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A new species of *Xenasmatella* (Polyporales, Basidiomycota) from southern China

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Abstract

A new species of *Xenasmatella*, based on morphology, and molecular analysis (using the nuc rDNA ITS1-5.8S-ITS2 (ITS) dataset), was found growing on the underside of rotten bamboo in Yunnan Province, China. *X. roseobubalina* is characterized by annual, resupinate basidioma with a pinkish buff hymenophore, a monomitic hyphal system, thin-walled generative hyphae with clamp connections, abundant crystalline matter among the hyphae, the absence of cystidia and cystidioles, and broadly ellipsoid to subglobose basidiospores measuring 3.8–5 × 3.3–4 µm. We present the new species with illustrated description and comparisons to closest species as well as a key to the species of *Xenasmatella* known from China.

Keywords: A new taxon, polypore, taxonomy, wood-inhabiting fungi

Introduction

Phlebiella P. Karst. (1890: 271) was erected by Karsten (1890) and typified by *P. vaga* (Fr.) P. Karst. (1890: 271) [= *Xenasmatella vaga* (Fr.) Stalpers (1996: 37)]. However, Karsten (1890) did not give a generic description, so Donk (1963) specified that the genus *Phlebiella* was invalidly published. However, taxonomists continued using *Phlebiella* (the invalid name) for decades (Duhem 2010). Piątek (2005) noticed Donk's opinion and realized that the first available generic name for species in *Phlebiella* was *Xenasmatella* Oberw. (1966: 28). *Phlebiella* is now regarded as an invalid name and species in the genus have been moved to *Xenasmatella* (Duhem 2010). For example, Zong & Zhao (2021) described two new species, *Phlebiella gossypina* C.L. Zhao (2021: 505) and *P. wuliangshanensis* C.L. Zhao (2021: 508). However, Gruhn *et al*. (2021) transferred the two species to the genus *Xenasmatella* as *Xenasmatella gossypina* (C.L. Zhao) G. Gruhn & Trichies (2021: 40) and *Xenasmatella wuliangshanensis* (C.L. Zhao) G. Gruhn & Trichies (2021: 40).

Xenasmatella was typified by *X. subflavidogrisea* (Litsch.) Oberw. ex Jülich (1979: 335) (Oberwinkler 1966), however, the taxonomic position of the genus *Xenasmatella* at higher ranks is not clear and it is listed as *incertae sedis* (Larsson 2007). The genus is characterized by a resupinate, ceraceous to subgelatinous basidioma with a smooth, porulose, reticulate or grandinioid hymenophore, a monomitic hyphal system with generative hyphae bearing clamp connections, the presence of pleural basidia, and hyaline, warted, thin- to thick-walled, subglobose to globose, ellipsoid or cylindrical basidiospores (Oberwinkler 1966; Bernicchia & Gorjón 2010; Zong *et al.* 2021). So far 19 species have been accepted in the genus worldwide (Oberwinkler 1966; Stalpers 1996; Hjortstam & Ryvarden 2005; Bernicchia & Gorjón 2010; Duhem 2010; Huang *et al.* 2019; Larsson *et al.* 2020; Maekawa 2021; Zong *et al.* 2021).

During investigations on wood-inhabiting fungi from South China, a resupinate specimen was collected from Yunnan Province, and its morphology corresponded to the concept of *Xenasmatella*. Phylogenetic analysis based on the ITS rDNA sequences confirmed its affinity. Both morphological and molecular evidence demonstrated the specimen represents an undescribed species of *Xenasmatella* which we present in this paper with illustrated description and comparisons with related or similar species.

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Materials and methods

Site description

The type specimen was collected from China, Yunnan Province, Mengla County, Menglun, lowlands, Tropical Rain Forest Park. The vegetation is virgin tropical forest dominated by *Altingia chinensis* (Champ.) Oliver ex Hance, *Gironniera subaequalis* Planch., *Knema furfuracea* (Hook. f. et Thoms.) Warb., *Neolamarckia cadamba* (Roxb.) Bosser, *Parashorea chinensis* Wang Hsie. The annual rainfall is 1193.7–2491.5 mm. The average temperature is 18–22 ℃. E 101°29′, N 21°32′, elev. 1000 m.

