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***Skeletocutis mopanshanensis* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis**

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With 3 figures and 1 table

Abstract: A new poroid wood-inhabiting fungal species, *Skeletocutis mopanshanensis* sp. nov., is proposed based on morphological and molecular characters. The species is characterized by resupinate, white to cream basidiocarps, a dimitic hyphal system with unbranched generative hyphae and big, ellipsoid basidiospores measuring $4.7\text{--}6.6 \times 3.2\text{--}4.5 \mu\text{m}$. The internal transcribed spacer (ITS) and the large subunit (LSU) regions of nuclear ribosomal RNA gene sequences of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analysis based on molecular data of ITS+nLSU sequences showed that *Skeletocutis mopanshanensis* belonged to the tyromyces clade, formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and was closely related to *S. yunnanensis*, and then grouped with *S. portrosensis* and *S. sp* with a lower support. Both morphological and molecular characters confirmed the placement of the new species in *Skeletocutis*.

Key words: Phylogenetic analysis, Polypores, taxonomy, Tyromyces clade, wood-rotting fungi.

Introduction

Skeletocutis Kotl. & Pouzar (Polyporaceae, Polyporales) was erected by Kotl. & Pouzar (1958) and typed by *S. amorpha* (Fr.) Kotl. & Pouzar. It is a large, cosmopolitan genus

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characterized by a combination of annual to perennial, resupinate or pileate basidiocarps with white, cream pink to lilac, often slightly discoloured and resinous when dry; a di- to trimitic hyphal structure in which generative hyphae with clamp connections and skeletal hyphae hyaline; both types of hyphae encrusted in the dissepiments; cystidia absent, cystidioles present in most species; and hyaline, thin-walled, allantoid, cylindrical to ellipsoid basidiospores, which are negative in Melzer's reagent. In addition, the genus causes a white rot (Niemelä 1998, Núñez & Ryvarden 2001, Dai 2012, Ryvarden & Melo 2014).

Recently, Binder et al. (2013) employed molecular study based on multi-gene datasets and demonstrated that the type species of *Skeletocutis* (*S. amorpha*) belongs to the tyromyces clade and appeared to be grouped with *Tyromyces chioneus* (Fr.) P. Karst., and *Piloporia sajanensis* (Parmasto) Niemelä by using ribosomal DNA sequences. Bian et al. (2016) described a new poroid species in *Skeletocutis* based on its morphological characters and rDNA sequences, and this species also belonged to the tyromyces clade, and was related to *S. amorpha* and *S. portcrossensis* A. David.

Although *Skeletocutis* is a cosmopolitan genus, most species in the genus were found in boreal and temperate forests from the northern hemisphere (Gilbertson & Ryvarden 1986, Núñez & Ryvarden 2001, Ryvarden & Melo 2014). A comprehensive study on the *Skeletocutis* was made by Niemelä mostly based on morphological characteristics and ecological habits, and several new species were described from Europe (Niemelä 1998). Previously 23 species including five species described from China were recorded in the country (Dai 1998, 2012, Cui 2013, Bian et al. 2016). During investigations on the diversity of polypores in southwestern China, an additional undescribed species corresponding to *Skeletocutis* was found. To confirm the affinity of the undescribed species of *Skeletocutis*, phylogenetic analysis was carried out based on the ITS and nLSU sequences.

Materials and methods

The studied specimens are deposited at the herbarium of Southwest Forestry University (SWFC). Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2010). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, IKI = Melzer's reagent, IKI- = both inamyloid and indextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications that a small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer, and incubated in a 65°C water bath for 60 min. After that, 0.4 ml phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13 000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for a centrifugation at 12000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of adsorbed film to elute

the genome DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min (Chen et al. 2015). The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Katoh & Toh 2008; <http://mafft.cbrc.jp/alignment/server/>) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 21462). Sequences of *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. obtained from GenBank were used as outgroups to root trees following Binder et al. (2013) in the ITS+nLSU analysis.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Zhou et al. (2016), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAXML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller et al. 2009). Branch support for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 5 million generations (ITS+nLSU), and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BP) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (BP) and 0.95 (BPP) were considered as significantly supported, respectively.

