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Leucocoprinus beninensis Sarawi, sp. nov., a new species of Agaricaceae from Benin

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ABSTRACT
Leucocoprinus beninensis Sarawi, sp. nov., is described here as a new species from Benin, West Africa, based on morphological and phylogenetic investigation. *Leucocoprinus beninensis* Sarawi, sp. nov., differs from known species of *Leucocoprinus* Pat. and closely related species of *Leucoagaricus* (Locq. Ex) Singer by the combination of basidiomata with reddish brown, light brown to brown squamules on a white background, a change of colour of the basidiomata to blue-green or grey-blue when dried, the absence of cheilocystidia, and small amygdaliform to ellipsoid basidiospores. Molecular phylogenetic analyses of the nrDNA ITS region reveal that sequences of *Lc. beninensis* Sarawi, sp. nov., cluster with sequences of *Lc.* cf. *caerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu, *Lc. cinnamomeodiscus* Asif, Saba & M. Raza, *Lc. irinellus* (Chalange) Kun L. Yang, Jia Y. Lin & Zhu L. Yang, *Lc. sericifer* (Locq.) Asif, Saba & Vellinga, and *Lc. viriditinctus* (Berk. & Broome) Asif, Saba & Vellinga.

KEY WORDS
Agaricales,
lepiotaceous fungi,
molecular phylogeny,
tropics,
new species.

RÉSUMÉ
Leucocoprinus beninensis *Sarawi, sp. nov., une nouvelle espèce d'Agaricaceae du Bénin.*
Leucocoprinus beninensis Sarawi, sp. nov., est décrit ici comme une nouvelle espèce du Bénin, en Afrique de l'Ouest, sur la base de données morphologiques et phylogénétiques. *Leucocoprinus beninensis* Sarawi, sp. nov., diffère des espèces connues de *Leucocoprinus* Pat. et d'espèces voisines de *Leucoagaricus* (Locq. Ex) Singer par la combinaison de basidiomes avec des squamules brun rougeâtre, brun clair à brun sur un fond blanc, un changement de couleur des basidiomes en bleu-vert ou gris-bleu lorsqu'ils sont séchés, l'absence de cheilocystides, et de petites basidiospores amygdaliformes à ellipsoïdes. Les analyses phylogénétiques moléculaires de la région ITS de nrADN révèlent que les séquences de *Lc. beninensis* Sarawi, sp. nov., se regroupent avec celles de *Lc.* cf. *caerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu, *Lc. cinnamomeodiscus* Asif, Saba & M. Raza, *Lc. irinellus* (Chalange) Kun L. Yang, Jia Y. Lin & Zhu L. Yang, *Lc. sericifer* (Locq.) Asif, Saba & Vellinga et *Lc. viriditinctus* (Berk. & Broome) Asif, Saba & Vellinga.

MOTS CLÉS
Agaricales,
champignons lépiotes,
phylogénie moléculaire,
tropiques,
espèce nouvelle.

INTRODUCTION

The genus *Leucocoprinus* Pat. (Agaricaceae) includes 50 known species with a worldwide distribution (He *et al.* 2019). Morphologically, species of the genus *Leucocoprinus* are characterized by basidiomata with coprinoid shape, with sulcate pileus margin, free lamellae, white spore print, dextrinoid basidiospores, and absence of clamp connections (Singer 1986; Vellinga 2001).

Due to similar morphological characteristics, species of the genera *Leucocoprinus* and *Leucoagaricus* (Locq. ex) Singer were grouped in the older genus *Leucocoprinus* in the past, but *Leucocoprinus* species were separated from *Leucoagaricus* species based on fragile, coprinoid basidiomata with striate pileus margin and pseudoparaphysis in the hymenium (Singer 1986). However, phylogenetic studies have demonstrated that the genera *Leucocoprinus* and *Leucoagaricus* are polyphyletic (Johnson & Vilgalys 1998; Johnson 1999; Vellinga 2004; Vellinga *et al.* 2011; Ge *et al.* 2015; Ma *et al.* 2022). Subsequently, Redhead (2023) and Asif *et al.* (2024) recombined the majority of *Leucoagaricus* species to *Leucocoprinus*. However, Yang *et al.* (2024) recognize both genera and described several new genera based on multilocus phylogenetic analyses, and we follow this approach.

In temperate regions, the genus *Leucocoprinus* has been extensively studied (Candusso & Lanzoni 1990). Recently, several new species of *Leucocoprinus*, named as species of *Leucoagaricus*, have been described from the subtropics and tropics, suggesting the presence of a great diversity of species in these regions (Dennis 1952; Heinemann 1973, 1977; Pegler 1977, 1983; Vellinga *et al.* 2011; Jabeen *et al.* 2020; Ma *et al.* 2022; Sysouphanthong & Thongklang 2022; Ashraf *et al.* 2023; Rehman *et al.* 2023). For Benin, West Africa, only *Leucoagaricus americanus* (Peck) Vellinga and *Leucocoprinus cretaceus* (Bull.) Locq. have been reported, but no *Leucoagaricus* or *Leucocoprinus* species was yet described from Benin (Piepenbring *et al.* 2020).

