

FULL PAPER

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Three new species of *Annulatascus* (Ascomycetes) from Hong Kong freshwater habitats

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Abstract *Annulatascus joannae*, *A. lactus*, and *A. tropicalis* are described and illustrated from decaying woody substrata in freshwater habitats in Hong Kong. *Annulatascus joannae* is distinguished by ellipsoidal and thick-walled ascospores whereas *A. lactus* has milky ascomata and *A. tropicalis* has relatively large, fusiform, 1–3-septate ascospores. *Annulatascus biatriisporus* is reported as a new record in Hong Kong. A key to and a synoptic table of *Annulatascus* species are provided.

Key words Annulatascaceae · Aquatic fungi · Streams · Systematics

Introduction

Annulatascus K.D. Hyde was introduced for taxa having black, superficial ascomata with a long neck, cylindrical, unitunicate asci with relatively massive, refractive apical apparatus, and fusiform ascospores with appendages or sheaths (Hyde 1992). The genus has since been modified, with *Annulatascus bipolaris* K.D. Hyde, having unique bipolar thread-like appendages, being transferred to *Cateractispora* K.D. Hyde, S.W. Wong & E.B.G. Jones (Hyde et al. 1999b).

Currently there are nine accepted species in *Annulatascus* (Fröhlich and Hyde 2000; Ho et al. 1999a,b; Hyde 1992, 1995; Hyde and Wong 2000; Hyde et al. 1998; Wong et al. 1999) (Table 1). Species are separated on the basis of ascospore morphology and the presence or absence of a mucilaginous sheath. All species possess asci with bipartite apical apparatus, and some have verrucolous

ornamented ascospores as seen at the ultrastructural level (Ho et al. 1999a; Wong et al. 1999). Two species have been identified from terrestrial habitats on palm rachides (Fröhlich and Hyde 2000). Recently, molecular data showed that the genus is polyphyletic and that certain taxa may be conspecific (Campbell and Shearer 2001), and therefore a revision of the genus and an investigation of the relationship between aquatic and terrestrial taxa with sequence data and morphological characters is ongoing.

All previously described species of *Annulatascus* have been reported from tropical Australia, South Africa, and tropical Southeast Asia, but recently some representatives have been reported from the Great Smoky Mountains in the United States (Raja and Shearer 2001), suggesting the cosmopolitan distribution of the genus. During an ongoing investigation of freshwater ascomycetes on submerged wood in Hong Kong freshwater habitats (Tsui et al. 2001), we have identified three new *Annulatascus* species. They are illustrated here and compared with described species. *Annulatascus biatriisporus* is also reported as a new record for Hong Kong.

Samples of submerged woody substrata were collected from different freshwater habitats in Hong Kong, taken back to the laboratory in plastic bags, and processed following the methods described in Tsui et al. (2001). Fungi were isolated by using single-spore isolation (Choi et al. 1999) and, where successful, cultures have been deposited in the Hong Kong University Culture Collection (HKUCC).

Annulatascus lacteus K.M. Tsui, I.J. Hodgkiss & K.D. Hyde, sp. nov. Figs. 1–10

Ascomata 140–200 µm alta, 100–130 µm diam, superficialia, subglobosa vel pyriformia, papillata, ostiolata, gregaria, nigra, paraphysata, lactea usque pallide brunnea. Rostrum cylindraceum, 50 µm longum, 40–50 µm diam, periphysatum. Peridium ca. 20 µm crassum, externe visum *textura angularis*, ex cellulis 3–4-stratosis pseudoparenchymaticis hyalinis compressis compositum. Asci 130–170 × 9–10.5 µm, octospori, unitunicati, cylindrici, pedicellati, apparatusu apicali non amyloidei refractivo 3–4 µm alto 4–5 µm diam praediti. Ascospores 24–28 × 6–8 µm, imbricate

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Table 1. A synopsis of *Annulatascus* species

