

Massarina ingoldiana, a new ascomycete from freshwater habitats

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Abstract: A species of *Massarina* (Pleosporales) was found on woody debris in aquatic habitats in several countries. This new species is characterized by its transversely septate ascospores that are surrounded by a large, gelatinous sheath. The pattern of ascospore appendage enlargement is very similar to that of another freshwater ascomycete, *Pleospora scirpicola*.

Key Words: Ascomycetes, aquatic, Pleosporales, systematics

INTRODUCTION

An ascomycete with a very distinctive ascospore sheath was encountered in a variety of freshwater habitats in Australia, Brunei Darussalam, Malaysia, and North America. The morphological features of this fungus support its placement in the genus *Massarina* Sacc. (Lophiostomataceae, Pleosporales, Loculoascomycetes). The unusual ascospore sheath distinguishes it from other species of *Massarina* and it is therefore described as a new species.

MATERIALS AND METHODS

Submerged woody and herbaceous debris was collected from aquatic habitats and returned to the laboratory for incubation in moist chambers (Shearer, 1993). After initial examination, samples were incubated at room temperature and examined periodically over 6–12 mo. Abbreviations for collectors names are JLC for J. L. Crane, KDH for K.D. Hyde, and CAS for C.A. Shearer.

To observe details of ascomal anatomy and centrum structure, ascomata were fixed in 4% glutaraldehyde, embedded in ultra-low viscosity resin (Huhn-

dorf, 1991) and sectioned at 6 μm . Thin sections were mounted on gelatin coated microscope slides and permanently preserved using a 1:1 mixture of Permunt and Xylene. Squash mounts of ascomata in water were made to observe the ascomal wall in face view, asci, paraphyses and ascospores. India ink was added to water mounts to observe the ascospore sheath. Measurements of ascomata were made from thin sections or whole mounts in water. All other measurements were made in lactic acid with azure A.

Single spore isolates were obtained according to the procedures of Shearer (1993). To confirm identities of cultures, isolates were induced to fruit by growing them on alfalfa stems submerged in water and then incubating the colonized alfalfa stems in moist chambers. To test for the presence of an Ingoldian hyphomycete anamorphic state, thin strips of colonized cornmeal agar (Difco) were submerged in sterile, distilled water and incubated at 17 C.

TAXONOMY

Massarina ingoldiana Shearer *et* Hyde, *sp. nov.*

FIGS. 1–13

Ascomata 180–310 μm alta, 185–500 μm diam, subglobosa, immersa vel superficialia, nigra, ostiolata, sub clypeo immersa, solitaria vel gregaria. *Rostrum* cylindricum, centrale, periphysatum, 65–113 \times 73–122 μm . *Asci* fissitunicati, octospori, anguste oblongi vel cylindrici, stipitati, cavernula apicali praediti vel non, 98–250 \times 20–44 μm . *Pseudoparaphyses* septatae, anastomosantes, 1.5–3 μm diam. *Ascosporae* 3–4 seriatae, 1-septatae, tum 3-subinde 5-septatae, fusiformes, hyalinae, serius palide brunneae, 38–70 \times 7–16 μm , involucreo gelatinoso circumfusae in aqua dilatato.

HOLOTYPE. ILLS.

Etymology. Named in honor of Professor C. T. Ingold for his pioneering work with freshwater ascomycetes.

Colonies on Emerson's yeast extract soluble starch agar immersed with a dense flattened mat of aerial hyphae; immersed hyphae brown-black, aerial hyphae dark golden brown; hyphae causing a brown pigmentation of the surrounding agar. *Ascomata* 180–310 μm high, 185–500 μm diam, solitary to clustered, immersed, becoming superficial by sloughing off of host, subglobose to flattened globose, ostiolate, papillate, \pm clypeate, black (FIGS. 1, 4). *Peridium*

