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Gametophyte and Embryo of *Microgonium tahitense* (Hymenophyllaceae)

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ゼニゴケシダの配偶体と胚

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Spores of *Microgonium tahitense* (Nadeaud) Tindale (Hymenophyllaceae), collected from Yonaguni Isl., Ryukyu, were cultivated for three years. The spore germination showed a Trichomanes-type by Nayar and Kaur (1971), and Ip and Ir types by the author (1972). In the early development, prothallial cells took an irregular form due to the appearance of the first rhizoidal cell. Gametophytes were branched filaments and formed antheridia and archegonia on archegoniophores. Young sporelings, which put on calyptras, were born by fertilization. Gemmae were formed actively on the filaments. The first frond divided soon into lateral veins and false veins from the midrib. A sporangium contained 64 spores and the count of gametic chromosome numbers was n = 34. This plant collected from Yonaguni Isl. was a sexual diploid as confirmed by the observation of both gametophyte and sporophyte.

Microgonium tahitense (Nadeaud) Tindale is a small, peltate, and epiphytic or saxifragous filmy fern growing on wet rocks in valleys and on the barks of trees in forests in the Ryukyus, the Bonins, Formosa, Java to New Guinea, eastwards to north-east Australia and Polynesia.

Since Bower (1888), sexual and apogamous growth in the Hymenophyllaceae has been described on several species by several workers. The embryological information in the family was very poor, including the recent works of Stone (1958) and Yoroi (1976), and *M. tahitense* has never been observed in its embryology.

In this paper, an account will be given on this species as to the developmental morphology in the gametophyte, the embryology in the sporophyte, and the chromosomal data.

Materials and Methods: The fertile fronds of *Microgonium tahitense* were collected in October 1974 and in March 1991 by the author on Mt. Urabu, Yonaguni Isl., Okinawa Pref. Voucher specimens (Yoroi 5435 and 6182) are deposited in the Herbarium of the University of Tokyo. The fertile fronds were washed with tap water, and the sporangia were removed from them to keep in clean drag-papers, where the spores were shed. They were preserved in a refrigerator after being dried for several hours in the shade.

The spores were sown on a medium with inorganic nutrient in Petri-dishes. The medium was

Meyer's solution solidified with 0.8% agar. The dishes were placed in a culture box kept at 23±1°C under 12 hours of illumination a day with white fluorescent tubes at 300–1000 lux. Five months later, the young gametophytes were planted on sterilized soil in small flower pots kept in the laboratory at 23–25°C under the same light conditions.

Embryos were fixed with Craf I (Sass 1958), embedded in paraffin, sectioned at 8 μm by microtome, and stained with Delafield's hematoxylin. The fronds with sporangia were fixed in Newcomer's solution in the field for cytological study. The gametic chromosome numbers were counted in the fronds with the acetocarmine squash methods of Manton (1950). Photographs and camera-lucida drawings were made on the microtome sections of both gametophyte and sporophyte, including those of gametic chromosomes.

Observations: The spores are green, tetrahedral-globose in shape with three ridges of laesurae on the spore coat (Fig. 1-1). The exine is thin and spiny. Usually, a sporangium contains 64 regular spores. The average diameter of the spores is 29.0 μm.

When the exnine splits at the laesurae as the spore swells, a cushion-like cell (basal cell) protrudes out of it. The cell is chlorophyllous and has four corners directed to the three side-walls and one basal wall of the spore coat. The formation of the basal cell takes place within two weeks after sowing. The fully swollen basal cell is two to three times as large as the original spore, but it is smaller as compared with the other previously reported species of the family.

About one month after sowing, the basal cell for the first time cuts off a rhizoidal cell, which contains a few pale chloroplasts. The rhizoidal cell is produced on an upper surface of the basal cell or some times at one corner of the basal cell (Fig. 1-2). A prothallial cell usually arises from one of the three corners directed to the former side-walls (Fig. 1-3, 4). The development of the prothallial cell at the corner directed to the basal wall is much less frequent.

