

Ascomycetes from freshwater habitats: *Ascolacicola aquatica* gen. et sp. nov. and a new species of *Ascotaiwania* from wood submerged in a reservoir in Hong Kong

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Abstract: *Ascolacicola aquatica* gen. et sp. nov., and *Ascotaiwania mitriformis* sp. nov., from decaying submerged wood collected in the Plover Cove Reservoir in Hong Kong are described and illustrated. *Ascolacicola aquatica* has characteristics that are common to both *Ascotaiwania* and *Savoryella*, but the anamorph is *Trichocladium uniseptatum*. A new genus is therefore introduced to accommodate this fungus. *Ascotaiwania mitriformis* sp. nov. is typical of the genus and differs from other species in having large brown fusiform ascospores with mitriform end cells. Its anamorph is an undescribed species of *Monotosporella* and both states of the fungus are described and illustrated.

Key Words: Ascomycetes, anamorph, lignicolous fungi, Sordariales, systematics

INTRODUCTION

During examination of freshwater fungi occurring on submerged wood in the Plover Cove Reservoir, The New Territories, Hong Kong, we identified two new species of ascomycetes with brown, septate ascospores which had paler end cells. Both fungi resemble species of *Ascotaiwania* Sivan. & H. S. Chang. However in pure cultures obtained from single ascospores, one fungus produced a *Trichocladium uniseptatum* Berk. & Broome anamorph, while the other produced a *Monotosporella* S. Hughes, anamorph. *Monotosporella* has previously been shown to be the anamorph of *Ascotaiwania sawada* H. S. Chang & Y. S. Hsieh (Sivichai et al., 1998). This latter species with large brown fusiform ascospores and hyaline mitriform end cells conforms to *Ascotaiwania*, and is therefore described as a new species, *A. mitriformis*. The other species has several characters atypical for *Ascotaiwania*; including ascomata with relatively short necks, filamentous, persistent paraphyses, asci with discoid refractive apical

rings, relatively small ellipsoidal ascospores and a *Trichocladium* anamorph. We therefore introduce a new genus to accommodate it.

MATERIALS AND METHODS

Submerged wood was collected from the Plover Cove Reservoir and returned to the laboratory in sterile plastic bags. Samples were incubated in moist plastic boxes at room temperature and examined periodically over 2–3 mo. Squash mounts of ascomata in water were made for microscopic examination. Measurements of ascomata were made from thin sections or whole mounts in water. The contents of the ascomata were removed from the ascomata and a spore suspension was made with sterile distilled water in a watch-glass and the suspension was pipetted onto potato dextrose agar (PDA). The single ascospores were allowed to germinate (ca 2 d) and then they were transferred to smaller PDA plates for further growth.

DESCRIPTION

***Ascolacicola* Ranghoo et K. D. Hyde, gen. nov.**

Ascomata superficialia, subglobosa, nigra, ostiolata, papillata, paraphysata, solitaria vel gregaria. Asci octospori, cylindrici, breviter pedicellati, unitunicati, J- apparatus apicale praediti. Ascospores uniseriatae, ellipsoideae, hyalinae, 4-cellulatae, cellulis centralibus brunneis, cellulis polaribus hyalinis vel pallide brunneis. Status anamorphosis: *Trichocladium uniseptatum*.

Ascomata superficial, subglobose, black, coriaceous, solitary to gregarious and beaked. Peridium comprising several layers of brown flattened angular cells. Paraphyses filamentous, hyaline, numerous, flexuous, septate, rounded at the ends. Asci 8-spored, cylindrical, short pedicellate, unitunicate, apically rounded, with a J-, refractive, discoid, apical ring. Ascospores overlapping uniseriate, ellipsoidal, 4-celled, not constricted at the septa, brown with hyaline to pale brown end cells, smooth walled. Anamorph: *Trichocladium uniseptatum*.

Etymology. From the Latin *lacus* meaning “reservoir” and *cola* meaning “dwelling.”

Species typica. *Ascolacicola aquatica* Ranghoo et K. D. Hyde.

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Ascolacicola aquatica Ranghoo et K. D. Hyde, sp. nov. FIGS. 1–11

Ascomata 250–375 μm alta, 225–275 μm diam, superficialia, subglobose, nigra, ostiolata, papillata, paraphysata, solitaria vel gregaria. Peridium 18–27.5 μm crassum. Asci 125–163 \times 10–12.5 μm , 8-spori, cylindrici, breviter pedicellati, apparato apicale 3.75 alto, 6.25 μm diam, praediti. Ascosporeae 12.5–16.5 \times 4–7.5 μm , uniseriatae imbricatae, ellipsoideae, 4-cellulatae, cellulis centralibus brunneis, cellulis polaribus hyalinis vel pallide brunneis.

