JOURNAL OF THE COLLEGE OF SCIENCE, TOKYO IMPERIAL UNIVERSITY.
VOL. XL., ARTICLE 5.

Physiological Studies on *Schistostega osmundacea* (Dicks.) Mohr.

By

Viscount Yasumochi TODA,
Rigakushi.

With two plates and two text-illustrations.

Introduction.

Schistostega osmundacea, the luminous moss, was first discovered[1] at the end of the eighteenth century in England and in Germany, and since that time it has been found to exist in various parts of Europe[2] and also in North America.[2]

In Japan,[3] the moss was collected for the first time in the year 1910 by K. Koyama, in the cave "Senjōjiki," at Iwamurada, in the province of Shinano.

Since then, the moss has been repeatedly found in various other places[4] in the same province, about half of which are

---

[1] cf. Wilson, W.: Bryologia Brittanica, p. 300. 1855, and Roth, G.: Die europäischen Laubmoose, I, p. 555. 1904.

[2] Engler, A. und Prantl, K.: Die natürlichen Pflanzenfamilien, 1. Teil, Abt. 3, 1. Hälfte, pp. 531—532. 1903: Paris, E. G.: Index bryologicus, pp. 226—227. (Editio secunda); Lesquereux, L. and James, T. P.: Manual of the Mosses of North America, p. 189. 1895.

[3] Miyoshi, M.: Über die Kultur der *Schistostega osmundacea*, Schimp. (Bot. Mag., Tokyo. Vol. XXVI., 1912.)

[4] According to Yagi, (Journal of Education of Shinano, vol. 335. 1914) the moss was found in twenty-seven places; now, however, it is known to exist in more than seventy localities.

situated in the mountainous region bordering on the province of Kōzuke.

It has also been found in a few places in the provinces of Kōzuke, Shimotsuke, Yamato, Yamashiro, and Musashi.

The first important contribution to the study of the physiology of *Schistostega osmundacea* was made by F. NOLL (**4**) in the year 1888. He has shown that the curious phenomenon of the moss emitting an emerald green light, depends on the peculiar structure of the cells of the protonema. The latter are somewhat ovoid and have on the elongated side from four to six chlorophyll bodies, the main central part being filled with transparent, colourless cell-sap. The light passing into the cell along the optical axis is so refracted that it falls on the chromatophores on the paraboloidal wall, so that it becomes brighter than when it comes in.

The light that enters the cell outside the optical axis, however, is not refracted in such a way that it directly reaches the chromatophores, but makes a total reflection twice on the paraboloidal wall, and is refracted out again from the cell, taking a direction parallel and opposite to that of its incidence.

As the outcoming light passes through the chromatophores, it appears not white, but strongly green. That the green light given out by the moss is of a nature quite different from that of phosphorescent light, has been fully established by NOLL.

After making further investigations on this phenomenon in 1908, SENN (**7**) came to the conclusion that the angle of incidence made by the total reflection of the outer and the inner rays of light on the back wall is 65° with the former, and 45° with the latter, when the paraboloidal part of this cell wall has an angle of 42°–43°, and that, when the rays are reflected by the opposite wall and sent out of the cell in the direction of the incident rays, the former make an angle of 35° and the latter an angle of 56°. By calculating the index of the refraction of light of the cell, he obtained $v=1.50$.

As to the culture of this moss MIYOSHI (3) made a series of experiments in 1912, and ascertained that it can easily be cultivated.

As the *Schistostega* is always found in a cave where there is but little light, it is interesting to ascertain the intensity of light most suitable for the moss, and the degree of temperature and percentage of humidity required for its growth. It would further be of interest to know in what artificial nutritive solutions the luminous moss grows best, and lastly, whether this moss, in common with some other species, dislikes lime.

The present work has been carried on in the Botanical Institute of the Science College of the Imperial University of Tokyo from August, 1914, to July 1915, under the guidance of Prof. M. MIYOSHI, to whom I beg to express my sincere thanks. Acknowledgments are also due to Mr. T. KOBAYASHI, Principal of the Heion Primary School; to Mr. T. KUROIWA, Head-man of the same village; to Mr. R. KONO, Principal of the Ina Agricultural School; to Mr. R. KAKEGAWA; Principal of the Iwamurada Primary School; and to Mr. G. YUMOTO, all of whom have greatly assisted me in making observations of the moss in its native place. I am also greatly obliged to Mr. T. YAGI, teacher of Natural History at the Nagano Girls' High School, and to Dr. S. OKAMURA, both of whom have furnished me with valuable material for my studies.

I. Natural Occurrence of *Schistostega osmundacea*.

On an excursion to Mt. Komagadake, to the Nakabusa Hot Springs, and to the Yutanaka Hot Springs, in the summer of 1914, where the *Schistostega* was known to thrive, I made some observations on the natural conditions of these places, all of which are in the province of Shinano. The differences in the intensity of light, in the humidity, and in the temperature in and out of the caves were carefully estimated.

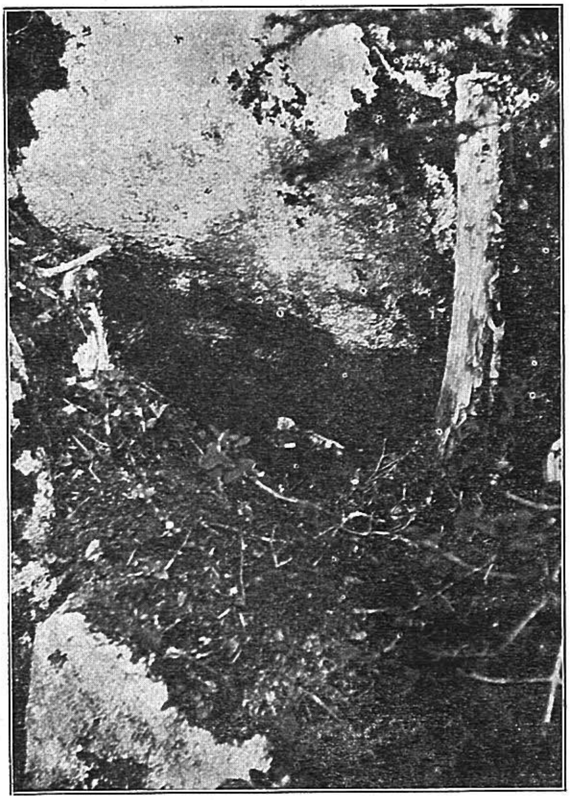


Illustration 1. A crevice under a crag of biotite granite in Mt Komagadake, at an elevation of 1900 m. showing the natural occurrence of *Schistostega osmundacea*. × shows the spot where Vouk's Photometer was placed.

Natural Conditions of the Caves. — The caves in which the moss is found, consist of augite andesite (in 21 places), of granite (in 12 places), of clay-slate (in 9 places), of shale (in 3 places), of diluvium formation (in 3 places), and of other kinds of rock (in about 14 places). Possibly a volcanic rock like andesite, which is very easily weathered, affords the best conditions for the growth of the moss.

1. Mt. Komagadake.—In this granitic mountain the moss occurs in small caves as well as in holes formed at the foot of fallen dead trees, at a height of 1850–2400 m. above the sea-level (see illustration 1).

The soil of these places consists of weathered rocks and decomposed vegetable matter. The caves mostly face a direction from the south-east to the south-west, only one of them facing due north.

2. Nakabusa Hot Spring and Mt. Ariyake.—On the way from Miyashiro to Nakabusa, the moss is found in successive small caves for a distance of about 5 miles. On Mt. Ariyake, near Nakabusa, it occurs in caves and in holes at the foot of fallen dead trees, at a height of about 2250 m. above sea-level. The rock consists of biotite granite, and the caves face various directions, only a few looking north. It was only in the above-mentioned locality, near Nakabusa, that I found much of the moss furnished with sporangia; in other places the moss was almost always found in a sterile condition.

