

# A new species of *Perenniporia* (Polyporales, Basidiomycota) described from southern China based on morphological and molecular characters

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**Abstract** A new poroid wood-inhabiting basidiomycete, *Perenniporia nanlingensis*, collected in Guangdong Province, southern China, is described and illustrated on the basis of three collections. Both the morphology and phylogenetic analysis of rDNA ITS sequences support this new species. Macroscopically, the new species is characterized by an annual growth habit, resupinate basidiocarps with pinkish buff to cinnamon-buff pore surface when dry. Microscopically, it has a trimitic hyphal system, slightly dextrinoid and cyanophilous skeletal and binding hyphae, and its basidiospores are ellipsoid, truncate, strongly dextrinoid and cyanophilous,  $9.0\text{--}9.8 \times 5.0\text{--}5.9 \mu\text{m}$ .

**Keywords** Molecular phylogeny · Polypore · Wood-rotting fungi · Taxonomy

## Introduction

*Perenniporia* Murrill is a large, cosmopolitan genus. According to the modern definition it is characterized by ellipsoid to distinctly truncate basidiospores, which usually are thick-walled, cyanophilous and variably dextrinoid; its hyphal structure is di- to trimitic with clamp connections on generative hyphae and its vegetative hyphae are cyanophi-

lous and variably dextrinoid (Decock and Stalpers 2006). About ninety species have been described or transferred to the genus, and most of them occur in the tropics or subtropics (Cui et al. 2007; Dai et al. 2002; Decock 2001; Decock and Figueroa 2000; Decock and Ryvarden 1999, 2000, 2003; Decock et al. 2000, 2001; Gilbertson and Ryvarden 1987; Hattori and Lee 1999; Núñez and Ryvarden 2001; Ryvarden and Gilbertson 1994; Xiong et al. 2008).

Knowledge of *Perenniporia* in China was summarized by Dai et al. (2002). To date, 29 species of *Perenniporia* have been recorded from China (Cui et al. 2006, 2007, 2008, 2010; Dai and Penttilä 2006; Dai et al. 2003, 2004, 2007; Wang et al. 2009; Xiong et al. 2008). During an investigation of wood-inhabiting fungi in southern China, an additional undescribed species of *Perenniporia* was found. To confirm the affinity of the new taxon and infer its evolutionary relationships among similar species of *Perenniporia*, phylogenetic analyses were performed based on ITS sequences.

## Materials and methods

**Morphological studies** The studied specimens are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic routine used in the study follows Dai (2010). Sections were studied at magnification up to  $\times 1000$  using a Nikon Eclipse E 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements and drawings were made from slide prepa-

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rations stained with Cotton Blue and Melzer's reagent. Spores were measured from sections cut from the tubes. In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. In the text the following abbreviations are used: KOH=5% potassium hydroxide, CB=Cotton Blue, CB+=cyanophilous, 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=number of spores measured from given number of specimens. Special color terms are from Anonymous (1969) and Petersen (1996).

**Molecular procedures and phylogenetic analyses** The fungal taxa used in this study are listed in Table 1. Phire Plant Direct PCR Kit (Finnzymes) procedure was used to extract total genomic DNA from the fruitbody and for the polymerase chain reaction (PCR), and PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide (Stöger et al. 2006). DNA sequencing was performed at Beijing Genomics Institute. All newly generated sequences have been submitted to GenBank and are listed in Table 1.

Sequences were aligned with additional sequences from GenBank (Table 1) using BioEdit (Hall 1999) and ClustalX (Thomson et al. 1997). Alignment was manually adjusted to allow maximum alignment and minimize gaps. In the study, nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5 (Gardes and Bruns 1993; White et al. 1990).

Maximum parsimony and MrBayesian analysis were applied to the ITS dataset. All characters were weighted and gaps were treated as missing data. Maximum parsimony analysis (PAUP\* version 4.0b10) was used (Swofford and PAUP\* 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed and all parsimonious trees were saved. Clade stability 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 all trees generated under different optimality criteria. Bayesian analysis with MrBayes3.1.2 (Ronquist and Huelsenbeck 2003) implementing the Markov Chain Monte Carlo (MCMC) technique and parameters predetermined with MrMODELTEST2.3 (Posada and Crandall 1998; Nylander 2004) was performed and the parameters in MrBayes were set as follows: lset nst=6, and rates=gamma.

Four simultaneous Markov chains were run with 1,500,000 generations, starting from random trees, and keeping one tree every 1,000th generation.

