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Short Communication

First record of the occurrence of *Sporodiniella umbellata* (Mucorales) in Taiwan

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During an investigation of mucoraceous fungi indigenous to soil of old-growth forest in Taiwan, an entomogenous fungus identified as *Sporodiniella umbellata* was encountered. It has a sporangiophore with a conspicuous umbellate habit with sterile spines and sporangia, and was found growing on dead insect, *Acraea issoria-formosana* and *Danaus plexippus*. Description of the specimens and living cultures are provided, along with illustrations of taxonomically important structures. Artificial inoculation of *S. umbellata* on larvae of mealworm resulted in the radial growth of young sporangiophores emerged from the test larvae.

Key Words——entomogenous fungus; facultative parasite; Mucorales; *Sporodiniella umbellata*.

The genus *Sporodiniella* was introduced by Boedijn (1959) with a single species, *Sporodiniella umbellata* Boedijn, based on a collection from an insect carcass from Sumatra. It was treated as a doubtful species by Zycha et al. (1969), because no type material was known to exist. Accordingly, it was invalidly published and was a designated neotype (R.S. 618 in herb, on *Umbonia* sp.), which was collected by H. C. Evans at Pichilingue in Ecuador in 1974 and published by Evans and Samson in 1977.

During a general survey of the mucoraceous fungi of Taiwan, an entomogenous species was encountered on a common insect host, *Acraea issoria-formosana* (Nymphalidae) attached to a leaf of *Miscanthus floridulus* (Labill) Warb. ex Schum. & Laut. located in grass on a river bank. *Sporodiniella umbellata* is unique, bearing sporangiophores umbellately that branch dichotomously, one branchlet terminating in a sterile spine and the other in a single sporangium. We have focused on the sporangiophores of this fungus and paid special attention to the conspicuous umbellate habit with a sterile spine bearing a single sporangium, unlike all known species of Mucorales in their structure.

The purpose of this paper is to compare the Taiwan collections (Taian 01 and 02, 1992; FML 01 and 02, 1993) with the original descriptions and with the living cultures derived from newly collected Shanshia specimens (FML 01 and 02), and to examine the host range extension for *Sporodiniella umbellata*.

