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***Boletus durhamensis* sp. nov. from North Carolina**

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ABSTRACT—A new bolete with cinnamon-brown pores, *Boletus durhamensis*, is described. Collected in northern North Carolina, it is possibly mycorrhizal with *Quercus* spp. Morphological and molecular characters support this taxon as a new species.

KEY WORDS—*Boletaceae*, ectomycorrhizal fungi, oaks, taxonomy

Introduction

Studies on boletes in North Carolina started with Coker & Beers (1943). Their work, which was concentrated in the Piedmont area of Orange County, presented morphological descriptions of 68 *Boletus*, four *Boletinus*, and one *Strobilomyces* species. Subsequent studies by Grand (1970a, b, c; 1981), which provided more information about the occurrence, distribution, and host associations of boletes in the mountains and Piedmont regions, added about 10 new records for the state. More recently as part of a workshop devoted to boletes, Justice (2008) listed species from North Carolina based on Bessette et al. (2000) and species reported by the Asheville Mushroom Club and North American Mycological Association's Wildacres Regional Forays.

In 2001, one of us (McConnell) found an unusual brown-capped bolete in a hardwood forest at the Edison Johnson Recreation Center in Durham, NC. The fruiting bodies consistently had brown pore surfaces in all developmental stages, from the very youngest button through maturity. Boletes with brown pore surfaces are rare in North Carolina, and despite careful evaluation of the

unknown bolete's macrofeatures and a search of recent mycological literature, it was not possible to identify the species. Ernst Both (Buffalo Museum of Science, NY) recognized the material as an undescribed species, tentatively naming it "*Boletus durhamensis*" based on its collection locality. Unfortunately, Both died before he was able to publish these findings.

Binion et al. (2008) published one image of *B. durhamensis* with a description (by McConnell), but the name was not validly published under the rules of the International Code of Nomenclature. Since the first collection and description of *B. durhamensis* were obtained when working in collaboration with Ernst Both, and because new collections have been made, his provisional species name is validated here.

Materials & methods

Morphological observation

Macroscopic descriptions are based on fresh and dried specimens, field notes, and color photographs. Color terms are general approximations, while numerical color designations are from Kornerup & Wanscher (1978). Macrochemical reactions were determined using 10% NH₄OH, 5% KOH, and 10% FeSO₄. Microscopic structures were observed with an Olympus BH-2 compound microscope; freehand sections of dried fungal material were rehydrated in 70% ETOH and mounted separately in 3% KOH and Melzer's reagent. In the description of basidiospores, *n* = number measured, followed by

TABLE 1. Taxa included in the study, with their vouchers and GenBank accession numbers. New sequences are indicated with bold font.

TAXON	VOUCHER	ITS	LSU	TEF
<i>Boletus aereus</i>	REH-8721	—	KF030339	KF030426
	SU07	DQ131619	—	—
<i>B. amygdalinus</i>	112605ba	—	JQ326996	JQ327024
	src491	DQ974705	—	—
<i>B. calopus</i>	Bc1	—	AF456833	JQ327019
	UF1401	HM347645	—	—
<i>B. durhamensis</i>	BOS-885	KM675997	KM675998	—
	Both-4561	KM675995	KM675996	KM668212
<i>B. edulis</i>	BD380	EU231984	HQ161848	—
	Be1	—	—	JQ327018
<i>B. luridus</i>	B12	—	AF139686	—
	AMB12640	KC734544	—	—
<i>B. pinophilus</i>	42/93	—	AF462359	—
	isolate 2163	KC750240	—	—
<i>B. satanas</i>	Bs2	—	AF042015	—
<i>B. subvelutipes</i>	RV98-102	—	AY612804	—
<i>B. tenax</i>	REH-6871	—	KF030320	KF030437

