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## ***Aliquandostipite crystallinus*, a new ascomycete species from wood submerged in freshwater habitats**

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**Abstract**—*Aliquandostipite crystallinus*, a new species of loculoascomycete from wood submerged in freshwater habitats in Costa Rica, Panama, USA (TN), and Venezuela, is described and illustrated. The characteristic features of the new species are sessile, light to dark brown, globose to subglobose, papillate ascomata; broad, brown, septate stoloniferous hyphae connecting adjacent ascomata; large, thin-walled peridial cells; clavate to cymbiform, fissitunicate asci; fusiform, one to three septate, multiguttulate, pale brown ascospores with short, cellular, hyaline, tapering, apical appendages; ascospores surrounded by a long, broad, fusiform gelatinous sheath that elongates in water; and refractive acicular crystals that form within the ascospores and hyphae upon preservation in lactic acid or glycerin. *Aliquandostipite khaoyaiensis* is reported for the first time from the Western Hemisphere in Costa Rica on wood submerged in freshwater streams.

**Keywords**—aquatic, fungi, Great Smoky Mountains National Park, Loculoascomycetes, taxonomy

### **Introduction**

As part of our ongoing inventories of freshwater filamentous ascomycetes along latitudinal gradients in the Western Hemisphere, collections of woody debris were made from various freshwater habitats. During these inventories, a fungus with morphological characteristics similar to those of the genus *Aliquandostipite* Inderb. (Inderbitzin et al. 2001) was found on submerged wood from Costa Rica, Panama, USA (TN), and Venezuela. Although this fungus is similar in most respects to *A. khaoyaiensis* Inderb., the type of the genus, it differs in lacking stalked ascomata and in having larger ascospores with apical ascospore appendages and an ascospore sheath that tapers at the ends and elongates extensively in water. In addition, refractive, acicular crystals form within the ascospores and hyphae when fixed with lactic acid or glycerin. This characteristic has not been reported nor have we observed it in our collections of *A. khaoyaiensis*. Based on the foregoing differences, the fungus is described and illustrated herein as a new species of *Aliquandostipite*.

## Materials and Methods

Submerged woody debris was collected from freshwater habitats and transported to the laboratory in plastic bags containing paper towels. After initial examination, woody debris was incubated in the laboratory in plastic boxes containing moistened paper towels at ambient temperatures (about 24° C) under 12/12h (light/dark) conditions. Samples were examined with a dissecting microscope immediately after collection and periodically for 6–12 months to locate fruiting bodies.

Ascomata were removed from the substrate and crushed in a drop of distilled water on a glass slide. India ink or aqueous nigrosin was added to the aqueous mounts to reveal gelatinous sheaths on or around the ascospores. Single spore isolates were obtained using the procedures of Shearer (1993). Measurements of the physes, asci and ascospores were made of material mounted in distilled water or fixed in lactic acid or glycerin.

Distilled water was replaced with lactic acid containing azure A or with glycerin for preservation using the double cover glass method (Volkman-Kohlmeier & Kohlmeier 1996). Ascomata were fixed and embedded using a modification of Huhndorf's technique (1991) suggested to us by Huhndorf (pers com): the osmium tetroxide step was deleted, and acetone was used instead of ethanol for dehydration and infiltration. Ascomata were sectioned following the procedures of Fallah and Shearer (2001). Digital images were obtained with a Spot RT digital camera using an Olympus microscope equipped with Nomarski interference and phase optics. Specimens were deposited in the Herbarium of the University of Illinois at Urbana Champaign (ILL).

