



Favolus gracilisporus (Polyporaceae, Basidiomycota), an East Asian polypore species new to the European mycobiota

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Abstract

The interesting finding of *Favolus gracilisporus* a recently described polypore species from East Asia is reported new to the European mycobiota from Hungary (Central Europe). This species has previously been known only from the type locality, Mt Hagga in South Korea. The ITS sequence, macro-, microscopical characteristics and photographs of the Hungarian specimen are given.

Key words – Central Europe – new record – *Polyporus* – phylogeny

Introduction

Europe is probably the best investigated area regarding the polypore mycobiota with approx. 400 poroid species (Ryvarden & Melo 2014). Nevertheless, new species (e.g. *Caudicicola gracilis* Kotir., Kulju & Miettinen, *Ceriporia pierii* Rivoire, Miettinen & Spirin) from the old continent have been described recently on rare occasions (Kotiranta et al. 2017, Miettinen et al. 2016) and new occurrences of extra-European (i.e. tropical or subtropical) polypores have also been reported sporadically, e.g. *Amyloporus campbellii* (Berk.) Ryvarden (Bernicchia et al. 2017). In 2016, a peculiar polyporoid species was found in Hungary (Central Europe) on the trunk of a planted hackberry tree (*Celtis* sp.). Macroscopically the basidiome is related to the favoloid morphotypes of *Polyporus* s. lato, but it differs from any known European species, viz. *Favolus pseudobetulinus* (Murashk. ex Pilát) Sotome & T. Hatt and *Neofavolus alveolaris* (DC.) Sotome & T. Hatt.

The sanctioned genus *Favolus* Fr. (non *Favolus* P. Beauv.) was established by Fries (1828) and later *F. brasiliensis* (Fr.) Fr. (\equiv *Daedalea brasiliensis* Fr.) was selected as a generic type (Donk 1960). In their monographic study, Núñez & Ryvarden (1995) treated “*Favolus*” as one of the six infrageneric groups of the genus *Polyporus* P. Micheli ex Adans., and characterized by the flabelliform to dimidiate pileus and the short lateral stipe without a dark crust. They listed four species to the “*Favolus*” group, namely *Polyporus alveolaris* (DC.) Bondartsev & Singer, *P. grammocephalus* Berk., *P. philippinensis* Berk., and *P. tenuiculus* (P. Beauv.) Fr. (as a synonym of *Favolus brasiliensis*).

Table 1 Species, specimens and sequences used in the phylogenetic analysis.

Species name	Sample no.	Locality	ITS	References
<i>Favolus acervatus</i>	MEL:2382618	Australia	KP012981	GenBank
<i>F. acervatus</i>	Cui11053	China	KU189774	Zhou et al. (2016)
<i>F. acervatus</i>	SFC20120725-05	Korea	KX792916	Kim et al. (2016)
<i>F. acervatus</i>	TFM:F-14764	Japan	AB735971	Sotome et al. (2013)
<i>F. brasiliensis</i>	TENN10242	Costa Rica	AB735976	Sotome et al. (2013)
<i>F. brasiliensis</i>	INPA:241452	Brazil	AB735977	Sotome et al. (2013)
<i>F. emerici</i>	MEL:2382691	Australia	KP013055	GenBank
<i>F. emerici</i>	Dai 7847	China	KM385430	GenBank
<i>F. gracilisporus</i> ^a	SFC20130704-40	South Korea	KY038472	Tibpromma et al. (2017)
<i>F. gracilisporus</i> ^a	LWZ 20130809-15	China	KM385429	GenBank
<i>F. gracilisporus</i>	BP 106942	Hungary	MF401551	In this study
<i>F. grammocephalus</i> ^b	WD2379	Japan	AB587628	Sotome et al. (2011)
<i>F. grammocephalus</i>	Cui10926	China	KU189776	Zhou et al. (2016)
<i>F. philippinensis</i> ^b	Cui10941	China	KX548976	GenBank
<i>F. philippinensis</i> ^b	Dai7959	China	KX548977	GenBank
<i>F. philippinensis</i> ^b	Dai10849	China	KX548978	GenBank
<i>F. pseudobetulinus</i> ^b	CulTENN10298	Finland	AF516571	Krüger (2002)
<i>F. pseudobetulinus</i> ^b	TRTC:51022	Canada	AB587629	Sotome et al. (2011)
<i>F. pseudobetulinus</i> ^b	TFM:F-27626	Japan	AB587645	Sotome et al. (2011)
<i>F. roseus</i>	UOC WASNP W13	Sri Lanka	KR049231	GenBank
<i>F. roseus</i>	IJV04	Tanzania	KM593876	Juma et al. (2016)
<i>F. tenuiculus</i>	Dai13615A	China	KU189775	Zhou et al. (2016)
<i>F. tenuiculus</i> ^b	ML284	Malaysia	JQ409357	GenBank
<i>F. tenuiculus</i> ^b	Mu_1	unknown	KC951263	GenBank
<i>F. tenuiculus</i> ^b	HE2934	unknown	KC505555	GenBank
<i>Neofavolus alveolaris</i>	KUC20130808-54	South Korea	KJ668478	Jang et al. (2015)
<i>N. mikawai</i>	TFM:F-27416	Japan	AB735962	Sotome et al. (2013)
<i>N. cremeoalbidus</i>	TUMH:50009	Japan	AB735980	Sotome et al. (2013)

