MYXOMYCETES OF TAIWAN VI.

Badhamia gracillis

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(Manuscript received 30 November, 1989; revised version accepted 11 March, 1990)

Abstract: *Badhamia gracilis* is reported for the first time from Taiwan. Morphological characteristics were examined with the aid of LM and SEM. Spores possess spines with the wall surface divided by ridges into large reticula.

Spore germinated by splitting, with two protoplasts issuing from smaller spores and four from larger ones. Cream-colored plasmodia were then found in single swarm cell cultures. This collection is therefore non-heterothallic. Fruiting bodies thus formed produced viable spores resembling those from field collections. A description of the species based on the Taiwan collection is also provided.

INTRODUCTION

In a field collection of 25 Oct., 1986, a large pile of logs was found along side the road close to the bus station in Chih-Nan-Kung, Mu-Cha District, Taipei City. Close examinations of the logs revealed fruiting bodies of Myxomycetes in abundance on the partially decayed logs. *Badhamia gracilis* (Macbr.) Macbr. (Physaraceae) (Martin and Alexopoulos, 1969) was one of the species found and is a new record for Taiwan (Liu, 1980–1984, Nakazawa 1929; Wang and Chien, 1988; Wei and Liu, 1989).

The abundant sporangia offered an excellent opportunity for developmental studies on this species which hitherto has been sparse. Capillitial development was described by Welden (1955) from moist chamber culture and spore germination was listed in a table compiled by Gray and Alexopoulos (1968) as being achieved. Blackwell and Gilbertson (1980) noted the spores were typical from agar culture, and Collins (1979) listed this species as being cultured from spore to spore, however, no other developmental features were mentioned by those investigators. In this report developmental features of the Taiwan collection are described from spore-to-spore in agar culture.

MATERIALS AND METHODS

Mature fruiting bodies were collected in the Chih-Nan-Kung area. For SEM examination, spores were pretreated by using the method described by Aldrich and Blackwell (1976).

For germination, spores were sown in small Petri-plates (30 mm×20 mm) containing liquid medium. Two types of media were used, bark extract and water. The bark extract was prepared by using 20 g of water willow (*Salix warburgii* 0.

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Seem) in 1 liter of water without any agar and following the instructions given by Gray and Alexopoulos (appendix 1. p. 247, 1968). The germinated cultures were then transfered to one-half strength corn meal agar (1/CMA) for further growth and developmental studies. In addition, spore suspensions were prepared simultaneously in a hemacytometer for observation of germination behavior and the subsequent development. Spore suspensions and all subsequent culture were incubated in darkness at 25°C unless under examination in lighted laboratory condition.

Clonal cultures were prepared by transferring a drop of swarm cell suspension to a small Petri plate containing distilled water, which was then diluted by subsequent transfer to new plates until only 2 or 3 swarm cells remained. Individual swarm cells were subsequently transferred by pipette onto a plate of 1/2 CMA and examined microscopically (×150) to confirm the presence of a single swarm cell or myxamoeba.

RESULTS AND DISCUSSION

Species Description

Badhamia gracilis (Macbr.) Macbr., in Macbride & Martin, Myxomycetes 35. 1934.

Sporangia stipitate, white, gregarious, occassionally two united at base of stalk, reniform, ovoid, or globose in some, umbilicate below, 0.30–0.35 mm in diameter, 0.55–0.75 mm in length; peridium membranous, lime granules scattered evenly and in scale-like patches on the peridium; stalk long, 0.75–1.0 mm in length, weak, sulcate and twisted, straw yellow (deep olive buff or dark olive buff, in Ridgway, 1912), darker (dark olive) close to the base, straight or repent on the substrate; hypothallus membranous, transparent, circular at each stalk base; capillitium physaroid, lime nodes large, angular, may connected by some short transparent thread, often massed at the center of sporangium; spores blackish brown in mass, violaceous brown by transmitted light, 15–16.5(–20) μm in diameter, consistently more or less angular in profile, walls with large reticulum, 1–6 in a hemisphere, prominently and evenly echinulate; plasmodium cream.