Morphological studies

Macro-morphological descriptions are based on voucher specimens and field notes. Microscopic structures were examined from slide preparations of dried tissues stained with Cotton Blue and Melzer's reagent as described by Dai (2010). Spore measurements include ornamentation. The following abbreviations are used in the description: CB = Cotton Blue; CB– = acyanophilous in Cotton Blue; IKI = Melzer's reagent; IKI– = neither amyloid nor dextrinoid in Melzer's reagent; KOH = 5 % potassium hydroxide; L = mean spore length (arithmetic average of basidiospores); W = mean spore width (arithmetic average of basidiospores); and Q = variation in the L/W ratios between the specimens studied, (n=a/b) = number of spores (a) measured from given number of specimens (b). When the variation in spore size is shown, 5 % of the measurements were excluded from each end of the range, and these values are shown in parentheses. Special color terms follow Petersen (1996) and herbarium abbreviations follow Thiers (2018). Voucher specimens from the study were deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC) and the herbarium of Southwest Forestry University (SWFC).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from dried specimens using a CTAB Rapid Plant Genome Extraction Kit (Aidlab Biotechnologies Company, Ltd., Beijing, China) according to the manufacturer's instructions with some modifications (Li *et al.* 2014). The ITS regions were amplified with primers ITS4 and ITS5 (White *et al.* 1990).

The polymerase chain reaction (PCR) procedure for the ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, 72 °C for 1 min, and a final extension of 72 °C for 10 min (Zhao *et al.* 2015). The purification and sequencing of the PCR products was conducted by the Beijing Genomics Institute, Beijing, China, with the same primers used in the PCR reactions. Species were identified by sequence comparison with accessions in the NCBI databases using the BLAST program.

Phylogenetic analyses

Phylogenetic trees were constructed using ITS rDNA sequences, and phylogenetic analyses were performed with the Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) methods. Sequences of the species and strains were primarily adopted from ITS-based tree topology as described by Zong & Zhao (2021). New sequences generated in this study, along with reference sequences retrieved from GenBank (Table 1), were aligned by MAFFT 7 (Katoh *et al.* 2019; http://mafft.cbrc.jp/alignment/server/) using the "G-INS-i" strategy and manually adjusted in BioEdit (Hall 1999). Unreliably aligned sections were removed before the analyses, and efforts were made to manually inspect and improve the alignment. The data matrix was edited in Mesquite v3.70 (Maddison & Maddison 2021). The sequence alignment was deposited at TreeBase (submission ID 29463). Sequences of *Dacryopinax spathularia* (Schwein.) G.W. Martin (1948: 116) and *Dacrymyces stillatus* Nees (1817: 90) obtained from GenBank were used as outgroups to root the trees in the ITS analysis following Zong & Zhao (2021).

Maximum Parsimony analysis was applied to the ITS dataset sequences. Phylogenetic analysis followed Liu & Dai (2021), and the tree was constructed using PAUP* version 4.0 beta 10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with tree bisection and reconnection (TBR) branch swapping, and 1000 random sequence addition maxtrees were set to 5000. Branches of zero length were collapsed, and all the parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics, including the Consistency Index (CI), Homoplasy Index (HI), Rescaled Consistency index (RC), Retention Index (RI), and tree length (TL), were calculated for each Maximum Parsimonious Tree (MPT) generated.

The analysis using ML was conducted using RAxML-HPC v. 8.2.3 (Stamatakis 2014) and RAxML-HPC through the CIPRES Science Gateway (Miller *et al.* 2009; http://www.phylo.org). Statistical support values (BS) were obtained

using nonparametric bootstrapping with 1000 replicates. The BI analysis was performed with MrBayes 3.2.7a (Ronquist & Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 3 million generations until the split deviation frequency value <0.01, and the trees were sampled at every 1000 generation. The first 25 % of the sampled trees were discarded as burn-in, and the remaining ones were used to reconstruct a majority rule consensus tree and calculate the Bayesian Posterior Probabilities (BPP) of the clades.

