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Melanomma dinghuense, a new loculoascomycete with Munk pore-like perforations from Dinghushan Biosphere Reserve in Southern China

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Melanomma dinghuense **found on decaying wood is described as a new species and illustrated. It differs from all other species of *Melanomma* by the presence of longitudinally ridged ascospores. Its fruitbody cells contain Munk pore-like perforations. An anamorph which can be placed in *Aposphaeria* was obtained in culture.**

Key Words——East Asia; Loculoascomycetes; Melanommatales; peridial pores; tropical mycology.

The genus *Melanomma* Nitschke ex Fuckel was established in 1870 (Fuckel, 1870). It is the type genus of the order Melanommatales in the Loculoascomycetes (Barr, 1987). Chesters (1938) studied morphology and ontogeny in *M. fuscidulum* Sacc. and *M. pulvis-pyrius* (Pers.: Fr.) Fuckel, the type species, and emended the description of the genus to include ascomycetes with the following characteristics: ascomata spherical or irregularly globose, gregarious, occasionally scattered, immersed becoming superficial on the host matrix or on a more or less well-developed subiculum, apex dome-shaped, papillate or conspicuously beaked, carbonaceous, smooth or rugose; paraphysoids persistent, branched, septate, hyaline. Asci cylindrical or clavate-cylindrical, eight-spored. Ascospores uni- or biseriate, spindle-shaped, often slightly curved, usually triseptate, width of the two anterior cells greater than that of the two posterior cells, yellowish or dark brown.

In a forest in subtropical southern China, we found a Loculoascomycete which matches well the above criteria for inclusion in the genus *Melanomma*. Since it cannot be attributed to any existing species of *Melanomma*, we describe it here as a new species.

Materials and Methods

Decaying corticated branches lying on the ground were collected, and returned in plastic bags to the laboratory for microscopic examination.

For semi-thin sectioning, ascomata from the wood were embedded in Jung tissue freezing medium (Leica Instruments GmbH, Germany) and sectioned with a Jung CM 1500 cryostat.

To prepare cultures, an ascospore suspension was plated out on both CMA and PDA Petri dishes, which were subsequently incubated at 25°C in the dark. Single germinated ascospores were transferred to new PDA Petri dishes by sterile forceps.

The pycnidia obtained in culture were embedded in plastic for sectioning in the following way: conidia-producing pycnidia were fixed from ca. 12 h up to several weeks in 2.5% glutaraldehyde buffered in 0.2 M sodium cacodylate buffer (pH 6.8) at 8°C. One day prior to dehydration, the material was transferred to aqueous sodium cacodylate buffer and left there overnight. Dehydration was carried out through a water-ethanol series to 100% ethanol. The material was then embedded in Spurr's resin using an ethanol-resin series involving the ratios of resin to ethanol of 1:2, 3:1, and 1:0. The material was left in the respective dilutions overnight, and placed in fresh resin prior to polymerization. About 1–4 μm thick sections were cut with a Reichert Om U3 ultramicrotome, stained with toluidine blue, and subsequently examined by light microscopy.

