PARASITISM OF BRITISH TERRESTRIAL ISOPODA BY DIPTERA

By

ROBIN ANTHONY BEDDING

B.Sc. A.R.C.S.

Thesis submitted for Ph.D. degree of London University and for the Diploma of Imperial College.

Imperial College of Science and Technology,

Zoology Department,
Prince Consort Road,
South Kensigton,
London, S.W.7.

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ABSTRACT

Seven species of Rhinophorinae (Calliphoridae) were found parasitising British woodlice : Styloneuria discrepans(Pand.), Melanophora roralis(L.), Plesina maculata (Fall.), Phyto melanocephala(Meig.), Rhinophora lepida(Meig.), Frauenfeldia rubicosa(Meig.), and Stevenia atramentaria(Meig.).

A study has been made of the morphology of immature stages with particular emphasis on the first stage larvae which are of two unusual types.

Contamination of the substrate by the viscous secretion produced by the exopodites of the uropods of woodlice was found to be the main stimulus inducing oviposition.

The first stage larvae lie in wait for their hosts; respond mainly to mechanical stimuli and vary from one species which will attach to anything moving to another which only readily attaches to Porcellio scaber L. which are about to moult. The sizes of host most frequently parasitised depends mainly upon the type of larva and its length.

Each species of parasite has an unusual and charateristic mode of entry.

Final digestion of the host by the third stage larva was found to be aided by a secretion of protease from the larval anus.

All species of parasite were found to be host specific.

Suppression of moulting is caused by at least one parasite species and suppression of ovariole development and of oostegite formation is characteristic of all species.

Superparasitism only occurs commonly in Plesina in the field. Physiological suppression of supernumerary parasites and some cannibalism does take place.

Dissections of over 20,000 woodlice from a variety of habitats have been made. The two commonest species of parasite, Plesina and Styloneuria, were rarely found in the same woodlouse population and their presence or absence was determined by the type of microhabitat of the host.

Obligatory diapause occurs in the first stage larva of one, and facultative diapause occurs in the second stage larvae of five of these parasite species.

CONTENTS

1. INTRODUCTION AND HISTORICAL

1a) Introduction

The Terrestrial Isopoda are unique in being the only members of the large class Crustacea to have become fully terrestrial. While they are not represented by great numbers of species, numerous individuals are found in a variety of terrestrial habitats, ranging from the upper sea shore, sand dunes, grassland, heath, hedgerow, and woodland to within human habitations and their surrounding gardens and rubbish heaps.

Despite the fact that this group evolved as far back as the Pleistocene period, none of the vast array of parasitic Hymenoptera has made use of these potential hosts. Only a small subfamily of Diptera, the Rhinophorinae (Calliphoridae), among the Insecta, have been found to parasitise them.

With the exception of morphological accounts of some of the larvae and adults, little was previously known about the Rhinophorinae. It was the aim of the present work to find out as much as possible of their general life histories, host relationships and ecology of the British representatives of this group and also to complete the morphological studies of immature stages.

1b) Historical

The earliest known record of a dipterous parasite of woodlice is to be found in a paper by V. Roser (1840) on the Diptera of Württemberg. A "Tachinia atramentaria" is mentioned here as being parasitic on a

woodlouse, possibly Oniscus asellus L. Brauer and Bergenstamm are then mistakenly quoted by Nielsen as stating that Stevenia umbrivaga Fall. was reared from Oniscus asellus by V. Roser. The mistake was copied by later authors forinstance by Baer (1920-1) and Lundbeck (1927) but in fact there is no evidence that this species has ever been reared from woodlice.

In 1903 C.T.Brues recorded in "Entomological News" that he had reared Melanophora roralis(L.) from a Porcellio species (probably P.scaber) in Massachusetts.

The "Entomologist Monthly Magazine" of 1908 contains an account by Donisthorpe of the rearing of two Phyto melanocephala(Meig.) from Oniscus asellus collected in the Isle of Wight. However, the claim of Wainright (1928) that Donisthorpe also reared Ptilocerina ornamentaria Meig. from Oniscus asellus, was refuted by Donisthorpe. W.R.Thompson (1917) published a preliminary note on some dipterous larvae found in woodlice from Haslar, Hants. Unfortunately, the material described as that of Phyto melanocephala was not of this species. Thompson (1920) suggested that material of the first stage larvae belonged to Phyto and later stages to Melanophora roralis, but subsequent rearing of the culture showed that only Styloneuria discrepans(Pand.) and Frauenfeldia rubicosa(Meig.) were present. From the study of the buccopharyngeal armature and skin fragments obtained of the first stage larva, Thompson considered that there was a similarity to Sarcophaga and Bigonichaeta, but also that the larvae of the woodlouse parasites are sufficiently different from all other known Muscid types, to constitute a unique type.

In 1920 a brief account of the rare Cyrillia augustifrons Rond., whichThompson had reared from some French Metophonorthus pruinosus, was published. W.R.Thompson (1934) published a long account (some 70 pages) in "Parasitology" titled "The Tachinid Parasites of Woodlice". Despite the fact that he states that "The study of these parasites has proved quite exceptionally difficult and laborious", Thompson has provided an account and figures which make it possible,with some experience to, identify almost any larva found parasitising a British woodlouse.

Detailed morphological descriptions and drawings are given of the three larval stages of Plesina maculata(Fall.) In Melanophora roralis, Styloneuria discrepans, Frauenfeldia rufiçosa and Cyrillia augustifrons, first stage larvae are described only from moulted skin fragments and buccopharyngeal armature, but second and third stages of these species are also described. Further, the first stage larva of one, and the first stage skin and second stage buccopharyngeal armature of another undetermined species (Species A and B) are also described.

Thompson was unable to get eggs from any adult parasites except from a single Plesina. The resulting larvae of these eggs died and were desiccated before they were seen by him. No first stage larva was seen alive outside its host, and entry into the host was not observed.

From dissections of females from the field, Thompson found no egg development within the ovary and correctly concluded that the eggs are deposited in an undeveloped state.

A summary of 1737 dissections is included in the work and mention is made of W.H.Thorpe's experiment using the biological indicator method with Polytoma cultures to show that cutaneous respiration occurs in the second stage larvae of Plesina maculata. The effect of parasitism on the host was found to be in degeneration of the ovaries and in suppression of oostegite formation.

Since Thompson's papers, little work on the Rhinophorinae has been published. Descriptions of new species of adult parasites are the only additions to be found in the literature since 1934. Thus in 1934 Villeneuve described a new species - Stevenia inops from Palestine, and in 1941 Styloneuria atrior from Morocco, and in 1953 Plesina fascipennis and P. clarripennis were described by Mensil.

2: GENERAL METHODS

2a) Collection of material

All the material used for this work was collected from the field or reared in the laboratory.

Woodlice were collected from diverse habitats and in great numbers so as to obtain as many different parasite species as possible. A large pooter which could hold several hundred woodlice was used. This has a glass entrance tube (internal diameter 1 cm) which accommodates almost all individuals encountered and has a terminal short rubber tube which aids the extraction of woodlice from cracks.

Large populations on the underside of stones or wood were brushed or knocked into collecting boxes or trays. From living trees woodlice were obtained by sounding the bark for hollows and then prizing them off with a screw-driver while a tray was held underneath.

Small airtight rectangular collecting tins (3·8 × 7·6 × 6·3 cm) (1.5 x 3 x 2.5 inches) were used. As many as 300 - 400 woodlice can be packed into these, and, given fresh grass as padding, will survive for a week or more. Some adult parasites of several species were collected from the field for breeding, although most were reared from the woodlouse cultures. Unless great care was taken during capture, internal damage often resulted and the flies died soon afterwards. Rhinophora and Styloneuria of both sexes were found commonly on flower heads of Umbelliferae and Compositae. With the former species it was usually found sufficient to place a large glass tube over the top of the specimens but Styloneuria was best collected with a very light dipterist's hand net.

Melanophora was taken on white walls or rocks in the sunlight and once again least damage resulted when a glass tube was placed

over the specimen. Stevenia males were occasionally found on Umbelliferous flower heads in one locality in Kent and were taken by net, but females were only seen while exhibiting oviposition behaviour, on logs inhabited by Porcellio rathkei. The female Stevenia were too agile to be taken directly with a tube and when a net is placed over them then, instead of flying up as will most species, they run out of the side. The best method of collecting this very rare fly was to put the net in front of it as it ran and then to blow it in!!

2b) Culturing of Woodlice

Most cultures were kept in a constant temperature of 25°C. This is about the optimum temperature for the production of adult parasites.

Isolated specimens were kept in glass petri-dishes (3.5 inches) in diameter, the floor of the petri-dish being lined with damp blotting paper. A little carrot was included as food. Perspex petri-dishes were found to be unsuitable since evaporation took place too rapidly. Flat transparent perspex boxes (7 x 4.5 x 1.5 inches) with airtight internally fitting lids were found to be ideal cages for large cultures and up to 200 specimens were kept in each box. The floors of the boxes were lined with several thicknesses of slightly damp blotting paper which produced almost 100% relative humidity without causing condensation. Pieces of bark were included to supply extra surface area, thus reducing cannibalism. The bark also helped to absorb excess moisture and provided the woodlice with an extra source of food. Carrot was the main source of food. This has been recommended by Heeley (1941) who reared woodlice through several generations on this diet.

At 25°C and 100 per cent relative humidity, carrot rapidly putrefies and a very high mortality rate results amongst the woodlice. To avoid this, only clean carrot freed from all decaying parts was used and as little of its surface area as possible was left in contact with the damp blotting paper.

As a result, conditions usually remained satisfactory for about one week, and then woodlice were transferred to cleaned and sterilised containers with fresh blotting paper and carrot.

Woodlice killed by third stage larval parasites were removed after daily inspection of cultures and isolated in (5 x 2.5 cm) (2 x 1 inch) tubes half filled with sterilized peat, moistened slightly with a 2 per cent solution of Nipagin. If puparia within woodlice were not maintained at a high humidity, considerable mortality resulted.

2c. Culturing Adult Parasites

Flies were kept in cylindrical cages of cellulose acetate and were (8.8 cm 3.5 inches) in diameter and (15.2 – 20.3 cm 6 – 8 inches) high. Perspex petri-dishes filled with damp plaster-of-paris were used as bases.

The cages had removable lids of petri-dishes with perforations for aeration and fitted with inverted (1 cm $\frac{3}{8}$ inch) tubes containing sugar solution and cotton wool wicks. The tubes were held in place with short rubber tube collars.

Flies were introduced into and removed from the cages by means of (2.5 cm 1 inch) diameter side apertures normally blocked by corks. It was also possible to introduce small pieces of oviposition material through these apertures.

In most cases the only food solution necessary was 5%

sucrose with 0.2% Nipagin as a preservative, but for some experiments marmite solution was introduced into feeders and small pieces of meat were hung in the cages.

3d) Methods of artificial infection of hosts.

a) Phyto, Melanophora, Styloneuria and Plesina

Only the larvae of Phyto melanocephala will successfully and indiscriminately enter their host (Armidillidium vulgare) irrespective of its condition. As to the other species of parasites, even when every individual of the host in culture (Porcellio scaber) is supplied with one or more first stage larvae, the resulting parasitism rarely exceeds five per cent.

There are two reasons for this:- A) the host is only vulnerable to attack at a certain stage of its moulting cycle and B) newly moulted hosts are highly cannibalised by non-moulting woodlice. Melanophora can only enter hosts within two or three days after their ecdysis. Suitable hosts can readily be selected by testing all individuals for softness by gently pressing them between the first finger and thumb. Great care is required since woodlice are very vulnerable to injury when in this state. From one fifth to one third of the individuals of a culture are usually suitable (at 25°C) and when these are confined together little or no cannibalism will occur as their mouth parts are still too soft.

When each of these suitable hosts is supplied with a single larva, 80% parasitism often results.

Suitable hosts for parasitism by Styloneuria can be selected by examining the anterior sclerites. White calcareous deposits are

present in large patches a few days preceeding moulting (the ideal time for infection with these species of parasites). About one third of a normal culture is usually suitable and the resulting parasitism varies between 30% and 50% after introduction of 1 - 2 first stage larvae onto each host. Ideal conditions for infection by Plesina larvae have not yet been found.

Melanophora larvae are best introduced on the underside of a host, with a sharpened match stick, but while the other species can occasionally be induced to attach to a moistened match, it is simpler to bring the host close to the larva which then attaches itself.

b) Frauenfeldia, Stevenia and Rhinophora larvae being comparatively large are quite easily "handled" using a moistened, sharpened match stick. The point can be inserted beneath the rearing end of the larva which can then be lifted off the substrate, by virtue of surface tension, and gently wiped off onto the sternites of the host which is held between fore finger and thumb of the other hand.

In the case of Stevenia larvae, any individual of P.rathkei over 8 - 9 mm in length is suitable for infection. For Frauenfeldia hosts of about 7 - 10 mm are selected and for Rhinophora those of 4 - 6 mm. (The larvae find difficulty in penetrating larger specimens but within the size ranges indicated, any individuals are suitable, independant of the state of their moulting cycle.

Handling the tiny woodlice necessary for infection by Rhinophora is difficult, so these are best temporarily stuck by the dorsum to a finger made sticky with starch paste, and then turned over!

3. MORPHOLOGY

3a) Preparation of first stage Larvae

Killing

Peterson (1948) in "Larvae of Insects" recommends several methods of killing insect larvae and three of his main mixtures were tried with varying success on the rather small, delicate first stage larvae of Rhinophorinae. Both the mixtures containing xylene (X.A. and X.A.A.D) produced excessive transparency and some distortion, but the K.A.A.D mixture (Kerosene 1 part, 95% ethyl alcohol 10 parts, glacial acetic acid - 2 parts, dioxane - 1 part), was used with some measure of success. When the amount of Kerosene was reduced by half to avoid explosion of the larvae, (Brauenfeldia, Rhinophora and Stevenia) were killed and fixed in an undistorted lifelike condition. With the other more delicate species, too great a proportion of distorted specimens resulted. It was found better to pour boiling water onto the larva while it was extended in a watch glass.

Preserving

Larvae killed in K.A.A.D. were left in it for 2 - 3 hours, washed in 90% ethyl alcohol and then transferred to fresh 90% ethyl alcohol in which they keep indefinitely.

Larvae killed in boiling water were slowly taken through the alcohols to Gisin's fixative (H.Gisin 1947) 90% ethyl alcohol, 750 ml., ether 250 ml., glacial acetic acid 30 ml., formalin 3 ml., and left for two days before transferring to 90% alcohol.

Staining

Specimens of Frauenfeldia and Stevenia are sufficiently pigmented to make staining unnecessary.

The cuticle of other species did not readily take up acid and Basic Fuschin, Orange G, Borax Carmine and Lignin pink, but chloramol black proved very successful. The best results were achieved with this stain incorporated into the mountant.

Mounting

In most mountants, larvae were found to be badly distorted. Out of Balsam, Euparal, Hoyers, Glycerine, Terpiniol and Polyvinyl lacto phenol, only the latter was at all satisfactory for whole mounts. Gurrs P.V.L.P. 10 parts plus 1 part glycerol was best. Only Stevenia and Frauenfeldia larvae could be transferred directly without immediate distortion. With other species, 10 per cent P.V.L.P. in 70% alcohol was added drop by drop to the specimen in 70% alcohol, with continual mixing. The resulting solution was then allowed to evaporate down for

several days and even then some specimens became distorted.

For examination of the posterior ends of most species, it was found most satisfactory to cut these off before adding them to the diluted mountant. This helped to avoid distortion.

Most satisfactory preparations were obtained with cavity slides but as these could not be examined under oil immersion, normal slides with supported coverslips also had to be used.

Even using this method, distortion results after a few weeks and so fresh preparations had to be made for each examination.

3b) Preparation of Mouthparts

In order to ensure the correct identity of the material used, pure cultures of each species were obtained by infecting unparasitised woodlice with first stage larvae bred from the easily identified adult parasites.

The buccopharyngeal armature of first and second stage larvae was either obtained from their moulted skins or by warming the anterior end of preserved larvae in sodium hydroxide for a few minutes. The best preparations of third stage larval mouthparts were obtained from the larva before pupation since those removed from within the puparium were usually distorted.

All mouthparts were mounted in Balsam with unsupported coverslips and considerable care was exercised to ensure that they were perfectly flat and correctly orientated before drawing. It was necessary

to ensure that the right half exactly covered the left and that all portions of the armature were in one plane. As it was rarely possible to ensure that preparations dried in exactly the correct position, they were drawn whilst the Balsam was still a little fluid, since this enabled manipulation.

3c) Description of Eggs

Thompson (1934) obtained a single batch of Plesina eggs. None of these was drawn and the only description given by him was that the eggs were elongate, fusiform and thin-shelled. Thompson also dissected out some eggs from Frauenfeldia. These he described as - 'elongate, spindle-shaped in form, with a rather thin, lightly sculptured, transparent, colourless cuticle'.

During the present work, the eggs of the seven species of Rhinophorinae which parasitise woodlice have been examined and drawn. (see figs. 1 - 7).

General description

Eggs basically of the normal Calliphorid type. Fusiform, thin, soft shelled, pearly white, either with hexagonal reticulations or with longitudinal ridges; median area bordered by hatching lines with wing-like extensions in some species.

i) Styloneuria (see fig. 4)

length: 0.5 mm

maximum breadth: 0.175 mm

maximum depth: 0.16 mm

Almost symmetrically oval viewed dorsally, boat-shaped viewed laterally. Posterior end rounded; anterior narrow, truncate bearing micropyle. Intermediate area: less than half the length of egg; few obvious reticulations, very narrow anteriorly but broadening a little posteriorly; bordered laterally by a low vertical flange of ~~cuticle~~ chorion which is a little deeper posteriorly. Longitudinal ridges with occasional branches over all but intermediate region of egg; transverse ridges not apparent.

ii) Phyto (see fig. 3)

length: 0.5 mm

maximum breadth: 0.17 mm

maximum depth: 0.17 mm

Very similar to eggs of Styloneuria although rather more boat-shaped than oval when viewed dorsally. Intermediate area only just over one-third of total egg length; rather broader than that of Styloneuria and boat shaped with several obvious reticulations; bordered laterally by a low flange of uniform depth. Longitudinal ridges with occasional branches over all but intermediate region of egg, but weak cross ridges also just visible.

iii) Melanophora

length: 0.46 mm

maximum breadth: 0.17 mm (including wings)

maximum depth: 0.15 mm

Greatly tapered anteriorly and posteriorly as viewed both laterally and dorsally. Posterior end pointed. Deepest a little more than one-third from posterior end.

Intermediate area: occupies full length of egg; with elongate, hexagonal reticulations throughout; bordered by conspicuous, winged, laterally directed flanges for full length of egg.

Wings: broadest medially and decrease in breadth anteriorly and posteriorly; with only minute reticulations. Ventral and lateral surface of egg with broad hexagonal reticulations.

iv) Plesina (see fig. 2)

length: 0.46 mm

maximum breadth: 0.14 mm

maximum depth: 0.125 mm

Dorsally appears lanceolate; posteriorly rounded but more tapered than anterior which is also rounded. Deepest just over one-third of the way from the anterior end but broadest about halfway along. Intermediate area: occupies full length of egg; anterior eigth with three pairs of longitudinal ridges but rest of area with broad hexagonal reticulations; bordered as in Melanophora by wing-like flanges for whole length of egg. Flanges directed latero-vertically; with only minute reticulations. Lateral and ventral surface of egg with hexagonal reticulations

Fig. 1 - Dorsal and lateral views of the egg of *Plesina maculata*

Fig. 2 - Dorsal and lateral views of the egg of *Melanophora roralis*

Fig. 3 - Dorsal and lateral views of the egg of *Phyto melanocephala*

Fig. 4 - Dorsal and lateral views of the egg of *Styloneuria discrepans*

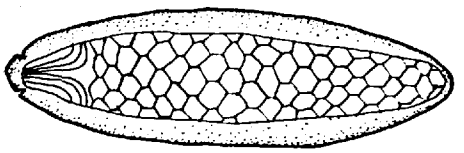
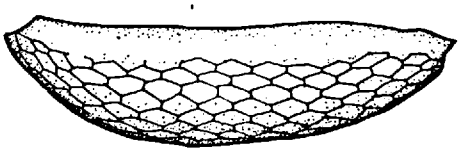


Fig. 1



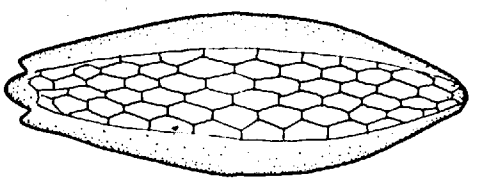
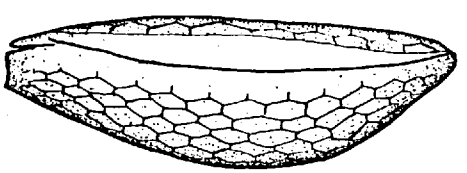


Fig. 2



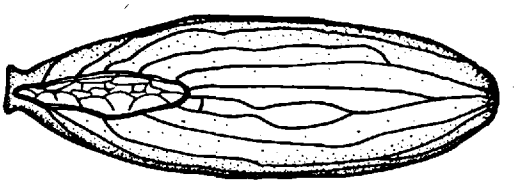
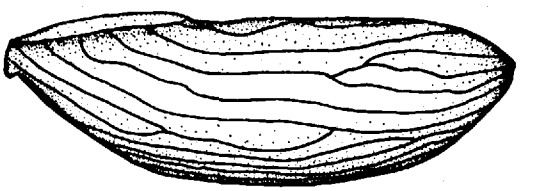


Fig. 3



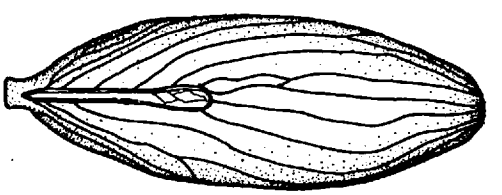
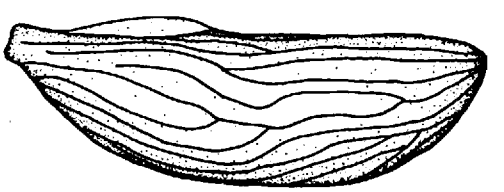


Fig. 4



v) Frauenfeldia (see fig 7)

length: 0.91 mm

maximum breadth: 0.22 mm

maximum depth: 0.20 mm

Boat shaped. Posterior end tapered but rounded. Anterior end with micropyle truncate. Intermediate area: extends for sixth-sevenths of total egg length; narrow; with elongate hexagonal reticulations; anterior third of area with three rows of small micropyles numbering eighteen in all; bordered by low flange of irregular depth. Remainder of egg surface with hexagonal reticulations.

vi) Rhinophora (see fig. 5)

length: 0.64 mm

maximum breadth: 0.17 mm

maximum depth: 0.19 mm

Boat shaped; pointed posteriorly when viewed laterally; dorsal surface dipping sharply anteriorly one-third of the distance from posterior end; egg with much softer chorion than other species; slightly opaque and iridiscent. Intermediate area: extending for full length of egg; parallel sided and about one-third width of egg for anterior two-thirds narrowed posteriorly; small elongate hexagonal reticulations over most of area; anterior two-thirds bordered by a very low flange, but flange almost non-existent for remaining third. Rest of egg hexagonally reticulated, but transverse ridges of reticulations only feeble.

Fig. 5 – Dorsal and lateral views of the egg of *Rhinophora lepida*

Fig. 6 – Dorsal and lateral views of the egg of *Stevenia atramentaria*

Fig. 7 – Dorsal and lateral views of the egg of *Frauenfeldia rubricosa*

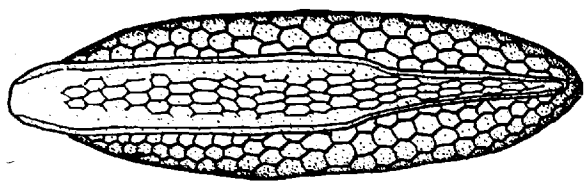


Fig. 5

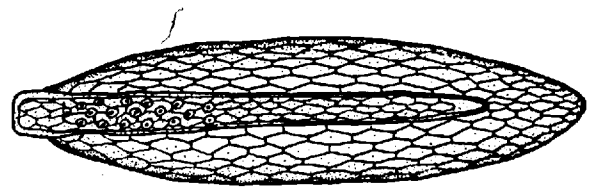
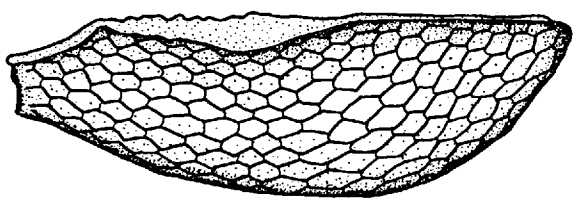


Fig. 7



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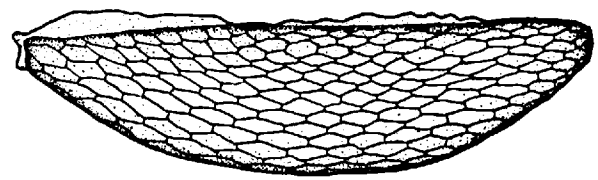

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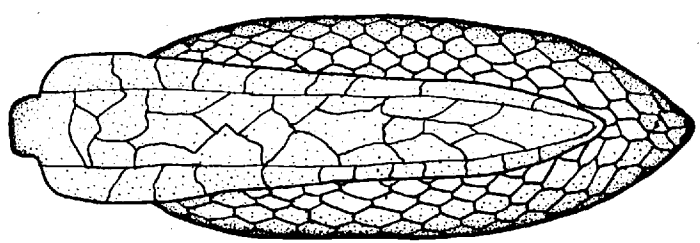
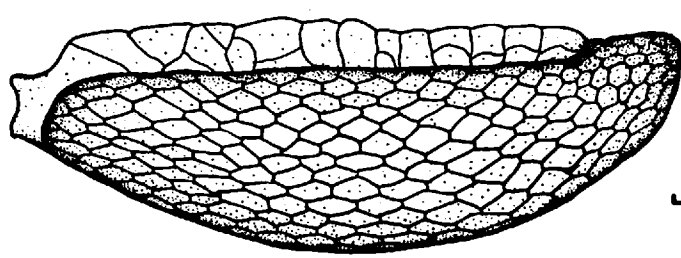


Fig. 6



·2 mm

vii) Stevenia (see fig. 6)

length:	0.7 mm
maximum breadth:	0.22 mm
maximum depth:	0.22 mm

Broadest and deepest halfway along the egg. Posterior end tapers to a point but the egg tapers only a little towards the anterior and then broadens dorsally. Intermediate area: broadest anteriorly; with large irregular reticulations; bordered laterally by a fairly deep latero-vertically directed flange. Flange with large irregular reticulations. Remainder of egg surface with hexagonal reticulations.

3d) Description of first stage larvae

i) Styloneuria

Although the first stage skin rarely remains attached to the second stage larva (because of the mode of entry peculiar to this species), Thompson (1934) did find it on several occasions.

From these remains Thompson was able to make an accurate drawing and description of the buccopharyngeal armature and antennae, but apart from stating that he could find no scales or spines on the fragments of cuticle, no further description of the first stage larva could be made by him. In fact, as can be seen from figs. 9 - 11, the cuticle is not bare but covered with spinose papillae similar to those described by Thompson (1934) in Plesina first stage larvae.

Description (see figs. 8 - 11)

length: 0.77 mm

maximum breadth: 0.14 mm

Elongate spindle shaped in form. Widest and deepest at fifth and sixth segment, tapering gradually anteriorly and posteriorly. Slightly more convex ventrally than dorsally. Unpigmented; pearly white in appearance.

Head

Partially telescopic into first thoracic segment. Dorsally appears semi-circular, but laterally trilobed. Ventrally mouth surrounded by lobed hood. No scales or nodules can be seen on the head cuticle.

Antennae:

length: 0.02 mm., cylindrical, rounded apically; directed antero-dorsally at about 45° to central line; arise from short cylindrical base.

Thorax

Segments conspicuously constricted from each other; of similar length; appear barrel shaped from dorsal and ventral aspects; increase in depth from anterior to posterior end so that in lateral aspect thorax appears sub-triangular.

First thoracic segment: antero-dorsal margin gently rounded but antero-lateral margin slightly concave and at 45° to the transverse. Segment dorsally appears almost parallel sided with the anterior third constricted a little. One pair of mid dorsal and one pair of mid ventral short setae. These arise from inconspicuous cylindrical bases.

Anterior half of segment with feeble rounded scales but rest of segment bare. No spinose papillae present.

Second and third thoracic segments: similar; each with two pairs of dorsal, 3 pairs of lateral and one pair of ventral, short inconspicuous perpendicularly directed setae arising from a short cylindrical base in the centre of a spinose papilla. On third segment setae are around the middle of the segment but on the second, setae arise just posterior to the anterior third of segment.

Anterior third of second thoracic segment covered with about six rows of feeble scales but rest of segment covered with small, spinose sub hemi-spherical protuberances similar to those described by Thompson (1934) in Plesina except that there is no larger central spine. The spines of each protuberance are numerous, evenly distributed, short stiff and vertical. Ventrally on both second and third thoracic segments, the cuticular armature is more scale-like but is nevertheless spinose. Between second and third thoracic segments cuticle bare for distance of about one quarter of a segment. Third thoracic segment with only two rows of scales anteriorly. Rest of segment clothed with typical papillae except for posterior eighth which is bare.

Abdomen

Laterally distinctly constricted from thorax but dorsally and ventrally fairly flush. Segmentation of first five abdominal segments obscure, but last three segments are conspicuously constricted from each other.

Apart from the last segment all abdominal segments appear to have two pairs of dorsal, one pair dorso-lateral, one pair ventro-lateral, and one pair of ventral setae (i.e. one pair less than thoracic segments two and three). These setae are very short (about half length of those on the thorax) and difficult to locate on the first five

abdominal segments although more conspicuous on the sixth and seventh segments.

The surface of each abdominal segment is covered with the type of spinose papillae already described. Ventrally these tend to be flatter than elsewhere but they are of similar structure. While there is a very narrow, intersegmental membrane between the first, second, and third abdominal segments, the boundary between third, fourth and fifth segments is almost indistinguishable. On either side of the conspicuous intersegmental membrane between the sixth, seventh and eighth abdominal segments, the spinose papillae are contracted longitudinally and usually bear only a single fringe of spines (see figs. 9 - 11).

Eighth abdominal segment. (see figs. 9-11). As in Plesina, Melanophora and Phyto, this is very highly modified to seat the rest of the larval body at right angles to the substrate and bears the posterior spiracles.

Ventrally and dorsally, the segment appears sub-triangular, narrowing posteriorly. While the first half of the segment lies almost in line with other segments, the posterior half is directed ventrally.

Dorsally and laterally, the anterior half of the segment has a similar cuticular armature to other segments. It bears dorsally a pair of strong acute divergent spines about one quarter of the way down the total segment. Just posterior to these are a pair of similar dorso-lateral, a pair of lateral and behind these a pair of ventro-lateral, strong acute spines. Ventrally on the anterior third of the segment there are flattened, rather scale-like spinose papillae.

The spiracles are cylindrical, rounded terminally, divergent and arising in the middle and on either side of the median

longitudinal third of the last segment. They are directed latero-posteriorly at about 45° to the midline of the larva.

Behind the spiracles, the cuticular armature is unlike that found elsewhere on the larva. Transverse rows of broken ridge bearing fringes of short spines on their crests, cover the surface of the segment posterior to the spiracles and anterior to the complex of lobes which is present at the extreme posterior end. Just behind the spiracles is a pair of small lateral setae, and a larger pair of acute dorsal divergent setae, the same distance apart as the spiracles lies half way between the spiracles and the end of the segment.

Ventrally, the longitudinal middle third of the segment is depressed from one third to two thirds of the way down the segment to form a U-shaped hollow. The rim of this hollow is ridged laterally and within it lie three further pairs of longitudinal bar-like ridges.

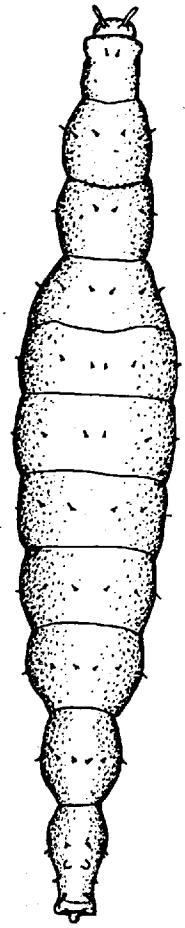
The extreme posterior end of the segment is composed of a number of unarmoured sac like lobes. Dorsally a single main swollen lobe fans out like a fish tail. This is posteriorly sub-divided into a small median semi-circular lobe with a pair of larger lobes on each side. From just beneath the small median lobe there arises a long mobile tongue-like structure which is usually extended ventrally. At the extreme end of the tongue there appear to be three small pores. Laterally there is a pair of smaller lobes and ventrally a single lobe. These lobes surround a central space in the middle of the posterior face. They appear to be sacs of cuticle probably distended with haemolymph and appear capable of considerable distortion when the animal is alive. Together with a copious secretion of muco-polysaccharride or muco-

Fig. 8 - Dorsal and lateral views of the first stage larva of Styloneuria discrepans

Fig. 9 - Lateral view of eighth abdominal segment of the first stage larva of Styloneuria discrepans

Fig. 10 - Ventral view of eighth abdominal segment of the first stage larva of Styloneuria discrepans

Fig. 11 - Dorsal view of eighth abdominal segment of the first stage larva of Styloneuria discrepans



·2 mm

Fig. 8

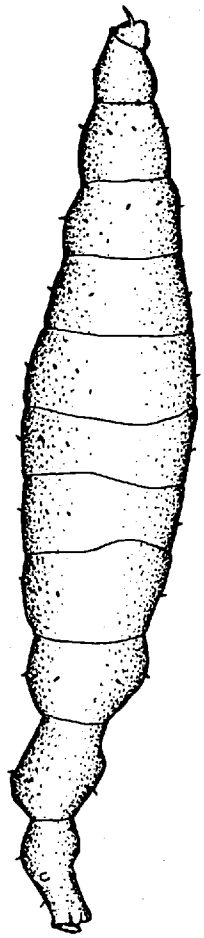
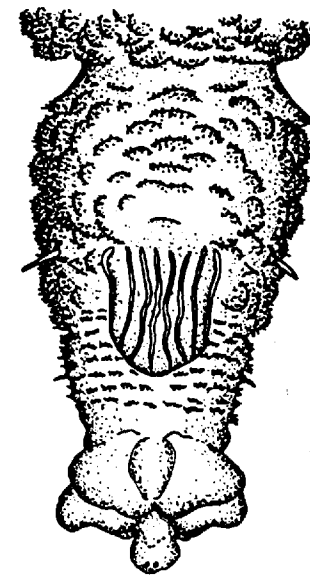
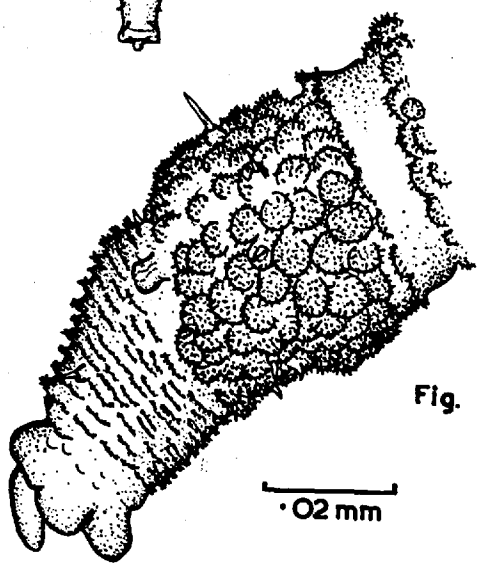


Fig. 10





·02 mm

Fig. 9

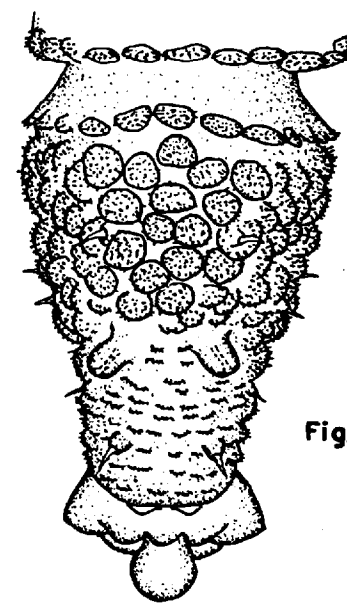


Fig. 11

protein they form an efficient adhesive mechanism enabling the larva to stand and rotate on its posterior end.

ii) Melanophora

Thompson (1934) described the buccophoryngeal armature of this species in some detail although very little material was available to him. He included a very brief and incomplete description of the larval skin but the complete larva was undescribed until now.

Description (see figs. 12 - 15)

length; 0.44 mm

maximum breadth: 0.074 mm (without setae)

Elongate spindle shaped in form. Widest and deepest at sixth segment, tapering gradually anteriorly and posteriorly. Almost straight dorsally; convex ventrally.

Head

Partially telescoped into first thoracic segment with which it forms a triangle. Dorsally appears almost parallel sided before bulging out slightly to meet first thoracic segment. Directed at 45° antero-dorsally; slightly bilobed antero-laterally. Ventrally mouth surrounded by a pear shaped hood which occupies anterior half of ventral surface. Antero-laterally, within the hood, there is a pair of spiny pads. Cuticle of head unarmoured.

Antennae: length: 0.01 mm, about five times as long as wide. Divergent: directed antero-laterally, gradually tapering. Inserted on short, broad ill-defined base.

Thorax

Thoracic segments not sharply divided from each other, bare of setae. First and second segments appear trapezium shaped

dorsally; third is dorsally sub-rectangular.

First thoracic segment: dorsally bare except for second quarter which bears irregularly shaped, tight fitting nodules, armed centrally with acute, long, pyramidal spikes directed posteriorly at 45°. Laterally bare for anterior third and posterior quarter of segment; rest covered with nodules similar to those on dorsum but with spikes of nodules at right angles. Ventrally, anterior half of first thoracic segment very feebly and sparsely scaled with round scales bearing single, weak spines directed posteriorly. Posterior ventral half of segment bare. Second and third thoracic segments: very similar to each other; dorsally and laterally completely covered with nodules except for narrow bare margin between segments; ventrally middle two-thirds scaled. Nodules similar to those of first thoracic.

Dorsally on second and third thoracic segments are a single pair of almost cylindrical processes of similar length to spikes of nodules, but not tapering terminally.

Abdomen

Laterally somewhat constricted from thorax but dorsally and ventrally flush. Pronounced lateral constrictions between abdominal segments, but ventrally and dorsally first five segments of abdomen flush. Ventrally, segments swell out to form belly; deepest at third and fourth segments, dorsally almost straight. Segments six to eight sharply constricted.

First seven segments each with a pair of very long finely tapering setae directed ventro-laterally and almost at right angles to the longitudinal axis of the larva; arise from inconspicuous base about two thirds way down lateral surface.

Setae of the first segment longest (0.056 mm); other setae about two thirds this length, decreasing a little in length towards the posterior end of the larva.

Median dorsal pair of small conical papillae present on abdominal segments one to seven on either side of middle third except sixth and seventh where separated by middle fifth.

Cuticular Armature: ventrally mainly confluent, forming weak transverse ridges with tiny scales. These become more and more nodule-like laterally. Segments one to four with a single median ventral transverse row and segments five to six with three median ventral transverse rows of feeble nodules. Seventh segment with many rows or nodules ventrally. Dorsally, first abdominal segment with five rows of nodules with small spines, but rest of segment with bare scales. Segments two to five with no scales but ridge like folds similar to ventral surface. Sixth segment with posterior half having scales with small spines dorsally. Seventh seg ment with irregular, usually spineless scales, as in eighth.

Laterally abdomen covered with irregular nodule like scales as on thorax, but each with single short papilla instead of sharp spines.

Eighth Abdominal Segment: (see figs. 13 - 15) Club shaped; greatly modified; long lateral setae present as on other segments, but dorsal papillae replaced by a pair of dorsal setae. Both are directed laterally, posteriorly and dorsally. Another pair of short dorso-lateral setae occurs two thirds way down the segment.

Spiracles: situated just above these setae; dorsal, cylindrical and divergent, arise on either side of middle longitudinal third. Between spiracles a single median posteriorly directed setae arises from

Fig. 12 - Dorsal and lateral view of the first stage larva of Melanophora roralis.

Fig. 13 - Lateral view of eighth abdominal segment of the first stage larva of Melanophora roralis

Fig. 14 - Ventral view of eighth abdominal segment of the first stage larva of Melanophora roralis

Fig. 15 - Dorsal view of eighth abdominal segment of the first stage larva of Melanophora roralis.

Fig. 12

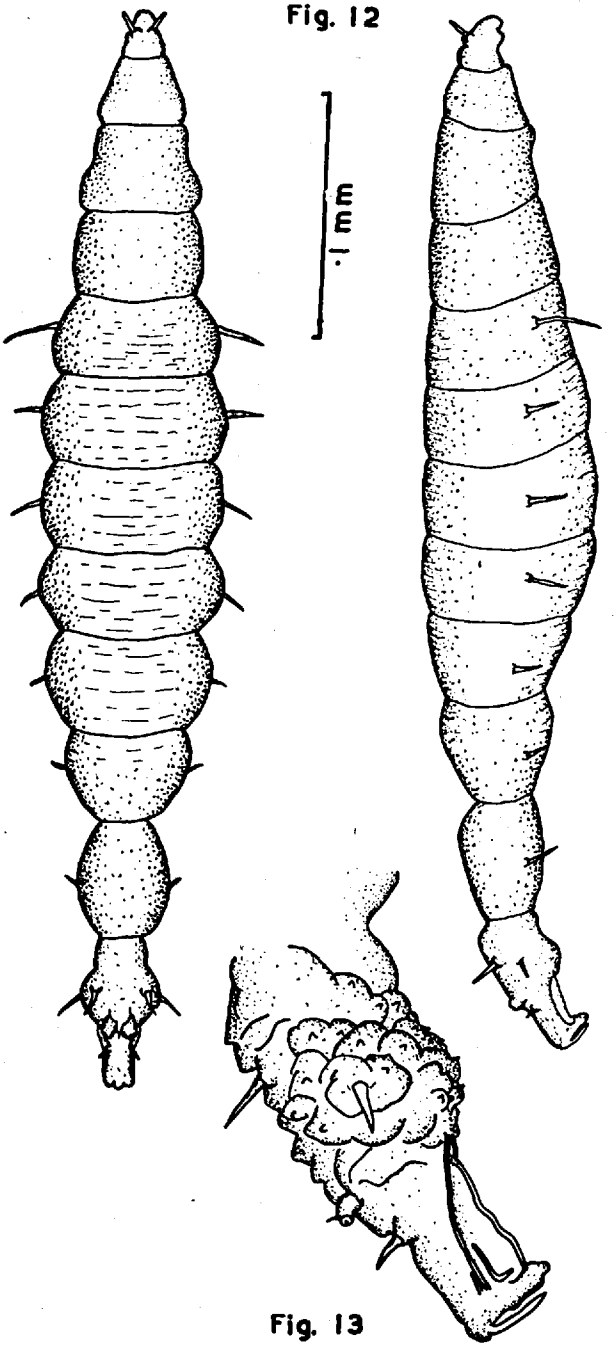
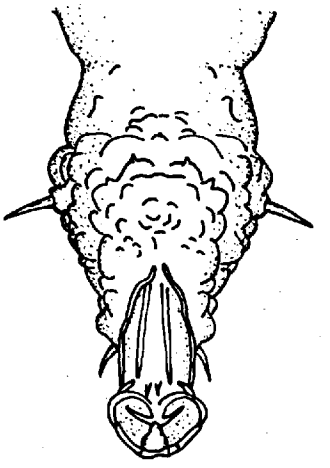


Fig. 13

Fig. 14



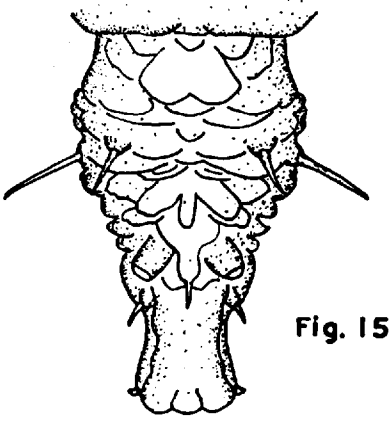


Fig. 15

·02 mm

posterior end of large Y shaped scale. Behind spiracles dorsal surface devoid of scales while anteriorly scales without spines are present.

Laterally and ventrally segment also scaled for anterior two thirds but these scales bear short papillae.

Pair of ridges lying on outer edge of dorsal surface extend from just behind spiracles almost to end of segment. Ventrally, a pair of strong ridges arise near middle line of ventral surface and diverge to occupy the whole of ventral surface and half of lateral surface posteriorly. Inside and just posterior to these arise a pair of almost parallel ridges which lie on either side of longitudinal middle third. Posteriorly inner ridges pointed and free, and lie outside pair of very short pointed parallel ridges. Posterior face of segment surrounded by two confluent ridges which turn inwards ventrally and bear a T shaped extension on posterior face. A tongue-like process arises dorsally and is directed ventrally.

iii) Plesina

Described by Thompson (1934) from dried up specimens. Thompson's general description of the first stage larval morphology and of the buccopharyngeal armature of this stage is accurate although probably because of the distorted condition of his specimens, he was unable to give a full description of the eighth abdominal segment.

