Mycoscience (2010) 51:208–214
DOI 10.1007/s10267-009-0027-x

FULL PAPER

Freshwater ascomycetes: *Wicklowia aquatica*, a new genus and species in the Pleosporales from Florida and Costa Rica

Huzefa A. Raja · Astrid Ferrer · Carol A. Shearer · Andrew N. Miller

Received: 24 August 2009 / Accepted: 13 November 2009 / Published online: 30 January 2010

Abstract During a latitudinal survey of freshwater ascomycetes, an unidentified fungus with bitunicate asci was found on submerged wood and herbaceous material from Florida and Costa Rica. Based on morphological characteristics and 28S rDNA large subunit (LSU) sequence data, this fungus is described as a new genus and species, *Wicklowia aquatica*, and placed in the Pleosporales (Pleosporomycetidae, Dothideomycetes). Phylogenetic analyses based on LSU sequences did not resolve the familial placement of *W. aquatica* within the Pleosporales. The characteristic features of *W. aquatica* are subglobose, dorsiventrally flattened, ostiolate, immersed to erumpent, black ascomata; a peridial wall composed of 4–5 layers of darkened pseudoparenchymatic cells; cellular pseudoparaphyses immersed in a gel matrix; broadly clavate, bitunicate asci; and cylindrical, hyaline, one-septate ascospores with rounded apices and surrounded by a gelatinous sheath that expands in water; ascospore sheath attached at the ascospore base with a gelatinous curtain extending from the base that fragments into basal filamentous appendages which radiate from the base of the ascospore.

Keywords Aquatic fungi · Dothideomycetes · LSU · Neotropics · Sequences · Systematics

H. A. Raja (✉) · A. Ferrer · C. A. Shearer
Department of Plant Biology, University of Illinois,
Room 265 Morrill Hall, 505 South Goodwin Avenue,
Urbana, IL 61801, USA
e-mail: raja@uiuc.edu

A. N. Miller
Illinois Natural History Survey,
University of Illinois,
Champaign, IL 61820-6970, USA

Introduction

During studies of freshwater ascomycetes along a latitudinal gradient through North and Central America, an unusual bitunicate fungus was found on submerged wood and herbaceous material in Florida (as F76 in Raja et al. 2009) and on submerged wood in Costa Rica. Morphological characters such as presence of a globose, immersed to erumpent pseudothecium with a pseudoparenchymatous peridium, interascal tissue of cellular pseudoparaphyses, fissitunicate asci, and thin-walled, hyaline, one-septate ascospores with a gelatinous sheath are characteristics of taxa in the order Pleosporales (Dothideomycetes). The fungus from Florida and Costa Rica, however, differs from other Pleosporalean genera with one-septate, hyaline ascospores surrounded by a gelatinous sheath (Zhang et al. 2009) in that it possesses filamentous appendages radiating around the ascospore base. Although common in marine taxa in Halosphaeriales (Kohlmeyer and Volkmann-Kohlmeyer 1991; Jones 1994, 2006; Jones et al. 2009), radiating ascospore appendages are rare in freshwater and terrestrial taxa in the Pleosporales. The goals of this study were (1) to fully characterize and describe the morphology of this novel fungus from Florida and Costa Rica and (2) to use morphological and phylogenetic analyses of large subunit 28S rDNA to elucidate the phylogeny and taxonomy of the new taxon.

Materials and methods

Morphological study

Methods for collection, isolation, morphological characterization, and illustration of freshwater ascomycetes used

