

Lucidascoarpha pulchella, a new ascomycete genus and species from freshwater habitats in the American tropics

Astrid Ferrer¹
Huzefa A. Raja
Carol A. Shearer

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

Abstract: A new fungus collected from submerged wood in Costa Rica and Ecuador has ascostromatic ascomata with fissitunicate asci and lacks pseudoparaphyses, characters that place it in the Dothideaceae (Dothideales). It is unusual in the order because it has white ascomata. Based on other morphological characters however this fungus could not be accommodated in any existing genus in the Dothideaceae and it is described herein as a new genus and species, *Lucidascoarpha pulchella*. These morphological features are characteristic of *L. pulchella*: ascomata glistening, white, each with a long, periphysate neck; a membranous peridium composed of 5–7 thin-walled, hyaline cells; pseudoparaphyses absent; asci fissitunicate, clavate, eight-spored; ascospores seven-septate, hyaline, multiguttulate, verruculose, surrounded by a large, regular, gelatinous sheath.

Key words: aquatic fungi, ascomycetes, Dothideales, Dothideomycetes, fungi, submerged woody debris, systematics

INTRODUCTION

During a latitudinal survey of freshwater ascomycetes in Central and South America an unusual ascomycete species was found on woody debris submerged in streams. This species can be placed within the Dothideaceae, Dothideales, a family of ascostromatic fungi that have fissitunicate asci but that lack pseudoparaphyses (Barr 1987, Eriksson 2006, Rossman 1987). This new species shares the diagnostic morphological characteristics of several genera in the Dothideaceae but is distinct from other

species in this family. We therefore describe this fungus as a new genus and species in the Dothideaceae.

MATERIALS AND METHODS

Collection techniques and fungal isolation methods are presented in Ferrer and Shearer (2005). Ascomata on wood were removed and crushed in a drop of distilled water on a glass slide. One percent aqueous nigrosin was added to the aqueous mounts to reveal gelatinous sheaths. Measurements of the asci and ascospores were made of material mounted in distilled water or material fixed in glycerin or lactic acid. Slides were preserved with the double cover glass method of Volkmann-Kohlmeyer and Kohlmeyer (1996). Digital images were shot with a Spot RT digital camera with an Olympus microscope equipped with Nomarski interference optics. Images were edited in Photoshop CS2. Specimens were deposited in the Herbarium of the University of Illinois at Urbana-Champaign (ILL).

TAXONOMY

Lucidascoarpha A. Ferrer, Raja & Shearer, gen. nov.

Type species: *Lucidascoarpha pulchella* A. Ferrer, Raja & Shearer

Etymology: From Latin *Lucido* = shining; *ascoarpha* = ascocarp

Ascomata alba, superficialis vel immersa, solitaria vel gregaria, globosa vel subglobosa, ostiolata. Peridium membranaceum. Asci fissitunicati, clavati, pedicellati, octospori, biseriati. Pseudoparaphyses absentia. Ascosporeae ellipsoideae-fusiformes, hyalinae, septatae, guttulate, verruculosae, circumcinctae vagina gelatinosa.

