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Borofutus, a new genus of *Boletaceae* from tropical Asia: phylogeny, morphology and taxonomy

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Abstract A new monotypic genus in the *Boletaceae*, *Borofutus*, typified by *B. dhakanus,* is described using morphological and molecular evidence. This is a putatively ectomycorrhizal fungus associated with *Shorea robusta*. *Borofutus* is characterized by the combination of the following characters: basidiomata small to medium-sized; pileus grayish brown to cocoa brown; hymenophore subdecurrent, cream then golden brown, with broad, nearly hexagonal pores; basidiospores purple to purplish red in H_2O, ornamented with irregular to regular shallow pits; cystidia lageniform, thick-walled. *Borofutus* is sister to *Spongiforma* in molecular phylogenetic analyses using DNA nucleotide sequences of single or multiple loci. A description, line drawings, phylogenetic placement and comparison with allied taxa are presented herein.

Keywords Boletes · *Dipterocarpaceae* · Multiple gene analysis · New fungal taxon

Introduction

Fungi in the *Boletaceae* have been widely studied by fungal taxonomists and molecular mycologists (Watling 1970; Smith and Thiers 1971; Besl and Bresinsky 1997; Binder and Hibbett 2007). However, research on samples collected from tropical regions, such as tropical Southeast Asia, South America, and Africa is limited (Corner 1972 in Malaysia; Heinemann and Rammeloo 1987a, b in Africa; Wolfe and Bougher 1993 in Australia; Halling and Mueller 2003 in Costa Rica; Ortiz-Santana et al. 2007 in the Caribbean region; Halling and Fechner 2011 in Australia). Recently, three new genera of *Boletaceae*, *Durianella* Desjardin et al., *Spongiforma* Desjardin et al., and *Corneroboletus* N. K. Zeng & Zhu L. Yang were described from tropical Southeast Asia (Desjardin et al. 2008, 2009; Zeng et al. 2012). Forests in this region are often dominated by *Shorea robusta*, a tree which plays an important role as ectomycorrhizal partner of boletes and other groups of fungi. During the last four monsoons (2009–2012), a broad survey of mushrooms was conducted in Bangladesh and several boletes were collected. One of the bolete species belongs to the porcini mushroom "*Alloboletus*" based on DNA sequence data (Feng et al. 2012). Recently, a bolete associated with *S. robusta* was collected and carefully examined. The broad-pores with subdecurrent hymenophore indicate that this bolete represents a new genus in the *Boletaceae*.

In this study, we used morphological data together with DNA nucleotide sequence analysis of multiple loci including the 5.8S region of ITS, the large subunit nuclear ribosomal RNA (nrLSU), translation elongation factor 1-alpha (*tef1*-α), the largest subunit of RNA polymerase II (*rpb1*) and the second largest subunit of RNA polymerase II (*rpb2*). Both morphological and molecular phylogenetic analyses are indispensable for understanding the taxonomic and phylogenetic relationships among the genera within the *Boletaceae* (Yang 2011). This work addresses the following two objectives: (i) to compare the morphological characters among *Borofutus* and the related genera, and (ii) to assess the phylogenetic position of *Borofutus*.

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Materials and methods

Sampling

Materials were collected by the first author from Bhawal National Park, Gazipur, Dhaka, Bangladesh during 2009–

2012 in forests dominated by *S. robusta*. Specimens examined are deposited in the Herbarium of Kunming Institute of Botany (KUN-HKAS) of the Chinese Academy of Sciences, China and two duplicates as SHAF 1 and SHAF 2 are kept in SAU Herbarium of Agarics Flora (SHAF), Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

Morphological studies

The macro-morphological features were described based on detailed field notes made from fresh basidiomata, and documented by photographs. Color codes are according to Kornerup and Wanscher (1981). Microscopic structures were revived in 5 % KOH. Radial-vertical sections of the pileipellis and longitudinal sections of the stipitipellis were made halfway the pileus and the stipe, respectively. All microscopic features were drawn by free hand. The notations '(n/m/p)' indicate that the measurements were made on 'n' basidiospores from 'm' basidiomata of 'p' collections with a minimum of 20 basidiospores per basidioma. Dimensions of basidiospores are presented in the following form (a) b–c (d); in which 'b–c' contains a minimum value of 90 % and extreme values 'a' and 'd' are kept in parentheses. Q = length/width ratio derived from each basidiospore measured; Q_m=Q ±SD, where Q is the average of basidiospores measurement and SD is the standard deviation. Basidiospores were also observed using a scanning electron microscope (SEM). Tiny pieces of hymenophoral fragments dried by silica gel were mounted on aluminum SEM stubs with double-sided adhesive tape. The samples were coated with gold palladium (thickness 10 nm) and provided 8,600 nA current flow at 10 s to enhance conductivity. Images were obtained via a SEM (HITACHI S-4800) using a working distance of 7,700 μm and accelerating voltage of 10.0 kV.

Molecular studies

DNA extraction and PCR amplification

Genomic DNA was extracted from silica-gel dried or herbarium materials using a modified Cetyltrimethylammonium bromide (CTAB) procedure of Doyle and Doyle (1987). ITS1/ITS4 (White et al. 1990), LROR/LR5 (Vilgalys and Hester 1990), *ef1*-983F/*ef1*-1567R (Rehner and Buckley 2005), *rpb1*-Af/*rpb1*-Cr (Dentinger et al. 2010) and *rpb2*-6F/*rpb2*-7.1R (Matheny 2005) were used for the amplifications of ITS, nrLSU, *tef1*-α, *rpb1* and *rpb2* fragments, respectively. In addition, we used the three primers pair *ef1*-BF/*ef1*-BR, *rpb1*-BF/*rpb1*-BR and *rpb2*-BF/*rpb2*-BR, newly designed by G. Wu (unpublished data), to amplify *tef1*-α, *rpb1* and *rpb2* fragments. PCR mixtures contained 1 μL of both forward and reverse primer, 2.5 μL PCR reaction buffer, 2.5 μL dNTP, 0.3 μL Taq polymerase, 1 μL BSA, and 1.5 μL DNA template. The final volume was adjusted to 25 μL by adding double distilled water (ddH_2O). PCR reactions were conducted on an ABI 2720 Thermal Cycler (Applied Biosystems, USA) and the reaction conditions were as follows: pre-denaturation at 94 °C for 5 min, then followed by 35 cycles of denaturation at 94 °C for 40s (ITS), 60s for (nrLSU, *rpb1*, *rpb*2 and *tef1*-α); annealing at 50 °C (ITS) for 40s, 53 °C (nrLSU, *rpb1*, *rpb*2 and *tef1*-α) for 60s; elongation at 72 °C (ITS) for 40s, 80s for (nrLSU, *rpb1*, *rpb*2 and *tef1*-α); a final elongation at 72 °C for 8 min was included after the cycles. PCR products were purified with a Gel Extraction & PCR Purification Combo Kit (Spin-column) (Bioteke, Beijing, China) and then sequenced on an ABI-3730-XL sequence analyzer (Applied Biosystems, USA) using the same primers as in the original PCR amplifications.

