

## The Young Stages in Some Species of the Japanese Aneuraceae (Hepaticae)

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**Abstract** Germination of spores or gemmae were studied on seven species of the Aneuraceae in Japan. Sporelings of *Aneura maxima*, *A. pinguis*, *Riccardia kodamae*, *R. miyakeana* and *R. planiflora* primarily develop thalloid protonemata, as do the gemmalings of *Riccardia miyakeana* and *R. planiflora*. Sporelings of *Riccardia crassa*, *R. jackii*, *R. miyakeana* develop filamentous-thalloid protonemata. The oil-bodies of young stages are similar to those of mature thalli, suggesting that the oil-bodies may be stabilized within species.

**Key words:** *Aneura*, *Riccardia*, oil-body, young stage.

Two patterns of the young stages, which occur through the germination of spores or gemmae, have been observed in the family Aneuraceae, as 1) primarily cylindrical protonema, and 2) primarily filamentous protonema and secondarily cylindrical protonema (Leitgeb, 1877; Clapp, 1912; Nehira, 1962). Nehira (1983) named the *Palavicinia*-type for the former and the *Riccardia*-type for the latter.

Although the oil-bodies of the thallus may be taken as an important taxonomic character in the family Aneuraceae (Mizutani and Hattori, 1957; Schuster, 1987), there has been little investigation of the oil-bodies of young stages except for Hässel de Menéndez (1967). She studied sporelings of some South American species of this family, and illustrated the oil-bodies of *Riccardia umbrosa*.

In the present study, germination of spores or gemmae in some species of the Japanese Aneuraceae are observed, and the comparison of oil-bodies of young stages with those of mature thalli is made.

### Materials and Methods

The materials used in this study are the ripe spores or gemmae of the following species.

1. *Aneura maxima* (Schiffn.) Steph.: Japan, Honshu, Hiroshima-ken, Ryûzu-kyo Gorge, April 18, 1987, coll. T. Furuki, no. 7106 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.
2. *Aneura pinguis* (L.) Dum.: Japan, Kyushu, Miyazaki-ken, May 3, 1988, coll. T. Furuki, no.

7300 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.

3. *Riccardia crassa* (Schwaegri.) Carr. et Pears.: Japan, Yakushima Isl., March 5, 1987, coll. T. Furuki, no. 7076 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.

4. *Riccardia jackii* Schiffn.: Japan, Honshu, Hiroshima-ken, Ryûzu-kyo Gorge, April 18, 1987, coll. T. Furuki, no. 7099 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.

5. *Riccardia kodamae* Mizut. et Hatt.: Japan, Yakushima Isl. March 5, 1987, coll. T. Furuki, no. 7079 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.

6. *Riccardia miyakeana* Schiffn.: Japan, Honshu, Hiroshima-ken, Ryûzu-kyo Gorge, coll. T. Furuki, no. 7106 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.

7. *Riccardia planiflora* (Steph.) Hatt.: Japan, Yakushima Isl., March 5, 1987, coll. T. Furuki, no. 7098 in HIRO, dup. in the herbarium of the Natural History Museum and Institute, Chiba.

The mature capsules were sterilized for one minute in 70 % ethyl alcohol, and washed with autoclaved water. The spores were tapped over 1% agar medium of one-half-strength Knop's medium (pH 6.0-7.0) in a test tube. The gemmae were washed with autoclaved water several times and tapped over the above medium. The experiment was carried out at a temperature of  $20 \pm 2^\circ\text{C}$  under a light intensity of 2000-2500 lux obtained from white fluorescent tubes with 12 hours diurnal light-dark cycle.

