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Autofluorescence and Ultrastructure in the Myxomycete *Diachea leucopodia* (Physarales)

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Abstract Autofluorescence is reported for the first time in Myxomycete fruiting bodies. Ultrastructure of stalked sporangia of *Diachea leucopodia* (Didymiaceae, Physarales) was studied using scanning and transmission electron microscopy, energy-dispersive X-ray microanalysis, and fluorescence microscopy. External and internal properties of the peridium that surround the spores and capillitium exhibit autofluorescence. The stalk is composed of calcareous granules and energy-dispersive X-ray microanalysis demonstrates that the elemental composition of the peridium, capillitium, and stalk has varying concentrations of calcium.

Introduction

Autofluorescence is reported here for the first time in fruiting structures of a Myxomycete. *Diachea leucopodia* (Didymiaceae, Physarales) belongs to the Myxomycetes and is characterized by sporangia with a thin, iridescent, transparent, external peridium enclosing dark brown spores, a branching and anastomosing capillitium, and a typically calcareous columella and stalk. This species was observed using different microscopic techniques including scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray microanalysis (EDX), and fluorescence. Little information [5] is available about the ultrastructure of this species. The important question of what constitutes a morphospecies in the Myxomycetes is based in part on the ultrastructural characters described here. Fruiting body morphology is used in generic and species descriptions to identify Myxomycetes. In addition, the structural property and the presence of autofluorescence are discussed as these relate to the Myxomycetes.

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Materials and Methods

Specimens examined: Argentina, Buenos Aires: Ezeiza, VII-1972 *D. leucopodia*, BAFC 22603; Florencio Varela, on bark of *Melia azedarach* and *Fragaria* sp, IX-1971, BAFC 22487; USA, Maryland: Takoma Park, VII-1962, BAFC 22576. Herbarium abbreviations follow Holmgren et al. [4].

Herbarium material was used for SEM and TEM studies. Sputter coating treatment was made using gold–palladium for 3 min. Scanning photomicrographs were taken with a Zeiss Supra 40 FESEM microscope. The EDX spectroscopy technique also was used in Myxomycetes by Nelson [9], Aldrich [1], and Schoknecht and Keller [12] to analyze the elemental composition of the peridium and stalk.

Material used for TEM was pre-fixed in 2.5 % glutaraldehyde in phosphate buffer (pH 7.2) for 2 h then post-fixed in OsO_4 at 2 °C in the same buffer for 3 h followed by

dehydration in an ethanol series and embedded in Spurr's resin. Thin sections were made on a Sorvall ultramicrotome, then stained with uranyl acetate and lead citrate [10]. The sections were observed and photographed with a JEOL-JEM 1200 EX II TEM at 85.0 kV. Observations were made under UV light with an Axioscope microscope (filter: 450–490 SB, FT 510, BP 515–565). The samples were also observed with an Olympus BX60 M Brightfield reflected light metallurgical microscope and the images were captured with a Photometrics CoolSnap$_{cf}$ camera.

Results

The sporangium of *D. leucopodia* is cylindrical with a thin, iridescent external peridium (Fig. 1a).

Peridium

The observations of the ultrastructure of peridia with SEM and TEM (Fig. 2a) agree with the description provided by Inchaussandague et al. [5]. Iridescence was observed only in mature sporangia and was mostly absent in immature structures.

The use of fluorescence microscopy without the presence of any fluorochrome shows the peridium as an autofluorescent structure (Fig. 2 b–d). Fluorescence appears in young sporangia whereas iridescence is more evident in mature sporangia.

The analyses with EDX show the presence of silica (Si), phosphorous (P), iron (Fe), potassium (K), and calcium (Ca) (Fig. 3c–f). However, not all of the studied samples exhibit the same elements, and their distribution throughout the peridium is not uniform.

Stalk

A cross section of the stalk shows an external layer that surrounds its body, where a high content of spherical-shaped calcium granules is found (Fig. 1b, c). The EDX analysis confirms this observation (Fig. 3a).

