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Hidden gems of Benin: Unravelling the diversity of *Lepiota* spp. through phylogenetic, morphological, and chemotaxonomic exploration

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Abstract: We investigated *Lepiota* species from Benin and reference species from Europe based on fieldwork, light microscopy, and the analysis of DNA sequence data. As a result, thirteen *Lepiota* species from Benin are described as new to science, whereas only one previously described species, *L. albogranulosa*, was found. An identification key to these species is provided. The taxonomical results are supported by a comprehensive multilocus phylogeny based on ITS, LSU, and *RPB2* sequences. In addition, the occurrence of amatoxins in basidiomata of *Lepiota* spp. was examined, in order to detect toxic mushrooms and for chemotaxonomic conclusions for the genus *Lepiota* in general. To this end, an UHPLC-HRMS² analysis process was applied and molecular networks were generated to obtain an overview of the chemical diversity encompassed by 46 *Lepiota* species, with particular emphasis on their amatoxin content, resulting in the first report of a *Lepiota* species containing amatoxin for Benin. The annotation coverage was extended using DEREPLICATOR and taxonomically informed metabolite annotation (TIMA). Birnbaumins were discovered in several species as well as other chemical features of possible chemotaxonomic interest. This study extends the knowledge about diversity, amatoxin occurrence, and possible chemotaxonomic markers in the genus *Lepiota*.

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INTRODUCTION

Lepiota (*Agaricaceae*) is a worldwide distributed genus which currently is one of the ten largest genera in *Agaricales* (Kalichman *et al.* 2020). Many more species remain to be discovered, especially in the tropics (Berkeley & Broome 1871, Beeli 1932, 1936, Dennis 1952, 1970, Pegler 1972, Vellinga 2004). Recently, several *Lepiota* species have been newly described from the tropics and subtropics of South America and Southeast Asia (Bashir *et al.* 2020, Hou & Ge 2020, Sysouphanthong *et al.* 2020, Asif *et al.* 2022, Haqnawaz *et al.* 2022, Ahamed *et al.* 2023, Liang *et al.* 2023, Mao *et al.* 2023, Roy *et al.* 2023, Asif *et al.* 2024, Azeem *et al.* 2024, Rehman *et al.* 2024). Despite the early work of Beeli and Heinemann in the Democratic Republic of Congo and Central Africa (Beeli 1932, 1936, Heinemann 1973, 1977, 1980), many *Lepiota* species in Africa remain undiscovered and undescribed. For West Africa, 18 species of *Lepiota* have been recorded, but not even one from Benin (Piepenbring *et al.* 2020). West Africa provides suitable habitats for a variety of fungal species due to its diverse ecosystems, ranging from tropical rainforests to savannas. However, mycological studies in the region remain limited, and Benin in particular, has been largely overlooked in fungal biodiversity research.

Lepiota species are characterized by basidiomata with universal and partial veils, free lamellae, whitish spore powder, predominantly dextrinoid basidiospores, and clamp connections (Vellinga 2003). According to Sarawi *et al.* (2025), the genus *Lepiota* can be divided into seven sections based on pileus covering and basidiospore shapes: section *Stenosporae*, with a trichoderm and spurred basidiospores; section *Helveolae*, with a trichoderm and ovoid to ellipsoid basidiospores; section *Christatae,* with a hymeniderm or a pileus covering consisting of (sub)globose cells arranged in chains, ovoid to ellipsoid or spurred basidiospores, and brownish pileus colours; section *Lilaceae*, with a hymeniderm pileus covering, ovoid to ellipsoid basidiospores, and violet or vivid orange-brown pileus colours; section *Lepiota*, with a trichodermal pileus covering and fusiform to penguin-shaped basidiospores; section *Fuscovinaceae*, with a cutis and ovoid to ellipsoid basidiospores; and section *Eriophorae*, with a pileus covering consisting of (sub)globose cells arranged in chains and ovoid to ellipsoid basidiospores. The phylogenetic positions of these sections and the species they include were examined using ITS and LSU, a low number of IGS sequences, *RPB2*, or mtSSU sequence data, and correlated with morphological character states (Vellinga 2003, Liang *et al.* 2018, Hou & Ge 2020, Sarawi *et al.* 2025).

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Certain *Lepiota* species are of medical concern due to the presence of amatoxins in their basidiomata. Amatoxins are bicyclic octapeptides that cause severe liver damage and even death by inhibiting RNA polymerase II (Wieland *et al.* 1981). They are responsible for 95 % of all fatal mushroom poisonings worldwide (Gicquel *et al.* 2014). Amatoxins also occur in two other, not closely related genera, viz., *Amanita* (*Amanitaceae*) and *Galerina* (*Strophariaceae*) (Luo *et al.* 2018). The distribution of amatoxins within *Lepiota* has long been unclear; however, a recent study demonstrated that fewer *Lepiota* species contain amatoxins than previously assumed and that they are apparently restricted to subsection *Helveolinae* (Sarawi *et al.* 2022).

In addition to amatoxins, another group of cyclic toxins, namely phallotoxins, exists. These bicyclic heptapeptides interact with the F-actin filaments and thus affect the cell structure by inhibiting the depolymerisation of F-actin (Wieland 1977). It has been demonstrated that phallotoxins are not absorbed via the gastrointestinal tract and are therefore not responsible for the toxicity of certain species (Wieland 1983). So far, phallotoxins have not been reported for *Lepiota* species (Sgambelluri *et al.* 2014, Sarawi *et al.* 2022).

So far, only one study is known concerning molecular network analysis for the genus *Lepiota*, in which *L. brunneoincarnata* and species belonging to the genus *Amanita* were analysed for amatoxins and phallotoxins; however, this study does not consider chemotaxonomy (Zhao *et al.* 2023). Expanding on this approach, Feature Based Molecular Networking enables the mapping of large quantities of tandem mass spectrometry data, such as those obtained by (U)HPLC-HRMS^2 of numerous samples. Compounds with similar fragmentation patterns cluster, and therefore probably correspond to structurally related structures (Yang *et al.* 2013). In the process, these tandem mass spectrometry data are also compared to reference MS/MS spectra held in the GNPS spectral repositories to annotate nodes that are analogous to spectroscopic data deposited in these libraries (Wang *et al.* 2016, Nothias *et al.* 2020). Molecular networks can be enriched with functional information relevant to a given study (e.g. bioactivity, phylogenetic information) to identify compounds with specific properties of interest in a given study and, potentially, to streamline subsequent isolation for full structural elucidation. Innovative chemoinformatic tools are continuously being developed to extend the annotation coverage to compounds for which experimental MS/MS data have not been uploaded yet to the GNPS spectral libraries such as DEREPLICATOR, a dereplication algorithm dedicated to the identification of peptide natural products from their tandem mass spectra (Mohimani *et al.* 2017). Recorded MS/MS spectra can also be compared against in silico MS/MS fragmentation database (ISDB), through tools like the TIMA v. 2.9.3 package and LOTUS database (Allard *et al.* 2016, Rutz *et al.* 2019, Rutz *et al.* 2022). In short, this latter strategy considers not only the spectral similarity score, but also the taxonomic distance between the biological source of the annotated compound and the biological source of the candidate structures, as well as the structural consistency between the different nodes of a group (structural consistency being expected within the different nodes of a group) (Rutz *et al.* 2019).

By integrating these techniques, molecular networking provides valuable insights into the chemical diversity of *Lepiota* species, facilitating the discovery of novel bioactive compounds and improving our understanding of fungal secondary metabolism.

MATERIALS AND METHODS

Fieldwork and morphological studies

Lepiota specimens were collected during mycological surveys in June and July 2022 at different sites in Benin, West Africa. They were found in gallery forests, woodlands, and savannahs with trees identified as *Berlinia grandiflora*, *Isoberlinia doka*, *I. tomentosa*, *Monotes kerstingii*, *Uapaca guineensis*, and/or *Uapaca togoensis*, among other species. The macroscopic characteristics of the freshly collected basidiomata were recorded in the field. Colour codes were assigned using Kornerup & Wanscher (1978). The basidiomata were air-dried on an electric food dehydrator at 40 °C. The microscopic features were examined using light microscopes. 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 metachromasy of the basidiospores. Measurements are given, with average value and extreme values in brackets. The basidiospore measurements are presented along with the information (n/m/p), denoting 'n' basidiospores from 'm' basidiomata of 'p' collections. The specimens from Benin are deposited in the Herbarium FR, Frankfurt am Main, Germany, and isotypes are deposited in the mycological herbarium of the University of Parakou, UNIPAR, Benin.

DNA extraction, PCR amplification and sequencing

For DNA extraction, small pieces of fresh basidiomata were stored in double cetyl-trimethylammonium bromide (CTAB). These pieces were later frozen with liquid nitrogen and ground to a fine powder using an MM301 Mixer Mill (Retsch GmbH, Germany). The extraction was performed using the innuPREP plant DNA kit (IST Innuscreen, Germany) following the manufacturer's instructions. The PCR was performed using VWR *Taq* DNA polymerase (VWR, Germany) in a peqStar gradient thermal cycler (PEQLAB, Germany). For the amplification of the nuclear ribosomal internal transcribed spacer (ITS) region, the primer pairs ITS1F or ITS5 together with ITS4B or ITS4 (White *et al.* 1990, Gardes & Bruns 1993) and for the amplification of the 28S nuclear ribosomal large subunit region (LSU), the primer pair LR0R/LR7 were used (Vilgalys & Hester 1990, Cubeta *et al.* 1991). For the protein encoding gene region RNA polymerase II second largest subunit (*RPB2*), the primer pair bRPB2-6F/rpb2-7.1R (Matheny 2005) was used. The PCR products were verified by gel electrophoresis using a 1 % (w/v) agarose gel and purified using the QIAquick PCR purification kit (QIAGEN, Germany). The purified PCR products were sequenced by Sanger sequencing at Microsynth SeqLab (Germany). All sequences generated are deposited in GenBank (Table 1).

Table 1. Specimens used for phylogenetic analyses and the corresponding GenBank accession numbers.

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
Cystolepiota bucknallii	SeSa12	Germany	OL527684	**PP594631**	—	This study/Sarawi *et al.* (2022)
Cystolepiota hetieri	KUN-HKAS 53554	China	MN810143	MN810102	MN820977	Hou *et al.* (2020)
Echinoderma asperum	SeSa369	Germany	**PP594613**	—	**PP841246**	This study
	SeSa62	Germany	**PP594540**	**PP594656**	**PP841262**	This study
Echinoderma perplexum	SeSa362	Germany	**PP594610**	**PP594733**	**PP841242**	This study
	KaiR1713	Germany	**PP594623**	**PP594751**	**PP841165**	This study
Lepiota alba	KUN-HKAS 90371	China	MN810115	MN810075	MN820946	Hou *et al.* (2020)
Lepiota albogranulosa	T14/LAH:10152012, holotype	Pakistan	LK932284	—	—	Qasim *et al.* (2015)
	SeSa342	Benin	**PP594605**	**PP594728**	**PP841237**	This study
	SeSa347	Benin	**PP594609**	**PP594732**	**PP841241**	This study
Lepiota alopochroa	MFLU 09-0178	Thailand	HQ647294	—	—	Sysouphanthong *et al.* (2011)
Lepiota andegavensis	8-X-1994, P.D.H. Roux 2121 (herb. Roux)	France	AY176461	—	—	Vellinga (2003)
Lepiota angusticystidiata	HKAS 50064, holotype	China	KP177192	KP177198	—	Liang *et al.* (2018)
Lepiota apatelia	hah5x1991	Netherlands	GQ203819	—	—	Vellinga (2010)
	BH20210810	Germany	**PP594619**	**PP594746**	**PP841157**	This study
Lepiota atrobrunneodisca	HSA 115, holotype	China	OP724226	OP724232	—	Mao *et al.* (2023)
Lepiota attenuata	HKAS50110, holotype	China	EU681776	GU199354	MK705816	Liang *et al.* (2011)
Lepiota aurantiicolor	SeSa184	Benin	**PP594570**	**PP594688**	**PP841195**	This study
	SeSa216	Benin	**PP594575**	**PP594695**	**PP841203**	This study
	SeSa181	Benin	**PP594567**	**PP594685**	**PP841192**	This study
	SeSa177	Benin	**PP594564**	**PP594682**	**PP841189**	This study
	SeSa176, holotype	Benin	**PP594563**	**PP594681**	**PP841188**	This study
	LAH37661, holotype	Pakistan	OQ282887	OQ282883	—	Asif *et al.* (2024)
Lepiota aureofulvella	HNL502948	Laos	KX711967	—	—	Sysouphanthong *et al.* (2011)
Lepiota bahawalnagarensis	LAH:37787, holotype	Pakistan	OQ658766	—	—	Asif *et al.* (2024)
Lepiota baiyunensis	B22052705, holotype	China	OQ547186	OQ547188	—	Liang *et al.* (2023)
Lepiota bengalensis	Iqbal 825 (GDGM 45684), holotype	Bangladesh	KU563148	KU563150	—	Hosen *et al.* (2016)
Lepiota beninensis	SeSa296	Benin	**PP594592**	**PP594713**	**PP841221**	This study
	FC-21-201, holotype	Benin	**PP594630**	**PP594755**	**PP841161**	This study
Lepiota boudieri	SeSa13	Germany	OL527685	**PP594636**	**PP841186**	This study/Sarawi *et al.* (2022)

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
	SeSa46	Germany	OL527692	**PP594644**	**PP841252**	This study/Sarawi *et al.* (2022)
Lepiota brunneoaurantia	UEH-F0006, holotype	Pakistan	OR464184	—	—	Azeem *et al.* (2024)
Lepiota brunneodisca	AKD 36/2019 (GUBH 20381), holotype	India	ON935795	—	—	Roy *et al.* (2023)
Lepiota brunneogranulosa	LAH:36803, holotype	Pakistan	NR_184481	NG_149011	—	Asif *et al.* (2022)
Lepiota brunneoincarnata	S8	Germany	OL527674	**PP594738**	**PP841177**	This study/Sarawi *et al.* (2022)
	SeSa54	Germany	OL527694	**PP594648**	**PP841256**	This study/Sarawi *et al.* (2022)
	SeSa4	Germany	OL527681	**PP594632**	**PP841250**	This study/Sarawi *et al.* (2022)
Lepiota brunneoolivacea	SeSa137	Benin	**PP594562**	**PP594680**	**PP841187**	This study
	SeSa214, holotype	Benin	**PP594574**	**PP594693**	**PP841201**	This study
Lepiota brunneopileata	LAH:37842, holotype	Pakistan	OQ970543	—	—	Rehman *et al.* (2024)
Lepiota brunneosquamulosa	HKAS:50142, holotype	China	NR_169900	—	—	Liang *et al.* (2018)
Lepiota castanea	SeSa43	Germany	**PP594531**	**PP594643**	—	This study
	SeSa30	Germany	**PP594527**	**PP594639**	**PP841222**	This study
	SeSa39	Germany	**PP594529**	**PP594641**	**PP841249**	This study
	S7	Germany	OL527673	—	—	Sarawi *et al.* (2022)
Lepiota castaneidisca	E.C. Vellinga 2300 (UC)	USA	AF391057	—	—	Vellinga (2001c)
Lepiota cf. *erythrostricta*	RC_MART_15.175	Martinique, France	**PP594629**	**PP594754**	**PP841169**	This study
	CL_MART_06.091	Martinique, France	**PP594625**	—	—	This study
Lepiota chiangraiensis	MFLU 20-0197, holotype	Thailand	MT020094	MT020099	—	Hyde *et al.* (2020)
Lepiota cholistanensis	LAH:35831, holotype	Pakistan	NR_173802	NG_088084	—	Bashir *et al.* (2020)
Lepiota cingulum	20-IX-1995, M. Enderle (L)	Germany	AY176359	AY176360	—	Vellinga (2003)
Lepiota citrophylla	MFLU 09-0172	Thailand	HQ647295	—	—	Sysouphanthong *et al.* (2011)
Lepiota clypeolaria	SeSa95	Germany	**PP594549**	**PP594667**	—	This study
	SeSa53	Germany	OL527693	**PP594647**	**PP841255**	This study/Sarawi *et al.* (2022)
	SeSa42	Germany	**PP594530**	**PP594642**	**PP841251**	This study
Lepiota condylospora	MFLU 090048, holotype	Thailand	JN224822	—	—	Sysouphanthong *et al.* (2020)
Lepiota cortinarius	HKAS:46095	China	EU416306	EU416307	—	Liang *et al.* (2010)
Lepiota cremea	OKA-TR11019, holotype	Turkey	OL630458	—	—	Kaygusuz (2022)
Lepiota cristata	SeSa70	Austria	**PP594542**	**PP594658**	**PP841265**	This study

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
	SeSa61	Austria	**PP594539**	**PP594655**	**PP841261**	This study
	SeSa55	Germany	OL527695	**PP594649**	**PP841257**	This study/Sarawi *et al.* (2022)
	SeSa59	Austria	**PP594537**	**PP594653**	**PP841260**	This study
Lepiota cristatoides	5-IX-1996, H.A. Huijser s.n. (herb. Huijser)	Netherlands	AY176363	AY176364	—	Vellinga (2003)
Lepiota cystophoroides	E.C. Vellinga 2142 (L)	France	AF391031	AY176365	—	Vellinga (2003)/Vellinga (2001b)
Lepiota echinacea	FH1	Germany	**PP594620**	**PP594747**	**PP841162**	This study
	KUN-HKAS 105582	China	MN810155	MN810104	MN820954	Hou *et al.* (2020)
Lepiota echinella	4-X-1998, H.A. Huijser s.n. (herb. Huijser)	Belgium	AY176366	AY176367	—	Vellinga (2003)
Lepiota echinobispora	SeSa115, holotype	Benin	**PP594560**	**PP594678**	**PP841184**	This study
	SeSa114	Benin	**PP594559**	**PP594677**	**PP841183**	This study
	SeSa345	Benin	**PP594607**	**PP594730**	**PP841239**	This study
Lepiota elaiophylla	SeSa88	Germany	**PP594545**	**PP594662**	**PP841270**	This study
	SeSa363	Germany	**PP594611**	**PP594734**	**PP841243**	This study
Lepiota elseae	AH:40487, holotype	Spain	NR_158471	—	—	Caballero *et al.* (2015)
Lepiota erminea	SeSa98	Germany	**PP594552**	**PP594670**	**PP841276**	This study
	RD21111001	Germany	**PP594617**	**PP594744**	**PP841170**	This study
	S38	Germany	OL527680	**PP594742**	**PP841175**	This study
Lepiota exocarpi	PERTH:08944946	Australia	MT571655	—	—	Unpublished GenBank, Lebel *et al.* (2020)
Lepiota farinolens	PR2021090701	Germany	**PP594618**	**PP594745**	**PP841167**	This study
	E.C. Vellinga 2146 (L)	France	AY176368	AY176369	—	Vellinga (2003)
Lepiota felina	S22	Germany	OL527677	**PP594741**	**PP841173**	This study/Sarawi *et al.* (2022)
	SeSa7	Germany	**PP594523**	**PP594633**	**PP841264**	This study
	SeSa57	Germany	**PP594535**	**PP594651**	**PP841258**	This study
Lepiota flavonigrescens	SeSa305, holotype	Benin	**PP594596**	**PP594717**	**PP841226**	This study
	SeSa306	Benin	**PP594597**	**PP594718**	**PP841227**	This study
Lepiota flavostipitata	SeSa182	Benin	**PP594568**	**PP594686**	**PP841193**	This study
	SeSa243	Benin	—	**PP594699**	**PP841207**	This study
Lepiota flavostipitata	SeSa326	Benin	—	**PP594724**	**PP841233**	This study
	SeSa203, holotype	Benin	—	**PP594690**	**PP841197**	This study
	SeSa215	Benin	—	**PP594694**	**PP841202**	This study

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
	SeSa112	Benin	**PP594558**	**PP594676**	**PP841182**	This study
Lepiota forquignonii	E.C. Vellinga 2284 (L)	Netherlands	AY176370	AY176371	—	Vellinga (2003)
Lepiota fuscovinacea	S36	Germany	OL527678	—	**PP841174**	This study/Sarawi *et al.* (2022)
	SeSa8	Germany	**PP594524**	**PP594634**	**PP841268**	This study
	S17	Germany	—	**PP594739**	**PP841171**	This study
Lepiota grangei	KaiR1768	Germany	**PP594624**	**PP594638**	**PP841166**	This study
	4-X-1998, H.A. Huijser (herb. Huijser)	Belgium	AY176471	—	—	Vellinga (2003)
Lepiota griseovirens	SeSa91	Germany	**PP594548**	**PP594665**	**PP841272**	This study
	SeSa90	Germany	**PP594547**	**PP594664**	**PP841271**	This study
Lepiota haroonabadensis	LAH:36801, holotype	Pakistan	MZ172897	—	—	Niazi *et al.* (2021)
Lepiota hystrix	25-X-1998, H.A. Huijser s.n (herb. Huijser)	France	AY176377	AY176378	—	Vellinga (2003)
Lepiota ignicolor	17-X1-999, H.A. Huijser (herb. Huijser)	Netherlands	AY176472	—	—	Vellinga (2003)
Lepiota ignivolvata	SeSa101	Germany	**PP594555**	**PP594673**	**PP841179**	This study
	SeSa99	Germany	**PP594553**	**PP594671**	—	This study
	SeSa97	Germany	**PP594551**	**PP594669**	**PP841275**	This study
Lepiota jacobi	SeSa94	Germany	—	**PP594666**	**PP841273**	This study
	SeSa76	Germany	**PP594543**	**PP594659**	**PP841266**	This study
	SeSa77	Germany	—	**PP594660**	**PP841267**	This study
	FH7	Germany	**PP594621**	**PP594748**	**PP841163**	This study
	KaiR1690	Germany	—	**PP594750**	**PP841164**	This study
	E.C. Vellinga 2257 (L)	Netherlands	AY176474	—	—	Vellinga (2003)
Lepiota lahorensis	T18/LAH:10002012, holotype	Pakistan	KT182475	—	—	Qasim *et al.* (2016)
Lepiota lilacea	SeSa25	Germany	OL527688	—	—	Sarawi *et al.* (2022)
	SeSa367	Germany	**PP594612**	**PP594735**	**PP841244**	This study
Lepiota lilaceostriata	SeSa241	Benin	**PP594577**	**PP594697**	**PP841205**	This study
	SeSa280, holotype	Benin	**PP594588**	**PP594709**	**PP841217**	This study
Lepiota longisterigmata	SeSa254	Benin	**PP594581**	**PP594702**	**PP841210**	This study
	SeSa253	Benin	**PP594580**	**PP594701**	**PP841209**	This study
	SeSa180	Benin	**PP594566**	**PP594684**	**PP841191**	This study
	SeSa179, holotype	Benin	**PP594565**	**PP594683**	**PP841190**	This study

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
	SeSa208	Benin	**PP594572**	**PP594691**	**PP841198**	This study
	SeSa295	Benin	**PP594591**	**PP594712**	**PP841220**	This study
	SeSa116	Benin	**PP594561**	**PP594679**	**PP841185**	This study
	SeSa183	Benin	**PP594569**	**PP594687**	**PP841194**	This study
	SeSa252	Benin	**PP594579**	**PP594700**	**PP841208**	This study
	SeSa185	Benin	**PP594571**	**PP594689**	**PP841196**	This study
Lepiota luteophylla	25-VIII-1972, H.V. Smith 284 (MICH)	USA	AY176475	—	—	Vellinga (2003)
Lepiota maerimensis	MFLU 12-2036 , holotype	Thailand	MW251839	MW251847	—	Tibpromma *et al.* (2017)
Lepiota magnispora	SeSa68	Austria	**PP594541**	**PP594657**	**PP841263**	This study
	SeSa48	Germany	**PP594532**	**PP594645**	**PP841253**	This study
	SeSa60	Germany	**PP594538**	**PP594654**	—	This study
	SeSa56	Germany	**PP594534**	**PP594650**	—	This study
Lepiota mandarina	HKAS:50028, holotype	China	KM214811	KM214816	—	Liang (2016)
Lepiota mengei	UTC00253524	USA	MN810131	MN810082	MN820942	Hou *et al.* (2020)
Lepiota metulispora	HMAS61859	China	EU681778	MK651673	—	Liang *et al.* (2011)
Lepiota minutisterigmata	SeSa343, holotype	Benin	**PP594606**	**PP594729**	**PP841238**	This study
	SeSa281	Benin	**PP594589**	**PP594710**	**PP841218**	This study
Lepiota minutoflava	SeSa283	Benin	**PP594590**	**PP594711**	**PP841219**	This study
	SeSa239, holotype	Benin	**PP594576**	**PP594696**	**PP841204**	This study
Lepiota neophana	E.C. Vellinga 3735 (UC)	USA	GQ203809	—	—	Vellinga (2010)
Lepiota nigrescentipes	5-X-1993, G. Riousset 93051001 (herb. Riousset)	France	AY176382	AY176383	—	Vellinga (2003)
Lepiota ochraceafulva	SeSa100	Germany	**PP594554**	**PP594672**	**PP841178**	This study
Lepiota omninoflava	KUN-HKAS 106734, holotype	China	MN810157	MN810092	MN820951	Hou *et al.* (2020)
Lepiota oreadiformis	S18	Germany	—	**PP594740**	**PP841172**	This study
	SeSa21	Austria	OL527686	**PP594637**	**PP841199**	This study/Sarawi *et al.* (2022)
	SeSa29	Germany	**PP594526**	—	—	This study
	SeSa96	Germany	**PP594550**	**PP594668**	**PP841274**	This study
Lepiota pakistanensis	LAH:37846, holotype	Pakistan	OQ954776	OQ954783	—	Rehman *et al.* (2024)
Lepiota pilodes	SeSa379	Germany	**PP594614**	**PP594736**	**PP841247**	This study
	SeSa382	Germany	**PP594615**	—	**PP841248**	This study

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
Lepiota pleurocystidiata	MFLU 09-0056, holotype	Thailand	MT020097	MT020102	—	Hyde *et al.* (2020)
Lepiota poliochloodes	MFLU 081272	Thailand	HQ647296	—	—	Sysouphanthong *et al.* (2011)
Lepiota psalion	WU 5152, holotype	Austria	MG581687	MG581699	—	Vizzini *et al.* (2019)
	FC20103101	Germany	**PP594616**	**PP594743**	**PP841160**	This study
Lepiota pseudovenenosa	SeSa304	Benin	**PP594595**	**PP594716**	**PP841225**	This study
	SeSa339	Benin	**PP594604**	**PP594727**	**PP841236**	This study
	SeSa302, holotype	Benin	**PP594593**	**PP594714**	**PP841223**	This study
	SeSa309	Benin	**PP594600**	**PP594721**	**PP841230**	This study
	SeSa303	Benin	**PP594594**	**PP594715**	**PP841224**	This study
Lepiota punaensis	HAW:F-00256, holotype	USA	NR_173815	NG_153999	OM215203	Stallman *et al.* (2020)
Lepiota pyrochroa	E.C. Vellinga 2006 (L)	Netherlands	AY176477	—	—	Vellinga (2003)
Lepiota rhodophylla	UC(USA-CA):1860004, holotype	USA	NR_119624	—	—	Schoch *et al.* (2014)
Lepiota rubella	F22	Germany	**PP594622**	**PP594749**	**PP841159**	This study
	SeSa87	Germany	**PP594544**	**PP594661**	**PP841269**	This study
Lepiota rufipes	9-X-1991, H.A. Huijser (herb. Huijser)	Netherlands	AF391066	—	—	Vellinga (2010)
Lepiota sanguineofracta	TO-HG2916, holotype	Italy	KF879620	—	—	Vizzini *et al.* (2019)
Lepiota scaberula	UC(USA-CA):1860006, holotype	USA	NR_119449	—	—	Schoch *et al.* (2014)
Lepiota sineorecondita	TR gmb 01481, paratype	Netherlands	MK508899	MK508901	—	Vizzini *et al.* (2019)
	HMJAU3799	China	GU199362	GU199355	—	Vizzini *et al.* (2019)
Lepiota sosuensis	JBSD:CA3, holotype	Dominican Republic	NR_184875	—	—	Justo *et al.* (2015)
Lepiota sp.	SeSa261	Benin	**PP594584**	**PP594705**	**PP841213**	This study
	SeSa262	Benin	**PP594585**	**PP594706**	**PP841214**	This study
	SeSa308	Benin	**PP594599**	**PP594720**	**PP841229**	This study
	SeSa325	Benin	—	**PP594723**	**PP841232**	This study
	SeSa318	Benin	**PP594601**	**PP594722**	**PP841231**	This study
	SeSa335	Benin	**PP594602**	**PP594725**	**PP841234**	This study
	SeSa278	Benin	**PP594587**	**PP594708**	**PP841216**	This study
	ANG 460	Dominican Republic	MN250954	–	–	Angelini *et al.* (2020)
	SeSa276	Benin	**PP594586**	**PP594707**	**PP841215**	This study

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
	SeSa346	Benin	**PP594608**	**PP594731**	**PP841240**	This study
	SeSa255	Benin	**PP594582**	**PP594703**	**PP841211**	This study
	SeSa110	Benin	**PP594556**	**PP594674**	**PP841180**	This study
	SeSa307	Benin	**PP594598**	**PP594719**	**PP841228**	This study
	SeSa336	Benin	**PP594603**	**PP594726**	**PP841235**	This study
	PA653	Panama	EF527379	—	—	Vo *et al.* (2009)
Lepiota spheniscispora	UC(USA-CA):1860005, holotype	USA	NR_119448	—	—	Schoch *et al.* (2014)
Lepiota spiculata	JBSD:127426	Dominican Republic	MK696156	MK696155	MK696576	Angelini *et al.* (2020)
	CL_MART_11.034	Martinique, France	**PP594626**	—	—	This study
	CL_MART_11.078	Martinique, France	**PP594627**	**PP594752**	**PP841158**	This study
	RC_MART_10.072	Martinique, France	**PP594628**	**PP594753**	**PP841168**	This study
Lepiota squamulodiffracta	CA21, holotype	Dominican Republic	KR022006	—	—	Justo *et al.* (2015)
Lepiota subalba	SeSa9	Germany	**PP594525**	**PP594635**	—	This study
Lepiota subcastanea	HKAS:45633, holotype	China	KM214812	KM214817	—	Liang (2016)
Lepiota subgracilis	HKAS:5802	China	EU416290	EU416291	—	Liang *et al.* (2010)
Lepiota subincarnata	SeSa28	Germany	OL527689	—	—	This study/Sarawi *et al.* (2022)
	SeSa32	Germany	**PP594528**	**PP594640**	—	This study
	S6	Germany	OL527672	**PP594737**	**PP841176**	This study/Sarawi *et al.* (2022)
	SeSa49	Germany	**PP594533**	**PP594646**	**PP841254**	This study
	SeSa89	Germany	**PP594546**	**PP594663**	—	This study
Lepiota subvenenata	170927-68, holotype	China	MK411234	—	—	Zhang *et al.* (2019)
Lepiota thiersii	E.C. Vellinga 2589 (UC)	USA	GQ203817	—	—	Vellinga (2010)
Lepiota tomentella	SeSa58	Germany	**PP594536**	**PP594652**	**PP841259**	This study
Lepiota tyrianthina	SeSa111	Benin	**PP594557**	**PP594675**	**PP841181**	This study
	SeSa213, holotype	Benin	**PP594573**	**PP594692**	**PP841200**	This study
Lepiota vellingana	LAH37033, holotype	Pakistan	HE974764	—	—	Nawaz *et al.* (2013)
Lepiota venenata	HKAS:101874, holotype	China	NR_160618	—	—	Cai *et al.* (2018)

Table 1. (Continued).

