
Aquatic fungi from Lake Fuxian, Yunnan, China

Lei Cai^{1*}, Clement K.M. Tsui², Keqin Zhang¹ and Kevin D. Hyde²

¹Key Laboratory of Microbial Fermentation of Yunnan Province, Yunnan University, Kunming, Yunnan, P.R. China; *e-mail: lei_cai@hotmail.com

²Centre for Research in Fungal Diversity, Department of Ecology & Biodiversity, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China

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Sixty-four higher fungi were recorded on submerged wood, bamboo and tree roots in Lake Fuxian, Yunnan, China. *Aniptodera chesapeakensis*, *Dictyosporium heptasporum*, and *Savoryella lignicola* were frequently collected on wood samples. The occurrence of *Halosarpheia retorquens* and *Halosphaeria cucullata*, which have previously been recorded from marine habitats, is interesting, while a species of *Lulworthia* is the first record of this genus from a lake. *Pseudohalonectria fuxianii* sp. nov. is described and illustrated and compared with similar species in the genus. This is the first report of aquatic fungal communities in a lake from mainland China and the data is compared with previous studies.

Key words: Ascomycetes, biodiversity, ecology, marine, mitosporic fungi, taxonomy.

Introduction

There have been many reports of aquatic fungi on wood submerged in rivers or streams in tropical and subtropical regions such as Hong Kong (Tsui *et al.*, 2000), north Queensland, Australia and the Seychelles (Hyde and Goh, 1997, 1998a). Fungi on submerged wood have also been reported from streams in temperate regions, particularly UK and USA (Shearer *et al.*, 1991; Shearer, 1993; Hyde and Goh, 1998b). There have however, been few reports of fungi occurring on submerged wood in temperate or tropical lakes (Shearer and Crane, 1986; Goh and Hyde, 1999; Hyde and Goh, 1999).

There are about 40 lakes in Yunnan Province, China and they have high economic importance for fishing and water supply (Whitmore *et al.*, 1997). In the present study we have investigated the fungi on submerged wood in Lake Fuxian, in Yunnan, China.

Materials and Methods

Lake Fuxian (24°35'N 102°50'E) is located in the south east of Kunming and is the second deepest lake in China (max. depth 155 m) (Whitmore *et al.*, 1997). It was formed by tectonic faulting and is supposed to be of the Pliocene

age because numerous fossil animals have been discovered from nearby localities. The climate in that region is warm temperate and the lake is unusual as run off from the surrounding mountains carry large quantities of sulphur into the waters (sulphur concentration in some inlets up to 37.6 mg/L).

Visits were made to the west-bank of Lake Fuxian in December 1999, August 2000 and April 2001. The banks of the lake are lined with trees of *Ficus virens* var. *sublanceollapa* and smaller shrub-like trees and plants. Woody samples, as well as submerged bamboo and tree roots were collected during each visit, and subsequently about 100 samples have been collected. These samples were collected from the pools in the small caves where water seeps from the surrounding cliffs. In general, the water from the pools is slightly warmer than the lake water. When the water level of the lake is low, the water flows from the pools into the lake. The pools are then distinct habitats from the main lake. When the water level of the lake is high, the lake water mixes with the pool waters.

Water samples were collected in August 2000 and the water temperature (23-25 °C), salinity (2.37‰), chloride concentration (4.2 mg/L) and sulphur concentration (37.6 mg/L) of the cave pools were measured in the region (data analysed by the Inspection Centre of Water Resource, Yunnan Provincial Government, China).

After collection in the field, all the samples were taken back to the laboratory, incubated individually in snap lock plastic bags for 1-3 weeks, and then examined under a dissecting microscope for the presence of fungi. Sporulating fungi were identified and their occurrence recorded. Material supporting fungi was air-dried and is held in YNU. Some duplicates and type material of new species are lodged at HKU(M). Frequency of occurrence for each fungus was calculated based on the number of samples on which a particular fungus was found as against the total number of samples examined (Tsui *et al.*, 2000). Species-area curve was also plotted to estimate the minimum number of woody substrates needed to collect most common fungi present in this habitat (Begon *et al.*, 1993).

Results

A total of 64 fungal taxa, comprising 35 ascomycetes and 29 mitosporic taxa were recorded (Table 1). Twenty-two taxa are new records for China and all are new records for organic substrata submerged in a lake in mainland China. The most common species identified in this study was *Dictyosporium heptasporum* occurring on 15% of samples. Other common species were *Aniptodera chesapeakeensis* (10%), *Coelomycete* sp. (6%), *Massarina thalassioidea* (6%), *Phaeoisaria clematidis* (6%), *Pseudohalonectria lignicola*

Table 1. Fungi collected on submerged wood at Lake Fuxian (percentage occurrence data from collections in April 2001).

