The Autecology of
Elachista fucicola (Vell.) Aresch.
and *Elachista scutulata* (Sm.) Duby.

by

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Thesis presented for the degree of Master of Science, in the University of St. Andrews.

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DECLARATION.

I hereby declare that the following Thesis is based on the record of work done by me and is my own composition and that it has not been presented previously for a Higher Degree.

The research was carried out in the Gatty Marine Laboratory of the University of St. Andrews, under the direction of Dr. M.C.H. Blackler.

CERTIFICATE.

I certify that Amy Dhunjishaw Katpitia, B.Sc. (Hons.) has spent four terms of research work under my direction and that she has fulfilled the conditions of Ordinance No. 51 (St. Andrews) and that she is qualified to submit the accompanying thesis in application for the Degree of Master of Science.

Supervisor of Research.

CONTENTS.

INTRODUCTION.

The genus Elachista was founded by Duby in 1830, and he placed Conferva scutulata Sm. in it. This is the type specimen of the genus and is figured in English Botany by Sowerby. The illustration is dated August 1st, 1811.

Elachista scutulata (Sm.) Duby was first found by Mr. Borrer at Brighton growing on Himanthalia elongata. The genus in Britain contains two other species, Elachista fucicola (Vell.) Aresch. and Elachista flaccida (Dillw.) Aresch. Elachista fucicola (Vell.) Aresch. is the oldest known and the commonest species of the genus Elachista in Britain. Colonel Velley first discovered this brown alga and he gave a correct representation and description of it growing on Fucus serratus as Conferva fucicola in the year 1795, in his book, Coloured Figures of Marine Plants Found on the Southern Coast of England.

Velley found this plant growing in great abundance, particularly in the spring under an elevated cliff near Weymouth called "The Look Out".

The plant Velley described and figured is in his herbarium in Liverpool Museum and this is the type specimen of Elachista fucicola. Areschoug in 1842 placed Velley's Conferva fucicola in the genus Elachista.

Elachista flaccida (Dillw.) Aresch. In 1843 Areschoug placed this in the genus Elachista ~~(Arn.)~~ ~~Harvey~~ (Am.) Harvey. Formerly there was a fourth species Elachista grevillei, now reduced to a form of Elachista fucicola. E. grevillei was found by Dr. Greville in July 1852 growing on Cladophora rupestris at Largs. In the same year, but in the month of August, it was found by Professor Walker Arnott at Corrighills, Arran.

The plant consists of a basal disc, from which assimilatary filaments and paraphyses arise.

AIM OF INVESTIGATION.

To investigate the life history of Elachista fucicola and to study Elachista scutulata to compare its morphology and anatomy with E. fucicola and to study E. fucicola form grevillei to determine whether it is a separate form or a distinct species.

LITERATURE.

There is very little literature on Elachista apart from distribution records. Kylin (1937) investigated the life-history of Elachista fucicola, while Sauvageau (1929) has described the genus in detail.

DISTRIBUTION.

Distribution of E. fucicola from British Phycological Society Record Cards and Herbarium Specimens which have been examined.

British Records:

Scotland Aberdeenshire : Cruden Bay - D. Stewart, Aug. 1956. Between Pittulie and Rosehearty - H. Blackler, July 1958. Cove Bay, south of Aberdeen,- H. Blackler, July, 1958.

Argyllshire : Near Polanach, east shore Loch Linnhe- A.R.A. Taylor, July 1960. Fintry Bay, Cumbrae! - E. Batters, Aug. 1891. Bute! - Greville 1840.

Ayrshire : Largs! Carmichael, 1852.

Fife : Balmerino on River Tay.

Hebrides : South Uist - L.S. Moore. Aug. 1950.

Caithness : Dunnet Bay, Castletown.

Sutherland : Melvisch, Port Skerry,- E. Conway, Aug. 1954.

Inverness-shire: Isle of Raasay - G. Russell, Sept.1954

Orkney : Kirkwall! - Dr. Pollexfen, 1836.

Isle of Mousa, West Burra! Lerwick!

Shetland : Eshaness! A. Katpitia, Aug. 1962.

Wales Pembrokeshire : West Marloes Bay, St. Brides Haven, Thorny Pit, Gamm estuary, - H. Blackler, Sept. 1956.

!.Exclamation marks indicate herbarium specimens which have been examined.

Caernarvonshire: Penhryn! Schiffner Aug. 1935.

Ireland Co. Clare : Caher Rush, Spanish Point - H. Blackler Aug. 1958.

Co. Cork : Cork Harbour! - I. Caroll, 1874.

Co. Donegal: Bundoran! - T.S. Lee, July 1891.

Co. Dublin : Killiney! - Mrs. Gatty.

Co. Galway : Carroroe, Salt Hill, Cashla Bay! - H. Blackler, 1958.

Co. Mayo : Clew Bay! H. Blackler, Aug. 1958.

Co. Antrim : Green Island, Belfast Lough,- H. Blackler Sept. 1935.

England Devonshire : Torquay! 1845.

Cumberland: St. Bees Head, - H. Blackler, Sept.1955.

Cornwall: Falmouth! T.H. Buffham, June 1884.

Kent : Dover! J.S. Maskelyne, Dec. 1933.

Northumberland : Berwick-on-Tweed! - E.A.L.Batters, July, 1884.

Isle of Man : Port St. Mary, Port Erin! Castletown! - A. Katpitia, March, 1962.

Foreign specimens examined in the Herbarium of Natural History Department of British Museum.

Denmark : Baltic Insula Stroma Vestmanhaven!

France : Cherburg!

Germany : Heligoland!

Greenland : North of Koromiut, Tasermiut Fjord!

Canada : Nova Scotia! {near Baffin Island}! Cumberland Strait!
Peggy's Cove, near Halifax!

U.S.A. : Sitka! Alaska - Blake's Point! Sullivan River Falls!
Connecticut - Long Island, Madison!, Rhode Island!
Massachussetts - Nepa Tree Point! Thimble Island!
Otter Cliff! Desert Island Maine.

Elachista fucicola occurs in the northern hemisphere from Cumberland Strait, Nova Scotia to Massachussetts and is on the Alaskan coast in North America. It occurs in Greenland, Iceland, Norway, all round the British Isles, and on the French coast to Bayonne.

Distribution of Elachista fucicola form grevillei.

Fife : St. Andrews!- A. Katpitia.

Ayrshire : Largs- Greville! Isle of Arran! - Walker Arnott.

Berwickshire: Dunbar!, Haddington!,

Argyllshire : Isle of Bute! and Cumbrae!

Northumberland: Berwick-on-Tweed! - E.A.L. Batters.

Recorded from France, Channel coast by Harmel (1939).

Distribution of E. scutulata from British Phycological Society Record Cards and Herbarium Specimens which have been examined.

England - Cornwall : Semmen Cove, Portreath!

Devonshire: Plymouth! Torquay! Sidmouth! The Lizard, coast of Devon!

Dorsetshire : Swanage! Durlston Head!

Yorkshire : Filey Brig! - Mrs. Gatty.

Isle of Man : Port St. Mary! - A. Katpitia

Ireland Co. Clare : Caher Rush! - H. Blackler

Co. Cork : Cork Harbour! - I. Caroll

Co. Donegal: Bundoran! - J.S. Lea

Co. Galway : Salt Hill opposite Mutton Island - H. Blackler

Co. Mayo : Clew Bay! - H. Blackler

Foreign specimens examined in the Herbarium of Natural History Department of British Museum.

France : Cherburg! - J. Chauvin

Norway : Christiansand! - Ekman

Spain : Rivadea! - M.C. Sauvageau.

Distribution ranges from Iceland to North Africa.

Distribution of E. flaccida from British Phycological Society Record Cards and Herbarium Specimens which have been examined at the Natural History Museum and the Herbarium, Kew.

Scotland Argyllshire : Bute

Fife : St. Andrews! - H. Blackler

Berwickshire : Dunbar!

Ireland Co. Clare : Clare Island, south shore!

Co. Donegal : Malin Head - H. Blackler

Co. Galway : Carroroe

England Devonshire : Sidmouth!

Dorsetshire: Weymouth'.

Northumberland : Berwick-on-Tweed!

Foreign distribution.

France : Cherburg!- L. Corbière; Port-en-Bessin!-Berlot;

Bordeaux Bay.

Canada : Vancouver Island; Davies Strait!-Dr. Lyall.

COLLECTION OF MATERIALS.

The collecting areas in which Fucus serratus and F. vesiculosus plants bearing the Elachista fucicola were found consist of a series of rocky ledges all along the east and west coasts of St. Andrews. It was also observed growing on these plants by the Maiden Rock area and the Step Rock pool, and at Fife-Ness, 9 miles south of St. Andrews, on the rocky ledges.

Trips to the collecting areas were made quite often, at low water of spring and neap tides, which occur during the morning at about 9 o'clock and at 3 o'clock in the afternoon.

Specimens of Fucus vesiculosus and F. serratus bearing the epiphyte were collected. Plants in the neighbourhood were inspected for any visible sign of Elachista fucicola. Quite often Fucus plants without any external infection were also brought to the laboratory to be examined under the microscope.

Elachista scutulata which grows on Himanthalia elongata plants was found in a shallow pool, just above low water of spring tides at Fife-Ness. The plants growing in the pool were efficiently aerated. Specimens of Elachista scutulata were collected in October. Plants of the Himanthalia elongata in the nearby vicinity were inspected without detaching.

Also plants of Himanthalia elongata bearing patches resembling those of Elachista scutulata were gathered and brought to the laboratory for examination. Excursions to the collecting areas were made quite frequently.

Elachista fucicola f. grevillei which grows on Cladophora rupestris occurs in shallow pools situated very close to the local landmark, the Maiden Rock. Frequent trips were made to the collecting area, generally at low water of spring tides.

Elachista fucicola on Fucus serratus and F. vesiculosus was collected also in the Isle of Man at Port Erin, Port St. Mary, Castletown and from Shetland at Eshaness, Isle of Mousa, Lerwick and West Burra.

EXAMINATION OF OTHER MATERIAL COLLECTED.

1. No sign of Elachista fucicola, E. scutulata, E. flaccida and E. fucicola f. grevillei, was found on other algae collected for examination.

2. Himanthalia elongata bearing E. scutulata with plurilocular sporangia was found at Fife-Ness in October. In Shetland in August, the fronds of H. elongata showed unilocular sporangia only. No plurilocular sporangia were observed. Plants with unilocular and plurilocular sporangia were found in March in the Isle of Man.

3. Young fronds of Fucus serratus, F. vesiculosus, Himanthalia elongata, and Cladophora rupestris were all free from the epiphyte.

METHODS OF INVESTIGATION.

Collected material was subjected to various methods of treatment in order to find out some of the facts concerning the morphology, anatomy, cytology and life-history of Elachista fucicola.

I. Morphology. Whole material.

(i) Harris's Haematoxylin

(ii) Hygro-butyl alcohol and Harris's Haematoxylin

(iii) Glycerin jelly method.

II. Anatomy. Sectioned material.

(a) Hand section

(i) Stained with safranin

(ii) Aceto-carmine stain.

(b) Microtome section.

III. Cytology.

IV. Life History.

Treatment of Material.

I. Morphology.

Preparation of whole material.

(i) Harris's haematoxylin method.

Material was fixed for 24 hours in chrom-acetic acid.

1% chromic acid	25 cc.
1% glacial acetic acid	20 cc.
Sea water	55 cc.

It was then washed in running water for 24 hours and tap water for one hour, and then transferred to Harris's Haematoxylin for 20 minutes and washed in distilled water. The material was then put into acid water in order to destain it (about 5 drops of hydrochloric acid to 100 cc. distilled water). It was kept in acid water for 2-3 minutes then washed in running tap water and dehydrated in turn in 70%, 85%, and 95% alcohol, and finally absolute alcohol. It was cleared in ~~clove oil~~ a mixture of clove oil, ~~and~~ absolute alcohol + xylol + ~~clove oil~~, in equal parts. Two changes of xylol were given and the material then mounted in Canada Balsam.

(ii) Hygro-butyl Alcohol Method with Harris's Haematoxylin

Fixed material was washed in running sea water for 24 hours and one hour in running tap water. The material was then transferred to Harris's Haematoxylin and left for 20 minutes, later washed in distilled water and put into acid water in order to destain it. It was kept in acid water for about 5 minutes and then washed and dehydrated in 15%, 30%, 50%, 70% ethyl alcohol, allowing 20 minutes in each. The container was always kept covered between

changes of fluids. The material was kept overnight in 85% ethyl alcohol, later replaced with fresh 95% ethyl alcohol and hygro-butyl alcohol was added by the gradual substitution method. Between every third and fourth addition some of the mixture was discarded. This was continued until the preparations were about 90 parts of hygro-butyl alcohol and 10 parts of ethyl alcohol. The mixture of hygro-butyl and ethyl alcohol was replaced immediately with Canada balsam, diluted 10 times with hygro-butyl alcohol. The container was then set aside, exposed to air, but in a warm place on the hot plate, so as to allow the canada balsam to attain a mountable consistency. Evaporation period was overnight. The material was then mounted in canada balsam.

(iii) Glycerin Jelly Method.

Fresh material was left in 50% glycerin for two to three days or more, thus gradually allowing the glycerin to ~~increase its own~~ concentration. The material was then transferred on to a clean slide and mounted in a drop of glycerin jelly. Glycerin jelly was melted previously by standing the tube of jelly in a beaker of boiling water. A few drops of aceto-carmine or aceto-orcein or safranin stains were added to the jelly. Slides prepared were left to dry at room temperature for about 6 to 9 days, and then ringed with asphaltum.

II. Anatomy.

Sectioned Material.

(a) Hand Sections.

Sections were fixed for an hour in 5% formalin in sea water. They were then passed through 2.5%, 5%, 7%, 10%, 15%, 20%, 50%, 70%,85%, 95% and absolute alcohol series used for dehydration; stained for 2-5 minutes in safranin stain of the following composition:

(i) Safranin

Powdered safranin	1 gram
95% alcohol	50 cc.
Distilled water	50 cc.

Stained sections were dehydrated in 95% alcohol and absolute alcohol, and counter stained for 1-2 minutes in a solution of light green in clove oil (1 gram/100 cc.). Sections were cleared in xylol and mounted in Canada balsam.

(ii) Sections were stained by immersion for 1 hour in aceto-carmine, to which a few crystals of ferric acetate had been added.

N.B. Very little ferric acetate was required, since too much caused precipitation of the stain.

Stained sections were dehydrated and cleared in the usual manner and mounted in canada balsam.

As some of the material was being prepared for microtoming, and as this material was being transferred from 30% to 85% alcohol, a few of the hand sections were cut from each lot and subjected to a variety of techniques as previously mentioned.

(b) Microtome sections.

Fixation and dehydration.

Pieces of Elachista fucicola, f. grevillei, E. scutulata and E. flaccida with their respective hosts, were cut off and fixed in the following fixatives.

Chrom acetic fixative

1% chromic acid	25 cc.
1% glacial acetic acid	20 cc.
Sea water	55 cc.

Chromic acid was prepared from chromium trioxide by dissolving 1 gram chromium trioxide in 100 cc. water. Fixation time of 24 hours was allowed, and the material was washed in running sea water for 24 hours and later in fresh water for one hour. It was dehydrated in the following series of aqueous alcohol solutions: 2.5%, 5%, 7.5%, 10%, 15%, 20%, 30%, 50%, 70%, 85%, 95% and two changes of absolute alcohol. Each change was given at an interval of 3 hours. No other fixative was used, since chromacetic fixative yielded good results.

Clearing. It was as gradual as the dehydration. The clearing agent used for all the fixed material was cedar wood oil. The material was then passed through each of the following absolute alcohol: cedar wood oil series at an interval of 3 hours: 2.5%, 5%, 7.5%, 10%, 15%, 30%, 50%, 70%, 85%, 95% and two changes of pure cedar wood oil.

Infiltration. After the pure clearing reagent, the material was ready for infiltration with paraffin wax (melting point 58°C.). It was carried out in the following way:

From time to time small shavings of paraffin wax were added to the clearing reagent, containing the material, until it was partially saturated with dissolved paraffin wax. The corks were then removed and the tubes were placed on the top of the oven. From time to time more paraffin shavings were added. The tubes were left overnight in the oven, and the next day, the mixture of paraffin wax and cedar wood oil was poured off and more melted paraffin wax was added to the tubes. Three to four changes of paraffin wax were given in order to get rid of the cedar wood oil.

Care was taken, not to add paraffin wax shavings in large quantities, as they might damage the tissues of the material.

Embedding: Material to be sectioned was made into blocks by pouring the contents of the tubes into small watch glasses which were previously smeared with a little glycerin. More paraffin wax (melted) was added to the watch glasses, and the material was arranged by means of heated needles. As soon as a thin film had formed on top of the wax, the watch glasses were put under running cold water and left until the blocks floated off free from the watch glasses.

Blocks to be cut were also prepared by pouring enough melted paraffin wax to cover the material in the rectangular casting formed by two 'L' shaped pieces of brass, set on a glass plate and immersing the pieces of material in it. A heated needle was used for arranging the material, and the whole thing was put into the sink in which cold water flowed, and plunged under water.

Microtoming: The larger blocks were cut into small portions and mounted on the microtome block in such a way that ribbons of transverse sections of Elachista fucicola, E. fucicola f. grevillei, E. scutulata and E. flaccida were cut at 2, 3 and 5 μ. The ribbons were placed on black paper and cut into suitable length for mounting on 3" x 1" glass slides.

Mounting: Clean slides were thinly smeared on one surface with Meyer's egg albumin. The ribbons were placed on the

slides and flooded with distilled water. It was ensured that no air bubbles remained between the ribbons and the slides. The ribbons were stretched by gently warming the slides over a spirit lamp. The excess of water was drained off and the slides were set on their edges to allow the adhesive to dry properly in a dust proof place for some hours or for a day or two.

Staining: Paraffin wax was removed before staining the sections by passing the slides through xylol. They were kept in the xylol for about 5 - 7 minutes. They were then passed through equal parts of xylol and absolute alcohol, 95%, 85%, 70%, 50%, 30%, 20%, 10%, 7.5%, 2.5% alcohol to distilled water.

(i) Heidenhains Iron Haematoxylin.

Slides were kept in the mordant 4% iron-alum for 30 to 60 minutes, in order to mordant the material. The excess of mordant was then removed by washing the slides for ½ to 1 hour or so in running water. The slides were then left in 0.5% haematoxylin solution overnight. The next day the slides were washed in running water to remove the excess stain, then differentiated in 2% iron-alum. The slides were continuously examined under the microscope until it was ascertained that they were sufficiently destained. Complete removal of iron-alum was done by keeping the slides in running water for an hour or so, then rinsing in distilled water.

Dehydration: This was carried out by immersing the slides for 3-4 minutes in each of the following alcohols: 2.5%, 5%, 7%, 10%, 15%, 20%, 30%, 50%, 70%, 85%, 95% and absolute alcohol.

Counter staining of the dehydrated material was carried out in Orange G in clove oil (1 gram of Orange G + 50 cc. clove oil + 50 cc. absolute alcohol). The slides were then passed through absolute alcohol + clove oil, equal parts, and then through equal parts of clove oil + xylol + absolute alcohol, and finally through two changes of xylol, and mounted in canada balsam.

(ii) Iron Haematoxylin with safranin as a counter stain. Slides were taken down the series to running water, and kept in 4% iron-alum for two hours. They were then washed in running water for ½ to 1 hour and rinsed in distilled water. The sections were left in the stain as long as they were left in the mordant. Differentiation was carried out in 2% aqueous iron-alum. The action was stopped by transferring the slides to water and washing them in running water for at least 1 hour.

The slides were left in safranin solution for 12 - 15 hours. Safranin solution was made up by taking equal quantities of distilled water, 95% alcohol and 1 gram of safranin.

Differentiation was carried out in 70% alcohol acidified with a few drops of concentrated hydrochloric acid. The slides were washed in running water for 1 hour, dehydrated in 50%, 70%, 95% alcohol; absolute alcohol + clove oil + xylol; and finally two changes of xylol and mounted in canada balsam.

