ON SPORE GERMINATION OF *SCHIFFNERIA VIRIDIS* STEPH. AND THREE SPECIES OF MUSCI

by

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The germination of the spores of three mosses, *Diphyscum involutum* Mitt., *Philonotis sp.*, *Vesicularia vesicularis* (Schwaegr.) Broth. and one hepatica, *Schiffneria viridis* Steph. has been attempted. The materials for this study were collected from Taiping Shan, alt. 2200 m., by C. C. Chuang and M. T. Kao, in November 1965. The specimens bearing mature sporophytes were selected and stored in a cool, moist place for a few days until they reached full maturity. The spores of each species were carefully gathered from the mature, unopened capsules and immediately sown into Hoagland's solution in pertri dishes which had been sterilized beforehand as described in (6). The plates were then placed in a controlled environment at 20°C, under 24 hours of illumination, approximately 1200 lux of light intensity.

The present study revealed that the spores of the above mentioned three mosses and one hepatica showed immediate germination into protonema in 24 hours after sowing. The protonema of the mosses did not develop into upright leafy gametophytes; they reached a stage of profuse branching system and advanced no further. *Diphyscum involutum* Mitt. is the first record of its collection on Taiwan and the germination of its spores is the first report to science. The germination of the spores of *Schiffneria viridis* Steph., however, showed not only a filamentous pattern of protonema but also manifested a leafy-thalloid gametophyte grown out from the protonema. Their differentiation into apical regions, lateral leaves, rhizoids and scales on the ventral surface was obvious; but the leaves on these gametophytes are more distantly arranged as compared with that of the parent plant, and sex organs are not developed on them.

The sporeling pattern of *Schiffneria hyalina* Steph. was described by Nehira, (16) revealing only a stage of filamentous protonema. The protonema of Taiwan *Schiffneria viridis* in this study exhibited a shorter filament but with more branches, and its later development, about 47 days after sowing of the spores, resulted in a many-celled thalloid structure, and in 100–128 days, a complete leafy plant (Plates V, VI, VII). The formation of this leafy gametophyte from the simple filamentous protonema has not been found nor reported before.

OBSERVATIONS AND RESULTS

The observations on the stages of development in the spore germination of three mosses and one liverwort and the results of the experiment can be summarized by the following table:

Name of species	Date of observations	Days and hours after germination	% of spores germinated	Stages of development
Vesicularia vesicularis (1)	Nov. 3 sowing of spores			
	Nov. 4	24 hrs.	5%	
	Nov. 5	48 hrs.	14%	Pl. II, (1) b-d
	Nov. 6	72 hrs.	16%	Pl. III, (1) a
	Nov. 11	8 days	58%	Pl. III, (1) b
	Nov. 18	15 days	98-100%	
Philonotis sp. (6)	Nov. 3 sowing of spores			
	Nov. 4	24 hrs.		
	Nov. 5	48 hrs.	6%	Pl. II, (6) e-g
	Nov. 6	3 days	60%, 98-100%	Pl. III, (6) a, b
	Nov. 8	5 days	98-100%	
	Nov. 10	7 days	98-100%	Pl. III, (6) c
	Nov. 18	15 days	98-100%	Pl. III, (6) c
Diphyscum involutum Mitt. (7)	Nov. 3 sowing of spores			
	Nov. 6	3 days	11%	Pl. II, (7) c, d
	Nov. 11	8 days	55%	
	Nov. 18-26	15-23 days	98-100%	Pl. III, (7) a, b
Schiffneria viridis Steph. (8)	Nov. 3 sowing of spores			Pl. II, (8) a
	Nov. 4-6	3 days	no germination	
	Nov. 11	8 days	3%	Pl. II, (8) b
	Nov. 18	15 days	61%	
	Nov. 26	23 days	54%	
	Dec. 20	47 days		Pl. IV, 1, 2, 3, 4
	Jan. 14	100 days		Pl. V, 1, 2, 3, 4
	Feb. 11	128 days		Pls. VI, VII & VIII

SUMMARY

1. The spores of all the three species of Musci tested are markedly enlarged at the onset of germination and two of them, *Vesicularia vesicularis* (Schwaeg.) Broth. and *Philonotis* sp. showed immediate germination in 24 hours after planting, but only 5% of the spores germinated. In every species studied a filamentous protonema is formed. The germination rate rapidly increased from 17–23 days and the elongation of branches of the protonema increased accordingly; thus a profuse growth of filamentous protonemata resulted (Plate III), but no further advancement toward the formation of an upright leafy gametophyte was observed.
2. *Schiffneria viridis* Steph. of Taiwan is a delicate hepatica with a leafy upper portion and thalloid basal portion where the margins are more or less overlapping. In its spore germination it showed excellent results in the present study. The spores are spherical in shape, about 13 μ in diameter, generally yellow-brown in color, and reticulate on their surfaces. They did not germinate readily, until three days after sowing. Short filamentous protonema composed of several irregular, spherical cells were formed when the sporelings were 14 to 47 days old (Plate IV, 1, 2, 3, 4). Then a simple ribbon-like thalloid structure with well developed rhizoids arising from ventral cells became prominent in about 100 days (Plate V, 1, 2, 3, 4); and a leafy gametophyte with marked differentiations of lateral leaves and growing tip specifying the apical region (Plates VI, VII, VIII) was completely formed in about 128 days. This last stage culminates the morphological features of a mature gametophyte grown in nature, except that the lateral leaves are more or less distantly placed (Plate VI), while in the latter they are more closely arranged, especially toward the basal portion of the plant, where it is somewhat confluent with wavy margins instead of separate leaves (Plate I, 3). However, in these gametophytes, there are no indications of sex organs, nor do they give rise to any further developments as observed in the present study.

