热带亚热带植物学报
JOURNAL OF TROPICAL AND SUBTROPICAL BOTANY

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引用本文:
朱孟丽, 赵欣, 王伟成, 等. 中国肉盘衣属地衣新记录种[J]. 热带亚热带植物学报, 2022, 30(1): 125–131.

在线阅读 View online: https://doi.org/10.11926/jtsb.4398

热带亚热带植物学报 2022, 30(1): 125 ~ 131
Journal of Tropical and Subtropical Botany

中国肉盘衣属地衣新记录种

朱孟丽[1*], 赵欣[1*], 王伟成[2,3], 贾泽峰[1**]

(1. 聊城大学生命科学学院，山东 聊城 252059；2. 中国科学院微生物研究所，北京 100101；3. 中国科学院大学，北京 100049)

摘要：在对海南省叶生地衣资源的调查研究中发现了中国肉盘衣属地衣新记录种：乳头肉盘衣[*Fellhanera mastothallina* (Vain.) Lücking & Sérus.]，描述了其形态学、解剖学及化学特征，提供了显微结构照片。主要识别特征为：地衣体壳状，布满灰绿色乳头状颗粒，子囊盘棕色，囊盘被外部由疏丝组织构成，子囊孢子(3~)7 隔，26~36 μm ×2~3 μm，呈末端变窄的细棍棒状。基于线粒体小亚基(mtSSU)序列构建的系统发育树表明该种与本属模式种 *F. fuscatula* (Müll. Arg.) Vězda 具有较近的亲缘关系。编制了中国该属地衣的分种检索表。

关键词：乳头肉盘衣；茶渍纲；地衣型真菌；mtSSU；分类学；新记录

doi: 10.11926/jtsb.4398

A Newly Recorded Species of *Fellhanera* from China

ZHU Mengli[1*], ZHAO Xin[1*], WANG Weicheng[2,3], JIA Zefeng[1**]

(1. *College of Life Sciences, Liaocheng University*, Liaocheng 252059, Shandong, China; 2. *Institute of Microbiology, Chinese Academy of Sciences*, Beijing 100101, China; 3. *University of Chinese Academy of Sciences*, Beijing 100049, China)

Abstract: *Fellhanera mastothallina* (Vain.) Lücking & Sérus. was reported as a newly recorded species from China based on the foliicolous lichen specimens collected from Hainan Province. The characteristics of morphology, anatomy and chemistry were provided with microstructure photos. It was characterized by the crustose thallus with light green to orange-red verrucosa, brown apothecia, external exciple prosoplectenchymatous and narrowly clavate ascospores tapering towards proximal end, (3–)7-septate, 26–36 μm ×2–3 μm. The phylogenetic tree based on the mtSSU region showed that it had close relationship with the type species *F. fuscatula* (Müll. Arg.) Vězda. A key to known Chinese *Fellhanera* species was also provided.

Key words: *Fellhanera mastothallina*; Lecanoromycetes; Lichenized fungi; mtSSU; Taxonomy; New record

The genus *Fellhanera*, belonging to family Pilocarpaceae, in order *Lecanorales*, class Lecanoromycetes was established in 1986 with its type *F. fuscatula* (Müll. Arg.) Vězda[1–2], including more than 90 species worldwide[3–4]. *Fellhanera* is the second largest genus among foliicolous lichens next to *Porina* Acharius[5] and quite diverse in morphological features[6]. The distinctive traits of this genus are the comparatively small apothecia with a thin margin; paraplectenchymatous excipulum; usually indistinct, branched, and sparsely to densely anastomosing paraphyses; *Byssoloma*-type asci; ellipsoid to cylindrical, transversely septate to muriform ascospores; and pycnidial conidiomata[6]. *Fellhanera* are mostly foliicolous, common in pan-tropical ecologies.

