Short title: *Spongiforma squarepantsii* from Borneo

Spongiforma squarepantsii, a new species of gasteroid bolete from Borneo

Dennis E. Desjardin[1]*

Kabir G. Peay[2]

Thomas D. Bruns[3]

[1]Dept. of Biology, San Francisco State university, 1600 Holloway Ave., San Francisco, California 94131; [2]Dept. of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108, USA; [3]Dept. Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, California 94720-3102

Abstract: A gasteroid bolete collected recently in Sarawak on the island of Borneo is described as the new species *Spongiforma squarepantsii*. A comprehensive description, illustrations, phylogenetic tree and a comparison with a closely allied species are provided.

Key words: Boletales, fungi, taxonomy

INTRODUCTION

An unusual sponge-shaped, terrestrial fungus was encountered by Peay et al. (2010) during a recent study of ectomycorrhizal community structure in the dipterocarp dominated forest of the Lambir Hills in Sarawak, Malaysia. The form of the sporocarp was unusual enough that before microscopic examination the collectors were uncertain whether the fungus was a member of the Ascomycota or the Basidiomycota. However, upon returning to the laboratory it was recognized as a species of the recently described genus *Spongiforma* Desjardin, Manf. Binder, Roekring & Flegel that was described from dipterocarp forests in Thailand (Desjardin et al. 2009). The Borneo specimens differed in color, odor and basidiospore ornamentation from

the Thai species, and subsequent ITS sequence analysis revealed further differences warranting its formal description as a new species.

MATERIALS AND METHODS

After collection, specimens were dried in the field and shipped back to UC Berkeley for morphological analysis and long-term storage. In the field, a small portion of tissue from each fruiting body was also removed and stored in 300 µl of 2X CTAB buffer (100 mM Tris-HCl (pH 8.1), 1.4 M NaCl, 20 mM EDTA, 2% cetyl trimethyl ammonium bromide) for later use in DNA extraction. CTAB preserved samples were kept refrigerated (except during transportation), shipped back to UC Berkeley, and then stored at –20 C until DNA extraction. DNA was extracted using the DNeasy Tissue Kit (Qiagen Sciences, Valencia, CA, USA) with slight modifications as in Peay et al. (2010). The internal transcribed spacer (ITS) regions and a portion of the 28S large subunit (LSU) of the nuclear rRNA genes were PCR amplified and sequenced in both directions using the primer pairs ITS1F / ITS4 and LROR / TW13, respectively (White et al., 1990; Gardes & Bruns, 1993) as in Peay et al (2010). To confirm phylogenetic placement we aligned the LSU sequences for both basidiomes along with those of *Spongiforma thailandica* and allied clades identified in Desjardin et al. (2009) using the program MAFFT v. 6.814b (Katoh et al. 2002). Phylogenetic trees were generated using neighbor joining, maximum likelihood (PHYMYL, GTR with fixed transition:transversion rates, proportion of invariable sites, gamma distribution parameter, optimized for tree/length/rate; Lefort et al. 2003) and Bayesian methods (Mr. Bayes, GTR with gamma rate variation, 1 100 000 generations, burn-in = 100 000, 4 heated chains, unconstrained branch lengths, exponential = 10, shape parameter = 10; Huelsenbeck and Ronquist 2001). All programs were implemented using the application Geneious Pro v. 5.1.7 (Biomatters, Auckland, NZ). The sequence alignment was

submitted to TreeBASE (#xxxx). For the scanning electron micrographs of basidiospores, small fragments of dried material were affixed to stubs with carbon tape, coated with 75 angstroms of Au/Pd alloy using a Gatan PECS 682 ion-beam sputter coater, and photographed using a Carl Zeiss SMT Ultra 55 FE-SEM.

TAXONOMY

Spongiforma squarepantsii Desjardin, Peay and T.D. Bruns sp. nov. FIGS 1–2

MycoBank MB 519524

Basidiomata epigaea, cerebriformia, suaveolens. Peridium nulla. Gleba loculis labyrinthiformibus, 2–10 mm lata, aurantiacis; columella dendroideis, alba. Basidiosporae 10–12.5 × 6–7 µm, amygdaliformae, rugulosae, inamyloideae, cyanophileae. Cystidia 12–40 (–60) × 4–8 µm, subcylindrica vel acuminata. Trama glebae gelatinosae. Fibulae nulla. Holotypus hic designatus: Malaysia, Sarawak, TDB 3541 (UC 1860255).

