Ascoyunnania aquatica gen. et sp. nov., a freshwater fungus collected from China and its microcylic conidiation

Lei Cai[1, 2*], Keqin Zhang[1] and Kevin D. Hyde[2]

[1]Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming, Yunnan, China
[2]Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong

Cai, L., Zhang, K.Q. and Hyde, K.D. (2005). *Ascoyunnania aquatica* gen. et sp. nov., a freshwater fungus collected from China and its microcylic conidiation. Fungal Diversity 18: 1-8.

Ascoyunnania aquatica gen. et sp. nov. is introduced to accommodate a remarkable ascomycete species collected from submerged bamboo in a small stream in Jinghong, Xishuangbanna, Yunnan, China. This fungus is characterized by deeply immersed ostiolate ascomata, unitunicate, cylindrical to clavate asci that lack an apical apparatus and ellipsoidal, unicellular, hyaline, guttulate ascospores that germinate to form dark brown to black, globose, tuberculate secondary spores. This species and its microcylic conidiation are illustrated and described in this paper. The ecological role of the microcylic conidiation, and the taxonomic affinity of *A. aquatica* are discussed.

Key words: ascomycete, bambusicolous fungi, freshwater fungi, lignicolous fungi, systematics.

Introduction

During studies on freshwater fungi in the tropics and subtropics (e.g. Ho *et al*., 2001; Cai *et al*., 2003; Fryar *et al*., 2004; Luo *et al*., 2004; Tsui and Hyde, 2004), an interesting ascomycete was collected. Under a dissecting microscope the fungus superficially resembles a sporodochial hyphomycete on the surface of the bamboo. The clustered 'conidia' on the surface were dark brown, globose and tuberculate. On careful examination it was found that the 'conidia' were attached to a large, hyaline, ellipsoidal sac-like apparatus. No conidiophores or conidiogenous cells could be found. When the surface of the substratum was cut using a razor blade, we found that an ascoma occurred deeply beneath the 'conidial' mass. The hyaline ascospores were slightly larger than the sac-like apparatus attached to the warted conidia. Subsequent experiments showed that the hyaline ascospores had germinated directly to

*Corresponding author: Cai Lei; e-mail: leicai@hkusua.hku.hk

form dark brown secondary spores within the asci (the germination from free ascospores was not observed despite several attempts) and the sac-like apparatus attached to the secondary spores was in fact the hollow remains of the ascospore.

The asci of this species were unitunicate, clavate to cylindrical, dextrinoid, with a short tapering base, and lacked an apical apparatus. Mature asci mostly floated freely in the ascomata. Ascospores were unicellular, hyaline, guttulate and ellipsoidal. The characters of this fungus do not fit any previously described genera. A new genus is, therefore, established to accommodate this fungus.

Materials and methods

Submerged substrata were collected by L. Cai from a small stream in a tropical forest in Jinghong, Xishuangbanna, Yunnan, China (N22°02′ E100°50′), and returned to the laboratory in zip lock plastic bags. The samples were processed and examined following the method described in Tsui *et al*. (2000). Type specimens were deposited in PDD and HKU(M). Observations and photographs were made from materials mounted in water. The widths of the conidia were measured at the widest point. The range between minimum and maximum values for microscopic measurements is given. Mean values are in brackets with 'n' being the number of items measured. The experiment to establish germination of ascospores was conducted by removing the contents of the ascomata and preparing a suspension of asci and ascospores in sterile distilled water in a watch-glass. The suspension was then pipetted onto potato dextrose agar (PDA) and examined hourly.

Taxonomy

Ascoyunnania L. Cai & K.D. Hyde, **gen. nov.**

Etymology: From 'asco' for ascomycete and 'Yunnania', referring to the Province where this fungus was collected.

Ascomata immersa, globosa vel subglobosa, atro-brunnea vel nigra, coriacea, gregaria, ostiolata. Rostrum cylindrici, longe, periphysata. Paraphyses filamentosae, septatae, hyalinae. Asci 8-spori, unitunicati, cylindrici vel clavati, pedicellati, dextrinoidea. Ascosporae 1-2-seriatae, ellipsoideae vel fusiformes, unicellularae, hyalinae, guttulatae, leptodermae. Secundarii sporae globosa, atro-brunnea vel nigra, tuberculatae.

