

Ecology of aquatic nematode-trapping hyphomycetes in southwestern China

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ABSTRACT: Aquatic nematode-trapping hyphomycetes have rarely been studied. The current study examined 1000 waterlogged soil samples in order to systematically investigate resources of aquatic nematode-trapping hyphomycetes and to compare species diversity and community similarities in polluted and unpolluted aquatic environments. Many nematode-trapping hyphomycetes live in shallow freshwater (at a water depth of 20 cm). No nematode-trapping hyphomycetes were isolated from the 20 samples collected at the bottom (at a water depth of 4 m) of Dianchi Lake. Among the 35 isolated species of predacious fungi, *Arthrobotrys oligospora*, *A. musiformis*, *Monacrosporium thaumasium* and *M. longiphorum* were the most common species. Species with adhesive networks were the most frequently isolated. The similarity indices between fungal communities living in similar aquatic environments were higher than those from dissimilar aquatic environments, and the level of species diversity for the brooks of the nature reserves was higher than that for the contaminated lake or river.

KEY WORDS: Aquatic fungi · Predacious hyphomycetes · Community similarity · Fungal diversity

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INTRODUCTION

Freshwater fungi are broadly defined as fungi which, for part or the whole of their life cycle, rely on free freshwater (Thomas 1996), including any species growing on substrata that are predominantly aquatic or semi-aquatic. Freshwater hyphomycetes are a main group of freshwater fungi and comprise 4 biological groups: the Ingoldian fungi, the aero-aquatic fungi, the terrestrial-aquatic hyphomycetes and the submerged-aquatic hyphomycetes (Goh et al. 2003). The Ingoldian fungi include a group of hyphomycetes that usually occur on herbaceous substrata, such as plant litter and leaves, and are readily trapped in foam; these fungi usually have tetra- or branched or sigmoid conidia. The aero-aquatic fungi are only capable of producing mycelium on submerged leaves or woody substrata. When the substrata are exposed to air, the fungi can sporulate and the conidia are dispersed by water flow when the substratum is submerged again. The terrestrial-aquatic hyphomycetes are characterized by production of staurosporous conidia similar in shape to

those of the Ingoldian fungi, but lacking conspicuous conidiophores. The fungi can be isolated from raindrops on intact terrestrial plant parts, such as on leaf-surfaces or in rainwater draining from intact tree trunks. The submerged-aquatic hyphomycetes represent a heterogeneous assemblage of fungi growing on submerged decaying plant materials. These fungi produce conidia when the substrata are no longer submerged. The conidia disperse in air and/or water. After Ingold published his first paper on the freshwater hyphomycetes isolated from England more than 60 yr ago (Ingold 1942), over 500 species of freshwater hyphomycetes have been reported (Wong et al. 1998) and the number is increasing at a rapid rate. In the last decade, much attention has been paid to the submerged-aquatic hyphomycetes, typically of the genera *Bactrodesmium*, *Camposporidium*, *Canalisporium*, *Dactylaria*, *Dictyochoeta*, *Exaerticlava*, *Monotoporiella*, *Nawawia*, *Phaeoisaria*, *Sporidesmiella*, *Sporidesmium*, *Sporoschisma*, *Sporoschismopsis* and *Xylomyces*; however, little research has been done on the aquatic nematode-trapping hyphomycetes such as

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Arthrobotrys Corda, *Monacrosporium* Oudem., and *Dactylella* Grove on submerged substrata. Currently, only 8 species of predacious hyphomycetes from aquatic environments have been recorded (Ingold 1944, Peach 1950, 1952, Johnson et al. 1961, Anastasiou 1964, Hao et al. 2004). Of the 8 species, *Dactylella submersa*, *Monacrosporium reticulatum*, *M. scaphoides*, *M. tentaculatum* and *D. dainchiensis* were first isolated from aquatic environments but the rest (*Arthrobotrys dactyloides*, *M. candidum* and *D. heptameris*) were first isolated from terrestrial environments. *A. dactyloides* was isolated from saltwater, whereas the other 7 species were isolated from freshwater.

It is known that various nematodes live in aquatic environments. More than 150 species have been recorded from freshwater in South Africa (Heyns 2002) and over 4000 species in saltwater (Zhang 2003). Aquatic nematodes are even used as bio-indicators of water pollution (Geetanjali et al. 2002). As a type of micro-fungi preying nematodes, there also should be corresponding communities of nematode-trapping hyphomycetes in aquatic environments. It is postulated that there must be many aquatic nematode-trapping hyphomycetes.