MATERIAL AND METHODS

Morphological examination

Specimens of *Leucocoprinus* sp. were collected during a mycological survey in June to July 2022 at different sites in Benin, West Africa. The macroscopic characteristics of the fresh basidiomata were recorded in the field and colour codes were assigned using Kornerup & Wanscher (1978). The basidiomata were air-dried on an electric food dehydrator at 40 °C. Microscopic features were examined using a Nikon Eclipse 80i light microscope. Microscopic characteristics of the dried material were studied in water or 5-10 % KOH. Some sections were stained with ammoniacal 10 % Congo Red. Melzer's reagent was used to study the dextrinoidity and Cresyl blue (0.1 % in H_2O) for the metachromasy of the basidiospores. Measurements are given with extreme values in brackets. The specimens are deposited in the Herbarium FR, Frankfurt am Main, Germany.

DNA extraction, PCR amplification and sequencing

For DNA extraction, small pieces of dried basidiomata were frozen with liquid nitrogen and ground to a fine powder using a MM301 Mixer Mill (Retsch GmbH, Germany). The extraction was performed using the innuprep plant DNA kit (IST Innuscreen, Germany) following the manufacturers' instructions. The PCR was performed using the VWR Taq DNA polymerase (VWR, Germany) in a peqStar gradient thermal cycler (PEQLAB, Germany). For the amplification of the nuclear ribosomal internal transcribed spacer (nrITS) region, the primer pairs ITS5/ITS4B were used (White *et al.* 1990; Gardes & Bruns 1993). PCR products were verified by gel electrophoresis using a 1 % (w/v) agarose gel. The purified PCR products were sequenced by Sanger sequencing at Microsinth SeqLab (Germany).

Phylogenetic analysis

The obtained DNA sequences were edited, assembled, and quality checked using Geneious Prime V2021.2.2 (Biomatters Ltd., New Zealand). Additional sequences of *Leucoagaricus*, *Leucocoprinus*, *Micropsalliota,* and *Cystolepiota* Singer species were obtained from GenBank (Table 1). The final data sets were then aligned in MAFFT v7 (Katoh & Standley 2013) using the E-INS-i algorithm for the nrITS dataset (Katoh & Toh 2008). The alignments were checked and pruned in Mega7 (Kumar *et al.* 2016). A Maximum-Likelihood analysis was conducted for each dataset using RAxML 8.2.12 (Stamatakis 2014) applying the GTRCAT model with 25 per site rate categories and 1000 bootstrap iterations (Felsenstein 1985). A Bayesian Markov Chain Monte Carlo (MCMC) analysis was performed using MrBayes 3.2.7a on XSEDE (Huelsenbeck & Ronquist 2001; Ronquist *et al.* 2012), with two runs of 10 million generations with four chains each, a sampling frequency every 1000 generations, and a stoprule for topological convergence diagnostics falling below 0.01. The GTR model and a gamma distribution was used. The first 25 % generations were discarded as burn-in. The Maximum-Likelihood and Bayesian Markov Chain Monte Carlo analyses were performed via Cipres Science Gateway (Miller *et al.* 2010). Phylogenetic trees were visualized using TreeGraph2 (Stöver & Müller 2010).

RESULTS

Phylogenetic results

The Maximum-Likelihood phylogeny (Fig. 1) based on ITS sequences of species of *Leucoagaricus*, *Leucocoprinus,* and further closely relates genera comprises 63 sequences, including 61 from GenBank. The sequences of the specimens from Benin cluster together with *Lc. cinnamomeodiscus* Asif, Saba & M. Raza, *Lc. irinellus* (Chalange) Kun L. Yang, Jia Y. Lin & Zhu L. Yang, *Lc. sericifer* (Locq.) Asif, Saba & Vellinga, *Lc. viriditinctus* (Berk. & Broome) Asif, Saba & Vellinga, and two sequences deposited as *Lc. caerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu (basionym: *La. coerulescens*) in GenBank.

