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Sutorius: a new genus for *Boletus eximius*

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Abstract: *Sutorius* is described as a new genus of Boletaceae to accommodate *Boletus robustus* originally named illegitimately by C.C. Frost from eastern North America. The legitimate name, *Boletus eximius*, provided by C.H. Peck, has been used since for a dark purple to chocolate brown bolete with finely scaly stipe and reddish brown spore deposit. This iconic taxon has been documented on five continents. Despite the straightforward species identification from morphology, the interpretation of stipe macromorphology and spore color has led to equivocal generic placement. Phylogenetic analyses of genes encoding large subunit rRNA and translation elongation factor 1α confirm *Sutorius* as a unique generic lineage in the Boletaceae. Two species are recognized based on multiple accessions: *S. eximius*, represented by collections from North America, Costa Rica, Guyana, Indonesia and Japan (molecular data are lacking for only the Guyanan and Japanese material); and *S. australiensis*, represented by material from Queensland, Australia. Additional collections from Zambia and Thailand represent independent lineages, but sampling is insufficient to describe new species for these entities.

Key words: biogeography, boletes, Boletineae, phylogeny, ribosomal DNA

Introduction

Boletus eximius Peck was proposed as a new name by Peck (1887) for *Boletus robustus* Frost (1874) non Fries (1851). Since then, this idiosyncratic bolete from northeastern North America has been placed in *Ceriomyces* (Murrill 1909), *Tylopilus* (Singer 1947) and *Leccinum* (Singer 1973). Because Murrill's concept of *Ceriomyces* can be discounted as a mixture of several modern genera, placement of *B. eximius* has been based primarily on either color of the spore deposit or the type of surface ornamentation of the stipe. Thus, Smith and Thiers (1971) were inclined to consider the spore color (reddish brown) more nearly like that of a *Tylopilus* whereas Singer (1973, 1986) judged that the stipe ornamentation was of a scabrous nature as in a *Leccinum*.

To anchor the name to a specimen, Halling (1983) designated a lectotype from among original Frost specimens and noted that descriptions published by Snell and Dick (1970), Smith and Thiers (1971) and Grund and Harrison (1976) adequately describe and illustrate the characters of the taxon. Treatments by Bessette et al. (2000) and Roody (2003) as a *Tylopilus* and Halling and Mueller (2005) as a *Leccinum* provide color photographs and updated descriptions. The latter publication extended the distribution to Central America. Fulgenzi et al. (2007) reported *T. eximius* from Guyana in northeastern South America.

Boletus eximius has been described from collections beyond the Americas, specifically Papua New Guinea (Hongo 1973, as *B. nigroviolaceus* Heim), Japan (Hongo 1975, 1979, 1980; Imazeki and Hongo 1989), China (Teng 1996) and Australia (Bougher and Thiers 1991, as *L. australiense*; Watling and Li 1999). Corner (1972) placed Heim's taxon as a questionable synonym of *Boletus alboater* (=*Tylopilus alboater*). Corner's description might circumscribe *T. alboater*, but Horak (2011) maintains that those interpretations are still in doubt.

Obvious patterns of amphi-Pacific disjunction of bolete morphotaxa have been cited and documented by Halling et al. (2008). Among these, four species of *Tylopilus* (*alboater*, *balloui*, *eximius* and the *chromapes*-group) were noted as particular examples of boletes

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exhibiting this disjunction. Those authors limited their study to the phylogeography of the *T. balloui* consortium and further suggested such disjunctions deserved additional examination from a molecular perspective. Critical morphological and molecular analyses of Chinese materials supported recognition of a new genus, *Zangia*, in the *T. chromapes*-group (Li et al. 2011). Our study of specimens morphologically identifiable as *Boletus eximius* from the Americas, eastern Asia, Indonesia, Africa and Australia with support from phylogenetic inference suggests recognition of a new genus, *Sutorius*, to accommodate that species plus *S. australiensis* from Queensland. Sufficient accession data is lacking to diagnose lineages properly from Thailand (*Sutorius* sp. 1) and Africa (*Sutorius* sp. 2).

MATERIALS AND METHODS

Morphological datasets.—Macromorphological data were derived from fresh specimens. General color terms are approximations, and the color codes (e.g. 7D8) are page, column and grid designations from Kornerup and Wanscher (1983). All microscopic structures were observed with an Olympus BHS compound microscope equipped with Nomarski differential interference contrast (DIC) optics and measured from dried material revived in 3% KOH. The abbreviation *Q* refers to the mean length/width ratio measured from *n* basidiospores, and *x* refers to the mean length × mean width. Herbarium codes (Thiers 2011) are cited for all collections from which morphological features were examined.

