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## Freshwater ascomycetes: a new genus, *Ocala scalariformis* gen. et sp. nov. and two new species, *Ayria nubispora* sp. nov. and *Rivulicola cygnea* sp. nov.

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**Raja, H.A. \*, Ferrer, A. and Shearer C.A.**

Department of Plant Biology, University of Illinois, Rm 265 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801.

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A new genus, *Ocala scalariformis* gen. et sp. nov. and two new species, *Ayria nubispora* sp. nov., and *Rivulicola cygnea* sp. nov., are described and illustrated from submerged woody debris collected during a distributional study of freshwater ascomycetes along a latitudinal gradient in North and Central America. *Ocala scalariformis* belongs to the Pleosporales, (Dothideomycetes) and its uncertain placement at the familial level is discussed. *Ayria nubispora* and *Rivulicola cygnea* belong to the Sordariomycetes and are discussed in relation to other species in their respective genera.

**Key words:** aquatic fungi, Costa Rica, Florida, submerged wood, fungal systematics

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\*Corresponding author: Raja, H.A.; e-mail: raja@illinois.edu

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### Introduction

The freshwater ascomycetes are an ecological group of fungi that occur on submerged or partially submerged plant substrates in aquatic habitats (Shearer, 1993, 2001; Vijaykrishna *et al.*, 2006). Our knowledge of the freshwater ascomycetes is fairly recent (Shearer, 1993, 2001; Goh and Hyde, 1996; Tsui and Hyde, 2003; Shearer *et al.*, 2007). This ecological group of fungi has been studied only in the last 50 years beginning with pioneering studies by Prof. C.T. Ingold (Ingold, 1951, 1954, 1955; Ingold and Chapman, 1952). Although our knowledge of the systematics of freshwater ascomycetes has increased dramatically in the last 30 years, it is still fragmentary at best.

Currently 561 taxa of freshwater ascomycetes have been reported; this number includes species collected and described exclusively from freshwater habitats as well as species reported from terrestrial and freshwater habitats. Collections from freshwater are predominantly from North America, Europe and South East Asia (<http://fungi.life.uiuc.edu/>).

To better understand the systematics and geographical distribution patterns of freshwater ascomycetes, we are investigating the latitudinal distribution patterns of these fungi in North and Central America (Ferrer and Shearer, 2007; Ferrer *et al.*, 2007, 2008; Raja and Shearer, 2008, Raja *et al.*, 2008, 2009, in press). During our continuing study, three novel taxa were encountered in Costa Rica and Florida. One taxon was unique at the generic level and required the establishment of a new genus, *Ocala* Raja & Shearer. The other two taxa are described herein as new species within two existing freshwater ascomycete genera, *Ayria* Fryar & K.D. Hyde and *Rivulicola* K.D. Hyde.

### Materials and methods

Methods for collection, characterization and illustration of the taxa are described in Fallah and Shearer (2001), Shearer *et al.* (2004) and Raja and Shearer (2006, 2008). Latitude, longitude, water temperature and pH were recorded in the field and are presented in the

specimen citation section.

## Taxonomy

### *Ocala* Raja & Shearer **gen. nov.**

Mycobank: MB 512703

*Etymology:* *Ocala* refers to the Ocala National Forest in Florida where the fungus was collected.

Ascomata minuta, brunneae vel atrobrunneae, superficialis vel immerses, globosa vel subglobosa, membranacea, ostiolata, papillata. Peridium tenuitunicati, composita textura angularis vel epidermoidea aspectu externo. Pseudoparaphyses sparsae, septatae. Asci fissitunicati, fasciculati, obclavati vel fusoidi, pedicellati, octospori, uniseriatis vel biseriatis. Ascospores hyalinae, ellipsoideae vel fusiformes, phragmoseptatae, pachydermae, cum vel ex gelatinosa appendicibus.

Typus: *Ocala scalariformis* Raja & Shearer

Ascomata small, brown to dark brown, semi-transparent, superficial or immersed, scattered, globose to subglobose, membranous, ostiolate, with a short papilla. Peridium thin-walled, *textura angularis* to *epidermoidea* in surface view. Pseudoparaphyses sparse, septate. Asci fissitunicate, fasciculate, obclavate to fusoid, apex flattened, with an elongate, tapered pedicel, containing eight uniseriate to biseriate overlapping ascospores. Ascospores hyaline, narrowly ellipsoidal to fusiform, phragmoseptate, thick walled, straight or slightly curved, with or without amorphous apical gelatinous appendages.

