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Morphology and Zoospore Ultrastructure of *Rhizophydium macroporosum* (Chytridiales)

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ABSTRACT : *Rhizophydium macroporosum* Karling is reported as a new record to Taiwan. It is isolated from mud of pond, and has been pure cultured on 1/4YpSs medium. A mature zoosporangium possesses one to six exit papillae. Zoospores emerge *en masse* from the discharged pores and are surrounded by gelatinous material. The ultrastructure of zoospore of this fungus is first reported here.

KEY WORDS : **Chytridiales, *Rhizophydium*, Ultrastructure, Zoospore.**

INTRODUCTION

Chytridiales is the largest order of the Chytridiomycetes with Rhizidiaceae as the largest family of 45 genera. *Rhizophydium* (Rhizidiaceae) has about 166 species (Karling, 1977), but many of which are incompletely known for their biology as well as life cycle, etc.. Species of *Rhizophydium* are known as their worldwide distribution. *R. macroporosum* Karling was described as saprophytic isolated from snake skin and bleached corn leaves from soil samples (Karling, 1967). Although it has been reported, our isolate is the only strain surviving in pure culture at the current time.

In recent years, many species of chytrids have been successfully grown in culture. Morphological variations in culture (Roane and patorson, 1974) and zoospore discharge mechanisms (Barr, 1975) were reported. Cultural studies have created more problems than they have solved (Barr and Hadland-Hartmann, 1978a). Recent fine-structural studies of chytridiomycete zoospores have led to a reevaluation of the taxonomy and phylogeny of these organisms (Powell, 1978; Barr, 1978; Lange and Olson, 1979). When Barr revised and redefined the Chytridiales (Barr, 1980), he recommended that the phylogenetic validity of genera in the Chytridiales should be confirmed by ultrastructural characters of zoospore (Longcore, 1992).

The purpose of this paper is to report our recent results on the investigation on morphology in culture of *R. macroporosum* and its zoospore ultrastructure to provide extra taxonomic criteria, and compare it with previously published informations.

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MATERIALS AND METHODS

Isolation and culture

This fungus was isolated by using standard methods (Barr, 1987). It was baited with pine pollen and grown on 1/4 Emerson' s YpSs agar. Morphological observations were made under the light microscope on 1/4YpSs agar and YPD broth. Developmental stages were recorded on Kodak T-MAX film by a Leitz Normaski microscope.

Preparation for electron microscopy

Zoospores were prepared for electron microscopy using a sequential glutaraldehyde-osmium tetroxide fixation method (Barr, 1980; Beakes *et al.*, 1988; Longcore, 1992). In brief, zoospores in suspension were fixed in 3% glutaraldehyde (0.05M s-collidine buffer, pH 7.4) for 2h, postfixed in 1% osmium tetroxide for 1h, washed in buffer, and embedded in 2% agar. The agar blocks were stained with 2% aqueous uranyl acetate for 2h, dehydrated in an ethanol series, and embedded in spurr' s low viscosity resin. Serial sections were cut with a diamond knife on a Sorvar MT-5000 ultramicrotome. Sections stained with lead citrate, and examined on a Hitachi HU-12A transmission electron microscope at 75KV.

Sporangia were obtained from 5-7 days old cultures on 1/4YpSs agar plate, following the initial fixation and buffer washing, dehydrated through a graded ethanol series, and then dried in a CAT-28000 critical-point drier via liquid CO_2. The specimens were sputte coated with gold before examination in a ABT DS-130S scanning electron microscope operated at 10KV.

RESULTS AND DISCUSSION

Rhizophydium macroporosum Karling, Sydowia 20: 76-77, 1967.

On 1/4YpSs agar: Sporangia sessile, spherical, 30-130 μm in diam., or pyriform, usually with one to six exit papillae that located on the upper port of the sporangium. Rhizoids arising from base of sporangia, and extending for distance up to 200 μm. Zoospores spherical, 4.5-6 μm in diam., with a minute refractive globule, emerging slowly from 2-4 exit papillae simultaneously and surrounded by a hyaline matrix. Colony with cream to brown in color.

