Journal of Parasitology 2021 107(5) 669–675
© American Society of Parasitologists 2021

Published 1 September 2021

DOI: 10.1645/20-144

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

NEW DEFINITIVE HOST RECORD FOR *CHORDODES MORGANI* (NEMATOMORPHA) IN NEBRASKA WITH NOTES ON ECOLOGY

T. Figueira[1], D. Owen[2], B. Hanelt[3], and J. F. Shea[2]

[1] Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.
[2] Department of Biology Creighton University, 2500 California Plaza, Omaha, Nebraska 68178.
[3] Center for Evolutionary and Theoretical Immunology at University of New Mexico, 230 Castetter Hall, MSC03-2020, 1 University of New Mexico, Albuquerque, New Mexico 87131.
Correspondence should be sent to John Shea (https://orcid.org/0000-0001-9434-539X) at: *Johnshea@creighton.edu*

KEY WORDS

Chordodes morgani
Horsehair worm
Parcoblatta
Wood cockroach
Nematomorpha
Life cycle
Pitfall traps
Ecology
Definitive host

ABSTRACT

The life cycle and ecology of the horsehair worm *Chordodes morgani* (Nematomorpha) in Nebraska remain unknown. To identify its definitive host, we installed a series of pitfall traps along 3 first-order streams at 4 sites: Elk Creek, Upper Elk Creek, Maple Creek, and West Oak Creek, all located northwest of Lincoln, Nebraska. In addition, we opportunistically hand-collected insects at these sites, including wood cockroaches (*Parcoblatta virginica*), and maintained them in the lab until they passed adult worms. Two of these field-collected wood cockroaches each yielded 1 adult worm, which was identified as *C. morgani* by microscopy, showing that *P. virginica* serves as a definitive host. Experimental infections of captive-reared *Parcoblatta americana* supported this result. The wood cockroach was found at all 3 creeks, but *C. morgani* was not found at West Oak Creek, suggesting that the definitive host does not limit the distribution of *C. morgani*. Physical properties of the streams were measured to examine how these properties influenced the distribution of the worm. Flow rate and pH differed between the 3 sites where *C. morgani* was found and the West Oak Creek site, suggesting an important role for these abiotic factors in the distribution of this horsehair worm species.

The ecology of nematomorphs, also known as gordian or horsehair worms, is poorly understood (Chandler, 1985; Poinar, 1991), in part because, unlike other parasites, adult horsehair worms exit their definitive host when they are sexually mature. They do this by manipulating their definitive arthropod hosts to enter water where the adults emerge and mate (Thomas et al., 2002, 2003). Females lay eggs in the water column, in vegetation, on sticks or rocks, or in the substrate. Larvae develop within eggs and encyst in an aquatic invertebrate host (Hanelt and Janovy, 1999, 2004; Schmidt-Rhaesa, 2001; Bolek et al., 2015). The life cycle continues when a terrestrial insect consumes the aquatic paratenic host (Hanelt and Janovy, 2003). This complex combination of free-living and parasitic stages makes the study of the horsehair worm difficult. Of the more than 360 described freshwater Nematomorpha species (Yadav et al., 2018), less than 30% of the definitive arthropod hosts are known (Harkins et al., 2016), and definitive host specificity has been tested only in *Paragordius varius*, *Chordodes morgani*, *Gordius robustus* (Hanelt and Janovy, 2003), and *Chordodes kenyaensis* (Bolek et al., 2013). In addition, few studies have examined the factors that correlate with free-living hairworm abundance in aquatic habitats, and such information is lacking for most species of gordiids, including most species of *Chordodes* (Poulin, 1996; Bolek and Coggins, 2002; Salas et al., 2011).

Seasonality impacts horsehair worm abundance in a variety of species in different locations, such as in Argentina among *Chordodes brasiliensis*, *Noteochorododes cymatium*, *Noteochorododes talensis*, and *Pseudochordodes dugesi* (Salas et al., 2011), in Wisconsin with *Gordius difficilis* (Bolek and Coggins, 2002), in France with *Paragordius tricuspidatus* and *Spinochordodes tellinii* (Schmidt-Rhaesa et al., 2005), and in New Zealand with *Gordius dimorphus* (Poulin, 1996). For example, Schmidt-Rhaesa et al. (2005) noted that the abundance of the 2 horsehair worms matches the abundance of their respective definitive hosts. However, Salas et al. (2011) noted that seasonal differences in abundance observed in subtropical Argentina, in contrast to the differences observed in temperate regions where water bodies freeze in the winter, suggest that horsehair worm abundance, in addition to the presence of their definitive hosts, could also be explained by the presence of their aquatic paratenic hosts.