## Taxonomic Description

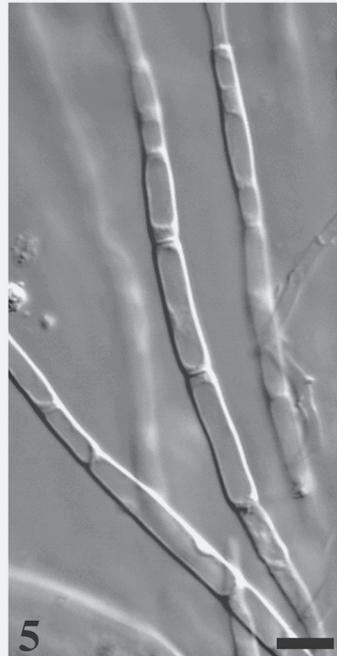
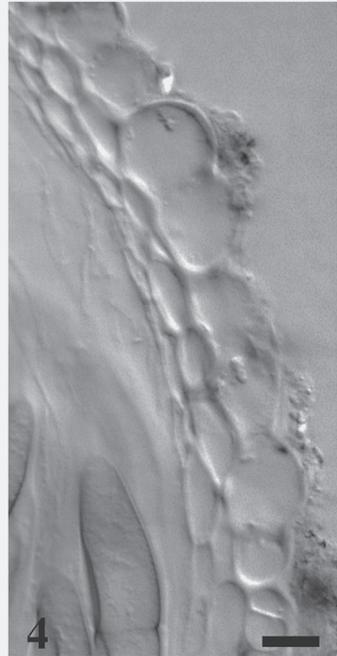
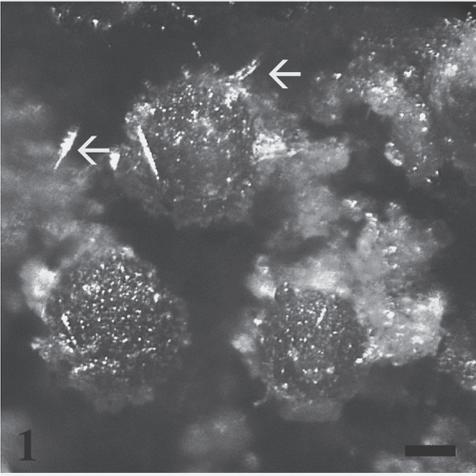
*Aliquandostipite crystallinus* Raja, A. Ferrer & Shearer

Figs. 1–13

*Ascomata lignatilia* 215–270 × 220–320 μm, *globosa ad subglobosa, papillata, immersa ad partim immersa vel superficialia, solitaria vel gregaria, primo hyalina demum atrobrunnea. Papilla ca.* 30–36 × 60–90 μm, *periphysibus. Peridium* 21–25 μm, *e textura angularis constitutum. Pseudoparaphyses septatae, hyalinae* 3–4 μm *lata. Asci fissitunicati, octospori, clavati ad cymbiformes, apici rotundato* 132–174 × 30–41 μm (*mean* = 160 × 35 μm, *n* = 15). *Ascosporae tri-vel tetraseriate, fusiforme, multiguttulatae* 70–86 × 9–15 μm (*mean* = 76 × 12 μm, *n* = 50), *primo uniseptatae, demum triseptatae, pallidae brunneae demum atrantes, appendiculatae, crystallinae, tunica grandi, fusiforma, mucilagina, extensa in aqua.*

Colonies on peptone yeast extract agar (PYG) immersed with a mat of aerial hyphae; hyphae septate brown (7–13 μm wide). Hyphal cells containing acicular crystals after storage in lactic acid or glycerin. Ascomata on wood 215–270 × 220–320 μm, hyaline when young becoming dark brown with age (Fig. 1), globose to subglobose, papillate, immersed to partially immersed, or superficial, scattered to clustered, connected by

Figs. 1–5. *Aliquandostipite crystallinus*. (Holotype R076-1). 1. Ascomata on wood, note the subtending hyphae and ascomata shedding wood particles. 2. Longitudinal section of ascoma. 3. Section of papilla showing apically attached pseudoparaphyses. 4. Longitudinal section of ascomal wall, note large hyaline peridial cells. 5. Pseudoparaphyses. Measure bars for Fig. 1 = 150 μm. Fig. 2 = 20 μm. Figs. 3, 4, 5 = 10 μm.



