

## ***Megalohypha*, a new genus in the Jahnulales from aquatic habitats in the tropics**

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**Abstract:** A new fungus collected from submerged wood in tropical forest streams in Panama and Thailand is described as a new genus and species in the Jahnulales. This fungus, *Megalohypha aqua-dulces*, is described based on ascospore morphology, which differs substantially from that of the other genera in the Jahnulales. It has these morphological features: ascomata hyaline, translucent, with subtending, wide, brown, septate, stoloniferous hyphae; peridium of large, thin-walled cells; hamothecium of septate pseudoparaphyses; asci clavate, fissitunicate, 8-spored; and ascospores 1-septate, brown, rough-walled, with longitudinal sulcate striations. An interesting feature of *Megalohypha aqua-dulces*, which it shares with some other taxa in Jahnulales, is the presence of both sessile and stalked fruiting bodies. In addition, the stalks have morphology that consists of a wide hypha with a narrower hypha apparently enclosed within and the stalks are strongly constricted at the septa.

**Key words:** euascomycetes, freshwater, fungal systematics, submerged wood

### INTRODUCTION

In our on-going inventory of freshwater filamentous euascomycetes, a new fungus with characteristics of the Jahnulales Pang, Abdel-Wahab, El-Sharouny, E.B.G. Jones & Sivichai was collected from streams in the tropical rain forests of Panama and from a stream in Thailand. The order Jahnulales was erected based on a well supported clade from molecular analyses and morphological characteristics

(Pang et al 2002) that include stalked/sessile ascomata, hyphal stalk cells that are ca. 40 µm wide, and 2-celled ascospores with or without appendages or sheaths (Pang et al 2002). While our new fungus presents the diagnostic morphological characteristics of the Jahnulales (Pang et al 2002) it is distinctively different from other genera in this order. Therefore the fungus is described as a new genus and species in the Jahnulales.

### MATERIALS AND METHODS

Samples of submerged, partially decomposed woody debris were collected from rivers and streams at Soberania National Park in lowland tropical forest in central Panama and Than Bok Khorani National Park in Thailand. Samples were placed in Ziploc™ plastic bags containing paper towels and returned to the laboratory. Samples were incubated in the laboratory in plastic boxes containing moistened paper towels at ambient temperature (about 24 C) and examined with a dissecting microscope immediately after collection and periodically for 9 mo.

Ascomata were placed on a cover glass on a microscope slide in a drop of distilled water and opened with dissecting needles to release asci and ascospores. Aqueous nigrosin or India ink was added to the water mounts to reveal appendages or gelatinous sheaths on the ascospores. Distilled water was replaced with glycerin for preservation using the double cover glass method (Volkmann-Kohlmeyer and Kohlmeyer 1996). Additional ascomata were fixed and sectioned with a modification of the technique outlined by Huhndorf (Huhndorf 1991, Fallah and Shearer 2001). Digital images were obtained with a Spot RT digital camera using an Olympus microscope equipped with Nomarski interference optics. Images were processed with Adobe Photoshop CS2 (Adobe Systems Inc., Mountain View, California). Specimens were deposited in the Herbarium of the University of Illinois at Urbana-Champaign (ILL) and the Herbarium of the University of Panama (PMA). Cultures were obtained and are maintained according to the procedures of Fallah and Shearer (2001) and are deposited in the University of Illinois Fungus Collection. Specimens collected and cultured in Thailand were deposited at BIOTEC Bangkok Herbarium (BBH) and BIOTEC Culture Collection (BCC), respectively.