Ascomata immersed, globose to subglobose, dark brown to black, coriaceous, usually gregarious, with central, periphysate ostiole. Beak long, brown, periphysate, apex visible at host surface, usually densely covered by numerous secondary spores. Peridium dark brown, composed of several layers of angular cells. Paraphyses rare, hypha-like, septate, unbranched, hyaline,

thin-walled, tapering distally. Asci 8-spored, unitunicate, clavate to cylindrical, dextrinoid, pedicel tapering, lacking any apical apparatus. Ascospores 1-2-seriate, ellipsoidal to fusiform, unicellular, hyaline, guttulate, thin-walled, lacking a mucilaginous sheath. Ascospores germinate in the asci to form globose, dark brown to black, regularly warted secondary spores.

Type species: *Ascoyunnania aquatica* L. Cai and K.D. Hyde.

Ascoyunnania aquatica L. Cai & K.D. Hyde, **sp. nov.** (Figs. 1-9)

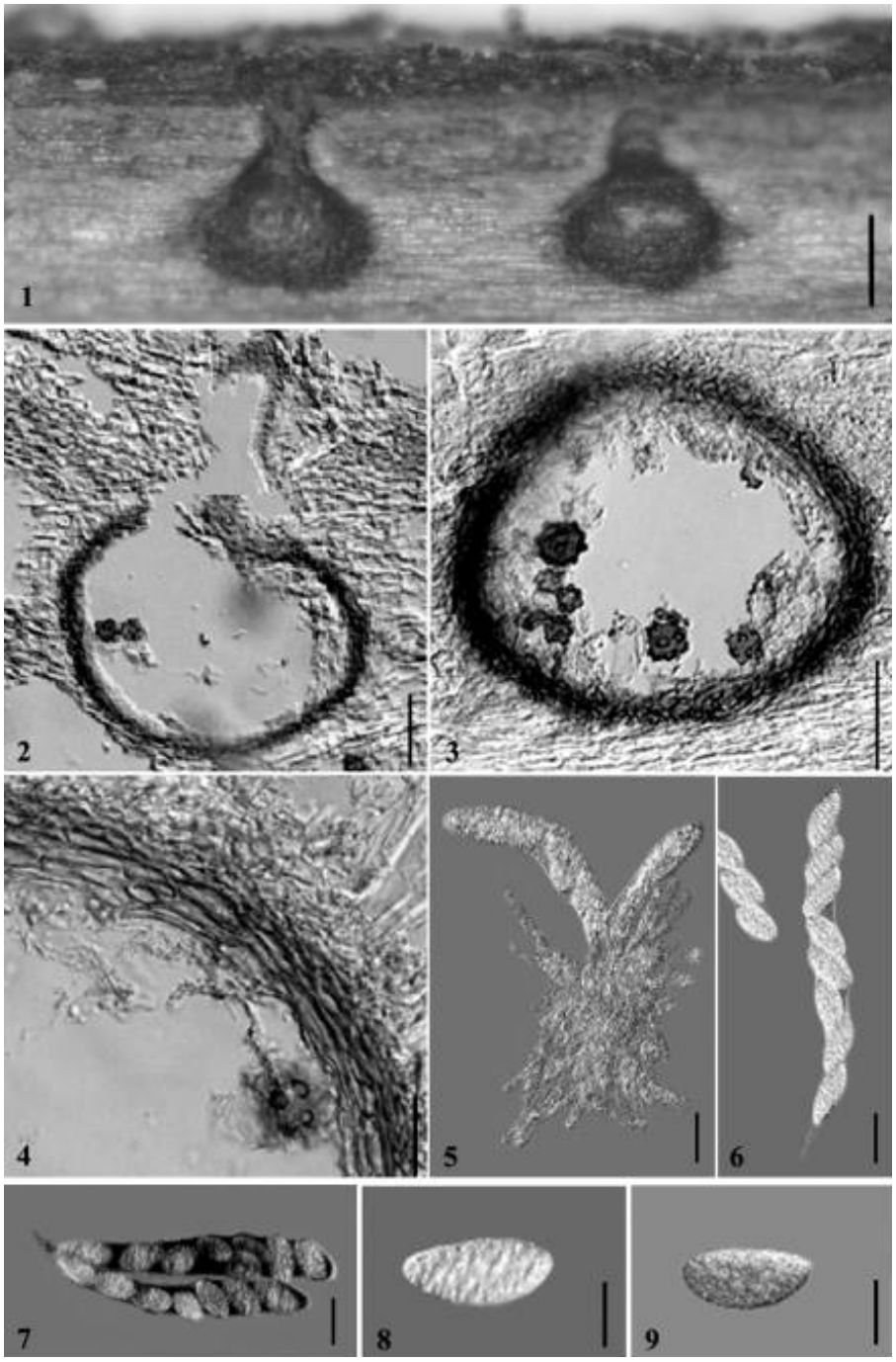
Ascomata 210-320 µm diam., 310-400 µm alta, immersa, globosa vel subglobosa, atro-brunnea vel nigra, coriacea, gregaria, ostiolata. *Rostrum* cylindrici, 150-220 × 40-60 µm, periphysata. *Peridium* 15-30 µm crassum. Paraphyses 3-5 µm diam., filamentosae, septatae, hyalinae. *Asci* 155-310 × 25-45 µm ($\overline{x}$ = 231 × 31 µm, n = 20), 8-spori, unitunicati, cylindrici to clavati, pedicellati. *Ascosporae* 32.5-52.5 × 17.5-26 µm ($\overline{x}$ = 43 × 21 µm, n = 30), 1-2-seriatae, ellipsoideae vel fusiformes, unicellularae, hyalinae, guttulatae, leptodermi, saepe tunica gelatinosa praeditae. *Secundarii sporae* globosa, atro-brunnea vel nigra, tuberculatae.