subtending superficial stoloniferous hyphae ca.  $144\text{--}170 \times 10\text{--}13 \mu\text{m}$ . Papilla ca.  $30\text{--}36 \times 60\text{--}90 \mu\text{m}$  (Figs. 2, 3). Peridium  $21\text{--}25 \mu\text{m}$  wide, *textura angularis-globosa* in surface view, in longitudinal section 2–3 cell layers wide, innermost layer of hyaline thin-walled cells compressed laterally, outermost layer of large thin-walled isodiametric cells  $17\text{--}22 \mu\text{m}$  wide (Fig. 4). Pseudoparaphyses septate, slightly constricted at the septa, sparsely branched above the asci, hyaline, without gel coating,  $3\text{--}4 \mu\text{m}$  wide (Fig. 5). Asci basal, fasciculate, fissitunicate, clavate to cymbiform, broad at the center and rounded towards the apex, 8-spored,  $132\text{--}174 \times 30\text{--}41 \mu\text{m}$  (mean =  $160 \times 35 \mu\text{m}$ ,  $n = 15$ ) with or without an apical chamber (Figs. 6, 7, 13). Ascospores 3–4 seriate, fusiform, multiguttulate, upper cell slightly broader than lower cell,  $70\text{--}86 \times 9\text{--}15 \mu\text{m}$  (mean =  $76 \times 12 \mu\text{m}$ ,  $n = 50$ ), one septate initially (Figs. 8–12) becoming three-septate with age (Fig. 9), slightly constricted at the septa, pale yellow brown becoming darker brown with age, with short, cellular, hyaline, tapering, apical appendages ca.  $2\text{--}5 \mu\text{m}$  long (Fig. 8); ascospores surrounded by a large, fusiform, mucilaginous sheath (Figs. 10, 11). Sheath at first appressed to the ascospore but elongating to form a sigmoid or C shape  $200\text{--}452 \mu\text{m}$  long,  $4\text{--}7 \mu\text{m}$  wide on either side of the ascospore at the midseptum, tapering towards the apices, surrounding the entire ascospore, expanding after ascospore is released from the ascus into the water; becoming extremely long and sticky after immersion in water (Fig. 10). Refractive acicular crystals formed within the ascospores and hyphae after preservation in lactic acid or glycerin (Figs. 12, 13).

**Habitat:** Saprobic on submerged wood in freshwater streams.

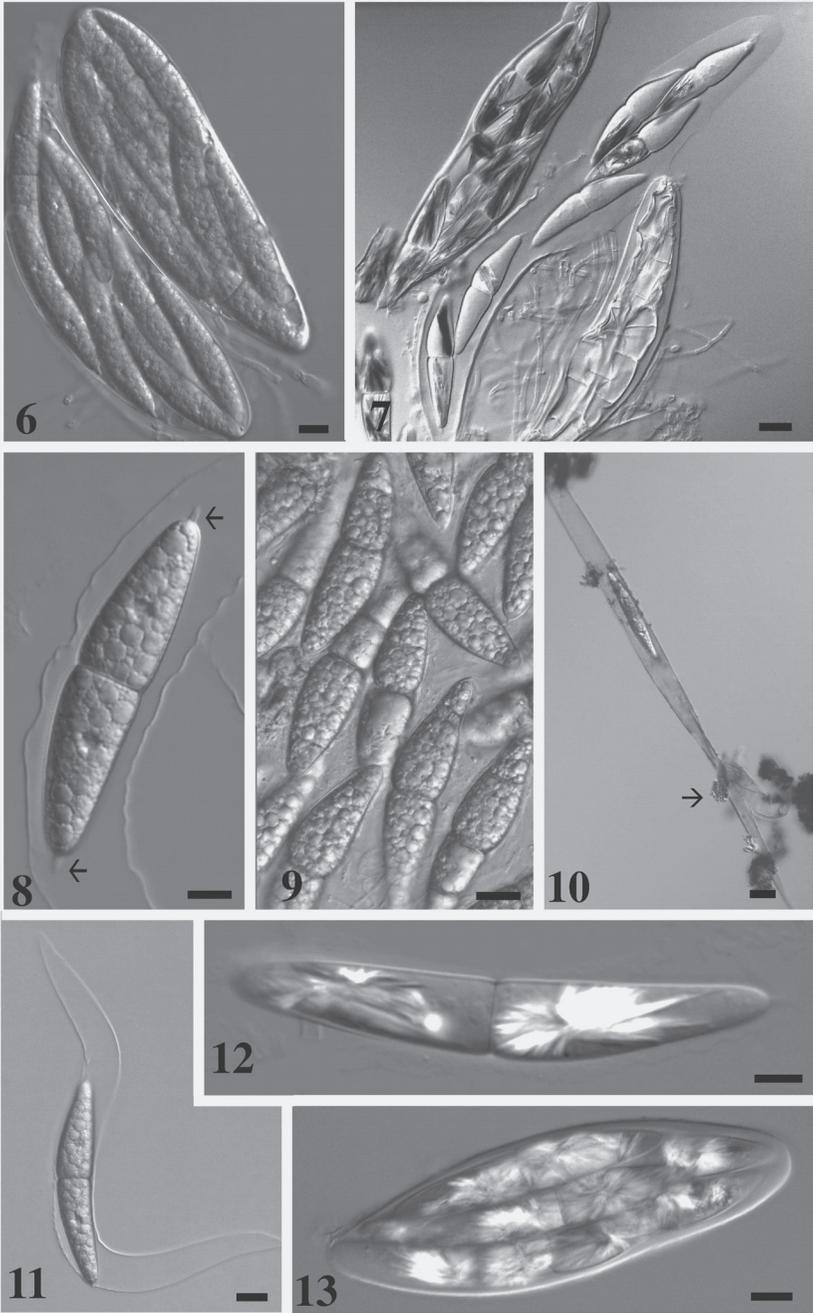
**Geographical distribution:** Costa Rica, Panama, USA (TN), Venezuela.

