THE GEOGRAPHICAL DISTRIBUTION AND GROWTH HABITS OF *HAPLOMITRIUM*[1]

by

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The genus *Calobryum* Nees 1836 belongs to the family *Calobryaceae* of the order *Calobryales* (Campbell, 1920) and is characterized by having a fleshy, rhizome-like basal portion, having three-ranked leaves, radially placed on erect stems, and lacking rhizoids.

A change in nomenclature, combining *Calobryum* with the monotypic genus *Haplomitrium* 1833 of the *Haplomitriaceae* and of the same order, was suggested by Schuster[26], based on the opinion that very little generic difference exists between them. This suggestion was supported by a group of hepaticologists: Fulford, Hattori, Inoue and Grolle who, the last named, transferred the species of *Calobryum* under *Haplomitrium*[9]. The seven species of *Haplomitrium* with their geographical distributions are listed as follows:

1. *H. hookeri* (Smith) Nees: Europe, Spitzbergen, USA (New Hampshire).
2. *H. intermedium* Berrie: South Australia
3. *H. gibbsiae* (Steph.) Schust: New Zealand
4. *H. blumii* (Nees) Schust: Java, Sumatra, New Guinea, Taiwan.
5. *H. andinum* (Spruce) Schust: Peru, Ecuador, Lesser Antilles.
6. *H. giganteum* (Steph.) Grolle: Phillippines.
7. *H. rotundifolium* (Mitt.) Schiffnia: Japan, Taiwan.

H. blumii was found on Ali-shan in 1952, 1965, and 1966 but it was not identified as such until the present time. So Taiwan has 2 species, *H. rotundifolium* and *H. blumii*. The former species is characterized by having rounded leaves with archegonia restricted to the lower calyptra; while the latter has slightly pointed leaves, especially the bracts, and with archegonia borne on both the lower and upper calyptra.

H. rotundifolium is found on Yang Ming Shan and at Chi-tou, at altitudes of 1000 m. and extending to 2200–2400 m. on Ali-shan and Taiping Shan. *H. blumii* occurs mostly on Ali-shan alt. 2400–2600 m and occasionally it has been also found on Taiping Shan. Both Taiwan species are found on moist forest floors, steep clay banks, in semi-shade or on sheltered ledges and along the steep-sloping roadsides leading to Chu-shan where the Forestry Experimental Station of NTU is located.

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H. rotundifolium and *H. blumii* are commonly associated with *Scapania bolanderii, S. ligulata* Steph., *Frullania tamariscii* (L.) Duns. subsp. *moniliata, F. squarrosa, Fissidens nagasakinus* Besck, *Hookeria acutifolia* Hook. ex Schwaegr., *Pogonatum Teysmannianum* (Doz. & Molk) Bryol. Jav. and *Sphagnum* sp.. The covering forests consists of *Yusania niitakaymeonsis,* at the back of the Ali-shan hostel and the trees represented there are *Chamaecyparis formosensis, C. taiwanensis* and *Cryptomeria japonicus.*

The average pH of 16 soil samples tested is between 5.4–5.6.

The germination of the spores of *Haplomitrium* has been studied by several workers. It was found that the development of the sporeling was slow and difficult. Campbell[4] found the 7-celled stage in her germination of the spores of *H. gibbsiae*; and Nehira[23] found a globose mass of 16 cells from the spores of *H. rotundifolium.* In the present study, the writer attempted the germination of the spores of *H. rotundifolium* collected from Taiping Shan, and they revealed a much further development of sporelings than those of the previous workers. Ours reached a larger, many celled thallus stage which is somewhat triangular in shape and with a slight differentiation of meristematic regions (Pls. II, III).

In order to know more about the growth habits of *Haplomitrium* the writer has been maintaining cultures of living specimens in a controlled laboratory.

MATERIALS AND METHODS

1. Materials of *H. rotundifolium* and *H. blumii* collected from Ali-shan have been used for establishing of a living culture for the present study. Spores of *H. rotundifolium* for germination were selected from the collection made in March 1966 from Taiping Shan.
2. The medium used for establishing the living culture was originally developed by White 1943, given in Hatcher's[10], while a nutrient solution, after Arnon and Hoagland's 1940 was used for the germination of the spores of *H. rotundifolium.*
3. In order to maintain the living specimens of *Haplomitrium* in good condition, material should be immediately cleaned and isolated from mixed plants or debris after being brought into the laboratory and then washed and flushed thoroughly with demineralized water before they are separated and planted into covered glass jars (4–7 inches in diameter and of various heights). Regular watering of a moderate amount should be applied once in every 3 days. All the specimens were placed on racks at 20°C, with 10 hours of daily illumination, and approximately 1200 lux of light intensity. Plants thus treated appear to be very fresh and green after a period of two weeks and have continued so thereafter.
4. Techniques for the selection and disinfection of the spores, as well as the petri-dishes and other articles to be used for germination have been described in Hatcher[10]. The sterilized spores were sown quickly on the surface of Hoagland's solution in sterilized petri-dishes to avoid any possible chance of contamination,