20–48 μm wide, inner layer of hyaline, elongate thin-walled cells, 20–30 \times 4–7 μm at sides, 12–16 \times 4–6 μm at base, outer layer of very small, thin-walled, isodiametric, often compressed cells, outer cells interspersed among host cells, outermost cells and cells of clypeus occluded with brown, amorphous material (FIGS. 1, 2). *Beak* central, cylindrical, periphysate, 65–113 \times 73–122 μm , wall thicker at base, outer cells at base darkened and occluded with brown amorphous material, forming a clypeus-like structure (FIGS. 1, 2). *Pseudoparaphyses* septate, 1.5–3.0 μm wide, hyphae-like, hyaline, anastomosing, embedded in a gelatinous matrix (FIGS. 5, 6). *Asci* fissitunicate, oblong to cylindrical, broadly rounded at apex, tapering to stipe at base, 98–250 \times 20–44 μm , (type specimen – 146–220 \times 188 \times 25–36, $x=28.3$ μm , $n=20$) thick-walled: wall at side 2–6 μm thick, wall at apex 8–22 μm thick, with or without a very short cylindrical apical chamber; stipe short, 14–22 \times 4–6 μm , often strongly curved (FIGS. 5–7). *Ascospores* 3–4 seriate, 1-septate, becoming 3–5-septate late in development, fusiform, slightly constricted at mid-septum, hyaline, becoming pale brown with age, 38–70 \times 7–16 μm (type specimen – 46–62 \times 53.7 \times 7–12 \times 9.83 μm , $n=30$), filled with small lipid droplets to give a foamy appearance (FIG. 8), often guttulate (FIG. 9), surrounded by a fusiform gelatinous sheath (FIGS. 3, 8) which expands to form a long, amorphous, sticky enveloping sheath (FIGS. 10–13).

HOLOTYPE. UNITED STATES. WISCONSIN: Adams County, Lemonweir River, 43°46'16"N, 89°53'10"W, on submerged, decorticated woody debris, 31 July 1992, *CAS & JLC A-39-1* (ILLS 52289); culture from the holotype, ATCC 200398 (ILLS 52290).