Ascomata 250–375 μm high, 225–275 μm diam, superficial, subglobose, black, coriaceous, solitary to gregarious. Neck 175–200 μm high, 62.5–75 μm diam (FIGS. 1, 2), periphysate, beaked, black. Peridium 18–27.5 μm wide, comprising of 5–7 layers of brown flattened angular cells (FIG. 3). Paraphyses 175–200 \times 2.5–3.8 μm , filamentous, hyaline, numerous, septate, apically rounded (FIG. 7). Asci 125–163 \times 10–12.5 μm , 8-spored, cylindrical, short pedicellate, apically rounded, with a J-, refractive, discoid, apical ring, 2.5–3.7 μm high, 6.2 μm diam (FIGS. 4–6, 8). Ascospores 12.5–16.5 \times 4–7.5 μm (mean = 15.9 \times 5.6 μm , n = 50), uniseriate, ellipsoidal, 4-celled, not constricted at the septa, brown with hyaline to pale brown end cells, smooth walled (FIGS. 9–11).

HOLOTYPE. HONG KONG. THE NEW TERRITORIES: The Plover Cove Reservoir, on submerged wood, March 1997, V. M. Ranghoo (HKU(M) 5243).

Additional material examined. HONG KONG. THE NEW TERRITORIES: Lam Tsuen River, Tai Po, on submerged wood, July 1997, K. M. Tsui (HKU(M) 5542).

Etymology. In reference to the freshwater habitat where the species was collected.

Habitat. Saprobic on submerged wood.

Known distribution. Hong Kong.

Description of anamorph. Colonies on PDA reaching 1 cm diam after 1 mo incubation at room temperature (25 C), with dark gray cottony mycelium, producing gray aerial mycelium on the surface, growing mainly in concentric rings. Black pigment diffusing into the agar, anamorph produced in culture.

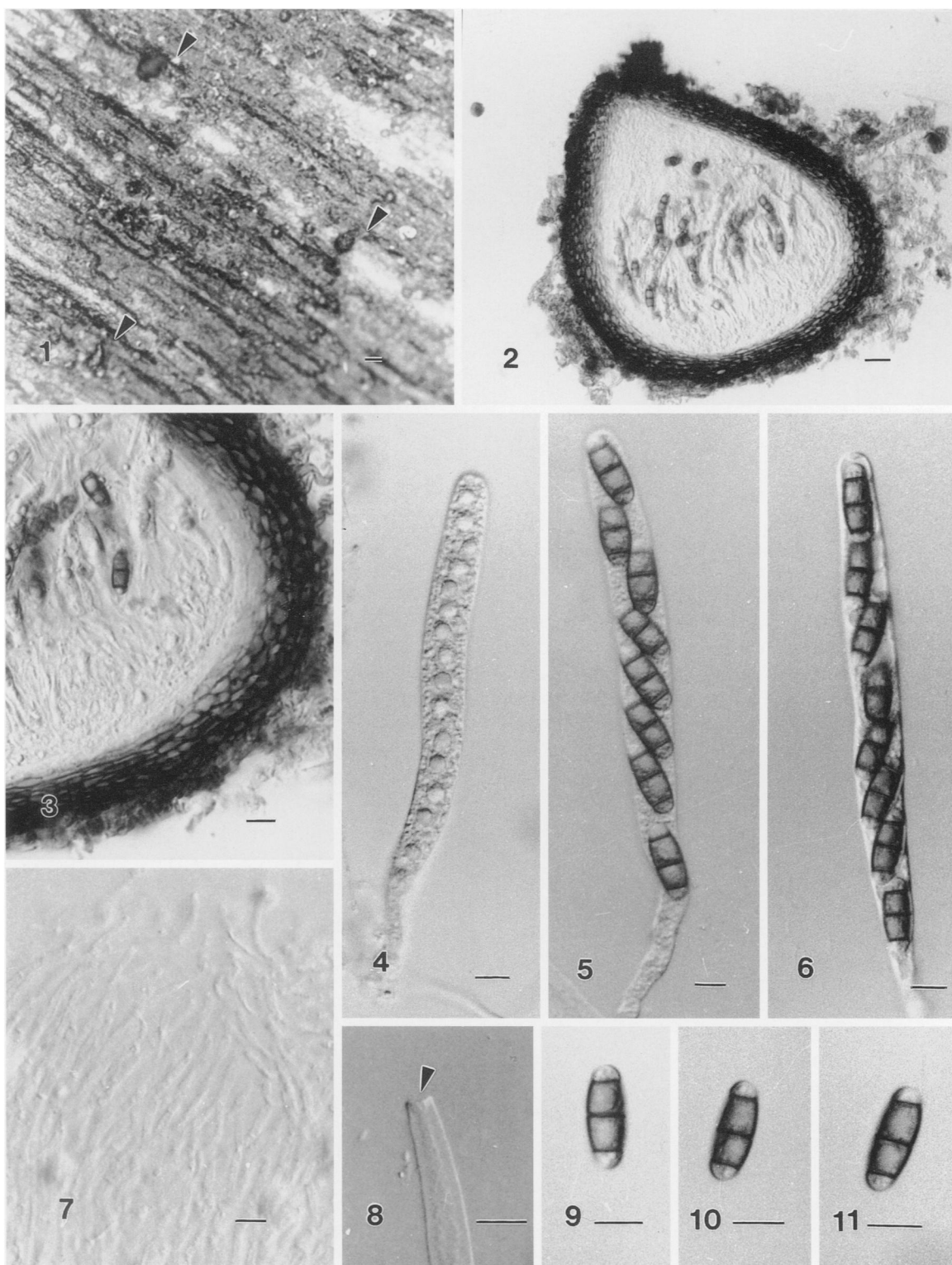
Trichocladium uniseptatum Berk. & Broome (Hughes and Pirozynski, 1972) FIGS. 12–21