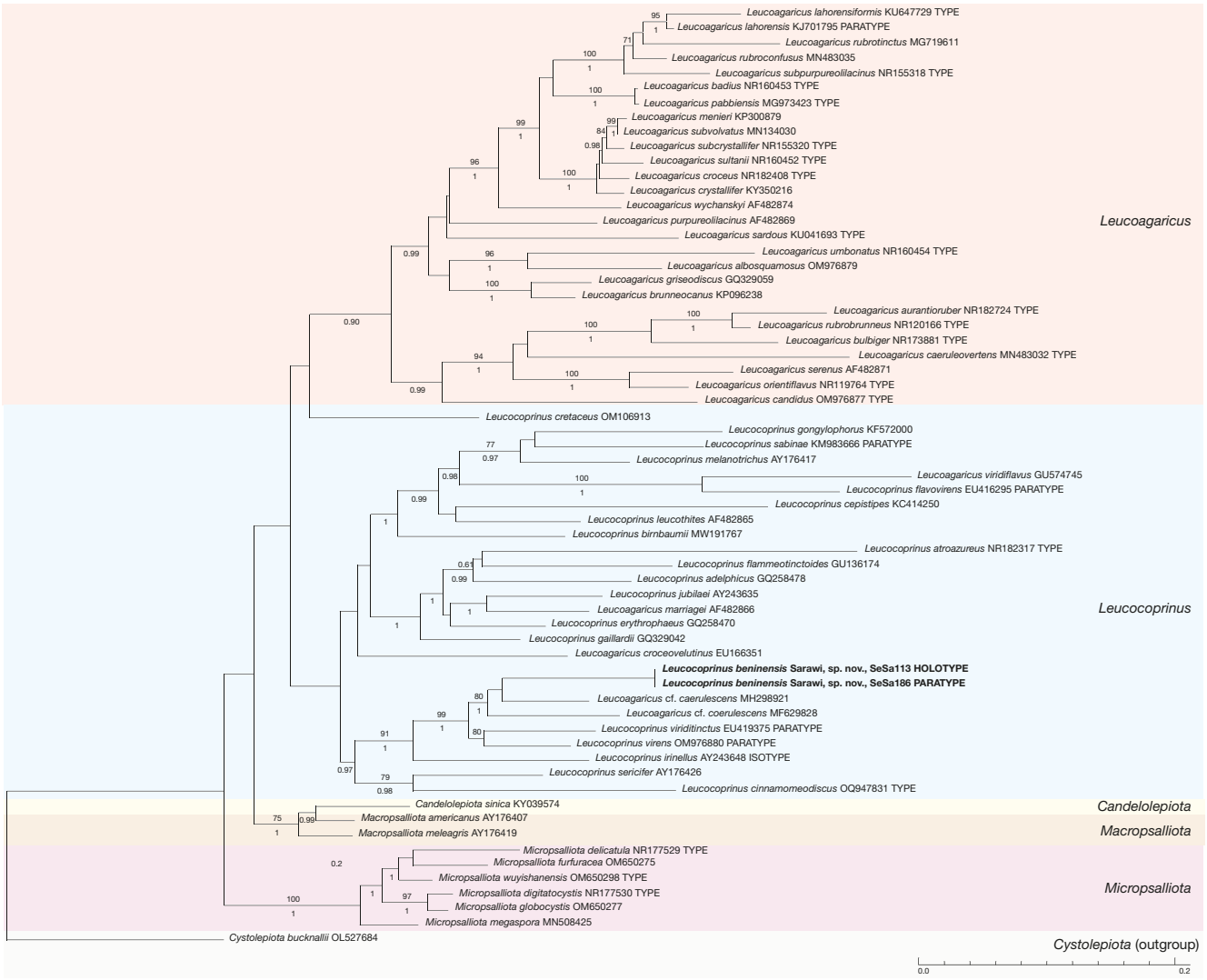

FIG. 1. — Maximum-Likelihood phylogram of *Leucocoprinus* Pat. species and the outgroup based on ITS sequences. Maximum-Likelihood bootstrap values (> 70) are shown above branches and posterior probabilities obtained from Bayesian analysis (> 90 %) are shown below. Scale bar: estimated changes per nucleotide.

The phylogenetic position of the specimens from Benin within the cluster is well supported by the classical bootstrap values as well as by the bayesian PP.

TAXONOMY

Family AGARICACEAE Chevall.
Genus *Leucocoprinus* Pat.

Leucocoprinus beninensis Sarawi, sp. nov.
(Fig. 2)

HOLOTYPE. — **Benin** • Borgou Department, Okpara, Forêt de Okpara; on sandy soil, in woodland dominated by *Isoberlinia doka* Craib & Stapf and *Uapaca togoensis* Pax; 9°14'13.2"N, 2°43'26.4"E; 200 m alt.; 12.VI.2022; *S. Sarawi, S. Badou, D. Dongnima, F. Hampe, C. Manz, B. Olou, A. Rühl SeSa113*; FR-0246101, isotype: UNIPAR; GenBank PX634060 (ITS).

ETYMOLOGY. — The epithet *beninensis* refers to Benin, the country in which the type specimens of this species were collected.

ADDITIONAL MATERIAL. — **Benin** • Collines Department, near Kilibo; on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*; 8°36'57.6"N, 2°39'54"E; 320 m alt.; 21.VI.2022; *S. Sarawi, D. Dongnima, F. Hampe, C. Manz, A. Rühl SeSa186*; FR-0246102; GenBank PX634061 (ITS).

MYCOBANK. — MB#861479.

ECOLOGY, HABITAT, AND DISTRIBUTION. — Basidiomata solitary or gregarious, pressumably saprotrophic, on soil, in woodland. Currently only known from Benin.

