

DESCRIPTION OF THE LIFE STAGES OF *MACROGLENES PENETRANS* (KIRBY)
(HYMENOPTERA: CHALCIDOIDEA, PTEROMALIDAE), A PARASITOID OF THE
WHEAT MIDGE, *SITODIPLOSI MOSSELLANA* (GÉHIN) (DIPTERA:
CECIDOMYIIDAE)

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Abstract

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The life stages of *Macroglenes penetrans* (Kirby), an egg–larval parasitoid of the wheat midge, *Sitodiplosis mosellana* (Géhin), are described. The mean length and width, respectively, of 25 parasitoid eggs deposited in host eggs were 0.105 ± 0.008 (SD) mm and 0.041 ± 0.006 mm. Mature oocytes were 0.131 ± 0.008 mm long by 0.038 ± 0.004 mm wide, slightly larger than laid eggs.

A frequency distribution of head width (HW) indicated two larval instars during the feeding period. During development, the HW of the first instar remains almost constant at about 0.03 mm whereas body length (BL) increases from about 0.15 to about 0.3 mm. Both HW and BL increase in the second instar from about 0.15 and 0.49 to 0.34 and 1.05 mm, respectively, for mature larvae. The third instar does not feed and is characterized by hook-like “pseudomandibles” and four incurving spines on the terminal segment. The pupa is aedeous exarate. Adults are about 2 mm long, shiny bluish-black, and have translucent wings. Males have prominent rust-coloured eyes; eyes of females are less prominent and fuscous.

Résumé

Les stades de développement de *Macroglenes penetrans* (Kirby), un parasitoïde ovarlaire de la cécidomyie orange du blé, *Sitodiplosis mosellana* (Géhin), sont décrits. Les oeufs pondus dans ceux de l'hôte mesurent $0,105 \pm 0,008$ mm (moyenne \pm écart-type, $n = 25$) de long et $0,041 \pm 0,006$ mm de large, alors que les oocytes matures sont légèrement plus grands avec respectivement $0,131 \pm 0,008$ mm et $0,038 \pm 0,004$ mm.

Pendant la période d'alimentation, la distribution des fréquences de la largeur de la tête du premier stade larvaire a une longueur constante d'environ 0,03 mm alors que la longueur de corps (BL) passe d'environ 0,15 mm à 0,3 mm en fin de croissance. Durant le deuxième stade larvaire, les deux paramètres HW et BL passent respectivement de 0,15 mm à 0,34 mm et de 0,49 mm à 1,05 mm en fin de croissance. Le troisième stade larvaire ne s'alimente pas et est caractérisé par des “pseudomandibules” unciformes et les quatre spinules incurvées du segment terminal. La nymphe est du type libre. Les adultes, d'environ 2 mm de long, sont de couleur bleu-noir métallique et possèdent des ailes translucides. Les mâles se distinguent par leur yeux proéminents de couleur rouille; les yeux des femelles sont moins proéminents et brun foncé.

Introduction

In 1984 a chalcidoid wasp, later identified as *Pirene penetrans* (Kirby), was observed emerging in some research plots used to study the wheat midge, *Sitodiplosis mosellana* (Géhin), in northeastern Saskatchewan. This parasitoid was first described by Kirby in 1800 as *Ichneumon penetrans*. The imago of this insect was later described under the generic name *Macroglenes* by Johansson (1936) as reviewed by Peck (1963). Synonymy and taxonomy have been given by Graham (1969). Although the generic name *Pirene* has been preferred and widely used, *Macroglenes* Westwood has precedence (Bouček 1988) and will be used here. The species *Macroglenes penetrans* (Kirby) is widely distributed and usually common in Europe (Doeksen 1938; Speyer and Waede 1956; Barnes 1956). Its distribution in Canada and the United States is unknown but it was collected in Manitoba

associated with the wheat midge outbreak that occurred there from 1954 to 1959 (Beirne 1971). Nine specimens from that collection were identified as *P. penetrans* by C. Yoshimoto at the Biosystematics Research Centre (Ottawa, Canada). In a concurrent study in Switzerland, 15 specimens of *M. penetrans* were identified by Z. Bouček at the Commonwealth Institute of Entomology, London.