Notes: new sequence is in bold.

^a as *Favolus* sp. in GenBank.

^b as *Polyporus* in GenBank.

The phylogenetic analyses proved by Sotome et al. (2013) reveal that the infrageneric group “*Favolus*” is divided into two main clades in generic level. Based on morphological observations and molecular phylogenetic evidence, they revised *Favolus* and proposed *Neofavolus* Sotome & T. Hatt. as a new genus, typified on *N. alveolaris*. In this study we aimed to clarify the species level identity of the Hungarian “favoloid” specimen, based on macro- and micro-morphological features and molecular phylogenetic analysis of the ITS region.

Materials & Methods

Isolates and morphology

The Hungarian specimen studied is deposited at the herbarium of Hungarian Natural History Museum (BP). Macromorphological descriptions are based on field notes. Micromorphological data were obtained from the dried specimens, and observed under a Zeiss Axio Imager.A2 light microscope, equipped with AxioVision Release 4.8.2. software. Measurements were done with a 100× oil immersion objective (1000× magnification). Drawings were produced with the aid of a drawing tube. Observations of microscopic features as well as measurements, and drawings were made from slide preparations stained with Melzer’s reagent. Spores were measured from sections cut from the tubes. The following abbreviations were used in the description of the basidiospores: IKI =

Melzer's reagent, IKI- = both inamyloid and indextrinoid, L = mean spore length, W = mean spore width, Q = variation in the L/W ratios, n = number of spores were measured.

