SYNCHRONIZATION OF LIFE CYCLES OF THREE NEW MERMITHIDS (NEMATODA) WITH THEIR CHIRONOMID (DIPTERA) HOSTS AND SOME OBSERVATIONS ON THE PATHOLOGY OF THE INFECTIONS

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ABSTRACT

At Portage Creek, in the Delta Marsh, Manitoba, one new Gastromermis and two new Hydromermis species (Nematoda: Mermithidae) infected Polypedilum simulans, Harnischia sp., and Cladotanytarsus sp. (Diptera: Chironomidae) larvae or adults. The parasites are described and illustrated. Each mermithid species infected mainly one chironomid species, though occasionally other chironomid species were infected. Parasites from these latter hosts showed variations in four morphometric characteristics compared to the same characteristics of the parasites from the main hosts.

The life cycles of each mermithid species differed in the time of emergence of the adults, the overwintering stage, and the number of broods per year. These differences were synchronized with events in the life cycles of the host chironomids. The frequency of male mermithids increased as the number of parasites per host increased. One Hydromermis species emerged from intersex Harnischia sp. adults, which are described and illustrated.

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INTRODUCTION

A large emergence of chironomids occurred during the first weeks of July, 1966, at the University of Manitoba's Delta Marsh field station. Some of these chironomids were observed to harbour nematode parasites. As a result, this project was outlined during the spring of 1967. The objectives were: (i) to find an infested larval population of chironomids; (ii) to investigate the formation of intersexes in parasitized adults; and (iii) to investigate the apparent environmental sex determination reported for mermithids.

An infested chironomid population was discovered at Portage Creek in June, 1967. After a month's study, two species of mermithids were found in the collections. A third species was discovered soon after. None could be identified with species previously described in the literature.

As three new mermithid species with unknown life histories were involved, it became necessary to change the objectives of the study. The new objectives were: (i) to describe the new mermithids; (ii) to determine their life cycles; (iii) to determine if the life cycles were synchronized with those of the hosts; (iv) to determine whether the sex of the parasites depended on the number of parasites in the host; and (v) to observe whether intersexes were present among parasitized adults.

REVIEW OF LITERATURE

CHIRONOMID LIFE HISTORY

Most publications on the Chironomidae are either taxonomic or morphological studies. Comparatively few are concerned with their ecology and life history. Many of the known life history studies involve the large mud-dwelling "bloodworms" of the genus Chironomus (= Tendipes). Table I lists some examples of chironomid life history studies.

TABLE I

EXAMPLES OF CHIRONOMID LIFE HISTORY STUDIES

SPECIES	LOCATION	INVESTIGATOR
Chironomus decorus Joh.	New York	Ping (1917)
Chironomus cristatus Fabr.	New York	Branch (1923)
Chironomus tentans Fabr.	New York	Sadler (1935)
Chironomus hyperboreus Staeg.	Saskatchewan	Rempel (1936)
Tanypus guttatipennis Goetgh.	Lk. Victoria Africa	MacDonald (1956)
Procladius umbrosus Goetgh.	Lk. Victoria Africa	MacDonald (1956)
Chironomus anthracinus Zett.	Denmark	Jónasson (1965)
Chironomus plumosus L.	Wisconsin	Hilsenhoff (1966)

Metamorphosis in chironomids is complete with the usual egg, larval,

pupal and adult stages present. In the majority of species, all stages, except the adult, are aquatic. The duration of these stages may vary considerably; some species breeding in warm water may have a number of generations per year, whereas the same species breeding in cold water may require a year for development (Wirth and Stone, 1963).

There are four larval instars, each of which can be determined by the size of the head capsule or by the size of structures within the head capsule (Ford, 1959). The duration of larval development depends on water temperature and oxygen content. Availability of food is also important, as some chironomid larvae can exist for relatively long periods with a minimum of food (Fellton, 1940; Jónasson, 1965). Once a larva reaches physiological maturity, it continues to feed but grows little.

Most chironomids overwinter as larvae, some of which are extremely resistant to freezing (Andersen, 1946; Smith, 1958). The larvae pupate when largely unknown stimuli are present. Spring circulation (Rempel, 1936), increased temperature (Sadler, 1935), longer hours of daylight (Englemann and Shappirio, 1965), lunar phases (MacDonald, 1956), and increased food supplies caused by phytoplankton blooms (Hilsenhoff, 1967) were proposed as possible stimuli for the pupation and emergence of various chironomids. The period of emergence may vary within any generation. Both Sadler (1935) and Fellton (1940) observed a spread of three weeks and more between the emergence of the first and last adults of a culture started from one egg mass. However, some species with a one year life cycle have a restricted period of emergence (eg. C. anthracinus emerges over a period of 4 - 5 days (Jónasson, 1965)). Adults rarely live longer than one week.

LIFE HISTORY OF THE MERMITHID PARASITES OF CHIRONOMIDS

Appendix II shows the general life cycle of mermithids parasitic in chironomids.

Thienemann (1954) reviewed all the chironomids which have been found parasitized by mermithids. Johnson(1955) summarized the data in Thienemann's monograph. Table II brings these data up to date.

Mermithids are obligate parasites that develop as juveniles in the haemocoele of insects (Welch, 1965). The life cycle of most mermithids is characterized by six stages (Johnson, 1955): egg; ovic larva; preparasitic larva; parasitic larva; postparasitic larva; adult. The stages designated as "larvae" are more correctly termed "juveniles " (Welch, 1963).

The preparasitic infective juvenile has a limited time to enter a host. The reported times vary from one day for the infective juveniles of Paramermis contorta in clean water (Comas, 1927; Svabenik, 1928) to about four weeks for those of P. rosea in water and mud (Wülker, 1961). These juveniles die without further development if they fail to find a host.

The preparasitic infective juvenile actively penetrates its host (Comas, 1927; Svabenik, 1928; Wülker, 1961, 1965). Both Svabenik and Wülker (op. cit.) reported that the infective juvenile would penetrate a host larva of any stage, while Comas felt that penetration required a relatively immobile early instar with a soft cuticle (immediately post moult). Wülker (1965) described infective juveniles paralyzing their hosts, probably by injecting a substance, and then penetrating the cuticle. He stressed that inducing host paralysis is restricted to certain

TABLE II

STUDIES ON CHIRONOMIDS PARASITIZED BY MERMITHIDS

1955 - 1968

CHIRONOMID	STAGE	MERMITHID	LOCATION	INVESTIGATOR
Chironomus plumosus	larva	Hydromermis contorta	Indiana	Johnson (1955)
Tanytarsus gregarius	adult	Paramermis contorta	Germany	Wülker (1958)
Chironomus plumosus	adult	Unident.	Estonia	Krall (1959)
Chironomus anthracinus	adult	Paramermis rosea	Germany	Wülker (1960)
Tanytarsus gregarius	adult	Paramermis contorta	Germany	Wülker (1961)
Chironomus anthracinus	adult	Paramermis rosea	Germany	Wülker (1961)
17 genera, 97 species	adult	Agamomermis sp.	Brazil; Argentina; Dutch Guiana	de Oliveira and Lent (1962)
Chironomus tentans	larva	Paramermis contorta	Italy; France	Parenti (1962 a; 1965 b)

TABLE II (Cont'd.)

CHIRONOMID	STAGE	MERMITHID	LOCATION	INVESTIGATOR
Chironomus tentans	larva	Paramermis contorta	Laboratory	Parenti (1962 b, c; 1965 a, c)
Chironomus plumosus	larva	Octomyomermis itascensis	Minnesota	Johnson (1963)
3 - 5 genera of Pelopiinae	adult	Unident.	Arizona; Michigan; Florida; Georgia	Roback (1963)
Cryptochironomus group	adult	Hydromermis sp.	Sudan	Wülker (1963 a)
Dicrotendipes sp.	adult	Hydromermis sp.	Sudan	Wülker (1963 a)
Chironomus, 9 spp.	larva	Paramermis rosea	Laboratory	Wülker (1963 b)
Chironomus sp.	adult	Mermis sp.	Calcutta	Das Gupta (1964)
Chironomus spp.	adult	Paramermis rosea Hydromermis contorta	Laboratory	Götz (1964)
Smittia sp.	larva	Orthomermis oedobranchus	England	Poinar (1964)

TABLE II (Cont'd.)

CHIRONOMID	STAGE	MERMITHID	LOCATION	INVESTIGATOR
Tendipes plumosus	adult	Filipjevimermis singularis	Russia	Strelkov (1964 a, b)
Glyptotendipes lobiferus	adult	Hydromermis itascensis	Minnesota	Johnson (1965)
Chironomus anthracinus	larva	Gastromermis rosea	Laboratory	Wülker (1965)
Chironomus costatus	adult	Unident.	Singapore	Karunakaran (1966)

mermithids and is applicable only to a certain number of host species. The locating of the host is not a directed behaviour (Wülker, 1961).

Only Strelkov (1964 a) described a passive infection. He reported that Filipjevimermis singularis infective juveniles are swallowed by fourth instar Tendipes plumosus larvae. These juveniles are also unusual in that they penetrate into the nervous system of the host and "hibernate" in the brain. Only after spending time in the brain do the parasites enter the haemocoele and grow.

Mermithids grow rapidly only during the later stages of their development (Welch, 1965). When they are ready to emerge, they occupy most of the host's haemocoele and are looped back on themselves several times. Usually, some of the parasite population emerges from larvae, while some is carried over to the adult insects. The parasites leave through the body wall at intra- or intersegmental folds or through natural openings (Welch, 1963). They kill their hosts upon emergence (Welch, 1965).

The postparasitic juveniles moult to the adult stage a short time after leaving their hosts. The longest time reported is that of Orthomermis oedobranchus, which usually completes its final moult four days after emergence (Poinar, 1964). Mating occurs after the final moult and oviposition quickly follows. Svabenik (1928) observed that the eggs of Paramermis contorta hatched after 4 - 30 days, depending on the temperature. All other species that have been investigated fall within these limits, except Orthomermis oedobranchus, whose eggs showed no sign of development after several weeks at room temperature (Poinar, 1964).

Little is known about the host specificity of mermithids parasitizing chironomids. Johnson (1955) reported that Hydromermis contorta parasitized Chironomus plumosus; though C. riparius and Cryptochironomus stylifera were also found infected, their incidence of infection was low and the nematodes never matured in them. Wülker (1963 a) found only one Hydromermis species parasitizing a group of closely related species of the Cryptochironomus group. He felt the worms may be restricted by host specificity to this group. This would agree with his 1961 observations that Paramermis rosea is infective only for Chironomus spp. and that P. contorta from Schluchsee parasitizes only Tanytarsus gregarius. Karunakaran (1966) examined 17 chironomid species from 6 genera and found an unidentified mermithid only in adults of Chironomus costatus. C. apicatus and C. javanus, which are closely related to C. costatus and occur at the same locality, were not infected. This suggests that the nematode is specific to C. costatus, though it is not clear if the specificity is physiological or ecological. As Welch (1960 c) stated in discussing mermithid parasites of mosquitoes, "specificity may arise less from adaptation and immunological processes than from the concurrence of the host and parasites at a time when the mermithids are seeking hosts."

CHIRONOMID INTERSEXES

Mermithids frequently cause the formation of intersexes in chironomids. These intersexes possess male and female structures composed of genetically identical cells as opposed to gynandromorphs, which possess male and female parts composed of genetically male and female cells (Brust, 1966; Smith and Perry, 1967). Wülker (1961, 1964) reviewed the

literature on chironomid intersexuality due to mermithid parasitism.

Rempel (1940) described the morphological aberrations in Chironomus rempeli Thien. intersexes: (i) male genital appendages are present; (ii) the tarsi of the forelegs have no long bristles (female - like); (iii) the antennae are short with six segments and small bristles (female - like); (iv) the cubital fork of the wing is distal to the cross vein between the radius and media (female - like); and (v) the eighth abdominal sternite is bifuricated (female - like). Some had no internal reproductive organs, though most possessed ejaculatory ducts. Two also had vasa deferentia. Two specimens had a complete male reproductive system with spermatogensis observed in one. There were also individuals classed as "aberrant females," which were morphologically females with internal reproductive organs partially or completely destroyed.

Wülker (1958) described Tanytarsus gregarius intersexes with female bodies and male hypopygia and eighth sternites. There were also many aberrant females sensu Rempel. Wülker (1960) examined Chironomus anthracinus intersexes and divided them into "gonopod" and "sternite" types. The gonopod intersexes were morphologically females except for male or intersexual eighth sternites and completely male gonopods. These individuals usually developed, in varying degrees, ejaculatory ducts and often vasa deferentia and testes. Sternite intersexes had female bodies and female or intersexual eighth and ninth sternites. The ovaries were reduced and there were no spermathecae or mucous glands. Wülker (1961) also reported that infested populations of Tanytarsus gregarius showed intersex antennae with all grades intermediate between normal male and female types. Intersex antennae could

also be induced in a few Chironomus species which were infected with mermithids from another host. De Oliveira and Lent (1962) observed intersexual antennae in some infected Neotropical chironomids, as did Roback (1963) in some infected Pelopiinae.

Rempel (1940) thought that intersexes were females which, through a loss of their reproductive glands, developed male genitalia and reproductive ducts, while males could not support a parasite and died as infected larvae. However, Wülker (1961) showed that infected male larvae develop into the typical gonopod intersex, while infected female larvae develop into the sternite type. Rempel et al (1962) verified that the gonopod intersex was genetically a male.

Rempel (1940) stated that the size, sex and number of parasites harboured are not related to the extent of the changes induced. Götz (1964) arranged the sternite and antennal intersexuality of various chironomids in scales and statistically analyzed the influence of various host-parasite relationships on the degree of sexual change. Wülker (1964) summarized Götz's significant results. In the populations studied, there were both positive and negative correlations between the intersexuality of various characters and such variables as the number and sex of parasites, their speed of development, and the time of infection.

The genesis of damage was traced to a total or partial suppression of the genital imaginal discs of the eighth and ninth larval abdominal segments (Wülker, 1964). In males, the sex appendages, ejaculatory duct, and vasa deferentia originate from the ninth segment. In females, only the gluten gland originates from the ninth

segment. The spermathecae and structures of the eighth sternite originate from the eighth segment. The determination of the imaginal organs is not rigid, but more or less labile, so that parasitic alterations are possible even in late stages of ontogenesis (Götz, 1964).

Wülker (1964) listed the possible mechanisms of parasitic damage in insects: (i) mechanical damage by direct feeding or pressure on the organs; and (ii) physiological damage by food deprivation, products of metabolism, or by secretions, antigens or toxins. He also stressed that the reactions of the host, as far as they are associated with the resulting sexual change, must be considered. Here, the reactions of the imaginal discs, the inner secretory glands, changes in normal metabolism, and reactions of specific physiological systems such as the formation of antibodies, must all be considered. All possibilities remain to be tested.

SEX DETERMINATION IN MERMITHIDS

The sexual differentiation of mermithid juveniles appears to be regulated by the environment, with the sex ratio a function of the number of parasites per host; high numbers result in the development of males; low numbers result in females. This was reported in both aquatic and terrestrial mermithids (Cobb, Steiner, and Christie, 1927; Caullery and Comas, 1928; Christie, 1929; Kaburaki and Iyatomi, 1933; Couturier, 1950, 1963; Johnson, 1955; Wülker, 1961; Parenti, 1962 a, 1965 b; Poinar and Gyrisco, 1962; Phelps and De Foliart, 1964). Environmental control of the sex ratio may be a mechanism to control

parasite numbers (Couturier, 1963).

Christie (1929) experimented quantitatively with Mermis subnigrescens and excluded differential mortality of the sexes as the cause of variation in the sex ratio. Parenti (1962 a, b, c, 1965 a, b, c) investigated Paramermis contorta (2n = 12 and no detectable sex chromosomes (Parenti, 1962 c)), a parasite of Chironomus larvae and showed that : (i) crowding plays a major part in determining the sex of the parasites (Parenti, 1962 a); (ii) at a given degree of infection (number of parasites per host) the relative frequency of males decreases with increasing lengths of larvae at the moment of the nematodes' penetration (Parenti, 1962 b); and (iii) a parasite that enters the host first can influence the sex of those entering later. If a female is the first to develop, it tends to influence the juveniles which penetrate later by favouring their differentiation as males. Similarly, if a male develops first, it tends to influence the development of juveniles which penetrate later in the female direction (Parenti, 1965 a).

Welch (1965) gave as possible hypotheses for the mechanism of environmental sex determination in mermithids: the effect of the host's sex; nutritive limitation; accumulation of contaminants (to which may be added oxygen deficiency). Of these, nutritive limitation has been the most favoured. Caullery and Comas (1928) felt that sex probably depends on the nutritional conditions in the host, with the several males encountered corresponding to some "sickly" (ie. under nourished) hosts. Poinar and Gyrisco (1962) discussed the possibility of limited nourishment causing a preponderance of males. Vandel (1932) stated that the responsible factor is concerned with nutrition, or better, is the same type as the factor "accumulation" in many rotifers and cladocerans,

where a substance provided by the animals themselves influences the sex of individuals. Johnson (1955) felt that an increase in parasitism causes a population pressure resulting in a decrease in the amount of food available per parasite and an increase in the amount of excreted material present.

Caullery and Comas (1928) studied the European form of Hydromermis contorta and found up to 17 parasites per host, with usually one or two females in each host. Johnson (1955) studied the American form of H. contorta and found up to 12 parasites per host, with an average of one female in each host and never more than two. He speculated that females secreted a substance which inhibited the production of other females. Parenti's (1965 a) results seem to add weight to this hypothesis. Also, Couturier (1963) observed that rarely more than two and never more than three female Pseudomermis hagmeieri were found in the same host. It was also rare to find only males in the same host, and when they were numerous (above 10), they were always accompanied by one to three females. It may also be significant that Comas (1927) noted that intersexuality affects only female mermithids and that Christie (1929) reported that such intersexes may develop from female juveniles that are under the influence of some environmental force that induces maleness.

An hypothesis that females secrete a substance which inhibits the production of other females does not explain the situation in all mermithids. Christie (1929) found that three Mermis subnigrescens or less per host were always females; between 3 and 23, a mixture of females and males occurred, with one or the other sex predominating (up to 14 females with no males occurred); more than 23 parasites per host

were always males. Similarly, Couturier (1950) observed that one or two Hexamermis sp. per host always resulted in females; above 6 always produced males; between 2 and 6, the sex ratio varied greatly. Poinar and Gyrisco (1962) recovered only one female Hexamermis arvalis out of 61. The hypothesis of crowding with the resulting nutritional limitation seems the best for these cases.

METHODS AND MATERIALS

STUDY AREA

The Delta Marsh is a freshwater marsh on the southern shores of Lake Manitoba. Covering 15,000 hectares (37,050 acres), it is an intricate network of various sized shallow bays of open water interconnected by channels and separated by vegetation (Hochbaum, 1944). Normally, the two most prevalent plant species in the marsh are Phragmites communis var. berlandieri and Scolochloa festucacea (Walker, 1966).

The main study area was at Portage Creek, which is a broad channel running in a north - south direction and carrying flood water to Simpson Bay in the Delta Marsh (Walker, 1959). The study area extended approximately 18 metres along the east shore of the channel in Township 13, Range 6, Section 32 NE 15. This northern part of the channel is permanently filled with water (Walker, 1959).

The substratum of the study area varied from grey sand to a black ooze with large soil particles, smelling of hydrogen sulphide. The water was approximately 20 - 75 cm. deep. Walker (1959) gave an account of the aquatic and emergent vegetation along Portage Creek.

FIELD PROCEDURE

Samples were taken with a 6" X 6" Eckman dredge. The sampling sites were on three lines which were perpendicular to the shore and approximately 9 metres apart; three sites were on each line. The sites

were about 4, 6, and 8 metres from the shoreline. The sample from each site was treated separately in both the field and the laboratory.

Sampling dates were July 21, August 15, September 20, and October 28, 1967, and April 26, May 22, and June 17, 1968. Incomplete survey samples were taken on December 29, 1967, and April 2, 1968, at three of the nine sites.

From July to October, 1967, each Eckman haul was washed ten times in a net of Nitex[1] monofilament (#405; aperture: 0.405 mm. square). Each washing consisted of submerging the net in the water, then withdrawing it. The remaining material was put in a labelled glass quart sealer with some water. The sealer top was fastened for transportation to the laboratory. On all other dates, each Eckman haul was put directly into a labelled 32" X 18" 50 lb. test polyethylene bag. The top of the bag was tied to prevent spilling during transportation.

All samples were taken to the laboratory in a Styrofoam cooler containing frozen "Freeze-Paks" during the summer and heated "Freeze-Paks" during the cold months.

Some qualitative samples were taken during the summers of 1967 and 1968 with a triangular Nitex monofilament (#405) net. The net was pushed into the bottom sediments, then pushed horizontally and lifted. The sample was washed by swirling the net in the water and put into a white 16½" X 12½" X 5" plastic basin. Water was added, and a sample of the chironomid larvae was removed with an eye dropper and put into 8 fluid ounce sample jars with screw tops. The jars were taken to the laboratory in the Styrofoam cooler.

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1. B & S. H. Thompson & Co. Montreal, Canada.

LABORATORY PROCEDURE

The bottom samples were kept exposed to the air in a cold room at 10° C. until they were processed. The qualitative samples were kept in a refrigerator at 4° C.

Separating Bottom Samples

Each Eckman sample was washed with dechlorinated water through three copper screens (mesh number 10, 40, and 80; smallest aperture: 0.177 mm.). The debris left on each screen was washed into separate white enamel 8" X 12" dissecting trays and examined. The chironomids and nematodes were removed with an eye dropper and put into water in an 8 fluid ounce sample jar. Most specimens were then preserved in 5% formalin (nematodes were first heat killed as in the section Killing and preserving and were stored separately for mounting) and stored in 19 X 65 mm. allergy vials[1] with neoprene stoppers.[2] Some specimens were kept alive in the 8 ounce jars and stored at 4° C.

Analysis of Preserved Chironomids

Each allergy vial was emptied into a syracuse dish and the contents examined with an Olympus stereo dissecting microscope. The chironomids were removed individually with surgical forceps and placed in 5% formalin in one of four syracuse dishes labelled "P. simulans", "Harnischia sp.", "Cladotanytarsus sp.", and "other." The "other" specimens were counted and their number recorded. The three known species were each grouped as pupae or by the width of the larval head capsules.

All larvae and pupae were examined for parasites with an Olympus

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1. O. H. Johns Glass Co., Toronto, Canada.
2. Hospital Equipment Corporation, East Orange, N. J., U. S. A.

monocular microscope with a 10X eyepiece and the 10X objective. Occasionally the 40X objective was used to verify presence of a parasite. The specimens were placed five at a time on a glass slide in a drop of 5% formalin and covered with a 22 mm. square No. 1 coverslip. They were recorded as pupae or by their instar and as unparasitized, parasitized by early stages of parasites (parasites that were the length of infective juveniles) or parasitized by late stages of parasites (parasites longer than infective juveniles).

A few head capsules of each instar were measured from each sample. The larvae were removed from the preservative, placed in water, and the head capsules removed. After about 15 minutes, the head capsules were placed ventral or dorsal side up in a smear of melted glycerine jelly on a glass slide and were measured (see Measuring and Drawing).

