

***MORGANELLA FIMBRIATA* (LYCOPERDACEAE, AGARICOMYCETES), A NEW SPECIES FROM CAMEROON**

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Morganella fimbriata, a new puffball species, is described. The main diagnostic features are: cellular subgleba, exoperidium with spines which leave an areolate pattern on endoperidium after they fall off, and fimbriate stoma. Comparison with the morphologically closed species as well as ITS rDNA phylogenetic tree are presented.

Keywords: Agaricaceae, biodiversity, fungi, gasteromycetes, ITS nrDNA, *Lycoperdon*, molecular genetic identification, *Morganella*, puffballs

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INTRODUCTION

The genus *Morganella* was described by Zeller with the one species *Morganella mexicana* Zeller (1948). Later *Morganella* was amended by Kreisel and Dring (1967), with segregation several species from *Lycoperdon* Pers. The main distinguishing features are: small basidiomes with compact or chambered subgleba (perhaps lacking in some species), without a diaphragm; mature gleba without capillitium but with abundant paracapillitium; spores globose to broadly ovoid, verruculose to spinulose. The type species is *M. mexicana*, a synonym of *Lycoperdon fuligineum* Berk. et M.A. Curtis (Kreisel, Dring, 1967). Most of the species of *Morganella* are growing on dead wood, except *Morganella stercoraria* P. Ponce de León described as growing on cow dung and *M. arenicola* Alfredo et Baseia often found on sandy soil. The *Morganella* species are distributed mostly in tropical and subtropical regions. According to the Index Fungorum database (2022) there are approximately 20 species are known without synonyms.

Lycoperdon sulcatostomum (C.R. Alves et Cortez) Baseia, Alfredo et M.P. Martín described as *Morganella sulcatostoma* C.R. Alves et Cortez has strike features such as sulcate peristome, exoperidium detaching as large plates in maturity like in *Lycoperdon marginatum* Vittad. (Alves, Cortez, 2013). As pointed in Alfredo et al. (2017), the capillitium is present together with the paracapillitium. On the phylogenetic tree this species did not group in any of the subgenera of *Lycoperdon* s.l., described by Larsson and Jeppson (2008) and probably is a member of undescribed genus.

Using the morphological and molecular genetics methods new subgenus *Apioperdon* was described in *Morganella* (Krüger, Kreisel, 2003). *L. pyriforme* Schaeff. has been transferred from the genus *Lycoperdon* as a type species and new combination *Morganella*

pyriformis (Schaeff.) Kreisel et D. Krüger was proposed. But this species is characterized by the presence of non-pitted capillitium along with paracapillitium and a number of other morphological features that distinguish it from *Morganella*. It was shown that *Lycoperdon pyriforme* has isolated position in *Lycoperdon* on *Lycoperdaceae* phylogenetic tree (Larsson, Jeppson, 2008; Bates et al., 2009). Based on this facts *Apioperdon* delimited as independent genus (Vizzini, Ercole, 2017).

Phylogenetic analysis based on ITS and LSU nrDNA sequences showed that *Morganella* species are formed a weakly supported subclade in a *Lycoperdon* s.l. clade (Larsson, Jeppson, 2008; Bates et al., 2009; Alfredo et al., 2017). Taking a broad concept of the genus *Lycoperdon*, the *Morganella* (as well as *Apioperdon*, *Bovistella*, *Vascellum*) were accepted as subgenera in *Lycoperdon* s.l. (Larsson, Jeppson, 2008). According to modern views, it is possible to restore genus *Morganella* as independent genus (Wijayawardene et al., 2020).

In the course of the exchange of gasteroid basidiomycetes specimens between the Fungarium of the Royal Botanic Gardens Kew (K) and the Yu. Rebriev personal collection, several specimens were obtained. One of them was collected by P.J. Roberts in Korup National Park (Cameroon) and primarily labelled as *L. fuligineum* (cf.) Berk. et M.A. Curtis. On the basis of absence of the capillitium and lignicolous habitat this specimen was identified by me as *Morganella* sp. *L. fuligineum* is a synonym of *Morganella fuliginea* (Berk. et M.A. Curtis) Kreisel et Dring and characterized by very small or almost lacking compact subgleba and spinulose basidiospores (Kreisel, Dring, 1967) while our specimen has cellular subgleba and finely verruculose basidiospores. Using the phylogenetic analysis, it was shown that the ITS original sequence is quite far from those available in the GenBank database.