these dishes were placed on racks as previously mentioned and grown under 24 hours of continuous illumination.

OBSERVATIONS AND RESULTS

1. The cultures established since March 1965 are still growing vigorously producing crops of young gametophytes in abundance, each arising asexually from the apices of the old branches. No sexual reproduction has been observed. However, this spring, two well formed sporophytes, fantastically appeared shooting up in two separate culture jars from the collection made October 1966. Besides these two, no other sporophytes or any indication of sexual reproduction was ever observed in these cultures.
2. Antheridia on male gametophytes in excellent condition at first, began to fade gradually. Archegonia borne on stem tips of the female gametophytes were also found. Some female plants developed the so called "shoot calyptra" derived from the calyptra, plus the stem immediately surrounding the fertilized archegonia, Fulford[6]. Several archegonia were observed, protruding here and there from the surface of the "shoot calyptra" which is usually about 5–10 mm. high. (Pl. IV, fig. 1, 2)

   Close observations revealed that most of these archegonia degenerated and showed no indication of growth at all. They are considered primitive archegonia and did not proceed to maturity.
3. When the spores were sown in the Hoagland's solution, no immediate, observable reactions could be seen. It was not until one week afterward, that they began to float on the surface of the solution, and became swollen. Their color turned from brownish to bright green with the appearance of the conspicuous chloroplasts. The first division of the cell into two almost equal sized-cells occurred about three weeks after the sowing of the spores (Pl. II, Fig. 2). In the germination of the spores of *H. blumii*, this 2-celled stage occurred only 4 days after sowing. Both Campbell[4] and Nehira[23] reported that the sporeling developed outside the exospore but in the present study this was not distinguishable due to too many cells in the sporeling, nor could I see the mucilage hair that Campbell referred to in her paper. Pls. II, III, figs. 1–17 show the series of developments that occurred in the sporelings of *H. rotundifolium* in our laboratory within a period of 87 days. The first stages showed very slow progress. It took 4–5 weeks to attain the globose-mass-cell stage. Then active divisions of the cells seemed to take place. They divided repeatedly until a thallus structure of triangular shape was formed at the end of 87 days. Differentiation of cells seemed to occur at the ends of the triangular thallus. Nevertheless, the activity of cells became retarded soon after reaching this stage, and finally they showed no further development beyond this although they remained alive for a long while.

DISCUSSION AND CONCLUSIONS

1. A living culture of *Haplomitrium* can be maintained in a controlled condition at 20°C and 10 hours daily illumination, approximately 1200 lux of light intensity.
2. Vigorous growth of these living cultures can be maintained indefinitely. Crops of young gametophytes have been produced in abundance, mostly arising asexually from the apices of female gametophytes or the branches of the rhizome-stem. They produced no sporophyte except in two occasions which were from a recent collection made last October. This may indicate that fertilization in *Haplomitrium* takes place in late summer or autumn and sporophytes do not develop until next spring[2]. But why no other fertilized eggs gave rise to sporophytes is not known. It is not likely, in a cluster of female plants, that only two of them were fertilized. Plants developed under such controlled conditions are much smaller than those growing in nature.
3. Primitive archegonia developed on the surface of the "shoot calyptra" were nonfunctional. They were found in *H. blumii*.
4. For the germination of spores of *H. rotundifolium*, Hoagland's nutrient solution seems to be a favorable medium. In addition to the development of a protonema of globose-mass-cell stage, a complex differentiating thallus has resulted. This stage of the sporeling has not been reported by previous investigators. It is far beyond the 7-celled stage found by Campbell[4] or the globose-mass cell stage by Nehira[23]. The question why there is no further development of the sporelings than to that of a triangular thallus resulting in the present study, can be answered perhaps by more replications of this experiment when more spores of *H. rotundifolium* are available. Spores of *H. blumii* are now in the process of germinating. The two-celled stage was found as soon as 4 days after sowing.
5. An examination of the chromosome number of *H. rotundifolium* in Taiwan has revealed that N=9 which agrees with *H. rotundifolium* in Japan (Tatuno, 28) and *H. gibbsiae* in New Zealand (Campbell, 4).
6. Based on the morphology of the oil bodies possessed in the leaf cells of *H. rotundifolium* and *H. blumii*, little or no differences can be drawn between them. But the age of plants does have a bearing on the presence of the types and the arrangement of oil bodies. In the younger leaf cells, both elongated and spherical bodies are present (Pl. V, figs. 4, 5) while in older cells only the spherical ones are present, and these are usually clustered toward the periphery of the cell, leaving a clear space in the center, the elongated ones are lacking. (Pl. V, 3, 6).
7. Both *H. rotundifolium* and *H. blumii* prefer wet habitats in deep shade and grow in Taiwan at high altitudes.
8. No sporophytes of *H. rotundifolium* or *H. blumii* were found in the material collected May 20–22 on Ali-shan. This may indicate that the fruiting season for *H. blumii* is between late February and March or early April.

