APOMICTIC DEVELOPMENT IN BRYOPHYTES OBSERVED IN VITRO CULTURES

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Abstract: This study concerns the apomictic development of six bryophytes which has been carried out in a series of experiments in vitro under a controlled enivronment. It revealed that the filamentous gemmae of both *Cyathophorella japonica* and *Trachyloma indicum* could produce protonemata and leafy gametophytes in aseptic cultures, in about 4 weeks. *Schistochila formosana*, a hepatic, generally reproducing by spores could also produce filamentous gemmae and from their tapering ends protonema was produced and thenceforth leafy buds. The seta of *Trematodon longicollis* and *Funaria hygrometrica* possess a high potential for developing apomictic gametophytes, so do the leaves of *Pottia recta* and *Funaria hygrometrica* and the calyptra of *Trematodon*. Products of these asexual bodies underline the capacity for apomixis, carrying with them their specific characteristics which are not easily changed by environmental factors. Actually no marked difference was found between the apomictic gametophytes as compared with those developed from spores.

Leafy gametophytes of *Funaria*, *Trematodon* and *Pottia* were also cultured from spores for the purpose of making comparisons between those produced by apomixis and by the normal method.

The apomictic development observed in the above 5 mosses and one hepatic is found to be the first report for these species in science.

INTRODUCTION

Bryophytes, in general, reproduce by means of spores. However, in this study, it was found that apomictic gametophytes were developed by six bryophytes, either by gemmae or by parts separated from the parent plants. Filamentous gemmae in *Cyathophorella japonica* Broth. and *Trachyloma indicum* Mitt. as well as *Schistochilla formosana*, Horik. a hepatic from Ali-shan, were found to be all alike in their early protonema stages. But in their later stages, buds of different types were formed. In *Cyathophorella japonica*, a large, many-celled bud was initiated from the protonema which gave rise to young leaves and thence leafy gametophytes; while in *Trachyloma indicum* the protonema gave rise to a ribbon-like "frondiform" stage which in turn initiated primordial leaves (Pl. II), and finally proceeded to a leafy gametophyte stage. From this difference one might postulate that there is a phylogenic relationship between this moss and *Tetraphis pellucida* which also forms "frondiform" structures in their gametophyte development[12].

As to the culture of the parts separated from parent plants in this study, high degrees of apomixis was evident. Their resulting gametophytes either reached

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mature stages of leafy gametophytes as shown in the seta cultures of *Trematodon* and *Funaria*, or were maintained in protonemata stages as were the leaf cultures of *Pottia* and *Trematodon*.

Experimental bryophytes have frequently been studied in recent years. However, the findings in this study are the first time reports for the following: (1) Apogamic development from filamentous gemmae of *Cyathophorella*, *Trachyloma* and *Schistochila*. (2) Seta cultures of *Trematodon* and *Funaria* resulting in leafy gametophytes. (3) Leaf cultures of *Pottia* and *Funaria* giving rise to protonema. (4) Calyptra culture of *Trematodon* resulting in a protonema stage. These add much knowledge to our understanding of the developmental phases of these bryophytes.

MATERIALS AND METHODS

Materials for this study include a random group of bryophytes:

Name	Locality	Collection date	Collectors & Nos.
1. *Cyathophorella japonica* Broth. (Hypopterygiaceae)	Wu-lai, Taipei Co. Growing on damp rock, mixed with other mosses.	Oct. 10, 1969	Liu, Chen, Kao (6012)
	Pei-cha-tien-shan, Taoyuan Co., On boulder, in partial shade.		Lai (1001)
2. *Trachyloma indicum* Mitt. (Pterobryaceae)	Pei-cha-tien-shan, Taoyuan Co., epiphytic on tree trunk.	Nov. 14, 1970	Lai (1002)
3. *Trematodon longicollis* Michx. (Dicranaceae)	Ali-shan, Chiayi Co.	Nov. 1969	Liu, Chen, Feng (9006)
4. *Funaria hygrometrica* Hedw. (Funariaceae)	Ali-shan, Guest House, on soil.	Nov. 1969	Liu, Chen, Peng (9005)
5. *Pottia recta* Mitt. (Pottiaceae)	NTU campus, on soil.	Nov. 1969	Chang (8005)
6. *Schistochila formosana* Korikawa (Schistochilaceae)	Ali-shan on way to Hostel partial shaded slope.	Nov. 1969	Liu and others (8016)

Media: (Liquid) 50% Hoagland's solution (22).
(Semi-solid) Hoagland's agar medium—50% Hoagland's solution and 1% agar add 0.25% activated charcoal.

