Reesimermis nielseni gen. et sp. n. (Nematoda: Mermithidae) Parasitizing Mosquitoes in Wyoming

YUAN-HWANG TSAI[1] AND ALBERT W. GRUNDMANN
Tropical Disease Research Center, St. Clare's Hospital, New York City, New York, and Department of Environmental Biology, University of Utah, Salt Lake City, Utah

There has been a renewed interest in the parasites of mosquitoes as the search for organisms that could prove valuable in biological control is pursued. As a result of such a study in northern Utah and southern Wyoming regions, a species of mermithid nematode was found inhabiting six mosquito species at a site near Lone Tree, Uinta County, Wyoming. Following an extensive study of the life history, pathogenesis in mosquitoes, and morphology of this organism, a new genus and species name is proposed.

Mermithid nematodes have been reported frequently from both larvae and adults of mosquitoes in North America and elsewhere. Jenkins (1964) listed four defined species, *Limnomermis aquatilis* Dujardin from larval Anophelines in France, *Agamomermis culicis* Stiles from adult *Aedes sollicitans* (Walker) from the United States, *Paramermis canadensis* Steiner from *Aedes vexans* (Meigen) and *A. strictus* in British Columbia, Canada, and a *Mermis* sp. from larval *Aedes aegypti* in Africa (Gendre 1909). Other records of *Mermis* sp. are: Muspratt (1945) in larval and adult Anopheles, Iyengar (1927) in India and Walandouw (1934) in Sumatra. At least fifteen additional occurrences in mosquitoes have been reported in North American literature.

Other mosquito-inhabiting species that have been described are *Hydromermis churchillensis* Welch 1960 from larval *Aedes communis* (De Geer) from Manitoba, Canada. This species was also reported from Canada by Jenkins and West (1954) from *A. communis, A. impiger* (Walker), *A. nigripes* (Zetterstedt), and *A. pionips* Dyar. Smith (1961) reported what is assumed to be this same species from larval *A. pullatus* in Colorado. Welch (1964) described *Romanomermis iyengari* from *Anopheles subpictus* Grassi in India. A species of *Gastromermis* was reported by Cox (1966) from *Anopheles funestus* Giles in west Africa.

Welch (1960) reviewed the status of reported mosquito-inhabiting mermithids from North America and proposed referring *Paramermis canadensis* to species *inquirendae* because it was indicated that the description had been based on immature specimens. He further stated that a definite status for *Agamomermis culicis* would be unwise at this time since the female was unknown and thus the generic diagnosis insufficient. Reviewing the world status of mermithids inhabiting mosquitoes, three species belonging to three genera currently are determined valid, while four may be determined only to genus.

The systematics of the Family Mermithidae Braun 1883 is difficult due to the scattered literature and observations on immature forms. Best references are Filipjev (1934), Filipjev and Schuurmans (1941), and the work of a number of Russian workers that were nicely reviewed by Welch (1965).

In North America, Johnson (1963) erected the genus *Octomyomermis* from Chironomids. Poinar (1964) established *Orthomermis*, also from Chironomids. Welch (1960a, 1962b, 1963a, 1964, 1965) has described species from six genera in Simulid larvae.

General Description
Genus *Reesimermis*, gen. n.

Reesimermis: Mature adult worms elongate, cylindrical, with obtuse ends in both males and females. Cephalic papillae consisting of a crown of six in a hexagonal position, which may represent two lateral and four submedial cephalic nerve endings. Eight longitudinal hypodermal chords in dorsal, ventral, lateral, and submedial positions (Fig. 10). Spicules paired, slightly curved, medium-sized, fused posteriorly for more than half of their length (Fig. 8). Vagina straight, of medium length, muscular and pear-shaped. Cuticle smooth, no

[1] Present address: Microbiological Department, Bristol Laboratories, Syracuse, New York 13201.

criss-cross striae on the middle cuticle layer. Mouth opening terminal. Postparasitic juveniles with tail appendage. Aquatic habitat.