Molecular datasets.—Eighteen sequences from 15 collections (TABLE I) that have been identified morphologically as *B. eximius* were newly generated, including nuclear large subunit ribosomal DNA (nuc-lsu) and translation elongation factor 1α (*tef1*). For the final data assembly, 26 additional species (TABLE I) were selected based on results from studies from which some nuc-lsu and *tef1* sequences were already available (Binder and Hibbett 2006, Binder et al. 2010). These represent the major lineages of Boletineae (e.g. *Boletus*, *Tylopilus*, *Leccinum*, *Xerocomus*) with an emphasis on taxa having affinities to *B. eximius* based on phylogenetic or morphological evidence. In addition, 11 nuc-lsu and 19 *tef1* sequences were generated for species other than *B. eximius* to maximize data overlap. BLAST queries were run with the *B. eximius* sequences as queries to confirm the selection for taxon sampling. The new sequences were deposited in GenBank (JQ326993–JQ327040, TABLE I).

DNA extraction, PCR amplification, sequencing and alignments.—DNA was extracted from herbarium specimens using phenol-chloroform based protocols (Lee and Taylor 1990). The samples were inspected under a dissecting microscope, and the pileipellis and the outer hymenial layer were removed with sterile scalpels. Up to 15 mg tissue was ground, adding liquid nitrogen three times, and the homogenized samples were resuspended in 2% CTAB or 3% SDS extraction buffer. The cell lysates were cleaned after 1 h at 60 C by adding 0.8 mL phenol-chloroform (1 : 1), followed by isopropyl alcohol and 3 M sodium acetate precipitation and a wash step in 70% EtOH. DNA samples were resuspended in up to 75 μL Tris-EDTA buffer, and the concentration was estimated on 1% agarose gels. In addition, the E.Z.N.A forensic DNA extraction kit (Omega Bio-tek, USA.) was used to remove excess pigments present in many of the *B. eximius* samples.

PCR mixtures contained 2.5 μL 10× PCR buffer, 5 μM dNTP, 12.5 pM of each PCR primer, 0.25 μL Paq5000 DNA polymerase (Agilent Technologies, Stratagene, Santa Clara, California) and 5 μL DNA template in 25 μL volumes. The amplification program for nuc-lsu using the primer combinations LR0R-LR7 and LR0R-LR5 (Vilgalys and Hester 1990) included 35 cycles of 94 C for 45 s, 50 C for 1 min 10 s and 72 C for 2 min. The *tef1* product 983F-2218R was amplified with the thermo-cycler protocol by Rehner and Buckley (2005). All PCR products were sequenced with the BigDye 3.1 terminator sequencing kit (Applied Biosystems, Foster City, California) with these primers: LR0R, LR7 (when applicable), LR5, LR3 and LR3R (Vilgalys and Hester 1990) for nuc-lsu products; 983F, 1577F, 1567R and 2212R (Rehner and Buckley 2005) for *tef1* products. Sequencing was conducted on an ABI 3130 genetic analyzer (Applied Biosystems), and the raw data were processed into contigs with Sequencher 4.7 (Gene Codes Corp, Ann Arbor, Michigan).

The finished nuc-lsu and *tef1* nucleotide datasets were uploaded to the MAFFT server 6 (http://mafft.cbrc.jp/alignment/server/) and aligned automatically with the Q-INS-i option. Both alignments were adjusted manually in MacClade 4.05 (Maddison and Maddison 2005) and concatenated into a single dataset. The alignments were deposited in TreeBASE (S12246, http://purl.org/phylo/treebase/phylows/study/TB2:S12246).

Phylogenetic analyses.—The individual nuc-lsu and *tef1* datasets as well as the combined nuc-lsu + *tef1* dataset were analyzed with maximum likelihood methods. Bootstrap support values (BS) were estimated with RAxML 7.2.6 (Stamatakis 2006) running 500 replicates under the GTR model employing rapid bootstrap inferences. Posterior probability values (PP) for the combined nuc-lsu + *tef1* dataset were estimated with PhyloBayes 3.2 (Lartillot et al. 2009). The runs were performed with the CAT-GTR model and were stopped after the largest and mean discrepancy observed across all bipartitions approximated zero. A phylogram with branch lengths inferred from the combined dataset with RAxML including the support values from both methods is included (FIG. 1).

RESULTS

DNA extraction and PCR.—Obtaining high quality DNA and PCR products was straightforward except for the *B. eximius* samples. The crude *B. eximius* DNA was heavily pigmented, which interfered with PCR; as a result LR0R-LR7 and *tef1* products were successfully

TABLE I. Voucher information and GenBank accession numbers (new submissions in boldface)

