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ENDOPARASITES OF SIMULIUM ORNATUM MG.
IN SOUTH ENGLAND,
WITH SPECIAL REFERENCE
TO LARVAL PARASITIZATION
(ILLUSTRATIONS)

Thesis submitted to University of London
for the degree of Ph.D.

by

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PLATES.
PLATE: I

Parasites of the body cavity of Simulium ornatum larvae: longitudinal sections of:

a)- A normal 4th. instar larva, showing well-developed pupal respiratory filaments (histoblasts) and imaginal discs of legs and wings, well formed fat body lobes and a normal size of spinning gland.
   (Giemsa stain)

b)- A 3rd. (probably 4th.*) instar larva infected with a Thelohania sp., showing the pseudocysts (or spore masses, LP) of the parasite replacing the fat body lobes throughout the larval haemocoele
   (H. & E. stain)

c)- A 3rd. (probably 4th.*) instar larva infected with a Chytrid, Coelomycidium sp., form "A"
   (Phycomycetes: Chytridiales), showing a strongly-positive PAS reaction given by the membranes of the newly formed zoospores within the sporangial membranes (PP).
   (Periodic Acid Schiff's reaction; counterstained with light green)

d)- A 3rd. (probably 4th.*) instar larva parasitized with a female juvenile mermithid nematode (JFP).
   (Giemsa stain)

*Note: I have referred to all parasitized larvae as 3rd. instar, because they lacked the imaginal discs of legs and wings and the pupal respiratory filaments of the 4th. instar larvae. Whether this is due to parasitism or to the age of the larva is not possible to decide. Retarded development is, however, shown by a certain percentage of parasitized larvae.
PLATE: I

Some parasites in the body cavities of *Simulium ornatum* larvae

---

*Fig. 1* Normal larva, *Thelohania* sp., *Chytrids*, juvenile female Mermithid nematode.
A live *Simulium ornatum* larva, from the Tilling Bourne, infected with a *Theloahania* sp.

Note the characteristic number (1-2) of "pseudocysts" (or spore masses) for infections of microsporidia in larvae from the Tilling Bourne. cf. with numerous septate pseudocysts of *Theloahania* infections in larvae from the River Lea (PLATE: I).

This larva also shows the absence of pupal respiratory filaments and imaginal discs of legs and wings of 4th. instar.
Simulium ornatum larva infected with Thelohania sp.
PLATE: III

Live material of *Thelohania* infections of *Simulium ornatum* larvae:

Fig. 1: A single parasitic pseudocyst (or spores mass) in the body cavity of the abdominal region of the larva; a feature (1-2 pseudocysts - cf. PLATE: II) characterising *Thelohania* infections of *Simulium ornatum* larvae from the Tilling Bourne.

Fig. 2: The "pseudocyst" dissected out from the larva in Fig. 1: Note spores dispersing from the broken end of the pseudocyst.

Fig. 3: The unusual shape - truncate oval - of spores found in one infection of *Thelohania*. Note the absence of the posterior vacuole seen in all other types of *Thelohania* spores (cf. Fig. 6).

Fig. 4: A tetranucleate sporont and three octosporous pansporoblasts.

Fig. 5: Octonucleate sporonts.

Fig. 6: Typical ovoid shape of the spore of the majority of *Thelohania* infections. Note the presence of a "posterior vacuole" in the broader end of the spore.

Fig. 7)

Fig. 8): Phase contrast photomicrographs, showing extruded polar filaments of the spores.
Some structural features of pseudocysts (spore masses) of, mainly, *Thelohania* & *Nosema*. All figures represent parts of longitudinal sections of infected *Simulium ornatum* larvae.

**Fig. 1** A small young pseudocyst of *Thelohania* (l), showing sporonts (S) and refractile spores (H. & E. stain).

**Fig. 2** Fat body cells (hc) packed with newly formed *Thelohania* sporonts (S) before the disintegration of the cell walls. Note the degenerate host cell nucleus (n) (H. & E.).