<i>B. variipes</i>	BD245	EU231958	EU232003	—
<i>B. variipes</i> var. <i>fagicola</i>	4249	—	JQ327014	JQ327017
	BD190	EU231963	—	—
<i>B. vermiculosus</i>	222/97	—	DQ534646	—
<i>Bothia castanella</i>	MB03-053	—	DQ867117	KF030421
	MB03-067	DQ867114	—	—
<i>B. fujianensis</i>	HKAS-82694	—	KM269193	—
	HKAS-82693	KM269196	—	—
<i>Butyriboletus appendiculatus</i>	Bap1	—	AF456837	JQ327025
	VDK-O429	HQ882194	—	—
<i>B. regius</i>	11265	—	KF030267	KF030411
	MG408a	KC584789	—	—
<i>Caloboletus firmus</i>	MB06-060	—	KF030278	KF030408
	Arora-13039	KM396278	—	—
<i>Sutorius australiensis</i>	REH-9280	—	JQ327005	JQ327031
<i>S. eximius</i>	REH-9400	—	JQ327004	JQ327029
	TH-8988	KT339268	—	—
<i>Tylophilus alboater</i>	TDB-1206	—	AF139708	—
<i>T. appalachiensis</i>	TENN61182	FJ596794	—	—
<i>T. atromicotianus</i>	Both s.n.	EU685114	EU685110	—
<i>T. badiceps</i>	78206	—	KF030335	KF030335
<i>T. felleus</i>	AT2001011	—	JQ326993	JQ327015
	HKAS-55832	—	HQ326934	—
	JMP0093	EU819449	—	—
<i>T. ferrugineus</i>	MB06-053	—	JQ326994	JQ327016
<i>T. indecisis</i>	98-98	—	AF456820	—
<i>T. microsporus</i>	HKAS-59661	—	KF112450	KF112225
<i>T. plumbeoviolaceus</i>	MB06-056	—	KF030350	KF030439
"T. <i>tabacinus</i> "	HN-2295	—	AY612837	—
	HN-2295 (CFMR)	KX925216	KX925217	—
<i>Xerocomellus chrysenteron</i>	HKAS-56494	—	KF112357	KF112172
	TENN60896	FJ596906	—	—
	AT2005034	—	KF030354	KF030417
<i>X. cisalpinus</i>	IB19980850	—	AF514815	—
	PDD94421	—	JQ924322	KF112171
	GS-10020	KT271743	—	—
	BB06304-Bv	HM190074	—	—
	REH-8724	—	KF030271	KF030416
<i>X. zelleri</i>	KGP68	DQ822794	—	—
	H126	—	AF514820	—
<i>Xerocomus fennicus</i>	H126	—	AF514820	—
"X. <i>perplexus</i> "	MB00-005	JQ003657	JQ003702	KF030438
<i>X. pruinatus</i>	IB19961055	—	AF514825	—
<i>X. ripariellus</i>	GR22465	—	AF514818	—
<i>X. subtomentosus</i>	strain Xs1	—	AF139716	JQ327035
	1549a-Q-6103	KM248935	—	—
<i>Chalciporus piperatus</i>	MB 04-001	—	DQ534648	GU187690
	2591	KM248949	—	—
<i>C. pseudorubinellus</i>	4302	—	KF030287	KF030441
<i>C. rubinellus</i>	2626	KM248951	—	—

the mean basidiospore lengths and widths \pm their standard deviations and the Q_m value, which represents the mean Q value \pm its standard deviation, where Q = length/width ratio. Specimens examined are deposited in the CFMR herbarium. Herbarium acronym follows Thiers (2016).

DNA extraction, PCR & sequencing

DNA sequences from two nuclear ribosomal DNA regions (LSU and ITS) and one protein-coding gene (TEF1-alpha) of *B. durhamensis* were generated in the present study. DNA extraction, amplification, and sequencing from dried specimens of *B. durhamensis* were conducted at the Center for Forest Mycology Research in Madison, following Palmer et al. (2008). The ITS region was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990), the 5' end of the LSU region was amplified using primer pair LROR/LR5 (Vilgalys & Hester 1990) and TEF1 was amplified using primer pair EF1-983/EF1-1567R (Rehner & Buckley 2005). For TEF PCR protocols see Minnis & Lindner (2013).

