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ORIGINAL ARTICLE

Paxilloboletus gen. nov., a new lamellate bolete genus from tropical Africa

Sylvestre A. Badou[1] · Brendan Furneaux[2,3] · André De Kesel[4] · Faheema Kalsoom Khan[2] · Roel D. Houdanon[1] · Martin Ryberg[2] · Nourou S. Yorou[1]

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Abstract
This study presents *Paxilloboletus* gen. nov., a new lamellate bolete genus represented by two tropical African species, *Paxilloboletus africanus* sp. nov. and *Paxilloboletus latisporus* sp. nov. Although the new taxa strongly resemble *Paxillus* (Paxillaceae), they lack clamp connections and form a separate generic clade within the Boletaceae phylogeny. The new species are lookalikes, morphologically only separable by their spore morphology. Descriptions and illustrations of the new genus and new species are given, as well as comments on ecology, distribution, and morphological differences with other gilled Boletaceae.

Keywords 3 new taxa · Boletaceae · Africa · Lamellate hymenophore · Morphology · Phylogeny · Taxonomy

Introduction

Over the years, our survey of bolete diversity in tropical Africa (here restricted to suborder Boletineae) has resulted in a collection of specimens both with poroid and with lamellate hymenophores. Among the stipitate-pileate Boletineae, poroid forms are the most common, but there are several well-separated lamellate clades dispersed among the clades with poroid hymenophores (Farid et al. 2018; Zhang and Li 2018). The genera *Paxillus* Fr. (Paxillaceae) and *Phylloporus* Quél. (Boletaceae) are the most well recognized and globally distributed Boletineae with lamellate hymenophores. Over the years several *Paxillus* and *Phylloporus* species have been recombined into new genera (e.g., Gilbert 1931; Singer 1942; Bresinsky et al. 1999; Farid et al. 2018; Vadthanarat et al. 2019), some outside the Boletineae, and a few new species have also been described in new lamellate genera (Singer and Digilio 1952; Zhang and Li 2018), resulting in a total of six lamellate lineages in the Boletineae: *Paxillus*, *Phylloporus*, *Phyllobolites* Singer (Boletaceae), *Phylloboletellus* Singer (Boletaceae), *Phylloporopsis* Angelini, Farid, Gelardi, ME Sm., Costanzo & Vizzini (Boletaceae), and *Erythrophylloporus* Ming Zhang & TH Li (Boletaceae). However, hitherto only *Paxillus* and *Phylloporus* are known from tropical Africa, with 6 of the 38 known *Paxillus* species and 15 of the 86 known *Phylloporus* species occurring in the region (Kirk 2021; Heinemann and Rammeloo 1986, 1987a). Based on a combination of phylogenetic and morphological characters, we here introduce the seventh lamellate genus in the Boletineae, *Paxilloboletus* gen. nov. (Boletaceae), so far only known from tropical Africa.

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✉ Martin Ryberg
martin.ryberg@ebc.uu.se

[1] Tropical Mycology and Plants - Soil Fungi Interactions, Laboratory of Ecology, Botany and Plant Biology, Faculty of Agronomy, University of Parakou, 03 Box 125, Parakou, Benin

[2] Systematic Biology Program, Department of Organismal Biology, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

[3] Evolutionary Biology Program, Department of Ecology and Genetics, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

[4] Meise Botanic Garden, Nieuwelaan 38, 1860 Meise, Belgium

Materials and methods

Sampling, microscopy and morphology

Basidiomata were collected in several tropical African countries (Bénin, Democratic Republic of the Congo, Guinea,

Tanzania, Togo and Zambia), during the rainy season. Specimens were photographed in situ and field notes were taken of their macroscopic characters. Sectioned specimens were dried for 24–48 h in a field drier at 40–60 °C and immediately stored afterwards in airtight bags (type Minigrip), with or without silica gel. All studied material is deposited in the herbaria of the University of Parakou (UNIPAR), the University of Uppsala (UPS), and Meise Botanic Garden (BR) (abbreviations in Index Herbariorum; Thiers, continuously updated).

Macroscopic descriptions are based on available field notes, photographs, and in some cases on observations made on the exsiccata (Badou et al. 2018). Color codes and names have been taken from Methuen Handbook of Color (Kornerup and Wanscher 1978). Macrochemical reactions (bluing) of the context and hymenophore of exsiccata were determined using Melzer's reagent. Microscopic structures were examined in 10% ammonia, with or without Congo red. Observations, measurements, and drawings of microscopic structures were carried out using an Olympus BX51 or a Leica DM 2700 M light microscope equipped with a digital camera and drawing tube.

Dimensions of microscopic structures are given as a range. Basidiospore dimensions are presented in the following format: **(a–) b– c– d(–e)**, where **c** is the average, **b = c – 1.96 * SD** (5th percentile) and **d = c + 1.96 * SD** (95th percentile), **a** the extreme minimum and **e** the extreme maximum value. **Q** is the length/width ratio based on at least 50 spores and is presented in the same format as the spore length and width. Pellis structures were studied from a fine surface scalp, taken either halfway between the center and the margin of the cap (pileipellis), and/or halfway up the stipe (stipitipellis).

For scanning electron microscopy (SEM), a portion of hymenophore was dried in a critical point dryer (Leica EP CDP 300), mounted on a SEM stub and coated with a layer of approximately 6 nm Pl/Pd using a High-Resolution Fine Sputter Coater for FE–SEM (JFC–2300HR Coating Unit, JEOL). Scanning electron microscopy was carried out with a JEOL JSM–7100FLV Field Emission SEM (Meise Botanic Garden).

DNA extraction, amplification, and sequencing

Genomic Deoxyribonucleic Acid (DNA) was extracted from dried specimens using the DNeasy Plant Kit (Quiagen). A total of five regions were sequenced, including the nuclear ribosomal internal transcribed spacer (ITS), partial nuclear ribosomal large subunit (LSU), and fragments of three protein-coding genes: the largest and second-largest subunits of RNA polymerase II (*RPB*1 and *RPB*2, respectively) and transcription elongation factor 1-α (*TEF*1–α). ITS sequencing was attempted for all studied specimens, and *RPB*2 sequencing was subsequently attempted for the majority, with special focus on specimens from the Democratic Republic of the Congo, where higher variability in ITS was observed. The remaining three genes were sequenced for four specimens with non-identical ITS sequences. The chosen regions were amplified using primer pairs ITS1 (White et al. 1990) and LB-w (Tedersoo et al. 2008) for ITS; LR0R and LR5 (Hopple and Vilgalys 1994) for LSU; RPB1–B–F and RPB1–B–R (Wu et al. 2014) for *RPB*1; fRPB2–5F (Liu et al. 1999) and bRPB2–7R (Matheny 2005) for *RPB*2; and EF1–983F and EF1–2218R (Rehner and Buckley 2005) for *TEF*1–α. Thermocycling protocols were according to Wu et al. (2014). After amplification, PCR products were cleaned with ExoSAP–IT. Sequencing was performed by Macrogen Europe (Amsterdam, the Netherlands) using the same primers as for amplification, except that ITS4 was used as a reverse sequencing primer for ITS, and internal primers bRPB2–6F (Matheny 2005) and bRPB2–6R2 (Matheny et al. 2007) were added for *RPB*2. Reads were trimmed and assembled using Prosed (version 3.2; Filatov 2002) or Staden (Staden et al. 2000). Sites where chromatograms show clear double peaks indicative of heterozygosity or other intragenomic variation were hand-annotated with IUPAC ambiguity codes.

Alignment and phylogenetic inference

The four-gene (LSU, *RPB*1, *RPB*2 and *TEF*1–α) data set of Gelardi et al. (2015) was used as a base alignment to place the specimens phylogenetically. Publicly available sequences of the lamellate genera *Phylloporopsis* (two specimens; Farid et al. 2018), *Phylloboletellus* (one specimen; Binder and Hibbett 2006) and *Erythrophylloporus* (six specimens; Zhang and Li 2018; Vadthanarat et al. 2019; Haelewaters et al. 2020) were added to the matrix, along with the sequences of our specimens. No sequence data is available for *Phyllobolites.* After preliminary results indicated the placement of our specimens in subfamily Boletoideae, we also added publicly available sequences from the additional genera *Afrocastellanoa*, *Durianella*, *Guyanaporus*, *Heliogaster*, *Indoporus*, *Mackintoshia* and *Nigroboletus* (one specimen each), all previously placed in subfamily Boletoideae (Desjardin et al. 2008; Gelardi et al. 2015; Henkel et al. 2016; Orihara et al. 2010; Orihara and Smith 2017; Parihar et al. 2018; Smith et al. 2015), which were not present in the base alignment. Accession numbers for these added sequences are in Table S1. Taxonomic identity of specimens was updated with reference to the current annotation of the sequences on GenBank, synonymy from Species Fungorum (Kirk 2021), as well as recent literature (Chai et al. 2019; Farid et al. 2018; Gelardi et al. 2015; Gelardi 2020; Henkel et al. 2016; Orihara et al. 2016;

Orihara and Smith 2017; Parihar et al. 2018; Smith et al. 2015; Zhu et al. 2015; Wu et al. 2016a, 2016b, 2019; Zhang et al. 2019; Vadthanarat et al. 2019).