However, human activities can cause aquatic environments to deteriorate. Because freshwater fungi are sensitive to changes in water quality (Tan & Lim 1984, Tsui et al. 1998), studies on the biodiversity of aquatic nematode-trapping hyphomycetes may help to show the effects of water pollution.

In this study, 3 genera (*Arthrobotrys* Corda, *Monacrosporium* Oudem., and *Dactylella* Grove) from freshwater environments are studied in order to systematically investigate resources of aquatic nematode-trapping hyphomycetes and to compare species diversity and community similarities in both polluted and unpolluted aquatic environments.

MATERIALS AND METHODS

Sampling sites. In this study, 21 sites were sampled. Of these sites, 16 were in the longitudinal range-gorge region (LRGR) in southwestern China, mainly the Yunnan Province. LRGR includes the basins of 4 major international rivers, which comprise the Yuanjiang-Red River, the Lancang-Mekong River, the Nujiang-Salween River, and the Irrawaddy River. This area is classified as one of the world's hotspots for biodiversity.

Another site, the Lotus Pool, is 2 km away from the City of Anglong County, southwest of Guizhou Province. The Lotus Pool covers 53 000 m² and only lotus is planted there.

The remaining 4 sites were Dianchi Lake, Panlong River, the Cangshan Nature Reserve, and the Kuankuoshui Nature Reserve. Dianchi Lake is 306 km² and lies in the south suburb of Kunming City, Yunnan Province. Its average depth is 4.4 m and the deepest 10 m. The water has been contaminated by urban sewage and industrial waste. Samples from Dianchi Lake were collected at the shallow water area (20 cm deep) and at 4 m depth. Panlong River, 105 km long, originates from Songhuaba Reservoir in the north suburb of Kunming City and flows into Dianchi Lake. Its water has long been contaminated by urban sewage. The Cangshan Nature Reserve lies in Dali District, Yunnan Province. The altitude of Cangshan ranges from 2200 to 4122 m. The coverage of Cangshan reaches 950 km² and 18 brooks flow through the mountain. The Kuankuoshui Nature Reserve covers 2450 km² and lies in Suiyang County, Guizhou Province. The altitude of Kuankuoshui ranges from 850 to 1700 m.

Sampling. The samples were collected randomly with a scoop or a grab sampler. Each sample, approx. 50 g, consisted of bottom sediment. Altogether 1000 samples were collected of which 20 samples were collected from the bottom of Dianchi Lake (24° 50' N, 102° 45' E), at a depth of 4 m, and the rest were collected from shallow depths (20 cm) at the 21 sampling sites. Of the 980 samples, 400 were collected in Dianchi Lake (24° 50' N, 102° 45' E), Panlong River (25° 22' N, 103° 15' E), and brooks of both the Cangshan Nature Reserve (25° 58' N, 100° 02' E) and the Kuankuoshui Nature Reserve (28° 13' N, 107° 10' E) in July 2003. In each of the above sites, 100 samples were collected. In Lotus Pool (25° 07' N, 105° 20' E), 100 samples were collected in September 2003. In April 2004, the rest of the samples were collected from 16 sampling sites in brooks of LRGR (WenShan 23° 32' N, 104° 26' E; MaGuan 23° 06' N, 104° 42' E; MengZi 23° 23' N, 103° 19' E; GeJiu 22° 34' N, 103° 15' E; HongHe 23° 37' N, 102° 39' E; SiMao 22° 83' N, 100° 94' E; JingHong 22° 03' N, 100° 87' E; TengChong 24° 95' N, 98° 43' E; GenMa 22° 71' N, 99° 80' E; LinCang 23° 88' N, 100° 11' E; BaoShan 24° 46' N, 99° 11' E; MangShi 24° 43' N, 99° 61' E; MengLun 21° 93' N, 101° 25' E; LanPing 26° 42' N, 99° 45' E; LiJiang 26° 88' N, 100° 23' E; ZhongDian 27° 88' N, 99° 66' E). At each of the above sampling sites, 30 samples were collected. All the samples were put into plastic bags and sealed and stored in a refrigerator at 4°C before being analyzed.

Isolation and morphological identification of species. The method employed in this research was modified from that of Liu & Zhang (2003). In their method, after the plate inoculated with a selected spore was incubated, a patch (1.5 × 0.6 cm) of culture medium on the plate was dug away to form a hole. Then nema-

todes *Panagrellus redivius* were added into the hole to observe what traps are produced by the hyphomycete species. In the present study, as soon as a spore was selected onto another plate with corn meal agar (CMA), a patch (1.5 × 0.6 cm) was dug beside the spore. Then the plate inoculated with the selected spore was placed in an incubator (25°C). In this way, the time used to observe traps was shortened because when nematodes were added to the hole for observation of trap formation, hyphae had already grown at the bottom of the hole.