DNA sequence alignments and dataset assembly

The nrLSU nucleotide sequences from *Borofutus* are compared with those submitted to GenBank database and a single 94 % match with the nrLSU sequence of *Spongiforma thailandica* (DED 7873) was identified. This level is consistent with other congeneric comparisons in the *Boletales* (Binder and Hibbett 2007). Subsequently, the nrLSU sequences of representatives of *Boletaceae* were downloaded from GenBank and combined with our own dataset. The sequences were aligned with MAFFT v6.8 (Katoh et al. 2005) and manually optimized on BioEdit v7.0.9 (Hall 1999) using default settings. To understand the relationships of our samples with the remaining genera in *Boletaceae*, 5.8S, *tef1*-α, *rpb1* and *rpb2* sequences of *Boletaceae* were also retrieved from GenBank and combined with selected nrLSU sequences. Procedures followed for the alignment and optimization of this dataset were identical to those of the nrLSU dataset.

In total 47 sequences were newly generated for this study and deposited in GenBank including nine sequences each for ITS and *tef1*-α, twelve for nrLSU, ten for *rpb1* and seven for *rpb2* (Table 1). The nrLSU and the combined datasets were complemented with selected published sequences (Binder and Fischer 1997; Bresinsky et al. 1999; Binder and Besl 2000; Binder and Bresinsky 2002a, b; Grubisha et al. 2002; Peintner et al. 2003; Leonardi et al. 2005; Matheny et al. 2006; Binder and Hibbett 2007; Halling et al. 2007; Matheny et al. 2007; Drehmel et al. 2008; Porter et al. 2008; Desjardin et al. 2009, 2011; Binder et al. 2010; Dentinger et al. 2010; Li et al. 2011; Lebel et al. 2012; Neves et al. 2012) and those from GenBank. To assemble the combined dataset, nrLSU, 5.8S, *tef1*-α, *rpb1* and *rpb2* sequences used in Binder et al. 2010 were retrieved and combined with our data. The resulting five alignments (nrLSU, 5.8S, *tef1*-α, *rpb1* and *rpb2*) were then concatenated using Phyutility (Smith and Dunn 2008) for further phylogenetic analysis.

Table 1 Specimens used in phylogenetic analyses and their GenBank accession numbers

Species Name	Isolate/Voucher/strain	Locality	GenBank Accessions				
			ITS, 5.8S	nrLSU	*tef1*-α	*rpb1*	*rpb2*
Aureoboletus auriporus	BDCR0431	Costa Rica		HQ161871			
Aureoboletus gentilis	Pug1	Germany		DQ534635			
Aureoboletus thibetanus	AFTOL-450	China	DQ200917	AY700189	DQ029199	DQ435800	DQ366279
Austroboletus dictyotus	HKAS 59804	China	–	**JX901138**	–	–	–
Austroboletus fusisporus	HKAS 75207	China	**JX889719**	**JX889720**	**JX889718**	**JX889721**	–
Austroboletus gracilis	112/96	USA		DQ534624			
Austroboletus niveus	312	New Zealand		DQ534622			
Boletellus ananas	TH6264	–		AY612799			
Boletellus exiguus	TH8809	Guyana		HQ161862			
Boletellus projectellus	AFTOL-713	–	AY789082	AY684158	AY879116	AY788850	AY787218
Boletellus shichianus	AFTOL-532	China	DQ200921	AY647211	DQ408145	–	DQ366280
Boletinellus merulioides	AFTOL-575	–	DQ200922	AY684153	DQ056287	DQ435803	DQ366281
Boletus edulis	Be3*	Germany	AY680988	AF456816	GU187682	GU187444	GU187774
Boletus inedulis	NCJ14	–		AY612803			
Borofutus dhakanus	HKAS 73785	Bangladesh	**JQ928605**	**JQ928615**	**JQ928577**	**JQ928585**	**JQ928596**
Borofutus dhakanus	HKAS 73789	Bangladesh	**JQ928606**	**JQ928616**	**JQ928576**	**JQ928586**	**JQ928597**
Borofutus dhakanus	HKAS 73792	Bangladesh	**JQ928607**	**JQ928617**	**JQ928575**	**JQ928587**	**JQ928598**
Bothia castanella	MB03-067	USA		DQ867115			
Bothia castanella	87/98	USA		DQ534660			
Calostoma cinnabarinum	AFTOL-439	–	AY854064	AY645054	AY879117	AY857979	AY780939
Chalciporus aff. *piperatus*	HKAS 50214	China	**JQ928610**	**JQ928621**	–	**JQ928594**	–
Chalciporus piperatus	MB 04-001*	USA	AF074922	DQ534648	GU187690	GU187453	–
Gomphidius roseus	AFTOL-1780	Germany	DQ534570	DQ534669	GU187702	GU187459	GU187818
Gyrodon lividus	REG G11	Germany	DQ534568	AF098378	GU187461	GU187786	GU187701
Heimioporus betula	DD9852			AY612797			
Heimioporus retisporus	MS6			AF050650			
Leccinellum sp.	HKAS 50221	China	**JQ928612**	**JQ928624**	**JQ928583**	**JQ928593**	–
Leccinum aff. *aurantiacum*	HKAS 57390	China	**JQ928611**	**JQ928625**	**JQ928581**	**JQ928591**	**JQ928602**
Leccinum aurantiacum				AF139689			
Octaviania asterosperma	Octa1	Germany		DQ534619			
Paragyrodon sphaerosporus	MB06-066	USA	GU187540	GU187593	GU187737	–	GU187803
Paxillus vernalis	AFTOL-715	China	DQ647827	AY645059	DQ457629	–	–
Phlebopus portentosus	HKAS 52855	China		**JQ928622**			
Phlebopus portentosus	REG Php1	–	DQ534569	AF336260	GU187735	GU187476	GU187801
Phylloporus bellus	MCA559	–		AY612817			
Phylloporus pelletieri	Pp1	Germany		AF456818			
Phylloporus pumilus	REH8062	Indonesia		JQ003681			
Pisolithus arrhizus	REG 588	USA	GU187538	AF336262	–	GU187473	GU187798
Porphyrellus porphyroporus	AFTOL-1779	Germany	DQ534563	DQ534643	GU187734	GU187475	GU187800
Porphyrellus sordidus	148/98	USA		DQ534644			
Retiboletus aff. *griseus*	HKAS 59460	China	**JQ928613**	**JQ928626**	**JQ928580**	**JQ928590**	**JQ928601**
Retiboletus aff. *nigerrimus*	HKAS 59699	China	–	**JQ928627**	**JQ928582**	**JQ928592**	**JQ928603**
Retiboletus ornatipes	215/97	–		AF456805			
Retiboletus retipes	96/97	–		AF456830			
Rhizopogon nigrescens	MB 06-070	USA	–	GU187594	GU187744	GU187478	GU187806
Rossbeevera griseovelutina	TNS-F-36992	Japan		HQ693882			
Royoungia boletoides	ACW 4137	Australia		DQ534663			

