

Annulatascus fusiformis sp. nov., a new freshwater ascomycete from the Philippines

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Abstract: *Annulatascus fusiformis* sp. nov. is described based on a specimen collected on submerged wood from a river in the Philippines. The species possesses globose to subglobose, black, immersed ascomata with long hyaline necks; wide, septate, tapering paraphyses, and cylindrical unitunicate asci with a relatively massive refractive apical ring. In this respect it is similar to species of *Annulatascus*, however, *A. fusiformis* differs from other species in the genus, as ascospores are provided with bipolar mucilaginous pad-like appendages. The species is illustrated with light, scanning and electron micrographs.

Key Words: Annulatascaceae, appendages, aquatic fungi, new species, taxonomy

In a continuing study of freshwater *Annulatascus*-like species in the tropics (Hyde et al 1998, Goh et al 1999, Wong et al 1999a), an undescribed species was collected on submerged wood in the Philippines. *Annulatascus* K. D. Hyde presently includes 7 species (Ho et al 1999a, b), all from freshwater habitats in the tropics. *Annulatascus fusiformis* sp. nov. differs from other *Annulatascus* species, as the ascospores have bipolar mucilaginous pad-like appendages. At the transmission electron microscope (TEM) level, the mucilaginous pad comprises electron-dense material that completely surrounds the ascospore, albeit thinner in the center of the ascospore. The morphological differences between *A. fusiformis* and other species in the genus are discussed.

Partially decomposed decorticated submerged wood was collected from Natigbasan Creek, Impalutao, Bukidnon, in the Philippines and returned to the laboratory in Hong Kong, where it was incubated at room temperature (~22 C) in moist plastic boxes and examined within 4 wk for fungal fruiting bodies. Fruiting structures were then prepared for further

study at the light, scanning and transmission electron microscope levels, following the methods of Wong et al (1999b). Single spore isolations were attempted, but the ascospores did not germinate.

Annulatascus fusiformis K. D. Hyde et S. W. Wong, sp. nov. FIGS. 1–19

Ascomata 170–220 µm diam, immersa vel semi-immersa, globosa vel subglobosa, nigra, coriacea, papillata, paraphysata, periphysata. *Asci* 141–235 × 7.5–10.5 µm, 8-spore, longe-cylindrici, pedicellati, apparato apicale, 4–5 µm longi, 3–4 µm diam praediti. *Ascosporae* 16.5–25.5 × 6–9 µm, uniseriatae, fusiformes, hyalinae, 1–5-septatae, verruculosae, appendiculatae.

Ascomata 170–220 µm diam, immersed or semi-immersed, globose to subglobose, black, coriaceous and solitary. *Neck* long, brown to black, periphysate, with a hyaline apex (FIG. 1). *Peridium* comprising several layers of thick, brown-walled compressed cells, hyaline and elongate inwardly. *Paraphyses* up to 7.5 µm wide, filamentous, numerous, constricted at the septa and tapering distally (FIG. 6). *Asci* 141–235 × 7.5–10.5 µm (\bar{x} = 180 × 8.8 µm, n = 25), 8-spored, long-cylindrical, with a tapering base (FIGS. 4, 5) and a relatively massive, refractive, bipartite, apical ring, 4–5 µm long, 3–4 µm wide (FIGS. 2, 3). *Ascospores* 16.5–25.5 × 6–9 µm (\bar{x} = 20 × 7 µm, n = 50), uniseriate or overlapping uniseriate, fusiform, hyaline, 1–5-septate, thick-walled, straight or slightly curved, with verruculose wall ornamentations at the apical and subapical regions (at TEM level) and with bipolar mucilaginous pad-like appendages (FIGS. 7–10).

Specimen examined. PHILIPPINES. MINDANAO: Bukidnon, Impalutao, Natigbasan Creek, on submerged wood, Jan 1994, K.D. Hyde (HOLOTYPE; HKU(M) 3102).

