Journal of Invertebrate Pathology **74,** 89–95 (1999)
Article ID jipa.1999.4860, available online at http://www.idealibrary.com on IDEAL®

New North American Records of Aquatic Insects as Paratenic Hosts of *Pheromermis* (Nematoda: Mermithidae)

Daniel P. Molloy,* William S. Vinikour,† and Richard V. Anderson‡

**Biological Survey, New York State Museum, Cultural Education Center, Albany, New York 12230; †Environmental Assessment Division, Argonne National Laboratory, Argonne, Illinois 60439; and ‡Department of Biological Sciences, Western Illinois University, Macomb, Illinois 61455*

Received November 16, 1998; accepted March 9, 1999

Several species of aquatic insects in Trout Park Nature Preserve (Elgin, IL) were observed to have small, black spots (<0.1 mm diameter) visible within their bodies. Microscopic examination revealed these spots to be coiled juveniles of a mermithid (Nematoda: Mermithidae). Based on host habitat (seepage areas and rivulets), host species (aquatic insects), and size (mean diameter of coiled juveniles = 79 μm), it is likely that these mermithids were in the genus *Pheromermis*. Since adult mermithids were not found, species determination was not feasible, and the possibility of a new species cannot be ruled out. *Pheromermis pachysoma* and *Pheromermis vesparum*, however, are two species known to use aquatic insects as paratenic (i.e., transport) hosts in order to reach their definitive hosts, vespid wasps. Wasp larvae are infected by consuming the flesh of adult aquatic insects that contain the coiled juveniles of these *Pheromermis* spp. Of the 19 macroinvertebrate species examined in this study, *Pheromermis* juveniles were found in 4 caddisfly species (*Hesperophylax designatus, Lepidostoma liba, Glossosoma intermedium,* and *Diplectrona modesta*) and in 2 stonefly species (*Clioperla clio* and *Amphinemura delosa*). In addition to all 6 insect species being new host records for *Pheromermis* infection, this also represents the first report of nematode infection in stoneflies within the Western Hemisphere and of a *Pheromermis* sp. in Illinois. Among trophic groups, insect detritivores have been frequently recorded infected with coiled *Pheromermis* juveniles because of their direct consumption of eggs, and we also observed this for detritivores in our investigation (e.g., *L. liba* and *A. delosa*). Because *C. clio* was intensively infected, however, our study also provided evidence that predatory insects can be paratenic hosts. Coiled juveniles were typically present in muscle and fat body and present in almost all body regions. Not every infected paratenic host had external signs of infection; thus, dissections are required for accurate determination of infection prevalence and intensity. Our findings, in conjunction with those made in previous studies, indicate that a wide variety of aquatic insects may be used as paratenic hosts by *Pheromermis*. Because of their small size, it is highly likely that coiled juveniles are either overlooked or mistaken for melanized integumental wounds during field studies of aquatic insects. A more careful inspection for these coiled juveniles in aquatic insects, especially detritivores and their predators in seepage areas, would probably reveal that *Pheromermis* is far more common than presently documented. © 1999 Academic Press

***Key Words:* *Hesperophylax designatus; Lepidostoma liba; Glossosoma intermedium; Diplectrona modesta; Clioperla clio; Amphinemura delosa; Pheromermis vesparum; Pheromermis pachysoma;* prevalence and intensity of infection.**

INTRODUCTION

Following an aquatic macroinvertebrate survey of Trout Park Nature Preserve, Elgin, Illinois (Vinikour and Anderson, 1984), some preserved specimens were noted to have small, black spots (<0.1 mm diameter) visible within their bodies. Microscopic examination revealed each spot to contain a nematode—identical to the coiled juveniles of *Pheromermis* (Mermithidae) as illustrated in Poinar (1981). As a result, this investigation was initiated to characterize this *Pheromermis* infection within this aquatic macroinvertebrate community.