Table 1 (continued)

Species Name	Isolate/Voucher/strain	Locality	GenBank Accessions				
			ITS, 5.8S	nrLSU	*tef1*-α	*rpb1*	*rpb2*
Spongiforma squarepantsii	LHFB14	Malaysia	HQ724511	HQ724509	–	–	–
Spongiforma thailandica	DED7873	Thailand	EU685113	EU685108	–	–	–
Strobilomyces floccopus	AFTOL-ID 716	–	AY854068	AY684155	AY883428	AY858963	AY786065
Strobilomyces sp.	RH4514	Australia		EU685109			
Suillus spraguei	AFTOL-717	–	AY854069	AY684154	AY883429	AY858965	AY786066
Tylopilus felleus	TM03_453	Canada		EU522827			
Xanthoconium affine var. *maculosum*	BD217	USA		HQ161854			
Xanthoconium purpureum	BD228	USA		HQ161864			
Xerocomus chrysenteron	IB19990951	–		AF514808			
Xerocomus subtomentosus	Xs1	Genmany		AF139716			
Zangia olivacea	HKAS 45445	China		HQ326945			
Zangia roseola	HKAS 52661	China	**JQ928614**	**JQ928623**	**JQ928584**	**JQ928595**	**JQ928604**

Accessions numbers in boldface indicate newly generated sequences. An asterisk (*) at the isolate number emphasizes that the sequences were represented by three strains for *Boletus edulis* and two strains for *Chalciporus piperatus*. The remaining ones were from previously selected published sequences and from GenBank

Unfortunately, we were unable to generate *tef1*-α, *rpb1* and *rpb2* sequences from *Spongiforma thailandica* using currently available and newly designed primers. Consequently, we treated the sequences of *tef1*-α, *rpb1* and *rpb2* for *Spongiforma* as missing data in the matrix for the combined analysis as done by Binder et al. (2010) and Li et al. (2011). The five gene dataset and the single gene dataset of the nrLSU have been deposited in TreeBASE (S13338, http://purl.org/phylo/treebase/phylows/study/TB2:S13338).

Phylogenetic analyses

Molecular phylogenetic analyses were conducted based on two non-protein coding (5.8S including ITS and nrLSU) and three protein coding genes (*tef1*-α, *rpb1* and *rpb2*). Two datasets were analyzed, one combining five genes and the other containing only nrLSU sequences. The two datasets were analyzed using RAxML v7.2.6 (Stamatakis 2006), MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) and PAUP* 4.0b10 (Swofford 2002) for Maximum Likelihood (ML), Bayesian Inference (BI) and Maximum Parsimony (MP) methods, respectively. For both BI and ML analyses of the combined dataset, a partitioned mixed model was selected by setting sequences of five gene markers as different partitions. The substitution model suitable for the nrLSU dataset and the five gene partitions of the combined dataset were determined using the Akaike Information Criterion (AIC) complemented in MrModeltest v2.3 (Nylander 2004). The chosen model for the nrLSU dataset was GTR+I+G, while models for the five partitions of the combined dataset were GTR+I+G for 5.8S, nrLSU and *tef1*-α; HKY+I+G for *rpb1* and *rpb2*. As RAxML only supports the GTR model, we used GTRGAMMAI for all partitions for our analysis based on the combined dataset while using ML algorithm. Default settings were used for all parameters in the ML analysis and statistical support values were obtained using nonparametric bootstrapping with 1,000 replicates. Bayesian analyses with four chains were conducted by setting generations to four million for nrLSU and two million for the combined dataset and using the stoprul command with the value of stopval set to 0.01. Chain convergence was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure sufficiently large ESS values (>200). Burn-ins were then determined by checking the –lnL trace plots in Tracer. Posterior probabilities were calculated using the sumt command implemented in MrBayes. Maximum parsimony analysis was estimated in PAUP* 4.0b10 followed those of Li et al. (2011). Gaps in the alignment were treated as missing data in both phylogenetic analyses. *Suillus spraguei* (Berk. & M. A. Crutis) Kuntze was selected as the outgroup for both the nrLSU and multilocus (5.8S, nrLSU, *tef1*-α, *rpb1* and *rpb2*) datasets.

Results

Molecular studies

The nrLSU dataset included 55 nrLSU sequences and the alignment contained 895 nucleotide sites (including gaps). In this alignment, 500 characters were constant, while 395 characters were variable, of which 295 characters were parsimony informative. The combined dataset consisted of 156,

891, 1,137, 1,297 and 1,268 nucleotide sites (including gaps) for 5.8S, nrLSU, *tef1*-α, *rpb1* and *rpb2*, respectively. In this alignment, 2,628 characters were constant, while 2,121 characters were variable, of which 1,677 were parsimony informative. For both datasets, phylogenetic trees generated from ML, MP and BI analyses were nearly identical with minimal variation in statistical support values. Phylogenetic trees generated from both nrLSU and the combined datasets showed that *Borofutus* formed independent clade in the family *Boletaceae* and were clearly divided by genetic distance and clustered with the gasteroid bolete *Spongiforma*. The results of the analyses are summarized in Figs. 1 and 2.