Analysis of Live Samples

Parasite analysis: The chironomids were removed from the 8 ounce jars with an eyedropper, placed on a glass slide in a drop of water two at a time, and covered with a 22 mm. square No. 1 coverslip. After examination at 100 magnification with the Olympus monocular microscope, the coverslip was removed and the specimens placed in water in one of the following groups of syracuse dishes: (i) P. simulans group; (ii) Harnischia sp. group; (iii) Cladotanytarsus sp. group; and (iv) "other" group. Each group contained three syracuse dishes: (i) parasitized by early stages of parasites; (ii) parasitized by late stages of parasites; and (iii) unparasitized. After all the chironomids in the jar were examined, each syracuse dish was examined with the dissecting microscope and the number of specimens, their stage (including larval instar), and

their parasite status were recorded. The specimens with early stages of parasites and the unparasitized second, third, and some fourth instars, were preserved in 5% formalin.

Rearing: Chironomids were reared individually in about 1 cm. of dechlorinated water in 7 dram glass polyshell vials[1] with plastic tops partially ajar. The vials were kept in 2 - 3 cm. of water in 16" X 11" white enamel photographic trays.

The chironomids with late stages of parasites were reared until the parasites emerged. The date of emergence, species, sex and number of parasites, and the species and stage of host, were recorded. Rearing of the parasites was continued until death or until the final moult was complete. The date of the start of the moult was recorded. If the host died before the parasites emerged, it was dissected in Insect Ringer's solution. The date and, if possible, the species, sex and number of parasites were recorded.

Some unparasitized fourth instar larvae and pupae were also reared. The dates of pupation, adult emergence, or death, were recorded.

Killing and preserving: The nematodes were killed by placing them in a drop of water on a glass slide and passing it through the flame of an alcohol lamp until heat rigor occurred (Goodey, 1963). They were preserved in 5% formalin or TAF (Courtney, Polley, and Miller, 1955). The adult chironomid hosts were preserved in 70% ethyl alcohol; larval and pupal hosts were discarded. Unparasitized adults with their larval and pupal skins were preserved in 70% ethyl alcohol one day after emergence.

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1. The Richards Glass Co. Ltd., Winnipeg, Canada.

All specimens were stored in 7 X 25 mm. microvials.[1] Each microvial contained nematodes from one host, one adult host, or an unparasitized adult with larval and pupal exuviae. Seven microvials were stored in one 19 X 65 mm. allergy vial.

Processing and Mounting Preserved Specimens

Nematodes: The nematodes were processed to pure glycerine by the glycerol-ethanol method (Seinhorst, 1959) or by a glycerol-methanol method. In the latter method, the nematodes were transferred from the fixative to a small dish containing about 1 ml. of a mixture of 95 parts methanol and 5 parts glycerol. They were left at 35° - 40° C. for about one hour, and then at room temperature in a desiccator for 24 hours.

The nematodes were permanently mounted in glycerine on individual 25 X 75 X 1 mm. glass slides under 18 mm. round No. 1 coverslips. Goodey (1963) gave the detailed process.

En face mounts and cross-sections of the body were prepared after the nematodes had been processed to glycerine. The sections were made by hand under a dissecting microscope, with the nematode in a drop of glycerine on a piece of Parafilm folded over on itself several times. A piece of stainless steel razor blade held in an Irex[2] 4½ inch "I be darned" was used as the cutting instrument. The sections were mounted in glycerine jelly by the method outlined by Goodey (1963).

Chironomids: Larval and pupal exuviae and adults were mounted in Turtox CMC - 10 mounting medium on 25 X 75 X 1 mm. glass slides and under 18 mm. round No. 1 coverslips. The unparasitized specimens

1. O. H. Johns Glass Co. Ltd., Toronto, Canada.
2. Irex Surgical Instruments, Box 788, Adelaide Street P. O., Toronto 1, Canada.

were removed from the alcohol preservative and placed in distilled water where the adult's head, abdomen, and wings were separated from the thorax. After about 15 minutes, the exuviae were mounted under separate coverslips on one slide, with the larval skin ventral side up and the pupal skin dorsal side up. The wings and head (dorsal side up) were mounted under separate coverslips on another slide, while the thorax with the legs attached and the abdomen with genitalia (dorsal side up) were similarly mounted on a third slide. The slides were labelled and stored for one week to allow the mounting medium to set and to clear the specimens.

Parasitized adults were placed in distilled water where the head and abdomen were separated from the thorax. After about 15 minutes, the head was mounted dorsal side up; the thorax, with the attached wings and legs, was mounted laterally under the same coverslip. The abdomen, with the genitalia, was mounted ventral side up under another coverslip on the same slide. The slides were labelled and filed away for one week.

Measuring and Drawing

Nematodes: The nematodes were measured directly with an Olympus filar micrometer eyepiece on a Leitz Ortholux microscope equipped for both bright light and phase contrast viewing, or from drawings made with the same microscope equipped with a Leitz drawing apparatus. A Wild M20 microscope with a Wild drawing apparatus was also used for drawings.

Chironomids: All chironomid head capsules were measured with the

Olympus micrometer on the Leitz microscope. The adult chironomids were drawn with the Wild M20 microscope.

IDENTIFICATION OF CHIRONOMIDS AND MERMITHIDS

CHIRONOMIDAE

The three species of parasitized chironomids belong to the subfamily Chironominae; two are of the tribe Chironomini, the third is of the tribe Tanytarsini. Dr. A. L. Hamilton[1] (per. comm.) identified the two Chironomini as Polypedilum simulans Townes, 1945, and a new Harnischia species, close to H. darbyi (Sublette, 1960). Dr. J. E. Sublette[2] (per. comm.) identified the third chironomid as a new species of Cladotanytarsus which he will describe in a forthcoming revision of the Nearctic Tanytarsini.

MERMITHID GENERA: Gastromermis AND Hydromermis

Gastromermis was first erected by Micoletzky (1923) as a subgenus of the genus Paramermis Linstow, 1898, for species with ventrally displaced mouths. Filipjev (1934 a) elevated the subgenus to a genus and diagnosed it in his key to genera of Mermithidae (1934 b) as: six cephalic papillae in one plane, cuticle thin with no criss-cross fibers, vagina S-shaped; one short spicule, eight longitudinal chords, and mouth displaced to the ventral side. Because a gradation exists from species having an almost terminal mouth opening to species having a mouth opening posterior to the head papillae and all other criteria conform to those of Hydromermis, Johnson (1965) made Gastromermis a subgenus of

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Hydromermis.

Micoletzky (1923) assigned four species to this taxon: Paramermis (Gastromermis) haempeli Micoletzky, 1923, Paramermis (Gastromermis) gastrostoma (Steiner, 1918) Micoletzky, 1923, Paramermis (Gastromermis) rosea (Hagmeier, 1912) Micoletzky, 1923, and Paramermis (Gastromermis) aquatilis (Dujardin, 1845) Micoletzky, 1923. Polozhentsev and Artyukhovskii (1959) recognized Gastromermis transsilvanica (Coman, 1953) as well as the above species. Other species described in this taxon are Gastromermis viridis Welch, 1962, Gastromermis boophthorae Welch and Rubzov, 1965, Gastromermis rosalbus Rubzov, 1967, Gastromermis virescens Rubzov, 1967, Gastromermis clinogaster Rubzov, 1967, Gastromermis odagmiae Rubzov, 1967, Gastromermis crassifrons Rubzov, 1967, Gastromermis minuta Rubzov, 1967, Gastromermis crassicauda Rubzov, 1967, and Gastromermis longispicula Rubzov, 1967. Johnson (1965) placed Hydromermis itascensis Johnson, 1965, in the taxon because its mouth is displaced ventrally. Similarly, Welch and Rubzov (1965) placed Paramermis fluviatilis Hagmeier, 1912, Paramermis macroposthia Steiner, 1919, and Paramermis steineri Kreis, 1924 in the taxon.

Since Johnson's (1965) paper, Welch and Rubzov (1965) described six varieties of a new Gastromermis species and Rubzov (1967) described eight new Gastromermis species. All have ventrally displaced mouths and a single spicule that is at least twice as long as the anus-to-terminus distance. Perhaps Gastromermis should remain a genus, differing from Hydromermis by its single elongate spicule. Forms with a shorter curved spicule and a ventrally displaced mouth may be placed in the genus Hydromermis, as Johnson (op. cit.) pointed out that other genera have species with terminal mouths and subventral mouths.

I have placed the following species in the genus Gastromermis: G. haempeli Micoletzky, 1923; G. gastrostoma (Steiner, 1918) Micoletzky, 1923; G. viridis Welch, 1962; G. boophthorae Welch and Rubzov, 1965; G. rosalbus Rubzov, 1967; G. virescens Rubzov, 1967; G. crassifrons Rubzov, 1967; G. minuta Rubzov, 1967; G. clinogaster Rubzov, 1967; G. odagmiae Rubzov, 1967; G. crassicauda Rubzov, 1967; and G. longispicula Rubzov, 1967. Although the number of longitudinal chords and the nature of the cuticle was not determined for G. haempeli, it is placed in this genus because Micoletzky (1923) stated that it was similar to G. gastrostoma.

Gastromermis rosea (Hagmeier, 1912) Micoletzky, 1923, has a short spicule and presumably six longitudinal chords and may be placed in the genus Limnomermis as noted by Hagmeier (1912 - addendum). Gastromermis transsilvanica (Coman, 1953) Polozhentsev and Artyukhovskii, 1959, also belongs to Limnomermis because it possesses six longitudinal chords (Johnson, 1965). Paramermis fluviatilis Hagmeier, 1912, has a short spicule, eight longitudinal chords, and a ventrally displaced mouth and should be placed in Hydromermis. Hydromermis itascensis Johnson, 1965, is a true Hydromermis. Paramermis macroposthia Steiner, 1919 and Paramermis steineri Kreis, 1924 may belong to Gastromermis but need re-examination with regard to the shape and structure of their lateral chords (Welch and Rubzov, 1965). Also, P. steineri has an unusual spicule. Gastromermis aquatilis (Dujardin, 1845) Micoletzky, 1923, described on the basis of females, has an unknown number of longitudinal chords. It is of historical value only, and should be designated a nomen nudum.

Gastromermis haempeli Micoletzky, 1923, is the type species of the genus Gastromermis by original designation (Welch and Rubzov, 1965).

Corti (1902) set up the genus Hydromermis, the third established in the family, after Mermis Dujardin, 1842, and Paramermis Linstow, 1898. Hagmeier (1912) did not recognize Hydromermis and assigned his species to Paramermis, though in a footnote to his paper he recognized that some of his species could be assigned to Hydromermis. Steiner (1919) did not recognize the genus at first, but in 1929 he placed a species in Hydromermis and in 1932 returned a species that he had previously removed. Filipjev (1934 b) gave a diagnosis for Hydromermis in his key to genera of Mermithidae.

Corti (1902) established Hydromermis to contain his species Hydromermis rivicola. Welch (1960 b) synonymized H. rivicola with Hydromermis contorta (Linstow, 1889) Hagmeier, 1912 and stated that H. contorta is the type of the genus.

Gastromermis deltensis new species (Figs. 1 - 7)

Mermithidae Braun, 1883. Gastromermis Micoletzky, 1923, emended Filipjev, 1934 a. Cuticle thin (4 - 7 μ near nerve ring), without criss-cross fibers. Eight longitudinal chords in dorsal, ventral, lateral and submedial positions. Chords bulge into pseudocoelom in vicinity of nerve ring, where dorso-lateral chords reduced and closely associated with dorsal chord, while ventro-lateral chords reduced and equidistant from lateral and ventral chords. In this region, lateral chords about 30 μ wide with two rows of cells; dorsal and ventral chords with one row of cells. Head homocephalic; slight neck constric-

Figs. 1 - 7. Gastromermis deltensis n. sp.

Fig. 1 Lateral view of male head.

Fig. 2 Ventral view of male head.

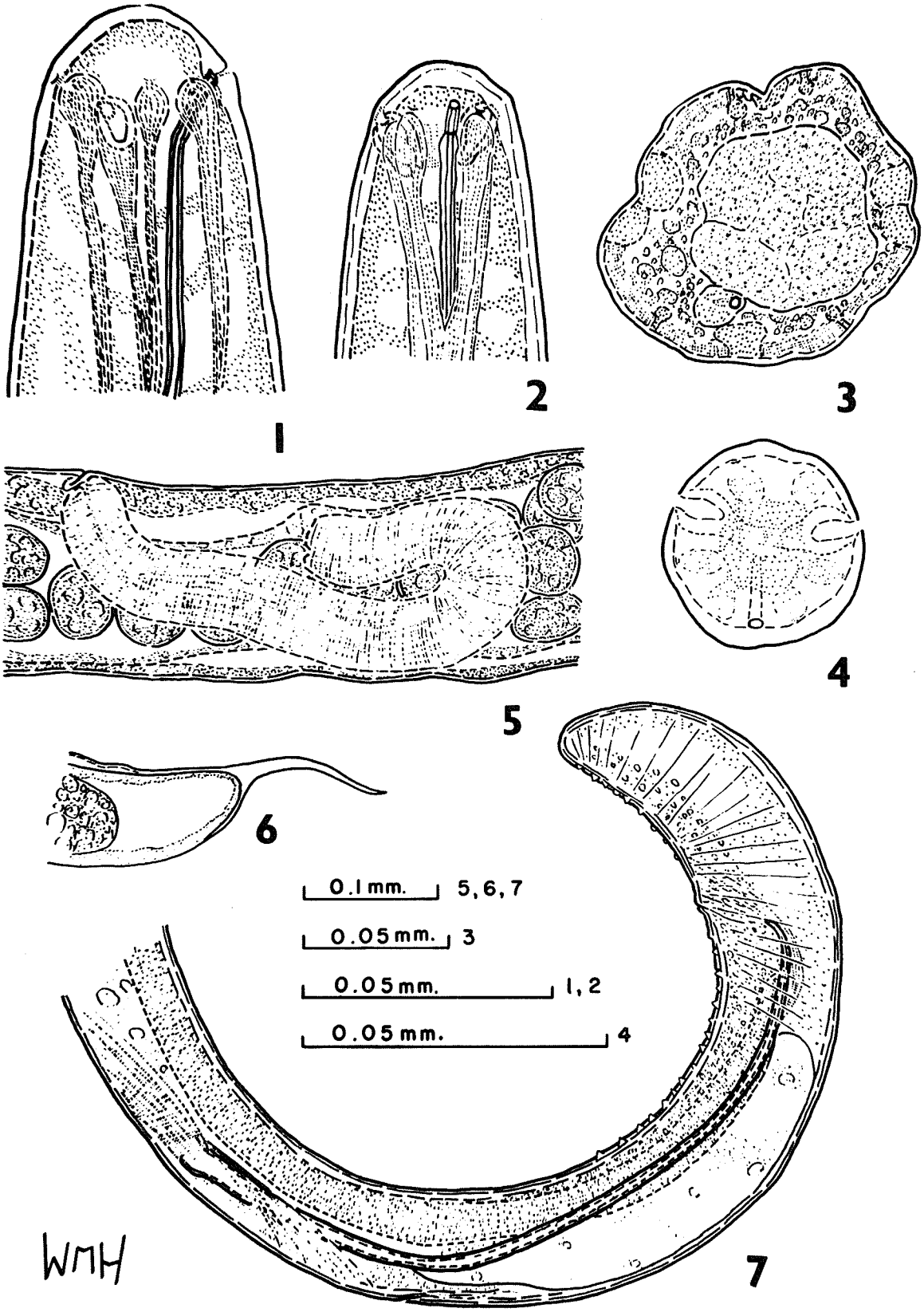
Fig. 3 Cross section of male body in anterior region.

Fig. 4 Head of male, end-on view.

Fig. 5 Lateral view of vulval region, head to left, ventral surface up.

Fig. 6 Lateral view of tail of postparasitic juvenile female, ventral surface up.

Fig. 7 Lateral view of male tail, ventral surface up.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0.1 mm. 5, 6, 7
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0.05 mm. 1, 2
0.05 mm. 4
WMH

tion. Head papillae in one plane, grouped on six cephalic papilla-tracts arranged submedially and laterally. Amphids dorsolateral, opening into ring of head papillae; dorsal commissure present. Excretory pore 0.25 - 0.30 mm. from apex. Mouth displaced ventrally, opening slightly anterior to or in ring of head papillae; esophageal collar present. Esophagus of uniform width, 2 - 4 μ for entire length, running ventrally and extending to approximately 1/3 of body length. Terminus rounded, tip directed slightly ventrally. Single spicule longer than twice anus-to-terminus distance.

Female: (16 specimens). Length 14.1 (7.5 - 14.4)[1] mm. Width at head papillae 0.041 (0.028 - 0.044) mm., and at vulva 0.14 (0.07 - 0.17) mm. Amphidial pouch 9 - 13 μ X 3 - 6 μ ; amphid, including walls, 11 - 16 μ X 6 - 8 μ ; elliptical pore 4 μ X 3 μ . Anterior end of body to: head papillae and amphid pore 17 (10 - 17) μ ; nerve ring 0.16 (0.13 - 0.18) mm.; trophosome 0.22 (0.18 - 0.26) mm.; anterior ovary 0.34 (0.34 - 0.75) mm. (12 specimens). Posterior end of body to: trophosome 0.11 (0.03 - 0.13) mm.; posterior ovary 0.23 (0.25 - 0.32)mm. (4 specimens). Vagina a reversed J-shape, 0.34 (0.18 - 0.29)mm. long (linear distance from anteriormost to posteriormost part), lying dorsally and extending posteriorly. Vulva a ventral transverse slit at a distance 41% (41% - 50%) of the body length. Eggs spherical to oval 46 - 57 μ in diameter, in situ, and embryonated.

Male: (15 specimens). Length 7.6 (5.6 - 11.2)mm. Width at head papillae 0.033 (0.028 - 0.037)mm., at midpoint 0.076 (0.056 - 0.105) mm., and at anus 0.077 (0.065 - 0.100)mm. Amphidial pouch 7 - 12 μ X 3 - 5 μ ;

1. First measurement is that of holotype or allotype; figures in parentheses give the range of measurements of paratypes.

amphid, including walls, 11 - 14 μ X 6 - 9 μ ; elliptical pore 4 μ X 3 μ . Anterior end of body to: head papillae and amphid pore 12 (10 - 16) μ ; nerve ring 0.12 (0.13 - 0.16) mm., trophosome 0.20 (0.15 - 0.22)mm.; anterior testis 0.59 (0.45 - 0.70)mm. (12 specimens). Posterior end of body to: trophosome 0.27 (0.25 - 0.33)mm. (11 specimens); anus 0.15 (0.14 - 0.19)mm. Single spiculum 0.5 - 0.7 mm. long with a basal width of 15 - 23 μ , a shaft width of 8 - 11 μ at midpoint, and tapering to a blunt point. Three rows of anal papillae; variable in number; median row 18 - 26 papillae anterior to anus, approximately 11 posterior to anus; lateral row 10 - 20 papillae anterior to anus, approximately 13 posterior to anus.

Free-living female juvenile: In 10 paratypes with partially-shed cuticles, the juvenile tail appendage measured 0.09 - 0.11 mm.

Free-living male juvenile: In 9 paratypes with partially-shed cuticles, the juvenile tail appendage measured 0.08 - 0.11 mm.

Type host: Harnischia sp. (close to H. darbyi (Sublette, 1960)).

Type locality: Portage Creek in the Delta Marsh, Manitoba.

Location: Juvenile stages of worm parasitic in the haemocoele of the chironomid larvae.

Diagnosis: This species stand closest to Gastromermis gastrostoma (Steiner, 1918) Micoletzky, 1923. However, it is much shorter, and the female's body is stouter (holotype: $a^1 = 100$; G. gastrostoma: a = 141.4)

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1. $a = \frac{\text{Total Length}}{\text{Greatest Width}}$

while the male's is thinner (allotype: a = 98.8; G. gastrostoma: a = 75.1 and 87.2). The amphids of G. gastrostoma are much larger (30 - 34 μ X 14 - 20 μ) as are the amphid pouches (28 μ X 10 - 16 μ). There are no drawings of the female genitalia for G. gastrostoma, but Steiner (1918) described the vagina as "S-shaped". The vagina of G. deltensis is best described as a "reversed-J-shape". The spicule lengths of G. deltensis are similar to those of G. gastrostoma, but if considered as a percent of body length, are larger (5.8% - 6.9% compared to 3.4% and 3.8%.

G. gastrostoma was recovered from bottom soil samples taken in Hamburg Harbour and the Elbe River near Hamburg and Altona, Germany. It was described on the basis of two juvenile males and a juvenile female. Its host is unknown.

G. deltensis Parasitic In Polypedilum simulans

G. deltensis rarely parasitized P. simulans. The four female worms recovered from this chironomid differed from the paratypes of G. deltensis in: (i) the distance from the apex to the vulva as a percent of body length (52% compared to 41 - 50%); (ii) the shorter juvenile tail appendage (0.05 - 0.06 mm. compared to 0.09 - 0.11 mm.); (iii) the slightly more ventrally displaced mouth; and (iv) the greater distance from the apex to the anterior ovary (0.99 and 1.11 mm. compared to 0.34 - 0.75 mm.).

Hydromermis variabilis new species (Figs. 8 - 14)

Mermithidae Braun, 1883. Hydromermis Corti, 1902. Cuticle thin, 2 - 5 μ ; no criss-cross fibers. Eight longitudinal chords in dorsal,

Figs. 8 - 14. Hydromermis variabilis n. sp.

Fig. 8 Dorsal view of female head.

Fig. 9 Lateral view of female head.

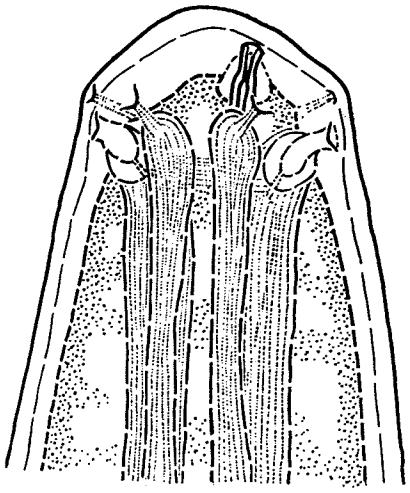
Fig. 10 Head of male, end-on view.

Fig. 11 Cross section of male body in anterior region.

Fig. 12 Lateral view of tail of postparasitic juvenile female, ventral surface to left.

Fig. 13 Lateral view of vulval region, head to right, ventral surface up.

Fig. 14 Lateral view of male tail, ventral surface up.