### *Ocala scalariformis* Raja & Shearer, **sp. nov.**

(Figs 1-10)

Mycobank: MB 512704

*Etymology:* *scalariformis* L. = ladder-like, referring to the appearance of the multiseptate ascospores.

Ascomata in ligno 165–195 × 130–138 μm, minuta, brunneae vel atrobrunneae, superficialis vel immerses, globosa vel subglobosa, membranacea, ostiolata, papillata; papillae 15–25 × 10–15 μm, brevis. Peridium tenuitunicati, composita textura angularis vel epidermoidea aspectu externo; cellulis 10–15 × 2–5 μm. Pseudoparaphyses 25–35 × 5–8 μm, sparsae, septatae. Asci 140–200 × 18–26 μm ( $\bar{x}$  = 168 × 22 μm, n = 20), bitunicati, fissitunicati, fasciculati, obclavati-fusoidi, pedicellati, octospori, uniseriatis vel biseriatis. Ascospores 38–45 × 9–10 μm ( $\bar{x}$  = 42 × 10 μm, n = 25), hyalinae, ellipsoideae vel fusiformis, 9–12 septatae,

pachydermae, appendices bipolaris, in aquo retorquentes, non visibiles in acido lacteo.

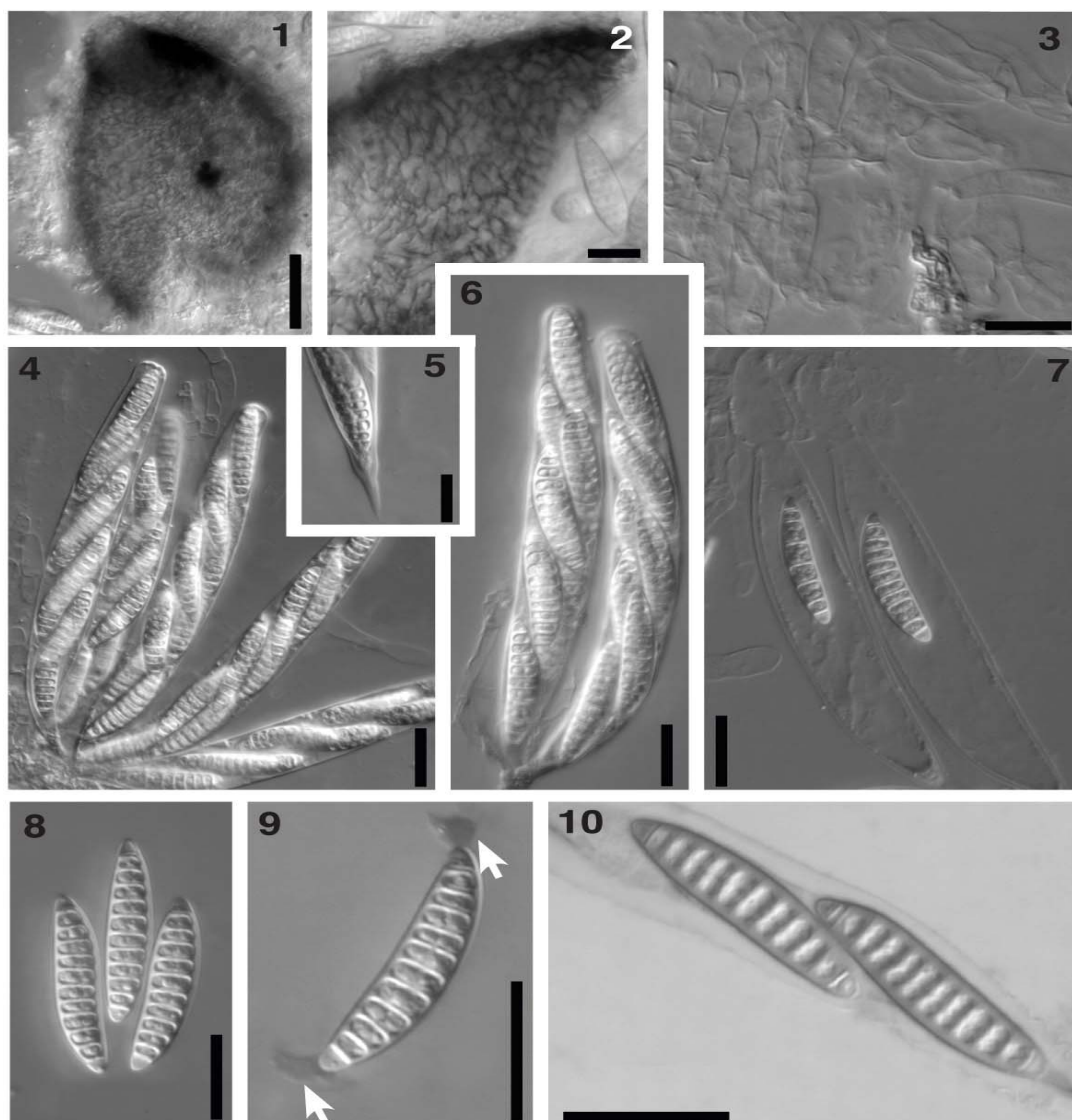
Ascomata on wood 165–195 × 130–138 μm, brown to dark brown, semi-transparent, superficial to immersed, scattered, globose to subglobose, membranous, ostiolate, with a short papilla; papilla 15–25 × 10–15 μm; aperiophysate. Peridial wall membranous, composed of cells forming a *textura angularis* to *epidermoidea* in surface view; cells 10–15 × 2–5 μm. Pseudoparaphyses 25–35 × 5–8 μm, sparse, broad, hyaline, septate, slightly constricted at the septa, branched. Asci 140–200 × 18–26 μm ( $\bar{x}$  = 168 × 22 μm, n = 20), obclavate-fusoid, bitunicate, fissitunicate; ectoascus rupturing at the ascus apex, fasciculate, with a flattened apex, containing eight overlapping to obliquely biseriate ascospores, pedicellate; pedicel tapering to a fine point. Ascospores 38–45 × 9–10 μm ( $\bar{x}$  = 42 × 10 μm, n = 25), 9–12 septate, hyaline, narrowly ellipsoidal to fusiform, slightly curved, thick-walled, accumulating at the tip of the papilla to form a white mass on the substratum, with ephemeral, amorphous bipolar gelatinous appendages ca. 2–5 × 4–7 μm, staining blue in aqueous nigrosin, extending in water, not visible in glycerin and lactic acid.