## Results

### Taxonomy

*Perenniporia nanlingensis* B.K. Cui & C.L. Zhao, sp. nov. (Fig. 1)

Mycobank no.: MB 561625

*Carpophorum annuum, resupinatum. Facies pororum cremae bubalina vel roseo-bubalina; pori rotundi, 6–7 per mm. Systema hypharum trimiticum, hyphae generatoriae fibulatae, hyphae skeletales subiculi 2.6–5.2 µm in diam. Sporae hyalinae, ellipsoideae, truncatae, dextrinoideae, CB+, 9.0–9.8 × 5.0–5.9 µm.*

*Type.* — CHINA. Guangdong Province, Ruyang County, Nanling Nature Reserve, on dead angiosperm tree, 16. IX.2009 Cui 7589 (holotype in BJFC); Cui 7541 & 7620 (paratype in BJFC).

*Etymology.* — *nanlingensis* (Lat.): referring to the mountain name in southern China.

*Fruitbody.* — Basidiocarps annual, resupinate, adnate, not easily separated from substrate, corky, without odour or taste when fresh, becoming hard corky upon drying, up to 35 cm long, 10 cm wide, 5.5 mm thick at center. Pore surface cream-buff to yellowish buff when fresh, pinkish buff to cinnamon-buff upon drying; pores round, 6–7 per mm; dissepiments thick, entire. Sterile margin narrow, cream-buff, up to 1 mm wide. Subiculum cream to buff, thin, up to 0.5 mm thick. Tubes concolorous with pore surface, hard corky, up to 5 mm long.

*Hyphal structure.* — Hyphal system trimitic; generative hyphae with clamp connections; skeletal and binding hyphae weakly detrinoid, CB+, tissues unchanged in KOH.

*Subiculum.* — Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2.5–3.8 µm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, branched, interwoven, 2.6–5.2 µm in diam.; binding hyphae hyaline, thick-walled, frequently branched, flexuous, interwoven, 1.5–2.5 µm in diam.

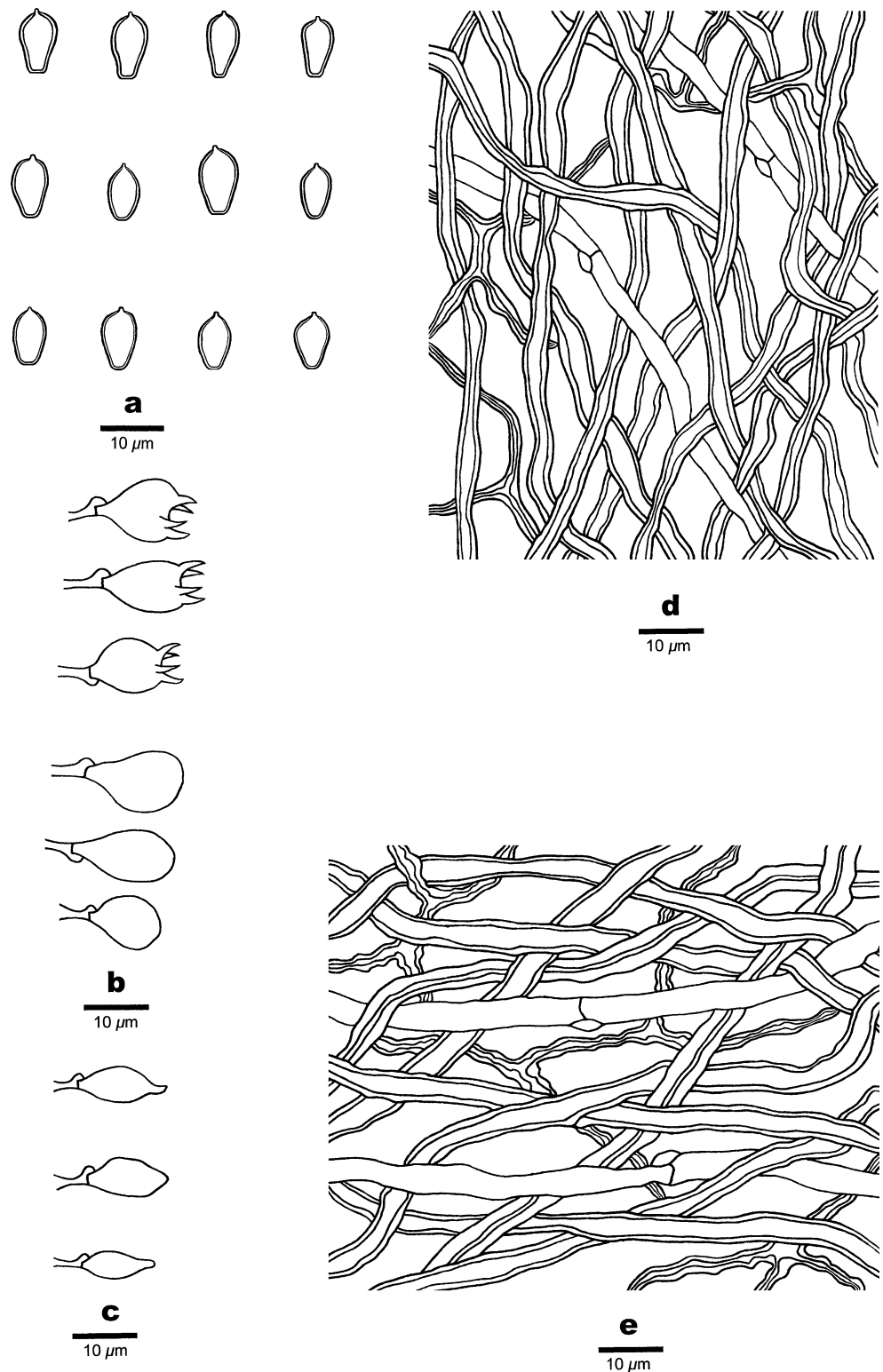
*Tubes.* — Generative hyphae infrequent, hyaline, thin-walled, unbranched, 2.8–3.5 µm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, branched, interwoven, 2.9–5 µm; binding hyphae hyaline, thick-walled, frequently branched, interwoven, 1.1–2.7 µm in diam. Cystidia absent, but fusoid cystidioles present, hyaline, thin-walled, 10–13 × 4.2–6 µm; basidia barrel-shaped, with four sterigmata and a basal clamp

