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Ultrastructural characters of a *Physarum melleum* on living leaves of *Dendrobium candidum* in China*

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Abstract: A known species, *Physarum melleum*, was found fruiting on living leaves of *Dendrobium candidum*, which was collected in China in 2004. Its morphological characters were revealed by light microscopy (LM), environmental scanning electron microscopy (ESEM) and scanning electron microscopy (SEM). Character variations were distinguished by its olive-yellow peridium and its always thinner capillitium containing globulose granular material between the large calcareous nodes. The calcium carbonate granules, deposited on stalks, peridium and hypothallus as well as within stalks, were globose and smooth.

Key words: Myxomycete, *Physarum*, Ultrastructure, Fruiting site, Peridium
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INTRODUCTION

Myxomycetes (plasmodial slime molds) are a wide group of soil amoebae which actively feed primarily on other protists, bacteria, and fungi in top soil and thatch. They are widespread and comprise more than 1000 described taxa (Schnittler and Stephenson, 2002**)**. They are characterized by a complex life cycle. Two haploid amoeboflagellates fuse to form a diploid plasmodium. At a certain point, the hidden plasmodium resurfaces from soil, creeps onto detritus or low-lying branches or other vegetation and forms sporophores on top of the plant surface. The plasmodium can reach conspicuous dimensions and the surface of their fruiting site may be covered with a translucent to creamy white or yellowish slimy growth (Couch, 1995). The sporophores dry up and release their spores either mechanically, as in *Trichiales*, or passively by using wind, rain, or insects.

Myxomycete sporophores may cover large portions of low-lying plants. Golenia and Rebendel (1970) and Filipowicz (1979) observed *Diachea leucopodia* and *Stemonitaceae* on young and old leaves of strawberry plants (*Fragaria ananassa* Duch.) in Poland. In North America, *Fuligo septica*, *Mucilago spongiosa*, and *Physarum cinereum* are known to colonize the surface of turfgrass leaves (Sharnoff, 1991; Stephenson and Stempen, 1994; Couch, 1995; Alexopoulos *et al*., 1996). More recently, some myxomycetes species were observed rather frequently on the living inflorescences of these large tropical herbs (Schnittler and Stephenson, 2002), while *P. pusillum* actually colonized on the body of a living cryptic lizard, the cryptic Neoptropical species *Corytophanes cristatus* (Townsend *et al*., 2005).

Recently, we encountered a species of *Physarum* on the living leaves of *Dendrobium candidum* Wall. ex Lindl. grown in a plot near the shrubs of hillside in Shaoxing District of Zhejiang Province, China. It was very similar to *P. melleum* (Berk. & Br.) Massee, but differed with respect to the capillitium. Otherwise, in this specimen calcium carbonate granules are typically regular and globose, characteristics not previ-

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ously reported in the genus *Physarum*. Accordingly, in this report, we describe this specimen as species *P. melleum*, and document its morphological and ultrastructural characters.

MATERIALS AND METHODS

Myxomycete samples were collected from the suburban area of Shaoxing City (30°00′ N, 120°38′ E, elevation 20 m), Zhejiang Province, China. Shaoxing has a subtropical humid monsoon climate and its average annual rainfall is 1438.9 mm, coming mostly in the wet season from May through July. A mean monthly temperature in Shaoxing is above 16.5 °C.

The species description was based on light microscopy (LM) and environmental scanning electron microscopy (ESEM) or scanning electron microscopy (SEM). Macroscopic measurements were taken from 100 mature sporophores. Sporophores were examined in wet mounts because lactophenol dissolves calcareous structures. For ESEM, fresh samples were mounted directly onto an aluminium stub with metal-containing glue, and examined and photographed in a PHILIPS XL30 ESEM (FEI Company, German). Samples for SEM were washed in phosphate buffer prior to post fixation in 1% (w/v) osmium tetraoxide in 0.1 mol/L phosphate buffer for 2 h, then dehydrated in a graded ethanol series. Myxomycete samples were fixed in 1% (w/v) glutaraldehyde at 4 °C for 1 h and then transferred to phosphate-buffed glutaraldehyde (pH 7.0) for 24 h at 4 °C. The samples were subsequently critical-point dried, mounted onto an aluminium stub with metal-containing glue, coated with gold and examined and photographed in an SEM as described above.