Taxonomy

Borofutus Hosen & Zhu L. Yang, **gen. nov**.
MycoBank: MB 800166
Etymology: *Borofutus* is from Bengali language, where 'boro' means large and 'futo' means pore, referring to the broad pores of the hymenophore.

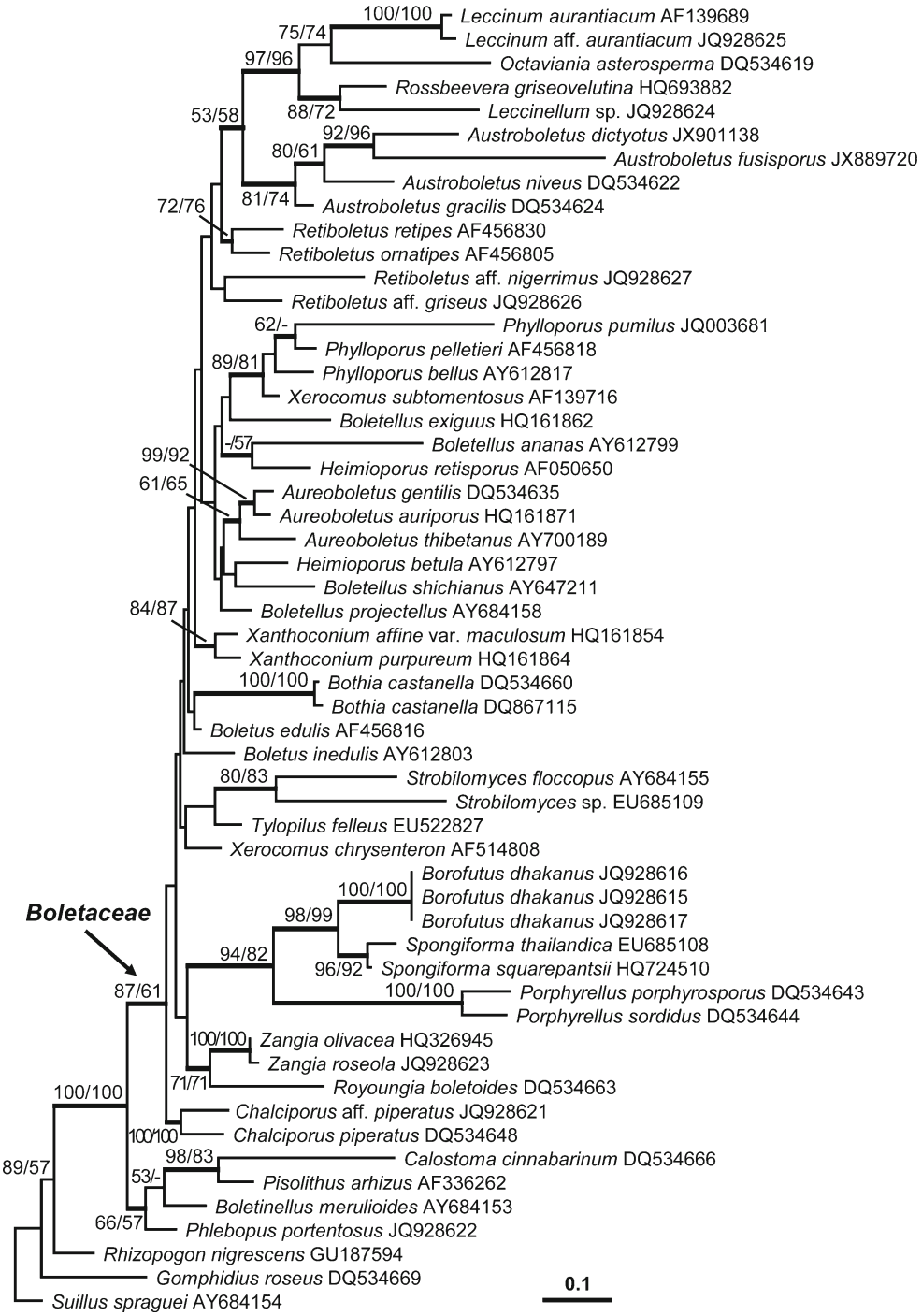

Fig. 1 Phylogenetic tree generated from nrLSU dataset of 55 sequences in the *Boletales* using ML method. Posterior probabilities from Bayesian inference (BI) (>0.94) are indicated as thick branches and bootstrap values derived from ML and MP analyses (BS> 50 %) are shown above or beneath the branches at nodes. Parsimony analysis resulted in two most parsimonious trees of 1,726 steps, with Consistency Index (CI)=0.314, Retention Index (RI)=0.506 and Rescaled Consistency Index (RC)= 0.182. GenBank accession number for each sequence is provided behind the species name