Methods:

1. **Gemmae cultures**—filamentous gemmae were taken from the attenuated tips of *Cyathophorella japonica* (Pl. I, 2) and *Trachyloma indicum* (Pl. II, 8) with sterilized forceps. Having been washed several times in sterilized distilled water they were planted in separate sterilized Petri dishes and kept covered. The gemmae culture of *Schistochila formosana* was prepared as above. All the above prepared cultures were placed in a controlled environment: Temperature 20°C±1, at 9hr light diurally and intensity of illumination about 1200 lux. After profuse protonemata were developed, the cultures were transplanted onto Hoagland's agar medium for further growth.

2. **Seta cultures**—seta of *Trematodon longicollis* and *Funaria hygrometrica* were selected for use from fresh specimens. After being washed in sterilized distilled water, they were cut into 3 segments (basal, middle and apical), and planted into respective Petri dishes with 50% Hoagland's solution and stored in a controlled environment as above.
3. **Calyptra cultures**—available calyptra still intact on fresh *Trematodon* were selected for this expeiment, after being cleaned in sterilized distilled water, planted in 50% Hoagland's solution and stored at the same controlled conditions as before.
4. **Leaf cultures**—healthy leaves selected from *Pottia* and *Funaria* were planted in agar medium with 0.25% activated charcoal. Cultures thus prepared were placed under the same controlled environment as above.
5. **Germination of spores**—available spores of *Funaria*[20], *Pottia* and *Trematodon* were harvested from mature capsules. The whole capsules with seta intact were immersed in distilled water and finally rinsed in sterilized distilled water. One capsule was placed in 50% Hoagland's solution in a Petri dish, then crushed open in water so as to avoid contamination. The Petri dishes were covered and placed under a controlled environment as before.

OBSERVATIONS AND RESULTS

1. **Gemmae cultures**—gemmae of *Cyathophorella japonica* are filamentous in structure. Its growth was by branching which occurred about one week after planting (Pl. I, 4, 5, 6) then budding followed in about 24 days. Branches usually originated from the upper portion of a protonema cell (Figs. 9, 6), and buds often protruded from the main filaments, rather than from the branches; generally these came from near the septa of a cell. The cells in the bud are large and aggregated somewhat like a strawberry (Fig. 7), but later the bud elongated and leaf primordia began to differentiate at its growing apex (Fig. 9). The size of the bud is much larger than that produced in *Funaria hygrometrica* or *Pottia recta* (Pl. IV, 4 from *Funaria* seta culture; or Pl. V, 5 from *Pottia* spore germination). Gemmae grow faster in Hoagland's solution than on agar medium. But when the growth of the protonema attained a certain stage, transplants from liquid to agar medium seemed to be necessary in order to induce further development, especially for the formation of a leafy gametophyte. The young apomictic gametophyte has 3 ranks of leaves, almost equal in size, while in wild type gametophytes, they are unequally developed (Pl. I, 1), the 2 lateral rows being equal in size and laterally spreading, but the 3rd row of leaves are much smaller, being hardly visible to the naked eye. The filamentous gemmae of *Trachyloma indicum* are likewise borne on the club-like tips of upper branches; they appear yellowish-brown under the microscope. Pl. II, Fig. 2

shows three filamentous gemmae, in dark color, sending out green protonemata from both ends as observed in a two-week culture. Its bud initiation (Pl. II, 3) being the same as seen in *Funaria* or other bryophytes (Pl. IV, 3), they mostly occurred near a septum. Leafy buds, however, were not observed but instead a ribbon-like frondiform-structure was found arising singly or in groups (Pl. II, 4, 5, 6, 7). These gave rise to primordial leaves and finally developed the leafy branches of the mature gametophyte (Pl. II, 6, 7, 8). This frondiform-stage resembles the frondiform stage in *Tetraphis pellucida* described by Schneider and Sharp[12], and that in *Schiffneria viridis* by Yang 1966[21] except that the former is cylindrical in shape while the latter two are more or less flat. *Schistochila formosana* produced filamentous gemmae on the tips of apical leaves when fresh specimens were kept in controlled conditions for several months. Each gemma consists of 3–7 cells with the end cells tapering, where the protonema usually developed (Pl. III, 1, 2). Pl. III, fig. 3 showed a culture of profuse protonemata produced from these gemmae about 4 weeks in culture. However, they remained in the same stage for a long period and no further changes were observed thereafter.