Results

The ITS+nLSU dataset included sequences from 66 fungal specimens representing 59 species. The dataset had an aligned length of 2415 characters, of which 1400 characters are constant, 288 are variable and parsimony-uninformative, and 727 are parsimony-informative. Maximum parsimony analysis yielded two equally parsimonious trees (TL = 5654, CI = 0.306, HI = 0.692, RI = 0.557, RC = 0.170). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.001417 (BI).

The phylogeny (Fig. 1) inferred from ITS+nLSU sequences demonstrated seven major clades for 59 species of the Polyporales. The new species clustered into the tyromyces

Table 1. A list of species, specimens, and GenBank accession number of sequences used in this study.

Species name	Sample no.	GenBank accession no.		References
		ITS	nLSU	
<i>Abortiporus biennis</i>	TFRI 274	EU232187	EU232235	Binder et al. 2005
<i>Antrodia albida</i>	CBS 308.82	DQ491414	AY515348	Kim et al. 2007
<i>A. heteromorpha</i>	CBS 200.91	DQ491415	AY515350	Kim et al. 2007
<i>Antrodiella americana</i>	Gothenburg 3161	JN710509	JN710509	Binder et al. 2013
<i>A. semisupina</i>	FCUG 960	EU232182	EU232266	Binder et al. 2005b
<i>Bjerkandera adusta</i>	NBRC 4983	AB733156	AF287848	Binder et al. 2005
<i>Ceriporiopsis alboaurantia</i>	Cui 4136	KF845948	KF845955	Zhao et al. 2015b
<i>C. balaenae</i>	H7002389	FJ496669	FJ496717	Tomšovský et al. 2010
<i>C. consobrina</i>	Rivoire 977	FJ496667	FJ496716	Tomšovský et al. 2010
<i>C. fimbriata</i>	Dai 11672	KJ698633	KJ698637	Zhao et al. 2015b
<i>C. gilvescens</i>	BRNM 710166	FJ496684	FJ496720	Tomšovský et al. 2010
<i>C. gilvescens</i>	BRNM 667882	FJ496685	FJ496719	Tomšovský et al. 2010
<i>C. guidella</i>	HUBO 7659	FJ496687	FJ496722	Tomšovský et al. 2010
<i>C. rosea</i>	Dai 13584	KJ698636	KJ698640	Zhao et al. 2015b
<i>C. semisupina</i>	Cui 10222	KF845949	KF845956	Zhao et al. 2015b
<i>C. semisupina</i>	Cui 10189	KF84595	KF845958	Zhao et al. 2015b
<i>Cinereomyces lindbladii</i>	KHL 12078	FN907906	FN907906	Binder et al. 2013
<i>Climacocystis borealis</i>	KH 13318	JQ031126	JQ031126	Binder et al. 2013
<i>Coriobolus caperata</i>	LE(BIN)-0677	AB158316	AB158316	Tomšovský et al. 2010
<i>Dacryobolus karstenii</i>	KHL 11162	EU118624	EU118624	Binder et al. 2005
<i>Daedalea quercina</i>	DSM 4953	DQ491425	DQ491425	Kim et al. 2007
<i>Earliella scabrosa</i>	PR 1209	JN165009	JN164793	Binder et al. 2005
<i>Fomitopsis pinicola</i>	CBS 221.39	DQ491405	DQ491405	Kim et al. 2007
<i>F. rosea</i>	ATCC 76767	DQ491410	DQ491410	Kim et al. 2007
<i>Fragiliporia fragilis</i>	Dai 13080	KJ734260	KJ734264	Zhao et al. 2015a
<i>F. fragilis</i>	Dai 13559	KJ734261	KJ734265	Zhao et al. 2015a
<i>F. fragilis</i>	Dai 13561	KJ734262	KJ734266	Zhao et al. 2015a
<i>Ganoderma lingzhi</i>	Wu 1006-38	JQ781858	–	Zhao et al. 2015a
<i>Gelatoporia subvermispora</i>	BRNU 592909	FJ496694	FJ496706	Tomšovský et al. 2010
<i>Grammothelopsis subtropica</i>	Cui 9035	JQ845094	JQ845097	Zhao et al. 2015a
<i>Heterobasidion annosum</i>	PFC 5252	KC492906	KC492906	Binder et al. 2013
<i>Hornodermoporus martius</i>	MUCL 41677	FJ411092	FJ393859	Robledo et al. 2009
<i>Hypochnicium lyndoniae</i>	NL 041031	JX124704	JX124704	Binder et al. 2005
<i>Junghuhnia nitida</i>	KHL 11903	EU118638	EU118638	Binder et al. 2005
<i>Mycoacia fuscoatra</i>	KHL 13275	JN649352	JN649352	Tomšovský et al. 2010
<i>M. nothofagi</i>	KHL 13750	GU480000	GU480000	Tomšovský et al. 2010
<i>Obba rivulosa</i>	KCTC 6892	FJ496693	FJ496710	Miettinen & Rajchenberg 2012
<i>O. valdiviana</i>	FF 503	HQ659235	HQ659235	Miettinen & Rajchenberg 2012
<i>Perenniporia medulla-panis</i>	MUCL 49581	FJ411087	FJ393875	Robledo et al. 2009
<i>Perenniporiella neofulva</i>	MUCL 45091	FJ411080	FJ393852	Robledo et al. 2009
<i>Phlebia livida</i>	FCUG 2189	AF141624	AF141624	Tomšovský et al. 2010