Description (see figs. 16 - 19)

length: 0.6 mm (not fully extended.)

maximum breadth: 0.095 mm.

Body tapers anteriorly and posteriorly from fifth and sixth segments; considerably less spindle shaped than shown by Thompson's figure where the larva was obviously greatly flattened by coverslip.

Segmentation distinct; rudimentary head, three thoracic, eight abdominal segments. Cuticular armature: like that of Stylonuria except that each convex protuberance bears a central well-developed spine as well as a covering of shorter spines; not pigmented as described by Thompson but transluscent.

Thoracic segments without setae.

First seven abdominal segments each with one pair of long, lateral setae directed latero-ventrally.

Eighth Abdominal Segment: (see figs. 17 - 19) greatly modified; divisible into a sub-spherical anterior half having a cuticular armature similar to that of other abdominal segments, and a cylindrical posterior half with cuticular armature differing from that of other segments.

Anterior half of segment with one pair of lateral latero-posteriorly directed setae, and one pair of dorsal, dorso-laterally directed setae.

Posterior half of segment with dorsal spiracles, anteriorly spiracles arise on either side of middle longitudinal third; cylindrical; divergent and with terminal dorso-lateral acute process. Behind the spiracles there is a pair of dorsal, dorso-latero-posteriorly directed setae. Dorsally and laterally there are a number of short, deep transverse ridges in the cuticle. Ventrally the cuticle is bare of armature, although mid ventrally there is a pair of stout, longitudinal ridges which arise near the anterior margin of the posterior half of the segment. These ridges posteriorly give rise to a pair of longitudinal, terminally acute spines which lie parallel to the underlying cuticle, but just clear of it, and extend up to the terminal lobes of the segment. A similar pair of spines without the accompanying ridges

Fig. 16 - Lateral view of the first stage larva of Plesina maculata

Fig. 17 - Lateral view of eighth abdominal segment of the first stage larva of Plesina maculata

Fig. 18 - Ventral view of eighth abdominal segment of the first stage larva of Plesina maculata

Fig. 19 - Dorsal view of eighth abdominal segment of the first stage larva of Plesina maculata

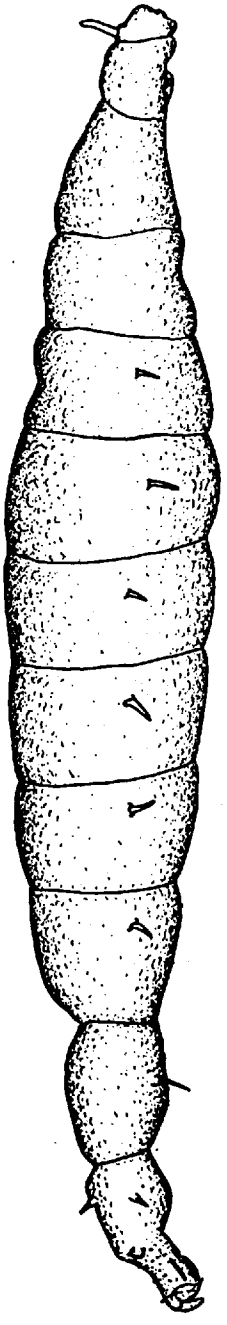


Fig. 16

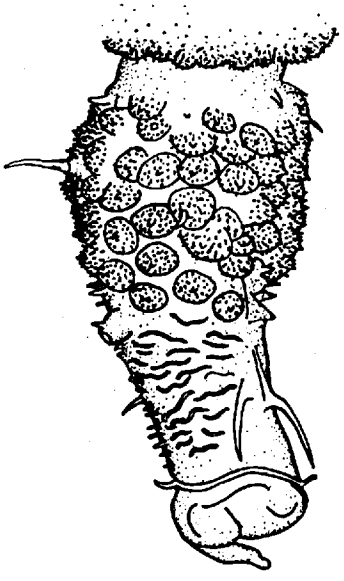


Fig. 17

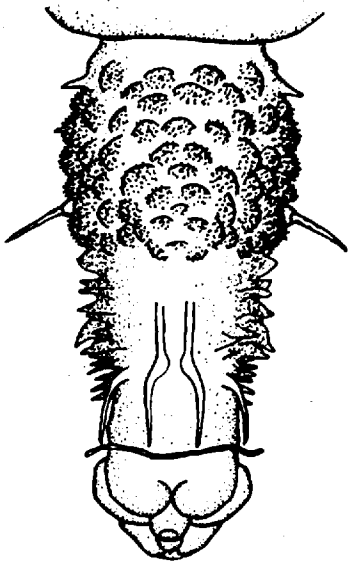


Fig. 18

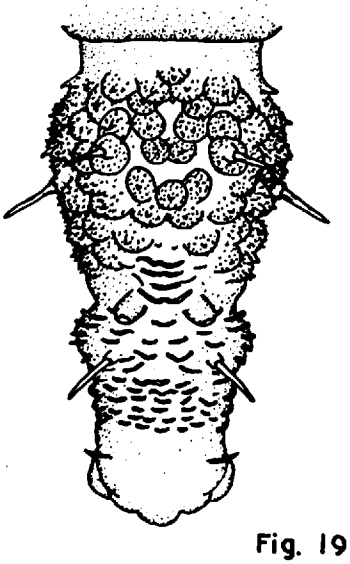


Fig. 19

lie ventro-laterally. Just posterior to the distal ends of these species there is a deep transverse ridge which extends from the ventral surface to terminate dorso-laterally.

Behind this ridge is a complex of lobes. As in Styloneuria, Melanophora and Phyto there is a sub-cylindrical tongue-like lobe which arises dorsally and is directed ventrally.

Above the tongue-like lobe there is a sub-semi-circular shaped flap which is tri-lobed. A pair of ventro-lateral lobes extend inside this and meet above the tongue, while inside these latter lobes there is a ventral bi-lobed structure.

iv) Phyto

Only the buccopharyngeal armature and antennae were previously described from moulted remains (Thompson 1934).

Descriptions: (see figs. 20 - 23)

General

length: 0.83 mm

maximum breadth: 0.13 mm

Larva resembles that of Melanophora, Styloneuria and Plesina. Body spindle shaped; tapers anteriorly and posteriorly from fifth and sixth segments. Cuticle armed with unpigmented, rounded, posteriorly directed, simple scales or dorsally and ventrally on some segments with transverse ridges. Last segment structurally complex and adapted for maintaining the larva in an erect posture.

Head

Simple, unarmoured; cuboidal; directed ventrally; telescopes into first thoracic segment. Dorsally appears truncate; broadest halfway down length. Ventrally mouth surrounded by hood on the inside

of which there are a few indistinct minute spines. Laterally hood appears terminally trilobed.

Antennae: of same length as head (0.024 mm)., narrow; cylindrical; rounded apically dorsally but laterally appearing to taper; slightly divergent; directed anteriorly; each inserted on a short broad base.

Thorax

Dorsally and laterally broadens from anterior to posterior. Segmentation distinct; no setae present.

First segment: laterally appears almost parallel sided although broadening slightly from anterior to posterior. Segment medially with conspicuous lateral bulges. Dorsally cuticle of anterior half and posterior quarter of segment bare; third quarter with three transverse rows of scales fused to form feeble ridges. Ventrally anterior half of segment with five rows of feeble ridges. Laterally rounded scales present for about the middle half of the segment.

Second and third segments: similar to each other although the third segment is a little broader and deeper than the second. Each almost twice the length of the first segment. Dorsally and ventrally with about twelve rows of feeble transverse ridges and only small bare intersegmental regions. Laterally with rounded scales.

Abdomen

First five segments of similar length; fairly flush with each other dorsally and ventrally, but lateral constrictions between segments obvious. Sixth segment considerably broader medially than anteriorly or posteriorly but gradually deepening from posterior to anterior. Seventh segment almost parallel sided in lateral view although broadening towards the middle of the segment; longer than other abdominal

segments. Cuticular armature of the first five segments similar to that of the second and third thoracic segments with weak transverse ridges, each partially broken three or four times, present dorsally and ventrally and with normal scales laterally. Scales encroach dorsally and ventrally on the sixth segment while the seventh segment is fully scaled both dorsally and ventrally as well as laterally. The first five abdominal segments have no setae, but the sixth and seventh segments each have a pair of short latero-ventral setae mounted on conical bases, about half-way down the segment.

Last abdominal segment: (see figs. 20 - 22) The eighth abdominal segment broadens gradually for its anterior half and then sharply narrows down to the spiracles which are two thirds of the way towards the posterior end of the segment. Remainder of the segment appears dorsally and ventrally almost parallel sided and is about half the maximum width of the segment. Laterally the segment presents a more complex shape; it is deepest about one third of the way down and gradually narrows towards the posterior end of the segment.

About halfway down the segment there is a pair of strong, finely tapering, acutely pointed lateral setae, each arising from a short cylindrical base, and directed laterally at right angles to the segment. Just anterior to this pair of lateral setae is another similar pair which are dorsal and lie either side of the mid dorsal half; these are slightly divergent and directed dorsally. There is a further pair of strong, divergent, dorsally and posteriorly directed setae which arise latero-dorsally just behind the spiracles. The spiracles are cylindrical, rounded, divergent, directed latero-dorso-posteriorly, and arise on either side of the dorsal surface about two thirds of the way

down the segment. Each spiracle bears subterminally a pair of short, tapering, anteriorly directed setae. The spiracular openings are not terminal but on the inner face of the spiracular process.

Between the spiracles there is a single large Y-shaped scale with a smaller posteriorly rounded scale on either side posteriorly.

Immediately posterior to these scales there are four pairs of longitudinal slightly inwardly curved, posteriorly acute, strong ridges occupying the whole breadth of the dorsal surface for half the remaining length of the segment. Two rows of small rounded scales behind this ridge precede a single, large, dorsal terminal lobe.

Four pairs of strong longitudinal ridges are also present ventrally, but these are almost three times the length of the dorsal ones and occupy a specialised region of the ventral surface. Just over one third of the way down the segment, the ventral surface curves sharply inwards forming a margin from which three of the pairs of longitudinal ridges arise. At this point about the middle three quarters of the segment is occupied by the unscaled cuticle bearing the ridges which curve slightly inwards for half their length before curving outwards again. Because of this, the middle of the inner pair almost meet medially. The second pair of ridges from the centre, arise a short distance behind the others, but all four pairs terminate the same distance from the posterior end of the segment. Ventrally, at the extreme posterior end of the segment there can be seen a complex of lobes.

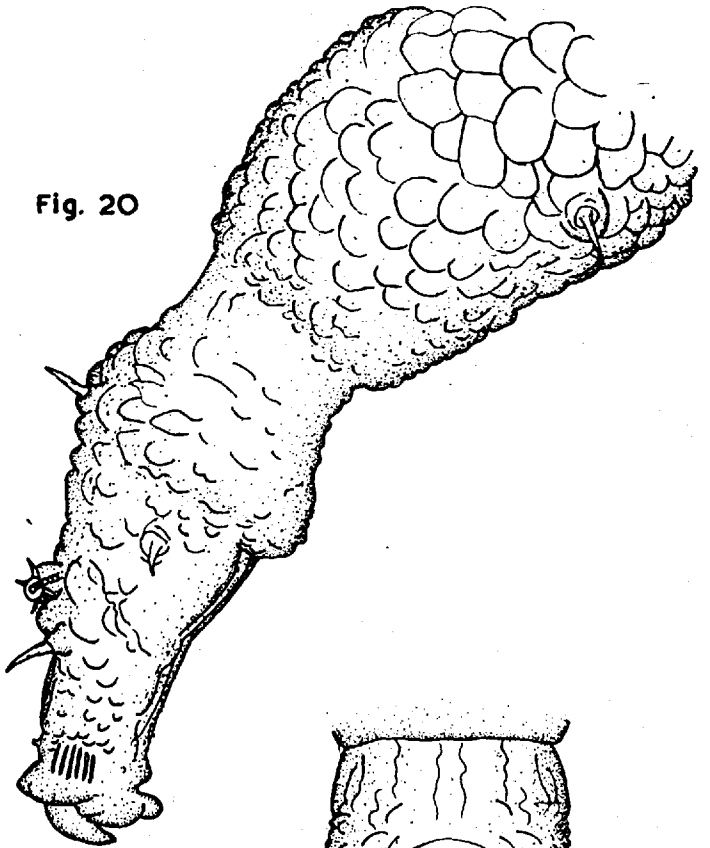
Beneath the dorsal lobe but above the other two pairs of lobes there is a median tongue-like lobe.

Fig. 20 - Lateral view of seventh and eighth abdominal segments of the first stage larva of Phyto melanocephala

Fig. 21 - Dorsal view of eighth abdominal segment of the first stage larva of Phyto melanocephala

Fig. 22 - Ventral view of eighth abdominal segment of the first stage larva of Phyto melanocephala

Fig. 20



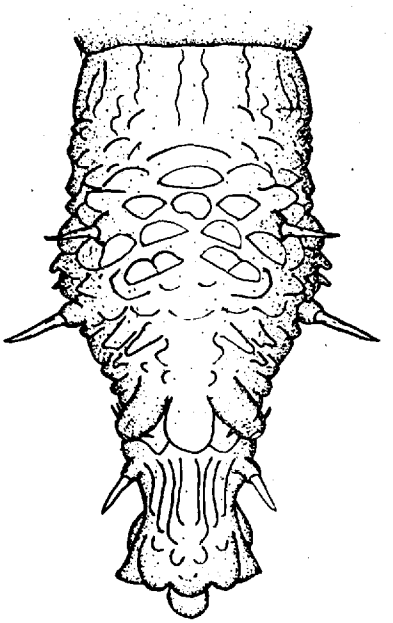


Fig. 21

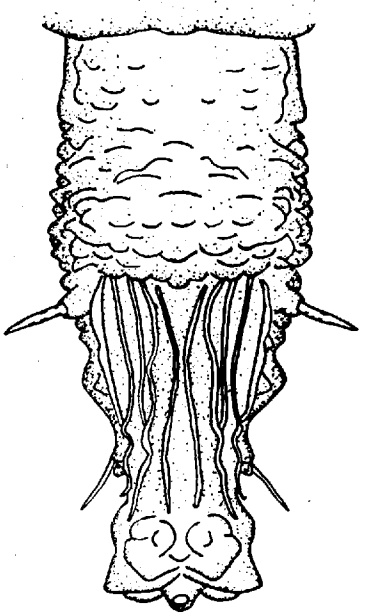


Fig. 22

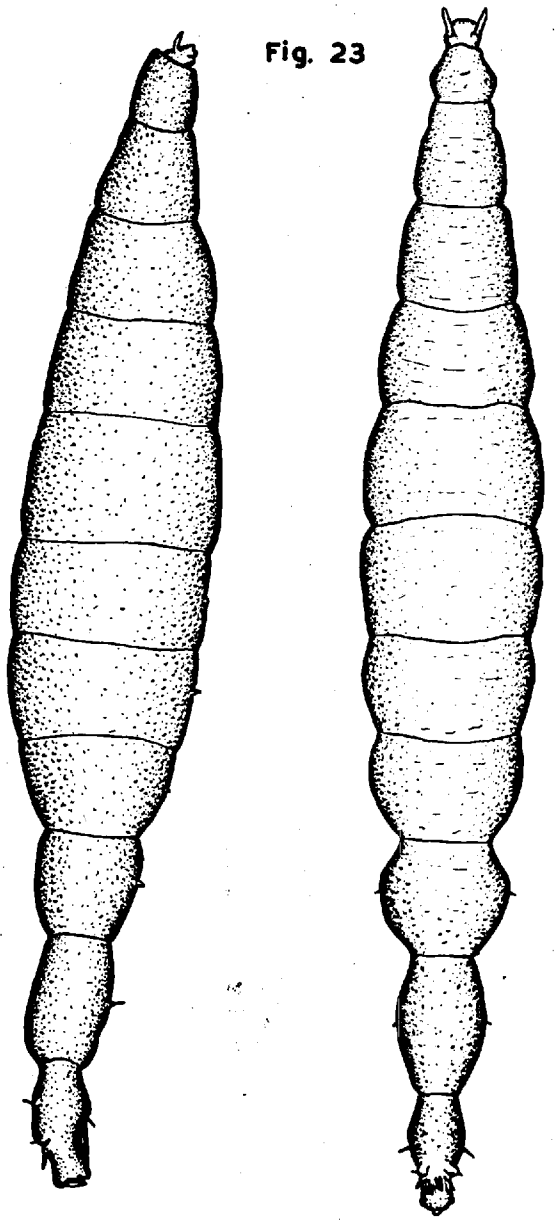
·02 mm

Fig. 23 - Dorsal and lateral views of first stage larva of Phyto melanocephala

Fig. 24 - Ventral abdominal "pseudopod" of the first stage larva of Frauenfeldia rubicosa

Fig. 25 - Lateral abdominal "pseudopod" of the first stage larva of Frauenfeldia rubicosa

Fig. 23



·1 mm

Fig. 24

·02 mm

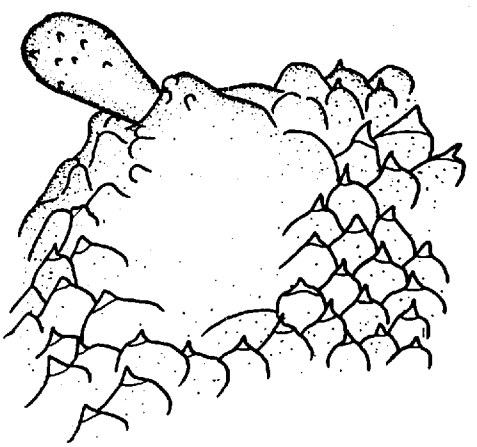


Fig. 25

The cuticle of the anterior fifth of the eighth abdominal segment is bare, but dorsally from this region up to the spiracles, posteriorly directed broad short rounded scales are present. Laterally feeble, ill-defined scales extend down almost to the posterior ends of the ventral ridges, but mid-ventral no scales occur behind the anterior ends of these ridges.

v) Frauenfeldia

The buccopharyngeal armature of this species was described by Thompson (1934) from moulted remains attached to second stage larvae, but the complete larva was previously undescribed.

Description: (see figs. 24 - 28)

length: 1.13 mm

breadth: 0.2 mm

Body tapers anteriorly and posteriorly from sixth and seventh segments. Flattened dorso-ventrally. Segmentation distinct: rudimentary head, three thoracic, eight abdominal segments. Conspicuous brown pigmentation. Numerous acute, triangular, posteriorly directed, pigmented scales. Large, globular, pigmented "pseudopods" around the equators of all but the last segment, each pseudopod arising from behind a large pigmented plate. (see fig.25).

Head. Simple structure: small; dorsally sub-triangular; bare of cuticular armature; telescoped into first thoracic segment. Pair of small palps present on anterior head border.

Antennae: long (0.04 mm) slender, gradually tapering from base to tip. Inserted dorso-latero posteriorly in short broad collar. Directed dorso-anteriorly; slightly divergent.

Thorax

Dorsally broadens from anterior to posterior end forming a triangle together with the head; depth of first segment little less than that of second and third, which are similar in depth.

First thoracic segment: small; broadest two-thirds way down segment where it is about twice breadth of anterior margin. Narrows posteriorly to meet second segment. Anterior margin of segment with a pair of dorso-lateral lobes. Halfway down the segment there occur a dorsal and a lateral pair of large brown pigmented plates which occupy about one quarter of the segment length. These merge into the general scaling anteriorly but are sharply delimited posteriorly where they are concave.

Within concavity of each plate there articulates a large "pseudopod" (length 0.019 mm, breadth 0.01 mm) bearing antero-terminally a tapering setae of about one half length of "pseudopod" itself. "Pseudopod" rounded apically, narrowing proximally; bearing only a very few feeble inconspicuous scales. Medially, dorsally and ventrally, segment bears small sparse, rounded backwardly projecting pigmented scales. Beneath "pseudopods" cuticle is bare.

Second thoracic segment: broadest half way down. One pair of dorsal, one pair of dorso-lateral, and one pair of lateral "pseudopods", arising about two thirds way down segment. "Pseudopods" similar to those of first segment; each associated with large, pigmented cuticular plate which is concave latero-posteriorly on the inner side.

Cuticle only scaled anterior to the "pseudopods". Scales larger than those of first segment and more densely packed; triangular and acute in dorsal region directed posteriorly and towards midline; rounded laterally ventrally triangular but smaller and more sparsely.

distributed than dorsally.

Third thoracic segment. Similar to second but "pseudopods" with only very short setae and less feebly scaled. Dorsal and lateral cuticular scales larger, more densely packed than those of second segment; distinctly triangular; scale apices directed posteriorly and towards midline. Ventral cuticular armature similar to that of second thoracic segment.

Abdomen

First five abdominal segments very similar in shape and structure although increasing in length towards the posterior end. Two pairs of dorsal, one pair of dorso-lateral and one pair of ventro-lateral "pseudopods" present (see fig. 25). These differ from thoracic "pseudopods" in having no terminal setae and in being more heavily scaled to spined especially in the more posterior segments. As in thoracic segments, a large cuticular plate is present in front of each "pseudopod".

All abdominal segments except last have one pair of mid ventral pseudopods which have no associated cuticular plates, and become progressively reduced in size towards the posterior end of the abdomen. (see fig. 24)

On the first five abdominal segments, the dorsal and lateral scales are larger, more densely overlapping and of more complex shape than those of the thorax. Basally each scale is rounded while apically it is acute triangular with the sides of the triangular portion being slightly concave. This type of scale is present in front of the dorsal and lateral pseudopods and between the meso-dorsal pseudopods. Between

other pseudopods the scales are small, sparse and rounded. Posterior to the pseudopods the cuticle is relatively unarmoured. Ventrally the scales are small, triangular and not so densely packed as those of the dorsal surface.

The sixth and seventh segments are globose; longer than other abdominal segments but not so wide. Pseudopods are more crowded; more spinose and tend to be spatulate in shape, but are similar in number to those of other abdominal segments. Cuticular scales become much more rounded and more disorganised towards the posterior end than those of other segments. Behind pseudopods of those segments there are numerous minute rounded scales.

Eighth abdominal segment: (see fig. 28) Almost spherical in shape. Scales round and organised in rows dorsally; extend only to just below halfway down segment. At the border between scaled and unscaled integument there is a pair of small, sessile, cylindrical, unpigmented dorso-lateral processes directed posteriorly.

The spiracles are dorsal, dome-shaped, divergent and arise about two thirds of the way down the segment. Distally around the spiracle opening there is a whorl of fine setae.

As in the first stage larvae of Rhinophora and Stevenia, there is a pair of large thin, transparent, inflated vesicles which occur latero-ventrally and occupy almost the total width of the segment ventrally. Latero-ventrally, just below the spiracles and on the posterior margin of the segment there is a pair of small conical two segmented processes. Mesally on the posterior border there is a similar but larger pair lying between the two inflated vesicles.

Fig. 26 - Dorsal view of the first stage larva of Frauenfeldia rubicosa

Fig. 27 - Dorsal view of head and first two thoracic segments of the first stage larva of Frauenfeldia rubicosa

Fig. 28 - Dorsal view of seventh and eighth abdominal segments of the first stage larva of Frauenfeldia rubicosa

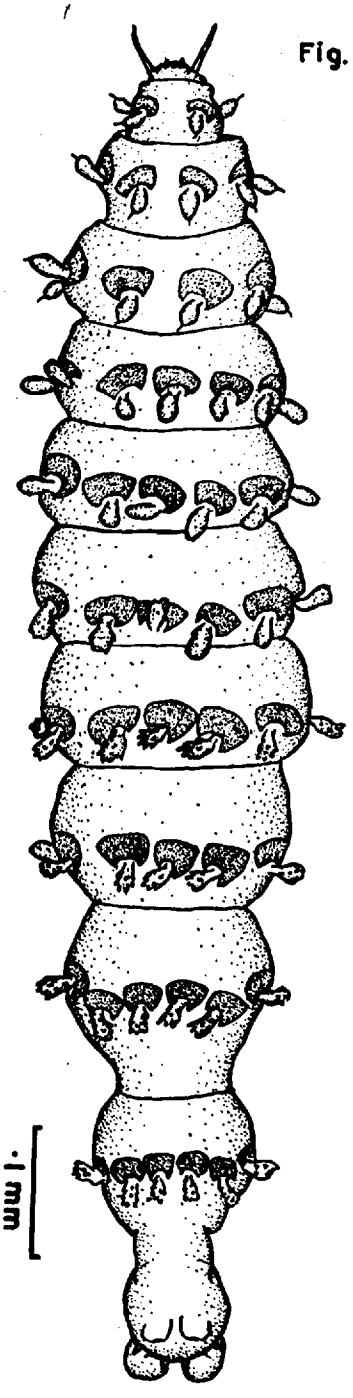


Fig. 26

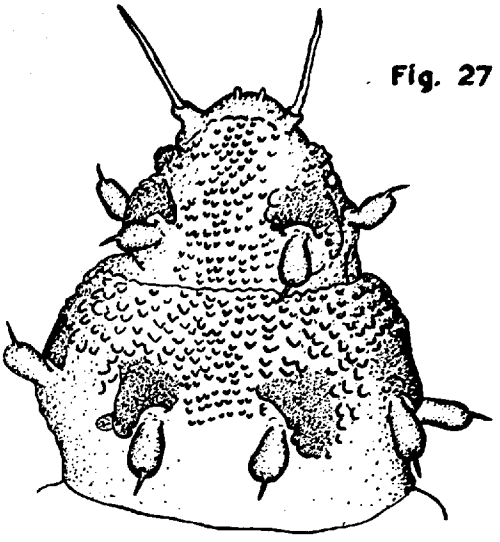


Fig. 27

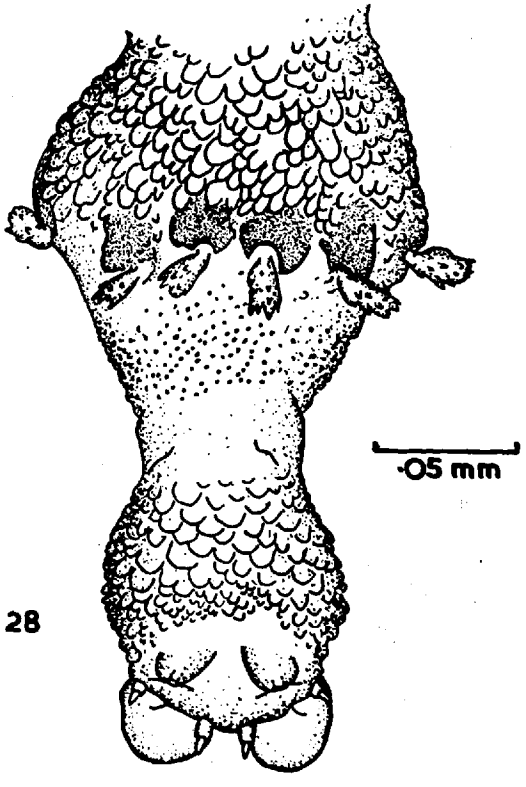


Fig. 28

vi) Stevenia

The only larval material, possibly of this species, which has been previously available consisted of a few fragments of skin and buccopharyngeal armature of a single first stage larva and an anterior spiracle of the third stage. W.R.Thompson (1934) described these fragments, which he received from Switzerland, as "speciesB". His drawings of a "cuticular organ" and the buccopharyngeal armature are sufficiently like those of the first stage larvae of Stevenia atramentaria, which I have reared, to be reasonably certain they are from a closely related,if not from the same, species.

Description (see figs. 29 - 35 and 47)

length: 1.0 mm

maximum breadth 0.24 mm (including "pseudopods")

Body tapers anteriorly and posteriorly from fifth and sixth segments. Segmentation distinct: rudimentary head, three thoracic, eight abdominal segments. Diffuse pale brown pigmentation, numerous acute posteriorly directed pigmented scales; large, globular, spinose, pigmented "pseudopods" around equators of all but the last segment (see figs. 29 - 34).

Head. Simple structure; cuboidal; dorsally rounded at apex, laterally apex appears truncate but bilobed; directed antero-ventrally; unarmoured; telescopes into first thoracic segment.

Antennae. Long, (0.07 mm), and whiplike; inserted into short wide collar situated posteriorly and latero-dorsally. Strongly divergent (45°C to midline); finely tapering.

Mouth. Occupies posterior half ventrally; one fifth width of head; rounded anteriorly; pair of small protuberances laterally.

Buccopharyngeal armature (see fig. 47) Length: 0.18 mm

Very similar to that described from single specimen by W.R.Thompson (1934) as "species B", and somewhat similar to that of Frauenfeldia.

Composed of complicated anterior sclerite, (differing in some respects from Thompson's description of "species B")and a fused posterior region consisting of an elongate intermediate and basal sclerite.

Anterior sclerite: Composed of left and right halves dorsally completely fused; basal portion of each half not flat but distinctly internally concave; each half initially diverging ventrally before converging somewhat to a ventral greatly thickened border. From anterior end of border arises a conspicuous ventro-posteriorly projecting sub-cylindrical sclerite. Two thirds of the way towards posterior of base of anterior sclerite is a median perforation coinciding with the origin of the antenna. Anteriorly fused dorsal surface of sclerites produced into single strong acutely pointed tooth which is antero-ventrally directed; length almost equal to that of basal portion. (This tooth may be formed from both left and right sclerites but no line of fusion can be distinguished in any specimens.) Each sclerite produced antero-ventrally into smaller acute tooth which diverges ventrally from the dorsal tooth.

Posteriorly, anterior sclerite is sub semi-circular with one dorsal and one ventral, small, posteriorly directed tooth both dorsally and ventrally.

Intermediate sclerite: composed of two unfused sclerites twice the length of anterior sclerite; nine times as long as deep; ventrally slightly concave; rounded anteriorly; fused to basal sclerite.

Basal sclerite: Dorsal wings similar in length to intermediate sclerite; produced and rounded anteriorly; finely acute posteriorly. Ventral wings spatulate posteriorly; ventral edge of spatula rounded, dorsal edge acute, almost meeting dorsal wing which converges towards it.

Thorax

Dorsally broadens from anterior to posterior end forming a triangle together with the head; depth of first segment little less than that of second and third, which are similar. In second and third segments, the areas around articulation of the"pseudopods"are produced into conical protuberances; laterally each protuberance occupies the total segment length; dorsally it is of half the segment length or less. All segments armoured with scattered triangular scales; more numerous and pronounced around base of "pseudopods"; sparse ventrally; inter-segmental areas dorsally bare of scales but ventral surface uniformly scaled.

First Segment narrows anteriorly to width of head; broadest at anterior of posterior third where there is one pair of "pseudopods" on lateral margin of dorsal surface; one pair of lateral "pseudopods"; both pairs like those of other segments but smaller, relatively sessile and not mounted on prominent cones.

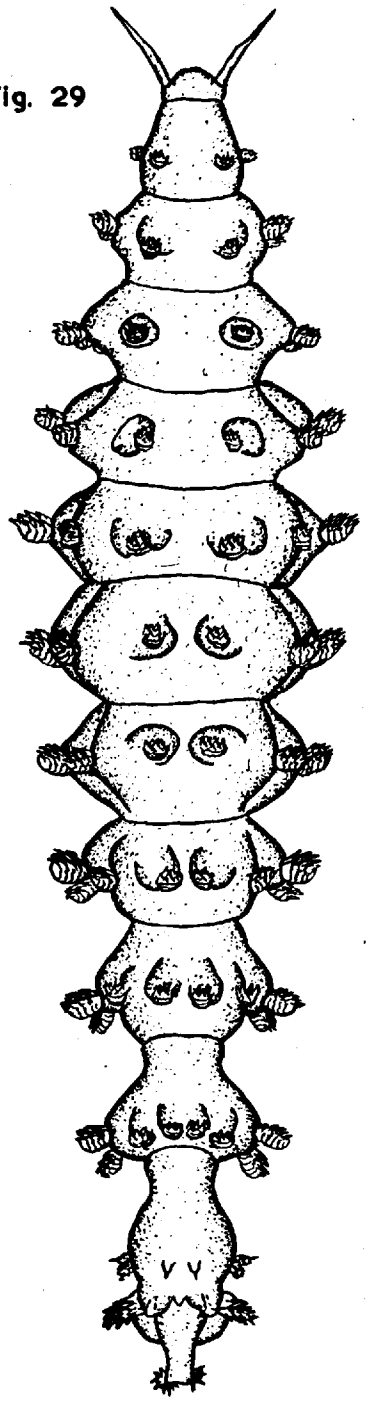
Ventrally there is a pair of minute papillae on either side of middle third segment.

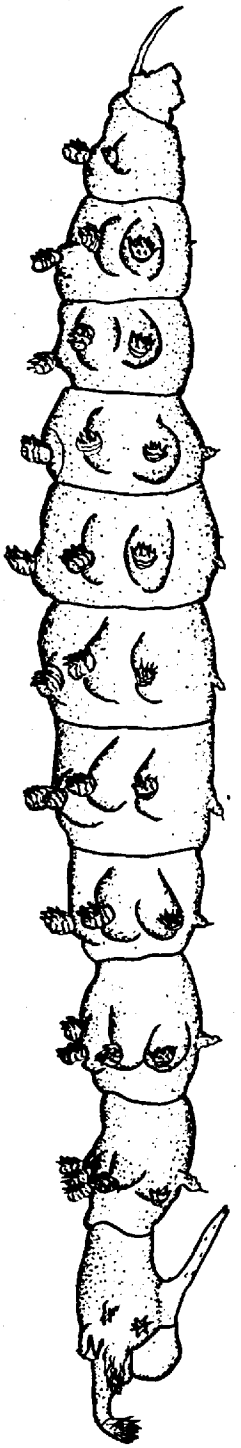
Second and third segments: Alike; shape basically rectangular with conical protuberances to receive two pairs of lateral and one pair of dorsal moveable pseudopods. "Pseudopods" globose; anteriorly with large strong acute spines; posteriorly and laterally with about six rows of smaller spines; terminally bare. Scales of third segment

Fig. 29 - Dorsal view of the first stage larva of Stevenia atramentaria

Fig. 30 - Lateral view of the first stage larva of Stevenia atramentaria

Fig. 29





1 mm

Fig. 30

more numerous dorsally than on second and distinctly more triangular and acute as opposed to rounded. Intersegmental region between third and fourth segment ill defined, with scales both dorsally and ventrally. There are no ventral "pseudopods".

Abdomen

Segments one to four; shape and size almost indentical and similar to second and third thoracic.

Segments five to eight: progressively narrowing posteriorly both laterally and dorso-ventrally with intersegmental area progressively more elongate. Segments one to seven: with three pairs of lateral, one pair of dorsal and one pair of ventral "pseudopods" with first lateral "pseudopods" of segments five to seven becoming dorso-lateral.

Ventral "pseudopods": (see fig. 35) mid ventral; fleshy with isolated scales; not mounted upon conical protuberances; relatively unpigmented. "Pseudopods" of segments one to four at about the middle of the segment; those of five to seven becoming progressively more posteriorly situated in relation to each segment and progressively more crowded.

Last abdominal segment. (see figs. 31 - 33) Greatly modified: dorsally broadly ovate, produced posteriorly into rectagonal extension of length similar to and one third length of rest of segment. Caudal extension antero-ventrally swollen. Conspicuous mid-ventral furca arises half way down the segment from a broad base which extends laterally and posteriorly and occupies full width of segment. Base almost semi-circular in ventral view but of uniform thickness; unscaled. Furca: directed antero-ventrally; extending to ventral "pseudopods" of segment

Fig. 31 - Dorsal view of seventh and eighth abdominal segments of the first stage larva of Stevenia atramentaria

Fig. 32 - Lateral view of seventh and eighth abdominal segments of the first stage larva of Stevenia atramentaria

Fig. 33 - Ventral view of seventh and eighth abdominal segments of the first stage larva of Stevenia atramentaria

Fig. 34 - Lateral "pseudopod" of the first stage larva of Stevenia atramentaria

Fig. 35 - Ventral "pseudopod" of the first stage larva of Stevenia atramentaria

Fig. 31

·05 mm

Fig. 32

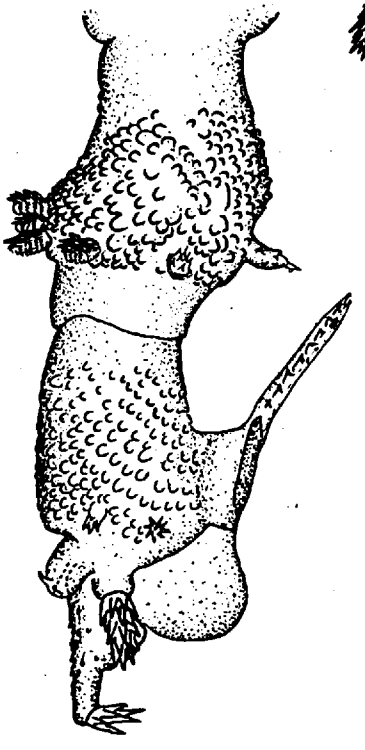


Fig. 33

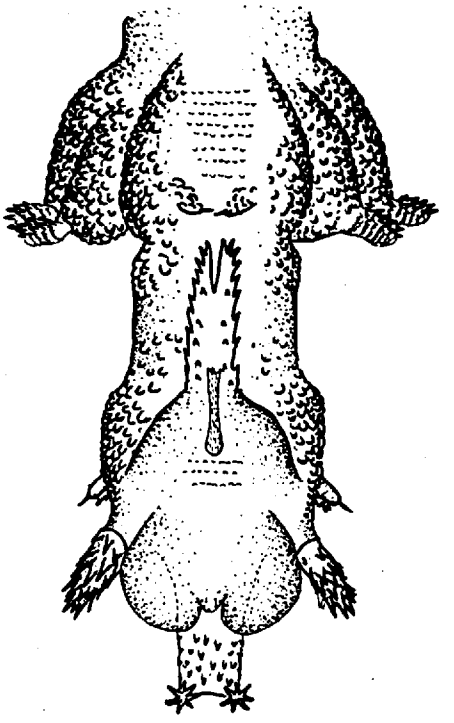
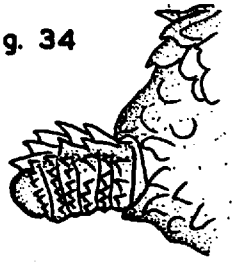


Fig. 34



·02 mm

Fig. 35

·02 mm

seven; tapers dorso-ventrally, from ventral view appears almost parallel sided and bifid for one third of its length; lateral and inner edges serrated; supported internally at base by strong sclerotised spicule about half the length of furca.

Ventral unscaled cuticle posterior to furca base swells into large bilobed vesicle extending posteriorly to half the length of caudal extension. Vesicle occupies full width of segment.

A large cylindrical densely spinose process directed latero-posteriorly is on either side of vesicle; it is more than half the length of caudal extension and of similar thickness. Similar but only half as large processes are situated at posterior corners of caudal extension; directed ventrally. Two pairs of processes present laterally half way down the segment; these are half the size of "pseudopods" of other segments. Dorsal "pseudopods" evenly and strongly spinose; directed latero-posteriorly. Ventral pair; perpendicular to base; weekly spinose; each terminating in a short setae. Between the dorso-lateral processes are a pair of small dorsal lobes with short setae. Spiracles; open dorsally into a pair of large mammilate, unscaled lobes which almost meet medially and extend laterally across breadth of dorsal surface; around spiracle openings there is a whorl of tapering setae.

vii) Rhinophora

The complete larva of this species was available to Thompson (1934) although he did not know which species it was, but was only obtained by dissection some time after entry into the host when larvae are swollen and distended with food. Thompson described fully

the buccopharyngeal armature, but only briefly described and did not draw the complete larvae which were not well preserved.

Description: (see figs. 36 - 40)

length: 0.95 mm

maximum breadth: 0.13 mm

Body only very slightly tapering anteriorly and posteriorly in both depth and breadth from the first and second abdominal segments. Segmentation distinct; small "pseudopods" present around equators of all segments but the last. Cuticular armature of small triangular to rounded backwardly directed, lightly brown pigmented scales.

Head

Cylindrical; about as wide as long; terminally rounded; cuticle unarmoured. Two pairs of small palps present, one pair antero-dorsally and the other ventrally.

Antennae long (0.043 mm), tapering; slightly divergent; directed anteriorly; curved ventrally

Thorax

1st thoracic segment: longer but narrower and less deep than any other segment. About twice as long as wide. Widens and deepends from anterior to posterior. One pair of dorsal (see fig. 38) and one pair of lateral "pseudopods" directed at right angles to the segment each with a long acute terminal seta, occur about two thirds of the way down the segment. Little protuberane of the body wall occurs beneath the "pseudopod".

Second and third thoracic segments: Similar to each other; wider than long; widest about three fifths of the way down segment where"pseudopods" occur. There are one pair of dorsal and two pairs of lateral

"pseudopods" similar to those of the first thoracic segment.

Abdomen

First seven segments similar: segments barrel-shaped; widest about three fifths of the way along the segment; each with one pair of dorsal, one pair of dorso-lateral, two pairs of lateral, and one pair of ventral "pseudopods".

Dorsal and lateral "pseudopods" of first and second abdominal segments with short terminal setae; other "pseudopods" with no setae. "Pseudopods" apparently formed of a conical mass of fused scales and are relatively sessile. (see figs. 39 - 40). Posterior ventral "pseudopods" larger than more anterior ones.

Dorsal "pseudopods" inserted on relatively flat well-scaled cuticle but dorso-lateral and lateral "pseudopods" are mounted on prominences which occupy almost the total segment length. Areas between these prominences are relatively little scaled whereas the prominences are densely scaled. Dorsally the full width of the segments in front of the "pseudopods" is covered with overlapping triangular scales, but behind the "pseudopods" only a few small rounded scales occur on otherwise bare cuticle.

Eighth abdominal Segment (see fig. 37) Roughly spherical in shape. Dorsally scales extend only to just behind halfway of the segment. Here there arise a pair of rounded, conical spiracles. Dorso-laterally just in front of the spiracles and on the border between scaled and unscaled cuticle there is a pair of short cylindrical processes each with a short terminal seta. These processes are directed latero-posteriorly.

Ventro-laterally as in Frauenfeldia and Stevenia larvae, there is a pair of swollen vesicles which extend over the full width of the segment. Between these on the posterior surface, lying close together are a pair of three jointed, quite large, cylindrical, terminally rounded, slightly divergent processes. On either side of these is a capitate seta of about half the length of the processes. There are no ventral processes and most of the ventral surface is bare of scales.

Fig. 36 - Dorsal and lateral views of the first stage larva of Rhinophora lepida

Fig. 37 - Dorsal view of seventh and eighth abdominal segments of the first stage larva of Rhinophora lepida

Fig. 38 - Lateral "pseudopod" of second thoracic segment of the first stage larva of Rhinophora lepida

Fig. 39 - Ventral abdominal "pseudopod" of the first stage larva of Rhinophora lepida

Fig. 40 - Lateral "pseudopod" of sixth abdominal segment of the first stage larva of Rhinophora lepida

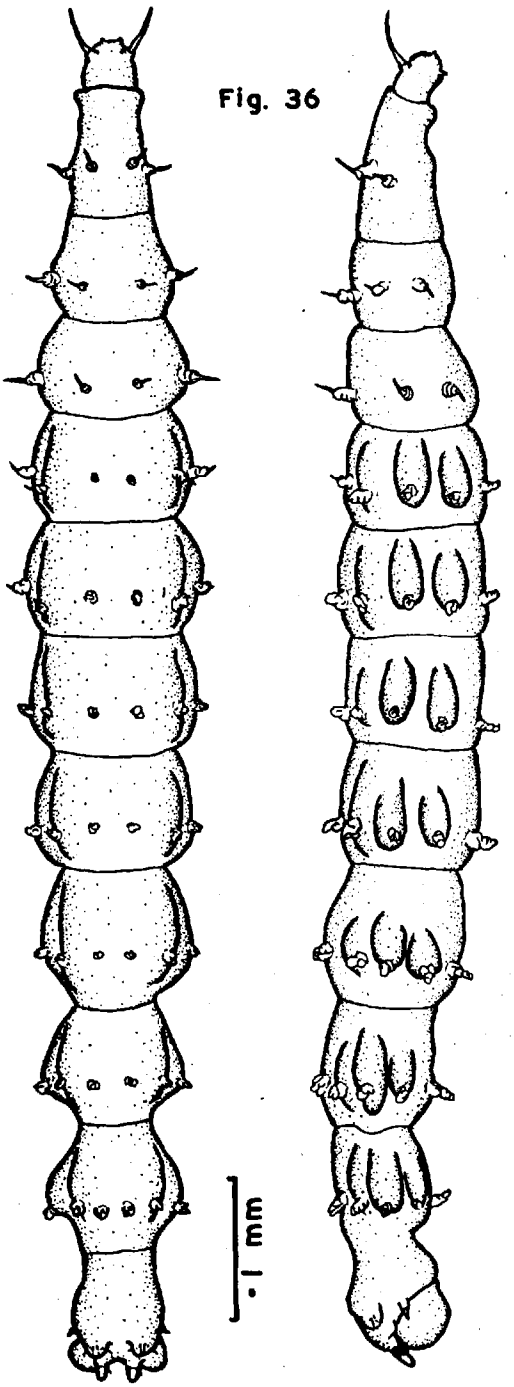


Fig. 36

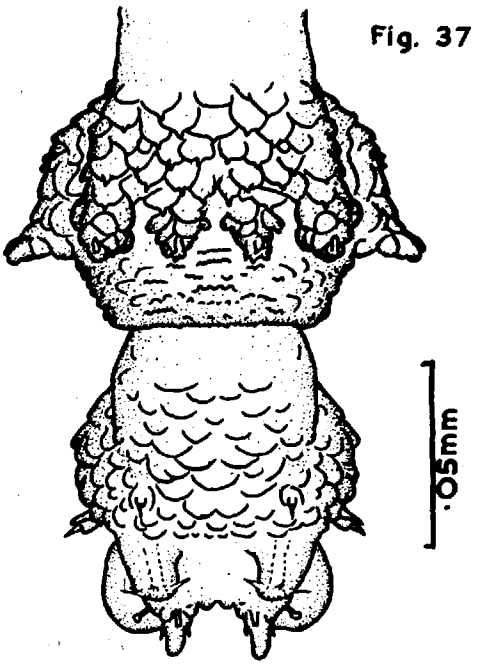


Fig. 37

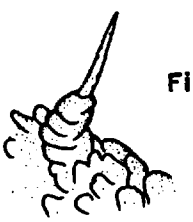


Fig. 38

Fig. 39

Fig. 40

Key to Buccopharyngeal Armature of First Stage Larvae.

