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Akanthomyces lepidopterorum, a new lecanicillium-like species

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Abstract

During a survey of entomopathogenetic fungi from Southwest China, a new species, *Akanthomyces lepidopterorum* was found on an undetermined lepidopteran larva. It differs from other species based on mostly smaller conidia, mononematous conidiophores and moderate length of phialide. Both the morphological identification and phylogenetic analysis of combined *ITS*, *LSU* and *RPB2* sequence data support *A. lepidopterorum* as a new species. A new combination is also proposed in the genus *Akanthomyces*.

Keywords: 1 new species, combination, mononematous, morphology, phylogeny

Introduction

The genus *Akanthomyces* Lebert was introduced with *A. aculeatus* Lebert as the type species. Kepler *et al*. (2017) treated *Lecancillium* W. Gams & Zare as a junior synonym of *Akanthomyces*, and combined *L. attenuatum* Zare & W. Gams, *L. lecanii* (Zimm.) Zare & W. Gams, *L. longisporum* (Petch) Zare & W. Gams, *L. muscarium* (Petch) Zare & W. Gams and *L. sabanense* Chir.-Salom., S. Restrepo & T.I. Sanjuan into *Akanthomyces*.

Shrestha *et al.* (2019) transferred *Lecanicillium araneogenum* Wan H. Chen *et al.* to *Akanthomyces neoaraneogenus* (Wan H. Chen *et al.*) W.H. Chen, Y.F. Han & Z.Q. Liang. Chen *et al*. (2020) reported a new verticillium-like species, *Akanthomyces neocoleopterorum* W.H. Chen *et al.* Currently, there are seven verticillium-like species in *Akanthomyces* (Kepler *et al*. 2017, Shrestha *et al*. 2019, Chen *et al*. 2020).

During a survey of entomopathogenetic fungi from Southwest China, a new species was found on a leptodopteran larva. It is described here as *Akanthomyces lepidopterorum sp. nov*. and is supported by morphological characters and phylogenic analysis.

Materials & methods

Specimen collection and isolation

The infected specimens (SD0515) were collected from Sandu City (25°57’22.21” N, 107°57’54.69” E), Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, on 1 May 2019. Isolation of strains were conducted as described by Chen *et al*. (2019). Fungal colonies emerging from specimens were isolated and cultured at 25 °C for 14 days under 12 h light/12 h dark conditions following protocols described by Zou *et al*. (2010). Accordingly, strains SD05151 and SD05152 were obtained. The specimens and the isolated strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China.

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Macroscopic and microscopic morphological characteristics of the fungi were examined and growth rates determined from cultures grown on potato dextrose agar (PDA) incubated at 25 °C for 14 d. Hyphae and conidiogenous structures were mounted in lactophenol cotton blue or 20% lactate solution and observed with an optical microscope (OM, BX35, Olympus, Japan).

DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out according to Liang *et al*. (2011). The extracted DNA was stored at −20 °C. RNA polymerase II largest subunit 2 (*RPB2*) was amplified according to van den Brink *et al*. (2012). The internal transcribed spacer (*ITS*) region and large subunit ribosomal RNA (*LSU*) genes were amplified by PCR according to the procedures described by White *et al*. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit [no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China] according to the manufacturer's protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

The DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of *ITS*, *LSU* rRNA and *RPB2* were selected based on Kepler *et al*. (2017), Wei *et al*. (2018), Zhou *et al*. (2018), Huang *et al*. (2018), Su *et al*. (2019), Ponizovskaya *et al*. (2020), Chen *et al*. (2020) and the results of a Blast search in GenBank. The sequence of *Simplicillium lanosoniveum* (J.F.H. Beyma) Zare & W. Gams (isolate CBS 704.86) was chosen as outgroup taxon. Multiple datasets of *ITS*, *LSU* and *RPB2* were aligned using MAFFT v7.037b (Katoh & Standley 2013) and alignments were edited with MEGA6 (Tamura *et al*. 2013). Sequences were concatenated with SequenceMatrix v.1.7.8 (Vaidya *et al*. 2011). Partition homogeneity test in PAUP4.0b10 (Swofford 2002) was performed by using the command 'hompart'.

The analysis was carried out using Bayesian inference (BI) and maximum likelihood (ML) methods. For BI, a Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 (Ronquist *et al*. 2012) for the combined sequence datasets. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 16,001 trees were used for calculating posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined using the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. ML analyses were constructed with RAxMLGUI (Silvestro & Michalak 2012). The GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. The final alignment is available from TreeBASE under submission ID: 26584 (http://www.treebase.org).