A total of 24 models of evolution were scored using PAUP* version 4.0 beta 10 (Swofford 2002). Optimal substitution models for the combined dataset were then determined using the Akaike Information Criterion (AIC) implemented in MrModeltest 2.3 (Posada & Crandall 1998; Nylander 2004). The model GTR + I + G was selected for use in the Maximum Likelihood (ML) and Bayesian Inference (BI) analyses.

TABLE 1. Taxa information and GenBank accession numbers of the sequences used in this study. New species is in bold.

Species name	Sample no.	Country of origin	GenBank accession no. ITS
Aleurobotrys botryosus	He 2712	China	KX306877
Bondarzewia berkeleyi	Dai 12759	USA	KJ583202
Brevicellicium exile	UC 2022824	USA	KP814539
Dacrymyces stillatus	TUFC 12835	Japan	AB712464
Dacryopinax spathularia	AFTOL-ID 454	-	AY854070
Laetisaria lichenicola	CBS 128705	Luxembourg	MH864964
Lignosus hainanensis	Dai 10670	China	NR154112
Phellinopsis asetosa	Dai 13553	China	KJ425524
Phellinus ellipsoideus	Cui 4270	China	JQ837948
Polyozellus multiplex	AFTOL-ID 677	-	DQ411528
Polyporus brumalis	KHL 8558	Sweden	AF347108
P. squamosus	Cui 12375	USA	KX851641
Porpomyces mucidus	KHL 11062	-	AF347091
Thelephora ganbajun	ZRL 20151295	-	LT716082
Trametes versicolor	ZRL 20151477	-	LT716079
T. versicolor	KHL 8559	Sweden	AF347107
Trechispora farinacea	KHL 8793	Sweden	AF347089
T. hymenocystis	KHL 8795	Sweden	AF347090
Xenasmatella ardosiaca	KHL 12928	Costa Rica	EU118658
X. ardosiaca	CBS 126045	USA	MH864060
X. christiansenii	TASM YG-G36	Uzbekistan	MT526342
X. christiansenii	KHL 11689	Finland	EU118659
X. gossypina	CLZhao 8233	China	MW545957
X. gossypina	CLZhao 4149	China	MW545958
X. rhizomorpha	CLZhao 9170	China	MT832955
X. rhizomorpha	CLZhao 9847	China	MT832953
X. roseobubalina	**Dai 20506**	**China**	**OM855607**
X. tenuis	CLZhao 4528	China	MT832960
X. tenuis	CLZhao 11258	China	MT832959
X. vaga	KHL 11065	Sweden	EU118660
X. vaga	CFMR DLL2011-290	USA	KJ140766
X. wuliangshanensis	CLZhao 4080	China	MW545962
X. wuliangshanensis	CLZhao 4308	China	MW545963
X. xinpingensis	CLZhao 2216	China	MT832961
X. xinpingensis	CLZhao 2467	China	MT832962

Branches that received bootstrap support for Maximum Likelihood (BS), Maximum Parsimony (BP), and Bayesian Posterior Probabilities (BPP) > 65 % (BS), 50 % (BP), and 0.90 (BPP) were significantly supported. In addition, the

ML analysis resulted in the best tree, and only the ML tree is shown along with the support values from the MP and BI analyses. FigTree v1.4.4 (Rambaut 2018) was used to visualize the resulting tree.

Results

The ITS dataset contained sequences from 37 fungal specimens representing nine *Xenasmatella* taxa. The dataset had an aligned length of 921 characters, of which 373 were constant, 146 were variable but parsimony-uninformative, and 402 were parsimony-informative. MP analysis yielded eight equally parsimonious trees (TL = 2108, CI = 0.501, RI = 0.570, RC = 0.286, HI = 0.499). And the average standard deviation of split frequencies was 0.008212 (BI).

The phylogeny (Fig. 1) inferred from the ITS sequences demonstrated that *Xenasmatella sp.nov.* clustered in the *Xenasmatella* clade and grouped with *X. gossypina*, with strong support (99 % BS, 100 % BP, 1.00 BPP).