DESCRIPTION

***Physarum melleum* (Berk. & Br.) Massee**

Sporangia gregarious, not closely crowded, stipitate, 0.75~0.90 mm in total height, spore case globose, 0.3~(0.435)~0.5 mm; peridium rugose, persistent, opaque, coarse when mature, stiff, olive-yellow to pale brown, encrusted with lime; stalk cylindric or tapering upward, stout, opaque, typically white, furrowed, embedded with calcareous globulose beads, height 0.35~0.45 mm, diameter at midsection 0.14~0.16 mm, expanded at base 0.34~0.47 mm; columella structurally similar to stalk, small, conical, white; hypothallus white or colourless, inconspicuous; capillitium always thinner between the large, angular and white calcareous nodes; spores globose, free, dark brow to black in mass, purplish brown with transmitted light, wall black, 1 m thick, body of spore 8.5~(9.0)~11.5 μm diameter, ornamentation unconspicuous under LM, but spore ornamentation with blunt warts under ESEM (Figs.1~3).

Materials examined

Specimens were collected from a plot, located in neighboring shrubs on a hillside in the suburban area of Shaoxing City, elevation 66 m. Living *Dendrobium candidum* were harvested on June 27th, 2004, HMAS 172269 (J.Z. Zhang et X.M. Xu) and MJ 2004-001 (deposited in Herbarium of Department of Plant Pathology of Zhejiang University, Hangzhou City, China).

The specimen is identified as a member of *Physarum* based on its thin capillitium and large calcareous nodes, and most closely resembles *P. melleum*. It mostly reminds of *P. melleum* (Berk. & Br.) Massee, with similar size of finely warted spores, and a white stalk, with a short and obtuse tip. However, it differs in the peridial color and the structure of the capillitium. The peridium is olive-yellow, whereas it is honey-yellow in *P. melleum*. Whitish calcareous nodes are large (up to 150 μm) (Fig.1d), while the capillitium is thin between the nodes. Under SEM, capillitium is a thin thread and seems to contain globulose granules (Fig.3d), which are hardly visible.

RESULTS

The surface of spore case, stalk and hypothallus was all covered by globose calcium carbonate granules (Figs.2a, 2c and 2f). When samples were treated for SEM, calcium carbonate granules flaked off, leaving numerous hemi-round pits on the furrowed surface of the stalk (Fig.3a). Similarly, the treated cross sections of stalk also left numerous hemi-round pits (Fig.3c). The stalk consists of organic matter and globose calcium carbonate granules (Fig.3c). In other taxa, such as *Clastoderma*, the organic matter may

Fig.1 Light microscopy (LM) of *Physarum melleum*. (a) Groups of stalked sporangia on young leaves of *Dendrobium candidum*; (b) Enlarged portion of (a); (c) Single or groups of sporangium under stereomicroscopy; (d) Single sporangium with broken spore case showing black spore mass intermingled with whitish capillitium. The enlarged calcareous nodes and character of the genus *Physarum* are clearly visible

