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## Freshwater Ascomycetes: *Hyalorostratum brunneisporum*, a new genus and species in the Diaporthales (Sordariomycetidae, Sordariomycetes) from North America

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*Hyalorostratum brunneisporum* gen. et sp. nov. (ascomycetes) is described from freshwater habitats in Alaska and New Hampshire. The new genus is considered distinct based on morphological studies and phylogenetic analyses of combined nuclear ribosomal (18S and 28S) sequence data. *Hyalorostratum brunneisporum* is characterized by immersed to erumpent, pale to dark brown perithecia with a hyaline, long, emergent, periphysate neck covered with a tomentum of hyaline, irregularly shaped hyphae; numerous long, septate paraphyses; unitunicate, cylindrical asci with a large apical ring covered at the apex with gelatinous material; and brown, one-septate ascospores with or without a mucilaginous sheath. The new genus is placed basal within the order Diaporthales based on combined 18S and 28S sequence data. It is compared to other morphologically similar aquatic taxa and to taxa reported from freshwater habitats that share affinities to the Diaporthales.

**Key words** – aquatic – fungi – phylogenetics –saprophyte –submerged wood – systematics

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### Introduction

Freshwater ascomycetes occur on a variety of submerged substrates in both lotic (creeks, rivers, streams) and lentic (bogs, lakes, ponds, swamps) habitats (Shearer 1993, Cai et al. 2003, Shearer et al. 2004, 2007, Raja et al. 2009, Shearer & Raja 2010). During an investigation of freshwater ascomycetes along a latitudinal gradient in North and Central America, we found a distinctive ascomycete from a lake in Alaska and again from a stream in New Hampshire. Based on its saprobic habit, perithecial ascoma, and unitunicate ascus, we could assign this fungus to the class Sordariomycetes. Additional morphological characters such as membranous ascomal wall, long

hyaline neck, presence of paraphyses, and unitunicate asci with a prominent apical ring indicated that the new fungus belongs in the subclass Sordariomycetidae (Eriksson & Winka 1997). However, due to a unique combination of morphological characters such as: J- apical ring covered apically by gelatinous material; and reniform to cylindrical, brown, one-septate ascospores with or without a gelatinous sheath, we could not unequivocally assign this fungus to any of the existing orders and families currently included in the Sordariomycetes (Lumbsch & Huhndorf 2007, Kirk et al. 2008) based on morphology alone.

The goals of this study, therefore, were to (1) analyze partial sequences of the nuclear

ribosomal 18S small subunit (SSU) and 28S large subunit (LSU) of the unidentified fungus and compare it with other SSU and LSU sequences from taxa currently included in the Sordariomycetes to determine its phylogenetic placement, and (2) describe and illustrate this distinctive fungus.

## Methods

### Sampling and morphological study

Submerged, dead woody and herbaceous debris was collected randomly from freshwater habitats following the procedures described in Shearer et al. (2004). Samples were placed in Ziplock® plastic bags lined with moist paper towels and transported to the laboratory. Collection site information and date of collection was recorded in the field and is provided in the specimen-examined section. In the laboratory, samples were placed in moist chambers and incubated at ambient temperature (~25°C) and 12/12 hr light and dark conditions. Samples were subsequently examined with a dissecting microscope within one week of collection and periodically over 6–12 months (Shearer 1993, Shearer et al. 2004). Methods for species' isolation are outlined in Fallah & Shearer (2001) and Shearer et al. (2004) and methods for morphological observation of specimens and embedding and sectioning procedures are described in Fallah & Shearer (2001) and Raja & Shearer (2008). The holotype and additional specimens examined are deposited at the University of Illinois Herbarium (ILL).

### Molecular study

Detailed methods for DNA extraction, PCR, sequencing, and alignment procedures for SSU and LSU genes are outlined in Campbell et al. (2007) and Shearer et al. (2009).

### Taxon sampling

In order to determine the phylogenetic relationships of the unidentified fungus we constructed phylogenetic trees with newly generated SSU and LSU sequences of isolates A573-2a and A573-2b (Table 1) and published sequences of taxa from the most recent phylogenetic classification of the Sordariomycetes by Zhang et al. (2006) (Table 2). We

established a dataset for each of the two genes and also a combined dataset using both genes. The datasets contained sequences from 76 taxa for the SSU and 77 taxa for LSU, while the combined dataset consisted of sequences from 77 taxa (Table 2). The datasets contained sequences of a wide array of taxa representing various orders from the three subclasses, Hypocreomycetidae, Sordariomycetidae, and Xylariomycetidae within the class Sordariomycetes (Eriksson & Winka 1997, Zhang et al. 2006). Members of the Leotiomycetes were used as outgroup taxa (Fig. 1).

### LSU dataset

In addition to the combined SSU and LSU dataset we also prepared a separate data matrix that consisted of LSU sequences from 58 taxa belonging to the Sordariomycetes (Zhang et al. 2006) as found in a Blast search (<http://blast.ncbi.nlm.nih.gov>). The smaller LSU dataset consisted of sequences from the blast search, as well as sequences of unitunicate taxa from GenBank that had morphological similarities to the undescribed fungus A573. The GenBank accession numbers for the 58 taxa LSU dataset are provided in Fig. 2.