For each of the four genes (LSU, *RPB*1, *RPB*2 and *TEF*1–α), new sequences were aligned separately using MUSCLE in Geneious (version 10.2.3; Drummond et al. 2010) with default settings, and trimmed to match the base alignment. The new sequences were then sequentially added to the base alignment using MAFFT with the –add option (version v7.453; Katoh and Frith 2012; Katoh and Standley 2013). These separate alignments of each gene were concatenated, and a maximum likelihood phylogenetic tree was built using the online version of RAxML XSEDE2 (version 8; Stamatakis 2006) in CIPRES portal (Miller et al. 2010) with rapid bootstrapping according to the MRE_IGN stopping criterion and the GTRGAMMA substitution model.

To more closely examine relationships among our specimens, we assembled a two-gene ITS and *RPB*2 dataset including our specimens. We also performed a BLAST search of publically available ITS sequences from GenBank and Unite, and added hits with > 90% sequence identity to any of our specimens across the full ITS region. In contrast to the four-gene dataset, where only approximately 570 bp of *RPB*2 between conserved domains 6 and 7 were included, the two-gene dataset included the full length of our *RPB*2 sequences, approximately 1, 100 bp from conserved domain 5–7. As outgroups, we included publically available ITS and *RPB*2 sequences from three additional members of Boletoideae for which corresponding long *RPB*2 sequences were available: *Boletus edulis*, *Porphyrellus porphyrosporus*, and *Strobilomyces strobilaceus* (Table S2). LSU sequences were also used for the outgroups to extend the ITS sequences to the LB–w binding site to match the sequences generated in this study. The two genes were aligned separately with MAFFT in generalized affine (e–ins–i) mode for ITS, and global (g–ins–i) mode for *RPB*2. The two alignments were trimmed and then concatenated, and a maximum likelihood tree was generated as above. Trees were visualized using the ggtree package (version 2.4.1; Yu et al. 2017) in R (version 4.0.3; R Core Team 2020).

Results

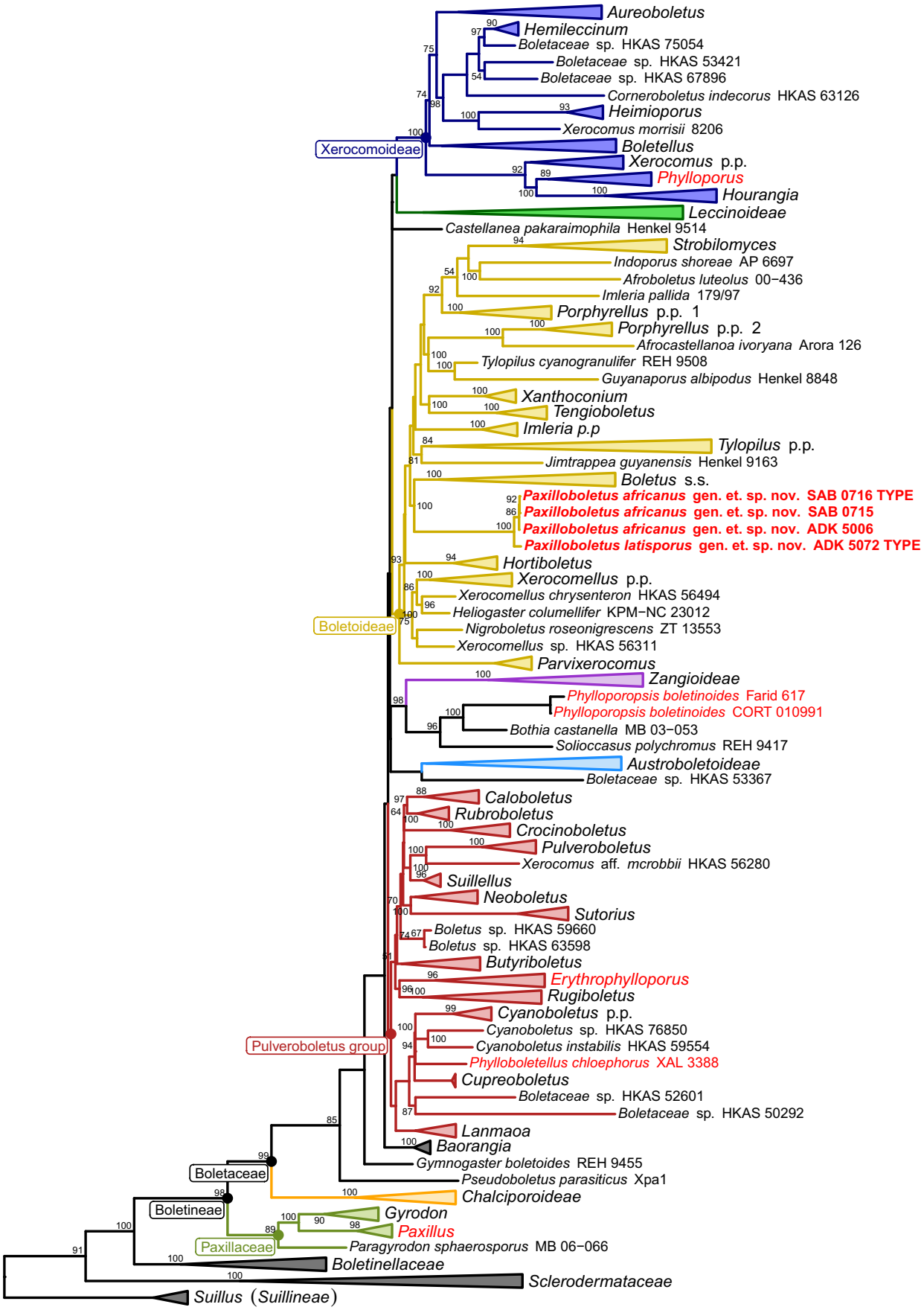
DNA analyses

We obtained ITS sequences for eleven specimens from four countries in West and Central Africa (Table 1). *RPB*2 sequences were obtained for a subset of eight specimens, and LSU, *RPB*1, and *TEF*1–α were obtained for a further subset of four specimens (Table 1). A BLAST search of GenBank revealed one additional ITS + LSU sequence (accession number FR731194; Tedersoo et al. 2011), from an ectomycorrhizal root tip in Madagascar, which was a 100% match to ADK–5006 from the Democratic Republic of the Congo. The ITS sequences fell into two clusters, each with greater than 99.5% sequence similarity, and with ~ 97% similarity between the two clusters. Variable sites within each cluster (max 2) were ambiguous in some of the specimens, indicating genetic mixing within clusters. *RPB*2 sequences had > 99.9% similarity within the same two clusters, and ~ 99% similarity between clusters. We consider this consistent clustering of the specimens using two unlinked loci to be evidence that the clusters represent two closely related but distinct species.

The topology of our four-gene ML–tree (Fig. 1; version with all tips expanded in Fig. S1) is in general agreement with previous results. Although our phylogeny places *Phylloboletellus* within *Cyanoboletus* Gelardi, Vizzini and Simonini with 94% bootstrap support, this result is based only on LSU, and the very short branch lengths at the root of *Cyanoboletus* suggest that this placement may not be reliable.

Table 1 Newly generated sequences. Holotype specimens are **bold**

Species	Specimen	Country	ITS	LSU	*RPB*1	*RPB*2	*TEF*1–α
Paxilloboletus africanus	ADK–2292	Benin	MZ702467	–	–	MZ707873	–
	ADK–3703	Benin	MZ702468	–	–	MZ707874	–
	ADK–4368	Togo	MZ702472	–	–	–	–
	ADK–5006	DR Congo	MZ702469	MZ702478	MZ707877	MZ707875	MZ707867
	KIT–00524	Guinea	MZ702474	–	–	–	–
	SAB–0715	Guinea	MZ702473	MZ702480	MZ707876	MZ707868	MZ707864
	SAB–0716	Guinea	MZ702470	MZ702479	MZ707878	MZ707869	MZ707865
	SYN–3877	Guinea	MZ702471	–	–	–	–
Paxilloboletus latisporus	**ADK–5072**	DR Congo	MZ702475	MZ702481	MZ707879	MZ707870	MZ707866
	ADK–5117	DR Congo	MZ702476	–	–	MZ707871	–
	ADK–5493	DR Congo	MZ702477	–	–	MZ707872	–