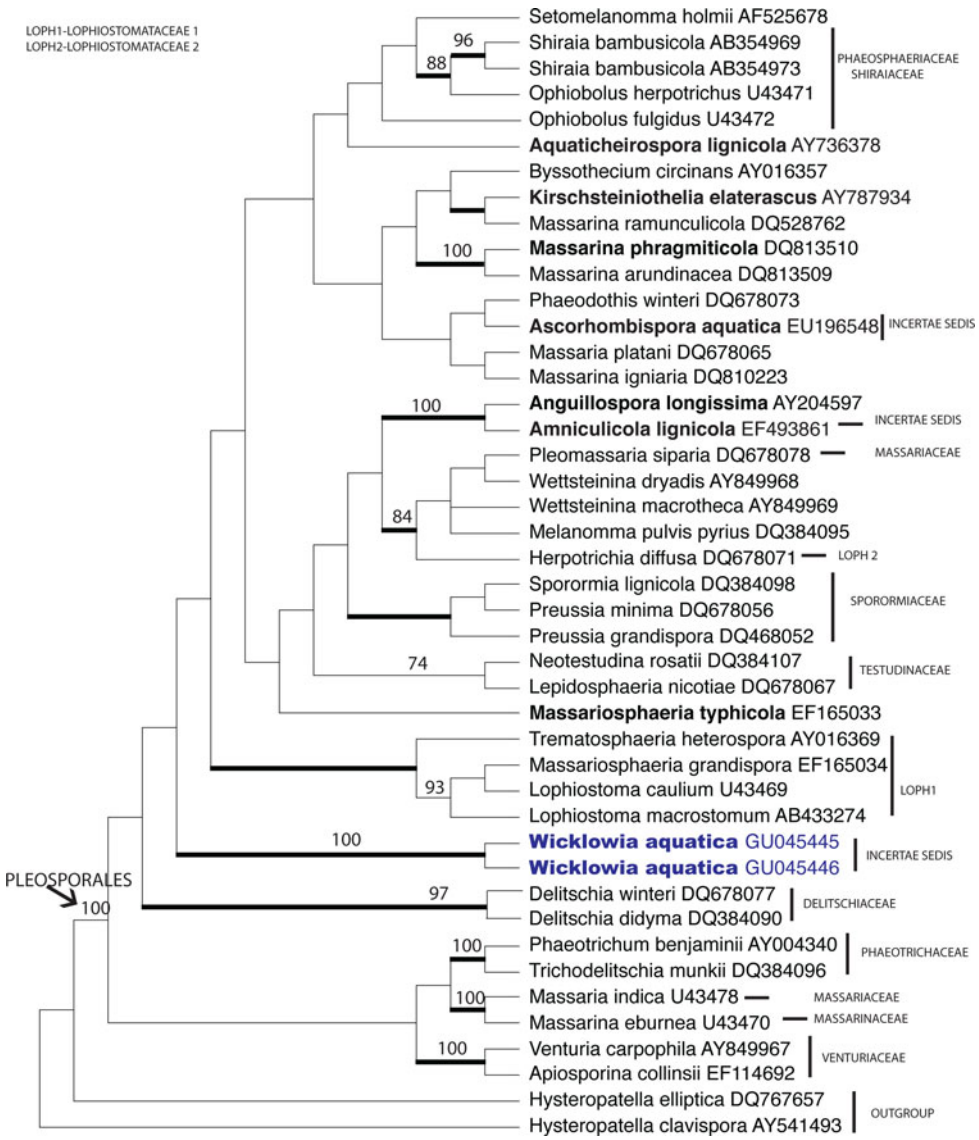


Fig. 1 Cladogram of one of the three most parsimonious trees from a heuristic analysis of 28S rDNA sequences from families currently in the Pleosporales (based on Lumbsch and Huhndorf 2007; Kirk et al. 2008). Taxa reported/described from freshwater are in *bold*. Tree length, 1133; consistency index (CI) = 0.499; retention index (RI) = 0.657. Bootstrap support values ≥70% from 1000 replicates are included *above* the branches. *Thickened branches* indicate posterior probabilities ≥95%. *Hysteropatella elliptica* and *Hysteropatella clavispora* were used as outgroup taxa

in this study are described by Shearer et al. (2004) and Raja et al. (2009). The holotype and additional specimens are deposited in the Herbarium of the University of Illinois at Urbana-Champaign (ILL).

Molecular study

Fungal isolates F76-2 (Florida) and AF289-1 (Costa Rica) were grown on peptone-yeast-glucose agar (PYG: 1.25 g peptone, 1.25 g yeast extract, 3.0 g D-glucose, 18 g Difco-Bacto agar, 1 l distilled water) at room temperature for 2–16 weeks. Mycelia were harvested using a sterile scalpel. Mycelia were then ground to a fine powder with pestle and mortar after freezing with liquid nitrogen. Total genomic DNA was extracted using the procedures of Campbell et al. (2007). The LROR–LR6 region of nuclear large subunit rDNA was amplified and sequenced using primers LROR, LR3, LR3R, and LR6 (Vilgalys and Hester 1990) at the University of Illinois Biotechnology Center with an ABI 373A automated sequencer. Detailed methods are outlined in Raja et al. (2008). Sequences were manually edited and assembled in Sequencher 4.7 (Gene Codes, Ann Arbor, MI, USA) with published sequence data.

To determine the familial placement of the new fungus, partial LSU (28S nrDNA) sequences of the new taxon were analyzed with LSU sequences for 42 other species obtained from GenBank that represent families currently circumscribed within the Pleosporales, Dothideomycetes (Lumbsch and Huhndorf 2007; Kirk et al. 2008). GenBank accession numbers follow taxon names on the tree (Fig. 1). *Hysteropatella elliptica* and *Hysteropatella clavispora* were used as outgroup taxa based on work by Schoch et al. (2006).