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

Fungal taxon	Specimen No.	GenBank No. ITS
<i>Perenniporia corticola</i> (Corner) Decock	Cui 2655	HQ654093
<i>P. corticola</i>	Dai 7330	HQ654094
<i>P. corticola</i>	Cui 1248	HQ848472
<i>P. detrita</i> (Berk.) Ryvarden	MUCL 42649	FJ411099 *
<i>P. fergusii</i> Gilb. & Ryvarden	Gilbertson 16116	HQ876607
<i>P. formosana</i> T.T. Chang	Dai 5245	HQ876612
<i>P. fraxinea</i> (Bull.) Ryvarden	Cui 7154	HQ654095
<i>P. fraxinea</i>	Cui 8885	HQ876611
<i>P. fraxinea</i>	DP83	AM269789 *
<i>P. japonica</i> (Yasuda) T. Hatt. & Ryvarden	Cui 7047	HQ654097
<i>P. latissima</i> (Bres.) Ryvarden	Cui 6652	HQ876604
<i>P. latissima</i>	Dai 9368	FJ617304
<i>P. maackia</i> (Bondartsev & Ljub.) Parmasto	Cui 8929	HQ654102
<i>P. martia</i> (Berk.) Ryvarden	Cui 7992	HQ876603
<i>P. martia</i>	MUCL 41677	FJ411092 *
<i>P. martia</i>	MUCL 41678	FJ411093 *
<i>P. medulla-panis</i> (Jacq.) Donk	Dai 10780	HQ654099
<i>P. medulla-panis</i>	Dai 8736	HQ654100
<i>P. medulla-panis</i>	MUCL 43250	FJ411087 *
<i>P. medulla-panis</i>	MUCL 49581	FJ411088 *
<i>P. minor</i> Y.C. Dai & H.X. Xiong	Cui 5738	HQ848475
<i>P. minor</i>	Cui 5782	HQ883475
<i>P. minutissima</i> (Yasuda) T. Hatt. & Ryvarden	Dai 11643	HQ876602
<i>P. nanlingensis</i> B.K. Cui & C.L. Zhao	Cui 7620	HQ848477
<i>P. nanlingensis</i>	Cui 7589	HQ848478
<i>P. nanlingensis</i>	Cui 7541	HQ848479
<i>P. narymica</i> (Pilát) Pouzar	Dai 10510	HQ654101
<i>P. ochroleuca</i> (Berk.) Ryvarden	Cui8817	HQ848476
<i>P. ochroleuca</i>	Dai 11486	HQ654105
<i>P. ochroleuca</i>	MUCL 39563	FJ411097 *
<i>P. ochroleuca</i>	MUCL 39726	FJ411098 *
<i>P. ohiensis</i> (Berk.) Ryvarden	Cui 5714	HQ654103
<i>P. ohiensis</i>	MUCL 41036	FJ411096 *
<i>P. rhizomorpha</i> B.K. Cui et al.	Cui 7507	HQ654107
<i>P. robiniophila</i> (Murrill) Ryvarden	Cui 7144	HQ876608
<i>P. robiniophila</i>	Cui 5644	HQ876609
<i>P. robiniophila</i>	Cui 9174	HQ876610
<i>P. robiniophila</i>	Dai 10416	HQ654096
<i>P. straminea</i> (Bres.) Ryvarden	Cui 8858	HQ654104
<i>P. straminea</i>	Cui 8718	HQ876600
<i>P. subacida</i> (Peck) Donk	Dai 8224	HQ876605
<i>P. subacida</i>	MUCL 31402	FJ411103 *
<i>P. subacida</i>	Cui 3643	FJ613655
<i>P. subadusta</i> (Z.S. Bi & G.Y. Zheng) Y.C. Dai	Cui 8459	HQ876606
<i>P. tenuis</i> (Schwein.) Ryvarden	Cui 5523	HQ848474
<i>P. tephropora</i> (Mont.) Ryvarden	Cui 6331	HQ848473
<i>P. tephropora</i>	Cui 9029	HQ876601
<i>P. truncatospora</i> (Lloyd) Ryvarden	Dai 5125	HQ654098
<i>Ganoderma applanatum</i> (Pers.) Pat.	BCRC 36235	EU232219 *
<i>Polyporus arcularius</i> (Batsch) Fr.	CulTENN7883	AF516524 *

