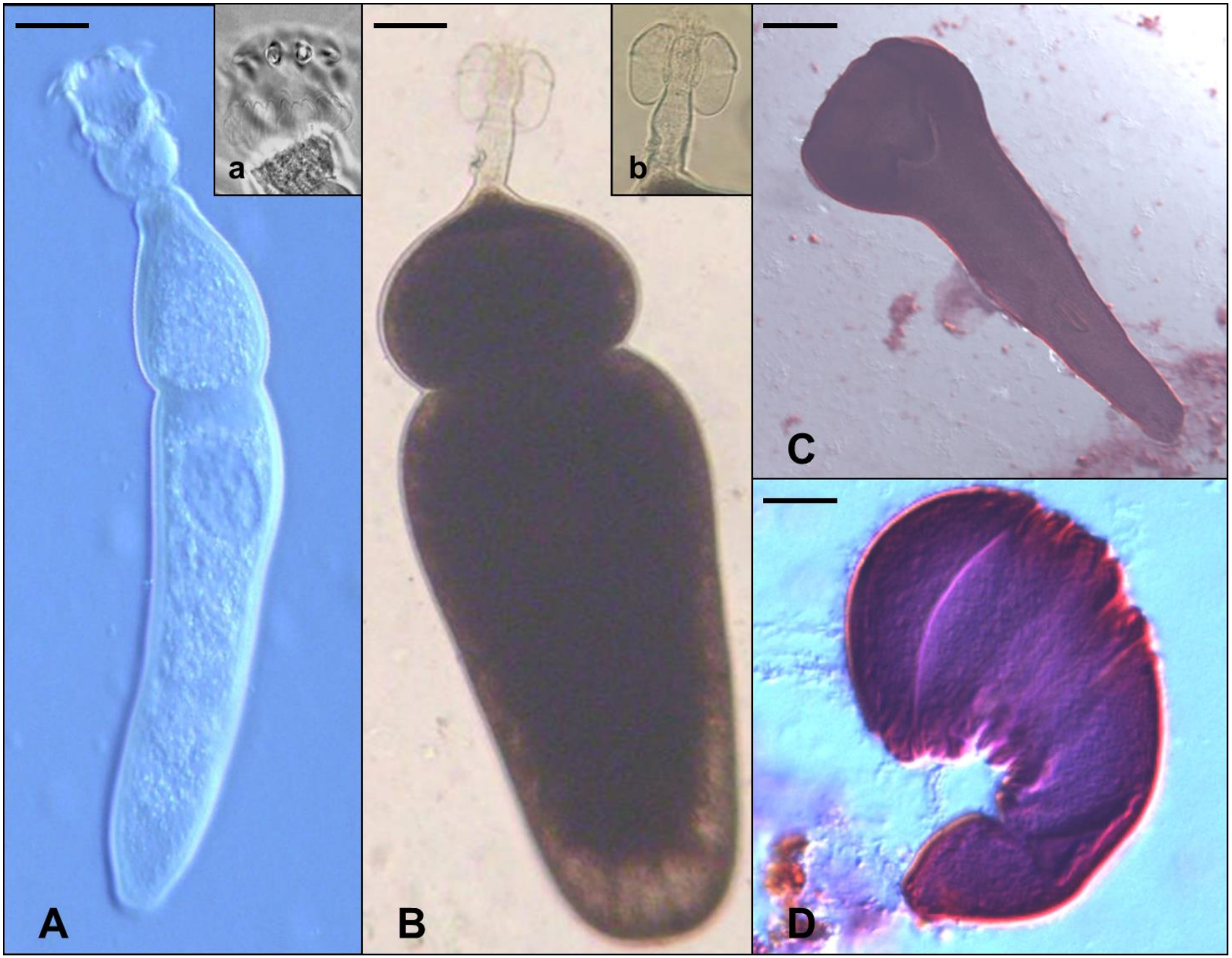
a
b
A
B
C
D

Figure 3. Differential interference contrast photomicrographs of oocysts of *H. acanthatholius* (A-B) and oocysts of an unknown gregarine (C-D). Scale bar = 7 µm for A. Scale bar = 3 µm for B-D.

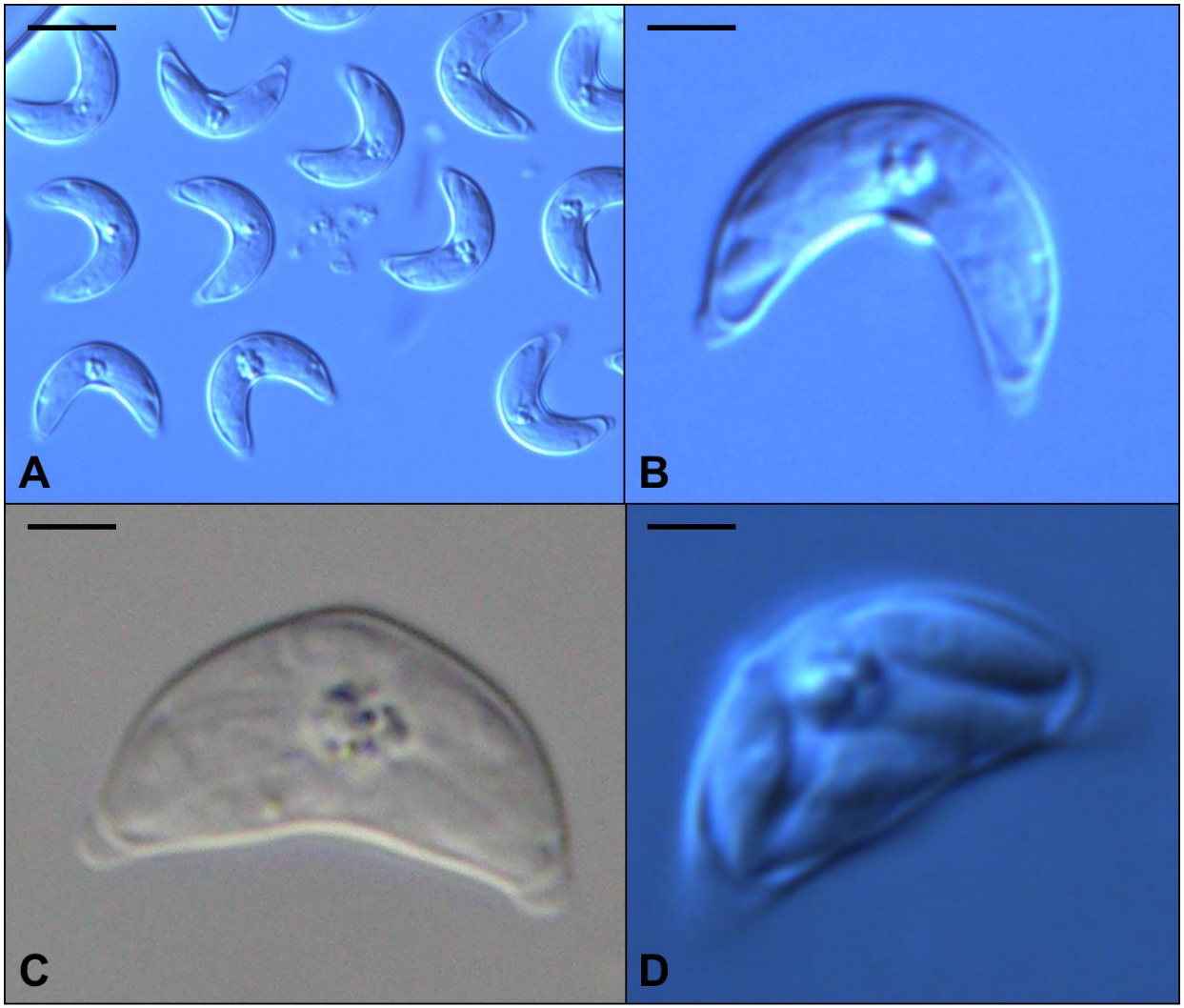
A
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TABLE I. Prevalence (Pr %), mean intensity (MI) ± 1 SD, and mean abundance (MA) ± 1 SD of gregarines recovered from the common bluet, *Enallagma civile*, citrine forktail, *Ischnura hastata*, eastern forktail, *Ischnura verticalis*, and southern spreadwing, *Lestes disjunctus australis*, from Teal Ridge, Stillwater Oklahoma.