Diagnosis

Reesimermis is the second genus to be described in the family Mermithidae possessing two spicules that are fused posteriorly for more than half their length. Four established genera are found that possess eight longitudinal chords, and *Reesimermis* differs from each of these in several distinct anatomical features. The genus *Hydromermis*, which contains *H. churchillensis* and *H. contorta*, differs from *Reesimermis* in the shape of the vagina which is S-shaped in *Hydromermis* but straight and pear-shaped in *Reesimermis*, and in the morphology of the spicules of which there are two fused for half of their length in the new genus and described as single in *Hydromermis*. However, *H. churchillensis* and *H. contorta* possess two spicules fused for more than half their length in contradiction to the generic characters but have the S-shaped vaginal characteristics of *Hydromermis*. These two species may be considered as intermediate evolutionary forms occurring between the separate, paired spicules of *Isomermis* Coman 1953 and the single spicule of *Hydromermis*. Both genera are similar in other characteristics.

The genus most closely related to the new genus is *Romanomermis* which differs primarily in having two completely separate spicules. Both genera inhabit mosquitoes. Another genus appearing in the literature that should also be considered is *Octomyomermis*, but when the published description was compared with that of *Romanomermis*, the two seemed to be almost identical. Since no specimens of either group were examined by the authors, it is not possible to accurately state that *Octomyomermis* should be synonomized with *Romanomermis*. Other genera possessing paired spicules, pear-shaped vagina, and eight longitudinal chords are *Allomermis* and *Orthomermis*, but these differ from each other and from *Hydromermis* in the arrangement of cephalic papillae. The following key separates these genera from *Reesimermis*:

1. Four cephalic papillae in one plane; two spicules completely separated; criss-cross striae on the cuticle; mouth opening ventral ..... *Allomermis*
   Different arrangement and number of cephalic papillae; two spicules separated or fused posteriorly for more than half of their length; no criss-cross striae on the cuticle; mouth opening terminal 2
2. Two lateral cepalic papillae in one plane with six papillae in ring underneath; two spicules separated ..... *Orthomermis*
   Six cephalic papillae in one plane; two spicules separated or fused more than half of their length 3
3. Paired spicules separated ..... *Romanomermis* (*Octomyomermis*)
   Paired spicules fused for more than half of their length ..... *Reesimermis*

Reesimermis nielseni sp. nov.

GENERAL: All following measurements are in millimeters unless otherwise noted. Worm long and slender with smooth cuticle. Criss-cross fibers in middle cuticular layers absent. Cuticle thin, 5.5–7.7 μ in adults, and 2–3 μ in postparasitic larvae. Head convex, rounded with six cephalic papillae consisting of four submedian and two lateral papillae arranged

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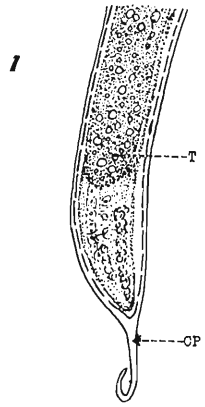
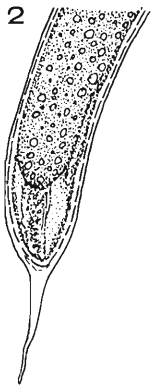
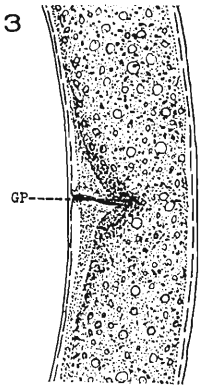
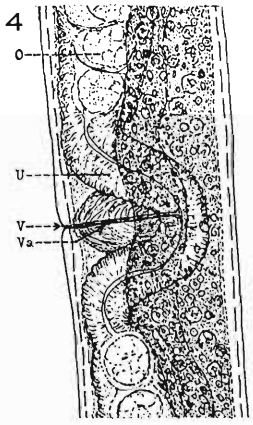
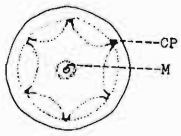
Figures 1–10. 1. Lateral view of postparasitic male juvenile tail. T, trophosome; CP, caudal appendage (× 100). 2. Lateral view of postparasitic female juvenile tail (× 100). 3. Lateral view of female postparasitic juvenile vagina. GP, genital primordium (× 150). 4. Lateral view of vagina (mature female worm). O, ova; U, uterus; V, vulva; Va, vagina (× 200). 5. Face view of mature worm head. CP, cephalic papillae; M, mouth opening (× 400). 6. Dorsal view of mature female head. CP, cephalic papillae; A, amphid (× 500). 7. Lateral view of mature female tail. T, trophosome (× 100). 8. Lateral view of mature male tail. Sp, spicule; Gp, genital papillae (× 100). 9. Ventral view of mature male tail. Sp, spicule; Gp, genital papillae (× 100). 10. Cross section in anterior region of female. D, dorsal hypodermal chord; L, lateral hypodermal chord; R, reproductive organ; S, submedial hypodermal chord; T, trophosome; V, ventral hypodermal chord (× 400).