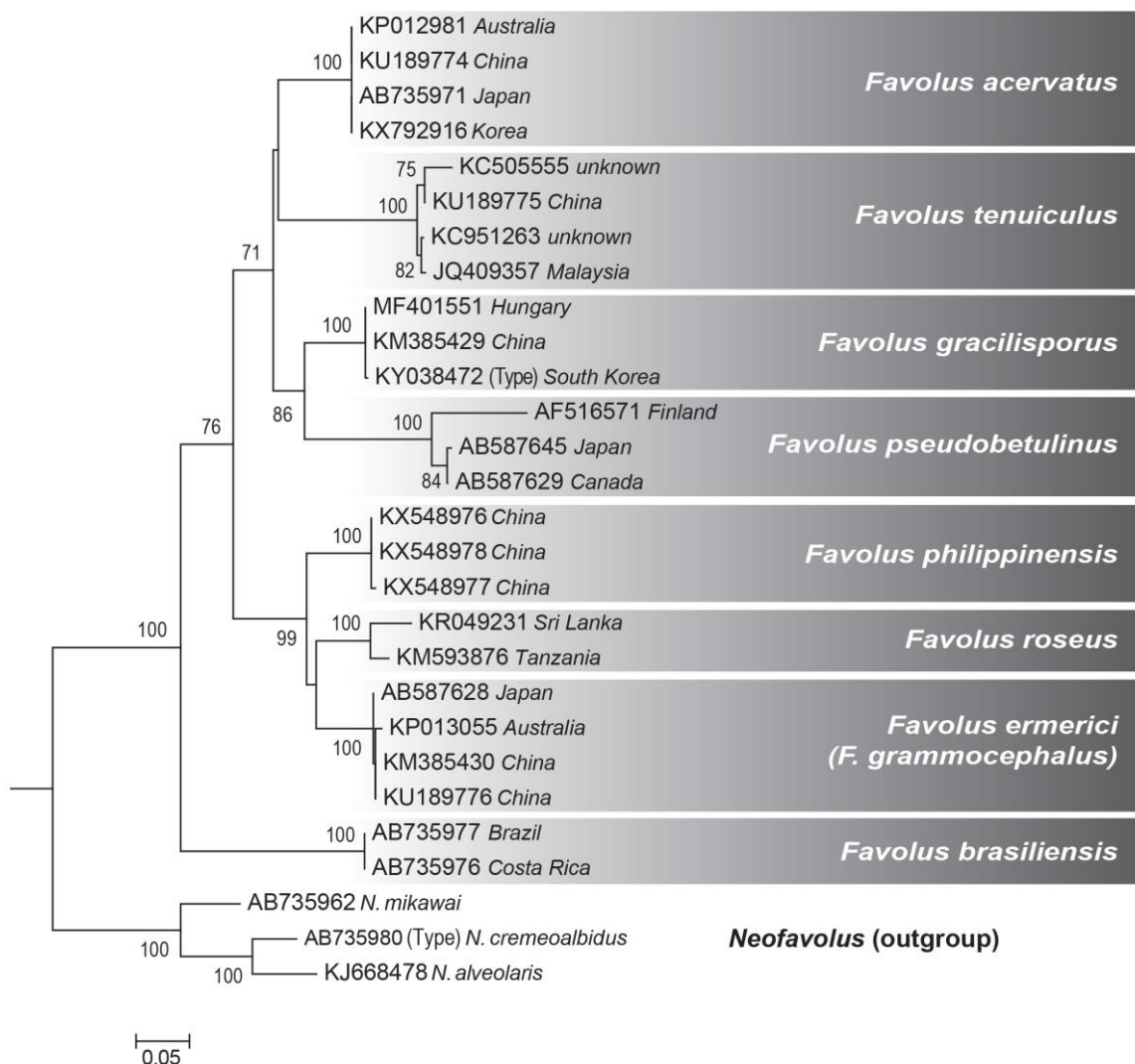


Figure 1 – Phylogenetic tree obtained from ITS sequences. Maximum likelihood bootstrap values >70% are given at the branches. Bar indicates 0.05 expected change per site per branch.

DNA isolation and PCR Analysis

We generated data for the ITS (internal transcribed spacer) nrDNA regions using the Phire® Plant Direct PCR Kit (Thermo Scientific, USA) and following the manufacturer's recommendations. The primer pairs ITS1F/ITS4 (Gardes & Bruns 1993, White et al. 1990) were used to amplify the ITS regions. PCR procedures were as follows: initial denaturation on 98 °C for 5 min, followed by 40 cycles denaturation on 98 °C for 5 s, annealing on 55 °C for 5 s, extension on 72 °C for 5 s, and a final extension on 72 °C for 1 min. PCR products were checked in 2% agarose gels. The amplicons were sequenced commercially at LGC Genomics (Berlin, Germany) with the same primers used in PCR reactions. The chromatograms were checked, assembled and edited with the CodonCodeAligner 7.0.1 (CodonCode Corporation, Centerville, MA, USA).