Species	*Enallgma civile* N= 278			*Ischnura hastata* N = 140			*Ischnura verticalis* N = 65			*Lestes disjunctus australis* N = 42		
	†Pr (%)	MI ± SD (Range)	MA ± SD	Pr (%)	MI ± SD (Range)	MA ± SD	Pr (%)	MI ± SD (Range)	MA ± SD	Pr (%)	MI ± SD (Range)	MA ± SD
Hoplorynchus acanthatholius	33 (12%)	5.9 ± 10.6 (0–51)	0.7 ± 4.1	1 (0.7%)	0.2 ± 0.4 (0–1)	0.7 ± 0.9	9 (14%)	3 ± 3.9 (0–13)	0.4 ± 1.7	2 (5%)	23 ± 3.5 (0–14)	0.6 ± 2.5
Nubenocephalus sp.	2 (0.72%)	1 ± 0 (0–1)	0.007 ± 0.09	2 (1%)	4 ± 1.4 (0–3)	0.03 ± 0.3	4 (6%)	1 ± 0 (0–1)	0.06 ± 0.2	0 (0%)	-	-
Steganorhynchus sp.	2 (0.72%)	2.5 ± 2.1 (0–4)	0.02 ± 0.3	4 (3%)	1 ± 0 (0–1)	0.04 ± 0.2	0 (0%)	-	-	0 (0%)	-	-
Unidentified Gregarine	1 (0.4%)	20 ± 0 (0–20)	0.1 ± 1.2	1 (0.7%)	2 ± 0 (0–2)	0.02 ± 0.2	0 (0%)	-	-	0 (0%)	-	-

† Number (%) infected.

Table II. Morphological characteristics of oocysts of *Hoplorhynchus acanthatholius* and an unknown gregarine recovered from *E. civile* and *L. d. austrails* collected from Keith Co., Nebraska and Teal Ridge Stillwater, Oklahoma.

Location	Teal Ridge, Oklahoma			Cedar Point, Nebraska	Statistics	
Host Species	*Enallagma civile*	*Lestes d. austrails*	*Enallagma civile*	*Enallagma civile*	H corrected value	P<
Gregarine Species	Unknown Gregarine	*Hoplorhynchus acanthatholius*	*Hoplorhynchus acanthatholius*	*Hoplorhynchus acanthatholius*		
N	30	30	30	30	NA	NA
Length Between Oocyst Knob	13.59 + 1.36^{A} (11.44–16.38)	10.47 + 1.76^{B} (5.95–13.58)	12.64 + 0.54^{C} (11.28–13.52)	13.07 ± 0.84^{D} (11.68–14.71)	65.415	0.0001
Length From Oocyst Equator to Oocyst Knob	6.41 + 0.86^{A} (4.95–7.92)	5.24 + 0.95^{B} (2.66–6.89)	6.18 + 0.32^{C} (5.51–6.84)	6.5 ± 0.46^{D} (5.53–7.6)	46.854	0.0001
Total Width of Oocyst Equator Edge to Terminal Knob	7.64 + 0.94^{A} (5.67–9.7)	8.46 + 1.38^{B} (4–10.6)	10.55 + 0.4^{C} (9.49–11.17)	10.87 ± 0.57^{C} (9.78–11.99)	92.673	0.0001

Oocyst Width at Equator	7.39 + 0.63^{A} (6.41–8.77)	4.3 + 0.7^{B} (3–5.35)	5.30 + 0.17^{C} (4.81–5.69)	4.99 ± 0.59^{D} (4.13–7.79)	92.585	0.0001
Width of Oocyst Midway between Equator and Terminal Knob	5.49 + 0.71^{A} (3.93–6.51)	3.65 + 0.6^{B} (2–4.56)	4.91 + 0.22^{C} (4.54–5.39)	4.29 ± 0.21^{D} (3.81–4.73)	94.156	0.0001
Oocyst Length from Equator to Terminal Knob	0.68 + 0.45^{A} (0.13–1.31)	4.44 + 1.12^{B} (3–8.79)	5.28 + 0.37^{C} (4.4–6.05)	5.99 ± 0.85^{D} (4.45–8.82)	88.397	0.0001
Terminal Knob Length	1.51 + 0.6^{A} (0.82–2.53)	1.11 + 0.23^{B} (0.82–1.77)	1.43 + 0.17^{C} (1.08–1.87)	0.97 ± 0.18^{D} (0.63–1.32)	63.402	0.0001
Terminal Knob Width	1.63 + 0.61^{A} (1.08–2.81)	1.3 + 0.41^{B} (0.88–2.19)	1.64 + 0.18^{C} (1.38–2.13)	1.48 ± 0.19^{D} (1.1–1.87)	22.956	0.0001
Diameter of Oocyst Residuum	2.13 + 0.76^{A} (1.01–3.25)	1.71 + 0.53^{B} (1–2.6)	2.69 + 0.26^{C} (2.1–3.09)	2.33 ± 0.36^{D} (1.82–3.16)	39.228	0.0001
Length from Oocyst Residdum to Inner Curve	3.56 + 0.65^{A} (2.41–4.63)	0.9 + 0.51^{B} (0–2.32)	1.59 + 0.28^{C} (1.1–2.26)	1.28 ± 0.41^{D} (0.57–2.1)	72.099	0.0001
Length from Oocyst	8.2 + 1.19^{A} (6.23–10.1)	7.74 + 1.5^{A} (5–10.63)	9.04 + 0.31^{C} (8.58–9.78)	9.61 ± 0.5^{D} (8.68–10.73)	50.324	0.0001