3. Yutanaka Hot Spring (Heion-mura).—On the banks of the river Yamase, which runs through the southern part of the village, there are four large caves (560 m. above sea-level) facing south-west, with gravel beds.

Intensity of Light.—For determining the intensity of light, I found Vouk's (**9**) photometer[1] very convenient and easily portable. With this apparatus I compared the intensity of light inside the caves with that outside. For example, at Mt. Komagadake: $L=\frac{1}{5}-\frac{1}{43.4}$ and at Nakabusa: $L=\frac{1}{94.2}-\frac{1}{289.4}$.

For the caves at Yutanaka I estimated the "Lichtgenuss" about once in two months, from August 1914 to April 1915. The results may be seen in the following Table I.

---

[1] Vouk, V.: Ein verbesserter, neuer Wiesnerscher Insolater zur Bestimmung des Lichtgenusses (Ber. d. deutsch. bot. Ges. Bd. XXX, pp. 391—394. 1912). Compare also Wiesner, J.: Der Lichtgenuss der Pflanzen (1907).

TABLE I.

Intensity of Light and relative "Lichtgenuss" in the caves at Yutanaka.

Time	Weather	Cave 1.				Cave 2.				Cave 3.				Cave 4.				Outside the Caves.
		About 1m. from the Entrance of the Cave.		Farther end of the Cave.		About 1m. from the Entrance of the Cave.		Farther end of the Cave.		About 1m. from the Entrance of the Cave.		Farther end of the Cave.		About 1m. from the Entrance of the Cave.		Farther end of the Cave.		
		I	L	I	L	I	L	I	L	I	L	I	L	I	L	I	L	I
10–11½. a.m. 2. X. 1914	Fine.	0.0182	$\frac{1}{16.1}$	0.003	$\frac{1}{98}$	0.0194	$\frac{1}{15.1}$	0.0013	$\frac{1}{226.2}$	0.0217	$\frac{1}{13.5}$	0.0025	$\frac{1}{117.6}$	0.0202	$\frac{1}{14.5}$	0.002	$\frac{1}{147}$	0.2941
10–11. a.m. 2. XII. 1914	Fine.	0.0229	$\frac{1}{17.9}$	0.0034	$\frac{1}{121}$	0.0133	$\frac{1}{30.9}$	0.0025	$\frac{1}{164.6}$	0.0245	$\frac{1}{16.8}$	0.0046	$\frac{1}{89.5}$	0.0294	$\frac{1}{14}$	0.0053	$\frac{1}{77.6}$	0 4117
1½–2½. p.m. 3. XII. 1914	Fine.	0.0388	$\frac{1}{8.8}$	0.008	$\frac{1}{42.8}$	0.0202	$\frac{1}{16.9}$	0.0052	$\frac{1}{65.9}$	0.0396	$\frac{1}{8.6}$	0 0083	$\frac{1}{41.3}$	0.0307	$\frac{1}{11.2}$	0.0047	$\frac{1}{72.9}$	0.3430
10–12. a m. 4. XII. 1914	Cloudy.	0.0396	$\frac{1}{4.7}$	0.0066	$\frac{1}{28.3}$	0.0178	$\frac{1}{10.5}$	0.0032	$\frac{1}{58.5}$	0 0396	$\frac{1}{4.7}$	0.0053	$\frac{1}{35.3}$	0.0332	$\frac{1}{5.6}$	0.0043	$\frac{1}{43.5}$	0 1872
10–11. a.m. 31. I. 1915	Fine.	0.0457	$\frac{1}{9}$	0.0081	$\frac{1}{50.8}$	0.0206	$\frac{1}{19.9}$	0.0027	$\frac{1}{152.4}$	0.0396	$\frac{1}{10.3}$	0.0048	$\frac{1}{85.7}$	0.0294	$\frac{1}{14}$	0.0032	$\frac{1}{128.6}$	0.4117
11–12. a.m. 1. II. 1915	Cloudy.	0.0412	$\frac{1}{4.1}$	0.0046	$\frac{1}{37.3}$	0.0343	$\frac{1}{5}$	0.0035	$\frac{1}{49}$	0.0332	$\frac{1}{5.1}$	0.0044	$\frac{1}{39}$	0.0412	$\frac{1}{4.1}$	0.0045	$\frac{1}{38.1}$	0.1716
10–11½. a.m. 31. III. 1915	Fine.	0.049	$\frac{1}{20.4}$	0.0042	$\frac{1}{238}$	0.0374	$\frac{1}{26.7}$	0.0031	$\frac{1}{322.5}$	0.0823	$\frac{1}{12.1}$	0.0028	$\frac{1}{357.1}$	0.0935	$\frac{1}{10.6}$	0.0111	$\frac{1}{90}$	1.
10–11½. a.m. 1. IV. 1915	Fine.	0.0438	$\frac{1}{22.8}$	0.0055	$\frac{1}{181.3}$	0.0219	$\frac{1}{45.6}$	0.0031	$\frac{1}{322.5}$	0.0457	$\frac{1}{21.8}$	0.0039	$\frac{1}{256.4}$	0.049	$\frac{1}{20.4}$	0.005	$\frac{1}{200}$	1.
10–11½. a.m. 17. VII. 1915	Fine.	0.0257	$\frac{1}{26.7}$	0.0048	$\frac{1}{142.6}$	0.0125	$\frac{1}{55.1}$	0.0023	$\frac{1}{295.4}$	0.0294	$\frac{1}{23.4}$	0.0035	$\frac{1}{196.9}$	0.0413	$\frac{1}{16.6}$	0.0037	$\frac{1}{183.8}$	0.6864
10–11½. a.m. 18. VII. 1915	Fine.	0.0241	$\frac{1}{34.2}$	0.0042	$\frac{1}{191.5}$	0.0153	$\frac{1}{53.9}$	0.0025	$\frac{1}{330.7}$	0.0317	$\frac{1}{26}$	0.0037	$\frac{1}{220.4}$	0.0275	$\frac{1}{30}$	0.0042	$\frac{1}{198.4}$	0.8234

I=Intensity of light. L=Lichtgenuss.

Humidity.—At Mt. Komagadake and at Nakabusa, I tested the humidity in the caves where the moss occurs, and found that the mean humidity was 94% and 100% respectively. At Yutanaka, I obtained the results as given in Table II, the mean humidity in the four caves being 93%.

TABLE II.

Humidity and Temperature in and out of the Caves at Yutanaka.