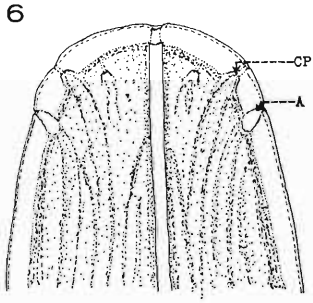


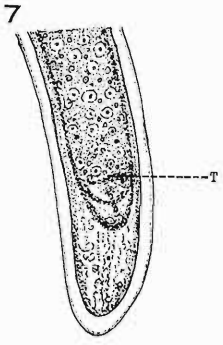


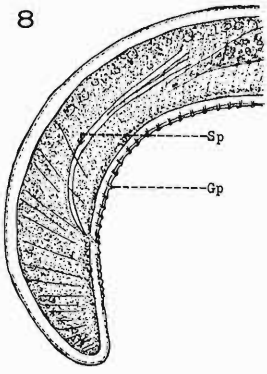


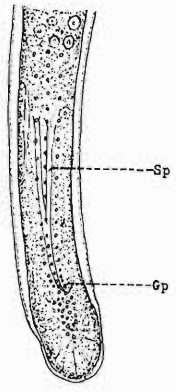


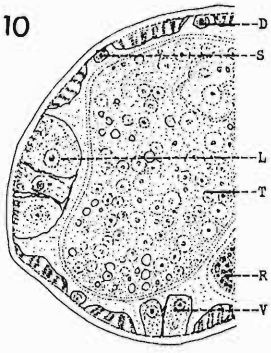












hexagonally in one plane and in a slight depression of cuticle (Fig. 5). Mouth terminal in slight depression of cuticle. Small neck constriction visible behind papillar depression. Amphids located behind cephalic papillae pouch-shaped, pores small, situated about 2–4 μ behind lateral papillae. Ligament connecting amphids absent. Oesophagus folded, diameter 4.5–5.5 μ (average 5 μ) near mouth opening, and 4 μ along main tract; extends over three-fourths of body length. Nerve ring 265–290 μ from mouth. Excretorylike glands 0.66–0.85 mm from mouth opening. Stichocytes beside oesophagus numerous and glandular-shaped. Lateral hypodermal chords with three cells (a few with paired cells). Male and female worms have obtuse terminus and are slightly curved ventrally. Free-living infective juveniles, postparasitic and parasitic juveniles retaining needle-shaped caudal appendage.

i. Female

Seventeen specimens. Body length 15.10 (11.0–21.2), stout, blunt at posterior end, tapering at anterior end. Greatest width of head at level of papillae 0.060 (0.055–0.065); at vulva 0.188 (0.160–0.225); and at tail measured at the terminus of trophosome 0.141 (0.125–0.195). Amphid 13 by 5 μ. Distance from anterior ovary to mouth opening 0.84 (0.32–1.33), that of posterior ovary to base of tail 0.57 (0.43–0.69).

Vulva, 45.7% (41.1–53.1%) of body length, a transverse opening slit (0.049) in ventral view, straightly located in lateral view, leading to muscular pear-shaped vagina that is straight thick-walled tunnel, 0.112 (0.095–0.130) in length, connected to opposed amphidelphic musculated uteri, 0.070 in width and 0.190 (0.180–0.198) in length.

Rate of anterior ovary to body length 41.8% (37.4–46.2%) and that of posterior ovary to length is 48.7% (42.3–53.9%), so $v = {}^{41.8}45.7^{48.3}$. Length of posterior end of trophosome to base of tail 0.150 (0.100–0.170), that of anterior end of trophosome to degenerate mouth opening 0.420; no anal opening (Figs. 4, 6, and 7).

ii. Male

Eleven specimens. Body length 10.2 (6.2–14.10). Head width measured at level of papillae 0.050 (0.048–0.053); width at middle body 0.144 (0.120–0.160); and width at anus 0.116 (0.096–0.130). Tail length 0.157 (0.140–0.175). Amphids same as in female. Paired spicules, fused posteriorly for 63–68% of their length, 250 (205–310) μ in length. Individual bases of the separated proximal ends 22–26 μ. Width of single separate spicule 4.4 μ at midpoint, and that of the middle fused part 6.5 μ; gently curved, tapering to sharp pin-point. Testes two parallel tubes running to middle of body possessing some flagellated sperm. Three rows of pregenital papillae. Median row with 19–22 papillae, lateral row with 16–19 papillae, postgenital papillae irregular, clustered and tiny. The two lateral papillar rows converge close to median row at anal area (Figs. 8, 9).