The first 60 bp of the 5′-end were excluded from all analyses because data were missing in most taxa. Six

ambiguously aligned regions were delimited, and characters in these regions were recoded and analyzed to recover their phylogenetic signal using the program INAASE (Lutzoni et al. 2000). The remaining unambiguously aligned regions were subjected to a symmetrical stepmatrix using STMatrix ver. 2.2 (François Lutzoni and Stefan Zoller, Biology Department, Duke University). An unequally weighted maximum-parsimony (MP) analysis was conducted using PAUP* 4.0b10 (Swofford 2002) as follows: constant characters were excluded, gaps were treated as missing data, 1000 random-addition replicates were implemented with TBR branch-swapping, MULTREES option was in effect, and zero-length branches were collapsed. Bootstrap support was estimated by performing 1000 bootstrap replicates (Felsenstein 1985) using these settings.

Modeltest 3.7 (Posada and Crandall 1998) was used to determine the best-fit model of evolution for the dataset. Maximum-likelihood (ML) analyses were performed using PAUP with 1000 stepwise random-addition replicates and tbr branch-swapping with a reconnection limit of 12 using the best-fit model, which was the GTR + I + G model with unequal base frequencies (freqA = 0.2390, freqC = 0.22980, freqG = 0.30160, freqT = 0.22960), a substitution rate matrix (A ↔ C = 1.0000, A ↔ G = 3.1004, A ↔ T = 1.2883, C ↔ G = 1.2883, C ↔ T = 8.0493, G ↔ T = 1.0000), a proportion of invariable sites = 0.5644, and a gamma distribution shape parameter = 0.7795.

Bayesian analysis employing Markov chain Monte Carlo (MCMC) was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Huelsenbeck and Ronquist 2001) as an additional means of assessing branch support. A comparable model to the ML analyses was used to run 10 million generations with trees sampled every 100th generation, resulting in 100,000 total trees. Two independent analyses were performed with four chains using default settings to ensure that trees were being sampled from the same tree space and that they converged on the same tree. The first 10,000 trees that extended beyond the burn-in phase in each analysis were discarded, and the remaining 90,000 trees were used to calculate posterior probabilities. The consensus of 90,000 trees was viewed in PAUP 4.0b10 (Swofford 2002).

Results

Morphological study

Examination of specimens and whole mounts of fresh material indicated that the new fungus fits well within the Pleosporales, but the combination of very small pseudothecia, asci, and ascospores and the presence of both an ascospore sheath and appendages ruled out placement of this taxon in any existing Pleosporalean family or genus.

Molecular study

Initial analyses were performed with LSU sequence data from Schoch et al. (2006) with representatives from various orders within the Dothideomycetes (data not shown). The two isolates of the new fungus were placed within the Pleosporales. Species that were phylogenetically distinct from the Pleosporales were removed, and further analyses were carried out with 42 taxa representing various families currently in the Pleosporales (Fig. 1). Maximum-parsimony (MP) analyses of the 42 LSU sequences recovered three most parsimonious trees [all with CI = 0.499, RI = 0.657, and homoplasy index (HI) = 0.328]; one tree is shown in Fig. 1. Maximum-likelihood (ML) analyses also generated three most likely trees (data not shown); ML trees did not differ in topology from MP trees. Phylogenetic analyses based on 28S rDNA sequences place the new fungus in the Pleosporales but in a lineage separate from other morphologically similar genera in the Pleosporales.