### TAXONOMY

***Megalohypha*** A. Ferrer et Shearer, gen. nov.

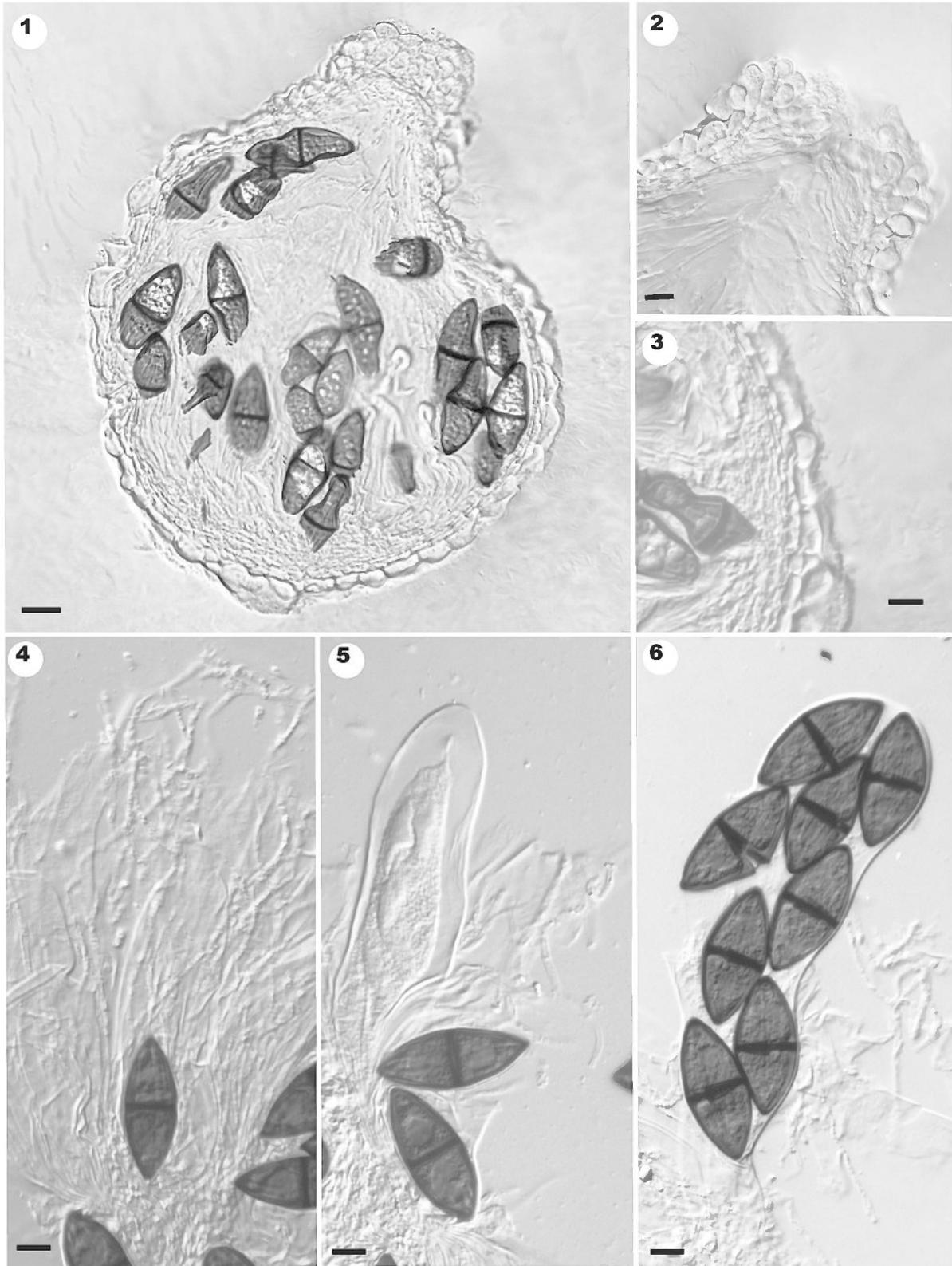
Type species: *Megalohypha aqua-dulces*

Etyymology: From the Greek “megalo” = big, large and “hypha” = hyphae

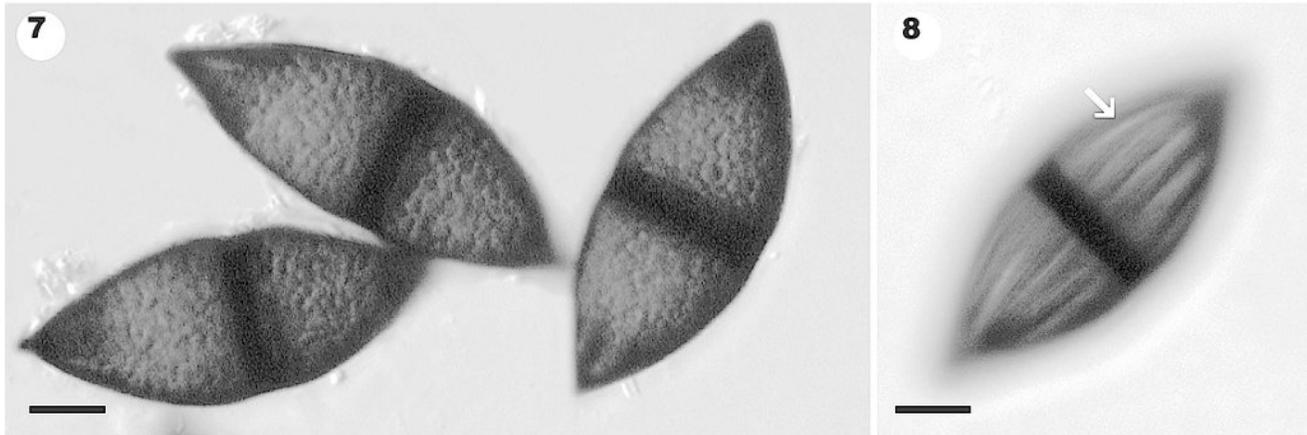
Ascomata superficialia, globosa vel obpyriformia, ostio-

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FIGS. 1–6. *Megalohypha aqua-dulces* collected from wood (Holotype AF005-1). 1. Longitudinal section through ascoma. 2. Neck showing subglobose divergent cells. 3. Longitudinal section showing cells of peridium. 4. Hamathecium. 5–6. Asci. Bars: 1 = 20  $\mu\text{m}$ , 2–6 = 10  $\mu\text{m}$ .



FIGS. 7–8. *Megalohypha aqua-dulces* collected from wood (Holotype AF005-1). 7. Ascospores. 8. Ascospore, arrow indicates sulcate striations. Bars: 7–8 = 10  $\mu\text{m}$ .

lata, papillata, habentes vel sine stipite, ad substratum connexi per hyphas latas, brunneas, septatas, stoloniformes. Paries peridii cellularis magnis, hyalinis, tenuitunicatis. Hamathecium pseudoparaphysibus. Asci fissitunicati, clavati, brevipedicellati, octospori. Ascosporeae brunneae vel fuscae, uniseptatae, symmetricae, tunicatae asperae, striis longitudinalis.

Ascomata superficial on wood, globose to obpyriform, ostiolate, papillate, stalked or sessile, connected to the substratum by broad, brown, septate, stoloniferous hyphae. Peridial wall of large, hyaline, thin-walled cells. Hamathecium pseudoparaphysate, pseudoparaphyses septate, branched, anastomosing above the asci. Asci fissitunicate, broadly clavate or fusiform, short pedicellate, 8-spored. Ascospores brown to dark brown, 1-septate, symmetrical, rough-walled, longitudinally striate.