Species	Voucher	Origin	GenBank accession numbers			References
			ITS	LSU	*RPB2*	
	KUN-HKAS 105751	China	MN810128	—	MN820958	Hou *et al.* (2020)
Lepiota viridigleba	OSC:56971, holotype	USA	NR_121542	—	—	Schoch *et al.* (2014)
Lepiota woehnertii	SeSa242	Benin	**PP594578**	**PP594698**	**PP841206**	This study
	SeSa256, holotype	Benin	**PP594583**	**PP594704**	**PP841212**	This study
Lepiota xanthophylla	E.C. Vellinga 2240 (L)	Netherlands	AY176405	AY176406	—	Vellinga (2003)

Phylogenetic analysis

The generated DNA sequences were edited, assembled and quality checked using Geneious Prime v. 2021.2.2 (Biomatters Ltd., New Zealand). Additional sequences from a previous study (Sarawi *et al.* 2022) supplemented with sequences from GenBank were used for the phylogenetic analysis (Table 1). To ensure comprehensive taxonomic and geographic representation, we selected sequences of all major sections of the genus *Lepiota*, with a particular emphasis on multilocus data. Furthermore, we included sequences from geographically diverse populations, covering both temperate and tropical regions, while retaining predominantly those sequences with high similarity (≥ 90 % identity) to our samples. The final single-gene datasets were then aligned separately in MAFFT v. 7 (Katoh & Standley 2013) using the E-INS-i algorithm for the ITS dataset and the FFT-NS-i algorithm for the LSU and *RPB2* datasets (Katoh & Toh 2008). The alignments were checked and pruned in MEGA v. 7 (Kumar *et al.* 2016). A Maximum-Likelihood analysis was conducted for each dataset using RAxML v. 8.2.12 (Stamatakis 2014) applying the GTRCAT model with 25 per site rate categories and 1000 bootstrap iterations (Felsenstein 1985). Since no major topological inconsistencies were found between the Maximum-Likelihood phylogenies of the individual gene loci, the alignments were concatenated and a Maximum-Likelihood analysis was performed as described above using a partitioned model with the three partitions, ITS, LSU, and *RPB2*. Transfer bootstrap expectations (TBE) were obtained for this analysis via BoosterWeb (Lemoine *et al.* 2018). A Bayesian Markov Chain Monte Carlo (MCMC) analysis was performed using MrBayes v. 3.2.7a on XSEDE (Huelsenbeck & Ronquist 2001, Ronquist *et al.* 2012), with two runs of 10 M 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 the Cipres Science Gateway (Miller *et al.* 2010). Phylogenetic trees were visualized using TreeGraph2 (Stöver & Müller 2010).

The alignments and trees are deposited in Figshare (https://doi.org/10.6084/m9.figshare.25827439).

Natural compound extraction and instrument conditions

For the extraction of natural compounds, dry material of each specimen was frozen in liquid nitrogen and subsequently grounded to a fine powder using an MM301 Mixer Mill (Retsch GmbH, Germany). The powder was then suspended in a methanol:dichloromethane (1:1; *v*:*v*) mixture, shaken and incubated at room temperature overnight. Supernatants were obtained by centrifugation at 4000 × g for 10 min and freeze dried using an RVC 2-33 CDplus Rotational vacuum concentrator with a CT 04-50 cold trap (both from Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The dried extracts were suspended in methanol to a final concentration of 2 mg/mL.

The LC-ESI-HRMS^2 analyses were performed on an Agilent 1290 Infinity II UHPLC coupled to a hybrid quadrupole time of flight (QTOF) mass spectrometer Agilent 6546 (Agilent Technologies) equipped with an ESI source, operating in positive ion mode. A BEH Waters Acquity C_{18} UPLC column (2.1 × 100 mm; 1.7 µm) was used, with water + 0.1 % formic acid (A) and acetonitrile + 0.1 % formic acid (B) being used as mobile phase. The flow rate was set to 0.5 mL/min. A linear gradient from 5 % to 100 % B in 12 min followed by 100 % B for 4 min was used. Source parameters were set as follows: capillary temperature at 320 °C, source voltage at 3500 V, sheath gas flow rate at 11 L/min. The MS scans were operated in full-scan mode from *m/z* 100 to 1200 (0.1 s scan time) with a mass resolution of 60.000 at *m/z* = 922. An MS1 scan was followed by MS2 scans of the three most intense ions above an absolute threshold of 3,000 counts. Selected parent ions were fragmented at a collision energy fixed at 25 eV and an isolation window of 1.3 *m/z*. The purine $(M+H)^+$ ion ($C_5H_5N_4^+$, *m/z* = 121.0509) and hexakis (^{1}H, ^{1}H, ^{3}H-tetrafluoropropoxy)phosphazene $(M+H)^+$ ion ($C_{18}H_{19}F_{24}N_3O_6P_3^+$, *m/z* = 922.0098) were used as internal lock masses and a permanent MS/MS exclusion list criterion was set to prevent oversampling of the internal calibrant. The LC-UV and MS/MS data acquisition and processing were performed using MassHunter Workstation software (Agilent Technologies).

MS/MS data preprocessing

The MS data were converted from the Agilent standard .d format to .mzXL format using MSConvert, part of the ProteoWizard package (Chambers *et al.* 2012). The .mzXL files were processed using MZMine 3 v. 3.9.0 (Schmid *et al.* 2023). The threshold for mass detection was set to 1.5E3 for the MS^1 and 7.0E2 for the MS^2. The ADAP chromatogram builder algorithm was used with a minimum group size of scans of three, a group intensity threshold of 2.0E3, a minimum height intensity of 3.0E4, and a *m/z* tolerance of 0.005 Da or 10 ppm. For chromatogram deconvolution, the Local minimum resolver algorithm was selected with a chromatographic threshold of 90 %, a minimum search range in retention time of 0.05 min, a minimum relative height of 0 %, a minimum absolute height of 3E4, a minimum ratio of peak top/edge of two, a peak duration range of 0–1 min, and a minimum of three data points. For pairing the MS^2 scans, a *m/z* tolerance of 0.02 Da or 10 ppm and a retention time tolerance of 0.15 min were set. Isotope grouping was performed using the ^{13}C isotope filter algorithm, with the most intense peak selected as the representative isotope, having a *m/z* tolerance of 0.001 or 3 ppm and a retention time tolerance of 0.05 min. The peak alignment was performed using the join aligner, with an *m/z* tolerance of 0.005 Da or 10 ppm, a retention time tolerance of 0.2 min, and equivalent weights for both. The feature list row filter was used to keep only datasets containing MS^2 scans. Gap filling was performed with an intensity tolerance of 20 %, an *m/z* tolerance of 0.002 or 10 ppm, a retention time tolerance of 0.05 min. The “Export/Submit for GNPS/FBMN” option was used to export the processed data in .mgf and .csv format.

Molecular Network Generation and DEREPLICATOR annotation

A feature-based molecular network (FBMN) analysis was performed with the exported files using the FBMN online workflow v_28.2 via GNPS (Wang *et al.* 2016, Nothias *et al.* 2020) with a precursor ion mass tolerance of 0.02 Da and a fragment ion tolerance of 0.02 Da. The molecular network was created where the edges were filtered to have a cosine score above 0.6 and more than five matched peaks. Edges between two nodes were kept only if each of the nodes appeared in the top 10 most similar nodes of the connected feature (network TopK = 10). Matches against the spectral libraries of the GNPS required a cosine score above 0.7 and at least 5 matched peaks. Cytoscape v. 3.10.2 was used to visualize and analyse the molecular networking data (Shannon *et al.* 2003). The GNPS job parameters and resulting data are available at the following address https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=e527f0adec444f6c9f639781e6616884 and the data resulting from the DEREPLICATOR job (variable dereplication process) are available at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=71fc9eb966da4de49c2c97c9038f5d09. Since the general molecular network could not discriminate between amaninamide (*m/z* = 903.3663, tR = 2.84 min) and γ-amanitin (*m/z* = 903.3661, tR = 2.67 min), a second FBMN workflow was undertaken to address this issue. These toxins could indeed be discriminated using identical processing parameters except for the retention time tolerance of the join aligner module where a more stringent value of 0.1 min was retained. This additional molecular network is made available at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=34abc1cb556f4a4d9b0359bab66502c0 and the data resulting from the DEREPLICATOR job (variable dereplication process) are available at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=80a5cd357c0d4401bc6f95dec578cb25.

ISDB annotation and taxonomically informed reconsideration

The taxonomically informed metabolite annotations were made using TIMA, following the documentation in this repository (v. 2.9.3) and re-ranking from the taxonomical information available on LOTUS (Allard *et al.* 2016, Rutz *et al.* 2019, Rutz *et al.* 2022, Rutz *et al.* 2024). The ISDB used for this process includes the combined records of the Dictionary of Natural Products (v. 32.2) and the LOTUS Initiative records (v. 4.1) (Allard *et al.* 2023). For the TIMA annotation-related parameters the number of final candidates was set to three, the minimal chemical consistency score to consider a class and the minimal similarity score were set to 0, while the minimal biological score and minimal chemical score to keep MS1 only annotation were set to 0.6. The weight for the biological information was set to 0.5, the weight for the chemical information was set to 0.166, and the weight for the spectroscopic matching was set to 0.333 (Hell *et al.* 2022). The biological score matches were set as follows: domain 0.1, kingdom 0.2, phylum 0.3, class 0.4, order 0.5, family 0.6, tribe 0.7, genus 0.8, subgenus 0.85, species 0.9, and variety 1. The chemical score matches were set as follows: kingdom 0.25, superclass 0.5, class 0.75, and parent 1. The option parameter “Summarise results to one row per feature” was chosen.

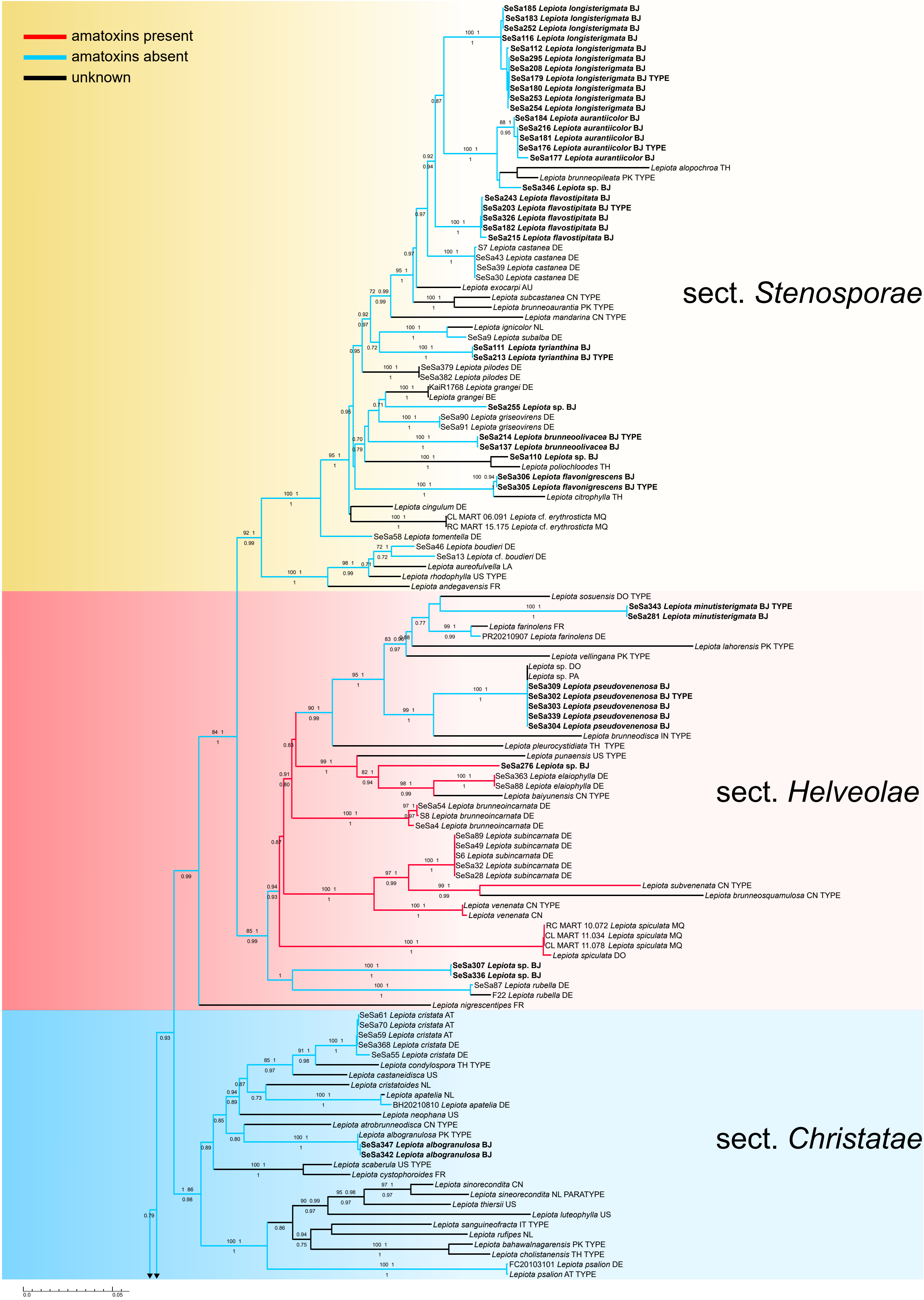


Fig. 1. Maximum-Likelihood phylogram of *Lepiota* species and the outgroup based on concatenated alignments of ITS, LSU, and *RPB2*. Maximum-Likelihood bootstrap values (> 70 %) and posterior probabilities obtained from Bayesian analysis (0.90) are shown on the upper side of each branch and transfer bootstrap expectations (> 70 %) are shown on the lower side. The geographic origin is indicated by two-letter codes according to ISO 3166. Sequences of *Lepiota* specimens from Benin are highlighted in **bold**. Information about amatoxin occurrence of *L. venenata* and *L. subvenenata* obtained from Cai *et al.* (2018) and Zhang *et al.* (2019). Scale bar = estimated changes per nucleotide.

RESULTS

Phylogenetic analysis

For this study, a total of 353 sequences of 60 specimens of *Lepiota* species recently collected in Benin and of 72 specimens of *Lepiota* recently collected in Europe were generated. They comprise 108 ITS sequences, 125 LSU sequences, and 119 *RPB2* sequences representing all sections in *Lepiota*. Single-locus trees derived from the sequence data did not exhibit conflicting phylogenies (Supplementary Figs S1–S3). However, the support values for the LSU single-locus tree were low compared to those of the other gene loci. Multilocus phylogenies, using concatenated alignments of ITS and *RPB2*, as well as ITS, LSU, and *RPB2* sequences, were constructed from the data (Supplementary Fig. S4). Once again, the tree topology and branch support were consistent, with no topological incongruence between Maximum-Likelihood and Bayesian analyses. The multilocus phylogeny delineated several clades, corresponding to the sections within the genus *Lepiota*, namely *Christatae*, *Eriophorae*, *Fuscovinaceae*, *Lepiota*, *Lilaceae*, *Helveolae*, and *Stenosporae*. However, the precise positions of the individual sections related to each other were not well supported. The positions of the sequences from specimens from Benin are well supported and they cluster among sequences obtained from specimens from various other geographical regions, without forming distinct clades.

Taxonomy

The morphological and molecular phylogenetic results revealed the recently described species *L. albogranulosa*, known from Pakistan and thirteen hitherto undescribed *Lepiota* species, belonging to the sections *Eriophorae*, *Helveolae*, *Lepiota*, *Lilaceae*, and *Stenosporae*.

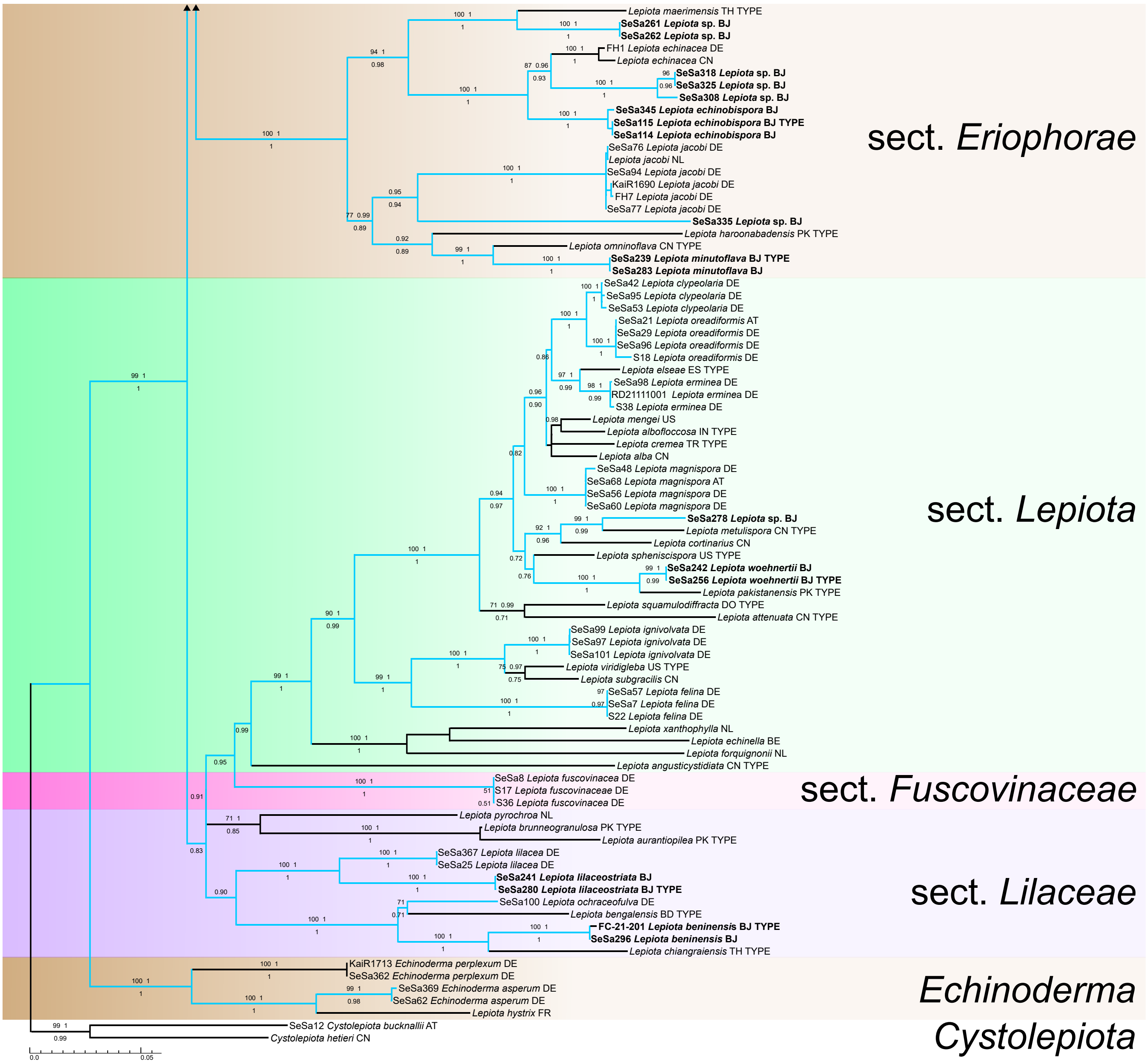


Fig. 1. (Continued).

Section *Eriophorae*

Lepiota echinobispora Sarawi & Reschke, ***sp. nov.*** MB 854014. Fig. 2.

Etymology: *echinos* (Greek) = spiny, *bi* (Latin) = two, *spora* (Greek) = spore; refers to the echinate pileus covering this species possesses and the predominantly bispored basidia.

Typus: **Benin**, Borgou Department, Okpara, Forêt de Okpara N9.237, E2.724, alt. 200 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 12 Jun. 2022, *S. Sarawi, S. Badou, D. Dongnima, F. Hampe, C. Manz, B. Olou & A. Rühl*, SeSa115 (**holotype** FR-0175201, **isotype** in UNIPAR). GenBank: ITS = PP594560, LSU = PP594678, *RPB2* = PP841184.

Basidiomata small to medium. *Pileus* 10–25 mm diam., initially hemispherical to conical, becoming convex to plano-convex, occasionally slightly umbonate, with concentrically arranged conical spines with curved tips, spines brown to dark brown or greyish brown (6E4–8, 6F4–8, 7E4–5), densely clustered at the centre, scattered towards margin; centre of the pileus with brown background (6E4–8, 6F4–8), paler, light brown to cream towards margin (6A2–3, 6D4–5). *Lamellae* free, moderately crowded to crowded, ventricose, 1–3 mm deep, white to cream; edge concolourous; lamellulae in up to two tiers. *Stipe* 10–35 × 1–3 mm, cylindrical, at apex whitish to cream or light brown (6A2–3, 6D4–5) gradually darker brown or greyish brown (6E4–E8, 6F4–8, 7E4–5) below annular zone, in lower half with indistinct spines that are concolorous with pileus spines. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (120/3/3) (3.5–)3.9–4.2–4.5(–5.6) × (2.4–)2.6–2.9–3.1(–3.8) µm, Q = 1.24–1.87, Q_{av} = 1.43–1.53, ovoid to ellipsoid, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (13–)15–19(–23) × (4.5–)5.4–6.6(–7.3) µm, narrowly clavate to clavate, hyaline, with oily content, predominantly 2-spored, but also frequently 4-spored, occasionally 1-spored, with up to 4 µm long sterigmata, when 1- or 2-spored with longer and thicker sterigmata up to 7 µm long. *Lamella edge* mostly sterile, sometimes heterogeneous with some fertile parts. *Cheilocystidia* (10–)13–20(–26) × (3.5–)4.6–6.0(–6.9) µm, cylindrical or narrowly clavate to clavate, hyaline. *Pleurocystidia* absent. *Pileus covering* composed of two types of agglutinated chains forming spines, i.e., chains formed by spherical cells and less abundant chains formed by cylindrical cells; terminal cells of chains formed by spherical cells, (16–)21–32(–41) µm in diam., (sub)globose; basal cells cylindrical or narrowly clavate to clavate. The other type of chains consists of cylindrical to elongated cells, (21–)28–40(–46) × (7–)8–12(–14) µm; terminal cells with rounded apex. Both type of chains with pale brown to brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary, probably saprotrophic, on soil, in woodland. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimens examined: **Benin**, Borgou Department, Okpara, Forêt de Okpara, N9.237, E2.724, alt. 200 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 12 Jun. 2022, *S. Sarawi, S. Badou, D. Dongnima, F. Hampe, C. Manz, B. Olou & A. Rühl*, SeSa114; Collines Department, between Djalloukou and Konkon, N7.730, E1.839, alt. 190 m.a.s.l., on sandy soil, in woodland with *Isoberlinia doka*, 3 Jul. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa345.

Notes: *Lepiota echinobispora* in section *Eriophorae*, is characterized by a light brown, brown to greyish brown coloured pileus with conical spines, dextrinoid, ellipsoid to ovoid basidiospores, predominantly 2-spored basidia, the presence of cheilocystidia, and the cellular structure of the spines, which are composed of chains consisting of either spherical or cylindrical cells. Phylogenetically, *L. echinobispora* is close to *L. echinacea*, with a p-distance of 6 % in the ITS sequence. Macroscopically, distinguishing *L. echinobispora* from *L. echinacea* can be challenging due to the latter's variable micromorphology. However, microscopically, *L. echinobispora* differs from *L. echinacea* by the presence of cheilocystidia, which are either absent or only present in tufts in *L. echinacea*, predominantly 2-spored basidia, compared to mostly 4-spored basidia in *L. echinacea*, and the pileus covering of *L. echinacea,* which is formed by only one type of chain formed by spherical cells (Lange 1940, Knudsen 1980, Vellinga 2001a).