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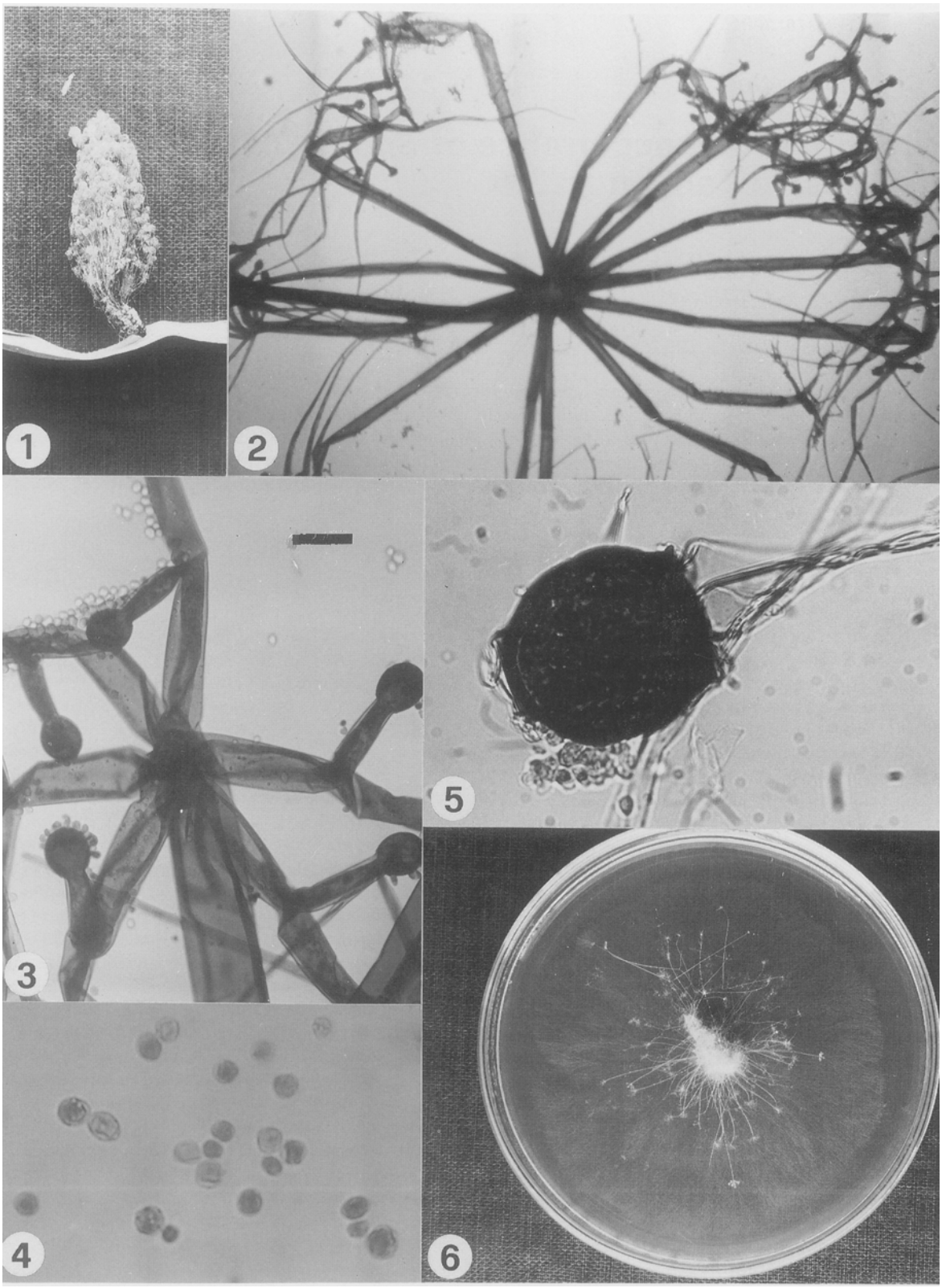
Materials and Methods

Taian specimens (Taian 01 and 02, 1992) were collected on insect pupae of *Acraea issoria-formosana* (Nymphalidae). The pupae were attached to the midribs of the lower leaf surface of *Miscanthus floridulus* growing in grass along a river bank in Taian County, Miauli Prefecture. Shanshia specimens (FML 01 and 02 ,1993) were collected on insect larvae of *Danaus plexippus* on the lower leaf surface of *Ficus septica* Burm, on the roadside at Full-Moon Lake, Shanshia County, Taipei Prefecture. The collections and living cultures were examined following routine procedure, and whole mounts were made and stained with cotton blue. Living cultures (CH 01 and 02) were successfully obtained from Shanshia specimens (FML 01 and 02) on carrot agar (CA, carrot 200 g, blended and boiled for 20 min, filtered through cheesecloth, agar 16 g/L) at 20°C in darkness. Living mealworms (*Tenebrio molitor*) were purchased from a pet shop and all tested worms were autoclaved before use. An artificial infestation of *S. umbellata* on larvae of mealworm on corn meal agar (CMA, corn grain 20 g, soaked in hot water 45 min, filtered though cheesecloth and diluted with water to 1 L, agar 16 g) at 24°C was examined 72 h after inoculation. All photographs were taken with a light microscope (Leitz, Orthoplan) using Kodak FX 402 films.

Results

Mycological Observations

Sporodiniella umbellata Boedijn ex Evans & Samson, Can. J. Bot. **55**: 2981–2984. 1977. Figs. 1–6
=*Sporodiniella umbellata* Boedijn, Sydowia **12**: 336.