9. It has been found that *H. blumii* is more commonly distributed in Taiwan than *H. rotundifolium*, yet it has not been previously recorded. And this is *H. blumii's* first record in Taiwan.
10. The species of *Haplomitrium* are widely distributed: ranging from the arctics to the antarctics. In the north they are found from Spitzbergen to Europe and North America (New Hampshire); in the tropics they are found in the West Indies, Taiwan and Philippines; and reaching to the equatorial region in Indonesia, Ecuador and Peru, and in the far south they are known from Southern Australia and New Zealand.

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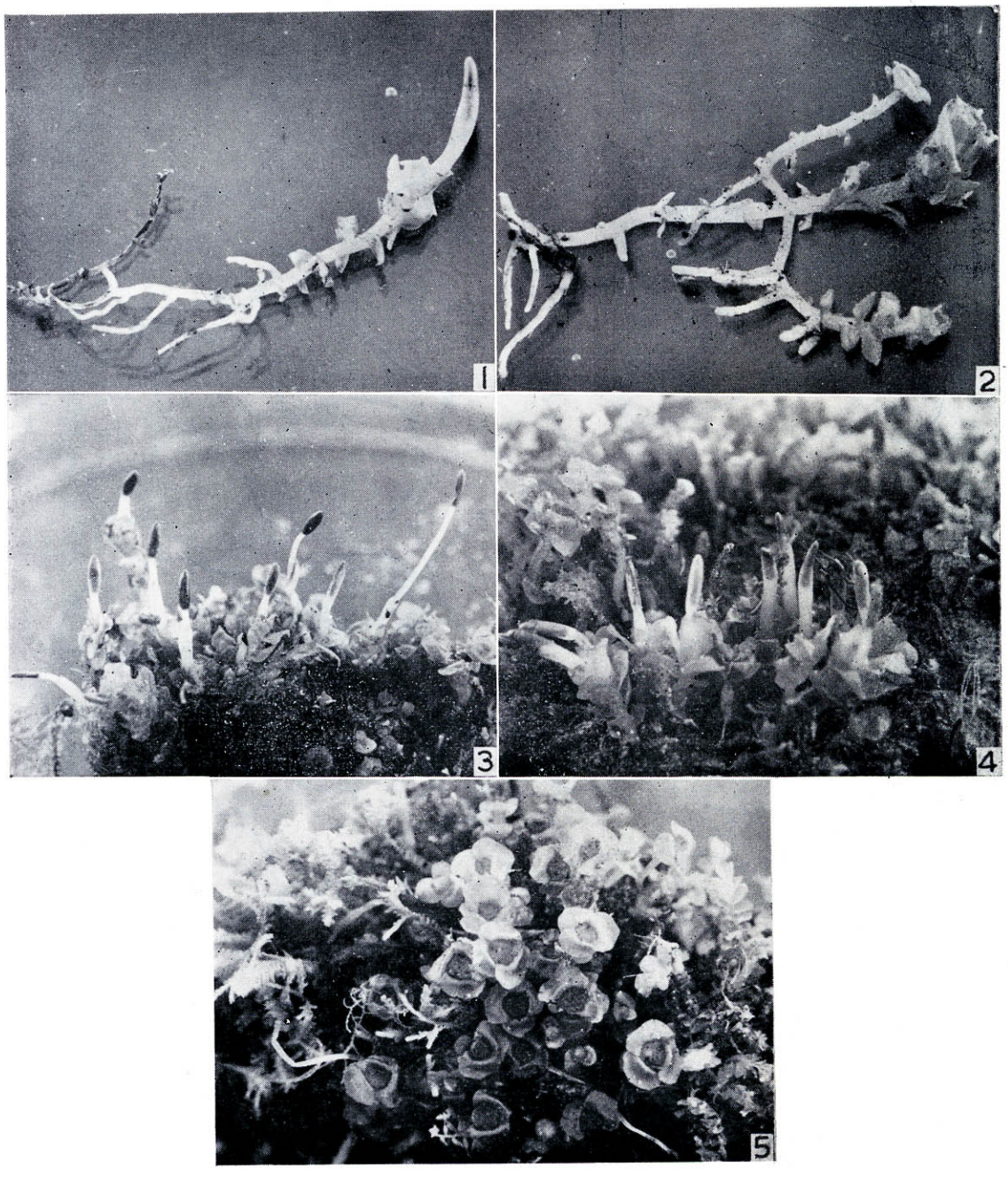
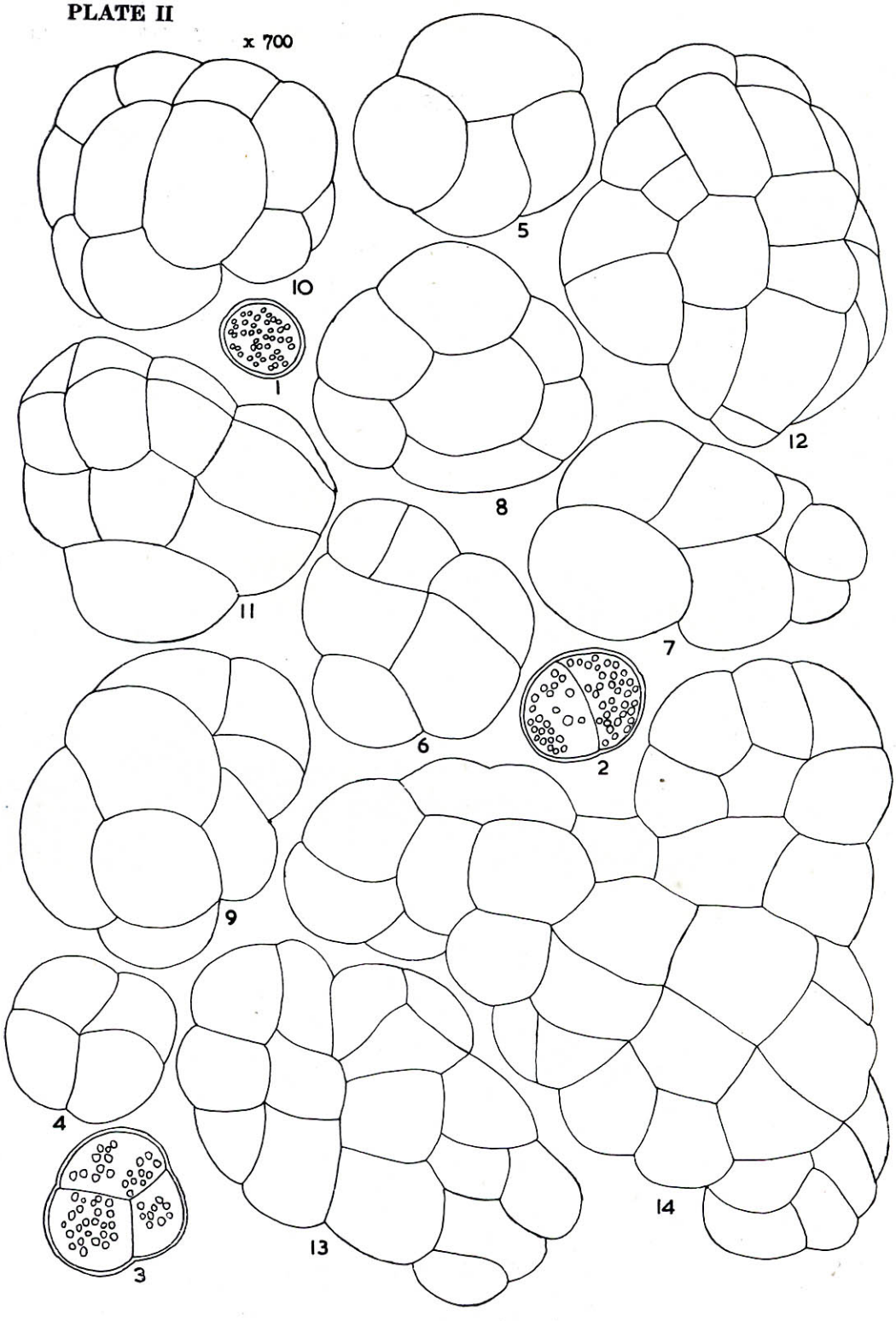
PLATE I



Haplomitrium blumii (Nees) Schust.

