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RESEARCH ARTICLE

Morphology and Zoospore Ultrastructure of *Chytriomyces multioperculatus* (Chytridiales)

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ABSTRACT: *Chytriomyces multioperculatus* Sparrow and Dogma is a new record of Taiwan. It was isolated from water of a pond, and pure cultured on 1/4YpSs medium. The mature zoosporangium produced numerous operculate discharge papillae. The opercula either separated from or remained attached to the rim of the exit orifices. The zoospore ultrastructure of C. *multioperculatus* is similar to the zoospore characteristic of *Lobulomyces* (Lobulomycetales).

KEY WORDS: Chytridiales, *Chytriomyces*, ultrastructure, zoospore.

INTRODUCTION

Some of the largest chytrid genera (ex. *Rhizophydium, Entophlyctis* and *Chytriomyces*) are based on few distinctive morphological characters and are in need of revision. Current efforts are focused on combining morphological, ultrastructural and molecular data for redefining genera of Chytridiales (James *et al*., 2006). *Chytriomyces* (Chytridiales) has about 34 species (Letcher and Powell, 2002). The ultrastructural and molecular features of only a few species have sufficient data. Only three species of the genus (*C. aureus, C. confervae, and C. hyalinus*) have well described zoospore ultrastructure (Barr and Hartmann, 1976; Dorward and Powell, 1982; Longcore, 1992). More than half the species have been observed and reported only upon their discovery (Letcher & Powell, 2002), perhaps indicating that many species are uncommon to rare. On the basis of genetic and ultrastructural data, two former members of the genus (*C. angularis* and *C. poculatus*) have been removed from *Chytriomyces* and placed in *Lobulomyces*, Lobulomycetales (Simmons *et al.*, 2009).

The purpose of this paper is to describe *C. multioperculatus* as a new record of Taiwan and to illustrate its zoospore ultrastructure.

MATERIALS AND METHODS

Isolation and culture

The water of a pond was collected and baited with pine pollen, and incubation in 20°C. Emerson's 1/4 YpSs medium (Barr, 1987) was used to isolate and culture this organism. Morphological observations were made with a Leica microscope on 1/4 YpSs agar (12 g agar/liter) and 1/4 YpSs slush (1 g agar/liter).

Preparation for electron microscopy

Scanning electron microscope: Sporangia were obtained from 5-7 days old cultures fixed with a sequential glutaraldehyde-osmium tetroxide fixation method (Chen and Chien, 1996), washed in cacodylate buffer solution, dehydrated through a graded ethanol series, and dried in critical-point dryer. The specimens were sputter-coated with gold before examination in a AKASHI (ABT) DS-130S scanning electron microscope at 10KV.

Transmission electron microscope: Zoospores were prepared for transmission electron microscopy using a sequential glutaraldehyde-osmium tetroxide fixation method (Chen and Chien, 1996). Fixed zoospores were placed in agar. The agar blocks were stained with uranyl acetate, dehydrated in an ethanol series, and embedded in spurr's low viscosity resin. Serial sections were cut with a diamond knife on an ultramicrotome. Sections were stained with lead citrate, and examined on a Hitachi HU-12A transmission electron microscope at 75KV.

RESULTS

Morphology

Chytriomyces multioperculatus Sparrow and Dogma, Arch. Microbiol. 89:193, 1973.

Fig. 1

In 1/4YpSs slush: Thallus monocentric, eucarpic. Sporangia spherical or subspherical, 16-53 μm in diam., usually with one to three (or more) slightly elevated discharge papillae, 5-13 μm in diam., each surmounted by an operculum. Rhizoids arising from one or more places on a subtriangular or spindle-shaped apophysis.

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Zoospores spherical, 2.5-4 μm in diam. Resting spore not observed.

On 1/4 YpSs agar: sporangia spherical. Basal rhizoidal axis 2.5-5 μm in diam. Zoospores spherical, discharged forcefully, upon dehiscence of opercula, motility extrasporangial. Opercula are saucer-shaped, smooth and 7-15 μm in diam., either separated from or hinged to the rim of the exit orifice.

Specimen examined: **Tauyun**: Tachi, pond, 2 Jan. 2004, *CHNA 2202a*. Isolated on pine pollen from water.