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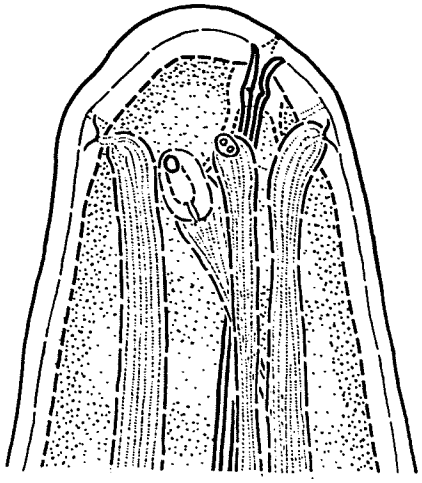

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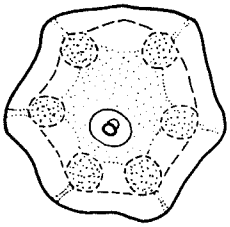

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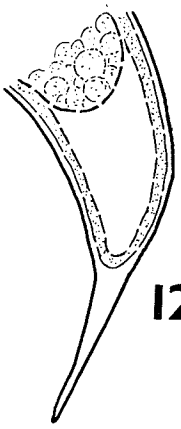

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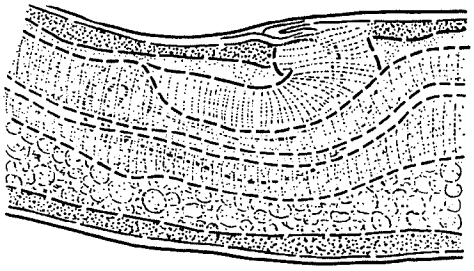

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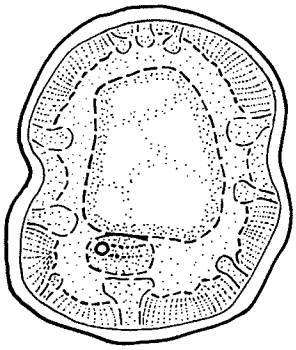

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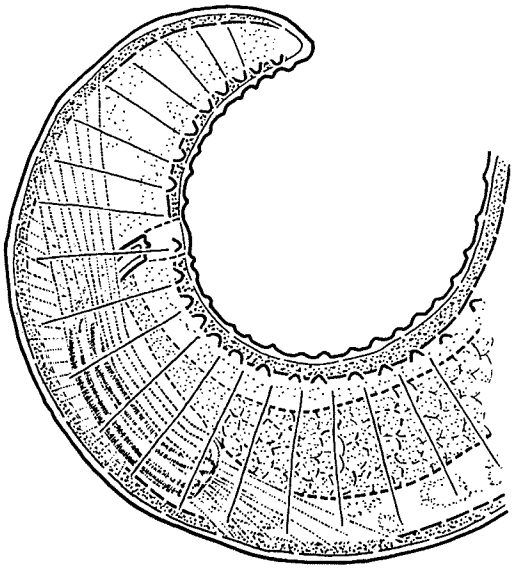


14

0.1 mm. 12,13

0.05 mm. 11

0.1 mm. 14

0.05 mm. 8,9,10

ventral, lateral and submedial positions; chords bulge into pseudocoelom near nerve ring. Lateral chords contain two rows of cells; ventral and dorsal chords contain one row of cells; dorso-lateral chords reduced and close to dorsal chord; ventrolateral chords reduced and equidistant from lateral and ventral chords. Head homocephalic; slight neck constriction. Head papillae in one plane, grouped on six cephalic papilla-tracts arranged submedially and laterally. Amphids dorsolateral, connected by dorsal commissure. Excretory pore 0.2 - 0.3 mm. from apex. Mouth displaced ventrally in varying degrees, to maximum of half distance to head papillae. Esophagus 3 - 4 μ wide running ventrally and extending about 1/3 of body length; esophageal collar present. Terminus rounded and directed slightly ventrally.

Female: (17 specimens). Length 15.2 (11.5 - 15.9)[1] mm. Width at head papillae 0.045 (0.036 - 0.050) mm. Amphid pouch 9 - 14 μ X 3 - 6 μ ; amphid, including walls, 11 - 18 μ X 6 - 9 μ ; amphid pore 4 X 3 μ . Anterior end of body to: head papillae 14 (7 -17) μ ; amphid pore 23 (13 - 23) μ ; nerve ring 0.14 (0.16 - 0.24)mm.; trophosome 0.20 (0.20 - 0.31)mm.; anterior ovary 0.38 (0.61 - 1.04) mm. (12 specimens). Posterior end of body to: posterior ovary 0.20 (0.22)mm. (3 specimens); trophosome 0.07 (0.05 - 0.11)mm. Vulva a ventral transverse slit at a distance 50 (44 - 51)% of body length. Body width increases slightly anterior to vulva and decreases posterior to vulva; 0.15 (0.12 - 0.17) mm. slightly anterior to vulva; 0.15 (0.09 - 0.16) mm. at vulva; 0.13 (0.08 - 0.15)mm. slightly posterior to vulva. Vagina S-shaped 0.18 (0.16 - 0.23) mm. long (linear distance from anteriormost

1. First figure is that of holotype or allotype; figures in parentheses give range of paratypes.

to posteriormost part), opening laterally into uterus. Eggs ellipsoid, 53 - 63 μ X 43 - 47 μ in situ and unembryonated.

Male: (20 specimens). Length 7.6 (6.6 - 12.3) mm. Width at: head papillae 0.038 (0.034 - 0.045) mm.; midpoint 0.102 (0.086 - 0.128) mm.; anus 0.087 (0.077 - 0.115) mm. Amphid pouch 8 - 12 μ X 3 - 8 μ ; amphid, including walls, 10 - 15 μ X 6 - 10 μ ; amphid pore 4 X 3 μ. Anterior end of body to: head papillae 11 (9 - 16) μ ; amphids 19 (15 - 22) μ ; nerve ring 0.18 (0.16 - 0.22) mm.; trophosome 0.24 (0.23 - 0.33) mm.; anterior testis 1.16 (0.53 - 1.37) mm. (14 specimens). Posterior end of body to: trophosome 0.39 (0.31 - 0.39) mm.; anus 0.12 (0.11 - 0.17) mm. Single curved spiculum 0.15 (0.12 - 0.17) mm. long, with a basal width of 20 (16 - 27) μ , a shaft width of 14 (12 - 18) μ at midpoint, and a tip that is flat with three small projections, or blunt. Anal papillae in three rows; number of papillae variable; 8 - 11 in median row posterior to anus and 17 - 20 anterior to anus; 21 - 25 in ventrolateral row.

Free-living female juvenile: In 13 paratypes with partially-shed cuticles, the juvenile tail appendage measured 0.06 - 0.11 mm.

Free-living male juvenile: In 14 paratypes with partially-shed cuticles, the juvenile tail appendage measured 0.06 - 0.11 mm.

Type host: Polypedilum simulans Townes, 1945; Harnischia sp. (close to H. darbyi (Sublette, 1960)).

Type locality: Portage Creek in the Delta Marsh, Manitoba.

Location: Juvenile stages parasitic in haemocoele of the chironomid

larvae or pupae and in abdomen of adult.

Diagnosis: Hydromermis leptoposthia Steiner, 1929, stands closest to H. variabilis. It can be distinguished by its terminal mouth, more spherical amphids, fewer anal papillae, a spicule with a poorly-defined basal swelling, and a large anterior overlapping vulva lip with no pre-vulvar expansion or post-vulvar constriction. H. fluviatilis (Hagmeier, 1912) Hagmeier, 1912, and H. itascensis Johnson, 1965, have ventrally displaced mouths and short spicules. H. fluviatilis is distinguished from H. variabilis by its larger amphids (23 X 20 μ), smaller spicule (70 μ), pointed terminus, and fewer anal papillae. H. itascensis is a larger species and is separated from the new species by a more ventrally displaced mouth, a shorter vagina with no pre-vulvar expansion or post-vulvar constriction, a longer spicule (170 - 210 μ) and a pointed terminus.

H. leptoposthia was collected in the U. S. S. R. and described on the basis of one male and one female. The host is unknown. H. fluviatilis was collected from chironomid larvae in Germany, while H. itascensis parasitized Glyptotendipes lobiferous (Chironomidae) adults at Lake Itasca, Minnesota.

Host-specific Variations in H. variabilis

Length of juvenile tail appendage: Most post-parasitic juvenile mermithids possess a tail appendage which is a projection of the parasitic juvenile cuticle. This appendage is shed with the cuticle at the moult to maturity. Figure 15 shows the frequency distribution of the lengths of 50 juvenile tail appendages of H. variabilis which

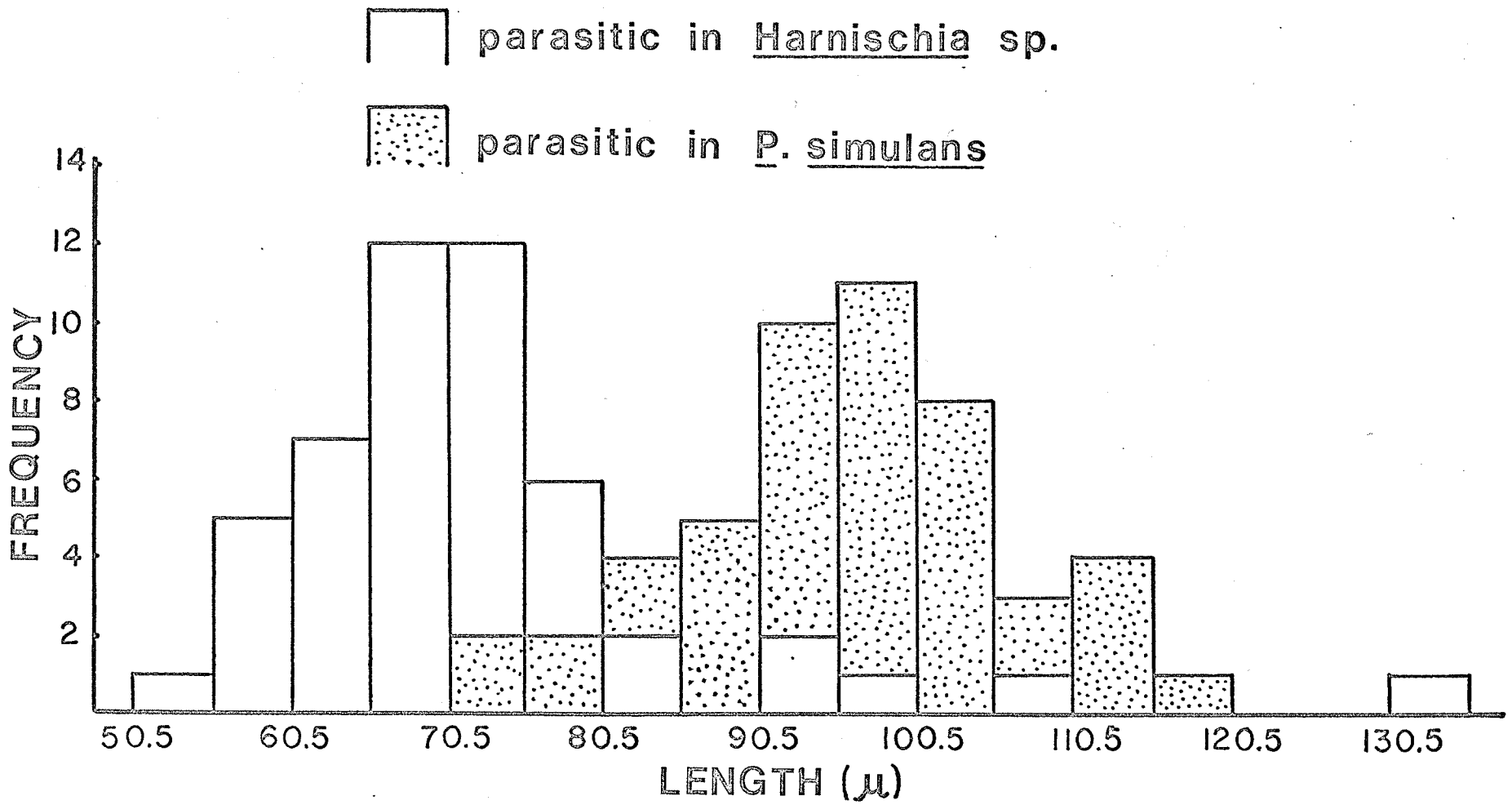


Fig. 15 Lengths of juvenile tail appendages of H. variabilis.

parasitized Polypedilum simulans and 50 tail appendages of specimens which parasitized Harnischia sp. Table III gives the statistical data on the lengths of the tail appendages.

Table III shows that an F test on the sample variances yields an F value below the critical value, indicating that the population variances can be assumed equal ($P < 0.05$). Therefore, a t-test may be used to analyze the means. A two-tailed t-test indicates no significant difference at the 0.05 level between the lengths of the juvenile tail appendages of males and females. When the lengths of the appendages of males and females from the same host are pooled and the means compared, a highly significant t-value is obtained. This indicates that the lengths of juvenile tail appendages of H. variabilis parasitizing P. simulans are significantly different from those of the same species parasitizing Harnischia sp.

Spicule structure: Figure 16 shows two types of spicules from H. variabilis males, one of which emerged from Harnischia sp., the other from P. simulans. The spicules are clearly different; with Harnischia sp. as host, the spicule is gently curved with a blunt distal end; with P. simulans as host, the spicule has a more severe curve and a flat distal end with three points. Of 29 spicules from males with P. simulans as a host, only 2 were spicules of the type from Harnischia sp. hosts. Twenty-one spicules of specimens from Harnischia sp. showed none of the Polypedilum type. Table IV presents the statistical data on the three parameters investigated for the two spicule types. The two Harnischia sp. type spicules from the males from P. simulans hosts are listed separately.

In all cases, an F test on the sample variances yielded an F value lower than the critical value, indicating that the assumption that the

TABLE III

STATISTICAL DATA ON THE LENGTHS OF JUVENILE TAIL APPENDAGES OF *H. variabilis*

VARIABLE	n	STATISTIC s^2	F	$\bar{x} \pm s$	t
Host: *P. simulans*					
Female Tail Appendage Length (μ)	8	154.9	1.53	96.0 ± 12.4	- 0.07
Male Tail Appendage Length (μ)	42	101.3	($F_{.025} = 2.62$)	95.7 ± 10.1	($t_{.025} = 2.31$)
Host: *Harnischia* sp.					
Female Tail Appendage Length (μ)	25	225.2	2.25	76.6 ± 15.0	- 2.28
Male Tail Appendage Length (μ)	25	100.6	($F_{.025} = 2.27$)	68.3 ± 10.0	($t_{.025} = 2.31$)
Pooled Male and Female Lengths					
Host: *P. simulans*	50	106.9	1.66	95.8 ± 10.3	- 9.79
Host: *Harnischia* sp.	50	176.9	($F_{.025} = 1.77$)	72.4 ± 13.3	($t_{.025} = 2.28$)

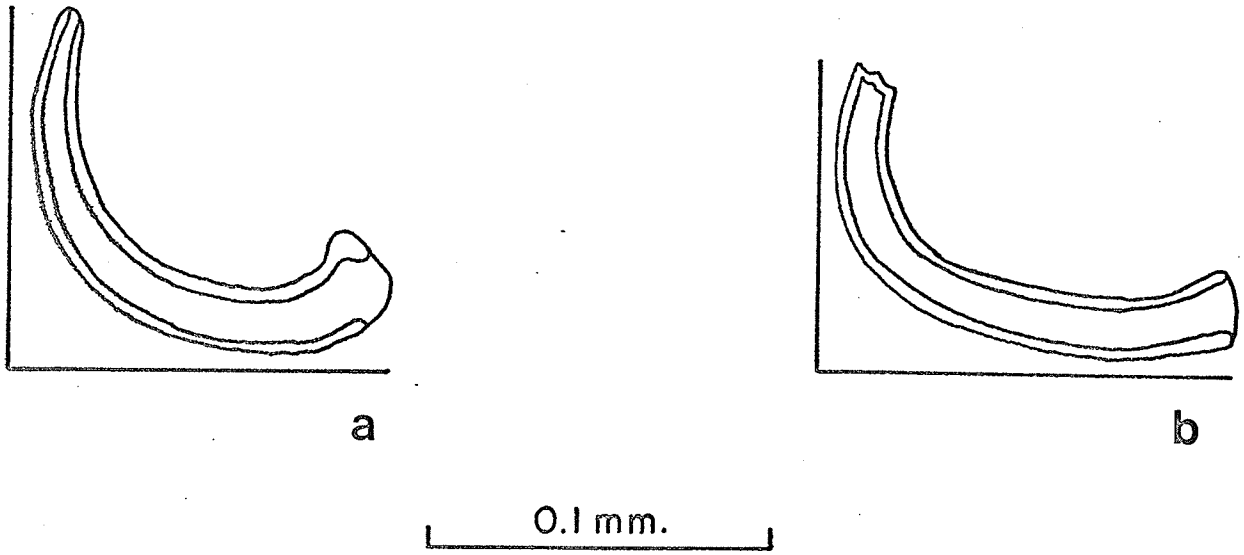


Fig. 16 Variation in H. variabilis spicule shape. a Harnischia sp. as host. b P. simulans as host.

TABLE IV

STATISTICAL DATA ON *H. variabilis* SPICULES

VARIABLE	n	STATISTIC s^2	F	$\bar{x} \pm s$	t
			($F_{.025} = 2.33$)		($t_{.025} = 2.33$)
Spicule length in μ					
Host: *Harnischia* sp.	21	132.85	1.03	151.4 ± 11.5	0.46
Host: *P. simulans*	27	129.34		153.0 ± 11.4	
Host: *P. simulans* (*Harnischia* sp. type spicule)	2	0		145.0 ± 0	
Spicule base width in μ					
Host: *Harnischia* sp.	21	8.72	1.45	25.1 ± 3.0	- 5.26
Host: *P. simulans*	27	6.03		21.0 ± 2.4	
Host: *P. simulans* (*Harnischia* sp. type spicule)	2	4.5		23.5 ± 2.1	
Spicule width at midpoint in μ					
Host: *Harnischia* sp.	21	3.34	1.01	15.4 ± 1.8	- 1.35
Host: *P. simulans*	27	3.30		14.7 ± 1.8	
Host: *P. simulans* (*Harnischia* sp. type spicule)	2	2.0		15.0 ± 1.4	

population variances are equal can be made at the 0.05 level of significance. This allows the use of the t-test for a comparison of the means. Table IV shows that the means of the lengths and of the midpoint widths of the two spicule types are not different ($P < 0.05$). However, the means of the base widths of the two spicule types are significantly different at the 0.05 level.

Ventral displacement of the mouth: The statistical data on the ventral displacement of the center of the mouth from the mid-point of a line joining the dorsal and ventral limits of the head papillae are given in Table V.

TABLE V

STATISTICAL DATA ON THE VENTRAL DISPLACEMENT OF THE MOUTH OF H. variabilis

	STATISTIC				
	n	s^2	F	$\bar{x} \pm s$	t
			($F_{.025} = 2.42$)		($t_{.025} = 2.33$)
Ventral displacement of the mouth in μ					
Host: Harnischia sp.	22	2.75	1.15	8.8 ± 1.7	9.26
Host: P. simulans	21	2.39		4.2 ± 1.5	

Analysis of the sample variances by an F test indicates that the population variances can be presumed equal ($P < 0.05$). A subsequent two-tailed t-test indicates that the mean of the ventral displacements of the mouths of H. variabilis parasitizing Harnischia sp. is significantly different at

the 0.05 level from that of the same species parasitizing P. simulans.

Hydromermis palustris new species (Figs. 17 - 22)

Mermithidae Braun, 1883. Hydromermis Corti, 1902. Cuticle thin 2 - 4 μ, without criss-cross fibers. Eight longitudinal chords in dorsal, ventral, lateral and submedial positions; lateral chords contain 2 rows of cells near nerve ring, 3 rows for most of body length; dorsal and ventral chords contain one row of cells; subventral and subdorsal chords reduced, present as breaks in musculature; subventral chord equidistant from lateral and ventral chords, subdorsal chord closer to dorsal than to lateral chord. Head homocephalic; neck constriction slight if present. Head papillae in one plane, grouped on 6 cephalic papilla-tracts arranged hexagonally in face view. Amphids lateral; dorsal commissure present. Mouth terminal, opening in a cone-shaped depression of cuticle. Esophagus 3 μ wide for most of length, extends past vulva to approximately 2/3 of body length. Excretory pore 0.13 - 0.35 mm. from apex. Terminus tapered to a fine point in both sexes.

Female: (11 specimens). Length 7.7 (6.9 - 11.8)[1] mm. Width at: head papillae 0.028 (0.025 - 0.036) mm.; vulva 0.11 (0.10 - 0.17) mm. Amphid pouch 7 - 9 μ X 3 - 5 μ; amphid, including walls: 8 - 9 μ X 4 - 9 μ in dorsal or ventral view; 9 - 19 μ X 8 - 14 μ in lateral view; amphid pore 4 X 3 μ. Apex to: head papillae 8 (7 - 10) μ; amphid openings 12 (12 - 18) μ; nerve ring 0.15 (0.12 - 0.19) mm.; trophosome 0.23 (0.19 - 0.30) mm.; beginning of anterior ovary 0.45 (0.38 - 0.85) mm. (7 specimens); end of anterior ovary (2.6 - 4.9) mm. (6 specimens); begin-

1. First figure is that of holotype or allotype; figures in parentheses give range of measurements of paratypes.

Figs. 17 - 22. Hydromermis palustris n. sp.

Fig. 17 Head of female, end-on view.

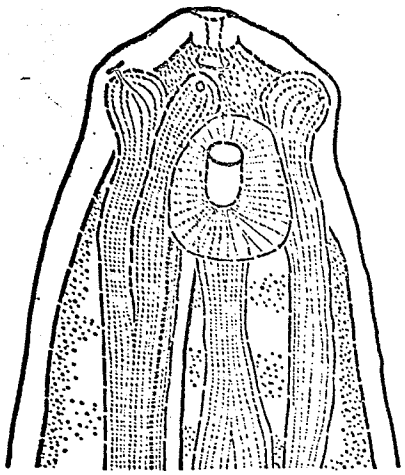
Fig. 18 Cross section of female body in anterior region.

Fig. 19 Lateral view of vulval region, head to right, ventral surface up.

Fig. 20 Lateral view of male tail, ventral surface up.

Fig. 21 Lateral view of female head.

Fig. 22 Ventral view of female head.



