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Cataractispora receptaculorum, a new freshwater ascomycete from Hong Kong

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Abstract: A new species of *Cataractispora, C. receptaculorum,* is described from freshwater habitats. This species is characterized by triseptate verruculose ascospores and polar appendages that unfurl in water. The ascospores lack polar chambers that enclose the appendages as in *C. bipolaris* and *C. viscosa*. An ultrastructural study of this species revealed that the ascus wall and apical ring of this species is typical of the Annulatascaceae, while the ascospore wall with verruculose ornamentations and the ontogeny of the ascospore polar appendages are similar to the other species of *Cataractispora. Cataractispora receptaculorum* is illustrated with interference light, scanning and transmission electron micrographs.

Key words: Annulatascaceae, aquatic fungi, electron microscopy, spore appendage, taxonomy

INTRODUCTION

During our studies of freshwater fungi in the tropics (Ho et al 1999c, 2001), an undescribed ascomycete was found on wood and bamboo submerged in a reservoir of Hong Kong. In this taxon, ascospores are triseptate and have unfurling polar appendages. Several ascomycete genera from aquatic habitats possess ascospores with unfurling appendages, including *Aniptodera* Shearer et M. Miller, *Cataractispora* S.W. Wong, K.D. Hyde et E.B.G. Jones, *Diluviocola* S.W. Wong, K.D. Hyde et E.B.G. Jones, *Halosarpheia* Kohlm. et E. Kohlm., *Halosphaeria* Linder, *Nais* Kohlm., and *Phaeonectriella* R.A. Eaton et E.B.G. Jones (Kohlmeyer and Kohlmeyer 1979; Kohlmeyer and Volkmann-Kohlmeyer 1991; Shearer 1993; Hyde et al 1997, 1999a, b; Hyde and Pointing 2000).

With the aid of light microscopy this taxon is best placed in the genus *Cataractispora* (Annulatascaceae; Hyde et al 1999b), which is characterized by immersed to superficial, globose, subglobose to ellipsoidal ascomata, cylindrical asci with bipartite apical ring (when seen by a transmission electron microscope [TEM]) and uniseriate, septate, hyaline ascospores with polar appendages that unfurl in water (Hyde et al 1999b). Hyde et al (1999b) described three species of *Cataractispora*: *C. aquatica* K.D. Hyde, S.W. Wong et E.B.G. Jones (type species), *C. appendiculata* K.D. Hyde, S.W. Wong et E.B.G. Jones, and *C. viscosa* K.D. Hyde, S.W. Wong et E.B.G. Jones; and transferred *Annulatascus bipolaris* K.D. Hyde to this genus as *C. bipolaris* (K.D. Hyde) K.D. Hyde, S.W. Wong et E.B.G. Jones. Our taxon also has immersed, globose to subglobose ascomata, cylindrical asci with a prominent apical ring and uniseriate, septate, hyaline ascospores with unfurling polar appendages. We also have examined the fungus with TEM and found that the ontogeny of the polar appendages is similar to that of *C. appendiculata* and *C. aquatica*. This fungus, therefore, is described as a new species of *Cataractispora, C. receptaculorum,* and is illustrated with light and electron micrographs.

