THE BEHAVIOUR AND BREEDING BIOLOGY OF THE RATFLEA *Nosopsyllus fasciatus* (Bosc).

By

QAZI JAVED IQBAL

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Supervisor: D.A. HUMPHRIES.

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SUMMARY

Various structural and behavioural aspects of the breeding biology of *Nosopsyllus fasciatus* (Bosc.) the European rat flea, were studied in the laboratory.

1. Mating is initiated when a contact-chemical stimulus is received by the male maxillary palps. The copulating pair is negatively photofactic. The general pattern of mating behaviour is similar to that found in other Ceratophyllid fleas.

2. The adhesive discs of the male antenna and the sculpturing of the female second abdominal sternite maximise the frictional forces necessary for antennal clasping during copulation.

3. Previous studies of the structure of male flea genitalia are extended by use of the scanning electron microscope, enabling a better understanding of their action. The two penis rods are shown to run one within the other, the inner rod emerging from an aperture in the washer-like end of the outer rod.

4. Exposure to a temporary rise in temperature is shown to be an important factor in mating behaviour and ovarian development. Both sexes normally require a bloodmeal before they will mate, but will mate unfed at temperatures between 30 and 35°C, or if temporarily exposed to this temperature range. A bloodmeal has a longer lasting effect on sexual activity than does a rise in temperature.

5. The mating pheromone is not secreted by unfed male or female fleas but is present on the cuticle of both sexes, when they have recently fed or experienced a temperature rise to 30°C. It is argued that the brief rise in temperature triggers a process of pheromone secretion which continues for several hours after the

temperature has fallen. The pheromone is shown to be transient.

6. Chilling below 20°C renders previous attractive females unattractive to males.

7. Females are attractive but non-receptive to males after their first mating. Receptivity cannot be induced again for 72 hours.

8. Females of *N. fasciatus* are in the second stage of ovarian maturity at emergence from the cocoon. Ovarian development is further stimulated by a brief exposure to 30°C, but a bloodmeal is necessary for full yolk deposition and for egg laying. Copulation also accelerates ovarian development.

9. The presence of males with the pregnant females stimulates egg productivity.

10. Feeding on the host lasts from two to three hours although it is physically possible for a full bloodmeal to be taken within ten minutes. Sectioning of the rat's skin and observation of behaviour while on the host showed this long period to result partly from the difficulty of striking a suitable blood vessel and partly from disturbance by the host's grooming.

11. Fleas are killed on areas of the host body which can be reached by its mouth, or which are allogroomed. It is suggested that displacement grooming may have a secondary anti-parasite function.

12. In a single bloodmeal, a female takes about nine times more blood than the male.

13. The adaptations of the larval mouthparts for rasping solid food particles, and for taking liquid faecal blood directly from the imaginal anus are described.

CONTENTS

CHAPTER I

INTRODUCTION

INTRODUCTION

Much work has been done on rat fleas in relation to their medical importance as vectors of disease, especially bubonic plague. The importance of rat fleas was first realized in the last years of the nineteenth century, when Ogata (1897) put forth the hypothesis that rat fleas act as vectors of bubonic plague. Later, Thompson (1903) and independently, Rayband and Virjbitski (1902-3), proved experimentally that plague in India is transmitted from rat to rat by the Oriental rat flea *Xenopsylla cheopis* (Roths.). Since that time, several other species of rodent fleas including *Nosopsyllus fasciatus* (Bosc.) have been reported to be capable of transmitting plague from rodent to rodent under certain conditions, (Goyle, 1928; Ioff, 1941 and Burroughs, 1947).

As a result of this epidemiological interest our knowledge of the biology of rat fleas has accumulated in certain limited areas, for example, taxonomy, anatomy, physiological studies of survival and development in relation to climate, population surveys and experiments on host preference. Relatively little is known aboutcertain other important aspects of rat flea biology, for example, much of basic physiology (apart from the work of Wigglesworth (1935) on respiration) and ecology which has been concerned mainly with surveys of population rather than with fundamental studies on factors affecting the fleas at various stages in their life cycle (apart from extensive studies on the effects of temperature and humidity (Hirst, 1953)). Behaviour too has received little attention until recently (Humphries, 1967).

The present project includes parts of all the above mentioned three areas, and sets out to describe basic physiological, structural and behavioural features which bear on the breeding ecology of *Nosopsyllus*

fasciatus both in their relevance to reproduction and in the part they play in the relationship with the host.

The choice of *N. fasciatus* arises not only from its cosmopolitan distribution and wide host preference, but also from the fact that ecologically and structurally it is a fairly typical representative of rodent fleas, so that knowledge of its basic biology may be expected to give some indication of general features of rodent flea biology.

CHAPTER 2

MATERIALS AND METHODS

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Six stock cultures were established. The fleas were obtained from a laboratory stock of *N. fasciatus* at the Molteno Institute, Cambridge. This stock had itself originated from fleas taken from wild brown rats *(Rattus norvegicus)* in central Birmingham. Polypropylene cages (approximately 43 cm by 28 cm, and 16 cm high) were used as culture cages, each standing in moat trays (79 cm by 48 cm and 4.5 cm high) filled with water to prevent the escape of fleas. The depth of sawdust bedding in each cage varied approximately from 4 cm to 7 cm. Originally one female and five male albino rats (Wistar strain), approximatley a month old were used as hosts, one in each cage. After the first month, the female was replaced by a male rat. Unless otherwise stated, all fleas used for experimental purposes were derived from cultures established on the male host. The cages were kept in a room whose temperature varied seasonally over the approximate range of 17°C - 23°C; the actual temperature at which the flea eggs and larvae developed was obviously considerably influenced by the rat occupying the cage. A typical temperature range beneath the surface of the sawdust was 22°C - 27°C. An appropriate humidity was maintained by the urine of the host, and by the water spilled by the rat while drinking from the water-bottle.

The tendency for a thick wet layer of sawdust to build up at the base was dealt with by replacing the sawdust every three months. Similarly, abundance of mites *(Tyroglyphus farinae)* which are predators on flea larvae was reduced by the same procedure. The adult flea's eggs and larvae were sieved out from the old sawdust and put into the new culture cages with fresh sawdust bedding. Every six months, the rats from the culture cages were replaced by a batch of six fresh male rats.

All fleas used for experimental purposes were taken as second and third instar larvae, sorted by inspection from the stock cultures, and reared to the imaginal state under controlled conditions. They were always kept in batches of 15, in bottles 7.5 cm high and 2.5 cm in diameter, each containing 1 g powdered rat's blood(air dried at about 23°C) and 1 g finely ground rat-cake all mixed well with about 3 g sawdust. Rat's blood was used to rear the fleas as it seemed appropriate to use blood of their natural host rather than horse blood used for example by Sharif (1948). Any possible dietary deficiences were avoided by using rat cake, the ingredients of which are barley, wheat, oats, dried yeast, vitamin and mineral supplements, dried milk powder and English white fish meal. The bottles were kept in a desiccator maintained at 80% relative humidity, according to the potassium hydroxide method of Madge (1961) and at a temperature of 23°C ±0.5°C, under dark conditions, in an incubator.

Development was usually complete within 12 days, after which the imagines would remain resting within their cocoons. Emergence was stimulated by shaking the bottles, this produced relatively uniform batches of imagines for experimental purposes, since there is evidence (Humphries, 1967, 1968) that emergence from the cocoon appears to be a critical event in determining the subsequent time course of physiological and behavioural processes.

Immediately after emergence fleas were transferred to a small polypropylene cage (29 cm by 22 cm and 10 cm high) and were sorted out into separate batches of males and females.

The fleas were handled for experimental purposes by sucking them into a suction bottle (fig. 1). For feeding the fleas, either partially

Figure 1 Suction Tube

A. Glass vial

B. Cork

C. Glass tube for sucking the fleas into the glass vial

D. Glass tube attached to rubber tubing

E. Rubber tubing

F. Glass tube used for sucking the air with fleas into the glass vial

G. Cotton gauze attached to glass tube D to prevent inhaling of any particles or fleas

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shaved adult rats or young rats 3 days to 6 weeks old were used. The body skin temperature of 3 to 15 day old rats, due to the scanty fur, fell from about 34°C to about 27°C within half an hour, if kept away from their mother. To maintain their body temperature they were kept in a glass jar warmed by a current of air rising from a thermostatically controlled heater, situated 32 cm below. Temperature was monitored by a thermistor probe, fixed in the jar at the same level as the young rat.

The general qualitative observations of behaviour were made in various situations.

(a) In the culture cages, where a wide range of behaviour is exhibited.

(b) Mating behaviour of fleas and feeding behaviour of larvae was observed in an arena 0.75 cm high and 1.5 cm in diameter, made of a celluloid ring stuck to a glass slide and roofed with a cover slip.

(c) Host-parasite relationships were observed in a small polypropelene rat cage, where a partially shaved male rat provided the blood meals.

(d) In the experiments on the effects of bloodmeal and temperature on mating behaviour, small bottles (7.5 cm high and 1.5 cm in diameter) were used as arenas and the floor of the bottle lightly covered with

a little sawdust. The sawdust covering was necessary because preliminary studies showed that on a smooth glass surface, both male and female *Nosopsyllus* exhibit few types of activity apart from vigorous jumping. These observations were carried out in a mixture of artificial room light and diffuse daylight. Temperature changes were induced either in a glass fronted incubator or by a current of warm air rising from a thermostatically controlled heater situated 32 cm beneath the arena. Temperature was monitored by a thermistor probe.

Whenever, in the results the number of observations made are not mentioned, the conclusions are derived from a study of twenty five or more specimens.

To prepare permanent slides of fleas in copula, they were put quickly and with minimum disturbance directly into frozen potassium hydroxide and immediately after,a drop of chloroform was added. This technique killed the fleas without causing separation. They were then transferred to fresh potassium hydroxide 10% and left for three days, after which the solution was removed with a syringe and the fleas were given two washes of distilled water (15 and 30 minutes). Then 10% acetic acid was added and the fleas left in it for one hour, after which again 15 and 30 minute washes in distilled water were given, followed by dehydration in alcohol, clearing in clove oil and mounting in Depex.

Photographs of permanently mounted fleas were taken with a Carl Zeiss photo-microscope. Green and blue filters were employed. For electron-microscope photographs, both conventional and scanning electron microscopes were employed.

In the legends for plates the magnification provided refers to the magnification as seen on the print.

CHAPTER 3

MATING BEHAVIOUR

A. MATING BEHAVIOUR

Introduction

The mating behaviour of fleas has been described in outline by several authors. The main publications describing the general sequence of actions during copulation are by Mitzmain (1910) on *Diamanus montanus* (Baker), Waterston (1912) on *Ceratophyllus farreni* (Rothschild), Lundblad (1927) on *C. gallinae* (Schrank) and *Xenopsylla cheopis* (Rothschild), Holland (1955) on various Ceratophyllinae, and Geigy and Suter (1960) on *Tunga penetrans*. Most recently Humphries (1967a) described the mating behaviour of the hen flea *C. gallinae* more extensively.

Details of the structure and mode of linkage of flea genitalia were partly described by Snodgrass (1946). He attempted to explain the mechanism of genital linkage on the basis of a slide preparation of a mated pair of *C. swansoni* (Liu). Further descriptions of linkage are given by Holland (1955) for Ceratophyllinae and by Humphries (1967b, 1968) for *C. gallinae* and *C. styx*.

No description of the mating behaviour of *N. fasciatus* has been published apart from a brief preliminary account by Iqbal and Humphries (1970).

This section introduces further details on the general sequence of actions seen during the mating of *Nosopsyllus* and provides the context for understanding the later sections on genital linkage and on the effects of temperature and other variables on mating behaviour.

Details in the following account are normally based on at least twenty observations, often many more.

An outline of mating behaviour

Mating behaviour is summarised diagramatically in fig. 2.

Pairs in copula are frequently observed in the stock cultures. Pair formation occurs on the surface of the sawdust. A male accidentally encountering a female, erects his antennae, inserts his head beneath the female's abdomen and establishes an antennal clasp on the second or third abdominal sternum of the female. The male then lifts the posterior part of his abdomen bringing his genitalia into alignment with those of the female to acheive the full copulatory posture. Immediately after linkage of genitalia has been established the pair burrows out of sight. Copulation while on the host occurs occasionally, but was only seen on infant rats, never on adults.

Initiation of mating by a contact-chemical stimulus.

The stimulus which initiates mating differs in different species of insects. It may be auditory as in *Deinacrites cancer.* (Downes, 1966), *Drosophila melanogaster* (Bastock and Manning, 1955), visual as in the grayling butterfly *Eumenis semele* (Magnus, 1958), olfactory as in *Lucila cuprina* (Bartell, Shorey and Browne, 1969) or contact-chemical as in *Blattella germanica* (Roth and Willis, 1954). A contact-chemical stimulus was found to be the initiator of the mating sequence in *C. gallinae* by Humphries (1967), though Rothschild (1965a) notes that in *Spilopsyllus cuniculi* the "characteristic zigzag approach made by the male on these occasions suggests that it is following some airborne trail of scent".

Initial observations indicated that the behaviour of *Nosopsyllus* resembled that of *B. germanica* and *C. gallinae* in depending normally

on actual contact between male and female, for neither males nor females were seen to react to one another at a distance as would have been expected had the initial attraction been visual or airborne olfactory. However when a male collided with a female by chance he often reacted by erecting his antennae and pushing his head at the female.

The evidence that a contact-chemical stimulus initiates mating is as follows.

1. Males do not attempt to mate with inanimate objects like large pieces of sawdust. This shows that it is not a simple tactile response.

2. Legs and flea-sized portions of the terga of freshly killed cockroaches were presented to 15 male fleas. On contact none of the males responded.

3. Fifteen freshly killed recently fed female *N. fasciatus* were presented to 15 different males. On contact all the males erected their antennae and attempted to mate. These results suggested that a specific contact-chemical stimulus is involved. This was tested as follows.

4. Twenty males were presented (to the maxillary palp region) with a glass rod which had been rubbed against the bodies of live freshly fed females. All of the males reacted to the rod by erecting their antennae.

The above evidence shows that mating in *N. fasciatus* is initiated by a contact-chemical stimulus received by the male when he by chance collides with a female.

The site of reception of the contact-chemical stimulus in males.

Taschenberg (1880) and Jordan and Rothschild (1908) suggested that the flea antenna is an organ of smell, and as such is concerned in mating. Humphries (1967) demonstrated that the maxillary palps receive the initial sexual stimulus in *C. gallinae* and observed no reaction when the stimulus was presented to the antennae.

The maxillary palps and antennae of *Nosopsyllus* bear setae of apparently chemo-receptor type, (Plates 1 and 2). Direct observations had suggested that the maxillary palps receive the sexual stimulus. Two sets of experiments were carried out to test whether the maxillary palps, or the antennae, or both, are responsible for reception of the initial contact-chemical stimulus.

1. Maxillary palps.

(a) Both maxillary palps of 25 freshly fed males were removed.

(b) In a second batch of 25 freshly fed males, only one maxillary palp was removed. Excisions were performed using Borradaile needles. The flea was held with one needle on a glass slide under the binocular microscope and the spreading out of the mouthparts was assisted by a drop of Ringer's solution. The operated fleas were isolated and left for half an hour to recover from possible injury effects as it was found that experimental manipulation can often temporarily supress mating behaviour. They were then allowed to come into contact with fed females in the arena and in small bottles. Each couple was observed for three hours. In experiment (a) none attempted to mate. In experiment (b) all twenty five males mated.

Further experiments were carried out on another 25 males to make sure that simple injury effects were not involved as the fleas

in experiment (a) had double the operative damage of those in experiment (b). One of the forelegs was sliced away from the upper end of the coxa, and they were then allowed to come into contact with fed females. Each couple was observed for a maximum of three hours. One male did not attempt to mate. Most formed pairs successfully. Simple injury effects therefore are unlikely to account for the striking difference between the results for experiments (a) and (b).

2. Antennae.

A finely drawn glass rod was rubbed against the bodies of freshly killed fed females and was then touched lightly against the antennae (and surrounding region) of a fed male. Out of a batch of twenty males none erected his antennae. In addition, the same glass rod was used to tcuch the forelegs of the fed males. None out of a batch of twenty reacted. Compare this result with that reported on p.9 when the rod was presented to the maxillary palps.

The above evidence establishes that the maxillary palps of the male are the organs receiving the contact-chemical stimulus from the female, and that the receptors on the antennae and forelegs are not involved at this stage.

There are two morphologically distinct types of receptors on the maxillary palps (Plates 2&3).

(a) Finely pointed sensillae which lie closely along the maxillary palp pointing towards its tip.

(b) Blunt sensillae which are borne predominantly on the distal segment of the maxillary palp, branching out at different angles and directions, but mainly anteriorly and laterally. It is suggested that

the blunt sensillae are more likely to be the chemo-receptors for the mating stimulus as they are distributed on the palp in such a way that when the palp is vibrating at a normal angle during locomotion they are first to touch objects (such as fleas) with which the male collides. They protrude further from the palp than do the finer setae and their form too is more similar to that of chemo-receptors on other insects, for example those on the antennae of *Pediculus* (Wigglesworth, 1941).

Sometimes the male loses contact before acheiving the antennal clasp, due to erratic jumping or moving by the female. The male then runs forward in a zigzag pattern with his antennae erect and maxillary palps quickly vibrating. The zigzags are short and frequent (Figs. 3-20). If the male does not encounter a female he may after 1 to 4 cm swerve sharply and continue to zigzag in the new direction. This behaviour is well suited to increase the chances of the male regaining contact with the female. If contact is not regained, the behaviour lasts for only few seconds, after which the antennae are withdrawn into the antennal grooves and the lowered head is raised to the normal posture. This behaviour is comparable to the behaviour shown by coccinellid larvae when searching for aphids (Banks 1957).

Linkage of genitalia.

After the establishment of the antennal clasp, the male lifts the posterior part of his abdomen until the distal part is brought anteriorly over his back and the exposed genitalia are just touching the female's genitalia. If the genitalia do not exactly align with each other the male waves the distal part of his abdomen to and fro,

dorso-ventrally and laterally, until the genitalia oppose each other precisely. In some cases it was observed that males which failed to acheive perfect alignment readjusted the antennal clasp and raised their genitalia again to acheive the correct alignment.

The main linkage between male and female terminalia is then acheived by the upwardly projecting claspers of the male. The movable processes of the claspers are pushed underneath the female's sternum 7, and the fixed processes of the claspers fit against the movable processes from the outside thus pinning the margin of sternum 7. The erected penis rod is then guided by the two flaps of sternum 9, and introduced into the female's vagina, after which the two aedegal hooks become fixed in the depression in the tenth tergum of the female, (Plates 4-8). A detailed account of the structure and mode of action of the genitalia is given in a later section.

Once copulation starts, the male becomes largely passive as far as locomotion is concerned and any locomotory movements are generally made by the female.

Reaction towards light.

In culture cages, immediately after copulation starts the female burrows into the sawdust dragging the male. In the experimental arena the female remains motionless most of the time (Figs. 3-20). To check whether the burrowing response seen in the culture is thigmotactic or due to negative phototaxis fifteen pairs in copula were put in petri dishes floored with slightly crumpled black paper, which was pierced by several holes. In the first experiment, light of 200 lumens/sq. ft. was thrown onto the dish from a bulb 60cm above. Within 25 seconds all the fifteen pairs had moved underneath the

paper through the holes. In the second experiment the paper was arranged over the pairs in copula and light was thrown from underneath the dish. Within 30 seconds, all the pairs had moved to the upper side of the paper through the holes. These results suggest that fleas in copula are negatively phototactic, but the possibility of thigmotoxis playing some part is not ruled out.

The function of the negative phototactic response during mating may be to reduce the pair's vulnerability to attack by the host. Leeson (1936) and Buxton (1938, 1948) reported the capture and killing of *X. cheopis* by their rodent hosts, and Humphries (1963) recorded similar anti-parasite activity against *C. gallinae*, *C. nobilis* and *Malaraeus penicilliger*.

The Copulatory phase.

During copulation the male performs regular thrusting strokes of his terminalia against the terminalia of the female. These actions stop only when the female starts moving. The male also makes rhythmic coiling and uncoiling movements of the aedegal tendons during copulation (Plates 7-8).

At the completion of copulation, which usually lasts two to three hours; the male releases his antennal clasp and the fleas then turn and walk in opposite directions thus withdrawing the penis rod of the male from the female. After withdrawal the temporarily straight penis rod is retracted into a curved-back position within about three seconds and comes to lie between the flaps of sternum 9 (Plates 7-11). Separation is usually initiated by the male but may occasionally be initiated by the female vigorously kicking the male with her legs.

Rejection by the female.

In the stock of cultures some females rejected males by moving or jumping away from them. In some cases when the male succeeded in acheiving the antennal clasp, the female broke the clasp by vigorously kicking the male with her metathoracic legs. Laboratory experiments showed that this rejection behaviour is affected by the female's previous mating experience and her experience of factors normally associated with the host, (see Chapter 4A).

Male to male encounters.

Humphries (1967) reported male to male encounters among *C. gallinae*. Similar attempts to mate were observed among *N. fasciatus*.

To remove the possibility of previous contact with the female's body contaminating the male with the female's mating pheromone, 29 pairs of freshly fed males which had been segregated from females since emergence from the cocoon were observed in the laboratory; all of them attempted to mate and in nine cases their mating attempts reached the stage of attempting to clasp with the claspers.

The presence of a sexually stimulating substance on male cuticle was confirmed by further observations. Fifteen freshly killed recently fed males were presented to fifteen fed males. Antennal erection and antennal clasping occured in all cases. Another fifteen males were presented with a fine glass rod rubbed against the bodies of freshly killed fed males. All of tem erected their antennae.

The simplest hypothesis is that the sexually stimulating substance on male cuticle is similar or identical to that on female cuticle.

Discussion.

In broad outline, the mating behaviour of *N. fasciatus* may be considered as consisting of three phases, preliminary orientation manoeuvres, sometimes taking as little as one second, linkage of genitalia occupying about five seconds, and a subsequent copulatory phase lasting two to three hours.

The importance of tactile and contact-chemical stimuli in the preliminary orientation manoeuvres may be related to the host's microhabitat. Fleas are most likely to make mating contacts where population density is high. Rat nests are normally in darkness, for example in holes or beneath buildings, where visual attraction would be of little help. There was no indication in the present study that visual stimulation plays any part in mating and Hanström (1927) has shown histologically that the flea's ocelli are probably inadequate for this task. The crowding of the fleas in the host's nest or upon the host's body is likely to facilitate the chance meeting of sexes. The present laboratory observations suggest that, in the wild, mating would usually occur in the rat's nest, though Iqbal and Humphries (1970) observed occasional pairing on the host's body and Poole and Underhill (1953) described similar behaviour in *Megabothris clantoni*.

Early descriptions of the mating behaviour of various species suggested that male's behaviour prior to linkage of genitalia is a unitary activity without any essential active participation by the female (Waterston, 1912; Holland, 1955 and Geigy and Suter, 1960). The mating of *Ceratophyllus* described by Humphries (1967) shows that

the male's behaviour consists of a series of fixed acts, each of which is under the strict control of stimuli automatically received from the female. The absence of any of these stimuli breaks the male's mating sequence. Bastock and Manning (1955) in the case of *D. melanogaster* and Barth (1964) in the case of the cockroach *Byrostria fumigata* have shown that these mating sequences are also under specific stimulus control. The present work demonstrates that the male *Nosopsyllus* also depends on stimuli from the female to initiate, orientate and complete the first phase of mating (see also sections B and C). The importance of the female's behaviour is also shown by the active rejection of males by unfed, and by previously mated females.

Humphries (1967) reported that in *C. gallinae* "vibratory activity of sternum 8, both up and down tapping and antero-posterior stroking is associated with locomotory movement by the female. There is a burst of tapping when the genitalia are linking, and subsequently almost every time that the female walks or shows active signs of doing so". He suggested that this stroking causes inhibition of movement in the female. In contrast, *Nosopsyllus* males perform rhythmic thrusting movements against the body of the female almost continuously when she is motionless. It is argued later that these thrusting strokes may assist the transfer of sperms.

Summary.

Mating starts when a male accidentally collides with a female. The contact-chemical stimulus is received by the maxillary palps of the male. A sexually stimulating substance (probably similar or identical in nature) is present on the cuticle of both sexes. It

causes the male to erect his antennae, push his head underneath the female's abdomen and clasp her abdominal sternum 2 or 3. If the male loses contact a searching behaviour is elicited. After the acheivement of the antennal clasp, he raises his genitalia and a genital linkage is acheived. Failure of perfect alignment may be corrected either by dorso-ventral and lateral waving of the male's abdomen or a reversion to an earlier stage in the mating sequence allowing the male to readjust the antennal clasp. A negatively phototactic behaviour is shown after the start of copulation which lasts from two to three hours.

PLATES 1 - 11

Plates 1 - 11.

Pl. 1. Male antenna in erected posture.

A. Setae

B. Origin of spread-out club from second segment.

Pl. 2. Tip of maxillary palp.

A. Blunt sensillae, probably contact chemo-receptors.

Pl. 3. Maxilla, showing finely pointed setae (A) on the palps.

Pl. 4. Male and female in copula. The male antennal clasp has become detached.

Pl. 5. Male antennae in clasping posture.

Pl. 6. Terminalia of fleas in copula.

A. Male's claspers gripping female's sternum.

B. Aedegal hooks.

C. Sternum 9 of male.

D. Depression in tergum 8 of female.

E. Penis rod inside bursa copulatrix and spermathecal duct. Inner rod just emerging.

Pl. 7. Male terminalia.

A. Erected penis rod.

B. Coiled aedegal tendons.

C. Sternum 9.

D. Aedegal hook.

Pl. 8. Fleas in copula, showing appearance of aedegal tendons when uncoiled (A).

Pl. 9. Male flea.

A. Penis rod partly retracted into curved back position.

Pl. 10 Aedegus

A. Penis rod in normal fully curved back resting position.

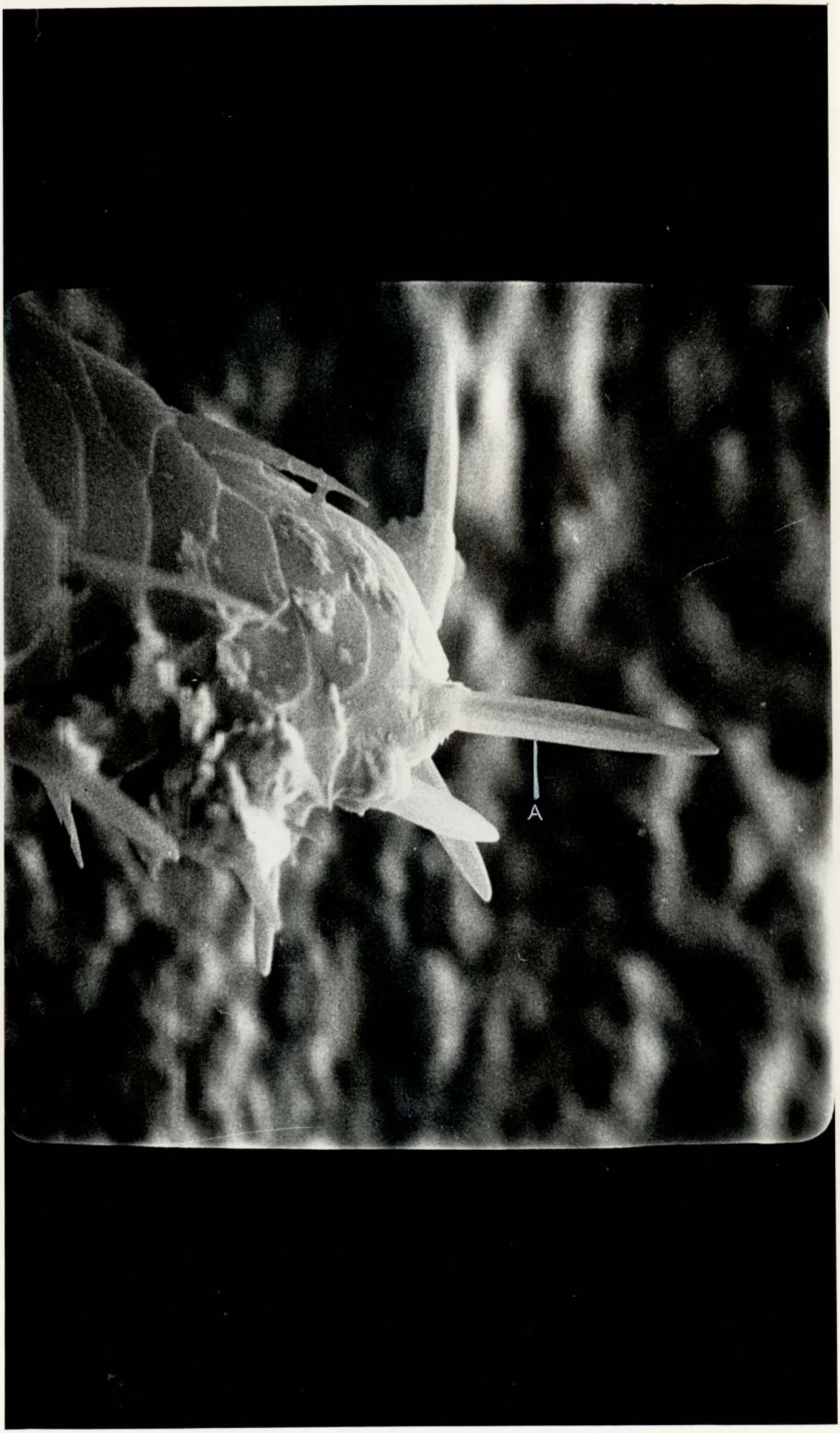
Pl. 11. Aedegus.

A. Penis rod in between the two flaps of sternum 9 (B).

C. Aedegal hooks.

PL.1

PL. 2



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PL. 3

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PL. 4

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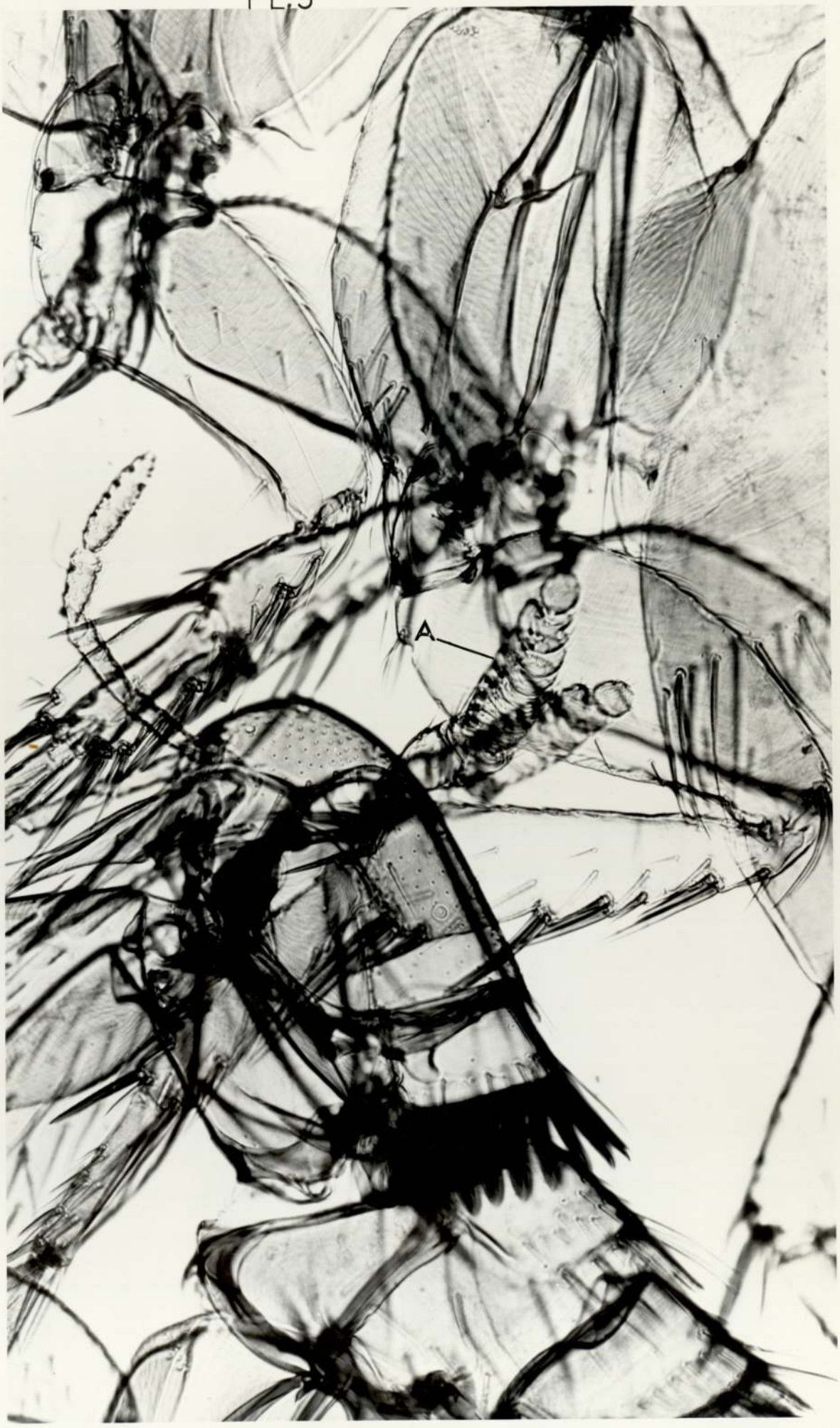
PL. 5



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PL.6

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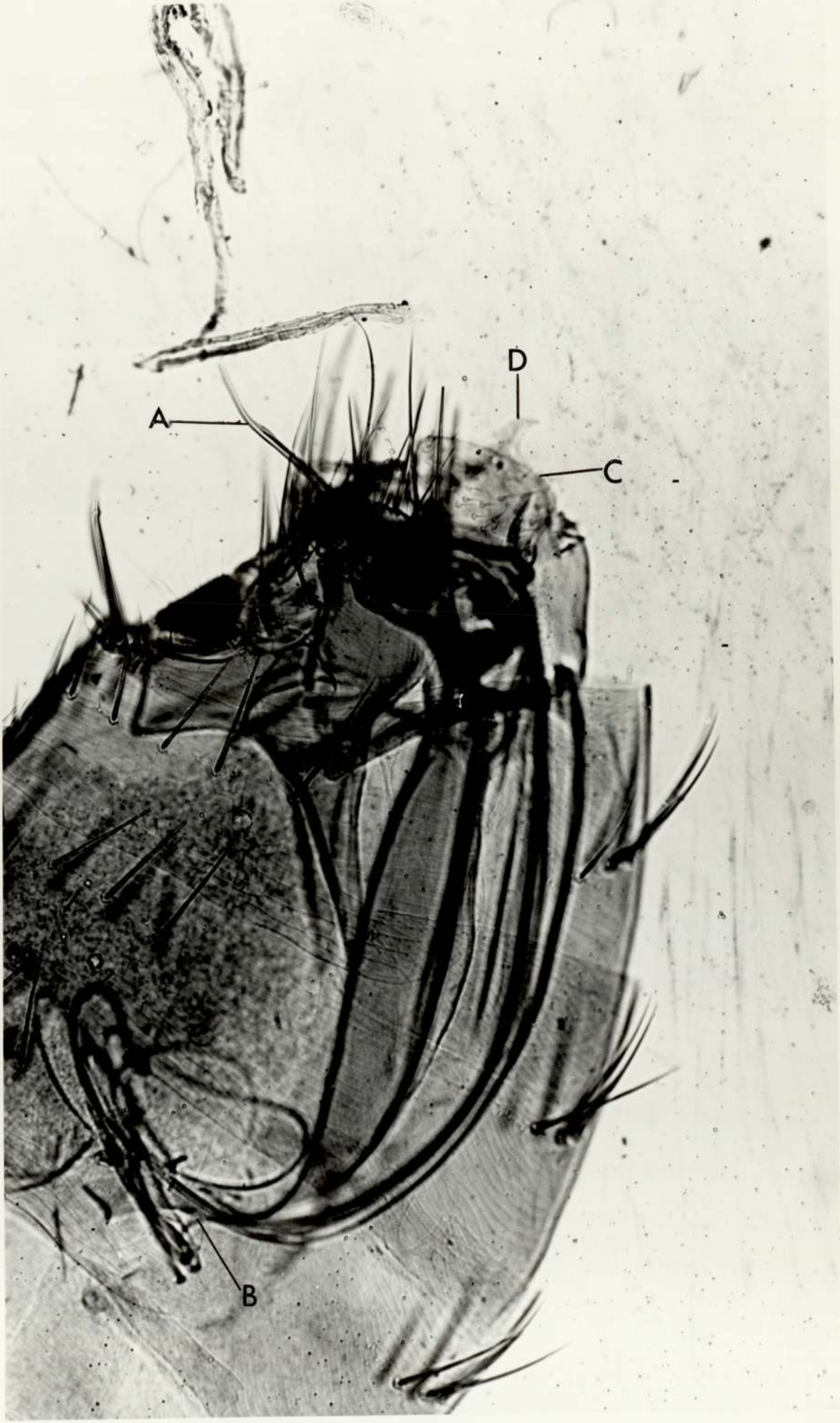
PL.7



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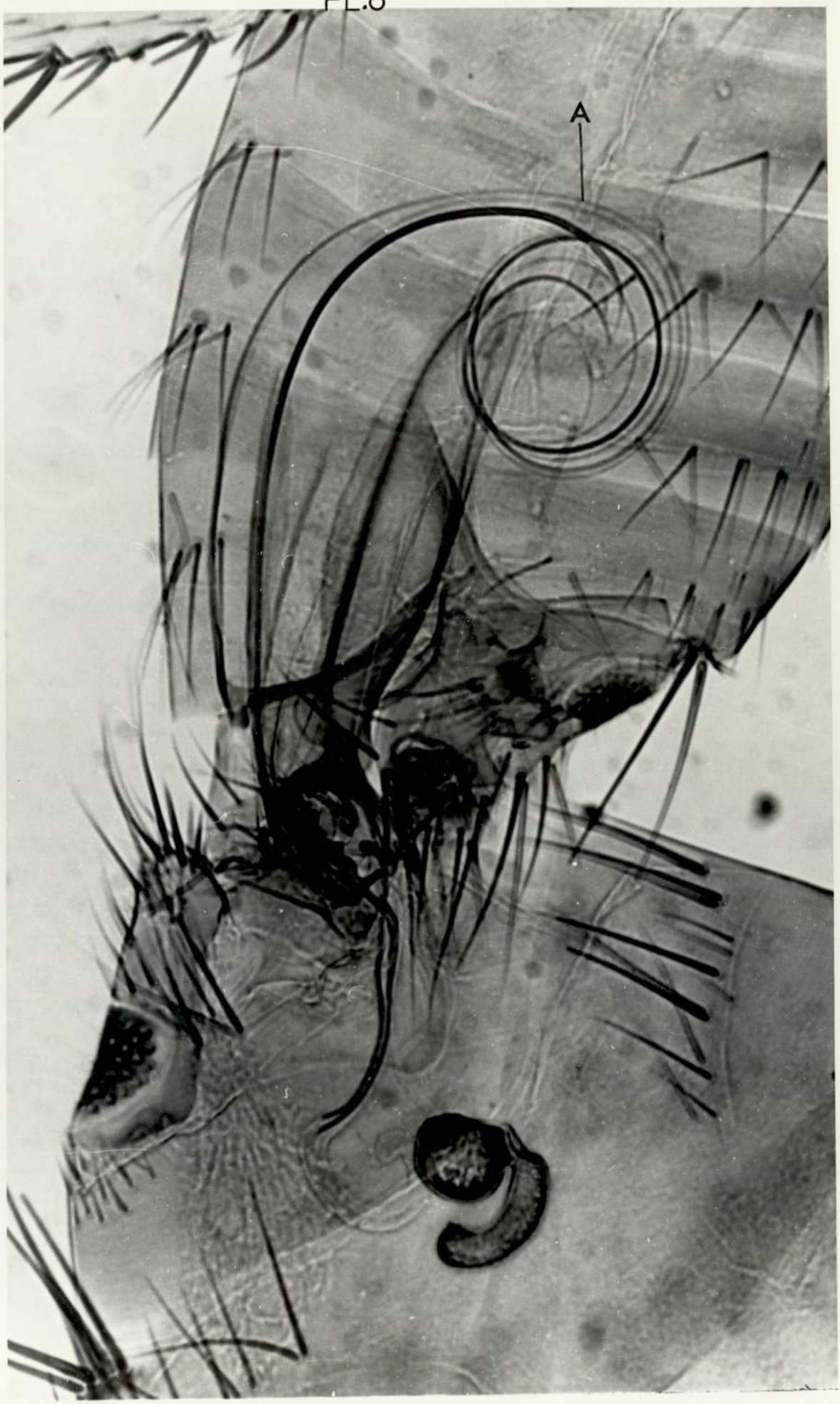

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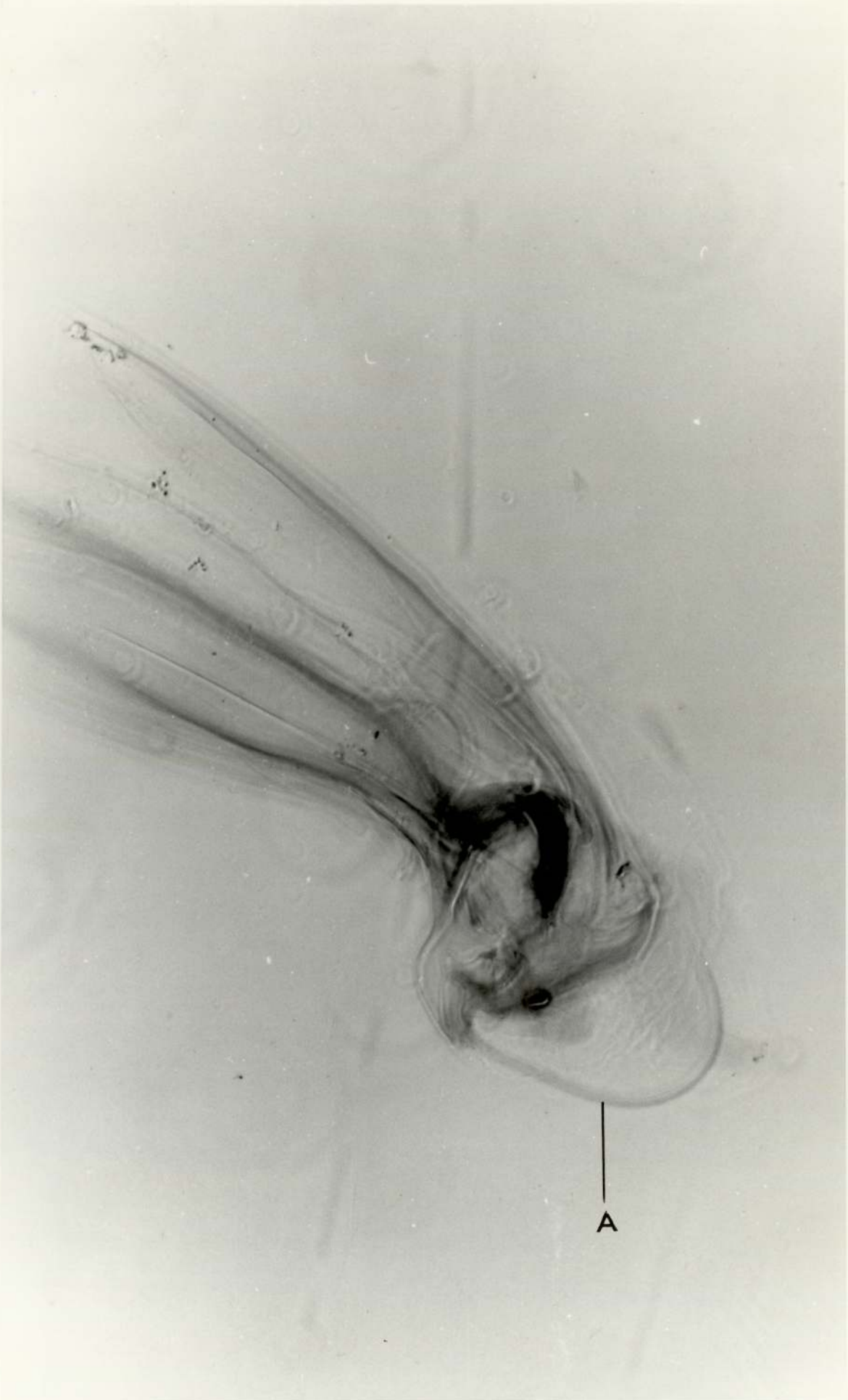
PL.9

PL.10



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PL. 11

Fig. 2.

