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***Aquaticola*, a new genus of *Annulatasceae* from freshwater habitats**

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*Aquaticola hyalomura* gen. et sp. nov. and *A. ellipsoidea* sp. nov. are described based on specimens found on submerged wood collected in streams and a reservoir in Hong Kong. *Aquaticola* species are similar to members of *Annulatasceae*, but differ in having smaller ascus apical rings, and overlapping uniseriate to biseriate ascospores that are also smaller in size. *Aquaticola* is compared with genera of *Annulatasceae* and other similar genera.

**Key words:** *Annulatasceae*, ascus ring, freshwater fungi, taxonomy

**Introduction**

During our study on the freshwater mycota in the tropics (e.g. Ho *et al.*, 1997, 1999a, 1999c; Goh *et al.*, 1998) we collected two undescribed ascomycetes on submerged wood in streams and a reservoir in Hong Kong. These taxa are characterized by hyaline to black, beaked ascomata, septate, unbranched, wide and tapering paraphyses, broadly oblong to long cylindrical, unitunicate asci with a relatively small, refractive J- apical ring, and hyaline, ellipsoidal ascospores. These ascomycetes are similar to genera placed in the *Annulatasceae* (Wong *et al.*, 1998), although the species cannot be accommodated in any described genus.

*Annulatasceae* was introduced to accommodate a group of genera reported from tropical rivers and lakes (Wong *et al.*, 1998). These genera are characterized by dark brown to black, beaked ascomata, septate, unbranched, wide and tapering paraphyses, long cylindrical, unitunicate asci, provided with a relatively massive, refractive apical ring. The fungi discussed in the present paper are, however, different to all of these genera and therefore a new genus *Aquaticola* is introduced to accommodate them.

## Materials and methods

Submerged wood was collected from streams and a reservoir in Hong Kong and examined as described in Ho *et al.* (1999b). All measurements given in this paper were made from material mounted in water. Material has been deposited in HKU(M).

## Taxonomy

### *Aquaticola* W.H. Ho, K.M. Tsui, Hodgkiss and K.D. Hyde, **gen. nov.**

*Etymology*: In reference to the aquatic habitat of the fungus.

*Ascomata* globosa vel subglobosa, immersa vel superficiales, coriacea, alba vel atrobrunnea, interspersa vel gregaria, ostiolata, papillata. *Papilla* cylindrica, periphysata. *Peridium* hyalina vel brunnea, textura angulatum. *Paraphyses* filamentosae, numerosis, non ramosae, septatae, constrictae ad septa, hyalinae, decrescentes ad apicem. *Asci* octospori, late oblongati vel late cylindrici, pedicellati, unitunicati, tenuitunicati, persistenti, rotundati vel truncati ad apicem, apparatu apicale J-. *Ascospores* uniseriatae imbricatas vel biseriatae, ellipsoideae, ad apices rotundatae, hyalinae, septatae vel aseptatae, laeviae, tenuitunicatae, guttulate, tunica gelatinosa praesens vel absens.