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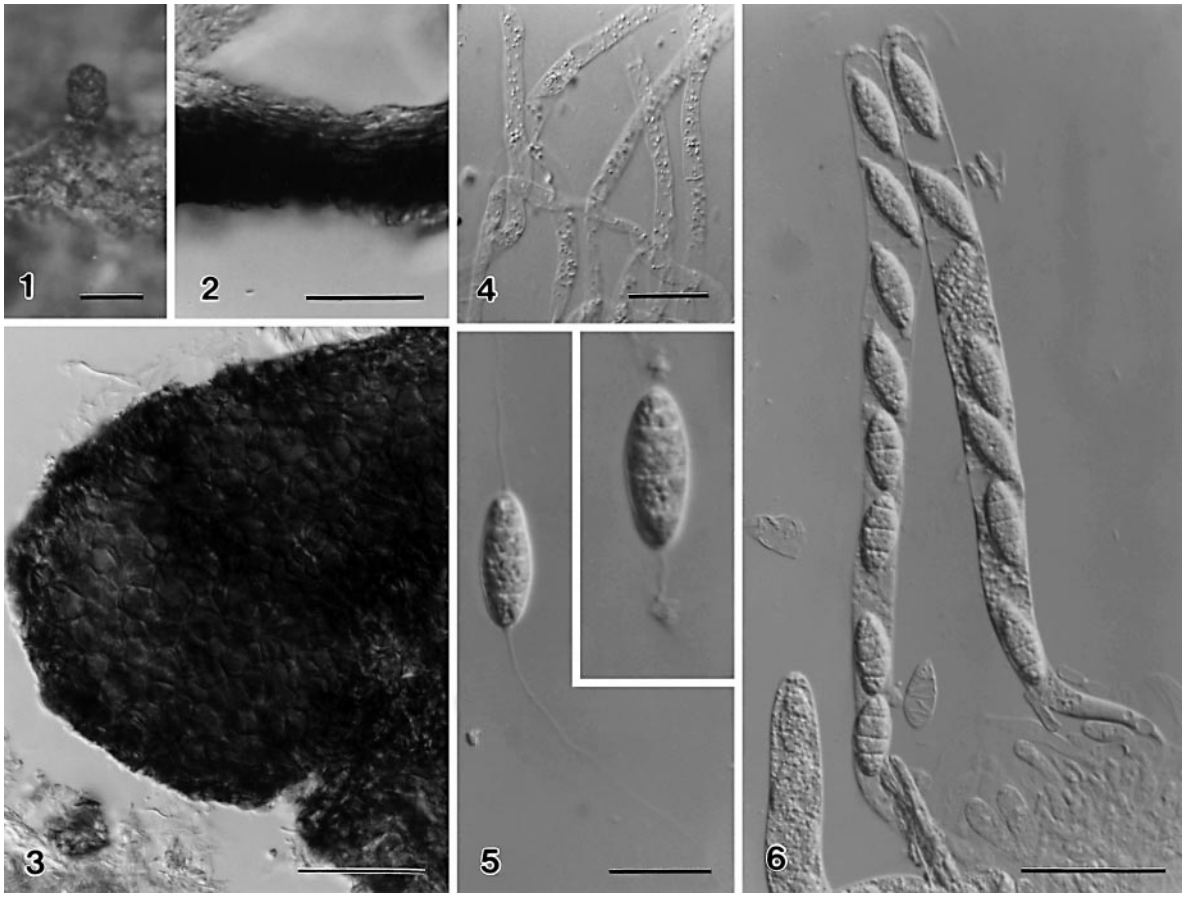
MATERIALS AND METHODS

Submerged wood and bamboo were collected from Plover Cove Reservoir in Hong Kong on 15 Nov 1997. Plover Cove Reservoir was built during the 1960s by joining several islands and a peninsula with dams and replacing seawater with freshwater. Several streams flow into the reservoir. Samples were returned to the laboratory and incubated in plastic boxes lined with moistened paper towels and examined periodically for 3 mo. Squash mounts and sections, using a freezing microtome at −20 C, of fungal fruiting bodies were prepared on slides, mounted with water for measurement and photographed under a differential interference contrast light microscope. Procedures for the preparation of the material examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) follow Ho et al (1999a). All material examined by TEM was fixed with 4% (v/v) glutaraldehyde with ruthenium red for 4 h, and post-fixed with 2% (w/v) OsO_4 at 4 C overnight.

TAXONOMY

Cataractispora receptaculorum W.H. Ho, K.D. Hyde et I.J. Hodgkiss, sp. nov. FIGS. 1–6.