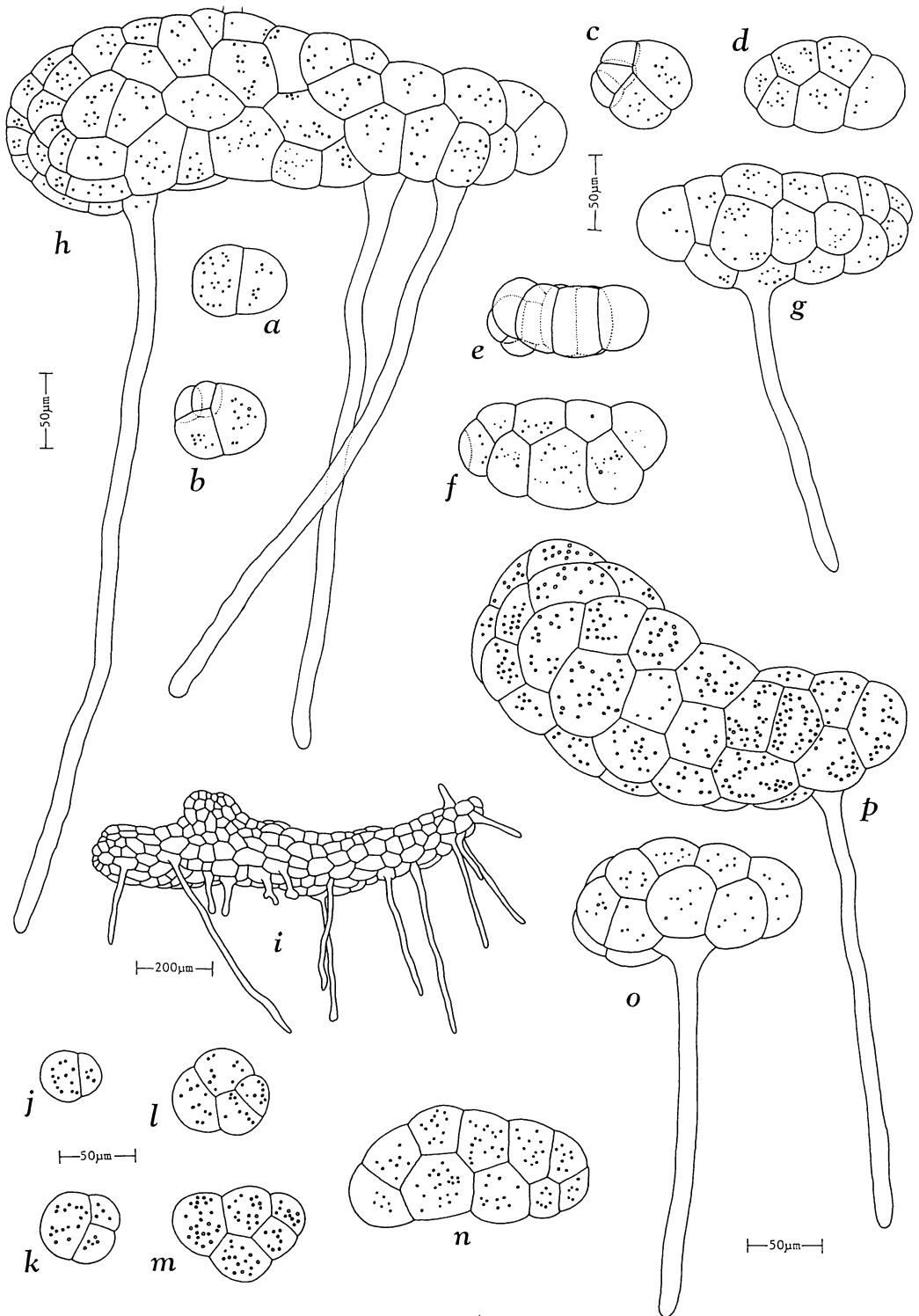


Fig. 1. Sporelings of *Aneura maxima* (a-i) and *Aneura pinguis* (j-p). (e, g-i, o, p, lateral view).

