

## Three new genera representing novel lineages of Sordariomycetidae (Sordariomycetes, Ascomycota) from tropical freshwater habitats in Costa Rica

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**Abstract:** Three new genera are established in the Sordariomycetidae based on morphological and molecular data (SSU and LSU nrDNA) to accommodate five ascomycete species collected from submerged woody debris in freshwater habitats from Costa Rica. The genus *Bullimyces* contains three new species, *B. communis*, *B. costaricensis* and *B. aurisporus*. *Bullimyces* is characterized by globose to subglobose, membranous, black, ostiolate ascomata; deliquescent, hyaline, globose cells that fill the center of the centrum; unitunicate asci that deliquesce early in some species; and septate, thick-walled ascospores with or without gelatinous sheaths or appendages. *Bullimyces* species form a well supported clade with 100% bootstrap support, but the position of the genus in the Sordariomycetidae remains unclear. The second genus, *Riomyces*, is represented by a single species, *R. rotundus*. *Riomyces* is characterized by globose to subglobose, membranous, black, ostiolate ascomata, unitunicate, cylindrical asci, hyaline, globose cells that fill the hamathecium and septate, thick-walled ascospores with a gelatinous sheath. Although *Riomyces* is morphologically similar to *Bullimyces*, the two genera did not group together with support in any analysis. The third genus, *Hydromelitis*, is represented by a single species, *H. pulchella*. *Hydromelitis* is characterized by pyriform, membranous, black, ostiolate ascomata, unitunicate asci lacking an apical structure, simple, thin-walled, septate paraphyses and hyaline to golden yellow,

multiseptate, thick-walled ascospores with a gelatinous sheath. *Bullimyces*, *Riomyces* and *Hydromelitis* were nested within an unsupported clade consisting of members of the Ophiostomatales, Magnaporthales and freshwater Annulataceae sensu lato and sensu stricto.

**Key words:** aquatic, ascomycetes, fungal systematics, LSU, phylogenetics, SSU

### INTRODUCTION

Aquatic ascomycetes are microscopic, saprobic fungi that colonize and decompose submerged substrates in freshwater and marine habitats. At present 278 freshwater and 275 marine species have been reported in the Sordariomycetes from aquatic habitats (Jones et al. 2009, <http://fungi.life.illinois.edu/>) and all the major lineages contain aquatic species (Zhang et al. 2006). Aquatic species share several morphological characters including the presence of elaborate gelatinous ascospore sheaths and appendages, which presumably function in attaching spores to substrates in water (Shearer 1993; Jones 1994, 2006). Phylogenetic studies have shown that these orders have evolved independently from terrestrial ancestors (Spatafora et al. 1998, Vijaykrishna et al. 2006). The position of various genera remains incertae sedis at the ordinal level as new species from the tropics are described. During a comparative survey across four sites in Costa Rica, we found five new species of freshwater ascomycetes representing three new genera. The genus *Bullimyces* contains three new species, *B. communis*, *B. costaricensis* and *B. aurisporus*, the genus *Riomyces* is based on a single species, *R. rotundus*, and *Hydromelitis* is represented by a single species, *H. pulchella*. We provide a description of these species and use both morphological and molecular sequence data from partial 18S small subunit (SSU) and 28S large subunit (LSU) nuclear ribosomal DNA to determine their placement as novel freshwater Sordariomycetidae lineages.

### MATERIALS AND METHODS

*Collection, isolation and morphological examination.*—Methods for the collection, isolation and morphological examination of specimens are presented in Ferrer and Shearer (2005). Specimens are deposited in the Fungarium of the University of Illinois at Urbana-Champaign (ILL).

*Molecular study.*—Fungal mycelia were removed from cultures grown on potato dextrose agar (PDA, Difco) and ground in liquid nitrogen. DNA was extracted with the

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TABLE I. Strains used in this study, their origin and GenBank accession numbers

Taxa	Isolate number	Origin	SSU	LSU
<i>Bullimyces aurisporus</i>	AF316-1a	Costa Rica: Alajuela	JF758615	—
<i>Bullimyces aurisporus</i>	AF316-1b	Costa Rica: Alajuela	JF758614	JF775590
<i>Bullimyces communis</i>	AF281-3	Costa Rica: Heredia	JF758617	JF775585
<i>Bullimyces communis</i>	AF281-4	Costa Rica: Alajuela	JF758618	JF775586
<i>Bullimyces communis</i>	AF281-5	Costa Rica: Alajuela	JF758619	JF775587
<i>Bullimyces costaricensis</i>	AF317-1a	Costa Rica: Limon	—	JF775591
<i>Bullimyces costaricensis</i>	AF317-1b	Costa Rica: Limon	JF758616	JF775592
<i>Hydromelitis pulchella</i>	AF284-2	Costa Rica: Alajuela	JF758613	JF775588
<i>Riomyces rotundus</i>	AF303-1	Costa Rica: Heredia	JF758612	JF775589

DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, California) according to the kit's instructions. Fragments of SSU and LSU were PCR amplified with PureTaq™ Ready-To-Go PCR beads (Amersham Bioscience Corp., Piscataway, New York) according to Promputtha and Miller (2010). Primers NS1 and NS4 (White et al. 1990) for SSU and LROR and LR6 for LSU (Rehner and Samuels 1994, Vilgalys and Hester 1990) were used for PCR amplification. PCR products were purified with the QIAquick PCR purification kit (QIAGEN Inc., Valencia, California) and subsequently used in 11 µL sequencing reactions with BigDye® Terminators 3.1 (Applied Biosystems, Foster City, California) and primers NS1, NS2, NS3 and NS4 for SSU and LROR, LR3, LR3R and LR6 for LSU. Sequences were generated at the University of Illinois Biotechnology Center using an ABI 3730XL high-throughput automated sequencer. Sequences were assembled and edited manually in Sequencher 4.7 (Gene Codes Corp. 1991).

*Phylogenetic analyses.*—DNA sequences for *Bullimyces*, *Hydromelitis* and *Riomyces* are provided (TABLE I). To determine the familial placement of the new genera, taxa sequenced in this study along with sequences obtained from GenBank from various subclasses currently circumscribed within the Sordariomycetes based on Zhang et al. (2006) and Réblová (2006, 2009) were aligned with MUSCLE® (Edgar 2004) as implemented in the program SeaView 4.1 (Galtier et al. 1996, Guoy et al. 2010). The SSU and LSU datasets were initially analyzed separately and then concatenated and analyzed as a single dataset after no conflict among the bootstrap values of well supported clades was observed (Wiens 1998). A second analysis was performed for LSU data with a more extensive taxon sampling and including members in the *Annulatascaceae* sensu lato and stricto clades (Réblová 2006, 2009). Models of evolutionary change were determined with Modeltest 3.7 (Posada and Crandall 1998). Maximum likelihood analyses (ML) were conducted with an online version of RAxML (<http://phylobench.vital-it.ch/raxml-bb/index.phpwith>) (Stamatakis 2008) under the GAMMA model with 100 bootstrap replicates. Bayesian analyses employing Markov chain Monte Carlo (MCMC) were performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001, Huelsenbeck and Ronquist 2001) as an additional means of assessing branch support. Bayesian analyses using a uniform model (GTR + I + G)

were conducted using 10 000 000 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. The first 10 000 trees, which 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 generated in PAUP 4.0b10 (Swofford 2002). Phylogenetic trees were drawn with Figtree 1.2.2.

## RESULTS

*Phylogenetic analyses.*—The combined SSU and LSU included 2407 characters from 46 taxa. A total of 364 ambiguously aligned characters were excluded, yielding a total of 2043 characters. The ML analysis resulted in a single most likely tree (FIG. 1), which was similar in topology and placement of the major orders of Sordariomycetes to the phylogeny presented by Zhang et al. (2006). *Bullimyces*, *Riomyces* and *Hydromelitis* grouped within an unsupported clade consisting of members of the Ophiostomatales and Magnaporthales along with species of Sordariomycetidae inc. sed. The *Bullimyces* clade was well supported with 100% maximum likelihood bootstrap (MLB) support and 100% Bayesian posterior probability (BPP); the three sequences of *B. communis* formed a well supported clade and were sister to *B. costaricensis* and *B. aurisporus*. *Riomyces* was basal to *Bullimyces* with no support. *Ceratostomella pyreneica* was basal to *Bullimyces* but was not supported by MLB. *Hydromelitis* formed a clade with *Lentomitella cirrhosa* with no support.