**Fig. 3** Part of a section of a late stage pseudocyst of *Nosema*, showing spores only. Note the strongly Feulgen +ve, comparatively compact and large nucleus of the spore. (Feulgen test for DNA: counterstain: fast green).

**Fig. 4** Section through pseudocyst of *Thelohania*, showing hypertrophied fat body cell nuclei (n) as compared, in size, with the spore nuclei (e) (HCl-Giemsa stain).

**Fig. 5** A x3.75 enlargement for part of the pseudocyst section from Fig. 5, to show ovoid shape and structure of the hypertrophied larval fat body cell nucleus, and the unusual double-cell thick wall surrounding the parasitic pseudocyst.

**Fig. 6** A second type of hypertrophied larval fat body cell nucleus (n) within a *Thelohania* pseudocyst. This fimbriated type of cell nucleus characterises all *Thelohania* infections of *Simulium ornatum* larvae from the Tilling Bourne (HCl-Giemsa stain).

**Fig. 7** A third type of hypertrophied larval fat body nucleus (n), showing enlargement of chromosomes without breakdown into fine mesh as in the other two types. This type is caused by the majority of *Thelohania* infections in *S. ornatum* larvae from River Lea. (Methyl green - Pyronin staining).
Some structural features of the "pseudocysts" of Thelohania infections. All figures (except 9 & 10) represent parts of longitudinal sections of infected Simulium ornatum larvae.

Fig. 1): A Thelohania infection, showing pansporoblasts and spores breaking away from the pseudocysts upon maturation, into the haemocoel (H. & E. stain).

Fig. 2): Thelohania infections, pseudocysts reaching all parts of the haemocoel of the infected larva, including the proleg (PR - arrowed), and the head capsule. Note the grouping of the matured spores (dark staining) on the periphery of the pseudocysts (H. & E.).

Fig. 3): A single non-septate pseudocyst confined to the bulbous part of the infected larva characterising Thelohania infections (1-2 pseudocysts - cf. Pl. III, Fig. 1 & Pl. II) of larvae from the Tilling Bourne. (Giemsa stain).

Fig. 4): Seasonal variation of the grouping of Thelohania pseudocysts within the haemocoel of the infected S. ornatum larvae from the River Lea (Giemsa).

Fig. 5): A x10 enlargement of a Thelohania pseudocyst from Fig. 6, showing the sporonts (S) within the larval fat cells, and the mature spores to the outside.

Fig. 6): Early infection of Thelohania in the fat body of the larva, showing separate pockets of spores (arrowed) surrounded by distinct walls (H. & E. stain).

Fig. 7): A smear of a Thelohania infection, showing a variety of spore shapes and sizes, characterising this particular infection. Note the comparatively darker staining older spores (Gram's stain with crystal violet).

Fig. 8): A smear of Thelohania infection, showing the usual shape type of the spores (Gram's stain with crystal violet).
PLATE: VI

Sporogony of *Thelohania* parasites of *Simulium ornatum* larvae:

Fig. 1) Mitosis in *Thelohania*; this is a rare infection, showing clear mitotic figures and individual chromosomes:
   (1) a trinucleate sporont,
   (2) a tetranucleate sporont
   (3) a hexanucleate sporont
   (4) an octonucleate sporont
   (5) an early octonucleate pansporoblast
   (6) a late octosporous pansporoblast
   (HCl - Giemsa stain).

Fig. 3) *Thelohania*, late pansporoblast, showing the usual type of spore with single irregular nucleus. This pansporoblast of this particular infection has a membrane that remains intact for a considerable length of time surrounding the newly formed spores
   (HCl - Giemsa stain).