Figs. 1–6. *Sporodiniella umbellata*.
1. Habit of colonized turf on natural host (Taian 01), bar = 10 mm. 2. Umbellate sporangiophore, bar = 120 μm. 3. Base of ultimate branches with fertile head, bar = 40 μm. 4. Mature sporangiospores, bar = 10 μm. 5. Zygospore from natural substrate, bar = 20 μm. 6. Artificial infestation of *S. umbellata* on a larva of mealworm (*Tenebrio molitor*) on CMA at 24°C 72 h after inoculation, showing the radial growth of young sporangiophores, emerged from the test larva, bar = 10 mm.

1959. (nomen invalidum, Art. 37, B) (Greuter, 1988).

Description of specimens on natural substrate

Aerial hyphae. Dimensions and morphology of Shanshia specimens from which living cultures (CH 01 and 02) derived were examined, and aerial mycelia were found to be more or less septate, yellowish brown, smooth-walled, and 9–18 μm in diam.

Sporangiophores. Arising directly from substrate hyphae or from aerial mycelia. The primary sporangiophores (stalk) and secondary sporangiophores (branched stipe) are yellowish brown to pale brown, smooth-walled, and the branched stipe usually terminates with 6–12 verticate dichotomous branches. Each branch bears a single sporangium and a spine, 350–600 μm in length.

Sporangia. The sporangia are globose to subglobose, gray-brown, thin-walled, 26–65 μm in diam.

Columellae. The columellae are globose to subglobose 15–25 μm in diam with poorly defined collar and with a conical stipe.

Sporangiospores. Hyaline to pale yellow, various in shape and size, globose to subglobose, 3–5.6 × 3–5 μm, mostly finely roughened but occasionally smooth-walled.

Zygospores. Zygospores formed in old collapsed colonies on the hosts (Taian 01 and 02) and on the surrounding leaf surface on which the insect pupae are attached, globose to subglobose (50–)55–82.5(–106)μm in diam, somewhat appressed, reddish brown or black in reflective light, ornamented with irregular warts.

Suspensors. Hyaline, more or less equal, smooth-walled, 33–46 μm wide (Fig. 5).

Neotype: R.S. 618 in herb. (CBS) on *Umbonia* sp. of the family Membracidae (Homoptera) associated with cocoa farms in Pichilingue, Ecuador.

Distribution: Indonesia, Ecuador, Taiwan.

Species examined: Taian specimens (Taian 01 and 02) on *Acraea issoria-formosana* of the family Nymphalidae, attached on the midrib, lower surface of *Miscanthus floridulus*, collected by Bij-Chyi Hwang, 29-VIII-1992, Taian County, Miauli Prefecture. Shanshia specimens (Full-Moon Lake, FML 01 and 02) on *Danaus plexippus* of the family Danaidae, attached on the lower leaf surface of fig (*Ficus septica*) collected by Bij-Chyi Hwang. Living cultures (CH 01 and 02) were obtained from Shanshia specimens on 9-VI-1993, Shanshia County, Taipei Prefecture.

Morphology of living cultures on agar media

Colonies on CA or malt extract agar (MEA) growing rapidly, completely filling a Petri dish within 5 d at 25°C, forming a yellow-brown tuft up to 2 cm high, aerial hyphae and sporangiophores abundant on both media, but more so on CA than MEA within one week at 20°C. On CMA, only substrate hyphae growing, and both aerial hyphae and sporangiophores scattered in cultural plates at room temperature.

Colonies on the host (mealworm, *Tenebrio molitor*) consisting of a number of erect, long, primary sporangiophores, 8–21 mm long, 45–76 μm wide, forming a yellowish brown tuft up to 15–20 mm high. Aerial hyphae present, thin-walled, smooth, 6.7–24.8 μm. Primary sporangiophores pale brown in age, smooth-walled, consisting of long unbranched secondary sporangiophores, 45–60 μm × 6–21 mm, terminating in an apical verticil; each ultimate branch being dichotomously divided with one arm bearing a sporangium and the other ending in a long sterile spine (Fig. 2). Sporangial stalk conical in shape, 21–45 × 18–25 μm. Sporangia globose, pale brown, 45–50 μm in diam. Columellae globose to subglobose, smooth-walled, 18–21 × 25–32.5 μm in diam, more or less with collar (Fig. 3). Sterile spine (80–)400–900 μm in length, 45 μm at the base and 5 μm width in distal end. Sporangiospores globose to subglobose, finely roughened, 3–5.5(–6) μm in diam (Fig. 4).

