

A new species of *Skeletocutis* (Polyporales, Basidiomycota) from Vietnam

Abstract

A new species of Polyporales, now named *Skeletocutis vietnamensis*, was collected on angiosperm trunks in Vietnam. It is described based on morphological characteristics and molecular evidence. The species has snow white pore surfaces, angular pores mostly 5–7 per mm with entire mouths, a dimittic hyphal structure throughout the trama, generative hyphae in all parts of the basidioma covered by fine crystals, skeletal hyphae slightly inflated in KOH, not agglutinated, and pyriform to ovoid basidiospores measuring 2.8–3.4×1.5–2.1 μm. The combination of these characters, spores in particular, separate this species from other species of *Skeletocutis*. Phylogenetic analysis based on the internal transcribed spacer (ITS) regions indicated that the new species grouped with *Skeletocutis* species and formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP).

Keywords: Phylogeny, taxonomy, wood, decaying fungi

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Introduction

The genus *Skeletocutis* Kotl. & Pouzar was established in 1958 and its type species is *Skeletocutis amorphia* Fr. Kotlába & Pouzar.¹ The genus is widely distributed around the world, but the majority of the species known so far are found in the Northern Hemisphere.^{2–5} Species in *Skeletocutis* cause white rot. They mostly have resupinate basidioma although the genus type has pileate or effused-reflexed basidioma. The generative hyphae, at least, partly covered by fine crystals and the tiny basidiospores are the most important characteristics of the genus.^{3,5} *Skeletocutis* is phylogenetically close to *Tyromyces* P. Karst., *Ceriporiopsis* Domański and *Piloporia* Niemelä, and they cluster within the *Tyromyces* clade.^{6,7} Currently only two species have been described from Southeast Asia, *Skeletocutis falsipileata* (Corner) T Hatt⁸ in Malaysia and *Skeletocutis bicolor* (Lloyd) Ryvarden⁹ in Singapore. Seven species (*Skeletocutis fimbriata* Juan Li & YC Dai, *S. luteolus* BK Cui & YC Dai, *S. substellae* YC Dai, *S. bambusicola* LW Zhou & WM, *S. inflata* BK Cui & YC Dai, *S. yunnanensis* LS Bian, *S. pseudo-odora* LF Fan & Jing Si) were described in five provinces in southern China.^{10–16} During a survey of lignicolous fungi in Vietnam in October 2017, two specimens were collected growing on angiosperm trunks, and have resupinate basidioma with a distinct cottony sterile margin, relatively small pores, plenty of hyphal pegs, a dimittic hyphal structure, generative hyphae bearing clamp connections and fine, sharp-pointed encrustations especially at the dissepiment edges, bottle-shaped cystidioles and pyriform to ovoid basidiospores. These characters fitted the genus of *Skeletocutis*, but we could not assign them to a name and so we describe the collections as a new species.

Materials and methods

Morphological studies

The studied specimens are deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic procedure followed Dai.¹⁷ Microscopic measurements were made from slide preparations stained with Cotton Blue, Melzer's reagent and 5% potassium hydroxide. In the text the following abbreviations were used: KOH=5% potassium hydroxide, IKI=Melzer's reagent, IKI=neither amyloid nor dextrinoid, CB =

Cotton Blue, CB=acyanophilous, L=mean spore length (arithmetic average of all spores), W=mean spore width (arithmetic average of all spores), Q=variation in the ratios of L/W between specimens studied, n=number of spores measured from given number of specimens. Color terms follow Petersen.¹⁸

Molecular procedures and phylogenetic analyses

The methods of DNA extraction and amplification in this study followed Chen et al.¹⁹ A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to extract total genomic DNA from dried specimens of the new collections according to the manufacturer's instructions with some modifications. The primer pair ITS5 /ITS4 was used for PCR amplifications (primer sequences used in this study were obtained from <http://www.biology.duke.edu/fungi/mycolab/primers.htm>). The PCR products were sequenced in the Beijing Genomics Institute, China, with the same primers. The newly generated sequences were deposited in GenBank and labeled in Figure 1.

