

***Donkioporiella mellea* gen. et sp. nov. (Polyporales, Basidiomycota) from Guangxi, China**

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Abstract – A monotypic genus *Donkioporiella* is newly introduced for a new species *D. mellea*. *Donkioporiella* is characterized by perennial, resupinate and woody basidiocarps, trimitic hyphal system with clamped generative hyphae, and broadly ellipsoid, pale yellowish to yellow, thick-walled, smooth, cyanophilous basidiospores. Morphologically, *Donkioporiella* resembles *Donkioporiella*, but differs in its thin-walled and acyanophilous basidiospores. *Donkioporiella* is composed of two rare species, viz. *D. albidofusca* and *D. expansa*; both species differ from *Donkioporiella mellea* in their larger pores and narrower basidiospores. The phylogenies inferred from nLSU and ITS datasets support that *Donkioporiella mellea* fell into the core clade of Polyporales and represented a distinct lineage. This lineage was most related with *Donkioporia*, but the weak statistical supports suggested it as a separate genus from *Donkioporia*.

Core clade of Polyporales / *Donkioporia* / Phylogeny / Polypore / Taxonomy

INTRODUCTION

Polypores, a group of wood-decaying basidiomycete fungi, are distributed in at least ten orders of Agaricomycetes and most species belong to Polyporales and Hymenochaetales (Dai, 2012). Recently, many new taxa in family, generic and species ranks have been described in these two orders from China (Li & Cui, 2013; Ariyawansa *et al.*, 2015; Zhao *et al.*, 2015; Zhou, 2015a, b; Zhou & Dai, 2012; Zhou *et al.*, 2016a, b, c). However, there are still many unidentified Chinese specimens deposited at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). When reexamining these specimens, three specimens collected from Guangxi, southern China, draw our attention. They resemble members of Hymenochaetales, Hymenochaetales, in macromorphology, but appear totally different in their microscopic characters. After phylogenetic analysis and more detailed morphological examination, the three specimens were identified to represent a previously unknown monotypic genus in the core clade of Polyporales. They are newly introduced in the present study.

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MATERIALS AND METHODS

Morphological examination –. The microscopic procedure followed Dai *et al.* (2014). Cotton blue (CB), Melzer's reagent (IKI) and 5% potassium hydroxide (KOH) were used to stain specimen sections, which were examined using a Nikon Eclipse 80i microscope under phase contrast illumination at magnification 1000 \times . Microscopic structures were drawn with the aid of a drawing tube and all measurements were taken from sections under CB. When presenting basidiospore size, 5% of the measurements from each end of the range are given in parentheses; L is short for mean basidiospore length (arithmetic average of all basidiospores), W for mean basidiospore width (arithmetic average of all basidiospores), Q for the variation of L/W ratio between the specimens studied and n for number of basidiospores measured from a given number of specimens. Special color terms for basidiocarps follow Petersen (1996).

Molecular sequencing –. Three herbarium specimens from Guangxi were directly employed as the template for PCR amplification using the Phire[®] Plant Direct PCR Kit (Finnzymes Oy, Finland) according to the manufacturer's instructions. The nuclear large subunit ribosomal DNA (nLSU) and internal transcribed spacer (ITS) sequences were, respectively, amplified with primer pairs LR0R and LR7 (Vilgalys & Hester, 1990) and ITS5 and ITS4 (White *et al.*, 1990). The PCR procedure was as follows: initial denaturation at 98°C for 5 min, followed by 39 cycles at 98°C for 5 s, 48°C (for nLSU region) / 59°C (for ITS region) for 5 s and 72°C for 5 s, and a final extension at 72°C for 10 min. The PCR products were sequenced at the Beijing Genomics Institute, China using primers LR0R, LR7, LR3 and LR3R (Vilgalys & Hester, 1990) for nLSU region and ITS5 and ITS4 for ITS region. All newly generated sequences were deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>).

