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NEW RECORDS OF NEMATOMORPH PARASITES (NEMATOMORPHA: GORDIIDA) OF GROUND BEETLES (COLEOPTERA: CARABIDAE) AND CAMEL CRICKETS (ORTHOPTERA: RHAPHIDOPHORIDAE) IN WASHINGTON STATE

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ABSTRACT: From 1998 to 2003, beetles and crickets infected with hairworms were collected from 4 localities within the Hanford Nuclear Site and the Hanford Reach National Monument, located in a shrub-steppe region of Washington State along the Columbia River. Infected hosts comprised 6 species of carabid beetles within 5 genera and 2 camel crickets within 1 genus; all are newly documented insect–nematomorph associations. A large proportion of the infected hosts (48%) were collected from a single site during a single collecting period. Of the 38 infected hosts, 32 contained a single worm, 4 hosts contained 2 worms, and 2 hosts contained 3 worms. Five of the hosts with multiple infections contained at least 1 male and 1 female worm. Camel crickets were infected with *Neochordodes occidentalis* while carabids were infected with an undescribed species of *Gordionus*. As the majority of hairworms are collected in the post-parasitic adult phase, host data and hairworm–arthropod associations remain poorly documented and our work adds new data to this area of nematomorph biology.

Hairworms (Nematomorpha: Gordiida) are cosmopolitan freshwater parasites with 18 species known from North America (Schmidt-Rhaesa et al., 2003; Poinar and Chandler, 2004; Poinar et al., 2004). Adults are free-living in freshwater environments while juveniles are obligate parasites in terrestrial arthropods with a paratenic aquatic host, e.g., insects and mollusks (Hanelt and Janovy, 2003). Paratenic hosts in freshwater environments become infected by consuming mobile, non-swimming gordiid larvae which cross the gut wall and encyst. The paratenic hosts are later ingested by the definitive host, typically a predatory or omnivorous terrestrial insect (Hanelt and Janovy, 1999, 2004; Poinar and Weissman, 2004). Species in the orders Orthoptera and Blattaria are the most frequent insect hosts, with ground beetles (Coleoptera: Carabidae) also commonly parasitized (Hanelt and Janovy, 2000; Schmidt-Rhaesa et al., 2003). Upon maturity, gordiids exit the definitive host and enter fresh water, where they mate and lay eggs within a few days (Hanelt and Janovy, 1999, 2000; Bolek and Coggins, 2002).

Several records of gordiid parasites in ground beetles (Coleoptera: Carabidae) have been published, primarily from Europe (Larochelle, 1978; Poinar et al., 2004). Poinar and Chandler (2004) summarize the known hosts for North American gordiids. Their review indicates that many hosts are still unknown, with several North American host associations discovered only recently (e.g., Hanelt and Janovy, 2000; Poinar et al., 2004). The present paper contributes observations of horsehair worms infecting 6 newly recorded carabid and 2 rhaphidophorid hosts from a shrub–steppe ecosystem in south-central Washington State.

MATERIALS AND METHODS

Study site descriptions

Collections: The present work was part of a larger biodiversity inventory of the Hanford Site, located in Benton and Franklin counties in south-central Washington State (Soll et al., 1999). We trapped insects as part of this survey between 1998 and 2003, collecting in numerous locations and habitat types across the site. The Hanford Site, including the Hanford Reach National Monument, encompasses slightly more than 150,000 ha of largely intact shrub–steppe habitat which has had limited public access since the 1940s (Soll et al., 1999). Water bodies on or near the site are restricted to the Columbia River and Yakima River, a few spring systems, an alkaline pond (West Lake), scattered vernal pools, and several wetlands fed by irrigation runoff north of the Columbia River (Fig. 1). We captured parasitized insects at 4 localities during the study, described briefly below (see Sakschewsky and Downs [2001] for a thorough treatment of plant communities of the site).