Taxa	Frequency of occurrence
<i>Dictyosporium heptasporum</i> (Garov.) Damon	15
<i>Savoryella lignicola</i> R.A. Eaton & E.B.G. Jones	10
<i>Aniptodera chesapeakeensis</i> Shearer & M.A. Mill.	9
<i>Coelomycete</i> sp.	6
<i>Massarina thalassioidea</i> K.D. Hyde & Aptroot	6
<i>Phaeoisaria clematidis</i> S. Hughes	6
<i>Pseudohalonectria lignicola</i> Minoura & T. Muroi	6
<i>Brachiosphaera tropicalis</i> Nawawi	5
<i>Jahnula poonythii</i> K.D. Hyde & S.W. Wong*	5
<i>Halosphaeria cucullata</i> (Kohlm.) Kohlm.*	4
<i>Pleospora</i> sp.	4
<i>Xylomyces chlamydosporis</i> Goos, R.D. Brooks & Lamore	4
<i>Dactylaria hoogi</i> R.F. Castañeda & W.B. Kendr.	3
<i>Diplodia</i> sp.	3
<i>Helicomycetes torquatus</i> Shearer	3
<i>Jahnula granulosa</i> K.D. Hyde & S.W. Wong*	3
<i>Kirschsteiniothelia elasterascus</i> Shearer	3
<i>Phoma</i> sp. 1	3
<i>Phomatospora berkeleyi</i> Sacc.	3
<i>Torula herbarum</i> (Pers.) Link	3
<i>Trematosphaeria</i> sp.	3
<i>Astrosphaeriella vesuvius</i> D. Hawksw. & Boise*	2
<i>Dactylaria uniseptata</i> Matsush.	2
<i>Leptosphaeria</i> sp.	2
<i>Lulworthia midwinteri</i> Cai <i>et al.</i> *	2
<i>Massarina peerallyi</i> K.D. Hyde & Aptroot*	2
<i>Mauritiana rhizophorae</i> Poonyth, K.D. Hyde, Aptroot & Peerally*	2
<i>Nais inornata</i> Kohlm.*	2
<i>Phaeosphaeria luctuosa</i> Otani & Mikawa*	2
<i>Phoma</i> -like sp.	2
<i>Pseudohalonectria lutea</i> Shearer*	2
<i>Alysidium</i> sp.	1
<i>Aniptodera margaritum</i> Shearer*	1
<i>Astrosphaeriella trochus</i> (Penz. & Sacc.) D. Hawksw.*	1
<i>Berkleasium corticola</i> R.T. Moore	1
<i>Beverwykella pulmonaria</i> (Beverw.) Tubaki*	1
<i>Camarosporium</i> -like sp.	1
<i>Diaporthe</i> sp.	1
<i>Diatrypella</i> sp.	1
<i>Dictyosporium lakefuxianensis</i> L. Cai, K.D. Hyde, McKenzie & K. Zhang *	1
<i>Didymostilbe</i> sp.	1
<i>Dictyosporium australiense</i> B. Sutton	1

* New records for China.

Table 1. (continued).