*Habitat:* lentic (lake).

*Known distribution:* USA (FL).

*Material examined:* UNITED STATES. FLORIDA: Ocala National Forest, Mary Lake, 29°4' 20.8"N, 81°49'57.8"W, water temperature 30C, pH 5, on submerged decorticated woody debris, 16 July 2006, Huzefa A. Raja and J.L. Crane, F121-1 (**HOLOTYPE, ILL40594**).

*Notes:* *Ocala scalariformis* possesses morphological characters that support its placement in the subclass Pleosporomycetidae and the order *Pleosporales* Luttrell ex M.E. Barr. These characters include superficial to erumpent, globose to subglobose perithecial ascomata with an ostiole; cellular pseudoparaphyses; fissitunicate asci; and hyaline or brown, septate, thin or thick-walled ascospores (Kirk *et al.*, 2008; Zhang *et al.*, 2008). At present 23 families are recognized within the *Pleosporales* (Kirk *et al.*, 2008). Among the known families in the *Pleosporales*, *O. scalariformis* is most similar to species in the *Phaeosphaeriaceae* in having globose to



Figs 1–10. *Ocala scalariformis* from the holotype. **1.** Ascoma squash mount; bar = 50  $\mu\text{m}$ . **2.** Peridial wall in surface view; bar = 10  $\mu\text{m}$ . **3.** Pseudoparaphyses; bar = 20  $\mu\text{m}$ . **4.** Asci in a fascicle; bar = 20  $\mu\text{m}$ . **5.** Ascus base with a pointed pedicel; bar = 10  $\mu\text{m}$ . **6.** Asci; bar = 20  $\mu\text{m}$ . **7.** Fissitunicate asci; bar = 20  $\mu\text{m}$ . **8.** Ascospores. **9.** Ascospore showing bipolar gelatinous appendages stained in aqueous nigrosin. **10.** Ascospores in glycerin; **8, 9, 10;** bars = 20  $\mu\text{m}$ .

subglobose, ostiolate, perithecial ascomata with soft membranous peridial walls; fissitunicate asci, and septate ascospores (Barr, 1979; Cannon and Kirk, 2007). Based on small, membranous papillate ascomata, it fits best in the *Phaeosphaeriaceae*, but the broad cellular pseudoparaphyses, and hyaline phragmoseptate, thick-walled hyaline ascospores with ephemeral, amorphous, bipolar gelatinous appendages differ from the 22 genera currently placed in this family (Lumbsch and Huhndorf, 2007) and hence a new genus is established herein.

