Journal of the American Mosquito Control Association, 13(4):398–401, 1997

EXPERIMENTAL HOST RANGE STUDIES WITH *HELEIDOMERMIS MAGNAPAPULA* (MERMITHIDAE), A PARASITE OF *CULICOIDES VARIIPENNIS* (CERATOPOGONIDAE)

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ABSTRACT. Infectivity of the mermithid nematode *Heleidomermis magnapapula*, a parasite of *Culicoides variipennis variipennis* and *C. v. sonorensis* in dairy wastewater pond habitats, was tested against larvae of aquatic Diptera in the laboratory. Observations were made on preparasite penetration. If the host species could be reared after parasitization, further observations determined nematode development and emergence. Genera in the families Syrphidae (*Eristalis*), Psychodidae (*Psychoda*), and Chironomidae (*Tanypus*), common in wastewater pond habitats, were not attacked. Larvae of *Bezzia* and *Dasyhelea* (Ceratopogonidae) also were not attacked. Larvae of *Chironomus* (Chironomidae) were penetrated, but rapidly encapsulated the nematodes. All *Culicoides* spp. exposed were readily penetrated by *H. magnapapula* preparasites. The nematodes successfully emerged from *C. v. occidentalis*. Some nematode maturation was seen in *C. lahontan*, and limited adult nematode emergence was seen in *C. boydi* and *C. cacticola*. No nematode development was observed in 5 other *Culicoides* spp. penetrated.

Mermithid nematodes are common parasites of aquatic Diptera (Rubtsov 1974, Poinar 1979). While mermithids are relatively more host-specific than commonly studied terrestrial insect parasitic nematodes in the genera *Steinernema* or *Heterorhabditis* (Poinar 1979, Gaugler and Kaya 1990), they often parasitize more than a single host species. Host species typically are fairly closely related (e.g., members of the same genus or family) and share similar habitats (Rubtsov 1974, Poinar 1979). For example, *Romanomermis culicivorax* (Ross and Smith) can naturally or experimentally parasitize multiple species and genera of Culicidae that differ in susceptibility (Petersen and Chapman 1979, Poinar 1979). Several *Culicoides* spp. are hosts of *Heleidomermis vivipara* Rubtsov in Eurasia; these include *C. circumscriptus* Kieffer, *C. desertorum* Gutsevich, *C. nubeculosus* (Meigen), *C. puncticollis* (Becker), *C. salinarius* Kieffer, and *C. stigma* (Meigen) (see Hribar and Murphree 1987). The other known member of the genus in the Old World, *H. ovipara* Rubtsov, is reported from *C. helveticus* Callot, Kremer, and Deduit, *C. pulicaris* (L.), *C. circumscriptus*, and *C. manchuriensis* Tokunaga (Rubtsov 1974).

The mermithid *Heleidomermis magnapapula* Poinar and Mullens has been reared from mature, field-collected larvae of biting midges in the *Culicoides variipennis* complex, including *C. v. variipennis* (Coquillett) from New York and *C. v. sonorensis* Wirth and Jones from California (Poinar and Mullens 1987). *Culicoides v. sonorensis*, in particular, is considered a primary vector of bluetongue virus to ruminants in North America (Tabachnick 1996).

Both the midge and the nematode are found primarily in sediments from aquatic habitats polluted by animal manure. To date, *H. magnapapula* has not been found in *C. v. occidentalis* Wirth and Jones, which inhabits saline and alkaline habitats (Paine and Mullens 1994). Salinity levels in such habitats are excessive for the nematode (Mullens and Luhring 1996), but it was not known whether *C. v. occidentalis* is capable of supporting the parasite. Few natural enemies are known for *Culicoides* spp. (Wirth 1977), and *H. magnapapula* can be produced *in vivo* in the laboratory (Mullens and Velten 1994). This makes the mermithid of some interest for potential biological control. We conducted host range studies to determine whether *H. magnapapula* could parasitize and develop in other Diptera commonly found in dairy wastewater habitats or in alternate species of Ceratopogonidae.

Two methods were used to obtain larvae of Diptera for exposure to *H. magnapapula* preparasites: 1) collection of habitat mud and extraction of larvae, and 2) rearing of *Culicoides* spp. larvae from eggs derived from field-collected, identified female midges.