Final alignment of the partial LSU sequences of nrDNA included 1179 characters from 67 taxa. A total of 154 ambiguously aligned characters were excluded, yielding a total of 1025 characters. The LSU data was expanded to include additional related species from GenBank, most of which lacked a corresponding SSU sequence. The tree obtained with the ML analysis showed a topology similar to the SSU and LSU tree

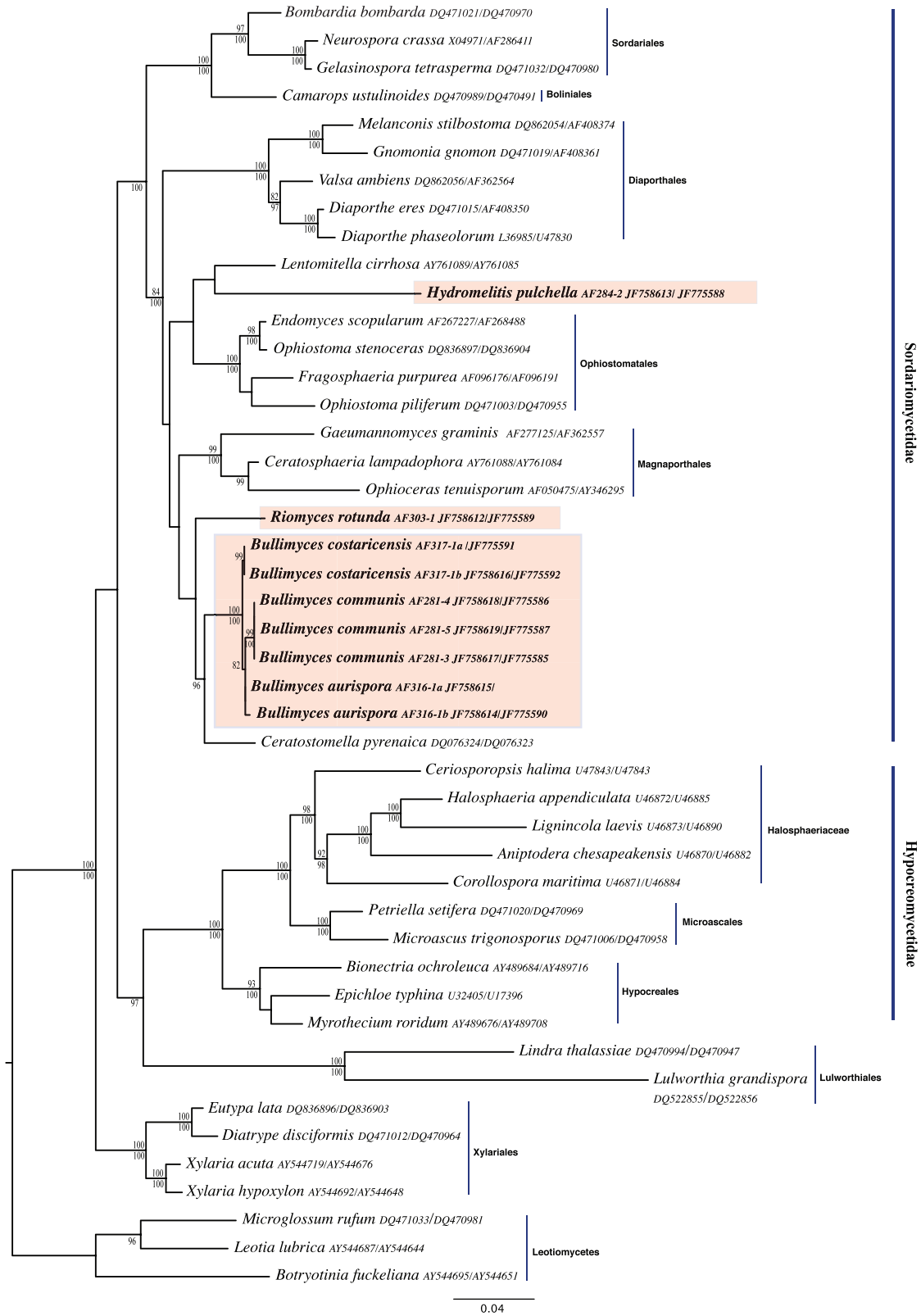


FIG. 1. Most likely tree based on a combined SSU and LSU nrDNA sequence data obtained with RAxML. Numbers above the branches indicate ML bootstrap support  $\geq 75\%$  and numbers below indicate Bayesian posterior probabilities  $\geq 95\%$ . GenBank accession numbers are given after taxon names. Species sequenced for this study are highlighted.

(FIG. 2), with *Bullimyces*, *Riomyces* and *Hydromelitis* within an unsupported clade consisting of members of the Ophiostomatales, Magnaporthales and freshwater Annulatascaceae sensu lato and sensu stricto. The three sequences of *B. communis* formed a monophyletic group with 99% MLB and 100% BPP values and formed a well supported clade to *B. costaricensis* and *B. aurisporus* with 98% MLB and 100% BPP. Because ordinal or familial placement for the three genera was not supported, they should remain in the Sordariomycetidae inc. sed.

#### TAXONOMY

***Bullimyces*** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer gen. nov.

MycoBank MB561094

Ascomata in ligno submerso, partim immersa vel immersa, globosa vel subglobosa, membranacea, nigra, ostiolati. Collum cylindricum, atrobrunneae. Centris ascocarpiorum cellulis pseudoparenchymaticis, globosis, hyalinis, deliquescentibus. Asci unitunicati, cylindrici, tenuitunicati, sine poro apicali, octospori, uniseriati, persistentes vel deliquescentes. Ascosporeae ellipsoideae-fusifformes, hyalinae vel luteae, septatae, ad septum non constrictae, pachydermaticae, cum vel sine tunica gelatinosa praeditae et appendages.

*Typus generic:* *Bullimyces communis* A. Ferrer, A.N. Mill., C. Sarmiento et Shearer

*Etymology:* From Latin *Bulla* = bubble; + Greek *Myces* = fungus, in reference to the round cells filling the hamathecium.

Ascomata on submerged wood immersed to erumpent in the substrate, globose to subglobose, membranous, black, ostiolate. Neck cylindrical, dark brown. Pseudoparenchyma of thin-walled globose, hyaline cells, filling the centrum, deliquescent. Asci unitunicate, cylindrical, thin-walled, lacking an apical pore and apical structures, eight-spored, uniseriate, deliquescing early in some species. Ascospores broadly ellipsoidal-fusiform to ellipsoidal, hyaline becoming dark yellow with age, septate, not constricted at septa, thick-walled, with or without a gelatinous sheath and appendages.

***Bullimyces communis*** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer sp. nov. FIGS. 3–16

MycoBank MB561095

Ascomata in ligno submerso, 350–650 × 340–660 μm, immersa, globosa, membranacea, nigra, ostiolati. Collum 100–170 × 60–80 μm, cylindricum, atrobrunneae. Centris ascocarpiorum cellulis pseudoparenchymaticis, 38–90 μm diam, globosis, hyalinis, deliquescentibus. Asci 310–430 × 18–22 μm, unitunicati, cylindrici, tenuitunicati, sine poro apicali, octospori, uniseriati, deliquescentes. Ascosporeae 40–48 × 19–22 μm, ellipsoideae-fusifformes, hyalinae, 3-septatae,

ad septum non constrictae, pachydermaticae, hyaline calyptra bipolare.

*Holotype:* COSTA RICA. ALAJUELA, Caño Negro Reserve, Rio Frio, 10°53'N, 84°45'W, water 27.5 C, pH 5, on submerged wood, 15 Dec 2005, Astrid Ferrer & Marlon Salazar, AF281-1 (ILL).

*Etymology:* From Latin *Communis* = common, in reference to being the most collected species in Costa Rica.

Ascomata on submerged wood, 350–650 × 340–660 μm, immersed in the substrate, globose, membranous, black, ostiolate, brown. Neck 100–170 × 60–80 μm, cylindrical, dark brown. Pseudoparenchyma of thin-walled cells 38–90 μm diam, globose, hyaline, filling the centrum, deliquescent. Asci 310–430 × 18–22 μm, unitunicate, cylindrical, thin-walled, lacking an apical pore and apical structures, floating free within the centrum, eight-spored, uniseriate. Ascospores 40–48 × 19–22 μm (mean = 43.70 × 20.20 μm, n = 30), broadly ellipsoidal-fusiform to ellipsoidal, hyaline, three-septate, not constricted at septa, thick-walled (2–3 μm), with hyaline, gelatinous caps at both apices, 2–3 μm long, staining in aqueous nigrosin, ascospores accumulate as a mass at tip of the neck after discharge.

