
***Arnium gigantosporum*, a new ascomycete species from fresh water in Florida**

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Arnium gigantosporum, a new ascomycete species found on submerged wood in a Florida lake, is described and illustrated. The characteristic features of this species are large, semi-transparent, membranous ascomata; a dark, opaque, neck covered with pointed, tapering, long tufts of agglutinated, rigid, septate, cylindrical, brown hairs at an angle of 30-45° to the peridium; broad, clavate, swollen asci with a long stipe; and large, aseptate, fusiform, dark brown ascospores, which are rough-walled in a horizontally striated pattern and surrounded by a striated gelatinous sheath.

Key words: aquatic, fungal systematics, *Lasiosphaeriaceae*, lentic, *Sordariales*

Introduction

During a latitudinal survey of freshwater ascomycetes in North America, an unusual species was found on woody debris submerged in a Florida lake. Features such as presence of subicular tissue; black, hairy, membranous, perithecial ascomata, presence of paraphyses; clavate asci; dark ascospores with a gelatinous sheath; and saprophytic, lignicolous habit are characteristic of many *Sordariales* (Kirk *et al.*, 2001). The large, semi-transparent, membranous ascomata; a dark opaque neck with agglutinated rigid brown cylindrical hairs; clavate, swollen asci; and dark brown aseptate, fusiform ascospores of this fungus agree with the characteristics of the genus *Arnium* Nitschke ex G. Winter (Lundqvist, 1972; Krug and Cain, 1972; Doveri, 2004) in the *Lasiosphaeriaceae*. This fungus fits best within the section *Echria* N. Lundq. (Lundqvist, 1972) and differs from other described species in having large, rough-walled, horizontally striated, fusiform ascospores with a striated gelatinous sheath. We therefore, describe this fungus as a new species of *Arnium* sect. *Echria*.

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Materials and methods

Collection techniques and single spore isolation methods are presented in Shearer *et al.* (2004). Slides were preserved using the double cover glass method of Volkmann-Kohlmeyer and Kohlmeyer (1996). Ascomata were fixed and embedded using a modification of Huhndorf's technique (1991) that is described in Raja *et al.* (2005). Ascomata were sectioned following the procedures of Fallah and Shearer (2001). Images were taken using an Olympus microscope with Nomarski differential interference contrast microscopy equipped with a Spot RT digital camera. Slides and dried cultures are deposited in the Herbarium of the University of Illinois at Urbana-Champaign (ILL) and the Illinois Natural History Survey Mycology Herbarium (ILLS).

Taxonomy

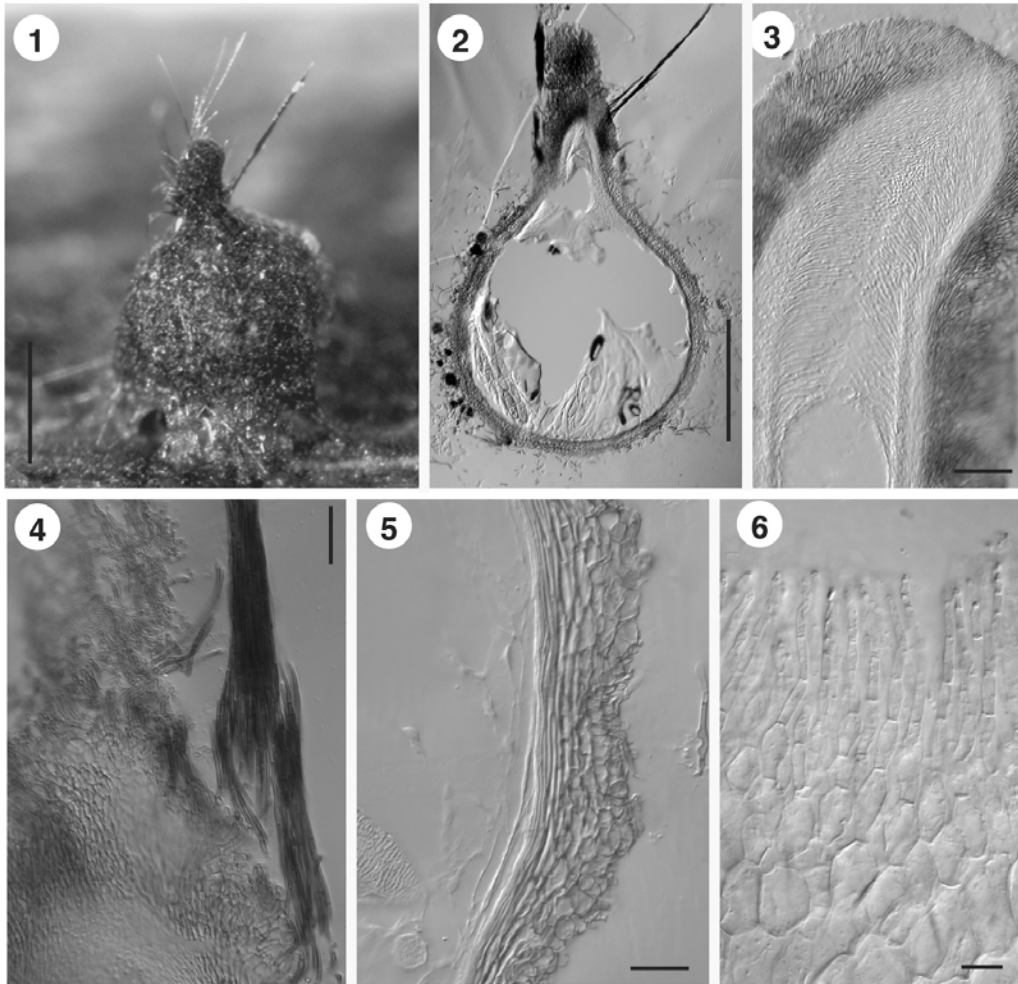
Arnium gigantosporum Raja & Shearer, **sp. nov.** (Figs. 1-15)

