

SOME OBSERVATIONS ON THE BIOLOGY AND MORPHOLOGY OF THE IMMATURE STAGES OF *PSYCHODOPYGUS WELLCOMEI* FRAIHA, SHAW AND LAINSON, 1971. (DIPTERA: PSYCHODIDAE)<sup>1</sup>

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(With 5 plates)

**SUMMARY:** Under laboratory conditions, the development from egg to adult of *P. wellcomei* takes an average of 42 days. The larval stages are similar to those of *P. arthuri*, described by Barretto (1941), but can be distinguished from this species by the ratio of the first to second antennal segment, by the form of the lateral head setae and prothoracic dorsolateral setae. The pupal stage of *P. wellcomei* is characterized by a trifid pre-alar seta and simple spine-like thoracic and abdominal setae.

THE immature stages of phlebotomine sandflies have been little studied in the past, due to the difficulty of finding natural breeding sites, and the problems involved in laboratory rearing. Bayma (1923), in a posthumous publication, was the first worker in South America to show interest in the immature stages of phlebotomines. As early as 1916 he successfully reared *Lutzomyia intermedia* (Lutz & Neiva, 1912), but mistakenly identified it as *Phlebotomus papatasi*. Following Costa Lima's correct identification of this material, the paper was republished (Bayma, 1936).

In a description of the immature stages of 12 phlebotomine species,

Barretto (1941), laid down the basis of larval and pupal taxonomy. Other workers, Lindquist (1936), Mangabeira, (1942, 8a-11a & 13a), Addis (1945), Sherlock (1957 & 1957a), Mangabeira & Sherlock (1962), Sherlock & Carneiro (1963), Carneiro & Sherlock (1964), Chaniotis & Anderson (1964), and Guitton & Sherlock (1969), have described various immature stages of a further 18 New World species.

Of some 250 species of phlebotomines known from North to South America, only 30 have been partially or fully described in the immature stages. Of these species, the genus *Brumptomyia* is most fully represented with 4 descriptions.

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It has been shown that there are marked specific differences in the larvae of most 3rd to 4th instars, and pupae of phlebotomines (Barretto, 1941 and Carneiro & Sherlock, 1964). However, Barretto, (loc. cit.) also showed that the immature stages of certain closely related species, *Lutzomyia intermedius* and *Lutzomyia whitmani*, are indistinguishable. Further descriptions of the immature stages will show to what extent larval and pupal morphology can help resolve taxonomic problems at the specific, and the generic level.

The present paper describes the morphology of the immature stages of *Psychodopygus wellcomei*, and some observations on the biology of these stages are made.

## MATERIALS AND METHODS

During epidemiological studies on cutaneous leishmaniasis in the "Serra dos Carajás", in the state of Pará, Brazil, numerous *Psychodopygus wellcomei* were collected biting man, during both night and day captures. Blood fed females were transported to Belém, Pará, for rearing and possible colonisation.

Each female is isolated in a 5.5 x 2.5 cm. plastic snap cap vial, the top of which is perforated by a 1 cm. diameter hole covered in nylon gauze. On top of the gauze is laid a small cotton wool ball, soaked in a 10% sucrose solution. The vials are lined with filter paper, which is dampened daily with distilled water. Tubes are kept at 26 degrees centigrade in an aquarium lined with damp cotton wool, and covered in damp lint. To prevent the entry of ants, the aquarium is placed in the centre of a water tray.

The eggs are removed from the filter paper and walls of the vials, and placed on small numbered filter paper discs, which are kept for 7 days in a dish lined

with dampened filter paper. Approximately 2 days before the minimum hatching time (9 days), the discs are transferred (100 eggs per dish), to another petri containing the matured food.

The petri dishes containing the matured food are prepared as follows: The dishes, which are 9 cms. in diameter are initially autoclaved to prevent subsequent fungal growth. A filter paper circle is placed in each dish, and moistened with 0.6 cc's of distilled sterile water, and sprinkled with 0.05 gms. of HERTIG & JOHNSON'S (1961) larval food mixture of ground rabbits faeces, ground decaying forest leaf litter and dried powdered insects. Finally 25 drops of a Baker's yeast paste, made from 1 gm. of yeast to 2 cc's of water is added to each dish (GEMETCHU, 1971). The dishes are then placed in a dampened cotton wool lined aquarium, which is sealed with a plate of glass and vaseline to prevent the entry of mites and fruit flies. Maturing dishes are left from 15-30 days. A mature plate is judged on a fungus free appearance and it's smell. During maturation a strong odour of ammonia is produced, which gives way in time to a richer, more organic smell, approaching that of the forest floor.

On hatching, the 1st instar larvae feed well on the matured yeast, though they die rapidly if the food is insufficiently mature. Small quantities of sterile, ground rodents, faeces were added to plates containing older instars. During the late 4th instar, dry forest leaf litter is added to the plates to provide elevated pupation sites. Throughout the course of development the plates are inspected daily, and moistened with sterile distilled water as required.

Eggs and immature stages were mounted in Berlese, and drawn with the aid of a camera lucida.

## RESULTS

*Behaviour of larval stages:* The times taken for the various stages of larval development are shown in Table I.

Eclosion occurs through a lateral split, which runs from the anterior egg pole along approximately one third of the egg length. The 1st larval instars, more than any other stage, are highly dependant on a ready, close source of food. They apparently wander completely at random, and if

the food is isolated to one small region of the dish many go in the wrong direction, and die, due either to an inability to locate the food source, or from dessication on the petri lid. This apparent initial lack of sensitivity disappears in the later stages, which quickly locate any food.