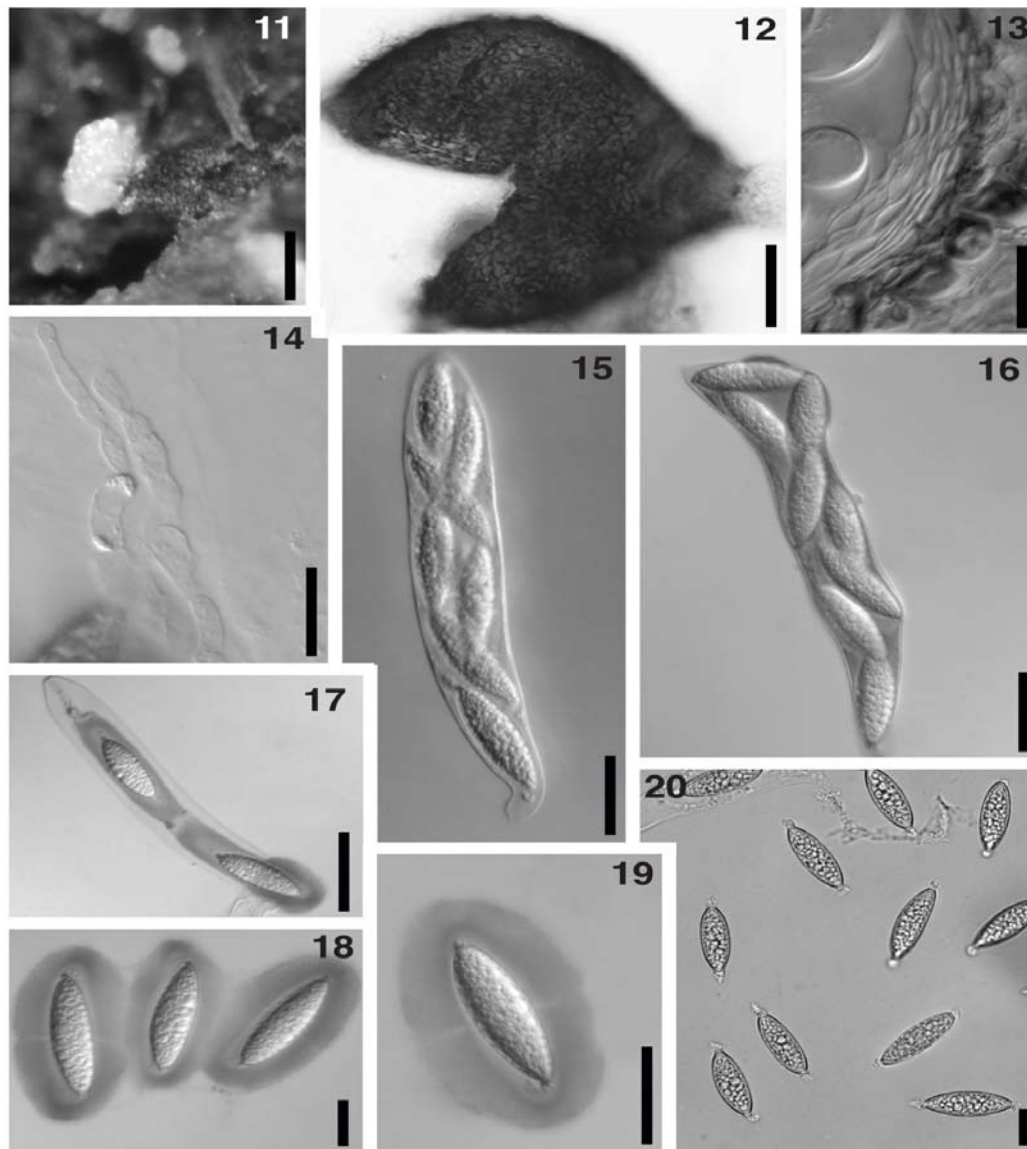
*Ayria nubispora* Raja, A. Ferrer & Shearer **sp. nov.**

(Figs 11–20)

Mycobank: MB 512705

*Etymology:* nubes L. = cloud and spora L. = spore, referring to the cloud-like sheath surrounding each ascospore.