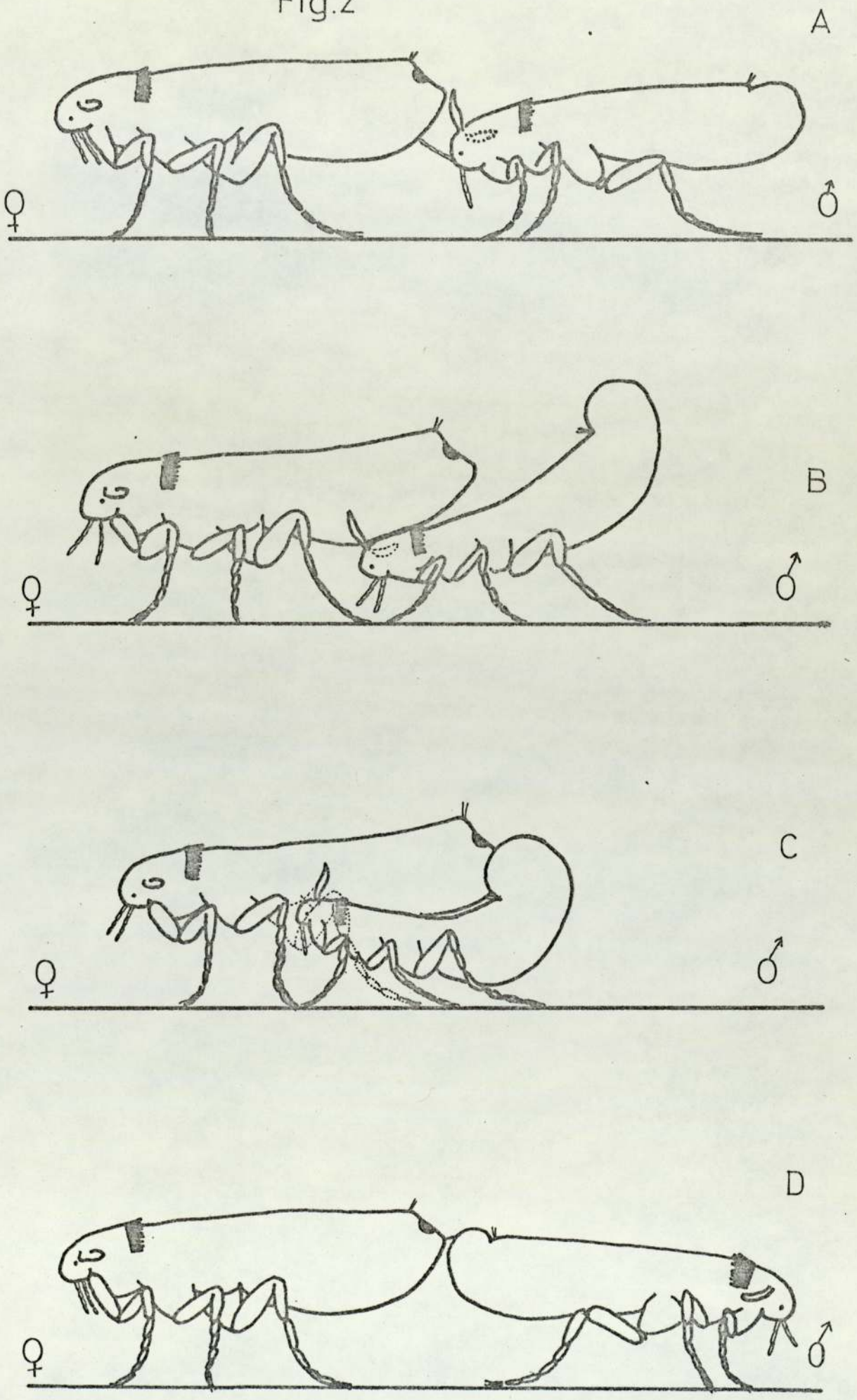
A. Male detects the female by touching her with his maxillary palps and erects antennae.

B. Male pushes his head under the abdomen of the female, clasps it and raises his genitalia.

C. Linkage of male and female genitalia.

D. Termination of copulation, the male after unclasping his antennae turns in the opposite direction to that of female and starts walking, thus withdrawing his penis rods from the female.

Fig.2



Figs. 3 to 20.

The first of the numbers given below and those on the diagrams refer to the order of the periods of immobility during copulation. The second number of each pair refers to the duration of each period in minutes. Dotted lines show jumping. Continuous lines show walking before commencement of copulation (note male zig-zagging).

Fig. 3.
1, ———— 7
2, ———— 2
3, ———— 9
4, ———— 21
5, ———— 17
6, ———— 2
7, ———— 6

Fig. 4.
1, ———— ½
2, ———— 3
3, ———— 7
4, ———— 28
5, ———— 19
6, ———— 12
7, ———— 4
8, ———— 2

Fig. 5.
1, ———— 2

Fig. 6.
1, ———— ½
2, ———— 5
3, ———— 11
4, ———— 7
5, ———— 21
6, ———— 7

Fig. 7.

1, —— 9
2, —— 20
3, —— 18
4, —— 23
5, —— 30
6, —— 14
7, —— 11
8, 9

Fig. 8.

1, —— 4
2, —— 7
3, —— 11
4, —— 9
5, —— 21
6, —— 17
7, —— 8
8, —— 3

Fig. 9.

1, —— 2
2, —— 2
3, —— 7
4, —— 8
5, —— 14
6, —— 11
7, —— 13
8, —— 8
9, —— 6

Fig. 10.

1, —— 6
2, —— 7
3, —— 20
4, —— 13
5, —— 7
6, —— 28
7, —— 11
8, —— 14
9, —— 9

Fig. 11.

1, —— 4
2, —— 7
3, —— 7
4, —— 11
5, —— 23
6, —— 13
7, —— 17
8, —— 6
9, —— 4

Fig. 12.

1, —— $\frac{1}{2}$
2, —— 4
3, —— 7
4, —— 24
5, —— 11
6, —— 10
7, —— 21
8, —— 13
9, —— 7
10, —— 3

Fig. 13. 1,——— 5

2,——— 6

3,——— 4

4,——— 9

5,——— 17

6,——— 11

7,——— 9

8,——— 11

9,——— 7

10,——— 7

Fig. 14. 1,——— 2

2,——— 6

3,——— 4

4,——— 9

5,——— 14

6,——— 38

7,——— 22

8,——— 17

9,——— 6

Fig. 15. 1,——— 9

2,——— 4

3,——— 18

4,——— 7

5,——— 4

6,——— 26

7,——— 13

8,——— 18

9,——— 4

10,——— 6

Fig. 16. 1,——— 7

2,——— 4

3,——— 2

4,——— 13

5,——— 28

6,——— 9

7,——— 6

Fig. 17. 1,——— 2

2,——— 8

3,——— 20

4,——— 11

5,——— 36

6,——— 9

7,——— 11

Fig. 18. 1,——— 1

2,——— 3

3,——— 9

4,——— 18

5,——— 7

6,——— 4

7,——— ½

Fig. 19. 1,——— 3

2,——— 7

3,——— 34

4,——— 20

5,——— 13

6,——— 12

7,——— 4

Fig. 20. 1,——— 3

2,——— 6

3,——— 11

4,——— 4

5,——— 18

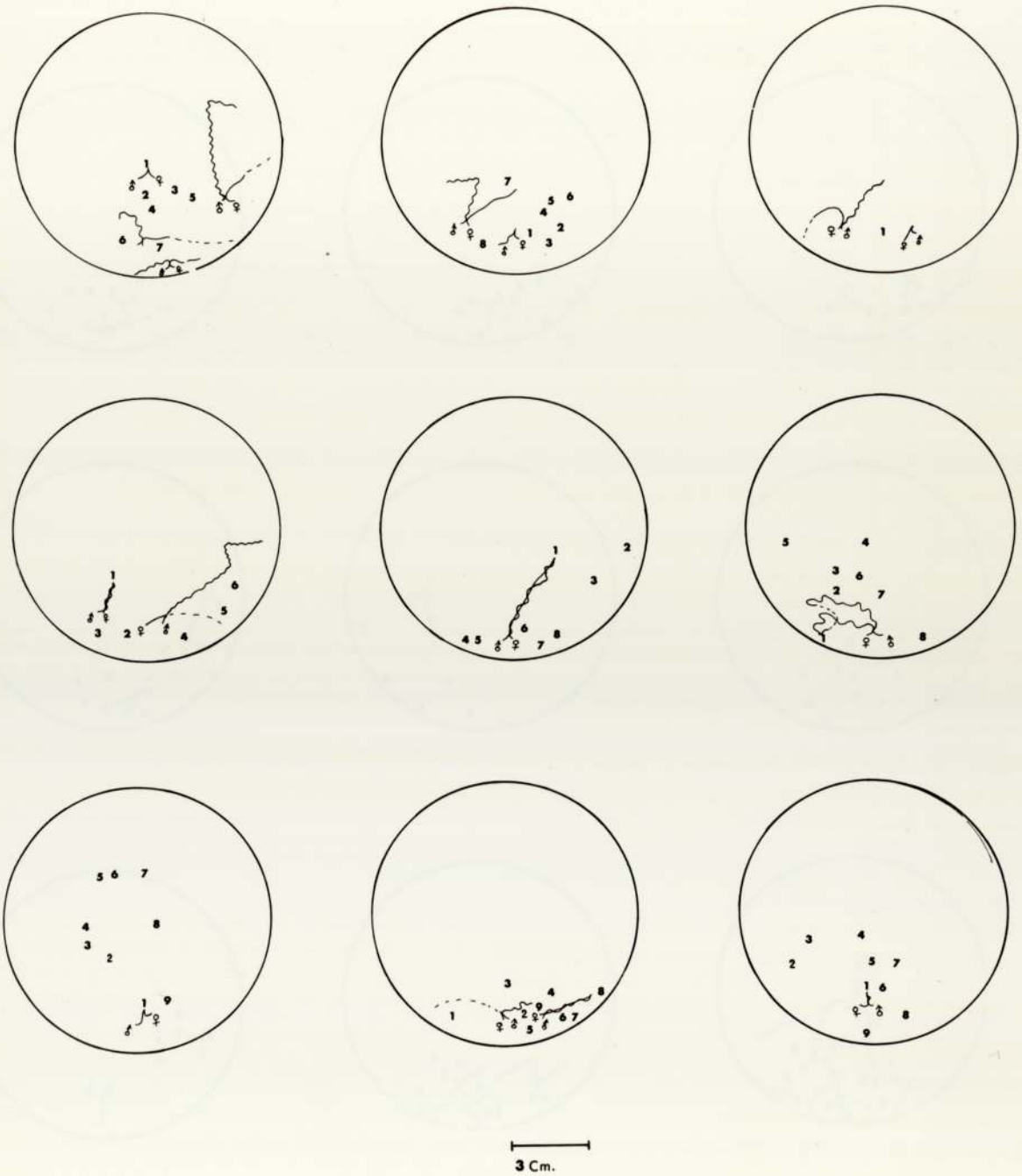
6,——— 27

7,——— 9

8,——— 13

9,——— 7

Figs. 3—11



B. THE STRUCTURE AND FUNCTION OF THE MALE ANTENNAE

Introduction

Flea antennae are sexually dimorphic. The dimorphism in *N. fasciatus* is illustrated in plates 12 and 13. Aspects of antennal structure have been described by various workers. Oudemans (1909) described the disc-shaped organs on the internal surface of the male antennae as bristles, or spatulate setae. Lundblad (1927) suspected the adhesive function of discs on the inner surface of the antennal club and Dampf (1910) figured them. According to Hopkins and Rothschild (1953) these setae, which they termed "holding structures", are present in all the seventeen families of the order Pulicoidea with the exception of the Ancistropsyllidea. Most recently Rothschild and Hinton (1968) have given a detailed account of the morphology of these structures, in *Archaeopsylla erinacei* (Bouché), *Xenopsylla brasiliensis* (Baker), *X. cheopis* (Rothschild), *Ceratophyllus fringillae* (Walker), *Cleopsylla monticola* (Smit), *Amphipsylla primaris* (Jordan and Rothschild), *Paraceras melis* (Walker), *Hystrichopsylla talpae* (Curtis) and *Ischnopsylla elongatus* (Curtis). There is no published account of the structures on *N. fasciatus* .

The action of clasping has been described by Mitzmain (1910). Wenk (1953) described antennal musculature. Humphries (1967) has given a detailed account of the clasping sequence by the antennae of male *C. gallinae* on the second sternite of the female and has suggested that the inner surfaces of the antennae also receive the stimulus which is responsible for the full raising of male genitalia. Rothschild and Hinton (1968) likewise suggested that the antennal discs might perform a tactile function in receiving the stimulus, triggering raising of the genitalia. They suggested further that there may be some correlation between the

type of adhesive organs in the male and the arrangement of the ridges on the second sternite of the female.

The present report describes the antennal structure of male *N. fasciatus* in detail and considers their mechanical and possible sensory function.

RESULTS.

The antennae of the male *N. fasciatus* measure approximately 46μ in length. They are at their broadest (averaging 11.1μ) about one third of the way up from the base of club and gradually taper towards the tip (plate 13). They consist of three sections:- segment one, segment two and the club (of eleven segments). Segments one and two lack adhesive discs.

The erection of the antennae is in two distinct stages (Fig 21), firstly as a lateral and dorsal movement from the antennal grooves at a postero-lateral angle of approximately 35° to the antero-posterior axis of the body and then as a dorsal and anterior erection into the final posture.

In the erected posture the antennae are held in a slightly curved fashion so that their anterior aspects in concave and the posterior aspect is convex (Plate 13). The inner aspect of the club normally looks flat and bears adhesive discs (Plate 14). The outer aspect of the club lacks adhesive discs. The eleven segments of each club are connected narrowly to one another on the anterior side (Plates 15 & 16). These segments may spread out fanwise or may be held so closely to each other that they give the club the appearance of a single structure bearing the adhesive discs. Each club segment is supplied with approximately 50 to 60 adhesive discs.

The adhesive discs are 0.77 - 0.84 μ in diameter and are borne on

long stalks approximately 0.63 - 1.0 μ arising at right angles to the club segment. Over most of the club, the discs are flat (Plates 17 & 18) but towards the apex they become convex (club shaped) and their stalks are even longer, approximately 3.8 - 4.1 μ (Plates 19 & 20).

Numerous finely pointed setae are present on the basal antennal segment (Plates 13, 15 and 16) and there is a row of three stout blunt setae, possibly chemo-receptors, on the antero-lateral margins of each club segment. These chemo-receptors arise from pit-like depressions and point away from the area covered by the adhesive discs (Plates 18 and 20).

To examine the possible correlation between the adhesive discs of the antenna and the spacing of striations on the second sternite of the female, measurements of the adhesive discs and the distances between two consecutive striations were taken.

As stated before, the maximum height of the erected antenna is approx. 46 μ , thus a zone of up to this height along each side of the female's second sternite may be clasped. Measurements were, therefore, concentrated on this zone. In practice a zone 57.7 μ high was examined, and measurements taken at three different positions, the anterior, middle and the posterior parts of this zone (Plate 21). At each position measurements of the ridges were taken at four different vertical points measured from 5 μ above the ventral extremity to 58 μ . See table 1.

Table 1. spacing of ridges on the second sternite of the female.

Measurement at 5	Measurement at 15	Measurement at 31	Measurement at 58
Anterior 3 μ	2 μ	2 μ	2 μ
Middle 2 μ	2 μ	2 μ	2 μ
Posterior 2 μ	2 μ	2 μ	2 μ

Plate 21 shows that the vertical striations on the second sternite are concavo-convex antero-posteriorly.

The detachment of the male antennae was observed in one hundred and twenty instances. At the completion of mating, the male drags his antennae downwards till their apices reach the lower end of the second abdominal sternite and become free.

The antennal clasp on the second sternite seems to be very firm as the male was never observed to lose his grip, when the female was dragging him along, underneath, above and through the sawdust in the culture cages.

DISCUSSION

During the clasping sequence the segments of the antennal club are spread out (Plate 15 and 20). The spreading out of the club segments occurs after the erection of the antennae at the moment of contact with the female sternite. This spreading of the club segments results in a larger area of the second sternite of the female being covered by the adhesive discs.

The different structure of the two types of adhesive discs may be related to the spreading of the club segments and to their central or marginal position. The main area of flat discs can be closely and densely applied to the specially sculptured second sternite. The longer-stalked club-shaped organs, marginal in position, meet the surface of the sternite at an angle, hence their relatively longer stalks. The club-shaped end may enable contact to be made at varying angles, providing attachment points in the spaces between the separated club segments.

The problem of how the antennal grip is achieved can be best discussed in relation to a review of adhesive mechanisms in the walking of certain

insects and lizards on smooth vertical surfaces, where the problem, as in the case of the flea's antennae, is to create a firm grip which can be easily released.

Dellit (1934) and Mahendra (1941) reviewed earlier work on the house-gecko and both discounted theories based on electrostatic charges or the secretion of adhesive substances. Mahendra suggested that the traction is initially provided by the setae present on the digits of the lizards making contact and gripping the irregularities of the substratum. Ruibal and Ernst (1965) have suggested that the adhesive of the setae to the substratum appears to be a surface phenomenon. They state 'Frictional force is directly proportional to the area of actual contact between two substances (not the total area of that substance which is actually in contact)'. The flattened spatulas, therefore, represent a mechanism that increases the total contact area between the setae and the substratum. According to Ruibal and Ernst, frictional force is believed to result from the molecular cohesion of the substances in contact, so the total frictional force exerted by the millions of spatulas provide the traction that allows geckos and anoles to easily climb smooth vertical surfaces.

Gillet and Wigglesworth (1932) described the tibial climbing organ of *Rhodnius prolixus (Stål)*. The climbing organ is a little oval sac of pliant chitin filled with blood, bearing on its lower surface about 5,000 tubular hairs about 1μ in diameter, which appear to be the outlet of unicellular glands producing oily secretion. This secretion, soluble in xylol, enables the insects to climb upwards on clean glass almost vertically. Edwards and Tarkanian (1970) experimented on the adhesive pads of *R. prolixus* and suggested that the setae of the tibial adhesive pads are similar in morphology and function to the digital setae of lizards. They stated that performance is considerably decreased when setae

and substratum are dehydrated and depleted of lipids, and suggested that in the case of insects, both frictional and meniscus effects are significant in developing traction.

The foregoing description of the adhesive discs of the antennae of the male flea are quite compatible with the hypothesis that they function in much the same way as the setae of the lizards and the climbing organs of *R. prolixus* . There is no evidence of the presence of secretory glands at the base of the stalks of adhesive discs in *N. fasciatus*. There would seem to be no need for such glands as the surface of the female sternite is itself waxy. However, according to Rothschild and Hinton (1968) these glands are present in the case of *A. erinacei* , *X. cheopis*, *X. brasiliensis*, *C. fringillae*, *C. monticola*, *A. primaris*, *P. melis*, *H. talpae and I. elongatus*.

It is suggested that the antennal club-segments in their extended posture with discs closely and densely applied to the second sternite achieve a firm grip due to surface phenomena, involving a) Frictional forces in much the same way as achieved by the setae of the lizards and the climbing organs of *R. prolixus*. The adhesive discs fit in closely against the irregularities in between the consecutive striations of the second sternite, thus offering greater friction. b) The waxy surface of the female cuticle which is likely to enhance adhesion as demonstrated in Rhodnius by Edwards and Tarkanian (1970).

The correlation between the adhesive discs and the ridges on the second sternite of the female was examined. The results suggest that two to three adhesive discs could easily fit in between the consecutive ridges. Secondly, the vertical striations on the second sternite are concavo-convex, antero-posteriorly, corresponding to the posture of the erected antennae.

Humphries (1967) has suggested that during copulation of the hen flea a tactile stimulus, received by the inner surface of the fully erect antennae, is responsible for initiating the raising of the male genitalia. Rothschild and Hinton (1968) suggested that possibly the adhesive discs perform some tactile function as well as a purely restraining action. There is no evidence to suggest this additional sensory function of the adhesive discs. Indeed there is no need for this hypothesis as the antennal club bears a number of finely pointed setae, possibly tactile in function, and the chemo-receptors described earlier, either or both of which may be concerned with eliciting or maintaining the raising of male genitalia after antennal clasping.

The detachment of the antennal clasp seems to be a viscous phenomenon as the surface of the flea is covered with wax so that the antennae can easily slip parallel to the surface but cannot be pulled away easily at right angles to the surface. It seems that the orientation of the ridges on the second sternite of the female plays an important role by not letting the antennal discs slip anteriorly or posteriorly by during copulation but allowing the discs to slip ventrally when the male pulls his antennae down (Plates 22 and 23).

SUMMARY

1. The male antenna has eleven club segments, each of which bears 50-60 adhesive discs borne on stalks.

2. The adhesive discs are of two types, central flat discs and marginal convex (club-shaped) discs.

3. Antennal clasping is achieved via the adhesive discs, possibly as a result of surface phenomena, involving frictional forces, in much the same way as the achievement of grip by the setae of lizards or the climbing organs

of *R. prolixus*. The waxy surface of the female cuticle probably assists adhesion.

4. There is a correlation between the arrangement of the ridges on the second abdominal sternite of the female and the curvature of the male antennae during clasping.

5. Approximately three adjacent discs can fit in between consecutive ridges on the female sternite.

6. The ridges on the second sternite of the female are orientated so that they are likely to stop the slipping of the antennal discs anteriorly or posteriorly during copulation but permit the unclasping of the antennae by letting the discs slip downwards.

7. The male unclasps the antennae by sliding them to the ventral edge of the second abdominal sternite.

8. The chemo-receptors on the antennal club segments probably detect second stage courtship signals as the antennal clasp is achieved.

PLATES 12 - 21

Plates 12-21

Pl. 12. Female antenna rounded and lacking adhesive discs.

Pl. 13. Male flea.

A. Elongated antenna bearing adhesive discs and finely pointed setae (B).

Pl. 14. Male antenna.

A. Adhesive disc.

Pl. 15. Male antenna, showing spreading and seperation of club segments (A).

A blunt sensilla (B) is visible at the right.

Pl.16. Male antenna.

A. Club segments, showing their attachment on the anterio-lateral aspect. Finely pointed setae (B) project laterally.

Pl. 17. Adhesive discs (A). X49,700.

Pl. 18. Terminal club segment. X16,800.

A. Club-shaped adhesive discs.

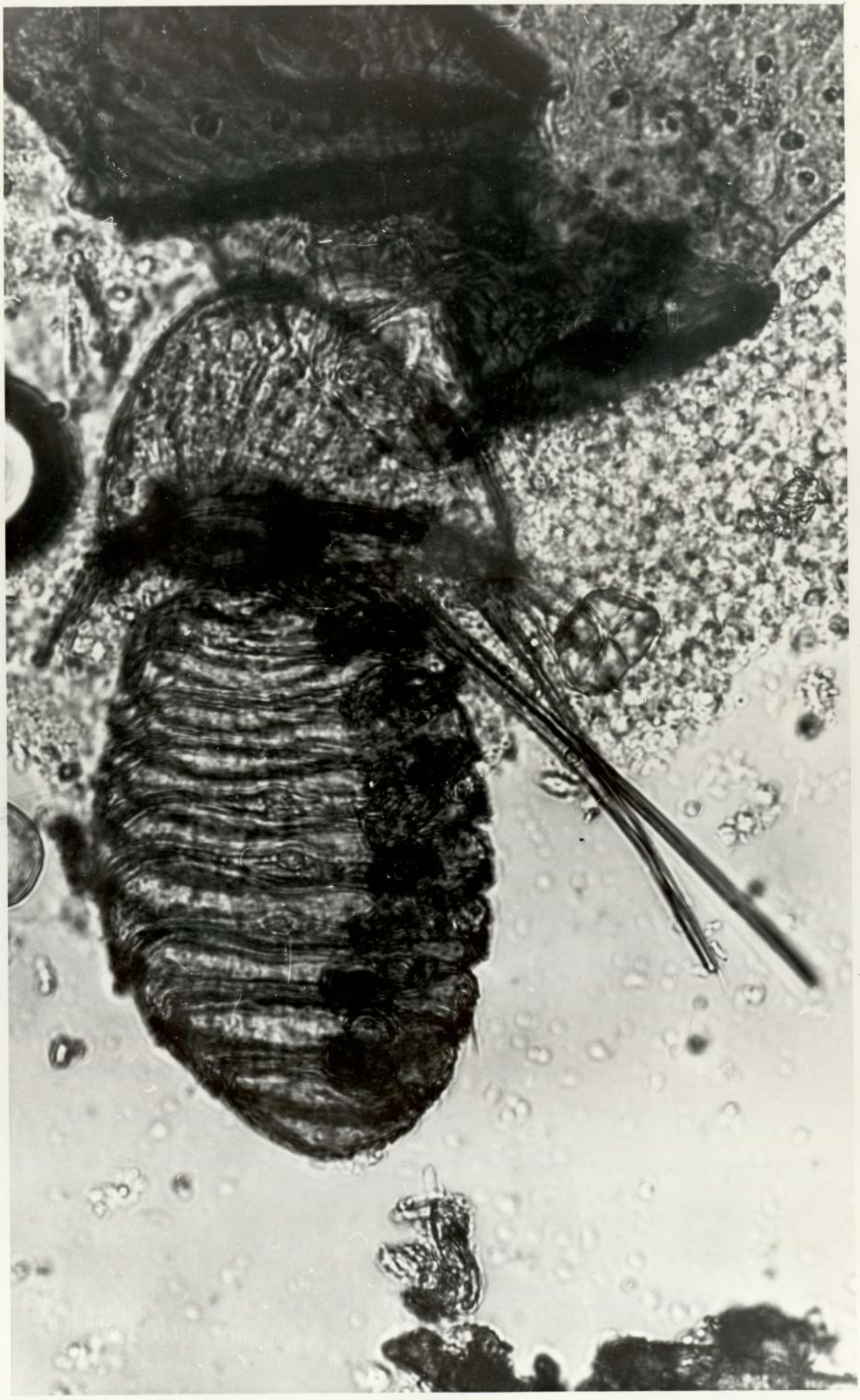
B. Stout blunt sensilla.

Pl. 19. Club-shaped adhesive disc (A) showing longer stalk. X42,000.

Pl. 20. Male antenna X 10,500, viewed from apex, showing stout blunt sensillae (A) projecting posteriorly, so that they would be parallel to the female's abdominal cuticle, and lightly touch it.

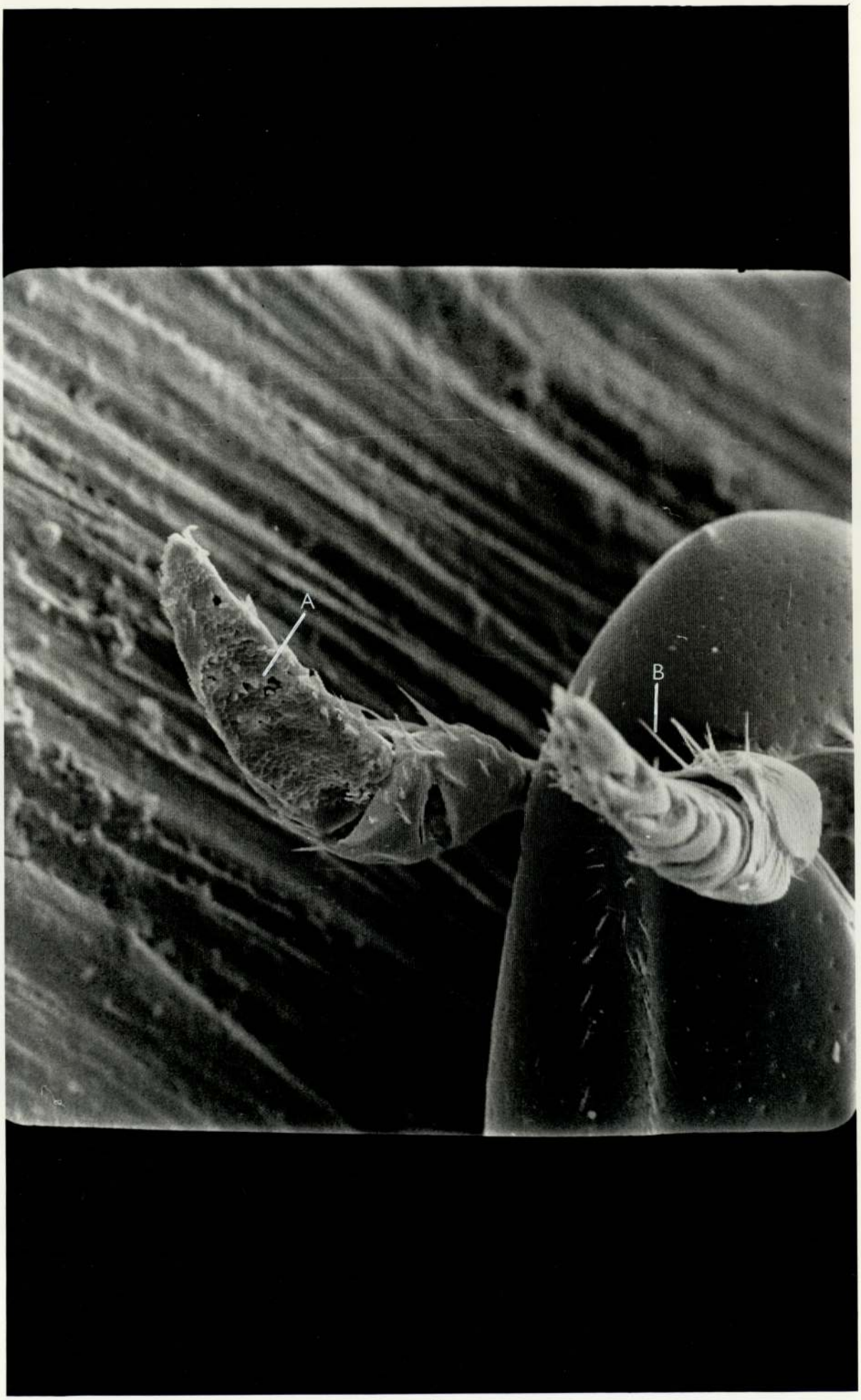
Pl. 21. Striations (A) on the female's second sternite. X 1,820.

PL .12



·03 mm.

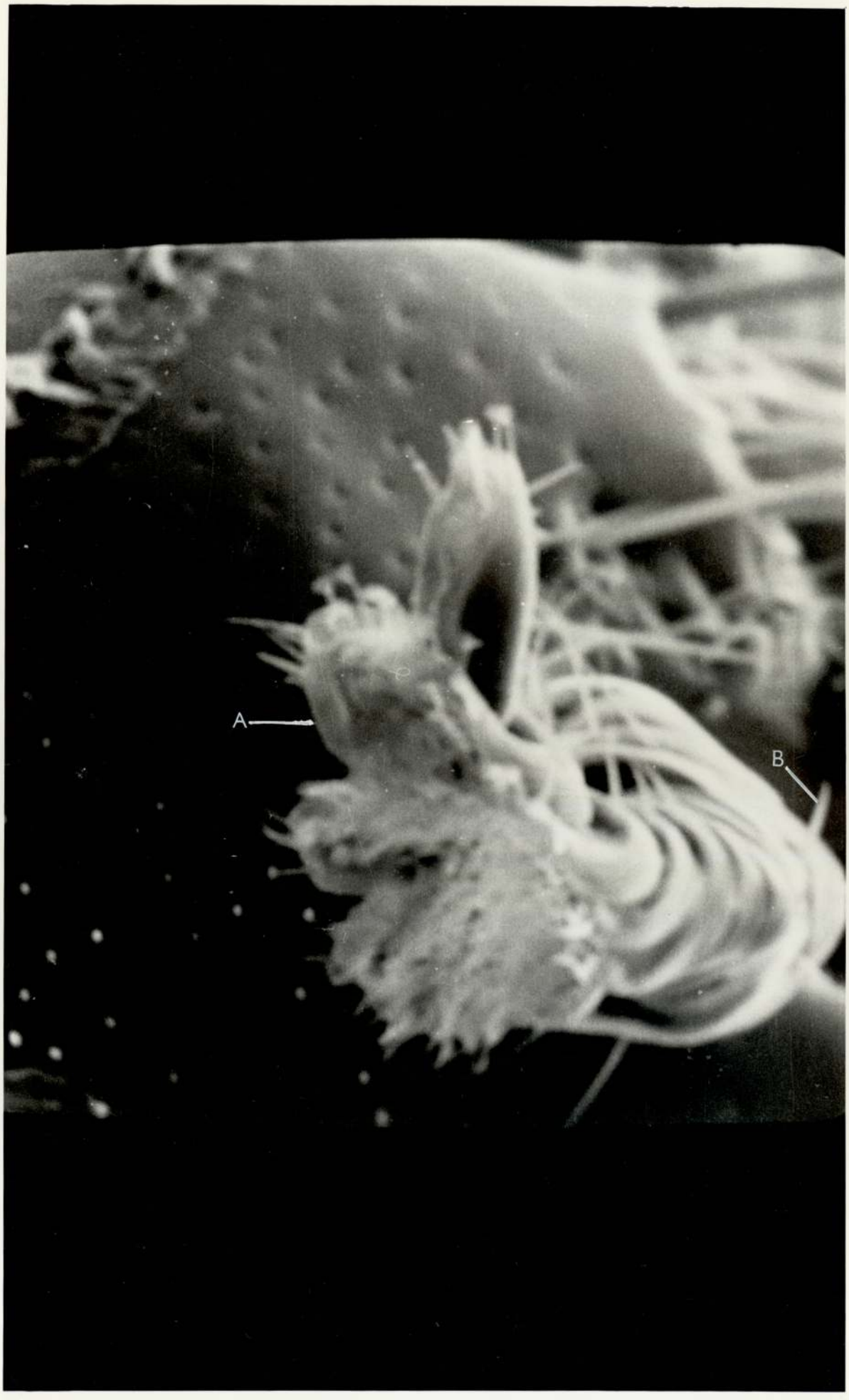
PL . 13



·07 mm.

PL. 14

PL.15

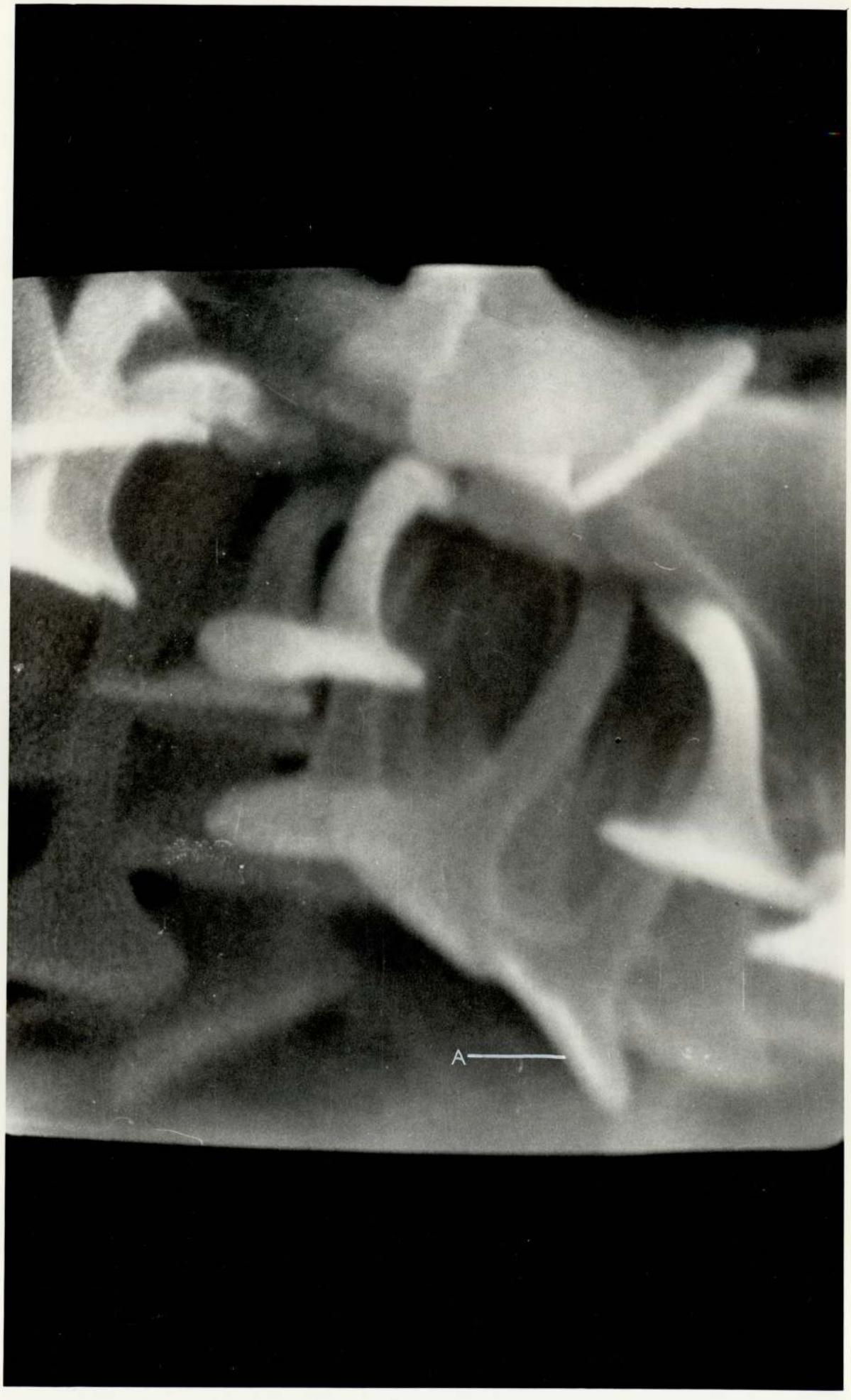


·02 mm.

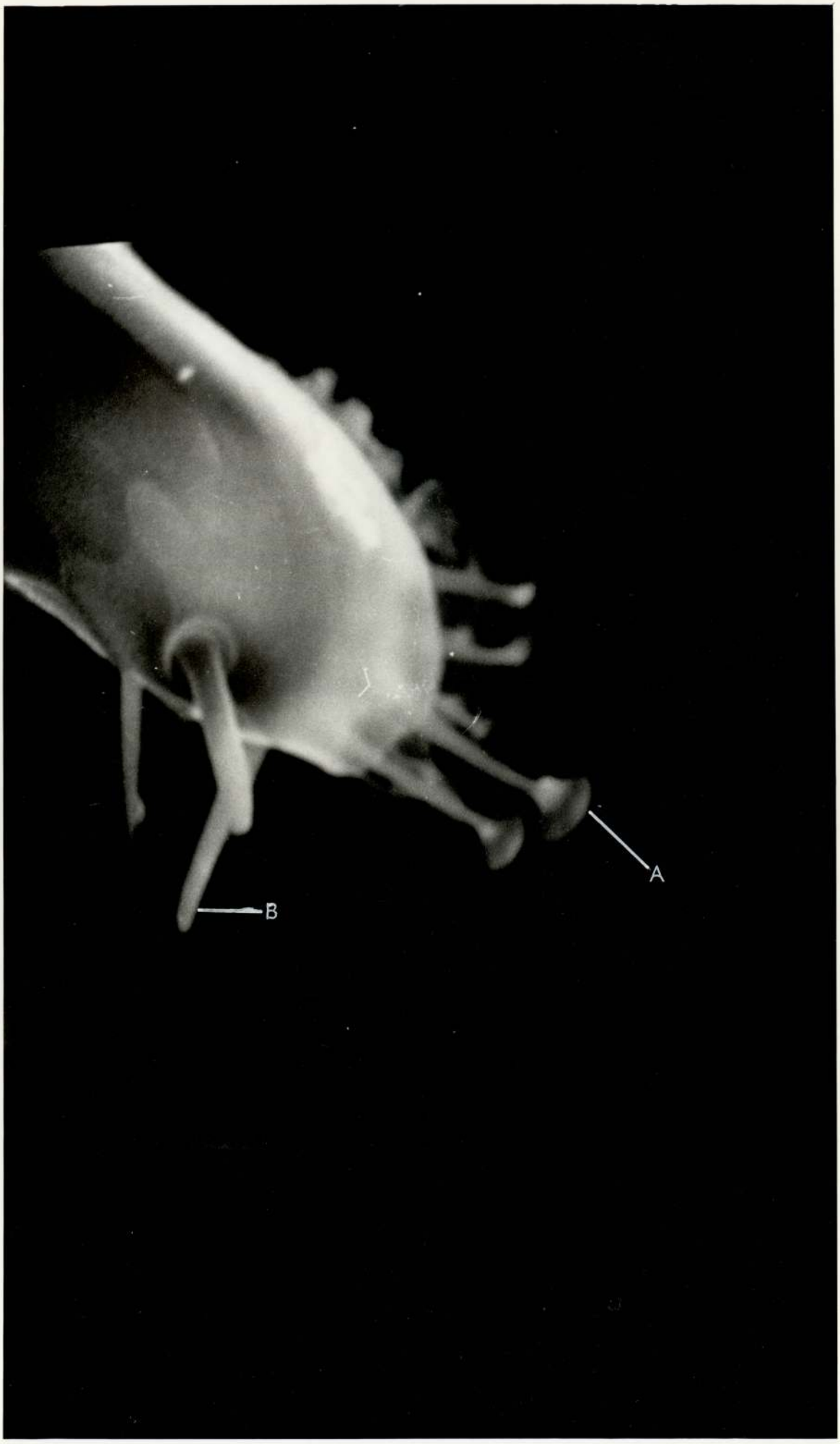
PL. 16

·02 mm.