*Known distribution:* COSTA RICA: Alajuela, Heredia.

*Habitat:* On submerged, dead woody debris.

*Additional specimens examined:* COSTA RICA. ALAJUELA: Caño Negro Reserve, Rio Frio, 10°53'N, 84°45'W, water 27.5 C, pH 5, on submerged wood, 15 Dec 2005, Astrid Ferrer & Marlon Salazar, AF281-4, AF281-5, AF281-6, AF281-7. HEREDIA: La Selva Biological Station, La Selva Stream, 10°25'N, 84°01'W, water 25 C, pH 5, on submerged wood, 10 Jan 2006, Marlon Salazar, AF281-3.

*Commentary:* *Bullimyces communis* was the most common freshwater ascomycete reported in this study from Costa Rica with six collections. The presence of globose cells in the hamathecium was observed consistently in all collections, although in water the globose, chain-like cells deliquesce promptly (FIGS. 11–16). Another interesting aspect of this fungus is that the asci were never observed to be arranged in a hymenial layer but instead floating among the globose cells. *Bullimyces communis* is characterized by three-septate ascospores with gelatinous caps at both apices. One collection, AF281-6, had hyaline ascospores that became dark yellow with age but was identical to the type specimen in every other feature.

***Bullimyces costaricensis*** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer sp. nov. FIGS. 17–24

MycoBank MB561097

Ascomata in ligno submerso, 450–490 × 450–500 μm, immersa, globosa vel subglobosa, membranacea, nigra, ostiolati. Collum 380–390 × 77–80 μm, cylindricum, atrobrunneae. Centris ascocarpiorum cellulis pseudoparenchymaticis, 35–55 μm diam, latae, globosis, hyalinis, deliquescentibus. Collum 280–390 × 77–80 μm. Asci 430–600 × 20–23 μm,

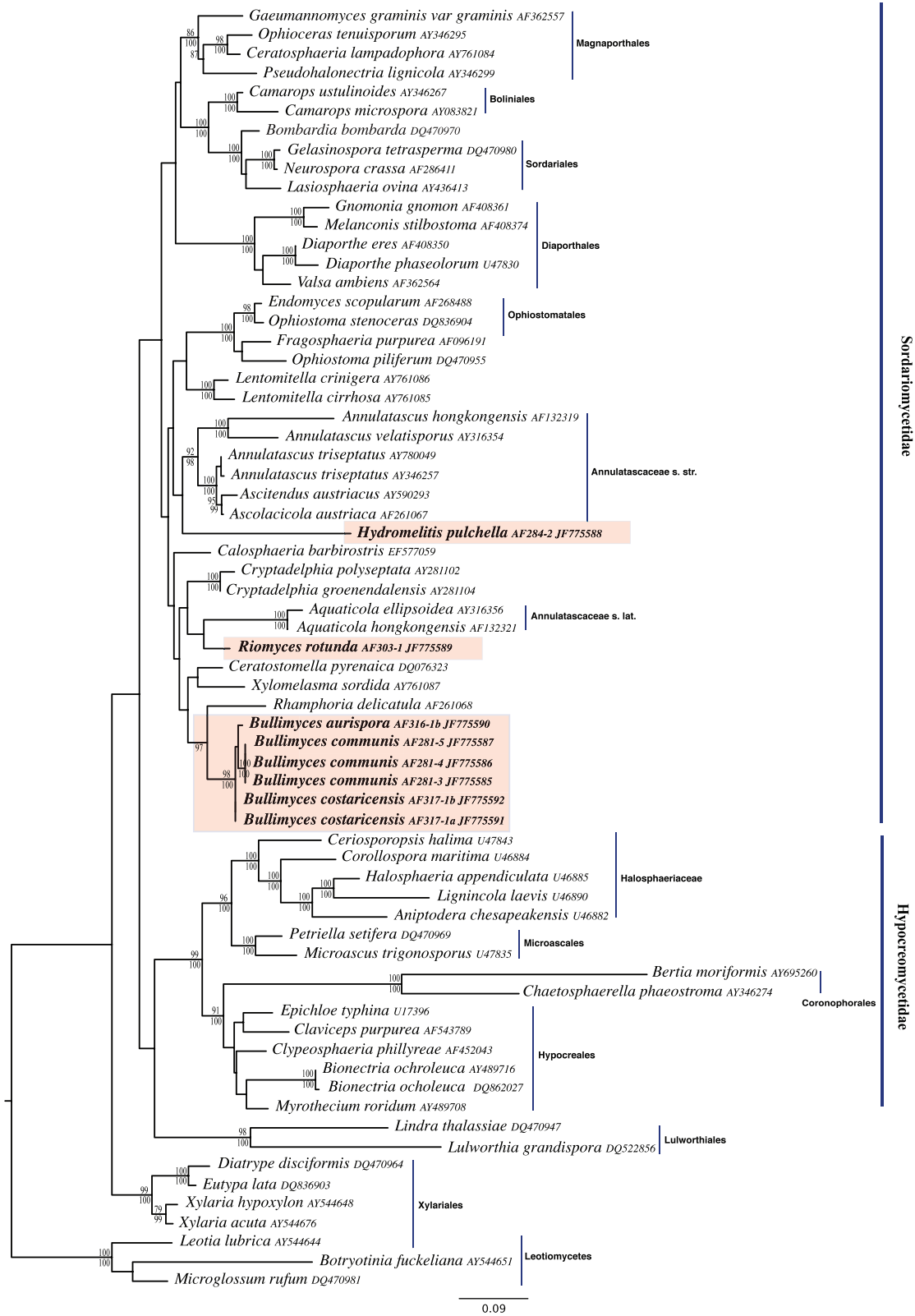
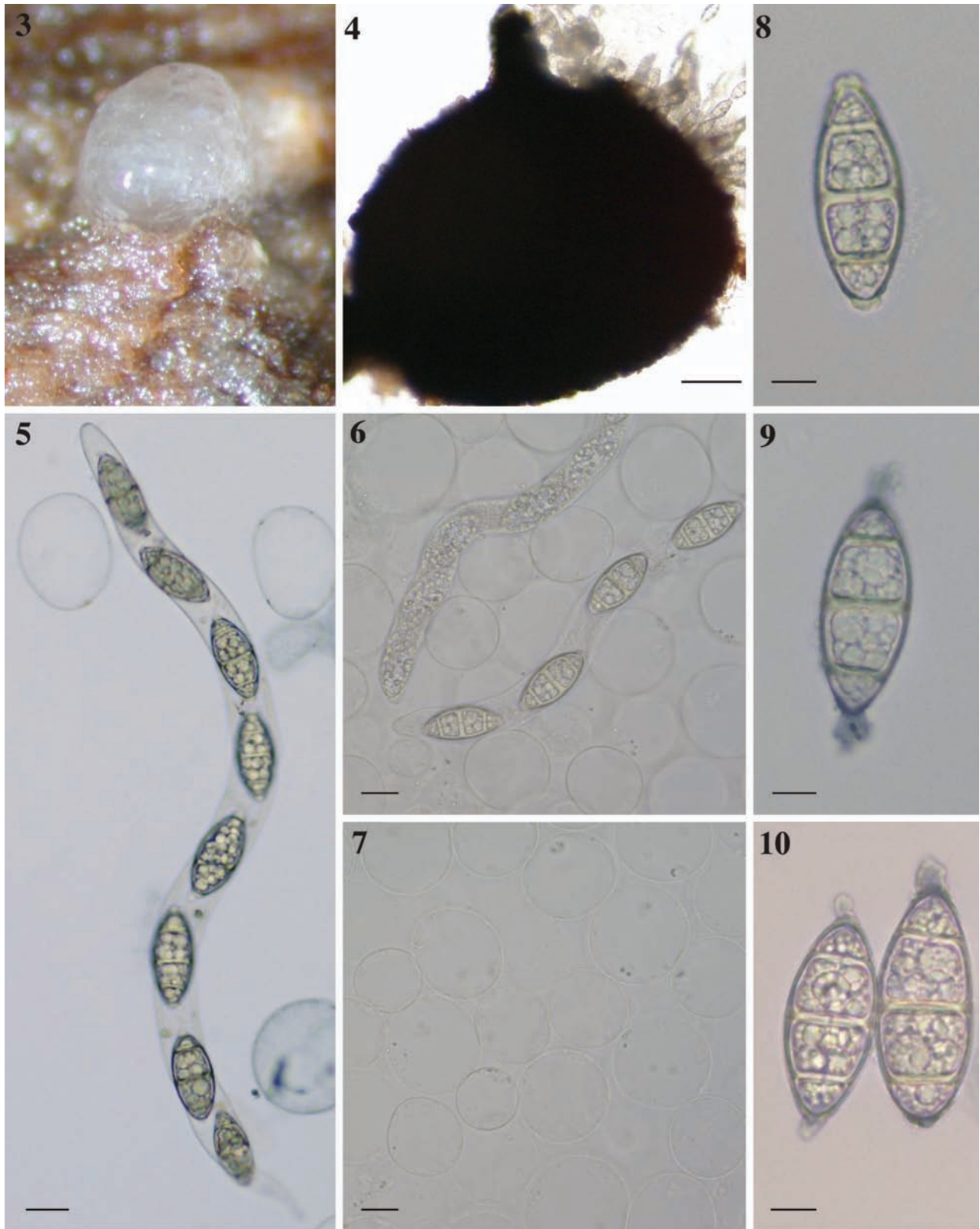
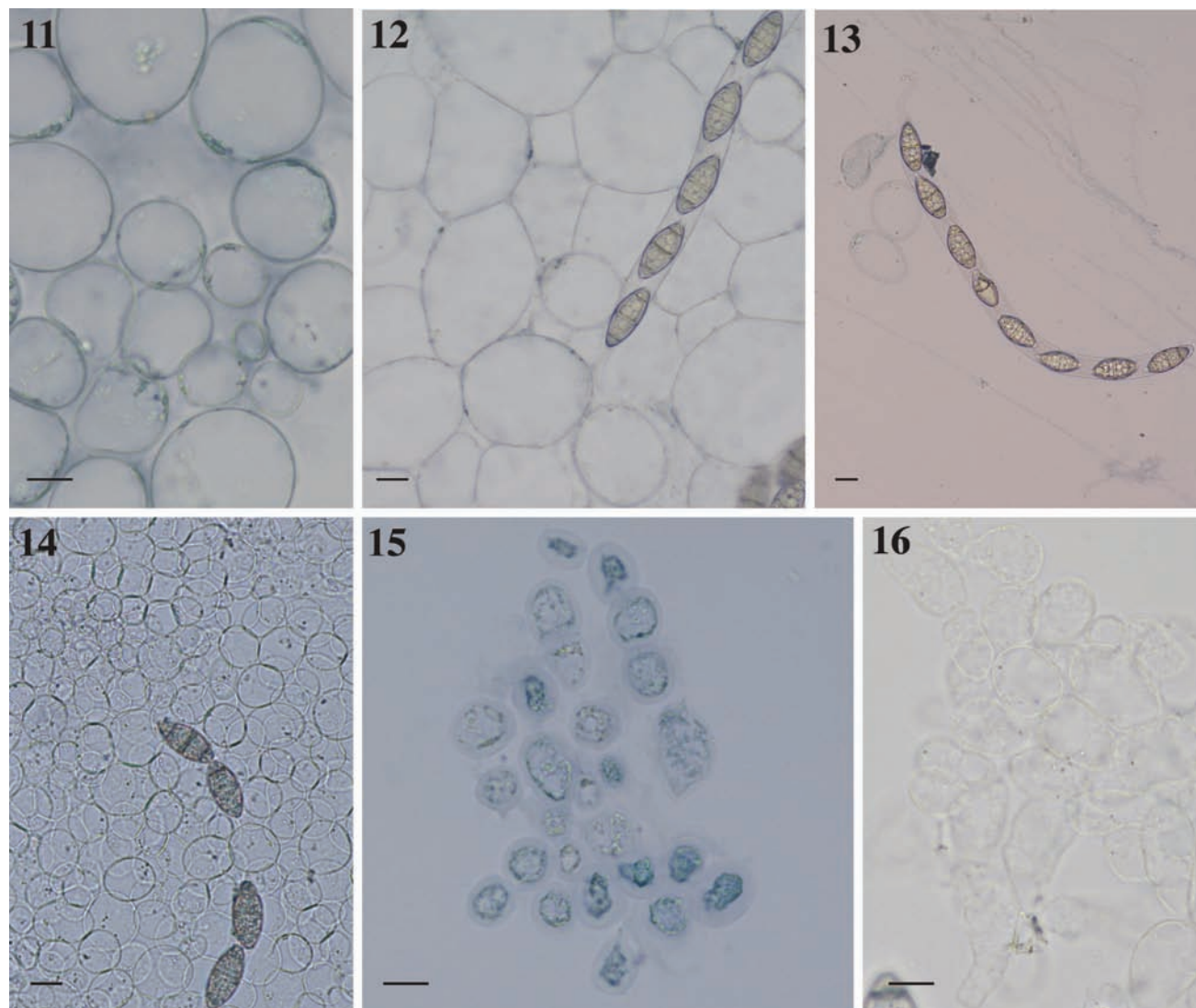