Specimen examined: TAIPEI HSIEN, Taishan lake, 2. Dec. 1993, *NTNUSO5a*. Isolated on pine pollen from mud.

Note: This species is recorded as new to Taiwan. This species possesses fairly large number and great size of the exit papillae. The slow emergence of the zoospores are simultaneously from several papillae. These characters are similar to Karling's (1967) description.

Morphology

R. macroporosum is eucarpic, epibiotic chytrid and the encysted zoospore gives rise directly to a sporangium. The thallus development is correspond to Whiffen's type 1,

Rhizophydium type (Whiffen, 1944). The upper 1/2 to 2/3 of sporangium has one to six dome-shaped or conical exit papillae, 7.5-12.5 μm in diam. (Figs. 3, 7). Rhizoidal system usually extensively develop up to 200 μm, main axis, 5-7.5 μm in diam., arising from a single point (Fig. 2) or rarely from several places (Fig. 3) at the base of the sporangium. Zoospores spherical, occasionally becoming amoeboid, the whiplash flagellum is typically positioned posteriorly (Fig. 1). Zoospores emerging in a small globular mass surrounded by a hyaline matrix (Fig. 4) and lying quiescent for a few moments before separating. It is similar to the species with *Chytridium* subtype zoospore (Longcore, 1995). A mass of zoospores at one orifice may reenter the sporangia, and emerge through another orifice (Figs. 5-6). After the initial masses of zoospores have dispersed and the pressure within the sporangium is apparently reduced. The zoospores remaining in the sporangium swarm violently, usually emerge singly and dart away. After this, the edge of the outer wall may occasionally be folded back to form a collar-like band at the base (Fig. 8).