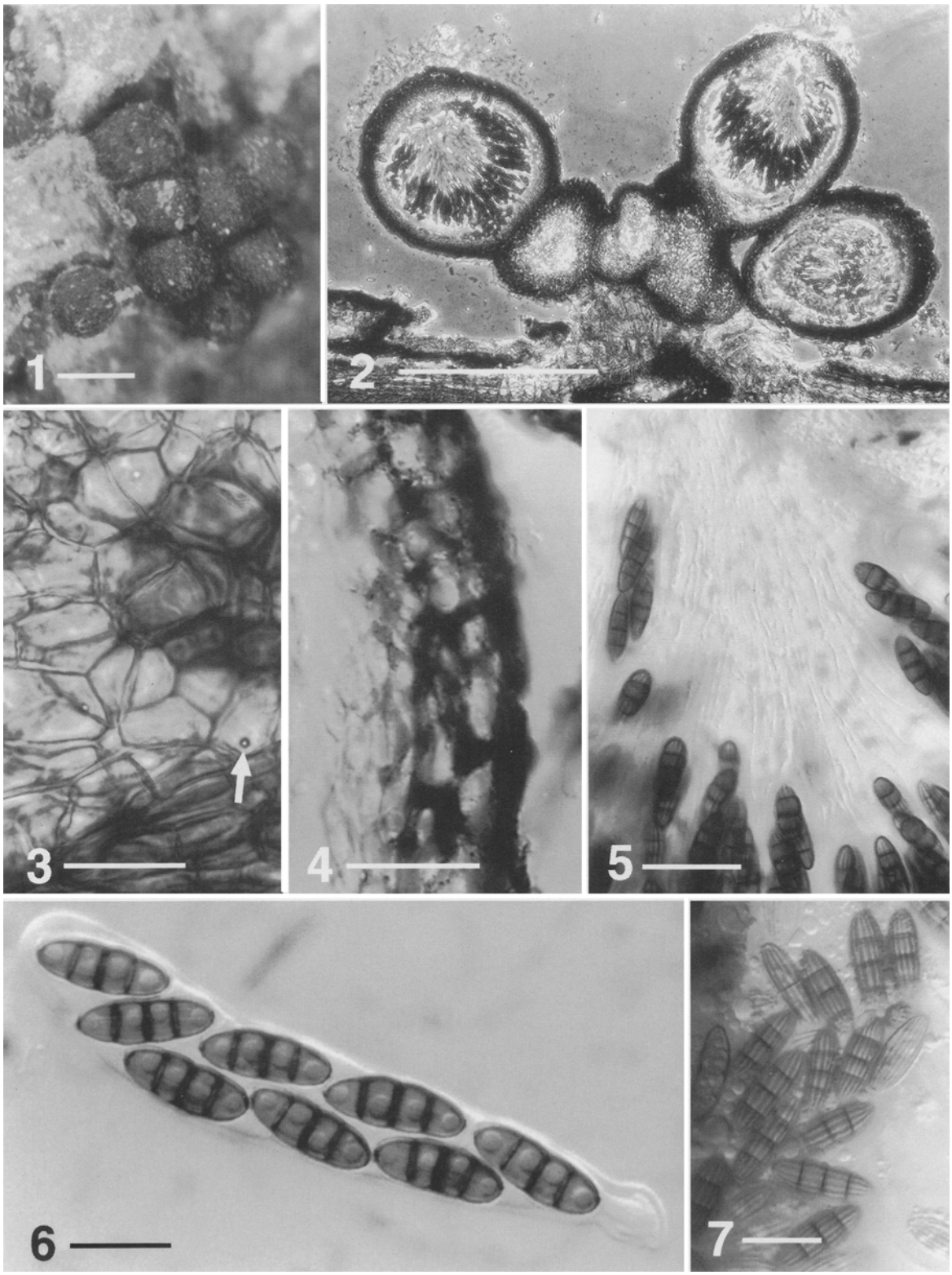
Results

Melanomma dinghuense Inderbitzin, sp. nov. Figs. 1–11

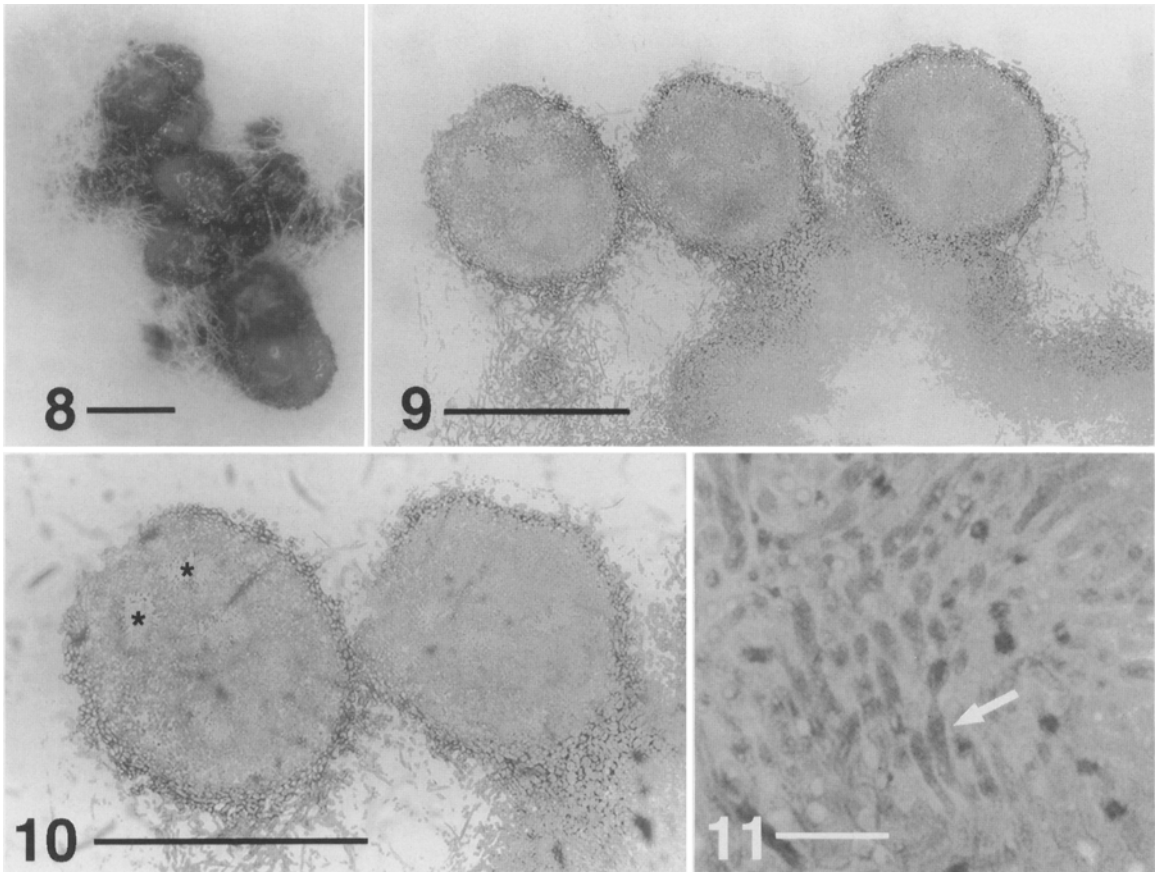
Ascomata 250–300 μm diam, globosa, coriacea, papillata, stromati in cortice immerso 2–25 aggragatim insidens. Cellulae stromatis "textura angularis", pallide brunneae, tenuiparietales, cum poris circularibus 0.5–1 mm diam perforatae. Paraphysoides 0.5–2 μm crassae, septatae, ramosae. Asci 50–78 × 8–15 μm, bitunicati, clavati, breviter stipitati. Ascosporae 10–17 × 3–6 μm, ovales, pallide brunneae vel olivaceae, triseptatae, leviter curvatae, longitudinaliter striatae.

Holotypus: specimen D1.1 in ligno emortuo, Dinghushan, Oct. 1998, M. A. Abdel-Wahab et P. Inderbitzin leg. (UBC F13996).

Etymology: referring to the type locality, Dinghushan Biosphere Reserve.



Figs. 1–7. *Melanomma dinghuense*. (1. Dissecting microscope. 2. Phase contrast. 3. Bright field. 4-7. Differential interference contrast.) 1. An aggregate of ascomata erumpent through the bark. 2. Vertical section through ascomata seated on a stromatal base. 3. Detail of stromatal tissue. Note Munk pore-like perforations in some cell walls (arrow). 4. Vertical section through ascomatal wall. The outer wall layer is irregularly incrusted with dark pigment. 5. Central cavity with thread-like paraphysoids. 6. Ascus with ascospores in optical section. 7. Ascospores in surface view. Scale bars = 300 μm for 1, 2; 10 μm for 3, 6, 7; 20 μm for 4, 5.



Figs. 8–11. Anamorph of *Melanomma dinghuense* obtained in culture. (8. Dissecting microscope. 9–11. Bright field micrographs of semi-thin plastic sections). 8. An aggregate of pycnidia exuding conidia as a slimy mass. 9. Vertical section through an aggregate of pycnidia. Note the stromatal base. 10. Vertical section through two pycnidia. Note the hymenial cavities (asterisk) produced within the pycnidia surrounded by dark cells. 11. Hymenium with a conidiogenous cell (arrow) producing a conidium. Scale bars = 200 μm for 8–10; 10 μm for 11.