PL .17



PL.18




A

PL 20

PL.21

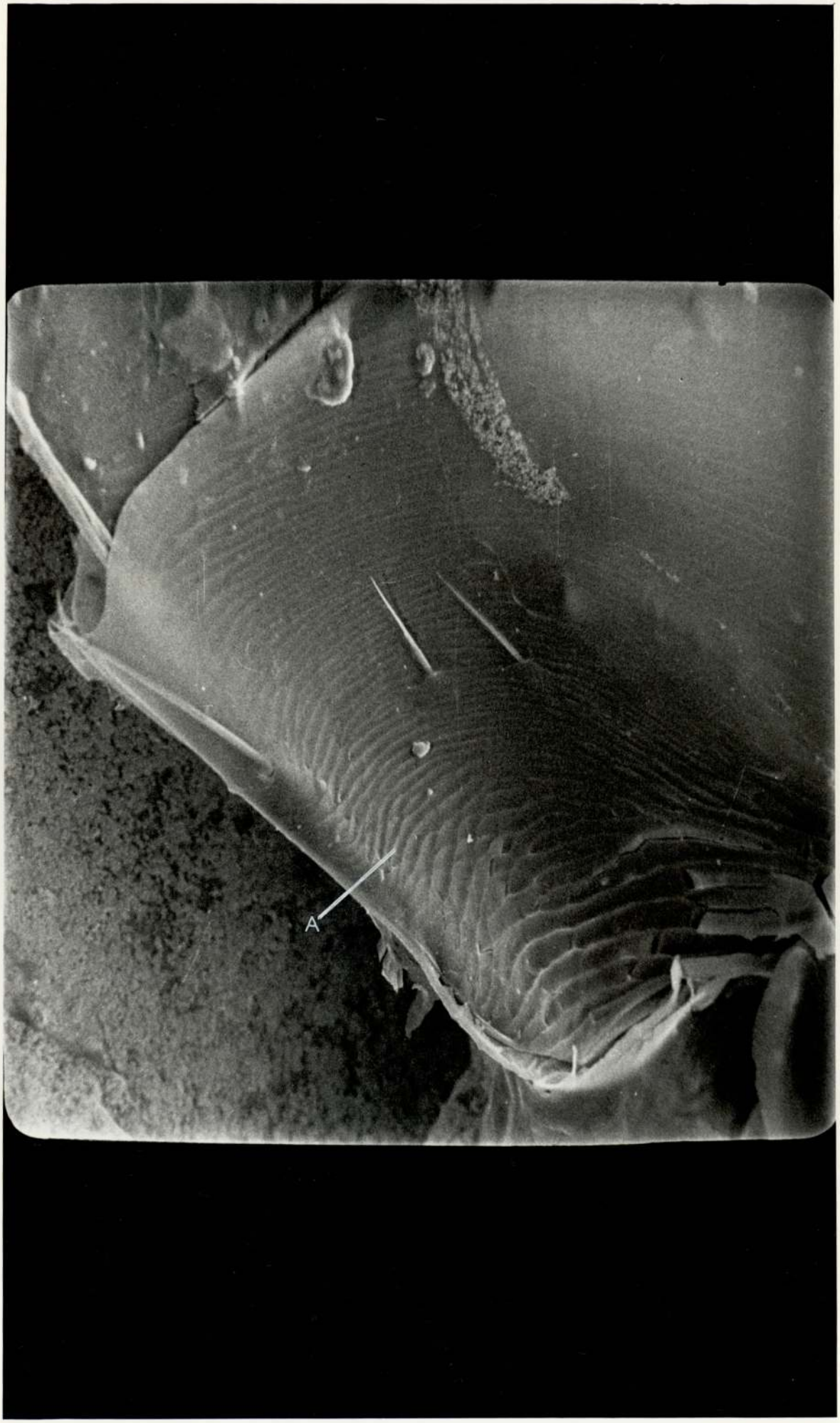
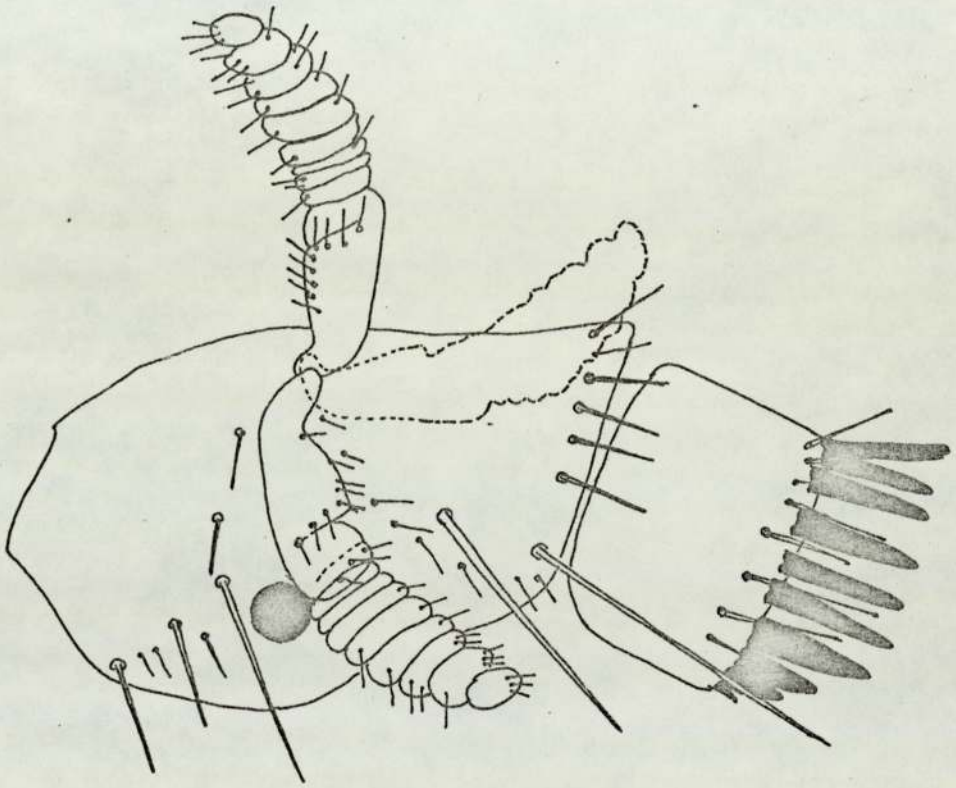


Fig. 21.

Erection of the male antenna.

Fig.21



C. THE STRUCTURE AND ACTION OF THE MALE GENITALIA

Introduction

The male genitalia of fleas form a complex system of which, despite numerous studies, our knowledge is still very limited. The basic morphology and nomenclature of the genital parts were established by Packard (1894), Rothschild (1898), Jordan and Rothschild (1908, 1912, 1922), Oudemans (1909), Minchin (1915), Jordan (1926, 1933, 1937, 1939, 1941, 1942 a, b, 1946, 1947, 1948, 1950), Sharif (1945), Snodgrass (1946), Traub (1950a, 1953-1954, 1957, 1963 a, b, 1965, 1968, 1969), Traub and Johnson (1952a, b), Wenk (1953). Hopkins and Rothschild (1953-1966), Smit (1954, 1960,1970), Johnson (1957) and Rothschild and Traub (1971) have extended this descriptive work.

The action of the genitalia has been studied both by inference from the anatomical relationships of the parts and by direct observation of stages in genital linkage. Mitzmain (1910) while working on rodent fleas described the movements and postures adopted by the copulating pairs, as well as the relationships of some of the anatomical parts. Sharif (1945) described the structure and musculature of the penis rod and from this inferred its mode of action in the cat-flea *Ctenocephalides felis orientalis* (Jordan). Günther (1961) gave an account of the functional anatomy of the copulatory apparatus of the Ceratophyllid fleas.
Snodgrass (1946) described the relations of the intromittant organ from a slide preparation of a mated pair of *Ceratophyllus rossittensis swansoni* (Lin). Holland (1955) on the basis of 23 slide preparations of mated pairs of *C. idius* (Jordan and Rothschild),

C. niger (Fox), *C. rossittensis swansoni*, *Monopsyllus wagneri* (Baker) and *Diamanus montanus* (Baker) and direct observations of the process of pairing, gave an account of the action and function of the parts involved in genital linkage. Most recently Humphries (1967 b) gave a detailed account of the action of male genitalia of the hen flea *C. gallinae*.

Nothing has been published on the action of male genitalia in *N. fasciatus*, and their morphology has been described only in outline in taxonomic studies such as that of Lewis (1967).

The present description of the structure and action of the male genitalia of *N. fasciatus*, may throw further light on the mechanism of copulation in fleas generally. The general methods are given in Chapter 2. The dissections of the fleas were performed on a drop of Ringer's solution on a glass slide, using Borradaile needles under a binocular microscope (Fig. 22).

The Claspers

Structure.

The two claspers lie posterior to their long apodemes which are derived from tergum 9; these apodemes are situated beneath tergum 8. The postero-dorsal aspect of the apodemes are loosely linked together by means of the sensilium (plates 22,23). Each clasper consists of a movable and a fixed process. These processes are not simple plates, rather in both cases their opposed edges are hollowed, somewhat resembling a reptilian jaw, with a single denture on each side. The fixed process has two broad teeth (one on each side) each of which bears a concavity (plates 24-26). The movable process has also a pair of pointed teeth (one on each side) which when the

processes are closed together exactly fit in and against the concavities in the broad teeth of the fixed process (plates 24-26).

The clasping manoeuvres of the movable process appear to be effected by a muscle (flexor muscle) situated in the fixed process, the tendon emerging from the proximal inner side of the fixed process close to the point of articulation and attached to the opposing hollowed interior proximal margin of the movable process (plate 27).

There are numerous long and short setae on the fixed and movable processes of the claspers.

Action.

Holland (1955) and Humphries (1967, 1968a) demonstrated the gripping function of the claspers. During copulation the female's abdomen fits in between the two claspers. The above description of their structure in *Nosopsyllus* and observation of their position in slides of fleas in copula enable a more detailed description to be given of how the grip is established.

The main linkage between male and female terminalia is effected by the grip of the male claspers on the posterior margins of sternum 7 of the female. After the alignment of the terminalia, the male pushes his terminalia vigorously against the female so that the movable process is pushed underneath the posterior margin of sternum 7 (plate 28). Humphries (1967b) appears to infer that it is the pushing of the male's terminalia against the female which closes the movable process against the fixed process like a pincer. The present work suggests that closure is effected by the flexor muscle of the clasper itself. The fixed process comes to overlap sternum

7 from the outside and the firm grip is established by the pointed teeth of the movable process being pushed into the concavities of the teeth of the fixed process with the sternal margins trapped in between them (Diagram 1a,b). The strength of the clasper grip has been remarked on by Humphries (1967b) who reported that the margin of sternum 7 of the female *Ceratophyllus* is sometimes broken at the places where the male claspers grip. Plate 28 shows that distortion of sternum 7 by the teeth of the claspers could easily produce such breakage.

The long and short setae present on the fixed and movable processes of the claspers may have a sensory function relating to alignment and achievement of a firm grip.

Sternum 9.

Structure

The form of sternum 9 of the male *Nosopsyllus* has as in many other genera of fleas been used as an important taxonomic character (for example Smit (1957) and Lewis (1967). However, very little is known about the detailed structure of sternum 9 of *Nosopsyllus*, or indeed about the relationship between its structure and function in this or other fleas.

Sternum 9 consists of two main cuticular flaps which are joined together at the base. Each of these flaps is in turn divided into two in such a way that both flaps remain joined on the internal side. This division allows the distal flaps to move freely towards the inner side, (Plates 7, 29).

Internally, sternum 9 is extended into two vertical "v" shaped proximal arms and one horizontal arm which has been described as an

apode mal tendon of the distal arm of sternum 9 (Smit, 1957), apodeme of pons coxalis (Sharif, 1945) and apodemal rod of sternum 9 (Holland, 1955) (Plates 30, 31).

In between the two flaps of sternum 9 lie the aedegal hooks, the distal part of the aedegus and the penis rod. Part of the function of sternum 9 may be to give mechanical protection to the aedegal hooks and penis rod.

The proximal arms and the apodemal rod are richly supplied with musculature. Sharif (1945) has described the musculature in *N. fasciatus* as follows.

(i) The retractor of the ninth sternite, extending from the lower anterior end of the ninth abdominal sternite to the proximal portion of the anterior apodemal rod.

(ii) The external dorso-ventral muscles, which are arranged into two broad sheets of numerous fibres arising from the apodemal rod and extending upto the lower margins of the tenth sternal apodeme.

Besides these muscles described by Sharif it was noted that the proximal arms are also supplied with musculature. These muscles arise from the lower part of the proximal arms and extend up to its dorsal scoop shaped end. These are suggested to be the ninth sternite protractor muscles (Plates 30,31).

Action

Humphries (1967) has given a brief account of the function of sternum 9 in *C. gallinae*. During copulation the distal edge of sternum 9 is brought into alignment with female's genital aperture, and protracted so that the arms engage with short stout setae on sternum 9 of the female and serve to establish correct alignment for

subsequent stages in copulation. The function of the distal arms of sternum 9 in *Nosopsyllus* appears to be similar and the present detailed observations on their structure together with Sharif's descriptions of the musculature enable a fuller picture to be proposed as to their mode of action. The protractor muscles push the distal flaps of sternum 9 into the female's genital chamber by pulling the proximal arms of sternum 9 downwards thus bringing the distal flaps inwards. Outwards lateral pressure by the arms of sternum 9 against the walls of the genital aperture can be acheived by the action of subsidiary muscles associated with the protractor muscles. These subsidiary muscles pull the proximal arms apart thus widening the gap between the two distal flaps of sternum 9. This ensures a clear aperture for the entrance of the penis rod. The semi-divided distal flaps also press tightly against the genital aperture as they curve towards the inner side or can be pulled by the muscles towards the interior. Sternum 9 also guides the penis rod to the female's genital aperture because the penis rod rests inbetween the two flaps of it. The withdrawal of sternum 9 after the commencement of copulation is effected by the retraction muscle of the 9th sternite and external dorso-ventral muscles.

The bristles on the distal flaps of sternum 9 maybe sensory in function and concerned with adjustment of alignment. For diagramatic illustrations of the action of sternum 9 see plates (6,29,32) and diagrams (2a, b).

Aedegal hooks

Structure

Lewis (1967) in his account of the taxonomic characters of the genus *Nosopsyllus* has given diagrams of the male genitalia which show the presence of aedegal hooks but he did not describe them.

The two aedegal hooks are the external extension of the aedegal crochets. Normally, they lie inbetween the two flaps of sternum 9 but during copulation the aedegal hooks become fitted into the cavity in tergum 8 of the female (Plates 7,29,33).

Action

Though the main linkage of the male and female genitalia is established by the male claspers, it is possible that during copulation the male's vigorous movement against the female may cause loss of correct alignment of the aedegus and penis rod; the function of the aedegal hooks appears to be to prevent this. After the introduction of the penis rod into the female's oviduct, the secured hooks move forward and fit into the cavity of tergum 8 of the female (Plates 6,7,29).

Penis rod

Previous accounts of penis rod structure in other flea species were based only on investigation under the light microscope. The present account based on electron microscopy, shows a much more complex structure than had previously been described. It may be partly because of this more detailed study that the penis rod of *Nosopsyllus* appears peculiar in shape and structure as compared to other species of fleas. There are however, certain important differences, for instance in *C. gallinae* and *S. cuniculi* the whole penis rod lies, at rest, inside the aedegus, and during copulation it is extruded and introduced into the female's oviduct, whereas in *Nosopsyllus*, the outer penis rod is not retracted inside the aedegus but lies in a folded-back position in between the two flaps of sternum 9 (plates 29,34).

The outer penis rod is unique in structure and although under the microscope it appears to be smoothly cylindrical, it is in fact a cylinder composed of a rolled up blade so that it gives a spiral impression in cross section (plates 35,36). Towards the tip it becomes narrow forming a neck which then broadens to form a funnel-like structure bearing a hole in the centre, from which the inner penis rod can be extruded or withdrawn (Plates 37,38,39).

The inner penis rod like the outer penis rod, is also an open tube horse-shoe shaped in cross section. The open side of it faces in the opposite direction to the open side of the outer penis rod (plates 36,40). The inner penis rod can be withdrawn inside the aedegus and only the tip of it lies outside the opening of the outer penis rod (Plates 38,41).

Action

For a better understanding of the action of the penis rod it is appropriate to give a short account of the musculature of the copulatory apparatus as described by Sharif (1945).

1. Protractors of the copulatory apparatus

(a) Upper protractor (b) Lower protractor.

"The contraction of both these sets of muscle fibres results in the protrusion of the copulatory apparatus from the abdomen.

2. The protractors of the lower copulatory rod which are also two in number.

"Their function of protration of the lower copulatory rod is intensified by the depression of the 10th sternal apodeme".

3. The extensor of the upper copulatory rod.

This muscle drags the upper copulatory rod along with the lower one, when the latter is protracted.

i. The extensor of the lower copulatory rod.

"Both the extensors prevent the unnecessary bending of the anterior one third of the copulatory rods which is likely to happen due to the contraction of the protractors of the lower copulatory rod. Thus they assist the protractors indirectly in their function of protraction of the copulatory rods."

Sharif did not describe any musculature within the penis rod; Humphries (1963) noticed that in the case of *C. gallinae*, "the tip of the pointed penis rod twitched from side to side with a snake like motion, although no movement was apparent in the more proximal parts of the rod." This suggests that there may be some sort of musculature within the penis rod; indeed in *Nosopsyllus* due to the folded back

position of the outer penis rod which lies distal to the aedegus and due to its stout structure, it seems difficult to conceive how the rod is straightened to enable copulation unless it is supplied with some intrinsic musculature.

Both outer and inner penis rods are not closed tubes as previously mentioned. The functional reasons for their peculiar structure appear to be connected with the need for the penis rod to bend, both at rest and during penetration of the spermathecal duct. Narrow plastic tubing of two different diameters was used to investigate the properties of closed versus longitudinally open tubes. The narrow tubing was inserted into the wider tubing. The observations showed that in the case of closed tubing the outer tube tended to compress tightly the inner one when bent thus hindering its movements, whereas in the case of open tubing the bent zone tended to broaden thus giving the inner tube free mobility. If the outer penis rod was a closed cylinder, then when bent at several places (as during copulation) it would not only hinder the free mobility of the inner penis rod but also by pressing the inner rod might impede the flow of the sperms. There is another functional reason why the penis rod should consist of open tubing. When the male flea after copulation turns around and starts walking in the opposite direction to the female, considerable stress could be imposed on a closed cylinder. This possibility of damage is avoided by the open structure of the tube which readily allows distortion by twisting. The open nature of the rods poses little problem of sperm leakage as the inner rod slides over the outer rod in such a position that the two form jointly a closed cylinder (plate 36).

During copulation the outer penis rod is erected and introduced into female's oviduct (Plate 42). The outer penis rod penetrates as far as the junction between the ductus copulatrix and the spermatheca (Plate 6). The tunnel like structure at the tip of the outer penis rod appears to fit against the wall of the ductus copulatrix at the point of origin of the spermathecal duct. The inner penis rod is extruded further and can thus enter the spermathecal duct. It travels through the whole length of the spermathecal duct and it may be assumed that the sperms are shed directly into the spermatheca.

Summary

1. The structure of claspers and of sternum 9 of the male is described in detail. Their mode of action is related to their structure and musculature.
2. The two aedegal hooks are the external extension of the aedegal crochets. Normally they lie in between the two flaps of sternum 9. During copulation the two recurved aedegal hooks fit into the cavity of tergum 8 of the female thus producing a correct alignment of the aedegus and penis rod.
3. The outer penis rod lies in a folded back position in between the two flaps of sternum 9. It is a cylinder composed of a rolled up blade. The inner penis rod is also an open tube and is horse-shoe shaped in cross section. The open side of it faces in the opposite direction to the open side of the outer penis rod. During copulation the outer penis rod penetrates as far as the junction between the ductus copulatrix and the spermathecal duct, while the inner penis rod is further extended and travels through the whole length of the spermathecal duct.

PLATES 22 - 42

Plates 22-42

Pl. 22. Claspers, lateral view.

A. Sensillium.

Pl. 23. Claspers, dorso-lateral view.

A. Sensillium.

Pl. 24. Claspers, lateral view.

A. Moveable process.

B. Fixed process.

Pl. 25. Claspers, lateral view.

A. Tooth of moveable process

B. Tooth of fixed process.

Pl. 26. Claspers, showed hollowed fixed process (A).

Pl. 27. Claspers, showing insertion of the flexor muscle (A).

Pl. 28. Fleas in copula, showing the gripping position (A) of the male claspers on the 7th sternum of the female.

Pl. 29.

A. Distal flaps of sternum 9.

B. Penis rod lying between the two flaps of sternum 9.

Pl. 30. Sternum of the male flea.

A. Flaps of sternum 9, separated from each other.

B. Proximal arms of sternum 9, separated from each other.

Pl. 31. Sternum 9 of the male flea.

A. Proximal arms.

B. Apodemal rod.

C. Basal flap of sternum 9.

D. Distal flap of sternum 9.

E. Setae on the flaps of sternum 9.

Pl. 32. Fleas in copula.

A. Sternum 9 in the female's genital chamber, the distal flaps against the aparture of the duct of the bursa copulatrix.

Pl. 33.

A. Aedegal hooks.

Pl. 34. Male terminalia.

A. Penis rod lying in between the two flaps of sternum 9 in normal resting position.

Pl. 35. Outer penis rod, showing ventral opening where the cylinder is incomplete.

Pl. 36. Transverse section of penis rods.

A. Outer penis rod.

B. Inner penis rod.

Pl. 37.

A. Penis rods.

Pl. 38. Tip of penis rods.

A. Neck of outer rod.

B. Outer penis rod.

C. Inner penis rod protruding from aparture in the funnel.

D. Funnel of outer rod.

Pl. 39. Penis rods.

A. Outer penis rod.

B. Neck.

C. Funnel.

D. Inner penis rod.

Pl. 40. Tip of penis rods viewed end on.

A. Inner penis rod showing its structure as an incomplete cylinder.

B. Opening of the outer penis rod, from which the inner rod protrudes.

Pl. 41. Penis rods.

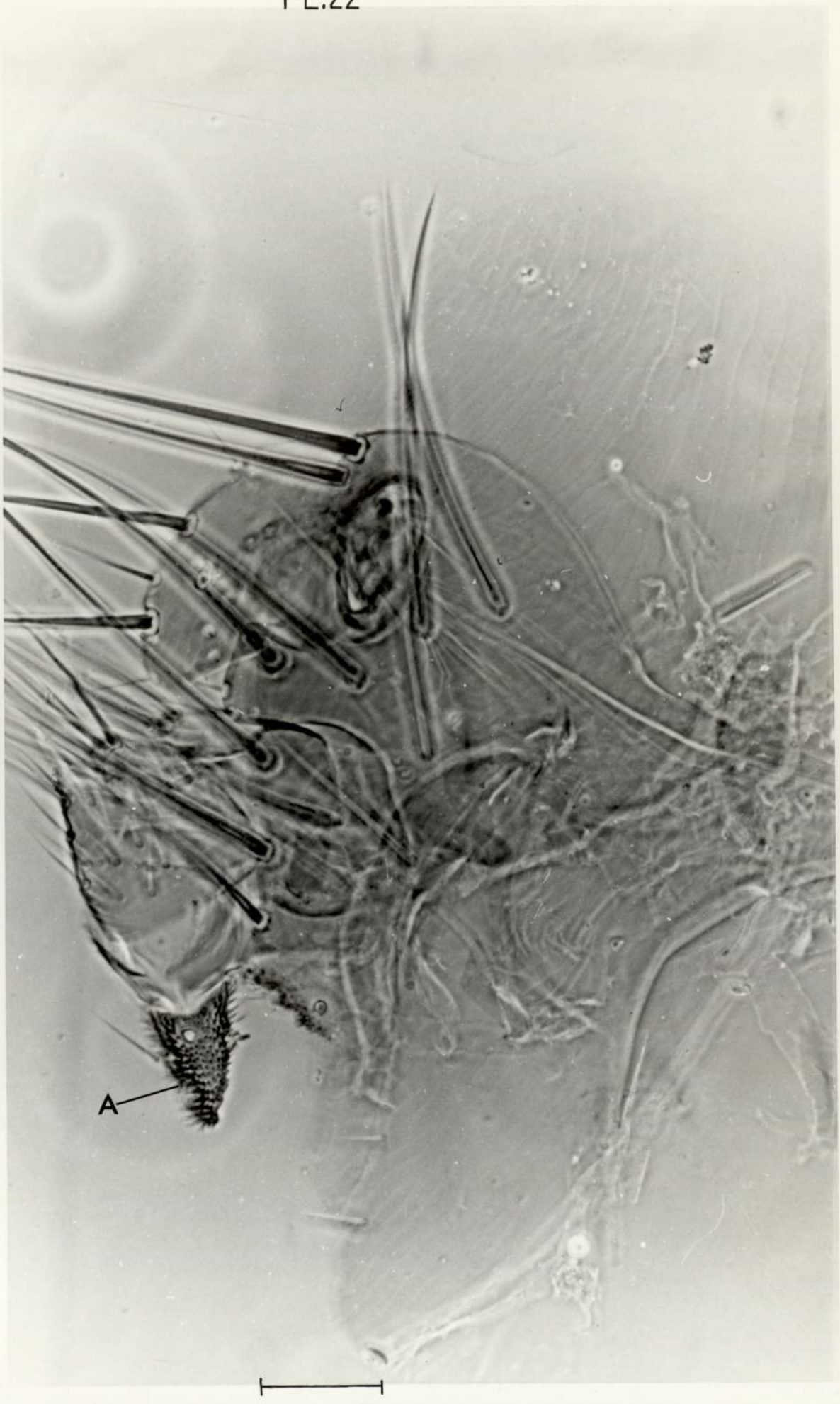
A. Outer penis rod.

B. Inner penis rod in an extruded position.

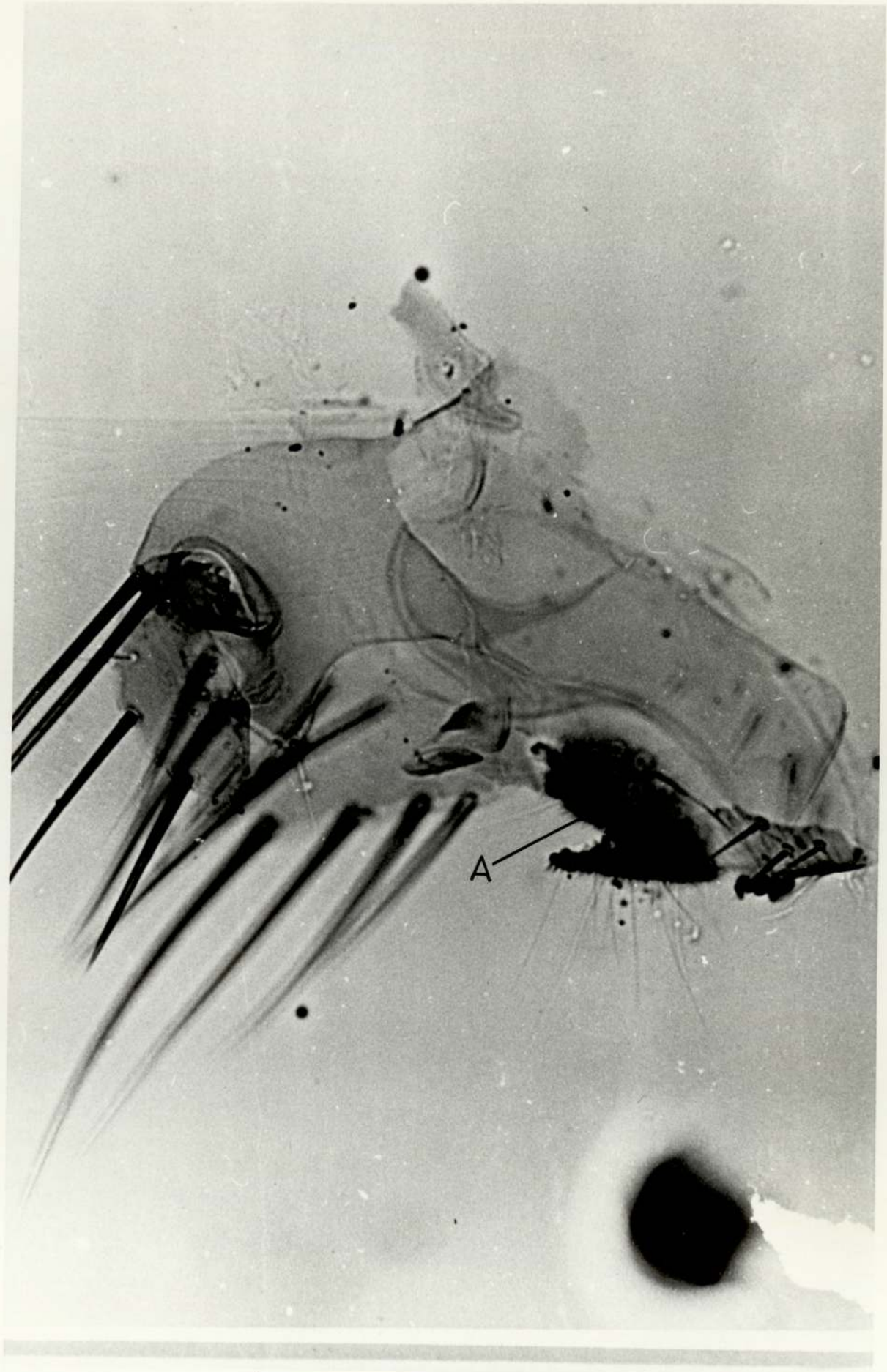
Pl. 42. Male flea.

A. Erected penis rod. The inner rod is just visible within the outer rod.

PL.22



PL.23



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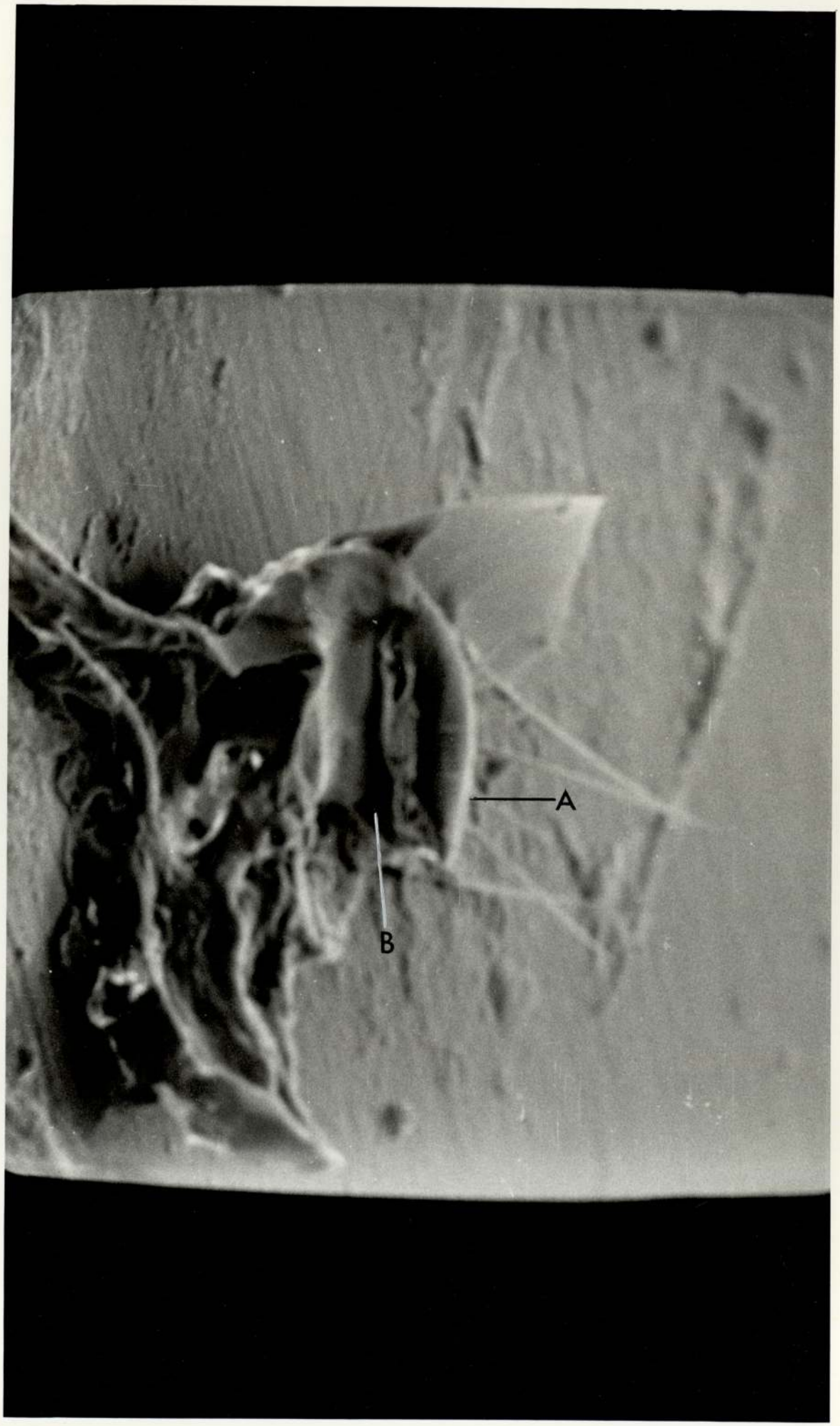
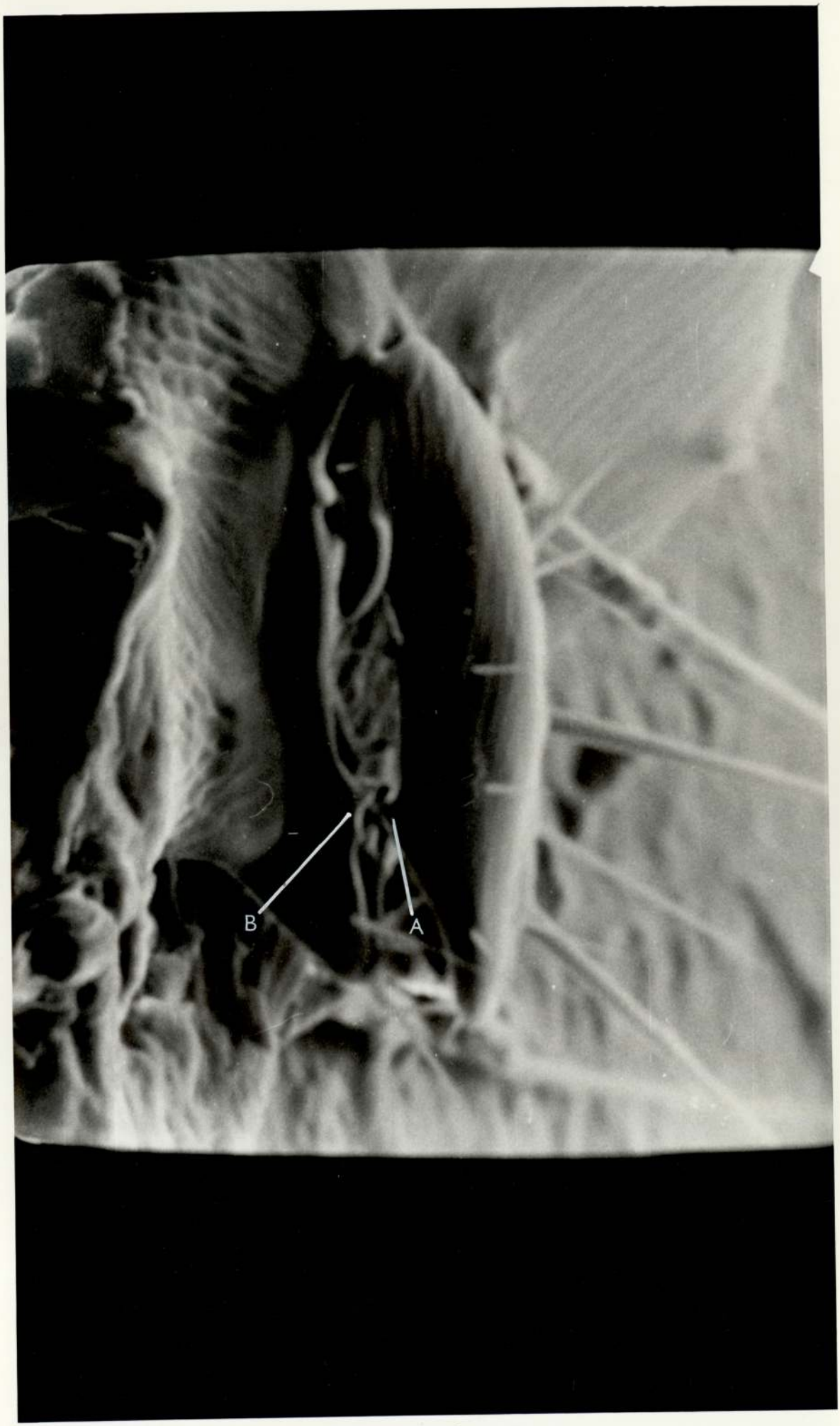
PL.24



PL.25



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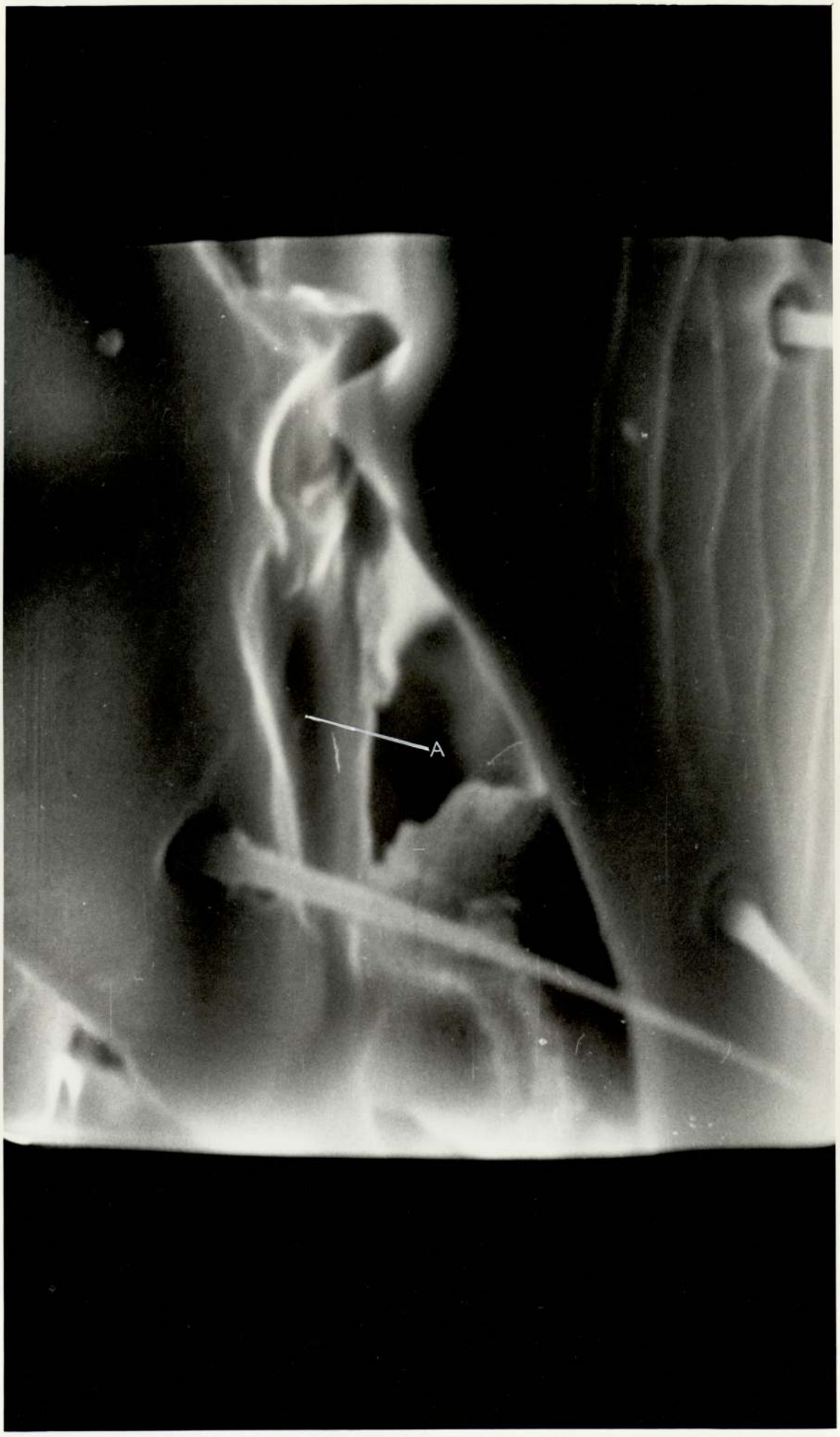
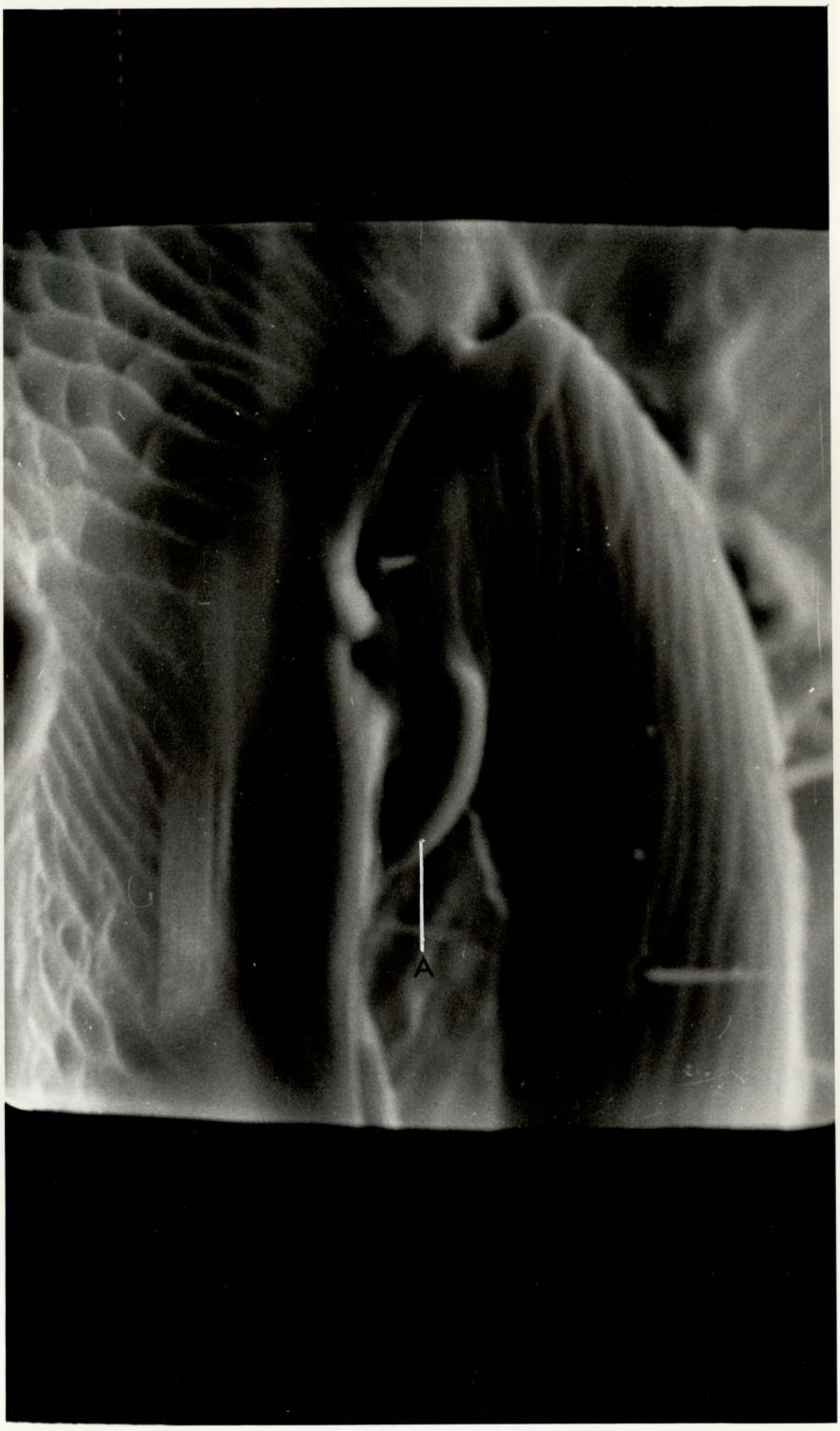
PL.26



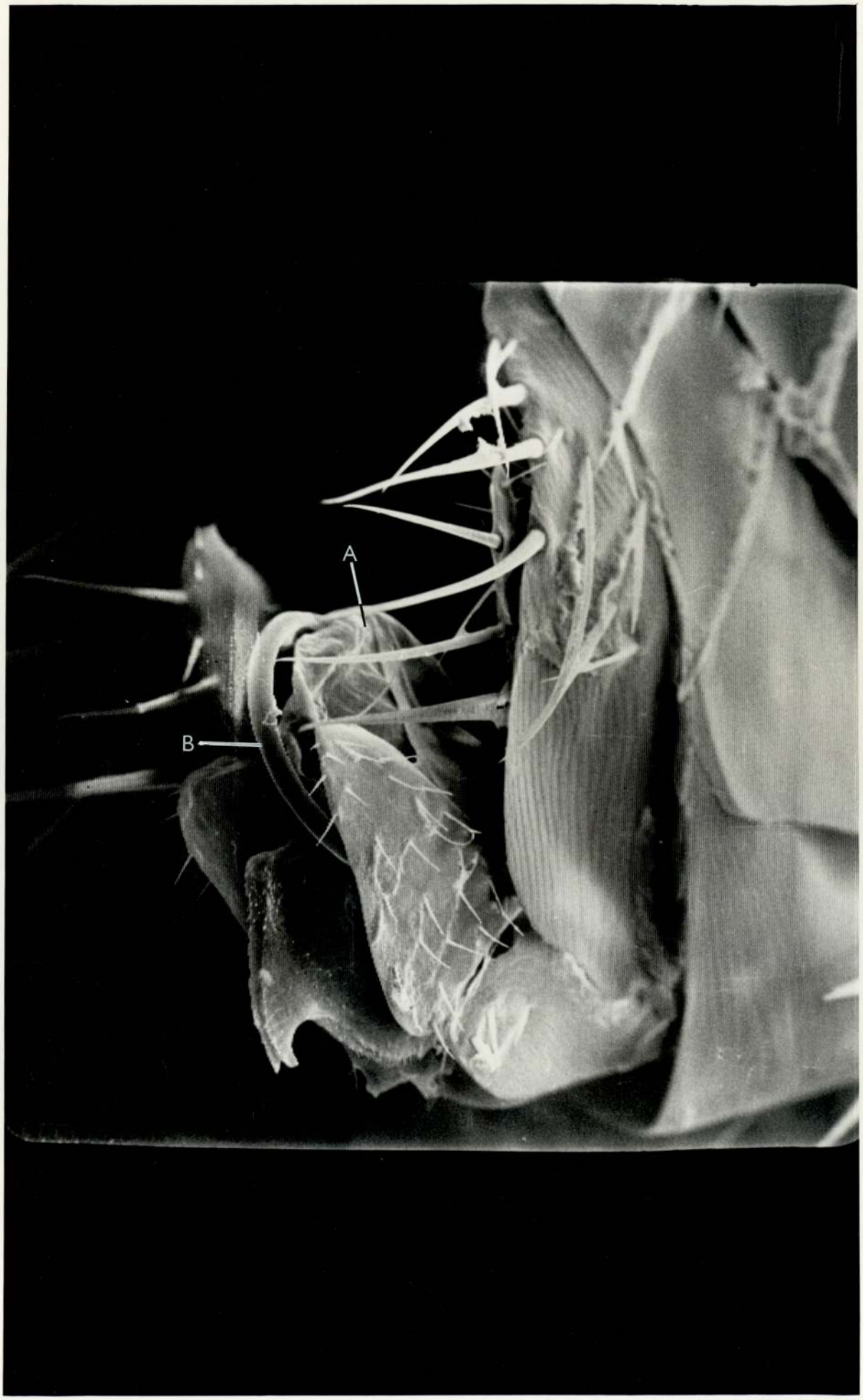
·01mm.