Habitat: Logs.

Specimen examined: Taipei City: Mu-Cha district, CHLB 536a, 538, 539b, 541a; Oct. 25, 1986.

Distribution recorded: North America (USA and Mexico), Panama, Puerto Rico, West Indies, Galapagos, England, Japan, and Taiwan.

The specimens fruited in abundance on a pile of decomposing shaded wood logs. It is not difficult to recognize the species by its characteristic sporangia and straw-yellow stalk which is long and twisted. Microscopic characters of spores are especially distinct. The large surface reticulum that makes the spores appeare angular in profile is actually formed by ridges on the wall when viewed under SEM (plate II, Fig. 1). The TEM photomicrographs (Scheetz and Alexopoulos, 1971) show that the nature of the ridges is not homologous with that of the reticulum of truely reticulate spores and that it may represent a distortion of the spores (angular protoplast with corners more or less coincide with the cross section of ridges).

Spore Germination and Growth Development

Spores germinate by splitting (Plate III, Fig. 3) in both distilled water and bark extract. Twenty-two hr after wetting in glass distilled water, large numbers of spores were found germinating, although much earlier (5 hr after wetting) 2 empty spore cases had already been observed in the culture.

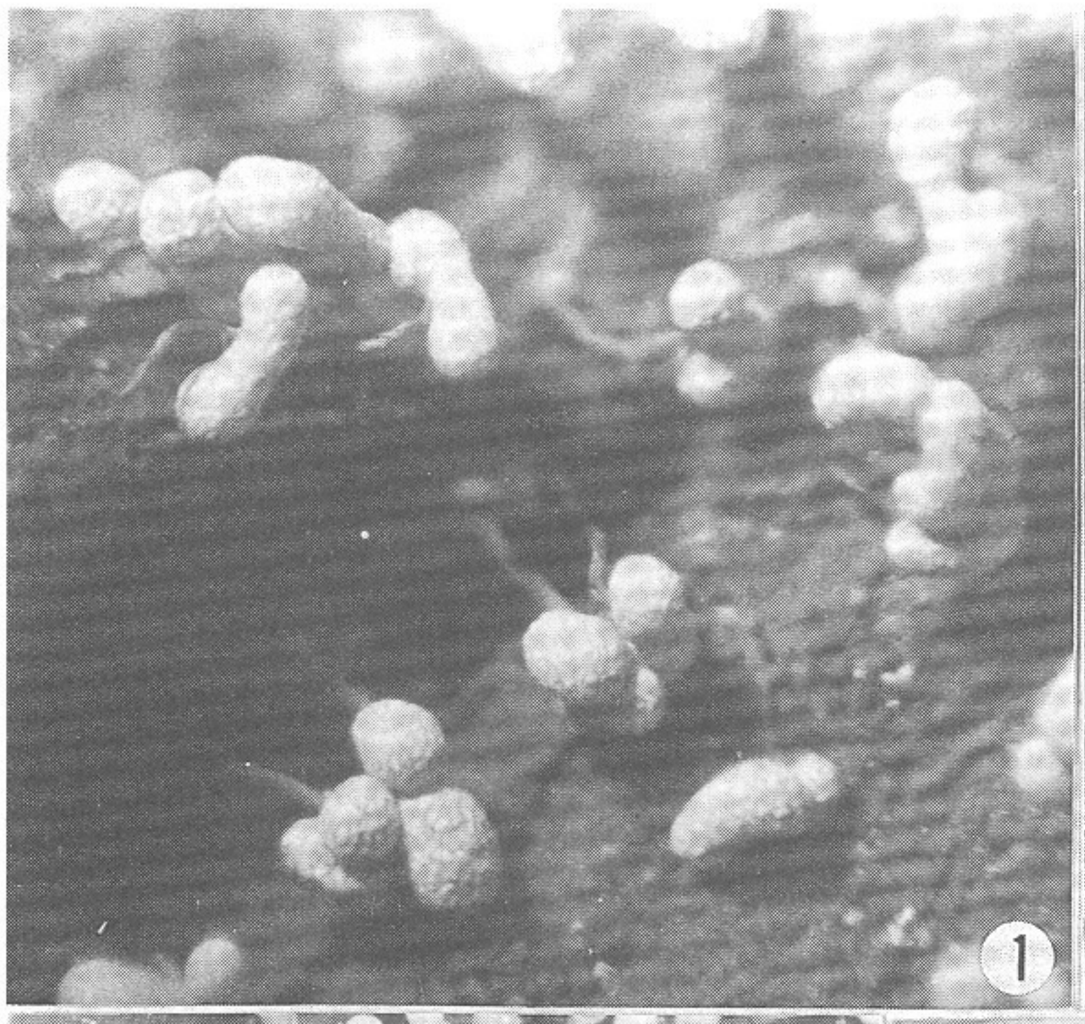
When wetted, the spores appeared angular and uninucleate. They soon became swollen and contained 2 (or more than 2 in larger spores) discernible nuclei. Spore walls then cracked (Plate III, Fig. 5) with the protoplast remaining inside while cleavage occured after wall cracking. Protoplasts then emerged from the spore 2-3 hr later, two from each smaller spore (15-16.5 μm) and 4 from larger ones (17.5-20 μm). Gray and Alexopoulos (1968) stated that the number of cells produced by a germinating spore varies from spore to spore even within the same species. In *Badhamia* spp. Gilbert (1928) and Smith (1929) found that 1-4 protoplasts emerged from each spore. It is unknown whether the smallest (12 μm) spores of *B. gracilis* emit one protoplast at germination since none of the Taiwan specimens yielded spores of this size. The reason for multiple cells forming and emerging from a single spore is also unclear. It has been reported in other species (Gray and Alexopoulos, 1968) that spores are uninucleate at maturity, but that mitosis and cytokinesis result in two protoplasts per spore. This has now been confirmed for *B. gracilis* (Plate III, Fig. 1).