(iii) Methyl-Green-Pyronin and Ribonuclease Method. Sections were brought down to running water and then left overnight in methyl-green-pyronin stain. They were then rinsed in distilled water for a few seconds. Dehydration was rapidly carried out in absolute acetone. Sections were then briefly rinsed in equal parts of acetone and xylol, and cleared in two changes of xylol and mounted in canada balsam.

0.4 grams of the pyronin-methyl-green was added to 250 ml. distilled water. To this solution an equal volume of M/5 acetate buffer, pH 4.8, was added.

III. Cytology.

(a) Squashes.

Material of all sizes, small as well as big, which was scraped off from the host tissue was treated in various ways as described below.

(i) Austin's Method (1958). Material fixed in 1:3 acetic alcohol, was washed thoroughly in water and then transferred to a dish containing 2% iron-alum. After about 15-30 minutes (mordanting time depends upon the nature of material), the material was washed for as long a time as the material was left in the mordant. The material was then placed on the slide in a few drops of aceto-carmine stain. One drop of saturated ferric acetate was added per 10 ml. stain solution. Material was teased with needles and slides continuously warmed. Care was taken to prevent aceto-carmine from drying up. Over staining of material was prevented by adding 1% iron-alum. The material having been slightly squashed, a cover-slip was placed on it. Then with pressure of the thumb, the material was completely squashed. The preparation was sealed by ringing with glycerin jelly, or the slide was inverted over 10% acetic acid, so as to let the cover slip float off with the material on it, since the coverslip was smeared with egg albumin. The material on the cover slip was passed through 95% and absolute alcohol and

and cleared in xylol and mounted in Euparal or canada balsam.

(ii) Aceto-carmine Method. Darlington & La Cour. Material was fixed in 1:3 acetic alcohol for 18 hours to 24 hours. Glass slides smeared with Meyer's egg albumin were baked in the oven at 57°C. for 24 hours. Fixed material was put on to the prepared slides, in a few drops of either aceto-carmine or aceto-orcein (already made up) and allowed to remain for 5 minutes covered with a clean cover slip. The material was squashed as firmly as possible with the thumb. The slide was then gently heated by passing over a spirit lamp flame, and then inverted in a dish of 10% acetic acid. The coverslip with the material floated off the slide and was dehydrated by passing through 95% and absolute alcohol, cleared in xylol and mounted in Euparal.

(iii) Naylor's Method. (1958).

Material was fixed in 1:3 acetic alcohol for 18-24 hours and washed in tap water and transferred to 30% hydrogen peroxide, for 4 hours. ~~If~~ When necessary the storage of material was done in fresh 1:3 acetic alcohol with a few drops of ferric acetate solution added. Material was stained with aceto-carmine. Before staining the fixing fluid was removed by washing to prevent blackening, and the material to be stained was warmed in a few drops of rather concentrated solution of

sodium carbonate (6% sodium carbonate as mentioned by Naylor was not useful for this particular material as the concentration was not strong enough to soften the cell wall). Material was squashed, irrigated with water, dehydrated as usual and mounted in Euparal or canada balsam.

In order to obtain satisfactory results, plants to be fixed were placed in the refrigerator, and then for 2 hours at room temperature under a bright light.

(iv) Austin's and Naylor's Methods. These were combined and made use of.

Material was kept in the refrigerator overnight and the next day, it was put in a petri dish in water under bright artificial light for two hours. It was then fixed in 1:3 acetic alcohol for 18-24 hours. Later transferred to 30% hydrogen peroxide for 4 hours and again removed to the fixative. For storage purposes, a few drops of ferric acetate were added to it. The material was washed in water before it was used. It was then transferred to 2% iron alum for 15 minutes, and washed for the same amount of time in running water. A few drops of 6% sodium carbonate were placed on the slide and the material to be squashed was placed in it. It was then slightly warmed, squashed and a few drops of aceto-carmine stain put on it. A clean coverslip smeared

with egg albumin was placed over the material, and gently pressed. The slide was then inverted into a dish of 10% acetic acid, so as to let the coverslip float off. The coverslip with the material was transferred to 95% alcohol and to absolute alcohol and mounted in Euparal, left on the hot plate and next day cleaned and labelled.

(v) Russell's Method, (1962) Material was fixed for 12-24 hours in 3:1 (95% alcohol, glacial acetic acid), to which is added sufficient quantity of aqueous ferric chloride to impart a straw colour to the fixative.

Material can be stored in 70% alcohol and can be left in the refrigerator for up to six months without serious deterioration.

Material to be stained was washed in distilled water. Small pieces were then arranged on the slide in a drop or two of 100 volume hydrogen peroxide and the slide then warmed gently for about 30 seconds. The specimen was then covered by an albuminised coverslip. The coverslip was first dried by passing over the spirit lamp flame, and the preparation was then squashed between two thicknesses of filter paper. The slide was inverted over ~~into~~ a dish containing 2% acetic acid until the coverslip dropped off and then ~~coverslip~~ it was placed in a watch glass containing aceto-carmine (made according to Darlington & La Cour)

which in turn was placed on a gauze and the whole was heated until just short of boiling point, by means of a gentle bunsen flame. The aceto-carmine was kept at this temperature for 5-10 minutes, when staining was completed. The coverslip was then placed in 95% alcohol, followed by two changes of absolute alcohol. The material was then cleared in Euparal Essence and mounted in Euparal.

(b) Macerating Method.

(i) Ammonium Oxalate Macerating Fluid. Fresh material was treated for 24 hours in a solution of acid alcohol, prepared by adding 10 cc. hydrochloric acid to 55 cc. 95% alcohol, and 25 cc. distilled water. The material was then washed in water and transferred to 0.5 cc. ammonium oxalate, and allowed to soak in it. The tissue was then mounted in a drop of the solution and gently tapped. No attempt was made to make a permanent preparation in this case.

(ii) α Bromo-naphthalene. Material was kept for an hour in α bromo-naphthalene and then left in the fixative, 1:3 acetic alcohol for about 18-24 hours. It was then washed in tap water and left in macerating fluid for 10 minutes (50 cc. hydrochloric acid and 50 cc. 95% alcohol). It was then stained in orcein for 30-60 minutes, gently heated over the spirit flame in a watch glass. The material was placed

on a clean slide smeared with egg albumin, and covered with a clean coverslip. It was then pressed between the thumb and finger, and then inverted into a dish containing 2% acetic acid, so as to let the coverslip come off. The slide with the material was then differentiated in 95% alcohol, followed by two changes of absolute alcohol, cleared and mounted in Euparal.

(iii) Leuco-basic Fuchsin . Material was fixed in 1:3 acetic alcohol for 4 - 24 hours, rinsed in 2-3 changes of water, and macerated by hydrolysis in N. hydrochloric acid at 60°C. for six minutes or so. The material was stained in leuco-basic fuchsin for 2-3 hours, and small pieces were teased out on a slide in a drop of 45% acetic acid. A coverslip was placed on it in position and pressure under several thicknesses of blotting paper was applied, allowing no sideways movement of the coverslip. The slide and the coverslip were separated by turning the slide face down in a smearing dish containing 40% alcohol. The coverslip was then passed through ~~alcohols:~~ 80% alcohol, and two changes of absolute alcohol. The slide and the coverslip were recombined by mounting in Euparal.

Microchemical test for Fucosan. The Vanillin test for fucosan was carried out using vanillin dissolved in hydrochloric acid. In this case the fucosan turned red.

Comments on different methods.

Out of the different methods used for whole mount preparations, namely the Harris's Haematoxylin, Hygro-butyl alcohol, and the glycerin jelly mount, the Hygro-butyl method afforded the best results. In the case of Harris's Haematoxylin and glycerin jelly preparations, the results obtained were not satisfactory.

Microtome sections: Chrom acetic fixative was used, as it yielded good results throughout.

Cedarwood oil was used as a clearing agent, as it caused no hardening of the tissues and there was no shrinkage or plasmolysis of the tissue noticed. Heidenhain's iron haematoxylin was used as a stain and it gave most satisfactory results. The nucleus was very well stained, although the chromosomes were not seen clearly. The material was counter stained in Orange G. The effect produced was quite good.

Safranin and iron-haematoxylin stain was used to differentiate between the nuclei which were stained bright red. These were easily made out, but the chromosomes were not seen at all.

Methyl-green-pyronin stain was used to differentiate the nucleus and its contents. It was found that this was an excellent stain for differentiation between the host and epiphyte tissue. It clearly showed the penetration of

the epiphyte into the host tissue as each was stained a different colour.

Squashes:- for chromosome count.

Austin's squash method was not found to be useful especially for Elachista fucicola since the cell wall and sporangial wall of this were very tough and resistant to squashing. In this method there was no kind of macerating agent used which at least makes the tissue soft.

The second squash method also did not work well, since the cell walls and sporangial walls were too tough, and there was a large number of chromatophores in the filaments making it difficult to make out the nuclei in the cells of the filaments.

The third method, where hydrochloric acid was used for maceration mixed with an equal parts 95% alcohol, was not efficient at all. It was soon discovered that the tissues shrank, and also the staining was not very clear.

With regard to Naylor's method, 6% solution of sodium carbonate was used as she suggested, as an aid for squashing purposes. This produced no effect on the cell wall or the sporangial wall of Elachista fucicola, so the concentration was increased with the result that a blurred effect was produced. It did not prove very useful.

Naylor's and Austin's methods were combined to improve the results, but the results obtained were not satisfactory.

Russell's technique gave quite satisfactory results. The method proved to be better than the other methods.

Maceration:

Ammonium oxalate was used as a macerating fluid, and the material was treated with hydrochloric acid. It gave most unsatisfactory results - the tissues were damaged.

Leuco-basic fuchsin was found quite satisfactory, compared with Ammonium oxalate method.

α -bromo-naphthalene method was quite good as it inhibited spindle formation and thus kept the chromosomes well apart.

IV. LIFE-HISTORY.

(i) Preparation of spore slides.

Clean slides were smeared with Meyer's egg albumin. Material was left in cold sea water for a while and as soon as the spores were liberated they were transferred to the smeared slides. Spores were killed by inverting the slides over 5% formalin and left for about 5-7 minutes. The slides were then allowed to dry slowly at room temperature for a period of 2 - 3 days. They were stained with Heidenhaen's iron-haematoxylin. The technique followed for staining was the same as for microtome sections. The slides were then

dehydrated and mounted in canada balsam.

(ii) Cultures.

All glass ware used was sterilised by dry heating for 2 - 4 hours in an oven at 200°C. Erd-Schreiber culture medium was prepared in the following way.

2 litres of Büchner and Berkefeld filtered outside sea water were heated in a flask to a temperature of 70°C., and then allowed to cool to room temperature. 100 cc. of a soil extract, prepared as below, was brought to boil in a small conical flask and 0.2 gm. of sodium nitrate and 0.04 gm. sodium hydrogen phosphate were added to the cooled sea water and the mixture once more cooled.

Soil extract : 500 gm. of garden soil, not heavily manured, were boiled with 1 litre of tap water, and allowed to simmer for 40 minutes. The mixture was covered and left aside for 2 days to allow the soil particles to settle. The liquid was then decanted and filtered through a double thickness of filter paper into a conical flask.

Fresh soil extract was prepared every month and fresh culture medium was also prepared every month. Bowls containing the material to be cultured were covered with glass plates and put in the culture room.

Some of the cushions of Elachista fucicola were placed in watch glasses containing Büchner and Berkefeld filtered sea water, and examined under the low power of the microscope for release of zoids from the unilocular sporangia. Some of the Elachista cushions were directly placed on a slide, and immersed in filtered sea water, and examined for release of zoids.

Several different methods were employed for the release of zoids from the unilocular sporangia, but until the beginning of December it appeared that all attempts proved fruitless, as there was no sign of zoids coming out of the sporangia, although the sporangia were fully matured. The following experimental methods were tried out.

Freshly collected material was brought into the laboratory, and examined for the presence of sporangia under a binocular microscope. The material was then put into a polythene bag and kept in the refrigerator overnight. The rest of the material was kept in constant running sea water. The next day both the lots of material were examined under low power of the microscope for the release of zoids. For several days the material was kept in such a condition and examined every day but there was no sign of the liberation of the zoids. In case the cold sea water might help the

liberation of zoids, some of the sea water was put into the refrigerator for overnight and next day the material was flooded ~~out~~ with ~~in~~ refrigerated sea water, but it did not help in releasing the zoids.

The next method employed was that ~~lots of~~ healthy Fucus plants were collected and chopped into pieces. About 150 ml. of sea water ~~was~~ were put into a beaker and the pieces of Fucus immersed in it. It was then boiled for about 15 to 20 minutes. The plant extract thus prepared was strained off and it was then made use of. A few drops of the extract were added to the sea water in a watch glass containing the Elachista cushions. It was then examined under the microscope for the release of zoids; and thus to see whether there was any substance present in the Fucus that had something to do with the release of zoids from the sporangia, but still there was no sign of liberation of zoids.

Another experiment was set up in a moist chamber. A moist chamber was set up in the following way. The moist chamber contained a mixture of sea water and fresh water in equal parts. Petri dishes were inverted in it and on these were placed cavity slides containing the sporangia. These were immersed in sea water and to it a drop or two of the

Fucus extract were added. The whole perspex box containing the slides was covered up with a lid and kept in a semi-lighted place. The slides were daily examined for the zoids, but there was no sign of liberation of them.

Another attempt was made. Freshly collected material, containing fully matured sporangia, was wrapped in a polythene bag and left in the culture room for 5 to 6 days. The material was then placed on the slide and flooded with filtered sea water, and it was then examined under the microscope. Masses of zoids were seen coming out of the sporangia. It is also interesting to note that some of the material was at the same time placed in the refrigerator, and when this material was examined under the microscope, masses of zoids were seen to be coming out of the unilocular sporangia.

Immediately the zoids were seen, they were pipetted out with as little as possible of the filtered sea water. Pipettes were sterilised before using. The zoids were transferred to petri dishes containing a quantity of the culture medium. Some of the liberated zoids were transferred to cavity slides and kept in the moist chamber. The petri dish and the cavity slides containing the zoids were examined microscopically for the signs of fusion between the zoids. Some of the dishes were placed in the culture room facing north light, while the other dishes were kept

on the window of a south facing room. To most of the cultures a few hand sections of healthy Fucus fronds free from infection and carefully washed in Büchner and Berkefeld filtered sea water, were added. Some cultures, however, were kept free from the host plant. Fresh culture medium was added to the dishes whenever required but the cultures were not aerated.

Many experiments were set up with the zoids, some with the Fucus sections, in the presence and absence of light. Some were set up with only Elachista cushions in the presence and absence of light, and some with only spores in the presence and absence of light. An experiment was set up in which artificial light was provided throughout 24 hours of the day.

In other experiments, a continuous flow of running sea water after passing ~~through~~ over healthy Fucus fronds, was supplied to the Elachista cushions. Continuous light was also provided to the cultures.

Description of Genus and Key for Identification of Species.

The genus Elachista consists of a basal disc from which arise brown coloured, unbranched, assimilatory filaments and short paraphyses. Both unilocular and plurilocular sporangia are found, the former much the commoner.

KEY.

1. Epiphytic on Fucales.....2.
   Epiphytic on Cladophora rupestris.....4.
2. Forming shield-like swellings on fronds of Himanthalia elongata.....E. scutulata.
   Occurring on other Fucales.....3.
3. Growing on Fucus serratus and F. vesiculosus.....E. fucicola.
   Growing on Cystoseira or Halidrys.....E. flaccida.
4. Growing on Cladophora rupestris.....E. fucicola f. grevillei.

Elachista scutulata forms dark coloured oblong shield-like thalli on Himanthalia elongata (Fig. 1). It penetrates into the tissue of the host through the conceptacles (Fig. 5). The tubercle is composed of dichotomously branched fibres. The paraphyses, assimilatory filaments and plurilocular and unilocular sporangia arise from the tubercle. (Fig.2).

Elachista fucicola forms a button-shaped cushion

attached to Fucus vesiculosus or Fucus serratus by a central part. (Figs. 3 & 4). It penetrates into the tissue of the host (Fig. 6). Unilocular sporangia are to be seen.

Elachista fucicola f. grevillei forms hemispherical tubercles on Cladophora rupestris. The tubercle is similar to that of Elachista fucicola, but smaller in size.

Elachista flaccida occurs on Cystoseira baccata and C. fibrosa and Halidrys siliquosa.

The genus Elachista was placed in Ectocarpeae by J.G. Agardh (1842), but in Harvey's opinion (1849) incorrectly. Harvey states "The structure of the tubercle in which the spores are lodged, is precisely that of the Chordarieae, and did this tubercle constitute the whole plant, no doubt Professor Agardh would associate the genus with the latter family, for the whole structure of this part is analogous to that of Leathesia, and the nature and position of the spores the same. But then there are the long pencilled filaments composing the largest part of the frond; and these are very unlike anything found elsewhere in Chordarieae, while they closely resemble the threads of an Ectocarpus in structure. Taking these filaments for the frond, Agardh

would be correct in referring the genus to Ectocarpeae. But, to my mind, the tubercle, as it contains the fructification, must be regarded as the most essential part of the structure; the filaments as an accessory part; and therefore I am of opinion that the genus is best placed in Chordarieae."

Kjellman (1890) founded the family Elachistaceae, and Fritsch (1945) put it under the Ectocarpales, while Papenfuss (1955) placed it in the Chordariales.

MORPHOLOGICAL AND ANATOMICAL STRUCTURE.

1. Elachista fucicola. It grows on Fucus vesiculosus and Fucus serratus. It consists of a basal disc of parenchymatous cells from 3 to 4 cm.* in diameter attached by a central point to the Fucus plant. Assimilatory filaments and paraphyses come off from it.

Assimilatory filaments form the brush-like tufts, rising from the hemispherical tubercle. They are elongate and brownish to olivaceous in colour and are attenuated towards the base, larger and submoniliform above it, and cylindrical. Filaments scarcely taper at the tip and the apex is obtuse if intact (Fig.7). From results of many measurements the mean is 1.5 - 2 cm. long. The cells in the upper part of the filament measure 52.2 μ in length and 47 μ in breadth, on an average, whereas the cells in the lower part of the filament are ~~about~~ 40.9 μ long and 38.7 μ broad. There are about 37 - 196 cells in a mature filament. Filaments are simple and unbranched.

The mature cell of the assimilatory filament contains numerous disc-shaped chromatophores which are dark brown in colour (Fig.8). The outer cell wall of the filament is rather thick - about 1 - 1.5 μ. In the young cells the discoid chromatophores are slightly elongated. Attached to the chromatophores by the cytoplasm are the pyrenoids.

*All figures given in this thesis are based on the average of 50 measurements.

The pyrenoids are dark brown deeply staining bodies which may be attached to the chromatophores. The nucleus of the cell is centrally suspended in the cytoplasm. There is one nucleolus in the nucleus. The filaments, when viewed separately, are yellowish in colour, but in mass their colour is dark brown.

Paraphyses arise as a result of dichotomous branching of the outer cells of the disc. They are decidedly clavate and moniliform above. (Fig.9). Paraphyses are about 126μ-164 long. There are about 5 - 10 cells in a paraphysis; the end cell is slightly swollen. The uppermost cells are usually as long as, or longer than, broad and measure 27.8μ in length to 23.3μ in breadth. The cells of the paraphyses are almost colourless and only a few chromatophores are present. (Fig.11).

The mature cell of a paraphysis is similar to the cell of the filament.

The Disc. This consists of colourless dichotomously branched fibres (Fig.10).

Sporangia. Unilocular sporangia spring from the basal cells of the paraphyses (Fig.13). They are olive green in colour, and when matured become brown in colour. The unilocular sporangia are club shaped, and are 37μ - 134μ long and 13.5μ - 32.8μ broad. The sporangia are found at

different stages of development in a single disc (Fig.12). Dehiscence of sporangia is by means of an apical pore. The contents shoot out as if under pressure, leaving the empty sporangium (Fig.14). The mature sporangium contains a large number of zoospores.