Each sample was prepared in triplicate. About 2 g of bottom sediment was spread onto every Petri dish with CMA using sterile toothpicks. Only one occurrence of the same species was recorded as long as it was isolated from any of the 3 replicates of 1 sample.

The isolated fungi in this study were identified according to the keys by Li et al. (2000).

Data analysis. Index of similarity was calculated using Sørensen's formula to determine similarity in species occurrences between stations (Odum 1971). The values for this index range from 0 to 1 (1 means very similar while 0 indicates no similarity) where:

$$S' = 2C / (A + B) \quad (1)$$

where S' is the degree of similarity, A and B are the number of species at Sites A and B, respectively and C is the number of species common to both collections.

The species diversity was measured using the Shannon-Wiener index (Shannon & Weaver 1963):

$$H' = -\sum_{i=1}^n P_i \log_e P_i \quad (2)$$

and Simpson index:

$$D = 1 - \sum_{i=1}^n P_i^2 \quad (3)$$

where H' (D) is the value of the species diversity, P_i is the proportion of the i th species and n the number of species at the site.

RESULTS

Nematode-trapping hyphomycetes

In total, 1000 samples were examined for aquatic nematode-trapping hyphomycetes. No species were isolated from the 20 samples collected from the bottom of Dianchi Lake, but from the remaining 980 samples 35 species were isolated (Table 1), 21 of which were of the genus *Monacrosporium*, 8 were *Arthrobotrys*, and 6 were *Dactylella*.

Of the 35 species, the most common were *Arthrobotrys musiformis*, *A. oligospora*, *Monacrosporium*

elliposporum, and *M. thaumasium*, which were isolated from 17, 18, 13 and 15 sites, respectively. *A. conoides* was the dominant species in BaoShan; *A. musiformis* was the dominant species in GeJiu and JingHong; *A. oligospora* was the dominant species in BaoShan, LanPing, Dianchi Lake and Panlong River; *M. elliposporum* was the dominant species in Wen-Shan and SiMao; and *M. megalosporum* was the dominant species in Lotus Pool. Of the 35 species, 17 produced adhesive networks, which amounted to 48.6% of the total isolated aquatic nematode-trapping hyphomycetes. More information on the type of traps observed for each recorded species is shown in Table 2.

Community similarities and species diversity

The samples used in the community similarities and species diversity study were collected in July 2003 from Dianchi Lake, Panlong River, and brooks of both the Cangshan Nature Reserve and the Kuankuoshui Nature Reserve. The first 2 sites belong to polluted aquatic environments. Brooks of the 2 reserves, however, belong to unpolluted aquatic environments. Fig. 1 presents species diversity for these 4 sites using the Shannon-Wiener index and Simpson index. The results of the 2 indices were almost identical; however, the value of Site 2 was higher than that of Site 1 according to the Shannon-Wiener index, whereas, the value of Site 1 was higher than that of Site 2 according to the Simpson index. This is because different diversity indices for a community may lead to different results (Patil & Paillie 1982, Tóthmérész 1995). The value of species diversity index for the Kuankuoshui

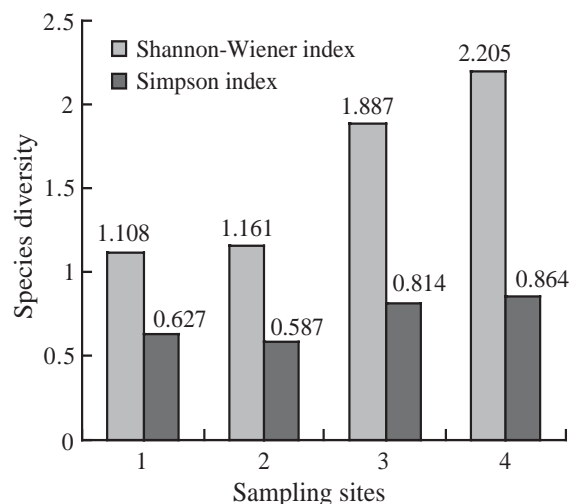


Fig. 1. Species diversity for 4 sampling sites. 1: Dianchi Lake; 2: Panlong River; 3: Cangshan; 4: Kuankuoshui

Table 1. Occurrence frequency (number of samples in which a certain species was isolated/total number of samples $\times 100\%$) of aquatic predacious fungi in the 21 sites