Species	Ascomata	Asci	Ascospores
<i>A. aquaticus</i> W.H. Ho, K.D. Hyde & Hodgkiss	220–600 µm high, 240–600 µm diam, dark brown	150–175 × 10–12 µm	19–24 × 6–7 µm, ellipsoidal with acute ends, aseptate, with a mucilaginous sheath at EM level
<i>A. biatriisporus</i> K.D. Hyde	195–325 µm high, 390–520 µm diam, black	210–260 × 12–17 µm	40–58 × 8–10 µm, fusiform with swollen ends, aseptate, with mucilaginous covering, relatively thick walled
<i>A. citriosporus</i> J. Fröhlich & K.D. Hyde	224–310 µm high, 350–485 µm diam, black	137.5–178.8 × 10–12 µm	22.5–30.5 × 6.4–8.5 µm, fusiform, aseptate, with mucilaginous covering, relatively thick walled
<i>A. fusiformis</i> K.D. Hyde & S.W. Wong	170–220 µm diam, black	141–235 × 7.5–10.5 µm	16.5–25.5 × 6–9 µm, fusiform, straight to slightly curved, 1–5-septate, with polar mucilaginous pad-like appendages, verruculous at EM level
<i>A. hongkongensis</i> W.H. Ho, Ranghoo, K.D. Hyde & Hodgkiss	210–250 µm high, 250–280 µm diam, dark brown	250–275 × 25–30 µm	35–37.5 × 12.5–15 µm, ellipsoidal with acute ends, 3-septate, with mucilaginous sheath, verruculous at EM level
<i>A. joannae</i> K.M. Tsui, Hodgkiss & K.D. Hyde	180–200 µm high, 150–200 µm diam, black	150–200 × 10–13 µm	20–28 × 9–12 µm, ellipsoidal to fusiform, thick walled, aseptate, with mucilaginous sheath, relatively thick walled
<i>A. lacteus</i> K.M. Tsui, Hodgkiss & K.D. Hyde	140–200 µm high, 100–130 µm diam, milky	130–170 × 9–10.5 µm	24–28 × 6–8 µm, fusiform, aseptate, without mucilaginous sheath or appendages
<i>A. licualae</i> J. Fröhlich & K.D. Hyde	77.5–95 µm high, 202–460 µm diam, black	88.8–125 × 5.8–7.8 µm	15–17.8 × 3.8–5 µm, fusiform-rhomboid, aseptate
<i>A. palmietensis</i> K.D. Hyde	150–440 µm diam, black	98–142 × 7–10.5 µm	20–26 × 6–7 µm, fusiform with blunt ends, 3-septate, no mucilaginous sheath
<i>A. triseptatus</i> S.W. Wong, K.D. Hyde, E.B.G. Jones & S.T. Moss	222–353 µm diam, black	140–218 × 9–14 µm	18–33 × 6–12 µm, fusiform, 3-septate, with mucilaginous sheath verruculous at EM level
<i>A. tropicalis</i> Ranghoo & K.D. Hyde	250–260 µm high, 250–270 µm diam, black	190–255 × 12–18 µm	42.5–52.5 × 7.5–10 µm, fusiform, 1–3-septate, lack mucilaginous sheath or appendages, relatively thick walled
<i>A. velatisporus</i> K.D. Hyde	450 µm high, 260–410 µm diam, black	220–290 × 12–18 µm	26–42 × 9–12 µm, fusiform, aseptate, with mucilaginous sheath, verruculous at EM level

A., *Annulatascus*; diam, diameter; EM, electron microscopy

uniseriatae, fusiformes, aseptatae, hyalinae, in massa aurantiacae.