2. Elachista fucicola f. grevillei. This grows on Cladophora rupestris in pools near high water of neap tides. On examination of the morphology and anatomy of the assimilatory filaments and paraphyses and unilocular sporangia, it is identical with Elachista fucicola only differing in its habitat and the smaller size of its disc.

Morphology and anatomy:

Assimilatory filaments - the dichotomously branched fibres bear at their tips the assimilatory filaments. They are 0.6 - 1.6 cm. long, and are rigid, elongate, slender and cylindrical. They are slightly attenuated at the base (Fig.15). The apex of the filament is obtuse if intact. The lower articulations are wider than they are long in the lower part.

The cell structure is the same as that of Elachista fucicola (Fig.16).

Paraphyses are borne at the ends of the branched fibres. They are moniliform, slightly curved (Fig.17). There is

decided variation in the size of the cells of the paraphyses. The upper cells are 33.5 μ long and 23.7 μ broad; the lower cells are 35.3 μ long and 12.5 μ broad.

The mature cell of the paraphyses is similar to that of Elachista fucicola (Fig. 18).

Sporangia. The unilocular sporangia are similar to Elachista fucicola (Figs. 19 & 20). They measure 68.9 μ in length and 22.3 μ in breadth. Dehiscence of sporangia is by means of an apical pore as in E. fucicola.

3. Elachista scutulata. This forms shield-shaped masses on Himanthalia elongata. The basal part is not a globular disc but a shield-shaped structure from 0.2 - 0.9 cms. in thickness to 1.5 - 3.9 cms. in length.

Assimilatory filaments are shorter than in Elachista fucicola (Fig. 21). There is hardly any difference in size of the cells of the filaments throughout. The upper cells are 30.5 μ in length and 12.1 μ in breadth, and the lower cells are about the same size. The number of cells in mature filaments vary from 18 to 80. The upper cells are filled with olive coloured chromatophores found in large numbers; the lower cells contain very few chromatophores.

The mature cell of the assimilatory filament is the same as the single cell of the filament of E. fucicola. (Fig. 22).

Paraphyses are borne at the ends of the dichotomously branched hyaline fibres comprising the shield. The upper cells of the paraphyses are moniliform and measure 27.5 μ in length and 17.7 μ in breadth, whereas the lower cells are 32.1 μ long and 16.1 μ broad. The approximate number of cells found in the paraphysis is 4 - 9. (Fig.23).

The single cell of a paraphysis is similar to the cell of the paraphysis of E. fucicola (Fig.24).

The basal disc consists of dichotomously branched hyaline fibres (Fig.25).

Sporangia. Unilocular and plurilocular sporangia occur in the species. Plurilocular sporangia are rare; they were first found at Plymouth by A.L. Smith in 1899. The specimen is in Batter's Herbarium, in the Natural History Department of the British Museum.

Plurilocular sporangia were first observed in October 1961 (Katpitia and Blackler 1962). They were again observed in October 1962 on E. scutulata growing on Himanthalia elongata in a shallow pool at Fife Ness, nine miles south of St. Andrews. It was noticed that these plants were devoid of unilocular sporangia. Plurilocular sporangia were also found on Elachista scutulata on Himanthalia elongata growing in a pool near Scarlet Point, Castletown, in the Isle of Man, in March 1962. These plants were also devoid of unilocular sporangia.

The plurilocular sporangia occur on long stalks. They are long and narrow and measure 62.3μ- 128μ in length, and 9.9μ- 12μ in breadth. The plurilocular sporangia show horizontal septation. The number of divisions in each sporangium varies from 30 to 50. They develop from the tips of the branched paraphyses, and reach almost the same height as that of the assimilators (Figs. 26 & 27).

The plurilocular sporangia are like those described and figured by Thuret (1850) for plants growing on the French coast. He found that plurilocular sporangia occurred in the winter months on plants without or with very few unilocular sporangia.

Dehiscence of a sporangium has not been observed.

Unilocular sporangia were observed on the material collected during the month of October 1962 at Fife Ness in a shallow pool. Unilocular sporangia are olive green coloured and occur on long stalks. Dehiscence of sporangia has not been observed. The unilocular sporangia measure 69.3μ long and 31.6μ broad. Mature sporangia contain large numbers of zoospores.

4. Elachista flaccida. Preserved material from the Channel Isles growing on Cystoseira baccata was examined.

Assimilatory filaments arise from the tips of the dichotomously branched fibres composing the tubercle. (Fig. 28). They

are brown coloured and form pencillate epiphytical tufts. The tufts are occasionally so numerous and so closely set as almost to conceal the supporting plant. Filaments are long, tapering very much to the base, then rapidly widening to the middle, from which they narrow very gradually to their upper extremity. The lower cells of the filament measure 28.2 μ in length and 81.3 μ in breadth, whereas the cells in the upper part of the filament are 75.8 μ long and 164.3 μ broad. The apex of the filament is obtuse. The filaments are much broader than the assimilatory filament of E. fucicola or E. scutulata.

The mature cell is like that of the other species of Elachista described.

Paraphyses arise between the filaments from the tips of the dichotomously branched fibres of the disc. They are linear, obovate, tapering at the base and gradually swelling upwards (Fig.29). Paraphyses have oval cells. The upper cells of the paraphyses are 63.8 μ long and 36.6 μ broad; the lower cells measure 41.7 μ in length, and 12.1 μ in breadth.

The single cell of a paraphysis is similar to that of Elachista fucicola.

Unilocular sporangia develop at the base of the paraphyses. They are ovate and dark brown coloured, and

are found in close association with the paraphyses (Fig.30). The sporangia are 49.9 μ to 136.6 μ long and 15.6 μ to 41.4 μ broad. Unilocular sporangia at different stages of development are found in some tubercles.

Measurements of the Assimilatory Filaments, Paraphyses, and Unilocular and Plurilocular Sporangia of species of Elachista.

	Elachista fucicola	E. fucicola f. grevillei	E. scutulata	E. flaccida
No. of cells of assimilatory filament	37 - 196		18 - 80	
No. of cells in paraphysis	5 - 10		4 - 9	
Assimilatory filament cell				
length	40.9-52.2 μ		30.1-30.5 μ	28.2-75.8 μ
breadth	38.7-47.3 μ		12.1-12.7 μ	81.3-164.3 μ
Paraphysis cell				
length	27.3 μ	33.5-35.3 μ	27.5-33.1 μ	41.7-63.8 μ
breadth	23.3 μ	12.5-23.7 μ	10.1-17.7 μ	12.1-36.8 μ
Unilocular sporangium				
length	37-134 μ	68.9 μ	63.6 μ	15.6-41.4 μ
breadth	13.5-32.8 μ	22.3 μ	31.6 μ	49.9-136.6 μ
Plurilocular sporangium				
length			62-128 μ	
breadth			9.9-12 μ	

LIFE HISTORY OF ELACHISTA FUCICOLA IN CULTURE.

Zoids from unilocular sporangia, when they had disentangled themselves from the ejected mass, swam about vigorously for a period varying between 15 and 40 minutes before settling down. They were pear-shaped, measured 4 - 6μ in diameter. Each of the zoids possessed two flagella attached to a blepharoplast, a long anterior one directed forward and a short posterior one directed backward during the movement of the zoid (Fig.31). It ~~is~~ was interesting to watch the movement of the zoid, usually at the beginning as soon as they were liberated out of the sporangium. They moved about in all directions very quickly, flapping the two flagella around. No sooner had they settled down than the shorter flagellum coiled round the body of the zoid, so that at first it appeared that the zoid possessed only one flagellum, while the longer flagellum still showed some movement, before completely coming to a resting stage. The posterior part of the zoid contained one disc-shaped chromatophore. The nucleus was partly obscured by the chromatophore. The anterior part of the zoid was filled with a clear matrix. The zoids were not photostatically sensitive as they do not possess an eye spot. Kylin (1937), observed the absence of an eye spot and lack of photostatic behaviour.

The liberated zoids showed no sign of fusion in pairs while they continued to move about in an erratic manner. Cultures were set up on 5th December and for 15-17 days zoids showed no germination at all. On germination they developed a germ tube. Development at first was very slow. Between December and the beginning of January only 1 - 6 celled filaments were observed (Figs 32,33). In five weeks old cultures there was good growth of filaments (Figs. 34,35). In most of the plants branching was noticed (Figs. 36,37), and the branches in turn again branched. (Figs. 38). This was observed by Kylin in 1937 in his cultures. The cell of the filament was divided by a longitudinal cell wall. The two cells arising from the division produced two short branches diverging away from the assimilatory filament. One of the branches was shorter than the other. The branches consisted of four cells and the divergence between the two ~~is~~ was almost 80° (Fig.39). In one case only one of the cells had produced a branch. The culture showed the branching of creeping filaments when examined on 18th January. In six weeks old cultures erect filaments were observed, developing on creeping or horizontal filaments (Fig.40). Erect filaments at various stages of development starting from little out-growths on the horizontal filaments were noticed (Fig.41).

The cultures grew in the same way as those recorded by Kylin (1937). There was no difference in growth of the culture noticed. The filaments grew by intercalary divisions, they possessed short jointed rhizoids at the base of the creeping filaments (Figs.42-46). At a later stage a protonema was found on which disc-like structures were formed and from which numerous erect filaments arose (Figs. 47-48). Then at a later stage plants bearing unilocular sporangia were seen (Figs. 49, 50 & 57).

On further examination of the cultures at a later stage, plurilocular sporangia were noticed. Different stages in development of plurilocular sporangia were found (Fig. 52). Empty plurilocular sporangia were also seen, but the liberation of zoids was not observed (Fig.53). This is the first observation of plurilocular sporangia in Elachista fucicola. The plurilocular sporangia observed in culture were transferred to another culture dish.

On re-examining the culture several spores had germinated - constituting the second generation in culture. (Fig. 54). Some of the germinated sporelings even showed branching (Fig.55). Some of the spores had germinated in the plurilocular sporangia.

The creeping filaments showed branches at a later stage up to 3 - 4 cells (Fig.56). Primary branches were noticed

on the creeping filaments and these branches in turn again showed secondary branches of 1-2 cells. At a later stage plurilocular sporangia were also noticed (Fig.57). The plants produced by the plurilocular sporangia were quite different from those produced by unilocular sporangia.

Culture set up from the spores from Unilocular Sporangia.

When the cultures were examined several sporelings were noticed. They were ~~of~~ at the 2-5 celled stage. On re-examining the culture, creeping filaments had much increased in size and showed development of erect filaments, some of which were branched.

Cultures of Elachista plants for four generations were grown. In the first generation small plants identical in form with the parents ~~are~~ were produced, but very small and producing unilocular sporangia (Fig.58). The spores from these sporangia germinated and grew the same way and produced another crop of similar plants bearing unilocular sporangia, the spores of which repeated the above cycle. This occurred for four generations, each producing unilocular sporangia.

Several cultures were set up of the material collected in St. Andrews and the Isle of Man. The plants produced from the Isle of Man material were exactly similar to those

produced by St. Andrews material. Both the cultures were set up on the same day, so as to notice if there was any difference in growth time. The plants that were produced in the culture were exactly similar to plants collected on the shore except they were much smaller.

The following culture experiments were set up and observed daily, and comparisons made to see which conditions most favoured the growth of Elachista fucicola.

1) With Fucus sections and facing the light.

Healthy Fucus sections were added to the culture set up and kept on window ledge with a north light. It was soon discovered that all these conditions favoured the growth of the sporlings. In contrast with this, another culture was prepared and it was kept away from natural light. Except for light, all the other conditions were the same as in the previous experiment. As a result very poor growth of the sporlings was observed.

2) In another experiment no Fucus was introduced into the culture dish, but plenty of natural light was provided.

In the case of the second set of cultures they were kept away from light and fresh Fucus sections were added to the culture. After some time both the cultures were examined and it was found that growth of the sporlings was

very poor in the case where no natural light was provided, and that growth occurred in the absence of Fucus.

3) An experiment was set up to observe whether any substance coming out of the host plant had any effect on the growth of the sporelings. It was set up in the following way.

Elachista cushions bearing matured sporangia were placed in the sterilised bowl containing the culture solution. Healthy Fucus fronds were kept in a big dish through which running sea water was allowed to pass and then to trickle into the bowl containing the cushions. The bowl was tilted so as to drain off the water.

A control experiment was set up in which the running sea water was directly allowed to trickle down into the bowl containing the Elachista fucicola cushions. The whole experiment was kept under constant light. The cultures were examined at a later stage. The experiment showed difference in density of the growth of sporelings. The bowl in which the water had passed through Fucus fronds contained more sporelings than the second bowl in which the sea water flowed directly.

4) In another experiment one culture bowl contained both Fucus fronds and Elachista cushions; a second bowl contained Elachista cushions only, while a third contained Fucus fronds and acted as the control. These bowls were kept under

constant light to see if light helped quicker growth of the sporelings. No growth occurred in any of the bowls.

5) An experiment was set up in the following way to see whether the infection of new healthy Fucus plants occurred when Elachista was grown in the same bowl.

A big sterile bowl was filled with culture solution and pieces of healthy Fucus fronds were introduced. A large beaker was then introduced into the bowl. On top of the beaker was a watchglass containing Elachista cushions, pieces of Fucus fronds and culture solution. The culture solution in the watch-glass and that in the beaker were in contact with each other. Every week one piece of Fucus frond was removed from the big beaker and transferred into a petri dish containing culture solution. For six weeks the fronds were transferred into separate culture dishes. These culture dishes were examined periodically to see if the filament of Elachista that was put into the watch-glass, and previously grown in culture, had grown and infected the healthy Fucus frond. There was no sign of infection at all. The pieces of Fucus frond were as they had been before the experiment had begun. They showed that infection of that plant had not taken place and the experiment was discarded late in April.

CYTOLOGY OF ELACHISTA FUCICOLA.

Nuclei in division were seen in unilocular sporangia of the material collected on the shore and grown in culture, fixed at different times during the day. It was impossible to ascertain if there was any period during which most of the nuclei divided. If material was collected at low tide and brought into the laboratory, kept in running sea water until the time the tide would reach the plants on the shore and then fixed, this was found to be the best method of obtaining figures in the sporangia. The number of chromosomes is the same in the parent and culture plants. Unilocular mother cells showing nuclei in the metaphase have been observed showing no evidence of reduction division. The size of the nuclei is the same in both kinds of material.

All stages in division were found in the same sporangium. (Fig. 59). During prophase the chromosomes lie in a well-defined area bounded by some substance. The chromosomes, during metaphase were small, even under the greatest magnification obtained. Anaphase and telophase were difficult to make out, because of small size of nuclei.

Eight chromosome were counted. Division of nuclei in the cells of the filaments was not observed.

DISCUSSION AND CONCLUSION.

Elachista fucicola, Elachista scutulata, Elachista flaccida and Elachista fucicola f. grevillei have not been recorded growing on other plants than those previously listed. It seems that E. fucicola f. grevillei growing on Cladophora rupestris is identical to Elachista fucicola except that it grows on a different alga.

E. fucicola has been previously recorded as having only unilocular sporangia, but when the cultures were set up from zoospores from unilocular sporangia, plurilocular sporangia were obtained in culture.

Plurilocular sporangia have also been found in Elachista scutulata in addition to unilocular sporangia. The plants bearing plurilocular sporangia were found on the Fife coast and in the Isle of Man, and have been recorded previously only from France and at Plymouth in 1899.

As Elachista fucicola was only known to bear unilocular sporangia, it was expected that the gametophyte generation bearing plurilocular gametangia would develop in culture, but spores from unilocular sporangia gave small plants bearing unilocular sporangia for four generations. Ruud (1934) in Sweden obtained a similar result with Dictyosiphon chordaria.

There has been no indication of reduction division in unilocular mother cells and chromosome number appears the

same in parent and in culture plants.

SUMMARY.

1. Reference is made to the original descriptions of Elachista scutulata, E. fucicola, E. fucicola f. grevillei, and Elachista flaccida.

2. The geographical distribution of the plants is given. The distribution in Great Britain is given by counties and generalisation is made.

3. The host plants on which the epiphytes have been found are mentioned.

4. The areas at St. Andrews in which Elachista scutulata, E. fucicola, E. fucicola f. grevillei, and E. flaccida occur are described and the method of collecting material for study in the laboratory is explained.

5. The techniques employed for staining and fixing whole material and microtome sections and for making squashes are given.

6. The method of setting up cultures and sub-cultures are given.

7. Description of filaments, paraphyses and sporangia are given.

8. Cell contents are described for the different species.

9. The method of dehiscence of unilocular sporangia and behaviour of the zoospores is described.

10. Cytological investigation showed that nuclei in the unilocular sporangia of parent plants and culture plants have the same number of chromosomes.

11. Plurilocular sporangia have been noted in the culture of Elachista fucicola and on plants of E. scutulata.

12. Zoospores from unilocular sporangia of E. fucicola have been grown in culture and found to produce new plants bearing unilocular sporangia similar to the parent, but much smaller. Four generations of such plants have been obtained in culture.

The presence of ~~the~~ Fucus is not necessary for the growth of Elachista fucicola in culture.

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PLATE I.

Fig. 1. Photograph of Elachista scutulata growing on Himanthalia elongata.

N.S. approx.

Fig. 2. Photograph of Elachista scutulata showing plurilocular sporangia, assimilatory filaments and paraphyses.

Magnification x 50 approx.

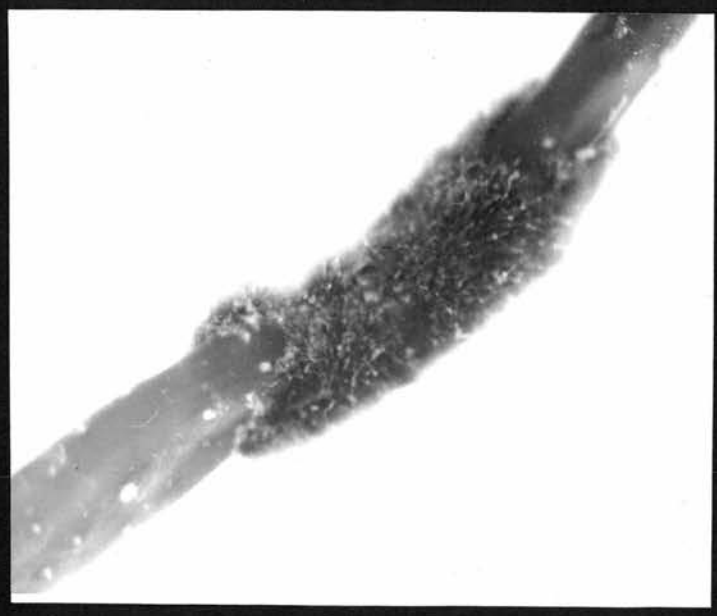

FIG. 1.

FIG. 2.

PLATE I.

PLATE II.

Fig. 3. Photograph of young plants of *Elachista fucicola* on *Fucus vesiculosus*.

Fig. 4. Photograph of older plants of *Elachista fucicola* on *Fucus vesiculosus*.

FIG. 3.

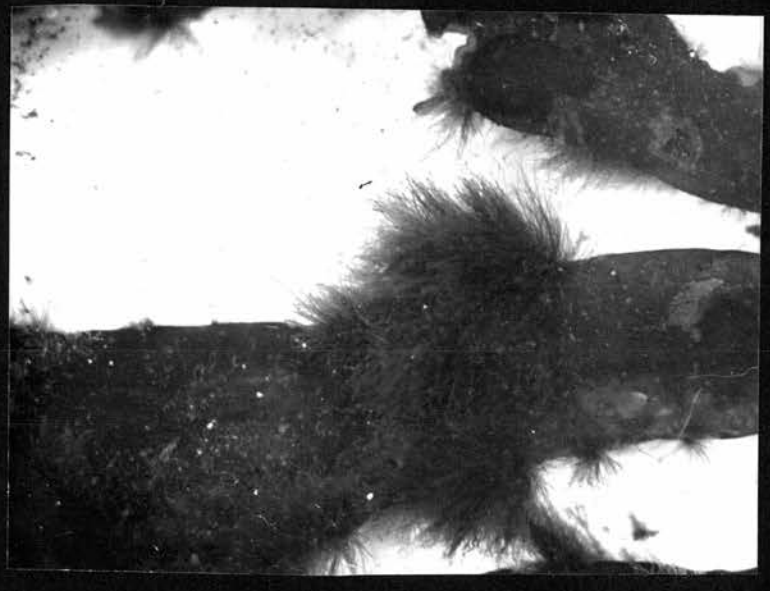

FIG. 4.