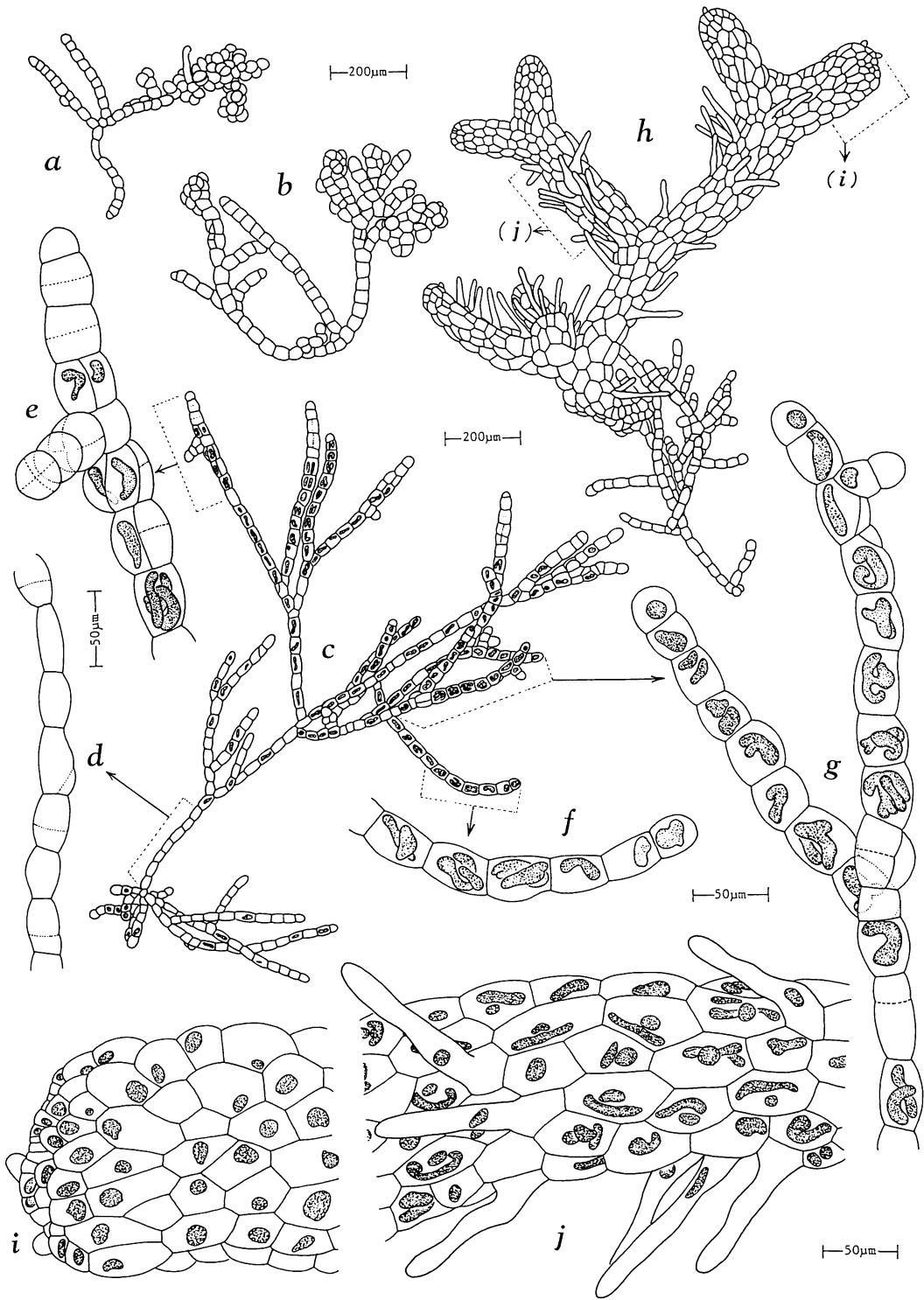


**Fig. 2.** Sporelings of *Riccardia crassa*. a, b, filamentous protonemata just developing into thalli. c, multi-branched filamentous protonema. d-g, parts of c. h, the phase of development into young plant. i, j, parts of h. (i, dorsal view, j, ventral view).

### Observations

The exosporous germination of spores is found in all species. The detailed descriptions of development of germination in *Aneura* and *Riccardia* are available in Leitgeb (1877), Clapp (1912), Nehira (1962, 1966) and Ono (1978). In the present study, I shall describe briefly the stages of germination of spores or gemmae.

#### 1. *Aneura maxima* (Fig. 1a-i)

On germination the spore enlarges slightly. The first wall is formed crossing the major axis of the ovoid cell (Fig. 1a). The second wall occurs obliquely to the preceding walls. By further division a cylindrical or ovoid protonema forms (Fig. 1b-i). This species is constantly thalloid at the young stages. The oil-bodies are spherical, drop-like, hyaline, ca.  $1\mu\text{m}$ , 5-30 in each cell. The rhizoids form from the ventral epidermal cells of thalli (Fig. 1g-i).