DESCRIPTION

Basidiomata small to medium sized. Pileus 5-25 mm diam., initially campanulate or hemispherical, then convex and eventually plano-convex, umbonate, pileus covered by almost erect, tomentose squamules, light reddish brown to light brown (7C2-4, D3-5, 8C2-4, D3-6), sometimes greyish-brown (7E2-3, F2-3) with a darker reddish brown to brown pileus

TABLE 1. — Specimens used for phylogenetic analyses and the corresponding GenBank accession 355 numbers.

Species	Voucher	Origin	ITS	References
Cystolepiota bucknallii (Berk. & Broome) Singer & Clémençon	SeSa12	Germany	OL527684	Sarawi *et al.* 2022
Candelolepiota sinica Kun L. Yang, Jia Y. Lin & Zhu L. Yang	HKAS 52738	China	KY039574	Yang & Ge 2017
Leucoagaricus albosquamosus Y.R. Ma, Z.W. Ge & T.Z. Liu	CFSZ20662	China	OM976879	Ma *et al.* 2022
Leucoagaricus aurantioruber Y.R. Ma, Z.W. Ge & T.Z. Liu	CFSZ:19756, holotype	China	NR182724	Ma *et al.* 2022
Leucoagaricus badius S. Hussain, Pfister, Afshan & Khalid	LAH:SH148, holotype	Pakistan	NR160453	Hussain *et al.* 2018
Leucoagaricus brunneocanus Fei Yu, J.F. Liang & Z.W. Ge	*Z. L. Yang 3972*	China	KP096238	Ge *et al.* 2015
Leucoagaricus bulbiger Justo, Bizzi & Angelini	JBSD:130924, holotype	Dominican Republic	NR173881	Justo *et al.* 2021
Leucoagaricus caeruleovertens Justo, Bizzi & Angelini	ANGE 734, holotype	Dominican Republic	MN483032	Justo *et al.* 2021
Leucoagaricus candidus Y.R. Ma, Z.W. Ge & T.Z. Liu	CFSZ20964, holotype	China	OM976877	Ma *et al.* 2022
Leucoagaricus cf. *caerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu	Mushroom Observer # 116307	Mexico	MH298921	Rockefeller 12.V.2018 GenBank direct submission
Leucoagaricus cf. *coerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu	Mushroom Observer # 115977	Mexico	MF629828	Rockefeller 16.VIII.2017 GenBank direct submission
Leucoagaricus croceus S.M. Tang & K.D. Hyde	MFLU:19-1331, holotype	Thailand	NR182408	Manawasinghe *et al.* 2022
Leucoagaricus crystallifer Vellinga	SFC 1010003-02	Spain	KY350216	Caballero *et al.* 2009 24.XII.2016 GenBank direct submission
Leucoagaricus griseodiscus (Bon) Bon & Migl.	MCVE:13719	Italy	GQ329059	Garbelotto *et al.* 2009 24.II.2010 GenBank direct submission
Leucoagaricus lahorensiformis S. Hussain, H. Ahmad, Afshan & Khalid	SHL2, holotype	Pakistan	KU647729	Hussain *et al.* 2018
Leucoagaricus lahorensis Qasim, Amir & Nawaz	LAH 2481336, paratype	Pakistan	KJ701795	Qasim *et al.* 2015
Leucoagaricus menieri (Sacc.) Singer	6275	Netherlands	KP300879	Vellinga 20.V.2015 GenBank direct submission
Leucoagaricus orientiflavus Z.W. Ge	*Z.W. Ge 2063*, holotype	China	NR119764	Schoch *et al.* 2014
Leucoagaricus pabbiensis Usman & Khalid	LAH35302, holotype	Pakistan	MG973423	Usman & Khalid 2018
Leucoagaricus purpureolilacinus Huijsman	*E.C. Vellinga 2291* (L)	Netherlands	AF482869	Vellinga *et al.* 2003
Leucoagaricus rubrobrunneus E.F. Malysheva, Svet. & E.M. Bulakh	LE262863, holotype	Russia	NR120166	Schoch *et al.* 2014
Leucoagaricus rubroconfusus Migl. & Coccia	82002	Italy	MN483035	Justo *et al.* 2021
Leucoagaricus rubrotinctus (Peck) Singer	420526MF0068	China	MG719611	Wang *et al.* 2017 31.XII.2018 GenBank direct submission
Leucoagaricus sardous (Zecchin & Migl.) Cons. & Contu	MCVE 20105, holotype	Italy	KU041693	Muñoz *et al.* 2012
Leucoagaricus serenus (Fr.) Bon & Boiffard	*E.C. Vellinga 1930* (L)	Belgium	AF482871	Vellinga *et al.* 2003
Leucoagaricus subcrystallifer Z. W. Ge & Zhu L. Yang	HKAS:49373, holotype	China	NR155320	Ge *et al.* 2015
Leucoagaricus subpurpureolilacinus Z.W. Ge & Zhu L. Yang	HKAS:48285, holotype	China	NR155318	Ge *et al.* 2015
Leucoagaricus subvolvatus (Malençon & Bertault) Bon	L1	Türkiye	MN134030	Dizkirici *et al.* 2019
Leucoagaricus sultanii S. Hussain, H. Ahmad & Khalid	LAH-SH115, holotype	Pakistan	NR160452	Hussain *et al.* 2018
Leucoagaricus umbonatus S. Hussain, H. Ahmad & Afshan	LAH:SHL1, holotype	Pakistan	NR160454	Hussain *et al.* 2018
Leucoagaricus viridiflavus (Petch) T.K.A. Kumar & Manim.	AK80a	India	GU574745	Liang *et al.* 2010
Leucoagaricus wychanskyi (Pilát) Bon & Boiffard	IX/X-1987	Netherlands	AF482874	Vellinga *et al.* 2003
Leucocoprinus adelphicus (Vellinga) Redhead	*E.C. Vellinga 3153*	United States	GQ258478	Vellinga *et al.* 2010
Leucocoprinus atroazureus (J.F. Liang, Zhu L. Yang & J. Xu) Asif, Saba & Vellinga	HKAS:42670, holotype	China	NR182317	Liang *et al.* 2010
Leucocoprinus beninensis Sarawi, sp. nov.	SeSa113, holotype	Benin	PX634060	This study
Leucocoprinus beninensis Sarawi, sp. nov.	SeSa186	Benin	PX634061	This study
Leucocoprinus birnbaumii (Corda) Singer	PDD:117582	New Zealand	MW191767	Johnston *et al.* 2020 04.XI.2020 GenBank direct submission
Leucocoprinus cepistipes (Sowerby) Pat.	A18	China	KC414250	Shang *et al.* 2012 25.IX.2013 GenBank direct submission
Leucocoprinus cinnamomeodiscus Asif, Saba & M. Raza	*Asif-SP14AS11*, holotype	Pakistan	OQ947831	Asif *et al.* 2024
Leucocoprinus cretaceus (Bull.) Locq.	JLF10042	United States	OM106913	Frank 2022 09.I.2022 GenBank direct submission
Leucocoprinus croceovelutinus Bon & Boiffard	*E.C. Vellinga 3131* (UC)	United States	EU166351	Vellinga & Sundberg 2008
Leucocoprinus erythrophaeus (Vellinga) Redhead	*E.C. Vellinga 3248* (UC)	United States	GQ258470	Vellinga *et al.* 2010