PLATE II.

PLATE III.

Fig. 5. Photomicrograph of Elachista scutulata showing basal tissue in a conceptacle of Himanthalia elongata.

Fig. 6. Photomicrograph of basal disc of Elachista fucicola showing filaments penetrating the thallus of Fucus sp.

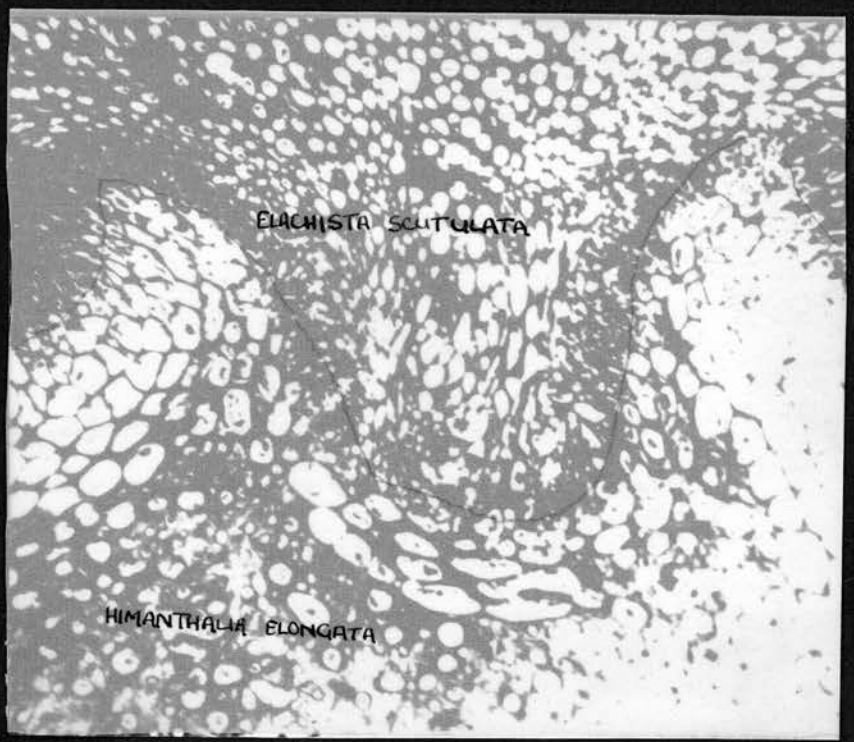

FIG. 5.

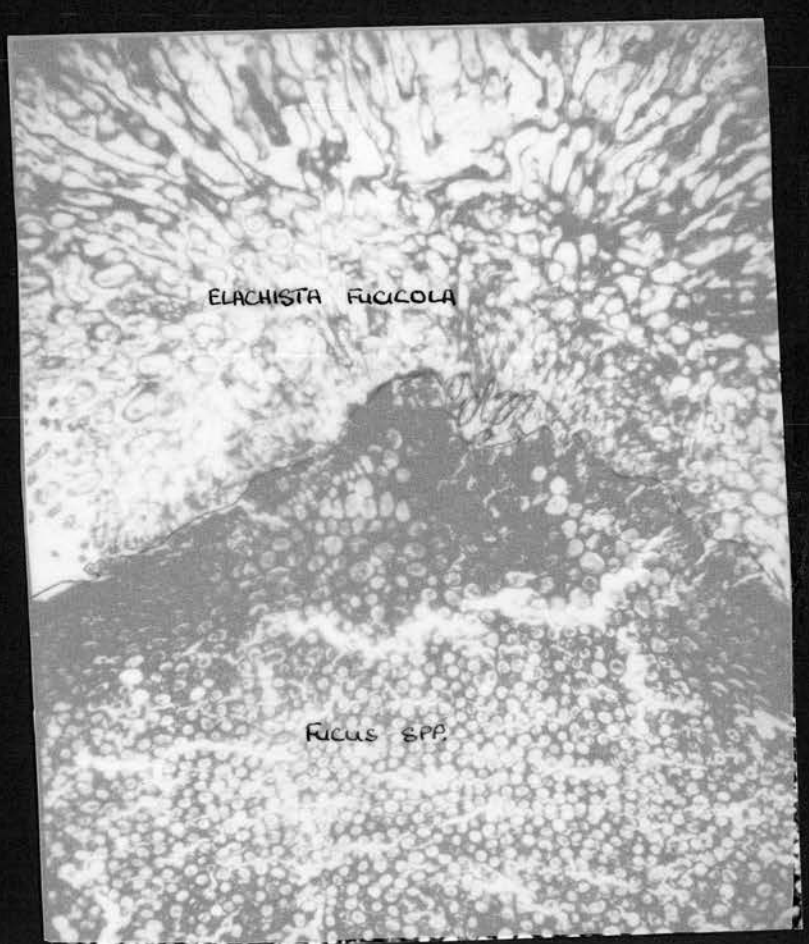

FIG. 6.

PLATE III

PLATE IV.

Fig. 7. Camera lucida drawing of assimilatory filament of Elachista fucicola.

Fig. 8. Camera lucida drawing of cell of assimilatory filament showing chromatophores, pyrenoids and the nucleus.

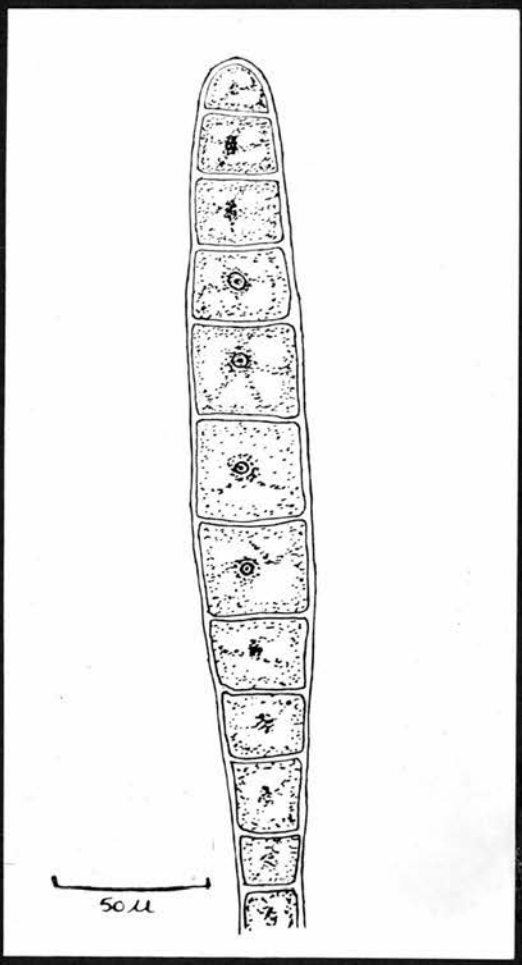

FIG. 7.

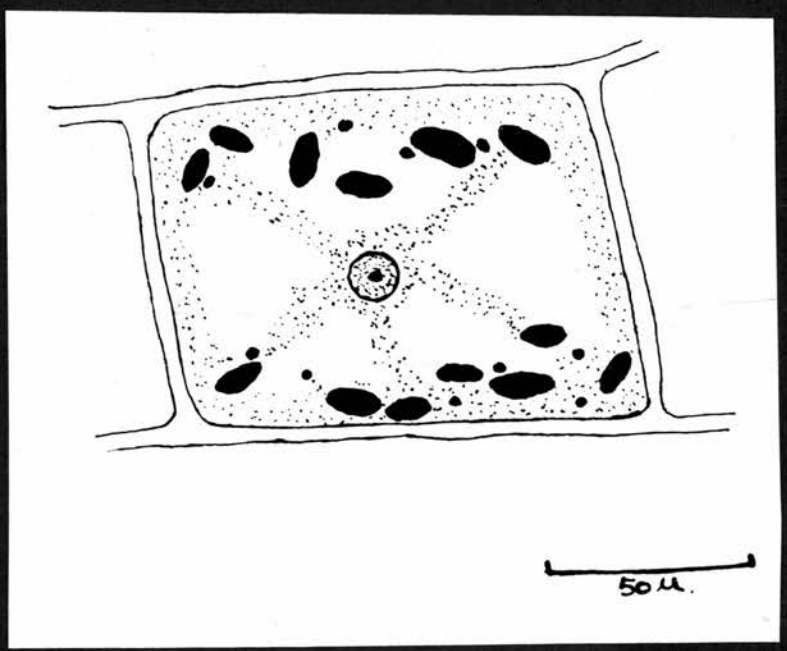

FIG 8

PLATE IV

PLATE V.

Fig. 9. Camera lucida drawing of paraphysis of *Elachista fucicola*.

Fig. 10. Camera lucida drawing of dichotomous branching of fibres from the basal disc.

Fig. 11. Camera lucida drawing of cell of paraphysis.

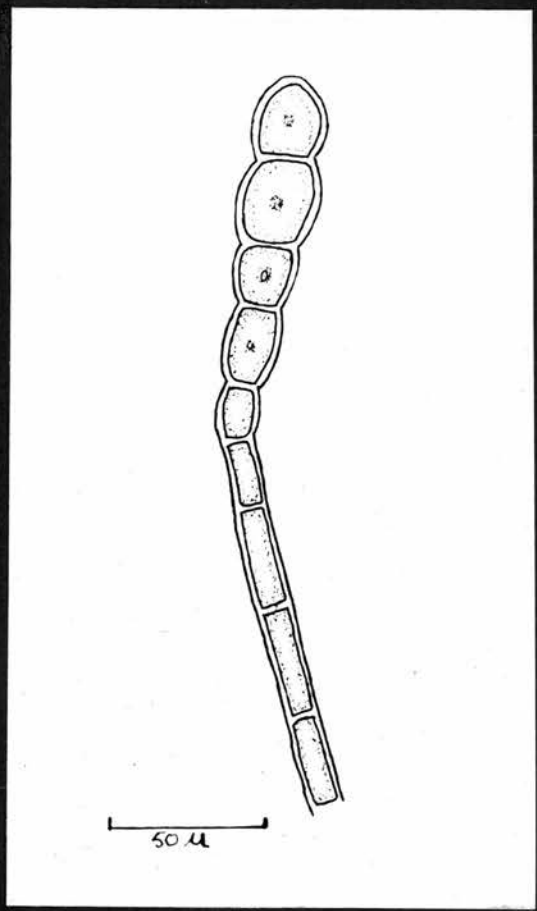

FIG. 9

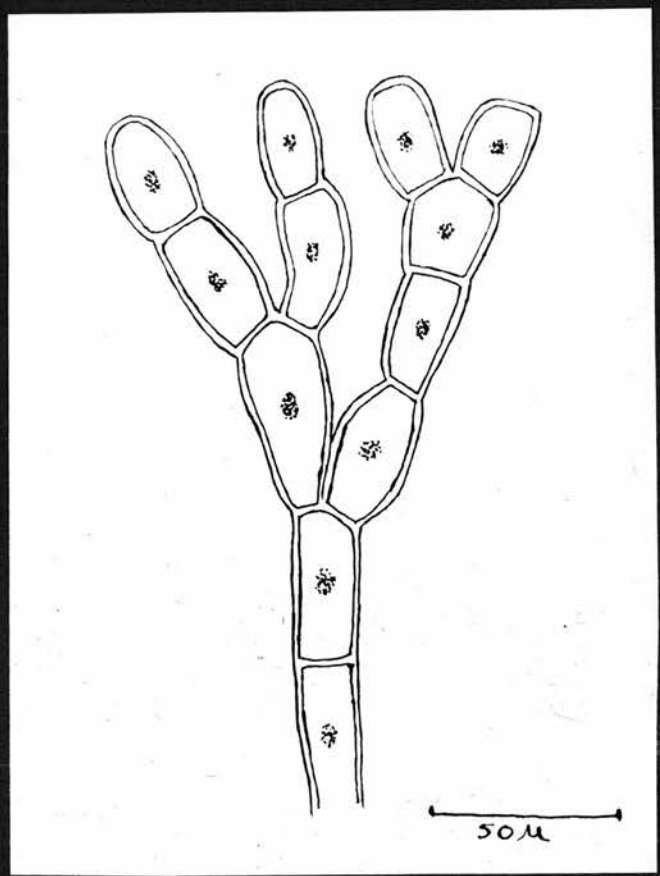

FIG. 10.

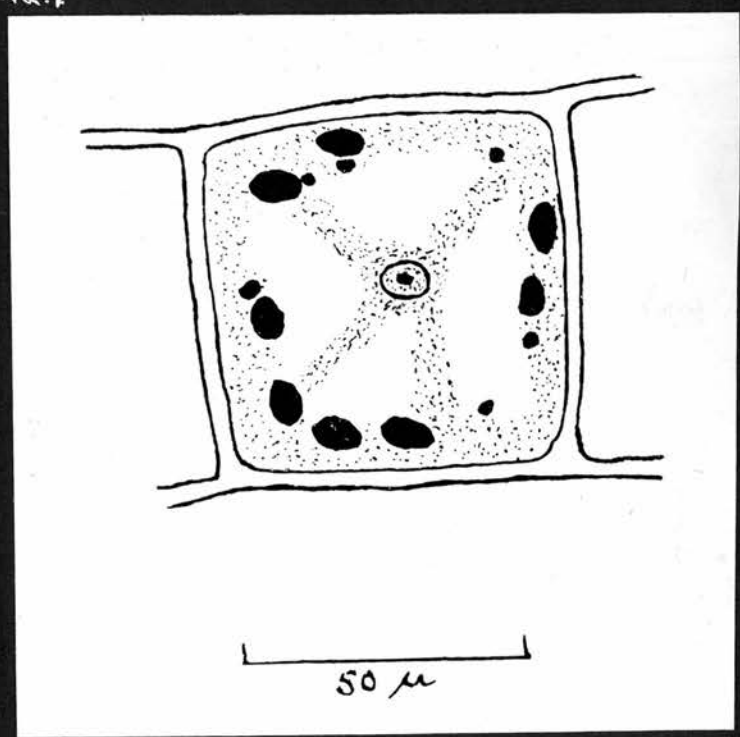

FIG. 11.

PLATE V.

PLATE VI.

Fig. 12. Photomicrograph of squashed preparation of Elachista fucicola, showing unilocular sporangia, paraphyses, and assimilatory filaments on the edge of the disc.

Magnification x 80.

Fig. 13. Camera lucida drawing of unilocular sporangium and paraphysis of Elachista fucicola.

Fig. 14. Camera lucida drawing of Elachista fucicola with mature unilocular sporangium with an apical pore through which the spores are liberated.

PLATE VI

FIG. 12.

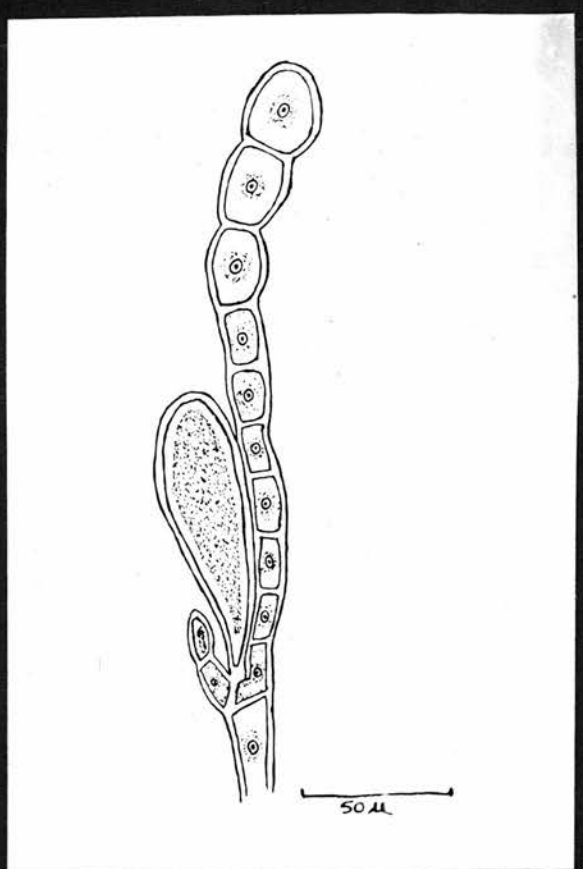

FIG. 13.

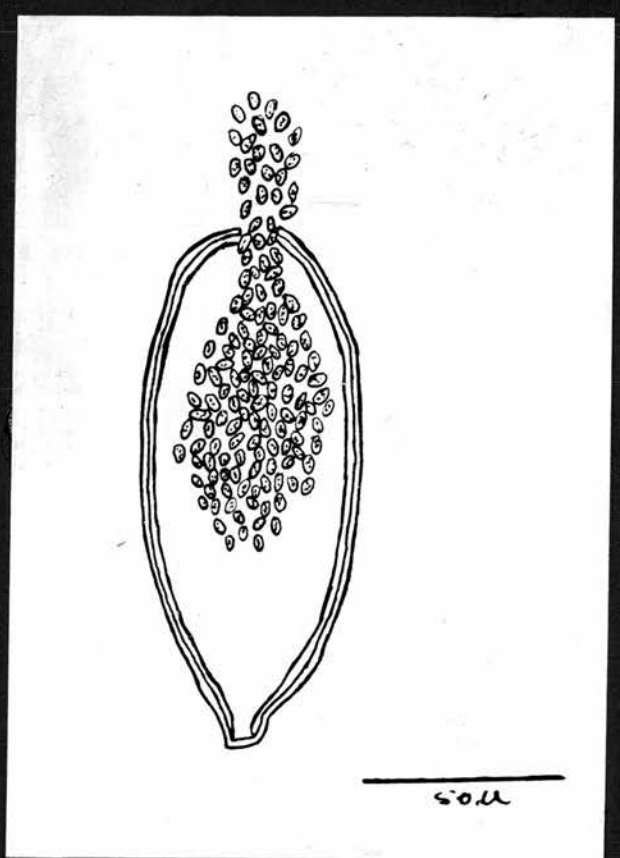

FIG. 14.

PLATE VII.

Fig.15. Camera lucida drawing of assimilatory filament of Elachista fucicola f. grevillei.

Fig.16. Camera lucida drawing of cell of assimilatory filament.

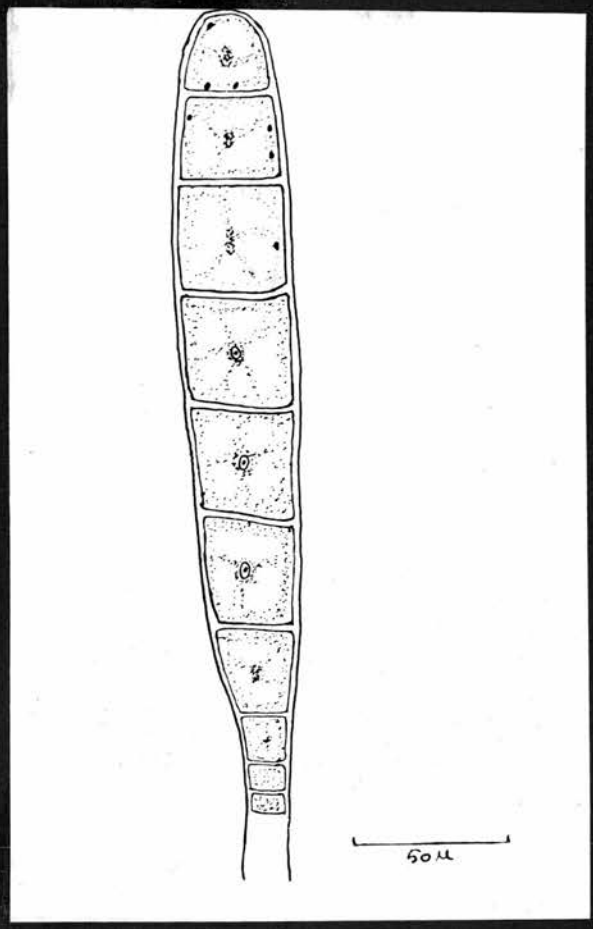

FIG. 15.

