DIDYMELLA APTROOTII SP. NOV. FROM BAMBOO SUBMERGED IN FRESHWATER

K.D. Hyde & S.W. Wong, Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong. Email kdhyde@hkucc.hku.hk

A new species of *Didymella* is described based on specimens occurring on bamboo in Hong Kong, Malaysia and the Philippines. *Didymella aptrootii* was collected on bamboo submerged in a lake and rivers. *Didymella aptrootii* is illustrated with light, scanning and transmission electron micrographs. It is compared with other similar *Didymella* species and similar taxa on bamboo.

Key words: aquatic fungi, bamboo fungi, new species.

Introduction

During studies on the fungi on submerged bamboo we made several collections of a loculoascomycete, that we could not accommodate in any existing species. This taxon was at first believed to be a species of *Massarina* (*sensu* Aptroot 1998), but on further reflection we place it in *Didymella*.

Material and methods

Scanning electron microscopy

An ascospore suspension was pipetted dropwise and slowly on to the polycarbonate membrane. Subsequently, the membranes were fixed in 2% (w/v) aqueous osmium tetroxide (OsO_4) at 4°C overnight. Fixed material was then dehydrated through a graded ethanol series from 10% to 90% (in 10% steps), then 95%, followed by three changes of absolute ethanol. Each of the above changes was for 15 minutes. After critical point drying and gold/palladium coating, the specimen was examined using a Leica Cambridge Stereoscan 440 SEM operated at 20kV.

Transmission electron microscopy

The procedures used followed Ho *et al.* (1999).

Taxonomy

Didymella aptrootii K.D. Hyde & S.W. Wong, sp. nov. Figs 1–25

Etymology: In honour of the mycologist André Aptroot.

Ascomata 200–270 μm diam., immersa globosa vel subglobosa, vel semi-immersa, erumpentes, nigra, solitaria vel gregaria, ostiolata. Asci 77–130 × 18–25 μm, 8-spori, clavati, pedicellati, fissitunicati. Ascosporae 20–30 × 8–11 μm, 3-seriatae, 1-septatae, asymmetricae, hyalinae, tunica gelatinosa praeditae. HOLOTYPUS: HONG KONG, New Territories, Plover Cove Reservoir, on submerged bamboo, 15 Nov. 1996, *K.D. Hyde* (HKU(M) 3333).

Ascomata 200–270 μm in diam, globose to subglobose, immersed or semi-immersed, becoming erumpent, black, solitary and scattered, or clustered, ostiolate (Figs 1, 2, 9). Peridium thin, comprising 3 or 4 layers of angular, brown walled cells (Fig. 2). Pseudoparaphyses sparse, filamentous, septate (Fig. 10). Asci 77–130 × 18–25 μm (x = 103 × 20 μm, n = 25), 8-spored, clavate, pedicellate, fissitunicate, with an ocular chamber (Figs 4–6, 10–13). Ascospores 20–30 × 8–11 μm (x = 21 × 9.5 μm, n = 50), 3-seriate, 1-septate, asymmetrical, septum nearer to the apex and constricted at the septum, hyaline, surrounded by large irregular mucilaginous sheath (Figs 7, 8, 14–19).

Figures 1–8. *Didymella aptrootii* (from holotype). Fig. 1. Appearance of fungus on bamboo host. Fig. 2. Section of an ascoma. Fig. 3. Peridum, comprising a few layers of *textura angularis*. Figs 4–6. Asci with the fissitunicate dehiscence. Figs. 7 & 8. Ascospores with mucilaginous sheath. (Fig. 8 in India Ink). **Bars:** 1 = 500 μm, 2–8 = 20 μm.

Other material examined: HONG KONG, New Territories, Plover Cove Reservoir, on submerged bamboo, 15 Nov. 1996, *K.D. Hyde* (HKU(M) 4725); *ibid.* (HKU(M) 4714). MALAYSIA, Lentang River, on submerged bamboo, 15 Oct. 1991, *K.D. Hyde* (HKU(M) 1595). PHILIPPINES, Mindanao, Bukidnon, Impalatao, Natigbasan Creek, Jan. 1994, on submerged bamboo, *K.D. Hyde* (HKU(M) 4715); Negros Occidental, Bario Alegria, Liput River, Lot 1320 (K.D. Hyde plot), on submerged bamboo, 27 Apr. 1997; *K.D. Hyde & V.A. Arimas* (HKU(M) Liput 2).

Known distribution: Hong Kong, Malaysia, The Philippines.

Known host: Bamboo.

Scanning electron microscopy

Mature ascospores of *D. aptrootii* were fusiform, and asymmetrical with an eccentric septum (Figs 20, 21). Each ascospore was surrounded by an irregular, rough and sticky mucilaginous sheath, which adhered the ascospore to the polycarbonate membrane.

