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# *Lyomyces fissuratus* and *L. fumosus* (Schizoporaceae, Hymenochaetales), new species from southern China

Xi Luo<sup>1,3</sup>, Yu-Hui Chen<sup>3</sup> & Chang-Lin Zhao<sup>1,2,\*</sup>

- <sup>1)</sup> Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, CN-650224 Kunming, P.R. China (\*corresponding author's e-mail: fungichanglinz@163.com)
- <sup>2)</sup> College of Biodiversity Conservation, Southwest Forestry University, CN-650224 Kunming, P.R. China

<sup>3)</sup> College of Life Sciences, Southwest Forestry University, CN-650224 Kunming, P.R. China

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Luo X., Chen Y.H. & Zhao C.L. 2021: *Lyomyces fissuratus* and *L. fumosus* (Schizoporaceae, Hymenochaetales), new species from southern China. – *Ann. Bot. Fennici* 58: 219–230.

Two new wood-inhabiting species of fungi, *Lyomyces fissuratus* C.L. Zhao and *L. fumosus* C.L. Zhao (Schizoporaceae, Hymenochaetales) are proposed based on a combination of morphological and molecular evidence. *Lyomyces fissuratus* has resupinate basidiomes with a tuberculate, cracking hymenial surface and slightly thick-walled basidiospores, and *L. fumosus* has farinaceous to pruinose basidiomes with a smoky grey hymenial surface and slightly thick-walled basidiospores. ITS sequences and LSU gene regions were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. According to a phylogenetic reconstruction of Schizoporaceae based on a concatenated ITS + LSU data set, the two new species were nested in *Lyomyces*. In a second phylogenetic analysis of the genus *Lyomyces*, multiple isolates of *L. fissuratus* formed a monophyletic lineage in a clade comprising *L. mascarensis*, *L. cremeus*, *L. microfasciculatus* and *L. wuliangshanensis*. *Lyomyces fumosus* was retrieved as a sister species of *L. fimbriatus*.

## Introduction

Corticioid fungi are a heterogeneous group containing mainly basidiomycete species. Basidiomes are generally resupinate and often simple, but in the more structured ones, the margins are usually reflexed, the surface pileate and the hyphae more or less sclerified (Gorjón 2020). Molecular phylogenetic studies elucidated the relationships among different taxa of corticioid fungi, which is now known to be a polyphyletic group (Larsson & Larsson 2003, Larsson *et al.* 2004, Binder *et al.* 2005, 2010, Larsson 2007, Sulistyo *et al.* 2021). Lyomyces is a small corticioid genus characterized by resupinate to effused basidiomes with smooth to granular or odontioid hymenophore, a monomitic hyphal system bearing clamp connections, and strongly encrusted generative hyphae. Cystidia are of several types, basidia clavate to suburniform, and basidiospores smooth, thin- to slightly thick-walled and cyanophilous (Karsten 1881, Bernicchia & Gorjón 2010). Species of Lyomyces are found on dead, still attached or fallen branches of angiosperms, on dead woody or herbaceous stems, or occasionally on gymnosperm wood (Yurchenko et al. 2017). World220

1903, Bourdot & Galzin 1911, Cunningham 1959, 1963, Wu 1990, Hjortstam & Ryvarden 2009, Xiong *et al.* 2009, Yurchenko *et al.* 2013, 2017, 2020, Gafforov *et al.* 2017, Riebesehl & Langer 2017, Chen & Zhao 2020).

Molecular studies by Riebesehl and Langer (2017) on Hyphodontia s. lato showed that it should be divided into the following genera: Hastodontia, Hyphodontia, Kneiffiella, Lagarobasidium, Lyomyces and Xylodon. They proposed 35 new combinations, including 14 in Lyomyces. Yurchenko et al. (2017) clarified the L. sambuci complex based on sequences of the internal transcribed spacer (ITS) and the nuclear large subunit (LSU) ribosomal DNA gene, and described four new species of Lyomyces. Riebesehl et al. (2019) emended the generic concept of Lvomvces and provided an identification key to all 22 species. Chen and Zhao (2020) plotted macro- and micromorphological characters onto an ITS-based phylogeny and found that none of them were phylogenetically informative within Lyomyces.