Residdum to Closest Terminal Knob						
Average Sporozoite Length	5.59 + 1.04^{A} (3.19–7.08)	4.64 + 0.92^{B} (3–5.85)	7.82 + 0.72^{C} (6.03–9.04)	7.22 ± 0.72^{D} (5.76–8.84)	69.903	0.0001
Average Sporozoite Width	1.9 + 0.35^{A} (1.28–2.44)	0.84 + 0.16^{B} (0.62–1)	2.17 + 0.43^{C} (0.98–2.94)	1.66 ± 0.19^{D} (1.18–2.13)	49.793	0.0001
Number of Sporozoites	(4)	(4)	(4)	(4)	NA	NA

Upper case letters represent significant differences among morphological characteristics of oocysts from different damselfly species and population combinations ($P < 0.04$ for all significant differences). NA = not applicable

CHAPTER IV

PARASITE COMMINITY STRUCTURE IN FOUR SPECIES OF DAMSELFLIES (ODONATA: ZYGOPTERA) FROM TEAL RIDGE, STILLWATER OKLAHOMA.

ABSTRACT: Few ecological studies exist on parasite community structure in insects and compared to other invertebrate and vertebrate groups, insects have been largely ignored in ecological studies on parasite community structure. This is surprising because some insects, such as odonates, have become model systems for studies on host parasite interactions, and there is a desperate need for descriptive studies on their parasite community structure. In this study I examined 525 individual tenerals and/or adults of four species of damselflies (*Enallagma civile, Ischnura hastata*, *Ischnura verticalis*, and *Lestes disjunctus australis*) that varied in their development time in the water, temporal and flying season, size and phylogenetic relationships. All damselflies were collected from Teal Ridge a semi-permanent wetland located in Stillwater, Oklahoma during the fall and summer of 2010–2012. The parasite compound community of this odonate assemblage consisted of at least seven taxa including five species of gregarines, two species of helminths, and one species of mite. A total of 549 individual parasites were recovered of which 57% were gregarines, 37% were mites and 6% were helminths. The nematode *Serpinema* cf. *trispinosum* and the gregarine protozoa, *Hoploryncus*

acanthatholius were considered generalists and infected all four species of damselflies. Additionally, none of the individual parasite species were host specific to a single damselfly species and all parasite species infected at least two species of damselflies, however in terms of frequency a majority of mites, *Arrenurus* sp. (94%) and gregarines (73%) infected *E. civile*. Average parasite species richness was low among the four species of damselflies ranging from 0.2 $\pm$ 0.4 (0–2) for *I. hastata* to 0.3 $\pm$ 0.6 (0–2) for *E. civile*. There was no relationship in damselfly size and parasite abundance, intensity, or species richness among any of the damselfly species examined. This study indicates that parasite community structure of damselflies was most similar to the only other study on parasite communities of insects and drastically different in terms of standard measures of parasite community structure in vertebrate hosts which are much higher in terms of parasite prevalence, mean abundance, mean intensity and species richness.

INTRODUCTION

Parasite communities can be classified into different hierarchical levels. An infracommunity includes all parasite infrapopulations within an individual host. The component parasite community includes all the infracommunities within a given host population, whereas the compound parasite community consists of all the parasited infracommunities within a community of hosts (Holmes and Price, 1986; Bush et al., 1997). The comprehensive review by Esch et al. (1990) on parasite community structure indicates that parasite communities of vertebrates and molluscs are highly variable, depauparate, and noninteractive in structure. These studies indicate that important features distinguish invertebrates from vertebrates as hosts for parasites.

First invertebrates are usually smaller than vertebrates, second the life span of most invertebrates is much shorter than vertebrate hosts, and third many invertebrates, particularly molluscs and arthropods, serve as intermediate hosts for parasites that complete their development in a vertebrate host (Kuris, 1990; Bush et al., 1993).

Although descriptive analyses of parasite community structure are now available for a wide variety of vertebrate hosts, few such studies have been conducted on invertebrates. Most studies on parasite communities of invertebrates have concentrated on molluscs and crustaceans and almost nothing is known on the parasite community structure in insect hosts (Kuris, 1990; Bush et al., 1993; Yoder and Coggins, 1998). Recently, Mariluan et al. (2012) examined a community of benthic insects (plecoptera, ephemeroptera, trichoptera and diptera) from Patagonia and reported community parameters for trematode and nematode infections in these hosts. However, what is needed now are studies on a broader range of insects that serve as hosts for a wide variety of endo-parasites and ecto-parasites that utilize arthropods not only as intermediate hosts but also as definitive hosts. One such group of insects is the Odonata suborder Zygoptera (Percival et al., 1995; Clopton, 1995; Clopton, 2004; Clopton et al., 2010; Hays, et al., 2007; Lajeunesse, 2007; Bolek and Janovy, 2007a; 2007b; Bolek et al., 2009; 2010; Wiles and Reyda, 2011a; 2011b).

Damselflies (suborder Zygoptera) are carnivorous aquatic based insects that spend part of their life cycle in the water and the other part of their life cycle in a terrestrial environment (Corbet, 1999). Their carnivorous nature and distribution in aquatic and terrestrial habitats makes damselflies ideal hosts for a wide range of endo and ecto-parasites. Surveys indicate that these insects serve as hosts for a wide

variety of protozoan, platyhelminth, nematode, nematomorph, mite and dipteran parasites (Corbet, 1999). However, most surveys of damselfly parasites have concentrated on specific parasite species descriptions such as gregarine protozoa (Cielocha et al., 2011), or in life cycle studies of digentic trematodes of vertebrate hosts (Bolek et al., 2009; 2010) and no studies have examined multiple species of damselflies from a single location for their parasite assemblages. A recent review by Baker (2011) summarized all the recent publications on odonate parasite life cycles, and the ecology and behavior of odonate parasite interactions. However, to my knowledge no parasite community studies exist on damselfly parasites.

Previous surveys of multiple species of damselflies for gregarines, digeneans and mites indicate that some parasite species appear to be specialists where as others are considered generalists (Lejeunesse, 2007; Bolek et al., 2009; 2010; Cielocha et al., 2011). These studies suggest that distinct life histories of damselflies and their parasites probably play a major role in parasite distribution in their zygopteran hosts. These studies also suggest that damselflies may be ideal insect hosts for comparative parasite community studies.