Fig. 5: Late pansporoblast of *Thelohania* infection, with spores showing either double nuclei or a single nucleus and a polar metachromatic granule
   (HCl - Giemsa stain).
Sporogony of *Thelohania* form "A", parasites of *Simulium ornatum* larvae:

Figs. A1) & Figs. A2):

1) tetranucleate sporont
2) octonucleate sporont
3) octonucleate pansporoblast
4) octosporous pansporoblast

(Dry-fixed - HCl- Giemsa stained smears).
The lophania spp.: Sporogony & spore stages

PLATE: VII

A1

0 - 20 µ
Plate: VIII

Sporogony of Thelohania form "B", parasites of Simulium ornatum larvae:

Figs. B1) B2)
    B3) : (1) tetranucleate sporont
    B4) (2) octonucleate sporont
          (3) 8-16-nucleate pansporoblast
          (4) octosporous pansporoblast

Note. the binucleate sporoblast of B2(3) and B3(3).

(Dry-fixed Giemsa-stained smears)
PLATE: IX

Sporogony of *Thelohania* form "C", parasites of *Simulium ornatum* larvae:

Figs. C1) C2) : (1) tetranucleate sporont
C3) (2) octonucleate sporont
C4) (3) octosporous pansporoblast
     (4) " pansporoblast

Note the *binucleate* sporoblasts of C2 (3) and *binucleate* spores C2 (4) and C3 (4).

(Dry-fixed Giemsa-stained smears).
PLATE: X

Sporogony of *Thelohania* form "D", parasites of *Simulium ornatum* larvae:

Figs. D1)   D2)   D3)   D4)  :  (1) tetranucleate sporonts
          :  (2) octonucleate sporonts
          :  (3) octonucleate and/or octosporous pansporoblasts
          :  (4) octosporous pansporoblast

(Dry-fixed - HCl-Giemsa stained smears).
PLATE: XI

Sporogony of Thelohania and Plistophora, parasites of Simulium ornatum larvae:

Fig. 1: Thelohania infections
(a) uninucleate sporont
(b) binucleate sporont
(c) tetranucleate sporont
(d) & (e) octonucleate sporonts
(f) octosporous pansporoblast

Fig. 2: Thelohania infection
(a) binucleate sporont
(b) tetranucleate sporont
(c), (d) & (e) octonucleate sporonts
(f) octosporous pansporoblast

Fig. 3: Plistophora form "A" infection
(a) early sporont
(b), (c) & (d) intermediate stages of multinucleate sporonts
(e) multinucleate sporont with late nuclear division
(f) multisporous pansporoblast.

(All smears: dry-fixed and Giemsa-stained Camera-lucida drawings).
Plate: XI: THELOHANIA (FIG. 1 & 2) & PLISTOPHORA (FIG. 3)

SPOROGONY

1

a b c
d e f

2

a b c
d e f

3

a b c
d e f

0 20μ

0 20μ 3 20μ
PLATE: XII

Sporogony of *Plistophora* infections of *Simulium ornatum* larvae:

A1: Early sporont (form "A")
A2, A3, A4 & A5: multinucleate sporonts with intermediate stages of nuclear division
A7: Multisporous (over 100 spores) pansporoblast

B1: Early sporont of another infection (form "B")
B2 - B6: Intermediate stages of sporonts
B7: Multisporous (16-32 spores) pansporoblasts

(All smears dry-fixed and Giemsa-stained)
PLATE: XII

Fig.: PLISTOPHORA SPP.
Sporogony

A

1
2
3
4
5
6
7

B

1
2
3
4
5
6
7

0 20μ
PLATE: XIII

Nosema infections of Simulium ornatum larvae

Fig. 1: Schizonts undergoing nuclear division

Fig. 2: Schizonts at final stage of nuclear division (32 daughter nuclei) and beginning of cytoplasmic division.

Fig. 3: As a final phase of development, a schizont producing, within its fine membrane, 16 uninucleate sporonts.

Fig. 4: The fine membrane finally breaks to liberate the enclosed sporonts. The sporont has a thin wall and a terminal irregular nucleus.