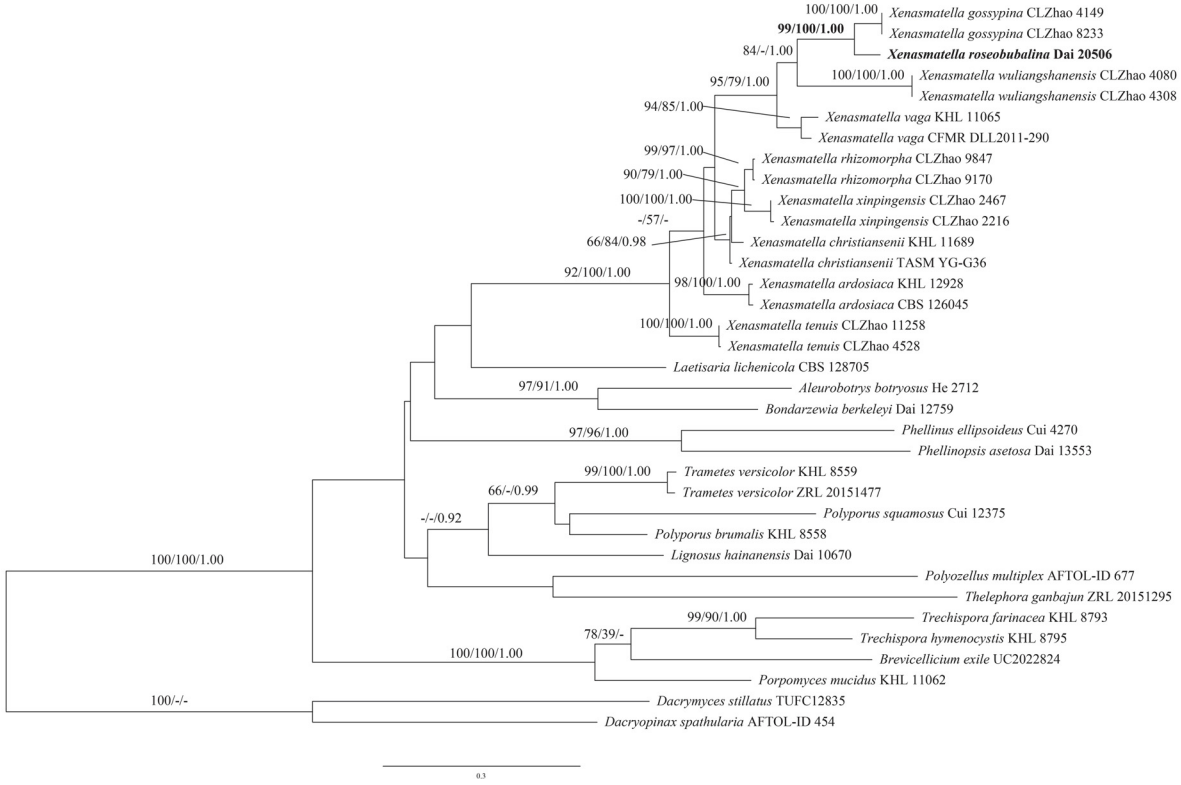

FIGURE 1. Phylogeny of *Xenasmatella* and related species by ML analysis based on ITS rDNA sequences. Branches are labelled with Maximum Likelihood bootstrap > 65 %, parsimony bootstrap proportions > 50 %, and Bayesian Posterior Probabilities > 0.90, respectively. New species is in bold.

Taxonomy

Xenasmatella roseobubalina Z.B. Liu & Yuan Yuan, *sp. nov.* (Figs. 2–3)
MycoBank no.—MB 843185

Etymology:—'*roseobubalina*' (Lat.): refers to the species having a pinkish buff hymenophore.

Type:—CHINA. Yunnan Province, Mengla County, Yulingu, on rotten bamboo, 18 August 2019, Y.C. Dai, *Dai 20506* (Holotype, BJFC 032174, isotype in SWFC).

Description:—Basidiomata annual, resupinate, adnate, detachable, membranaceous, without odor or taste when fresh, brittle when dry, up to 6.5 cm long, 2.5 cm wide and less than 0.1 mm thick. Hymenial surface smooth, pinkish buff (5A3), uncracked when fresh and dry, and with some scattered crevices upon drying. Sterile margin distinct, fimbriate and white; subiculum not found.