Mycobank number: MB500930.

Etymology: From the Greek "gigas" = giant and "spora" = seed or spore

Ascomata 760-970 × 415-560 µm, superficialia ad semiimmersa in ligno, obpyriforma, ostiolata, papillata. Rostrum 150-200 × 78-98 µm, cylindricum ad conicum, cum pilis longis, brunneis. Peridium 30-35 µm latum, membranaceum, duobus partibus instructum. Paraphyses filiformi-ventricosae. Asci 200-437 × 45-155 µm, octospori, clavati, longe stipitati, ad apicem rotundati. Ascospores 84-126 × 20-34 µm, monostichae vel distichae, fusiforme, brunneae, ad libellam cum concrecente vagina extensa in aqua. Holotypus F77-1.

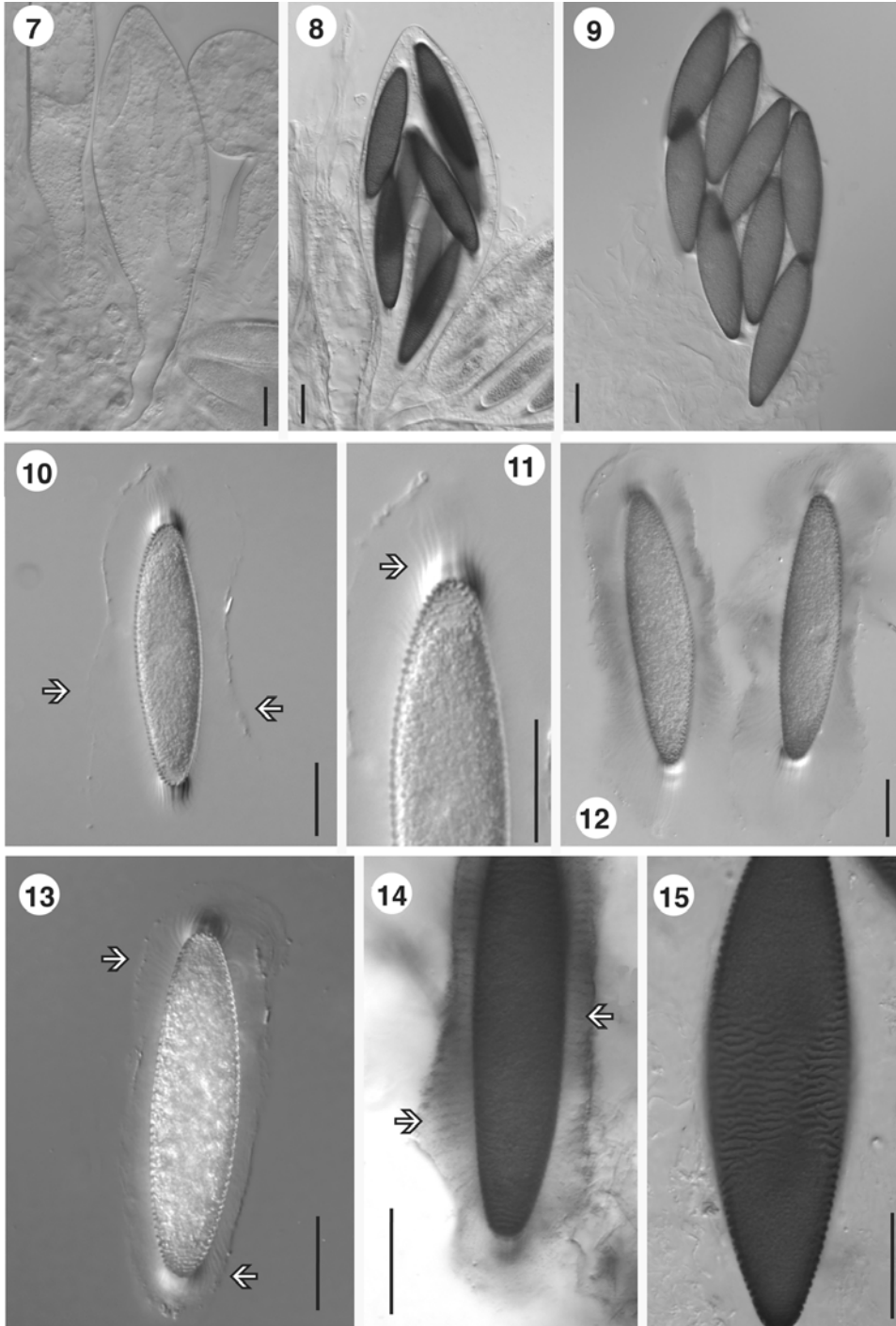
Ascomata on wood 760-970 × 415-560 µm, superficial to partially immersed, subiculate or not, tomentose, scattered, obpyriform, ostiolate, membranous (Figs. 1-2). *Neck* 150-200 × 78-98 µm, cylindrical to conical, opaque, black, inside lined with hyaline, filiform paraphyses (Fig. 3), covered with pointed, unbranched, tapering, long tufts of agglutinated, rigid, septate, cylindrical, brown hairs measuring *ca.