A number of hymenopterous parasitoids are known to attack the wheat blossom midges *S. mosellana* and *Contarinia tritici* (Kirby) in Europe (Barnes 1956). Both midge species were thought to be hosts for the same complex of parasitoids (Johannson 1936; Doeksen 1938; Basedow and Schütte 1982). However, a recent study indicates that *S. mosellana* and *C. tritici* have distinct complexes of parasitoids within the families Pteromalidae and Platygasteridae (Affolter 1987). Affolter (1987) lists parasitoids of *S. mosellana* as *M. penetrans*, *Platygaster* sp., and *Euxestonotus error* (Fitch); those ascribed to *C. tritici* are *Piestopleura* sp., *Isostasius* sp., and *Leptacis tipulae* (Kirby). Recently collected European specimens of *E. error* are considered to be conspecific with specimens described by Fitch in 1865 (Masner 1989). The rarity of *E. error* in North America suggests that it was accidentally introduced by earlier settlers but failed to become well established (Masner 1989). Another platygasterid wasp (*Inostemma horni* Ashmead) was reported to be a parasitoid of *S. mosellana* in Washington State by Reeher (1945).

Although *M. penetrans* is known as an important natural control agent of *S. mosellana* in Europe (Basedow and Schütte 1982; Affolter 1987), no detailed studies on its biology have been made; its immature stages have not been described. The objective of this study was to describe the life stages of this parasitoid, primarily to enable the separation of the immature instars from those of other parasitoid species known to occur in Europe and which may occur in North America. The major portion of the work was done in Saskatchewan supplemented with observations on the immature instars of the parasitoid from a population in Switzerland.

Materials and Methods

Adult. Adult *M. penetrans* were reared from field-collected wheat midge cocoons. The lengths of 50 *M. penetrans* males and 32 females were obtained.

Egg. Parasitoid females that emerged in the laboratory were held for a few days and then dissected to obtain oocytes. Oocytes were stained in 1% Eosin in water to improve microscopic examination. Twenty-five oocytes were measured for each of five females. Six to seven oocytes also were obtained for each of three females from the Swiss population. Eggs of *M. penetrans* were obtained by inducing female parasitoids to oviposit into wheat midge eggs in the laboratory. The lengths and widths of 25 parasitoid eggs dissected from midge eggs were recorded. Eggs and all subsequent stages were measured with an ocular micrometer and compound microscope.

Larva. To obtain a range in size of parasitoid larvae, soil samples containing wheat midge larvae were collected from the field each week from May to mid-October. An average of 25 (range 6–53) parasitoid larvae was dissected from the midge larvae each week to measure for parasitoid head width (HW), head length (HL), and body length (BL). An average of 17 (range 4–30) parasitoid larvae dissected from midge larvae collected at weekly intervals from wheat heads from mid-July to mid-September also was measured. A frequency distribution of head widths from 693 parasitoid larvae was used to separate the larval instars.

Observations were made on living larvae and whole mounts to determine the presence of mandibles in different instars and to describe morphological differences that could be used to separate instars. Larvae considered to be in the early and late second instar were fixed in Bouin's fixative (Baker 1958) overnight, stained with Rhodamine B (in 50% ethyl

alcohol) for 4 h during dehydration in a graded alcohol series, and then mounted in "Permount". Also, semi-permanent slides were made of late second-instar larvae that were cleared and mounted in de Faure's fluid under a cover slip.

Third-instar Larva and Pupa. Third-instar larvae and pupae were obtained by holding parasitized midge larvae that had broken diapause on damp blotting paper and allowing the parasitoids to develop. Measurements of HW and BL were obtained for 10 third-instar larvae and BL and thoracic width on seven pupae.

Results

Adult. Both sexes of *M. penetrans* (Fig. 1A and B) are shiny bluish-black with iridescent wings. Prominent rust-coloured eyes distinguish the males (Fig. 1A), the lower one-quarter of each eye being darker than the remainder. Eyes of the females (Fig. 1B) are less prominent than those of males and are brownish-black. The mean length \pm SD of 50 males and 32 females was 1.77 ± 0.119 and 1.80 ± 1.05 mm, respectively.

