Ultrastructural observations on *Oxydothis alexandrarum*

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Wong, S.W. and Hyde, K.D. (1999). Ultrastructural observations on *Oxydothis alexandrarum*. Fungal Diversity 2: 181-188.

Ultrastructural observations on the asci and ascospores of *Oxydothis alexandrarum* are presented. Asci are cylindrical, unitunicate, with a J+, subapical ring, and a faint canal leading to the apex. At the TEM level the subapical ring is an area of densely staining granular material. The faint canal comprises a region of less electron-dense granular material. Ascospores are filiform with bipolar mucilaginous appendages. The fine structure of the asci in *Oxydothis* is most similar to that found in the Diatrypaceae.

Key words: Amphisphaeriaceae, electron microscopy, Hyponectriaceae, taxonomy.

Introduction

The genus *Oxydothis* Penz. and Sacc. was revised by Hyde (1996) and 41 species were accepted. Species in the genus are found predominantly on palm leaves and rachides. Their ascomata usually develop beneath a darkened stroma, or beneath a raised blistering area, and may have their axis parallel, oblique or perpendicular to the host surface. Asci are cylindrical, unitunicate, usually with a J+, subapical ring, and a faint canal has been reported leading to the apex (Hyde, 1996). The ascospores are long fusiform to filiform, bicelled, and taper from the centre to spine-like, pointed or rounded processes (Hyde, 1996). The presence of apical mucilage has been reported (Hyde, 1996), but not illustrated. *Oxydothis* species are probably endophytes and at least one species has been isolated directly as an endophyte and has sporulated in culture (Taylor, 1998).

Oxydothis has been placed in different families at various times. Müller and Arx (1973) and Wehmeyer (1976) included *Oxydothis* in the Amphisphaeriaceae, Barr (1990) included it in the Hyponectriaceae, while Eriksson and Hawksworth (1991) preferred to keep *Oxydothis* in the Amphisphaeriaceae. Hyde (1993) was undecided to which of these families

Oxydothis should be placed, while Hawksworth *et al.* (1995) placed *Oxydothis* in the Hyponectriaceae.