### Phylogenetic analyses

Separate alignments were made for the larger SSU and LSU datasets. After ambiguous regions were delimited and excluded from both SSU and LSU datasets, maximum likelihood analyses (ML) using PhyML were run on each dataset using a GTR model including invariable sites and discrete gamma shape distribution with 1000 ML bootstrap replicates (BS) and with combined NNI and SPR tree searches in effect (Guindon & Gascuel 2003). Individual SSU and LSU phylogenies were then examined for conflict by comparing clades with bootstrap support  $\geq 70\%$  (Wiens 1998). On the final combined dataset (SSU and LSU), we performed a ML analysis using PhyML with the same parameters as above and 1000 ML BS were performed to assess clade support (Felsenstein 1985).

As an additional means of assessing branch support, Bayesian analysis employing a Markov chain Monte Carlo method (MCMC) was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001, Huelsenbeck & Ronquist

**Table 1** Isolates newly generated in this study.

Species	Voucher info*	GenBank Accession Numbers	
		nucSSU rDNA	nucLSU rDNA
<i>Hyalorostratum brunneisporum</i>	A573-2a	HM191721	HM191719
<i>Hyalorostratum brunneisporum</i>	A573-2b	–	HM191720

\*A, Carol A. Shearer

**Table 2** Species used in this study.

Species	Voucher info**	GenBank Accession Numbers	
		nucSSU rDNA	nucLSU rDNA
<i>Ambrosiella xylebori</i>	CBS 110.61	DQ471031	DQ470979
<i>Aniptodera chesapeakensis</i>	ATCC 32818	U46870	U46882
<i>Apiognomonina errabunda</i>	AR 2813	DQ862045	AF408334
<i>Balansia henningsiana</i>	GAM 16112	AY489683	AY489715
<i>Bionectria ochroleuca</i>	AFTOL 187	DQ862044	DQ862027
<i>Bombardia bombardia</i>	SMH 3391	DQ471021	DQ470970
<i>Botryotinia fuckeliana</i>	OSC 100012	AY544695	AY544651
<i>Camarops microspora</i>	CBS 649.92	DQ471036	AY083821
<i>Camarops ustulinoides</i>	DEH 2164	DQ470989	DQ470941
<i>Ceriosporopsis halima</i>	JK 5473F	U47843	U47844
<i>Ceratocystis fimbriata</i>	TCH C89	U32418	U17401
<i>Chromendothia citrina</i>	AR 3446	DQ862046	AF408335
<i>Claviceps purpurea</i>	GAM 12885	AF543765	AF543789
<i>Cordyceps capitata</i>	OSC 71233	AY489689	AY489721
<i>Cordyceps cardinalis</i>	OSC 93609	AY184973	AY184962
<i>Cordyceps ophioglossoides</i>	OSC 106405	AY489691	AY489723
<i>Corollospora maritima</i>	JK 4834	U46871	U46884
<i>Cryphonectria cubensis</i>	CBS 101281	DQ862047	AF408338
<i>Cryptodiaporthe aesculi</i>	CBS 109765	DQ836899	DQ836905
<i>Cryptosporella hypodermyia</i>	CBS 171.69	DQ862049	DQ862028
<i>Diaporthe eres</i>	CBS 109767	DQ471015	AF408350
<i>Diaporthe phaseolorum</i>	NRRL 13736	L36985	U47830
<i>Diatrype disciformis</i>	CBS 197.49	DQ471012	DQ470964
<i>Doratomyces stemonitis</i>	CBS 127.22	DQ836901	DQ836907
<i>Endothia gyrosa</i>	CBS 112915	DQ836898	DQ470972
<i>Epichloë typhina</i>	ATCC 56429	U32405	U17396
<i>Eutypa lata</i>	CBS 208.87	DQ836896	DQ836903
<i>Fragosphaeria purpurea</i>	CBS 133.34	AF096176	AF096191
<i>Gelasinospora tetrasperma</i>	CBS 178.33	DQ471032	DQ470980
<i>Glomerella cingulata</i>	CBS 114054	AF543762	AF543786
<i>Gnomonia gnomon</i>	CBS 199.53	DQ471019	AF408361
<i>Graphostroma platystoma</i>	CBS 270.87	DQ836900	DQ836906
<i>Haematonectria haematococca</i>	GJS 89-70	AY489697	AY489729
<i>Halosphaeria appendiculata</i>	CBS 197.60	U46872	U46885
<i>Hydropisphaera erubescens</i>	ATCC 36093	AY545722	AY545726
<i>Hypocrea americana</i>	OSC 100005	AY544693	AY544649
<i>Hypocrea lutea</i>	ATCC 208838	AF543768	AF543791
<i>Lasiosphaeria ovina</i>	SMH 4605	DQ836894	AY436413
<i>Leotia lubrica</i>	OSC 100001	AY544687	AY544644
<i>Leucostoma niveum</i>	AR 3413	DQ862050	AF362558
<i>Lignincola laevis</i>	JK 5180A	U46873	U46890
<i>Lindra thalassiae</i>	JK 5090A	DQ470994	DQ470947
<i>Lulworthia grandispora</i>	JK 4686	DQ522855	DQ522856
<i>Mazzantia napelli</i>	AR 3498	DQ862051	AF408368
<i>Melanconis alni</i>	AR 3500	DQ862052	AF408371
<i>Melanconis marginalis</i>	AR 3442	DQ862053	AF408373
<i>Melanospora tiffanii</i>	ATCC 15515	AY015619	AY015630
<i>Melanospora zamiae</i>	ATCC 12340	AY046578	AY046579
<i>Microascus longirostris</i>	CBS 267.49	DQ471026	AF400865