Another morphologically similar species, though not closely related, is *L. jacobi*. *Lepiota jacobi* can be distinguished from *L. echinobispora* by its darker brown basidiomata, 4-spored basidia, and absence of cheilocystidia (Knudsen 1980, Vellinga 1992, 2001a). *Lepiota carinii* can be differentiated from *L. echinobispora* by its yellow-brown colours, the distinctly spiny stipe, and 4-spored basidia (Bresadola 1930, Knudsen 1980, Vellinga 2001a).

Several other similar looking *Lepiota* species are known from older studies in East and Central Africa and other (sub-)tropical regions but these lack molecular sequence data. However, *L. kigumuensis* described from Kenya is morphologically similar to *L. echinobispora*. *Lepiota kigumuensis* differs from *L. echinobispora* by minute, loose, ochraceous brown squamules and significantly larger basidiospores, 4.7–6.5 × 3.0–4.3 (Pegler 1977).

Lepiota minutoflava Sarawi, ***sp. nov.*** MB 854015. Fig. 3.

Etymology: *minutus* (Latin) = very small, *flavus* (Latin) = yellow; refers to the yellow colour and the small size of the basidiomata.

Typus: **Benin**, Collines Department, Ekpa, N8.601, E2.600, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka*, 23 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa239 (**holotype** FR-0175168, **isotype** in UNIPAR). GenBank: ITS = PP594576, LSU = PP594696, *RPB2* = PP841204.

Basidiomata very small to small. *Pileus* 2–5 mm diam., initially hemispherical to conical or campanulate, becoming convex to plano-convex, at centre yellow to orange or orange-brown (5A7–8, 5B7–8, C6–8), gradually paler, light orange-brown to pale yellow towards margin (5A5–6, 5B4–6, 5C5–6), covered by minuscule, granulose, brown to orange-brown warts.

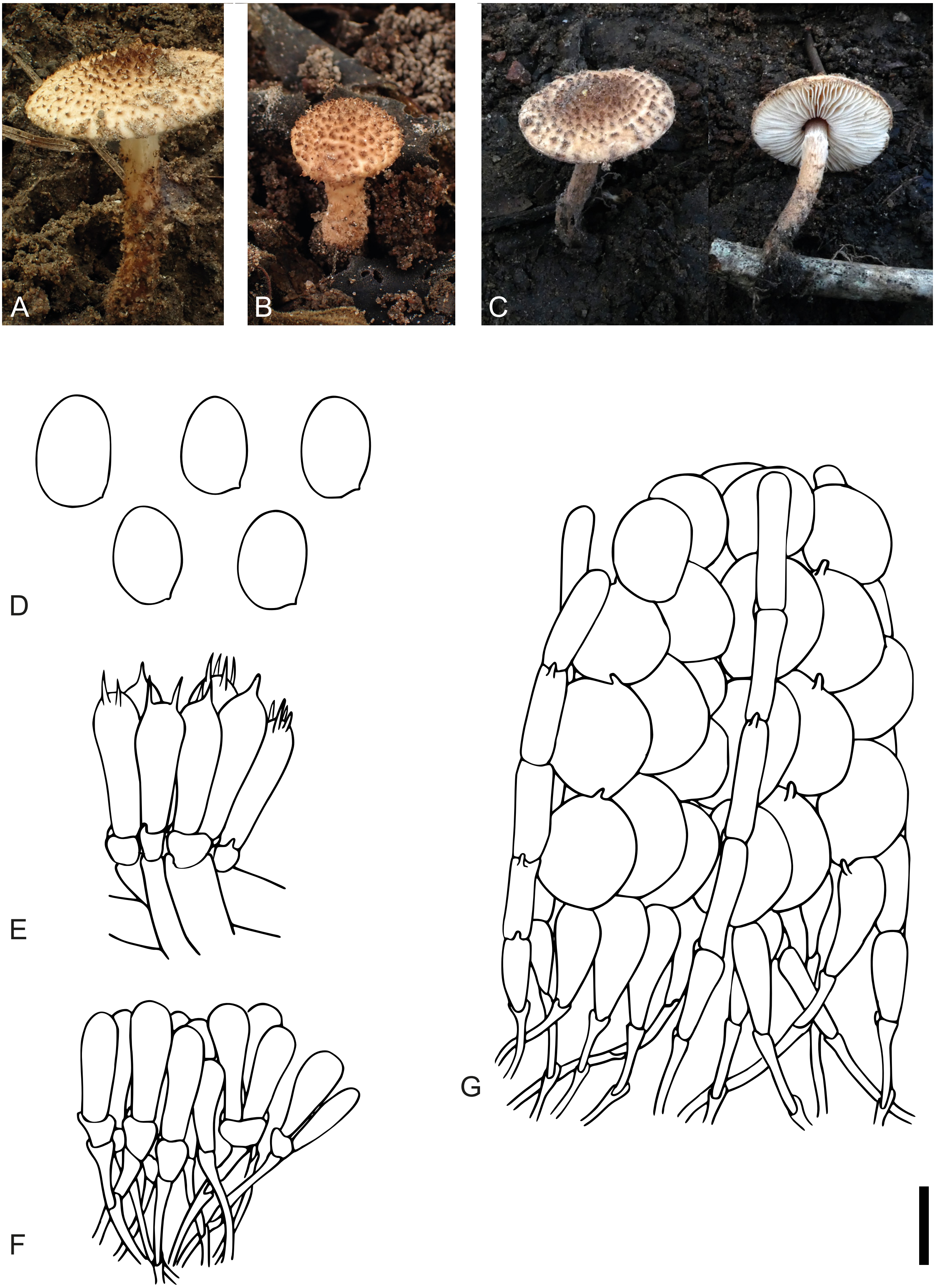


Fig. 2. Basidiomata and microstructures of *Lepiota echinobispora*. **A.** Basidioma (SeSa115, holotype). **B.** Basidioma (SeSa114). **C.** Basidioma (SeSa345), seen from above (left) and from below the pileus (right). **D.** Basidiospores (SeSa115). **E.** Basidia (SeSa115). **F.** Cheilocystidia (SeSa115). **G.** Pileus covering (SeSa115). Scale bar: A–C = 1 cm; D = 3 µm; E, F = 10 µm; G = 20 µm.

Lamellae free, moderately crowded to distant, ventricose, 0.5–1.5 mm deep, pale yellow to light orange (4A3–5, 5A4–5); edge concolourous; with one lamellula. *Stipe* 10–20 × 0.5–1 mm, cylindrical, at apex pale yellow to light orange or light brown (5A2–5, 5B3–5, 5C4–5), gradually darker, orange-brown (5A6–8, 5C5–8, 5D5–6) towards base, with minute orange-brown warts. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (3.8–)4.3–4.7–5.1(–5.6) × (2.6–)2.8–3.1–3.4(–3.7) µm, Q = 1.26–1.75, Q_{av} = 1.50–1.53, ovoid to ellipsoid, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)18–22(–25) × (5.5–)6.0–7.2(–8.0) µm, narrowly clavate to clavate, hyaline, with oily content, yellowish in water and KOH, 4-spored, occasionally 2-spored; sterigmata up to 5 µm long, sometimes relatively long and broad, up to 7 × 2 µm. *Lamella edge* sterile to heterogenous with abundant cheilocystidia and some basidia in between. *Cheilocystidia* (14–)19–30(–39) × (5.3–)6.4–10.4(–13.4) µm, cylindrical or narrowly clavate to clavate, hyaline. *Pleurocystidia* absent. *Pileus covering* made up of chains with terminal cells measuring (11–)12–24(–34) µm in diam., (sub)globose; basal cells narrowly clavate to clavate, with pale brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata gregarious in small groups, probably saprotrophic, on soil, in woodland and savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Atacora Department, Kota waterfalls, N10.212, E1.444, alt. 500 m.a.s.l., on sandy soil, in savannah with *Berlinia grandiflora*, *Isoberlinia tomentosa*, and *Uapaca guinensis*, 26 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa283.

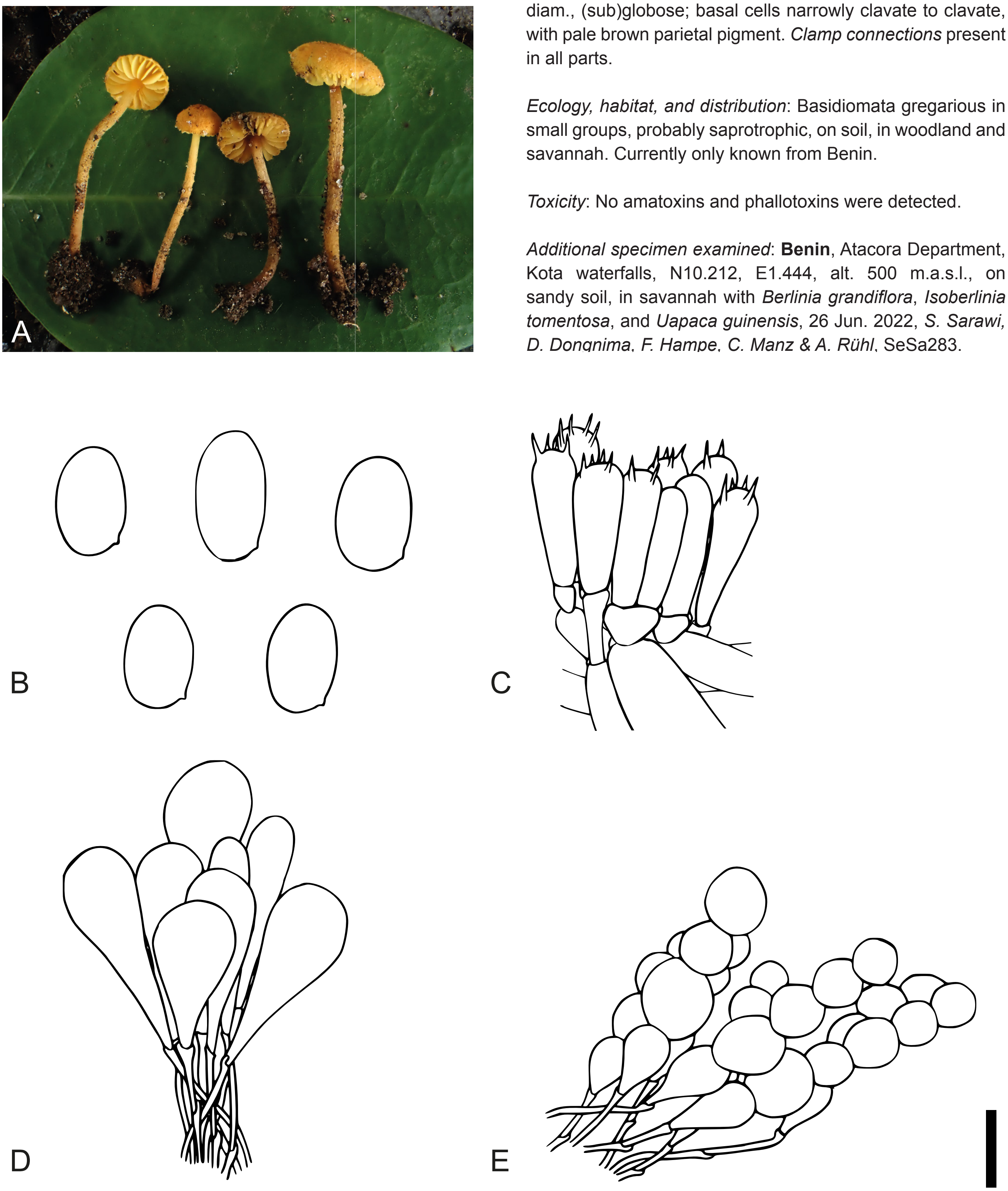


Fig. 3. Basidiomata and microstructures of *Lepiota minutoflava* (SeSa239, holotype). **A.** Basidiomata. **B.** Basidiospores. **C.** Basidia. **D.** Cheilocystidia. **E.** Pileus covering. Scale bar: A = 5 mm; B = 3 µm; C, D = 10 µm; E = 20 µm.

Notes: *Lepiota minutoflava*, section *Eriophorae*, is characterized by minuscule basidiomata with yellow to orange-brown colours and minute warts on the pileus, which are only clearly visible with a lens. Microscopically, *L. minutoflava* is characterized by dextrinoid, ellipsoid to ovoid basidiospores and a pileus covering consisting of chains of spherical cells. Phylogenetically, it is close to *L. omninoflava* known from China, with a p-distance of 11 % in the ITS sequence. However, *L. omninoflava* has larger and paler coloured basidiomata, with rather distinctive warts, and a fertile lamella edge (Hou & Ge 2020).

Compared to *Lepiota* species known from older studies in East and Central Africa and other (sub-)tropical regions, *L. minutoflava* with its yellow and minute basidiomata resembles only two species, namely *L. luteocephalata* known from Congo and Uganda (Beeli 1927, Pegler 1977) and *L. subflavescens* known from Martinique and the USA (Murrill 1914, Pegler 1983). However, *L. luteocephalata* is distinguished by its sulphur-yellow pileus with brown squamules and white-brownish stipe colouration and *L. subflavescens* by its brighter lemon-yellow pileus, pale yellow stipe, and the presence of an annulus (Beeli 1927).

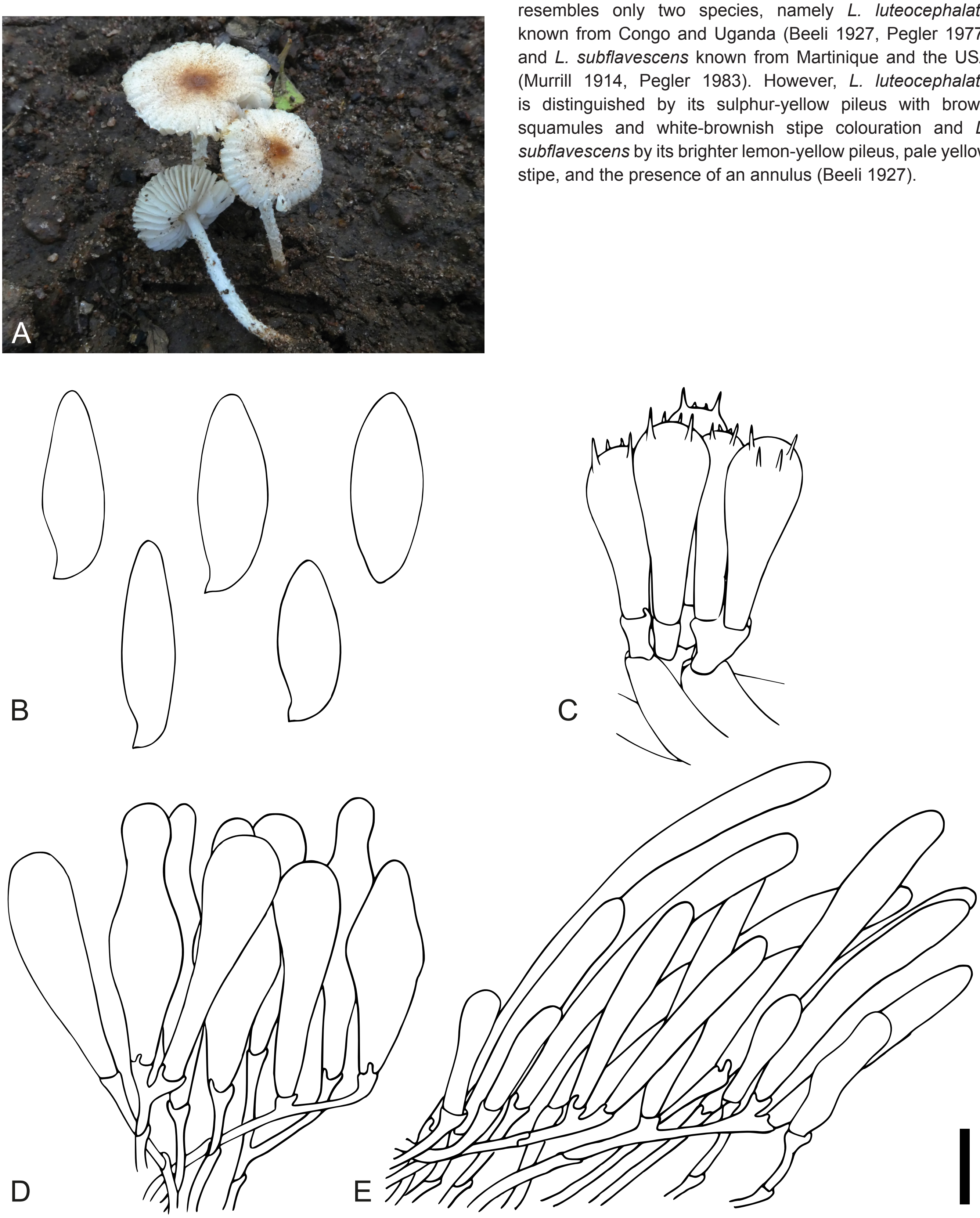


Fig. 4. Basidiomata and microstructures of *Lepiota woehnertii* (SeSa256, holotype). **A.** Basidiomata. **B.** Basidiospores. **C.** Basidia. **D.** Cheilocystidia. **E.** Pileus covering. Scale bar: A = 1 cm; B = 5 µm; C, D = 10 µm; E = 20 µm.

Section *Lepiota*

Lepiota woehnertii Sarawi, ***sp. nov.*** MB 854016. Fig. 4.

Etymology: Named in honour of Prof. Dr. Jens Wöhnert (Goethe University, Frankfurt am Main), a German biochemist and structural biologist, who kindly supported the present project.

Typus: **Benin**, Borgou Department, Forêt de Wari Maro, N9.183, E2.213, alt. 310 m.a.s.l., on sandy soil, in savannah with *Isoberlinia doka*, 25 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa256 (**holotype** FR-0175165, **isotype** in UNIPAR). GenBank: ITS = PP594583, LSU = PP594704, *RPB2* = PP841212.

Basidiomata small to medium. *Pileus* 5–15 mm diam., initially campanulate or hemispherical, then convex and eventually plano-convex, covered by slightly erect, granulate squamules, light orange brown to cream (6B2–3) with a darker brown disc (6D7–8, 6E7, 6F6) in the centre, on a striate, white, background, with white, floccose, velar remnants at outmost margin. *Lamellae* free, moderately crowded, ventricose, 1–3 mm deep, white edge concolourous; with one lamellula. *Stipe* 20–25 × 1–2 mm, cylindrical, white to yellowish white (4A2), golden brown (5D4) towards base, smooth, but white woolly floccose below the annular ring zone. *Annulus* not observed, but annular ring zone present. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (10.2–)12.0–13.3–14.6(–17.3) × (2.8–)4.0–4.5–5.0(–6.0) µm, Q = 2.35–3.81, Q_{av} = 2.77–3.23, in frontal view fusiform to amygdaliform, in side view with more or less curved abaxial side and adaxial suprahilar depression, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (22–)23–30(–39) × (7–)9–10(–12) µm, clavate, hyaline, with oily content, 4-spored, with up to 5 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (22–)25–32(–38) × (6–)8–11(–14) µm, narrowly clavate to clavate or (sub)utriform to fusiform, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm consisting of cylindrical, erect cells, (44–)60–107(–155) × (6–)8–12(–15) µm, with rounded apex, with pale brown to yellowish brown parietal pigment, in between with short, narrowly clavate to clavate cells. *Clamp connections* present in all parts.

Ecology, habitat, distribution: Basidiomata solitary or gregarious in small groups, probably saprotrophic, on soil, in gallery forests and savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Borgou Department, Tchatchou, Forêt de Tchatchou-Gokana, N9.026, E2.589, alt. 360 m.a.s.l., on sandy soil, in gallery forest with *Berlinia grandiflora*, 23 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa242.

Notes: *Lepiota woehnertii*, in section *Lepiota*, is characterized by basidiomata with light brown to brown squamules on a white background, a striate pileus, and a stipe that is woolly below the ring zone. *Lepiota woehnertii* is phylogenetically close to the recently described *L. pakistanensis*, with a p-distance of 4 % in the ITS sequence. However, *L. woehnertii* differs by its white pileus with a light brown to brown pileus centre and larger basidiospores (12.0–14.6 × 4.0–5.0 µm) in contrast to the ochre and cream colours and distinctly shorter and wider basidiospores (10.4–11.7 × 4.8–5.8 µm) of *L. pakistanensis* (Rehman *et al.* 2024). Other morphologically similar species are *L. attenuata*, known from China and *L. metulispora,* known from China, India, Sri Lanka, and Tanzania (Liang *et al.* 2011). *Lepiota attenuata* differs from *L. woehnertii* by yellow to yellow brown squamules on the pileus, greyish orange squamules at the base of the stipe, and significantly larger basidiospores (14.5–19.0 × 4.0–5.5 µm) that are distinctively narrower towards apex (Berkeley & Broome 1871, Liang *et al.* 2011). *Lepiota metulispora* differs from *L. woehnertii* by yellowish to yellow-brown colours on the pileus, lemon-yellow to brown colours on the stipe, the presence of an annulus, and slightly smaller basidiospores (13.5–15.5 × 4.5–5.5 µm) (Berkeley & Broome 1871, Liang *et al.* 2011). However, Pegler (1977) designated this species as *L. metulaespora*, collected in Tanzania, and provided a description that differed mainly in its larger basidiospores (11–19 × 3.7–5 µm) and its chrome-yellow discolouration when cut. Berkeley and Broome's (1871) description of *L. metulispora* included a stuffed stipe, a feature that was contrasted by Pegler (1977) and Liang (2011) who described the stipe as hollow. Therefore, further research is required to verify whether the species collected in Sri Lanka by Berkeley and Broome are distinct species compared to those from Tanzania, China, and India. Morphologically, *L. thrombophora* from Asia can be confused with *L. woehnertii*. However, it can be distinguished by the darker, rather dark brown to red-brown colours of its basidiomata (Berkeley & Broome 1871, Liang *et al.* 2011). Other species within the same clade as *L. woehnertii* are *L. cortinarius*, *L. magnispora*, and *L. spheniscispora*. These species have larger basidiomata than *L. woehnertii* and basidiospores strongly differ by their size and shape (Murrill 1912, Lange 1915, Vellinga 2001a, b), and these species occur mainly in temperate regions (viz. Europe or North America).

Section *Lilaceae*

Lepiota beninensis Sarawi & Manz, ***sp. nov.*** MB 854017. Fig. 5.

Etymology: The epithet *beninensis* refers to Benin, the country in which this species was collected.

Typus: **Benin**, Borgou Department, Béniakou, N9.486, E2.786, alt. 330 m.a.s.l., on loamy rocky soil, in savannah with *Isoberlinia tomentosa* and *Monotes kerstingii*, 29 Jun. 2021, *C. Manz, F. Hampe & N. S. Yorou*, FC-21-201 (**holotype** FR-0175167, **isotype** in UNIPAR). GenBank: ITS = PP594630, LSU = PP594755, *RPB2* = PP841161.

Basidiomata small to rather large and robust. *Pileus* 15–50 mm diam., convex to plano-convex; centre with a smooth, closed calotte, orange brown to brown or red brown (6C8–3, 6D8–6, 7E8–5, 7F8–5); surface around calotte splitting up into brown to light brown or red-brown (6C6–3, 6D6–4, 7D6–5, 7E6–4) squamules and patches with uplifted tips, on a striate, white to cream (6A1, 6B2) background. *Lamellae* free, moderately crowded to crowded, ventricose, 2–5 mm deep, white; edge concolourous; lamellulae in up to two tiers. *Stipe* 10–50 × 2–10 mm, cylindrical, at apex white, light yellow or pale orange (6A2–3, 6B1–4) towards base, with distinct girdles of few squamules at the base that are concolourous with pileus centre. *Annulus* persistent, white to cream; margin concolourous with pileus centre. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (6.4–)7.3–8.0–8.7(–10.3) × (4.3–)4.7–5.1–5.5(–6.0) µm, Q = 1.37–1.81, Q_{av} = 1.53–1.60, ellipsoid to ovoid in side view, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (22–)23–29(–36) × (7–)8–10(–11) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, with up to 5 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (15–)20–33(–42) × (6–)7–10(–12) µm, clavate, subcapitate to utriform, hyaline. *Pleurocystidia* absent. *Pileus covering* a hymeniderm consisting of clavate to narrowly clavate, sometimes irregularly shaped cells, (21–)30–51(–67) × (7–)9–14(–21) µm, with brown parietal pigment, some incrusting pigment present in the subpellis. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary, probably saprotrophic, on soil, in savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Borgou Department, near Kpessou, Forêt de l'Ouémé Supérieur, N 9.261 E 2.184,

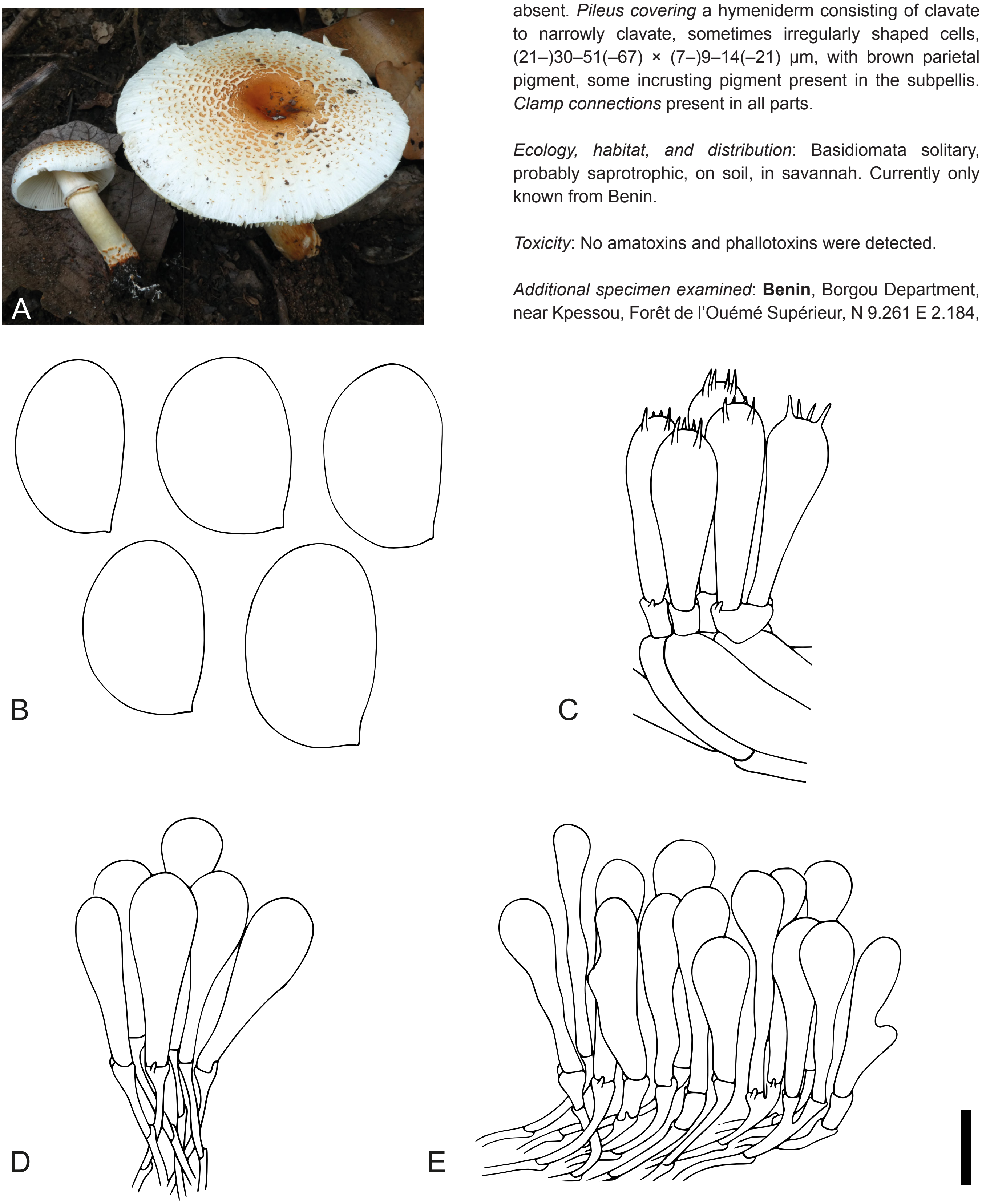


Fig. 5. Basidiomata and microstructures of *Lepiota beninensis* (FC-21-201, holotype). **A.** Basidiomata. **B.** Basidiospores. **C.** Basidia. **D.** Cheilocystidia. **E.** Pileus covering. Scale bar: A = 2 cm; B = 3 µm; C, D = 10 µm; E = 20 µm.

alt. 340 m.a.s.l., on sandy soil, in savannah with *Isoberlinia doka* and *I. tomentosa*, 28 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa296.

