An ultrastructural study of the asci and banded ascospores of *Fasciatispora petrakii*

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Ascospores of *Fasciatispora petrakii* are brown and unicellular and are provided with pallid central bands with unknown function. Ascospores were examined at the electron microscopic level in order to establish their fine structure. The bands are narrow regions of the ascospore wall with less pigmentation. They may act as areas of weakness through which spores can germinate. Ascospores are surrounded by a mucilaginous sheath, which is also illustrated at ultrastructural level. The asci are similar to those found in species of *Xylaria* (Xylariaceae) and placement of *Fasciatispora* in the Xylariaceae is considered most appropriate.

Key words: electron microscopy, saprobic, taxonomy, Xylariaceae.

Introduction

In introducing the new genus *Rikatlia,* Cannon (1992) reviewed ascomycetes with banded spores, including their anamorphs. Many of these species belonged to the Phyllachoraceae, while others were members of the Melanconidaceae, Hyponectriaceae and Dothideales and one (*Fasciatispora* K.D. Hyde) a member of the Xylariales. Banded ascospores have therefore arisen in several unrelated groups of fungi, and are presumably related to function rather than taxonomic relationships. Cannon (1992) concluded that with the exception of *Fasciatispora* and most species of the *Beltrania* group, all taxa were parasitic, either on plants or other ascomycetes.

Cannon (1992) was unaware of any literature on the function of these pallid bands. He suggested that "the bands act as germ slits, forming weak points in the predominantly thick and strongly pigmented walls from which germ tubes can grow." This is supported by evidence that ascospores often break at the bands, when gentle pressure is applied to the coverslip (Fig. 3).

We have also been fascinated by the presence of pallid bands in spores. The genus *Fasciatispora* was introduced for *F. nypae* K.D. Hyde and now includes

seven species (Hyde, 1995), all with pallid bands, occurring on palms and *Pandanus* species. We therefore decided to investigate germination in one of these species, and to examine their spores at the ultrastructure level, in order to establish the structure of these pallid bands.

Materials and methods

A suspension of ascospores of *Fasciatispora petrakii* was prepared by placing the contents of several ascomata in a drop of sterile distilled water. The suspension was then dropped on a petri-dish containing Potato Dextrose Agar with streptomycin, dried in a laminar air-flow cabinet and left overnight at room temperature to germinate. The position of the germ tubes was observed under a light microscope on the next day.

Scanning electron microscopy

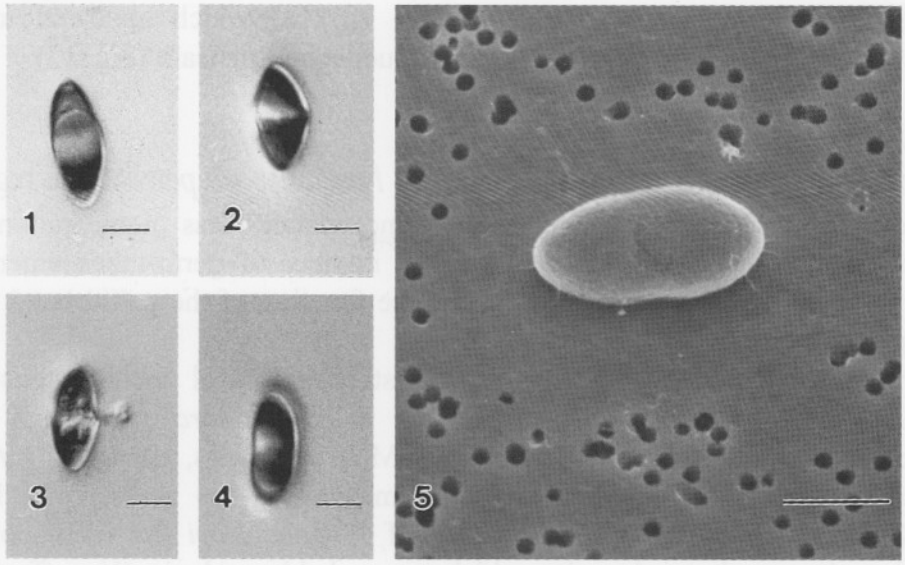
An ascospore suspension was settled on to a polycarbonate membrane (Nucleopore) with pore size 5 µm. The membranes were fixed in 2 % (w/v) aqueous osmium tetroxide at 4 C overnight, dehydrated through a graded ethanol series, critical point dried and sputter coated with gold/palladium. Finally, the material was examined in a Leica Cambridge Stereoscan 440 scanning electron microscope operated at 20 kV.