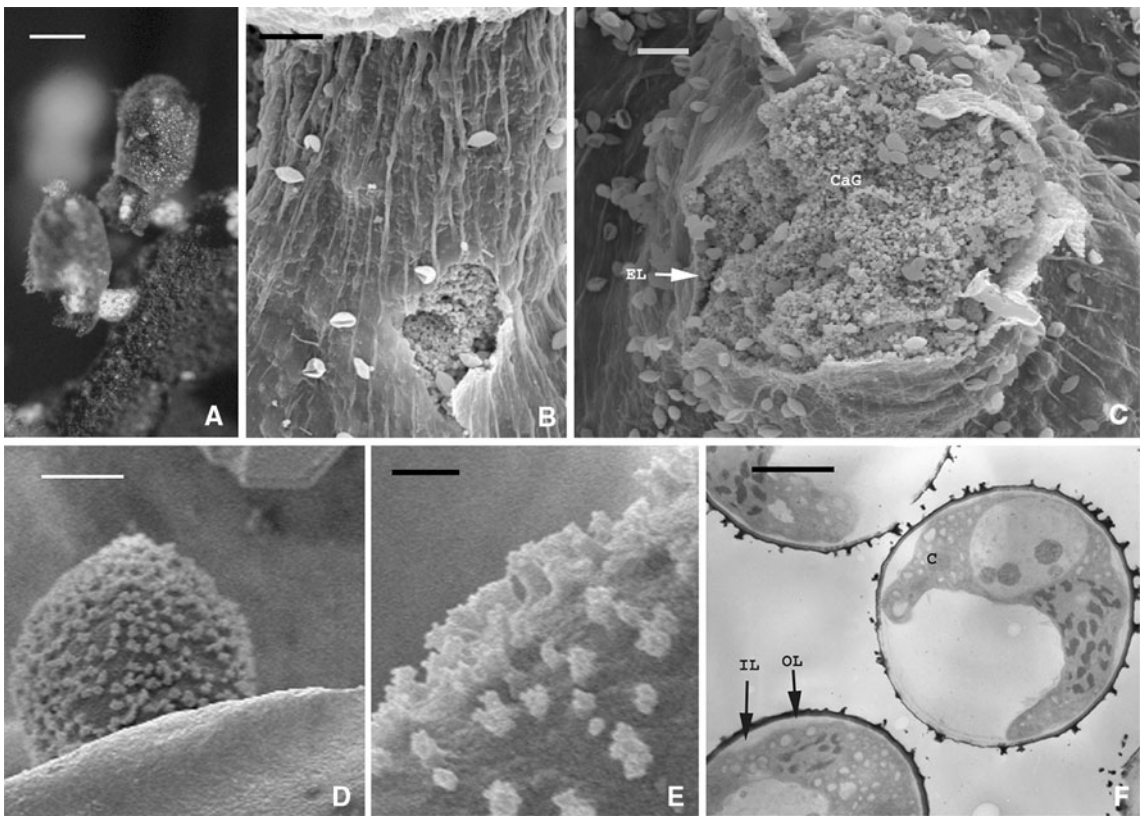

Fig. 1 *Diachea leucopodia.* A Sporangia. **a** photomicrograph showing an iridescent external peridium; **b**, **c** SEM photomicrograph showing stalk. **c** Cross section of the stalk showing a fine external layer and a higher content of calcium granules in the inner part. **d–f** Spores. **d**, **e** surface SEM photomicrograph. **d** Spore surface with irregularly distributed projections. **e** Detail of **d**. **f** TEM photomicrograph showing two layers of spore wall and cytoplasm. *EL* external layer, *CaG* calcium granules, *OL* outer spore wall layer, *IL* inner spore wall layer, *C* cytoplasm. *Bar* **a** 144 μm; **b** 100 μm; **c** 20 μm; **d** 5 μm; **e** 2 μm; **f** 5 μm

