

Acetylacetone-Dimethylaminobenzaldehyde Reagent of

Block, Durrum and Zweig (1958) p. 209, which reveals free hexo-
samines as cherry-red spots and N-acetylglucosamine as purple-
violet spots.

p-Anisidine HCl for revelation of uronic acids see Block,
Durrum and Zweig (1958) p. 182.

C. Culturing the Fungus.

Except for experiments in Section VI, (iv), the special medium developed for the Sirex fungus, NDY/6 with 2 p.p.m. of o-phenyl phenol, has been used in all culturing experiments.

All experiments were carried out at a constant temperature of 22°C.

(1) Cultures made from the Larva.

(a) The Gut.

In an attempt to verify the claim of Clark (1933) that there were glands in the hind gut of female larvae which carried the Sirex fungus, cultures were made from twenty females and ten male larvae. The larvae were rinsed three times in sterile water, and then dissected dry. The gut was cut off anteriorly across the narrow oesophagus and posteriorly in front of the anus. The hind-gut was cultured separately from the mid-gut in an attempt to see which regions were giving results.

(b) The Hypo-pleural Organ.

To obtain cultures from the hypo-pleural organs of female larvae, the organ was cut out. Underlying fat body was scraped off before the organ was cultured. No attempt was made to sterilise the external surface of the organ.

(c) Larval tunnels.

Cultures were made from slivers of wood sliced from the larval tunnels with a sterile scalpel.

(ii) Pupa and Adult.

(a) Wood scrapings in pupal chamber, the pre-pupal skin.

Cultures were made from the wood lining the pupal chamber, from wood scrapings, from the surface of the frass and sometimes from the pre-pupal skin.

(b) Ovipositor and inter-segmental sacs.

Very few specimens were available for these experiments and inevitably some were contaminated.

To study the onset and development of fungal inoculation of the inter-segmental sacs, female pupae and adults at different stages of development were cut out of the wood.

Cultures were made from the head, thorax, abdominal sclerites, tip of the abdomen and ovipositor. The ovipositor was cut off at the junction of the saw sheath and its base. The sheath was cultured separately from the distal end of the valvulae. The proximal parts of the valvulae were lifted out of the sheath base and cut off as close to the body as possible.

The membranous and muscular connexions between the sub-genital plate and the inter-segmental sacs were severed, so that the sacs could be used for culturing too. In this way it was possible to see from which areas of the body and which regions of the ovipositor the fungus could be cultured at the different stages of development of the female.

(iii) Germination of Wax Packets.

To see whether the individual wax packets would germinate when ruptured, ten packets were cultured on the special medium for the Sirex fungus. Five of these packets were damaged with a sharp needle. The rough maps drawn of each plate, showing the relative positions of the wax packets, made the subsequent inspection of these plates easier.

(iv) Effect of mucous and oily secretions on the fungus.

(a) Stock cultures.

The effect of these secretions on the vegetative growth of the fungus was investigated as follows:

Stock cultures of the fungus were established on NDY/6. Isolates punched out of stock cultures with a cork borer of diameter 0.3 mm were sub-cultured on plates of 2% water agar, on which the vegetative growth is sparse.

Three days later, a sparse corona of fungal threads of radius two to three millimeters, could be seen growing around each isolate. At this stage, secretions of the accessory glands were placed 2 cm

away from each isolate. Altogether there were 4 different treatments replicated four times in this experiment-

- (1) only the oily secretion was used
- (2) " " mucus " " "
- (3) a mixture of the two secretions was used
- (4) there were no secretions on the control plates.

The plates were examined daily for a week.

A similar experiment was carried out using macerated club glands. The presence of cellular material not normally accessible to the fungus made it difficult to assess the results. Ideally, the oily droplets occurring in the inter-segmental sacs of teneral adults should have been used. The shortage of females at this stage made this approach impossible.

(b) Wax packets.

In an attempt to see whether undamaged wax packets could be stimulated to germinate by these secretions, twelve packets were placed on water agar and left for three days before they were given four different treatments. This method ensured that no damaged packets were tested with the secretions.

- (1) The packets were coated with drops of the oily secretion.
- (2) They were covered with mucus.
- (3) They were coated with a mixture of oil and mucus.
- (4) They were not treated at all.

The plates were examined daily for two weeks.

D. Infective Behaviour.

A teneral adult female, which had recently emerged from a pupa placed in a small test tube lined with filter paper, was kept under close observation for three hours to see whether there was a display of adaptive infective behaviour.

IV.

ANATOMY OF SIREX NOCTILIO(1) Larva.

(a) Dissections and Serial Sections.