Parasitism of aquatic insects by mermithid nematodes has been frequently reported, and the dipteran families Chironomidae, Simuliidae, and Culicidae have been especially well studied in this regard (Poinar, 1979). Mermithids typically have a single host in their life cycle, and development commences soon after egg hatch when the second-stage juvenile (i.e., one molt occurs inside the mermithid egg) comes into contact with a suitable host and penetrates into its hemocoel. Following completion of their parasitic development, mermithids typically emerge from their hosts, molt to the adult stage, mate, and lay eggs. Poinar (1976) and Poinar *et al.* (1976), however, reported that the mermi-

0022-2011/99 $30.00

thid *Pheromermis pachysoma* had an unusual life history requiring two hosts—an aquatic paratenic and a terrestrial definitive host. Paratenic (i.e., transport) hosts have been documented in the life cycles of other helminths (e.g., hairworms (Nematomorpha); Poinar and Doelman, 1974), but were previously unknown among the Mermithidae. Incorporation of a paratenic host into an aquatic nematode's life cycle is advantageous in that it allows exploitation of terrestrial hosts which these parasites would otherwise be unable to contact (Poinar *et al.*, 1976). Paratenic hosts also assist in spatial and temporal dispersal of the parasites since the paratenic host acts as an accumulator of the parasitic organisms (Kennedy, 1975).

Based on aquatic habitat and documented paratenic hosts of described *Pheromermis* spp., these mermithids from Trout Park could have been *P. pachysoma* or *Pheromermis vesparum*, but the possibility of a new species cannot be ruled out. The overall life cycle of *P. pachysoma* or *P. vesparum* is similar (Fig. 1) and can be summarized as follows (Poinar *et al.*, 1976; Kaiser, 1987). An egg containing a second-stage juvenile is typically eaten by an aquatic insect. Following egg hatch within the gut, the juvenile migrates to various host tissues and remains in a coiled, quiescent stage without any further development. These coiled mermithids typically appear as black spots in tissues underlying a host's integument. Since there is no evidence of host tissue consumption, infection of paratenic hosts by *Pheromermis* spp. is normally benign. Following its metamorphosis to the adult stage and the start of its terrestrial existence, the paratenic host may then be captured by a wasp (Hymenoptera: Vespidae) and brought back to its nest. Wasp larvae are infected by consuming the flesh of such infected prey. Following completion of its parasitic development within the hemocoel of a wasp, the postparasitic stage emerges from an adult wasp when it is visiting a water source or wet area. Upon their return to water, these two *Pheromermis* spp. complete their life cycle by molting to the adult stage, mating, and laying eggs. Virgin females and released eggs can remain viable in nature for a year or more.

The present study of Trout Park macroinvertebrates further expands the list of paratenic hosts for *Pheromermis* and includes the first record of nematode infection in stoneflies (Plecoptera) within the Western Hemisphere. In addition, it traces the dynamics of *Pheromermis* infection in one of the paratenic host populations from early through late instars and documents the location of the coiled mermithids within the body regions of their paratenic hosts.

MATERIALS AND METHODS

Trout Park Nature Preserve is located on the heavily wooded eastern bluff of the Fox River in the northern