With few exceptions, the genus *Chordodes* is predominantly restricted to tropical and neotropical regions of the world (Schmidt-Rhaesa, 2002; Achiorno et al., 2008; Chiu et al., 2011;

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021

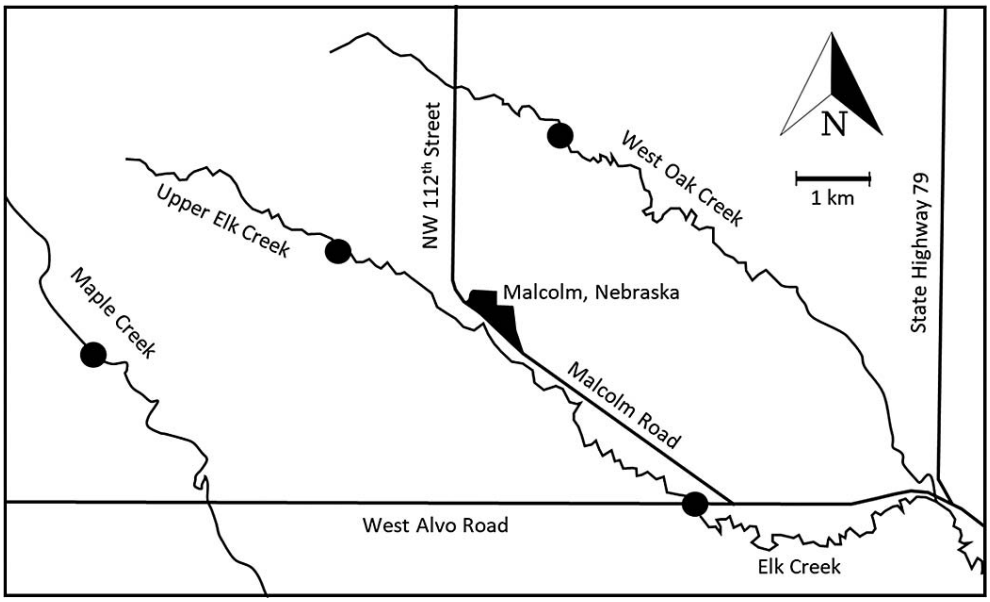

Figure 1. Map of the 4 sampling sites, representing 3 different first-order streams: Elk Creek, Upper Elk Creek, Maple Creek, and West Oak Creek. The wood roach (*Parcoblatta virginica*) was found at all 4 sites, but *Chordodes morgani* was not found at West Oak Creek.

Schmidt-Rhaesa and Lalramliana, 2011; Bolek et al., 2013; Schmidt-Rhaesa et al., 2015; Schmidt-Rhaesa and Perissinotto, 2016). Although little documentation exists on definitive host use for most species of *Chordodes*, when known, mantids, roaches, or orthopterans have been documented as definitive hosts (Montgomery, 1907; Studier et al., 1991; Poinar, 1999; Schmidt-Rhaesa and Ehrmann 2001; Schmidt-Rhaesa, 2002; Chiu et al., 2011; Bolek et al., 2013). However, and as mentioned above, it remains unclear as to the range of species of definitive hosts most species of *Chordodes* can infect.

Chordodes morgani has been found in eastern North America and as far west as Nebraska (Studier et al., 1991; Poiner and Chandler, 2004; Robison et al., 2012). It has been reported from orthopterans and cockroach definitive hosts across its range (Schmidt-Rhaesa et al., 2003). Specifically, Studier et al. (1991) reported cave crickets, *Hadenoecus subterraneus*, and camel crickets, *Ceuthophilus stygius*, as definitive hosts for *C. morgani* from Kentucky. In contrast, Montgomery (1907) and Schmidt-Rhaesa et al. (2003) reported *C. morgani* from an unidentified cockroach (location unknown) and from the smoky brown cockroach, *Periplaneta fuliginosa*, from South Carolina and nymphs of an unidentified species of wood cockroaches in the genus *Parcoblatta* from South Carolina and Missouri, respectively. Finally, McAllister and Hanelt (2012) found *C. morgani* in the oriental cockroach, *Blatta orientalis*, in Oklahoma. The paratenic host remains unknown.

Chordodes morgani adults were found in Elk Creek near Lincoln, Nebraska, between 1999 and 2000 (Hanelt et al., 2001), wrapped around dead twigs, laying eggs, but not in nearby creeks in the same county of Lancaster (B. Hanelt, pers. comm.). This fact allows us to ask what factors correlate with the presence of *C. morgani*. To answer this, we sampled Elk Creek and nearby creeks for adult horsehair worms and terrestrial insects in July 2017 and 2018. We measured the chemical and physical parameters of each creek as well as vegetation mass. We hypothesized that the presence of the definitive arthropod host will correlate with the presence of *C. morgani*.

MATERIALS AND METHODS

Study sites

We sampled 4 sites in Lancaster County, Nebraska, representing 3 different creeks. Elk Creek (40°53′11.1″N, 96°50′4.5″W) is located 5.8 km downstream from Upper Elk Creek (40°54′56.8″N, 96°53′29.0″W). Maple Creek (40°54′12.0″N, 96°55′47.5″W) is located 8.2 km northwest of Elk Creek, while West Oak Creek (40°55′49.36″N, 96°51′16.7″W) is located 5.1 km north of Elk Creek (Fig. 1). These sites were chosen because they are all first-order streams that flow into the Platte River via Salt Creek and are within 10 km of each other, and 2 of the 4 sites have historical data (Hanelt et al., 2001).

Collection efforts

To collect insects that are potentially infected with *C. morgani*, we placed 74 pitfall traps and 10 baited traps at the 4 sites in July of 2017. Pitfall traps consisted of polyethylene containers (15 × 15 × 5 cm) filled with a mixture of 3:1 water:propylene glycol solution following Ernst et al. (2016). Traps were placed in 7-cm-deep holes, made flush with the ground, and covered with cardboard squares (16 × 16 × 1 cm) secured with 4 steel nails (10 cm). Traps were serviced daily to remove specimens and debris and to refill the mixture as needed. Specimens collected from the traps were placed into plastic bags and identified to genus (Froeschner, 1954; Bland, 2003; Brust et al., 2008; Evans, 2014). They were then dissected and examined for the presence of horsehair worms.