Vegetative hyphae partly superficial and partly immersed, branched and septate, not constricted at the septa, hyaline, 1–3 μm wide (FIGS. 12–14). Conidigenous cells thin-walled, hyaline, sometimes thickened and pigmented near the base, subglobose, ampulliform, or irregularly cylindrical, 5–7 \times 5–8 μm (mean = 6 \times 7 μm , n = 50), bearing a single terminal conidium (FIGS. 15, 16, 18, 19). Chlamydo-

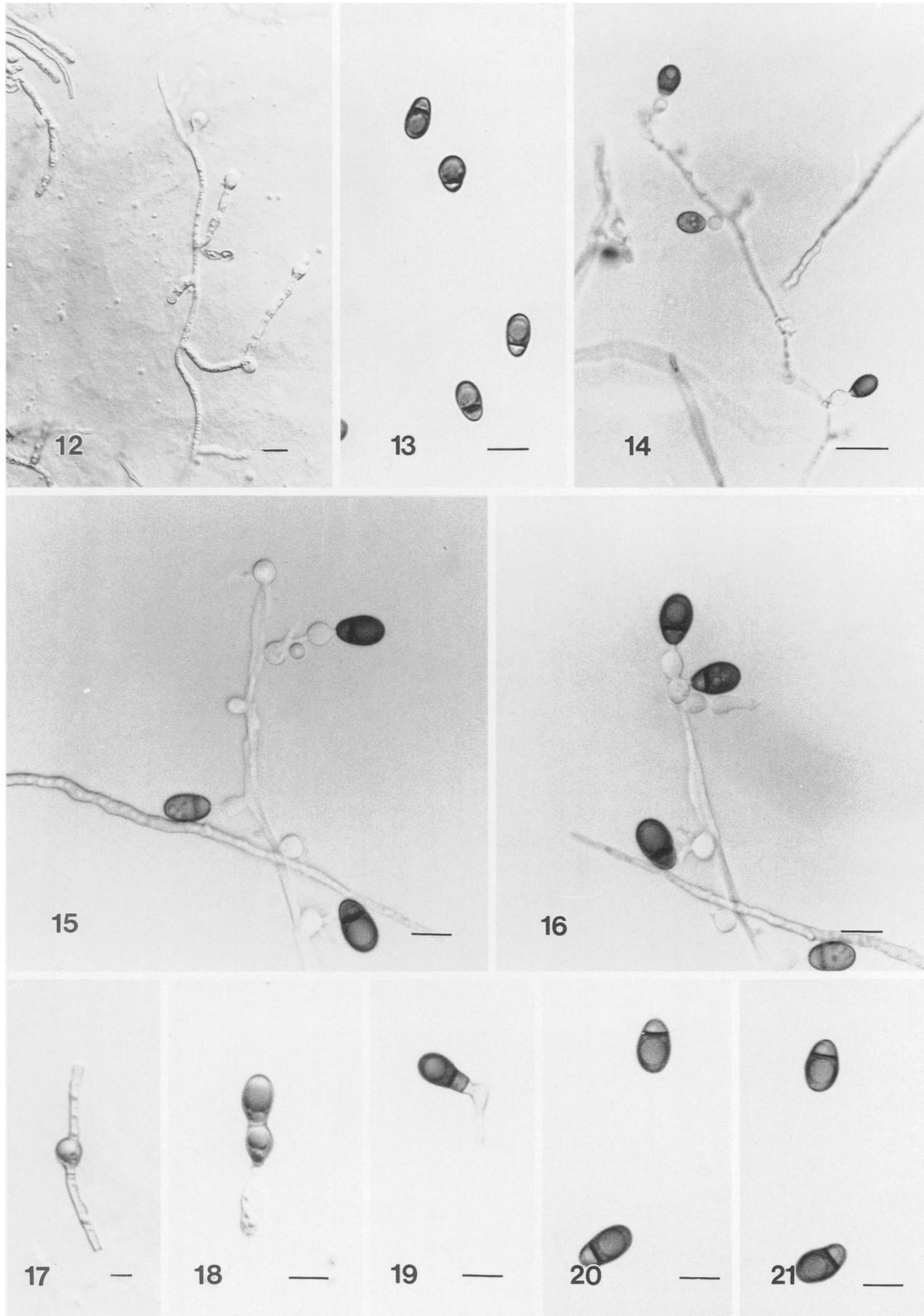
spores produced in culture (FIG. 17). Conidia broadly obovoid, 1-septate near the base, slightly constricted at the septa when mature, 3.8–6.3 \times 6.5–10 μm (mean = 5 \times 7.5 μm , n = 50) with the upper cell rounded, thick-walled, dark-brown (FIGS. 20, 21).