Egg. Eggs of *M. penetrans*, an egg-larval parasitoid, are laid into the eggs of the host wheat midge (Fig. 1C). Oviposition was observed in the laboratory and parasitized eggs also were found in the field (unpublished data). Mean length (\pm SD) and width (\pm SD) of 25 eggs laid by *M. penetrans* was 0.105 ± 0.008 and 0.041 ± 0.006 mm, respectively. The eggs are glistening creamy white, slightly translucent, and have no noticeable sculpturing at magnifications of 100–200 times.

Mature oocytes are milky white and have a stalk more prominent than on the laid egg (Fig. 2A). Mean length and width of 125 oocytes from the Saskatchewan population was 0.131 ± 0.008 and 0.38 ± 0.004 mm, respectively. Mean length and width of 20 oocytes from the Swiss population was 0.115 ± 0.009 and 0.033 ± 0.003 mm, respectively, slightly smaller than those measured for the Saskatchewan population.

Larval Instars. The cumulative frequency distribution of the HW clearly separated the first and second instars (Fig. 3). Early and late development of each of these instars were distinguishable, so this designation was made for convenience in separating larvae of different maturity within instars. The third instar does not feed and is of short duration.

First-instar larva. Newly hatched *M. penetrans* larvae have a prominent head with an elongated translucent body (Fig. 2B and C). The body widens slightly behind the head and tapers to the end of the abdomen. Eleven segments usually are visible in newly hatched larvae; a small spine or bristle marks each of the nine abdominal segments dorsally (Fig. 2B). A pair of terminal ventrally curving hooks are borne on the abdomen (Fig. 2B). Small sickle-shaped mandibles that move independently also are evident (Fig. 2C). Mean length and width of four mandibles from two individual larvae from the Swiss population were 8.36 and 3.93 μm , respectively. As the first-instar larva grows it becomes tear-drop-shaped (Fig. 2D). Although the body length increases from about 0.144 to 0.302 mm, the width of the head remains almost constant (Fig. 3). In late first-instar larvae segmentation and bristles on the dorsum are not evident; the caudal hooks are still present. The gut, filled with a pale yellowish substance, can be seen through the body wall. The large, more opaque larva is, therefore, more easily seen when dissected from the host. A pair of small internal lobes, probably brain lobes, are present behind the head abutting the gut (Fig. 2D).

Second-instar larva. Unlike the first instars, the head of second instars does not protrude anteriorly and caudal hooks are not present (Fig. 2E and F). There is also a progressive increase in HW as development continues (Fig. 3). Mean HW, HL, and BL of early second-instar *M. penetrans* dissected from midge larvae from overwintering cocoons in early May were 0.188 ± 0.025 ($n = 29$), 0.074 ± 0.016 ($n = 27$), and 0.514 ± 0.075 ($n = 36$) mm. Usually 13 body segments, including the head, are evident, with the thoracic segments being most prominent. The exuviae of first-instar larvae often