2. **Seta cultures**—The seta of both *Trematodon longicollis* and *Funaria hygrometrica* produced aposporous gametophytes rather successfully in this study. The apical segments of the seta showed better results in protonema growth than those from the middle or basal parts. Tufts of protonema usually appeared in about 10 days after planting, at the upper end of the apical segment (Pl. III, 5). Fig. 4 of Pl. III is a mature aposporous gametophyte in the midst of an abundant growth of protonema which originated from *Trematodon* seta. The experiment on *Funaria* seta exemplified a more complete series of development, most of which had been reported in a previous paper, Yang 1970[23]. In this study five more figures are added to our knowledge of the potential of *Funaria* seta for aposporous development. Prior to the appearance of the protonema in the seta culture, the first change observed was the enlargement of the cut end, and then the expansion of the cut surface as is shown in Pl. IV, Figs. 1, 2. An examination of a part of the protonema thus developed, as seen under a microscope, shows the cross and oblique septa and occasionally a bud initial protruding from the wall of a protonema cell, usually close to an oblique septum (Pl. IV, 3). When this bunch of protonema was subjected to 9 hr exposure of red light, several changes occurred: a gradual increase in the width of the protonema filament, its surface became roughened and papillate, and finally proceeded to the formation of a bud which consisted of a clump of small, transparent cells (Pl. IV, 4). The surface of the bud was not smooth, as if the outer cells were piled up in a clump. One or more basal cells of the bud was connected to the

protonema and immediately below it, there arose rhizoids, while 3 cells at the top of the bud began to extend and unfold as leaf primordia—which later developed into 3 ranks of leaves (Pl. IV, 5) as is usually found in a mature leafy gametophyte of spore origin. Figures showing the developement of *Funaria* spore germination is represented in Pl. IV, 6. Besides their differences in origin, there is no other obvious morphological difference between them, as observed in this study.

3. **Calyptra cultures**—the calyptra of *Trematodon* after 2 weeks in culture in Hoagland's solution, showed that they were able to grow and develop into protonema from its surface cells but they did not proceed any further—this may have been due to the lack of nutrition from the calyptra cells.
4. **Leaf cultures**—leaves of *Pottia* and *Funaria* were selected for this experiment. It was found that among the 60 *Funaria* leaves planted on the agar medium, only a few showed some signs of life, but they did not maintain it long enough to show any development. However, *Pottia* leaves did live and produce tufts of protonema from the marginal cells (Pl. V, 4). The cells from the costa however, did not show much sign of regeneration. This agrees with what Schneider and Sharp reported[12].
5. **Spore germination**—when spores of *Trematodon, Pottia* and *Funaria* were sown in 50% Hoagland's solution, they germinated readily in about a week[13]. The results obtained from *Funaria* has been reported previously[23]. Spores of *Trematodon* did not develop further than just a few protonema. This may be due to the age of the spores and time of maturity, or other unknown factors. Pl. V, 1, 2, showed mature leafy gametophytes of *Pottia* developed in culture with their protonemata still attached. The bud formed in this culture (Pl. V, 5) appeared to have the same morphological features as that in *Funaria* or that of other mosses.

DISCUSSION AND CONCLUSION

1. Apomictic development of 6 bryophytes, *Cyathophorella japonica* Broth., *Trachyloma indicum* Mitt., *Schistochilla formosana* Horik., *Trematodon longicollis, Funaria hygrometrica* Hedw. and *Pottia recta* (Sm.) Mitt. have been investigated in vitro cultures under controlled conditions.
2. Sporophytes of *Cyathophorella japonica* have been reported previously but the two collections used in this work were fruitless, bearing only filamentous gemmae. Newly collected epiphytic *Trachyloma indicum* were also found to have filamentous gemmae only on their branch tips. *Schistochila formosana*, a leafy hepatic, ordinarilly reproduces by spores but the present material, when kept in a culture room under controlled conditions for certain lengths of time likewise, produced filamentous gemmae on their leaf apices.

3. It has been found that the gemmae of both *Cyathophorella* and *Trachyloma* possess potentials for apomictic development in artificial culture. The former produces cylindrical gametophytes with leaves radially spaced on the stem unlike those grown in nature, with flat features. The cause for this change is not known. The occurrence of a "frondiform" stage in the life cycle of *Trachyloma* is a distinct character. It may indicate a phylogenetic relationship between *Trachyloma indicum* and *Tetraphis pellucida* whose life cycle also involves a "frondiform" structure reported by previous workers[12].
4. It is interesting to note that *Schistochila formosana*, being a leafy hepatic has undergone the same course of gametophyte development with that of *Cyathophorella japonica*, a hepatic-like moss. Superficially, there is a resemblance between these two bryophytes—the plant body is somewhat flat, bearing two lateral ranks of leaves, and the size, and the general features of their plant bodies are similar. Finally their power of developing apomictic gametophytes by means of filamentous gemmae is also similar. We may be justified in considering their position as intermediate between mosses and liverworts.
5. The seta cultures in this study gave successful results. Both seta of *Trematodon longicollis* and *Funaria hygrometrica* develop aposporous gametophytes, completing their leafy gametophyte stage. The leaf and calyptra cultures proved to have power of apomictic development to a certain extent. But their failure in reaching the leafy gametophyte stage may be due to the scanty nutrition contained in the cells of the calyptra and leaves.
6. It was found that the 9 hr red light exposure stimulated the bud formation in *Funaria* protonema development, but it inhibited the growth and development of the bud, if the same exposure was continued. Besides, red light did not bring any effect on the growth and development of *Trematodon* culture.
7. This experimental study on apomictic development in the concerned bryophytes, adds somewhat to our limited knowledge of the bryophytes.