In Oklahoma, the common bluet, *Enallagma civile* (Hagen, 1861), the citrine forktail, *Ischnura hastata* (Say, 1839), the eastern forktail, *Ischnura verticalis* (Say, 1839), and the northern spreadwing, *Lestes disjunctus australis* (Selys, 1862), occur sympatrically. Among the four species of damselflies examined in this study, three species are considered pond damselflies (family Coenagrionidae); whereas one species belongs to the spreadwings (family Lestidae). Both families of damselflies

and all four species differ significantly in the biology and life histories. Therefore these species make ideal subjects for comparative parasite community studies.

Most coenagrionids over winter as larvae and emerge once or twice a year (Dmitriew and Rowe, 2005). In contrast, lestids only emerge once a year and over-winter as eggs in the aquatic vegetation, their larvae developing much more rapidly than larvae of coenagrionids, spending only 2–3 months in their larval stage (Krishnaraj and Pritchard, 1995). Mature adult males of coenagrionids and lestids tend to spend most of their time around the water searching for mates and/or mating; whereas adult females of these two families of damselflies spend the majority of their time away from the water where they forage for food (Forbes, 1991).

The northern spreadwing *L. d. australis*, was the largest damselfly sampled during this study. *Lestes d. australis* is found across the United States at temporary ponds and has a flying season from March to December (Abbott, 2005). Common bluets, *E. civile*, are medium sized bright blue colored damselflies, have a cosmopolitan distribution across the United States. Their flight season lasts all year, flying around ephemeral and permanent ponds, lakes, and slow moving streams. In contrast, the citrine forktail, *I. hastata*, damselfly is the smallest of all the damselflies sampled in this study and has a flight season of year round being found at heavily vegetated lakes and ponds across the United States (Abbott, 2005). Finally, the eastern forktail, *I. verticalis*, a brightly colored blue, black, and green damselfly is smaller than the common bluet but larger than the citrine forktail. One of the most commonly found damselflies in the United States, *I. verticalis* can be found at ponds, lakes, slow moving streams, and marshes habitats. These damselflies have one of the

shortest flight seasons, flying only from April to September (Abbott, 2005). Since these four damselfly species vary in body size, habitat preference in the larval and adult stages, and flight season (Abbott, 2005), I was interested in elucidating what role, if any, differences in larval development time in the pond, host habitat, age, size, and flight season play in determining parasite populations and communities of these Oklahoma damselflies. In addition, I was interested in comparing characteristics of parasite communities of this damselfly assemblage with other studies on vertebrate and invertebrate parasite communities.

MATERIALS AND METHODS

Description of damselfly collection, mite collecting and necropsy

During September 2010–September 2012 525 teneral and adult damselflies of four species representing two families were collected from Teal Ridge Stillwater, Payne County, Oklahoma (N 36° 6' 1.44" W 97° 4' 51.405; Table I). All damselflies were collected between 9:00 AM and 6:00 PM with an aerial net and place in a 1 liter plastic jar and stored on ice until brought back to the laboratory. In the laboratory all damselflies were identified to species, stage and sex based on descriptions and keys in Abbott (2005; 2011), Westfall and May (2006), and May and Dunkle (2007).

After identification total body length (TL) and head width (HW) were recorded for each individual damselfly to the nearest 1.0 mm and 0.1 mm, respectively. Damselflies were then examined for mites under a stereo microscope. Any mites were removed with forceps and placed into vials of 70% ethanol. Each damselfly was then placed individually, abdomen down, in glass vial filled with two ml of water in order to collect damselfly fecal matter for gregarine gametocysts.

After 24 to 36 hours passed each teneral or adult zygopteran was then removed from the vile and killed by removing the head. At necropsy, individual damselflies were placed in odonate saline and the abdominal sterna were peeled back. The entire gut was removed and gently teased apart with forceps on a microscope slide, and examined for intestinal parasites. The rest of the body of individual damselflies was then divided into three regions: the head, thorax including the legs, and the abdomen; each body region was teased apart with forceps and examined for parasites.

All gregarine trophozoites were prepared as permanent slides by drying the gut content on slides and fixing the in alcohol–formaldehyde–acetic acid (AFA); slides were then stained with acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam. All nematodes and mites were fixed in 70% ethanol cleared in glycerol and examined as temporary mounts; and all trematodes were fixed in hot AFA or formalin and trematodes were stained with acetocarmine, dehydrated in a graded ethanol series (70–100%), cleared in xylene, and mounted in Canada balsam according to Pritchard and Kruse (1982).

Damselfly Processing for Gametocysts and Oocysts

After 36–48 hrs, damselfly fecal content was searched for gametocysts and any gametocysts found were removed and measured with a ruler to the nearest mm and photographed placed. All gametocysts were then placed in 1.5 ml plastic wells filled with aged tap water and watched daily for oocyst dehiscence. Any oocysts that were collected from the dehised gameotcysts were placed onto a wet mount slide and photographed with an Olympus BX-51 upright research microscope. All measurements are reported in chapter III.

Morphological characteristics for identification and damselfly parasite community infection parameter

All gregarines were identified based on descriptions in Hays et al. (2007) and Percival et al. (1995); all trematode metacercariae were identified based on descriptions in Bolek et al. (2010); nematodes were identified based on descriptions by Baker (1979), Bartlett and Anderson (1985), and Moravec and Vargas-Vasquez (1998a; 1998b) and mites were identified to genus based on descriptions in Zawal (2008).

Prevalence, mean intensity, and mean abundance are according to Bush et al. (1997). Mean parasite species richness is the sum of parasite species per individual damselfly, including noninfected individuals, divided by the total sample size. All values are reported as the mean $\pm$ 1 SD. Because variances were heteroscedastic, the Kruskal–Wallis test and the Kolmogorov–Smirnov 2-sample test were used to compare differences in mean abundance, mean intensity and mean parasite species richness among different host species. The chi-square test for independence was calculated to compare differences in prevalence; whereas Student's *t*-test was used to compare differences in mean abundance, mean intensity and mean parasite species richness between male and female damselflies. Man-Whitney U tests were calculated when variances were heteroscedastic (Sokal and Rohlf, 1981). Pearson's correlation was used to determine relationships among host TL and HW and intensity and abundance of parasites. Pearson's correlation was calculated for host TL and HW and parasite species richness per individual damselfly. Voucher and type specimens of parasites and damselfly hosts will be deposited in the H. W. Manter Parasitology

Collection, University of Nebraska, Lincoln, Nebraska and the K.C. Emerson Entomology Museum, Oklahoma State University-Stillwater, respectively.