Ascomata 275–300 μm diam, 250–275 μm alta, globosa vel subglobosa, immersa, coriacea, atrobunnea, solitaria, os-



FIGS. 1–6. Interference light micrographs of *Cataractispora receptaculorum*. 1. Ascomatal neck on wood. 2. Longitudinal section of peridium. 3. Squash mount of peridium. 4. Paraphyses. 5. Ascospores with polar appendages unfurling at different stages. 6. Mature asci with eight uniseriate ascospores and an apical ring. Note that the polar appendages of the ascospores are intact and pad-like. Also note the immature ascus and numerous primordial asci at the base of the mature asci. Scale bars: 1 = 0.2 mm, 3 = 100 μm, 2, 4, 5 = 20 μm, 6 = 50 μm.

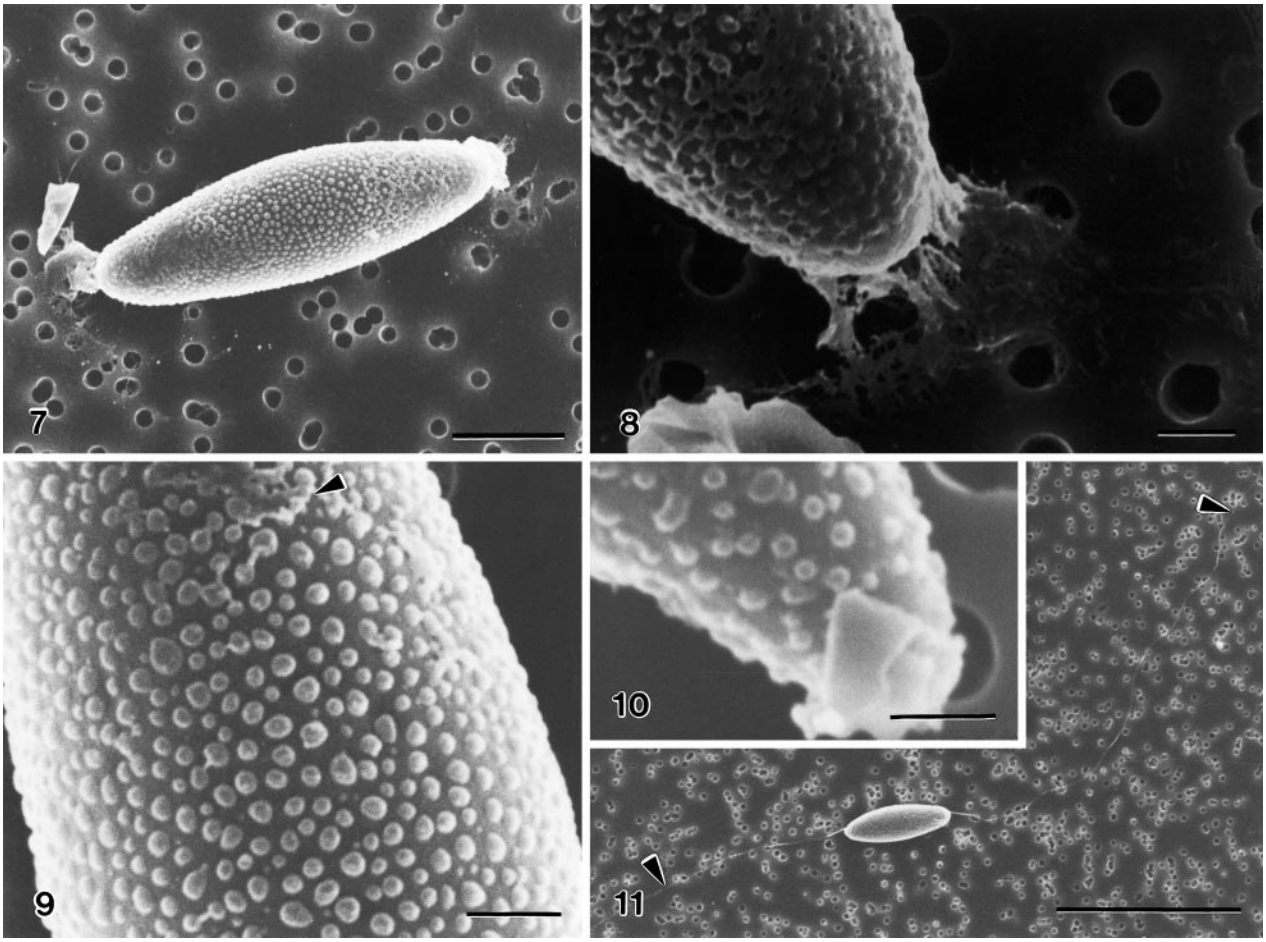
tiolata. *Colla* 113–125 μm diam, 250–275 μm alta, cylindrica, atrobunnea. *Peridium* 17.5–23 μm crassum, textum angulatum. *Paraphysibus* 175–200 × 4–5 μm, septatis, angustatis. *Asci* 213–233 × 15–22.5 μm, octospori, cylindrici, unitunicati, persistenti, pedicellati, apparato apicale praediti. *Ascosporae* 27.5–31 × 7.5–10 μm, uniseriatae vel imbricatae, hyalinae, ellipsoideae, guttulatae, tenuitunicatae, 3-septatae, appendiculatae.