The combination of morphological and molecular data made it possible to conclude that the studied specimen belongs to a new to science species.

MATERIALS AND METHODS

Morphology. The type material is deposited in the Komarov Botanical Institute of the Russian Academy of Sciences herbarium (LE). The examination of microstructures under the light microscope Mikmed-6 was made after boiling for a short time in lactophenol cotton blue. Scanning electronic microphotographs were taken using the Carl Zeiss EVO-40 XVP in the South Science Center of the Russian Academy of Sciences Center of Collective Use (no. 501994). Microscopic measurements were made by means of the specialized program Scandium 5.0. Light microphotographs as well as fruitbody photographs were taken using equipment of the Core Facility Center "Cell and Molecular Technologies in Plant Science" at the Komarov Botanical Institute of the Russian Academy of Sciences (BIN RAS).

Phylogenetic analysis. Genomic DNAs were isolated using CTAB method (Doyle, Doyle, 1987). ITS regions were PCR amplified and sequenced using primer pair ITS1F and ITS4 (White et al., 1990; Gardes, Bruns, 1993). The programs used for PCR amplifications are as following: pre-denaturation at 94°C for 5 min, then followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 50 s and elongation at 72°C for 50 s, followed by a final elongation at 72°C for 8 min. PCR products were cleaned and sequenced in the BIN RAS.

50 ITS sequences, including one newly generated from type specimen of *Morganella fimbriata*, were used (Table 1). *Disciseda* spp. were chosen as the outgroup taxa. All ITS sequences were aligned using the MAFFT on-line server, setting the Q-INS-i strategy (Katoh et al., 2019), and manually adjusted with MEGA 11 (Tamura et al., 2021). Phylogenetic differences were measured using BLAST (2022).

The ITS rDNA phylogenetic tree (Fig. 1) was obtained using MrBayes v. 3.2.1 (Ronquist et al., 2012) using the GTR + I + G model with 1.5 M. The best tree was visualized in FigTree v. 1.2.3 and edited in Adobe Illustrator CS5 (Fig. 1).

RESULTS

Our molecular data based on ITS sequences (Fig. 1) showed that the genus *Morganella* formed own clade (PP = 0.96). These data are inconsistent with previous phylogenetic reconstructions of the *Lycoperdaceae* (Larsson, Jeppson, 2008; Alfredo et al., 2017; Rebriev et al., 2020).

Taxonomy

Morganella fimbriata Rebriev sp. nov. (Fig. 2).

MycoBank no.: MB 844813

Diagnosis. Stoma dentate-fimbriate; exoperidium of slender spines up to 0.5–1.5 mm, clustered in stellate groups and leaving an inconspicuous reticulate pattern on the endoperidium when drop; subgleba cellular; basidiospores globose, 2.9–3.4 µm, punctate to almost smooth under the LM, often with a pedicel up to 5–7 µm.

Description. *Basidiomes* turbinate to pyriform, 10–15 mm high and 11–16 mm diam. *Stoma* wide, dentate-fimbriate, covered by a thin hyaline layer which can become detached in maturity. *Exoperidium* of dark-brown to cream-brown slender spines (up to 0.5–1.5 mm in upper part), clustering and joining at their tips forming stellate groups; finely granulose and echinulose in between; leaving at maturity an inconspicuous dark-brown reticulate pattern on the endoperidium when the spines drop. *Endoperidium* light greyish- to cream-brown. *Gleba* cream-brown. *Subgleba* white, prominent, cellular, occupying up to a third of the basidiome.

Basidiospores globose, pale brown, 2.9–3.4 µm, punctate to almost smooth under the LM and verruculose under the SEM, often with a pedicel up to 5–7 µm. *Capillitium* absent. *Paracapillitium* abundant, hyaline, incrustated, rarely bifurcated, 5–7 µm diam.