TABLE I

The duration of immature stages of laboratory reared *Psychodopygus wellcomei* at 26° C

Stage	Number observed	Average duration in days	Range
Blood Meal-Egg	20	4.5	4-8
Eggs (Number laid per female 32-40, average 35)	350	12	9-15
1st Instar	180	5	3.5-6
2nd Instar	112	5	3.5-6
3rd Instar	65	4	3.5-7
4th Instar	43	8	7-15
Pupa	33	8	7-10
Total Development Time	—	46.5	—

## DESCRIPTION OF IMMATURE STAGES

### 1. The Egg.

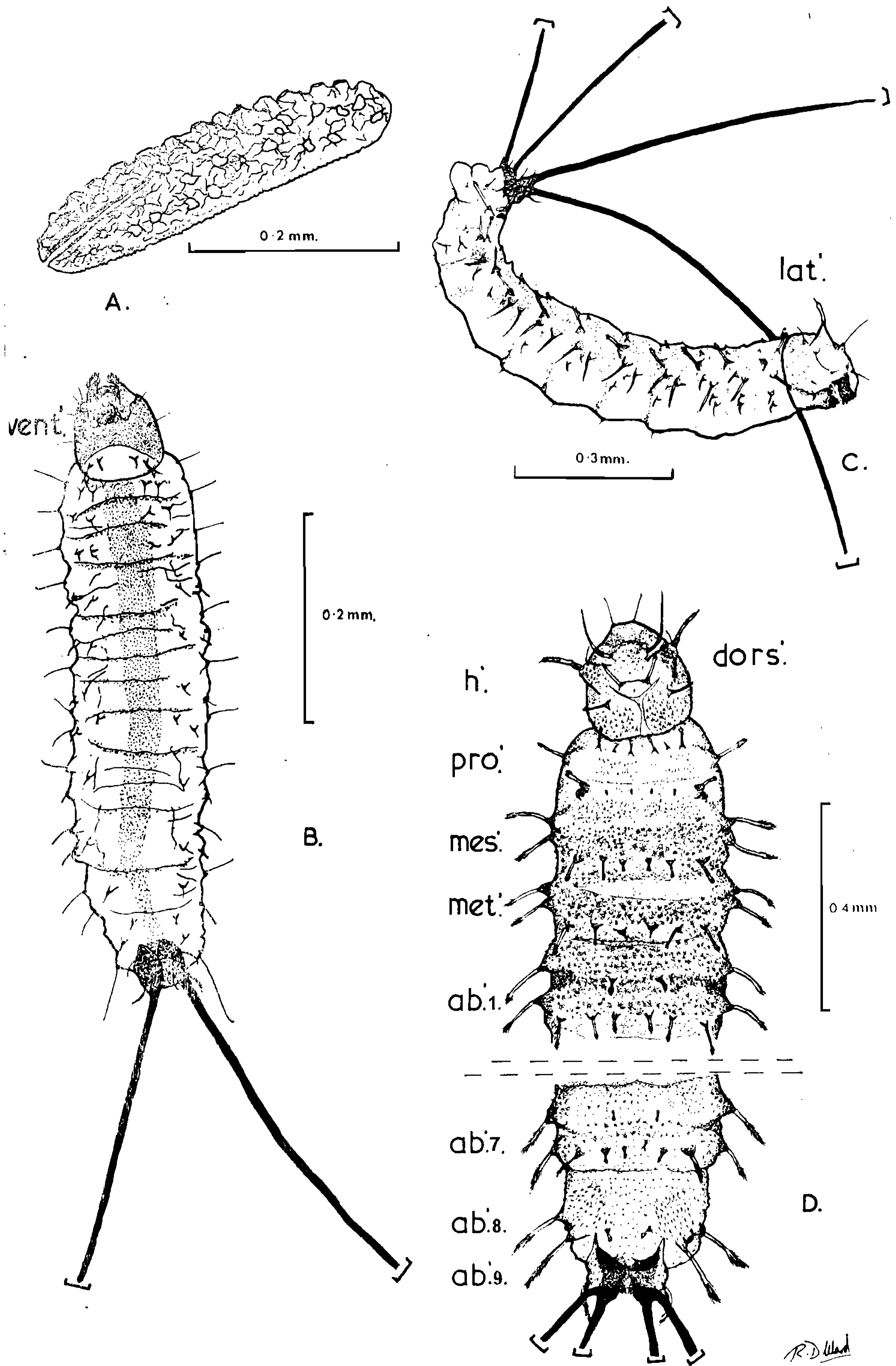
The eggs are typically ellipsoidal in shape, with flattened ventral and convex dorsal surfaces (fig. A). The eggs measure 312  $\mu$  - 344  $\mu$  with an average length of 327.6  $\mu$  (6 measured). The exochorion is sculptured with small "mountainous" formations, distributed over the dorsal and

dorsolateral surfaces. The mature eggs are dark brown to black when tanned. Following eclosion the egg shell is light brown in appearance, and the sculpturing becomes more apparent than in the entire egg.

### 2. The Larval Stages.

The setal terminology, used in the present work (fig. J), is a modification of that used by BARRETTO (1941). To avoid





*Psychodopygus wellcomei* Fraiha, Shaw and Lainson, 1971.

Fig. A — Egg; lateral face. Fig. B — 1st larval instar; ventral face.  
 Fig. C — 2nd larval instar; lateral face. Fig. D — 3rd larval instar;  
 dorsal face.

unnecessary repetition, the morphology of the 1st, 2nd and 3rd instars is only described briefly where it appears to differ from that of the 4th instar, which is described in full. Except where otherwise stated, measurements are based on 6 specimens.

a) *1st Instar.*

The larva is figured ventral face upwards, as it is in the ventral setae that variation from later instars is most apparent (fig. B). The total body length of this stage, from the front of the head capsule to the base of the two caudal setae is 550-558  $\mu$  (2 measured).

I — *Head*

The head capsule is somewhat squarely shaped lightly sclerotised, and measures from 90-95  $\mu$  in length and 86-90  $\mu$  in width. The antennae measure 36-40  $\mu$ , and arise from a large spineless tubercle. The lightly pigmented first antennal segment is approximately 1/5 the length of the second, which gives rise to a relatively shorter, and more rounded apical process than in subsequent instars. On the dorsal surface is the heavily chitinized egg breaker.

II — *Prothorax.*

The anterior spiracle is small and inconspicuous. Dorsal internal prothoracic setae are comb-tipped, fine, and measure 12  $\mu$ , reaching to the head capsule. It appears that the accessory setae are absent. The dorsolateral setae are barbed apically and measure 6  $\mu$ .