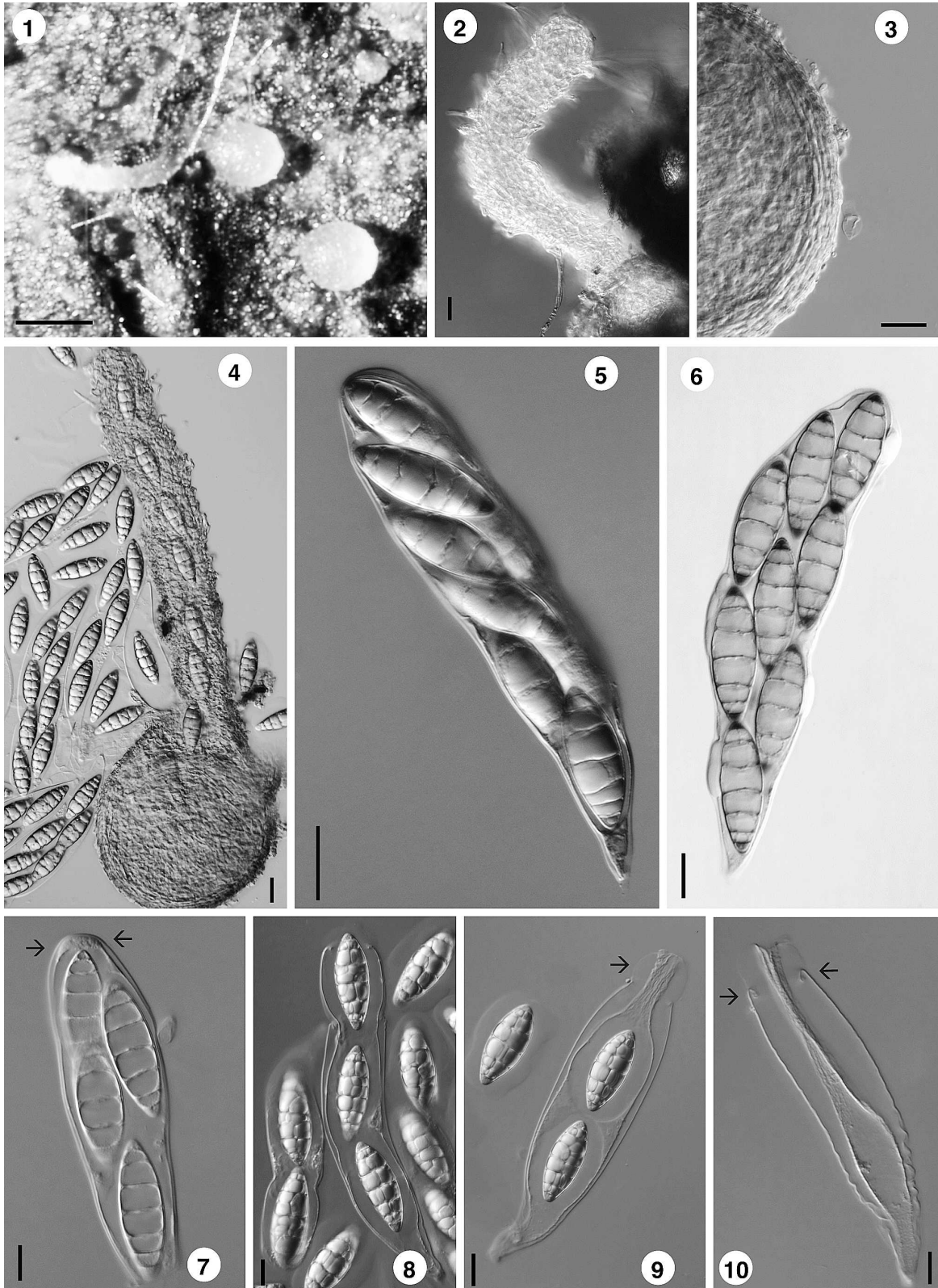
Ascomata white, superficial or immersed, scattered or clustered, venter globose to subglobose. Neck long, cylindrical, white, periphysate. Peridium membranous, composed of 5–7 elongated, thin-walled, hyaline cells; tissue of *textura angularis* in surface view. Pseudoparaphyses absent. Asci fissitunicate, clavate, pedicellate, with gelatinous material around the apex of the endoascus, containing eight overlapping, biseriate ascospores. Ascospores ellipsoidal to fusiform, seven-septate, hyaline, multi-

Accepted for publication 29 April 2008.

