Mycoscience (2007) 48:395–398
DOI 10.1007/s10267-007-0375-3

SHORT COMMUNICATION

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Freshwater ascomycetes: *Aliquandostipite minuta* (Jahnulales, Dothideomycetes), a new species from Florida

Received: March 28, 2007 / Accepted: June 22, 2007

Abstract An undescribed ascomycete similar to species in the Aliquandostipitaceae (Jahnulales, Dothideomycetes) was collected from submerged wood in a freshwater swamp in Big Cypress National Preserve, Florida. The characteristic features of the new species are as follows: (i) ascomata are small, sessile, light brown, globose to subglobose, papillate, and anchored to the substrate by wide, brown, septate and subtending hyphae; peridial wall is composed of 1 to 2 layers of large, angular cells with large lumens; (ii) asci are ovoid to broadly clavate, and fissitunicate; (iii) ascospores are one-septate, fusiform, multiguttulate, pale brown, surrounded by a fusiform gelatinous sheath, and equipped with numerous filamentous appendages around the midseptum. The new fungus is most similar to *Aliquandostipite crystallinus*, from which it differs in overall smaller size and morphology. This new fungus is described and illustrated herein as *A. minuta.*

Key words Aquatic · Fungi · Submerged wood · Swamp · Systematics

During ongoing studies of freshwater ascomycetes along a latitudinal gradient in North America (Raja et al. 2003, 2005; Raja and Shearer 2006a,b), we found an undescribed ascomycete fungus on submerged wood in a freshwater swamp in Big Cypress National Preserve, Florida. This fungus has characteristics similar to taxa in the Jahnulales (Dothideomycetes) that include (i) hyaline, globose to subglobose, superficial to partially immersed ascomata attached to the substrate by wide (5–15 μm), brown, subtending hyphae; peridial walls 1 to 2 cell layers thick, composed of large, angular to globose cells with large lumens; (ii) clavate to ovoid asci that separate readily from the ascogenous hyphae; (iii) fusiform, smooth-walled, one-septate ascospores surrounded by a gelatinous sheath. Within the Jahnulales, these morphological characteristics fit the generic concept of *Aliquandostipite* Inderb. (Inderbitzin et al. 2001).

The overall small size of the ascomata, asci, and ascospores, and ascospore characteristics such as the presence of a fusiform gelatinous sheath constricted at the midseptum and numerous filamentous appendages radiating out at the ascospore midseptum, distinguish this ascomycete from all other described species of *Aliquandostipite* (Inderbitzin et al. 2001; Raja et al. 2005), and it is herein described and illustrated as a new species.

Specimen collection methods for freshwater ascomycetes are outlined in Shearer et al. (2004). With the aid of a dissecting microscope, ascomata on incubated wood were located and removed with fine needles and placed in a drop of distilled water on a glass slide. Ascomata were teased open with fine needles to release asci, ascospores, and pseudoparaphyses. India ink or aqueous nigrosin was added to the aqueous mounts to reveal gelatinous sheaths on or around the ascospores. Measurements of the pseudoparaphyses, asci, and ascospores were made of material mounted in distilled water. For preservation, distilled water was replaced with lactic acid containing azure A or with glycerin using the double cover glass method (Volkmann-Kohlmeyer and Kohlmeyer 1996). Holotype material was deposited in the Herbarium of the University of Illinois at Urbana Champaign (ILL). Digital micrographs were obtained with a Spot RT digital camera using an Olympus microscope equipped with Nomarski interference and phase optics. Digital images were edited using Adobe Photoshop CS2.

Aliquandostipite minuta Raja & Shearer, sp. nov.

