STUDIES ON SPORE GERMINATION AND GEMMAE DEVELOPMENT OF *RICCARDIA MULTIFIDA* (L.) S. F. GRAY, *DUMORTERIA HIRSUTA* (SW.) REINW., BL. ET NEES, *XENOCHILA INTEGRIFOLIA* (MITT.) INOUE AND *MARCHANTIA POLYMORPHA* L.

BAO-YU YANG and FANG-MEI HSU

I. INTRODUCTION

This is one of the series of studies on the germination of spores and the cultivation of gemmae of bryophytes. The purpose of such studies is to investigate the possible phylogenetic relations among Hepaticae by comparing their early stages of development either through the germination of spores or the cultivation of their gemmae. All experiments described in this paper have been conducted in a controlled laboratory from the spring of 1966 to summer of 1967.

Previous studies on the germination of spores and cultivation of gemmae include: On Spore Germination of *Schiffneria virides* Steph.[21], The Ontogeny of the Gemmae of *Hyophila Tortula*[22] and The Geographical Distribution and Growth Habits of *Haplomitrium*[23]. This paper reports the results of the investigation on the germination of spores and gemmae development on four Hepaticae, namely *Riccardia multifida, Dumorteria hirsuta, Xenochila integrifolia* and *Marchantia polymorpha.*

II. MATERIALS AND METHODS

Most of the materials for this study were collected by the authors themselves and several other members of the Botany Department of National Taiwan University from different localities:

Riccardia multifida from Yang-ming Shan on March 15, 1966.

Dumorteria hirsuta from Wu-lai, on May 1, 1966.

Xenochila integrifolia from Chi-tou, on Oct. 25, 1966, by Dr. Inoue and Miss Hsu.

Marchantia polymorpha from Ali Shan on Oct. 26, 1966 and March 15, 1965.

The nutrient solution used in this study is mainly Hoagland's solution, though Knop's solution was used on a few occasions but did not show good results.

Spores of *Riccardia multifida* and *Dumorteria hirsuta* were taken from respective capsules which had been sterilized in 0.1% bleaching solution before they were sown in 50% Hoagland's solution and Knop's solution in sterilized petri dishes, which

in turn were placed in the controlled laboratory under constant light exposure, about 1200 lux, and at 20°C.

Gemmae of *Xenochila integrifolia* were taken from the apices of scale-like small leaves of gemmiparous shoots, and germinated readily in 50% Hoagland's solution.

Gemmae of *Marchantia polymorpha* taken from gemma cups were planted on filter paper supported by glass rods, 5 mm. in diameter and the nutrient medium 50% Hoagland's solution was added just sufficient to cover the gemmae in the solution. Solutions were changed at intervals of 3–4 weeks, accompanied by a thorough wash of the gemmalings in distilled water in order to keep them from contamination and from deterioration or decay. Other details of this culture were based on the methods given in Voth *et al.*[21]. The technique for the harvest of gemmae from gemma cups was after the method described by Miller[12].

III. OBSERVATIONS AND RESULTS

1. *Riccardia multifida* (L.) S. F. Gray—

Spores, yellowish brown and spherical about 15 μ in diameter, were sown in Knop's solution on March 28 and April 1, 1966. The early development from the germinating spores consists of a filament of four cells by successive divisions in one plane (Pl. I, Figs. 1a, 1b). Subsequently from the filamentous protonema, there arises a thalloid protonema by vertical divisions of the cells of the filament at right angles to the first three divisions (Pl. I, Figs. 1c, 1d). Nehira observed that later development of the filamentous protonema much resembles to that of *Schiffneria viridis*[15][24], but in the present study the Schiffneria type of protonema has not been found. In general, the germination procedure of sporelings agrees with what Nehira reported, but it has been further found, the time required between the filamentous stage and the thalloid was about a month. Further development of the thalloid stage, however, has not been able to follow due to bacterial contamination.

The gemmae of *Riccardia multifida* (L.) Gray are green, two-celled, ellipsoidal bodies (Pl. I, Fig. 2a) arising from the margins of the thalloid gametophyte. As soon as three days after sowing of the gemmae in 50% Hoagland's solution one of the cells became enlarged (Pl. I, Fig. 2b) and then divided into 2 cells and the other cell of the gemma began to divide likewise; consequently, in 3 to 7 days, the 4-celled and the 8-celled stages toward the formation of thalloid protonema were observed (Pl. I, Figs. 2c, 2d), and in 10 to 14 days a multicellular thalloid gametophyte resulted (Pl. I, Figs. 2e, 2f) by repeated divisions of the cells in 2 planes.