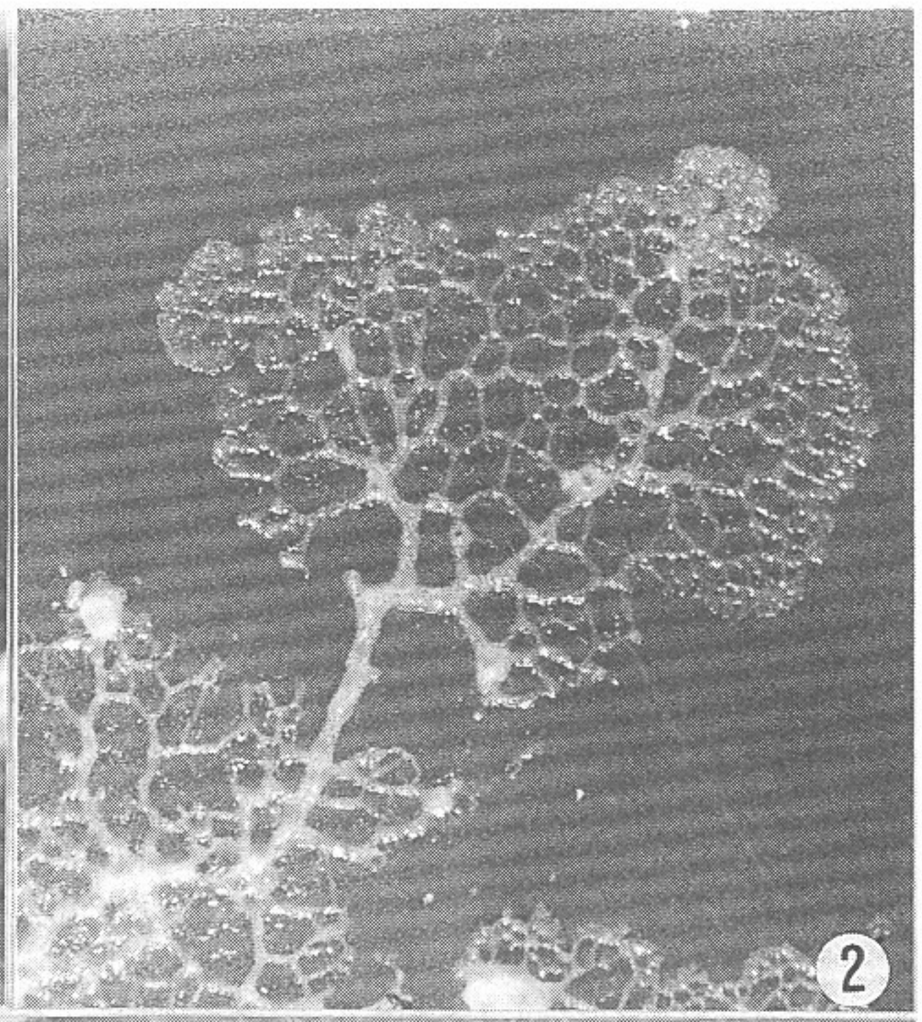
Upon emergence from a spore the protoplast became amoeboid and produced either fine pseudopodia or a single flagellum. The presence of a second flagellum could not be determined by the light microscope. These cells eventually assumed the shape of a typical swarm cell which remained amoeboid for about 10 mins with the anterior flagellum slowly moving while crawling forward. About 40 mins after the protoplast emerged the swarm cells were actively swimming. A second protoplast emerged shortly afterward and followed the same behavioral pattern.