Figures 9–19. *Didymella aptrootii* (from HKU(M) 4714). Fig. 19. Appearance of solitary ascomata on bamboo host. Figs 10–13. Various stages of fissitunicate dehiscence in asci. Note the ascospores being released in 13. Figs 14–18. Ascospores with mucilaginous shealth. Fig. 19. Ascus with long pedicel. **Bars:** 9 = 500 μm, 10–12 = 20 μm, 13–19 = 10 μm.

Figures 20–25. *Didymella aptrootii* (from holotype). Figs 20–21. Scanning electron micrographs of the mature ascospore. Mature ascospores are fusiform, centrally constricted, asymmetrical and surrounded by an irregular, rough and sticky fibrillar sheath. Figs 22–25. Transmission electron micrographs of mature ascospores of *Didymella aptrootii*. Fig. 22. Longitudinal section of the whole ascospore illustrating each cell comprising a large lipid globule and several smaller guttules (LG). The ascospore is surrounded a wide fibrillar sheath (FS) with membrane residues (Mb) present at the margin. Figs 23–25. Ascospore wall comprising a thick mesosporium (M) and a thinner electron-dense episporium (E). The mesosporium is continuous with the septum (Sp) of the spore. The fibrillar material of the sheath (FS) is derived from the episporium. **Bars:** 20–22 μm, 23–25 = 100 nm.

Transmission electron microscopy

Each cell of mature bi-celled ascospores was filled with a central large lipid globule (LG) surrounded by several smaller lipid guttules (Fig. 22). Mature ascospores were surrounded by a wide fibrillar sheath (FS; Figs 22–25), with a residual perispore (p) at the margin (Fig. 22). The ascospore wall comprised a thick mesosporium (M, about 60 nm thick) and a thinner electron-dense episporium (E; 30–40 nm; Figs 23–25). The mesosporium formed the septum (Sp; Fig. 24) and the condensed fibrillar material of the sheath appeared to have been derived from the electron-dense projections of the episporium (Figs 23, 25). The electron-dense projections were formed all around the ascospore wall, except at the septum (Fig. 24).

Discussion

Six species of *Didymella* have been described from bamboo, including *D. eutypoides* Rehm, *D. maculosa* Penz. & Sacc., *D. pseudosasae* I. Hino & Katum., *D. seriata* Rehm, *D. tenuispora* I. Hino & Katum., and *D. yezoënsis* I. Hino & Katum. (Eriksson & Yue 1998). None of these species are similar to *D. aptrootii* in their shape, width or length of ascospores (Hino & Katumoto 1958, 1960; Penzig & Saccardo 1897; Rehm 1916).

Didymella aptrootii should also be compared with species of *Guignardia*, *Botryosphaeria* and *Mycosphaerella* described from bamboo. *Botryosphaeria* is represented by two species from bamboo. *Botryosphaeria arundinariae* Earle has narrower ascospores, and *Botryosphaeria oblongula* Sacc. is a synonym of *Anthostomella grandispora* Penz. & Sacc. (Earle 1898; Eriksson & Yue 1998; Saccardo 1917). There are three species of *Guignardia* known from bamboo. In *Guignardia bambusae* I. Miyake & Hara, the ascospores have a small dwarf cell (Hino & Katumoto 1965), in *G. bambusina* Rehm they are differently shaped (*i.e.* reniform) and 1-celled, and in *G. dinochloae* Rehm they are also 1-celled (Rehm 1916).

There are five species of *Mycosphaerella* described from bamboo. *Mycosphaerella bambusae* Hara and its varieties have smaller ascospores (Fröhlich & Hyde 1998), *M. bambusifolia* I. Miyake & Hara also has smaller ascospores (Saccardo 1926), *M. inequalis* I. Hino & Katum. has apiospores, while *M. phyllostachydicola* Tomilin and *M. shibataeae* I. Miyake & Hara is a synonym of *Guignardia bambusae* (Eriksson & Yue 1998; Hino & Katumoto 1961). We can find no other species resembling *D. aptrootii* and therefore this taxon is described as new.

The long pedicel in the ascus of *D. aptrootii* (Fig. 19) is similar to that found in *Kirschsteiniothelia elaterascus* Shearer (Shearer 1993). Both of these fungi occur in freshwater and it would be interesting to establish if this character has developed as an adaptation for life in water. A less likely scenario is that the species may be related.

The ontogeny of ascospore appendages, including sheaths, are considered to be important in the delineation of genera (Hyde *et al.* 1998, 1999a; Jones 1995). In *D. aptrootii* the fibrillar sheath is derived from the episporium. There are no ultrastructural data of the ascospore sheath ontogeny in other *Didymella* species.