Time	Weather	Cave 1.		Cave 2.		Cave 3.		Cave 4.		Outside the Caves.	
		H	*T*	*H*	*T*	*H*	*T*	*H*	*T*	*H*	*T*
5–6 p.m. 6. VIII. 1914	Fine.	90%	21°C.	99%	19.9°C					60.5%	29.5°C.
10–12 a.m. 7. VIII. 1914	Fine.	100%	23.5°C.	100%	19.5°C.	95%	21.7°C	96%	21.2°C.	66%	28.5°C.
10½–11½ a.m. 8. VIII. 1914	Fine.	98%	21.7°C.	98%	20.4°C.	99%	20.7°C.	98%	21.2 C.	75%	28.7°C.
2–3 p.m. 30. IX. 1914	Fine.	93%	17.2°C.	96%	17.3°C.	94%	16.5°C.	100%	16°C.	94%	14.8°C.
10–11½ a.m. 2. X. 1914	Fine.	90%	15.1°C.	94%	16.2°C.	95%	14.5°C.	98%	14.9°C.	77%	16.7°C.
10–11 a.m. 2. XII. 1914	Fine.	83%	11.7°C	89%	11°C.	92%	9.6°C.	100%	9°C.	74‰	11.7°C.
1½–2½ p.m. 3 XII. 1914	Fine.	85%	10 6°C.	90%	10.1°C.	86%	9.5°C.	91%	9.1°C.	77%	7°C.
10–12 a.m. 4. XII. 1914	Cloudy.	84%	9.7°C.	88%	9.4°C.	87%	8.2°C.	88%	8°C.	86%	6.8°C.
10–11 a.m. 31. I. 1915	Fine.	84%	4.8°C.	89.5%	4.5°C.	87%	2.9°C.	94%	2.7°C.	80%	1.9°C.
11–12 a m. 1. II. 1915	Cloudy.	93%	4.6°C.	95%	4.8°C.	96%	3.7°C.	93%	4.2°C.	100%	2.7°C.
10–12 a.m. 2. II. 1915	Cloudy.	90%	5.2°C.	91%	4.7°C.	96%	3.3°C	96%	3.2°C.	82%	3°C.
10–11½ a.m. 31. III. 1915	Fine.	95%	6.2°C.	92%	5.7°C.	91%	5°C.	95%	4.8°C.	74%	4.2°C.
10–11½ a.m. 1. IV. 1915	Fine.	90%	8.7°C.	97%	7°C.	95%	7.3°C.	97%	6.7°C.	76.5%	9.5°C.
10–11½ a.m. 17. VII. 1915	Fine.	93%	22.2°C	100%	17.8°C.	92.5%	20.5°C	96%	20.5°C.	69%	29.5°C.
10–11½ a.m. 18. VII. 1915	Fine.	93%	22.2°C.	100%	18°C.	94%	20.6°C.	93%	21°C.	60%	31°C.
Mean Humidity.		90.7%		94.5%		92.8%		95%			

H=Humidity. *T*=Temperature.

From August to October, the soil in the cave was wet, and the growth of the shoots and of the protonemata was very active. From December to January, on the contrary, owing to the combined effect of lower temperature and decreased humidity, the protonema was in an inactive condition and the reflection of light from it was hardly perceptible. On the approach of spring, however, the moss again began to thrive with the increase of temperature and humidity of the soil and air, forming sporangia at the end of March.

Temperature.—In August, the moss attained its full growth, producing protonemata and shoots in abundance, and the phenomenon of the reflected light was most remarkable during this season. 20° to 25°C. seems to be the most suitable temperature for the development of this moss, the mean temperature observed at Yutanaka being 24°C. in August. Towards November, when the mean temperature was 9.1°C., the growth of the moss gradually declined, and the reflection of light greatly diminished. In January and February, when the mean temperature was as low as $-1.4°$ and $-0.9°$C. respectively, the growth of the moss naturally ceased. From the middle of March on, the growth of the protonema was renewed and the reflected light could gradually be noticed. In winter, I observed that the temperature in the cave was always from 2° to 5°C. higher than that at the Meteorological Station in this village. As the lowest temperature in the village was $-11.5°$C. this year, the minimum temperature in the cave can be estimated as not lower than $-9.5°$C.

Assuming that in all the caves where the moss occurs, the winter temperature inside the cave is always higher than that outside, then the temperature in these caves would probably never be lower than $-20°$C.[1] Moreover, most of these caves being covered with snow during the whole winter, the temperature of the ground on which the moss grows cannot be even so low as $-20°$C. In proof of this fact the following observations made at Kashiwabara (Shinano) are given.

Temperature in the snowdrift at Kashiwabara.			
	Max. temp.	Min. temp.	Difference
Temp. of Air.	1.9°C.	−5.1°C.	7.0°C.
Temp. on the Surface of Snow.	2.1	−7.1	9.2
Temp. of Snow at 15 cm. below the Surface.	−0.2	−5.1	4.9
Temp. of Snow at 30 cm. below the Surface.	−1.2	−2.1	0.9
Temp. of Snow at 15 cm. above the Ground.	—0.2	−0.5	0.3
Temp. on the Ground.	0.0	0.0	0

As to the cave (Senjōjiki) at Iwamurada; on the 3rd April, 1915, I made the following observations for temperature, humidity, and intensity of light.

---

[1] The minimum temperatures of some localities, near which the luminous moss is found, are as follows:

Iwamurada (Shinano),	−16.4°C	Feb. 1914.
Mt. Asama ,,	−19.5°C	Nov. 1914—Mar. 1915.
Matsumoto ,,	−22.9°C	Jan. 1913.
Nagano ,,	−11.4°C	Jan. 1913.
Karuizawa ,,	−21.5°C	Jan. 1913.

Weather	Humidity		Temperature		Intensity of Light and relative "Lichtgenuss"						
	In the Cave.	Outside the Cave.	In the Cave.	Outside the Cave.	Farther end of the Cave.		On the side of the Cave.		About 1m. fr. the Entrance of the Cave.		Outside the Cave.
Cloudy	91%	90%	6.6°	9.2°	$I=$ 0.0008	$L=\frac{1}{128.6}$	$I=$ 0.0017	$L=\frac{1}{60.5}$	I= 0.0143	$L=\frac{1}{7.2}$	$I=$ 0.1029

I=Intensity of light. L=Relative "Lichtgenuss."

II. Materials and Methods.

The materials for the present studies were almost all collected from the caves at Yutanaka. The spores used for germination were obtained at Nakabusa.

In making the various experiments mentioned below I found it convenient to cultivate the moss in a Petri dish and to put it in a wooden box of 33 cm. × 25 cm. × 20 cm. capacity, with an aperture of 13 cm. square in front. The moss thus gets as much light as in its native place, its beautiful emerald green light being easily seen from outside through the aperture.

I then put into the Petri dish a piece of brick (or tile) which had been completely sterilized, and covered this lightly with the soil on which the moss had been growing. In a short time protonemata were seen to grow all over the brick (or tile), and in about two months many shoots were produced.

Instead of using a Petri dish I put a whole piece of sterilized brick into a basin with a little water and deposited on it the protonema-laden soil. The whole was placed in the box as described above.

III. Influence of Light on the Growth of the Moss.

Thanks to the researches of Servettaz (8), we now know that for each sort of moss and at each stage of its development, there is an optimum light. If we wish to obtain a good result in culture, we must device an arrangement which will allow the moss to get as near an amount of light as the plant obtains naturally.

Schistostega thrives well in a cave where the light is so feeble that many other, common, species of moss could not live. The classical researches of Noll and the recent studies of Senn, show that the cell of the protonema, being lens-shaped, can effect assimilation in a feeble light quite as well as other species do in much brighter places.

1. Optimum Intensity of Light and optimum "Lichtgenuss."—In my present study I first estimated the optimum intensity of the light and the optimum "Lichtgenuss" at Yutanaka. Results of which are given in Table I.

At the farther end of the cave, the intensity of light was between 0.0013 and 0.0111 (Bunsen's unit), while near the entrance, it was between 0.01 and 0.09. Outside the cave, it was between 0.17 and .1.

In the cave, the shoots grow most abundantly near the entrance, while the protonemata thrive best at the farther end. It seems, therefore, that the optimum intensity of light for the development of the protonema is between 0.008 and 0.002, and that for the shoot is somewhat below 0.09. It will also be seen that the optimum intensity of light for the moss is between 0.02 and 0.002.

The relative "Lichtgenus" of the moss at about 1 m. from the entrance of the cave is observed to be: $L=\frac{1}{4.1}-\frac{1}{55.1}$ and at the farther end of the cave: $L = \frac{1}{28.3} - \frac{1}{357.1}$. According to WIESNER (**10**), the relative "Lichtgenuss" in the light and in the shade is in most cases $\frac{1}{30}$, but in our caves, at the farther end of the latter as mentioned above, the maximum "Lichtgenuss" was $\frac{1}{4.1}$ and the minimum $\frac{1}{357.1}$, and the optimum "Lichtgenuss" of the moss between $\frac{1}{50}$ and $\frac{1}{200}$.