**Table 2 (Continued)** Species used in this study.

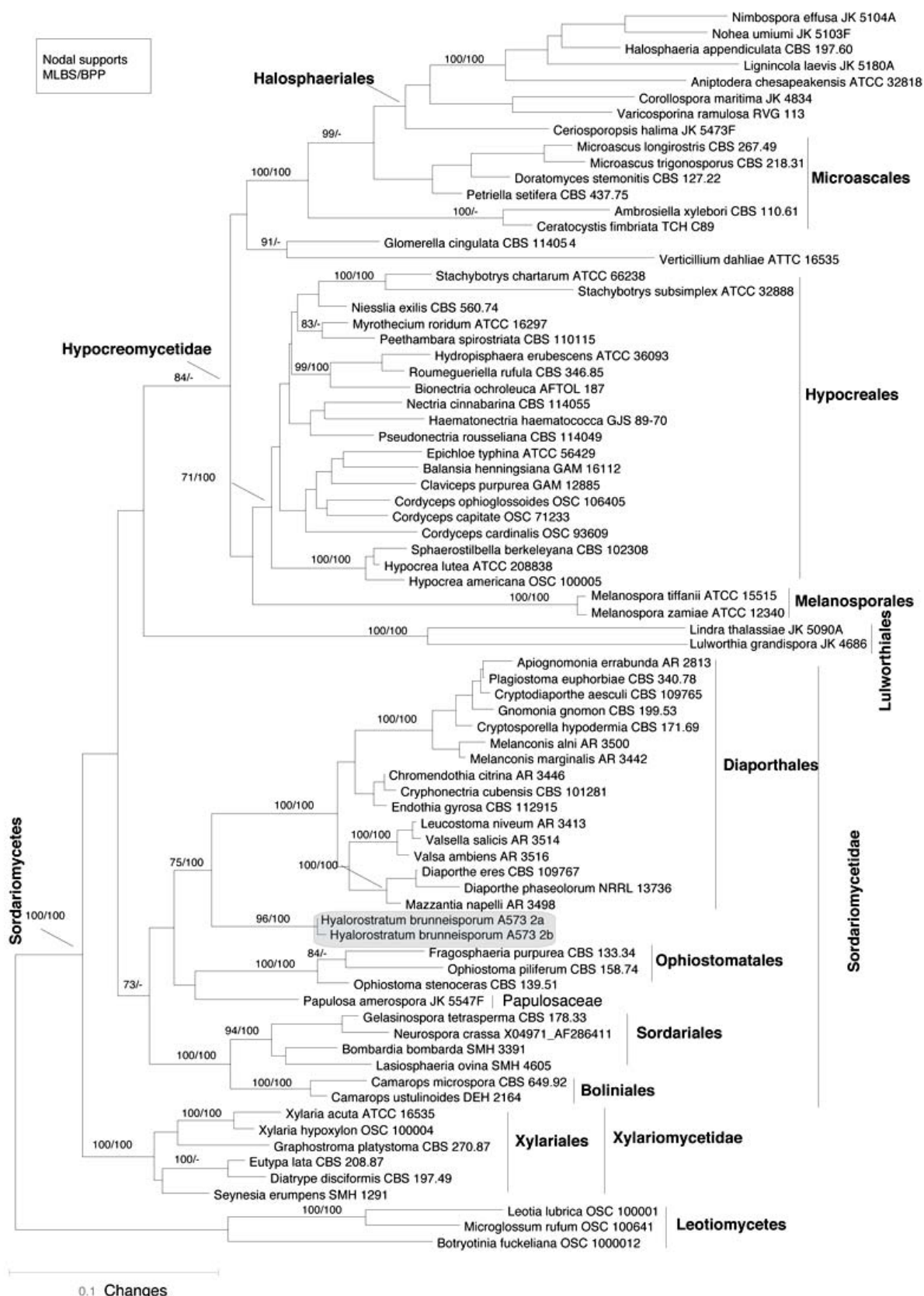
Species	Voucher info**	GenBank Accession Numbers	
		nucSSU rDNA	nucLSU rDNA
<i>Microascus trigonosporus</i>	CBS 218.31	DQ471006	DQ470958
<i>Microglossum rufum</i>	OSC 100641	DQ471033	DQ470981
<i>Myrothecium roridum</i>	ATCC 16297	AY489676	AY489708
<i>Nectria cinnabarina</i>	CBS 114055	U32412	U00748
<i>Neurospora crassa</i>		X04971	AF286411
<i>Niesslia exilis</i>	CBS 560.74	AY489688	AY489720
<i>Nimbospora effusa</i>	JK 5104A	U46877	U46892
<i>Nohea umiuni</i>	JK 5103F	U46878	U46893
<i>Ophiostoma stenoceras</i>	CBS 139.51	DQ836897	DQ836904
<i>Ophiostoma piliferum</i>	CBS 158.74	DQ471003	DQ470955
<i>Papulosa amerospora</i>	JK 5547F	DQ470998	DQ470950
<i>Peethambara spirostriata</i>	CBS 110115	AY489692	AY489724
<i>Petriella setifera</i>	CBS 437.75	DQ471020	DQ470969
<i>Plagiostoma euphorbiae</i>	CBS 340.78	DQ862055	AF408382
<i>Pseudonectria rousseliana</i>	CBS 114049	AF543767	U17416
<i>Roumegueriella rufula</i>	CBS 346.85	DQ522561	DQ518776
<i>Seynesia erumpens</i>	SMH 1291	AF279409	AF279410
<i>Sphaerostilbella berkeleyana</i>	CBS 102308	AF543770	U00756
<i>Stachybotrys chartarum</i>	ATCC 66238	AY489680	AY489712
<i>Stachybotrys subsimplex</i>	ATCC 32888	AY489679	AY489711
<i>Valsa ambiens</i>	AR 3516	DQ862056	AF362564
<i>Valsella salicis</i>	AR 3514	DQ862057	AF408389
<i>Varicosporina ramulosa</i>	RVG-113	U43846	U44092
<i>Verticillium dahliae</i>	ATCC 16535	AY489705	AY489737
<i>Xylaria acuta</i>	ATCC 56487	AY544719	AY544676
<i>Xylaria hypoxylon</i>	OSC 100004	AY544692	AY544648