iii. Eggs

Flattened ellipsoid shaped, 84 by 74 (80–90 by 70–80) μ, with cross-section 74 by 68 (70–80 by 63–70) μ. Egg shell thin, transparent and sticky outside, with very thin vitelline membrane beneath the shell (visible only in preserved specimens). Eggs unembryonated with one egg cell when deposited. Size of eggs in ovaries smaller and irregular in shape, crowded side by side.

iv. Preparasitic juveniles

Body length 0.56–1.20; width at head region 6–7 μ, width at the middle of body trunk 15–17 μ. Onchostylet sharp and thin, about 12–14 μ in length. Numerous granular cells in body. Head rounded, tail slender, long, whiplike.

v. Parasitic juveniles

Body length and width vary with state of development. In 28 specimens measured, length 0.88–13.4; greatest width 0.034–0.125. Head rounded, tail long, slender.

vi. Postparasitic juveniles

Body longth 7.4–25.5; greatest width 0.103–0.190. Ratio of head to body width 0.31–0.44. Anterior trophosome arising 0.370 (0.310–0.420) from head end, posterior trophosome arising 0.085–0.200 from base of caudal appendage. Caudal appendages 0.150–0.190 long. Sexes are distinguishable (Figs. 1, 2).

SPECIMENS DEPOSITED: Holotype—10 female adults and 10 male adults in the University of Utah Parasitological Collection.

Table 1. Seasonal fluctuation in female–male ratio (FMR) for postparasitic juvenile populations of *Reesimermis nielseni*.

Date collected	No. of worms	Female		Male		FMR*
		No.	Per cent	No.	Per cent	
21 May 1966	142	32	22.53	110	77.47	0.29
12 June 1966	611	327	53.50	284	46.50	1.15
19 June 1966	277	105	37.90	172	62.10	0.61
28 June 1966	61	29	47.54	32	52.46	0.91
Total worms examined	1,091	493	45.20	598	55.80	0.824

* FMR is obtained by dividing number of females by number of males.

TYPE HOST: *Aedes communis* (De Geer).

ADDITIONAL HOSTS: *Aedes cinereus* Meigen, *A. fitchii* (Felt and Young), *A. increpitus* Dyar, *A. pullatus* (Coquillet), and *Culiseta impatiens* (Walker).

LOCATION: Haemocoel of host larvae, occasionally in the pupae and adults of *Aedes increpitus* and *A. pullatus*.

LOCALITY: Lone Tree, Uinta County, Wyoming. Elevation 8,000 feet. Collected May–June 1965–66.

Morphological differences between the male and female postparasitic juveniles

The chief morphological differences between premale and prefemale postparasitic juveniles obtained from mosquito larvae have been briefly described by Welch (1960) as being the larger size of the female and presence of the genital primordium of the vagina in the middle of the female body. Knowledge concerning the differential diagnosis of the male and female free-living postparasitic stage is inadequate because in cases of superinfection, male and female worms are often hard to separate on the basis of size, and in cases of single or double infections, the males are quite large. The presence of the genital primordium of the vagina is difficult to observe in preserved material and may be very difficult to detect in live specimens. Therefore, an attempt was made to distinguish the sexes of the postparasitic juveniles obtained following emergence from the bodies of mosquito larvae by means of the morphology of the caudal appendages and primordium of the vagina.

The caudal end of the postparasitic larvae was found to be characteristically different in males and females, with that of the premale being thin and short (150–160 μ) and that of the prefemale stouter at the base and longer (170–190 μ). Also the distance from the end of the terminal trophosome to base of the caudal appendage in the premale is much greater (170–200 μ, Fig. 7) than in the prefemale (85–130 μ, Fig. 8). The morphology of the caudal end is thus a useful character that can be observed under the low power of a dissecting microscope.

The primordium of the vagina, located near the middle of the body (Fig. 3) has been previously used to separate premale and prefemale worms. It is recognizable as a small slit appearing beneath the juvenile cuticle that does not open to the outside at 46.5% of the distance from the anterior end. This feature is difficult to recognize in active specimens.