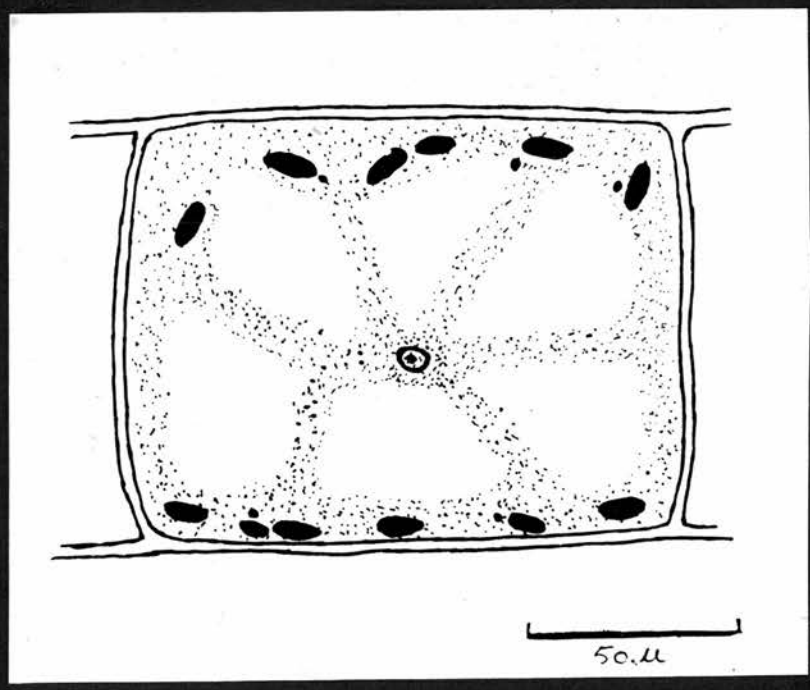

FIG 16.

PLATE VII

PLATE VIII.

Fig.17. Camera lucida drawing of paraphysis of *Elachista fucicola* f. *grevillei*.

Fig.18. Camera lucida drawing of cell of paraphysis.

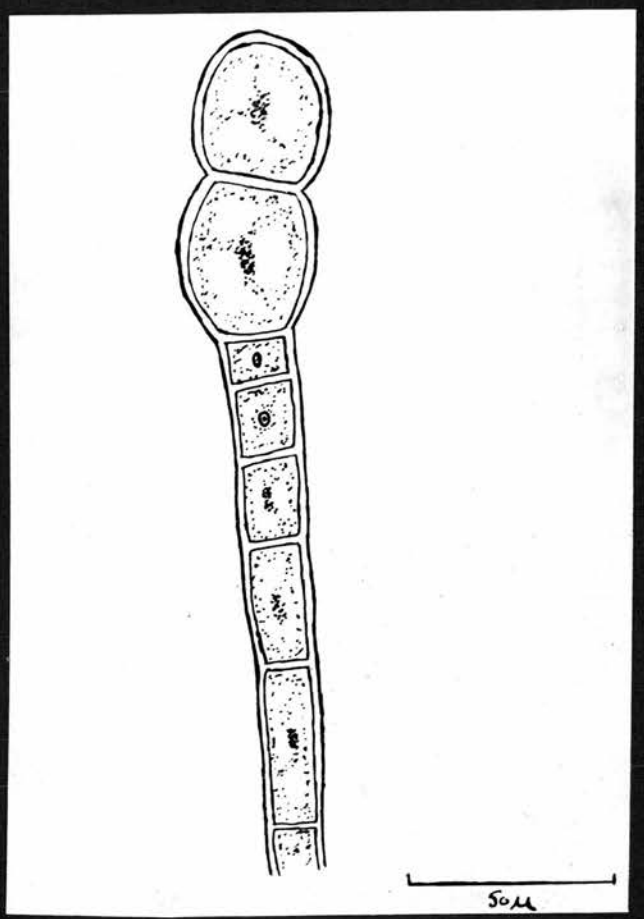

FIG. 17.

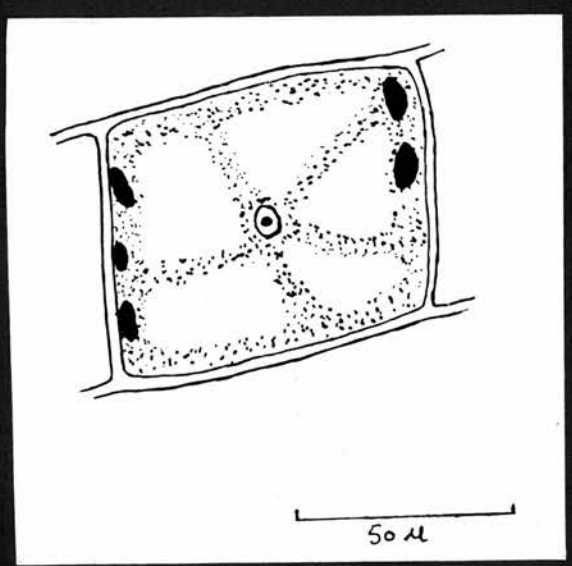

FIG. 18.

PLATE VIII

PLATE IX.

Fig.19. Camera lucida drawing of unilocular sporangium of Elachista fucicola f. grevillei.

Fig.20. Camera lucida drawing of young unilocular sporangium of Elachista fucicola f. grevillei.

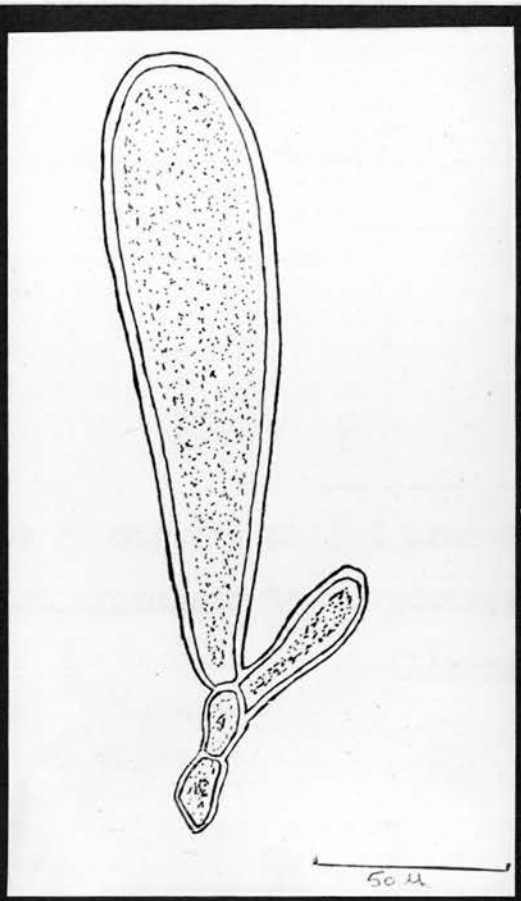

FIG. 19

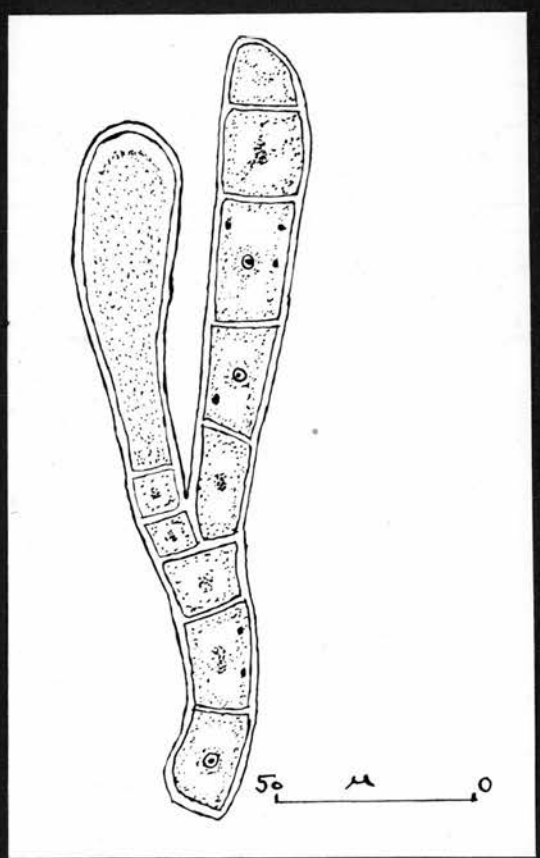

FIG. 20.

PLATE IX

PLATE X.

Fig.21. Camera lucida drawing of assimilatory filament of Elachista scutulata.

Fig. 22. Camera lucida drawing of cell of assimilatory filament of Elachista scutulata.

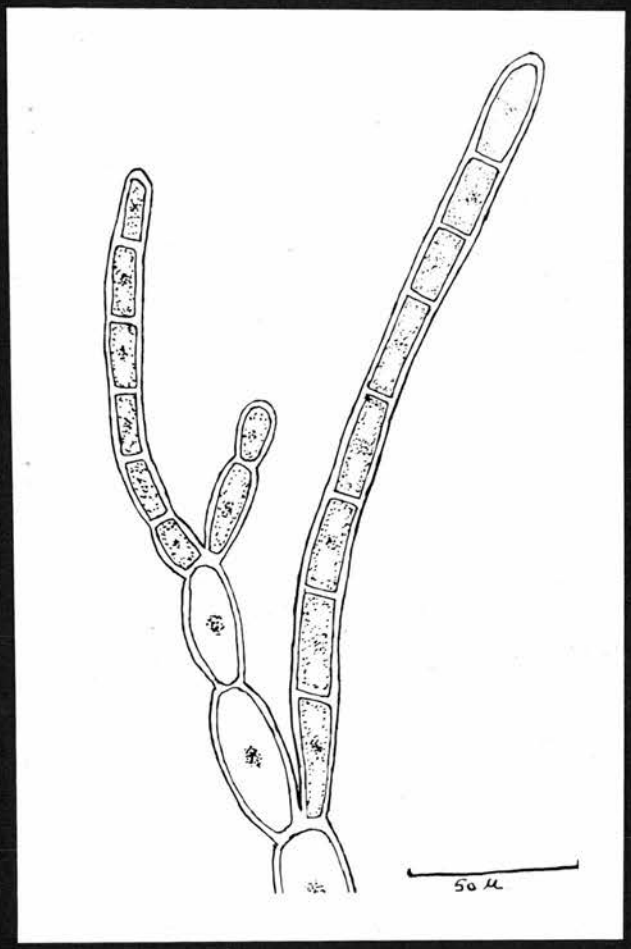

FIG 21

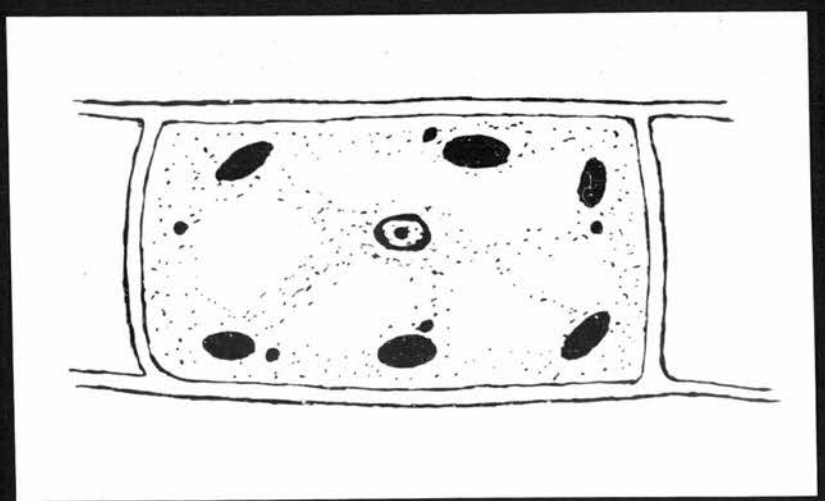

FIG. 22.

50 μ.

PLATE X.

PLATE XI.

Fig. 23, Camera lucida drawing of paraphysis of *Elachista scutulata*.

Fig. 24. Camera lucida drawing of cell of paraphysis.

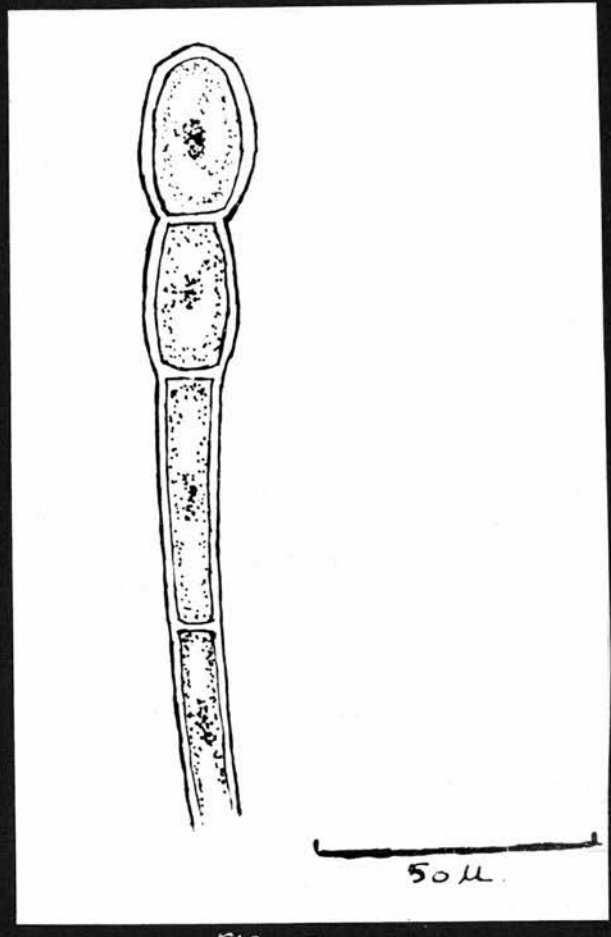

FIG 23

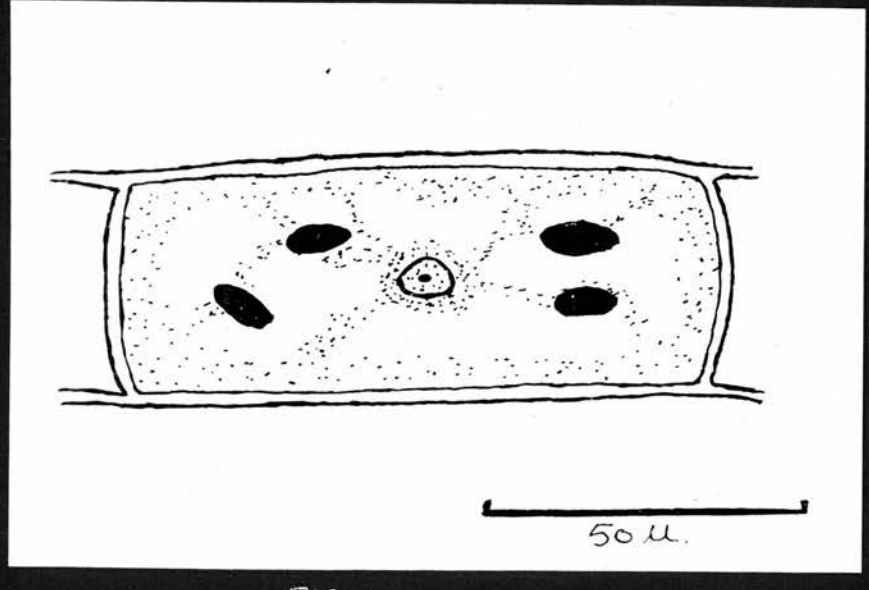

FIG. 24.

PLATE XI

PLATE XII.

Fig.25. Camera lucida drawing of branching of hyaline fibres of Elachista scutulata.

Fig.26. Camera lucida drawing of plurilocular sporangia, paraphyses and assimilatory filaments of Elachista scutulata.

Fig.27. Camera lucida drawing of mature plurilocular sporangia of Elachista scutulata.

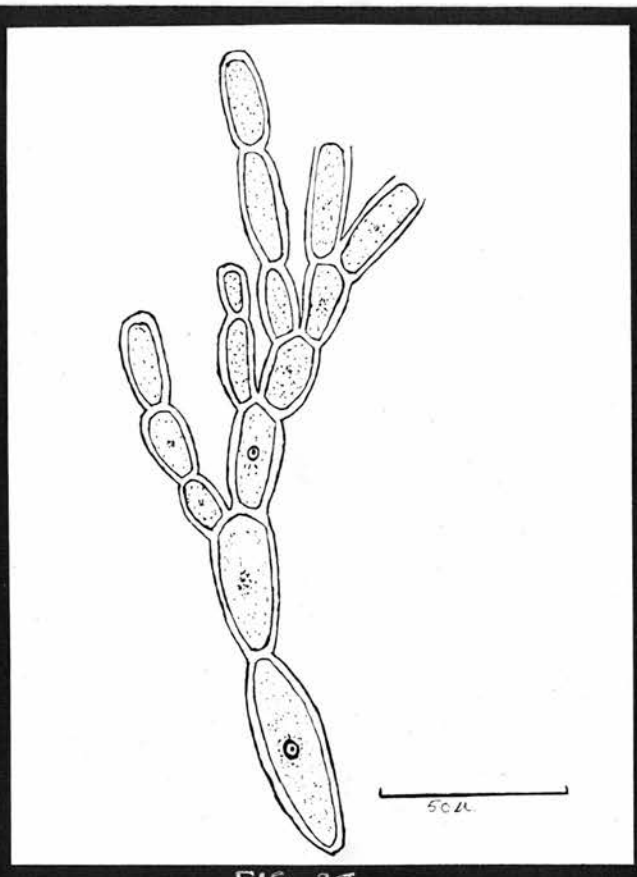

FIG 25

PLATE XII

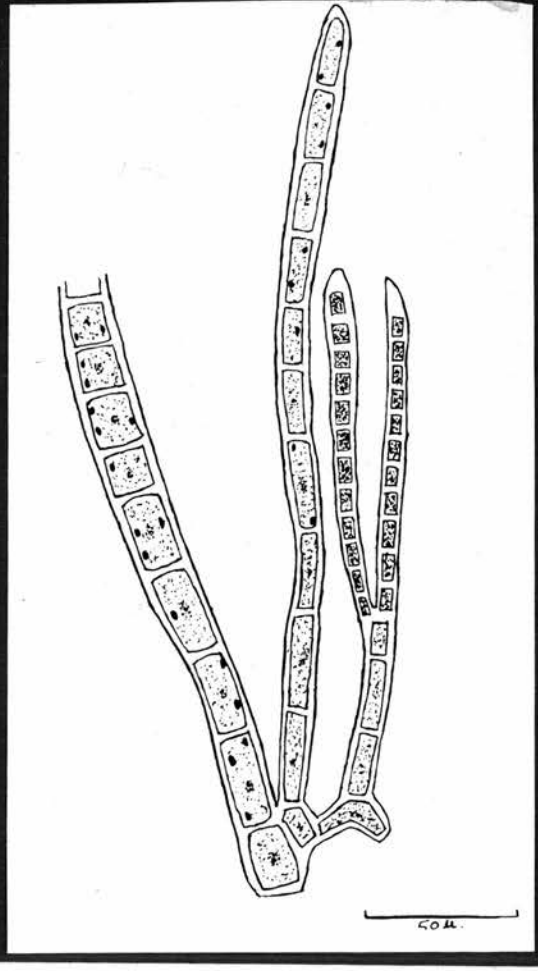

FIG 26.