Fig. 5: Reduction in size of the sporont and compactness of the nucleus in a central position and thickening of the wall to produce a sporoblast.

Fig. 6: Transformation of sporoblast into a spore. Note the large dark-staining nucleus of the spore in comparison to that of the spores of Thelohania and of Plistophora.

(All smears stained with HCl-Giems strain)
PLATE: XIV

Microsporidia-like infections of the mid-gut epithelium of Simulium ornatum larvae

Arrows indicate the spores, the only stages of the microorganism seen, aggregated around the cell nuclei:

Fig. 1): Shows close aggregation of spores

Fig. 2): Shows comparatively small-sized spores, which are not closely grouped as the others.

Fig. 3): Shows indication of spore breaking into the gut lumen (G).

Fig. 4): Another example of indication of spore dispersion into the gut lumen. This also shows the rod-shaped strongly Feulgen +ve nuclei of the spores (Feulgen reaction).

Fig. 5): Shows enlargement of the nucleus of the infected cell (H. & E. stain)

(All sections, except where indicated, are Giemsa-stained)
Degenerating gut epithelial cells and gregarines, seen in lumens of *Simulium ornatum* larval guts, in longitudinal sections:

**Fig. 1** Degenerate gut epithelial cells normally seen in the posterior part of the lumen of the mid-gut (G), presumably pushed out during moulting cycles of the larva (H. & E. stain).

**Fig. 2** The same zone of the posterior part of the mid-gut of the larva, but showing a higher activity of degeneration of a whole layer of epithelium at that region, and production of pedunculate cells, all pushed into the gut lumen (G).

**Fig. 3** Shrunken epithelial cells associated with gregarines.

**Figs. 4, 7 & 8**: Showing different stages of degenerating gut cells and transformation of their cytoplasm into small dark-staining granules, which coalesce into larger granules (Fig. 9).

**Fig. 5** Production, presumably in the course of degeneration, of fan-like structures which are also pushed into the gut lumen (G).

**Fig. 6** Two Eugregarine "sporadins" free in the larval gut lumen, in a pre-conjugation stage - apart from the typical gregarine nuclei, note also the similarity to the degenerating gut cells in Fig. 1.

(All sections, except where indicated, are Giemsa-stained)
A mature trophozoite or "sporadin" of the Eugregarine endocommensals in the mid-gut of *Simulium ornatum* larvae.

ES = epimerite scar.
Different stages of the parasite in part longitudinal sections of Simulium ornatum larvae, and in smears:

**Fig. 1**: Early stages, seen as small multinucleate bodies, each infecting one of a group of neighbouring fat body cells.

**Fig. 2**: Increase in size of the "bodies" without any appreciable further nuclear division.

**Fig. 3)**: Increase in size with further nuclear division of the "bodies" to produce multinucleate sporangia, released into the coelomic fluid (Fig. 4).

**Fig. 5**: Posterior part of an infected larva, showing numerous round sporangia filling the body cavity.

**Fig. 6)**

**Fig. 6A)**: Enlargement of sporangia from Fig. 5, Fig. 6A, shows a vacuolated sporangium (V).

**Fig. 7**: Cytoplasmic division of sporangia and fragmentation to produce uninucleate zoospores (as shown in Figs. 10 & 11).

**Fig. 8**: Empty sporangial cases, contents discharged by pressure (Smear - Giemsa stain).

**Fig. 9**: Hypha-like structure occasionally seen surrounding some sporangia (Smear - Giemsa stain).

**Fig. 10**: Zoospores, in smear under phase contrast microscopy.

**Fig. 11**: Zoospores, in smear (Giemsa stain).

(All sections - Giemsa-stained)
PLATE: XVII

Coelomycidium sp.
Form "A"

Enlargements of Figs.: 6A, 10 & 11 of:

PLATE: XVI.
PLATE: XVIII

Coelomycidium sp.
Form "C"

Thick-walled sporangia seen in sections of infected Simulium ornatum larvae:

Fig. 1: Young sporangia in the fat body cells (Giemsa stain).