The following experiments made in the laboratory of the Botanical Institute show that the intensity of light given above is just what the moss requires.

1) On the 15th of September, 1914, I placed, in a dark room, seven PETRI dishes, each containing the moss, at 15 cm., 30 cm., 45 cm., &c. distance from a small window, and repeatedly observed the intensity of light in every position. The following results were obtained. (Table III., A.)

TABLE III., A.

Intensity of Light and relative "Lichtgenuss."

Time	Weather	45 cm. from Window of the Dark room.		30 cm. from Window.		15 cm. from Window.		Outside
		I	*L*	*I*	*L*	*I*	*L*	*I*
10–12 a.m. 27. XI. 1914.	Fine.	0.0006	$\frac{1}{571.6}$	0.0025	$\frac{1}{137.2}$	0.0057	$\frac{1}{60.1}$	0.343
11–12 a.m. 13. I. 1915.	Fine.	0.0007	$\frac{1}{420.1}$	0.0027	$\frac{1}{108.9}$	0.0069	$\frac{1}{42.6}$	0.2041
10–12 a.m. 28. I. 1915.	Fine.	0.0008	$\frac{1}{367.6}$	0.0027	$\frac{1}{108.9}$	0.0076	$\frac{1}{38.6}$	0.2941
10–12 a.m 1. III. 1915.	Fine.	0.0008	$\frac{1}{643.2}$	0.0027	$\frac{1}{190.5}$	0.0115	$\frac{1}{44.7}$	0.5146
11–12 a.m. 29. IV. 1915.	Fine.	0.0015	$\frac{1}{666.}$	0.0057	$\frac{1}{175.4}$	0.0229	$\frac{1}{43.6}$	1.

In the Petri dish, placed at 15 cm. from the window, the moss began to show its emerald green light in one week, and its protonemata increased greatly in number during one month. At 30 cm. distance, the growth of the protonemata was even better. But at 45 cm., though the moss throve well at the beginning of November, it gradually ceased to develop as the days went on, hardly any growth being observable in the months of December and January, and no emerald green light being then seen. The light was observed to reappear at the beginning of April. The moss in the Petri dishes placed farther back ceased altogether to grow. When they were brought to 30 cm. distance from the window, however, the moss began to develop actively and to send out a reflected light, L for this distance being $\frac{1}{108.9}-\frac{1}{190.5}$. From these experiments, it follows that 0.01—0.002 is the proper intensity of light for the growth of the protonema, and that no growth is possible when it is below 0.000 5.

2) By the culture of the moss in the Petri dish (page 10), I observed the different degrees of the intensity of light in the three boxes, and noticed 0.0069—0.0008 as the most suitable for the development of the moss. The "Lichtgenuss" of the moss in these boxes was $\frac{1}{81.3}-\frac{1}{384.6}$. (Table III., B.)

TABLE III., B.

Intensity of Light and relative "Lichtgenuss."

Time	Weather	No. 1. Wooden box.		No. 2.		No. 3.		Outside
		I	*L*	*I*	*L*	*I*	*L*	*I*
2–3 p.m. 27. XI. 1914.	Fine.	0.0013	$\frac{1}{158.3}$	0.0008	$\frac{1}{257.2}$	0.0015	$\frac{1}{137.2}$	0.2058
2–3 p.m. 13. I. 1915.	Fine.	0.0023	$\frac{1}{81.3}$	0.0011	$\frac{1}{179.2}$	0.0021	$\frac{1}{89.1}$	0.1872
1–3 p.m. 1. III. 1915.	Fine.	0.0069	$\frac{1}{42.6}$	0.0023	$\frac{1}{127.8}$	0.003	$\frac{1}{98}$	0.2941
2–3 p.m. 28. III. 1915.	Fine.	0.0022	$\frac{1}{133.6}$	0.0011	$\frac{1}{267.3}$	0.0021	$\frac{1}{140}$	0.2941
2–3 p.m. 29. III. 1915.	Fine.	0.0029	$\frac{1}{142.4}$	0.0022	$\frac{1}{187.1}$	0.0049	$\frac{1}{84}$	0.4117
1–2 p.m. 29. IV. 1915.	Fine.	0.0081	$\frac{1}{123.4}$	0.0028	$\frac{1}{357.1}$	0.0026	$\frac{1}{381.6}$	1.

3) To ascertain whether the above result was constant, I observed the intensity of light by the culture of the moss on a whole brick (page 10) on three different parts of it.

A. At the edge of the brick.

B. At $11\frac{1}{2}$ cm. from the edge.

C. At $16\frac{1}{2}$ cm. from the edge.

Between $1\frac{1}{2}$ cm. and 9 cm. from the edge, the shoots grew well; between $6\frac{1}{2}$ cm. and $11\frac{1}{2}$ cm. the protonemata grew well and gave out their peculiar light. From $16\frac{1}{2}$ cm. on, where the intensity of light was below 0.0006 and the "Lichtgenuss" less than $\frac{1}{490}$, the moss, and indeed the protonemata, could not even continue their growth. Thus the proper intensity of light seems to be 0.006—0.002 for the growth of the protonemata, and 0.01—0.004 for that of the shoot. (Table IV.)

TABLE IV.

Intensity of Light and relative "Lichtgenuss."

Time	Weather	*A.*		*B.*		*C.*		Outside
		I	*L*	*I*	*L*	*I*	*L*	*I*
3–4½ p.m. 1. III. 1915	Fine.	0.0069	$\frac{1}{42.6}$	0.0022	$\frac{1}{133.6}$	0.0006	$\frac{1}{490.1}$	0.2941
3–4½ p.m. 29. III. 1915	Fine.	0.0172	$\frac{1}{24}$	0.0041	$\frac{1}{100.4}$	0.0006	$\frac{1}{686.1}$	0.4117

From the above experiments, I have come to the conclusion that *the optimum intensity of light for the development of the moss is 0.02—0.002; that the protonema can grow in a light even as feeble as 0.0008; that the shoot ceases to grow where the intensity is 0.001, and that the required minimum "Lichtgenuss" of the moss is about* $\frac{1}{500}$.

2. Effect of Light on the Moss.—When the filaments of the protonemata, after growing over the surface of the soil or any other object on which the moss grows, send new filaments up into the air, they are easily acted on by light, curving towards the direction from which it comes. These growing filaments possess a positive heliotropism. Their orientation may be changed by turning the culturing dish 180 degrees. The full-grown filaments are not bent in the new direction of light, but new branches are given out from each filament in the direction of the light. (Fig. 19.) The growing shoots, too, have been observed to possess a positive heliotropism. During my experiments, I turned a culturing dish 180 degrees on the 4th of April, and found on May 13th that the

shoots were bent towards the light: on May 13th I again turned the dish 180°, and found on the 26th of May that the shoots again were bent towards the light. (Figs. 1 and 2.)

It is already known that in uniform light, the leaves of *Schistostega* generally turn in all directions, but that when the light is unilateral, their divergence is $\frac{1}{2}$. After putting a culturing dish in a dark room for seven months, beginning with November 14th, I found that only the protonemata were alive. Not a single leafy shoot had been produced; but the green colour of the chromatophores had not wholly disappeared. It is also interesting to observe that the shoot is shorter and the leaf larger in a feeble than in a comparatively intense light. Placed in a position where the intensity of light is above 0.1, the colour of the moss gradually disappears from all its parts, changing into a silvery white after the lapse of from seven to ten days. This is owing to the disappearance of the colour of the chromatophores and of the destruction of the lens-shaped cells. Under such circumstances the moss soon loses its vitality. In comparing the formation of starch in the lens-shaped cells of the protonema with that in the filamentous cells, we find that it is greater in the former than in the latter. From this fact and also from that, that a fairly large percentage of sugar exists in the lens-shaped cell, it is inferred, that by virtue of these lens-shaped cells this moss can effect assimilation in a feeble light quite as well as other species do in a stronger light.