2. *Dumorteria hirsuta* (Sw.) Reinw., Bl. et Nees—

The spherical spores of *D. hirsuta* measuring about 22–28 μ in diameter, are ornamented with dark, distinct, reddish papillae (Pl. I, Fig. 3a). When treated

in 50% Hoagland's solution and also 50% Knop's solution, they protruded a long, filamentous germ tube (Pl. I, Figs. 3b, 3c). Soon, the chloroplasts richly supplied in the tube migrated toward the tip as the tube elongated. At the tips of the elongating tubes, now filled with chloroplasts, cell divisions take place. At first two transverse divisions occurred (Pl. I, Fig. 3d) followed by vertical divisions at right angles of the two terminal cells (Pl. I, Fig. 3e), thus assuming a thalloid sporeling which is the typical pattern of the Marchantiales[3][5].

3. *Xenochila integrifolia* (Mitt.) Inoue—

The multicellular gemmae of *X. integrifolia*, (each with a unicellular stalk elliptical in outline) with numbers of cells varying between 20 to 40 were planted in 50% Hoagland's solution; they germinated immediately. It took about 20 days to complete the development of a young leafy gametophyte from a compound gemma. The primary growth initiated from one of the cells in the gemma was observed on the next day after planting (Pl. II Figs. 2, 3p).

The rhizoids are nonseptate and arise from the ventral surface of the leaves. They extend rapidly in the culture in 2-4 days (Pl. II, Figs. 4, 5, 6). In 18 days leaf primordia began to develop at one end while rhizoids still are elongating from other cells (Pl. II, Fig. 7).

The formation of a young leafy gametophyte with leaf primordia and young leaves growing at the apex is shown in Pl. II, Figs. 8, 9. Finally, the completion of a well developed mature plant with leaves broadly inserted in about 20 days, is illustrated in both Pl. II, Fig. 8 and Pl. III, Fig. 6. Underleaves were not developed in the present specimen. All the above characters agree exactly with plants growing in the wild.

4. *Marchantia polymorpha* L.—

The gemmae of *M. Polymorpha* grow readily in a moist chamber when supplied with appropriate nutrient solution. The experiment described here was conducted in a controlled laboratory since spring of 1966. New growth initiated from either of the two opposite notches or from the sides of the gemmae.

The rhizoids appeared first ventrally from some of the cells of the compound gemmae. It took only about two weeks for the ribbon-like gametophyte to develop into the characteristic form with numerous rhizoids of both types, smooth and tuberculate, tangled in culture[3].

The gemmalings of several weeks old in the laboratory culture appeared green and slender, some of them vertical in position instead of being prostrate. No sexual growth was observed in all the gemmalings grown in the culture. However, gemma cups with gemmae developed within them were found on two gemmalings; these gemmae, when cultivated in nutrient solution also gave rise to normal gemmalings and gametophytes vegetatively. Patches of gemmalings were found de-

veloped at apical portions of several ribbon-like gametophytes. Obviously they were produced from gemma cups while the latter withered away.

Internal anatomy revealed a slight disorganization of the tissues while epidermal pores, typically with 4 surrounding cells, were easily recognized. Ultra violet radiation was applied to the gemmae of *M. Polymorpha* and to a related species, *Reboulia hemisphaerica* (L.) Raddi. The results will be reported in a separate paper.

IV. DISCUSSION AND CONCLUSION

In the present study, the germination of spores and the cultivation of gemmae of four Hepaticae, *Riccardia multifida, Dumorteria hirsuta, Xenochila integrifolia* and *Marchantia polymorpha* were studied and described.

1. *Riccardia multifida* is a delicate thalloid hepatic whose reproduction is carried out sexually in the production of spores and asexually in the production of gemmae.

   The present study revealed that the protonema of *R. multifida* has two types of patterns, the filamentous and the thalloid. Nehira, however, observed only the filamentous protonema in the sporelings of *R. multifida,* and he did not mention about the thalloid protonema of the gemmalings. Furthermore, it was previously reported that the large thalloid protonema was found in *R. pinguis,* and the smaller filamentous protonema in three other species: *R. multifida, R. sinuata* and *R. nagassakiensis*[18]. The authors, however, found that both filamentous and thalloid protonema occurred in the sporeling development, while the gemmalings gave rise directly to thalloid only, skipping entirely the filamentous stage.