Ascomata 140–200 µm high, 100–130 µm diameter, superficial, subglobose to pyriform, papillate, ostiolate, gregarious, membranous, milky to pale brown (Fig. 1). Beak ~50 µm long, 40–50 µm diameter, periphysate (Fig. 2). Peridium ~20 µm thick, *textura angularis* in surface view; in section composed of several layers of hyaline, compressed pseudoparenchymatic cells (Fig. 2). Paraphyses 2–3 µm wide, numerous, septate, unbranched, hyaline, tapering distally (Fig. 3). Asci 130–170 × 9–10.5 µm (\bar{x} = 150 × 9.5 µm, n = 20), 8-spored, unitunicate, thin walled, cylindrical, pedicellate, with a nonamyloid, refractive apical apparatus, 3–4 µm high × 4–5 µm wide (Figs. 8–10). Ascospores 24–28 × 6–8 µm (\bar{x} = 27 × 6.5 µm, n = 45), overlapping uniseriate, fusiform with acute ends, aseptate, hyaline, without mucilaginous sheath or appendages (Figs. 4–7).

Etymology: From the Latin *lacteus*, referring to the milky color of the ascomata.

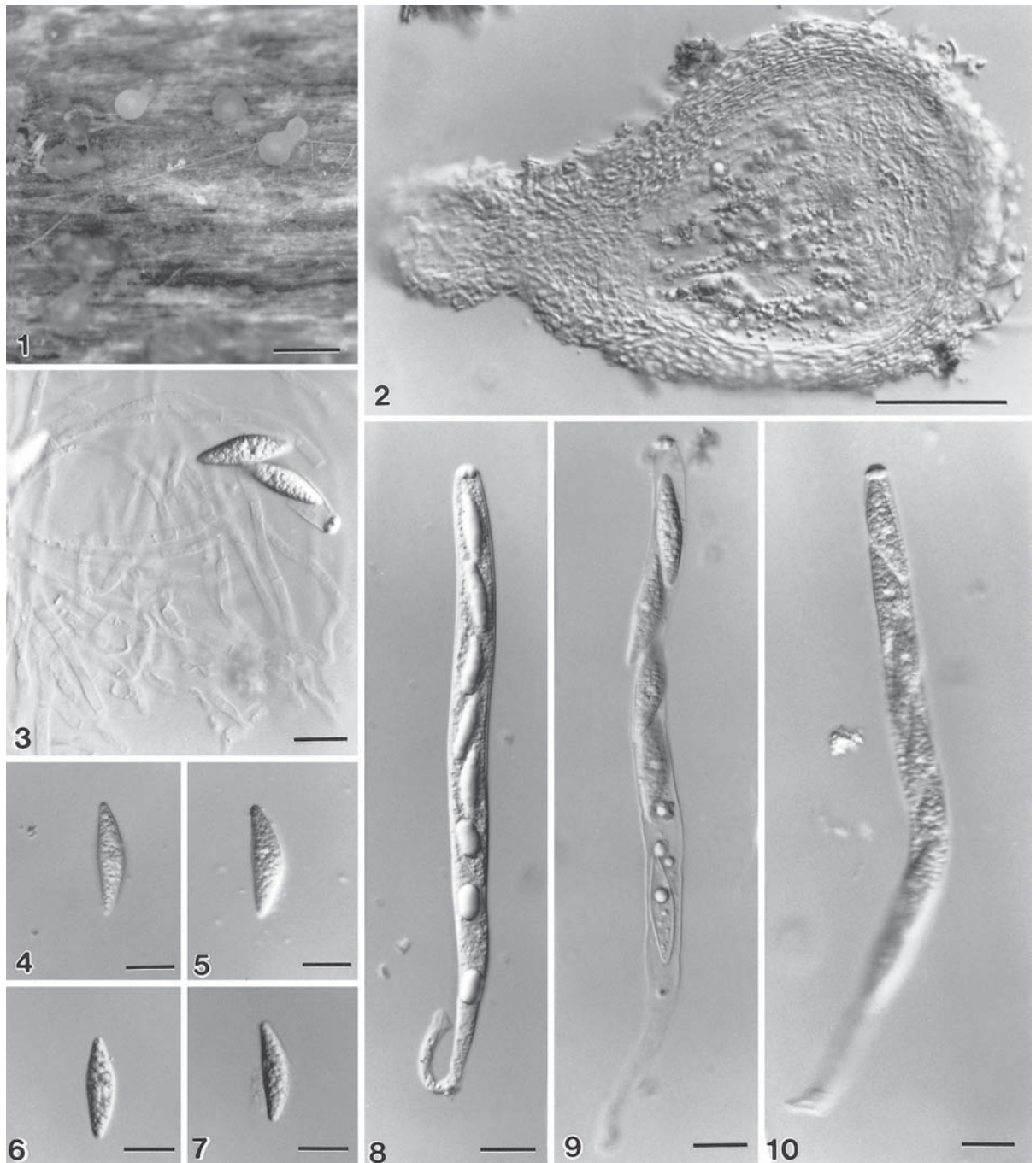
Holotype: China, Hong Kong, Tai Po, Lam Tsuen River, on submerged wood, September 1996, K.M. Tsui, KM313 (HKU(M) 4623).