Parkin (1942) drew attention to the structure of the gut of Siricid larvae which is much simpler than is generally found in larvae digesting wood. The possibility that the larvae of Siricidae feed either on the fungus lining their tunnels, or on wood which has been attacked by fungus, made the digestive system the focus of attention during these studies which have revealed that there are extensive, branched salivary glands, throughout the body cavity (see Figure 1a). There is considerable variation in the size of the salivary reservoirs, some being wider than the mid-gut, others being as narrow as the oesophagus. A possible interpretation of this variation is that the reservoirs discharge their entire contents in one dose. Morgan (pers. comm.) has suggested that the mandibles fit together to form a cup in which scrapings of wood and fungus might be pre-digested by saliva. Although Francke-Grosmann (1939) found traces of mycelium in the gut of larvae, the gut contents of specimens of Sirex noctilio examined in this study can best be described as a milky fluid containing coarse particles.

There are differences between the larvae of S. noctilio

Figure 1.

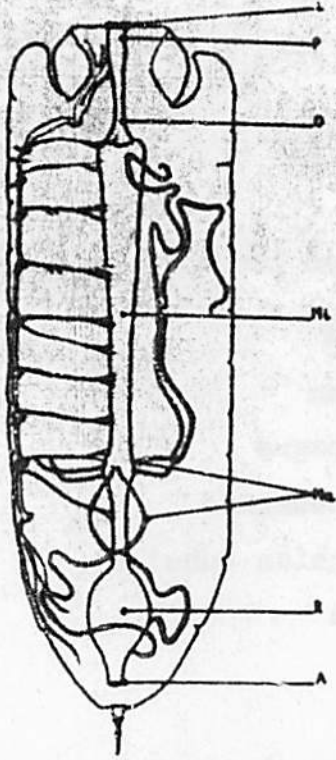
a. Gut and main tracheae of larva of *S. noctilio*.

- l labrum
- p pharynx
- o oesophagus
- Mi mid-intestine
- Ma malpighian tubules
- R rectum
- A anus

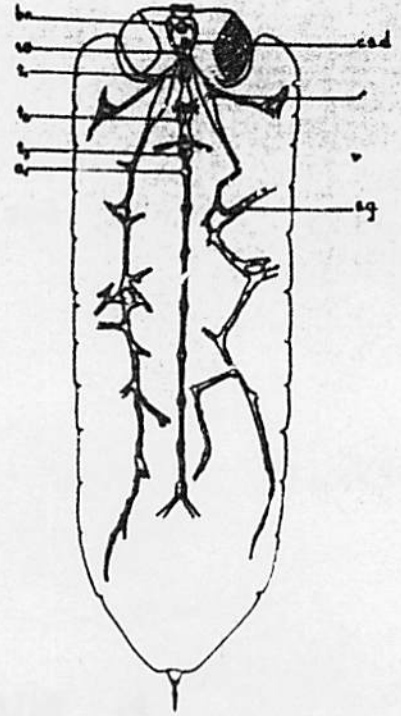
b. Salivary glands and central nervous system of larva of *S. noctilio*.

- csd central salivary duct
- r salivary reservoir
- sg branched salivary gland
- br brain
- so sub-oesophageal ganglia
- t₁ first thoracic ganglia
- a₁ first abdominal ganglia

c. Gut and salivary gland of larva of *S. noctilio* after Maxwell.