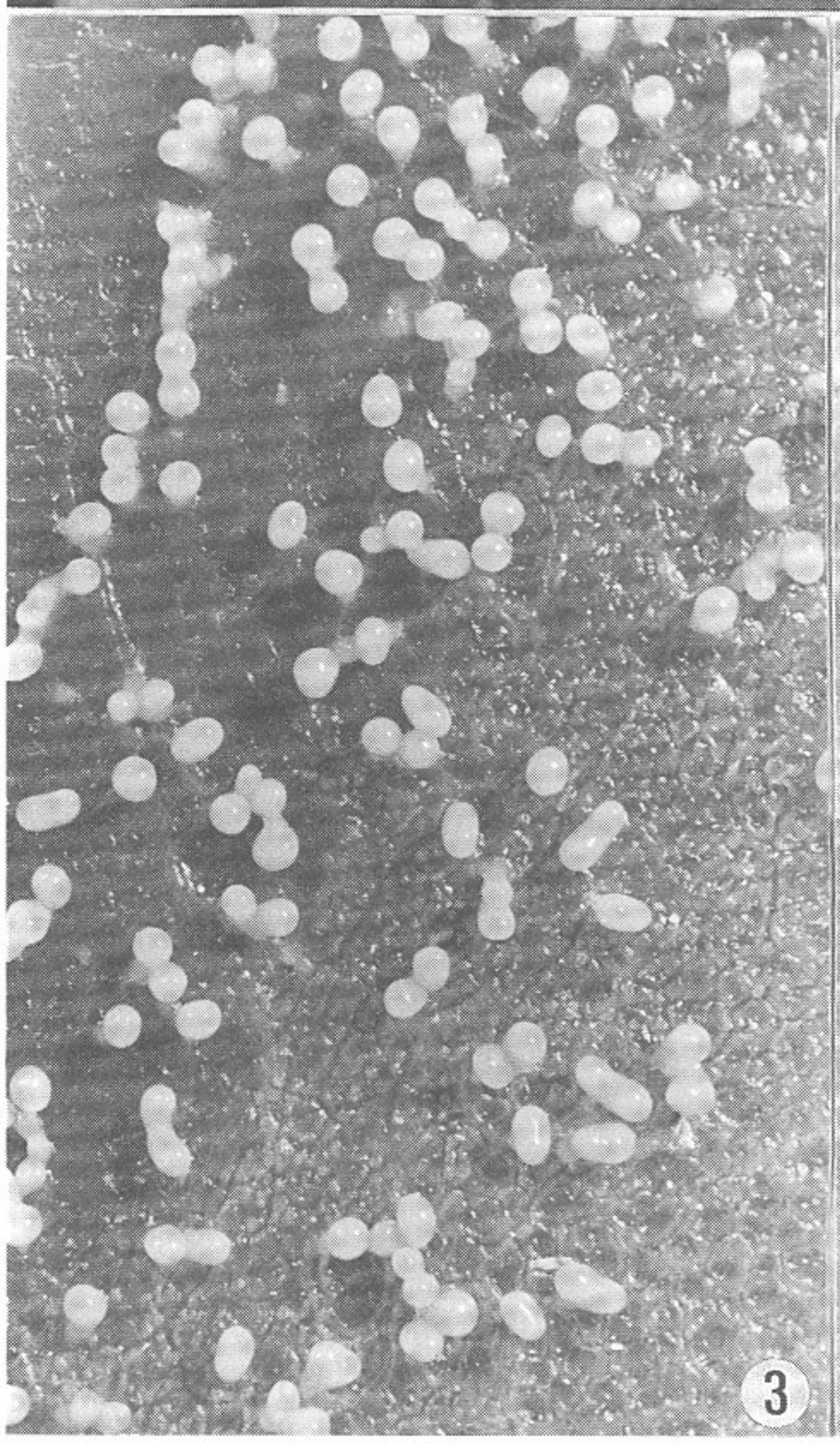
Fusion of swarm cells or myxamoebae was not observed either in gross culture or clonal culture, although large numbers of myxamoebae were observed on the second day following preparation. Plasmodia formed on the third day in both cultures, increased in size and were cream color with a typical phaneroplasmodium appearance (Plate I, Fig. 2). The fact that plasmodia appeared in the clonal cultures suggests the species is not heterothallic. This result provides another new member for the non-heterothallic group of Myxomycetes (Collins and Betterley, 1982).