Ascomata in ligno 250–270  $\times$  158–168  $\mu\text{m}$ , superficiales vel partim immerses, globosa vel subglobosa, atrobrunnea, membranacea, ostiolata; collum atrum ad basem pallidum versus ad apicem. Collum 40–50  $\times$  30–60  $\mu\text{m}$ , cylindricum, aperiphysatum. Peridium 8–20  $\mu\text{m}$  crassum, textura angularis aspectu externo. Paraphysibus 90–120  $\times$  7–9  $\mu\text{m}$  crassum ad basem, hyalinum, cylindricum. Asci 212–250  $\times$  34–40  $\mu\text{m}$ , unitunicati, cylindrici vel clavati, octospori, uniseriati vel biseriati, deliquescentes in aqua. Ascosporae 48–56  $\times$  16–20  $\mu\text{m}$ , hyalinae, multiguttulatae, ellipsoideae-fusiformes, appendicibus filamentosis et vagina gelatinosa.



Figs 11–20. *Ayria nubispora*. **11.** Ascoma on wood with a mass of white ascospores; bar = 150  $\mu\text{m}$  (AF 268-1). **12.** Squash mount of ascoma showing peridium in surface view; bar = 40  $\mu\text{m}$  (AF 268-1). **13–19** (from the holotype). **13.** Peridium. **14.** Paraphyses. **15, 16.** Ascus. **17.** An ascus deliquescing in water. **18, 19.** Ascospores stained with aqueous nigrosin showing gelatinous sheath and bipolar appendages. **20.** Ascospores showing bipolar appendages (AF 268-1); **13–20;** bars = 20  $\mu\text{m}$ .

Ascomata 250–270  $\times$  158–168  $\mu\text{m}$ , superficial or partially immersed, scattered on membranous, ostiolate with a hyaline neck. Neck 40–50  $\times$  30–60  $\mu\text{m}$ , cylindrical, aperiphysate. Peridium 18–20  $\mu\text{m}$  wide, tissue composed of *textura angularis* in surface view; in medial longitudinal section composed of a dark brown outer layer consisting of isodiametric cells occluded by brown amorphous material and a hyaline inner layer made up of elongated, thin-walled cells. Paraphyses 90–120  $\times$  7–9  $\mu\text{m}$ , sparse, hyaline, septate, somewhat constricted at the septa, attached at the base, cylindrical, slightly tapering towards the apex. Asci 212–250  $\times$  34–40  $\mu\text{m}$  ( $\bar{x}$  = 226  $\times$

36  $\mu\text{m}$ , n = 20), unitunicate, cylindro-clavate with a short pedicel, thin-walled, tapering to a rounded apex, lacking an apical pore or ring, deliquescing in water, with eight overlapping uniseriate to biseriate or irregularly arranged ascospores. Ascospores 48–56  $\times$  16–20  $\mu\text{m}$  ( $\bar{x}$  = 52  $\times$  18  $\mu\text{m}$ , n = 25), hyaline, one-celled, ellipsoidal to fusiform, flattened on one side, finely multiguttulate, with bipolar gelatinous appendages at first hood or cap like, unfurling to a long thread-like structure, and with an oval to circular gelatinous sheath that surrounds the ascospores; sheath ca. 10–18  $\mu\text{m}$  wide, staining blue in aqueous nigrosin. Colonies on PYG (Difco) slow-growing,

approximately 20 mm diameter in 30 d, grey in the center, black towards the margins; margins uneven, colonies black in reverse. Hyphae hyaline to dark brown, septate, ca. 2  $\mu\text{m}$  wide. Colonies on CMA (Difco) with balsa, effuse, mycelium dark brown, black in reverse, hyphae septate, dark brown, ca. 1–2  $\mu\text{m}$  wide, forming ascomata in culture. Ascomata in culture 260  $\mu\text{m}$  long, and 160  $\mu\text{m}$  wide, scattered, submerged in the media, globose to subglobose, black, ostiolate. Asci 150–170  $\times$  40–50  $\mu\text{m}$  ( $\bar{x}$  = 160  $\times$  43  $\mu\text{m}$ ,  $n$  = 20), unitunicate, clavate, pedicellate, thin-walled, with eight irregularly arranged ascospores, deliquescing in water. Ascospores 38–46  $\times$  16–18  $\mu\text{m}$ ,  $n$  = 20), ellipsoidal, multiguttulate, surrounded by an oval to circular gelatinous sheath ca. 6–8  $\mu\text{m}$  wide, staining blue in aqueous nigrosin; bipolar appendages present, but not readily visible.