China had previously reported nine *Fellhanera* species, which are distributed in Yunnan, Hainan, Hongkong and Taiwan[7–13]. Based on specimens

Received: 2021-03-01 **Accepted:** 2021-04-19
This work was supported by the National Natural Science Foundation of China (Grant No. 31700018; 31800010; 31750001).
ZHU Mengli (Born in 1994), Undergraduate, interesting in taxonomy of lichenous fungi. E-mail: 982340973@qq.com
* Co-first author
** Corresponding author. E-mail: zfjia2008@163.com

collected from Hainan Province, *Fellhanera mastothallina* (Vain.) Lücking & Sérus. is found new to China.

1 Materials and methods

1.1 Specimens and morphology

The specimens of new record are deposited in the Fungarium of College of Life Sciences, Liaocheng University (LCUF) and Herbarium Mycologicum Academiae Sinicae-Lichenes (HMAS-L). A dissecting microscope (Olympus SZX16) and a light microscope (Olympus BX53) were used for the morphological and anatomical studies. Measurements were taken from mature vertical sections of fruit bodies mounted in water.

1.2 Chemistry

Amyloidity of the ascospores was tested using Lugol's solution. Spot tests with K (10% aqueous solution of potassium hydroxide), C (saturated solution of aqueous sodium hypochlorite), and P (saturated solution of *p*-phenylenediamine in 95% ethyl alcohol) were performed on the thallus surface. The lichen substances were detected and identified by thin-layer chromatography, using solvent C[14–16].

1.3 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from ascomata of the specimens using the Hi-DNAsecure Plant Kit (Tiangen, Beijing, China) according to the manufacturer's protocol. PCR amplification was performed using the mtSSU1 and mtSSU3R primer pair for mtSSU[17]. The 25 μL PCR reaction system containing 1 μL each primer solution (10 μmol/L), 0.5 μL genomic DNA, 10 μL ddH_2O, and 12.5 μL 2 ×*Taq* PCR Master-Mix (Tiangen, Beijing, China). Thermocycling conditions comprised initial denaturation at 95 ℃ (5 min); 35 denaturation cycles at 94 ℃ (45 s), annealing at 50 ℃ (1 min), extension at 72 ℃ (1.5 min) and a final extension at 72 ℃ (10 min). The target product of PCR was affirmed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai). Nine newly generated sequences were submitted to GenBank. The sequences obtained were evaluated using BLASTn and combined with selected sequences of Pilocarpaceae from GenBank (Fig. 1), *Micarea adnata* and *M. micrococca* were used as the outgroup based on Ekman et al. [18]

1.4 Phylogenetic analysis

Contigs were assembled and edited using the program Geneious v. 6.1.2 (Biomatters Ltd., Auckland, NZ). A total of 39 sequences were aligned using MAFFT v. 7[19]. The program Gblocks v. 0.91b was used to delimit ambiguous regions, implementing all the options for a less stringent selection (http://molevol.cmima.csic.es/castresana/Gblocks_server.html)[20], which yielded final alignment of 670 bp. Maximum likelyhood (ML) and Bayesian inference (BI) were performed using the CIPRES Scientific gateway portal (http://www.phylo.org/portal2/)[21]. Maximum likelihood bootstrapping analysis was performed with RAxML-HPC v. 8[22], using the default parameters as implemented on the CIPRES, NSF XSEDE resource with bootstrap statistics calculated from 1 000 bootstrap replicates. For the Bayesian analysis, the best substitution model was estimated using jModelTest 2.1.6[23]. Based on the results, we used GTR+I+G model. Bayesian analysis was performed using MrBayes v. 3.2.2 on CIPRES with 2 independent runs, searching for 10 000 000 generations with four independent chains and sampling every 1000th tree[24]. After discarding the burn-in, the remaining 7 500 trees of each run were pooled to calculate a 50% majority rule consensus tree. Generated phylogenetic tree was visualized under Figtree v. 1.4.2[25].