DISCUSSION AND CONCLUSIONS

1. The Hoagland's solution used in this study was found very favorable as a medium for spore germination in testing the bryophytes studied.
2. The three species of Musci developed filamentous protonema which gave rise to profuse growth of protonemata but they did not develop leafy, erect gametophytes.
3. It is found that *Diphyscum involutum* Mitt. is the first record of this moss in Taiwan, and the spore germination of the three tested mosses is also the first time they have been reported.

4. In the spore germination of *Schiffneria viridis* Steph., a brief stage of filamentous pattern, same as in that of *S. hyalina* Steph. reported by Nehira, was obtained, and besides, a leafy gametophyte was properly developed. This is the first report of the gametophyte development of *Schiffneris viridis* Steph. in artificial cultures. The occurrence of a thalloid portion of the young gametophyte which gave rise to a flat axis, generally of 7–8 cells broad with relatively small lateral leaves, may suggest a transition or an intermediate stage between thalloid and leafy developments in hepaticae, as suggested by previous workers.
5. Stephania included two species in the genus *Schiffneria* established in 1894, *Schiffneria hyalina* Steph. 1894 and *Schiffneria viridis* Steph. 1908 (7) (8). *Schiffneria viridis* was found several times from Taiwan, on Ali-shan and Tai-ping shan, in 1963 and 1965 respectively. According to Dr. H. Inoue's opinion, in a discussion with the writer, *Schiffneria viridis* is synonym of *Schiffneria hyalina*. If this is confirmed, the leafy gametophyte developed from the spores of *Schiffneria viridis* in the present study is also the gametophyte of *Schiffneria hyalina* which showed a filamentous protonema previously, in Nehira's experiment.
6. The specimens of the protonema of the above mosses and the gametophyte of *Schiffneria viridis* cultured in this study are preserved in solution for further research. Judging from the repeated rejuvenation found in culture and the materials collected since the fall of 1965 are still growing in good condition, *Schiffneria viridis* Steph. of Taiwan is probably a perennial plant.
7. The above experiment, on the spore germination of *Schiffneria viridis* Steph. has been repeated from material recently collected from Alishan and the results were just the same as herein described.

Appreciation is herewith expressed to Miss C. S. Ho, a former assistant in the Botany Department of National Taiwan University, for preparing all of the original drawings and photographs in this paper, and also for her assistance in the germination of spores.