FIGURE 2. Basidiomata of *Xenasmatella roseobubalina* (Holotype, Dai 20506). Scale bar = 1.0 cm. Photo by: Yu-Cheng Dai.

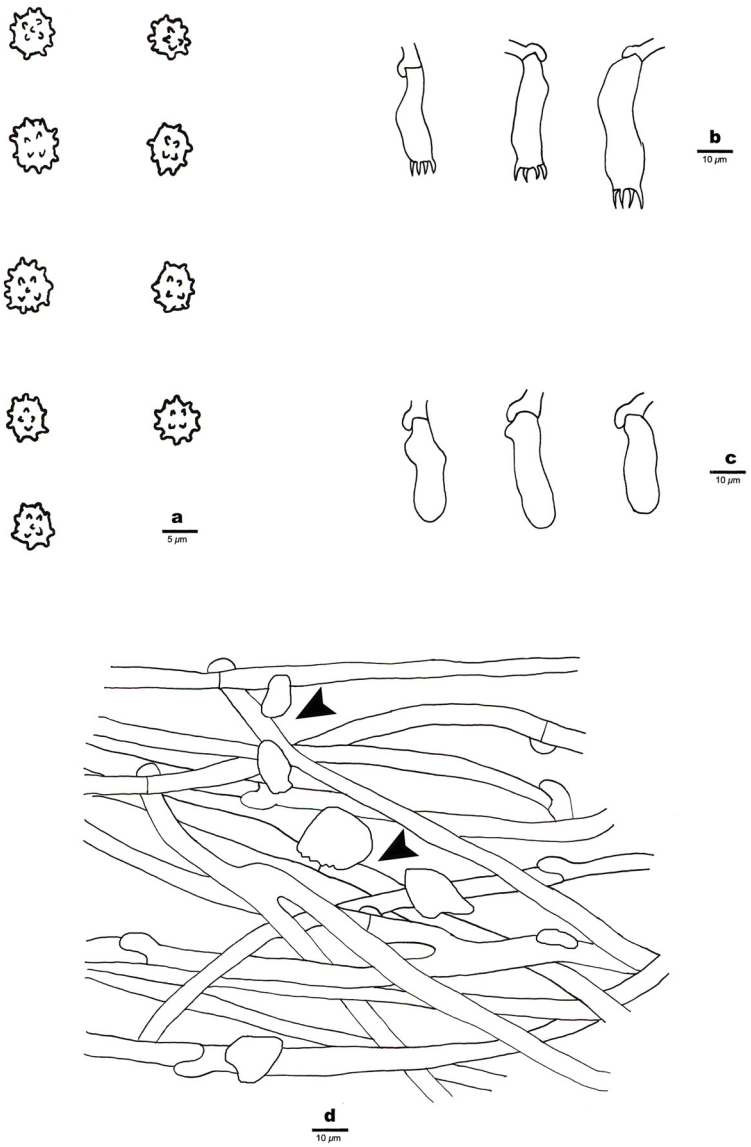

FIGURE 3. Microscopic structures of *Xenasmatella roseobubalina* (Holotype, Dai 20506). **a** Basidiospores; **b** Basidia; **c** Basidioles; **d** Hyphae from subhymenium and crystals (black arrow) among hyphae. Drawings by: Zhan-Bo Liu.

Hyphal structure:—Hyphal system monomitic; clamped, hyaline, thin-walled generative hyphae in subhymenium, frequently branched, 2–8 μm in diameter, IKI–, CB–. Abundant crystalline matter present among hyphae. Tissues unchanged in KOH.

Hymenium:—Cystidia and cystidioles absent; basidia pleural or clavate, with 4 sterigmata and a basal clamp connection, 20–26 × 5–8 μm; basidioles in shape similar to basidia, but shorter than basidia.

Basidiospores:—Broadly ellipsoid to subglobose, hyaline, thin-walled, warted, IKI–, CB–, (3.5–)3.8–5(–6) × 3.3–4(–5) μm, L = 4.43 μm, W = 3.9 μm, Q = 1.14 (n = 60/1).