Fruiting bodies appeared on the 12th day. At the beginning of fruiting the plasmodia became concentrated into numerous small masses or so-called "sporangial initials" (Plate I, Fig. 3). Color of the plasmodia remained unchanged at this time, then gradually became darker as the sporangia matured. White lime granules dispersed on the surface of the mature sporangia were then evident (Plate I, Fig. 4). This is in general agreement with Welden's description (1955). Viable spores obtained from the F-1 generation, however, exhibited a low germination rate.

In general, the Taiwan specimens conformed to published descriptions of *B. gracilis* (Hagelstein, 1944; Martin and Alexopoulos, 1969; Farr, 1976), with a life-cycle pattern typical of that reported (Alexopoulos and Mims, 1979) for other endogenous Myxomycetes.







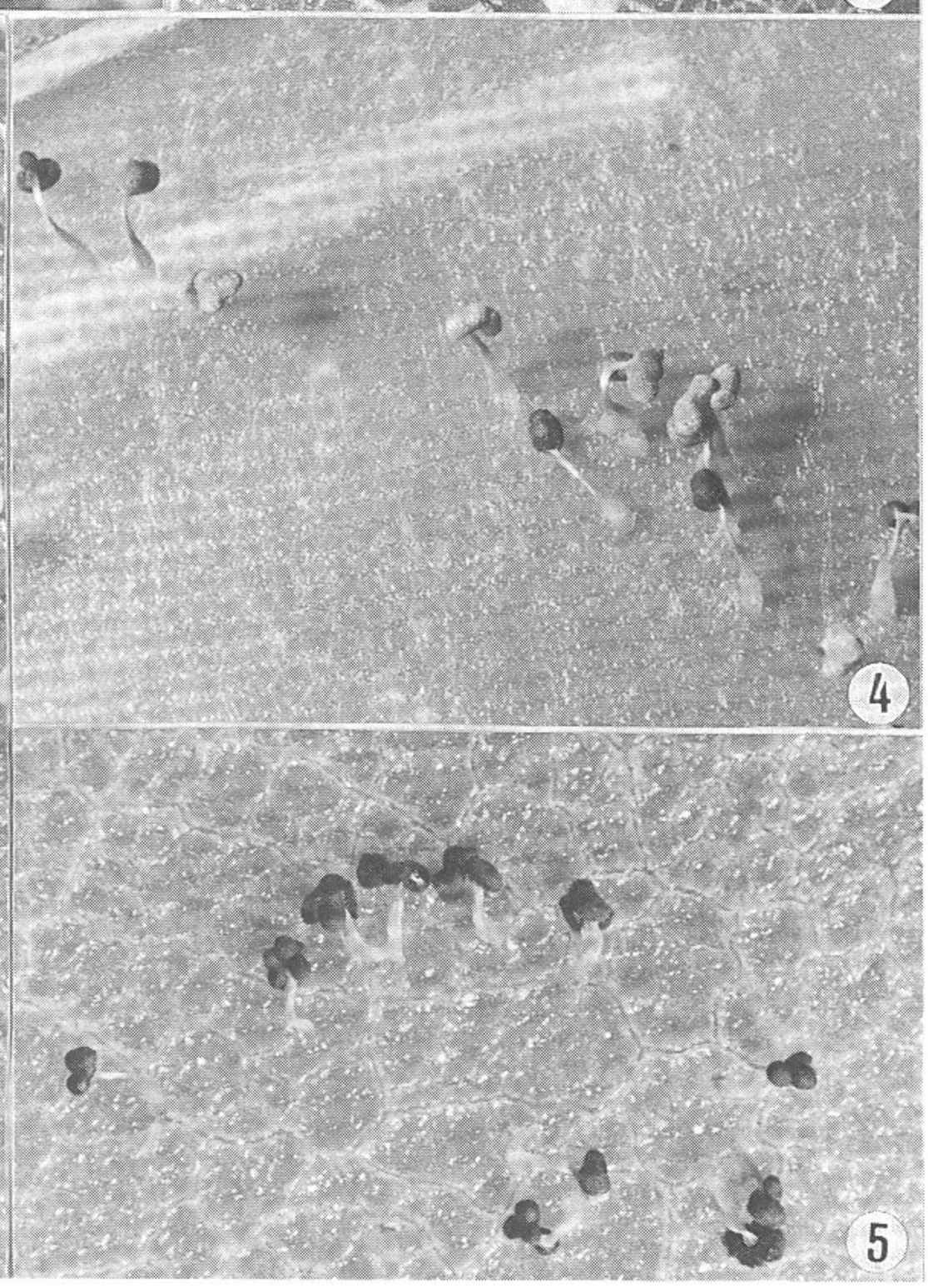


Plate I.

Fig. 1. Sporangia, ×15.
2. Plasmodium, ×6.
3. Sporangial initial, ×6.
4. Sporangia (with white lime) from spore cultures, ×6.
5. Young sporagia (no lime discernible) from spore culture, ×6.