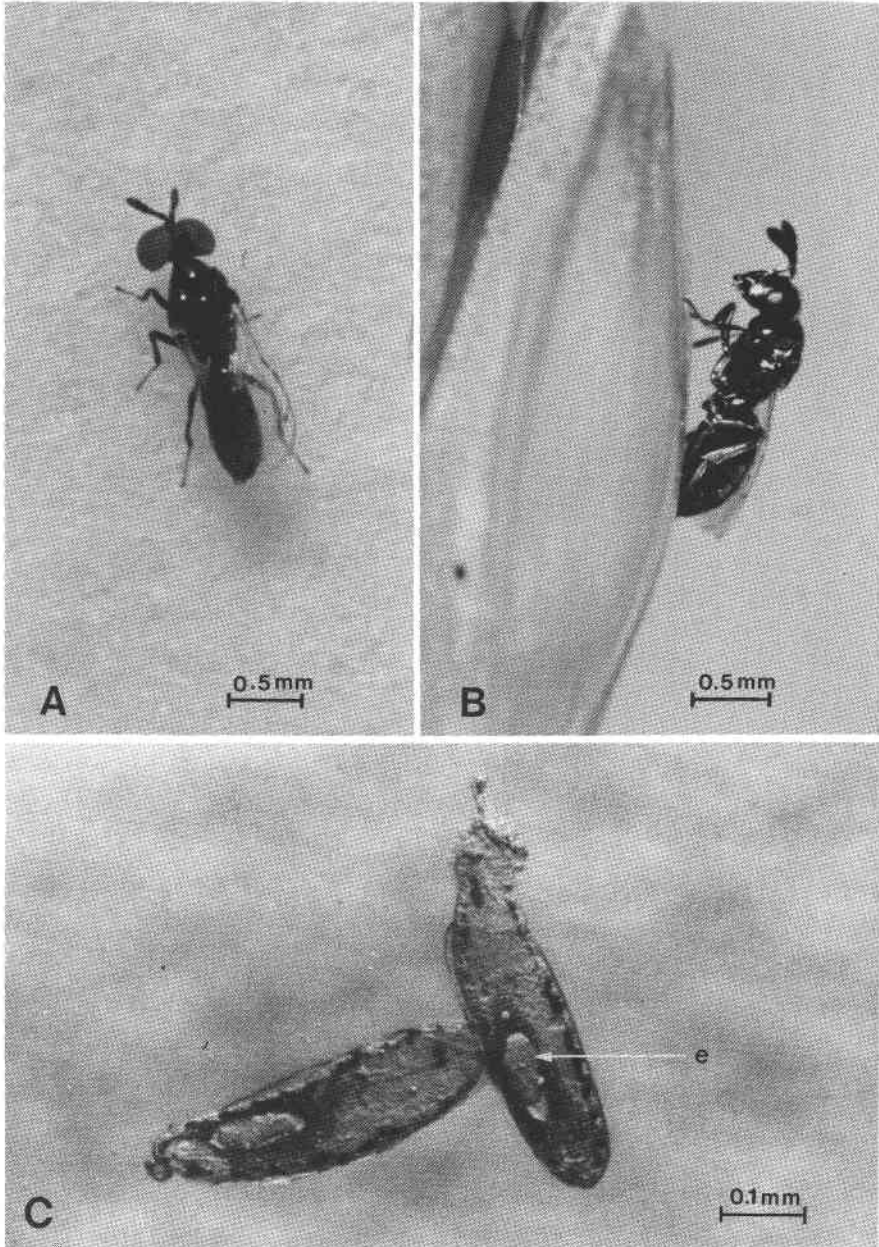


FIG. 1. Adult male (A) and female (B) *M. penetrans* and (C) *S. mosellana* eggs containing eggs (e) of *M. penetrans*.

are observed attached to the end of the abdomen when the larva is dissected from the host (Fig. 2E). This detaches when the larva is placed in distilled water.

Through the body wall, the gut and what appear to be brain lobes can be seen. The gut has a central core filled with dense orange material. The esophagus often can be seen (Fig. 2F).