During studies on wood-inhabiting fungi in southern China, we found specimens of *Lyo-myces* that could not be assigned to any of the described species.

# Material and methods

## **Morphological studies**

The studied specimens are deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. The colour terms follow Petersen (1996). Macromorphological descriptions are based on field notes. Micromorphological examination of dried specimens was carried out under a Nikon Eclipse E100 light microscope following Dai (2012) and Cui *et al.* (2019). The following abbreviations are used in the paper: KOH = 5% potassium hydroxide, CB = cotton blue, CB+ = cyanophilous, IKI = Melzer's reagent, IKI- = non-amyloid and non-dextrinoid, L = mean spore length (arithmetic mean), Q = L/W ratio, n =

number of measured spores/number of specimens.

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#### **DNA extraction and sequencing**

CTAB rapid plant genome extraction kit DN14 (Aidlab Biotechnologies Co. Ltd., Beijing) was used to obtain genomic DNA from dried specimens following the manufacturer's instructions. The ITS region was amplified with the primer pair ITS5 and ITS4 (White *et al.* 1990), and the LSU region with primer pair LR0R and LR7 (Vilgalys & Hester 1990, Hopple 1994).

The PCR cycling 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 followed Shen *et al.* (2019).

The PCR procedure for LSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min.

The PCR products were purified and directly sequenced at the Kunming Tsingke Biological Technology Ltd. Co. (Yunnan, P.R. China). All newly generated sequences were deposited in GenBank (Table 1).

## **Phylogenetic analyses**

Sequencher ver. 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to assemble and edit the generated sequences, which were then were aligned in MAFFT ver. 7 (https://mafft.cbrc.jp/ alignment/server/) using the G-INS-I strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in Tree-Base (submission ID 27305).

Two data sets were compiled. The first one, consisting of the ITS + LSU sequences, was used to position the new species among genera related to *Lyomyces* in Schizoporaceae, and the second, comprising the ITS data only, was used to place the new taxa among previously described species of *Lyomyces*. The outgroups were *Oxyporus populinus* for ITS + LSU, and *Palifer verecundus* and *Xylodon asperus* for ITS.