The posterior ventrolateral setae are fine, sparsely barbed and relatively longer than in subsequent instars (12  $\mu$ ).

III — *Meso and Metathorax and Abdominal Segments 1-7.*

The dorsolateral setae are stoutly built, barbed along the apical 1/3, and measure 18  $\mu$ . The basal setae appear to be absent.

As in the prothorax the posterior ventrolateral setae are relatively longer than in older instars, measuring 12-18  $\mu$ .

IV — *Abdominal Segment 8.*

The dorsal submedian setae are short, (6  $\mu$ ), equal in length to the internal posterior dorsals. These two pairs of setae are borne on a small chitinized area, bearing 4-5 rows of small spine-like setae.

V — *Caudal Setae.*

The two caudal setae measure from 1.08-1.140 mm. in length.

b) *2nd Instar.*

The total body length of the 2nd instar (fig. C), is from 960  $\mu$ -1.20 mm. (2 measured).

I — *Head.*

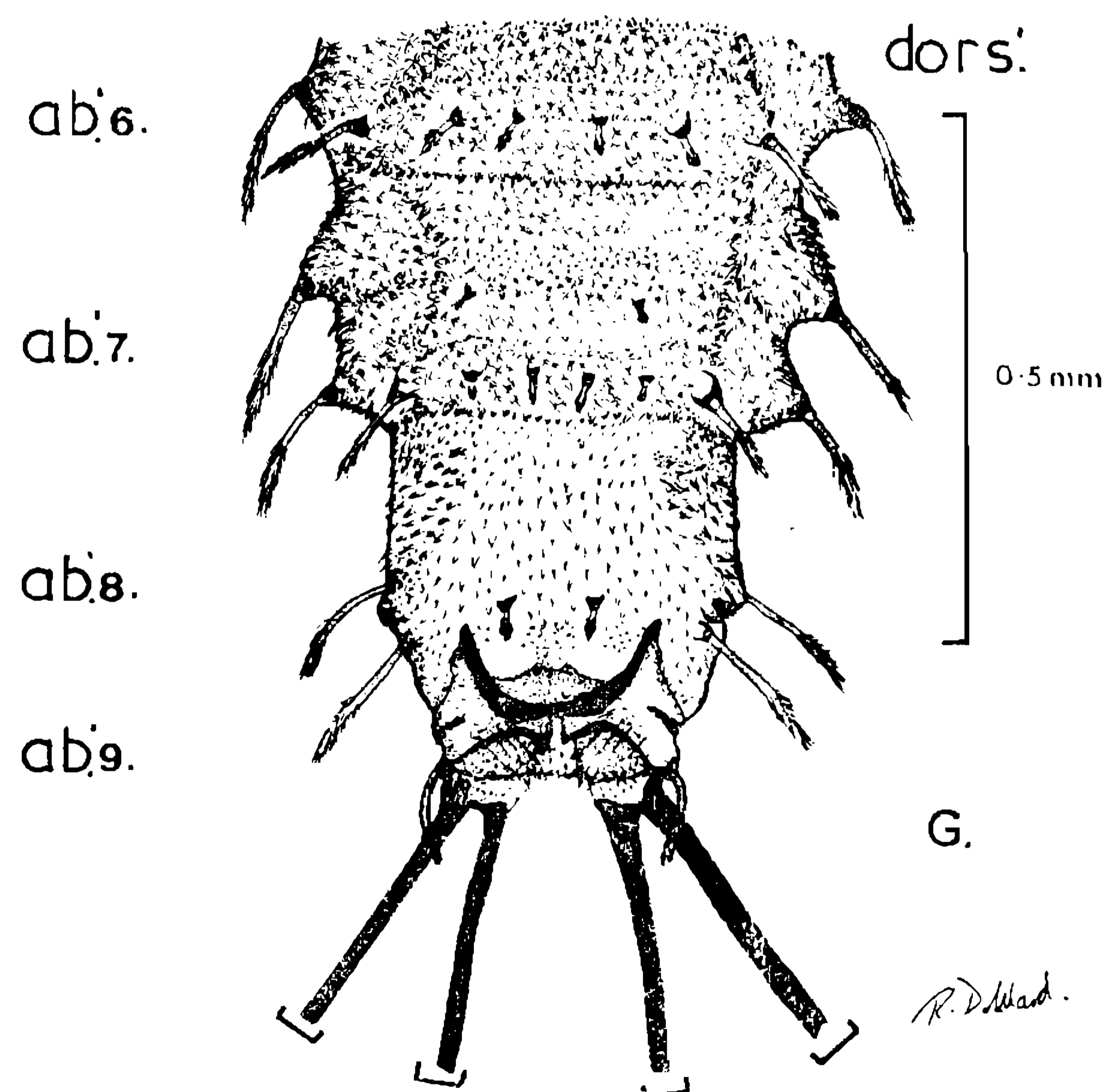
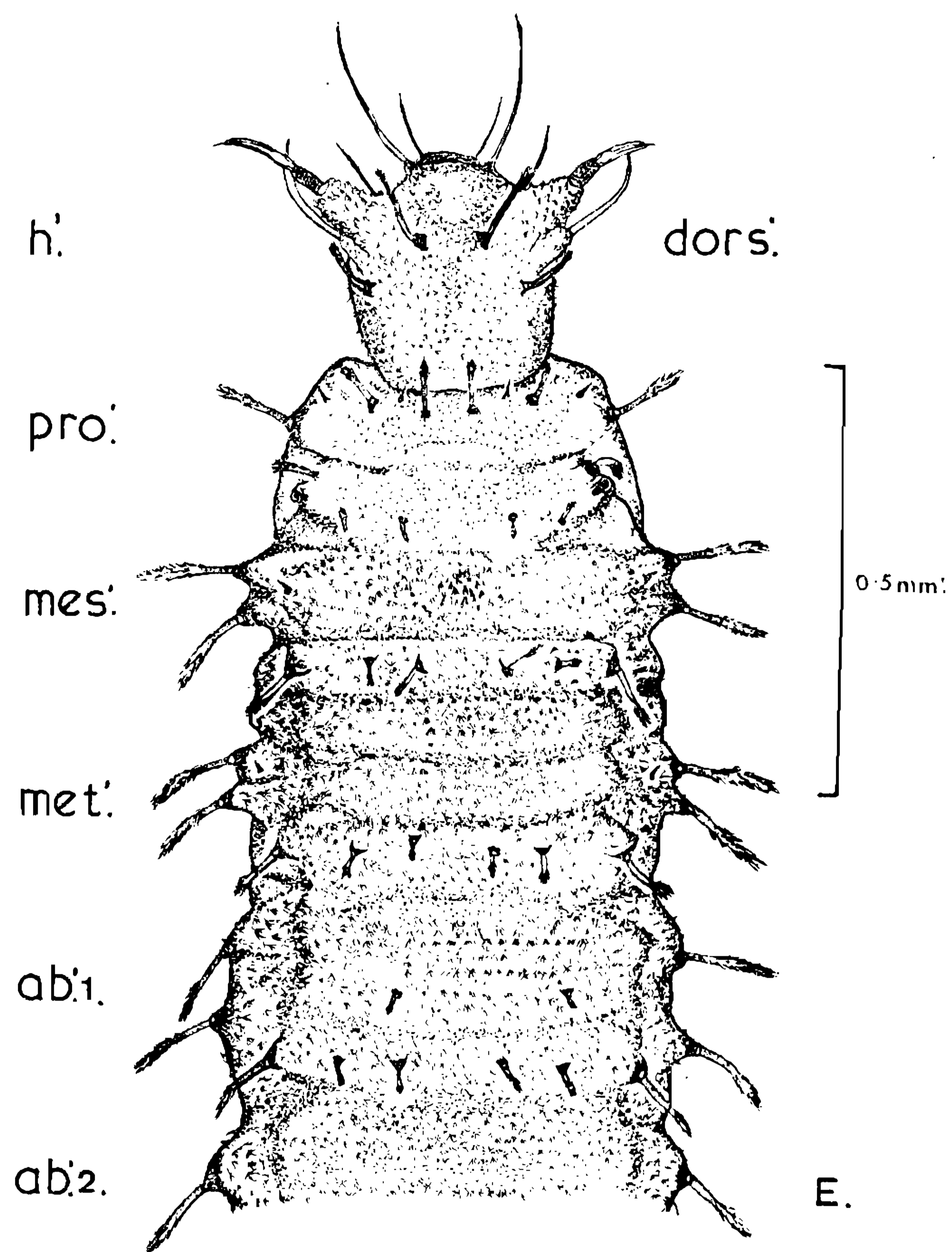
The head capsule ranges in size from 132-150  $\mu$  long, and 126-138  $\mu$  wide. The antennae which arise from prominent, sparsely spined tubercles, measure from 42-45  $\mu$  in length. The first segment is darkly pigmented, and under 1/2 the length of the second, which bears a short spine-like apical process.