Sand dunes: A large area of sand dunes lies along the western margin of the Columbia River and for several kilometers inland (46°31.369′N, 119°21.192′W). The dunes occupy approximately 3,100 ha and are of Holocene and Recent origin. Vegetation is typical of active Columbia Basin sand dunes. We placed 15 pitfall traps in a T-shaped formation, running the traps through areas of the dunes with little or no vegetation and through a draw with a shrub overstory. The trapping site is approximately 4 km from the Columbia River, the nearest water body. We maintained traps from 14 March 1998 through 28 May 1999.

Rattlesnake Ridge, northeast slope: Two pitfall-trap sites were located on the northern slope of Rattlesnake Ridge (46°22.57′N, 119°31.64′W), which demarks the southern border of the Hanford Site. Widespread fire in the early 1980s destroyed most of the dominant sagebrush in this part of the reserve (Johansen et al., 1993), leaving post-burn sites dominated by native bunchgrass or introduced cheatgrass (*Bromus tectorum* L.) with interspersed patches of un-burned climax sagebrush. We sampled a large cheatgrass stand and an adjacent stand of climax big sagebrush (*Artemisia tridentata*). These adjacent sites were separated by the Rattlesnake Ridge Road which is approximately 20 m wide. The closest water body is the Yakima River, about 5.6 km away. A series of 10 pitfall traps spaced 10 m apart was placed perpendicular to the road in each plant community, beginning 20 m from the road. Traps were maintained from 23 May 1998 through 14 November 1999.

West Lake: West Lake is a relatively large alkaline pond located within the central Hanford Site and surrounded by salt- and alkali-tolerant vegetation. Historically, West Lake was highly intermittent, with water depth dependent upon groundwater levels. Hanford operations raised the local water table, increasing and stabilizing West Lake's depth for several decades (Emery and McShane, 1978; Poston et al., 1991). Recent changes in wastewater management have somewhat restored the historic seasonal fluctuation (Poston et al., 1991). We placed pitfall traps in a transect running from the surrounding shrub–steppe community into and across the shallow basin containing West Lake. This transect contained 24 traps spaced 15 m apart. Traps were maintained from 20 March 1998 to 26 August 1999.

White Bluffs Ferry: The White Bluffs Ferry site (46°40.541′N, 119°26.949′W, 128 m elevation) was located in a shallow depression approximately 50 m from the Columbia River. This is near the White Bluffs Ferry landing, established in the 1880s and used until the early 1940s. There are no longer remnants of the ferry landing or buildings, although the site is still used as a boat launch. Traps were installed in a depression created by road construction and maintenance that was