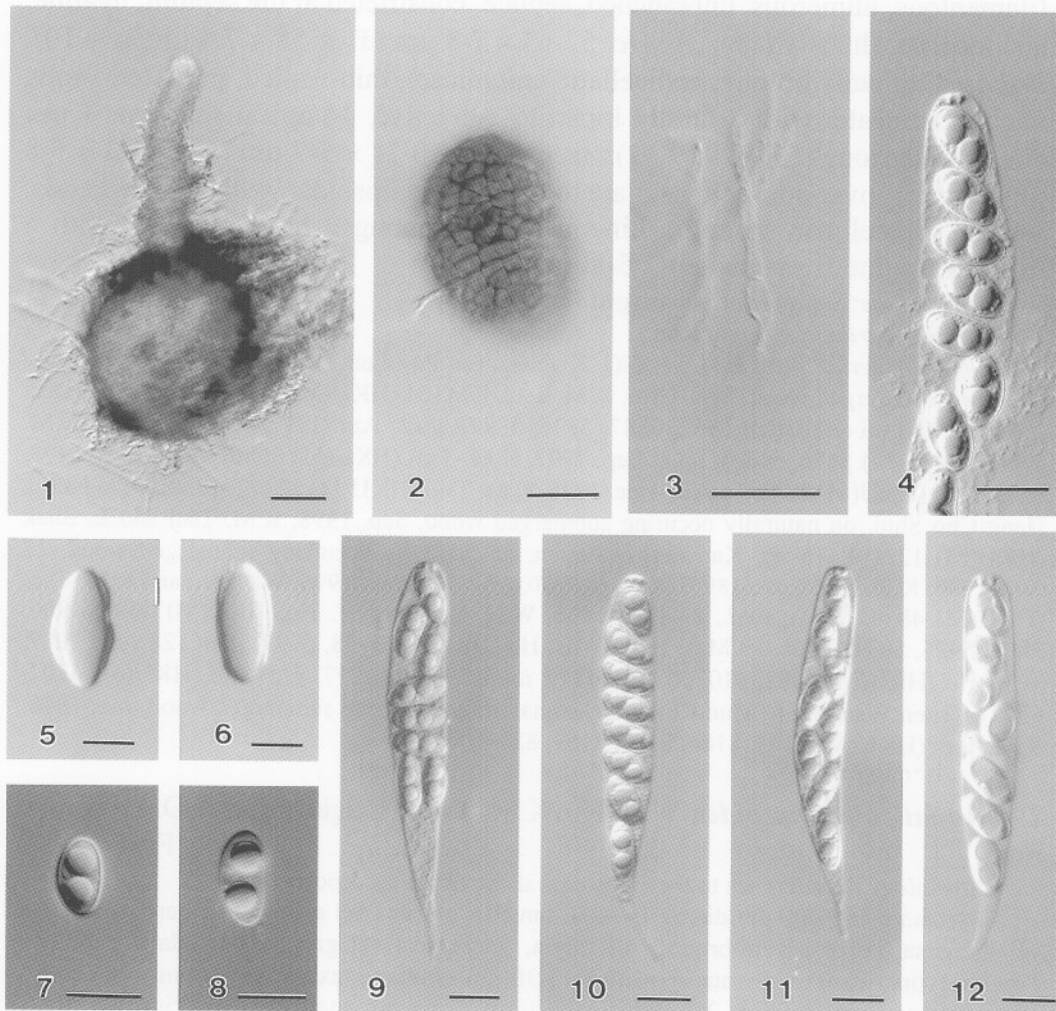
*Ascomata* globose to subglobose, immersed to superficial, coriaceous, white to dark brown, scattered to gregarious, ostiolate, papillate. *Beaks* cylindrical, periphysate. *Peridium* hyaline to brown, tissues of *textura angularis* in surface view. *Paraphyses* hypha-like, filamentous, numerous, unbranched, septate, constricted at the septum, hyaline and tapering distally. *Asci* 8-spored, broadly oblong to long cylindrical, pedicellate, unitunicate, thin-walled, persistent, apex rounded to truncate, with a small refractive J- apical ring. *Ascospores* overlapping uniseriate to biseriate, ellipsoidal, with rounded ends, hyaline, septate or non-septate, smooth, thin-walled, guttulate, with or without a mucilaginous sheath.

*Type species*: *Aquaticola hyalomura* W.H. Ho, K.M. Tsui, Hodgkiss and K.D. Hyde.

### 1. *Aquaticola hyalomura* W.H. Ho, K.M. Tsui, Hodgkiss and K.D. Hyde, **sp. nov.** (Figs. 1-12)

*Etymology*: From Latin *hyalo* meaning "hyaline" and *murus* meaning "wall", referring to the colorless peridium.