Phylogenetic analysis

DNA sequences were used primarily for molecular identification and were compared with other sequences available in GenBank via BLASTn search (Benson et al. 2013). DNA sequences were also used to infer the phylogenetic relationship among *B. durhamensis* and other members of the family *Boletaceae*. Considering the BLASTn search results and the main morphological characters of *B. durhamensis*, sequences of species pertaining to the genera *Boletus*, *Bothia*, *Butyriboletus*, *Caloboletus*, *Sutorius*, *Tylopilus*, *Xerocomellus*, and *Xerocomus* were retrieved from GenBank (TABLE 1). *Chalciporus piperatus* (Bull.) Bataille, *C. pseudorubinelus* (A.H. Sm. & Thiers) L.D. Gómez, and *C. rubinelus* (Peck) Singer were used as outgroup in the phylogenetic analyses. Sequences were edited with Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan), and aligned using MAFFT v.7 (Katoh & Standley 2013). The alignment was manually adjusted using MacClade 4.08 (Maddison & Maddison 2002). ITS, LSU, and TEF datasets were compiled and evaluated with Maximum likelihood (ML) analysis run in RAXML server, v.7.7.1 (Stamatakis et al. 2008) under GTR GAMMA model with 100 rapid bootstrap replicates.

Taxonomy

Boletus durhamensis B. Ortiz, Bessette & McConnell, sp. nov.

PLATES 1–2

MYCOBANK MB 810115

Differs from *Boletus vermiculosoides* and *B. vermiculosus* by its lack of a bluing reaction when its flesh is exposed, its partial veil covering the immature pores, and its smaller basidiospores.

TYPE: USA. North Carolina: Durham Co., Edison Johnson Recreational Center, 11 August 2001, O.L. McConnell, Both 4561 (**Holotype**, CFMR, GenBank KM675995, KM675996, KM668212).

ETYMOLOGY: *durhamensis* refers to Durham, North Carolina, where this bolete was first collected by mycologist Owen L. McConnell.



PLATE 1. *Boletus durhamensis* (BOS 885). Basidiomata. Photo by OL McConnell.

ICONES: Macrofungi associated with oaks of eastern North America (Binion et al. 2008: 48).

PILEUS 4–8 cm diam., hemispherical at first, becoming convex to broadly convex at maturity; surface dry, subtomentose to subvelutinous, medium brown to cinnamon-brown (near 5D7-5D5), becoming paler brown to yellowish brown (near 4B6) in age; margin incurved when young, typically yellowish, with a slight overlapping band of sterile tissue. FLESH white, slightly tinged with yellowish tan, unchanging when exposed; odor pleasant and somewhat fruity when fresh, strong and disagreeable in dried specimens; taste not distinctive. HYMENOPHORE tubular; tubes 2–6 mm deep, straw-colored (near 3B4), not staining when bruised but becoming brown in dried specimens (6D7); pores 2–3 per mm in immature specimens, ≤ 1 mm diam. in mature specimens; pore surface adnate to narrowly depressed near the stipe, uniformly deep cinnamon-brown (near 6D6) in mature specimens, unchanging or slightly darker brown when bruised; pores in immature specimens covered by a layer of whitish hyphae (partial veil or conspicuously developed cheilocystidia), this layer becoming greyish yellow (4B4-4C4) in dried specimens. STIPE 2.8–7.5 cm long, 1–2 cm broad, nearly equal or slightly enlarged downward, solid; surface dry and smooth, with a very pale yellow ground color (3A3-4B3) that typically becomes covered with cinnamon-brown (near 6D6) pruinosity, the pruinosity being sparse on young boletes and heavier on mature ones, and the stipe sometimes becoming vertically streaked with pruinosity; true reticulation

is often absent on young specimens, and when found on older stipes consisting of a fine network typically restricted to the uppermost centimeter or two. Basal mycelium white. STIPE FLESH solid, concolorous with the pileal flesh. SPORE PRINT olivaceous gray-brown (near 4D7) or olive-brown in fresh deposit.