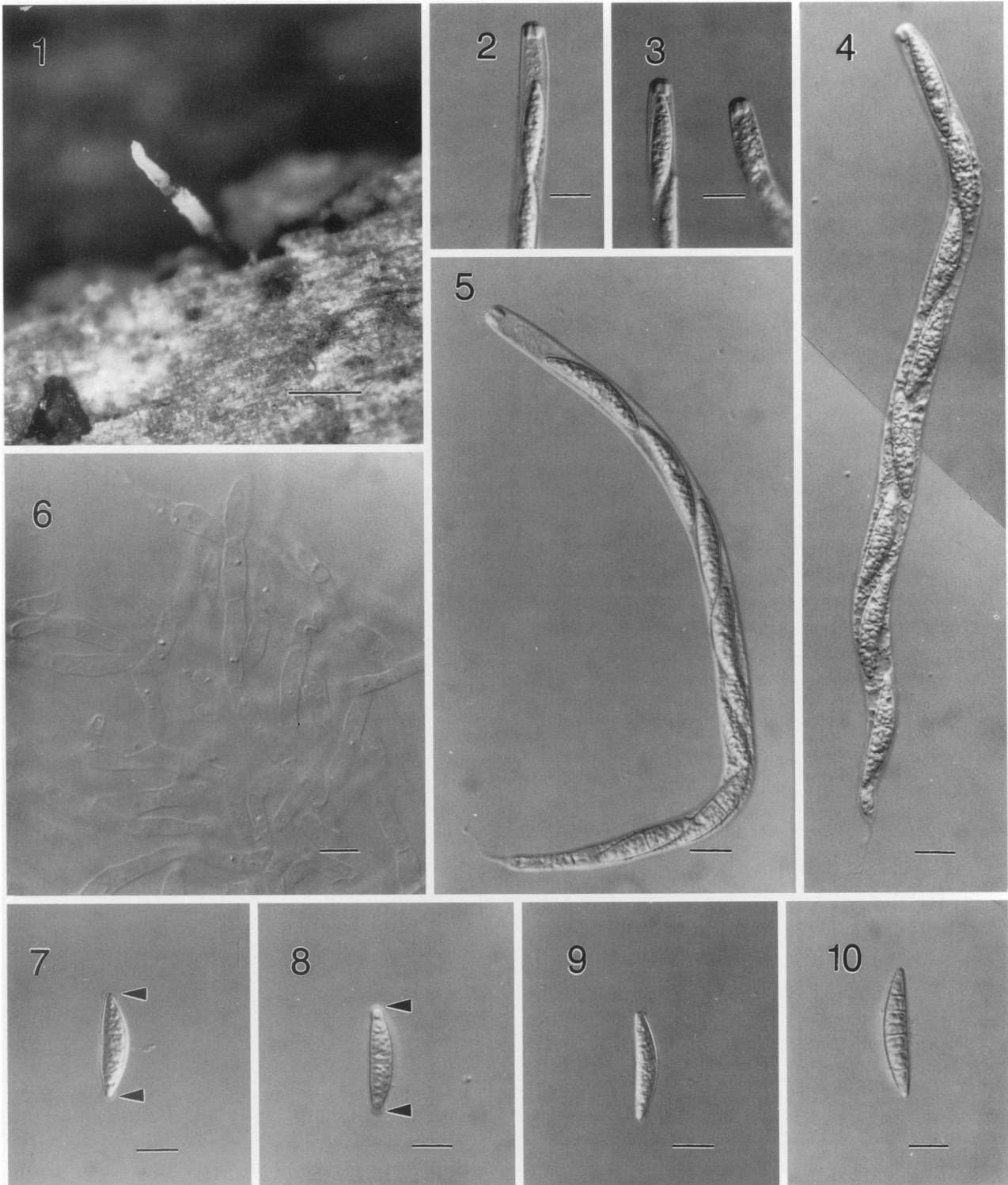
Etymology. In reference to the fusiform ascospores.

Habitat. Saprobic on wood submerged in freshwater.