Phylogenetic analysis –. Nucleotide blast searches using the new sequences as queries show that the newly sequenced specimens have an affinity to members of Polyporales but not Hymenochaetaeae. Therefore, to exactly explore the phylogenetic position of the newly sequenced specimens in Polyporales, their nLSU sequences and those representing species from all known clades in Polyporales were compiled for the current phylogenetic analysis. Species from Theleporales were selected as the outgroup (Hibbett *et al.*, 2007). ITS dataset was used to further elucidate the phylogenetic relationship of the newly sequenced specimens with previously known species based on the phylogenetic results inferred from the nLSU dataset, and *Sebipora aquosa* Miettinen and *Gelatoporia subvermispora* (Pilát) Niemelä were accordingly selected as the outgroup.

The two datasets were aligned using MAFFT 7.110 (Katoh & Standley, 2013) with the Q-INS-i option (Katoh & Toh, 2008). The resulting alignments were deposited in TreeBASE (<http://www.treebase.org>; accession numbers S19298), and their best-fit evolutionary models were estimated using jModelTest 2.1.4 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012). According to the evolutionary models, maximum likelihood (ML) and Bayesian inference (BI) algorithms were used to phylogenetically analyze the two alignments. ML analysis was performed using raxmlGUI 1.2 (Stamatakis, 2006; Silvestro & Michalak, 2012) under the auto FC option for bootstrap (BS) replicates (Pattengale *et al.*, 2010). BI analysis was conducted using MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) with two independent runs, each including four chains and starting from random trees. Ten million generations were set and trees were sampled every 1000th generation. The first 25%

of sampled trees were discarded as burn in, whereas all remaining trees were used to construct a 50% majority consensus tree and for calculating Bayesian posterior probabilities (BPPs). Chain convergence was judged using Tracer 1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>).

RESULTS

Molecular phylogeny – Three nLSU sequences and two ITS sequences were newly generated (Table 1). The nLSU dataset has 66 sequences and resulted in an alignment with 902 characters, while the ITS dataset, including 29 sequences and resulted in an alignment with 675 characters. The best-fit evolutionary model for both alignments were estimated as GTR + I + G. The BS searches in ML analyses stopped after 400 and 350 replicates, respectively, for nLSU and ITS datasets. All chains converged in BI analyses for both datasets after ten million generations, where the effective sample sizes of all parameters were more than 3000 and the potential scale reduction factors were close to 1.000. ML and BI analyses generated congruent topologies in main lineages for nLSU and ITS datasets, and thus only the topologies from the ML analyses are presented along with statistical values from both analyses at the nodes.

In the phylogeny inferred from the nLSU dataset (Fig. 1), three newly sequenced specimens, viz. LWZ 20140622-11, LWZ 20140622-12 and LWZ 20140622-15, formed a strongly supported terminal lineage (98%/1) nested within the core clade of Polyporales. This lineage was related to *Donkioporia expansa* (Desm.) Kotl. & Pouzar without reliable statistical support (69%/1; Fig. 1). The phylogeny inferred from the ITS dataset (Fig. 2), concentrating the core clade of Polyporales, also show the newly sequenced specimens LWZ 20140622-12 and LWZ 20140622-15 formed a distinct lineage with full statistical supports (100%/1) that has a weakly supported relationship with *Donkioporia albidofusca* (Domański) Vlasák & Kout and *D. expansa* (72%/0.88). Combined with distinct morphological characters, the three newly sequenced specimens are described as a new species representing a new genus.

Taxonomy

Donkioporiella L.W. Zhou, gen. nov.

MycoBank no.: MB 817113

Type species: *Donkioporiella mellea* L.W. Zhou (described below).

Etymology. *Donkioporiella* (Lat.): referring to the similarity to the genus *Donkioporia* Kotl. & Pouzar.

Basidiocarps perennial, resupinate, sometimes with elevated margins, inseparable from substrate, woody. **Hyphal system** trimitic; **generative hyphae** with clamp connections, hyaline thin-walled; **skeletal hyphae** dominant, yellow to brown, thick-walled; **binding hyphae** hyaline, thick-walled, frequently branched. **Basidiospores** broadly ellipsoid, pale yellowish to yellow, thick-walled, smooth, inamyloid, indextrinoid, cyanophilous.

Donkioporiella mellea L.W. Zhou, sp. nov.