3. Movement of the Chromatophore.—By the studies of Noll and Senn, it has been fully established, that the light which is reflected from the lens-shaped cells is caused by its peculiar optical structure. The reflected emerald green-light which reappears after the culturing dish has been turned 180 degrees,

gives place to the silvery white light, if the dish is placed in its former position. This depends on the peculiar form of the cell of the protonema. As the result of my experiments I have found that the angle of equal intensity of the reflected emerald green light is 50°—70°. I am, therefore, inclined to think that the reflected light is emitted from the cell parallel to the incident rays, not only when the latter is paralled to the optical axis of the cell, but also when it makes with the axis an angle not exceeding 35°. On this point, however, much further study is necessary before a safe conclusion can be arrived at.

In addition to what SENN (7) has done in regard to the movement of the chromatophore I have made the following observation:—

In unilateral light, the chromatophores in the cell of the protonema are gathered in its paraboloidal part, which arrangement SENN calls "Escharostrophe" (Fig. 20). If the culturing dish is turned 180 degrees, the chromatophores change their positions in the direction of the new light in one week; but when the dish is placed in a light whose intensity is from 0.1 to 0.5, the chromatophores are scattered round the cell wall in one day, this arrangement being called "Apostrophe" by SENN (Fig. 3).

Four weeks after I put the PETRI dish in a dark room (May 3rd), I observed the chromatophores in the lens-shaped cell of the protonema to be in "Apostrophe" (Fig. 4). In the case of liquid culture in unilateral light, the chromatophores of the filamentous cells of the protonema are arranged on the side farthest from the light. This SENN calls "Antistrophe."

As regards the emerald green light reflected from the lens-shaped cells, which is again seen after the direction of the light is changed, I suspect that this phenomenon, besides being dependent on the movement of the chromatophores, is also caused by the action of the filament which contains a group of spherical cells, that is, the cells of the protonema spread on the surface, appear to change their orientation on the turning of the filament.

On December 10th, 1914, I took five wooden boxes, 15 cm. square, with a piece of red, yellow, green, blue, and violet glass respectively on the front, and covered the Petri dishes containing the moss with these boxes. In comparing the results of different cultures in different rays, all of unilateral light, I found on the 24th of February, 1915, that the moss throve best in the violet, the blue, and the yellow light. In the red, it did not thrive well, and in the green least of all. The formation of starch in the chloroplast in the cell of the protonema I found to be greatest in the blue and in the yellow light, the red ranking next, the green following.

In the violet and the blue, 7—10 days were required for the reflection of the emerald green light to reappear after the change in the direction of the light; in the yellow 10—14 days; in the red a little more; while in the green hardly any light was seen reflected after the lapse of even as many as 20 days. I used for my experiment two sets of the double glass cylindrical apparatus designed by Prof. Miyoshi1) to suit the bubble-counting method, filling one set with an ammoniacal solution of copper hydrate, and the other with a solution of potassium bichromate. In each set, I placed, on the 16th of April, 1915, a Petri dish containing the protonema, covering the former set with a piece of blue glass and the latter with red glass, and pasting black paper on the back of each, I let the light come only from the front. On May 16th, the

---

1) Miyoshi M.: Experimental botany (Japanese edition). 2nd Ed. 1908. p. 415. Fig. 219.

heliotropism of the filaments of the protonema was clearly observable in the dish put in the former bottle (Fig. 5), while in the latter no such phenomenon could be seen. This result coincides with the well-known fact that the heliotropic curvature occurs more strongly in blue light than in any other coloured light.[1]

IV. Influence of Temperature.

Camille Servettaz (**8**) found that a temperature between 16° and 25°C. is most suitable for the species of moss he studied; that those species cease to grow when the temperature is above 45°C.; and that a temperature between 15° and 16°C. is required for the formation of buds and leafy shoots; more heat being required for this than for the development of the protonema. In my observations of *Schistostega* in its natural state, a temperature between 19° and 21°C. seems to suit it best. The temperature in September and October, 1914, was between 15° and 25°C. in the laboratory, and the moss was observed to produce leafy shoots abundantly. The moss placed in my hot house, the temperature of which was carefully kept between 16° and 25°C., continued to produce shoots. I have observed the development to stop at 2°–6°C.

Resistance to lower temperature.—To ascertain the lowest temperature the moss can stand, I took a wide-mouthed cylindrical Dewar flask and put a test tube into it containing the moss with a thermometer inserted. Filling the flask with a mixture of snow and salt, and covering it with a close-fitting lid so as to allow the upper half of the thermometer to protrude from the lid, the temperature of the test-tube began to fall. When it reached

---

[1] Pfeffer, W.: Pflanzenphysiologie, II. Aufl. Bd. II., p. 577. 1904.

$-15°$C., the cells of the protonema were seen to shrink (Fig. 6), but the filamentous cells did not die even at $-20°$C. At about $-18°$, however, the cells of the leaf began to freeze, and finally the shoot itself died. Keeping the temperature from $-12°$ to $-20°$C. for 44 hours; from $-16°$ to $-19°$C. for 20 hours, from $-14°$ to $-20°$C. for 24 hours; and from $-19°$ to $-20.5°$C. for 72 hours by the above arrangement; then taking the moss out and placing it in a hot-house (16°—25°C.): in each case the moss showed again the phenomenon of reflected light after a lapse of 20 or 25 days. From the above experiments, we can infer that *the filamentous protonema has a strong power of resisting low temperatures, and that it can continue to live even at* $-20.5°$*C.*

V. Influence of Humidity.

The humidity of the air has a close relation to the growth of the moss, which thrives best when the air has a humidity of above 90%. When tho air is dry, the shoots die and the growth of the protonema ceases. When the soil is too damp, however, the formation of shoots is very slow. I took two pieces of brick, and covering them with the soil on which the moss had been growing, put each of them in a PETRI dish, filling one with just enough water to keep the brick moist and the other with much water. I then placed the dishes in a hot-house in which the temperature was kept between 16° and 25° (Feb. 1). In the first dish the protonema began its growth in about 10 days. On April 12th, I observed that the shoots had grown over the brick in the first dish, but in the second dish only a few protonemata were visible. As for the shoots, there were so few of them that it was very hard to find them. From the above results, it can be inferred that *the more damp the air is, the better the growth of the* *moss, but that too much dampness of the soil is injurious to its development.*

Resistance to desiccation.—I took a piece of almost entirely dried-up soil from the cave at Yutanaka and examining it under a microscope obtained the pictures as shown in Figs. 7, 8 and 9. In this condition, the filamentous cell of the protonema is very thin, the chromatophores have lost their colour, and the spherical cells are shrunk, with their chromatophores broken or becoming a shapeless mass. The shoots are all dead through desiccation.

I kept a culturing dish in a H_2SO_4 desiccator for 4 weeks, until the reduction of its weight had entirely ceased. Taking it out and placing the dish in a hot-house for three months, supplying it with water all that time, I found the protonema giving out the reflected light again and renewing its development.

This fact has been further ascertained by the recent study of B. M. Bristol (2), who observed that when protonema-laden soil was stored in a sealed bottle so as to prevent the loss of moisture therein contained, the moss became dormant, developing resting filaments. He also observed that when the conditions suitable for the development of the protonema were given,—by putting the soil into nutritive solution,—young colourless filaments were produced from the walls of the resting filamentous cells and small discoid chromatophores appeared with the increase of the length of the filaments. This remarkable rejuvenescence took place even after the moss had been bottled for nearly fifty years.

I put a quantity of soil laden with protonema of the luminous moss in a Petri dish, and kept it there without any supply of water for nearly a year. At the end of that time when I examined the protonema under a microscope, I observed the protonema to have the structure of resting filaments. The cells of these filaments looked like those in Illustration 2: a thickening of the cell walls and the existence of a number of oil globules in the cytoplasm as illustrated in the figure were observed. No spherical cells could be found.