Aureoboletus
Hemileccinum
Boletaceae sp. HKAS 75054
Boletaceae sp. HKAS 53421
Boletaceae sp. HKAS 67896
Corneroboletus indecorus HKAS 63126
Heimioporus
Xerocomus morrisii 8206
Xerocomoideae
Boletellus
Xerocomus p.p.
Phylloporus
Hourangia
Leccinoideae
Castellanea pakaraimophila Henkel 9514
Strobilomyces
Indoporus shoreae AP 6697
Afroboletus luteolus 00−436
Imleria pallida 179/97
Porphyrellus p.p. 1
Porphyrellus p.p. 2
Afrocastellanoa ivoryana Arora 126
Tylopilus cyanogranulifer REH 9508
Guyanaporus albipodus Henkel 8848
Xanthoconium
Tengioboletus
Imleria p.p
Tylopilus p.p.
Jimtrappea guyanensis Henkel 9163
Boletus s.s.
Paxilloboletus africanus gen. et. sp. nov. SAB 0716 TYPE
Paxilloboletus africanus gen. et. sp. nov. SAB 0715
Paxilloboletus africanus gen. et. sp. nov. ADK 5006
Paxilloboletus latisporus gen. et. sp. nov. ADK 5072 TYPE
Hortiboletus
Xerocomellus p.p.
Xerocomellus chrysenteron HKAS 56494
Heliogaster columellifer KPM−NC 23012
Boletoideae
Nigroboletus roseonigrescens ZT 13553
Xerocomellus sp. HKAS 56311
Parvixerocomus
Zangioideae
Phylloporopsis boletinoides Farid 617
Phylloporopsis boletinoides CORT 010991
Bothia castanella MB 03−053
Solioccasus polychromus REH 9417
Austroboletoideae
Boletaceae sp. HKAS 53367
Caloboletus
Rubroboletus
Crocinoboletus
Pulveroboletus
Xerocomus aff. mcrobbii HKAS 56280
Suillellus
Neoboletus
Sutorius
Boletus sp. HKAS 59660
Boletus sp. HKAS 63598
Butyriboletus
Erythrophylloporus
Rugiboletus
Cyanoboletus p.p.
Cyanoboletus sp. HKAS 76850
Pulveroboletus group
Cyanoboletus instabilis HKAS 59554
Phylloboletellus chloephorus XAL 3388
Cupreoboletus
Boletaceae sp. HKAS 52601
Boletaceae sp. HKAS 50292
Lanmaoa
Baorangia
Gymnogaster boletoides REH 9455
Boletaceae
Pseudoboletus parasiticus Xpa1
Chalciporoideae
Boletineae
Gyrodon
Paxillus
Paxillaceae
Paragyrodon sphaerosporus MB 06−066
Boletinellaceae
Sclerodermataceae
Suillus (Suillineae)

◂**Fig. 1** ML tree of Boletineae based on LSU, *TEF*1–α, *RPB*1 and *RPB*2. Bootstrap support values > 50 are shown near nodes. Names of lamellate taxa are red, and new sequences for this study are **bold**. Monophyletic taxa represented by multiple specimens, and not containing mixed lamellate and non-lamellate taxa, are collapsed. Naming and coloring of subfamily-level clades within Boletaceae, as well as rooting, follow Wu et al. (2014)

Our four specimens SAB–0715, SAB–0716, ADK–5006, and ADK–5720 clustered together with 100% bootstrap support in the four-gene phylogeny. They were placed within subfamily *Boletoideae*, clearly separated from the other lamellate genera. Within *Boletoideae*, the new specimens were placed as sister to *Boletus* L., sensu stricto (but including "*Alloboletus*", "*Inferiboletus*", and "*Obtextiporus*" sensu Dentinger et al. 2010), also with 100% bootstrap support. The two-gene phylogeny (Fig. 2) separates the new specimens, along with the environmental sequence, into the same two clusters identified on the basis of sequence similarity, with 100% and 99% bootstrap support, supporting the hypothesis that these clusters represent phylogenetically distinct species. Because these specimens are not phylogenetically nested within any known genus, and their lamellate hymenium clearly distinguishes them from all closely related species, we describe them as a new genus with two species.

Taxonomy

Paxilloboletus **Furneaux, De Kesel & FK Khan gen. nov.,**

Figures 3a–e, and 4

Mycobank MB840710

Etymology: *Paxilloboletus*–the name combines *Paxillus* and *Boletus* because morphologically the specimens resemble *Paxillus* but genetically they are more related to *Boletus*.

Description

Basidiomata epigeal, putrescent, pileate, stipitate, evelate, with lamellate hymenophore, medium to small sized; pileus convex to slightly depressed, tomentose, usually with persistently incurved margin; hymenophore easily separated from context of pileus, strongly decurrent, with lamellae regularly bifurcating and anastomosing, yellow, becoming yellowish brown; stipe solid, dry, tomentose, with or without ridges or reticulation in its uppermost part; basal mycelium whitish; context whitish to yellowish white throughout, unchangeable when exposed, strongly amyloid in the lamellae; taste fungal, insignificant; odor weak, insignificant; spore print yellowish brown, without olivaceous tint; basidiospores ellipsoid-fusiform, smooth under SEM; caulo-, cheilo- and pleurocystidia of similar shape, the latter much more abundant than cheilo- and caulocystidia; pileipellis a tomentum; hymenophoral trama divergent near the pileal context, subregular to regular toward the lamella edge, gelatinized; clamp connections absent.

Typus generis *Paxilloboletus africanus* sp. nov.

Paxilloboletus africanus **Badou, De Kesel & Yorou sp. nov.,**

Figures 5 and 6a–e

Mycobank MB840711.

Holotype: Guinea, Prefecture of Kankan, road Kankan-Kouroussa, Levari village, 10°12′15″N, 9°11′40″W, Baro forest reserve dominated by *Uapaca togoensis* Pax and *Anthonotha crassifolia* (Baill.) J.Léonard—[Chevalier]., 02/07/2018 leg. SA Badou, (SAB–0716) (UNIPAR).

Etymology: refers to its distribution throughout tropical Africa.

Habitat and distribution

P. africanus has this far been collected in West Africa, specifically Benin (gallery forest with *Berlinia grandiflora* and *Uapaca guineensis*), Togo (miombo forest with *Uapaca togoensis* and *Monotes kerstingii*) and Guinea (woodland with *Isoberlinia* spp., *Uapaca togoensis* and *Anthonotha crassifolia*); in Central Africa, specifically the Democratic Republic of the Congo (miombo dominated by *Julbernardia globiflora*) and Zambia (soil and relic miombo woodland); and in East Africa in Tanzania (primary miombo forest). It is also known in Madagascar from sequence data (EcM root tip of *Uapaca bojeri* in littoral forest).

Description

Basidiomata epigeal, solitary or in small groups of 2–3, pileate; hymenophore lamellate, bifurcating, anastomosing, decurrent, sometimes leaving fine, low ridges or a thin network on the upper stipe, centrally stipitate, putrescent, gymnocarpic. Spore print yellowish brown.

Pileus 30–75 mm diam., convex, with incurved margin at first, becoming slightly depressed in center, entirely smooth, sometimes ruptured or cracked at maturity, tomentose to velvety, dry, matte, at first pure white, showing fine yellowish-brown fibrils (hand lens!), becoming cream to slightly yellowish (2A2–3A2), margin concolorous, persistently incurved or rarely straight, wavy, smooth, tomentose.

Stipe 20–35(45) × 10–12(15)mm, cylindric, tapering downwards, shorter than pileus diameter, smooth, rarely tomentose, matte, at first white to yellowish white, becoming cream to yellowish (2A2–3A2), sometimes finely ridged or beset with a network in upper third, non-rooting; basal mycelium white, clasping soil, sometimes with fine mycelial strands.

Lamella decurrent, easily separable from pileus context, narrow, up to 4–5 mm high at mid radius, often crowded, arcuate to straight, frequently forked (1–4 ×), often anastomosing or with distinct interconnecting veins near the stipe, yellow (2A5–7), becoming yellowish brown with age (5DE6–7), edge smooth and concolorous, unchanged when bruised, dark blackish blue with Melzer's reagent.

Context 3–6 mm thick in the center of the pileus, gradually thinner toward the margin, pure white throughout,

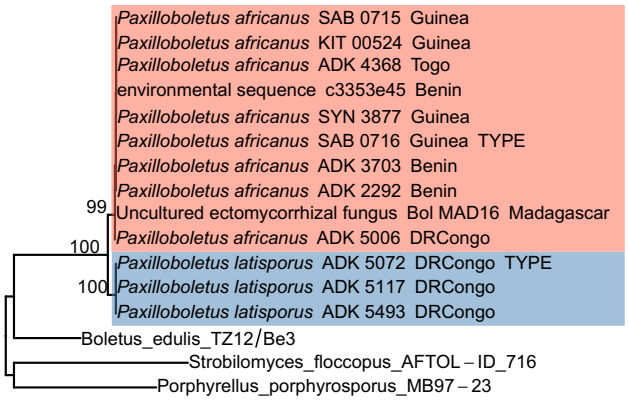


Fig. 2 ML tree of *Paxilloboletus* based on ITS and *RPB*2. Bootstrap support values > 95 are shown near nodes. Rooting based on 4–gene tree in Fig. 1

becoming dirty yellowish white in the stipe of older specimens, unchanging when exposed. Context strongly amyloid (Melzer's reagent), at least in lamella (especially the hymenium itself).

Taste fungal, insignificant. Odor feeble, insignificant.

Basidia clavate, (27.3–)47.6 × (7.9–)11.7 µm, hyaline, 4-spored, sterigmata 3.8–4.3(5.2) µm long.

Basidiospores (7.3–)7.6–8.9–10.2(–11.1) × (3.8–)3.9–4.4–4.8(– 5.1)µm with Q = (1.66–)1.72–2.04–2.36(–2.45) {N = 381; all 6 collections} and (7.3–)7.3–8.4–9.5(–9.9) × (3.9–)4–4.5–4.9(–5.1)µm with Q = (1.66–)1.65–1.87–2.09(–2.37) {N = 101; only holotype SAB–0716}, ellipsoid to slightly fusiform, sometimes with a slight adaxial (supra-hilar) depression, thin-walled, smooth under SEM, with a small hilar appendage, without apical pore, slightly cyanophilic, slightly amyloid (grayish walls). Pleurocystidia (54.5–)91.2 × (8.4–)13.2 µm, frequent, born deep in subhymenium, mostly fusiform, rarely septate in the lower third, with rounded apex, hyaline, thin walled, smooth, inamyloid. Gill edge mostly fertile, caulo- and cheilocystidia less frequent, (57.5–)75.7 × (8.9–)11.2 µm, similar to pleurocystidia.