Species name	Specimen	GenBank accession no.		References
		ITS	nLSU	
Fasciodontia brasiliensis E. bugellensis	MSK-F 7245a KAS-FD 10705a	MK575201 MK575203	MK598734 MK598735	Yurchenko <i>et al.</i> 2020 Yurchenko <i>et al.</i> 2020
Hastodontia hastata	KHL 14646	MH638232	MH638232	Viner <i>et al.</i> 2018
Hyphodontia alutaria	KHL 11978	EU118631	EU118631	Larsson 2007
Kneiffiella barba-iovis	KHL 11730	DQ873609	DQ873610	Larsson <i>et al.</i> 2006
K. palmae	KAS-GEL 3456	DQ340333	DQ340369	Yurchenko <i>et al</i> . 2020
Lyomyces allantosporus	KAS-GEL 4933	KY800401	KY795965	Yurchenko <i>et al.</i> 2017
L. allantosporus	FR 0249548	KY800397	KY795963	Yurchenko <i>et al.</i> 2017
L. bambusinus	CLZhao 4808	MN945970	-	Chen & Zhao 2020
L. bambusinus	CLZhao 4831	MN945968	MW264919	Chen & Zhao 2020
L. cremeus	CLZhao 4138	MN945974	MW264922	Chen & Zhao 2020
L. cremeus	CLZhao 8295	MN945972	-	Chen & Zhao 2020
L. crustosus	YG-G 39	MF382993	-	Gafforov et al. 2017
L. crustosus	UC 2022841	KP814310	-	Rosenthal <i>et al</i> . 2017
L. crustosus	KHL 11731	DQ873614	DQ873614	Larsson <i>et al</i> . 2006
L. erastii	MA-Fungi 34336	JX857800	-	Unpublished
L. erastii	YG 022	MF382992	-	Gafforov et al. 2017
L. fimbriatus	Wu 910620-7	MK575209	-	Yurchenko <i>et al</i> . 2020
L. fimbriatus	Wu 911204-4	MK575210	MK598740	Yurchenko <i>et al.</i> 2020
L. fissuratus	CLZhao 4291	MW713738	MW713730	This study
L. fissuratus	CLZhao 4299	MW713739	MW713731	This study
L. fissuratus	CLZhao 4352	MW713742	MW713732	This study
L. fissuratus	CLZhao 4394	MW713741	MW713733	This study
L. fissuratus	CLZhao 4577	MW/13/40	MW/13/34	This study
L. fissuratus	CLZhao 9784	MW/13/43	MW/13/35	This study
L. TUMOSUS	CLZnao 8188	NIVV/13/44	MW/13/36	I NIS STUDY
L. TUMOSUS	GLZNa0 17855	NIW/13/45	MW/13/3/	I his study
L. griseliniae	KHL 12971	DQ873651	DQ873651	Larsson <i>et al.</i> 2006
L. juniperi	KAS-GEL 4940	DQ340316	-	Dishasehl & Langer 2017
	CL Zhao 4516	MN045077	-	Chap & Zhao 2020
L. macrosporus	CLZ1140 4510	MN045075	10100204920	Chen & Zhao 2020
	KAS-GEL 1833	KV800300	- KV705061	Vurchenko et al 2020
	KAS-GEL 4008	KV800400		Vurchenko et al 2017
L microfasciculatus	CL Zhao 5109	MNQ54311		Chen & Zhao $2020$
L microfasciculatus	TNM F24757	.IN129976	_	Yurchenko & Wu 2014
L organensis	MSK-F 7247	KY800403	KY795967	Yurchenko et al 2017
L orientalis	KAS-GEL 3376	DQ340325	DQ340351	Yurchenko et al. 2017
L. orientalis	KAS-GEL 3400	DQ340326	_	Yurchenko <i>et al.</i> 2017
L. pruni	Ryberg 21018	DQ873624	DQ873625	Yurchenko et al. 2020
L. pruni	KAS-GEL 2327	DQ340312	DQ340349	Yurchenko et al. 2020
L. sambuci	80 SAMHYP	JX857721	-	Yurchenko <i>et al.</i> 2017
L. sambuci	83 SAMHYP	JX857720	-	Yurchenko <i>et al.</i> 2017
L. sambuci	KAS-JR 7	KY800402	KY795966	Yurchenko <i>et al.</i> 2017
L. vietnamensis	Wu 9807-88/TNM F9073	JX175044	KX857814	Yurchenko & Wu 2014
L. wuliangshanensis	CLZhao 4167	MN945979	-	Chen & Zhao 2020
L. wuliangshanensis	CLZhao 4108	MN945980	-	Chen & Zhao 2020
Oxyporus populinus	CBS 218.39	MH855986	MH867486	Vu <i>et al</i> . 2019
Palifer verecundus	KHL 12261	DQ873642	-	Larsson <i>et al</i> . 2006
Xylodon asperus	UC 2023169	KP814365	-	Unpublished
X. exilis	TUB-FO 42565	MH880198	MH884898	Riebesehl <i>et al.</i> 2019
X. flaviporus	MA-Fungi 79440	MH260071	MH260066	Fernández-López et al. 2018
X. hyphodontinus	LIP GG-MAR12-238	MH880207	MH884905	Riebesehl <i>et al.</i> 2019
X. nespori	B.Nordon 30915	DQ873622	DQ873622	Larsson <i>et al</i> . 2006
X. quercinus	CBS 333.62	MH858169	MH869761	Vu <i>et al.</i> 2019
X. rimosissimus	Ryberg 21031	DQ873627	DQ873628	Larsson <i>et al</i> . 2006