1. Each mandibular sclerite with only two main teeth; sclerite heavily pigmented. 2.

Each mandibular sclerite with three or more teeth; sclerite feebly pigmented. 4.

2. Accessory sclerite present between mandibular and intermediate sclerites. (see fig. 45)

Rhinophora lepida

No accessory sclerite present between mandibular and intermediate sclerites. 3.

3. Dorsal tooth of mandibular sclerite about twice as long as, and twice as broad at the base as ventral tooth. (see fig. 46)

Frauenfeldia rubicosa

Dorsal tooth of mandibular sclerite less than one and half times as long as, and little wider at the base than the ventral tooth. (see fig. 47)

Stevenia atramentaria

4. Dorsal wing of basal sclerite almost absent (see fig. 43)

Styloneuria discrepans

Dorsal and ventral wings of basal sclerite of similar length. 5.

5. Six teeth present on each mandibular sclerite (see fig. 41)

Phyto melanocephala

Less than six teeth present on each mandibular sclerite. 6.

6. Mandibular sclerite with ventral hair-like process; anterior angle of dorsal wing with many unpigmented spots. (see fig. 44)

Plesina maculata

Mandibular sclerite without ventral hair-like process; anterior angle of dorsal wing with not more than two or three unpigmented

Buccopharyngeal Armature of First Stage Larvae.

Fig. 41 - Lateral view of armature of *Phyto*.

Fig. 42 - Lateral view of armature of *Melanophora*.

Fig. 43 - Lateral view of armature of *Styloneuria*.

Fig. 44 - Lateral view of armature of *Plesina*.

Fig. 45 - Lateral view of armature of *Rhinophora*.

Fig. 46 - Lateral view of armature of *Frauenfeldia*.

Fig. 47 - Lateral view of armature of *Stevenia*.

Fig.41

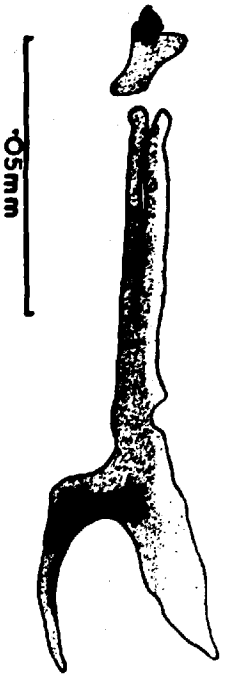


Fig.42

Fig.43

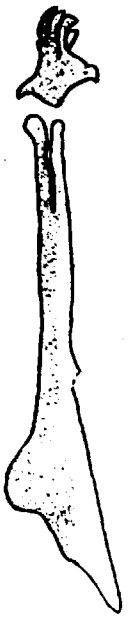


Fig. 44

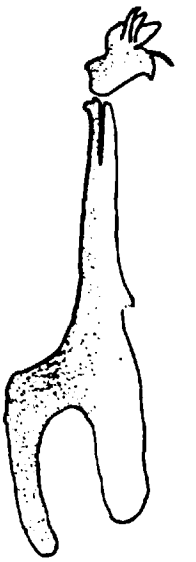


Fig. 45

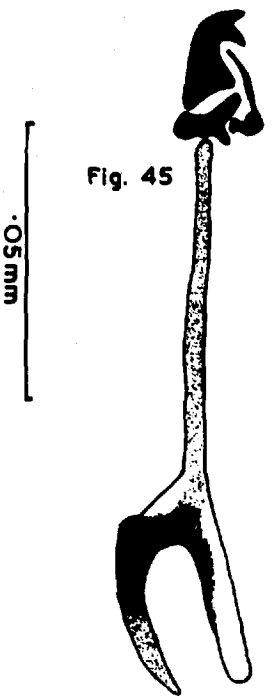


Fig. 46

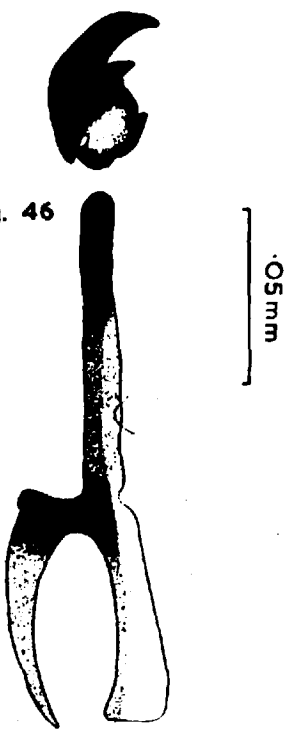
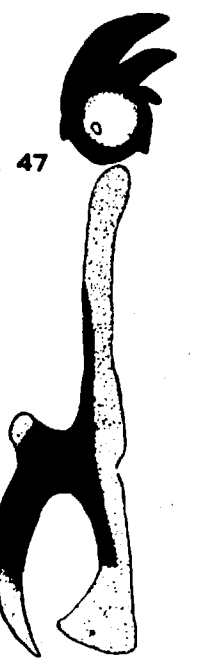


Fig. 47



spots. (see fig. 42)

Melanophora roralis

3e. Description of second stage larva of Stevenia

(previously undescribed)

The general appearance resembles that of the second stage larvae of other species described by Thompson (1934). Spindle shaped. Cuticle thin transparent clearly revealing internal organs and colourless body fluids; reddish brown mid intestine and yellowish white Malphigian tubules. On all segments but last, cuticle bare except for occasional small circular organs. Last segment posterior dorsally club shaped; scattered short feebly chitized spines directed anteriorly (probably aiding the anchoring of the larva to the host tissues).

Head with antennary and maxillary sensoria present dorso-laterally. Respiratory system metapneustic. Posterior stigmata cylindrical ventro-posterior; converge and almost touch in older specimens at least; each with three oval stigmatic papilla orange brown in colour.

Buccopharyngeal armature: (see figs. 48-50)

length: 0.236 mm

Similar to that of Frauenfeldia. Some variation between different specimens. Anterior or mandibular sclerites separated from each other. Unlike any other species in that left mandibular sclerite is distinctly different from right.

Base of both mandibular sclerites sub quadrilateral. Right sclerite: produced dorsal-anteriorly into acute pointed, ventrally and laterally curving tooth which is somewhat longer than base.

Left sclerite: with very short acute tooth dorso-anteriorly. Ventral anterior corner of base of both sclerites right angled. Dorsal posterior corner sometimes rounded, sometimes pointed and produced posteriorly. An additional conspicuous irregular sclerite present at ventral-anterior corner of each sclerite.

Dorsal and ventral inverted U-shaped sclerites present between mandibular sclerites but neither is fused to the main sclerites.

Intermediate sclerite of similar length to right anterior sclerite but in others almost indistinguishably fused.

Usually dorso-posteriorly produced along anterio-dorsal edge of vasal sclerite. Intermediate sclerites of each side joined by weakly sclerotised bridge for about the middle half of the sclerites.

Basal sclerite usually produced ventro-anteriorly. Dorsal wing conspicuously deeper and longer than anterior. Dorsal edges of both wings irregularly sclerotised.

3f) Buccopharyngeal Armature of Second Stage Larvae previously described by Thompson (1934)

The buccopharyngeal armature of the second stage larvae of most species are readily identifiable from Thompson's descriptions and figures but some discrepancies do exist between his figures and my preparations. An attempt has also been made during the present work to consider the variations between individuals of the same species.

i) Styloneuria (see fig. 53)

length: 0.12 mm

The buccopharyngeal armature of this species is exactly as described by Thompson and easily identifiable. There is very little variation.

ii) Plesina (see figs. 56 - 57)

length: 0.159 mm

In this species the buccopharyngeal armature varies and positive identification is sometimes difficult. The mandibular sclerites vary from specimen to specimen but not to the extent of those of the third stage. Occasionally, the ventral tooth is distally produced a little posteriorly like that of Melanophera which makes confusion with this latter species possible. The shape of the notch behind the intermediate sclerites is very variable and often the ventral surface of the ventral wing of the basal sclerite is flush with the dorsal floor of this notch. When viewed ventrally the mandibular sclerites and intermediate region present a characteristic shape making the armature easily distinguishable from that of Melanophora and Phyto. (see fig. 57) The dorsal wing of the basal sclerites usually arises rather more posteriorly than is shown in Thompson's figure, and the ventral wing

rarely terminates as indicated in his figure. The angle between the ventral and dorsal wings shows some variation in different specimens.

iii) Melanophora (see figs. 54 - 59)

length; 0.146 mm

Whilst the buccopharyngeal armature of this species varies considerably from specimen to specimen, Thompson's figure is much more different from the basic type than any specimens I have examined. Further, although in his text Thompson states that it is difficult to tell this armature from that of Plesina, his figures of the two species bear no resemblance.

The armature as a whole is much more like that of Plesina, Styloneuria and Phyto than is apparent from Thompson's figure.

A characteristic of the mandibular sclerite of this species is the posterior extension of the ventral tooth which is shown in Thompson's figure. The narrow anterior end of the intermediate sclerite is also characteristic of this species although the ventral edge of this sclerite is rarely so conspicuously concave as shown in Thompson's figure.

The dorsal wing of the basal sclerite usually arises much more posteriorly than shown in Thompson's figure, and the angle between the dorsal and ventral wings of this sclerite is usually considerably narrower. Further, while Thompson shows no hypopharyngeal sclerite in his figure, there is one in all the specimens I have examined. The whole armature is usually less well scleritised than that of Plesina, Styloneuria or Phyto.

iv) Phyto (see figs 58 - 59)

length: 0.16 mm

Easily identifiable from Thompson's figure and description, However, the angle between the dorsal and ventral wings of the basal sclerite is shown by Thompson to be acute whereas this is broadly rounded in most specimens. Ventral teeth of the mandibular sclerite are widely divergent when compared with Plesina and Melanophora. (see fig. 59.) Relative length of intermediate sclerite greater than either Plesina or Melanophora.

v) Frauenfeldia (see fig. 51)

length: 0.21 mm.

Identifiable from Thompson's figure but specimens are much less irregular than his figure suggests. The ventral tooth of the mandibular sclerite is usually represented by a right angled corner and there is a separate small angled sclerite just posterior to this. Neither of these features are apparent from Thompson's figure. The basal and intermediate sclerites are similar to those drawn by Thompson.

vi) Rhinophora (see fig. 52)

length: 0.165 mm

Thompson's figures the buccopharyngeal armature of the second stage larvae of this species (of which he had only a single specimen) as "Species A". From this and his description and figures of the first stage larvae of "Species A" it is apparent that his "Species A" is in fact Rhinophora lepida. However, his figure of the second stage armature is not fully accurate particularly with respect to the

Buccopharyngeal Armature of Second Stage Larvae

Fig. 48 - Lateral view of armature of Stevenia.

Fig. 49 - Lateral view of left mandibular sclerite of Stevenia.

Fig. 50 - Ventral view of mandibular and other sclerites of Stevenia.

Fig. 51 - Lateral view of armature of Frauenfeldia.

Fig. 52 - Lateral view of armature of Rhinophora.

Fig. 53 - Lateral view of armature of Styloneuria.

Fig. 54 - Lateral view of armature of Melanophora.

Fig. 55 - Ventral view of mandibular and other sclerites of Melanophora.

Fig. 56 - Lateral view of armature of Acroneuria

Fig. 57 - Ventral view of mandibular and other sclerites of Plesina.

Fig. 58 - Lateral view of armature of Phyto.

Fig. 59 - Ventral view of mandibular and other sclerites of Phyto.

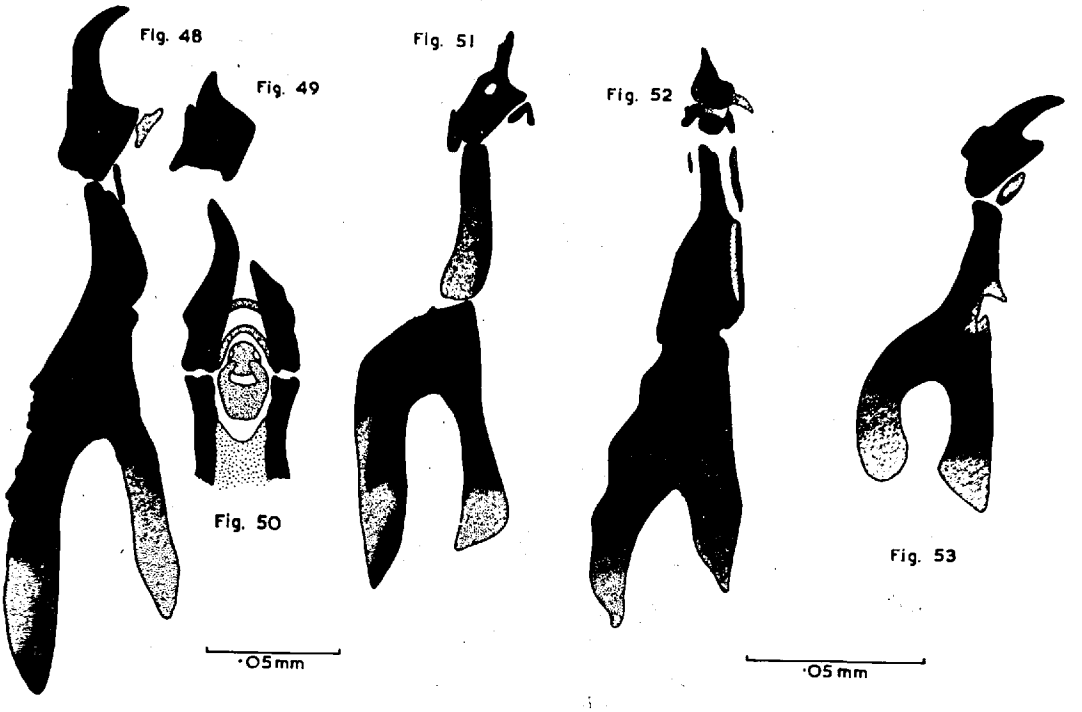


Fig. 48
Fig. 49
Fig. 50
Fig. 51
Fig. 52
Fig. 53
·05mm
·05 mm

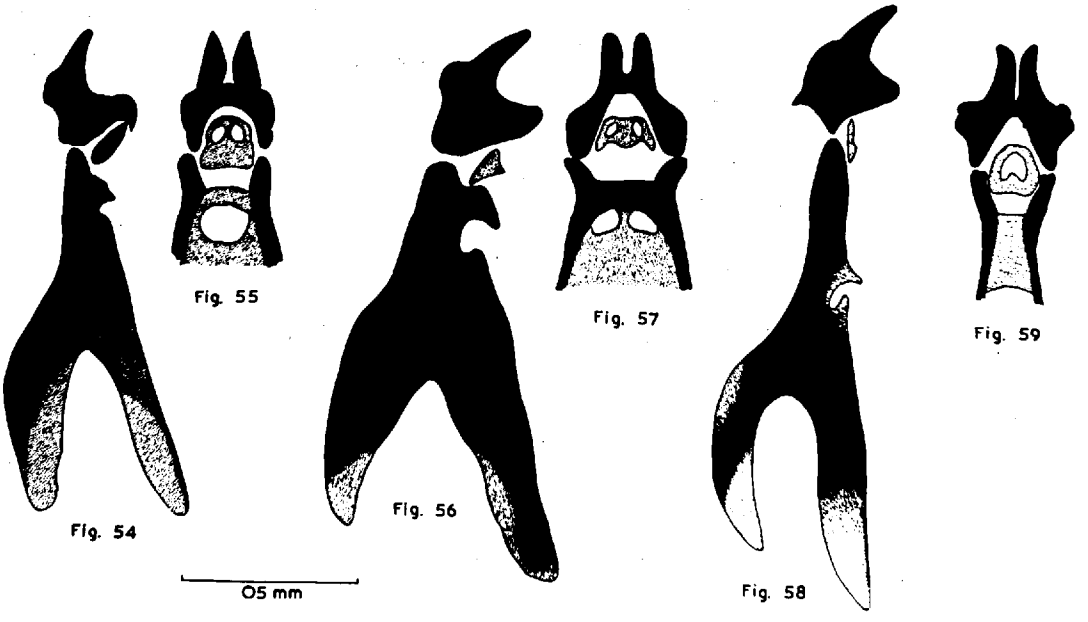


Fig. 54
Fig. 55
Fig. 56
Fig. 57
Fig. 58
Fig. 59
05 mm

mandibular sclerite. This is actually composed of a number of differentially sclerotised portions rather like the mandibular sclerite of the third stage larva of this species.

The intermediate sclerite is similar to that figured by Thompson although whilst his figure shows a posterior ventral portion separated from the main body of the sclerite this is in fact fused to the main sclerite by a feebly sclerotised portion. The angle between the dorsal and ventral wings of the basal sclerite is in fact acute and not rounded as shown by Thompson, although the comparatively short, dorsal and ventral wings shown by him are characteristic of this armature.

The intermediate and basal sclerites are usually fused although the line of fusion is always apparent.

Key to Second Stage Larvae

1. Intermediate and basal sclerites indistinguishably fused. 4.
   Intermediate and basal sclerites either completely separate or incompletely fused. 2.

2. Dorsal and ventral wings of basal sclerite no longer than remainder of this sclerite (see fig. 52)
   Rhinophora lepida
   Dorsal and ventral wings of basal sclerite considerably longer than the remainder of this sclerite. 3.

3. Left mandibular sclerite without a long dorsal tooth while right sclerite has a ventrally curved tooth which is longer

than the remainder of the sclerite. (see fig. 48 - 50)

Stevenia atramentaria

Both mandibular sclerites similar (see fig. 51)

Frauenfeldia rubicosa

4. Intermediate sclerite at least one third of the length of the basal sclerite. 5.

Intermediate sclerite less than one third of the length of the basal sclerite. 6.

5. Dorsal wing of basal sclerite distally greatly rounded, almost semi-circular; ventral wing distally obliquely truncate; malpighian tubules almost white.

(see fig. 53) Stylocneuria discrepans

Dorsal wing of basal sclerite distally rather pointed; ventral wing distally tapering; malpighian tubules usually sulphur yellow.

(see figs. 58 - 59) Phyto Melanocephala

6. Anterior end of intermediate sclerite narrower than distal end of ventral tooth almost invariably produced posteriorly; malpighian tubules always white; last segment without spines or scales.

(see figs. 54 - 55) Melanophora roralis

Anterior end of intermediate sclerite broader than distal end of ventral tooth of mandibular sclerite; ventral tooth rarely produced posteriorly; malpighian tubules usually sulphur yellow; last segment with forwardly directed spines and scales.

(see figs. 56 - 57) Plesina maculata

3g) Description of Third Stage larvae of Stevenia and Rhinophora.

i) Stevenia

Previously undescribed. General structure similar to that of other Rhinophorinae third stage larvae described by Thompson (1934). Cuticle: thin transparent, almost bare of spines or scales except for a few weakspines on the seventh and eighth abdominal segments. Tracheal system: metapneustic; anterior spiracles prominent, each bearing about twelve spherical respiratory papillae; posterior spiracles situated at extreme end of narrow cylindrical last segment and opening or two conical protuberances each of which has three narrow, oval spiracular slits.

Buccopharyngeal armature: (see fig. 64)

length: 0.53 mm.

Somewhat similar to that of Frauenfeldia.

Mandibular sclerites: roughly square in form, each with a single tooth, as long as the rest of the sclerite, arising antero-dorsally.

Tooth directed forwards but curved downwards; with an acute tip, sclerites joined by a very narrow bulge dorsally at base of tooth. Intermediate sclerite, viewed laterally is roughly T-shaped. Three distinct accessory sclerites lie in the ventral region between the mandibular and intermediate sclerites. The dorsal linings of the basal sclerite are distinctly broader and longer than the ventral lining. The dorso-anterior angle of the dorsal wing is distinctly produced as in the third stage buccopharyngeal armature of Frauenfeldia, but is feebly chitinized.

Puparium

length: 4.5 -6 mm

breadth: 1.5 - 2 mm.

Light golden brown in colour. Developing imago easily visible through puparium.

ii) Rhinophora

(Previously undescribed)

General structure similar to that of other Rhinophorinae third stage larvae described by Thompson (1934).

Cuticle: thin, transparent, bare of spines and scales except for a few weak spines on the seventh and eighth abdominal segments.

Tracheal system: metapneustic; anterior spiracles prominent, each bearing twelve respiratory papillae; posterior spiracles situated at extreme end of narrow cylindrical last segment and opening on two conical protuberances each of which has three narrow, oval spiracular slits.

Buccopharyngeal armature: (fig. 66)

length: 0.44 mm

Very considerable variation between different specimens. Mandibular sclerites:

sclerotised. Each sclerite with a single antero-dorsal fully sclerotised but variably shaped tooth arising from remainder of sclerite which is also variably and irregularly shaped. Posterior portion of mandibular sclerite fully sclerotised but irregular and very variable. Between this posterior sclerotised area and the anterior tooth, the mandibular sclerite is weakly and peculiarly sclerotised, having a "fluffy" appearance. This "fluffy" area is produced into a variable, irregularly shaped ventral tooth in most specimens.

Intermediate sclerites: variable, but less so than mandibular and basal sclerites. Roughly T-shaped when viewed laterally.

Basal sclerites: variable. Dorsal and ventral wings usually of similar length but dorsal wing occasionally longer; both wings taper posteriorly; dorsal wing produced a little anteriorly. Dorsal edge of dorsal wing very irregular in outline.

Puparium

Light olive brown in colour. Developing imago easily visible through puparium.

3b) Buccopharyngeal Armature of Third Stage Larvae(previously described by Thompson (1934).)

Thompson (1934) described and figured the third stage larvae of Plesina, Phyto, Melanophora, Styloneuria, Frauenfeldia and Cyrillia. Whilst the third stage larvae of most of these species are fairly easily identifiable from Thompson's descriptions, there are certain discrepancies in some of his figures of buccopharyngeal

armatures.

There is a considerable variation in the buccopharyngeal armature of individuals of the same species and so at least ten specimens of each species were studied. Some of the types of variation found are figured and discussed below.

i) Plesina: (see figs. 62 & 68)

There is a much greater difference between figure 18 (Thompson 1934) of the buccopharyngeal armature of the third stage of this species and any of my specimens of this, than between Thompson's figure and my specimens of any other species. In fact, it is almost impossible to identify his figure with any of my specimens which were obtained from a variety of habitats.

length: 0.39 mm

Mandibular sclerites: variable. Left and right sclerites of same specimen usually similar but occasionally very different from each other (see fig. 68). Long acute dorsal tooth directed anteriorly and slightly ventrally, its length often exceeding that of the rest of this sclerite. Ventral tooth directed ventrally and its length may or may not exceed that of dorsal tooth; sometimes tapered distally but often broadens distally forming a posteriorly directed appendix. Remainder of sclerite usually more narrow than long; posterior usually concave both dorsally and ventrally; posteriorly considerably narrowed. Mandibular sclerites almost invariably connected by a weakly sclerotised narrow bridge dorsally.

Intermediate sclerite: T-shaped. Vertical arm constructed medially and only slightly produced posteriorly rather than greatly produced

and a tapering as described by Thompson (1934)

Basal sclerite: dorsal wing directed backward and upward for one quarter of its length and not for one half of its length as described by Thompson; arises one third of the way along ventral sclerite. Remainder of dorsal wing directed and gradually tapering posteriorly; not perforated as shown in Thompson's figure; ventral wing posteriorly directed but not tapered.

ii) Melanophora (see fig. 61)

length: 0.39 mm.

All specimens easily identifiable from Thompson (1934).

The third stage buccopharyngeal armature is characterised by complete fusion of the imtermediate and basal sclerites.

Mandibular sclerite: variable.

Basal and intermediate sclerite: quite variable

Most specimens differ a little from Thompson's figure in that the antero-dorsal angle of the intermediate sclerite is produced much more than is shown by him.

iii) Phyto (see fig. 63)

length: 0.468 mm

Specimens easily identified from fig. 33 (Thompson 1934)

Mandibular sclerite: shows little variation; specimens differ from Thompson's figure in having a shorter, broader ventral tooth and the dorsal tooth curves round more towards this.

Buccopharyngeal Armature of Third Stage Larvae.

Fig. 60 - Lateral view of armature of *Styloneuria*.

Fig. 61 - Lateral view of armature of *Melanophora*.

Fig. 62 - Lateral view of armature of *Plesina*.

Fig. 63 - Lateral view of armature of *Phyto*.

·1 mm

Fig. 60

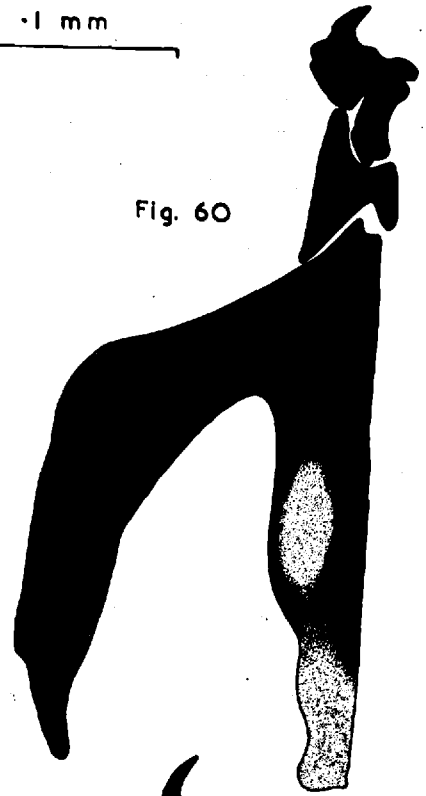


Fig. 61

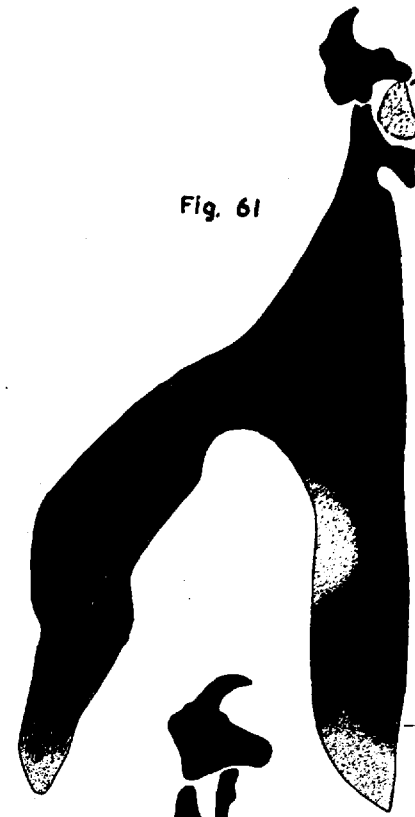


Fig. 62

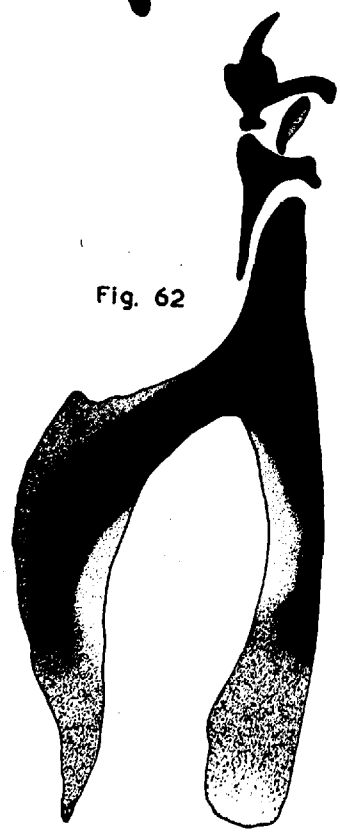


Fig. 63

Buccopharyngeal Armature of Third Stage Larvae.

Fig. 64 - Lateral view of armature of *Stevenia*.

Fig. 65 - Lateral view of armature of *Frauenfeldia*.

Fig. 66 - Lateral view of armature of *Rhinophora*.

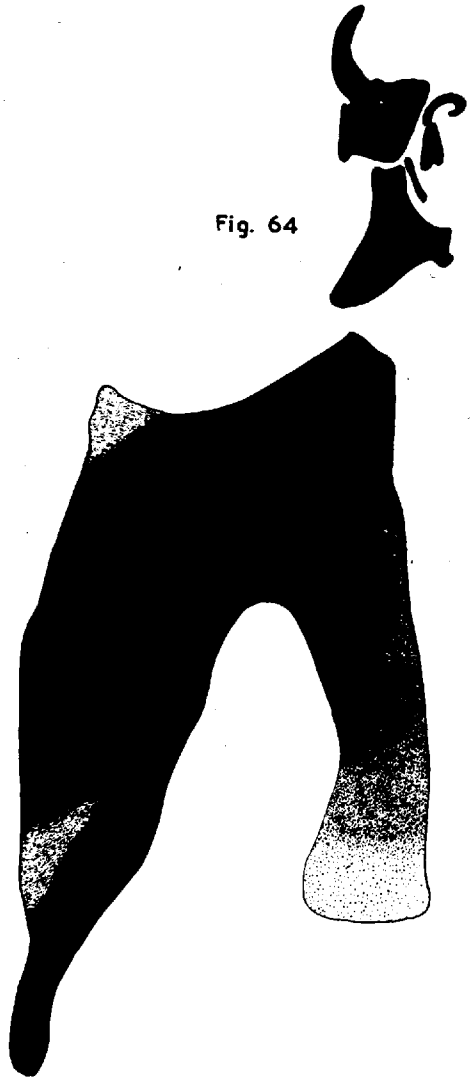


Fig. 64

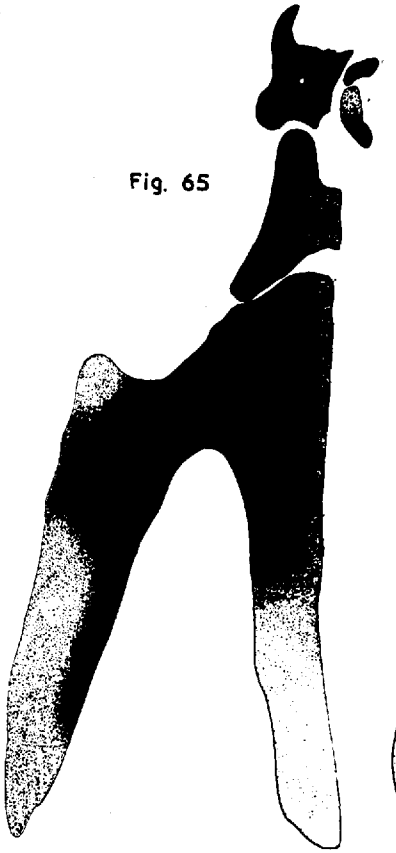


Fig. 65

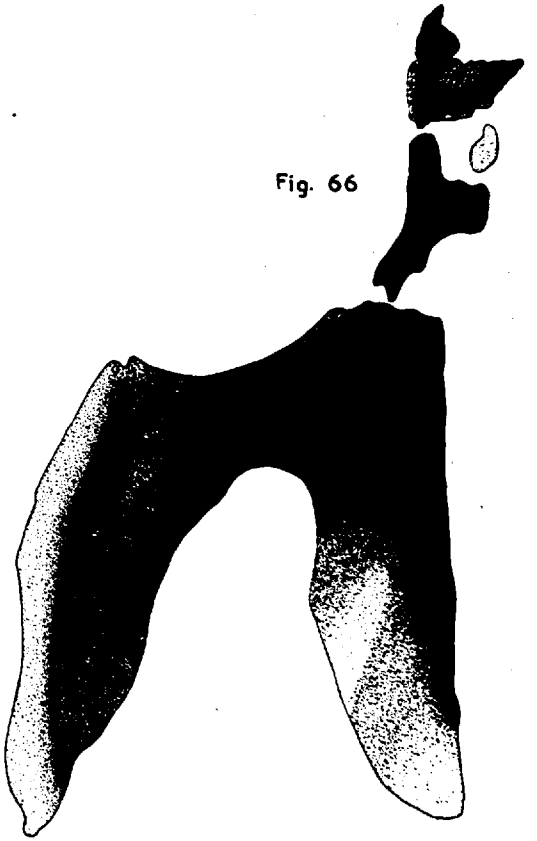


Fig. 66

·1 mm

iv) Styloneuria (see figs. 60 & 67)

length: 0.373 mm.

Specimens easily identified from fig. 29 (Thompson 1934)

There is considerable variation between mandibular sclerites of different specimens and often between the left and right sclerite of the same specimen. (see fig. 57.)

v) Frauenfeldia (see fig. 65)

length: 0.44 mm

Specimens easily identifiable from fig. 65 (Thompson 1934)

Mandibular sclerite: vary somewhat but differ from Thompson's figure in having only one ventral tooth at the antero-ventral corner of the sclerite.

Key to Third Stage Larvae using the Buccopharyngeal Armature.

1. Intermediate and basal sclerites fused. (see fig. 61)

Melanophora roralis

Intermediate and basal sclerites articulated. 2.

2. Mandibular sclerite with only dorsal tooth and basal portion heavily sclerotised, the middle of the sclerite being of a "fluffy" appearance. (see fig. 66) Rhinophora lepida

Mandibular sclerite evenly and heavily sclerotised. 3.

3. Mandibular sclerite with ventral tooth almost as long as, or longer than dorsal tooth and directed ventrally; rest of

sclerite not rectangular. 4.

Mandibular sclerite with ventral tooth almost absent and much smaller than dorsal tooth; directed antero-ventrally; remainder of sclerite rectangular and usually perforated medially. 6.

4. Both dorsal and ventral wings of basal sclerite grandually and smoothly tapering posteriorly. Angle between axes of dorsal and ventral teeth of mandibular sclerite less than 60°

(see fig. 63) Phyto melanocephala

Only dorsal wing of basal sclerite gradually tapering posteriorly. Angle between axes of dorsal and ventral teeth of mandibular sclerite usually more than 80°. 5.

5. Dorsal and ventral wings of basal sclerite usually convergent posteriorly. Base of ventral tooth of mandibular sclerite not, or little, broader than base of dorsal tooth.

(see fig. 62) Plesina maculata

Dorsal and ventral wings of basal sclerite usually slightly divergent Base of ventral tooth of mandibular sclerite considerably broader than base of dorsal tooth. (see fig. 60) Styloneuria discrepans

6. Dorsal tooth of mandibular sclerite longer than the remainder of mandibular sclerite. (see fig. 64) Stevenia atramentaria

Dorsal tooth of mandibular sclerite about half the length of rest of sclerite. (see fig. 65) Frauenfeldia rubicosa

Fig. 67 - Various mandibular sclerites from third stage larvae of Styloneuria. Each pair of sclerites from the same larva and all three larvae were from the same population of hosts.

Fig. 68 - Various mandibular sclerites from third stage larvae of Plesina. Each pair of sclerites was from the same larva and all three larvae were from the same population of hosts.

These figures are intended to show that the detailed structure of a particular sclerite, at least in third stage larvae, is unreliable as a guide to speciation.

Fig. 67

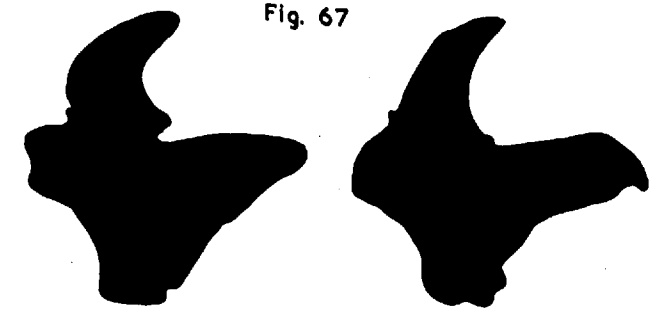
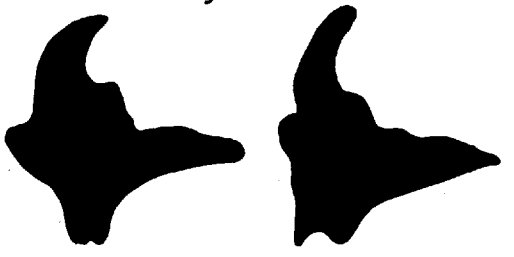


Fig. 68



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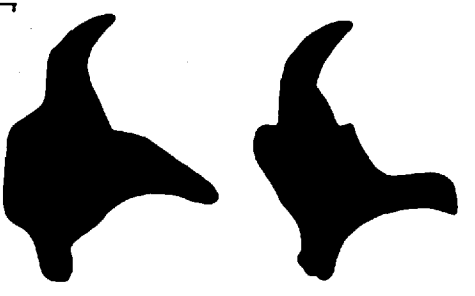
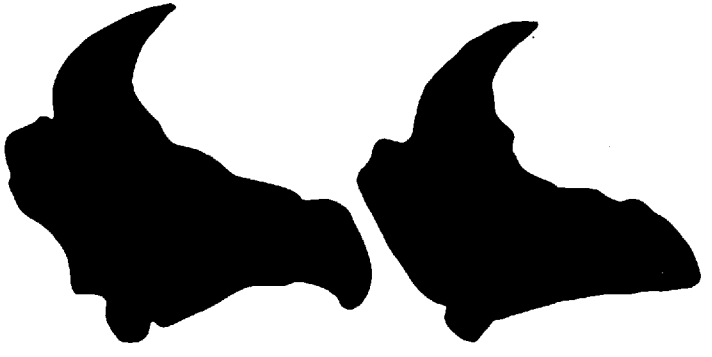
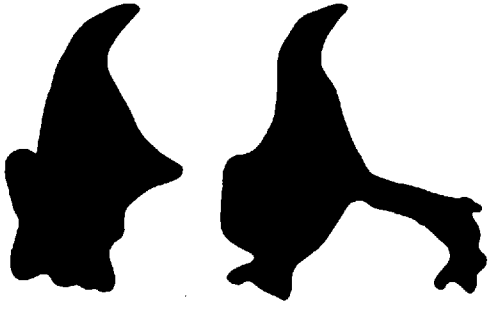






4. MATING AND OVIPOSITION BEHAVIOUR.

i) Styloneuria

Mating (Number of observations exceeded 100)

Under laboratory conditions it was at first found very difficult to obtain mated females of Styloneuria even when several males and females were left together for days in various sizes of cages and provided with a variety of feeding materials, light intensities and temperatures.

It was found best, finally, to confine a single female a few hours after emergence with five to ten males at least 36 hours old in small cylindrical cages (4 in. diameter x 8 in. (10·2cm diam. x 20·4cm.)). When the cages are brought close to an artificial sunlight tube, both sexes exhibit a rapid jerking of the body up and down by flexing of the legs. After a few minutes of this, one of the males usually makes a rapid dart at the female and the two fall to the cage floor in copula.

Copulation usually lasts from twenty minutes to one hour although a pair occasionally separate within a minute or so. Only in these cases have females been observed to copulate again before oviposition.

It has not for some reason been possible to induce copulation by leaving flies in bright natural sunlight, even at high temperature.

In the field both male and female Styloneuria have been observed feeding on flowers, but copulation has only been observed in the laboratory.

Oviposition (Number of observations exceeded 100)

Styloneuria females require two to three days at 25°C after emergence before they are ready to oviposit, and they need only sucrose solution during this period.

Given the correct stimuli, both mated and virgin females will now lay eggs, although in the latter the ovaries are never fully emptied. When it has been possible to induce copulation in females of this age, oviposition often took place within an hour under the requisite conditions which are described below. Dissections of newly laid eggs had shown that no appreciable development takes place within the parent.

Unlike Melanophora, female Styloneuria never lay eggs unless substrate previously contaminated by woodlice is present. Styloneuria was the first species studied in which this was found to be essential and much experimentation was required to find this out.

When mated female Styloneuria were kept in cages with no contamination, they died after two or three days with greatly distended abdomens and extended ovipositors and although they had previously explored all cracks and crevices with their ovipositors, no eggs were laid. Unmated females under similar conditions also failed to oviposit, but often survived for two or more weeks at 20°C.

Even when P.scaber were introduced into the cages, no eggs were laid, although the flies were subjected to a variety of temperatures, humidities and degrees of illumination and the females were fed on sucrose, marmite solution and fresh meat; flowerheads were also left in the cages.

Eventually it was found that when some woodlice were introduced into a cage together with pieces of bark on which they had been living for some weeks, eggs were deposited all over this. Subsequently, such bark even without woodlice was found to provide the essential stimulus to oviposition. To confirm this, numerous pieces of damp uncontaminated bark were scattered on the sand floor of a large cage (1 x 1 x 2 ft) (31·5 × 31·5 × 63 cm) together with a single piece of a similar sized contaminated bark and a mated female Styloneuria which was showing beginnings of oviposition behaviour was released into the cage.

The female did not fly directly to the contaminated bark but ran rapidly round the cage crawling under any pieces of bark it came across and explored the cracks with her ovipositor. Some minutes were spent examining each of the pieces of bark in this way before the female ran to another and repeated the performance. Eventually, on reaching the contaminated bark the female behaved in the same manner, but here a number of eggs were laid before she left for another piece. Each time on arriving randomly at the contaminated bark, eggs were laid here although none were deposited elsewhere.

The experiment was repeated twelve times with different females and the same result was obtained. Females ready to oviposit rarely flew, although while running, they frequently sprang into the air a few inches, often landing in the opposite direction. Unlike males and non-ovipositing females which are attracted to a light source, ovipositing females are distinctly photonegative.

It was assumed that some substance was left by the woodlice which induced oviposition. Initially, pieces of bark were smeared with faeces of woodlice, but this did not induce egg laying.

Dr. H. Gorvett (personal communication) has observed that when Porcellio scaber touches any object with the tip of the antennal flagellum it often leaves a minute droplet of secretion. This is the only record of undisturbed woodlice leaving a secretion and initially I considered that this substance could have been the sign stimulus which released oviposition behaviour. However, when woodlice from which the antennae had been amputated were left on fresh bark for a number of days, this bark still stimulated oviposition.

The woodlouse produces two other main secretions but only under conditions of extreme stress or injury (Gorvett 1956). Secretions from the lateral plate glands wiped on to bark did not induce oviposition, but when the viscous secretion from the uropods was smeared on to bark female Styloneuria readily oviposited. A copious secretion is produced from the uropods when a woodlouse is squeezed or injured. This can be drawn out into silk-like threads (see plate 1). If these are wrapped around one half of a fresh piece of bark but not the other, Styloneuria lay almost all their eggs on this half. This experiment, which was repeated ten times, indicated that uropod secretion provides a stimulus for oviposition. However, Gorvett (1956) states that he has been unable to find any evidence that woodlice leave secretions from the uropods under normal conditions and do so only when they are attacked by a predator or are injured in some other way.