Holotype. Cameroon; South West Province, Korup National Park, trail from Mana Bridge to Transect P, N 5.20°, E 8.92°, on rotten wood, 05 04 1997, leg. P.J. Roberts (LE 253894; holotype). ITS sequence: GenBank MT250089.

Etymology. Name refers to the presence of a fimbriate stoma.

Habitat and distribution. The specimen was found on rotten wood in tropical forest, in a group of several basidiomes. Until now the known distribution is restricted to Central Africa, Cameroon.

DISCUSSION

On the basis of its small-size basidiomes, absence the capillitium and abundant the paracapillitium in combination with lignicolous habit the new species clearly refers to the genus *Morganella*.

Morphologically *M. fimbriata* is close to *M. compacta* (G. Cunn.) Kreisel et Dring, known from New Zealand (Cunningham, 1926), and *M. costaricensis* M.I. Morales, known from central and South America (Suárez, Wright, 1996), by showing a cellular subgleba and an areolate pattern on the endoperidium once the spines of the exoperidium have dropped in maturity. One else taxon from Africa named *Lycoperdon fuliginum* sensu Dring (Dring, Rayner, 1967) and described later as *Morganella afra* Kreisel et Dring (Kreisel, Dring, 1967) has minutely chambered subgleba, minutely granular exoperidium and minutely asperulate to short-spined basidiospores. However, *M. fimbriata* is distinct from all known species of *Morganella* in having a fimbriate stoma. The comparison of discussed species is presented in Table 2.

Based on ITS nrDNA phylogenetic analyses, *M. fimbriata* is far from all sequenced taxa. The sequence of the new species differed from nearest *M. puiggarii* KX064241 by 11%, where 46 substitutions, four 1 bp insertions and 4 deletions (two 1bp, one 2bp and one 3bp).

Morganella clade has a good support in a *Lycoperdon* s.l. clade (PP = 0.96) (Fig. 1). This contradicts

Table 1. Taxa, vouchers, locations, and GenBank accession numbers of DNA sequences used in the study