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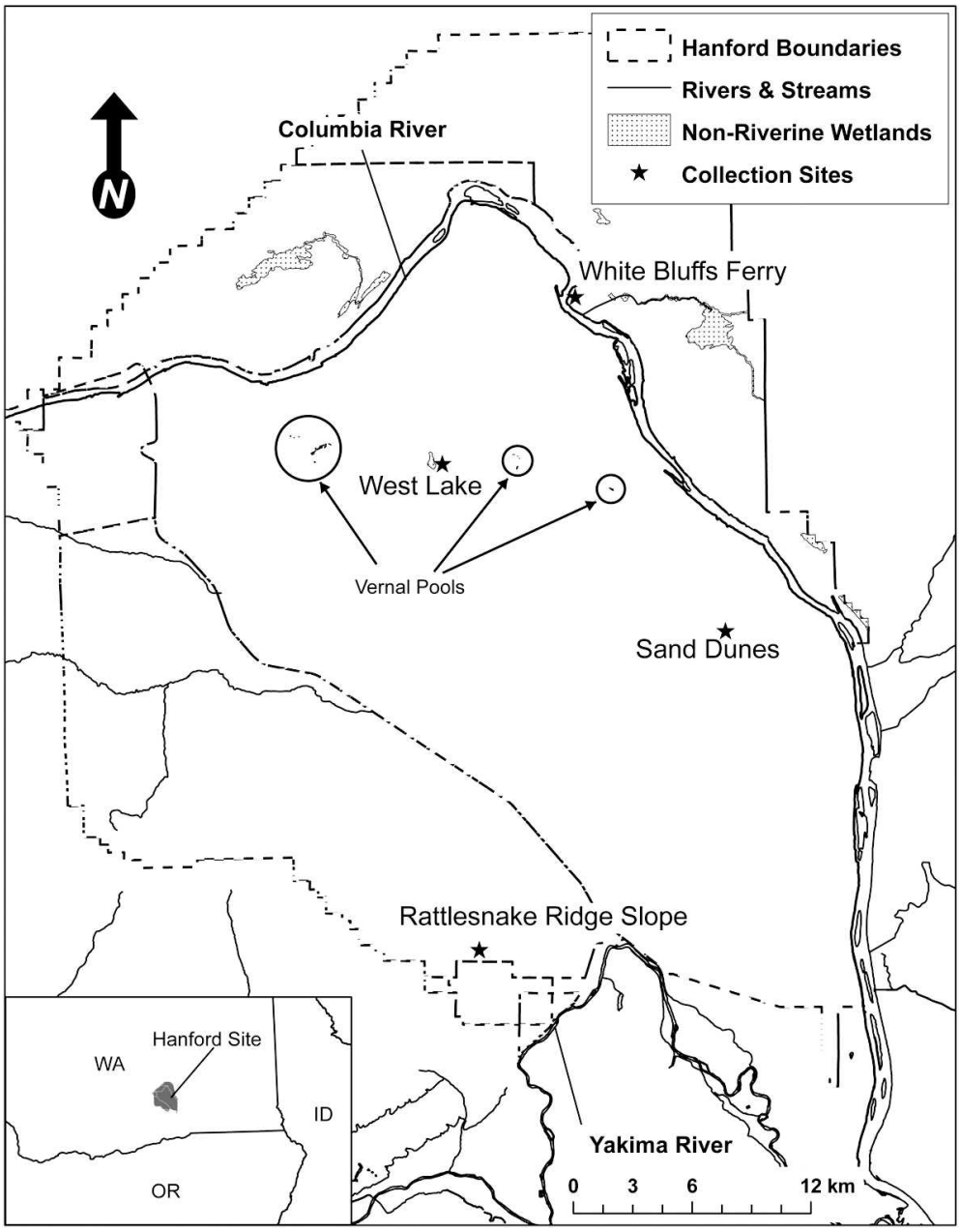

FIGURE 1. Map of the Hanford Site (including the Hanford Reach National Monument and other federally managed properties) and collecting localities, depicting all known ephemeral and permanent water bodies.

approximately 55 m long, 15 m wide, and 3–4 m deep. Ten pitfall traps were set at 10-m intervals and maintained from April 2002 through April 2003.

Trapping method

We used un-baited pitfall traps constructed from 500-ml deli cups containing a 1:1 mixture of propylene glycol:water as a preservative and killing agent. Each trap included a baffle system to increase the effective trap diameter, after Morrill et al. (1990). Traps were covered with a square, flat lid to minimize debris, disturbance by predators, and flooding from precipitation.

Trap containers were removed from the field every week or 2 (depending on weather conditions) and replaced with fresh containers. Samples were processed in the lab. Parasitized hosts were detected when worms partly emerged from the abdomen while in the preservative in traps. These specimens were preserved in vials with 70% EtOH for further study. Ground beetles were identified to species using keys found in Lindroth (1961, 1966, 1968, 1969) and rhaphidophorids identified using keys in Vickery and Kevan (1985).

Parasite material and microscopy

Worms were carefully removed from their hosts and the anterior, posterior, and a 15–20-mm midsection of each was placed in fresh 70% ethanol. The remaining parts of each worm were placed in 100% ethanol at −80 C for genetic analysis. The posterior end of each worm was examined for species-level characters. In addition, a cuticle preparation was made from each specimen for study by light microscopy. A 0.5-mm section was taken from the worm's midsection and the underlying tissue was removed using a razor blade. The cuticle was placed onto a slide with water, ensuring that part of the cuticle was folded under to allow for

TABLE I. Collection data for gordiids and hosts.