RESULTS

A total of 278 adult common bluet damselflies *E. civile* (138 female and 140 male), 140 citrine forktailk damselflies *I. hastata* (82 adult females and 58 adult males), 65 adult eastern forktail damselflies *I. verticalis* (41 female and 24 male), and 42 adult northern spreadwing damselflies, *L. d. australis* (14 female and 45 male) were collected from Teal Ridge. Of the four damselfly species collected *L. d. australis* was the longest (39.73 mm $\pm$ 2.84; 30–44) followed by *E. civili* (32.86 mm $\pm$ 2.29; 22–40), then *I. verticalis* (24.19 $\pm$ 3.07, 15–35) whereas *I. hastata* was the shortest in length (22.95 mm $\pm$ 1.93; 15–27). *Lestes d. australis* had the widest heads (4.47 mm $\pm$ 0.58; 3–5) followed by *E. civili* (3.48 mm $\pm$ 0.47; 2–4.5), then *I. verticalis* (2.79 $\pm$ 0.39, 2–4) whereas *I. hastata* had the shortest HW (2.44 mm $\pm$ 0.45; 2–3.5). The Kruskal–Wallis 1-way analysis of variance revealed significant differences in BL and HW among species of damselflies (H corrected = 389.48; $P <$ 0.001; H corrected = 309.803; $P < 0.001$). The Kolmogorov–Smirnov 2-sample tests showed that all possible host species pairs differed significantly ($P < 0.05$) in TL and HW.

There was no statistically significant difference in the average TL of female and male *L. d. australis* (t = -0.87; P = 0.39) or female and male *I. verticalis* (t = 0.79; P = 0.43). However, there was a statistically significant difference in the average TL of female and male *I. hastata* and female and male *E. civile* (t = 2.003; P = 0.047; t = -2.23; P = 0.03), with female *I. hastata* being significantly longer (23.2 mm $\pm$ 2.2;

15–27) than male *I. hastata* (22.6 mm ± 1.4; 20–27) and male *E. civile* being significantly longer (33.2 mm ± 2.1; 23–38) than female *E. civile* (32.6 mm ± 2.5; 22–40). There was no statistically significant difference in the average HW of female and male *L. d. australis* (t = 1.36; P = 0.18), female and male *E. civile* (t = -1.386; P = 0.17), or female and male *I. verticalis* (t = -0.64; P = 0.52). However, there was a statistically significant difference in the average HW of female and male *I. hastata* (t = 4.432; P = 0.0001), with female *I. hastata* having significantly wider heads (2.6 mm ± 0.45; 2–3.5) than male *I. hastata* (2.3 mm ± 0.37; 2–3).

The parasite compound community of this damselfly assemblage consisted of at least seven species of parasites including four species of gregarines, two species of helminths, and one species of mite (Table I). A total of 549 parasites were collected from these damselflies, consisting of five species with direct life cycles and two species with indirect life cycles. Of the 525 damselflies collected 116 (22%) were infected with at least one species of parasite. Of the 278 *E. civile* examined 76 (12 tenerals and 266 adults; 27%) were infected with parasites. The component community consisted of six parasite species; four species of gregarines, one species of helminth, and one species of mite. The overall parasite mean abundance and mean intensity per infracommunity was 1.5 ± 5.1 and 6.8 ± 9.0 respectively. Totals of 228 individual gregarines (53%), 189 mites (44%) and 11 helminths (3%), were found with infracommunities being dominated by gregarines. Prevalence was highest (11%) for the gregarine *Hoplorynchus acanthatholius*, and lowest for an unidentified gregarine at 0.04% (see Table I). The mean species richness for *E. civile* was 0.3 ±

0.6. Multiple parasite species infections were uncommon with 0, 1, and 2 species occurring in 202, 69, and 7 damselflies, respectively.

Of the 140 *I. hastata* examined 17 (12 %) were infected with parasites. The component community consisted of six parasite species; four gregarine species and two helminths. The overall parasite mean abundance and mean intensity per infracommunity was 0.3 $\pm$ 0.9 and 1.9 $\pm$ 1.9 respectively. Totals of 29 individual gregarines (78%) and 8 helminths (22%) were found with infracommunities being dominated by gregarines. Prevalence was highest (4%) for the gregarine *Hoplorynchus acanthatholius*, being 4%, and lowest (0.01%) for an unidentified gregarine (Table I). The mean species richness for *I. hastata* was 0.2 $\pm$ 0.4. Multiple parasite species infections were uncommon with 0, 1, and 2 species occurring in 123, 15, and 2 damselflies, respectively.

Of the 65 *I. verticalis* collected 15 (23 %) were infected with parasites. The component community consisted of five parasite species; two gregarines, two helminths, and one mite species. The overall parasite mean abundance and mean intensity per infracommunity was 0.8 $\pm$ 2.2 and 4.1 $\pm$ 4.3 respectively. Totals of 30 individual gregarines (60%), 13 mites (26%) and 7 helminths (14%), were found with infracommunities being dominated by gregarines. Prevalence was highest (14%) for the gregarine species *Hoplorynchus acanthatholius*, and lowest (0.01%) for metacercariae of *Halipegus* sp. (Table I). The mean species richness for *I. verticalis* was 0.3 $\pm$ 0.6 with 0, 1, and 2 species occurring in 50, 9, and 6 damselflies, respectively.

Of the 42 *L. d. australis* collected 8 (19 %) were infected with parasites. The component community consisted of two parasite species; one gregarine species and one helminth species. The overall parasite mean abundance and mean intensity per infracommunity was 0.8 $\pm$ 2.6 and 4.3 $\pm$ 4.8 respectively. Totals of 25 individual gregarines (74%) and 9 helminths (26%) were found with infracommunities being dominated by gregarines. Prevalence was highest (10%) for the nematode *Serpinema* cf. *trispinosum* and lowest (5%) for the gregarine *Hoplorynchus acanthatholius* (Table I). The mean species richness for *L. d. australis* was 0.3 $\pm$ 0.6, with 0 and 1 species occurring in 34 and 8 damselflies, respectively.

There were no significant correlations in damselfly total body length or head width and intensity or abundance for any parasite species or for parasite species richness in any of the four species of damselflies ($P > 0.05$). However, the Kruskal–Wallis 1-way analysis of variance revealed significant differences in total parasite mean intensity among species of damselflies (H corrected = 8.518; $P < 0.036$). The Kolmogorov–Smirnov 2-sample tests showed that the only species pair that differed significantly was *E. civile* and *I. hastata* ($\chi 2 = 9.979$; $P < 0.01$).

There were no significant differences in prevalence, mean abundance, or mean intensities for any parasite species, total parasites, diet parasites, non-diet parasites or mean parasite species richness between male and female damselflies for any of the damselfly species ($P > 0.05$; Table II). However, except for mean intensity in *L. d. australis* prevalence, mean abundance and mean intensity was always higher in female damselflies for diet parasites than male damselflies (Table II).

In terms of the parasites recovered, mites were restricted to two species of damselflies in the family coenagrionidae (*E. civile* and *I. verticalis*), whereas the nematode *Serpinema* cf. *trispinosum* infected all four species of damselflies and was considered a generalist parasite. Among the gregrine protozoa, *Hoploryncus acanthatholius* was considered a generalist and infected all four species of damselflies from both families; whereas the other three groups of gregarines and the trematode *Halipegus* sp. were restricted to two or three species of coenagrionid damselflies and did not infect *L. d. australis* (see Table I).