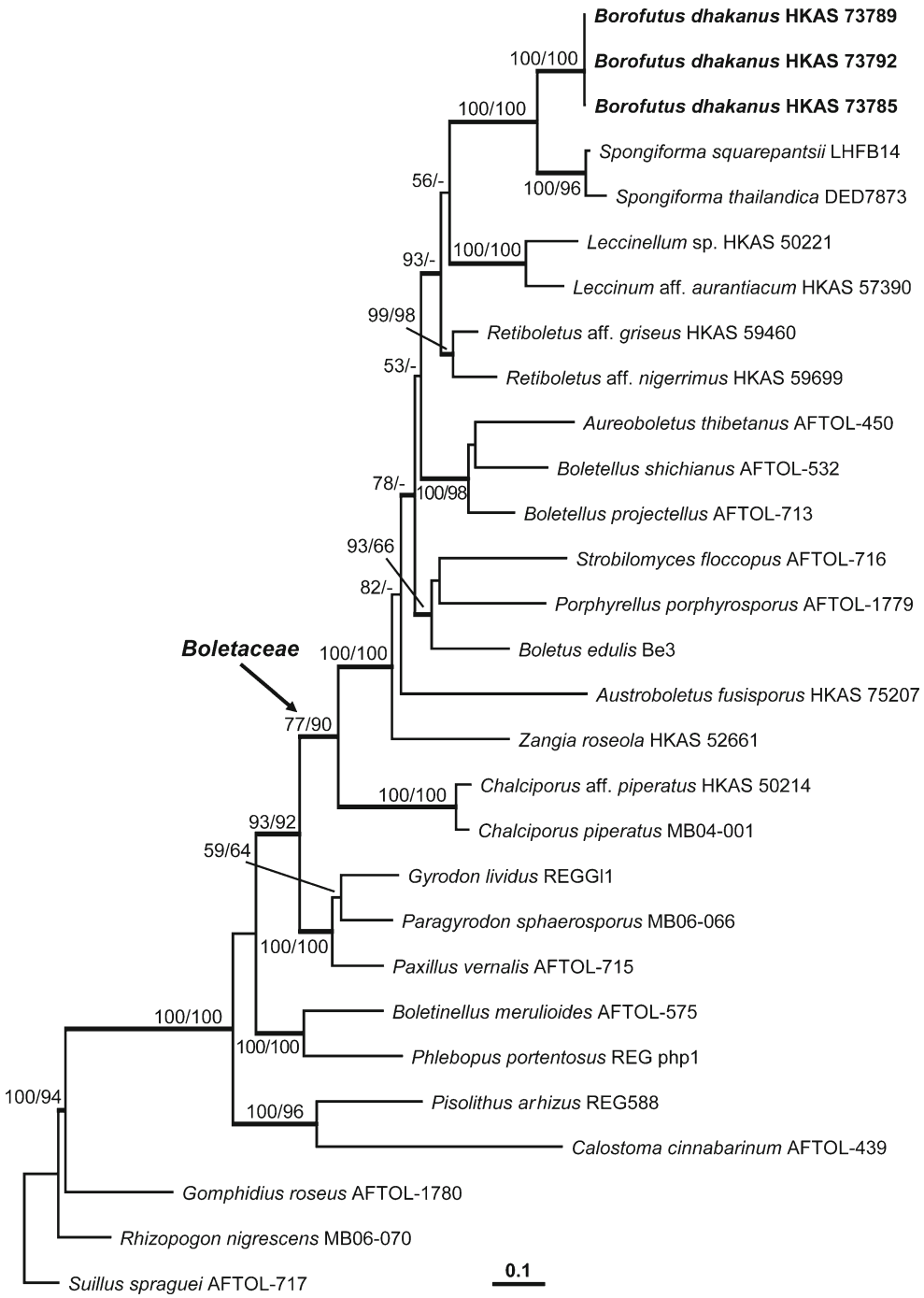

Fig. 2 Phylogenetic tree generated from the combined 5.8S, nrLSU, *tef1-α*, *rpb1* and *rpb2* dataset using ML method. Posterior probabilities from BI (>0.98) are indicated as thick branches, and bootstrap values derived from ML and MP analyses (BS>50 %) are shown above or beneath the branches at nodes. Parsimony analysis resulted in two most parsimonious trees of 7,140 steps, with Consistency Index (CI)=0.445, Retention Index (RI)=0.488 and Rescaled Consistency Index (RC)=0.239. *Borofutus dhakanus* is highlighted in boldface. Herbarium voucher or isolate number is provided behind the species name

Basidiomata epigeous, stipitate-pileate with tubular hymenophore. Pileus covered with squamules. Hymenophore subdecurrent, broadly tubular; pores up to 2–6 mm wide, pallid to cream when young, becoming yellowish to golden brown at maturity. Stipe central, covered with squamules but apical part glabrous, upper half ribbed by the subdecurrent lines of the hymenophore or confined to apex; basal mycelium whitish. Context pallid to light yellowish, usually unchanging in color when cut but turning pale reddish to pale reddish purple in some areas over the course of 1–2 h. Basidiospores purple to purplish red in H_2O, purplish violet in 5 % KOH, boletoid to somewhat amygdaliform, slightly thick-walled; minutely verrucose under light microscope but with regular to irregular shallow pits under SEM. Cheilocystidia and pleurocystidia lageniform, thick-walled. Pileipellis a trichoderm, becoming a subcutis when mature. Clamp connections absent.

