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A new genus of Boletaceae from eastern North America

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Abstract: *Bothia* is described as a new genus in the Boletaceae based on *Boletinus castanellus* described by C.H. Peck from eastern North America. A widespread, occasionally encountered taxon, *Bothia castanella* possesses a combination of macro- and microscopic features that has prompted past placement in seven different genera. Yet, as a species it is readily recognizable with its chestnut brown, dry pileus, decurrent, pale brown hymenophore with radially elongated tubes, a short, sometimes eccentric, exannulate stipe, yellow brown spore deposit and constant association with *Quercus*. Phylogenetic analyses of large subunit rDNA and BLAST searches using the ITS region confirm the placement of *B. castanella* as a unique generic lineage in the Boletaceae.

Key words: boletes, phylogeny, *Quercus*, ribosomal DNA

INTRODUCTION

C.H. Peck (1900) described *Boletinus castanellus* as a new species of Boletaceae based on a collection gathered in Sep (1889, fide Both 1993) in New Jersey by E.B. Sterling. The whereabouts of this collection is presently unknown. Nevertheless, the name for this taxon was adopted by Murrill (1909), who had not seen the type, but transferred it to *Boletinellus*. Murrill (1909) also distinguished the latter genus and *Boletinus* with radiating rows of tubes but noted that *Boletinus* differed by possessing an annulus. In a later treatment (Murrill 1914), *B. castanellus* remained in *Boletinellus*.

When Snell (1936) originally compared *Boletinus squarrosoides* Snell & Dick to *B. castanellus*, he noted that the former taxon differed by ''reddish brown color, terete scaliness, and yellow colors of the flesh, tubes and stipe.'' Coker and Beers (1943) maintained the two taxa as distinct but admitted that *B. squarrosoides* might be just a variety. Singer (1938) recognized both species as distinct and placed *B. castanellus* in *Gyrodon* and *B. squarrosoides* in *Phylloporus*. Later he (Singer 1945) noted that *B. castanellus* was not a *Boletinus* (a suilloid bolete in his classification) and a type no longer was preserved. Eventually Snell and Dick (1958) considered *B. squarrosoides* to be a synonym of *B. castanellus* after examination of additional collections and suggested that the former was just a manifestation of extremely variable morphology. In that paper, Snell indicated he had seen Peck's type of *B. castanellus* 20 y earlier but the type apparently was unavailable at that moment. Earlier Snell (1945) had examined Pennsylvanian specimens collected by Michener, which had been determined questionably as *Boletus pocono* Schwein. These specimens, at the time, were deposited in the ''… Division of Mycology and Disease Survey at Washington'' (Snell 1945, p 385). A search for this taxon name in the specimen database at Beltsville (BPI) did not result in any matches. However, Snell concluded that the Michener material he saw was attributable to *B. castanellus*, and if identical to Schweinitz's taxon, could be an earlier name for *B. castanellus*. Snell (1945) was also unable to locate type material among the Schweinitz collections in Philadelphia (PH). Queries to DWC, a known repository for Michener and Schweinitz collections, also did not turn up any collections (S. Bartholomew-Began pers com). In any event, application of the name *B. pocono* remains dubious because the inadequate protologue of Schweinitz (1832) is equivocal, and type or other original material is apparently not extant. Below we propose a new genus in the Boletaceae to accommodate *B. castanellus*. The combination of macro- and micromorphological features is foreign to any known bolete genus, and generic recognition is supported further with molecular phylogenetic data.

MATERIALS AND METHODS

Color terms in capital letters (e.g. Rood's Brown) are those of Ridgway (1912); in the case of spore deposits we used Kornerup and Wanscher (1983) with the color code 5C6. All microscopic structures were observed and measured from dried material revived in 3% KOH. Encrusting pigments on hyphae of the pileus were determined by reviving paradermal sections in 95% ethyl alcohol and

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mounting in distilled H_2O. The letter abbreviation *Q* refers to the mean length/width ratio measured from *n* basidiospores, and *x* refers to the mean length × mean width. Herbarium acronyms are from Holmgren et al (1990).