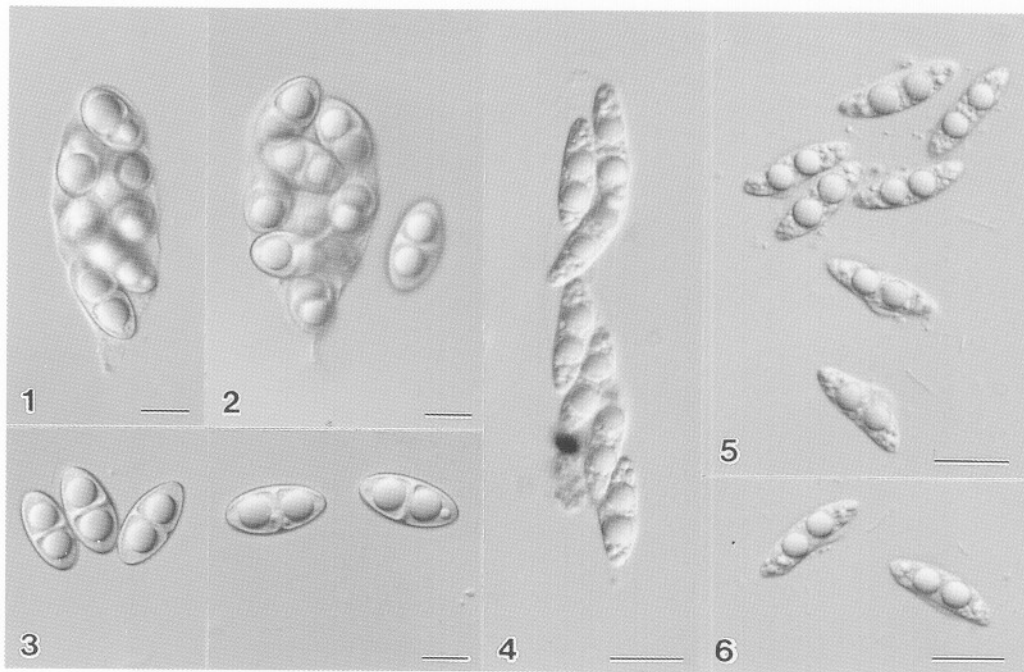
Taxa	Frequency of occurrence
<i>Dyrithiopsis lakefuxianensis</i> L. Cai, K.D. Hyde, Jeewon, E.C.Y. Liew & K. Zhang*	1
<i>Ellisembia adscendens</i> Subram.	1
<i>Eutypa</i> sp.	1
<i>Helicomyces roseus</i> Link	1
<i>Halosarpheia retorquens</i> Shearer & J.L. Crane*	1
<i>Massarina corticola</i> (Fuckel) L. Holm	1
<i>Massarina ingoldiana</i> Shearer & K.D. Hyde	1
<i>Massarina</i> sp.	1
<i>Nais aquatica</i> K.D. Hyde	1
<i>Ophioceras commune</i> Shearer, J.L. Crane & W. Chen	1
<i>Ophioceras tenuisporum</i> Shearer*	1
<i>Periconia minutissima</i> Corda	1
<i>Phaeonectriella appendiculata</i> K.D. Hyde & W.H. Ho*	1
<i>Phoma</i> sp. 2	1
<i>Phomopsis</i> sp.	1
<i>Pseudohalonectria fuxianii</i> L. Cai, K. Zhang & K.D. Hyde*	1
<i>Sporidesmiella hyalosperma</i> var. <i>hyalosperma</i> (Corda) P.M. Kirk*	1
<i>Sporoschisma saccardoi</i> E.W. Mason & S. Hughes	1
<i>Trichocladium lignicola</i> I. Schmidt*	1
<i>Xylomyces pusillus</i> Goh, W.H. Ho, K.D. Hyde & K.M. Tsui	1
<i>Verticillium</i> sp.	1
<i>Zopfiella latipes</i> (N. Lundq.) Malloch & Cain*	1

(6%), and *Savoryella lignicola* (9%). Thirty-three species were recorded on one occasion. The average number of fungi recorded on each sample was comparatively high, with an average of 1.61 species per sample. The fungi occurring on these wood samples were characteristic aquatic species and not indicative of aerial contamination.

Halosarpheia retorquens, *Halosphaeria cucullata*, and *Lulworthia* sp. are probably some of the most interesting taxa as these are normally found in marine or estuarine environments (Hyde and Pointing, 2000). Other interesting taxa were *Dyrithiopsis lakefuxianensis*, a new amphisphaeriaceous genus producing a *Monochaetia* anamorph in culture and a new species of *Pseudohalonectria*. *Pseudohalonectria fuxianii* sp. nov. is described and illustrated in this paper, and the details of some interesting taxa are also described below.

Aniptodera margarition Shearer, Mycologia 81: 142 (1989). (Figs. 1-3)

Notes: This is the first report of the taxon from Asia. It was described and has been collected from USA and England (Shearer, 1989a).



Figs. 1-6. *Aniptodera margarition* (1-3) and *Halosarpheia retorquens* (4-6). 1-2. Asci. 3. Ascospores. 4. Asci. 5-6. Ascospores. Bars: 1-3 = 10 μ m; 4-6 = 15 μ m.