Species	Isolate ID	Location	Date	Collector	nuc-lsu	*tef1*
Sutorius australiensis	9056A	Fraser Island, Queensland	9 Feb 2009	R. Halling	**JQ327011**	—
Sutorius australiensis	9205	Fraser Island, Queensland	9 Jun 2009	R. Halling	**JQ327012**	—
Sutorius australiensis	9441	Cooloola, Queensland	20 Feb 2011	R. Halling	**JQ327006**	**JQ327032**
Sutorius australiensis	9280	Fraser Island, Queensland	26 Mar 2011	R. Halling	**JQ327005**	**JQ327031**
Sutorius eximius	8594	Jardín de Dota, Costa Rica	5 Jun 2004	R. Halling	**JQ327008**	**JQ327027**
Sutorius eximius	995	San Gerardo de Dota, Costa Rica	15 Jun 2004	T. Osmundson	**JQ327010**	**JQ327030**
Sutorius eximius	986	La Chonta, Costa Rica	14 Jun 2004	T. Osmundson	**JQ327009**	**JQ327028**
Sutorius eximius	9400	Ulster County, NY, USA	24 Sep 2010	R. Halling, G. Lincoff	**JQ327004**	**JQ327029**
Sutorius eximius	3136	Unknown	Unknown	E. Both	—	—
Sutorius eximius	8069	Java, Indonesia	16 Jan 2001	R. Halling	**JQ327003**	—
Sutorius eximius	8600	Potaro-Siparuni, Guyana	23 Jul 2003	T. Henkel	—	—
Sutorius eximius	88-144	Tottori, Japan	3 Aug 1988	E. Nagasawa	—	—
Sutorius sp. 2	01-515	Mutinondo, Zambia	4 Jan 2001	D. Arora	—	—
Sutorius sp. 2	01-528	Mutinondo, Zambia	4 Jan 2001	D. Arora	**JQ327002**	—
Sutorius sp. 1	ECV3603	Bai Mae Sae, Thailand	4 Jul 2007	E. C. Vellinga	**JQ327000**	**JQ327033**
Aureoboletus thibetanus	AFTOL-450	Kunming, Yunnan, China		Z.-L. Yang	AY700189	DQ029199
Boletellus projectellus	AFTOL-713	Cape Cod, MA, USA	14 Sep 2003	M. Binder	AY684158	AY879116
Boletellus sichianus	AFTOL-532	Yunnan, China	2003	L. Wang	AY647211	DQ408145
Boletus amygdalinus	112605ba	Mendocino County, CA, USA	26 Nov 05	B. Neill	**JQ326996**	**JQ327024**
Boletus appendiculatus	Bap1	Bavaria, Germany	10 Aug 1995	J. Schreiner	AF456837	**JQ327025**
Boletus bicolor var. *borealis*	2858	Erie County, NY, USA	—	E. Both	**JQ326998**	**JQ327021**
Boletus calopus	Bc1	Bavaria, Germany	7 Sep 1994	N. Arnold	AF456833	**JQ327019**
Boletus carminipes	MB 06-061	Erie County, NY, USA	4 Aug 2006	M. Binder, E. Both	**JQ327001**	**JQ327022**
Boletus edulis	Be1	Bavaria, Germany	14 Sep 1994	M. Binder	AF050643	**JQ327018**
Boletus inedulis	MB 06-044	Erie County, NY, USA	3 Aug 2006	M. Binder, E. Both	**JQ327013**	**JQ327020**
Boletus luridiformis	AT2001087	Berkshire, England, UK	—	A. F. S. Taylor	**JQ326995**	**JQ327023**
Boletus peckii	3959	Erie County, NY, USA	4 Aug 1995	A. R. Clark, E. Both	**JQ326999**	**JQ327026**
Boletus variipes var. *fagicola*	4249	Cheboygan County, MI, USA	10 Aug 1968	A. H. Smith	**JQ327014**	**JQ327017**
Buchwaldoboletus lignicola	Pul1	Maindreieck, Germany	9 Sep 1995	J. Schreiner	**JQ326997**	**JQ327040**
Chalciporus piperatus	MB 04-001	Rutland, MA, USA	28 Sep 2004	M. Binder	DQ534648	GU187690
Hemileccinum impolitum	Bim1	Bavaria, Germany	21 Sep 1995	J. Schreiner	AF139715	**JQ327034**
Leccinum albellum	MB 06-040	Erie County, NY, USA	30 Jul 2006	M. Binder	**JQ327007**	**JQ327038**
Leccinum scabrum	Ls1	Austria	14 Sep 1995	M. Binder	AF139705	**JQ327039**
Paxillus filamentosus	Pf1	Bavaria, Germany	21 Aug 1995	L. Krieglsteiner	AF167680	GU187736
Paxillus vernalis	Pv2	Canada	31 Aug 1997	T. Lohmeyer	AY645059	DQ457629
Phylloporus pelletieri	Pp1	Bavaria, Germany	9 Sep 1995	M. Kronfeldner	AF456818	**JQ327036**
Porphyrellus porphyrosporus	MB 97-023	Bavaria, Germany	9 Sep 1996	M. Beisenherz	DQ534643	GU187734
Strobilomyces floccopus	Sf1	Bavaria, Germany	12 Aug 1995	J. Enzmann	DQ534626	**JQ327037**
Tylopilus felleus	AT2001011	Stadsskogen, Uppsala, Sweden	17 Sep 2001	A. F. S. Taylor	**JQ326993**	**JQ327015**
Tylopilus ferrugineus	MB 06-053	Erie County, NY, USA	3 Aug 2006	E. Both	**JQ326994**	**JQ327016**
Xerocomus subtomentosus	Xs1	Bavaria, Germany	10 Aug 1995	J. Enzmann, A. Bresinsky	AF139716	**JQ327035**