#### Table 1. List of species, specimens and GenBank accession numbers of sequences used in this study.

The ITS and ITS + LSU sequences were analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). Approaches to phylogenetic analyses followed Zhao and Wu (2017). MP analysis was performed in PAUP\* ver. 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 most-parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 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 most-parsimonious tree generated. ML was inferred with RAxML-HPC2 using the CIPRES Science Gateway (http://www.phylo.org/sub sections/portal/). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates and evaluated under the gamma model.

MrModeltest ver. 2.3 (https://github.com/ nylander/MrModeltest2) was used to determine the best-fit evolution model for the data set for Bayesian inference (BI), which was performed using MrBayes ver. 3.1.2 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 used in each of two runs from random starting trees for  $300 \times 10^3$  and  $1050 \times 10^3$  generations, with trees and parameters sampled every 100 generations. The first 25% of the generations was discarded as burn-in. A majority rule consensus tree of all remaining trees and posterior probabilities were calculated.

#### Results

#### Molecular phylogeny

The ITS + LSU data set (Fig. 1) included sequences from 37 fungal specimens representing 29 species belonging to 6 genera related to *Lyomyces* in the family Schizoporaceae. The data set had an aligned length of 1720 characters, of which 1013 characters were constant and 466 parsimony-informative. The MP analysis yielded seven equally parsimonious trees (TL = 2397, CI = 0.4631, HI = 0.5369, RI = 0.5602, RC = 0.2594). The best-fit model for ITS + LSU alignment estimated and applied in BI was GTR + I + G. At the end of the BI runs, the average standard deviation of split frequencies was 0.009721. The tree topology obtained by BI was similar to the one from MP and ML. In the phylogenetic reconstruction (Fig. 1) of Schizoporaceae, the two new species were nested into *Lyomyces* and both formed a well-supported lineage.

The ITS-only data set (Fig. 2) included sequences from 41 fungal specimens representing 21 species within Lyomyces. The aligned length of the data set was 632 characters, of which 284 characters were constant and 264 parsimony-informative. The MP analysis yielded 12 equally parsimonious trees (TL = 1120, CI =0.4955, HI = 0.5045, RI = 0.7025, RC = 0.3481). The best-fit model for the ITS alignment estimated and applied in BI was GTR + I + G. At the end of the BI runs, the average standard deviation of split frequencies was 0.009908. The tree topology obtained by BI was similar to the one from MP and ML. In the phylogenetic reconstruction based on the ITS-only data set (Fig. 2) our isolates of L. fissuratus formed a monophyletic lineage and grouped within a clade comprising L. mascarensis, L. cremeus, L. microfasciculatus and L. wuliangshanensis. Lyomyces fumosus was a sister species of L. fimbriatus.

#### Taxonomy

# *Lyomyces fissuratus* C.L. Zhao, *sp. nova* (Figs. 3 and 4)

MycoBank MB 839023. — HOLOTYPE: China. Yunnan Province: Puer, Jingdong County, Wuliangshan National Nature Reserve, 23.95°N, 100.37°E, on fallen branch of angiosperm, 5 October 2017 *CLZhao 4291 (SWFC004291).* — PARATYPES: Same locality as holotype, on trunk of angiosperm, 5 October 2017 *CLZhao 4299 (SWFC004299)*, *CLZhao 4352 (SWFC004352)*; 6 October 2017, *CLZhao 4394 (SWFC004394)*, *CLZhao 4577 (SWFC004577)*; on the fallen branch of angiosperm, 6 January 2019, *CLZhao 9784* (*SWFC009784*).

ETYMOLOGY: Referring to the cracking on the hymenial surface (Lat. *fissuratus* = fissured).