Results

Phylogenetic analyses

Statistical supports of ML bootstrap support/BI posterior probabilities are at the nodes in the phylogenetic tree (Fig. 1). The concatenated sequences included 31 taxa, and consisted of 2,030 (*ITS*: 540, *LSU*: 618 and *RPB2*: 872) characters with gaps, respectively.

The final value of the highest scoring tree was –13,074.918382, which was obtained from the ML analysis of the dataset (*ITS+LSU+RPB2*). The parameters of GTR model to analysis of the dataset were estimated base frequencies; A = 0.252363, C = 0.257868, G = 0.276099, T = 0.213670; substitution rates AC = 1.247761, AG = 2.433446, AT = 0.873335, CG = 0.829516, CT = 6.076763, GT = 1.000000; gamma distribution shape parameter α = 0.418749.

In the phylogenetic tree (Fig. 1), *Akanthomyces lepidopterorum* clustered with *A. neocoleopterorum* in a subclade, and grouped with *A. attenuatus*, *A. neoaraneogenus* and *Lecanicillium pissodis* Kope & I. Leal in a clade.

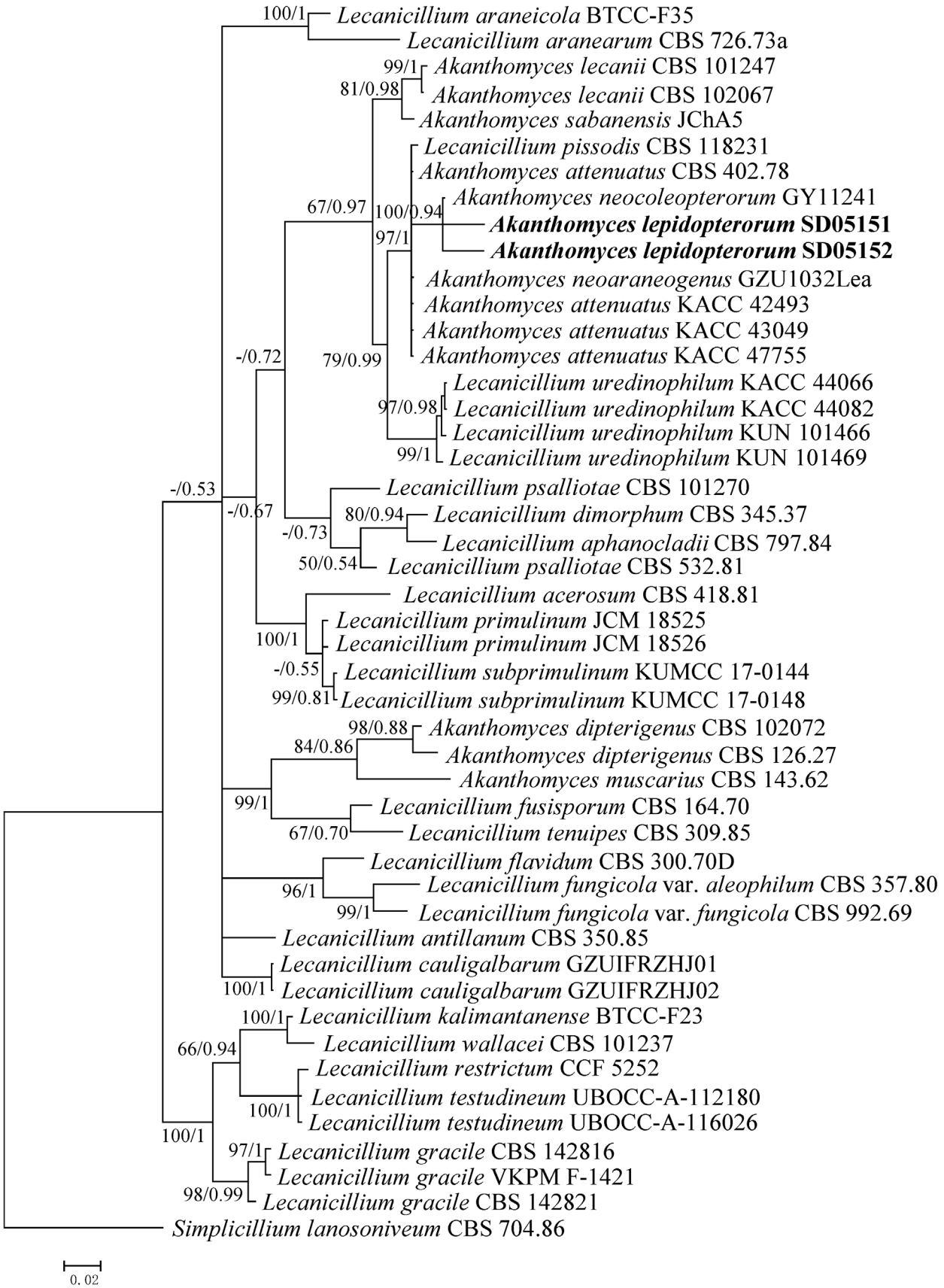

FIGURE 1. Phylogenetic analysis of *Akanthomyces lepidopterorum* and related species in *Akanthomyces* and *Lecanicillium* based on combined partial *ITS+LSU+RPB2* sequences. Statistical support values (≥50%) are shown at nodes, and presented as bootstrap values/ Bayesian posterior probabilities.