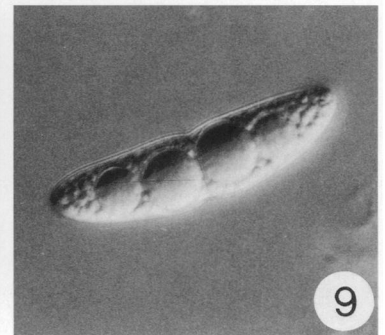
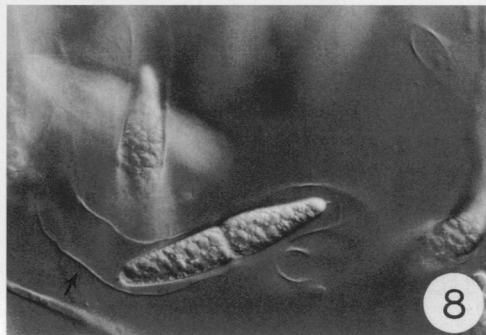
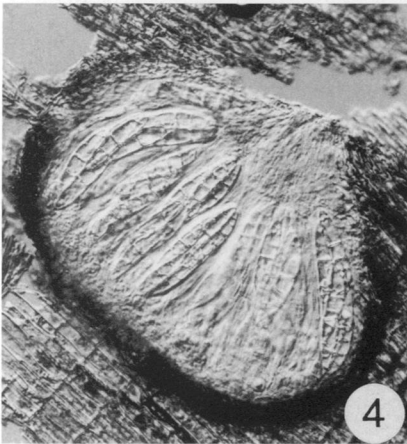
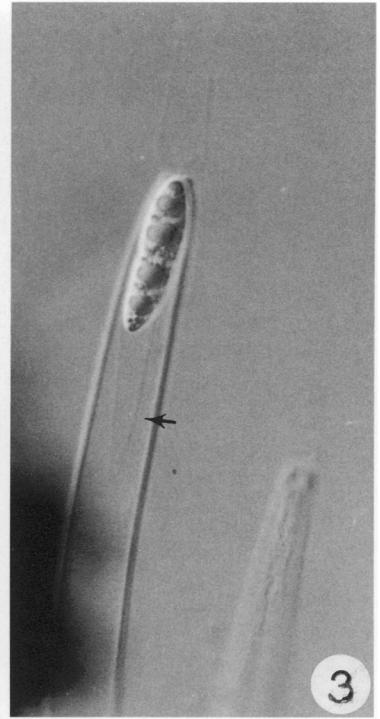
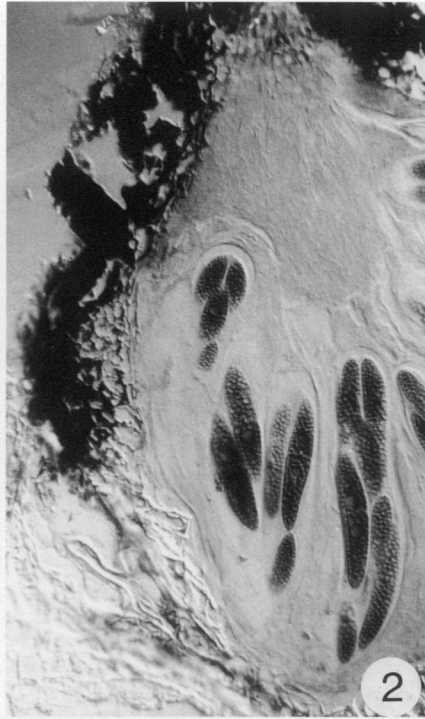
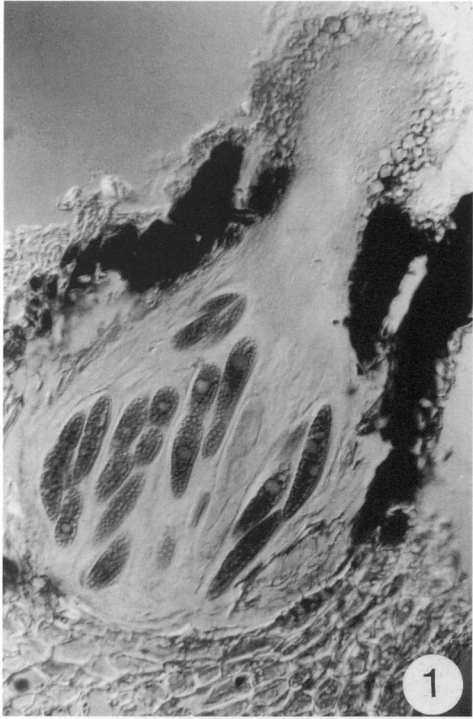
Additional material examined. AUSTRALIA. NORTH QUEENSLAND: Kuranda, Top of the range, on submerged wood, Dec. 1991, *KDH* [HKU(M) 1552]; Babinda, Boulders National Park, on submerged twig, 30 Dec. 1991, *KDH* [HKU(M) 879]. BRUNEI DARUSSALAM. Temburong, Sungai Belalong, Sungai Esu, on submerged wood in rainforest stream, 28 Feb 1994, *KDH & W. S. Wong SE14*, [HKU(M) 1938]; July 1993, *KDH* [HKU(M) B166]. MALAYSIA. Sungai Lipur Lentang, Oct. 1991, *KDH* [HKU(M) 1573]. UNITED STATES. FLORIDA: Big Cypress Nature Preserve, Monroe Station, 25°48'16.3"N, 81°6'2.7"W, on bark of submerged corticated wood, 29 Dec. 1994, *CAS & JLC A-39-4* (ILLS 52296). ILLINOIS: Alexander County, Horeshoe Lake, 37°6'59"N, 89°19'25"W, on partially decorticated submerged woody debris, 11 Mar. 1994, *CAS & JLC A-39-3* (ILLS); Johnson County, Elvira Cypress Swamp, Deer Pond, 37°25'43.2"N, 88°56'2.7"W, on bark of submerged corticated wood, 3 Sept. 1994, *CAS & JLC A-39-6* (ILLS). MAINE: York County, Shaker Pond, 43°29'33"N, 70°43'15"W, on bark of submerged corticated wood, 3 July, 1994, *CAS & JLC A-39-5* (ILLS 52297). VIRGINIA: Great Dismal Swamp, 36°N, 76°31'42"W, on submerged herbaceous stem, 17 May 1993, *CAS & JLC A-140-1* (ILLS