Lamellar trama divergent near pileus, with compact mediostratum and gelatinized lateral strata, subregular to regular toward the lamella edge, in the center composed of thin-walled, hyaline hyphae (diam. 4–5 µm) with locally roughened surface, beneath the subhymenium mixed with conspicuously inflated (diam. 8–16 µm) hyphae, constricted at the septa. Pileipellis a tomentum composed of intermixed, hyaline and pigmented hyphae, all smooth, thin-walled, with cylindrical, non-inflated end-cells of 5.2–9.8 µm diam; the pigmented pileal hyphae normally septate, filled with a yellowish to pale brownish thrombomorphic deuteroplasm, unchanged in KOH, becoming only slightly browner in Melzer's reagent. Stipitipellis in the upper part of the stipe with sparse hymenial elements, elsewhere composed of parallel hyphae supporting a collapsed tomentum, similar to the pileipellis. Clamp-connections absent in all tissues.

Additional materials:

Benin, Atacora Prov., Natitingou, Kota falls, 10°12′48.12″N, 1°26′42.72″E, alt. 510 m, gallery forest with *Uapaca guineensis*, 23/09/2000 leg. A. De Kesel, (ADK–2922) (BR, BR5020129133262); ibidem, 10°12′24.00″N, 1°26′53.30″E, alt. 470 m, Kota falls along Perma river, gallery forest with *Berlinia grandiflora* and *Uapaca guineensis*, 19/06/2004 leg. A. De Kesel, (ADK–3703) (BR, BR5020157097543); ibidem, 10°12′33.3 N, 1°26′47.6″E, alt. 450 m, 26/06/2004 leg. A. De Kesel, (ADK–3762) (BR).

Democratic Republic of the Congo, Katanga Prov., Kisangwe (28 km NE of Lubumbashi), Mikembo sanctuary, 11°28′47.24″S—27°39′42.45″E, alt. 1180 m, miombo dominated by *Julbernardia globiflora*, 31/01/2012 leg. A. De Kesel, (ADK–5006) (BR, BR5020212111214V).

Guinea, Prefecture of Kankan, road Kankan-Kouroussa, Levari village, 10°12′17″N–9°11′40″W, Baro forest reserve, woodland with *Isoberlinia* spp., *Uapaca togoensis* and *Anthonotha* sp., 02/07/2018 leg. SA Badou, (SAB–0712, SAB–0715, SAB–0717) (UNIPAR); ibid., 10°31′03.7″N, 9°32′39.5″W, 02/07/2018 leg. S.N. Yorou, (SYN–3877) (UNIPAR); ibid., 10°53′03″N, 9°32′29″W, 02/07/2018 leg. K.I. Tchan, (KIT–00534) (UNIPAR); Kankan, 10°11′20.0″N, 9°11′02.9″W, Bissantougou forest reserve, woodland with *Isoberlinia* spp. and *Uapaca togoensis*, 02/07/2018 leg. K.I. Tchan, (KIT–00524) (UNIPAR).

Tanzania, Kigoma Province, Jakobsen beach, near Kigoma, 4°54.55′S, 29°36.03′E, alt. 790 m, primary miombo forest, 26/03/2011 leg. De Crop E, (EDC11–096) (BR, BR5020185167676).

Togo, Centrale, Kparatao (29 km NW of Sokodé, towards Bassar), 9°11′37.80″N, 0°59′8.04″E, alt. 580 m, 14/07/2007 leg. A. De Kesel, (ADK–4368) (BR, BR5020163838055).

Zambia, Copperbelt Province, Riverside, Kitwe, growing together on soil at top of trench, relic miombo woodland, 27/12/1974 leg. Ivory 2, R. Watling, (as *Phylloporus albocarnosus* in Watling and Turnbull 1993) (FP335/14) (NDO and K, only file at BR, BR021948–26).

***Paxilloboletus latisporus* De Kesel, Furneaux & Ryberg sp. nov.,**

Figures 7 and 8a–e

Mycobank MB840712.

Holotype: Democratic Republic of The Congo, Katanga Prov., Kisangwe (28 km NE of Lubumbashi), 11°29′4.44″S, 27°40′10.80″E, alt. 1210 m, Mikembo sanctuary, muhulu with *Julbernardia globiflora*, *Brachystegia microphylla* and *Uapaca kirkiana*, 08/02/2012 leg. A. De Kesel, (ADK–5072) (BR, BR5020212110187V).

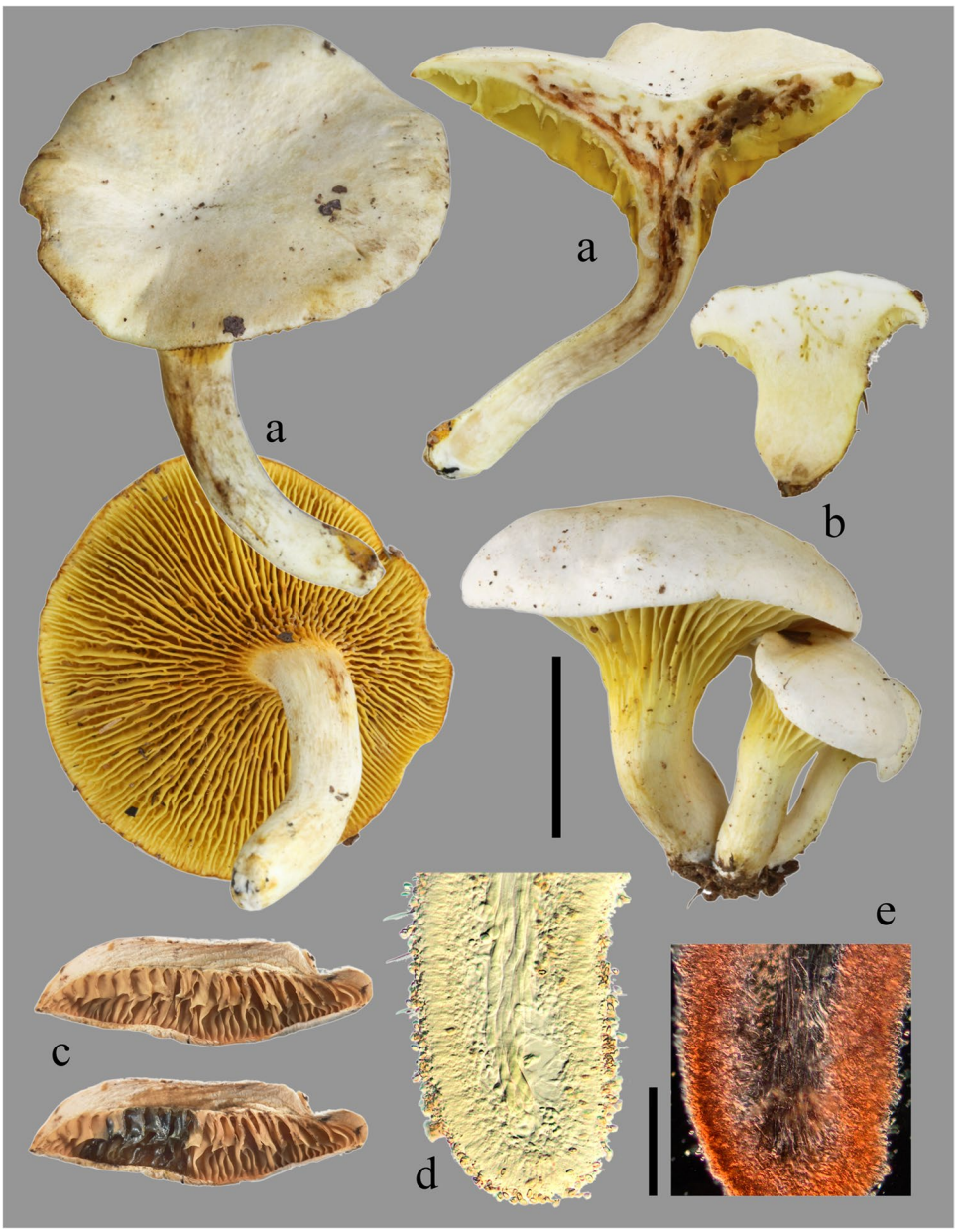


Fig. 3 *Paxilloboletus* gen. nov., with: a. *Paxilloboletus africanus* sp. nov. mature basidiomes (ADK–5006); b. *Paxilloboletus latisporus* sp. nov. mature basidiomes (ADK–5072); c. amyloid reaction on exsiccatum of *P. africanus* (ADK–2922; upper image = before; lower image = 2 min. after contact with Melzers' reagent); d. gill trama of *P. africanus* with inflated hyphae (ADK–2922); e. gill section of *P. latisporus* dyed in Congo red showing strongly gelatinized trama, DIC optics (ADK–5117). Scale bars: a, b, c = 20 mm; d, e = 100 µm

Etymology: latisporus means "with wider spores" and refers to the fact that this taxon is morphologically separated from the typus generis by this character.

Habitat and distribution

P. latisporus sp. nov. has only been collected from a single locality in Central Africa, specifically in the Democratic Republic of the Congo (Mikembo sanctuary, muhulu type of miombo with, *Julbernardia globiflora*, *Brachystegia microphylla*, *Brachystegia spiciformis*, *Marquesia macroura* and *Uapaca kirkiana*).