Ascomata growing gregariously on a ca. 1 cm thick corticated branch of a dicotyledonous tree, immersed-erumpent, globose, 250–300 μm in diam, dark brown to black, rugulose, coriaceous, papillate, in groups of 2–25 on an immersed, up to 150 μm thick stromatal base (Figs. 1, 2). Stromatal cells in *textura angularis*, light brown, thin-walled, some with circular perforations 0.5–1 μm in diam similar to Munk pores in the Nitschkeaceae (Fig. 3). Ascomatal wall in surface view a *textura angularis*, in transverse section a *textura angularis-globulosa*, bilayered (Fig. 4): outer layer dark, 15–20 μm thick, 3–5 cells wide, cell walls up to 3 μm thick, irregularly incrusted with dark-brown pigment; inner layer hyaline, 7–15 μm thick, and 2–6 cells wide except at apex where many-layered and up to 50 μm thick, cell walls ca. 1 μm thick, refractive, innermost cells elongate, except at apex where also innermost cells rounded; paraphysoids 0.5–2 μm wide, branched, septate, no gel coating observed in either toluidine or aniline blue (Fig. 5). Asci (50–)52–69(–78) × 8–13(–15) μm (n = 30), clavate, bitunicate, fissitunicate, short-stalked, with a small ocular chamber (Fig. 6), forming a crozier at the base, lining the ascomatal cavity up to ca. 3/4th of the height of the ascoma (Fig. 2), leaving a central cavity which contains relatively few paraphysoids. Ascospores (10–)12–15(–17) × (3–)4–5.5(–6) μm (n = 30), biseriate in the central part of the ascus, oval, light brown to olive brown, 3-septate, constricted at the septa, at times slightly curved or with asymmetric hemispores, longitudinally striate (Figs. 5–7). Striae originating near poles, ca. 18 per ascospore, hemispherical in transverse section and ca. 0.5 μm in diam, appearing before secondary septa are formed (Fig. 7).

Colonies derived from single ascospore isolates in light on PDA reached 2 cm in diam after 10 d. Mycelium white, dense, cottony. After 50 d conidia-exuding anamorph present. Conidiomata suspended in the mycelium or immersed in agar and becoming erumpent, covered by mycelium or not, consisting of one to ca. 10 pycnidia clustered on a stromatic base (Figs. 8, 10). The latter may be absent, especially in solitary pycnidia. Stromatic base up to 250 μm high, comprising thin-walled cells in *textura angularis-globulosa* (Fig. 10). Pycnidia epapillate, black, 200–250 μm in diam, globose, with single os-

tiole. Pycnidial wall 4–20 μm thick, 1–6 cells wide, continuous at the inside, irregularly roughened at the outside (Fig. 10). Wall cells in transverse section thick-walled, pigmented, in *textura globulosa*, at the area of attachment to the stroma sparser at times, or extending up to 75 μm into the stroma (Fig. 9). Tissue between pycnidial wall and hymenium a loose *textura globulosa*, comprising thin-walled cells of similar dimension as wall cells (Fig. 10). Hymenium convoluted, consisting of conidiogenous cells (Fig. 10). Conidiogenous cells flask-shaped, unbranched, determinate, 5–12 × 2–3 μm, no collarette seen (Fig. 11). Conidiogenesis apical, unilocal. Conidia solitary, blastic, hyaline, thin-walled, smooth, ovoid with flattened base to oblong, up to 3 × 2 μm, exuded through ostiole as a slimy, gray-yellowish mass (Figs. 8, 11). Conidiae germination was not investigated. The teleomorph was not formed in culture.

Specimens examined: D1.1 (UBC F13996), holotype, on decaying corticated branch on the ground, Dinghushan Biosphere Reserve, Zhaoqing, Guangdong Province, People's Republic of China, October 1998, M. A. Abdel-Wahab and P. Inderbitzin. Habitat and distribution: On corticated branch of a dicotyledonous tree on the ground of a monsoon evergreen broad-leaved forest along a stream in Dinghushan Biosphere Reserve, China.

Discussion

The sexual state of *Melanomma dinghuense* matches the generic concept of *Melanomma* given by Chesters (1938), except for the morphology of the ascospores, and the presence of Munk pore-like perforations in the cell walls of both stroma and ascomatal wall.

