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A new species of *Hansfordia* isolated from the marine brown alga, *Colpomenia sinuosa*

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ABSTRACT — A new species of *Hansfordia* was described and illustrated from the marine brown alga *Colpomenia sinuosa* at the coast of Weihai, China. It is *Hansfordia sinuosae*, which differs from other species in the genus *Hansfordia* based on the characters of conidia.

KEY WORDS - hyphomycetes, taxonomy

Introduction

During an investigation of fungi associated with marine macroalgae, an interesting hyphomycete species from the alga *Colpomenia sinuosa* was found at the coast of Weihai, China. It is described here as a new taxon in the genus *Hansfordia*.

The genus *Hansfordia* was established by Hughes (1951) with *H. ovalispora* as type species. The fungi in this genus are characterized by their conidia borne in acropetal succession on conspicuous denticles (Barron 1968). Von Arx (1982) considered this genus as a synonym of *Dicyma* Boulanger. However, at present they are regarded as two different genera by the presence of separating cells in *Hansfordia* which are absent in *Dicyma*. Twenty-one species names in the genus *Hansfordia* have been recorded (http://www.indexfungorum.org/Names/Names.asp 2011), but it seems that some species are misplaced (Kirk 1986; Hu & Guo 2007).

Materials & methods

Sampling and isolation

The strain of *Hansfordia sinuosae* initially was isolated by K.M. Sun in June 2010 from the marine brown alga, *Colpomenia sinuosa*, on the coast of Weihai, China. Algae

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collected from the intertidal zones were placed into sterile plastic bags and carried back to laboratory. The samples were washed with sterile seawater and immersed in 75% ethanol for 1 min. Each algal thallus was cut into approximately 2×2 mm segments. These were finely ground in sterile seawater with mortars and pestles, and 100 µl of supernatant solution was spread over culture media. For the isolation, 9 cm Petri dishes containing potato dextrose agar (PDA) supplemented with 1 g/l Penicillin G and Streptomycin sulphate were prepared. Petri dishes were then sealed, incubated at 20 °C and examined periodically. When colonies developed, they were transferred to fresh media with PDA immediately to obtain pure isolates. All pure isolates were stored at 4 °C in the dark.

Incubation and observation

The fungal strain used in this study was cultured on PDA in the dark at 20 °C. Macroscopical characters and general growth rates were reported from pointinoculated media in Petri dishes (9 cm diam.) incubated for 14 days. In order to observe microscopical morphology, a new method for microscopical slide preparation was developed. Firstly, a square block $(1.6 \times 1.6 \text{ cm})$ was moved from sea water agar (SWA) and a groove formed; then, a patch of thalli were inoculated onto the medium block $(0.4 \times 0.4 \text{ cm}, \text{SWA})$ and placed at the centre of the groove; finally, a cover slip (1.8) \times 1.8 cm) was placed above the groove, which was examined after 3 days for the first time and observed continuously every two days by making temporary or permanent slides. The method is convenient for observation of the formation of conidia and conidiophores with natural morphology. Observations, measurements and photographs were carried out in seawater mounts using an Olympus BX51 microscope (PLATE 1) and scanning electron microscope (SEM) for particular ultrastructure observation (PLATE 2). All microscopic characters were measured from more than 50 individuals. The type specimen (dried culture) and living culture are deposited in the Herbarium of Ocean University of China Marine Biology (OUCMB). The ex-type culture is kept in China General Microbiological Culture Collection Center (CGMCC).

Taxonomy

Hansfordia sinuosae Wei Li & X.L. Cheng, sp. nov.

PLATES 1-2

МусоВанк МВ 519457

Coloniae on PDA post 14 dies 20°C 13 mm diam.. Mycelium partim superficiale, partim in substrato immersum, ex hyphis laevibus vel crudus, ramosis, septatis, hyalinae, postea brunneis, 1–2 µm latis compositum. Conidiophora macronematosa, mononematosa, erecta vel repentia, recta vel flexuosa, hyalinae, postae brunneae, ad apici non setiformia, asepta. Cellulae conidiogenae in conidiophoris incorporatae, terminales, geniculatae. Conidiorum secessio rhexolytica, fracta ab cellula intercalaria. Conidia acrogena vel acropleurogena, ellipsoidea ad late sub-globosa, aspectu laevia, sine septa, pallidissima brunnea, 4–6 × 3–4 µm, basi hilo circa 0.5 µm lato leniter promineti.

TYPE: China. Shandong Province: Weihai, intertidal zone, isolated from living *Colpomenia sinuosa (Phaeophyceae*), 5 June 2010, K.M. Sun (OUCMBI₁₀1190, dried agar culture, **holotype**; CGMCC 3.14278, ex-type culture).