Notes: *Lepiota beninensis*, section *Lilacea*, is characterized by robust basidiomata with an orange-brown calotte at the pileus centre, which splits into brown to light-brown squamules and patches on a white background, along with an annulus with brown margin, dextrinoid, ellipsoid to ovoid basidiospores, and a hymeniderm as pileus covering. Phylogenetically, *L. beninensis* forms a distinct clade together with *L. chiangraiensis*, *L. bengalensis*, and *L. ochraceofulva*. Macroscopically, *L. chiangraiensis* known from Thailand and *L. bengalensis* known from Bangladesh, can be easily distinguished from *L. beninensis* by the less intense orange colours on pileus and stipe, as well as yellowish to yellowish white lamellae (Hosen *et al.* 2016, Hyde *et al.* 2020). Microscopically, the basidiospores of *L. beninensis* (7.3–8.7 × 4.7–5.5 µm) are slightly smaller than the non-dextrinoid basidiospores of *L. chiangraiensis* (8.5–10.2 × 5–6.2 µm), and larger than those of *L. bengalensis* (6.0–6.6 × 3.0–3.6 µm) (Hosen *et al.* 2016, Hyde *et al.* 2020). *Lepiota beninensis* can be differentiated from the European species *L. ochraceofulva* which has less yellowish to ochraceous colours on the pileus and stipe, and it lacks a persistent annulus (Orton 1960, Vellinga 2001a).

Lepiota lilaceostriata Sarawi, ***sp. nov.*** MB 854018. Fig. 6.

Etymology: *lilaceus* (Latin) = lilac-coloured, *striatus* (Latin) = striate; refers to the purplish colours, which are similar to those of *Lepiota lilacea*, and the striate pileus.

Typus: **Benin**, Atacora Department, Kota waterfalls, N10.212, E1.444, alt. 500 m.a.s.l., on sandy soil, in savannah with *Berlinia grandiflora*, *Isoberlinia tomentosa*, and *Uapaca guinensis*, 26 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa280 (**holotype** FR-0175191, **isotype** in UNIPAR). GenBank: ITS = PP594588, LSU = PP594709, *RPB2* = PP841217.

Basidiomata small. *Pileus* 10–15 mm diam., umbonate or convex to plano-convex; centre with a smooth, closed calotte, dark purple, almost black to purplish grey (14F2–3) or brownish grey to grey (8E1–2); surface around calotte splitting up into purplish squamules with uplifted tips, on a striate, white to cream (8A1, 8B1) background. *Lamellae* free, moderately crowded to crowded, ventricose, 1–2 mm deep, white edge concolourous; with one lamellula. *Stipe* 20 × 1–2 mm, cylindrical, annulate, greyish yellow (4C3) above the ring, olive-brown towards base (4D3). *Annulus* persistent; underside concolorous with pileus centre; upper side whitish to cream. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (3.6–)4.0–4.4–4.8(–5.4) × (2.1–)2.6–2.9–3.2(–3.7) µm, Q = 1.26–1.73, Q_{av} = 1.44–1.57, ellipsoid to ovoid in side view, hyaline, smooth, non-dextrinoid, non-metachromatic. *Basidia* (13–)16–19(–21) × (5.0–)5.3–6.3(–7.3) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, with up to 3 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (15–)17–23(–27) × (7–)8–11(–14) µm, clavate to broadly clavate, hyaline. *Pleurocystidia* absent. *Pileus covering* a hymeniderm consisting of cylindrical to narrowly clavate cells, (11–)17–31(–45) × (4–)6–11(–13) µm, hyaline, lacking any pigment; young cells with light brown, and hyphae of the underlying layer with brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary, probably saprotrophic, on soil and decayed wood, in rather moist tree savannah and gallery forests. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Borgou Department, Tchatchou, Forêt de Tchatchou-Gokana, N9.026, E2.589, alt. 360 m.a.s.l., on decayed wood, in gallery forest with *Berlinia grandiflora*, 23 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa241.

Notes: *Lepiota lilaceostriata*, section *Lilaceae*, is characterized by a striate cap with a dark purple to black, sometimes rather brownish grey calotte, which breaks up into radial patches towards the edge, an annulus which is coloured on the underside, and non-dextrinoid basidiospores. This species resembles *L. lilacea*, known from America and Europe, due to the pileus colour and the coloured annulus, as well as non-dextrinoid ellipsoid to ovoid basidiospores and a hymeniderm as pileus covering (Bresadola 1892, Vellinga 2001a). Distinguishing these two species morphologically can be challenging; however, *L. lilacea* typically displays a less striate cap and fleshier basidiomata compared to *L. lilaceostriata* (Bresadola 1892, Vellinga 2001a). Microscopically, there are no evident differences. Phylogenetic studies suggest that the species most closely related to *L. lilaceostriata* is *L. lilacea*, with a p-distance of 8 % in the ITS sequence. These two species form a cluster, distinct from all other representatives of the section *Lilaceae*. To differentiate *L. lilaceostriata* from *L. lilacea* conclusively, consulting molecular sequence data is recommended. Other species resembling *L. lilaceostriata* include *L. felina* and *L. pseudolilacea*, which also have an annulus that is coloured on the underside (Karsten 1879, Huijsman 1947). However, they differ in the trichodermal pileus covering and basidiospore size, and are phylogenetically distant, belonging to section *Lepiota* (Vellinga 2001a, 2003, Sarawi *et al.* 2025).

Section *Helveolae*

Lepiota minutisterigmata Sarawi, ***sp. nov.*** MB 854019. Fig. 7.

Etymology: *minutus* (Latin) = very small, *sterigma* (Greek) = small, stalk-like structure; refers to the small sterigmata of this species.

Typus: **Benin**, Collines Department, between Djalloukou and Konkon, N7.730, E1.839, alt. 190 m.a.s.l., on sandy soil, in woodland with *Isoberlinia doka*, 3 Jul. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa343 (**holotype** FR-0175197, **isotype** in UNIPAR) GenBank: ITS = PP594606, LSU = PP594729, *RPB2* = PP841238.

Basidiomata small to medium. *Pileus* 5–15 mm diam., convex to plano-convex, with umbo, covered by concentrically arranged, pyramidal, brown (6F8–3, 6E8–5) squamules on a light brown to white (6A1–2, 6C2–4, 6D3–5) background. *Lamellae* free, moderately crowded to crowded, ventricose, 1–3 mm deep, white; edge concolourous; with one lamellula. *Stipe* 15–30 × 1–2 mm, cylindrical, at apex white floccose, red-golden to light brown (6C4–2, 6D4–2, 7D5–3) towards base. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (5.1–)6.4–7.0–7.6(–8.2) × (3.5–)4.8–5.3–5.9(–6.4) µm, Q = 1.12–1.49, Q_{av} = 1.24–1.39, ellipsoid to ovoid, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)18–24(–29) × (8–)9–11(–12) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, rarely 2-spored, mostly with short, up to 0.5(–1) µm long sterigmata, occasionally up to 4 µm long. *Lamella edge*

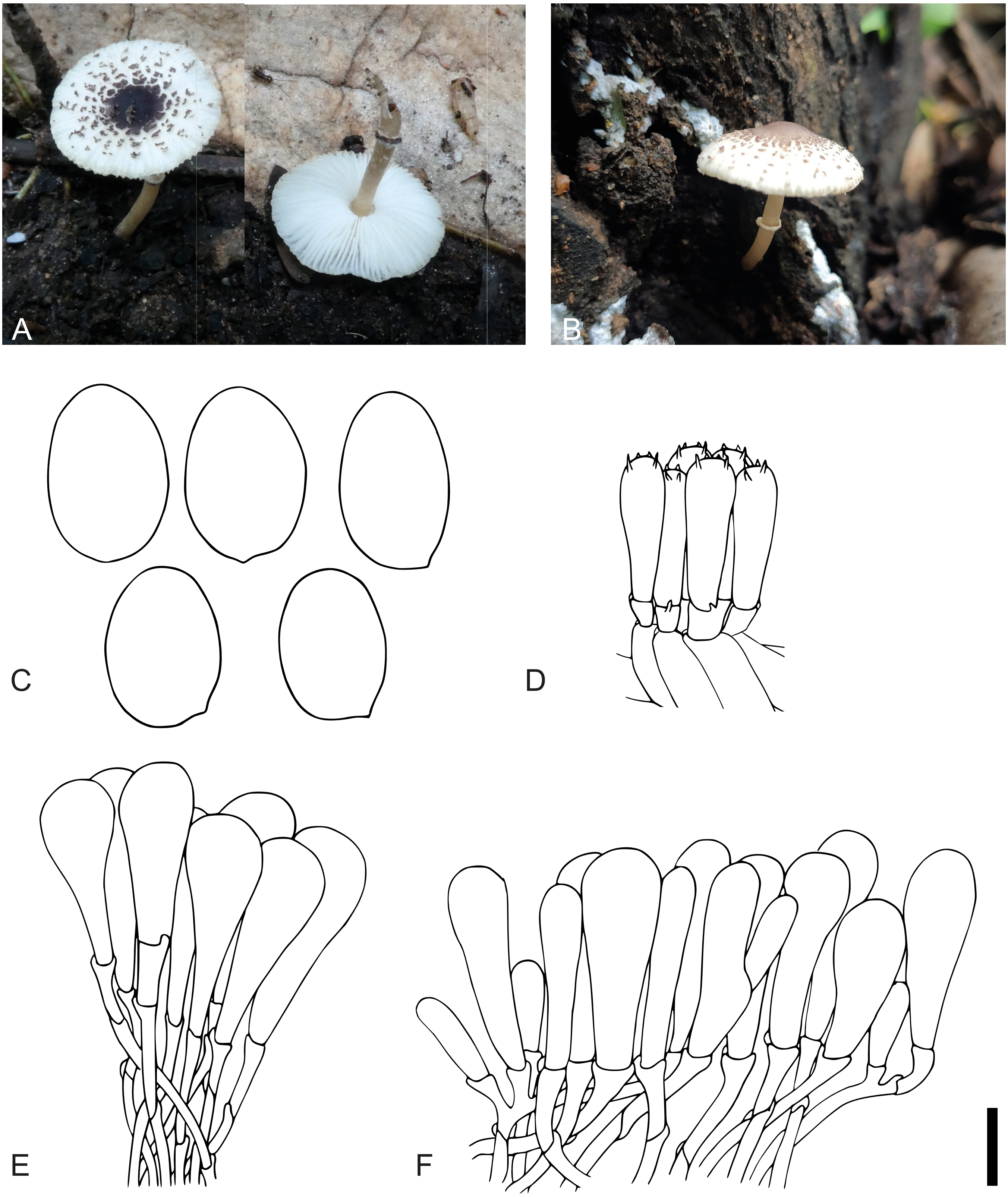


Fig. 6. Basidiomata and microstructures of *Lepiota lilaceostriata*. **A.** Basidioma (SeSa280, holotype), seen from above (left) and from below the pileus (right). **B.** Basidioma (SeSa241). **C.** Basidiospores (SeSa280). **D.** Basidia (SeSa280). **E.** Cheilocystidia (SeSa280). **F.** Pileus covering (SeSa280). Scale bar: A, B = 7 mm; C = 2 µm; D, E = 10 µm; F = 20 µm.

sterile. *Cheilocystidia* (12–)16–27(–43) × (6–)7–12(–21) µm, narrowly clavate or clavate to broadly clavate or slightly inflated, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm consisting of inflated clavate cells, (18–)28–46(–57) × (9–)14–21(–26) µm, with pale brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary or gregarious in small groups, probably saprotrophic, on soil, in woodland and savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Atacora Department, Kota waterfalls, N10.212, E1.444, alt. 500 m.a.s.l., on sandy soil, in savannah with *Berlinia grandiflora*, *Isoberlinia tomentosa*, and *Uapaca guinensis*, 26 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl,* SeSa281.

Notes: This species is characterized by light brown to brown colours and pyramidal squamules on a white background, ellipsoid to ovoid basidiospores, basidia with predominantly minute sterigmata and a trichoderm formed by inflated cells. *Lepiota minutisterigmata* forms a clade together with the tropical species *L. lahorensis*, *L. sosuensis*, *L. vellingana*, and the European species *L. farinolens*. The relationships within this clade are not resolved. *Lepiota minutisterigmata* differs from *L. lahorensis* by its pyramidal squamules and basidiospores that are smaller (6.4–7.6 × 4.8–5.9 µm) than those of *L. lahorensis* (8.3–11.6 × 6.1–8.3 µm) (Qasim *et al.* 2016). Macroscopically, *L. sosuensis* can be easily distinguished from *L. minutisterigmata* by pure white basidiomata and the lack of pyramidal squamules (Justo *et al.* 2015). *Lepiota vellingana* differs from *L. minutisterigmata* by lacking pyramidal squamules and the absence of an annulus (Nawaz *et al.* 2013). Additionally, *L. minutisterigmata* differs by short sterigmata and a pileus covering consisting of short, inflated cells (Nawaz *et al.* 2013, Justo *et al.* 2015, Qasim *et al.* 2016). The European species *L. farinolens* can be distinguished macroscopically from *L. minutisterigmata* by basidiomata with ochre to pink to pink-brown colours,

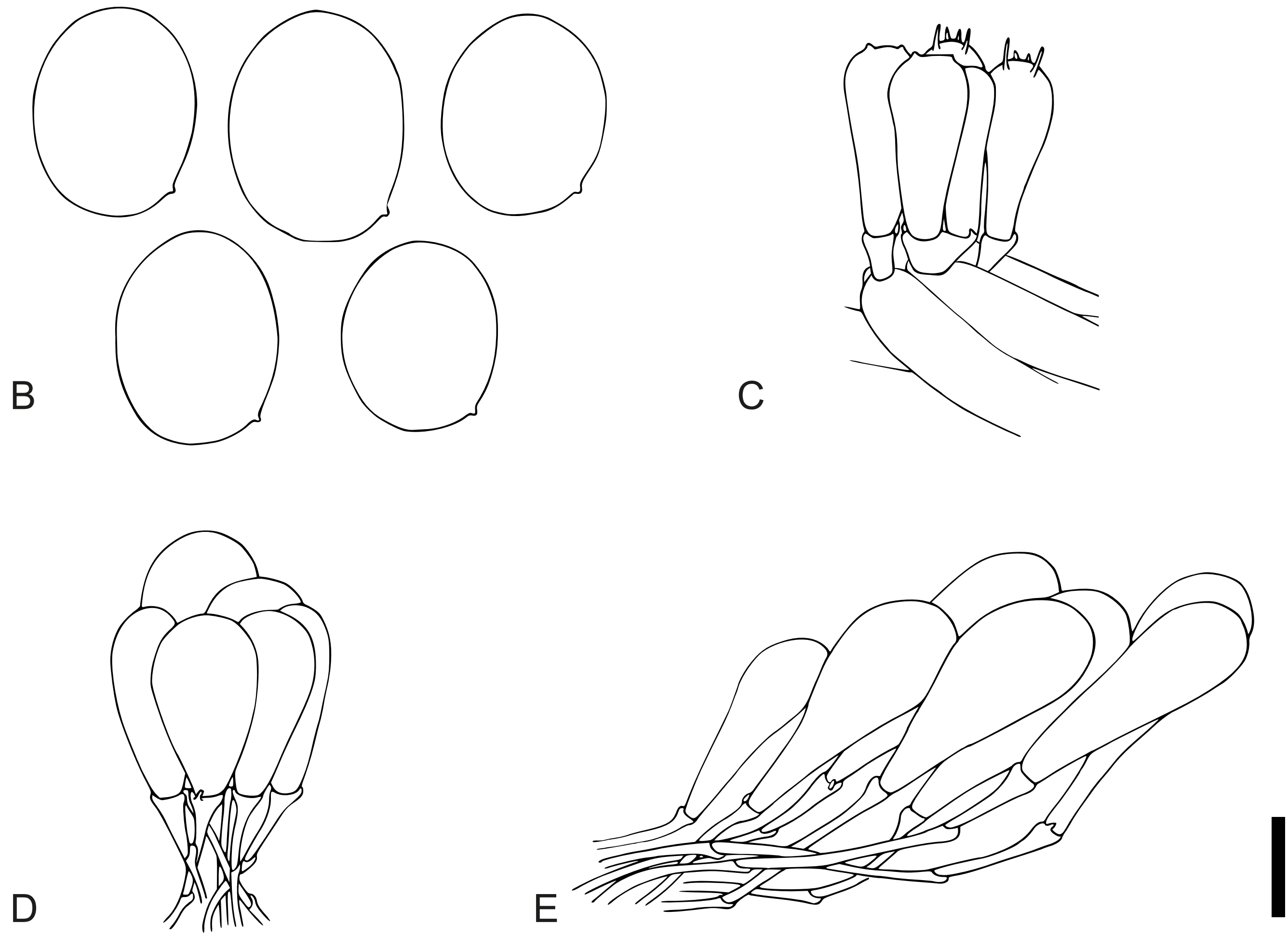


Fig. 7. Basidiomata and microstructures of *Lepiota minutisterigmata* (SeSa343, holotype). **A.** Basidiomata. **B.** Basidiospores. **C.** Basidia. **D.** Cheilocystidia. **E.** Pileus covering. Scale bar: A = 1 cm; B = 3 µm; C, D = 10 µm; E = 20 µm.

plane squamules, and an annulus. Microscopically it differs by slightly larger basidiospores (8.0–9.0 × 4.5–5.0 µm), significantly larger cheilocystidia (40–50 × 8–12 µm), and much longer and narrower cells of the pileus covering (100–160 × 10–15) (Bon & Riousset 1992).

Lepiota pseudovenenosa Sarawi & Reschke, ***sp. nov.*** MB 854020. Fig. 8.

Etymology: *pseudes* (Greek) = deceptive, *venenosa* (Latin) = poisonous; refers to the species' appearance, which is similar to poisonous *Lepiota* species, such as *L. subincarnata* and *L. brunneoincarnata*, yet this species does not contain amatoxins or phallotoxins.

Typus: **Benin**, Donga Department, Bassila, N9.001, E1.649, alt. 360 m.a.s.l., on sandy soil, in dense gallery forest with

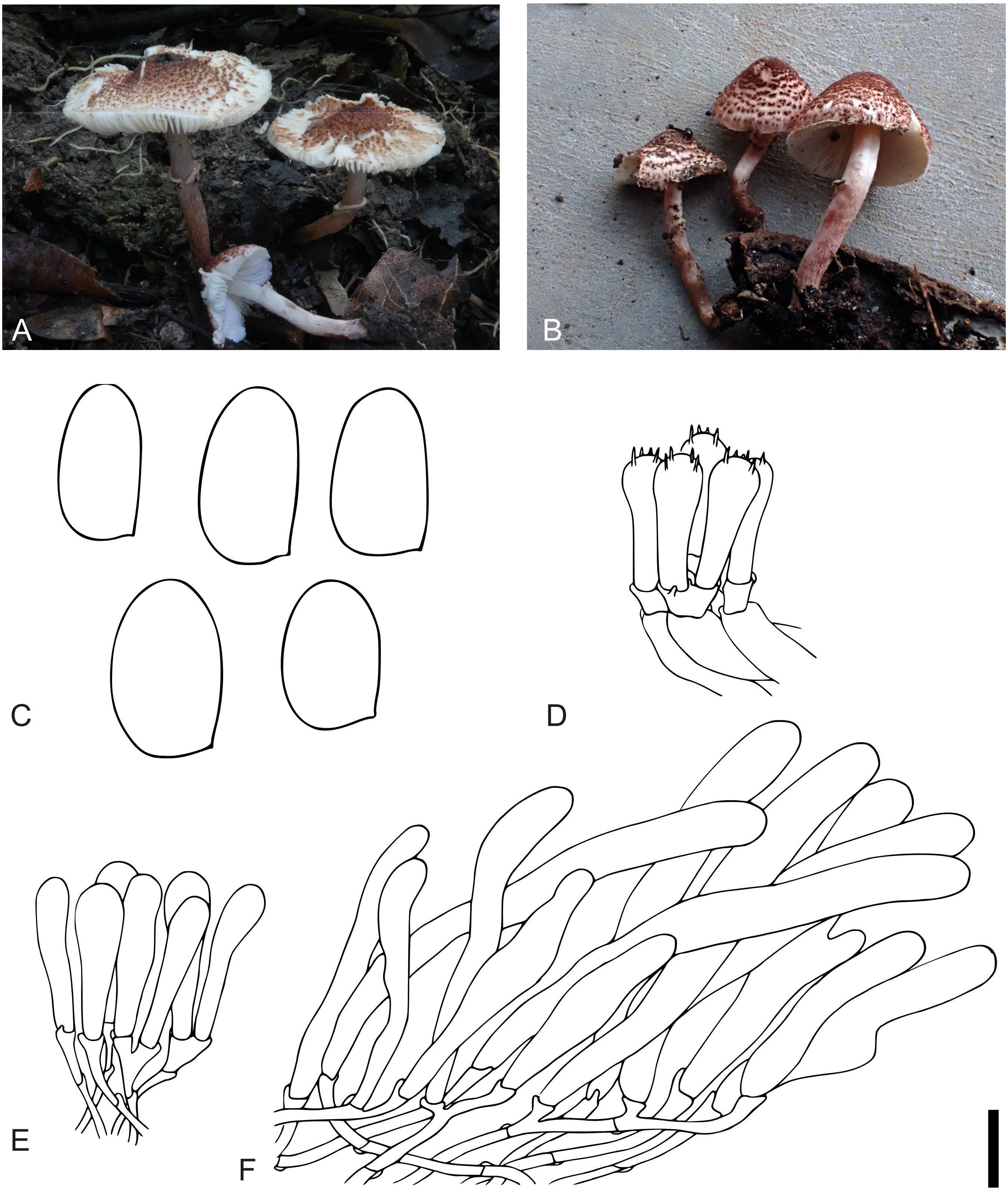


Fig. 8. Basidiomata and microstructures of *Lepiota pseudovenenosa*. **A.** Basidiomata (SeSa302, holotype). **B.** Basidiomata (SeSa339). **C.** Basidiospores (SeSa302). **D.** Basidia (SeSa302). **E.** Cheilocystidia (SeSa302). **F.** Pileus covering (SeSa302). Scale bar: A = 1 cm; B = 2 cm; C = 2 µm; D, E = 10 µm; F = 20 µm.

Berlinia grandiflora, 30 Jun. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa302 (**holotype** FR-0175172, **isotype** in UNIPAR). GenBank: ITS = PP594593, LSU = PP594714, *RPB2* = PP841223.

Basidiomata small to medium. *Pileus* 10–25 mm diam., first campanulate to hemispherical or conical, becoming convex to plano-convex or plano-concave, with umbo; at centre dark brown to nearly black (7F7–8, 8F7–8) to sometimes vinaceous pink, dark pink or red-brown (9F7–8, 10F7–8), paler towards margin (7D5–8, 7E7–8, 9D5–7, 9E6–8, 10D5–7), covered by concentrically arranged, pyramidal or tomentose squamules that are concolourous with pileus centre, on a pale brown to vinaceous pink or white background. *Lamellae* free, moderately crowded to crowded, ventricose, 1–2 mm deep, white; edge concolourous; lamellulae in up to two tiers. *Stipe* 10–25 × 2–3 mm, cylindrical, annulate, whitish to cream above the annulus, gradually darker, from pale brown or pale vinaceous pink to reddish brown, dark brown or vinaceous pink to dark pink (7D4–8, 7E7–8, 7F6–8, 9D4–7, 9E6–8, 9F7–8, 10D5–7, 10F7–8) towards base, smooth. *Annnulus* non-persistent; margin concolourous with pileus centre; upper side whitish to cream. *Odour* and *taste* not recorded. *Basidiospores* (200/5/5) (3.0–)4.0–4.4–4.7(–5.4) × (2.0–)2.4–2.7–3.1(–3.8) µm, Q = 1.32–1.95, Q_{av} = 1.53–1.71, ellipsoid to ovoid, hyaline, smooth, non-dextrinoid or at most weakly dextrinoid, non-metachromatic. *Basidia* (14–)16–19(–23) × (4.0–)5.0–6.5(–8.0) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, occasionally 2-spored, with up to 2–3 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (12–)17–25(–36) × (3.0–)4.5–6.3(–7.9) µm, cylindrical or narrowly clavate to clavate, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm consisting of cylindrical, erect cells, (12–)42–93(–153) × (3.6–)5.5–8.7(–12.1) µm, with rounded apex, with pale brown to brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary or gregarious in small groups of three to six basidiomata, probably saprotrophic, on soil and dead wood, in gallery forests. Currently known from Benin, as well as the Dominican Republic and Panama according to GenBank sequences.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimens examined: **Benin**, Donga Department, Bassila, N9.001, E1.649, alt. 360 m.a.s.l., on sandy soil, in dense gallery forest with *Berlinia grandiflora*, 30 Jun. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa303; ibid., on sandy soil, in dense gallery forest with *Berlinia grandiflora*, 30 Jun. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa304; ibid., on sandy soil, in dense gallery forest with *Berlinia grandiflora*, 30 Jun. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz, A. Rühl*, SeSa309; ibid., on dead wood, in dense gallery forest with *Berlinia grandiflora*, 2 Jul. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa339.

Notes: *Lepiota pseudovenenosa* in section *Helveolae*, is characterized by light brown, brown to pink- or red-brown colours, pyramidal squamules in the pileus centre, which flatten towards margin, an annulus with coloured margin, non-dextrinoid, ellipsoid to ovoid basidiospores, narrow cheilocystidia and a trichoderm as pileus covering. The closest related species is *L. brunneodisca*, with a p-distance of 13 % in the ITS sequence. *Lepiota brunneodisca*, known from India, differs from *L. pseudovenenosa* by light brown to orange brown or yellowish white colours, appressed squamules, and an annulus without coloured margin (Roy *et al.* 2023). The basidiospores of *L. pseudovenenosa* (4.0–4.7 × 2.4–3.1 µm) are smaller than those of *L. brunneodisca* (6.2–7.7 × 3.8–4.5 µm) and the cheilocystidia of *L. pseudovenenosa* are narrower (Roy *et al.* 2023). The non-dextrinoid basidiospores of *L. pseudovenenosa* are a distinctive characteristic to differentiate it from other representatives of section *Helveolae*, as no species with non-dextrinoid basidiospores were known within this subsection up to now. However, the dextrinoidity of *L. brunneodisca* has not been examined, thus it cannot be used here as a characteristic to distinguish the species (Roy *et al.* 2023).