Taxon	ID (Specimen)	Country	GB no. ITS	Reference
<i>Apioperdon pyriforme</i> (Schaeff.) Vizzini	YuR1858	Russia	MH628571	GenBank
<i>Bovista aestivalis</i> (Bonord.) Demoulin	YuR1600	Russia	MH628569	Rebriev et al. (2020)
<i>B. acuminata</i> (Bosc) Kreisel	YuR0946	" "	MH628567	" "
" "	YuR1993	" "	MH628573	" "
<i>B. furfuracea</i> Pers.	MJ5435	Sweden	DQ112622	Larsson, Jeppson (2008)
<i>B. nigrescens</i> Pers.	S&JJ980905	" "	DQ112612	" "
<i>B. plumbea</i> Pers.	NYGD01	Pakistan	JX183694	Yousaf et al. (2013)
<i>Calvatia candida</i> (Rostk.) Hollós	MJ3514	Hungary	DQ112624	Larsson, Jeppson (2008)
<i>Disciseda candida</i> (Schwein.) Lloyd	STB304	USA	EU833654	Bates et al. (2009)
<i>D. hyalothrix</i> (Cooke et Masee) Hollós	NSK1014099	Russia	MN151399	Vlasenko et al. (2020)
<i>Lycoperdon albastipitatum</i> (Baseia et Alfredo) Baseia, Alfredo et M.P. Martín	UFRN-Fungos2249	Brazil	KU958361	Alfredo et al. (2017)
<i>L. albastipitatum</i> *	INPA239563	" "	KU958363	" "
<i>L. arenicola</i> (Alfredo et Baseia) Baseia, Alfredo et M.P. Martín*	UFRN-Fungos1006	" "	KU958303	" "
" "	UFRN-Fungos655	" "	KU958307	" "
<i>L. echinatum</i> Pers.	MJ6498	Sweden	DQ112578	Larsson, Jeppson (2008)
<i>L. fuligineum</i> Berk. et M.A. Curtis	UFRN-Fungos371	Brazil	KU958353	Alfredo et al. (2017)
" "	UFRN-Fungos1971	" "	KU958321	" "
" "	UFRN-Fungos1972	" "	KU958323	" "
" "	UFRN-Fungos2575	" "	KU958325	" "
" "	UFRN-Fungos2562	" "	KU958337	" "
<i>L. molle</i> Pers.	YuR2024	Russia	MH628574	Rebriev et al. (2020)
<i>L. nigrescens</i> Pers.	MJ5376	Sweden	DQ112577	Larsson, Jeppson (2008)
<i>L. nudum</i> (Alfredo et Baseia) Baseia, Alfredo et M.P. Martín	UFRN-Fungos1766	Brazil	KU958315	Alfredo et al. (2017)
<i>L. nudum</i> *	UFRN-Fungos1765	" "	KU958319	" "
<i>L. oblongatum</i> Accioly, Baseia et M.P. Martín *	UFRN-Fungos2570	" "	KU958355	" "
<i>L. perlatum</i> Pers.	YuR597	Russia	MH445551	Crous et al. (2019)
" "	MJ4684	Sweden	DQ112630	Larsson, Jeppson (2008)
<i>L. pratense</i> Pers.	YuR0554	Russia	MH445550	Crous et al. (2019)
" "	YuR1788	" "	MH445554	" "
<i>L. sulcatostomum</i> (C.R. Alves et Cortez) Baseia, Alfredo et M.P. Martín	ICN177032	Brazil	KU958373	Alfredo et al. (2017)
" "	ICN177033	" "	KU958369	" "
<i>Lycoperdon</i> sp. 1	CMU55-Ly1	Thailand	KC414581	GenBank
<i>Lycoperdon</i> sp. 2	HKAS 88251	China	MH311863	" "
<i>Morganella fimbriata</i>*	LE 253894	Cameroon	MT250089	Current study
<i>M. fuliginea</i> (Berk. et M.A. Curtis) Kreisel et Dring	TFB11275	Argentina	KY352656	GenBank
" "	TENN59070	Paraguay	AF485065	Krüger, Kreisel (2003)
" "	AMP051	Malaysia	KY777487	GenBank
<i>M. puiggarii</i> (Speg.) Kreisel et Dring	CMU-Mor3	Thailand	KX064241	Kumla et al. (2017)
<i>M. sosinii</i> Rebriev et Bulakh*	YR-2013	Russia	KC591769	Rebriev, Bulakh (2015)

Table 1. (Contd.)

Taxon	ID (Specimen)	Country	GB no. ITS	Reference
“ ”	YuR3730	“ ”	OP161223	Current study
“ ”	VLA19 09 97	“ ”	OP161221	“ ”
<i>M. subincarnata</i> (Peck) Kreisel et Dring	414	Germany	AJ237626	Krüger et al. (2001)
“ ”		USA	MN964264	GenBank
“ ”	TNS Kasuya B286	Japan	KF551244	Kasuya et al. (2013)
“ ”	R-83	Mexico	KR135347	GenBank
<i>Morganella</i> sp.	LE287320	Vietnam	OP161220	Current study
“ ”	YuR3049	“ ”	OP161222	“ ”
“ ”	YuR3054	Russia	OP161224	“ ”
<i>Vascellum curtisii</i> (Berk.) Kreisel	HIP1	USA	HQ235043	Miller et al. (2011)
<i>V. intermedium</i> A.H. Sm.	STB091	“ ”	EU833667	Bates et al. (2009)

Note. The new sequences are given in bold. Type specimens are marked with asterisk.

Table 2. The comparison of morphological characters used for the delimitation of *Morganella fimbriata* from similar species