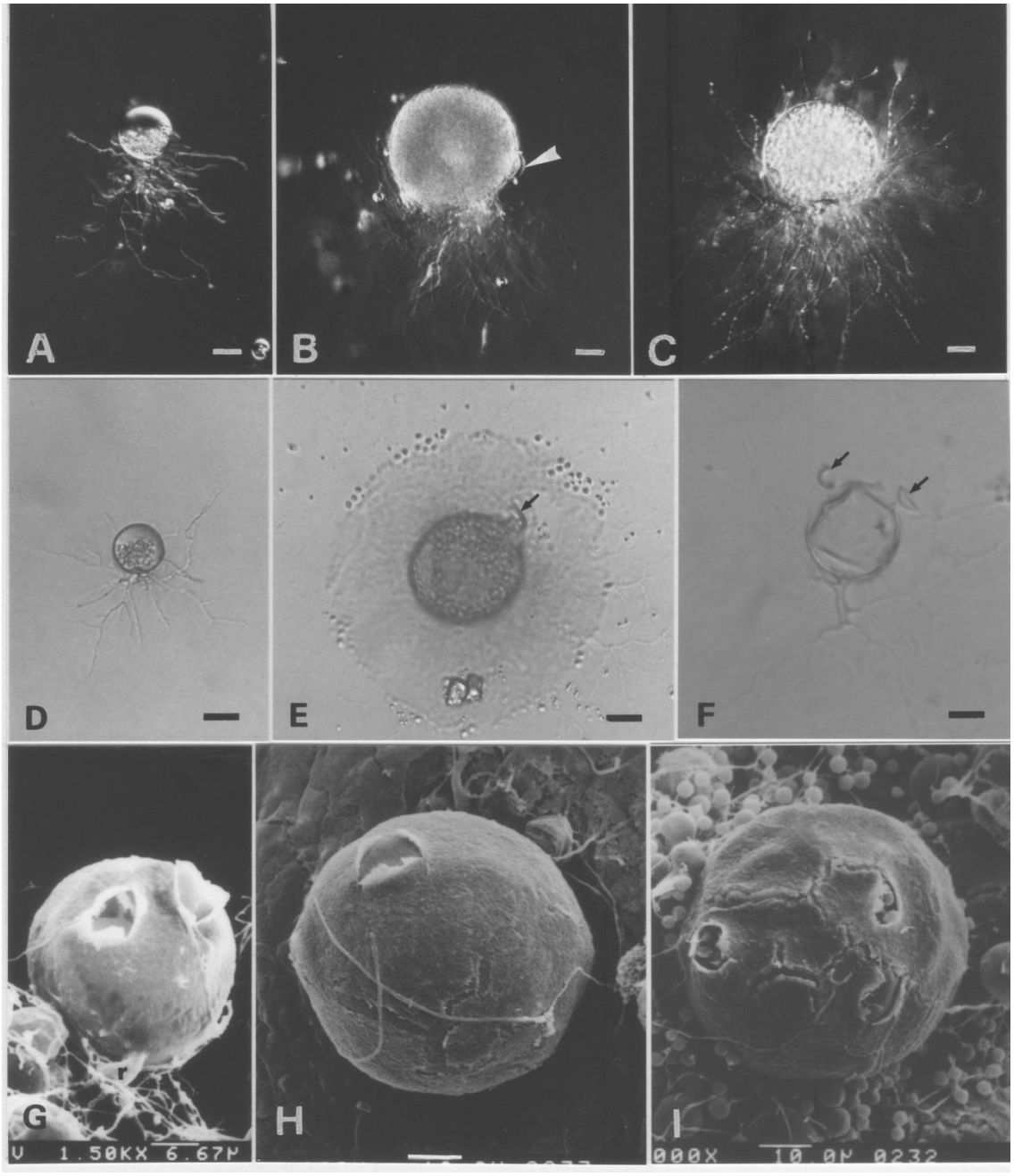


Fig. 1. Morphology of *Chytriomyces multioperculatus*. A-C: In 1/4YpSs slush. D-F: On 1/4YpSs agar, Normaski microscopy. G-I: On 1/4YpSs agar, scanning electron microscopy. A: Young sporangium. B: Mature sporangium with exit papilla (arrowhead). C: Mature sporangium. D: Young sporangium. E: Swimming zoospores around sporangium, with an operculum (arrow). F: Two saucer shaped opercula (arrows) persist outside the empty sporangium. G: The rhizoidal system with a spindle-shaped apophysis (r). H: Young sporangium with an operculate papilla. I: Mature sporangium with two exit pores and one inside operculum. Scale bar for all =10 μm, except in G =6.67 μm.