Species	Wen-Shan	Ma-Guan	Meng-Zi	Ge-Jiu	Hong-He	Si-Mao	Jing-Hong	Teng-Chong	Gen-Ma	Lin-Cang	Bao-Shan	Mang-Shi	Meng-Lun	Lan-Ping	Li-Jiang	Zhong-Dian	Dianchi-Lake	Panlong-River	Cang-shan	Kuankuo-shui	Lotus-Pool	
<i>Arthrobotrys brochopaga</i>																					8	
<i>A. conoides</i>	10		3	3	3	3	47	3	3	3	3	3	3	3								5
<i>A. dactyloides</i>									3										3			
<i>A. javanica</i>			3	3	3						7	20										
<i>A. musiformis</i>	13	23	10	43	30	37	50	10	13	13	30	7	7	31	13	10	54	31	16	22	8	
<i>A. oligospora</i>	23		33	20	10	20	17	13	13	33	50	17	7	56	3	10	54	56	14	14	12	
<i>A. superba</i>	3	3	3					3					30	1	3	20						
<i>A. vermicola</i>												13	3									
<i>Dactylella clavata</i>									3													
<i>D. dianchiensis</i>																	11					
<i>D. leptospora</i>																	11					
<i>D. multiformis</i>																3						
<i>Dactylella</i> sp.											3											
<i>D. yunnanensis</i>									3						3							
<i>Monacrosporium aphrobrochum</i>																				2	3	
<i>M. bembicoides</i>																						0.4
<i>M. candidum</i>								3														0.4
<i>M. cionopagum</i>								3												6	2	
<i>M. coelobrochum</i>												3								15	1	
<i>M. cystosporium</i>									3													0.4
<i>M. doedycoides</i>													3									
<i>M. drechsleri</i>		20						7								23						
<i>M. elegans</i>								3														
<i>M. elliposporum</i>	40	3	3	3	63	10	7	37	7	7	33	3	20	7	3	3	31	31	13	4		
<i>M. eudermatum</i>	7	3									3	3	3									
<i>M. longiphorum</i>		3																	17	9	2	
<i>M. megalosporum</i>									7	3										1	71	
<i>M. microscaphoides</i>																			0.4			
<i>M. parvicolle</i>												3										
<i>M. sclerothyphum</i>																		3				
<i>Monacrosporium</i> sp. 1																						6
<i>Monacrosporium</i> sp. 2																						1
<i>Monacrosporium</i> sp. 3																				5	1	
<i>M. sphaeroides</i>					7	3						3										1
<i>M. thaumasiom</i>	37	27	13	27	27	13	27	13	23	30	33	17	11	3	4	5	24	3	6	19		
Species richness (S)	7	7	6	3	6	4	6	8	6	8	4	7	11	7	4	5	4	6	9	9	18	5

Table 2. Type of traps produced by isolated species

Type of traps	Species isolated	No. species	Overall %
Adhesive nets	<i>Arthrobotrys conoides</i> , <i>A. javanica</i> , <i>A. musiformis</i> , <i>A. oligospora</i> , <i>A. superba</i> , <i>A. vermicola</i> , <i>Dactylella dianchiensis</i> , <i>D. multififormis</i> , <i>Monacrosporium cystosporium</i> , <i>M. elegans</i> , <i>M. eudermatum</i> , <i>M. longiphorum</i> , <i>M. megalosporum</i> , <i>M. microscephoides</i> , <i>Monacrosporium</i> sp. 3, <i>M. sphaeroides</i> , <i>M. thaumasium</i>	17	48.6
Constricting rings	<i>M. doedycooides</i> , <i>A. dactyloides</i> , <i>M. aphrobrochum</i> , <i>M. bembicodes</i> , <i>M. coelobrochum</i> , <i>A. brochopaga</i> ,	6	17.1
Adhesive knobs	<i>M. drechsleri</i> , <i>M. ellipsosporum</i> , <i>M. parvicolle</i> , <i>Monacrosporium</i> sp. 1, <i>M. sclerohyphum</i>	5	14.3
Adhesive knobs and non-constricting rings	<i>Monacrosporium</i> sp. 2, <i>D. leptospora</i> , <i>M. candidum</i>	3	8.6
Adhesive branches	<i>M. cionopagum</i>	1	2.9
No traps	<i>D. clavata</i> , <i>Dactylella</i> sp., <i>D. yunnanensis</i>	3	8.6

Nature Reserve was the highest, followed by that for the Cangshan Nature Reserve, for Panlong River, and for Dianchi Lake. Table 1 shows species richness for the 4 sampling sites. The species richness (S) for the Kuankuoshui Nature Reserve or the Cangshan Nature Reserve was higher than that for Dianchi Lake or Panlong River. The value of species diversity index for each of the nature reserves was higher than that for the contaminated lake or river.