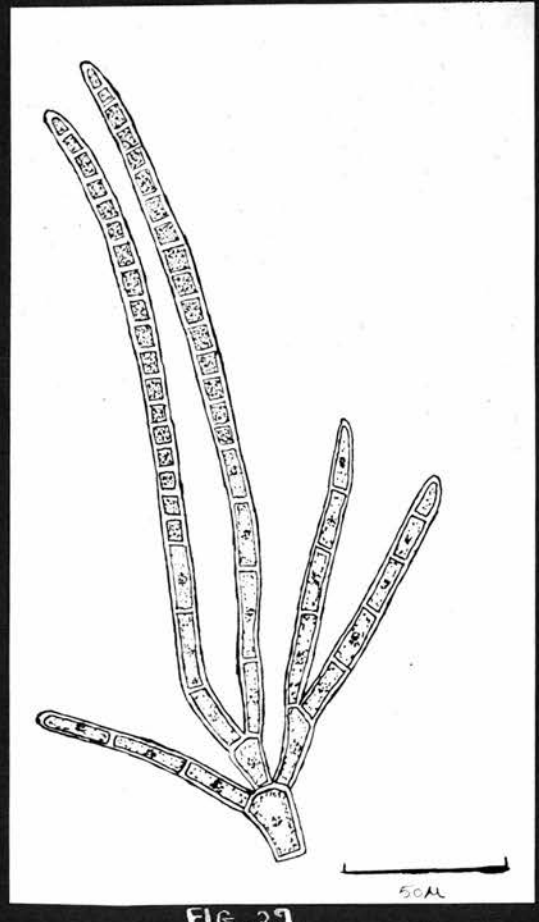

FIG. 27.

PLATE XIII.

Fig. 28. Camera lucida drawing of assimilatory filament of Elachista flaccida.

Fig. 29. Camera lucida drawing of paraphysis of Elachista flaccida.

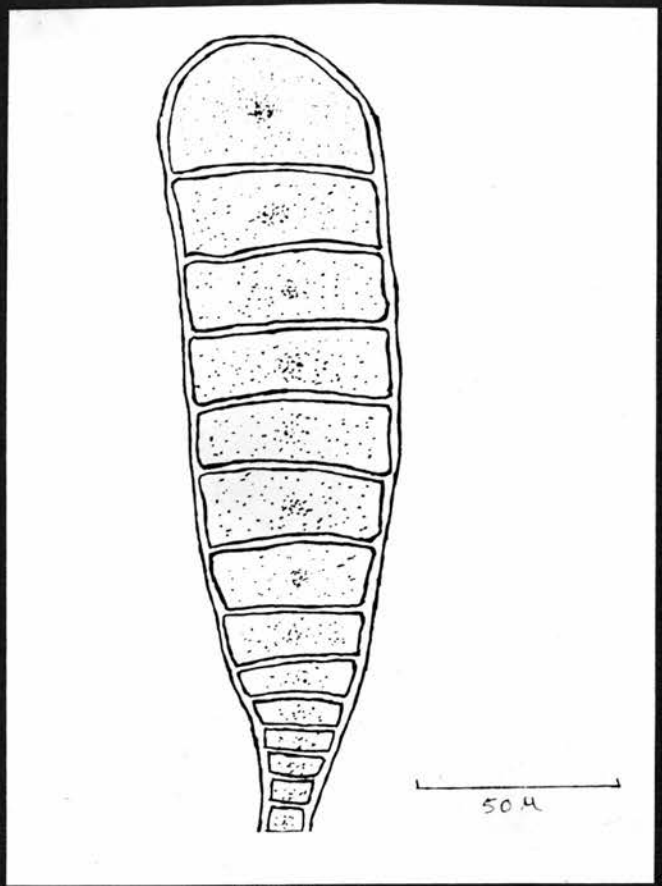

FIG. 28.

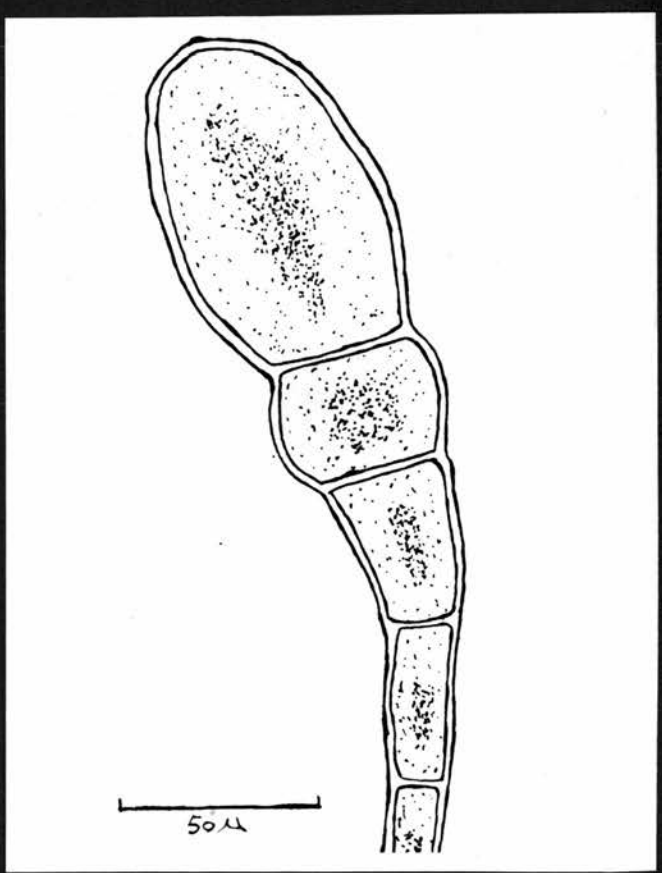

FIG. 29.

PLATE XII

PLATE XIV.

Fig. 30. Camera lucida drawing of unilocular sporangium and paraphysis of Elachista flaccida.

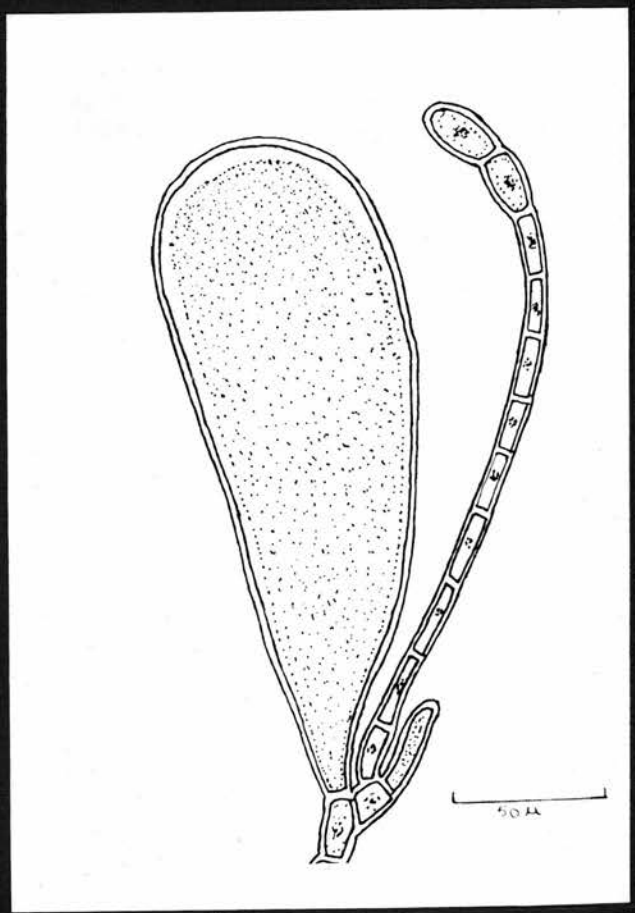

FIG. 30.

PLATE XIV

PLATE XV.

Fig. 31. Camera lucida drawing of zoospores of Elachista fucicola.

Fig. 32. Camera lucida drawing of sporelings, two-three celled long.

Fig. 33. Camera lucida drawing of six-celled sporeling.

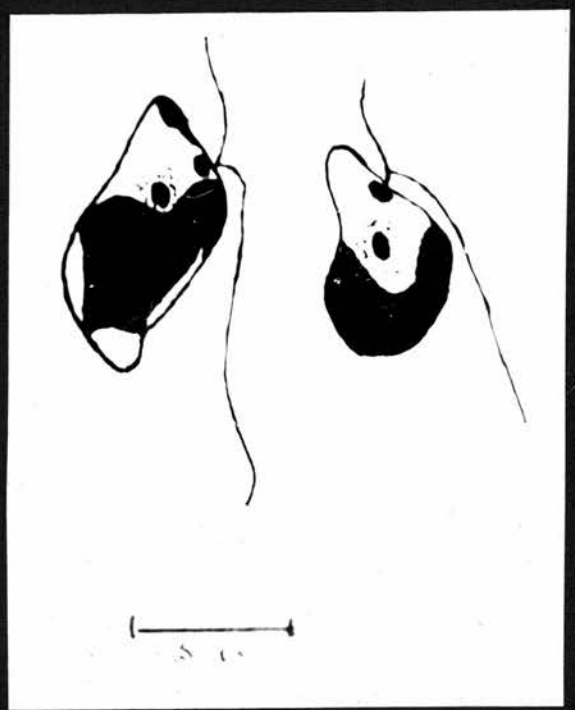

FIG 31

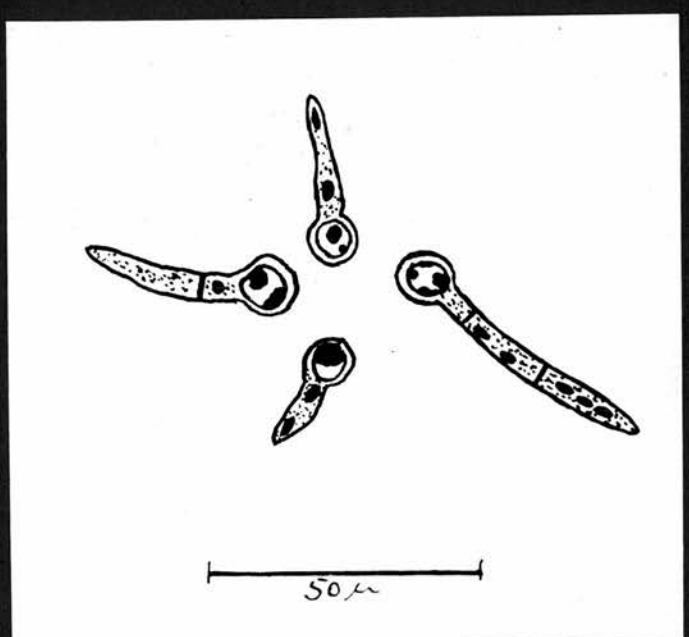

FIG 32.

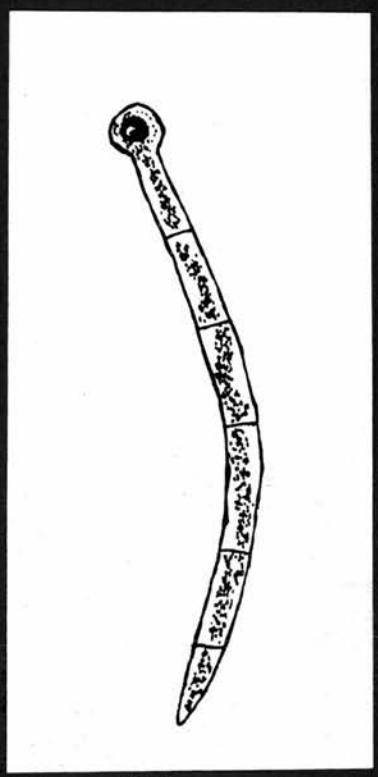

FIG 33.

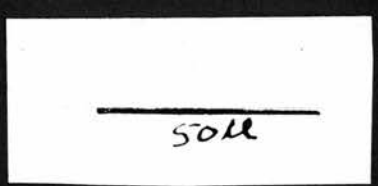

PLATE XV

PLATE XVI.

Fig. 34. Camera lucida drawing of sporling of Elachista fucicola showing branches.

Fig. 35. Camera lucida drawing of older branched sporling of Elachista fucicola.

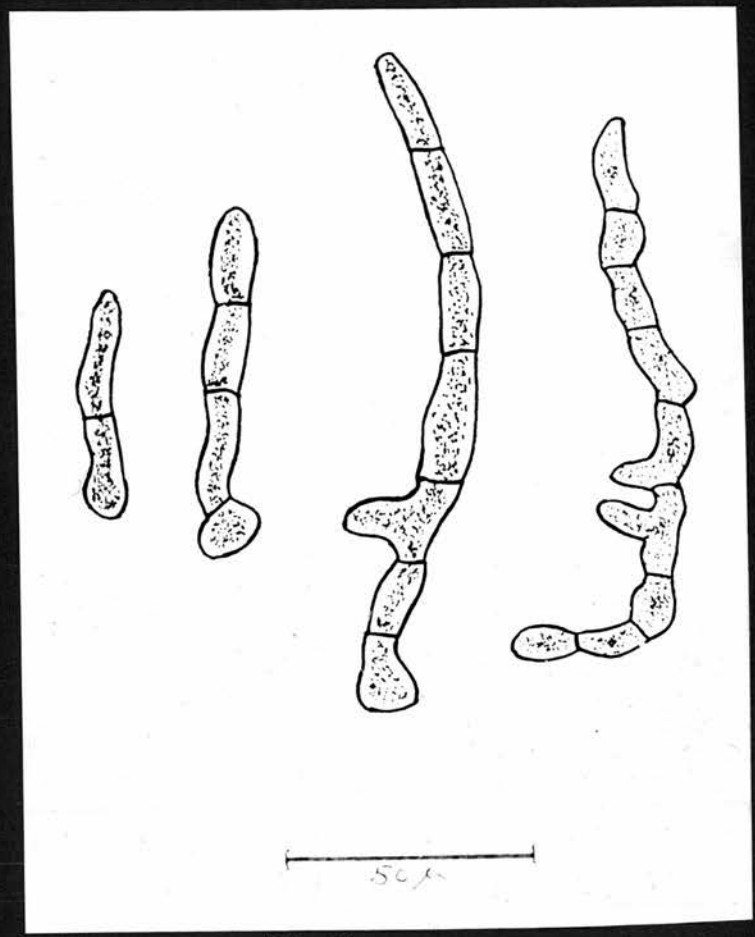

FIG. 34.

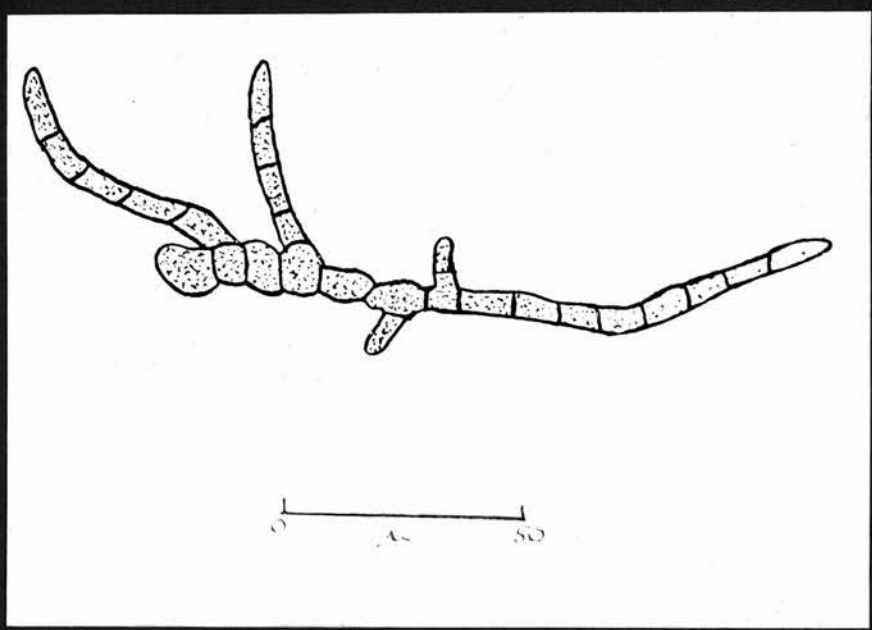

FIG. 35.

PLATE XVI

PLATE XVII.

Figs. 36 and 37. Camera lucida drawings of development of erect filaments from creeping ones in Elachista fucicola.

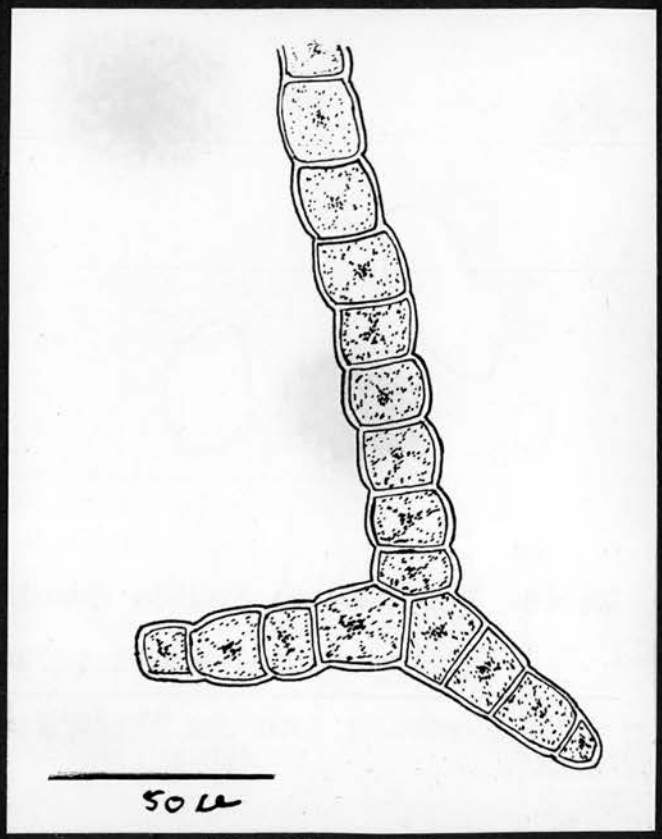

FIG. 36.

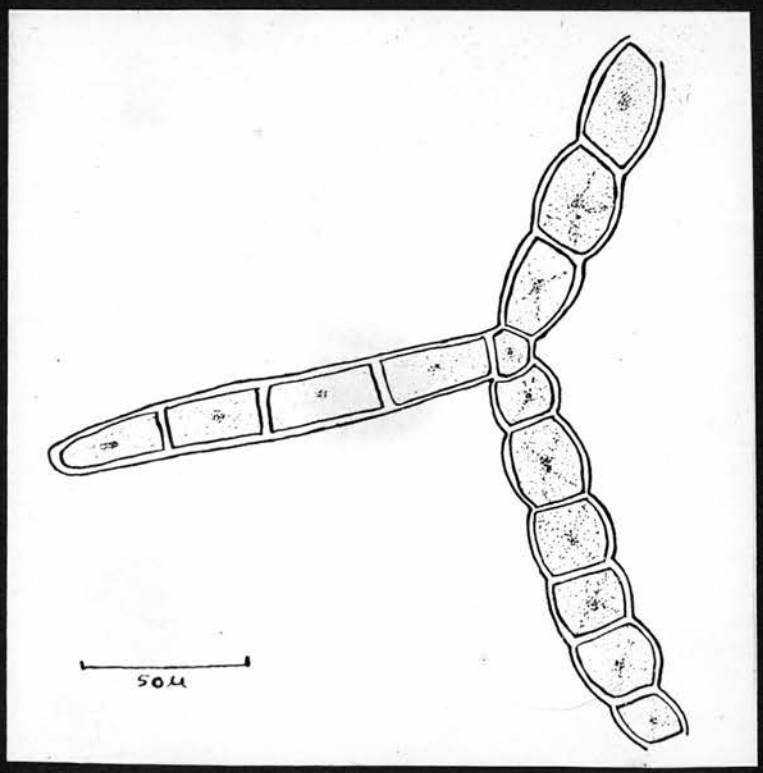

FIG. 37.

PLATE XV

PLATE XVIII.

Fig. 38. Camera lucida drawing of creeping filaments with erect branches, one of which has branched, of Elachista fucicola.

Fig. 39. Camera lucida drawing of branched filaments shown in Fig. 38.