   Fulford[4] pointed out that the sporeling pattern in hepaticae is constant within a genus, probably a family and she also recognized the exceptions in Riccardia. The present authors further verify that the exceptional condition involving the occurrence of both filamentous and thalloid stages in the development of sporelings while the gemmaling development of the same species giving rise to thalloid protonema. Both of these exceptional conditions are reported as the first time in this study.
2. In *Dumorteria hirsuta,* a germ tube protrudes out from the spore upon its germination. It is from the tip of that elongated tube that a thalloid gametophyte develops, very similar in form to the sporeling of *Ricciocarpus natans*[3]; this tube formation is probably typical in the members of Marchantiales. The dark red papillae distributed on the surface of the spore is another special character of this species. Gemmae were not found in this species.
3. In *Xenochila integrifolia,* no sexual growth was observed in the present study. It reproduces mainly by large numbers of compound gemmae; each cell of the

gemma is able to develop into a new plant. The rapid development of the gemmaling into a mature gametophyte is successfully demonstrated in the present work. The experiment has been repeated with similar results. Pl. III, Fig. 6 shows the well-developed gametophyte with the gemma still attached at its base. Plants with spore-bearing sporophytes have rarely been collected. *Hyophila tortula*, a gemmae producing moss, reproduces similarly by gemmae, probably not by spores.

4. *Marchantia polymorpha*—In the ontogeny of gemmae, the ribbon-like thallus became elongated, thin and light colored. When the thallus produces gemma cups, it is found to be deep green, thick and healthy. No sexual reproduction ever occurred in any of the cultures.

V. SUMMARY

1 The thalloid *Marchantia polymorpha* reproduces sexually by spores and vegetatively by gemmae. The gemmalings usually develop from two notches situated at the opposite ends. But the present study revealed that they may also develop from the sides between or near the notches. All the gemmalings are thalloid in pattern, no filamentous ones have been found in the present experiment.

2. The sporeling development of *Dumorteria hirsuta* is distinguished by having a long germ tube protruding out from a germinating spore; immediately from the tip of this germ tube a mature gametophyte is developed. This type of sporeling is similar to that of *Ricciocarpus natans*[3] and other members in Marchantiales[10].

3. *Xenochila integrifolia* is a leafy hepatica belonging to *Plagiochilaceae*[7]. Its life cycle is probably continued by the vegetative production of gemmae. Each cell in the compound gemma is able to develop into a gametophyte. In Pl. II, Figs. 2, 3, at "p" is the differentiating cell from which the leafy gametophyte is developed as in Pl. III, Fig. 6. This ontogeny of the gemmae of *Xenochila integrifolia* is the first report in science.

4. Generally, the sporeling pattern in the Hepaticae is constant within a genus, probably within a family[4]. However, in *Riccardia* it is found variable, especially in *R. multifida;* the occurrence of both filamentous and thalloid protonema is found in the present study and considered very exceptional. The gemmaling development directly in the thalloid stage is also first time reported here.

LITERATURE CITED

(1) ANTHONY, R. E. 1962. Greenhouse cultures of Marchantia polymorpha and induction of sexual reproductive structures. Turtox News **40** (1): 2-5.

(2) BENSON-EVANS, K. 1964. Physiology of the reproduction of Bryophytes. Bryologist **67** (4): 431-445.

(3) BOLD, H. C. 1967. Morphology of Plants. 2nd. Ed. Harper and Brothers Publishers, New York. p. 189, fig. 15-12.

(4) FULFORD, M. 1956. The young stages of the leafy Hepaticae. Phytomorphology **6**: 199-235.

(5) GOEBEL, K. 1930. Organographie der Pflauzen. pp. 830, Fig. 850 H.

(6) HATCHER, R. E. 1965. Towards the establishment of a pure culture collection of Hepaticae. Bryologist **68** (2): 227-230.

(7) INOUE, H. 1963. Contribution to the knowledge of the Plagiochilaceae of Southeastern Asia. IV. The genus *Xenochila*. Bull. Nat. Sci. Mus. (Tokyo) Vol. 6, No. 4 (No. 53) A commemorative No. 1.