Other material examined: China, Hong Kong, Tai Po, Lam Tsuen River, on submerged wood, December 1998, K.M. Tsui, KM313 (HKU(M) 12209).

Notes: The presence of milky ascomata in *A. lacteus* is unique among all *Annulatascus* species. It may be necessary to introduce a new genus to accommodate this species; however, this would be premature at this stage. *Annulatascus velatisporus* K.D. Hyde also produces fusiform and aseptate ascospores, which are, however, relatively greater in width and are surrounded by a mucilaginous sheath. Several freshwater fungal taxa in *Aniptodera* Shearer & M.A. Mill. and *Halosarpehia* Kohlm. & E. Kohlm. also have milky ascomata, but their asci are clavate and have no massive and refractive apical rings (Hyde et al. 1999a).

Annulatascus joannae K.M. Tsui, I.J. Hodgkiss & K.D. Hyde, sp. nov. Figs. 11–20

Ascomata 180–200 µm alta, 150–250 µm diam, immersa, globosa vel subglobosa, papillata, ostiolata, gregaria, coriacea, nigra. Rostrum cylindricum, 150–200 × 40–60 µm, periphysatum. Paries ascomatis bistratosus, 20 µm crassus; stratum exterius *textura angularis*, materia nigra amorph



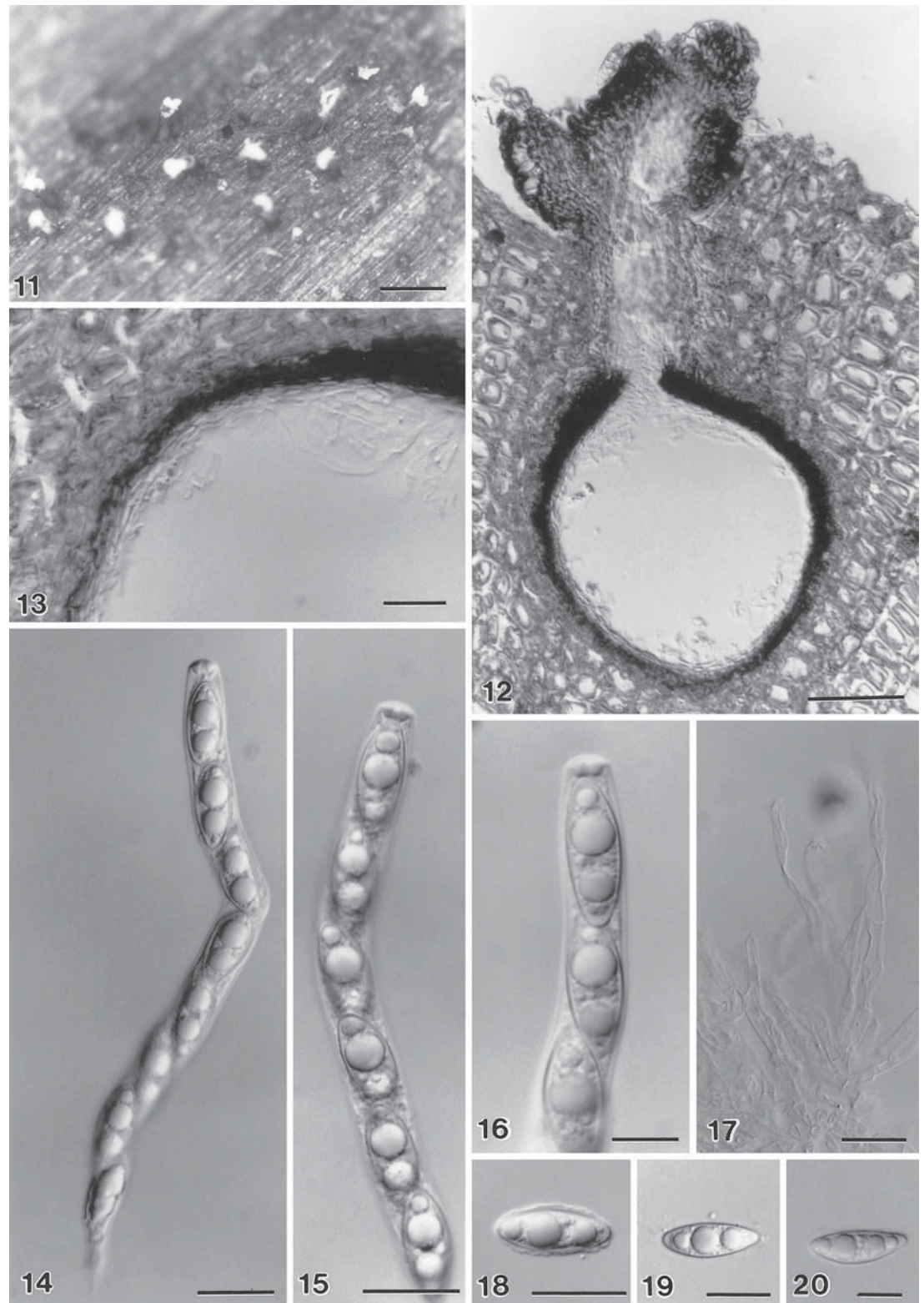