Sequence alignment and phylogenetic analysis

The newly generated *Favolus* sequence is deposited in GenBank (Benson et al. 2017) and the accession numbers is included in Table 1. For the phylogenetic analysis, similar sequences were searched from GenBank using the BLASTn search tool (Altschul et al. 1990). The ITS region was aligned with PRANK (Löytynoja & Goldman 2005, 2008) as implemented in its graphical interface (PRANKSTER) using default settings. SeaView (Gouy et al. 2010) was used to visually inspect and

improve the alignment. Gaps were coded following the simple indel coding algorithm (Simmons et al. 2001) with the program FastGap 1.2 (Borchsenius 2009). The nucleotide and the binary data sets resulted an alignment length of 773 characters. Maximum Likelihood (ML) phylogenetic analysis was carried out using RAxML (Stamatakis 2014) in raxmlGUI (Silvestro & Michalak 2012). For the nucleotide partition the GTRGAMMA substitution model, while for the indel partition the RAxML default set for binary characters were applied. Rapid bootstrap analysis with 1,000 replicates was performed using sequences of the genus *Neofavolus* as outgroup. The alignment and phylogenetic tree is deposited in TreeBASE under S21252.

Results

ITS sequence analyses

Altogether 25 ITS sequences of *Favolus* were included in our ML phylogenetic analysis. Eight strongly supported species level clades were recognized. The Hungarian specimen (MF401551) clustered together with *Favolus* sp. collected in China (KM385429) and the holotype of *F. gracilisporus* H. Lee, N.K. Kim & Y.W. Lim (KY038472), which is a recently described species from South Korea (Tibpromma et al. 2017). The closest species based on our ITS analysis is *F. pseudobetulinus* with only 89 % identity (564/636). Other morphologically somewhat similar species, i.e. *F. emerici* (Berk. ex Cooke) Imazeki, *F. philippinensis* (Berk.) Sacc. and *F. roseus* Lloyd cluster in a separate, strongly supported clade (BS=99%).

Taxonomy

Favolus gracilisporus H. Lee, N.K. Kim & Y.W. Lim, in Tibpromma et al., Fungal Diversity 83: 219, 2017. Figs 2–3

Basidiomes annual, attached by a short, rounded and undifferentiated lateral stipe, solitary or several form a branched base; pilei flabelliform to circular, up to 60 mm from the base to margin, up to 110 mm in width and 7 mm thick; upper surface azonate, fibrillose, radially striate, ivory to pale buff; margin concolorous or darker with age; hymenophore poroid, whitish or cream to pale brown, pores radially elongated, 2–2.5 mm long and 0.5–1 mm wide; dissepiments entire to slightly lacerate; tubes pale tan to ivory, corky, brittle when dry, up to 0.7 mm thick; context concolorous, continuous with the tube, homogenous, up to 5.5 mm thick. Stipe short, laterally attached to the pileus, buff, glabrous, with decurrent pores. Hyphal system dimitic with generative hyphae and skeleto-binding hyphae; generative hyphae hyaline in KOH, thin-walled, 3–4 µm in diam., septa with clamps, the thick-walled skeleto-binding hyphae aseptate, much branched, dominating in the basidiocarp, 3–6 µm in diam. Cystidia and cystidioles absent. Basidia clavate, 4–sterigmate, difficult to observe, 19–23 × 5–6 µm, with a basal clamp connection. Basidiospores cylindric to slightly allantoid, hyaline, smooth, IKI–, (7.40–)7.72–8.38(–8.76) × (3.14–)3.27–3.5(–3.69) µm, L = 8.1 µm, W = 3.3 µm, Q = 2.39 (n = 30).

Specimen examined – Hungary, Central Transdanubia, Komárom–Esztergom County, Komárom (N 47°43'15", E 18°8'37"), leg. P. Jakab, 27 May 2016, on *Celtis* sp. trunk (BP 106942).