Besides the newly generated ITS sequences, the current ITS dataset included sequences from all available species of three genera (*Piloporia* Niemelä, *Skeletocutis* and *Tyromyces* P. Karst.) in the *Tyromyces* clade.⁷ The ITS sequence of *Cinereomyces lindbladii* (Berk.) Jülich²⁰ was selected as the outgroup.⁷ This dataset was aligned using MAFFT 7.110²¹ with the Q-INS-I opinion.²² Maximum parsimony analysis was applied to the ITS dataset sequences (Table 1). Approaches to phylogenetic analysis followed Zhou et al.,²³ and the tree construction procedure was performed in PAUP* version 4.0b10.²⁴ 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.²⁵ 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.²⁶

Branch support for ML analysis was determined by 1000 bootstrap replicate. 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 50% (BP) and 0.95 (BPP) were considered as significantly supported respectively.

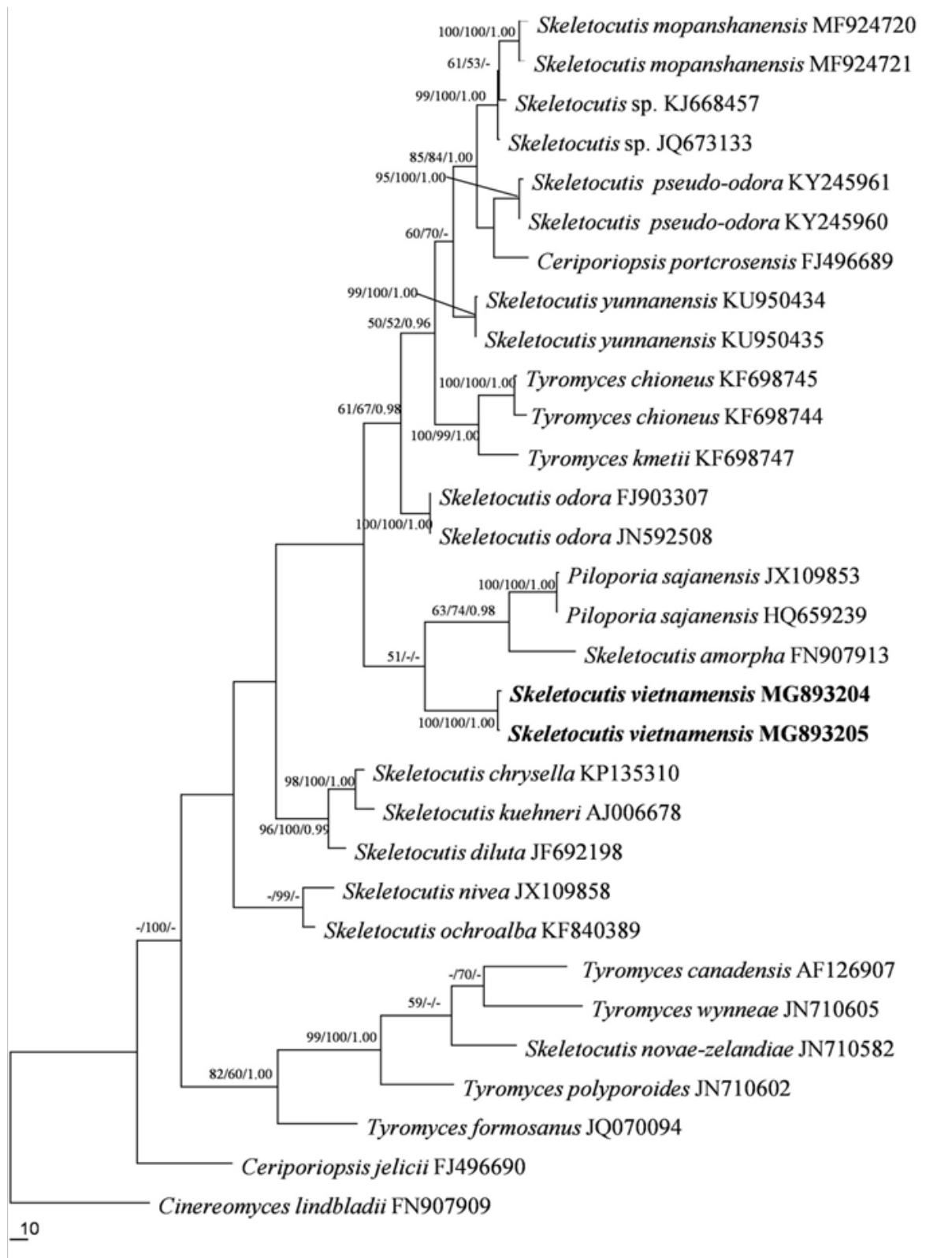


Figure 1 Maximum Parsimony strict consensus tree illustrating the phylogeny of *Skeletocutis vietnamensis* and related species based on the combined dataset (ITS). Branches are labeled with maximum likelihood bootstrap higher than 50%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively. The newly sequenced specimens are labeled in boldface.