1. A female gametophyte ×1. 2. Male gametophyte with 2 young plants arising from the rhizome-like branches ×1. 3. Female plants with mature sporophytes ×1. 4. Ditto, sporophytes in younger stage ×1. 5. Male plants showing distinct antheridial heads surrounded by 3 perichaetial leaves ×1.

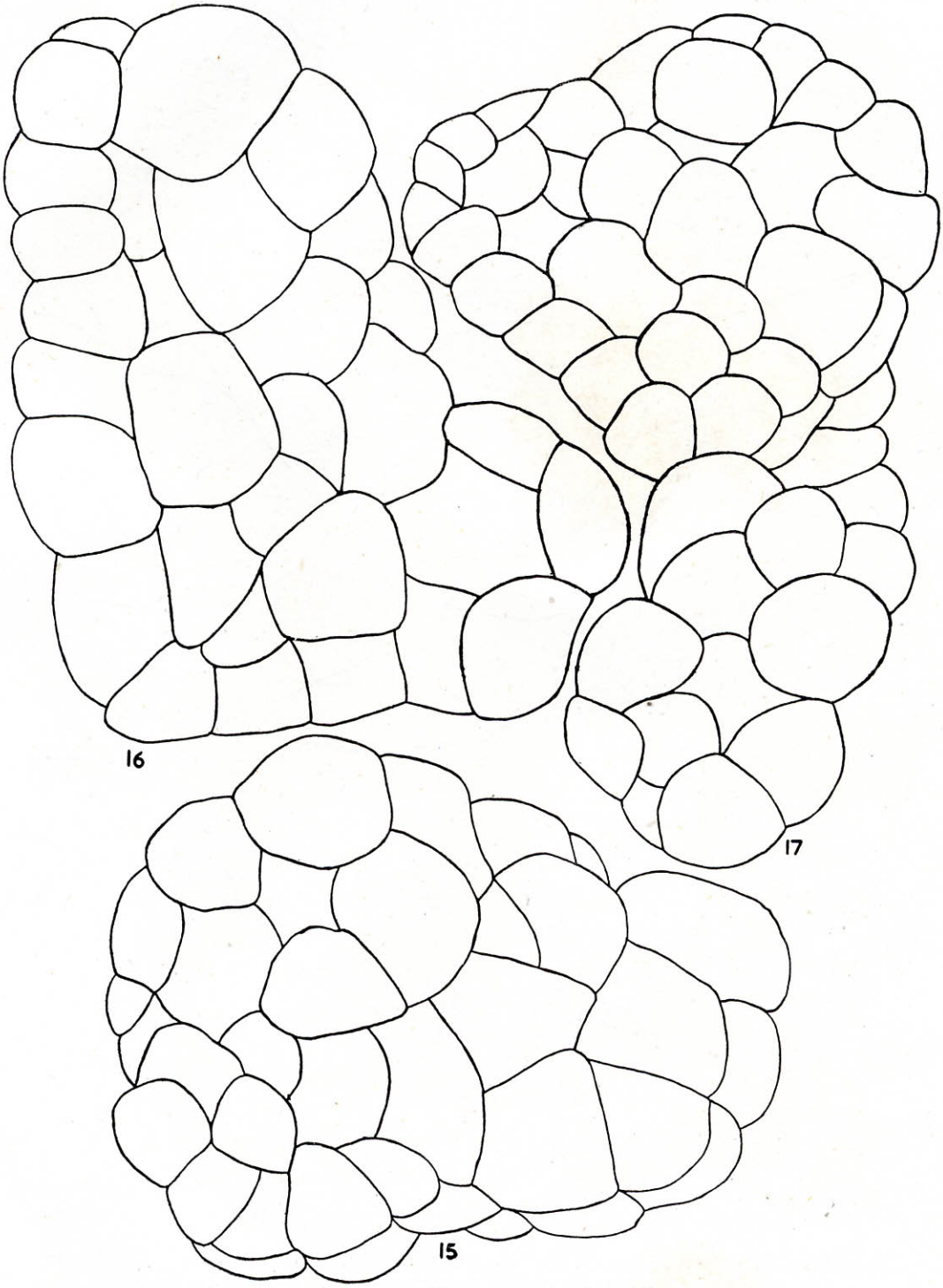
PLATE II



Haplomitrium rotundifolium, germination of spores

1. A spore, one week after planting showing conspicuous chloroplasts. 2. 2-celled stage. 3, 4. After second division resulting 4-celled stage. 5-8. A globose-mass-celled stage. 9-13. Further divisions of the spore, resulting a mass of many-celled sporelings. 