BASIDIOSPORES $8.1\text{--}10.8 \times 3.6\text{--}4.5 \mu\text{m}$ ($n = 30$, $9.33 \pm 0.96 \times 3.9 \pm 0.35 \mu\text{m}$; $Q_m = 2.40 \pm 0.15$), fusoid to subcylindrical, smooth, grayish yellow in KOH; pale yellowish or with brighter wall in Melzer's. BASIDIA $22.5\text{--}27$ (-41.4) $\times 7.2\text{--}8.1 \mu\text{m}$, mostly clavate, few cylindro-clavate (2) 4-sterigmate, hyaline in KOH. BASIDIOLES $18\text{--}27$ (-38.7) $\times 6.3\text{--}8.1 \mu\text{m}$, mostly clavate. PLEUROCYSTIDIA $24.3\text{--}48.6 \times 5.4\text{--}11.7 \mu\text{m}$, most frequently lageniform, but also fusoid to fusoid-ventricose or ventricose-rostrate, few cylindrical, mostly with rounded tip, smooth and thin-walled, hyaline or with yellow contents in KOH, non-reactive in Melzer's. CHEILOCYSTIDIA $22.5\text{--}52.2 \times 5.4\text{--}9 \mu\text{m}$, fusoid, ventricose-rostrate, smooth, thin-walled, hyaline or with pale yellow contents in KOH, non-reactive in Melzer's; terminal elements covering the immature pores longer, $45\text{--}78.3 \times 5.4\text{--}8.4 \mu\text{m}$, cylindrical to sub-constricted, hyaline in KOH, non-reactive in Melzer's. PILEIPELLIS a tangled layer of erect to repent hyphae, $3.6\text{--}7.2 \mu\text{m}$ diam., hyaline to grayish yellow in KOH; end cells cylindrical. PILEUS TRAMA hyphae moderately to tightly interwoven, $4.5\text{--}19.8 \mu\text{m}$ diam., hyaline in KOH, non-reactive in Melzer's, smooth, thin-walled. STIPITIPELLIS hyphae $3.6\text{--}11.7 \mu\text{m}$ diam., parallel to subparallel to interwoven, hyaline in KOH, yellowish in Melzer's. CAULOCYSTIDIA $27\text{--}97.2 \times 6.3\text{--}9 \mu\text{m}$, clavate, fusoid, cylindrical, sub-lageniform, in clusters (fasciculate), hyaline in KOH, yellowish in Melzer's, thin-walled. DERMATOBASIDIA present, $19.8\text{--}28.8 \times 7.2\text{--}9 \mu\text{m}$, hyaline in KOH, contents non-dextrinoid to weakly dextrinoid in Melzer's. CLAMP CONNECTIONS absent.

MACROCHEMICAL REACTIONS: Pileipellis staining red-orange with KOH, slightly dull vinaceous with NH_4OH , and negative with FeSO_4 ; flesh not reacting (negative) with KOH, NH_4OH , and FeSO_4 .

ECOLOGY & DISTRIBUTION: solitary, scattered, or in groups on the ground in a sparsely grassy area near a mixed broadleaf woods with willow oak (*Quercus phellos*), white oak (*Quercus alba*), red maple (*Acer rubrum*), sweetgum (*Liquidambar styraciflua*), and dogwood (*Cornus florida*); August; reported only from the eastern Piedmont of North Carolina.

ADDITIONAL SPECIMENS EXAMINED: USA. NORTH CAROLINA: Granville Co., Town of Butner, John Umstead Hospital picnic area, under white oak, 29 July 2014, O.L. McConnell BOS 885 (CFMR; GenBank KM675997, KM675998); Durham Co., Edison Johnson Recreational Center, under willow oak, 7 August 2014, O.L. McConnell BOS 886 (CFMR); Duke Forest Jogging Trail, October 1993, HN 2295 (DUKE, as "*Tylophilus tabacinus*").