*Habitat*: fresh water (lotic and lentic).

*Known distribution*: Costa Rica, USA (FL).

*Material examined*: UNITED STATES. FLORIDA: Marion County, Ocala National Forest, Fore Lake, 29°16' 15"N, 81°55'02"W, water temperature 18C, pH 7, on submerged decorticated woody debris, 9 February 2006, *Huzefa A. Raja and J.L. Crane, F99-1 (HOLOTYPE, ILL40594)*.

*Additional material examined*: COSTA RICA. Heredia, La Selva, La Selva stream, 10° 25'0.12"N, 84°1'0.45"W, water temperature, 25C, pH 5, on submerged decorticated woody debris, 10 January 2006, *Astrid Ferrer and Marlon Salazar, AF268-1*; Heredia, La Selva, Arboleda stream, 10°26'0.75"N, 84°0'0.62"W, water temperature 25C, pH 7, on submerged decorticated wood, 9 January 2006, *Astrid Ferrer and Marlon Salazar, AF268-2*; Limon, Barra del Colorado and Tortuguero National Park, Las Palmas Stream, 10°35'0.94"N, 83°31'0.87"W, water temperature 25C, pH 5, on submerged decorticated wood, 18 December 2005, *Astrid Ferrer and Marlon Salazar, AF268-3*.

*Notes*: The genus *Ayria* Fryar & K.D. Hyde is characterized as having superficial, membranous, globose to subglobose, light to dark brown ascomata, sparse septate paraphyses, eight-spored, thin-walled, unitunicate, clavate, pedicellate asci, and hyaline, unicellular, ellipsoidal ascospores with appendages at the apices (Fryar and Hyde, 2004). *Ayria nubispora* fits well within the general concept of the genus with respect to ascomal, ascus and ascospore morphology. The Florida material agrees in all respects with the protologue of *A. appendiculata* Fryar & K.D. Hyde, the type species of the genus, but differs as follows. The

ascospores of *A. nubispora* are larger in size (48–56  $\times$  16–19  $\mu\text{m}$ ) compared to those of *A. appendiculata* (21–26  $\times$  7.5–10  $\mu\text{m}$ ). Ascospores of both *A. appendiculata* and *A. nubispora* have apical appendages but a prominent gelatinous sheath surrounds ascospores of *A. nubispora*. These differences warrant the establishment of a new species.

The new species from Florida was found on submerged decorticated woody debris in a lake in Ocala National Forest and in three lotic habitats in Costa Rica. The ascospores of the Costa Rican material are smaller in size (34–40  $\times$  12–14  $\mu\text{m}$ ) compared to the Florida specimen. In addition, we also found that ascospores produced in the ex holotype culture F99-1 on CMA with balsa were smaller in size (38–46  $\times$  16–18  $\mu\text{m}$ ) than those found in the holotype material.

The taxonomic position of the genus *Ayria* is currently unknown, but based on morphological characteristics, Fryar and Hyde (2004) placed it in the family *Annulatascaceae incertae sedis*. The *Annulatascaceae* includes numerous taxa reported from woody debris submerged in freshwater habitats from temperate and tropical latitudes (<http://fungi.life.uiuc.edu/>).

### *Rivulicola cygnea* Raja & Shearer **sp. nov.**

(Figs 21-30)

Mycobank: MB 512706

*Etymology*: cygneus L. = swan-like, referring to the long curved neck of the ascomata.

Ascomata in ligno 470–680  $\times$  140–170  $\mu\text{m}$ , dispersa vel gregaria, horizontalia vel substrata, hyalina vel brunnea, membranacea; centra globosa vel subglobosa 150–200  $\times$  140–160  $\mu\text{m}$ , ostiolata cum longi collum. Collum 240–500  $\times$  30–40  $\mu\text{m}$ , hyalinum, periphysatum. Peridium circa 10–12  $\mu\text{m}$  crassum, 4–5 cellulae. Paraphysibus septatum, ca. 3  $\mu\text{m}$  latum, ad basum. Asci 122–155  $\times$  7–8  $\mu\text{m}$ , copiosi, fasciculati, unitunicati, cylindrici, octospori, ad apicem rotundati, longi pedunculati. Ascospores 20–24  $\times$  6–7  $\mu\text{m}$ , 3-septatae, ellipsoideae, multiguttulatae, circumcinctae ad tunica gelatinosae.