#### 2. *Aneura pinguis* (Fig. 1j-p)

The protonema development is similar to that of the previous species. The young protonemata become constantly cylindrical thalloid (Fig. 1j-p). The oil-bodies are spherical, drop-like, hyaline,  $1-2\mu\text{m}$ , 10-30 in each cell. The rhizoids only form from ventral epidermal cells of thalli.

#### 3. *Riccardia crassa* (Fig. 2a-j)

The spore stretches slightly and the first wall forms two equal cells. A second division occurs in each daughter cell with formation of a transverse wall. Therefore the protonemata become filamentous (Fig. 2a-g). The filamentous protonema is much branched (Fig. 2a-c) and the cells of filamentous protonemata, either intercalary or terminally, are sometimes divided by transverse, longitudinal or oblique walls (Fig. 2d, e, g). An apical cell of a filamentous protonema begins to function and gradually develops into a thalloid protonema through both transverse and longitudinal divisions (Fig. 2h). The thalloid protonemata have rhizoids on both dorsal and ventral surfaces (Fig. 2h, j). The oil-bodies of filamentous protonemata are usually ellipsoidal to vermiform, brown,  $25-50 \times 7.5-12.5\mu\text{m}$ , 1-3 in each cell (Fig. 2e-g). The dividing cells frequently lack oil-bodies (Fig. 2d, e, g). The oil-bodies of thalloid protonemata are the same as those of the filamentous ones (Fig. 2i, j).

#### 4. *Riccardia jackii* (Fig. 3a-j)

The filamentous protonemata composed of several cells are formed first (Fig. 3a-c). Secondly, cylindrical protonemata develop in the sporeling. The oil-bodies are spherical, drop-like, hyaline, 2-10 in each cell of filamentous protonemata and protonemata are cylindrical at first (Fig. 3a-d). The cylindrical protonemata gradually develop into young plants. The large, ovoid oil-body is found in the young plants bearing mucilage papillae on the ventral surface of the thalli (Fig. 3e). The young plants initially have a large, ovoid oil-body and drop-like oil-bodies in each cell (Fig. 3f, g). However, the epidermal cells of the midhallus gradually lack oil-bodies (Fig. 3h-j). The rhizoids only form from ventral epidermal cells of thalli.

#### 5. *Riccardia kodamae* (Fig. 4a-g)

This species shows unistratose thalloid protonemata (Fig. 4a-f) that gradually develop into multicellular young thalloid plants (Fig. 4g). The oil-bodies are spherical to ellipsoidal, brown, composed of indistinct to distinct globules,  $7.5-10 \times 7.5-15\mu\text{m}$ , 1-2 in each cell. The rhizoids form from the ventral epidermal cells of thalli.

#### 6. *Riccardia miyakeana* (Fig. 5a-k)

This species can have both thalloid (Fig. 5b, c, i) and filamentous (Fig. 5d, e, g, h) sporelings and gemmalings. The daughter cell within the sporecoat contains an oil-drop which is ovoid, homogeneous, hyaline,  $7.5-12.5 \times 7.5-15\mu\text{m}$  (Fig. 5a, d). The oil-bodies are spherical, drop-like, hyaline,  $1\mu\text{m}$ , 5-20 in each cell of filamentous or thalloid protonemata.

#### 7. *Riccardia planiflora* (Fig. 5l-s)

The thalloid protonemata are initially developed in both sporelings (Fig. 5o-r) and gemmalings (Fig. 5s). The oil-bodies are homogeneous or composed of indistinct globules, hyaline, spherical to ovoid,  $1-2 \times 1-5\mu\text{m}$ .