**Etymology:** “*crystallinus*” in reference to the refractive acicular crystals formed within the ascospores and hyphae after preservation in lactic acid or glycerin.

**Holotype: USA, Tennessee:** Great Smoky Mountains National Park, Elkmont Campground, a small stream between Jakes Creek and Little River,  $35^{\circ}39'512''\text{N}$ ,  $83^{\circ}34'881''\text{W}$ , water temperature 23 C, pH 5, on submerged, decorticated wood, 5 July 2002, *Huzefa A. Raja & Nate Hamburger*, R076-1. (ILL).

**Additional Specimens examined:** COSTA RICA. Salto, La Selva Biological Station,  $10^{\circ}25'28''\text{N}$ ,  $84^{\circ}0'17''\text{W}$ , water temperature 25 C, pH 7, on submerged decorticated wood, 18 May 2000, *Jennifer L. Anderson & Rebecca Wulffen*, A514-1; Arboleda Bridge, freshwater stream,  $10^{\circ}25'48''\text{N}$ ,  $84^{\circ}0'34''\text{W}$ , water temperature 25 C, pH 6, on submerged monocotyledonous debris, 20 May 2000, *Jennifer L. Anderson & Rebecca Wulffen*, A514-6; Arboleda 30, freshwater stream,  $10^{\circ}25'47''\text{N}$ ,  $84^{\circ}0'39''\text{W}$ , water temperature 26 C, pH 7, on submerged decorticated wood, 17 May 2000, *Jennifer L. Anderson & Rebecca Wulffen*, A514-2. PANAMA. Colon Province, Soberania National Park, Frijoles River,  $9^{\circ}08'0''\text{N}$ ,  $79^{\circ}43'0''\text{W}$ , on submerged decorticated wood, 12 January 2003, *Astrid Ferrer* AF-007-1; Juan Grande River, on submerged corticated wood, 12 January 2003 *Astrid Ferrer* AF-007-2; Limbo River,  $9^{\circ}09'0''\text{N}$ ,  $79^{\circ}44'0''\text{W}$ , on submerged corticated wood, 18 May 2003, *Astrid Ferrer* AF-007-3. USA, Tennessee. Outskirts of the Great Smoky Mountains

[6–13. *Aliquandostipite crystallinus*. 6. Asci. 7. Dehisced asci and acicular crystals in ascospores. 8. Ascospore showing sheath, arrow indicates the tapering apical appendages. 9. Three-septate ascospores. 10. Ascospore showing sheath extending in water, arrow indicates debris adhering to the sticky sheath. 11. Ascospore with fusiform, sigmoid sheath. 12–13. Ascospore and ascus showing acicular crystals after storage in lactic acid and glycerin. Measure bars for Figs. 6, 7, 8, 9, 11, 12, 13 =  $10 \mu\text{m}$ . Fig. 10 =  $20 \mu\text{m}$ . Figs. 6, 8, 9 (AF007). Fig 7 (A514-7). Figs. 10, 11, 12, 13 (R076-1).