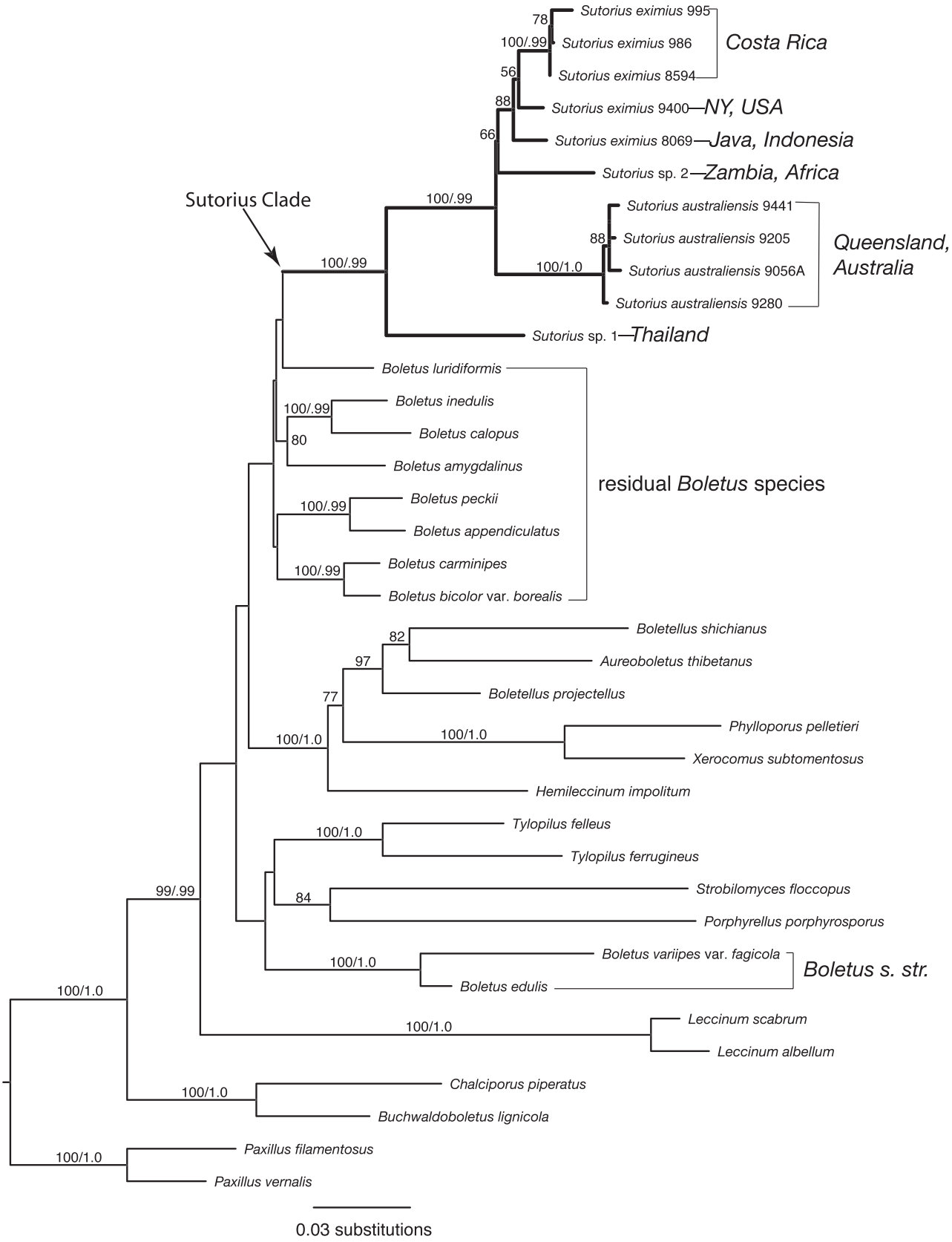

FIG. 1. Phylogenetic relationships and placement of *Sutorius eximius* within the Boletaceae inferred from a combined nuc-lsu + tef1 dataset (2245 bp) using RAxML and PhyloBayes. The tree topology corresponds to the optimal maximum likelihood tree calculated by RAxML. Support values ≥50% BS and 0.95 PP are shown.

amplified from only six samples (Osmundson 986, 995; Halling 9441, 8594, 9280; ECV3606). Success increased after a treatment with the E.Z.N.A forensic DNA extraction kit and a switch to the LR0R-LR5 primer combination; however, *tef1* products were not obtained for three *B. eximius* collections (Halling 9056A, Halling 9205, Arora 01-528). Similar difficulties obtaining good quality DNA from North American *B. eximius* were reported (Binder 1999), suggesting that Tylopilosins (yellow di-phenolic compounds with uncharacterized bioactivity) might be inhibitors of PCR. No PCR products were amplified from a Japanese collection (Nagasawa 88-144A), which had been treated and stored with naphthalene, or from a second African collection (Arora 01-515). Two collections (Both 3136, Henkel 8600) turned out to be contaminated by foreign fungal DNA after sequencing and were omitted from further analyses.