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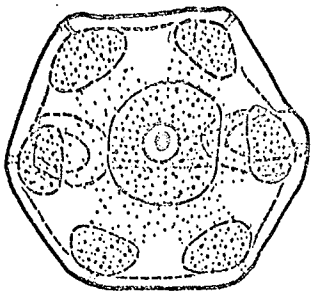

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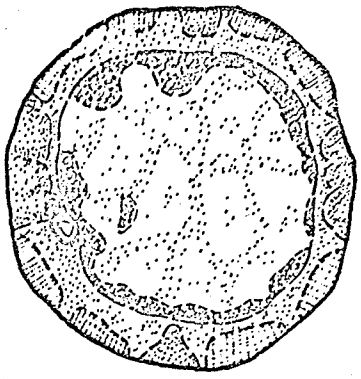

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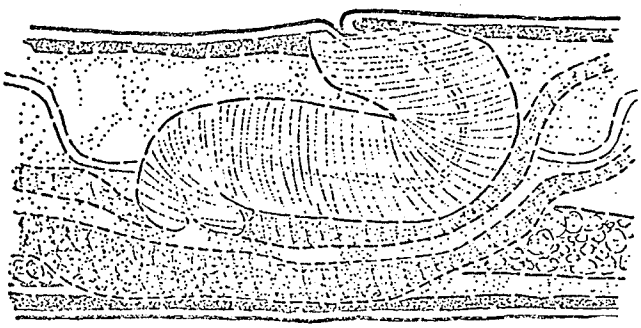

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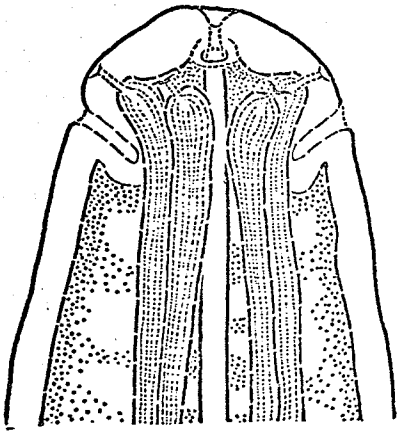

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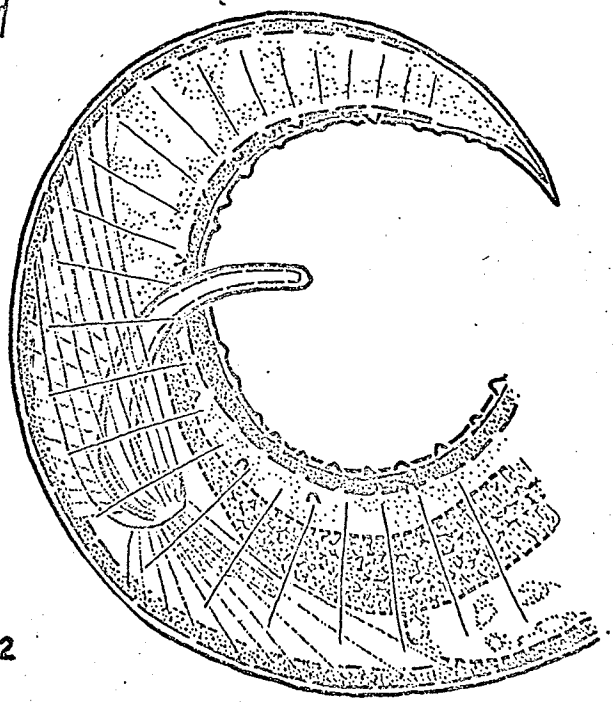
WMH



20

0.05 mm. 18

0.1 mm. 19

0.1 mm. 20

0.05 mm. 17

0.05 mm. 21, 22

ning of posterior ovary (3.8 - 5.8) mm. (6 specimens). Vulva a ventral transverse slit with an overlapping anterior lip, at a distance 45 (43 - 54) % of total length. Vagina S-shaped, 0.14 (0.14 - 0.26) mm. long (linear distance from anteriormost to posteriormost part), opening ventrolaterally into uterus. Terminus to: posterior ovary 0.23 (0.07 - 0.36) mm. (7 specimens); trophosome 0.09 (0.08 - 0.14) mm. (7 specimens). Eggs ellipsoid to almost circular, 65 - 73 μ X 54 - 67 μ *in situ*, and embryonated

Male: (6 specimens). Length 6.1 (3.3 - 5.9) mm. Width at: head papillae 0.026 (0.020 - 0.026) mm.; mid-point 0.079 (0.056 - 0.075) mm.; anus 0.063 (0.053 - 0.061) mm. Amphid pouch 7 - 9 μ X 3 - 4 μ ; amphid, including walls 10 - 12 μ X 8 - 10 μ ; amphid pore 3 X 3 μ . Apex to: head papillae 6 (6 - 8) μ ; amphid openings 11 (9 - 13) μ ; nerve ring 0.16 (0.10 - 0.15) mm.; trophosome 0.26 (0.17 - 0.24) mm.; anterior testis 0.78 (0.46 - 0.84) mm. Terminus to: anus 0.15 (0.12 - 0.17) mm.; trophosome 0.25 (0.20 - 0.27) mm. (5 specimens). Single spicule, 0.11 (0.09 - 0.11) mm. long, gently curved, with a blunt distal end, a base width 15 (10 - 15) μ , and a shaft width at midpoint 10 (10 - 13) μ . Anal papillae in three rows vary in number; 9 - 10 papillae in lateral rows; median row has 10 - 12 papillae posterior to anus and 14 - 18 papillae anterior to anus.

Free-living juveniles: Both male and female free-living juveniles do not possess a tail appendage.

Type host: *Cladotanytarsus* sp.

Type locality: Portage Creek in the Delta Marsh, Manitoba.

Location: Juvenile stages parasitic in haemocoele of the chironomid larvae.

Diagnosis: Hydromermis churchillensis Welch, 1960 a, Hydromermis contorta (Linstow, 1889) Hagmeier, 1912, Hydromermis fluviatilis (Hagmeier, 1912) Hagmeier, 1912, and Hydromermis palustris are distinguished from other Hydromermis species by the pointed termini of both sexes. H. contorta is distinguished from the new species by its larger size (females: 26.2 ± 1.6 mm. (Welch, 1960 b)), larger spicule (0.2 - 0.3 mm.), and amphids opening farther back from the apex (26 μ), larger (males: 17 X 19 μ), with a larger pore (8 μ). H. fluviatilis is also larger (females: 13 - 16 mm.), has a slightly ventrally displaced mouth, larger amphids in lateral (23 X 20 μ) and ventral (15 X 4 μ) view and set farther back from the apex (30 μ), and a shorter (0.066 mm.) more robust spicule with a thickened distal end. H. churchillensis is larger (females: 12 - 21 mm.) than H. palustris, has narrower amphids (females: 2 μ ; males: 4 μ), a vulva without a prominent overlapping anterior lip, and paired fused spicules that are longer (0.22 mm.), have a greater maximum width (21 μ) and a second widened portion at the point of fusion.

Chironomids are recorded as hosts of H. fluviatilis and H. contorta, the former from Germany and the latter from Austria, Germany, and the United States (Welch, 1960 a). Aedes communis (Diptera: Culicidae) is the type host of H. churchillensis.

Morphological Variation in H. palustris

Three male and one female H. palustris emerged from Cladotanytarsus

sp. larvae but differed morphologically from the paratypes. The males differed in that they showed: (i) a slight ventral displacement (3 - 6 μ) of the mouth; (ii) a rounded or slightly-pointed terminus; (iii) a slightly shorter anus-to-terminus distance (0.09 - 0.12 mm. compared to 0.12 - 0.17 mm.); (iv) a greater width at the anus (0.066 - 0.071 mm. compared to 0.053 - 0.063 mm.); and (v) a shorter terminus to trophosome distance (0.15 - 0.20 mm. compared to 0.20 - 0.27 mm.). The females showed: (i) a slightly ventrally displaced (3 μ) mouth; (ii) a rounded terminus; (iii) a shorter length (6.3 mm. compared to 6.9 - 11.8 mm.); and (iv) a shorter vagina length (0.11 mm. compared to 0.14 - 0.26 mm.).

One female H. palustris emerged from a P. simulans or Harnischia sp. larva. It differed from the paratypes in: (i) length (12.1 mm. compared to max 11.8 mm.); (ii) a greater apex to nerve ring distance (0.22 mm. compared to max 0.19 mm.); and (iii) a greater apex to anterior ovary distance (1.42 mm. compared to max 0.85 mm.).

OBSERVATIONS AND RESULTS

CHIRONOMID LIFE CYCLES

Table VI gives the head capsule widths of the larvae of the three chironomid species. No first instar larvae were taken.

TABLE VI

HEAD CAPSULE WIDTHS OF THE CHIRONOMID LARVAE

SPECIES	INSTAR	HEAD CAPSULE WIDTH IN MM.				DYAR'S RATIO[1]
		n	$\bar{x} \pm s$	Narrowest	Widest	
P. simulans	2	30	0.11 ± 0.00	0.10	0.12	0.58
	3	50	0.18 ± 0.00	0.17	0.20	0.61
	4	50	0.30 ± 0.01	0.27	0.34	
Harnischia sp.	2	24	0.10 ± 0.01	0.07	0.11	0.61
	3	50	0.16 ± 0.00	0.14	0.17	0.66
	4	50	0.24 ± 0.02	0.21	0.27	
Cladotanytarsus sp.	2	12	0.09 ± 0.00	0.08	0.10	0.64
	3	50	0.15 ± 0.00	0.13	0.16	0.70
	4	50	0.21 ± 0.00	0.19	0.23	

1. $\frac{\text{width of head of any instar}}{\text{width of head of preceding instar}}$ = constant (Dyar, 1890)

Dyar's Ratio, multiplied by the mean head capsule widths of the second instar, gives the following approximate widths of the first instar head cap-

sules: P. simulans: 0.06 - 0.07 mm.; Harnischia sp.: 0.06 - 0.07 mm.; Cladotanytarsus sp.: 0.06 mm.

Figures 23 - 25 show the percent of second, third and fourth instar larvae and pupae of each species taken in 1967 on July 21, August 15, September 20, and October 28, and in 1968 on April 26, May 22, and June 17. The figures indicate that the three species overwintered mainly as third instar larvae and that the overwintering populations were present by September 20. The chironomids spent part of the winter in the frozen bottom sediments, as a sample chopped from the bottom on April 2, 1968, yielded the three species.

Cladotanytarsus sp. (Fig. 23)

This was the first of the three species to moult in the spring. In April, 42% of Cladotanytarsus sp. was present as fourth instars, while the other two species had not yet moulted. No Cladotanytarsus sp. larvae were taken in May, and only two fourth instars were taken in June; none could be found thereafter. However, the presence of mainly fourth instars and some pupae in July and August, 1967, indicates that emergence and egg-laying probably occur during most of the summer.

Harnischia sp. (Fig. 24)

Harnischia sp. was the second to moult in the spring; most of the overwintering third instars developed into fourths and a few pupated by mid-May. Twenty of 44 larvae from this sample pupated in the laboratory from May 26 to June 1. The June 17 sample, and an extra sample on June 26, yielded mainly fourth instars and pupae, with some fourths pupating in the collection vessels. As the July and August 1967 samples also contained

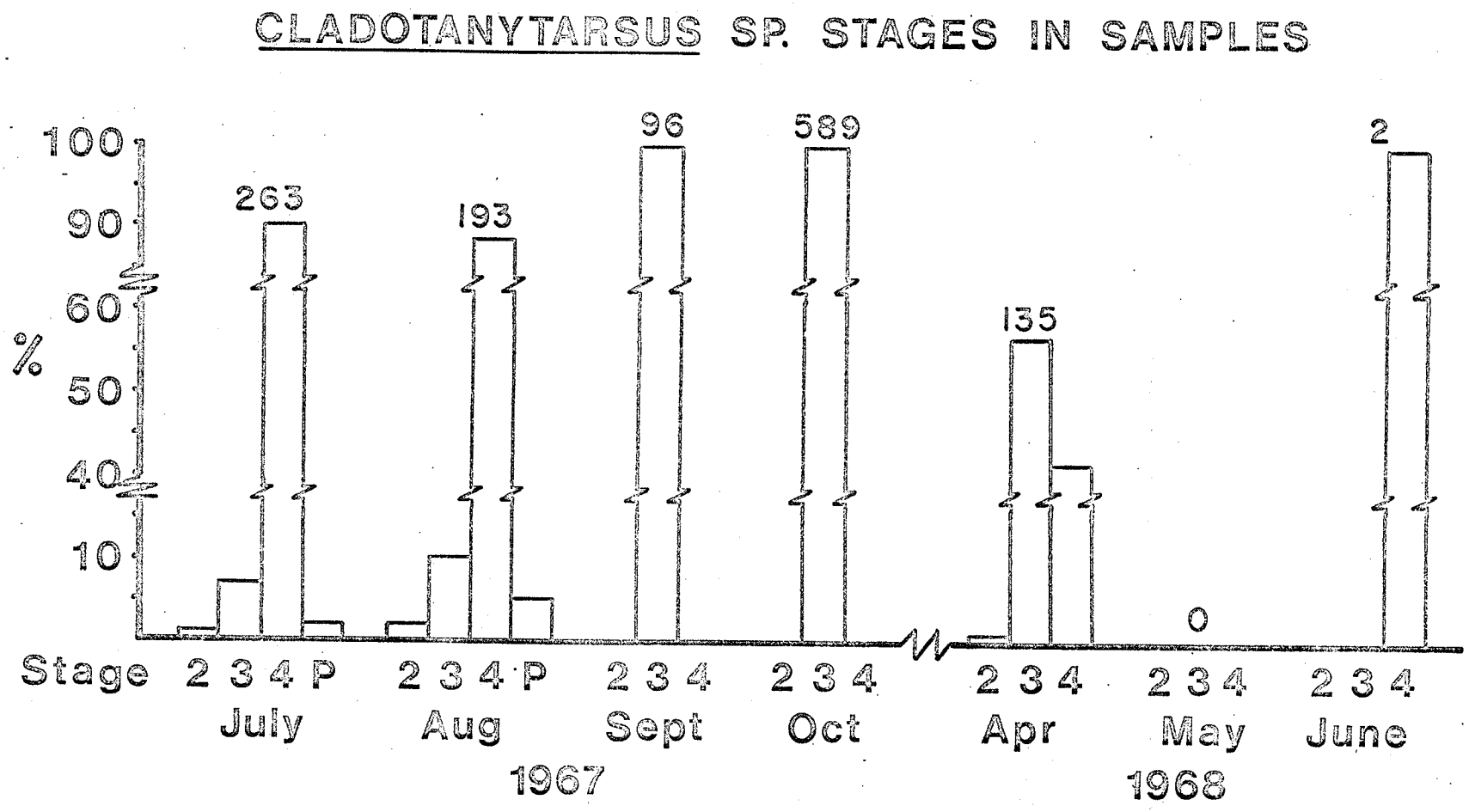


Fig. 23 Cladotanytarsus sp.: Percent of second, third and fourth instar larvae and of pupae in the monthly samples. (numbers = sample size)

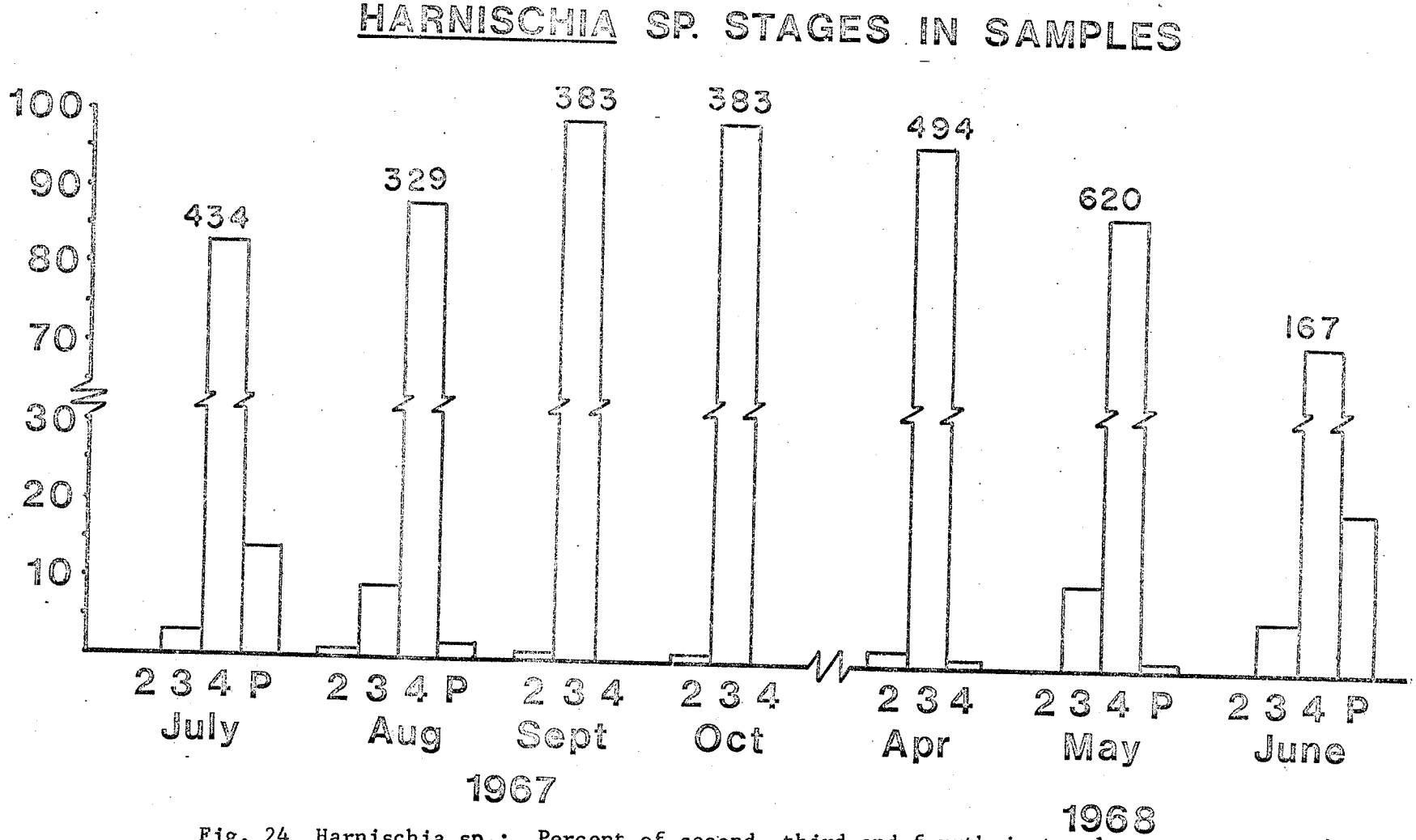


Fig. 24 Harnischia sp.: Percent of second, third and fourth instar larvae and of pupae in monthly samples. (numbers = sample size)

mainly fourth instar larvae and some pupae, Harnischia sp. probably emerges and oviposits during most of the summer.

The pupal life of 67 Harnischia sp. larvae captured at various times throughout the year and reared in the laboratory at room temperature was 1.7 ± 0.6 days.

Polypedilum simulans (Fig. 25)

The overwintering population of P. simulans was the last to moult in the spring. Only 17% of the May sample was fourth instars, compared to 99% on June 17. Repeated sampling on June 26, yielded 7 P. simulans larvae. Similarly, in 1967, 87 larvae were taken in two nettings on June 22, compared to 6 in 5 nettings on July 5. This indicates a fairly synchronized emergence of the overwintering population during the last two weeks of June. Mainly fourth instars with some pupae were present in July, 1967, while mainly third instars with some fourths and pupae were present in August, 1967. This indicates that emergence and oviposition of P. simulans during the summer is limited to a period of about one month from the end of July to the end of August.

MERMITHID LIFE CYCLES

Figures 26 - 28 show the percent of parasitized chironomid larvae of each species in the monthly samples. The larvae were divided into two groups: (i) those with early stages of parasites (parasites that have not increased in length after penetrating; and (ii) those with late stages (parasites that have increased in length after penetrating). The early stages of the parasites could not be identified as to their species.

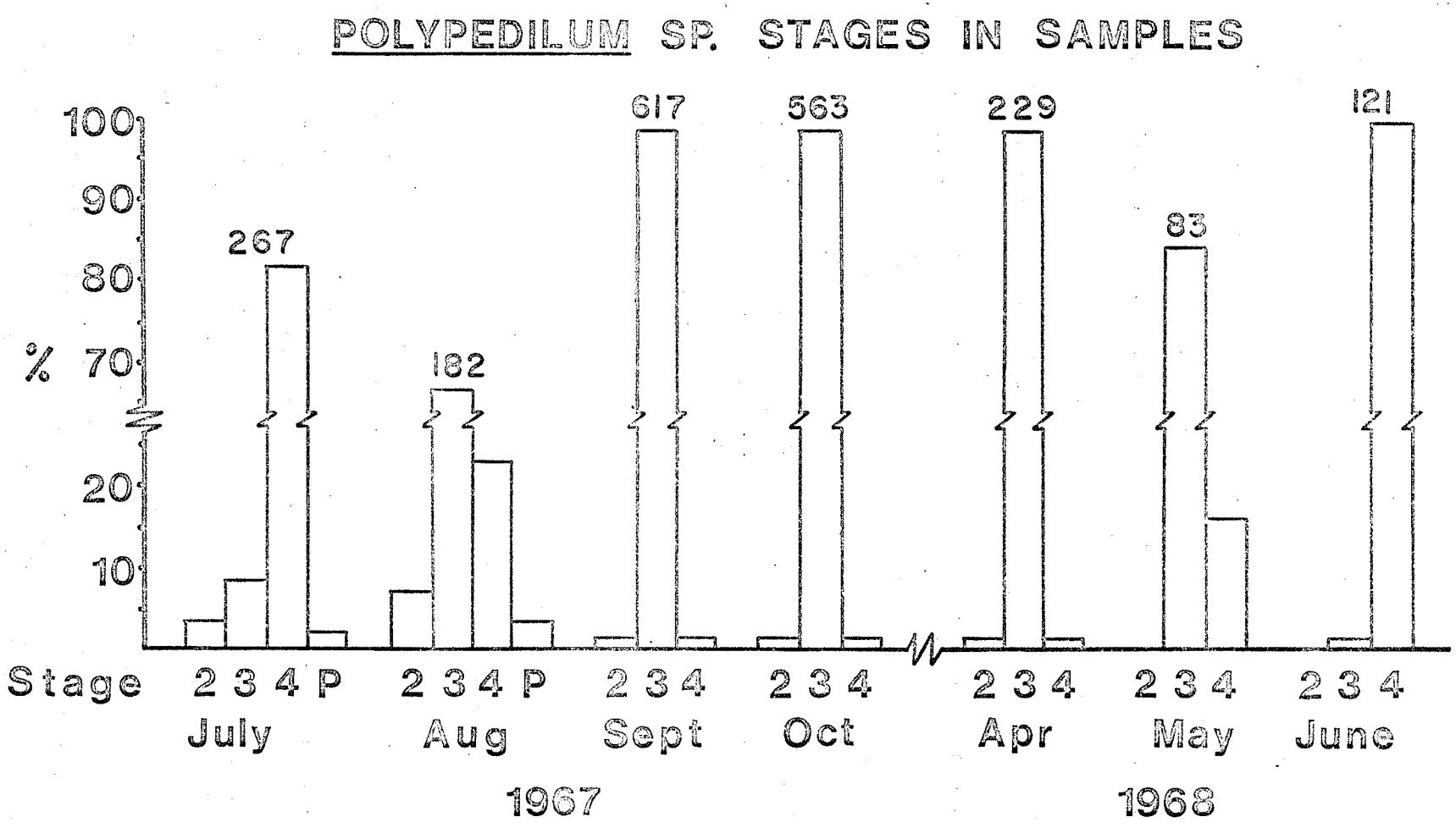


Fig. 25 Polypedilum simulans: Percent of second, third and fourth instar larvae and of pupae in monthly samples. (numbers = sample size)

Hydromermis variabilis

H. variabilis emerges primarily from the adults of P. simulans and Harnischia sp. As H. variabilis probably overwinters as an egg which hatches in the spring, figures 26 - 28 show a large increase in parasitism by early parasites in the April sample. These nematodes were long, thin and active (typical of the free-living infective juvenile), indicating that infection occurred close to April 26, 1968. They thickened without increasing in length and assumed the typical sickle-shape of the early stage of mermithid parasitism. This was the stage present on May 22 in P. simulans and in some Harnischia sp.