Key to the nine species of *Xenasmatella* known from China. The new species is in bold.

1. Tissues turning dark red or purplish in KOH ..... *X. vaga*
1. Tissues unchanged in KOH. 2
2. Rhizomorphs present ..... *X. rhizomorpha*
2. Rhizomorphs absent 3
3. Basidia often bifurcate at base ..... *X. insperata*
3. Basidia not bifurcate at base 4
4. Basidiomata less than 100 μm thick 5
4. Basidiomata more than 100 μm thick 6
5. Basidiospores 3.3–4 μm wide; crystals present among hyphae ..... ***X. roseobubalina***
5. Basidiospores 2.3–3.4 μm wide; crystals absent ..... *X. tenuis*
6. Generative hyphae slightly thick-walled ..... *X. xinpingensis*
6. Generative hyphae thin-walled 7
7. Generative hyphae unbranched ..... *X. wuliangshanensis*
7. Generative hyphae branched 8
8. Generative hyphae 1.5–2.5 μm in diameter; basidiospores 4–5 × 3.5–4.5 μm ..... *X. ailaoshanensis*
8. Generative hyphae 2.5–6 μm in diameter; basidiospores 3.3–4.4 × 2.8–4 μm ..... *X. gossypina*

Discussion

Larsson (2007) proposed a phylogenetic classification for corticioid fungi at the family level. In his 5.8S+nLSU phylogenetic analysis, *Phlebiella ardosiaca* (Bourdot & Galzin) K.H. Larss. & Hjortstam (1987: 316) [= *Xenasmatella ardosiaca* (Bourdot & Galzin) Stalpers (1996: 37)], *P. christiansenii* (Parmasto) K.H. Larss. & Hjortstam (1987: 316) [= *X. christiansenii* (Parmasto) Stalpers (1996: 37)] and *P. vaga* (= *X. vaga*) nested in the *Phlebiella* (= *Xenasmatella*) clade and grouped with Trechisporales K.H. Larss. and Polyporaceae Fr. ex Corda.

In the present study, a new species, *Xenasmatella roseobubalina sp. nov.*, is described based on phylogenetic analyses and morphological characters. Morphologically, *X. roseobubalina* and *X. xinpingensis* C.L. Zhao (2020: 118) share the same size of basidiospores (3.8–5 × 3.3–4 μm *vs.* 3.5–4.9 × 3–4.2 μm), but the basidioma of *X. roseobubalina* is thinner than that of *X. xinpingensis* (less than 100 μm *vs.* 100–300 μm). Besides, the basidia of *X. roseobubalina* are larger than those of *X. xinpingensis* (20–26 × 5–8 μm *vs.* 15.5–20 × 4.5–6.5 μm, Zong *et al*. 2021). Phylogenetically, *Xenasmatella roseobubalina* is nested in the *Xenasmatella* clade based on ITS sequence data (Fig. 1) and grouped with *X. gossypina* with strong support (99 % BS, 100 % BP, 1.00 BPP). Morphologically, *X. gossypina* has gossypine to byssaceous hymenial surface, while the hymenial surface of *X. roseobubalina* is smooth. *X. roseobubalina* can also be distinguished from *X. gossypina* by its larger basidiospores (3.8–5 × 3.3–4 μm *vs.* 3.3–4.4 × 2.8–4 μm, Zong & Zhao 2021). In addition, a minimum of >1.5% nucleotide differences in the ITS regions may be indicative of a new species (Jeewon & Hyde 2016). Nucleotide differences in the ITS regions between *X. gossypina* and *X. roseobubalina* are up to 8 %.

To date, in geographical distribution, nine species of *Xenasmatella* have been reported from China (Dai 2011, Huang *et al*. 2019, Zong & Zhao 2021, Zong *et al*. 2021, this study). An identification key to these species is provided.

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Raw Data

The following information was supplied regarding data availability:

The sequence alignment was deposited at TreeBase (submission ID 29463; http://purl.org/phylo/treebase/phylows/study/TB2:S29463?x-access-code=2e9de0503bece9217a6a389aac442e5c&format=html).

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