a



b



c

FIG. 1

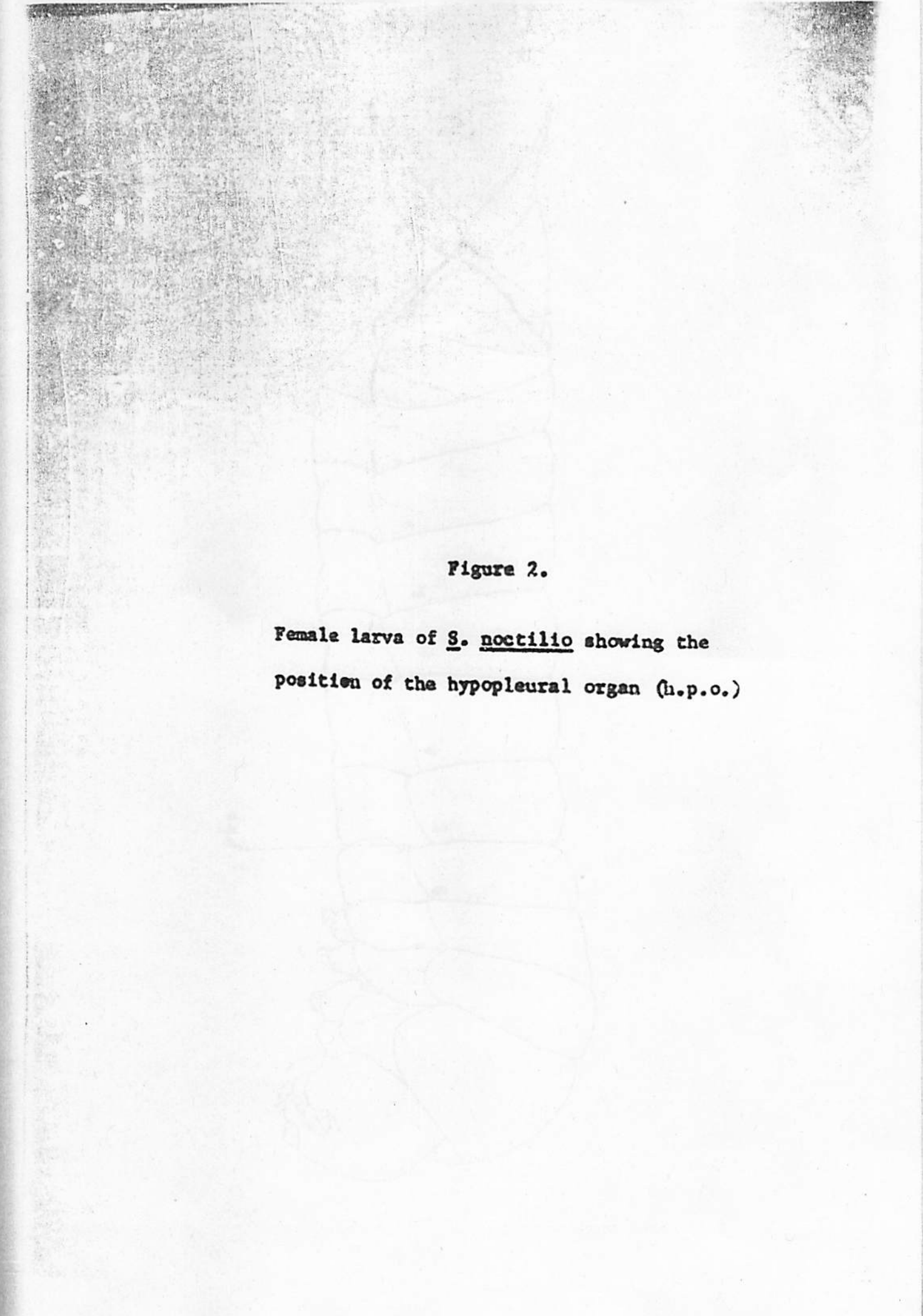
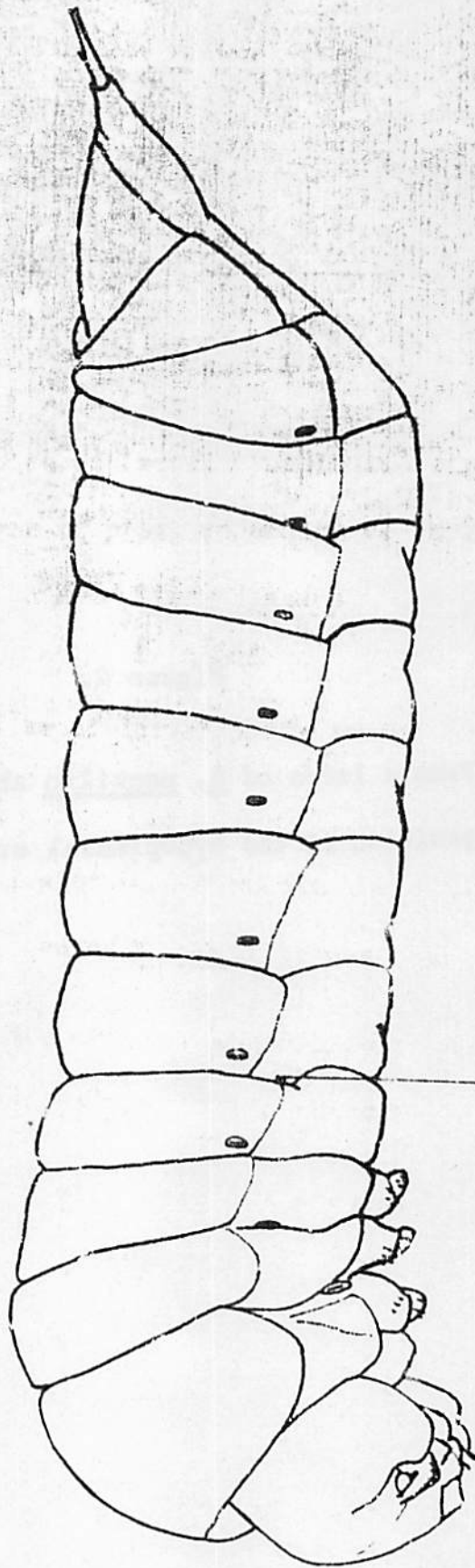


Figure 2.

Female larva of S. noctilio showing the
position of the hypopleural organ (h.p.o.)



h.p.o

Fig.

Figure 3.

- a. Surface view of larval hypopleural organ showing arrangement of pits, according to Parkin.
- A Sirex cyaneus
B S. gigas
- b. Surface view of larval hypo-pleural organ of S. noctilio mag. 90x.
- c. Dark bundles of fungus within the pits of the hypo-pleural organ of S. noctilio mag 330x.
- d. Surface view of two damaged pits of the hypo-pleural organ of S. noctilio. The third pit is lying on its side.
- e. An isolated pit, showing a projection of the scalloped base partially dividing it. Mag. 330x.

