# *MELANOPSAMMA RANJANII* SP. NOV.: A NEW PARASITE OF *SELAGINELLA**

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## Introduction

The fungi so far recorded on the various species of *Selaginella* have been listed by Gregor (1938) and by Mitra (1943). The specimen to be described here, differs from all the fungi mentioned in the above lists. It was found growing on living *Selaginella chrysocaulos* in a shaded place in the Lloyd Botanical Gardens, Darjeeling, in the month of September 1938. Even after a very careful search, however, not more than two infected plants could be found. But these infected plants showed the black perithecia of the fungus at the tip of almost every branch and spike, many of which had well-developed micro- and megasporangia (Fig. 1). These fructifications were not found on the branches near the base of the plant nor on other parts, such as stem and leaves. The only other external symptom of these plants was a little drooping of the infected branches. They were green and apparently were not killed by the parasite at the stage at which the material was collected. Unfortunately both the infected plants were preserved in formalin-acetic-alcohol, so that no cultural studies or inoculation experiments could be made. The writer had thus to be content with a study based on teasings and microtome sections of the original material.

## Observations

### *Host-Parasite Relation*

A longitudinal section through the infected tip (Fig. 1) revealed the very interesting feature that the hyphæ of the parasite were present only in the xylem of the vascular bundle. To ascertain the extent of penetration, transverse sections of the stem at various heights were examined. Sections of the stem at the very base did not show infection. The presence of the fungus was first detected in sections about 1·8 cm. below the lowest branch whose tip bore the perithecia. Above this portion every section up to the very top showed the presence of the parasite.

A careful examination of the transverse section of the infected part of the main stem or the branches (Figs. 3 and 4) shows the presence of hyphæ inside the xylem only, the phloem, pericycle, endodermis and cortex being quite free from the parasite. The hyphæ are found

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in quite large numbers in all parts of the xylem, including protoxylem and many of the tracheids are even found to be clogged (Fig. 4). Near the tip of the branches where the xylem breaks up into isolated strands separated by parenchyma, the hyphæ are found inside the xylem as well as the parenchymatic cells, but even here they do not grow into the pericycle or parts of the cortex. At the very tip of the branch, however, all the undifferentiated parenchymatic cells are attacked and this infected part gradually merges into a pseudo-parenchyma formed entirely of fungal hyphæ, on which the perithecia are situated (Fig. 1). The infected tracheids of the stem are continuous with those of the branch trace. The hyphæ also enter the xylem of the leaf trace but do not affect any other part of the leaf (Fig. 1). The growth of the hyphæ inside the xylem evidently interferes with the flow of water and this explains the drooping of the infected branches already referred to. A longitudinal section of the stem (Fig. 5) shows a luxuriant development of the hyphæ within the tracheids. They are fairly thick and frequently show branching, anastomoses and H-pieces.

*Description of the Parasite*

Except the perithecia no pycnidial or other imperfect conidial stages have been found.

*Perithecia.*—At the tips of vegetative shoots or sporangiferous spikes one can see minute globose, carbonous perithecia present superficially in groups of two to five, solitary ones being extremely rare. They are hard, smooth and devoid of hairs. These seem to be situated directly on the tips of branches but a longitudinal section through the infected region shows that they are seated on a small pseudoparenchymatous base which does not form a well-marked external stroma. This pseudoparenchymatic base, comes out with the perithecia if they are separated (Fig. 6 A). The venter of the perithecium is spherical (Fig. 2). There is no beak, but the ostiole is situated as a clearly defined pore in a minute round papilla at the top (Fig. 6 A). Inside the perithecium are asci and paraphyses originating from the basal region and the sides. They are absent in the region of the neck where they are replaced by periphyses. The size of the perithecia varies greatly and sometimes younger perithecia are found attached to the same pseudoparenchymatic base as the mature ones. The mature perithecia range from 209 $\mu$ to 383 $\mu$ in diameter. A perithecium of average size measures about 300 $\mu$.

*Asci.*—The asci are cylindrical or club-shaped. They have got short, tapering stalks and possess slightly flattened bases for attachment (Fig. 6 B). Each ascus contains eight ascospores generally arranged in a single series (monostichous), but here and there some of the ascospores show a distichous arrangement (Fig. 6 B). The asci are hyaline to somewhat translucent and contain oil globules, which come out when teased. The asci are 80–88 $\mu$ long and 12–14 $\mu$ broad.

