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Spongiforma squarepantsii, a new species of gasteroid bolete from Borneo

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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 placement and a comparison with a closely allied species are provided.

Key words: Boletales, fungi, taxonomy

INTRODUCTION

An unusual sponge-shaped (cf. *Spongia,* Porifera), terrestrial fungus was encountered by Peay et al. (2010) during a recent study of ectomycorrhizal (ECM) 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 on laboratory examination it was recognized as a species of the new genus *Spongiforma* Desjardin, Manf. Binder, Roekring & Flegel that was described from dipterocarp forests in Thailand (Desjardin et al. 2009). The Borneo specimens differed from the Thai species in color, odor and basidiospore ornamentation, and subsequent ITS and LSU nrDNA sequence analyses revealed further differences warranting its formal description as a new species.

MATERIALS AND METHODS

After collection the macromorphological features of each specimen were documented and the specimens were placed in a drying oven and shipped back to UC for micromorphological analysis and long-term storage. For documentation of micromorphological features small thin hand-sections of the dried basidiomes were mounted in ethanol followed by distilled water, 3% KOH, Melzer's reagent or cotton blue, and examined with a Nikon Optiphot-2 compound microscope with DIC optics. Spore statistics include x, the arithmetic mean of the spore length × spore width (± standard deviation) for n spores measured; Q, the quotient of spore length by spore width in any one spore, indicated as a range of variation in n spores measured; Q_m, the mean of Q-values. Herbarium acronyms are those of Thiers (continuously updated), and color terms and notations are those of Kornerup and Wanscher (1978). For the scanning electron micrographs of basidiospores small fragments of dried material were affixed to aluminum stubs with carbon tape, coated with 75 Å of Au/Pd alloy with a Gatan PECS 682 ion-beam sputter coater and photographed with a Carl Zeiss SMT Ultra 55 FE-SEM.

In the field a small portion of tissue from each basidiome (one from UC 1860255 and one from UC 1860254) also was removed and stored in 300 µL 2× 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 to UC Berkeley and stored at −20 C until DNA extraction. DNA was extracted with the DNeasy Tissue Kit (QIAGEN Sciences, Valencia, California) with slight modifications as in Peay et al. (2010). The internal transcribed spacer (ITS 1 and 2) regions and a portion of the 28S large subunit (LSU) of the nuclear rDNA genes were amplified by PCR and sequenced in both directions with primer pairs ITS1F/ITS4 and LROR/TW13 respectively (White et al. 1990, Gardes and Bruns 1993) as described in Peay et al. (2010). All sequences were submitted to GenBank. To infer phylogenetic placement we aligned the LSU sequences that we generated for both basidiomes along with LSU sequences of *Spongiforma thailandica* Desjardin, Manf. Binder, Roekring & Flegel and allied boletoid clades (downloaded from GenBank) as identified in Desjardin et al. (2009) with the program MAFFT 6.814b (Katoh et al. 2002). The sequence alignment was submitted to TreeBASE (ID 11126). Phylogenetic trees were generated with 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, four heated chains, unconstrained branch lengths, exponential = 10, shape parameter = 10; Huelsenbeck and Ronquist 2001). All programs were implemented with the application Geneious Pro 5.1.7 (Biomatters, Auckland, New Zealand).

TAXONOMY

Spongiforma squarepantsii Desjardin, Peay and T.D. Bruns FIGS. 1–3

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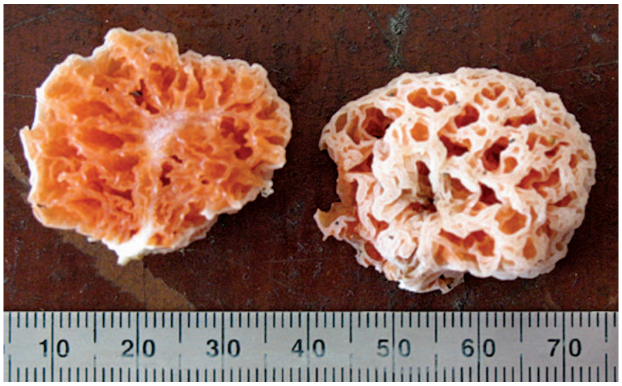

FIG. 1. Basidiome of *Spongiforma squarepantsii* (Holotype) in longitudinal section, showing the interior (left) and exterior (right) surfaces.