Alignments and phylogenetic analyses.—The final nuc-lsu alignment included 995 positions with 335 distinct alignment patterns and a proportion of gaps and undetermined characters of 0.112345 in RAxML. The full length LR0R-LR7 sequences were trimmed to fit the majority of LR0R-LR5 sequences. The *tef1* alignment is 1250 nucleotides long including introns and third positions, with 607 distinct alignment patterns and a proportion of gaps and undetermined characters of 0.053864 in RAxML. The nuc-lsu and *tef1* alignments were combined without additional modifications. PhyloBayes analyses were run on the combined nuc-lsu + *tef1* with four MCMC chains, sampling data every 100th cycle. The maximum difference in split frequency between runs dropped to zero after approximately 37 000 per chain, and the analyses were stopped. The chains were analyzed with the readpb program, removing 10% of the samples as burn-in. A total of 37 144 trees were used to estimate posterior probabilities. The tree inferred from the combined dataset and the support values estimated with RAxML and PhyloBayes is illustrated (FIG. 1).

The phylogenetic placement of Boletus eximius.—The Boletaceae received maximum support in all analyses (BS = 100%, PP = 1), but the backbone of the Boletaceae is poorly resolved. The genus *Boletus* is not monophyletic, as has been shown with nuc-lsu (Binder 1999) and multilocus data (Binder and Hibbett 2006, Dentinger et al. 2010), and splits into at least three groups: the *B. edulis*, the *B. eximius* and a group of weakly resolved *Boletus* species termed ''residual.'' *Boletus eximius* is not closely related to the genera *Tylopilus* or *Leccinum*, which earlier served as alternative placements for *B. eximius*. The phylogenetic tree inferred from nuc-lsu data places *B. eximius* without support as sister group of a larger clade including *Xerocomus*, *Phylloporus*, *Aureoboletus*, *Boletellus* pro parte and *Hemileccinum* species. This result is consistent with Binder (1999) and Binder and Hibbett (2006). The *tef1* phylogeny places *B. eximius* close to the ''residual'' *Boletus* species outside the *B. edulis* group. This relationship is not supported by bootstrap but holds up in the combined nuc-lsu + *tef1* phylogenetic analyses. The *B. eximius* clade, including ECV3603 from Thailand, is supported in all analyses and receives 68% and 100% BS from nuc-lsu and *tef1* analyses respectively and 100% BS and 1.0 PP in the combined analyses (FIG. 1).

TAXONOMY

Sutorius Halling, Nuhn & Fechner gen. nov.

A generibus Boletacearum sporis in cumulo rubrobunneis, inamyloideis, oblongis, levibus; contexto pallido, lilaceo vel roseobrunneo colubrino; fibulae nullis; pileo sicco, viscido ubi humido, fuscolilaceobrunneo o violaceobrunneo vel cacaino, raro margine sterili; hymenophoro adnexo, fuscoviolaceobrunneo o theobromino vel vinaceobrunneo ad extremum; stipite sicco, transverse scissuris squamuloso Leccino dissimilo, squamis fuscoviolaceobrunneo distinguenda.

Typus: Boletus robustus Frost *non* Fr.

Etymology: sutor(*-ius*) = Latin (m.) for cobbler; specifically Charles C. Frost, a Vermont shoemaker, who described the species *Boletus robustus* non Fr.

MycoBank: MB563942

Sutorius eximius (Peck) Halling, Nuhn, & Osmundson. comb. nov. FIGS. 2–3

Boletus eximius Peck, J. Mycol. 3:54. 1887, nom. nov. for *Boletus robustus* Frost, Bull. Buffalo Soc. Nat. Sci. 2: 104. 1874, *non* E. M. Fries, Nova Acta Regiae Soc. Sci. Upsal. ser. III, 1:46. 1851.

Ceriomyces eximius (Peck) Murrill, Mycologia 1:148. 1909.

Tylopilus eximius (Peck) Singer, Amer. Midl. Nat. 37:109. 1947.

Leccinum eximium (Peck) Pomerleau, Bol. du Cercle des Mycol. Amat. de Québec 6:117. 1959, *com. inval.* Art. 33.3.

Leccinum eximium (Peck) Singer, Persoonia 7:319. 1973.

MycoBank: MB563943

As noted above, *S. eximius* has been sufficiently described and well illustrated. Variations in spore dimensions are given (TABLE II).

Habit, habitat, distribution: Reported or observed among litter, on soil in forests associated with *Dicymbe*, *Dipterocarpus*, *Fagus*, *Hopea*, *Quercus*, *Shorea*, *Tsuga*. North America: eastern Canada to Georgia, west to

FIGS. 2–4. *Sutorius* habit images. 2. *S. eximius*, REH7798, Costa Rica (×1.5). 3. *S. eximius*, REH8069, Indonesia (×2). 4. *S. australiensis*, REH9411, Queensland (×1).