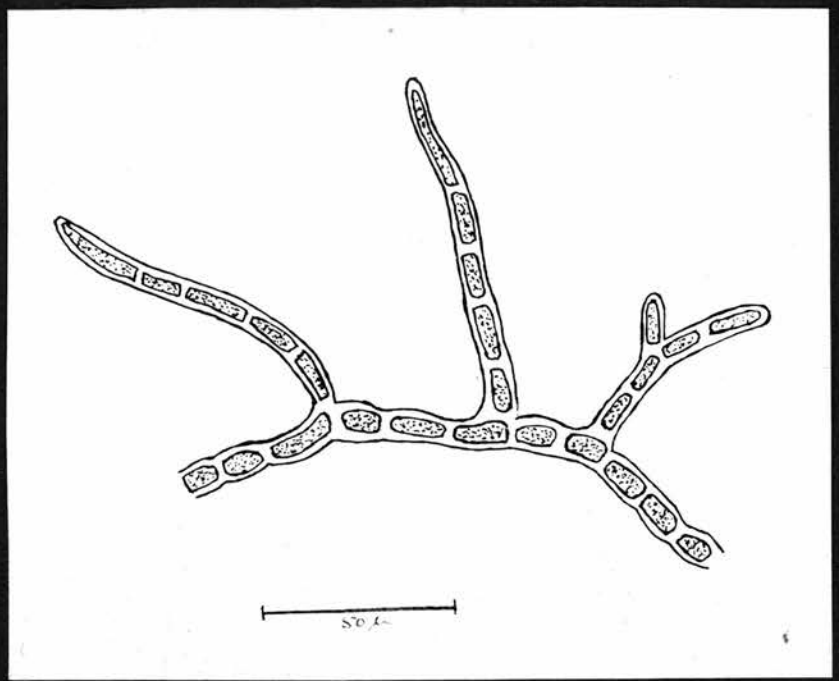

FIG. 38.

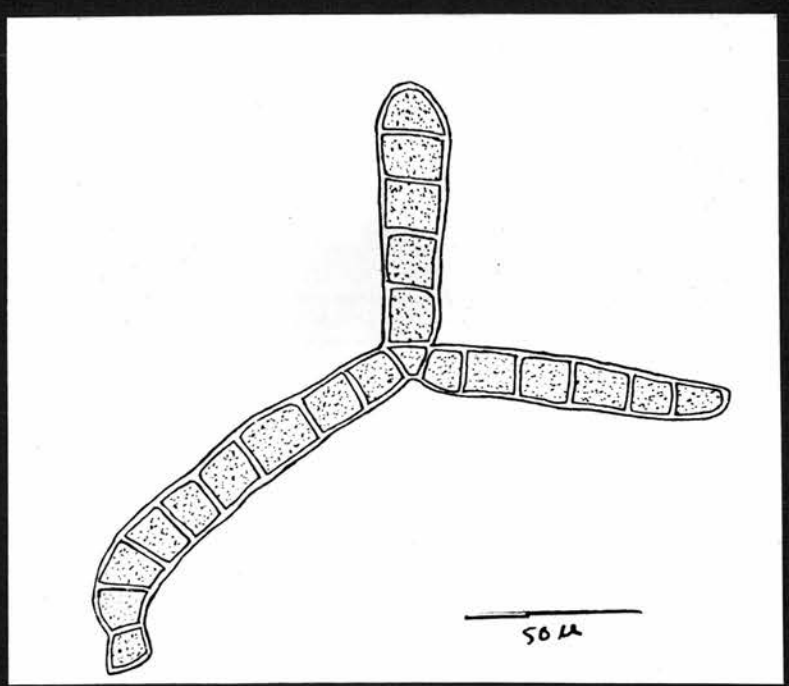

FIG. 39.

PLATE XVIII

PLATE XIX.

Fig. 40. Camera lucida drawing of erect branches on creeping filament of Elachista fucicola.

Fig. 41. Camera lucida drawing of erect branches on creeping filament of Elachista fucicola.

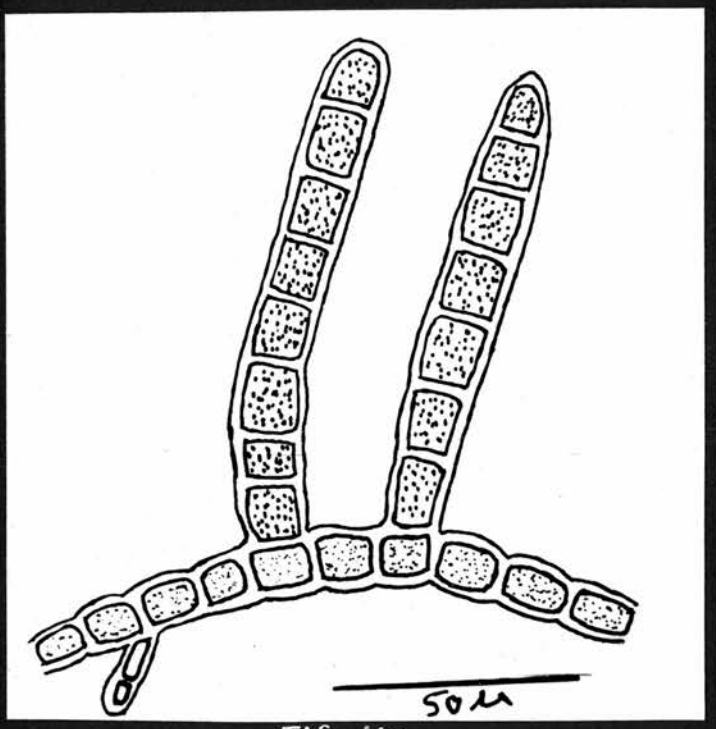

FIG 40

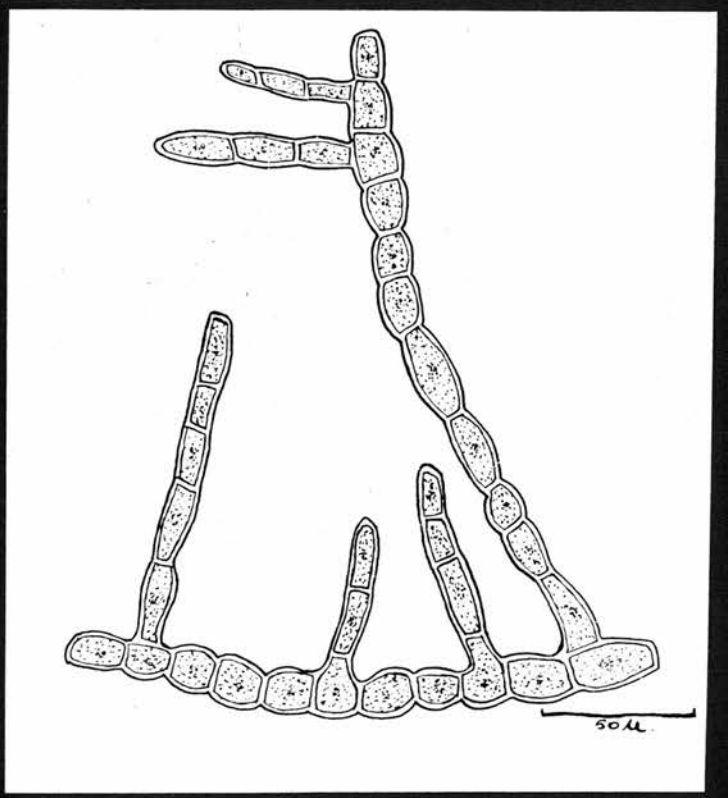

FIG 41

PLATE XIX

PLATE XX.

Figs. 42, 43 and 44. Camera lucida drawings of rhizoids arising from the creeping filament of Elachista fucicola.

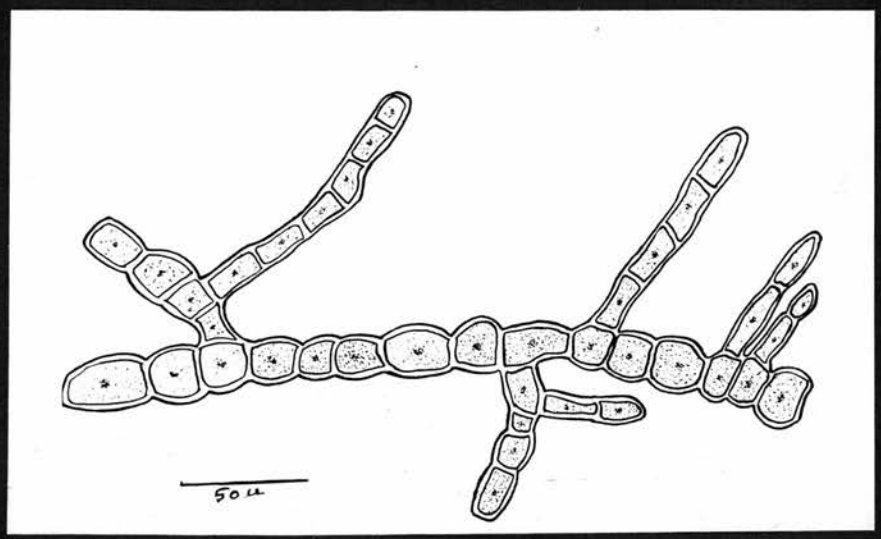

FIG. 42.

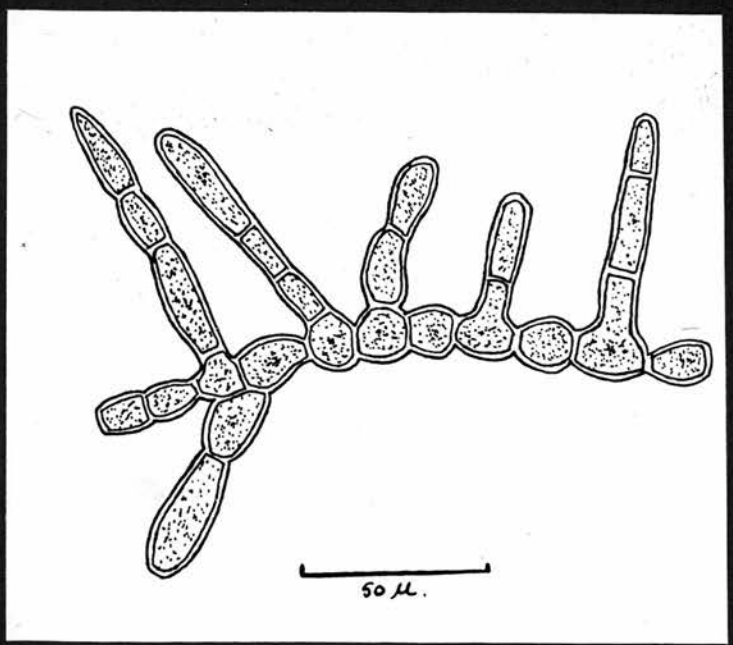

FIG. 43.

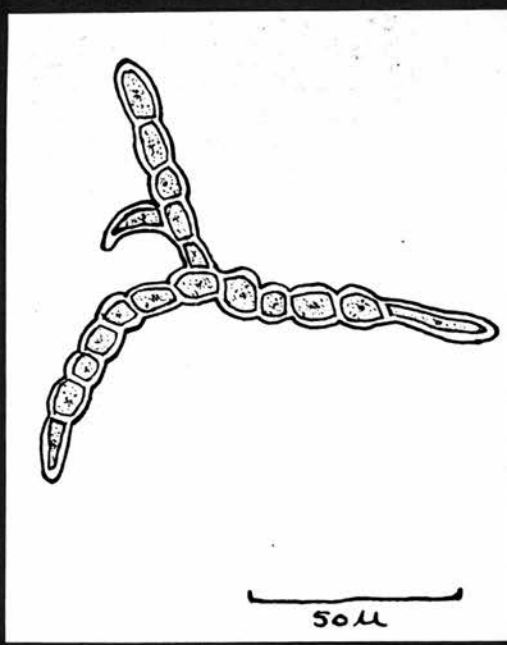

FIG. 44.

PLATE XX

PLATE XXI.

Figs. 45 and 46. Camera lucida drawings of erect filaments and rhizoids arising from creeping filaments of Elachista fucicola.

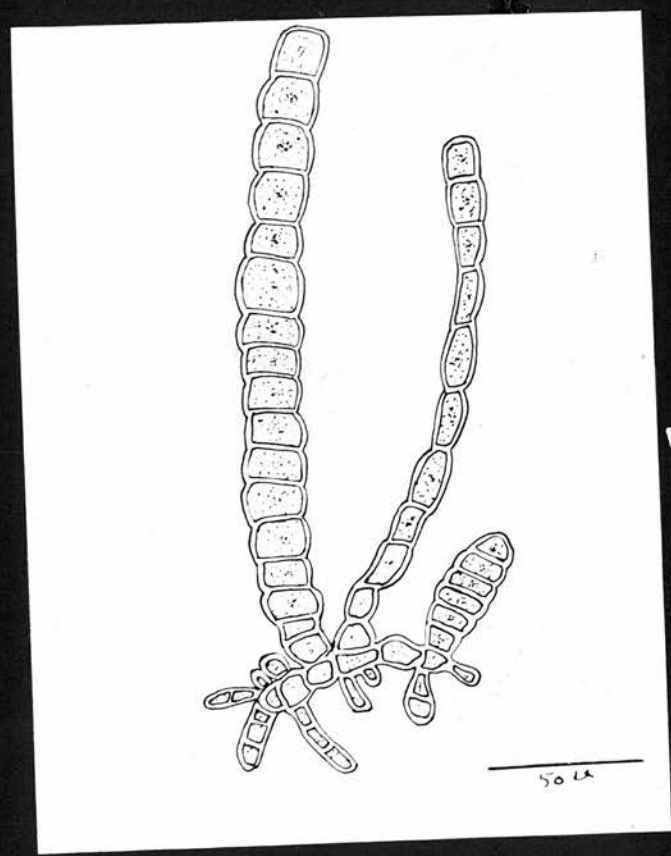

FIG. 45.

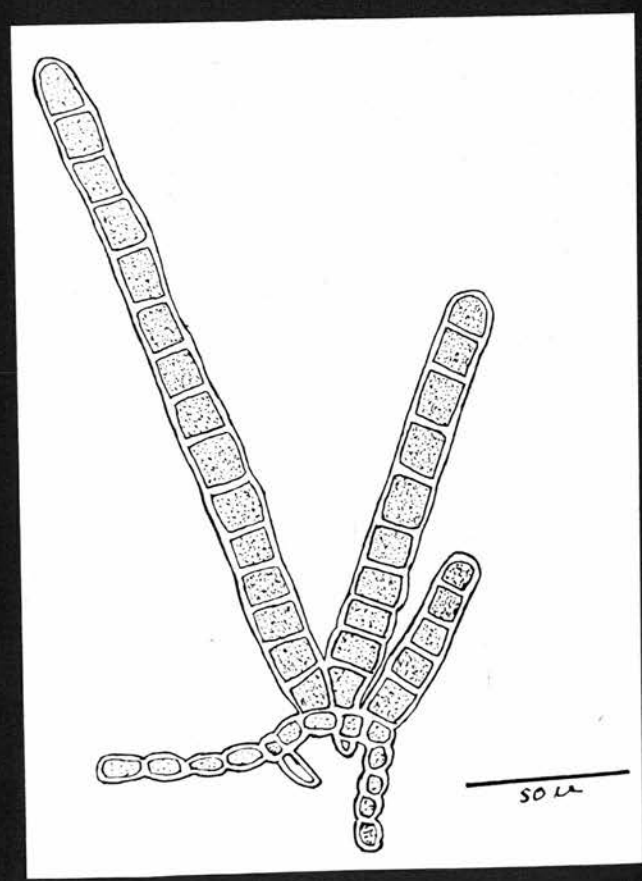

FIG. 46.

PLATE XX

PLATE XXII.

Fig. 47. Camera lucida drawing of protonema of Elachista fucicola showing erect filaments and rhizoids.

Fig. 48. Camera lucida drawing of well developed protonema of Elachista fucicola with erect filaments and rhizoids.

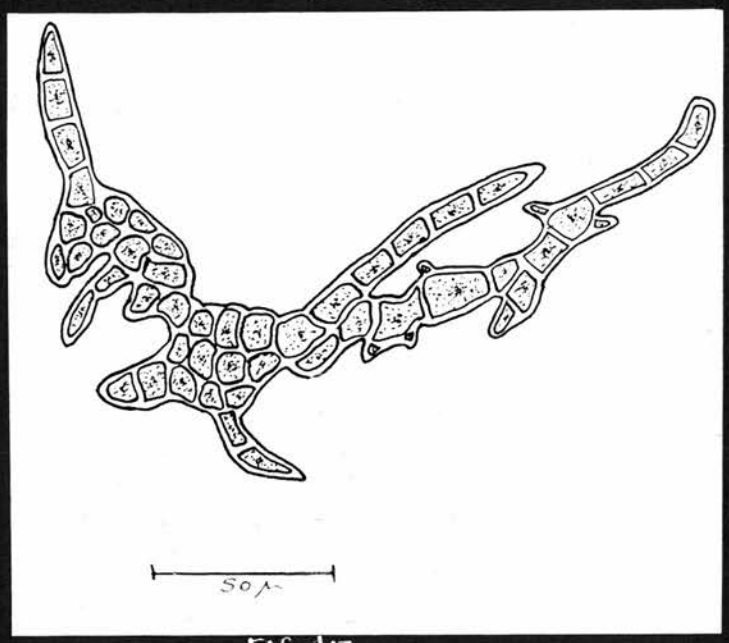

FIG-47.

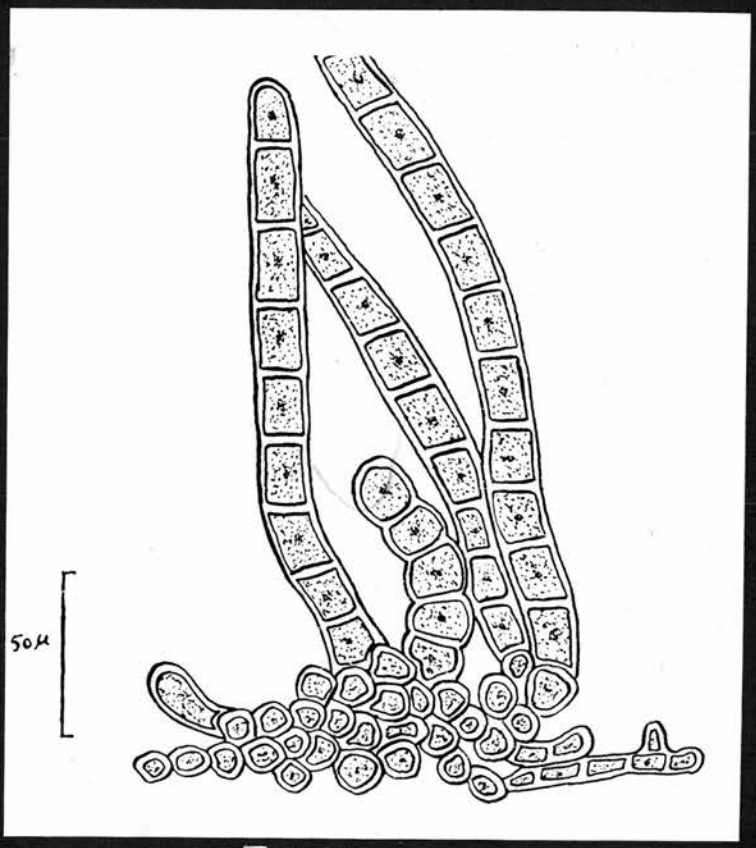

FIG 48

PLATE XXI

PLATE XXIII.

Figs. 49, 50 and 51. Camera lucida drawings of small plants of Elachista fucicola grown from spores in culture, and bearing unilocular sporangia.