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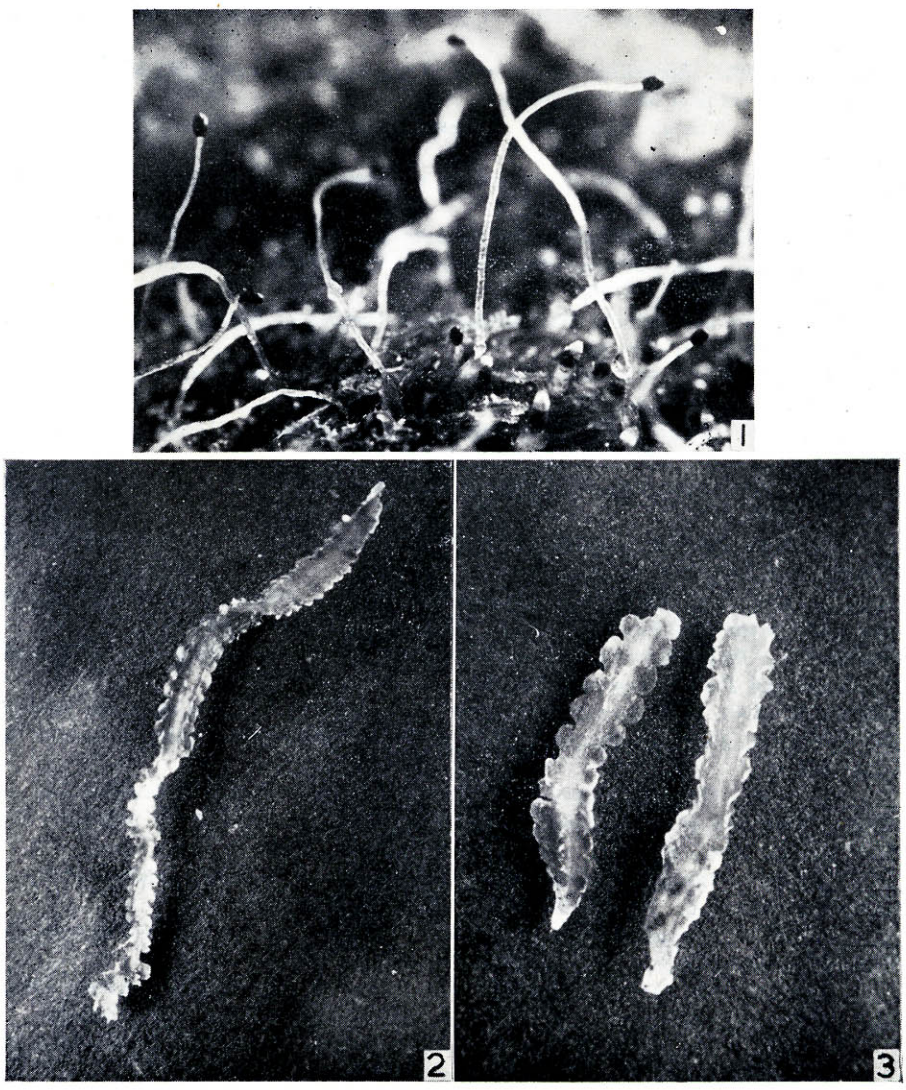
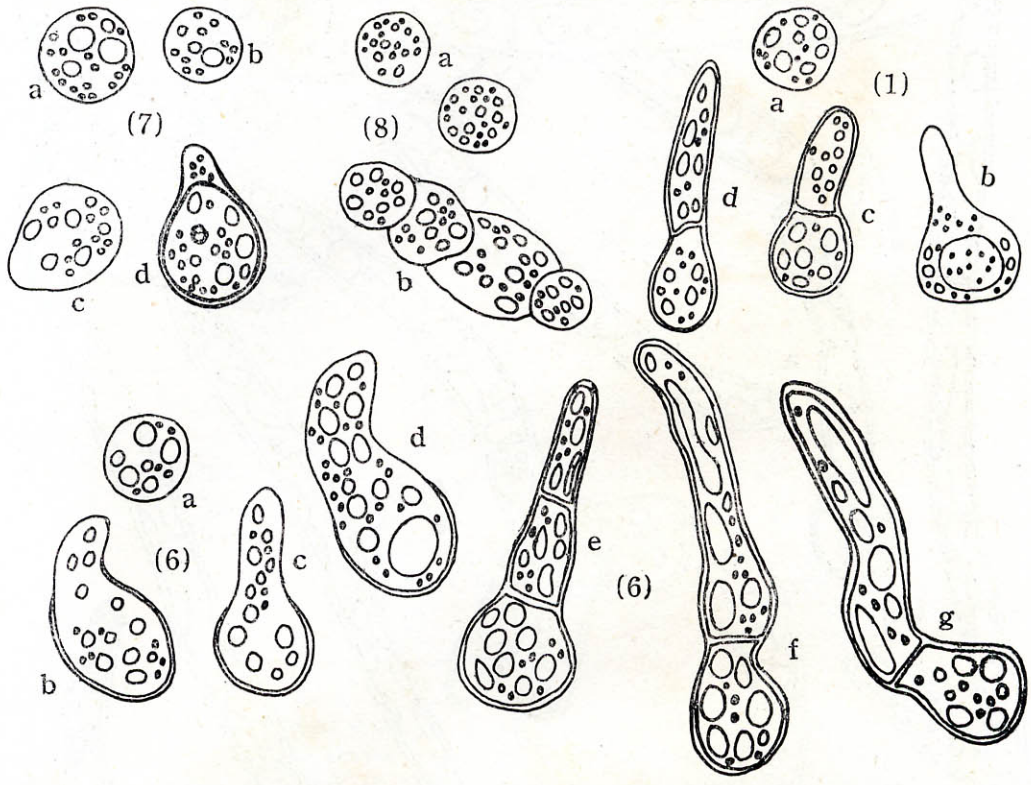
PLATE I



(8) *Schiffneria viridis* Steph.

1. Habit with mature sporophytes ×1.
2. Plant growing from laboratory, showing 2 new portions arising from the old plant ×2.
3. Plants newly collected from the field showing the leafy upper portion and thalloid lower portion ×2.

PLATE II



Early Stages of Spore Germination of four testing Bryophytes

(1) *Vesicularia vesicularis* (Schwaegr.) Broth. a. spore × 900. b-d. Early germination and first cell divisions × 900.