Figs. 1–13

Ascomata lignatilia 130–150 × 150–160 μm, globosa vel subglobosa, ostiolata, papillata, immersa ex parte immersa vel superficiala, gregaria, primo hyalina demum atrobrunnea, hyphis latis brunneis subtentia. Peridium e textura

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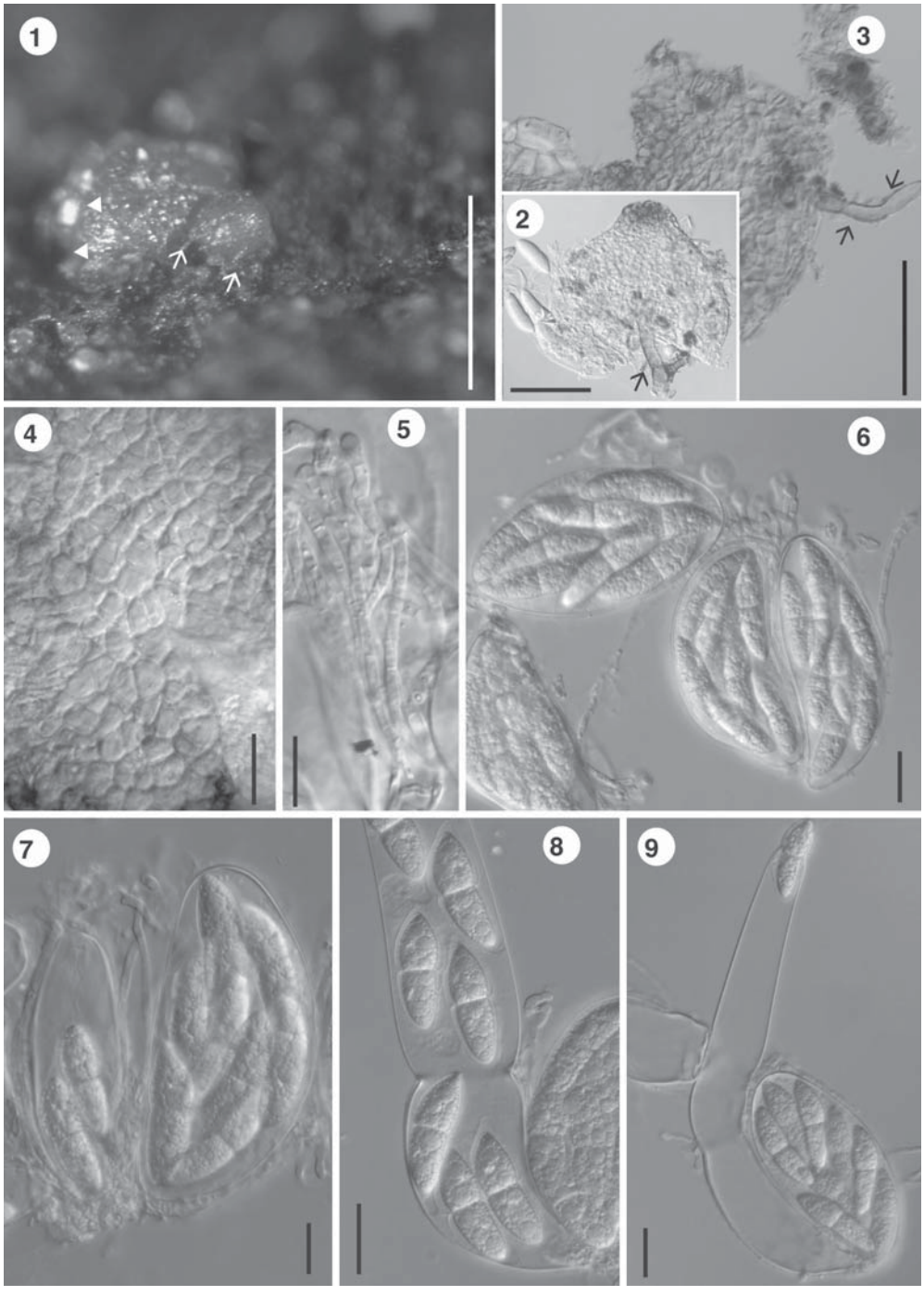