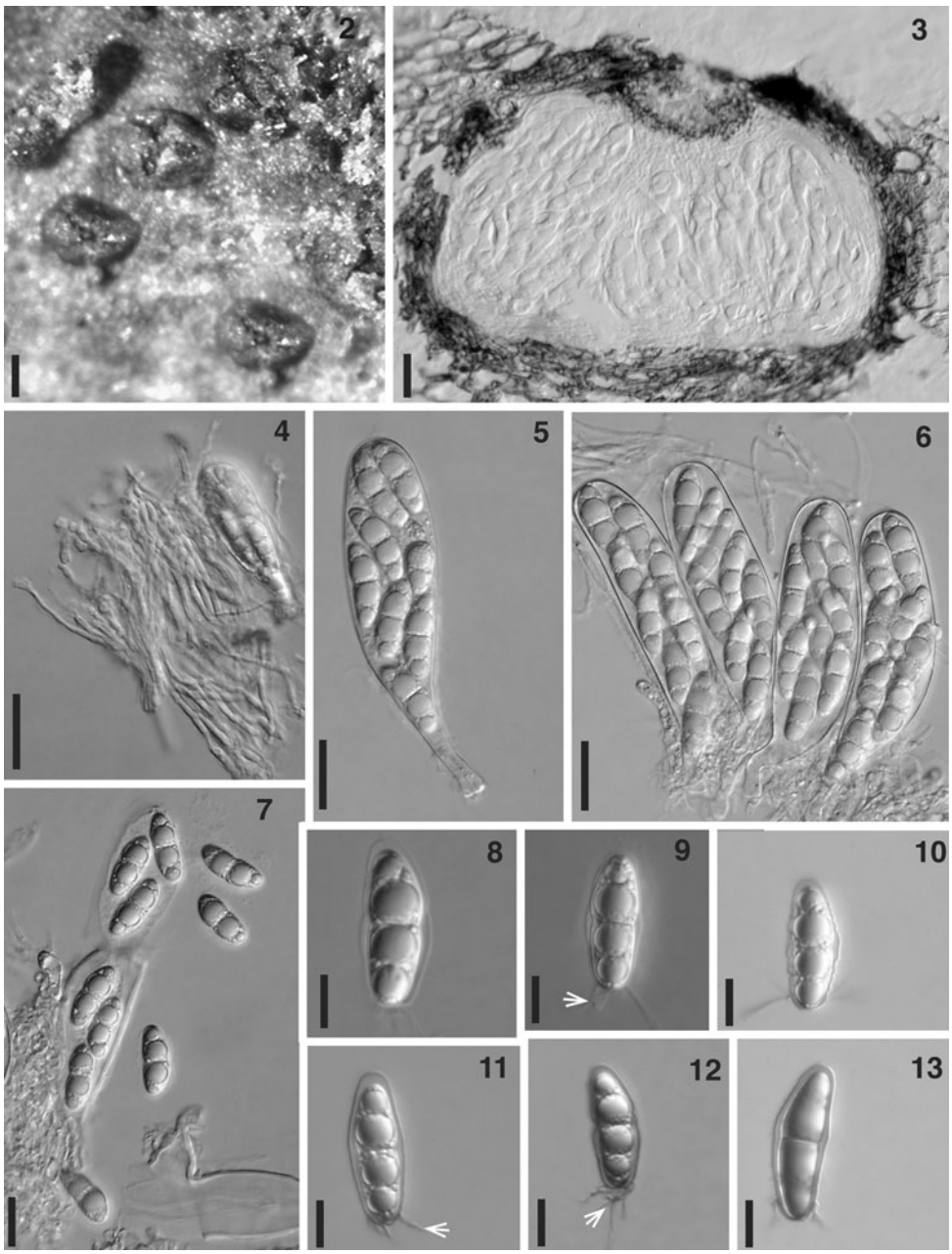
Although the new fungus is placed in the Pleosporales based on molecular sequence analyses (Fig. 1) as well as morphological data (Figs. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13), its relationships to other families and genera in the Pleosporales are unclear. It forms a clade basal to most of the families of the Pleosporales represented in the analyses except for the Delitschiaceae, Phaeotrichaceae, Massariaceae, Massarinaceae, and Venturiaceae. Given the lack of molecular data to provide support for inclusion of this new fungus in any existing Pleosporalean family or genus, we therefore erect a new genus to accommodate this fungus.

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

MycoBank No. 515225

Ascomata subglobosa complanata dorsiventraliter, ostiolata, immersa vel erumpentia, solitaria vel gregaria, formantia depressio, super substratum, facie atra, ovalia vel circularia, tenuia crateriformia. Paries peridii 4–5 stratorum cellulis parvis pseudoparenchymaticis. Pseudoparaphyses septatae, sparsae. Asci fissitunicati, late clavati, ad apices rotundati, matrice gelatinosa. Ascosporae cylindricae, tenuitunicatae ad apices rotundatae, hyalinae, uniseptatae, leviter constrictae ad septum, circumcinctae vagina gelatinosa expansa aqua. Vagina affixa ad basim, plagula gelatinosa extensa deorsum ex basi, frangens fimbria subapicalis circa basis ascosporae.

Ascomata subglobose, flattened dorsiventrally, ostiolate, immersed becoming erumpent, solitary to gregarious, appearing as a black oval to circular, shallow, crater-like depression on the substrate. Peridial wall of 4–5 layers of small pseudoparenchymatic cells. Pseudoparaphyses septate, sparse. Asci fissitunicate, in a gel matrix, broadly