Basidiomes (FIG. 1) epigeous, 30–50 mm diam × 20–30 mm tall, astipitate, irregularly globose to ovoid, cerebriform to sponge-like, rubbery-pliant. *Peridium* absent. *Hymenophore* composed of ridges or folds delimiting empty locules; locules 2–10 mm diam, irregular in outline, lined with a well-developed hymenium, minutely ciliate, orange (6A4-8) to deep orange (7A5-8); sterile ridges ciliate, pale orange white (5-6A2-3) or paler. *Spores* (from dried basidiomes) reddish brown to deep mahogany (8E7-8). *Columella* poorly developed, as a narrow, dendritic cord of tissue running through the center of the basidiome, white; attached to a white rhizomorphic strand. *Odor* vaguely fruity or strongly musty, not of coal tar. *Sterile ridges* composed of a trichoderm of erect, chains of cylindrical hyphae 4–6 µm diam, hyaline, inamyloid, thin-walled, terminated by cystidia. *Cystidia* 12–40(–60) × 4–8 µm, subcylindrical to fusoid or acuminate, hyaline, inamyloid, thin-walled; abundant on the sterile locule edges and

scattered amongst basidia in the hymenium. *Hymenophoral trama* of subparallel to interwoven hyphae 2.5–7(–13) µm diam, cylindrical, branched, septate, not inflated or rarely slightly inflated at seprum, strongly gelatinous, hyaline, inamyloid, thin-walled. *Subhymenium* pseudoparenchymatic, composed of inflated to ovoid or vesiculose cells 10–24 × 8–20 µm, hyaline, inamyloid, thin-walled, non-gelatinous. *Basidia* statismosporic, 28–40 × 8–9.7 µm, clavate, 4-spored with straight sterigmata up to 9.5 µm long. *Basidioles* subclavate. *Basidiospores* (FIG. 2) (9.5–)10–12.5 × 6–7 µm [x = 10.9 ± 0.8 × 6.4 ± 0.3 µm; Q = 1.5–2, Q_m = 1.65 ± 0.14, n = 25 spores], amygdaliform to subfusoid with a small central apiculus,, distal end rounded or subtruncate with a small central pore, coarsely verrucose and rusty brown in dist. water, finely roughened or nearly smooth and pale lilac grey in 3% KOH, coarsely verrucose and deep reddish brown in Melzer's reagent, cyanophilic, thick-walled (0.5–1.2 µm); ornamentation forms swollen pustules that loosen and dissolve in 3% KOH. Clamp connections absent in all tissues.

Habit, habitat and known distribution: Solitary, epigeous on ground under undetermined Dipterocarp trees, in Lambir Hills National Park, northern Borneo, Malaysian state of Sarawak. Lambir is an aseasonal, tropical rainforest, receiving c. 3000 mm of rainfall per year, with maximum and minimum daily temperatures between 32 C and 24 C (Lee et al., 2002). The forest at Lambir contains >1000 tree species but is dominated by the family Dipterocarpaceae. Detailed descriptions of the neighboring plot are available in Lee et al. (2002) and Davies et al. (2005).

Material examined: MALAYSIA. BORNEO ISLAND, SARAWAK: Lambir Hills National Park, about 0.5 km from road on trail to 52-hectare long-term forest dynamics research

plot, 4º20'N, 113º50'E, 28 May 2008, collected by *T.D. Bruns ,TDB 3541* (HOLOTYPE: UC 1860255); ibid., 25, May, 2008 (UC 1860254).

Genbank Accession numbers: UC 1860254 – LSU: HQ724510. UC 1860255 (Holotype) – LSU: HQ724509; ITS: HQ724511

Etymology: Named in honor of the famed cartoon character SpongeBob SquarePants, whose sponge shape shares a strong resemblance to the new fungus. Moreover, the hymenium when observed with scanning electron microscopy (FIG. 2) looks like a seafloor covered with tube sponges, reminiscent of the fictitious home of SpongeBob.

Commentary. *Spongiforma squarepantsii* is characterized by rather small, sponge-like and rubbery basidiomes that are externally pale orangish white and internally deep orange, with small empty locules lined with sporogenous tissue; no stipe but with a narrow dendritic white columella attached to a coarse white rhizomorph; a vaguely fruity-musty and pleasant odor; amygdaliform basidiospores with an apical pore that are coarsely verrucose and reddish brown in water but become lilac grey and nearly smooth in 3% KOH; an absence of clamp connections; and an association with members of the Dipterocarpaceae. It differs macromorphologically from the only other known species in the genus, *S. thailandica* Desjardin, Manf. Binder, Roekring & Flegel, described recently from central Thailand (Desjardin et al. 2009), in forming smaller basidiomes with a deep orange gleba and a rather pleasant fruity-musty odor. In comparison, *S. thailandica* forms basidiomes 50–100 mm broad by 40–70 mm tall, has a gleba that is pale greyish orange to brownish grey when young and darkens to reddish brown or dark brown in age, and has a strong odor of coal tar. Micromorphologically, *S. squarepantsii* differs from *S. thailandica* in forming more coarsely verrucose basidiospores (compare FIG. 2 herein with FIG. 2c–d of Desjardin et al 2009).