2 Results

2.1 Phylogenetic analysis

The final alignment consisted of 9 newly generated mtSSU sequences and 30 sequences downloaded from NCBI (Fig. 1). The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian inference analysis (BI) exhibited the same topology; we therefore present only the ML tree. The molecular phylo-

geny based on the mitochondrial small subunit marker (mtSSU) of Pilocarpaceae exhibits a well-supported monophyletic lineage containing the genera *Byssolecania*, *Byssoloma*, *Calopadia*, *Fellhanera*, *Lasioloma*, *Sporopodium* and *Tapellaria*. The tree shows *Fellhanera* is polyphyletic in its current delimitation. *Fellhanera mastothallina* is revealed as a sister clade to the type species *F. fuscatula.* These two species together with *F. microdiscus*, *F. paradoxa* and *F. rhapidophylli* form a monophyletic lineage without good support (BS = 59%, PP = 0.85). *Aquacidia antricola*，*A. trachona*, *F. bouteillei*, *F. subtilis* and *Sporopodium antoninianum* cluster together and get a high support (BS = 96%, PP = 1.0). While another *Fellhanera* species, *F. viridisorediata* shows a close relationship with *Byssoloma leucoblepharum*.

2.2 Taxonomy

Fellhanera mastothallina (Vain.) Lücking & Sérus, in Lichenologist **33**(3): 192 (2001) Fig. 2