Transmission electron microscopy

Asci and ascospores were embedded in 2 % (w/v) Ion agar and then fixed in 4 % (v/v) glutaraldehyde with added ruthenium red in 0.1 M sodium cacodylate buffer at pH 7.2 for 4 hours at room temperature and postfixed in 2 % (w/v) osmium tetroxide with added ruthenium red in 0.1 M sodium cacodylate buffer at pH 7.2 overnight at 4 C. The material was dehydrated through a graded ethanol series and finally transferred to absolute acetone. Dehydrated material was then embedded in Möllenhauer's resin (Möllenhauer, 1964). Ultrathin sections were stained with lead citrate (Reynolds, 1963) for 15 minutes and uranyl acetate solution for 30 minutes. Finally, the specimens were examined using a JEOL 100SX transmission electron microscope operated at 80 kV.

Results

Ascospores of *Fasciatispora petrakii* are brown with central pallid bands and are surrounded by a mucilaginous sheath (Figs. 1-4). The position of germ-tube emergence was variable. In some cases, the germ-tubes did arise from the centre of the ascospores, but in an equal number of cases they arose from the apex or sub-apical region.



Figs. 1-4. Ascospores of *Fasciatispora petrakii.* Note the central pallid bands and surrounding mucilaginous sheath. When gentle pressure is applied to the ascospore it will break in the centre (seen in 3). **Fig. 5.** Scanning electron micrograph. Mature ascospore. Ascospore surrounded by a wide sheath. Note the pores in the polycarbonate membrane are covered by sheath material. Bars = 5 μm.

At the scanning electron microscope level, the ascospores were ellipsoidal, but slightly constricted at the centre, and surrounded by a wide sheath (Fig. 5). At the Transmission Electron Microscope level mature asci were provided with a sub-apical ring which comprised electron-dense material (Fig. 6), with a less electron-dense region between the sub-apical ring and the wall at the ascus apex. The ascus wall comprised a single electron-dense layer, *ca* 50 nm thick, continuous over the ascus apex (Fig. 6). Immature ascospores were ellipsoidal and were not constricted at the centre (Fig. 7). A wide mucilaginous sheath was already well-developed in immature ascospores (Figs. 7, 8), and was composed of condensed fibrillar material (Figs. 8, 9). The immature ascospore wall comprised an thick outer electron-dense episporium and an inner thin electron-transparent mesosporium (0.15-0.2 μm). The thickness of the episporium increased towards the ascospore ends (Fig. 8). Single-membrane layers were present inside the mesosporium (Fig. 9). In mature ascospores, the mid-region was slightly constricted (Fig. 10). The episporium was thick at the polar regions (*ca* 0.5 μm) and became thinner (0.1 μm) in the mid-region (Figs. 10, 11). The thickness of the electron-transparent mesosporium also decreased to 25-50 nm in the mid-region (Fig. 12). The outer surface of the episporium possessed

deposits of electron-dense material (Figs. 12, 13) which appeared to be associated with the fibrillar material of the mucilaginous sheath (Fig. 13).

