



## *Mallochybe africana* (Inocybaceae, Fungi), the first species of *Mallochybe* described from Africa

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### Abstract

The family Inocybaceae has been poorly studied in Africa. Here we describe the first species of the genus *Mallochybe* from West African and Zambian woodlands dominated by ectomycorrhizal trees of Fabaceae and Phyllanthaceae. The new species *M. africana* is characterized by orange-brown fruitbodies, a fibrillose pileus, a stipe tapered towards the base and large ellipsoid basidiospores. It resembles many north and south temperate species of *Mallochybe* but is most closely related to the southeast Asian tropical species, *M. errata*. *M. africana* is widely distributed in West Africa (Benin, Togo, Burkina Faso and Ivory Coast) extending to South-eastern Africa in Zambia. Phylogenetic analyses based on 5.8S rDNA, nLSU and RPB2 sequence data confirm that *M. africana* is nested within *Mallochybe*. A complete morphological description and illustrations, including photographs and line drawings, are presented.

**Keywords:** African woodland, Agaricales, morphology, systematics, taxonomy

### Introduction

Systematic studies of macrofungi in West Africa are increasing. However, despite taxonomic progress in some groups documented from West Africa during the last twenty years (Bâ *et al.* 2013, Maba *et al.* 2014, 2015, Rooij & Verbeken 2003, Yorou *et al.* 2011, Yorou & Agerer 2008) the tropical African ecozone, and particularly West Africa, remain poorly explored. In Soudano-Guinean forest ecosystems the family Inocybaceae Jülich (1982: 374) is among the least studied of the agaric fungi. Aside from some recent studies conducted in Africa (Matheny & Bougher 2006, Matheny *et al.* 2017) most identifications of African species of Inocybaceae (Buyck & Eyssartier 1999, Watling 2001) have been made based on morphology using literature from Europe. It is highly likely that Inocybaceae species from Africa differ from those of other continents and many collections are probably misidentified. The lack of taxonomic studies on the diversity of Inocybaceae in Africa has probably led to it being underestimated (Buyck & Eyssartier 1999, Hennings 1901, Watling 2001), therefore, it is essential to increase sampling efforts to identify and describe species of Inocybaceae in African ecosystems.

*Mallochybe* (Kuyper) Matheny, Vizzini & Esteve-Rav (2020:12) was first described as a subgenus of *Inocybe* (Fr.) Fr. (1863:346) but recently Matheny *et al.* (2020) elevated *Mallochybe* to one of seven genera in Inocybaceae. The genus is a monophyletic group of some 55 species (Matheny *et al.* 2020) distributed over much of the globe but predominantly occurs in north and south temperate regions and is not well-known outside of Europe and North

America (Matheny *et al.* 2009, 2020). A species of *Mallocybe* was recorded from Sub-Saharan Africa, but it remains undescribed (Matheny *et al.* 2009, 2020).

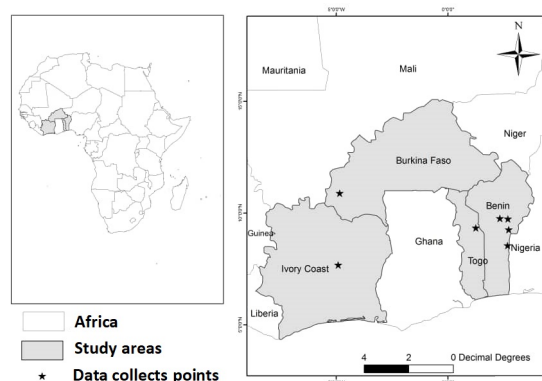
Species of *Mallocybe* are generally characterized by coarsely fibrillose, tomentose, or squamulose basidiomata, a dark reaction to weak alkaline solutions like 5% potassium hydroxide, the presence of necropigmented basidia, absence of pleurocystidia, and cheilocystidia present as, often short (<50 µm long), terminal elements of hyphae of the hymenophoral trama (Cripps *et al.* 2010, Jacobsson 2008, Kuyper 1986, Matheny *et al.* 2020).

In this paper we present the first species of *Mallocybe* described from the African continent. Morphological and multigene molecular analysis of 5.8S rDNA, nLSU and RPB2 sequences data support *Mallocybe africana* as a species new to science.

## Material and methods

### *Study area and specimen sampling*

Specimens were collected between 2013 and 2017 in Benin, Burkina Faso, Ivory Coast and Togo in woodland dominated by ectomycorrhizal trees such as *Isoberlinia doka* Craib & Stapf (1912: 94), *Isoberlinia tomentosa* Hutch. (1928: 203), *Uapaca togoensis* Pax (1904: 371) and *Berlinia grandiflora* Hutch. & Dalziel (1928: 398). Specimens were dried with an electric dryer (type Stöckli Dörrex) for 24 hours at 45° C. All studied materials, including the holotype, are deposited at the Mycological herbarium of Parakou University (UNIPAR).



**FIGURE 1.** Study area.

### *Morpho-anatomical analyses*

Specimens were photographed with a Sony FE digital camera. Color codes were described according to Kornerup & Wanscher (1978). Samples of the dried specimens were rehydrated and examined in 3% KOH and Congo Red. Drawings of microscopic characters were made with the aid of a drawing tube attached to a Leica DM2700. Microscopic characters were drawn at 1000× magnification. For each microscopic element observed, 40 measurements were made from three samples from three collections. We measured length (L) and width (W) of the basidiospores and calculated the ratio  $Q = L/W$ . Measurements of basidiospores and basidia excluded the apiculus and sterigmata respectively. Spore measurements are given as (a)–b–c(–d), where (a) = extreme minimum value, range b–c contains the minimum of 90% of the calculated values and (d) = extreme maximum value, as indicated by Jabeen & Khalid (2020).

### *DNA extraction, PCR and sequencing*

Genomic DNA was extracted from dried specimens using a QIAGEN® plant mini kit. Three nuclear gene regions, internal transcribed spacer (ITS), portions of the large subunit ribosomal RNA gene (nLSU) and RNA polymerase II subunit (RPB2), were amplified using the following primers: ITS1F and ITS4 for ITS (Gardes & Bruns 1993, White *et al.* 1990), LR0R, LR7, LR5 and LR3R for nLSU (Cubeta *et al.* 1991, Rehner & Samuels 1995, Vilgalys & Hester 1990) and bRPB2-6F, bRPB2-7.1R for the most variable region of RPB2 (Matheny 2005). PCR products were cleaned and sequenced at Macrogen Inc. (Macrogen Europe B.V., Amsterdam, Netherlands) using the same primers as those used for PCR.