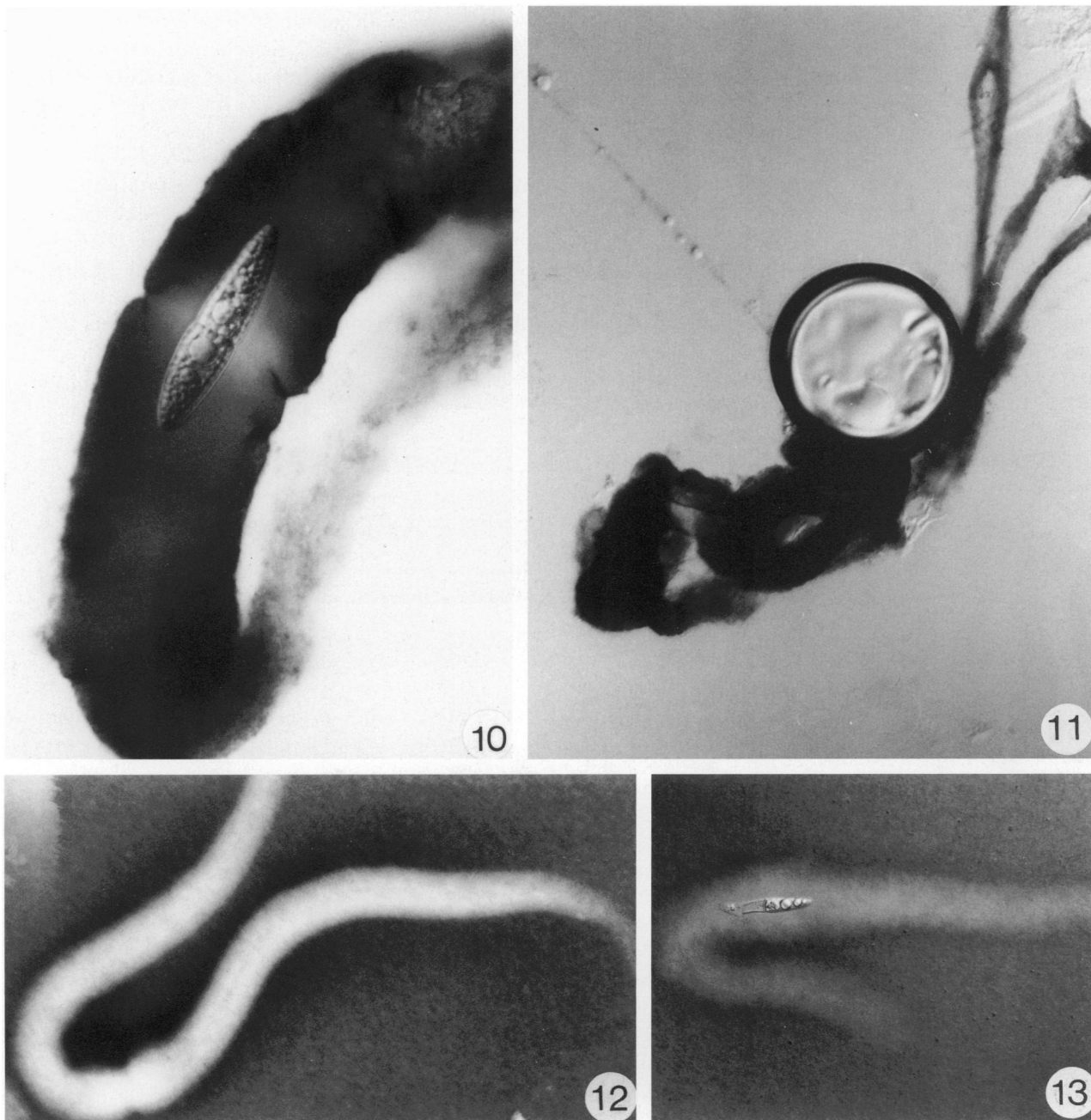
52295). WISCONSIN: Adams County, Lemonweir River, 43°46'16"N, 89°53'10"W, on bark of submerged, corticated woody debris, 25 Sept. 1993, *CAS & JLC A-140-2* (ILLS 52291); on bark of submerged, corticated woody debris, 18 May 1995, *CAS & JLC A-39-13* (ILLS); Richland County, Wisconsin River, Lower Wisconsin River State Wildlife Area, 43°12'40"N, 90°20'8.9"W, on partially decorticated submerged woody debris, 29 Oct. 1993, *CAS & JLC A-140-3* (ILLS 52293).