DISCUSSION

Only one other study has examined the parasite community structure in insects (Mariluan et al., 2012). The parasite communities of Oklahoma damselflies examined in this study was similar to the previous study by Mariluan et al. (2012) in that the parasite communities of these insect species were depauperate and isolationist in nature. Most parasite species recovered in this study did not show strict host specificity and have been reported in a range of damselfly species (Bolek et al., 2010; Cielocha et al., 2011; Lajeunesse, 2004; Lajeunesse, 2007). This is similar to previous studies on helminth communities of vertebrate hosts were generalist parasites dominated the host parasite communities (Esch et al., 1990).

However, when comparing the parasite community structure of damselflies from this study to previous studies on parasite communities in vertebrate hosts, parasite communities of damselfly hosts were vastly different for standard measures of parasite community structure in vertebrate hosts. For example, average species

richness, for helminths parasites of fish, amphibians, reptiles, birds and mammals range from 1.2 for freshwater fish to as high as 7.4 for birds (Esch, et al., 1990). Additionally, overall parasites prevalence and mean abundance is also much higher in vertebrate hosts than in insect hosts such as damselflies, ranging from 49% and 34 for freshwater fish to as high as 99% and 400 for birds respectively (Esch et al., 1990; Kennedy et al., 1986; Mariluan et al., 2012; this study). One explanation for this is that vertebrate hosts are much larger than damselflies, and are at the top of the food chain and commonly serve as definitive hosts for helminth parasites (Esch et al., 1990). Unlike vertebrate hosts, few helminths (with the exception of nematomorphs and some nematodes) mature in odonate and other groups of insects in general suggesting that insect host captured a number of trematode species from vertebrates (see Bolek, 2006; Bolek et al., 2010). Finally, although damselflies and other insect groups are also top predators in aquatic ecosystems, the breath of diet that these invertebrates sample is much narrower compared to their vertebrate counterparts suggesting that these innate differences in insect and vertebrate predators may explain differences in their parasite community structure (see Esch et al., 1990).

Overall there were few differences found in the community structure between the four species of damselflies examined at this wetland. The one exception to this was that there was a significant difference in overall parasite mean intensity among two damselfly species. However, this pattern was not straight forward because a significant difference only occurred between the smallest damselfly, *I. hastata,* having a significantly lower overall parasite mean intensity from *E. civile* a medium sized species which had a significantly higher overall parasite mean intensity.

Additionally, none of the individual parasite species were host specific to a single damselfly species and all parasite species infected at least two species of damselflies. Therefore, the distribution of parasite community structure in terms of overall parasite prevalence, mean intensity, mean abundance or species richness did not clearly differ in damselfly species that differed in their size, larval developmental time in the pond or flight season at Teal Ridge. However, in terms of frequency a majority of mites, *Arrenurus* sp. (94%) and gregarines (73%) infected *E. civile*. These data are similar to the only other study on parasite community structure in aquatic insects by Marilium, et al. (2012). For example, of the nine insect taxa examined by Marilium et al. (2012) not a single taxan harbored more than one helminth species. Prevalence and mean intensities was also low in these insect hosts ranged from 0–7% and 0–4.9 for the nine taxa examined.

One interesting observation in this study was the lack of host specificity exhibited by damselfly gregarine parasites. Most of the gregarine parasites from this studied infected at least two of the four damselfly species examined, with *H. acanthatholius* infecting all four damselfly species. For most of the past century gregarines have been hypothesized as being host specific, with the possibility that every insect species could harbor their own unique host specific gregarine, thereby potentially making gregarines the most specious parasite (Levine, 1979). While laboratory cross-infections of these parasites in some insect groups such as beetles and cockroaches have yielded results indicating strict host specificity (Clopton et al., 1992; Clopton and Janovy, 1993) there have been arguments against the gregarines host specific traits in some field studies. Recent field studies on damselfly gregarines

indicate that some species of gregarine can infect multiple species of damselflies in a single genus (Cielocha et al., 2011; Clopton, 2009). My study adds to this observation and indicates that some greagrine species can infect multiple genera and families of damselflies.

The second group of parasites that exhibited interesting distribution on damselfly hosts in this study was mites. Mites infected two species of damselflies (*E. civile* and *I. verticalis*) at Teal Ridge. However, in terms of frequency most mites (94%) were recovered from *E. civile* and only three *I. verticalis* were infected with mites. Although host specificity cannot be ruled out, one explained for this observation may be the fact that some mite species prefer the most populous damselfly species at the pond they reside in (Lajeunesse, 2004). It is also possible that mite emergence and the emergence of *E. civile* also overlapped more so than the emergence times of the other species of damselflies at Teal Ridge, therefore making *E. civile* individuals more susceptible to mite infestations (Rolff et al., 2001).

There were also no significant differences in parasite community structure among male and female damselflies for any parasite community parameters. However, although not significant, female damselflies had a higher prevalence, mean abundance and mean intensity (except for *L. d. australis*) for diet parasites than male damselflies. One explanation for this observation is that female damselflies consume more food than male damselflies for egg production (Anholt, 1997). Studies on differences in feeding rates of male and female odonates have suggested that due to the higher feeding rate of female damselflies, females have a higher risk of exposure to parasitism especially if the parasites are acquired through the diet (Corbet, 1999).

My study does not support this hypothesis, because no significant differences were found in any parasite community parameters among male and female damselflies in this study. However, due to the very low infection rate of most parasites in damselflies at Teal Ridge more studies need to be conducted in other regions and species of damselflies to confirm this observation.

The individual parasite species and overall parasite prevalence, mean abundance, and mean intensity were very low at this pond. These observations are in contrast to previous studies on gregarines, helminths and mites of damselflies which reported much higher prevalence, mean intensities and mean abundances than in the current study (Locklin and Vodopich, 2010, Cielocha *et al*., 2011, Bolek *et al*, 2010, Mariluan, *et al.*, 2012). Many of these studies found that prevalence of parasites in damselflies ranged from 20% to 100%. One explanation for these drastic differences in the low prevalence, mean intensity and mean abundance of parasites of damselflies from Teal Ridge and previous studies could be that one of the most severe droughts occurred in Oklahoma during my study. Most ponds at Teal Ridge dried up during the summer months, stayed dried during the summer and only contained water in early spring and again in fall (personal observations). A previous study by Gerard (2001) indicated how droughts can affect parasite community structure. In his study Gerard (2001) examined the composition of trematode communities in an aquatic snail host before and during a major drought in France. His study indicated that the drought not only affected the snail population but also affected the trematode community structure in their snail hosts. In his study, trematode communities decreased from 10 species to four species during pre-drought and drought conditions,

respectively. Additionally, overall trematode prevalence decreased in the snail hosts from 5% during pre-drought conditions to a low of 0.7% during the drought. Gerard (2001) hypothesized that the caused for the decrease in prevalence and species richness was due to unfavorable environmental conditions during the drought that increased mortality rates in the snail hosts and providing a much smaller host population for these trematodes to infect and continue their life cycle. This previous study suggests that environmental changes such as droughts may reduced the transmission rates of parasites in the damselfly system at Teal Ridge during my study. The low prevalence and intensities of *Halipagus* sp. metacercariae infecting damselflies compared to other similar studies (Bolek et al., 2010) suggest that this pond experienced a reduction in snail population, decreasing the transmit of *Halipegu*s sp. to the paratenic damselfly hosts. Clearly, studies on damselfly parasite communities during no-drought years at Teal Ridge need to be conducted to test this hypothesis.