¹ Corresponding author. E-mail: aferrer@life.uiuc.edu

→

FIGS. 1–10. *Lucidascoarpha pulchella* from the holotype. 1. Hyaline ascomata on surface of wood. 2. Neck with hyaline hyphae. 3. Peridium of an ascoma. 4. One ascoma in optical section showing discharge of ascospores through the neck. 5. Clavate ascus. 6. Ascus stained with 1% nigrosin showing the staining reaction of the ascospore end cells. 7. Ascus fixed with



lactic acid. Arrows indicating the gelatinous material between the ecto- and endoascus at ascus apex. 8. Fissitunicate ascus dehiscence. 9. Partially empty ascus with arrows indicating the gelatinous material surrounding the apex of the endoascus. 10. Released ascus with arrows indicating the inward curling of the ectoascus wall. Bars: 1 = 500 μ m; 2, 3 = 10 μ m; 4, 5, 7, 8, 9, 10 = 10 μ m; 6 = 20 μ m.

guttulate, verruculose, surrounded by a large gelatinous sheath.

Lucidascoarpha pulchella A. Ferrer, Raja & Shearer, sp. nov.

Ascomata 105–200 × 90–180 µm, solitaria vel gregaria, superficialis vel immersa, albidus, globosa vel subglobosa, rostrata, membranacea. Rostrum 160–280 × 38–50 µm, cylindraceum, periphysatum, albidum. Peridium 10–14 µm crassum. Asci 145–150 × 28–30 µm, fissitunicati, clavati, pedicellati, octospori, biseriatati. Pseudoparaphyses absentia. Ascospores 36–50 × 12–16 µm, 7-septatae, hyalinae, ellipsoideae-fusiformes, multiguttulatae, verruculosae, circumcinctae vagina gelatinosa, grandia, extensa in aqua.

Ascomata on wood, scattered to aggregated, numerous, superficial to partially immersed with white, glistening necks protruding from the substrate, venter globose to subglobose, 105–200 × 90–180 µm diam, white, glistening (FIG. 1). *Neck* 160–280 × 38–50 µm, periphysate, cylindrical, curving irregularly, short hyphae protruding from the surface of the neck (FIGS. 1, 2, 4). *Peridium* 10–14 µm wide, composed of 5–7 elongated, thin-walled, hyaline cells (FIG. 3), tissue of *textura angularis* in surface view. *Pseudoparaphyses* absent. *Asci* 145–150 × 28–30 µm (mean = 144 × 29 µm, n = 20), clavate, pedicellate, straight or at times slightly curved toward the base (FIGS. 5, 6), with gelatinous material deposited around the apex of endoascus (FIGS. 7, 9), containing 8 overlapping, obliquely biseriate ascospores (FIGS. 5, 6). Dehiscence fissitunicate, ectotunica rupturing at the apex and becoming strongly wrinkled and curled inwards, endotunica elongating to a quarter length of the ascus (FIGS. 8, 9, 10). *Ascospores* 36–50 × 12–16 µm (mean = 40 × 13 µm, n = 50), seven-septate, hyaline, ellipsoidal to fusiform, verruculose (FIGS. 11–13, 15), multiguttulate in a dictyose pattern (FIGS. 11, 12), end cells lacking guttules, staining blue in aqueous nigrosin (FIGS. 6, 11–13), surrounded by a large gelatinous sheath; gelatinous sheath ca. 2 µm wide in ascus (FIGS. 8, 9, 11), expanding in water to 28–60 µm wide at the mid region, 68–128 µm long at ascospore apices (FIG. 14), germinating from ascospore apices (FIG. 16). Colonies on potato dextrose agar (Difco), slow growing, 0.5 cm diam in 4 wk at 24 C, dark brown, compact, with little aerial mycelium. Anamorph not observed.

Known distribution: Costa Rica and Ecuador.

Etymology: From Latin *Pulchella* = very pretty.

Habitat: lotic.

HOLOTYPE: ECUADOR. NAPO: Yasuni National Park, Laguna-Tiputini River, 0°46'S, 79°51'W, water 26 C, pH 5.5–6, on submerged, decorticated wood, 4 Apr 2004, AF178-2. (ILL 40558).