This fungus fits within the generic concept of *Massarina* in having pseudothecia of pseudoparenchymatous cells, cellular pseudoparaphyses, and one septate hyaline ascospores, tardily becoming 3–5 septate and darkened, and surrounded by a gelatinous sheath. Munk (1956) noted the presence of small darkened round cells around the ostiole found in the type species of *Massarina*, *M. eburnea* (Tul. & C. Tul.) Sacc. which he referred to as the *inter alia*. Later Munk (1957) referred to this region as a "clypeus-stroma". Whether or not the darkened tissue at the base of the beak of *M. ingoldiana* (FIGS. 1, 2) is homologous to the "clypeus-stroma" of *M. eburnea* is not clear. Collections that developed under the epidermis of corticated wood were usually found immersed in a common clypeus-like tissue. *Massarina ingoldiana* differs from other *Massarina* species in that the ascospores are constricted only at the mid-septum rather than at the midseptum and in both hemispheres as well, in the foamy appearance of the ascospores, and in the extremely large ascospore sheath.

Several other *Massarina* species have been reported from freshwater (Magnes and Hafellner, 1991; Leuchtmann, 1984), including some with aquatic hyphomycete anamorphs (Webster, 1965; Willoughby and Archer, 1973; Webster and Descals, 1979; Scheuer, 1991); five species have been reported from marine habitats (Kohlmeyer and Volkmann-Kohlmeyer, 1991). Barr (1987) questioned the placement of species with aquatic hyphomycete anamorphs in *Massarina* given that other anamorphs reported for the genus are coelomycetous. Several different isolates of *M. ingoldiana* were tested for the production of aquatic hyphomycete anamorphs, but none was observed and no other anamorphs were produced in culture either. Whether the group of species with aquatic hyphomycete anamorphs represents an evolutionary line distinct from *Massarina*, whether or not *M. ingoldiana* belongs in such a group, and how closely the marine *Massarina* species are related to the freshwater and terrestrial species are interesting questions worthy of additional study.

Considerable morphological variation occurred among the different populations of *M. ingoldiana*





FIGS. 10-13. *Massarina ingoldiana*. 10-12. From type specimen, ILLS. 10, 11. Material has been mounted in water, stained with India ink, and then rinsed with water. 10. Ascospore surrounded by a gelatinous sheath, $\times 860$. 11. Sticky sheaths of several ascospores adhering to an air bubble, $\times 150$. 12. Ascospore with sheath immersed in India ink, $\times 180$. 13. From specimen A-140-2. Ascospore with sheath immersed in India ink, $\times 135$.