## Sequence alignment and phylogenetic analysis

We generated fourteen new sequences that have been submitted to GenBank (Table 1), but the accession numbers MT458693, MT509392, MT458692 and MT476161 are not presented in Table 1 because they contain only short fragments of ITS or LSU and were excluded from the phylogenetic analysis. The new sequences were subjected to a BLAST search and relevant related sequences retrieved from GenBank (Benson *et al.* 2010). These ITS, LSU and RPB2 sequences were aligned separately using MAFFT V7.464 (Katoh *et al.* 2019). The ITS1 and ITS2 regions of the internal transcribed spacer (ITS) contained highly variable sites, which were difficult to align correctly and were excluded. For phylogenetic analysis, we used 5.8S rDNA, LSU and RPB2. Thus, the 5.8S rDNA partition included 29 taxa with 156 sites, the LSU partition included 49 taxa with 1524 sites and RPB2 included 27 taxa with 772 sites. The final concatenated data set of 5.8S rDNA, LSU and RPB2 was generated using Geneious 7.0.2 (Kearse *et al.* 2012) and included 49 taxa and 2452 sites.

The dataset was partitioned in 5.8S rDNA, LSU, RPB2 codon position 1, RPB2 codon position 2, RPB2 codon position 3 and the intron in RPB2 separately. We tested for the best partitioning scheme and best model for each partition using ModelFinder (Kalyaanamoorthy *et al.* 2017). It indicated that keeping all the partitions was the best. Maximum Likelihood (ML) analysis was performed with IQTREE 1.6.12 (Nguyen *et al.* 2015). Bootstrap support was assessed with 1000 replicates of ultrafast bootstrap resamplings (Hoang *et al.* 2017). Sequences from *Tubariomyces sp.* BB6018, *T. hygrophoroides* Esteve-Rav., P.-A. Moreau & C.E. Hermos (2010: 1394), *T. inexpectatus* (M. Villarreal, Esteve-Rav., Heykoop & E. Horak) Esteve-Rav. & Matheny (2010: 1390) and *T. similis* Della Magg., Tolaini & Vizzini (2013: 377) were used as outgroup taxa based on Matheny *et al.* (2020).

For Bayesian Inference (BI) analyses, GTR models with gamma distributed rate heterogeneity and a proportion of invariant sites parameter were assigned to each partition as indicated above, using MrBayes 3.2.7 (Ronquist *et al.* 2012), set as follows: lset applyto = (all), nst = 6, rates = invgamma, ngammacat = 4, sampling frequency = 1000, and the command “unlink” was used to unlink parameters across characters on partitioned datasets. Two independent Markov Chain Monte Carlo (MCMC) processes were executed, each in 4 chains for 20 million generations. Posterior probabilities (BPP) were calculated after burning the first 25% of the posterior sample and ensuring that this threshold met the convergence factors described above.

**TABLE 1.** List of taxa used in the molecular analyses along with vouchers, accession numbers and geographic origin. The new species is in bold.

Species	Voucher	Country	ITS	LSU	RPB2	References
<i>Mallocybe africana</i>	Aignon, Yorou & Ryberg	Benin		MK908842		Unpublished
	<b>HLA0462</b>	<b>Benin</b>	<b>MT458691</b>	<b>MT456364</b>		<b>This study</b>
	<b>MR00369</b>	<b>Burkina Faso</b>	<b>MT476162</b>	<b>MT509361</b>		
	<b>MR00385</b>	<b>Togo</b>	<b>MN096194</b>	<b>MN097886</b>	<b>MT465593</b>	
	<b>MR00358</b>	<b>Benin</b>	<b>MT476160</b>	<b>MT509360</b>	<b>MT628398</b>	
	PC 96204	Zambia		EU569871		Matheny <i>et al.</i> 2009
	PC:0088767	Zambia	MN178510	MN178542		Unpublished
<i>M. agardhii</i> (N. Lund)	Matheny & Esteve-Rav.	Finland		AY380365	AY333772	Matheny 2005
<i>M. althoffiae</i> (E. Horak)	Matheny & Esteve-Rav.	Papua New Guinea	NR_163748	EU555444		Matheny <i>et al.</i> 2009
<i>M. arenaria</i> (Bon)	Matheny & Esteve-Rav.	France	FN550937	FN550937		Matheny <i>et al.</i> 2019
<i>M. arthrocytis</i> (Kühner)	Matheny & Esteve-Rav.	Norway		AY380394	AY337402	Matheny 2005
<i>M. crassivelata</i>	Ferisin, Bizio, Esteve-Rav., Vizzini & Dovana	Slovenia	MN536812	MN537138		Crous <i>et al.</i> 2020

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TABLE 1. (Continued)