Molecular techniques.—Approximately 10–20 mg of fungal tissue (herbarium materials) were ground in liquid nitrogen and extracted in 600 µL of extraction buffer (1% SDS, 0.15 M NaCl, 50 mM EDTA) at 65 C for 1 h, purified with phenol-chloroform-isoamyl alcohol (25:24:1) and precipitated with 95% ethanol and 3 M NaCl. Crude DNA extracts were purified with GeneClean (Bio 101, La Jolla, California) and diluted with distilled water up to 500-fold for use as PCR templates. Sequence data of five *B. castanella* isolates (*MB03-053*, *MB03-067*, *Halling 6889*, *NY#28002*, *Halling 6636*) were generated from two regions: partial nuclear large subunit (nuc-lsu) rDNA bounded by primers LR0R and LR5 (Vilgalys and Hester 1990) and complete internal transcribed spacers 1 and 2 and the 5.8S rDNA (nuc-ITS rDNA) bounded by primers ITS1 and ITS4 (White et al 1990). Sequences generated in this study were submitted to GenBank (accession Nos. DQ867110-DQ867119). PCR reactions had a final volume of 25 µL and included 5 µL DNA solution, 2.5 µL PCR reaction buffer, 0.5 µL dNTP mix (0.2 mM), 25 pmol per primer and 0.3 U *Taq*DNA polymerase (Promega, Madison, Wisconsin). The amplifications were run in 37 cycles on a PTC-200 thermal cycler (MJ Research, Waltham, Massachusetts) with these parameters: denaturation 95 C (1 min), annealing 50 C (45 s), extension 72 C (1.5 min). PCR products were purified with Pellet Paint (Novagen, EMB Biosciences, San Diego, California). Sequencing reactions were set up with the same primer combinations used for PCR reactions using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v.3.1 (Applied Biosystems, California). Each reaction mix included 0.5 µL BigDye, 4 pmol primer, and 1.0 µL PCR template. The cycling program was: 96 C denaturation (2 min), 50 C annealing (15 s) and 60 C extension (4 min) in 35 cycles. Cycle sequencing products were purified with Pellet Paint and run on an ABI 3130 automated DNA sequencer (Applied Biosystems). Contiguous sequences were edited with Sequencher version 3.1 (GeneCodes Corp., Ann Arbor, Michigan).

Phylogenetic analyses.—ITS sequences of *B. castanella* were too divergent to be aligned to other species across the Boletaceae. BLAST searches were performed in the UNITE database (http://unite.zbi.ee; Kõljalg et al 2005) using the INSD option (blasting UNITE, GenBank, EMBL, and DDBJ simultaneously). Blasting the internal transcribed spacer 1 and internal transcribed spacer 2 separately resulted in ambiguous hits or no matches, the highly conserved 5.8S gene yielded inconclusive results across Boletales, and the complete ITS sequences closest to *B. castanella* were *Boletus erythropus* (score 377/727 bits, E value = e-104) and *Xerocomus pruinatus* (score 365/727 bits, E value = e100). Regions of high similarity mostly concerned the 5.8S gene and therefore we abandoned the assembly of an ITS dataset in the absence of reference data.