Host species	Collection site	Date	No. infected hosts	Nematomorphs		No. uninfected hosts*
				F	M	
A. *Gordionus* sp.						
Calosoma luxatum Say	Sand dunes	28-Mar-98	1	0	1	32
Poecilus lucublandus Say	West Lake	18-Apr-98	1	0	1	35
Cymindis planipennis Le Conte	Rattlesnake Ridge	4-Apr-98	1	0	1	9,020
Amara discors Kirby	Rattlesnake Ridge	18-Apr-98	1		1, unknown sex	37
Amara quenseli Schöenherr	Rattlesnake Ridge	9-Apr-99	1†	1	1	1,373
A. quenseli Schöenherr	White Bluffs Ferry	22-Apr-02	2	1	1	10
Calathus ruficollis (Dejean)	White Bluffs Ferry	22-Apr-02	22‡	9	15	4,063
C. ruficollis (Dejean)	White Bluffs Ferry	4-Apr-03	6§	6	4	4,063
C. ruficollis (Dejean)	White Bluffs Ferry	11-Apr-03	1	0	1	4,063
B. *Neochordodes occidentalis*						
Ceuthophilus sp.	White Bluffs Ferry	4-May-02	1	0	1	no data
Ceuthophilus vicinus Hubbell	White Bluffs Ferry	8-Jun-02	1†	1	1	no data

* Total uninfected hosts are given for the indicated collection locality only and includes all specimens collected at that site throughout the study. The total number of each species collected during the entire study is much higher.
† Specimen with 2 emergent worms.
‡ Two specimens with 2 emergent worms.
§ Two specimens with 3 emergent worms.

investigation of cuticle structures from a lateral view. Worms were identified by their posterior and cuticle structures based on the key and photomicrographs provided by Schmidt-Rhaesa et al. (2003).

Molecular methods

Molecular work was conducted on 2 individuals of each of the 2 types of worms collected. From each worm, a 0.5-cm section was cut, dried at room temperature, and used for DNA extraction using the E.Z.N.A.® Mollusc DNA Kit (Omega Bio-tek, Norcross, Georgia) following the manufacturer's instructions. Extracted DNA was stored at −70 C. Partial sequences of 18S and 28S rDNA, and complete *cox*1, were amplified using GoTaq® Flexi DNA Polymerase (Promega Corp. Madison, Wisconsin). The primers used were: HHW28S-001F: CCG ATT TCC GAC CTC AGA T; HHW28S-1175R: ACC CAG GTT TGA CGA TCG ATT TGC; G18S1F, TAC CTG GTTGAT CCT GCC AGT AG; 18S1018R, TAT CTG ATC GCC TTC GAA CC; LCO1490: GGT CAA CAA ATC ATA AAG ATA TTG G; HCO2198: TAA ACT TCA GGG TGA CCA AAA AAT CA, using standard PCR protocols. PCR reactions were analyzed by agarose gel electrophoresis, with the use of 1.0% agarose gels, stained with 0.5% GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California) and visualized on a UV transilluminator. Amplicons were purified by ethanol precipitation and sequenced using the BigDye® version 3.1 kit (Applied Biosystems, Foster City, California) on an ABI 3130× sequence analyzer (Applied Biosystems). Both strands of the amplified DNA fragments were sequenced, edited in Sequencer version 4.10.1 (Gene Codes, Ann Arbor, Michigan), and manually corrected for ambiguous base calls.

DNA sequences were compared to other sequences in the GenBank database using BLAST (Altschul et al., 1990), applying the distance tree of results utility employing the Fast Minimum Evolution model (Jukes and Cantor, 1969) associated with BLASTN to infer phylogenetic relationships. For *cox*1, sequences were aligned by eye, along with 30 other unpublished nematomorph and outgroup *cox*1 sequences, with third position codons removed. jModelTest (Posada, 2008) was used to select the best fitting nucleotide substitution model using the Akaike information criterion. Mega 5.0 (Tamura et al., 2007) was used to produce a maximum likelihood (ML) tree.