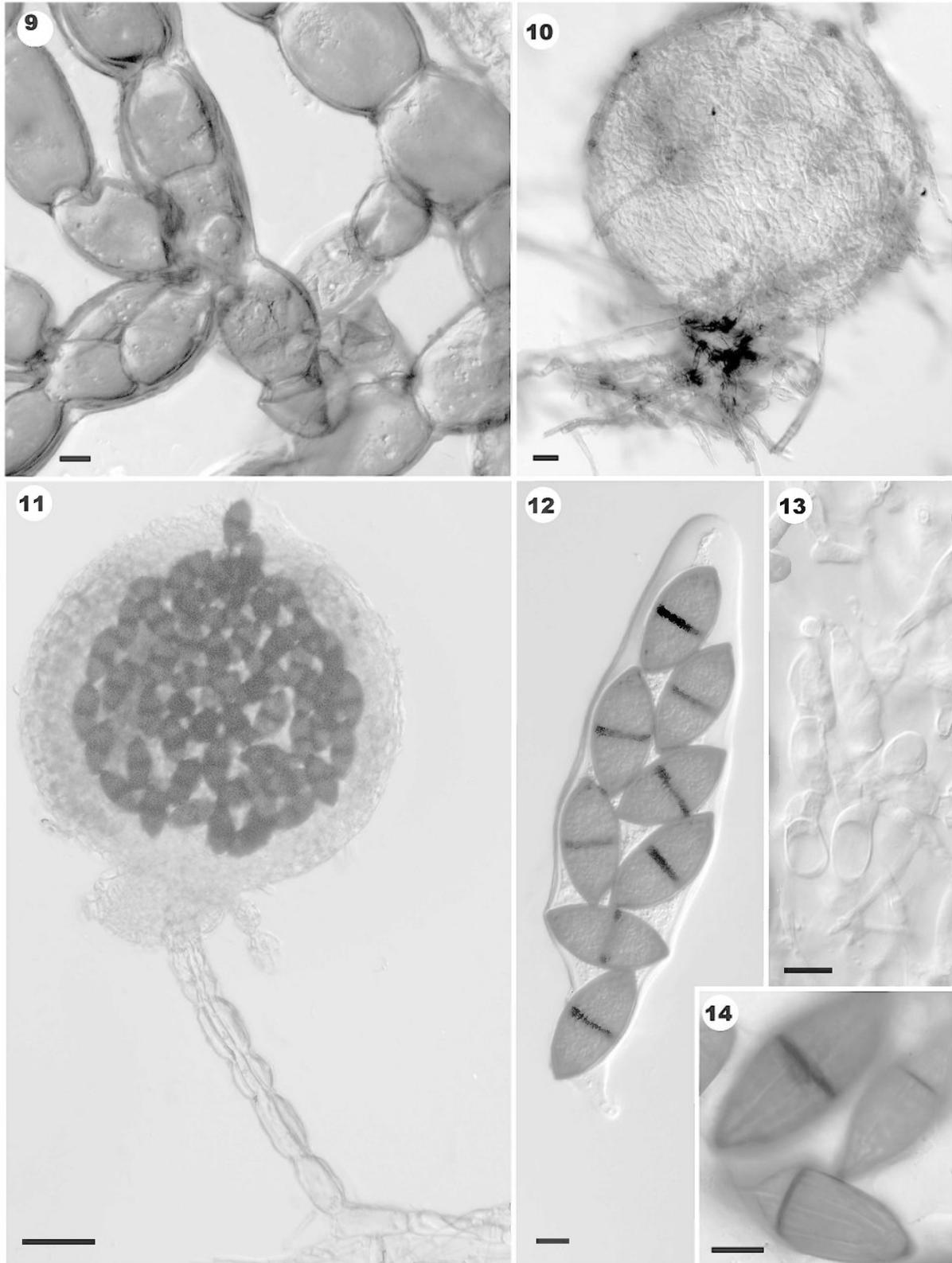
*Megalohypha aqua-dulces* A. Ferrer et Shearer, sp. nov. FIGS. 1–14

Ascomata 240–300  $\times$  200–250  $\mu\text{m}$ , superficialia, globosa vel obpyriformia, ostiolata, papillata, hyalina, membranacea, habentes vel sine stipite, ad substratum connexi per hyphas superficiales, latas, brunneas, septatas, stoloniformes. Papillae 55  $\times$  45  $\mu\text{m}$ , breve, cylindraceum, periphysibus hyalinis. Paries rostri e cellulis divergentibus extrinsecus, globosis, tenuitunicatis compositum. Peridium circa 15  $\mu\text{m}$  crassum, sectione longitudinali e cellulis 1–2 stratis hyalinis, tenuitunicatis compositum. Pseudoparaphyses, 3–4  $\mu\text{m}$  latae, filamentosae, septatae, hyalinae. Asci 110–160  $\times$  35–60  $\mu\text{m}$ , fissitunicati, clavati, brevipedicellati, octospori. Ascosporeae 40–55  $\times$  19–22  $\mu\text{m}$ , dispositae irregulariter, ellipsoideae, apicibus acutis, brunneae vel fuscae, uniseptatae, utraque cellula spatii aequi, tunicatae asperae, stria sulcata longitudinali, exappendiculatae.