*Ascomata* 160-290  $\mu\text{m}$  diam., 160-300  $\mu\text{m}$  alta, globosa vel subglobosa, immersa vel superficiales, coriacea, alba vel atrobrunnea, solitaria, ostiolata, papillata. *Papilla* 100-150  $\times$  45-65  $\mu\text{m}$ , cylindrica, periphysata. *Peridium* hyalina vel atrobrunnea, textura angulatum. *Paraphyses* 2-3.5  $\mu\text{m}$  crassae ad basim, filamentosae, numerosis, non ramosae, septatae, constrictae ad septa, hyalinae, decrescentes ad apicem. *Asci* 52-63  $\times$  7-8  $\mu\text{m}$ , octospori, late oblongati, pedicellati, unitunicati, tenuitunicati, persistenti, truncati ad apicem, apparatu apicale J-, ca. 4  $\mu\text{m}$  diam., 2  $\mu\text{m}$  longi. *Ascospores* 10-14  $\times$  5-7  $\mu\text{m}$ , uniseriatae imbricatas vel biseriatae, ellipsoideae, ad apices rotundatae, hyalinae, unicellulares, laeviae, tenuitunicatae, guttulate, foveolatae et tunica gelatinosa praeditae.



**Figs. 1-12.** Light micrographs of *Aquaticola hyalomura* (from holotype). **1.** Ascoma. **2.** Peridium comprising angular cells in surface view. **3.** Paraphyses which are septate, wide and taper distally. **4.** Ascus with faintly bipartite apical ring. **5-6.** Ascospores with thin mucilaginous sheaths. **7-8.** Ascospores with two large guttules. **9-12.** Asci with apical rings and short pedicels. Bars: 1 = 100  $\mu\text{m}$ , 2-4, 7-12 = 10  $\mu\text{m}$ , 5, 6 = 5  $\mu\text{m}$ .

*Holotype:* HONG KONG, Tai Po Kau Forest Stream, on submerged decaying wood, 27 June 1996, K.D. Hyde, WH189 (HKU(M) 2969).

*Ascomata* 160-290  $\mu\text{m}$  diam., 160-300  $\mu\text{m}$  high, globose to subglobose, immersed to superficial, coriaceous, white to pale brown, mostly solitary, ostiolate, papillate (Fig. 1). *Beaks* 100-150  $\times$  45-65  $\mu\text{m}$ , cylindrical, periphysate. *Peridium* hyaline or pale brown, tissues of *textura angularis* in surface view (Fig. 2). *Paraphyses* 2-3.5  $\mu\text{m}$  wide at the base, hypha-like,

filamentous, numerous, unbranched, septate, constricted at the septum, hyaline, and tapering distally (Fig. 3). *Asci* 52-63 × 7-8 μm ( $\bar{x}$  = 58 × 7.4 μm, n = 10), 8-spored, broadly oblong, pedicellate, unitunicate, thin-walled, persistent, apex slightly truncate, with a faintly bipartite, refractive, J-, apical ring (ca. 4 μm diam., 2 μm long) (Figs. 4, 9-12). *Ascospores* 10-14 × 5-7 μm ( $\bar{x}$  = 12.2 × 5.8 μm, n = 25), overlapping uniseriate to biseriate, ellipsoidal, with rounded ends, hyaline, unicellular, smooth, thin-walled, containing 2 large guttules, with a thin mucilaginous sheath (Figs. 5-8).

*Mode of life*: Saprobic on submerged wood.

*Known distribution*: Hong Kong.