II — *Prothorax.*

The brush-tipped, external prothoracic setae measure 18  $\mu$ , twice the length of the internal setae. The prothoracic dorsolateral setae are short (9  $\mu$ ) and brush-tipped.

III — *Meso and Metathorax.*

Both the meso and metathoracic and abdominal dorsolateral setae are well developed measuring 30  $\mu$ , equivalent in length to the abdominal basal setae. The meso and metathoracic basals measure 15  $\mu$ .



*Psychodopygus wellcomei* Fraiha, Shaw and Lainson, 1971.

Fig. E — Head, thorax and abdominal segments 1-2 of the 4th larval instar; dorsal face. Fig. G — Abdominal segments 6-9 of the 4th larval instar; dorsal face.



IV — *Caudal Setae*.

There are four caudal setae, the original pair of the first instar having become bifurcated. They measure from 1.326-1.378 mm. in length.

c) *3rd Instar*.

The total body length of the 3rd instar larva (fig. D) measures approximately 2.184 mm from the front of the head capsule to the base of the caudal setae (1 measured).

I — *Head*.

The head capsule measures approximately 210  $\mu$  long and 198  $\mu$  wide. The antennal segments, excluding the basal tubercle measure 72  $\mu$ . The first antennal segment is darkly pigmented, and under half the length of the second, which is translucent.

II — *Caudal Setae*.

The four caudal setae measure approximately 1.742 mm.

d) *4th Instar*.

The total body length of the 4th instar larva (figs. E-H), is from 2.416-3.856 mm., an average of 3.406 mm.. The body is darker in colour than in previous instars, and the cuticle more heavily sculptured over the dorsal surface, which is covered in rows of small mountainous tubercles terminating in spines. This sculpturing consists of both small and large spines, which when viewed dorsally appear star-like. The true outline of the spines is best viewed along the anterior dorsolateral edges of the body segments, where they achieve their greatest development.

I — *Head*.