PL.27

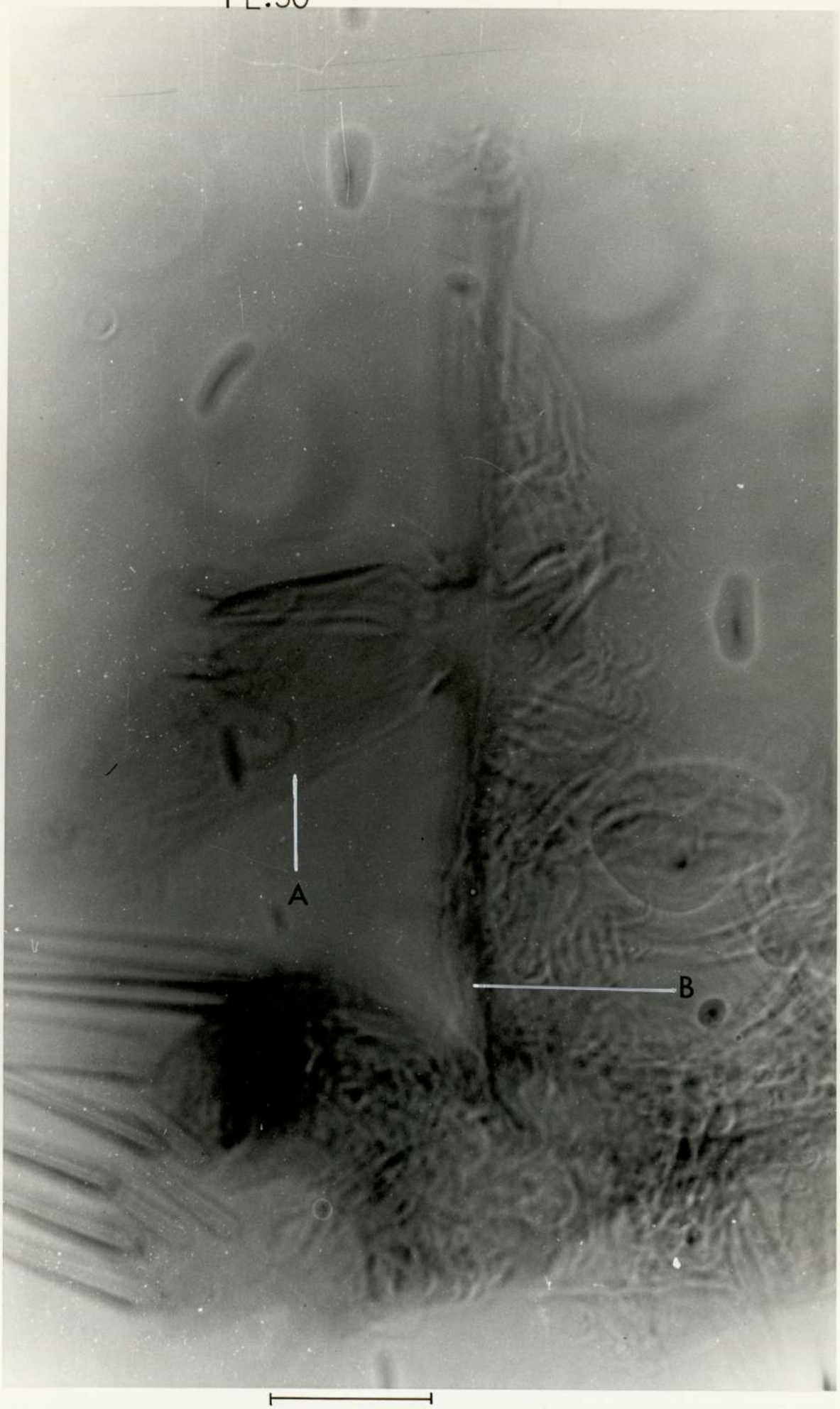


PL.28

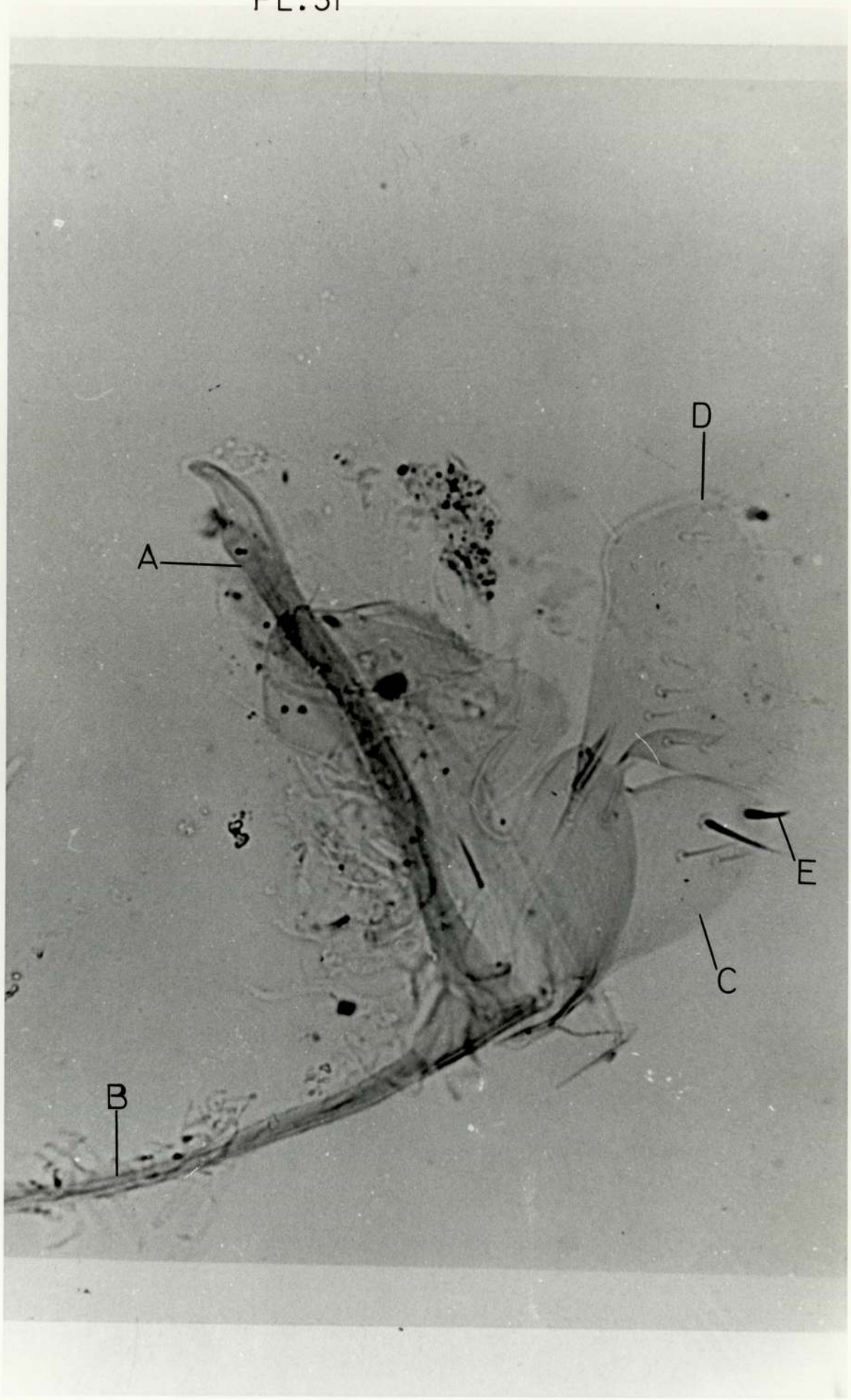
PL.29



PL.30

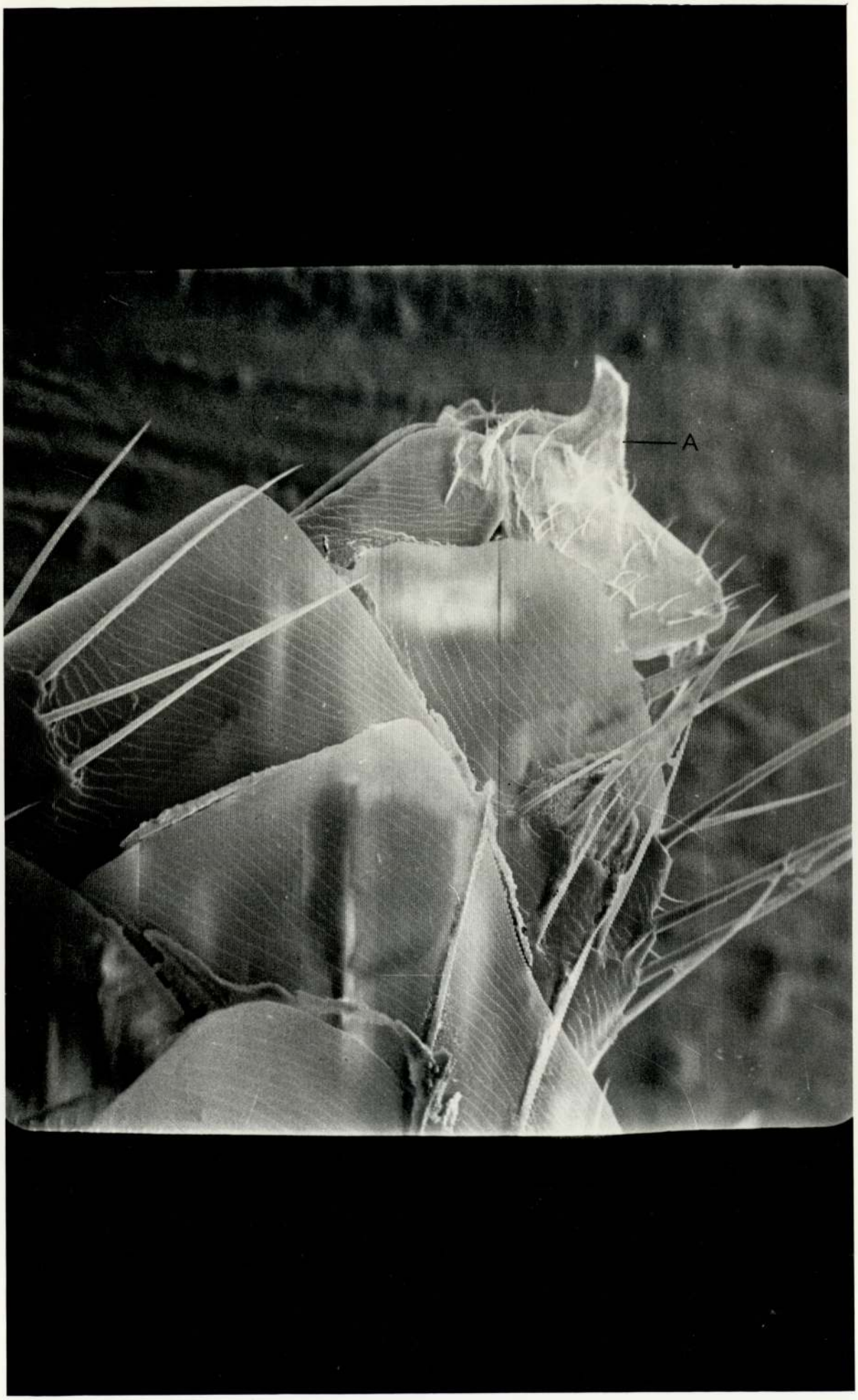


PL. 31



PL. 32

PL.33

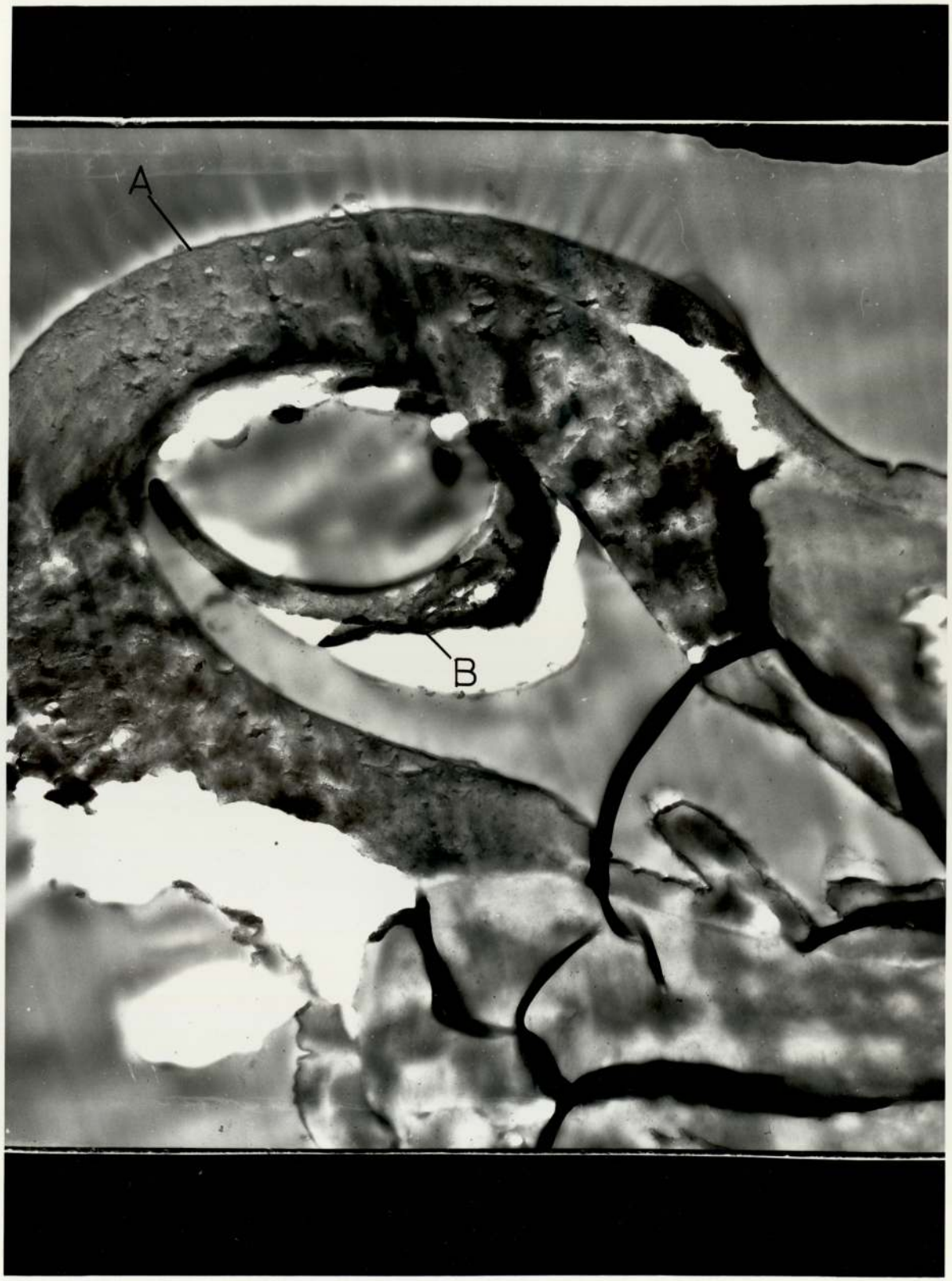


PL. 34

.15 mm.

PL. 35

PL. 36



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PL.37

PL.38

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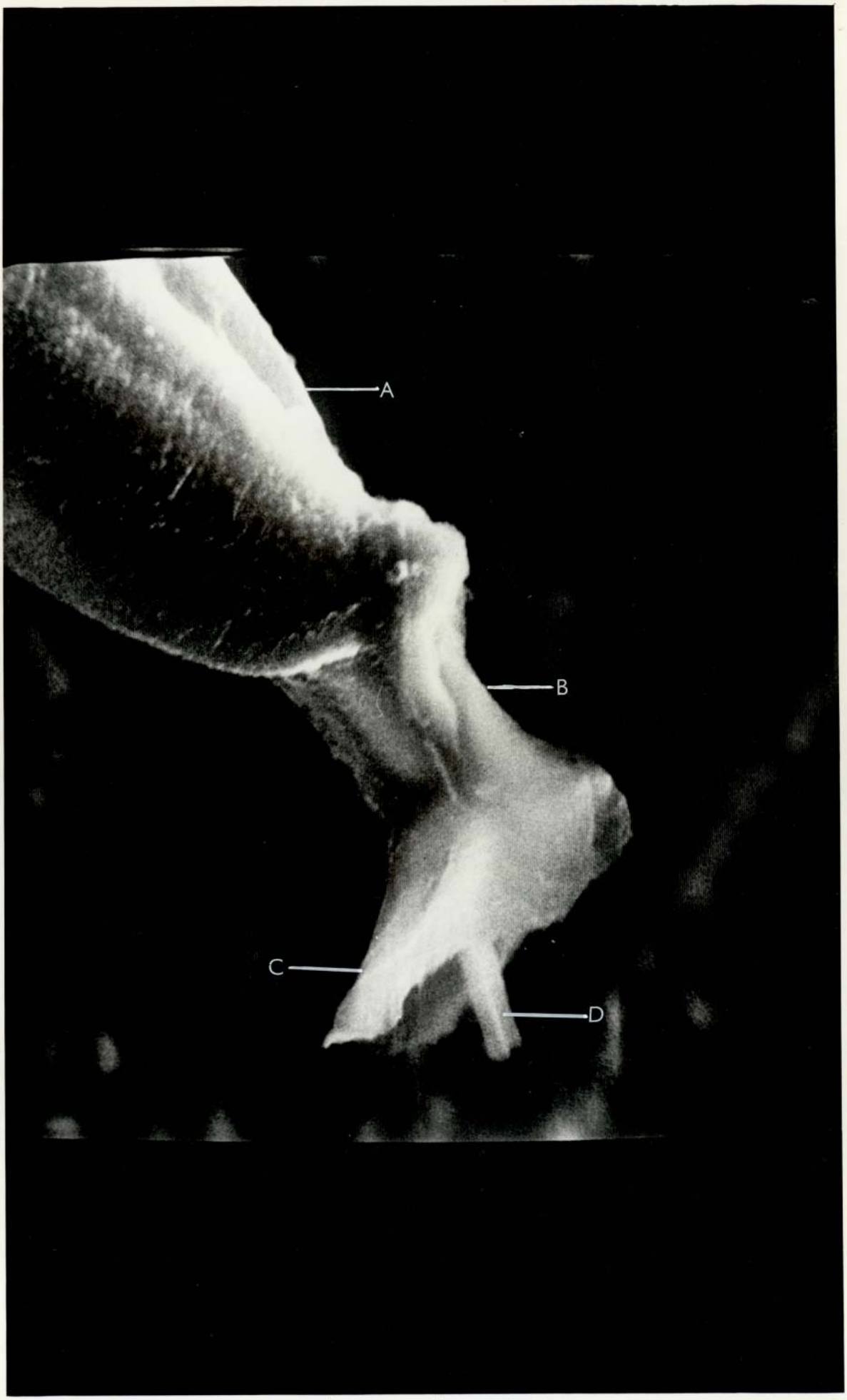
PL.39



PL.40

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PL.41

·01 mm.

PL.42

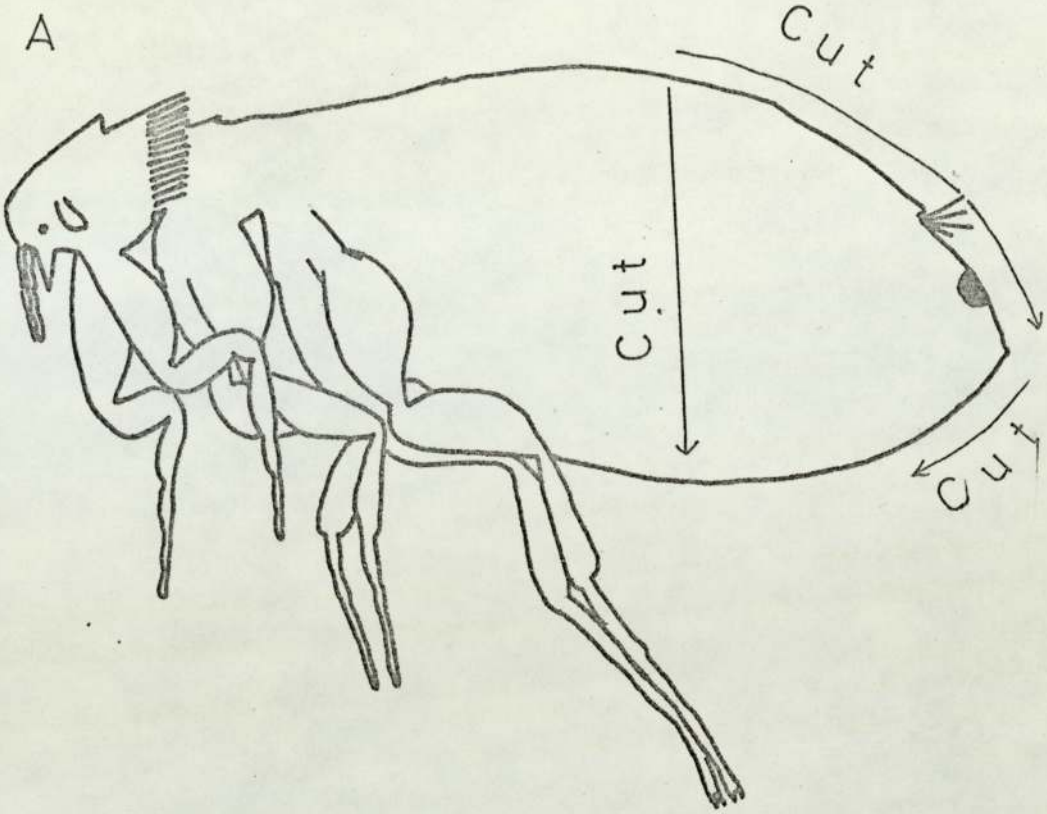
·2mm.

Fig. 22. Dissection of the flea.

A. Method of making incisions.

B. Opening of the abdominal flaps of one side for taking out the genitalia.

Fig. 22



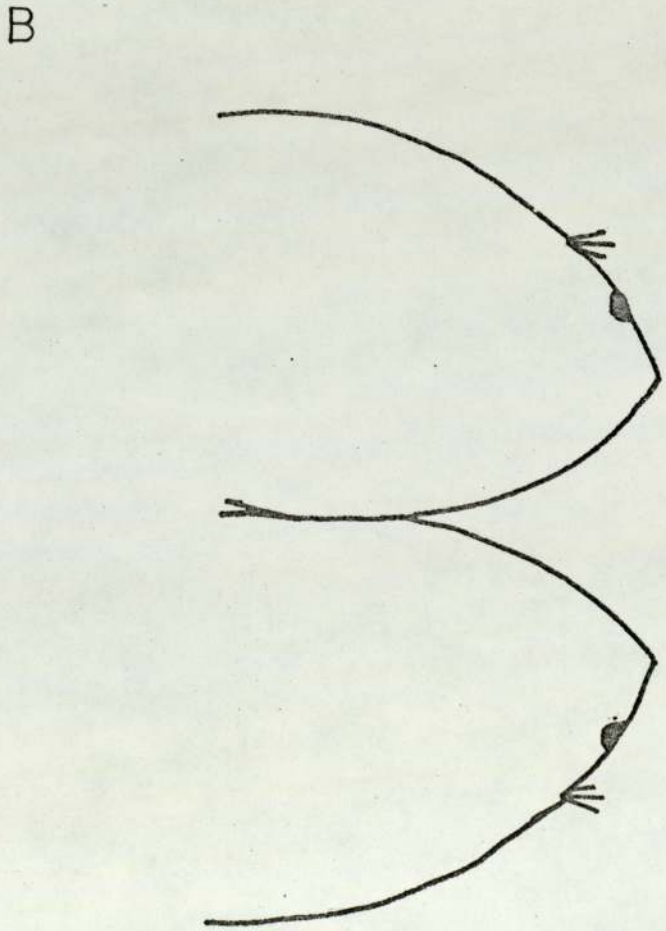


Diagram I. Clasping of the 7th sternum of the female by the male claspers.

(a). Lateral view.

A. Tooth of movable process.

B. Tooth of fixed process.

C. Flap of sternum 7 of the female pressed in between the teeth A and B.

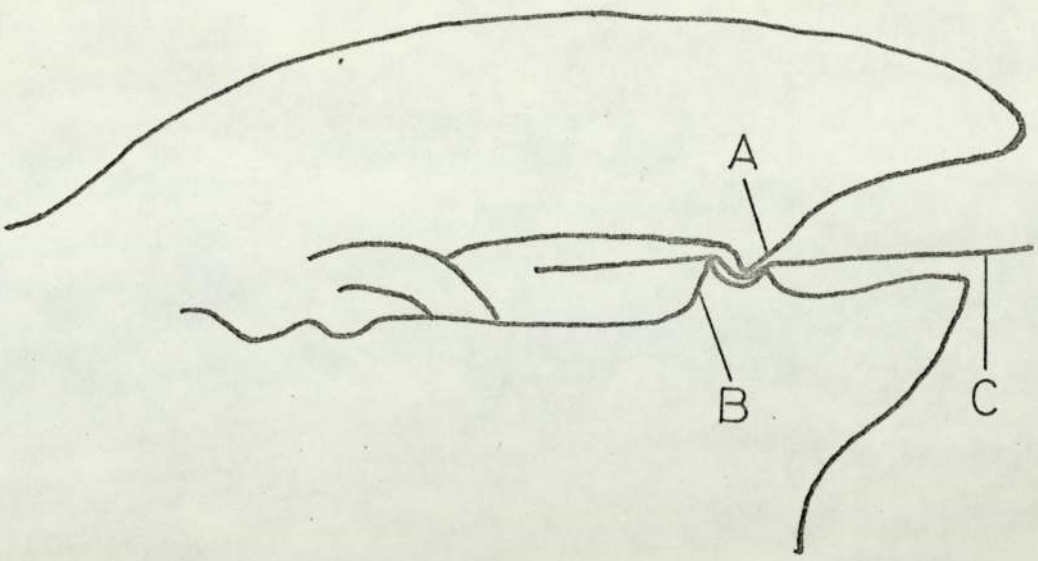
(b). Anterior view.

A. Teeth of movable process

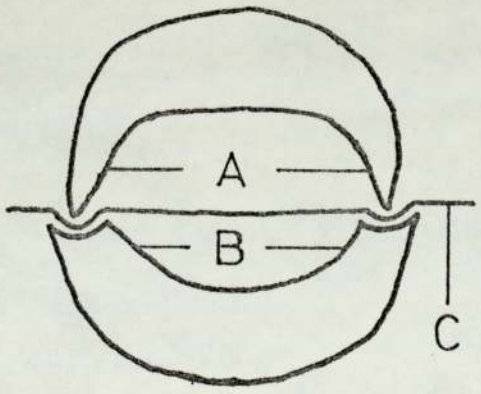
B. Teeth of fixed process

C. Flap of the sternum 7 of the female pressed in between the teeth A and B.

DIAGRAM.1



a.



b.

Diagram 2. Action of sternum 9 of the male flea.

(a). Preliminary alignment.

A. Female genital chamber (vagina).

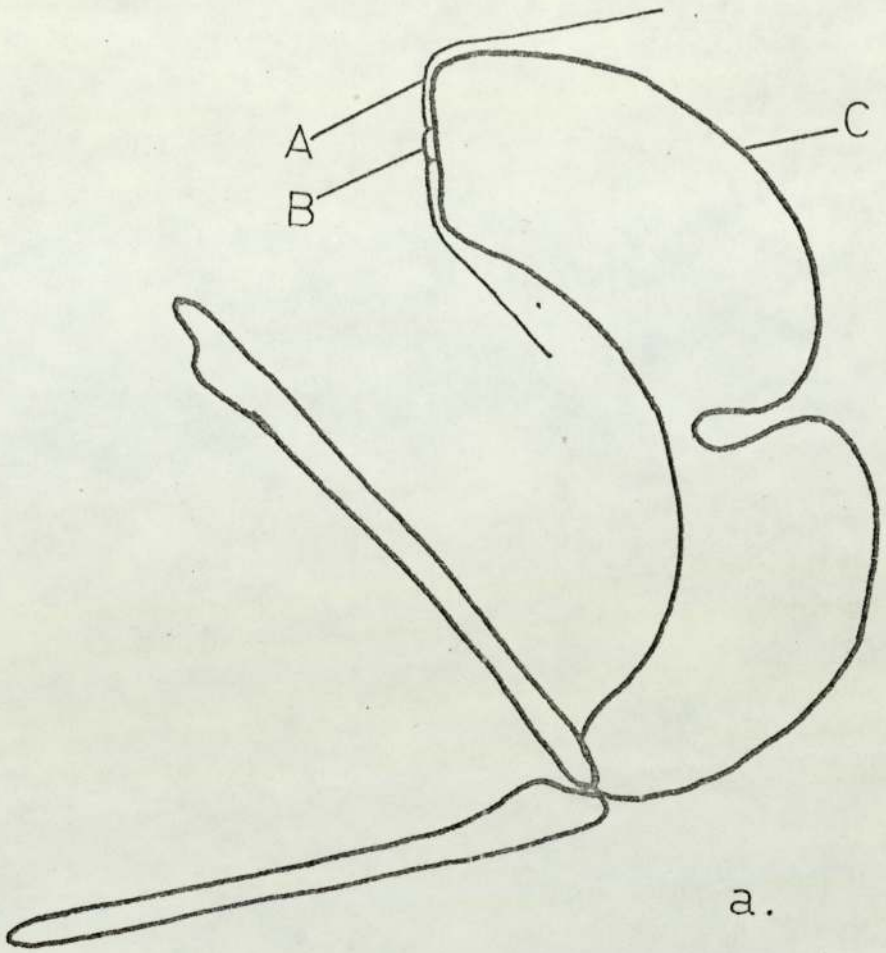
B. Aperture of duct of bursa copulatrix.

C. Flaps of sternum 9 pressing against aperture of duct of bursa copulatrix.

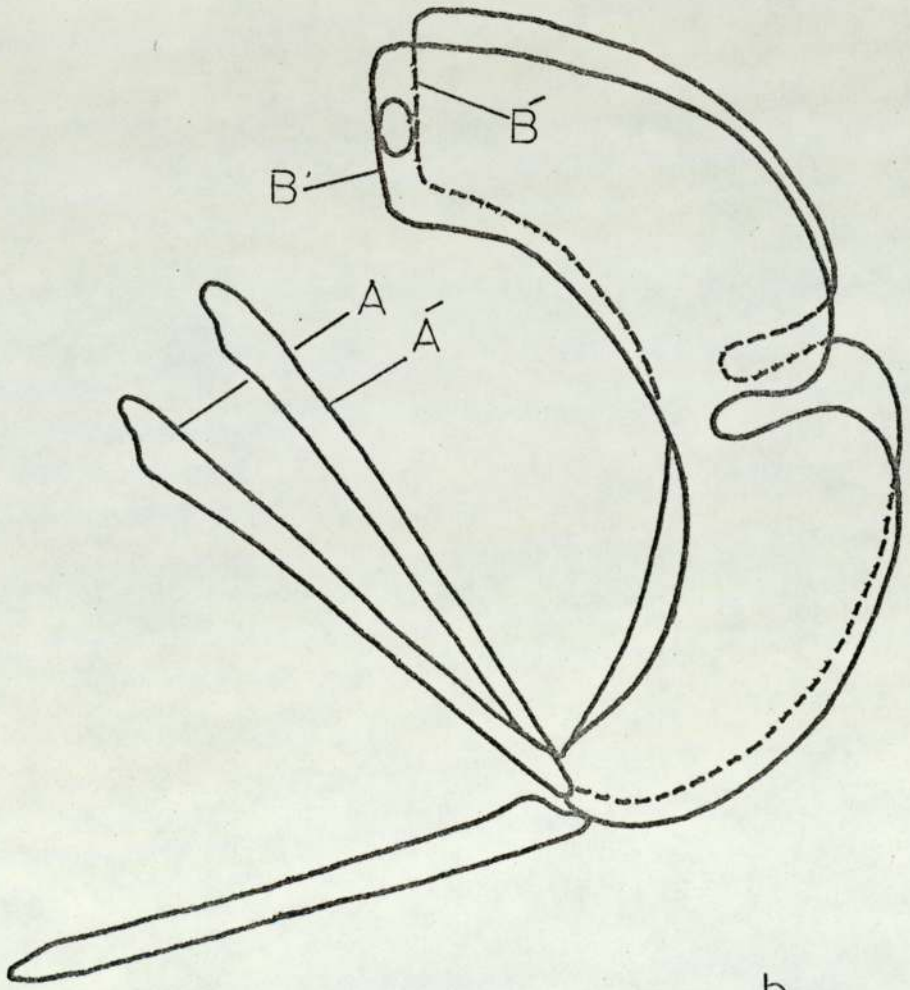
(b). After alignment, flaps of sternum 9 (B,B) pressing outwards, opening the aperture for insertion of the penis rod.

A. The two proximal arms of sternum 9 which are pulled apart to cause the separation of the two flaps of sternum 9.

DIAGRAM.2



a.



b.

CHAPTER 4.

READINESS TO MATE.

A. TEMPERATURE AS A CRITICAL FACTOR IN THE MATING BEHAVIOUR

Several species of fleas parasitizing mammals have been reported to require a blood meal before they will mate. Mitzmain (1910) states that mammalian blood is necessary for copulation in *P. irritans* and in *D. montanus*, Poole & Underhill (1953) found a similar requirement in *M. claytoni*. In several ceratophyllid bird fleas, however, a blood meal is not an essential preliminary to mating, as shown by Humphries (1963, 1969) in *C. gallinae* , *C. garei* Rothschild and *C. fringillae* (Walker), by Waterson (1912) in *C. farreni* Rothschild, and by Holland (1955) in *C. niger* Fox, *C. idius* Jordan and Rothschild and *C. riparius* Rothschild. However, this is not to say that a blood meal has no effect on mating in *Ceratophyllus;* groups of unfed *C. gallinae* which have, after several days, ceased to mate, will resume mating activity immediately after blood meal on the human arm.

The evidence is conflicting regarding *fasciatus* also a ceratophyllid flea. Strickland, (1914) reports that it does not copulate unfed and in any case never in the first week after emerging from its cocoon. Bacot (1914) asserts that it will pair when unfed, indeed shortly after emergence.

The precise physiological manner in which the blood meal affects mating behaviour is not known. The effects of the quality of the blood meals taken by the European rabbit flea *Spilopsyllus cuniculi* (Dale) on the maturation of the ovaries and the probability of insemination have been the subject of much recent research (Mead-Briggs, 1964; Mead-Briggs & Vaughan, 1969; Rothschild & Ford, 1966) and hormones from the anterior lobe of the pituitary of the host have been implicated as critical factors. The reproductive dependence of *Spilopsyllus* on hormonal changes in its host is,

however, to be regarded as a specialization to its very unusual ecology (Mead-Briggs , 1964) and these findings therefore have little predictive value for reproductive processes in rodent fleas.

THE EFFECT OF A BLOOD MEAL

Preliminary observations indicated that *N. fasciatus* will not mate in the experimental arena when unfed. Fleas which had taken a blood meal were seen to mate soon afterwards, even when the blood had been ingested immediately after emergence from the cocoon.

In order to determine whether the stimulating effect of the blood meal, allowing mating to occur, was on the male or on the female a series of tests were set up. Males and females were sorted into separate batches directly after emergence from their cocoons. Immediately after emergence half the males and half the females were allowed a blood meal for up to 3 h. After this feeding period males and females were placed together in groups of ten (five pairs) in the experimental arenas at about 23°C, combining unfed and fed males with unfed and fed females in all four possible ways. Twenty-five pairs were used in each combination. They were kept under continuous observation for 3 h and instances of mating were counted (Table 2).

Table 2 Numbers of pairs (out of 25) which mated when fed and unfed fleas were variously combined.

	Unfed males	Fed males
Unfed females	0	0
Fed females	0	25

The results indicate that both sexes require a blood meal before mating will occur. If one of the partners has not had a blood meal, mating cannot take place.

There are several factors associated with the taking of a blood meal which via nervous or endocrine processes might conceivably activate mating behaviour. They include abdominal distension, a nutritional factor in the imbibed blood, the performance of the sucking act, and experience of a rise in temperature while on the host's body. The factor which is simplest to investigate is temperature, and the following experiments were performed to examine first the effect of ambient temperature, and second the effect of experiencing a previous temporarily maintained rise in temperature equivalent to that which might be encountered while taking a blood meal.

THE EFFECT OF AMBIENT TEMPERATURE

Males and females were paired in three combinations, unfed males with fed females, fed males with unfed females and both unfed. The sexes were again kept separate until introduced into their arenas. The initial temperature for the several groups of five pairs varied from 22 to 24°C and was allowed to rise steadily at the rate of 1°C/3h period to a maximum of 31°C.

No mating activities at all were observed up to and including 29°C. Most of the fleas paired during the 3 h period at 30°C. The sudden and almost complete onset of mating at this temperature was very striking. The detailed results are shown in Table 3. An additional group of 25 unfed pairs all mated when moved from room temperature directly to 31°C for a period of 3 h.

The upper temperature limit for mating was also investigated. Five groups each consisting of 20 unfed males and 20 unfed females were tested for 1 h in arenas at 33, 34, 35, 36 and 37°C, respectively. At 36 and 37°C no mating occurred, although the fleas were extremely active.

Eleven pairs mated at 35°C, 14 pairs at 34°C and all 20 mated at 33°C. Two additional batches of 20 pairs were tested at 35 and 36°C for 3 h. Again no mating occurred at 36°C; at 35°C seven pairs mated.

These results show that mating of unfed *N. fasciatus* will take place in the range of temperature between 30 and 35°C inclusive.

Table 3 Number of pairs commencing to mate at each 1°C rise in temperature from 29°C.

	29°C	30°C	31°C	Total pairs formed
25 fed males/25 unfed females	0	25	0	25
25 fed females/25 unfed males	0	24	0	24
26 unfed females/26 unfed males	0	25	1	26

THE EFFECT OF A PREVIOUS TEMPORARY RISE IN TEMPERATURE

In view of the similarity of the effects of a blood meal and of a rise in temperature in initiating mating, a further set of experiments was made to investigate whether a temporary rise in temperature, such as might be experienced during the taking of a blood meal, would later enable mating to occur at room temperature.

Unfed newly emerged fleas were sorted into monosexual groups of 25 males and 25 females which were then placed in separate containers and subjected to a temperature of 30°C for 1 h. The containers were then removed from the incubator and kept at room temperature for 30 min. after which their internal temperature was 23°C $\pm$ 1°C. Males and females were then placed together for the first time, in arenas at this temperature in five groups of ten. All fleas formed mating pairs within 3 h.

DISCUSSION

The results of the foregoing experiments show that a temporary rise in temperature to between 30 and 35°C will stimulate both male and

female unfed *Nosopsyllus* to mate, even after temperature has again fallen below this critical temperature range. This effect is equivalent to that of taking a blood meal, which also stimulates mating in both sexes. Since the taking of a blood meal is inevitably associated with a rise in temperature while on the host, it is suggested that the stimulating effect of the blood meal derives primarily from this temperature factor. At a room temperature of 22°C the temperature at a point about 1 mm above the skin surface on the lumbar region of an adult male laboratory rat is about 34°C. Thus even neglecting the heat of the imbibed blood it is clear that while taking a blood meal a flea will reach the critical range of temperature here shown to be concerned in enabling mating to occur. It is possible that the contradictory findings of Bacot (1914) and Strickland (1914) may be explicable in terms of differences in the temperature experiences of the fleas they observed.

In view of the present findings it should be noted that previous studies on the factors governing readiness to mate in fleas (Rothschild & Ford, 1966; Mead-Briggs & Vaughan, 1969; Humphries, 1963) have not explicitly controlled for temperature as a critical variable. It would be interesting for example to investigate the possibility that the 'nestling factor' discovered by Mead-Briggs & Vaughan (1969) to be essential for copulation in the rabbit flea *Spilopsyllus* might include temperature as one of its components.