In the vegetative green protonema-filaments, starch is stored for reserve food, while in the cells of the resting filaments, fatty oil, instead of starch, is stored for the retention of vitality.

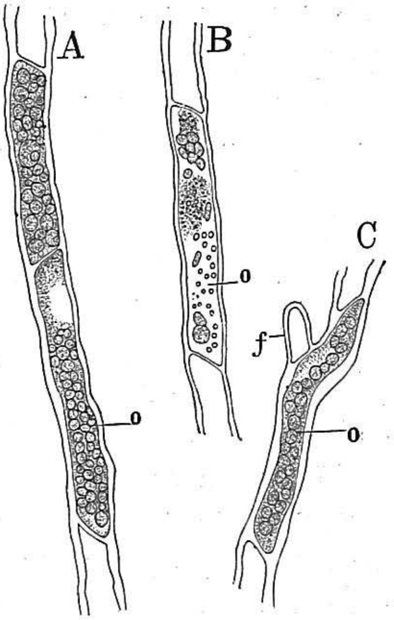


Illustration 2. A. B. Resting filamentous cells filled with oil globules. O. oil globules. C. Resting filamentous cell with a young colourless filament. F. young vegetative filament. ×580.

That the resistance of the luminous moss to desiccation is very great, is evident from the fact that after placing the Petri dish mentioned above, in a hot-house for 20 days, supplying it all the while with sufficient water, young colourless filaments were seen produced from the walls of the resting filamentous cells (Illustration 2, C).

VI. Cultural Experiments.

Camille Servettaz **(8)**, in one of his recent papers, treats of the development and nutrition of various species of moss in sterilized media. To determine what is the best nutritive solution for the culture of *Schistostega*, I took a number of Petri dishes and filled half their depth with quartz sand carefully washed first in fuming HCl; and then in water from the conduit tap, for 24 hours.

I then poured in each dish a different kind of nutritive solution, and planting the moss on the sand, examined its growth in each dish. The kinds of nutritive solution used were:—

a) Solution of Elie and Émile Marchal.[1]

b) Pfeffer's solution.

c) Detmer's solution.

d) Knop's solution.

On the 10th of March I put the above-mentioned Petri dishes, each in a wooden box as described on page 10. On the 15th of May, I observed that the development of the moss in the dish containing the Marchal solution was the best, the protonema producing a large number of shoots and spreading over the whole surface of the sand. In the dishes containing the Knop and Detmer solutions the growth of the moss was not so good, the protonema producing fewer shoots and growing over only half its surface. In the dish with the Pfeffer solution the growth of the moss was observed to be the worst. In all these cases, however, the plant was seen to be thriving, showing that each solution contained more or less the kind of nourishment it required. Again I poured the four different kinds of nutritive solution each on a small quantity of soil taken from the place where the moss had been growing, and found, as I had expected, that the moss throve best on the soil nourished with the Marchal solution.

Liquid culture.—I put the moss into four Erlenmeyer bottles of 100 c.c., with 20 c.c. of the four kinds of solution respectively. From 7 to 10 days later, many adventive protonemata were seen on the various parts of the stem. The shoots floating on the surface of the liquid had produced, down in the liquid, adven-

---

[1] This solution should be neutralized before use.

tive protonemata with only a few spherical cells, and up in the air many lateral innovations. No lateral innovations were observed in the liquid. The Knop and Detmer solutions gave the best result for the growth of the moss.

In comparing the shape of the aerial protonema with that of the aquatic one, I noticed that the filamentous cell was somewhat longer and thinner, and the chlorophyll grain of the cell smaller in the latter than in the former (Fig. 10).

VII. Effect of Lime on the Growth of the Moss.

By the studies of H. Paul (5 and 6), it has been ascertained that all kinds of lime salt are not equally injurious to the *Sphagnum*, an alkaline salt of lime, such as calcium carbonate, being most harmful. Boas (**1**) ascertained that 0.05—0.28% solution of calcium nitrate and of calcium chloride facilitates the growth of some species of moss. We have not been told yet of any case of *Schistostega* growing in a calcareous cave, either in Europe or in Japan. In order to ascertain whether the moss really dislikes lime or not, I put the shoots of the moss in solutions of different concentration, of calcium nitrate, calcium sulphate, calcium chloride, and calcium carbonate, and examined their growth in the each case.

1) Calcium nitrate.—In 0.3% and 0.05% solutions the moss produced a few adventive protonemata in 2 weeks, and in 2 months a few lateral innovations from its stem. The development of the moss was better in a 0.05% solution than in a 0.3% one. In 0.1% and 0.2% solutions, it made the greatest growth, bringing out many adventive protonemata in 2 weeks, and producing numerous lateral innovations from the stem in 2 months. The development was better in a 0.1% than in a 0.2% solution.

2) Calcium sulphate.—In 0.2 and 0.3% solutions, the moss did not produce a single adventive protonema in 2 weeks. After 2 months had passed, it was observed to have produced a few protonemata, but no lateral innovations whatever. In 0.05 and 0.1% solutions, it produced a few adventive protonemata in 2 weeks and a few lateral innovations in 2 months.

3) Calcium chloride.—In a 0.05% solution, the moss produced a few adventive protonemata in two weeks, but then it almost ceased to grow. In 0.1%, 0.2% and 0.3% solutions, the moss produced no adventive protonemata at all in 2 weeks, but after 2 months, the moss in the 0.3% solution was seen to have produced a few lateral innovations. In a 0.2% solution the moss produced more lateral innovations and in 0.1% still more (Fig. 21). Thus 0.1% of calcium nitrate, of calcium sulphate, and of calcium chloride seem to stimulate the growth of the moss to the highest degree.

4) Calcium carbonate.—All the shoots put in 0.3, 0.2, 0.1 and 0.05% solutions produced a few adventive protonemata in two weeks. The moss grew best in the 0.1% solution. After 3 weeks, the shoots in 0.05 and 0.1% solutions were seen to have produced a large number of adventive protonemata. The shoots in 0.2 and 0.3% solutions produced only a few adventive protonemata, and did not make any growth even after 5 weeks; in 0.05 and 0.1% solutions, they produced many adventive protonemata and a few lateral innovations from the stem floating on the surface of the liquid. From these experiments I infer that in this case also 0.05 and 0.1% are the most suitable degrees of concentration.

It seems probable that *Schistostega does not dislike lime, at least not in the above-mentioned concentrations.*

VIII. Germination of the Spore and the Formation of the Protonema.

We already know that with regard to the duration of the germination of the spore there are two kinds of moss, one, whose spore takes from two to six months to germinate, and one, whose spore germinates in a few days after it comes out of the sporangium (C. SERVETTAZ.). *Schistostega* belongs to the former kind, its spores germinating in a month after their dispersal from the sporangium. The spores that I sowed on earthen plates in my hot-house (16°—25°C.) on the 26th of September almost all began to germinate on the 26th of October. The first filament which issued from the spore had a cylindrical form (Fig's. 12 and 13, drawn Nov. 30), and grew by the division of the terminal cell. When this filament consists of two or three cells, a spherical cell is produced from the terminal cell (Fig's. 14 and 15, drawn Jan. 30). Many spherical cells of the same form are produced in a row by budding (Fig. 16, drawn Feb. 13). These spherical cells begin to ramify, producing filaments or cells like themselves (Fig. 18, drawn Mar. 3). It is by this process of ramification that the surface of the substratum is covered with filaments and groups of spherical cells.

At times, groups of cells of the shape of a tadpole and containing many chlorophyll grains are to be found. Some of them are seen separated from the filamentous cells, which, I suppose, can make an independent growth by their own division (Fig. 22).

Summary.