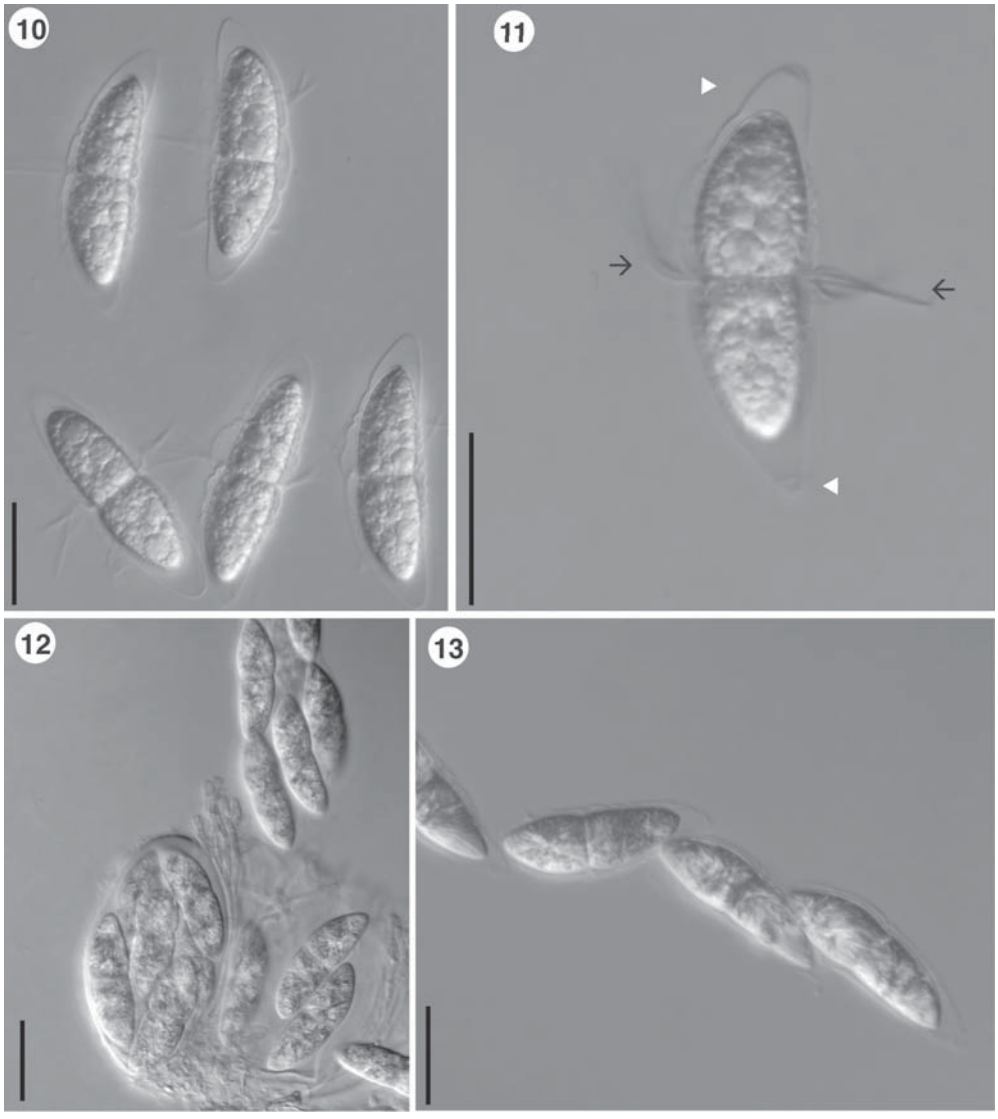
Figs. 1–13. *Aliquandostipite minuta* from the holotype. **1** Ascoma on wood. Note the large fruit body of *A. khaoyaiensis* (*arrowheads*) and smaller fruit body of *A. minuta* (*arrows*). **2, 3** Crush mount of ascomata; note *arrows* showing the wide, brown subtending hypha. **4** Peridium in surface view. **5** Pseudoparaphyses. **6**, **7** Ovoid to broadly clavate asci. **8**, **9** Fissitunicate ascus dehiscence. **10** Ascospores with gelatinous sheaths constricted at the midseptum. **11** Ascospore showing fusiform sheath (*arrowheads*) and filamentous appendages (*arrows*). **12**, **13** Ascospores fixed in glycerin showing refractive acicular crystals. *Bars* **1** 1 mm; **2** 50 µm; **3** 100 µm; **4–13** 20 µm