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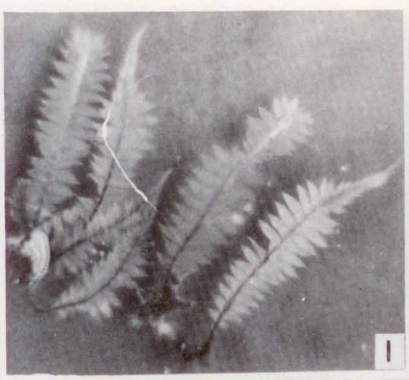
Plate I

Cyathophorella japonica Broth. (Figs. 1-10)

1. *Cyathophorella japonica* in habit ×1.5.
2. Ditto, a plant tip bearing filamentous gemmae ×6.3.
3. Gemmae separated from a parent plant ×40.
4. Gemmae branching ×100.
5. Protonemata developed in culture, 2-week old ×40.
6. Portion of protonema with septate branches ×375.
7. Bud formation and origin of branches, 3-week old ×375.
8. A young leafy gametophyte developed from protonema, showing 3 rhizoids extending downward ×40.
9. An older bud (2-3 weeks old) showing initiation of primordial leaves ×375.
10. Same as 8 (2-3 weeks old), showing 2 leafy buds in culture ×62.
11. Same as 8 (2-3 weeks old) ×100.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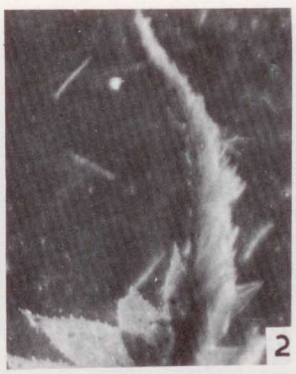
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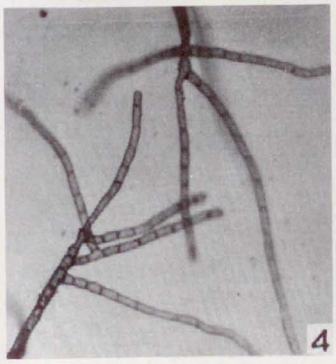
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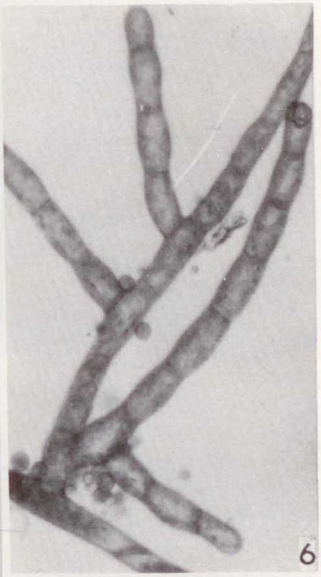
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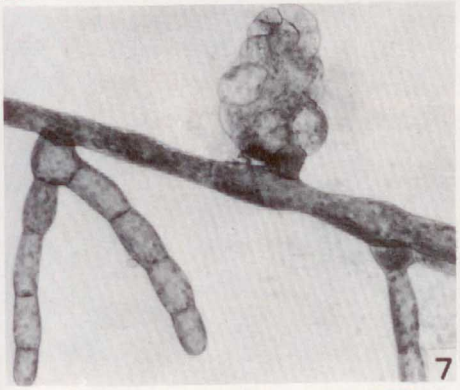
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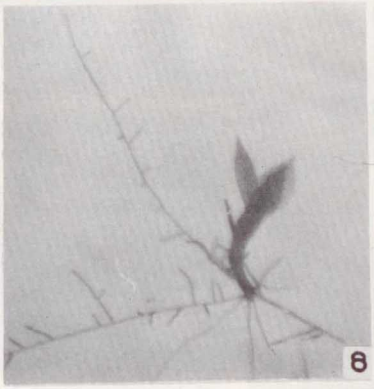
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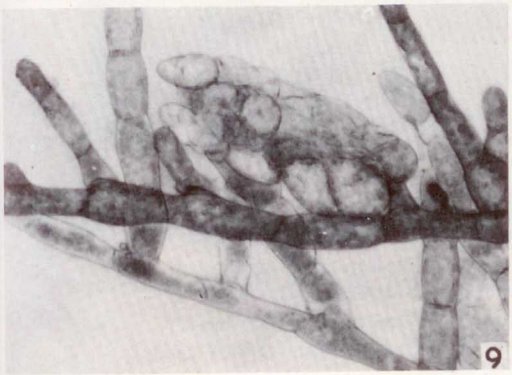
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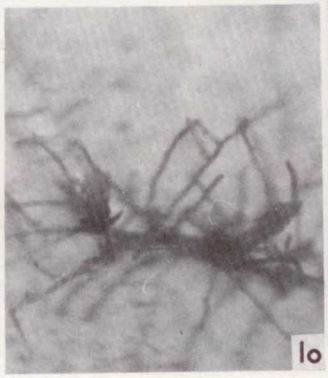
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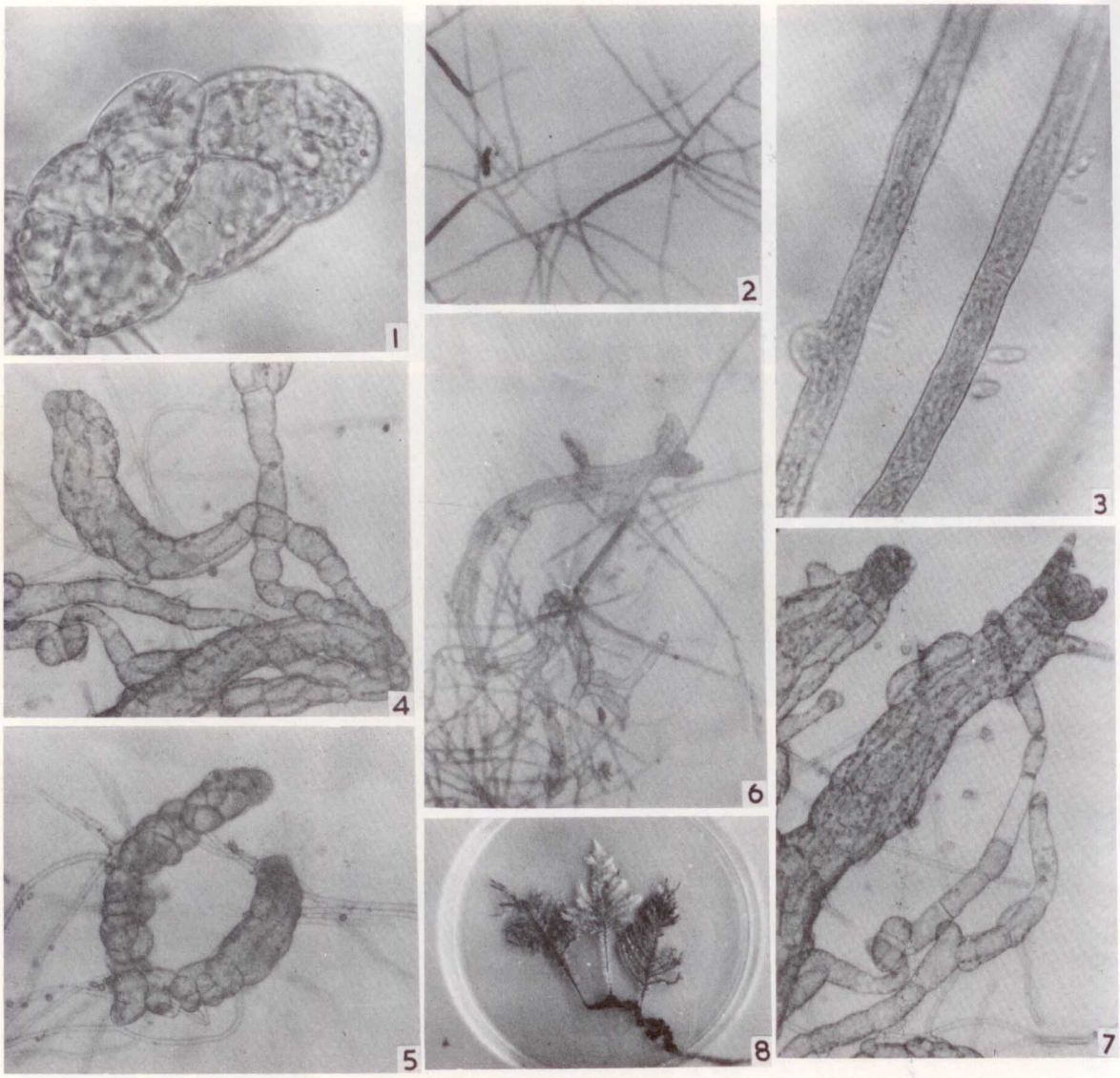