<i>P. radiata</i>	UBCF 19726	HQ604797	HQ604797	Binder et al. 2013
<i>P. subserialis</i>	FCUG 1434	AF141631	AF141631	Tomšovský et al. 2010
<i>Piloporia sajanensis</i>	Mannine 2733a	HQ659239	HQ659239	Tomšovský et al. 2010
<i>Podoscypha venustula</i>	CBS 65684	JN649367	JN649367	Binder et al. 2013
<i>Polyporus tuberaster</i>	CulTENN 10197	AF516596	AJ488116	Binder et al. 2013
<i>Postia guttulata</i>	KHL 11739	EU11865	EU11865	Kim et al. 2007
<i>Sebipora aquosa</i>	Miettinen 8680	HQ659240	HQ659240	Miettinen and Rajchenberg 2012
<i>Skeletocutis amorpha</i>	Miettinen 11038	FN907913	FN907913	Tomšovský et al. 2010
<i>S. jelicii</i>	H 6002113	FJ496690	FJ496727	Tomšovský et al. 2010
<i>S. mopanshanensis</i>	CLZhao 1152	MF924720	MF924722	Present study
<i>S. mopanshanensis</i>	CLZhao 1184	MF924721	MF924723	Present study
<i>S. nivea</i>	ES 2008	JX109858	JX109858	Tomšovský et al. 2010
<i>S. ochroalba</i>	JK 1208/8	KF840389	–	Tomšovský et al. 2010
<i>S. portcrossensis</i>	LY 3493	FJ496689	FJ496689	Tomšovský et al. 2010
<i>S. sp.</i>	NMM-2009	FJ791129	–	Tomšovský et al. 2010
<i>S. yunnanensis</i>	Dai 15709	KU950434	KU950436	Bian et al. 2016
<i>S. yunnanensis</i>	Dai 15712	KU950435	KU950437	Bian et al. 2016
<i>Steccherinum fimbriatum</i>	KHL 11905	EU118668	EU118668	Tomšovský et al. 2010
<i>S. ochraceum</i>	KHL 11902	JQ031130	JQ031130	Tomšovský et al. 2010
<i>Stereum hirsutum</i>	NBRC 6520	AB733150	AB733325	Tomšovský et al. 2010
<i>Truncospora ochroleuca</i>	MUCL 39726	FJ411098	FJ393865	Robledo et al. 2009
<i>Tyromyces chioneus</i>	Cui 10225	KF698745	KF698756	Zhao et al. 2015b
<i>Xanthoporus syringae</i>	Gothenburg 1488	JN710607	JN710607	Tomšovský et al. 2010

clade and was formed a monophyletic lineage with a high support (100% BS, 95% BP, 0.99 BPP), and was closely related to *Skeletocutis yunnanensis* L.S.Bian, C.L.Zhao & F.Wu with a high support (96% BS, 87% BP, 0.98 BPP), and then grouped with *S. portcrossensis* and *S. sp.* with a lower support.