RESULTS

We captured 36 carabid individuals that belonged to 6 species, 1 rhaphidophorid species, and 1 rhaphidophorid identifiable only to genus, that were infected with horsehair worms (Table I). Parasitized specimens were obvious due to the worm protruding from the host's abdomen (Fig. 2). The majority of parasitized individuals (n = 29) were from 1 beetle species, *Calathus ruficollis* DeJean, predominantly collected during a single trapping interval. All parasitized beetles were captured in the spring, between late March and April. However, most species were active throughout the year and actual peak seasonal activity varied by species. For instance, *Calosoma luxatum* Say was collected most frequently in the spring, while peak activity period for the other species ranged from early summer (*Poecilus lucublandus* Say) through late fall (*Amara discors* Kirby) (see Looney [2000] for detailed carabid seasonality data). One parasitized rhaphidophorid was captured in early May and the other in June. We did not collect data for non-infected rhaphidophorids.

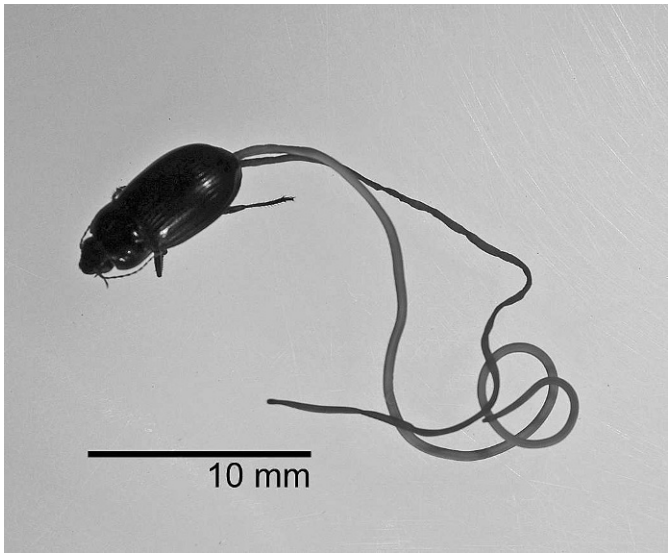

FIGURE 2. *Amara quenseli* with protruding *Gordionus* sp. This host contained 2 worms, a female (thicker, lighter color) and a male (thinner, darker color).

A total of 46 worms was collected from the 38 hosts. Multiple infections were observed in 5 beetles and 1 camel cricket (Table I); 4 hosts each with 2 worms and 2 hosts each with 3 worms. Five cases of multiple infections were composed of at least 1 male and 1 female worm. The frequency of emerging worms was generally low for the total number of carabid beetles captured; data were not collected for camel crickets (Table I).

Worms collected from the cricket hosts contained only a single type of cuticular areole. The male posterior was undivided, without tail lobes, and some specimens contained a slight ventral groove. Based on these characters, we determined this species to be *Neochordodes occidentalis* (Montgomery, 1898). Worms from the beetles contained a single type of cuticular areole with interareolar tubercles. Male worms from the beetles possessed 2 tail lobes, precloacal rows of bristles, postcloacal spines, and adhesive warts. Based on these characters, this is a *Gordionus* species. However, the presence of distinct interareolar structures makes this a new species which will be formally described elsewhere.

Amplification products of appropriate size were obtained for all 3 genes for both individuals of *N. occidentalis*. However, for *Gordionus* sp., PCR with *cox*1 failed to produce bands for either worm individual. Genetic data were deposited into GenBank (GenBank JN881995–JN882004). The amplified fragments were about 1,125 base pairs (bp) for 28s, about 950 bp for 18S, and 700 bp for *cox*1.

Presently, GenBank contains very little information for the Nematomorpha (20 sequences for 18S, 5 sequences for 28S, and 1 *cox*1 sequence). This data limitation, especially for 28S and *cox*1, does not allow us to place either of these species into a phylogenetic context, but it does allow us to check our genetic data against contamination and broadly place these species into the phylum. Furthermore, the limitation of *cox*1 sequences makes the BLAST tool unsuitable for this gene. For 28S, the BLAST search and distance tree revealed that the sequences obtained for *N. occidentalis* and *Gordionus* sp. were most closely related to the only 3 hairworm 28S sequences presently in GenBank. For 18S, the closest matches were *N. occidentalis* (AF421768; max score: 1655, max identity: 100%, e-value: 0) and *Gordionus wolterstorffii* (AF421765; max score: 1646, max identity: 99%, e-value: 0) for *N. occidentalis* and *Gordionus* sp., respectively. The model chosen by the jModelTest for *cox*1 was TIM1+I+G. The ML tree showed that *N. occidentalis* was placed among other *N. occidentalis* from New Mexico and Arizona, whereas the *Gordionus* sp. was placed between the larger group of *Chordodes* species and *Gordius* species (data not shown). All of these results taken together strongly suggest that DNA sequences were from hairworms and not contaminants.