Discussion

Several recent phylogenetic studies confirmed that *Polyporus* is a polyphyletic genus, and treated *Favolus* and *Neofavolus* as separate genera (e.g. Dai et al. 2014, Seelan et al. 2015, Sotome et al. 2013, Zhou et al. 2016, Zmitrovich & Kovalenko 2016). The genus *Neofavolus* comprises four species, out of which the subporoid lamellae forming *N. suavissimus* (Fr.) J.S. Seelan, Justo & Hibbett (Seelan et al. 2015) and *N. alveolaris* have been found in Europe (Ryvarden & Melo 2014). *Neofavolus alveolaris* has similar diamond-shaped pores and a laterally stipitate basidiocarp as *Favolus gracilisporus*, but it can be distinguished by the solitary, typically smaller basidiocarp which has orange-yellow pileus with brown triangular squamules when young, as well as the larger basidiospores [10–13(–14.5) × (3.5–)4–5 µm] measured in European specimens, and the preference

of growing on small branches of different deciduous trees (Bernicchia 2005, Piątek 2004, Ryvarden & Melo 2014).

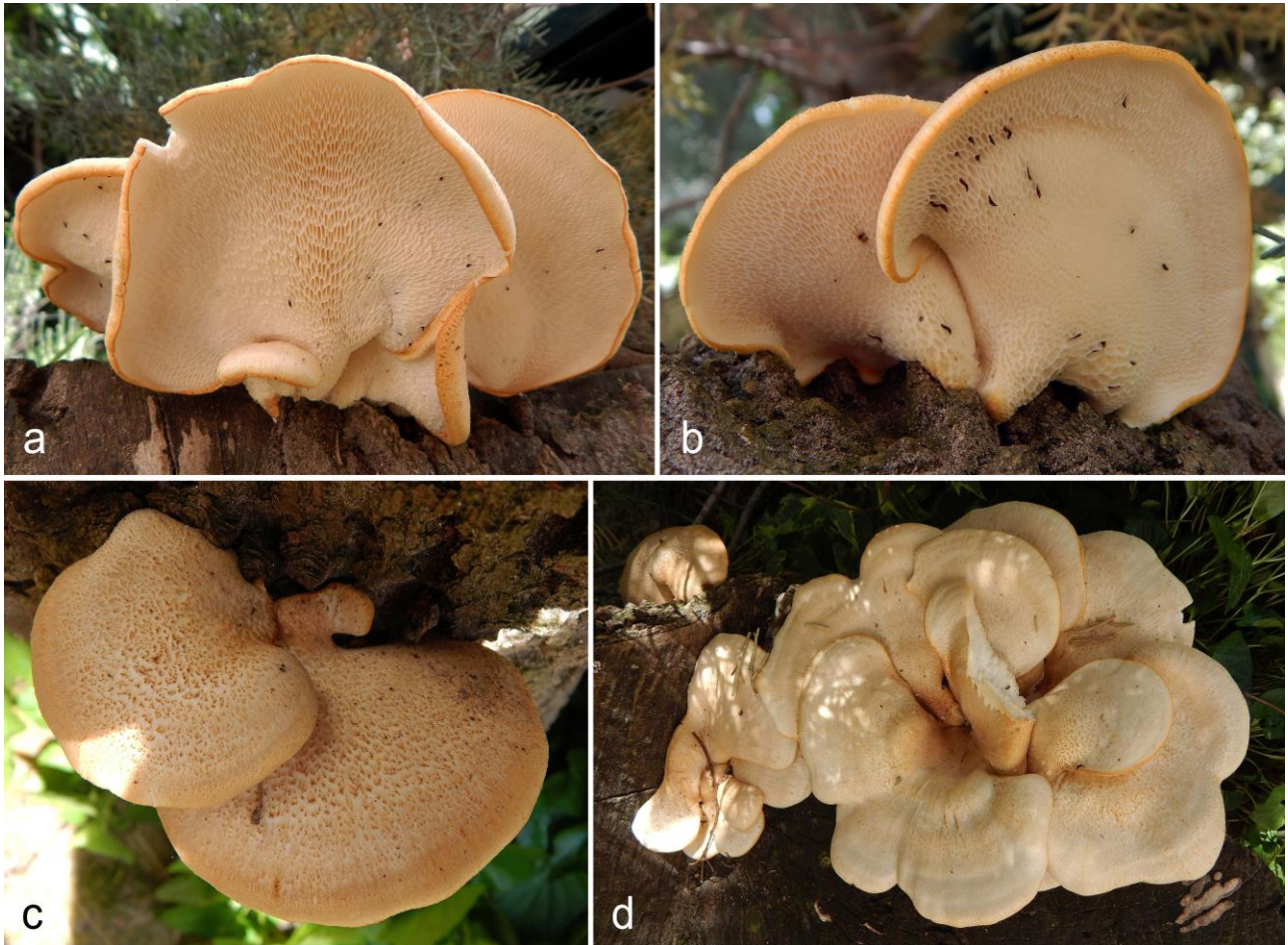


Figure 2 – *Favolus gracilisporus* basidiomes in the habitat (BP 106942). a–b pore surface. c–d pileal surface. Photographs by György Vrba

Most of the species accepted in the genus *Favolus* s. str. are mainly distributed in tropical areas (Sotome et al. 2013), and only *F. pseudobetulinus* is known from Europe, with a boreal distribution (Ryvarden & Melo 2014, Dai 2012). This species is closely related to *F. gracilisporus* in the phylogenetic analysis based on ITS sequences, but it has smaller angular pores (1–2 per mm) and simple septate tramal generative hyphae (Sotome et al. 2011). *Favolus roseus* distributed in tropical areas of Southeast Asia has similar radially elongated pores (1–3 × 0.5–2 mm) as *F. gracilisporus*, but it differs by the small basidiocarps with yellowish to brownish orange pore surface (Sotome et al. 2013). *Favolus elongoporus* (Drechsler-Santos & Ryvarden) Zmitr. & Kovalenko was described from Brazil that also has large irregularly elongated pores (0.5–2 per mm radially), but the hyphal pegs, the arboriform binding hyphae and the distinctly smaller basidia (10–15 × 3–7 μm) and basidiospores (6–8 × 2–3 μm) distinguished it from *F. gracilisporus* (Drechsler-Santos et al. 2008). Based on the morphological study of the holotype of *F. philippinensis*, Sotome et al. (2013) noted that this species is characterized by the large angular and elongated pores (1–4 mm long and 0.5–1.5 mm wide), the brownish orange pileus and the leathery context. *Favolus philippinensis* is widely accepted as a separate species (Coelho & da Silveira 2014, Gomes-Silva et al. 2012, Louza & Gugliotta 2007, Sotome et al. 2013, Silveira & Wright 2002) and it has several synonyms as well proposed by Ryvarden (1989) based on type studies of the favoloid species described by Hennings (1900) and Lloyd (1922, 1923) from Southeast Asia and Oceania (i.e. *F. glandulosus* Lloyd, *F. lagunae* Lloyd, *F. samoensis* Lloyd, *F. scabrolineatus* Lloyd and *Polyporus tomohoniensis* Henn.), although, these synonymizations lack molecular phylogenetic investigations.