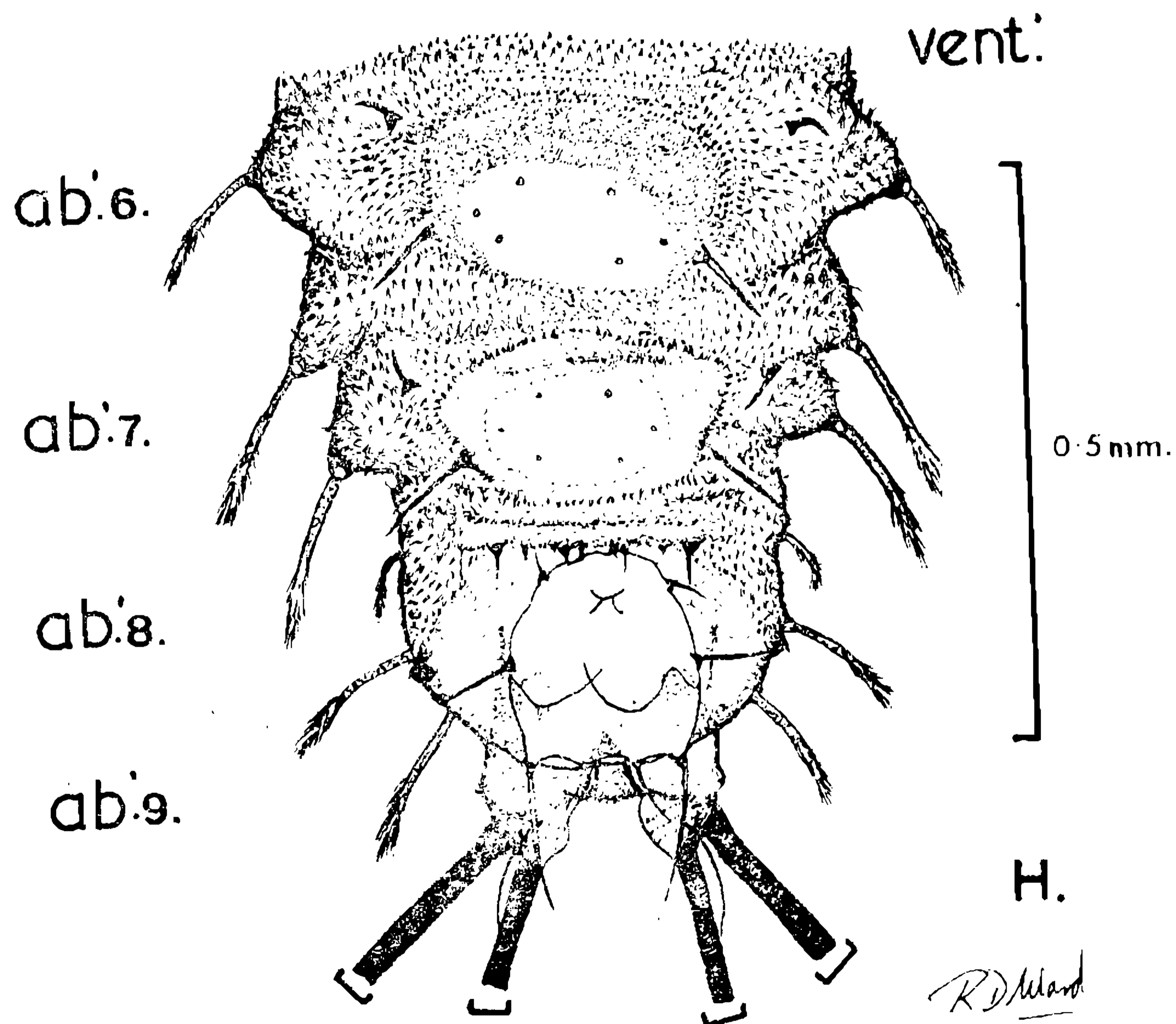
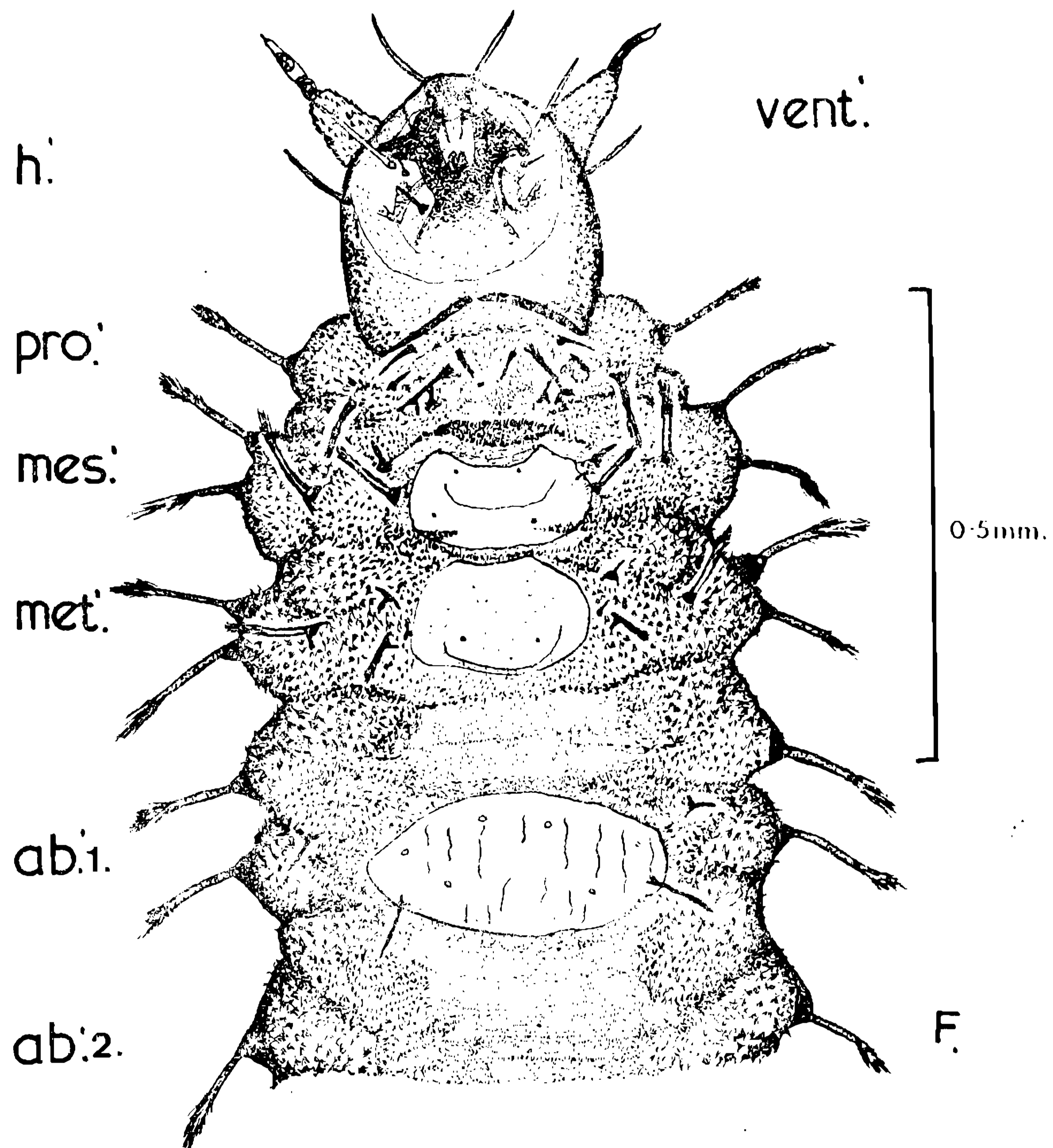
The head capsule measures from 240-312  $\mu$  in length (average 273.6  $\mu$ ), by 264-288  $\mu$  in width (average 270  $\mu$ ). The antennae arise from a large heavily spined