Ascomata 210-320 µm diam., 310-400 µm high, immersed to 250-350 µm deep, globose to subglobose, dark brown to black, coriaceous, usually gregarious, with central, periphysate ostiole. *Beak* cylindrical, 150-220 × 40-60 µm, brown, periphysate (Figs. 1-2), apex visible as minute black dots or small papilla on the host surface, usually densely covered by numerous secondary spores (Figs. 1, 17). *Peridium* 15-30 µm wide, outwardly brown or dark brown, composed of 3-4 layers of angular cells; inwardly pale brown, composed of several layers of compressed cells (Fig. 4). *Paraphyses* sparse, 3-5 µm wide at base, hypha-like, septate, unbranched, tapering distally, usually dissolving at maturity with remnants of short rows of cells. *Asci* 155-310 × 25-45 µm ($\overline{x}$ = 231 × 31 µm, n = 20), 8-spored, unitunicate, cylindrical to clavate, dextrinoid, with a tapering pedicel, apex rounded, lacking any apical apparatus (Figs. 5-7). *Ascospores* 32.5-52.5 × 17.5-26 µm ($\overline{x}$ = 43 × 21 µm, n = 30), overlapping uniseriate to biseriate, ellipsoidal to fusiform, unicellular, hyaline, with numerous small guttula, thin-walled, wall minutely verrucose, lacking mucilaginous sheath (Figs. 8-9).