Description

Basidiomata epigeal, solitary or in small groups of 2–3, pileate; hymenophore lamellate, bifurcating, anastomosing, decurrent, with fine, low ridges or a thin network on the uppermost part of the stipe, centrally stipitate, putrescent, gymnocarpic. Spore print yellowish brown.

Pileus 20–60 mm diam., convex, with incurved margin at first, becoming broadly convex to plane, smooth, not ruptured or cracked at maturity, tomentose to velvety, dry, matte, at first pure white, sometimes with very fine yellowish-brown fibrils (hand lens!), becoming whitish cream (2A2), margin concolorous, persistently incurved (rarely straight), wavy, smooth, tomentose.

Stipe (15–)31 × (8–)11 mm, cylindric, fragile, tapering downwards, shorter than pileus diameter, smooth, tomentose, matte, white to yellowish white, becoming cream

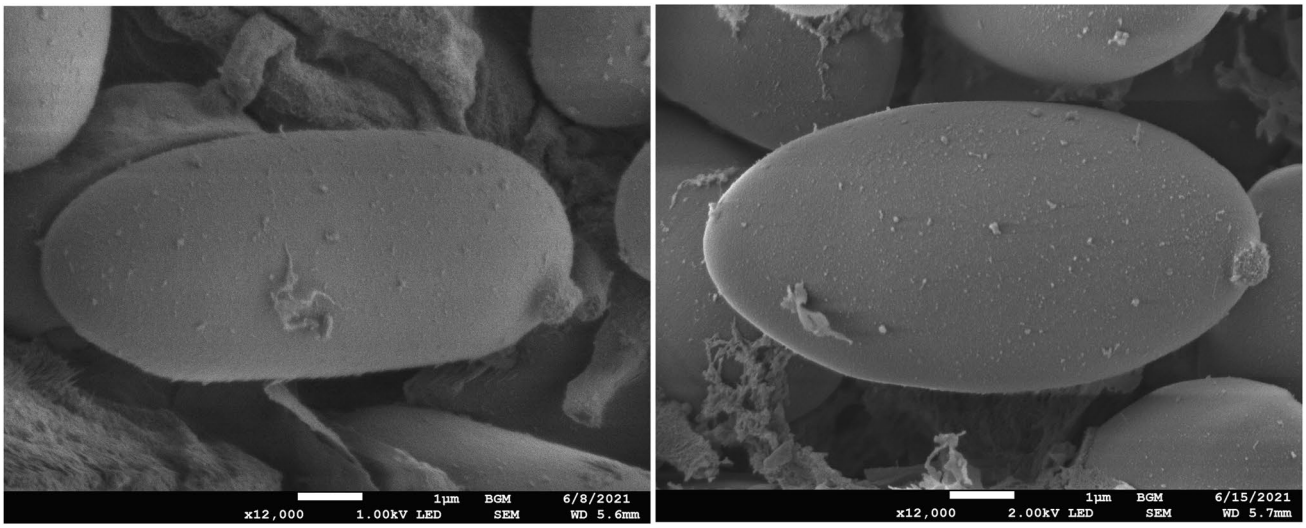


Fig. 4 SEM photographs showing smooth basidiospores of *Paxilloboletus* gen. nov., with: (left) *Paxilloboletus africanus* sp. nov. (ADK–5006) and (right) *Paxilloboletus latisporus* sp. nov. (ADK–5072)

(2A2), finely ridged or beset with a network in the upper third, non-rooting; basal mycelium white.

Lamella decurrent, easily separable from the pileus, narrow, up to 3–4 mm high at mid radius, fairly thin and crowded, arcuate to straight, frequently branched (1–3×), often anastomosing or with distinct interconnecting veins near the stipe, yellow (2A5–7), becoming yellowish brown with age (5DE6–7), edge smooth and concolorous, unchanged when bruised, dark blackish blue with Melzer's reagent.

Context 5–8 mm, very thick in the center of the pileus, thinner toward the margin, pure white throughout, becoming dirty yellowish white in the stipe of older specimens, unchanging when exposed. Context of exsiccata moderately amyloid (Melzer's reagent), at least in the lamella, especially the hymenium.

Fig. 5 *Paxilloboletus africanus* sp. nov. in situ habitus of young and mature basidiomata (SAB–0712 and SAB–0716)

Taste fungal, insignificant. Odor weak, insignificant.

Basidia clavate, (36.4–)53.6×(9.9–)12.1 µm, hyaline, 4–spored, rarely 1–2 spored, sterigmata 4–4.9(5.6)µm long.

Basidiospores (7.3–)7.5–8.8–10.1(–11.1)×(4.3-)4.3–4.8–5.3(–5.5)µm with Q=(1.48–)1.6–1.81–2.02(–2.16) {N=259; all 3 collections} and (7.3–)7.3–8.5–9.7(–11.1)×(4.3–)4.3–4.7–5.1(–5.2) µm with Q=(1.48–)1.57– 1.79–2.01(–2.16) {N=92; only holotype ADK–5072}, ellipsoid to slightly fusiform, sometimes with slight adaxial (supra-hilar) depression, thin-walled, smooth under SEM, with small hilar appendage, without apical pore, slightly cyanophilic. Pleurocystidia (50.3–)86.4×(9.6–)15.4 µm, frequent, born deep in the subhymenium, mostly fusiform, rarely septate in the lower third, with broad rounded apex, hyaline, thin-walled, smooth, inamyloid. Gill edge mostly fertile, cheilo- and caulocystidia infrequent, (49.8–)66.2×(9.2–)13.3 µm, mostly identical to pleurocystidia.

Lamellar trama divergent near the pileus, with compact mediostratum and gelatinized lateral strata, subregular to regular toward the lamella edge, in the center composed of thin-walled, hyaline hyphae (diam. 4–5 µm) with locally roughened surface, beneath the subhymenium mixed with conspicuously inflated hyphae (diam. 7.8–16(–17.2)µm), constricted at the septa. Pileipellis a tomentum composed of intermixed, hyaline and optically dense hyphae, all smooth, thin-walled, with cylindrical, non-inflated end-cells of (4.8)5.2–7.15(9.8)µm diam.; optically dense hyphae filled with a hyaline or pale yellowish thrombomorphic deuteroplasm, normally septate, unchanged in KOH and slightly browner in Melzer's reagent. Stipitipellis in the upper part of the stipe with hymenial elements, elsewhere similar to the pileipellis. Clamp–connections absent in all tissues.

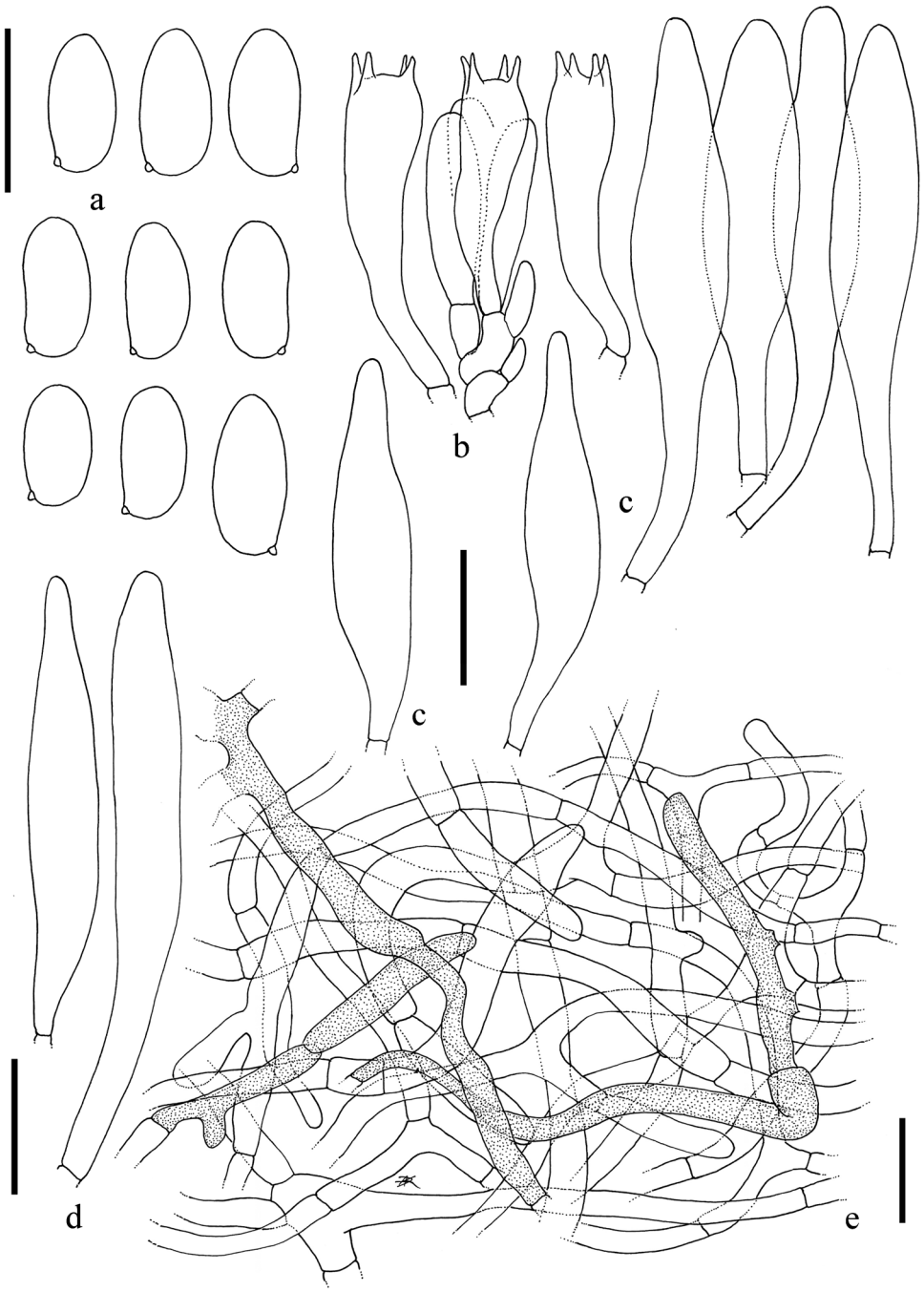


Fig. 6 *Paxilloboletus africanus* sp. nov. a. basidiospores; b. basidia; c. pleurocystidia; d. cheilocystidia; e. pileipellis a tomentum with sparse, yellowish to pale brownish hyphae with thrombomorphic deuteroplasm. Scale bars a = 10 µm and b, c, d, e = 20 µm (all from SAB–0716, holotype)