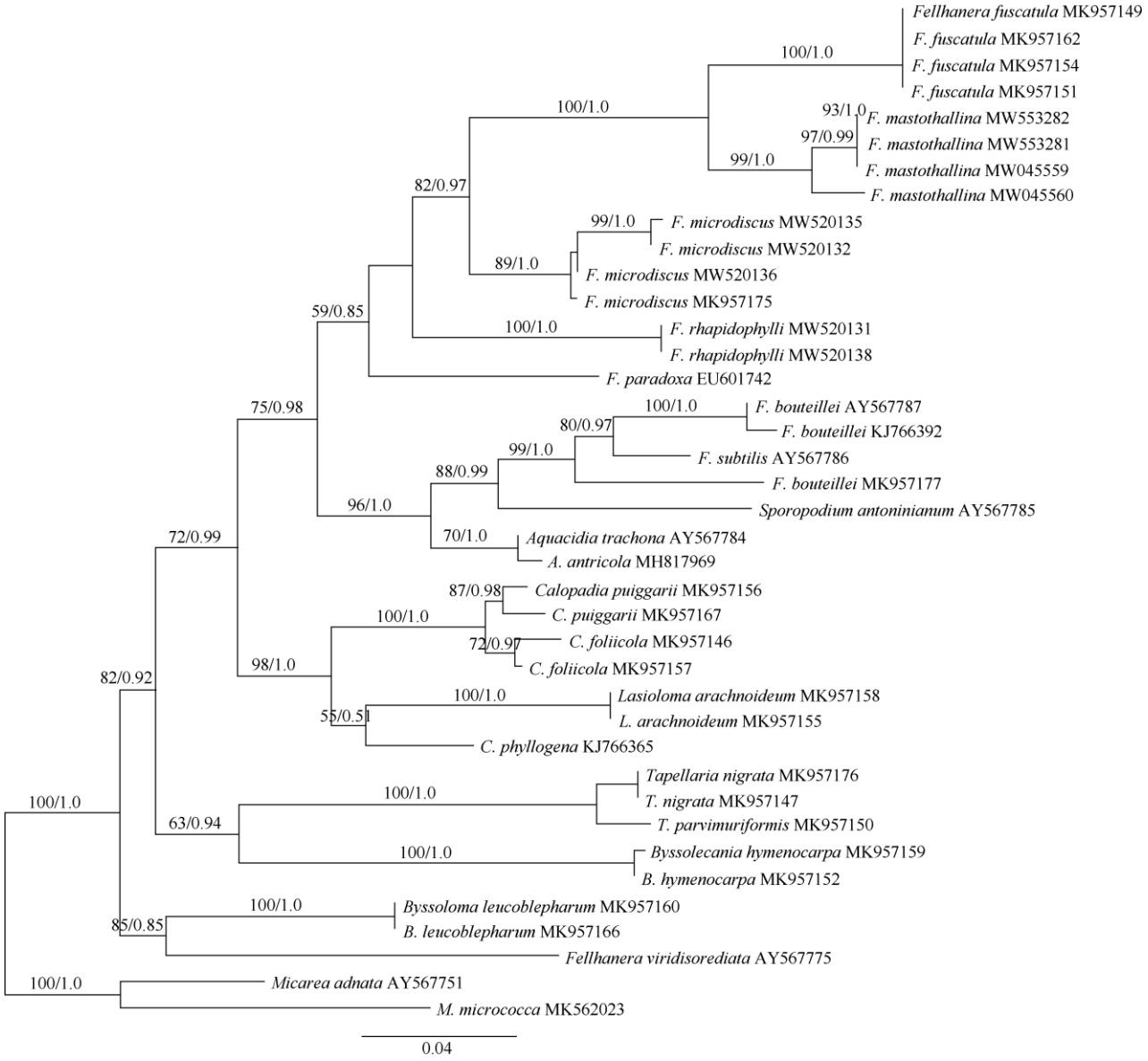

Fig. 1 Maximum likelihood tree of *Fellhanera mastothallina* and related species within Pilocarpaceae based on the mitochondrial small subunit marker (mtSSU). ML bootstrap values and MCMC posterior probabilities (second value) are displayed above each branch. Branches recovered with ML-BS support ≥ 70% and BI-PP support ≥ 0.95 were regarded as strongly supported. GenBank accessions are attached to the sequences. Newly generated sequences are shown in bold.

≡ *Bacidia mastothallina* Vain., in Ann. Acad. Sci. Fenn., ser. A, **15**: 64 (1921)

≡ *Bacidina mastothallina* (Vain.) Vězda, in Vězda et al., Ann. Naturh. Mus. Wien **99**B: 738 (1997)

Type: Philippines, Robinson & Ramos 11900 (TUR-holotype).

Description: Thallus foliicolous, crustose, continuous, verrucose, 10–40 mm across and 10–15 μm thick, greyish-green, rough, often irregular in outline, with light green to orange-red verrucosa of 0.01–0.03 mm diam. Apothecia when mature sessile, rounded, 0.2–0.5 mm diam. and 150–200 μm high; disc plane to slightly convex, brown to dark brown; margin distinct and thin, about 0.1 mm wide, usually accompanied by a white pruina. Excipulum light grey to light brown, 7–15 μm thick, internal parts appear paraplectenchymatuous and external parts prosoplectenchymatous. Hymenium 40–66 μm high, colourless. Hypothecium 26–74 μm high, brown to dark brown. Asci clavate, 36–48 μm ×4–9 μm. Ascospores 8 per ascus, narrowly clavate, and tapering towards proximal end, (3–)7-septate, 26–36 μm ×2–3 μm, about 10–13 times as long as broad; I+ violet. Pycnidia not observed.

Chemistry: Trace amounts of substances were detected by TLC. Spot tests on thallus: K+ dark brown, C–, P–, KC+ dark brown.

Ecology and distribution: The species is a typically foliicolous lichen, grows mainly in tropical Asia and also reported in Papua New Guinea, Australia and New Caledonia[26]. New to China.

Specimen examined: CHINA. Hainan: Wuzhishan City, Wuzhishan Nature Reserve, on leaves, 18°54′27″ N, 109°40′48″ E, elev. 730 m, 12 Dec. 2019, Y.H. Ju HN19446-a (GB accession No.: MW045559), HN19458 (GB accession No.: MW045560), HN19459 (LCUF). Changjiang County, Bawangling Nature Reserve, Baishitan Scenic Area, on leaves, 19°7′17″ N, 109°4′53″ E, elev. 700 m, 4 Sep, 2017, W. C. Wang HN20170025 (HMAS-L 139457, GB accession No.: MW553282); Yajia Scenic Area, on leaves, 19º7′17″N, 109º4′53″, elev. 550 m, 5 Sep. 2017. W. C. Wang HN20170124