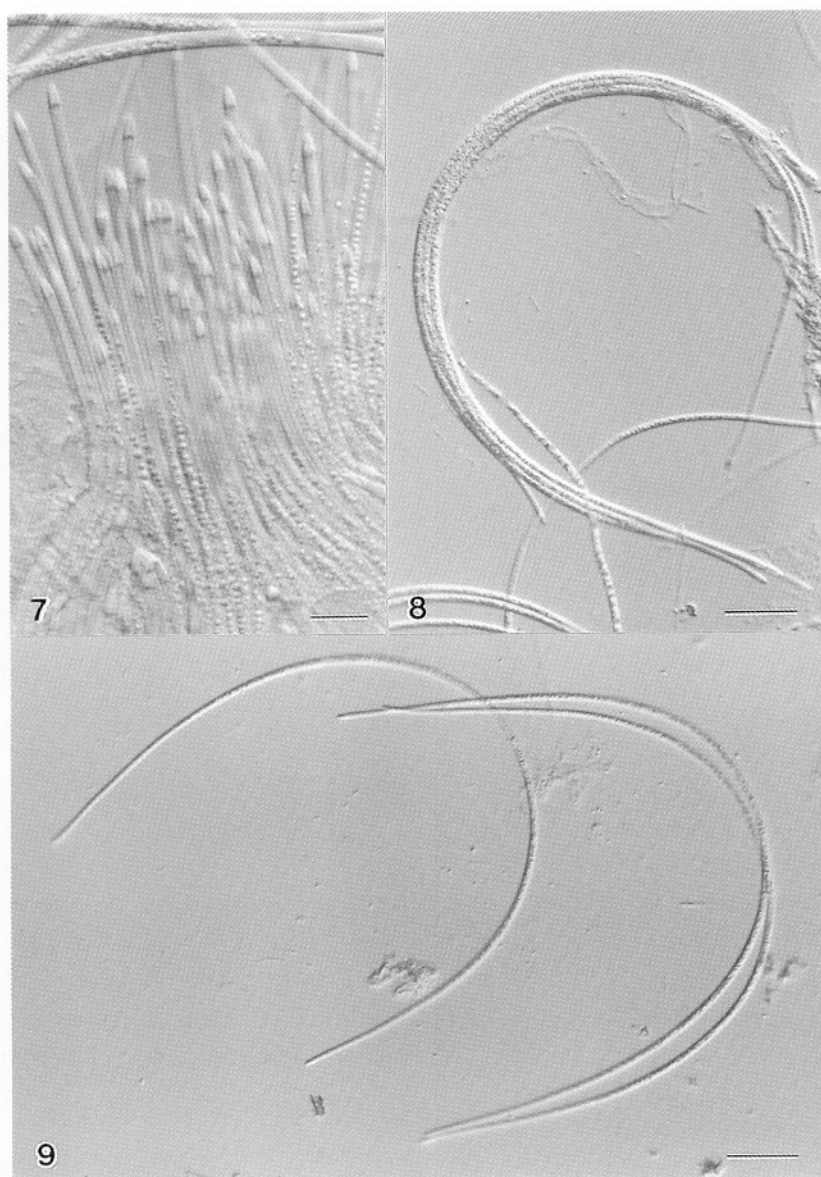
Halosarpheia retorquens Shearer & J.L. Crane, *Botanica Marina* 23: 608-609 (1980). (Figs. 4-6)

Ascomata ca. 140-200 μ m diam., gregarious, globose or subglobose, immersed or superficial, dark brown to black, ostiolate, periphysate. *Catenophyses* present. *Asci* ca. 110 \times 30 μ m, unitunicate, 8-spored, clavate, pedicellate, apically truncate, thin-walled, deliquescing early. *Ascospores* 22-25 \times 6.5-8 μ m, 2-3-seriate, hyaline, ellipsoidal with acute ends, 1-septate and constricted at the septum, smooth, thin-walled, with polar appendages extending from the end to the septum.

Material examined: CHINA, Yunnan Province, Cheng Jiang, Lu Chong, 1 km north on bank of Fuxian Lake, on submerged wood, 26 December 1999, L. Cai and K.M. Tsui (HKU(M) 16107).

Halosphaeria cucullata (Kohlm.) Kohlm., *Canadian Journal of Botany* 50: 1956 (1972).

Ascomata semi-immersed, becoming superficial, subglobose to obpyriform, black, coriaceous, solitary or clustered, with a short neck, ostiole periphysate. *Paraphyses* not seen, catenophyses-like structures present. *Asci* 80-87.5 \times 17.5-27.5 μ m (\bar{x} = 83.5 \times 22 μ m, n = 5), 8-spored, cylindric-clavate



Figs. 7-9. *Lulworthia* sp. Ascospores. Note the end chambers which are clearly visible in 7. Bars = 20 μ m.

to clavate, unitunicate, pedicellate, lacking an apical apparatus, deliquescing early. *Ascospores* 22-61 \times 5-7 μ m (\bar{x} = 45 \times 6 μ m, n = 25), fasciculate, cylindrical, hyaline, 0-1-septate, smooth-walled, lacking a sheath.

Material examined: CHINA, Yunnan Province, Cheng Jiang, Lu Chong, 1 km north on bank of Fuxian Lake, 24°35'N 102°50'E, on submerged twig, 26 December 1999, L. Cai and K.M. Tsui (HKU(M) 16108); *ibid.*, 28 August 2000, L. Cai and K.D. Hyde (HKU(M) 16128); *ibid.*, 5 April 2001, L. Cai and K.D. Hyde (HKU(M) 16129).