Figs. 1–10. Light (1) and differential interference contrast (2–10) micrographs of *Annulatascus lacteus* (from holotype). 1 Appearance of ascomata on wood. 2 Section of the ascoma. 3 Asci with paraphyses. 4–

7 Ascospores. 8–10 Asci showing apical ring. Bars 1 200 μm ; 2 50 μm ; 3–10 10 μm

completum; stratum interius ex cellulis angularibus brownish compositum. Asci 150–200 \times 10–13 μm , cylindrici, pedicellati, apparatu apicali non amyloidea refractivo 2.5–4 μm alto 5–6.5 μm diam praediti. Ascospores 20–28 \times 9–12 μm , imbricate uniseriatae, ellipsoideae vel fusiformes, crassotunicatae, guttulateae, aseptatae, hyalinae, vagina mucosa tenuicircumdantes.

Ascomata 180–200 μm high, 150–250 μm diam, immersed, globose to subglobose, papillate, ostiolate, gregari-

ous, coriaceous, black (Figs. 11, 12). Beak cylindrical, 150–200 \times 40–60 μm , periphysate (Fig. 12). Peridium \sim 20 μm thick, *textura angularis*, comprising several layers of outer, melanized angular cells, and inner, pallide brunneis angular cells (Figs. 12, 13). Paraphyses \sim 4 μm wide, numerous, septate, unbranched, hyaline, tapering distally (Fig. 17). Asci 150–200 \times 10–13 μm (\bar{x} = 176 \times 11.5 μm , n = 20), 8-spored, unitunicate, thin walled, long cylindrical to broadly cylindrical, pedicellate, with a nonamyloid, refractive apical appa-