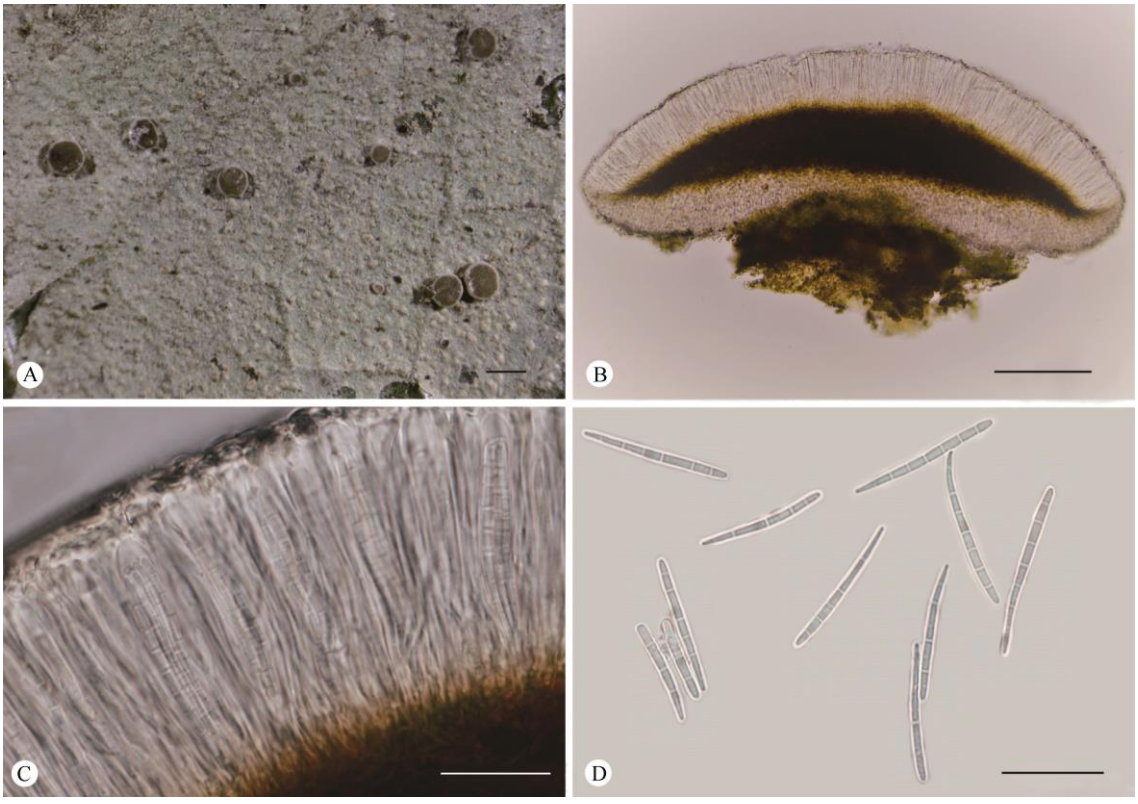

Fig. 2 *Fellhanera mastothallina* (Y.H. Ju HN19458). A: Thallus with apothecia; B: Vertical section of an apothecium; C: Part of a vertical section of an apothecium; D: Ascospores. Bars: A = 0.5 mm, B = 100 μm, C–D = 20 μm

(HMAS-L 139601, GB accession No.: MW553281).

3 Discussion

Phylogenetically *Fellhanera mastothallina* is close related to the type species *F. fuscatula*, this result confirms the hypothesis that *F. mastothallina* belonged to *F. fuscatula* group by Lücking based on evident from thallus and apothecial characters[6]. *Fellhanera fuscatula* can be distinguished by reddish brown apothecia, paraplectenchymatous exciple and shorter ascospores (18–24 μm ×3–4.5 μm). The morphological characteristics of our specimens collected from Hainan are almost identical with the type specimen from Phillippines and materials from Papua New Guinea and New Caledonia except for the longer and narrower ascospores (the latter are 22–32 μm ×3–4 μm)[26–27]. Our phylogenetic result that *Fellhanera* being heterogeneous in its current circumscription is coincident with the preliminary studies of the family Pilocarpaceae[13,28–29]. Some taxa within *Fellhanera* can be placed in other genera once sufficient evidence obtained in the future molecular studies with larger taxon sampling. Comparisons of the characteristics of the known Chinese species of *Fellhanera* are shown in Table 1.