Known habitat: On wood in mostly marine habitats.

Despite many attempts, it was not possible to isolate the taxon in culture.

***Lulworthia* sp.**

(Figs. 7-9)

Ascomata 250-300 μm high, 200-250 μm diam., semi-immersed, becoming superficial, subglobose to globose, black, coriaceous, solitary, ostiole periphysate. *Paraphyses* lacking. *Asci* 240-430 \times 6-9 μm (\bar{x} = 305 \times 8 μm , n = 5), 8-spored, cylindrical, unitunicate, thin-walled, deliquescing early. *Ascospores* 210-405 \times 2-3 μm (\bar{x} = 295 \times 2.5 μm , n = 25), fasciculate, filiform, hyaline, unicellular, smooth-walled, with tapering end chambers, provided with mucilaginous appendages.

Material examined: CHINA, Yunnan Province, Cheng Jiang, Lu Chong, 1 km north on bank of Fuxian Lake, 24°35'N 102°50'E, on submerged twig, 28 August 2000, L. Cai and K.D. Hyde (HKU(M) 16127).

Known habitat: On various aquatic substrata, usually in marine habitats.

Notes: Despite many attempts, it was also not possible to isolate the taxon in culture.

***Pseudohalonectria fuxianii* L. Cai, C.K.M. Tsui, K. Zhang & K.D. Hyde, sp. nov.**

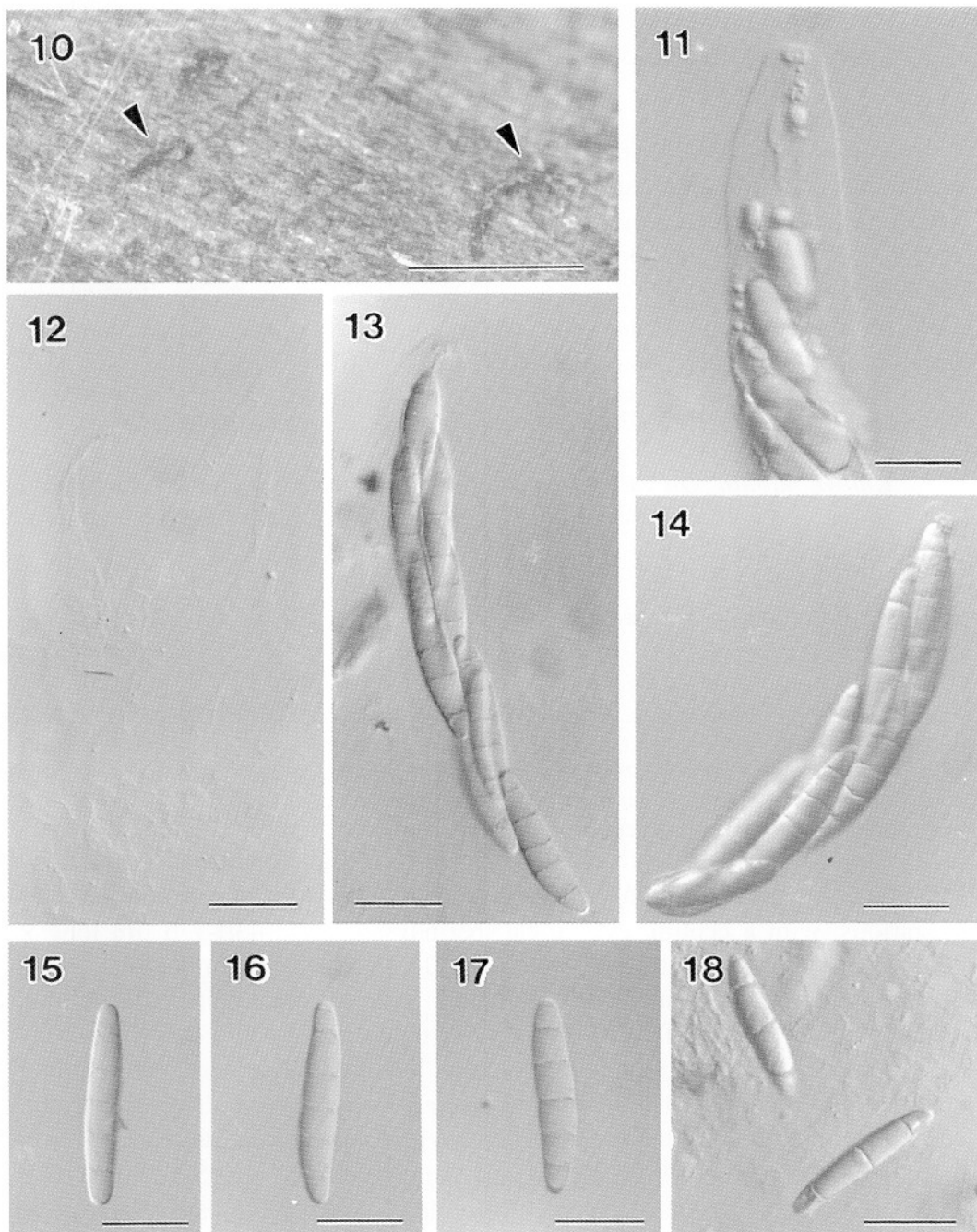
(Figs. 10-19)