Species	Voucher	Country	ITS	LSU	RPB2	References
<i>M. errata</i> (E. Horak, Matheny & Desjardin)	DED8022			EU569844		Matheny <i>et al.</i> 2009
Haelew	ZT10072	Thailand		GQ892936		Horak <i>et al.</i> 2015
	ZT 9238	India		EU569845		Matheny <i>et al.</i> 2009
	ZT10108	Thailand		GQ892935		Horak <i>et al.</i> 2015
<i>M. fibrillosa</i> (Peck) Matheny & Esteve-Rav.	LVK14390	USA		MN178527	MN203518	Matheny <i>et al.</i> 2019
<i>M. fulvipes</i> (Kühner) Matheny & Esteve-Rav.	EL8307	Sweden	FN550935	FN550935		Cripps <i>et al.</i> 2010
<i>M. fuscomarginata</i> (Kühner) Matheny & Esteve-Rav.	BJ890718	Sweden	GU980656	GU980656		Cripps <i>et al.</i> 2010
<i>M. gymnocarpa</i> (Kühner) Matheny & Esteve-Rav.	SJ980707	Sweden	AM882866	AM882866		Ryberg <i>et al.</i> 2008
<i>M. heimii</i> (Bon) Matheny & Esteve-Rav.	JV 14932F	Italy		AY380379	AY337380	Matheny 2005
<i>M. isabellina</i> (Matheny & Bougher) Matheny & Esteve-Rav.	PERTH:07712758	Australia	MN178501	MN178528	MH618212	Matheny <i>et al.</i> (2019)
<i>M. latispora</i> (Bon) Matheny & Esteve-Rav.	JV19640F	Finland	MN178503	MN178529	MN203520	Matheny <i>et al.</i> 2019
<i>M. leucoblema</i> (Kühner) Matheny & Esteve-Rav.	PBM1522	USA		MN178533	MH577511	Cripps <i>et al.</i> 2010
<i>M. leucoloma</i> (Kühner) Matheny & Esteve-Rav.	CLC1869	USA	GU980618	GU980618		Cripps <i>et al.</i> 2010
<i>M. malenconii</i> (R. Heim) Matheny & Esteve-Rav.	JV5498A	Finland		EU569870	EU569869	Horak <i>et al.</i> 2015
<i>M. multispora</i> (Murrill) Matheny & Esteve-Rav.	CO4248	USA	MN178509	MN178540		Matheny <i>et al.</i> 2019
<i>M. myriadophylla</i> (Vauras & E. Larss.) Matheny & Esteve-Rav.	JV 19652F	Finland		AY700196	AY803751	Matheny <i>et al.</i> 2009
<i>M. pygmaea</i> (J. Favre) Matheny & Esteve-Rav.	EL48-05	Norway	GU980628	GU980628		Cripps <i>et al.</i> 2010
<i>M. pyrhopoda</i> (Matheny & Bougher) Matheny & Esteve-Rav.	PERTH:08557764	Australia	KP308815	KP170986	KM406226	Horak <i>et al.</i> 2015
<i>M. sabulosa</i> (Matheny & Bougher) Matheny & Esteve-Rav.	PERTH:07680775	Australia	KP308823	KP170995	KM406236	Horak <i>et al.</i> 2015
<i>Mallocybe</i> sp.	ADP060305	USA	MN178513	EU600877	EU600876	Matheny <i>et al.</i> 2009
<i>Mallocybe</i> sp.	BK 6-June-97-24	USA		MN178541	AY337408	Matheny 2005
<i>Mallocybe</i> sp.	PBM 1922	USA		MN178543	MN203527	Unpublished
<i>Mallocybe</i> sp.	PBM 2290	USA		EU555446	EU555445	Matheny <i>et al.</i> 2009
<i>M. siciliana</i> (Brugaletta, Consiglio & M. Marchetti) Brugaletta, Consiglio & M. Marchetti	AMB 18274	Italy	MG757417	MG757419		Brugaletta <i>et al.</i> 2018
<i>M. squarrosoannulata</i> (Kühner) Matheny & Esteve-Rav.	SJ84030	Sweden		GU980609		Cripps <i>et al.</i> 2010
<i>M. subdecurrens</i> (Ellis & Everh.) Matheny & Esteve-Rav.	REH10168	USA	MH024850	MH024886	MH577503	Matheny <i>et al.</i> 2020

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TABLE 1. (Continued)

Species	Voucher	Country	ITS	LSU	RPB2	References
<i>M. subflavospora</i> (Matheny & Bougher) Matheny & Esteve-Rav.	E5880	Australia		AY380396	AY337404	Matheny 2005
<i>M. substraminipes</i> (Kühner) Matheny & Esteve-Rav.	CLC1731	USA	GU980603	GU980603		Cripps <i>et al.</i> 2010
<i>M. subtilior</i> (Matheny & Bougher) Matheny & Esteve-Rav.	OKM 24631	Australia		AY380398	AY337406	Matheny 2005
<i>M. terrigena</i> (Fr.) Matheny, Vizzini & Esteve- Rav.	JV 16431	Sweden		AY380401	AY333309	Matheny 2005
<i>M. tomentosula</i> Matheny & Esteve-Rav.	PBM4138	USA	MG773814	MK421969	MH577506	Matheny <i>et al.</i> 2020
<i>M. unicolor</i> (Peck) Matheny & Esteve-Rav.	TENN:06355	USA	MN178525	MN178554	MN203534	Matheny <i>et al.</i> 2019
<i>Tubariomyces</i> sp.	BB6018	Zambia	MK421965	EU600887	EU600886	Matheny <i>et al.</i> 2009
<i>T. hygrophoroides</i> Esteve-Rav., P.-A. Moreau & C.E. Hermos.	P05112008	France	GU907097	GU907094	GU907090	Alvarado <i>et al.</i> 2010
<i>T. inexpectatus</i> (M. Villarreal, Esteve-Rav., Heykoop & E. Horak) Esteve-Rav. & Matheny	AH25500	Spain	GU907095	GU907091	GU907088	Alvarado <i>et al.</i> 2010
<i>T. similis</i> Della Magg., Tolaini & Vizzini	RFS0805	Spain	GU907096	GU907092	GU907089	Alvarado <i>et al.</i> 2010

## Results

### Phylogenetic analyses

The ML and BI phylogenies show the placement of the investigated specimens within *Mallocybe* (Fig 2). The eight African collections clustered together with strong support (96% ML bootstrap, 1 BPP) to form a unique lineage, which we recognize as a new species, *M. africana*. Within *Mallocybe*, the new species *M. africana* formed a subgroup with the southeast Asian tropical species, *M. errata* (E. Horak, Matheny & Desjardin) Haelew (2020: 24), with strong support (99% ML bootstrap, 1 BPP).

