the period 1967–1969. Difficulties in the establishment of this colony are reported and the rearing methods found successful are described.

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PARASITISM OF *ANOPHELES ANNULIPES* WALKER BY A MERMITHID NEMATODE

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INTRODUCTION

Numerous examples of the parasitism of mosquitoes by mermithid nematodes have been reported (Jenkins, 1964). The only previous Australian observation was made in Townsville, Queensland, where 9 out of 13 third and fourth instar *Anopheles annulipes* larvae were found to be infected with an unidentified mermithid nematode (Laird, 1956).

OCCURRENCE

In 1967 and 1968 *A. annulipes* were collected in considerable numbers from the Nattai River near Mittagong, New South Wales. A large proportion of the larvae collected in November and December 1967, never reached maturity because they were killed by the emergence of nematode parasites, frequently during the journey back to Sydney. The coiled nematodes were visible lying ventrally in the thoracic and abdominal haemocoel of infected third and fourth instar larvae, which were slightly swollen ventrally and moved sluggishly.

The incidence of parasitism was measured on returning to the laboratory by counting the number of live parasitised larvae and the number of larvae killed during the journey by emerging nematodes. Table 1 shows the rate of infection on four successive collection days.

Conditions for the breeding of *A. annulipes* became increasingly favourable after December, resulting in greater numbers of larvae in January. Approximately 1500 larvae, most of them first and second instar, were collected on both 4 Jan. and 9 Jan.; the proportion of these larvae infected with mermithids could not be determined directly as the larvae were too small for the parasites to be visible, but very few parasites later emerged from

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TABLE 1. Infection Rate

	16 Oct.	20 Nov.	30 Nov.	7 Dec.
No. larvae infected	162	289	174	12
Total no. of larvae	201	532	384	72
% infected	80.5%	54.5%	45.4%	16.7%

these larvae. There was some difference in the incidence of parasitism in different pools, the highest rate (90 percent) being found in a pool isolated from the flushing action of the main river.

At the Nattai River this nematode parasite is apparently limited to *A. annulipes* as its host species. No nematodes were found in *A. pseudostigmaticus* larvae which are present in large numbers during winter months when *A. annulipes* is scarce. The few culicine species found in this part of the Nattai River were likewise free of nematode infection.

DESCRIPTION

The full-grown white nematode which emerges from the host larva is covered by a thin cuticle which extends posteriorly into a terminal spine. The cuticle and spine are lost during a moult a few days after emergence, after which the nematode is sexually mature. It has a long oesophagus with reduced musculature, a rather blunt anterior end and a more pointed posterior end; the intestine is adapted as a food storage organ. All these features are characteristic of nematodes belonging to family Mermithidae. The sexes are separate, the average length of the female being 22.5 mm, and of the male 13.2 mm. The vulva of the female lies about halfway along its body and opens into a short vagina. The male has a curved posterior end with a single spicule.

The exact classification of the species is uncertain. A number of juvenile and adult mermithids were sent to G. O. Poinar, Jr. (Assistant Invertebrate Pathologist Berkeley), who considered they belonged in either genus *Hydromermis* or genus *Limnomermis*, depending on the number of hypodermal chords present. This detail is unknown due to lack of material for sectioning.

LIFE HISTORY IN THE LABORATORY

EMERGENCE. This was witnessed several times. The mermithid moved vigorously within the motionless mosquito/ larva before penetrating the body wall and coiling out quickly. Some were seen emerging through cuticular folds in the terminal abdominal region, one was seen entering the hindgut and leaving the body through the anus. When more than one mermithid was present in the same host they emerged in quick succession though not always at the same place. Host larvae always died soon after emergence had taken place. The stimulus for emergence is uncertain, but the heat and light of the microscope lamp seemed to stimulate the process, and the jolting journey in a crowded container on the way back to the laboratory from the Nattai River certainly resulted in many parasites emerging.

MATURATION AND REPRODUCTION. Postparasitic juvenile mermithids coiled into a tight mass when placed in clean river water at 26 C. Moulting commenced 4–6 days after emergence, and copulation commenced as soon as the cuticle was cast. The female began ovipositing a minimum of 2 days afterwards, continuing for 7–8 days. The female died after laying hundreds of sticky eggs; the exact number could not be determined as single pairs of mermithids did not breed successfully in the laboratory. The male mermithid died soon after copulation.