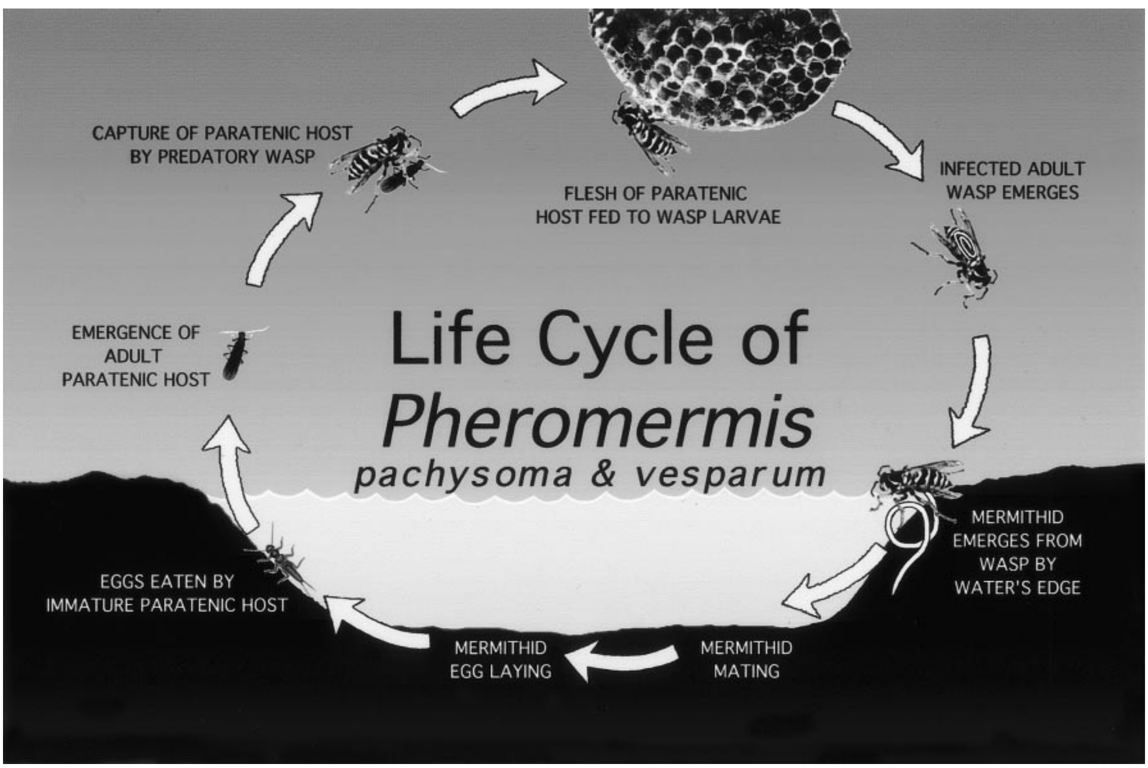

FIG. 1. Life cycle of *Pheromermis* spp. infecting aquatic insects and wasps based on life histories described in Poinar *et al.* (1976) and Kaiser (1987).

part of the city of Elgin, Illinois. The bluff slopes steeply, with park elevations ranging from 220 m at the base to 245 m at the bluff top. The preserve contains two steep ravines and several shallow depressions along the slope. Five major brooks are contained within the 10.5-hectare preserve and each brook has spring- and seepage-fed rivulets feeding into them. The major brook is about 670 m long. Spring and seep temperatures are 11°C, whereas temperature extremes in the lower portion of the main brook range from 4 to 18°C. Depths range from <3.0 cm to about 1 m in some pooled areas. Brook widths range from 0.3 to <3.0 m, with most averaging 1–2 m wide. Substrates in all the brooks are predominantly cobble interspersed with smaller particles ranging to silts. Marl deposits are also extensive throughout the park, as are leaf packs and wood debris.

Aquatic macroinvertebrates were collected and preserved monthly from October 1979 through May 1980 from brooks, rivulets, and seepage areas. Invertebrates were hand collected from all major habitat types including cobble, gravel, sand/silt, marl, wood debris, aquatic vegetation (algae and watercress), leaf litter packs, and artificial substrates (e.g., bricks, cans, and other trash). Coiled nematodes were noted in some specimens during the identification phase of the survey. Thus, a number of alcohol-preserved macroinvertebrates (19 species) were dissected and examined for infection (70×).

