

Ascominuta lignicola, a new loculoascomycete from submerged wood in Hong Kong

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Ascominuta lignicola gen. et sp. nov. is described and illustrated based on a specimen collected on submerged wood in Hong Kong. *Ascominuta lignicola* is characterised by relatively small ascomata, globose, 4-spored asci, and ascospores with an elaborate sheath. *Ascominuta lignicola* is compared with *Massarina*, *Mycosphaerella* and *Wettsteinina*. The placement of *Ascominuta* in the *Ascomycetes incertae sedis* is discussed.

Key Words—Ascomycetes *incertae sedis*; freshwater ascomycetes; systematics.

We have been investigating the fungi occurring on wood submerged in freshwater (Hyde et al., 1998; Lee et al., 1998; Wong et al., 1999) and in this paper we describe a new Loculoascomycete. *Ascominuta lignicola* gen. et sp. nov. has a unique combination of morphological characters, including relatively small ascomata, globose, 4-spored asci, and 1-septate hyaline ascospores with an elaborate mucilaginous sheath. This fungus cannot be referred to any genus within the loculoascomycetes and *Ascominuta* gen. nov. is introduced to accommodate this remarkable species.

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. All measurements of fungal structures were made in water. Single spore cultures were obtained as follows: a suspension of the ascospores in sterile distilled water was pipetted onto potato dextrose agar (PDA) plates. Single ascospores were allowed to germinate (ca. 2d) and transferred to PDA plates for further growth. Ascospores were prepared for SEM following the methods used by Ho et al. (1999).

Taxonomy

Ascominuta V. M. Ranghoo et K. D. Hyde, gen. nov.

Ascomata partim immersa vel superficialia, globosa, atrobrunnea vel nigra, solitaria vel aggregata. Peridium peritheci, 3–5-stratosum, ex cellulis hyalinis extrinsecus *textura angularis* fuscaris compositum. Pseudoparaphyses cellulosa, breviter catenulatae, ad septa constrictae. Asci 4-spore, globosi, bitunicati, sine apparatus apicali. Ascosporeae biseriatae, late fusiformes, 1-septa-

tae, ad septum constrictae, hyalinae, muris laevigatae, tunica gelatinosa praeditae.

Etymology: From “Asco” and the Latin *minutus* = small, in reference to the small-sized ascomata and asci of this taxon.

Typus generis: *Ascominuta lignicola* V. M. Ranghoo et K. D. Hyde.