angularis compositum. Pseudoparaphyses septatae, hyalinae, ca. 2 µm latae. Asci fissitunicati, octospori, ovoidei vel clavati, apice rotandati, 74–112 × 36–58 µm. Ascosporae uniseptatae, fusiformes, multiguttulatae, 42–54 × 10–14 µm, dispositae irregulariter, pallide brunneae, vagina fusiformi mucilagini 5–18 µm longi circumcinctae, ad septum appendicibus numerosis 15–20 µm longis cinctae.

Etymology. From Latin *minutus* in reference to the small size of ascomata, asci, and ascospores compared to those of other described species of *Aliquandostipite*.

Ascomata on wood about 130–150 × 150–160 µm, hyaline to light brown (Fig. 1), globose to subglobose, ostiolate; darkened around the ostiole, papillate (Fig. 2), immersed to partially superficial, scattered, with wide, brown subtending hyphae approximately 5–15 µm wide (Figs. 2, 3). Peridial wall composed of large cells, lumen of the cells about 5 × 15 µm wide, *textura angularis* in surface view (Fig. 4). Pseudoparaphyses 2 µm wide, septate, hyaline, slightly swollen at the apex (Fig. 5). Asci 74–112 × 36–58 µm (mean = 96 × 44 µm; n = 20), sparse, ovoid to broadly clavate (Figs. 6, 7), broadly rounded at the apex, fissitunicate (Figs. 8, 9); endoascus wall 3–5 µm thick, and extending to 110–120 µm from the opening of the ectoascus, with or without an apical chamber, stalk absent or very short; ascus separating from

Figs. 1–13. Continued



the ascogenous hyphae at maturity (Figs. 6, 7), 8-spored. Ascospores 42–54 × 10–14 µm (mean = 47 × 12 µm; *n* = 40), hyaline, becoming pale brown; irregularly arranged, fusiform, multiguttulate, one-septate; upper cell slightly broader than basal cell, ascospores surrounded by a fusiform mucilaginous sheath about 5–18 µm long at the ascospore apices and constricted at the midseptum, with numerous filamentous appendages, unleafing from the ascospore sheath in water and forming a fringe around the midseptum (Figs. 10, 11); appendages about 15–20 µm long, staining in aqueous nigrosin, not visible in glycerin or lactic acid. Refractive acicular crystals present in cells of the ascospores after preservation in glycerin or lactic acid (Figs. 12, 13).

Colonies on peptone yeast extract glucose (PYG) agar growing slowly, reaching about 25 mm in 10 days; aerial hyphae white, immersed hyphae brown, effuse; hyphae wide, about 8–16 µm, thick-walled, septate, deeply constricted at the septa; producing a light pinkish brown pigment that diffuses into the surrounding agar; no anamorph observed.

Habitat: Saprobic on submerged decorticated wood in a swamp.

Material examined: USA: Florida, Monroe County, freshwater swamp at Big Cypress National Preserve, 25°46′53″ N, 81°05′31″W, on submerged decorticated wood, water temperature 20°C, pH 7, February 17, 2006. H.A. Raja and J.L. Crane (holotype: ILL 40108; = H.A. Raja and J.L. Crane F117-1). Ex-holotype culture, H.A. Raja F117-1.

Known distribution: USA (Florida).