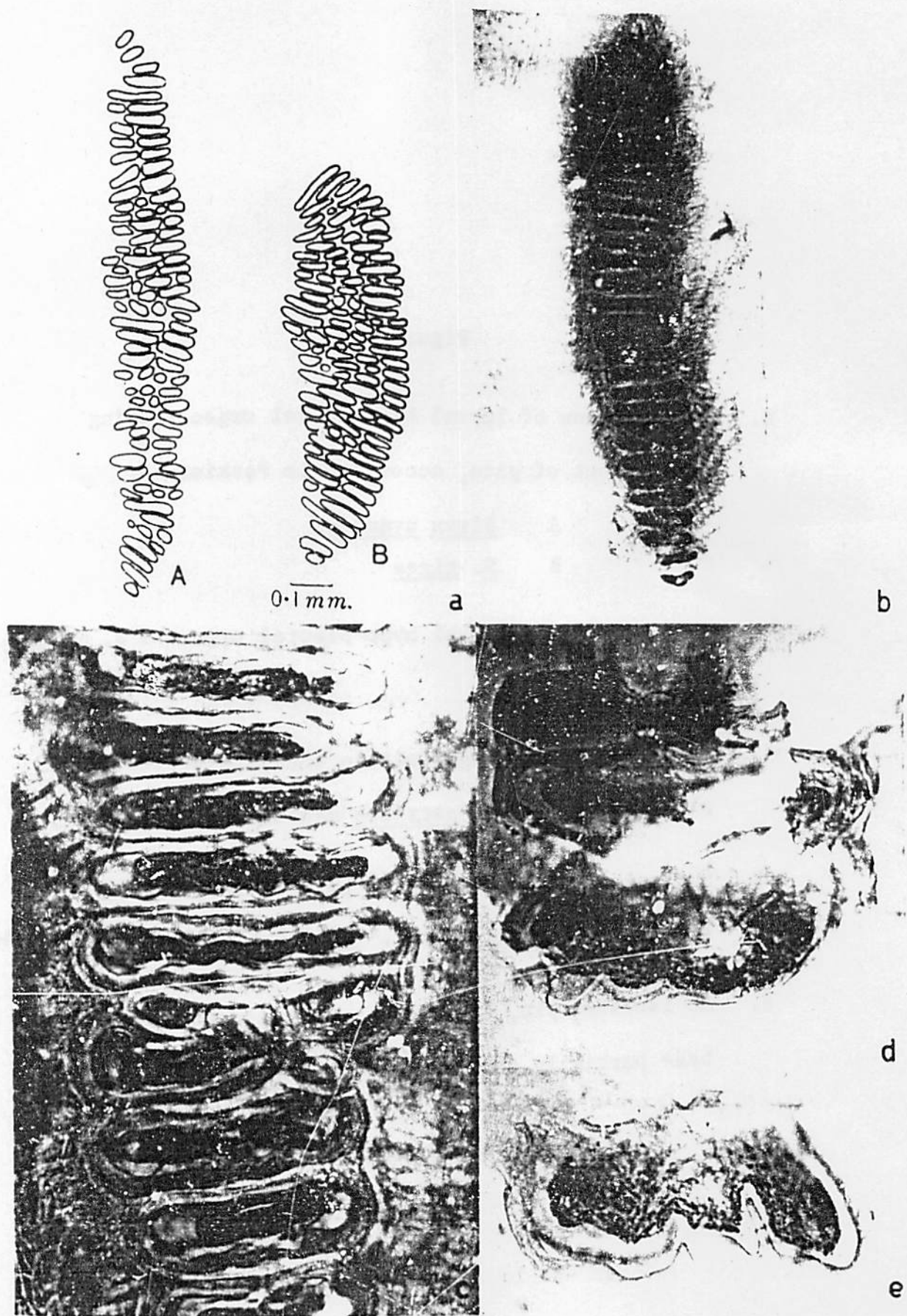


FIG. 3

examined during this study and those described by Maxwell (1955). Whereas the larvae obtained from logs of Pinus radiata in Tasmania have six malpighian tubules and extensive branched salivary glands, those obtained from Pinus sp. in England and described by Maxwell, have eight malpighian tubules and "slender squared salivary ducts widening posteriorly into rectangular glandular body". (Compare Fig. 1a with Fig. 1c).

Attempts to locate the glands in the hind-gut described by Clarke (1933) were also unsuccessful. Serial sections show there are six longitudinal pads lining the internal surface of the rectum which has a rich tracheal supply. Considering the dryness of the frass and the fluid condition of the contents of the mid-gut, these pads might be the sites at which water is resorbed.

The structure of the hypo-pleural organ, which was worked out from serial sections cut vertically, sagittally and horizontally, will be discussed in (1)b.

(b) The Hypo-pleural Organ.

1. The position of the hypo-pleural organ on the posterior surface of the pleuron of first abdominal segment of female larvae is shown in Figure 2.

Compared with the drawings of the hypo-pleural organ of U. gigas and S. cyaneus (Fig. 3a) first published by Parkin (1942),

the surface appears to be a series of narrow pits. Under high magnification (Fig. 3c) the darkened coils of fungus are clearly visible within the pits.