The ascospores of *M. dinghuense* are *Melanomma*-like in that they are 3-septate, constricted at the septa, straight to slightly curved, with the upper hemispore being wider than the lower hemispore in some ascospores. However, the presence of conspicuous longitudinal ridges on ascospores has not been reported in *Melanomma* before (Fig. 7).

Since Holm (1957) partly revised the genus *Melanomma* recognizing 12 species, several authors have added species to the genus (Holm, 1991; Vassilieva, 1987; Vasyagina *et al.*, 1987; Ramesh, 1993; Yuan and Barr, 1994). *Melanomma dinghuense* differs from all species in the literature by the presence of conspicuously ridged ascospores.

Munk pores are circular perforations in fruitbody cell walls, and are characteristic of the fungi in the Nitschkeaceae. There, the pores are ca. 1 μm in diam, surrounded by a ring-like thickening, and are present as one to several in the cell walls of two adjacent cells (Nannfeldt, 1975a, 1975b).

The Munk pore-like perforations in *M. dinghuense* are 0.5–1 μm in diam, surrounded by a ring-like thickening or not (Fig. 3), and are a lot less abundant than in members of Nitschkeaceae. In a vertical section of the stroma of *M. dinghuense*, only a few pores can be observed, whereas in the cells surrounding the centrum cavity only one pore in total could be detected with certainty (not illustrated). The cells surrounding the centrum cavity are laterally compressed, their walls are thickened, and irregularly incrusted with a dark pigment, possibly obstructing most of the perforations present.

Apart from fungi in the Nitschkeaceae, Munk pore-like perforations have also been found in a representatives of the following families (Cannon, 1995): Lasiosphaeriaceae, Melanconidaceae, Valsaceae, Dothideaceae, Xylariaceae, Ceratostomataceae, Hyponectriaceae, and Vialaeaceae. Thus, Munk-pore like perforations are widely distributed in a variety of ascomycete families, and do not suggest any particular phylogenetic relationship of *M. dinghuense*.

Chesters (1938) attributed the anamorph of *M. pulvis-pyrius* to *Aposphaeria agminalis* (Sacc.) Sacc., and described it as follows: pycnidia scattered or crowded together in small groups, superficial or immersed around the base, globose-conical, about 200–300 μm in diam, black, carbonaceous, shortly papillate. Pycnidial wall in transverse section consisting of an outer zone of four to six layers of dark brown, thick-walled cells enclosing a pseudoparenchymatous layer of hyaline cells. Flask-shaped conidiogenous cells project from the inner surface of this layer to form a zone lining the pycnidial cavity. The apex of the young conidiogenous cells is rounded, and a collarette is evident only after the first conidium has been shed. Conidia hyaline, ellipsoid or somewhat oblong, rounded at each end, 2–3.5 × 1.5–2.5 μm, exuded in a cream-colored mass.

The conidiomata obtained from single ascospore isolates of *M. dinghuense* are morphologically similar to the ones of *M. pulvis-pyrius* described above. Differences include the presence of a stromatic base in *M. dinghuense*, and the arrangement of the conidiogenous cells: in *M. pulvis-pyrius* they form a hymenium delimiting one rounded pycnidial cavity, whereas in *M. dinghuense* the hymenium is convoluted, lining several pycnidial cavities (Fig. 10).

In conidiogenous cells of *M. dinghuense*, no collarettes were observed (Fig. 9). Chesters (1938) noted that the collarettes are most obvious when freezing microtome sections are stained in Congo red, and mounted in water. Sections of conidiomata of *M. dinghuense* of different ages were treated in this way, but collarettes were not evident.

The type material of *M. dinghuense* was collected along a small stream in a monsoon evergreen broad-leaved forest in Dinghushan Biosphere Reserve in subtropical southern China. The dominant tree species in the area were *Castanopsis chinensis* Hance, Cheng & Hwa, *Cryptocarya concinna* Hance, and *Syzygium levinei* Merr. & Perry.

Research in various disciplines has been carried out in Dinghushan Biosphere Reserve since the 1950's, accumulating a wealth of scientific data. For example, more than 500 species of macromycetes are known to occur in the area (Kong et al., 1993). However, the microfungi have received little attention. According to our knowledge, *M. dinghuense* is the first lignicolous microfungus to be reported from the reserve.

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