*Other material examined*: HONG KONG, Tai Po, Lam Tsuen River, on naturally occurring submerged wood, Sep. 1996, K.M. Tsui, KM104 (HKU(M) 4628, 4652); *ibid.*, Dec. 1996, K.M. Tsui (HKU(M) 5362, 5387, 5396, 5399); *ibid.*, Apr. 1997, K.M. Tsui and K.M. Wong (HKU(M) 5436, 5460); *ibid.*, Sep. 1997, K.M. Tsui (HKU(M) 8086); *ibid.*, May 1998, K.M. Tsui (HKU(M) 12158); *ibid.*, Dec. 1998, K.M. Tsui (HKU(M) 12217, 12222); Sai Kung, Hang Cho Shui, on naturally occurring submerged wood, Sep. 1998, K.M. Tsui and E. Shek (HKU(M) 12280); Tai Po Kau Forest Stream, on naturally occurring submerged wood, 27 June 1996, K.D. Hyde, WH189 (HKU(M) 2970); *ibid.*, 21 Sep. 1996, W.H. Ho and K.M. Tsui (HKU(M) 4830, 4841); *ibid.*, 29 Dec. 1996, W.H. Ho and S.Y. Ho (HKU(M) 4896, 4900, 5909, 5926, 5931); *ibid.*, 29 Mar. 1997, W.H. Ho (HKU(M) 5998, 5999); *ibid.*, 28 June 1997, W.H. Ho (HKU(M) 6082, 6103, 6104, 6112); *ibid.*, 27 Dec. 1997, W.H. Ho (HKU(M) 6202, 6203); Tsuen Wan, Shing Mun Reservoir, on naturally occurring submerged wood, Jan. 1998, K.M. Tsui (HKU(M) 8151, 8166, 8167, 8169, 8202).

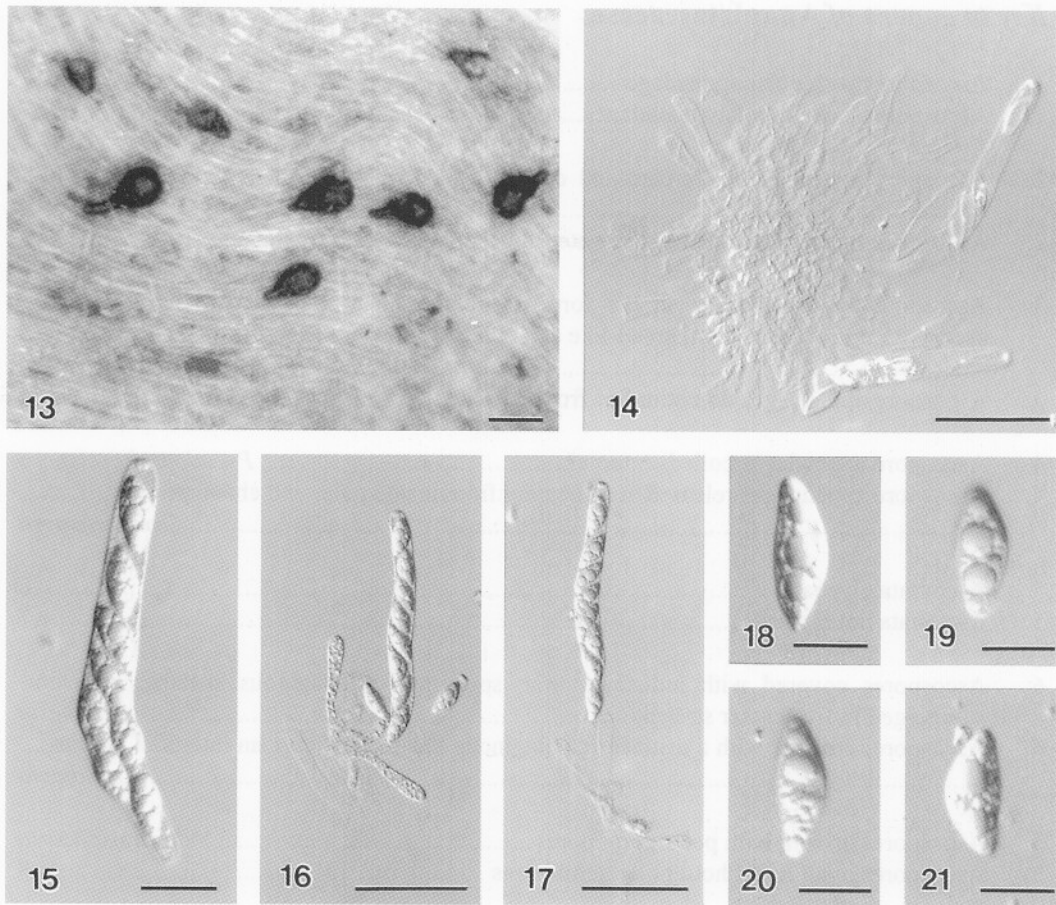
2. *Aquaticola ellipsoidea* W.H. Ho, K.M. Tsui, Hodgkiss and K.D. Hyde, **sp. nov.** (Figs. 13-21)