MycoBank no.: MB 817114

Figs 3, 4

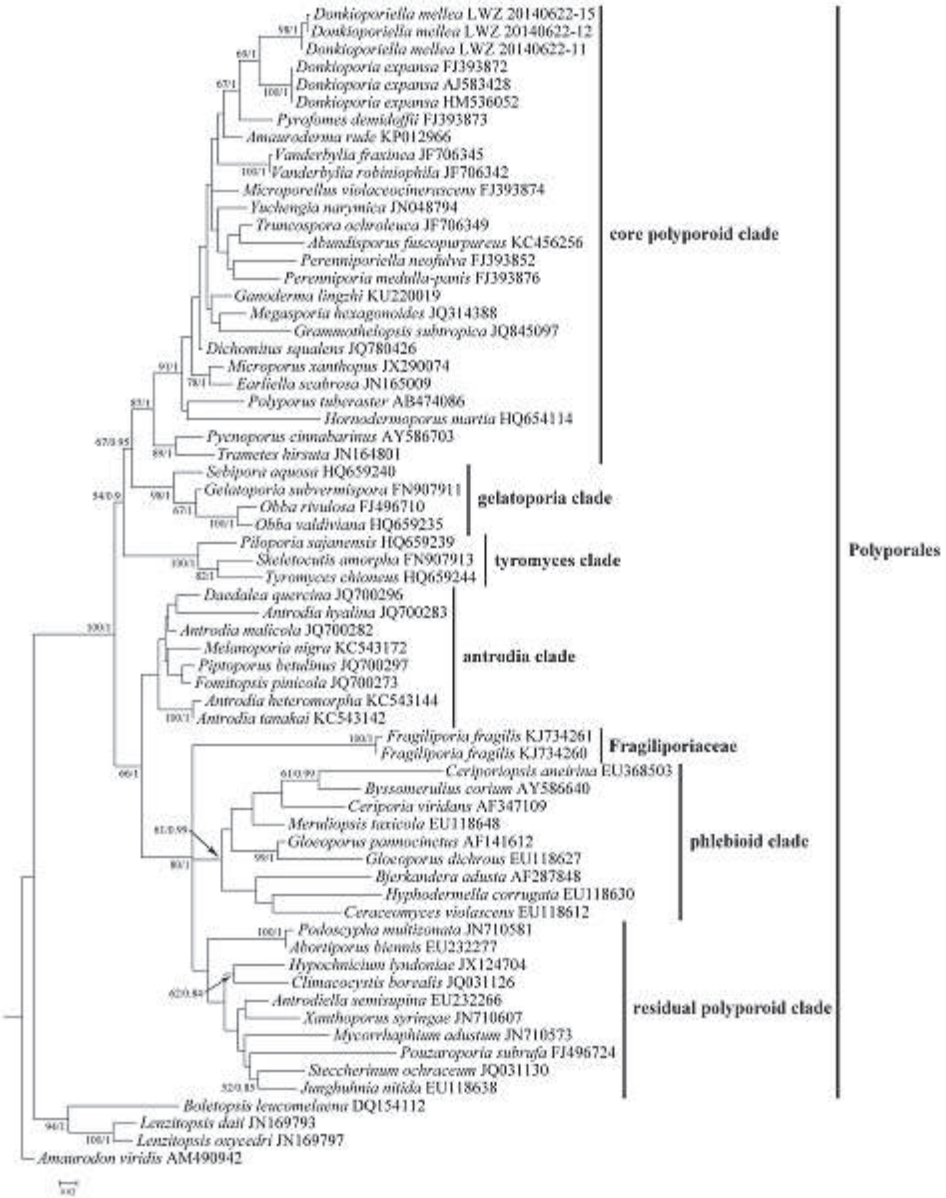


Fig. 1. Phylogenetic position of *Donkioporiella mellea* in Polyporales inferred from the nLSU dataset. The topology is from the maximum likelihood analysis with statistical values, respectively, from maximum likelihood and Bayesian inference analyses, if simultaneously above 50% and 0.8, at the nodes.