basal tubercle. The first antennal segment is darkly pigmented and half the length of the second, which is lighter in colour and terminates in a short spine-like apical process. The antennae are 96-102  $\mu$  in length (average 97.2  $\mu$ ). The clypeal setae are simple, spine-like and measure 90  $\mu$ . The anterior dorsal setae are simple, arise from prominent tubercles, and measure 180  $\mu$ , extending beyond the tips of the antenna. The posterior dorsal setae, are half the length of the anterior dorsals, brush-tipped, and sparsely barbed along the apical third. The posterior antennal setae are approximately two thirds the length of the posterior dorsals, and are similar in structure. The lateral setae arise from prominent tubercles which are slightly ventro-posterior to the antennal tubercles, and are somewhat obscured by them. They are long, measuring approximately 150  $\mu$ , and are finely barbed. The dorsal genal setae measure 78  $\mu$ , extend to the end of the first antennal segment, and are simple. The lateral genals are similar to the dorsals, and the ventral genal setae half the size of the dorsal and lateral setae.

II — *Dorsal and Ventral Prothorax*.

The prothorax appears to be lighter in colour than the subsequent body segments as it has fewer cuticular spines. The dorsal internal and external prothoracic setae measure 24  $\mu$ , are brush-tipped and pigmented. Between the internal and external setae there is a short, unpigmented, brush-tipped anterior accessory seta which measures 12  $\mu$ . Lateral to the external seta there is a short spine-like accessory shoulder seta measuring 21  $\mu$ . The dorsal submedian and internal posterior dorsal setae are short, measuring 6-9  $\mu$ , brush-tipped and pigmented. The dorsolateral setae, measure 30  $\mu$ , have a flared brush tip, and lie anterior to the prothoracic spiracles.

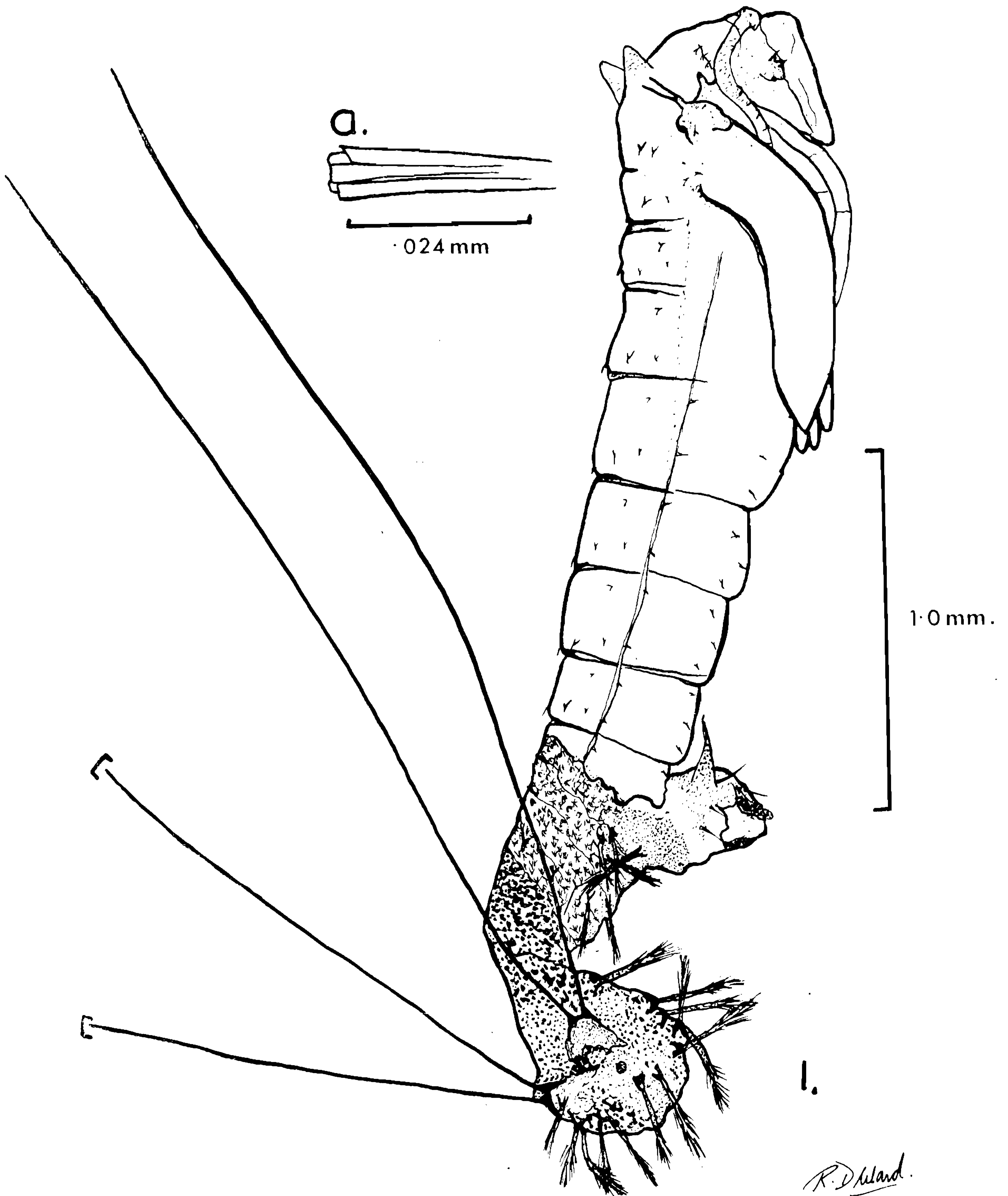
The heavily barbed anterior ventrolateral setae are the largest setae on this segment, measuring 105  $\mu$ . The ventral internal and external prothoracic setae measure 36  $\mu$  and 45  $\mu$  respectively, and are fine and simple. Arising from large tubercles, the ventral submedian setae measure 60  $\mu$ , are stoutly



*Psychodopygus wellcomei* Fraiha, Shaw and Lainson, 1971.