**Fig. 2.** Maximum parsimony strict consensus tree illustrating the phylogeny of two new species and related species in *Lyomyces* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50% and Bayesian posterior probabilities > 0.95, respectively.

Basidiomes annual, resupinate, subceraceous to membranaceous when fresh, becoming membranaceous and cracking upon drying, up to 16 cm long and 4.2 cm wide, 60–100  $\mu$ m thick. Hymenial surface tuberculate, white to cream when fresh, turning cream upon drying. Margin narrow, concolorous with hymenial surface. Hyphal system monomitic; generative hyphae with clamp connections, colourless, thick-walled, rarely branched, 2–3  $\mu$ m in diameter, IKI–, cyanophilous; tissues unchanged in KOH. Hymenium three kinds of cystidia: (1) capitate, rare, colourless, thin-walled, smooth or slightly encrusted, 17–30.5 × 1.8–5.5  $\mu$ m; (2) tapering, colourless, thin-walled, smooth or slightly encrusted,  $15.5-24.5 \times 3-4.5 \mu m$ ; (3) submoniliform, colourless, thin-walled, smooth or slightly encrusted,  $15.5-22 \times 2.7-4 \mu m$ ; cystidioles absent. Basidia clavate, thin-walled, with four sterigmata and a basal clamp connection,  $14.7-23.3 \times 2.9-4.8 \mu m$ ; basidioles abundant, in shape similar to basidia, but slightly smaller. Basidiospores broadly ellipsoid to subglobose, colourless, slightly thick-walled, smooth, IKI–, cyanophilous, with irregular-shaped inclusions or with single oil-like globule, (3.8)4–6(6.5)  $\times$  (3.4)3.6–5.3(5.5)  $\mu m$ ,  $L = 5.02 \mu m$ ,  $W = 4.38 \mu m$ , Q = 1.1-1.2 (n = 60/2).

HABITAT AND DISTRIBUTION: Lignicolous, causing a white rot. Thus far known only from China.



**Fig. 3.** Basidiomes of *Lyomyces fissuratus* (holotype).

# *Lyomyces fumosus* C.L. Zhao, *sp. nova* (Figs. 5 and 6)

MycoBank MB 839024. — HOLOTYPE: China. Yunnan Province: Yuxi, Xinping County, the ancient Tea Horse Road scenic area, 23.97°N, 101.52°E, on trunk of angiosperm, 21 August 2018 *CLZhao 8188* (SWFC008188). — PARA-TYPE: China. Yunnan Province: Honghe, Pingbian County, Daweishan National Nature Reserve, 22.95°N, 103.7°E, on fallen branch of angiosperm, 1 August 2019 *CLZhao 17855* (SWFC017855).

ETYMOLOGY: Referring to the fumate hymenial surface (Lat. *fumosus* = smoky).

Basidiomes annual, resupinate, farinaceous when fresh, becoming pruinose upon drying, up to 11 cm long and 2 cm wide,  $40-80 \mu$ m thick.

Hymenial surface smooth, white to smoky grey when fresh, turning to smoky grey upon drying. Margin narrow, smoky grey. Hyphal system monomitic; generative hyphae with clamp connections, colourless, thick-walled, rare branched,  $1.8-3.7 \mu m$  in diameter, IKI–, cyanophilous; tissues unchanged in KOH. Hymenium three kinds of cystidia: (1) capitate, colourless, thin-walled, smooth or slightly encrusted,  $15.4-48.7 \times$  $4-4.2 \mu m$ ; (2) tapering, colourless, thin-walled, smooth or slightly encrusted,  $18.2-50.6 \times 3.8 4.5 \mu m$ ; (3) moniliform, colourless, thin-walled, smooth or slightly encrusted,  $8.5-22.7 \times 2.5 3.7 \mu m$ ; cystidioles absent. Basidia clavate to barrel-shaped, with four sterigmata and a