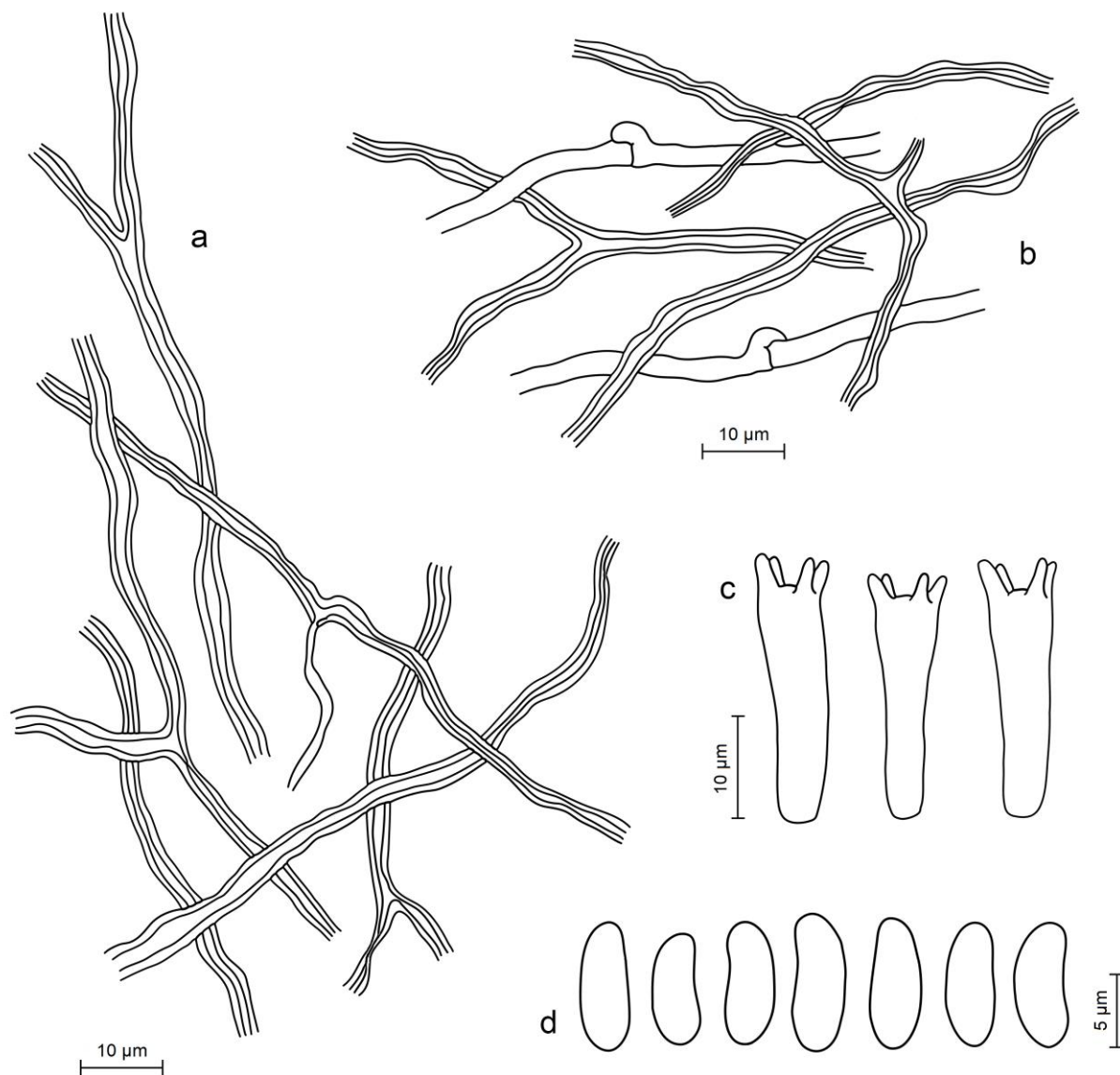


Figure 3 – Microscopic structure of *Favolus gracilisporus* (BP 106942). a contextual skeletal-binding hyphae. b clamped generative hyphae and skeletal-binding hyphae in the trama. c basidia. d basidiospores.

Conclusion

Molecular data confirm that the collection from Hungary is identical with the holotype of *Favolus gracilisporus*, although according to the description proved by Tibpromma et al. (2017) the Korean specimen has somewhat larger spores ($7.9\text{--}9.7 \times 2.5\text{--}3.3 \mu\text{m}$) and basidia ($27\text{--}35 \times 5.7\text{--}8 \mu\text{m}$) and distinctly thinner basidiocarp (up to 3.5 mm). The study of more collections of this species is needed to better estimate the morphological variability and real distribution of this taxon. Several species classified in *Favolus* by morphological features have not been studied by molecular methods (e.g. Zmitrovich & Kovalenko 2016) and a robust phylogeny including more specimens (e.g. type specimens) and species are needed to estimate a better knowledge of the genus. According to the present knowledge, *F. gracilisporus* is a separate species in the genus *Favolus* and the Hungarian findings is a new representative of this species to the European mycobiota.

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