RESULTS AND DISCUSSION

Mermithid Species Determination

The melanized cavities containing the nematodes were round and laterally flattened, apparently due to the pressure exerted within by the coiled juvenile. Mean cavity diameter was 79 µm (range 60–89 µm, $n = 10$). Poinar (1981) had reported a range in diameter of 60–100 µm for coiled juveniles of *P. pachysoma*, but similar published information is unavailable for *P. vesparum*. It is likely, however, that the diameter of cavities containing coiled juveniles of both species would be similar since the size of infective-stage juveniles of *P. pachysoma* ($L \times W \approx 300 \times 12$ µm, Fig. 6 in Poinar *et al.*, 1976) falls within the range for *P. vesparum* ($L \times W = 245–300 \times 12–13$ µm; Kaiser, 1987). Unfortunately the length of the coiled *Pheromermis* juveniles from Trout Park could not be reliably measured due to the rigidity of the alcohol-preserved specimens. In any case, determination of mermithid species is based primarily on adult morphology. Poinar *et al.* (1976) had indicated that free-living stages of *P. pachysoma* in California could be observed in the spring in clusters just under the surface of the soil or under the gravel forming the bottom of streamlets. Adult *P. pachysoma* and *P. vesparum* are relatively large, wide, white worms, and adult females have been recorded to have a mean length and width, respectively, of 6.7 cm and 0.9 mm (Poinar *et al.*, 1976) and 7.9 cm and 0.9 mm (Kaiser, 1987). Such mermithids, however, were not observed during a search of rivulets and seepage areas within Trout Park in May 1997, and thus no specific determination of this *Pheromermis* sp. was possible for this study.

Paratenic Host Range

P. pachysoma has been reported to have both a wide range of paratenic hosts and a wide geographical distribution, including North American and European records (Poinar, 1981). Field reports of its paratenic hosts (Poinar *et al.*, 1976; Poinar, 1981) include Trichoptera (*Gumaga griseolum*, *Limnephilus peltus*, and *Hesperophylax* sp.), Diptera (*Tipula* sp.), Coleoptera, and Ephemeroptera. In addition, dipteran larvae and ephemeropteran nymphs have been successfully infected as paratenic hosts in the laboratory (Poinar, 1981; Kaiser, 1987). North American collection records for *P. pachysoma* include the states of Arizona, California, Colorado, Louisiana, Nevada, New Mexico, North Carolina, Oregon, South Dakota, Tennessee, Utah, and Wyoming and the Canadian provinces of British Columbia, Ontario, and Saskatchewan (Poinar, 1981). *P. vesparum* has been reported only from Austria (Kaiser, 1987) where its paratenic hosts included Trichoptera (Limnophilidae) and Diptera (Tipulidae), but since surveys of *Pheromermis* spp. are so rarely conducted, the geographical distribution of *P. vesparum* is likely much more extensive than implied by this single published report.

Of the 19 macroinvertebrate species examined from the Trout Park study, *Pheromermis* juveniles were found in four caddisfly species (*Hesperophylax designatus*, *Lepidostoma liba*, *Diplectrona modesta*, and *Glossosoma intermedium*) and in two stonefly species (*Clioperla clio* and *Amphinemura delosa*) (Table 1). In addition to all six insects being new host species records for *Pheromermis* infection, this also represents the first report of *Pheromermis* in the state of Illinois and of nematode infection in stoneflies within the Western Hemisphere. Unidentified nematodes, possibly *Pheromermis*, however, have been reported from European stoneflies. Kuhtreiber (1934) indicated that unidentified nematodes were found inside the intestines of stonefly nymphs and adults in the Tyrolean Alps. Schoenemund (1924) indicated that there were unidentified coiled nematodes present in stonefly intestines quite often in Germany and that young and older nymphs of *Perla* and *Perloides* had unidentified mermithids in their body cavity, antennae, caudal filaments, and "back vessels." Winkler (1956) reported an 11-cm-long *Mermis* sp. in the abdominal cavity of a stonefly. Although the stonefly in the latter reference does not