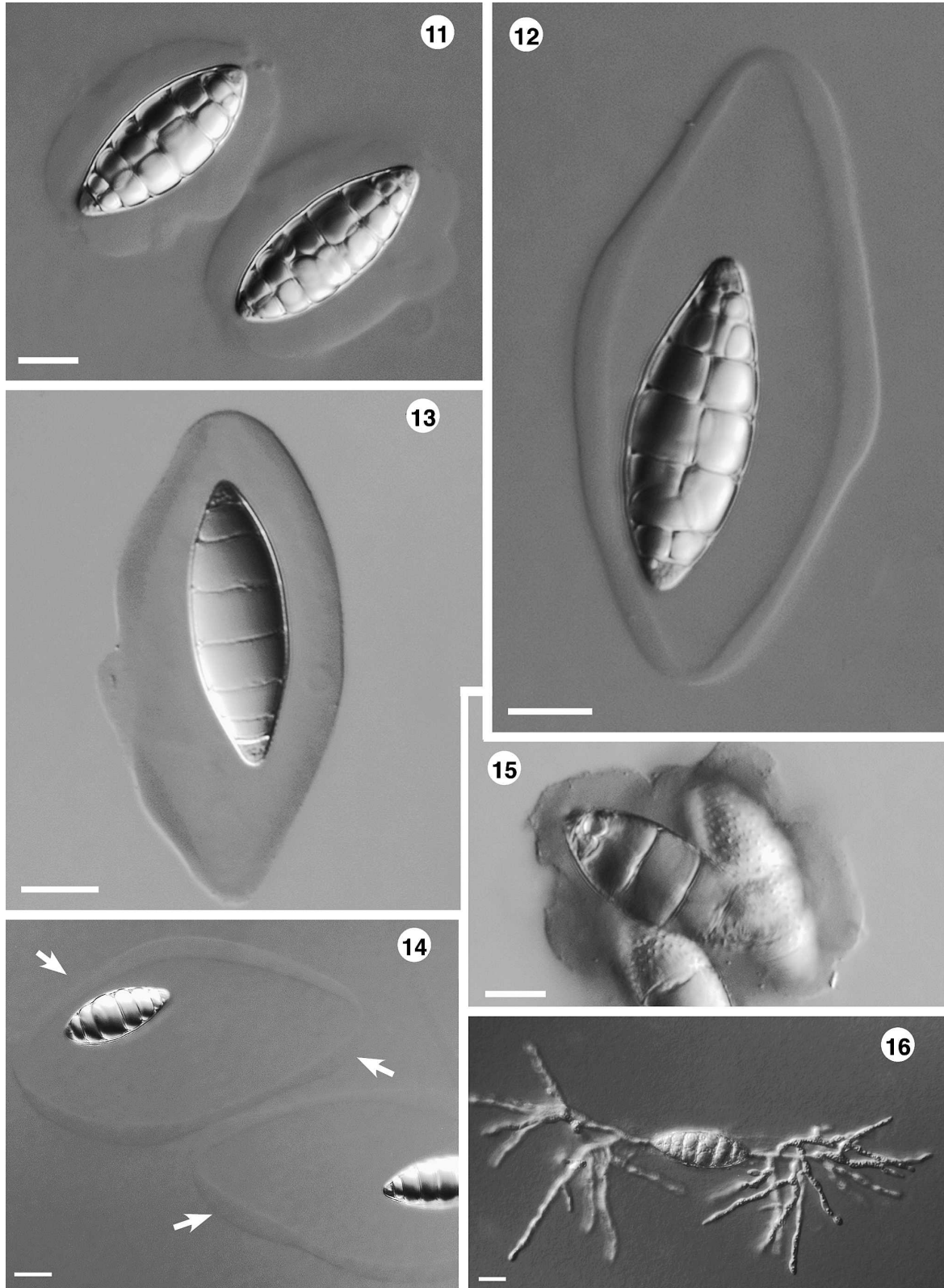
Additional specimens examined: COSTA RICA. ALAJUELA: Caño Negro Reserve, Frio Stream, on submerged, decorticated wood, 10°53'N, 84°45'W, water 28 C, pH 5, 15 Dec 2005, A. Ferrer & M. Salazar, AF280-1. (ILL 40555); LIMON: Barra del Colorado and Tortuguero National Park, Las Palmas stream, on submerged, decorticated wood, 10°35'N, 83°31'W, water 25 C, pH 5, 18 Dec 2005, A. Ferrer & M. Salazar, AF280-2. (ILL 40556); HEREDIA: La Selva, Sarapiquí River, on submerged, decorticated wood, 10°26'N, 84°01'W, water 25 C, pH 5, 10 Jan 2006, M. Salazar, AF280-5. (ILL 40557).