Figs. 11–20. Light (11) and differential interference contrast (12–20) micrographs of *Annulatascus joannae* (from holotype). 11 Appearance of ascomata on wood. 12 Section of the ascoma. 13 Section of the peridium. 14, 15 Asci. 16 Closeup of the ascus apex. 17 Paraphyses. 18–20 Ascospores. Bars 11 200 μ m; 12 50 μ m; 13–15, 18–20 20 μ m; 16, 17 10 μ m

ratus, 2.5–4 µm high × 5–6.5 µm wide (Figs. 14–16). Ascospores 20–28 × 9–12 µm (\bar{x} = 24 × 9.5 µm, n = 35), overlapping uniseriate, ellipsoidal to fusiform, thick walled, with two to three large lipid globules, aseptate, hyaline, surrounded by a thin mucilaginous sheath (Figs. 18–20).

Colonies on potato dextrose agar (PDA) slow growing, reaching 3 cm diameter after 1 month. Mycelium white, woolly, dome-shaped with entire edges, reverse dark brown. No fructifications produced.

Etymology: The Latin *joannae*, derived from Dr. Joanna F. Chu, for her encouragement to the first author in post-graduate research and for stimulating his interests in mycology and ecology.

Holotype: China, Hong Kong, Tai Po, Lam Tsuen River, on submerged wood, December 1998, K.M. Tsui, KM 164 (HKU(M) 12177, ex-type culture from the holotype in HKUCC 4370).

Other material examined: China, Hong Kong, Tai Po, Lam Tsuen River, on submerged wood, December 1998, K.M. Tsui, KM164 (HKU(M) 12225).

Notes: *Annulatascus joannae* is different from other species of *Annulatascus* because it has aseptate, ellipsoidal, and thick-walled ascospores. *Annulatascus joannae* shows some resemblance to *A. biatriisporus* in having ascospores surrounded by an inconspicuous mucilaginous sheath. However, *A. biatriisporus* has consistently larger ascospores with swollen ends (Hyde 1995).

Annulatascus tropicalis Ranghoo & K.D. Hyde, sp. nov.

Figs. 21–29

Ascomata 263–275 µm diam, 250–263 µm longa, globosa vel subglobosa, substrato partim immersa, coriacea, atrobrunnea vel atra, aggregata. Rostrum 100–150 × 30–50 µm, periphysatum. Parietis peritheciis 20–30 µm crassus, *textura angularis*, ex cellulis externe 5–7-stratosis elongatis brunneis et interne 2–3-stratosis hyalinis compositus. Asci 190–255 × 12–18 µm, octospori, cylindrici, pedicellati, unitunicati, annulo apicali 3–5 µm alto et 3–5 µm lato distincto refractivo iodo haud coerulescente praediti. Ascospores 42.5–52.5 × 7.5–10 µm, uniseriatae vel imbricate uniseriatae, fusiformes, 1–3-septatae, hyalinae, glabrae.

Ascomata 263–275 µm diameter, 250–263 µm high, globose or subglobose, semiimmersed, coriaceous, black, mostly gregarious (Fig. 21). Beak 30–50 µm diameter, 100–150 µm long, black, periphysate (Figs. 22, 25). Peridium 20–30 µm thick, in surface view of *textura angularis*, in section composed of 5–7 layers of dark brown walled elongated cells with 2–3 inner layers of hyaline cells (Fig. 22). Paraphyses 163–200 × 6–7.5 µm, septate, tapering toward the apex, nonbranched (Fig. 26). Asci 190–255 × 12–18 µm, 8-spored, cylindrical, pedicellate, unitunicate, apically truncate, with a large, nonamyloid, refractive apical ring, 3–5 µm high × 3–5 µm diameter (Figs. 23, 24). Ascospores 42.5–52.5 × 7.5–10 µm, uniseriate to overlapping uniseriate, fusiform, 1–3-septate, not constricted at septum, hyaline, lacking a sheath or appendages (Figs. 27–29).

Etymology: From the Latin *tropicalis*, referring to its discovery in tropical habitats.