Mucilaginous sheaths are commonly present surrounding the ascospores of many loculoascomycetes (Jones 1994). These have been illustrated in *Massarina* species (Read *et al.* 1997; Shearer & Hyde 1997), *Vaginatispora aquatica* K.D. Hyde (Hyde 1995) and *Mamillisphaeria dimorphospora* K.D. Hyde, S.W. Wong & E.B.G. Jones (Hyde *et al.* 1999b). The ontogeny of the sheaths in many genera of loculoascomycetes is still unclear. In *Pleospora gaudefroyi* Pat. the sheath is exosporial in origin (Yusoff *et al.* 1993), whereas in *D. aptrootii* the sheath is derived from the episporium.

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THE POISONOUS GREEN-GILLED FUNGUS *CHLOROPHYLLUM MOLYBDITES* IN SOUTH WESTERN AUSTRALIA

Neale L. Bougher
CSIRO Forestry and Forest Products, Private Bag, P.O. Wembley, Western Australia 6014.

Chlorophyllum molybdites (G. Mey.: Fr.) Massee is a large and distinctive fungus aptly referred to as the 'green-gilled parasol'. It is a poisonous but not deadly species with a thermolabile poison, and is reportedly harmless for most people if eaten well-cooked (Southcott 1996). *Chlorophyllum molybdites* produces robust, large fruit bodies easily mistaken for attractive and edible species of *Macrolepiota* such as *M. rachodes* (Vittad.) Singer. Traditionally the monotypic genus *Chlorophyllum* has been separated from *Macrolepiota* by its greenish mature lamellae and spore deposit, and a few microscopic characters such as rarity of clamp connections and poorly metachromatic spores (Reid & Eicker 1991). However, *Chlorophyllum molybdites* has been recently re-named as *Macrolepiota molybdites* (G. Mey.: Fr.) G. Moreno, Bañares & Heykoop. These authors suggest that similarity in macroscopic and microscopic characters of *Chlorophyllum* and *Macrolepiota* support their synonymy (Moreno *et al.* 1995). They propose a new Section—*Chlorophyllum* within the genus *Macrolepiota*. For the purposes of this article I prefer to retain the more widely recognised and distinctive name—*Chlorophyllum molybdites*.

Chlorophyllum molybdites is reportedly most common in tropical/subtropical regions of the world, but it has also been recorded in New Zealand, South Africa, Japan, Canada, England and other temperate regions (Reid & Eicker 1991). In Australia, it is widely known from the Northern Territory (*e.g.* Darwin 12° 27' S), Queensland (over large range of latitudes), South Australia (*e.g.* Woomera 31° 10' S), and New South Wales (*e.g.* Sydney 33° 52' S, Broken Hill 31° 58' S) (Grgurinovic 1997; Reid & Eicker 1991; Young 1989). Numerous cases of poisonings attributed to *C. molybdites* have been reported, particularly in northern Australia (Southcott 1996). In Western Australia the fungus is commonly found in the north, such as in the Kimberley Region (*e.g.* Derby 17° 18' S), and only once recorded south of Carnarvon (24° 53' S)—in Perth in April 1981 collected by L. van der Pennen (locality not known, no site or descriptive notes provided). A more recent, large occurrence of *C. molybdites* in the metropolitan area of Perth (31° 57' S) during February 1998 (and same location in April 1999) was therefore considered a noteworthy record for the south-west region of Australia. Furthermore it provided an opportunity to test on recently collected south-western Australian material the validity of using Congo Red on spores (Weresub 1971) as a method to distinguish *C. molybdites* from *Macrolepiota*.

Description of *Chlorophyllum molybdites* from Perth 1998 and 1999

Large numbers of fruit bodies occurred during 1998 and 1999—up to 50 fruit bodies each year over an area of about 5 m^2—providing ample material of all developmental stages for descriptive purposes (Figures 1, 2 [on back cover]). All colour codes quoted are from Kornerup & Wanscher (1978). Some of the main distinctive characteristics of the fruit bodies were:

1. Pileus brown when very young, then the surface soon develops coarse brown scales upon a cream background.
2. Lamellae cream then sometimes with a dull greenish tinge, finally dull greenish grey when fully mature.
3. Stipe tall, fibrous, hollowed, with a brown region below the ring.
4. Ring membranous, superior, ragged, brown underneath.
5. Flesh cream discoloring red-brown.
6. Spore print dull, smoky, green (only when fresh, see spore deposit notes below).