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FIGS. 1-9. *Massarina ingoldiana*. 1, 2. From type specimen, ILLS. 1. Median section through ascoma, $\times 325$. 2. Median section through an ascoma showing darkened clypeus-like region around wall of beak, $\times 335$. 3. From specimen A-140-2. Ascospore with fusiform sheath (arrow) being discharged from endoascus, $\times 440$. 4. From specimen HKU(M) 1552. Partial section through an ascoma, $\times 660$. 5. From specimen A-140-2. Asci and paraphyses from fresh material, $\times 304$. 6. From specimen HKU(M) 1552. Asci showing thick wall, $\times 450$. 7, 8. From specimen A-140-2. 7. Endoascus emerging from ectoascus, $\times 315$. 8. Ascospore showing foamy appearance of ascospore and unexpanded ascospore sheath (arrow), $\times 390$. 9. From specimen HKU(M). Guttulate ascospore in water; sheath is no longer visible, $\times 720$.

sampled. The thickness of the ascomal wall and the length of the papilla/beak varied among populations and with substratum. Lengths and widths of ascospores and the degree of secondary septation and pigmentation also varied among populations. In some cases, secondary septa were observed in ascospores within asci, while in most cases, they were observed only in ascospores that had been released from asci.

The most consistent and distinguishing morphological characters are the ascospore sheath and the foamy appearance of the ascospore due to the presence of small lipid droplets. The ascospore sheath is extremely similar to that of *Pleospora scirpicola* (D. C.) Karst. (Ingold, 1955). Immediately after ascospore discharge, the sheath appears as a well-defined, fusiform covering with definite margins (FIGS. 3, 8). Soon thereafter, the sheath rapidly enlarges to an enormous size without definite margins and appears to be sticky, adhering to debris particles, air bubbles and glass microscope slides (FIGS. 10–13). Unfortunately, the sheath is completely invisible and the lipid droplets are cleared in lactic acid, and therefore, cannot be seen in slides deposited in herbaria or in fresh or dried material mounted directly into lactic acid. The sheath, once hydrated, cannot be seen in water (FIG. 9) unless India ink is added to the slide (FIGS. 10–13). The elongated, sticky sheath probably enables this fungus to attach to substrata in moving water.

Hyde (1995a) provided a description of *Massarina eburnea* (Tul. & C. Tul.) Sacc, the type of *Massarina*. Ascospores in this species have a narrow sheath and are four-celled. In fact, mucilaginous sheaths are a common feature in species of *Massarina* in marine, freshwater and terrestrial habitats. In the marine species *M. thalassiae* Kohlm. & Volkm.-Kohlm. and *M. velatasporea* K. D. Hyde & Borse the ascospores are surrounded by regular, wide, spreading sheaths (Hyde and Borse, 1986; Kohlmeyer and Volkmann-Kohlmeyer, 1987), while in *M. armatispora* K. D. Hyde et al. the sheath is drawn out at the ends (Hyde et al., 1992). In the freshwater species *M. bipolaris* K. D. Hyde and *M. fronsisubmersa* K. D. Hyde appendages are drawn out at the ends and contain an inner spine-like structure (Hyde, 1994, 1995b). Many of the terrestrial species of *Massarina* from palms also have sheaths. In *M. palmetta* (Cooke) M. E. Barr the ascospores are surrounded by a condensed sheath, 2 µm wide, while in *M. palmicola* K. D. Hyde & Aptroot, the sheath is invaginated at the central septum but swells around the ends of the spore (Hyde and Aptroot, 1996). The morphology of the sheath in *M. ingoldiana*, which enlarges to enormous size, is unlike that found in any other *Massarina* species. Our

experience indicates there to be little overlap among the species of *Massarina* occurring in freshwater, marine or terrestrial habitats, and therefore, we do not consider that *M. ingoldiana* has been described previously.

Massarina ingoldiana appears to be widely distributed geographically and occurs in both lotic and limnetic habitats. It occurs primarily on corticated and decorticated woody debris; only one collection, A-140-1, was found on nonwoody plant debris. This fungus is present in the mature ascomal stage on debris when it is removed from water. This indicates that it is actively reproducing in the aquatic environment.

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