Zoospore ultrastructure

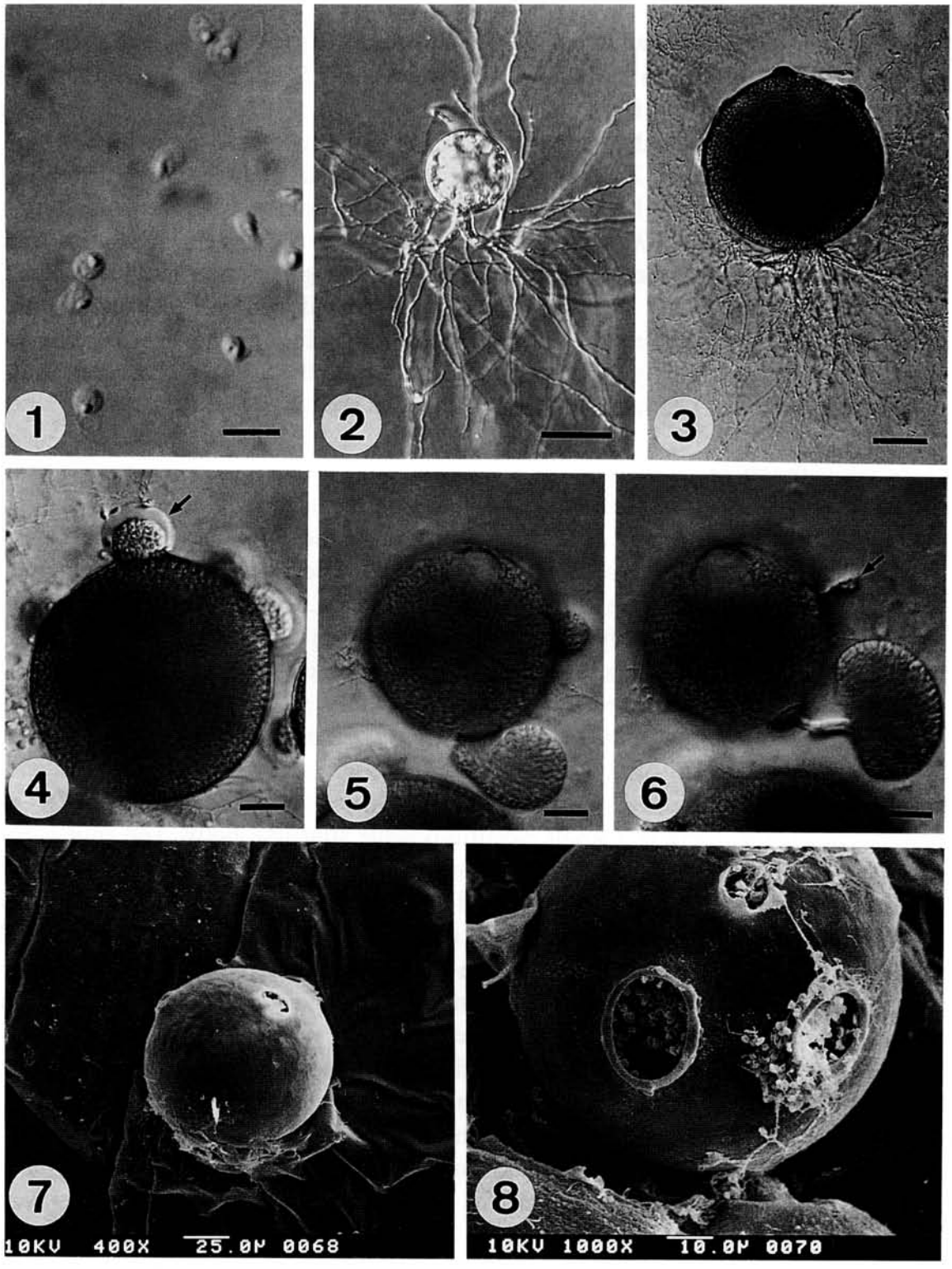
Longitudinal sections of zoospores show a single nucleus. The nucleus is located laterally position in the zoospore (Fig. 11). It is usually close to the ribosomal aggregate. The abundant ribosomes are aggregated and are partially delimited from the remainder of the cytoplasm by mitochondria, nucleus and vesicles (Figs. 9, 10). A microbody partially encapsulates the large lipid body, and separates it from the nucleus (Fig. 12). The rumposome is a honeycomb-like arrangement of membranes. It lies on the other side of the lipid body facing the outside of the cell (Fig. 11), next to the plasmalemma.

The basal kinetosome terminates adjacent to the endoplasmic reticulum cisternum delimiting the "core" region (Figs. 13-15). The kinetosome and the nonfunctional centriole lie side by side and are orientated approximately parallel to each other (Figs. 14, 18). On one side of the kinetosome, there is a small electron-opaque spur-like body. The spur is always on the same side as the kinetosome-associated microtubules (Figs. 14, 15). The microtubule bridge is between the rumposomal structure and the functional kinetosome (Figs. 16, 17).