* 100-500 µm long and 2 µm wide, occurring at an angle of 30-45° to the peridium (Fig. 4). *Peridium* 30-35 µm thick, pseudoparenchymatous in surface view, semitransparent; in longitudinal section, peridial wall 2-layered, inner layer composed of 2-3 rows of elongated, flattened, thin-walled, hyaline to brown cells; outer layer composed of 3-4 rows of brown, thin-walled, angular cells 10-20 × 3-5 µm wide (Fig. 5). *Paraphyses* hyaline, filiform-ventricose, 100-120 µm long, *ca.* 10-12 µm wide at the base, 3-4 µm wide at the apex, septate, abundant (Fig. 6). *Asci* 200-437 × 45-155 µm (\bar{x} = 281 × 90 µm, n = 25), eight-spored, unitunicate, thin-walled, broadly clavate, with a long stipe *ca.* 50-100 µm long, swelling in water; ascus apex narrowly rounded, without an apical ring (Figs. 7-9). *Ascospores* 84-126 × 20-34 µm (\bar{x} = 109 × 27 µm, n = 50), irregularly arranged, one-celled, hyaline when young (Figs. 10-11, 13), ranging from golden to dark brown at maturity



Figs. 1-6. *Arnium gigantosporum* (from holotype). 1. Ascomata on wood. 2. Longitudinal section of ascomata. 3. Periphysate neck. 4. Neck with long tufts of rigid agglutinated brown hairs. 5. Longitudinal section of the peridium. 6. Paraphyses, with swollen cells at the base and tapering towards the apex. Bars. Figs. 1-2 = 200 μm ; 3, 4, 5 = 20 μm ; 6 = 10 μm .