Morphologically, two further species, *L. erinana*, known from Trinidad and *L. flagellata*, known from Kenya, Sri Lanka, and Uganda, resemble *L. pseudovenenosa*. However, *L. erinana* differs from *L. pseudovenosa* by the absence of an annulus and a scaly stipe (Dennis 1970) and *L. flagellata* differs by the absence of an annulus, dextrinoid basidiospores, and inflated cheilocystidia (Pegler 1977).

Section *Stenosporae*

Lepiota aurantiicolor Sarawi, ***sp. nov.*** MB 854021. Fig. 9.

Etymology: *aurantius* (Latin) = golden, *color* (Latin) = colour; refers to the colours of the pileus.

Typus: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa176 (**holotype** FR-0175175, **isotype** in UNIPAR). GenBank: ITS = PP594563, LSU = PP594681, *RPB2* = PP841188.

Basidiomata small to medium. *Pileus* 5–20 mm diam., first hemispherical to conical and then becoming convex to plano-convex or plano-concave, covered by slightly erect, tomentose squamules, at centre orange brown or reddish brown to brown (7C7–8, 7D7–8, 7E7–8, 8D7–8, 8E7–8), light orange, light orange red or light brown (7A5–7, 7B5–7, 7C5–7, 7D4–7) towards margin, on a white background, with velar remnants at outmost margin. *Lamellae* free, moderately crowded to crowded, ventricose, 1–4 mm deep, white to cream edge concolourous; lamellulae in up to two tiers. *Stipe* 10–35 × 1–3 mm, cylindrical, at apex white to cream, gradually more intensely coloured, pale yellow, pale orange, orange-brown or reddish brown (5A2–4, 5B3–5, 6A3–5, 6B4–6, 7B3–5, 7C5–7, 7D4–8) towards base, in lower half with distinct girdles of few squamules that are concolourous with pileus centre. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (200/5/5) (4.9–)6.0–6.7–7.4(–8.4) × (2.4–)3.1–3.5–3.9(–4.6) µm, Q = 1.58–2.26, Q_{av} = 1.78–2.03, ellipsoid in front view and subtriangular in side view, with an indistinct spur, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)17–20(–23) × (6.1–)7.0–

8.1(–8.6) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, frequently 2- or 1-spored, with up to 3–6 µm long, straight, non-bulbous sterigmata, when 2- or 1-spored with up to 10 µm long, irregularly shaped, bulbous sterigmata. *Lamella edge* sterile. *Cheilocystidia* (16–)19–26(–31) × (5–)6–7(–8) µm, narrowly clavate to clavate or cylindrical, occasionally subutriform to narrowly utriform, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm made up of (37–)46–81(–118) × (8–)10–14(–19) µm, clavate or cylindrical, erect cells, with rounded apex, with pale brown to brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary or gregarious, probably saprotrophic, on soil, in woodland. Currently only known from one locality in Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimens examined: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa177; ibid., in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa181; ibid., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa184; ibid., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 22 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa216.

Notes: *Lepiota aurantiicolor*, section *Stenosporae*, is characterized by orange brown basidiomata, indistinctively spurred basidiospores, mostly 4-spored basidia, occasionally 2-spored with longer sterigmata, and a trichodermal pileus covering. *Lepiota aurantiicolor* has strong macroscopic similarity to *L. longisterigmata* and *L. flavostipitata* and often co-occurs in the same habitat. Accurate identification mainly relies on microscopic characteristics of the sterigmata and molecular sequence data. *Lepiota longisterigmata* can be easily distinguished from *L. aurantiicolor* by its long sterigmata. *Lepiota flavostipitata* differs from *L. aurantiicolor* by deeper, darker orange-brown colours and a yellowish stipe apex. Further related species are *L. alopochroa* and *L. brunneopileata*. *Lepiota alopochroa*, known from New Zealand and Thailand, can be differentiated from *L.*

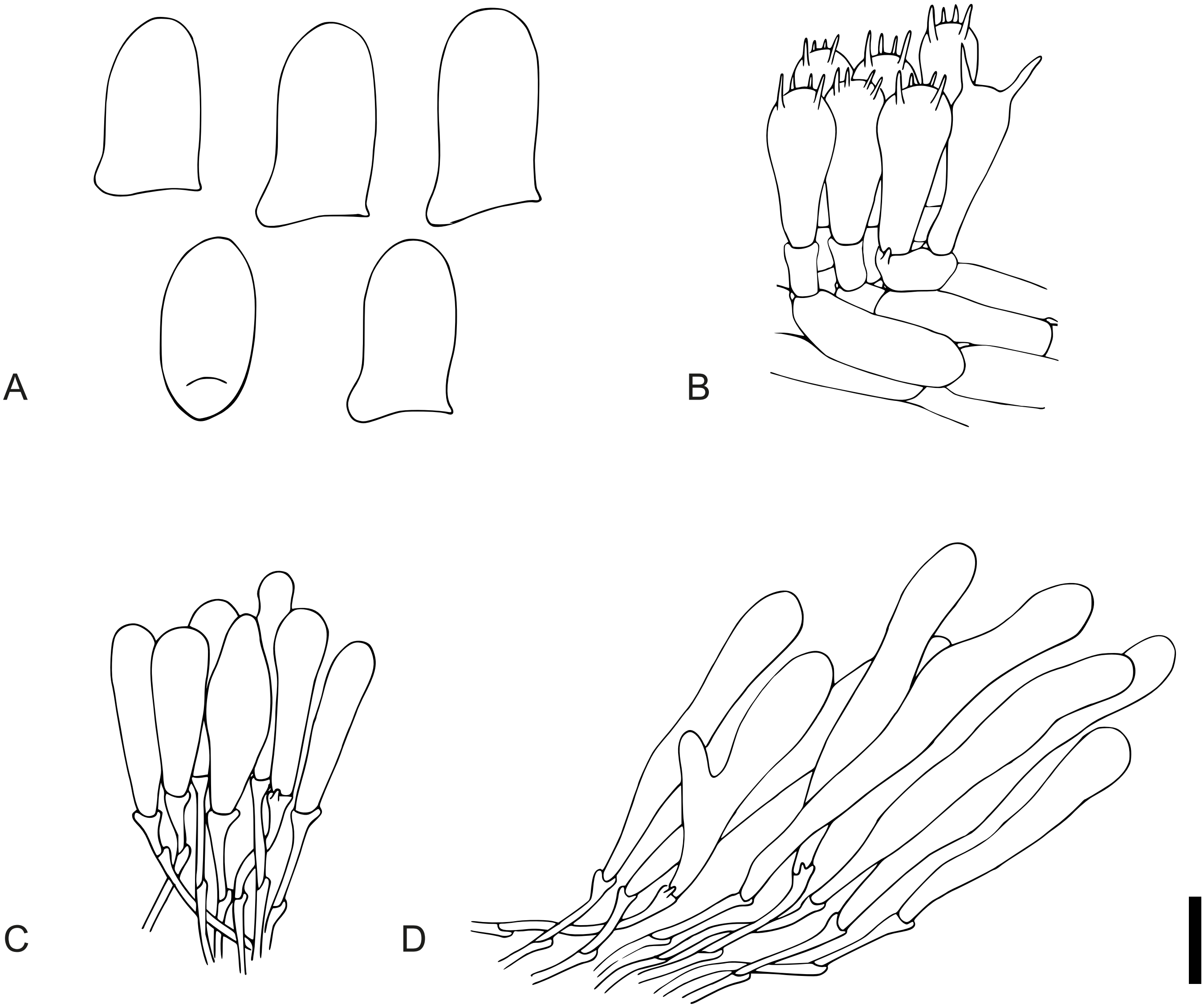


Fig. 9. Microstructures of *Lepiota aurantiicolor* (SeSa176, holotype). **A.** Basidiospores. **B.** Basidia. **C.** Cheilocystidia. **D.** Pileus covering. Scale bar: A = 3 µm; B, C = 10 µm; D = 20 µm.

aurantiicolor by serrulate, yellow to orange-white lamellae and larger basidiospores (7.5–9.3 × 3.8–4.0 µm) compared to *L. aurantiicolor* with basidiospores measuring (6.0–7.4 × 3.1–3.9 µm) (Sysouphanthong *et al.* 2011). Furthermore, the lighter coloration of *L. aurantiicolor* and the non-metachromatic basidiospores differentiate it from *L. brunneopileata*, known from Pakistan (Rehman *et al.* 2024). Given the complexity of this species complex, molecular sequence data are recommended for accurate identification.

Further, *L. aurantiicolor* morphologically resembles *L. pseudoignicolor*, known from Guadeloupe and Venezuela, by the colours of the basidiomata and the basidiospore shape

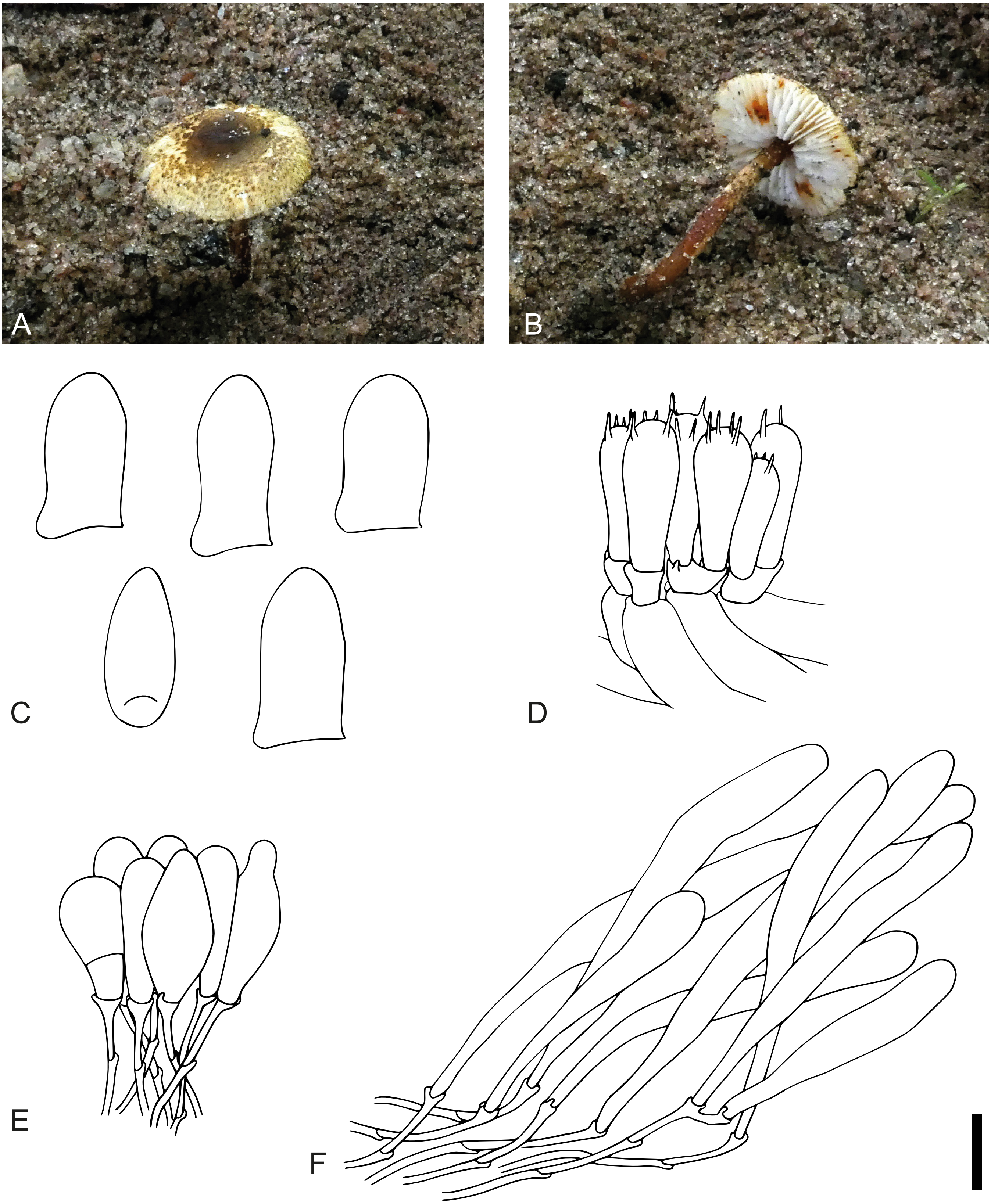


Fig. 10. Basidiomata and microstructures of *Lepiota brunneoolivacea* (SeSa214, holotype). **A.** Basidioma, seen from above the pileus. **B.** Basidioma, seen from below the pileus. **C.** Basidiospores. **D.** Basidia. **E.** Cheilocystidia. **F.** Pileus covering. Scale bar: A, B = 8 mm; C = 3 µm; D, E = 10 µm; F = 20 µm.

and size, but lacks molecular sequence data. However, *L. pseudoignicolor* differs by yellow lamellae and larger basidiospores (9–11.5 × 3–4 µm) (Dennis 1970, Pegler 1983).

Lepiota brunneoolivacea Sarawi, ***sp. nov.*** MB 854022. Fig. 10.

Etymology: *brunneus* (Latin) = brown, *olivaceus* (Latin) = olive-coloured; refers to the brown to olive brown basidiomata.

Typus: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 22 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa214 (**holotype** FR-0175163, **isotype** in UNIPAR). GenBank: ITS = PP594574, LSU = PP594693, *RPB2* = PP841201.

Basidiomata small to medium. *Pileus* 5–15 mm diam., convex to plano-convex, with or without umbo, at centre with a dark yellow brown (5F8–4) calotte; surface around calotte splitting up into yellow brown to light brown or olive brown (5D8–4, 5E8–4) patches, towards the margin sometimes with a slight red tinge, on a light yellow brown (5D4) background. *Lamellae* free, moderately crowded to crowded, ventricose, 2–4 mm deep, white to cream, discolouring brownish red with age; edge concolourous; lamellulae in up to two tiers. *Stipe* 15–35 × 2–3 mm, cylindrical, at apex yellow brown (5E8–4, 5D8–4), more brown to reddish brown (6F8–4, 7F8–4, 8F8–6) towards base. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (5.4–)6.0–6.6–7.1(–7.8) × (2.5–)2.9–3.2–3.5(–4.2) µm, Q = 1.69–2.48, Q_{av} = 2.05–2.08, amygdaliform in front view to triangular in side view, with an indistinct spur, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)17–20(–24) × (5.6–)6.3–7.5(–8.5) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, occasionally 2-spored, with up to 4 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (13–)19–25(–29) × (5–)6–9(–11) µm, narrowly clavate to clavate or narrowly fusiform, occasionally subutriform to narrowly utriform, rarely septate, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm made up of (46–)63–108(–131) × (8–)10–15(–18) µm, clavate or cylindrical, erect cells, with rounded apex, with pale brown to brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary, probably saprotrophic on, soil, in woodland and savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Atacora Department, Kota waterfalls, N10.212, E1.444, alt. 500 m.a.s.l., on sandy soil, in savannah with *Berlinia grandiflora*, *Isoberlinia tomentosa*, and *Uapaca guinensis*, 17 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa137.

Notes: *Lepiota brunneoolivacea* in section *Stenosporae*, is characterized by yellow-brown, light brown to olive-brown basidiomata, with a stipe that is reddish brown towards the base, dextrinoid, indistinctively spurred basidiospores, and a trichodermal pileus covering. Phylogenetically, *L. brunneoolivacea* clusters together with further species which form basidiomata with greenish colours, but the support of this clade is not significant and further studies are needed to analyse the phylogeny of those species. Species closely related to *L. brunneoolivacea* include *L. grangei*, *L. griseovirens*, and *L. poliochloodes*. *Lepiota grangei*, known from Europe, is distinguished from *L. brunneoolicaceae* based on darker and more intense green to blue-green colours, much larger basidiospores (8.0–13.0 × 3.4–4.6 µm), and septate pileus covering cells (Vellinga & Huijser 1993). *Lepiota* griseovirens, known from North Africa and Europe, can be distinguished from *L. brunneoolivacea* by grey-black or greyish olive-brown basidiomata with green, blue hues towards the pileus margin, slightly larger basidiospores (6.4–9.5 × 3.3–4.3 µm), and by pigments encrusted in the subpellis (Vellinga & Huijser 1993). The macromorphologically most similar species is *L. poliochloodes*, also described from Europe, with green grey, grey olive, grey brown, light olive brown colours; however, *L. poliochloodes* differs from *L. brunneoolivacea* by the pileus covering with short clavate cells, septa, and pigments encrusted in the subpellis (Vellinga & Huijser 1993, Sysouphanthong *et al.* 2011).

Lepiota flavonigrescens Sarawi & Reschke, ***sp. nov.*** MB 854023. Fig. 11.

Etymology: *flavus* (Latin) = yellow, *nigrescens* (Latin) = turning black; refers to the yellow basidiomata of this species which turn black after drying.

Typus: **Benin**, Donga Department, Bassila, N9.001, E1.649, alt. 360 m.a.s.l., on sandy soil, in dense gallery forest with *Berlinia grandiflora*, 30 Jun. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa305 (**holotype** FR-0175181, **isotype** in UNIPAR). GenBank: ITS = PP594569, LSU = PP594717, *RPB2* = PP841226.

Basidiomata small to medium, staining dark brown to black when dried. *Pileus* 15–25 mm diam., convex to plano-convex or plano-concave, with umbo; at centre dark orange brown to dark brown (6C8–7, 6D8–4, 6F8–4, 7F8–6), splitting up outwards into orange brown to light brown (6C8–6, 6D8–5, 6E8–5, 7D8–6, 7E7–5) patches, on a light yellow to greyish yellow (4C5–4) background, with velar remnants at outmost margin. *Lamellae* free, moderately crowded to crowded, ventricose, 3–4 mm deep, greyish yellow (4C5–4); edge whitish; lamellulae in up to two tiers. *Stipe* 35–50 × 2–3 mm, cylindrical, at apex yellow (5A4–2), more brown (5D8–4, 6D8–4) towards base, in lower half with distinct girdles of squamules that are concolourous with pileus centre. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (5.9–)6.7–7.3–7.9(–8.9) × (2.5–)3.0–3.3–3.6(–4.0) µm, Q = 1.82–2.70, Q_{av} = 2.21–2.28, cylindrical or amygdaliform in front view and triangular in side view, with or without slight lateral knobs, with a distinct spur, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)17–20(–24) × (5.6–)6.3–7.5(–8.5) µm, narrowly clavate to clavate, with oily content, 4-spored, occasionally 2-spored, with up to 5 µm long sterigmata, basidia and sterigmata strongly incrusted with brown to dark brown necropigment dissolving in KOH. *Lamella edge* sterile. *Cheilocystidia* (14–)18–26(–31)

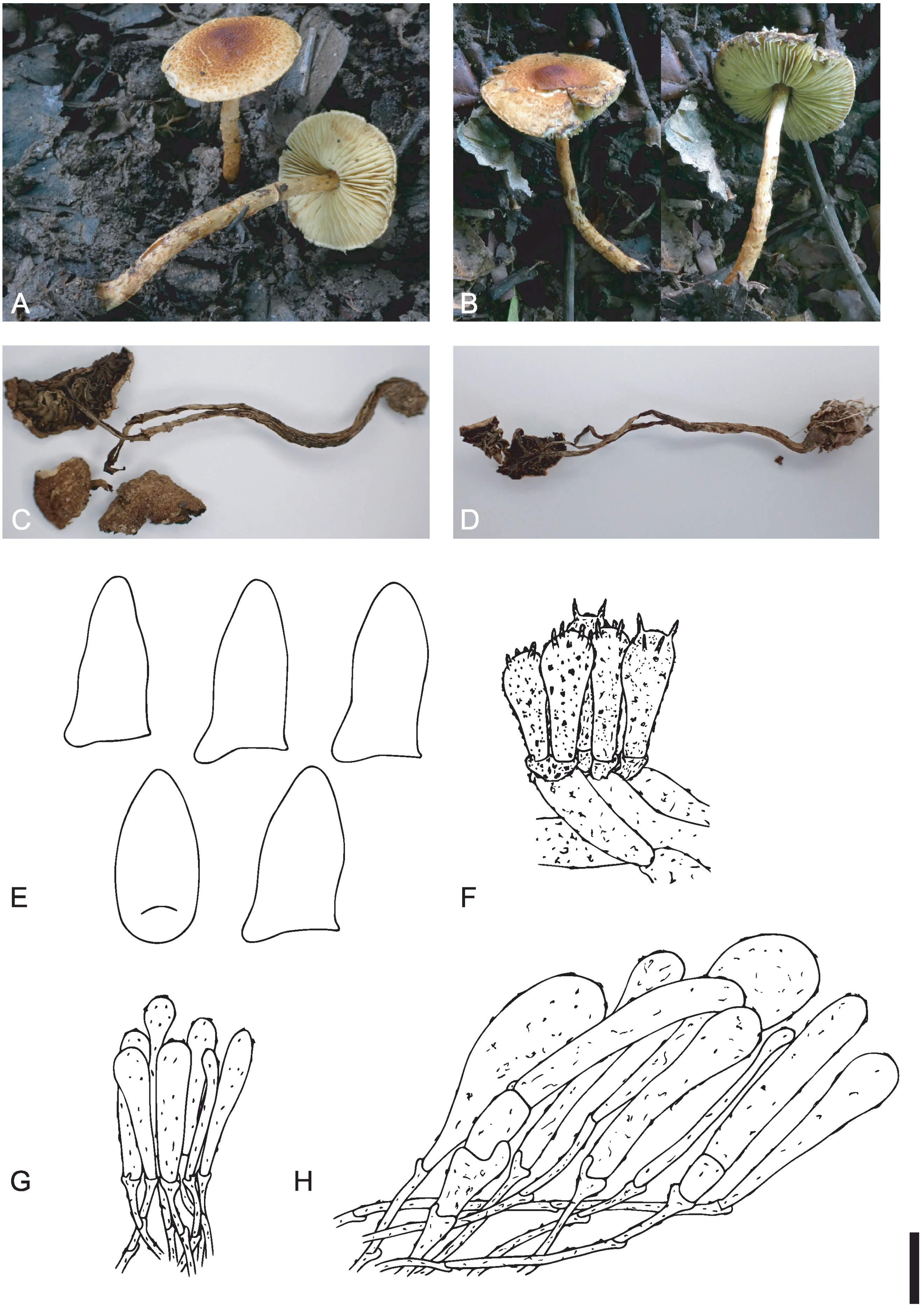


Fig. 11. Basidiomata and microstructures of *Lepiota flavonigrescens*. **A.** Basidiomata (SeSa305, holotype). **B.** Basidioma (SeSa306), seen from above (left) and from below the pileus (right). **C, D.** Dried basidiomata (C. SeSa305; D. SeSa306). **E.** Basidiospores (SeSa305). **F.** Basidia (SeSa305). **G.** Cheilocystidia (SeSa305). **H.** Pileus covering (SeSa305). Scale bar: A = 8 mm; B = 1.2 cm; C, D = 1 cm; E = 3 µm; F, G = 10 µm; H = 20 µm.

× (3–)4–7(–9) µm, mainly subcylindrical-capitate to narrowly clavate or somewhat tibiiform, sometimes broadly clavate to almost ovoid, rarely irregularly fusiform, slightly incrusted with brown necropigment dissolving in KOH. *Pleurocystidia* absent. *Pileus covering* a trichoderm made up of (26–)42–68(–83) × (7–)11–17(–21) µm, cylindrical, erect cells or clavate to inflated cells, with rounded apex, rarely septate, strongly incrusted with brown necropigment dissolving in KOH. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary or gregarious in small groups, probably saprotrophic, on soil, in gallery forests. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimen examined: **Benin**, Donga Department, Bassila, N9.001, E1.649, alt. 360 m.a.s.l., on sandy soil, in dense gallery forest with *Berlinia grandiflora*, 30 Jun. 2022, *S. Sarawi, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa306.

Notes: *Lepiota flavonigrescens*, section *Stenosporae*, is characterized by yellowish basidiomata with brown to orange brown squamules and yellow lamellae. Notably, these basidiomata turn black after drying. Microscopically, *L. flavonigrescens* exhibits encrusted pigmentation of the basidia, cheilocystidia, and pileus covering cells, a distinctive feature uncommon in species of the genus *Lepiota*. Encrusted pigments are known only from *L. nigrescentipes*, which differs from *L. flavonigrescens* by its white basidiomata and non-dextrinoid, ellipsoid to ovoid basidiospores (Bon & Riousset 1992, Salom & Siquier 2009). Other species that may resemble *L. flavonigrescens* in appearance include the yellowish species *L. baiyunensis*, *L. citrophylla*, *L. elaiophylla*, *L. luteocastanea*, *L. luteophylla*, *L. subcitrophylla*, and *L. xanthophylla*. However, these species can be distinguished by the absence of encrusted pigments and the lack of a black staining reaction upon drying (Berkeley & Broome 1871, Hongo 1956, Orton 1960, Sundberg 1971, Horak 1980, Vellinga & Huijser 1997, Sysouphanthong *et al.* 2011, Liang *et al.* 2023). Furthermore, *L. baiyuensis*, *L. elaiophylla*, *L. punaensis*, and *L. xanthophylla* can be distinguished from *L. flavonigrescens* by ellipsoid to ovoid basidiospores (Orton 1960, Vellinga & Huijser 1997, Stallman *et al.* 2020, Liang *et al.* 2023).

Lepiota flavostipitata Sarawi, ***sp. nov.*** MB 854024. Fig. 12.

Etymology: *flavus* (Latin) = yellow, *stipitatus* (Latin) = stalked; refers to the yellow stipe apex of the basidiomata.

Typus: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 22 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa203 (**holotype** FR-0175179, **isotype** in UNIPAR). GenBank: LSU = PP594690, *RPB2* = PP841197.

Basidiomata small to medium. *Pileus* 5–20 mm diam., first hemispherical to conical and then becoming convex to plano-convex or plano-concave, covered by slightly erect, tomentose squamules; at centre orange-brown or reddish brown to brown (7B7–8, 7C7–8, 7D7–8, 7E7–8, 8D7–8, 8E7–8), light orange, light orange red or light brown (7A5–7, 7B5–7, 7C5–7, 7D4–7) towards margin, on a white background, with velar remnants at outmost margin. *Lamellae* free, moderately crowded to crowded, ventricose, 1–4 mm deep, white to cream; edge concolourous; lamellulae in up to two tiers. *Stipe* 10–50 × 1–3 mm, cylindrical, at apex white to pale yellow (6A1–3), gradually darker, pale orange, orange-brown or reddish brown (5A2–4, 5B3–5, 6A3–5, 6B4–6, 7B3–5, 7C5–7, 7D4–8) towards base, in lower half with distinct girdles of few squamules that are concolourous with pileus centre. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (160/4/4) (5.0–)5.9–6.3–6.8(–7.5) × (2.7–)3.1–3.5–3.8(–4.4) µm, Q = 1.59–2.10, Q_{av} = 1.77–1.89, ellipsoid in front view and subtriangular in side view, with an indistinct spur, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (14–)16–20(–23) × (5.5–)6.9–8.2(–9.2) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, occasionally 2-spored, with up to 3–6 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (15–)19–24(–26) × (5–)6–8(–9) µm, narrowly clavate to clavate or cylindrical, occasionally subutriform to narrowly utriform, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm made up of (47–)58–95(–116) × (8–)9–16(–23) µm, clavate or cylindrical, erect cells, with rounded apex, with pale brown to brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary or gregarious, probably saprotrophic, on soil, in gallery forests and savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimens examined: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa182; ibid., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 22 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa215; Borgou Department, Tchatchou, Forêt de Tchatchou-Gokana, N9.026, E2.589, alt. 360 m.a.s.l., on sandy soil, in gallery forest with *Berlinia grandiflora*, 23 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa243; Donga Department, near Manigri, Forêt des Monts Kouffé, N8.992, E1.823, alt. 360 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Monotes kerstingii*, 1 Jul. 2022, *S. Sarawi, D. Dongnima, R. Dramani, F. Hampe, C. Manz & A. Rühl*, SeSa326.