\*\*AR, Amy Rossman; AFTOL, Assembling the fungal tree of Life; ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; DEH, Don E. Hemmes; GAM, Julian H. Miller Mycological Herbarium; JK, Jan Kohlmeyer; NRRL, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL; OSC, Oregon State University Herbarium; RVG, Robert V. Gessner; SMH, Sabine M. Huhndorf.

2001). Constant characters were included. Modeltest 3.7 (Posada & Crandall 1998) as well as JModeltest (Posada 2008) were used to determine the best-fit model of evolution for the dataset. In both programs, the GTR+I+G model was selected by Akaike Information Criterion (AIC) (Posada & Buckley 2004). Likelihood model assumptions used were: unequal base frequencies (freqA = 0.2448, freqC = 0.2297, freqG = 0.2950, freqT = 0.2305), a substitution rate matrix (A<->C = 1.0327, A<->G = 2.6031, A<->T = 1.4127, C<->G = 0.7741, C<->T = 6.2081, G<->T = 1.0000), a proportion of invariable sites of 0.4160 and a gamma distribution shape parameter of 0.5230. The above model was then implemented in a Bayesian analysis of 10 million generations with trees sampled every 1000th generation resulting in 10 000 total trees. The first 1000 trees that extended beyond the burn-in phase in each analysis were discarded and the remaining 9000 trees were used to calculate posterior probabilities (PP).

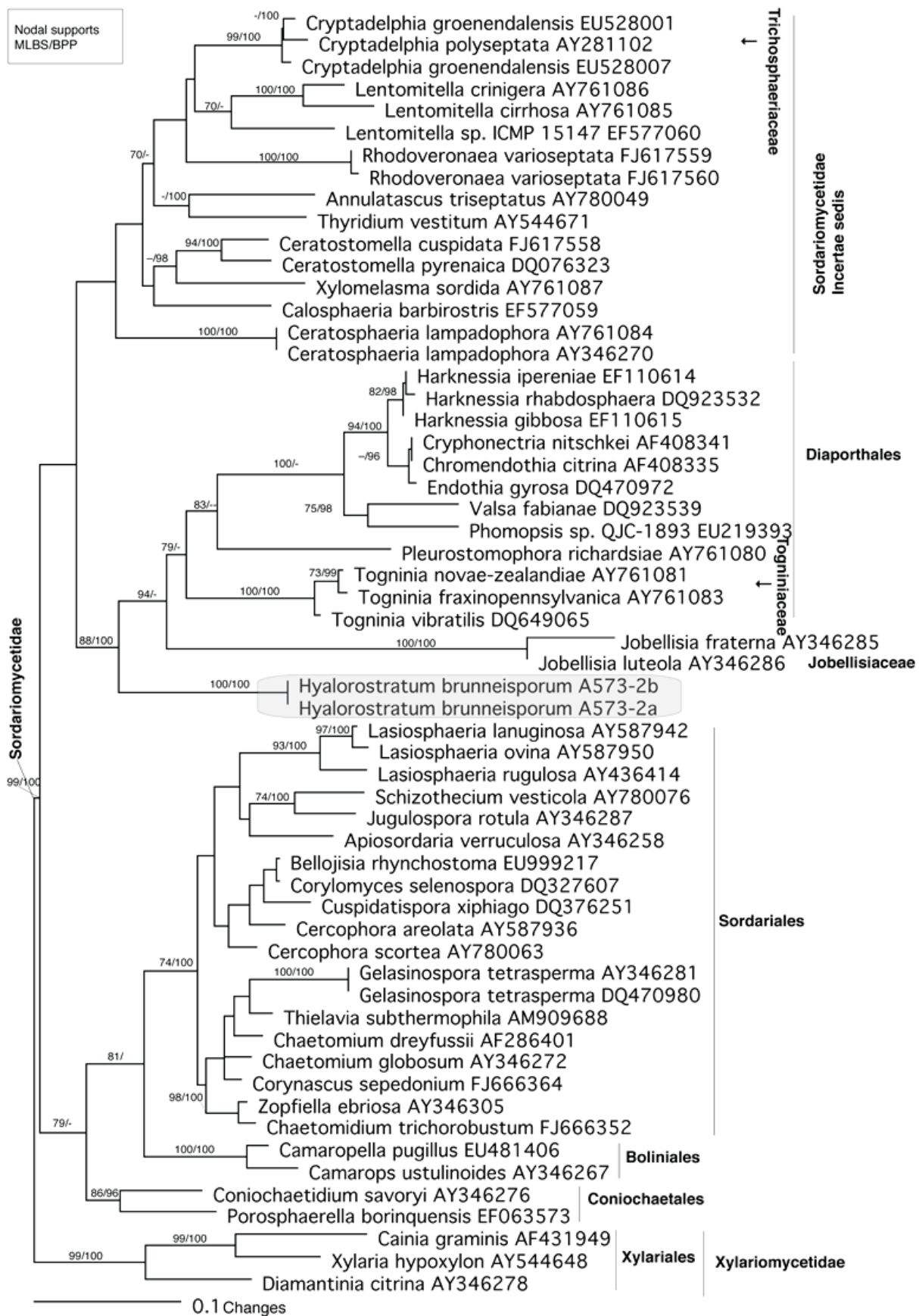
The consensus of 9000 trees was viewed in PAUP 4.0b10 (Swofford 2002). The Bayesian analysis was run twice starting from a different random tree each time, to ensure that trees from the same tree space were being sampled each time.