Newly generated nuc-lsu sequences of *B. castanella* specimens were aligned manually into the nuc-lsu dataset of Binder and Hibbett (2006) that includes 301 species of Boletales or roughly 30% of the described species in Boletales. This preliminary dataset was narrowed by consecutively running heuristic searches in PAUP* 4.0b10 (Swofford 2002), pruning redundant sequences until a comprehensive dataset including 168 species remained. The alignment was submitted to TreeBase (SN2982). The nuc-lsu dataset was analyzed on a Microway Linux Pro 9.2 Opteron AMD 246 cluster (Plymouth, Massachusetts) using PAUP* and MrBayes v3.1.1 (Ronquist and Huelsenbeck 2003). After the exclusion of 74 ambiguous characters the alignment consisted of 932 characters, 335 of which were parsimony informative. Heuristic searches under the parsimony criterion used equally weighted characters, 1000 random taxon addition sequences, tree bisection reconnection (TBR) branch swapping, MAXTREES set to 10 000. Constrained analyses, forcing *Bothia* isolates and the *Xerocomus chrysenteron* group to form a single clade, were run under the same conditions (see Commentary). Parsimony bootstrap analyses were performed for 1000 replicates, employing TBR branch swapping and 10 random taxon addition sequences. The best-fit likelihood model for the Bayesian approach was estimated with MODELTEST 3.06 (Posada and Crandall 2001) following the Akaike Information Criterion (AIC). Two parallel Metropolis-coupled Markow chain Monte Carlo (MC^3) analyses were run under the GTR + Γ + I model using one cold and three heated chains employing 3×10^6 generations, saving trees every 100th generation. A 50% majority rule consensus tree was used to calculate posterior probabilities in PAUP* including the stationary proportion of trees saved after likelihood scores converged to a stable equilibrium. The average standard deviation of split frequencies was 0.002763 at the end of the runs. The burn-in phase of excluded trees was evaluated at the end of each run by plotting log-likelihood scores as a function of generation time in Microsoft Excel 98 (Redmond, Washington).

TAXONOMY

Bothia Halling, T.J. Baroni, et Binder, gen. nov.

A generibus Boletacearum sporis in cumulo flavobrunneis, inamyloideis, levibus; hymenophoro decurrenti, boletinoido, brunneo pallido; contexto pallido immutabili; cystidiis nonfasciculatis; fibulae nullis; Querco consociato constanter in combinatum distinguenda.

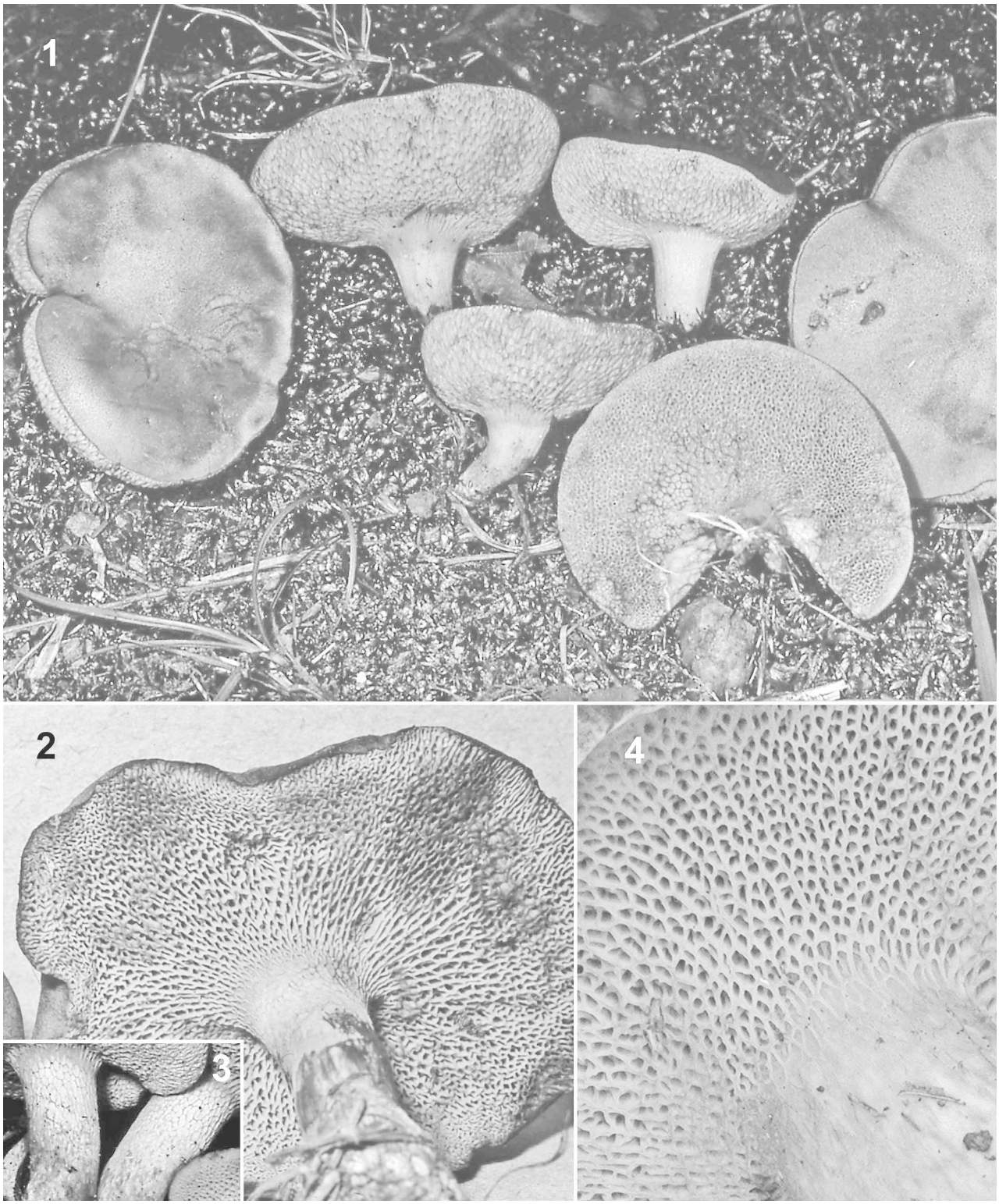
Typus: *Boletinus castanellus* Peck.