1) The optimum intensity of light for *Schistostega osmundacea* is 0.02—0.002 (BUNSEN's unit), the protonema thriving well even in so feeble a light as 0.0008. The shoot, however, never grows where light is 0.001 or below it. The relative "Lichtgenuss" is $\frac{1}{4}$—$\frac{1}{500}$ and the optimum "Lichtgenuss" of the moss is $\frac{1}{50}$—$\frac{1}{200}$.

2) In a dark place, the protonema can live without producing, for seven months at least, a leafy shoot. In a place where the intensity of light is over 0.1, the moss cannot live.

3) The movement of chromatophores requires a considerable length of time. When the protonema is placed in the light, the chromatophores are scattered in a day, and when the direction of light is changed, they all turn towards it in 7—10 days at a temperature of 15°—25°C.

4) The spherical cell of the protonema seems to enable the moss to effect assimilation in a feeble light.

5) The moss thrives better, and the movement of the chromatophore is faster in a blue and a violet light than in any other visible light except a white light.

6) The optimum temperature for the development of the shoot is 16°—25°C. The protonema will not die so long as the temperature is above −20.5°C., the shoot, however, dying at −18°C.

7) The optimum humidity of air is 90%—100%.

8) The protonema has a strong power of resisting desiccation.

9) ELIE and ÉMILE MARCHAL's solution is the best cultural medium for the moss.

10) Calcium nitrate, calcium sulphate, calcium chloride, and calcium carbonate, each in 0.1% solution, are not only not injurious to *Schistostega osmundacea*, but stimulate its growth to a certain extent.

11) The spore, at a temperature of 16°—25°C., germinates in one month, and its first filament has a cylindrical shape, producing many spherical cells soon after.

Tokyo, June, 1915.

Literature.

1) BOAS, F., (1913), Zur Physiologie einiger Moose. (Hedwigia, Bd. LIV. pp. 14–21.)

2) BRISTOL, M., (1916), On the remarkable retention of vitality of moss protonema. (New Phytologist. Vol. XV, No. 7. pp. 137–143.)

3) MIYOSHI, M., (1912), Über die Kultur der Schistostega osmundacea Schimp. (Bot. Mag. Tokyō. Vol. XXVI, pp. 304–306.)

4) NOLL, F., (1888), Über des Leuchten der Schistostega osmundacea Schimp. (Arb. d. bot. Inst. in Würzburg. Bd. III, pp. 477–488.)

5) PAUL, H., (1906), Zur Kalkfeindlichkeitsfrage der Torfmoose. (Ber. d. deutsch. bot. Ges. Bd. XXIV, pp. 148–154.)

6) ——, (1908), Die Kalkfeindlichkeit der Sphagna und ihre Ursache. (Mitt. der Königl. Bayr. Moorkulturanstalt, Stuttgart. pp. 63–118.)

7) SENN, G., (1908), Gestalts- und Lageveränderung der Pflanzen-Chromatophoren.

8) SERVETTAZ, C., (1913), Recherches expérimentales sur le développement et la nutrition des mousses en milieux stérilisés. (Annales d. sciences naturelles. Tome XVII, pp. 110–221.)

9) VOUK, V., (1912), Ein verbesserter, neuer Wiesnerscher Insolator zur Bestimmung des Lichtgenusses. (Ber. d. deutsch. bot. Ges. Bd. XXX, pp. 391–394.)

10) WIESNER, J., (1907), Der Lichtgenuss der Pflanzen.

Contents.

Published March 31st, 1918.

Y. TODA:
PHYSIOLOGICAL STUDIES ON SCHISTOSTEGA OSMUNDACEA.

PLATE I.

Plate I.

Fig's. 1 & 2. Heliotropism of the shoot of *Schistostega osmundacea* (DICKS.) MOHR.; ↙ first direction of rays; ↙ direction of rays after being turned 180°; ↙ direction of rays after being turned 180° again.

Fig. 3. Position of the chromotophores in the cell of the protonema when placed in the light (Apostrophe).

Fig. 4. Position of the chromotophores in the cell of the protonema when put in a dark room (Apostrophe).

Fig. 5. Heliotropism of the protonema in a blue light; ↙ direction of rays.

Fig. 6. State of the cell of the protonema shrinking at −15° C.

Fig's. 7, 8 & 9. Protonema in a state of desiccation.

7. filamentous cell; 8. tadpole-shaped protonema;
9. lens-shaped cell.

Fig. 10. Protonema cultured in KNOP'S solution.

Fig. 11. Spore.

Fig's. 12 & 13. Cylindrical filament issuing from the spore.

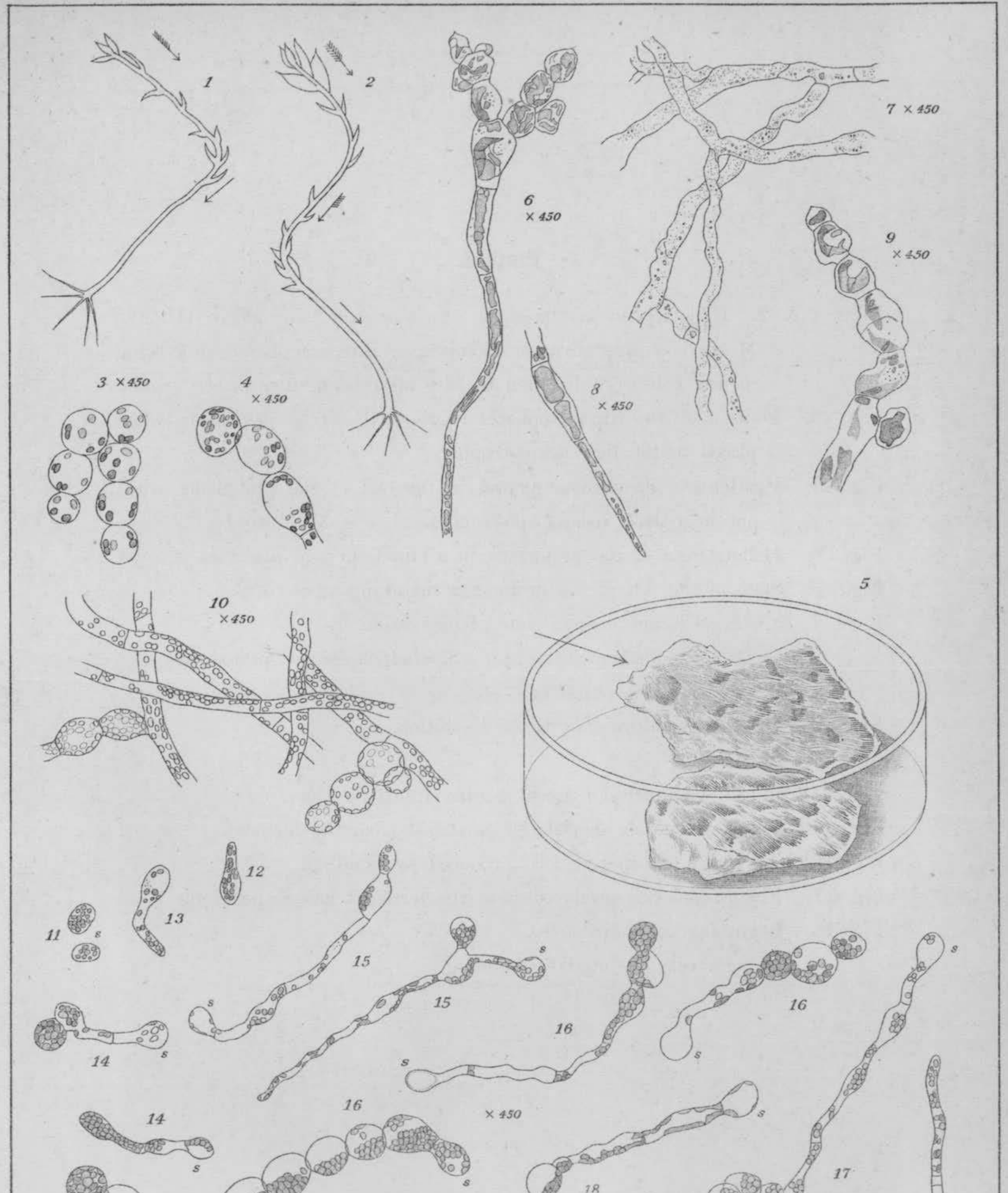
Fig's. 14 & 15. First lens-shaped cell produced from the filament.