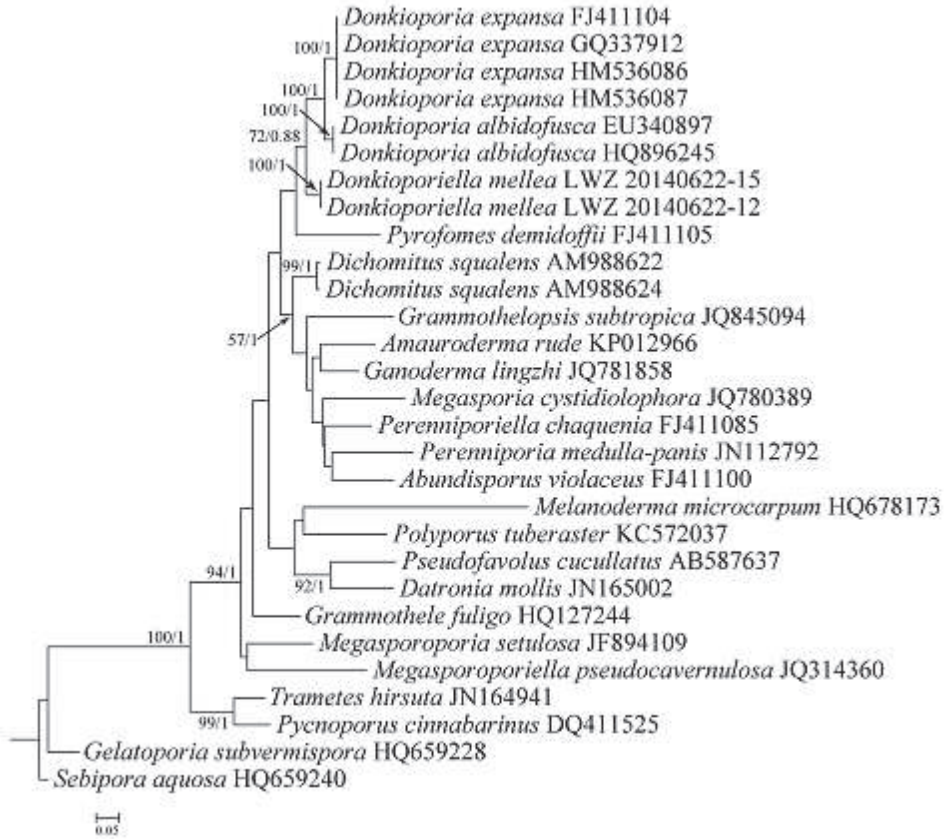


Fig. 2. Phylogenetic relationship of *Donkioporiella mellea* with other species in the core clade of Polyporales inferred from the ITS dataset. The topology is from the maximum likelihood analysis with statistical values, respectively, from maximum likelihood and Bayesian inference analyses, if simultaneously above 50% and 0.8, at the nodes.