Table 1. — Continuation

Species	Voucher	Origin	ITS	References
Leucocoprinus flammeotinctoides (Vellinga) Redhead	*E.C. Vellinga 3247* (UC)	United States	GU136174	Vellinga 2010 04.VI.2012
Leucocoprinus flavovirens (J.F. Liang, Zhu L. Yang & J. Xu) Kun L. Yang, Jia Y. Lin & Zhu L. Yang	HKAS 50024, paratype	China	EU416295	Liang *et al.* 2010
Leucocoprinus gaillardii (Bon & Boiffard) Asif, Saba & Vellinga	MCVE:736	Italy	GQ329042	Garbelotto *et al.* 2009 24.02.2010 GenBank direct submission
Leucocoprinus gongylophorus (Möller) Heim	Cylo005	Panama	KF572000	Kooij *et al.* 2015
Leucocoprinus irinellus (Chalange) Kun L. Yang, Jia Y. Lin & Zhu L. Yang	*Chalange 97082101*, isotype	France	AY243648	Vellinga 04.VI.2012 GenBank direct submission
Leucocoprinus jubilaei (Joss.) Wasser	*J. & A. Guinberteau 99101101*	France	AY243635	Vellinga 04.VI.2012 GenBank direct submission
Leucocoprinus leucothites (Vittad.) Redhead	*E.C. Vellinga 2050* (L)	Netherlands	AF482865	Vellinga *et al.* 2003
Leucocoprinus marriagei (D.A. Reid) Bon	*E.C. Vellinga 2005* (L)	Netherlands	AF482866	Vellinga *et al.* 2003
Leucocoprinus melanotrichus (Malençon & Bertault) Migl. & Donato	*E.C. Vellinga 2262* (L)	Netherlands	AY176417	Vellinga 2004
Leucocoprinus sabinae (Angelini, Justo & Vizzini) Kun L. Yang, Jia Y. Lin & Zhu L. Yang	ANGE305, paratype	Dominican Republic	KM983666	Justo *et al.* 2015
Leucocoprinus sericifer (Locq.) Asif, Saba & Vellinga	*E.C. Vellinga 2116* (L)	Netherlands	AY176426	Vellinga 2004
Leucocoprinus virens (Y.R. Ma, Z.W. Ge & T.Z. Liu) Asif, Saba & Vellinga	CFSZ19794_2, paratype	China	OM976880	Ma *et al.* 2022
Leucocoprinus viriditinctus (Berk. & Broome) Asif, Saba & Vellinga	HKAS 50033, paratype	China	EU419375	Liang *et al.* 2010
Macropsalliota americanus (Peck) Kun L. Yang, Jia Y. Lin & Zhu L. Yang	*E.C. Vellinga 2454* (UCB)	United States	AY176407	Vellinga 2004
Macropsalliota meleagris (Gray) Kun L. Yang, Jia Y. Lin & Zhu L. Yang	*E.C. Vellinga 1990* (L)	Netherlands	AY176419	Vellinga 2004
Micropsalliota delicatula R.L. Zhao, J. Xin Li & M.Q. He	HMAS:290752, holotype	China	NR177529	Li *et al.* 2021
Micropsalliota digitatocystis R.L. Zhao, J. Xin Li & M.Q. He	HMAS:290750, holotype	China	NR177530	Li *et al.* 2021
Micropsalliota furfuracea R.L. Zhao, Desjardin, K. Soytong & K.D. Hyde	HFJAU1570	China	OM650275	Yan *et al.* 2022
Micropsalliota globocystis Heinem.	HFJAU1518	China	OM650277	Yan *et al.* 2022
Micropsalliota megaspora R.L. Zhao, Desjardin, K. Soytong & K.D. Hyde	HFJAU0712	China	MN508425	Yan *et al.* 2022
Micropsalliota wuyishanensis J.Q. Yan	HFJAU3048, holotype	China	OM650298	Yan *et al.* 2022