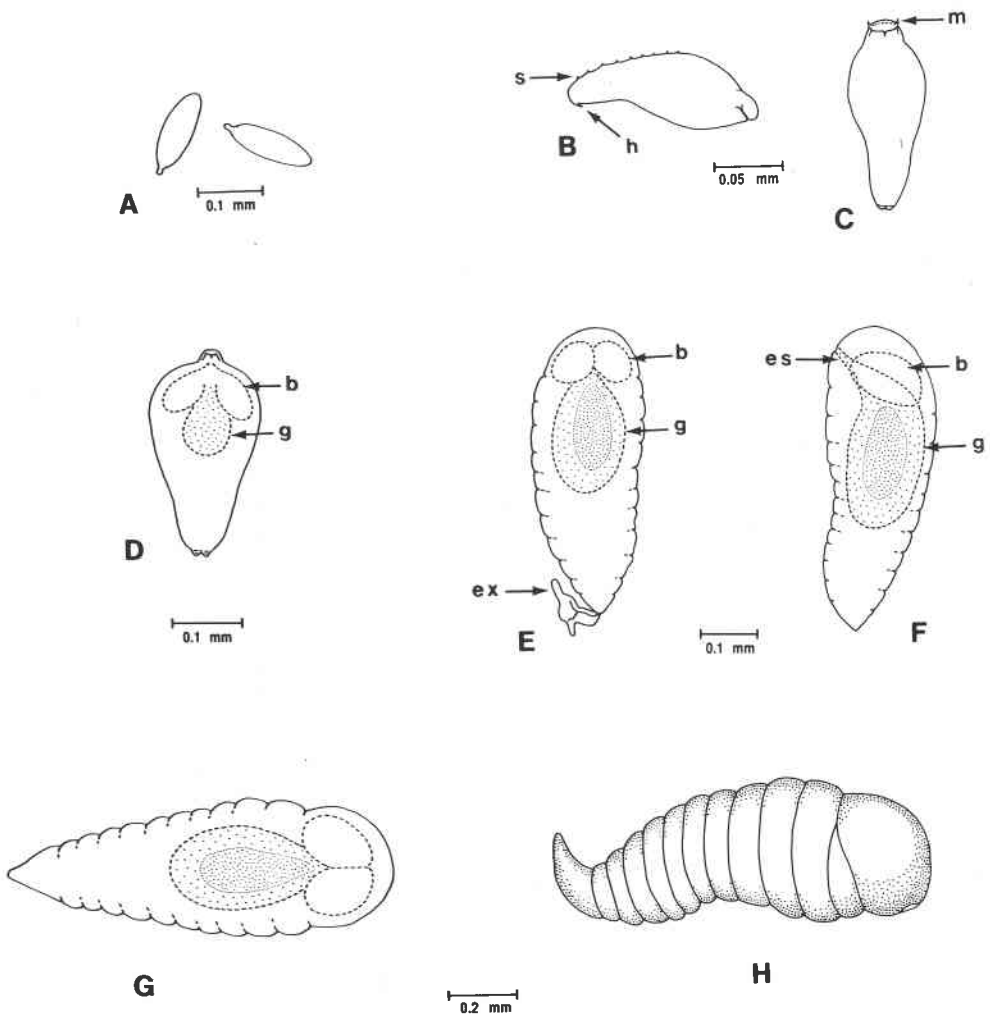


FIG. 2. Oocyte, and larval stages of *M. penetrans*: A, stalked oocytes; B (lateral) and C (ventral), views of an early first-instar larva showing dorsal spines (*s*), terminal hooks (*h*), and mandibles (*m*); D, ventral view of late first-instar larva showing brain lobes (*b*) and gut (*g*); E (dorsal) and F (lateral), views of an early second-instar larva showing brain lobes (*b*), gut (*g*), exuviae of first instar (*ex*), and esophagus (*es*); G, dorsal view of late second-instar larva showing brain lobes (*b*) and gut (*g*) seen through body wall; H, 3-dimensional lateral view of late second-instar larva with characteristic tail curve.

Older second-instar larvae are longer, wider, and more robust than younger ones. Fully mature larvae dissected from wheat midge larvae collected from the field about mid-June had attained HW's, HL's, and BL's of 0.338 ± 0.053 ($n = 22$), 0.261 ± 0.042 ($n = 21$), and 1.052 ± 0.25 ($n = 25$) mm, respectively.

Through the body wall, orange gut and brain lobes are apparent (Fig. 2G). Imaginal discs of antennae and mouth parts are evident as more-or-less circular areas beneath the cephalic cuticle. Segmentation is more pronounced and the terminal segments of the abdomen typically curve upward (Fig. 2H). No mandibles were observed in whole mounts of second-instar larvae but they were seen occasionally in living larvae during microscopic examination. They were unsclerotized and made noticeable only by their movement.

