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Myxarium and Myxarium porosum –New Records of Genus and Species for China (Post-print)

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Abstract

This study conducted a taxonomic investigation on a morphologically distinct specimen of the genus *Carcinomyces* collected from Liancheng National Nature Reserve in Gansu Province, using morphological characterization and molecular phylogenetic analysis based on ITS sequences. The results indicate that the specimen represents *Carcinomyces polyporina* (newly proposed), belonging to Basidiomycota, Tremellomycetes, Tremellales, and *Carcinomycetaceae* (newly proposed). This species represents a new record genus and species for China, and constitutes the first report of this genus in East Asia. *Carcinomyces polyporina* parasitizes the fruiting bodies of polypore fungi, forming gelatinous galls, with basidia that split longitudinally, and basidiospores that often germinate to produce conidia. The studied specimen is deposited in the Cryptogamic Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences, with the accession number HKAS 115765.

Full Text

New Records of the Genus *Carcinomyces* and Species *Carcinomyces polyporina* in China

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Abstract

This study reports a macrofungus specimen with distinctive morphology collected from Liancheng National Nature Reserve in Gansu Province. Morphological characterization and molecular phylogenetic analysis based on internal transcribed spacer (ITS) sequences identified the specimen as *Carcinomyces polyporina*, representing a newly recorded genus and species in China and the first report of this genus in East Asia. *Carcinomyces polyporina* is characterized by its parasitism on polypore fruiting bodies, forming colloidal mycocecidia, basidia with longitudinal septation, and conidia commonly gemmating from basidiospores. The specimen is deposited in the Herbarium of Cryptogamic Flora, Kunming Institute of Botany, Chinese Academy of Sciences (accession number HKAS 115765).

Keywords: new generic record, new species record, *Carcinomyces polyporina*, Liancheng National Nature Reserve, systematic classification

Introduction

The genus *Carcinomyces* (proposed herein) was established by Oberwinkler and Bandoni in 1982 and initially placed within the Heterobasidiomycetes, family Carcinomycetaceae (proposed herein), with its ordinal position undetermined at that time (Oberwinkler & Bandoni, 1982). Species in this genus typically parasitize certain basidiomycete fruiting bodies or plants (Fungsin et al., 2002; Crous et al., 2019), often inducing tumor-like growths in host tissues and forming transparent to translucent gelatinous mycocecidial structures that appear colorless to pale yellow when fresh. The hyphal tips can simultaneously produce conidiophores and basidia.

Carcinomyces polyporina was first described in 1970 and originally placed in the genus *Tremella* (Reid, 1970). In 1982, Oberwinkler and Bandoni transferred it to *Carcinomyces* based on its mycocecidial fruiting bodies (Oberwinkler & Bandoni, 1982). However, in 1986, Ginns merged the type species *Carcinomyces effibulatus* into *Syzygospora* (Ginns, 1986), sparking controversy over the validity of *Carcinomyces* and leaving the taxonomic position of *C. polyporina* unresolved.

Recent molecular phylogenetic studies of the Tremellomycetes have demonstrated that *Carcinomyces effibulatus* (Ginns, 1978), *Carcinomyces arundinariae* (Fungsin et al., 2002), and *C. polyporina* belong to the Basidiomycota, Tremellomycetes, Tremellales, family Carcinomycetaceae, genus *Carcinomyces*, and are phylogenetically distant from *Syzygospora* (Liu et al., 2015; Crous et al., 2019). Crous et al. (2019) described *Carcinomyces nordestinensis* based on molecular and morphological evidence, and their phylogenetic trees supported the recognition of *Carcinomyces* as a distinct genus within the Tremellales and Carcinomycetaceae. No species of *Carcinomyces* had previously been reported from China. This study presents morphological and molecular systematic

research on a specimen of this genus collected in China.

Materials and Methods

1.1 Specimen Information The specimen was collected on August 1, 2019, in Pengzigou, Liancheng National Nature Reserve, Yongdeng County, Lanzhou City, Gansu Province, within a mixed coniferous-broadleaf forest at an elevation of 1,941 m (coordinates: 102°44' 55.257 E, 36°37' 42.288 N). The dominant vegetation included *Picea crassifolia*, *Picea wilsonii*, and *Betula albosinensis*. The field collection number was ye629, and the herbarium accession number is HKAS 115765.