Notes: Distinctive characteristics of *L. flavostipitata* are orange brown basidiomata, a yellowish stipe apex, and indistinctly spurred basidiospores. *Lepiota flavostipitata* is part of a clade that includes the (sub-)tropical species *L. alopochroa*, *L. brunneopileata*, *L. longisterigmata* and *L. aurantiicolor*. The differentiation of *L. flavostipitata* from *L. longisterigmata* and *L. aurantiicolor* is complex due to their shared habitats and the macromorphological similarity. However, *L. flavostipitata* stands out by its deeper, darker orange-brown colour and the yellowish stipe apex. Additionally, it lacks the unusually long sterigmata which are typical for *L. longisterigmata*. *Lepiota* *alopochroa* can be distinguished from *L. flavostipitata* by greyish orange to brownish orange colour of the stipe, by serrulate yellow to orange-white lamellae and larger basidiospores (7.5–9.3 × 3.8–4.0 µm) (Sysouphanthong *et al.* 2011). The differentiation from *L. brunneopileata* is based on the stipe colour, which is consistently orange brown to brown in *L. brunneopileata,* but becomes yellowish towards the apex in *L. flavostipitata*, along with the metachromatic basidiospores of *L. brunneopileata* (Rehman *et al.* 2024). Given the complexities of this species complex, molecular sequence data is recommended for accurate identification.

Lepiota pseudoignicolor, known from Guadeloupe and Venezuela resembles *L. flavositpitata*, by the colours of the basidiomata and the basidiospore shape, but lacks molecular sequence data. However, *L. pseudoignicolor* differs by yellow lamellae and larger basidiospores (9–11.5 × 3–4 µm) (Dennis 1970, Pegler 1983).

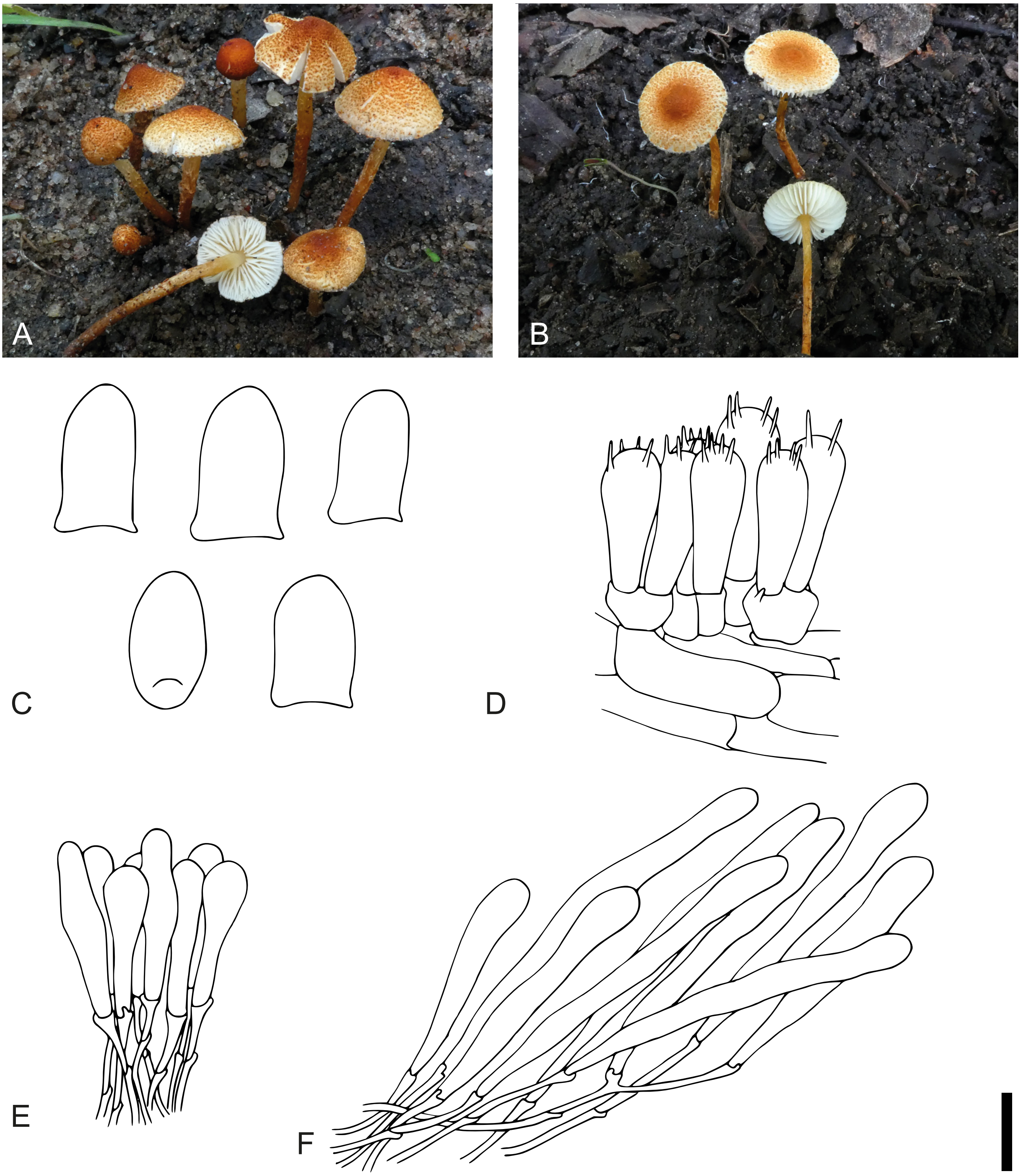


Fig. 12. Basidiomata and microstructures of *Lepiota flavostipitata*. **A.** Basidiomata (SeSa203, holotype). **B.** Basidiomata (SeSa182). **C.** Basidiospores (SeSa203). **D.** Basidia (SeSa203). **E.** Cheilocystidia (SeSa203). **F.** Pileus covering (SeSa203). Scale bar: A = 1.5 cm; B = 1 cm; C = 3 µm; D, E = 10 µm; F = 20 µm.

Lepiota longisterigmata Sarawi, ***sp. nov.*** MB 854025. Fig. 13.

Etymology: *longus* (Latin) = long or elongated, *sterigma* (Greek) = small, stalk-like structure; refers to the long sterigmata of the basidia.

Typus: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa179 (**holotype** FR-0175196, **isotype** in UNIPAR). GenBank: ITS = PP594565, LSU = PP594683, *RPB2* = PP841190.

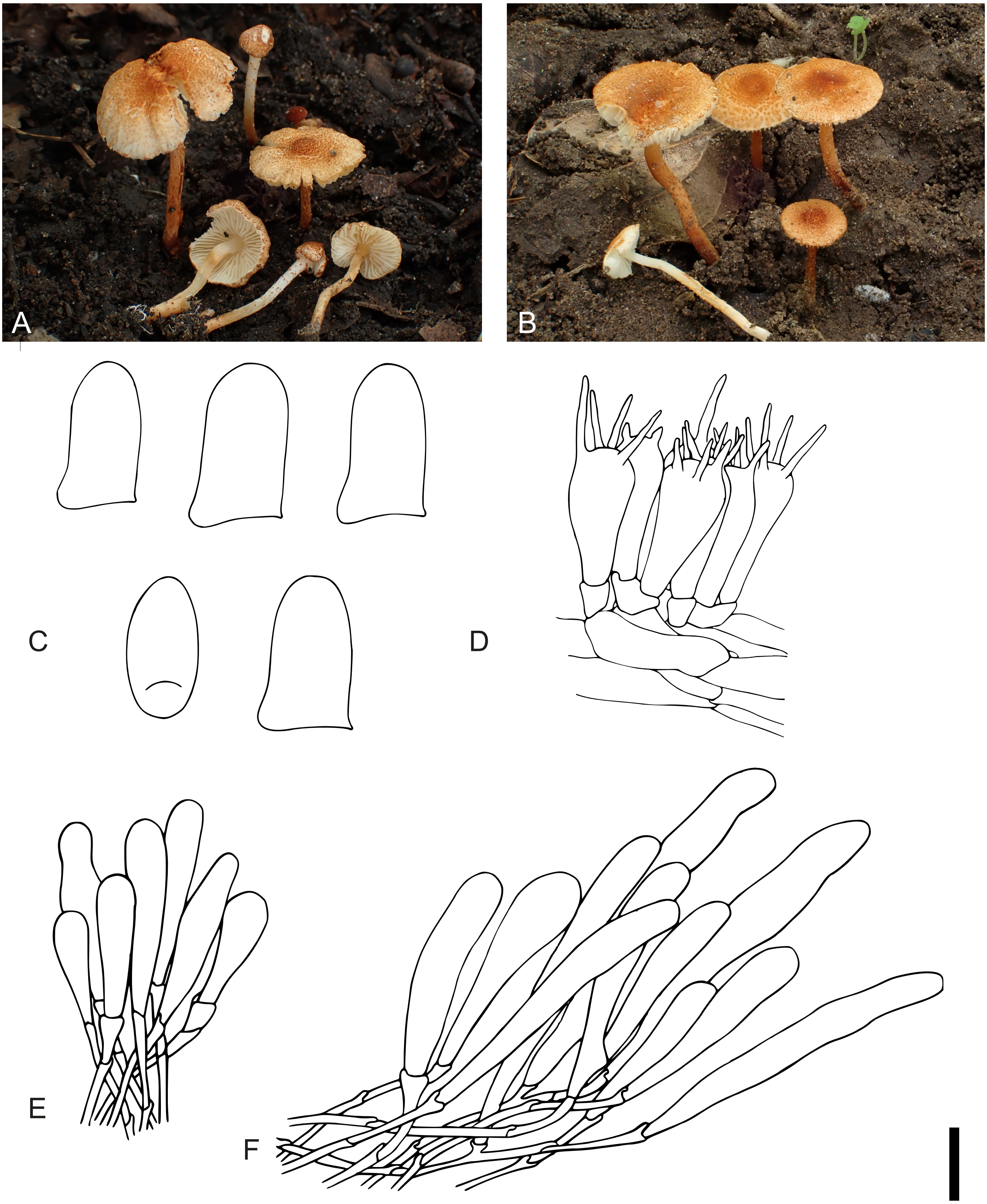


Fig. 13. Basidiomata and microstructures of *Lepiota longisterigmata*. **A.** Basidiomata (SeSa179, holotype). **B.** Basidiomata (SeSa112). **C.** Basidiospores (SeSa179). **D.** Basidia (SeSa179). **E.** Cheilocystidia (SeSa179). **F.** Pileus covering (SeSa179). Scale bar: A = 2 cm; B = 1 cm; C = 5 µm; D, E = 10 µm; F = 20 µm.

Basidiomata small to medium. *Pileus* 5–25 mm diam., at first hemispherical to conical, then becoming convex to plano-convex or plano-concave, covered by slightly erect, tomentose squamules; at centre orange-brown or reddish-brown to brown (7C7–8, 7D7–8, 7E7–8, 8D7–8, 8E7–8), paler, light orange, light orange-red or light brown (7A5–7, 7B5–7, 7C5–7, 7D4–7) towards margin, on a white background, with velar remnants at outmost margin. *Lamellae* free, moderately crowded to crowded, ventricose, 1–4 mm deep, white to cream; edge concolourous; lamellulae in up to three tiers. *Stipe* 10–45 × 1–3 mm, cylindrical, at apex white to cream, gradually darker, pale yellow, pale orange, orange-brown or reddish brown (5A2–4, 5B3–5, 6A3–5, 6B4–6, 7B3–5, 7C5–7, 7D4–8) towards base, in lower half with distinct girdles of few squamules that are concolourous with pileus centre. *Annulus* not observed. *Odour* and *taste* not recorded. *Basidiospores* (210/9/9) (5.3–)6.2–6.9–7.5(–10.0) × (2.9–)3.4–3.7–4.1(–4.7) µm, Q = 1.54–2.13, Q_{av} = 1.74–1.92, ellipsoid in front view and subtriangular in side view, with an indistinct spur, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)17–21(–25) × (6.2–)7.1–8.4(–9.5) µm, narrowly clavate to clavate, hyaline, with oily content, 2- to 4-spored, occasionally 1-spored, with 5–10(–20) µm long, irregularly shaped sterigmata. *Lamella edge* sterile. *Cheilocystidia* (12–)18–27(–40) × (4–)5–7(–9) µm, narrowly clavate to clavate or cylindrical, occasionally subutriform to narrowly utriform or fusiform, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm made up of (28–)51–89(–118) × (6–)11–15(–19) µm, clavate or cylindrical to sometimes rather subfusiform, erect cells, with rounded apex, occasionally somewhat thick-walled, with pale brown to brown parietal pigment. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary or gregarious, probably saprotrophic, on soil, in woodland and savannah. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Additional specimens examined: **Benin**, Borgou Department, Okpara, Forêt de Okpara, N9.237, E2.724, alt. 200 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 12 Jun. 2022, *S. Sarawi, S. Badou, D. Dongnima, F. Hampe, C. Manz, B. Olou & A. Rühl*, SeSa112; ibid., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 12 Jun. 2022, *S. Sarawi, S. Badou, D. Dongnima, F. Hampe, C. Manz, B. Olou & A. Rühl*, SeSa116; Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa180; ibid., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 21 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa185; ibid., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 22 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa208; Borgou Department, Forêt de Wari Maro, N9.183, E2.213, alt. 310 m.a.s.l., on sandy soil, in savannah with *Isoberlinia doka*, 25 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa252; ibid., on sandy soil, in savannah with *Isoberlinia doka*, 25 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa253; ibid., on sandy soil, in savannah with *Isoberlinia doka*, 25 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa254; Borgou Department, near Kpéssou, Forêt de l'Ouémé Supérieur, N9.261, E2.184, alt. 340 m.a.s.l., on sandy soil, in savannah with *Isoberlinia doka* and *I. tomentosa*, 28 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa295.

Notes: *Lepiota longisterigmata*, section *Stenosporae*, is characterized by orange brown basidiomata, indistinctively spurred basidiospores, basidia with exceptionally long irregular sterigmata, narrowly clavate, clavate to cylindrical cheilocystidia, and a trichodermal pileus covering. *Lepiota alopochroa*, *L. brunneoaurantia*, *L. brunneopileata*, *L. castanea*, *L. mandarina*, *L. subcastanea*, and species described above, *L. aurantiicolor* and *L. flavostipitata* are all macroscopically and phylogenetically close to *L. longisterigmata*. *Lepiota longisterigmata* differs from all these species particularly in its very long sterigmata, measuring 5–10(–20) µm, a characteristic that has been observed consistently in various collections from different locations. In addition to this *L. alopochroa*, known from tropical Asia, differs from *L. longisterigmata* by serrulate, pale yellow to orange-white lamellae, and relatively small basidiospores (7.5–9.3 × 3.8–4.0 µm) (Sysouphanthong *et al.* 2011). *Lepiota brunneoaurantia*, known from Pakistan, differs from *L. longisterigmata* by darker and more intense colours of the basidiomata, a distinct umbo, a darker stipe, and slightly smaller basidiospores (5.2–6.2 × 2.4–2.8 µm), and significantly smaller cheilocystidia (8.9–13.1 × 4.2–5.9 µm) (Azeem *et al.* 2024). Distinctive characteristics of *L. castanea* are the dark chestnut-brown colours of the basidiomata, the presence of an annulus, and the significantly larger and distinctively spurred basidiospores (7.0–14.0 × 3.0–5.5 µm) (Quélet 1881, Vellinga 2001a). *Lepiota mandarina*, known from China, presents red brown, orange red colours of the pileus and stipe, though it is microscopically similar to *L. longisterigmata*, except in the shape of the sterigmata (Liang 2016). Macroscopically, *L. subcastanea*, known from China, differs from *L. longisterigmata* by yellowish to yellowish brown, mustard-brown, sometimes orange or reddish colours of the pileus and stipe as well as, larger distinctively spurred basidiospores (8.5–11.0 × 3.5–4.5 µm) (Liang 2016). It is a challenge to distinguish *L. longisterigmata* from *L. aurantiicolor* and *L. flavostipitata*. These three species often grow together, intermixed, and are very similar macroscopically. The main distinguishing characteristic are the long sterigmata of *L. longisterigmata*. *Lepiota flavostipitata* can be distinguished by stronger orange, orange-brown colours of the basidiomata and a yellowish stipe apex. Given the complexities of this clade, molecular sequence data are needed for accurate identification.

Further, *L. longisterigmata* morphologically resembles *L. pseudoignicolor*, known from Guadeloupe and Venezuela, by the colours of the basidiomata and the basidiospore shape and size, but unfortunately, that species lacks molecular sequence data. However, *L. pseudoignicolor* differs by yellow lamellae and significantly larger basidiospores (9–11.5 × 3–4 µm) (Dennis 1970, Pegler 1983).

Lepiota tyrianthina Sarawi, ***sp. nov.*** MB 854026. Fig. 14.

Etymology: The epithet refers to the colour of the basidiomata, reminiscent of Tyrian purple, a deep purple hue historically obtained from certain species of mollusks in the Muricidae.

Typus: **Benin**, Collines Department, near Kilibo, N8.616, E2.665, alt. 320 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 22 Jun. 2022, *S. Sarawi, D. Dongnima, F. Hampe, C. Manz & A. Rühl*, SeSa213 (**holotype** FR-0175205, **isotype** in UNIPAR).

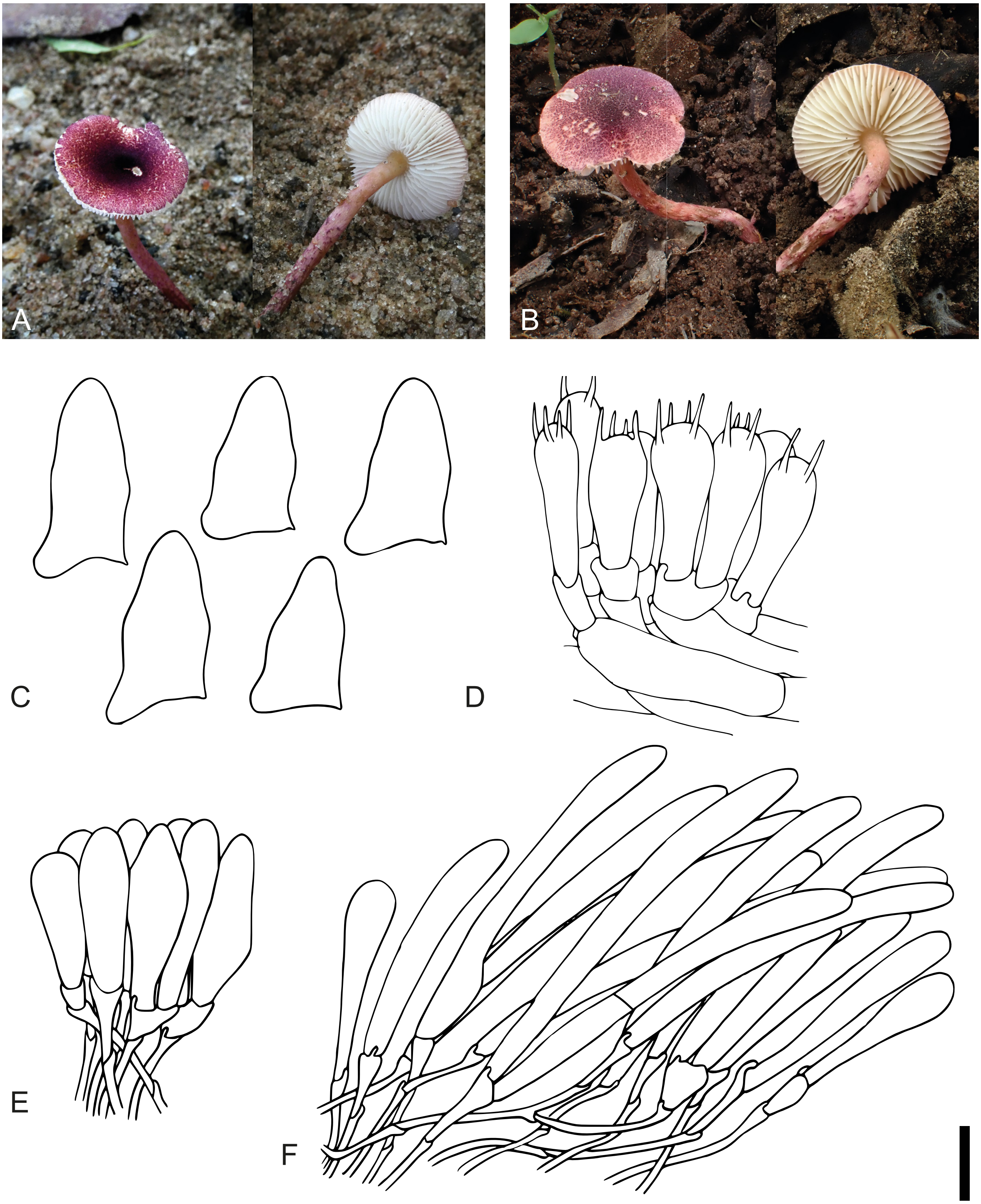


Fig. 14. Basidiomata and microstructures of *Lepiota tyrianthina*. **A.** Basidioma (SeSa213, holotype), seen from above (left) and from below the pileus (right). **B.** Basidioma (SeSa111) seen from above (left) and from below the pileus (right). **C.** Basidiospores (SeSa213). **D.** Basidia (SeSa213). **E.** Cheilocystidia (SeSa213). **F.** Pileus covering (SeSa213). Scale bar: A = 1 cm; B = 6 mm; C = 3 µm; D, E = 10 µm; F = 20 µm.

GenBank: ITS = PP594573, LSU = PP594692, *RPB2* = PP841200.

Basidiomata small to medium. *Pileus* 10–15 mm diam., convex to plano-convex, with or without umbo; at centre with a dark purple to almost blackish (14F8–7) calotte; surface around calotte splitting up into magenta to violet (14C8–7, 14D8–6) patches, on a white to cream background, or not splitting up and hardly showing the background. *Lamellae* free, moderately crowded to crowded, ventricose, 2–3 mm deep, white to cream; edge concolourous; lamellulae in up to two tiers. *Stipe* 30–35 × 1–2 mm, cylindrical, curved at base, at apex cream to yellowish (3B2–1), more pinkish, magenta to purple (14C8–3, 14D8–6) towards base, in lower half with distinct girdles of squamules that are concolourous with pileus centre. *Odour* and *taste* not recorded. *Basidiospores* (80/2/2) (5.4–)6.3–7.0–7.7(–8.6) × (2.8–)3.1–3.4–3.7(–4.1) µm, Q = 1.72–2.37, Q_{av} = 2.00–2.10, cylindrical in front view, triangular in side view, with two lateral knobs, with a distinct spur, hyaline, smooth, dextrinoid, non-metachromatic. *Basidia* (15–)17–22(–25) × (6.1–)6.5–7.7(–8.4) µm, narrowly clavate to clavate, hyaline, with oily content, 4-spored, occasionally 2-spored, with up to 6 µm long sterigmata. *Lamella edge* sterile. *Cheilocystidia* (15–)18–25(–31) × (5–)6–7(–8) µm, narrowly clavate to clavate or cylindrical, occasionally subutriform to narrowly utriform or fusiform, hyaline. *Pleurocystidia* absent. *Pileus covering* a trichoderm made up of (44–)60–105(–151) × (6–)8–13(–18) µm, cylindrical, erect cells, with rounded apex, mainly non septate, rarely with up to 2 septa, with pale brown parietal pigment and pale purple intracellular pigment; purple pigments dissolving in KOH. *Clamp connections* present in all parts.

Ecology, habitat, and distribution: Basidiomata solitary, probably saprotrophic, on soil, in woodland. Currently only known from Benin.

Toxicity: No amatoxins and phallotoxins were detected.

Further specimen examined: **Benin**, Borgou Department, Okpara, Forêt de Okpara, N9.237, E2.724, alt. 200 m.a.s.l., on sandy soil, in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, 12 Jun. 2022, *S. Sarawi, S. Badou, D. Dongnima, F. Hampe, C. Manz, B. Olou & A. Rühl*, SeSa111.

Table 2. Peptide natural products (PNP’s) matches of DEREPLICATOR, order by increasing p-value.

	Compound	Score	p-value	Peptide mass	Observed mass [M+H]$^+$
1	Cyclolinopeptide B	24	1.40E-61	1057.60	1023.61
2	Cyclolinopeptide A	16	7.80E-42	1039.65	992.65
3	Massetolide F	18	1.30E-41	1125.69	1144.71
4	Massetolide F	18	5.10E-39	1125.69	1127.70
5	Cyclolinopeptide B	16	9.60E-35	1057.60	1022.61
6	Pseudodesmin A	16	2.00E-33	1124.71	1126.70
7	Massetolide F	11	2.40E-25	1125.69	1145.71
8	Surugamide_A	11	6.50E-25	911.62	784.53
9	Argyrins 4’’-Demethoxy	13	6.50E-25	794.30	826.41
10	Citrusin VI	12	1.40E-24	794.47	807.53
11	Massetolide E	12	1.70E-24	1111.67	1112.68
12	Cyclolinopeptide D	11	1.90E-24	1063.56	940.54
13	Massetolide J	9	6.70E-23	1111.67	1130.69
14	Peptidolipin F	11	2.10E-21	781.46	668.41
15	Dolastatin G 55-Demethyl	9	4.70E-20	1044.67	941.54
16	Massetolide E	9	7.00E-20	1111.67	1113.68
17	Citrusin VI	8	1.10E-19	794.47	851.54
18	Massetolide H	9	3.10E-19	1153.72	1154.73
19	b-Amanitin	8	5.60E-18	919.34	920.36
20	Microphycin AL828	10	9.20E-18	828.44	812.38
21	Phomafungin	10	1.20E-17	1029.56	920.38
22	Massetolide F	7	2.10E-16	1125.69	1126.70
23	Pimaydolide	7	2.20E-16	653.42	639.43
24	b-Amanitin-41-Amide	7	3.50E-15	918.35	919.36
25	Sporidesmolide V	6	6.00E-14	652.44	653.45
26	Citrusin VIII	8	7.60E-14	821.48	855.38
27	Destruxins 2-Valine analogue	6	7.00E-12	579.36	512.32
28	Cyclolinopeptide A	6	7.70E-11	1039.65	935.53

Notes: *Lepiota tyrianthina*, section *Stenosporae*, is characterized by dark purple, magenta to violet basidiomata, the lack of an annulus, and distinctly spurred, dextrinoid, basidiospores with lateral knobs. Phylogenetically close or morphologically similar species are *L. erythrostricta*, *L. ignicolor*, *L. pilodes*, and *L. subalba*. *Lepiota tyrianthina* differs from *L. ignicolor*, *L. pilodes*, and *L. subalba* by the purple to violet coloured basidiomata (compare with descriptions by Bresadola 1892, Orton 1960, Vellinga & Huijser 1993). *Lepiota erythrosticta* Berk. & Broome (1871) was described from Sri Lanka and is the morphologically closest species to *L. tyrianthina* because of its purple to violet basidiomata and similar micro-characteristics. Pegler (1972) examined the type specimen of *L. erythrosticta* and extended the description of Berkeley & Broome (1871). *Lepiota tyrianthina* differs from *L. erythrosticta* by more fragile basidiomata, the absence of an annulus, and basidiospores with lateral knobs. *Lepiota erythrosticta* has been reported several times from different geographical regions, however, the descriptions differ from those of Berkeley & Broome (1871) and Pegler (1972) in colour and basidiospore-characteristics for specimens from Sri Lanka (Dennis 1952, Sysouphanthong *et al.* 2011, Liang 2016). None of these specimens resemble *L. tyrianthina*. Two ITS sequences of specimens from the herbarium Lille (LIP), France, deposited as *L.* cf. *erythrosticta* collected in Martinique, are close to those of *L. tyrianthina*, however, they differ by a p-distance of 14 %. *Lepiota erythrosticta* as currently treated by various authors includes several species, and further investigations are needed. Unfortunately, sequence data of the type specimen or authentic material is not available.