Voucher specimens for parasites are deposited at the Museum of Southwestern Biology, Division of Parasitology, University of New Mexico, Albuquerque, New Mexico. (accession numbers MSB: Para: 941-942). Insect host specimens are tagged as voucher specimens and have been deposited in the James Entomological Collection, Washington State University, Pullman, Washington.

DISCUSSION

This study presents an informative dataset focused on the terrestrial definitive hosts, with host data available for each worm individual. Since most hairworms are collected in their post-parasitic, free-living form, knowledge of host associations remains sparse. In fact, host data are only available for 8 of the 18 described North American species (Schmidt-Rhaesa et al., 2003; Poinar and Chandler, 2004; Schmidt-Rhaesa et al., 2009). This report adds 1 species, *Ceuthophilus vicinus*, to the known hosts of *N. occidentalis*. Previous host reports were of a ''large grasshopper'' (Montgomery, 1900), an acridid (Ward and Whipple, 1918), and the katydid genus *Pediodectes* and multiple species of *Stenopelmatus* (Jerusalem crickets) (Poinar and Wiessman, 2004). Of the 34 *Gordionus* species recorded from Europe, hosts include myriapods, coleopterans, and dermapterans (Schmidt-Rhaesa, 1997; Poinar et al., 2004). This paper identifies a third host record for a North American *Gordionus*, in addition to *Stenopelmatus* sp. (Poinar and Wiessman, 2004) and the millipede *Cambala annulata* (Schmidt-Rhaesa et al., 2009).

Although our data are too sparse for statistical analysis, we are intrigued by the observation that 5 of the 6 multiple-worm infections included both male and female specimens. This sample size is quite small and does not account for sex differences in development and emergence but, even so, raises questions about nematomorph ecology including mate-finding and effects on host behavior. Multiple infections of nematomorphs occur in from 8–15% of hosts (Poulin, 1995; de Villalobos et al., 1999; Thomas et al., 2002), and multiple-sex infections could increase the likelihood of finding a mate after exiting the developmental host.

Nematomorphs instigate erratic locomotory behavior and a greater propensity to enter water in their hosts (Thomas et al., 2002). The majority of parasitized beetles recognized in this study were trapped at 1 site near water during a single collecting period, perhaps indicating an aggregative effect of hairworms on their hosts (Table I). On the other hand, several other hosts were captured in collecting localities relatively far (~5 km) from the nearest water (Fig. 1; Rattlesnake Ridge slope, Sand Dunes). Five of the carabid host species are normally capable of flight, indicative of the important role carabids may play in nematomorph dispersal, though it is not known if parasitization by hairworms impairs flight. Even if flight ability is curtailed when parasitized, carabids may still contribute to wide nematomorph dispersal; the host *Calosoma luxatum*, trapped at the Sand Dunes site several kilometers from water, is a flightless species.

Although we only report obvious cases of parasitism, i.e. emergent, mature nematomorphs, prevalence seems to be quite low. Confirmed hosts comprised only a tiny fraction of all beetles of each parasitized species. Based on beetles captured at a specific locality, observed parasitism by host species ranged from 0.01% (*Cymindis planipennis*) to 16% (*Amara quenseli*) (Table I). These numbers are considerably lower if calculated for the total number of each host species captured during the entire study, with the highest observed parasite frequency dropping to 3.5% (for *Poecilus lucublandus*) and the lowest to 0.007% (for *Calathus ruficollis*).