TABLE 1

Pheromermis Infection in Macroinvertebrates in Trout Park Nature Preserve

Species	Principal habitat	Trophic relationship[a]	No. examined	Mean intensity[b]	Prevalence[c] (%)
Trichoptera					
Hesperophylax designatus	Wood debris	Shredder, detritivore, herbivore	30	7.3	40.0
Lepidostoma liba	Wood debris	Shredder, detritivore	15	18.7	100
Neophylax concinnus	Wood debris, cobble	Scraper	34	0	0
Wormaldia moesta	Cobble	Collector, filterer	21	0	0
Diplectrona modesta	Wood debris, leaf litter, cobble	Collector, filterer	18	1.3	16.7
Glossosoma intermedium	Cobble, wood debris	Scraper	70	1.0	1.4
Plecoptera					
Clioperla clio	Leaf litter, wood debris	Engulfer, predator	264	23.8[d]	42.4–100[e]
Amphinemura delosa	Cobble, wood debris	Shredder, detritivore, collector, gatherer	31	6.7	100
Nemoura trispinosa	Cobble, wood debris	Shredder, detritivore	5	0	0
Ephemeroptera					
Baetis vagans	Cobble, wood debris	Collector, gatherer, scraper	27	0	0
Diptera					
Euparyphus sp.	Wood debris	Collector, gatherer, scraper	14	0	0
Dicranota sp.	Leaf litter	Engulfer, predator	3	0	0
Tipula abdominalis	Leaf litter, wood debris	Shredder, detritivore, herbivore	11	0	0
Coleoptera					
Agabus sp.	Wood debris	Piercer, predator	2	0	0
Optioservus fastiditus	Wood debris	Collector, gatherer, scraper	7	0	0
Cymbiodyta sp.	Wood debris	Collector, gatherer	2	0	0
Turbellaria					
Dugesia dorotocephala	Cobble, wood debris	Engulfer, predator	7	0	0
Isopoda					
Caecidotea intermedius	Cobble, leaf litter, wood debris	Shredder, detritivore	17	0	0
Amphipoda					
Gammarus pseudolimnaeus	Cobble, watercress, leaf litter, wood debris	Shredder, detritivore	31	0	0

[a] Merritt and Cummins (1996) and Pennak (1978).
[b] Coiled juveniles per infected host; except for *C. clio,* data presented represent pooled monthly dissections throughout study.
[c] No. infected/no. examined.
[d] Range in mean infection intensity in *C. clio* during individual monthly samples = 1–30; see Fig. 2.
[e] October 1979–May 1980; monthly $n = 33$.

represent a paratenic host, it is particularly curious since the size of this mermithid is unusually long, and fully developed *Pheromermis* spp. within the hemocoels of their definitive hosts are relatively large mermithids. *P. pachysoma* and *P. vesparum*, for example, have been reported up to 9.0 and 10.2 cm in length, respectively (Poinar *et al.*, 1976; Kaiser, 1987).

Prevalence and Intensity

Prevalence and intensity of infection were particularly high among *C. clio, L. liba,* and *A. delosa* (Table 1). *C. clio* was the only species collected in sufficient numbers to examine infection on an individual monthly basis. Both prevalence and intensity of infection in this species were observed to increase throughout the study period (Table 1, Fig. 2). Soon after hatching in October, early instar *C. clio* nymphs (head capsule width ≈ 1.0 mm) became lightly infected. Mean infection intensity, as noted externally in the body wall, in such early instar *C. clio* was less than 1/stonefly in October, but peaked in April at >30 in late instars (head capsule width ≈ 3.0 mm) (Fig. 2).Likewise, maximum intensity of infection in *C. clio* was 2 in October/November, 34 in December/March, and 101 in April/May. Following discovery of *Pheromermis* infection in these 1979–1980 survey samples, 57 *C. clio* previously collected in 1977 in Trout Park were examined and all of them were also infected (mean infection intensity = 11). Thus, *Pheromermis* infection was well established in Trout Park preceding the study.