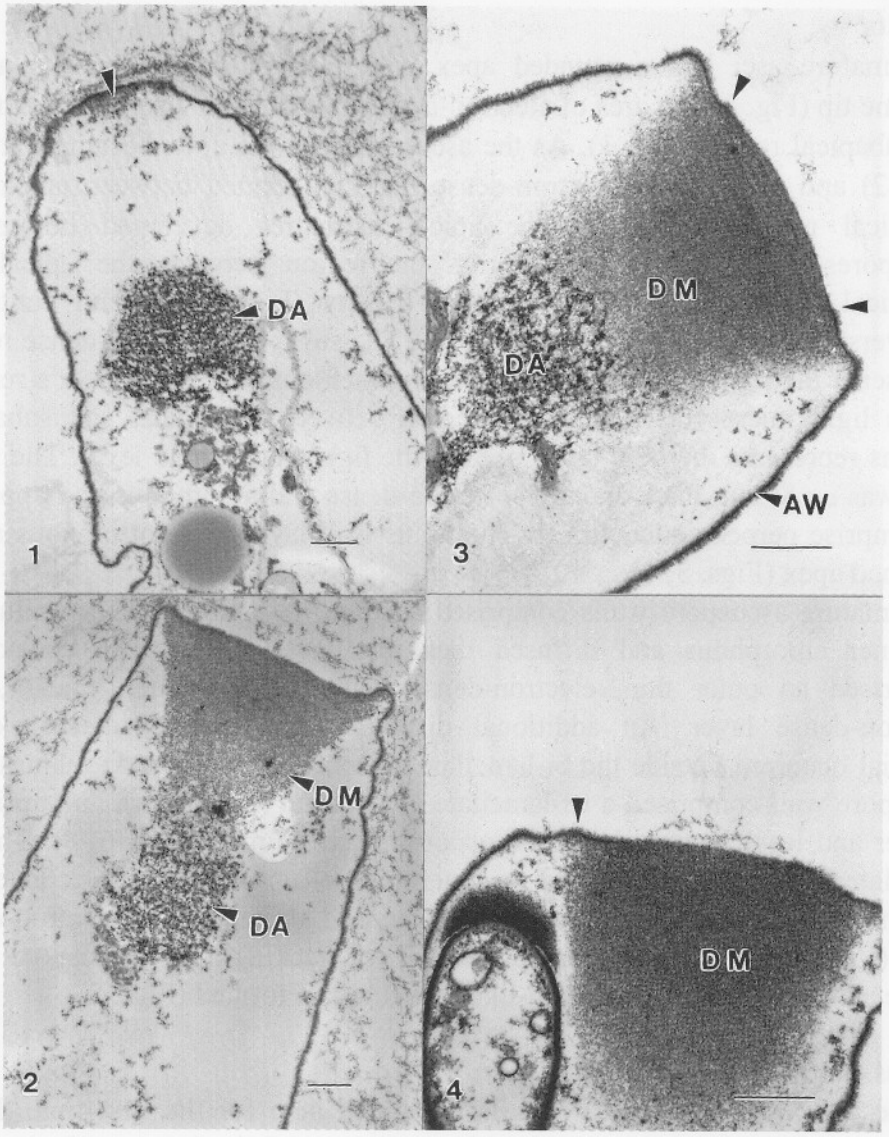
The anamorph of *Oxydothis selenosporellae* Samuels and Rossman is reported to be a *Selenosporella* sp. (Samuels and Rossman, 1987), which indicates a lack of affinity towards the genus *Amphisphaeria,* which is reported to have a *Pestalotiopsis*-like anamorph (Nag Raj, 1977; Kang, Hyde and Kong, 1999). The anamorphs in the Hyponectriaceae are reported to belong to the hyphomycetous form-genera, although many of the genera included in the Hyponectriaceae by Barr (1990) have now been arranged elsewhere. We have isolated numerous *Oxydothis* species in culture, but although an occasional species will produce its teleomorph in culture, we have observed no anamorph. Our knowledge of anamorphic states, is therefore unclear in reference to establishing the affinities of *Oxydothis*.

Molecular studies based on the analysis of the 5.8S rRNA gene and internal transcribed spacer sequences (Kang, Kong and Hyde, 1998) have also so far failed to establish the relationships of *Oxydothis.* Although *Oxydothis* previously appeared to have phylogenetic relationships with the Amphisphaeriaceae (*sensu lato*), it did not cluster with amphisphaeriaceous genera with *Pestalotiopsis*-like anamorphs (e.g. *Amphisphaeria, Discostroma* Clem.), which indicates it is not closely related to the Amphisphaeriaceae (*sensu* stricto). Of the fungi sequenced *Oxydothis* also proved to be as dissimilar to *Clypeosphaeria* as it was to *Amphisphaeria.* Kang *et al.* (1998) placed *Oxydothis* in the Clypeosphaeriaceae, but admitted this family was heterogenous. The Xylariaceae were found to be unrelated to the Amphisphaeriaceae and *Oxydothis*.

Both morphological and molecular studies on the genus *Oxydothis* have so far failed to establish its close relatives. Hyde (1993) illustrated *Lasiobertia* Sivan. and found that it was closely related to *Oxydothis* and should be included in the same family. This has generally been overlooked in the literature (Hawksworth *et al.*, 1995). The purpose of this paper is to examine the asci and ascospores of *Oxydothis* at the ultrastructural level in order to gain a better understanding of the biology and phylogenetic relationships of this genus. The nature of the subapical ring and faint canal leading to the apex and the presence of mucilage at the ascospore ends will be investigated.

Materials and methods

The methods used for preparation of material for Electron Microscopy follow those of Wong, Hyde and Jones (1998). The terminology for the ascospore wall layers is that of Jones (1995).