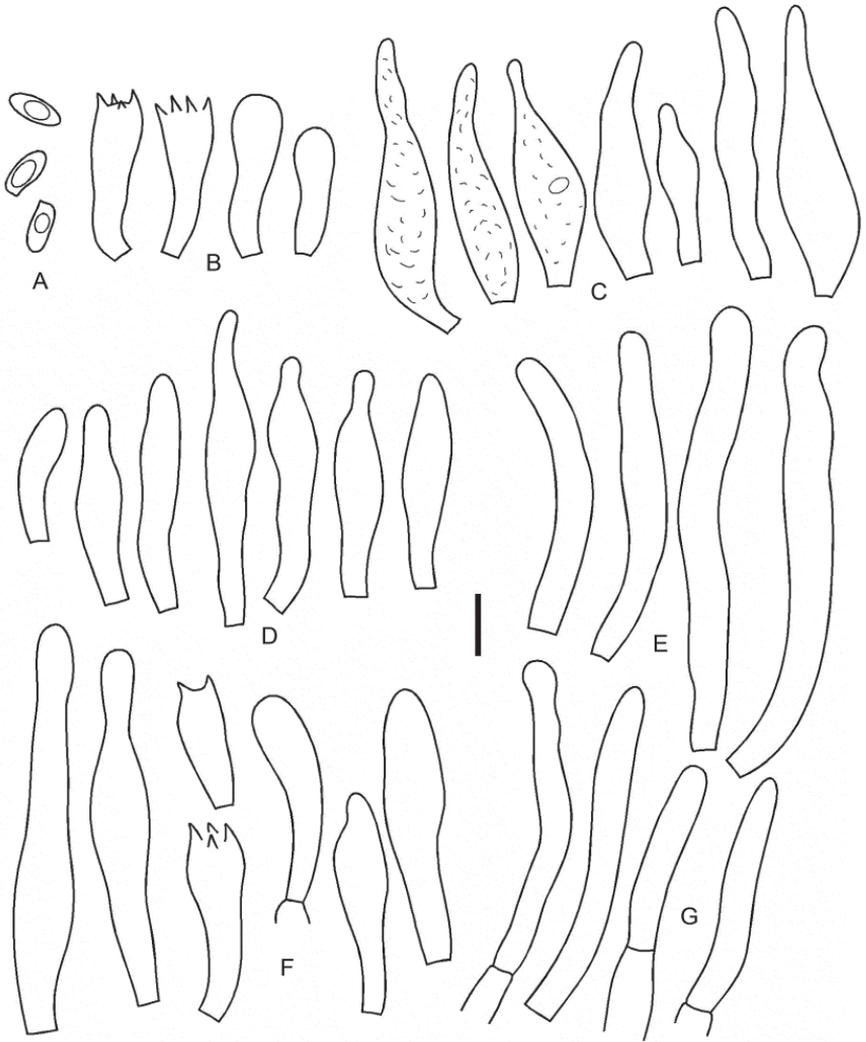


PLATE 2. *Boletus durhamensis* (holotype Both 4561; BOS 855). A. basidiospores; B. basidia and basidioles; C. pleurocystidia, some with yellow contents; D. cheilocystidia; E. cheilocystidia covering the immature pores; F. caulocystidia and dermatobasidia; G. terminal elements of the pileipellis. Scale bar = 10 μ m.

Additional characters of *B. durhamensis* based on dried specimens of collection HN 2295 (initially misidentified as “*Tylopilus tabacinus*”): PILEUS ≤ 12 cm diam., plane, uplifted, smooth to finely velutinous, pale brownish orange (5C5). FLESH cream. TUBES ≤ 11 mm deep, cinnamon-brown to raw sienna (6D7); pores

1–2 per mm yellowish brown (5D8), adnate to decurrent. STIPE 10 cm long, 3.5 cm diam., concolorous with pileus; finely reticulate at apex (11 mm from apex), otherwise smooth; caespitose. BASIDIOSPORES 9–10.8 × 3.6–4.5 μm, fusoid, smooth. BASIDIA 20.7–26 × 8.1–9 μm, clavate, 4-sterigmate, hyaline in KOH. PLEUROCYSTIDIA 49.5–62.1 × 9–9.9 μm, most frequently lageniform. CHEILOCYSTIDIA 24.3–31.5 × 6.3–9.9 μm, fusoid, ventricose-rostrate.

COMMENTS—The cinnamon-brown or brown pores would place *B. durhamensis* in *Boletus* sect. *Luridi* (Singer 1986), while the presence of a hyphal layer covering the immature pores (partial veil) and lack of any bluing reaction would place it in *B.* sect. *Boletus* (Singer 1986). Within *B.* sect. *Luridi*, a brown pore surface is also found in *B. vermiculosoides* A.H. Sm. & Thiers and *B. vermiculosus* Peck, which differ by turning blue when their flesh is exposed, the absence of a partial veil covering the immature pores, and their larger basidiospores. Within *B.* sect. *Boletus*, the presence of the partial veil on immature pores is shared with several species including *B. aereus* Bull., *B. edulis* Bull., *B. pinophilus* Pilát & Dermek, and *B. variipes* Peck, all of which can be distinguished from *B. durhamensis* by their possession of a true reticulum and larger basidiospores.