While the effect of a rise in temperature on the male *Nosopsyllus* is to enable it, in the presence of a fed female, to perform mating behaviour, the precise way in which the female is enabled to mate by a rise in temperature is uncertain and two hypotheses are possible. If the female is considered to be initially unreceptive the blood meal may render her behaviourally receptive to the male; alternatively it

may enable an initially receptive but unattractive female to provide an adequate sexual stimulus to the male. The second hypothesis is favoured by the present finding that when unfed females are paired with fed males, not only is there no mating, there are no attempts to mate. As the male plays the active role in initiating mating between fed fleas, it would be expected on the first hypothesis that in those instances in which the male had fed and the female had not there would have been attempts by the male to mate and that these attempts would have been unsuccessful due to the non-receptive state of the female. That such mating attempts were not observed suggests that the male is able to distinguish between temperature-stimulated and non-temperature-stimulated females by some stimulus received before mating behaviour begins.

SUMMARY

1. Both male and female *N. fasciatus* normally require a blood meal before they will mate.
2. Fed males do not attempt to mate with unfed females. It is suggested that the taking of a blood meal enables the female to provide a stimulus necessary for the male to show mating behaviour.
3. Unfed *Nosopsyllus* of both sexes will mate if subjected to a temperature between 30 and 35°C inclusive. Above 35°C mating does not occur.
4. Below 30°C mating occurs only if the fleas have previously been subjected to a temperature of 30°C or above. A temperature rise to the critical point thus acts as a trigger for an enabling process which continues after temperature has again fallen.
5. It is suggested that the effect of a blood meal in enabling mating to occur may be explained by the fleas' experience, while on the host, of a rise in temperature to the level critical for mating.

B. FURTHER INVESTIGATIONS ON FACTORS AFFECTING THE OCCURRENCE OF MATING BEHAVIOUR

Duration of the Effect of a Blood Meal and of Temperature Rise

The effects of a blood meal and of temperature were studied in more detail. Firstly, the duration of the effect of a blood meal and the duration of the effect of a temperature rise were compared.

Isolated male and female imagines were allowed to mate after specified periods of time had elapsed since their first blood meal (taken immediately after emergence).

In this experiment and in the following experiments fleas were observed five pairs at a time at $23^{\circ}C \pm 1^{\circ}C$ for three hours. The observations were repeated on four further groups, making twenty five pairs in all. The time of emergence of the different groups was adjusted so that all twenty five pairs were of the same age at the time of observation. Pairing was scored in instances where genital linkage lasted for 30 secs or longer. The results are shown in Table 4.

Table 4 Number of pairs, out of twenty five, formed during a three hour period at various times after the taking of a blood meal (add one hour for age as from time of emergence from the cocoon).

Hours	1	5	10	15	20	25	30
Pairs Formed	25	25	24	20	16	12	0

It appears from these results that the effect of a blood meal in stimulating mating begins to decline at about ten to fifteen hours and has vanished at thirty hours.

The group of fleas which failed to mate thirty hours after their blood meal were given a rise of temperature (30°C) for one hour before again introducing the sexes for mating. Further batches were fed on emergence, isolated for periods of thirtyfive to seventy three hours, then given a similar experience of temperature rise. The results are given in Table 5.

Table 5. Number of pairs out of twenty five, formed at various times after a temperature rise at thirty three hours.

Hours	1	2	7	12	17	22	27	32	37	40
Number of Pairs Formed	25	25	25	25	25	24	17	10	6	0

The results suggest that even if the effect of the blood meal had apparently vanished at thirty hours, an experience of temperature rise would again trigger mating (Fig. 23). This stimulation by temperature begins to decline at fifty five to sixty hours and has vanished at seventy three hours after completing the original blood meal.

In the above exploratory experiment the effect of a blood meal and temperature rise cannot be directly compared, due to the variables of age and previous experience. Therefore to study the effect of temperature without previous experience of a blood meal, unfed isolated newly-emerged male and female imagines were given a rise of temperature (30°C) for one hour and were then allowed to mate with recently-emerged freshly-fed imagines of the opposite sex, after specified periods

of time. These experiments also revealed the effect specific to each sex. The results are shown in Tables 6 and 7.

Table 6 Numbers of pairs formed out of twenty five, by unfed 'temperature rise' females when allowed to mate with freshly fed males.

Hours after the end of the temperature rise	5	10
Number of pairs formed	25	0

Table 7 Number of pairs formed out of twenty five, by unfed 'temperature rise' males when allowed to mate with freshly fed females.

Hours after the end of the temperature rise	5	10	15
Number of pairs formed	25	8	1

As these results show that in temperature rise females there is a quick decline in mating activity (Table 6), quicker than in the male and, more importantly, quicker than would have been expected if the effect of temperature rise is exactly equivalent to the effect of a blood meal (compare with Table 4); an additional comparative experiment between the effect of a blood meal and the effect of a temperature rise was carried out. Three sets of females were used, a) unfed females (control), b) unfed temperature rise females and c) fed females. The females were kept in isolation for ten hours and were then allowed to mate with freshly fed males. The results are given in Table 8.

Table 8 Number of pairs formed by twenty five females when allowed to mate with freshly fed males.

Control Unfed females 10hr after emergence	Unfed females 10hr after a temperature rise	Fed females 10hr after a bloodmeal
0	3	23

It appears therefore, that the effect of a temperature rise (as measured by mating success) on both male and female begins to decline after five hours, but in the case of males the stimulating effect is longer lasting, though much less so than the effect of a bloodmeal (compare tables 4 and 7) which is still visible at twenty five hours even when the males are provided with females also fed twenty five hours previously.

In all the previous experiments the fleas were given a 'temperature rise' for one hour as compared to those fleas which spent up to three hours on the host for a blood meal, so the quick decline in mating may have been due to the shorter duration of the rise in temperature. In an additional experiment, where twenty five unfed females, after emergence, were given a temperature rise for three hours, isolated for ten hours and then allowed to mate with fresh fed males, the results - only one pair formed - still indicated a decline in mating activity much more rapid than that seen after a blood meal.

In the preliminary experiment on the effect of a bloodmeal (Table 4), the activity of mating (in both sexes) was assessed against a partner which was not freshly fed and which had been deprived of food for the stated period. This may have reduced the chances of mating, and prevents direct comparison between the results in table 4 and the results in table 6 and 7. So, in a further experiment newly emerged fleas were given a bloodmeal, kept in isolation for specified periods of time and then allowed to mate with freshly fed fleas of the opposite sex. The results are given in Tables 9 and 10.

Table 9. Number of pairs formed by twenty males, at various times after the taking of a bloodmeal.

Hours	16	26	36	46
Number of pairs formed	20	18	7	0

Table 10. Number of pairs formed by twenty females at various times after the taking of a bloodmeal.

Hours	16	26	36
Number of pairs	19	14	0

Comparison of the tables 6.7.8.9. and 10 summarised in fig. 24, shows that a bloodmeal has a longer lasting effect in stimulating mating in both males and females as compared to the effect of a temperature rise. Fig. 24 also suggests that in both cases (bloodmeal and temperature rise) the mating activity of females ceases before the stimulating effect in males has subsided.

Since the female takes the passive part in sexual pairing there appeared to be two possible explanations for the reduction in her mating activity; loss of attractiveness to the male, or development of rejection mechanisms. Experiments were carried out to test these possibilities. Three sets of five females of the same age were individually presented to randomly chosen single fresh fed males in glass tubes by holding them withh fine forceps. If a male did not respond to a female after touching her three times, no mating attempts were recorded. The criterion of response was antennal erection. The three sets of females were presented to the males. (a) Ten hours after having a bloodmeal (b) Five hours after the end of a period of temperature rise (c) Ten hours after the end of a period of temperature rise. The results are given in table II.

Table II Number of males (out of five) showing mating activity towards each group of females.

	MATING ATTEMPTS		
10hr fed females	5	5	5
5hr temperature rise females	5	5	5
10hr temperature rise females	0	0	1

It appears therefore, that those females which fail to pair ten or more hours after a temperature rise have lost their attractiveness to males. This is sufficient explanation for the reduction in mating activity seen in tables 6 and 8. It is difficult to assess the receptivity of females which are unattractive to males; no instances of persistent

rejection of males by unmated females have been observed, whereas as shown later, such rejection occurs in females which have already mated. Unmated females which are attractive are also receptive.

DISCUSSION

The fact that a bloodmeal has been found to be unnecessary for the initiation of mating (Iqbal and Humphries, 1970) correlates well with the subsequent work of Rothschild and Ford (in press) who demonstrated that newly emerged females (unfed) are in the early stages of maturation (unspecified) and the males on emergence are fully matured. The details of development reported in Chapter 4 show that females are in the second stage of maturity (scanty yolk deposition in oocyte 1) on emergence. Several workers have described the dependence of different species of fleas on their host for providing a bloodmeal before mating. Mead-Briggs and Rudge (1960), Mead-Briggs (1964a). Mead-Briggs and Vaughan(1969), Rothschild and Ford (1964a,b, 1966), Rothschild (1965a,b) and Rothschild, Ford and Hughes (1970) have reported that the rabbit flea *S. cuniculi* is dependent for its reproduction on the pregnant doe and on the nestling. Rothschild, Ford and Hughes (1970) in *X. cheopis* have shown that a bloodmeal is necessary for mating to occur. Maddrell (1966) in the case of *Rhodnius* suggested that the stimuli to the nervous system necessary to cause plasticiation of the abdominal wall are the sensations which accompany feeding and that these have a cumulative effect.

The foregoing quantitative effect of bloodmeal and temperature rise are compared, and show that a bloodmeal has a longer lasting effect on sexual activity as compared to a temperature rise alone. The longer lasting effect of the bloodmeal could be due to many factors associated with the host such as olfactory experience, probing activities and

sensory and motor aspects of imbibing blood. Other possible factors could be extension of the gut after a bloodmeal, nutritional aspects such as the supply of energy enabling physiological activities to persist at high level for a longer period of time, or the greater metabolic activities occurring as a result of the digestion of the bloodmeal (reflected in the different pattern of spiracular opening). Any of the above factors could conceiveably act by triggering some neuro-endocrine mechanism, but the last two factors being themselves long lasting, might presumably have a longer effect on such an intermediary mechanism.

It was also observed that in both cases (bloodmeal and temperature rise) the females become unreceptive before the males cease their sexual activity but both sexes resume their sexual activity if stimulated by temperature rise. This resuming of sexual activity is possibly an effective way of ensuring impregnation.

Summary

1. A blood meal has a longer lasting effect on sexual activity as compared to a temperature rise.

2. When mating is stimulated either by a blood meal or by a temperature rise, the females become unreceptive before the males cease their sexual activity.

3. Both sexes which have ceased to mate resume their sexual activity, if given a temporary temperature rise (30°C) thus ensuring impregnation.

Fig. 23. Readiness to copulate.

A. Comparative graph of tables 4 and 5.

○ Fed pairs.

▽ Restimulation by temperature rise.

Fig.23

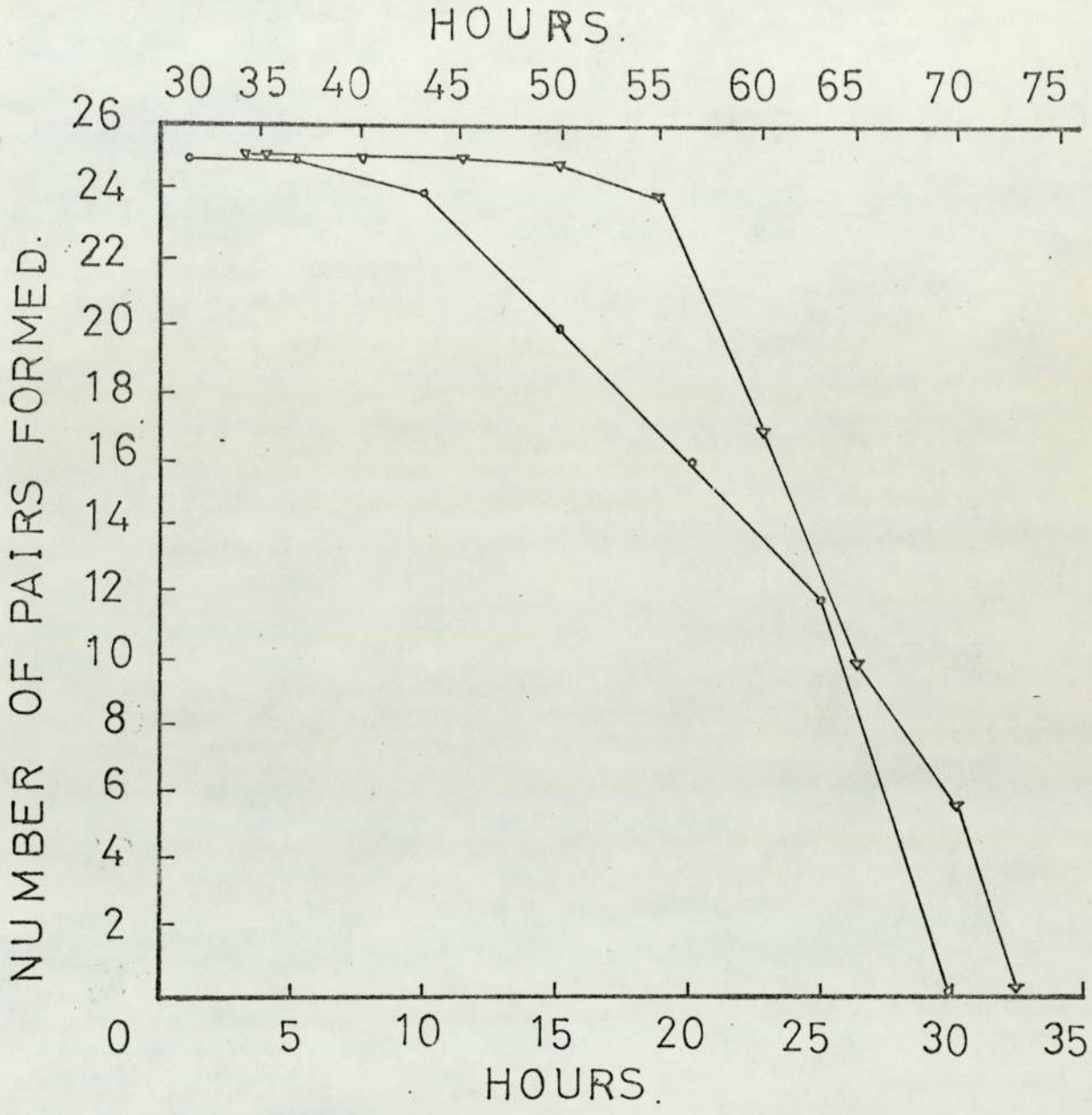

HOURS.
30 35 40 45 50 55 60 65 70 75
NUMBER OF PAIRS FORMED.
26 24 22 20 18 16 14 12 10 8 6 4 2 0
5 10 15 20 25 30 35
HOURS.

Fig. 24. Readiness to copulate.

A. Comparative graph of tables 6,7,8, 9 and 10.

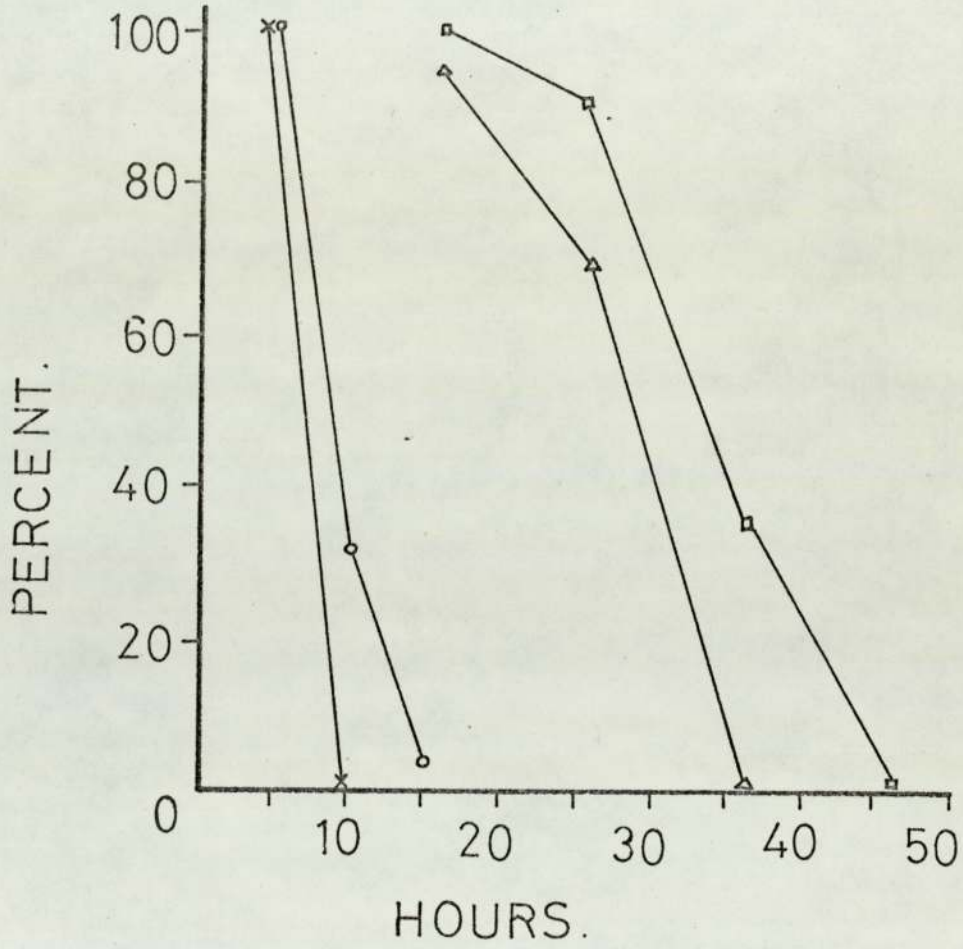
X Pairs formed by unfed temperature rise females.

O " " " " " " males.

Δ Pairs formed by fed females.

□ Pairs formed by fed males.

Fig.24



C. THE MATING PHEROMONE

Transfer from flea to flea.

Previously, during the experiments on mating behaviour (chapter 3) it was shown that a substance could be rubbed onto a glass rod from the body of the both male and female to stimulate male antennal erection. Although observations indicated that this pheromone acts only on contact between the male maxillary palp and the partner's cuticle, the fact that it could be transferred to a glass rod suggested the possibility that it might be transferred from one female flea to another.

Unfed temperature rise (30°C) females were kept at room temperature for ten hours (i.e. until in an "unattractive" state) and were then kept for half an hour with fresh fed females in a narrow glass vial (5cm high and 1cm in diameter) so that the bodies of the two batches would rub against each other. After that they were given a good shaking to ensure more rubbing of their bodies. The two groups of females were then separated and were allowed to mate with fresh fed males. All the fed females mated. None of the unfed females mated and no mating attempts were observed.

It appears therefore that the pheromone present on the cuticle of the fleas is not significantly transferrable by normal contact.

Presence of pheromone in the male flea.

It has already been demonstrated in chapter 3 that a pheromone is present on the cuticle of freshly fed males which will stimulate male mating attempts. The following experiment indicated that the pheromone is also induced on male cuticle by temperature rise to

30°C, as in the female. One hundred newly emerged unfed males were given a one-hour temperature rise (30°C) and were then kept together in batches of ten in ten glass vials at room temperature for approximately one hour. Thirtyfive mating attempts were observed. These attempts were defined by antennal clasping of another male, at which point the pair was removed from the vial. It appears therefore that the pheromone is induced by temperature rise and by blood meal in both males and females.

The presence of a pheromone on both male and female cuticle was further confirmed by an experiment in which a finely-drawn glass rod was rubbed against the body of a flea of a particular batch and then offered to males of different batches. Unfed females, fed females, temperaturerise females, unfed males, fed males and temperaturerise males were, in a randomised order, used as subjects on which the glass rod was rubbed, the rod then being presented to one of the following unfed male, freshly fed male, or temperature rise male. For each batch of subjects a different glass rod was employed; the rod was cleaned after each presentation to the maxillary palps of the test male. Antennal erection was the criterion for response, each test male being given five presentations of the rod for each sampling of a member of a subject batch. Each subject batch contained ten fleas each in a separate container.

The results (table 13) indicate that the pheromone is not secreted by unfed fleas, males or females, and also confirms the previous findings.

Table 13. Antennal erection responses (Out of 10 in each case).

	Unfed females	Fed females	Temperature rise (30°C) Unfed females	Unfed males	Fed males	Temperature rise (30°C)
Unfed males	0	0	0	0	0	0
Fed males	0	9	10	0	7	9
Temperature rise (30°C) Unfed males	0	10	10	0	9	9

Summary.

1. The mating pheromone is not secreted by unfed fleas, male or female.

2. The mating pheromone is present on the cuticle of both male and female fleas, when they have recently fed or have been subjected to a temperature rise ($30^{\circ}C$).

3. The mating pheromone is not significantly transferrable from flea to flea by normal contact.

D. THE CHILLING EFFECT AS A CRITICAL FACTOR IN THE MATING ACTIVITY OF *NOSOPSYLLUS*.

Previously, it was shown that after a lapse of time both fed and temperature rise fleas lose their sexual activity. This could be due to many factors, one obvious possibility being the cooling down of the fleas. To study if cooling could make the pheromone inactive or unattractive to males, twenty five temperature rise ($30^{\circ}C$) females were chilled in the cooled incubator to a temperature of $10^{\circ}C$ for half an hour and thereafter they were kept in the open at room temperature for one hour. Then these females were allowed the opportunity to mate with freshly fed males, but none did so.

A similar experiment was performed on twenty five fed males, and again no mating occured.

The results suggest either that cooling destroys the attractiveness of the mating pheromone on the female cuticle or that an intermediate process (neuroendocrine) in production of a transient pheromone is switched off.

To observe if the chilled female's attractiveness would return, they were given a rise of temperature ($30^{\circ}C$) for one hour and were then allowed to mate with freshly fed males. All twenty five mated.

This demonstrates that the pheromone or the capacity to produce the pheromone is not permanantly destroyed by cooling to $10^{\circ}C$.

To observe the effect of darkness, if any, on the fleas when kept in the incubator, five freshly fed females were kept in the dark (in the incubator regulated at room temperature $23^{\circ}C \pm 1^{\circ}C$) for

1 hr. and were then allowed to mate with freshly fed males. All of them formed pairs. The same result was obtained when the incubator temperature was 25°C.

It appears therefore that darkness has little or no effect on mating.

To determine the temperature at which cooling begins to affect mating twenty five unfed temperature rise (30°C) females were cooled to 20°C for ½ hr. in the cooled incubator, after which they were returned to room temperature for one hour, then allowed to mate with freshly fed males. Two pairs were formed.

The limiting temperature therefore appears to be between 20°C and 23°C.

To observe whether the mating pheromone was transient and continuous, or if the mating pheromone was long lasting, ten fed and ten temperature rise females were killed by chopping their heads off, after which they were presented to freshly fed males, (a) after an interval of ½ hr. and (b) after 1 hr. All the males responded in test (a). There was no response in test (b).

Discussion

The results suggest that the pheromone present on the cuticle of the fleas, which is active and attractive to males at 30°C becomes unattractive or has vanished after exposure to temperatures below 20°C. Two kinds of explanations are possible. That some physiological process could be involved controlling the pheromone secretion by the fleas. Alternatively, low temperature might directly affect the physical availability of pheromone to the male.

A direct effect might for example concern the waxes on the female epicuticle, which might possibly melt at a high temperature (30°C), making the female attractive and would solidify at a low temperature to render her unattractive again. Chibnell (1934) described that in some insect waxes at high temperature e.g. 40°C the iodine number of the lipids is changed. Such reversible temperature-induced changes might be responsible for the changes in female attractiveness according to temperature. However it is difficult to reconcile this kind of direct effect explanation, and the long duration of attractiveness even when temperature falls after a blood meal or temperature rise, with the very short duration of the pheromone on dead cuticle.

The surprisingly clear-cut results obtained with the dead female cuticle suggest that after stimulation by a blood meal or rise in temperature to 30°C, an internal process (possibly neuro-endocrine) is set in motion leading to the secretion of a transient mating pheromone. The pheromone is effective to male fleas for about half an hour. Secretion is however continuous so that normally at room temperature an effective pheromone is present for several hours. Chilling below 20°C appears to switch off the internal process which maintains secretion, and therefore renders the female unattractive within an hour.

Hanson (1965) showed that phase transition in water at certain temperatures (e.g. 15°C and 30°C) plays an important role in biological processes. He suggested (personal communication) that the dependence of *Nosopsyllus* mating on temperature might be such a phase-transition phenomenon. However there is no clear

evidence for this. If phase-transition effects were involved one would not expect females to remain attractive below 30°C.

Summary.

1. Chilling below 20°C renders previous attractive females unattractive to males when returned to room temperature.

2. The capacity to secrete a pheromone is not destroyed permanently if the females are temporarily chilled to 10°C.

3. The pheromone is transient, being effective for little more than half an hour. Normally it must therefore be secreted continuously for the several hours that a blood meal or temperature rise induce attractiveness.

4. Darkness has little or no effect on mating.

E. REMATING

Introduction

Multiple mating is known to occur in several groups of insects. For example, Mayer, (1946) reported that females of *D. melanogaster* would accept another male 24 hours after the first mating. The multiple mating behaviour shown by several cockroach species has been investigated in detail (Hunter-Jones, 1960; Clarke and Sheppard, 1962; Taylor, 1966). The present chapter describes multiple mating in *Nosopsyllus*.

Seven sets of experiments were designed and for every experiment twenty five fleas of both sexes were used; observations were made for 3 hours. Five couples were kept together in a bottle and if a couple started mating, the pair was removed to a petri dish.

OBSERVATIONS

Experiment 1.

Newly emerged females were given a bloodmeal and were then immediately allowed to mate with fed males of the same age. Just after the cessation of this first mating, these females were returned to a fresh fed batch of males. None mated.

The females rejected the males which frequently attempted to mate. The rejection behaviour was shown either by kicking the male vigorously with the hind legs if the male had succeeded in attaching his antennae to her abdomen or by jumping away from the male. The females become sluggish after mating and lie burrowed for most of the time in the sawdust.

Experiment 2.

Females were given a second blood meal, after the completion of the first mating and were then kept with fresh fed males. Again there were no pairs formed, though the males did attempt to clasp.

Experiment 3.

The females were given a one hour rise of temperature after the completion of the first mating. Again there was no mating due to active rejection of the males' clasping attempts.

Experiment 4.

Females which had mated were given a blood meal every day and kept in glass vials over a filter paper on which they laid eggs, beginning on the second day. After 72 hours, directly after their bloodmeal, they were introduced to fresh fed males. Twenty one pairs were formed.

Experiment 5.

Males which had completed their first mating, were immediately afterwards placed with a fresh batch of newly - emerged recently - fed females. No pairs were formed and no mating attempts were observed.

Experiment 6.

Males were given a blood meal after their first mating and were then kept with freshly emerged fed females. All mated.

Experiment 7.

Males were given a rise of temperature ($30^{o}C$) after their first mating and were then kept with freshly fed females. Seventeen pairs were formed.

DISCUSSION

After the first mating the females are still attractive to males. as evidenced by the males' attempts to mate in experiments 1,2, and 3. Pheromone production is therefore, not switched off in the female by the act of mating. Mating does, however, induce in the female a period of non-receptivity, characterised by the active rejection of the male.

After 72 hours, receptivity has been largely restored.

In males the first mating is followed by a sexual quiescent period, though experiments 6 and 7 show that mating activity can immediately be restored as soon as they are given a bloodmeal or a temperature rise to 30°C.

SUMMARY

1. Females are attractive but non-receptive to males after their first mating but could be stimulated to remate after 72 hours.

2. Males also cease mating activity after their first copulation, but unlike the females, can immediately be stimulated to remate after a bloodmeal or temperature rise (30°C).

CHAPTER 5

OVARIAN DEVELOPMENT AND THE STRUCTURE OF THE FEMALE REPRODUCTIVE ORGANS

OVARIAN DEVELOPMENT AND THE STRUCTURE OF THE FEMALE REPRODUCTIVE ORGANS

Introduction

The structure of the female reproductive organs, apart from the ovaries, was first described by Minchin (1915) in *N. fasciatus*. Smit (1957) and Lewis (1967) figure parts of the female system but again give no account of the ovaries. Our main understanding of the female reproductive system is due to the work of Mead-Briggs (1962), who described the structure of the female reproductive organs of the European rabbit flea, *S. cuniculi*, and also gave an account of the ovarian development of the flea. Mead-Briggs and Rudge (1960) first reported that the rabbit flea *S. c uniculi* needs a bloodmeal from a pregnant rabbit for ovarian maturation. This dependence on the host for maturation, copulation and impregnation has been further investigated and confirmed by Mead-Briggs (1964, 1969); Rothschild and Ford (1964, 1964a, b, 1966, 1969) Exly, Ford and Rothschild (1965); Rothschild (1965, 1967) and Rothschild, Ford and Hughes (1970). In a much less detailed study of *Echidnophaga gallinacea* (Westwood) Geigy and Suter (1960) showed that before copulation the female must feed for two to three days during which time the ovaries reach a certain but undefined stage of maturity. Suter (1964) implied that the females of all species of fleas must undergo a period of maturation after emergence from the cocoon before they are ready for copulation and become attractive to males. In *Spilopsyllus* a bloodmeal containing appropriate hormones must normally be taken during their maturation period (Mead-Briggs and Vaughan, 1969). In *X. cheopis* a bloodmeal is required though there is no dependence on the hosts' hormones (Rothschild, Ford and Hughes, 1970). *N. fasciatus* is not dependent on a bloodmeal for mating (Iqbal and Humphries, 1970). This flea will also mate immediately after emergence from the cocoon, and

and provides a striking contrast to *Spilopsyllus* .

As no work has been done in *Nosopsyllus* on ovarian development and structure, the present work was undertaken.

THE FEMALE REPRODUCTIVE ORGANS

The Ovary of the Newly Emerged Flea

Each of the two ovaries consists of three ovarioles. In the thirty specimens studied there were consistantly six ovarioles; in contrast Mead-Briggs (1962) reported variation of from four to eight ovarioles in each ovary in *S. cuniculi*. The ovarioles of *Nosopsyllus* are of panoistic type, and each has a terminal filament, germarium, vitellarium and pedicel (Fig. 25).

In a newly emerged flea the length of the ovariole varies from 627 μ to 680μ. The primary oocyte (OO_1), varies in size from 132 x 99μ to 165 x 132μ. It is shining yellow in colour and the germinal vesicle is clearly visible and varies in size from 43 x 49μ to 49 x 52μ (Fig.25).

There is at this stage no or little yolk deposition in $oocyte_1$ and at the time of emergence, the ovary is in stage 2 of maturity as defined by Mead-Briggs (1962) for *Spilopsyllus*. The number of distinct oocytes in each ovariole varies from one to three. Observations on unfed females dissected after twenty four hours showed that there had been no significant ovarian development.

The pedicel of the ovariole is short and before the first ovulation it is closedby a transverse septum at the vitellarial end and the epithelial plug is present between the septum and $oocyte_1$.

Ovarian development was studied in four batches (A to D) which received treatments which preliminary observations suggested might be affecting reproductive physiology. For each batch twenty five females were used.

A. Newly emerged fleas were given a blood meal and after twenty four hours were dissected to study the ovaries.

B. Treatment as for batch A, but allowed to mate just after the bloodmeal and dissected after twenty four hours.

C. Females (unfed) were given a one-hour rise of temperature to 30± 1°C and after twenty four hours they were dissected.

D. Unfed females were given a one-hour rise of temperature and were then allowed to mate with fed males. After twenty four hours, they were dissected to study the ovaries.

RESULTS

Table 14 Batch A

Length of ovariole	No.of oocytes per ovariole	Oocyte$_1$	
		Size of oo$_1$	Size of germinal vesicle
790-960µ	18 - 23	165 x 100µ 200 x 130µ	47 - 53µ

The above results show that one day after feeding the ovaries have developed considerably. Oocyte$_1$ has become dull yellow due to scanty yolk deposition and the germinal vesicle is still visible. This stage of maturation corresponds to a point intermediate between stages 2 and 3 in *Spilopsyllus*.

Table 15 Batch B

Length of Ovariole	No. of oocytes per ovariole	Oocyte$_1$		Oocyte$_2$		Oocyte$_3$	
		Size of oo$_1$	Size of germinal vesicle	Size of oo$_2$	Size of germinal vesicle	Size of oo$_3$	Size of germinal vesicle
1580 - =2240µ	19 - 23	460 x 260µ -760 x 330µ	not visible	230 x 165µ 460 x 200µ	47-57µ	200 x 130µ- 300 x 165µ	45-55µ

The ovarioles have grown extensively in length and the oocytes are larger than in batch A. Out of six oocytes$_1$ three oocytes$_1$ of one ovary were always bigger than the other three. The colour of oocyte$_1$ is grey in transmitted bright light and shining white in reflected light. The germinal vesicle of oocyte$_1$ is not visible due to yolk deposition. Oocytes 2 and 3 are dull yellow with scanty deposition of yolk granules and the germinal vesicle is clearly visible.

The maturation stage is comparable with stage 3 defined by Mead-Briggs (1962).

Table 16 Batch C

Length of Ovariole	No. of oocytes per ovariole	Oocyte$_1$	
		Size of oo$_1$	Size of germinal vesicle
760- 990µ	13-20	165 x 100µ 200 x 130µ	46 - 58 µ

In the temperature rise females the ovarioles have become longer and the number of oocytes has also increased, but the colour of the oocytes was still shining, although there were traces of yolk granules in $oocyte_1$.

This experiment suggests that temperature acts as a stimulating trigger in the development of the ovaries.

Table 17 Batch D

Length of ovariole	No. of Oocytes per ovariole	$Oocyte_1$		Oocyte2		$Oocyte_3$	
		Size of oo_1	Size of germinal vesicle	Size of oo_2	Size of germinal vesicle	Size of oo_3	Size of germinal vesicle
1000-1580μ	17-21	260x 200μ-400 x 200μ	51-53μ	200 x 130μ	46-51μ	165x 100μ	43-49μ

In the above experiment, although the ovarioles, $oocyte_1$, oo_2 and oo_3, had grown considerably, $oocyte_1$ had scanty deposition of yolk changing its colour to dull yellow and the germinal vesicle was still visible, whereas in fed and mated females, the germinal vesicle is not visible due to yolk deposition.

A granular, yellowish white (fluid) secretion similar to the one mentioned by Mead-Briggs (1962) is present in the cavity of the pedicel and lateral oviducts, when the ovaries reach the 3rd stage of maturity i.e. when $oocyte_1$ has been fully impregnated with yolk and the germinal vesicle is no more visible. This oviducal fluid disappears within twenty four hours after the female has stopped laying eggs. Its function is probably to assist the passage of eggs.

DISCUSSION

The results show that at the time of emergence from the cocoon *Nosopsyllus* females are in stage 2 of maturity. This has subsequently been confirmed by Rothschild and Ford (personal communication). Whereas, *Spilopsyllus* is dependent for ovarian development on an appropriate bloodmeal, *Nosopsyllus* will mate just after emergence even without a bloodmeal. However, Rothschild (personal communication) reported a population of *N. fasciatus* which does not mate on emergence and requires a bloodmeal to do so. One of the reasons for not requiring a bloodmeal and being in an advanced stage of maturity of emergence, could be that in the present work dietary requirements for the host's blood were made available to the larvae during rearing.

Experiment C, demonstrates that temperature acts as a stimulating trigger for ovarian development as it is scarcely conceivable that the direct effect of temperature on metabolism for a single hour could account for the striking development which occurred, causing enlargement of ovarioles, increasing the number of oocytes and even traces of yolk deposition.

Another factor which is shown to be involved in ovarian development is a "copulatory factor" (experiments B and D). This copulatory factor appears to be responsible for extensive growth of ovarioles, larger oocytes than batches A and C but not maturity of the ovaries, because thereis no increase in yolk deposition.

A bloodmeal seems essential for full yolk deposition as shown by Prasad (1969) in *X. cheopis*. Unfed females which had experienced a temperature rise did not slow full yolk deposition even when they had mated.

SUMMARY

1. Females of *N. fasciatus* are in the second stage of ovarian maturity when they emerge from the cocoon.
2. *Nosopsyllus* is not host dependent for mating or for partial ovarian development but a bloodmeal is essential for full deposition.
3. A temporary rise in temperature to 30°C acts as a stimulating trigger for ovarian development.
4. A "copulatory factor" also stimulates ovarian development but does not effect yolk deposition.

Fig. 25. Ovary.

A. Oocyte 1.

B. Pedicel

C. Germinal vesicle.

D. Ovariole.

E. Vitellarium

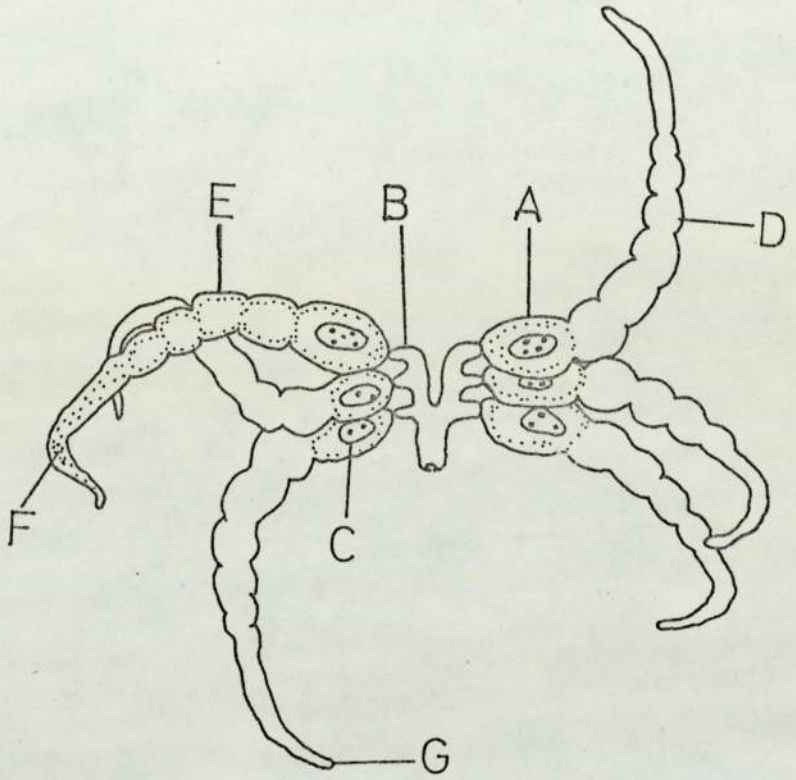
F. Germarium.

G. Terminal filament.

H. Oocyte.

I. Yolk.

Fig.25



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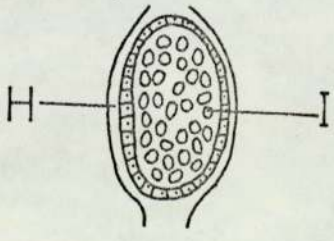

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CHAPTER 6

EGG PRODUCTIVITY

EGG PRODUCTIVITY

Introduction.

The egg productivity and hatching of eggs of fleas has been described by several workers. Strickland (1914) described the eggs of *N. fasciatus* and experimented with the ovum and its hatching under different conditions of temperature and humidity. Bacot and Ridewood (1914) also described the eggs of *N. fasciatus* and *X. cheopis*. According to Bacot (1914) females of *P. irritans*, when fed twice each day laid far more eggs than those fed only once. Mitzmain (1910) worked on the egg productivity of *C. acutus* and suggested that "mammalian blood appears essential for fleas to partake of copulation and oviposition". He also described the process of hatching. Sharif (1937, b) reported that at 23°C and 80% R.H. the eggs of *N. fasciatus* hatched in $3\frac{1}{2}$ to $4\frac{1}{2}$ days. Buxton (1948) while working on *X. cheopis* reported that a higher temperature gives rise to egg laying on an earlier day. Hirst (1927) studied the egg productivity of *X. cheopis* and *X. astia*. Humphries (1963) described the process of hatching in *C. styx* and *C. gallinae*. Cotton (1969) studied oviposition in *Megabothris turbis* (Rothschild) and in 1970 reported that eggs of *C. gallinae* developed in 3 to 4 days at 28°C and in 6 days at 21°C. According to Samarina, Alexeyer and Shiranovich (1968) *N. fasciatus* and *X. cheopis* start laying eggs earlier when fed on the golden hamster as compared to those fed on the white mouse and white rat, they also reported that the females of both species lay eggs rhythmically, dispersing them in a wave like pattern.

The present work deals with the egg productivity and hatching of *N. fasciatus*.

General account of hatching

The egg varies in size from 660 X 363 μ to 726 X 363 μ. They are shiny white and sticky and adhere to the substratum or paper on which they are laid. In some cases a blood spot is visible on the egg. The hatching of thirty eggs was observed under the binocular microscope, in a petridish. The eggs hatched in 4 to 5 days at room temperature (23 ± 1°C). As the egg is opaque and the contents are invisible, the observations were made when the larva had made a slit in the egg with hatching spine.

The slit is enlarged by nodding strokes against the shell with the hatching spine, followed by a quiescent period after which a new pattern of movements is adopted, with the hatching spine striking against the edges of the slit so that it elongates parallel to the longitudinal axis of the egg, although it may also tear around the equator. Sometimes the abdomen of the larva may emerge first from the slit but usually it is the head which is first freed from the shell.

After wriggling out of the shell the larvae may immediately feed. Mitzmain (1910) reported that *C. acutus* females furnish tiny blood particles on the egg shell and the larvae feed on these particles after emerging. Although such blood spots were occasionally observed on the eggs of *Nosopsyllus* they were found on very few eggs, which suggests that they are not left regularly by the mother and do not usually form the first blood meal of larvae.

Egg Productivity

Four experiments were designed and for each experiment twenty females were used. All the experiments were performed at room temperature (23 ± 1°C).

(1) Newly emerged females were given a blood meal and were then allowed to mate. After twenty four hours the eggs laid by each female were counted. The mean number of eggs laid per female was 3.6.

(2) After mating females were given a blood meal daily but were kept without males. The results are given in table 18.

Table 18

Days

1	2	3	4	5	6	7	8	9	10	11
3.6	7.2	7.2	7.0	7.0	5.8	4.6	4.6	4.6	4.4	—

Mean No. of eggs laid

The maximum number of eggs laid by a female was 61. The fleas laid at the maximum rate on days two to six after which egg laying declined. After the tenth day no eggs were laid.

(3) Females which had already mated were given a blood meal daily and were kept with males. The results are given in table 19.

Table 19

Days

1	2	3	4	5	6	7	8	9	10
3.6	7.2	7.2	7.2	6.6	6.5	6.5	6.5	6.2	6.2

Mean No. of eggs laid

The maximum number of eggs laid by a female was 70. After ten days the experiment was discontinued.

(4)

(a) Females were given a blood meal once before mating and were then kept unfed. On the first day mean number of eggs laid was 3.6 and on the second day 7.2 but after forty eight hours none of the females laid eggs.

(b) The fleas which had stopped laying eggs after forty eight hours were after seventy two hours again given a blood meal daily. The results are given in table 20.

Table 20

Days

	1	2	3	4	5	6	7	8	9	10	11
Mean No. of eggs laid	3.6	7.2	—	4.6	4.6	4.6	4.6	4.6	4.1	4.1	—

Females resumed laying eggs with a maximum of 6 eggs on the sixth day and stopped after the tenth day. The maximum number of eggs laid by a female was 48.

Eggs were always laid during the night and never in day light. Regulation of oviposition activity by photoperiods is known in other insects for example the sugar cane borer *Diatraea saccharalis* (Miskimen, 1966).