Taxonomy

Skeletocutis mopanshanensis C.L.Zhao, **sp. nov.**

Figs 2, 3

Mycobank no.: MB 823081

DIAGNOSIS: The species is distinct by its resupinate basidiocarps with white to cream hymenophores, round pores measuring 4–5 per mm, a dimitic hyphal system with unbranched generative hyphae, tramal hyphae subparallel along the tubes and covered by fine crystals, and ellipsoid basidiospores measuring 4.7–6.6 × 3.2–4.5 µm.

HOLOTYPE: CHINA. Yunnan Prov., Yuxi, Xiping County, Mopanshan National Forest Park, on fallen branch of *Pinus yunnanensis*, 18 January 2017, CLZhao 1184 (SWFC).

ETYMOLOGY: *Mopanshanensis* (Lat.): referring to the locality (Mopanshan) of the type specimen.

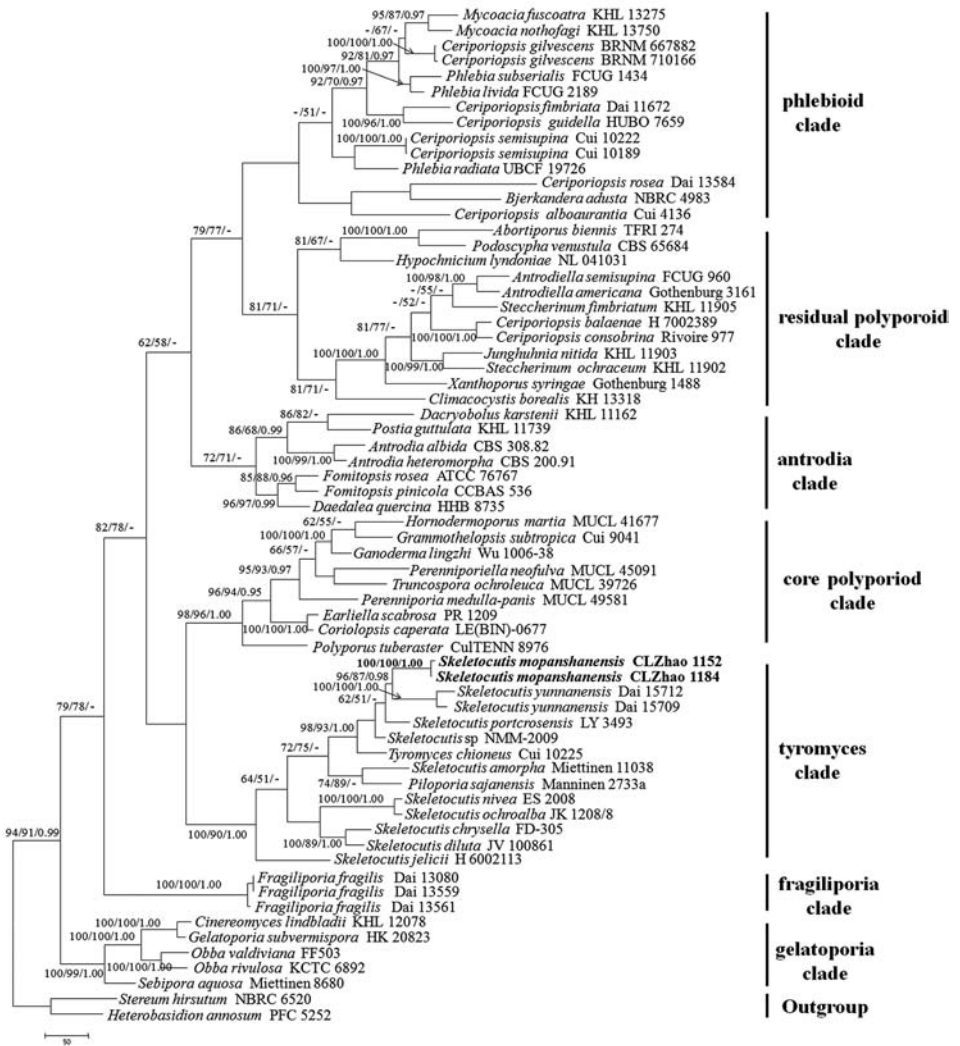


Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Skeletocutis mopanshanensis* and related species in Polyporales based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. Clade names follow Binder et al. (2013).

FRUITING BODY: Basidiocarps annual, resupinate, very difficult to separate from substrate, soft leathery, without odour or taste when fresh, becoming soft corky to fragile upon drying, up to 7 cm long, 4 cm wide and 1 mm thick at centre. Pore surface white when fresh, white to cream upon drying; pores round, 4–5 per mm; dissepiments thin, entire. Sterile margin distinct, white, up to 2 mm wide. Subiculum white to cream, soft corky,



Fig. 2. Basidiomata of *Skeletocutis mopanshanensis*. Bars: 1.5 cm (holotype).