In conclusion this study has revealed many new questions about the parasite community structure in damselfly hosts. This survey has expanded the range for gregarine, mite and helminth parasites in Oklahoma damselflies and has expanded the known host range for many of these parasites including *L. d. australis* as a new host for the gregarine *H. acanthatholius* and all four species of damselfly as new paratenic hosts for the nematode *S.* cf. *trispinosum.* Finally, this study puts into question studies on gregarine host specificity indicating that gregarine species can not only infect multiple species of damselflies in the same genus, but also multiple genera of damselflies within a single family and across different families of damselflies. It is

my hope that the new questions that have arisen from this study will stimulates further studies on parasites communities of damselflies. One major problem in our understanding of the organization of parasite communities in damselflies is the lack of knowledge on life cycles of damselfly parasites. For example, the life cycle of *S. trispinosum* and all of the gregarine species discovered during this study have yet to be completed in the laboratory. Such life cycle studies would allow us to test host specificity of the parasites of damselflies and to test some of the observations on the distribution of parasites in damselflies made in the present study.

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TABLE I. Prevalence (Pr %), mean intensity (MI) ± 1 SD, and mean abundance (MA) ± 1 SD of parasites recovered from the common bluet, *Enallagma civile*, citrine forktail, *Ischnura hastata*, eastern forktail, *Ischnura verticalis*, and northern spreadwing, *Lestes disjunctus australis*, from Teal Ridge, Stillwater Oklahoma.

Species	*Enallgma civile* N= 278			*Ischnura hastata* N = 140			*Ischnura verticalis* N = 65			***Lestes disjunctus australis*** **N = 42**		
	†Pr (%)	MI ± SD (Range)	MA ± SD	Pr (%)	MI ± SD (Range)	MA ± SD	Pr (%)	MI ± SD (Range)	MA ± SD	Pr (%)	MI ± SD (Range)	MA ± SD
Gregarine												
Hoplorynchus acanthatholius	33 (12%)	5.9 ± 10.6 (0–51)	0.7 ± 4.1	1 (0.7)	0.2 ± 0.4 (0–1)	0.7 ± 0.9	9 (14)	3 ± 3.9 (0–13)	0.4 ± 1.7	2 (5)	23 ± 3.5 (0–14)	0.6 ± 2.5
Nubenocephalus sp.	2 (0.72)	1 ± 0 (0–1)	0.007 ± 0.09	2 (1)	4 ± 1.4 (0–3)	0.03 ± 0.3	4 (6)	1 ± 0 (0–1)	0.06 ± 0.2	0 (0)	-	-
Steganorhynchus sp.	2 (0.72%)	2.5 ± 2.1 (0–4)	0.02 ± 0.3	4 (3)	1 ± 0 (0–1)	0.04 ± 0.2	0 (0)	-	-	0 (0)	-	-
Unidentified Gregarine	1 (0.4%)	20 ± 0 (0–20)	0.1 ± 1.2	1 (0.7)	2 ± 0 (0–2)	0.02 ± 0.2	0 (0)	-	-	0 (0)	-	-
Trematoda												
Halipegus sp.	0 (0)	-	-	3 (2)	3 ± 0 (0–1)	0.04 ± 0.2	1 (2)	1 ± 0 (0–1)	0.02 ± 0.1	0 (0)	-	-
Nematoda												
Serpinema cf. *trispinosum*	7 (3%)	1.6 ± 1.5 (0-5)	0.04 ± 0.3	4 (3)	5 ± 0.5 (0-2)	0.04 ± 0.2	5 (8)	0.3 ± 0.5 (0-2)	0.1 ± 0.3	4 (10)	2.3 ± 1.5 (0-4)	0.2 ± 0.8

Mites												
Arrenurus sp.	30 (11%)	6.3 ± 5.8 (0-19)	0.7 ± 2.7	0 (0)	-	-	3 (5)	4.3 ± 1.5 (0-10)	0.2 ± 1.2	0 (0)	-	-

† Number (%) infected.

TABLE II. Prevalence (Pr %), mean intensity (MI) ± 1 SD, mean abundance (MA) ± 1 SD of diet, non-diet and total parasites and mean parasite species richness (SR) + 1 SD recovered from female and male common bluets, *E. civile*, citrine forktails, *I. hastata*, eastern forktails, *I. verticalis*, and northern spreadwings, *L. d. australis*, from Teal Ridge, Stillwater Oklahoma.

Species	*Enallagma civile*		*Ischnura hastata*		*Ischnura verticalis*		*Lestes disjunctus australis*	
	Male	Female	Male	Female	Male	Female	Male	Female
	N= 140	N= 136	N= 58	N= 82	N= 24	N= 41	N=25	N= 17
Diet Parasites								
Pr (%)†	18 (13%)	28 (20%)	5 (9%)	16 (20%)	6 (25%)	12 (29%)	3 (12%)	5 (29%)
MA + SD	0.4 ± 2.0	1.3 ± 5.7	0.1 ± 0.4	0.4 ± 1.2	0.3 ± 0.9	0.7 ± 2.2	0.8 ± 2.9	0.9 ± 2.2
MI ± SD (Range)	3.3 ± 4.9 (0–20)	6.4 ± 11.5 (0–51)	1.2 ± 0.5 (0–2)	2.1 ± 2.1 (0–9)	2 .0± 1.4 (0–4)	3.2 ± 4.0 (0–13)	6.3 ± 6.8 (0–14)	3.0 ± 3.5 (0–9)
Non-Diet Parasites (Mites)								
Pr (%)†	15 (11%)	15 (11%)	0	0	3 (13%)	0	0	0
MA + SD	0.6 ± 2.4	0.8 ± 3.0	-	-	0.5 ± 2.1	-	-	-
MI ± SD (Range)	5.7 ± 5.0 (0–16)	6.9 ± 6.7 (0–19)	-	-	4.3 ± 4.9 (0–10)	-	-	-
Total Parasites								
Pr (%)†	33 (24%)	43 (31%)	5 (9%)	16 (20%)	9 (38%)	12 (29%)	3 (12%)	5 (29%)
MA + SD	1.0 ± 3.3	2.1 ± 6.4	0.1 ± 0.4	0.4 ± 1.2	0.9 ± 2.2	0.7 ± 2.2	0.8 ± 2.9	0.9 ± 2.2
MI ± SD (Range)	4.4 ± 5.0 (0–20)	6.6 ± 10.0 (0–51)	1.2 ± 0.5 (0–2)	2.1 ± 2.1 (0–9)	3.5 ± 3.3 (0–10)	3.2 ± 4.0 (0–13)	6.3 ± 6.8 (0–14)	3.0 ± 3.5 (0–9)
SR ± 1 SD	0.3 ± 0.5	0.3 ± 0.5	0.1 ± 0.4	0.2 ± 0.5	0.4 ± 0.7	0.3 ± 0.6	0.1 ± 0.3	0.3 ± 0.5

† Number infected.