Ascomata 275–300 μm diam, 250–275 μm high, globose to subglobose, immersed, coriaceous, dark brown, solitary, ostiolate (FIG. 1). *Neck* 113–125 μm diam, 250–275 μm long, cylindrical, dark brown (FIG. 1). *Peridium* 17.5–23 μm thick, composed of 2–3 layers of compressed, angular brown pigmented cells and a thin inner layer of light brown compressed cells (FIGS. 2, 3). *Paraphyses* 175–200 × 4–5 μm, septate, persistent, tapering toward the apex (FIG. 4). *Asci* 213–233 × 15–22.5 μm ($\bar{x}$ = 220 × 20 μm, n = 25), 8-spored, cylindrical, unitunicate, persistent, pedicellate, with a large, refractive apical ring (3.8–4 μm diam, 5–7.5 μm long) (FIG. 6). *Ascospores* 27.5–31 × 7.5–10 μm ($\bar{x}$ = 29 × 9.4 μm, n = 25), uniseriate or overlapping uniseriate, hyaline, ellipsoidal, guttulate, thin-walled, 3-septate, with polar pads that unfurl in water into long thin filamentous appendages (FIG. 5).

Colonies on PDA slow growing, attaining 15 mm diam after 6 mo at 24 C, dark greenish gray with pale grayish intervals, undulating in outline, reverse dark brown. Mycelium superficial and immersed in agar, branched, septate, thin-walled and smooth. Aerial mycelium abundant and gray. Fruiting bodies not seen.

Specimen examined. HONG KONG: Plover Cove Reservoir, on bamboo submerged in freshwater, 15 Feb 1997, *V. M. Ranghoo PC38* (HOLOTYPE HKU(M) 5239; HKUCC 3710); *ibid.* on wood submerged in freshwater, 15 Nov 1996, *K. M. Wong et K. D. Hyde PC21* (HKU(M) 4702; HKUCC 1358, 1359).

Etymology. In Latin ''receptaculorum'' means reservoir.