Table 1 A list of species, specimens and GenBank accession numbers of sequences used in this study. New sequences are shown in bold.

Species	Specimen no.	Locality	GenBank accession no. ITS
<i>Ceriporiopsis jelicii</i>	H 6002113	Finland	FJ496690
<i>Ceriporiopsis portcrossensis</i>	LY 3493	France	FJ496689
<i>Cinereomyces lindbladii</i>	H 19911	Finland	FN907909
<i>Piloporia sajanensis</i>	Mannine 2733a	Finland	HQ659239
<i>Piloporia sajanensis</i>	JX 109853	Russia	JX109853
<i>Skeletocutis amorphia</i>	Miettinen 11038	Finland	FN907913
<i>Skeletocutis chrysellia</i>	FD 305	USA	KP135310
<i>Skeletocutis diluta</i>	JV 100861	USA	JF692198
<i>Skeletocutis nivea</i>	ES 2008	Sweden	JX109858
<i>Skeletocutis ochroalba</i>	JK 1208/8	Czech Republic	KF840389
<i>Skeletocutis kuehneri</i>	Sample 115		AJ006678
<i>Skeletocutis mopanshanensis</i>	MF 924720	China	MF924720
<i>Skeletocutis mopanshanensis</i>	CLZhao 1184	China	MF924721
<i>Skeletocutis novae-zelandiae</i>	X 489	New Zealand	JN710582
<i>Skeletocutis odora</i>	B 40	Latvia	FJ903307
<i>Skeletocutis odora</i>	JV 1007-7	Czech Republic	JN592508
<i>Skeletocutis pseudo-odora</i>	KY 245961	China	KY245961
<i>Skeletocutis pseudo-odora</i>	KY 245960	China	KY245960
<i>Skeletocutis</i> sp.	KUC 20121109-07	South Korea	KJ668457
<i>Skeletocutis</i> sp.	DLL 2010-112	—	JQ673133
<i>Skeletocutis vietnamensis</i>	Dai 18378	Vietnam	MG893204
<i>Skeletocutis vietnamensis</i>	Dai 18374	Vietnam	MG893205
<i>Skeletocutis yunnanensis</i>	Dai 15709	China	KU950434
<i>Skeletocutis yunnanensis</i>	Dai 15712	China	KU950435
<i>Tyromyces canadensis</i>	Pentill 1321	Finland	AF126907
<i>Tyromyces chioneus</i>	Cui 10225	China	KF698745
<i>Tyromyces chioneus</i>	Cui 7191	China	KF698744
<i>Tyromyces formosanus</i>	MYA-262	—	JQ070094
<i>Tyromyces kmetii</i>	Dai 12403	China	KF698747
<i>Tyromyces polyporoides</i>	X 510	Venezuela	JN710602
<i>Tyromyces wynneae</i>	X 1217	Denmark	JN710605

Results

Phylogeny

The ITS dataset, including 31 sequences, resulted in an alignment with 666 characters, of which 246 characters are constant, 88 variable characters are parsimony-uninformative and 332 characters are parsimony-informative. MP analysis yielded seven equally parsimonious trees (TL=1361, CI=0.554, HI=0.446, RI=0.665, RC=0.369). Best model for the ITS 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=0.009615 (BI). The new species clustered in the *Tyromyces* clade and formed a monophyletic lineage with a high support (100% BS, 100% BP, 1.00 BPP).

Taxonomy

Skeletocutis vietnamensis Rui Du & XH Ji, sp. nov. (Figure 2 & Figure 3)



Figure 2 Basidiomata of *Skeletocutis vietnamensis* (Holotype). Photo by Yu-Cheng Dai—Scale bar=1 cm.