Table 1 Comparisons of the characteristics of the known Chinese species of *Fellhanera*

Species	Disc color	Ascospores			Lichen compound	Locality
		Number	Size (μm)	Length/width		
F. bouteillei	Yellow to orange-yellow	1, septate	10–17 ×3–6	2.5–3.5	Usnic, isousnic, zeorin, asemone[30]	Yunnan[12], Taiwan[11], Hong Kong[7,9,10]
F. fuscatula	Ochraceous yellow to (reddish) brown	7, septate	18–24 ×3–4.5	5–6	–	Yunnan[12]
F. mastothallina	Brown to dark brown	7, septate	26–36 ×2–3	10–13	–	**Hainan**
F. microdiscus	Reddish brown	(3–)5, septate	14–21 ×3.5–5	3.5–4.5		Hainan[13]
F. rhaphidophylli	Reddish brown to dark greyish brown	3, septate	12–18 ×3–4	4–5	–	Taiwan[11], **Hainan**
F. semecarpi	Ochraceous yellow to reddish brown	1, septate	10–16 ×4–5	2.5–3.5	–	Yunnan[8], Taiwan[12]
F. subfuscatula	Brown	(3–)5, septate	14–26 ×3–5	4–6	–	Taiwan[11]
F. subternella	Yellow to orange-yellow	3(–4), septate	10–16 ×3–4.5	3–4	Usnic, isousnic, zeorine	Taiwan[11]
F. subtilis	Orange	3, septate	11–16 ×2.5–4.5	3.5–4.5	–	Taiwan[11]
F. viridisorediata	Dark brown	(0–)l, septate	(12–)14–17 ×3–5.5	3–4	–	Taiwan[11]

Localities newly recorded are shown in boldface.

Key to the known Chinese species of *Fellhanera*

1a. Lichen compounds present, mainly usnic, isousnic and zeorin acids ……2

1b. Lichen compounds absent ……3

2a. Ascospores 1-septate, 10–17 μm ×3–6 μm, 2.5–3.5 times as long as broad ……*F. bouteillei*

2b. Ascospores 3(–4)-septate, 10–16 μm ×3–4.5 μm, 3–4 times as long as broad ……*F. subternella*

3a. Ascospores l-septate or non-septate ……4

3b. Ascospores more than 1-septate ……5

4a. Ascospores oblong-ovoid, 1-septate, with constriction at septa, 10–16 μm ×4–5 μm, 2.5–3.5 times as long as broad ……*F. semecarpi*

4b. Ascospores elongate ellipsoid, (0–)l-septate, without constriction at septa, (12–)14–17 μm ×3–5.5 μm, 3–4 times as long as broad ……*F. viridisoredi*

5a. Ascospores only 3-septate ……6

5b. Ascospores 5 or 7-septate, rarely 3-septate ……7

6a. Disc orange; ascospores 11–16 μm ×2.5–4.5 μm, 3.5–4.5 times as long as broad ……*F. subtilis*

6b. Disc reddish brown to dark greyish brown; ascospores 12–18 μm ×3–4 μm, 4–5 times as long as broad ……*F. rhaphidophylli*

7a. Ascospores 5-septate, rarely 3-septate ……8

7b. Ascospores 7-septate, rarely 3 or 5-septate ……9

8a. Ascospores ellipsoid, usually slightly curved and attenuated at one end, 14–21 μm ×3.5–5 μm, 3.5–4.5 times as long as broad ……*F. microdiscus*

8b. Ascospores oblong, with slight constrictions at septa, 14–26 μm ×3–5 μm, 4–6 times as long as broad ……*F. subfuscatula*

9a. Ascospores oblong, 7-septate, with constrictions at septa, 18– 24 μm ×3–4.5 μm, 5–6 times as long as broad ……*F. fuscatula*

9b. Ascospores clavate, (3–)7-septate, rarely 3 or 5-septate, without constriction at septa, 26–36 μm ×2–3 μm, 10–13 times as long as broad ……*F. mastothallina*

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