Figs. 1-4. *Oxydothis alexandrarum*. Transmission electron micrographs. **1.** Immature ascus with an aggregation of electron-dense material at the rounded apex (arrowed). A region of electron-dense material (DA) has also accumulated in the subapical region. **2.** Prior to the formation of the ascospores, the ascus apex becomes flattened and electron-dense material (DM) has been deposited between the subapical region (DA) and the ascus apex. **3, 4.** Apex of mature asci. The mature ascus wall (AW) is thin, and comprises irregular horizontal fibrils and an inner electron dense region. The ascus wall is discontinuous at the ascus apex (arrowed). The electron dense canal between the granular subapical region (DA) and the ascus apex, contains transverse aggregations of granular material near the apex (arrowed in 3). Note the ascospore tip in 4. Bars = 0.5 μm.

Results

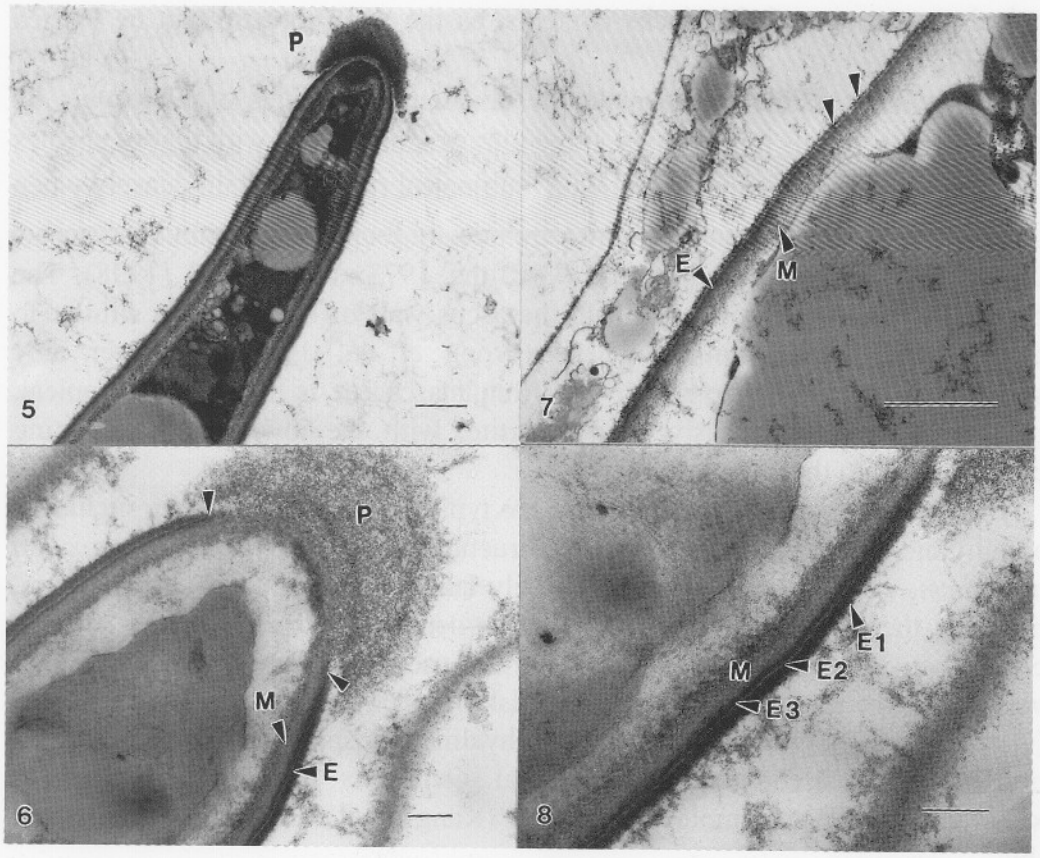
Immature asci had a rounded apex with electron-dense deposits at the extreme tip (Fig. 1). An area of electron-dense material had also accumulated in the subapical region (Fig. 1). As the ascus matured the apex became flattened (Fig. 2) and a region of electron-dense material formed between apical and subapical regions (Fig. 2). The apical structures developed before the ascospores had formed. At maturity, the region between the apical and subapical regions increased in electron-density (Figs. 3, 4) and contained transverse fibrils near to the apex (Fig. 3). The subapical electron-dense region represents the amyloid subapical ring seen when observed in Melzer's reagent at the light microscope level. The region between the apical and subapical regions represents the faint canal seen at the light microscope level. The ascus wall was *ca* 80 nm thick and was electron-dense and amorphous and appeared to comprise perpendicular fibrils. The ascus wall layer was not obvious at the flattened apex (Figs. 3, 4).