TABLE II. Spore dimensions and statistics for specimens and literature cited

Locality	Spore measurements	Cited documentation
Australia	(12–)12.5–15 × 3.5–4.5 µm n = 30, x = 12.93 × 4.03, Q = 3.21	Bougher and Thiers 1991
Australia (*Halling 9441*)	11.9–15.4(–16.8) × 3.5–4.9 µm n = 15, x = 13.9 × 4.1 µm, Q = 3.38	this paper
Costa Rica	10.5–13.3 × 4.2–4.9 µm Q = 2.55	Halling and Mueller 2005
Guyana	9.7– 12 × 4.2–5.3 µm, Q = 2.18	Fulgenzi et al. 2007
Japan	10.5–15.5 × 4–5.5 µm	Hongo 1975
Papua New Guinea	10–18 × 4.5 µm	Heim 1963
Papua New Guinea	11–16.5 × 4.5–5.5 µm	Hongo 1973
Thailand (*ECV3603*)	9.8–11.6 × 3.5–4.6 µm n = 15, x = 10.7 × 4.27 µm, Q = 2.51	this paper
USA (*Halling 9400*)	12.6–16.8 × 4.2–4.9 µm n = 15, x = 15.1 × 4.6 µm, Q = 3.29	this paper
USA	13.5–23.5 × 3.5–5.5 µm	Singer 1947
USA	11–17 × 3.5–5 µm	Smith and Thiers 1971
Zambia (*Arora 01-528*)	12.6–15.4 × 4.9–5.6(–6.3) µm n = 15, x = 13.95 × 5.4 µm, Q = 2.6	this paper

Wisconsin. Costa Rica. Indonesia. Possibly Japan, China and Guyana.

Specimens examined: COSTA RICA. ALAJUELA. Grecia: Grecia. Bosque del Niño, 10°9′4″N, 84°14′42″W, 1900 m, 29 Jun 1995, *Halling 7490* (NY, USJ), 31 May 1996, *Halling 7598* (NY, USJ),15 Jun 1996, *Halling 7688* (NY, USJ); SAN JOSÉ. Dota: Jardín. ± 3.5 km W of Interamerican Highway at Empalme, 9°42′52″N, 83°58′28″W, 2220 m, 5 Jun 2004, *Halling 8594* (NY, USJ); La Chonta, S of Interamerican Highway near km 54 toward Laguna/Cerro Chonta, 9°41′58″N, 83°56′31″W, 2400 m, 11 Jul 2000, *Halling 8020* (NY, USJ), 14 Jun 2004, *Osmundson 986* (NY, USJ); San Gerardo. ± 5 km SW of Cerro de la Muerte, Albergue de Montaña, Savegre, 2200 m, 9°33′2″N, 83°48′27″W, 2200 m, 20 Jun 1994, *Halling 7308* (NY, USJ), 8 Jun 1996, *Halling 7643* (NY), 5 Jul 2001, *Halling 8252* (NY, USJ), 15 Jun 2004, *Osmundson 995* (NY, USJ); Finca El Jaular, between km 66/67 of Interamerican Highway, 9°39′35″N, 83°52′6″W, 2234 m, 1 Jul 1998, *Halling 7798* (NY, USJ). GUYANA. REGION 8 POTARO-SIPARUNI. Pakaraima Mountains, Upper Potaro River, 4 km SW Potaro base camp *Dicymbe* plot 3, 5°18′5″N, 59°54′40″W, 710–750 m, 23 Jul 2003, *Henkel 8600* (NY). INDONESIA. JAVA. West Java, Haurbentes Park, 6°32′29″S, 106°26′16″E, ± 250–300 m, 16 Jan 2001, *Halling 8069* (NY). JAPAN. TOTTORI. Mount Daisen, 3 Aug 1988, *E. Nagasawa 88-144* (TMI 13038, dupl. NY). THAILAND. CHIANG MAI PROV. Ban Mae Sae, Highway 1095 at km 55, 19°14′32.6″N, 98°38′29.4″E, 10 Jun 2006, 990 m, *Osmundson 1171* (MFLU, NY); 4 Jul 2007, *Vellinga 3603* (MFLU, UC). USA. [One from among 60 specimens in NY] NEW YORK. Ulster County, Minnewaska State Park, upper trail near parking lot, 41°43′47″N, 74°14′11″W, 495–510 m, 24 Sep 2010 *Halling 9400* (leg. G. Lincoff) (NY). ZAMBIA. Mutinondo Wilderness, 12°27′S, 31°17′E, 4 Jan 2001, *D. Arora 01-515, 01-542* (CUW).

Commentary: Based on the phylogram (FIG. 1), a true *S. eximius* clade is well supported by molecular data and geography (Java, USA, Costa Rica). The Japanese material was not placed based on molecular inference, but it could be either *S. eximius* (consistent with other patterns of Laurasian disjunctions) or an Asian group near *Sutorius* sp. 1 (Thailand). It is not clear where the Guyanan entity would place in a molecular analysis, even though it occurs in northeastern South America, but mycorrhizal partnership with legumes might be consistent with an African grouping near *Sutorius* sp. 2 (Zambia). Except for variation of spore dimensions (TABLE II), there is little if any morphological difference in the specimens examined. The differences between the Costa Rican and USA measurements probably represent a clinal variation well documented by Halling and Mueller (2002, 2005) and Osmundson and Halling (2010) for many agarics and boletes.

Sutorius australiensis (Bougher & Thiers) Halling & Fechner comb. nov. FIG. 4

Leccinum australiense Bougher & Thiers, Mycotaxon 42:256. 1991.