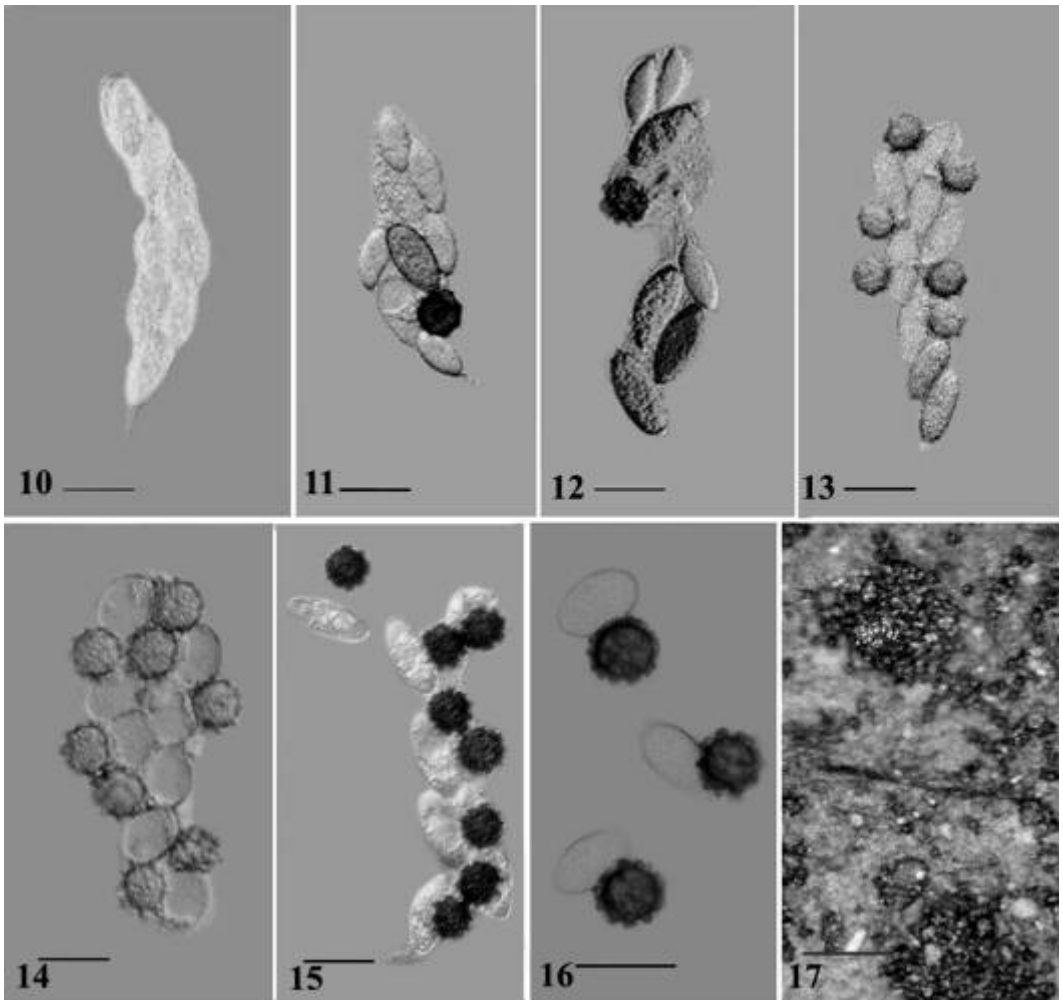
Ascospores germinate within the asci to form dark brown to black, warted secondary spores. Secondary spores 25-35 µm in diam. ($\overline{x}$ = 30 µm, n = 30), forming clusters at the apices of the ostioles on the surface of the bamboo, globose, dark brown to black, uniformly warted at the surface, bearing a large, hyaline, thin-walled, smooth, non-guttulate, ellipsoidal sac-like structure (the remains of the ascospore), measuring 27.5-50 × 15-22.5 µm ($\overline{x}$ = 40 × 19 µm, n = 30), slightly smaller than the ascospores (Fig. 16).

Etymology: Meaning 'aquatic' in relation to the habitat of this species.

Specimens examined: CHINA, Yunnan, Jinghong, on submerged bamboo in a small forest stream, 8 August 2001, L. Cai (**holotype designated here**, PDD 75039; Isotype HKU(M)17155).



Figs. 1-9. *Ascoyunnania aquatica* (from holotype). **1.** Side view of ascomata. **2-3.** Sections of the ascomata. **4.** Peridium. **5.** Squash mount illustrating asci and paraphyses. **6-7.** Asci. Note the tapering pedicel. **7.** Asci in Melzer's reagent. **8-9.** Ascospores. Bars: 1= 200 µm; 2, 3 = 100 µm; 4, 8-9 = 20 µm; 5-7 = 40 µm.



Figs. 10-17. *Ascoyunnania aquatica* (from holotype). Sequence of germination of ascospores in asci to produce secondary spores. **10.** Ungerminated ascospores in asci when first placed on PDA. **11.** A single ascospore has germinated at its base to form a brown secondary spore. **12.** Several ascospores have germinated or are about to germinate. **13.** Germinating ascospores with immature secondary spores. **14.** All ascospores in the ascus have germinated to produce secondary spores. Most of the cytoplasm has disappeared from the germinated ascospores. **15.** Germinated ascospores with secondary spores. The ascospores still contain cytoplasm. The ascus wall has deliquesced. **16.** Mature secondary spores. The sac-like structures are the remains of the ascospores. **17.** Black mass of secondary spores on the host surface around the ascoma ostioles. Bars: 10-16 = 40 µm; 17 = 200 µm.