(8) _____ 1958. Studies on Spore Germination of Hepaticae. 4. *Makinoacrispata* (Steph.) Miyake. Bot. Mag. Tokyo. **71**: 214-217.

(9) _____ 1955. Studies on Spore Germination of Hepaticae. 5. *Fossombronia jayonica* Schiffn. Bot. Mag. Tokyo. **73**: 131-136.

(10) _____ 1960. Studies in Spore Germination and the Earlier Stages of Gametophyte Development in the Marchantiales. Journ. Hattori Bot. Lab. **23**: 148-191. p. 170, Fig. IX. 8, 9; p. 176, Fig. XIII. 12, 13.

(11) LERSTEN, N. R. 1961. A comparative study of regeneration from isolated gametophytic tissue. Bryologist **64**: 37-47.

(12) MILLER, M. W. 1964. Technique for isolating and culturing gemmae of *Marchantia polymorpha* L. under aseptic conditions. Bryologist **67** (3): 317-320.

(13) MILLER, SPARROW and ROGERS 1965. The radiosensitivity of gemmae of *Marchantia polymorpha* to acute gamma irradiation. Bryologist **68** (1): 31-46.

(14) MILLER, M. W., GARBER, E. D. and VOTH, P. D. 1962. Nutritionally deficient mutants of *Marchantia polymorpha* induced by X-rays. Bot. Gaz. **124**: 94-102.

(15) MIZUTANI, M. and HATTORI, S. 1957. On etude on the systematic of Japanese Riccardias. Journ. Hattori Bot. Lab. **18**: 27-64.

(16) MULLER, K. 1954. Kryptogamen Flora Bd 6. Die Lebermoose Europas. 429-506.

(17) NEHIRA, K. 1962. The germination of spores in Hepaticae. 3. A comparative study on the filamentous protonema in some Hepaticae. Hikobia **3** (1): 4-9.

(18) _____ 1962. The germination of spores in Hepaticae. 4. Two types of sporeling pattern in the *Riccardia*. Hikobia **3** (2): 96-101.

(19) TASEN, N. 1958. Factors regulating the initial development of gemmae in *Marchantia polymorpha*. Bryologist **61** (3): 191-204.

(20) VOTH, P. D. and HOMNER, K. C. 1940. Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. Bot. Gaz. **102** (1): 169-205.

(21) Votch, P. D. 1943. Effects of nutrient-solution concentration on the growth of *Marchantia polymorpha*. Bot. Gaz. **104**: 591-601.

(22) YANG, B Y. 1965. Ontogeny of the Gemmae of *Hyophila tortula*. Taiwania, **11**: 35-40.

(23) _____1966. The geographical distribution and growth habits of *Haplomitrium*. Taiwania **12**: 9-20.

(24) _____1966. On spore germination of *Schiffneria viridis* Steph. Taiwania **12**: 21-34.

(25) _____1967. Spore germination and development of leaf gametophyte of *Haplomitrium rotundifolium* in culture. Present issue, Taiwania 13.

EXPLANATION OF FIGURES

PLATE I

Riccardia multifida and *Dumorteria hirsuta*

Fig. 1. *Riccardia multifida*—Stages in sporeling development.
a. single spore. b. 4-celled filamentous protonema. c, d. stages in thalloid protonema.

Fig. 2. *Riccardia multifida*—Stages in gemmaling development.
a. two-celled gemma. b. one of the 2 cells enlarging, ready for division. c-f. stages approaching a mature thalloid gametophyte.

Fig. 3. *Dumorteria hirsuta*—Stages in sporeling development.
a. spore with reddish papillae. b. the germ tube protruding from a germinating spore. c. the first septum formed at the tip of the germ tube. d. two transverse divisions formed at the same tip. e. two longitudinal divisions formed at the tip of the germ tube toward the formation of a thalloid gametophyte.

PLATE II

Xenochila integrifolia, stages in gemma development

Fig. 1. A mature gemma.

Figs. 2, 3. "p" Primary growth initiated from one of the cells observed on the next day after planting.

Figs. 4, 5. Extension of rhizoids, 2 days later.

Fig. 6. Further elongation of the rhizoid, 4 days later.

Fig. 7. 18 days after planting, leaf primordia at the upper end while long rhizoids at the lower.