Holotype: China: Hong Kong, Tai Po, Plover Cove Reservoir, on submerged wood, January 1997, V.M. Ranghoo (HKU(M) 5253).

Notes: *Annulatascus tropicalis* resembles *A. biatriisporus* and *A. fusiformis* in having long fusiform ascospores (Hyde 1995; Hyde and Wong 2000). *Annulatascus biatriisporus* differs in producing ascospores with bipolar swollen ends, whereas the ascospores in *A. fusiformis* are smaller, 1–5-septate, and have bipolar pad-like appendages. *Annulatascus tropicalis* also resembles *Cateractispora aquatica* K.D. Hyde, S.W. Wong & E.B.G. Jones in having fusiform ascospores, but differs in lacking polar chambers and thread-like apical appendages (Hyde et al. 1999b).

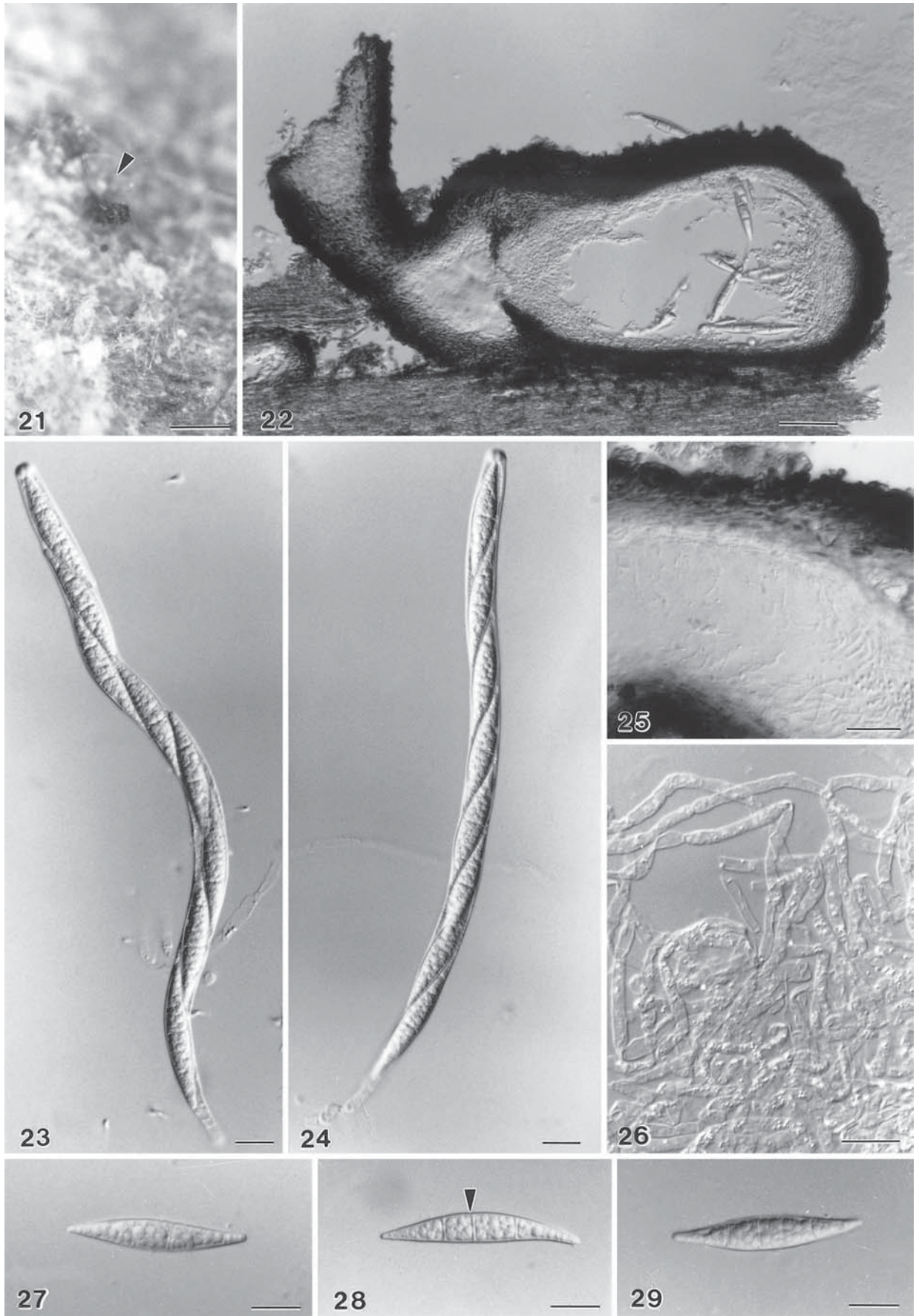
Annulatascus biatriisporus K.D. Hyde, Nova Hedwigia 61:120 (1995).