Plate II

Trachyloma indicum Mitt. (Figs. 1-8)

1. Tip of a frondiform ×1000.
2. Filamentous gemmae in dark brown and their green developing protonema, two weeks old ×100.
3. Origin of a branch, note the septa on the protonema ×100.
4. Frondiform structures in various stages, showing rhizoids arising from some surface cells ×250.
5. Two frondiform structures developed from protonemata and colorless rhizoids of smooth type ×250.
6. Ribbon-like frondiform structures developing leaf primordia ×250.
7. Frondiform showing apical bud with leaf primordia ×250.
8. In habit, with gemmae borne on the tips of branches ×0.5.

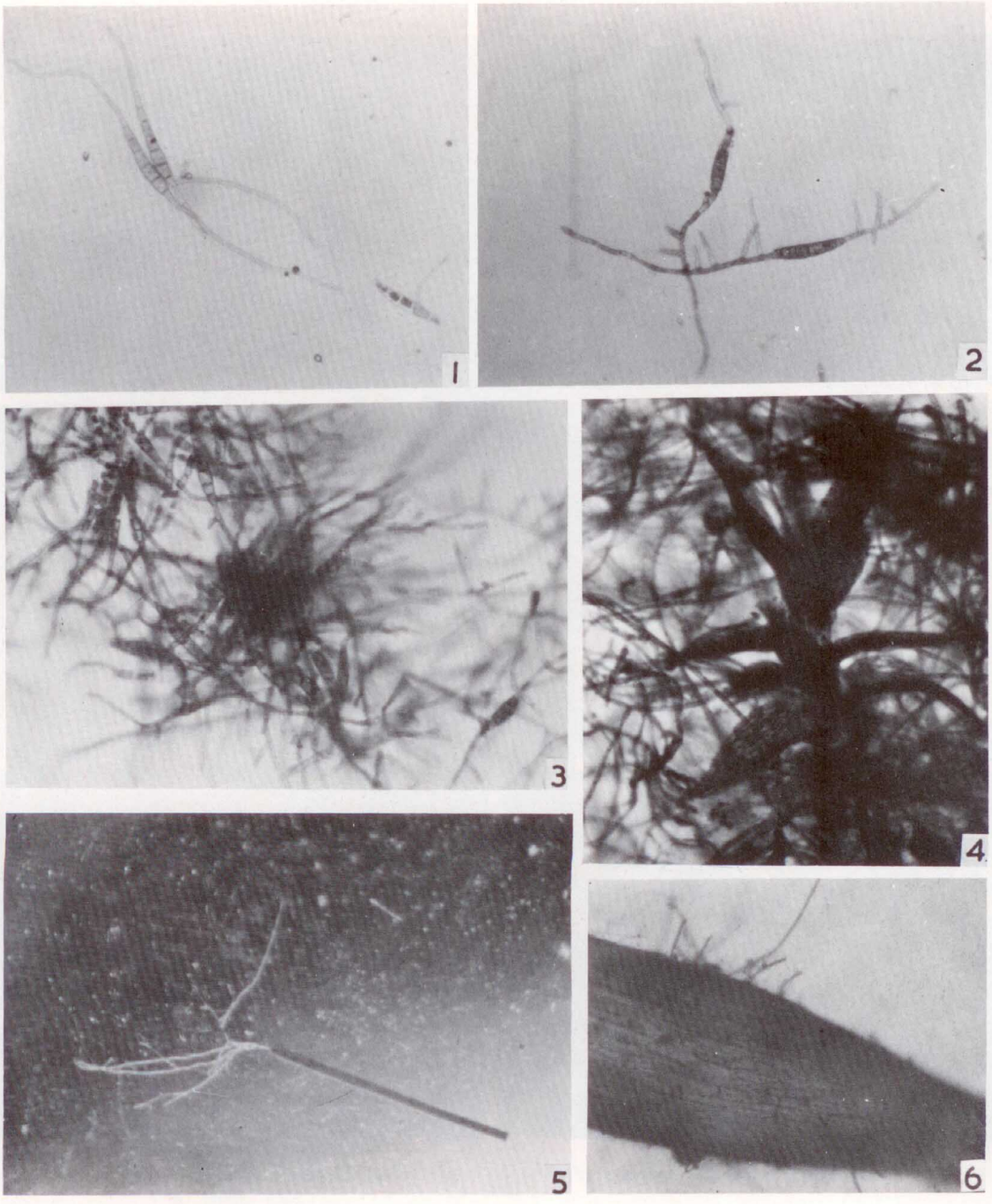

Plate III

Schistochila formosana (Figs. 1-3)

1, 2. Two filamentous gemmae with tapering ends and developing protonema ×100.