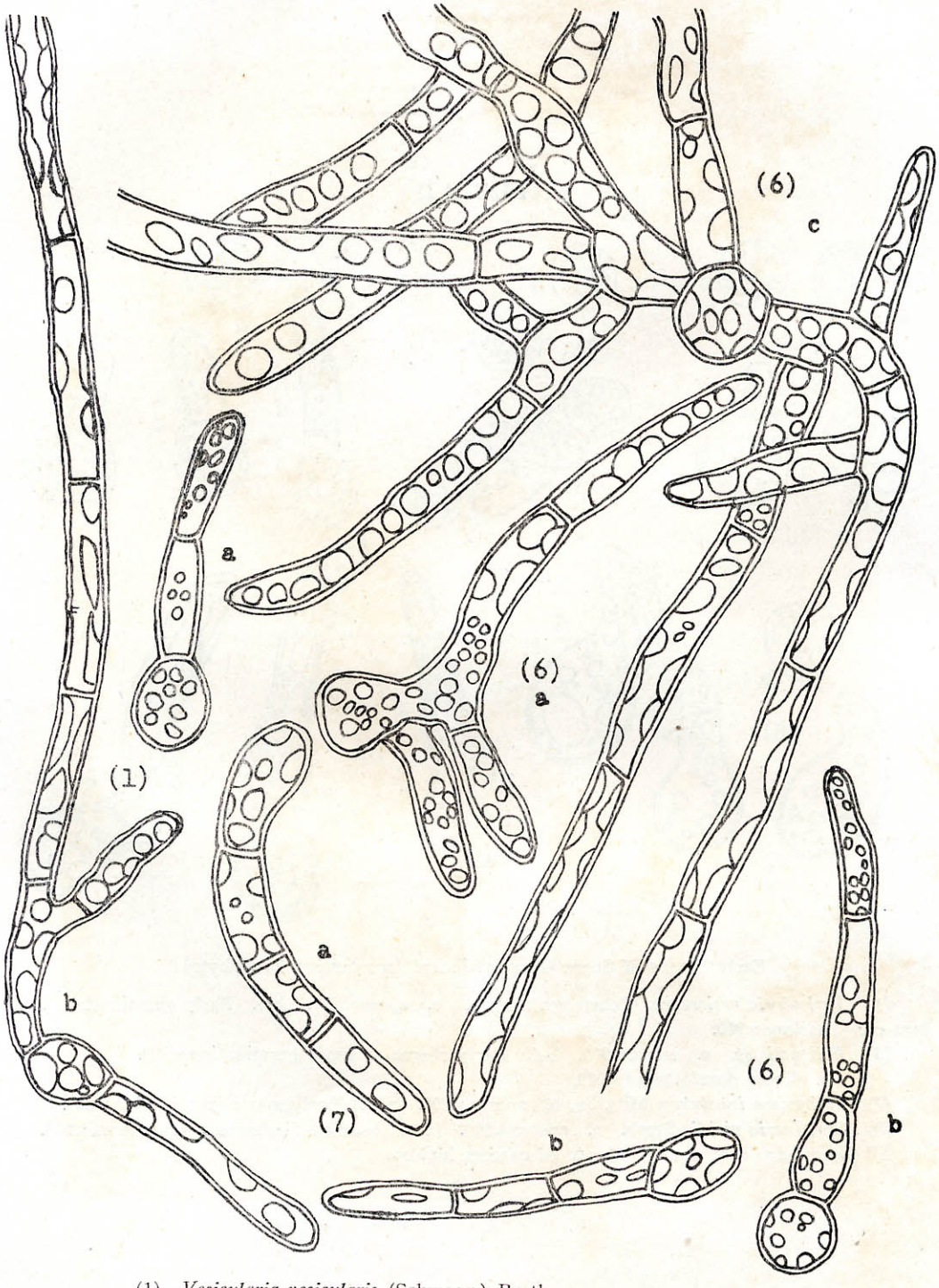
(6) *Philonotis sp.* a. spore × 900. b-d. Early stages of spore germination × 900. e-g. Later development × 900.

(7) *Diphyscum involutum* Mitt. a, b. spores × 900. c, d. Early spore germination × 900.

(8) *Schiffneria viridis* Steph. a. spores × 900. b. A 4-celled filamentous protonema × 900.