Etymology: In honor of Ernst E. Both, promoter, facilitator and consummate student of boletology.

Bothia castanella (Peck) Halling, T. J. Baroni, & Binder, comb. nov. FIGS. 1–7

Boletinus castanellus Peck, Bull Torrey Bot Club 27:613. 1900.

Boletinellus castanellus (Peck) Murrill, Mycologia 1:8. 1909.



FIGS. 1–4. Habits of *Bothia castanella*. 1. *Baroni 5331*. × 1/2. 2. Decurrent and bruised hymenophore. *Halling 6636* (Neotype) × 1/2. 3. Reticulate stipe, from *Roody s.n.* × 1/3. 4. Close-up of boletinoid hymenophore with compound pores. *Halling 8669* × 2.

Gyrodon castanellus (Peck) Singer, Rev Mycol (Paris) 3:172. 1938.

Xerocomus castanellus (Peck) Snell & Dick, Mycologia 50:58. 1958.

Suillus castanellus (Peck) Smith & Thiers, Contr monogr N Amer sp *Suillus*. 26. 1964.

Chalciporus castanellus (Peck) L.D. Gómez, Rev Biol Trop 44(4):78. 1996 (1997) *nom. inval.* (Art. 33.3).

Holotype: USA. New Jersey. September, E.B. Sterling (NYS), apparently missing. NEOTYPUS: *Halling 6636* (NY), here designated.

Boletinus squarrosoides Snell & Dick in Snell, Mycologia 28:468. 1936.

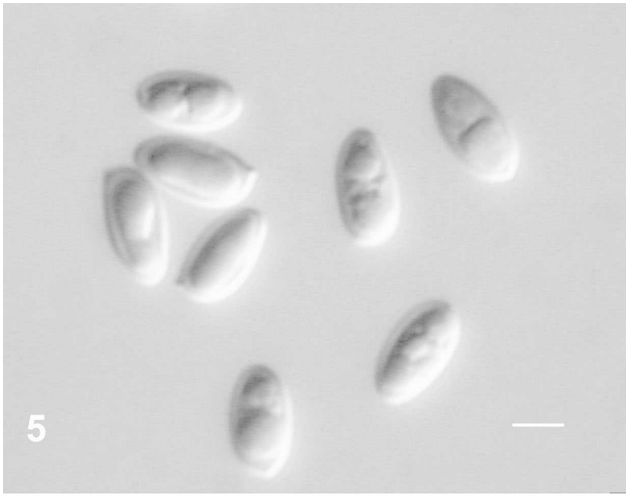
Phylloporus squarrosoides (Snell & Dick) Singer, Rev Mycol (Paris) 3:170. 1938.