centre (7E5-6, 8E4-6, F4-6), sometimes purplish-brown or grey (9E3-5, F1-5), on a slightly striate, white background. Lamellae free, moderately crowded, ventricose, 1-3 mm deep, white. Stipe 10-30 × 1-3 mm, cylindrical, equal, not bulbous at the base, smooth, white, towards the base yellowish to light brown (6A1-3; B1-2). Annulus persistent, ascending, white. Odour and taste not recorded. Basidiospores (80/2/2) (5.3–)5.8–6.4-6.9(–7.6) × (3.8–)4.1–4.3-4.5(–4.8) µm, Q = 1.33-1.69, Q_{av} = 1.46-1.51, amygdaliform, ellipsoid to ovoid, hyaline, smooth, without germ pore, dextrinoid, non-metachromatic. Basidia (16–)17-20(–23) × (6.7–)7.6-9.0(–10.0) µm, clavate, hyaline, with oily content, mostly 4-spored, sometimes 1- or 2-spored, with up to 5 µm long sterigmata, pseudoparaphyses absent. Lamella edge fertile, consisting of a homogeneous mixture of basidia and basidioles. Cheilocystidia absent. Pleurocystidia absent. Pileus covering a hymeniderm made up of chains of cylindrical to subglobose elements, sometimes disorderly arranged, terminal elements narrowly clavate to clavate, sometimes with acute apex, (23–)29-43(–47) × (6.5–)8.5-16.0(–20.0) µm, with pale brown to brown intracellular pigment and sometimes with encrusted brown pigments. Clamp connections absent in all parts of the basidiomata.

DISCUSSION

In this study, a species of *Leucocoprinus* from Benin is described as new to science based on morphological and phylogenetic studies for the first time. Basidiomata of *Leucocoprinus beninensis* Sarawi, sp. nov., are characterized by reddish brown, light brown to brown squamules on a white background on the pileus. Notably, these basidiomata, predominantly the lamellae, turn greenish-blue to greyish-blue after drying. Further, they exhibit an ascending annulus, dextrinoid, non-metachromatic, amygdaliform to ellipsoid basidiospores, and lack cheilocystidia.

Phylogenetically, *Lc. beninensis* Sarawi, sp. nov., forms a distinct clade together with *Lc. cinnamomeodiscus*, *Lc. irinellus*, *Lc. sericifer*, *Lc. viriditinctus*, and two sequences deposited in GenBank as *Lc. caerulescens* (Fig. 1). The latter two sequences are labelled as "*Lc.* cf. *caerulescens*" probably due to their change of colour, basidiospore size, and cheilocystidia. No sequence data of the holotype of *La. caerulescens* is available, however, Liang *et al.* (2010) investigated the holotype and provided a detailed morphological description. Morphologically, *Lc. beninensis* Sarawi, sp. nov., differs from most known species by the colour of its basidiomata, absence of cheilocystidia, and

TABLE 2. — Morphological differences matrix of *Leucocoprinus beninensis* Sarawi, sp. nov., and closely related species. — indicates missing information.