Discussion. *Ascotaiwania* and *Savoryella* E. B. G. Jones & R. A. Eaton are freshwater ascomycete genera with unitunicate asci producing brown ascospores with hyaline end cells. Four other terrestrial genera of unitunicate ascomycetes producing the same type of ascospores are *Ascocodinaea* Samuels, *Botanamphora* Nogrased & Scheuer, *Melanamphora* Lafl., and *Melanochaeta* E. Müll., Harr & Sulmont. These genera can easily be separated from *Ascolacicola* using several important morphological characters, including the presence or absence of particular anamorphs, and these are shown in TABLE I.

Ascolacicola aquatica has some characters that are typical of *Ascotaiwania*; namely immersed ascomata; a peridium of brown walled flattened cells; cylindrical asci; and brown septate ascospores with hyaline end cells. *Ascolacicola aquatica*, however, differs in having ascomata with shorter necks, asci with discoid apical rings which are relatively flattened as compared to the large bipartite refractive apical rings typical of the genus *Ascotaiwania* (Sivanesan and Chang, 1992; Chang et al., 1998). The large refractive apical ring in the type species of *Ascotaiwania*, *A. lignicola* described by Sivanesan and Chang (1992) was one of the characteristics which distinguished it from other related genera and the taxonomic placement of *Ascotaiwania* at the family level was also based on this morphological character. Later studies carried out on the large bipartite ring at the ultrastructural level as described by Chang et al. (1998) gave evidence for the transfer of *Ascotaiwania* to the Annulatascaceae, a family comprising genera with similar apical rings and ascospore morphology (Wong et al., 1998). The flattened apical ring of *Ascolacicola* is typical of the Sordariales (Barr, 1990) but distinctly different from that of *Ascotaiwania* and until now ascal apical ring structure variability has not been considered as a variable morphological character of the genus *Ascotaiwania* (Chang et al., 1998). Moreover, the ascospores in *A. aquatica* are relatively small, lighter in color and have rounded end cells. The paraphyses in *A. aquatica* are also thin, filamentous, being numerous and persistent, as compared to the sparse and early deliquescing paraphyses which are typical of *Ascotaiwania* (Sivanesan and Chang, 1992; Chang et al., 1998). Until now only 2 species of *Ascotaiwania* have been reported to produce the *Monotosporella* anamorph in culture (Sivichai et al., 1998) and the anamorph *Trichocladium uniseptatum* produced in culture of *Asco-*



FIGS. 1-11. *Ascolacicola aquatica* (from HKU(M) 5243). 1. Ascomata on wood (arrowed). 2. Section through ascoma. 3. Peridium consisting of 5-7 layers of brown flattened angular cells. 4. Ascus with immature hyaline ascospores. 5, 6. Mature asci with brown ascospores. 7. Numerous thin, filamentous paraphyses. 8. Ascus apical ring (arrowed) which is discoid. 9-11. Ascospores with rounded end cells. Scale bars: 1 = 200 μm , 2 = 20 μm , 3, 4 = 10 μm , 5-7 = 100 μm , 8-11 = 10 μm .