When the prothallial cell develops into a short prothallial filament with two or more transverse wall formations, the second and the third prothallial cells begin to arise from the remaining corners of the basal cell (Fig. 1-6, 7, 8). The prothallial cells often produce lateral branches (Fig. 1-5, 8). The cells are small, compared with those of the other Trichomanoid ferns. They show variously shaped barrel-forms and contain much less chloroplasts than the basal cell. Transformation to the rhizoidal cell is recognized by the disappearance of the chloroplasts included in the distal cell of the prothallial filament.

Within six months, a branched prothallium grows to a size of about 1.5 mm in diameter (Fig. 1-9). In about eleven months, the profusely branched prothallium seems roughly to be a sphere of about 5 mm in diameter with radially emitting filaments and rhizoidal cells in all directions. No longitudinal cell division occurs in the filaments until the formation of sex organs.

In eighteen months' culture, the formation of antheridium occurs on the prothallial filament. The antheridium arises from the filament cell as an outgrowth, as is shown in the formation of antheridia of *Polyphlebium venosum* (R. BR.) Copeland (Stone 1958). The outgrowth is rich in chloroplasts and produces a stalk cell and a spherical cell. The spherical cell cuts off two ring cells, one dome-shaped cell with an operculum and one central cell. By a succession of cell divisions the central cell produces numerous spermatozoids, which move out actively when the operculum is shed. During the cell divisions, the stalk cell, the ring cells and the dome-shaped cell are filled with