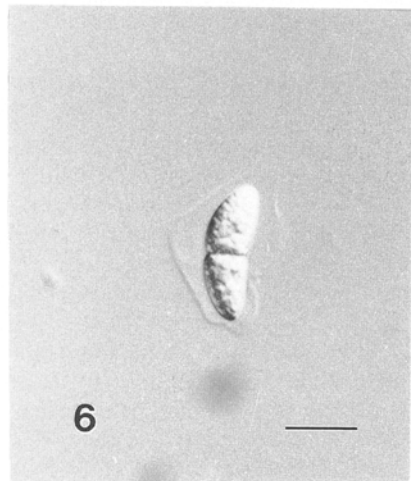
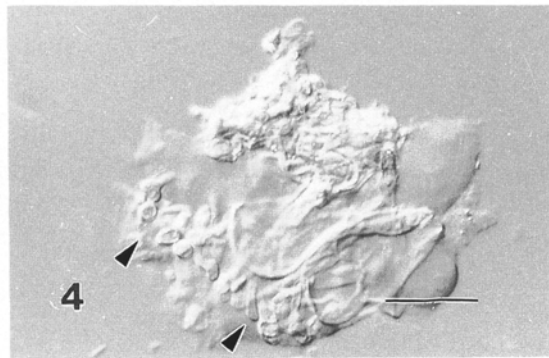
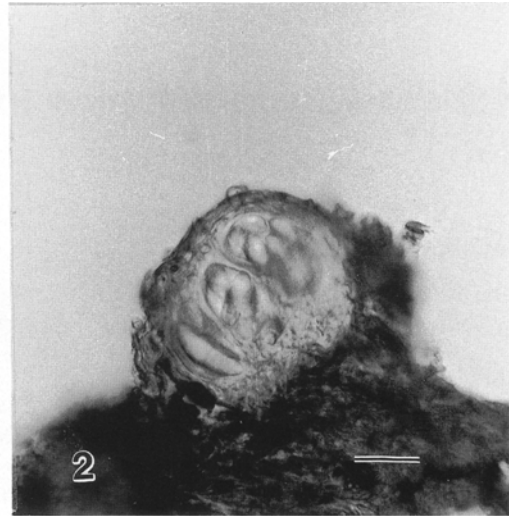
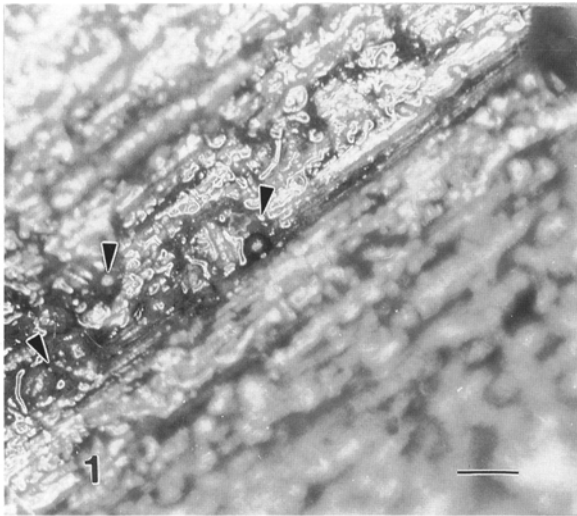
Ascomata superficial to partly immersed in the host substrate, globose, dark brown to black, solitary or aggregated, ostiole periphysate. *Peridium* composed of 3–5 layers of hyaline compressed cells, the outer layer of dark brown *textura angularis*. *Pseudoparaphyses* comprising short chains of globose cells, often breaking up into individual cells, constricted at the septa. *Asci* 4-spored, globose, pedicellate, bitunicate, lacking an apical apparatus. *Ascospores* biseriatae, broadly fusiform, somewhat curved, 1-septate, constricted at the septum, hyaline, smooth-walled, with an irregular mucilaginous sheath.

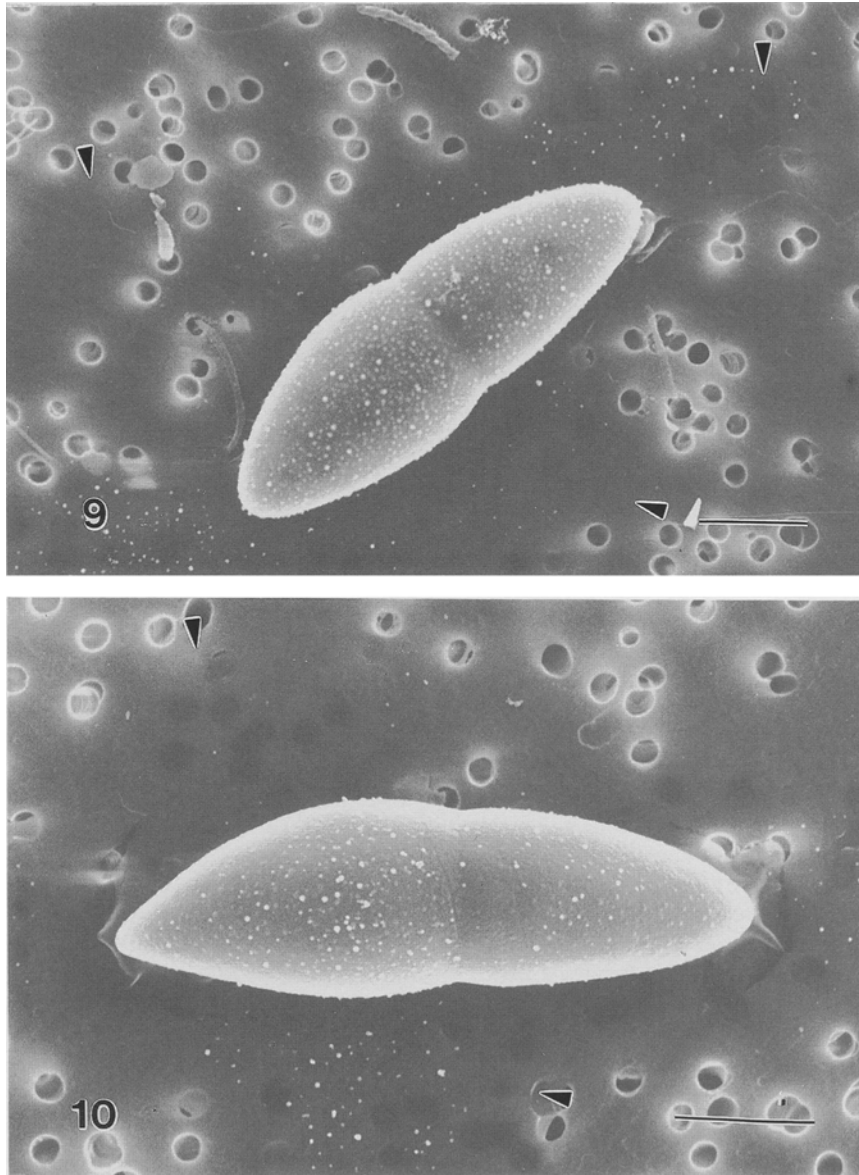
Type species: *Ascominuta lignicola* V. M. Ranghoo et K. D. Hyde.