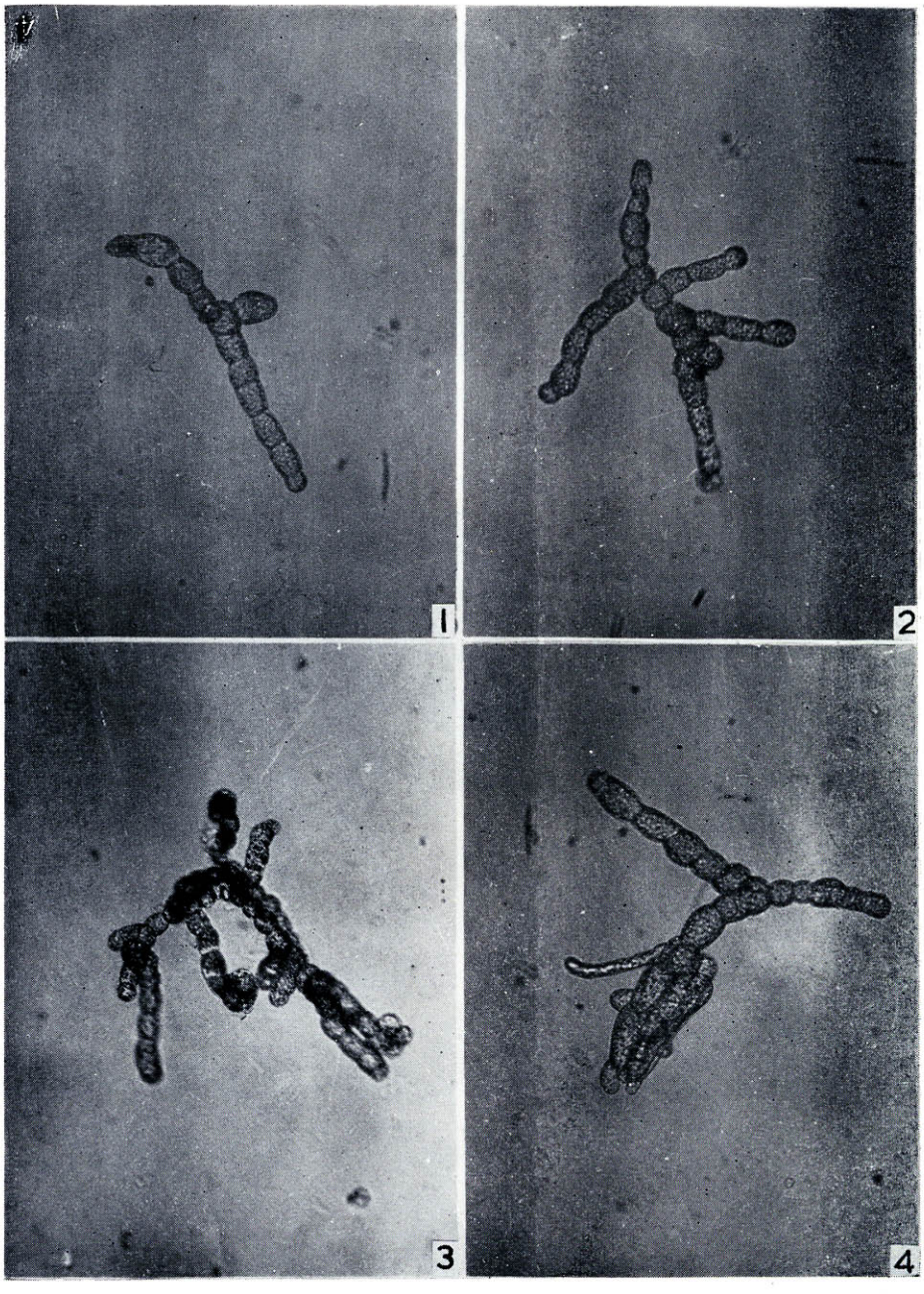
All figures are drawn with the aid of camera lucida.

PLATE III



(1) *Vesicularia vesicularis* (Schwaeg.) Broth.
a. Protonema of 3 days old. b. Ditto, 8 days old. ×900
(6) *Philonotis sp.*
a, b. Protonema of 3 days old. c. Ditto, 7-15 days old. ×900
(7) *Diphyscum involtum* Mitt. a, b. Protonema of 15-23 days old. ×900