Immature ascospore walls comprised an outer bi-lamellate episporium and an inner amorphous and diffused mesosporium (Fig. 7). The episporium comprised an outer thin, electron-dense layer and an inner, thicker, less electron-dense layer. An additional discontinuous layer of electron-dense material occurred outside the bi-lamellate episporium (Fig. 7). At maturity the ascospore wall comprised a tri-lamellate episporium (*ca* 35 nm), and an inner, thicker and less electron-dense mesosporium (*ca* 30 nm) (Figs. 6, 8). The tri-lamellate episporium comprised an outermost, electron-dense, thick layer; an intermediate, thin, electron-transparent layer; and an inner, thin, electron-dense layer (Fig. 8). The tri-lamellate episporium was lacking at the ascospore apex, where an amorphous, diffuse, pad-like structure had formed (Figs. 5, 6).

Discussion

The pad at the ascospore ends is illustrated here for the first time. Many filiform ascospores are provided with mucilage at their tips (e.g. *Linocarpon* spp., *Lulworthia* spp.) and the mucilage is thought to be associated with ascospore attachment (Hyde and Jones, 1989). It is not clear from the micrographs exactly how the mucilaginous appendages are formed. In Fig. 5 and 6 the mucilage appears to have passed through the mesosporium. However, as the episporium is lacking at the tips (Fig. 5) it may be that the mucilage has developed from the episporium. The episorium of ascospores of *Oxydothis* is unusual in that it is made up of three layers.

The asci of *Oxydothis* species as seen with the light microscope comprise a subapical ring which is usually amyloid, with a faint canal leading to the apex



Figs. 5-8. *Oxydothis alexandrarum*. Transmission electron micrographs. **5.** Apex of mature ascospore with rounded pad-like appendage (P) at the tip. Bar = 0.5 μm. **6.** Mature ascospore wall comprising an outer trilamellate episporium (E) and an inner less electron-dense mesosporium (M). The wall is poorly developed, lacking or poorly fixed at the ascospore tip (arrowed) where this is a polar pad (arrowed). Note the polar pad is amorphous and diffuse. **7.** Immature ascospore wall comprising an outer bilamellate episporium (E) and an inner diffuse mesosporium (M). An additional discontinuous layer of electron-dense material (arrowed) occurs at the ascospore tip. **8.** Mature ascospore wall comprising an outer trilamellate episporium and an inner mesosporium (M). The trilamellate episporium comprises an outer, thick, electron-dense layer (E1); an intermediate, thin, electron-transparent layer (E2); and an inner, thin, electron-dense layer (E3). Bars = 0.1 μm.

(Hyde, 1996). This canal is thought to be hollow as ascospores pass through this region when ejected. At the TEM level it was observed that the subapical ring is an area of densely staining granular material. The faint canal comprises a region of less electron-dense granular material. A hollow region is lacking in both the subapical ring and faint canal. This may be due to the orientation of the section (i.e. not median). The electron transparent region surrounding these

apical structures probably lacks contents, as the ascospore tip can be lodged here (Fig. 4).

It may be possible to elucidate the taxonomic affinities of *Oxydothis* by comparing the ascus morphology at the ultrastructural level with that of species in other families. Unitunicate asci with (sub)apical rings that stain densely when fixed in glutaraldehyde and osmium tetroxide are found in the Annulatascaceae, Lasiosphaeriaceae and Xylariaceae (Griffiths, 1973; Wong *et al.*, 1998). The perpendicular fibrillar orientation in the ascus wall of *Oxydothis* is similar to that found in the Annulatascaceae (Wong *et al.*, 1998). However, any similarities end here. The ring in the Annulatascaceae is non amyloid, apical, refractive, relatively massive and bipartite, with the lower part elongating downwards into the cytoplasm.