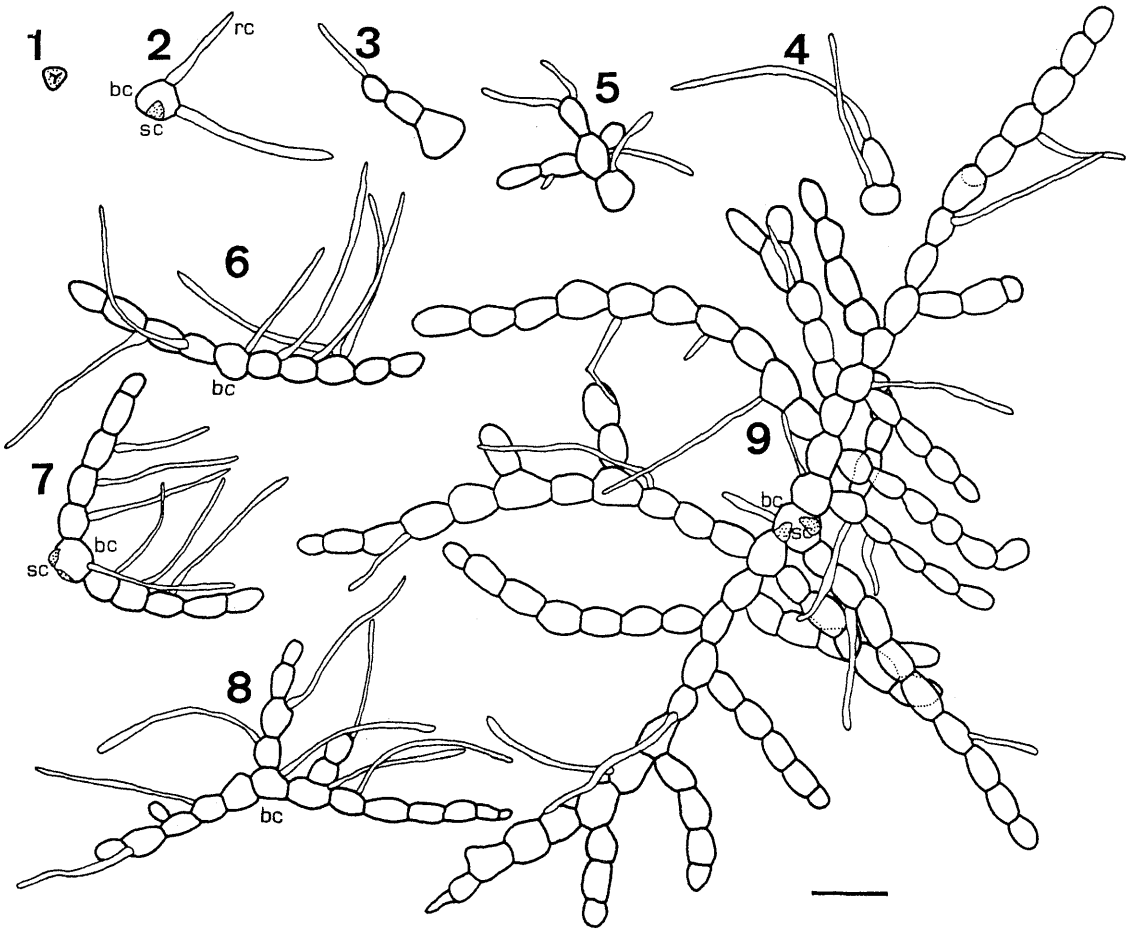

Fig. 1. Germination of spores and young gametophytes. 1. Spore. 2–4. One month after sowing. 2. First rhizoidal cell protruding. 3. First prothallial cell protruding. 4. Basal cell protruding a prothallial cell and a rhizoidal cell. 5–7. Two months. 5. Basal cell with a branched filament. 6–7. Basal cell with two filaments. 8. Three months. Basal cell with three filaments. 9. Six months. Prothallium with branched filaments and rhizoidal cells. bc: basal cell, sc: spore coat, rc: rhizoidal cell. Bar = 100 μm.

chloroplasts, but these disappear at maturity. Figure 2-1 shows two mature antheridia.

In a few weeks after the formation of antheridium, an apical cell is produced by the repeated oblique wall-formations on the prothallial filament. The apical cell loses its activities soon, resulting in the formation of a mass of cells like a sphere, as is shown in the formation of the archegoniophores of *Crepidomanes bilabiatum* (N. et Bl.) Copel. (Stokey 1948). The archegoniophore is usually found at the distal end of the lateral filament near to the basal portion of the mother filament, and sometimes midway on it. The archegoniophore is stalked and bears several archegonia of various ages. Figure 2-2 shows a small archegoniophore protruding three old and one young archegonia. Each archegonium bears an egg cell, a ventral canal cell, neck canal cells and four neck cells in tiers of four to five cells. The neck cells stand vertically, as is seen in the Osmundaceae (Fig. 3-1).