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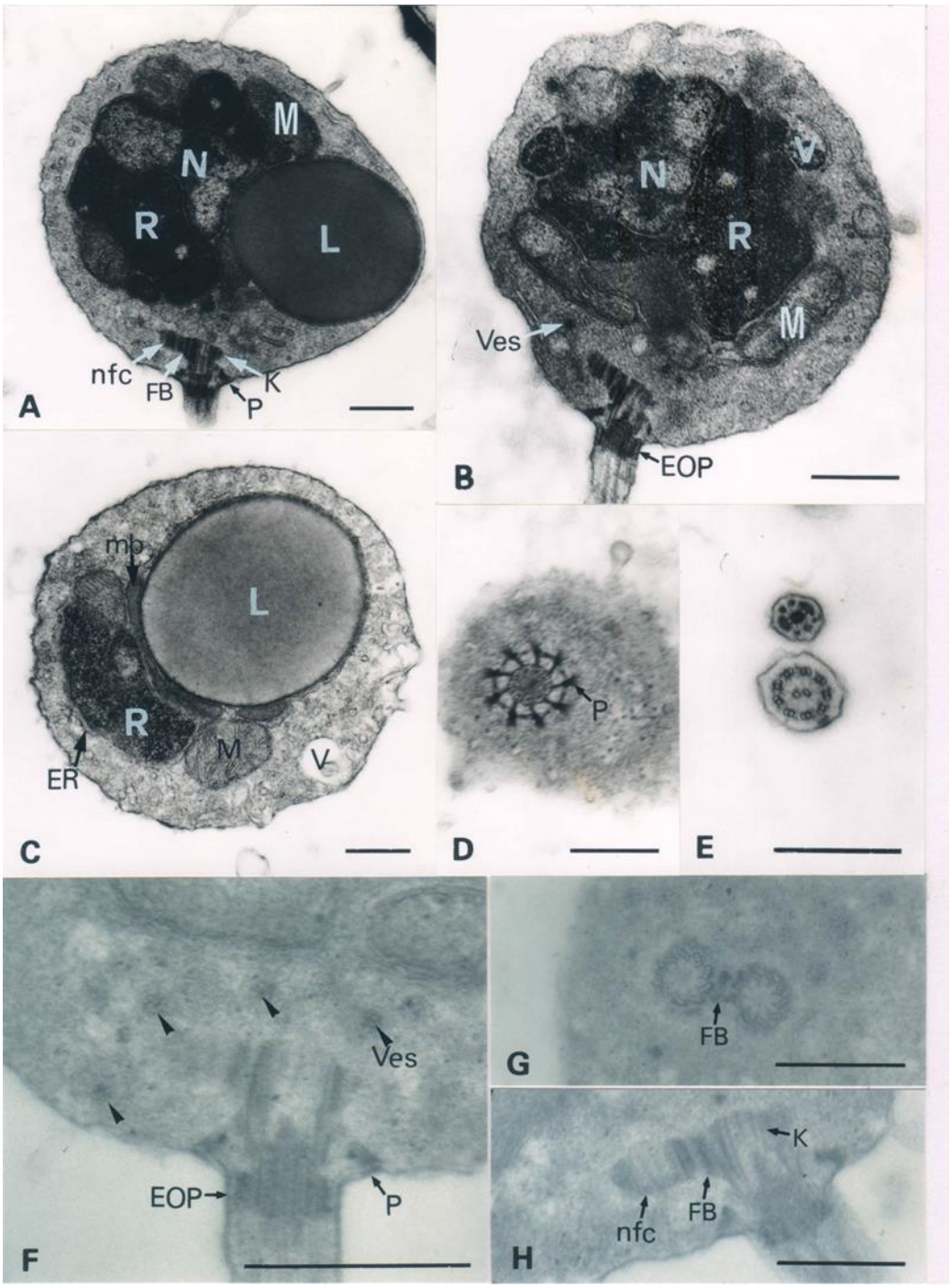