Similarity indices of fungal communities between different sites are shown in Table 3. Values between the Cangshan Nature Reserve and the Kuankuoshui Nature Reserve or between Dianchi Lake and Panlong River were higher than those between Dianchi Lake and the Cangshan Nature Reserve, between Dianchi Lake and the Kuankuoshui Nature Reserve, between Panlong River and the Cangshan Nature Reserve, or between Panlong River and the Kuankuoshui Nature Reserve. The values between fungal communities living in similar aquatic environments were higher than those from dissimilar aquatic environments. Similar aquatic environments refer to polluted or unpolluted environments and dissimilar aquatic environments mean a polluted environment and an unpolluted one.

Table 3. Similarity indices of fungal communities between different sites

Site	Sørensen's index (S')		
	Panlong River	Cangshan	Kuankuoshui
Dianchi Lake	0.4	0	0.18
Panlong River		0.13	0.25
Cangshan			0.52

DISCUSSION

The isolated fungi in this study show that there are plenty of nematode-trapping hyphomycetes in freshwater environments. Except for *Dactylella dianchiensis* (Hao et al. 2004) and the 4 unidentified species, the rest of the 35 species have been described and recorded from terrestrial samples before. The results are in accordance with the broad definition of freshwater fungi (Thomas 1996). Other researchers also found similar results before. For example, *D. heptameris* was isolated from decaying leaves in a pond (Peach 1950) but this species was originally isolated from terrestrial samples (Drechsler 1943). Furthermore, Cai et al. (2003) noted 'Of the 58 taxa identified from bamboo submerged in the Liput River, 18 species overlapped with those on terrestrial bamboo in the Philippines, Hong Kong and mainland China.' The above examples demonstrate that many species from aquatic environments also exist in terrestrial systems.

No species was isolated from the bottom (4 m depth) of Dianchi Lake, while from all other sites (20 cm depth) a large number of species were isolated. Two explanations are offered: (1) the inefficiency of our sampling methods and (2) that aquatic nematode-trapping hyphomycetes numbers decrease with increased water depth. The latter case has been found for terrestrial samples. Persmark et al. (1996) reported a reduction in the number of nematode-trapping fungi in 4 soil layers (10, 20, 30 and 40 cm depth) and there were few nematophagous fungi below 40 cm. Further study needs to be conducted to find out whether numbers of aquatic nematode-trapping hyphomycetes decrease with increased water depth and whether the current isolation meth-

ods should be improved in order to investigate predacious fungi in deep waters.

Some isolated species produce adhesive networks, adhesive branches, adhesive knobs, constricting rings and non-constricting rings. Species with adhesive networks were the most frequently isolated. A similar result was reported by Gray (1987) and Persmark et al. (1997) when they investigated land samples. They speculated that species which produce adhesive networks grow quickly, need less nutrition and saprotrophic ability of these species is strong.

Several studies have suggested that differences in riparian vegetation structure and substrate preferences of freshwater fungi are important factors in determining the variations of fungal communities among different aquatic environments or even in the different regions in the same habitat, such as upstream and downstream of a river (Fabre 1996, Tsui et al. 2000). In this study it was also found that the similarity index of fungal communities between similar aquatic environments is broadly higher than that between dissimilar aquatic environments.

The distribution and species diversity of land nematode-trapping fungi are affected by such factors as altitude, habitat, soil moisture, organisms, pH, nematode density, etc. (Gray 1987), but whether those of nematode-trapping hyphomycetes in aquatic environments are affected by such factors is unknown. The species diversity of nematode-trapping hyphomycetes in aquatic environments may be affected by the ecological conditions of aquatic environments or water quality (unpolluted or polluted). In this study, although consideration was given to the time of collecting samples, the depth of sampling, the amount of samples, and the method of counting species, no attention was paid to the water temperature and the altitude of the sampling sites. Despite this, the results indicate that water pollution may exert an adverse effect on aquatic nematode-trapping hyphomycetes. Indeed, the fact that water pollution has an adverse effect on fungal diversity has been reported widely. Tsui et al. (2001) reported that aquatic fungi diversity gradually declines with increased water contamination. The contamination of heavy metals and organisms generally lowers the fungal biodiversity in terrestrial and aquatic ecosystems (Raviraja et al. 1998, Ledin 2000, Lovely 2000, Prasad 2001). Au et al. (1992) also indicated that many species will disappear when water is polluted. Pollution is a severe problem faced by all living beings on earth and is rapidly reducing the bio-resources. With the deterioration of water quality, aquatic fungal resources are becoming threatened so much that numerous species may become extinct before they can be discovered. Protecting the abundant resources of aquatic predacious fungi is necessary.

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