Baited traps consisted of polyethylene containers (25 × 25 × 13 cm) placed in 15-cm-deep holes, made flush with the ground, and covered with cardboard squares (26 × 26 × 1 cm) secured with 4 steel nails (16 cm). The bait mixture consisted of fermented beer, garlic, onions, oats, and peanut butter (modified from Greenslade and Greenslade, 1971). Live specimens collected from the baited traps were placed in plastic vials for examination in the lab.

While servicing the traps, arthropods were also collected opportunistically by hand, maintained in the lab in separate vials

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021

(100 ml culture container, Carolina Biological Supply Co., Burlington, North Carolina), and provided with a moist cotton ball, pieces of autoclaved bark for cover, and cat food. We maintained all arthropods until either worms emerged from the arthropods or they died. If they died, we dissected the remains to check for the presence of horsehair worms. We made no effort to prompt the worms to exit the roach (i.e., we did not submerge the roaches), and worms that emerged from living arthropods were identified by microscopy following descriptions and keys (Schmidt-Rhaesa et al., 2016).

We also collected aquatic snails (*Physa* sp.) from each site using strainers and by hand. These were dissected and cysts were counted (Hanelt et al., 2001) but not identified to genus based on morphology (Szmygiel et al., 2014).

To collect adult *C. morgani* from the streams, we took advantage of their tendency to wrap around sticks to lay their eggs by constructing artificial stick traps. These consisted of 6 wooden skill sticks (Creatology, 11.4 × 0.9 × 0.2 cm) tied with fishing line, which served to collect both adult *C. morgani* and floating vegetation. We secured one end of the line to a steel garden stake, which was placed in the creek, and the other end was free to float in the water. Each day, we checked both the artificial stick traps as well as naturally occurring sticks and vegetation for horsehair worms. After 18 days, 10 artificial stick traps, with their accumulated vegetation, were randomly selected from each site, placed in a paper bag, and dried in an oven for 24 hr at 60 C. The dried organic matter was pulled or scraped off the stick traps and weighed to the nearest 0.01 g (Scout Pro SP202, Ohaus Corp., Pine Brook, New Jersey) to estimate the amount of vegetation as available habitat for adult horsehair worms. To account for the bounded and continuous nature of the variable vegetation dry weight, we fit a generalized linear model with a gamma error structure using the glmmTMB package in R (Brooks et al., 2017; R Core Team, 2020). In cases where the recorded values were 0 (n = 3), we added 0.0001 because the gamma error structure allows only for positive values. Confidence intervals were extracted with the ggeffects package (Lüdecke, 2018).

Site properties

To measure the physical properties of the stream we constructed a 10-m transect and measured the width of the creek at 0, 5, and 10 m. We then measured depth at 3 equidistant points (0, 5, and 10 m) and measured surface velocity by recording the time a fishing buoy takes to float 5 m, repeated 5 times. The average depth and width were used to calculate the cross-sectional area of the creek, which was multiplied by a velocity adjustment coefficient of 0.8 and the average surface velocity to determine the creek's flow according to the float method (Harrelson et al., 1994). A YSI-Xylem ProPlus probe (Yellow Springs, Ohio) measured pH, dissolved oxygen, nitrates, salinity, and temperature at each site once a week for 4 wk in July. Data were averaged (n = 4) and analyzed for differences among sites using a 1-factor ANOVA.

In the summer of 2018, we sampled for *C. morgani* adults from each of the 4 sites and measured the diameter of the sticks and vegetation upon which they are found. Measurements were taken in the field with calipers to the nearest 0.01 mm (UBANTE digital calipers ISO9001:2000, Shenzhen, China).

Experimental infection

In the lab, 34 captive-reared wood cockroaches (*P. americana*), purchased from a roach supplier who had maintained them until most were second-generation subadults, were individually housed in vials, provided with a moist cotton ball and a cat food pellet. Roaches were starved 24 hr before exposure to snails (*Physa*) collected from Elk Creek. Microscopy first confirmed that snails contained *C. morgani* cysts using identifying characteristics described in Szmygiel et al. (2014). The soft parts of the remaining snails were removed, homogenized, and evenly deposited among the roaches. Multiple exposures were necessary because we failed to find cysts in most snails. Repeat exposures occurred 4, 8, 17, 23, 30, 38, 45 and 52 DPE (days post-exposure).

RESULTS

Collection efforts

Over the 25 days of sampling in 2017, we collected 54 *C. morgani* adults: 26 from Elk Creek, 19 from Maple Creek, and 9 from Upper Elk Creek. We did not collect *C. morgani* adults from West Oak Creek. We collected 15 *C. morgani* adults from artificial stick traps, while the rest were collected from vegetation and naturally occurring twigs. Of the 40 worms identified by microscopy, 8 were too decayed to allow proper identification, 5 were *Gordius* cf. *robustus*, and 27 were *C. morgani*. Samples were deposited at the Museum of Southwestern Biology, Parasitology Division, University of New Mexico, Albuquerque, New Mexico, MSB: PARA:31791–31796, for parasites and MSB: HOST:24226–24231.