Fig. 2. Ultrastructure of *Chytriomyces multioperculatus* zoospore. A&B: Longitudinal sections through zoospore illustrating the typical arrangement of organelles. C: Cross section through zoospore. The ribosomes are surrounded by endoplasmic reticulum, and the ribosomal core is adjacent to mitochondria and the microbody-lipid globule complex. D: Cross section through the base of kinetosome, props attached to the hooked doublet. E: Cross section through the basal electron-opaque plug of flagellum (upper) and cross section of flagellum (lower). F: Longitudinal section with vesicles (arrowheads) and the electron-opaque plug in base of flagellum. G: Cross section through the kinetosome-nonflagellated centriole and fibrillar bridge. H: Longitudinal section through the kinetosome-nonflagellated centriole, the two connected by a fibrillar bridge. Abbreviations, EOP: electron-opaque plug; ER: endoplasmic reticulum; FB: fibrillar bridge; K, kinetosome; L, lipid globule; M, mitochondria; mb, microbody; N, nucleus; nfc, nonflagellated centriole; P, props; R, ribosomes; V, vacuole; Ves, vesicles. Scale bar for all =0.5μm.

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Ultrastructural features of *C. multioperculatus* are similar to those of the Group V type zoospore (Longcore 1992; Letcher and Powell, 2005). The zoospore is spherical, with a single, large lipid globule occupying a lateral position (Figs. 2A, C). Ribosomes are aggregated in a membrane-bound core. Mitochondria are outside the endoplasmic reticulum that delineates the ribosomal core (Figs. 2A, B, C). The nucleus is positioned near the edge of the ribosomal aggregation and is not associated with the kinetosome. A microbody surrounds part of the lipid globule forming a microbody-lipid globule complex (MLC) (Fig. 2C). Vesicles are present in the peripheral cytoplasm (Figs. 2B, F).

The nonflagellated centriole is parallel to the kinetosome, and a dense fibrillar bridge connects the kinetosome and nonflagellated centriole (Figs. 2A, G, H). An electron-opaque plug extensions anterior and posterior (Figs. 2B, F), present in the transition region of the flagellum (Fig. 2E). Flagellar props extend from the kinetosome to the plasma membrane (Figs. 2A, F) and are interconnected by fibrillar material (Fig. 2D).

DISCUSSION

Chytriomyces multioperculatus is an epibiotic chytrid. The thallus development is corresponds to Whiffen's type 2, Chytrid type (Whiffen, 1944). This species is a new record for Taiwan.

The zoospore of *C. multioperculatus* lacks microtubular root, kinetosome-associated structures, a striated inclusion, and a fenestrated MLC cisterma, features that are the same as those of the *C. angularis* (Longcore, 1992). Genetic analyses, ultrastructural data, and morphology supported the establishment of a new order *Lobulomycetales*, and placement of *C. angularis* in a new genus *Lobulomyces* (Simmons *et al*., 2009). *C. multioperculatus* exhibits operculation, and non-vesicular zoospore discharge, morphological characters similar to *Lobulomyces*. But, *C. multioperculatus* has a rounded sporangium with numerous discharge papillae, which is different from the angular zoosporamgium that is longer than wide of *Lobulomyces* species. The ultrastructural evidence indicates that *C. multioperculatus* is not related to the type species of *Chytriomyces*, but may be related to Lobulomycetales. Additional sampling might locate other isolates with this zoospore morphology and help clarify taxonomic ambiguities.

Although zoospore ultrastructure predicts affinities of an isolate, it is zoospore ultrastructure combined with molecular data that confirms an organism's phylogenetic and taxonomic placement. Further research is necessary to obtain molecular sequences to reliably remove *C. multioperculatus* from *Chytriomyces* and possible placement in *Lobulomyces.*

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多囊蓋壺菌 (*Chytriomyces multioperculatus*) 形態及游孢子超微結構研究

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摘 要:本文描述一種臺灣新紀錄多囊蓋壺菌(*Chytriomyces multioperculatus*)，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，游孢子囊具有 2 個以上的釋放孔，游孢子釋出後囊蓋脫落或垂掛在孔緣。游孢子超微結構為本菌種首次報導，顯示基本上與 *Lobulomyces* (Lobulomycetales)游孢子之特徵相似，不具有微小管束與梳狀體。

關鍵詞：壺菌目、壺菌屬、超微結構、游孢子。

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