Although *A. minuta* is similar to *A. crystallinus* Raja, A. Ferrer & Shearer in having globose to subglobose, ascomata, fusiform ascospores and producing refractive acicular crystals in ascospores fixed in glycerin and lactic acid (Raja et al. 2005), it differs from *A. crystallinus* in size of ascomata, shape and size of asci, ascospore sheath morphology, and the presence of median, filamentous ascospore appendages. The ascomata of *A. minuta* are smaller in size (130–150 × 150–160 µm) compared to the larger ascomata of *A. crystallinus* (215–270 × 220–320 µm) (Raja et al. 2005). The

asci of *A. minuta* are small, 74–112 × 36–58 µm, ovoid to broadly clavate, whereas those of *A. crystallinus* are larger in size, 132–174 × 30–41 µm, and clavate to cymbiform. The ascospore sheath in *A. minuta* is small, fusiform, and constricted at the midseptum, whereas in *A. crystallinus*, the ascospore sheath is longer, sigmoidal, or C-shaped and not constricted at the midseptum. In addition, ascospores of *A. crystallinus* possess two short, cellular, hyaline, tapering appendages at the ascospores apices, a character not seen in *A. minuta*. *Aliquandostipite minuta*, on the other hand, possesses numerous median filamentous appendages (Figs. 10, 11), a character absent in *A. crystallinus*.

Aliquandostipite minuta differs considerably from *A. khaoyaiensis* Inderb., the type species of the genus *Aliquandostipite* and the family Aliquandostipitaceae (Inderbitzin et al. 2001) in overall size of ascomata, asci, and ascospores as well as in ascus and ascospore sheath morphology. The ascomata of *A. minuta* are smaller in size (130–150 × 150–160 µm) compared to larger ascomata of *A. khaoyaiensis* (319 × 232 µm) (see Fig. 1). The asci of *A. minuta* are ovoid to broadly clavate, and shorter (74–112 µm), whereas asci in *A. khaoyaiensis* are clavate and longer (136–194 µm) (Inderbitzin et al. 2001). The ascospores in *A. minuta* are smaller in size (42–54 × 10–14 µm), and are surrounded by a fusiform sheath, which is constricted at the midseptum, and bear numerous median filamentous appendages, while in *A. khaoyaiensis* the ascospores are larger (49.6–70 × 12.8–20 µm), surrounded by a large, thick-walled, slug-like unconstricted sheath, and do not bear filamentous appendages.

All the species of the genus *Aliquandostipite* have a gelatinous sheath surrounding the ascospores (Inderbitzin et al. 2001; Raja et al. 2005); however, only *A. minuta*, in addition to having a fusiform gelatinous sheath, possesses numerous filamentous appendages around the ascospore midseptum, a rare feature among freshwater ascomycetes, but well known in marine ascomycetes in genera such as *Corollospora* Werderm. and *Nereiospora* E.B.G. Jones, R. G. Johnson & S.T. Moss (Kohlmeyer and Volkmann-Kohlmeyer 1991; Jones 1995, 2006). Because *A. minuta* occurs in the Dothideomycetes and *Corollospora* and *Nereiospora* are in the Sordariomycetes, this is likely a case of convergent evolution. Transmission and scanning electron microscopy studies are required, however, to determine whether the median appendages in the freshwater and marine fungi are homologous with respect to development, structure, and composition.

Acknowledgments We appreciate the comments provided by the two anonymous reviewers and a corresponding editor, who greatly improved this paper. We would like to thank Dr. J.L. Crane for assistance with collecting. Appreciation is expressed to the personnel of Big Cypress National Preserve for permission to collect within the park. Financial support for this study by the National Science Foundation Grant (DEB-0316496), and National Institute of Health (NIH Grant No. R01GM-60600) is gratefully acknowledged. This paper represents a portion of a thesis (H.A.R.) in partial fulfillment of the requirements for the doctoral degree at the Graduate College of the University of Illinois at Urbana-Champaign.

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