Size differences between premale and prefemale worms can be used for rapid identifications under some conditions, but should be applied in conjunction with the previous methods. Postparasitic juvenile females are longer and stouter, averaging 25 mm in length while juvenile males are shorter and thinner (average 15 mm in length). Sometimes two prominent incisions holding big excretorylike gland cells are visible on the ventral side, just under the stichosome about one-tenth to one-twelfth distance from the anterior end. These are far more prominent in premales.

Female–male ratio (FMR) in postparasitic juveniles

Wide fluctuation appeared in sex ratios when postparasitic worms were sexed at different times during the season (Table 1). Males made up 77.4% of the population on 21 May 1966, dropped to 46.5% on 12 June, increased to 62.1% 19 June, and 52.46% on 28 June. The fluctuations correlated well with the size of worm burdens per host with the

worms being predominately males when the worm burden was greater than four and almost exclusively female when less than four were present. This phenomenon has been described previously by a number of workers (Christie, 1929; Johnson, 1955; Couturier, 1963; and Parenti, 1965) who pointed out that sex determination in mermithids may be environmentally controlled with crowding being the primary factor. Another factor was introduced by Strelkov (1964) who showed that sex of the parasite corresponds to that of the host in *Filipjevimermis singularis*. No attempt was made to resolve the latter in this study.

Female-male ratios were obtained both by examination of free-living postparasitic juveniles that had emerged spontaneously from hosts in the laboratory and through dissection of infected hosts. FMR were similar in both phases done on the same days, indicating that it would be possible to predict population changes through examination of free-living individuals in soil samples from the bottom of ponds, and thus to provide data of value to potential biological control of mosquitoes. FMR ratios are calculated by dividing the number of females by the number of males, with a low FMR being less than one (Table 1).

Life history

The life history of *R. nielseni* is similar to that described by Welch (1960a) for *Hydromermis churchillensis*, a parasite of *Aedes communis* in Canada.

Unembryonated eggs are deposited in the bottom soil of shallow pools. Observations showed embryonation to be evident in 5–10 days and the eggs contained fully developed larvae seven days later. The larvae do not hatch upon completion of development under cold dry field conditions* but remain dormant until favorable conditions are present. The eggs are vulnerable to desiccation so that drying is immediately fatal if the duration is more than a few seconds, but eggs appear to be resistant to freezing winter temperatures in soil.

In the type locality, snow is present from October to April when melting snow water forms the pools in which the mosquito hosts develop. Approximately 2 weeks later the larvae emerge from the eggs and use their long, slender tails for swimming in search of a host. The larvae can exist for about 4 days under April and May temperatures and readily attach by their onchostylets to the mosquito larvae that appear at this time. Penetration into the host body cavity takes several minutes after which the larvae gradually become motionless.

The parasitic juveniles grow rapidly and are late stage juveniles in 10–15 days. Emergence from the host occurs soon after this stage of development is reached. The worms emerge by pushing the anterior end through the conjunctivum between the segments of the thorax, abdomen and neck. Complete emergence takes about 1 minute and is fatal to the host. The free-living postparasitic juveniles burrow into the bottom soil where they become motionless for 2–4 weeks. Molting occurs at the end of this period with the males preceding the females. Development and sexual differentiation are slow and it is usually 1–2 months before copulation occurs. Another 10–30 days elapse before egg production occurs and the number of eggs produced depends upon the female size and the size of the trophosome which was determined by the conditions of parasitism depending mainly on the number of larvae that inhabit a single host. A large female can produce as many as a thousand eggs. After oviposition, the female dies in 5–6 days, having exhausted the food stored in the trophosome. Males survive longer than the females since they require little stored material for sperm production.

Reesimermis nielseni has one generation per year, a condition well adapted to the life cycles of its hosts that are "single brooded" mountain *Aedes* species confined to the higher altitudes.

Summary

All life stages of *Reesimermis nielseni*, gen. et sp. n., a parasite of mosquitoes from Lone Tree, Wyoming, are described. A key to the closely allied genera of Mermithidae is included. The type host is the larva of *Aedes communis* De Geer but the species was also found parasitizing *Aedes cinereus* Meigen, *A. fitchii* (Felt and Young), *A. increpitus* Dyar, *A. pullatus* (Coquillett), and *Culiseta im-*

* The eggs, upon completion of development, could hatch after 1.5–2 months in soil water at 18–20 C in the laboratory.

patiens (Walker). A method of sexing post-parasitic juveniles is described as well as the seasonal fluctuation obtained in the female–male sex ratios during May and June of 1966. The life history is included and shows one generation per year.