Ascominuta lignicola V. M. Ranghoo et K. D. Hyde, sp. nov. Figs. 1–10

Ascomata 60–65 μm alta, 70–75 μm diam, superficialia vel partim immersa, globosa, atrobrunnea vel nigra, solitaria vel aggregata. Peridium peritheci, 10–15 μm latum, 3–5-stratosum, ex cellulis hyalinis, extrinsecus *textura angularis* fuscis compositum. Paraphyses ad basim 5–15 \times 2–3 μm latae, cellulosa, breviter catenulatae, ad septa constrictae. Asci 25–30 \times 25–30 μm , 4-spore, globosi, pedicellati, bitunicati, sine apparatus apicali. Ascosporeae 22–25 \times 8–10 μm , biseriatae, late fusiformes, 1-septatae, constrictae, hyalinae, muris laevigatae, tunica gelatinosa praeditae.

Etymology: From the Latin, *lignum* = wood, and *-cola* = dweller, in reference to the habitat on wood.





Figs. 9, 10. Scanning electron micrographs of *Ascominuta lignicola*. Figs. 9, 10. Ascospores with large mucilaginous sheaths, that occlude pores in the polycarbonate membrane (arrowed). Scale bars: 9, 10=6 μm .

Holotype. HONG KONG, New Territories, Plover Cove Reservoir: on submerged wood, Jan. 1997, V. M. Ranghoo (HKUM 5246); culture ex type HKUCC 3709.

Ascomata 60–65 μm high, 70–75 μm diam, no visible necks even in section, superficial to partly immersed in the host substrate, globose, dark brown to black, solitary or aggregated (Fig. 1). Ostiole periphysate. Peridium 10–15 μm wide, comprising three to five layers of hyaline compressed cells, with an outer layer of dark brown *textura angularis* (Fig. 2). Pseudoparaphyses 5–15 μm long, 2–3 μm wide at base, comprising short

chains of globose cells, often breaking up into individual cells, constricted at the septa (Fig. 4). Asci 25–30 \times 25–30 μm , 4-spored, globose, pedicellate, bitunicate (Figs. 3, 5). Following the dehiscence of the ectotunica, the endotunica extends and finally the ascospores are released. Ascospores 22–25 \times 8–10 μm , biseriolate, broadly-fusiform, 1-septate, constricted at the septa, hyaline, surrounded by an irregular mucilaginous sheath (Figs. 6–10). Colonies on PDA slow-growing, attaining 25 mm after one month at room temperature (25°C), dark grey, growing in concentric rings, with greyish

Figs. 1–8. Light micrographs of *Ascominuta lignicola*. Fig. 1. Superficial ascomata (arrowed). Fig. 2. Section through ascoma. Figs. 3, 5. Asci with 4-spores. Fig. 4. Pseudoparaphyses (arrowed). Figs. 6–8. One-septate ascospores with large mucilaginous sheaths. Scale bars: 1=100 μm , 2, 3, 5=20 μm , 4, 6–8=10 μm .

Table 1. Synopsis of the main characters of the genera *Ascominuta*, *Massarina*, *Mycosphaerella* and *Wettsteinina*.