Ascomata on wood 240–300  $\times$  200–250  $\mu\text{m}$ , superficial, globose to obpyriform, ostiolate, papillate, hyaline, membranous, connected to substrate by a brown, septate stalk strongly constricted at the septa,

20–25 wide and up to 450  $\mu\text{m}$  long; sessile ascomata connected to the substrate by superficial, wide, brown, septate, stoloniferous hyphae (FIG. 1). Papillae 55  $\times$  45  $\mu\text{m}$ , short, cylindrical, with inwardly growing, hyaline periphyses; wall of the papilla composed of outwardly diverging globose, thin walled cells (FIG. 2). Peridium about 15  $\mu\text{m}$  thick, in longitudinal section composed of 1–2 layers of hyaline, thin-walled cells (FIG. 3). Pseudoparaphyses 3–4  $\mu\text{m}$  wide, filamentous, septate, hyaline, branched and anastomosing above the asci (FIG. 4). Asci 110–160  $\times$  35–60  $\mu\text{m}$ , fissitunicate, clavate or ellipsoid, short pedicellate, with or without an apical chamber, 8-spored (FIGS. 5, 6). Ascospores 40–55  $\times$  19–22  $\mu\text{m}$  (mean = 48.3  $\times$  18.8  $\mu\text{m}$ , SD = 2.8  $\times$  0.91  $\mu\text{m}$ , n = 30), irregularly arranged, ellipsoidal, acutely tapered at apices, brown to dark brown, 1-septate, septum appearing as a dark band, both cells of equal shape and size, rough walled (FIG. 7) with longitudinal sulcate striations (FIG. 8) lacking appendages or a gelatinous sheath.

Colonies on potato-dextrose agar (PDA; Difco) effuse, with little aerial mycelium, slow growing, reaching 1.2 cm diam in 4 wk, dark brown and producing a pale yellow pigmentation of the surrounding agar. Hyphae brown, smooth, strongly constricted at the septa, 25–40  $\mu\text{m}$  wide (FIG. 9). No anamorph was observed. Stalked and sessile ascomata formed on PDA slants after 1 mo incubation at 24 C. Ascomatal stalks of wide septate hyphae strongly constricted at the septa with a narrower hypha contained within (FIGS. 10, 11). Asci and ascospores were formed; asci were equipped with an apical chamber (FIG. 12) and ascospores were a lighter brown color (FIG. 14) than those formed on natural substrates; wider pseudoparaphyses (4–9  $\mu\text{m}$ ) were observed (FIG. 13) in ascomata formed in culture than in those formed on natural substrates.



FIGS. 9–14. *Megalohypha aqua-dulces* grown in culture (AF005-2). 9. Mycelium. 10. Sessile ascomata. 11. Stalked ascomata. 12. Asci. 13. Hamathecium. 14. Ascospores. Bars: 9, 12, 13 and 14 = 10  $\mu$ m; 10 = 20  $\mu$ m; 10 = 20  $\mu$ m; 11 = 50  $\mu$ m.

*Specimens examined.* PANAMA. COLON PROVINCE: Soberania National Park, Juan Grande River, 9°8'N, 79°43'W, 12 Jan 2003 on submerged decorticated wood, A. Ferrer, AF005-1. (ILL). HOLOTYPE.

*Additional specimens examined.* PANAMA. COLON PROVINCE: Soberania National Park, Frijoles River, 9°8'N, 79°43'W, on submerged decorticated wood, 12 May 2003. A. Ferrer, AF005-2. THAILAND. KRABI PROVINCE: Than Bok Khorani National Park, Stream at Nature Trail, 8°23.6'N, 98°44.1'E, on submerged wood, 26 Jan 2006. S. Sivichai, SS3792.

*Known distribution.* PANAMA (COLON), THAILAND (KRABI)

*Etymology.* *Aqua-dulces* (*aqua* = water, *dulces* = sweet) in reference to its freshwater habitat.