Figs. 2–13 *Wicklowia aquatica* from the holotype (F76-2). **2** Erumpent ascomata on wood. **3** Longitudinal section through the ascoma. **4** Pseudoparaphyses. **5** Pedicellate ascus with 8 ascospores. **6** Asci with pseudoparaphyses. **7** Bitunicate ascus. **8** Ascospores from ex-type culture (F76-2) mounted in water. **9** Ascospores from ex-type culture (F76-2) mounted in water (*arrow*). **10** Ascospores in water stained with aqueous nigrosin, note the gelatinous sheath, and basal appendages. **11** Ascospores in water stained with aqueous nigrosin: note the gelatinous sheath, and basal appendages (*arrow*). **12** Ascospores in water stained with aqueous nigrosin: note the gelatinous sheath, and basal appendages (*arrow*). **13** Ascospore in glycerin showing gelatinous sheath and appendages. *Bars* **2** 100 μm; **3–7** 20 μm, **8–13** 10 μm

clavate, rounded at the apex. Ascospores cylindrical, thin walled, rounded at the apices, hyaline, one-septate, slightly constricted at the septum, surrounded by a gelatinous sheath that expands in water; sheath attached at the base with a gelatinous curtain extending downward from the base that fragments into filamentous appendages forming a subapical fringe around the ascospore base.

Type species: *Wicklowia aquatica* Raja, A. Ferrer & Shearer

Etymology: Honoring Dr. Donald T. Wicklow for his outstanding studies of the nature and role of fungal secondary compounds.

Wicklowia aquatica Raja, A. Ferrer & Shearer sp. nov., Figs. 2–13

MycoBank No. 515226

Ascomata 140–145 × 265–270 μm, subglobosa, complanata dorsiventraliter, osteolata, atra, immersa vel erumpentia, solitaria vel gregaria, formentia depressio super substratum, fascie atra, ovalia vel circularia, tenuia crateriformia. Paries peridii 4–5 stratorum cellulis parvis pseudoparenchymaticis. Pseudoparaphyses septatae, sparsae, 2 μm latae, matrice gelatinosa. Asci 60–95 × 18–26 μm, fissitunicati, matrice gelatinosa, late clavati, ad apices rotundati, pedunculo arcuato, attenuato, octospori biseriati

vel triseriatis, imbricati. Ascosporae 25–30 × 7–9 µm (modus 25 × 8 µm, $n = 25$), cylindricae, tenuitunicatae, ad apices rotundatae, applanatae citro, hyalinae, uniseptatae, laeviter constrictae ad septum, unaqaeque cellula biguttulata, circumcinctae vagina gelatinosa expansa aqua ca. 2 µm lata. Vagina affixa ad basim, plagula gelatinosa extensa deorsum ex basi, frangens appendix filamentosa ca. 3–6 µm longa, faciens fimbria subapicalem, vagina et appendices nigrosin ope caerulescens.

Ascomata 140–145 × 265–270 µm, subglobose, flattened dorsiventrally, ostiolate, black, immersed to erumpent, solitary to gregarious, appearing as a black oval to circular, shallow, crater-like depression on the substrate. Peridial wall ~25 µm wide, composed of 4–5 layers of small pseudoparenchymatic cells. Pseudoparaphyses septate, sparse, 2 µm wide, in a gel matrix. Asci 60–95 × 18–26 µm, fissitunicate, in a gel matrix, broadly clavate, rounded at the apex, apical chamber not observed, tapering to a curved stalk, with eight overlapping biseriate to triseriate ascospores. Ascospores 25–30 × 7–9 µm (mean, 25 × 8 µm; $n = 25$), thin walled, cylindrical, rounded at the apices, slightly flattened on one side, hyaline, one-septate, slightly constricted at the septum, with two large lipid globules in each cell, surrounded by a gelatinous sheath that expands in water to ~2 µm wide on each side of the ascospore; sheath attached at the base, with a gelatinous curtain extending downward from the base that fragments into filamentous appendages ~3–6 µm long, forming a subapical fringe; sheath and appendages staining blue in aqueous nigrosin.

Characteristics in culture: colonies on PYG slow growing, floccose, dark grey, reverse black. Mycelium immersed in the agar, composed of septate brown hyphae. Colonies on corn meal agar (CMA; Difco, Detroit, MI, USA) slow growing, grey; reverse hyaline to grey; mycelium diffuse, immersed to aerial. Hyphae hyaline to light brown, thin walled, 1–2 µm wide, septate. Ascomata formed in culture after about 3 months around the periphery of the growing colony on CMA, partly superficial to immersed in the agar. Colonies on potato dextrose agar (PDA; Difco) slow growing, grey in the center, black toward the periphery; dark black in reverse. Hyphae light brown. Forming ascomata in culture after 3–4 months on the center of the agar inoculum plug.

Etymology of species epithet: in reference to the aquatic habitat of the fungus.

Material examined: United States. Florida: Apalachicola National Forest, Apalachicola River at Fort Gadsden Landing, 29°56′00″N, 85°0′00″W, water temp. 9°C, pH 6, on submerged decorticated woody debris, 14 January 2006, Huzefa A. Raja and J.L. Crane, F76-2 (holotype: ILL 40790 = H.A. Raja and J.L. Crane F76-2). Ex-holotype culture, H.A. Raja F76-2.