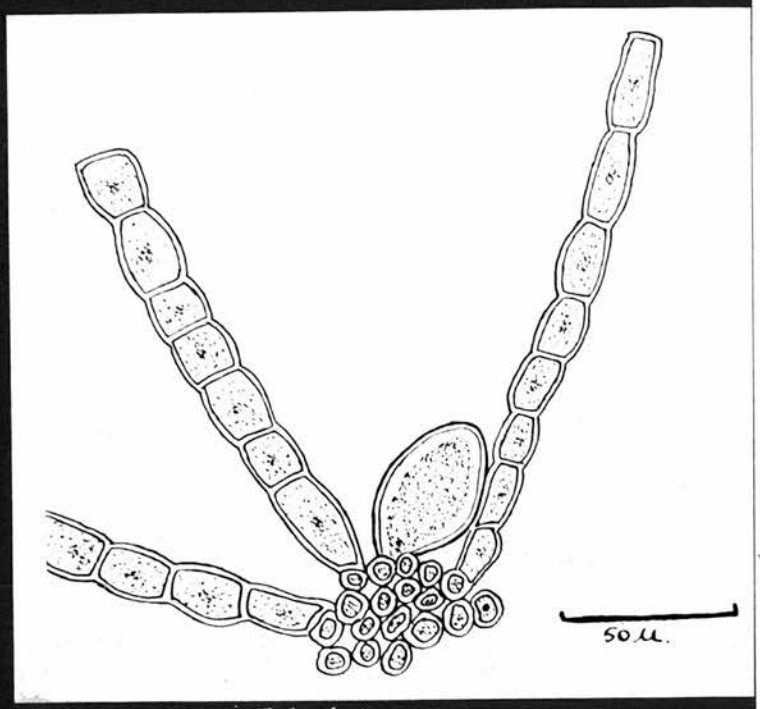

FIG. 49.

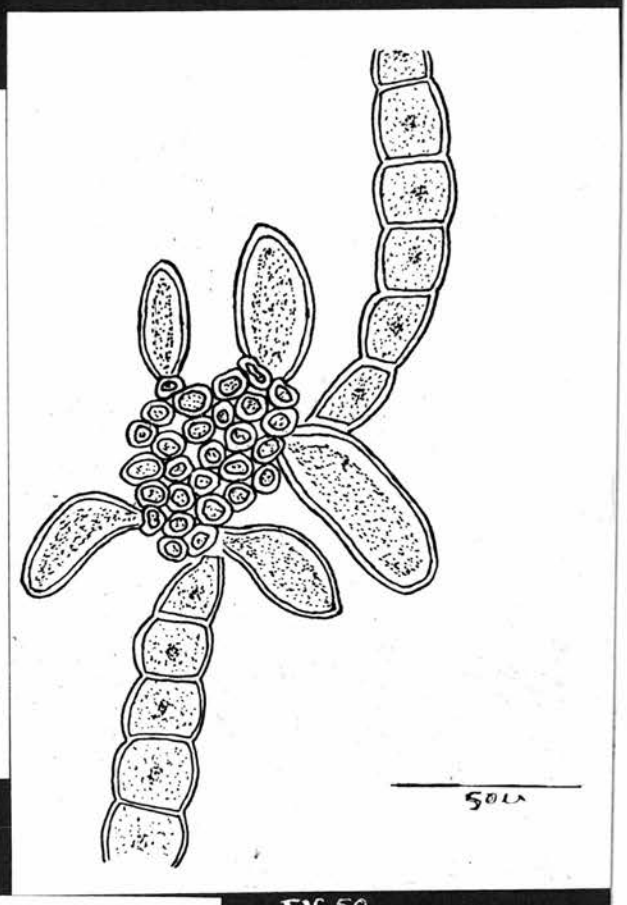

FIG. 50

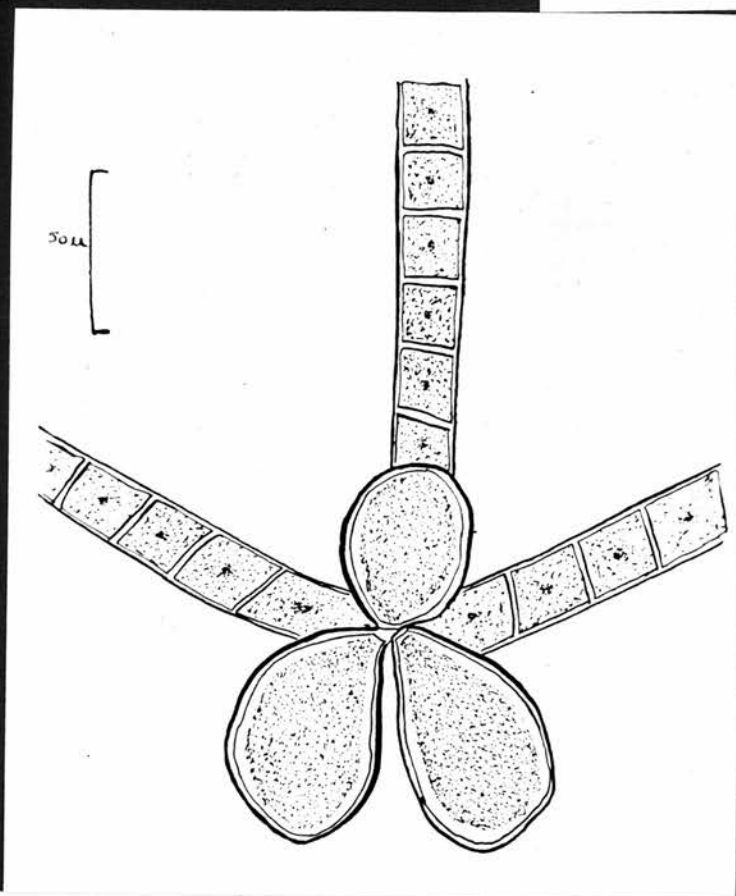

FIG. 51.

PLATE XXII

PLATE XXIV.

Fig. 52. Camera lucida drawing of plurilocular sporangium on plant of Elachista fucicola grown in culture.

Fig. 53. Camera lucida drawing of empty plurilocular sporangium of Elachista fucicola.

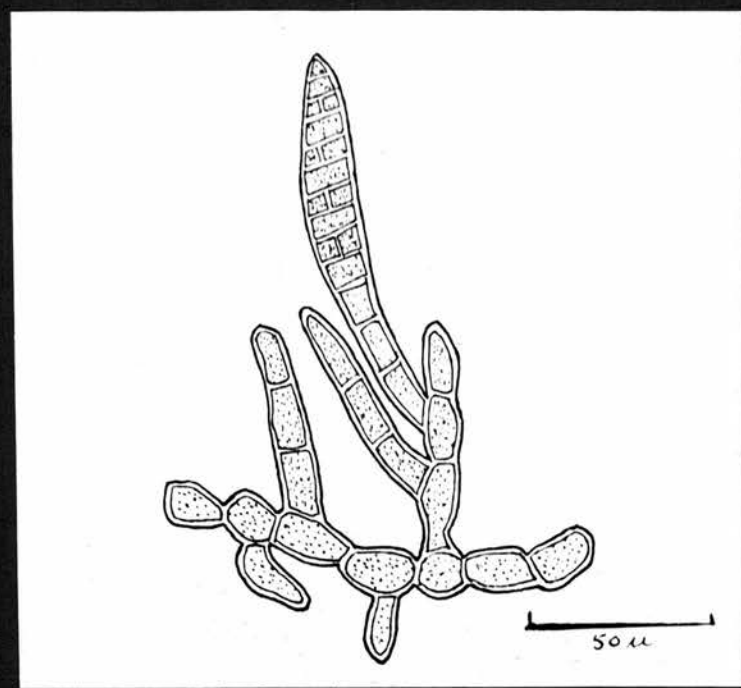

FIG. 52.

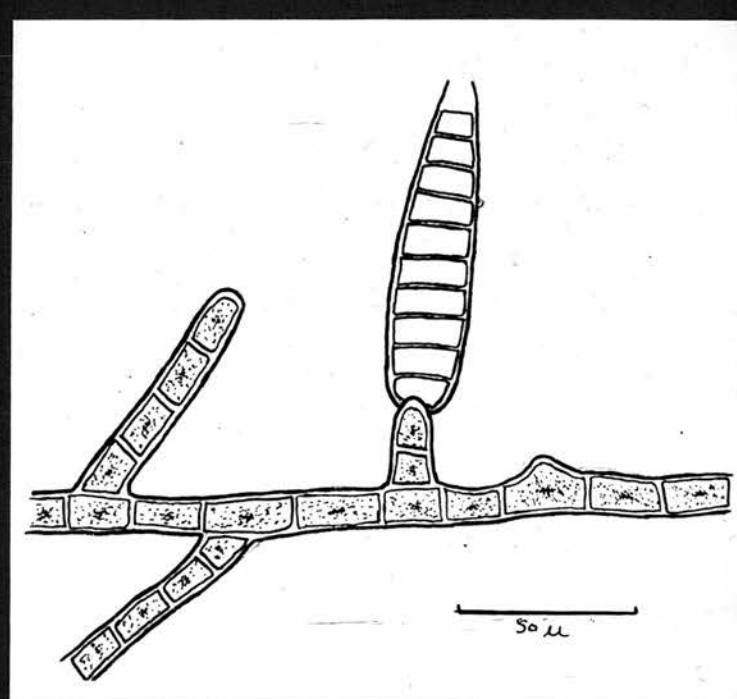

FIG. 53.

PLATE XXIV

PLATE XXV.

Fig. 54. Camera lucida drawing of germinating spores from plurilocular sporangium of Elachista fucicola.

Fig. 55. Camera lucida drawing of erect filaments of Elachista fucicola showing branches of 1-2 cells.

Fig. 56. Camera lucida drawing of later stage in development of Elachista fucicola sporelings.

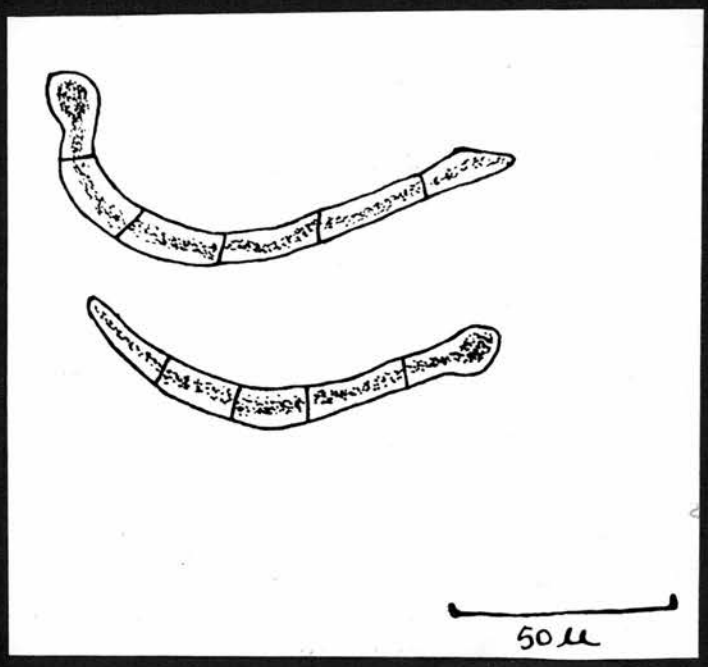

Fig. 54

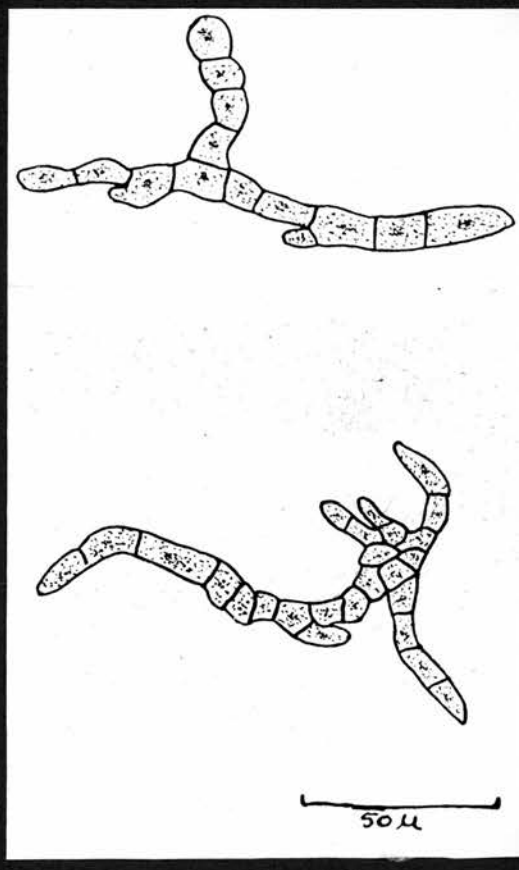

Fig. 56

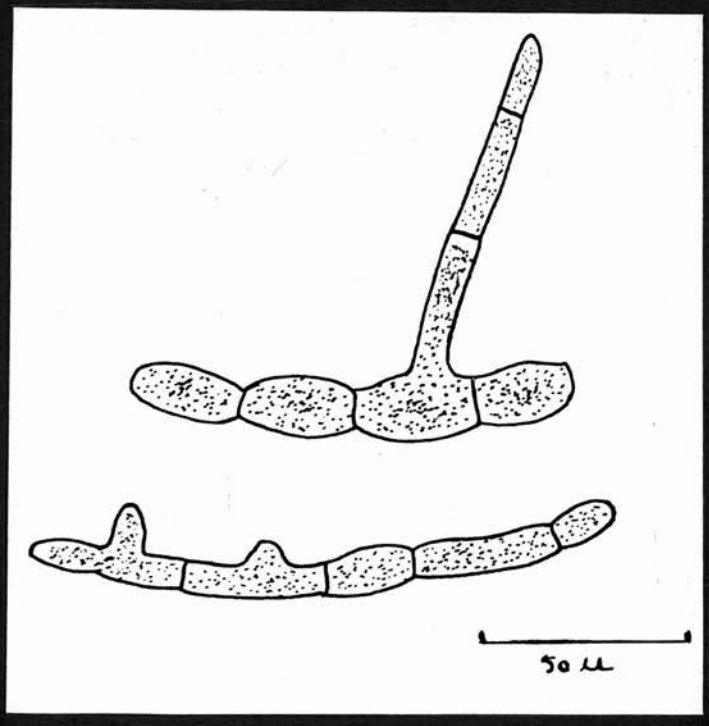

Fig. 55

PLATE XXV

PLATE XXVI.

Fig. 57. Camera lucida drawing of second generation of Elachista fucicola plants grown in culture from zoospores from plurilocular sporangia of first generation. (Fig. 53).

Fig. 58. Photomicrograph of young plants of Elachista fucicola grown in culture from zoospore from a unilocular sporangium. Plants show assimilatory filaments diverging in all directions from a basal disc on which are borne unilocular sporangia.

Magnification x 60.

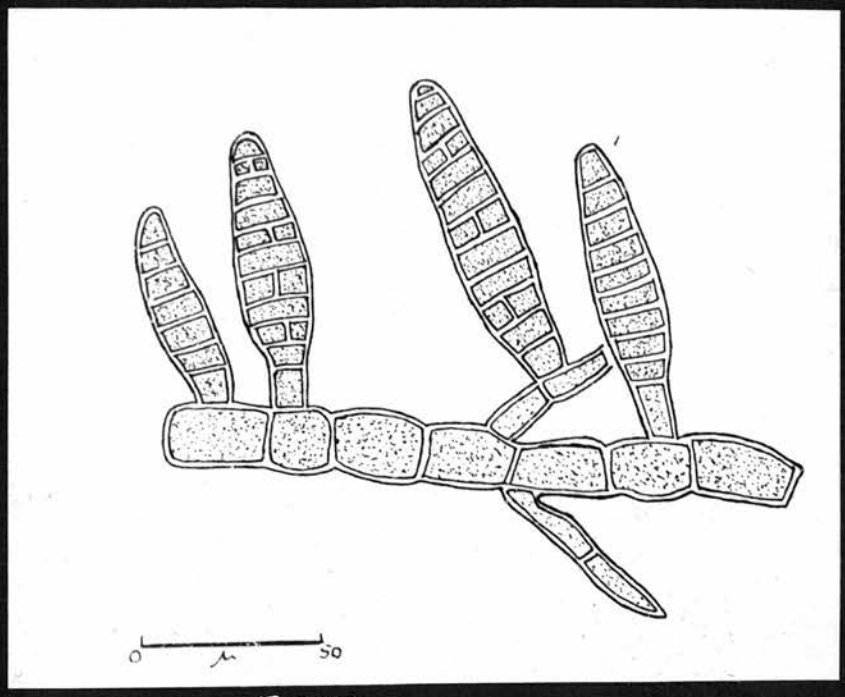

FIG. 57.

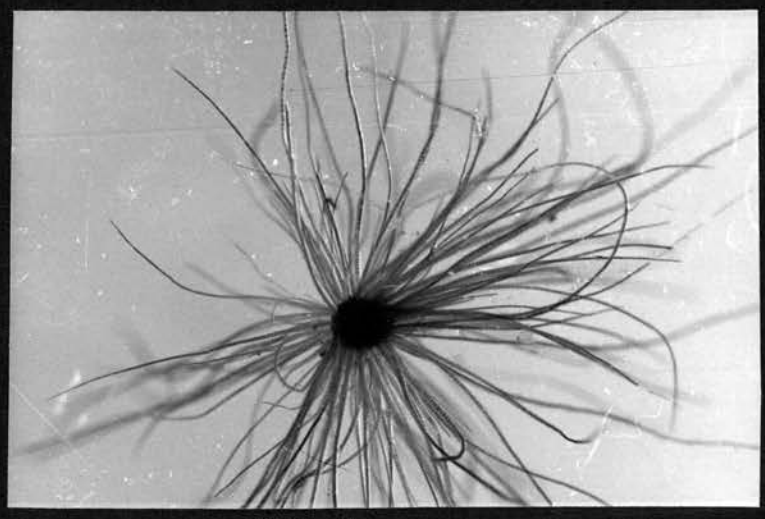

FIG. 58.

PLATE XXVI

PLATE XXVII.

Fig. 59. Camera lucida drawing of unilocular sporangium of Elachista fucicola plant as shown in Fig. 58, showing nuclei in various stages of mitosis.

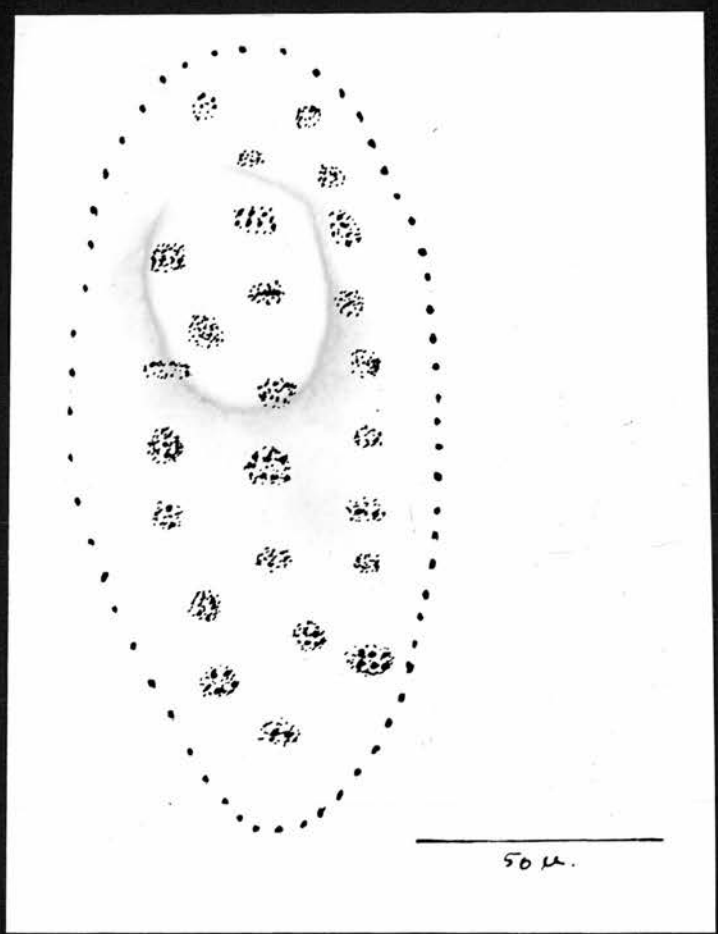

FIG. 59.

PLATE XXVII