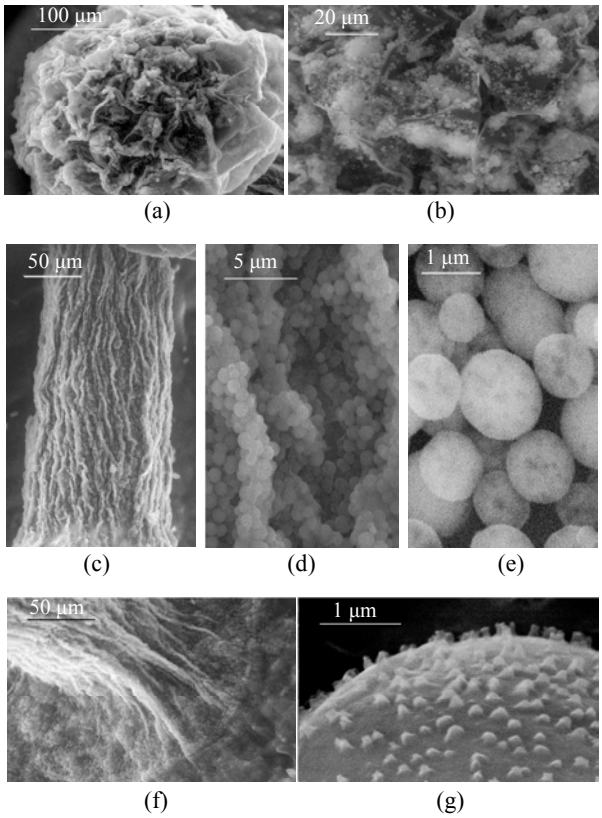

Fig.2 Environmental scanning electron microscopy (ESEM) of *Physarum melleum* of sporangia. (a) A spore case covering calcium carbonate granules wrinkled peridium; (b) Calcium carbonate globulose granules on wrinkled peridium; (c) Stalk with irregular furrows; (d) A layer of calcium carbonate globulose granules covering on the surface furrows and ridges of the stalk; (e) High magnification showing individual calcium carbonate globulose granules on the surface of the stalk; (f) Basal part of furrowed stalk showing calcium carbonate globulose granules on hypothallus; (g) Various pattern of spore ornamentation with blunt warts

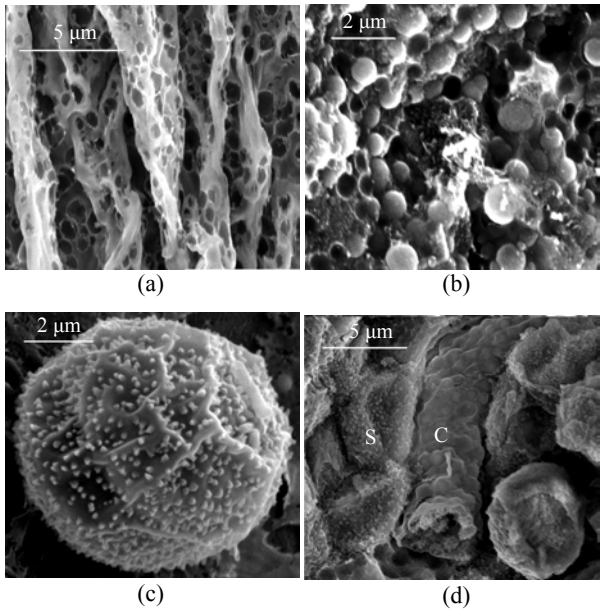

Fig.3 Scanning electron microscopy (SEM) of *Physarum melleum* of sporangia. (a) Furrowed surface of stalk without calcium carbonate globulose granules. Calcium carbonate globulose granules flaked off, leaving numerous pits; (b) Cross section of stalk showing individual globulose granules. Some of calcium carbonate globulose granules flaked off, leaving a lot of pits; (c) Spore with warty ornamentation and irregular protuberant ridges; (d) Capillitium (C) intermingled in spore (S) mass. A thin capillitium contained globulose granular material

include fungal spores, algae, or bacteria that were trapped in the plasmodium (Keller and Braun, 1999). Calcium carbonate granules were either distributed on the surface of the sporangium or in the interior. Calcium carbonate granules were globulose, of a diameter of 0.75~2.0 μm. Spore surface had blunt warty ornamentation. Under SEM, the capillitium apparently contained globulose granular material.

DISCUSSION

Physarum is the myxomycete genus containing the largest number of species (Clark and Stephenson, 2003). Members of the genus deposit amorphous calcium carbonate (lime) on their stalks and peridium and within the sporangia as limy knots (Townsend *et al*., 2005). However, in this specimen calcium carbonate granule differs from the above description and its different sized carbonate granules are in a uniform globose shape. Otherwise, the ultrastructure of capillitium showed that it also contained globulose granules, but these granular compositions are still not determined yet. These details are described for the first time for *Physarum*.

Ecologically speaking, some species of *Physarum* seem to prefer certain substrates for fruiting, *Physarum cinereum* fruits on living St. Augustine grass (*Stenotaphrum secundatum*), whereas *P. bivalve* prefers living herbaceous plants (Townsend *et al*., 2005). *Physarum melleum* is frequently seen on decaying leaf litter and wood fragments and is the most common species in the midwest of North America (Keller and Braun, 1999) and in a few provinces in China, including Jilin, Heilongjiang, Jiangsu, Anhui, Fujian, Taiwan, Hubei, Hunan, Honghang, Guangxi, Sichuan, and Yunnan (Chen *et al*., 1999). Nevertheless, in this study, we have observed that *Physarum melleum* fruits on young living leaves of *Dendrobium candidum*, which is cultivated as a traditional Chinese medicinal herb (Zhang *et al*., 2007).

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