Typus generis: *Borofutus dhakanus* Hosen & Zhu L. Yang

Borofutus dhakanus Hosen & Zhu L. Yang, **sp. nov.** Figures 3, 4a–d, 5

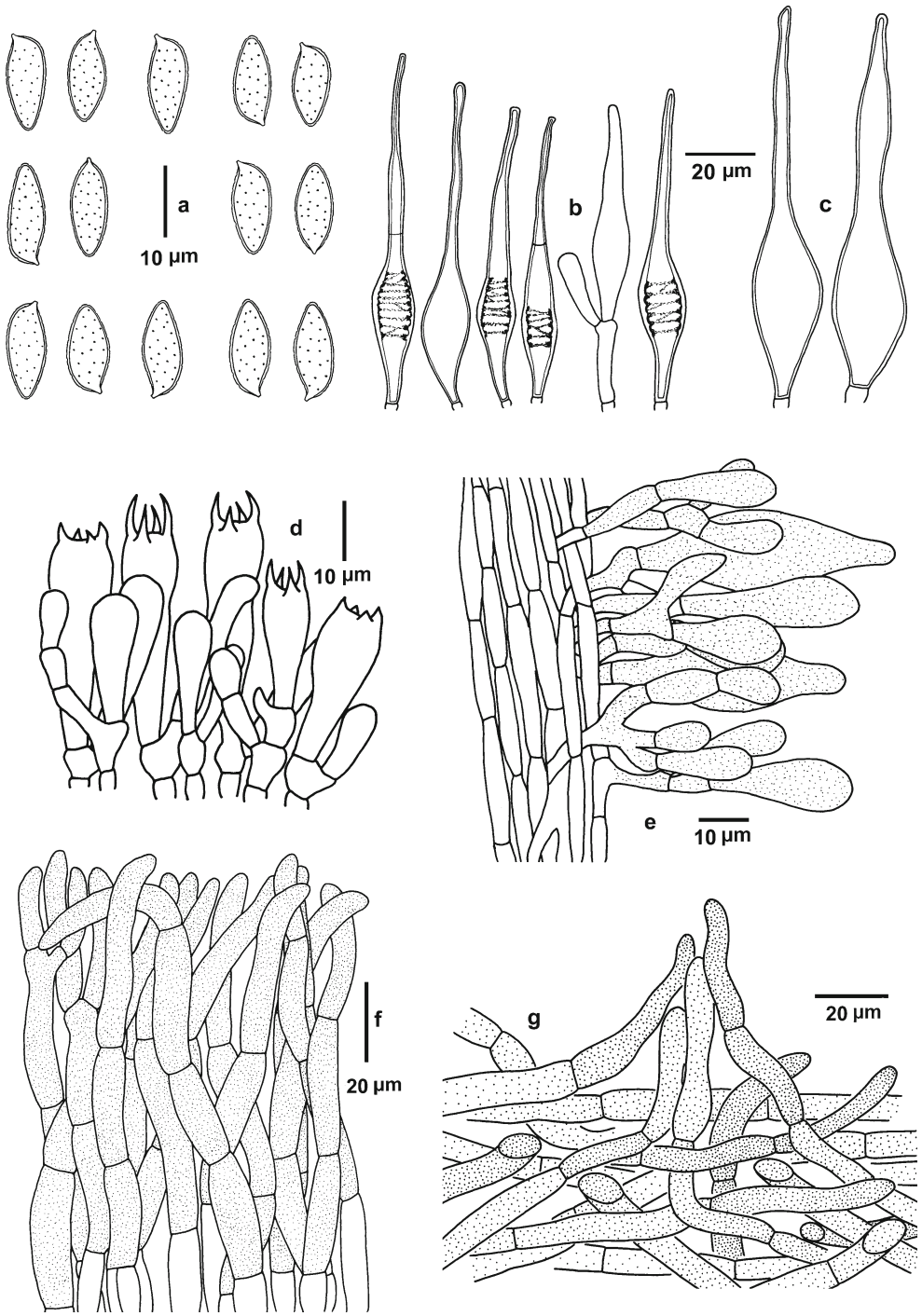

Fig. 3 Microscopic features of *Borofutus dhakanus* **a** Basidiospores. **b** Cheilocystidia. **c** Pleurocystidia. **d** Basidia with basidioles. **e** Surface of stipe in longitudinal section showing caulocystidia. **f–g** Pileipellis. **f** Trichoderm pileipellis from young basidiome. **g** Subcutis-like pileipellis from mature basidiome. (**a**, **c**, **d**, **f** and **g** from holotype, HKAS 73785; **b** from HKAS 73793; **e** from HKAS73784)

MycoBank: MB 800167

Etymology: *dhakanus* refers to the name of type locality (Dhaka).

Holotypus: BANGLADESH, Dhaka division, Gazipur, Bhawal National Park, 24°45′N 90°50′E, 20 m, 6 July 2011, M. I. Hosen 176 (HKAS 73785).

Basidiomata small to medium-sized. *Pileus* 30–65 mm, convex when young, becoming plane with age, covered with light brown (6D8) to cocoa brown (6F7), grayish brown (6F3–4) to dark grayish brown (6F6–7) squamules, which become grayish black (6F1) at maturity; center sometimes umbilicate, depressed, not glabrous, becoming rimose with age, dry, somewhat slightly tacky when wet, margin occasionally uplifted. *Hymenophore* tubular, subdecurrent, pallid to creamy to yellowish (4A1–2), turning pale reddish to pale reddish purple on exposure to air for long time when young, becoming light brown (6D6–8) to golden brown (5D7–8) at maturity; pores 5–11 mm depth, 4–10 mm length and 2–6 mm wide, sometimes double-pored, mostly hexagonal, relatively broader