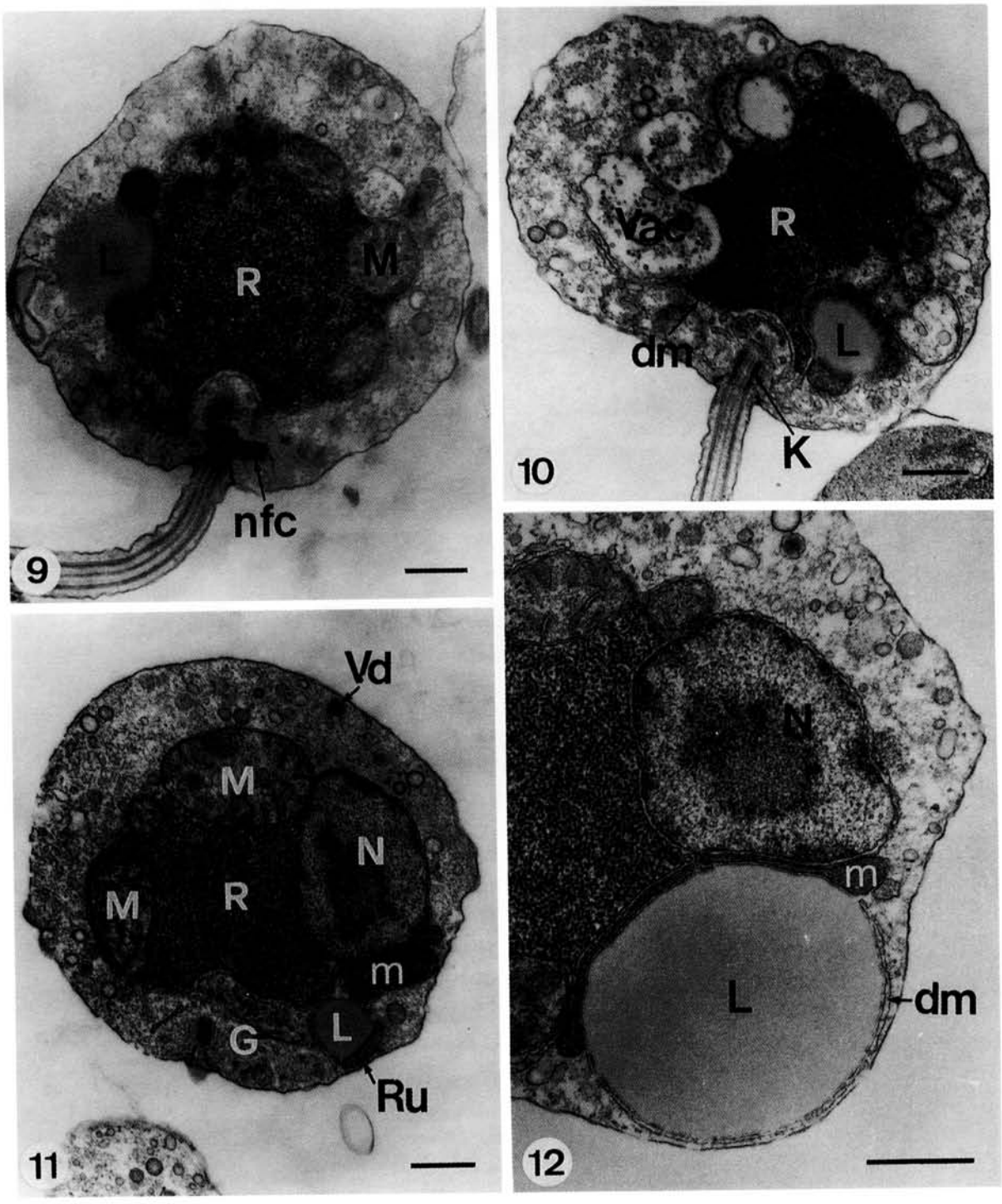
In the peripheral area of the zoospore, there are a few globular vesicles, about 0.2-0.3 μm in diameter which contain very electron-opaque material (Figs. 11, 16). Near the kinetosome, there is a cluster of vesicles or smooth endoplasmic reticulum (Figs. 16, 19) which buds off from the double membrane system.

The zoospore of *R. macroporosum* is fundamentally similar to that of *Rhizophydium* type (Barr and Hadland-Hartmann, 1978a) but with some variations. These variations include the Golgi cisternae that are not seen in distinct profile but lie near the kinetosome. Another difference in the location of the nucleus also exist among species with a *Chytridium* zoospore subtype. In *Chytridium confervae* the nucleus abuts on the lipid globule (Barr and Hadland-Hartmann, 1976), and in *Entophlyctis luteolus* the nucleus consistently surrounds a portion of the lipid globule (Longcore, 1995). The spur associated with the kinetosome may be an additional criterion (Barr and Hadland-Hartmann, 1978b). It also exist among *Phlyctochytrium arcticum, P. irregulare, P. plurigibbosum* (Barr and Hadland-Hartmann, 1979), and one of two isolates of *R. sphaerotheca* (Barr and Hadland-Hartmann, 1978a).