	<i>Ascominuta</i>	<i>Massarina</i>	<i>Mycosphaerella</i>	<i>Wettsteinina</i>
Ascomata	Non stromatic and non-clypeate 70–75 μm diameter	Sometimes stromatic and clypeate 100–1000 μm diameter	Sometimes aggregate to form stroma-like structures 47–155 μm diameter	Non stromatic and non-clypeate 80–550 μm diameter
Asci	4-spored Globose	8-spored Narrowly to broadly clavate	8-spored Clavate to obclavate	8–16-spored Saccate to broadly fusiform
Ascospores	1-septate Hyaline	1–3(7)-septate Hyaline, pale to dark brown	1-septate Hyaline to green	1–7-septate Hyaline to brown
Pseudoparaphyses	Few and composed of short chains of globose cells	Filiform and numerous	Absent	Filiform and numerous
Anamorphs	Unknown	<i>Acrocalymma</i> , <i>Chaetophoma</i> , <i>Diplodia</i> , <i>Periconia</i> , <i>Phoma</i> , <i>Stagonospora</i> , <i>Tetraploa</i> , <i>Tumularia</i>	<i>Cercoseptoria</i> , <i>Cercospora</i> , <i>Cladosporium</i> , <i>Passalora</i> , <i>Polythrincium</i> , <i>Ramularia</i> , <i>Ovularia</i> , <i>Cercospora</i> , <i>Phoma</i> , <i>Ascochyta</i> , <i>Septoria</i> , <i>Phloeospora</i>	Unknown

white aerial hyphae, reverse black in colour. No anamorph or teleomorph produced in culture.

Mode of life: Saprobic on submerged wood.

Known Distribution: Hong Kong.

Ascominuta lignicola is unique among the Loculoascomycetes because of its unusual combination of morphological characteristics. These include: (1) 4-spored, globose asci; (2) 1-septate ascospores surrounded by an irregular mucilaginous sheath; (3) sparse pseudoparaphyses comprising short chains of globose cells; and (4) the relatively small size of the ascomata.

Genera of Loculoascomycetes with which *Ascominuta lignicola* should be compared are *Massarina* Sacc. and *Wettsteinina* Höhn.

Massarina species are characterised by immersed, erumpent or superficial, clypeate to non-clypeate ascomata and a hamathecium of relatively wide septate (cellular) pseudoparaphyses and are often found on wood (Hyde, 1995; Hyde and Aptroot, 1998; Aptroot, 1998). Asci are 8-spored, bitunicate, narrowly to broadly clavate usually with a shallow ocular chamber, and ascospores are 1–3(–7)-septate, fusiform to long ellipsoidal, often with appendages or mucilaginous sheaths (Aptroot, 1998). Although the ascospores of *Ascominuta lignicola* are similar to some species of *Massarina* (e.g. *M. velatipora* K. D. Hyde) in having ascospores surrounded by a mucilaginous sheath, it cannot be accommodated in *Massarina* because of the globose asci and sparse pseudoparaphyses comprising short chains of globose cells.

Ascominuta lignicola resembles some species of *Wettsteinina* (e.g. *W. savilei* Shoemaker et Babcock) in producing hyaline, 1-septate ascospores, surrounded by a mucilaginous sheath. Species of *Wettsteinina*, however, are usually pathogenic or saprobic on plant material (not wood) and asci are globose to broadly fusiform with 8(16) ascospores. The ascospores in *Wettsteinina* are 1–7-septate and usually brown when mature (Shoemaker and Babcock, 1987). Pseudopara-

physes are numerous and filiform in *Wettsteinina* as compared to sparse and composed of short chains of globose cells in *Ascominuta*. *Wettsteinina* does therefore not appear to be a suitable genus to accommodate *Ascominuta*.

Ascominuta lignicola should also be compared with *Mycosphaerella* Johanson (*Mycosphaerellaceae*) as the ascomata of *Mycosphaerella* species generally lack pseudoparaphyses. *Ascominuta* is similar to several *Mycosphaerella* species in having small globose ascomata and 2-celled ascospores (Corlett, 1988; Hanlin, 1990; Fröhlich and Hyde, 1998). *Ascominuta* differs from *Mycosphaerella* in having 4-spored globose asci, while those of *Mycosphaerella* are 8-spored and clavate to obclavate. A synopsis of the differences between *Ascominuta*, *Massarina*, *Mycosphaerella* and *Wettsteinina* is provided in Table 1.

Ascominuta lignicola is presently best placed within the Ascomycetes *incertae sedis*.

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