?*Boletus nigroviolaceus* Heim, Rev. Mycol. (Paris) 28:282. 1963.

MycoBank: MB563944

Pileus (3–)7.5–9(–11) cm broad, convex to plano-convex to plane, dry or viscid (in wet weather), finely matted to matted subtomentose, sometimes finely velutinous with a subtle to distinct hoary bloom at first, brown (7E6-5), dark (chocolate) brown (7,8,9F8,7), reddish brown (8E8), violet brown

(10D4-11E4) to lilac brown (11D4) (especially toward margin), to nearly black in some, becoming brown (7E6,5), even at margin or sometimes with a slight sterile extension. Flesh white to pale lilac, with pinkish brown to brownish lilac marbling/mottling, with mild odor and flavor that is mild to slightly unpleasant, slightly bitter. Tubes adnexed to deeply depressed, lilac whitish when young, soon flesh (6A-B3) to light brown (6D4), with pores stuffed and violet brown (11F5) when young, becoming brown (7E6,5) to cocoa brown (6E7) with age, bruising a cinnamon brown. Stipe (2.5–)4–6(–8.5) cm long, 1–2 cm broad, strict or curved, equal to subclavate, dry, finely subsquamulose to finely scabrous-scissurate on a pale lilac ground (16D3) or very nearly white, with scales a pinkish brown to pinkish lilac to violet brown or a dull brown (7E6-5, 8E3), with interior whitish to lilac brown to grayish lilac and mottled, becoming streaked with pale brown or light brownish orange staining, white and matted to tomentose at base with white basal mycelium or occasionally mixed with a short brown, ocher tomentum, sometimes ''brownish tomentose'' (Hongo 1973, PNG).

Spores red brown in deposit, 11.9–15.4(–16.8) × 3.5–4.9 μm (n = 15, x = 13.9 × 4.1 μm, Q = 3.38), light brown in KOH, smooth and thin-walled, ellipsoid to subfusoid to fusoid, inamyloid. Basidia 20–34 × 8–11 μm, clavate, hyaline, four-sterigmate. Hymenial cystidia 20–40 × 6–8 μm, scattered and uncommon, thin-walled, with hyaline to granular and golden to pale brown contents, narrowly fusoid. Tube trama boletoid and divergent, with central stratum brown to golden yellow; the lateral strata elements hyaline, 3.5–8.4 μm wide, subgelatinous with age, often with amorphous dark lilac to pinkish orange-brown pigment deposits. Pileipellis hyphae a trichodermium, in KOH yellow ochraceous, inamyloid; elements 3.5–6 μm wide, elongated to cylindrical or obtuse, encrusted with pigment (but dissolving in KOH), thin-walled, not gelatinized. Pileus trama interwoven, hyaline, inamyloid, thin-walled. Stipitipellis hyphae vertically oriented, parallel, giving rise to clusters of caulocystidia, 20–30 μm × 5–15 μm wide, cylindrical to clavate to subfusoid, hyaline to brown contents, with encrusting pigment present (with dark brown or lilac to purple acerose crystals dissolving in KOH). Stipe trama hyphae parallel, cylindrical, hyaline, inamyloid, often with amorphous dark lilac to pinkish orange-brown pigment deposits. Clamp connections absent.

Specimens examined: AUSTRALIA. QUEENSLAND: Koombooloomba area, [label data: ''Red Road, Tully Falls area''], 4 May 1988, *Bougher & Malajczuk E4010* (ACIAR E4010, BRIP 17542, Holotype: *Leccinum australiense*, BRI). Davies Creek Road, 5 Apr 1991, *M. Castellano E4095* (Paratype: *Leccinum australiense*, BRIP 17543, BRI). Wide Bay District, Great Sandy National Park, Cooloola section, Freshwater Road, 25°56′37″S, 153°7′24″E, 154 m, 20 Feb 2011, *Halling 9441* (BRI, NY), 23 May 2011, *Halling 9543* (BRI, NY); Fraser Island, road from Central Station to Eurong, 25°29′6″S, 153°5′18″E, 75 m, 11 Feb 2009, *Halling 9056* (BRI, NY); road from Wanggoolba Creek Ferry landing to Central Station, 25°27′39″S, 153°1′26″E, 90 m, 7 Jun 2009, *Halling 9190* (BRI, NY); Ungowa Road 25°27′31″S, 153°0′40″E, 24 m, 9 Jun 2009, *Halling 9205* (BRI, NY); road from Eurong to Central Station, about 1 km W of Eurong, ± 25°29′S, ± 153°6′E, 26 Mar 2010, *Halling 9280* (BRI, NY); road from Eurong to Central Station, 25°30′1″S, 153°6′19″E, 51 m, 17 May 2011, *Halling 9485* (BRI, NY); 4 km along Woralie Road, at Knifeblade Sandblow car park, 25°13′25″S, 153°13′46″E, 121 m, 18 May 2010, *Halling 9315* (BRI, NY).