Additional specimens examined: United States. Florida: Ocala National Forest, Alexander Springs, 29°04′52″N, 81°33′57″W, water temp. 22°C, pH 5, on submerged herbaceous material, 3 March 2005, Huzefa Raja and J.L. Crane, F76-1; channel of Apalachicola River, 29°45′01″N, 85°00′34″W, water temp. 28°C, pH 6.5, on submerged corticated wood, 16 June 1997, *Kevin Robertson, A419-1*; Costa Rica. Alajuela, Caño Negro Reserve, Rio Frio, 10°53′00″N, 84°45′00″W, water temp. 27°C, pH 5, 15 December 2005, A. Ferrer and M. Salazar, AF289-1; Caño Negro Reserve, Rio Frio, 10°53′00″N, 84°45′00″W, water temp. 28°C, pH 5, on submerged wood, 15 December 2005, A. Ferrer and M. Salazar, AF289-2; Heredia, La Selva stream, 10°25′00″N, 84°0′00″W, water temp. 25°C, pH 5, on submerged wood, 9 January 2006, M. Salazar, AF289-4; Limon, Las Palmas stream, 10°35′00″N, 83°31′00″W, water temp. 25°C, pH 5, on submerged wood, 18 December 2005, A. Ferrer and M. Salazar, AF289-6.

Known distribution: Costa Rica, USA (Florida).

Discussion

Wicklowia aquatica has several morphological characters that support its placement in the Pleosporales (Pleosporomycetidae, Dothideomycetes) (Figs. 2–13). These characters include globose, erumpent, ostiolate, perithecial pseudothecia with a pseudoparenchymatous peridium, cellular pseudoparaphyses, fissitunicate asci, and hyaline, one-septate, thin-walled ascospores with a gelatinous sheath (Kirk et al. 2008). Based on morphological characters, *W. aquatica* is superficially similar to members of the genus *Massarina* Sacc. in having clavate, short-stalked asci, and one-septate, hyaline ascospores with a gelatinous sheath (Bose 1961; Hyde 1995; Aptroot 1998; Tanaka and Harada 2003; Zhang et al. 2009). *Wicklowia*, however, is much smaller in ascomal, ascus, and ascospore dimensions, and has a distinctly different ascospore sheath, i.e., it is attached at the ascospore base and has a curtain-like structure extending downward from the base that fragments into filamentous appendages which form a subapical fringe around the ascospore base (Figs. 8, 9, 10, 11, 12, 13). Extremely small size and ascospore sheath characteristics are not reported for any member of *Massarina* (Aptroot 1998; Tanaka and Harada 2003).

Wicklowia aquatica shares some similarities with the recently described freshwater ascomycete species *Amniculicola lignicola* Y. Zhang & K.D. Hyde (Zhang et al. 2008) in having hyaline, one-septate ascospores surrounded by a gelatinous sheath and occurrence on wood in freshwater habitats. *Wicklowia aquatica* differs from *A. lignicola* in that the asci of *W. aquatica* are clavate with

eight overlapping biseriate to triseriate ascospores, whereas asci in *A. lignicola* are cylindrical to narrowly fusiform, with obliquely uniseriate and partially overlapping ascospores (Zhang et al. 2008). The ascospores of *W. aquatica* have a sheath as well as basal filamentous appendages, whereas the sheath of *A. lignicola* surrounds the ascospores and is not anchored at the base of the ascospore. Radiating filamentous basal appendages are not reported for *A. lignicola*. Furthermore, parsimony analyses of partial 28S rDNA sequences also indicate that *W. aquatica* does not cluster with either *Massarina eburnea* (Tul. & C. Tul.) Sacc. (the type species of *Massarina*) or with *Amniculicola lignicola* (the type species of *Amniculicola*) (see Fig. 1). In addition, our analyses did not provide any support for its placement into other recognized genera included in the tree (Fig. 1).

Wicklowia aquatica should also be compared with *Ascominuta lignicola* Ranghoo & K.D. Hyde, a bitunicate genus described from wood in freshwater (Ranghoo and Hyde 2000). The two taxa are similar in that they have small ascomata and hyaline, one-septate ascospores with a gelatinous sheath that expands in water. However, *W. aquatica* can be distinguished readily from *Asco. lignicola* in ascus morphology. The asci of *Wicklowia* are clavate, broadly rounded at the apex (60–95 × 18–26 μm), and contain eight ascospores per ascus (see Figs. 5, 6), whereas asci in *Asco. lignicola* are globose and four spored (25–30 × 25–30 μm) (Ranghoo and Hyde 2000). A 28S rDNA sequence of *Asco. lignicola* from GenBank (AF132335) was included in a larger preliminary analysis, but it did not show any phylogenetic affinities with *W. aquatica* (data not shown). The 28S rDNA sequence of *Asco. lingicola* (AF132335) also had very few base pairs in common with other taxa in the Dothideomycetes alignment.