Oxydothis has some characters that are typical of the Xylariaceae, including an amyloid subapical ring. At the ultrastructural level (illustrated in *Hypoxylon multiforme* (Fr.) Fr.) both have a densely staining granular subapical region, however there is a region of less electron-dense granular material (the faint canal) between the subapical ring and the ascus apex in *Oxydothis*. In *H. multiforme* the upper part comprises a disk of electron-dense granular material (Griffiths, 1973). Other differences (e.g. hyaline ascospores lacking a germ slit, non stromatic ascomata), indicate that *Oxydothis* may be unrelated to xylariaceous taxa and molecular studies (Kang *et al.*, 1998) have confirmed this.

Although the asci in *Lasiosphaeria spermoides* (Hoffm.) Ces. and De Not. (Lasiosphaeriaceae) have rings with densely staining parts (at the TEM level), they differ distinctly from *Oxydothis* as the ascus rings are refractive, J- and apical (Griffiths, 1973).

Representatives from several other unitunicate ascomycetes families have been examined at the ultrastructural level (Griffiths, 1973; Samuels, McKenzie and Buchanan, 1981; Read *et al.*, 1993a; Read, Jones and Moss, 1993b; Hsieh *et al.*, 1995). Griffiths (1973) illustrated the fine structure of the asci in seven unitunicate ascomycetes belonging to different genera. The fine structure of the ascus of *Oxydothis* is unlike that found in *Ceratostomella, Cordyceps, Diaporthe* or *Nectria*. It is superficially similar to that found in *Eutypella quaternata* (Pers.) Schroet., in having a subapical region (which is faintly amyloid) of densely staining granular material, and an upper less electron-dense granular region. However, the ascus wall in *E. quaternata* is made up of longitudinally orientated fibrils, whereas is *Oxydothis* the fibrils are orientated perpendicular to the ascus wall. The relationships of *Oxydothis* with the Diatypaceae certainly warrant further investigation.

The fine structure of the asci of *Neurospora lineolata* Frederick and Uecker (Sordariaceae) was illustrated by Hohl and Streit (1975). The apical ring comprises a short cylinder of wall-like material, which is continuous with the ascus wall and lacks the electron-dense deposits found in *Oxydothis.* In *Apiospora* (Apiosporaceae) the ascus apical ring is highly reduced and lacks any densely staining regions (Samuels *et al.*, 1981). This is also true of the Halosphaeriaceae (e.g. *Halosarpheia*; Hseih *et al.*, 1995) and Chaetosphaeriaceae (e.g. *Chaetosphaeria chaetosa* Kohlm., Read *et al.*, 1993a), or in the genus *Savoryella* (e.g. *S. appendiculata*; Read *et al.*, 1993b).

Species of *Oxydothis* are extremely common on palms (Hyde, Fröhlich and Taylor, 1997) and are invariably one of the earliest colonisers of dead palm leaves and fronds. Species rarely occur on older decaying material, but have been isolated as an endophyte on at least one occasion (Taylor, 1998). It is probable that *Oxydothis* species are initially endophytes in leaves and rachides of palms and once the palm structure dies, convert to a saprobic lifestyle. It is not clear whether species are host specific or generalists. However, considering the numbers of species (presently 41), and the numbers waiting description (more than 50 in our laboratory), we suspect that *Oxydothis* species are at least specific at the host genus level. This would make sense if the endophytes had evolved with their individual hosts. If it is the case, then we suspect that several hundred *Oxydothis* species await discovery.

Acknowledgements

S.W. Wong thanks Dr. S.T. Moss for his teaching of electron microscope techniques. We should like to acknowledge Mr. Y.C. Mok, Mr. W.S. Lee and Ms. S.L. Amy Wong in the Electron Microscopy Unit, Queen Mary Hospital, Hong Kong for their technical help. Thanks are also extended to Mr. Lee and Mr. Cheung for photographic assistance. The Hong Kong Research Grants Council are thanked for funding this work.

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