Fig. 2): Young (arrowed, top left Fig. 2) and late (arrowed, top right, Fig. 3) sporangia. Note the thick wall (W) of the mature sporangium (Feulgen reaction).

Fig. 4: Showing the association between Thelohania pseudocysts (Th) and this type of sporangia (arrowed) (Giemsa stain).

Fig. 5: An enlargement of part of Fig. 4, showing dark-staining, RNA-rich thick walls of sporangia (P). H = haemocytes. (Toluidine blue-methyl green-orange G staining).

Fig. 6: Vacuolation and rare uncompleted division of this type of sporangia (Giemsa stain).
Non-pathogenic fungi (Phycomycetes: Trichomycetes) endocommensals in the hind-gut of *Simulium ornatum* larvae.

Fig. 1: Basal pad-like attachment of mycelium to host gut epithelium - arrowed.

Figs. 2 & 6: Young vegetative hyphae.

Fig. 3: Zygospore.

Fig. 4: Ovoid pedunculate conidium.

Fig. 5: Ampulliform sessile conidium.

Fig. 7: Later stage of branching of hyphae.

Fig. 8: Earlier stage of branching of hyphae.

Figs. 9, 10 & 11: Longitudinal sections of *Simulium* larvae at the posterior end of the hind-gut, showing thread-like hyphae in the rectum. Fig. 9 also shows an ampulliform conidium (a). (Giemsa stain).
Non-pathogenic FUNGI parasitic in the hind-gut of the larva.
Mermithid nematode: post-parasitic juvenile female from *Simulium ornatum* larva, showing tail end before the last moulting stage prior to the adult stage of the worm.

(Carmine-borax stain).
Male and female genitalia of *Simulium ornatum* (adults):

Figs. 1, 2 & 3: male terminalia
C = cerus       Cx = coxite     Ms = median sclerite
P = paramere    PH = parameral hooks
Sp = apical spines       St = style
V = ventral plate       Vs = ventral plate support
9th. = 9th. abdominal segment
10s = 10th. sternite    10t = 10th tergite

Figs. 4 & 5: longitudinal sections at the abdominal region of the adult female fly
10 = cercus       GO = genital opening
Pp = paraproct    Rec = receptaculum seminis

---

Camera Lucida drawings of:

Figs. 1, 2, 3, 5, & 6: male terminalia

Fig. 7: female terminalia:
GF = genital fork  GP = gonapophysis
R = receptaculum seminis
10 = cercus.
Section through the walls of two neighbouring Thelohania "pseudocysts" or spore masses, showing the indefinite structure of these walls, suggesting their evolution from the remnants of the disintegrated fat body cells of the parasitized larva.

Note the presence of degenerating host cell nuclei (N), and mitochondria (M). Black round bodies (B) are concentrated along the periphery of the walls.
EMG: 1

x 16,500
Low magnification view, showing different stages of dividing Thelohania sporont (1, 2, & 3), young sporoblast (4), late sporoblast (5) and mature spores (6). Early dividing sporonts show structures that look like Golgi apparatus, or possibly spore polaroplast primordia (PP).
Section of a young dividing sporont of *Thelohania*, showing cytoplasmic furrows formed by the constricting sporont membrane (SW) around the daughter nuclei (N). The cytoplasm is traversed by a dense network of endoplasmic reticulum (ER).

Degenerating host cell nuclei (HN) and mitochondria (M) are visible.
EMG: 3

x16,500
Late lobulate dividing sporont of Thelohania, showing one of the deep furrows that, later, separate the lobes (8 lobes), each to develop into a uninucleate (N) sporo-blast. What looks like a secretion granule (SG) is seen.