Colonies on PDA 13 mm diam. in 14 days at 20°C, obverse black green with obvious white margin, reverse olive-green. Mycelium superficial and immersed,

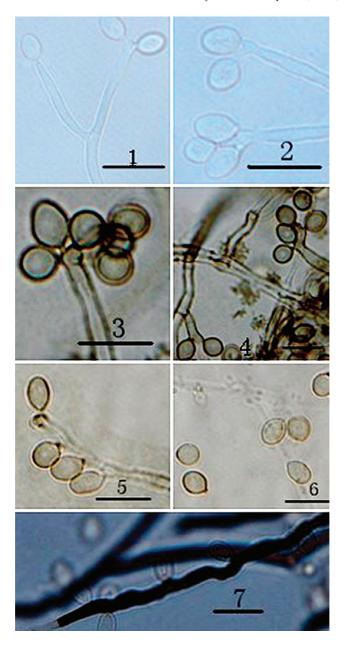


PLATE 1. *Hansfordia sinuosae* (Scale bars = 10 μm). 1–2: conidia and conidiophores after 4 days; 3–6: conidia and conidiophores after 7 days; 7: conidiophores after 12 days.

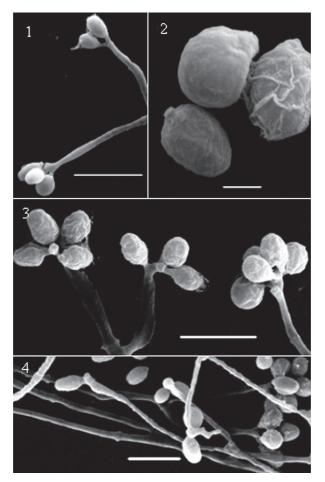


PLATE 2. *Hansfordia sinuosae* (Scale bars: 1, $3-4 = 10 \mu m$; $2 = 2 \mu m$). 1, conidia and conidiophores after 4 days; 2-4, conidia and conidiophores after 7 days.

composed of septate, branched hyphae. Hyphae hyaline, smooth initially, gradually turning brown and often rough later, $1-2 \mu m$ wide. Conidiophores erect or repent, straight or flexuous, early smooth and hyaline, $10-30 \mu m$ long, $1.0-1.5 \mu m$ wide, and gradually turning brown, elongating up to $140-150 \mu m$ later, apices not setiform. Conidiogenous cells integrated, terminal, slightly geniculate. Conidia secession rhexolytic by fracture of the wall of a small separating cell. Conidia acrogenous, later acropleugenous, solitary, ellipsoidal to broadly subglobose, appearing smooth, pale brown, aseptate, $4-6 \times 3-4 \mu m$, with a slightly protruding basal hilum about 0.5 μm wide.

Discussion

The new taxon differs from most other species in the genus Hansfordia in conidium size, which is smaller than that of *H. alba* (7–9 \times 2.5–3 µm; Meyer 1959), H. arborescens (9.5–10 µm; Hughes 1958), H. biophila (6–12 × 2.5–3.5 μm; Ellis 1976), *H. caricis* (6–8 × 3.5–4.5 μm; Kirk 1986), *H. catalonica* (8–12.5 \times 6–9 µm; Gene et al. 2000), *H. cinnamomi* (14–20 \times 10–12 µm; Deighton 1960), H. grewiae (7–9 μ m; Hughes 1951), H. ovalispora (8–11 × 4.5–6 μ m; Hughes 1951), *H. pallens* (8–13.5 × 5.5–7.5 μm; Hu & Guo 2007), *H. parasitica* (8–10 \times 3–4 µm; de Hoog 1974), and *H. togoensis* (14–17 \times 4.5–6 µm; Hughes 1951) but larger than that of *H. indica* $(2.5-4.5 \times 2-3 \mu m; Rao \& Rao 1980)$ and *H.* triumfettae (3-4 µm; Hughes 1951). Hansfordia sinuosae is similar in conidium size to *H. pulvinata* (5.4–6 μ m; Hughes 1958), *H. nebularis* (3–5 × 3–4 μ m; Ellis 1976), and H. canescens (3.5-5 μ m diam or 5-6 \times 4 μ m; Hughes 1951), but they can be distinguished from each other by their conidial morphology. The conidia of *H. pulvinata* and *H. nebularis* are spherical and verrucose, while the conidia of *H. canescens* are broadly globose and hyaline. Based on the features of conidial shape, size and smooth surface, H. sinuosae is depicted as a new species.

Considering that there are distinct differences in the hyphae and conidiophores between the earlier and later growth stages of *H. sinuosae*, we attempted to examine slides continuously in order to fully document and understand the growth and morphological changes. The results suggest that the method of slide preparation and examination mentioned above is suitable for this purpose.

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