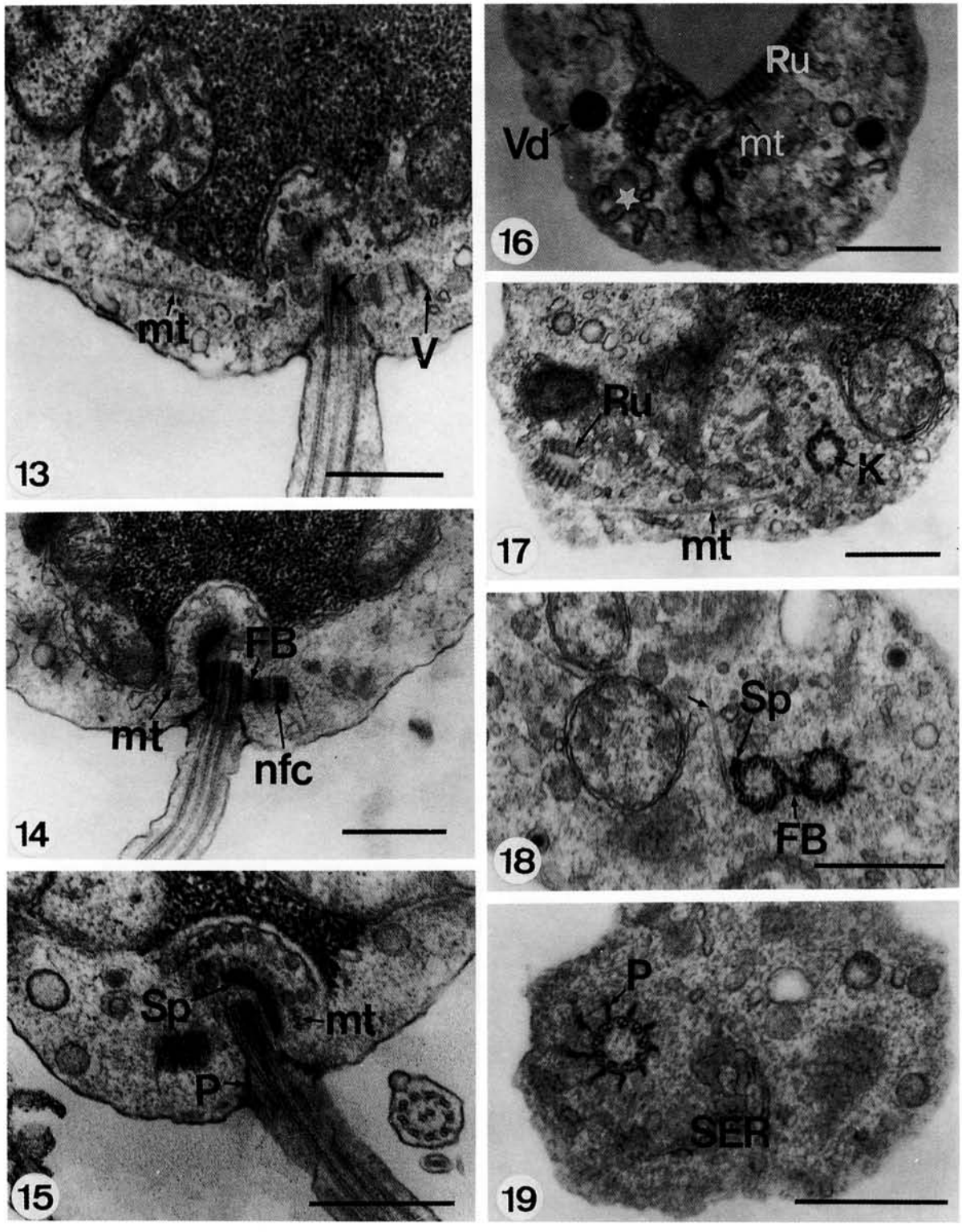
This fungus has several characters common to *Rhizophydium* and *Chytridium.* Thus, based on zoospore ultrastructure, and also on thallus development, *R. macroporosum* is a valid taxon.