Habitat mud known to contain *Culicoides* spp. larvae was collected from Deep Creek, located at the University of California Boyd Deep Canyon Research Center near Palm Desert, Riverside County, CA, and from dairy wastewater ponds in the Chino Basin, Riverside County, CA. Larvae of *C. v. occidentalis* were collected from mud in the Bolsa Chica Marsh, Orange County, CA. Dipteran larvae were extracted from field mud by direct saturated $MgSO_4$ flotation or by pouring a 2% agar layer onto mud and allowing the larvae to migrate into the agar for later separation (see Hribar 1990). With the exception of *C. v. occidentalis*, other field-collected Diptera larvae were identified to the genus level (Merritt and Cummins 1996). Larvae were exposed within 24 h after extraction. When possible, early-stage larvae were used. All exposed larvae were held in plastic dishes with nutrient-enriched 1.5% noble agar (DIFCO Laboratories, Detroit, MI)

Table 1. Results of experimental exposures of aquatic Diptera larvae to the mermithid nematode parasite *Heleidomermis magnapapula.*

Family	Genus	Number exposed	Penetrated	Developed	Emerged
Psychodidae	*Psychoda*	4	—	—	—
Syrphidae	*Eristalis*	5	—	—	—
Chironomidae	*Chironomus*	10	+	—	—
	Tanypus	10	—	—	—
Ceratopogonidae	*Bezzia*	4	—	—	—
	Dasyhelea (*mutabilis* gp.)	2	—	—	—
	Culicoides				
	C. v. occidentalis	350	+	+	+
	C. boydi	12	+	+	+
	C. lahontan	17	+	+	—
	C. freeborni	11	+	—	—
	C. brookmani	17	+	—	—
	C. jacksoni gp.	4	+	—	—
	C. cacticola	19	+	+	+
	C. n. sp. (*nr. lahillei*)	10	+	—	—
	C. n. sp. (*biguttatus* gp.)	37	+	—	—

until death, pupation, or adult nematode emergence occurred. While on the agar, larvae were provided with nutrient-rich water and the bacterial feeding nematodes *Pelodera* sp. and *Panagrellus redivivus* (L.) as potential food.

To obtain known *Culicoides* larvae for exposure, host-seeking females were collected using CO_2-baited (dry ice), battery-powered suction traps with fine-mesh catch bags. The traps were deployed 1–3 h prior to sunset and collected the next morning 1–2 h after sunrise. Insects collected in Morongo Valley, San Bernardino County, were placed on ice and transported back to the laboratory for bloodfeeding. Insects collected at Deep Canyon Reserve either were given the opportunity to bloodfeed at the research station or were transported back to the laboratory. Midges were allowed to feed on defibrinated sheep blood through a Parafilm or chickskin membrane (Hunt 1994). Engorged females were separated and placed in a holding chamber with a 10% sucrose wick. They were held for 7 days at 21°C and then decapitated to stimulate oviposition. Eggs from individual females were placed on moist filter paper on a 1.5% noble agar layer in a Petri dish, and the female was saved in 70% EtOH for later slide-mounting and identification. Larvae were provided with algae and *Pelodera* sp. and *P. redivivus* nematodes for food. Larvae were exposed to *H. magnapapula* preparasites at 3–10 days of age.

Heleidomermis magnapapula were reared in the laboratory in larvae of *C. v. sonorensis* as described by Mullens and Velten (1994). Preparasites were collected in dechlorinated tap water from 5–10 females that were actively producing preparasites. Preparasites were <2 h old when they were used in the experiment, because infectivity declines rapidly after preparasite emergence (unpublished data). Preparasites were placed within a drop of water on a glass depression slide, and an individual host larva was pipetted onto the slide. The host and preparasites were watched continuously for 10 min using a dissecting microscope. Larvae remained with the preparasites until a preparasite successfully attached, 5 preparasites had rejected a potential host, or the 10 min had elapsed. If preparasites showed no interest in the host, the original host was removed, and a young laboratory-reared *C. v. sonorensis* larva was offered to confirm preparasite infectivity.

After 1–3 preparasites attached, the larva was removed to another spot on the slide to prevent hyperparasitization. The larva was observed until all the attached preparasites penetrated. Larvae were reared on agar in plastic sorting trays and fed as described above. Larvae were observed every 1–2 days until death or pupation occurred. If multiple parasitized larvae were alive after 2–3 days, at least one individual was placed in a depression slide and observed using a compound microscope to determine whether the nematode within was alive or had developed. When larvae pupated, the pupae were removed from the agar and placed in individual containers on moist cotton until the adult flies emerged. The dead pupae or emerged flies were dissected to determine if there was any sign of nematode development.

Results of the laboratory exposures are presented in Table 1. Preparasites of *H. magnapapula* did not respond to larvae of *Psychoda* sp. (Psychodidae), *Eristalis* sp. (Syrphidae), *Tanypus* sp. (Chironomidae, subfamily Tanypodinae), or ceratopogonid larvae in the genera *Dasyhelea* (*mutabilis* group) or *Bezzia*. Early-instar *Chironomus* sp. (Chironomidae, subfamily Chironominae) were attacked but invariably melanized the preparasites within a few minutes, and no nematode development was observed.