Fig. F — Head, thorax and abdominal segments 1-2 of the 4th larval instar; ventral face. Fig. H — Abdominal segments 6-9 of the 4th larval instar; ventral face.





*Psychodopygus wellcomei* Fraiha, Shaw and Lainson, 1971.

Fig. I — Pupa; lateral face. Fig. I-a — Pre-alar seta.

Fig. J — Setal nomenclature. *P. wellcomei*.*Head.*

c	.....	— clypeal.
ad	.....	— anterior dorsal.
pd	.....	— posterior dorsal.
ant t	.....	— antennal tubercle.
ant I-2	.....	— antennal segments.
ap	.....	— apical process.
l	.....	— lateral.
pa	.....	— posterior antennal.
dg	.....	— dorsal genal.
lg	.....	— lateral genal.
vg	.....	— ventral genal.

*Prothorax.*

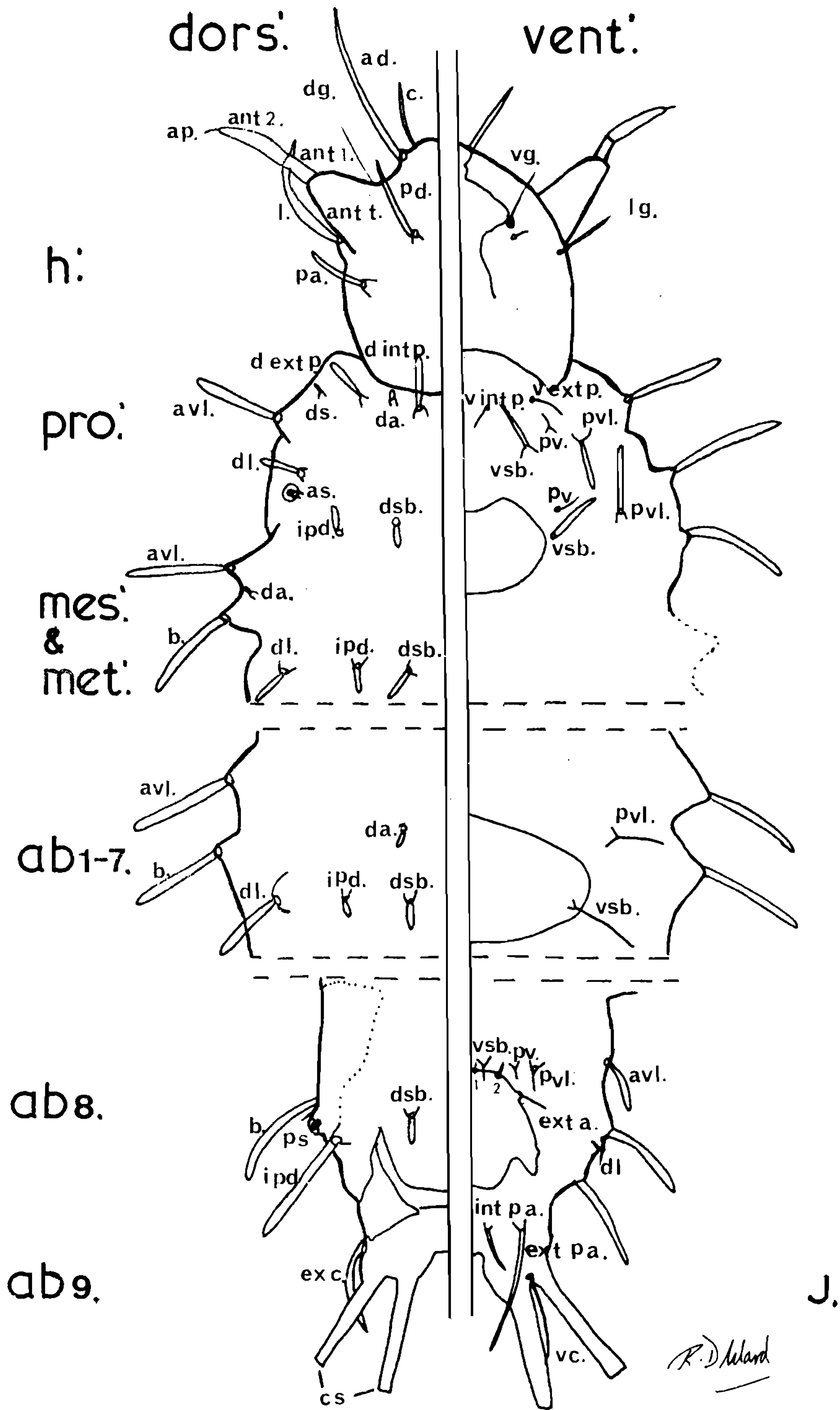
d int p	.....	— dorsal internal prothoracic.
d ext p	.....	— dorsal external prothoracic.
avl	.....	— anterior ventrolateral.
ds	.....	— dorsal shoulder accessory.
da	.....	— dorsal accessory.
d sb	.....	— dorsal submedian.
ipd	.....	— internal posterior dorsal.
dl	.....	— dorsolateral.
as	.....	— anterior spiracle.
v int p	.....	— ventral internal prothoracic.
v ext p	.....	— ventral external prothoracic.
vsb	.....	— ventral submedian.
pv	.....	— posterior ventral.
pvl	.....	— posterior ventrolateral.

*Meso & Metathorax and abdominal segments I-8.*

b	.....	— basal.
ps	.....	— posterior spiracle.
Rest of terminology as above.		

*Abdominal segment. 9.*

I	.....	— internal pre anal.
2	.....	— external pre anal.
ext a	.....	— external anal.
int p a	.....	— internal post. anal.
ext p a	.....	— external post anal.
vc	.....	— ventral caudal.
ext c	.....	— external caudal.
c s	.....	— caudal setae.