FIG. 2. Most likely tree from LSU nrDNA sequence data obtained with RAxML. Support values and highlighting as illustrated (FIG. 1).



FIGS. 3–10. *Bullimyces communis* (from Holotype). 3. Ascogonia on the substrate. 4. Whole mount of ascogonia. 5. Asci stained with aqueous nigrosin. 6. Asci and globose cells. 7. Globose cells filling the venter of the ascogonia. 8. Ascospore in water. 9, 10.



FIGS. 11–16. *Bullimyces communis* (from Holotype). 11, 12. Globose cells stained with aqueous nigrosin (AF281-1). 13. Globose cells stained with aqueous nigrosin, and remains of stained, deliquescent globose cells (AF281-1). 14. Ascospores and globose cells (AF281-4). 15. Deflated globose cells stained with aqueous nigrosin (AF281-4). 16. Globose cells (AF281-5). Bar = 10  $\mu$ m.

unitunicati, cylindrici, tenuitunicati, sine poro apicali, octospori, uniseriati, deliquescentes. Ascosporae 40–68  $\times$  15–21  $\mu$ m, ellipsoideae-fusifor-mes, hyalinae demum atrantes, 5-septatae, ad septum non constrictae, pachydermaticae.

**Holotype:** COSTA RICA. LIMON: Barra del Colorado and Tortuguero National Park, Las Palmas Stream, 10°35'N, 83°31'W, water 25 C, pH 5, on submerged wood, 18 Dec 2005, Astrid Ferrer & Marlon Salazar, AF317-1 (ILL).

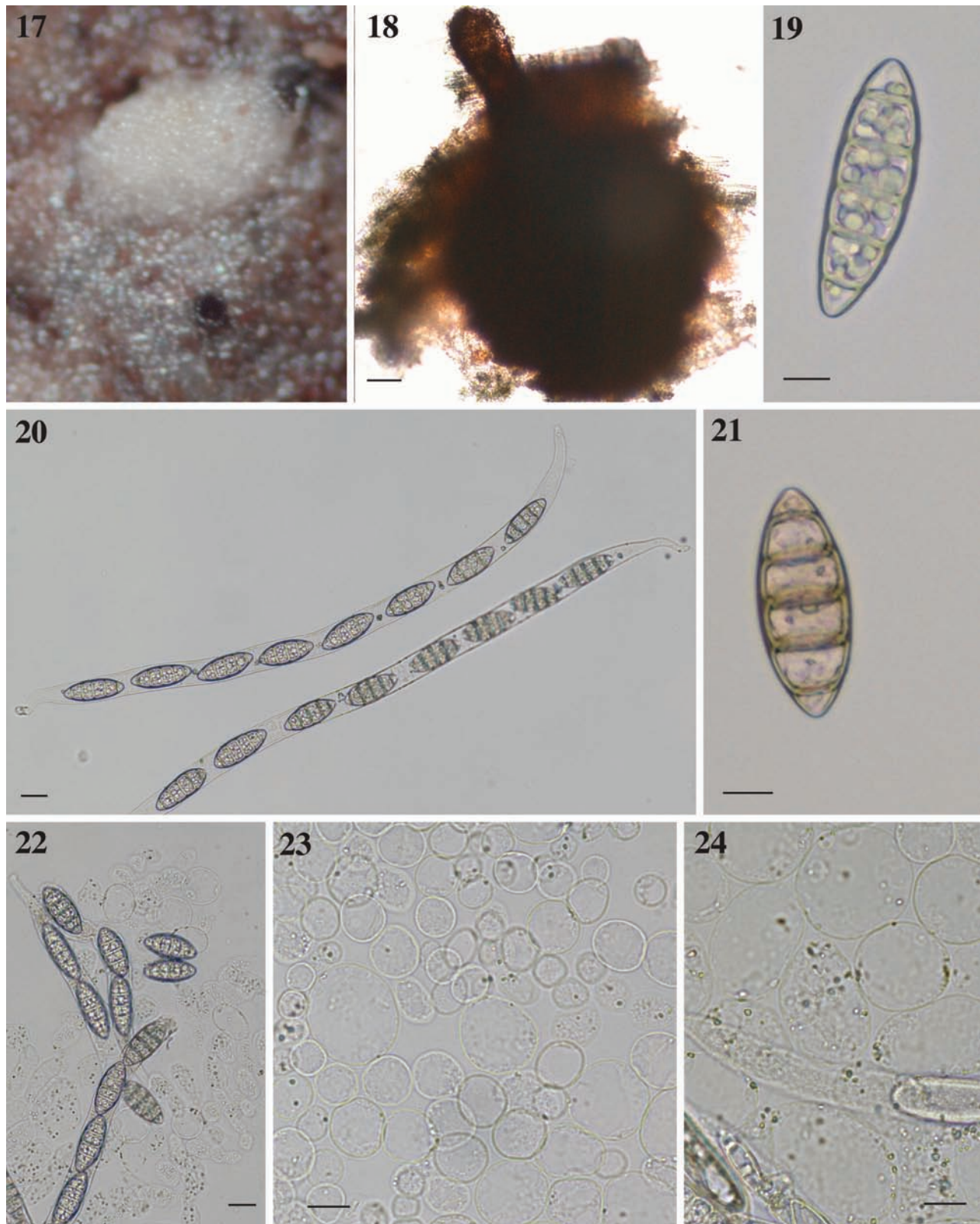
**Etymology:** *Costaricensis*, in reference to the country (Costa Rica), where this fungus was collected.