Species	<i>Morganella fimbriata</i>	<i>M. afra</i>	<i>M. compacta</i>	<i>M. costaricensis</i>
Basidiome size	10–15 × 11–16 mm	10–25 mm	up to 4 mm	up to 12 mm
Stoma	dentate-fimbriate	torn	plane, torn, small	irregular
Exoperidium	slender spines up to 0.5–1.5 mm, clustered in stellate groups; leaving an inconspicuous reticulate pattern on the endoperidium when drop	minutely granular	spines 3–4 mm, surrounded by a ring of minute brown granules; leaving an conspicuous reticulate pattern on the endoperidium when spines drop	simple pyramidal or groups of spines up to 1 mm long
Basidiospores	globose, 2.9–3.4 µm diam., punctate to almost smooth, with pedicels up to 5–7 µm	globose, 3.5–4.5 µm, minutely asperulate to short-spined (to strongly echinulate in Dring, Rayner, 1967), with the stump of a pedicel	globose, 3.5–4.5 µm diam., asperulate, with caducous pedicels up to 5 µm	slightly ovate, 3.6–4.0 × 3.2–3.6 µm, smooth, with pedicels 5.4–12.6 µm
Paracapillitium	rarely bifurcated	undescribed	sparsely branched or simple	distinctly branched

other publications, where *Morganella* has a weak support (Larsson, Jeppson, 2008; Bates et al., 2009; Alfredo et al., 2017). However, *M. fimbriata* take a separate position on the tree. If we consider this taxon as a sister group, then the rest of the *Morganella* clade has very weak support (PP = 0.64).

It is highly probably that there are several genera mentioned as *Morganella* s.l. on the basis of morphology. The *Morganella* s.l. is divided into the few groups (Fig. 1). The subclade A has high support (PP = 0.99) and consist of type species *M. fuliginea* as well as *Lycoperdon nudum* (Alfredo et Baseia) Baseia, Alfredo et M.P. Martín, *L. albotipitatum* (Baseia et Alfredo) Baseia, Alfredo et M.P. Martín and one non-identified sequence KR135347. All these species are character-

ised by compact subgleba and respond to the sect. *Morganella* Ponce de León (1971). Another species are distributed among weakly supported subclades B (PP = 0.88), C (PP = 0.65) and singleton with the new species *Morganella fimbriata*. All of them are characterised by chambered subgleba and respond to the sect. *Subincarnata* Ponce de León (1971). However *Lycoperdon arenicola* (Alfredo et Baseia) Baseia, Alfredo et M.P. Martín characterized by growing on soil basidiomes with the capillitium what does not correspond to the classical understanding of *Morganella*.

Subclade B has many unidentified sequences named as “*Morganella* sp.” or “*Lycoperdon* sp.”. Some of them are undescribed taxa probably.