Etymology: referring to the place where this fungus was collected.

Ascomata 240-320 μm diam., 300-400 μm alta, immersa vel erumpentes, brunneoaurantiaca, globosa vel subglobosa, solitaria vel gregaria, paraphysaticum. *Collum* 430-570 μm longum \times 70-110 μm diam. *Asci* 90-187.5 \times 17.5-30 μm , 8-spore, cylindrici vel clavati, apparatu apicale 2.5-3 μm diam. \times 2-2.5 μm alta. *Ascosporeae* 30-52.5 \times 7.5-12.5 μm , multi-seriatae, ellipsoideae, 3-5-septatae, hyaline to subhyalinae.

Ascomata 240-320 μm diam., 300-400 μm high, immersed or partially immersed, erumpent, orange-brown, globose to subglobose, solitary to gregarious, with a central, cylindrical, periphysate neck, 430-570 μm long \times 70-110 μm diam. (Figs. 10, 19). *Peridium* 10-24 μm wide, membranous, tissue of *textura angularis*, outer cells darkened yellow, inner cells pale yellow, pseudoparenchymatic (Fig. 19). *Paraphyses* numerous, up to 6 μm wide at the base, filamentous, tapering distally, septate, hyaline, unbranched, same length as asci (Fig. 11). *Asci* 90-187.5 \times 17.5-30 μm , 8-spored, cylindrical to clavate, with a refractive, thimble-shaped, nonamyloid apical apparatus, 2.5-3 μm diam. \times 2-2.5 μm high (Figs. 12, 13). *Ascospores* 30-52.5 \times 7.5-12.5 μm (\bar{x} = 38.5 \times 9 μm , n = 20), multi-seriate, ellipsoidal, 3-5-septate, mostly 5-septate at maturity, hyaline to subhyaline, apex round or slightly curved, without sheath or appendages (Figs. 14-18).

Colonies on potato dextrose agar growing slowly, up to 1.5 cm diam. in two weeks at room temperature (\sim 25 C), floccose, appressed, aerial mycelium present, pale brown in centre and pale yellow towards the margin from above;



Figs. 10-18. Light and differential interference contrast micrographs of *Pseudohalonectria fuxianii* (from holotype). **10.** Appearance of ascomata on wood (arrowed). **11.** Apical portion of the ascus showing the refractive apical ring. **12.** Paraphyses. **13-14.** Asci. **15-18.** Ascospores. Bars: 10 = 1200 μm ; 12-13, 18 = 20 μm ; 14 = 22 μm ; 15-17 = 18 μm .

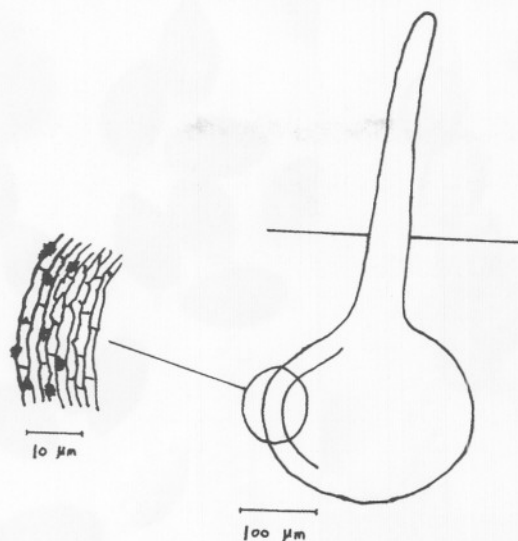


Fig. 19. *Pseudohalonectria fuxianii* (from holotype). Diagram of ascoma and peridium.

reverse brown to dark brown, margin regular. The medium around the colonies was stained dull yellow. Hyphae 2-2.5 μm wide, pale yellow, septate, branched and smooth-walled, not sporulating in culture.