There are perhaps several ways in which woodlice may leave uropod secretion under field conditions, any or all of which may contribute to contamination of the substrate. These are:-

1. Females with brood pouches readily secrete at the slightest disturbance.

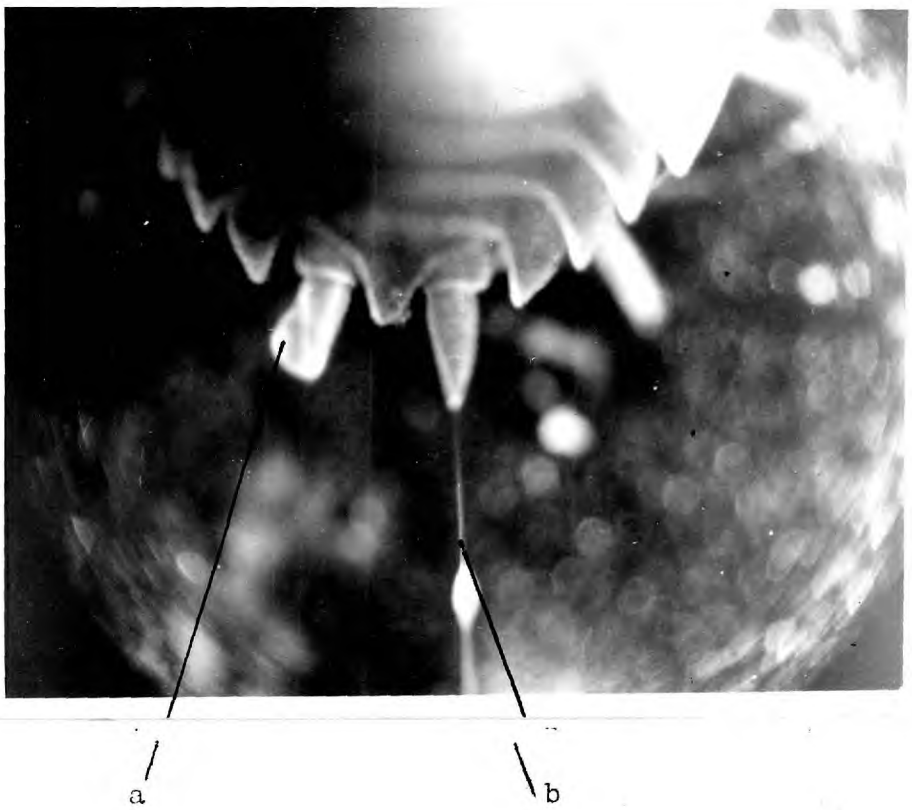


Plate 1: Posterior end of a woodlouse which has secreted from the exopodite of its uropods in response to injury.

a - exopodite of left uropod with secretion in globule as it normally appears.
b - secretion from exopodite of right uropod drawn into a thread which can be a foot or more in length.

2. Cannibalism, at least in cultures, is rife and presumably woodlice are just as likely to secrete when being eaten alive by other woodlice as when they are injured in any other way. (Thus when a culture of initially 475 Porcellio scaber were kept for ten days at 25°C in a (31·5 × 31·5 × 22·8 cm) (12 x 12 x 9) cage, with a floor covering of (2·5 cm one inch) deep, damp sawdust, with many pieces of bark and the woodlice were provided with surplus food and 100 per cent. humidity, only 370 were left, although no cause of mortality other than cannibalism was obvious. Large cultures in smaller containers are depleted even more rapidly and woodlice were observed attacking others which appeared to be healthy but often freshly moulted).

3. While woodlice walk, they trail their uropods and may leave invisible traces of secretion as they do so.

4. When the pleopods of a P.scaber become excessively wet, a characteristic behaviour is exhibited. The pleon is actively wiped repeatedly against the substrate and the uropods trail along behind.

During this process it is possible that although the uropod secretion itself is not readily soluble, some soluble active component is washed from the uropods and deposited on the substrate.

The last possibility seems quite likely in view of the fact that while bark on which Oniscus has lived for some time does not promote oviposition, bark with Oniscus uropod secretion does so. Oniscus does not wipe its pleon when its gills have excess of water. The wiping mechanism aids Porcellio in clearing the pseudotracheae which are not found in Oniscus.

At 20° C eggs are laid in batches of 12 to 20 with

intervals of 10 to 40 seconds between each egg of a batch. Between successive ovipositions females exhibit the running and jumping movements already described, for one to five minutes.

Neither oviposition nor egg batches have been seen in the field.

ii) Melanophora

Mating (Number of observations exceeded 150)

While female Melanophora will copulate within an hour or so of emerging from pupation, the males usually require at least twelve hours (at 25°C) before they will attempt copulation. It is of interest to note in this connection that both in culture and in the field males do emerge one or two days earlier than females.

When a female Melanophora is ready for copulation, a characteristic rapid fluttering of wings takes place as she walks about. This is conspicuous even at a distance, as the wing which is mainly black, has a white tip where there is greatest amplitude during the fluttering movements, and the female chooses a light background on which to flutter. When the female ceases walking the fluttering often stops for a few seconds. Bright illumination stimulates continuous and rapid fluttering movements as the female walks in circles, and under these circumstances, copulation most readily occurs.

Male flies are also stimulated by illumination to exhibit the same fluttering movements and these are accentuated by the presence of the female, but in the absence of bright illumination the male only occasionally flutters for a few seconds.

When a female is confined with a male, the male is immediately attracted to her fluttering but "loses interest" as soon as the female ceases these movements.

The male may follow the female for a minute or two before actually copulating and during this period flutters his wings continuously. The male edges himself on top of the female rather than jumping on as in some other species.

Copulation lasts from five to twenty minutes during which time both male and female occasionally flutter their wings. Other males, when present, very often try to copulate with the female at the same time and this frequently results in the original male being superseded by another.

Whilst the male ceases wing-fluttering after copulation, the female continues to do so and begins flexing movements with the ovipositor.

Unlike other species of this group, virgin Melanophora females will readily copulate at any age.

Although the wing flutterings during the oviposition period appear to be of an identical pattern to those preceding copulation, males are only occasionally attracted to them. It seems, therefore, that some other stimulus, probably a chemostimulant, may also be required to induce attempts at copulation. However, females may occasionally copulate for a second time during the period preceding oviposition.

The behaviour of adult Melanophora in the field has been observed on several occasions. Both males and females are generally attracted to light-coloured vertical surfaces to the sunlight.

Males are much more commonly found than females and often several were found on a single post or rock. When a female lands near a male, the latter follows her and mounts within a few seconds (unlike the behaviour in the laboratory). Females were more often observed alighting on surfaces with no males nearby, but almost invariably within a minute or two a male was attracted to her and copulation ensued.

That the attraction of the male was to the female rather than just to the favourable situation is indicated by the fact that when the same situations were observed after the original pair had departed other males did not usually arrive even after an hour or more.

Oviposition (Number of observations exceeded 100)

Unfed newly emerged females will lay eggs within a few minutes of copulation if provided with pieces of bark on which Porcellio scaber have been living for some days.

As described for Styloneuria it was found experimentally that uropod secretion is almost certainly the active ingredient of this required contamination, and in fact previously uncontaminated bark on which uropod secretion had been wiped repeatedly, proved in choice chamber experiments to be more attractive for oviposition than bark on which woodlice had been living. While Styloneuria oviposits strictly only on the contaminated bark, Melanophora will lay some eggs on bark nearby which is uncontaminated.

When provided with contaminated bark, the female fly, continually fluttering the wings, explores this with her extended ovipositor seeking cracks and recesses in which she deposits eggs. The eggs are laid intermittently in batches of four to eight, the eggs

of each batch being laid at intervals of 5 - 15 seconds at 25°C . The female shifts her position slightly between laying each egg of a batch. There is a pause of 30 - 90 seconds while the fly runs, without fluttering, or it will fly short distances before another batch is laid. This behaviour produces a distribution of well-scattered eggs over the available substrate.

The eggs, although usually laid in crevices, lie with their longitudinal axes parallel to the substrate surface, with the flatter winged surface uppermost. When all crevices are filled, the fly will lay in recesses or on flat surfaces.

After 2 - 3 hours, the female gradually slackens its pace of laying until eggs are laid at the rate of only one every few minutes.

Usually all eggs are laid within six hours. The total number of eggs laid by each female varies from 150 - 450 and seems to be roughly proportional to the size of the fly which is in turn proportional to the size of host in which pupation has occurred; this is indicated by the following table:-

Table showing the relationship between host size and the number of eggs laid by the emerging parasite

	No. of eggs laid by female from host length 7 mms	No. of eggs laid by female from host length 9 mms	No. eggs laid by female from host length 11 mms
1	277	324	328
2	195	344	393
3	230	387	415
4	281	289	360
5	315	340	356
Total:	1298	1684	1852

Female hosts were used throughout as there is a difference in width between males and females of the same length.

The female fly is markedly photonegative or at least it avoids bright illumination while ovipositing so that almost all eggs are laid on the shaded side of a piece of contaminated bark even when all crevices on this side are filled with eggs.

Out of the many dozens of female Melanophora observed, only one batch of eggs was recorded from each female although mating did sometimes occur after a female had laid all her eggs. Usually the female dies within one to three days after oviposition, and dissections of these females show that even when fed on marmite and sucrose solution or when fresh meat is present in the cages, no further development will take place.

In the field it is probable that Melanophora does not even feed on plant nectar; I have never seen Melanophora on flowers myself, even in vicinities where this species of fly is common, and Day (1948) who lists large numbers of flowers for almost all Tachinids and Calliphorids keyed by him, lists none for Melanophora. One of the most common habitats for Melanophora is on the rocky sea shore at base of cliffs, where few of any suitable flowers are available and, so perhaps the fact that this species has no nutritional requirements for oviposition is an adaptation to this habitat. Certainly none of the other species which do have nutritional requirements is commonly found in this type of habitat.

When a newly emerged female is mated but confined away from any substrate contaminated by woodlice, no eggs are laid for two to three days. When oviposition commences, the fly once again lays eggs in crevices and even on the sides of the container but at a somewhat

slower rate than when a contaminated substrate is present. Nevertheless all the eggs are usually laid within a period of 12 hours.

When female Melanophora are unmated and kept away from substrate contaminated by woodlice, no eggs are laid and the females remain alive for as long as three weeks at 20°C, but, if at any time after two to three days they are mated, oviposition takes place within a few minutes independent of the presence of woodlouse contamination.

Virgin females will lay eggs on contaminated substrate after they reach an age of about three days, but the ovaries are never emptied. While ovipositing on a contaminated substrate, the female fly does not seem to be attracted to the woodlice themselves, if these are present, but if a woodlouse is introduced into a container where a female has commenced to oviposit on a substrate without the uropod secretion, there is some attraction and the female will settle on the woodlouse and lay eggs between the epimerites although it will also continue to lay eggs in uncontaminated places. Whether this is an attraction of the fly to a suitable substrate by sight or a chemostimulant attraction is difficult to determine. Reactions to Oniscus which is not a host of the parasite, are the same, while those to the smoother Philoscia are not. If a woodlouse is killed thus causing copious secretion to exude from lateral plate and uropod glands, no eggs are laid on it, and this indicates that the parasite is more repelled by the pungent lateral plate secretion than it is attracted by the uropod secretion. However, the dried shell left of a woodlouse after a parasite has emerged from it and which is presumably relatively odourless, is readily oviposited upon. Two or three days after the eggs are laid they drop off the woodlouse.

It is just possible that when a mated female is unable to find a contaminated habitat in the field but can find a host, she oviposits on a woodlouse, which may return to a colony where the eggs drop off. However, since many thousands of woodlice in Melanophora habitats have been examined during the oviposition period without finding a single egg on them, this possibility does not seem likely.

The wing flutterings of Melanophora may aid in host location since chemoreceptors are present on the wings and the fluttering causes a rapid flow of air over these. In spite of this possibility it has not been possible to produce any evidence to this effect. Specimens from which wings were removed readily oviposited on contaminated bark soon after emergence and mating; winged females, when released in a large cage with numerous pieces of bark one of which is contaminated, do not fly straight to the piece of contaminated bark, but explore all pieces with the ovipositor until the contaminated one is encountered and eggs are then laid on it. Winged females have no apparent tendency to follow the path of individual woodlice released in large cages. Why the female continues to exhibit flutterings throughout oviposition with what must be a great loss of energy remains a mystery.

iii) Plesina

Mating (Number of observations - 50)

Female Plesina will readily copulate from an hour or so after emergence, but males will not usually do so until at least twelve hours have elapsed. Older virgin females will also readily copulate.

Courtship is similar to that of Melanophora. When exposed

to a bright light, female Plesina flutter their wings while walking about in circles. This behaviour attracts the males which follow the females and flutter their wings. Within a few minutes the male edges himself on top of the female and copulation takes place. Unlike other species of Rhinophorinae, a pair of Plesina usually remains in copula for at least an hour and often for several hours. Frequently a few minutes after separation, courtship and copulation begin again, so that one pair may spend most of a day or more in copula.

Unlike Melanophora males, male Plesina often attempt copulation while the females flutter their wings during oviposition, but copulation rarely ensues as a result of this.

Mating of this species has not been observed in the field.

Oviposition. (Number of observations - 30)

Female Plesina begin oviposition two or three days after emergence if they are mated and a substrate previously contaminated by Porcellio scaber is present. Provided that the females are of the required age, they will lay eggs within a few minutes of copulation.

As in other species, uropod secretion appears to be the active ingredient of the woodlouse contamination although it was found that bark on which woodlice had been living for several days proved a little more attractive than fresh bark spread with uropod secretion. In fact, although experimentation on this was limited, bark on which large quantities of secretion had been spread usually proved less attractive than bark with only a little secretion. If no contamination was present, females though mated did not begin to oviposit until about one week after copulation and then the eggs were laid at a much slower rate than otherwise.

Oviposition behaviour is similar to that of Melanophora. When supplied with contaminated substrate the ovipositing fly continually flutters her wings, and explores this with her extended ovipositor seeking cracks and recesses in which she deposits eggs horizontally with the winged surface outermost. A distinct preference is shown for vertical surfaces and while the female will lay some eggs when exposed to bright light, she prefers the dark side of objects for oviposition and will crawl between two pieces of bark to lay eggs. Unlike Melanophora which lays eggs rapidly at the beginning of the oviposition period, Plesina commences to oviposit by laying one egg every few minutes until a dozen or so eggs are laid. Then the speed of oviposition increases so that eggs are laid intermittently in groups of two to eight, the eggs of each batch being laid at intervals of ten to thirty seconds at 25°C. The fly then runs or sometimes flies around for one to five minutes before laying another batch.

All the eggs are usually laid within two days, the total for each female varying from 250 to 400 and depending mainly on the size of the fly which in turn usually depends on the size of its host.

Virgin females will lay eggs after about a week if provided with contaminated substrate, but these are often laid in a single bunch.

Whilst it has been relatively easy to obtain eggs from this species in culture, only a very small proportion of them finally produced larvae. Initially, this was thought to be due to the attacks of mites and fungi to which the eggs are supposedly more vulnerable than those of other species since they take considerably longer to hatch (12 days at 25°C). Mites were eliminated but the eggs still did not develop and

and became infected by fungi although various concentrations of Nipagin were applied to prevent this. Recently it was found that when Plesina pupae, flies and eggs were kept at room temperature (approx. 20°C), more eggs were viable.

It is well known that the males of some species of insects often become sterile at much lower temperatures than females. Thus when the Chalcid Euchalcidia caryoberi was exposed to 16°C for ten days by Hanna (1935) females were unaffected but 70 per cent of the males were sterile. When pupae of Ephestia Kuehniella are kept at temperatures above 27°C spermatogenesis is retarded and the spermatozoa lose their mobility (Raichoudhury 1936).

As there was a possibility that Plesina males were being similarly affected, males emerging from puparia at 20°C were mated with females emerging from puparia at 25°C. Many of the resulting eggs produced larvae although large numbers of eggs still failed to develop. Obviously considerably more work is required to establish whether failure of eggs to develop is caused by the sterility of male flies reared at 25°C.

Plesina eggs collected from the field were almost 100% viable.

Oviposition has not been observed in the field although on two occasions eggs have been found.

From egg distribution in the field it seems that the ovipositing Plesina crawls right under the bark of trees and lays eggs throughout the microhabitat of the host. Two trees with loose bark were seen, but when several square feet of bark was stripped from the trees,

dozens of eggs were found laid on the tree trunk, often a foot or more from any entrance hole in the bark. In the situations examined, the eggs were fairly evenly distributed, about one or two to every two square inches of trunk examined. Everywhere beneath the bark that was accessible to the woodlice themselves appeared to have been also accessible to the ovipositing fly.

iv) Phyto

Mating (Number of observations - 40)

The mating behaviour of Phyto melanocephala is similar to that of Styloneuria, but takes place more readily.

Females from a few hours old onwards and males at least one day old readily exhibit the jerking behaviour described in Styloneuria when they are exposed to bright light of any kind. Even when only a single male and a female are confined together, the male soon springs on to the female and the pair fall to the ground in copula. Mating is quite short, lasting from two to ten minutes. No females have been observed to copulate more than once although males readily do so.

The species has not been observed in the field.

Oviposition (Number of observations - 40)

If provided with the essential stimuli, mated females will commence ovipositing three days after emergence.

Feeding on sucrose solution satisfies the nutritional requirements, while additional feeding on marmite solution or fresh meat does not seem to increase fecundity.

As in Styloneuria, egg laying will not normally take place in the absence of substrate contaminated by the normal host. Bark on which Armadillidium vulgare had been living for some time induced

rapid oviposition. However, Phyto does not appear so rigidly to depend upon this stimulus as does Styloneuria, since a few eggs are sometimes deposited on the uncontaminated substrate near to any contaminated regions. Further, the occasional egg is laid when a mated female is confined for several days under uncontaminated conditions, and a dozen or so eggs are sometimes laid on bark treated with P.scaber uropod secretion. Unmated females will lay about half their eggs if confined with contaminated substrate for several days.

The presence of Armadillidium vulgare itself, on uncontaminated bark, does not induce oviposition.

As with Styloneuria, female Phyto seem to be negatively phototaxic during the oviposition period. They crawl under all objects available feeling all round with their ovipositors. In contaminated situations eggs are laid in cracks and crevices, with the long axis of the egg generally horizontal to the substrate.

The pattern of egg laying is somewhat sporadic. Batches of eggs varying from two to ten in number are laid at intervals varying from one to five minutes, the time elapsing between the laying of each egg of a batch being from six to thirty seconds. Between laying each batch, besides the running and jumping behaviour described in Styloneuria, Phyto also flies around a great deal. This is probably connected with the generally much more sparse distribution of its host, namely Armadillidium.

Once again, the total number of eggs laid by each female varies greatly, usually with the size of the fly which in turn depends upon the size of its host. Two hundred to five hundred eggs may be laid within one to two days and the fly usually then dies (with exhausted ovaries). Unmated Phyto females which are kept in cages uncontaminated

by Armadillidium will, however, survive for as long as two weeks.

v) Rhinophora

Mating (Number of observations exceeded 50)

Under laboratory conditions it is difficult to obtain mated females of this species. However, female Rhinophora are most receptive a few hours after emergence. When confined with a number of males several days old, and subjected to bright light one of them usually eventually springs onto the female without any apparent prior courtship.

Mating usually lasts from 5 - 15 minutes. No female has been observed to copulate more than once.

Rhinophora is one of the commonest and widely distributed of the Rhinophorinae to be found as an adult in the field and during the present study has been seen on numerous occasions. Males are found far more commonly than females and seem to be attracted to anything white. Although found commonly on Umbelliferae and often in large numbers, males will also settle on white paper or on stones. Copulation has only been observed on three occasions and then a female settled near males on an umbelliferous head in the sunlight and was pounced on within a few seconds in each case.

Oviposition. (Number of observations - 40)

Rhinophora females will lay eggs three days after emergence (at 20°C) but as only freshly emerged females could be mated it is not known whether or not this period is also required after mating. However, when laid, eggs show no signs of previous development.

When ready to oviposit the female runs about probing into all crevices and cracks with her long ovipositor. However, except under

certain circumstances, oviposition does not take place. Initially, contaminated bark such as was found to induce oviposition in Styloneuria was left with the searching females, but generally no eggs were laid. When rubbish consisting of small pieces of broken bark and wood dust on which woodlice had been living was put in a cage with a Rhinophora female it was found that it completely buried its ovipositor, which when extended is as long as the rest of the abdomen, and after feeling round for some time, laid an egg. While the ovipositor is actually inserted, the wings of the female flutter slightly as in Melanophora. It was later found that females will lay on contaminated bark but only if very deep contaminated crevices, in which the female can bury her ovipositor, are available. The eggs are laid vertically in the crevices with the micropyle end uppermost. Possibly this is an important mechanism for ensuring the availability of suitable hosts, as only in such crevices would woodlice small enough to be parasitised by the larvae when they hatch, be frequently found. Further, only woodlice small enough to crawl into the crevices could contaminate the inside of them.

In Rhinophora oviposition extends over a week or more. Ten to fifty eggs are laid a day usually two or three eggs being laid within a few minutes, this followed sometimes by hours of flying around before more eggs are laid.

On one occasion, a few eggs of this species were found in the field, buried deeply in the soft wood beneath some loose bark of an elm tree. These eggs were scattered and from the position of the entrance holes in the bark, it was obvious that the fly had had to crawl some distance beneath the bark before ovipositing.

vi) Frauenfeldia

Mating

Mating behaviour has not been observed although three pairs were seen in copula in the laboratory where they had been exposed to artificial sunlight.

Oviposition (Number of observations - 9)

No eggs were laid until one week after emergence. Ten to thirty eggs were laid daily after this time and no female laid more than a total of 130 eggs. All flies were fed on a mixture of sucrose and marmite to ensure oviposition, although whether or not this was essential was not determined.

Bark on which P.scaber had been living for some time provided the necessary stimulus for oviposition. Eggs were laid singly, at intervals varying from several minutes to several hours, in deep crevices, vertically with the micropyle outermost.

Eggs of this species were found in the field on two occasions and as with Rhinophora these were well scattered deep in the soft wood beneath loose bark and the fly must have crawled for several inches beneath the bark as eggs were found a long way from any entrance holes.

vii) Stevenia

Mating (Number of observations - 12)

Females mated quite readily from two or three hours after emergence and in one case, two weeks afterwards, but males would not mate until they were at least a day old.

As in Melanophora, the female flutters her wings as she walks about before mating and this attracts the male who follows her path

fluttering his wings in a like manner. (While this is usually the only occasion when males flutter, females flutter for much of the time from emergence to mating and again during the oviposition period). The male shows no interest in the female as soon as she ceases the wing fluttering.

After a courtship lasting a minute or so, the male edges on top of the female, and copulation ensues. This lasts from five to fifteen minutes.

Bench lamp illumination was sufficient to induce courting behaviour which led to copulation.

While none of the females reared in culture were seen to mate more than once, two females collected in the field which laid fertile eggs before being confined with a male in the laboratory, did afterwards mate again.

Although male Stevenia was caught on flowers in the field on three occasions and observed there several other times, females were only seen and caught on logs inhabited by P.rathkei where no males were present, and were found resting on grass blades and other foliage nearby (there were no flowerheads in the vicinity of the logs).

It is not known where copulation occurs as it has not been observed in the field.

Oviposition. (Number of observations - 16)

The majority of the few females obtained from culture mated soon after emergence, but laid no eggs for at least five days at 25°C. Whether some embryological development takes place within the egg before oviposition was not definitely determined owing to the shortage of material, but the short period of two and a half to three days (at 25°C) required between oviposition and egg hatching suggests that this is

probable.

Two females, obtained from the field and which had already laid some fertile eggs resumed laying shortly after copulation in the laboratory, but there was no reason to believe that these eggs were not fertilised by the sperm of the field male.

When females were fed throughout their life only on sucrose solution and kept in clean cages, they still laid some eggs when they were provided with the necessary stimulus, and feeding on marmite solution and fresh meat apparently did not increase their fecundity.

Bark on which Porcellio rathkei had been living for some time proved to be a necessary stimulus for oviposition. In the presence of this material, a female Stevenia walks around and over it with fluttering wings and tries to crawl under it or into crevices. If able to do this, the fly feels around with its ovipositor for some time before eventually depositing a single egg. The fly then runs and flies. Occasionally it rests for several minutes up to an hour or even more and then begins to search and to flutter its wings once again.

In contrast to Rhinophora and Frauenfeldia, the eggs are not usually laid in deep crevices but on the flat underside of bark with the winged surface outermost.

The fly showed photonegative behaviour during oviposition and no eggs were laid in situations exposed to light. No oviposition took place on uncontaminated bark whether or not P.rathkei were present, and the flies would not oviposit on bark contaminated by P.scaber. It was not possible to test whether as in other species it was the uropod secretion which provided the essential stimulus to oviposition as specimens of P.rathkei were extremely rare at the time. However, it seems

most probable that once again the uropod secretion is essential.

Although this parasite species is extremely rare, it was possible to observe both its oviposition and egg distribution in the field in June 1964 in the only habitat where P.rathkei was at all common and parasitised.

Observations, lasting several days (20 - 26.6.64) were made on a number of logs and a fallen tree trunk inhabited by one to two hundred P.rathkei and several hundred P.scaber. On several occasions female Stevenia were seen to fly from many feet up in the sky straight on to the logs. On all occasions when the actual landing place was noticed, the fly alighted on patches of the tree trunk on logs where the bark was stripped and the white wood underneath was visible. On alighting, the flies fluttering their wings rapidly run with short jerky movements to the sides of the tree trunk or logs and tried crawling under any loose bark. Large cracks were entered and in these the females were seen feeling around with their ovipositors, and laying eggs on two occasions. Invariably the female fly made for the underside of logs and pieces of bark.

Later, all the logs and pieces of bark were examined. Only a few pieces had eggs attached to their undersides. No more than one or two eggs to a piece of bark were found and they were usually associated with the presence of P.rathkei.

It is difficult to say whether female Stevenia are attracted by the sight of barked trees and logs, but they landed on the logs from all directions, apparently independantly of the prevailing wind.

5. BEHAVIOUR OF FIRST STAGE LARVAE

i) Melanophora

(Number of larvae observed exceeded 2,000)

At 25°C hatching of eggs occurs 7 days after oviposition. The emerging larva splits the egg down both hatching lines for about a third of their length and the intermediate area is lifted up in the fashion of a trap door as the larva squirms out until it's posterior end reaches the anterior of the egg. It now stands erect inside the egg on its posterior end and may remain in this position for some time, although usually it somersaults out of the egg soon after hatching. The whole process takes from two to three minutes. If undisturbed, the larvae remains quite near the egg seated on its posterior end with its body at right angles to the substrate and in a relatively contracted condition. At the slightest mechanical disturbance from the substrate of surrounding air, the larva pivoting on its fixed posterior end, describes circular, and often figures of eight movements seeking in all directions with its anterior end. The larva elongates to about one and a half times its original length during this activity, but it usually returns to its erect contracted static position a few seconds after the cessation of the stimulation, although sometimes it may somersault a few milimetres after being disturbed. When mechanical stimulation is continued for more than a few seconds, the larva ceases to respond and contracts to the resting position. Violent mechanical stimulation also causes active larvae to contract and become sessile and after such treatment larvae may not respond normally for several minutes. If larvae are confined in a small airtight container, such as a sealed watch glass, they are much less

liable to activation by mechanical stimulation. This is possibly because the larvae are mainly sensitive to air currents and these are less easily set up by vibrations in a small airtight compartment. As soon as the watch glass is opened the larvae are again active. Possibly the very long lateral abdominal setae of these larvae are vibrated by even slight air currents.

Complete somersaulting is the only method of locomotion under normal circumstances. The larva feels the substrate in front of it for a hold with its anterior end, and then swings the posterior end over. It then retains a hold on the substrate and releases the anterior end. A full somersault takes about two seconds.

Holding of the substrate by both anterior and posterior ends is aided by secretion of an adhesive material which readily stains with Alcian blue indicating its muco-polysaccharide or muco-protein nature. This secretion is left whenever the larva somersaults or is removed from the substrate and is so effective that larvae can readily somersault and maintain their erect postures on glass.

A complex of structures at the posterior end of the larva (see fig. 13) seems to play a part in maintaining this posture.

The mode of action of the fixed posterior end was observed by transferring a larva to a coverslip and observing the underside of the slip under oil immersion. The function of the long tongue-like lobe which seems to bear two secretary pores, may be complex. It appears to enable the larva to cling to the substrate on the dorsal side, while the two pronounced ridges on the ventral side tend to prop up the larva. At the anterior end the oral hood becomes filled with secretion and acts as a sucker.

If larvae are killed with alcohol or K.A.A. they usually remain fixed even to glass. Thompson (1934) considered that the first stage larvae of Rhinophorinae in all probability went in search of their hosts but numerous experiments which I carried out indicate that this is certainly not so. Firstly, the larvae seem to be in no way directly attracted to woodlice; a number of larvae varying from 1 - 20 were introduced into the centre of graduated glass tubing of internal diameter of one cm., by means of a glass rod. At one end a number of P.scaber were confined by means of muslin and the tube corked at both ends. Although a little movement of larvae by somersaulting did occur, this was non-directional. Most larvae remained where introduced even after being left for periods of 3 days or more. The tubing was tried horizontal, erect with woodlice below the larvae, and erect with woodlice above the larvae, and the tubing was also left at various angles. Various humidities were maintained from 100 per cent to 0 per cent and the tube was left in light and dark conditions. Humidity gradients with woodlice and water soaked cotton wool at one end of the tube and solid KOH at the other, also produced no reaction from the larvae.

The distance of hosts from the larvae was varied from 10 cm. to .5 cm but there was no evidence of attraction.

One possible reason for this lack of locomotion by the larvae could have been an inability to move on glass although on the occasion that the larvae do somersault on glass there appears to be no difficulty in doing so. However, when pieces of bark with larvae on them were wedged into the tube and similar experiments to those described above were tried, the same results were obtained. Even when the woodlice

are held just out of reach of the larvae, the latter shows no tendency to migrate towards them. It also seems that the larvae are not attracted to the conditions in which their hosts live. No humidity gradients tried caused locomotion and the larvae do not show geotactic or phototactic activity. Although vibrations of the substrate caused seeking movements already described, larvae do not move towards the source of vibration and even when the substrate is gently or heavily scraped within a few millimetres of the larva it does not somersault towards the scraping. Further, the seeking movements with the anterior end are not directed particularly towards the disturbance but are just as random. Sudden illumination and darkening of the larvae causes no activation of the seeking response.

When the seeking anterior end of the larva contacts anything that is moving, it clings on to this with its anterior end and either somersaults on to it or brings up the posterior end behind the anterior. If this moving object is not a woodlouse, then the larva will again readily, after a few seconds, cling on to anything else moving past it, including the substrate, but it will not readily leave a woodlouse once it has attached itself to it. Frequently when larvae are crowded, one larva may become attached to the anterior end of another and remain there, sometimes for hours. Continual transference from one object to another eventually results in the loss of the ability of the larva to transfer itself, although it may attempt to do so. It is probable that there is a limit to the amount of secretion that a larva can produce as, eventually the parasite is much more easily dislodged from the substrate and cannot retrieve its erect posture.

Generally larvae will cling to any moving object, but sometimes when touched even by woodlice they retract and lie against the substrate and will not respond for some minutes. Repeated experimentation indicates that this reaction is due to a number of causes. Thus if larvae are repeatedly mechanically stimulated they no longer respond and then will lie flat when touched or just remain contracted in the erect position but immobile. That is, the larvae may show prolonged sensory adaptation or habituation to this stimulus. However, even if larvae have not been disturbed for a long period, they occasionally respond in the same way. Thus if a larva is touched suddenly without previous stimulation which evokes seeking movements, it often retracts immediately although some seconds afterwards when the same larva is disturbed by air currents or vibrations it readily clings on to a moving object. Larvae are also more inclined to retract if touched well below the anterior end.

In order to determine whether humidity plays any part in the occasional rejection by larvae of moving objects, larvae on bark were confined in an airtight celluloid compartment and left for 30 minutes at 0 per cent and 100 per cent humidity maintained by either solid KOH or a wad of cotton wool soaked in water put in at one end of the compartment. A large pin inserted through a rubber diaphragm at the side of the compartment could be manipulated to reach any of the larvae without disturbing the humidity.

It was found that at 100 per cent humidity most larvae readily attached to the pin when touched by it, while at 0 per cent humidity the majority of larvae either lay flat or just vibrated if touched.

When the needle was moistened, most larvae readily attached themselves whatever the surrounding humidity, and similarly under conditions of 0 per cent humidity larvae attached readily to the leg of a woodlouse (from which evaporation also takes place continually).

Larvae will survive for as long as two weeks at 20°C, 100 per cent humidity, but usually die within 12 hours at 0 per cent humidity.

Attachment to host (Number of observations exceeded 200).

Melanophora seems to be the only one of these parasites which will attach to anything moving which it can reach. The larvae somersault onto moving objects grasping onto them with the anterior end and quickly swinging over the posterior end to maintain a hold.

Usually when a woodlouse moves over them the larvae manage to catch hold of the tibia but with very small hosts they can sometimes reach straight onto the sternites.

As soon as the larva is attached it proceeds to somersault towards the sternites of its host and then makes its way to the inter-segmental membrane. Here it comes to lie in the fold of the membrane and moves along this. Movement now is by hauling, the anterior end digging in and the posterior end being pulled along for short distances. The larva usually makes its way to somewhere in the middle third of the membrane and remains there beginning to penetrate.

Although as stated below, Melanophora larvae only manage to penetrate freshly moulted woodlice, no preference in attachment could be found experimentally. This was hardly expected, since no preference was found between woodlice and other animals.

There was, however, a difference in attachment to

hosts of varying sizes. Large hosts of over 1.1 cm in length usually carry their tibia at a greater distance from the substrate than the length of the larvae and so the parasite cannot reach them. Larvae only managed to reach these hosts when they (the larvae) were attached to projections of the substratum, or when they were touched by the hosts tarsus. Often a large host was found to walk over numerous larvae without any being able to reach it.

Entry (Number of observations exceeded 100)

It was found that whilst larvae will easily make their way to the intersegmental membrane of the host, they usually remained there without entering and soon died. The larvae were often able to insert one or more segments into some hosts but died without getting any further. On other hosts, the whole larval body entered, but the process of entry was as long as two to three days.

First infections of woodlice by Melanophora resulted in 5 per cent and less parasitism. Later, it was found that larvae entered, quite rapidly, newly moulted hosts and experiments showed that the time taken for the larvae to enter was roughly proportional to the time which had elapsed since the host moulted. Thus on hosts which had moulted only two to three hours previously larvae entered within two to four hours. On hosts which had moulted twelve hours previously larvae took four to eight hours to enter. Forty eight hours after the host has moulted the larvae did not complete their entrance for two to three days. Larvae could not enter hosts which had moulted longer than three to four days.

The time for which the host was vulnerable after moulting was affected by its size. Smaller hosts were numerable for longer periods of time.

After entry the larvae protrude vertically from the sternites into the host's coelom, with the last one or two segments protruding from the entry hole. They feed on the host's blood and swell to about one and a third times their previous greatest diameter. When the first stage larva moults after 5 days at 25°C, the second stage larva often retains this posterior connection with the exterior, although some larvae may migrate.

ii) Styloneuria (Number of larvae observed exceeded 2,000)

At 25°C hatching occurs 5 days after oviposition. The hatching lines of the egg are usually split along the whole of their length so that the small intermediate area often drops away from the egg and the larva emerges in the same way as Melanophora.

As in Melanophora the larva is normally seated on its posterior end and at right angles to the substrate and when mechanically undisturbed is in a contracted sacklike condition. (see plate 2). However, unlike Melanophora larvae those of Styloneuria show a preference for attachment to vertical surfaces or to hanging downwards, so that if the larvae hatch from eggs lying on the upper surface of an object they usually migrate to the sides of the object or the sides of ridges on the surface of it. If they hatch from eggs on the underside they remain near the eggs. This migration is independent of illumination or humidity as it will take place in darkness, with illumination from either side or from beneath, and in a range of natural humidities up to 100 per cent. Mechanical stimulus as in Melanophora produces the characteristic exploratory movements but these are slower than those of Melanophora. Gentle air currents seem to provide much more stimulation for the seeking movements than

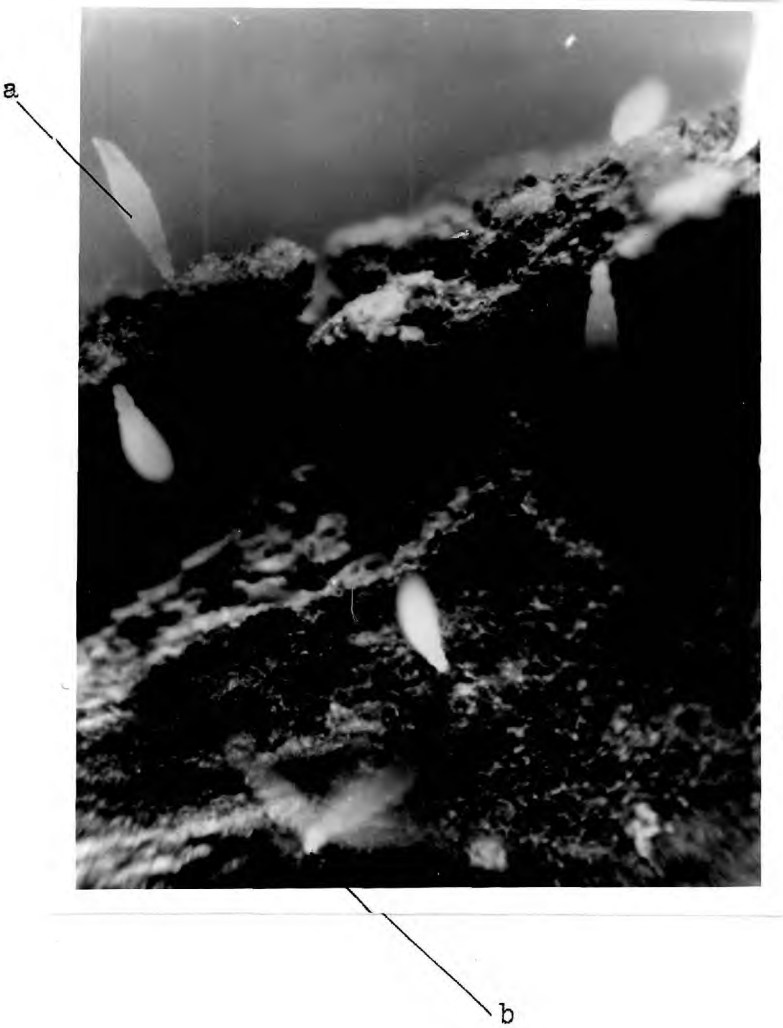


Plate 2 : (x 30), Styloneuria first stage larvae on bark.

a - larva in typical resting position.

b - larva showing typical pivoting on posterior end

jolting the substrate and beside being more rapid the seeking movements continue for longer intervals of time (20 - 45 seconds as compared to 5 - 20 seconds).

As with Melanophora violent jolts of the substrate or strong air currents cause contraction and sessility.

When the anterior end of the parasite contacts a moving object, . if this is not a woodlouse, the whole larva retracts sharply and lies against the substrate. Only rarely when a moistened object is moved past the larva, the larva may somersault onto it.

Experiments similar to those with Melanophora indicate that there is no phototactic response and flashing light has no effect in stimulating the larva. The parasites do not move towards their hosts.

Under experimental conditions Styloneuria larvae did not react to various humidity gradients but on a single occasion a very marked behaviour pattern was observed. Several hundred larvae on pieces of bark were being kept in an airtight plastic box at one end of which was a pad of wet blotting paper inserted to maintain high humidity which is essential for normal longevity of the larvae. After a day the culture was inspected and at least three quarters of the larvae numbering several hundred were found surrounding the wet blotting paper in a dense band although many must have migrated several inches. Despite numerous repetitions with many other larvae not the slightest tendency to this behaviour has again been found and in more subtle humidity experiments, larvae have shown no response to humidity although various ages of larvae previously left for 1 - 4 days at 0 per cent relative humidity and 100 per cent relative humidity were used in the experiments.

As in Melanophora, the posterior end of Styloneuria secretes a mucoprotein or mucopolysaccharide which aids the complex structures of the posterior end in maintaining the larva in an erect posture. This secretion seems to have somewhat stronger powers of adhesion than that of Melanophora so that larvae killed in alcohol have to be heated for some time with KOH before the posterior end becomes free from the substrate even if this is glass.

At 20° C in 100 per cent relative humidity larvae remain alive for as long as one month and even at 0 per cent relative humidity, the larvae live for several days. Even when dropped into 90 per cent alcohol they survive for as long as 3 hours.

Attachment to host (Number of observations exceeded 200)

As has been described above, Styloneuria larvae, when mechanically stimulated by any moving object, will explore in all directions with their anterior ends, but when they contact any object other than a woodlouse (earwigs, beetles and centipedes were tried as well as inanimate objects) they usually retract and often lie flat against the substratum

When the anterior end comes into contact with a leg of a suitable woodlouse host the parasite does not retract but holds on with its anterior end, releases its posterior end from the substratum and then attaches it to the woodlouse leg by somersaulting. It then releases its anterior end.

However, if the tibia of the woodlouse, which is carried horizontally, hits the larva well below its anterior end (as does occur with small woodlice), the parasite lies flat, as it does when touched by objects other than woodlice. A small, and therefore unsuitable, host

thus usually passes over the parasite without the latter attempting to attach itself.

When the larva has attached itself to the host but is considerably disturbed by the movements of the latter (as it generally is when it is attached to the usual position of the host's tibia), it somersaults upward until it reaches a position where it is less disturbed; that is, until it reaches the femur, coxa or sternite of the host, where it remains.

The larvae can only enter a moulting host and an experiment was corducted to test whether larvae attached themselves more readily to woodlice about to moult.

Experiment - to determine whether larvae attach more readily to hosts about to moult.

Method

A few days before moulting, woodlice form conspicuous white patches of calcium deposit on their sternites (Heeley 1941.) It is thus possible by inspection to sort out these woodlice from others.

A number of woodlice with white patches were mixed with woodlice without them (all were females of 1.3 cms in length) and were introduced into a large (1.5 x 4 in ; 3·8 × 8·8 cm tube filled with bark on which a large number of Styloneuria larvae were fairly evenly distributed. The tube was occasionally agitated and after five minutes all the woodlice were removed and the number of parasites on each host was recorded.

The experiment was then repeated with females of 1.0 c ms length.

Results

	with white patches		without white patches	
	HOSTS	PARASITES	HOSTS	PARASITES
A	23	56	19	22
B	15	39	12	10
A + B	38	95	31	32

X^2 = 20.6 shows this result to be very highly significant.

These results indicate only that larvae have preference for woodlice with white patches. This preference for woodlice about to moult however may be much greater. Thus the possible recognition of woodlice about to moult, but yet lacking white patches is not taken into account.

Once the larvae become attached they do not migrate to another host, even if the original one is "unsuitable".

Experiments have shown that even when an unsuitable host had dozens of larvae attached to it none of these would pass onto suitable hosts even if these were kept in close contact for hours.

When the parasite has reached a position on the coxa, femur or sternite, it may remain there sessile for as long as two to three weeks (at room temperature). The body becomes contracted and the larva is so fixed to its host's cuticle that if pulled with forceps it will break at the base.

In connection with this, it was noticed that if larvae become attached to the very thin cuticle of the female oostegite in a short time an opaque patch appears in the otherwise transparent cuticle. Also sometimes, the posterior end of the larva will sink into the cuticle. Possibly a weak chitinase is present in the muco-protein or muco-

polysaccharide secretion from the posterior end and these together fuse the larva to the cuticle. Further evidence of this fusion is in that the larvae remain firmly attached to the host even after they are killed in alcohol etc. They do drop off after immersion for about 30 minutes in 10 per cent Potassium hydroxide.

Entry (Number of observations exceeded 100)

Much time was spent in observing and experimenting with Styloneuria larvae which made no attempt to enter the host. Various temperatures, humidities, surplus water, different sizes of host, newly moulted hosts and wounded hosts, were tried to induce the larvae to enter, but entry was not observed for some time. It was obvious that the larvae were requiring some special conditions to enter the host. Early in the work on this species it was considered probable that the larva was "waiting" for the host to moult, but woodlice which had larvae attached before moulting were still seen externally after the host's moult and although the larvae appeared to squirm in the moulting fluid they did not enter even when the cuticle of the host was again quite hard. Eventually, it was found that these first stage larvae never in fact enter the host, although they are actually "waiting" for the host to moult. The woodlouse moults in two halves. First the posterior half of the cuticle is shed and then the anterior with a period of two to three days between the two stages of ecdysis. As one half is being moulted the larvae migrate from the exuviae onto the wet new cuticle by somersaulting and squirm through the moulting fluid to their place of penetration, which is usually somewhere in the longitudinal middle third of the sternum and usually in the intersegmental membrane although sometimes directly into a sternite.

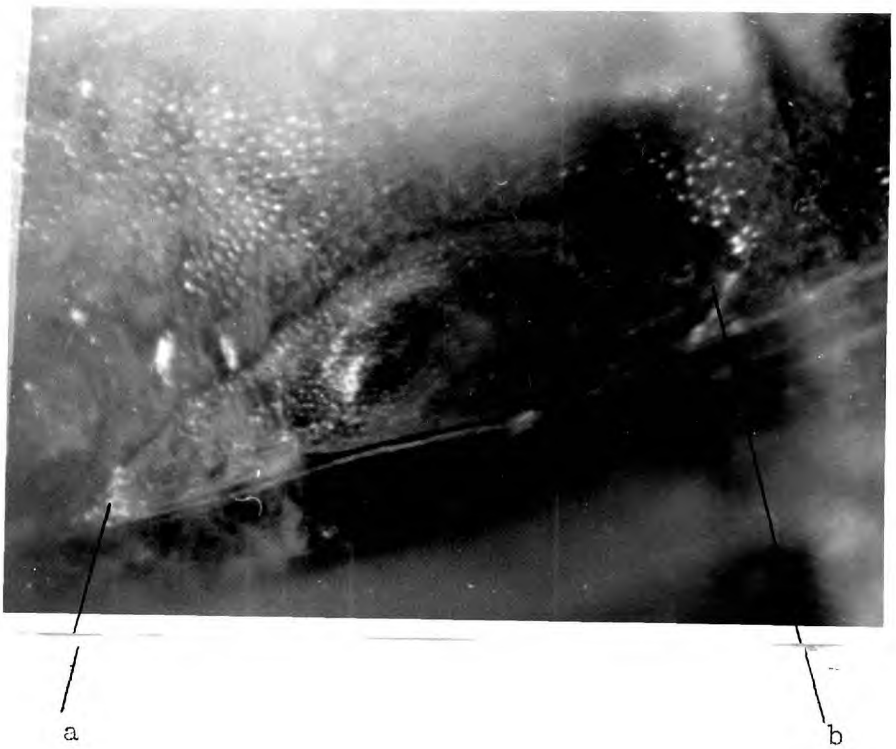


Plate 3 : (x 130) Styloneuria first stage larva penetrating intersegmental membrane of freshly moulted host. The first stage larva never enters any further than this.

a - Posterior end attached to membrane of host.

b - Anterior end penetrating membrane.