## Taxonomy

*Mallocybe africana* Aignon, Yorou & Ryberg *sp. nov.* Figures 3 & 4

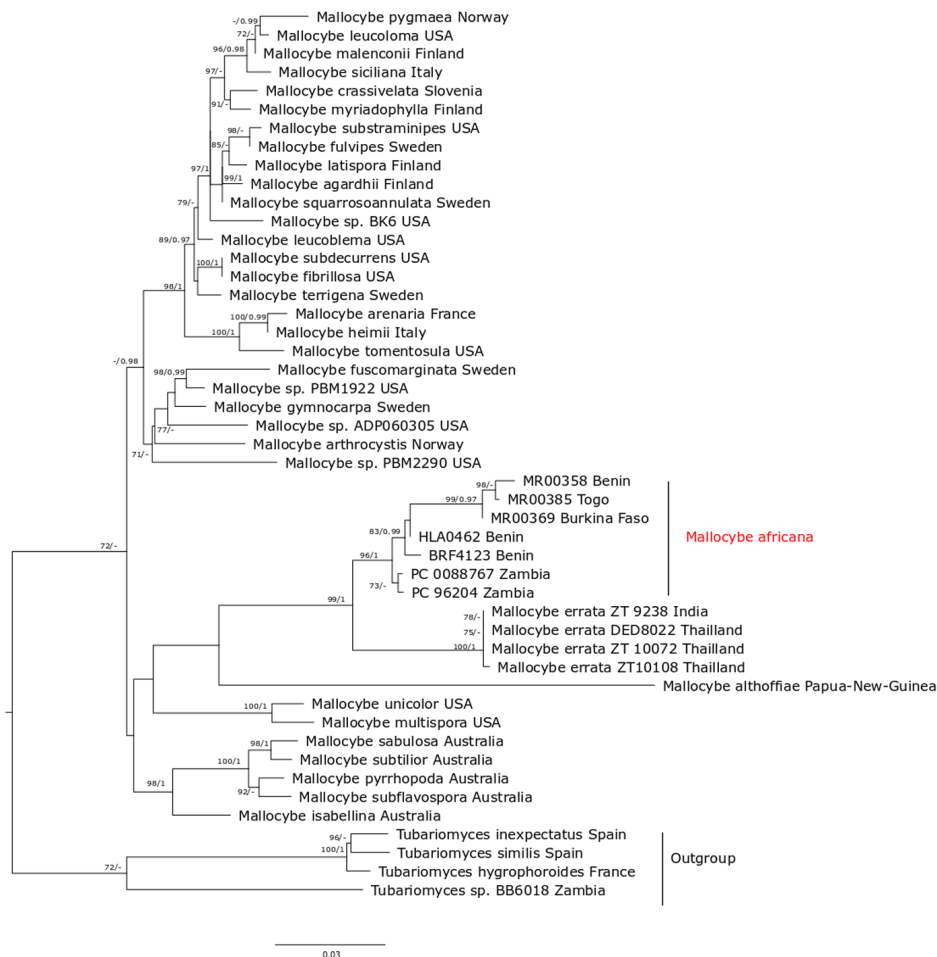
MycoBank No:—835658

Diagnosis:—*Mallocybe africana* is most closely related to *M. errata* from southeast Asia but differs from it by the smaller size of the basidiomata, larger basidiospores, and ecological association with Fabaceae Lindley (1836: 148) and/or Phyllanthaceae Martynov (1820: 369). *Mallocybe errata* is associated with Pinaceae Spreng. ex F. Rudolphi, (1830: 35) and Dipterocarpaceae Blume (1825: 222).

Holotype:—BENIN. Borgou, North region: Village Gando, 09°45'43.8"N, 002°19'56.2"E, 08 July 2013, on soil in woodland dominated by *Isoblerlinia doka* and *I. tomentosa*, *M. Ryberg* (MR00358), deposited in Parakou University, GenBank accession: ITS (MT476160), LSU (MT509360) and RPB2 (MT628398).

Etymology:—*africana*, refers to the distribution in Africa.

Description:—*Pileus* 7–20 mm diam, hemispherical when young, expanding to convex or applanate when mature, margin inflexed, surface dry, fibrillose to tomentose, at times scaly, disc occasionally with a grayish velipellis, otherwise orange-brown (5B4) to brown, sometimes yellowish (5B2) towards the margin when young, flesh yellowish white (4A2), pale brown (4C4) to grayish white, 1–2 mm thick. *Lamellae* adnate, close, with 30–40 L and several tiers of lamellulae, dark yellowish brown to dark brown (6B5), 2–4 mm deep, edges paler and fimbriate. *Stipe* 11–25 × 2–4 mm, tapered towards the base, dry, woolly or felty from veil remnants, becoming coarsely fibrillose or developing appressed fibrillose scales, almost cinnamon to orange-brown (5B4), buff to brown, paler yellow at top, flesh pale yellow to white, becoming hollow.



**FIGURE 2.** ML tree of 5.8S rDNA, LSU and RPB2 sequences showing the placement of *Mallocybe africana*. Values above or below branches indicate bootstrap proportions. BS values  $\geq 70\%$ /Bayesian posterior probabilities  $>0.95$  are shown. Origin of species is given after the name of each taxon. The new species is in red.

*Basidiospores* (8–)8.9–13.5(–14) × (4–)4.5–7.3(–8)  $\mu\text{m}$ ,  $\text{avl} \times \text{avw} = 11.2 \times 5.6 \mu\text{m}$  Q: (1.4–)1.6–2.6(–3),  $\text{avQ} = 2.0$ , ellipsoid, smooth, thick-walled, yellowish brown.

*Basidia* 20–47 × 5–14  $\mu\text{m}$ , usually with 4 sterigmata, cylindric to slenderly clavate, hyaline becoming ochraceous (necropigmented). *Cheilocystidia* (25–42 × 10–25  $\mu\text{m}$ , clavate, some almost pyriform or cylindrical and attenuated, thin-walled, clamped. *Pleurocystidia* absent. *Pileipellis* a cutis of dense layers of branched hyphae, with fusiform terminals, granular internal pigment brown, 5–12  $\mu\text{m}$  wide, hyphae internally incrustated, clamped. *Stipitipellis* a cutis made up of filamentous, branched, septate clamped hyphae 5–15  $\mu\text{m}$  wide, thin- or thick-walled, hyaline. *Caulocystidia* 32–45 × 10–12  $\mu\text{m}$ , cylindric to bacilliform, walls hyaline, scattered at stipe apex, infrequent, clamped.

Habit:—Solitary, scattered or in small clusters on soil.

Habitat:—Woodland dominated by *Isberlinia doka*, *I. tomentosa*, *Uapaca guineensis* Müller Argoviensis. (1864: 517), *U. togoensis* or *Berlinia grandiflora*. Occurring from June to September.

Geographical distribution: West Africa–Benin, Togo, Burkina Faso, Ivory Coast, and in south-eastern Africa–Zambia.