### Discussion

*Riccardia miyakeana* exhibits both thalloid and filamentous sporelings and gemmalings. In *Riccardia* the period of the filamentous stages seems to be variable, such as *R. miyakeana*, *R. crassa* and others (Nehira, 1962). The developmental patterns seem to be modified by environmental conditions (Leitgeb, 1877; Clapp, 1912; Fulford, 1956; Schuster, 1966; etc.), and Inoue (1959) discussed that the modification may be induced by

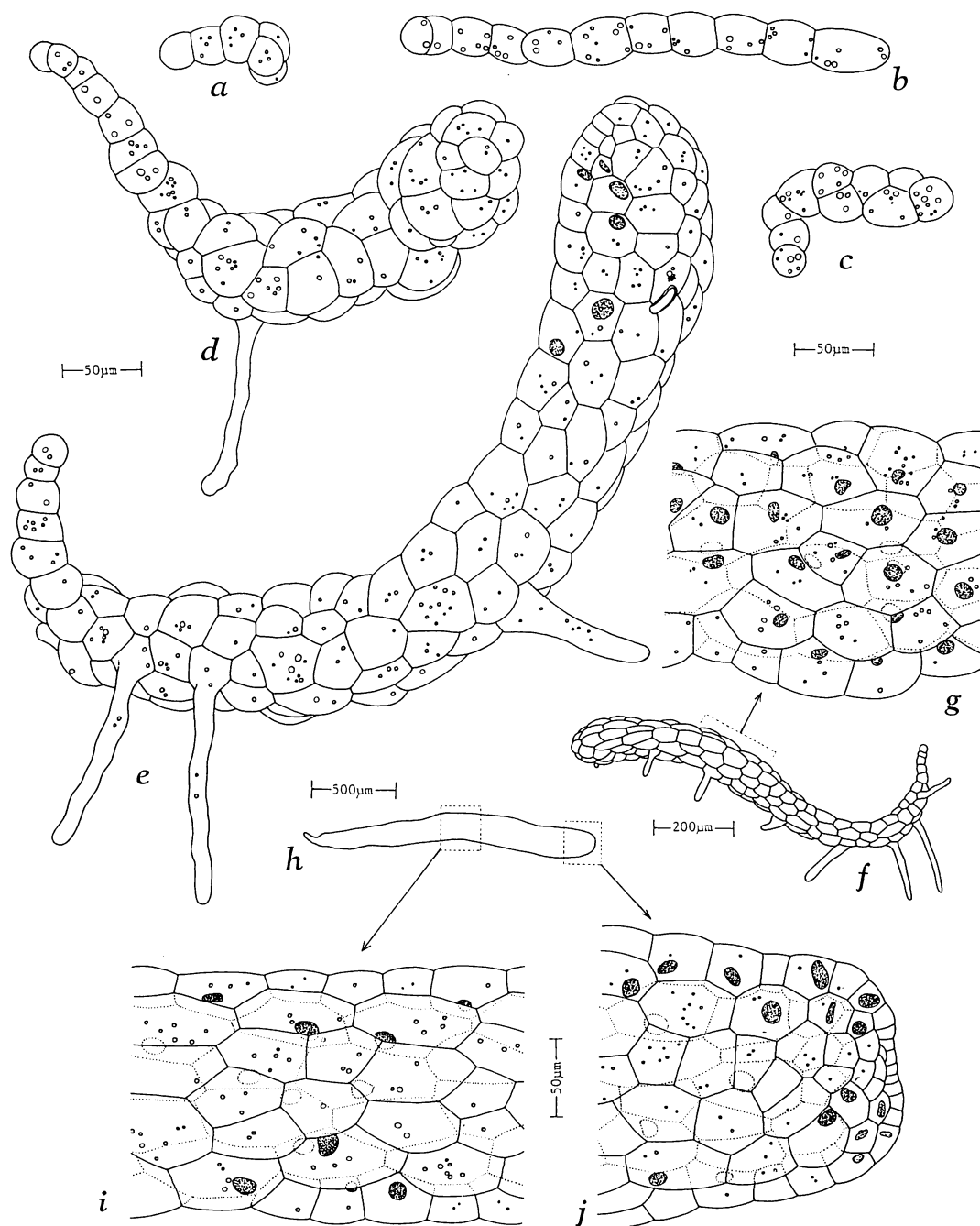


**Fig. 3.** Sporelings of *Riccardia jackii*. a-d, development from filamentous protonema into thaloid protonema (d, lateral view). e, the phase of development into young plant (lateral view). f, h, young plants. g, part of f (dorsal view). i-j, part of h (dorsal view).