This was demonstrated repeatedly by infecting Porcellio about to moult with some twenty larvae to each host. As moulting occurs, all the larvae on the moulting half can be seen somersaulting over the old cuticle and onto the wet new cuticle over which they squirm. The actual moulting only takes a few minutes but invariably all the larvae leave the old cuticle before it is discarded. Larvae on the non-moulted cuticle at the anterior end remain there until this half moults. This, together with the fact that the front legs usually pick up any larvae before the back legs reach them, explains why under natural conditions most larvae were found to have entered the anterior half of the host.

On reaching the intersegmental membrane the larva inserts its head or, at the most, one or two segments, through the soft cuticle and begins feeding on the host's blood. (see plate 3). Over a period of three days it swells very gradually changing from opaque white to slightly translucent. The increase in size is hardly noticeable, the larvae becoming about a third as wide again at its widest point. Whilst feeding, the larva has its posterior end attached to the sternite in front.

After three days the second stage larva "moults into" the host, the cuticle of the first stage remaining outside and uncollapsed, but the second stage larva entering the host until only its last segment bearing the spiracles protrudes to the outside. The whole process resembles an injection into the host of the second stage larva by the resistence set up by the cuticle of its first instar to increase in size.

iii) Plesina

At 25° C hatching occurs twelve days after oviposition. As in Melanophora, the emerging larva splits the egg down both hatching lines

for about a third of their length and the intermediate area is lifted up as the larva squirms out until its posterior end reaches the anterior of the egg. It may then somersault out of the egg but often remains for hours with its posterior end attached to the anterior inside part of the egg and its body laid against the substrate unlike the other larvae of the Styloneuria, Melanophora, Phyto group. Larvae found in the field were often still attached to the egg shell in this manner.

Basically the resting posture of this larva is similar to that of Styloneuria, Melanophora and Phyto. The tongue and lobes at the extreme posterior end of the larva clasp the substrate with the help of a secretion and the posterior few segments are held at right angles to the substrate. The rest of the larva bends at right angles to these posterior few segments so the main body rests against the substrate when the larva is not stimulated.

When stimulated by air currents, the whole larva becomes erect and pivoting on its posterior end describes circular movements, seeking in all directions with its anterior end. After a few seconds of this the larva slowly lowers itself into the resting position.

Larvae very rapidly become habituated to stimulation and after only a few seconds they cease to respond to further stimulation and maintain the resting position.

When larvae are confined in a covered, solid watch glass, they do not respond to mechanical stimulation, but as soon as the cover is removed they begin their characteristic seeking movements. It thus seems that air currents are the main stimulating factors.

As with other Rhinophorine larvae, Plesina larvae are not attracted to the host either from several centimetres or a few milimetres.

Larvae show no tendency to migrate along a humidity gradient although they live for much longer at higher humidities. They are not negatively or positively phototactic and are not stimulated by a flashing light. They do, however, tend to migrate from the under side of objects and to vertical surfaces and migration is by somersaulting.

When the seeking anterior end of the larva contacts a mobile object, it usually retracts and lies flat if this is not a woodlouse, as do Styloneuria larvae. However, occasionally when another object is moistened, larvae will attach to it.

Insufficient larvae were available to determine whether there is any preference for attachment to woodlice at different stages of moulting cycle. However, few larvae would attach to the legs of freshly moulted hosts.

When contacting the leg of a host, the larva usually somersaults onto it, although sometimes it will lie flat and not attempt to attach itself.

Entry (number of observations - 30)

Because of a shortage of first stage larvae of this species (see oviposition section) and the fact that only a very small proportion of the larvae introduced to hosts actually entered, much less has been found out about the entry of this species than about any of the other six.

Only about thirty hosts in all were successfully infected out of several hundreds to which one or more larvae were introduced.

It had previously been found that Melanophora larvae would only enter hosts which had recently moulted and that Styloneuria larvae

would only enter hosts in the process of moulting. Such hosts were, therefore, tried with Plesina larvae. At various times some fifty freshly moulted Porcellio scaber were each supplied with one or more larvae and most of these were individually observed for several days. Although many of the larvae disappeared, when the woodlice were dissected from one to two weeks afterwards, not a trace of a larva was found in any one of them.

About a hundred Porcellio scaber with "white patches" were each supplied with one or more Plesina larvae and most of these were individually observed for several days. Nine larvae were observed in the act of entering before the host moulted. Three entered the intersegmental membrane between the anterior sternites; two entered the membrane between the epimerites; and four crawled between the pleopods before entering the membranes at the base of these. Entry in all cases took at least one day. Larvae entering between the epimerites had their posterior segments at right angles to the membrane protruding into the air while the exposed segments of those larvae entering between the sternites lay horizontally in the membrane fold. Dissections of these hosts after one week showed that all larvae had then moulted into the second instar. All the remaining hosts infected were dissected but only five were found to be successfully parasitised. Although larvae often remain attached to a leg or sternite for one or two days, they usually disappear after this time and do not hang onto the host until the moult as do Styloneuria larvae.

About two hundred Porcellio scaber of various sizes, none of which had either recently moulted or was about to moult, were each supplied with one or more larvae. Five larvae were observed to enter the intersegmental membrane between epimerites of their host and larvae took one or two days to penetrate fully. The hosts of these latter were

dissected after three days and two of the larvae were found to be in their second instar while the rest were still first instars.

Only thirteen of the remaining woodlice were found to be successfully parasitised when dissected two weeks after infection. There were four second stage larvae in one of these, two in another and one in all the rest.

These results suggest that entry of Plesina larvae is independant of the moulting cycle of the host and that the reasons for successful entries are yet unknown.

iv) Phyto (Number of observations exceeded 500)

At 25° C hatching of eggs occurs five days after oviposition. As in Styloneuria, the hatching lines of the egg are usually split along the whole of their length and the small intermediate area often drops away from the egg as the larva emerges.

At relative humidity near to 100%, the larvae usually remain near the eggs although they migrate by somersaulting to the sides and undersides of objects if they are not already there.

As in Melanophora and Styloneuria, the larva is normally seated on its posterior end and at right angles to the substrate in a relatively contracted sac like condition. Slight mechanical stimuli particularly air currents, cause rapid, circular, seeking movements as in Melanophora, Styloneuria and Plesina and like these latter species, violent mechanical stimuli cause contraction and sessility and the larvae often lie horizontally against the substrate for several seconds.

Experiments similar to those used with other species indicate that larvae are not attracted to their hosts; they show no

phototactic responses and they are not stimulated by flashing light.

However, unlike Melanophora, Styloneuria and Plesina larvae, Phyto larvae readily migrate along a humidity gradient produced by solid potassium hydroxide at one end of a tube and cotton wool soaked with water at the other. Although ten repetitions of the experiment were made, using five larvae in each case, all larvae migrated by somersaulting, up to the end of the tube containing wet cotton wool.

The presence of a humidity response in larvae of this species, when it is absent in others, is surprising since Armadillidium vulgare, the host of Phyto, is much less dependant on high humidity for its distribution than is Porcellio scaber.

Attachment to host.

Like Styloneuria, Phyto larvae will only attach themselves to their normal hosts and although they will explore with their anterior ends in all directions if mechanically stimulated, they will retract and lie flat against the substrate if they contact anything other than Armadillidium.

In contrast to Styloneuria which usually attaches to the leg of its host, Phyto larvae become attached to the epimerites, uropods and sometimes the antennae and pleopods as often as to the legs. This probably occurs because these structures tend to be carried much closer to the substrate in Armadillidium than in Porcellio and so are within easy reach of the larvae.

When attaching themselves the larvae somersault onto the host as does Styloneuria. Woodlice of any species other than Armadillidium are definitely rejected, the parasite lying flat when these foreign hosts pass over.

Once attached to the host, the larva somersaults its way to the sternum, if not already there, and then seems to wander at random on the underside of its host exploring all around it with its anterior end. Finally, often after a period of several hours, the larva finds its way to the base of the penis if the host is a male or to the corresponding position on the female, which is immediately in front of the first pair of pleopods in the midline.

It was often observed that when the host rolled into a ball, as it does when disturbed, any larvae at the anterior end took a short cut directly onto the pleopods of the host.

Entry (No. of observations - 50)

Usually larvae on males were observed to approach the base of the penis by squirming between the penal lobes and pleopods. On reaching the soft membranous area on the underside of the penis the larvae begin penetration. When the host is a female the larvae find their way between the anterior pleopods and burrow into the membrane.

Within 24 hours the whole body except for the last segment enters the haemocoele and projects into it. A respiratory connection is maintained with the exterior by the protrusion of the last segment which bears the spiracles.

The larvae feed on the blood and moult five days after entry, the second stage maintaining, at least at first, a respiratory connection through the entry hole.

v) Rhinophora (Number of observations exceeded 500)

At 25°C hatching of eggs occurs five days after oviposition provided that the relative humidity is near to 100%. At low humidities

the developing embryos die.

During emergence, one or both hatching lines of the egg are split along about one third of the egg length. The emerging larva waves around its anterior end until the substrate is reached. It obtains a purchase possibly with the mandibular sclerites, and then the rest of the body is hauled out of the egg.

This is also the normal method of locomotion, the larva stretching out its anterior end and then hauling the rest of its body up behind so that its anterior half is raised into a loop.

When at rest, the larvae lie flat against the substrate unlike those of the Melanophora - Styloneuria group.

However, together with the other species, the main stimuli to which Rhinophora larvae respond are mechanical. When stimulated by slight vibrations of the substrate or by gentle air currents, the anterior or posterior three quarters of the larva is reared into the air and waves about through a sector of about 120° in front or behind its attached end.

Whether one or the other end is reared up appears to depend on chance when the stimulus is vibration of the substrate, but when this behaviour is stimulated by air currents the larva frequently explores with the end nearest to their origin.

When kept at 20°C and 100% relative humidity, Rhinophora larvae will live for a week or more, but at relative humidities much less than this, they die within a few hours. Despite this, it has not been possible to detect any humidity response in the larvae.

The larvae do not migrate towards woodlice of any size or condition, and they show no phototactic response although a certain

amount of random locomotion does occur.

Although they will rear up in response to mechanical stimulation, they do not cling to objects other than woodlice of a certain size.

Attachment to host.

When lying on the substrate the larvae of Rhinophora are rarely able to attach to any woodlice although they do rear up either the anterior or posterior ends in attempts to attach themselves. A Rhinophora larva cannot cling to the legs of a woodlouse but must reach the sternites before it can maintain a hold. As the larva can only rear to a height which is much less than its own length (9 mm), it cannot attach itself to woodlice in which the sternites are held further than this distance above the substrate.

Only when a suitable size of woodlouse (length 3 - 5 mm) remains over a larva for sometime does the larva manage to cling on to it. Larvae appear to be able to attach themselves much more easily to woodlice when these are crawling on the underside of objects and the larvae are hanging downwards.

Once on the host the larva crawls along a ventral inter-segmental membrane. Its seticular processes ("pseudopods") appear to be used to wedge it between the sternites so that even if the host is active and dorsal side up, the larva once in position does not fall off.

Entry (Number of observations exceed 100)

In this way the larva slowly crawls to a base of a leg where it punctures the thin basal membrane and over a period of two or three days penetrates segment by segment until it has completed entrance into

the haemocoel of the host.

The seticular processes, probably serve as barbs securing the larvae into the membrane of the host while the latter is very active. The posterior portion continues to be wedged between the sternites before complete entry of the parasite.

The larva eventually lies parallel to its original position before entry, and along the inside of the intersegmental membrane and it does not project at right angles into the haemocoel.

It then feeds on blood and slowly increases in size before an obligatory diapause sets in.

vi) Stevenia (Number of observations 60)

The hatching of eggs in this species occurs in three days at 25°C after oviposition, i.e. sooner than in any of the other Rhinophorinae described.

As the larva emerges it usually splits the egg down both hatching lines for about one third of their length. The larva stretches its anterior end out, gains a purchase on the substrate with its mandibles and then hauls the rest of its body from the egg.

Just as Stevenia Stage 1 larvae resemble those of Rhinophora and Frauenfeldia in structure, so is the general behaviour similar.

The mode of locomotion is by the looping method, the larva obtains a purchase on the substrate with its mandibular sclerite and head and then draws up the rest of the body into a loop behind, before it again extends its anterior end.

The most obvious response of the larva is to mechanical stimuli. The larva rears up its anterior or posterior half in the

presence of vibrations of the substrate or slight air currents. The posterior end is reared up more frequently, presumably in order to make use of the complicated posterior furca. The larva will not cling to anything except their normal host.

Because of the great rarity of Stevenia, comparatively few replicates of experiments with the larvae were possible. As far as it was possible to determine, the first stage Stevenia larvae are not attracted from a distance by their hosts (Porcellio rathkei). The tests used were the same as those adopted for the other species.

When larvae were exposed to a sharp humidity gradient (0% to 100%), they eventually migrated towards the saturated end. Generally however, in medium to high relative humidities they tend to take up positions on vertical surfaces and then to remain quite still unless they are mechanically disturbed.

At a high humidity and at temperatures varying between 20 to 25° C, larvae of one culture remained alive for two weeks.

Attachment to host.

Stevenia larvae will only readily attach to Porcellio rathkei. While similar in behaviour to Frauenfeldia in many respects, rearing in Stevenia is more often with the posterior end than with the anterior end. The mid-ventral moveable projection of the last abdominal segment which has no homologue in the other species, is used for the attachment to the host.

As with Frauenfeldia and Rhinophora this species cannot, or does not, attach itself to the legs of its hosts but only to the sternites.

In the field, most female Porcellio rathkei have eggs in their brood pouches when the first generation of Stevenia larvae are hatching. This considerably lowers the sternum of the woodlouse so that Stevenia larvae can reach up and catch on to the brood pouch of most specimens. The mid-ventral projection mentioned above appears to be dug into the soft brood pouch, acting as a barb while the larva hauls itself up. At the time when the second generation larvae appear in the field there are only a few female hosts with brood pouches and therefore the larvae can only attach themselves to small hosts.

Once on the brood pouch or sternites of its host, the larva may remain there for one to three days often staying in one place for a few hours and then moving to another. The reason why some larvae begin penetration after one day and others await two or three days has not been discovered. Possibly feeding takes place during this pre-entry phase. Certainly when a brood pouch is present some nutrient fluid exudes between the oostegites of the host and sometimes a little blood is present at the leg bases and on intersegmental membranes.

Entry

After the variable period of inactivity the larva makes its way to the base of the front right leg and begins penetration. Several dozen penetrating larvae have been observed and yet invariably this is the place of larval entry. There is no obvious internal or external structural difference between the base of the front legs and any other legs and no possible reason for this peculiar behaviour can be suggested. Possibly the front legs may be more suitable in females with brood pouches since the front legs are left free of it, but parasites on hosts

without brood pouches will also enter the front right leg.

It is also interesting that on the few occasions when Stevenia larvae have been found to enter Porcellio scaber on no occasion were the front legs the areas of penetration.

The actual time taken for complete entry varies from two to four days. The first few segments enter more rapidly than the others and these swell quite considerably more than the posterior ones. Eventually, the whole larva except for the last segment enters and projects into the haemocoel. Moulting takes place after six days, the second instar maintaining its respiratory connection with the outside.

vii) Frauenfeldia (Number of observations - 40)

At 25° C hatching occurs after an undetermined period which is less than six days.

Frauenfeldia larvae have a general behaviour which is very similar to that of Rhinophora. They are not attracted to their hosts; they show no phototactic or humidity responses, but they do tend to migrate to the sides and undersides of objects. Locomotion is by means of looping as in Rhinophora and Stevenia.

As in the other Rhinophorine first stage larvae, the most obvious response is to mechanical stimuli. The larvae flick their anterior or posterior ends in response to slight vibrations of the substrate or to slight air currents, but remain in a contracted condition when exposed to violent stimulation.

Larvae have remained alive for three weeks in 100 per cent relative humidity but unlike larvae of Rhinophora they take several days to die when left at much lower relative humidities.

Attachment to host

Like the larvae of Rhinophora, those of Frauenfeldia ca nnot attach themselves to the legs of woodlice but only to the sternites of their host. Woodlice of length greater than 9 mm are not vulnerable since the larvae cannot reach their sternites and woodlice less than 5 - 6 mm in length also do not prove suitable presumably because the parasite has no room to extend itself underneath the host and cannot, therefore, get a firm hold.

Entry (Number of observations - 30)

After attaching itself the larva rapidly assumes a position between two sternites, wedging itself between these with the aid of its "pseudopods". It then moves slowly along between the two sternites until it reaches a leg base. Here it begins to penetrate the thin, basal membrane and throughout a period lasting between one and three days, the first five segments enter the host and project into the haemocoele (see plate 4). The six segments remaining outside are held between the sternites by the seticular processes and never ir fact enter the host (see plate 5). These segments do not noticeably swell throughout the 17 - 19 days which precede moulting. However, periodic dissections of the infected host show that the anterior portion is at its widest part some three times the diameter of the posterior portion.

Moulting now occurs and the posterior half of the larval cuticle remains uncollapsed and attached to the basal membrane. The second stage larva does not maintain a respiratory connection at its place of entry, but migrates into the haemocoele with the anterior half of the first stage cuticle attached around its last segment.

It is of interest to note that the seticular processes of the anterior five segments which enter the host are different from those on the posterior six in having strong setae mounted terminally. Possibly these setae act as barbs during entry.

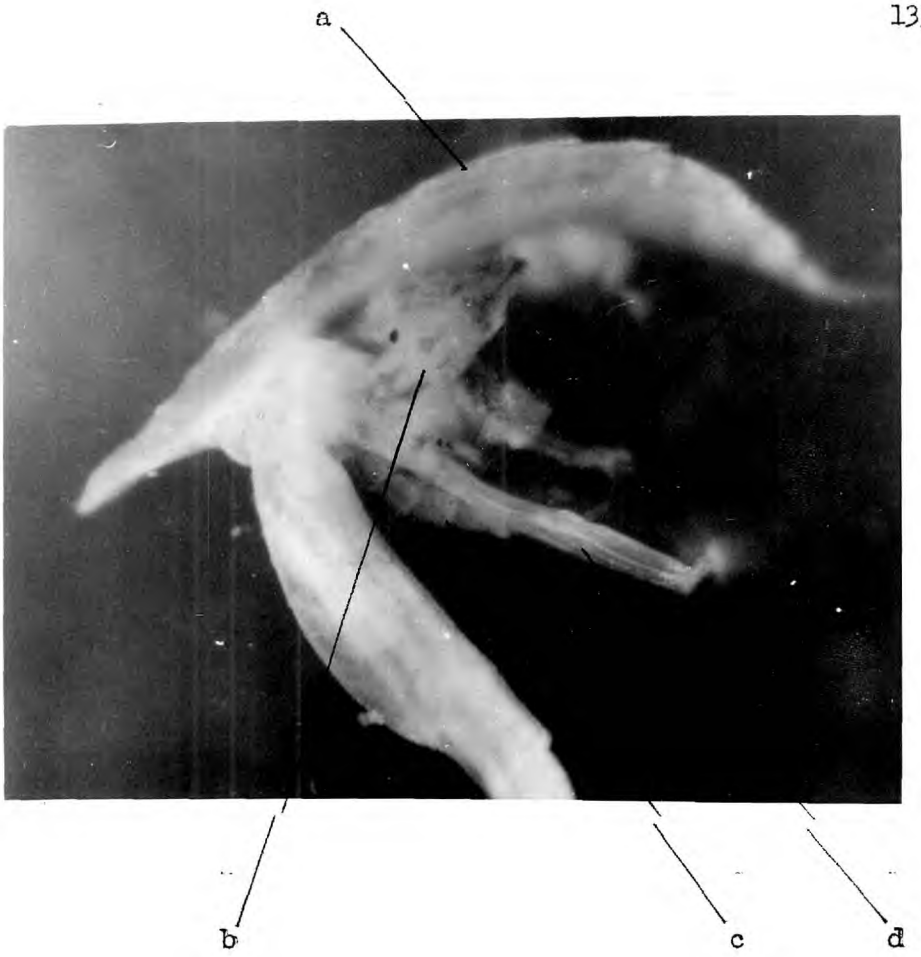


Plate 4 : (x50) Section of woodlouse with Frauenfeldia larva stage 2 penetrating one week after infection.

a - tergum of host

b - anterior half of larva within host considerably distended

c - posterior half of larva outside host not distended

d - sternum of host

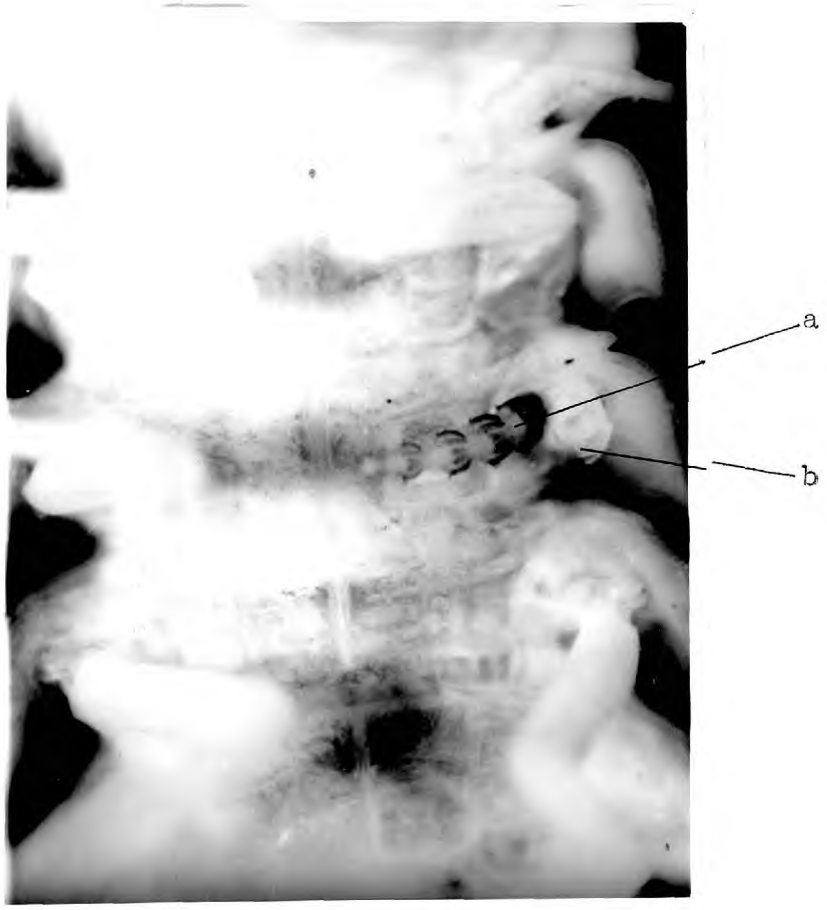


Plate 5 : (x 50) Ventral view of a woodlouse with Frauenfeldia first stage larva penetrated. The larva remains like this until it moults.

a - posterior half of larva
b - leg base of host

6. RESPIRATION AND NUTRITION OF SECOND AND THIRD STAGE LARVAE.

6a) Respiration

W.H.Thorpe tested a number of Plesina second stage larvae by the "biological indicator" method using a culture of Polytoma (Thorpe 1932) for W.R.Thompson (1934), and showed conclusively that cutaneous respiration was going on in this species.

Thompson (1934) considers that "it is possible that the larva obtains the greater part of its oxygen supply from cutaneous respiration".

While Thompson (1934) states "that the larvae often do lie in the integumental funnel with their posterior spiracles in direct communication with the external air, there is no doubt", he makes no distinction between the different species of parasites concerned.

During the present investigation it was found that the second stage larvae of some species almost invariably have an external respiratory connection while others almost invariably do not.

Thus while the second stage larvae of Styloneuria and Melanophora almost always have, and those of Phyto and Stevenia usually have, their posterior spiracles protruding from the host, the second stage larvae of Plesina, Frauenfeldia and Rhinophora rarely have any direct connection with the exterior.

In the four former species, the second stage larva usually maintains its external respiratory connection through the opening made by entry of the first stage larva. The almost spherical posterior end of a Styloneuria second stage larva may protrude right through one of the

ventral sclerites of its host, but more usually protrudes through an intersegmental membrane. More often than not the respiratory connection of this species is found at the anterior half of the host and very rarely as far posterior as the pleopods. Melanophora larvae have their external posterior connection through the intersegmental membrane or sometimes between the pleopods, but never directly through the sclerite. In both these species active respiratory movements of the protruding last segment continually take place, the posterior face of the segment being contracted inwards and then relaxed. When the posterior end of a larva is covered with water, quite vigorous movements occur until the posterior end is once more in contact with the air.

The external respiratory connection of Phyto larvae is through the thin membrane at the penis base or through the corresponding position in the female host and is usually covered by the pleopods. Sometimes the posterior end of a Phyto second stage larva becomes dislodged from this place of first stage larval entry, and becomes attached ventrally elsewhere but then it never penetrates to the exterior.

Stevenia larvae are usually found with their posterior ends either applied to the inside of, or actually penetrating the basal membrane of the first right leg of their host.

While only twenty second stage larvae of this species have been examined, it does seem that older larvae are less likely to maintain an external connection than younger ones.

Second stage larvae of Frauenfeldia and Plesina lose contact with the exterior at the beginning of the instar. Plesina second stage larvae are very rarely found with an external posterior

connection although a few have been found with spiracles protruding through the membrane between the epimerites. Usually the last segment of this species is anchored in the ventral connective tissue or quite often it is attached to the thin membrane above the pleopods. Presumably the forwardly-directed spines found on the last segment of the second stage larva of this species and of Frauenfeldia aid in anchoring the posterior end to the inside of the host. Frauenfeldia second stage larvae sometimes have their posterior ends anchored in the ventral connective tissue of the sternites, but are often found anchored to the inside of the tergites. Rhinophora second stage larvae are usually anchored to the ventral sclerites of their host. Neither of the second stage larvae of the latter two species, nor the fully entered first stage larvae of Rhinophora have ever been found with an external respiratory connection.

The second stage larvae of Frauenfeldia, Rhinophora and Plesina must obtain most of their oxygen through their general body surface by diffusion from the blood of their host although some oxygen may be obtained via the posterior spiracles which are closely applied to the inside of the fairly permeable host cuticle.

In all species the posterior spiracles of the third stage larvae are in direct communication with the exterior soon after the beginning of the third instar. Where a respiratory connection with the exterior was maintained by the second stage larva, the spiracles of the third stage larva usually remained in the same position. In species where the spiracles of the second stage larva are not protruded to the exterior, the third stage larva usually protruded its spiracles through the pleopods of its host.

6b) Nutrition Similar for all species.

(Detailed observations on Styloneuria and Frauenfeldia larvae).

As was confirmed by adding carmine solution, the second stage larva observed in blood outside the woodlouse only feeds spasmodically and little, but the third stage larva is very vigorous and continues to feed throughout the instar. Even at the beginning of the third instar, the larva can often be observed through the intersegmental membrane of its host probing with its anterior end in all directions of the haemocoele and continuously protracting and retracting its mandibular sclerites.

Numerous dissections have revealed that for at least the first half of its instar, the third stage larva feeds only on blood. All the organs and fat body of the host then appear to be intact although little or no food is found in the gut. However, two or three days before pupation the third stage larva begins to feed on the gonads and fat body of the host as well as on blood. If a larva is removed from its host at this time and supplied with fat body in blood, it rapidly drills through this with its mandibular sclerites moving several times per second. Such behaviour is not observed in the young third stage larvae.

Usually about one day before pupation of the parasite, the host becomes more and more sluggish and finally dies. At the same time the posterior end of the parasite is withdrawn into the host.

Soon after the death of the woodlouse, its dorsal tergites gradually tend to become somewhat transparent centrally due to the loss of the integumental pigment. Gradually during the time before pupation of the parasite the whole cuticle of the host loses its pigment (which is found in the hypodermal cells of the integument) and the squirming

larva can be seen quite clearly through the now semi-transparent cuticle. Waves of contraction continually pass from the posterior end of the larva anteriorly and the larva squirms in all directions so that the contents of the host appear to be continuously circulated by the parasite.

When the host is dissected at this stage, all the body contents except the gut are found to be liquefied, even the muscles of the appendages. The gut remains semi-digested and resembles a tarry strand which adheres to the integument of the host. This is a characteristic of all the seven species of parasites examined.

The liquefaction of the host body contents suggest that the parasitic larva was probably secreting a proteolytic enzyme.

The Charney and Tomarelli (1947) method of testing for a protease was used.

Method

Azo-albumin was made from the fresh white of egg. The body contents of a woodlouse liquefied by Styloneuria parasite were tested for protease by adding them to the Azo-albumin using a phosphate buffer of 7.5 pH. However, despite repeated attempts, no significant release of dye was observed.

The body contents of a dead parasitised woodlouse were then measured for their pH value and were found to be 9 - 10.

The experiment was then repeated with a phosphate and borax buffer of pH 9 and yielded a significant release of dye after an incubation period of 18 hours.

Using a reagent blank, which had been incubated with boiled experimental solution, as a control, the Eel photometer was calibrated to

give a reading of zero with this and then the following experimental solutions, incubated with azo albumin for 18 hours were tested.

1) A solution of the contents from 5 woodlice killed by third stage Styloneuria larvae.

2) Water in which 5 washed three-day-old third stage Styloneuria larvae had been left for one hour.

3) Washings of the black fluid faeces formed at the anus of 5 third stage larvae in one hour.

4) A control of washings from the larvae used for solution 2.

Results

Solution 1) Produced a deflection of 13 from the zero of reagent blank.

2) " " 10 " " " "

3) " " 9 " " " "

4) No deflection (control)

The experiment was repeated and similar results were obtained.

It thus seems probable that a protease is released from the anus in the faeces of the third larval stage when this is withdrawn into the host, and that this enzyme is responsible for liquefaction of the host contents which precedes pupation of the parasite. Possibly the action of the protease is also responsible for the death of the woodlouse.

Such behaviour enables the larva to utilise otherwise inaccessible food material even from the appendages and hypodermis of its host. Almost every drop of fluid is eventually imbibed by the larva so that it pupates within the completely empty cuticle of its host.

6c) Method of Establishing Duration of Larval Instars.

First Instar.

Batches of ten hosts were dissected at intervals of twelve hours after first stage larval penetration of Melanophora, Phyto, Plesina and Stevenia. It was possible, with the first stage larvae of Styloneuria and Frauenfeldia, which do not fully enter the host before moulting, to observe the time of moulting without dissection. Hosts parasitised by Rhinophora first stage larvae were dissected less frequently, since the first instar of this species lasts for considerably longer than that of the other species.

Second Instar.

The duration of this instar can obviously be found by subtracting the length of the first and third instars from the total time taken from penetration of the first instar to pupation of the third.

Third Instar.

Determination of the length of the third instar of Styloneuria larvae is relatively easy since the dark gut of the parasite, which can usually be seen through the host's sternites facilitates finding its posterior spiracles which protrude from the host. As the posterior end of the third instar is conspicuously different from that of the second, the change from one to another is immediately apparent. The duration of the third instar was taken as being the length of time from this change of the posterior end, until pupation of the parasite. With this species, several dozen specimens were observed, although little variation in duration occurred.

In the second stage larvae of Melanophora, the gut is very pale and rarely visible through the sternites of the host, but sometimes the posterior end can be seen slightly protruding, and can thus be observed to

change to third instar. Some twenty parasitised specimens were observed in this way.

The second stage larvae of Plesina occasionally protrude their spiracles between the epimerites and the change can then be observed, but although hundreds of host specimens were inspected, only three such cases were seen. One method used for finding the change from second to third instar larvae of Plesina, Stevenia, Rhinophora and Frauenfeldia, was to search through cultures for hosts in which the parasite could be seen through the cuticle and the change was then observed. However, such specimens were rare and often lost their value when the host cuticle lost its transparency before completion of the parasite second instar.

In the species where the posterior spiracles do not usually protrude until the third instar, suspected hosts were continuously inspected until these appeared. As the parasite does not invariably protrude its spiracles as soon as it moults, the longest times observed from protrusion of spiracles to pupation, were taken to be most reliable.

It is almost impossible to ever see the second stage larvae of Phyto through the thick cuticle of its host Armadillidium. However, it was sometimes possible to see the posterior spiracles of the second stage larvae by lifting the host's pleopods. The posterior spiracles of the parasite are often applied to the thin cuticle beneath these.

6d) Table showing time in days required for completion of various stages in the life history of Rhinophorinae. (at 25°C)

Spp.	Minimum age at oviposition	Oviposition to eclosion	Duration of 1st instar after penetration.	Minimum duration of 2nd instar	Duration of 3rd instar	Pupation to emergence
S.	2-3	5	5	20	5	11-12
M.	0	7	5	10	4.5	12-13
P.	2-3	12	3	?	4-7?	12-13
Ph.	3	5	5	8	5	11-12
R.	3 (20°C)	5	diapause	2-6?	3-5?	12-13
F.	7	6	17-19	?	?	12-13
Ste.	5	3	6	3.5	4-5	10-11

Key to abbreviations

S - Styloneuria

M - Melanophora

P - Plesina

Ph - Phyto

R - Rhinophora

F - Frauenfeldia

Ste - Stevenia

7. HOST SPECIFICITY

Thompson (1934) records the results of dissections of 1290 Porcellio scaber, 347 Oniscus asellus, 100 Metaponorthus pruinosus, and an unstated number and, therefore, presumably few Ligia oceanica, Philoscia muscorum, Porcellio dilatatus, Cylisticus convexus and Armadillidium vulgare.

He found Porcellio scaber to be parasitised by six species:- Plesina maculata, Styloneuria discrepans, Frauenfeldia rubicosa, his species A (Rhinophora lepida), Phyto melanocephala and Melanophora roralis in that order of commoness, and although from some localities only small numbers were dissected, Porcellio scaber were parasitised in 13 out of the 15 localities.

In Oniscus asellus he found 11 out of 151 specimens parasitised by Styloneuria discrepans or Plesina maculata in one locality (from which he also collected a large number of parasitised Porcellio scaber) but in the other 8 localities from which a total of 196 Oniscus were dissected, none was found to be parasitised.

The only other species of woodlice from which Thompson obtained a parasite was Metaponorthus pruinosus. From 16 specimens collected in France, one Cyrilla augustifrons was dissected although a further 84 dissected from 2 localities in Britain yielded no parasites. Thompson was also aware that Donisthorpe (1908) had reared Phyto melanocephala from Armadillidium vulgare.

On the basis of his results and evidence that Cercarea larvae of the same species were found in Oniscus and Porcellio, a Coccidian in Porcellio, Oniscus and Philoscia, and Echinorhynchus larvae of the same

were dissected from P.scaber, A. vulgare and Philoscia muscorum, Thompson (1934) suggests that "the dipterous parasites of woodlice could develop in individuals of almost any terrestrial isopod and that the prevalence of most species in Porcellio scaber and Oniscus asellus is due principally to the fact that these species live under conditions favouring the attack of dipterous parasites". This statement is made despite the fact that later, he mentions that the larvae of Plesina maculata and Styloneuria discrepans found in Oniscus asellus are more frequently surrounded by phagocytic envelopes than in Porcellio scaber.

Results from the present work lead to conclusions which are in opposition to those of Thompson.

7a) Field populations

In all,23,184 woodlice have been dissected from many diverse habitats and 2,482 parasites have been recorded from these, (see tables of the appendix).

Summary of the results of dissections of different species of woodlice.

No. of woodlice dissected		No. of parasites found	
17,055	Porcellio scaber	1,653	Plesina
		411	Styloneuria
		218	Melanophora
		90	Rhinophora
		39	Frauenfeldia
		2	Phyto
2,677	Oniscus asellus	14	Plesina
		11	Styloneuria
1,758	Armadillidium vulgare	35	Phyto
117	Porcellio rathkei	9	Stevenia
516	Metaponorthus pruinosus	0	
383	Philoscia muscorum	0	
212	Ligia oceanica	0	

No. of Woodlice dissected	No. of parasites found
(Continued)	
187 Metaponorthus cingendus	0
100 Armadillidium depressum	0
53 Cylisticus convexus	0
26 Porcellio laevis	0

In the present set of dissections, out of the seven parasites recorded from woodlice in Britain, Stevenia atramentaria has only been found on Porcellio rathkei (a previously unrecorded host of Rhinophorinae), Phyto melanocephala has only (with the exception of two second stage larvae found in P.scaber) been dissected from Armadillidium vulgare, and Rhinophora lepida, Melanophora roralis and Frauenfeldia rubicosa have not been taken from any species except Porcellio scaber. The remaining two species, Styloneuria discrepans and Plesina maculata, while normally found in Porcellio scaber were occasionally present in Oniscus asellus.

Other species of woodlouse which have been dissected are Philoscia muscorum, Porcellio laevis, Ligia oceanica, Cylisticus convexus, Metaponorthus pruinosus and Metaponorthus cingendus. No dipterous parasites were found in these although in many cases they were found living in the same habitat and often in the same microhabitat as parasitised Porcellio scaber.

Results of dissections relevant to observations on host specificity are found in tables of the appendix. As can be seen from these, when Oniscus asellus has been collected from habitats where few or no Porcellio scaber parasitised by either Plesina maculata or Styloneuria discrepans are present, it is extremely rare for Oniscus to be

parasitised. When Oniscus is found living with Porcellio parasitised by either of these two species, generally a comparatively low percentage of parasitised Oniscus are also found. From this it cannot be concluded that Oniscus is a successful host to these species since most parasites, even at the time of collection, are usually heavily encapsulated and moribund and, although cultures of Oniscus parasitised by both Plesina maculata and Styloneuria discrepans have been kept in captivity, no single parasite has ever been found to survive longer than the second instar.

Thus when 100 Oniscus from a population at Beddington Park (1d) (of which 25% were parasitised by Plesina) were kept at 25°C for 3 months, it was found on dissection that all parasites had been heavily phagocytised and killed in the 2nd larval stage, despite the fact that Porcellio scaber collected at the same time from the same habitat produced the expected numbers of parasites within a few weeks.

Similarly, 250 Oniscus from Woolwich from a population with about 5% parasitism, when dissected after 10 weeks at 25°C were found to contain only dead encapsulated Styloneuria larvae at stage two. In both these cases, however, the larvae had reached about three quarter full size before being killed.

On other occasions (see appendix tables) other species of woodlice, particularly Oniscus, have been found intermixed with Porcellio scaber which was parasitised by Melanophora, Rhinophora and Frauenfeldia, and yet none of these species has been found in any host other than Porcellio scaber.

Thus when Porcellio scaber (usually well parasitised by

is found beneath rocks on the sea shore it is often accomp-

anied by large numbers of Ligia oceanica which show no trace of parasitism.

With regard to Phyto melanocephala of which Thompson (1934) found 13 from a total of 1,290 Porcellio scaber, I, however, only found two specimens (2nd stage) in all of the several thousand Porcellio scaber dissected. This is despite the fact that from some habitats (9, 11, 48, 50, 55, see tables of the appendix) from which a total of 483 P.scaber were dissected, the P.scaber were actually mixed with parasitised Armadillidium. Experimental results given below further indicate that P.scaber is an unsuitable host for Phyto. In the above examples it should be noted that the Porcellio scaber mixed with the Armadillidium were themselves usually parasitised by other species none of which were present in Armadillidium.

Apparently the only record of Stevenia atrementaria being reared from a woodlouse host is that of V.Roser (1840) who records a "Tachinia" atramentaria as being reared from a woodlouse uncertainly identified as Oniscus asellus. However, many hundreds of Oniscus were dissected from many localities near Belvedere and Abbey Wood (the only areas cited for this rare parasite in keys and British museum collection), and no parasites were obtained. Eventually when the parasite was found in Porcellio rathkei from Cliffe (Kent) and two other areas nearby, several hundred Oniscus from the same habitat were dissected without a trace of the parasite as were similar numbers of Porcellio scaber with which Porcellio rathkei were mixed under bark of logs; (see tables of the appendix)

7b) Infection Experiments.

Melanophora larvae have been used to attempt infection of Oniscus asellus, Porcellio rathkei, Porcellio laevis, Porcellio scaber, Ligia oceanica, Cylisticus convexus, Philoscia muscorum and Armadillidium vulgare.

Batches of 50 hosts were used except Cycisticus of which only 15 hosts were available.

Experiments on Oniscus, Armadillidium and Philoscia have been repeated with lesser numbers (20) several times.

In Porcellio scaber, the normal host of Melanophora in the field, penetration is only affected when the host has recently moulted and so in the other species of woodlice larvae were put onto the hosts soon after moulting.

Apart from Porcellio scaber, Cylisticus convexus was the only other species in which Melanophora was able to complete its life history. Puparia were formed in 2 out of the 15 specimens of this species infected.

With Philoscia muscorum the parasite was able to enter some specimens quite readily even some days after moulting of the host had taken place, and the parasite developed into the 2nd instar. At this stage, however, it was found that the parasite usually became moribund, and its growth stunted. In all cases where the parasite had entered, the host died within two weeks although other unparasitised Philoscia in the same culture survived.

As with Porcellio scaber, larvae were only able to enter P.rathkei and P.laevis when these had recently moulted but in these hosts all larvae were killed by encapsulation soon after moulting into stage 2.

Larvae made no attempt to enter even small specimens which had recently moulted of Oniscus, Armadillidium or Ligia, and so larvae were introduced artificially by removing a leg and inserting them through the resulting aperture. (If even a small hole is made elsewhere the

woodlouse bleeds to death, but it can control bleeding from the base of the leg). The larvae so introduced were encapsulated and killed before reaching the second stage.

Styloneuria larvae while they do not usually attach themselves to other species of woodlice besides Porcellio, occasionally become attached to Oniscus and if the host moults soon afterwards the larva penetrates and the second stage later enters. As described earlier, however, the 2nd stage larva is eventually encapsulated and dies before reaching the 3rd stage. When Styloneuria larvae can be persuaded to attach to Armadillidium Philoscia, Ligia or P.rathkei, however, they do not remain in a contracted condition waiting for the host to moult, as in Oniscus and P.scaber, but readily migrate back to the substrate or onto P.scaber if this is present. Although when first stage larvae of Styloneuria are experimentally introduced into these other species they are killed by ~~phagocytes~~ encapsulation, this is not significant since this also happens when they are so introduced to their normal host P.scaber.

Phyto first stage larvae would usually only attach themselves to Armadillidium vulgare, but when they do attach to P.scaber or Oniscus asellus they somersault about on the host presumably because they are disturbed by the host's movements, and eventually migrate onto the substrate. Two hundred Phyto larvae were left with 100 P.scaber in culture but when these woodlice were dissected one week later not a single larva was found to have entered although when Armadillidium is present all the larvae enter. However, when 100 Oniscus were each supplied with a single Phyto first stage larva, about one quarter of these woodlice became infected. Second stage larvae developed but were finally encapsulated and killed and no third stage larvae were produced.

Stevenia atramentaria presents an interesting case of host specificity in which it was found that specimens of P.rathkei are vulnerable to attack by this parasite no matter at what stage of the moulting cycle the host is. Larvae of Stevenia would only attack P.scaber and Oniscus asellus when these had recently moulted. This is possibly because P.rathkei has a considerably softer cuticle than the other two species. Also the parasite did not restrict itself to the anterior right leg base as it does with P.rathkei but entered any leg base when attacking Porcellio or Oniscus.

After entry into either of these two species, the parasite was found to be killed by phagocytes during the first instar and before it had grown to any extent.

This evidence together with that given above indicates that the woodlouse from which V.Roser (1840) reared Stevenia atramentaria was probably wrongly identified as Oniscus asellus. It should be mentioned that when dead, P.rathkei looks very like Oniscus because its pseudotracheae are then obscure while the patterning on the dorsal surface is very similar.

Conclusions

While not all species of these parasites have been tested for host specificity, experiments on those that have, together with evidence from field indicate that generally the Rhinophorinae are strictly host specific by virtue of their physiological incompatibility, lack of attraction by any other than the usual host, as well as by barriers presented by differences in habitats of the hosts.

8. EFFECT OF PARASITISM ON HOST.

8a) Effect on Host Reproductive System.

In his paper on these parasites, Thompson (1934) records that parasitism has a marked effect on host ovaries and suppresses the formation of oostegites. He was unable to find any change in the internal or external characters of parasitised male woodlice.