\* Sequences downloaded from Genbank

**Fig. 1** Microscopic structures of *Perenniporia nanlingensis* B.K. Cui & C.L. Zhao (drawn from the holotype). **a** Basidiospores. **b** Basidia and basidioles. **c** Cystidioles. **d** Hyphae from trama. **e** Hyphae from subiculum



connection,  $10.2\text{--}11.1 \times 8.2\text{--}9.3 \mu\text{m}$ ; basidioles dominant, in shape similar to basidia, but slightly smaller.

**Spores.** — Basidiospores ellipsoid, distinctly truncate, hyaline, thick-walled, smooth, strongly dextrinoid, CB+,  $(8.8\text{--}9.0\text{--}9.8\text{--}10.0) \times (4.9\text{--}5.0\text{--}5.9\text{--}6.0) \mu\text{m}$ ,  $L=9.32 \mu\text{m}$ ,  $W=5.39 \mu\text{m}$ ,  $Q=1.7\text{--}1.76$  ( $n=90/3$ ).

#### Molecular phylogeny

The ITS dataset included sequences from 50 fungal specimens representing 26 taxa. The dataset had an aligned length of 577 base pairs with 303 parsimony informative positions. Parsimony analysis yielded one parsimonious

tree (TL=362, CI=0.503, RI=0.765, RC=0.385, HI=0.497) that is shown in Fig. 2. Bayesian analysis resulted in average standard deviation of split frequencies=0.006778. In phylogenetic reconstruction, the sequences of *Polyporus arcularius* (Batsch) Fr. obtained from GenBank (AF516524) was used as outgroup. The ITS strict consensus tree (Fig. 2) generated by Bayesian analysis and Maximum Parsimony showed sequences of *Perenniporia nanlingensis* were grouped together with other species of *Perenniporia* as a monophyletic cluster with strong support.

**Discussion**

The phylogenetic analysis showed that the three samples of *Perenniporia nanlingensis* formed a distinct lineage and showed phylogenetic distance from other taxa. Both morphology and rDNA data confirmed that the three samples represent a new species in *Perenniporia*.

*Perenniporia nanlingensis* is characterized by an annual growth habit, resupinate basidiocarps with pinkish buff to cinnamon-buff pore surface, a trimitic hyphal system with slightly dextrinoid and cyanophilous skeletal and binding