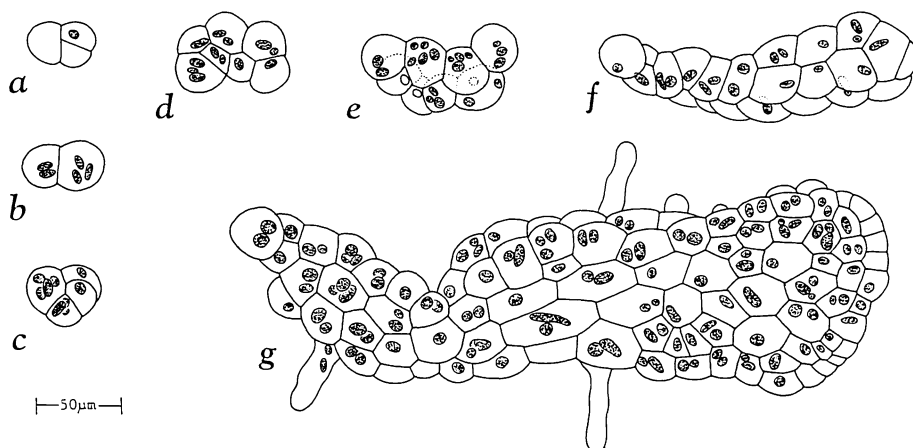


Fig. 4. Sporelings of *Riccardia kodamae*.

light intensity. In the gemmalings in species of *Riccardia*, Ono (1978) observed that two patterns occurred within the same species, and concluded the two patterns, *Pallavicinia*-type and *Riccardia*-type, were identical and the second pattern was caused by rather high intensity of light.

The sporelings of *Aneura maxima* and *Riccardia kodamae*, which are of the *Pallavicinia*-type, are similar to the *Metzgeria*-type. Nehira (1966, 1983) stated that the germination of *Metzgeria* differs from that of *Aneura* by a strap-shaped, two-cell-wide protonema with a two-cutting-faced apical cell. However, the protonemata of *A. maxima* and *R. kodamae* are sometimes initially unistratose with two cells in breadth. The mature thalli of *Aneura* and *Riccardia* also have an apical cell with two cutting faces (Renzaglia, 1982). The mature thalli of *Metzgeria* have a unistratose wing with a narrow multicellular midrib, although those of *Aneura* are multicellular, with or without a unistratose wing. In most thalloid hepatics the protonemata develop gradually into the gametophores, and it is difficult to distinguish protonemata from gametophores (Schuster, 1966). I consider that *Metzgeria* has a longer phase of unistratose thallus condition than does *Aneura* or *Riccardia* at young stages. Nehira (1966, 1983) also described that *Metzgeria* sometimes has filamentous protonemata with few cells. I feel there are no fundamental differences between young stages of *Metzgeria* and those of Aneuraceae.

The multi-branched protonemata, found in

some species of *Riccardia*, are very unique in Hepaticae. Schuster (1966) stated that in all thallose and all specialized leafy genera, each protonema normally produces only a single mature gametophyte. However, the protonemata of *Riccardia crassa* and several other species tend to branch frequently, and apical cells of branches develop into young plants (Fig. 2b). I conclude that sporelings of Aneuraceae are exosporous, and the plants develop through the thalloid stage with or without branched filamentous stages.

In *Aneura maxima*, the oil-bodies (ca.  $1\mu\text{m}$ ) in young stages are slightly smaller than those of mature thalli ( $2\text{--}4\mu\text{m}$ ), but they are nearly homogeneous, as are those of mature thalli. The oil-bodies of young stages of *A. pinguis* are smaller and more homogeneous than those of mature thalli ( $2\text{--}8\mu\text{m}$ , composed of indistinct numerous globules). The oil-bodies of *A. maxima* tend to be smaller and more homogeneous than those of *A. pinguis* in both young and mature thallus, although it is known that the oil-bodies of *Aneura* subgen. *Aneura* (including *A. maxima* and *A. pinguis*) are very variable in morphology (Mizutani and Hattori, 1957; Inoue, 1976). It seems that the oil-bodies of *Aneura* may be stabilized in shape, and nature except for number.