The presence of the partial veil covering the immature pores is also found in *Butyriboletus* (*Boletus* sect. *Appendiculati* Lannoy & Estadès; Šutara 2014). Šutara (2014), who refers to this veil as a layer of conspicuously developed cheilocystidia, indicates its presence in several *Butyriboletus* species: *B. appendiculatus* (Schaeff.) D. Arora & J.L. Frank, *B. fechtneri* (Velen.) Arora & J.L. Frank, *B. regius* (Krombh.) Arora & J.L. Frank, *B. roseogriseus* (J. Šutara et al.) Vizzini & Gelardi, and *B. subappendiculatus* (Dermek et al.) Arora & J.L. Frank. Šutara also mentions that it is not clear whether this cheilocystidia arrangement also occurs in American and Chinese *Butyriboletus* species, since neither Arora & Frank (2014) nor Li et al. (2014) mention it in their descriptions. When comparing *Butyriboletus* species with *Boletus durhamensis*, we found that most of those species have pileus with reddish tones, a yellow hymenophore that turns blue after bruising, and a stipe finely or strongly reticulate with reddish tones, and somewhat larger basidiospores. Other species that also appear to have this partial veil are *Sutorius australiensis* (Bougher & Thiers) Halling & N.A. Fechner and *S. eximius* (Peck) Halling et al.; however both species differ from *B. durhamensis* in their overall basidiocarp colors (which range from reddish brown to purple brown and dark gray), ornamented stipes, reddish brown spore print color, and larger basidiospores (Bessette et al. 2000, Halling et al. 2012).

BLASTn searches on GenBank were performed independently with the newly generated sequences. ITS and TEF BLASTn queries of *B. durhamensis*

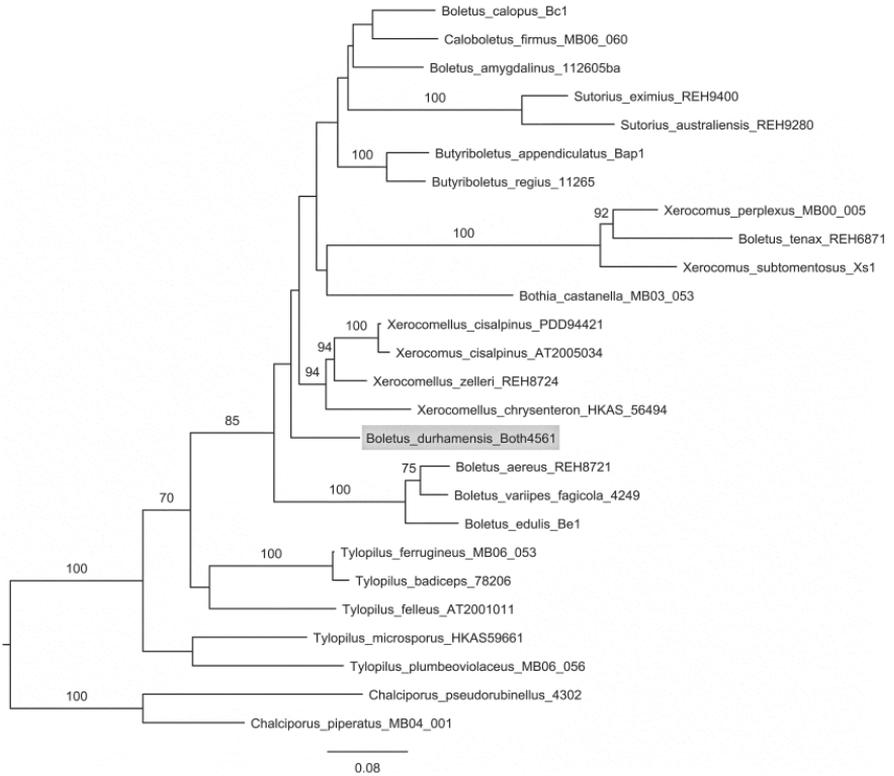


FIGURE 1. ML tree based on TEF sequences. Bootstrap values >70% are included above branches.

were uninformative at the generic level, with none of the searches exceeding 95% similarity with any available sequence. The nearest matches obtained were isolates representing the genus *Xerocomellus*. When comparing LSU sequences, the closest matches found were a sequence labelled as “*Tylophilus tabacinus*” (isolate HN2295) and a sequence of *Xerocomellus fennicus* (Harmaja) Šutara (isolate H126). In view of the search results and certain morphological characters (e.g., spore print color, presence of partial veil on immature pores), different bolete taxa were selected for additional sequence analyses to test the relationship of *B. durhamensis* with other boletes.