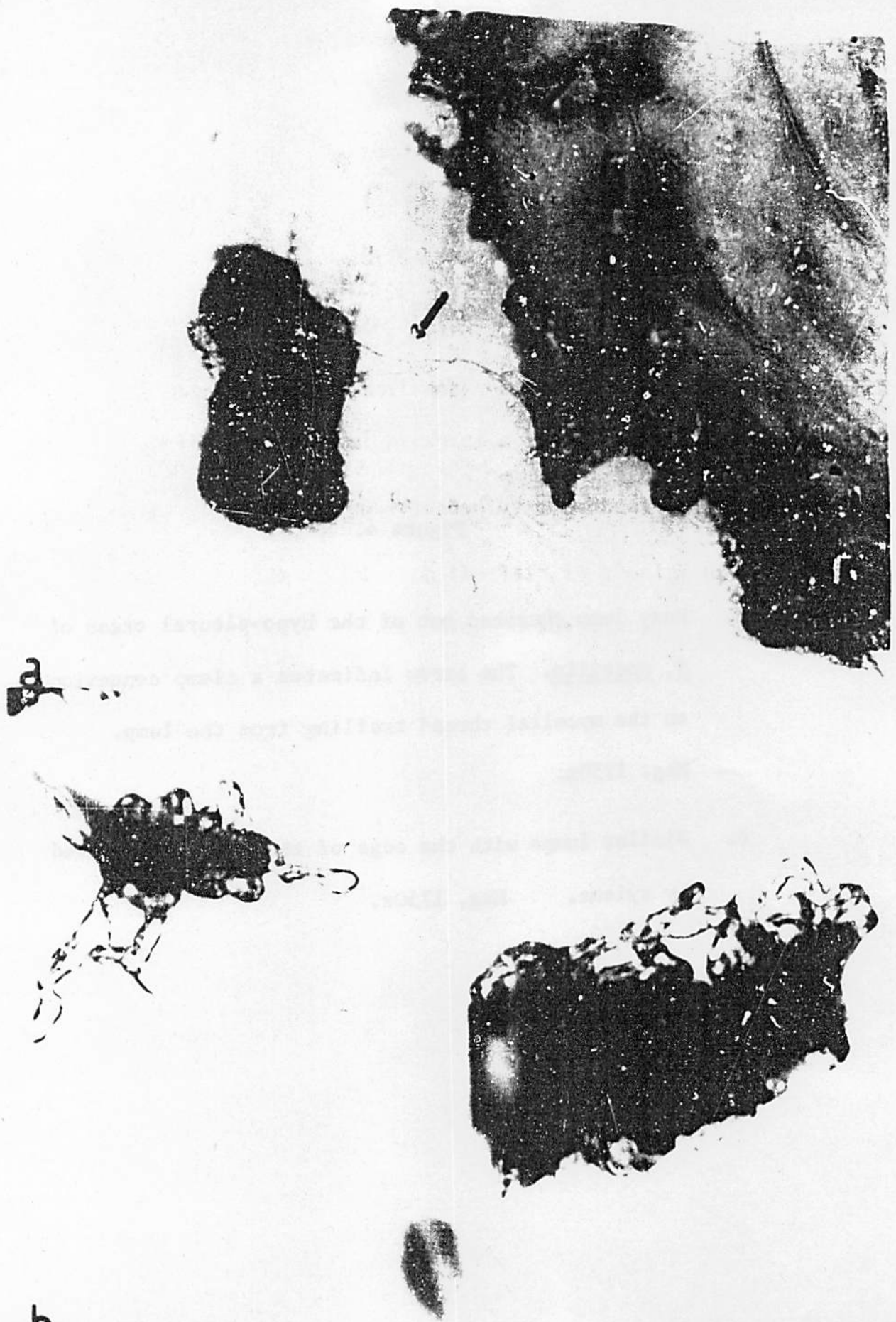
Parkin (1942) suggested that larvae of closely related woodwasp species could be identified from the arrangement of the pits and the ratio of the length to the width of the hypo-pleural organ. He gave the measurements of the hypo-pleural organ of almost fully grown larvae of S. cyaneus and U. gigas. From these measurements it can be seen that the length/width ratio would be approximately 5:1 for S. cyaneus and 3:1 U. gigas.

Rawlings (1953) has shown that this ratio is 6:1 for larvae of S. noctilio.

Morgan F.D. (Manuscript) has shown that the size of the larva varies with the moisture content of the wood. As the hypo-pleural organ is extended with each moult and its size is also related to the size of the larva, only when rearing conditions are identical, are comparisons between the hypo-pleural organs of similar-sized larvae of different woodwasp species possible. As environmental conditions vary widely, the usefulness of this method of identification is limited.

Figure 4.

- a. Waxy lump squashed out of the hypo-pleural organ of S. noctilio. The arrow indicates a clamp connexion on the mycelial thread trailing from the lump.
Mag. 1750x.
- b. Similar lumps with the edge of the matrix dissolved by xylene. Mag. 1750x.



b

FIG. 4

Figure 5.