Characteristics of Suitable Paratenic Hosts

Among trophic groups, insect detritivores are frequently infected with coiled *Pheromermis* juveniles because of their direct consumption of eggs (Poinar, 1981). We also observed this for detritivores in our investigation (e.g., *L. liba* and *A. delosa*, Table 1). Because *C. clio* was intensively infected (Table 1), however, our study also provided evidence that predatory insects can be paratenic hosts. As an early instar, *C. clio* probably ingested some *Pheromermis* eggs while grazing during the autumn months (October/Novem-

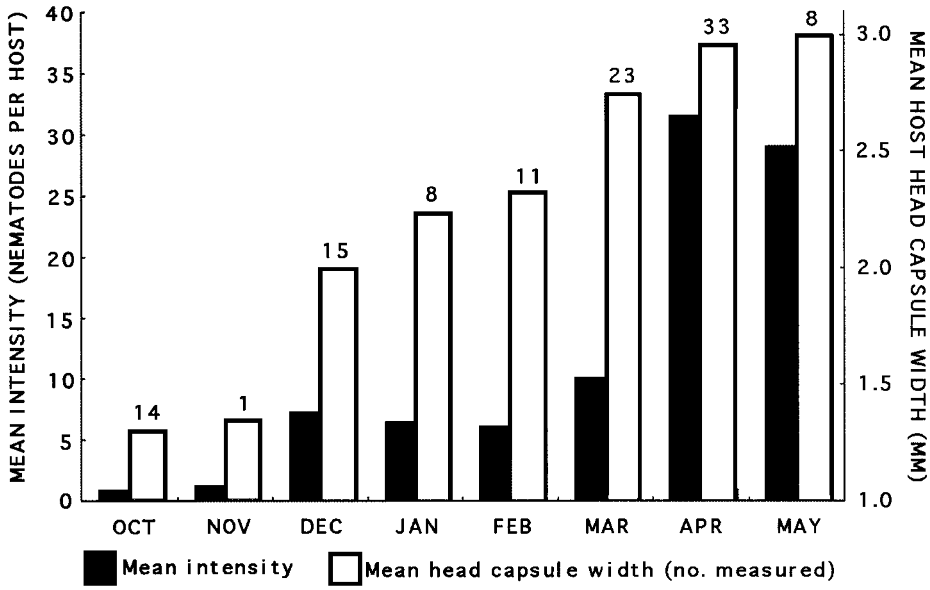

FIG. 2. Intensity of externally visible *Pheromermis* infection in *Clioperla clio* nymphs during the study period.

ber, Fig. 2), but we suggest that the sharp rise in infection intensity recorded during the following spring (April/May, Fig. 2) resulted from the probable consumption of infected prey. Poinar *et al.* (1976) observed experimentally that coiled juveniles removed from a host were capable of infecting new paratenic hosts. It is hypothesized, therefore, that following capture and consumption of other paratenic hosts, *C. clio* nymphs similarly became infected or, if already infected, increased their intensity of infection.

In addition to trophic status, habitat is a key factor determining which aquatic insects may become infected. Rivulet/seepage areas are believed to be highly suitable locations where wasps (the probable final host of this *Pheromermis* sp.) land to drink and, therefore, also ideal areas for nematode emergence, molting, mating, and egg laying. Because of low water velocity, *Pheromermis* eggs likely accumulate in such habitats, increasing the probability of their ingestion by detritivores. We suggest that *C. clio* became heavily infected because of its preference for leaf litter and its predation on detritivores in such microhabitats.

A large body size probably contributes to higher prevalence and intensity rates among suitable hosts due to increased food consumption, including *Pheromermis* eggs, and *C. clio* was one of the largest insects encountered at Trout Park. In this regard, however, it is surprising that the largest insect in the survey, *Tipula abdominalis*, was not found to be infected (Table 1). This species is known in part as a detritivore, and tipulids have been recorded as paratenic hosts for both *P. pachysoma* (Poinar *et al.*, 1976) and *P. vesparum* (Kaiser, 1987). Possibly low sample size (i.e., 11) was a contributing factor.

FIG. 3. Adult *Clioperla clio* with over 70 *Pheromermis* coiled juveniles visible as black spots through integument.