In about twenty months' culture, the formation

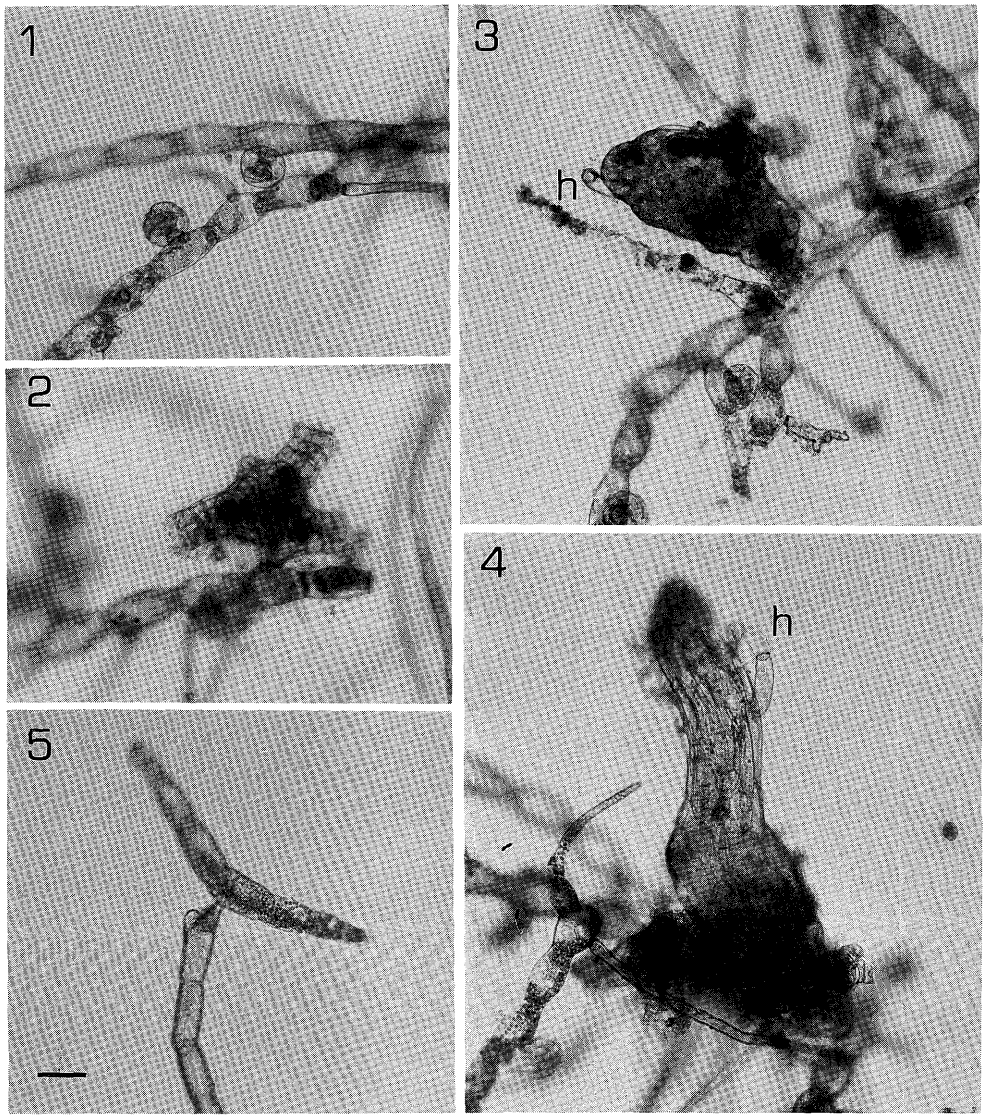

Fig. 2. Sex organs, young sporelings and a gemma. 1–2. 18 months after sowing. 1. Antheridia. 2. Archegonia on an archegoniophore. 3–4. 20 months. 3. Early stage of a sporeling, with a hair. 4. Later stage of a sporeling, with hairs. h: hair. 5. 21 months. A gemma on a gemmifer. Bar = 100 μm.

of a young sporeling occurs for the first time. Figure 2-3 shows an external view of the young sporeling of about 300 μm in length, which bears a bicellular hair at the tip. Figures 3-2a and b show slightly oblique microtome cross sections of the younger embryo of 100 μm in diameter. The embryo is wrapped in a calyptra, which has been broken by this time, as is shown in the embryo of *Polyphlebium venosum* (Stone 1958). The embryo grows soon to a young sporeling, which is accompanied by several multicellular (between two and four cells) hairs (Fig. 2-4). The calyptra is still observed in the cross section of the young sporeling (Fig. 3-3). Naturally, the first frond is formed at the distal end of the sporeling, while the first rhizoid of the frond comes from near the proximal