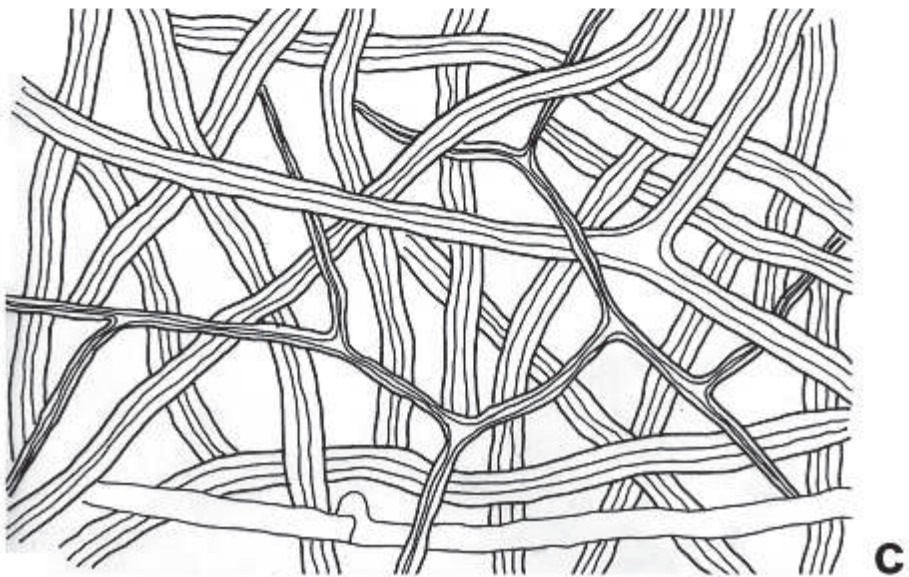
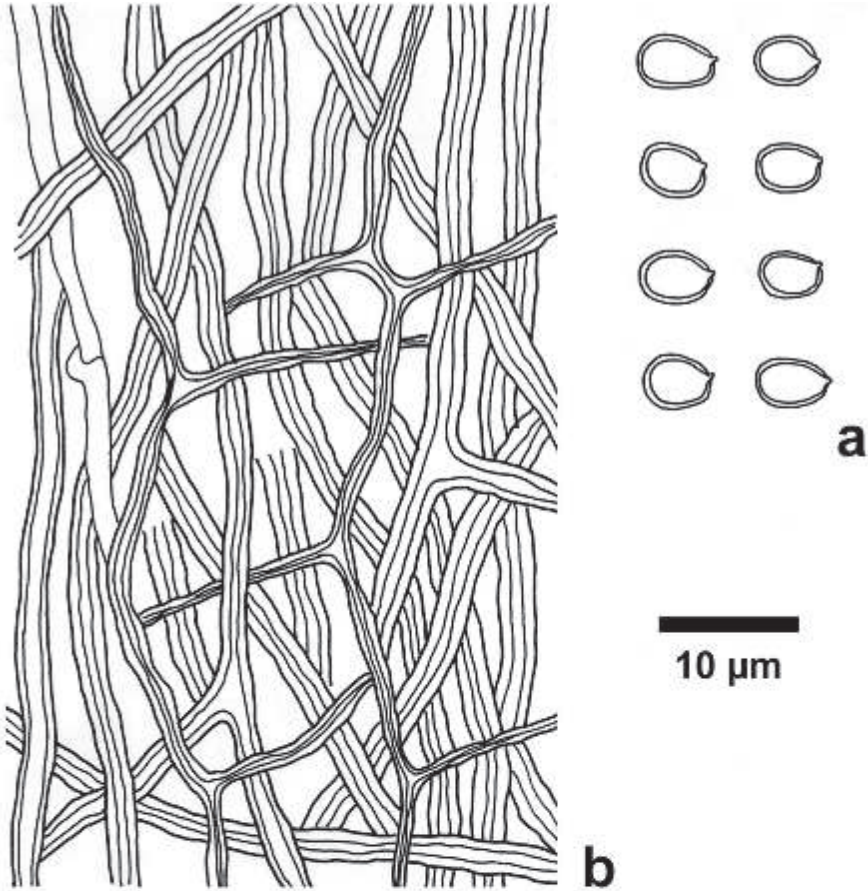
Table 1. Information for the newly generated sequences

Species name	Specimen no.	GenBank Accession no.	
		nLSU	ITS
<i>Donkioporiella mellea</i> L.W. Zhou	LWZ 20140622-11 (IFP)	KX258954	–
<i>D. mellea</i>	LWZ 20140622-12 (IFP)	KX258955	KX258957
<i>D. mellea</i>	LWZ 20140622-15 (IFP)	KX258956	KX258958



Fig. 3. Basidiocarps of *Donkioporiella mellea*. **a.** LWZ 20140622-15 (the holotype). **b.** LWZ 20140622-12. Scale bars: 5 cm.

Fig. 4. Microscopic structures of *Donkioporiella mellea* (drawn from the holotype). **a.** Basidiospores. **b.** Hyphae from trama. **c.** Hyphae from subiculum. Scale bar: 10 μ m. ▶



Holotypus: China, Guangxi, Jinxiu County, Dayaoshan National Nature Reserve, on dead standing angiosperm tree, 22 June 2014, LWZ 20140622-15 (IFP).

Etymology. *mellea* (Lat.): referring to the cinnamon-buff to honey-yellow color of the pore surface.

Basidiocarps perennial, resupinate, sometimes with elevated margins, inseparable from substrate, without odor or taste when fresh, woody, up to 40 cm long, 15 cm wide and 1 cm thick. **Pore surface** cinnamon-buff to honey-yellow, shining; **sterile margin** distinct, honey-yellow, up to 3 mm wide. **Pores** round, 7-9 per mm; **dissepiments** thick, entire. **Subiculum** yellowish brown, woody, up to 3 mm thick. **Tubes** cinnamon-buff, woody, up to 7 mm long.