Fig. 4. Microscopic structures of *Lyomyces fissuratus* (from the holotype). - A: Basidiospores. - B: Basidia and basidioles. -C: Capitate cystidia. - D: Tapering cystidia. - E: Submoniliform cystidia. -F: Section of hymenium. Scale bars: 5  $\mu$ m for A, 10  $\mu$ m for B-F.

basal clamp connection,  $11.5-17.5 \times 3-5 \mu m$ ; smooth, thin-walled, guttulate; basidioles abundant, in shape similar to basidia, but slightly smaller. Basidiospores broadly ellipsoid to globose, colourless, slightly thick-walled, smooth, IKI–, cyanophilous, with one to three oil-like globules,  $(3.4)3.8-6.2(7.1) \times 3.3-5.4(5.7) \mu m$ ,  $L = 4.99 \mu m$ ,  $W = 4.35 \mu m$ , Q = 1.25-1.3 (n = 60/2).

HABITAT AND DISTRIBUTION: Lignicolous, causing a white rot. Thus far known only from China.

#### Discussion

Prior to this study, the following nine Lyomyces species were reported from China: L. albus, L. bambusinus, L. capitatocystidiatus, L. cremeus, L. macrosporus, L. microfasciculatus, L. sambuci, L. tenuissimus and L. wuliangshanensis (Xiong et al. 2009, Yurchenko et al. 2013, Yurchenko & Wu 2014, Riebesehl & Langer 2017, Chen & Zhao 2020). Based on our morphological and phylogenetic analysis, all those can be separated from the two species described in this paper (Figs. 1 and 2).

The phylogenetic reconstruction of the ITS + LSU data set by Yurchenko *et al.* (2020) revealed that *Fasciodontia*, *Hastodontia*, *Hyphodontia*, *Lyomyces*, *Tubulicrinis* and *Xylodon* grouped together with *Lyomyces* and *Xylodon* being sister genera. In our analyses, *Lyomyces* grouped with *Fasciodontia*, *Hastodontia*, *Hyphodontia* and *Xylodon*, and the two new species were nested in *Lyomyces* based on ITS and LSU (Fig. 1). According to the ITS topology (Fig. 2) *L. fissuratus* is closely related to *L. mascarensis*, *L. cremeus*, *L. microfasciculatus* and *L. wuliangshanensis*, and *L. fimbriatus* and *L. fumosus* are sister species.



**Fig. 5.** Basidiomes of *Lyomyces fumosus* (holo-type).

Morphologically, *L. mascarensis* differs from *L. fissuratus* by having an odontioid hymenophore (Yurchenko *et al.* 2017), *L. cremeus* by having a smooth hymenophore and wider generative hyphae (3–5  $\mu$ m; Chen & Zhao 2020), *L. microfasciculatus* by having an odontioid hymenophore and presence of minute peg-like fascicles of hyphae (Yurchenko & Wu 2014), and *L. wuliangshanensis* by having a smooth hymenophore and narrower basidiospores (3.5–5.3 × 2.8–4  $\mu$ m; Chen & Zhao 2020).

*Lyomyces fimbriatus* differs from *L. fumosus* by having an odontioid hymenophore with fimbriate aculei (Wu 1990).

The family Schizoporaceae is an extensively studied group of Hymenochaetales (Dai 2012, Zhao *et al.* 2014, Viner *et al.* 2018, Cui *et al.* 2019, Riebesehl *et al.* 2019, Shi *et al.* 2019, He *et al.* 2020, Xu *et al.* 2020), but the species diversity in China is still not well known, especially in the subtropical and tropical areas. The two *Lyomyces* species here described are from the subtropics.

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Fig. 6. Microscopic structures of *Lyomyces fumosus* (from the holotype). – A: Basidiospores. – B: Basidia and basidioles. – C: Capitate cystidia. – D: Tapering cystidia. – E: Moniliform cystidia. – F: Section of hymenium. Scale bars: 5  $\mu$ m for A, 10  $\mu$ m for B–F.

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