Material examined: China, Hong Kong, Tsuen Wan, Shing Mun Reservoir, on submerged wood, October 1998, K.M. Tsui and T. Umali, KM 299 (HKU(M) 12310).

Notes: This is the third collection in the tropics and the first report in Hong Kong. The ascospores (48–65 × 7.5–10 µm) were relatively longer than those found in the type description (40–58 × 8–10 µm) (Hyde 1995).

Key to species of *Annulatascus*

1. Ascomata milky, ascospores 24–28 × 6–8 µm, fusiform *A. lacteus*
1. Ascomata black or dark brown 2
2. Ascospores aseptate 3
2. Ascospores septate 8
3. Ascospores small, 15–17.8 × 3.8–5 µm, fusiform-rhomboid, found on palms *A. licualae*
3. Ascospores larger, width often greater than 5 µm .. 4
4. Ascospores ellipsoid to citriform 5
4. Ascospores mostly fusiform 6
5. Ascospores ellipsoid, 20–28 × 9–12 µm .. *A. joannae*
5. Ascospores citriform, 22.5–30.5 × 6.4–8.5 µm, found on palms *A. citriosporus*
6. Ascospores 40–58 × 8–10 µm, long fusiform with swollen ends *A. biatriisporus*
6. Ascospores smaller 7
7. Ascospores 26–42 × 9–12 µm, fusiform, surrounded with thick, conspicuous mucilaginous sheath *A. velatisporus*
7. Ascospores 19–24 × 6–7 µm, fusiform with acute ends, with 2–4 large lipid globules *A. aquatica*
8. Ascospores 16.5–25.5 × 6–9 µm, fusiform, 1–5-septate, with bipolar pad-like appendages *A. fusiformis*
8. Ascospores mostly 1–3-septate, lacking bipolar pad-like appendage, but may be surrounded by a mucilaginous sheath 9
9. Ascospores with mucilaginous sheaths 10
9. Ascospores without mucilaginous sheaths 11
10. Ascospores 35–37.5 × 12.5–15 µm, 3-septate, ellipsoid with acute ends *A. hongkongensis*



Figs 21–29. Light (21) and differential interference contrast (22–29) micrographs of *Annulatascus tropicalis* (from holotype). 21 Appearance of ascoma on wood. 22 Section of an ascoma. 23, 24 Asci. 25 Section of the peridium. 26 Paraphyses. 27–29 Ascospores. Bars 21 200 μ m; 22 35 μ m; 23, 24, 27–29 10 μ m; 25, 26 20 μ m

10. Ascospores $18\text{--}33 \times 6\text{--}12\ \mu\text{m}$, 3-septate, fusiform
 *A. triseptatus*
11. Ascospores $20\text{--}26 \times 6\text{--}7\ \mu\text{m}$, 3-septate, fusiform with
 blunt ends *A. palmietensis*
11. Ascospores relatively large, $42.5\text{--}52.5 \times 7.5\text{--}10\ \mu\text{m}$, 1–3-
 septate, fusiform *A. tropicalis*

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