H. variabilis was ready to emerge on June 17. It was dissected from P. simulans larvae and pupae during the last two weeks of June and emerged from Harnischia sp. pupae and adults from June 18 - 27, with the peak from June 23 - 26. Emergence from the adult hosts occurred 1.3 ± 0.9 days ($n = 32$) after the host emerged from the pupal stage. Eight of 173 (4.6%) Harnischia sp. pupae and fourth instars and 0 of 7 P. simulans taken in an extra sample on June 26 were parasitized. They yielded H. variabilis from June 30 to July 3. In 1967, this species emerged from P. simulans pupae and adults from June 23 to July 6, with the peak from June 27 to July 4. It emerged from Harnischia sp. pupae and adults from June 29 to July 17, with the peak from June 29 to July 12.

The moult of 26 H. variabilis started 3.7 ± 0.8 days after emergence from Harnischia sp.. These nematodes probably mate and oviposit during the summer, as the three free-living females found in 1967 (one on July 21, two on August 15) were gravid. The parasite probably overwinters as as egg.

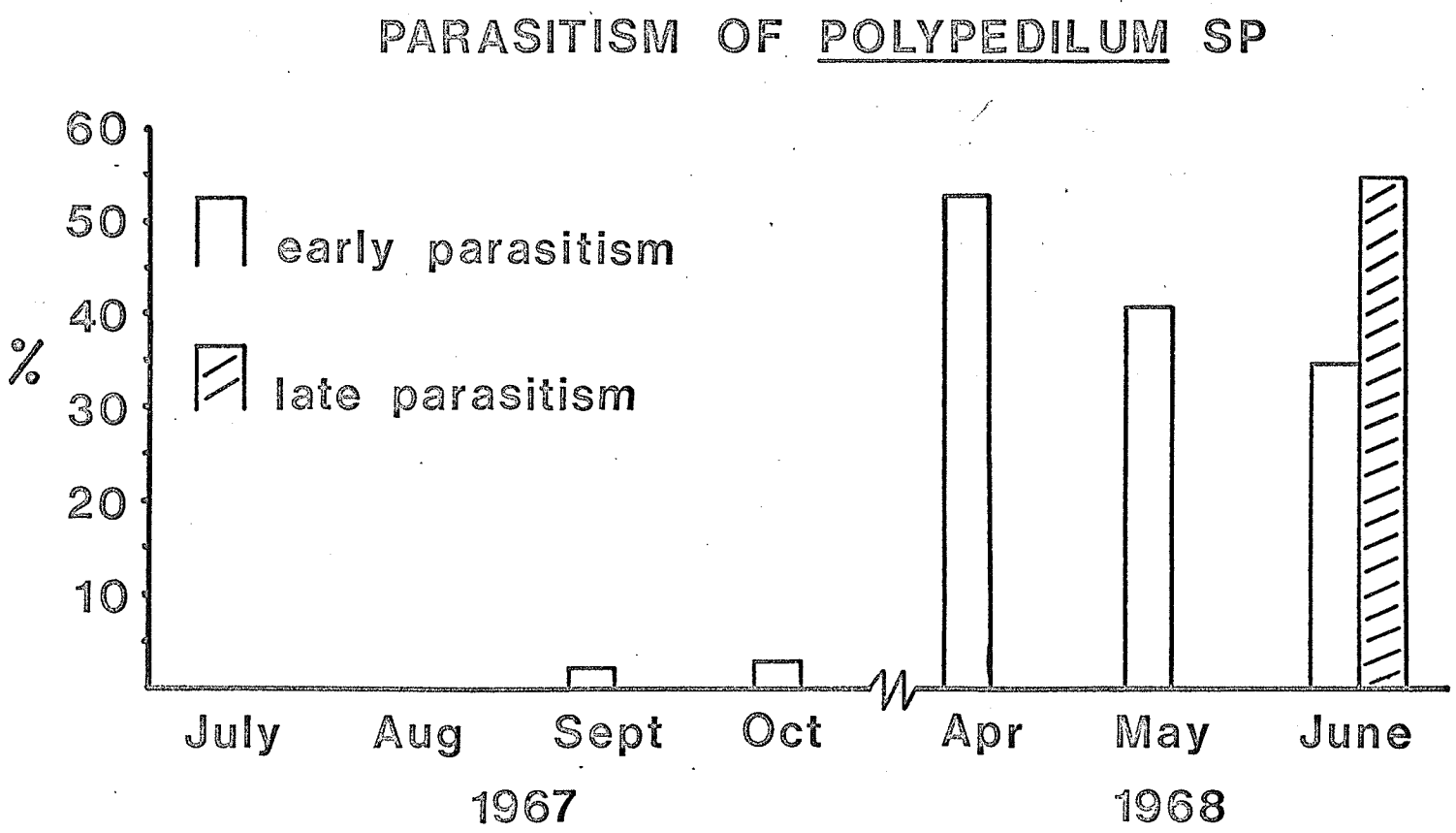


Fig. 26 Polypedilum simulans: Percent of larvae and pupae parasitized by early and late stages of parasites.

Gastromermis deltensis

G. deltensis emerges mainly from the larvae of *Harnischia* sp.. The worm overwinters as an early parasite with about 25% of the overwintering *Harnischia* sp. larvae infected. Two or three percent of the overwintering *P. simulans* larvae also harboured parasites that were similar in size to those in *Harnischia* sp. These were probably *G. deltensis*. Thirty-three *Harnischia* sp. specimens with these sickle-shaped parasites were cultured from the April 26, 1968 sample; only 4 yielded *G. deltensis* between May 8 and May 14. The hosts were all third instars.

On May 22, *Harnischia* sp. fourth instar larvae contained *G. deltensis*. These worms emerged in the laboratory from May 26 to May 29 and moulted 2.2 $\pm$ 0.8 days (n = 25) after emergence. Some hosts contained both late stages of *G. deltensis* and sickle-shaped stages of worms that were probably *H. variabilis* which died with the host when *G. deltensis* emerged.

G. deltensis infects new larvae, grows, and emerges during the entire summer. The sickle-shaped stages in *P. simulans* and *Harnischia* sp. on June 17 probably were *G. deltensis*; two *Harnischia* sp. larvae from this sample yielded two *G. deltensis* on June 21; four larvae had the sickle-shaped parasites together with the large *H. variabilis*. The hosts and the small parasites died when the larger parasites emerged. *G. deltensis* emerged from *Harnischia* sp. larvae collected from July 17 to August 30, 1967. From July 26 to August 28, sickle-shaped parasites were also present in *Harnischia* sp. Late stages of *G. deltensis* and early stages of parasites were also observed in an extra sample of *Harnischia* sp. larvae taken on August 11, 1968.

During July and August, 1967, no *P. simulans* larvae were infected.

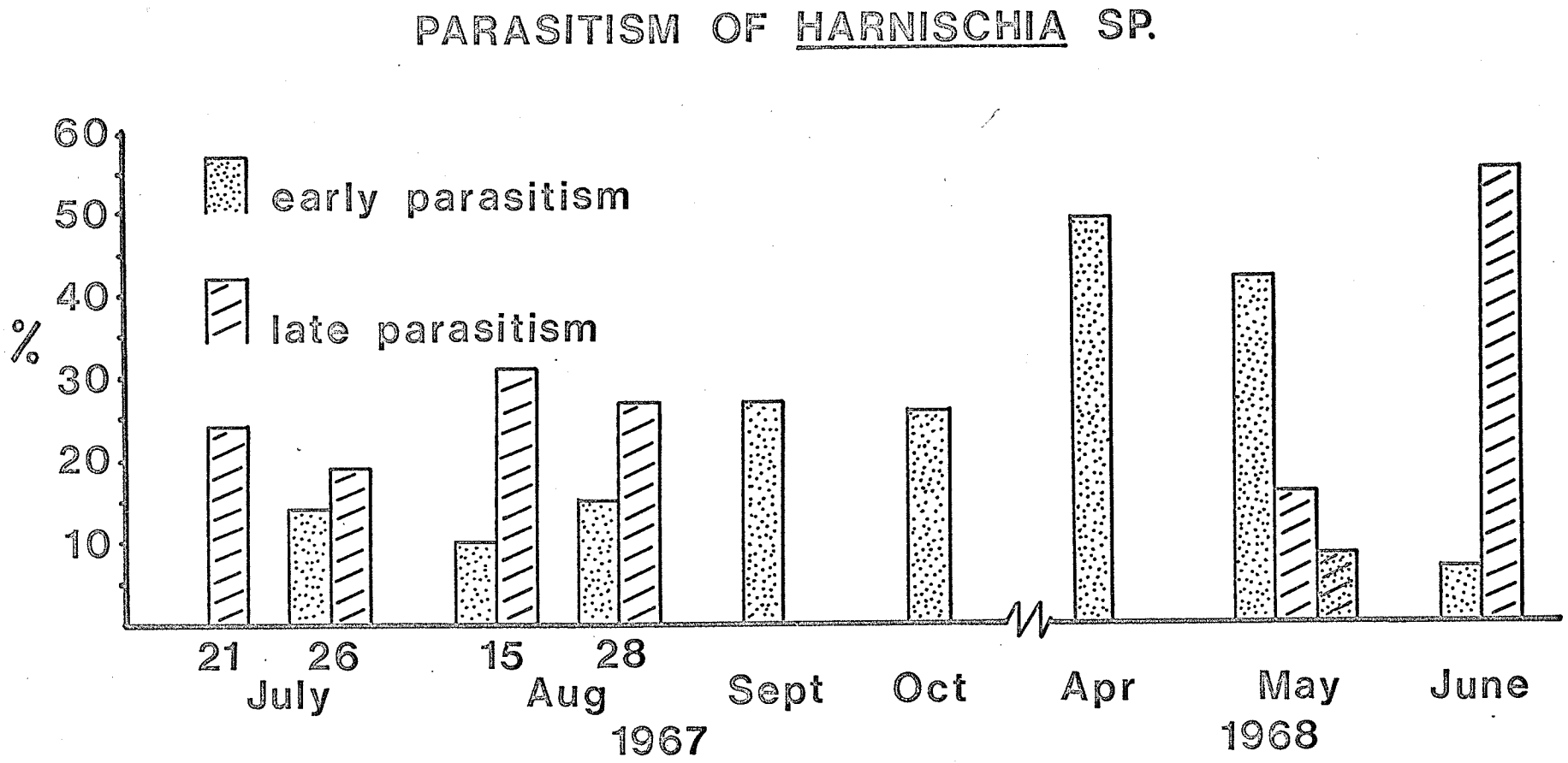


Fig. 27 Harnischia sp.: Percent of larvae and pupae parasitized by early and late stages of parasites.

However, on August 11, 1968, four of 152 (2.6%) P. simulans larvae were parasitized and yielded 4 G. deltensis females. Three of the parasites were preserved 4 days, 9 days, and nearly 3 months respectively after emergence, but none had moulted. The fourth started to moult 3 months and 9 days after emergence, but died the next day without completing the moult.

An attempt was made to infect Harnischia sp. artificially with infective G. deltensis juveniles. There seemed to be no orientation to a host; juveniles placed in a depression slide together with a larva swam past both a non-moving and a moving larva. Upon touching the larva, a juvenile would halt for a short moment with its anterior end against the chironomid's cuticle, and then swim away. No prolonged attachment or penetration was observed.

In a second attempt, infective juveniles and active embryonated eggs were placed in a vial with 2 third and 8 early fourth instar Harnischia sp. larvae. Within two days, all the larvae were infected. The number of parasites varied from 1 to 11 per host. The juveniles were observed to enter the larvae through the cuticle, with penetration occurring at any abdominal segment. The period of infectivity seemed to be limited, as 4 P. simulans and 4 Harnischia sp. larvae added to the same vial 6 days after the start of the experiment were not infected. Of the infected larvae, 5 died on the third day after infection, 2 died on the fifth day, and 3 were killed and preserved on the seventh day.

The frequency of G. deltensis in 219 Harnischia sp. larvae taken in one Ekman haul on May 22, 1968 was as follows: number per host 0, frequency 155; 1,60; 2,3; 3,1. This distribution fits a Poisson distribution

at the 5% level of significance ($\chi^2 = 4.75$; $\chi^2_{0.95} = 5.99$), indicating that G. deltensis is uniformly distributed with each larva equally susceptible to attack.

Hydromermis palustris

H. palustris parasitizes mainly Cladotanytarsus sp.. About 25% of the overwintering Cladotanytarsus sp. larvae were infected by sickle-shaped stages by mid-September. On October 28, 1967, the parasites were about half the length of a mature adult. They overwinter in this advanced state. A survey sample on April 2, 1968, yielded 43 (32%) larvae parasitized by advanced parasites. The majority of these larvae died by April 14; all were dead by April 27. No parasites emerged from these larvae. The third and fourth instar larvae taken on April 26 were parasitized mostly by worms that were ready to emerge. Of 20 such larvae cultured in the laboratory, 8 had their parasites emerge from May 3 to May 8; 12 died without having their parasites emerge.

The stages of parasite present in May and June are unknown. However, starting on July 26, 1967, samples showed both early and late stages of parasitism, and larvae yielded H. palustris from July 26 to August 28. This suggests that H. palustris infects new larvae, grows, and emerges during most of the summer.

The sample of April 2, 1968, showed a frequency of H. palustris in 134 Cladotanytarsus sp. larvae from 3 Ekman hauls as follows: number per host 0, frequency 91; 1,23; 2,8; 3,7; 4,4; 5,0; 6,1. This distribution shows a significant departure at the 5% level ($\chi^2 = 46.91$; $\chi^2_{0.95} = 11.1$) from a Poisson distribution and suggests that the distribution of the

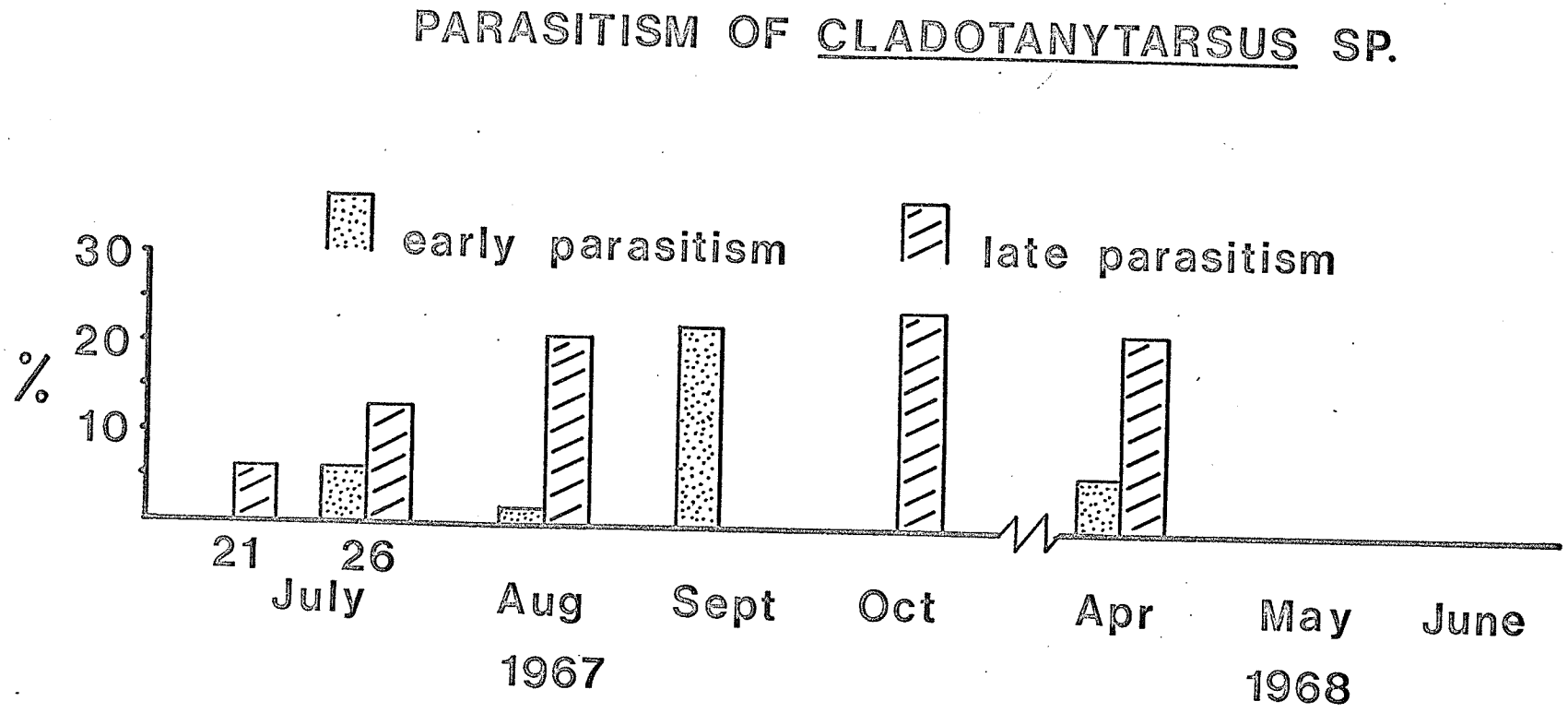


Fig. 28 Cladotanytarsus sp.: Percent of larvae and pupae parasitized by early and late stages of parasites.

parasite is uneven, and that once a larva is attacked, it is susceptible to a second attack.

An attempt was made to infect Cladotanytarsus sp. larvae artificially with H. palustris active embryonated eggs. When the eggs were presented in a tangle of organic debris to two larvae, the larvae ate from the debris. They could not ingest the eggs, which were too large to pass through the mouth. No infection occurred.

Summary

Table VII summarizes the life history of the three mermithids.

HOST SPECIFICITY

The following facts favour an hypothesis that the three mermithids are ecologically specific (both hosts and parasites must be present when the parasites are seeking hosts) to their hosts: (i) when the overwintering eggs of H. variabilis hatched, parasitism of the three chironomid species increased; (ii) 2 - 3% of the overwintering P. simulans larvae (main host for H. variabilis which overwinters as an egg) were infected, and four G. deltensis females emerged from P. simulans collected in August, 1968; (iii) an H. palustris female emerged from a P. simulans or Harnischia sp. larva, moulted, and was gravid when she was preserved; and (iv) H. variabilis consistently emerged from P. simulans and Harnischia sp.. However, these facts do not exclude an hypothesis that the parasites may also be physiologically adapted to certain hosts. Hence, though Cladotanytarsus sp. was newly infected in the spring, it never yielded H. variabilis, the only parasite that infects new larvae in the early spring. Also, only one of the four G. deltensis females that

TABLE VII

SUMMARY OF THE MERMITHIDS' LIFE HISTORY

MERMITHID	EMERGES FROM		PARASITE OVERWINTERS AS:	PARASITE FIRST EMERGES:	HOST FIRST MOULTS TO 4th INSTAR	PARASITE INFECTS NEW HOSTS	SUMMER EMERGENCE OF HOST
	SPECIES	STAGE					
G. deltensis	*Harnischia* sp.	larvae	early parasite	late May	mid-May	June - Sept.	several months
H. variabilis	*P. simulans* *Harnischia* sp.	adults adults	egg ?	late June	early June mid-May	April	≲ 1 month several months
H. palustris	*Cladotanytarsus* sp.	larvae	advanced parasite	early May	late April	late Spring to September	several months

emerged from P. simulans started to moult (more than three months after emergence, compared to the usual 2.2 ± 0.8 days); she died before completing the moult.

SEX OF THE MERMITHIDS

Multiple parasitism was common in H. variabilis and H. palustris, but was uncommon in G. deltensis. Tables VIII to XI present data on the relationship between the number of parasites per host and the sex of the parasites.

TABLE VIII

SEX OF H. variabilis PARASITIZING P. simulans

PARASITES PER HOST	CASES OBSERVED	FEMALES	MALES
1	47	29[1]	18[1]
2	24	2[2]	46
3	7	0	21
4	3	0	12
5	2	0	10
6	2	0	12

1. $\chi^2 = 2.56$ for 1 male : 1 female
2. Never more than 1 female per host

TABLE IX

SEX OF G. deltensis PARASITIZING Harnischia sp.

PARASITES PER HOST	CASES OBSERVED	FEMALES	MALES
1	162	92[1]	70[1]
2	5	2[2]	8
3	1	0	3

1. χ^2 = 2.98 for 1 male : 1 female
2. Never more than 1 female per host

TABLE X

SEX OF H. palustris PARASITIZING Cladotanytarsus sp.

PARASITES PER HOST	CASES OBSERVED	FEMALES	MALES
1	15	8[1]	7[1]
2	9	0	18
3	6	0	18
4	1	0	4

1. χ^2 = 0.07 for 1 male : 1 female

TABLE XI

SEX OF H. variabilis PARASITIZING Harnischia sp.

PARASITES PER HOST	CASES OBSERVED	FEMALES	MALES	IMMATURE
1	69	47[1]	22[1]	0
2	8	3[2]	10	3
3	3	0	7	2
4	1	0	4	0
5	0	0	0	0
6	2	0	12	0

1. $\chi^2 = 9.10$ for 1 male : 1 female
2. Never more than 1 female per host

Tables VIII - XI show that more females than males are produced when there is one parasite per host. This difference is significant only in Table XI, where the hypothesis that the ratio of males to females is one at an infection of one parasite per host is rejected ($\chi^2_{0.95} = 3.84$) at the 5% level. This hypothesis cannot be rejected at the 5% level for Tables VIII, IX, and X. At infections of 2 parasites per host, the frequency of males increases; 3 parasites per host or more always result in males.

Immature unidentified parasites were found together with almost mature forms of H. variabilis in Harnischia sp. This occurred in 5 cases; 4 from larvae collected on June 17, 1968; one from larvae collected June 26, 1968. These immature worms were early sickle-shaped stages and were probably part of the summer infection of G. deltensis.

Observations or dissections of all the hosts in Tables VIII - XI failed to indicate that the results can be attributed to the differential mortality of female juveniles under conditions of crowding.

PATHOLOGY OF THE INFECTIONS

The percentages of parasitism are indications of mortality in the chironomids, as most hosts died a few hours after the parasites emerged. However, it was common for Harnischia sp. larvae to live several days after G. deltensis emerged (3 lived until 12 days after the parasites' emergence) though none ever pupated. Also, two adult Harnischia sp. lived until 3 days after H. variabilis emerged.

The nematodes emerged from larvae by pushing against the anterior proleg until they broke through (5 cases observed). The host avoided losing an excess of haemolymph by retracting the proleg. The mermithids emerged from pupa and adult hosts through abdominal intersegmental membranes or by breaking open the adult's abdomen.

The fat bodies of the hosts with advanced parasites were depleted, so that the parasites could easily be seen through the transparent cuticle. Although no melanization and encapsulation of nematodes was observed, a few P. simulans and Harnischia sp. with early parasites had haemolymph filled with tiny black particles. None of these hosts survived more than a few days; no host with a large parasite and similar inky haemolymph was ever observed.

The most striking pathological effect was the formation of gonopod and sternite intersexes (Wülker, 1960) in adult Harnischia sp.. Of 42 adults parasitized by H. variabilis, 24 were sternite intersexes and 18 were gonopod types. One other specimen was a normal male with no intersexual characteristics. A Chi-square on the hypothesis that the ratio of the two types of intersexes is one, yields $\chi^2 = 0.86$; the hypothesis can-

not be rejected at the 5% level of significance ($\chi^2_{0.95} = 3.84$).