Ascomata on submerged wood, 450–490  $\times$  450–500  $\mu$ m, immersed to erumpent in the substrate, globose to subglobose, membranous, black, ostiolate. Neck 280–390  $\times$  77–80  $\mu$ m, cylindrical, dark brown. Pseudoparenchyma of thin-walled cells, 35–55  $\mu$ m

diam, globose, hyaline, filling the centrum, deliquescent. Asci 430–600  $\times$  20–23  $\mu$ m, unitunicate, cylindrical, short pedicellate, thin-walled, lacking an apical pore, floating free within the centrum, eight-spored, uniseriate, irregularly arranged, early deliquescent. Ascospores 40–68  $\times$  15–20  $\mu$ m (mean = 48  $\times$  19  $\mu$ m, n = 30), broadly ellipsoidal-fusiform to ellipsoidal, hyaline, becoming dark yellow with age, five-septate, not constricted at septa, thick-walled (3–5  $\mu$ m), ascospores accumulate in a mass at the tip of the neck after discharge.

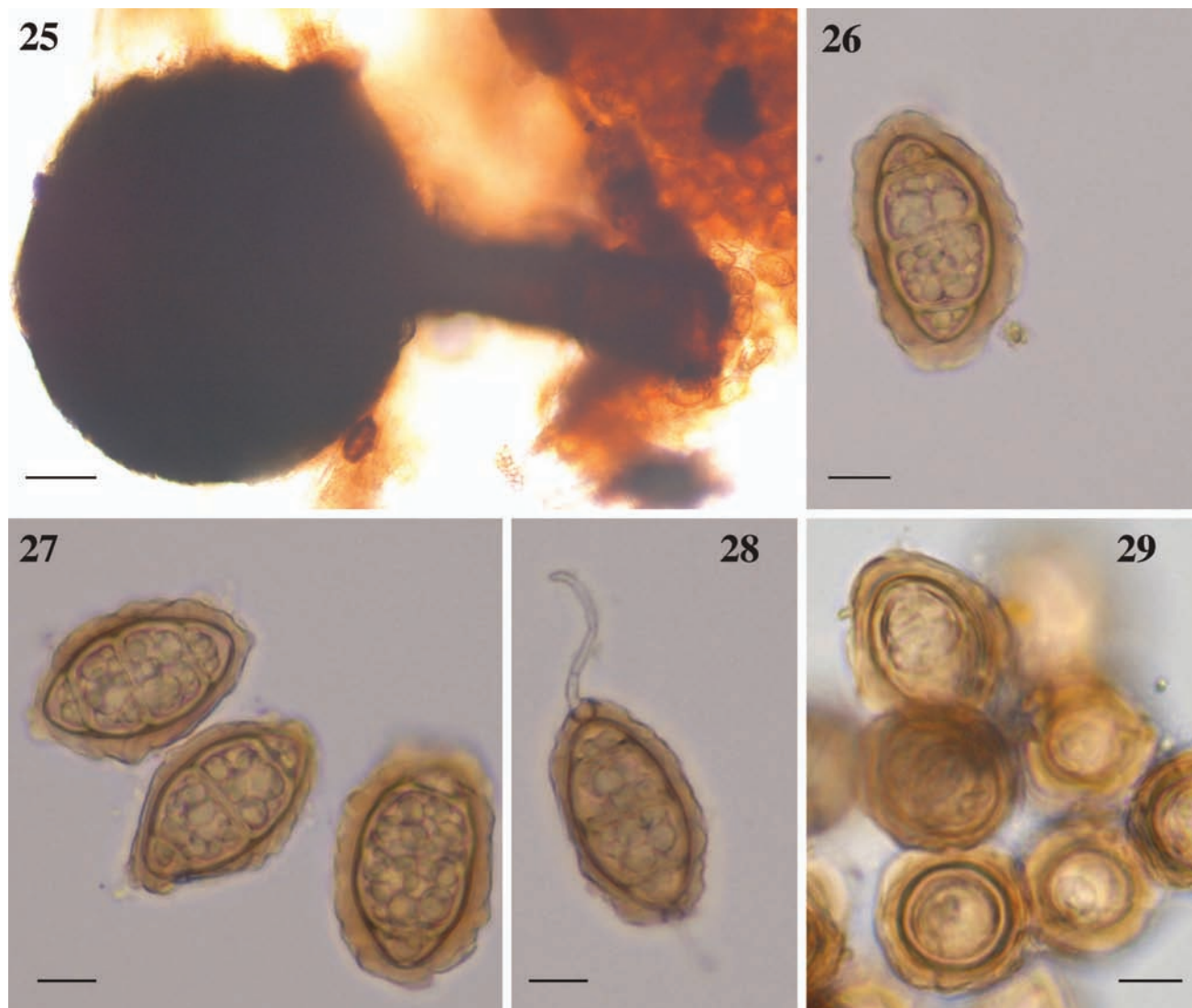
**Known distribution:** COSTA RICA: Limón (known only from the type specimen).

**Habitat:** On submerged, dead woody debris.



FIGS. 17–24. *Bullimyces costaricensis*. (AF317-1). 17. Ascomata on the substrate (note mass of hyaline ascospores. 18. Whole mount of ascoma. 19. Ascospore in water. 20. Elongated asci with eight ascospores. 21. Ascospore becoming pale brown with age. 22, 23, 24. Globose cells. Bar = 10  $\mu$ m.





FIGS. 25–29. *Bullimyces aurisporus* (AF316-1). 25. Whole mount of ascoma. 26 & 27. Ascospores in water showing gelatinous sheath. 28. Germinating ascospores. 29. Top view of ascospores. Bar = 10  $\mu\text{m}$ .

*Commentary:* *Bullimyces costaricensis* is morphologically similar to the type species *B. communis*, with dark ascomata, globose hamathecial cells, thin-walled asci lacking an apical pore, and thick-walled, hyaline, multi-septate ascospores. However *B. costaricensis* differs from *B. communis* in ascus and ascospore morphology. The asci of *B. costaricensis* are particularly long (430–600  $\mu\text{m}$  compared to 310–430  $\mu\text{m}$  in *B. communis*), and the ascospores of *B. costaricensis* are larger (40–68  $\mu\text{m}$  long in *B. costaricensis* compared to 40–48  $\mu\text{m}$  in *B. communis*), have more septa and lack appendages.