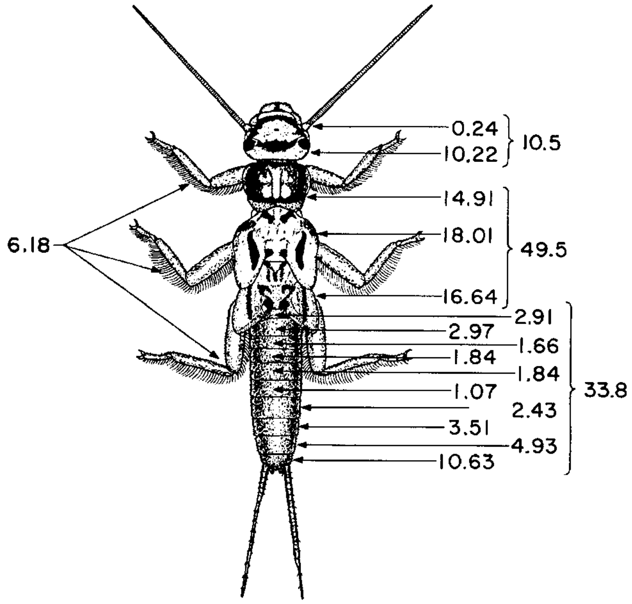

FIG. 4. Distribution of *Pheromermis* coiled juveniles ($n = 2689$) externally visible in *Clioperla clio* nymphs ($n = 113$). [Redrawn from Frison (1935).]

Affect of Infection on Host

In short-term, laboratory infection trials of *Culex pipiens* with *P. pachysoma*, Poinar *et al.* (1976) observed that infection intensities of more than 100 juveniles caused host death. However, *C. clio,* possibly due in part to its large size and the long-term exposure period (i.e., months), appeared unharmed by the accumulation of such high densities of juveniles. Poinar (1981) reported up to 200 *P. pachysoma* in adult caddisflies and indicated that such densities were not detrimental since hosts readily underwent metamorphosis. Since adult *C. clio* are over twice the size of adult caddisflies, they might be expected to support an even greater burden of nematodes, and active, heavily infected *C. clio* adults were observed in Trout Park (Fig. 3).

Location of Coiled Juveniles

Poinar *et al.* (1976) reported *P. pachysoma* in gut wall, muscles, and fat body of field-collected insects. In our study, coiled juveniles were typically present in muscle and fat body within every body region. The juveniles observed externally in *C. clio* specimens, for example, were present in virtually every body segment (Fig. 4). These black spots were almost invariably coiled nematodes and not integumental wounds. Depending on host species, however, certain body regions appeared to be preferred sites. The majority of juveniles were observed in the abdomen of *L. liba,* in the legs of *H. designatus,* and in the thorax of both *C. clio* and *A. delosa* (Table 2). Poinar (1981) indicated that coiled juveniles in caddisflies were primarily visible through the integument in the abdomen, with fewer in the thorax and legs.

Dissections revealed that not every infected paratenic host had external signs of infection. Although 100% (15/15) of infected *L. liba* had at least one coiled juvenile externally visible, 9.7% (3/31) and 25.0% (3/12), respectively, of infected *A. delosa* and *H. designatus* showed no external signs of infection. Among all coiled juveniles within a host's body, the percentage that were visible externally was similar among the three hosts for which sufficient data were available: 66.0% (138/209), 62.5% (175/280), and 65.5% (57/87), respectively, in *A. delosa, L. liba,* and *H. designatus.* Thus, accurate determinations of infection prevalence and intensity does require that dissections be performed.

Research Recommendation

Our findings, in conjunction with those made in previous studies, have shown that a wide variety of aquatic insects are used as paratenic hosts by *Pheromermis.* Because of their small size (≤0.1 mm), it is highly likely that coiled juveniles are being frequently overlooked or mistaken for melanized integumental wounds on aquatic insects during field studies. A more careful

TABLE 2

Distribution of *Pheromermis* Coiled Juveniles in Individual Body Regions of Paratenic Hosts

Species	Total no. infected hosts examined	Total no. coiled juveniles observed	% Distribution			
			Legs	Abdomen	Thorax[a]	Head
Plecoptera						
Clioperla clio[b]	113	2,689	6.2	33.8	49.5	10.5
Amphinemura delosa	31	209	0.0	42.7	57.3	0.0
Trichoptera						
Lepidostoma liba	15	280	8.0	77.8	14.2	0.0
Hesperophylax designatus	12	87	63.1	12.4	17.6	6.9

[a] Excluding legs.