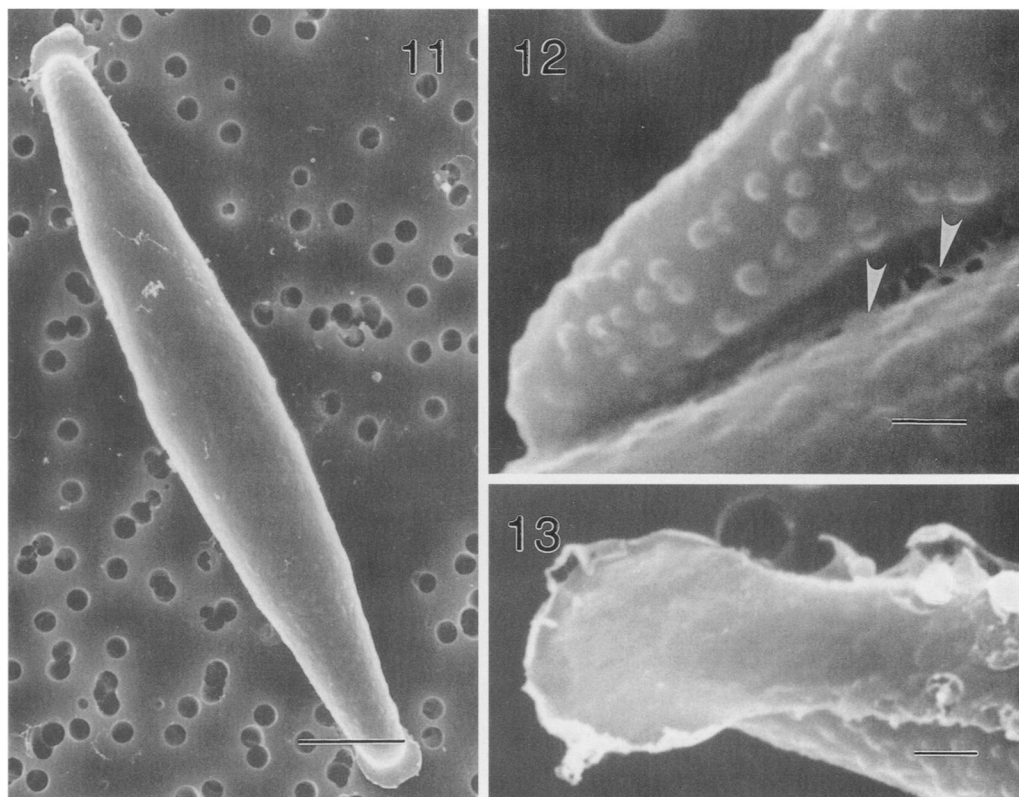
Known distribution. The Philippines Islands.

Mature ascospores of *A. fusiformis* are fusiform with rounded mucilaginous pads at each end (FIGS. 11, 13). These pads appear to be sticky and may aid in ascospore attachment (FIG. 12). Verruculose wall ornamentations are present at the apical and subapical regions of ascospores (FIGS. 11, 12), but are less well developed in the center (FIG. 11).

The wall of a mature ascus is bilamellate comprising an outer electron-dense amorphous layer (OA;



FIGS. 1-10. *Annulatascus fusiformis* (from HOLOTYPE). Interference light contrast and stereo-micrographs. 1. Immersed ascoma with long neck. 2, 3. Ascus apical rings. 4, 5. Asci, note the relatively massive refractive apical rings. The ascospores are uniseriate or overlapping uniseriate in the asci and the pedicel tapers to a point. 6. Wide, hyaline, septate paraphyses. 7-10. Hyaline septate ascospores with bipolar mucilaginous pad-like appendages (arrowed). Bars: 1 = 100 μm , 2-10 = 10 μm .



FIGS. 11–13. *Annulatascus fusiformis* (from HOLOTYPE). Scanning electron micrographs. 11. Fusiform ascospores with polar mucilaginous pads. 12. Two ascospores sticking together due to polar mucilaginous material (arrowed). Note the verruculose wall ornamentations at higher magnification. 13. Polar rounded mucilaginous pad of ascospore. Bars: 11 = 5 μm , 12, 13 = 1 μm .