Taxonomy

Akanthomyces lepidopterorum W.H. Chen, Y.F. Han & Z.Q. Liang, *sp. nov.* (Fig. 2)
Mycobank No.: MB 836089

Type:—CHINA. Guizhou Province: Qiannan Buyi and Miao Autonomous Prefecture, Sandu (25°57'22.21" N, 107°57'54.69" E), on a pupa, 1 May 2019, Wanhao Chen, holotype GZAC SD0515; ex-type culture GZAC SD05151. Sequences from the strain SD05151 have been deposited in GenBank with accession numbers: *ITS*=MT705971, *LSU*=MT705973, *RPB2*=MT727044.

Colonies on PDA, attaining a diameter of 28–30 mm after 14 days at 25 °C, white, powdery, thin; reverse yellowish. *Hyphae* septate, hyaline, smooth-walled, 1.3–1.9 µm wide. *Conidiophores* mononematous, hyaline, smooth-walled, with single phialide or 2 phialides. *Phialides* consisting of a cylindrical, somewhat inflated base, 12.7–25.8 × 1.4–1.7 µm, tapering to a thin neck. *Conidia* hyaline, smooth-walled, aseptate, mostly cylindrical, 3.5–5.6 × 1.4–2.1 µm, forming mostly globose heads. In culture both phialides and conidia were of similar general shape and size to those found on the pupa.

Etymology:—referring to the fungus colonizing pupa in the order Lepidoptera.

Additional strains examined:—CHINA. Guizhou Province: Qiannan Buyi and Miao Autonomous Prefecture, Sandu (25°57'22.21" N, 107°57'54.69" E), on a pupa, 1 May 2019, Wanhao Chen (SD05152). Sequences from this strain have been deposited in GenBank with accession numbers: *ITS*=MT705972, *LSU*=MT705974, *RPB2*=MT727045.

Known distribution:—Sandu, Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, China.

In the phylogenetic analysis, the ex-type strain (CBS 118231) of *Lecanicillium pissodis* was in the *Akanthomyces* clade. Hence a new combination is made.

Akanthomyces pissodis (Kope & I. Leal) W.H. Chen, Y.F. Han & Z.Q. Liang, *comb. nov.*
Mycobank No.: MB 836090

Basionym: *Lecanicillium pissodis* Kope & I. Leal, *Mycotaxon* 94: 334 (2006).

Discussion

Phylogenetic analysis based on the combined datasets of *ITS+LSU+RPB2* suggested that strains SD05151 and SD05152 belong to the genus *Akanthomyces*, clustering with *A. neocoleopterorum* in a subclade, and grouping with *A. attenuatus*, *A. neoaraneogenus* and *Lecanicillium pissodis* in a clade. When compared with the typical characteristics of these four species (Table 1), *A. lepidopterorum* could be distinguished by having a combination of moderate length of phialide and smaller conidia in globose heads. Therefore, based on the combined analysis of morphological characters and the phylogenetic results, *A. lepidopterorum* is supported as a new species.

Lecancillium was treated as a junior synonym of *Akanthomyces* by Kepler *et al*. (2017), and only five *Lecanicillium* species were transferred to *Akanthomyces*. However, *Lecanicillium pissodis* has a close relationship with *A. attenuatus*, *A. neocoleopterorum*, *A. neoaraneogenus* and the new species in the phylogenetic tree (Fig. 1). Thus, *Akanthomyces pissodis* is proposed as a new combination.

TABLE 1. Morphological comparison of *Akanthomyces lepidopterorum* with related species.