Holotype designated here: CHINA, Yunnan Province, Chengjiang, Lake Fuxian, on submerged wood, 5 April 2001, L. Cai and K.D. Hyde (HKU(M) 16126), **isotype** in (HKUCC 8619).

Habitat: Saprobic on submerged wood.

Known distribution: China.

Notes: *Pseudohalonectria fuxianii* is distinguished by its ellipsoidal ascospores (Shearer, 1989b; Hyde *et al.*, 1999). It is most similar to *P. adversaria* Shearer which also have ellipsoidal 5-septate ascospores. *Pseudohalonectria fuxianii* differs however, in having much wider ascospores (7.5-12.5 μm , vs. 4.5-7 μm).

Zopfiella latipes (Lundqvist) Malloch & Cain, Canadian Journal of Botany 49: 876 (1971) (Figs. 20-22)

Notes: This is fairly common species found on various herbaceous and woody substrata in both marine and terrestrial environments (Guarro *et al.*, 1991).

Discussion

The fungi comprised taxa which are common on wood in both tropical and temperate aquatic habitats, e.g. *Aniptodera chesapeakeensis*, *Helicomycetes torquatus*, *Pseudohalonectria lignicola* and *Savoryella lignicola* (Shearer,



Figs. 20-22. *Zopfiella latipes*. Immature asci and ascospores. Bars = 15 μ m.

1993; Goh and Hyde, 1999; Tsui *et al.*, 2000). This shows that these fungal taxa are cosmopolitan in distribution. However the occurrence of *Aniptodera margaritum* and *Diatrypella* sp. is indicative of the temperate climate of the region.

Lake Fuxian itself is not fully freshwater and can be considered brackish (salinity = 2.37‰). This study showed the existence of *Halosarpheia retorquens*, *Halosphaeria cucullata*, and *Lulworthia* sp. in a brackish lake located more than 600 km from the ocean. This observation is not unusual because certain typical marine taxa, such as *Lulworthia* sp., *Halosarpheia* spp. and *Nais inornata* have also been reported from a brackish lake in Italy (Grasso and La Ferla, 1982) and a saline lake in Wyoming (Davidson, 1974). Recently a *Lulworthia* sp. has also been isolated in a Montana river in the USA (Cai and Hyde, unpublished). This indicates that typical marine taxa can be isolated from brackish or saline habitats, remote from the sea and ocean, or even freshwater habitats. Currently both freshwater fungi and marine fungi are defined ecologically, for fungi which grow and reproduce predominantly or exclusively in freshwater or marine habitats (Kohlmeyer and Kohlmeyer, 1979; Shearer, 1993). Fungi isolated from brackish or salt lakes are excluded from these definitions because of inadequate information. Davidson (1974) proposed

Table 2. Number of taxa, average number of taxa identified and most common species identified in similar studies on freshwater fungi in lakes.

Location	Lake Barrine, North Queensland	Plover Cove Reservoir Hongkong	Lake Fuxian Yunnan, China	Swamps in North America
Climate	Tropical	Subtropical	Warm temperate	Temperate
Number of taxa identified	39 (15 ascomycetes, 23 mitosporic fungi and 1 basidiomycete)	57 (17 ascomycetes and 40 mitosporic fungi)	64 (35 ascomycetes and 29 mitosporic fungi)	134 (45 ascomycete, 88 hyphomycetes and 1 basidiomycetes), and 13 myxomycetes
Average number taxa per sample	1.4 (1.7 ^a)	2.4	1.6	Not provided
Three most common species	<i>Candelabrum brocciatum</i> (41%) <i>Trichocladium linderi</i> (11%) <i>Canalisporium pulchrum</i> (9%)	<i>Kirschsteiniothelia elasterascus</i> (70%) <i>Didymella aptrooti</i> (14%) Unidentified coelomycetes (12%)	<i>Dictyosporium heptasporum</i> (15%) <i>Aniptodera chesapeakeensis</i> (10%) <i>Savoryella lignicola</i> (9%)	Not provided
Reference	Hyde and Goh (1998)	Goh and Hyde (1999)	This paper	Shearer and Crane (1986)

^a after 6 months incubation.

the term "salt water fungi" for those typical marine taxa isolated from saline inland aquatic environments. However we considered this definition was also arbitrary and a meaningful definition could only be described with additional physiological and molecular studies on their ability to survival and reproduce, and the phylogenetic relationships between representatives isolated in different habitats.

There are some possible explanations for the occurrence of these typical marine fungi in inland lakes (Davidson, 1974). During the process of tectonic movement, the formation of Lake Fuxian may have kept the salts and the "relic marine fungal population" from the geological past. Also the fungi could have been introduced by migratory birds or air currents (Davidson, 1974). Since the ponds of the Lake Fuxian in the caves, where run off from the surrounding mountains occurs, contain high levels of salts and minerals, these introduced fungi are able to survive.