Feature-based molecular networking

The feature-based molecular network was generated from UHPLC-HRMS² data of 96 extracts belonging to 46 species of *Lepiota* and one extract from *Echinoderma asperum*. To map the chemical diversity of the 97 fungal extracts, these data were pre-processed using MZMine 3 and the resulting 4356 mass features were organized into a molecular network, consisting of 177 clusters with at least two nodes (Fig. 15). To visualize the distribution of the metabolites across the samples, the molecular network was mapped at different taxonomic levels (sections or subsections), using a colour tag. At first glance, the molecular network reveals that some groups of chemical compounds are restricted to

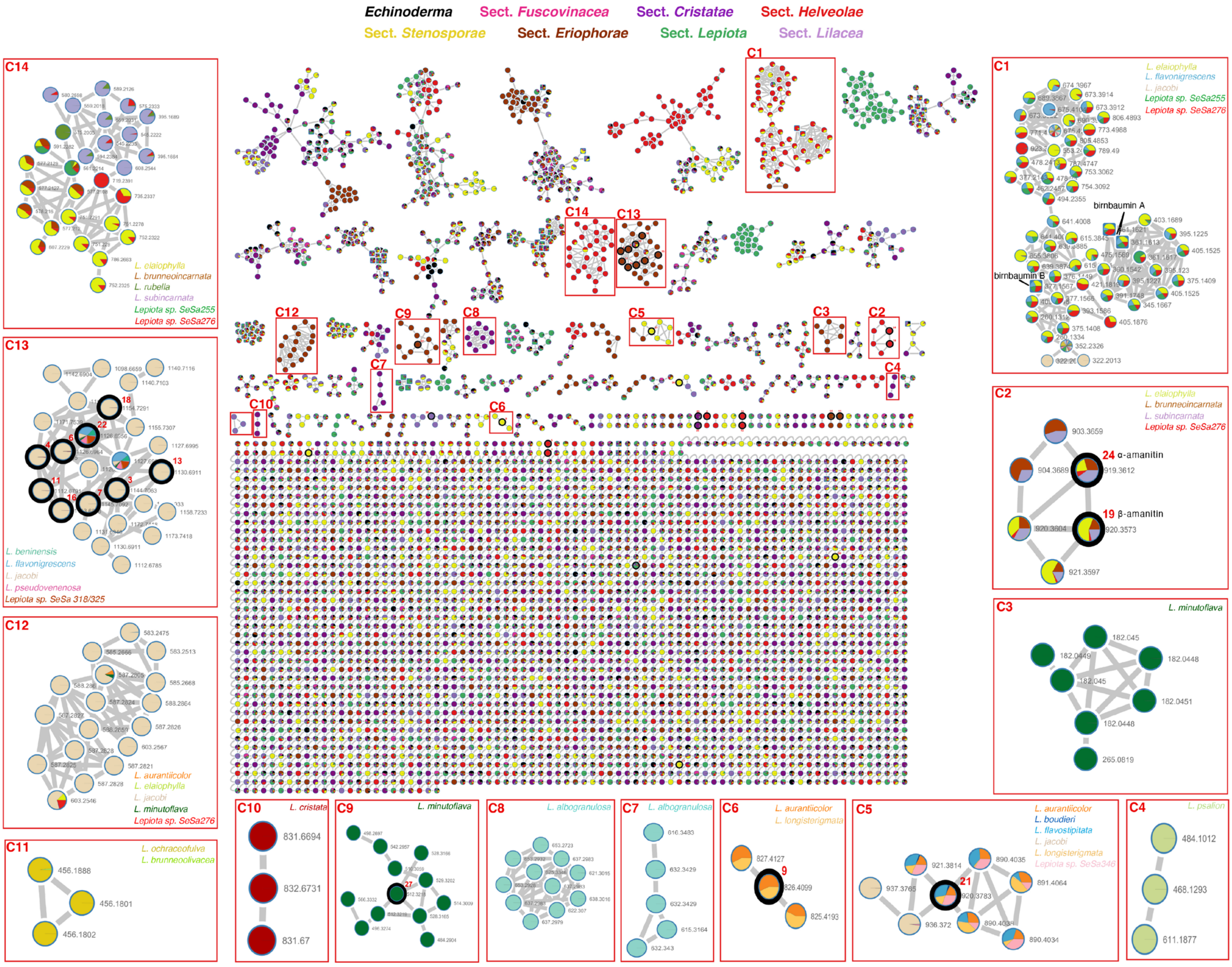


Fig. 15. Feature-based molecular network generated from the UHPLC-HRMS² data of 46 *Lepiota* extracts and one *Echinoderma asperum* extract. The network is colour coded on section level. Fourteen clusters are highlighted in red boxes (C1–C14) and colour coded on species level. Parent masses are indicated at the nodes. Nodes dereplicated against GNPS spectral libraries are indicated by blue rectangles. Black circles and red numbers indicate compounds proposed by DEREPLICATOR and cited in Table 2.

specific sections of *Lepiota*, suggesting that closely related organisms produce closely related chemical compounds that may be specific to a section/subsection.

The MS/MS data were dereplicated against the GNPS spectral libraries, resulting in 57 hits in total. These compounds mainly correspond to acylamides, diketopiperazines, phosphatidylcholines, or phosphatidylethanolamines, but also to *N*-hydroxyoxoamidines-containing alkaloids, namely birnbaumins A–B.

Birnbaumin cluster C1

Birnbaumins A–B occur in cluster C1 (Fig. 15) which refers to compounds from species of *Lepiota* belonging to the sections *Eriophorae*, *Stenosporae*, and *Helveolae*. Most of these species, viz., *L. elaiophylla*, *L. flavonigrescens*, *L. jacobi*, *Lepiota* sp. (SeSa255), and *Lepiota* sp. (SeSa276), develop yellow basidiomata, consistent with the colour of these pigments.

Amatoxin cluster C2

Amatoxin-like peptides are thought to be present in some of the species studied, so the variable dereplication mode of DEREPLICATOR was applied to allow the putative identification of unknown peptide natural products from known analogues. This dereplication process resulted in 28 peptide spectrum matches (PSM) (Table 2). Twenty-eight

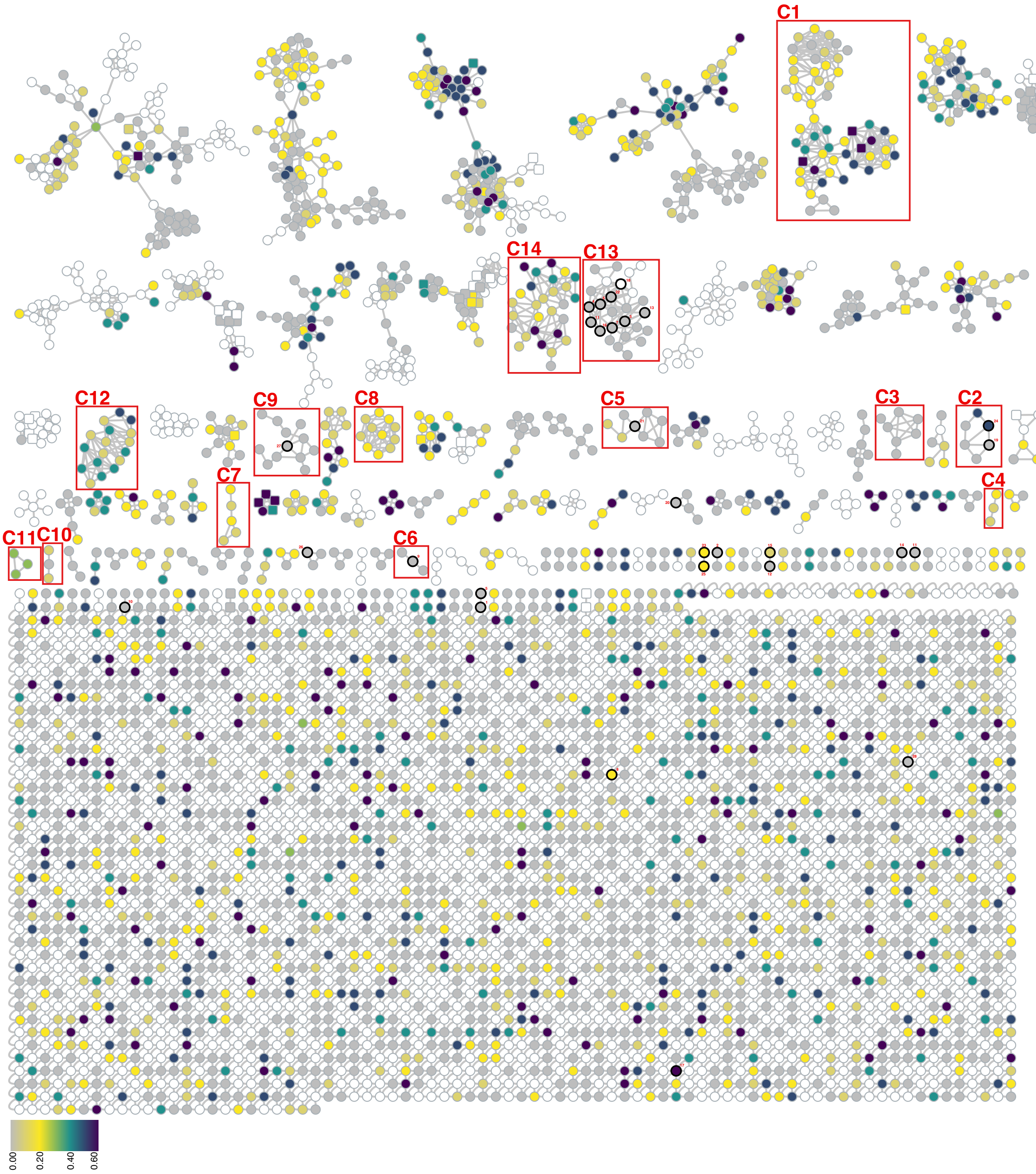


Fig. 16. Feature-based molecular network generated from the UHPLC-HRMS² data of 46 *Lepiota* extracts and one *Echinoderma asperum* extract. TIMA highest biological scores are indicated for each node by a colour gradient. White nodes refer to compounds not annotated by TIMA.

fungal peptide products had been proposed, including two amatoxin-type bicyclic peptides (α- and β-amanitin) (Table 2). Six nodes tentatively assigned to amatoxins belong to cluster C2 referring to species of section *Helveolae*. Notably, two features of this cluster were annotated as putative amatoxins by the TIMA workflow, namely α-amanitin (initial score: 0.20, final TIMA score: 0.48) and amaninamide (initial score: 0.18, final TIMA score: 0.48). In order to strengthen our confidence in these assignments, the samples had been co-chromatographed against extracts of *Amanita phalloides* and *A. virosa* for direct comparison against standards (see below, Fig. 19) with the same results.

Cyclic-peptide-containing cluster C5

Cluster C5 reflects the presence of compounds from fungi belonging to sections *Eriophorae* and *Stenosporae*. These compounds had a mass range from *m/z* 890 to *m/z* 937, somewhat similar to the mass of amatoxins. One MS/MS feature appearing in *L. aurantiicolor*, *L. boudieri*, *L. flavostipitata*, *L. jacobi*, *L. longisterigmata*, and *Lepiota* sp. (SeSa346) was annotated by DEREPLICATOR as a phomafungin analogue, a cyclic depsipeptide formerly reported from a fungal source (*Phoma* sp.).

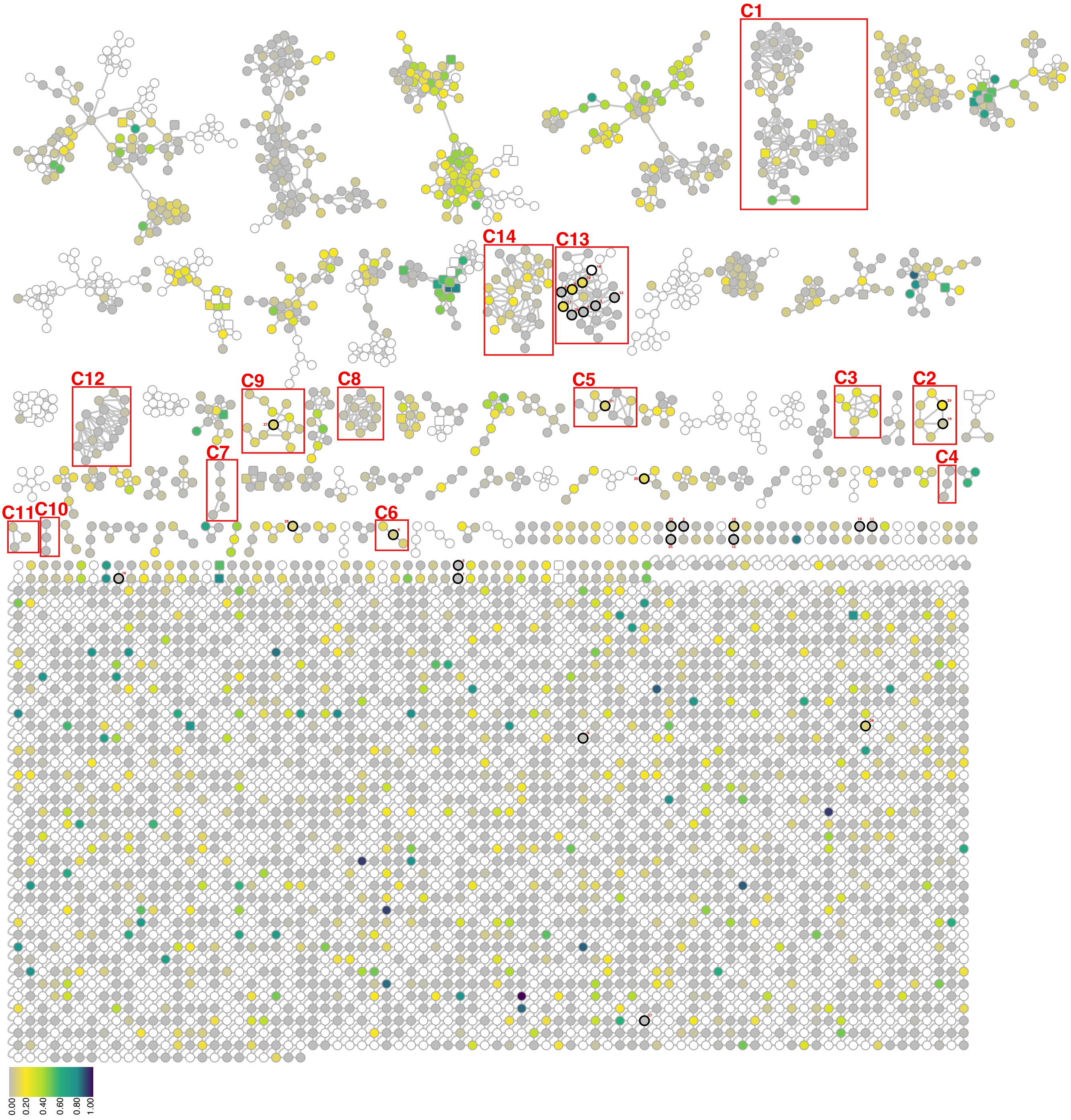


Fig. 17. Feature-based molecular network generated from the UHPLC-HRMS² data of 46 *Lepiota* extracts and one *Echinoderma asperum* extract. TIMA highest initial scores are indicated for each node by a colour gradient. White nodes refer to compounds not annotated by TIMA.

Stenosporae cluster C6

Compounds in cluster C6 were exclusively found in species of section *Stenosporae*. For cluster C6 no hits by DEREPLICATOR were obtained would be prefered.

Eriophorae clusters C3 and C9

The clusters C3 and C9 both refer to compounds of *L. minutoflava* in section *Eriophorae*. Most nodes in cluster C3 disclose a mass-to-charge ratio of *m/z* 182.045, consistent with a molecular formula of $C_8H_7NO_4$. Some other nodes were tentatively assigned to benzoxazinones, pyridine carboxylic acids, and/or benzoxazole. In cluster C9, one node was annotated by DEREPLICATOR as a destruxin analogue. Based on TIMA, some peptide compounds were tentatively proposed for nodes of this cluster with cosine scores between 0.06 and 0.08.

Massetolide-tagged cluster C13

Five nodes in cluster C13 were tentatively assigned to cyclic depsipeptides related to massetolides by DEREPLICATOR. TIMA confirmed this result for some nodes in this group, for other nodes massetolide derivatives or viscosin derivatives were proposed, with an acceptable degree of spectrometric correspondence (cosine values close to 0.15 for some of them).

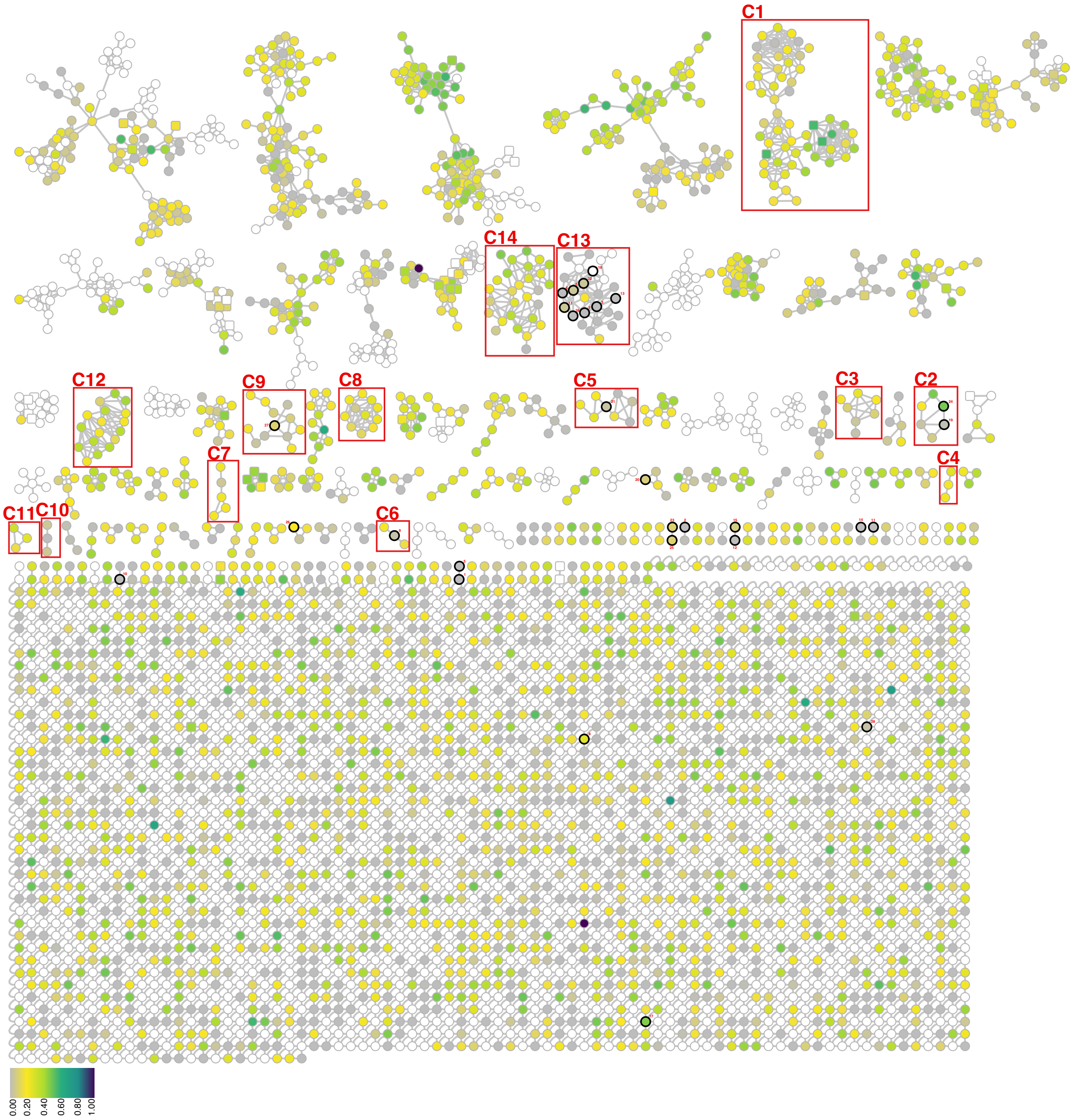


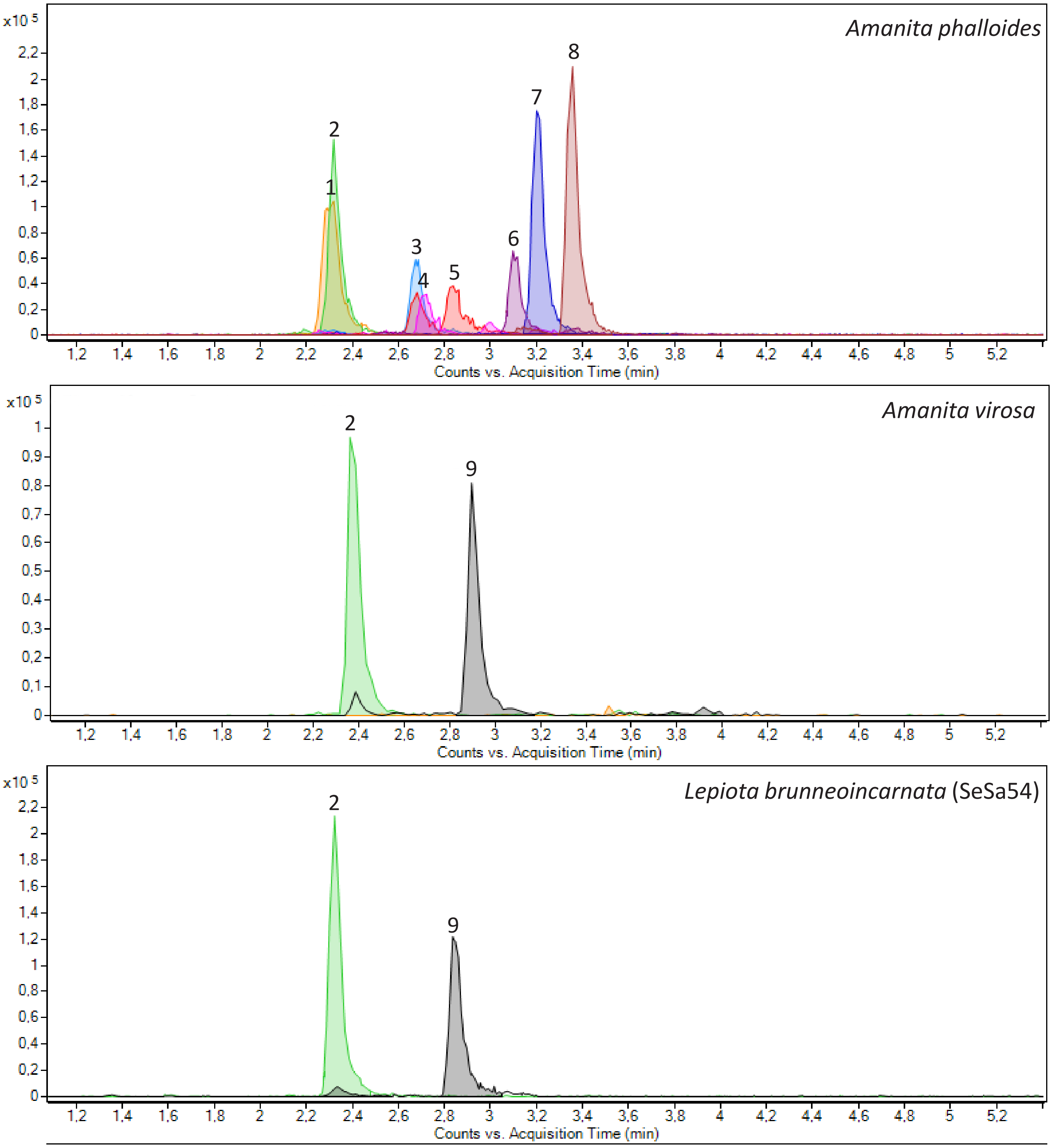
Fig. 18. Feature-based molecular network generated from the UHPLC-HRMS² data of 46 *Lepiota* extracts and one *Echinoderma asperum* extract. TIMA highest final scores are indicated for each node by a colour gradient. White nodes refer to compounds not annotated by TIMA.

Eriophorae dominated cluster C12

Most compounds clustering in C12 were found in basidiomata of *L. jacobi* (section *Eriophorae*), however, some nodes referred to sections *Lilacea*, *Stenosporae*, or section *Helveolae*. These compounds apparently are closely related to each other, as their masses span a narrow mass range from *m/z* 583.24 to *m/z* 603.2546, including various isomeric forms. For cluster C12, DEREPLICATOR could not lead to any annotation. The MS/MS features in this cluster did not lead to good spectrometric matches against TIMA (initial score constantly below 0.03), precluding any attempt to assign a chemical class to these compounds. Many of these results reveal a spectral similarity score of zero.

Lilaceae clusters C4, C7, C8, C10, and C11

Clusters C4, C7, C8, C10, and C11 were detected for species of section *Lilaceae*; however, cluster C11 corresponds to sections *Lilaceae* and *Stenosporae*. At species level, cluster C4 was exclusively assigned to *L. psalion*. Clusters C7 and C8 were assigned to *L. albogranulosa*. The compounds found in these two groups have the same mass range (with *m/z* ranging from 615 to 653) and disclose similar TIMA-based


Amanita phalloides

Amanita virosa

Lepiota brunneoincarnata (SeSa54)

Fig. 19. Reconstructed Ion Chromatograms (RIC) for the precursor masses of amatoxins and phallotoxins in the benchmark extracts of *Amanita phalloides* and *A. virosa*, as well as selected extracts of *Lepiota* specimens investigated for amatoxins and phallotoxins. Numbers at peaks refer to compounds listed in Table 2.

annotation trends. The spectrometric-based annotation of these clusters provides cosine scores constantly below 0.05 (Fig. 17). Nevertheless, consistent with their hydrophobic nature, nodes belonging to this group have been repeatedly annotated as terpene compounds, and plant-derived limonoids or diterpene alkaloids, such as aconitane. The annotations within these clusters resulted in better TIMA final scores, sometimes reaching values up to 0.3, however with low spectrometric support.

Cluster C10 was exclusively assigned to *L. cristata*. The nodes appearing in this cluster are related to highly hydrophobic compounds which could not be satisfyingly annotated by TIMA but a possible glycosphingolipid or a steroidic nature has been suggested by this tool, with low confidence.