Discussion

A blood meal seems essential for oviposition in *Nosopsyllus* as suggested by Minchin (1910) in *C. acutus*. Strickland (1914) on *N. fasciatus*, suggested "the effect of the rat's blood on the female with regard to egg laying seems to be rather stimulating than nutritive, as fleas that have been without food for many months will lay eggs immediately after they are fed". In *Nosopsyllus*, in the previous chapter on ovarian development, it was shown that the unfed female's oocytes lack yolk deposition and even after temperature induced mating, there is only a scanty deposition of yolk granules. The present results show that even if the starved females are given a blood meal they will not lay eggs until nightfall, which suggests that, though a blood meal may be a stimulus for egg laying, it is not the only one. Further, in the previous chapter it was shown, contrary to Strickland's suggestion, that a blood meal has a striking effect on yolk deposition and egg maturity, an effect which appears to be partly nutritive, as the effects of a rise in temperature are not so pronounced.

The maximum number of eggs were usually laid between days 2 and 4 inclusive but the surprising aspect of egg laying was that in females fed daily, but kept without males, it always stopped abruptly on the tenth day, after which no eggs were laid. Even those females which were starved and after seventy two hours were given a blood meal laid eggs only for ten days. This was unexpected as the non-production of eggs on day 3 might have been expected to prolong egg laying for an extra day. The continued presence of oocytes in the ovaries on and after the tenth day poses the problem of what

factor so abruptly causes the cessation of egg laying.

The presence of males seems to have a stimulating effect as the females always laid more eggs, probably due to remating.

Summary.

1. A blood meal is essential for egg laying.

2. With daily feeding but no opportunity for remating egg production ceases by the tenth day after mating.

3. Presence of males with the pregnant females stimulates egg productivity.

4. Eggs are always laid during the night.

CHAPTER 7.

Host-Parasite Relationship.

Host-Parasite relationship.

Introduction.

Little is known about fleas in their natural habitat and about their host-parasite relationships, although population dynamics and host specificity have attracted much research (for example, Buxton, 1932, 1938, 1948; Sharif 1948; Hirst, 1953; Rothschild, 1952; Smit, 1957b; Hopkins, 1957; Benton and Altmann, 1964; Haas, 1965, 1966a, b). This apparent contradiction arises because there are relatively few direct observations on the behaviour of fleas in relation to their host and to features in their general environment. Studies involving direct observations include those by Mead-Briggs (1964), Humphries (1969) and Cotton (1970).

The present work describes the behavioural interaction of *Nosopsyllus* with its rat host as observed in the culture cages, on the young rats and on partially shaved rats.

A. Host-finding behaviour of *Nosopsyllus*.

Three rats of different age groups (ten weeks, seventeen days and seven days old) were used as hosts. Each rat was placed in a standard plastic rat cage 15cm from twenty unfed, newly emerged fleas which were kept clustered at one end of the cage by their negative phototactic reaction to daylight from the window. In all three cases the fleas had jumped onto the host within five minutes. Reaction appeared to occur at a distance of several centimetres.

During these preliminary experiments it was noticed that those fleas which had started feeding on seven and seventeen days old rats, would start jumping away from the host without full bloodmeal after about half an hour. A thermistor probe showed that the body temperature of the young rats begins to fall soon after they are removed from their mother. In the seven day old rat, a skin-surface temperature of 27°C was recorded after half an hour, about 7°C below the adult skin-surface temperature. This suggested that temperature may be a factor in host-finding. When seven and seventeen day old rats which had cooled were gently warmed the fleas started returning to their bodies when skin temperature reached 34°C. Below this temperature fleas which accidentally encountered the host, or which were placed on its body rapidly left again.

During the experiments it was noticed that bright light from one side has inhibiting effects on the fleas's host perception, negative phototaxis tending to over-ride other responses.

The foregoing observations suggest that host perception in *Nosopsyllus* is in two stages, firstly orientation towards the host

and secondly once on the body of the host, whether it should stay on the host or leave. The second stage seems to depend to an important extent on temperature; if the host skin temperature falls much below normal the fleas start leaving the host. This may be related to the fact that many workers have reported that fleas abandon their host after its death (Rothschild and Clay, 1952). Cowx (1967) has shown that small mammals trapped alive lose more fleas in cold conditions.

Ioff (1941) reported that rodent fleas leave the host immediately after its death. The present work shows that the reactions of *Nosopsyllus* to host temperature would be expected to cause the fleas to leave the host soon after its death.

Reaction to the host from a distance suggests that possibly olfactory responses may be involved in the first, orientation, stage of host finding. Different factors are involved in the host perception of various other species of fleas, depending on their particular ecological conditions and the characteristic odour and behaviour pattern of their specific hosts. These factors include various combinations of olfactory responses, anemotaxis, warmth, collection of fleas on horizontal surfaces, and responses to light, shade, and mechanical vibration (Bates, 1962; Benton and Lee, 1964; Sgonina, 1935, 1939; Shlov and Naor. 1964, Strickland, 1914; Humphries, 1967c, 1968b).

B. Feeding behaviour of *Nosopsyllus*.

The feeding behaviour of many hundreds of unfed newly emerged fleas was observed by placing groups of five or ten at a time with partially shaved adult rats. Their pattern of movements and feeding was observed all the time until they left the host. The fleas were found to spend from two to three hours on the host, mostly remaining on or transferring to the sacral and pelvic (usually preanal) regions of the host. It is at this posterior position on the host's body that they usually imbibe blood. Occasionally fleas would take blood from the lumbar and thoracic regions but very rarely from head or neck. Seldom do fleas transfer anteriorly from the body to the neck or head of the host unless forced to move forward by the antiparasite activity of the host, fig. (26). Almost all flea movements tended to be directed posteriorly on the host. In addition the anterio-ventral regions were rarely traversed. The fleas move on or through the fur of the host by holding the hair with their claws. While on the host, searching for an appropriate site to imbibe blood, the fleas walk with their heads down and the abdominal region slightly raised.

They probe the host's skin frequently, with the stylet bundle and labial palps, even when in motion but when still, the probing is more frequent and the flea tosses its head both left and right to probe at different sites. If the site is not appropriate, the fleas move to another place shortly after probing that area until they find a suitable place to imbibe blood. Usually half or two-thirds of the lacinia is inserted into the rat's skin while feeding, and the insertion depth is controlled by the forelegs (fig 27).

Some fleas were observed to have imbibed a full bloodmeal and to leave the host within ten minutes. Others took up to three hours to obtain a bloodmeal, spending most of their time moving about probing different points on the host. To understand why some fleas spent so much time on the host and why often a relatively small proportion of time was spent imbibing blood, a study of sections of rat's skin in relation to stylet penetration was made.

Plates (44-47) show that arteries and veins are found in a fairly distinct layer, immediately above the adipose tissue, usually no less than about 130μ below the surface, and extending to a depth of about 230μ. These arteries and veins vary in diameter but there are two particularly common size types, large arteries (39 to 44μ in diameter) and small arteries (14 to 15μ in diameter); similarly large veins (29 to 30μ in diameter) and small veins (13 to 14μ in diameter). Skin was examined particularly in the pelvic and lumbar regions. In both regions the abundance of blood vessels varied. See table 21.

Table 21. Number of blood vessels (above the adipose tissue) beneath a line 250μ long on the skin surface.

Skin from pelvic region.				Skin from lumbar region.			
No. of blood vessels in a typical abundant supply area.		No. of blood vessels in a typical scarce supply area.		No. of blood vessels in a typical abundant supply area.		No. of blood vessels in a typical scarce supply area.	
Arteries	Veins	Arteries	Veins	Arteries	Veins	Arteries	Veins
37	7	17	3	23	3	11	1

A typical lacinia is approximately 519 μ in length and 27 to 33 μ in diameter. Its probable maximum penetrating length is about 430 μ, which means that it can just penetrate the adipose tissue lying underneath the layer of blood vessels. Plate 43 shows that the lacinia is serrated on both sides and acts like a saw during penetration through the rat's skin. According to Deoras and Prasad (1967), the laciniae, while piercing, work in alternate thrusts. The epipharyx is about 500 μ in length and 17-20 μ in diameter while its spatulate tip is upto 30 μ in diameter. The epipharyngeal tip is also serrated, which suggests that the epipharynx is also used to puncture the blood vessels.

Sometimes a flea would stay at one place for a long time (up to 40 min) and still be unable to imbibe a full bloodmeal, only traces of a bloodmeal being found in the gut on examination under a microscope. Such fleas usually move to another place and there may imbibe blood successfully. Other fleas may probe for only a few seconds before moving elsewhere. This suggested that after the stylets have penetrated a stimulus is necessary to keep the flea feeding at the same point. This stimulus may be provided by the host's blood when vessels are ruptured. The difficulty experienced by some fleas in imbibing blood is probably due to puncturing small blood vessels which yield an insufficient flow. This assumption is supported by the fact that there is in a single thrust, about a 95% probability of hitting small blood vessels as compared to about a 20% probability of striking large blood vessels. These observations appear to explain why it is necessary that a flea which is capable of taking a full bloodmeal within ten minutes nevertheless normally spends from one to three hours on the host during which time it is vulnerable to

attack by the host. Further delays in feeding are caused by frequent antiparasite activities by the host which often appear to be triggered by stylet penetration. Snodgrass (1946) for *C. felis* and Deoras and Prasad (1967) in *X. cheopis* and *X. astia* also described that the fleas interupt their bloodmeal at intervals.

A female imbibes about nine times more blood (6.21×10^{-4} cu mm) than the male (0.7059×10^{-4} cu mm). This could be due to two reasons. Firstly, a female ejects many blood droplets from the anus, occasionally during feeding, but usually during the following few hours, after leaving the host. The male also ejects blood, but in smaller quantities. Secondly the female needs more blood, as it must provide nutrients for the growth and maturation of many eggs.

PLATES 43 - 47

Plates 43 - 47

Pl. 43. Lacinia of flea.

A. Lacinia.

B. Central channel.

C. Serrations on the tip of Lacinia.

Pl. 44. Rat's skin (Transverse section of pelvic region).

A. Large artery.

B. Large vein.

C. Small artery.

D. Small vein.

E. Hair papilla.

F. Broken vein.

Pl. 45. Rat's skin (T.S. Pelvic region) stained in haematoxylin and eosin.

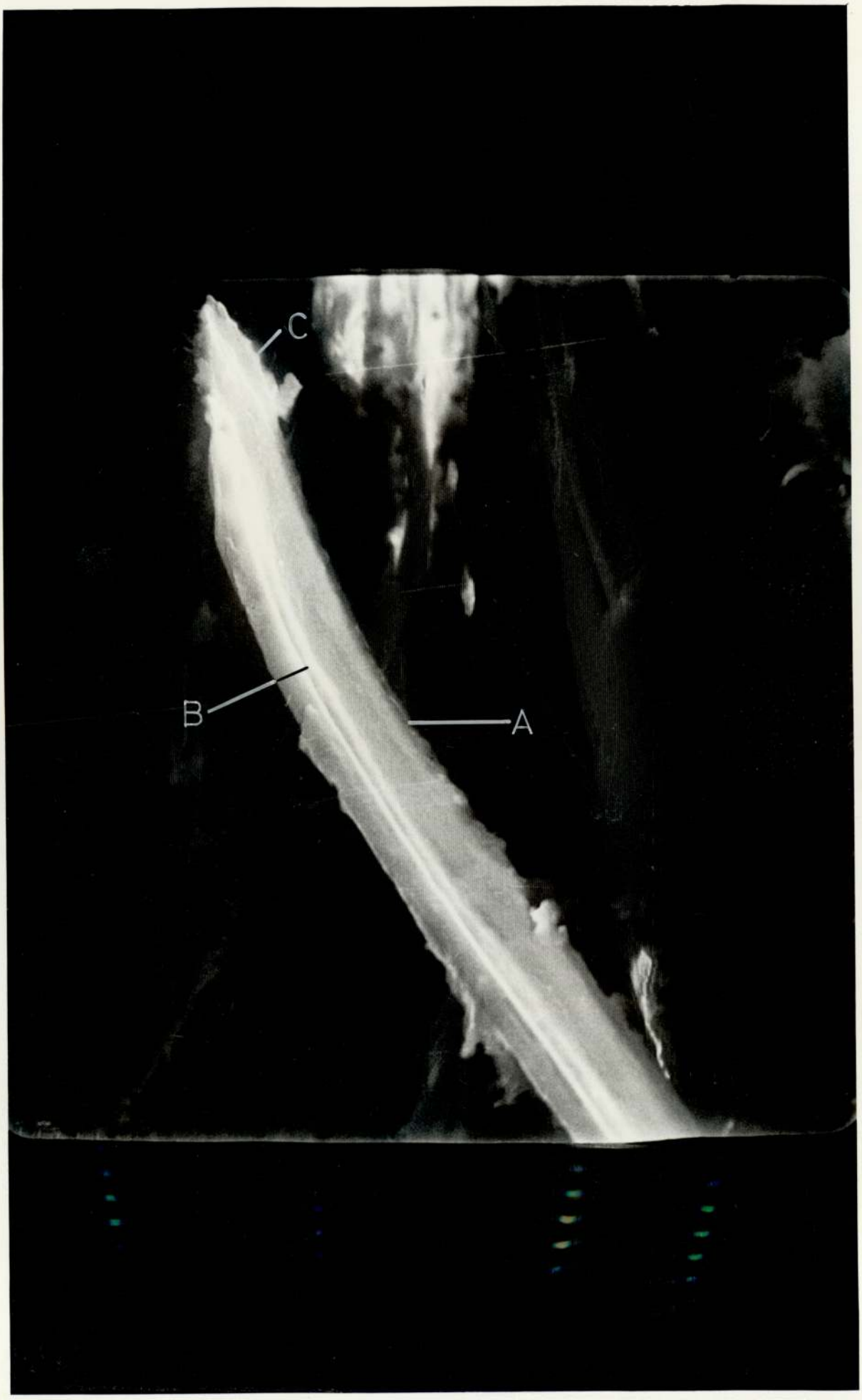
Distribution of blood vessels.

Pl. 46. Rat's skin (T.S. Lumbar region)

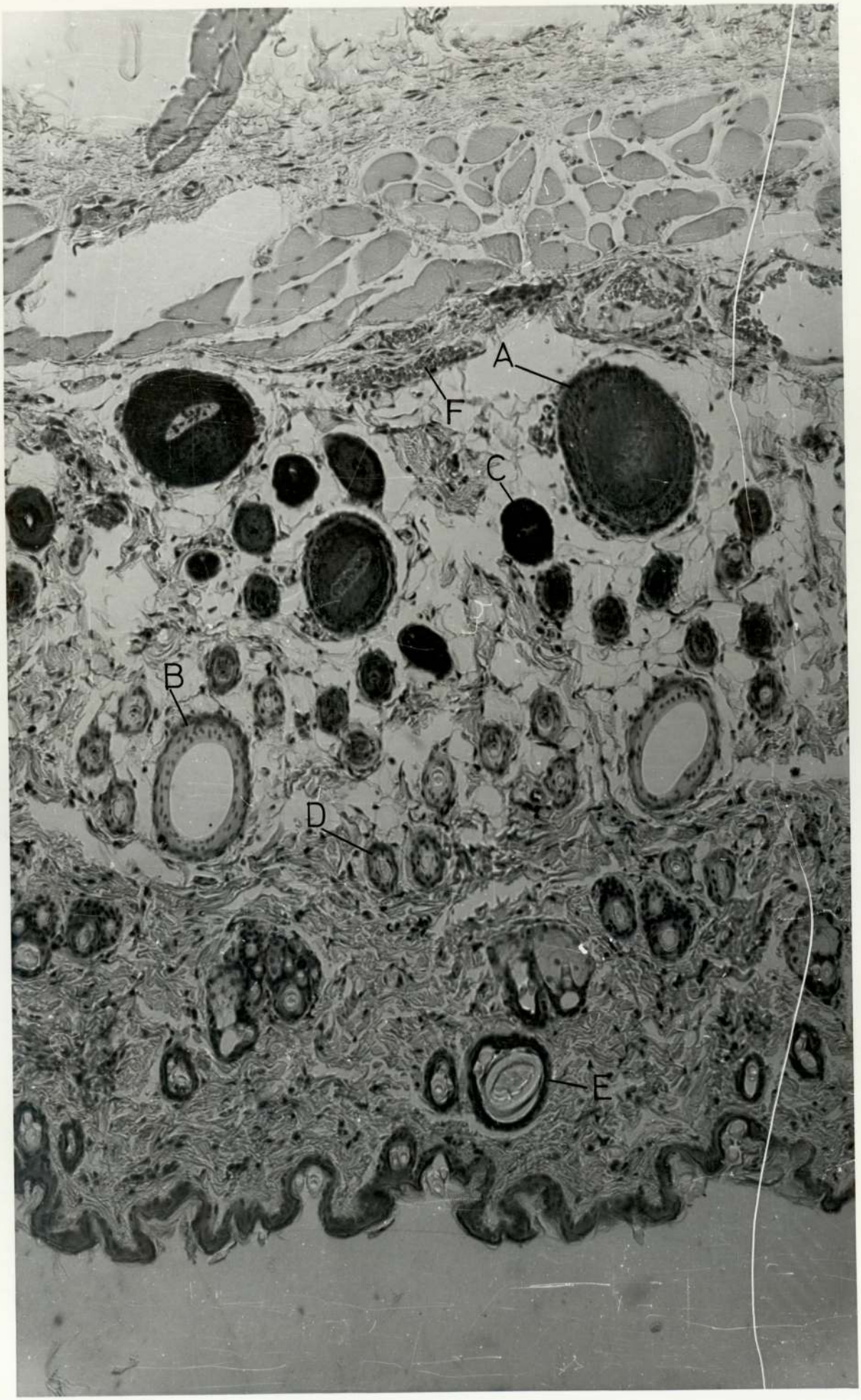
Distribution of blood vessels.

Pl. 47. Rat's skin (T.S. Lumbar region).

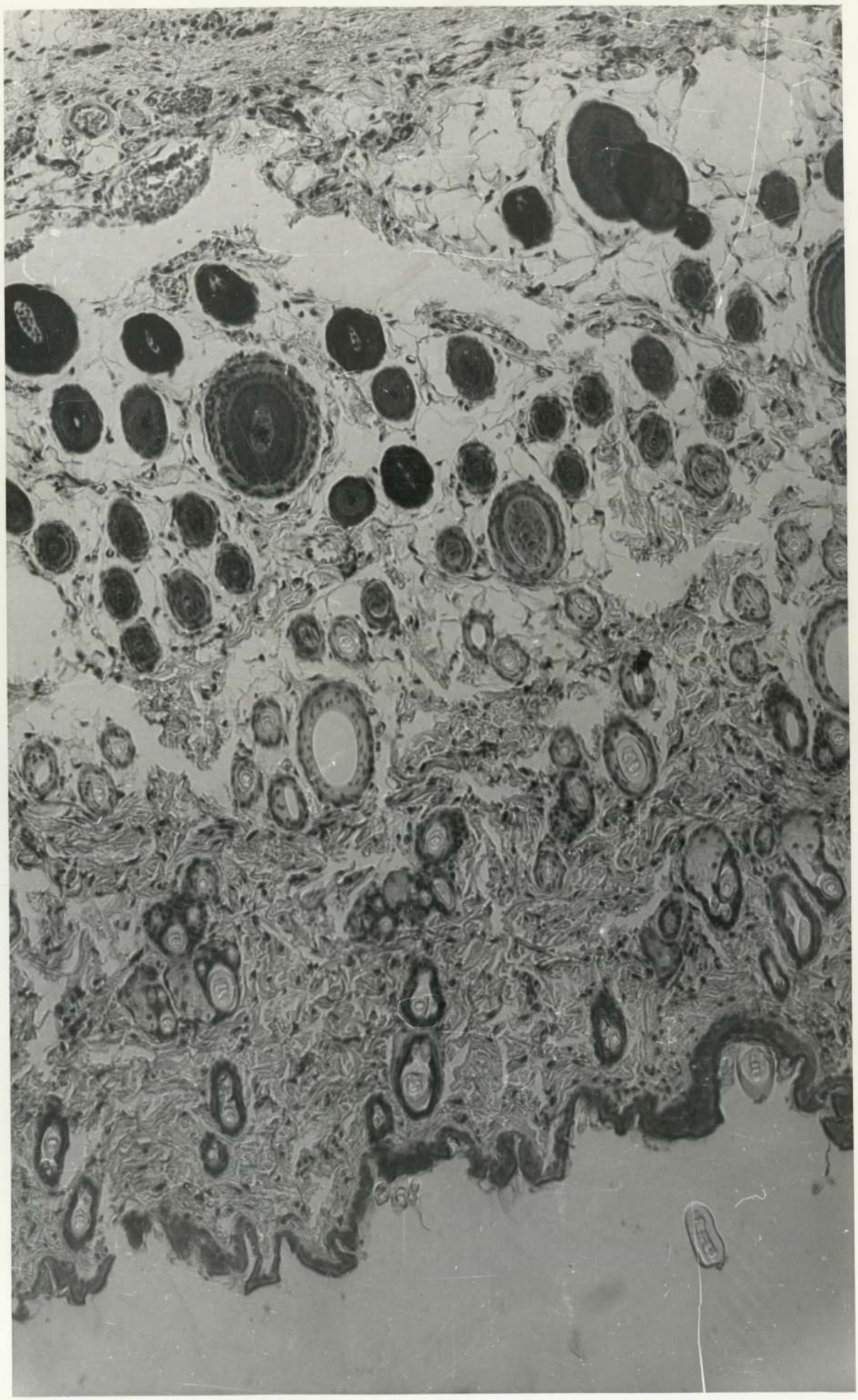
PL. 43



PL. 44

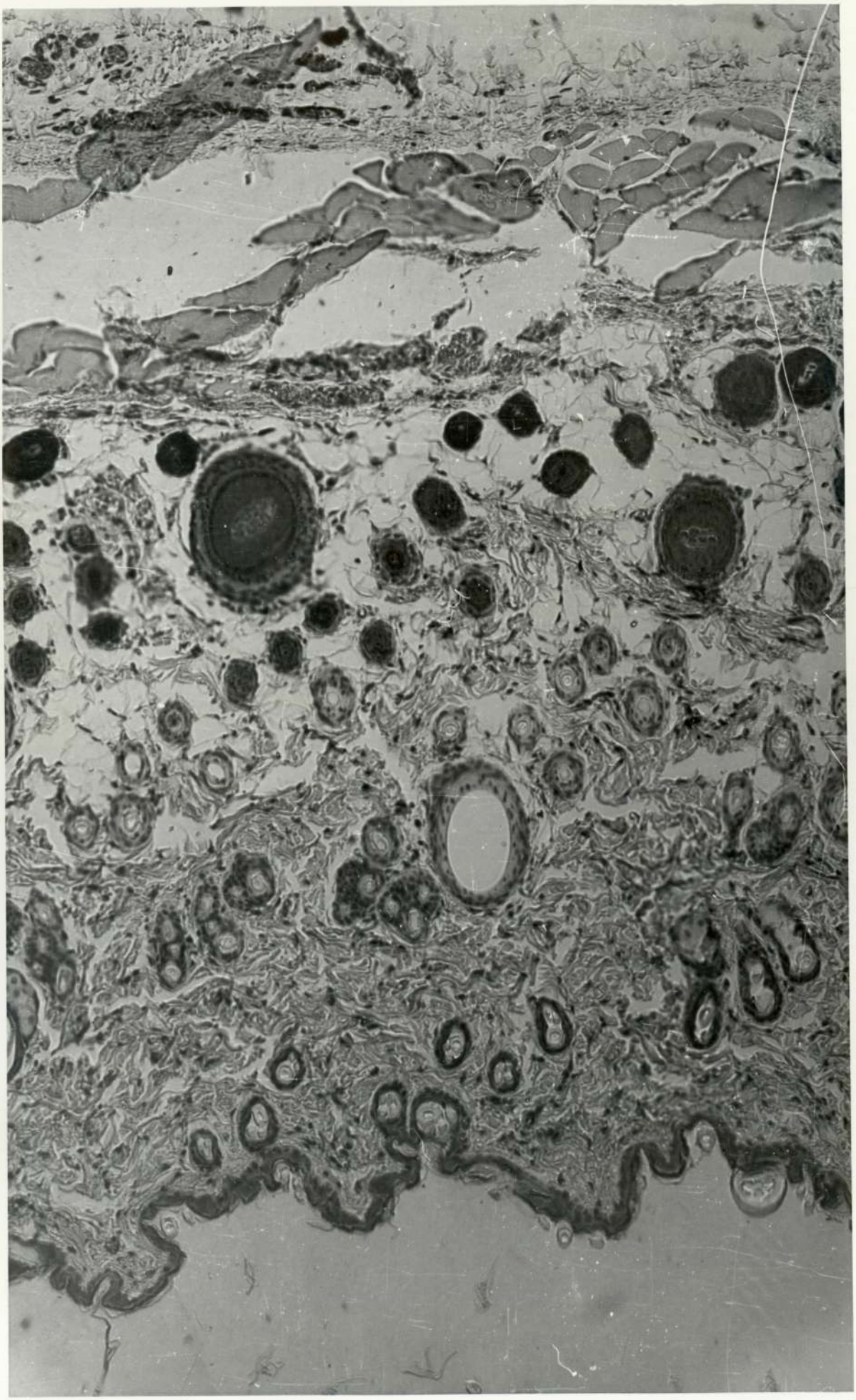


PL. 45



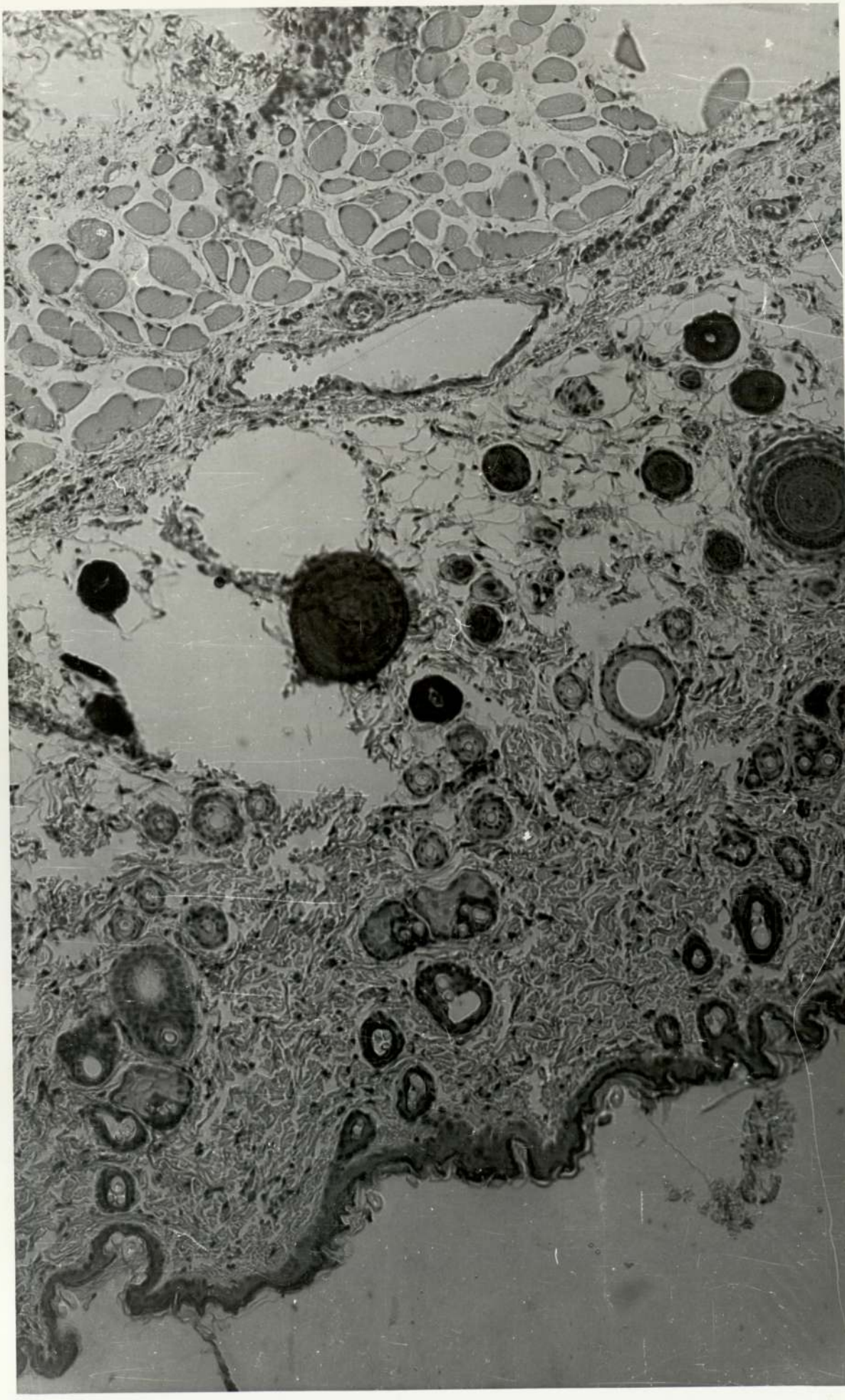
·04 mm.

PL. 46



·04 mm.

PL.47



·04 mm.

Fig. 26. Feeding points on the host most frequented by the fleas, indicated by the density of crosses.

FIG.26

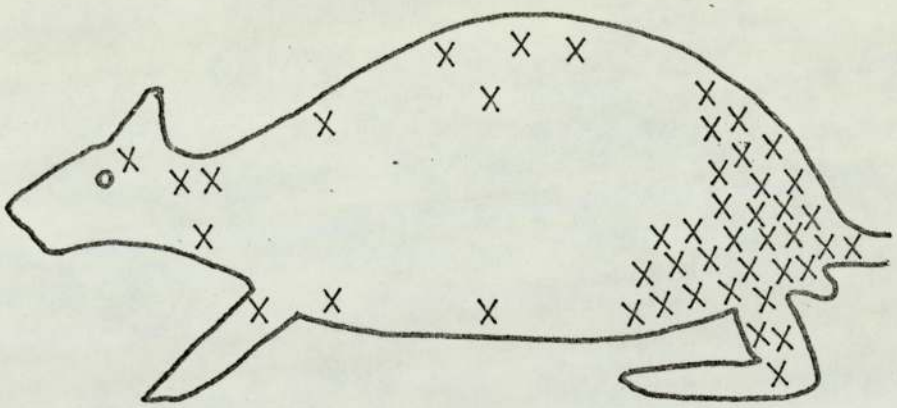
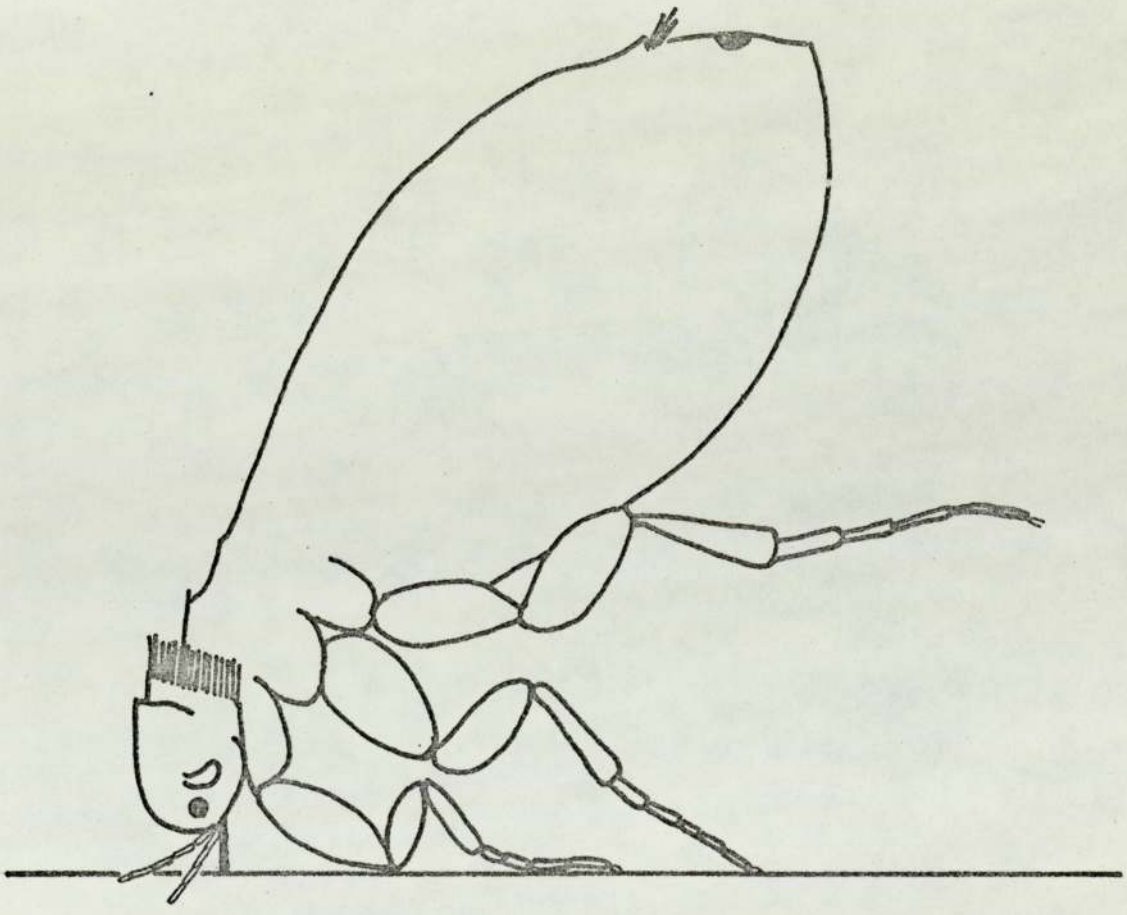


Fig. 27. Feeding posture of a flea on the host.

Fig.27



Antiparasite activity of the host and escape behaviour of the rat flea *N. fasciatus*.

It has long been known that one of the main causes of flea mortality is predation by the host (Leeson, 1936; Buxton, 1938, 1948; Humphries, 1966; Cotton, 1970). During the present investigations the fleas, while feeding on the partially shaved rats were often disturbed by the antiparasite activity of the host. To investigate this situation further, three partially shaved rats were infested with ten unfed fleas each and surviving fleas were recovered after a three hour period. The experiment was repeated several times. The overall results are given in table 22.

Table 22. Survival of fleas during a three hour period on a single host.

Rat	Total no. of fleas placed on the rat	Total no. of fleas recovered.	Anti-parasite activity of the host	Percentage of recovery of fleas
A	50	48	Normal	96%
B	100	78	Vigorous	78%
C	150	87	Vigorous	59%

In a further experiment two rats were kept in a single cage, each initially infested with ten fleas to observe the effect of grooming by the partners. The overall survival of two hundred fleas during a three hour period was 30%. This high percentage of killing of fleas by the host compared to that shown in table 22 could be due to two factors. Firstly, an increased mortality rate would be expected because two hosts, rather than one, were available

to kill the fleas. Secondly, the rats frequently groom their partner, particularly in the lumbar region clawing with fore paws close to the point at which the teeth and tongue agitate the partner's fur. This allogrooming can be directly observed to lead to the killing of fleas, which are usually eaten. During a number of earlier experiments when the rats were infested with one to five fleas, the antiparasite activity of the host was never vigorous and the fleas were rarely killed. This suggests that the antiparasite activity of the host depends on the density of fleas infestation.

It was noticed that the rat reacts to flea infestation by more frequent nibbling, biting and licking, clawing with the fore paws and scratching with the hind feet and by shaking. The areas most frequently groomed by various specific methods are shown in fig. 28. Occasionally some types of grooming may extend beyond the indicated areas, but this appears to be of relatively slight importance. Allogrooming is frequently seen during social behaviour particularly during and following agonistic interactions (Grant, 1963); it occurs in both sexes and is by no means limited to the agonistic context. Whatever its social functions may be, it seems to play an important role in antiparasite activity.

Another way in which the host's social behaviour relates to its antiparasite activity may well be the phenomenon of displacement grooming. Clarke (1956) in his report on the aggressive behaviour of the vole, *Microtus agrestis*, described that inbetween the successive approaches of an aggressive dominant male the subordinate shows displacement movements such as brief washing motions of fore-paws. Similar displacement grooming movements are shown by other

rodents (Grant and Mackintosh, 1963). It is reasonable to suppose that displacement grooming increases the hazards to the fleas. A displacement movement usually occurs when the animal is in behavioural conflict such as simultaneous attack and escape motivation, each component of the conflict preventing the other from being expressed. Displacement activities usually appear irrelevent to the social context in which they occur. Their possible social functions have been the subject of various theories (Lorenz, 1941; Tinbergen, 1952; McFarland, 1965; Chance, 1962). Bastock, Morris and Moynihan (1953) also point out that displacement activity may serve subsidiary functions such as acting as signals, or assisting nest building. In the present instance it is suggested that displacement grooming activities in addition to their social function are the principal form of displacement activity in rodents at least partly because they have a subsidiary antiparasite function and are therefore likely to be favoured by natural selection over displacement activities which have no subsidiary advantageous function.

Killing of the fleas can only be effected by the biting activity of the host which is not prevalent on many parts of the body. It seems that as a result of the various frequent grooming activities by the host, the disturbed fleas generally enter a grooming region where the host's biting is effective, such as the lateral and ventral areas of the abdomen and thorax, or the fleas may enter the dorsal area where allogrooming is effective.

A comparison of figures 26 and 28d shows that the fleas usually end up in those regions of the host's body which are rarely

self groomed and especially where biting is not effective.

It was observed that fleas which are disturbed by a stimulus such as a glass rod or the antiparasite activity of the host travel a long distance on the surface of the host as compared to the short journeys when finding a suitable place to imbibe blood. After such disturbance *Nosopsyllus* travels in a zigzag pattern, showing in this respect behaviour similar to that described for the duck flea *C. garei* by Humphries (1971). It would appear therefore that erratic movement is of advantage to the fleas both on avian and mammalian types of pelage. Such protean behaviour is common in many small arthropods when disturbed by a predator (Humphries and Driver, 1970). The fleas after disturbance by the antiparasite activity of the host remain immobile for a relatively long time and their probing activity is much reduced.

Summary.

1. Host-finding in *Nosopsyllus* is in two stages (a) A distinct orientation towards the host from a distance of several centimetres. This may be influenced by olfactory responses.

(b) Behaviour concerned with remaining on the host, which is affected by the body temperature of the host.

2. Feeding on the host lasts from two to three hours, although it is physically possible for a flea to obtain a full bloodmeal within ten minutes, the long stay on the host appears to be partly due to the probability of striking a suitable blood vessel being about 20% and partly due to frequent disturbance by the host's antiparasite activity.

3. The pattern of grooming activities of the host is described and related to flea mortality while on the host. Killing of fleas only occurs on areas of the body which can be reached by the host's mouth, or which are allogroomed by a partner.

4. It is suggested that grooming is an important displacement activity in rodents partly because of its antiparasite function.

5. The antiparasite activity of the host and the mortality of the fleas appears to increase with an increase in the flea infestation. The flea's escape behaviour towards host antiparasite activity involves travelling long distances through the fur in a zig-zag pattern, remaining immobile for long periods and temporary cessation of probing the host's skin.

6. In a single blood meal, a female imbibes more blood (6.21×10^{-4} cu mm) than a male (0.7059×10^{-4} cu mm).

Fig. 28. Rat grooming and anti-parasitic activity.

a. ⋀. Clawing.

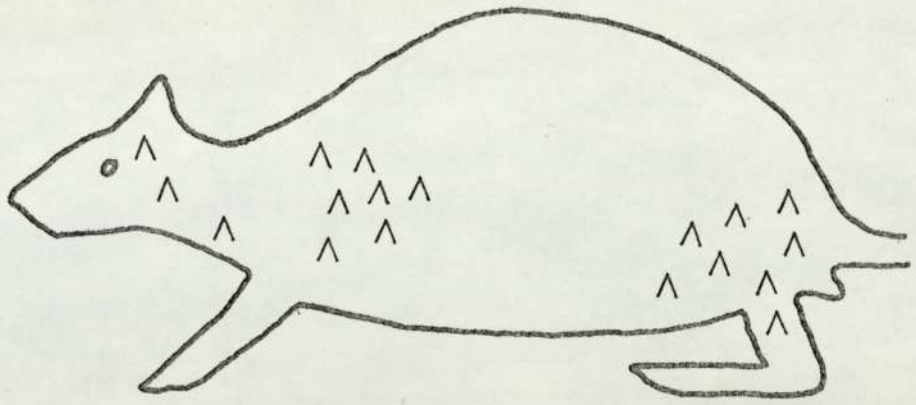
b. ≤. Wash

c. X. Scratch

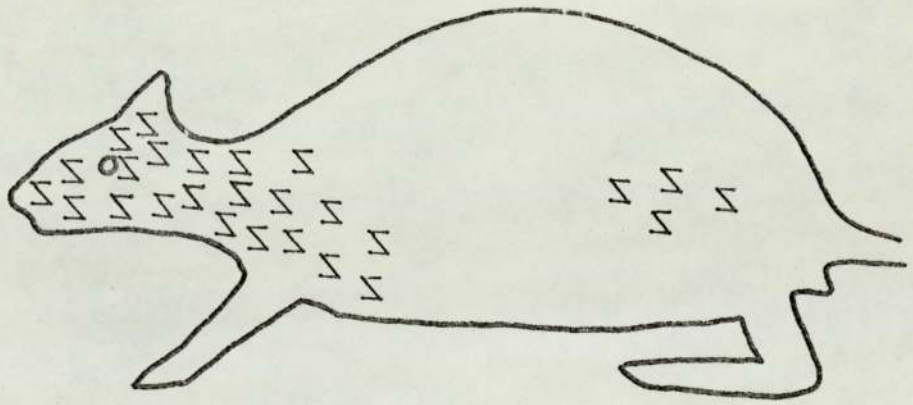
d. ○. Nibbling and biting.

e. ≲. Partner's grooming activity (allogroomed).

FIG.28



a.



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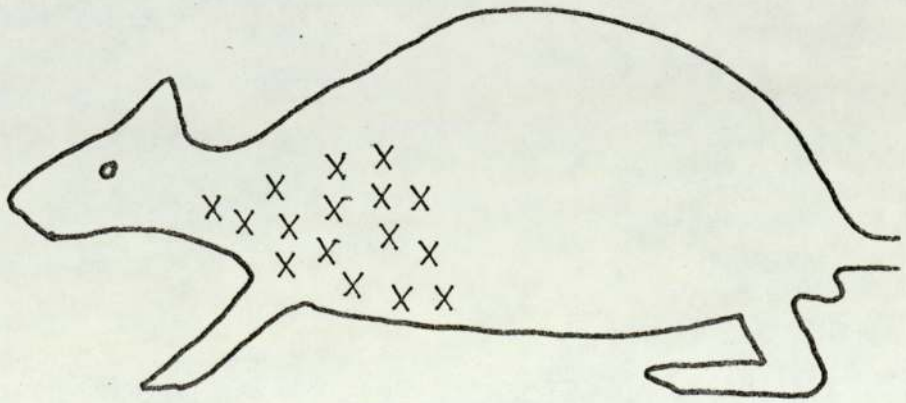
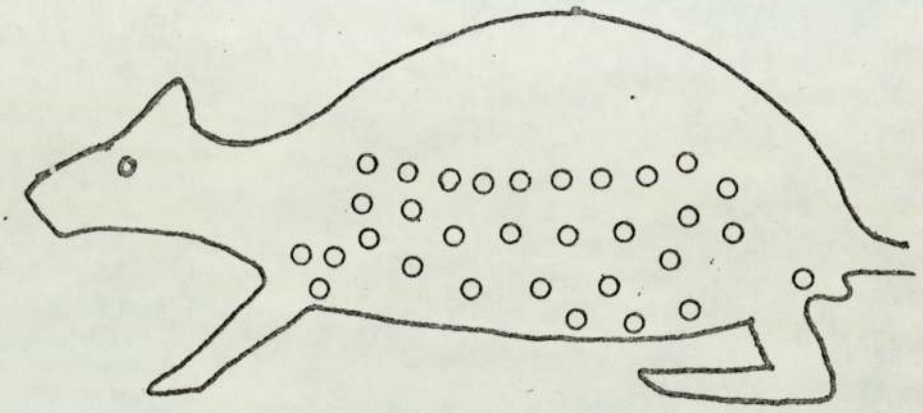
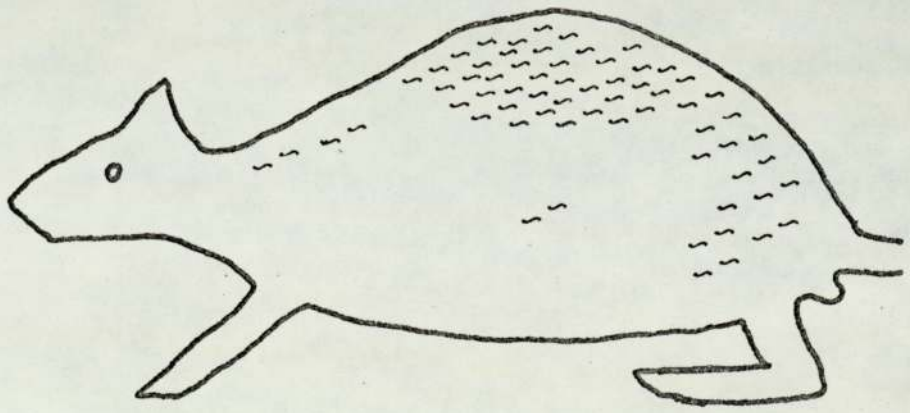


c.