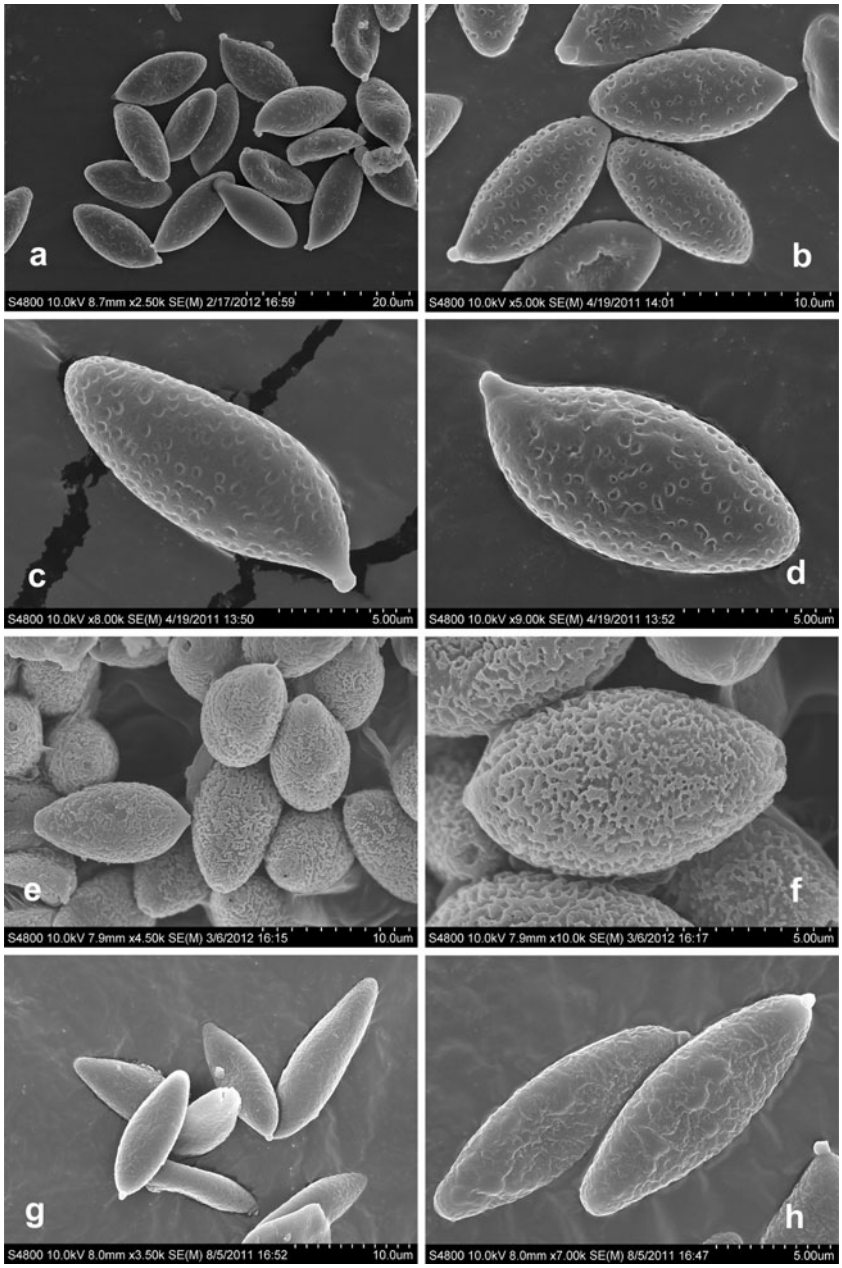

Fig. 4 SEM of basidiospores is from dried specimens of *Borofutus dhakanus*, *Spongiforma thailandica* and *Austroboletus tristis* **a–d** *Borofutus dhakanus* spores with scattered shallow pits. Note: **a** SEM of basidiospores dipped in 5 % KOH for 2 min remaining its natural behavior. **e–f** *Spongiforma thailandica* basidiospores with verrucose surface, subtruncated apex with pore. **g–h** *Austroboletus tristis* basidiospores with complex roughened surface of basidiospores. (**a** from holotype, HKAS 73785; **c–d** from HKAS 73777; **e–f** from isotype, DED 7873; **g-h** from holotype, FH, Sheet 3712)

towards the center then gradually narrower towards the margin, with reddish powdery mass inside the tubes when aged. *Stipe* 25–40×6–12 mm, central, cylindrical, narrowly tapering upwards, occasionally slightly swollen to the base, mostly curved, covered with purplish to grayish to cocoa brown squamules; apex glabrous, whitish to creamy to yellowish; upper half ribbed by the subdecurrent lines of the hymenophore; basal mycelium whitish. *Context* 5–15 mm thick in the center of the pileus, solid, yellowish to creamy, usually unchanging when bruised but in some specimens turn pale reddish to pale reddish purple when exposed to air for long time. *Taste* unknown and *odor* mild.

Basidiospores [320/16/16] (10)11–13(14)×(4.5)5–6(6.5) μm, [Q=(1.97)2.08–2.52(2.92), Q_m=2.29±0.16], boletoid to somewhat amygdaliform, slightly thick-walled (0.7 μm), brown-violet to purple to purplish red (11C6–8, 11D6–8) in H_2O, purplish violet (11E5–8, 11F5–8) in 5 % KOH; surface finely verrucose under light microscope (LM), but ornamented with irregular to regular, conspicuous shallow pits under SEM. *Basidia* (20) 31–33(39)×(7) 9–10(12) μm, narrowly clavate to clavate, hyaline to pale yellowish in H_2O and glycerin, thin-walled, tetrasporic, occasionally 2- or 3-spored, bearing relatively long sterigmata (5.0–8.5 μm long). *Hymenophoral trama* 110–170 μm wide, bilateral; hyphae cylindrical, hyaline, 7–20 μm wide. *Cheilocystidia* abundant, (45)70–80(100)×7–14 μm, narrowly lageniform to lageniform to broadly lageniform, sometimes narrowly utriform to mucronate, rostrate, slightly thick-walled (0.6–1.2 μm thick), with an attenuate

Fig. 5 *Borofutus dhakanus* in its natural habitat **a–b** Immature and mature basidiomata with their broad-pored hymenophoral surface. **c** Small group of basidiomata with their pileus surface. **d** Unchanged context (image taken immediately after sectioning). **e** Hymenophoral surface slightly turning pale reddish in some areas over the course of 60–120 min. (**a–b** from holotype, HKAS 73785; **c–e** from SHAF 2)

appendage, sometimes with a secondary septum; inner surface often with brown to greenish brown pigmented ornamentations. *Pleurocystidia* 85–105×12–20 μm, thin-walled to slightly thick-walled (up to 0.5 μm thick), scattered, narrowly lageniform to broadly lageniform with an appendage-like apex, without encrustation. *Pileipellis* a trichoderm when young, becoming a subcutis at maturity, composed of 4–5 long cylindrical cells; terminal cells 25–60(−105)×5–10(−13) μm, with brown to chocolate brown vacuolar pigments. *Stipe trama* composed of vertically arranged hyphae. *Stipitipellis* a sterile hymenium-like structure, composed of subclavate to clavate to fusiform cells with projecting cystidia (40–50×8–15 μm) with yellowish brown to pale brown vacuolar pigmentation. *Clamp connections* absent in all tissues.

Habit and habitat: Mostly solitary or often in small groups, and usually found growing in clayey soil rich in Fe in pure stands of *Shorea robusta*.

Known distribution: Currently known only from Bhawal National Park, Gazipur, Dhaka division, Bangladesh at 20 m elevation.

Specimens examined

Borofutus dhakanus: BANGLADESH, Dhaka Division: Gazipur, Bhawal National Park, latitude 24°45′N 90°50′E, altitude 20 m, 17 October 2009, M. I. Hosen 465, 467 and 468 (HKAS 73778, 73779 and 737780, respectively); same location, 24 October 2009, M. I. Hosen 508 and 523 (HKAS 73793 and 73777, respectively); same location, 5 July 2011, M. I. Hosen 158 (HKAS 73781), M. I. Hosen 160 (HKAS 73782 and SHAF 1); same location, 6 July 2011, M. I. Hosen 169 and 171 (HKAS 73783 and 73784, respectively); same location, 6 July 2011, M. I. Hosen 176 (HKAS 73785, **holotype**); same location, 7 July 2011, M. I. Hosen 180 and 189 (HKAS 73786 and HKAS 73787, respectively); same location, 8 July 2011, M. I. Hosen 198 (HKAS 73789); same location, 29 July 2011, M. I. Hosen 297 (HKAS 73791) and same location, 30 July 2011 M. I. Hosen 306 (HKAS 73792); same location, 8 August 2012, M. I. Hosen 583 (HKAS 75444 and SHAF 2).