Over the 25 days of sampling in 2018, we collected 71 *C. morgani* adults: 17 from Elk Creek, 38 from Maple Creek, and 16 from Upper Elk Creek. We did not collect *C. morgani* adults from West Oak Creek. We collected 16 *C. morgani* adults from artificial stick traps, while the rest were collected from naturally occurring sticks. The average diameter of sticks with *C. morgani* adults (n = 28) was 6.9 mm.

Pitfall traps yielded 476 terrestrial arthropods from the 4 sites (Table I; 235 Coleopterans, 135 Orthopterans, and 106 assorted arthropods such as spiders, flies, millipedes, ants, and isopods). None were infected with *C. morgani*. Baited traps yielded 5 wood cockroaches (*Parcoblatta virginica*) collected from all 4 sites, and none were infected with *C. morgani*. Two hand-collected wood cockroaches from Elk Creek were identified as *P. virginica* (Froeschner, 1954) and maintained in the lab until, 3 days later, each yielded 1 adult worm, which was confirmed as *C. morgani* by microscopy. Lab-infected wood roaches (*Parcoblatta americana*) yielded 2 adult worms 133 DPE and 178 DPE. Dissected snails (*Physa*) from Elk Creek averaged 10 cysts (n = 15, range = 0–74), while snails from Maple Creek averaged 18 cysts (n = 18, range = 0–3). Snails from West Oak Creek (n =28) had no cysts, and we failed to collect any snails from Upper Elk Creek.

Site properties

Sites did not differ in dissolved oxygen, temperature, or salinity (Table II). The flow rate was significantly slower at West Oak Creek than at both Elk Creek and Maple Creek but did not significantly differ with Upper Elk Creek (ANOVA, $P < 0.01$). The pH at Upper Elk Creek was significantly higher than West Oak Creek (ANOVA, $P < 0.05$). Nitrate level at Elk Creek was

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021

Table I. Numbers and types of Arthropods sampled in pitfall traps at 4 sites in eastern Nebraska. The category "Other" includes ants, spiders, flies, isopods, and millipedes.

Order	Genus	Elk Creek	Maple Creek	Upper Elk Creek	West Oak Creek	Total
Coleoptera	*Pterostichus*	61	7	8	1	77
	Ceuthophilus	30	8	2	0	40
	Harpalus	35	0	0	1	36
	Chlaenius	21	0	3	1	25
	Galerita	22	2	0	0	24
	Nudobius	14	0	0	0	14
	Copris	3	3	1	0	7
	Photinus	4	0	0	0	4
	Platydracus	3	0	0	0	3
	Ontholestes	1	0	1	0	2
	Otiorhynchus	1	0	0	0	1
	Scarabaeidae	1	0	0	0	1
	Scarites	1	0	0	0	1
	Total	196	21	15	3	235
Orthoptera	*Gryllus*	83	21	3	0	107
	Melanoplus	21	0	0	0	21
	Dichromorpha	6	0	0	0	6
	Total	110	21	3	0	134
Other		82	22	2	0	106

higher than at Maple Creek, but Maple Creek had a lower nitrate level than Upper Elk Creek (ANOVA, $P < 0.05$). West Oak Creek significantly differed from Upper Elk Creek in vegetation dry weight ($P = 0.00357$, glmmTMB).

DISCUSSION

Although *C. morgani* has been reported in Nebraska (Robison et al., 2012), the biotic and abiotic factors that correlate with the presence of this species remain mostly unknown. The emergence of 1 *C. morgani* adult from each of the 2 field-collected wood cockroaches (*P. virginica*) shows that the worms use these roaches as their definitive host and are the first confirmed species of a wood roach acting as a host for *C. morgani*. *Parcoblatta americana* occurs in the same genus as the field-collected roaches, and both are found in North America (Hebard, 1917). Exposure of these captive-reared wood cockroaches to aquatic snails from Elk Creek supports the suitability of the wood cockroach as a definitive host for *C. morgani*, which is consistent with the findings of Schmidt-Rhaesa et al. (2003), who report *C. morgani* in a *Parcoblatta* sp. nymph as well as in the more distantly related *Periplaneta fuliginosa*. It is also consistent with previous observations of 4 other *Chordodes* spp. using roaches as a definitive host (Poinar, 1999). Failure to find other arthropods infected with *C. morgani* does not leave out the possibility that other arthropods could also serve as a definitive host. Likewise, failure to find *C. morgani* adults or cysts at West Oak Creek after 2 summers of sampling does not necessarily mean this horsehair worm is absent from West Oak Creek. More extensive sampling, especially during other parts of the year, is required. Given that we collected wood cockroaches from all 4 sites but did not collect adult *C. morgani* at West Oak Creek suggests that the presence of the definitive host does not necessarily correlate with the presence of *C. morgani*.

Since *C. morgani* lays its eggs on sticks, some vegetation must be present in its habitat. Stream vegetation occurred at all 4 sites, and its accumulation on the artificial stick traps varied as measured by its dry weight from 0 to 49.7 g among the 4 sites. Despite the statistically significant difference between Upper Elk Creek and West Oak Creek, Elk Creek averaged the largest amount of stream vegetation with West Oak Creek having the second-largest amount (Table II), suggesting a poor correlation between vegetation and *C. morgani* abundance. Other factors such as the paratenic host or abiotic factors may limit the horsehair worm's distribution.