Eight consecutive horizontal sections taken near the center of the hypo-pleural organ of a large larva of S. noctilio.

- a. A major pit sub-divided by a central partition.
- b. The sub-division on the left is closing up. A third sub-division is forming on the right.
- c & d. The closure of the left sub-division is completed.
- e. Another sub-division is forming on the right.
- f. Three sub-divisions are of approximately equal size.
- g. The left sub-division has closed up, the right sub-division is partially obscured.
- h. The thin wall blocks off the entire pit.

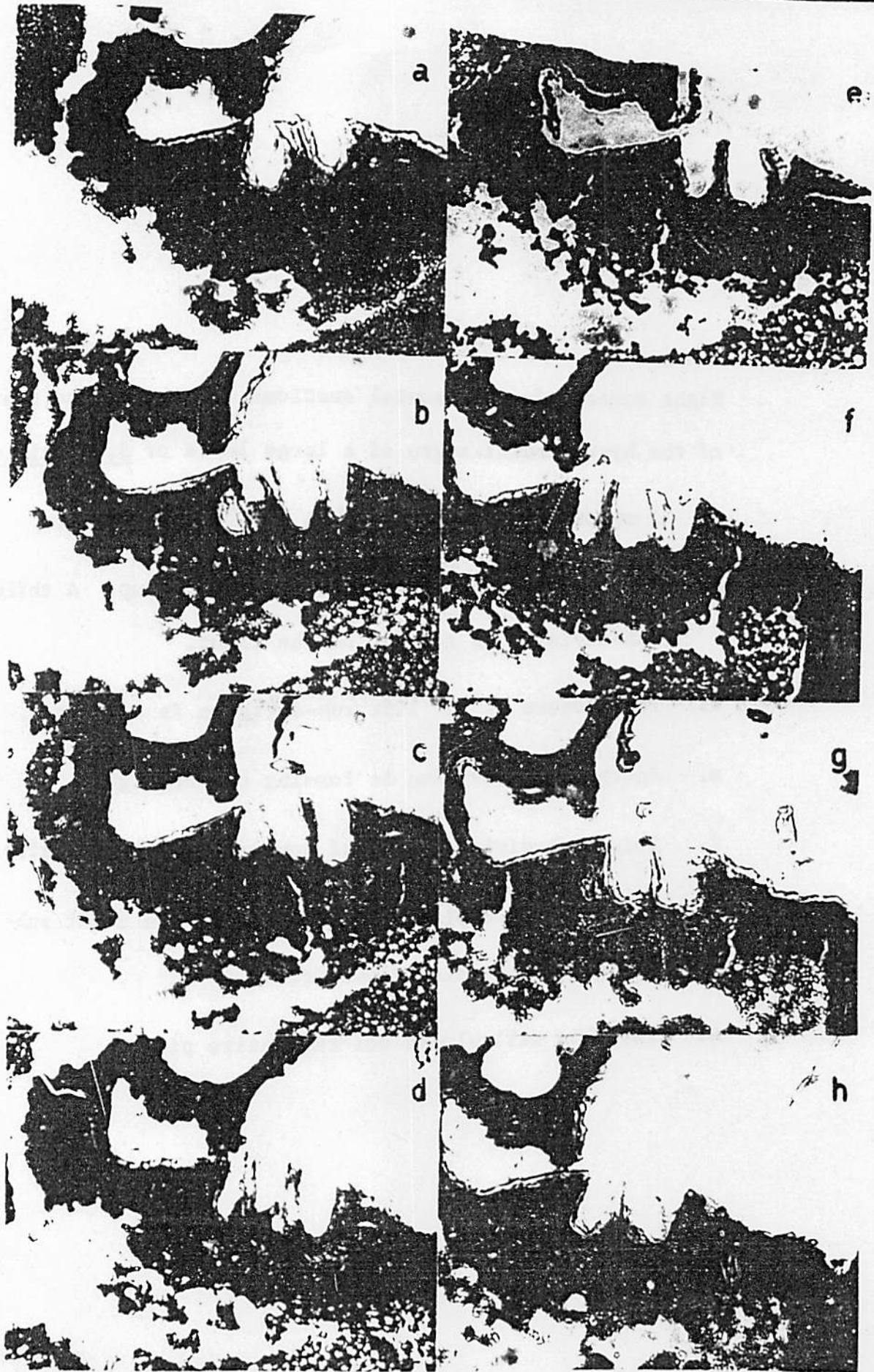


FIG. 5

Figure 6.

- a. Vertical longitudinal section of the hypopleural organ of a second instar larva of S. noctilio, showing eight major pits. The thick septa carry stout spines. Mag. 400x.
- b. Vertical longitudinal section of the hypo-pleural organ of a large larva of S. noctilio showing twenty-five pits, most of them carrying fungus. Mag. 80x.
- c. Vertical longitudinal section of the hypo-pleural organ of a pre-pupa of S. noctilio, showing the shrunken septa without any lumen. Mag. 160x.