up to 0.3 mm thick. Tubes concolorous with pore surface, soft corky to fragile, up to 0.7 mm long.

HYPHAL STRUCTURE: Hyphal system dimitic; generative hyphae with clamp connections, hyaline, thin-walled; skeletal hyphae thick-walled with a wide lumen; all hyphae IKI-, CB-, unchanged in KOH.

SUBICULUM: Generative hyphae hyaline, unbranched, covered by fine crystals, interwoven, 2.5–4 µm in diameter; skeletal hyphae dominant, hyaline, thick-walled with a wide lumen, occasionally branched, interwoven, 3–5 µm in diameter.

TUBES: Generative hyphae hyaline, thin-walled, unbranched, covered by fine crystals, interwoven, 2–3 µm in diameter; skeletal hyphae dominant, hyaline, thick-walled with a wide lumen, occasionally, interwoven, 2.5–4.5 µm in diameter. Cystidia absent, but fusoid cystidioles occasionally present, hyaline, thin-walled, 7–10 × 2–3 µm; basidia barrel-shaped to pyriform, with four sterigmata and a basal clamp connection, 9–13 × 5.5–8 µm; basidioles dominant, in shape similar to basidia, but slightly smaller.

SPORES: Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI-, CB-, (4.5–)4.7–6.6(–6.8) × (3–)3.2–4.5(–4.8) µm, L = 5.51 µm, W = 3.69 µm, Q = 1.41–1.65 (n = 60/2).

ROT TYPE: A white rot.

ADDITIONAL SPECIMEN (PARATYPE) EXAMINED: CHINA. Yunnan Prov., Yuxi, Xiping county, Mopanshan National Forest Park, on fallen branch of *Pinus yunnanensis*, 18 January 2017, CLZhao 1152 (SWFC).