Zygospores were examined only from Taian specimens (Taian 01 and 02) (Fig. 1) and the zygosporic state was not obtained in living cultural strains (CH 01 and 02) or matings.

Discussion

In 1958 Boedijn established *Sporodiniella umbellata*, a unique entomogenic fungus which was characterized by having dichotomously branched sporangia with spines, which was thought to resemble the genus *Syzygites* (Hesseltine, 1957). However, Zycha et al. (1969) treated this fungus as a doubtful species, and Hesseltine and Ellis (1973) recognized the genus *Sporodiniella* as being related to *Syzygites* but distinguished by having one arm ending in a long sterile spine. Recently, Benny (1992) has suggested *Syzygites* and *Sporodiniella*, as facultative parasites of basidiomycetes or insects, should be included in the Dicranophoraceae, because of the similarities in the branching pattern of the sporangiophores and distinctive zygospores.

The asexual state of *S. umbellata* on insect pupae of *Acraea issoria-formosana* persists to form sporangiophores (stalk) and aerial hyphae. Although stolons are rarely observed, but sometimes sterile spines may do instead of it in cultural plate. On the host pupae, the aerial hyphae are slender and really distinguished from the stout hyphae destined to become sporangiophores. Our observations showed that the fungus in many aspects resembles mucoraceous fungi. Its sporangiophore pattern strongly resembles the umbellate habit of *Mortierella umbellata* (Chien, 1972), the sterile spine of *Chaetocladium brefeldii* van Tieghem & Le Monnier (Zycha et al., 1969), the columella shape of *Actinomucor elegans* (Eidam) Benjamin & Hesseltine (Indoh, 1962) and zygosporangial wall ornamentation of *Mucor hiemalis* Wehmer (Zycha et al., 1969). Evans and Samson (1977) indicated that the zygosporic state only occurred in old, collapsed specimens on the natural substrate and could not be induced from living culture (Samson et al., 1988). Zygospores were observed on both old collapsed specimens (Taian 01 and 02) on the natural substrate (Fig. 5). The mature zygosporic state appeared, characterized mainly by the ornamentation of black warts by reflective light. Unfortunately, we could not obtain zygospores by mating living cultures (CH 01 and 02) and derived from Shanshia speciemens (FML 01 and 02).

However, Ingold (1978) stated that the sexual state of Mucorales is rarely seen on natural substrates.

A large population of *Miscanthus floridulus* is located in Taian County, Miauli Prefecture and in a mixed with bamboo in Shanshia County, Taipei Prefecture. Both localities are in common, hardwood habitat bordering one side of a river. The climate is strongly seasonal: 60–80% of the yearly precipitation falls in a four-month period (June to September). It is generally agreed that insect larvae and pupae may develop before the rainy season. *Sporodiniella umbellata*, the entomogenic fungus in Mucorales, is the only known species that attacks pupae or larvae especially on suitably shaded substrates in spring weather and flourished within this season.

Artificial infestation of *S. umbellata* on larvae of mealworm on CMA at 24°C was observed 72 h after inoculation. The fungus appeared as radial growth of young sporangiophores emerging from a test larva (Fig. 6). In conclusion, this fungus is considered as a facultative parasite that is able to live as a saprotroph, and can be cultured on CA, CMA and MEA media. All examined slides, specimens (Taian 01 and 02, FML 01 and 02) and living cultures (CH 01 and 02) have been deposited in the Mycological Collections, Institute of Biological Sciences, National Taiwan Normal University, Taipei, Taiwan, R.O.C.

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