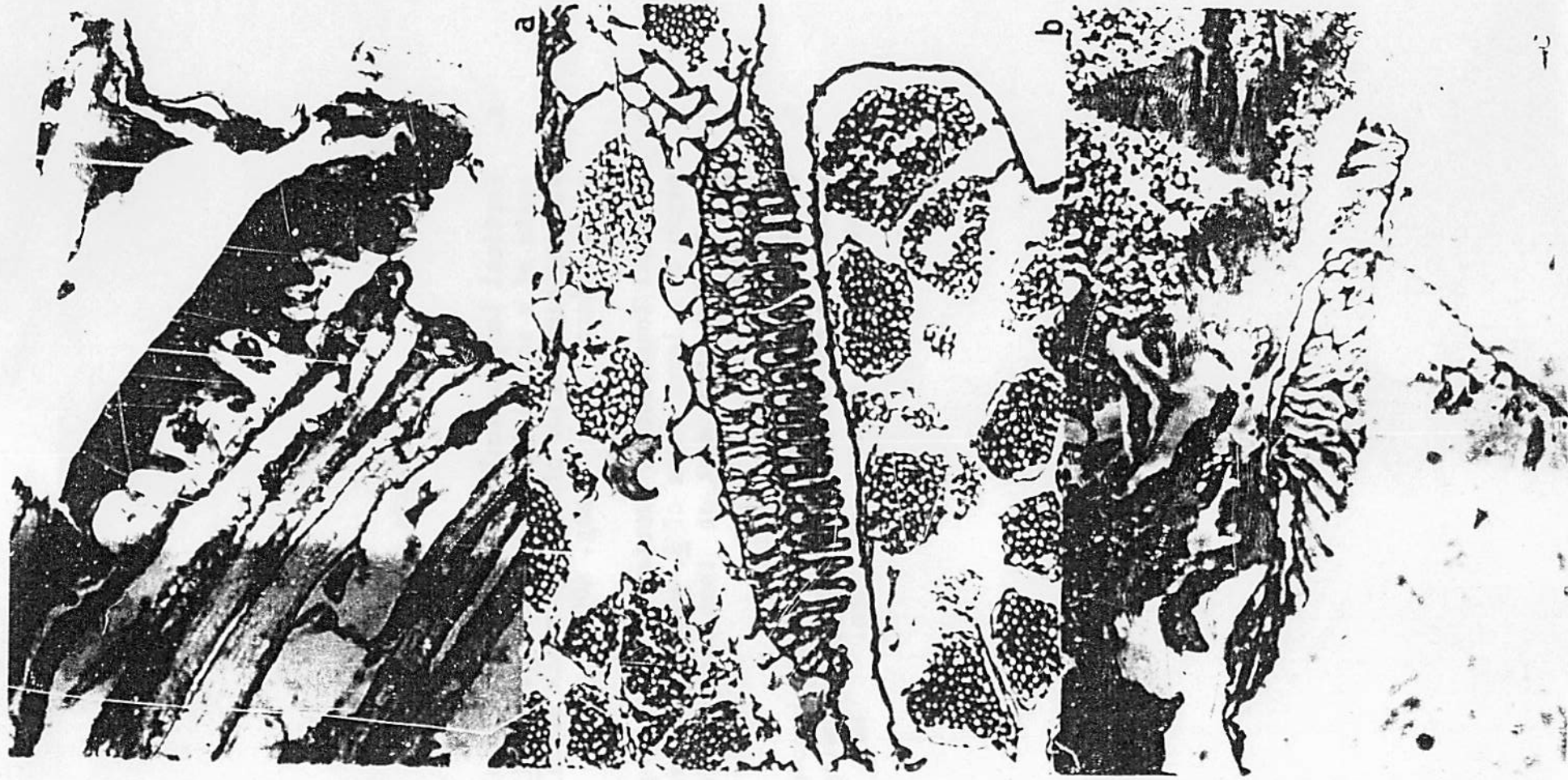


FIG. 3

Figure 6d.

Longitudinal section of female larva of
S. noctilio showing the main hypo-pleural
organ and a smaller organ on the anterior
segment.