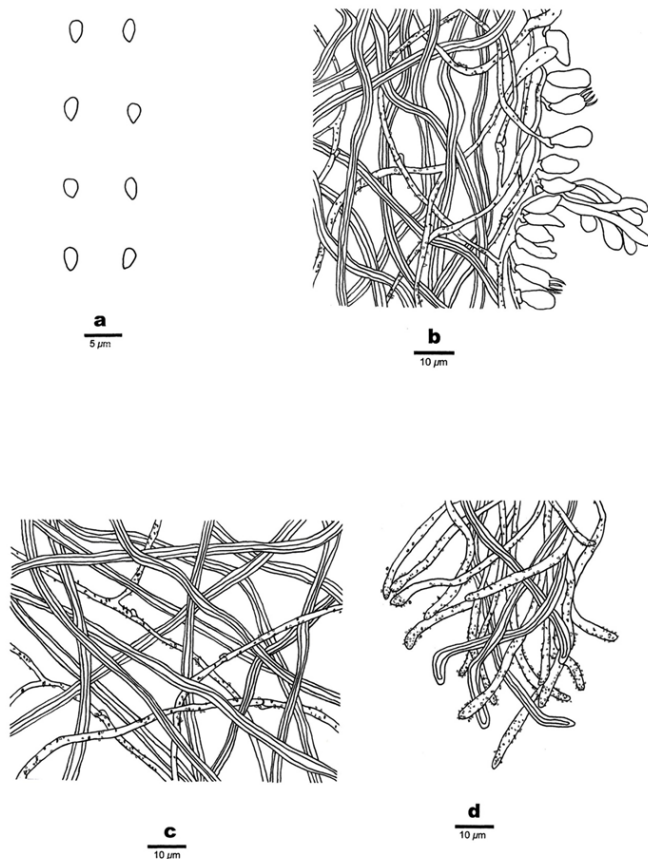


Figure 3 Microscopic structures of *Skeletocutis vietnamensis* (Holotype). a) Basidiospores, b) Section from tube trama, c) Hyphae from subiculum, d) Section of dissepiment edge. Scale bars: a) 5µm; b–d) 10µm.

Mycobank number MB 824183;

Holotype –Vietnam. Lam Dong Province, Lac Duong District, Bidoup Nui Ba Park, on rotten angiosperm trunk, 15 October 2017, Dai 18378 (Holotype in BJFC 0024888).

Etymology–*Vietnamensis* (Lat.): refers to the locality of the type species in Vietnam.

Fruiting body – Basidioma perennial, resupinate, very difficult to separate from substrate, soft leathery, and without odour or taste when fresh, becoming hard corky upon drying, up to 7.5cm long, 5cm wide, and 2.4mm thick at center. **Pore surface** snow white when fresh, honey yellow when bruised, white to cream upon drying, bruised part becoming buff-yellow when dry; sterile margin distinct when juvenile, byssoid to cottony, consistently white, up to 1.5mm, becoming narrow to almost lacking when mature; pores angular, freely arranged, mostly 5–7 per mm; dissepiments thin, entire. Subiculum white, hard corky, up to 0.4mm thick. Tubes darker than poroid surface, hard corky, up to 2mm long.

Hyphal Structure–Hyphal system dimitic, generative hyphae with clamp connections, hyaline, thin-walled, dominant at dissepiment edge; skeletal hyphae thick-walled with a narrow lumen to subsolid; IKI–, CB–, slightly inflated in KOH.

Context –Generative hyphae frequent, hyaline, thin- to slightly thick-walled, rarely branched and bearing fine crystals, 1.5–2.5µm in diameter; skeletal hyphae in trama dominant, thick-walled, flexuous, unbranched, interwoven, 2–3µm in diameter.

Tubes–Generative hyphae frequent, thin- to slightly thick-walled, occasionally branched, usually covered by fine, sharply pointed encrustations, especially at dissepiment edge, 1.5–2.5µm in diameter, and occasionally branched; Skeletal hyphae dominant, thick-walled with a narrow lumen to subsolid, unbranched, subparallel along the tubes, not agglutinated, 2–3µm in diameter. Dissepiment edge dimitic with smooth skeletal hyphae, and dominant winding, encrusted generative hyphae. Cystidia absent, cystidioles abundant, bottle-shaped, with a conical apex, almost the size of the basidia, 8–12×3–4.5µm; basidia infrequent, broadly clavate with a basal clamp connection and four sterigmata, 10–11×3–4.5µm; basidioles in shape similar to basidia, but slightly smaller. Hyphal pegs glomeratus and frequent.