*Etymology*: In reference to the ellipsoidal shape of the ascospores.

*Ascomata* 150-200 μm diam., 150-250 μm alta, globosa vel subglobosa, partim immersa vel immersa, coriacea, atrobrunnea vel nigra, interspersa vel gregaria, ostiolata, papillata. *Papilla* cylindrica, periphysata. *Peridium* 15-30 μm crassum, textura angulatum, ex duobus stratis compositum, exterius 2-3 stratis cellularum brunneum angularum, interius 4-8 stratis cellularum hyalinarum angularum. *Paraphyses* ca. 5 μm crassae ad basim, filamentosae, numerosis, non ramosae, septatae, constrictae ad septa, hyalinae, decrescentes ad apicem. *Asci* 90-125 × 10-12.5 μm, octospori, late cylindrici, late pedicellati, unitunicati, tenuitunicati, persistenti, truncati ad apicem, apparatu apicale J-. *Ascospores* 12-14 × 5-7 μm, uniseriatae imbricatas, ellipsoideae, ad apices rotundatae, curvae, hyalinae, unicellulares, laeviae, tenuitunicatae, guttulatae, foveolatae et tunica gelatinosa praeditae.

*Holotype*: HONG KONG, Tai Po, Tai Po Kau Forest Stream, on *Machilus velutina* wood bait, 29 Mar. 1997, W.H. Ho (HKU(M) 6033).

*Ascomata* 150-200 μm diam., 150-250 μm high, globose to subglobose, partly immersed to immersed, coriaceous, dark brown to black, scattered to gregarious, ostiolate, papillate (Fig. 13). *Beaks* cylindrical, periphysate, with setae at the tips. *Peridium* 15-30 μm wide, tissues of *textura angularis* in



**Figs. 13-21.** Light micrographs of *Aquaticola ellipsoidea* (from holotype). **13.** Ascomata on woody subst. rate. **14.** Squash mount of asci and paraphyses. **15-17.** Asci. **18-21.** Ascospores. Bars: 1 = 100  $\mu\text{m}$ , 2, 4-5 = 50  $\mu\text{m}$ , 3 = 20  $\mu\text{m}$ , 6-9 = 10  $\mu\text{m}$ .

surface view, two layered; outer layer composed of 2-3 rows of brown, angular cells; inner layer composed of 4-8 rows of hyaline, angular cells. *Paraphyses* hypha-like, filamentous, numerous, unbranched, septate, constricted at the septum, hyaline, ca. 5  $\mu\text{m}$  wide at the base, tapering distally (Fig. 14). *Asci* 90-125  $\times$  10-12.5  $\mu\text{m}$  ( $\bar{x}$  = 105  $\times$  11  $\mu\text{m}$ , n = 10), 8-spored, long cylindrical, long pedicellate, unitunicate, thin-walled, persistent, apex slightly truncate, with a small refractive J- apical ring (Figs. 15-17). *Ascospores* 12-14  $\times$  5-7  $\mu\text{m}$  ( $\bar{x}$  = 13  $\times$  7  $\mu\text{m}$ , n = 25), overlapping uniseriate, ellipsoidal, curved, hyaline, unicellular, smooth, thin-walled, guttulate, without a mucilaginous sheath (Figs. 18-21).