Additional specimens examined: BENIN. Collines, central region: Forest reserve of Toui-Kilibo, 8°33'38.15"N, 002°36'5.44"E, 12 August 2017, on soil in woodland dominated by *Isoberlinia doka*, L.H. Aignon (HLA0378), GenBank accession: ITS (MT458692). Borgou, North region: Forest reserve of N'dali, 09°44'55.73"N, 002°41'40.51"E, 30 August 2017, on soil in woodland dominated by *Isoberlinia doka* and *I. tomentosa*, L.H. Aignon (HLA0462), GenBank accession No.: ITS (MT458691) and nLSU (MT456364). Donga region, Igbere village: Wari- Marou forest reserve, 8°59'36.1" N, 1°58'10.4" E, 30 August 2018, on soil in woodland dominated by *Isoberlinia doka* and *Uapaca togoensis*, L.H. Aignon (HLA0623). BURKINA FASO. Bobo-Dioulasso: Forest reserve of Dan, 10°53'39.7" N, 004°50'17.6" W, 12 July 2013, on soil in woodlands and gallery forests dominated by *Berlinia grandiflora* and *Uapaca guineensis*, M. Ryberg (MR00369), GenBank accession: ITS (MT458691) and nLSU (MT509361). IVORY COAST. Bouake, Gbêkê region: District of Bandama Valley, 7°40'31.4" N, 004°54'29.2" W, 11 July 2018, on soil in woodland dominated by *Berlinia grandifolia*, L.H. Aignon (HLA0561), GenBank accession: ITS (MT476161). TOGO. Central region, prefecture of Assoli: reserve forest of Aledjo, 09°20'25.1" N, 001°14'66.6" E, 17 July 2013, on soil in Woodland dominated by *Isoberlinia tomentosa*, M. Ryberg (MR00385), GenBank accession: ITS (MN096194), LSU (MN097886) and RPB2 (MT465593). ZAMBIA. 6 kilometers before the Gibbon's farm, 17 November 1996 on soil in miombo woodland (Phyllanthaceae, Fabaceae), 08 February 1996, *G. Eyssartier* (EG96012ter!). (Phyllanthaceae, Fabaceae), *G. Eyssartier* (EG96012!). Luanshya-Ibenga road, on soil in miombo woodland, 03 February 1996, *G. Eyssartier* (EG96012bis!). Lusaka, on soil in miombo woodland dominated by species of Phyllanthaceae and Fabaceae.



**FIGURE 3.** A–D= Basidiomes of *Mallocybe africana*, A= MR00358, B= HLA0462 C=HLA0399 and D= HLA0561. Bar = 1 cm. Photos by: H.L. Aignon.

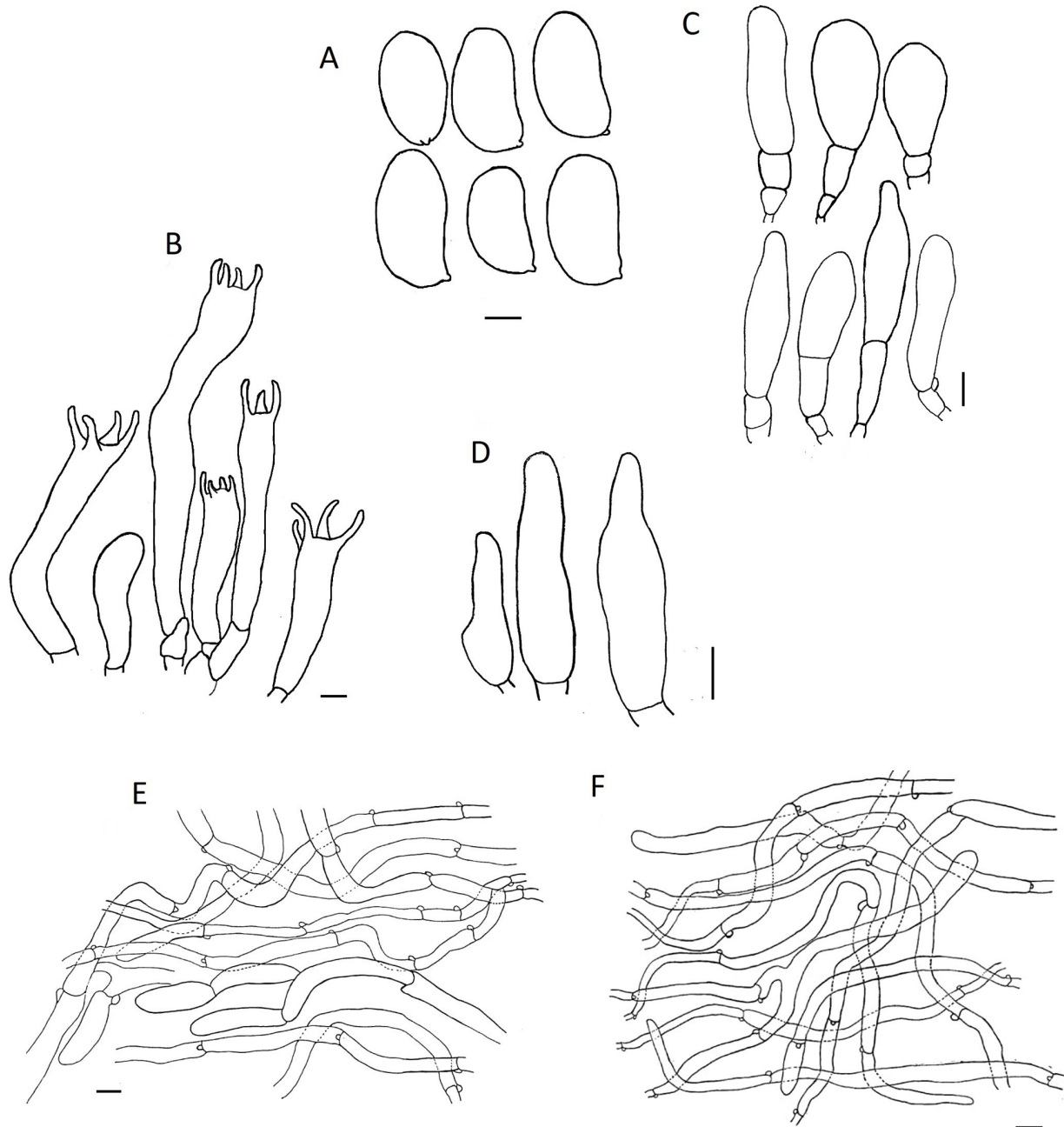
## Discussion

Previously, *M. africana* was provisionally referred to as “*Inocybe microdulcamara*” in Matheny *et al.* (2009) based on collections made by Bart Buyck and Guillaume Eyssartier in Zambia. Here we have detected the same species from other regions of tropical Africa, where it is widely distributed in different habitats including woodlands dominated by Fabaceae (*Isoberlinia doka*, *I. tomentosa* and *Berlinia grandiflora*) and of Phyllanthaceae (*Uapaca togoensis* and *U. guineensis*).