[b] Only data on externally visible juveniles are available for *C. clio;* a more extensive breakdown of distribution of externally visible juveniles is presented in Fig. 4.

inspection for these coiled juveniles in aquatic insects, especially detritivores in seepage areas, probably would reveal that *Pheromermis* is far more common than presently documented.

ACKNOWLEDGMENTS

We thank Mike Griffin (Bright Star Productions) and Kyle Alderman (CTC Vermont Color Photo Lab) for assistance with illustrations, Bryan Goodwin (NYS Museum) for dissections, and George Poinar (Oregon State University) for manuscript review. W. S. Vinikour's involvement in this research project was not funded by Argonne National Laboratory. We gratefully acknowledge Western Illinois University's Kibbe Field Station for support of publication charges. Contribution number 790 of the New York State Museum and Science Service.

REFERENCES

Frison, T. H. 1935. The stoneflies, or Plecoptera of Illinois. *III. Nat. Hist. Surv. Bull.* **20,** 281–471.

Kaiser, H. 1987. Biologie, Ökologie und Entwicklung des europäischen Wespen-Parasitoiden *Pheromermis vesparum* n. sp. (Mermithidae, Nematoda). *Zool. Jahrb. Abt. Syst. Ökol. Geogr. Tiere.* **114,** 421–449.

Kennedy, C. R. 1975. "Ecological Animal Parasitology." Wiley, New York.

Kuhtreiber, J. 1934. Die Plekopterenfauna Nordtirols. *Ber. Naturwiss. Med. Ver. Innsb.* **43/44,** 1–219.

Merritt, R. W., and Cummins, K. W. 1996. "Aquatic Insects of North America," (3rd ed.) Kendall/Hunt, Dubuque, IA.

Pennak, R. W. 1978. "Fresh-Water Invertebrates of the United States," (2nd ed.) Wiley, New York.

Poinar, G. O., Jr. 1976. Presence of Mermithidae (Nematoda) in invertebrate paratenic hosts. *J. Parasitol.* **62,** 843–844.

Poinar, G. O., Jr. 1979. "Nematodes for Biological Control of Insects." CRC Press, Boca Raton, FL.

Poinar, G. O., Jr. 1981. Distribution of *Pheromermis pachysoma* (Mermithidae) determined by paratenic invertebrate hosts. *J. Nematol.* **13,** 421–424.

Poinar, G. O., Jr., and Doelman, J. J. 1974. A reexamination of *Neochordodes occidentalis* (Montg.) comb. n. (Chordodidae: Gordioidea): Larval penetration and defense reaction in *Culex pipiens* L. *J. Parasitol.* **60,** 327–335.

Poinar, G. O., Jr., Lane, R. S., and Thomas, G. M. 1976. Biology and redescription of *Pheromermis pachysoma* (v. Linstow) n. gen., n. comb. (Nematoda: Mermithidae), a parasite of yellowjackets (Hymenoptera: Vespidae). *Nematologica* **22,** 360–370.

Schoenemund, E. 1924. Plecoptera—Steinfliegen. *In* "Biologie de Tiere Deutschlands" (P. Schulze, Ed.), pp. 32.1–32.34. Gebruder Borntraeger, Berlin.

Vinikour, W. S., and Anderson, R. V. 1984. Macroinvertebrates and fishes of Trout Park Nature Reserve, Elgin, Illinois. *Trans. Ill. Acad. Sci.* **77,** 95–102.

Winkler, O. 1956. Zwei Fälle von abnormer Entwicklung des Flügelgeäders bei Plecopteren. *Beitr. Entomol.* **6,** 240–243.