The pH at Upper Elk Creek was significantly higher than the site without *C. morgani* (West Oak Creek). This result is consistent with Salas et al. (2011), who found that the abundance of *Chordodes brasiliensis* in Argentina was greatest in autumn, which also had a high pH. The impact of pH on the free-swimming horsehair worm adult, its egg, or larval stages has not been studied. Adult horsehair worms spend relatively little time in the water, especially compared to its other life cycle stages. However, pH can impact possible paratenic hosts. For example, lab experiments showed that caddisflies are more tolerant of low pH than mayflies (Bell, 1971; Gaufin, 1973). The paratenic host of *C. morgani* may prefer a high pH environment.

Flow rate at Elk Creek and Maple Creek, where *C. morgani* was abundant, was significantly faster than at West Oak Creek, suggesting that flow rate may correlate with *C. morgani* abundance. This result is consistent with Salas et al. (2011), who found that the abundance of *C. brasiliensis* in Argentina was

Table II. Summary statistics with the mean (and 95% confidence interval) of the physical and chemical parameters at each of the 4 sites. Significant differences between sites are indicated with symbols (*, †, ‡, §, ||). The total number of adult horsehair worms collected in 25 days in 2017 and 2018 is also reported.

Parameter	Sample size	West Oak Creek	Elk Creek	Upper Elk Creek	Maple Creek
Salinity (ppt)	4	0.36 (0.33–0.39)	0.37 (0.32–0.41)	0.33 (0.32–0.33)	0.34 (0.31–0.36)
Dissolved oxygen (mg/L)	4	4.1 (2.8–5.3)	4.2 (3.1–5.3)	3.9 (2.3–5.6)	3.7 (1.9–5.5)
Temperature (C)	4	20.2 (18.7–21.7)	20.9 (19.3–22.4)	21.1 (20–22.1)	20.7 (19.6–21.8)
Nitrates (mg/L)	4	1.7 (1.5–2.0)	2.1 (1.9–2.3)*	2.0 (1.4–2.5)†	1.1 (0.8–1.4)*†
pH	4	7.9 (7.8–8.0)*	8.1 (8–8.2)	8.4 (8.0–8.9)*	8.1 (8.0–8.2)
Flow rate (L/sec)	5	16.0 (11.1–20.8)*||	54.1 (50.9–57.4)*‡§	17.0 (13.8–20.3)†‡	36.2 (32.9–39.6)†§||
Vegetation dry weight (g)	10	3.02 (1.12–8.09)*	9.47 (3.53–25.4)	0.38 (0.14–1.02)*	2.71 (1.01–7.27)
2017 no. of adult horsehair worms		0	26	9	19
2018 no. of adult horsehair worms		0	17	16	38

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021

greatest in autumn, which also had the fastest flow rate. Flow rate correlates with dissolved oxygen, and lab experiments show that low oxygen impacts the survival and emergence of mayflies, midges, and caddisflies (Nebeker, 1972), which often serve as paratenic hosts for horsehair worms (Schmidt-Rhaesa, 2012). However, we failed to find differences in dissolved oxygen among the 4 sites. Flow rate, along with other interconnected factors such as food quantity and river depth, also impacts the distribution of filter-feeding insects (Richardson and Mackay, 1991), which could serve as paratenic hosts. Identification of the paratenic host would assist future studies on the relationship between flow rate and the abundance of *C. morgani*.

Various stages of at least 1 species (*Chordodes nobilii*) are susceptible to herbicides and other toxins (Achiorno et al., 2008, 2009, 2010). Nitrates, like herbicides, are associated with agriculture, and excessive nitrate levels can lead to algal blooms, eutrophication, degradation of aquatic systems (Pauwels and Talbo, 2004; Xu et al., 2016), and negatively impact human health (Ward et al., 2005). The impact of nitrates on the adult, egg, or larval stages of horsehair worms has not been studied. However, nitrates' negative effects on aquatic insects, which could serve as paratenic hosts, have been demonstrated only in levels that exceed 4.5 mg/L (Camargo and Ward, 1995), which is higher than that observed in this study (Table II). Further, the higher nitrate level at Elk Creek does not seem to correlate with horsehair worm presence since we also found them at the site with the lowest nitrate level (Maple Creek; Table II), but not at the site with the second-lowest nitrate level (West Oak Creek).

Water temperature, pH, flow rate, and other abiotic factors change with the seasons, especially in temperate regions. Knowing how these abiotic factors impact the abundance of horsehair worms and their definitive and paratenic hosts will help clarify the relationship between horsehair worms and water quality. For example, *P. varius* requires high-quality water to maintain water balance (Yoder et al., 2014). The complex life cycle of Nematomorpha may make it more susceptible to environmental degradation as has been observed in larval trematodes (Huspeni and Lafferty, 2004; Huspeni et al., 2005; Shea et al., 2012). Ehlke (1979) found that Trichopterans, Ephemeropterans, and Nematomorphs were eliminated from a stream that was downstream from a landfill, but this observation was based on the collection of a genus that was not identified. Thus, we do not know if this horsehair worm species relied upon the presence of Trichopterans, Ephemeropterans, or both to complete its life cycle.