3. Ditto with profuse protonema ×100.

Trematodon longicollis (Figs. 4-6)

4. Young leafy gametophyte developed from *Trematodon* seta ×40.

5. Protonema developing from one end of a segment of *Trematodon* seta ×40.

6. Protonema developed from a Trematodon calyptra ×100.

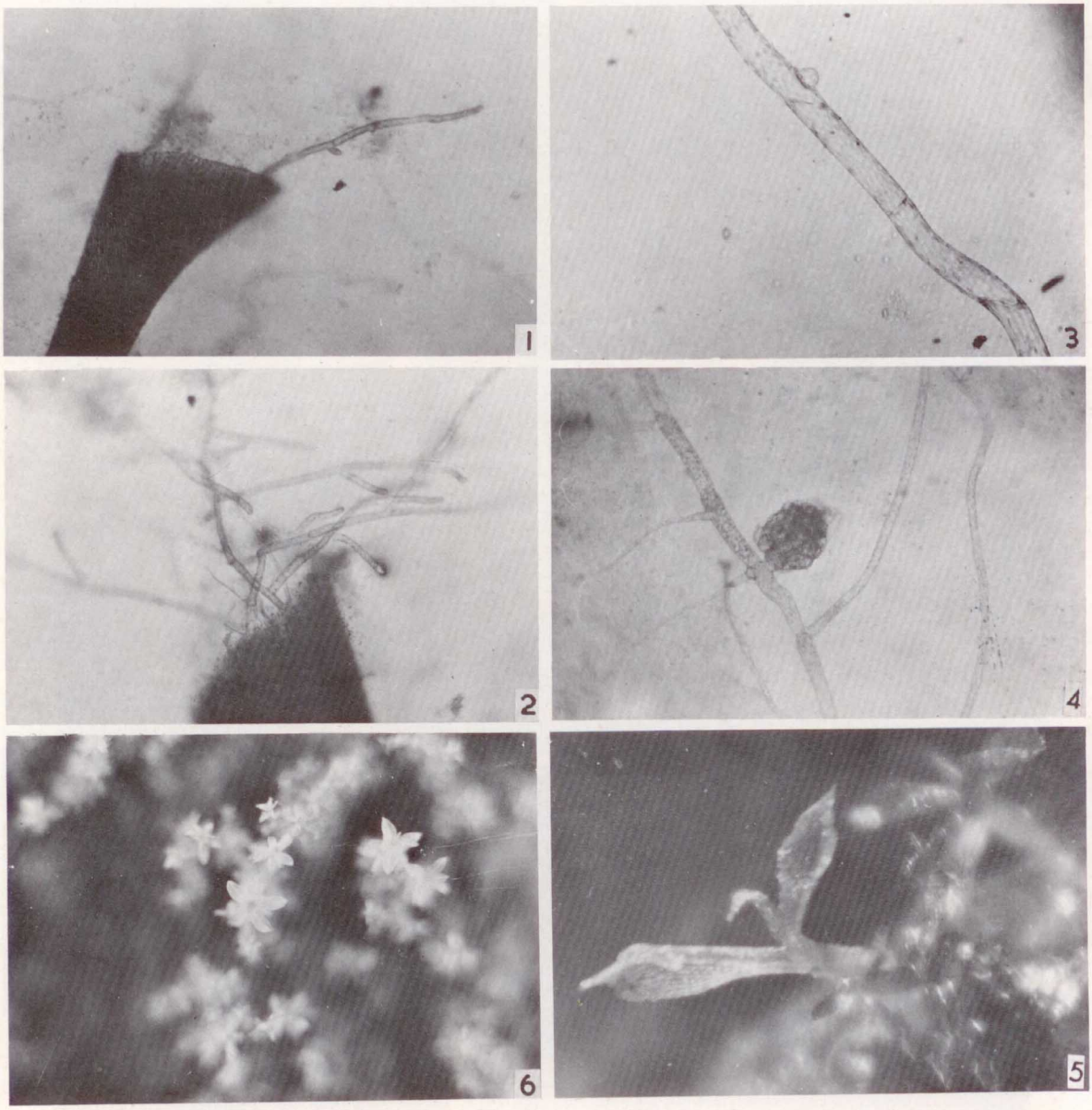

Plate IV

Funaria hygrometrica Hedw. (Figs. 1-6)

1. A segment of seta showing protonema from the expanded cut surface ×100.
2. Ditto ×100 (12 days after planting in 50% Hoagland's solution).
3. Showing oblique septum and initiation of a young bud ×250.
4. The 1st bud observed after 9 hr exposure of red light for 5 days ×250.
5. Leafy gametophyte produced from seta protonema, a culture on agar medium ×25 (Photo taken with Olympus steroscopic microscope Model X-Tr and adapter PM 7).
6. Leafy gametophytes produced from spore germination on agar medium ×2 (Photo taken with Asahi Pentax with extension tube No. 1).