## Key to genera of Annulatascaceae

1. Ascospores with polar appendages.....2
1. Ascospores lacking polar appendages.....5
2. Ascospore appendages bifurcate and curling backwards to form an irregular cup-like structure..... *Fluminicola*
2. Ascospore appendages uncoiled in water to become long and filamentous.....3
3. Ascospore appendages are in the form of polar conical caps, that detach from the ascospore tip in water, with thread-like appendages unfurling from inside the caps.....  
..... *Diluviocola*
3. Ascospore appendages do not unfurl from detached caps .....4
4. Ascospore appendages coiled, external..... *Pseudoproboscispora*
4. Ascospore appendages released as a filament from inside polar end chambers.....  
..... *Cateractispora*
5. Ascomata clypeate.....6
5. Ascomata not clypeate .....7
6. Ascospores covered with indistinct, thin, spreading mucilaginous sheaths, on wood submerged in freshwater streams ..... *Clohiesia*
6. Ascospores covered with a distinct, mucilaginous sheath, on *Nypa*, an intertidal species....  
..... *Frondicola*
7. Ascospores brown, with polar germ pores ..... *Submersisphaeria*
7. Ascospores hyaline, without polar germ pores .....8
8. Ascomata white to dark brown, asci broadly oblong to long cylindrical, with a small apical ring (ca. 4 µm diam., 2 µm long), ascospores overlapping uniseriate to biseriate .....  
..... *Aquaticola*
8. Ascomata dark brown to black, asci long cylindrical, with a massive apical ring (ca. 4.5-6 µm diam., 3.5-9 µm long), ascospores uniseriate or overlapping uniseriate.....  
..... *Annulatascus*

*Mode of life:* Saprobic on submerged wood.

*Known distribution:* Hong Kong.

*Other material examined:* BRUNEI DARRUSALAM, Temburong, KBFR, Sungai Sitam, on submerged decaying wood, 24 Oct. 1995, W.H. Ho & K.D. Hyde (HKU(M) 2924, 2930); HONG KONG, Tai Po, Tai Po Kau Forest Stream, on *Machilus velutina* wood bait, 23 June 1996, W.H. Ho (HKU(M) 4557, 4558); *ibid.*, 21 Sep. 1996, W.H. Ho and K.M. Tsui (HKU(M) 4851, 4858, 4863, 4869); *ibid.*, 29 Dec. 1996, W.H. Ho & S.Y. Ho (HKU(M) 5948, 5949, 5951, 5959); *ibid.*, 29 Mar. 1997, W.H. Ho (HKU(M) 6035, 6040, 6043, 6045, 6047); *ibid.*, 28 June 1997, W.H. Ho (HKU(M) 6122, 6124, 6125, 6127, 6134, 6138, 6139, 6140); *ibid.*, 28 Sep. 1997, W.H. Ho (HKU(M) 8652, 8655, 8658, 8660, 8662, 8665, 8667); *ibid.*, 27