National Park, Chilhowee Lake, 35°32'45N, 83°59'32W, water temperature 34 C, pH 5, submerged decorticated wood, 19 July 2000, *Jinx Campbell*, A514-3, VENEZUELA, Estado Portuguesa, mountain stream 2 miles from La Estacion, water temperature 28 C, pH 6, on submerged monocotyledonous debris, 16 January 1993, *J. L. Crane, J. D. Schoknecht & Francisco Ortega*, A514-7.

**Comments:** The genus *Aliquandostipite* was established by Inderbitzin et al. (2001) for an unusual ascomycete found on decaying wood in a rain forest in Thailand. This fungus produces dimorphic ascomata, i. e., sessile and stalked, and unusually large hyphae up to 50  $\mu\text{m}$  wide. Inderbitzin et al. (2001) described two species, *Aliquandostipite khaoyaiensis*, the holotype of the genus, and *A. sunyatsenii* Inderb. Recently, Pang et al. (2002) transferred *A. sunyatsenii* to the genus *Jahnula* Kirschst. based on molecular phylogenetic analyses of 18S rDNA data.

The general characteristics of *A. crystallinus* fit within the generic concept of *Aliquandostipite*. *Aliquandostipite crystallinus* however, differs from *A. khaoyaiensis* in several ways. The ascomata of *A. khaoyaiensis* are sessile or stalked, whereas, in *A. crystallinus* only sessile ascomata have been observed in collections from four different geographical locations. The ascospores of *A. khaoyaiensis* are more rounded towards the apex and are shorter (49.6–70  $\times$  12.8–20  $\mu\text{m}$ ) (Inderbitzin et al. 2001), whereas, the ascospores of *A. crystallinus* are more tapered and longer (70–86  $\times$  9–15  $\mu\text{m}$ ). The gelatinous sheath around the ascospore, a feature common to both species, also varies. The sheath is rounded and balloon shaped in *A. khaoyaiensis*, (Fig. 18) whereas in *A. crystallinus* the sheath is sigmoidal or C-shaped and tapering towards the apices (Fig. 11). The ascospores of *A. crystallinus* possess a short, cellular, hyaline, tapering apical appendage at each ascospore apex (Fig. 8) and refractive acicular crystals form within the ascospores and hyphae after preservation in lactic acid or glycerin (Figs. 12–13). These characters are not found in *A. khaoyaiensis*. Considerable variation in the mean lengths and widths of ascospores and asci was observed among the populations of *A. crystallinus*. Costa Rica: ascospores = 75  $\times$  18  $\mu\text{m}$ , asci = 225  $\times$  44  $\mu\text{m}$ ; USA (TN) ascospores = 76.5  $\times$  12  $\mu\text{m}$ , asci = 160  $\times$  35  $\mu\text{m}$ ; Panama: ascospores = 69  $\times$  13  $\mu\text{m}$ , asci = 209  $\times$  42  $\mu\text{m}$ ; Venezuela: ascospores = 80  $\times$  19  $\mu\text{m}$ , asci = 233  $\times$  48  $\mu\text{m}$ . These differences may be due to variation among specimens from different geographical locations.

The manner in which the ascospore sheath of *A. crystallinus* expands (Fig. 10) superficially resembles that of the ascospore sheath found in *Massarina ingoldiana* Shearer & K. D. Hyde (Shearer and Hyde 1997), and *Phaeosphaeria vilasensis* Fallah, Shearer & Leuchtm. (Fallah et al. 1999). All the aforementioned species belong to the loculoascomycetes, are found in freshwater habitats, and produce ascospore sheaths that extend upon contact with water. The presence of an ascospore sheath is considered to be an adaptation to the aquatic habitat, better enabling the ascospores to attach to substrates in water (Shearer 1993).