Additional materials:

Democratic Republic of the Congo, Katanga Prov., Kisangwe (28 km NE of Lubumbashi), 11°29′0.44″S, 27°40′0.46″E, alt. 1200 m, Mikembo sanctuary, miombo with *Julbernardia globiflora* and *Brachystegia spiciformis*, 15/02/2012 leg. A. De Kesel, (ADK–5117) (BR, BR5020212113270V); ibidem, 11°29′5.27″S, 27°40′20.09″E, alt. 1223 m, Mikembo sanctuary, miombo forest dominated by *Marquesia macroura*, 26/01/2013 leg. A. De Kesel, (ADK–5493) (BR, BR5020212112242V).

Discussion

The basidiomata of *Paxilloboletus* resemble taxa in *Paxillus* Fr. (Paxillaceae). This is mainly due to the persistently incurved margin, tomentose cap, and separable, decurrent, lamellar hymenophore with yellow hymenium. However, the absence of clamp connections and the phylogenetic position outside Paxillaceae (Fig. 1) exclude a close relationship with *Paxillus*.

Fig. 7 *Paxilloboletus latisporus* sp. nov. in situ habitus of young and mature basidiomata (ADK–5072, holotype)

Within the Boletaceae phylogeny, *Paxilloboletus* spp. take a position outside all known genera, and distant from other lamellate lineages. Neves et al. (2012) stated that the lamellate hymenophore configuration is a synapomorphy that distinguishes *Phylloporus* from the other genera in the family Boletaceae. The separate placement of lamellate genera of boletes within Boletaceae, including *Paxilloboletus*, clearly suggests homoplasy for the presence of a lamellate hymenophore. In particular, the phylogenetic position of *Paxilloboletus* as a lamellate sister of *Boletus* has the potential to set a clear boundary for both genera in a natural classification.

Within the Boletaceae, the basidiomata of *Paxilloboletus* spp. resemble the ones in *Phylloporus*, Sect. *Immutabiles* Heinem and Rammeloo (1987b) because of an unchanging context and smooth spores under SEM. Heinemann and Rammeloo (1987a) and later Watling and Turnbull (1993) studied a white capped *Phylloporus* from Zambia (collection FP335, deposited at NDO and K) and placed it in this section, under *Phylloporus albocarnosus* Heinem. Re-examination of the data available from FP335 shows that it is different from the type of *Phylloporus albocarnosus* (Goossens-Fontana 935, BR) by its very pale almost white pileus, tomentum-like pileipellis, and slightly wider spores with internal amyloid granulation (P. Heinemann notes). Re-examination of the pileipellis of the type of *Phylloporus albocarnosus* shows a pellis with clearly inflated to globular terminal elements (physalotrichoderm) and inamyloid lamella. Collection FP335 belongs to *Paxilloboletus* (*P. africanus*) and is not conspecific with *P. albocarnosus*. The latter is now only known from the type material.

From taxa in *Phylloporus* Quél. (Boletaceae) and *Phylloporopsis* Angelini, A. Farid, Gelardi, M.E. Sm., Costanzo and Vizzini (Boletaceae), the basidiomata of *Paxilloboletus* spp. differ by a tomentum-type of pileipellis without inflated end-cells and sparse, yellowish thromboplerous hyphae, an unchanging white to yellowish-white context, and lacking olivaceous tints in the spore print. *Erythrophylloporus* Ming Zhang and T.H. Li (Boletaceae) differs from *Paxilloboletus* by its yellowish-orange to red pileus, orange to red lamellae, vivid yellow context and ovoid to broadly ellipsoid spores (Vadthanarat et al. 2019). *Paxilloboletus* differs from the lamellate *Phylloboletellus* Singer (Boletaceae) by smooth, non-winged, ellipsoid to slightly fusiform basidiospores and from the ill-known velate genus *Phyllobolites* Singer (Boletaceae) by its non-verrucose spores and complete lack of velum (ring) on the stipe.

All collections of *Paxilloboletus africanus* and *P. latisporus* systematically show a moderate to strong amyloid reaction in the entire hymenophore, a characteristic that is missing in all other lamellate Boletaceae. On exsiccata, regardless of their age (3–21 years), this reaction is immediate and gains intensity for about 2 min. However, after 15 min, the dark blackish-blue stain starts to fade and disappears within the following hour. Fleeting-amyloid reactions in the trama have been reported from many boletes (Bozok et al. 2019), but the reaction in both *Paxilloboletus* is much stronger and not localized in the trama of the gills. In fact, seen under the microscope, the amyloid reaction (on a perradial section of a gill) mainly takes place in the hymenium and not, or hardly, in the lamellar trama. This is possibly due to the gelatinization of the gill trama of *Paxilloboletus*. In only few boletes, such as *Caloboletus calopus* (Pers.) Vizzini and *Suillellus luridus* (Schaeff.) Murrill, strong amyloid reactions have been reported (Bozok et al. 2019). In boletes the taxonomic value of this characteristic is considered either unclear, controversial or important (Watling 1971). Notwithstanding this situation, and because all of our collections respond homogeneously, we here consider this feature diagnostic for *Paxilloboletus*.

Due to their identical macro morphology, basidiomes of *Paxilloboletus africanus* and *P. latisporus* are difficult to set apart in the field. Microscopically, the taxa can only be separated by measuring a large number of spores, at least 50–100 and preferably from a spore print. While the average spore length is not different between the two species, the average spore width ranges from 4.1 to 4.5 µm in *P. africanus* and from 4.7 to 5.0 µm in *P. latisporus*. Although less pronounced, the average Q value also differs, namely Q = 1.8 in *P. latisporus* and Q = 2 in *P. africanus*.

Paxilloboletus is most likely ectomycorrhizal, and an ITS sequence matching *P. africanus* has previously been isolated from ectomycorrhizal root tips of *Uapaca bojeri* (Phyllanthaceae) in Madagascar (L. Tedersoo, GenBank accession number FR731194). In the absence of data