Studies on the fungal diversity in lakes and reservoirs have been conducted in the tropics and subtropics (Hyde and Goh, 1998; Goh and Hyde, 1999) (Table 2). Fifteen ascomycetes, 23 mitosporic fungi and 1 basidiomycete

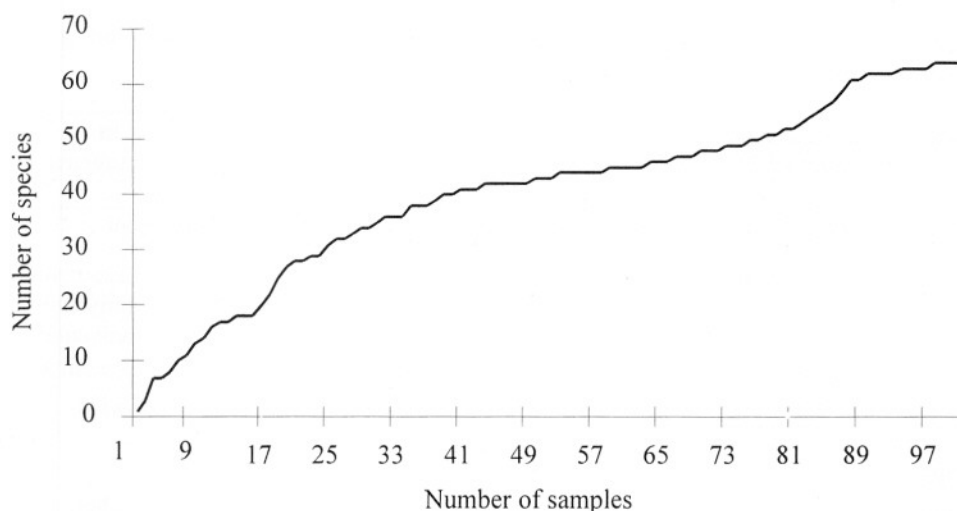


Fig. 23. Cumulative graph of number of fungi identified vs wood examined in Lake Fuxian.

were identified in Lake Barrine, with an average of 1.4 fungi identified per sample (Hyde and Goh, 1998). In the Plover Cove Reservoir, 17 ascomycetes and 40 mitosporic fungi were identified with an average of 2.4 fungi per sample (Goh and Hyde, 1999). In addition, Shearer and Crane (1986) found 134 fungal taxa, comprising 45 ascomycetes and 88 hyphomycetes, and 13 myxomycetes in a freshwater swamp in North America. Apparently the fungal diversity of the swamps appeared to be greater, possibly related to a more substantial collection of samples collected in more than one swamps, whereas the number of species in this study is similar to that from the Plover Cove Reservoir in Hong Kong.

Fungal communities in the Lake Fuxian are different to those from tropical and subtropical lakes (only 5 taxa and 4 taxa overlap with that in the Plover Cove Reservoir, and Lake Barrine respectively). There is also no overlap in the three most common species at each site. Also only nine fungal taxa in Lake Fuxian were in common with the fungal assemblages in swamps of USA (Shearer and Crane, 1986). These data show that the fungi communities in lotic habitats vary greatly between continents and different habitat types. A comparison of the number of taxa, average number of taxa, and most common species identified in the above studies are listed in Table 2. The frequency of occurrence of the most common species in Lake Fuxian (*Dictyosporium heptasporum* 15%) was distinctly lower than that in Lake Barrine (*Candelabrum brocciatum* 41%) and Plover Cove Reservoir (*Kirschsteiniothelia elasterascus* 70%). More ascomycetes (54.7%) than mitosporic taxa were identified in the present study as compared to previous

studies (Lake Barrine, 15 ascomycetes and 23 mitosporic fungi; Plover Cove Reservoir, 17 ascomycetes and 40 mitosporic fungi).

The results presented here go some way towards determining the assemblage of fungi occurring on submerged wood in a warm temperate lake. Direct incubation has been shown to yield more ascomycetes and less aquatic hyphomycetes when compared to bubble chamber incubation (Shearer and Webster, 1991) and therefore our technique may exclude certain Ingoldian fungi.

A curve was plotted to indicate the cumulative number of fungi identified against the number of woody samples examined (Fig. 23). The number of fungi increased quickly at first and then reached asymptote at around 88 samples. This indicates that collecting 100 wood samples can provide an accurate estimate of the fungal diversity in Fuxian Lake, as has been shown in other studies (e.g. Ho *et al.*, 2001).

Acknowledgements

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