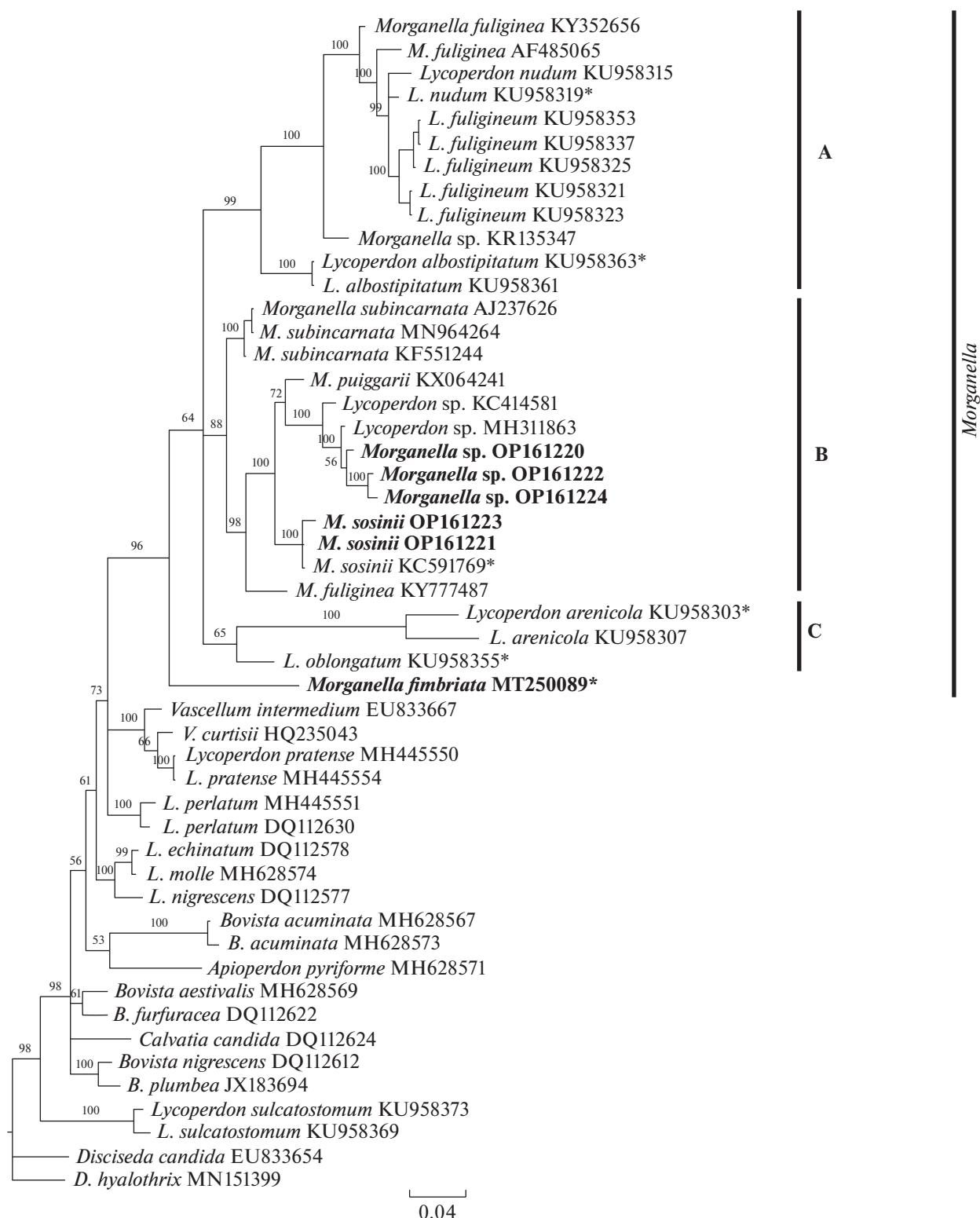


Fig. 1. ITS phylogenetic tree generated using MrBayes v. 3.2.6 under GTR + I + G model for 1.5 M generations. The GenBank accession numbers are indicated after each species name. Support values are indicated on the branches (posterior probabilities). The novel sequences is shown in bold text. Type specimens are pointed with asterisk.

It is notable that the sequences named as *Morganella fuliginea* are placed in two subclades of *Morganella* clade. In additional, type examination of *M. puiggarii*

clearly establishes its synonymy with *M. fuliginea* (Suárez, Wright, 1996). Sequence KX064241 named *M. puiggarii* is grouped with unidentified sequences.