On the basis of our multi-gene (5.8S rDNA, LSU and RPB2) phylogenetic analyses (Fig. 2), *M. africana* is subdivided into two subclades, with separation of the samples from West Africa from those in Zambia. However, the

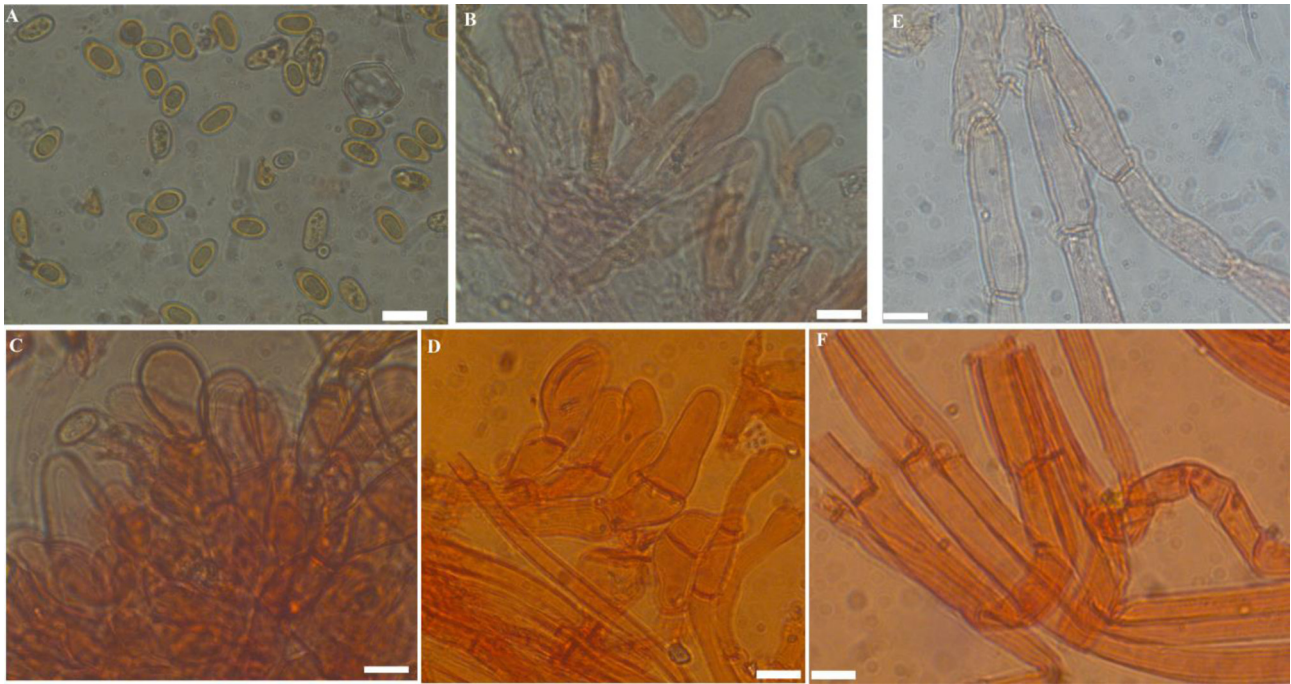
low sequences divergence in ITS and LSU between the clades (0.4% and 0.5% respectively) leads to us conclude that they belong to the same species.

In the phylogenetic tree, *M. africana* is most closely related to *M. errata* with strong bootstrap support. *M. errata* is a species from northwest Thailand and India (Kerala) found in tropical forests mainly dominated by *Pinus kesiya subsp. szemaoensis* Silba (2009:52) and dipterocarp forest dominated by *Dipterocarpus obtusifolius* Teijsm. ex Miq. (1864: 214) reported by Horak *et al.* (2015). The phylogenetic affinities of the Zambian collections of *M. africana* with *M. errata* have long been recognized, and, according to Matheny *et al.* (2009) and Horak *et al.* (2015), both were strongly supported as phylogenetically related to *M. heimii*. However, our phylogeny reveals that *M. africana* and *M. errata* may be more closely related to *M. althoffiae* (E. Horak) Matheny & Esteve-Rav (2020: 105), *M. unicolor* (Peck) Matheny & Esteve-Rav. (2020: 109) and *M. multispora* (Peck) Matheny & Esteve-Rav. (2020: 107).



**FIGURE 4A–F.** Micromorphology of *Mallocybe africana*, **A.** Basidiospores **B.** Basidia **C.** Cheilocystidia **D.** Caulocystidia **E.** Pileipellis **F.** Stipitipellis. Scale bars: **A**=3  $\mu$ m, **B**=5  $\mu$ m, **C, D, E, F** = 10  $\mu$ m. Drawings by: H.L. Aignon.





**FIGURE 5.** A–F. *Mallocybe africana*, microscopical characters in Congo Red (MR00358), **A.** Basidiospores **B.** Basidia **C.** Cheilocystidia **D.** Caulocystidia **E.** Pileipellis **F.** Stiptipellis. Scale bars: A–F = 10  $\mu$ m. Photos By: H.L.Aignon.

*Mallocybe africana* shares some morphological similarities with *M. errata* as both have a brown to orange-brown pileus with radially fibrous, fibrillose squamules or scales (Horak *et al.* 2015). However, *M. africana* differs from *M. errata* by the larger basidiospores (8–14  $\times$  4–8  $\mu$ m for *M. africana* and 8.5–10  $\times$  4.5–5  $\mu$ m for *M. errata*), plant association and geographic distribution. During this study, *M. africana* has been collected from tropical Africa and associated with Fabaceae and Phyllanthaceae species while *M. errata* is distributed in tropical Asia and associates with Dipterocarpaceae and *Pinus* (Horak *et al.* 2015). *Mallocybe africana* appears to be a common and widespread species in African woodland savannas, and its description may therefore potentially resolve the identification of many collections. Additional surveying is needed though, to determine exactly how widespread and common it is.

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## References

- Alvarado, P., Manjón, J.L., Matheny, P.B. & Esteve-Raventós, F. (2010) *Tubariomyces*, a new genus of Inocybaceae from the Mediterranean region. *Mycologia* 102: 1389–1397.  
<https://doi.org/10.3852/10-041>