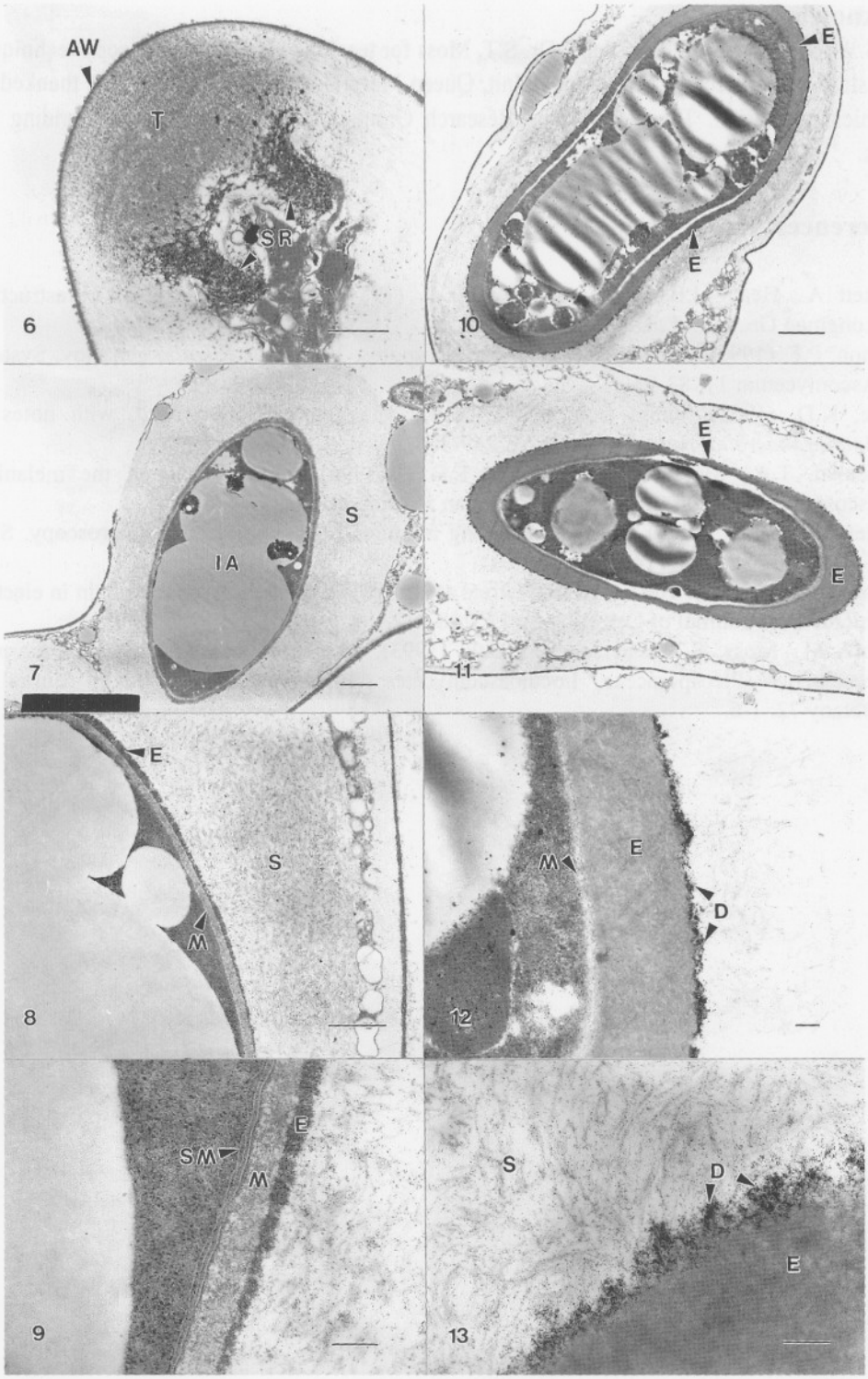
Discussion

The pallid central bands in ascospores of *Fasciatispora petrakii* are regions where the ascospore wall is both thinner and contains less pigmentation. As germination experiments showed an equal number of germ-tubes emerging from the polar regions of the ascospores, the function of the pallid band does not appear to be solely germ-tube related.

Brown ascospores studied at the ultrastructural level include those of *Corollospora fusca* Nakagiri and Tokura and *Pleospora gaudefroyi* Pat. (Yusoff, Moss and Jones, 1993; McKeown, Moss and Jones, 1996). In *C. fusca* ascospores possess longitudinal striae of melanin in the mesosporial layer (McKeown *et al.*, 1996). In ascospores of *P. gaudefroyi* the mesosporium contains electron-dense deposits, which are probably melanin (Yusoff *et al.*, 1993). In ascospores of *F. petrakii* the episporium (which is also presumably melanized), and the mesosporium are thinner at the mid-region.

The substructure of the ascus of *Fasciatispora petrakii* is most similar to that found in species in the Xylariaceae (Beckett, Heath and McLaughlin, 1974). In *Xylaria* the apical ring inverts following ascospores discharge (Beckett *et al.*, 1974), however, this has not been observed in *F. petrakii*. Nevertheless, these observations constitute further evidence for the placement of *Fasciatispora* in the Xylariaceae.

Figs. 6-13. Transmission electron micrographs. Longitudinal sections of mature asci and ascospores. **6.** Ascus apex with a sub-apical ring (SR) comprising dark electron-dense material. Less electron-dense deposits (T) occur between the ascus wall (AW) and the subapical ring (SR). Note the ascus wall is single-layered and is continuous over the ascus apex. **7.** Ellipsoidal immature ascospore (IA) surrounded by a condensed wide mucilaginous sheath (S). **8.** Ascospore wall which comprises an inner electron-transparent mesosporium (M) and an outer electron-dense episporium (E). The thickness of the episporium (E) increases towards the ascospore tip. Note the sheath (S) is composed of condensed fibrillar material. **9.** Several layers of single-walled membrane (SM) (endoplasmic reticulum?) located inside the mesosporium (M). Note the episporium (E) which varies in thickness and the fibrillar material within the sheath. **10, 11.** Mature ascospores, which are ellipsoidal, but constricted at the centre. The episporium (E) is thicker at the ascospore tip and thinner at the mid-region. **12.** The mesosporium (M) is highly-reduced. Some electron-dense material (D) is deposited at the surface of the episporium (E). **13.** The fibrillar material of the sheath (S) appears to be associated with the electron-dense material (D) deposited on the episporium (E). Bars: 10, 11 = 1 μm, 6, 7, = 0.5 μm, 8, 9, 12, 13 = 0.1 μm.


AW
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SR
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E
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10
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