***Bullimyces aurisporus*** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer sp. nov. FIGS. 25–29  
Mycobank MB561098

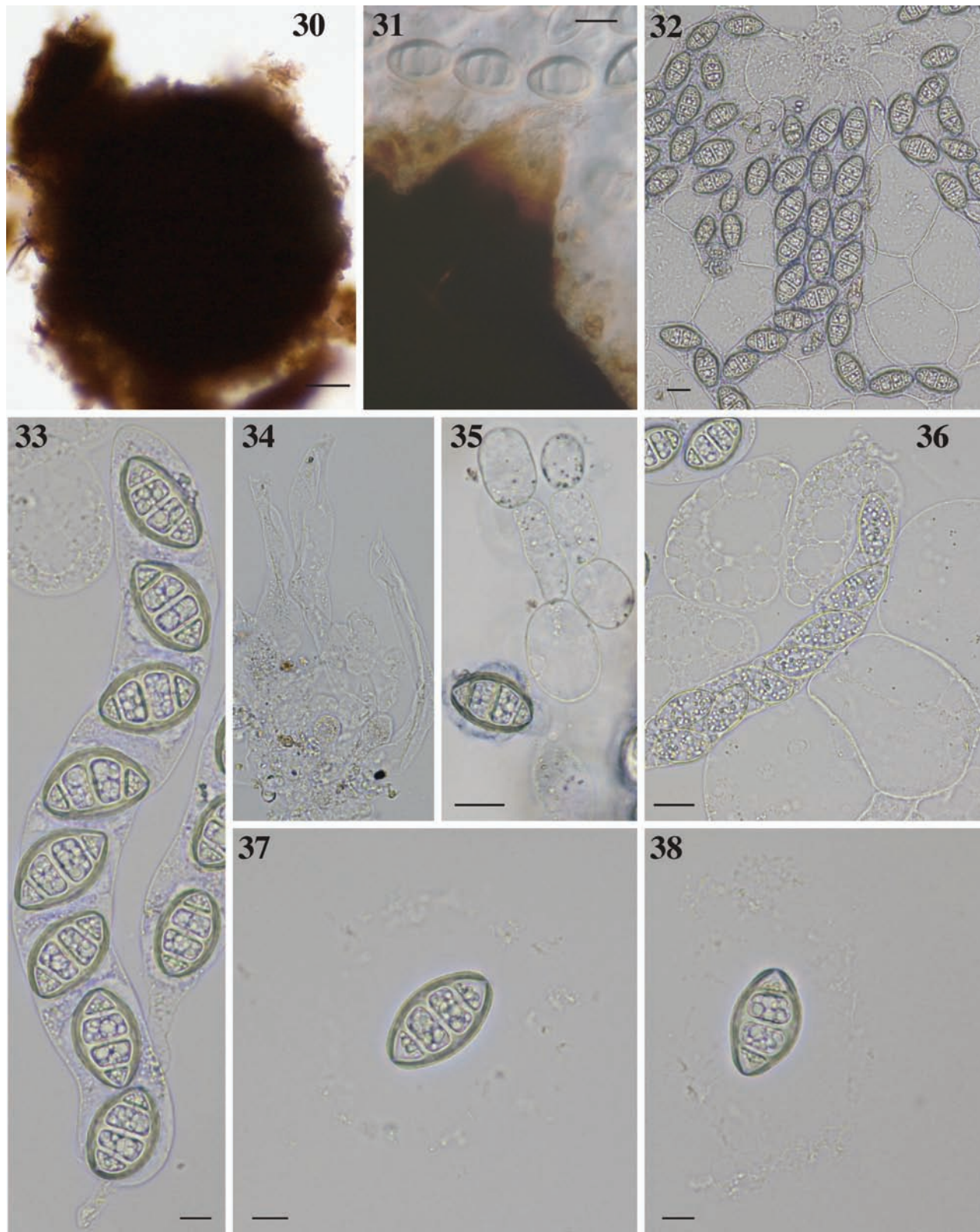
Ascomata in ligno submerso, 260–310  $\times$  290  $\mu\text{m}$ , partim immersa vel immersa, subglobosa vel obpyriforma, mem-

branacea, nigra, ostiolati. Collum 224  $\times$  62  $\mu\text{m}$ , cylindricum, atrobrunneae. Asci deliquescentes. Ascosporae 34–38  $\times$  17–20  $\mu\text{m}$ , ellipsoideae, lutea, three-septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

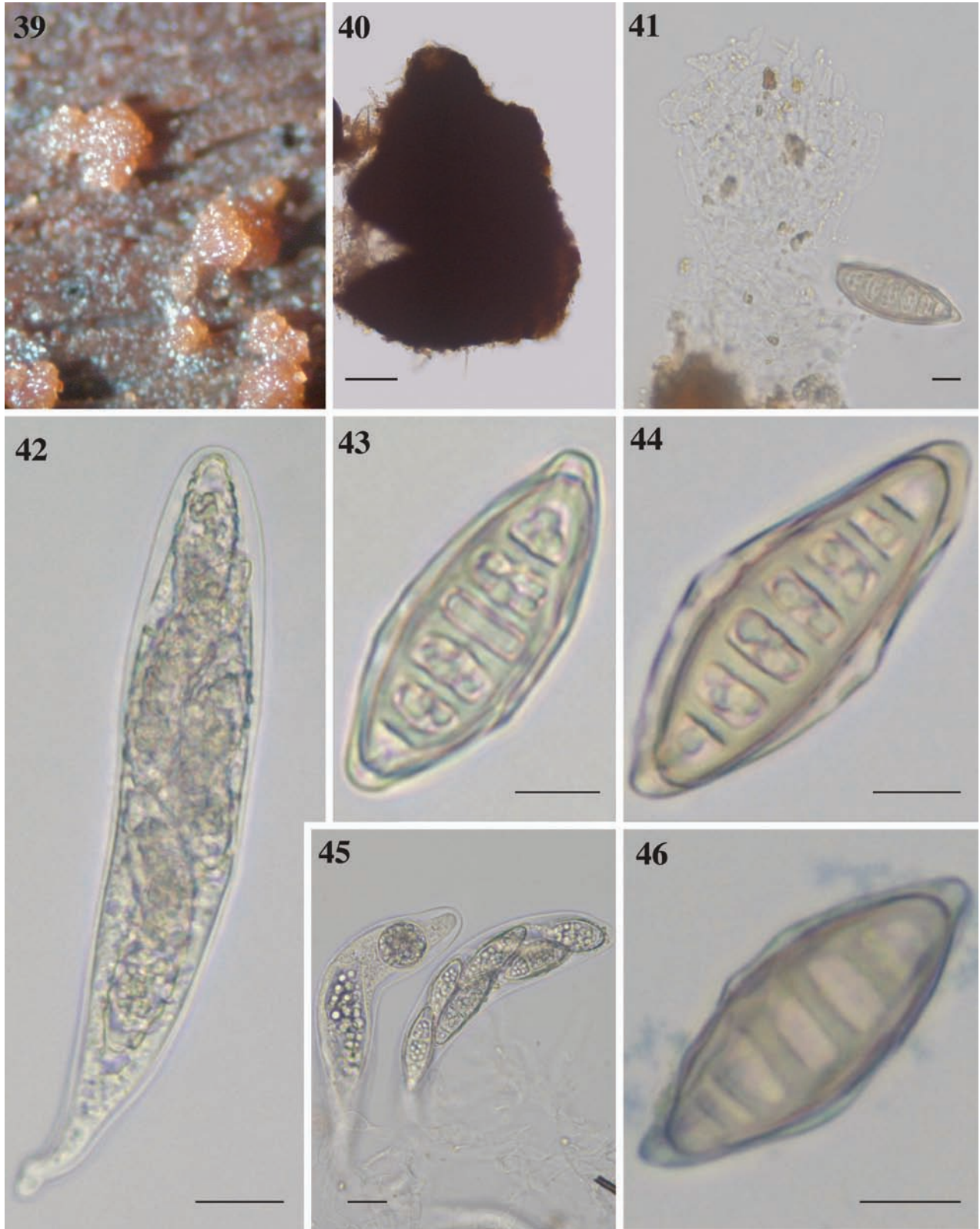
*Holotype:* COSTA RICA. ALAJUELA: Caño Negro Reserve, Caño Blanco stream 10°50'N, 84°7'W, water 26 C, pH 5, on submerged wood, 16 Dec 2005, Astrid Ferrer & Marlon Salazar, AF316-1 (ILL).

*Etymology:* From Latin *Aurum* = gold; *Spora* = spore, in reference to the color of the ascospores.