On the LSU dataset with 58 taxa, we performed a ML analysis using PHYML with the same parameters as with the combined SSU and LSU dataset and branch support was estimated by running 1000 MLBS. We used Gblocks (Castresana 2000, Talavera & Castresana 2007) to exclude introns and ambiguously aligned regions. Modeltest 3.7 (Posada & Crandall 1998) was used to determine the best-fit model of evolution for the dataset. The GTR+I+G model was selected using the AIC criterion. Likelihood model assumptions used were: unequal base frequencies (freqA = 0.2396, freqC = 0.2315, freqG = 0.3118, freqT = 0.2172), a substitution rate matrix (A<->C = 0.7262, A<->G = 2.4932, A<->T = 2.3185, C<->G = 0.7384, C<->T = 7.0633, G<->T =



**Fig. 1** – Phylogram of the most likely tree (lnL = -26258.181) using PhyML based on the combined dataset of 18S and 28S nrDNA. Maximum likelihood bootstrap support values  $\geq 70\%$  and Bayesian inference posterior probability  $\geq 95\%$  are shown above the branches. The new genus is shaded. Members of the Leotiomycetes are used as outgroup taxa.





**Fig. 2** – Phylogram of the most likely tree (lnL = -7393.723) using PhyML based on a dataset of 58 LSU taxa. Maximum likelihood bootstrap support values  $\geq 70\%$  and Bayesian inference posterior probability  $\geq 95\%$  are shown above the branches. The new genus is shaded. Members of the Xylariomycetidae are used as outgroup taxa.

1.0000), a proportion of invariable sites of 0.552 and a gamma distribution shape parameter of 0.4818. Bayesian analysis was run on the LSU dataset using the GTR+I+G model with similar run parameters as used for the combined dataset. In the 58 taxa LSU dataset we used members of the Xylariomycetidae as outgroup taxa.

## Results

### Morphological study

Examination of the morphology using fresh material indicated a unique combination of characters including: an immersed, black ascoma with a long, emergent, hyaline, periphysate neck bearing a tomentum of irregularly shaped hyaline hyphae; numerous long, septate paraphyses; long, cylindrical, unitunicate asci with a prominent J-, bipartite apical ring and gelatinous material above the ascus apical ring; and reniform to cylindrical, brown, bi- to multiguttulate, one-septate ascospores, with or without a gelatinous sheath.

### Sequence alignment and phylogenetic analyses of combined dataset

There were no significant conflicts among the clades in the separate SSU and LSU analyses based on PHYML bootstrap replicates (data not shown). Subsequent analyses were then performed on the combined SSU + LSU dataset. The combined SSU and LSU alignment consisted of 77 taxa (Table 1) and 3329 base pairs including introns and ambiguously aligned regions. After the removal of introns and ambiguously aligned regions, the combined alignment consisted of 3136 base pairs. The 5' and 3' ends of both SSU and LSU were excluded from the combined analyses due to missing data in most sequences. Since no discernible conflicts were observed in the separate SSU and LSU phylogenies, we present only the combined SSU and LSU PhyML tree. The combined matrix analyzed in this study produced a single most likely tree (Fig. 1).

The original 58 taxa-LSU dataset consists of 2696 base pairs. After the 5' and 3' ends were excluded due to missing data in most sequences and after the removal of ambiguous regions with Gblocks using the default para-

meters; the final LSU dataset consisted of 1061 characters.

Although our results provided strong support for the placement of A573 as basal to Diaporthales, Sordariomycetidae, based on our review of the literature we were unable to find a suitable genus within which we could include A573. We therefore describe it as a new genus and species.

### *Hyalorostratum* Raja et Shearer **gen. nov.**

Mycobank 518893

Ascomata immersa dein erumpentia, dispersa; venteribus globosis vel subglobosis, membranacea, laterica vel brunnea, ostiolata, rostrum, hyalinum, periphysatum; rostrum. Pseudoparaphysatum, numerosum, hyalinum, septatum. Asci unitunicati, cylindrici, pedicellati, octospori, cum apparatu apicali bipartis, ad apicali sin gelatinosi. Ascosporae ellipsoideae, reniforme, 1-septatae, hyalinae, serius brunnea, cum vel sine vagina mucilagina.

Type species – *Hyalorostratum brunneisporum*

Ascomata immersed, becoming erumpent, scattered; venter globose to subglobose, membranous, reddish to light brown, ostiolate with a long, emergent, hyaline, periphysate neck; neck covered with a tomentum of hyaline, irregularly shaped hyphae. Pseudoparaphyses numerous, hyaline, septate, filamentous, broader at the base than apex. Asci unitunicate, cylindrical, pedicellate, with bipartite apical apparatus, apical ring apex covered with gelatinous material, containing eight overlapping, uniseriate ascospores. Ascospores ellipsoidal to reniform, 1-septate, hyaline when young, becoming dark brown; multiguttulate.