Ascospores of *Massarina lunispora* K. D. Hyde & Goh are surrounded by a C-shaped sheath and have short (2  $\mu\text{m}$  long) cellular spines (Hyde and Goh 1998) similar to those of *A. crystallinus*. However, *A. crystallinus* differs from *M. lunispora* in ascomal color and wall structure, absence of a clypeus, and in the ascus and ascospore dimensions (larger asci and ascospores in *A. crystallinus*).

*Aliquandostipite khaoyaiensis* Inderb. American Journal of Botany 88: 54. 2001

Figs. 14–18

Ascomata globose to oval, tapering towards the peduncle (mean =  $319 \times 232 \mu\text{m}$ ,  $n = 10$ , ascomal stalk  $41\text{--}50 \mu\text{m}$  wide (Figs. 14, 15). Papilla ca.  $50 \mu\text{m}$  high and  $110 \mu\text{m}$  wide. Peridial wall made of large,  $6\text{--}10 \mu\text{m}$ , thin-walled hyaline cells  $13\text{--}19 \mu\text{m}$  wide (Fig. 16). **Asci** (mean =  $179 \times 44 \mu\text{m}$ ,  $n = 20$ ), clavate, with a small stalk (Fig. 17). Ascospores 2–3 seriate, oval in outline, multiguttulate, one-septate, slightly constricted at the septum, pale brown, upper cell broader than the lower cell (mean =  $77 \times 17 \mu\text{m}$ ,  $n = 20$ ), surrounded by a balloon shaped sheath (Fig. 18).

**Habitat:** Saprobic on decaying wood in a rain forest and on submerged wood in freshwater streams.

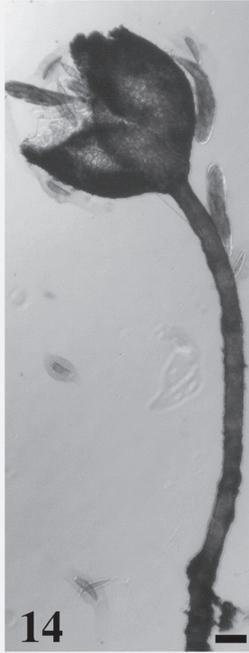
**Geographical distribution:** Costa Rica, Thailand.

**Specimens examined:** COSTA RICA. Sura 60, La Selva Biological Station,  $10^{\circ}25'48\text{N}$ ,  $84^{\circ}1'32\text{W}$ , water temperature  $25 \text{C}$ , pH 5, on submerged decorticated wood, 18 May 2000, Jennifer L. Anderson & Rebecca Wulffen, A441-1; Panteno,  $10^{\circ}25'8\text{N}$ ,  $84^{\circ}0'22\text{W}$ , water temperature  $25 \text{C}$ , pH 5.5, on submerged decorticated wood, 19 May 2000, Jennifer L. Anderson & Rebecca Wulffen, A441-2.

**Comments:** Collections of *Aliquandostipite khaoyaiensis* from freshwater in Costa Rica agree with the original protologue of *A. khaoyaiensis* from Thailand. Measurements of all the morphological features are within the range of those found in the type specimen collected from Thailand, except those of the asci and ascospores. The asci and ascospores of *A. khaoyaiensis* from Costa Rica are longer than those found by Inderbitzin et al. (2001). The mean of asci and ascospores for *A. khaoyaiensis* from Costa Rica are  $179 \times 44 \mu\text{m}$  and  $77 \times 17 \mu\text{m}$  respectively, whereas the mean of asci and ascospores from the type material are  $166.67 \times 45.57 \mu\text{m}$  and  $61.80 \times 16.27 \mu\text{m}$ . *Aliquandostipite khaoyaiensis* is reported for the first time from the Western Hemisphere from wood submerged in freshwater.

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Figs. 14–18. *Aliquandostipite khaoyaiensis*. (A441-1,-2). 14. Stalked ascoma. 15. Longitudinal section of ascoma. 16. Longitudinal section of peridial wall. 17. Asci and pseudoparaphyses. 18. Ascospores showing balloon shaped sheath (stained with India ink). Measure bars for Fig. 14 = 40  $\mu\text{m}$ . Figs. 15, 17, 18 = 20  $\mu\text{m}$ . Fig. 16 = 10  $\mu\text{m}$ .