Fertilised females did not survive for more than 2 weeks after oviposition, but several unfertilised females survived for 11 weeks in the absence of males; when males were put in with them, copulation and oviposition occurred.

EGGS. The sticky transparent eggs measured 50.6–64.4 x 59.9–64.4μ. At oviposition they were at the one cell-stage, and development began immediately at 26 C. The majority of eggs hatched 6–7 days after oviposition, although hatching was spread over several days.

INFECTIVE STAGE. Newly-hatched preparasitic juveniles measured approximately 580 x 11μ. They were very active swimmers which survived in the absence of a host for only 3–7 days.

INFECTION. Penetration of *A. annulipes* larvae by juvenile mermithids was observed in the laboratory when the newly hatched larvae were partly immobilised under a coverslip, in water containing infective juveniles. Entry occurred through the cuticle between the head capsule and the thorax in one larva; in another the mermithid coiled around one of the long thoracic hairs and entered the haemocoel near its shaft. When newly hatched *A. annulipes* larvae were able to swim freely in water containing infective juveniles, frequent contacts were observed between the two species but the mosquito larvae usually moved away on contact.

Young anopheline larvae left in water containing infective mermithids usually exhibited high mortality; as many as seven mermathids were seen moving inside the bodies of recently dead larvae. If larvae were transferred to clean water after 12 hours, most survived to the fourth instar when one or more full-grown mermithids emerged.

PARASITIC STAGE. In the laboratory mermithids emerged 5–10 days after infection. Table 2 shows the size of the parasites in relation to the duration of the parasitic stage.

TABLE 2. Length of Parasites at Emergence

Duration of parasitic stage in days	Mean length of mermithid on emergence (mm)
5	4.9 mm
7	14.5
8	20.1
9	32.3
10	29.8

MULTIPLE INFECTION

Naturally infected larvae and those infected in the laboratory frequently contained more than one mermithid, the maximum number seen being four. The mean number of parasites per host larva was 1.8 in larvae infected at the Nattai River, and 1.5 in experimentally-infected larvae.

HOST REACTION

The encapsulation and melanisation of mermithid parasites by their mosquito hosts has been reported previously (Welch, 1960; Petersen, 1968). One *A. annulipes* larva from the Nattai River was found to contain a partly melanised mermithid, just less than 7 mm in length. Six out of 40 experimentally infected larvae of *A. annulipes* showed some degree of reaction to their parasite. Four larvae which were able to develop and pupate normally had one or two blackened mermithids, similar in size to infective juveniles, visible beneath the thoracic or abdominal cuticle. One larva killed and dissected during the third instar had a partly-grown blackened mermithid in the thorax. In the sixth larva, a large black mermithid was visible lying alongside the gut; this larva pupated but the adult died while emerging from the pupal case and the black mermithid was dissected from the thorax.

Apart from the larva above which contained a black mermithid, no parasitised larvae pupated in the laboratory, and no adult mosquitoes dissected have contained mermithids. It is probable that adult infection is rare or non-existent in *A. annulipes*.

DISCUSSION

The effect of this mermithid parasite on the numbers of *Anopheles annulipes* in the summer of 1967–1968 was slight, despite the heavy infection rate in some pools at the beginning of the summer. While the mosquito population from individual pools would have been reduced by the heavy infection rates, the overall population was not affected as sufficient adult mosquitoes were able to emerge and reproduce. In the favourable conditions prevailing at the river this season, larval numbers built up quickly. The mermithids had no means of migrating to new breeding pools when their original pools stagnated or dried up; adult parasitism, which would achieve this better distribution, has not been observed.

The complete life cycle lasts 20–29 days; it is similar in many respects to that of other mermithids parasitising larval mosquitoes, such as *Hydromermis churchillensis* (Welch, 1960) and *Romanomermis* sp. (Petersen *et al.,* 1968). Slight delays were observed in the laboratory in the hatching of some eggs and long survival of adult parasites before copulation could be significant for the overwintering of the mermithid population. From May–September *A. annulipes* are very scarce, and *Anopheles pseudostigmaticus* is not susceptible to infection.

This mermithid is probably a different species to that described by Laird (1956), in which the post-parasitic mermithids did not possess a terminal extension of the cuticle which is present in the Nattai River mermithid.

SUMMARY

A case of parasitism of *Anopheles annulipes* larvae by a mermithid nematode is described, with some details of the life history of the mermithids in the laboratory.

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