Radiating filamentous appendages on ascospores are a common feature in marine and mangrove ascomycetes mostly belonging to the order Halosphaeriales (Hyde and Jones 1989; Jones 1994, 2006). This character, however, is a rare feature in freshwater ascomycetes belonging to the Dothideomycetes (see http://fungi.life.uiuc.edu/). Freshwater Dothideomycetes with radiating ascospore appendages include *Aliquandostipite minuta* Raja & Shearer (Raja and Shearer 2007), a freshwater ascomycete described from submerged wood in freshwater from Florida. *Wicklowia aquatica* is, however, quite different, and belongs in the Pleosporales, whereas *A. minuta* belongs in the Jahnulales, an order characterized by very wide hyphae, a feature not present in *W. aquatica*. *Heleiosa barbatula* Kohlm., Volkm.-Kohlm. & O.E. Erikss. in the Dothideomycetes, but reported from *Juncus roemrianus* Scheele in a salt marsh (Kohlmeyer et al. 1996), also has appendages similar to *W. aquatica* (see Figs. 10, 11, 12) (Kohlmeyer et al. 1996). However, *W. aquatica* and *H. barbatula* differ in that the ascospore appendages in *W. aquatica* are basal and ascospores are hyaline, whereas in *H. barbatula* the appendages occur at each end of brown ascospores. The two fungi also differ in their ascus morphology, substrate, and habitat. Another dothideomycete fungus with appendages superficially similar to *W. aquatica* is *Tirisporella beccariana* (Ces.) E.B.G. Jones, K.D. Hyde & Alias, which is reported from the mangrove palm *Nypa fruticans* (Thunb.) Wurmb (Jones et al. 1995). The appendages in *W. aquatica* occur at the base of the ascospores, whereas appendages in *T. beccariana* occur at the apex of the ascospores. *Wicklowia aquatica* also differs from *T. beccariana* in ascospore color, septation, and ascus morphology.

Wicklowia aquatica produces novel bioactive compounds. Three new nonadride analogues were isolated from the liquid growth medium from the ex-type isolate of *W. aquatica* (F76-2), along with the known nonadrides, epiheveadride, dihydroepiheveadride, and deoxoepiheveadride (Hosoe et al. 2007). Two additional new C-9 compounds were also obtained from the extract (Hosoe et al. 2007). The three new nonadride analogues showed no activity against *Aspergillus fumigatus* and *Fusarium verticillioides*, but the known nonadride compounds showed potent antifungal activities against the test fungi. Dihydroepiheveadride induced swelling of the hyphal terminus in cultures of *Aspergillus fumigatus*. A number of previously described freshwater aquatic fungi belonging to the Pleosporales have shown interesting chemistry, and several novel bioactive compounds have been isolated from freshwater ascomycetes in the Dothideomycetes (see Poch et al. 1992; Harrigan et al. 1995; Jiao et al. 2006a,b; Madur et al. 2006).

Acknowledgments We thank Chris Brown, Dr. Kevin Robertson, and Dr J.L. Crane for their assistance with collecting. We also thank the rangers at Apalachicola National Forest and Ocala National Forest for permission to collect within the forest. The Keck Center, UIUC, is acknowledged for sequencing. Financial support from the National Science Foundation and National Institutes of Health under (NSF grant No. DEB 03-16496, DEB 08-4472, and NIH grant No. R01GM-60600) helped make this research possible. 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 and National Institutes of Health.

References

Aptroot A (1998) A world revision of *Massarina* (Ascomycota). Nova Hedwigia 66:89–162

Bose SK (1961) Studies on *Massarina* Sacc. and related genera. Phytopathol Z 41:151–213

Campbell J, Ferrer A, Raja HA, Sivichai S, Shearer CA (2007) Phylogenetic analyses among taxa in the Jahnulales inferred from 18S and 28S nuclear ribosomal DNA sequences. Can J Bot 85:873–882

Felsenstein J (1985) Confidence intervals on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Harrigan GG, Armentrout BL, Gloer JB, Shearer CA (1995) Anguillosporal, a new antibacterial and antifungal metabolite from the freshwater fungus *Anguillospora longissima*. J Nat Prod 62:497–501

Hosoe T, Gloer JB, Raja HA, Shearer CA (2007) New nonadride analogues from a freshwater isolate of an undescribed fungus belonging to the order Pleosporales. American Society of Pharmacognosy, 48th Annual Meeting, Portland, Maine