In *Riccardia crassa* and *R. miyakeana*, the oil-bodies of young stages agree considerably with those of their mature thalli. In *Riccardia planiflora* the oil-bodies of young stages are smaller than those of mature thalli, and drop-like oil-bodies are found only in young stages. However,

the large oil-bodies are similar to those of mature thalli except for size. The young stages of *R. kodamae* have oil-bodies in all cells, although the oil-bodies of *R. kodamae* are described as 1-7 in number only in inner cells (Mizutani and Hattori, 1957). However, the mature thallus of *R. kodamae*

sometimes have oil-bodies in epidermal cells, especially in those of young prostrate thalli, but mature erect branches have no oil-bodies in epidermal cells. Thus these changes suggest that the distribution pattern of oil-bodies may be correlated to the development of thalli. It is more

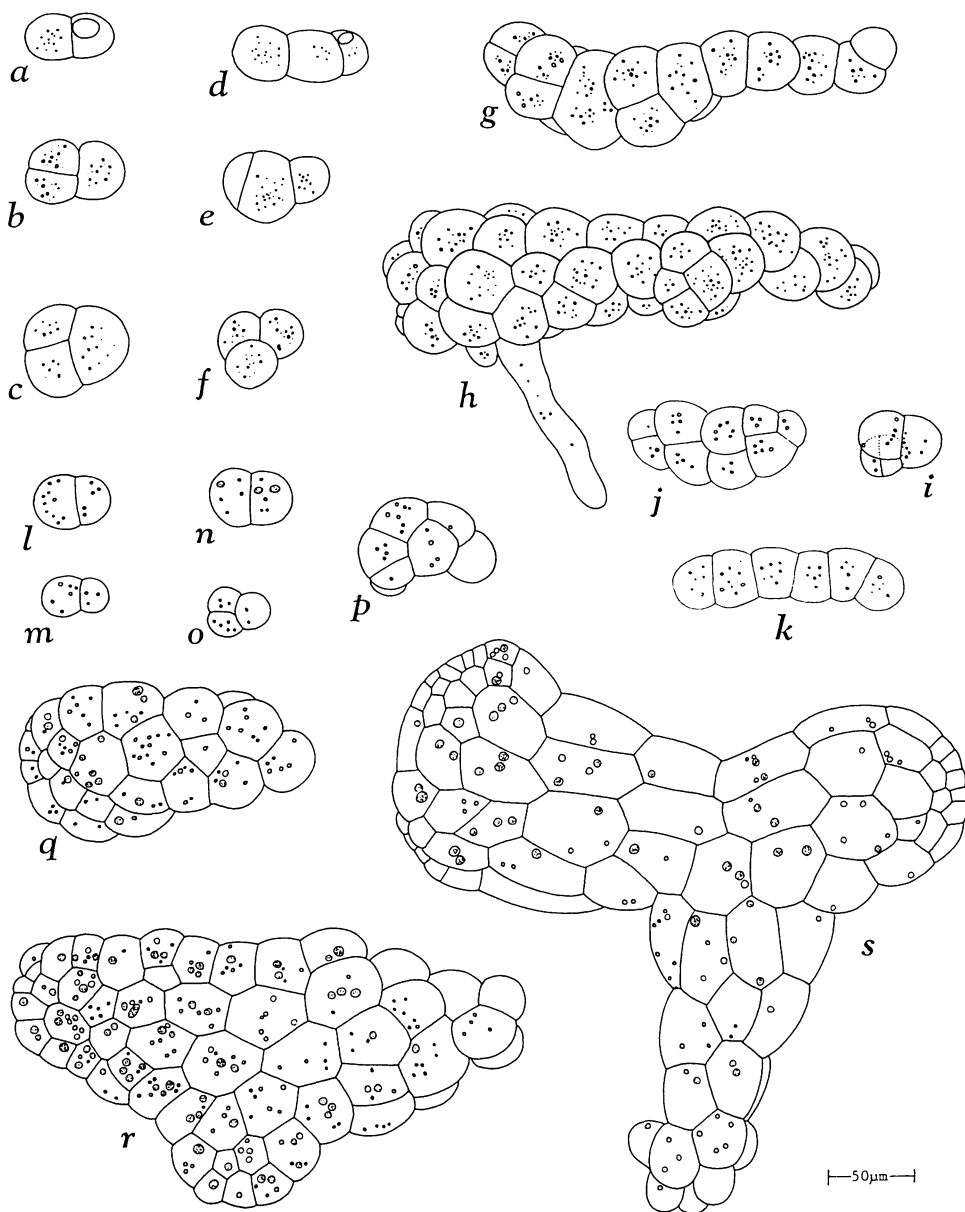


Fig. 5. Germinations of *Riccardia miyakeana* (a-k) and *Riccardia planiflora* (l-s). a-h, sporelings of *R. miyakeana* (g, h, lateral view). i-k, gemmalings of *R. miyakeana*. l-r, sporelings of *R. planiflora* (dorsal view). s, gemmalings of *R. planiflora* (dorsal view).