Remnants of host-cell endoplasmic reticulum (HER) and mitochondria (M) are visible.
Different stages of development, of *Thelohania*, with a sporoblast showing developing polar filament (PF) and polar filament base (PFB). The wall is still thin and composed of one unit.
EMG: 6

Longitudinal ultrathin section of a *Thelohania* spore, showing a thick spore wall composed of an outer layer (OC), a middle thick layer (ML), and an inner layer (IC). Arrays of endoplasmic reticulum (ER) are parallel to the outline of the spore. A polar filament (PF) with its straight anterior end terminating in a vesicular base (PFB) separated from the considerably thinned anterior part of the spore wall by a sporoplasmic cap (SC), and a coiled posterior end (PF). The polaroplast (P) lies immediately below the polar filament base and surrounds the anterior straight part of the polar filament. A large compact nucleus (N) with double membrane (NM) is centrally situated.
A few Thelohania spore sections like this, show double nuclei (N).
An ultrathin cross-section through the anterior part of a Thelohania spore, to show the circular cross-section of the polaroplast (P), with the polar filament (PF) running down its centre.

Sp = sporoplasm            SW = spore wall

PW = polaroplast wall
A large proportion of Thelohania spores in ultrathin sections show a further thinning of the anterior part of the spore wall and of the sporoplastic cap, to form a kind of a micropyle (Mp) or a weaker spot for the eversion of the polar filament.
High magnification for two types of polar filaments structures, seen in ultrathin sections of different Thelohania spores.

(A) shows a solid core, (B) showing a hollow centre, giving a tubular appearance.
Ultradifferent section of a spore of another form of Thelohania, showing a difference in electron-density of the spore wall from that of the spore wall of the spore in EMG:6.

Note also the difference in the number of coils formed by the polar filament.
A high magnification view of the anterior part of the L.S. of the spore in EMG: 11, to show the polaroplast (P) and the polar filament base (PFB).
An ultrathin L.S. of a spore of Plistophora, mainly to show the difference in structure of spore wall from that of the spore of Thelohania. Note the comparatively thinner middle layer (ML) and the microvilli-like processes forming the outer layer (OC) of the spore wall.
Low magnification view, showing sections of *Plistophora* spores, with the micro-villi-like processes composing the outer layer of the spore wall. Note intertwining of processes of neighbouring spores.
EMG: 14

x 22,000

spore
DIAGRAM 1. Two dimension plot of Thelohania spores, from measurements recorded in TABLE V.

- Smear Number
- Spore Size Class

A

B

C

D
Diagram 2 Frequency diagram of Thelohania form "A" spore measurements, based on Table IX.
Diagram: 3

Frequency diagram of Thelohania form "B" spore measurements, based on Table: IX
Diagram: 4  Frequency diagram of Thelohania Form "C"
spore measurements, based on Table: IX
Diagram: 5 Frequency diagram of Thelohania form "D" spore measurements, Based on Table: IX
Diagram: 6 Histograms showing percentage monthly incidence of protozoan infections:
1- Thelohania (Microsporidia), and
2- Gregarines, of Simulium ornatum larvae from Rivers Lea and Tilling-Bourne. Based on histological results.

For Simulium ornatum larvae from Tilling Bourne, the %age monthly incidence of Thelohania was always less than 1%...
Diagram: 7 Histogram showing percentage monthly incidence of Microsporidia-like infections of Simulium ornatum larvae from the Tilling Bourne. Based on histological results...

N.C. = No collection.
Diagram: 8 Histograms showing percentage monthly incidence of *Coelomycidium sp.* (Phycocystes - Chytridiales) infections of *Simulium ornatum* larvae from Tilling Bourne. Based on histological results.

1- Form "B"

2- Form "A"

n.c. = no collection
Diagram: 9 Histograms showing percentage monthly incidence of Trichomycetes (Fungi-Phycomycetes) infections of Simulium ornatum larvae from Rivers Lea and Tilling Bourne. Based on histological results.

R. Lea

Tilling Bourne

n.c. = no collection