The LSU nucleotide sequences from the two specimens of *Spongiforma squarepantsii* are a 98% match to the LSU sequence of the holotype specimen of *S. thailandica*. This is a level that is consistent with many other congeneric comparisons in the Boletales (Binder and Hibbett 2006), and in combination with the strikingly similar macro- and micromorphology it supports our conclusion that the Borneo specimens are members of the genus *Spongiforma*. In addition, all phylogenetic reconstruction methods strongly supported the node separating *S. thailandica* from *S. squarepantsii* and a monophyletic clade of *Spongiforma* sister to *Porphyrellus* (FIG. 3). The ITS sequence from the holotype specimen of *S. squarepantsii* matched that of the holotype specimen of *S. thailandica* at only the 90 % level. This is far below the typical 97–98 % ITS infraspecific variation reported from a wide range of Basidiomycota (Horton 2002; Hughes et al. 2009). This result in combination with the differences in color, odor, basidiospore ornamentation and geographic location between these specimens and *S. thailandica* leads us to conclude that the Borneo fungus is a distinct species.

The presence of the genus *Spongiforma* with dipterocarps in mainland Southeast Asia and on the island of Borneo means that we might expect to find the genus in additional parts of the range of dipterocarp forests. The lack of ballistospore discharge and presence of distinctive odors of the two species suggests that animal dispersal is likely, and animal dispersal in combination with island populations would likely limit gene flow much as it does with *Rhizopogon* spp. (Grubisha et al 2007). This may explain the high ITS divergence between the two species and would predict that other isolated tracts of dipterocarp forests may harbor additional species in the genus.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Andrew Ichimura (Electron Microscope Facility, San Francisco

State University) for taking the SEM micrographs, and NSF–MRI 0821619 and NSF–EAR 0949176 awards to Dr. Ichimura for purchase of the FESEM and supporting equipment. The fieldwork was supported by a grant to KGP and TDB from the Center for Tropical Forest Science – Arnold Arboretum Asia program of the Smithsonian Tropical Research Institute and Harvard University. Sequence analysis was made possible by NSF grant 0742696 to TDB and Peter Kennedy. The 52 Hectare Long-Term Ecological Research Project is a collaborative project of the Forest Department of Sarawak, Malaysia, Harvard University, USA (under NSF awards DEB-9107247 and DEB-9629601 to P.S. Ashton) and Osaka City University, Japan (under Monbusho grant 06041094 to T. Yamakura, 08NP0901 to S. Tamura and 09NP0901 to S. Sasaki). The authors would like to thank the Sarawak Forest Research Corporation for granting permission to conduct research in Lambir Hills National Park, and in particular we thank Lucy Chong and Kamal Abdullah for their assistance on the ground.

LITERATURE CITED

Binder M, Hibbett DS. 2006. Molecular systematics and biological diversification of Boletales. Mycologia 98:971–981.

Davies S, Tan S, LaFrankie J, Potts M. 2005. Soil-related floristic variation in the hyperdiverse dipterocarp forest in Lambir Hills, Sarawak. In: Roubik D, Sakai S, Hamid A, eds. Pollination Ecology and Rain Forest Diversity, Sarawak Studies. New York: Springer-Verlag, pp. 22–34.

Desjardin DE, Binder M, Roekring S, Flegel T. 2009. *Spongiforma*, a new genus of gasteroid boletes from Thailand. Fung Div 37:1–8.

Grubisha LC, Bergemann SE, Bruns TD. 2007. Host islands within the California Northern Channel Islands create fine-scale genetic structure in two sympatric species of the symbiotic ectomycorrhizal fungus *Rhizopogon*. Mol Ecol 16:1811–1822.

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704.

Horton TR. 2002. Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. Pl Soil, 244:29–39.

Hughes KW, Petersen RH, Lickey EB. 2009. Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. New Phytol 182:795–798.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinform 17:754–755.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nuc Acids Res 30:3059–3066.

Lee HS, Davies SJ, LaFrankie JV, Tan S, Yamakura T, Itoh A, Ohkubo T, Ashton PS. 2002. Floristic and structural diversity of mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia. J Trop For Sci 14:379–400.

Peay KG, Kennedy PG, Davies SJ, Tan S, Bruns TD. 2010. Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. New Phytol 185:529–542.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gefland DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to method and applications. San Diego: Academic Press, pp. 315–322.