1.2 Morphological Observation Ecological photographs were taken using a Panasonic Lux 10 camera. Dry specimens were examined for color and shape using a Motic SMZ-171 dissecting microscope. Hand sections were prepared and mounted in 5% KOH solution for microscopic examination with a Zeiss AX10 light microscope; micrographs were captured with a Canon 70D camera. Twenty mature conidia and basidiospores were measured, with dimensions expressed as (a)b-c(d), where a and d represent the minimum and maximum values, and b-c represent the 90% confidence interval. The length-to-width ratio (Q) was calculated, with Qm representing the mean Q value \pm standard deviation (Wu et al., 2016).

1.3 Molecular Phylogenetic Analysis Total DNA was extracted using the CTAB method. The internal transcribed spacer (ITS) region was amplified using primers ITS5 (5'-GGAAGGTA AAAGTCAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). PCR products were sequenced by Beijing Aoke Dingsheng Biotechnology Co., Ltd. The resulting sequences were manually edited and adjusted using BioEdit (Hall, 1999), subjected to BLAST analysis in NCBI, and submitted to GenBank.

Based on Liu et al. (2015), relevant ITS sequences were retrieved from GenBank and combined with our newly obtained sequence to construct a dataset. Maximum likelihood (ML) phylogenetic analysis was performed using RAxML 8.1.24 with the GTRGAMMA1 nucleotide substitution model and default parameters (Stamatakis, 2014). Neighbor-joining (NJ) analysis was conducted using MEGA 7.0.26. Bootstrap support was assessed with 1,000 replicates for both methods. Sequence information, including species names, origins, and GenBank accession numbers, is provided in Table 1.

Table 1 Information on ITS sequences used for phylogenetic tree construction

Sample name	Specimen No.	Location	GenBank accession No.
<i>Carcinomyces polyporina</i>		Spain	JN053501
<i>Carcinomyces polyporina</i>	HKAS 115765	China	MZ198241
<i>Carcinomyces effibulatus</i>		Spain	JN053499

Sample name	Specimen No.	Location	GenBank accession No.
<i>Carcinomyces arundinariae</i>	CBS: 9931	Netherlands	KY102550
<i>Carcinomyces nordestinensis</i>	BRT-317	Brazil	MK659873
<i>Kockovaella machilophila</i>			AF444477
<i>Fellomyces penicillatus</i>			AF444337
<i>Sterigmatosporidium polymorphum</i>	CBS: 8607	USA	AF444320
<i>Fibulobasidium murrhardtense</i>	CBS: 9190	Netherlands	KY103412

Note: Bold indicates newly submitted sequences from this study.

Results

Phylogenetic trees constructed using ML and NJ methods based on ITS sequences showed essentially identical topologies with only slight differences in support values. The ML tree is presented in Figure 1, with bootstrap percentages from ML (BP1) and NJ (BP2) analyses shown on branches. Our sequence clustered with the Spanish *C. polyporina* specimen AM20 with high support (BP1 = 100, BP2 = 100) and formed a well-supported clade (BP1 = 99, BP2 = 98) with other *Carcinomyces* species including the type species *C. effibulatus*.

Morphological examination of specimen HKAS 115765 revealed the following characteristics. The fungus parasitizes the hymenium of polypores (Figure 2), forming translucent to transparent gelatinous mycoecidia that appear slightly milky when fresh and dry into thin, membranous, brown to dark brown films. Conidiophores and conidia are common; conidiophores are short and club-shaped. Conidia are long-elliptical to fusiform, smooth, thin-walled, measuring (3.5)4.4-5.4(8.0) × (1.5)2.5-3.0(4.5) μ m with $Qm = 1.80 \pm 0.12$. Basidia are subglobose, 9.8-12.8 × 8.8-11.0 μ m, with longitudinal septation; sterigmata are 2- or 4-celled, up to 8 μ m long. Basidiospores are globose to subglobose, (4.8)5.0-5.5(6.0) × (4.5)4.6-4.8(5.1) μ m with $Qm = 1.10 \pm 0.01$, and commonly germinate to produce conidia (Figure 3). Clamp connections are present.

Based on the combined morphological and molecular evidence, we confirm that this specimen represents *Carcinomyces polyporina* (D.A. Reid) A.M. Yurkov, 2015 *Tremella polyporina* D.A. Reid, Trans. Br. Mycol. Soc. 1970.

Distribution: United Kingdom (Reid, 1970; Roberts, 2007), North America (Setliff, 1982), Spain (Millanes et al., 2011), Poland (Karasiński & Wołkowycki, 2015), China.

Figure 1. Molecular phylogenetic tree based on ITS dataset using maximum likelihood. Bootstrap percentages (BP) from ML and NJ analyses (1,000 replicates) are shown left and right on major branches, respectively.