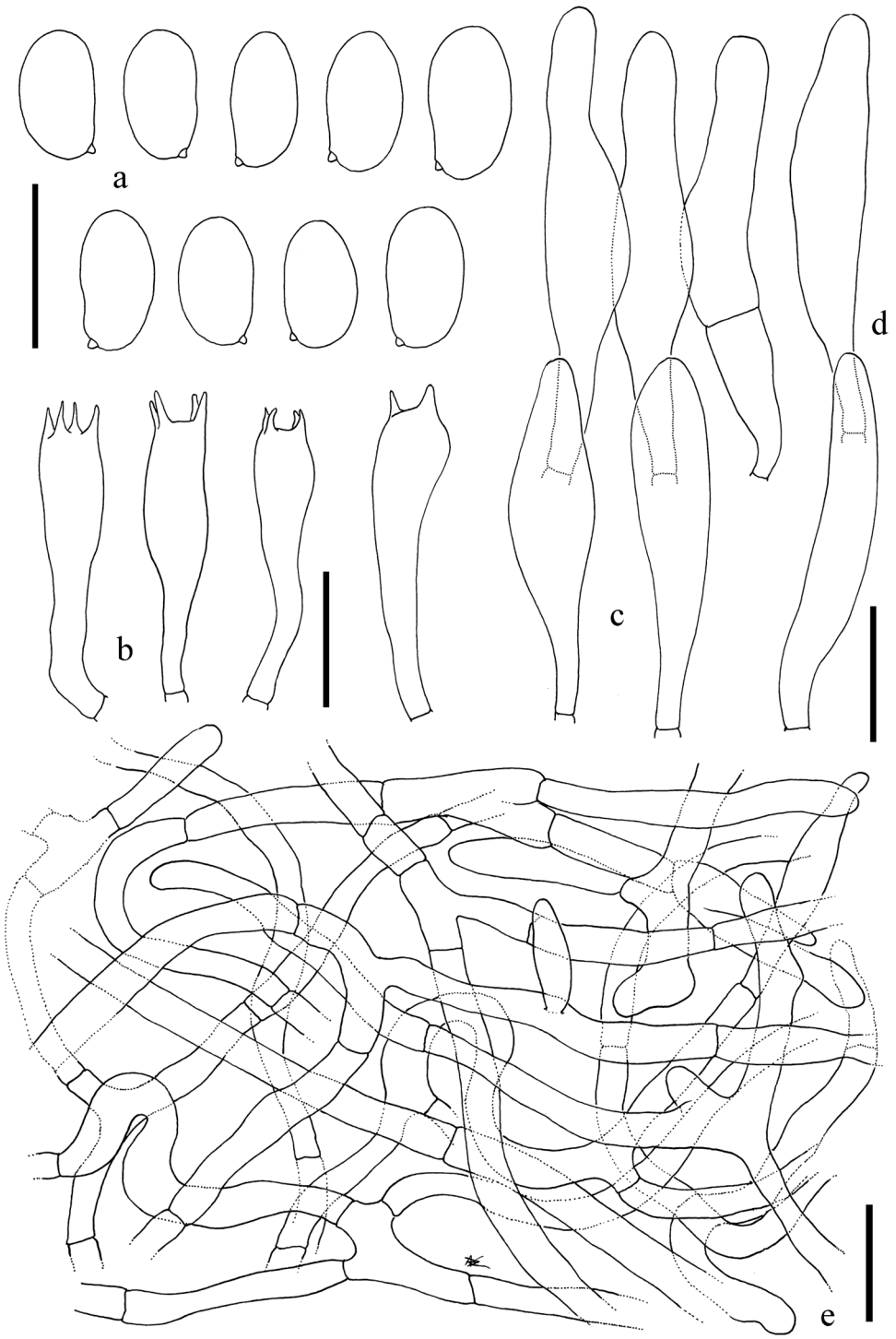


Fig. 8 *Paxilloboletus latisporus* sp. nov., a. basidiospores; b. basidia; c. cheilocystidia; d. pleurocystidia; e. pileipellis. Scale bars a = 10 µm and b, c, d, e = 20 µm (all from holotype ADK–5072)

directly linking the new taxa to associate host trees in continental Africa, we can only work with field observations. In almost all collections, regardless of the species, the field data indicate the proximity of *Uapaca* spp. (Phyllanthaceae) and a set of trees of subfamily Detarioideae of the Fabaceae. In West Africa (Guinea, Togo, Benin), these Detarioideae belong to *Isoberlinia* and *Berlinia*, while in Eastern Africa (DR Congo, Zambia and Tanzania) they belong to *Brachystegia*, *Julbernardia* and *Isoberlinia*. Compared to *P. africanus*, *P. latisporus* has a more restricted distribution which is potentially tied to a specific host. In fact, it was only found in mature miombo forests of DR Congo (Lubumbashi, Mikembo sanctuary) with *Uapaca*, *Brachystegia* and *Julbernardia*, and also large specimens of the ectomycorrhizal tree *Marquesia macroura* (Dipterocarpaceae).

In recent years, we observe more and more that ectomycorrhizal taxa are separated based on a combination of molecular and very subtle morphological characteristics (Delgat et al. 2019; De Kesel et al. 2016; Vadthanarat et al. 2021). Sibling species typically show few and feeble morphological differences. They are thought to be the result of recent divergence, likely involving an EcM host switch, in which sporocarp morphologies haven't had the time to diverge as well. Since *P. africanus* and *P. latisporus* resemble each other so strongly, we consider them pseudocryptic species. Without molecular data (Fig. 2), these taxa would not have been separated, simply because subtle differences in spore width are — traditionally — not considered strong enough characteristics to separate taxa.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-021-01756-y.

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Author contribution SAB planned the research, collected specimens, performed microscopic investigations, and wrote the initial draft of the manuscript. BF planned the research, performed phylogenetic analyses, and edited the manuscript. ADK collected specimens, located additional specimens in herbaria, performed microscopic investigations, wrote the final descriptions, edited the manuscript and applied for funding. FKK performed phylogenetic analyses. RDH performed initial phylogenetic analyses and coordinated research efforts between groups. MR applied for funding, planned the research, and supervised BF and FKK. NSY applied for funding, planned the research, and supervised SAB and RDH. All authors contributed to and approve the final manuscript.

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Data availability All studied materials are deposited in the herbaria of the University of Parakou (UNIPAR), the University of Uppsala (UPS) and/or Meise Botanic Garden (BR). Newly generated DNA sequences are available from GenBank (accession numbers MZ702467–MZ702481 and MZ707864–MZ707879). Alignments and trees are available at TreeBase (project number S28627; http://purl.org/phylo/treebase/phylows/study/TB2:S28627).

Code availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Badou AS, De Kesel A, Raspé O, Ryberg MK, Guelly AK, Yorou NS (2018) Two new African siblings of *Pulveroboletus ravenelii* (Boletaceae). MycoKeys 43:115–130. https://doi.org/10.3897/mycokeys.43.30776

Binder M, Hibbett DS (2006) Molecular systematics and biological diversification of Boletales. Mycologia 98:971–981. https://doi.org/10.1080/15572536.2006.11832626

Binder M, Larsson K-H, Matheny PB, Hibbett DS (2010) Amylocorticiales ord. nov. and Jaapiales ord. nov.: Early diverging clades of Agaricomycetidae dominated by corticioid forms. Mycologia 102:865–880. https://doi.org/10.3852/09-288

Bozok F, Assyov B, Taşkın H (2019) First records of *Exsudoporus permagnificus* and *Pulchroboletus roseoalbidus* (Boletales) in association with non-native Fagaceae, with taxonomic remarks. Phytol Balc 25(1):13–27. http://www.bio.bas.bg/~phytolbalcan/PDF/25_1/PhytolBalcan_25-1_02_Bozok_&_al.pdf

Bresinsky A, Jarosch M, Fischer M et al (1999) Phylogenetic relationships within *Paxillus* s. l. (Basidiomycetes, Boletales): separation of a southern hemisphere genus. Plant Biol (stuttg) 1:327–333. https://doi.org/10.1055/s-2007-978523

Chai H, Liang ZQ, Xue R, Jiang S, Luo SH, Wang Y, Wu LL, Tang LP, Chen Y, Hong D, Zeng NK (2019) New and noteworthy boletes from subtropical and tropical China. MycoKeys 46:55–96. https://doi.org/10.3897/mycokeys.46.31470

De Kesel A, Amalfi M, Kasongo B, Yorou NS, Raspé O, Degreef J, Buyck B (2016) New and interesting *Cantharellus* from tropical Africa. Cryptogam Mycol 37(3):283–327. https://doi.org/10.7872/crym/v37.iss3.2016.283

Delgat L, Dierickx G, De Wilde S et al (2019) Looks can be deceiving: the deceptive milkcaps (Lactifluus, Russulaceae) exhibit low morphological variance but harbour high genetic diversity. IMA Fungus 10:14. https://doi.org/10.1186/s43008-019-0017-3

Dentinger BTM, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau P-A, Nagasawa E, Soytong K, Taylor AF, Watling R, Moncalvo J-M, McLaughlin DJ (2010) Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*).