(Figs. 12, 14, 15); fusiform, rough walled in a horizontal striated pattern (Fig. 15); surrounded by a striated gelatinous sheath, which expands in water to *ca.* 5-8 μm wide at the mid region, and *ca.* 10-20 μm long at the ascospore apices (Figs. 10-15); sheath staining in aqueous nigrosin and India ink, absent in older ascospores; germ pores not observed.

Colonies on corn meal agar (CMA, Difco) grown at 25°C, hyaline to dark green. Mycelium effuse, immersed in agar, composed of branched, septate, pigmented hyphae. Colonies on potato dextrose agar (PDA, Difco) grown at 25°C, dark green in the centre and hyaline towards the periphery. Ascomata



Figs. 7-15. *Arnium gigantosporum* (from holotype). 7. Young ascus showing a long stipe. 8-9 Mature asci. 10. Young hyaline ascospore, note arrows showing gelatinous sheath expanded in water. 11 and 13. Ascospore showing striated sheath at the apex. 12. Ascospores showing sheath stained in India ink. 14. Ascospores with a striated gelatinous sheath stained with aqueous nigrosin; arrows indicate striations. 15. Mature ascospore showing a horizontal striated pattern on the ascospore wall. Bars. Figs. 7-15 = 20 μ m.

formed at the centre of colonies after 2-3 weeks on CMA and PDA, superficial to partially immersed in the agar. As the ascomata mature, the ascospores are shot out forcefully from the neck of the ascomata and adhere to the lid of the culture plate where they germinate. Some ascospores that are shot out to the periphery of the agar plate germinate and give rise to new ascomata within 2-3 weeks.

Anamorph: not seen.

Habitat: lentic.

Known distribution: Florida.

Material examined: USA, Ocala National Forest, Lake Kerr, on submerged, decorticated wood, 29°21'19"N, 81°48'45"E, UTM Zone 17, 17421130mE, 3247637mN, water temperature 21 C, pH 7, 3 March, 2005, *Huzefa A. Raja, Andrew N. Miller, and J.L. Crane*, F77-1. (ILL, slide made from a specimen on submerged, decorticated wood, F77-1. **holotype**).

Discussion

Currently, 34 species are included in the genus *Arnium* (Doveri, 2004). Morphological features that appear to distinguish *A. gigantosporum* from other taxa in the genus are the large size of the ascospores, and an ascospore wall roughening in a horizontal striated pattern (Figs. 10-15).

Arnium gigantosporum is placed within the sect. *Echria*, which contains four species (Lundqvist, 1974; Jeng and Krug, 1977). *Arnium gigantosporum* is most similar to *A. macrotheca* (H. Crouan & P. Crouan) N. Lundq., the type species of the sect. *Echria*, in having ascospores with a striated gelatinous sheath (Figs. 10-14), long-stipitate, clavate asci (Fig. 7) and presence of rigid agglutinated, brown, cylindrical hairs on an opaque, black neck (Figs. 2, 4). *Arnium gigantosporum*, however, differs from *A. macrotheca* in the size and morphology of ascospores and texture of the ascospore wall.

Most species in the genus *Arnium* grow on various kinds of herbivore dung, while a few species are herbicolous (Lundqvist, 1972). Other than *A. gigantosporum* only two species, *A. olerum* (Fr. : Fr.) N. Lundq. & J.C. Krug and *A. tomentosum* (Speg.) N. Lundq. & J.C. Krug, have been reported from a lignicolous substrate (Lundqvist, 1972; Lorenzo and Havrylenko, 2001). *Arnium gigantosporum* is the second species to be reported from an aquatic habitat. Only one other *Arnium* species, *A. apiculatum* (Griffiths) N. Lundq.,

has been collected from an aquatic habitat (Shearer *et al.*, 1980). *Arniium gigantosporum* differs from *A. apiculatum* in ascus and ascospore size and morphology.

It is plausible that the piece of wood from which *A. gigantosporum* was isolated could have been impregnated with herbivore dung before it got washed into the lake. Jeng and Krug (1977) have reported a number of *Arniium* spp. on herbivore dung collected from close proximities to water bodies, such as lakes and streams where many herbivores come to graze. It is also likely that the enzymes that *Arniium* spp. use to decompose ligno-cellulose substrates such as those in herbivore dung allowed it to colonize wood in water. At this time it is not clear if *A. gigantosporum* is an aquatic species; additional collections of this fungus will help understand the true habitat of this remarkable ascomycete.

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