Fig. 16. A row of lens-shaped cells produced by budding.

Fig. 17. Filamentous cell produced from the terminal lens-shaped cell.

Fig. 18. Beginning of ramification.

b) new cell produced by budding.


1
2
6
× 450
7 × 450
9
× 450
3 × 450
4
× 450
8
× 450
5
10
× 450
12
11
s
13
15
15
s
s
14
s
16
s
16
s
16
s
14
s
16
× 450
s
18
17
16
s
16
18
s
b
s

Y. Toda del.

Y. TODA:
PHYSIOLOGICAL STUDIES ON SCHISTOSTEGA OSMUNDACEA.

PLATE II.

Plate II.

Fig. 19. Photomorphic action of the filamentous protonema of *Schistostega osmundacea* (Dicks.) Mohr.; ↙ first direction of rays; ⇙ direction of rays after being turned 180°.

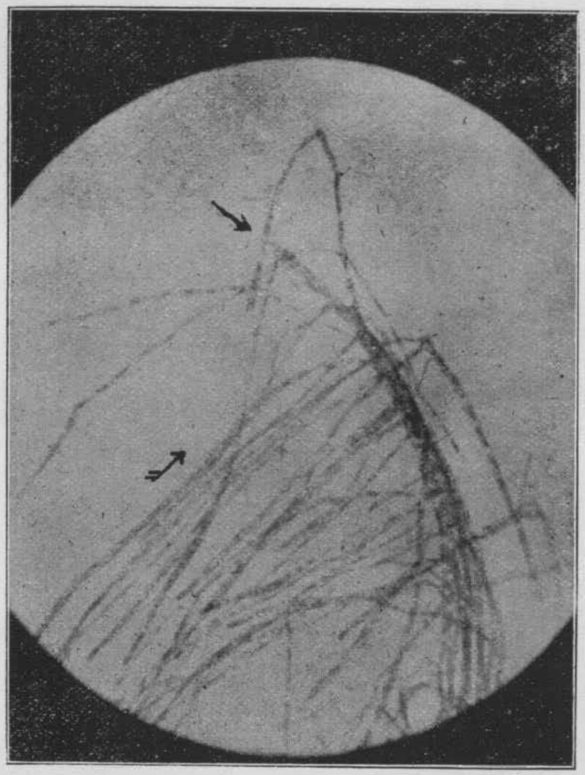
Fig. 20. Normal position of the chromatophores in the cell of the protonema in its native place (Escharostrophe).

Fig. 21. Shoot put in a 0.1% solution of calcium chloride for 2 months.

Fig. 22. Tadpole-shaped protonema.

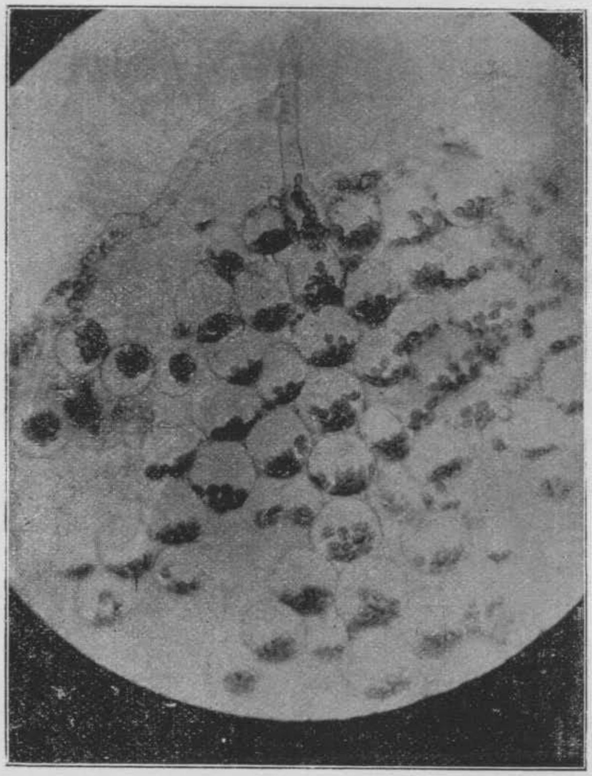
a) one that is separated from its mother cell.

19.



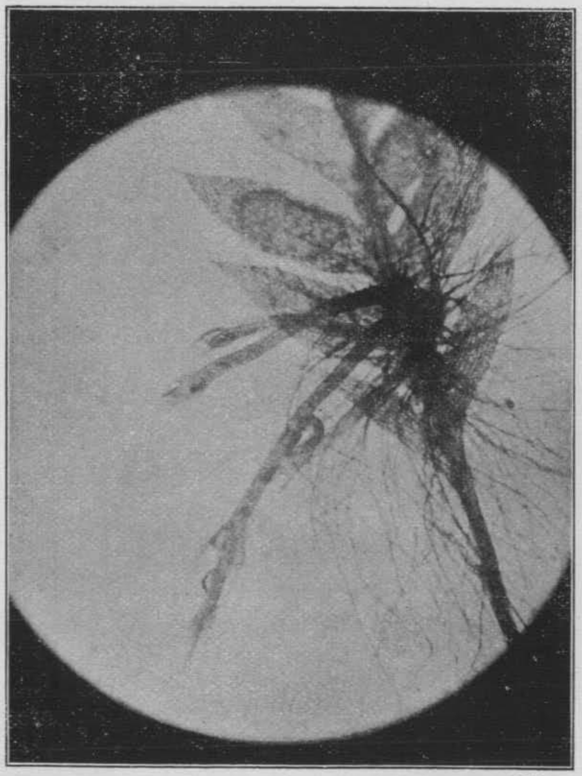
×325

20.



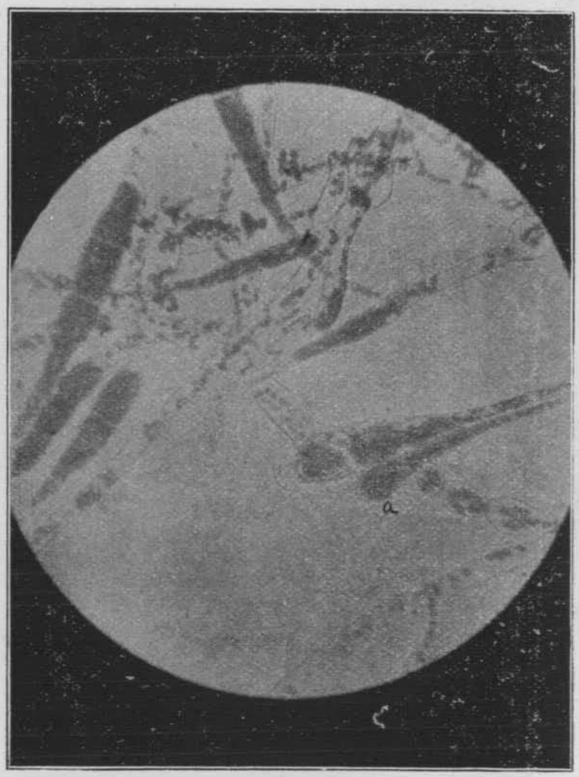
×580

21.



×131

22.



×508

Y. Toda photo.