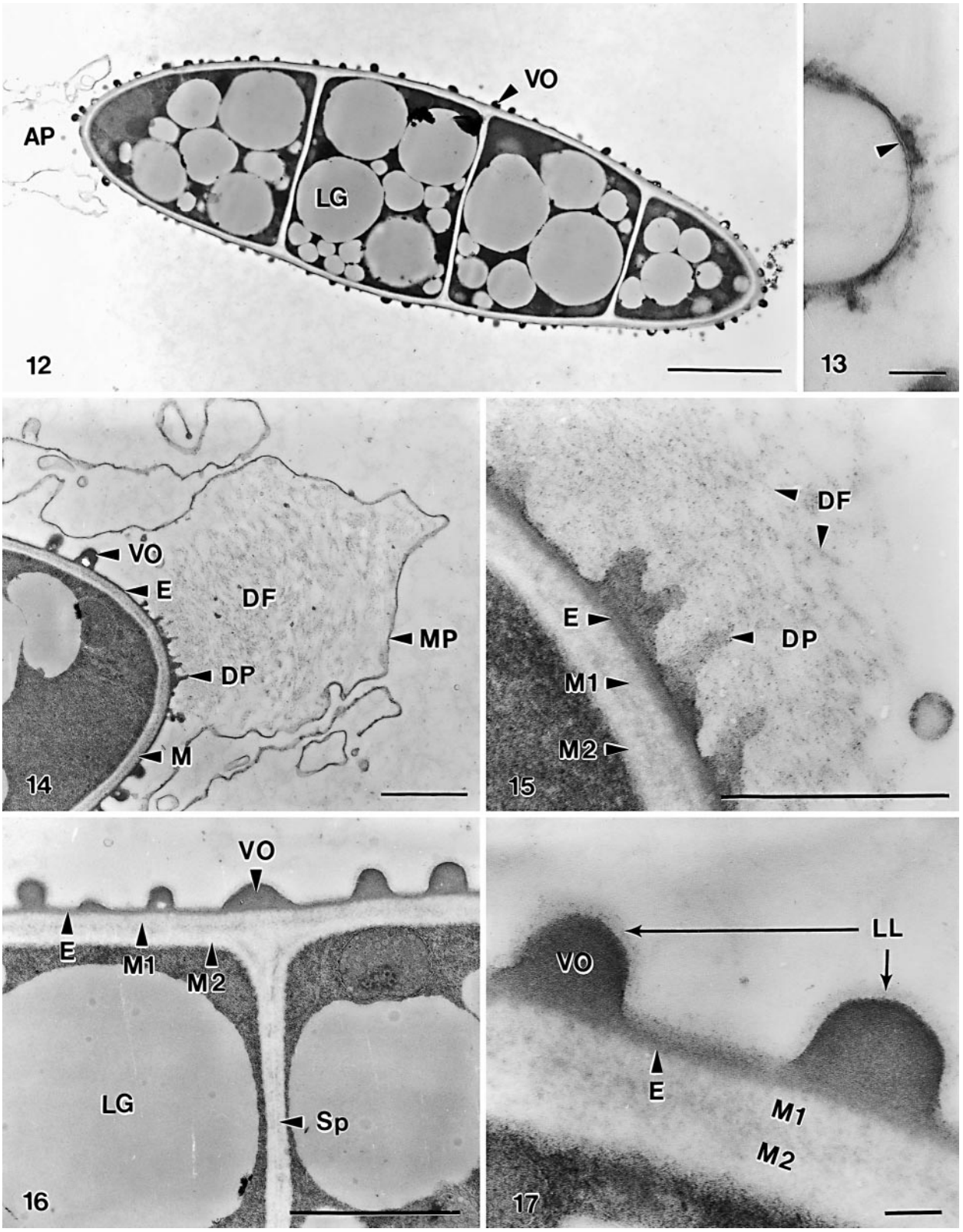
FIGS. 7–11. Scanning electron micrographs of *Cataractispora receptaculorum*. 7. Ellipsoidal ascospore polar appendages. 8. Ascospore tip with an appendage. 9. Globular verruculose wall ornamentations on the surface of ascospore. 10. Folded membrane at the polar region of ascospore. 11. An ascospore with unfurled appendages (arrowheads). Scale bars: 7 = 5 μm, 8–10 = 1 μm, 11 = 50 μm.

Habitat: Saprobic on wood and bamboo submerged in fresh water.

Known distribution: Hong Kong.

Based on SEM observations, mature ascospores of *Cataractispora receptaculorum* are ellipsoidal with polar appendages (FIG. 7). The polar appendages were initially in the form of mucilaginous pads (FIG. 8).The ascospore was evenly coated with hemispherical ornamentations 0.1–0.3 μm diam (FIG. 9). Remnants of the membrane complex mostly were found at the polar regions of the ascospore (FIG. 10). The mucilaginous pad unfurled in water to form long threads measuring up to 200 μm long (FIG. 11).