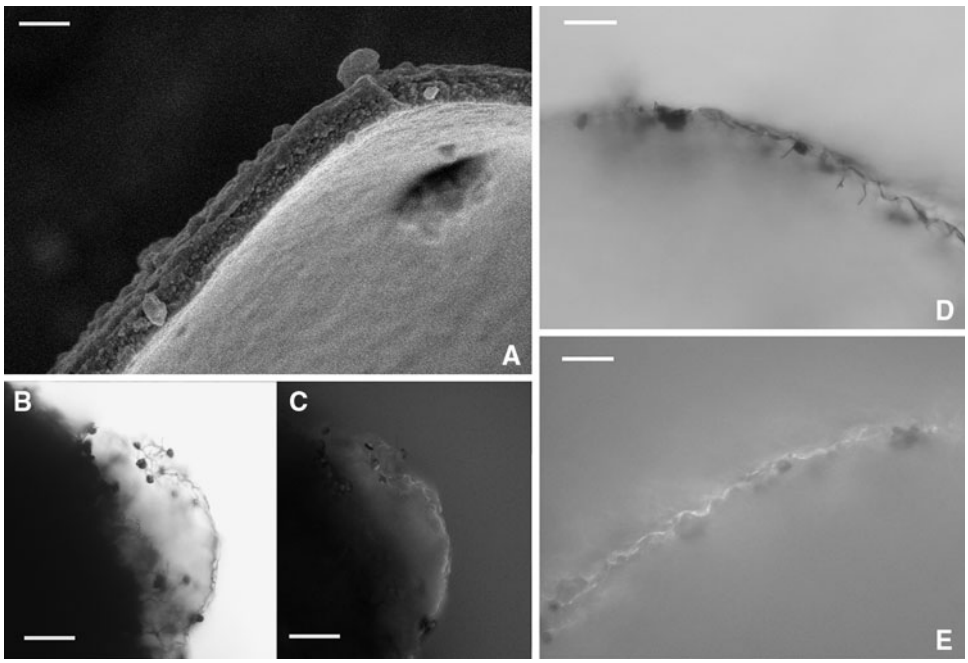

Fig. 2 *Diachea leucopodia*: **a–d** Transverse section of peridium. **a** Microphotography with SEM: peridium surface **b–d** light microscopy. **c**, **d** Peridium showing autofluorescence. *Bar* **a** 100 nm; **b** 0.4 μm; **c–d** 20 μm

Spores

The spores of *D. leucopodia* are roughly spherical, usually 8 μm in diameter, and have a dark brown color in mass. Spore ornamentation observed by SEM exhibits tips that form an irregular pattern on the spore surface (Fig. 1d–f). These tips result from vertical processes as seen in spore profile. Palynological terminology has been used in describing spore wall ornamentation and this species was assigned to the verrucate type by Rammeloo [11]. The sections of the spore wall observed with TEM show two layers, a thin external component with a high electron density, and an internal component, which is slightly thicker and has lower electron density. The spore wall is evenly thickened with no evidence of a germination pore. This spore ultrastructure is typical of the Physarales. The cytoplasm is very dense with several organelles (Fig. 1f).

Discussion

The results obtained provide new information about the ultrastructure and unreported features of *D. leucopodia* sporangia. The autofluorescence observed in the peridium is a previously unreported property of a Myxomycete fruiting structure. Iridescence appears as a feature of mature sporangia [5], meanwhile our data indicate that the fluorescence appears to be present in the earliest stages of sporangial development. It is possible to hypothesize that fluorescence is somehow involved in the protection of young spores from the adverse effects of ultraviolet light during development, providing in some measure a physiological advantage for these organisms. More direct observations in the field and laboratory experiments are needed to elucidate the functional role of iridescence and fluorescence in the Myxomycetes. Variation in the amount of moisture that occurs during development at the time of fruiting could explain the observed differences in iridescence, taking into account that water content in structures can promote changes in the color observed [13]. In contrast these changes would not affect the fluorescence observed.

In 1989 Steglich [14], working on crude extracts from sporophores of *Arcyria* species, observed fluorescence from these extracts. He determined that the substance responsible for this effect was quinolonocarbazole carboxylic acid. There was no mention of fluorescence in structural components. More studies are necessary to determine if the fluorescence of *D. leucopodia* corresponds to a similar component.

The presence of traces of Mn, P, S, and Si or Fe was previously reported and this could be a consequence of local accumulations [1, 6, 8, 9]. Similar results were observed using EDX dot maps and spectral analysis that determined the presence of varying amounts of intermixed calcium and silicon deposits in the peridium of *Didymium saturnus* [12].

SEM observations of *D. leucopodia* showed stalks filled with spherical granules of calcium carbonate similar to *D. subsessilis* [3]. In contrast *D. arboricola* had calcium carbonate crystals with euphedral rhombohedron shapes [7]. This raises questions about the genetic and environmental factors that influence formation of calcium carbonate since the granular and crystalline character has been used at various taxonomic levels.