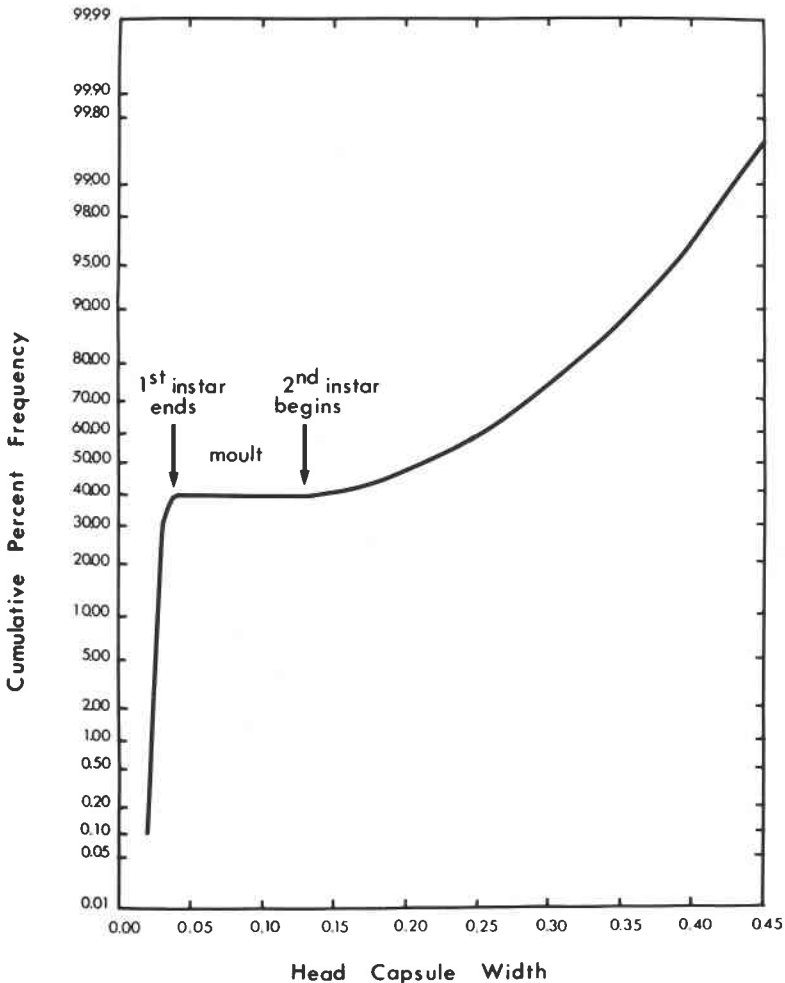


FIG. 3. Cumulative frequency distribution of head capsule width showing the end of the first instar and beginning of the second instar.

Third-instar larva. Parasitized larvae spin an elongate cocoon that appears to be identical to that spun by the unparasitized larvae. Within the cocoon the third-instar larva forms, enclosed by the second larval skin which is ruptured as the third instar develops. On moist blotting paper in the laboratory, parasitized and unparasitized larvae sometimes develop through the third instar and pupate without forming a cocoon. The third instar required only 2 days to complete development and transform to the pupa in the laboratory.

Mean third-instar HW and BL ($n = 10$) were 0.330 ± 0.086 and 1.403 ± 0.300 mm, respectively. Including the head, 13 segments were visible in dorsal view (Fig. 4A). Anteriorly, the head has two protruberances that disappear during transformation from third instar to pupa (Fig. 4A and B). The mouth area is bordered by two hook-like structures that appear to be mandibles (Fig. 4C). However, as the third-instar larva transforms to the pupa, these hooks are seen to be situated just below the developing pupal eye and were not associated with the mouth region so have been termed "pseudomandibles". They were no longer observed after the complete transformation from the third instar to the pupa. A