Fig. 8. A well developed gametophyte with stem, leaves, leafy apex and rhizoids. ×135.

Fig. 9. 20 days old, a multicellular young plant with numerous rhizoids, and young leaf initials surrounding a growing apex. ×314.

All figures are drawn with camera lucida ×314.

PLATE III

Xenochila integrifolia

Fig. 1. A mature gemma.

Fig. 2. Rhizoid begins to appear from one of the cells.

Fig. 3. Ditto, showing later stage.

Figs. 4, 5. Rhizoids, elongating from many cells.

Fig. 6. A mature leafy gametophyte well established, with the gemma still attached at the base of the plant.

PLATE I

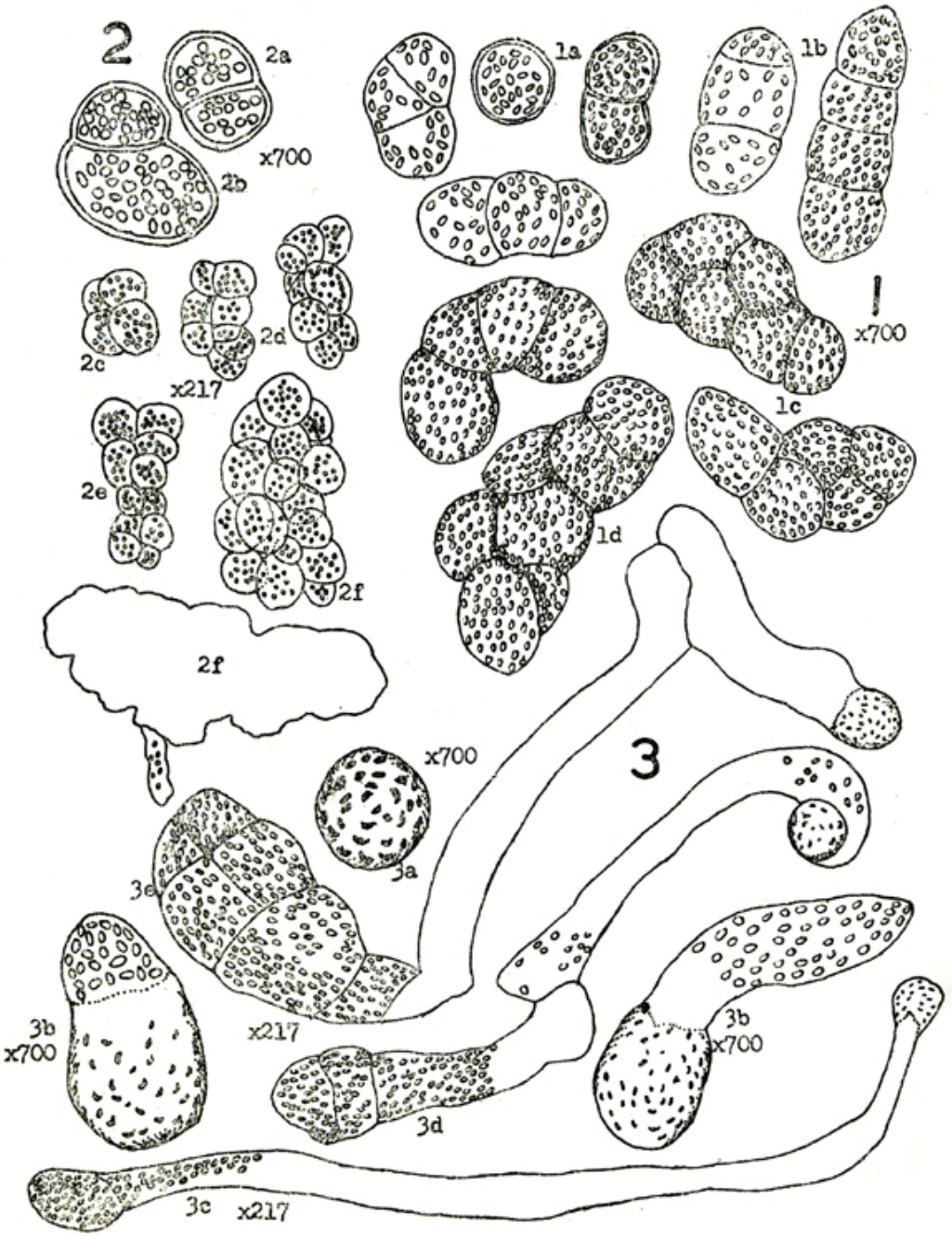
2
2a
x700
2b
2c
2d
x217
2e
2f
2f
1a
1b
1
x700
1c
1d
3
x700
3a
3c
x217
3b
x700
x217
3d
3b
x700
3c x217