Literature Cited

Christie, J. R. 1929. Some observations on sex in Mermithidae. J. Exp. Zool. 53: 59–76.

Couturier, A. 1963. Recherches sur des Mermithidae, nematodes parasites du henneton commun (*Melolontha melolontha* L.). Ann. Epiphities 14: 203–267.

Coz, J. 1966. Contribution a l'etude du parasitisme des adultes d' *Anopheles funestus* par *Gastromermis* sp. (Mermithidae). WHO/EBL/66.76, pp. 1–11.

Filipjev, I. N. 1934. The classification of the free-living nematodes and their relation to the parasitic nematodes. Smithsonian Misc. Coll. 89(6): 1–63.

———, **and J. H. Schuurmans Stekhoven, Jr.** 1941. A manual of agricultural helminthology (E. J. Brill, Leiden, Netherlands.) 878 pp.

Gendre, E. 1909. Sur des larves de *Mermis* parasites des larves du *Stegomyia faciata*. Bull. Soc. Path. Exct. 2: 106–108.

Iyengar, M. O. T. 1927. Parasitic nematodes of Anopheles in Bengal. Far Eastern Assoc. Trop. Med., Trans. 7th Congr. Brit. India. 3: 128–133.

Jenkins, D. W. 1964. Pathogens, parasites and predators of medically important arthropods. Wld. Hlth. Org. Organisation Mondiale de la Sante, 150 pp.

———, **and A. S. West.** 1954. Mermithid nematode parasites in mosquitoes. Mosq. News, 14: 138–143.

Johnson, A. A. 1963. *Octomyomermis itascensis* gen. et sp. nov., a parasite of *Chironomus plumosus* (L.). Trans. Am. Micr. Soc. 82: 237–241.

———. 1965. *Hydromermis itascensis* sp. n. (Nematoda: Mermithidae), a parasite of *Glyptotendipes labiferus* (Say) (Diptera: Chironomidae) and the taxonomic status of *Gastromermis*. J. Parasit. 51: 53–56.

Muspratt, J. 1945. Observation on the larvae of tree hole breeding Culicini (Diptera: Culicidae) and two of their parasites. J. Entomol. Soc. S. Africa 8: 13–20.

Parenti, U. 1965. Male and female influence of adult individuals on undifferentiated larvae of the parasitic nematode *Paramermis contorta*. Nature 207(5001): 1105–1106.

Poinar, G. O. 1964. A new nematode, *Orthomermis oedobranchus* gen. nov. (Mermithidae), parasitizing *Smittia* larvae (Chironomidae) in England. Nematologica 10: 501–506.

Smith, M. E. 1961. Further records of mermithid parasites of mosquito larvae. Mosq. News, 21: 344–345.

Strelkov, A. 1964. Biology of *Filipjevimermis singularis* sp. nov. found in the Rybinski Reservoir. Vestnik Leningr. Univ. No. 3, Ser. Biol. 19(1): 55–69.

Walandouw, E. K. 1934. Nematoden als bestrijders von anopheles larven. Geneesk Tijdschr. Ned.-Ind. 74(19): 1219–1224.

Welch, H. E. 1960a. *Hydromermis churchillensis* n. sp. (Nematoda: Mermithidae) a parasite of *Aedes communis* (Deg.) from Churchill, Manitoba, with observations on its incidence and bionomics. Can. J. Zool. 38 (3): 465–474.

———. 1960b. Notes on the identities of mermithid parasites of North American mosquitoes, and a redescription of *Agamomermis culicis* Stiles, 1903. Proc. Helmin. Soc. Wash. 27(2): 203–206.

———. 1962. New species of *Gastromermis, Isomermis,* and *Mesomermis* from black fly larvae. Ann. Ent. Soc. Am. 55: 535–542.

———. 1963. *Amphimermis bogonae* sp. nov. and *Hexamermis cavicola* sp. nov. from the Australian bogong moth, *Agrotis infusa* (Boisd.) with a review of the genus *Amphimermis* Kaburaki and Imamura 1932. (Nematoda: Mermithidae). Parasitology 53: 55–62.

———. 1964. *Romanomermis iyengari* n. sp., Pilot Register of Zool. Card No. 4.

———. 1965. Entomophilic nematodes. Ann. Rev. Ent. 10: 275–302.