- Bâ, A.M., Duponnois, R., Diabaté, M., Diédhiou, A.G., Sanon, K., Nérée & Dreyfus, B. (2013) *Diversité des champignons ectomycorhiziens et des ectomycorhizes des arbres forestiers natifs d'Afrique de l'ouest*. IRD. Marseille. pp. 1–105.
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J. & Sayers, E.W. (2010) GenBank. *Nucleic Acids Research* 38: 46–51.  
<https://doi.org/10.1093/nar/gkp1024>.
- Blume, C.L. (1825) *Dipterocarpaceae Blume, Bijdragen tot de Flora van Nederlandsch Indie*. 4. pp. 1–284.  
<https://doi.org/10.5962/bhl.title.6656>
- Brugaletta, E., Consiglio, G. & Marchetti, M. (2018) *Inocybe siciliana*, una nuova specie del Sottogenere *Mallocybe*. *Rivista di Micologia* 60 (3): 195–209.
- Buyck, B. & Eyssartier, G. (1999) Two new species of *Inocybe* (Cortinariaceae) from African woodland. *Kew Bulletin* 54: 675–681.  
<https://doi.org/10.2307/4110863>.
- Craib, W.G. & Stapf, O. (1912) *Isoblerinia doka* Craib & Stapf. *Bulletin of Miscellaneous Information (Royal Gardens, Kew)* 2: 93–94.
- Cripps, C.L., Larsson, E. & Horak, E. (2010) Subgenus *Mallocybe* (*Inocybe*) in the Rocky Mountain alpine zone with molecular reference to European arctic-alpine material. *North American Fungi* 5 (5): 97–126.  
<https://doi.org/10.2509/naf2010.005.0057>
- Cubeta, M., Echandí, E. & Albernethy, T. (1991) Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* 81: 1395–1400.  
<https://doi.org/10.1094/Phyto-81-1395>
- Crous, P.W., Wingfield, M.J., Chooi, Y.-H., Gilchrist, C.L.M., Lacey, E., Pitt, J.I., Roets, F., Swart, W.J., Cano-Lira, J.F., Valenzuela-Lopez, N., Hubka, V., Shivas, R.G., Stchigel, A.M., Holdom, D.G., Jurjević, Ž., Kachalkin, A.V., Lebel, T., Lock, C., Martín, M.P., Tan, Y.P., Tomashevskaya, M.A., Vitelli, J.S., Baseia, I.G., Bhatt, V.K., Brandrud, T.E., De Souza, J.T., Dima, B., Lacey, H.J., Lombard, L., Johnston, P.R., Morte, A., Papp, V., Rodríguez, A., Rodríguez-Andrade, E., Semwal, K.C., Tegart, L., Abad, Z.G., Akulov, A., Alvarado, P., Alves, A., Andrade, J.P., Arenas, F., Asenjo, C., Ballarà, J., Barrett, M.D., Berna, L.M., Berraf-Tebbal, A., Bianchinotti, M.V., Bransgrove, K., Burgess, T.I., Carmo, F.S., Chávez, R., Čmoková, A., Dearnaley, J.D.W., de A. Santiago, A.L.C.M., Freitas-Neto, J.F., Denman, S., Douglas, B., Dovana, F., Eichmeier, A., Esteve-Raventós, F., Farid, A., Fedosova, A.G., Ferisin, G., Ferreira, R.J., Ferrer, A., Figueiredo, C.N., Figueiredo, Y.F., Reinoso-Fuentealba, C.G., Garrido-Benavent, I., Cañete-Gibas, C.F., Gil-Durán, C., Glushakova, A.M., Gonçalves, M.F.M., González, M., Gorczak, M., Gorton, C., Guard, F.E., Guarizo, A.L., Guarro, J., Gutiérrez, M., Hamal, P., Hien, L.T., Hocking, A.D., Houbraken, J., Hunter, G.C., Inácio, C.A., Jourdan, M., Kapitonov, V.I., Kelly, L., Khanh, T.N., Kislo, K., Kiss, L., Kiyashko, A., Kolařík, M., Kruse, J., Kubátová, A., Kučera, V., Kučerová, I., Kušan, I., Lee, H.B., Levicán, G., Lewis, A., Liem, N.V., Liimatainen, K., Lim, H.J., Lyons, M.N., Maciá-Vicente, J.G., Magaña-Dueñas, V., Mahiques, R., Malysheva, E.F., Marbach, P.A.S., Marinho, P., Matočec, N., McTaggart, A.R., Mešić, A., Morin, L., Muñoz-Mohedano, J.M., Navarro-Ródenas, A., Nicolli, C.P., Oliveira, R.L., Otsing, E., Ovrebo, C.L., Pankratov, T.A., Paños, A., Paz-Conde, A., Pérez-Sierra, A., Phosri, C., Pintos, Á., Pošta, A., Prencipe, S., Rubio, E., Saitta, A., Sales, L.S., Sanhueza, L., Shuttleworth, L.A., Smith, J., Smith, M.E., Spadaro, D., Spetik, M., Sochor, M., Sochorová, Z., Sousa, J.O., Suwannasai, N., Tedersoo, L., Thanh, H.M., Thao, L.D., Tkalčec, Z., Vaghefi, N., Venzhik, A.S., Verbeken, A., Vizzini, A., Voyron, S., Wainhouse, M., Whalley, A.J.S., Wrzosek, M., Zapata, M., Zeil-Rolfé, I. & Groenewald, J.Z. (2020) Fungal Planet description sheets: 1042–1111. *Persoonia* 44: 301–459.  
<https://doi.org/10.3767/persoonia.2020.44.11>
- Fries, E.M. (1863) *Monographia Hymenomycetum Sueciae*. C.A. Leffler, Uppsala, pp. 1–355.
- Gardes, M. & Bruns, T. (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2 (2): 113–118.  
<https://doi.org/10.1080/10635150802429642>
- Hennings, pp. (1901) *Fungi camerunenses novi*. III. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 30: 39–57.
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2017) UFB002: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution* 35: 518–522.  
<https://doi.org/10.1093/molbev/msx281>
- Horak, E., Matheny, P.B., Desjardin, D.E. & Soyong, K. (2015) The genus *Inocybe* (Inocybaceae, Agaricales, Basidiomycota) in Thailand and Malaysia. *Phytotaxa* 230: 201–238.  
<https://doi.org/10.11646/phytotaxa.230.3.1>
- Hutchinson, J. & Dalziel, J.M. (1928) *Flora of West Tropical Africa* 1 (2). London. pp. 1–544.
- Hutchinson, J. (1928) *Isoblerinia tomentosa* Hutch. In Notes on the forest flora of South Central Africa. *Bulletin of Miscellaneous Information (Royal Gardens, Kew)* 5: 193–208.  
<https://doi.org/10.2307/4107184>
- Jabeen, S. & Khalid, A.N. (2020) *Pseudosperma flavorimosum* sp. nov. from Pakistan. *Mycotaxon* 135: 183–193.  
<https://doi.org/10.5248/135.183>