CHAPTER V

CONCLUSION

Although descriptive analyses of parasite community structure are now available for a wide variety of vertebrate hosts, few such studies have been conducted on invertebrates like damselflies. Surveys indicate that these insects serve as hosts for a wide variety of protozoan, platyhelminth, nematode, nematomorph, mite and dipteran parasites (Corbet, 1999). However, most surveys of damselfly parasites have concentrated on specific parasite species descriptions such as gregarine protozoa (Cielocha et al., 2011), or in life cycle studies of digentic trematodes of vertebrate hosts (Bolek et al., 2009; 2010) and no studies have examined multiple species of damselflies from a single location for their parasite assemblages. However, to my knowledge no parasite community studies exist on damselfly parasites. During the course of this study I collected 525 damselflies representing four species of damselflies across two different families. A total of 549 parasites were collected from these damselflies, consisting of five species of parasites with direct life cycles and two species of parasites with indirect life cycles.

In chapter II, I provide new host and geographical distribution information for juvenile and adult *S.* cf. *trispinosum* from damselfly and turtle hosts from Oklahoma

along with new morphological measurements for juvenile and adult male and female *S. trispinosum*. My study reports *S.* cf. *trispinosum* in damselflies for the first time. Along with infecting a wide range of turtle definitive hosts that vary in their diet and habitat, this nematode also infects a wide range of paratenic hosts including fish, frogs, snails, and damselflies. Dietary studies of the 18 known turtle definitive hosts indicate that all four groups of paratenic hosts have been reported in the diet contents of most of these turtles. However, larval and adult odonates are the most commonly reported food item in 14 of the 18 turtle species reported as definitive hosts for this nematode and odonates can make up to 50% of the frequency of the diet in some species of turtles which serve as definitive hosts for this nematode. Additionally, microcrustaceans, such as copepods which serve as first intermediate hosts for *S. trispinosum*, are the predominant food items of larval damselflies (Corbet, 1999; Bolek et al., 2010) suggesting that these insects come in contact with *S. trispinosum* more commonly than snail or amphibian paratenic hosts which feed on microcrustaceans less commonly. Finally, this study found that both teneral and adult damselflies were infected with this nematode in this study, indicating that these nematodes survive the molting process from larval damselfly to adult damselfly and provides a more plausible mechanism of how semi-terrestrial turtles such as *Terrapene carolina* become infected with this nematode. While fish do consume microcrustaceans as apart of their diet, most turtle diet studies reveled that most species of turtles which serve as definitive hosts for *S. trispinosum* rarely consume fish or feed on fish carrion. My study provides more information on this life cycle and indicates that this life cycle needs to be worked out in the laboratory.

In chapter III I provide new information on hosts, geographical distribution and morphological data on oocysts of gregarine parasites. My study provides new information on gregarine parasites of Oklahoma damselflies and expands the known host range for many of these. This study puts into question previous studies on gregarine host specificity indicating that gregarine species infect multiple species of damselflies in the same genus, damselflies in different genera and across different families of damselflies. Additionally, my work indicates that gregarine oocyst may be polymorphic, similar to their coccidian relatives. My morphological analysis of oocysts of *H. acanthatholius* from different species and populations of damselflies suggests that a molecular approach along with life cycle studies may be necessary for species identification of damselfly gregarines.

In chapter IV I describe the parasite community structure in four species of damselflies which differed in their size, time spent in the pond during their larval stage, habitat, and flight season. In comparison to the only other study on parasite communities of insects (Mariluan et al., 2012) my study was similar in terms of overall low parasite prevalence, intensities and parasite species richness. These studies suggest that parasite communities of insects are depauparate and isolationist in nature. Additionally, my study indicates that there was no host specificity for any of the parasites found in the four damselfly species examined. Comparisons of parasite communities of this damselfly assemblage with other studies on vertebrate parasite communities suggests similarities to other vertebrate helminths community studies, were generalist parasites are more common in host parasite communities. However, there were major differences between this study and previous studies on

parasite communities of vertebrates. Overall parasites prevalence and mean abundance was much higher in vertebrate hosts ranging from 49% and 34 for freshwater fish to as high as 99% and 400 for birds than for parasite communities of damselflies in this study (Esch et al., 1990; Kennedy et al., 1986). One explanation for this drastic difference is that vertebrate hosts are much larger than damselflies, and are at the top of the food chain and commonly serve as definitive hosts for helminth parasites (Esch et al., 1990). Unlike vertebrate hosts, few helminths (with the exception of nematomorphs and some nematodes) mature in odonate and insect hosts in general suggesting that damselflies host captured a number of trematode species from vertebrates (see Bolek, 2009; Bolek et al., 2010). Finally, although damselflies, like many vertebrates, are top predators in aquatic ecosystems, the range of their diet is much narower compared to their vertebrate host counterparts suggesting that these innate differences in insect and vertebrate predators may explain some of the drastic differences in their parasite community structure (see Esch et al., 1990).

Overall, I report five new host records and one new locality records for parasites of Oklahoma damselflies. It is my hope that the new questions that have arisen from this study will stimulates further studies on the distribution and identification of damselfly parasites and studies on parasite community structure of these fascinating insects.

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VITA

Crystal Wiles

Candidate for the Degree of

Master of Science

Thesis: PARASITES AND PARASITE COMMUNITY STRUCTURE IN DAMSELFLIES (ODONATA: ZYGOPTERA) FROM TEAL RIDGE, STILLWATER OKLAHOMA.

Major Field: Zoology

Biographical:

Education:

Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in December, 2013.

Completed the requirements for the Bachelor of Science/Arts in Biology at State University of New York College at Oneonta, Oneonta, New York in Year May, 2010.

Experience:

Aug 2011–May 2013- T.A. – Animal Biology, OSU Zoology Department
Aug 2010–Spring 2011- T.A. – Introduction to Biology, OSU Zoology Department

Professional Memberships:

American Society of Parasitologists
Southwestern Association of Parasitologists
Rocky Mountain Conference of Parasitologists