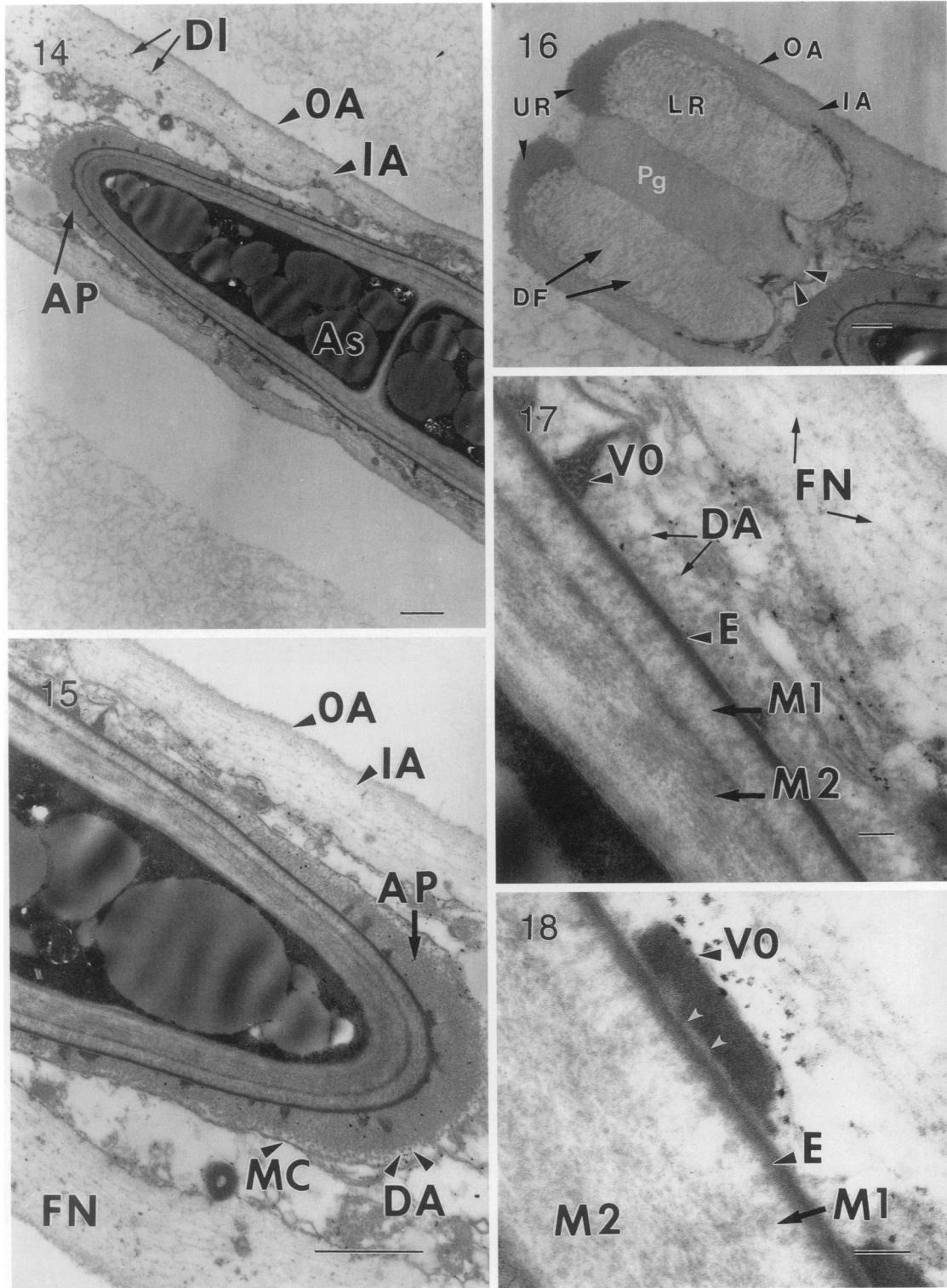
ca 54 nm thick), and a thicker inner electron-transparent layer (IA; 0.65–1.80 μm thick; FIGS. 14, 15). The inner wall layer comprises a network of fibrillar material (FN; FIGS. 15, 17) and some electron-dense inclusions (DI; FIG. 14). The apical ring is cylindrical and bipartite (FIG. 16) comprising an upper electron-dense part (UR), which appears to be an extension of the outer ascus wall; and a lower electron-transparent part (LR) with horizontally orientated electron-dense fibrils (DF; FIG. 16). A plug (Pg) is present at the level just below the upper part of the apical ring, and extends downwards at its base to form a conical protrusion. The plug is amorphous with similar electron density to that of the inner ascus wall, and both contain fibrillar material (FN) of the same electron density (FIG. 16).