FIG.28



d.



e.

CHAPTER 8

LARVAL BEHAVIOUR

LARVAL BEHAVIOUR

Introduction

The earliest description of flea larvae was given by Leuwenhoeck (1683). He tried to feed them on flies but said that they caused, "such a steam on the glass that the worms being hairy, were entangled in the moisture and remained motionless till they died." He also observed older fed larvae spinning their cocoon, pupating and emerging. Cestone (1699) noticed larvae curling up like watch springs. He fed them on epidermal debris derived from puppies by combing their fur.

Defrance (1824) suggested that the black grains on which the larvae feed were dried blood particles which flowed from the wounds when the fleas sucked the host. Bacot (1914) observed that "when the flea is in a gluttonous mood, it discharges, not small grains of uniform size, but drops of red liquid and scarcely digested blood", and these, "on drying assume irregular shapes". On this ejected blood the larvae of many species feed. Strickland (1914) experimented with the larvae of *N. fasciatus* under different conditions. According to him the most favourable food of the larvae is dried blood. He also showed that a low temperature combined with high degree of humidity is most favourable for the longevity of the larvae and that they are strongly negatively phototactic especially in their younger stages. Exposure to direct sunlight is rapidly fatal. Sharif (1937, 1948) reported that dried horse blood alone is an inadequate diet for the larvae of *N. fasciatus*, yeast supplement being necessary for successful rearing. He showed

that the iron contained in the haemoglobin was essential to the larvae. He also noted (Sharif, 1948a) that the mortality rate was high when a large amount of dry food was added to the test tube containing larvae as compared to that where a small quantity of dried food was added. This demonstrated the importance of water content of the diet.

Recently, Molyneux (1967) reported that the larva of *N. fasciatus* "attaches itself by the mandibles usually to the posterior end, particularly in the pygidial region", of the imago and "when the adult defaecates the larva releases the grip with the mandibles and imbibes blood passed out by the adult flea". He did not discuss the importance of such behaviour. In the culture cage there is an abundance of fleas and larvae and they live in close proximity, so that theoretically there would be an ample supply of such liquid food. It is difficult to assess the importance of this habit under natural conditions, where the fleas are usually less frequent and chances of finding both dried and ejected blood particles and adult fleas are reduced. From the aspect of water balance direct imbibition from the adult anus is advantageous. The probability of finding dried particles of blood is however likely to be higher than that of encountering adult fleas, simply because any one adult will eject many blood spots. It should also be noted that imaginal population of a rat's nest is likely as in other rodents (Cotton, 1965) to disappear still leaving a developing larval population whose supply of blood must now come entirely from dried faecal particles on the nest material.

The present work deals with various aspects of larval behaviour in *Nosopsyllus*, particularly feeding and also gives details of morphological and anatomical features of the mouth parts in relation to their function.

Feeding behaviour.

Feeding behaviour of the larvae was observed in the laboratory by putting blood drops or dried blood particles in a petri dish or in the celluloid arena over a graph paper.

Whenever a larva came across a blood particle or liquid drop, it immediately started feeding. While observing the larval behaviour in the culture cages, it was observed that enormous numbers of larvae attach themselves to the anal region of the adult flea (a maximum number of seven larvae attached to a single flea was recorded) and imbibe blood ejected by the adult flea as reported by Molyneux (1967).

Among earlier workers Heymons (1899) described the mandibles of *Nosopsyllus* as of chewing type; Bacot and Ridewood (1914) described the function of mandibles as of biting and nibbling. According to Perfiljew (1926) "Mandibles play the role of rasping and scrubbing the food and then pushing it into mouth". Sikes (1930) reported that mandibles can break up food into small palatable particles. Sharif (1937b) supported the view of Perfiljew (1926) and described that the hairs on the inner side of the maxilla and labrum, assist in pushing the food into mouth. He also described the larval mouth parts and their musculature.

The head appendages. It seems necessary to review the morphology and function of the mouth-parts and related organs, especially when the larvae are known to imbibe blood from the anus of the adult flea.

The antenna: consists of a long shaft arising from a dome-shaped mound as described by Sharif (1937b). Actually this mound is

dome-shaped only when in a protruded position. If some injury is induced or the antenna contacts some adverse stimulus e.g. a hot object, the mound is retracted and becomes a concavity into which the antennal shaft is also retracted to some extent(plates 56-59.) The outer part of the mound bears six cones arranged in a semicircle. At the tip, each cone bears a papilla, probably sensory in function, (plates 58-59). Three of the six are smaller than the other three and alternate with them. The antennal shaft is cylindrical. According to Sharif (1937b) the pseudojoint is not visible in the first and second instar lavae, but in the present work under high magnification it was visible in the second instar (plate 57). The sensory pits mentioned by Sharif (1937b) are actually longitudinal constrictions of the cuticle, around the neck of the antennal shaft. The distal one third of the shaft is narrow and bears a long seta at its tip surrounded by four sensory cones (Plates 60-61). The antennal mound is surrounded by four setae which are probably sensory in function.

Labrum; is semicircular, hanging transversely in front of the mouth. It is quite strongly sclerotised and the outer margin is tuberculated (plate 56). The posterio-dorsal area of labrum is devoid of any setae or tubercles (plate 62). The long and short hairs, spine-like hairs and spinules of the labrum have been described by Sharif (1937b).

Maxilla and Labium. The description of the maxillae, maxillary palps and labium given by Sharif (1937b) was confirmed (plates 49, 62-69).

Mandibles The mandibles of *Nosopsyllus* have been vaguely described by Bacot and Ridewood (1914), Perfeljew (1926) and Sharif (1937b).

Plates (49,70) show that the mandibles are narrower towards the distal end and bear teeth (usually six). The teeth are directed mesially upwards and cannot meet those on the opposite mandible.

The teeth are not conical in shape as described by Sharif (1937 b) but they are actually bi or trifurcated at the tip. The teeth on each mandible are accompanied by three sensory bristles.

Feeding mechanism.

Following account of the feeding mechanism is based on both direct observations and photographs of the mouth parts, using over one hundred larvae.

Feeding on dried particles , when a larva comes in contact with a blood particle it reacts only if the maxillary palps touch the particle. The blunt cone-like sensillae at the tip of the palp appear to be responsible for detecting the blood. Reaction does not occur at a distance therefore these sensillae are contact chemo-receptors.

The mandibles immediately begin to rasp and scrub at the food particle with the blunt teeth at their distal end. Close behind the mandibular teeth, the ventro-posterior surface of each mandible bears three short sensillae which are ideally placed to guide the teeth to scrape at food particles and not other undesirable objects. If the blood particle adheres firmly to the substratum the scraping action continues. Usually, however, it breaks free and is then picked up and held between the two mandibles by the teeth. It is crushed into small pieces by the forward pushing action of the labium grinding the particle against the mandibular teeth. The mandibles themselves are anatomically incapable of crushing food as

the toothed ends cannot fully meet each other. The resulting small pieces of dried blood are pushed into the mouth accompanied by the movements of the labial area and the areas bearing hairs on the inner side of the maxillae and labrum.

Feeding from the imaginal anus. Although the precise details are not known as to how the larval grip is acheived on the anus of the adult flea to imbibe blood, light can perhaps be thrown on some features of the mechanism. The usual site of the larval grip is the area around the anus. Plate 62 shows clearly the sucker-like appearance of the larval mouthparts viewed from the ventral surface. The tuberculated cuticle of the "sucker" ring is broken narrowly at three points, opposite each maxillary palp, and also a single gap just posterior to the origin of the labial palps. It can be seen that pressure on the maxillary and labial palps will slide them into the broken areas, neatly completing the "sucker" ring. Direct observation shows that the initial grip on the imago is acheived by the mandibles and labrum. Whether or not the tuberculated cuticle of the "sucker" ring is concerned with gripping, or whether it merely serves as a seal to prevent blood leaking past the mouth parts is uncertain. The convuluted cuticle could be contracted to acheive suction grip on the anal area, but direct observations to check this were not possible. The tubercles on the labrum may be additionally involved in providing a frictional adhesion.

The stimulating effect of the larval grip.

It was noticed that the adult flea, if recently fed, would almost immediately defaecate when seized by a larva. Thirty fed fleas were stimulated on and around the anal region with a stiff

bristle of a paint brush. On stimulation the fleas always ejected blood. So it seems likely that the larval grip provides a tactile stimulus for the flea to eject blood.

It is worth pointing out that the habit of taking liquid blood directly from the imaginal anus may have medical implications, since it provides an obvious opportunity for disease organisms to be transmitted directly from one flea generation to the next.

Locomotion.

According to Bacot and Ridewood (1914) "when the movements are more vigorous and in travelling over rough surfaces, the larva bends down the head, hooking what one may call its chin over some relatively fixed object, and then by contraction of the longitudnal muscles draws up the rest of the body". Sikes (1930) and Sharif (1937b) described that the mandibles assist in locomotion by grasping the surface with the terminal teeth.

However the present observations on *Nosopsyllus* do not support the account of locomotion given by Sikes and Sharif in respect of the function of the mandibles. Plates (48, 49) show that the mandibles and their teeth lie far behind and dorsal to the overhanging lip of the labrum. The labrum and the maxilla are tuberculated which can provide a frictional grip on the normally rough substratum.

Stereoscan photographs of the trunk cuticle of the larva show it to be tuberculated, and in between the tucercles, the cuticle is broken into finer flaplike plates (plate 52). On each segment arises a row of long setae and, anterior to these, a row of short setae (plates 50,51). Bacot and Ridewood (1914) mentioned the

independent movements of the long setae during locomotion. In fact, the movements of these long setae are alternated with the movements of the short setae and occur when the larva cannot flex the body to pull itself forwards with the labrum eg; when constricted under a coverslip or among fibrous debris. When the long setae are in an erect position, the short setae lie parallel to the larval body. The long setae then move backwards pushing the larva forward; the short setae are then erected and appear to act as anchors, preventing the body slipping backwards as the long setae withdraw and aquire an erect position (plate 51).

When the larva is travelling normally and on a smooth surface, usually the anal struts are not employed but during a hesitant movement or on a rough surface the anal struts are used for doing fast movement. Plates (53-55) show that the anal struts are without setae but the anal comb is present. The surface of the anal struts is rough due to scanty distribution of rows of small tubercles. The tips of the anal struts are blunt. During locomotion the anal struts are bent at right angles against substratum and then pushed backwards, thrusting the larva forwards. At the base of each anal strut, there is a conspicous pit, the function of which is unknown (plates 53 and 54).

Summary.

1. The larval mouthparts are adapted both for rasping solid particles, and for taking liquid faecal blood directly from the imaginal anus. The tuberculated cuticle around the mouth parts appears to act as a seal or sucker.

2. Blood particles are detected by sensillae on the maxillary palps.

3. The recently fed imago is stimulated to defaecate by a tactile stimulus close to the anus.

4. Locomotion variously involves the action of the labrum, long and short setae on the trunk segments, and the anal struts. The mandibles are not used in locomotion.

PLATES 48-70

A. Antennal Shaft

B. Antennal seta.

C. Antenna in a retracted position.

D. Constrictions of the cuticle.

Pl. 58. Larval antenna.

A. Antenna.

B. Dome-shaped mound.

Pl. 59. Larval antenna.

A. Large sensilla.

B. Small sensilla.

Pl. 60.

A. Tip of sensory cone of larval antenna.

B. Sensory cone of larval antenna X 29,750.

Pl. 61.

A. Tip of sensory cone.

Pl. 62. Larval mouth parts.

A. Posterio-dorsal part of labrum, without bristles.

B. Maxillary palp.

Pl. 63. Maxilla X5,670.

A. Maxillary palp.

B. Long setae.

C. Short setae.

Pl. 64.

A. Larval maxillary palp X 11,620.

B. Probable chemo-receptor.

Pl. 65. Maxilla with long (A) and short (B) setae. X 11,620.

Plates 48 - 70

Pl. 48. Larval mouth parts.

A. Mandibles.

Pl. 49. Larval mouth parts.

A. Mandibles.

B. Teeth.

C. Labrum.

Pl. 50. Larval body.

A. Long setae.

Pl. 51. Larval body

A. Long setae.

B. Short setae.

Pl. 52. Larval body X24,700.

A. Plate like flaps.

B. Tubercles.

Pl. 53.

A. Anal struts.

B. Anal Comb.

Pl. 54.

A. Anal Struts.

B. Anal Comb.

Pl. 55.

A. Tubercles on the anal structs.

Pl. 56. Larval mouth parts.

A. Antenna.

B. Labrum.

Pl. 57. Larval Antenna.

Pl. 66. Tubercles (A) around the base of maxillary palp X 11,620.

Pl. 67. Tip of maxillarypalp X 11,550.

A. Probable chemo-receptor.

Pl. 68. Labium. X 11,550.

A. Labial palp.

Pl. 69. Labium.

A. Area covered with short setae.

B. Labial palp.

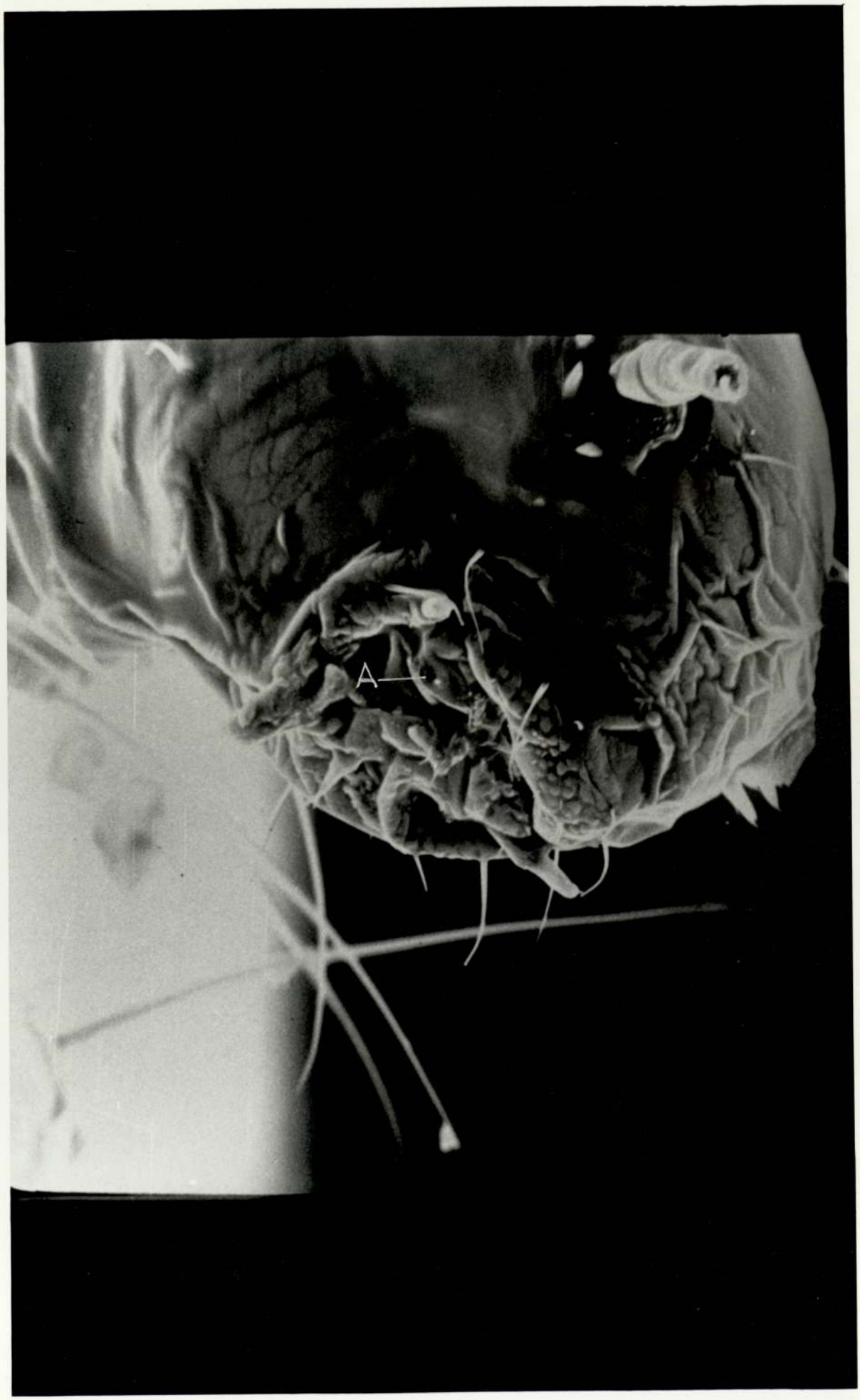
Pl. 70. Mandibles.

A. Main body of mandible

B. Tooth of mandible

C. Sensory bristles.

PL. 48



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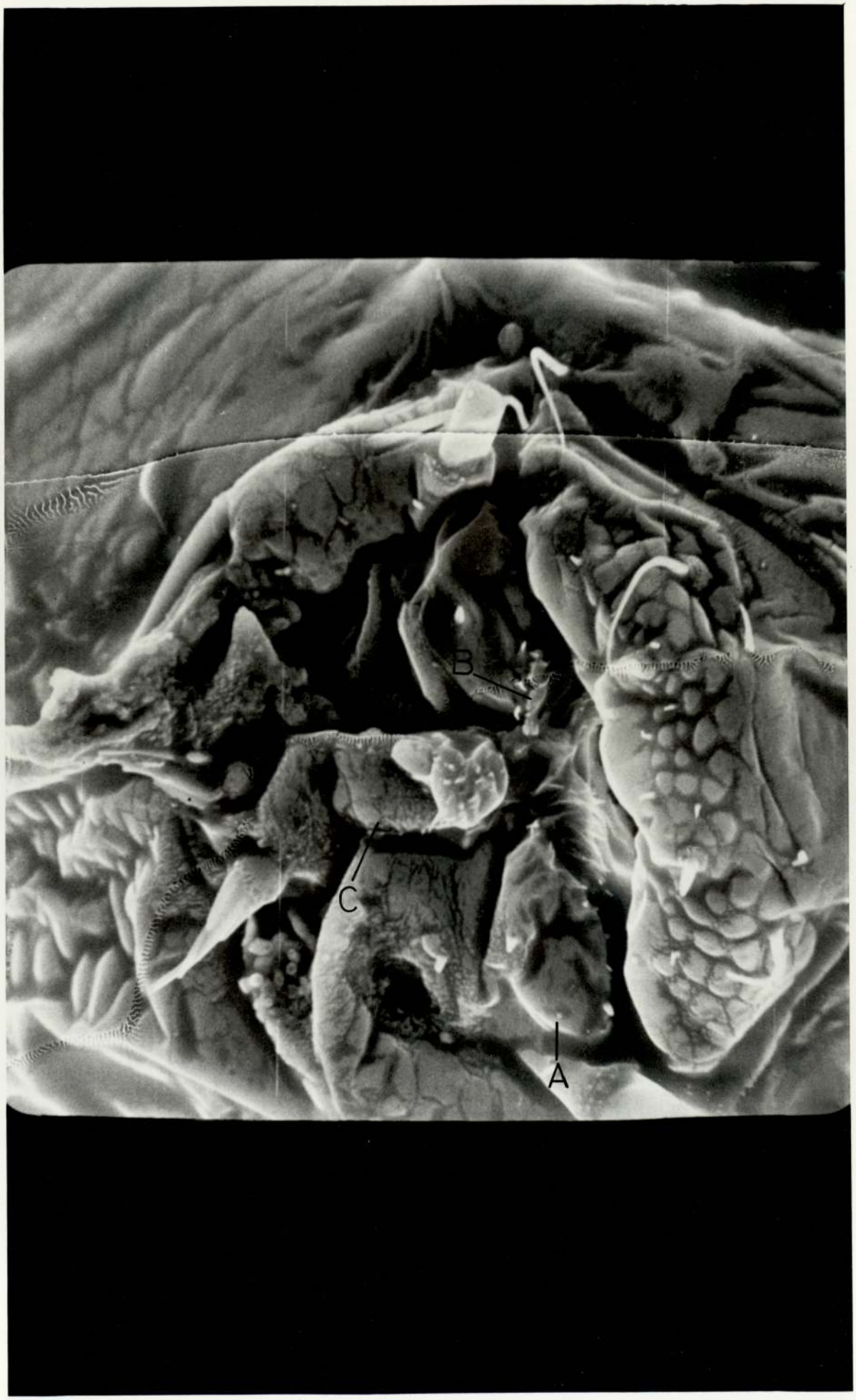
PL.49



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PL.50

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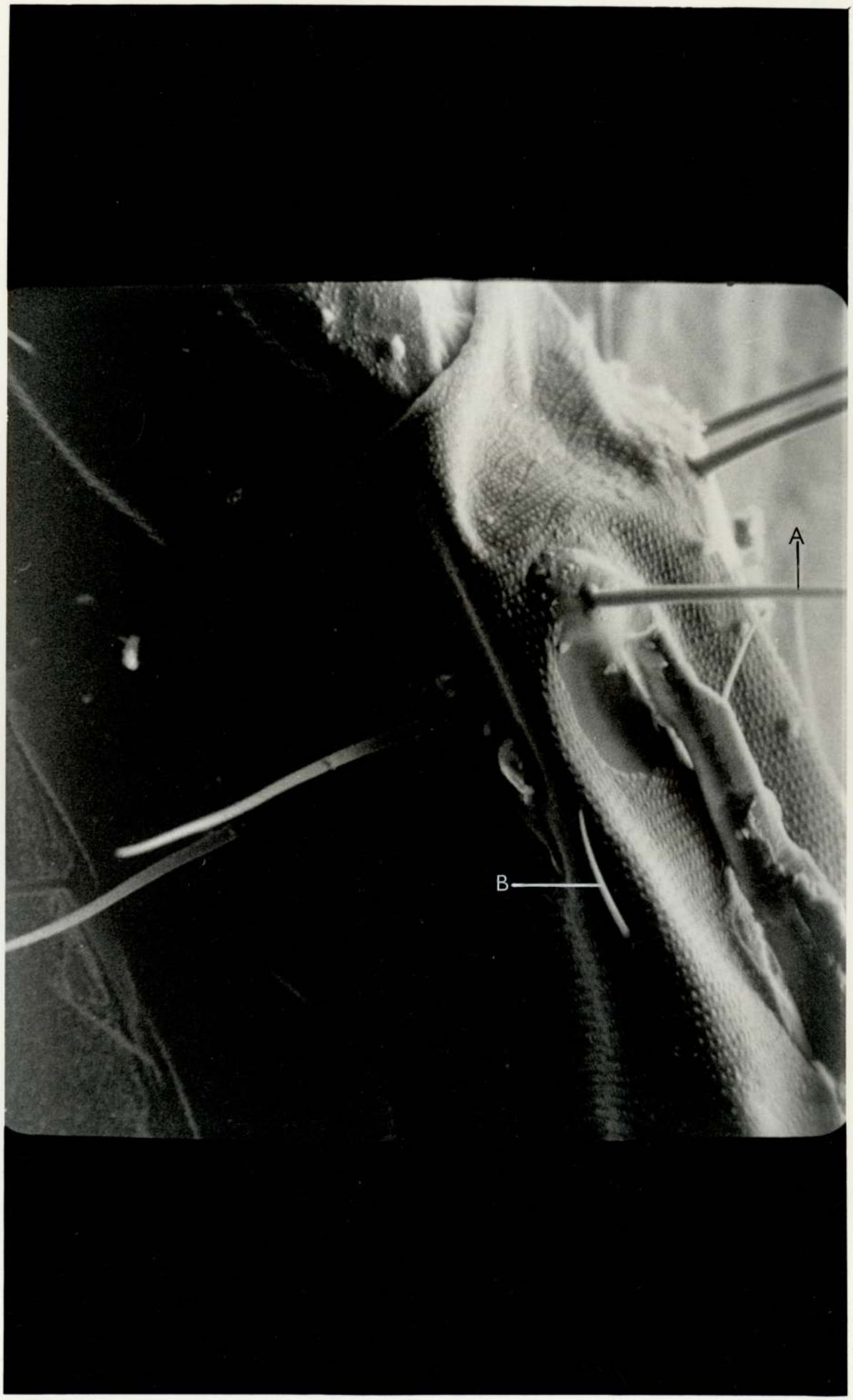
PL. 51



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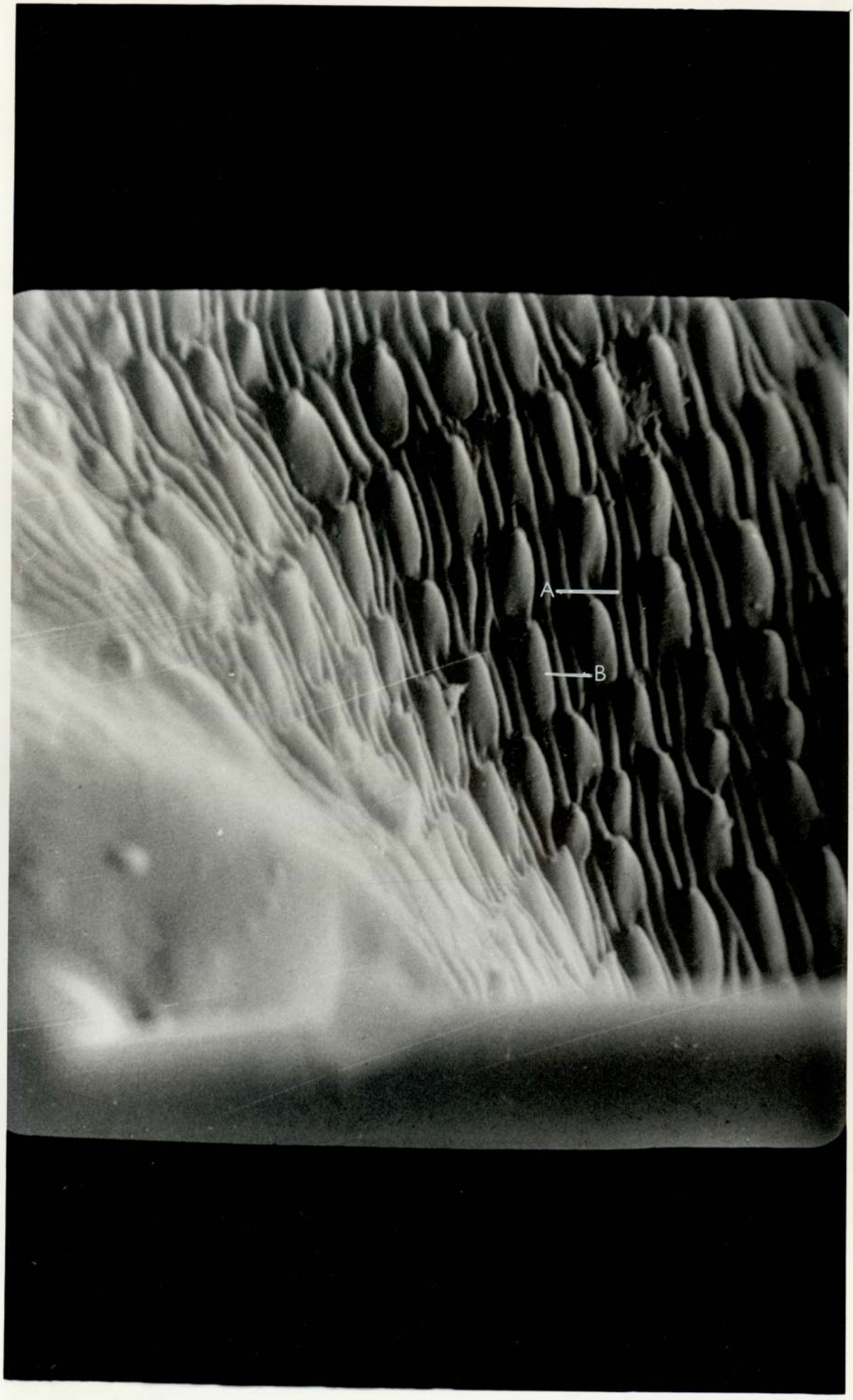
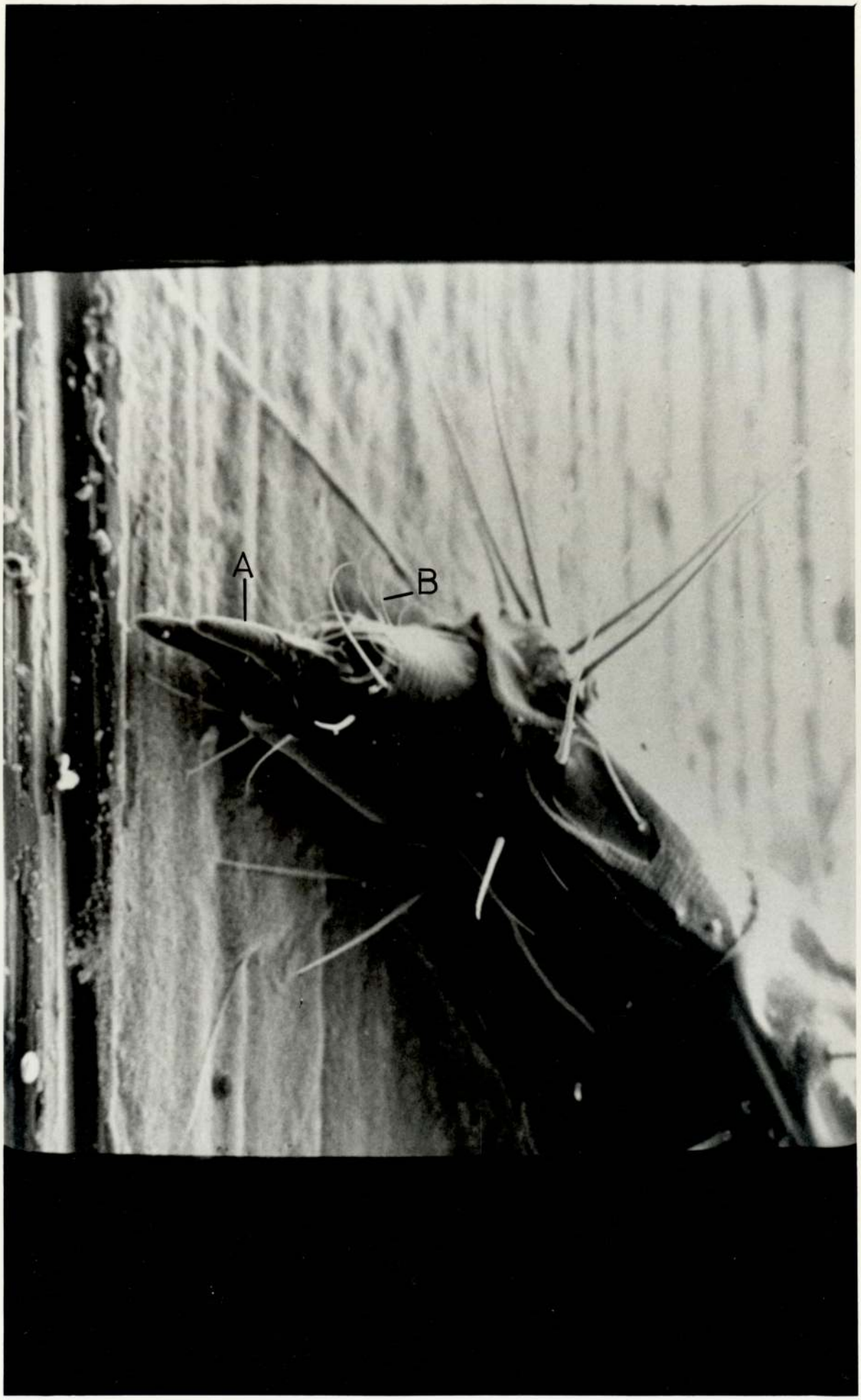
PL. 52



PL · 53



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PL . 54

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PL . 55

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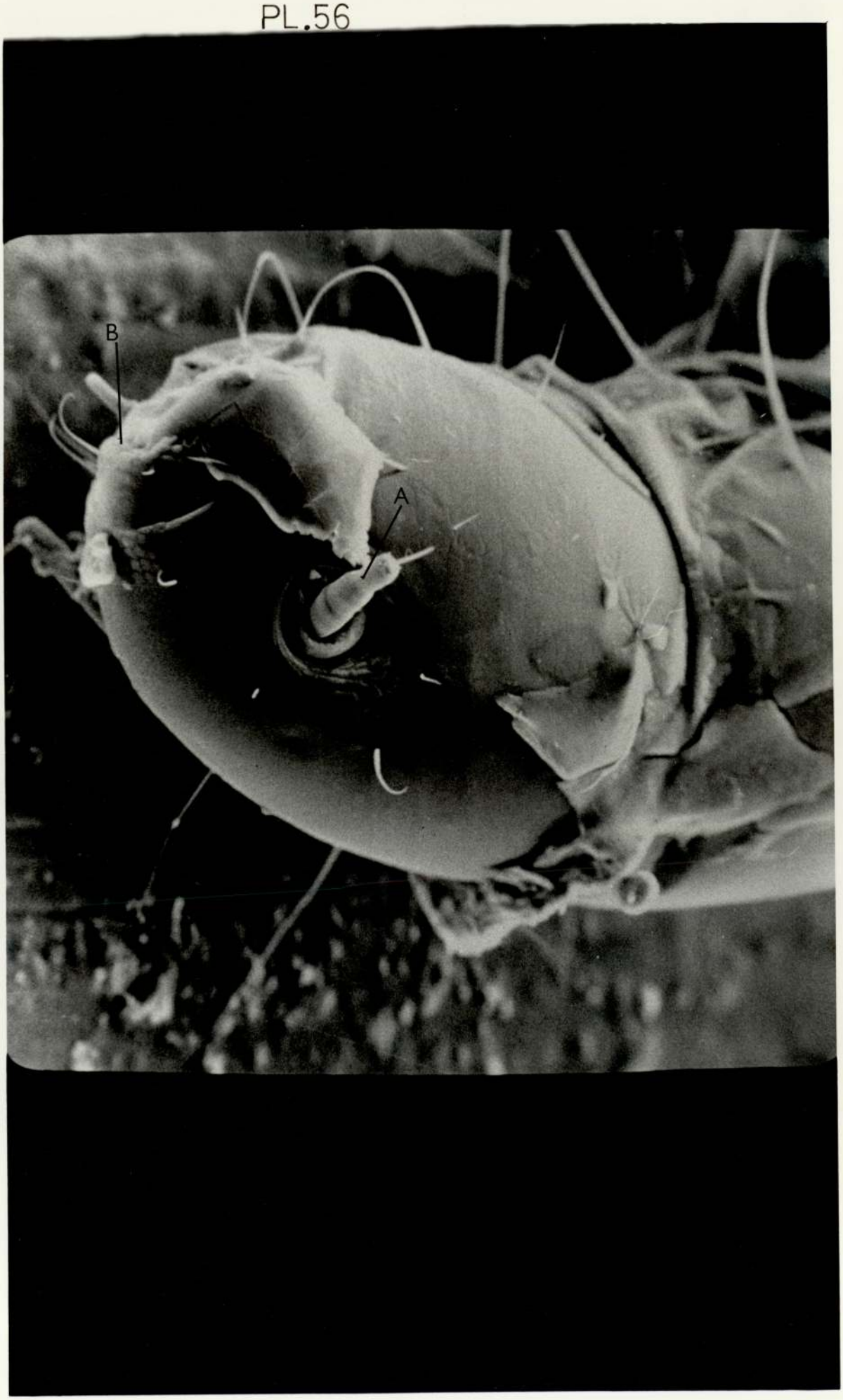
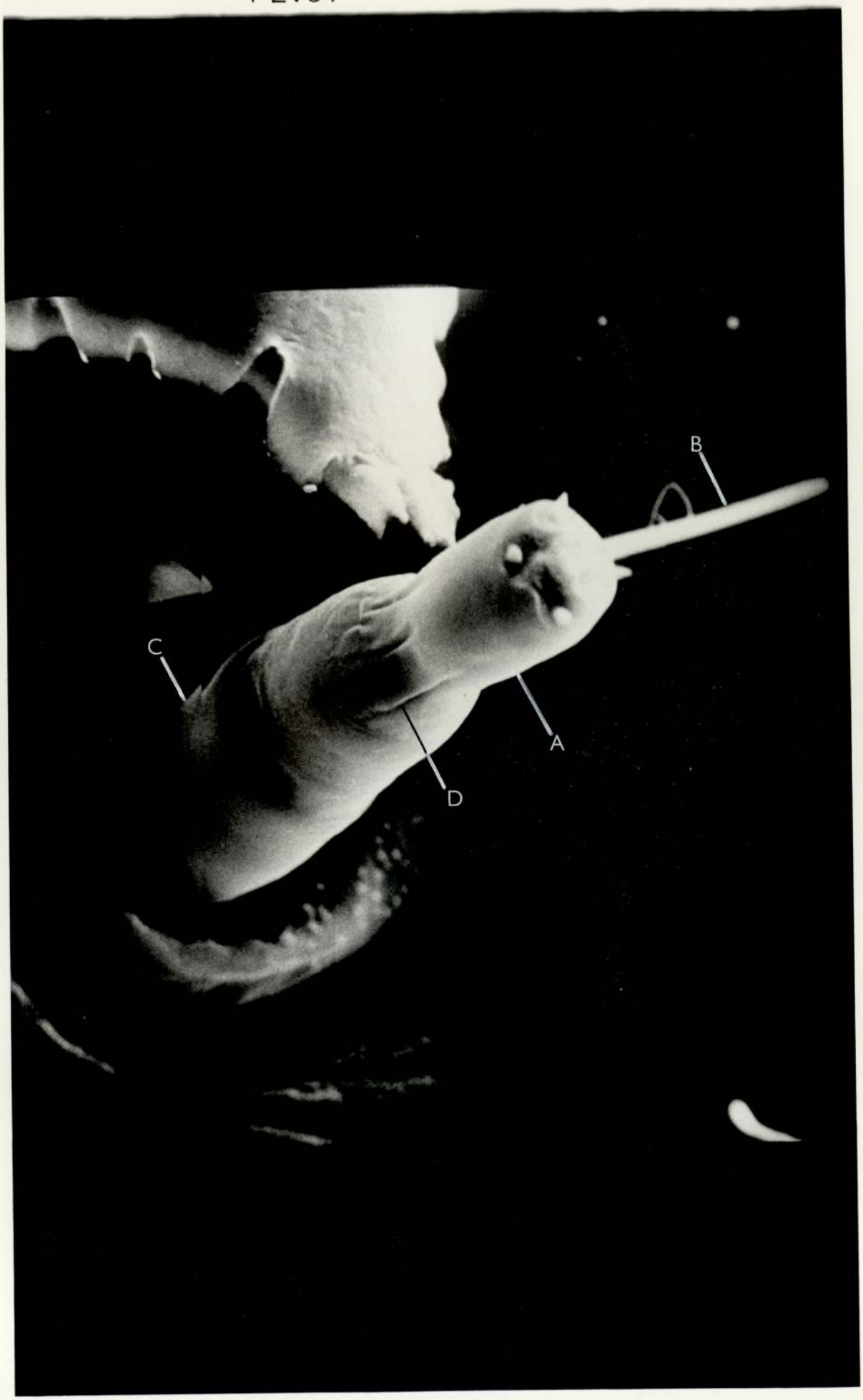
PL.56



·13 mm.