On the other hand, Hanelt et al. (2001) found no correlation between horsehair worm prevalence and land use and human population density, suggesting that Nematomorpha is resistant to anthropocentric disturbances. In a sampling of snails in Oklahoma, cysts and larvae of *P. varius* were found at 50% of the sites, while cysts and larvae of *C. morgani* were found at 11% of sites (Harkins et al., 2016), suggesting large species differences in prevalence. To identify areas suitable for snail infections with gordiids, these data were then used with the environmental variables of elevation, stream flow direction, streamflow accumulation, land use data, and distance from stream to construct an ecological niche model. Although the model successfully predicted the presence of encysted snails, Harkins et al. (2016) did not include measures of water quality.

Collecting more data on the abiotic and biotic factors associated with horsehair worm abundance will assist in elucidating its complex life cycle, allow researchers to better predict its presence in an environment, and help researchers rear horsehair worms in the lab.

ACKNOWLEDGMENTS

We thank Yoonjae Im, Amy Tanoue, Janet Huynh, Ralph Mendez, Chris Smith, and Izzy Utschig for helping to check traps and take measurements in 2017. We also thank McKay and Kane Carstens, Simeon Gboun, Sydney Dang, Quinn Sano, Elijah St. John, and Patrick Kuwong for helping to check traps and take measurements in 2018. This study was partially funded by a Center for Undergraduate Research and Scholarship Faculty grant. We thank Jon Larson and Kyle Koch of University of Nebraska–Lincoln Extension for confirming the insect identifications. Fieldwork was conducted under Nebraska Scientific and Educational Permits 1030 and 1103. The authors would also like to thank the 2 anonymous reviewers for feedback that improved this paper.

LITERATURE CITED

Achiorno, C. L., C. de Villalobos, and L. Ferrari. 2008. Toxicity of the herbicide glyphosate to *Chordodes nobilii* (Gordiida, Nematomorpha). Chemosphere 71: 1816–1822.

Achiorno, C. L., C. de Villalobos, and L. Ferrari. 2009. Sensitivity of pre-parasitic stages of *Chordodes nobilii* (Gordiida, Nematomorpha) to malathion. Ecotoxicology 18: 594–599.

Achiorno, C. L., C. de Villalobos, and L. Ferrari. 2010. Validation test with embryonic and larval stages of *Chordodes nobilii* (Gordiida, Nematomorpha): Sensitivity to three reference toxicants. Chemosphere 81: 133–140.

Bell, H. L. 1971. Effect of low pH on the survival and emergence of aquatic insects. Water Research 5: 313–319.

Bland, R. G. 2003. The Orthoptera of Michigan: Biology, Keys, and Descriptions of Grasshoppers, Katydids, and Crickets. Extension Bulletin E-2815. Michigan State University Extension, East Lansing, Michigan, 223 p.

Bolek, M. G., and J. R. Coggins. 2002. Seasonal occurrence, morphology, and observations on the life history of *Gordius difficilis* (Nematomorpha: Gordioidea) from southeastern Wisconsin, U.S.A. Journal of Parasitology 88: 287–294.

Bolek, M. G., A. Schmidt-Rhaesa, L. C. De. Villalobos, and B. Hanelt. 2015. Phylum Nematomorpha. *In* Ecology and General Biology: Thorp and Covich's Freshwater Invertebrates, J. Thorp and D. C. Rodgers (eds.). Academic Press, London, U.K., p. 303–326.

Bolek, M. G., C. Szmygiel, A. Kubat, A. Schmidt-Rhaesa, and B. Hanelt. 2013. Novel techniques for biodiversity studies of gordiids and description of a new species of *Chordodes* (Gordiida, Nematomorpha) from Kenya, Africa. Zootaxa 3717: 23–38.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Mächler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R Journal 9: 378–400.

Brust, M. L., W. W. Hoback, and R. J. Wright. 2008. The Grasshoppers (Orthoptera: Acrididae and Romaleidae) of

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021

Nebraska. University of Nebraska Extension, Lincoln, Nebraska, 138 p.

Camargo, J. A., and J. V. Ward. 1995. Nitrate (NO_3-N) toxicity to aquatic life: A proposal of safe concentrations for two species of Nearctic freshwater invertebrates. Chemosphere 31: 3211–3216.

Chandler, C. M. 1985. Horsehair worms (Nematomorpha, Gordioidea) from Tennessee, with a review of taxonomy and distribution in the United States. Journal of the Tennessee Academy of Sciences 60: 59–62.

Chiu, M. C., C. G. Huang, W. J. Wu, and S. F. Shiao. 2011. A new horsehair worm, *Chordodes formosanus* sp. n. (Nematomorpha, Gordiida) from Hierodula mantids of Taiwan and Japan with redescription of a closely related species, *Chordodes japonensis*. ZooKeys 160: 1–22.

Ehlke, T. A. 1979. Effects of landfill leaching on water quality and biology of a nearby stream, South Cairo, Greene County, New York. Water-Resources Investigations Report 79–113. U.S. Geological Survey, Water Resources Division, Washington, DC, 28 p. doi:10.3133/wri7913.

Ernst, C. M., B. Hanelt, and C. M. Buddle. 2016. Parasitism of ground beetles (Coleoptera: Carabidae) by a new species of hairworm (Nematomorpha: Gordiida) in Arctic Canada. Journal of Parasitology 102: 327–335.