DISCUSSION

Noteworthy features of *Lucidascoarpha pulchella* include the white, glistening ascomata with long necks (FIGS. 1–2) that are easily seen on the substratum, the fissitunicate ascus in which the endoascus apex is covered with gelatinous material (FIGS. 7–9) and the hyaline, phragmoseptate, rough-walled ascospores (FIG. 15) that have guttules in a dictyose pattern (FIGS. 11, 12). Apical cells of the ascospores have fewer guttules than the other cells of the ascospore (FIGS. 11, 12) and they stain darkly in nigrosin (FIGS. 5, 6). The ascospore sheath maintains a regular edge and shape as it enlarges in water (FIGS. 11, 12, 14) and is difficult to observe once expanded. The sheath of ascospores mounted directly on lactic acid or nigrosin remains unexpanded (FIGS. 13, 15). Some sheaths that were ruptured appeared to adhere to other materials in the slide preparation by sticky material within the sheath. This feature could help the spores attach to substrata in flowing water.

Among the Dothideomycetes with light colored ascocarps *Lucidascoarpha* is similar to *Aliquandostipite* Inderb., a genus in the Jahnulales (Pang et al 2002) in having translucent, light-colored fruiting bodies and septate ascospores surrounded by a broad gelatinous sheath (Inderbitzin et al 2001). However *Lucidascoarpha* does not have pseudoparaphyses or the wide, brown, septate hyphae that characterize the Jahnulales (Campbell et al 2007).

Lucidascoarpha can be accommodated in the Dothideales (*sensu* Barr 1987) because it has fissitunicate asci and lacks pseudoparaphyses. It is unusual in the order because of its white ascomata. Among the Dothideales *Lucidascoarpha* shows some similarities to the genus *Hyalocrea* H. Sydow & Sydow in the Dothideaceae (Rossman 1987), in having light colored ascomata. However the ascomata of *Lucidascoarpha* have a distinctive long cylindrical neck rather than a short ostiole and lack hairs present in most *Hyalocrea* species. In addition the centrum of the ascomata of *Lucidascoarpha* is filled with numerous asci while *Hyalocrea* species have few asci



FIGS. 11–16. *Lucidascocarpa pulchella* from the holotype. 11. Ascospores with guttules in a dictyose pattern, surrounded by a gelatinous sheath. 12. Ascospores with expanded gelatinous sheaths in water. 13. Ascospores with gelatinous sheaths in 1% nigrosin. 14. Ascospores with gelatinous sheaths expanded in water. Arrows indicating the expanded gelatinous sheaths. 15. Ascospores stained with 1% nigrosin and illustrating verruculose walls. 16. Germinating ascospore. Bars = 10 μ m.

per ascomata (Rossman 1987). Although ascospores of some *Hyalocrea* species have cellular appendages at each end, none of the species have verruculose ascospores surrounded by a broad gelatinous sheath as observed in *Lucidascoarpha*. Most *Hyalocrea* species have been reported growing on living leaves and one has been found growing on the stroma of other fungi (Rossman 1979, 1987) while *Lucidascoarpha pulchella* was found on submerged, decorticated wood.