Huelsenbeck JP, Ronquist FR (2001) MrBayes: Bayesian inference of phylogenetic trees. Biometrics 17:754–755

Huelsenbeck JP, Mark PVD, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. 3.1.2. http://mrbayes.csit.fsu.edu/download.php. Accessed January 2007

Hyde KD (1995) The genus *Massarina,* with a description of *M. eburnea* and an annotated list of *Massarina* names. Mycol Res 99:291–296

Hyde KD, Jones EBG (1989) Observations on ascospore morphology in marine fungi and their attachment to surfaces. Bot Mar 32:205–218

Jiao P, Gloer JB, Campbell J, Shearer CA (2006a) Altenuene derivatives from an unidentified freshwater fungus in the family Tubeufiaceae. J Nat Prod 69:612–615

Jiao P, Swenson DC, Gloer JB, Campbell J, Shearer CA (2006b) Decasporins A–E, bioactive Spirdodioxynapthalenes from the freshwater aquatic fungus *Decaisnella thyridioides*. J Nat Prod 69:1667–1671

Jones EBG (1994) Ultrastructure and taxonomy of the aquatic ascomycetous order Halosphaeriales. Can J Bot 73(suppl 1): S790–S801

Jones EBG (2006) Form and function of fungal spore appendages. Mycoscience 47:167–183

Jones EBG, Hyde KD, Read SJ, Moss ST, Alias SA (1995) *Tirisporella* gen nov., an ascomycete from the mangrove palm *Nypa fruticans*. Can J Bot 74:1487–1495

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Divers 35:1–187

Kirk PM, Cannon PF, David JC, Stalpers JA (2008) Ainsworth and Bisby's dictionary of the Fungi, 10th edn edn. CAB International, Wallingford

Kohlmeyer J, Volkmann-Kohlmeyer B (1991) Illustrated key to the filamentous higher marine fungi. Bot Mar 34:1–61

Kohlmeyer J, Volkmann-Kohlmeyer B, Eriksson OE (1996) Fungi on *Juncus roemerianus.* 8. New bitunicate ascomycetes. Can J Bot 74:1830–1840

Lumbsch HT, Huhndorf SM (ed) (2007) Outline of ascomycota—2007. Myconet 13:1–58

Lutzoni F, Wagner P, Reeb V, Zoller S (2000) Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst Biol 49:628–651

Madur SV, Swenson DC, Gloer JB, Campbell J, Shearer CA (2006) Heliconols A–C: antimicrobial hemiketals from the freshwater aquatic fungus *Helicodendron giganteum*. Org Lett 8:3191–3194

Poch GK, Gloer JB, Shearer CA (1992) New bioactive metabolites from a freshwater isolate of the fungus *Kirschsteiniothelia* sp. J Nat Prod 55:1093–1099

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 49:817–818

Raja HA, Shearer CA (2007) Freshwater ascomycetes: *Aliquandostipite minuta* (Jahnulales, Dothideomycetes), a new species from Florida. Mycoscience 48:395–398

Raja HA, Miller AN, Shearer CA (2008) Freshwater ascomycetes: *Aquapoterium pinicola*, a new genus and species of Helotiales (Leotiomycetes) from Florida. Mycologia 100:141–148

Raja HA, Schmit JP, Shearer CA (2009) Latitudinal, habitat and substrate distribution patterns of freshwater ascomycetes in the Florida Peninsula. Biodivers Conserv 18:419–455

Ranghoo VM, Hyde KD (2000) *Ascominuta lignicola*, a new loculoascomycete from submerged wood in Hong Kong. Mycoscience 41:1–5

Schoch CL, Shoemaker RA, Seifert KA, Hambelton S, Spatafora JW, Crous PW (2006) A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98:1041–1052

Shearer CA, Langsam DM, Longcore JE (2004) Fungi in freshwater habitats. In: Mueller GM, Bills GF, Foster MS (eds) Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam, pp 513–531

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland

Tanaka K, Harada Y (2003) Pleosporales in Japan (3). The genus *Massarina*. Mycoscience 44:173–185

Vilgalys R, Hester M (1990) Rapid identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246

Zhang Y, Jeewon R, Fournier J, Hyde KD (2008) Multi-gene phylogeny and morphotaxonomy of *Amniculicola lignicola:* a novel freshwater fungus from France and its relationships to the Pleosporales. Mycol Res 112:1186–1194

Zhang Y, Wang HK, Fournier J, Crous PW, Jeewon R, Pointing SB, Hyde KD (2009) Towards a phylogenetic clarification of *Lophiostoma/Massarina* and morphologically similar genera in the Pleosporales. Fungal Divers 38:225–251