Evans, A. V. 2014. Beetles of Eastern North America. Princeton University Press, Princeton, New Jersey, 560 p.

Froeschner, R. C. 1954. The grasshoppers and other Orthoptera of Iowa. Iowa State College Journal of Science 29: 163–354.

Gaufin, A. R. 1973. Water quality requirements of aquatic insects. Ecological Research Series, United States, EPA-660/3-73-004. Environmental Protection Agency, Washington, DC, 88 p.

Greenslade, P., and P. J. M. Greenslade. 1971. The use of baits and preservatives in pitfall traps. Australian Journal of Entomology 10: 253–260.

Hanelt, B., L. E. Grother, and J. Janovy Jr. 2001. Physid snails as sentinels of freshwater nematomorphs. Journal of Parasitology 87: 1049–1053.

Hanelt, B., and J. Janovy Jr. 1999. The life cycle of a horsehair worm, *Gordius robustus* (Nematomorpha: Gordioidea). Journal of Parasitology 85: 139–141.

Hanelt, B., and J. Janovy Jr. 2003. Spanning the gap: Experimental determination of paratenic host specificity of horsehair worms (Nematomorpha: Gordiida). Invertebrate Biology 122: 12–18.

Hanelt, B., and J. Janovy Jr. 2004. Life cycle and paratenesis of American gordiids (Nematomorpha: Gordiida). Journal of Parasitology 90: 240–244.

Harkins, C., R. Shannon, M. Papeş, A. Schmidt-Rhaesa, B. Hanelt, and M. G. Bolek. 2016. Using Gordiid cysts to discover the hidden diversity, potential distribution, and new species of Gordiids (Phylum Nematomorpha). Zootaxa 4088: 515–530.

Harrelson, C. C., C. L. Rawlins, and J. P. Potyondy. 1994. Stream Channel Reference Sites: An Illustrated Guide to Field Technique. U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, 61 p.

Hebard, M. 1917. The Blattidae of North America north of the Mexican boundary. Memoirs of the American Entomological Society, American Entomological Society 2: 74–75, 84–88.

Huspeni, T. C., R. F. Hechinger, and K. D. Lafferty. 2005. Trematode parasites as estuarine indicators: Opportunities, applications and comparisons with conventional community approaches. *In* Estuarine Indicators, S. Bortone (ed.). CRC Press, Boca Raton, Florida, p. 297–314.

Huspeni, T. C., and K. D. Lafferty. 2004. Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. Ecological Applications 14: 795–804.

Lüdecke, D. 2018. ggeffects: Tidy data frames of marginal effects from regression models. Journal of Open Source Software 3: 772. doi:10.21105/joss.00772.

McAllister, C. T., and B. Hanelt. 2012. New host and geographic distribution record for *Chordodes morgani* (Nematomorpha: Gordiida), from Oklahoma. Proceedings of the Oklahoma Academy of Science 92: 75–76.

Montgomery Jr., T. H. 1907. The distribution of the North American Gordiaceae, with description of a new species. Proceedings of the Academy of Natural Sciences of Philadelphia 59: 270–272.

Nebeker, A. V. 1972. Effect of low oxygen concentration on survival and emergence of aquatic insects. Transactions of the American Fisheries Society 101: 675–679.

Pauwels, H., and H. Talbo. 2004. Nitrate concentration in wetlands: Assessing the contribution of deeper groundwater from anions. Water Research 38: 1019–1025.

Poinar, G. 1999. *Paleochordodes protus* n.g., n.sp. (Nematomorpha, Chordodidae), parasites of a fossil cockroach, with a critical examination of other fossil hairworms and helminths of extant cockroaches (Insecta: Blattaria). Invertebrate Biology 118: 109–115.

Poinar, G. O. 1991. Hairworm (Nematomorpha: Gordioidea) parasites of New Zealand wetas (Orthoptera: Stenopelmatidae). Canadian Journal of Zoology 69: 1592–1599.

Poinar, G. O., and C. M. Chandler. 2004. Synopsis and identification of North American hairworms (Gordioidea: Nematomorpha). Journal of the Tennessee Academy of Sciences 79: 1–7.

Poulin, R. 1996. Observations on the free-living adult stage of *Gordius dimorphus* (Nematomorpha: Gordioidea). Journal of Parasitology 82: 845–846.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/. Accessed 27 January 2021.

Richardson, J., and R. Mackay. 1991. Lake outlets and the distribution of filter feeders: An assessment of hypotheses. Oikos 62: 370–380.

Robison, H. W., C. T. McAllister, and B. Hanelt. 2012. New geographic distribution records for horsehair worms (Nematomorpha: Gordiida) in Arkansas, including new state records for *Chordodes morgani* and *Paragordius varius*. Journal of the Arkansas Academy of Science 66: Article 35. Available at: http://scholarworks.uark.edu/jaas/vol66/iss1/35. Accessed 10 January 2021.

Salas, L., C. de Villalobos, and F. Zanca. 2011. Sexual size dimorphism, sex ratio and the relationship between seasonality and water quality in four species of Gordiida (Nema-

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021

tomorpha) from Catamarca, Argentina. Journal of Helminthology 85: 319–324.

Schmidt-Rhaesa, A. 2001. The life cycle of horsehair worms (Nematomorpha). Acta Parasitologica 46: 151–158.