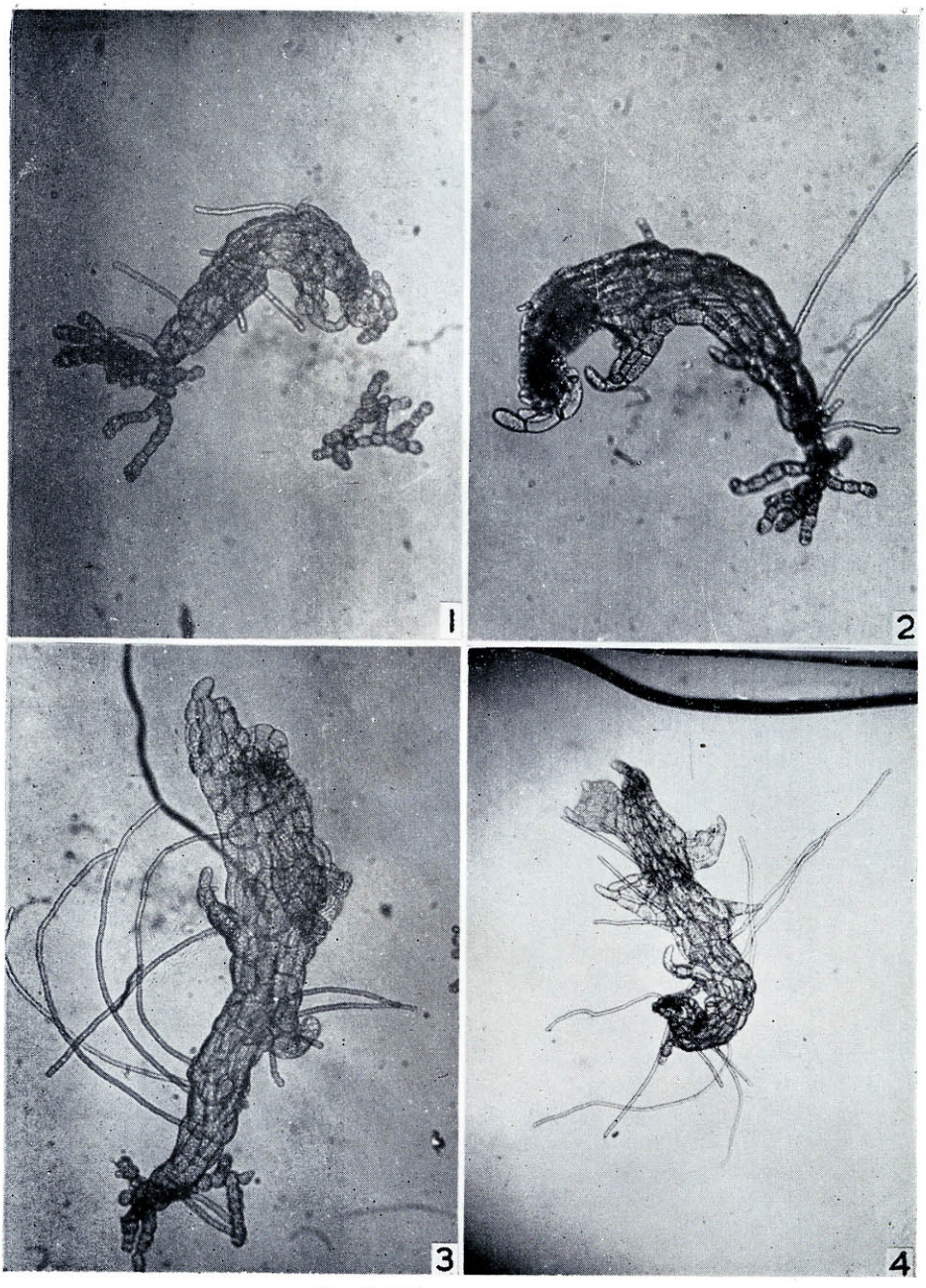
PLATE IV



(8) *Schiffneria viridis* Steph.

1, 2. Filamentous protonema composed of irregular spherical cells, about 47 days old. **3, 4.** in later stages transforming into thalloid structure.