Mature ascospores are fusiform, septate, and are surrounded by a mucilaginous sheath that is thicker at the ends, where they have the appearance of mucilaginous pads (AP; FIGS. 14, 15). In the ascus the mucilaginous pad is closely adpressed to the ascospore wall and is covered by the membrane complex (AP, As, MC; FIGS. 14, 15). The appendage comprises closely packed electron-dense aggregates (DA; FIG.

15). Some of these aggregates arise from the edge of the mucilaginous pad and are exosporial in origin (DA; FIGS. 15, 17).

The ascospore wall comprises a thin electron-dense epispodium (E; ca 40 nm thick); and a bipartite mesospodium including an outer electron-transparent layer (M1; ca 115 nm) with some electron-dense deposits; and an inner more electron-dense layer (M2; ca 346 nm; FIGS. 17, 18). The verruculose wall ornamentations (VO) at the apical regions are conical, electron-dense, scattered and may incorporate some smaller and electron-transparent zones (FIG. 17). In the subapical regions, the verruculose wall ornamentations may be flattened and electron-dense throughout (FIG. 18). A less electron-dense layer occurs at the interface between the wall ornamentations and ascospore wall (FIG. 18). The origin of these ornamentations is unknown.

Genera in the *Annulatascaceae* possess asci with a bipartite apical apparatus (Hyde et al 1998, Ho et al 1999a, b, Wong and Hyde 1999, Wong et al 1998, 1999b). Most genera also possess ascospores with verruculose wall ornamentations and appendages or sheaths (Wong et al 1998, 1999a). The wall ornamen-



FIGS. 14–18. *Annulatasacus fusiformis* (from HOLOTYPE). Transmission electron micrographs. Labels: Ascospore (As), outer thin electron-dense layer of ascus (OA), inner thick electron-transparent layer of ascus (IA), electron-dense inclusions (DI), fibrillar material (FN), mucilaginous pad (AP), membrane complex (MC), electron-dense aggregates (DA), upper electron-dense part of ascus ring (UR), lower electron-transparent part of ascus ring (LR), horizontally orientated fibrillar material (DF), electron-dense amorphous plug material (Pg), conical protrusion at base of plug (arrowed), episporium (E), outer electron-transparent layer of mesosporium with some electron-deposits (M1), inner thick layer of mesosporium with amorphous material (M2), wall ornamentations (VO). Bars: 14–16 = 1 μm , 17, 18 = 0.1 μm .

tations can usually only be seen at the SEM level. In *Diluviocola* K. D. Hyde, S. W. Wong & E. B. G. Jones (Hyde et al 1998) and *Pseudoproboscispora* (Wong & K. D. Hyde) Punith. (Wong and Hyde 1999) ascospores possess bipolar thread-like appendages. In *Annulatascus* species, ascospores are surrounded by a mucilaginous sheath and therefore *A. fusiformis* is better accommodated here (Wong et al 1998).

Hyde and Jones (1989) categorized the ascospore adhesion type of *Lautisporopsis circumvestita* (Kohlm.) E. B. G. Jones, Yusoff & S. T. Moss, *Ceriosporopsis tubulifera* (Kohlm.) Kirk and *Ondiniella torquata* (Kohlm.) E. B. G. Jones, R. G. Johnson & S. T. Moss as pad-like. The ascospore appendages of these species either form as direct outgrowths of the epispodium (*O. torquata*), elaboration of the mesospodium (*L. circumvestita*) or are of unknown origin (*C. tubulifera*). In *A. fusiformis* ascospores have nonextendable pad-like polar appendages. These appendages are continuous around the ascospore, although they cannot be seen in the center at the LM level. At the TEM level, the mucilaginous sheath of the ascospores of *A. fusiformis* comprises electron-dense material. No association of the mucilage material with any ascospore wall layers has been observed in *A. fusiformis* and thus, the appendages are considered to be exosporial in origin.

Annulatascus fusiformis differs from other species in the genus by the conspicuous pad-like appendages (Hyde 1992, 1995, Ho et al 1999a, b, Wong et al 1999b). It also has unusual verruculose flattened wall ornamentations, as compared to hemispherical in other *Annulatascus* species (Ho et al 1999a, b, Wong et al 1999b). Thin layers of mucilage occur at the ends of the ascospores of *A. biatriisporus* K. D. Hyde (Hyde 1995), and these could also be described as pad-like. The ascospores in *A. biatriisporus*, however differ in shape and are larger.

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