Pileus up to 100 mm diam.; ovoid at first with margin clasping to the stipe and firmly joined to a membranous partial veil; expanding to conical then umbonate with a thin, increasingly ragged, deeply split margin. Surface dry at all times, smooth or minutely areolate/cracked, entirely warm brown (near 5E5 to 6E5) in unexpanded buttons. Brown layer soon fragmenting into appressed, loose and recurved brown coarse scales on a cream background (which is radially silky-fibrillose), some scales imbricate especially near the pileus margin. The brown layer covers the ring as well, which later forms a brown undersurface on the mature ring. In mature pilei the brown scales are fewer and smaller near the pileus margin, but the centre often retains a dark dull brown

(near 6F3) patch. *Lamellae* free, deep (to 15mm), ventricose at maturity; edge smooth, entire; crowded; white in button, then cream with a very faint pinkish tinge in some bruising pinkish brown. Mature lamellae develop a dull greenish tinge (near 27C3 to 28C3). *Stipe* to 120 × 12 mm; cylindrical with a minor swelling at base; tough/fibrous but with a hollow centre. Surface dry; appressed longitudinally silky (see under × 10 lens); white with a dark brown region especially below the ring. *Ring* superior, membranous, not easily moveable; brown underneath, cream above; margin ragged, flaring out at right angles from the stipe. *Flesh* cream, discoloring red-brown. *Basal mycelium* white, fine. *Odour* pleasant mushroom. *Taste* mild, sweet. *Spore deposit* dull green (29E4–5 to 29D5–6) when fresh, becoming more smoky or greyish upon drying (29D3–D4), and ochre or bright yellow-brown (5C5 to 5D6) upon storage (*e.g.* after six months).

Specimens examined: Western Australia, Perth, no details provided of location or habitat, 1 April 1981, coll. *L. van der Pennen s.n.* (PERTH 908258). Western Australia, Perth, Floreat Park, campus of CSIRO (corner of Underwood Ave and Brockway Rd), on grass lawn, 25 Feb. 1998, coll. *N. Bougher s.n.* (CSIRO Forestry and Forest Products Mycology Herbarium, Perth, WA CSIRO E884). (Also observed at same location, April 1999).

Further notes

The local prevailing weather condition at the time *C. molybdites* was observed in 1998 was warm (maximum temperatures low 30s or high 20s ^{o}C) with two days of rain during the fortnight before the appearance of large numbers (40–50) of fruit bodies. The grassed area had been watered once-weekly for several months. Urea fertilizer had been applied onto the grass about one month before fruit bodies were observed. Fruit bodies were observed again in early April 1999 during a period of unseasonably warm weather (*e.g.* maximum daily temperature for Perth up to 34^{o}C). Grass lawn and paddocks are common habitats for *C. molybdites* (Grgurinovic 1997; Reid & Eicker 1991; Young 1989).

In view of the poisonous nature of *C. molybdites*, particular care needs to be taken when interpreting colour of the lamellae. Most specimens observed in Perth by the author during days of dry weather in 1998 had white to dull pinkish or greyish lamellae. Most specimens observed during rainy weather had distinctly and consistently greenish lamellae. The former perhaps did not attain full maturity owing to unfavorable weather conditions during their development, and they could easily be mistaken for edible *Macrolepiota* species—particularly *M. rachodes* which can also occur on lawns in Perth and many other parts of Australia (*e.g.* see illustration of south-western Australian representatives of *M. rachodes* in Bougher & Syme (1998)).

In cases of doubtful identity such as with specimens having whitish or cream gills, discrimination can be achieved by microscopic examination of spores. Spores of *Macrolepiota*, but not *Chlorophyllum*, become stained in Congo Red (Weresub 1971). When tested using 3% KOH to which a drop of Congo Red (1% aqueous) was added, almost all spores of air-dried *C. molybdites* specimens (CSIRO E884) with green gills were unstained, except for a low proportion of stained immature spores. Specimens of *C. molybdites* with cream gills (from the same patch, as described above) had some unstained spores but most were stained immature spores such as those adhering in quads or attached to basidia. Relatively few spores in total were present in whitish/cream-gilled specimens compared with green-gilled specimens, owing to immaturity of the former. In contrast, all spores of air-dried Australian and American specimens of *M. rachodes* (CSIRO E5628 and E3207 respectively) were heavily stained. These results support the premise that the Congo Red spore test (Weresub, 1971) is an accurate and rapid diagnostic method of discriminating between *C. molybdites* and *M. rachodes*. Fresh specimens are not required, as the test is applicable with air-dried specimens. However, results of tests conducted on the Western Australian material emphasise that when applying the test it is important to take into account the presence of immature, staining spores which may be predominant in whitish/cream-gilled and immature specimens of *C. molybdites*, and occur in low amounts in green-gilled specimens.

It is not known how far south *C. molybdites* occurs in south-western Australia. The current paucity of occurrence records for the species suggests that the fungus may not be widespread or abundant in the region. It not surprising therefore that to date there have been no confirmed reports of poisonings due to *C. molybdites* in Perth or the south-west of Australia. Never the less, an ever-expanding human population in the region no doubt will increase the likelihood of such cases in future years.

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