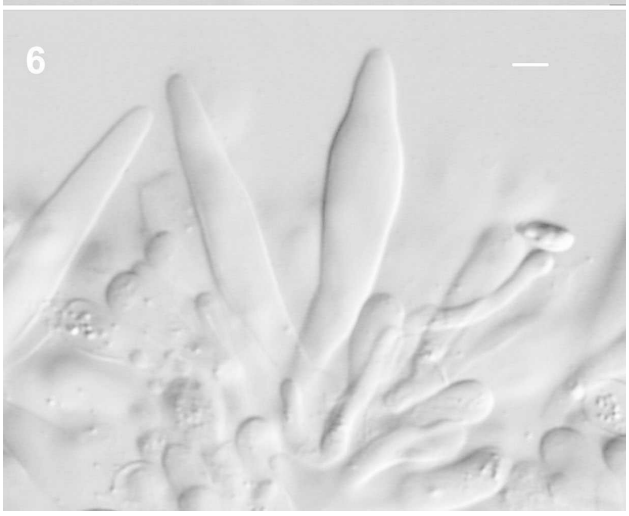
Xerocomus squarrosoides (Snell & Dick) Singer, Farlowia 2:295. 1945.

Holotype: USA. Pennsylvania. Reading, Mt Neversink, *E.A. Dick 532* (BPI! #783691).

The following description of macroscopic features includes observations compiled by Ernst Both, for whom the new genus is named.

Pileus 30–90(–100) mm broad, convex to plano-convex, becoming depressed on the disk in age, dry, coarsely tomentose to granular tomentose or with tomentum aggregated into fibrils, at times ±appressed fibrillose, sometimes with an irregular to wavy margin, dark reddish brown becoming dark brown to very dark brown (between Russet and Cinnamon Brown) or very dark chocolate brown (Carob Brown to Chestnut Brown) when young, fading at times to Rood's Brown or Mikado Brown to Sayal Brown, sometimes with margin nearly blackish brown. *Flesh* soft textured, white to dingy whitish, unchanging or slowly becoming a dingy pale rust color, with odor absent and taste mild or ±acidulous. *Hymenophore* tubulose, decurrent when young, shallowly depressed around stipe with age but still decurrent, with tubes 4–5(–10) mm long; pores boletinoid, coarse and angular to hexagonal, usually compound, 3–4 mm broad, sublamellate at the stipe, dull pallid brown to cinnamon brown with pinkish cinnamon tones (near Cinnamon Buff to Clay Color), usually staining brown to dark brown or dark rusty brown (Cinnamon Brown, Cinnamon, Sayal Brown). *Stipe* 20–60 mm long, (10–)13–18 mm broad above, 8–16 mm broad below, equal or tapering downward, occasionally broader at apex and base, central or eccentric, dry, usually paler than pileus and approaching pale vinaceous brown or spotted sooty brown, sometimes stained reddish at the base, otherwise bruising blackish brown when fresh, coarsely and shallowly reticulate in upper half or confined to apex, pseudoreticulate to striate ridged and minutely tomentose to densely and finely furfuraceous below, with reticulum darker brown than background color, with white basal mycelium, with interior solid, brownish to pale cinnamon toward the base.





FIGS. 5–6. Microscopic features. 3. Basidiospores. 4. Hymenial cystidia. *Halling 6636* Bar = 5 μm.

Spores yellow brown (5C6) in fresh deposit, (7.7–)8.4–10.5(–11.3) × (3.5–)4.2–4.9(–5.6) μm, n = 20, x = 8.8 × 4.5 μm, Q = 1.98, ellipsoid to long ovoid, inamyloid, hyaline to pale brownish yellow, smooth, thin-walled. *Basidia* 25–35 × 7–9 μm, clavate, 4-sterigmate, hyaline or rarely with brownish yellow contents, with coagulated dextrinoid content in Melzer's. *Hymenial cystidia* present and conspicuous as pleuro- and cheilocystidia, the latter especially abundant, fusoid to fusoid ventricose or sometimes ventricose rostrate, hyaline or rarely with pale brownish yellow content, inamyloid, smooth, thin-walled, 45–70 × 7–12 μm. *Hymenophoral trama* bilateral, with lateral elements 5–9 μm wide, hyaline, or rarely with oleiferous elements, subgelatinized with age; a differentiated central zone not apparent. *Pileus*