We did not examine any of these specimens for evidence of internal or already emerged parasites, nor did we likewise examine any of the other 36,533 carabid beetles captured during this study. Thus, these observed parasitization rates are likely underestimates. The 6 carabid species discussed here are out of a total captured carabid fauna of 93 species, 2 of which are also known hosts for nematomorphs (Leffler, 1984; Bolek and Coggins, 2002). This suggests that the hosts we observed were but a serendipitous

subsample of the total potential host population and that several other beetle species may have harbored nematomorphs as well.

Larochelle (1978) summarized the existing knowledge of carabid parasites worldwide in 1978, noting several gordiid host records. Poinar et al. (2004) expanded upon that list, adding 3 new host records and the first parasite records for a species of *Parachordodes* in North America. Currently, carabid genera in North America with documented gordiid parasites include *Amara* (present report), *Calathus* (present report), *Calosoma* (present report), *Chlaenius* (Leffler, 1984; Hanelt and Janovy, 2000), *Cymindis* (present report), *Gastrellarius* (Leidy, 1856), *Harpalus* (present report; Tomlin, 1975), *Poecilus* (present report), and *Pterostichus* (Goulet, 1974; Bolek and Coggins, 2002; Poinar et al., 2004). The most gordiid hosts recorded are from *Pterostichus*.

Including the species presented in this report, there are at least 79 known carabid hosts of horsehair worms. Sixty-three of these, or 80%, are known from Europe. However, the paucity of North American records surely indicates a lack of study rather than a diminished number of suitable carabid hosts. The present report and a handful of papers published in the past 25 yr in the United States add 11 carabid hosts to the 3 documented before 1984, a dramatic and relatively recent increase in our knowledge of North American carabid–gordiid interactions. Similarly, Poinar and Weissman (2004) reported several new host records for horsehair worms and mermithids parasitizing orthopterans, including the first records of nematodes parasitizing the species of Stenopelmatidae (Orthoptera). Taken together, these papers and our results indicate that nematomorph–arthropod associations remain poorly documented in North America, with many more likely to be discovered.

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LITERATURE CITED

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology **215:** 403–410.

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, United States. Journal of Parasitology **88:** 287–294.

de Villalobos, L. C., I. Ribera, and I. S. Downie. 1999. Hairworms found in Scottish agricultural land, with descriptions of two new species of *Gordionus* Muller (Nematomorpha: Gordiidae). Journal of Natural History **33:** 1767–1780.

Emery, R. M., and M. C. McShane. 1978. Comparative ecology of nuclear waste ponds and streams on the Hanford Site. PNL-2499, Pacific Northwest Laboratory, Richland, Washington, 17 p.

Goulet, H. 1974. Biology and relationships of *Pterostichus adstrictus* Eschscholtz and *Pterostichus pensylvanicus* LecConte (Coleoptera: Carabidae). Quaestiones Entomologicae **10:** 3–33.

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

———, and ———. 2000. New host and distribution record of *Gordius difficilis* (Nematomorpha: Gordioidea) from a vivid metallic ground beetle *Chlaenius prasinus* (Coleoptera: Carabidae) from western Nebraska, USA. Comparative Parasitology **67:** 107–108.

———, and ———. 2003. Spanning the gap: Identification of natural paratenic hosts of horsehair worms (Nematomorpha: Gordioidea) by experimental determination of paratenic host specificity. Invertebrate Biology **122:** 12–18.

———, and ———. 2004. Life cycle and paratenesis of American gordiids (Nematomorpha: Gordiida). Journal of Parasitology **90:** 240–244.

Johansen, J. R., J. Ashley, and W. R. Rayburn. 1993. Effects of rangefire on soil algal crusts in semiarid shrub-steppe of the Lower Columbia Basin and their subsequent recovery. Great Basin Naturalist **53:** 73–88.

Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Academic Press, New York, New York, p. 21–132.

Larochelle, A. 1978. Catalogue des parasites et phoretiques animaux des Coleopteres Carabidae (les Cicindelini compris) du monde. Cordulia **4:** 1–7.