Ascomata on wood 470–680  $\times$  140–170  $\mu\text{m}$ , scattered to gregarious, venter horizontal to the host surface, subiculate or not, hyaline to light brown, membranous; venter globose to subglobose, 150–200  $\times$  140–160  $\mu\text{m}$ , ostiolate, with a long neck. Neck 240–500  $\times$  30–40  $\mu\text{m}$ , hyaline, periphysate, covered with short hyaline, protruding hyphae ca. 10–15  $\mu\text{m}$  long

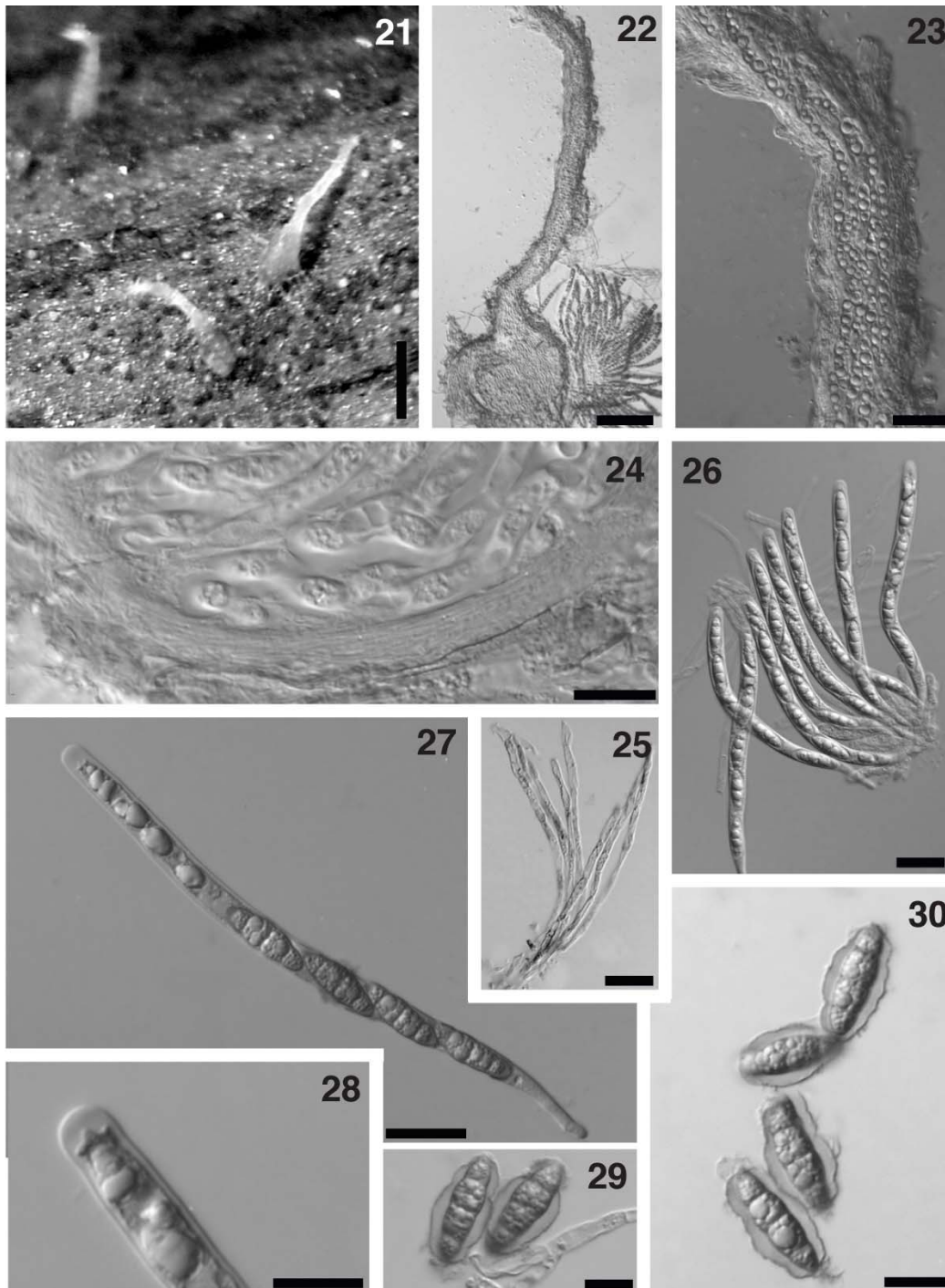


Fig 21–30. *Rivulicola cygnea* from the holotype. **21.** Hyaline, membranous ascomata on wood; bar = 250  $\mu\text{m}$ . **22.** Squash mount of ascoma; bar = 50  $\mu\text{m}$ . **23.** Hyaline, hairy neck with periphyses, note ascospores in the ostiolar canal; bar = 20  $\mu\text{m}$ . **24.** Peridium; bar = 10  $\mu\text{m}$ . **25.** Paraphyses; bar = 20  $\mu\text{m}$ . **26.** Fascicle of asci, note thick, round ascus apex; bar = 10  $\mu\text{m}$ . **27.** Ascus showing uniseriate ascospores; bar = 20  $\mu\text{m}$ . **28.** Ascus apex; bar = 20  $\mu\text{m}$ . **29, 30.** Ascospores stained in aqueous nigrosin showing gelatinous sheath; bar = 10  $\mu\text{m}$ .

Peridium 10–12  $\mu\text{m}$  wide, comprising 4–5 layers of hyaline to light brown, elongated, flattened cells. Hamathecium composed of paraphyses ca. 3  $\mu\text{m}$  wide, simple, septate, filamentous, attached at the base. Asci 122–155  $\times$  7–8  $\mu\text{m}$ , numerous, basal, fasciculate, unitunicate, cylindrical, elongating in water, rounded at the apex, apical wall thick,

with a non-amyloid apical ring, tapering to a short pedicel at the base, with eight, overlapping, hyaline, uniseriate ascospores. Ascospores 20–24  $\times$  6–7  $\mu\text{m}$ , ( $\bar{x}$  = 22  $\times$  6  $\mu\text{m}$ ,  $n$  = 40), three septate, ellipsoidal, multiguttulate, forcibly discharged from the asci, surrounded by a mucilaginous sheath ca. 2–3  $\mu\text{m}$  wide at the sides; sheath constricted at

the first ascospore septum, staining blue in aqueous nigrosin.

*Habitat*: fresh water (lentic).

*Known distribution*: USA (FL).