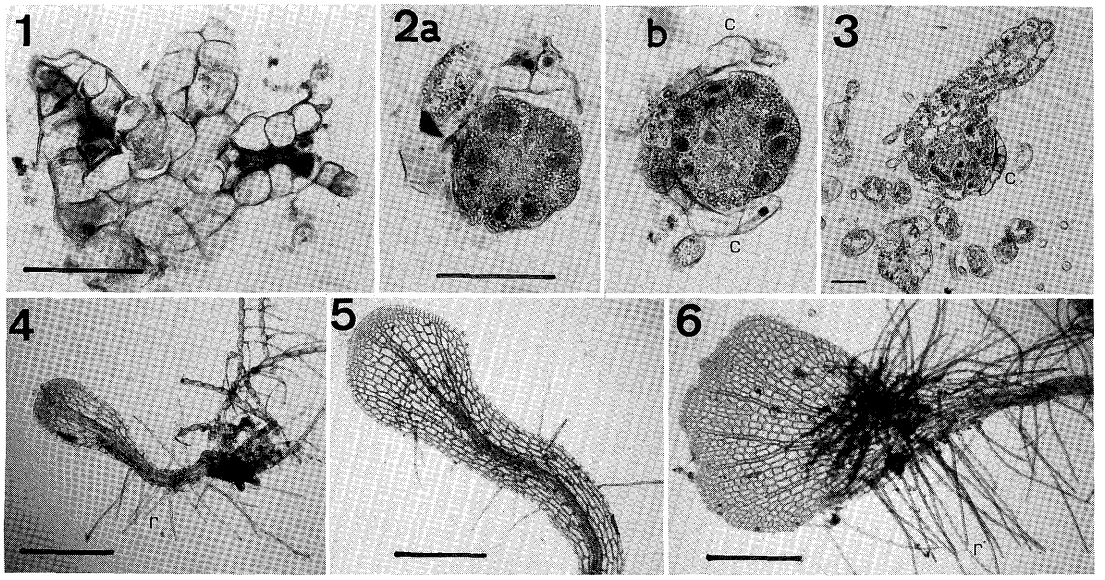

Fig. 3. Sections of archegoniophores and embryos, and first fronds. 1. Archegoniophore with three archgonia. 2. Two sections of an embryo with calyptra. 3. A section of a sporeling with calyptra. c: calyptra. 4. 21 months after sowing. First frond with a midrib and rhizoids. 5–6. Two years. 5. Early stage of the development of the first frond. 6. Later stage. Midrib dividing lateral veins and false veins, and lower side of the frond protruding rhizoids. r: rhizoid. Bars = 100 μm in the upper figures and bars = 1 mm in the lower figures.

portion (Fig. 3-4). The first frond makes up a midrib and a marginal meristem in the early stage of the development. As cells of the marginal meristem are active in the first frond, the midrib begins to divide into lateral veins and false veins (Fig. 3-5). Figure 3-6 shows a further grown-up stage of the first frond, from which arises a number of rhizoids from the margin and the lower surface of the proximal portion, as is shown recently in the sporophyte of this species by Hagemann (1988). The root is not recognized at all.

After one year and nine months from the beginning of the culture, the vegetative reproduction is accomplished by a gemma, as is shown in the formation of gemma of *Gonocormus minutus* (Bl.) v. d. Bosch. (Yoroi 1972). The gemma appears at the distal end of the prothallial filament and is a short one composed mostly of eight to nine cells. The gemma develops first from a terminal cell, which takes a flask-like shape. Then the terminal cell is cut off at the place of constriction, giving rise to an initial cell of the gemma, leaving the lower cell as a gemmifer. The initial cell is divided by transverse walls to form the gemma (Fig. 2-5). When both terminal cells of the gemma begin to form two rhizoidal primordia, the gemma falls on the ground to grow a new prothallial filament. The formation of gemmae continues for one year till the gametophytes form a mat, and then it ceases. The shape of gemmae is regular, as is shown in that of *Gonocormus minutus* (Bl.) v. d. Bosch. (Yoroi 1972).

The gametic chromosome number of n = 34 has been confirmed for this species (Fig. 4). In this species the size of chromosomes is very small, comparing with that of the other Trichomanoid ferns.

Discussion: The type of spore germination in this species corresponds to "Trichomanes type" according to Nayar and Kaur (1971), and to "Type

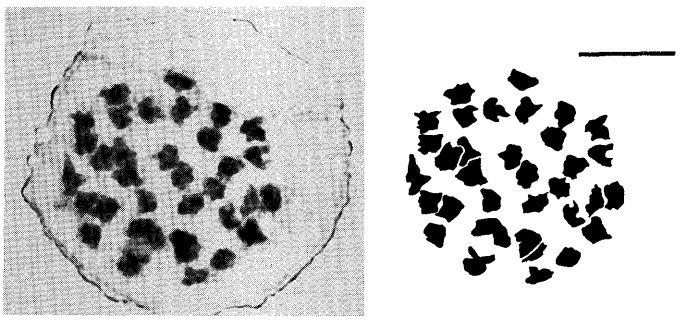

Fig. 4. Gametic chromosomes. n=34. Bar=10 μm.