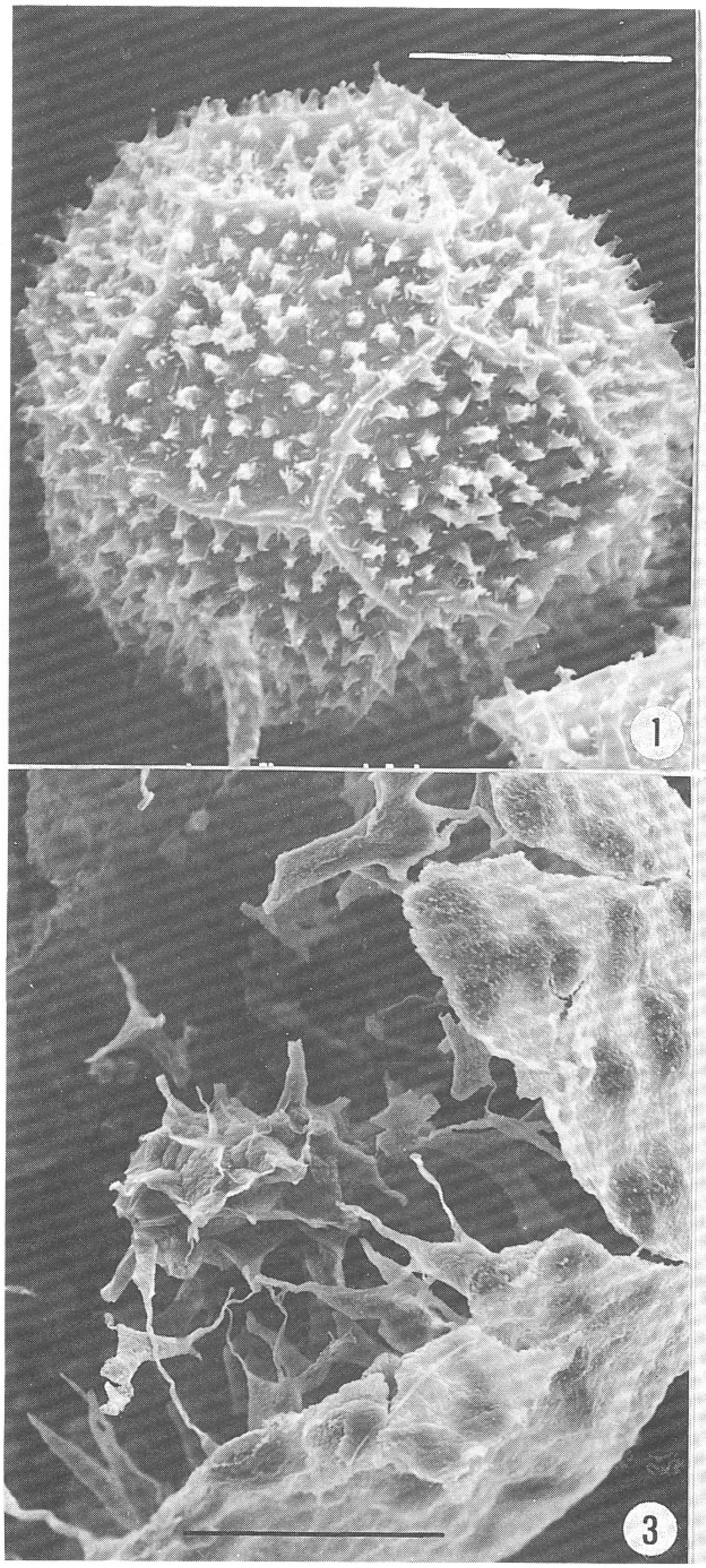


Plate II.

Fig. 1. SEM view of spores showing angular ridges.
2. Spores, average and large sized, showing the large reticulum on the surface, ×2010.
3. Portion of sporangium, showing capillitium.
4. Spores, optical section, showing the spines, ×804.
scale in 1=6 μm and in 3=100 μm.