Etymology – hyalo = L. for hyaline, and rostratum = L. for beak, referring to the hyaline ascomal beak.

Type species – *Hyalorostratum brunneisporum*

### *Hyalorostratum brunneisporum* Raja et Shearer **sp. nov.**

Figs 3–12

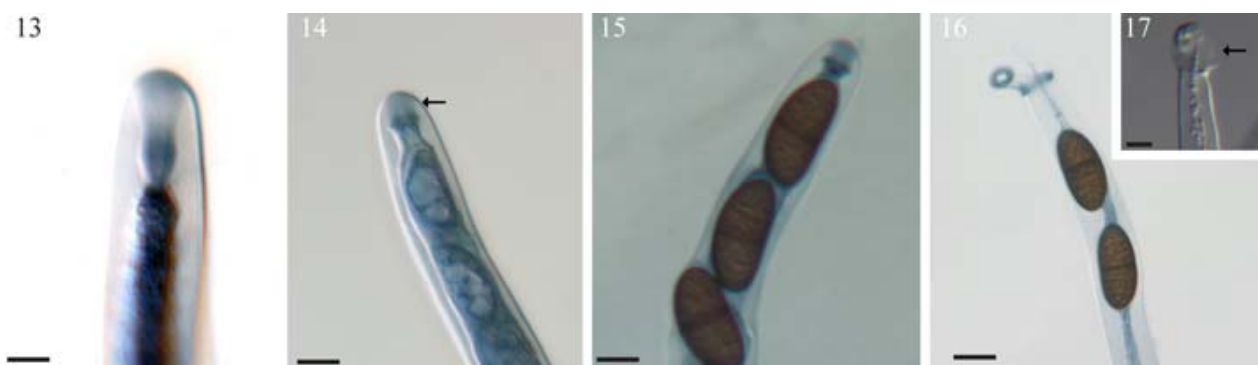
Mycobank 518894

Ascomata 500–620 × 200–290 μm, immersa dein erumpentia, dispersa; venteribus globosis vel subglobosis, membranacea, laterica vel brunnea, ostiolata, rostrum 220–235 ×



**Figs 3–12** – *Hyalorostratum brunneisporum* from the holotype (A573-1). **3.** Squash mount of an ascoma showing hyaline neck (note dark brown ascospores inside the neck). **4.** Longitudinal section through the ascoma. **5.**Periphysate neck. **6.** Longitudinal section of peridium. **7.** Paraphyses. **8.** Young and mature asci (note ascus extending in water). **9.** Mature ascus showing uniseriate, brown ascospores. **10.** Young ascospores in water surrounded by a gelatinous sheath. **11.** Mature, multiguttulate brown ascospores surrounded by a gelatinous sheath. **12.** Ascospore in glycerin. Bars 3 = 50  $\mu\text{m}$ ; 4, 5, 6, 8, = 20  $\mu\text{m}$ ; 7, 9–12 = 10  $\mu\text{m}$ .





**Figs 13–17 (A573-2)** – **13.** Young ascus showing developing apical apparatus. **14.** Young ascus with bipartite apical ring (note arrow showing mucilaginous material above ascus apical ring). **15.** Mature ascus showing bipartite apical ring and mucilaginous material present above the ring. **16.** Ascospores being discharged from ascus apex. **17.** Ascus apex showing mucilaginous material (indicated by arrow) after ascospores have been discharged. Bars 13–17 = 10  $\mu\text{m}$ .

55–60  $\mu\text{m}$ , hyalinum, periphysatum. Paries ascomatis duostratus, ca. 20–27  $\mu\text{m}$  latum, stratum exterior e cellulis brunneis 7–9  $\times$  2–3  $\mu\text{m}$ , pseudoparenchymaticis, stratum interior e, hyalinum compressum, 5–10  $\mu\text{m}$  alta. Paraphysatum numerosum, 160–170  $\mu\text{m}$  alta and 2–3  $\mu\text{m}$  diametrum, basum hyalinum, septatum. Asci 185–240  $\times$  10–20  $\mu\text{m}$ , unitunicati, cylindrici, pedicellati, octospori, cum apparatus apicali bipartis, ad apicali sin gelatinosi. Ascospores 22–32  $\times$  9–13  $\mu\text{m}$ , ellipsoidae, reniforme, 1-septatae, multiguttulatae vel biguttulatae, hyalinae, serius brunnea, cum vel sine vagina mucilagina.

Ascomata 500–620  $\times$  200–290  $\mu\text{m}$ , immersed, scattered, venter globose to subglobose, rounded at the base (Figs 3–4), membranous, reddish to light brown, ostiolate, with a long, erumpent neck. Neck cylindrical, periphysate, hyaline above and darkened below, 220–235  $\times$  55–60  $\mu\text{m}$ , in longitudinal section comprised of divergent hyphae ca 2–3  $\times$  5–7  $\mu\text{m}$  (Fig. 5). Peridium ca. 20–27  $\mu\text{m}$  wide, composed of two layers; outer layer of dark brown pseudoparenchymatic cells ca. 7–9  $\times$  2–3  $\mu\text{m}$ , inner layer of hyaline, flattened cells ca. 5–10  $\mu\text{m}$  long (Fig. 6). Paraphyses numerous, extending from the ascomal base into the ostiole, hyaline, septate, unbranched, filamentous, slightly broader at base than apex, 160–170  $\mu\text{m}$  long and 2–3  $\mu\text{m}$  wide at the base (Fig. 7). Asci 185–240  $\times$  10–20  $\mu\text{m}$ , ( $\bar{x}$  = 210  $\times$  15  $\mu\text{m}$ ,  $n$  = 25), unitunicate, cylindrical, rounded at the apex and tapering towards the base,