The gonopod intersexes had male genitalia in a female body. Seventeen had normal genitalia; one had atypical genitalia, with no anal point. All had typical short-haired female fore-tarsi rather than the long-haired male type. Most had typical female antennae, but three (16.7%) had intersexual types (Fig. 30 b, c). All showed modification of the eighth abdominal sternite (Fig. 29).

The sternite intersexes were typical females externally, except for absence of egg guides, a fusion of the ninth apodeme in most, and modification of the eighth abdominal sternite (Fig. 29). One specimen had intersexual antennae of the type with fusion of the two distal segments (Fig. 30 b). One other Harnischia sp. sternite intersex was host for G. deltensis.

No correlation appeared to exist between the degree of modification of the eighth sternites and the number or sex of the parasites.

Fig. 29 Genitalia and eighth abdominal sternites of normal and parasitized *Harnischia* sp. adults (ventral view).

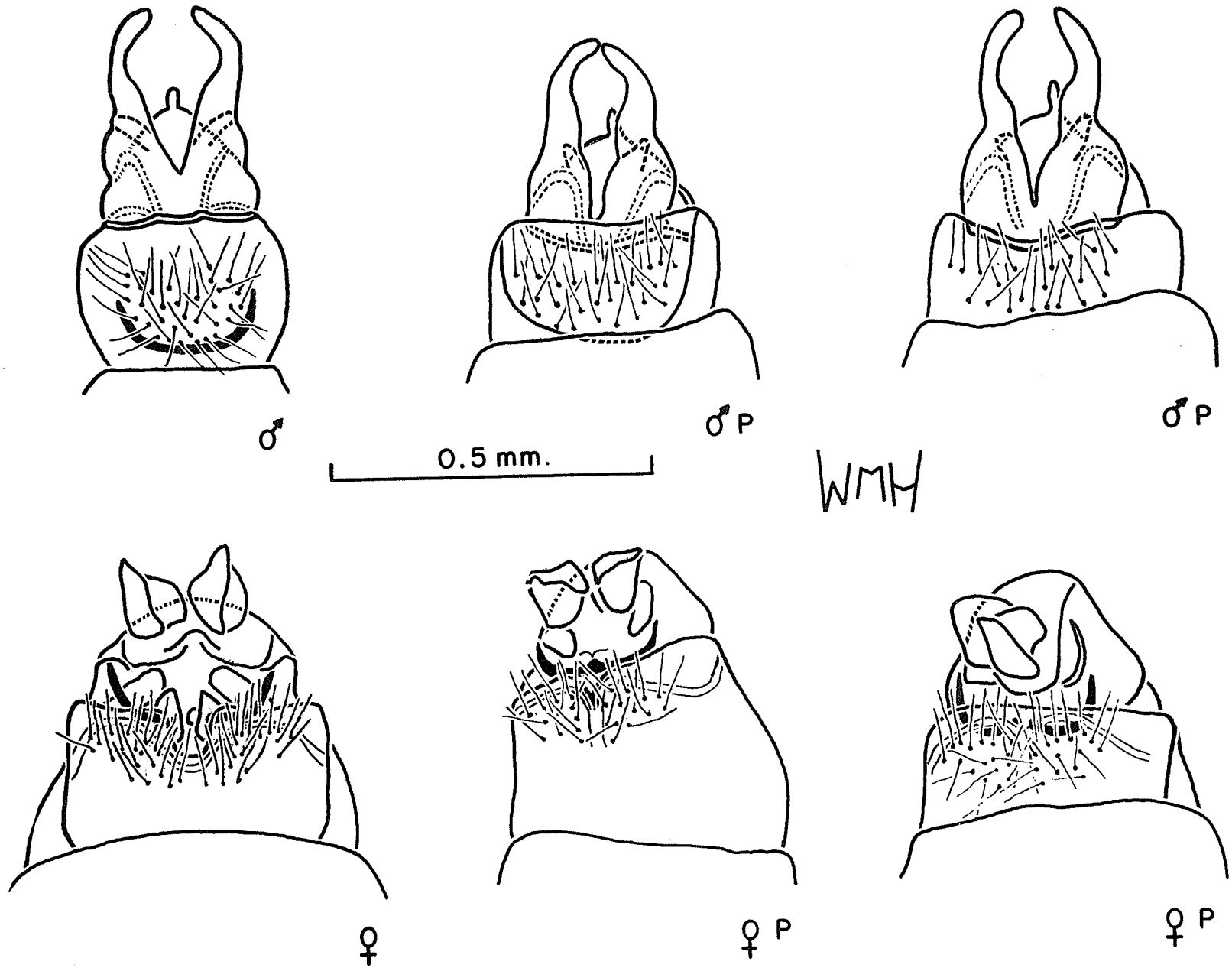

♂
♂P
♂P
0.5 mm.
WMH
♀
♀P
♀P

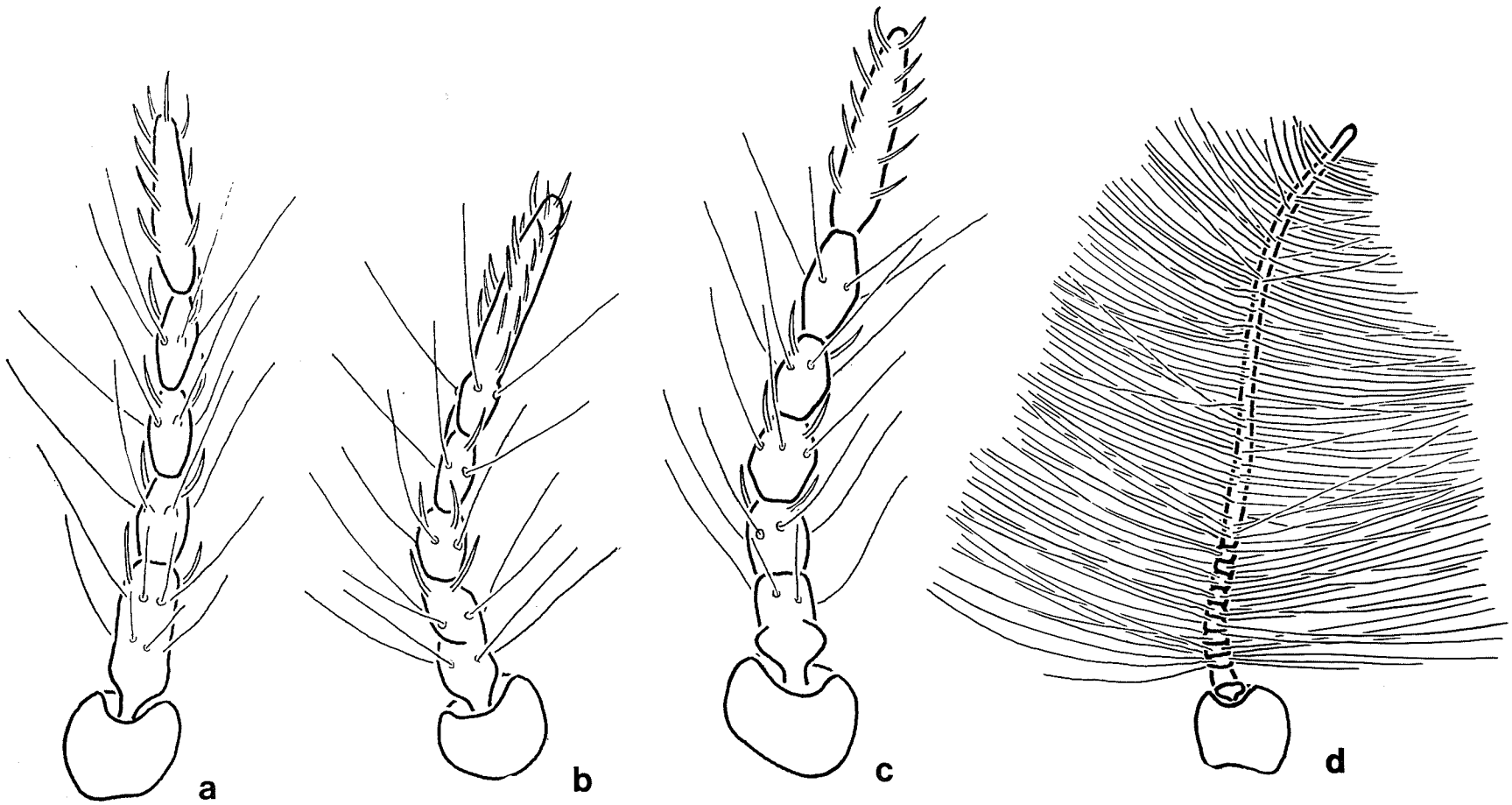
Fig. 30 Antennae of Harnischia sp.

Fig. 30 a Normal female.

Fig. 30 b Parasitized male and female.

Fig. 30 c Parasitized male.

Fig. 30 d Normal male.


a
b
c
d
0.1 mm. a,b,c
0.1 mm. d
WMH

DISCUSSION

SAMPLING ERROR

The diameters of head capsules of dipteran larvae determine whether all the larvae are retained by a sieve or not; only a fraction of the true population is captured if the head capsules can pass through the meshes (Jónasson, 1955). All instars of the three chironomid species could pass through the Nitex monofilament (aperture 0.405 mm.). All instars except fourths, most P. simulans thirds and some Harnischia sp. thirds could pass through the copper screens (smallest aperture 0.177 mm.).

The actual numbers of each instar present per unit area could not be determined because of the error introduced during separation. Data were presented therefore as percentages of each stage present. As the same separation error was carried throughout the sampling period, the percentages of each stage can be compared on a month-to-month basis; the changes in these percentages are therefore reasonably valid indications of changes in population structure.

TAXONOMY

Only Welch and Rubzov (1965) and Rubzov (1967) described morphological variation within mermithid species. Welch and Rubzov (op. cit.) recognized six varieties of G. boophthorae differing in the morphology of the post-parasitic free-living juvenile stages. Adults could not be separated on the basis of known taxonomic characters. The infraspecific categories were accepted as "varieties" (the authors noted that the term is seldom

used in Nematology and is unprotected nomenclatorially) because they were sympatric and could not be considered subspecies, and because at least five of the six varieties were found in the same river and host species and therefore could not be considered local ecological races. When they did occur in other hosts, they still retained their distinct characters. Rubzov (1967) described two varieties of G. virescens.

Hydromermis variabilis consistently occurred as two different forms in two different hosts in the same location. The hypothesis is presented that the two forms are host-induced variants of the same population, and hence one species. The similarity of the life cycles of the two forms and the variation of the same taxonomic characters in G. deltensis from the uncommon host P. simulans support the hypothesis. Cross-infections would be necessary to prove that the differences can be attributed to the different hosts.

MORPHOLOGICAL VARIATION

Appendix I discusses the use of ratios of certain morphometric characteristics in nematode taxonomy and presents data from the present study for the taxonomic validity of the ratio "distance of the vulva from the anterior / total length."

The literature on host-induced morphological variation in nematodes is confined to a few references to plant parasitic forms. Bird and Mai (1967) gave typical results, showing that host species influenced morphometric and allometric characteristics in Trichodorus christiei.

Five of the six varieties of G. boophthorae described by Welch and Rubzov (1965) occurred in the same host, but retained their distinct

characters when they occurred in other hosts. Rubzov (1967) indicated that his two varieties of G. virescens occurred in the same host. The present report is the first record of morphological variation in a mermithid from two different hosts.

The antennal lengths of Lecanium corni Bouché, a scale insect, grown on apricot or Christmasberry, fell into two slightly overlapping normal distributions if placed in separate frequency distributions based on the host, and into a bimodal distribution if the lengths were combined into one frequency distribution (Ebeling, 1938). The lengths of the juvenile tail appendages of H. variabilis have a similar distribution. Ebeling (op. cit.) showed that this was a host-determined variation; transfer of adult L. corni from apricot to Christmasberry provided offspring of the latter type.

Identification of immature mermithids has been hindered by a lack of definite morphological characters (Welch, 1962). Welch (op. cit.) described three new mermithids from black flies and noted that the shape of larval tail appendages is one character of immature mermithids that may aid their identification. As the length of the tail appendages of both G. deltensis and H. variabilis was influenced by the host, its value as a taxonomic character may be limited to specimens obtained from previously-recorded hosts.

The position of the mouth is important in mermithid taxonomy; it was the only character known to separate Gastromermis from Hydromermis (Polozhentsev and Artyukhovskii, 1959; Johnson, 1965). The degree of ventral displacement, together with other characters, was used to separate species in the genus Gastromermis (Polozhentsev and Artyukhovskii,

1959; Johnson, 1965). As the position of the mouth varied in G. deltensis, H. variabilis, and H. palustris, emphasis on mouth position may not be justified.

Variation in the spicule shape of H. variabilis and in the shape of the terminus of H. palustris is noteworthy, as both are important in identifying mermithid species. The differences in spicule shape may be attributed to host differences. However, the variation in the shape of the terminus of H. palustris occurred in worms which emerged from the type host species. The reason for this variation is unknown.

PENETRATION OF INFECTIVE JUVENILES

Observations on the artificial infections of Harnischia sp. larvae with G. deltensis juveniles are similar to those reported previously. The locating of the host did not seem to be a directed behaviour (Johnson, 1955; Wülker, 1961). The worms entered the host by penetrating through the cuticle (Comas, 1927; Svabenik, 1928; Wülker, 1961, 1965). The infective juveniles penetrated a host larva of any stage (Svabenik, 1928; Wülker, 1961); Comas (1927) had reported that penetration required an early instar with a soft cuticle (immediately post-moult). The juveniles had a limited period of infectivity (Comas, 1927; Svabenik, 1928; Johnson, 1955; and Wülker, 1961). Wülker (1965) described the paralysis of hosts by infective juveniles, probably by injection of a substance before penetrating the cuticle. This may apply to G. deltensis, whose juveniles were observed to touch a larva, halt for a moment with their anterior end against the cuticle, and then swim away.

NUMBER OF PARASITES PER HOST

Frequency distributions of the number of mermithids per host were given and analyzed by Sugiyama (1956), Welch (1960 a), and Wülker (1961); departures from a chance distribution were shown in all cases (Welch, 1963). Our results for H. palustris parasitic in Cladotanytarsus sp. are similar. These results suggest that the nematode distribution was contagious and was not continuous throughout an area of host distribution (Welch, 1963). Hence, exposure or susceptibility to parasitism was uneven within the insect population.

The frequency distribution of the number of G. deltensis in Harnischia sp. ($n = 219$) is the first to fit a Poisson distribution. This suggests that G. deltensis was uniformly distributed in the study area and that each Harnischia sp. larva was equally susceptible to attack.

SYNCHRONIZATION OF LIFE CYCLES

As the infective juveniles have a limited period of infectivity, one would expect the life cycles of the parasite and host to be closely synchronized. Such was the case for Hydromermis churchillensis and its mosquito host Aedes communis DeG.; both had one generation per year and overwintered as eggs. The coincidence of life cycles at the time of nematode attack on young mosquito larvae was critical for parasitism to occur (Welch, 1960 a). Gastromermis boophthorae had one generation per year and probably overwintered as an egg, but was not restricted to a specific blackfly host; the juveniles were found from the time of appearance of the host larvae in May to their disappearance in late August (Welch and Rubzov, 1965). The life cycles of G. viridis and Isomermis wisconsinensis were

closely synchronized with that of Simulium vittatum; in overwintering larval populations of S. vittatum, G. viridis was present; in small, temporary or permanent creeks harbouring S. vittatum parasitized by I. wisconsinensis, both parasite and host overwintered in the egg stage (Anderson and DeFoliart, 1962). The life cycles of H. contorta and Chironomus plumosus, which overwinters as a larva and is generally thought to have one generation per year, were synchronized. The parasite had two generations per year, and infected new hosts in November and February. However, from mid-May to mid-November (the host emerged and oviposited during September) it was free-living (Johnson, 1955). The life cycles of G. deltensis, H. variabilis and H. palustris were also synchronized with those of their principal hosts (Table VII).

HOST SPECIFICITY

Mermithid specificity to certain hosts may be physiological (arising from adaptation and immunological processes) or ecological (concurrence of the hosts and parasites at a time when the mermithids are seeking hosts) (Welch, 1960 c). Although Wülker (1961, 1963 a) and Karunakaran (1966) indicated that some mermithids may be restricted to certain chironomid hosts, it was not clear if the specificity was ecological or physiological. Johnson (1955) observed that H. contorta occasionally infected Chironomus riparius and Cryptochironomus stylifera, but never matured in these unusual hosts. In this study, four G. deltensis females emerged from P. simulans larvae but didn't moult to maturity.

Some of the evidence in the present study favours an hypothesis of ecological specificity, which agrees with the scant evidence from mermi-

thid parasitism of other dipterans. Rubzov (1964) stated that some mermithids at least do not exhibit strict host specificity; the different invasion patterns in various host species during a particular season were explained by the different dates of appearance of the various species of mermithids concerned. Welch (1960 a) cited two Aedes species besides A. communis as host records for H. churchillensis and noted that three species of Aedes larvae found in the same pools as infected A. communis were not infected. Similarly, six varieties of G. boophthorae did not require specific blackfly hosts, but were never found in two blackfly species which coexisted in large numbers with the parasitized species (Welch and Rubzov, 1965).

SEX DETERMINATION AND EVOLUTION IN THE MERMITHOIDEA

The apparent control of the sexual differentiation of mermithid juveniles by the environment is a well-documented phenomenon. The sex ratio seems to be a function of the number of parasites per host; low numbers result in the production of mainly females, while high numbers result in more males. The present study verifies this phenomenon. Although more females than males were produced when there was one parasite per host, the difference did not depart significantly from a 1:1 ratio (except for H. variabilis parasitic in Harnischia sp.). This suggests that the infective juveniles of G. deltensis, H. variabilis, and H. palustris have their sex genetically determined, but the sex genes can be influenced by the environment when more than one parasite occurs in a host.

Environmental sex determination in the Mermithidae may be of two

types. In the first type, low numbers of parasites per host usually result in females, while higher numbers result in mainly males with one or two females usually present. This was documented for H. contorta (Caullery and Comas, 1928; Johnson, 1955), P. contorta (= H. contorta) (Parenti, 1965 b) and Pseudomermis hagmeieri (Couturier, 1963). Significantly, Parenti (1965 a) showed that the sex of P. contorta juveniles could be influenced after they penetrated a host; in the laboratory females which penetrated first tended to influence juveniles penetrating later in the male direction, and males which penetrated first tended to influence later penetrating juveniles in the female direction. In the second type, a high number of parasites per host results in exclusively male parasites, as reported for Mermis subnigrescens (Cobb, Steiner, and Christie, 1927; Christie, 1929), Hexamermis sp. (Couturier, 1950), and the species in the present study.

Little is known about sex determination in the Tetradonematidae, the second family of the Mermithoidea. Hungerford (1919) noted that 2 to 20 specimens of Tetradonema plicans Cobb, 1919 of both sexes were found per host. In a table of 11 hosts, he showed 2 to 7 parasites per host, 10 of which had one female parasite each; one host had 2 females and 5 males. He also observed that the newly-hatched juveniles were of two types in terms of length and shape. Ferris and Ferris (1966), in the second record of T. plicans, noted that 1 to 12 worms per host occurred, and that all juveniles were of one type. Corethrellonema grandispiculosum Nickle, 1969 and Aproctonema chapmani Nickle, 1969 penetrated into their hosts as infective juveniles; usually one female and 2 or 3 male C. grandispiculosum were found in one host, though one dissection yielded 13 males and no fe-

males; one to three female and one to three male A. chapmani were usually found per host (Nickle, 1969).

Most tetradonematids are fertilized before leaving the host; parthenogenesis has not been recorded. It seems unlikely that most hosts should contain at least one female and usually several males if the sex of the juveniles is fixed genetically. It is more likely that, as in the Mermithidae, the sex of the juveniles can be altered depending on conditions occurring after penetration into a host. Possibly, the first one or two juveniles to enter a host usually develop as females, while the sex of those penetrating after is influenced in the male direction. This would insure that both females and males occurred in hosts harbouring several parasites, a condition necessary for fertilization of the female.

Cameron (1956) stated that the Mermithoidea can be arranged in a sequence according to the details of individuals' biology. First in the sequence are the Tetradonematidae, whose adults usually are found within the host. Tetradonema plicans may oviposit in the host (Hungerford, 1919), though the gravid female usually leaves the host before ovipositing (Ferris and Ferris, 1966). Most other tetradonematids also leave the host before ovipositing (Keilin and Robinson, 1933; Nickle, 1969) though Rubzov (1966) described some tetradonematids whose adults were not present in their hosts. The mermithid H. contorta is next in the sequence. It moults in the host but emerges before fertilization occurs (Kohn, 1905). Finally, the majority of mermithids complete their final moult after emerging and continue their life cycle as free-living stages.

This apparent sequence in life cycle types, together with the data on sex determination, tempts speculation on the evolution of sex determination

within the Mermithoidea. The ancestor of the group may have been a form with a definitive host (where the sexual life of the parasite occurs) as is the case for the majority of parasitic nematodes. As the host mass was probably not much larger than the parasite mass (the usual condition for mermithid parasites), a mechanism for insuring that the few parasites that could be harboured were of opposite sexes may have evolved. This mechanism could have been based on the ability of the first one or two juveniles to enter the host to develop as females, and others penetrating after to develop as males. Such a mechanism may have depended on nutritive limitation (a favoured hypothesis to explain environmental sex determination in the Mermithidae) as male members of the Mermithoidea are smaller than females.

The proposed ancestor may have given rise to the T. plicans type of parasite which mates and sometimes lays its eggs in the host, but usually leaves before oviposition. Most of the present-day tetradonematids probably evolved from the T. plicans type. They seem to show the type of sex determining mechanism proposed for their ancestors.

Tetradonematids similar to the present-day types may have been the ancestors for two lines of further evolution. The first line contains forms of the type described by Rubzov (1966), which are not found as adults in the host. The second line gave rise to the mermithids, of which H. contorta's life history is similar to the basic type, as it moults before emerging from its host. Sex determination in H. contorta is also similar to the proposed ancestral type. These lines may have evolved during times of low parasite density compared to that of the host. During these times, many hosts would contain one parasite, and the necessity of

leaving the host to find a mate becomes imperative.

The majority of mermithids moult after leaving their host. These forms probably evolved through adaptation from forms having the H. contorta type of life history. Sex determination in these forms is similar to that of H. contorta, as in Pseudomermis hagmeieri, or is a modification of this type, as in M. subnigrescens.

Cameron's (1956) sequence of the biology of the Mermithoidea implies that the group's ancestor was a form with a definitive host. The majority of nematodes with definitive hosts parasitize vertebrates; the mass of the parasites compared to that of the hosts is small. However, the mass of members of the Mermithoidea is similar to that of their invertebrate hosts; this similarity increases the probability of the mature parasite killing the host. It therefore seems improbable that the Mermithoidea evolved from forms which remained in the host to mature, mate, and oviposit.