*Paraphyses and Periphyses.*—Paraphyses are free, persistent, unbranched, non-septate and do not anastomose with each other (Fig. 6 C). They are hyaline to translucent and contain oil globules. The paraphyses are shorter and narrower than the asci, but like them possess a

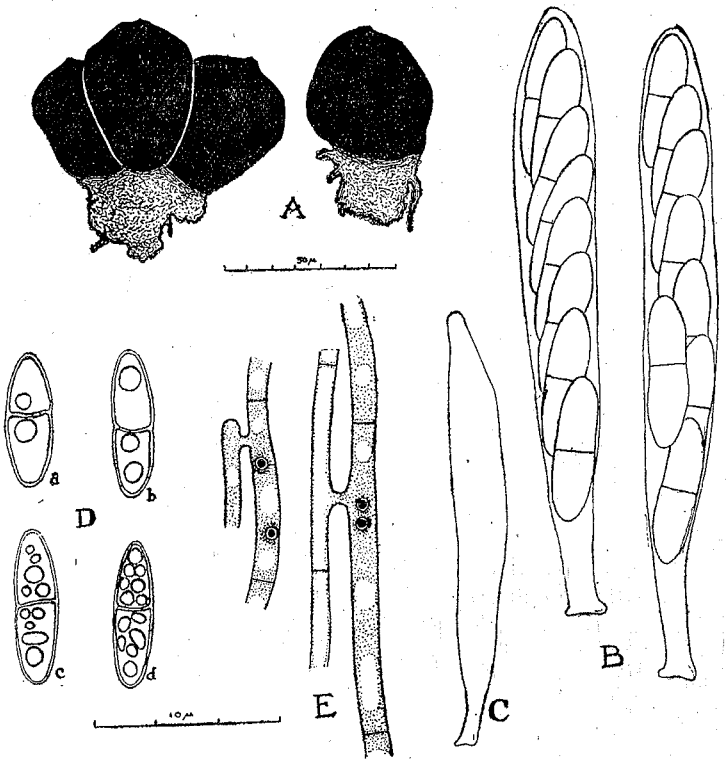



Fig. 6. *Melanopsamma Ranjanii* sp. nov.—Camera lucida drawings of perithecia, asci, etc. Fig. A, Perithecia with basal pseudoparenchymatic mass generally found in groups but in rare cases single. × 64. Fig. B, Asci with monostichous ascospores. × 800. Fig. C, Paraphysis. × 800. Fig. D, Bi-celled ascospores showing stages in maturation and increase of guttulæ in older spores. × 800. Fig. E, Binucleate hyphæ and H-pieces from L.S. of stem in xylem region. × 800.

tapering stalk, a flattened base and a rounded apex. They measure on an average 72 $\mu$ × 10·5 $\mu$. The periphyses never come out of the ostiole.

*Ascospores.*—The ascospores are ovoid to spindle-shaped, sometimes slightly curved, hyaline and bicelled. The equatorial septum divides the spore into two almost equal halves with a very slight constriction in the middle, which may sometimes be absent. Their ends are gradually rounded although in a few cases they are more pointed than usual. At first each cell of the ascospore is uni-guttulate but as it ages the guttulæ increase in number, so that the cells of the mature spore become multiguttulate (Fig. 6 D). This makes the septum difficult to see without proper staining. The ascospores vary from 20 to 28 $\mu$ in length and 7 to 8 $\mu$ in breadth. The average size of the ascospore is 26 $\mu$ × 7 $\mu$.

*Mycelium.*—The loose hyphæ inside the tracheids are branched and consist of elongated cells which are 30–60 $\mu$ long and 2·5–5 $\mu$ broad.

They frequently show anastomoses and H-pieces (Fig. 5). These cells contain a number of vacuoles. The cells near the pseudoparenchymatic base are rectangular to iso-diametric.

*Cytology.*—The mycelium, which shows fusions and H-pieces at various places, shows two nuclei in each cell. These nuclei may lie very close to each other or may be more or less apart (Fig. 6 E). They measure about 2 $\mu$ in diameter and consist of a deeply staining central body with a white halo around it. In a few cases very much elongated nuclei were found. These were probably in the course of division. Youngest perithecia are uniformly pseudo-parenchymatous with outer layers of thicker cells. In slightly older ones the centre is occupied by a mass of deeply staining hyphæ, the cells of which are bi-nucleate. The nuclei in this case were much smaller. No definitive nucleus or other stages in the cytology of the ascus were observed. Each cell of the ascospore contains a single nucleus.