Based on TEM observations, the mature 3-septate ascospores contained several lipid globules (LG) in each cell and were covered with wall ornamentations (VO) and provided with polar appendages (AP; FIG. 12). Remnants of a folded unit membrane profile (MP) were present at the polar regions, with the polar appendages remaining covered (FIGS. 13, 14). The ascospore wall was two-layered (viz. an outer electron-dense episporium [E; 30–35 nm thick] and an inner, bilamellate electron-transparent mesosporium [M; 120–180 nm thick]) (FIGS. 15–17). The episporium was contiguous with the electron-dense projections (DP) at the polar region, which in turn was contiguous with the electron-dense fibrillar appendage matrix (DF; FIG. 15). The dome-shaped electron-dense wall ornamentations (VO; 150–240 nm diam) also were contiguous with the episporium (FIG. 17). The episporium and the verruculose wall ornamentations were covered with a continuous, less electron-dense layer (LL; 20–30 nm thick; FIG. 17), except at the polar region (FIG. 15). In the bilamellate mesosporium, the boundary between the outer mesosporium layer (M1, 70–90 nm thick) and the inner mesosporium layer (M2, 110–125 nm thick) was electron dense (FIGS. 15–17). The septa (Sp; 130–150 nm thick) were continuous with the inner mesosporium (M2) of the periclinal spore wall (FIG. 16).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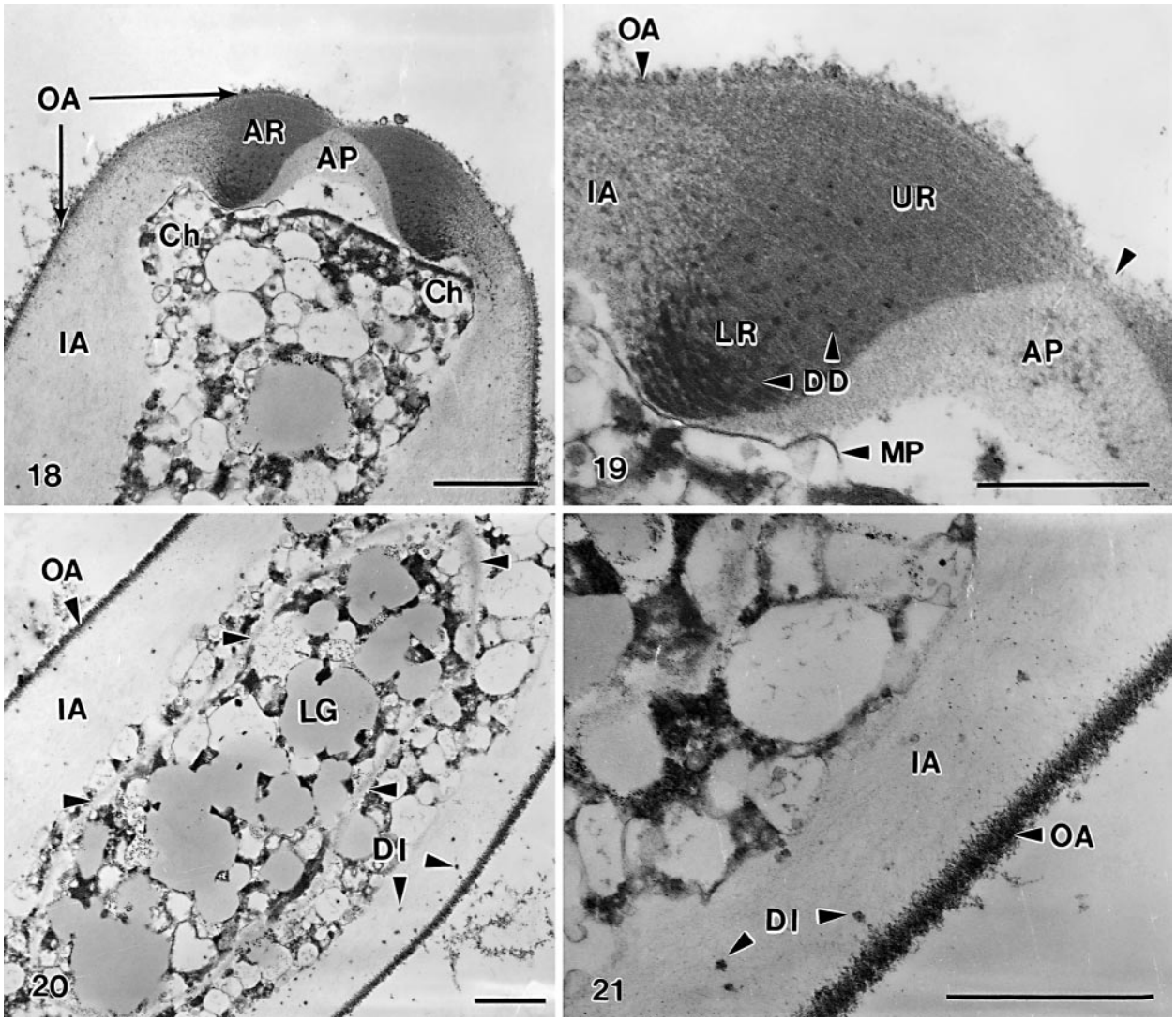