The phylogenetic relationship was inferred using three different datasets (ITS, LSU, TEF) and one phylogenetic analysis (ML). Results were based on the topology of the best-scoring ML tree. Datasets comprised: (ITS) 26 ingroup sequences, 578 characters; (LSU) 42 ingroup sequences, 875 characters;

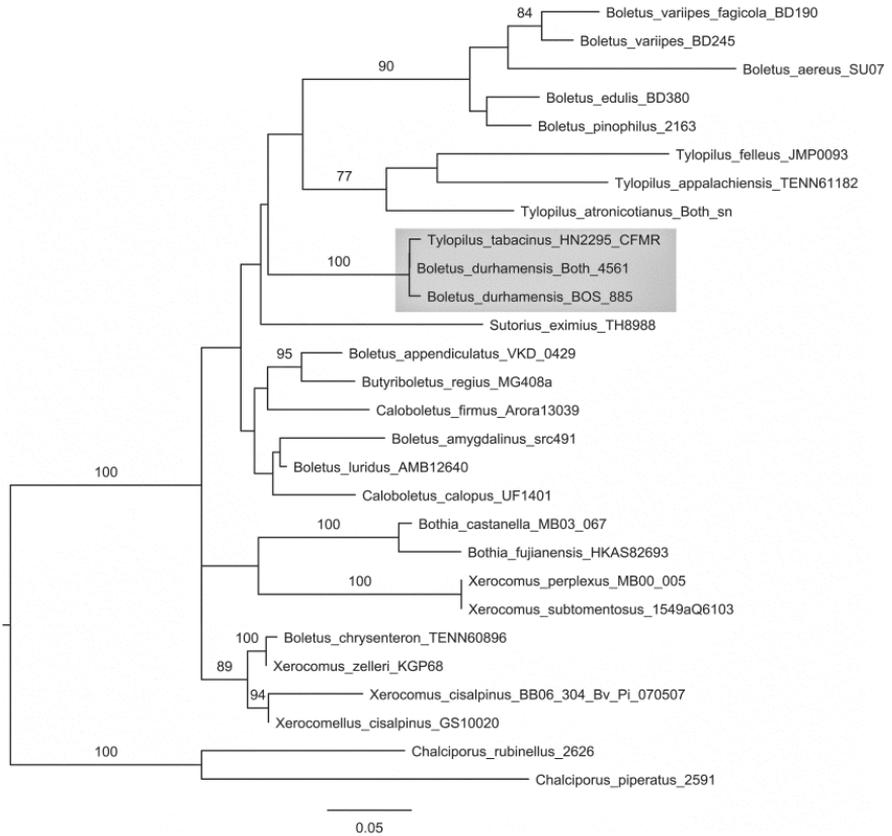


FIGURE 2. ML tree based on nrITS sequences. Bootstrap values >70% are included above branches.

(TEF) 24 ingroup sequences, 592 characters. The TEF analysis (FIG. 1) placed *B. durhamensis* on an independent branch within a moderately supported clade comprising species of *Boletus* s.s., *Bothia*, *Butyriboletus*, *Caloboletus*, *Sutorius*, *Xerocomellus*, and *Xerocomus*; this clade appears as a sister clade of *Tylopilus* s.s. The ITS and LSU analyses (FIGS. 2, 3) clustered *B. durhamensis* with isolates labelled as “*Tylopilus tabacinus*” (HN2295) in an independent clade and not grouping with any of the other bolete genera mentioned above.

Because of the similarity of these LSU sequences, we obtained the specimen of HN2295, which we examined morphologically and from which we generated ITS and LSU sequences. Comparison of the morphological and sequence data confirms that *B. durhamensis* and HN2295 are similar and that neither

of new genera may differentiate the well-supported groups obtained within each of them. Therefore, we suggest that more molecular data are still needed to clarify the evolutionary relationship and history within *Boletaceae*. Not all the described bolete species have been studied from a molecular perspective, and there are characteristics, such as the partial veil on the immature pores or the brown pore surface, that appear to have evolved independently in several different lineages of the family. In conclusion, further molecular studies of boletes are needed to determine whether *B. durhamensis* should remain in *Boletus* or be transferred to a new and as yet undescribed genus.

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