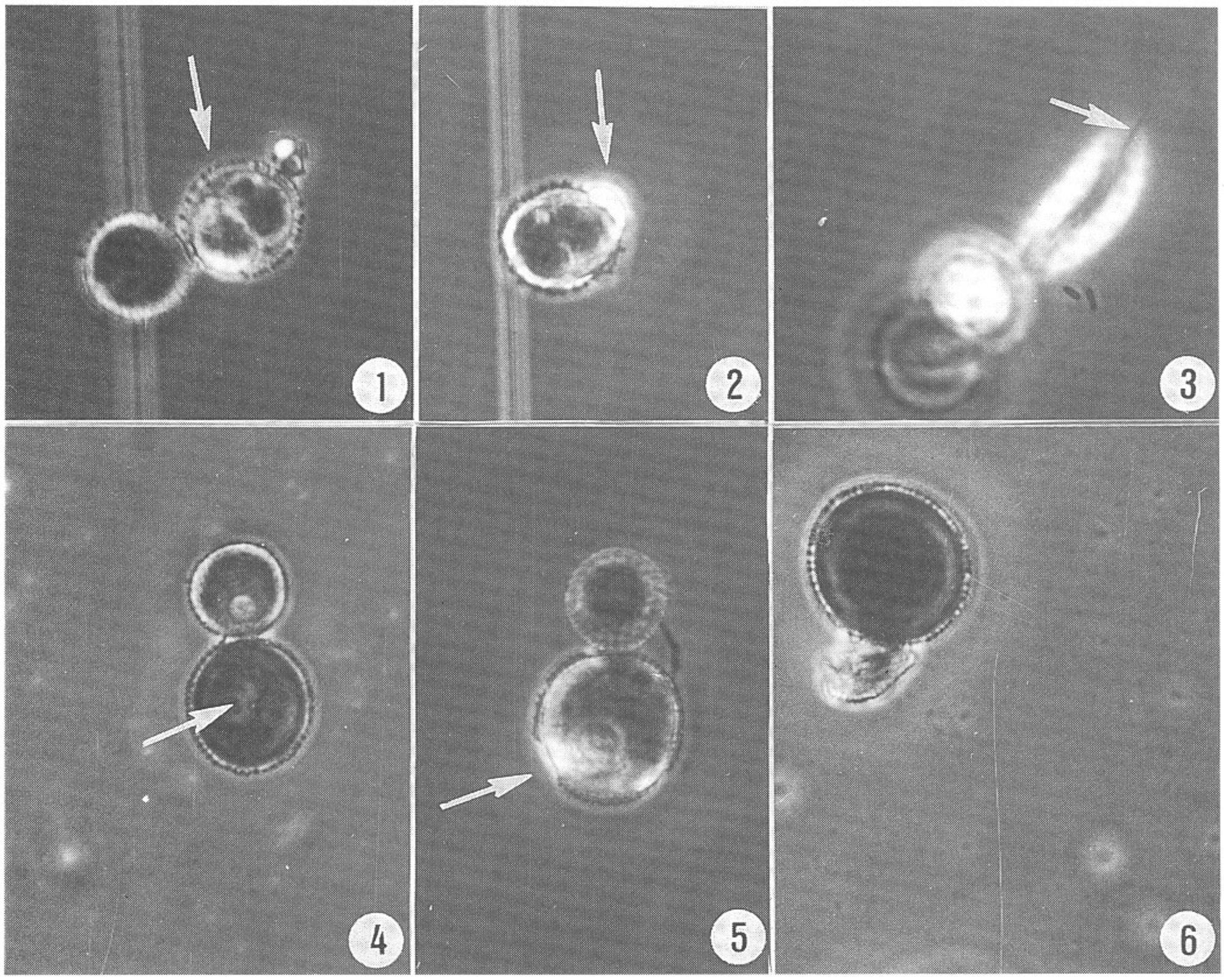


Plate III.

Figs. 1-3. Small spore germination, ×804.

1. Spore with protoplast cleaved into 2 cells.
2. Protoplast emerging from spore.
3. Second protolast emerging from spore, first protoplast now a uniflagellate (arrow) swarm cell.

Figs. 4-6. Large spore germination, ×804.

4. Spore wall beginning to split.
5. Split enlarged with discernible pore.
6. Emerging protoplast.

ACKNOWLEDGEMENTS

This research was finacially supported by the National Science Council of R. O. C. The author thanks Ms. Fu-Ya Yeh for preparing cultures for developmental studies. Special thanks are due to Dr. Roland Seymour of the Ohio State University, for his comment, critically reading and correcting the manuscript.

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臺灣黏菌 (6)

Badhamia gracilis

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摘 要

本篇描述臺灣黏菌新記錄種鈣絲屬黏菌 *Badhamia gracilis* (Macbr.) Macbr.。以光學顯微鏡和掃描式電子顯微鏡下檢視其特性，孢子表面分區成角形的大網格，而且密佈明顯的針狀突起。

孢子以開裂方式萌芽。比較小型的孢子產生兩個原生質團，而比較大型孢子則產生四個原生質團。培養單隻游走細孢，結果也形成乳白色的原生質體，因此該黏菌是「非雌雄異株」。所形成的孢子和親代一樣，而且能發芽。