#### DISCUSSION

Characteristics of this newly discovered fungus place it within the order Jahnulales. We have established a new genus for this fungus, *Megalohypha*, based on morphological differences with the existing genera in the Jahnulales, *Aliquandostipite*, *Jahnula*, and *Patescospora*. In addition molecular studies provide strong support for the placement of *M. aqua-dulces* on a single clade within the Jahnulales separate from the clades containing *Aliquandostipite*, *Jahnula* and *Patescospora* (Campbell J, Ferrer A, Raja HA, Sivichai S, Shearer CA unpublished data).

*Megalohypha* differs from all genera currently included in the Jahnulales in having vegetative hyphae and ascotal stalks that are strongly constricted at the septa (FIGS. 9, 11). In addition the ascospores have bipolar symmetry, acute as opposed to rounded apices, a dark band at the midseptum (FIGS. 7, 8, 14) and sulcate striations on the ascospore wall (FIGS. 8, 14). In addition mature ascomata of *Jahnula* species are more strongly pigmented brown and have a thicker peridium than those of *Megalohypha*. Ascomata of *Megalohypha* however resemble those of *Aliquandostipite* in gross morphology, but ascospores of *Aliquandostipite* are more elongated in outline, lighter (hyaline to pale brown), the upper cell is slightly larger than the lower cell, are surrounded by a large gelatinous sheath (Inderbitzin et al 2001, Raja et al 2005) and do not have sulcate wall striations.

A unique characteristic among the Jahnulales is the presence of narrower hyphae enclosed within the hyphal stalk cells and vegetative hyphae that are the largest (10–40 µm wide) observed in the euascomycetes (Hyde and Wong 1999, Inderbitzin et al 2001, Pang et al 2002). Pang et al (2002) suggested that the wide vegetative hyphae might be an apomorphic character in the Jahnulales. The function of the wide vegetative hyphae is not yet understood, but because

all species in the Jahnulales have been described from aquatic or wet habitats it might reflect an adaptation in this group to such habitats. Raja and Shearer (2006) and Vijaykrishna et al (2006) proposed that the broad, stoloniferous hyphae might play an important role in colonization and holding fungi onto softened wood in aquatic habitats.

Species in the order Jahnulales may play a role in decomposing organic matter in aquatic habitats; a recent study (Simonis J, Raja HA, Shearer CA unpublished data) shows that *Megalohypha aqua-dulces* can produce soft rot cavities in balsa wood.

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#### LITERATURE CITED

- Fallah PM, Shearer CA. 2001. Freshwater ascomycetes: new or noteworthy species from north temperate lakes in Wisconsin. *Mycologia* 93:566–602.
- Huhndorf SM. 1991. A method of sectioning ascomycete herbarium specimens for light microscopy. *Mycologia* 83:520–524.
- Hyde KD, Wong SW. 1999. Tropical Australian freshwater fungi XV. The ascomycete genus *Jahnula*, with five new species and one new combination. *Nov Hedwig* 68:489–509.
- Inderbitzin P, Landvik S, Abdel-Wahab A, Berbee ML. 2001. *Aliquandostipitaceae*, a new family for two new tropical ascomycetes with unusually wide hyphae and dimorphic ascomata. *Am J Bot* 88:52–61.
- Pang KL, Abdel-Wahab MA, Sivichai S, El-Sharouney HM, Jones EBG. 2002. *Jahnulales* (*Dothideomycetes*, *Ascomycota*): a new order of lignicolous freshwater ascomycetes. *Mycol Res* 106:1031–1042.
- Raja HA, Ferrer A, Shearer CA. 2005. *Aliquandostipitate crystallinus*, a new ascomycete species from wood submerged in freshwater habitats. *Mycotaxon* 91:207–215.
- , Shearer CA. 2006. *Jahnula* species from North and Central America, including three new species. *Mycologia* 98:319–332.
- Vijaykrishna D, Jeewon R, Hyde KD. 2006. Molecular taxonomy, origins and evolution of freshwater ascomycetes. *Fungal Divers* 23:367–406.
- Volkman-Kohlmeier B, Kohlmeier J. 1996. How to prepare truly permanent microscope slides. *Mycologist* 10:107–108.