Spores–Basidiospores mostly pyriform to ovoid, hyaline, thin-walled, smooth, CB–, IKI–, (2.6–)2.8–3.4×1.5–2.1(–2.2)µm, L=2.87µm, W=1.72µm, Q=1.67 (n=10/1).

Notes–*Skeletocutis vietnamensis* is characterized by resupinate, snow white pores when fresh but white to cream when dry, distinct byssoid to cottony sterile margin, small pores 5–7 per mm with entire dissepiments, a dimitic hyphal structure with skeletal hyphae in all parts of the basidioma, the presence of hyphal pegs, all generative hyphae covered by fine crystals, skeletal hyphae slightly inflated in KOH, pyriform to ovoid basidiospores measuring 2.8–3.4×1.5–2.1µm and growing on angiosperm wood. Microscopically *S. vietnamensis* is similar to *S. krawtzevii* (Pilát) Kotl. & Pouzar. But *S. vietnamensis* can be distinguished by having pyriform to ovoid spores and bottle-shaped cystidioles with a conical apex.

Additional specimen examined (paratype)–Vietnam. Lam Dong Province, Lac Duong District, Bidoup Nui Ba Park, on fallen angiosperm trunk, 15 October 2017, Dai 18374 (BJFC 024887).

Discussion

Phylogenetically *Skeletocutis* is a diverse genus, with *Tyromyces* and *Piloporia* in the same clade; *Tyromyces* is deeply embedded in a big clade which is mainly composed of *Skeletocutis* species.²⁷ *Tyromyces* fruit bodies are annual, resupinate to pileate, with a pileus that is finely tomentose to smooth, usually white, more rarely bluish, reddish to brownish, pore surface white to cream, rarely greyish

brown or reddish by drying, pores regular, round to angular, context or subiculum white to light brown when dry, consistency soft when fresh, mostly fragile and light weighted when dry. The hyphal system is usually monomitic, rarely dimitic, generative hyphae are hyaline, normally with clamps and lack crystals, cystidia are absent or present, when dimitic either with rather few binding hyphae or hyaline skeletal hyphae, spores are allantoid, cylindrical to ellipsoid, hyaline, smooth, and thin-walled.^{5,28} *Piloporia* basidioma are pileate, effused-reflexed to resupinate with a tomentose dark brown upper surface, a pore surface that is whitish to cork-colored with concolorous tubes; a duplex context with a black line separating the lower cork-colored part from the upper rusty brown part; a dimitic hyphal system; generative hyphae with clamps; skeletal hyphae hyaline to brown in upper part of context and finely encrusted in the dissepiments; absence of cystidia, and basidiospores that are allantoid, hyaline, and thin-walled.²⁹

Skeletocutis basidioma are pileate, effused-reflexed to resupinate with a dark brown tomentose upper surface, a whitish to cork-colored pore surface with concolorous tubes, a duplex context with a black line separating the lower cork-colored part from the upper rusty brown part; a dimitic hyphal system; generative hyphae with clamps; skeletal hyphae hyaline to brown in upper part of context and finely encrusted in the dissepiments and absence of cystidia. The generative hyphae are at least partly covered by fine crystals and the basidiospores are allantoid, hyaline and thin-walled.^{3,29} The new species we describe fits the characteristics of *Skeletocutis*, so we have named it *S. vietnamensis*. Microscopically *S. vietnamensis* is very similar to *S. krawtzevii*, and both species have frequent hyphal pegs. However, *S. krawtzevii* has rounded spores, amygdaliform cystidioles, and a monomitic hyphal structure which only contain generative hyphae at the dissepiment edge.^{3,30} In contrast, *S. vietnamensis* has pyriform to ovoid spores, bottle-shaped cystidioles with a conical apex, and a dissepiment edge with skeletal hyphae. Phylogenetically, *S. vietnamensis* is related to *S. amorpha* and *Piloporia sajanensis* (Parmasto) Niemelä,³¹ but *S. amorpha* can be distinguished by the pileate or effused-reflexed basidioma and the cartilaginous, pinkish buff to reddish orange pore surface.¹ *Piloporia sajanensis* differs from *S. vietnamensis* by the pileate basidioma, duplex context, absence of hyphal pegs and allantoid basidiospores measuring 3.5–4.0×0.8–1µm.³¹

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Conflicts of interest

Authors declare that there is no conflict of interest.

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