Austroboletus tristis: SINGAPORE, Botanical Garden, August 1917, C.F. Baker 4995 (Sheet 3712, **holotype** FH).

Spongiforma thailandica: THAILAND, Nakorn Nayok Province, Khao Yai National Park, Princes Trail ca 2 km from Visitor Center, N1426.142, E10123.080, altitude 750 m, 7 July 2005, D.E. Desjardin (DED 7873, **isotype** SFSU).

Discussion

Results drawn from phylogenetic analyses infer that *Borofutus* is sister to the gasteroid bolete *Spongiforma*. In the nrLSU analysis, the sister group relationship of *Borofutus* and *Porphyrellus* E. J. Gilbert was supported with 94 % ML BS, 82 % MP BS and PP=1.0 respectively, (Fig. 1). In the multilocus analysis, *Borofutus* and *Spongiforma* were also clustered within a clade with high statistical support values (100 % ML BS, 100 % MP BS and PP=1.0) but their relationships to other boletes remain unclear. Although *Porphyrellus porphyrosporus* was clustered with *Borofutus* in the tree based on the nrLSU dataset

(Fig. 1), they were separated from each other in the analysis of the combined dataset (Fig. 2). The incongruent results from different datasets have also been reported previously, and it is hypothesized that protein coding genes, such as *rpb1* can provide more phylogenetic information than the non-protein coding genes (Dentinger et al. 2010). The phylogenetic position of *Borofutus* is not well resolved based on the nrLSU analysis, but it clustered with *Spongiforma* in all of our analyses even though sequence data was lacking for *Spongiforma* for the three protein coding genes (*tef1*-α, *rpb1* and *rpb2*) in the multigene analysis.

Within the genus *Spongiforma* two species, *S. thailandica* and *S. squarepantsii*, were described (Desjardin et al. 2009, 2011). Both species have basidiospores turning violet gray in 3 % KOH. Although they are also associated with *Dipterocarpaceae*, they form sessile, sponge-like basidiomata producing reddish brown basidiospores with a subtruncated distal end with a tiny hole at the apex (Fig. 4e–f). The trichodermal pileipellis with cylindrical terminal cells in *Borofutus* is somewhat similar to those of some *Porphyrellus* species. However, species of *Porphyrellus* have reddish brown, smooth basidiospores, and an ixotrichoderm to subhymeniform pileipellis (Thiers 1975; Wolf 1979; Singer 1986).

The morphology and the chemical reaction of basidiospores in KOH of *Borofutus* resemble those of a few taxa of *Austroboletus* (Corner) Wolfe, viz. *A. longipes* var. *longipes* (Massee) Wolfe and R. H. Petersen, *A. longipes* var. *albus* (Corner) E. Horak (Corner 1972; Wolfe and Petersen 1978; Wolfe 1979; Horak 2011), and *A. tristis* (Pat. & Baker) Wolfe, which was regarded as a possible synonym of *A. longipes* var. *longipes* by Corner (1972) and Horak (2011). We have included *A. dictyotus* (Boedijn) Wolfe, the type species of the genus, in our molecular phylogenetic analysis of the nrLSU dataset. Our data indicated that *A. dictyotus*, together with a few other taxa of the genus, formed a separate clade (Fig. 1). Morphologically, the genus *Austroboletus* has a tomentose to subtomentose pileus with appendiculate remnants at the pileal margin, pruinose to alveolate reticulations on the stipe surface, vinaceous pink basidiospores with deep pits, warts or reticulations, and an ixotrichodermium to ixocutis pileipellis (Corner 1972; Wolfe 1979; Halling et al. 2006; Fulgenzi et al. 2010; Takahashi and Degawa 2011). *Austroboletus longipes* var. *longipes* has slightly sinute or nearly adnate tubes, slender boletoid basidiospores which are smooth or marbled verruculose under LM but minutely perforate under SEM (Corner 1972; Wolfe 1979; Horak 2011). *Austroboletus longipes* var. *albus* has large basidiomata up to 95 mm, a stipe with irregularly reticulations, slender fusoid verruculose basidiospores, and a pileipellis composed of erect cylindrical hyphae (Corner 1972; Horak 2011). Re-examination of the type material of *A. tristis* showed that the spores are [80/4/1] (13) 15–16 (20)×(4) 4–4.5 (5) μm [Q=(2.79) 3.43–3.66 (4.35), Q_m=3.54±0.41], slender boletoid, brown to deep brown in H_2O but becoming light bluish to light purple to violet-purple in 5 % KOH, verrucose under LM and conspicuous roughened under SEM (Fig. 4g–h).

Basidiospores ornamented with shallow to deep pits are not unique for *Borofutus* and are present in several species of *Austroboletus*, viz. *A. subflavidus* (Murrill) Wolfe (Fig. 14 in Watling and Hollands 1990, as *Boletellus subflavidus*), and *A. mutabilis* Halling et al. (Figs. 5–8 in Halling et al. 2006). *Austroboletus subflavidus* has a nearly circular pored hymenophore, a stipe with pinkish gray, strong and coarse reticulation, and a host association with *Pinaceae* and *Fagaceae* (Singer 1945; Ortiz-Santana et al. 2007). *Austroboletus mutabilis* has a dark red to brownish red to orangish yellow pileus, basidiospores with a conspicuously eroded suprahilar plage, and a stipe with an alveolate reticulate to lacerate surface (Halling et al. 2006).

The morphology of the basidiospores of *Borofutus* is somewhat similar to that of *Heimioporus betula* (Schwein.) E. Horak (Fig. 13 in Watling and Hollands 1990, as *Heimiella betula*). However, *H. betula* has a brownish red to orange red pileus, a stipe with longitudinally raised ornamentations, longer and wider olive brown basidiospores measuring 18–22× 8–11 μm, an ixotrichoderm pileipellis and associated with *Pinaceae* and *Fagaceae* (Horak 2004; Takahashi and Degawa 2011). The brown to cocoa brown pileus, the subdecurrent hymenophore, and the host specificity of *Borofutus dhakanus* are somewhat similar to those of *Phylloporus pumilus* M. A. Neves & Halling originally described from Indonesia by Neves et al. (2012). However, the latter taxon has diminutive basidiomata (with a 5–9 mm broad pileus), and smooth basidiospores.

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