The ovaries of overwintering female woodlice are thick, divided into distinct clear cut areas representing the ovules which are yellow or whitish and opaque owing to the presence in the egg cells of large masses of fat or yolk globules. Thompson (1934) states that "in parasitised specimens the ovaries are usually thin and flattened and more or less transparent owing to the absence of fat globules. As the parasite larva develops, the ovaries become more and more transparent until eventually they become practically invisible in situ" and "the ovary appears often to be partly empty containing only scattered ovules, usually circular instead of polygonal in form, and with transparent cytoplasm". Thompson considered the reason for this degeneration to be that the parasite larva requires a large amount of fat for its development and draws on the supply of fat contained in the ovaries.

These observations are supported by the present work, although it was found that the degree of degeneration differed considerably from one individual host to another and was also dependant upon the species of parasite present.

Thus, female Porcellio scaber parasitised by Plesina usually have almost completely developed ovaries even when they are heavily super parasitised. Although the ~~ovules~~ oocytes are a little more disordered than

usual, they are full sized thick and opaque. Within the ovary an occasional ~~ovule~~ oocyte appears to be disintegrated, but no other difference appears to exist.

At the other extreme, hosts parasitised by Rhinophora, Frauenfeldia and Stevenia have almost completely "degenerate" ovaries. In the two species which infect only small woodlice even uninfected woodlice have poorly developed ovaries. With parasitism by Styloneuria and Melanophora the host ovaries are usually almost completely degenerate from early infection onwards, but occasionally hardly affected. The ovaries of hosts parasitised by Phyto vary from complete degeneracy to being almost unaffected.

On the basis of this evidence together with information obtained on the time of parasite entry, a theory can be suggested as to what happens to the ovaries after parasitism. Thompson (1934) considered that the ~~ovules~~ oocytes were usually fully developed at the time of entry and then became gradually more and more degenerate as the parasite absorbed more and more fat.

This does not really account for the lack of ovary degeneration in hosts parasitised by Plesina even when these are super-parasitised, or for the very great variation in degree of degeneration caused by other species of parasites.

I suggest that the state of the ovary during parasitism depends mainly upon the state of ovarial development at the time of parasite entry. The parasite suppresses development of the ovary by absorption of fat from the blood and perhaps causes only a little actual degeneration.

Melanophora and Styloneuria larvae enter only just after the host moults. As moulting of the female host is linked with the reproductive cycle Heeley (1941), a moult occuring just before liberation of the eggs into the brood pouch and just after liberation of the brood, it can be seen that these larvae normally enter the host when the ovaries are comparatively undeveloped. Woodlice dissected soon after entry of these species have degenerate ovaries and it must be assumed that the ovaries develop only a little and irregularly after entry. This would explain why ovaries of hosts parasitised by Melanophora and Styloneuria are similar to ovaries of woodlice which have recently liberated eggs into the brood pouch. The former differ from these in being disorganised and with a few ovules partially developed and others not so. The occasional host parasitised by Melanophora and Styloneuria which has almost fully developed ovaries was probably infected during one of the less common moults which was not part of the breeding cycle. When hosts are parasitised by Rhinophora, Frauenfeldia or by second generation of Stevenia, they are immature and the development of their ovaries is suppressed by the presence of the parasite. First generation of Stevenia parasitise large females with brood pouches and these thus have ovaries containing undeveloped eggs at the time of penetration.

The variation of ovarial development in Armadillidium vulgare females parasitised by Phyto larvae is probably related to the fact that these larvae can enter at any time during the breeding cycle.

Plesina larvae occur in the field during early July when most P. scaber have finished with their first brood and have well-developed ovaries preceding production of the second brood, and again in September when the second brood is finished and the ovaries are again developed

Heeley (1941).

It would thus seem that the Dipterous parasites, while capable of suppressing ovarial development do not actually cause much degeneration as was suggested by Thompson (1934).

In woodlice infected by other parasites such as Acanthocephala and also bacterial parasites, the ovaries were also found to be "degenerate", but it is probable that the hosts become infected when they are most vulnerable, i.e. just after moulting.

As shown by Thompson, no oostegites are produced by parasitised females although parasites are, of course, found in hosts with oostegites if the former entered after the host moult producing these.

On no occasion has a parasitised female woodlouse been found with sperm within her spermatheca, although the spermathecae of most adult non-parasitised woodlice do contain conspicuous deposits of sperm. Thus secretion of hormones responsible for mating behaviour is probably inhibited by parasitism.

8b) Effect on moulting of Host.

The only previous reference to any effect these parasites may have on the moulting of their hosts is found in the paper by Thompson (1934). Here the author merely states that "moulting may be somewhat delayed by the feeding of the parasite larva".

The situation is somewhat more complex than this and varies according to the species of parasite. When Porcellio scaber is parasitised by non-diapausing Styloneuria larvae, moulting of the host and the formation of white calcareous patches which precedes moulting

rarely takes place. In the few hosts which do moult, this coincides almost exactly with the moult of the second instar parasite larva into third instar.

Three hundred Porcellio scaber of which about 15% were parasitised by Styloneuria were collected from a Woolwich garden during January 1963. They were each marked along their dorsal surface with a stripe of white paint. After three weeks at 25° C, 220 of them had moulted. These were dissected but none contained parasites. After another week, a further 25 had moulted and were dissected. Two of these contained parasite larvae in their third instar. Forty-four parasite pupae were formed during the next two weeks and all these were within woodlice which were still marked, i.e. which had not moulted.

A further fifty P.scaber were parasitised in the laboratory and then marked. Forty-four of these parasites pupated after four to five weeks at 25° C without the host moulting, but moulting occurred in the other six before the parasites pupated. In these specimens the larvae were observed to change to third instar within one to two days. Unparasitised Porcellio scaber moulted every 2 to 3 weeks.

When 500 P.scaber containing Styloneuria larvae were collected during October to November 1963, and kept at 25°C, most larvae did not pupate for 2 to 3 months. However, moulting of the hosts with these dispausing larvae was not completely suppressed and took place after four to five weeks.

No suppression of moulting was found in P.scaber infected by Melanophora, and Armadillidium vulgare infected by Phyto. Melanophora larvae enter the host just after its moult and usually pupate 18 to 22 days afterwards (at 25°C). In over a 100 marked Porcellio parasitised

by Melanophora, it was observed that moulting of the host approximately coincided with the moult of the parasite from second to third instar and pupation of the parasite took place 4 to 5 days later. Phyto enters Armadillidium during any stage of the host's moulting cycle. However, from a limited number of observations of this aspect (30 parasitised and marked Armadillidium) it seems that pupation again takes place just a few days after moulting of the host so that the duration of the second instar varies from 2 to 3.5 weeks and depends on the stage of the moulting cycle of the host at the time of larval entry.

No significant effect on moulting was observed in hosts parasitised by diapausing larvae of Plesina, Rhinophora and Stevenia.

From these observations it can be seen that only non-diapausing Styloneuria larvae effect host moulting significantly, but in Phyto and Melanophora there is a close coincidence in moulting between moulting of host and parasite.

It is possible that the host moulting hormone in some ways permeates the larval parasites and induces the final larval moult. Knowles and Carlisle (1959) state that Skinner and Carlisle (unpublished) have found that the insect moulting hormone is active in Crustaceans, and that the Crustacean moulting hormone promotes moulting in insects. Karlson (1959) has found the empirical formula of this hormone to be $C_{18}H_{30}O_4$, the unpolarised structure of which would facilitate passage through a lipoid membrane.

The length of larval instars of Styloneuria are longer than the normal period between moults of Porcellio scaber and may have evolved some suppressive factor which stops the host forming the moulting hormone before the parasite larva is itself ready to moult.

If it was just a matter of the effect of a parasite feeding within it which suppresses the host moult, it is difficult to see why no suppression occurs with Melanophora and Phyto larvae, which complete development in half the time of those of Styloneuria.

9. REACTIONS OF HOST TO PARASITE.

Thompson (1934) in writing about "the phagocytic reaction of the host" in his paper on "The Tachinid Parasites of Woodlice" gives only a little information on the reactions of woodlice to their dipterous parasites, but expounds his theory "that phagocytes do not in actual fact possess any inherent disposition to attack invaders". He considered that "the accumulation of phagocytes around a parasite indicates either that it is attached to a tissue in pathological condition or is itself in an unhealthy state". This statement is made despite mention that "larvae infesting Oniscus asellus seem to be more frequently and more thickly coated with phagocytes than those infesting Porcellio scaber" and that larvae in Oniscus often have a rather unhealthy appearance, and may even be dead.

This theory first proposed by Thompson (1930) is now almost entirely discredited. Besides the existence of a formidable amount of circumstantial evidence showing that death of some parasites is due to defence reactions - Strickland (1923), Meyer (1926), Paillot (1928), Lartschercko (1936), Boese (1936), Bass (1939), Schneider (1950), Muldrav (1953) and Walker (1959) - (all quoted from Salt 1963), the considerable amount of experimental data obtained by Salt (1956, '57, '60) provides conclusive evidence that death of at least some insect parasites is caused by encapsulation.

During the present work, it was found that the reactions of woodlice hosts to their parasites are usually conspicuous only when the parasite is in an unusual host although in certain circumstances there is a considerable reaction by the normal host to its parasite.

9a) Reaction of Usual Host to its Parasites

While the great majority of tachinid maggots in their second and third instars are partly or wholly enveloped in a haemocytic sheath by their usual hosts (Salt 1963) this is not true of the dipterous parasites of woodlice.

Thompson (1934) states that "the larvae of dipterous parasites of woodlice appear to pass the greater part of their existence in an "integumental sheath" formed by an invagination of the cuticula and epithelium of the body wall around the posterior extremity of the larva". Salt (1963) quotes Thompson (1934) as saying that accumulations of haemocytes commonly occurred around maggots in their delicate respiratory sheath. This latter statement may give the impression that the whole parasite larva within the woodlouse is completely or almost completely surrounded by a respiratory sheath as is found around the second and third stages of most tachinid parasites of insects. In fact only the last and sometimes part of the second to last segment of those larvae which maintain a respiratory connection with the exterior, are at all enclosed. The rest of the larval body usually remains completely free of any kind of sheath. Around the base of larvae with an external respiratory connection there is often a slight accumulation of phagocytes and often, but by no means always, the integument of the host also invaginates and grows around the base of the larva for a short distance forming a short integumental funnel proper which is quite thick though transparent. Thompson refers to this both as an integumental funnel and an integumental sheath, but the word "integumental" clarifies his view as to its origin. Nielsen (1909) distinguished clearly between

funnel and sheath and it is now generally agreed that the term "funnel" be applied to the structure formed by the epidermal cells of the host and "sheath" reserved for a structure formed by the blood of the host (Salt 1963).

With larvae which do not usually have a respiratory connection with the exterior such as Plesina, Frauenfeldia and Rhinophora, there is no respiratory funnel for most of the larval life although there often is one for a short time after the entry of the parasite when a respiratory connection with the exterior does exist. It thus seems that the usual woodlouse host may often have no reaction at all to the parasite. Phagocytes around the base of the larva probably accumulate around the host's wound, while formation of the respiratory funnel may be a cuticular ingrowth resulting from injury and persisting as the ingrowing edges of cuticle fail to meet because they are forced apart by the posterior end of the parasite.

While there is normally no other reaction to the parasite by the usual host, partial or total encapsulation by phagocytes does sometimes occur. Thus when first stage larvae are artificially introduced into their normal host, they are rapidly completely and thickly coated with phagocytes that kill the parasite within a day or so. This occurs even if the first stage larva is pushed deeply into the haemocoele and well clear of the wound. Normally when first stage larvae enter naturally, there is no accumulation of phagocytes except just around the wound made during entry, although Frauenfeldia first stage larvae are occasionally lightly encapsulated anteriorly.

Superparasitism often leads to suppression of larvae during the early second instar by other larvae especially in Plesina. The

suppressed larvae die and become opaque although usually there is no sign (such as scars) of active cannibalism. However, unlike almost all Tachinid larvae which die within their hosts these dead larvae remain completely free of phagocytes (see plate 7). When two or more well grown second instar larvae occur within a host they often bite when they can reach one another, and although this does not usually lead to the death of either larva, there is often a large collection of phagocytes about the wounded areas (see plate 7). These phagocytes do not appear to have an adverse effect on the larvae.

When the larvae of Melanophora are introduced onto freshly moulted Porcellio scaber hosts of length greater than 13 mm, they are able to enter the host readily and they moult into the second stage larva which commences to grow normally. However, as the second stage larva continues to grow in such a large host it often becomes heavily encapsulated, particularly around the head region and it is sometimes killed. Such instances are recorded from insect hosts where Meteorus Strickland (1923) and Hyposoter Puttler (1961) are encapulated in old but not in young hosts. As the larvae cannot easily reach and attach themselves to such large hosts, this probably does not happen frequently in the field.

When quiescent, Porcellio scaber containing diapausing Plesina larvae are brought into warm conditions, encapsulation of the larvae begins within a week or so, usually starting around the head region and probably suppressing feeding. After a few weeks, the larvae are often very thickly coated with phagocytes, and it seems probable that these are capable of finally removing all traces of the larva. Thus several hundred P.scaber of which at least thirty per cent were parasi-

tised by Plesina collected during December and January 1963 - '64 - '65 yielded only one or two puparia after being kept in culture for several weeks and when all these remaining woodlice in the culture were dissected not a trace of any parasite could be found.

With Melanophora and Styloneuria this does not occur probably because diapause in these species is not so strong as in Plesina and neither does this occur in Phyto since this species does not appear to diapause.

Phyto second stage larvae are, however, sometimes killed by their normal host Armadillidium vulgare. When this species of woodlouse is collected fromthe field and dissected, it occasionally contains a full grown second stage larva which is dead, with its cuticle uniformly blackened. This is frequently found in insect parasites and it has been shown, Eckstein (1931), to result from melanisation. When Armadillidium are parasitised by Phyto in the laboratory, the initial cultures are often very highly parasitised and sometimes over fifty per cent of the hosts yield puparia. However, when the remaining hosts are again infected, the percentage which yield puparia is very much fewer (10 - 20%). Dissection of the remaining hosts show that these contain a very high proportion of dead melanised larvae. It is possible that some genetic strains of Armadillidium vulgare do kill the parasite while others do not, as was found, Muldrew (1953), in the usual host of Mesoleius and by Walker (1959) with the usual host of Pseudeucoila.

Armadillidium does not encapsulate larvae dead or alive with phagocytes as does Porcellio scaber, and melanization was not observed within Porcellio scaber. No reaction of the usual host was observed to Frauenfeldia, Rhinophora or Stevenia.

Nematodes and Echinorhynchus larvae, occasionally found in Porcellio scaber were never encapsulated, nor were Echinorhynchus larvae encapsulated in Armadillidium vulgare.

Reactions of Unusual hosts to parasites.

As might be expected there is usually a considerably greater reaction by unusual hosts to these parasites. From the field, apart from the usual hosts, only Oniscus asellus was ever found parasitised and then only when it occurred intermixed with Porcellio scaber and the latter was parasitised by Plesina or by Styloneuria. The second stage larvae of these species within Oniscus were almost invariably encapsulated at least partially, and usually dwarfed, unhealthy and sometimes dead. This was found even when the Oniscus were collected during the early winter. When parasitised field Oniscus were kept in culture, periodic dissections revealed that the second stage larvae of the parasite finally become completely and thickly encapsulated and invariably died before reaching the third instar.

Salt (1963) considers that "the immediate cause of the death of encapsulated larvae appears to be suffocation", but this may not be the only factor with the parasites of woodlice. Death occurred even in Styloneuria larvae, which maintain a respiratory connection with the exterior and larvae become unhealthy while only a small proportion of body surface was encapsulated.

Frequently encapsulation was found to have commenced around the head region of the larva and it is possible that this may greatly impair the feeding ability of the parasite and thus be at least partially responsible for its death.

It is most probable that encapsulation is the cause of death of larvae within Oniscus. It was not possible to prove this by stripping the haemocytes from larvae and injecting them into their usual host Porcellio scaber as was first done by Salt (1956) with Nemeritis larvae, since the woodlice die from the wounds impaired in introduction of the parasite.

When larvae were induced to infect other species of unusual woodlice hosts, they were usually killed by complete encapsulation during their first instar. Thus when Melanophora larvae infected cultures of Porcellio rathkei, P.laevis, Metaponorthus pruinosus, M. cingendus, Ligia oceanica and Oniscus asellus, they failed to reach second instar. However, in Cylisticus convexus larvae were not encapsulated and reached pupation. In Philoscia muscorum, larvae reached second instar without encapsulation, but these hosts died soon afterwards containing second instar larvae which were usually not at all encapsulated.

Stevenia larvae which entered Porcellio scaber and Oniscus asellus, invariably died during the first instar, but were not heavily encapsulated. Only a thin film of haemocytes surrounded the dead larvae.

10. MULTIPARASITISM AND SUPER PARASITISM.

10a) Multiparasitism

There is no previous record in any aspect of multiparasitism of woodlice.

Despite the many thousands of field woodlice dissected, only a single specimen was found containing larvae of more than one species of parasite. A small female Porcellio scaber from Bromley, Kent, was found to contain a large second stage larva of Melanophora and a second stage larva of similar size of Plesina. (see plate 6). Both larvae were completely healthy in appearance and despite the fact that they were within easy reach of one another, neither had any scar marks.

That only this single instance of multiparasitism in the field has been found is not really extraordinary even though first stage larvae cannot discriminate between parasitised and unparasitised hosts.

Most woodlouse populations are parasitised by only one species or if by two, one species parasitises large hosts and the other small ones. Usually parasitism by all species except Plesina is less than twenty per cent, and when two species of parasite are present in a population percentage parasitism by the less common species is considerably less than this. Further, with Styloneuria and Melanophora, which rely on host moulting for larval penetration, unless larvae of two of these species are in the habitat at about the same time, they will not be able to enter the same host.

Experimental

a) Hanging drop experiments

Various combinations of different species and different

Plate 6 : Dissection of host showing the only woodlouse obtained from the field with multiparasitism. No scars of cannibalism are visible although both larvae are within reach of each other, and both larvae were healthy.

a - Melanophora second stage larva with pearly white adipose tissue characteristic of this species in diapause, or after diapause.

b - Plesina second stage larva

stages of larvae have been left together in hanging drops of woodlouse blood in order to determine whether active cannibalism takes place readily. Approximately 0.2% penicillin was included to decrease bacterial infection of the blood.

A small drop of 0.2% penicillin solution was added to a clean 2 mm square coverslip and allowed to dry and then a drop of woodlouse blood of approximately the same volume was added to this. The blood was obtained by a sterile, finely drawn cut pipette which was dipped into the woodlouse haemocoele after removal of a leg. The blood, after entering the pipette by capillary action was squeezed out into the coverslip, and the larvae were then added. The coverslip was then inverted over a cell of damp blotting paper on a slide and the whole slide put into a glass petri dish with a floor covering of damp filter paper. The hanging drop was not allowed to contract the blotting paper cell and neither the cell nor the filter paper were excessively moist. In this way the hanging drop neither tended to evaporate nor to absorb moisture from the surroundings. Generally it was possible to maintain the larvae in a healthy condition for at least three days, but occasionally fungus infection set in. Although various concentrations of Nipagin were tried to combat the fungus, these were either insufficient, or they killed the larvae.

Only those larvae which have Porcellio scaber as their usual host were experimented with: of the ten possible combinations of two different first stage larvae, eight were tried. First stage larvae of Frauenfeldia and Rhinophora, and Frauenfeldia and Plesina were never available at the same time. No cannibalism occurred in the hanging drops in three days in any of the other pairs of first stage larvae, although at least five sets of experiments were done with each pair. Generally

two or three first stage larvae of each species were included in each hanging drop and they were orientated to be within easy reach of the other species. That the larvae were usually active in the hanging drop was seen by their active feeding which greatly increased their size in most cases. Melanophora larvae retrieved two or three days after infecting hosts were actually able to moult into second stage larvae when left in hanging drops for two or three more days. Each of the four other species of second stage larvae were left in hanging drops with numbers of Melanophora or Styloneuria first stage larvae; Rhinophora first stage larvae were left with Melanophora, Styloneuria and Plesina second stage larvae, and Frauenfeldia first stage larvae were left with Plesina second stage. However, in none of these cases was cannibalism observed.

The second and third stage larvae, Plesina, Melanophora and Styloneuria were each tried with one another without results. It is quite probable that third stage larvae, at least, change their behaviour considerably with age, but unfortunately no distinction was made between ages in these experiments.

As these experiments produced only negative results, they are very inconclusive especially since it has been found from field dissections that cannibalism between second stage larvae of at least the same species does often occur, although it was not possible to observe this in hanging drop experiments.

While no attempts at cannibalism were actually observed in the experiments it is possible that they were in fact made but were not successful because the whole larva was floating freely in the blood instead having a fixed posterior end as within a host. Hanging drops also present another artificial condition in that oxygen content of the

drops probably remains relatively high and carbondioxide content low. If either reduced oxygen or increased carbon dioxide in the host blood, produced by super or multiparasitism, promotes cannibalism, then this would not occur in hanging drop experiments. On the other hand larvae in a hanging drop do not have their posterior spiracles in contact with the air and so may be suffering from lack of oxygen and hence may be less active.

Host Infection

Relatively little experimental infection by different species of the same host, have been attempted because of the difficulty of obtaining large numbers of larvae of different species at the same time. However, it has been relatively easy to get larvae of Melanophora and Styloneuria and to infect the same host at the same time and to infect hosts containing Plesina second stage larvae with Melanophora and Styloneuria larvae, and those containing Rhinophora first stage larvae with Melanophora larvae.

At least two dozen freshly moulted hosts with first stage Styloneuria larvae in the process of entering, were each successfully infected with a single Melanophora larva.

After four, eight and twelve days, four hosts were dissected in each set. In all cases both species of larvae were found to be developing normally and no sign of cannibalism or physiological suppression was observed. However, after a period between sixteen and twenty two days at 25°C, in all but three of the remaining hosts Melanophora pupae were formed. When the remaining three were dissected only Styloneuria second stage larvae were found, and there was no

apparent trace of Melanophora larva, presumably because these had never succeeded in entering.

While the numbers used for this experiment were very limited it appears that because of the shorter time required for their development, Melanophora larvae are able to dominate those of Styloneuria. While it is unlikely that any cannibalism occurred between the second stage larvae, the third stage larvae of Melanophora may have cannibalised the second stage larvae of Styloneuria. However, though no dissections were made in order to determine this, it seems more likely that the Melanophora third stage larva just continue to develop and kill the second stage Styloneuria by the secretion of the protease which digests the inside of the host. Such a situation often occurs when hosts are superparasitised.

Similarly, a culture of about fifty small Porcellio which had been infected with Rhinophora larvae, were infected with Melanophora larvae after each of the hosts moulted. After ten days at 25°C half of the culture were dissected and eight were found to contain both Rhinophora first stage larvae and Melanophora second stage larvae. (The rest contained no larvae on either Rhinophora or Melanophora larvae alone because double infection had been unsuccessful.) In none of these cases was either larva apparently affected by the presence of the other. The rest of the culture was kept at 25°C and three weeks after infection fourteen Melanophora puparia were formed and on dissection, the rest of the culture was found to contain either first stage Rhinophora larvae, or no parasites at all. Once again it appears that Melanophora larvae successfully dominated those of Rhinophora probably because they develop more rapidly (Rhinophora larvae undergo an initial obligatory diapause).

Several Porcellio cultures collected from the field in December to February 30 - 50% parasitised by Plesina larvae, were infected by Styloneuria and Melanophora larvae when the hosts were at a suitable stage in the moulting cycle. Dissection of some of these woodlice showed that no cannibalism occurred between the first or second stage larva of any of these species. After Melanophora and Styloneuria puparia were produced from the cultures, the remaining woodlice were dissected and found to contain either no parasites or only Plesina with no trace of either Melanophora or Styloneuria larvae.

10b) Superparasitism.

No instance of superparasitism of woodlice by their dipterous parasites has been previously recorded in the literature although in the field superparasitism by some species is not rare.

Field observations.

Superparasitism in the field was not observed in Stevenia or Phyto, and only in a single instance in Rhinophora and in Frauenfeldia. However, since in the field percentage parasitism by any of these species rarely exceeded ten and since comparatively low numbers of woodlice parasitised by them were examined, many records of superparasitism were not to be expected.

Only about two dozen field Porcellio superparasitised by second stage Styloneuria were dissected. Three of these woodlice contained three parasites and the rest only two. Where only two parasites were present in one host both parasites were usually well developed and of a similar size, although often where one parasite could reach the other,

one or both bore scars presumably inflicted by cannibilistic attacks. Usually one or both parasites were larger than single parasites obtained at the same time from the same population. However, in each instance where three parasites were found in the same host, one parasite was distinctly dwarfed and in one case one was completely suppressed and dead. While this latter parasite appeared white and fluffy internally, there was no sign of encapsulation by the host (see plate 7).

Four woodlice out of a field sample were superparasitised by two Styloneuria third stage larvae, while those Styloneuria which occurred singly in woodlice from the same population were all in the second instar. In the same sample several weeks elapsed before puparia were produced within woodlice containing a single parasite. In these and other cultures obtained from the field which contained diapausing Styloneuria, two Styloneuria puparia were occasionally formed within one host and this always occurred a week to several weeks before single puparia were formed within other hosts of the same culture.

It thus seems that superparasitism by Styloneuria may have the effect of breaking its diapause. One possible mechanism for this is the attempted cannibalism of Styloneuria which causes wounding, a well known breaker of diapause. Varley and Butler (1933) found that diapausing larvae of Lipara lucens are sometimes caused to pupate by pricking and, pricking can terminate diapause in Lucilia (Roubaud 1922)

Superparasitism by Melanophora parasites has also been found in the field, but dissections of field populations have never yielded more than two parasites to a host. No sign of attempted cannibalism was ever found in this species. Two puparia were often found within one host, but the host was then always comparatively large (9 - 11 mm).

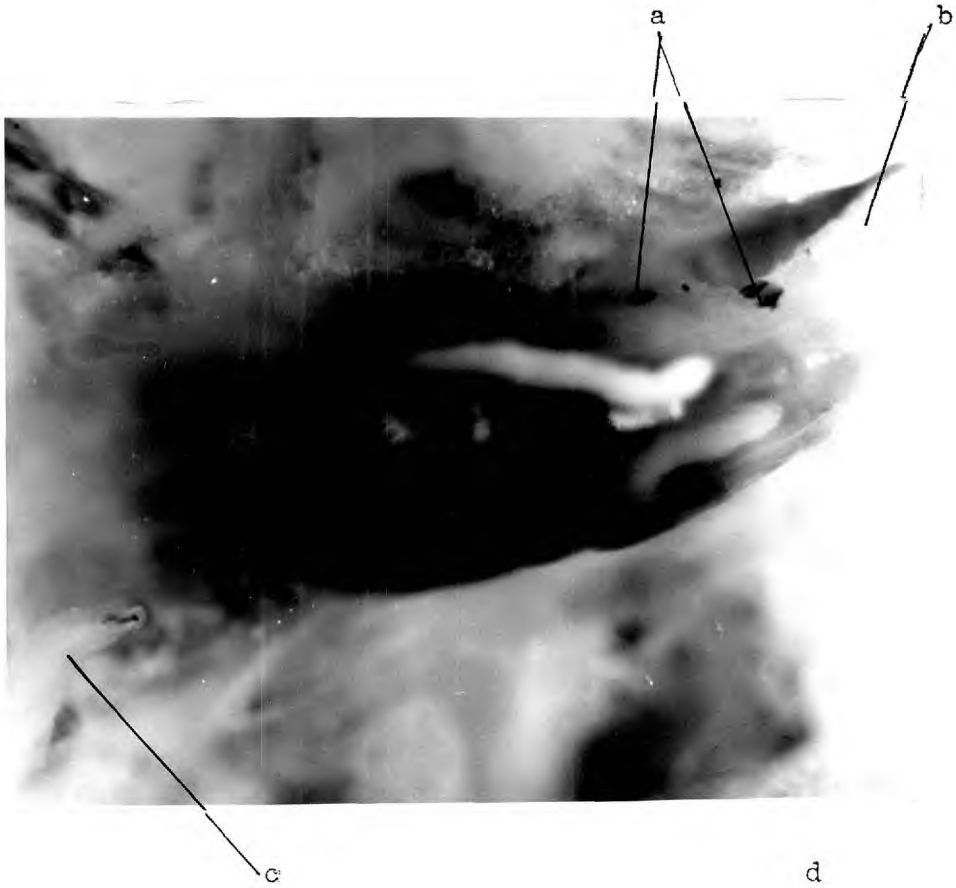


Plate 7 : Dissection of woodlouse showing superparasitism by Styloneuria

a - scars from cannibalistic attacks of another larva (not in the photograph) which bore similar scars.
b - encapsulation around wounded area of otherwise healthy larva.
c - dead, unscarred larva with no signs of encapsulation.

As no samples of populations containing diapausing Melanophora, showing superparasitism were cultured, it was not possible to observe whether superparasitism in this species breaks diapause. However, Melanophora superparasites did not pupate any earlier than single parasites.

Superparasitism in the field by Plesina second stage larvae is very common. This is correlated with frequent high percentages of parasitism. In some populations many more female woodlice of certain sizes were superparasitised then singly parasitised but this in some sizes of host was correlated with percentages of parasitism exceeding eighty. As with lower percentages of parasitism by this species, it was found that within one population the larger the host the more frequently they were superparasitised. Thus in the population from 1 c) while 48 parasites were obtained from 25 hosts of size 14 - 15 mm, no superparasites were found in 50 hosts of sizes ranging between 8 and 9 mm.

While the number of superparasites found in one host varied from two to five, only one or two of these were ever well developed. The remainder were either dead or moribund and were very small second stage with a white fluffy appearance, (see plate 7). They were never in the slightest degree encapsulated by the host. Occasionally these dwarfed dead larvae bore scar marks of cannibalism. The two normal larvae were often scarred if their posterior attachments allowed for reaching one another. Presumably most of the dead larvae had been physiologically suppressed, possibly as suggested by Fischer (1964) by the lack of oxygen during a phase of rapid growth.

Dissections of large numbers of woodlice superparasitised by Plesina show that while many second stage larvae of this species may be found in one host, it is very rare for more than one third stage larva

to be present. Frequently one fully developed third stage larva was found in a host which also contained one or more second stage larvae. Although several heavily superparasitised cultures were kept, only on a single occasion were two puparia found within one host.

Even when almost fully developed third stage larvae were dissected from woodlice also containing second stage larvae, no signs of successful cannibalism were even observed. Presumably one larva dominates another by faster development, and supernumary second stage larvae are finally killed either by the third stage larval protease or by desiccation after the pupation of the third stage larva.

As with superparasitism by Styloneuria, third stage larvae of Plesina are usually found several weeks earlier in superparasitised hosts than in hosts with only single parasites and this is probably also the result of cannibalistic wounding.

Experimental

Various combinations of different larval stages of the same species were left in hanging drops of host blood but in no case was active cannibalism observed. However, as discussed in the section on Multiparasitism, the absence of cannibalism within the hanging drop does not necessarily prove that this does not occur within a host.

First stage larvae of all species were kept in groups of three to six in hanging drops of host blood for two to three days. First stage larvae of Styloneuria, Melanophora, Phyto and Plesina were kept with second stage larvae of their own species, and second stage larvae of each of the above species were kept with third stage larvae of the same species. At least four replicates of each combination were tried but there was no indication at all of any attempted cannibalism although

the hanging drops were continually observed.

More information was obtained by multi-infection of hosts with first stage larvae. This was done most extensively with Melanophora and Styloneuria since it was easiest to obtain large numbers of first stage larvae of this species. Dozens of hosts were successfully superparasitised by varying numbers of first stage larvae of both these species. In all cases the results were similar. All larvae which succeeded in penetrating eventually moulted into second stage larvae. If only two larvae were present both of these continued normal development, but if several larvae succeeded in penetrating, generally only two succeeded in developing beyond the early second instar. The rest remained dwarfed, and died a few days after the first larval moult and appeared white and "fluffy" internally, but they were never encapsulated by the host. That this dwarfing was a result of superparasitism is born out by the fact that monoparasitic larvae which occur singly, only extremely rarely die early in the second instar and then they do not have this appearance. Scars left by cannibalism were only found when superparasitism was by Styloneuria and by Plesina and then usually not on the dwarfed larvae, but on the two developing ones (as described above from field populations). As the first stage larvae of both Styloneuria and Melanophora can only enter freshly moulted hosts and the hosts of both these species do not usually moult after entry of the parasite in the case of Styloneuria or until the second instar parasite moults in the case of Melanophora, it was impossible to infect hosts already containing second stage larvae with first instar larvae.

As most eggs obtained from Plesina were infertile, only

a relatively few first stage larvae were available, and only ten hosts were successfully experimentally superparasitised. In these hosts, larvae behaved exactly as did larvae of Styloneuria and Melanophora.

Only eight Porcellio rathkei were each infected with two larvae (because of the scarcity of both parasite and host), but in no instance did more than one larva succeed in entering. This was possibly because larvae of this species normally only enter the first right leg base. As in three out of the eight hosts, both larvae failed to penetrate before dying, it is possible that it was just chance that no two larvae entered the same host.

About thirty Armadillidium vulgare were each infected with three to five first stage larvae of Phyto, but no more than two larvae actually succeeded in entering any one host. This was possibly related to the small size of the area where penetration normally takes place. Five hosts containing two first stage larvae were dissected, but showed no sign of cannibalism although they could easily reach one another from their point of entry. While two second stage larvae at various stages of growth were dissected from eight superparasitised hosts, no scars resulting from cannibalism could be found. However, when the remaining parasites pupated, in no case was more than one puparium found in a single host.

Table showing degree of Superparasitism by Plesina in the field and effect of host length.

Habitat No.	Host length in mm.	Host sex	Ration of parasites to hosts	No. hosts each having given No. of parasites 0	1	2	3	4	5
1a)	13-14	female	11/10	3	4	2	1	0	0
		male	8/10	3	6	1	0	0	0
	12	female	11/10	1	8	0	1	0	0
		male	8/10	3	6	1	0	0	0
	11	female	11/10	3	4	2	1	0	0
		male	3/10	7	3	0	0	0	0
	10	female	8/10	3	6	1	0	0	0
		male	5/10	5	5	0	0	0	0
	7-9		10/30	20	10	0	0	0	0
			12/30	18	12	0	0	0	0
1d)	14-15	female	48/25	1	6	14	2	2	0
		male	24/20	6	7	4	3	0	0
	13	female	29/25	9	9	4	1	1	1
	12	female	25/25	3	19	3	0	0	0
		male	9/20	12	7	1	0	0	0
	10	female	14/25	12	12	1	0	0	0
		male	11/20	11	8	0	1	0	0
	8-9	female	12/50	38	12	0	0	0	0
		male	13/40	27	13	0	0	0	0
16d)	10-14	female	40/61	29	25	6	1	0	0
		male	24/53	29	24	0	0	0	0
4b)	10-13	female	38/89	55	30	4	0	0	0
		male	20/66	47	18	1	0	0	0

(for habitat quoted see tables of the appendix)

11. OBSERVATIONS ON PARASITISM IN NATURAL POPULATIONS OF WOODLICE.

11a) Percentage parasitism of different sizes of Porcellio scaber.

The only reference made to any host size preference in these parasites is by Thompson (1934) who states that "Species A has always been found in very small specimens of Porcellio." (Species A is in fact Rhinophora lepida).

In the present work size preferences have been investigated in some detail. Firstly it was considered of interest to see whether perhaps different species of the parasites of Porcellio scaber tend to avoid competition with each other by preferring different sizes of host. Secondly, for detailed ecological comparisons of percentage of parasitism of different woodlouse populations, it is important to know whether the size of the hosts has to be taken into account.

Rhinophora lepida has perhaps the most conspicuous size preference of these parasites. Thus, in the thousands of dissections made during this work, no larva of this species has been taken from a host of greater length than 9 mm and usually it is restricted to hosts of length of 5 - 8 mm. From tables and histograms of Ashtead and Basingstoke populations this preference can be seen to be very marked. As Porcellio scaber of this size range are less commonly parasitised by other species, Rhinophora normally has little competition, while tending to monopolise that size section of the population which is most numerous. As previously explained, Rhinophora is restricted to this size range of hosts initially because the first stage larvae cannot reach sternites of larger specimens which it must do to become attached.

Dissections of field populations of woodlice containing

Fig. 69 - Histogram showing the result of dissections of part of a population of Porcellio scaber from habitat No. 28 (see tables of appendix). Similar results were obtained from all other populations of Porcellio scaber which were found to contain Rhinophora larvae.

Fig. 70 - Histogram showing the result of dissections of part of a population of Porcellio scaber from habitat no. 25a). Similar results were obtained from the few other populations of Porcellio scaber which were found to contain Frauenfeldia larvae.

Fig. 71 - Histogram showing the results of dissections of part of a population of Porcellio scaber from habitat No. 43a). Whilst Melanophora larvae have been taken from several other populations only relatively small numbers were found. All these other larvae occurred within the restricted size range of hosts shown by the histogram.

DIFFERENTIAL PARASITISM BY RHINOPHORA WITH RESPECT TO HOST SIZE

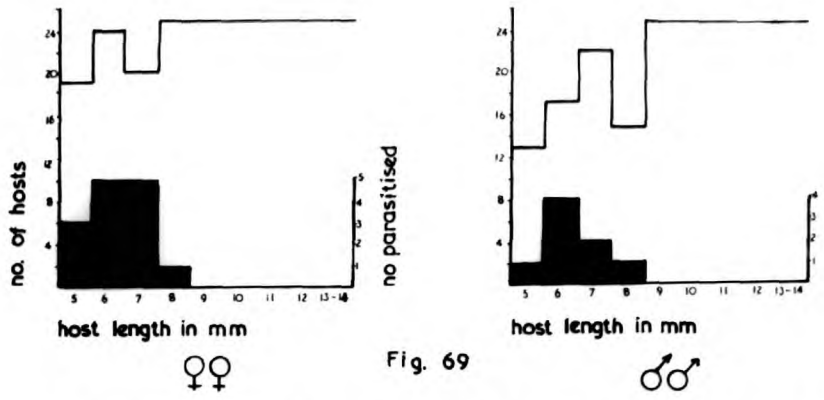


Fig. 69

DIFFERENTIAL PARASITISM BY FRAUENFELDIA WITH RESPECT TO HOST SIZE

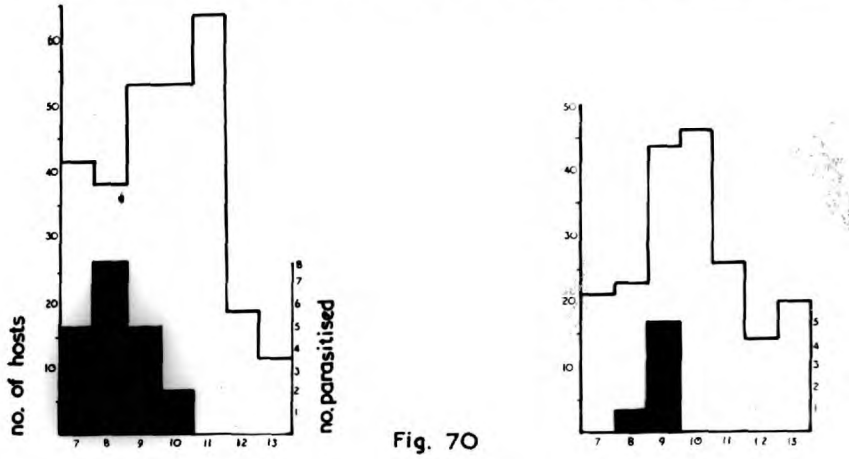


Fig. 70

DIFFERENTIAL PARASITISM BY MELANOPHORA WITH RESPECT TO HOST SIZE

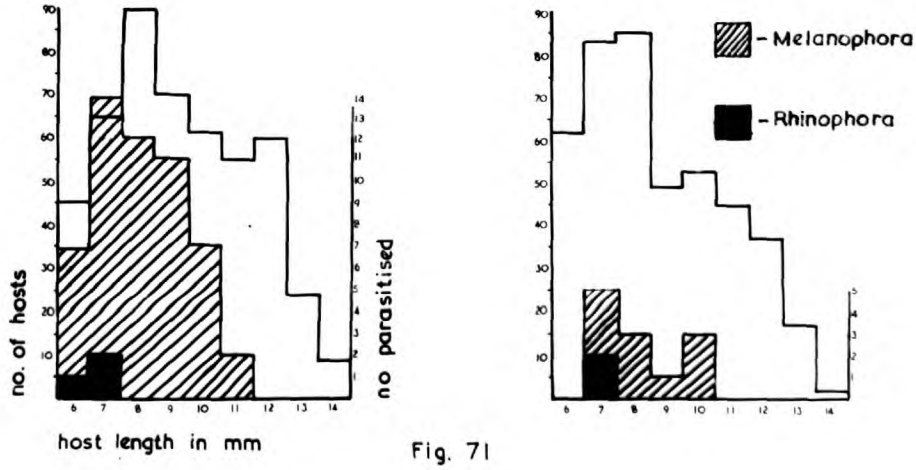


Fig. 71

Frauenfeldia show a similar restriction to host size except that these parasites prefer the range of hosts which are 6 - 10 mm long, although as this parasite is rare, few studies have been made (see fig. 70). The first stage larvae, as in Rhinophora must reach the host's sternites before they can attach themselves, but as the larva is longer than that of Rhinophora, it can reach larger hosts. Although there is a slight overlap in size preference between Rhinophora and Frauenfeldia, the rarity of the latter, and the normally fairly low percentage parasitisms of both mean that only very rarely will a larva from each compete for the same host. With the other type of parasitic larvae, (Melanophora, Styloneuria and Plesina group), a greater range of size tolerance is found in field populations.

These parasitic larvae remain in one place until walked over by a potential host and then usually cling onto its leg or any other part they can reach. The tibiae of Porcellio at least, are carried almost horizontally to the substrate and thus as the woodlouse walks, a large area is swept over at a particular height from the substrate. The height mainly depends on the size of the woodlouse.

Provided that this height does not exceed the length of the parasitic larva, then in general, one expects that the larger the woodlouse the greater is its chance of picking up a larva, since: a) The wider the woodlouse, the wider the path swept as it walks; b) the larger the woodlouse, the faster it can move and it will thus cover longer distances than smaller woodlice, provided that it exhibits the same degree of activity.

If the percentage of larval entries is proportional to the percentage of larval attachments with respect to any particular size group

of hosts, then according to the above hypothesis, dissections of field populations should show that the ratio of parasites to hosts increases proportionally with the size of the host. The ratio of the number of parasitised to non-parasitised woodlice would be complicated by super parasitism at high percentages of parasitism. Dissections of several field populations of woodlice parasitised by Plesina confirm that the ratio of parasitised hosts does increase markedly with host size. (see figs. 72, 73 and 74)

Laboratory observations show that even on a flat substrate first stage larvae of Plesina are able to reach the legs of even the largest Porcellio scaber available because the larvae are capable of considerable extension. Comprehensive experiments on the effect of size of host on the readiness with which larvae are picked up, were not carried out because of the great difficulty of handling larvae to obtain the sparse random distributions required.

Although several field populations containing Melanophora parasites have been examined, this parasite has never been found in woodlice of over 1.1 mm in length, and was found most commonly in 7 - 9 mm. size groups (see fig . 71).

The situation here is complicated because:-

a) these parasites will only enter newly moulted hosts and smaller hosts moult more frequently than the larger ones.Heeley (1941)

b) The parasite first stage larva is shorter than that of Plesina and much less capable of extension and cannot reach the legs of large hosts.

However, when in the laboratory, all sizes of Porcellio

Variation of Differential Parasitism by Plesina with respect to Host size.

Habitat No.	No. parasites found / Total No. females dissected.		No. parasites found / No. hosts of particular size. host length in mm.								
			7	8	9	10	11	12	13	14	15
1 a)	50/70	(71%)	1/10	3/10	6/10	7/10	11/10	11/10		11/10	-
1 b)	48/108	(44%)	-	4/17	12/30	18/33	11/18	3/10	-	-	-
1 d)	140/175	(80%)	-	5/25	8/25	14/25	12/25	25/25	28/25	48/25	
1 e)	76/150	(51%)	-	5/25	11/25	11/25	12/25	17/25		22/25	-
1 f)	54/107	(50%)	-	1/11	5/12	10/25	15/32	7/15		16/12	-
4 b)	51/137	(37%)	-	6/28	8/21	16/40	10/26	9/15	2/7	-	-
5 a)	49/150	(33%)	-	10/45	12/35	8/29	6/16	10/20	-	3/5	-
6 a&b)	41/133	(31%)	-	0/8	2/21	7/31	5/26	13/17	10/16	4/14	-
10 d&e)	52/147	(35%)	-	1/10	8/25	10/33	16/35	17/34	-	-	-
11 b)	11/88	(13%)	-	1/14	1/12	3/19	5/22	1/17	0/4	-	-
16 a)	62/184	(34%)	1/72	10/37	16/67	12/25	9/27	12/21	3/7	-	-
16 d)	53/105	(50%)	1/11	4/19	8/14	6/13	10/18	13/15	6/12	5/3	-
17	40/174	(23%)	5/30	10/53	4/23	6/27	4/19	7/13		4/9	

	8	9	10	11	12	13 - 15
Over 40%	22/107	50/116	66/131	67/128	76/100	136/97
	21%	43%	50%	52%	76%	140%
Under 40%	38/195	51/204	62/204	55/171	69/137	26/62
	20%	25%	30%	32%	50%	42%

In most habitats, unequal numbers of different size groups were dissected, as only small numbers of certain sizes occurred. A summation of the same-size groups from different habitats may, therefore, be a little misleading.