Ascomata on submerged wood, 260–310  $\times$  290  $\mu\text{m}$ , immersed to erumpent in the substrate, subglobose to obpyriform, membranous, black, ostiolate. Neck cylindrical, dark brown, 224  $\times$  62  $\mu\text{m}$ . Asci not observed, early deliquescent. Ascospores 34–38  $\times$  17–20  $\mu\text{m}$ , ellipsoidal, dark yellow, three-septate, not constricted at septa, thick-walled (2.0  $\mu\text{m}$ ), surround-



FIGS. 30–38. *Riomyces rotundus* from the holotype (AF303-1). 30. Whole mount of ascoma. 31. Neck. 32. Asci surrounded by globose cells. 33. Unitunicate ascus. 34. Empty Asci. 35, 36. Globose cells. 37, 38. Ascospores showing gelatinous sheath expanding in water. Bar = 10  $\mu$ m.



FIGS. 39–46. *Hydromelitis pulchella* from the holotype (AF284-1). 39. Ascomata on the substrate. 40. Whole mount of ascoma. 41. Paraphyses. 42, 45. Young ascus. 43. Ascospores in water. 44. Ascospore becoming pale yellow with age. 46. Ascospore stained with aqueous nigrosin. Bar = 10  $\mu$ m.

ed by an irregular gelatinous sheath about 3–5  $\mu\text{m}$  wide, ascospores accumulate in a mass at the tip of the neck after discharge.

*Known distribution:* COSTA RICA: Alajuela (known only from the type specimen).

*Habitat:* On submerged, dead woody debris.

*Commentary:* *Bullimyces aurisporus* is morphologically similar to the type species of the genus, *B. communis*, with dark ascomata and thick-walled, three-septate ascospores. However the ascospores of *B. aurisporus* are shorter (34–38  $\mu\text{m}$  in *B. aurisporus* compared to 40–48  $\mu\text{m}$  in *B. communis*) and have a thick, irregular mucilaginous sheath surrounding the ascospore. Although we do not have information about ascus and hamathecium morphology, molecular data support its placement in this genus. We were not able to induce sexual reproduction in culture for *B. aurisporus*.

**Riomyces** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer gen. nov.  
Mycobank MB561099

Ascomata in ligno submerso, partim immersa vel immersa, globosa ad subglobosa, membranacea, nigra, ostiolati. Collum breve, atrobrunneae. Hamathecium ascocarpiorum cellulis psedoparenchymaticis, globosis, hyalinis, deliquescentibus. Asci unitunicati, cylindrici, tenuitunicati, breve deicellati, sine poro apicali, octospori, uniseriati. Ascosporae, ellipsoideo-fusifformes, hyalinae, septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

*Typus generic:* *Riomyces rotundus* A. Ferrer, A.N. Mill., C. Sarmiento et Shearer

*Etymology:* From the Spanish word *Río* = river + Greek *Mycos* = fungus, in reference to the freshwater habitat of the fungus.

Ascomata on submerged wood, immersed to erumpent in the substrate, globose to subglobose, membranous, black, ostiolate. Neck short, dark brown. Hamathecium composed of globose, hyaline, deliquescent cells. Asci unitunicate, broadly cylindrical, thin-walled, short-pedicellate, lacking an apical pore and other apical structures, eight-spored, uniseriate. Ascospores ellipsoidal-fusiform, hyaline, septate, not constricted at septa, thick-walled, surrounded by a gelatinous sheath.

**Riomyces rotundus** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer sp. nov. FIGS. 30–38  
Mycobank MB561100

Ascomata in ligno submerso, 440–720  $\times$  440–600  $\mu\text{m}$ , partim immersa vel immersa, globosa vel subglobosa, membranacea, nigra, ostiolati. Collum 76  $\times$  84  $\mu\text{m}$ , breve, atrobrunneae. Hamathecium ascocarpiorum cellulis psedoparenchymaticis, 35–100  $\mu\text{m}$  diam, globosis, hyalinis, deli-

quescentibus. Asci 250–340  $\times$  25–29  $\mu\text{m}$ , unitunicati, cylindrici, tenuitunicati, breve deicellati, sine poro apicali, octospori, uniseriati, deliquescentes. Ascosporae 30–40  $\times$  20–25  $\mu\text{m}$ , ellipsoideo-fusifformes, hyalinae, 3-septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

*Holotype:* COSTA RICA. HEREDIA: La Selva Biological Station, Arboleda Stream 10°25'N, 84°00'W, water 25 C, pH 7, on submerged wood, 9 Jan 2006, Marlon Salazar, AF303-1 (ILL).

*Etymology:* From Latin *rotunda* = round, globular, in reference to the cells of the hamathecium.

Ascomata on submerged wood, 440–720  $\times$  440–600  $\mu\text{m}$ , immersed to erumpent in the substrate, globose to subglobose, membranous, black, ostiolate. Neck short, dark brown, 76  $\times$  84  $\mu\text{m}$ . Hamathecium composed of globose, wide, hyaline, deliquescent cells, 35–100  $\mu\text{m}$  diam. Asci 250–340  $\times$  25–30  $\mu\text{m}$ , unitunicate, broadly cylindrical, thin-walled, with a short pedicel, lacking an apical pore and other apical structures, eight-spored, uniseriate. Ascospores 30–40  $\times$  20–25  $\mu\text{m}$  (mean = 35  $\times$  22  $\mu\text{m}$ , n = 30), broadly ellipsoidal-fusiform, hyaline, three-septate, not constricted at septa, thick-walled (4–5  $\mu\text{m}$ ), surrounded by an irregular gelatinous sheath extending about ca. 10–20  $\mu\text{m}$  from the ascospore wall; sheath margins stain in aqueous nigrosin, ascospores accumulate in a mass at the tip of the neck after discharge.

*Known distribution:* COSTA RICA: Alajuela and Heredia.

*Habitat:* On submerged, dead woody debris.

*Additional specimens examined:* COSTA RICA. HEREDIA: La Selva Biological Station, Esquina Stream, 10° 24' N, 84° 00' W, water 25 C, pH 5, on submerged wood, 9 Jan 2006, Marlon Salazar, AF303-2. ALAJUELA: Caño Negro Reserve, Río Frio, 10° 53' N, 84° 45' W, water 27 C, pH 5, on submerged wood, 15 Dec 2005, Astrid Ferrer & Marlon Salazar, AF303-3.

*Commentary:* *Riomyces rotundus* is morphologically similar to species in the genus *Bullimyces* in having dark ascomata, the unique hyaline, globose cells filling the centrum, thin-walled asci lacking an apical pore, and thick-walled, hyaline, multiseptate ascospores. It was observed in this fungus that the asci were attached to a hymenial layer but with time the asci become unattached and surrounded by the globose cells filling the centrum, some empty asci were observed still attached to the hymenium (FIG. 34).

**Hydromelitis** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer gen. nov.  
Mycobank MB561101

Ascomata in ligno submerso, partim immersa vel immersa, pyriformia, membranacea, nigra, ostiolati. Paraphysibus simplicis, sepatatis. Asci unitunicati, clavati, tenuitunicati, breve deicellati, sine poro apicali, octospori, biseriati vel

interdum triseriati. Ascospores, ellipsoideae, hyalinae vel flavus, septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

Ascomata on submerged wood, immersed to erumpent, pyriform, membranous, black, ostiolate. Paraphyses simple, thin-walled, septate. Asci unitunicate, clavate, thin-walled, short-pedicellate, lacking an apical pore and other apical structures, eight-spored, biseriata. Ascospores ellipsoidal, hyaline to golden yellow, multiseptate, septa with or without a central channel, not constricted at septa, thick-walled, surrounded by a gelatinous sheath.

*Typus generic:* *Hydromelitis pulchella* A. Ferrer, A.N. Mill., C. Sarmiento et Shearer.

*Etymology:* From Latin *Hydromelitis* = honey-water, in reference to the color of the ascospores and the spore mass on the wood.

**Hydromelitis pulchella** A. Ferrer, A.N. Mill., C. Sarmiento et Shearer sp. nov. FIGS. 39–46 MycoBank MB561102

Ascomata in ligno submerso, 220–280 × 190–250 μm, partim immersa vel immersa, pyriformia, membranacea, nigra, ostiolati. Paraphysibus 3–5 μm diam, simplicis, sepatatis, hyalinis. Asci 150–170 × 30–33 μm, unitunicati, clavati, tenuitunicati, breve deicellati, sine poro apicali, octospori, biseriati vel interdum triseriati. Ascospores 35–51 × 13–18 μm, ellipsoideae, hyalinae vel flavus, 6-septatae, ad septum non constrictae, pachydermaticae, tunica gelatinosa praeditae.