FIGS. 18–21. Transmission electron micrographs of the longitudinal sections of asci of *Cataractispora receptaculorum*. Labels: Apical plug (AP), apical ring (AR; lower portion: LR; upper portion: UR), subapical chamber (Ch), electron-dense deposits (DD), electron-dense inclusion (DI), ascus wall layer (inner layer: IA; outer layer: OA), lipid globule (LG), membrane profile (MP). 18. Oblique, median section of the apical region of an ascus. 19. Higher magnification of the ascus apical ring in FIG. 18. Note that the outer ascus wall layer is discontinuous and absent at the central region of the ascus tip (arrowhead). 20. Immature ascus with a primordial ascospore (arrowheads). 21. Mature ascus. Scale bars: 18, 20, 21 = 1 μm, 19 = 0.5 μm.

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FIGS. 12–17. Transmission electron micrographs of the longitudinal sections of the ascospores of *Cataractispora receptaculorum*. Labels: Polar appendage (AP), electron-dense fibrillar appendage (DF), electron-dense projections (DP), episporium (E), lipid globule (LG), less electron-dense layer (LL), mesosporium (M; outer mesosporium: M1; inner mesosporium: M2), membrane profile (MP), septum (Sp), verruculose wall ornamentations (VO). 12. A triseptate mature ascospore. 13. Unit membrane (arrowhead) that covers the ascospore appendage. 14. Polar region of an ascospore. 15. A higher magnification of the polar region of the ascospore in FIG. 14. 16. Middle region of an ascospore. 17. Higher magnification of the verruculose wall ornamentations in FIG.16. Scale bars: 12 = 5 μm, 13, 17 = 0.1 μm, 14, 16 = 1 μm, 15 = 0.5 μm.

The ascus wall was bilamellate, comprising an outer electron-dense layer (OA; 110–120 nm thick) and an inner electron-transparent layer (IA; 700–1200 nm thick) (FIGS. 18, 19). The inner ascus wall layer narrows beneath the apical ring (AR) and forms a subapical chamber (Ch; FIG. 18). Abundant electron-dense fibrillar material and electron-dense inclusions (DI; 35–55 nm diam) were embedded in the inner wall layer in immature asci (FIG. 20). Fewer electron-dense inclusions were found in mature asci (FIG. 21). The outer wall layer comprised tightly packed electron-dense fibrils oriented perpendicular to the ascus wall (FIGS. 18, 21). The outer ascus wall layer was discontinuous at the ascus apical region and absent at the opening of the apical ring (FIG. 19).