*Psychodopygus wellcomei* Fraiha, Shaw and Lainson, 1971.

Fig. J — Setal nomenclature of 4th larval instar; dorsal and ventral faces.



built and comb-tipped. The posterior ventral setae are short, measuring  $12\ \mu$ , spine-like, and lie anterior to the submedian setae. The posterior ventrolateral setae measure  $51\ \mu$ , arise from a slightly ridged tubercle, are stoutly built and brush-tipped.

### III — *Meso and Metathorax.*

**Dorsal setae.** The dorsal submedian, and internal posterior dorsal setae arise from prominent chitinized tubercles, measure  $12-18\ \mu$  and are brush-tipped. The dorsolateral setae arise from similar tubercles, measure  $60\ \mu$ , are barbed along the apical third of the shaft, and brush-tipped.

**Ventral setae.** The anterior ventrolateral, and posterior ventral setae are similar to those of the prothorax. Posterior, and slightly dorsal to the anterior ventrolateral seta there is a long ( $108\ \mu$ ), heavily barbed basal seta. These two setae arise from a lateral extension of the body wall, forming a bifurcated tip to the wall. Mesad of these two setae, and slightly dorsally, is a small inconspicuous spine-like dorsal accessory seta. This is possibly homologous with the more mesal dorsal accessory seta of the abdominal segments.

### IV — *Abdominal Segments 1-7.*

The abdominal segments 1-7 have identical setal patterns.

**Dorsal setae.** The dorsal accessory setae are brush-tipped, arise from small tubercles and are short, measuring  $12\ \mu$ . Other dorsal setae are as described for the meso and metathorax.

**Ventral setae.** The anterior ventrolateral setae are as described for the meso and metathorax. The relative positioning of the ventral setae, suggests that the posterior ventral setae are absent; that the ventral submedians

persist as long, fine, spine-like setae, lying at the edge of the abdominal "feet"; and that the posterior ventrolaterals are reduced to short spine-like setae. The basal setae of the meso and metathorax appear to persist in the same position, lying posterior, and slightly dorsal to the anterior ventrolaterals throughout the abdomen.

### V — *Abdominal Segment 8.*

There are two dorsolateral, heavily chitinized areas, bearing numerous small spines. The dorsal accessory setae are absent, and the brush-tipped dorsal submedian setae, short measuring  $13.5\ \mu$ . The internal posterior dorsal setae are well developed, measuring  $126\ \mu$ . The dorsolateral setae are reduced to short spines, measuring  $12\ \mu$ , and characterized by their anterior position in relation to the spiracles.

The anterior ventrolateral setae are medium in length, measuring  $36\ \mu$ , and barbed along the apical third. The remaining setae are all short and spine-like, not exceeding  $21\ \mu$ .

### VI — *Caudal Segment (Abdominal Segment 9).*

The caudal segment bears two large bifurcated caudal setae measuring from  $3.146-3.248\ \text{mm}$ . (average  $3.185\ \text{mm}$ ). The external caudal setae are of medium length, measure  $75\ \mu$ , are barbed, and tend to curve inwards above the caudal setae.

The ventral caudal setae are approximately the same length as the externals, but are finer and lie ventral to the caudal setal fork. The internal and external pre-anal setae are short, measuring  $6\ \mu$ , and  $24\ \mu$  respectively, and are spine-like. The external anal setae measure  $90\ \mu$  and are simple and spine-like. The internal posterior anal setae measure  $45\ \mu$  and the external post anal setae  $180\ \mu$ . The typical bifurcated foot of phlebotomine larvae is figured in the contracted state, (fig. H).

### 3. *The Pupa.*

With the exception of the pre-alar setae, the thoracic and abdominal setae are simple and spine-like (fig. I). The pre-alar setae (fig. Ia) are long, measuring 96  $\mu$ , and almost reach the mesonotal tubercle. Apically each seta is trifid with flattened blades; the middle of which is marginally ribbed. The mesonotal tubercles are covered in numerous small rounded protuberances.

### DISCUSSION

The only other description of the immature stages of a member of the genus *Psychodopygus* is that of *P. arthuri* which was described by Barretto in 1941. From this description it is clear that the setal pattern of the larval stages of *P. wellcomei* and *P. arthuri* is very similar. They are, however, separable on a number of small structural differences.

The eggs of *P. arthuri* are larger than those of *P. wellcomei*, and the exochorionic sculpturing of *P. arthuri* is in the form of longitudinal crystals, whilst that of *P. wellcomei* has a 'mountainous' appearance.

In the 4th instar larvae the following differences are apparent. The ratio between the 1st and 2nd antennal segments is 1:3.4–4 in *P. arthuri*, and 1:2 in *P. wellcomei*. The lateral head setae in *P. arthuri* are heavily barbed, whilst those of *P. wellcomei* are only lightly barbed apically. The posterior dorsal setae of *P. arthuri* are 1.75 times the length of those in *P. wellcomei*. The dorsolateral setae of the prothorax of *P. wellcomei* appear to be shorter than

those of *P. arthuri*. In the pupal stage of *P. wellcomei* the pre-alar setae are trifid, in contrast to the bifid form in *P. arthuri*. A further distinguishing feature is the simple, spine-like form of the thoracic and abdominal setae in *P. wellcomei*, and their rounded apices in *P. arthuri* (Carneiro & Sherlock, 1964).

### SUMÁRIO

Algumas observações sobre a biologia e morfologia dos estágios imaturos de *Psychodopygus wellcomei* Fraiha, Shaw and Lainson 1971 (Diptera: Psychodidae)

Em condições de laboratório, o desenvolvimento dos ovos à forma adulta do *P. wellcomei* leva, em média, 42 dias.

Os estádios larvários são semelhantes àqueles do *P. arthuri*, descrito por Barretto (1941), mas podem ser distinguidos deste último pelo tamanho do primeiro e segundo segmentos antenais e pela forma das cerdas laterais da cabeça e dorso-lateral do protórax. O estágio pupal do *P. wellcomei* é caracterizado por uma cerda pré-alar trifurcada e por simples cerdas, torácica e abdominal, semelhantes a um espinho.

### ACKNOWLEDGMENTS

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