Schmidt-Rhaesa, A. 2002. Australian species of *Chordodes* (Nematomorpha) with a description of two new species, remarks on the genus *Chordodes* and its life history. Journal of Natural History 36: 1569–1588.

Schmidt-Rhaesa, A. 2012. Nematomorpha. *In* Handbook of Zoology. Gastrotricha, Cycloneuralia and Gnathifera, Vol. 1, Nematomorpha, Priapulida, Kinorhyncha and Loricifera, A. Schmidt-Rhaesa (ed.). De Gruyter, Berlin, Germany, p. 29–145.

Schmidt-Rhaesa, A., D. Biron, C. Joly, and F. Thomas. 2005. Host–parasite relations and seasonal occurrence of *Paragordius tricuspidatus* and *Spinochordodes tellinii* (Nematomorpha) in southern France. Zoologischer Anzeiger 244: 51–57.

Schmidt-Rhaesa, A., L. C. de Villalobos, F. Zanca, B. Hanelt, and M. G. Bolek. 2016. Phylum Nematomorpha. *In* Thorp and Covich's Freshwater Invertebrates, 4th ed.: Keys to Nearctic Fauna, vol. 2, J. H. Thorp, and D. C. Rogers (eds.). Academic Press, London, U.K., p. 181–188.

Schmidt-Rhaesa, A., and R. Ehrmann. 2001. Horsehair worms (Nematomorpha) as parasites of praying mantids with a discussion of their life cycle. Zoologischer Anzeiger 240: 167–179.

Schmidt-Rhaesa, A., B. Hanelt, and W. K Reeves. 2003. Redescription and compilation of Nearctic freshwater Nematomorpha (Gordiida), with the description of two new species. Proceedings of the Academy of Natural Sciences of Philadelphia 153: 77–117.

Schmidt-Rhaesa, A., and Lalramliana. 2011. *Chordodes mizoramensis* (Nematomorpha, Gordiida), a new species of horsehair worm from Mizoram, North-East India. ZooKeys 75: 1–8.

Schmidt-Rhaesa, A., L. Limatemjen, and A. K. Yadav. 2015. *Chordodes combiareolatus*, a new species of horsehair worms (Nematomorpha) from Nagaland, India, with further comments on *Chordodes moutoni*. Zootaxa 2925: 202–210.

Schmidt-Rhaesa, A., and R. Perissinotto. 2016. *Chordodes ferox*, a new record of horsehair worms (Nematomorpha, Gordiida) from South Africa. ZooKeys 566: 1–11.

Shea, J. F., G. J. Kersten, C. M. Puccia, A. T. Stanton, S. N. Stiso, E. S. Helgeson, and E. J. Back. 2012. The use of parasites as indicators of ecosystem health as compared to insects in freshwater lakes of the Inland Northwest. Ecological Indicators 13: 184–188.

Studier, E. H., K. H. LaVoie, and C. M. Chandler. 1991. Biology of cave crickets, *Hudenoecus subrerraneus*, and camel crickets, *Ceuthophilus stygius* (Insecta: Orthoptera): Parasitism by hairworms (Nematornorpha). Journal of the Helminthological Society of Washington 58: 248–250.

Szmygiel, C., A. Schmidt-Rhaesa, B. Hanelt, and M. Bolek. 2014. Comparative descriptions of non-adult stages of four genera of Gordiids (Phylum: Nematomorpha). Zootaxa 3768: 101–118.

Thomas, F., A. Schmidt-Rhaesa, G. Martin, C. Manu, P. Durand, and F. Renaud. 2002. Do hairworms (Nematomorpha) manipulate the water-seeking behavior of their terrestrial hosts? Journal of Evolutionary Biology 15: 356–361.

Thomas, F., P. Ulitsky, R. Augier, N. Dusticier, D. Samuel, C. Strambi, D. G. Biron, and M. Cayre. 2003. Biochemical and histological changes in the brain of the cricket *Nemobius sylvestris* infected by the manipulative parasite *Paragordius tricuspidatus* (Nematomorpha). International Journal for Parasitology 33: 435–443.

Ward, M. H., T. M. deKok, P. Levallois, J. Brender, G. Gulis, B.T. Nolan, and J. VanDerslice. 2005. International Society for Environmental Epidemiology. Workgroup report: Drinking-water nitrate and health—Recent findings and research needs. Environmental Health Perspectives 113: 1607–1614.

Xu, S., P. Kang, and Y. Sun. 2016. A stable isotope approach and its application for identifying nitrate source and transformation process in water. Environmental Science and Pollution Research 23: 1133–1148.

Yadav, A. K., Z. J. C. Tobias, and A. Schmidt-Rhaesa. 2018. *Gordionus maori* (Nematomorpha: Gordiida), a new species of horsehair worm from New Zealand. New Zealand Journal of Zoology 45: 29–42.

Yoder, J. A., B. W. Nelson, A. E. Rosselot, and A. J. Jajack. 2014. Knot formation reduces water exchange by adult males of the hairworm, *Paragordius varius* (Nematomorpha: Gordioidea). Marine and Freshwater Behaviour and Physiology 47: 299–311.

Downloaded from http://meridian.allenpress.com/journal-of-parasitology/article-pdf/107/5/669/2896801/i0022-3395-107-5-669.pdf by guest on 23 October 2021