MycoBank MB519524

Basidiomata epigaea, cerebriformes, suaveolentes. Peridium nullum. Gleba loculis labyrinthiformibus 2–10 mm latus, aurantiaca; columella dendroidea, 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 nullae. 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

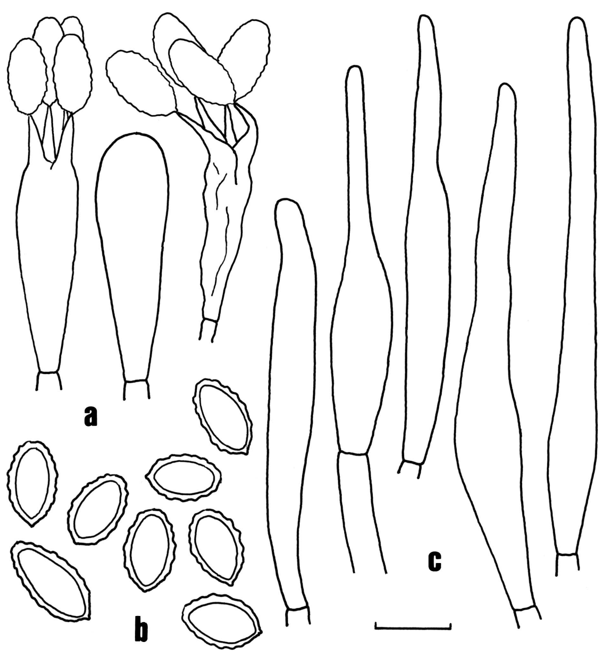

FIG. 2. Micromorphological features of *Spongiforma squarepantsii* (Holotype). A. Mature basidium, basidiole and collapsed basidium. B. Basidiospores, illustrating shape and wall thickness; surface ornamentation illustrated in FIG. 3. C. Cystidia from sterile ridges. Bar = 10 μm.

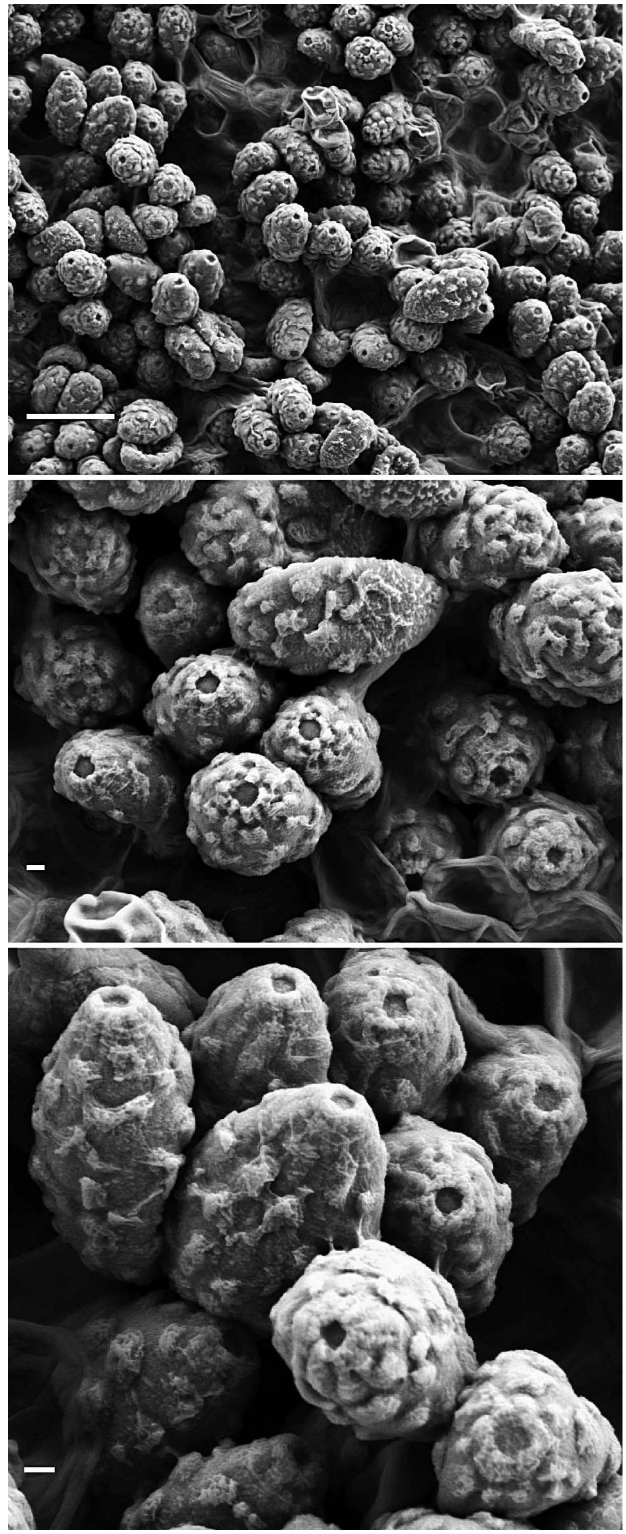

FIG. 3. Scanning electron micrographs of basidiospores of *Spongiforma squarepantsii* (Holotype). Scale bar: top = 10 μm; middle and bottom photos = 1 μm.