*Identity of the Fungus.*—The superficial, glabrous and unbeaked nature of the carbonous perithecia, which are not situated on a distinct stroma, together with the presence of hyaline, ovoid, bi-cellular spores indicate that this fungus belongs to the genus *Melanopsamma* Niessel (Fam. Sphæriaceæ). The fungus also agrees with the description given for *Melanopsamma* by Saccardo (1887) and by Winter (1887). A large number of species of *Melanopsamma* is known and many of them grow on rather primitive phanerogams, such as the Archichlamydeæ, Gymnosperms, etc. But none has been recorded so far on *Selaginella.* The habitat of the fungus together with the measurements of perithecia, asci, ascospores, etc., show that it is a new species. I have much pleasure in naming it *Melanopsamma Ranjanii* sp. nov. after Dr. Shri Ranjan, Professor of Botany, University of Allahabad. It may be noted here that this is also the first record of *Melanopsamma* on any host in India (*cf.* preliminary note by Mitra, 1943). Neither Butler and Bisby (1932) nor Mundkur (1938) mention anything about this genus. This is rather surprising as species of *Melanopsamma* are well represented in the tropics—a large number having been recorded from the Philippines. Gwynne-Vaughan and Barnes (1922, p. 153) also remark in connection with the Sphaeriales that "there is no doubt that a study of the tropical forms at present very incompletely known, will greatly increase their number."

## Discussion

A great deal of difference of opinion exists as to the limits of the Sphæriales. Petrak (1924) has shown that many species till then regarded as simple Sphæriales really belonged to the Pseudosphæriales, which are related to the Dothideales as their perithecia are in reality unilocular stroma. Many modern writers (Miller, 1928; Theissen and Sydow, 1918) have recognised this differentiation and have given certain criteria for distinguishing between a simple perithecium and an unilocular stroma. According to Miller (1928) the attempt by Petrak (1924) and others to separate these two on the basis of thickness of wall is not fruitful. On the other hand he considers the presence of a true perithecial wall to be a fundamental criterion of a true perithecium,

Chesters (1938) agrees with this. Presence of a true perithecial wall is correlated with other characters. The centre of the perithecium is not pseudoparenchymatous, asci do not ripen in series so that ripe and unripe asci are not found together, and true paraphyses and periphyses occur. If we apply the above criteria we see that *Melanopsamma Ranjanii* possesses true perithecia and so belongs to the Sphæriales. Chesters (1938) in his studies on two species of *Melanomma* came to the conclusion that these two species ought to be placed in the Pseudosphæriales, and opines that "It is probable that species of *Bertia* and *Melanopsamma* will be found to have a similar development to that of *Melanomma* and to belong to the Pseudosphæriales." The present study shows that Chesters' (1938) prediction is not true for all the species of *Melanopsamma.*

*Diagnosis of* Melanopsamma Ranjanii *sp. nov.*

Pērithecia superficialia, in summitate ramorum, gregaria, 2–5 simul, spherica, carbonacea, lævia, ostiolum in minuta papilla, 209–383 $\mu$ diam.; asci cylindrici vel clavati, octo-spori, hyalini, 80–88 $\times$ 12–14 $\mu$; paraphyses clavatæ; sporidia monosticha, ovoida vel fusoidea, apice rotundo, hyalina, uniseptata, multi-guttulata dum matura, 20–28 $\times$ 7–8 $\mu$; mycelia in xylemo caulis hospitis tantum, sed inficientia alias parenchymaticas cellas summitatis, bi-nucleata, cum frequentes anastomoses et H-partes.

*Hab.*—In summitate ramorum et spicis *Selaginellæ chrysocaulis*, Darjeeling, India, September 1938.

Perithecia superficial, at tips of branches, gregarious, in groups of 2–5, spherical, carbonous, smooth, ostiole in a minute papilla, 209–383 $\mu$ in diameter; asci cylindrical or club-shaped, eight-spored, hyaline, 80–88 $\times$ 12–14 $\mu$; paraphyses clavate; ascospores monostichous, ovoid to spindle-shaped, ends rounded, hyaline, uniseptate, multi-guttulate when mature, 20–28 $\times$ 7–8 $\mu$; mycelia only in xylem of the stem of the host but infects other parenchymatous cells at the tip, bi-nucleate, shows frequent anastomoses and H-pieces.

*Hab.*—At the tips of branches and spikes of *Selaginella chrysocaulos* in Darjeeling, India, September 1938.