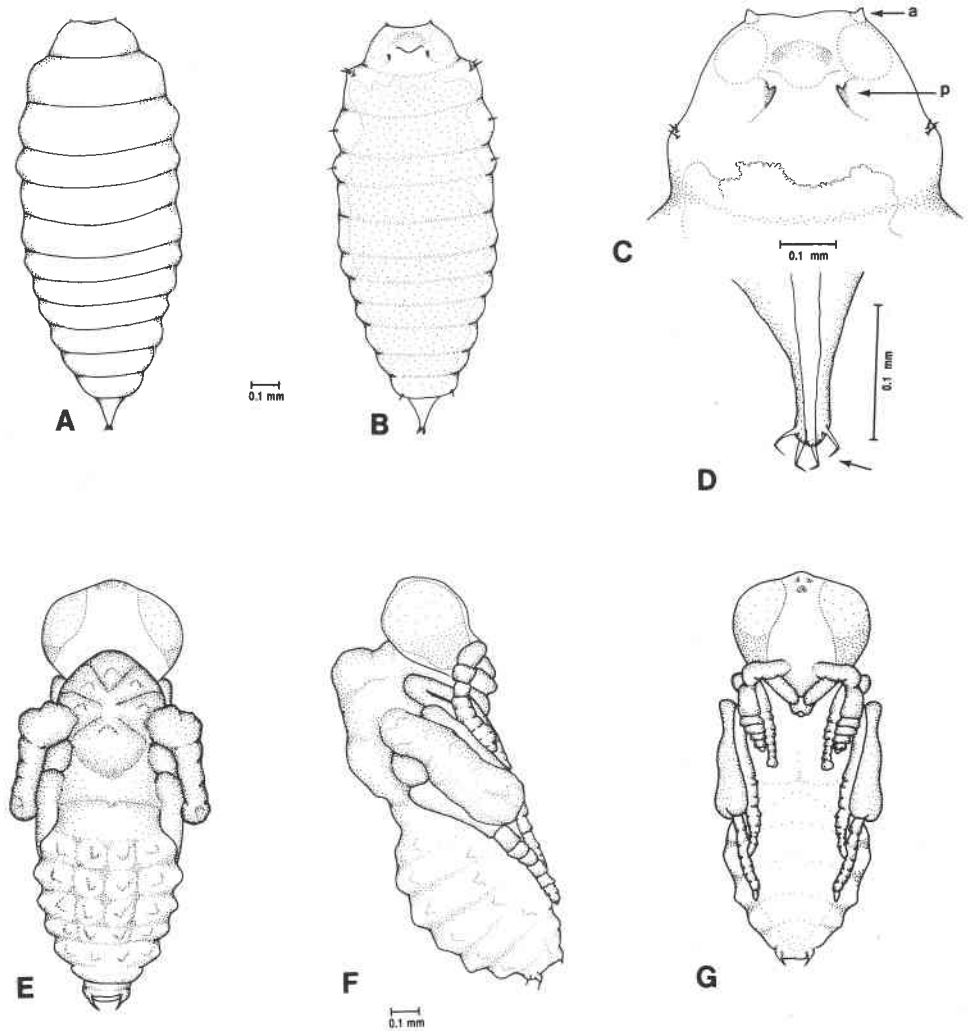


FIG. 4. A (dorsal) and B (ventral), views of a third-instar larva; C, an enlarged ventral view of the head showing the precursory antennal discs (*a*) and eyes and pseudomandible (*p*); D, terminal segment with incurving spines (*s*); E (dorsal), F (lateral), and G (ventral), views of a pupa.

yellowish food pellet, taking up most of the gut region, is seen through the body wall (Fig. 4B). The terminal segment of the abdomen usually has four or occasionally five hook-like spines (Fig. 4D) which are also lost when the prepupa transforms to the pupa.

Pupa. The pupa of *M. penetrans* is aedeous and exarate and is slightly larger than the third instar and slightly smaller than the adult. Mean length of seven specimens was 1.68 ± 0.19 mm. During development, the pupa is initially light in colour and then the eyes, ocelli, wings, and legs begin to pigment, followed by the remainder of the body. In males, the ventral approximately one-quarter of the eye darkens first and this portion remains darker than the remainder of the eye in the adult.

Discussion

Kutter (1934) found that *Pirene gramineae* Haliday, a species closely related to *M. penetrans*, parasitized the larvae of its host, *Contarinia pisi* Winnertz, and not the egg. Otherwise the two species appear to be similar in the morphology of their immature stages. Freshly deposited eggs of *P. gramineae* were slightly larger (0.16–0.17 mm long by 0.06–0.08 mm wide) than those of *M. penetrans*. *Pirene gramineae* also has two distinct larval stages during the feeding period. Mandibles in first-instar *P. gramineae* appear to be identical in form with those of *M. penetrans*. Kutter illustrated internal mandibles in late second-instar *P. gramineae* similar to those observed in *M. penetrans*. Also, Kutter describes a prepupae which Clausen (1940) considered to be a third-instar larva similar to the third-instar larva we describe for *M. penetrans*.

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