	***Lc. beninensis* Sarawi, sp. nov. (this study)**	***Lc. cinnamomeodiscus* Asif, Saba & M. Raza (Asif *et al.* 2024)**	***Lc. irinellus* (Chalange) Kun L. Yang, Jia Y. Lin & Zhu L. Yang (Chalange 1999)**	***Lc. sericifer* (Locq.) Asif, Saba & Vellinga (Vellinga 2001)**	***Lc. virens* (Y.R. Ma, Z.W. Ge & T.Z. Liu) Asif, Saba & Vellinga (Ma *et al.* 2022)**	***Lc. viriditinctus* (Berk. & Broome) Asif, Saba & Vellinga (Liang *et al.* 2010)**	***Lc. caerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu (Liang *et al.* 2010)**	***La.* cf. *caerulescens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu (MH298921)**	***La.* cf. *coerulenscens* (Peck) J.F. Liang, Zhu L. Yang & J. Xu (MF629828)**
Pileus centre colour	Reddish brown, light brown, brown	Cinnamon-like or dull orange	Ochraceous brown to brown	Pale ochraceous cream to yellowish	Brown to grey-brown	Pale brown, reddish brown to dark brown	Brownish	Reddish brown to brown	–
Basidio-spore	5.8-6.9 × 4.1-4.5	4.7-5.5 × 3.4-3.8	8.0-9.0 × 4.5-5.0	6.0-9.5 × 3.0-4.5	8.0-10.0 × 4.5-5.0	7.0-8.5 × 4.0-5.0	7.5-9.5 × 4.0-5.0	–	7.0-8.0 × 4.0-4.5
Basidio-spore properties	Amygdaliform to ellip-soid, with-out germ pore, dex-trinoid, non-metachro-matic	Ellipsoid to oblong, without germ pore, dextrinoid	Ellipsoide to subovoid, metachro-matic	Oblong amygda-liform to cylindrical-amygdali-form, with-out germ pore, dex-trinoid, metachro-matic	Amygdaliform to ellipsoid, without germ pore, dextrinoid, metachro-matic	Amygdaliform to ovoid, without germ pore, dextrinoid, metachro-matic	Amygdaliform to ovoid, without germ pore, dextrinoid, metachro-matic	–	Amygdali-form to ellip-soid, without germ pore, dextrinoid, metachro-matic
Cheilocys-tidia	Absent	Present	Absent	Present	Present	Present	Present	Present	Present
Colouring reactions	Greenish-blue to greyish-blue when dried	–	With ammonia first turning purple and than getting bluish-grey	–	Dark green to light green when bruised or dried	Dark blue when bruised or dried	Bluish green when dried	–	Vinaceous red then greenish when bruised and dark olive-grey when dried

basidiospore size (Table 2). *Leucocoprinus beninensis* Sarawi, sp. nov., has significant smaller basidiospores than *Lc. irinellus*, *Lc. caerulenscens, Lc. sericifer*, and *Lc. viriditinctus* and has larger basidiospores than *Lc. cinnamomeodiscus*. The absence of cheilocystidia is a characteristic only shared by the closely related species *Lc. irinellus*, which differs by the colour of the basidiomata and larger basidiospores. Additionally, basidiomata of *Lc. beninensis* Sarawi, sp. nov., have a less intense change in colour after drying than the closely related species.

Several further species of *Leucoagaricus*, *Leucocoprinus,* and *Lepiota* as described in older studies in East and Central Africa and other (sub-)tropical regions are morphologically similar to *Lc. beninensis* Sarawi, sp. nov., but lack molecular sequence data. According to information available, basidiomata of these species do not change in colour by bruising or drying. *Leucoagaricus striatulus* Heinem., known from Central Africa, is macromorphologically similar to *Lc. beninensis* Sarawi, sp. nov., but differs by pinkish brown colours, when dried cream with dark brown squamules, and more distinctive amygdaliform, metachromatic basidiospores (Heinemann 1973). *La. fuligineus* Pegler and *La. rhodocephalus* (Berk.) Pegler known from East Africa are also morphologically similar. *Leucoagaricus fuligineus* differs from *Lc. beninensis* Sarawi, sp. nov., by more robust basidiomata, a non-striate pileus margin, and metachromatic basidiospores (Pegler 1977). *Leucoagaricus rhodocephalus* differs from *Lc. beninensis* Sarawi, sp. nov., by pinkish to reddish colours, more robust basidiomta, and strongly amygdaliform basidiospores (Pegler 1977). Another morphologically similar species is *Lc. venezuelanus* Dennis, known from Martinique, Venezuela, and Zaire (Central Africa). *Leucocoprinus venezuelanus* differs from *Lc. beninensis* Sarawi, sp. nov., by its greyish brown to orange-brown colours, minute brown squamules on the annulus, and the presence of a germ pore (Dennis 1952). Beeli (1932) recorded *Lepiota montagnei* (Kalchbr.) Sacc. for Central Africa based on two collections. Later, Beeli separated them due to morphological differences by introducing *L. montagnei* var. *congolensis* Beeli (Beeli 1936). Later Heinemann (1977) renamed one of these two collections as *Lc. submontagnei* Heinem. based on studies of the holotype and a watercolour drawing. *Leucocoprinus submontagnei* (former *L. montagnei* var. *congolensis*) resembles *Lepiota micropholis* (Berk. & Broome) Sacc., known from Trinidad and Venezuela, but differ by larger basidiospores (Dennis 1952). *Lepiota micropholis* differs from *Lc. beninensis* Sarawi, sp. nov., by its dark purplish brown to black colours and smaller basidiospores (Dennis 1952). *Leucocoprinus submontagnei* differs