The apical ring (AR) was cylindrical (1.2 μm long, 2.5 μm diam, with 0.7 μm thick rim) and bipartite (FIG. 18). The upper portion of the ascus ring (UR) contained horizontally oriented electron-dense fibrillar material; the lower portion of the ascus ring (LR) contained similar horizontally oriented electron-dense fibrillar material with electron-opaque granular deposits (DD; FIGS. 18, 19). Both the upper and the lower portions of the ascus ring were continuous with the inner ascus wall layer (FIGS. 18, 19). An electron-transparent apical plug (AP; 1.5 μm diam at the base) was found within the channel of the apical ring (FIGS. 18, 19). The plug was thickest (0.5 μm) beneath the opening of the apical ring and became gradually thinner toward the base of the apical ring (FIG. 19). Unit membrane profiles (MP) were present at the interface between the epiplasm and at both the inner ascus wall layer at the subapical chamber and the apical plug (FIGS. 18, 19).

DISCUSSION

Genera of the Annulatascaceae are characterized by asci with a bipartite apical apparatus, ascospores with verruculose wall ornamentations and appendages or sheaths (Ho and Hyde 2000). In *Annulatascus,* ascospores are surrounded by a mucilaginous sheath (Wong et al 1999b, Ho et al 1999a, b). In *Diluviocola,* ascospores have polar conical caps that detach in water and thread-like appendages unfurl from within the cap. Ascospore appendages of *Diluviocola capensis* comprise electron-dense rod-like fibrils that are derived from exosporial material (Hyde et al 1998). In *Fluminicola,* ascospore appendages are crown-like and comprise electron-dense amorphous material that is formed from the mesosporium at the ascospore tips (Wong et al 1999a). In *Pseudoproboscispora* S.W. Wong & K.D. Hyde (as *Proboscispora* in Wong and Hyde 1999), ascospore appendages are at first coiled, proboscis-like at each end and then uncoil in water to form long threads. The ascospore appendages of *Pseudoproboscispora* comprise electron-dense amorphous material and electron-transparent zones and are not contiguous with the episporium as those of *Cataractispora* species. In species of *Cataractispora,* the appendage precursor material accumulates within polar chambers, cells or membrane. The appendages unfurl readily in water and do not coil as a proboscis. The ascospore appendages of *Cataractispora* comprise electron-dense fibrillar material that are contiguous with the episporium (Hyde et al 1999b, Ho and Hyde 2000).

Cataractispora receptaculorum is best included with other species in the genus *Cataractispora* because they share similar morphological characters of ascomata, paraphyses, asci and ascospores when viewed with a light microscope and the ultrastructural details of the asci and ascospores when viewed with an electron microscope l (Hyde et al 1999b, Ho and Hyde 2000).

Cataractispora receptaculorum can be distinguished from other *Cataractispora* species without difficulty with a light microscope. *Cataractispora receptaculorum* differs from *C. bipolaris* (0-septate) and *C. viscosa* (5-septate) in ascospore septation and differs from *C. aquatica* and *C. appendiculata* mainly in lacking ascospore polar chambers. The presence or absence of ascospore polar chambers is not considered a criterion in the delineation of ascomycetes at the generic level.

C. aquatica, C. appendiculata and *C. receptaculorum* also are similar when viewed with an electron microscope. Similarities include thickness of ascus and ascospore wall layers, sequence of formation of ascospore wall layers, ontogeny of wall ornamentations and ascospores appendages. The taxonomic significance of the ascus apical plug, which is present in the three *Cataractispora* species examined, is not known. However, the ontogeny of ascospore appendages is considered taxonomically significant generically and specifically (Moss and Jones 1977; Jones et al 1986; Jones and Moss 1987; Yusoff et al 1993a, b, 1994a, b, 1995; Jones 1995). In short, the similarity of the ultrastructural details, especially the ontogeny of ascospore appendages, together with the similarity of the morphological characters in *Cataractispora,* confirm its being a well-delineated genus.

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