FIG. 7. Phylogenetic relationships of *Bothia castanella* inferred from nuc-lsu sequences. Shown is one of the equally parsimonious trees (3222 steps, CI = 0.228, RI = 0.598). The tree is rooted with four *Athelia* species. Bootstrap frequencies >50% are provided at supported branches. Posterior probabilities of 0.95–1.0 obtained in the Bayesian analyses are indicated by bold nodes, lower posterior probabilities were not considered to suggest significant support.

trama interwoven, hyaline, inamyloid, with elements 5–14 μm wide, smooth, thin-walled. *Pileipellis* a trichodermium, with erect to suberect, cylindric hyphae, smooth, hyaline or sometimes with yellowish brown contents in KOH, hyaline or with orange brown contents and occasional orange brown encrusting pigment in Melzer's, inamyloid, with dark brown, dense, plaque-like and spiral encrustations in dH_2O, dissolving in dilute alkali, thin-walled, 4–7 μm wide. *Stipitipellis* hymeniform, with clavate cells and occasional basidia, rarely with scattered caulocystidia. *Stipe trama* parallel, cylindrical, vertically oriented, hyaline, inamyloid, with scattered oleiferous elements. *Clamp connections* absent.

Habit, habitat, and distribution: solitary to gregarious, rarely cespitose, under oaks, with birch, beech, hickory, eastern white pine, and hemlock sometimes present. Currently known in the eastern United States from the Carolinas northward to New York and New England and westward to Minnesota, appearing Jul–Sep. William Roody (pers com) has recorded eight specimens from West Virginia on deposit in DEWV.

Specimens examined: USA. CONNECTICUT. Old Lyme, *H.L. Wells* (NY). MASSACHUSETTS. Middlesex County, Stow, 3 Sep 1910, *S. Davis* (det. C.H. Peck) (NYS); Hampshire County, Amherst, Wildwood Cemetery, 23 Jul 1958, *H.E. Bigelow 7014* (NY); Canton, 4 Aug 1933, *D.H. Linder* (FH); Lincoln, De Cordova Museum, 15 Aug 2003, *M. Binder 03-067* (CUW); Paxton, Rutland State Park, 13 Aug 2003, *M. Binder 03-053* (CUW). MINNESOTA. Minneapolis, *M.S. Whetstone* (*C.G. Lloyd 57479*) (BPI #840783). NEW YORK. Bronx County: Bronx, New York Botanical Garden, 40°51′29.2″N, 73°52′39.7″W, 55 ft, 12 Sep 1985, *R.E. Halling 4582c* (NY), 30 Aug 1991, *R.E. Halling 6636* (NEOTYPE: NY), 40°51′40.5″N, 73°52′44″W, 92 ft, 9 Sep 2005, *R.E. Halling 8669* (NY), Bronxwood Park, 8 Aug 1908, *W.A. Murrill* (NY), Van Cortlandt Park, 31 Aug 1991, *S. Stein* (NY); Cornwall, Black Rock Forest, 12 Jul 1933 *A.B. Hatch 601* (det. R. Singer) (FH); Erie County, Town of Orchard Park, Chestnut Ridge Park, 12 Aug 1983, *E.E. Both 2470* (BUF, NY), 10 Jul 1987, *E.E. Both s.n.* (NY #28002), 23 Jul 1994, *E.E. Both 3655* (BUF, NY), 31 Jul 1994, *E.E. Both s.n.* (BUF, NY), 9 Aug 1995, *E.E. Both 3799* (BUF, NY), 28 Jul 1986, *T. J. Baroni 5331* (CORT); St. Lawrence County, Cranberry Lake, 2 Sep 1985, *S. Stein* (NY). NORTH CAROLINA. Asheville, *H.C. Beardslee* (NY); Macon County, near Franklin, Coweeta Hydrologic Research Area, Ball Creek Road, 23 Jul 1987, *T.J. Baroni 5566* (CORT), Coweeta Hydrologic Research Area, 14 Aug 1987, *T.J. Baroni 5712* (CORT). OHIO. Columbia County, Fredericktown, Vodry Estate, 10 Aug 1984, *R.E. Halling 3787* (NY). PENNSYLVANIA. Warren County, 4 mi S of Youngsville, 17 Sep 1947, *L.K. Henry* (NY). SOUTH CAROLINA. Oconee County, off Hwy 197, Elliott Rock Trail behind Fish Hatchery, 12 Aug 1987, *T.J. Baroni 5714* (CORT); Oconee State Park, 18 Aug 1992, *R.E. Halling 6889* (NY). VIRGINIA. Falls Church, 31 Jul 1908, *W.A. Murrill* (NY); Giles County, Mountain Lake 2–4 Sep 1936, *D.H. Linder* (fide W.H. Snell) (FH). Locality unknown: Boston Mycological Club, Sep 1936, det. *W.H. Snell* (FH).