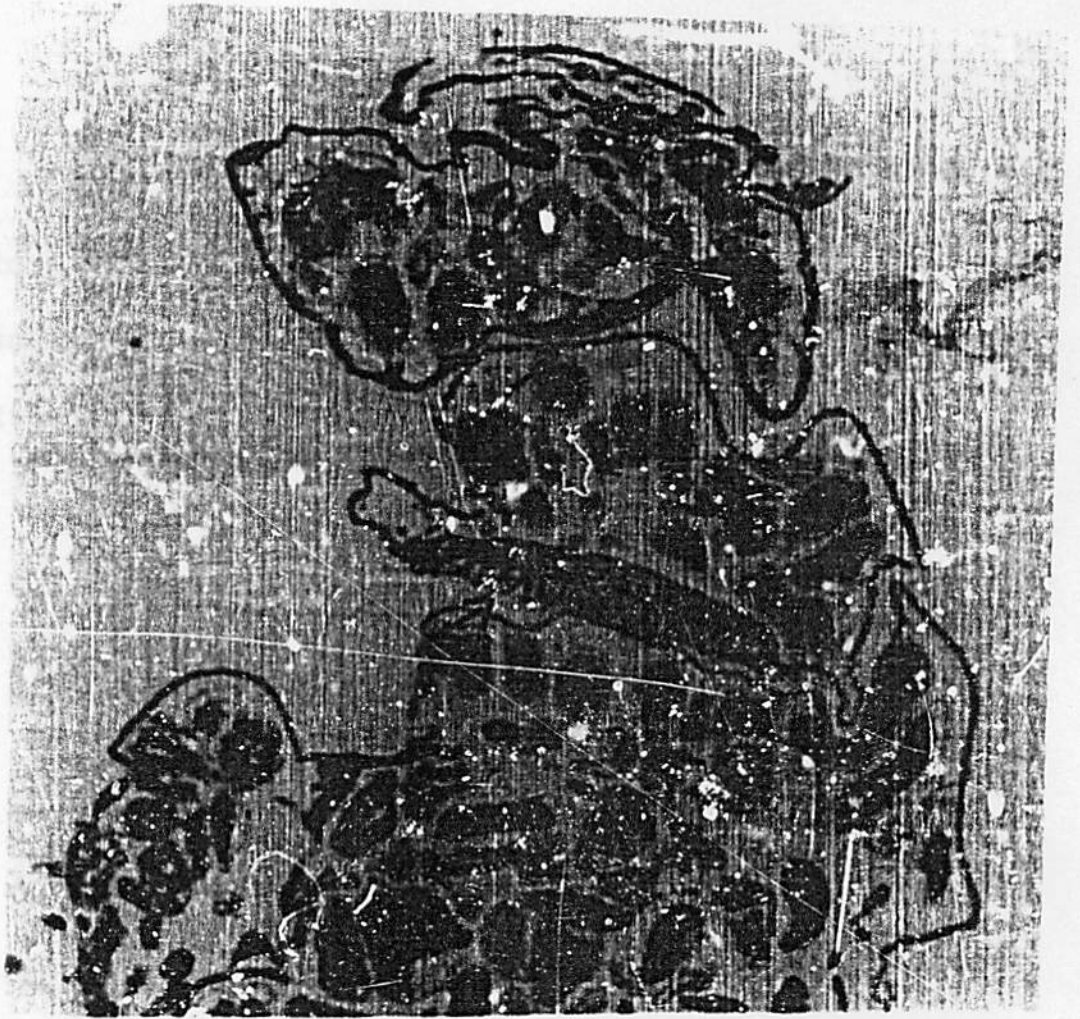


Table I indicates the variation in length/width ratio of the hypo-pleural organ of a few different sized larvae of S. noctilio.

TABLE I.

S. noctilio - measurements of hypo-pleural organ.

♀ larva	length	width	ratio	
1	4.1 units	0.9	4.5	} from same small larva
2	4	0.85	5.1	
3	8.4	1.2	7	
4	9.0	1.0	9	
5	5.5	0.9	6	
6	8.6	1.2	7.1	
7	7.6	1.2	6.3	

Gross appearance of the fungus.

Squashes of the organ, show the contents as waxy lumps, discoloured with a tangle of fungus. The wax readily colours with Sudan IV and Sudan Brown. After some of the wax has been dissolved away the fungus can be stained with Aniline Blue and Phloxine B. Fig. 4a, shows a strand of fungus with clamp connexions, extending from a waxy lump squeezed out of the organ.

On one occasion, the secretion within the pits came streaming out as oily droplets, and not as a firm lump.

In serial sections of the organ, from which all wax has been removed, a few growing tips can sometimes be seen protruding from the tightly-coiled, mycelial balls. Fig. 5b.

2. As would be expected from the external appearance, in vertical longitudinal sections the organ resembles a comb, the

teeth of the comb being the septa between the pits (Fig. 6b). Horizontal longitudinal sections made during this study indicate that towards the centre of the organ, the pits usually have two internal partitions. (Fig. 5). There may be as many as four of these partitions. Parkin (1942) has shown that similar subdivisions of the major pits occur in S. cyaneus.

There is often considerable variation in the structure of the organs, not only from larva to larva, but also between the two organs on a single larva.

Stillwell (1965) described an additional pair of hypo-pleural organs in T. columba situated on the posterior fold of the metathorax. These organs were smaller than the principal pair on the first abdominal segment and were not as heavily infected with fungus. In only one of the seventeen female larvae of S. noctilio could an additional pair of hypo-pleural organs be detected.

(Fig. 6d). It was also situated on the posterior fold of the metathorax. It is doubtful whether these smaller organs would be visible in external examinations, as they could be overlooked even in sections. There was no sign of fungus in the cavities which in these sections, did not appear to open to the exterior.

This discovery leads to the suggestion that perhaps there were once cuticular organs on all body segments of larval Siricidae. Although Yuasa (1923) lists the types of glands opening to the