Leffler, S. R. 1984. Record of a horsehair worm (Nematomorpha: Gordiidae) parasitizing *Chlaenius sericeus* Forster (Coleoptera: Carabidae) in Washington. Coleopterists Bulletin **38:** 130.

Leidy, J. 1856. A synopsis of Entozoa and some of their ectocongeners, observed by the author. Proceedings of the Academy of Natural Sciences of Philadelphia **8:** 42–58.

Lindroth, C. H. 1961. The ground beetles of Canada and Alaska, Part 2. Opuscula Entomologica Supplementum 20. Lund, Sweden, 200 p.

———. 1966. The ground beetles of Canada and Alaska, Part 4. Opuscula Entomologica Supplementum 29. Lund, Sweden, 240 p.

———. 1968. The ground beetles of Canada and Alaska, Part 5. Opuscula Entomologica Supplementum 33. Lund, Sweden, 296 p.

———. 1969. The ground beetles of Canada and Alaska, Part 6. Opuscula Entomologica Supplementum 34. Lund, Sweden, 248 p.

Looney, C. 2000. Seasonality and distribution of ground beetles (Coleoptera: Carabidae) on the Hanford Nuclear Reservation. M.S. Thesis. Washington State University, Pullman, Washington, 136 p.

Montgomery Jr., T. H. 1898. The Gordiacea of certain American collections with particular reference to the North American fauna. Bulletin of the Museum of Comparative Zoology **32:** 23–59.

———. 1900. Gordiacea from the Cope Collection. Biological Bulletin **3:** 95–98.

Morrill, W. L., D. G. Lester, and A. E. Wrona. 1990. Factors affecting efficacy of pitfall traps for beetles (Coleoptera: Carabidae and Tenebrionidae). Journal of Entomological Science **25:** 284–293.

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

———, J. Rykken, and J. LaBonte. 2004. *Parachordodes tegonotus* n. sp., (Gordioidea: Nematomorpha) a hairworm parasite of ground beetles (Carabidae: Coleoptera) with a summary of gordiid parasites of carabids. Systematic Parasitology **58:** 139–148.

———, and D. B. Weissman. 2004. Hairworm and nematode infections of North American Jerusalem crickets, field crickets, and katydids (Orthoptera: Stenopelmatidae, Gryllidae and Tettigoniidae). Journal of Orthoptera Research **13:** 143–147.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution **25:** 1253–1256.

Poston, T. R., K. R. Price, and D. R. Newcomer. 1991. An evaluation of the chemical, radiological, and ecological conditions of West Lake on the Hanford Site. PNL-7662, Pacific Northwest Laboratory, Richland, Washington, 85 p.

Poulin, R. 1995. Hairworms (Nematomorpha: Gordioidea) infecting New Zealand short-horned grasshoppers (Orthoptera: Acrididae). Journal of Parasitology **81:** 121–122.

Sakschewsky, M. R., and J. L. Downs. 2001. Vascular plants of the Hanford Site, PNNL-13688. Available at: http://ecology.pnl.gov/Library/pnnl13688.pdf. Accessed 7 March 2010.

Schmidt-Rhaesa, A. 1997. Nematomorpha. Gustav Fischer Verlag, Stuttgart, Germany, 128 p.

———, M. A. Farfan, and E. C. Bernard. 2009. First record of millipedes as hosts for horsehair worms (Nematomorpha) in North America. Northeastern Naturalist **16:** 125–130.

———, 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.

Soll, J., J. A. Hall, R. Pabst, and C. Soper. 1999. Biodiversity inventory and analysis of the Hanford Site—Final Report 1994–1999. The Nature Conservancy of Washington, Seattle, Washington, 179 p.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution **24:** 1596–1599.

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

Tomlin, A. D. 1975. Notes on the biology and rearing of two species of ground beetles, *Pterostichus melanarius* and *Harpalus pensylvanicus* (Coleoptera Carabidae). Canadian Entomologist **107:** 67–74.

Vickery, V. R., and D. K. McE. Kevan. 1985. The insects and arachnids of Canada, part 14. The grasshoppers, crickets, and related insects of Canada and adjacent regions. NRC Research Press, Ottowa, Ontario, Canada, 919 p.