Dec. 1997, W.H. Ho (HKU(M) 8759, 8760); *ibid.*, on *Pinus massoniana* wood bait, 27 June 1996, W.H. Ho (HKU(M) 4578, 4579, 4582); *ibid.*, 21 Sep. 1996, W.H. Ho & K.M. Tsui (HKU(M) 4871, 4872, 4874, 4876, 4877, 4878, 4879, 4881, 4882, 4883, 4887, 4888); *ibid.*, 29 Dec. 1996, W.H. Ho & S.Y. Ho (HKU(M) 5961, 5966, 5967, 5968, 5969, 5971, 5975, 5978, 5979); *ibid.*, 29 Mar. 1997, W.H. Ho, 6053, 6057, 6059, 6060, 6061, 6064, 6067, 6068); *ibid.*, 28 June 1997, W.H. Ho (HKU(M) 6142, 6146, 6148, 6151, 6152, 6153, 6156, 6158); *ibid.*, on submerged decaying wood, 27 June 1996, K.D. Hyde (HKU(M) 2947, 2948, 2949, 2952, 2953, 2957, 2960, 2961, 2964, 2966, 2967, 2971, 2975, 4520, 4521, 4539, 4545); *ibid.*, 21 Sep. 1996, W.H. Ho & K.M. Tsui (HKU(M) 4596); *ibid.*, 29 Dec. 1996, W.H. Ho & S.Y. Ho (HKU(M) 4894, 4895, 5901, 5915, 5923, 5935, 5936); *ibid.*, 29 Mar. 1997, W.H. Ho (HKU(M) 5982, 5983, 5996, 6000, 6002, 6004, 6005); *ibid.*, 28 June 1997, W.H. Ho (HKU(M) 6071, 6073, 6088, 6105, 6107, 6109, 6117, 6118, 6119); *ibid.*, 28 Sep. 1997, W.H. Ho (HKU(M) 6163, 6178, 6183, 6187, 6188, 6190, 6193, 6196, 6197, 8643); *ibid.*, 27 Dec. 1997, W.H. Ho (HKU(M) 8691, 8696, 8698, 8703, 8709, 8712, 8713, 8722, 8723, 8725, 8726, 8727); Tai Po, Lam Tsuen River, on submerged decaying wood, 9 Dec. 1998, K.M. Tsui, KM307 (HKU(M) 12178).

### Discussion

*Aquaticola* is similar to *Annulatascus*, the type genus of *Annulatascaceae* (Wong *et al.*, 1998), and when first encountered was thought to be this genus. *Aquaticola* and *Annulatascus* are similar in having globose, beaked ascomata; septate, unbranched paraphyses, which are wide at the base, and taper towards the apex; unitunicate, persistent asci with a refractive, non-amyloid apical ring; hyaline ascospores; and in terms of their habitat, i.e. on decaying wood submerged in freshwater (Hyde, 1992b). *Aquaticola* however, can be distinguished from *Annulatascus* by the nature of its broadly oblong to long cylindrical asci, which have a relatively smaller apical ring and rounded to truncate apex, overlapping uniseriate to biseriate ascospores and their smaller ascospore size. *Aquaticola* is best placed in the *Annulatascaceae* (Sordariales) based on morphological characteristics. The generic placement of *Aquaticola* is further supported by molecular studies indicating that *Aquaticola* is closely related to genera of the *Annulatascaceae* (Ranghoo *et al.*, 1999).

There have been 9 genera assigned to the *Annulatascaceae* (Hyde, 1992a, b, 1995, 1996b; Hyde *et al.*, 1998, 1999b; Wong *et al.*, 1998, 1999a, b; Wong and Hyde, 1999), of which *Ascotaiwania* was excluded based on molecular studies (Ranghoo *et al.*, 1999). *Aquaticola* differs from *Cateractispora*, *Diluviocola*, *Fluminicola*, and *Pseudoproboscispora* in producing ascospores without polar appendages. *Clohiesia* and *Frondicola* are distinct from *Aquaticola* and other genera of *Annulatascaceae* in producing clypeate ascomata, while *Submersisphaeria* is distinct in producing brown ascospores