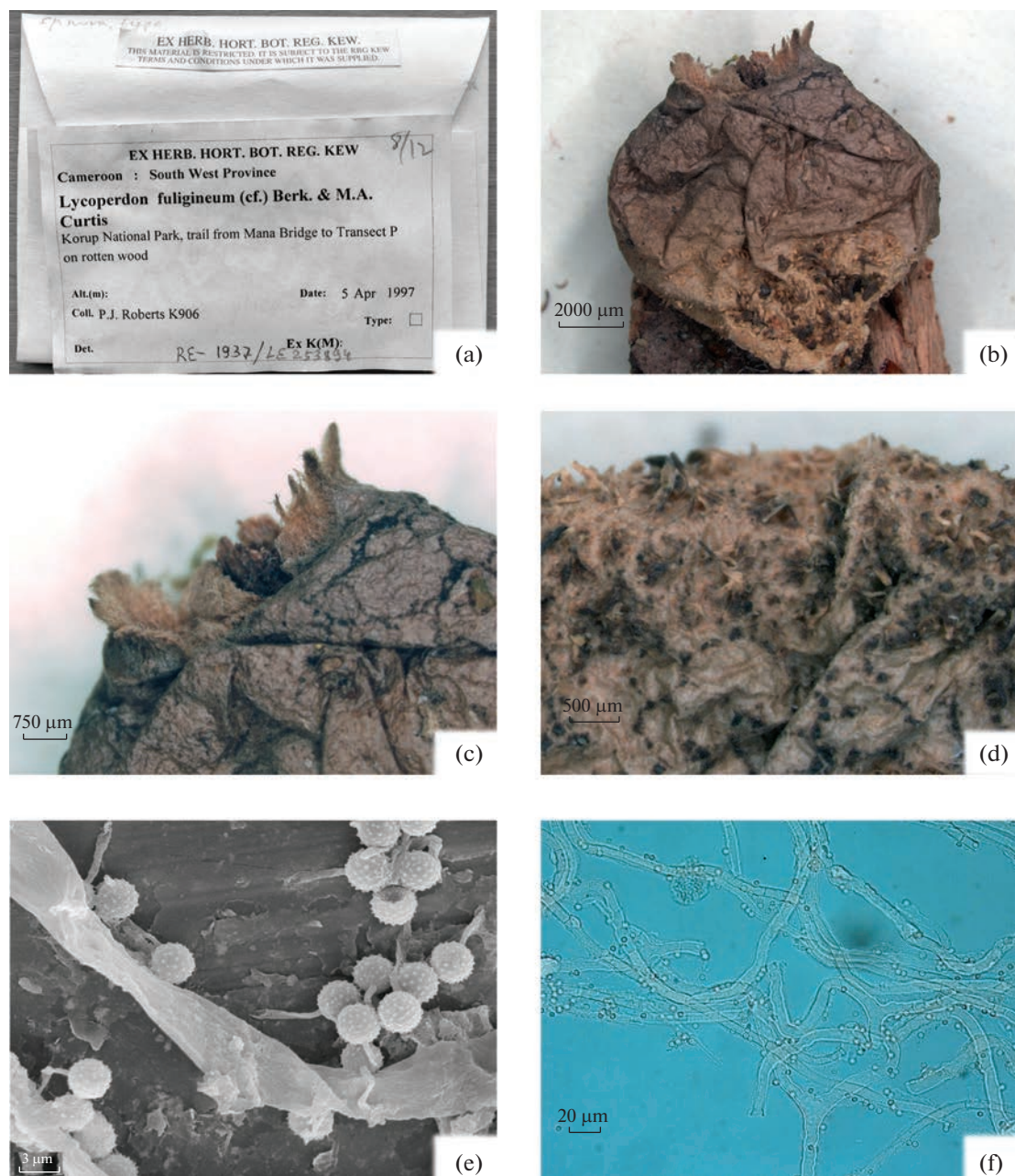


Fig. 2. *Morganella fimbriata*, holotype: a – the original envelope with the specimen received from the exchange fund of the Kew herbarium; b – matured basidiome; c – fimbriate stoma; d – spines of exoperidium and endoperidium with areolate pattern after spines falling, upper part of basidiome; e – pedicellate basidiospores and paracapillitium under SEM; f – basidiospores and paracapillitium under LM.

The vouchers sequences of the *M. fuliginea* KY777487 and *M. puiggarii* KX064241 origin is Malaysia and Thailand respectively. *M. fuliginea* areal is in the tropics and subtropics of North and South America (Ponce de León, 1971). So it is highly probably that vouchers discussed was misidentified. This confirms the importance of molecular genetic studies in clarifying the identification of doubtful specimens (Brock et al., 2009).

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Morganella fimbriata* (Lycoperdaceae, Agaricomycetes) — новый вид из Камеруна*Ю. А. Ребриев^{а, #}**^аЮжный научный центр РАН, Ростов-на-Дону, Россия[#]e-mail: rebriev@yandex.ru

Публикуется описание нового вида дождевика *Morganella fimbriata*. Главными диагностическими признаками являются ячеистое стерильное основание (субглеба), экзоперидий с тонкими шипами до 0.5–1.5 мм в верхней части плодового тела, после опадения которых на поверхности эндоперидия остается сетчатый рисунок, и бахромчатое устье. Проведено сравнение нового вида с морфологически близкими таксонами, а также с использованием нуклеотидных последовательностей ITS рДНК построено филогенетическое древо.

Ключевые слова: биоразнообразие, грибы, гастеромицеты, ДНК, дождевики, молекулярно-генетические методы идентификации, *Agaricaceae*, ITS, *Lycoperdon*, *Morganella*