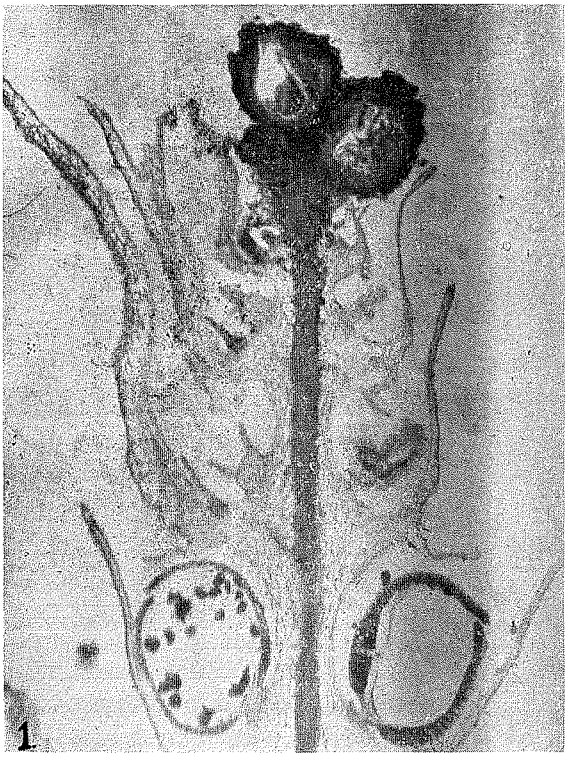
Type specimen deposited in the Herbarium of the Botany Department, Universtiy of Allahabad, India.

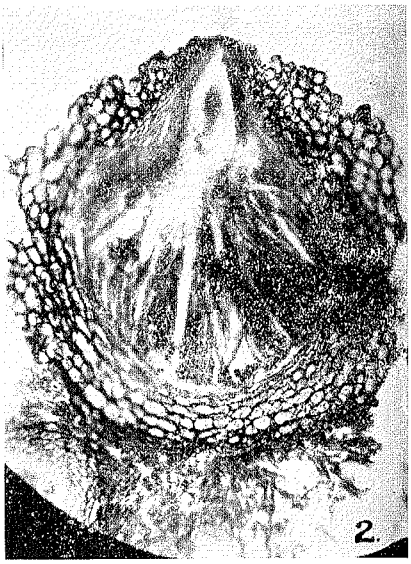
## Summary

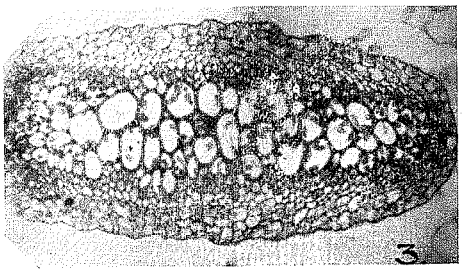
1. *Melanopsamma Ranjanii* sp. nov. is recorded as a new parasite of *Selaginella chrysocaulos*. This is also the first record of *Melanopsamma* on any host in India.

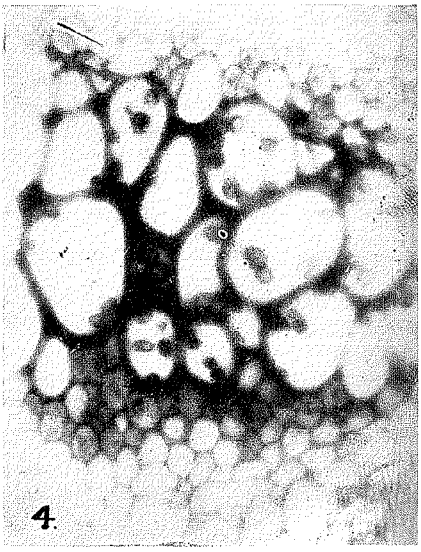
2. The black perithecia occur only at the tips of branches which do not show any other symptom except drooping.

3. In the stem of host, the parasite is present only in the xylem region without affecting other parts. Some tracheids are found to be totally clogged by the parasite. Here many hyphæ showing H-pieces and binucleate cells are found.





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4. At the tip of branches the young parenchymatous cells are attacked and this infected part gradually merges into a pseudo-parenchyma formed entirely of fungal hyphæ.

5. It has been shown that *Melanopsamma Ranjanii* possesses true perithecia and so belongs to the true Sphæriales.

6. A full description of the parasite is given.

In conclusion, the author wishes to express his thankfulness to Professor Shri Ranjan, D.Sc., Head of the Department of Botany and Dean of the Faculty of Science, University of Allahabad, for his valuable suggestions and for kindly going through the slides and manuscript. The author is also indebted to Father Jerome of St. Joseph's Seminary, Allahabad, for help in translating the diagnosis into Latin.

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## EXPLANATION OF PLATE

*Melanopsamma Ranjanii* sp. nov. on *Selaginella chrysocaulos*

Fig. 1. L.S. through tip of an infected spike with mature sporangia of the host, showing terminal perithecia, affected conducting strand of stem and leaves.

Fig. 2. L.S. through a perithecium showing asci, paraphyses, wall and spherical venter.

Fig. 3. T.S. through the vascular bundle of the stem in the infected region showing hyphæ in xylem.

Fig. 4. Hyphæ in xylem. Note their absence in phloem and pericycle.

Fig. 5. L.S. of the stem showing anastomosing hyphæ in xylem.