Habit, habitat, distribution: Reported or observed among litter, on soil or sand in forests associated with *Allocasuarina*, *Corymbia*, *Eucalyptus*, *Lophostemon*, *Syncarpia*. Queensland, Australia. Reported from Victoria and the Australian Capital Territory (Watling and Li 1999 as *T. eximius*). Possibly in Papua New Guinea with Fagaceae.

Commentary: As near as we can tell, R. Heim's specimen of *B. nigroviolaceus* from Papua New Guinea is not available from PC. The original description of *B. nigroviolaceus* (Heim 1963) and color illustration in Heim (1965) portray a deeply pigmented *Sutorius* recalling material collected in Queensland. Hongo's (1973) report of *B. nigroviolaceus* from PNG was decreed by him (Hongo 1975) to be nothing more than *S. eximius* (as a *Tylopilus*). Additional material from PNG could add support for the Austral lineage. For the specimens supporting the description of *Leccinum australiense* (Bougher and Thiers 1991), a spore print is included with the holotype specimen along with a Kodachrome transparency showing three, intensely colored basidiomes (one cut lengthwise), a bit on the young side and just approaching maturity. The paratype also includes a Kodachrome illustrating a *Sutorius*. The microscopic features presented by Bougher and Thiers (1991) sufficiently show those features. At present, an Australian indigenous *Leccinum* in the classical/typical sense of Lannoy and Estades (1995) or Den Bakker and Noordeloos (2005) is still unknown.

A geographical structuring (FIG. 1) recalls somewhat the one observed for *Boletus* sect. *Boletus* (Dentinger et al. 2010, fig. 4). This latter is based on material with morphological differences and using mostly different gene loci from localities comparable to ones in this study. The spore dimensions and statistics of the Australian material are most nearly like those from the USA (TABLE II), but the support from the molecular inference and geographic structuring

FIG. 5. Habit image, *Leccinum versipelle*, REH8498, Valday District, Russia (×0.5).

lend weight to our hypothesis that the Australian lineage is distinct. In the description above there is notice of different colors and morphology on the exterior of the stipe base. These features sometimes are not easy to discern because of adhering or embedded substrate but might be useful for finer distinctions when additional material is available.

In general, one feature specifically mentioned in the field description of *Vellinga 3603* and in the protolog (Heim 1963) and French description (Heim 1965) of *B. nigroviolaceus* is the nature in which the stipe ornamentation manifests itself. The protolog states ''… transverse scissuris albis et acuminatus striatus … ;'' in French, ''… formant à la loupe une sorte de *tigrure* complex, …'', and Vellinga noted ''… upper part [of stipe] surface breaking up and becoming fine tiger-patterned.'' In fact, this manifestation has been overlooked or perhaps not properly described in documenting the macroscopic features of this taxon (FIGS. 2–4). These transversely scissurate scales can be found in the freshest, well preserved material and are present in such specimens cited above (including those of the senior author). An image showing this feature also can be found on Mushroomobserver.org (Image 95313 of Observation 49122 by Damon Brunette). This manner of appearance of the surface ornamentation is fundamentally different from that of a *Leccinum* in the strict sense (FIG. 5).

DISCUSSION

Sutorius eximius is one of a number of boletes having a combination of morphological characteristics that have led to alternative classifications depending on character-weighting judgments by different authors; other prominent examples include *Tylopilus chromapes* (which has also been placed in *Leccinum*), *Tylopilus balloui* (also placed in *Rubinoboletus* and *Gyroporus*) and *Bothia castanella* (also placed in *Boletinus*, *Boletinellus*, *Chalciporus*, *Gyrodon*, *Suillus*, *Xerocomus*). Molecular data recently have proven useful in resolving these taxonomic quandaries. In the case of *T. ballouii*, molecular data support placement in *Tylopilus* despite its basidiospore morphology that is uncharacteristic for the genus (Osmundson and Halling 2011). In the case of *B. castanella* and the *T. chromapes*-like *Zangia* spp. (Li et al. 2011) and now *Sutorius eximius* these taxonomic enigmas are shown to be phylogenetically distinct from all genera in which they were formerly placed. Aligned with *Tylopilus* on the basis of spore deposit color (Smith and Thiers 1971), *S. eximius* is an uneasy match to the former due to having reddish brown instead of truly pinkish spores in deposit. Here we present morphological evidence that the nature of the stipe ornamentation of *S. eximius* superficially resembles *Leccinum* but appears to lack developmental homology in the genus. The molecular data presented here confirm what we therefore can conclude (if with some degree of uncertainty) from morphology: that *S. eximius* should not be placed in *Tylopilus* or *Leccinum*; *Sutorius* does not share a recent common ancestor with either of these genera in our phylogenetic analysis. Specimens sharing the unique morphology of *Sutorius* from North America, Asia, Africa and Australia form a monophyletic clade; however, the broader phylogenetic affinity of this group is as yet unknown due to poor backbone resolution, a problem observed in both single-gene (e.g. Halling et al. 2007) and multilocus (Binder and Hibbett 2006) analyses of the Boletineae (sensu Binder and Bresinsky 2002). Despite lacking this broader phylogenetic context, both the morphological and molecular distinctiveness of these fungi warrant generic recognition.

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