absent. Hymenophore composed of ridges or folds delimiting multiple, deep locules; locules 2–10 mm diam, irregular in outline, lined with a well developed hymenium, minutely ciliate; immature hymenium orange (6A4-8) to deep orange (7A5-8); sterile ridges ciliate, pale orange white (5-6A2-3) or paler. Spores (as observed on the hymenium of mature dried basidiomes) reddish brown to deep mahogany (8E7-8). Columella poorly developed, as a narrow, dendritic cord of tissue running longitudinally 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, intermixed with erect cystidia, hyaline, inamyloid, thin-walled. Cystidia (FIG. 2) 20–60(–73.5) × 4–9 µm, subcylindrical to fusoid or acuminate, hyaline, inamyloid, thin-walled, easily collapsed; abundant on the sterile locule edges and scattered among 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 septa, strongly gelatinous, hyaline, inamyloid, thin-walled. Subhymenium pseudoparenchymatous, composed of inflated to ovoid or vesiculose cells 10–24 × 8–20 µm, hyaline, inamyloid, thin-walled, non-gelatinous. Basidia (FIG. 2) statismosporic, 28–40 × 8–9.5 µm, clavate, four-spored with straight sterigmata up to 9.5 µm long; few seen because they collapsed soon after spore development with spores initially still attached to collapsed sterigmata. Basidioles clavate to subclavate. Basidiospores (FIGS. 2, 3) (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, broadest at proximal end, distal end rounded or subtruncate, with a small central apiculus, bilaterally symmetrical, coarsely verrucose and rusty brown in distilled water, finely roughened to pustulate and pale lilac gray in 3% KOH, coarsely verrucose and deep reddish brown, dextrinoid, 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 distribution: Solitary, epigeous on ground under undetermined dipterocarp trees, known only from the holotype locality in Lambir Hills National Park, northern Borneo, Malaysian state of Sarawak. Lambir is an aseasonal, tropical rainforest, receiving ca. 3000 mm rainfall per year, with maximum and minimum daily temperatures 32–24 C (Lee et al. 2002). The forest at Lambir contains > 1000 tree species but is dominated by the ECM family Dipterocarpaceae. Detailed descriptions of the neighboring plot are available in Lee et al. (2002) and Davies et al. (2005).

Specimens 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, 4°20′N, 113°50′E, 28 May 2008, collected by T.D. Bruns, *TDB 3541-B* (Holotype, UC 1860255); same location, 28 May 2008, collected by T.D. Bruns, *TDB 3541-A* (UC 1860254). Half of each specimen was deposited with the Sarawak Forest Department under permit NPW.907.4.4(III)-26 to K.G. Peay.

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 shape shares a strong resemblance to the new fungus. Moreover the hymenium when observed with scanning electron microscopy (FIG. 3) looks like a seafloor covered with tube sponges, reminiscent of the fictitious home of SpongeBob.

Commentary: Spongiforma squarepantsii is characterized by small, sponge-like and rubbery basidiomes that are externally pale orangish white and internally deep orange, with small deep locules lined with sporogenous tissue; the lack of a 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 gray and pustulate 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*, described recently from central Thailand (Desjardin et al. 2009), in forming smaller basidiomes with a deep orange gleba and a pleasant fruity-musty odor. In comparison *S. thailandica* forms basidiomes 50–100 mm broad × 40–70 mm tall, has a gleba that is pale grayish orange to brownish gray 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 to pustulate basidiospores (compare FIG. 3 herein with FIG. 2c, d of Desjardin et al. 2009). For a comparison of the genus *Spongiforma* to other boletoid genera see Desjardin et al. (2009). *Spongiforma* is unique among sequestrate boletoid genera in forming epigeous basidiomes that lack a peridium and have many exposed locules lined with statismosporic basidia.