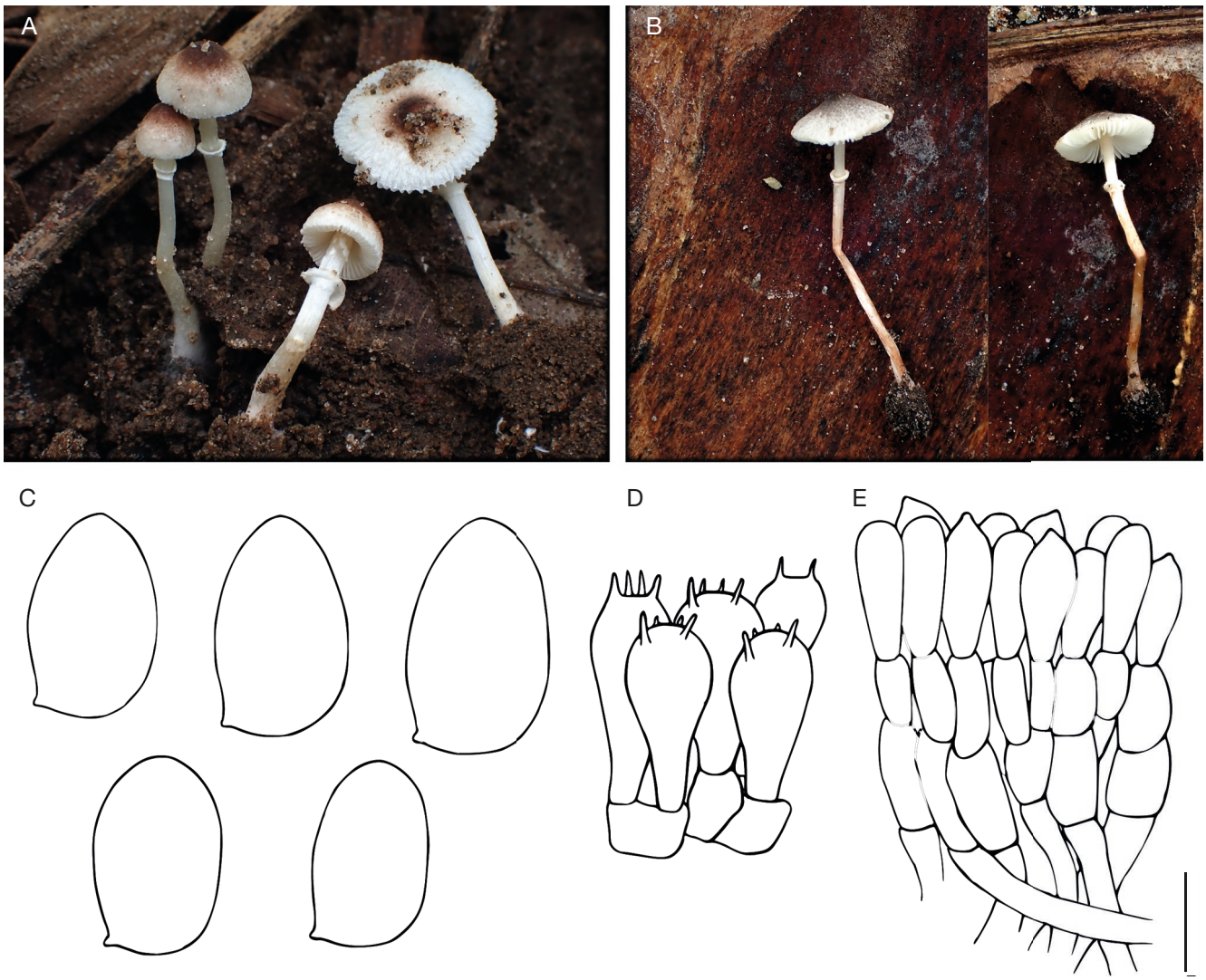

Fig. 2. — Basidiomata and microstructures of *Leucocoprinus beninensis* Sarawi, sp. nov. (SeSa113, holotype): **A**, basidiomata (SeSa113, holotype); **B**, basidioma (SeSa186), seen from above (**left**) and from below the pileus (**right**); **C**, basidiospores; **D**, basidia; **E**, pileus covering. Scale bars: A, 1.5 cm; B, 0.5 cm; C, 3 µm; D, 10 µm; E, 20 µm. Photo credits: Cathrin Manz.

from *Lc. beninensis* Sarawi, sp. nov., by basidiomata with dark brown-violet colours when fresh and ochre-brown colours when dried and slightly larger, metachromatic basidiospores (Heinemann 1977).

These results highlight the need for further exploration of unstudied fungal species in the forests of Benin. Additionally, they emphasise the importance of re-investigating and re-collecting previously documented African species to enhance our understanding through modern molecular techniques.

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