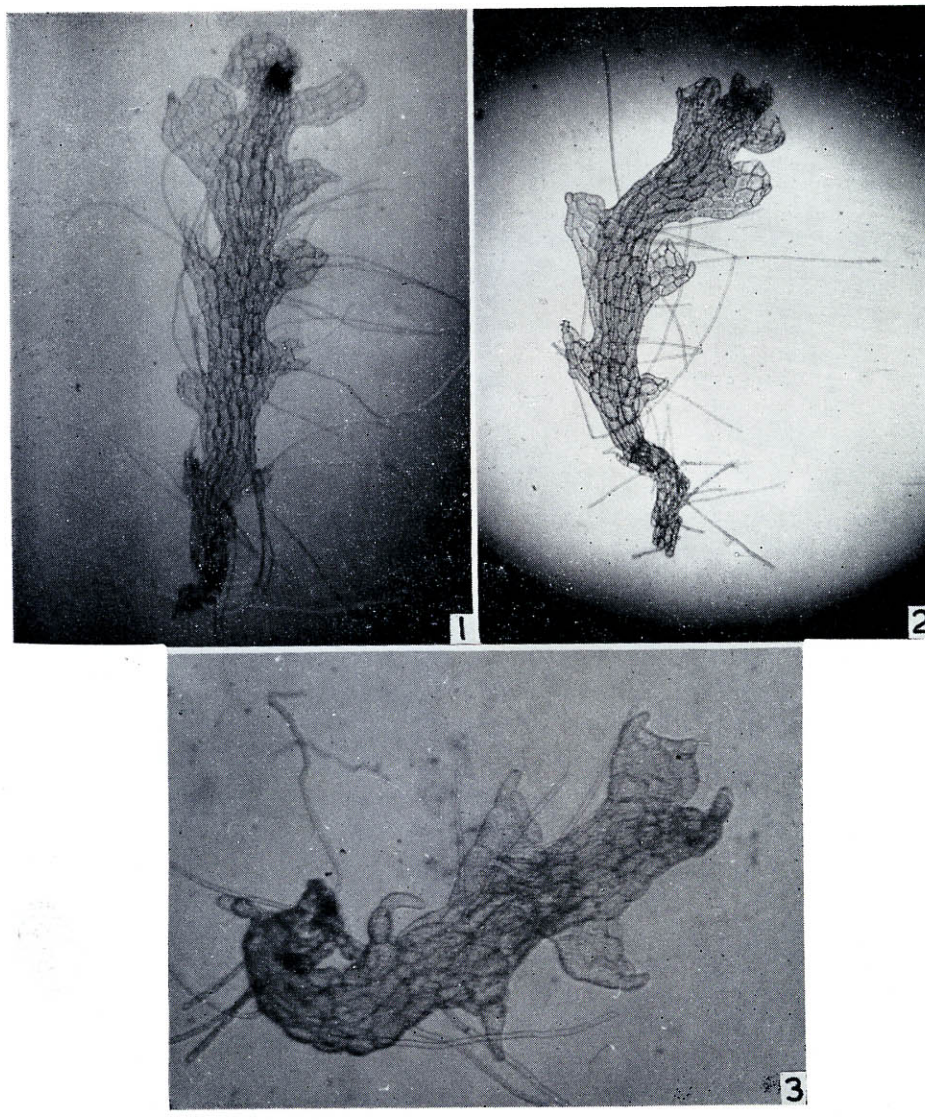
PLATE V



(8) *Schiffneria viridis* Steph.

Formation of thalloid structures in about 100 days. **1, 2.** Showing early stages and the beginning of leaf formation. **3.** Showing the broad axis with numerous rhizoids and young lateral leaves. **4.** Ditto, showing apical region and more developed leaves.

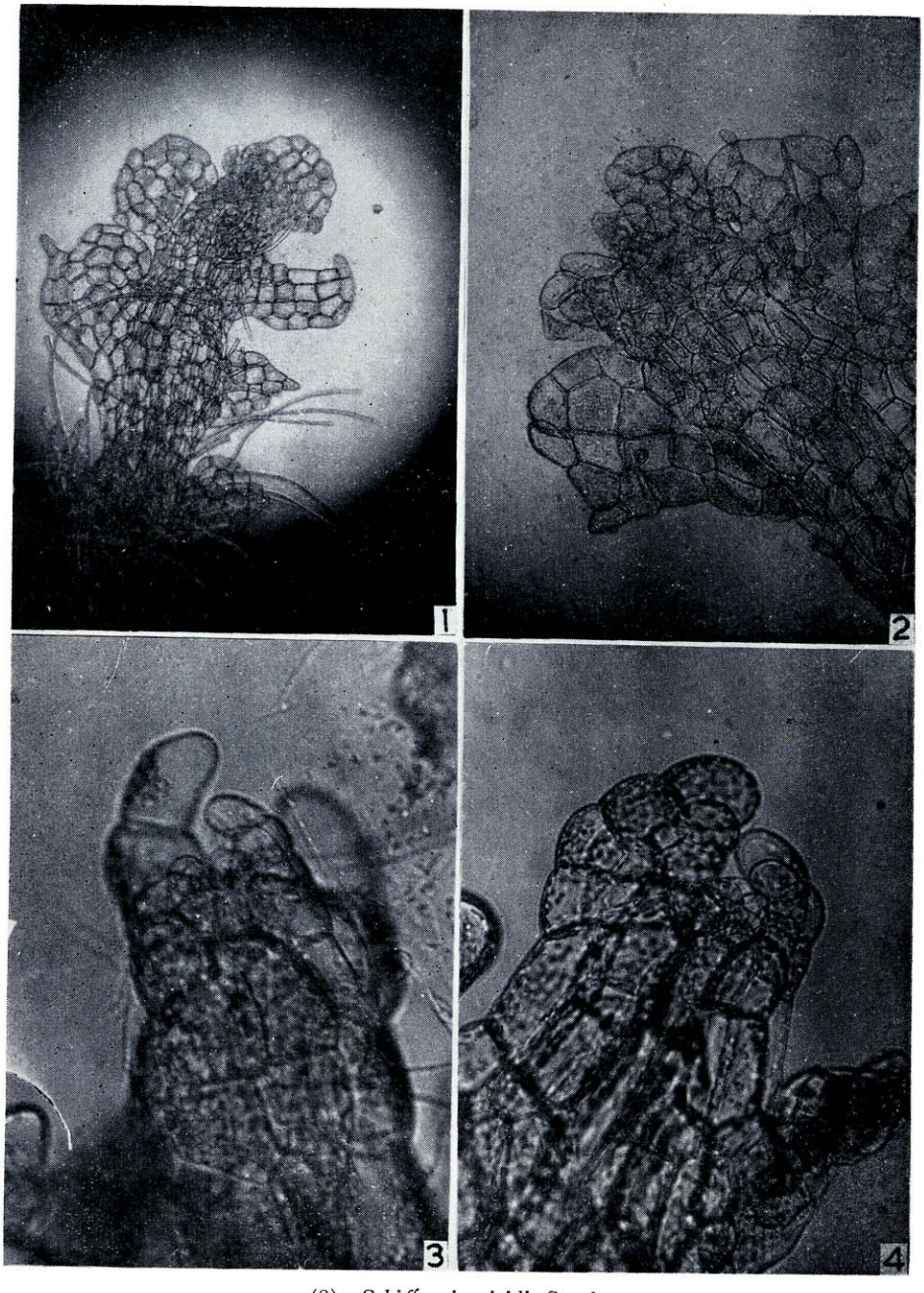
PLATE VI



(8) *Schiffneria* varidis Steph.