*Holotype:* COSTA RICA. ALAJUELA: Caño Negro Reserve, Río Frío 10°53'N, 84°45'W, water 27.5 C, pH 5, on submerged wood, 15 Dec 2005, *Astrid Ferrer & Marlon Salazar, AF284-2* (ILL).

*Etymology:* From Latin *Pulchella* = beautiful, in reference to the ascospores.

Ascomata on submerged wood, 220–280 × 190–250 μm, immersed to erumpent in the substrate, pyriform, membranous, black, ostiolate. Paraphyses cylindrical, 3–5 μm diam, simple, thin-walled, septate, hyaline. Asci 150–210 × 30–33 μm, unitunicate, clavate, thin-walled, with a short pedicel, lacking an apical pore and other apical structures, eight-spored, biseriata, occasionally triseriate. Ascospores 35–51 × 13–18 μm, ellipsoidal, hyaline to golden yellow, mostly six-septate (3–7), with or without a central channel in the septal walls, not constricted at septa, thick-walled (up to 5 μm); ascospores surrounded by a gelatinous sheath that stains in aqueous nigrosin, 3–7 μm wide at lateral walls of ascospores, tapering toward the apices, ascospores accumulate in a yellow mass at the tip of the neck after discharge.

*Known distribution:* COSTA RICA: Alajuela and Heredia.

*Habitat:* On submerged, dead woody debris.

*Additional specimens examined:* HEREDIA: La Selva Biological Station, El Surá Stream 10°25'N, 84°00'W, water 25 C, pH 7, on submerged wood, 10 Jan 2006, *Marlon Salazar, AF284-1*; La Selva Biological Station, Panteno, 10°25'8"N, 84°0'22"W, water 25 C, pH 5.5, on submerged decorticated wood, 19 May 2000, *Jennifer L. Anderson, Rebecca Wulffen, A468-1*; La Selva Biological Station, Rio Puerto Viejo, 10°25'48"N, 84°0'17"W, water 23 C, pH 5.5, on submerged, soft, decorticated wood, 20 May 2000, *Jennifer L. Anderson, Rebecca Wulffen, A468-2*; La Selva Biological Station, Piper, 10°25'57"N, 84°1'44"W, on submerged decorticated wood, 6 Feb 2001, *Cathy Pringle, A468-3*; La Selva Biological Station, Arboleda 30, 10°25'47"N, 84°0'39"W, submerged, soft, decorticated wood, 17 May 2000, *Jennifer L. Anderson, Rebecca Wulffen, A468-4*.

*Commentary:* *Hydromelitis pulchella* is morphologically similar to the type species of *Bullimyces*, *B. communis*, with dark ascomata, thin-walled asci lacking an apical pore, and thick-walled, multiseptate ascospores with a sheath. However *H. pulchella* differs from *B. communis* in ascomal and hamathecial morphology. The ascomata of *H. pulchella* are pyriform compared to globose in *B. communis*, and the hamathecium of *H. pulchella* is formed by simple, thin-walled, septate, hyaline paraphyses, while *B. communis* has hyaline, globose cells filling the centrum.

## DISCUSSION

*Bullimyces*.—This genus with three new species represents a distinct taxonomic entity based on morphological and molecular data. The three new species placed within *Bullimyces* share several morphological characteristics including globose to subglobose, membranous, black, ostiolate ascomata, hyaline, globose cells filling the centrum, unitunicate asci; and multiseptate, thick-walled ascospores, which darken with age into a golden color, with or without a gelatinous sheath or appendages. Nonetheless *Bullimyces* species can be identified based on ascospore morphology and septation. *B. communis* is characterized by three-septate ascospores with gelatinous caps at both apices; ascospores of *B. costaricensis* are five-septate and lack appendages, and *B. aurisporus* has three-septate ascospores and a mucilaginous sheath surrounding the ascospore.

*Bullimyces* is very similar to *Riomyces* described herein in ascomata and ascus morphology, but the cells filling the centrum in *Riomyces* are irregular and have thicker walls. *Bullimyces* and *Riomyces* both group with members of the Sordariomycetidae but were not sister taxa. *Bullimyces* shows affinities to *Ceratostomella pyrenaica* based on SSU and LSU data and to *Ceratostomella pyrenaica*, *Rhamphoria deliculata* and *Xylomelasma sordida* based on LSU data. These genera have similar ascomatal morphology to *Bulli-*

*myces* with dark, nonstromatic ascomata with a cylindrical neck, but otherwise they differ greatly in that these genera have asci with prominent apical rings, septate paraphyses that are wide at the base and tapering at the apex and mostly thin-walled ascospores (Réblová 2006). Furthermore *Bullimyces* and *Riomyces* differ in habitat from these other genera because *Bullimyces* and *Riomyces* occur only in freshwater while *Ceratostomella pyrenaica*, *Rhamphoria deliculata* and *Xylomelasma sordida* have been collected from decayed wood in terrestrial habitats.

The species of *Bullimyces* have a unique combination of morphological characters, which makes it difficult to place them taxonomically within the Sordariomycetes without supporting molecular data. For instance *Bullimyces* shares some striking morphological similarities to the freshwater genus *Luttrellia* Shearer, a member of the Halosphaeriaceae (Hypocreomycetidae) (Shearer 1978). The genus *Luttrellia* currently accommodates four aquatic species (Shearer 1978, Ferrer and Shearer 2007), with similar ascomatal and ascospore morphology, however the hamathecium of *Luttrellia* is composed of septate, hyaline catenophyses, with the exception of *Luttrellia halonata*, where catenophyses are hyaline, wide and globose (Ferrer and Shearer 2007). Unfortunately no molecular data are available at present for *Luttrellia*. Future molecular phylogenetic analysis may clarify whether *Luttrellia halonata* shares affinities with the genus *Bullimyces*.

Globose cells that fill the centrum of *Bullimyces* and *Riomyces* have been reported by Nakagiri and Tokura (1987) in the marine genus *Corollospora* (Halosphaeriaceae). *Corollospora* has pseudoparenchyma of hyaline, thin-walled, rounded cells filling the center of young ascocarps. Nonetheless they are distinct from those of *Bullimyces* and *Riomyces* because the pseudoparenchyma cells of *Corollospora* are joined one cell to another by pit connections (Nakagiri and Tokura 1987), a feature not seen in *Bullimyces*.

*Riomyces*.—The position of *Riomyces* remains incertae sedis because *Riomyces* was basal to *Bullimyces* and grouped with other members of the Ophiostomatales and Magnaporthaceae without support (FIGS. 1, 2). *Riomyces* and *Bullimyces* may be closely related based on their morphological similarities. However additional taxon sampling in the LSU analyses revealed that *Riomyces* and *Hydromelitis* also could be related to freshwater ascomycetes in the *Annulatascaceae* sensu lato and stricto. Unfortunately no SSU data are available for the *Annulatascaceae*, perhaps due to the difficulty in sequencing this group (Ferrer pers comm).

*Hydromelitis*.—Among the Sordariomycetidae *Hydromelitis* shows some similarities to the genus *Bullimyces* in

having multiseptate, thick-walled ascospores surrounded by a gelatinous sheath that darkens into a golden color with age. However the ascomata of *Hydromelitis* are distinctly pyriform rather than globose as in most *Bullimyces* species. In addition the hamathecium of *Hydromelitis* are formed by simple, thin-walled, septate, hyaline paraphyses while *Bullimyces* species have hyaline, globose cells filling the centrum.

The placement of the new genera at the family level is uncertain. The combination of morphological characteristics found in *Hydromelitis* including the membranous, pyriform ascomata and phragmoseptate, thick-walled ascospores with a sheath are not present in any family currently included in the Sordariomycetidae.

Freshwater ascomycetes possess unique morphological characteristics that might be adaptive to freshwater habitats, such as ascospores with elaborate appendages and sheaths (Shearer 1993, Jones 2006). Also in the aquatic Dothideomycetes order Jahnulales the vegetative hyphae are almost 10 times wider than other hyphae reported in the Ascomycota (Inderbitzin et al. 2001, Raja and Shearer 2006). We are of the opinion that these wide hyphae aid the attachment of the ascomata to the soft, decorticated, woody substrates while in water, analogous to an anchor. *Bullimyces* and *Riomyces* possess wide, globose cells in the hamathecium that might be adaptive in providing buoyancy to the asci and the ascospores when they are discharged into water. The functional significance of these traits remains unclear, but we hypothesize that these globose cells may be important for dispersion and establishment of fungi on wood in flowing water.

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