All species of *Culicoides* exposed were penetrated by *H. magnapapula* preparasites. Larvae of *C. v. occidentalis,* exposed in tap water and reared on agar, were excellent hosts. Nematodes emerged, mated, and produced progeny as well, as we have seen in *C. v. sonorensis* under similar rearing conditions (Mullens et al. 1995). Three other species, *C. boydi* Wirth and Mullens, *C. cacticola* Wirth and Hubert, and *C. lahontan* Wirth and Blanton, supported at least some mermithid development, and a few small males emerged successfully from late-instar larvae of *C. boydi* and *C. cacticola.* Some of the *Culicoides* spp. apparently died quickly as a result of parasitism and could not be found 2–3 days later. Others, particularly *C. lahontan, C. brookmani* Wirth, and *C.* n. sp. near *lahillei* (Iches) overcame the parasites, pupated, and emerged with no sign of the nematodes. The *Culicoides* spp. screened represented several subgenera. The normal hosts, *C. variipennis variipennis* and *C. v. sonorensis,* are in the subgenus *Monoculicoides.* Of other subgenera that supported at least some parasite development, *C. boydi* is in the subgenus *Avaritia, C. cacticola* is in the subgenus *Drymodesmyia,* and *C. lahontan* is in the subgenus *Culicoides.*

In the dairy wastewater sediments that are normal habitat for *H. magnapapula,* it is likely that the only hosts used regularly are *C. v. variipennis* and *C. v. sonorensis.* In southern California, other *Culicoides* spp. are rarely found in such habitats. *Culicoides v. occidentalis* also is a good host, provided it is parasitized and reared in a relatively nonsaline habitat. The lack of *H. magnapapula* in *C. v. occidentalis* populations is probably a result of the saline habitats (Tabachnick 1996, Mullens and Luhring 1996), rather than inherent host physiological factors. The nematode does not parasitize alternate fly genera, with the exception of *Chironomus.* This genus has been shown earlier to be susceptible to penetration by *H. magnapapula* (Poinar and Mullens 1987), but encapsulated and killed the nematodes, as seen again in the present study.

There are a number of reports in the literature of unknown mermithids from *Culicoides* (Wirth 1977). Smith and Perry (1967), for example, reported fairly high levels of mermithid parasitism in adults (often intersexes) of *C. stellifer* (Coquillett), *C. crepuscularis* Malloch, and *C. haematopotus* Malloch. These *Culicoides* spp., while they generally avoid heavy pollution, can be found in similar eastern U.S. habitats also used at times by *C. variipennis* (Blanton and Wirth 1979). *Heleidomermis magnapapula* normally is a parasite of *C. variipennis* larvae but can carry over into the adult midge in low numbers to aid in dispersal (Mullens and Velten 1994, Paine and Mullens 1994). It is tempting to speculate that the mermithids reported from *C. crepuscularis, C. stellifer,* and *C. haematopotus* might be *H. magnapapula* in an unusual host, resulting in intersex formation.

Certainly, *H. magnapapula* preparasites readily attack other *Culicoides* spp. regardless of their taxonomic affinity with *C. variipennis. Culicoides variipennis* is among the largest species in the genus. We have shown successful development in the desert cactus-breeding *C. cacticola* (about half the size of *variipennis*) and in the minute *C. boydi* (less than one third the size of *variipennis*). Emerging nematodes have been only males, however, and their fitness has not been assessed.

All the *Culicoides* spp. listed in Table 1 have been reared on agar from egg to adult (Breidenbaugh, unpublished data), but not all species (e.g., *C.* n. sp. *biguttatus* gp.) thrive on this substrate. Starved mosquito hosts parasitized by *R. culicivorax* tend to produce male parasites (Petersen 1972), and this might be the case with some of the *Culicoides* spp. we tested here. The acceptable host range also should be viewed cautiously, because species exposed may have died from causes unrelated to parasitism. In other species, host death in the first 1–2 days probably resulted from parasitism. Nevertheless, several species reared fairly well and obviously overcame the parasites, particularly *C. brookmani, C. lahontan,* and *C.* n. sp. near *lahillei.* While all *Culicoides* tested could be parasitized easily, at least these three can be considered physiologically unsuitable as hosts. Complete development in certain other *Culicoides* spp., however, demonstrates that the host range is likely not strictly limited to *C. variipennis* group midges.

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