Mol Phylogenetics Evol 57:1276–1292. https://doi.org/10.1016/j.ympev.2010.10.004
Desjardin DE, Wilson AW, Binder M (2008) Durianella, a new gasteroid genus of boletes from Malaysia. Mycologia 100:956–961. https://doi.org/10.3852/08-062
Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious 5.3. http://www.geneious.com/
Farid A, Gelardi M, Angelini C, Franck AR, Costanzo F, Kaminsky L, Ercole E, Baroni TJ, White AL, Garey JR, Smith ME, Vizzini A (2018) *Phylloporus* and *Phylloboletellus* are no longer alone: *Phylloporopsis* gen. nov, (Boletaceae), a new smooth-spored lamellate genus to accommodate the American species *Phylloporus boletinoides*. Fungal Syst Evol 2:341–359. https://doi.org/10.3114/fuse.2018.02.10
Filatov DA (2002) ProSeq: a software for preparation and evolutionary analysis of DNA sequence data sets. Mol Ecol Notes 2(4):621–624. https://doi.org/10.1046/j.1471-8286.2002.00313.x
Gelardi M (2020) Diversity, Biogeographic Distribution, Ecology, and Ectomycorrhizal Relationships of the Edible Porcini Mushrooms (*Boletus* s. str., Boletaceae) Worldwide: State of the Art and an Annotated Checklist. In: Pérez-Moreno J, Guerin-Laguette A, Flores Arzú R, Yu FQ, (Eds) Mushrooms, Humans and Nature in a Changing World: Perspectives from Ecological, Agricultural and Social Sciences. Springer, Cham, pp 223–271. https://doi.org/10.1007/978-3-030-37378-8_8
Gelardi M, Vizzini A, Ercole E, Horak E, Ming Z, Li TH (2015) Circumscription and taxonomic arrangement of *Nigroboletus roseonigrescens* Gen. Et. Sp. Nov., a new member of Boletaceae from Tropical South-Eastern China. PLoS One 10:e0134295. https://doi.org/10.1371/journal.pone.0134295
Gilbert É-J (1931) Les Bolets. Les livres du Mycologue III. Le François, Paris
Hahn C, Agerer R (1999) Studien zur Systematik der Paxillaceae (Boletales, Basidiomycota). Sendtnera 6:115–133
Haelewaters D, Dima B, Abdel-Hafiz AII, Abdel-Wahab MA, Abul-Ezz SR, Acar I, Nagy A, Papp V, Péter Á, Pfliegler WP, Sándor AD, Szentiványi T, Krisai-Greilhuber I (2020) Fungal Systematics and Evolution: FUSE 6. Sydowia 72. 10.12905/0380.sydowia72-2020–0271
Heinemann P, Rammeloo J (1986) Paxillaceae (Boletineae). Flore Illustrée des champignons d'Afrique Centrale, Fascicule 12. Ministère de l'Agriculture, Jardin Botanique National de Belgique, Meise, pp 275–310
Heinemann P, Rammeloo J (1987a) *Phylloporus.* Flore Illustrée des Champignons d'Afrique Centrale, Fascicule 13. Ministère de l'Agriculture, Jardin Botanique National de Belgique, Meise, pp 275–210
Heinemann P, Rammeloo J (1987b) Novitates generis Phyllopori. Bulletin Du Jardin Botanique National De Belgique 57:272–274
Henkel TW, Obase K, Husbands D, Uehling JK, Bonito G, Aime MC, Smith ME (2016) New Boletaceae taxa from Guyana: *Binderoboletus segoi* gen. and sp. nov., *Guyanaporus albipodus* gen. and sp. nov., *Singerocomus rubriflavus* gen. and sp. nov., and a new combination for *Xerocomus inundabilis*. Mycologia 108:157–173. https://doi.org/10.3852/15-075
Hopple JS, Vilgalys R (1994) Phylogenetic relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86:96–107. https://doi.org/10.2307/3760723
Katoh K, Frith MC (2012) Adding unaligned sequences into an existing alignment using MAFFT and LAST. Bioinformatics 28:3144–3146. https://doi.org/10.1093/bioinformatics/bts578
Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software Version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010
Kirk PM (2021) Species Fungorum. Royal Botanic Gardens, Kew. http://www.speciesfungorum.org. Accessed 19 Feb 2021
Kornerup A, Wanscher JH (1978) Methuen Handbook of Colour (3rd edn). Eyre Methuen Ltd, London, p 252
Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16:1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092
Matheny PB (2005) Improving phylogenetic inference of mushrooms with *RPB*1 and *RPB*2 nucleotide sequences (Inocybe; Agaricales). Mol Biol Evol 35:1–20. https://doi.org/10.1016/j.ympev.2004.11.014
Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Henrik Nilsson R, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Frøslev T, Ge Z-W, Kerrigan RW, Slot JC, Yang Z-L, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS (2007) Contributions of *RPB*2 and *TEF*1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol Biol Evol 43:430–451. https://doi.org/10.1016/j.ympev.2006.08.024
Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Institute of Electrical and Electronics Engineers (ed) Proceedings of the Gateway Computing Environments Workshop (GCE) 14 Nov 2010 New Orleans 45–52
Neves MA, Binder M, Halling R, Hibbett D, Soytong K (2012) The phylogeny of selected *Phylloporus* species, inferred from NUC-LSU and ITS sequences, and descriptions of new species from the Old World. Fungal Divers 55:109–123. https://doi.org/10.1007/s13225-012-0154-0
Nuhn ME, Binder M, Taylor AF, Halling RE, Hibbett DS (2013) Phylogenetic overview of the Boletineae. Fungal Biol 117:479–511. https://doi.org/10.1016/j.funbio.2013.04.008
Orihara T, Sawada F, Ikeda S, Yamato M, Tanaka C, Shimomura N, Hashiya M, Iwase K (2010) Taxonomic reconsideration of a sequestrate fungus, Octaviania columellifera, with the proposal of a new genus, Heliogaster, and its phylogenetic relationships in the Boletales. Mycologia 102:108–121. https://doi.org/10.3852/08-168
Orihara T, Lebel T, Ge ZW, Smith ME, Maekawa N (2016) Evolutionary history of the sequestrate genus *Rossbeevera* (Boletaceae) reveals a new genus *Turmalinea* and highlights the utility of ITS minisatellite-like insertions for molecular identification. Persoonia 37:173–198. https://doi.org/10.3767/003158516X691212
Orihara T, Smith ME (2017) Unique phylogenetic position of the African truffle-like fungus, *Octaviania ivoryana* (Boletaceae, Boletales), and the proposal of a new genus, *Afrocastellanoa*. Mycologia 109:323–332. https://doi.org/10.1080/00275514.2017.1301750
Parihar A, Hembrom ME, Vizzini A, Das K (2018) *Indoporus shoreae* gen, et sp, nov, (Boletaceae) from Tropical India. Cryptogam Mycol 39:447–466. https://doi.org/10.7872/crym/v39.iss4.2018.447
R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
Rehner SA, Buckley E (2005) A Beauveria phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia 97:84–98
Singer R (1942) Type studies on Basidiomycetes. I. Mycologia 34(1):64–93
Singer R, Digilio APL (1952) Pródromo de la flora Agaricina Argentina. Lilloa 22:5–462

Smith AH, Thiers HD (1971) Boletes of Michigan, 1st edn. University of Michigan Press, Ann Arbor, Michigan

Smith ME, Amses KR, Elliott TF, Obase K, Aime MC, Henkel TW (2015) New sequestrate fungi from Guyana: *Jimtrappea guyanensis* gen. sp. nov., *Castellanea pakaraimophila* gen, sp, nov., and *Costatisporus cyanescens* gen. sp. nov, (Boletaceae, Boletales). IMA Fungus 6:297–317. https://doi.org/10.5598/imafungus.2015.06.02.03

Staden R, Beal KF, Bonfield JK (2000) The Staden Package, 1998. Bioinformatics Methods and Protocols 115–130. https://doi.org/10.1385/1-59259-192-2:115

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U (2008) Strong host preference of Ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytol 180:479–490. https://doi.org/10.1111/j.1469-8137.2008.02561.x

Tedersoo L, Bahram M, Jairus T, Bechem E, Chinoya S, Mpumba R, Leal M, Randrianjohany E, Razafimandimbison S, Sadam A, Naadel T, Kõljalg U (2011) Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar. Mol Ecol 20:3071–3080. https://doi.org/10.1111/j.1365-294X.2011.05145.x

Thiers B (continually updated) Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden, New York. http://sweetgum.nybg.org/science/ih/

Vadthanarat S, Amalfi M, Halling RE, Bandala V, Lumyong S, Raspé O (2019) Two new *Erythrophylloporus* species (Boletaceae) from Thailand, with two new combinations of American species. MycoKeys 55:29–57. https://doi.org/10.3897/mycokeys.55.34570

Vadthanarat S, Halling RE, Amalfi M, Lumyong S, Raspé O (2021) An unexpectedly high number of new *Sutorius* (Boletaceae) species from northern and northeastern Thailand. Front Microbiol 12:643505. https://doi.org/10.3389/fmicb.2021.643505

Větrovský T, Kolařík M, Žifčáková L, Zelenka T, Baldrian P (2016) The *RPB*2 gene represents a viable alternative molecular marker for the analysis of environmental fungal communities. Mol Ecol Resour 16:388–401. https://doi.org/10.1111/1755-0998.12456

Watling R, Turnbull E (1993) Boletes from South and East Central Africa – I. Edinb J Bot 49(3):343–361

Watling R. (1971) Chemical tests in agaricology. In: Booth, C. (Ed.). Microbiol Methods 4:567–597

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand D, Sninsky J, White T (eds) Innis M. A Guide to Methods and Applications. Academic Press Inc, PCR Protocols, pp 315–322

Wu G, Feng B, Xu J, Zhu XT, Li YC, Zeng NK, Hosen MI, Yang ZL (2014) Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Divers 69:93–115

Wu G, Li YC, Zhu XT, Zhao K, Han LH, Cui YY, Li F, Xu JP, Yang ZL (2016a) One hundred noteworthy boletes from China. Fungal Divers 81:25–188. https://doi.org/10.1007/s13225-016-0375-8

Wu G, Zhao K, Li YC, Zeng NK, Feng B, Halling RE, Yang ZL (2016b) Four new genera of the fungal family Boletaceae. Fungal Divers 81:1–24. https://doi.org/10.1007/s13225-015-0322-0

Wu LL, Liang ZQ, Xue R, Fan YG, Jiang S, Fu YQ, Zeng NK, Su MS (2019) The genus *Crocinoboletus* (Boletaceae, Boletales): a new species and updated information for previously described species. Phytotaxa 419:91–99. https://doi.org/10.11646/phytotaxa.419.1.6

Yu G, Smith D, Zhu H, Guan Y, Lam TTY (2017) Tree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol 8:28–36. https://doi.org/10.1111/2041-210X.12628

Zhang M, Li TH (2018) *Erythrophylloporus* (Boletaceae, Boletales), a new genus inferred from morphological and molecular data from subtropical and tropical China. Mycosystema 37:1111–1126

Zhang M, Li TH, Wang CQ, Zeng NK, Deng WQ (2019) Phylogenetic overview of *Aureoboletus* (Boletaceae, Boletales), with descriptions of six new species from China. MycoKeys 61:111–145. https://doi.org/10.3897/mycokeys.61.47520

Zhu XT, Wu G, Zhao K, Halling RE, Yang ZL (2015) *Hourangia*, a new genus of Boletaceae to accommodate *Xerocomus cheoi* and its allied species. Mycol Prog 14:1–10. https://doi.org/10.1007/s11557-015-1060-0