Fig. 72 - Histograms showing the result of dissections of two populations of Porcellio scaber each heavily infected by Plesina larvae. Population A was obtained from habitat no. 1d) and population B from 1e). (See tables of the appendix.

Fig. 73 - Histograms showing the combined results of dissections from the only populations of Porcellio scaber where equal numbers of each size of host were dissected. Population C was obtained from habitat no. 1a).

Fig. 74 - Histograms showing the combined results of dissections of female Porcellio scaber from all populations containing Plesina larvae where differential parasitism according to host size were recorded. (see adjoining table). It can be seen that differential parasitism of female woodlice according to host size was much more marked in populations where overall parasitism exceeded 40 per cent. However, one factor to be considered is that all but one of such populations were obtained from the same locality where some special factor may have operated.

Fig. 75 - Histogram showing the result of dissections of part of a population of Porcellio scaber from habitat no. 17. This population was unusual in having four parasite species present.

DIFFERENTIAL PARASITISM BY PARAFEBURIA WITH RESPECT TO HOST SIZE.

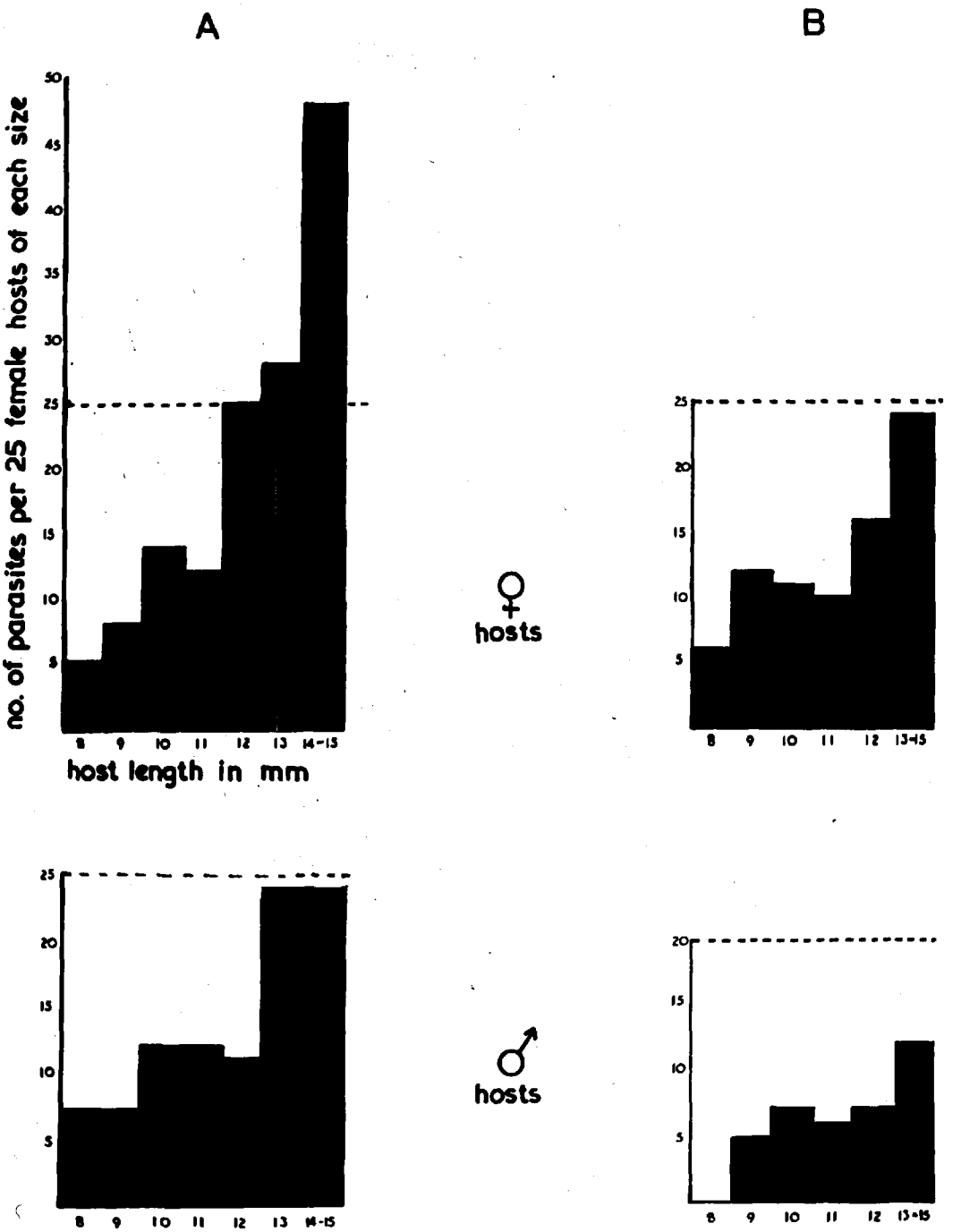


Fig. 72

DIFFERENTIAL PARASITISM BY PLESINA WITH RESPECT TO HOST SIZE

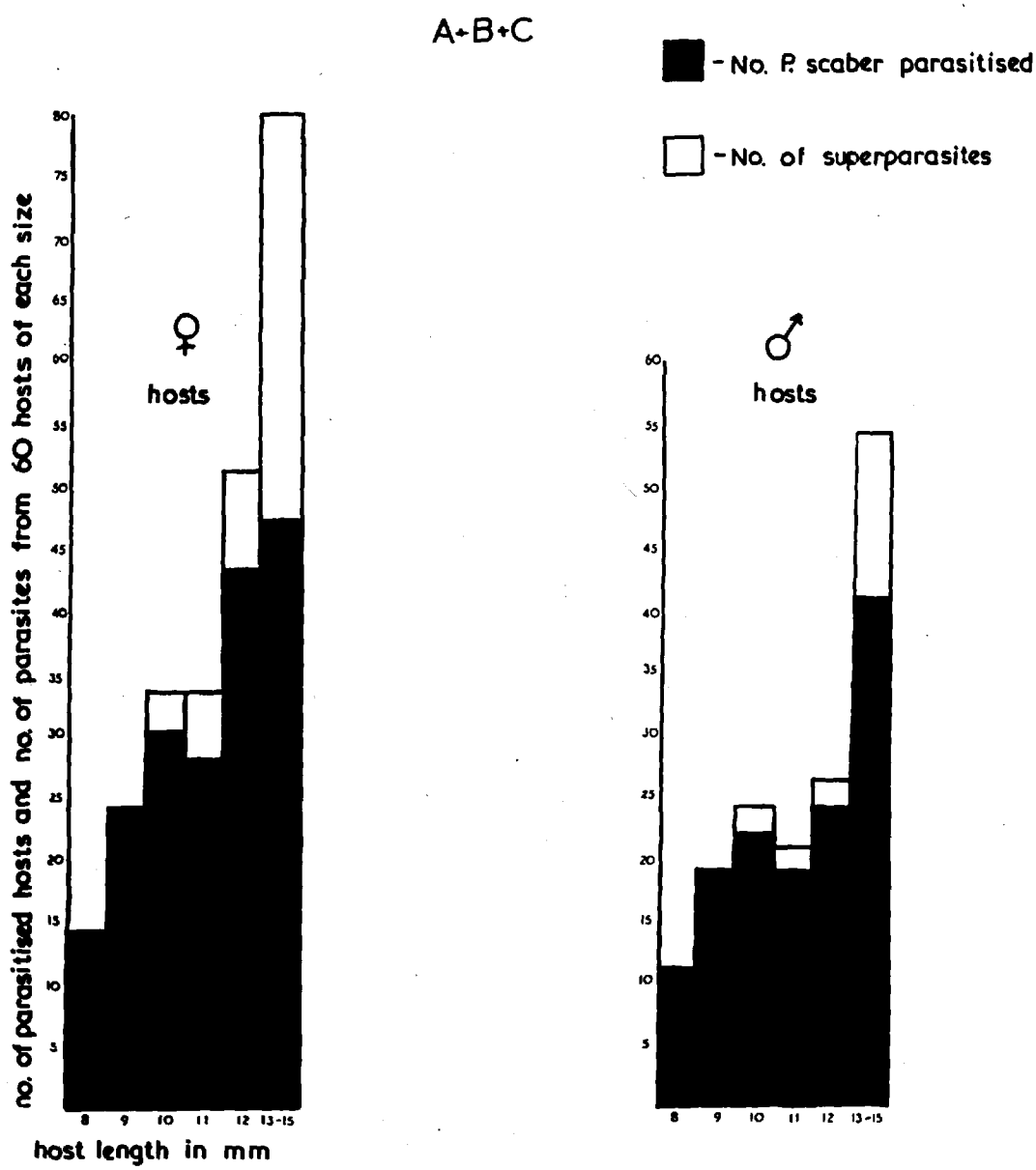


Fig. 73

over 40 per cent parasitised by Plesina

under 40 per cent parasitised by Plesina

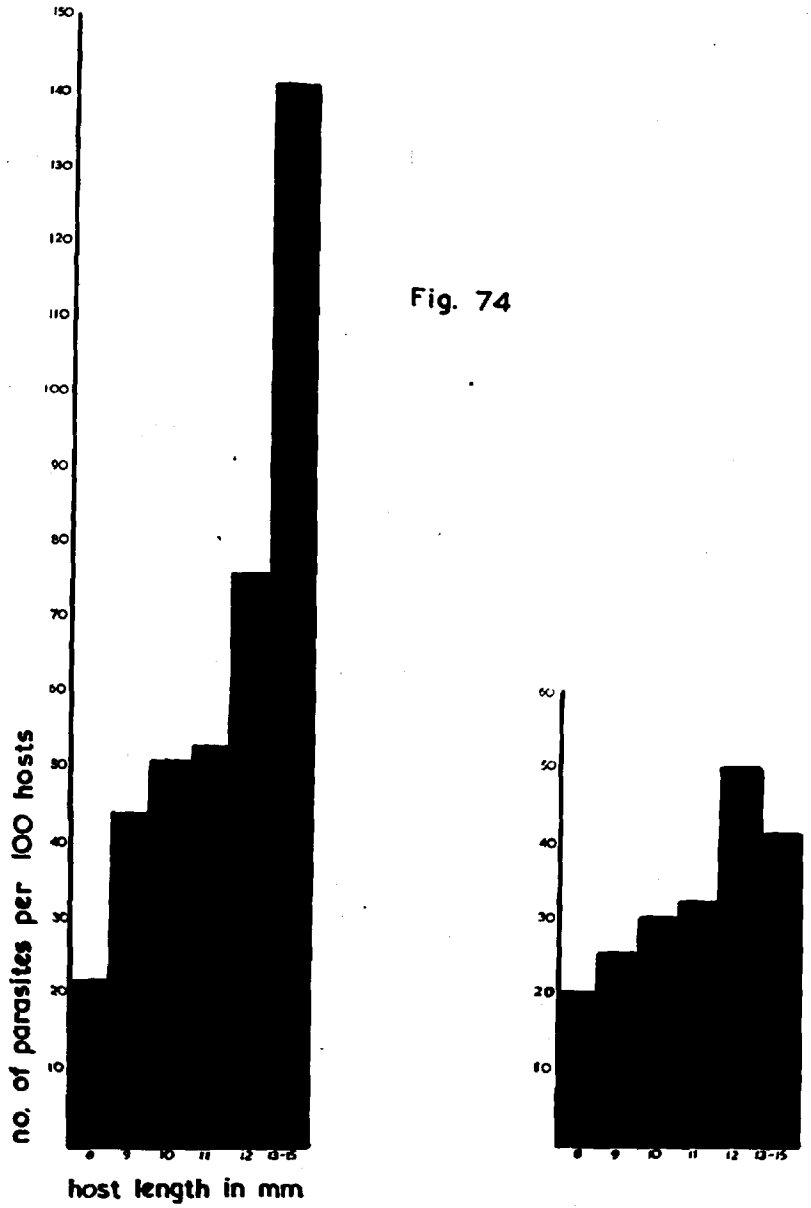


Fig. 74

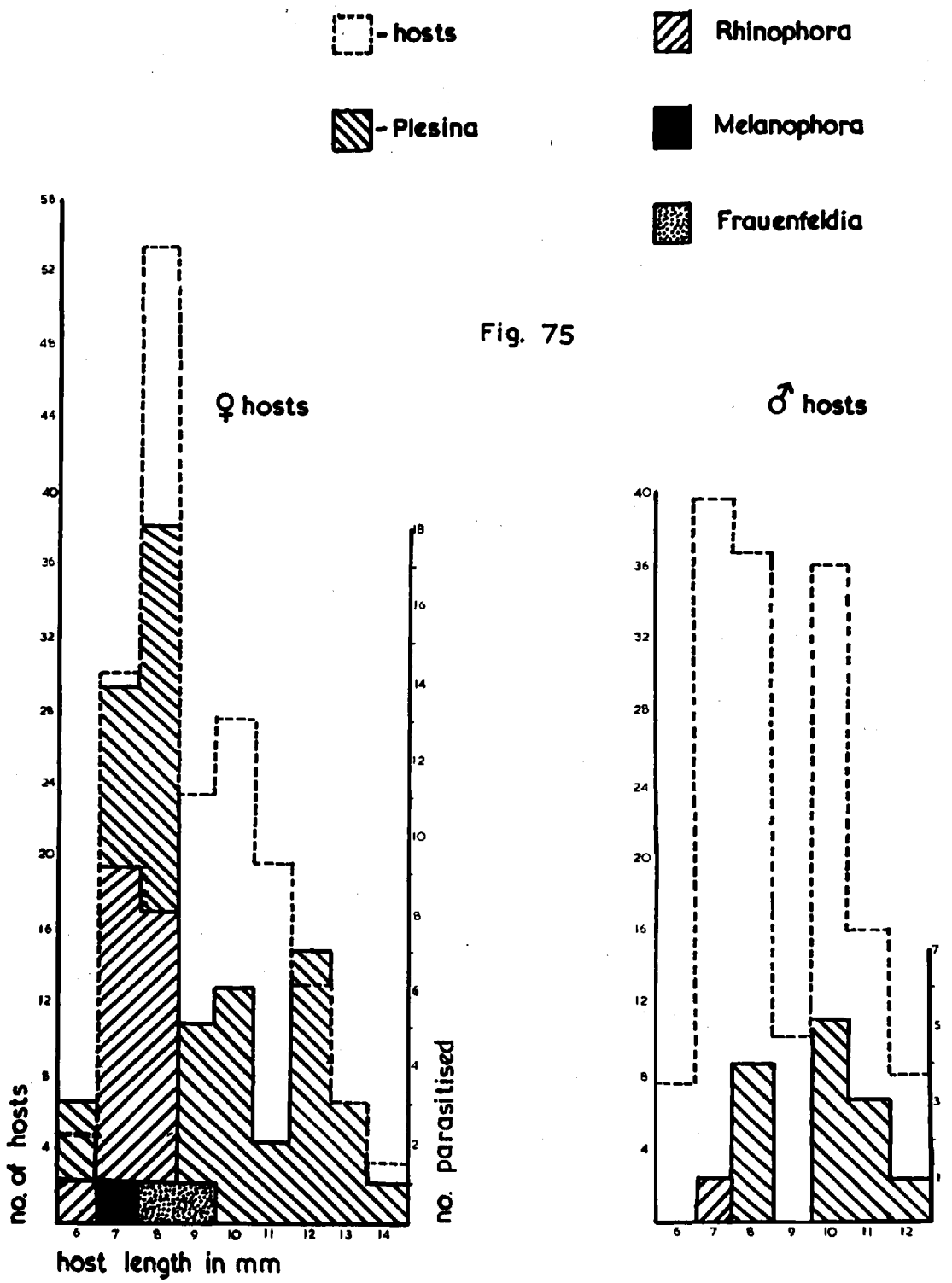

- hosts
- Plesina
Rhinophora
Melanophora
Frauenfeldia
♀ hosts
♂ hosts
no. of hosts
no. parasitised
host length in mm

Fig. 75

scaber which had newly moulted were infected, the parasite usually successfully completed its life cycle.

The fall off in percentage parasitism by Melanophora in the 1.0 and 1.1 sizes of hosts may well be because larvae can usually only reach these when they are situated on slight projections of the substrate, and because hosts of this size group moult less frequently than smaller hosts.

With parasitism by Styloneuria, the situation is again complicated. Although larvae can reach up to the legs of most hosts, they will only readily attach themselves to hosts which will moult within about a week or so.

Further, when hosts of under about 9 mm walk over them, the legs hit the larva well below its anterior end which usually causes it to lie flat against the substrate without attaching itself to the host. Thus woodlice of less length than 9 mm are rarely attacked. Apart from males being less frequently parasitised than females, there seems to be a significant difference between the sexes in the change of percentage parasitism with respect to host size (see figs. 76, 77 & 78). In fact while with the female hosts there is a marked decrease in percentage parasitism with decrease in size, there is little such tendency observable with the male hosts. This is probably connected with the great difference in the frequency of moulting between the sexes. The adult female woodlice have several moults connected with the breeding cycle (Heeley 1941), but only the younger male woodlice moult at all frequently. Thus in the male, the greater tendency of larger woodlice to pick up Styloneuria larvae, is offset by the more frequent moulting of smaller male woodlice.

Fig. 76 - Histogram showing the result of dissecting 30 hosts of each size and sex from a population of Porcellio scaber found in habitat No. 30a).

Fig. 77 - As above, but population found in habitat No. 30b).

Fig. 78 - Histogram showing the combined results of dissections from 30a) and 30b).

Several other populations of Porcellio scaber parasitised solely by Styloneuria larvae were dissected to investigate differential parasitism by Styloneuria according to host size, and similar results were obtained.

DIFFERENTIAL PARASITISM BY STYLONEURIA WITH RESPECT TO HOST SIZE

♀ hosts

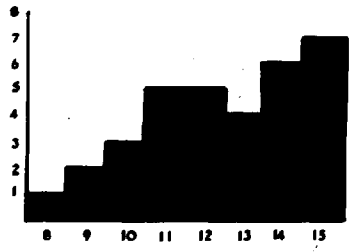
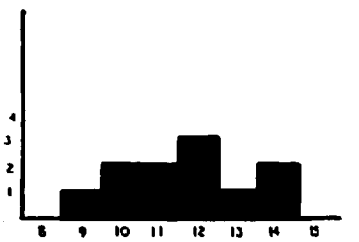


Fig. 76

♂ hosts



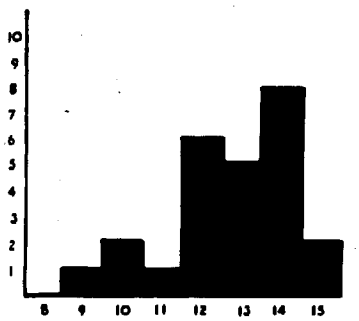
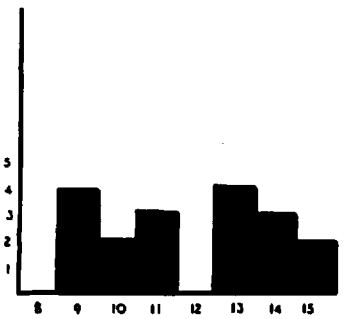


Fig. 77



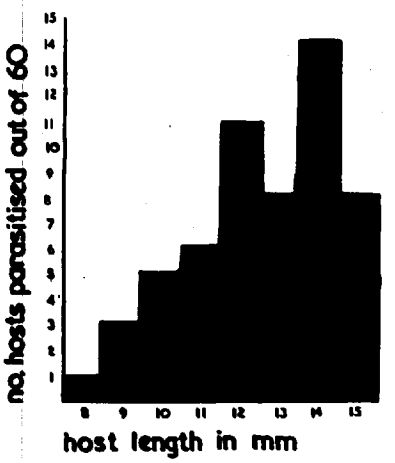
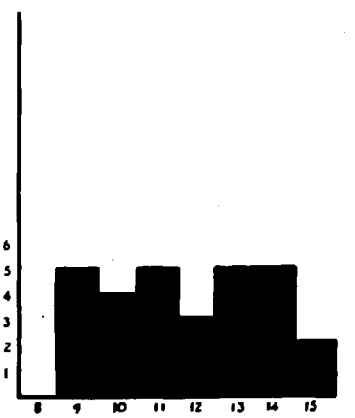


Fig. 78



As can be seen from section IIc) when two parasite species are found commonly in the same population of woodlice, generally one species dominates the larger host size groups (either Styloneuria or Plesina) and the other is found in the smaller size groups (Melanophora, Rhinophora and Frauenfeldia).

It was noted that when woodlice of 9 mm or less are parasitised by Styloneuria, even at high temperatures the parasites take several weeks or months longer to develop than parasites in larger woodlice and often the parasites of a small host are phagocytised and killed before developing. The same is true of Plesina in hosts of 8 mm or less. In both these species generally the larger the host, the greater the fecundity of the resulting female.

In contrast, Melanophora larvae when experimentally introduced to freshly moulted large hosts are often killed by encapsulation unless super parasitism occurs.

With the other two species of parasite studied (Phyto in Armadillidium, and Stevenia in P.rathkei), insufficient numbers have been dissected from the field to draw any definite conclusions. However, no pupae of Phyto were formed in Armadillidium of less than about 10 mms and most pupae occurred in larger hosts.

Experiments with the larvae of Stevenia indicate that these will only attach themselves to large hosts (10 mm and over) when a brood pouch is present, because otherwise they cannot, as is necessary for them, reach the sternites of their host. The first generation of first stage larvae in the field seems to coincide with the time when the majority of P.rathkei possess brood pouches. During the second generation of this parasite few females have brood pouches and so larvae can only attach

themselves to smaller woodlice (in which they diapause, while presumably the host increases in size).

11b) Percentage Parasitism of Males as Compared with Females.

There is no previous record of any difference in percentage parasitism between the sexes of woodlice, although in fact female woodlice are often considerably more highly parasitised than males.

Field observations

The greatest difference, in percentage parasitism between males and females was found in a population of Porcellio scaber from Ashtead, Surrey, parasitised partly by Rhinophora lepida (see fig. 75) Here, within the size range parasitised, 16 of the 89 females dissected contained Rhinophora larvae, whereas only one out of the 73 males dissected was parasitised by this species.

A considerable difference between parasitism of males and females was also found in a population of Porcellio scaber parasitised by Melanophora roralis. These woodlice were collected from Hugh Town beach in the Scilly Isles, (see fig. 71). Here, within the size range parasitised, 50 females out of 387 contained parasites while out of 374 males, only ten were parasitised, (i.e. percentage parasitism of females was about five times that of the males). With other Porcellio populations parasitised by Melanophora and Rhinophora however, the differences in percentage of parasitism between males and females was not so great.

In populations parasitised by Styloneuria, there was a significant difference in percentage parasitism of the sexes (see figs. but this was not so marked as in the two populations cited above, and as with Melanophora and Rhinophora, the ratio varied in different

populations. Usually with Styloneuria, females were about twice as heavily parasitised as males, but the situation was complicated by differences in percentage parasitism of hosts of different sizes, (see figs. 76, 77 and 78). Thus while percentages of parasitism of males of most different host sizes were not significantly different, those of the females were.

The greatest number of populations of Porcellio scaber, dissected to investigate differential parasitism of sexes, were parasitised by Plesina (see figs. 72, 73 and 74). It was found that proportionately more females were parasitised in populations with low percentages of parasitism and the reverse was true. The reasons for this are not clear although generally in other insect parasites discrimination is less between one host and another when percentage of parasitism is high.

Ratios of numbers of parasites per hundred males plotted against the number of parasites per hundred females give almost a straight line, (see fig. 79). Points were much more scattered when the percentages of parasitism of each sex were plotted against each other and superparasitism was not accounted for (see fig. 80). Percentage of parasitism by Plesina increased with host size in both sexes, unlike that in Styloneuria.

Insufficient numbers of P.scaber parasitised by Frauenfeldia of P.rathkei by Stevenia and of Armadillidium vulgare by Phyto were dissected from the field to determine whether there is any differential parasitism of sexes by these flies.

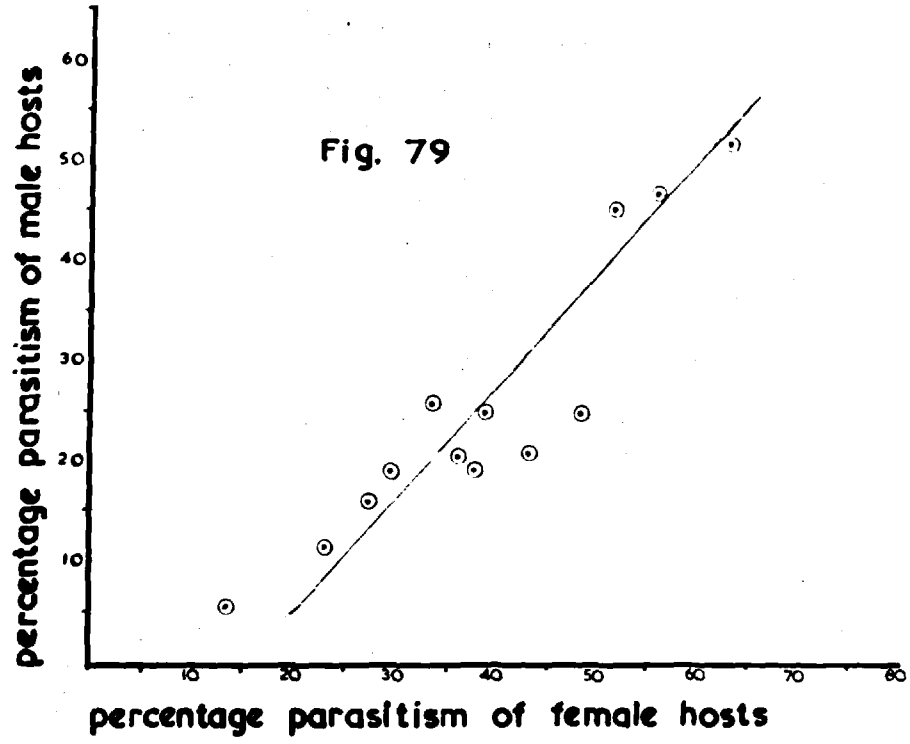


Fig. 79
percentage parasitism of male hosts
percentage parasitism of female hosts
10
20
30
40
50
60
70
80

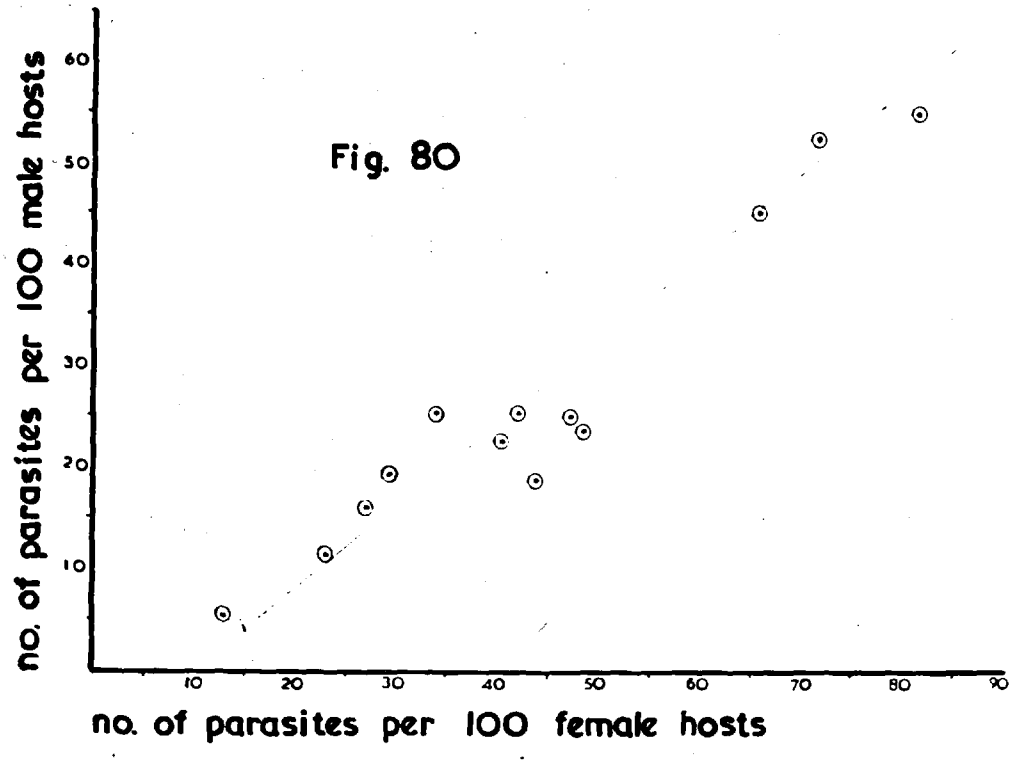


Fig. 80
no. of parasites per 100 male hosts
no. of parasites per 100 female hosts
10
20
30
40
50
60
70
80
90

Experimental

The differential parasitism of sexes caused by some species of parasite in the field could have been due to a number of factors. Some of these were tested experimentally with Melanophora and Styloneuria.

1). Melanophora larvae readily and invariably attach themselves to either male or female woodlice when they contact these and Styloneuria larvae also show no discrimination in attaching themselves to either male or female Porcellio when they touch them, provided that the woodlice are near to moulting. Several hundred male and female Porcellio were readily infected by both species of parasites.

2). When cultures of freshly moulted Porcellio scaber were infected with one Melanophora larva per host, the percentage of larvae which successfully entered and reached second instar was similar in both sexes of woodlice. Thus when 50 individuals of each sex from one culture were dissected eight to ten days after infection, 29 females and 25 males were parasitised. Similarly, in a culture of selected hosts each of which was infected with a Styloneuria larva, it was found two weeks later that 28 out of 63 females and 16 out of 38 males contained parasites.

3. Similar numbers of pupae were obtained from each sex of experimentally infected woodlice.

4. When exposed to cold conditions (5° C) for some weeks mortality of parasitised males was no greater than that of the females.

5. Dissections of parasitised cultures at all stages of parasitism show that only very rarely does the parasite die after entry, unless there is super parasitism, and that death occurs no more readily in male hosts than in females.

It thus seems that the difference in the parasitism of sexes of hosts in the field (caused by Melanophora and Styloneuria) is due to other causes. There are various possibilities:-

1. Both species rely on moulting of the host before entry and it is that adult females moult more frequently than the males (Heeley 1941).
2. The moulting cycles of adult female woodlice in the field tend to synchronise, because of the breeding cycle, whereas this is not so in the males. (Heeley 1941). If the parasite is in some way able to synchronise its life history with that of its host, females would be more vulnerable to parasitism. Such synchronisation is likely with Melanophora at least.
3. During the summer months, colonies of Porcellio scaber beneath stones and rubble sometimes consist almost entirely of females, as males have a greater tendency to aestivate.
4. Adult female woodlice sweep out a wider path as they walk since they are a little wider than the males.

While these factors seem to be the most likely causes of the differences in percentage parasitism between male and female hosts, other differences in the behaviour and ecology of the sexes could well play a part. Differential parasitism of the sexes by Plesina is probably not due to the factors mentioned above concerning moulting of the host. However, with Rhinophora it is unlikely that any of these factors play a part, since this species only attacks very small woodlice which show no obvious differences in behaviour, and the parasitic larva can enter a host at any stage of its moulting cycle. No experimentation has been attempted on differential parasitism of sexes of hosts by this species.

Laboratory infections of Armadillidium vulgare by Phyto

Melanocephela and of Porcellio rathkei by Stevenia atramentaria show that these parsitic larvae are equally capable of entering and surviving in hosts of either sex.

11c) Relationship between the habitat of the host and the species of parasite present.

While colonies of Porcellio scaber can be found in a great variety of different habitats, not all of the five species of Rhinophorinae which parasitise this woodlouse are to be found in any one kind of habitat.

Previous records of the occurrence of these parasites in the field have only been concerned with information about the adult flies; the time of their appearance in the field; the kind of flowers on which they feed; their commonness or rareness and the localities from which the rarer species had been taken. Day (1948) includes some brief references to habitats. Thus he mentions that adult Melanophora are sometimes found in the vicinity of houses; Styloneuria and Phyto near the sea; Rhinophora on waste ground and Plesina and Frauenfeldia in woodland.

During the present work woodlice from many different habitats and localities have been dissected and cultured (see tables of the appendix) in order to determine whether there is any relationship between the type of habitat in which the host lives, and the species of parasite found within it, and to gain some information as to the factors causing the commonness or rareness of a species of parasite.

Collections of woodlice from the field for ecological investigations were only made from October to early May since during other months there was a possibility that some parasites would be free living either as adults, eggs or first stage larvae.

Most of the populations of P.scaber obtained from certain types of habitat were parasitised by only one species of Rhinophorinae. Thus, only Styloneuria was obtained from the many hundreds of woodlice collected from beneath stones and rubbish on demolition sites, rubbish dumps,

gardens and other waste ground in and around London, and all populations of more than about fifty Porcellio scaber obtained from such habitats were parasitised by this species (see tables of the appendix). Woodlice from similar habitats in Kent, Surrey, Essex and Cornwall, while commonly parasitised by Styloneuria, rarely had any other species of parasite. The old walls constructed of mud and granite which are so common in Cornwall often house vast numbers of Porcellio scaber. Styloneuria was the only species found parasitising these populations.

While Styloneuria was found to be a very common parasite of woodlice in the types of habitat referred to above, woodlice from other types of habitats were rarely parasitised by this species.

Large populations of P.scaber commonly occur beneath loose bark of dead or living trees and logs, and larvae of Plesina maculata were usually found parasitising them. More often than not, especially where host colonies were found in woods, Plesina was the only parasite present, but from some populations, even those very heavily parasitised by Plesina, a low percentage parasitism by Melanophora roralis was recorded, while in host populations composed of a large proportion of very small woodlice, there was usually some parasitism by Rhinophora. (see tables of appendix).

While Plesina was rarely found parasitising any woodlice other than those living beneath bark, both Melanophora and Rhinophora did occur elsewhere. Melanophora was found most commonly parasitising woodlice living on the upper seashore region beneath rocks and between vegetation and rocks in Cornwall and within outhouses and gardens nearby. (see tables of appendix). However, it is possible that this is a different subspecies or even a different species from the Melanophora found parasitising woodlice beneath bark. Rhinophora also parasitise

those woodlice found in the seashore although it is not so common as Melanophora.

Frauenfeldia rubicosa is a rare species and too few populations of woodlice parasitised by it were investigated to make any generalisations about its habitat preferences. This species was only taken from a few populations of woodlice found in and around Silwood Park near Ascot and from Studland, Dorset. In the former locality woodlice collected from the litter at the base of some oak trees and others beneath the bark of those trees were parasitised by it. However, in contrast the woodlice parasitised by Frauenfeldia from Studland, were living in a sand dune habitat.

Large populations of P.scaber not parasitised by any species were rare. They were usually collected from habitats exposed to the wind and were completely isolated from other populations. Thus, as can be seen from the table summarising dissections of P.scaber from the field, only one parasite was found in woodlice from heathland.

While parasitism of Armadillidium vulgare by Phyto melanocephala seldom exceeded three to four per cent, wherever large populations of this woodlouse occurred, there was invariably some parasitism. A.vulgare was collected from grassland, bneath stones, in litter and beneath tree bark and was found to be parasitised in all these habitats. Phyto thus has a wider range of habitats than any of the parasites of Porcellio scaber. Adult Stevenia atramentaria are recorded by Wainright (1928) and Day (1948) as very rare and before the present investigation, were only taken in England from Belvedere and Abbey Wood, Kent. although the host species Porcellio rathkei is widely distributed throughout the British Isles. I have only found three populations of P.rathkei outside Kent and these were

all in Berkshire. None of them were parasitised. Two populations of this host species from Cliffe and one from Badger's Mount, Kent, were the only others found and all the three populations had Stevenia atramentaria parasites. The habitats of these three parasitised colonies differed in that one was found under stones and rubbish by a marsh, another beneath bark litter and under the bark of a dead tree in the middle of a field and the Badgers Mount population was found beneath the bark of logs in a wood. Despite very careful examination no other P.rathkei were found within miles of these three colonies. Obviously no inferences as to the habitat preferences of this parasite can be drawn from these limited observations. However, if indeed Stevenia atramentaria is limited in distribution to that region of Kent close to the Thames, there is the possibility that it has been introduced by shipping from the Continent where it is common.

Table of Summary of Dissections of Porcellio scaber from various types of Habitats. (see tables of the appendix).

Habitat type.		No. P.scaber	No. P.scaber parasitised and Species of Parasite found.					
1	2	dissected	P.	S.	M.	R.	F.	Ph.
A	T	3279	1019	2	9	17	2	0
A	F	2280	305	0	34	12	23	0
A	G	404	98	0	8	2	0	0
B	T	830	85	0	12	4	0	0
B	F	714	5	1	6	11	2	0
B	G	50	3	0	0	0	3	0
C	T	497	78	1	1	0	0	0
C	F	347	14	0	12	10	2	0
C	G	47	6	0	0	0	0	1
D	F	248	0	2	2	0	0	0
D	G	3939	9	384	17	0	0	0
D	S	1758	0	0	100	7	0	0
D	G	1998	0	1	0	0	0	0
D	D	330	0	0	0	3	0	1
Total								
A		5163	1422	2	51	31	25	0
B		1694	93	1	18	15	5	0
C		891	98	1	13	10	2	1
D		7309	9	387	119	10	0	1

Habitat type: Column 1 - microhabitat. Column 2 - macrohabitat

Key to lettering: (As for appendix tables)

1. Microhabitat

A - Beneath loose bark of living trees.

B - Beneath loose bark of logs.

C - Beneath bark and amongst wood of dead erect trees.

D - Beneath stones and rubbish.

2, Macrohabitat

T - Woodland

G - Gardens and wasteland

F - Fields

S - Seashore

D - Sand dunes

H - Heathland

C - Sea cliffs.

Key to abbreviations

Parasites:

P. - Plesina

S. - Styloneuria

M. - Melanophora

R. - Rhinophora

F. - Frauenfeldia

Ph. - Phyto

11 d) Factors effecting percentage parasitism.

As can be seen from the tables of the appendix which summarise the results of the dissections of many populations of woodlice from a variety of habitats, parasitism by various species varies between 0 and 50 per cent.

Whilst investigations into the problems involved have been relatively superficial, some information has emerged about the factors which determine the percentage of parasitism.

Parasites in general have long been considered to act in a density dependent way on populations of their hosts. (Nicholson 1933). Thus the greater the density of host in generation n the greater would be the expected percentage parasitism in generation n + 1. The effect is referred to as delayed density dependence (Varley 1948, 1957).

In colonial woodlice it is rather difficult to define "population density". For instance, there may be several hundreds of woodlice beneath a few square inches of bark on a particular tree whilst there may be no woodlice on rest of the tree or on other trees nearby. Similarly, large numbers of woodlice are often found under one stone whilst there are few or none under others around. In other habitats equally large numbers of woodlice often occur in small or large scattered groups over a wide area. Where the habitat consists of heaps of rubble, woodlice are found not only just beneath the surface but for a foot or more below this.

To compare population densities of colonial woodlice from diverse habitats was, therefore, considered of less relevance than the size of the population and the degree of its isolation from other populations. The latter, which is of course one aspect of host density, was found to be one of the main factors determining percentage parasitism by Plesina at least. Thus as can be seen from the tables of the appendix, most colonies of woodlice of host species in similar habitats in the same locality are parasitised to a similar degree. Generally woodlice in localities where there are many large colonies within at least a few hundred yards of each other are more highly parasitised than woodlice in more isolated colonies.

Superimposed upon this, however, is the ease of access of the adult parasite to the colony available. Thus woodlice beneath large unbroken areas of loose bark on trees or logs are usually much less highly

parasitised than woodlice under small pieces of loose bark on trees. In the latter case adult parasites are easily able to crawl from the surface of the tree and underneath the bark, to any part of the colony, whereas in the former case, adult parasites can probably only reach the outskirts of the colony easily.

As can be seen from the tables of the appendix and the table summarising these, most woodlice taken from living trees (usually Elm trees) are usually at least 20 per cent parasitised by Plesina. This is almost certainly because the loose bark on these trees is usually divided into small sections which have large edge circumferences compared to their area.

Another factor probably affecting the degree of parasitism is the amount of exposure of a habitat to winds. Thus while many thousands of woodlice were found beneath stones and vegetation on walls on Cornish cliffs where there are colonies every few yards for hundreds of yards, parasitism by Styloneuria was very low. These habitats are almost continuously swept by very strong sea or offshore winds and observations on other insects show that they often have great difficulty in settling. This factor might also account for the surprisingly low parasitism of woodlice collected from heathland, (only one Styloneuria larva from a total of 1928 P. scaber). However, the absence of parasites in this type of habitat might also be the result of the absence of suitable flower heads on which Styloneuria adults feed.

The preference of certain parasites for various sizes of hosts has already been discussed. Obviously if the ratio of a particular size group of hosts to other sizes is low and the parasite is able to parasitise only this size group, the percentage parasitism of the

whole population will in turn be low. This is particularly true of parasitism by Rhinophora lepida. Often populations of Porcellio scaber contain no, or only a few, individuals smaller than 9 mm. These populations are then either not parasitised by Rhinophora or parasitism by this species is very low. However, the degree of parasitism by Rhinophora of the particular size group appears to vary very little from habitat to habitat.

In dealing with percentaage parasitism by Rhinophora it is of little use to compare parasitism of whole colonies, but it is better to consider only the size groups of hosts which it is able to parasitise.

Too few populations of woodlice parasitised by Frauenfeldia have been found to draw any conclusions as to factors which may effect parasitism. This parasite, however, is restricted to certain sizes of hosts.

Whenever Armadillidium vulgare has been found in numbers exceeding about one hundred, it was usually found to be parasitised by Phyto, but in almost all cases the percentage parasitism has deviated little from one to three per cent.

Only three populations of Porcellio rathkei were found in Kent, the county to which its parasite Stevenia atramentaria is restricted. Each of these populations was found to be isolated by several miles from the others, and a very intensive search was made to determine this. However, despite the apparent isolation and the small size of the colonies, two of which contained less than 100 individuals, parasitism was found to be of the same order.

It has unfortunately not been possible to compare parasitism in many populations for two or more years. There were a number of reasons for this. Firstly, in London where many of the populations parasitised by Styloneuria were investigated, habitats studied in one year were usually destroyed by building or cleaning up operations by the next. Secondly, examination of populations on trees necessitated stripping of bark, thus destroying the habitat.

However, as can be seen from the tables a few habitats were examined for two years. How far the collecting of large numbers of woodlice from a habitat affects the degree of parasitism is difficult to determine but of 350 woodlice collected from habitat 1d (see tables of the appendix) during January 1964 when this habitat contained at least 2000 woodlice and there were several other populations near by, 171 hosts were parasitised by Plesina) compared with only 17 Plesina found in 100 woodlice at the same time the following year when the population was greatly reduced and isolated.

12. DIAPAUSE OF PARASITIC LARVAE

Thompson (1934) states that when woodlice were 'brought indoors' during early December, development of both hosts and parasites was definitely accelerated so that adult flies of some parasite species emerged as early as the middle of January. Apart from mentioning this and also that Rhinophora overwinters as first stage larvae and that other species parasitising woodlice overwinter during their second instar, he offers little information on the diapause in Rhinophorinae.

Only a preliminary investigation into the diapause of these parasites has been undertaken in the present work. The evidence obtained suggests that whilst there is no diapause in the hosts, there is an obligatory diapause of the second stage larvae of Styloneuria, Plesina, Frauenfeldia, Stevenia and some strains of Melanophora. There appears to be no diapause of Phyto larvae.

Rhinophora lepida

Experiments to investigate the diapause of this species are summarised in the table below. These indicate that a cold period of a month was sufficient to break the diapause in Rhinophora first stage larvae.

Porcellio scaber parasitised by this species were obtained from the field at various times of the year. Generally it was found that Rhinophora larvae in woodlice collected from February to May were not in diapause, while those collected from August to January were, i.e. they remained in the first instar. The second and third stage larvae of Rhinophora are not generally found in the field until April and it is thus difficult to explain why the first stage larvae kept for one month at a cold temperature in experimental conditions moulted into the second stage

before being subjected to warmer conditions.

Table of Experiments to Investigate the Diapause of Rhinophora lepida.