Cameron's (1956) sequence may also be criticized because it doesn't explain how the parasitic mode of life evolved. Current hypotheses favour the evolution of parasitic nematodes from terrestrial free-living groups (Osche, 1963; Inglis, 1965). Osche (op. cit.) stated that some free-living Rhabditida live on saprobiotic substrates (eg. carrion, feces). As the environments are temporary and far apart, the necessity for transport to new substrates frequently recurs. When the substrate is exhausted, many rhabditids form a resistant third larval stage ("dauerlarva") which contacts other animals (mainly insects) and is carried to a new substrate. Osche (1963) listed four steps in life histories which lead from this contact to parasitism and gave examples of rhabditid species at each step: (i) only certain insect species are used as carriers; (ii) the dauer-

larva penetrates into the insect and is protected from desiccation but takes no nourishment from the transport host; (iii) the dauerlarva penetrates into the host, acquires nourishment, and develops to the fourth stage before it leaves and completes development externally; and (iv) the larva grows and matures within the host.

The Mermithoidea are thought to be related to the Dorylaimata (Steiner, 1917, 1929; Filipjev, 1934 a; Filipjev and Schuurmans Stekhoven, 1941) which are principally free-living in soil and fresh water. If we assume a free-living Dorylaim-type ancestor for the Mermithoidea, and the various stages of association proposed for rhabditids (Osche, 1963), the most primitive Mermithoidea are those that are parasitic as juveniles and free-living as adults. Hence, evolution within the Mermithoidea would produce a sequence in their biology which is the exact reverse of that given by Cameron (1956).

The critical factor for the evolution of the mermithoid type of sex determination in this scheme may have been the similarity in the masses of the parasites and hosts. As the hosts could harbour few parasites, a large increase in the number of nematodes would result in the destruction of the host population, and hence of the parasites. Perhapes a selection pressure for a mechanism to control parasite numbers resulted in delayed sex determination. If an excess of parasites resulted in mainly males, the parasite biomass would decrease in the next generation. A production of an excess of males may rely upon the following.

The early parasitic stages of mermithids are similar in size to infective juveniles. For a time after penetration, these early stages grow little, probably because they must first physiologically adapt from a free-

living to a parasitic existence. Also, it is probable that the parasites' growth is synchronized with that of the host. Thus, these early small stages probably start growing at about the same time. As growth is rapid during the later stages of development (Welch, 1965), the parasites may compete for nutritional substances. If sex is determined during this period of rapid growth, and if development of females depends on a specific amount of a certain substance, an excess of parasites would result in few, if any, females. Such a competition for limited food would also result in a selection pressure for smaller individuals in conditions of crowding, and may account for the smaller size of male mermithids compared to females.

Once the mermithid sex determination mechanism evolved, a modification may have occurred whereby at least one of the parasites in each host was a female (eg. H. contorta). In conditions of crowding, continuation of the species is assured, but production of new juveniles is limited to those produced by the one or two females in each host. This situation allows the final adaptation in the Mermithoidea; some tetradonematids mature and mate within the host. In these cases, one would expect a selection pressure for further reduction in the size of mature males so that the necessary presence of males and females does not cause severe crowding and death of the host. Significantly, tetradonematid males are 1/3 - 1/7 the length of mature females.

CHIRONOMID INTERSEXES

The intersexuality of Harnischia sp. adults parasitized by H. variabilis is similar to the intersexuality of other chironomids summarized by Wülker (1961, 1964). Wülker (1961) and Götz (1964) compared normal and

intersexual eighth sternites; those of gonopod intersexes commonly showed a slight to marked tendency towards pairing of the bristle-field (female-like), while those of sternite intersexes showed a less distinct pairing of the bristle-fields, absence of the genital opening and a tendency towards fusion or deformation of the bracket-like ninth sternite. The Harnischia sp. gonopod intersexes showed modification of the eighth sternite, but no tendency towards pairing of the bristle-field. The eighth sternites of the sternite intersexes were similar to those drawn by Wülker (op. cit.) and Götz (op. cit.).

Intersex antennae of parasitized chironomids were drawn by Wülker (1961), Roback (1963) and Götz (1964) and include all types intermediate between normal male and female types. Their drawings include the types encountered in Harnischia sp. intersexes.

Wülker (1960) found close to a 1:1 ratio of sternite to gonopod Chironomus anthracinus intersexes. In 1961, he showed that the gonopod intersexes were genetically males, while the sternite intersexes were females. As the ratio of sternite to gonopod intersexes of Harnischia sp. was also 1:1, it is probable that the gonopod intersexes were genetically males, while the sternite intersexes were genetically females.

Although the genesis of damage has been traced to a total or partial suppression of the genital imaginal discs (Wülker, 1964), the mechanism remains unknown.

CONCLUSIONS

Three new species of mermithids, Hydromermis palustris, Hydromermis variabilis and Gastromermis deltensis were found. Studies showed that taxonomic characteristics such as the length of the juvenile tail appendage, the ventral displacement of the mouth, and the shape of the spicule, were influenced by the species of the host.

The life cycles of the parasites differed in several respects. H. palustris overwintered as an advanced parasitic stage and G. deltensis as an early parasitic stage, in overwintering larvae; H. variabilis probably overwintered in the egg stage. H. palustris first emerged in early May, G. deltensis in late May, and H. variabilis in late June and early July. H. palustris and G. deltensis emerged from fourth instar larvae until late August; H. variabilis emerged from adults over a period of about two weeks.

The life cycle of each parasite was synchronized with that of the principal host. H. palustris parasitized Cladotanytarsus sp. whose overwintering larval population was the first to moult in the spring. G. deltensis parasitized Harnischia sp. which was the second to moult in the spring. H. variabilis parasitized Polypedilum simulans which was the last of the three chironomids to moult. H. variabilis also parasitized Harnischia sp., but emerged at the same time as those parasitizing P. simulans. Cladotanytarsus sp. and Harnischia sp. emerged and oviposited during most of the summer, while P. simulans appeared to have a limited period of summer emergence and oviposition.

The sex of the parasites was correlated with their number per host. More females than males occurred for one parasite per host, though a significant departure from a 1:1 ratio was found only for H. variabilis parasitic in Harnischia sp.. Males usually occurred when there were two or more parasites in a host.

Intersexes were produced in Harnischia sp. adults parasitized by H. variabilis. The gonopod intersexes were probably genetically males, while the sternite intersexes were probably genetically females.

REFERENCES

Andersen, F. S. 1946. East Greenland lakes as habitats for chironomid larvae. Studies on the systematics and biology of Chironomidae II. Vidensk. Meddelelser om Gronland (Bd.) 100: 3-65.

Anderson, J. R., and DeFoliart, G. R. 1962. Nematode parasitism of black fly (Diptera: Simuliidae) larvae in Wisconsin. Ann. Entomol. Soc. Am. 55: 542-546.

Bird, G. W., and Mai, W. F. 1967. Morphometric and allometric variations in Trichodorus christiei. Nematologica 13: 617-632.

Branch, H. E. 1923. The life history of Chironomus cristatus Fabr. with description of the species. J. N. Y. Ent. Soc. 31: 15-30.

Braun, M. 1883. Die thierischen Parasiten des Menschen nebst einer Anleitung zur praktischen Beschäftigung mit der Helminthologie für Studierende und Aerzte. Wurzburg. 233 pp.

Brust, R. A. 1966. Gynandromorphs and intersexes in mosquitoes (Diptera: Culicidae). Can. J. Zool. 44: 911-921.

Cameron, T. W. M. 1956. Parasites and parasitism. Wiley, New York, 322 pp.

Caullery, M., and Comas M. 1928. Le déterminisme du sexe chez un nématode (Paramermis contorta) parasite de larves de chironomes. Compt. Rend. 186: 646-648.

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

Cobb, N. A. 1919. Tetradonema plicans nov. gen. et spec. representing a new family, Tetradonematidae, as now found parasitic in larvae of the midge-insect Sciara coprophila Lintner. J. Parasitol. 5: 176-185.

_____, Steiner, G., and Christie, J. R. 1927. When and how does sex arise? U. S. Dep. Agr. Official Record 6: 6.

Coman, D. 1953. Mermithide freatice. In: Fauna Republicii Populare Rômane. Acad. Rep. Populare Romine Studii Cercetari Stiint. 4: 123-152.

Comas, M. 1927. Sur le mode de penetration de Paramermis contorta. V. Linst. dans la larve de Chironomus thummi Kief. Compt. Rend. Soc. Biol. 96: 673-675.

Corti, E. 1902. Di un nuovo nematode parassita in larva di Chironomus. R. Inst. Lomb. Sci. e Lett. Rend. 2 s. 35: 105-113.

Courtney, W. D., Polley, D., and Miller, U. L. 1955. TAF an improved fixative in nematode technique. Plant Disease Reptr. 39: 570-571.

Couturier, A. 1950. Biologie d'un Hexamermis (Nematoda: Mermithidae) parasite des insectes défoliateurs de l'osier. Ann. Epiphyties 1: 13-37.

_____ 1963. Recherches sur des Mermithidae nématodes parasites du hanneton commun (Melolontha melolontha L.). Ann. Epiphyties 14: 203-267.

Dujardin, F. 1842. Mémoire sur la structure anatomique des Gordius et d'un autre Hélminthe, le Mermis, qu'on a confondu avec eux. Ann. Sci. Nat. 2nd series: 17-18; 129-142.

_____ 1845. Histoire naturelle des hélminthes ou vers intestinaux. Paris. 654 pp.

Dyar, H. G. 1890. The number of moults of lepidopterous larvae. Psyche 5: 420-422.

Ebeling, W. 1938. Host-determined morphological variation in Lecanium corni. Hilgardia 11: 613-631.

Englemann, W., and Shappirio, D. G. 1965. Photoperiodic control of the maintenance and termination of larval diapause in Chironomus tentans. Nature 207: 548-549.

Fellton, H. L. 1940. Control of aquatic midges with notes on the biology of certain species. J. Econ. Entomol. 33: 252-264.

Ferris, J. M., and Ferris, V. R. 1966. Observations on Tetradonema plicans, an entomoparasitic nematode, with a key to the genera of the family Tetradonematidae (Nematoda: Trichosyringida). Ann. Entomol. Soc. Am. 59: 964-971.

Filipjev, I. N. 1934 a. The classification of the free-living nematodes and their relation to the parasitic nematodes. Smithsonian Inst. Misc. Collections 89: 1-63.

_____ 1934 b. (Harmful and useful nematodes in rural economy). Moscow and Leningrad. 440 pp. (In Russian).

_____, and Schuurmans Stekhoven, J. H. Jr. 1941. A manual of agricultural helminthology. E. J. Brill, Leiden. 878 pp.

Ford, J. B. 1959. A study of larval growth, the number of instars, and sexual differentiation in the Chironomidae (Diptera). Proc. Roy. Entomol. Soc. London. (A). 34: 151-160.

Goodey, J. B. 1963. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries, and Food. Tech. Bull. No. 2. London. 72 pp.

Götz, P. 1964. Der Einfluss unterschiedlicher Befallsbedingungen auf die mermithogene Intersexualität von Chironomus (Dipt.) Z. Parasitenk. 24: 484-545.

Gupta, S. K. Das. 1964. Mermithid infections in two nematoceran insects. Current Sci. 33: 55.

Hagmeier, A. 1912. Beiträge zur Kenntnis der Mermithiden. I. Biologische Notizen und systematische Beschreibung einiger alter und neurer Arten. Zool. Jahrb., Abt. Syst. 32: 521-612.

Hilsenhoff, W. L. 1966. The biology of Chironomus plumosus (Diptera: Chironomidae) in Lake Winnebago, Wisconsin. Ann. Entomol. Soc. Am. 59: 465-473.

_____ 1967. Ecology and population dynamics of Chironomus plumosus (Diptera: Chironomidae) in Lake Winnebago, Wisconsin. Ann. Entomol. Soc. Am. 60: 1183-1194.

Hochbaum, H. A. 1944. The canvasback on a prairie marsh (revised). Am. Wildl. Inst. Washington, D. C. 182 pp.

Hungerford, H. B. 1919. Biological notes on Tetradonema plicans Cobb, a nematode parasite of Sciara coprophila Lintner. J. Parasitol. 5: 186-192.

Inglis, W. G. 1965. Patterns of evolution in parasitic nematodes. In: Evolution of parasites. Edited by A. E. R. Taylor. Blackwell Scientific Publications, Oxford. pp. 79-124.

Johnson, A. A. 1955. Life history studies on Hydromermis contorta (Kohn) a nematode parasite of Chironomus plumosus L. Doctoral Thesis, Univ. Ill. Urbana, Illinois.

_____ 1963. Octomyomermis itascensis gen. et sp. nov. (Nematoda: Mermithidae) a parasite of Chironomus plumosus L. Trans. Am. Microscop. Soc. 82: 237-241.

_____ 1965. Hydromermis itascensis n. sp. (Nematoda: Mermithidae) a parasite of Glyptotendipes lobiferus Say. (Diptera: Chironomidae) and the taxonomic status of Gastromermis. J. Parasitol. 51: 53-56.

Jónasson, P. M. 1955. The efficiency of sieving techniques for sampling freshwater bottom fauna. Oikos 6: 183-207.

_____ 1965. Factors determining population size of Chironomus anthracinus in Lake Esrom. Mitt. Intern. Ver. Limnol. 13: 139-162.

Kaburaki, T., and Iyatomi, K. 1933. Notes on sex in Amphimermis zuimushi Kab. et Im. Proc. Imper. Acad. Tokyo 9: 333-336.

Karunakaran, L. 1966. Parasitism of Chironomus costatus Joh. (Diptera: Chironomidae) by a mermithid. Nematologica 12: 172-174.

Keilin, D., and Robinson, V. C. 1933. On the morphology and life history of Aproctonema entomophagum Keilin, a nematode parasite in the larvae of Sciara pullula Winn. (Diptera: Nematocera). Parasitology 25: 285-295.

Kohn, F. G. 1905. Einiges über Paramermis contorta (v. Linstow) = Mermis contorta v. Linstow. Arb. Zool. Inst. Univ. Wien 15: 213-256.

Krall, E. 1959. (Hairworms and mermithids-parasites of insects). Eesti Loodus 4: 216-220. (In Estonian).

Kreis, H. A. 1924. Contribution à la connaissance des nématodes libres du Surinam (Guyane hollandaise). Ann. Biol. Lacustre 13: 123-136.

von Linstow, O. F. B. 1889. Bemerkungen über Mermis. Nachtrug zu "Ueber die Entwicklungsgeschichte und die Anatomie von Gordius tolosanus." Arch. Mikroscop. Anat. u. Entwicklungsmech. 34: 390-396.

_____ 1898. Das genus Mermis. Arch. Mikroscop. Anat. 53: 149-168.

MacDonald, W. W. 1956. Observations on the biology of Chaoborids and Chironomids in Lake Victoria and on the feeding habits of the "Elephant-snout fish" (Mormyrus kannume Forsk.). J. Anim. Ecol. 25: 36-53.

Micoletzky, H. 1923. Mermithiden und freilebende Nematoden aus dem Grundschlamm des Attersees in Oberösterreich. Zool. Anz. 55: 240-245.

Nickle, W. R. 1969. Corethrellonema grandispiculosum n. gen., n. sp. and Aproctonema chapmani n. sp. (Nematoda: Tetradonematidae), parasites of the Dipterous insect genera, Corethrella and Culicoides in Louisiana. J. Nematology 1: 49-54.

de Oliveira, S. F., and Lent, H. 1962. Preliminary notes on chironomid (Diptera) intersexuality induced by Agamomermis (Nematoda). Rev. Brasil. Biol. 22: 357-365.

Osche, G. 1963. Morphological, biological and ecological considerations in the phylogeny of parasitic nematodes. In: The lower Metazoa: Comparative biology and phylogeny. Edited by: E. C. Dougherty, Z. N. Brown, E. D. Hanson, and W. D. Hartman. Univ. of California Press, Berkeley. pp. 283-302.

Parenti, U. 1962 a. Richerche sulla sessualità dei Mermitidi. I. Rapporte sessi in una popolazione di Paramermis contorta. La variabilità sessuale in due popolazioni di P. contorta Linst. di Modena e Parigi. Arch. Zool. Ital. 47: 209-224.

_____ 1962 b. Richerche sulla sessualità dei Mermitidi. II. Infestazione sperimentale di Paramermis contorta in Chironomus tentans. Boll. di Zool. 29: 453-462.

_____ 1962 c. Fecondazione e corredo cromosomico di Paramermis contorta parassita di Chironomus tentans. Accad. Nazion. Lincei 32: 699-702.

_____ 1965 a. Male and female influence of adult individuals on undifferentiated larvae of the parasitic nematode Paramermis contorta. Nature 207: 1105-1106.

_____ 1965 b. Razze sessuali di Paramermis contorta. Monitore Zool. Ital. 73: 147-155.

_____ 1965 c. Genotipi sessuali multipli in Paramermis contorta. Boll. di Zool. 32: 1051-1057.

Phelps, R. J., and De Foliart, G. R. 1964. Nematode parasitism of Simuliidae. Wisconsin, Univ. Agr. Expt. Sta. Res. Bull. 245, 78 pp.

Ping, C. 1917. Observations on Chironomus decorus Johannsen. Can. Entomologist 49: 418-426.

Poinar, G. O. 1964. A new nematode, Orthomermis oedobrahchus gen. n. sp. n. (Mermithidae) parasitizing Smittia larvae (Chironomidae) in England. Nematologica 10: 501-506.

_____, and Gyrisco, G. G. 1962. A new mermithid parasite of the alfalfa weevil Hypera postica (Gyllenhal). J. Insect Pathol. 4: 201-206.

Polozhentsev, P. A., and Artyukhovskii, A. K. 1959. (On the systematics of the family Mermithidae Braun, 1883 (Dorylaimata, Enoplida)). Zool. Zh. 38: 816-828. (In Russian).

Rempel, J. G. 1936. The life history and morphology of Chironomus hyperboreus J. Biol. Bd. Can. 2: 209-221.

_____ 1940. Intersexuality in Chironomidae induced by nematode parasitism. J. Exp. Zool. 84: 261-289.

_____, Naylor, J. M., Rothfels, K., and Ottonen, B. 1962. The sex chromosome constitution of chironomid intersexes parasitized by nematodes. Can. J. Genet. Cytol. 4: 92-96.

Roback, S. 1963. Nematode parasites of Pelopiinae (Diptera: Tendipedidae). Entomol. News. 74: 143-149.

Rubzov, I. A. 1964. Observations on mermithid nematodes parasitizing blackflies WHO/EBL/19. 6 pp.

_____ 1966. (Nematodes of the family Tetradonematidae parasitizing in members of the family Simuliidae (Diptera)). Helminthologica 7: 165-198. (In Russian).

_____ 1967. (Mermithidae (Nematoda) endoparasites of blackflies (Diptera, Simuliidae). II. New species of genus Gastromermis Micoletzky, 1923). Proc. Zool. Inst. Acad. Sci. U. S. S. R. 63: 59-92. (In Russian).

Sadler, W. O. 1935. Biology of the midge Chironomus tentans Fabr. and methods for its propagation. Cornell Univ. Agr. Exp. Sta. Mem. 173: 1-25.

Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67-69.

Smith, A. U. 1958. The resistance of animals to cooling and freezing. Biol. Rev. Cambridge Phil. Soc. 33: 197-253.

Smith, W. W., and Perry, V. G. 1967. Intersexes in Culicoides spp. caused by mermithid parasitism in Florida. J. Econ. Entomol. 60: 1025-1027.

Steiner, G. 1917. Über die Verwandschaftensverhältnisse und die systematische Stellung der Mermithiden. Zool. Anz. 48: 263-267.

_____ 1918. Studien an Nematoden aus der Niederelbe. I. Teil, Mermithiden. Mitt. Zool. Museum Hamburg 35: 75-99.

_____ 1919. Die von A. Monard gesammelten Nematoden der Tiefenfauna des Neuenburgersees. Bull. Soc. Neuchâtel Sci. Nat. 43: 142-240.

Steiner, G. 1929. On a collection of mermithids from the basin of the Volga River. Zool. Jahrb. Jena Abt. Syst. 57: 303-328.

_____ 1932. Die arktischen Mermithiden, Gordioiden, und Nectonematoiden. Fauna Arctica (Roemer u. Schaudinn) 6: 161-174.

Strelkov, A. 1964 a. (On the biology of a new mermithid from midges of river reservoir). Vestn. Leningr. Univ. No. 3, Ser. Biol. 1: 55-69. (In Russian).

_____ 1964 b. (Intersexes in Tendipedidae arising as a result of their parasitization by mermithid worms). Dokl. Akad. Nauk. S. S. S. R. 158: 745-748. (In Russian).

Sublette, J. E. 1960. Chironomid midges of California I. Chironominae, exclusive of Tanytarsini (= Calopsectrini). Proc. U. S. Nat. Mus. 112: 197-226.

Sugiyama, K. 1956. Effects of the parasitism by a nematode on a grasshopper Oxya japonica II. On the distribution pattern of a nematode parasitic in grasshoppers. Dobutsugaku Zasshi 65: 386-391 (Japanese text; English summary).

Svabenik, J. 1928. Aus dem Leben der Paramermis contorta (v. Linstow). Zool. Anz. 77: 259-267.

Thienemann, A. 1954. Chironomus, Leben, Verbreitung und wirtsschaftliche Bedeutung der Chironomiden. Die Binnegewässer. Band 20. Stuttgart, E. Nägele.

Townes, H. K. 1945. The Nearctic species of Tendipedini (Diptera: Tendipedidae (= Chironomidae)). Am. Midland Naturalist 34: 1-206.

Vandel, A. 1932. Le sexe des parasites, dépend-il du nombre d'individus renfermés dans le même hôte? Soc. Ent. France, Livre du Centenair: 245-252.

Walker, J. M. 1959. Vegetation studies on the Delta Marsh, Delta, Manitoba. M. Sc. Thesis, University of Manitoba, Winnipeg.

_____ 1966. Vegetation changes with falling water levels in the Delta Marsh, Manitoba. Doctoral Thesis, University of Manitoba, Winnipeg.

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

Welch, H. E. 1960 b. The taxonomy of Hydromermis contorta (Linstow, 1889) Hagmeier, 1912, and its synonym Hydromermis rivicola Corti, 1902 (Nematoda: Mermithidae) with notes on some diagnostic characters. Nematologica 5: 92-97.

_____ 1960 c. Potentialities of nematodes in the biological control of insects of medical importance. In. Tech. Rept. Biol. Control Insects Med. Importance, 67-75.

_____ 1962. New species of Gastromermis, Isomermis, and Mesomermis from blackfly larvae. Ann. Entomol. Soc. Am. 55: 535-542.

_____ 1963. Nematode infections. Chapt. 11 In: Insect Pathology. Edited by: E. A. Steinhaus. Academic Press, New York. pp. 363-392.

_____ 1965. Entomophilic nematodes. Ann. Rev. Entomol. 10: 275-302.

_____, and Rubzov, I. A. 1965. Mermithids (Nematoda: Mermithidae) parasitic in blackflies (Insecta: Simuliidae). I. Taxonomy and bionomics of Gastromermis boophthorae sp. n. Can. Entomologist 97: 581-596.

Wirth, W. W., and Stone, A. 1963. Aquatic Diptera. Chapt. 14 In: Aquatic Insects of California. Edited by: R. L. Usinger, Univ. of California Press, Berkeley. pp. 372-482.