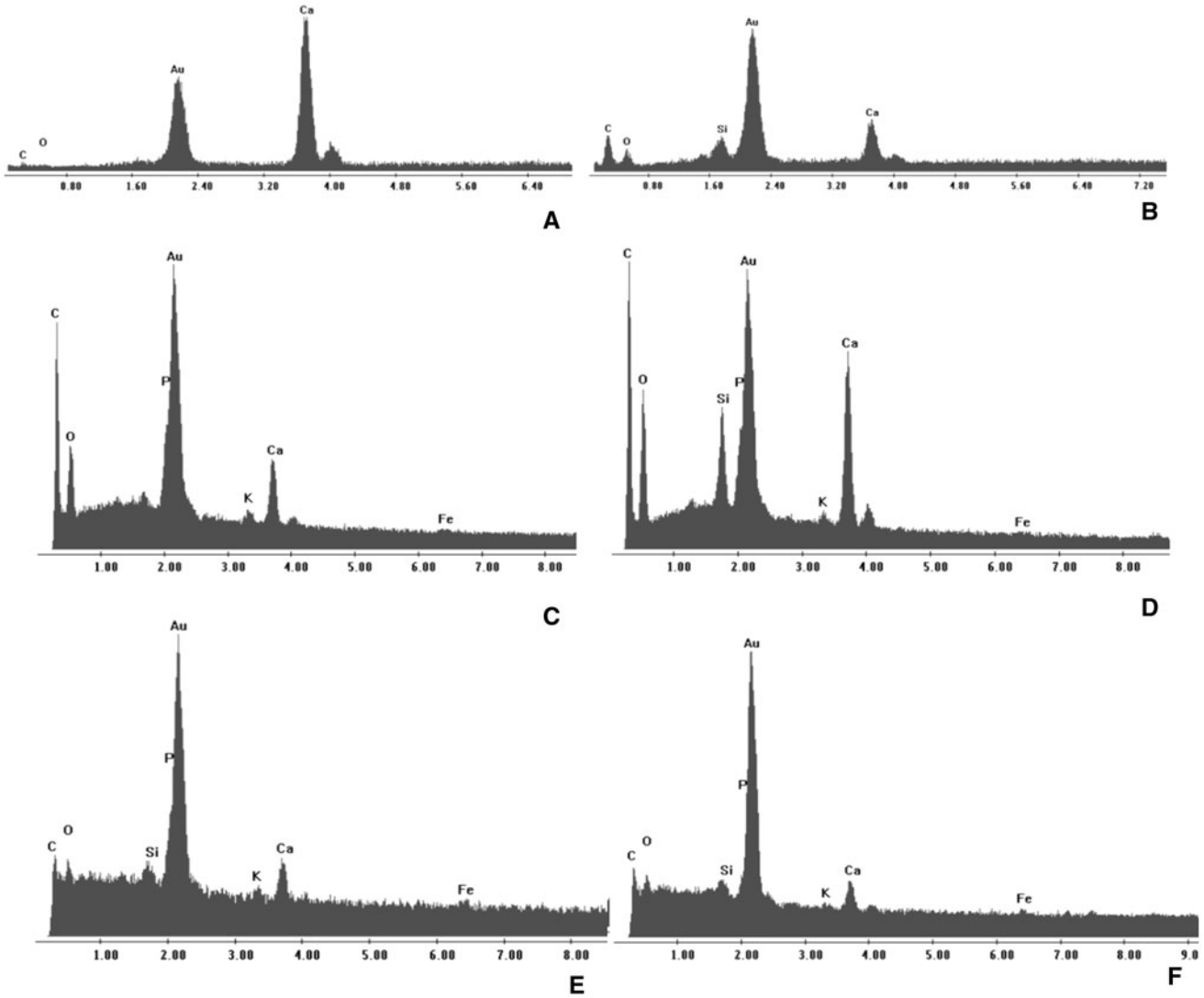

Fig. 3 Spectra recorded from **a** stalk, **b** capillitium; **c**–**f** peridium of *Diachea leucopodia* (**c**, **d** spectra corresponding to different peridial sections of BAFC 22603; **e**, **f** Spectra corresponding to different peridial sections of BAFC 22576). Different X-ray emission peaks labeled as Au correspond to the sputter coating with gold–palladium

In addition, the spore surface of *D. leucopodia* has a similar morphology to that described by Keller et al. [7] for *D. arboricola.*

Gaither and Keller [3] observed that the peridium is colorless when immersed in an aqueous medium. These authors suggested for the first time that the bright colors observed on the external peridial surface were not related to pigments but rather are a result of interference effects in a completely transparent material. Inchaussandague et al. [5] presented an optical model to explain the observed iridescence in *D. leucopodia*, and more recently Dolinko et al. [2] used a novel simulation tool to investigate the electromagnetic response of the peridia in more detail, taking into account the inhomogeneous nature of the peridium and its curvature. However, the significance of these new data was not discussed in a biological framework. The conjunction of iridescence and fluorescence observed raises new and unexplored ecological perspectives.

Additional ultrastructural studies of the peridia and stalks of *Lamproderma* and *Diachea* and other Myxomycete taxa may provide characters useful in taxonomy and answer more questions about evolutionary and ecological significance of fluorescence and iridescence. This study represents an important addition to the knowledge of peridial composition and structure in Myxomycetes particularly as it relates to fluorescent properties.

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