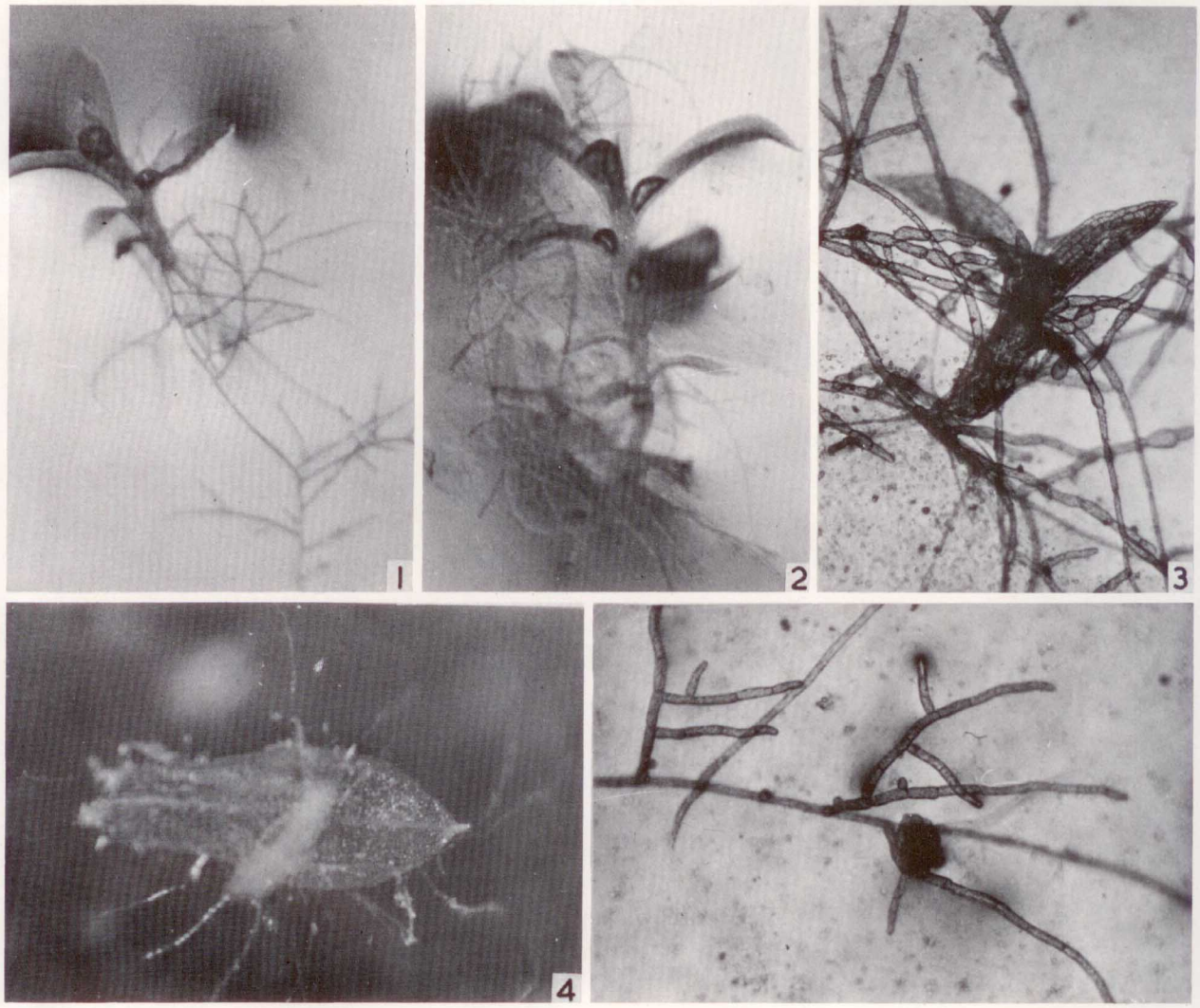

Plate V

Pottia recta (Figs. 1-5)

1. *Pottia recta*, protonema and gametophyte developed from spore germination ×40.
2. Ditto ×40.
3. Ditto ×250.
4. Protonoema developed from *Pottia* leaf ×100.
5. *Pottia* protonema and bud from spore germination ×250.