Figure Legends

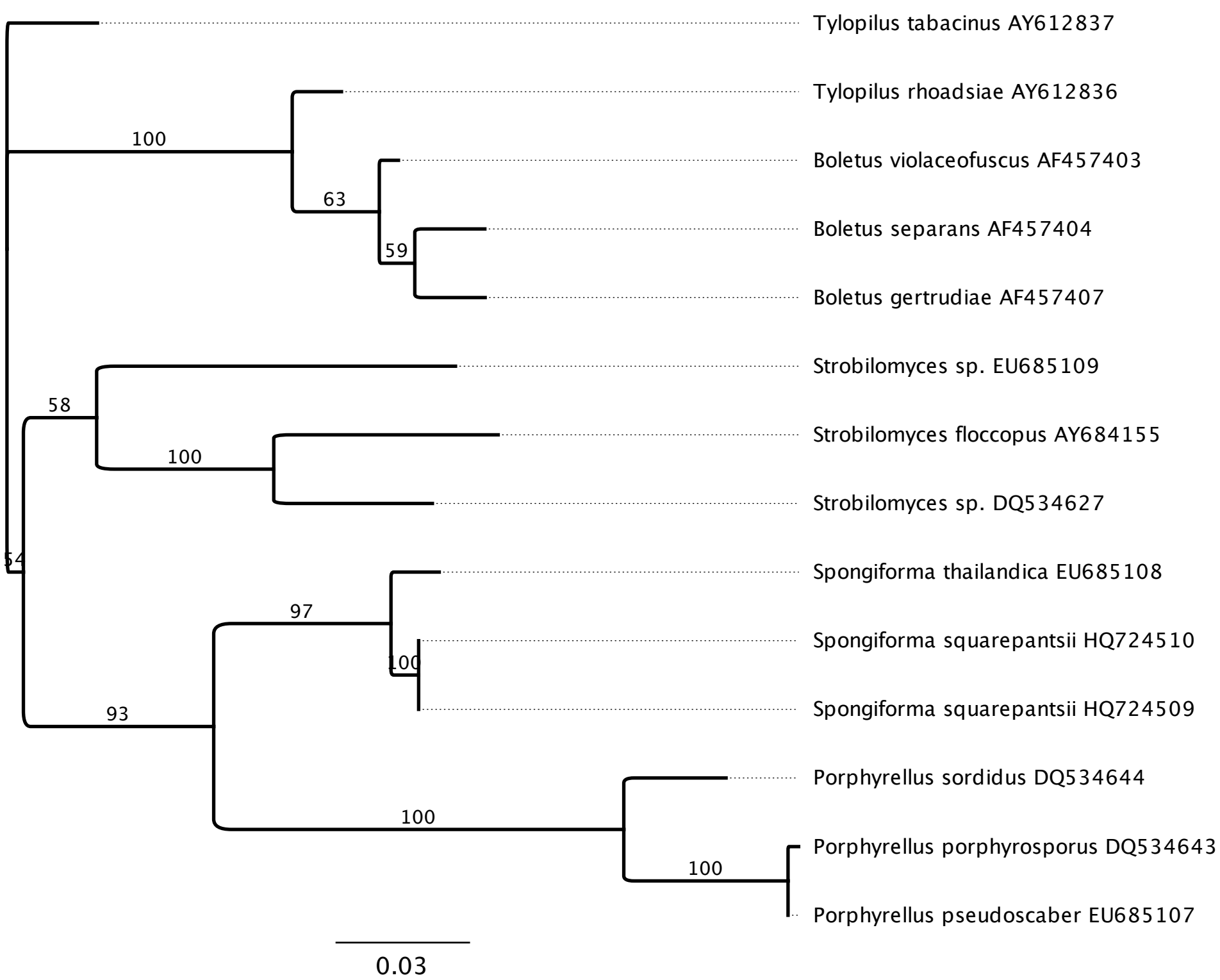
FIG. 1. Basidiome of *Spongiforma squarepantsii* (HOLOTYPE).

FIG. 2. Scanning electron micrographs of basidiospores of *Spongiforma squarepantsii* (HOLOTYPE). Scale bar: 10 µm – top photo; 1 µm – middle and bottom photos.

FIG. 3. Phylogenetic placement of *Spongiforma squarepantsii* inferred from nuclear LSU rDNA using maximum likelihood reconstruction. Numbers indicate node bootstrap support values.

* Corresponding author: ded@sfsu.edu


10
20
30
40
50
60
70


Tylopilus tabacinus AY612837
Tylopilus rhoadsiae AY612836
Boletus violaceofuscus AF457403
Boletus separans AF457404
Boletus gertrudiae AF457407
Strobilomyces sp. EU685109
Strobilomyces floccopus AY684155
Strobilomyces sp. DQ534627
Spongiforma thailandica EU685108
Spongiforma squarepantsii HQ724510
Spongiforma squarepantsii HQ724509
Porphyrellus sordidus DQ534644
Porphyrellus porphyrosporus DQ534643
Porphyrellus pseudoscaber EU685107
100
63
59
58
100
54
97
100
93
100
100
0.03