Species	Phialides (µm)	Conidia heads	Conidia size (µm)	Conidial shape
Akanthomyces attenuatus	9–15.5 × 1–2	1–4 conidia in dry clusters	4.5–6.5 × 1.5–2.0	cylindrical, attenuate base, uniform shape, occasionally 2-celled
A. lepidopterorum	12.7–25.8 × 1.4–1.7	globose heads	3.5–5.6 × 1.4–2.1	cylindrical
A. neoaraneogenus	30–64 × 1.1–3.2	globose heads	3.2–8.6 × 1.3–1.6	cylindrical
A. neocoleopterorum	19.9–29.6 × 1.6–2.0	absent	3.3–6.6 × 1.5–1.8	cylindrical
A. pissodis	(16–)18–28(–38) × 1–2	globose droplets with up to 50 or more conidia	4.0–9.2 × 1.6–2.4	cylindrical to ovoid or oval, variable shape

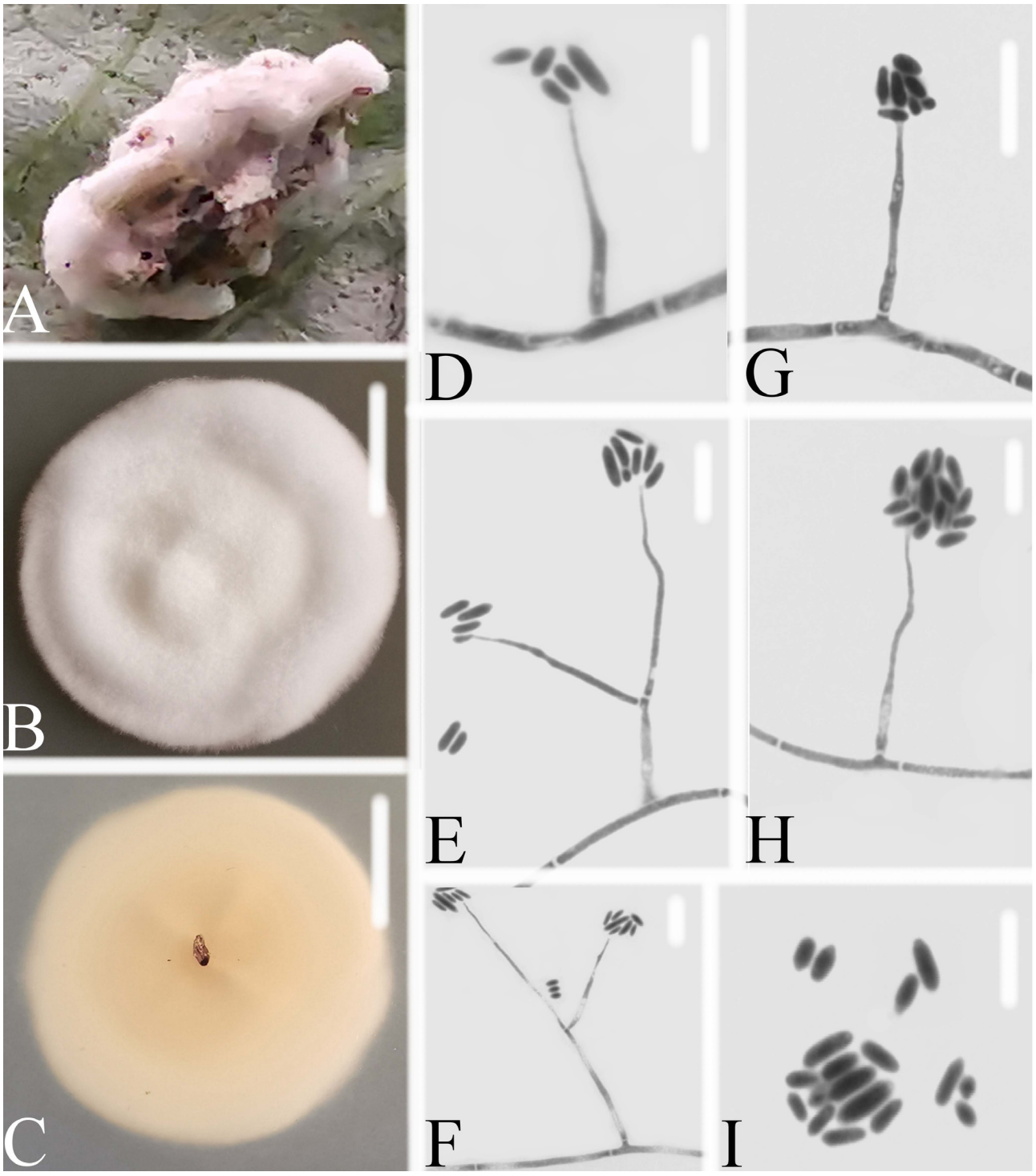

FIGURE 2. *Akanthomyces lepidopterorum*. **A**. Infected pupa. **B–C.** Culture on PDA, showing the top (B) and the underside (C). **D–H.** Phialides and conidia in globose heads formed on PDA. **I.** Conidia formed on PDA. Scale bars: B, C = 10 mm, D–I = 10 µm.

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