Figs. 1-8. Morphology of *R. macroporosum.* 1-2. in YPD broth; 3-6. on 1/4YpSs agar, Normaski microscopy. 7-8. on 1/4YpSs agar, scanning electron microscopy. 1. Zoospores with one lipid globule and posterior flagellum. (bar = 10 μm). 2. Young sporangium with extensive rhizoids (bar = 40 μm). 3. Nearly mature sporangium with conical exit papillae. 4. Emerging zoospore mass covered by a layer of hyaline material (arrow). 5-6. Sequential photomicrographs of two globular mass of zoospores escaping simultaneously from the sporangium. 6. A mass of zoospores at one orifice (arrow) reenter the sporangium (3-6. bars = 20 μm). 7. The upper portion of sporangium with three discharged pores. 8. The edge of the discharged pores folded back to form a collar-like band.



Figs. 9-12. Ultrastructure of *R. macroporosum* zoospore (all bars = 0.5μm). 9, 10. Longitudinal section through a zoospore; the aggregated ribosomes (R) were surrounded by mitochondria (M), lipid globule (L), vacuoles (Vac) and double membrane (dm). Note the kinetosome (K) area. 11. Longitudinal section showing nucleus (N), lipid globule, mitochondria(M), rumposome (Ru), microbody (m) and aggregated ribosomes(R). The electron-dense vesicles (Vd) in the peripherial cytoplasm, Golgi cisternae (G) lie near the kinetosome. 12. A microbody partially encapsulates the lipid body, and seperates it from the nucleus. The double membrane (dm) surrounding the microbody-lipid globule complex.



Figs. 13-19. Ultrastructure of *R. macroporosum* zoospore. (all bars = 0.5 μm). 13-15 Longitudinal sections through kinetosomal area showing kinetosome-associated microtubules (mt), props (P), an electron-opaque spur (Sp) proximal to the kinetosome, and veil (V) on one side of the nonfunctional centriole (nfc). 16, 17. Cross sections showing the kinetosome-associated microtubule running to rumposome, the electron-dense vesicles and smooth endoplasmic reticulum (star) in the periphery. 18. Cross section through the kinetosome and nonfunctional centriole showing the fibrous bridge (FB) connecting these organelles, the spur, and one kinetosome-associated microtubule (arrow) run from the side of the kinetosome. 19. Cross section through the basal of kinetosome, props (P) attached to the hooked doublets, and smooth endoplasmic reticulum (SER) in the peripheral cytoplasm.

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大孔根生壺菌 (*Rhizophydium macroporosum*) 形態及游孢子超微結構之研究

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摘　　要

本文描述一種臺灣新紀錄大孔根生壺菌 (*Rhizophydium macroporosum*)，使用光學顯微鏡及掃描式電子顯微鏡觀察菌體外部形態，並以穿透式電子顯微鏡觀察游孢子內部的微細構造。經由純培養，游孢子發育為成熟的菌體，游孢子囊大型，成熟的游孢子同時從多個釋放孔擠出於透明膠狀物質中，隨後游孢子散開。游孢子超微結構為該菌種首次報導，顯示基本上具有根生壺菌型游孢子之特徵。

關鍵詞：壺菌目，根生壺菌屬，超微結構，游孢子。

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