PLATE II

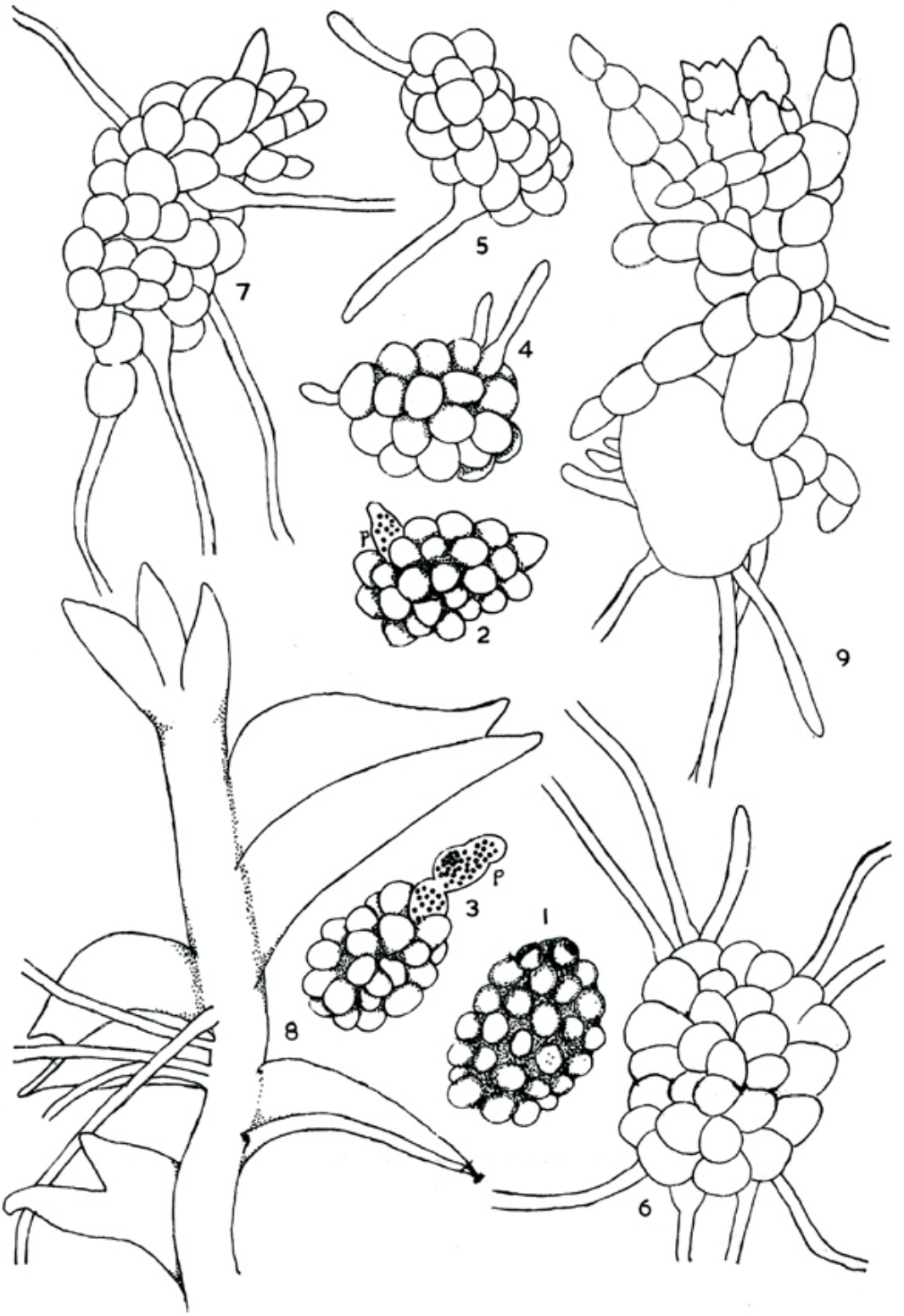

PLATE III