Figure 2. Macroscopic morphology of *Carcinomyces polyporina* and its host. A. Dorsal view of host; B. Ventral view of host; C. Host hymenium with gelatinous

mycocecidia of *C. polyporina*. a. Gelatinous mycocecidium of *C. polyporina*. Bars: A, B = 5 mm, C = 2 mm.

Figure 3. Microscopic structures of *Carcinomyces polyporina* and its host. A. Probasidium of *C. polyporina*; B. Mature basidium with longitudinal septation of *C. polyporina*; C. Basidiospore germination of *C. polyporina*; D. Conidio-phore of *C. polyporina*; E. Elliptical conidia of *C. polyporina*; F. Subglobose basidiospores of *C. polyporina*; G. Botuliform basidiospores of host. Bars = 5 m.

Discussion

This study confirms specimen HKAS 115765 collected from Liancheng National Nature Reserve, Gansu Province, as *Carcinomyces polyporina*, representing a new generic and specific record for China. The genus name *Carcinomyces* derives from the Greek *karkinos* (cancer, tumor), and we propose the Chinese name “胶瘤菌” (jiāo liú jūn, gelatinous tumor fungus) based on its gelatinous fruiting bodies. The specific epithet *polyporina* indicates its common parasitism on polypore hymenia, leading us to propose “孔生胶瘤菌” (kǒng shēng jiāo liú jūn, pore-born gelatinous tumor fungus) as the Chinese species name. Wang et al. (2010) mentioned the family Carcinomycetaceae in their report of *Syzygospora mycetophila* in China but did not provide a Chinese name; we propose “胶瘤菌科” (jiāo liú jūn kē, gelatinous tumor fungus family) based on its etymology.

Regarding systematic classification, the type species *Carcinomyces effibulatus* was previously transferred to *Syzygospora* by some researchers (Ginns, 1986). *Syzygospora* is currently placed in the Filobasidiales, Filobasidiaceae (Millanes et al., 2011; Liu et al., 2015), and Index Fungorum follows this classification, placing *Carcinomyces* in Filobasidiales, Filobasidiaceae (<http://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=823057>). However, the phylogenetic tree of Tremellomycetes constructed by Liu et al. (2015) shows that *C. effibulatus*, *C. arundinariae*, and *C. polyporina* form a highly supported monophyletic clade constituting a monotypic family—Carcinomycetaceae—within the Tremellales. In contrast, the type species of *Syzygospora*, *S. alba*, is positioned within the Filobasidiales, Filobasidiaceae, phylogenetically distant from *Carcinomyces*. Morphologically, *Carcinomyces* species predominantly parasitize basidiomycete fruiting bodies, forming mycocecidia with subglobose to cylindrical basidia that develop longitudinal or cruciate septa upon maturity. In contrast, Filobasidiaceae species mostly parasitize lichens and possess tubular or long-clavate basidia without septation. Therefore, both molecular and morphological evidence support the placement of *Carcinomyces* in the Tremellales, Carcinomycetaceae, rather than in the Filobasidiales, Filobasidiaceae, necessitating a taxonomic revision in the Index Fungorum database.

The genus *Carcinomyces* currently comprises only four recognized species: *C.*

effibulatus (type species) (Ginns, 1978), *C. arundinariae* (Fungsin et al., 2002), *C. nordestinensis* (Crous et al., 2019), and *C. polyporina* (Reid, 1970). These parasitic fungi exhibit complex and unique host relationships. For example, *C. effibulatus* is known only from *Collybia dryophila* fruiting bodies (Ginns, 1986); *C. nordestinensis* parasitizes leaves of *Bromelia antiacantha* while also occurring as an endophyte in *Handroanthus impetiginosus* (Crous et al., 2019); and *C. polyporina* has been recorded on *Tyromyces lacteus* and *Antrodia sinuosa* (Reid, 1970; Roberts, 2007). We were unable to obtain host DNA from our specimen, but based on morphological characteristics such as blue bruising of the fruiting body and botuliform basidiospores, we hypothesize the host belongs to *Tyromyces* or a related genus, though definitive identification requires additional specimens and study.

Previous records of *C. polyporina* were limited to Europe and North America (Reid, 1970; Setliff, 1982; Roberts, 2007; Millanes et al., 2011). Its discovery in China represents the first report from East Asia. As fungal diversity research continues to expand in scope and depth, additional *Carcinomyces* species will likely be discovered in more locations, clarifying their distribution patterns and ecological characteristics.

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