clearly shown in sporelings of *R. jackii*. At first the young stages of *R. jackii* have only drop-like oil-bodies in all cells, but they bear large oil-bodies in all cells after production of mucilage papillae. They gradually lack oil-bodies in epidermal cells and agree with the oil-bodies of mature thalli described by Mizutani and Hattori (1957). The oil-bodies of the young stages observed in this study agree with those of mature thalli for the most part. It suggests that the oil-bodies are stabilized, and they are taxonomically important characters within the family.

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### References

- Clapp, G. L. 1912. The life history of *Aneura pinguis*. Bot. Gaz. 54: 177-193.
- Fulford, M. 1956. The young stages of the leafy Hepaticae. Phytomorphology 6: 199-235.
- Hässel de Ménéndez, G. G. 1967. Estados inciales de especies Sudamericanas del género *Riccardia*. Bol. Soc. Arg. Bot. 11 (2/3): 97-104.
- Inoue, H. 1959. Studies on spore germination of Hepaticae 5. *Fossombronia japonica* Schiffn. Bot. Mag. Tokyo 72: 131-136.
- Inoue, H. 1976. Illustrations of Japanese Hepaticae-2. 193 pp. Tsukiji Shokan, Tokyo.
- Leitbeb, H. 1877. Untersuchungen über die Lebermoose III. Die Frondosen Jungermannieen. 144 pp., 9 pls. O. Deistung's Buchuhandlung, Jena.
- Mizutani, M. and S. Hattori. 1957. An etude on the systematics of Japanese Riccardias. J. Hattori Bot. Lab. 18: 27-64.
- Nehira, K. 1962. The germination of spores in Hepaticae 4. Two types of sporeling pattern in the *Riccardia*. Hikobia 3(2): 4-9.
- Nehira, K. 1966. Sporelings in the Jungermanniales. J. Sci. Hiroshima Univ. Ser. B, div. 2, 11: 1-49.
- Nehira, K. 1983. Spore germination, protonema development and sporeling development. In Schuster, R. M. (ed.), New Manual of Bryology, 1: 343-385. Hattori Botanical Laboratory, Nichinan, Japan.
- Ono, S. 1978. Gemmalings in two species of *Riccardia* (Hepaticae). J. Jap. Bot. 53 (3): 92-96. (In Japanese with English summary)
- Renzaglia, K. S. 1982. A comparative developmental investigation of the gametophyte generation in the Metzgeriales (Hepatophyta). Bryophytorum Bibliotheca 24: 1-358, 84 pls. J. Cramer, Vaduz.
- Schuster, R. M. 1966. The Hepaticae and Anthocerotae of North America, vol. 1. xvii+802 pp. Columbia Univ. Press, New York.
- Schuster, R. M. 1987. Studies on Metzgeriales : I. North American Aneuraceae. J. Hattori Bot. Lab. 62: 299-329.

### 本邦産ミドリゼニゴケ科数種における初期発生

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本邦産ミドリゼニゴケ科7種、ミズゼニゴケモドキ *Aneura maxima*, ミドリゼニゴケ *Aneura pinguis*, カネマルテングサゴケ *Riccardia crassa*, ナガサキテングサゴケ *Riccardia jackii*, コダマテングサゴケ *Riccardia kodamae*, ミヤケテングサゴケ *Riccardia miyakeana*, ヒメテングサゴケ *Riccardia planiflora* について胞子発芽の、またミヤケテングサゴケとヒメテングサゴケについては無性芽発芽の観察をおこない、それらの発芽やその後の発達、油体について記載し、考察した。発芽時に見られる油体は成熟した葉状体に見られるものとほぼ一致し、油体は安定した形質として分類学的に重要であることが示唆された。