Cluster C11 was constrained to *L. ochraceofulva,* and one node was additionally assigned to *L. brunneoolivacea*. Compared to C10, the C11 cluster appears to benefit from a slightly better TIMA annotation, especially when considering the final score. The DEREPLICATOR workflow did not provide any hit for any node of the clusters C4, C7, C8, C10, and C11.

Helveolae **and** ***Stenosporae*** **cluster C14**

Cluster C14 corresponds to compounds of basidiomata of *Lepiota* species belonging to section *Helveolae* and section

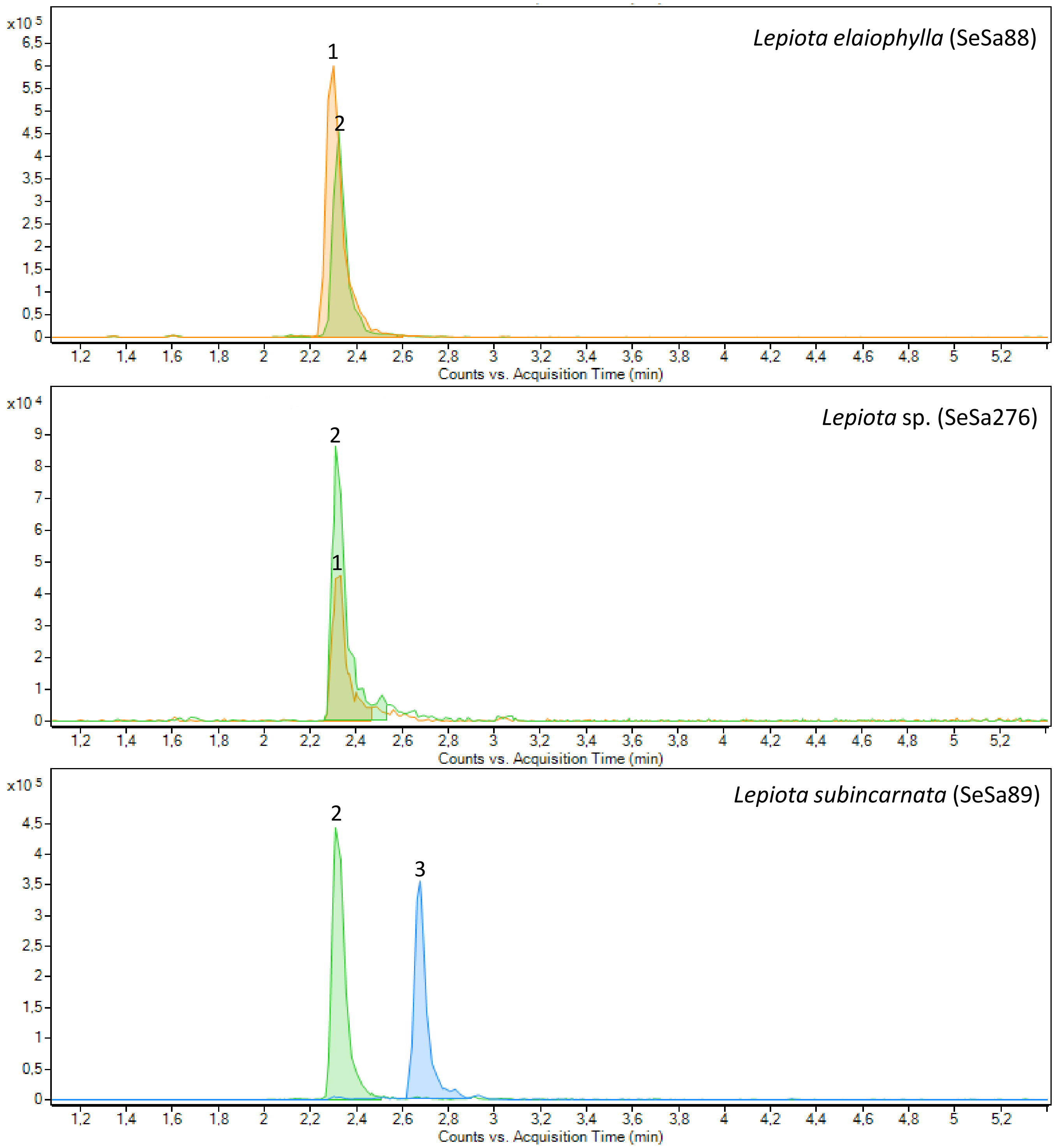


Fig. 19. (Continued).

Stenosporae. It presents at least two subclusters, which mainly stem from yellow species *L. elaiophylla* and *Lepiota* sp. (SeSa276) or from *L. rubella* and *L. subincarnata*. No annotation had been proposed by DEREPLICATOR. TIMA-based annotations for this group sometimes suggested the presence of Basidiomycota-derived compounds (e.g. terphenyl quinones, naphthoquinone-type dimers, etc.) with final scores of 0.4 to 0.5 for some nodes. However, these annotations rely predominantly on biological score and MS1-derived annotation and lack convincing MS/MS spectral similarity to *in silico* predicted spectra, resulting in low overall confidence.

Amatoxins in *Lepiota* species

In order to increase confidence in the annotations of amatoxins and phallotoxins in *Lepiota* spp. resulting from the DEREPLICATOR-based annotation and to identify amatoxin analogues that had not been annotated, all extracts were screened for the occurrence of amatoxins and phallotoxins by UHPLC-HRMS². Extracts of the benchmark species *Amanita phalloides* and *A. virosa* were used to compare the retention times in our chromatographic conditions and MS/MS fragmentation patterns (Fig. 19).

Reconstructed Ion Chromatograms (RIC) generated for the amatoxins and phallotoxins precursor masses in these two *Amanita* species provided reference retention time that supported the occurrence of α-amanitin and amaninamide in *L. brunneoincarnata*, of α-amanitin and β-amanitin in *L. elaiophylla* and *Lepiota* sp. (SeSa276), and of α-amanitin and γ-amanitin in *L. subincarnata*. The distinction of the isomeric γ-amanitin and amaninamide is further supported by the inspection of their MS² spectra that revealed diagnostic *m/z* ions at 243.13 and 357.18 for the former and at *m/z* 259.13 and 373.15 (Fig. 19, Table 3; Sarawi **et al.** 2022, Zhao **et al.** 2023).

To confirm the absence of amatoxins and phallotoxins as determined by the FBMN, the presence of amatoxins and phallotoxins in all the investigated *Lepiota* species was studied at MS1 level for the masses of all amatoxins and phallotoxins. No amatoxins or phallotoxins were detected in *L. apatelia*, *L. albogranulosa*, *L. aurantiicolor*, *L. beninensis*, *L. boudieri*, *L. brunneoolivacea*, *L. castanea*, *L. clypeolaria*, *L. cristata*, *L. echinobispora*, *L. erminea*, *L. farinolens*, *L. felina*, *L. flavonigrescens*, *L. flavostipitata*, *L. fuscovinacea*, *L. griseovirens*, *L. ignivolvata*, *L. jacobi*, *L. lilacea*, *L. lilaceostriata*, *L. longisterigmata*, *L. magnispora*, *L. minutisterigmata*, *L. minutoflava*, *L. ochraceofulva*, *L. oreadiformis*, *L. psalion*, *L. pseudovenenosa*, *L. rubella*, *Lepiota* sp. (SeSa255), *Lepiota* sp. (SeSa261), *Lepiota* sp. (SeSa262), *Lepiota* sp. (SeSa278), *Lepiota* sp. (SeSa307), *Lepiota* sp. (SeSa308), *Lepiota* sp. (SeSa318), *Lepiota* sp. (SeSa325), *Lepiota* sp. (SeSa335), *Lepiota* sp. (SeSa336), *Lepiota* sp. (SeSa346), *L. subalba*, *L. tomentella*, *L. tyrianthina*, and *L. woehnertii*. Additionally, no amatoxins or phallotoxins were detected in *Echinoderma asperum*.

DISCUSSION

In this study, 13 new *Lepiota* species were described as new to science whereas only one previously described species, *L. albogranulosa*, was found among our 104 collections from Benin made in 2022. This demonstrates the large potential for *Lepiota* species yet to be discovered in West Africa, simultaneously highlighting a significant gap in our current understanding of *Lepiota* diversity in the region. Moreover, the herein generated sequence data yielded the first multilocus tree for *Lepiota* species including numerous sequences from West Africa with 353 newly generated sequences from three different gene loci (ITS, LSU, and *RPB2*).

Occurrence of amatoxins within section *Helveolae*

A recent UHPLC-HRMS² study demonstrated that there are fewer amatoxin-containing *Lepiota* species than previously assumed and that these are restricted to section *Helveolae* (Sarawi *et al.* 2022). These results are confirmed in the present study. However, due to a larger sampling and examination of more species, chemotaxonomic relationships can now be defined more precisely. Amatoxins apparently are only present in section *Helveolae* but are absent within two subclades, one around *L. rubella* and one around *L. pseudovenenosa* (Fig. 1). This hypothesis must be investigated by further UHPLC-HRMS² or other methods to detect amatoxins and by phylogenetic analyses, as not all

Table 3. Masses and sum formulas of amatoxins and phallotoxins observed in extracts of the benchmark *Amanita phalloides* as well as *Lepiota brunneoincarnata*, *L. elaiophylla*, *Lepiota* sp. (SeSa276), and *L. subincarnata*.

Compound	Retention Time (min)	Detected Mass $[M^+H]^+$	Calculated Mass $[M^+H]^+$	Error (ppm)	Sum formula $[M^+H]^+$
β-amanitin (1)	2.32	920.3452	920.3455	+0.3	$C_{39}H_{54}N9O_{15}S$
α-amanitin (2)	2.23	919.3624	919.3614	–1.1	$C_{39}H_{55}N_{10}O_{14}S$
γ-amanitin (3)	2.68	903.3667	903.3665	–0.2	$C_{39}H_{55}N_{10}O_{13}S$
phallisin (4)	2.72	805.3180	805.3185	+0.6	$C_{35}H_{49}N_{8}O_{12}S$
amanin (5)	2.84	904.3515	904.3505	–1.1	$C_{39}H_{54}N_{9}O_{14}S$
phallisacin (6)	3.10	863.3236	863.3240	+0.5	$C_{37}H_{51}N_{8}O_{14}S$
phalloidin (7)	3.20	789.3244	789.3236	–1.0	$C_{35}H_{49}N_{8}O_{11}S$
phallacidin (8)	3.56	847.3287	847.3291	+0.5	$C_{37}H_{51}N_{8}O_{13}S$
amaninamide (9)	2.84	903.3664	903.3665	+0.1	$C_{39}H_{55}N_{10}O_{13}S$

the species in this subsection have been analysed for their amanitin content. As long as such data are not available, the three species placed within the amatoxin containing clade, *L. brunneosquamulosa*, *L. baiyunensis*, and *L. punaensis*, but also *L. pleurocystidiata*, which is placed in between the amatoxin containing clade and one of the two other clades, should be suspected to contain amanitin.

First amatoxin-containing *Lepiota* species from Benin

The first amatoxin containing *Lepiota* specimen (SeSa276) from West Africa is reported here. It is closely related to *L. elaiophylla* and contains α-amanitin and β-amanitin based on UHPLC-HRMS² results, a DEREPLICATOR-annotated feature-based molecular network (FBMN), and comparison against extracts of *Amanita phalloides* and *A. virosa* which revealed similar retention times and identical fragmentation patterns. There were no amatoxin containing *Lepiota* species known from West Africa before this study. This is the only investigated *Lepiota* species in Benin containing amatoxins. However, there still is a high risk of fatal poisoning due to the high number of unknown *Lepiota* species in West Africa and the incomplete knowledge about the occurrence of amatoxins.

Chemotaxonomy of *Lepiota* species

Through FBMN, chemotaxonomical information is provided for *Lepiota* by some clusters of compounds being restricted to certain phylogenetic groups.

Amatoxins as a chemotaxonomic character

The amatoxin cluster C2 of the FBMN confirmed that amatoxins occur exclusively in section *Helveolae* but not in all species of this group. *Lepiota* species, however, are not referenced as amatoxin-producers in LOTUS resulting in a low TIMA biological score of 0.6 for α-amanitin and of 0 for amaninamide (Fig. 16). The occurrence of amatoxins is supported by the TIMA annotation based on the initial score and final score for α-amanitin (initial score: 0.20, final TIMA score: 0.48) and amaninamide (initial score: 0.18, final TIMA score: 0.48). The exclusivity of the amatoxins in section *Helveolae* is further supported by the targeted search for masses of all known amatoxins (viz. Reconstructed Ion Chromatogram generation) of all other *Lepiota* extracts revealing the absence of amatoxins and phallotoxins outside section *Helveolae*.

Sarawi *et al.* (2022) recently demonstrated the presence of natural compounds with masses similar to those of amatoxins in *L.* cf. *boudieri* of section *Stenosporae*. In addition, previous studies have discussed the toxicity of *L. boudieri* and other *Lepiota* species of section *Stenosporae*, based on thin-layer chromatographic results (Gérault & Girre 1975, Enjalbert *et al.* 2002). It seems that these compounds occur in cluster C5, representing compounds from species in sections *Eriophorae* and *Stenosporae*, including *L. aurantiicolor*, *L. boudieri*, *L. flavostipitata*, *L. longisterigmata,* and *Lepiota* sp. (SeSa346). As previously indicated, the precursor masses within the cluster are reminiscent to those of amatoxins as they range between *m/z* 890 and *m/z* 937, although they belong to a distinct structural class as they clustered apart from these toxins and had not been dereplicated as such by DEREPLICATOR and TIMA. Notably, one of the nodes appearing in cluster C5 was tentatively annotated through the variable mode of the DEREPLICATOR workflow as an analogue of phomafungin, a cyclic lipodepsipeptide isolated from *Phoma* sp. (Herath *et al.* 2009). The TIMA annotation, however, resulted in poorly convincing annotations, based on both spectrometric and taxonomical scoring systems.

Putative chemotaxonomic characteristics of the *L. longisterigmata* clade

Stenosporae cluster C6 revealed nodes that are restricted to species in section *Stenosporae*, some of them being morphologically difficult to distinguish. Chemotaxonomic characteristics may be used to distinguish similar species from each other.

Birnbaumins as a chemotaxonomic character

Cluster C1 mainly includes species with yellow-coloured basidiomata. The molecular network resulted in annotating some of these nodes against the GNPS spectral libraries as birnbaumins A–B, with excellent confidence (cosine score of 0.97 for both). Birnbaumins are structurally intriguing indole derivatives reported as yellow pigments from *Leucocoprinus birnbaumii* (Bartsch *et al.* 2005). The current molecular networks indicate the presence of birnbaumins A–B in the extracts of *L. elaiophylla*, *L. flavonigrescens*, *Lepiota* sp. (SeSa255), and *Lepiota* sp. (SeSa276), but not in the extracts of *L. jacobi*. However, based on the FBMN results, the presence of birnbaumin-like structures in *L. jacobi* seems possible as nodes associated with this species are found in the same cluster. Apparently, birnbaumins are responsible for the yellow colour of some *Lepiota* species. Moreover, it appears that in addition to these compounds, many unknown derivatives of birnbaumins are present in *Lepiota* species.

Chemotaxonomic approaches to distinguish *L. rubella* and *L. bettinae*

Cluster C14 comprises nodes that are restricted to section *Helveolae* and some nodes referring to section *Stenosporae*. This cluster is split into two subclusters, corresponding to *L. elaiophylla* and *Lepiota* sp. (SeSa276) and to *L. rubella* and *L. subincarnata*.

The systematic position of *L. rubella* is controversial, since Dörfelt (1982) described a morphologically similar species by a different name, *L. bettinae*, and Bon (1993) assigned it to the genus *Echinoderma*. However, Vellinga *et al.* (1998) demonstrated that *L. bettinae* and *L. rubella* are synonyms and questioned the assignment of this species to the genus *Echinoderma* by Bon (1993). Based on morphological characteristics and the multilocus phylogeny presented in the context of the current study, the position of *L. rubella* is evidently within section *Helveolae* and not in *Echinoderma*. However, it was not possible to obtain sequence data from the type specimen of *L. bettinae* conserved in HAL and it was not possible to obtain material of *L. rubella* conserved in S. Further chemotaxonomic investigations of authentic material of *L. bettinae* and *L. rubella* could help, in addition to sequence data, to support the synonymy of *L. bettinae* and *L. rubella*.

The second subcluster of cluster C14 is mainly restricted to *L. elaiophylla* and *Lepiota* sp. (SeSa276). *Lepiota elaiophylla* and *Lepiota* sp. (SeSa276) belong to a group of species with yellow basidiomata within section *Helveolae*. However, despite of good TIMA final scores of 0.4 to 0.5 in both subclusters, the corresponding annotations are hardly convincing. It should be noted that the few nodes with a correct degree of spectrometric match refer to sesquiterpene lactones in this group, but these have no chemotaxonomic support, and the good overall score is related to multiple nodes being tagged consistently throughout the cluster.

Chemotaxonomy of section *Eriophorae*

Some further clusters were restricted to section *Eriophorae* but had no link with the genus *Echinoderma*. However, *E. asperum* was the only representative of the genus in our study. To obtain accurate conclusions about the chemotaxonomic differences between *Echinoderma* and section *Eriophorae*, a larger sampling size is required.

Two of these clusters, C3 and C9, were dominated by *L. minutoflava* with a large number of exclusive nodes. Phylogenetically and morphologically, *L. omninoflava* is most closely related to *L. minutoflava*, however, no chemical investigations were performed for *L. omninoflava* (Hou & Ge 2020). In this context, the cluster C9 may be of chemotaxonomic interest. The occurrence of a DEREPLICATOR-tagged node in this cluster, dereplicated as a destruxin analogue indicates the likely peptide constitution of the compounds. TIMA annotated some nodes of this cluster as diverse peptides with low confidence. To investigate the chemotaxonomic differences of *L. minutoflava* and *L. omninoflava*, further investigations of *L. omninoflava* are necessary. Thus, phylogenetically and morphologically similar species described in the future, may be distinguished based on chemotaxonomic results in addition to morphology and sequence data.

Cluster C12 is also mainly restricted to section *Eriophorae* and dominated by compounds obtained from *L. jacobi*, but none of the corresponding nodes could be annotated. DEREPLICATOR did not result in a single hit, which suggests that these compounds are not peptidic in nature. The MS/MS features in this cluster did not lead to good spectrometric matches against ISDB (initial score constantly below 0.03), precluding any attempt to assign a chemical class to these compounds. Many of these results reveal a spectral similarity score of zero. Some nodes were individually assigned to various structural groups when using TIMA comprising lanostanes, limonoids, or prenaphthoquinones dehydrodimers. In this context, a provisional assignment is nothing more than a query based on MS1 (i.e. the exact mass of the precursor) that may yield a few results with some degree of chemotaxonomic similarity, but a low overall confidence. This could pave the way for subsequent mycochemical investigations geared towards the isolation of these molecules of possible chemotaxonomic interest to distinguish *L. jacobi* from the morphologically similar species *L. echinacea* and *L. echinobispora*. The limited material of *L. echinacea* available did not allow a UHPLC-HRMS² analysis here.

Cluster C13 appears to be mostly constricted to compounds from species of section *Eriophorae* with additions from sections *Lilaceae*, *Stenosporae*, and section *Helveolae*. It is intriguing to note that DEREPLICATOR provisionally annotated five nodes of this cluster as cyclic depsipeptides corresponding to four putative massetolides and a pseudodesmine A derivative. These five compounds are antimicrobial cyclic depsipeptides with potent surfactant properties, produced by *Pseudomonas* species (Gerard *et al.* 1997, De Bruijn *et al.* 2008). These tentative annotations were supported by TIMA that also proposed some nodes to correspond to cyclic depsipeptides with spectrometric scores with values near 0.15 for some of them. The taxonomic distance between the *Lepiota* species studied here and the known massetolide producers (*Pseudomonas* bacteria) prevents a high final TIMA score from being achieved, due to poor chemotaxonomic consistency. Further investigations of the respective species are thus necessary on a larger scale to analyse whether *Lepiota* species can synthesize these natural compounds, which have so far only been known from bacteria, or whether it is a matter of contamination by bacteria, considering that there are bacteria that grow in and on fungal basidiomata or in symbiosis with fungi (Bonfante & Desirò 2017).

Chemotaxonomy of section *Lilaceae*

Some clusters were restricted to section *Lilaceae* and could help to resolve the polyphyletic position of this section divided into two clades. Four of five clusters, C4, C7, C8, and C10, are restricted to section *Christatae* and one cluster, C11, is restricted to section *Lilaceae*. The molecular network annotated using the TIMA biological score indicated poor overall relevance for the different hits. However, some entries performed slightly better, particularly those derived from fungi. Such nodes appear to have been tagged as chlorophyll derivatives reported from lichenized ascomycetes so that the biological relevance of such hits is probably lower than expected (Xu *et al.* 2017).

Cluster C11 has a good final score, due to one feature dereplicated as a metabolite obtained from a fungus-infected plant, namely makomotin B, but with a low corresponding spectrometric score, making this attribution very tentative (Choi *et al.* 2014).

Clusters referring to section *Lilaceae* are mainly restricted to single species and do not reflect the relationship of the two clades of section *Lilaceae*. These clusters might be used at species level as chemotaxonomic characteristics but not at section level. Hence, more species of section *Lilaceae* need to be investigated by UHPLC-HRMS² to obtain more information on possible groups of natural compounds restricted to the two clades of this section.

Application of DEREPLICATOR and TIMA

This study is one of the first to apply different in silico tools, namely DEREPLICATOR and TIMA, to the annotation of tandem mass spectrometry data from samples of *Basidiomycota*. As might be expected, the number of annotations obtained by data in GNPS spectral repositories was low, due to the low number of fungal metabolites uploaded to them. Therefore, annotation coverage has been extended using DEREPLICATOR and TIMA. It is difficult to evaluate the quality of the information retrieved by these tools, as it is hardly possible to compare these annotations with the real compounds, which would require isolating them and elucidating their structure.

The present study, however, reveals interesting information for some specific features. Some amatoxins were annotated using both DEREPLICATOR and TIMA workflows, with this latter reaching the commonly admitted cosine threshold of 0.2 against the MS/MS spectra comprised in the ISDB database. The final TIMA score of 0.48 for these features, while convincing, is an underestimate, as LOTUS does not list any *Lepiota* species as amatoxin producers.

Birnbaumins A and B could be annotated with very high confidence by comparison with the experimental MS/MS spectrum comprised in the GNPS spectral libraries. These specific features confirm the value of TIMA-derived annotations, as both were suggested as the preferred candidate with good confidence (spectrometric scores against ISDB MS/MS spectra = 0.26 and 0.24 and final TIMA scores = 0.55 and 0.54, respectively).

In some cases (e.g. massetolide-type cyclic depsipeptides), similar annotations have been proposed by both DEREPLICATOR and TIMA, which reinforces confidence in a given putative annotation. In other cases, hypotheses are presented about the possible nature of the compounds present in a given cluster, even if the structural accuracy is often limited.

Overall, these elements should encourage the use of TIMA even when annotating MS/MS data from organisms for which there is currently poor chemical information in LOTUS.

CONCLUSION

During 22 days of fieldwork in the small West African country of Benin, an immense diversity of fungi was discovered, with 96 % of the species of *Lepiota* being new to science. For each species, an overwhelming diversity of natural compounds was observed, with most of them being still unknown to science. Tropical Africa has great potential, not only in terms of species diversity but also in terms of the diversity of natural compounds. This study only scratches the surface of this great diversity and there are still many hidden gems to be discovered. We recommend using a chemotaxonomical approach to deal with this challenge, as this is most likely to yield scientifically sound results. Therefore, taxonomy and natural compound chemistry must go hand in hand in the future.

Preliminary key to *Lepiota* species known for Benin

1. Basidiomata yellow, turning black when dry .... *L. flavonigrescens*
1. Basidiomata brown, orange, olive-brown, orange-brown, red-brown, purple or white 2

2. Basidiomata purple with spurred spores .... *L. tyrianthina*
2. Basidiomata brown, orange, olive-brown, orange-brown, red-brown or white, with amygdaliform to fusiform, ellipsoid to ovoid, or spurred spores. 3

3. Spores non-dextrinoid and ellipsoid to ovoid 4
3. Spores dextrinoid and amygdaliform to fusiform, ellipsoid to ovoid, or spurred 6

4. Pileus covering a trichoderm; basidiomata brown, red-brown to pink-brown, with annulus .... *L. pseudovenenosa*
4. Pileus covering a hymeniderm or made up of chains of (sub)spherical cells; basidiomata white, or white with purple to black or greyish brown pileus centre. 5

5. Basidiomata white with purple to black or greyish brown pileus centre and under side of the annulus; pileus covering a hymeniderm .... *L. lilaceostriata*
5. Basidiomata completely white; pileus covering made up of chains of (sub)spherical cells .... *L. albogranulosa*

6. Spores ellipsoid to ovoid 7
6. Spores amygdaliform, fusiform, spurred, or penguin-shaped in side view 9

7. Pileus covering a trichoderm .... *L. minutisterigmata*
7. Pileus covering made up of chains of (sub)spherical or elongated cells 8

8. Basidiomata orange-yellow, with yellow to orange-yellow lamellae, with minuscule warts on pileus .... *L. minutoflava*
8. Basidiomata brown, with white lamellae, with conical warts on pileus .... *L. echinobispora*

9. Spores spurred or amygdaliform in side view 10
9. Spores fusiform or penguin-shaped in side view; basidiomata white, with light brown to brown pileus centre; stipe woolly .... *L. woehnertii*

10. Basidiomata with brown to olive-brown or somewhat greenish colours .... *L. brunneoolivacea*
10. Basidiomata with orange to orange-brown colours 11

11. Basidia with up to 20 µm long sterigmata .... *L. longisterigmata*
11. Basidia with up to 6 µm long sterigmata 12

12. Stipe towards apex yellow .... *L. flavostipitata*
12. Stipe towards apex white to cream........ *L. aurantiicolor*

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SUPPLEMENTARY MATERIAL

Fig. S1. Maximum-Likelihood phylogram mapping the phylogenetic relationships of *Lepiota* species and the outgroup based on ITS sequences. Maximum-Likelihood bootstrap values (> 70 %) are shown on the branches. The geographic origin of each specimen that yielded a sequence is indicated by two-letter codes according to ISO 3166. Sequences of Beninese *Lepiota* species are highlighted in **bold**. — Scale bar = estimated changes per nucleotide.

Fig. S2. Maximum-Likelihood phylogram mapping the phylogenetic relationships of *Lepiota* species and the outgroup based on LSU sequences. Maximum-Likelihood bootstrap values (> 70 %) are shown on the branches. The geographic origin of each specimen that yielded a sequence is indicated by two-letter codes according to ISO 3166. Sequences of Beninese *Lepiota* species are highlighted in **bold**. — Scale bar = estimated changes per nucleotide.

Fig. S3. Maximum-Likelihood phylogram mapping the phylogenetic relationships of *Lepiota* species and the outgroup based on *RPB2* sequences. Maximum-Likelihood bootstrap values (> 70 %) are shown on the branches. The geographic origin of each specimen that yielded a sequence is indicated by two-letter codes according to ISO 3166. Sequences of Beninese *Lepiota* species are highlighted in **bold**. — Scale bar = estimated changes per nucleotide.

Fig. S4. Maximum-Likelihood phylogram mapping the phylogenetic relationships of *Lepiota* species and the outgroup based on concatenated alignments of ITS and *RPB2*. Maximum-Likelihood bootstrap values (> 70 %) are shown on the branches. The geographic origin of each specimen that yielded a sequence is indicated by two-letter codes according to ISO 3166. Sequences of Beninese *Lepiota* species are highlighted in **bold**. — Scale bar = estimated changes per nucleotide.