FIGS. 12–21. *Trichocladium uniseptatum*. 12. Developing conidia. 13. Conidia. 14–16. Mature conidia with attached conidiogenous cells. 17. Chlamyospore produced in culture. 18. Conidium produced in chains. 19. Conidium attached to conidiogenous cell. 20, 21. Fully developed conidia. Scale bars: 12–14 = 10 μm , 15–21 = 5 μm .

TABLE I. A synopsis of the genera of unitunicate ascomycetes which produce brown ascospores with hyaline end cells

Genus	Ascomatal characters	Ascus apical ring	No. of septa in ascospore	Anamorph	Family
<i>Ascocodinaea</i>	Presence of sterile hyphae, no stroma	J-, discoid	3	<i>Codinaea</i>	Lasiosphaeriaceae
<i>Ascolacicola</i>	Beaked, no stroma present and small neck	J-, 2-3.5 × 5-6 μm, discoid	3	<i>Trichocladium</i>	Lasiosphaeriaceae
<i>Ascotaiwania</i>	No stroma, long and periphysate neck	J-, 5-7 × 6-10 μm, bipartite	3-7	<i>Monotosporella</i>	Annulatascaceae
<i>Botan amphora</i>	Reduced stroma present	J+, thin, discoid	5	Unknown	Melanconidaceae
<i>Melan amphora</i>	Well developed stroma present	J+, 3 × 5-6 μm, discoid	7	<i>Cytosporina</i>	Melanconidaceae
<i>Melanochaeta</i>	Basal stroma present	J+, thin, discoid	3-5	<i>Sporoschisma</i>	Lasiosphaeriaceae
<i>Savoryella</i>	No stroma, relatively long periphysate neck	J-, 1.5-2 × 4-5 μm, discoid	3	Unknown	Lasiosphaeriaceae

laticola leaves no doubt in our minds that *Ascolacicola* and *Ascotaiwania* are distinct genera.

Ascolacicola aquatica shares some features with the genus *Savoryella*; namely the discoid apical ring and the similar shape of the 3-septate ascospores (Ho et al., 1997). The ascospore end cells in *A. aquatica*, however, are hyaline to pale brown, and differ in shape from those found in species of *Savoryella*, which are hyaline, comparatively smaller and are more constricted at the septa. The paraphyses in the genus *Savoryella* are also sparse, while in *A. aquatica* they are numerous, flexuous and filamentous.

Ascolacicola aquatica differs from species of *Botan amphora* in having generally smaller ascomata which are not embedded in a stroma, smaller asci, and ascospores which are 5-septate and more elongate. It also differs from species of *Melan amphora* in having ascomata which are not embedded in any type of stromatic tissue. *Melan amphora* species also have ascospores which are 5- and 7-septate, larger, curved and cylindrical in shape, and asci which are considerably larger. *Melan amphora* species also differ from *Ascolacicola* in that they produce a *Cytosporina* Sacc. anamorph.

Ascolacicola aquatica also resembles *Melanochaeta* in having multiseptate brown ascospores with hyaline end cells. However, the absence of setae or hair like structures on the ascomata, the shape of the ascospores and the absence of a *Sporoschisma* Berk. & Broome anamorph eliminates *A. aquatica* from the genus (Goh et al., 1997).

Ascolacicola aquatica can also be compared with *Ascocodinaea* Samuels. *Ascolacicola* resembles *Ascocodinaea* in having 3-septate brown ascospores, but differs