14. Later development showing slight different differentiations into a triangular form.

PLATE III



Haplomitrium rotundifolium, germination of spores

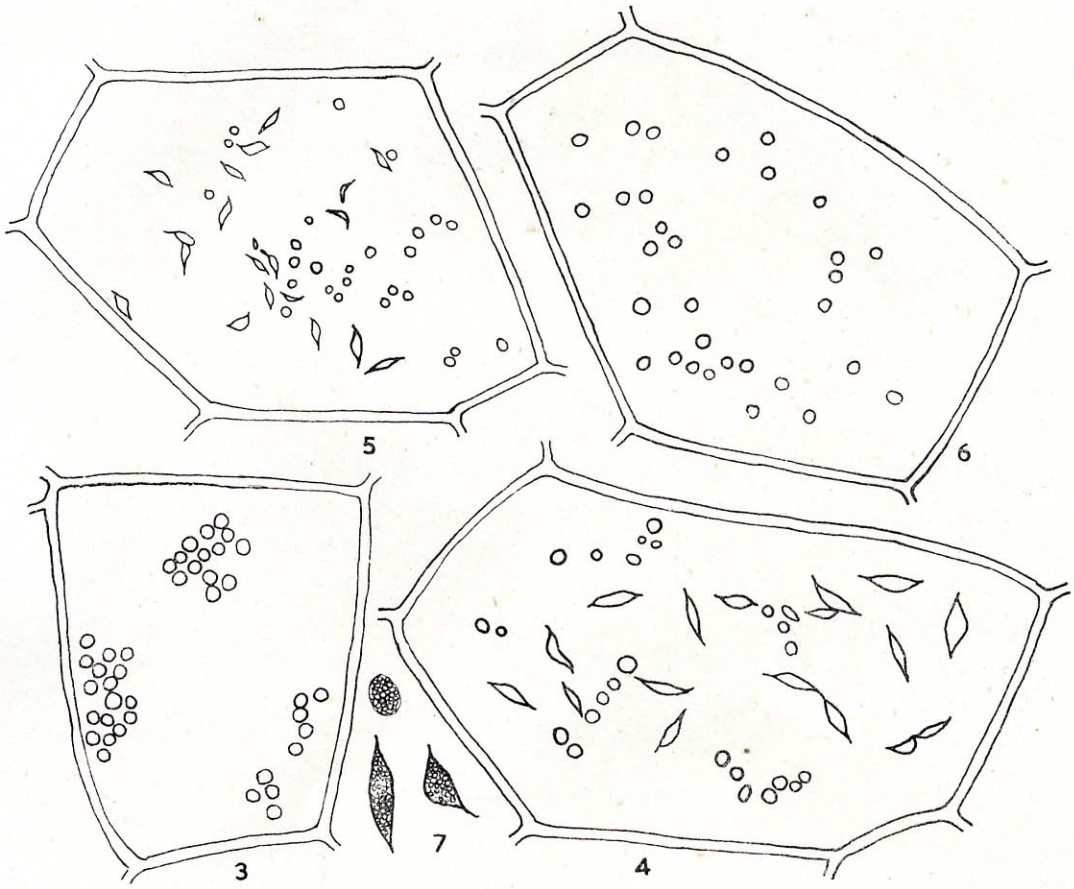
15–17. Later development showing various triangular-shaped sporelings.

PLATE IV

Haplomitrium blumii

1, 2. Apices of two female plants. a. shoot calyptra. b. primitive archegonia.

PLATE V



Heplomitrium rotundifolium, showing oil bodies in leaf cells.

3, 6. showing spherical oil bodies in older cells ×1080. 4, 5. younger cells containing both spherical and elongated bodies ×1080. 7. oil bodies ×2700.