The placement of the new fungus at the family level is uncertain. The combination of morphological characteristics found in *L. pulchella* are not present in any family currently included in the Dothideales (Barr 1987, Eriksson 2006); these characteristics include the white, membranous ascomata with a long, periphysate neck and multiguttulate, rough-walled, phragmoseptate ascospores with a thick-walled sheath that enlarges to become balloon-like. It also is possible that in adapting to the freshwater environment this fungus might have lost the ability to form pigmented, thick-walled ascomata, pigmented ascospores or pseudoparaphyses, and this could be misleading with respect to taxonomic placement. Molecular sequence data are necessary to determine where this fungus belongs within the Dothideomycetes. Although we have been able to culture this fungus we have been unable to obtain sequence data from DNA extracts.

Approximately 17 aquatic ascomycete genera, including *Lucidascoarpha*, and 70 other freshwater ascomycete taxa cannot as yet be accommodated in any existing orders or families based on morphological characteristics (see <http://www.life.uiuc.edu/plantbio/fungi/>). These anomalous genera highlight the diversity of aquatic fungi while also emphasizing the relatively low collecting activity for freshwater ascomycetes when compared to their terrestrial counterparts. Certainly when phylogenetic tools are applied to freshwater ascomycetes in a concerted way new evolutionary lineages will be revealed.

ACKNOWLEDGMENTS

We thank Marlon Salazar, Jim Dalling and Carolina Sarmiento for great help with collecting and lab work. We also thank Dr Margaret Barr for her helpful comments about *L. pulchella*. We also thank Drs G.J. Samuels and J.L. Crane for their comments on the manuscript. We appreciate the help of the Organization for Tropical Studies (OTS) for their logistic support to collect in Costa Rica and Pontificia Universidad Catolica del Ecuador (PUCE) and Ministerio del Ambiente for their logistic support to collect in Ecuador. Financial support by the National Science Foundation (NSF Grant No. DEB 0316496) is gratefully acknowledged. Any opinions, findings and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the National Science Foundation.

LITERATURE CITED

- Barr ME. 1987. Prodrromus to class Loculoascomycetes. Amherst, Massachusetts: Newell. 168 p.
- Campbell J, Ferrer A, Raja HA, Sivichai S, Shearer CA. 2007. Phylogenetic relationships among taxa in the Jahnulales inferred from 18S and 28S nuclear ribosomal DNA sequence. *Can J Bot* 85:873–882.
- Eriksson OE, ed. 2006. Outline of Ascomycota 2006. *Myconet* 12:1–82.
- Ferrer A, Shearer CA. 2005. New records and a new species of *Canalispodium* from aquatic habitats in Panama. *Mycotaxon* 93:179–188.
- Inderbitzin P, Landvik S, Abdel-Wahab MA, Berbee ML. 2001. Aliquandostipitaceae, a new family for two new tropical ascomycetes with unusually wide hyphae and dimorphic ascospores. *Am J Bot* 88:52–61.
- Pang K-L, Abdel-Wahab MA, Sivichai S, El-Sharouny HM, Jones EBG. 2002. Jahnulales (Dothideomycetes, Ascomycota): a new order of lignicolous freshwater ascomycetes. *Mycol Res* 106:1031–1042.
- Rossman AY. 1979. A preliminary account of the taxa described in *Calonectria*. *Mycotaxon* 8:485–558.
- . 1987. The Tubeufiaceae and similar Loculoascomycetes. *Mycol Pap* 157:1–71.
- Volkman-Kohlmeyer B, Kohlmeyer J. 1996. How to prepare truly permanent microscopic slides. *Mycologist* 10:107–108.