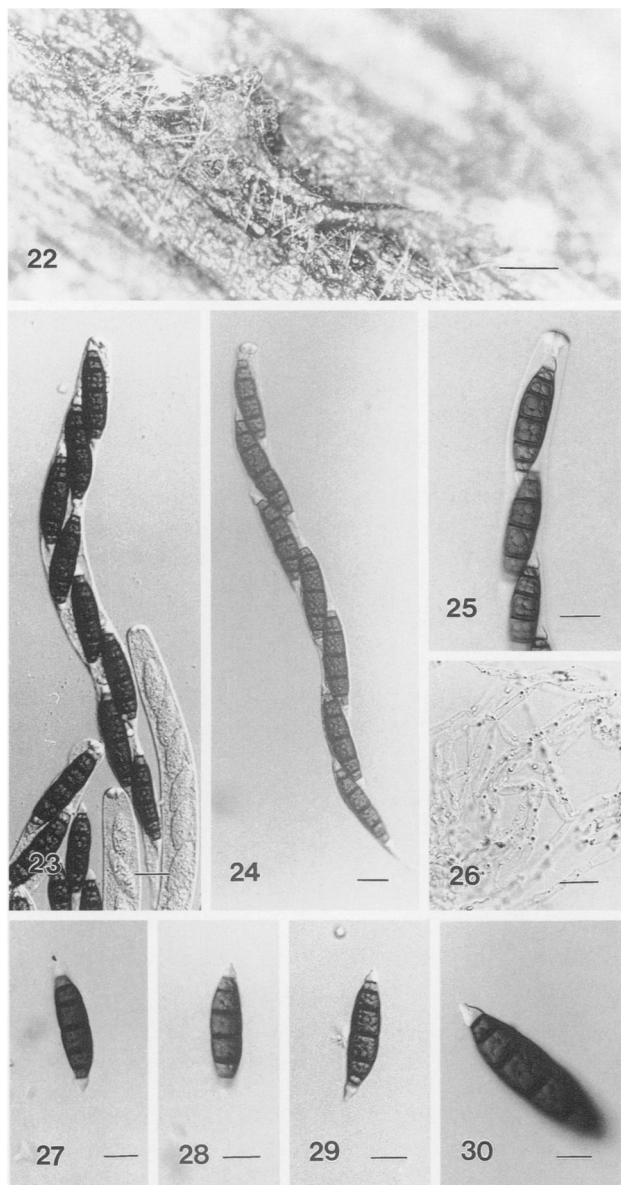
in lacking setae on the ascomata. The *Codinaea* Maire anamorph also eliminates *Ascolacicola* from the genus (Samuels et al., 1997).

Ascolacicola is best placed in the Lasiosphaeriaceae, Sordariales (sensu Barr, 1990) due to the presence of a combination of morphological characters typical of the family. These include dark, membranous ascomata, long cylindrical asci having apical rings, and a centrum interspersed with paraphyses (Barr, 1990).

***Ascotaiwania mitriformis* Ranghoo et K. D. Hyde, sp. nov.** FIGS. 22-30

Ascomata 400-450 μm alta, 500-560 μm diam, semi-immersa, subglobosa, nigra, ostiolata, papillata, paraphysata, solitaria vel gregaria. Asci 325-375 × 25-35 μm, octospori, cylindrici, breviter pedicellati, unitunicati, apparato apicale 7.5-10.0 μm alto, 12.5-15.0 μm diam, praediti. Ascosporae 62.5-72.5 × 12.5-17.5 μm (mean = 66.4 × 15.1 μm, n = 25), uniseriatae imbricatae, ellipsoideae, 7-septatae, cellulis centralibus brunneis, cellulis polaribus hyalinis vel pallide brunneis.

Ascomata 400-450 μm high, 500-560 μm diam, semi-immersed, subglobose, black, coriaceous, solitary to gregarious (FIG. 22). Neck 450-500 μm high, 125-150 μm diam, black, periphysate. Peridium 20-45 μm, composed of 5-7 layers of brown angular cells. Paraphyses 250-325 × 3.8-5 μm, hypha like, septate, branched and tapering towards the apex (FIG. 26). Asci 325-375 × 25-35 μm, 8-spored, cylindrical, short pedicellate, apically rounded with a relatively massive, J- apical ring, 7.5-10 μm high, 12.5-15 μm wide (FIGS. 23-25). Ascospores 62.5-72.5 × 12.5-17.5 μm (mean = 66.4 × 15.1 μm, n = 25),



FIGS. 22–30. *Ascotaiwania mitrifomis* (from HKU(M) 5224). 22. Superficial ascoma on wood. 23, 24. Mature asci. 25. Apical ring of ascus. 26. Deliquescing paraphyses. 27–29. Ascospores. 30. Close-up of an ascospore illustrating the mitriform end cell. Scale bars: 22 = 100 μm , 23–30 = 20 μm .

overlapping uniseriate, fusiform, 7-septate, not constricted at the septa, brown with hyaline end cells which are mitriform in shape, smooth-walled (FIGS. 27–30).

HOLOTYPE. HONG KONG. THE NEW TERRITORIES: The Plover Cove Reservoir, on submerged wood, 26 Jan. 1997, V. M. Ranghoo (HKU(M) 5224).

Etymology. From the Latin, *mitrifomis* in reference to the mitriform end cells of the ascospores.

Habitat. Saprobic on submerged wood.

Known distribution. Hong Kong.

Description of anamorph. Colonies on PDA immersed, reaching 1 cm diam after 1 mo at room temperature (25 C), with a dense mass of gray cottony aerial hyphae, with immersed hyphae grayish black and gray aerial hyphae, growing in concentric rings. Hyphae causing a black pigmentation in the surrounding medium.