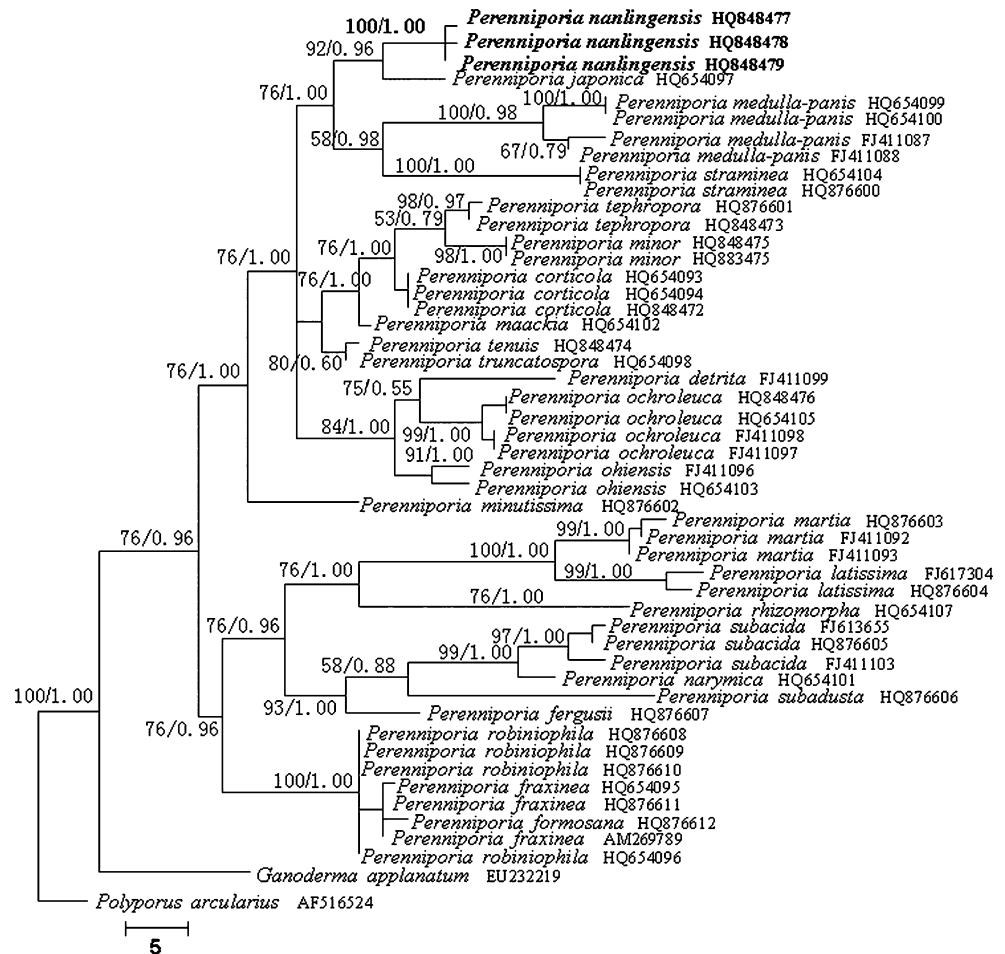
hyphae, and its basidiospores are ellipsoid, truncate, strongly dextrinoid and cyanophilous,  $9.0\text{--}9.8 \times 5.0\text{--}5.9 \mu\text{m}$ .

*Perenniporia roseoisabellina* (Pat. & Gaillard) Ryvarden and *P. nanlingensis* share similar truncate basidiospores, but the former differs from the latter in larger pores (2–3 per mm) and nondextrinoid hyphae (Hattori and Lee 1999). *Perenniporia subaurantiaca* (Rodway & Cleland) P.K. Buchanan & Ryvarden resembles *P. nanlingensis* in both having similar sized pores and basidiospores, but the former has a pale brown pore surface and non-truncate basidiospores (Hattori and Lee 1999).

*Perenniporia nanlingensis* may be confused with *P. subacida* (Peck) Donk by sharing resupinate basidiocarps and a buff to cinnamon-buff pore surface (Núñez and Ryvarden 2001), but the latter species is distinguished from *P. nanlingensis* by having perennial basidiocarps and smaller basidiospores ( $4.3\text{--}5.4 \times 3.2\text{--}4.1 \mu\text{m}$ , Dai et al. 2002).

*Perenniporia straminea* (Bres.) Ryvarden is similar to *P. nanlingensis*, both having an annual growth habit, resupinate basidiocarps, similar pores (6–7 per mm), and truncate basidiospores. However, *P. straminea* is distinguished from

**Fig. 2** Strict consensus tree illustrating the phylogeny of *Perenniporia nanlingensis* B.K. Cui & C.L. Zhao generated by Maximum Parsimony and Bayesian analysis based on ITS sequences, Parsimony bootstrap and Bayesian posterior probabilities more than 50% are shown the above or below branches



*P. nanlingensis* by having distinctly smaller basidiospores ( $3.2\text{--}4 \times 2.4\text{--}3 \mu\text{m}$ , Cui et al. 2010).

Phylogenetically, *Perenniporia japonica* (Yasuda) T. Hatt. & Ryvarden is so far the most closely related species to *P. nanlingensis*. The former species, however, differs from *P. nanlingensis* by having rhizomorphs and smaller basidiospores,  $4\text{--}5.2 \times 3\text{--}3.9 \mu\text{m}$  (Dai et al. 2002).

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