Commentary: *Bothia* can be characterized most easily by the more or less uniformly brown to dark brown colors, soft texture, conspicuously boletinoid and decurrent, pale brown hymenophore, reticulate/pseudoreticulate stipe, and pale brown spore deposit. Basidiome appearance in consistent association with oak seems diagnostic also. An excellent color illustration appears in Roody (2003).

Phylogenetic analyses unequivocally place *Bothia castanella* in the Boletaceae (FIG. 7) with strong support (bootstrap = 96%, posterior probability = 1.0) and relationships to Paxillaceae, Boletinellaceae and Suillaceae can be rejected. A sister group of *B. castanella* was not resolved in this study; however the monotypic genus formed a clade with the *Xerocomus chrysenteron* group that receives no statistical support. We ran constrained analyses forcing *Bothia* and the *X. chrysenteron* group (*X. chrysenteron*, *X. cisalpinus*, *X. dryophilus*, *X. porosporus*, *X. pruinatus*, *X. riparielllus*, *X. truncatus* and allies in FIG. 7) into a single clade and evaluated monophyly comparing constrained and unconstrained trees with the KH-test (Kishino and Hasegawa 1989). Although a relationship was not rejected statistically ($P = 0.204$), only a few morphological characters (e.g. epicuticular hyphae with spiral incrustations) would support a connection between *Bothia* and the *X. chrysenteron* group.

The morphological features displayed by *B. castanella* have contributed to its placement in several genera. Overwhelming reliance on one feature over another (e.g. hyphal arrangement in the hymenophoral trama) apparently seems to have played a part as well. First, the radially elongated tubes is a feature seen in some *Suillus* species (*Boletinus* is considered a synonym), *Gyrodon* and *Boletinellus*. However *Bothia castanella* lacks the clustered hymenial and caulocystidia with amorphous pigment seen in *Suillus* and ecologically is not associated with Pinaceae. Unlike *Gyrodon* and *Boletinellus*, *B. castanella* does not produce olive brown spores in deposit, is not cyanescent, lacks clamp connections and the hymenophore is a pale brown (not yellow). The arrangement of the tube trama lacks a central zone and thus would be of the *Phylloporus*-type in the sense of Singer (1986), an overriding factor directing placement in *Xerocomus* for some (Singer 1986, Snell and Dick 1958). Morphologically *Xerocomus* produces an olive brown spore deposit and lacks other cohesive features (Oolbekink 1991). Thus the unique combination of morphological features and apparent mycorrhizal association exhibited by *Bothia* further support generic recognition.

The dense dark brown encrustations of the pileus

surface hyphae dissolve readily in 3% KOH and 10% NH_4OH, producing only brownish or pale vinaceous discolorations in the mounting medium. These pigments slowly dissolve leaving hyaline or yellowish brown intracellular pigments in the surface hyphae. This reaction is useful in helping to identify dried herbarium materials of this species.

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