**Table 1.** Comparison between *Aquaticola* and similar genera.

	<i>Aquaticola</i> (this study)	<i>Arecomyces</i> (Hyde 1996a)	<i>Aniptodera</i> (Hyde <i>et al.</i> , 1999)	<i>Annulataascus</i> (Hyde 1992b; Wong <i>et al.</i> , 1998c; Yanna, pers. comm.)	<i>Lignincola</i> (Kohlmeyer and Kohlmeyer, 1979)	<i>Physalospora</i> (Barr, 1970, 1976; Hanlin, 1990)
Ascomata	Immersed or superficial	Immersed	Immersed or superficial	Immersed or superficial	Immersed or superficial	Immersed
	Non-clypeate	Clypeate	Non-clypeus	Non-clypeus	Non-clypeus	Non-clypeus
	White to dark brown	Brown	White to brown	Dark brown to black	Light-coloured to black	Brown
	Coriaceous	Coriaceous	Coriaceous	Carbonaceous to coriaceous	Coriaceous	Coriaceous
Asci	8-spored	(2-)4-8-spored	8-spored	8-spored	8-spored	8-spored
	Broadly oblong to cylindrical	Broadly cylindrical	Clavate or cylindrical	Long cylindrical	Clavate or subfusiform	Cylindro-clavate
	With a J- apical ring (1.2-2.5 $\mu\text{m}$ long, 2.5-4 $\mu\text{m}$ diam.)	With a J- apical ring (0.6-2 $\mu\text{m}$ long, 3-5 $\mu\text{m}$ diam.)	With a J- apical thickening and a simple pore	With a J- apical ring (3.5-9 $\mu\text{m}$ long, 4.5- 8 $\mu\text{m}$ diam.)	With a J- apical thickening	With or lacking a J- apical ring
	With rounded to truncate apex	With rounded to truncate apex	With rounded to truncate apex	With rounded apex	With rounded apex	With rounded apex
	Without cytoplasm retraction	Without cytoplasm retraction	Cytoplasm retracted below the apex	Without cytoplasm retraction	With or without cytoplasm retraction	Without cytoplasm retraction



Table 1. (continued).

	<i>Aquaticola</i>	<i>Arecomyces</i>	<i>Aniptodera</i>	<i>Annulatascus</i>	<i>Lignincola</i>	<i>Physalospora</i>
Ascospores	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline to lightly pigmented
	0-septate	0-septate	1-septate	0-3-septate	1-septate	0-septate
	Smooth-walled	Smooth-walled or echinate	Smooth-walled	Wall verruculose at SEM level	Smooth-walled	Wall verruculose or smooth
	With or lacking a mucilaginous sheath Lacking appendages	With a mucilaginous sheath Lacking appendages	Lacking a mucilaginous sheath With or lacking appendages	With a mucilaginous sheath Lacking appendages	Lacking a mucilaginous sheath Lacking appendages	With or lacking a mucilaginous sheath Lacking appendages
	Uniseriate to biseriate	Uniseriate to overlapping uniseriate	Biseriate to triseriate	Uniseriate to overlapping uniseriate	Biseriate	Uniseriate to biseriate
	16-58 × 5-15 µm	7.5-19 × 4-8 µm	15-55 × 7-22 µm	6.5-20 × 4-8.5 µm	12.5-24 × 5-8 µm	Longer than 20 µm
Habitat	Freshwater	Terrestrial	Freshwater, brackish water and marine	Freshwater and terrestrial	Marine, brackish	Terrestrial
Mode of nutrition	Saprophytic	Saprophytic	Saprophytic	Saprophytic	Saprophytic	Parasitic or saprophytic
Host/substrata	Wood	Palms	Wood	Wood, bamboo and palms	Wood, bark, roots	Palms, leaves

with polar germ pores. A key to the nine genera of *Annulatascaceae* is provided.

*Aquaticola* is also comparable to species of *Aniptodera*, but the latter differs in having asci with an apical thickening and a simple pore, and 1-septate ascospores which may have appendages (Hyde *et al.*, 1999a). *Aquaticola* is also comparable to *Arecomyces*, *Lignincola* and *Physalospora* in having asci with a J-, relatively small apical ring, and hyaline, ellipsoidal to fusiform ascospores. *Arecomyces* is saprobic on palms and the ascomata are clypeate (Hyde, 1996a), while *Physalospora* is parasitic or saprobic on leaves (Barr, 1970; Hanlin, 1990). In *Lignincola*, the ascospores are 1-septate and occur on substrata submerged in marine water (Kohlmeyer and Kohlmeyer, 1979). A synopsis of the morphological characteristics of *Aquaticola* and similar genera are compared in Table 1.

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