PL. 57



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PL.58

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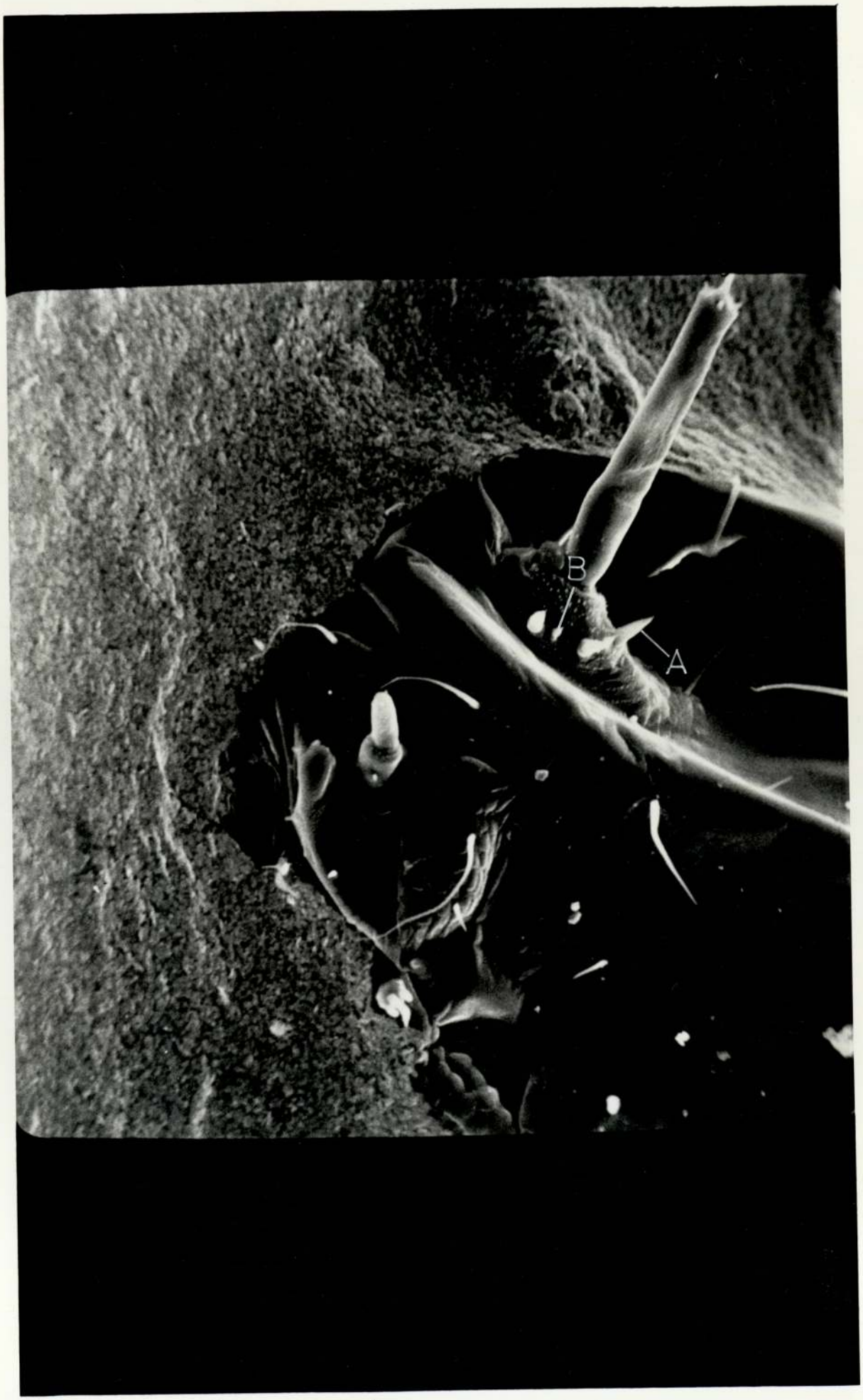
PL.59



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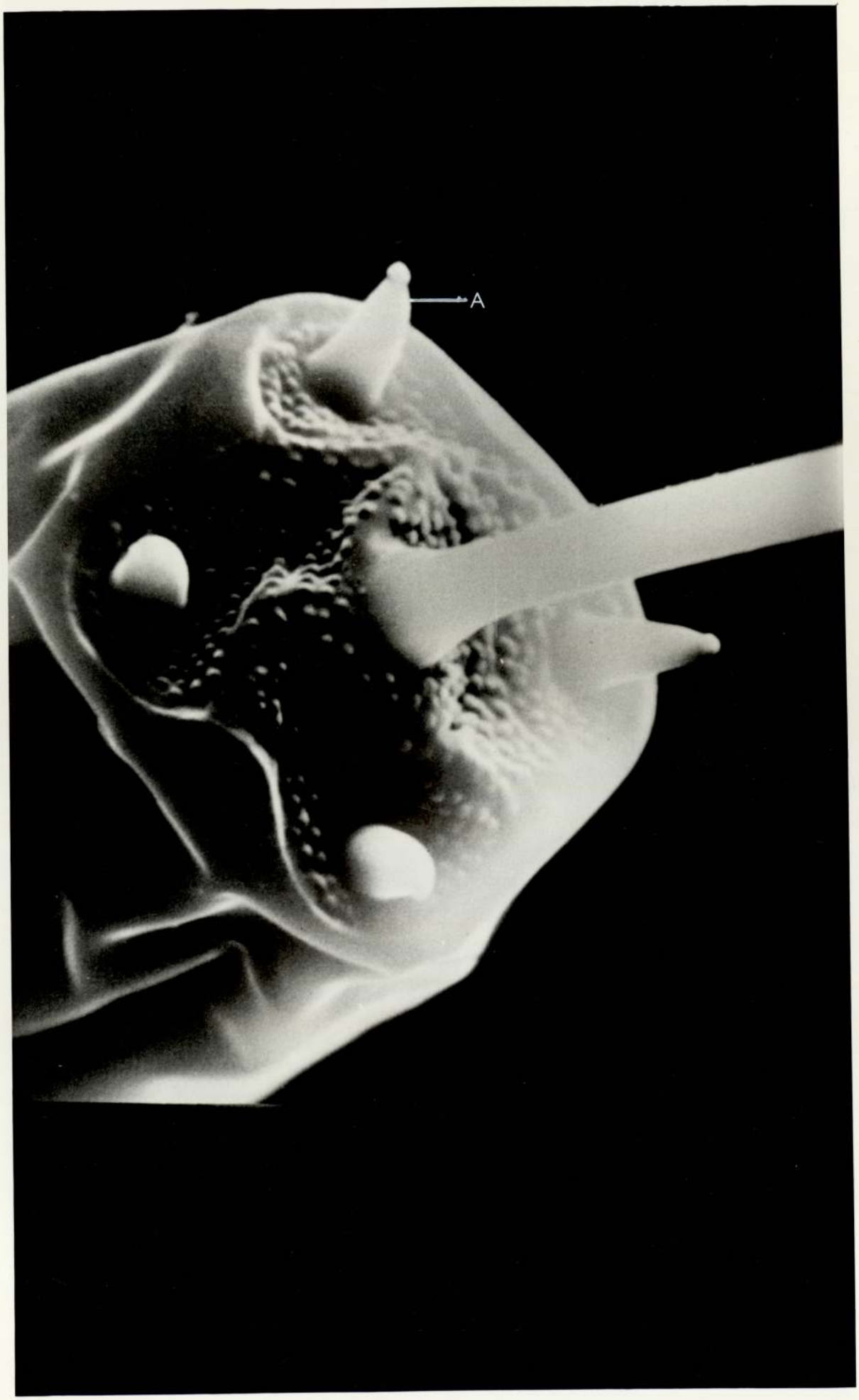

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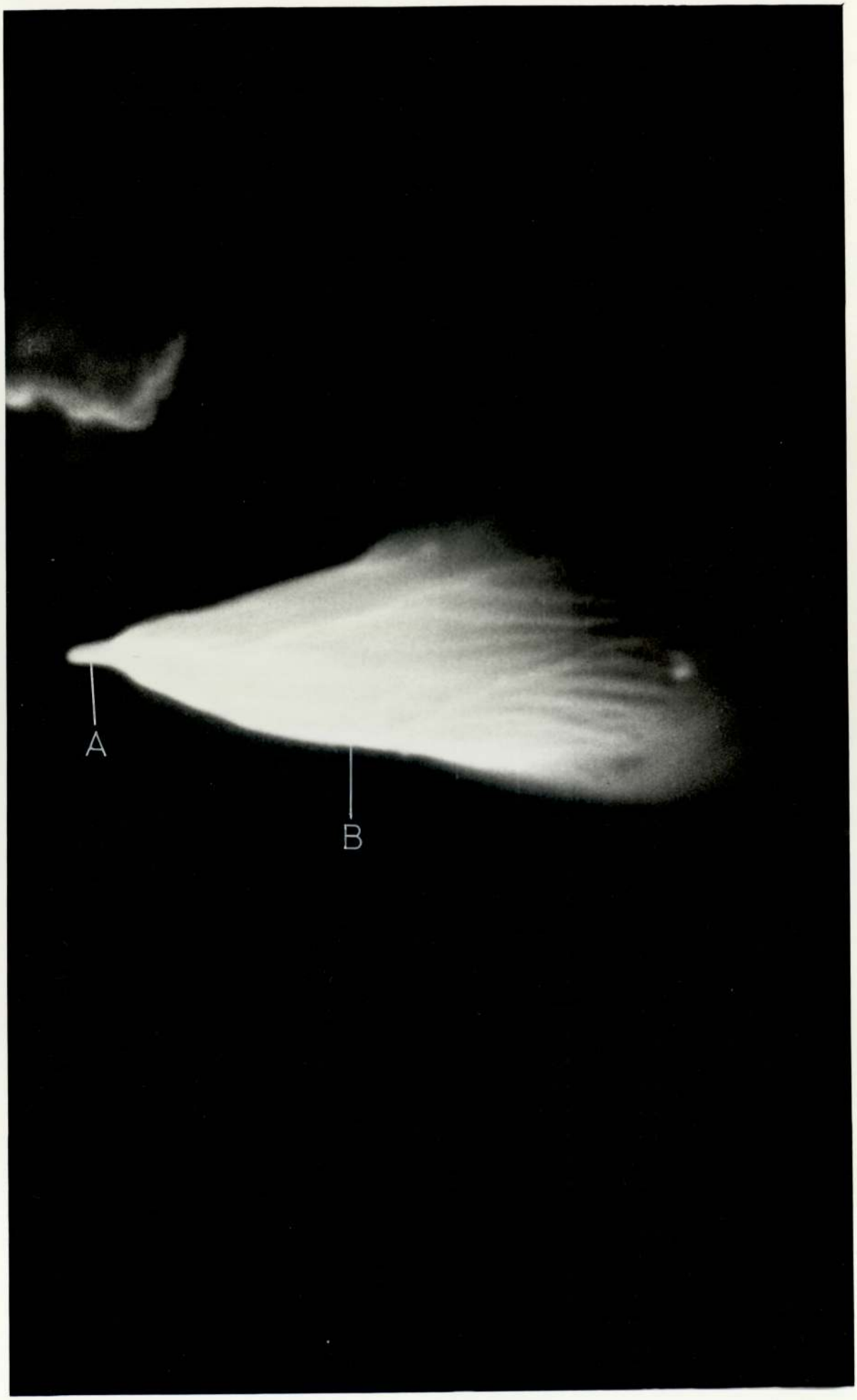
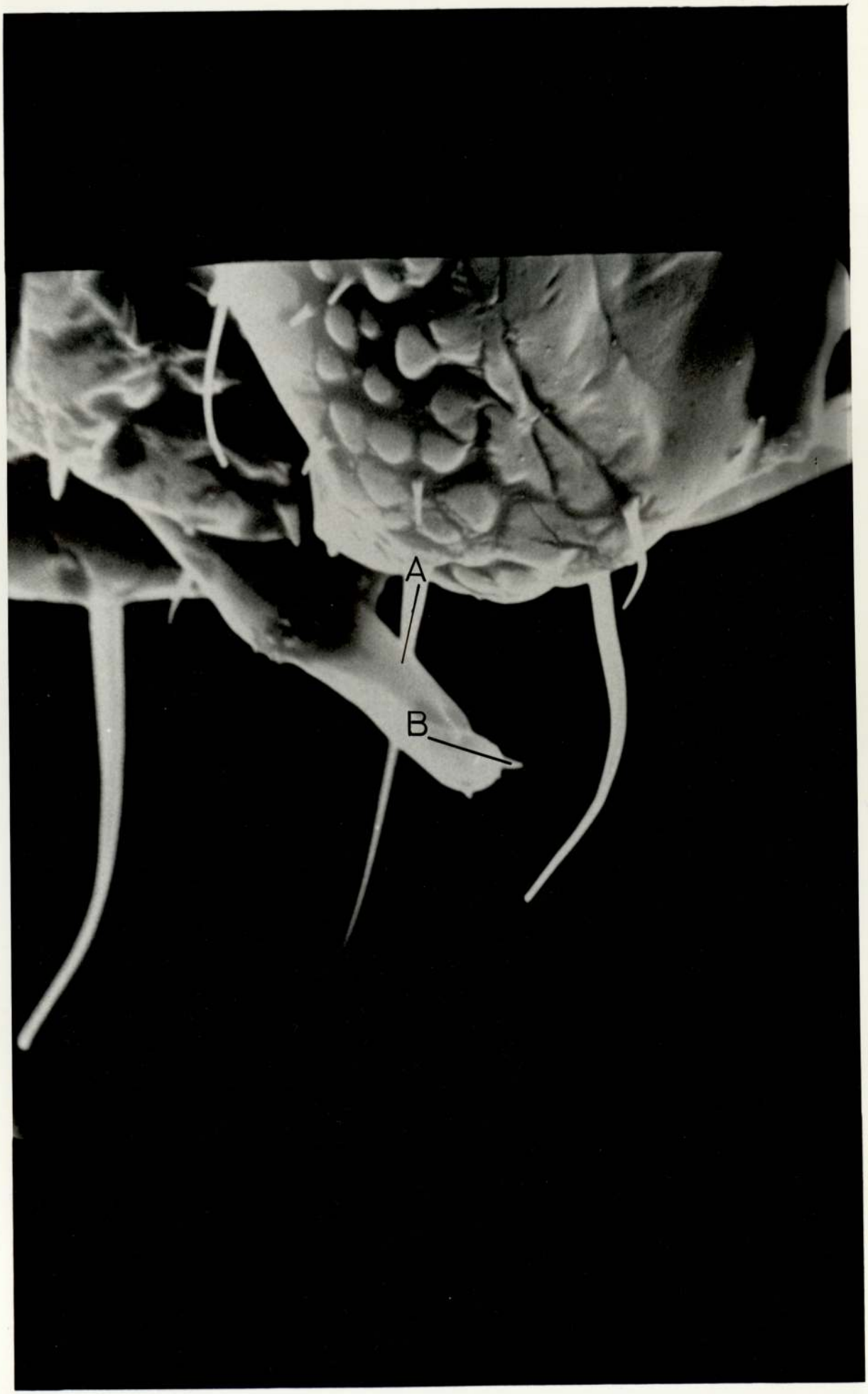


PL.62

·06 mm.

PL.63

PL.64



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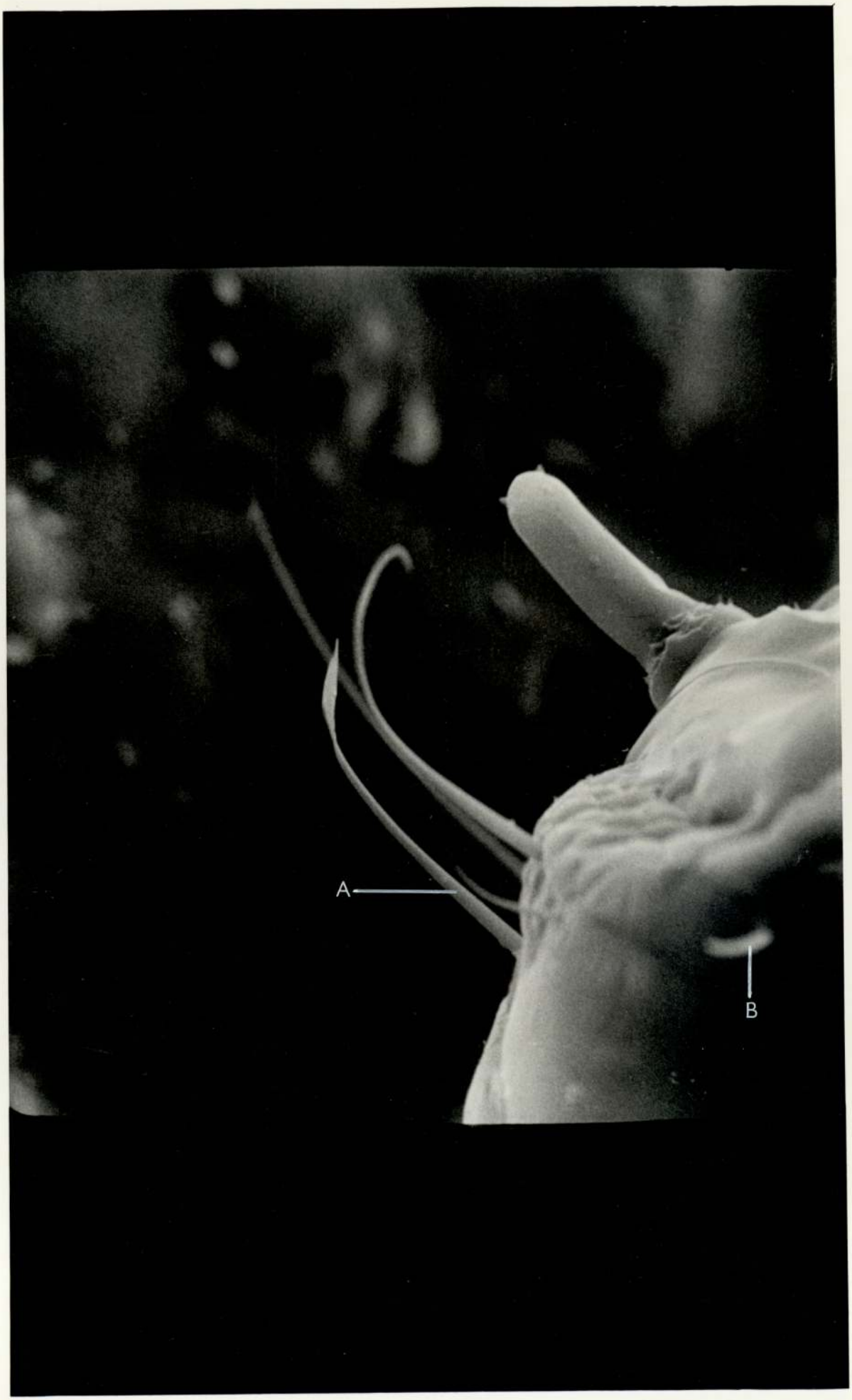
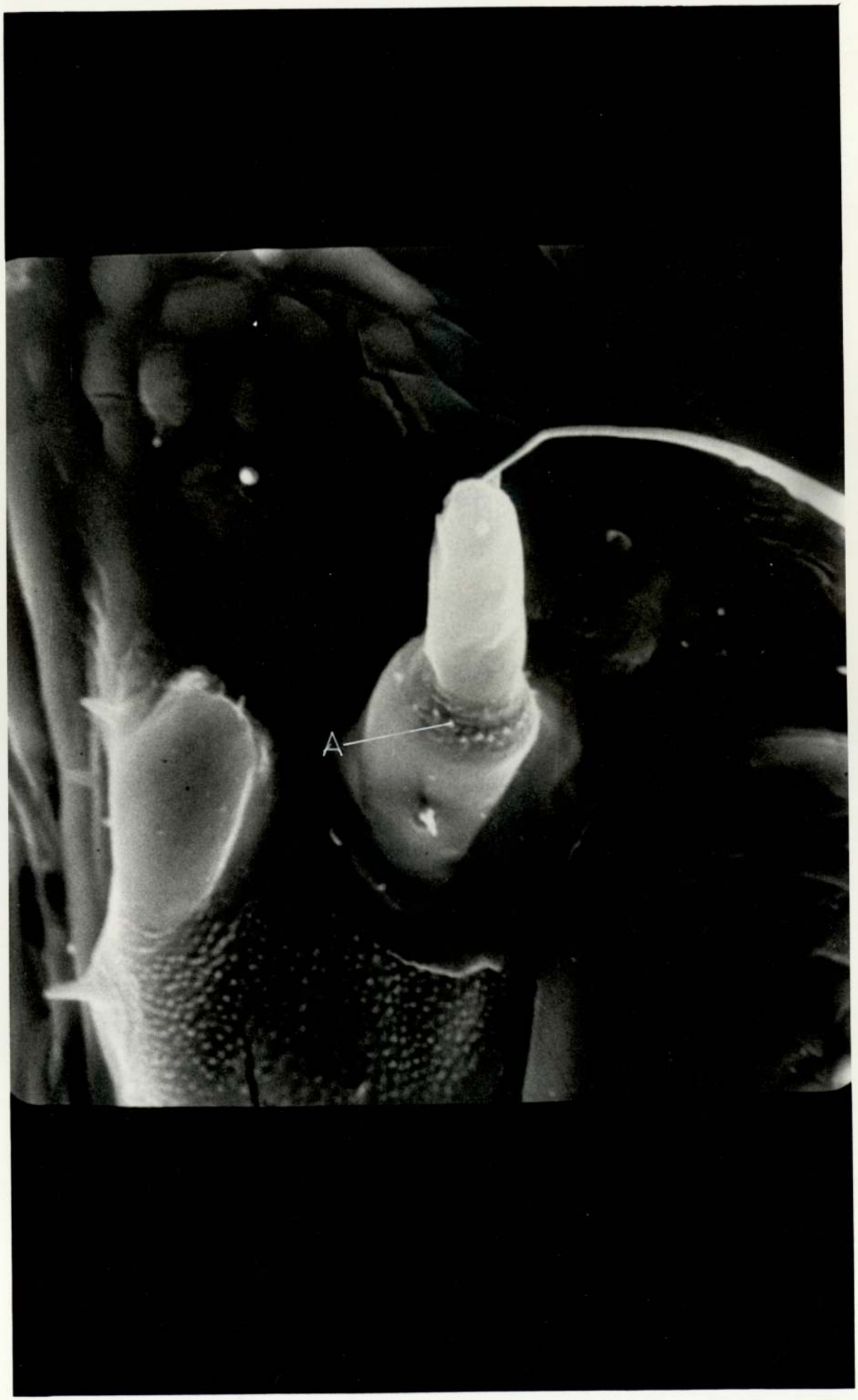
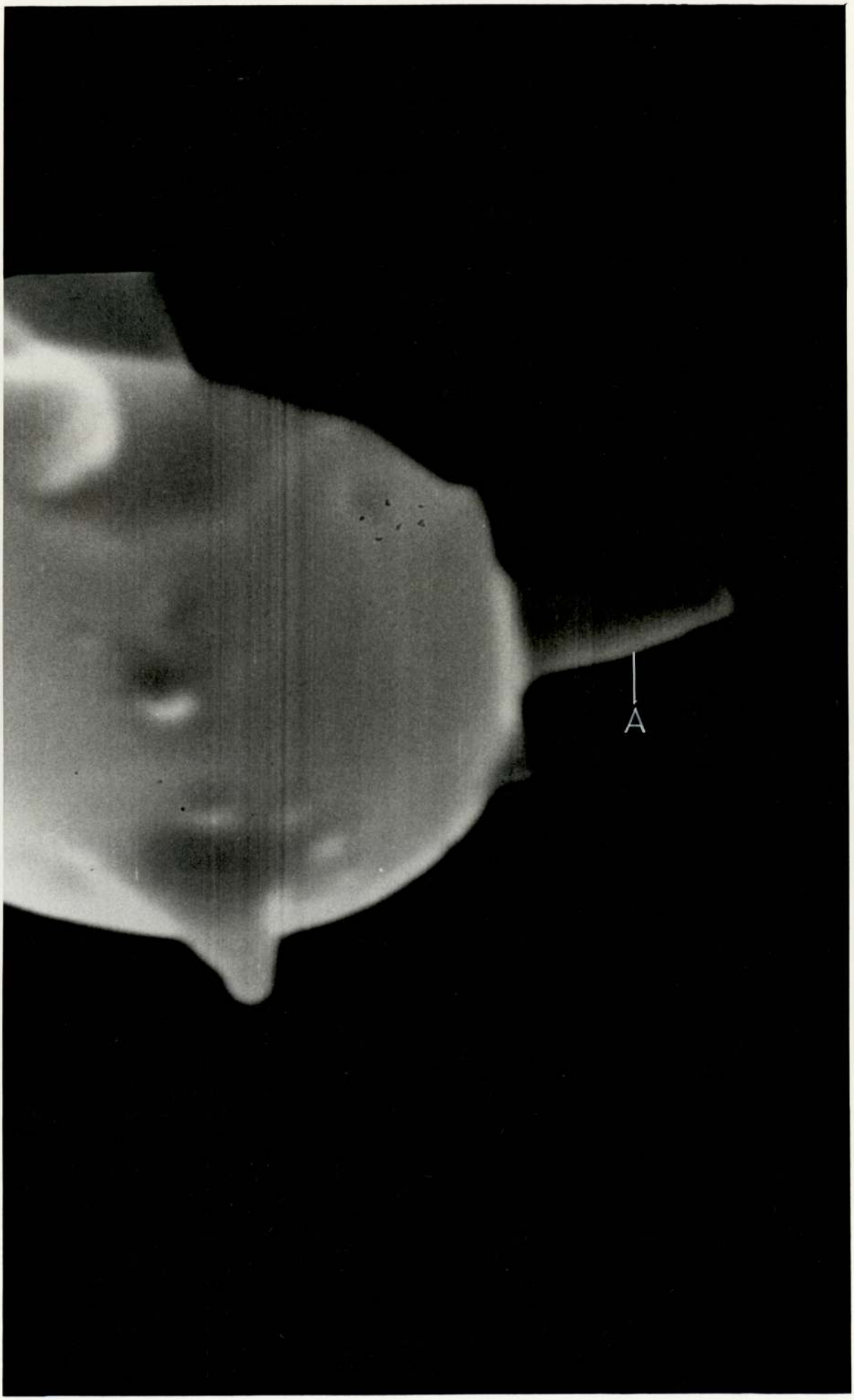


PL.66

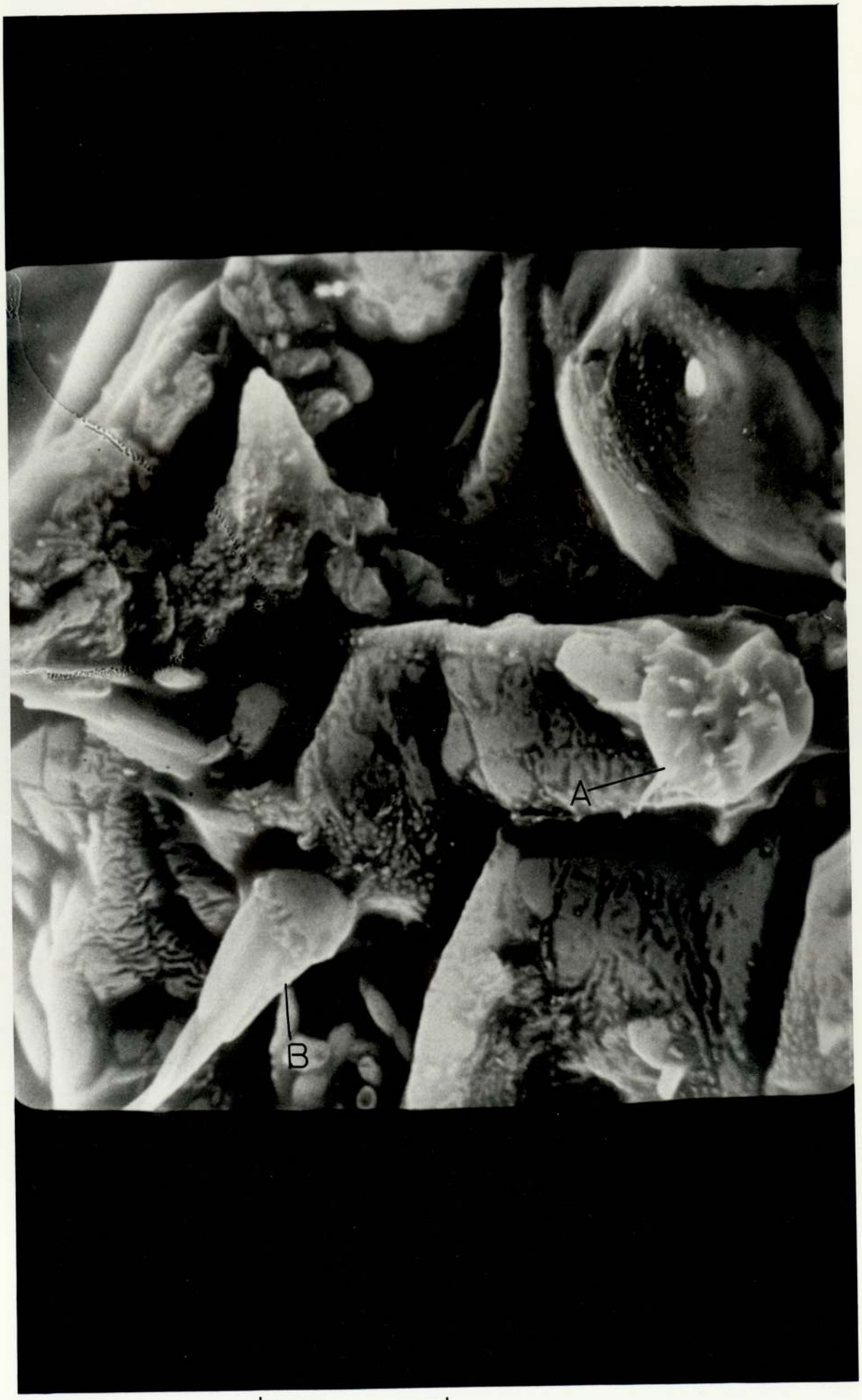


PL.67



PL.68

PL. 69



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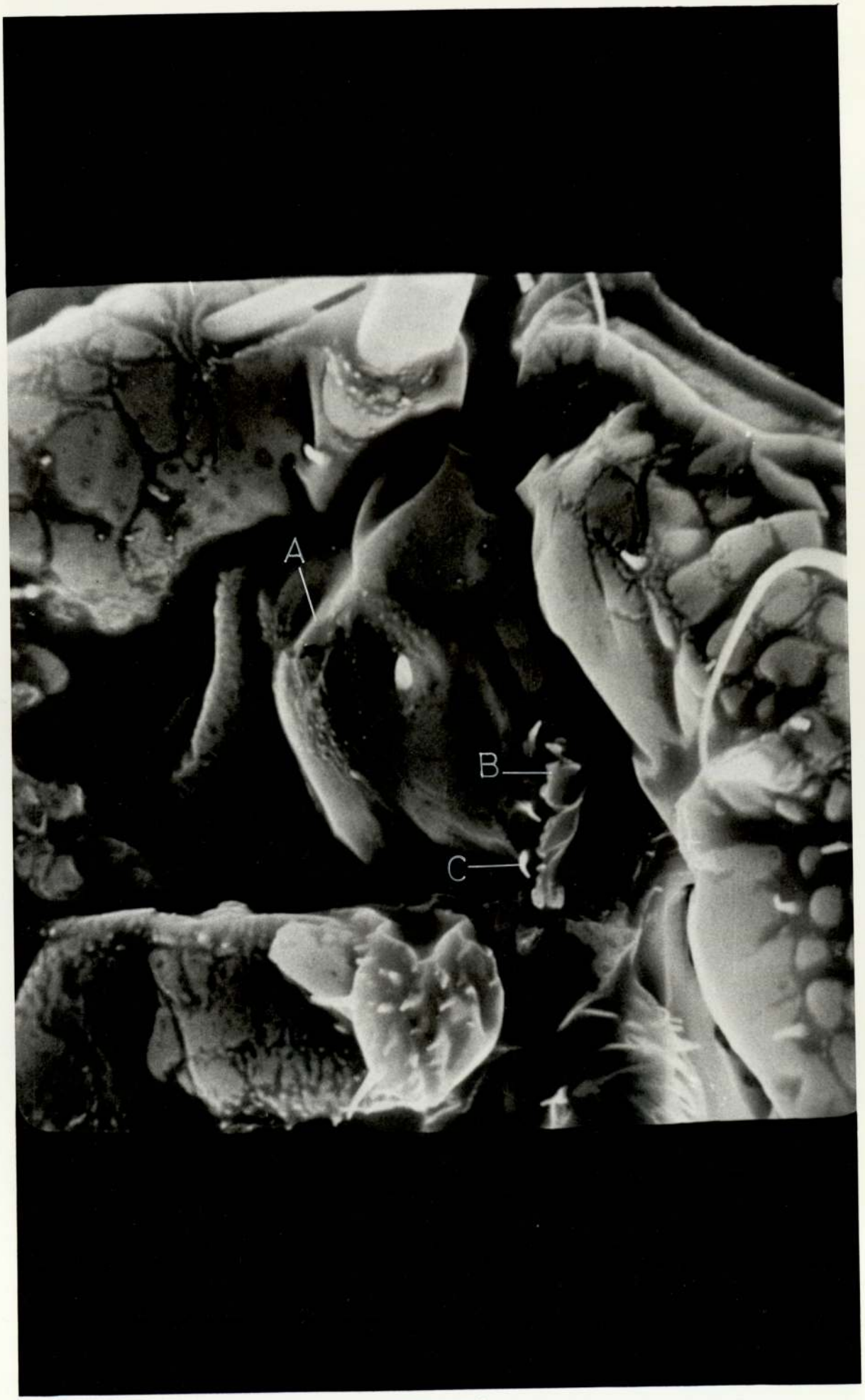
PL.70



CHAPTER 9.

General Discussion and Conclusions

General discussion and conclusions.

The behaviour and breeding biology of *Nosopsyllus* was studied in various behavioural, structural and physiological aspects. It is considered that such basic laboratory studies are required before it will be possible to explain the population dynamics observed (Cotton, 1965) in rodent fleas in the field.

In view of the hazards to survival of the imago once it has emerged from the cocoon, important factors for population dynamics are the chances that the fleas will find a mate, their readiness to mate, the state of ovarian maturation of the females, and the relationship between the number of matings and number of eggs produced.

The probability that a flea will find a mate is higher the denser the flea population. In bird fleas of the genus *Ceratophyllus* temporarily dense imaginal populations occur as a result of synchronised emergence from cocoons in the host's abandoned nests, and several species have been shown to mate readily immediately after emergence without requiring the presence of the host (Holland, 1955; Humphries, 1967a). In contrast the mating of fleas of mammals has almost always been reported to require the taking of a blood meal, and usually a period of maturation after emergence from the cocoon is needed before mating will take place (Poole and Underhill, 1953; Rothschild, 1965a). It is here suggested that the ecological function of this behaviour is probably the restriction of mating to situations where a host has been recently present, thus tending to ensure that eggs develop in inhabited nests rather than disused nests where there would be no fresh supply of faecal blood for the larvae

and the old blood spots in the nest would probably have been used by earlier larval populations. If this is the basic ecological function of the requirement of rodent fleas for a bloodmeal before mating, then the case of the rabbit flea *S. cuniculi* can be seen as an extreme specialization of the same pattern, where suitable rearing conditions for the larvae are found mainly in the breeding nest of the host and the flea's nutritional and hormonal requirements for ovarian maturation and mating (Mead-Briggs, 1964, 1969; Rothschild and Ford, 1964, 1964a, b, 1966, 1969; Exly, Ford and Rothschild, 1965; Rothschild, 1965, 1967; Rothschild, Ford and Hughes, 1970) ensure that production of fertilized eggs is most likely to occur in the breeding nest, not in the rabbit warren generally where there would be little blood for the larvae. The finding by Mead-Briggs and Vaughan (1969) that a 'nestling-factor' is important in triggering mating in *Spilopsyllus* is a further behaviour specialization serving the same ecological function.

Earlier findings (Strickland, 1914) that rodent fleas will not mate unfed had led to concentration on the bloodmeal itself as the physiological trigger which enables mating to occur. Although in the present work the nutritional effect of the blood meal is shown to be important in yolk deposition, and to have some effect on the duration of readiness to mate in both male and female, the new discovery is that exposure to the host's normal fur temperature is the critical event which makes mating possible, and that it also sets off an acceleration in ovarian maturation. It is evident therefore that the temperature experiences of the fleas must be monitored very closely in future work on rodent flea reproductive

biology; some earlier work can be criticised in this respect, for example the striking differences found by Buxton (1948) in the egg productivity of *Xenopsylla cheopis* reared on adult mice compared with those reared on baby mice may well have been partly due to the poorer temperature regulation of the baby mice. The effect which falling temperature has in causing fleas to stop feeding is another way in which it may indirectly affect breeding performance.

The temporary nature of the period of mating readiness induced by a temperature rise or by a bloodmeal in *Nosopsyllus* is another interesting feature from the ecological point of view. Females which have fed, mature oocyte$_1$ with full yolk deposition within twentyfour hours, yet these same females, if they fail to encounter a sexually active male within the same twentyfour hour period will not subsequently mate (unless re-stimulated by bloodmeal or rise in temperature). It appears therefore that mating and therefore the production of fertile eggs is restricted to a relatively short period following contact with the host, so decreasing the chances that larvae will develop in unfavourable situations. The almost complete cessation of egg production ten days after mating is compatible with this idea. Where the nest is still inhabited by the host, egg production will resume and extend beyond ten days because of the re-mating which is possible at three day intervals. The larval population therefore continues to grow in situations where there is likely to be an adequate blood supply, but is likely to soon stop growing in the absence of a host.

Another important factor in the control of readiness to mate is the transient pheromone mechanism. The pheromone is effective

for little more than half an hour. An effective pheromone is therefore present only during periods of continuous pheromone secretion. The female is rendered unattractive soon after the pheromone secretion stops. The attractiveness of the female can therefore be easily altered according to the availability of the host.

It becomes increasingly evident that larval requirements are a key factor in flea ecology, not only their humidity preferences (Strickland, 1914) but their nutritional requirements (Sharif, 1948). In the present study the arrangement of the larval mouth parts, as revealed by the stereoscan electron microscope, was related to the demands of contrasting modes of larval feeding - on dried blood particles and on liquid faecal blood direct from the imago.

Stereoscan studies were also made on the male antennae and on the exceedingly complex male genitalia. The high magnification made possible considerably more detailed descriptions of their structure than in previously published accounts (Holland, 1955; Humphries, 1967), and this has thrown new light on their mode of action. The structure of the penis rods in particular is shown to be surprisingly complex and is related to the successive penetrations of the vagina (by the aedegus), the bursa copulatrix (by the outer penis rod) and the spermathecal duct (by the inner penis rod).

As the principal cause of mortality in imaginal fleas is probably the host (Buxton, 1948) and as exposure of fleas to the host is greatest while feeding, the study of flea behaviour while on the host's body was undertaken to examine the means by which the

host captures and kills the fleas and to examine the means by which *Nosopsyllus* can reduce mortaility due to the host. Various forms of grooming by the host have been shown to be important both in delaying feeding, and in disturbing fleas, so increasing the likelihood that they will move to an area of the body where killing by host biting or allogrooming is possible. The relatively long period spent on the host body, exposed to these hazards, is partly due to repeated disturbance, and partly due to the difficulty of striking a suitably large blood vessel. The brief penetration of the stylets at a series of different sites is in this way explained.

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Temperature as a critical factor in the mating behaviour of the rat flea, *Nosopsyllus fasciatus* (Bosc.)

By Q. J. IQBAL and DAVID A. HUMPHRIES
Department of Biological Sciences, University of Aston in Birmingham

(*Received* 16 *April* 1970)

Several species of fleas parasitizing mammals have been reported to require a blood meal before they will mate. Mitzmain (1910) states that mammalian blood is necessary for copulation in *Pulex irritans* L. and in *Diamanus montanus* (Baker). Poole & Underhill (1953) found a similar requirement in *Megabothris clantoni* Hubbard. In several ceratophyllid bird fleas, however, a blood meal is not an essential preliminary to mating, as shown by Humphries (1963, 1969) in *Ceratophyllus gallinae* (Schrank), *C. garei* Rothschild and *C. fringillae* (Walker), by Waterston (1912) in *C. farreni* Rothschild, and by Holland (1955) in *C. niger* Fox, *C. idius* Jordan and Rothschild and *C. riparius* Rothschild. However, this is not to say that a blood meal has no effect on mating in *Ceratophyllus*; groups of unfed *C. gallinae* which have, after several days, ceased to mate, will resume mating activity immediately after a blood meal on the human arm.

The evidence is conflicting regarding the ceratophyllid flea *Nosopsyllus fasciatus* (Bosc.), the usual host of which is the brown rat, *Rattus norvegicus*. Strickland (1914) reports that it does not copulate unfed and in any case never in the first week after emerging from its cocoon. Bacot (1914) asserts that it will pair when unfed, indeed shortly after emergence.

The precise physiological manner in which the blood meal affects mating behaviour is not known. The effects of the quality of the blood meals taken by the European rabbit flea *Spilopsyllus cuniculi* (Dale) on the maturation of the ovaries and the probability of insemination have been the subject of much recent research (Mead-Briggs, 1964; Mead-Briggs & Vaughan, 1969; Rothschild & Ford, 1966) and hormones from the anterior lobe of the pituitary of the host have been implicated as critical factors. The reproductive dependence of *Spilopsyllus* on hormonal changes in its host is, however, to be regarded as a specialization to its very unusual ecology (Mead-Briggs, 1964) and these findings therefore have little predictive value for reproductive processes in rodent fleas.

Ecologically and structurally *N. fasciatus* is a fairly representative example of a mammal flea, the great majority of which are not semisedentary on the host like *Spilopsyllus*. An understanding of the reproductive biology of *Nosopsyllus* is therefore likely to illuminate reproduction in rodent fleas generally. The findings presented in this paper arose from attempts to clarify the mating requirements of *Nosopsyllus* and in particular to determine which of the several possible factors associated with the taking of a blood meal are critical for the initiation of mating.

THE EFFECT OF A BLOOD MEAL

Preliminary observations indicated that *N. fasciatus* will not mate in the experimental arena when unfed. Fleas which had taken a blood meal were seen to mate soon afterwards, even when the blood had been ingested immediately after emergence from the cocoon.

In order to determine whether the stimulating effect of the blood meal, allowing mating to occur, was on the male or on the female a series of tests were set up. Males and females were sorted into separate batches directly after emergence from their cocoons. Immediately after emergence half the males and half the females were allowed a blood meal for up to 3 h. After this feeding period males and females were placed together in groups of ten (five pairs) in the experimental arenas at about 23 °C, combining unfed and fed males with unfed and fed females in all four possible ways. Twenty-five pairs were used in each combination. They were kept under continuous observation for 3 h and instances of mating were counted (Table 1).

Table 1. *Numbers of pairs (out of 25) which mated when fed and unfed fleas were variously combined*

	Unfed males	Fed males
Unfed females	0	0
Fed females	0	25

The results indicate that both sexes require a blood meal before mating will occur. If one of the partners has not had a blood meal, mating cannot take place.

There are several factors associated with the taking of a blood meal which via nervous or endocrine processes might conceivably activate mating behaviour. They include abdominal distension, a nutritional factor in the imbibed blood, the performance of the sucking act, and experience of a rise in temperature while on the host's body. The factor which is simplest to investigate is temperature, and the following experiments were performed to examine first the effect of ambient temperature, and second the effect of experiencing a previous temporarily maintained rise in temperature equivalent to that which might be encountered while taking a blood meal.

THE EFFECT OF AMBIENT TEMPERATURE

Males and females were paired in three combinations, unfed males with fed females, fed males with unfed females and both unfed. The sexes were again kept separate until introduced into their arenas. The initial temperature for the several groups of five pairs varied from 22 to 24 °C and was allowed to rise steadily at the rate of 1 °C/3 h period to a maximum of 31 °C.

No mating activities at all were observed up to and including 29 °C. Most of the fleas paired during the 3 h period at 30 °C. The sudden and almost complete onset of mating at this temperature was very striking. The detailed results are shown in

dictory findings of Bacot (1914) and Strickland (1914) may be explicable in terms of differences in the temperature experiences of the fleas they observed.

In view of the present findings it should be noted that previous studies on the factors governing readiness to mate in fleas (Rothschild & Ford, 1966; Mead-Briggs & Vaughan, 1969; Humphries, 1963) have not explicitly controlled for temperature as a critical variable. It would be interesting for example to investigate the possibility that the 'nestling factor' discovered by Mead-Briggs & Vaughan (1969) to be essential for copulation in the rabbit flea *Spilopsyllus* might be a temperature factor.

While the effect of a rise in temperature on the male *Nosopsyllus* is to enable it, in the presence of a fed female, to perform mating behaviour, the precise way in which the female is enabled to mate by a rise in temperature is uncertain and two hypotheses are possible. If the female is considered to be initially unreceptive the blood meal may render her behaviourally receptive to the male; alternatively it may enable an initially receptive but unattractive female to provide an adequate sexual stimulus to the male. The second hypothesis is favoured by the present finding that when unfed females are paired with fed males, not only is there no mating, there are no attempts to mate. As the male plays the active role in initiating mating between fed fleas, it would be expected on the first hypothesis that in those instances in which the male had fed and the female had not there would have been attempts by the male to mate and that these attempts would have been unsuccessful due to the non-receptive state of the female. That such mating attempts were not observed suggests that the male is able to distinguish between temperature-stimulated and non-temperature-stimulated females by some stimulus received before mating behaviour begins.

SUMMARY

1. Both male and female *N. fasciatus* normally require a blood meal before they will mate.

2. Fed males do not attempt to mate with unfed females. It is suggested that the taking of a blood meal enables the female to provide a stimulus necessary for the male to show mating behaviour.

3. Unfed *Nosopsyllus* of both sexes will mate if subjected to a temperature between 30 and 35 °C inclusive. Above 35 ° mating does not occur.

4. Below 30 °C mating occurs only if the fleas have previously been subjected to a temperature of 30 °C or above. A temperature rise to the critical point thus acts as a trigger for an enabling process which continues after temperature has again fallen.

5. It is suggested that the effect of a blood meal in enabling mating to occur may be explained by the fleas' experience, while on the host, of a rise in temperature to the level critical for mating.