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

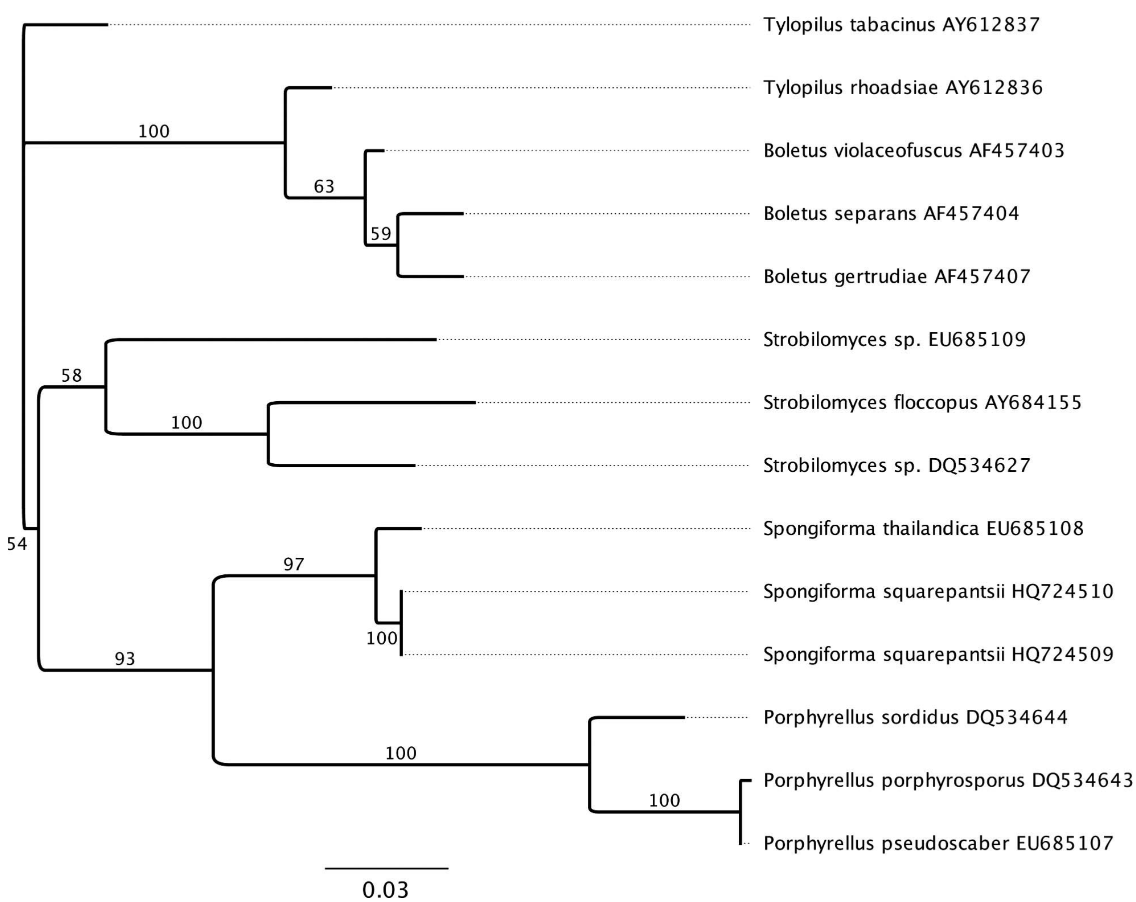

FIG. 4. Phylogenetic placement of *Spongiforma squarepantsii* inferred from nuclear LSU rDNA with maximum likelihood reconstruction. Numbers indicate node bootstrap support values.

the strikingly similar macro- and micromorphology 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 the tubulate bolete genus *Porphyrellus* (FIG. 4). The ITS sequence from the holotype specimen of *S. squarepantsii* matched that of the holotype specimen of *S. thailandica* at only 90%. 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 macromorphology, odor, basidiospore ornamentation, and in addition to the long distance separating the known localities of *S. squarepantsii* and *S. thailandica*, that leads us to conclude that the Borneo fungus is a distinct species. As was reported in the protolog of *S. thailandica* (Desjardin et al. 2009), ITS sequences of *Spongiforma* are highly divergent from those of the sister taxa *Porphyrellus* and *Strobilomyces* (as determined from LSU analyses) and we were unable to align the ITS sequences of *S. squarepantsii* and *S. thailandica* to those of *Porphyrellus* and *Strobilomyces* or to any other Boletaceae. Consequently an ITS phylogeny is not presented.

The occurrence of *Spongiforma* with dipterocarps in mainland southeastern 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 ballistosporic discharge and presence of distinctive odors of the two species suggests that animal dispersal is likely, and animal dispersal in combination with island populations likely would limit gene flow much as it does with *Rhizopogon* species (Grubisha et al. 2007). This may explain the high ITS divergence between the two species and leads us to predict that other isolated tracts of dipterocarp forests might harbor additional species in the genus.

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