- Jacobsson, S. (2008) Key to *Inocybe*. In: Knudsen, H. & Vesterholt, J. (Eds.) *Funga Nordica. Agaricoid, boletoid and cyphelloid genera*. Nordsvamp, Copenhagen. pp. 868–906.
- Jülich, W. (1982) Higher taxa of Basidiomycetes. *Bibliotheca Mycologica* 85: 1–485.
- Kalyanamoothy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermini, L.S. (2017) modelfinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* 14 (6): 587–589.  
<https://doi.org/10.1038/nmeth.4285>
- Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166.  
<https://doi.org/10.1093/bib/bbx108>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, pp. & Drummond, A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.  
<https://doi.org/10.1093/bioinformatics/bts199>
- Kornerup, A. & Wanscher, J.H. (1978) *Methuen handbook of colour*. 3rd ed. E. Methuen, London. pp. 1–252.
- Kuyper, T.W. (1986) A revision of the genus *Inocybe* in Europe I. Subgenus *Inosperma* and the smooth-spored species of subgenus *Inocybe*. *Persoonia* 3: 1–247.
- Lindley, J. (1836) Natural system of botany. Longman, London. pp.1–526.
- Maba, D.L., Guelly, A.K., Yorou, N.S., Kesel, A. De, Verbeken, A. & Agerer, R. (2014) The genus *Lactarius* s. str. (Basidiomycota, Russulales) in Togo (West Africa): phylogeny and a new species described. *IMA Fungus* 5: 39–49.  
<https://doi.org/10.5598/imafungus.2014.05.01.05>
- Maba, D.L., Guelly, A.K., Yorou, N.S., Verbeken, A. & Agerer, R. (2015) Diversity of *Lactifluus* (Basidiomycota, Russulales) in West Africa: 5 new species described and some considerations regarding their distribution and ecology. *Mycosphere* 6 (6): 737–759.  
<https://doi.org/10.5943/mycosphere/6/6/9>
- Martynov, I.I. (1820) Description: VI, In Cyrillic characters. In: *Tekno-Botanical Dictionary*. pp. 1–682.
- Matheny, P.B. (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). *Molecular Phylogenetics and Evolution* 35 (1): 1–20.  
<https://doi.org/10.1016/j.ympev.2004.11.014>
- Matheny, P.B., Aime, M.C., Bougher, N.L., Buyck, B., Desjardin, D.E., Horak, E., Kropp, B.R., Lodge, D.J., Soyong, K., Trappe, J.M. & Hibbett, D.S. (2009) Out of the Palaeotropics? Historical biogeography and diversification of the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. *Journal of Biogeography* 36: 577–592.  
<https://doi.org/10.1111/j.1365-2699.2008.02055.x>
- Matheny, P.B. & Bougher, N.L. (2006) The new genus *Auritella* from Africa and Australia (Inocybaceae, Agaricales): Molecular systematics, taxonomy and historical biogeography. *Mycological Progress* 5: 2–17.  
<https://doi.org/10.1007/s11557-005-0001-8>
- Matheny, P.B., Henkel, T.W., Séné, O., Korotkin, H.B., Dentinger, B.T.M. & Aime, M.C. (2017) New species of *Auritella* (Inocybaceae) from Cameroon, with a worldwide key to the known species. *IMA Fungus* 8: 287–298.  
<https://doi.org/10.5598/imafungus.2017.08.02.06>
- Matheny, P.B., Hobbs, A.M. & Esteve-Raventós, F. (2020) Genera of Inocybaceae: New skin for the old ceremony, *Mycologia* 112 (1): 83–120.  
<https://doi.org/10.1080/00275514.2019.1668906>
- Müller Argoviensis, J. (1864) *Neue Euphorbiaceen des Herbariums Hooker in Kew, auszugsweise vorläufig mitgeteilt aus dem Manuscript für De Candolle's Prodromus Flora* 33: 513–520.
- Nguyen, L.-T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution* 32: 268–274.  
<https://doi.org/10.1093/molbev/msu300>
- Pax, F.A. (1904) *Uapaca togoensis* Pax. In: *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*. pp. 1–741.
- Rehner, S. & Samuels, G. (1995) Molecular Systematics of the Hypocreales: a teleomorph gene phylogeny and the status of their anamorph. *Canadian Journal of Botany* 73: 816–823.  
<https://doi.org/10.1139/b95-327>
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.  
<https://doi.org/10.1093/sysbio/sys029>

- Rooij, P. Van & Verbeke, A. (2003) Studies in tropical African *Lactarius* species (Russulales, Basidiomycota) 11. Records from Benin. *Nova Hedwigia* 77 (1–2): 221–251.
- Ryberg, M., Nilsson, R.H., Kristiansson, E., Töpel, M., Jacobsson, S. & Larsson, E. (2008) Mining metadata from unidentified ITS sequences in GenBank: A case study in *Inocybe* (Basidiomycota). *BMC Evolutionary Biology* 8: 1–14.  
<https://doi.org/10.1186/1471-2148-8-50>
- Saba, M., Haelewaters, D., Pfister, D.H. & Khalid, A.N. (2020) New species of *Pseudosperma* (Agaricales, Inocybaceae) from Pakistan revealed by morphology and multi-locus phylogenetic reconstruction. *MycKeys* 69: 1–31.  
<https://doi.org/10.3897/mycokeys.69.33563>
- Silba, J. (2009) *Pinus kesiya* subsp. *szemaoensis* Silba, Revised version. *Journal of the International Conifer Preservation Society* 16 (1): 45–56.
- Rudolphi, F.K.L. (1830) *Systema orbis vegetabilium*. Kunike, Greifswald, pp. 1–80.
- Teijsm, S. (1864) *Dipterocarpus obtusifolius* Teijsm. ex Miq. *Annales Musei Botanici, Lugduno-Batavi* 1: 161–331.
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.  
<https://doi.org/10.1128/JB.172.8.4238-4246.1990>
- Vizzini, A., Maggiora, M.D., Tolaini, F. & Ercole, E. (2013) A new cryptic species in the genus *Tubariomyces* (Inocybaceae, Agaricales). *Mycological Progress* 12: 375–381.  
<https://doi.org/10.1007/s11557-012-0843-9>
- Watling, R. (2001) An unusual *Inocybe* sp. from West Africa. *Czech Mycology* 52: 329–334.  
<https://doi.org/10.33585/cmy.52413>
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) PCR protocols: a guide to methods and applications*. New York: Academic Press. pp. 315–322.  
<https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Yorou, N.S. & Agerer, R. (2008) *Tomentella africana*, a new species from Benin (West Africa) identified by morphological and molecular data. *Mycologia* 100: 68–80.  
<https://doi.org/10.1080/15572536.2008.11832499>
- Yorou, N.S., Diabaté, M. & Agerer, R. (2011) Phylogenetic placement and anatomical characterization of two new West African *Tomentella* (Basidiomycota, Fungi) species. *Mycological Progress* 11: 171–180.  
<https://doi.org/10.1007/s11557-011-0739-0>