Wülker, W. 1958. Parasitäre Intersexualität bei Chironomiden des Schluchsees. Gewässer und Abwässer 20: 61-67.

_____ 1960. Neue Ergebnisse zur parasitären Intersexualität der Chironomiden (Dipt.). Naturwissenschaften 47: 21-22.

_____ 1961. Untersuchungen über die Intersexualität der Chironomiden (Dipt.) nach Paramermis--Infektion. Archiv. Hydrobiologie Supplement 25: 127-181.

_____ 1963 a. Prospects for biological control of pest Chironomidae in the Sudan. WHO/EBL 11, 23 pp.

_____ 1963 b. Parasitologische und biochemische Verwandtschaft in der Gattung Chironomus (Dipt.). Naturwissenschaften 50: 49-50.

_____ 1964. Parasite-induced changes of internal and external sex characters in insects. Exp. Parasitol. 15: 561-597.

_____ 1965. Der Mechanismus des Eindringens parasitaerer Mermithiden (Nematoda) in Chironomus-Larven (Diptera: Chironomidae). Z. Parasitenk. 26: 29-49.

APPENDIX I

USE OF V% IN MERMITHID TAXONOMY

INTRODUCTION

Descriptions of nematodes often give the ratio between two well-defined distances rather than the absolute measurements of the same distances, with the idea that ratios diminish (but do not exclude) variability between individuals (Geraert, 1968). The most widely-used ratios are *a*, *b*, and *c*[1] of de Man (1880); the practice of indicating vulva position in terms of percent of body length (V%) is also common (Wu, 1960). Some early workers in mermithid taxonomy used de Man's *a* ratio and *c* for males. Many also expressed the position of structures as

$$\frac{\text{distance from anterior as \% body length}}{\text{width at structure as \% body length}}$$

Only V% is still consistently used in mermithid taxonomy.

Recently several authors discussed morphometric relations in plant parasitic nematodes. Wu (1960) concluded that her data for *Ditylenchus destructor* did not support the ratios *a*, *b*, and *c* in females and *a* and *b* in males, while it did support V%. Clark (1962) stated that the de Man formula must be regarded as an approximation. Coomans (1962) showed that deviation from the mean was highest for *c* and intermediate for *a* and *b* for

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1. $a = \frac{\text{Body length}}{\text{Body width}}$ $\quad b = \frac{\text{Body length}}{\text{distance from ant. to end of esophagus}}$

$$c = \frac{\text{Body length}}{\text{Tail length}}$$

Rotylenchus goodeyi, while the position of the vulva showed the least variability. The b and c values of four Pratylenchus species had wide ranges of variability (Taylor and Jenkins, 1957). Brzeski (1963) concluded that a had little taxonomic value, but that b, c, and V% were more constant for Eudorylaimus silvaticus. Fisher (1965) noted that the position of the vulva in females of Paratylenchus nanus did not vary sufficiently to warrant analysis. Geraert (1968), using measurements of several species of Tylenchida, concluded that : (i) the ratios a and b had no meaning; (ii) the ratio c was of limited value; and (iii) V% could be used only when the tail length was independent of the body length. He stressed that before a ratio can be used, it must be proved that it represents a constant value, independent of the entities used to calculate it.

It has never been shown that the two entities that form the ratio V% in mermithid taxonomy are correlated, and that the quotient between the two remains constant. Our measurements for three mermithid species will be used to discuss the validity of this ratio.

METHOD

The distance of the vulva from the anterior end, and the body length of 36 Gastromermis deltensis, 33 Hydromermis variabilis, and 23 H. palustris were measured and plotted. The regression line relating the two sets of measurements was calculated and drawn for each species.

RESULTS

Figures 1 to 3 show the distance of the vulva from the anterior plotted

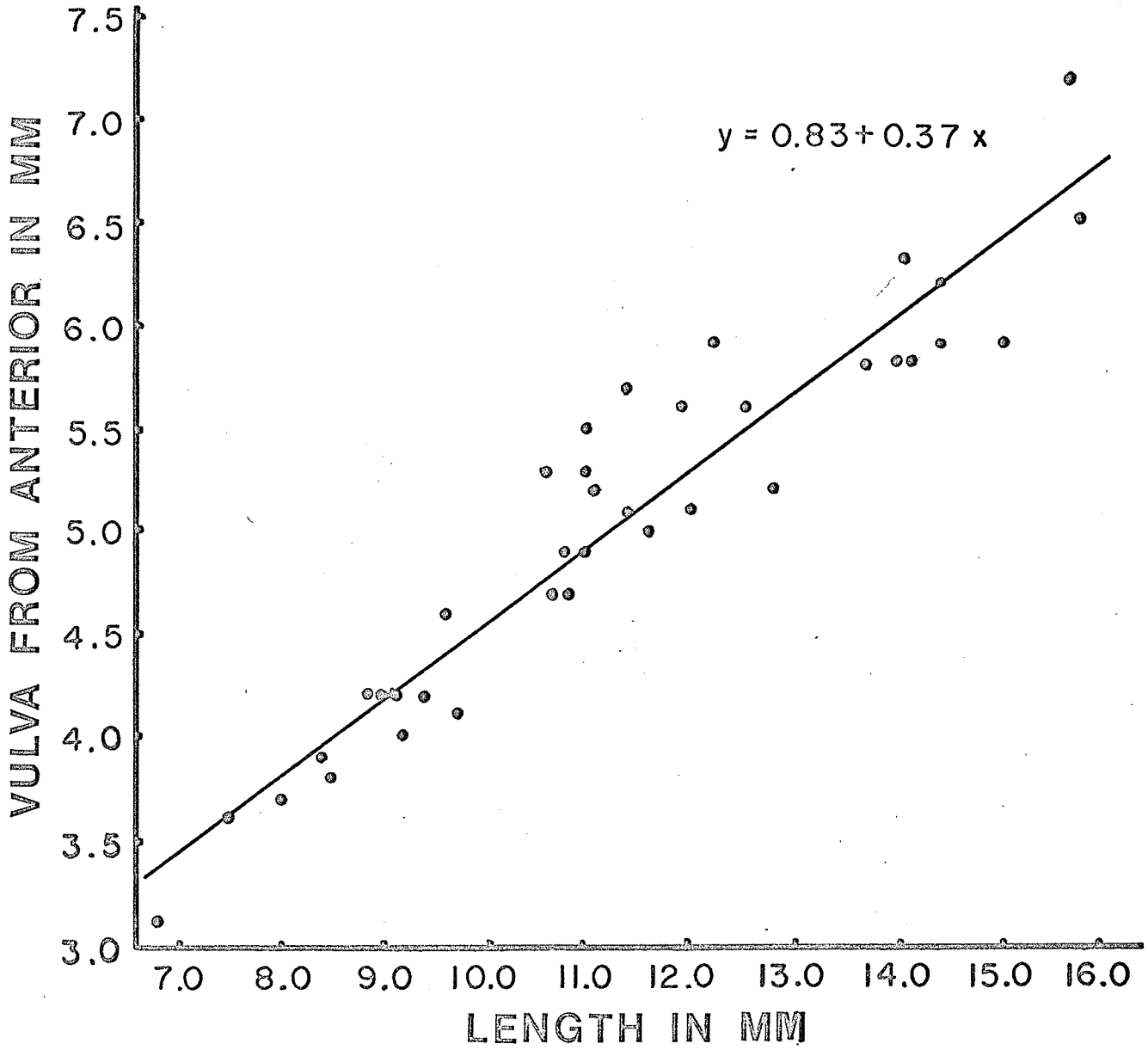


Fig. 1 G. deltensis: Relation between body length and length from vulva to anterior end.

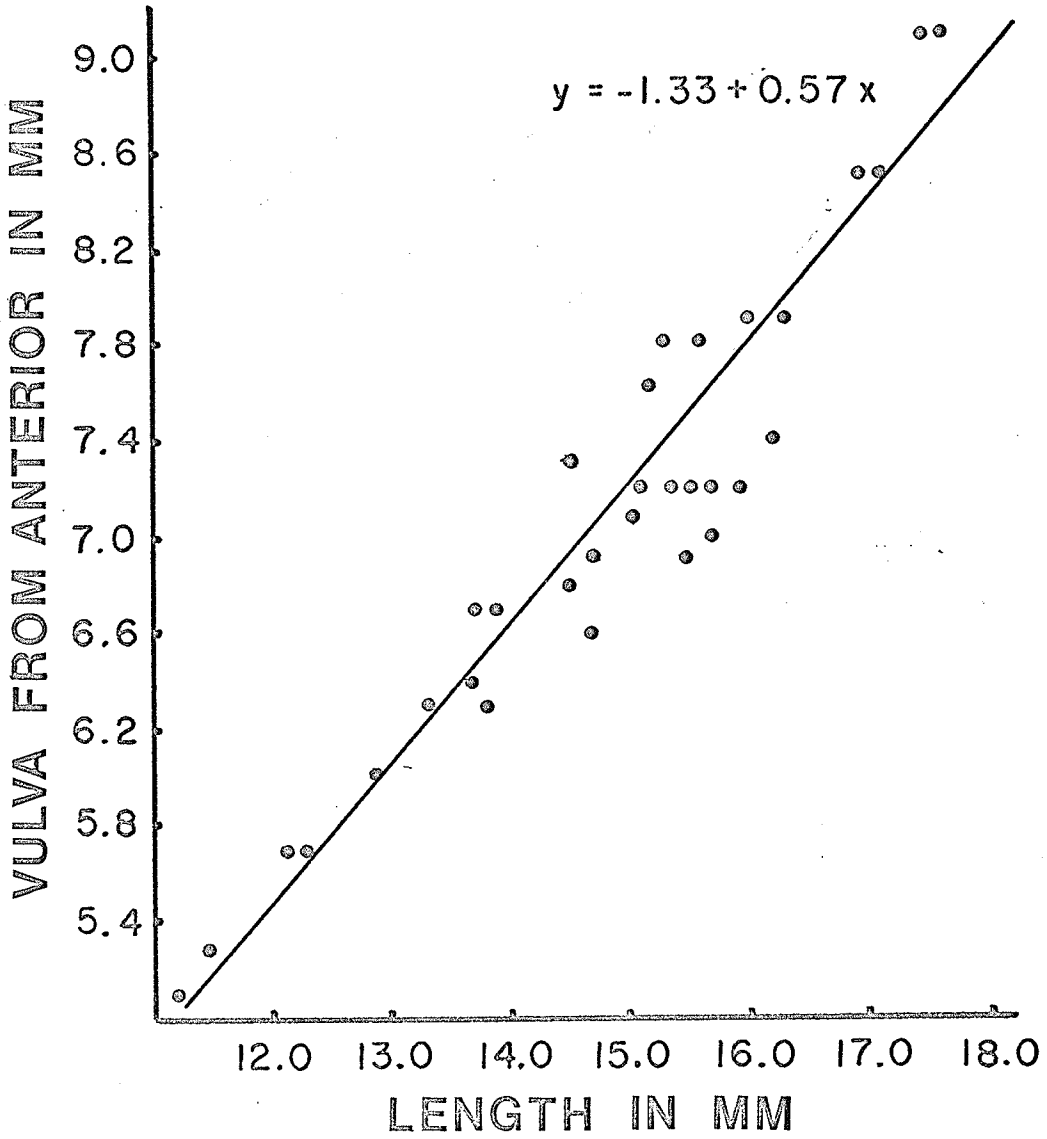


Fig. 2 H. variabilis: Relation between body length and length from vulva to anterior end.

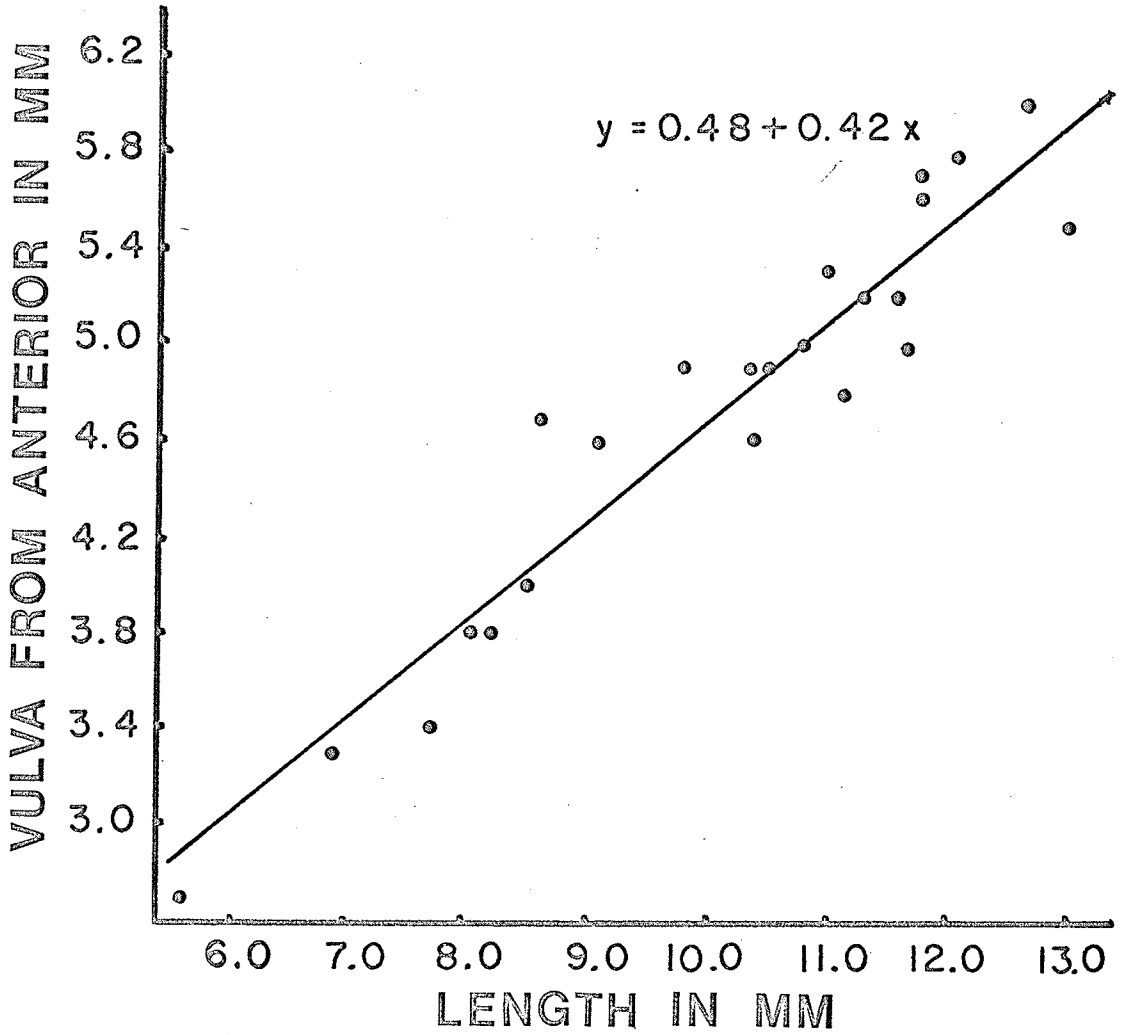


Fig. 3 H. palustris: Relation between body length and length from vulva to anterior end.

against the total length for G. deltensis, H. variabilis and H. palustris. There is an obvious high correlation between the two measurements. The following data apply to the figures:

G. deltensis: x-value at origin 6.6 mm.; $\hat{y}$ (estimated y-value for x = 6.6) 3.3 mm.; 95% confidence interval for $\hat{y}$ (2.7, 3.9).

H. variabilis: x-value at origin 11.0 mm.; $\hat{y}$ (for x = 11.0) 4.9 mm.; 95% confidence interval for $\hat{y}$ (4.3, 5.5).

H. palustris: x-value at origin 5.4 mm.; $\hat{y}$ (for x = 5.4) 2.8 mm.; 95% confidence interval for $\hat{y}$ (2.3, 3.3).

In all cases, the 95% confidence interval for the estimated y-value where the regression line crosses the ordinate, encloses the y-value at the origin.

DISCUSSION

A ratio can be used only when it is proved that both entities that form the ratio are correlated, and that the quotient between both remains constant. Hence, $\frac{y}{x} = c$ must apply, which is a straight line passing through the origin. If the two entities are related by a straight line of the form $y = A + Bx$, then $\frac{y}{x} \neq$ constant, and cannot be used as a ratio (Geraert, 1968).

Wu (1960) and Geraert (op. cit.) presented figures for the plot of vulva distance from the anterior against body length. Their criteria for the validity of the ratio V were that the two measurements be correlated, and that the regression line pass through the origin of the graph. If

these two criteria are satisfied, the ratio of the two measurements takes on the general form $\frac{y}{x} = c$ for the range of the data, and the ratio is valid.

It may be argued that the requirement for the regression line to pass through an apparently artificial origin is not valid, as the position of the origin can be changed by extending one or both axes closer to the true origin (0,0). However, the origin is not entirely artificial. It could be called a "biological origin," as it starts slightly before the smallest measurement in the range of data. In the case of V, one would expect a lower limit to the lengths of the animals, and hence a limit to the distance of the vulva from the anterior. This lower limit or sample origin is therefore set close to the smallest measurement in the sample. The population may contain an even smaller animal, but presumably it would fall within the confidence interval of the regression line when the axes are extended close to (0,0), as the plot of the confidence interval forms an hyperbola whose axes are the regression line and a line perpendicular to the regression line passing through $(\bar{x}, \bar{y})$.

CONCLUSIONS

In *G. deltensis*, *H. variabilis*, and *H. palustris*, the distance of the vulva from the anterior end is correlated with the body length, and the quotient between the two measurements remains constant over the sample range. The use of V% is therefore justified. Results from studies on de Man's ratios *a* and *c* in plant parasitic nematodes indicate that these ratios should not be given much importance in mermithid taxonomy until their validity is tested.

REFERENCES

Brzeski, M. 1963. Morphological studies on Eudorylaimus silvaticus Brzeski (Nematoda: Dorylaimidae). Bull. Acad. Polon. Sci. Sér. Sci. Biol. 11: 133-136.

Clark, W. C. 1962. Measurements as taxonomic criteria in nematology. Nematologica 7: 10.

Coomans, A. 1962. Morphological observations on Rotylenchus goodeyi Loof and Oostenbrink, 1958. I Redescription and variability. Nematologica 7: 203-215.

Fisher, J. M. 1965. Studies on Paratylenchus nanus. I Effects of variation in environment on several morphometric characters of adults. Nematologica 11: 269-279.

Geraert, E. 1968. Morphometric relations in nematodes. Nematologica 14: 171-183.

de Man, J. G. 1880. Die einheimischen, frei in der reinen Erde und im sussen Wässer lebenden Nematoden. Tijdschr. Ned. Dierk. Vereen. 5: 1-104.

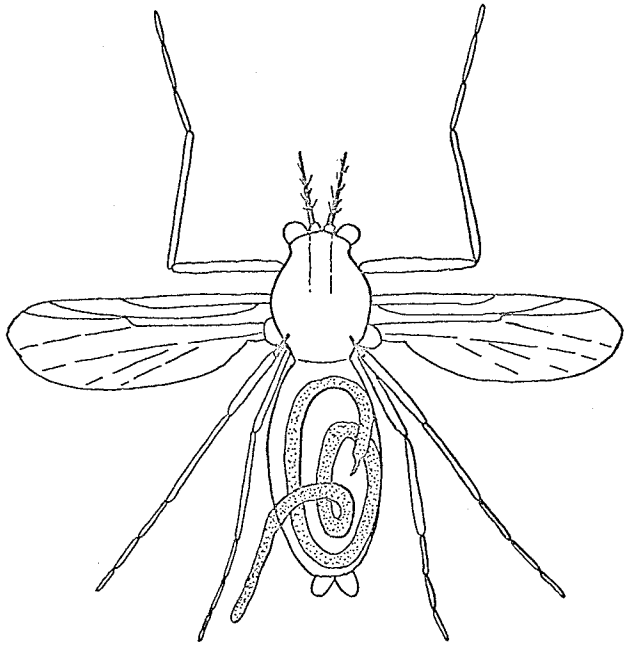
Taylor, D. P., and Jenkins, W. R. 1957. Variations within the nematode genus Pratylenchus, with the descriptions of P. hexincisus n. sp. and P. subpenetrans n. sp. Nematologica 2: 159-174.

Wu, L-Y. 1960. Comparative study of Ditylenchus destructor Thorne, 1945 (Nematoda: Tylenchidae), from potato, bulbous Iris and Dahlia, with a discussion of de Man's ratios. Can. J. Zool. 38: 1175-1187.

APPENDIX II

Fig. 1 Generalized life cycle of mermithid parasites of chironomids. (Cycle proceeds clockwise)

MERMITHID LIFE CYCLE



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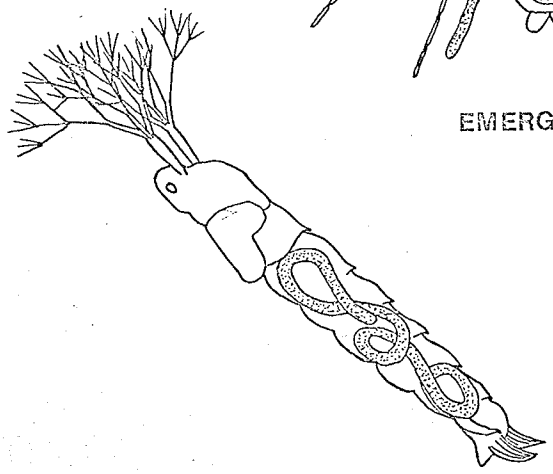
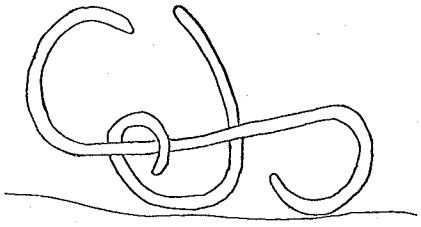
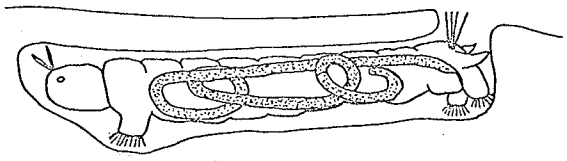




MATING



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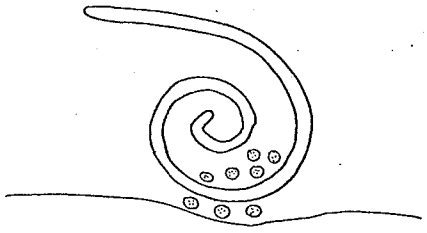
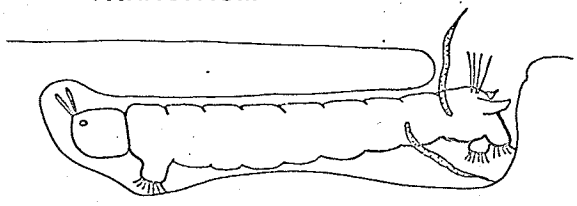


OVIPOSITION



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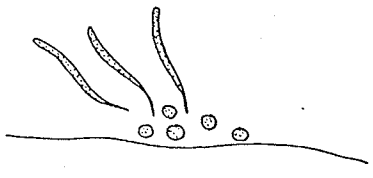

HATCHING