Hyphal system trimitic; generative hyphae with clamp connections; tissue darkening but otherwise unchanged in KOH. **Subiculum:** generative hyphae very rare, hyaline, thin-walled, unbranched, 2-3 μm in diam; skeletal hyphae dominant, brown, thick-walled with a narrow lumen to subsolid, occasionally branched, aseptate, loosely interwoven, 2-4 μm in diam; binding hyphae infrequent, hyaline, thick-walled with a narrow lumen to subsolid, frequently branched, 1-2 μm . **Tubes:** generative hyphae very rare, hyaline, thin-walled, unbranched, 1.5-3 μm in diam; skeletal hyphae dominant, yellow, thick-walled with a wide to narrow lumen, occasionally branched, aseptate, interwoven, 1-3 μm in diam; binding hyphae hyaline, thick-walled with a narrow lumen to subsolid, frequently branched, 1-2 μm . **Hymenium** collapsed, cystidia, cystidioles, basidia, basidioles not seen. **Basidiospores** broadly ellipsoid, pale yellowish to yellow, thick-walled, smooth, inamyloid, indextrinoid, cyanophilous, $(4.4\text{-})4.7\text{-}5.8(-6) \times (3.6\text{-})3.7\text{-}4.5(-4.8) \mu\text{m}$, $L = 5.13 \mu\text{m}$, $W = 4.08 \mu\text{m}$, $Q = 1.24\text{-}1.28$ ($n = 90/3$).

Additional specimens studied (paratypes): China, Guangxi, Jinxiu County, Dayaoshan National Nature Reserve, on dead standing angiosperm tree, 22 June 2014, LWZ 20140622-11 (IFP), on fallen angiosperm trunk, 22 June 2014, LWZ 20140622-12 (IFP).

Other specimens studied: *Donkioporia expansa*. China, Jilin, Huadian County, Dongxing, on fallen trunk of *Ulmus*, 17 October 1993, Dai 1674 (IFP). Belgium, Louvain-la-Neuve, Campus of University, on fallen angiosperm trunk, 3 December 2005, Dai 7466 (IFP).

DISCUSSION

In the field, *Donkioporiella mellea* can be confused with resupinate members of several genera in Hymenochaetaceae, including *Fomitiporia* Murrill (Dai, 2010), *Inonotus* P. Karst. (Ryvarden, 2005), *Tropicoporus* L.W. Zhou, Y.C. Dai & Sheng H. Wu (Wu *et al.*, 2015), because of the resupinate and woody basidiocarps with cinnamon-buff to honey-yellow colored pore surface. However, *D. mellea* can be easily distinguished in its trimitic hyphal system with clamped generative hyphae. These macro- and microscopic characters are reminiscent of genus *Donkioporia*, including two rare species *D. albidofusca* and *D. expansa* (Vlasák *et al.*, 2010).

Donkioporia was established by Kotlaba & Pouzar (1973) as a monotypic genus. Vlasák *et al.* (2010) recombined a second species *D. albidofusca*, transferring it from *Dichomitus* D.A. Reid based on morphology and an ITS-based phylogeny. The thin-walled and acyanophilous basidiospores make *Donkioporia* distinct from *Donkioporiella*, which has thick-walled and cyanophilous basidiospores (Vlasák

et al., 2010). At the species level, both species of *Donkioporia* differ from *Donkioporiella mellea* in their larger pores (5-7 per mm in *D. albidofusca* and 4-5 per mm in *D. expansa*) and thin-walled, narrower and acyanophilous basidiospores (2.5-3 µm wide in *D. albidofusca* and 3.2-3.7 µm wide in *D. expansa*; Ryvarde & Melo, 2014).

Phylogenetically, *Donkioporiella mellea* fell into the core clade of Polyporales and represented a distinct lineage (Figs 1 and 2). This lineage was most related with *Donkioporia*, but the weak statistical supports suggested it as a separate genus from *Donkioporia*. Therefore, based on both morphological and phylogenetic evidence, we consider that *Donkioporiella* is a new monotypic genus with a new species *D. mellea* as the generic type.

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