Ip" and "Ir" of the author (1972). No ribbon-like plates are discovered in this species, though the gametophyte is of the same type as usual in the other Trichomanoid ferns (Farrar and Wagner 1968, Yoroi 1985). If the appearance of rhizoidal cells is suppressed on the basal cell, later development of the prothallial filaments is directed in straight lines, keeping the angle of 120° regularly between them. Such a case is usual in *Gonocormus minutus* (Bl.) v. d. Bosch. and *Crepidomanes insigne* (v. d. Bosch.) Fu. (Yoroi 1972). However, the usual case for this species is that the rhizoidal cell develops in an earlier stage, and the prothallial filaments take an irregular disposition. The irregularity aforesaid is also observed in *Vandenboschia radicans* (Sw.) Copel. var. *orientale* (C. Chr.) H. Ito, *V. auriculata* (Bl.) Copel., *Selenodesmium obscurum* (Bl.) Copel. and *S. siamense* (Christ) Ching et Wang (Yoroi 1972, 1985). The irregularity and regularity in the earlier development seems to depend mainly on the specific nature of spores in those species, notwithstanding influence from the culture's condition.

The formation of sex organs on the gametophytes has been commonly observed in the Hymenophyllaceae (Mettenius 1864, Bower 1888, Goebel 1892, Stokey 1948, Holloway 1944, Stone 1958, Farrar and Wagner 1968, Yoroi 1972, Bierhorst 1975). In *Microgonium tahitense,* the young sporophyte has developed from an egg cell in the archegonium, which forms a calyptra after fertilization. In this species, as far as the author has observed, an apogamous embryo takes place neither on the archegoniophore nor on the archegonial jacket cell, as is shown in *Vandenboschia auriculata* (Stokey 1948), in *Trichomanes* [s. lat.] *pinnatum* (Bierhorst 1975) and in *Crepidomanes latemarginale* (Eaton) Copel. (Yoroi 1976).

Since Bower (1888) described gemmae, the vegetative reproduction of the gametophytes has been known in the Hymenophyllaceae. The reproduction has been also observed in the Vittariaceae (Farrar 1974, Yoroi 1975, Sheffield and Farrar 1988) and in the Grammitidaceae (Stokey and Atkinson 1958). Although the development of gemmae usually takes place in parallel with that of mother gametophytes, it has begun after the formation of sex organs in this species. The gametophytes have resulted in a mat condition by continuous gemma-formations. These facts might be adapted to the environment of extremely moist air or of wet conditions, in which this species lives.

The chromosome number of n=34 found in plants from Yonaguni Isl. agrees with the count by Braithwaite (1975). He also reported the count of n=34 for *M. motleyi* v. d. Bosch and that of n=68 for *M. bimarginatum* v. d. Bosch (Braith-

waite 1969, 1975). As for chromosome numbers, plants in this genus have either 34 or a multiple of this number.

Considering the developmental morphology of both gametophytes and sporophytes, the average number of spores in the sporangium, and the chromosome numbers of this genus, the plant collected by the author is considered to be a sexual diploid.

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要 旨

小笠原, 琉球列島から台湾, ジャワを経て, ニューギニア, オーストラリア東部からポリネシアにまで広く分布するゼニゴケシダの配偶体と胚発生を胞子（与那国島で採集）からの培養実験（3年）によって観察した. 胞子の発芽様式は Nayar と Kaur の類型では Trichomanes-type を, 著者 (1972) の分類では Ip 型と Ir 型を示すが, 初生仮根の発生が早いため, その後の発達は不規則になりがちである. 前葉体は糸状体で, 造精器と造卵器の両生殖器官が発達すると, 若い胞子体が生じる. 胚発生の初期にはカリプトラが観察されることから, この胚は有性生殖によって生じたと考える. 生殖器官の発達後に無性芽による増殖が盛んになり, 前葉体はマット状になる. とりわけ小形な前葉体が環境のなかで生きるのにこのことは適している. 第一葉では展開の早い時期に中肋から

側脈や偽脈を分化し，胞子葉に顕著に見られる仮根の発生も観察された．大きさの揃った胞子は1胞子嚢あたり64個で染色体数はn＝34であった．

配偶体と胞子体の観察結果から，与那国島のゼニゴケシダは2倍体の有性生殖種であると認めた．