Results and discussion

Germination of ascospores into secondary spores is illustrated in Figs. 10-16. The time required for germination varied. Some ascospores germinated within 2 hours, most germinated within 6 hours, while some had not germinated within 3 days. Germination was only observed within the asci, i.e. germination from free ascospores was not observed. The sequence of germination was as follows: (i) There was a swelling in one part of the ascospore forming a hyaline, rounded, immature secondary spore. The secondary spores usually formed laterally on the ascospores, but occasionally formed from the apex of the ascospore. (ii) The secondary spores became pale brown and tuberculate, while the contents in the ascospores apparently moved into the secondary spores. (iii) The guttules in the ascospores disappeared with time. The secondary spores became dark brown at maturity and the wall of the ascospore remained attached to the secondary spores appearing as an empty sac-like structure. (iv) The asci dissolved after germination.

In suspensions plated onto PDA that contained the contents of ascomata, the ascospores within asci germinated directly to form secondary spores. The free ascospores and secondary spores however, were never observed to form germ tubes. Secondary spores scraped from the surface of the host were used for single spore isolation. Despite several attempts, it was not possible to obtain isolates from either kind of spores.

Hanlin (1994) reviewed microcycle conidiation, the phenomenon in which spores directly form secondary spores, without formation of mycelium. It is a special method of asexual spore formation in which the normal life cycle of the fungus is bypassed. Numerous species of fungi in diverse taxa possess this capability, but most of the reported microcycle conidiation were induced through manipulation of particular environmental conditions, especially temperature (Smith *et al*., 1981; Hanlin, 1994). In only a few cases, microcycle conidiation occurs under natural conditions. Microcycle conidiation is thought to be a mean of species adaptation when unfavourable conditions, such as drought, reduced nutrition, change in pH, reduced humidity and temperature, are encountered (Hanlin, 1994). The direct formation of secondary spores in *Ascoyunnania aquatica* appears to be a natural part of the life cycle of this fungus. Since the collection site is small forest stream and may have periodical drought, the microcycle conidiation is possibly a method of adaptation of this fungus. The globose, warted, dark brown secondary spores are likely to be more resistant to desiccation and UV light. They may be able to withstand periods of drying or submersion before becoming entrapped to new substrata. It may also be possible that the brown secondary ascospores could disperse

better during periods when the stream dries out, or function in aerial dispersal (Hyde and Goh, 2003).

The phylogenetic affinity of this fungus is uncertain. The perithecioid, deeply immersed, dark brown, globose to subglobose, ostiolate ascomata and the unitunicate, freely floating asci may link it to the order *Diaporthales*. Other morphological characters such as lacking paraphyses at maturity and the hyaline ascospores are also features for *Diaporthales*. This species however, also showed some resemblances to the *Halosphaeriales* as it lacks an apical apparatus in the ascus and early disappearing paraphyses. Other morphological characters, such as the deeply immersed ascomata, persistent asci and ascospores lacking sheaths or appendages, however, probably exclude it from *Halosphaeriales* (Barr, 1978, 1990; Kirk *et al*., 2001). Although asci of the *A. aquatica* lack an apical apparatus which is untypical for *Diaporthales*, we prefer to place this taxon in the *Diaporthales* incertae sedis at this time. Because this taxon is aquatic and has most likely evolved from terrestrial ancestors, its characteristics may have been modified. Thus using morphological characteristics in determining the phylogenetic position of this taxon is possibly difficult, as these characteristics would be modified if *A. aquatica* was derived from a lineage of terrestrial ascomycetes. Cain (1972) pointed out that the morphological features of some fungi are often unreliable as indicators of phylogenetic relationships. The taxonomic affinity of this fungus is not confirmed pending further collections and molecular analysis.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (NSFC 3026002) and International Cooperation Research Foundation, Yunnan Province (2000C002). Pipob Lumyong, Department of Plant Pathology, Chiang Mai University is thanked for providing laboratory space to carry out part of this work. Dr. Eric H.C. McKenzie is thanked for helpful suggestions. V. Dhanasekaran is thanked for discussion. Helen Leung is thanked for technical assistance. Lei Cai thanks the University of Hong Kong for providing a postgraduate scholarship.

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(Received 9 September 2004; accepted 31 October 2004)