Monotosporella state of *Ascotaiwania mitrifomis*

FIGS. 31–34

Conidiophores erect, pale brown to brown, unbranched, 1- to 3-septate, 25–75 \times 6.25–8.75 μm (mean = 50 \times 7.5 μm , n = 25), with 2–4 percurrent proliferations (FIGS. 31, 32). Conidiogenous cells erect, dark brown to black, septate, smooth and thick walled. Conidia 10–20 \times 30–35 μm (mean = 15 \times 32.5 μm , n = 25), obovate to oval, solitary, dry, smooth, thick walled, 1–3 septate, basal cell dark-brown, upper cell black, slightly constricted at the septa (FIGS. 31–34).

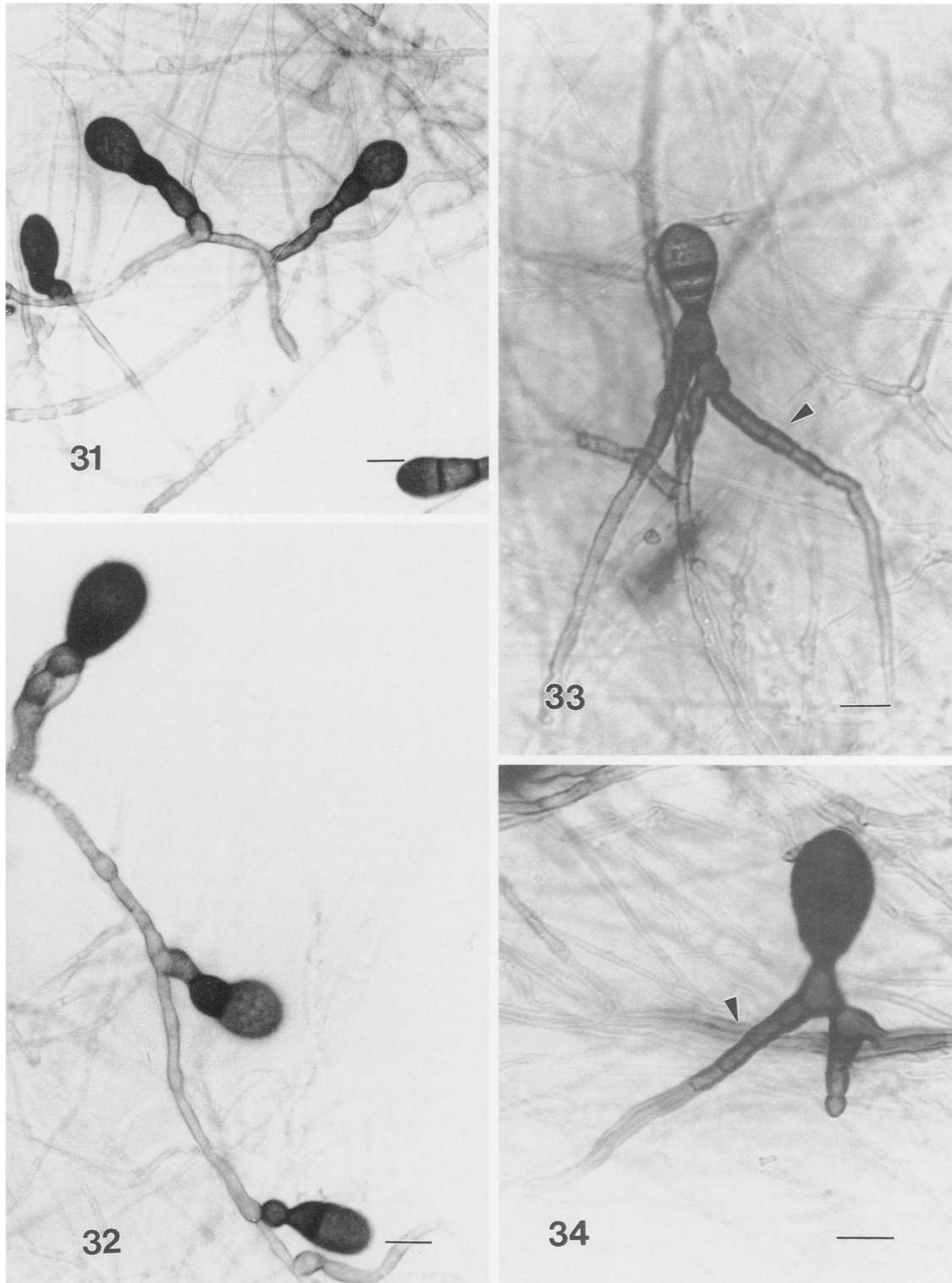
Discussion. Sivanesan and Chang (1992) established the genus *Ascotaiwania* for the type species *A. lignicola* Sivan. & H. S. Chang, an aquatic ascomycete with 7-septate brown ascospores with hyaline end cells. A second species, *A. palmicola* K. D. Hyde, was described for an ascomycete with 3-septate ascospores from palms. Chang et al. (1998) also described 3 additional species in the genus, namely *A. hsilio* H. S. Chang & Y. S. Hsieh, *A. sawada* H. S. Chang & Y. S. Hsieh, *A. wulai* H. S. Chang & Y. S. Hsieh.