pedicellate, extending in water to ca. 350–400  $\mu\text{m}$  in length, with a large, bipartite apical ring ca. 7  $\mu\text{m}$  long and 3  $\mu\text{m}$  wide, staining blue in aqueous nigrosin; apex of the apical ring covered with gelatinous material (Figs 8, 9, and Figs 13–17), containing eight overlapping, uniseriate ascospores; ascospores shot forcefully through the apical ring when the ascus wall extends in water. Ascospores 22–32  $\times$  9–13  $\mu\text{m}$  ( $\bar{x}$  = 25  $\times$  11  $\mu\text{m}$ ,  $n$  = 50), ellipsoid to reniform, 1-septate, hyaline when young (Fig. 10), becoming dark brown when old, multiguttulate becoming biguttulate, with or without a gelatinous sheath; sheath extending to ca. 2–3  $\mu\text{m}$  around the ascospore (Figs 11, 12).

Etymology – *L. brunnei* + *sporus*, referring to the brown ascospores of the fungus.

Known distribution – Alaska, New Hampshire (USA)

**HOLOTYPE.** USA. ALASKA: Headquarters, Lake Kenai Wildlife Refuge, Soldotna, 60°27.806N, 151°04.260W, on submerged woody debris, 5 September 2004, Wendy and John Witmer, A573-1 (HOLOTYPE, ILL 40792).

Anamorph – unknown.

Additional specimen examined – New Hampshire, Hubbard Brook Forest stream, on submerged woody debris, 10 September 2008, Rosalind Lowen, A573-2.

### Comments

The ascospores of *H. brunneisporus* are discharged through the bipartite ascus apical

ring (Figs 13–17). An apical ring begins to form early during ascus development (Fig. 13). Gelatinous material is produced within the ascus above the bipartite apical ring (Fig. 14). After ascospores mature, the bipartite apical ring is pushed upwards, which in turn pushes against the gelatinous material within the ascus apex (Fig. 15); the ascus elongates in water to twice its size, and subsequently, the ascospores are forcibly shot out (Fig. 16). Gelatinous material is seen around the outside of the ascus tip after the ascospores have been released (Fig. 17). Although bipartite apical rings occur commonly in species of Annulatasceae (Wong 1998, Ho & Hyde 2000, Campbell et al. 2003), and some species within the family show extension of asci in water (Campbell & Shearer 2004), gelatinous material above the ascus apical ring has not been reported for any species in the family; a feature to our knowledge, unique to the new genus, *H. brunneisporum*.

## Discussion

Several major clades presented in the multi-gene phylogeny of Zhang et al. (2006) such as the three subclasses, Hypocreomycetidae, Sordariomycetidae, and Xylariomycetidae, including their respective orders were recovered in our combined SSU and LSU phylogeny. *Hyalorostratum brunneisporum* showed strong affinities with taxa in the subclass Sordariomycetidae, and the two isolates of the new fungus (A573-2a and A573-2b) form a highly supported monophyletic clade (96% MLBS and 100% BPP) basal to the Diaporthales with 75% MLBS and 100% BPP (Fig. 1).

Phylogenetic analyses of LSU and combined SSU and LSU data showed that *H. brunneisporum* is related to taxa in the Diaporthales, as it occurs as basal to other diaporthalean taxa included in the analyses with strong MLBP and BPP support (Fig. 1). These results are supported by some morphological characters seen in *H. brunneisporum* such as black, perithecial, immersed to erumpent fruit bodies, with periphysate necks, and asci with a J- apical ring (Barr 1990, Samuels & Blackwell 2001, Castelbury et al. 2002, Rossman et al. 2007). In addition, the ascus base of *H. brunneisporum* detaches

readily from the subhymenium allowing the asci to float freely in the centrum as in some species of Diaporthales (Barr 1990, Barr 2001). *Hyalorostratum brunneisporum*, however, differs from diaporthalean taxa in many aspects, such as: its submerged aquatic habitat rather than terrestrial habitat; the ascomal neck covered with a tomentum of hyaline hyphae; presence of paraphyses at maturity; cylindrical ascus that elongates in water to twice its length (Fig. 8) as opposed to asci in Diaporthales that do not elongate to twice their length, and presence of a bipartite ascus apical ring with gelatinous material at the apex (Figs 13–17) compared to a non-bipartite apical ring lacking gelatinous material at the ascus apex in other diaporthalean taxa.

*Hyalorostratum brunneisporum* is morphologically similar to a recently described unitunicate freshwater ascomycete, *Paoayensis lignicola* Cabanela et al. (Cabanela et al. 2007), which also has brown ascospores and is placed in the Sordariomycetes. *Paoayensis lignicola* has ascomata that are grouped and have fused necks with a common ostiole, whereas, ascomata of *H. brunneisporum* have a single, long, hyaline, unfused neck. The asci in *P. lignicola* are short and clavate, but those of *H. brunneisporum* are cylindrical, long and extend in water. Although both species possess brown ascospores, the morphology of their ascospores differs. The ascospores of *P. lignicola* are 0–3-septate and limoniform, whereas those of *H. brunneisporum* are consistently one-septate and ellipsoidal to reniform.