Formation of leafy gametophytes in about 128 days, approaching mature stages. 3 × 50. 2 and 1 × 4.

PLATE VII



(8) *Schiffneria viridis* Steph.

Figures showing portions of thallus and the enlarged apical region. **1.** ×50. **2.** ×70. **3.** ×280. **4.** ×280.

PLATE VIII *Schiffneria viridis* Steph.

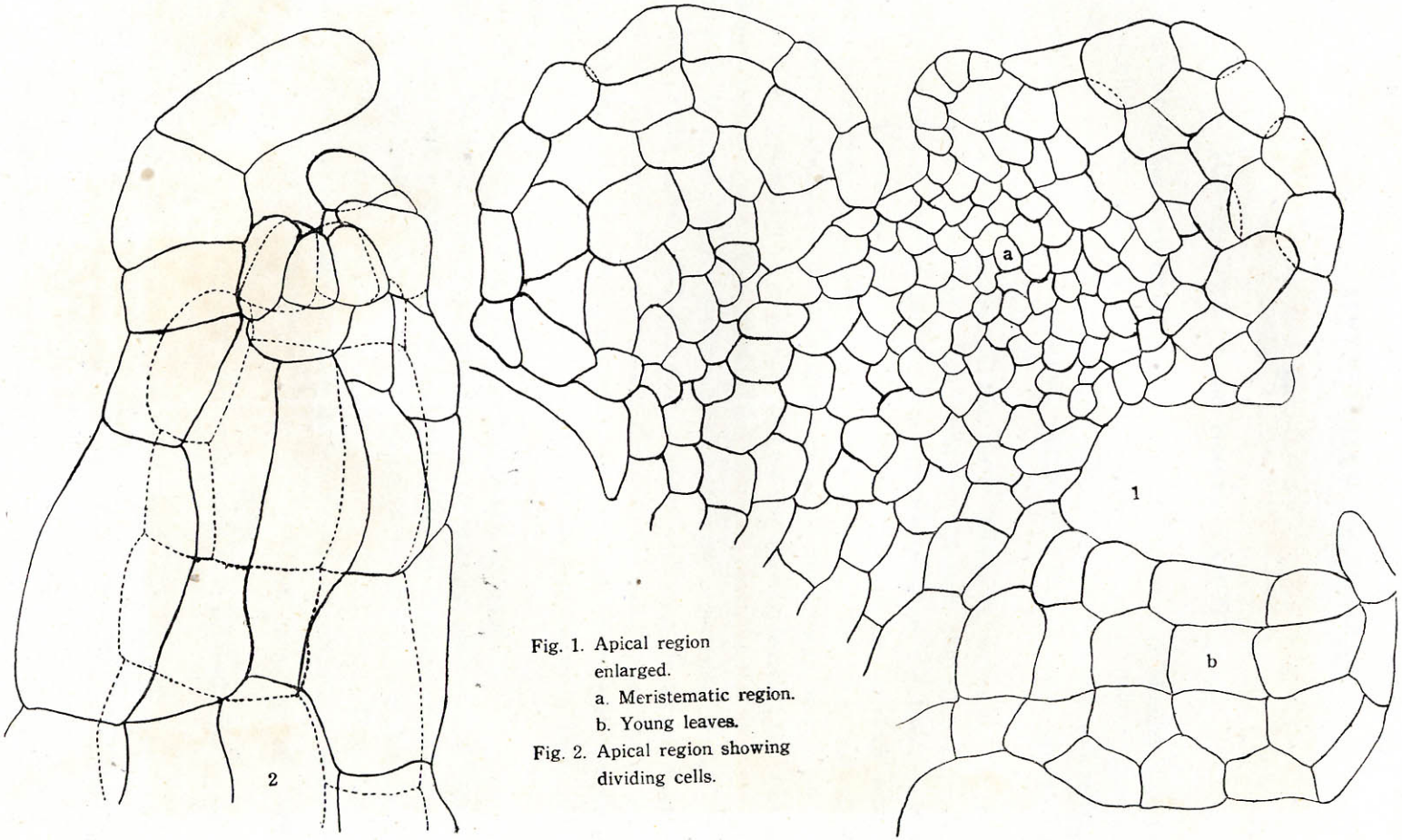


Fig. 1. Apical region enlarged.
a. Meristematic region.
b. Young leaves.
Fig. 2. Apical region showing dividing cells.