Ascotaiwania mitrifomis is very similar to *A. lignicola* and *A. wulai* in having 7-septate ascospores with hyaline end cells and a relatively massive refractive apical ring and we are confident of its inclusion in *Ascotaiwania*. The ascospores in *A. mitrifomis*, however, are the largest in the genus, as compared to those in *A. lignicola* (42–55 \times 8–13 μm) and *A. wulai* (53–62 \times 14–16 μm). The ascospore end cells in *A. mitrifomis* are also mitriform in shape, while in the other species they are truncate. A synopsis of the characters of the species of *Ascotaiwania* is given in TABLE II.

Sivichai et al. (1998) have recently reported a species of *Monotosporella* in single spore isolations of *A. sawada*. The *Monotosporella* species reported in the culture of *Ascotaiwania mitrifomis* differs from the anamorph of *A. sawada* and also differs from the other described species of *Monotosporella*.

KEY TO ASCOTAIWANIA SPECIES

1. Ascospores 7-septate, longer than 50 μm 4
1. Ascospores with fewer than 7 septa, less than 50 μm long 2



FIGS. 31–34. *Monotsporella* state of *Ascotaiwania mitriformis* in culture. 31, 32. Immature conidia growing in series along the mycelium. 33, 34. Mature conidia with conidiophores proliferating percurrently (arrowed). Note the rhizomorphs. Scale bars: 10 μm .

TABLE II. A synopsis of the characteristics of species of *Ascotaiwania*

	<i>A. palmicola</i> (Hyde, 1995)	<i>A. sawada</i> (Chang et al. 1998)	<i>A. hsilio</i> (Chang et al. 1998)	<i>A. mitriformis</i>	<i>A. lignicola</i> (Sivanesan and Chang, 1992)	<i>A. wulai</i> (Chang et al. 1998)
Ascomata	200–250 × 210–350 μm Immersed	242–278 × 541–564 μm Partly to fully immersed	205–273 × 307–410 μm Partly to fully immersed	400–450 × 500– 560 μm Semi- immersed	300–600 × 190–230 μm Partly to fully immersed	480–744 × 297–518 μm Partly to fully immersed
Asci	150–175 × 7.5–8 μm	180–187 × 13.3–15.5 μm	120–140 × 12.3–13.4 μm	325–375 × 25–35 μm	234–290 × 13– 19 μm	274–308 × 17– 21 μm
Aprical ring	5 × 4 μm	4.8–5.1 × 7.1– 10.3 μm	3.8–4.5 × 5.8– 7.2 μm	7.5–10 × 12.5–15 μm	7–9.2 × 5.3– 6.7 μm	7.5–9.7 × 11.4–13 μm
Ascospores	3-septate 17.5–20 × 5–6.5 μm	3-septate 25.4–44.6 × 7.1–10.3 μm	5-septate 25.3–29 × 7.1–7.7 μm	7-septate 62.5–72.5 × 12.5–17.5 μm	7-septate 42–55 × 8– 13 μm	7-septate 53–62 × 14– 16 μm
Shape of end cells	Rounded	Rounded	Rounded	Mitriiform	Rounded	Rounded
Habitat	Palm	Submerged wood	Submerged wood	Submerged wood	Dead wood	Submerged wood

2. Ascospores 3-septate 3
 2. Ascospores 5-septate *A. hsilio*
 3. Ascospores longer than 30 μm *A. sawada*
 3. Ascospores less than 30 μm long *A. palmicola*
 4. Ascospores with mitriiform end cells, longer than
 62.5 μm *A. mitriformis*
 4. Ascospores lacking mitriiform end cells, shorter
 than 62.5 μm 5
 5. Ascospores mostly 42–55 μm long *A. lignicola*
 5. Ascospores mostly 53–62 μm long *A. wulai*

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