*Material examined*: UNITED STATES. FLORIDA: Apalachicola National Forest, Unnamed Lake east of Lost Lake, 30°21'48"N, 84°22'56"W, water temperature 35C, pH 7, on submerged decorticated wood, 13 July 2004, Huzefa A. Raja and Christopher Brown, F41-1 (HOLOTYPE, ILL40111).

*Notes*: The new species from Florida fits the concept of the genus *Rivulicola* K.D. Hyde in having hyaline ascospores that occur horizontally on the substratum; asci that are cylindrical, with an apical ring and a pedicel; and uniseriate ascospores that are ellipsoidal, multi-septate and surrounded by a gelatinous sheath (Hyde *et al.*, 1997). In addition, the ascospores of the new species from Florida are forcefully ejected through the ascus apical apparatus, similar to those of the type species, *R. incrustata* K.D. Hyde, a phenomenon that was termed by Hyde *et al.* (1997) as semifissitunicate dehiscence (*sensu* Samuels and Rossman, 1987). Currently, two species are described in the genus *Rivulicola*; viz *R. incrustata*, the type species (Hyde *et al.*, 1997), and *R. aquatica* Ranghoo & K.D. Hyde (Ranghoo *et al.* 2000). Between the two species, *Rivulicola cygnea* is most similar to the type species, *R. incrustata*, in having a superficial ascoma, with a hyaline, periphysate neck covered with long, hyaline, filiform hyphae. However, it differs from *R. incrustata* in that the ascospores of *R. cygnea* do not stain the wood green as reported for the holotype collection of *R. incrustata* from Australia (Hyde *et al.*, 1997). Ascospores in *R. cygnea* are consistently three septate, and surrounded by a gelatinous sheath constricted at the first ascospore septum, whereas, ascospores of *R. incrustata* are multiseptate (3-4- or 5 septate) and are surrounded by an irregular granular sheath. *Rivulicola cygnea* differs from *R. aquatica* (Ranghoo *et al.*, 2000) in the size and pigmentation of the ascospores, as well as in the morphology of ascospores. The ascospores in *R. aquatica* are 1–3 septate, with thin fibrillar ornamentation seen on the ascospores, whereas ascospores of *R. cygnea* are always 3-septate, smooth-walled and are surrounded by a mucilaginous sheath.

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