No. of P.scaber infected	No. remaining unparasitised	Treatment	Dissections. No. and time.	Stage of parasite found
30	7	At 25°C, in 24 hr.daylength for 6 weeks	30, 5 per week dissected	1st stage larvae
30	2	a) at 25°C for 1 week 20°C for 1 day 15°C for 1 day 6 - 7°C for 4 weeks	10 dissected	2nd stage larvae
	6	b) Remaining hosts placed at 25°C	-	Puparia formed in 1 - 2 weeks
30	7	At 25°C in total darkness for 12 weeks	30, 5 per week dissected	1st stage larvae
30	5	At room temperature (18-23°C) for 12 weeks	30, 5 per week dissected.	1st stage larvae

Phyto melanocephala

Because Armadillidium vulgare usually hibernates in the soil during the winter months, it has not been possible to collect large numbers of this species from October to March and to determine whether or not Phyto larvae parasitising them during this time were in diapause or not. A number of cultures of Armadillidium parasitised by Phyto were collected in March, April, May, August and September and all the Phyto larvae pupated within two weeks at 25°C. However, there is also no diapause at these times of the year in the other parasitic species.

Attempts to induce diapause in Phyto larvae were unsuccessful as can be seen from the table below.

Table of experiments on the induction of Diapause in Phyto melanocephala

No. A. vulgare infected.	No. remaining unparasitised.	Treatment	Dissections No. at time.	Stage of Parasite Found.
500	230	25°C for 4 weeks	230 after 4 weeks	puparia formed in 2 - 3 wks.
50	18	20°C for 5 days 5°C for 3 " then at 25°C	18	puparia formed after 2-3 wks. at 25°C
50	13	20°C for 5 days 6°C for 5 " then at 25°C	13	As above
50	21	20°C for 5 days 6°C for 10 " then at 25°C	21	As above
50	30	Larvae before infection at 8°C for 3 days. At 25°C after infection	30	As above
100	37	Larvae before infection in 8 hr. 25°C for 4 days in same condition after infection	37	As above

Plesina maculata

Insufficient numbers of first stage Plesina larval penetrations were obtained to use infected hosts for diapause studies. However, large numbers of parasitised field hosts have been cultured.

Populations of several hundred Porcellio, recently parasitised by Plesina were obtained from the field during the months of July, August and September. These woodlice had been freshly parasitised, and this was inferred from the fact that while previous samples of populations from that habitat contained only fully grown second stage or third stage larvae during May and June, woodlice collected during the later months contained only very small second stage larvae. Further, free living first stage larvae were observed in these habitats one to two weeks before making the later collections. These woodlice were cultured and their parasites produced puparia after four to six weeks at 25°C and only the unparasitised woodlice remained alive, as was shown by dissections.

Porcellio populations which were parasitised by Plesina that were collected from the field from the end of January to March and kept at 25°C produced puparia after two to six weeks and dissections at the end of this time showed that all larvae had pupated.

A collection of some 300 Porcellio with about 10 per cent parasitism by Plesina was made throughout October 1964. Half of them were kept at 25° C and the other half at 6 - 7°C for a month. Only two puparia were formed in the first set, although the culture was kept for four months, while twelve puparia were formed in the second set a month to six weeks after it was removed from low temperature and placed into 25°C.

Melanophora roralis

It proved impossible to collect populations of Porcellio highly parasitised by Melanophora in September, October and early December as such populations were only found in Cornwall. However, 60 Porcellio were collected in Penzance, Cornwall, in the middle of December 1962, and three Melanophora puparia were formed after three weeks at 25°C.

Several samples of populations, only very lightly parasitised by this species, were obtained from Kent, Berkshire and Surrey in these months and they were kept at 25°C. A total of 24 puparia were formed after 2 - 3 months at this temperature.

Cultures of 250 Porcellio were infected with first stage larvae hatching from the eggs of the flies from these puparia and then kept at 25°C, and puparia were formed within 2 - 3 weeks. Dissections of the remaining hosts showed that all larvae had pupated by then.

Dissections of woodlice collected from September to April from the above three counties showed that all the second stage Melanophora larvae obtained from these woodlice were characterised by the conspicuous white colour of the adipose tissue (see plate 6). These larvae were, or had been in diapause. However, second stage larvae obtained by infecting woodlice with first stage larvae reared from these habitats had transparent adipose tissue. These larvae were not in diapause.

None of the numerous Melanophora second stage larvae dissected from Cornish woodlice collected during March and April (see Appendix tables) had the pearly white adipose tissue characteristic of the above larvae and neither did five Melanophora second stage larvae dissected from a small population of Porcellio collected from Cornwall during the middle of December 1962.

Whilst there were insufficient numbers of Melanophora from counties apart from Cornwall to allow experiments on diapause, numerous unsuccessful attempts were made to induce diapause in Cornish Melanophora. The experiments are summarised in the table below.

Table of Experiments on Induction of Diapause in Melanophora.

No. of freshly moulted Porcellio infected.	Treatment	Result
5 cultures of 50 - 100 hosts	Three days at 20°C, 2,4,6,8, or 10 days at 8°C. Then at 25°C	All larvae formed puparia after 2-3 weeks at 25°C
5 cultures of 50 - 100 hosts	Three days at 20°C 2,4,6,8 or 10 days at 5°C. Then at 25°C	As above
150 hosts	1st stage larvae at 5°C for 2 days before infection of Porcellio then at 5°C for 5 days. Then at 25°C.	As above
200 hosts	1st stage larvae at 8 hr. day for 4 days before infection of Porcellio also at short day and 25°C	As above

These experiments suggest that there is possibly no diapause in Melanophora collected in Cornwall, but more experiments are needed to prove this.

Styloneuria discrepans

About 200 Porcellio which were about to moult, have at various times been infected by Styloneuria larvae and then kept at 25°C. All larvae which had entered hosts of length greater than 9 mm pupated in 28 - 35 days.

A culture of 300 Porcellio 12 per cent parasitised by Styloneuria collected at the beginning of October 1962 and kept at 25°C. No puparia were formed until four months later (February) and all larvae from this culture had pupated after four and a half months. A further 500 Porcellio were collected from the same habitat at the beginning of November 1962 and half of these were left for six weeks at 6 to 7°C and then transferred to 25°C and the other half was kept at 25°C from the beginning. Both cultures produced puparia at the same time, which was eleven weeks later, and puparia were produced for a further two weeks.

A further collection of over 1000 Porcellio was made from the same habitat at the beginning of December 1962 and the first puparia were formed after only one month and then throughout two weeks afterwards. One hundred Porcellio from another habitat collected at the end of January 1963 produced parasitic puparia after two weeks at 25°C.

These results suggested that there was a facultative diapause in the second stage larva. Dissection and external examination of parasitised woodlice showed that the length of the third instar was constant and that while cold treatment was not essential it considerably speeded up development.

Attempts to induce diapause in Styloneuria were successful. Cultures of 25 - 50 Porcellio were infected by first stage larvae and left at 20°C for five days so that the larvae moulted into the second

stage. Infected woodlice were left at 6°C for 2,4,6,8 or 10 days, and diapause was induced in some larvae in all these cultures. While eight larvae pupated within one to two months at 25°C all the rest remained as second stage larvae for four months before forming puparis at 25°C. A culture of 300 first stage Styloneuria larvae was kept at 6°C for 2 days before these larvae were used to infect Porcellio, about to moult. The infected hosts were kept at 25°C and the larvae pupated within a month to six weeks.

As might be expected in a larva which parasitises hosts living in almost total darkness, short photoperiod had no effect in initiating diapause. Fifty Porcellio were infected with Styloneuria larvae which had been subjected to two days of 8 hours light and 16 hours darkness at 25°C and the unaffected hosts were then themselves subjected to this photoperiod and temperature. Puparia were produced within the same period as in non-diapausing larvae.

Frauenfeldia rubicosa

Little material of this species has been available, but from a culture of 150 Porcellio about 9 per cent parasitised by this species and collected at the beginning of December 1962, two puparia were produced within one month while the other larvae remained in instar two to three months.

Porcellio collected at the end of May 1963 from the same habitat produced Frauenfeldia puparia after two weeks at 25°C.

When 25 Porcellio were infected with Frauenfeldia first stage larvae, puparia were produced 5 - 7 weeks afterwards at 25°C.

Stevenia atramentaria

It has not been possible to collect P.rathkei parasitised by

Stevenia from the field in the winter as this woodlouse hibernates in inaccessible places.

Thirty P.rathkei all with brood pouches were infected with first stage larvae of Stevenia and all the larvae pupated within two to three weeks at 25°C. However, when a further culture of forty P.rathkei (only two of which had brood pouches) were infected and kept at 25°C, only two puparia were produced within two to three weeks and dissections of five hosts after four weeks showed that other larvae were still in the second instar. No further puparia were formed after six weeks and as this culture was the only one available, all specimens were then left at 6 - 7°C for one month. When the culture was then placed into 25°C two puparia were produced after twelve days, but it was one month before all larvae had pupated.

These results show little except that Stevenia has a facultative diapause possibly associated with the breeding cycle of the host and Melanophora can only enter freshly moulted hosts and the hosts of both these species do not usually moult after entry of the parasite in the case of Styloneuria or until the second instar parasite moults in the case of Melanophora.

13. DISCUSSION

Very little work has been done in the past on this interesting group of Diptera although they are the only insects which are known to parasitise Crustacea.

Not only is their behaviour of interest, but also the morphology of immature stages has a number of peculiarities.

Thompson (1934) considers that 'the Dipterous parasites of woodlice fall naturally into three main groups based mainly on the structure of the buccopharyngeal armature of the first stage larvae'.

In his first group Thompson included Plesina, Melanophora, Phyto and Styloneuria. During the present work it has been possible to compare not only the buccopharyngeal armature of the first stage larvae of these species but also their external morphology and behaviour. In these respects the larvae are also very similar to one another, and differ from the other species. The general body forms are alike, as is the structure of the last segment, which in each species is so modified as to enable the larva to stand erect from the substrate for much of its life.

Further, the peculiar seeking and somersaulting movements of these larvae are characteristic of this group. As pointed out by Thompson, the buccopharyngeal armature is slender, delicate in general construction, having only a single articulation and with an anterior region rather short, sub-quadrangular and bearing several more or less distinct teeth.

Thompson divides the remaining larvae into two groups: one containing Frauenfeldia, Cyrillia and 'species B' and the other his 'species A' (which is Rhinophora lepida). He separates Rhinophora from the other species because it has two articulations and two distinct anterior sclerites in the buccopharyngeal armature of the first larval

stage as compared with the single articulation and partially fused anterior sclerites of Frauenfeldia, Cyrillia and his 'species B'.

The latter three species are grouped together by Thompson because the buccopharyngeal armature is stout, heavily sclerotised and deeply pigmented, having only a single articulation but with one large and one rather small tooth.

Another factor which might be taken into consideration in separating Rhinophora from other species is that it is the only one which diapauses in the first instar. However, in external morphology it is very similar to Frauenfeldia and Stevenia, having articulated 'pseudopods' and even the same number of 'pseudopods' on each segment which are absent from the first group. The posterior end of first stage larva of Rhinophora is also similar to that of Frauenfeldia and Stevenia and is characterised by the presence of a pair of large swollen vesicles.

In behaviour patterns the first stage larvae of Frauenfeldia, Stevenia and Rhinophora also fall into one group; they have a looping method of locomotion, ability to rear up either anterior or posterior ends, and enter their hosts at the leg base membrane. Thus on the above characters the larvae could possibly be divided into two and not into three groups.

While very similar in external appearance the second stage larvae can readily be divided into two groups on their buccopharyngeal armature which has only one articulation in Plesina, Melanophora, Styloneuria and Phyto but two in Frauenfeldia, Stevenia and Rhinophora. This supports the grouping of the first stage larvae.

The third stage larvae are, however, all fairly similar, except that of Melanophora, which has only one articulation in buccopharyngeal

armature compared with the two of other species.

The Rhinophorinae in common with other Calliphoridae lay undeveloped eggs, so that these do not hatch until some days after oviposition. The eggs are neither attached to or injected into the host as in many Tachinid and Hymenopterous parasites but are laid just in the vicinity of the host.

Whether or not the parasites of woodlice would have been more successful had they evolved one of the perhaps more advanced means of oviposition, is debatable. Thompson (1934) considered that because Porcellio and Oniscus pass the greater part of their lives in protected situations, under stones or bark, they are inaccessible to direct attack by a parasite without a piercing ovipositor. However, during the present work it was found that in the field, Plesina, Frauenfeldia and Stevenia at least, usually crawled right into these "protected situations" before laying eggs throughout the microhabitats of the woodlice. Nevertheless, in spite of this, it is doubtful that the adult parasites would be able to reach the majority of their hosts in this way. Potentially, the habit of laying many eggs in the easily accessible portions of the microhabitat of the host and thus exposing the hatched parasitic larvae to moving woodlice may be the most successful method of infection. Certainly the high percentages of parasitism recorded from many field habitats would suggest this to be so.

The comparatively long period required for the hatching of the parasite eggs may seem disadvantageous. However, there is a mechanism which ensures that the eggs are laid in much frequented habitats of the hosts. As has been shown, the adult parasite does not oviposit merely in the presence of the host or in its vicinity. The necessary stimulus is

provided by the substrate which has been contaminated for at least a number of days by the secretions of woodlice. The fact that woodlice have already been in a microhabitat for some time indicates that it is a suitable one for them and that they will probably still be there some days after oviposition by the parasite. There are several advantages to the parasite in laying undeveloped eggs namely that these can be laid soon after emergence (e.g. Melanophora will lay most of its eggs on the day of its emergence) which minimises the chances of adult mortality before oviposition. Also the adult parasite does not have to feed and can complete its oviposition in areas, such as woodland and seashore, where there is little or no food supply. There are no records in the literature of Melanophora on flower heads and the actually very common species - Plesina has been recorded as "rare" because it has only rarely been found on flowers. However, the long period required for development of the eggs does subject these to the predation of mites and even to that of the woodlice themselves.

Whether or not these advantages outweigh the disadvantages is difficult to determine, but as these parasites have not evolved a mechanism for the retention of eggs during development as have many other parasitic Diptera, e.g. Bigonichaeta (Sweetman 1958), the evolutionary pressure for the development of this was presumably not very great. On the other hand as the deposition of undeveloped, unspecialised eggs in the vicinity of hosts is probably the most primitive method of dipterous parasitic oviposition, and as the Rhinophorinae for other reasons are believed to belong to the mainly non-parasitic Calliphoridae, it is possible that this group has only recently evolved parasitic habits and has not evolved any of the more complex methods of host infections

characteristic of other parasitic Diptera.

However, the development in the Rhinophorinae of two unique types of first stage larvae, each type and species being characterised by specialised structures, by specialised behaviour and mode of entry into the host, does suggest that a considerable degree of evolution away from the normal Calliphorid type has taken place in the larvae at least.

The larval behaviour is perhaps a little surprising in some respects, and in fact like the Echinomyiine first stage larvae, those of the Rhinophorinae do not go in search of their hosts, but usually remain close to where the eggs were deposited, until the host walks past them.

It is the mechanical stimuli which mainly activate the larvae to attach themselves to a host. This type of larval behaviour may be more primitive than the active searching behaviour in the Dexiids, since presumably in the latter, development of a complex specific chemosensitivity to the host is required. Nevertheless, in the case of the parasites of woodlice, the method of remaining stationary, so that the host must come to the parasite before infection is possible, may well be most effective since Porcellio scaber, the host of most of these species is usually found in large colonies, and both P.rathkei and Armadillidium vulgare, are to a certain extent colonial. Within a colony of woodlice it is quite likely that the parasite larva will be walked over by a host, especially since the nocturnal movements of the woodlice known at least in P.scaber cause many of them to vacate and to enter their shelters throughout the night (P.J.Den Boer (1961), Le Gay Brereton (1957) and thus much of the area within the shelter is traversed by potential hosts.

If a parasite has to search for its hosts it must use up food and water reserves and thus decrease its potential longevity as well as

increase the chance of superparasitism.

The degree of host selection by the parasite larvae varies from Melanophora which attach themselves to anything mobile within their reach, to Styloneuria which prefer Porcellio scaber that are about to moult. As Melanophora larvae can only enter those hosts which have recently moulted, numerous larvae of this species must be wasted, since once attached to a woodlouse, however unsuitable it is for penetration, they do not migrate to another more suitable host. It is possible, however, that the appearance of the first stage larvae of the parasite may coincide with the moulting of the hosts, which apparently is synchronised in the field (Heeley 1941). Thus at 25° pupation of Melanophora takes place about eighteen days after larval entry, and this is approximately the length of time between moults of Porcellio scaber females at this temperature. Melanophora puparia then take 12 - 13 days to hatch and eggs are usually laid on the day of adult emergence. The eggs take six days to hatch at this temperature. Larvae thus emerge at approximately the same time as the synchronised host moulting at 25° C. If host moulting and parasite pupal and egg development are affected to the same degree by changes of temperature, then it is possible that a similar situation may occur in the field.

Styloneuria on the other hand requires longer (at least 28 days) to develop after larval penetration into the moulting host, to pupation, and so the hatching of first stage larvae does not coincide with the moulting of the majority of hosts. The fact that Styloneuria first stage larvae are much more resistant to dessication and live longer than Melanophora, and can discriminate between suitable and unsuitable hosts probably compensates for the lack of a synchronisation of larval emergence with the moults of the host.

The situation is observed in the field in Plesina, Frauenfeldia, and Stevenia, where the eggs are sparsely and widely distributed throughout the shelter of the hosts and laid over a long period of time, the larva remaining near the egg after hatching, probably restricts superparasitism to a minimum.

Each of these dipterous parasites has a different and characteristic method of entering its host. However, one feature which they have in common is the relatively long period (from several hours to several days) required for completion of entry. This is in contrast to most insect entomophagous parasites where larval entry is usually rapid. Thus Bigonichaeta spinipennis may take only a few minutes to complete its entry. (Sweetman 1958)

While the sclerites of woodlice are thick and tough, the intersegmental membranes and membranes at the leg bases are relatively thin and delicate. It is thus difficult to see why larval entry should take such a long time and why the larvae of some species will not or cannot commence to enter until just after the host has moulted. A further problem is created by Styloneuria and Frauenfeldia, where only the anterior part of the first stage larva is inserted into the host and complete entry is only accomplished by the second stage larva.

It could be assumed that the reason why larvae of Styloneuria and Melanophora can only enter just after a host moult is because only at this time is the cuticle soft enough to allow penetration. Plesina and Phyto larvae however, will enter their hosts at any stage of the moulting cycle. When first stage larvae of Melanophora and Styloneuria were injected into hosts which had moulted a week or more before, these larvae were heavily encapsulated and died before reaching the second instar.

This encapsulation may have been stimulated by the wounding of the host necessary for artificial introduction of the larvae or by the fact that no posterior respiratory connection by the larvae with the exterior was possible during their early growth. However, it is also possible that while the host is usually capable of encapsulating larvae of these species, it is either not sensitive to larvae or cannot encapsulate them just after moulting. Unfortunately, it was not possible to inject larvae into freshly moulted hosts without the latter dying from loss of blood.

The very slow entry of the first stage larvae may have evolved to avoid encapsulation. Thus the larval cuticle is only very gradually exposed to the host blood.

It is difficult to relate the specialised sites of entry to the above consideration. On morphological grounds, the first instar larvae of the Rhinophorinae are divisible, into two groups, the first consisting of Stylonevria, Plesina, Melanophora and Phyto, and the second of Rhinophora, Frauenfeldia and Stevenia. The first stage larvae of the second group all enter through the membranes at the bases of the host's legs and larvae of the first group enter through the intersegmental membranes or in the case of Phyto through the membrane at the base of the penis or corresponding position on the female.

There are morphological differences between the first stage larvae of the two groups which are possibly relevant to the different sites of entry. Thus the three species with the largest first stage larvae enter the membranes at the leg bases. These membranes present a larger circular area than the intersegmental membranes. The membranes at the leg base of the host appear to be more difficult to penetrate than the intersegmental membranes since they are subjected to much

greater stresses than the latter during leg movements. While the buccopharyngeal armature of the second group of larvae which enter the membra at leg bases is strongly sclerotised and has strong acute teeth, the buccopharyngeal armature of the first group of larvae is only weakly sclerotised and the teeth are feeble. Possibly this is connected with a difference in toughness of the different membranes concerned. The 'pseudopods' which are only present on those larvae which enter the basal leg membranes act as barbs during entry and aid in holding the larva firmly between the sternites. They may thus help in preventing the larva being torn from its site of entry as the host walks.

The only statements to be found in the literature regarding the control of woodlice by their parasites is in the paper by Thompson (1934). In this paper, he suggests that "while one would expect the insect parasites of woodlice to be of considerable importance in regulating the numbers of these organisms, the data at present available do not at present suggest this". Thompson points out that most species do not appear to be attacked at all while others such as Armadillidium vulgare and Metoponorthus pruinosus are only rarely parasitised. He records from his dissections of 1737 Porcellio scaber and Oniscus asellus that "the average parasitism is relatively low". An average of only 9.1 per cent for Porcellio and 3.1 per cent for Oniscus, and maxims of 25.2 per cent for Porcellio and 7.2 per cent for Oniscus led Thompson to say that "It is thus evident that the dipterous parasites are not factors of major importance in the control of woodlice. Nevertheless, since under certain conditions as many as a quarter of the host population succumbs to the paracitic attack, it seems at first sight, that the Tachinids must play a very real part in control". The former sentence of this statement

appears to be at least partially contradicted by the latter.

The term "control" has often been used very loosely. Thus it could be supposed from the above statements of Thompson (1934) that it is the degree of mortality which is all important in determing whether or not a factor is a controlling agent. However, as Nicholson (1933) and many other authors have since emphasised, the degree of mortality caused by a particular factor is not evidence in itself of whether or not this factor is of importance in population control.

Solomon (1964) defines natural control as "The process(es) of keeping the numbers of animals, in a population not controlled by man, within the limits of fluctuation observed over a sufficiently representative period". It is now widely accepted that to exercise control of a population, a factor must have a density dependant or delayed density dependant action.

To determine whether or not certain parasites exercise important controlling actions, observations should be made for a number of years and preferably the relative importance of different factors causing mortality analysed by the key factor analysis (see Morris 1959, Varley and Gradwell 1960).

No such observations and analyses have been made on populations of woodlice and their parasites and therefore conclusions on the controlling effect of these parasites are premature.

Some information which may be of use to future workers on population control of woodlice has however, been forthcoming from the present study although little evidence has been obtained about density dependance of the Rhinophorinae.

My investigations indicate that many British species of woodlice do not seem to be hosts of dipterous parasites. Oniscus asellus, although occasionally parasitised when it is intermixed with Porcellio scaber, is usually able to kill the parasite by encapsulation before being killed itself. In this species, therefore, it is very unlikely that any degree of control of its population size is exercised by dipterous parasites.

Porcellio scaber, Porcellio rathkei and Armadillidium vulgare are the only species of woodlice which I found to be effectively parasitised and in which parasitic control is, therefore, possible.

In considering the control and mortality of P.scaber, Thompson (1934) was not in possession of certain facts which may have an important bearing on the problem. Firstly, while he considered that each species of parasite completed only one generation per year, I found that all species except Rhinophora have at least two and usually more generations in a single year. Further, species which may be the most efficient parasites, namely Plesina, Styloneuria, Melanophora, usually parasitise a considerably higher proportion of female hosts than males. In certain habitats, populations frequently have parasitism greater than 30 per cent, and in one large population parasitism of female hosts was as high as 82 per cent. Plesina which is the commonest species produces the highest percentage of parasitism and tends to attack larger woodlice which usually have larger broods than smaller ones and which also have more broods per year.

In general, where populations have become large and where they are in close proximity to other populations, the percentage of parasitism is relatively high. Thus an average percentage parasitism of all woodlice

collected from different habitats such as is quoted by Thompson (1934), tends to be misleading

Heeley (1941) published an account of the reproductive capacity of Porcellio scaber (among other species) in the field. In this species, the females produce per annum one brood averaging 36 young and 10 to 30 per cent of them produce a second brood averaging 12 young. Most females are capable of producing broods for at least two years in succession and in some cases for three. Heeley (1941) found that Porcellio scaber must overwinter twice before they could produce broods, but Verhoeff (1920) considers that only one overwintering is required.

I have found that Plesina, Styloneuria and Melanophora produce 150 - 450 eggs, and Frauenfeldia 50 - 150 eggs in each generation and that these parasites have at least two generations a year in the field, while Melanophora may possibly have twice this number of generations. Rhinophora females produce at least a 100 eggs but have only one generation a year.

Parasitised female hosts can produce a brood only extremely rarely. The fact that the reproductive capacity of males appears to be unaffected until they are finally killed by the parasite probably has little influence on the effectiveness of parasitic control especially since usually a much higher proportion of females than males are parasitised.

In considering the greater reproductive capacity of the parasites compared with that of the hosts, it must be born in mind that Styloneuria and Melanophora can attack hosts only at the appropriate stage of their moulting cycle. Although the field individuals of Porcellio scaber are said to possess a common synchronised rhythm of

moulting activity (Heeley 1941), there is little evidence at the moment of parasite synchronisation with the host and the fact that the more vulnerable newly moulted woodlice are also exposed to cannibalism, may be one reason why parasitisation by Styloneuria and Melanophora does not generally exceed ten per cent.

Styloneuria which is usually found in populations of woodlice without other parasites and which usually has only two generations a year does not cause very high mortalities in host populations. As moulting is generally suppressed when woodlice are parasitised by Styloneuria, the comparatively low percentage of parasitism by this species is even less effective than it appears since mortality by cannibalism occurs during moulting.

Percentage parasitism by Melanophora is usually insignificant except in the sea shore habitats where it is the only common Rhinophorine species. In such habitats where parasitism reaches 10 per cent, and where the parasites may have as many as four generations a year, they may cause high mortality of hosts.

Plesina seems to be the only species which may exercise really considerable mortality. As a species which is present in almost every woodland and tree habitat of woodlice, causing parasitism which often exceeds 30 per cent and occasionally reaches 80 per cent of the female population, its two generations a year must invariably kill off a very large proportion of each population of its hosts.

However, even this does not necessarily indicate that this parasitism is the main controlling factor since cannibalism is rife in Porcellio scaber colonies. Woodlice are also dependant on high humidities and there may well be a shortage of suitable sites as soon as a population

increases excessively. Thus in the absence of the parasite, the size of the population would be eventually limited, although excessive competition for space would probably also cause much successful migration to other habitats.

Only relatively low percentages of parasitism have been found in Armadillidium vulgare parasitised by Phyto melanocephala and in Porcellio rathkei parasitised by Stevenia atramentaria.

Finally, while only seven species of Rhinophorinae have been recorded from British woodlice, several other species of this sub family are found in Britain and in most cases their hosts are unknown, for instance, Morinia nana is quite common. It is likely that one or more of these other species is actually parasitic on woodlice although probably not on Porcellio scaber. The results of dissections of several thousands of this species have shown that Frauenfeldia occurred in only three localities and only comparatively small numbers of other species of woodlice were dissected.

It is considered that only a superficial survey of an exciting subject has been made and that the field is open for intensive investigations of many aspects of parasitism by these interesting insects.

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APPENDIX

Explanation of tables of dissections of woodlice from various habitats.

1) Habitat No: Each habitat is numbered for reference and probable separate or partly separate populations are lettered with small letters.

2) Habitat Type: denoted by capital letters. The left hand letter applies to the microhabitat and the right hand letter to the macrohabitat.

Key to lettering:

Microhabitat

A - Beneath loose bark of living trees.

B - Beneath loose bark of logs.

C - Beneath bark and amongst wood of dead erect trees

D - Beneath stones and rubbish

E - Amongst tree litter

F - Amongst roots of grass, etc.

G - Beneath vegetation on rocks

W - Within stonework of walls

Macrohabitat

T - Woodland

G - Gardens and wasteland

S - Seashore

D - Sand dunes

F - Fields

H - Heathland

C - Sea cliff

3) Estimated population size: Usually given to the nearest hundred woodlice. Where the population is definitely greater than five thousand it is classed as inf. - (infinite). The number given indicates the number of Porcellio scaber unless otherwise stated.

4) Estimated degree of isolation: A population of woodlice is classed as "isolated" if there are no other populations of the same species within a few hundred yards at least. Where this column is left blank it has been impossible to estimate the degree of isolation because of the proximity of gardens, etc. which may or may not contain woodlice.

5) Access to parasite: Classed as very good, good, medium, poor and very poor.

6) Key to abbreviations

Hosts

O. - Oniscus asellus

A.v. - Armadillidium vulgare

A.d. - A. depressum

P.r. - Porcellio rathkei

P.l. - P.laevis

Ph. - Philoscia muscorum

C. - Cylisticus convexicus

L. - Ligia oceanica

Am. - Amphipods

M.p. - Metaponorthus pruinosus

M.c. - Metaponorthus cingendus

T.a. - Trichoniscoides albidus

Parasites

P. - Plesina

S. - Styloneuria

M. - Melanophora

R. - Rhinophora

F. - Frauenfeldia

Ph. - Phryxe

Ste. - Stevenia

A. - Acanthocephala larvae

Only results obtained from dissecting woodlice collected from November to April are included because during other months some parasites occur as adults, eggs or free living first stage larvae.

The numbers of parasites reared in culture from field collections are not included firstly because cannibalism amongst hosts occurs frequently but also because diapausing parasitic larvae are often killed by their hosts.

Locality	Date	Habitat No.	Habitat Type	Estimated Population Size	Estimated Degree of Isolation	Access to Parasite	No. P. acuber Dissected	No. P. acuber Parasitised	No Other Species Dissected	No Other Species Parasitised
Beddington Pary Surrey	Jan-Feb 1964	1a)	AT	500	95 yd b)	v.good	140	74.P	24.O	2.P.
		1b)	AT	200	95 yd a)	good	157	49.P	-	-
		1c)	CT	300	200 yd a)	poor	125	28.P	-	-
		1d)	AT	2000	1000 yd a)	v.good	350	171.P	46.O	12.P
		1e)	AT	300	40 yd d)	good	270	102.P	-	-
		1f)	AT	200	75 yd d) between a) and d)	good	184	76.P 1.M 1.R	-	-
		1g)	AT	30		good	23	2.P	4.O	0
		1h)	AT	20	do.	good	15	3.P	-	-
		1i)	AT	20	do.	good	18	1.P	7.O	0
		1j)	AT	10	do.	good	8	0	-	-
		1k)	DT	100.O	200 yd d)	medium	-	-	60.O	0
		1l)	DT	40.O	80 yd a)	medium	-	-	34.O	0
	Jan 1965	1d)	AT	200	isolated	v.good	100	17.P	-	-
Mitcham Common,Sy.	Jan.1964	2a)	CT	60	isolated	poor	57	4.P	12.O	0
		2b)	AT	30	isolated	good	25	0	-	-
Hayes Common,Kent	Feb.1964	3a)	AT	150	15 yd b)	v.good	116	62.P	8.O	0
		3b)	AT	80	15 yd a)	v.good	72	33.P	-	-
		3c)	BT	30,30.O	10 yd b)	poor	26	3.P	30.O	0
Egham, Surrey	Jan.1964	4a)	AF	100	10 yd b)	good	61	15.P	-	-
		4b)	AF	300	10 yd a)	v.good	269	77.P 3.M	-	-
Chalk, Kent.	Feb.1964	5a)	AG	300	25 yd b)	good	258	84.P 7.M	-	-
		5b)	AG	80	25 yd a)	medium	65	14.P	-	-
		5c)	DG	24,109.O	10 yd b)	medium	24	0	50.O	0
Seale, Kent.	Feb.1964	6a)	AF	120	35 yd b)	good	104	24.P	18.A.v. 17. Ph	0
		6b)	AF	200	35 yd c)	good	169	42.P	-	-
Bexley, Kent.	Mar.1964	7	AT	100	isolated	good	83	19.P	-	-
Buckland, Kent.	Mar.1964	8	AF	200	isolated	good	134	18.P 13.M 5.R	-	-
Cliffe, Kent.	Mar.1963	9a)	DG	inf.O 30 P.r	150 yd d)	-	-	— —	250.O 18.P.r	2 Ste
	Feb. - Mar..1964	9b)	AG	90	100 yd c)	poor	81	2.P 2.R 1.M	-	-
		9c)	CG	100	100 yd b)	poor	47	6.P 1.Ph	36.M.r	0
		9d)	DG	inf. O. 100 P.r.	—	medium	26	0	300.O	0
									25.P.r	2 Ste
									100.A.v	2 Ph
									23 O.	0
									8 P.l	0
		9e)	DG	150 M.r	50 yd d)	-	-	-	80.M.r	0
		9f)	DG	—	50 yd b)	medium	-	-	200.A.v	3 Ph.
		9g)	CG	200.A.v	200 yd d)	poor	-	-	150.A.v	1 Ph.
Cattons. Nr.Cliffe, Kent.	Mar.-Apr. 1963	10	BF	2000	isolated	v.poor	100	1.P 2.M	30.P.r	3 Ste
	do. 1964	10	BF	1000	isolated	v.poor	120	1.P 2.M 1.S 1.R	10.P.r	1 Ste
	Feb.1965	10	BF	1000	isolated	poor	100	9.R 2.M	17.P.r	0
Higham, Kent	Apr.1963	11a)	DT	500.A 200.O	20 yd.b)	medium	-	-	100.A.v 50.O.	1 A
		11b)	BT	350 200 A	20 yd a) 25 yd d)	v.poor	100	8.P	150.A.v	2 Ph.
	Mar.1964	11b)	BT	150	do.	poor	100	13.P 1.M	—	-
		11c)	AT	200	10 yd b) 15 yd d)	good	117	46.P	—	-
		11d)	CT	200	15 yd c)	poor	171	32.P 1.M	—	-
		11e)	AT	50	40 yd b) & a)	medium	39	8.P 2.M	—	-

Locality	Date	Habitat No.	Habitat Type	Estimated Population Size	Estimated Degree of Isolation	Access to Parasite	No. P. scober Dissected	No. P. scober Parasitised	No. Other Species Dissected	No. Other Species Parasitised
Oakleigh Nr.Higham Kent.	Mar.1964	12a)	AF	40	100 yd b)	good	34	14.P	-	-
		12b)	AT	300	100 yd a)	medium	178	24.P 1.M 1.P 2.P	-	-
Cuxton, Kent.	Apr.1964	13	BT	600	isolated	v.poor	100	7.P	24.Ph.	-
Frogbole, Kent.	Apr.1963	14	BF	800	isolated	v.poor	250	0	-	-
Otford, Kent.	Dec.1963	15a)	CF	inf.	20 yd b)	poor	100	8.P 3.M	-	-
		15b)	AF	200	20 yd a)	medium	100	5.P 6.M	50.Ph	-
	Mar.1964	15a)	CF	inf.	isolated	poor	50	2.P 6.M	-	-
Bromley, Kent.	Apr.1964	16a)	AT	300	30 yd b)	v.good	352	103.P 4.M	-	-
		16b)	AT	250	30 yd a)	good	75	19.P	-	-
		16c)	AT	60	35 yd b)	good	50	12.P	-	-
		16d)	AT	800	150 yd A,b & C)	v.good	190	76.P	-	-
		16e)	AT	100	65 yd d)	good	68	20.P 1.S	-	-
		16f)	BT	150	10 yd e)	poor	70	27.P	-	-
		16g)	BT	80	15 yd e)	v.poor	63	15.P	-	-
	Feb.1965	16a)	AT	30	130 yd h)	v.good	18	5.P	-	-
		16d)	AT	30	120 yd h)	v.good	22	3.P	-	-
		16h)	AT	500	10 yd i)	good	54	18.P	-	-
		16i)	AT	80	10 yd h)	medium	35	5.P	-	-
		16j)	AT	100	10 yd h)	medium	46	10.P	-	-
		16k)	AF	50	200 yd h)	v.good	33	14.P	-	-
		16l)	AF	60	25 yd k)	good	57	17.P 1.M	-	-
		16m)	AF	100	10 yd l)	poor	89	9.P	-	-
Ashtead, Surrey	Jan.1964	17	AT	500	—	medium	334	58.P.16.M. 2.P 1.M	-	-
Aynsford, Kent.	Dec.1963	18a)	CT	60	20 yd b)	good	44	11.P	-	-
		18b)	BF	50	20 yd a)	poor	38	2.P	-	-
Bamsfield, Kent.	Mar.1964	19	BT	300	isolated	poor	221	4.P.9.M	-	-
Badgers Mount.Kent.	Dec.1963	20	BT	300	isolated	v.poor	100	9.P	17 P.r 50 Q	1 Ste 0
Keston, Kent.	Sept.1963	21	BT	50	—	poor	30	2.P	-	-
Abbey Wd. Kent.	Oct.1963	22	BT	300.Q	—	poor	-	-	200.Q	0
Bostall Wd. Kent.	Oct.1963	23	BT	100.Q	—	poor	-	-	78.Q	0
Lesnell Abbey,Kt.	Nov.1963	24	DT	5000 500 M.P	isolated	medium	200	3.P	100 M.P	0
	Feb.1964	24	DT	200 M.P	isolated	medium	50	0	100 M.P	0
Silwood Park, Berks.	Nov.-Dec 1962	25a)	EG	200	70 yd c) 250 yd e)	good	50	7.P 5.P	-	-
		25b)	BT	150.Q	200 yd a)	poor	-	-	100. Q	0
	Mar.1963	25c)	EG	100	70 yd a)	poor	50	3.P 3.P	20. Q	0
		25d)	BT	40	150 yd k)	poor	20	2.M 1.P	30. Q	0
	Apr.1963	25e)	AF	1000	250 yd a) 100 yd g)	poor	860	24.P 20.P 9.M 2.P	-	-
		25f)	AF	300	20 yd e)	poor	50	2.P 5.P	-	-
		25g)	AF	500	100 yd e)	poor	20	3.P	-	-
		25h)	BF	150	40 yd g)	poor	40	3.P	-	-
		25i)	CF	1000	70 yd g)	poor	100	5.P 3.M 2.P	-	-
		25j)	BF	60	90 yd e)	v.poor	56	1.P	25. Q	0
	Oct.1963	25e	AF	200	—	medium	100	7.P 1.P	-	-
		25i)	CF	500	—	poor	50	3.P	-	-
	Mar.1964	25k)	AF	400	400 yd a) 500 yd e)	good	110	18.P 2.M	-	-

Locality	Date	Habitat No.	Habitat Type	Estimated population Size	Estimated Degree of Isolation	Access to Parasite	No. P. scobar Dissected	No. P. scobar Parasitised	No. other species Dissected	No. other species Parasitised
New Lodge Berks.	Apr.1963	26	BF	250	isolated	poor	50	2.P 1.R	—	—
Frogmore, Berks.	Apr.1963	27a)	BF	250.A.V	continuous	poor	-	-	150 A.V	3 Ph
		27b)	BT	150	isolated	poor	50	4.P	—	—
		27c)	FF	inf.A.V	continuous	-	-	-	100 A.V	2 Ph
									100 Ph	0
	Mar.1964	27a)	BF	50.A.V	continuous	medium	-	-	47 A.V	2 Ph
		27d)	AF	100	isolated	good	90	20.P	—	—
Basing-stoke, Hants.	Apr.1963	28	BF	1000	isolated	poor	405	22.R	—	—
Bristol	Apr.1965	29	DG	-	-	-	-	-	100 A.d	—
Shepherds Bush, London.	Nov.Dec. 1962	30a)	DG	2000	50 yd. b)	medium	480	44. S	100 O	3 S
		30b)	DG	5000	50 yd. a)	medium	480	39. S	30. O	2.S
									26.P.l	0
Woolwich London	Sept.Oct 1963	31a)	DG	1000	-	medium	645	115. S	47. O	2.S
		31b)	DG	100	100 yds.c)	medium	50	11. S	—	—
Belvedere, Kent.	Nov.Dec. 1963	32a)	DG	500	200 yds.b)	medium	410	59. S	25. O	1.S
	Apr.1964	32b)	DG	150	200 yds.a)	medium	95	16. S	—	—
New Cross London	Jan.1964	33	DG	300	-	medium	235	28. S	—	—
Kensington London	Oct.1962	34	DG	inf. O	-	medium	-	-	200.O	0
Notting Hill, London	Oct.1962	35	DG	500	-	medium	150	13. S	50. O	0
Fulham, London.	Dec.1962	36	DG	2000	-	medium	200	18. S	50. O	2. S
Lewisham, London.	Mar.1965	37	DG	250	-	medium	50	2. S	100 T.s	0
Blackbush Surrey.	Mar.1964	38	DE	500	isolated	medium	200	0	—	—
Gallywood, Essex	Dec.1963	39a)	DG	200 inf. O	isolated	medium	100	5. P	100. O	0
		39b)	DG	300 M.R	50 yds.a)	medium	-	-	200 M.R	0
Chelsea, London	Dec.1963	40	DG	200	isolated	medium	100	7. S	50 A.V	0
Feltham Middx.	Nov.1963	41	DG	200	isolated	medium	75	3. P	20. O	0
Sidcup, Kent.	Apr.1965	42a)	DG	350. O	isolated	medium	-	-	200. O	0
		42b)	RG	inf. Ph	continuous	-	-	-	150 Ph	0
Old Town, St.Marys, Scilly Isles.	Apr.1963	43a)	DS	1500	300 yds.b)	medium	808	60.M 5.R	20 A.V	
									30 C	0
		43b)	DS	inf.	continuous	medium	200	6.M	30.L	
									20 Am	0
Woolpack, St.Marys	do.	43c)	DS	1000	isolated	poor	100	2.M	57.L	0
Inland, St.Marys.	do.	43d)	DF	500	isolated	poor	150	2.M	—	—
		43e)	FF	-	continuous	-	-	-	50 M.R	0
Garrison, St.Marys.	do.	43f)	DG	200 A.V	isolated	poor	24	0	145 A.V	0

Locality	Date	Habitat No.	Habitat Type	Estimated Population Size	Estimated Degree of Isolation	Access to Parasite	No. P. scelar Dissected	No. P. scelar Parasitised	No. other Species Dissected	No. other Species Parasitised
Coverack, Cornwall.	Apr.1964	44a)	DS	inf.	continuous	medium	100	23. M. 6. R	30 Am	0
		44b)	GS	inf.	continuous	poor	100	8. M	—	—
	Apr.1965	44a)	DS	inf.	continuous	medium	50	9. M. 1.R	40. L	0
		44b)	GS	inf.	continuous	poor	50	5. M	—	—
		44c)	FS	—	20 yd. a)	—	-	-	42 Ph	0
Penzance, Cornwall.	Sept.1962	45a)	DH	500	isolated	medium	300	0	—	—
		45b)	DG	450	isolated	medium	200	6.M 1.S	—	—
St.Ives Cornwall	Apr.1964	46	DS	inf.	continuous	medium	500	0	—	—
Scorrier, Cornwall	Apr.1964	47a)	DG	100	isolated	medium	65	12.S	23.O	0
	Apr.1965	47b)	AT	50	-	medium	40	3.P	—	—
Mawnporth, Cornwall	Apr.1964	48a)	DG	2000 A.V	continuous	medium	23	0	100 A.V	2. Ph
		48b)	DG	200, 200 A.V	20 yds. a)	medium	114	2.S	86 A.V 50.O	4. Ph 0
	Apr.1965	48a)	DG	1000 A.V	continuous	medium	-	-	50.A.V	2. Ph
Porthtowan, Cornwall	Apr.1964	49a)	WG	[illegible]	continuous	medium	100	9. S	—	—
		49b)	GC	inf.	continuous	poor	100	3. S	—	—
		49c)	DG	500	200 yds.a & b)	medium	113	5.S	34. O 15 M.c	0
		49d)	DF	-	200 yds a)	medium	48	0	70 A.V 100 O	0
		49e)	DS	-	500 yds a)	poor	-	-	85 L	0
		49f)	FS	-	200 yds a)	medium	-	-	72 M.c	0
	Apr.1965	49a)	WG	inf.	continuous	medium	50	2. S	—	—
		49b)	GC	inf.	continuous	poor	50	3. S	—	—
Godrevy, Cornwall	Apr.1964	50a)	DG	-	200 yd. b)	medium	36	0	—	—
		50b)	DD	inf.	continuous	medium	50	0	—	—
		50c)	WD	200	300 yd. b)	medium	72	0	—	—
	Apr.1965	50b)	DD	inf.	continuous	medium	220	3.A 1.R	100 A.V	3.Ph
Hayle, Cornwall	Apr.1964	51	DD	-	isolated	medium	60	3. R	—	—
Roscourne, Cornwall	Apr.1963	52	DH	200	—	medium	184	0	—	—
Camborne, Cornwall	Dec.1962	53a)	DG	1000	—	medium	200	11. M. 5.S 1.P	40.O	0
	Apr.1963	53b)	DH	inf.	continuous	medium	100	0	—	—
	Apr.1964	53c)	DG	350	—	medium	80	7.S	30. O	1. S
		53d)	DH	500	400 yd. b)	medium	200	1.S	—	—
		53e)	DF	500	200 yd. c)	medium	50	2.S	—	—
Lands End, Cornwall	Apr.1965	54	DH	inf.A.V	continuous	medium	-	-	72.A.V	1 Ph
Cape Cwll. Cornwall	Apr. 1965	55a)	DH	inf.	continuous	medium	50	0	—	—
		55b)	WH	inf. A.V	continuous	medium	-	-	50.A.V	6 Ph
Trebarwith, Cornwall	Apr. 1965	56	GS	inf.	continuous	medium	82	4.M 1.S	30 M.c	0
Illogan, Cornwall	Apr. 1965	57	CT	600	-	poor	100	3.P 1.S	—	—