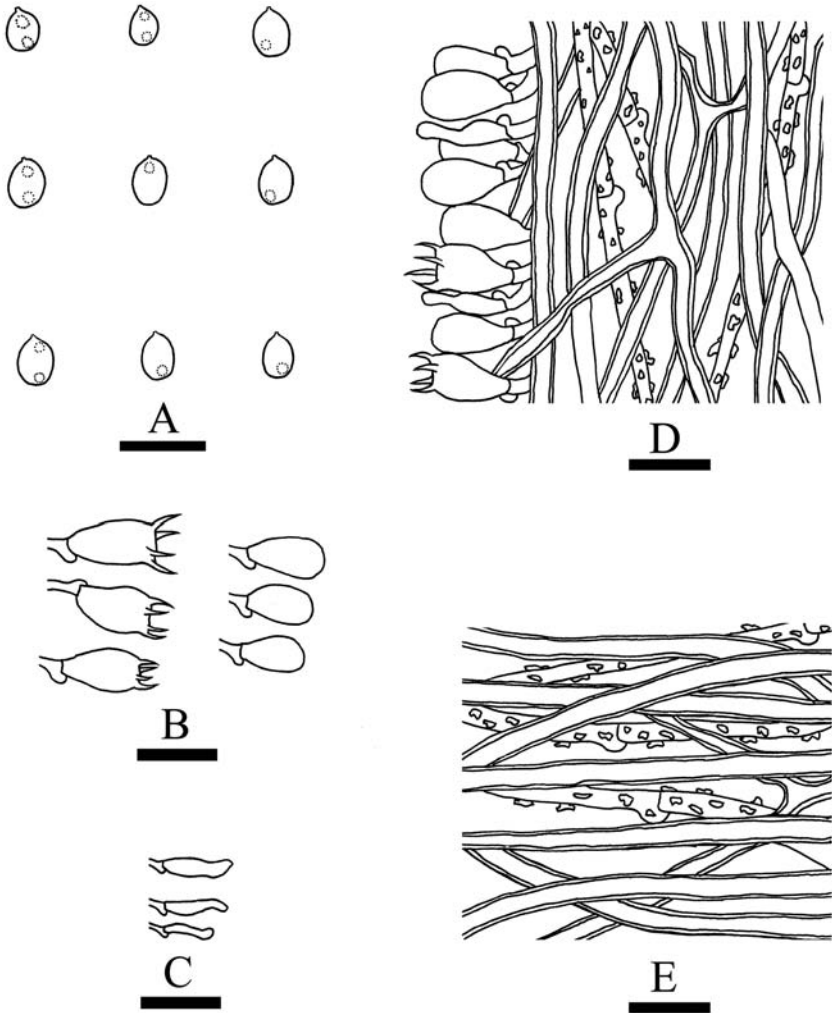


Fig. 3. Microscopic structures of *Skeletocutis mopanshanensis* (drawn from the holotype). a. Basidiospores; b. Basidia and basidioles; c. Cystidioles; d. Hyphae from trama; e. Hyphae from subiculum. Bars: A, B, C, D = 10 μ m.

Discussion

In the present study, a new species, *Skeletocutis mopanshanensis*, is described based on phylogenetic analysis and morphological characters. The species has unique morphological characters in *Skeletocutis* and forms a monophyletic lineage within the tyromyces clade.

Previously, seven clades were found in the Polyporales: antrodia clade, core polyporoid clade, fragiliporia clade, gelatoporia clade, phlebioid clade, residual polyporoid clade and tyromyces clade (Binder et al. 2013). According to our result based on the combined ITS+nLSU sequence data (Fig. 1), *Skeletocutis mopanshanensis* is nested into the tyromyces clade with strong support (100% BS, 100% BP, 1.00 BPP).

In our analysis (Fig. 1), *Skeletocutis mopanshanensis* grouped with *S. yunnanensis*, and then grouped with *S. portcrossensis* inferred from the ITS+nLSU analysis. However, morphologically *S. yunnanensis* differs from *S. mopanshanensis* by its angular pores and smaller and cylindric basidiospores ($3.5\text{--}4.5 \times 1\text{--}1.2 \mu\text{m}$; Bian et al. 2016). *Skeletocutis portcrossensis* can be distinguished by arachnoid margins and a monomitic hyphal system and smaller, cylindric basidiospores ($4\text{--}5 \times 1.5\text{--}1.8 \mu\text{m}$; Ryvar den & Melo 2014).

Having wider basidiospores ($> 2 \mu\text{m}$ in width) reminds of two similar species in the *Skeletocutis*: *S. percandida* (Malençon & Bertault) Jean Keller and *S. sensitiva* (Lloyd) Ryvar den. The former differs from *S. mopanshanensis* by cottony basidiocarps with white rhizomorph and cylindrical basidiospores; in addition, it grows on hardwood (Ryvar den & Melo 2014). The latter is separated from *S. mopanshanensi* by its resupinate to effused-reflexed basidiocarps with orange pore surface and smaller basidiospores ($4\text{--}4.5 \times 3\text{--}3.5 \mu\text{m}$; Núñez & Ryvar den 2001).

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