Other unitunicate taxa reported from aquatic habitats that have one-septate brown ascospores and occur within the Sordariomycetidae include: *Brunneisporella aquatica* V.M. Raghoo & K.D. Hyde (Raghoo et al. 2001), *Paraniesslia tuberculata* K.M. Tsui, K.D. Hyde & Hodgkiss (Tsui et al. 2001), *Phaeonectriella lignicola* R.A. Eaton & E.B.G. Jones (Eaton & Jones 1970), and *Submersisphaeria aquatica* K.D. Hyde (Hyde 1996). However, ascospores of *H. brunneisporum* differ from each of the above-mentioned taxa. The ascospores of *H. brunneisporum* are ellipsoid to reniform and  $22\text{--}32 \times 9\text{--}13 \mu\text{m}$ , while those of *B. aquatica* are ellipsoid to fusiform and smaller in size ( $17.5\text{--}20 \times 9\text{--}10 \mu\text{m}$ ). Ascospores of *H. brunneisporum* are smooth-walled

and posses a gelatinous sheath, whereas those of *P. tuberculata* are ornamented and lack a sheath. Ascospores of *H. brunneisporum* differ from those of *P. lignicola* in that they do not possess a terminal germ pore at each end. In addition, *H. brunneisporum* has a gelatinous sheath, but in *P. lignicola*, a gelatinous sheath is absent. The ascospores of *H. brunneisporum* do not posses small hyaline, cap-like appendages at both ends of the ascospores, which is characteristic of *S. aquatica* (Hyde 1996, Campbell et al. 2003).

Among the aforementioned taxa, *H. brunneisporum* most closely resembles *P. lignicola* in its ascomal morphology in that both taxa have a hyaline, periphysate neck and a venter that is immersed in the substrate (Fig. 1, plate 1, Eaton & Jones 1970). *Hyalorostratum brunneisporum*, however, can easily be distinguished from *P. lignicola* based on its hamathecium and ascus morphology. The hamathecium of *P. lignicola* consists of catenophyses while that of *H. brunneisporum* consists of long, slender, tapering, septate paraphyses. The asci of *H. brunneisporum* are cylindrical and elongate in water and have an apical ring with gelatinous material at the apex (Figs 8, 9, 13–17), whereas those of *P. lignicola* are clavate, somewhat thin-walled with a subapical retraction of cytoplasm and an apical pore.

Thus far 12 taxa belonging to the Diaporthales have been described or reported from freshwater habitats (Shearer & Raja 2010). Among those reported from decayed wood and bark, *H. brunneisporum* most closely resembles the genus *Jobellisia* in having a long neck, an ascus with a large, bipartite apical ring, and brown ascospores with a septum (Barr 1993, 1994, Huhndorf et al. 1999, Ranghoo et al. 2001). Recently, Réblová (2008) described a new family, *Jobellisiaceae*, in the Diaporthales for the genus *Jobellisia*. Two *Jobellisia* species, *J. viridifusca* K.M. Tsui & K.D. Hyde and *J. luteola* (Ellis & Everh.) M.E. Barr) have been reported previously from submerged wood in freshwater habitats (Ranghoo et al. 2001, Raja et al. 2009). The ascomata of *H. brunneisporum* are not formed in a stroma and are immersed to erumpent in the woody substrate, whereas ascomata in species of *Jobellisia* are surrounded by stromatic tissue (Barr 1993, 1994) and are mostly superficial on the

substrate. *Hyalorostratum brunneisporum* also differs in having a hyaline neck covered with a tomentum of hyaline hyphae compared to a black colored neck in *Jobellisia* species. In addition, a gelatinous sheath surrounds the ascospores of *H. brunneisporum* but not those of *Jobellisia* species, and no germ pores were observed in ascospores of *H. brunneisporum*, while germ pores are present in species of *Jobellisia* (Huhndorf et al. 1999). In the 58 taxa LSU dataset, *H. brunneisporum* was placed within the subclass Sordariomycetidae, with the two isolates forming a highly supported monophyletic clade (100% MLBS and 100% BPP) (Fig. 2), basal to the clade consisting of two *Jobellisia* species. This indicates that *H. brunneisporum* might be a sister group species of *Jobellisia* or that important related taxa may not be present in the sequence database used herein.

Recently two freshwater ascomycetes, *Phruensis brunneispora* Pinruan (Pinruan et al. 2004) and *Thailandiomyces bisetulosus* Pinruan et al. (Pinruan et al. 2008), as well as an interesting terrestrial ascomycete, *Lollipopaia minuta* Inderbitzin (Inderbitzin & Berbee 2001), which occurs in a tropical rainforest in Thailand, have been placed in the Diaporthales based on nrDNA sequence data, but these species also showed no affinities to any known diaporthalean families (Rossman et al. 2007). According to Rossman et al. (2007) the above taxa are referred to as diaporthalean fungi of unknown affinities. Our study also suggests that *H. brunneisporum* may be referred to the order Diaporthales based on results of our molecular phylogenetic analyses, but we could not assign the new fungus to any of the nine families currently included in the Diaporthales (Castlebury et al. 2002, Rossman et al. 2007). *Hyalorostratum brunneisporum* may represent a new lineage of fungi in the Sordariomycetidae showing affinities to the Diaporthales.

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