Ingoldian fungi in Lam Tsuen River and Tai Po Kau Forest Stream, Hong Kong

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Ingoldian fungal communities in Lam Tsuen River and Tai Po Kau Forest Stream were explored using three sampling techniques: foam collection, collection of naturally submerged leaves and leaf baiting. A total of 41 species belonging to 26 genera of Ingoldian fungi were identified. The common species were *Anguillospora* and *Triscelophorus* spp. Differences in species diversity were found using different sampling techniques. Foam collection was found to be the most efficient sampling technique of the methods used in studying fungal communities. There was no significant difference in species diversity either monthly or between the two sampling streams.

Key words: aquatic hyphomycetes, biodiversity, techniques.

Introduction

Ingoldian fungi play an important role in the decomposition of organic material, especially leaves in freshwater ecosystems (Au *et al.*, 1992a,b). They occur on plant debris in streams and rivers and sporulate underwater. This has been known since the pioneering studies of Ingold (1942). Studies of Ingoldian fungi have since been carried out in many countries of the world, but mostly in temperate regions (Goh, 1997).

The main purpose of this study was to investigate the biodiversity of Ingoldian fungi in Hong Kong freshwater ecosystems. Lam Tsuen River and Tai Po Kau Forest Stream were chosen as representative streams. Three sampling methods were used to study the biodiversity of Ingoldian fungi at the two sampling sites from September 1998 to December 1998. Other objectives of this research included: (i) To compare the abundance and biodiversity of Ingoldian fungi in Lam Tsuen River and Tai Po Kau Forest Stream; (ii) To compare the abundance and biodiversity of Ingoldian fungi during each of the three months studied; (iii) To compare the efficiency of the three methods in collecting fungal species.

Previous studies of Ingoldian fungi in Hong Kong streams have been carried out with a comparison of the fungi on specific leaf types in the polluted Lam Tsuen River and an unpolluted Tai Po Kau Forest Stream (Au *et al*., 1992a,b). Results indicated that Lam Tsuen River with organic pollution from nearby farms, had a comparatively poor species diversity and sporulation of aquatic hyphomycetes, when compared to Tai Po Kau Forest Stream, which pass through a managed nature reserve on the southwestern shore of Tolo Harbour (Fig. 1).

Since the implementation of the Tolo Harbour and Channel WCZ in 1987, there has been a marked improvement in the water quality of Lam Tsuen River. In 1996, the water quality of seven of 9 monitoring stations established by Environmental Protection Department Hong Kong were classified as excellent (Environmental Protection Department, 1996). As a result, the biodiversity of Ingoldian fungi in the "clean" Lam Tsuen River may be different from that in the past.

Materials and methods

Foam collection

This method involved the examination of persistent foam that developed below rapids, around barriers, or clinging behind boulders and fallen logs. Air bubbles rising through water efficiently capture fungal spores, and examination of foam is believed to give a reasonably complete list of aquatic hyphomycetes occurring in a given stream (Ingold, 1975). Foam from each stream were scooped up with a spoon into clean specimen bottles and kept in a portable icebox for transport to the laboratory. The foam samples were immediately kept in the refrigerator in the laboratory. The samples were usually kept in 3 C to 7 C. The purpose was to prevent the conidia in the foam from germinating. Ten drops of foam, one from each random sample, were examined for the presence of Ingoldian fungi. The fungi were then counted and identified under the microscope.

Substrate collection

To investigate the biodiversity of Ingoldian fungi in the two streams, submerged leaves accumulating between the rocks forming the rapids were collected randomly. Old and colonized leaves were selected and placed into clean polythene bags for transport to the laboratory. In the laboratory, the leaves were washed with distilled water and cut up into discs with a diameter of 0.8 mm. Eighty leaf discs from each stream were cut from as many different leaves as possible, then placed into small conical flasks containing 30 mL of water.

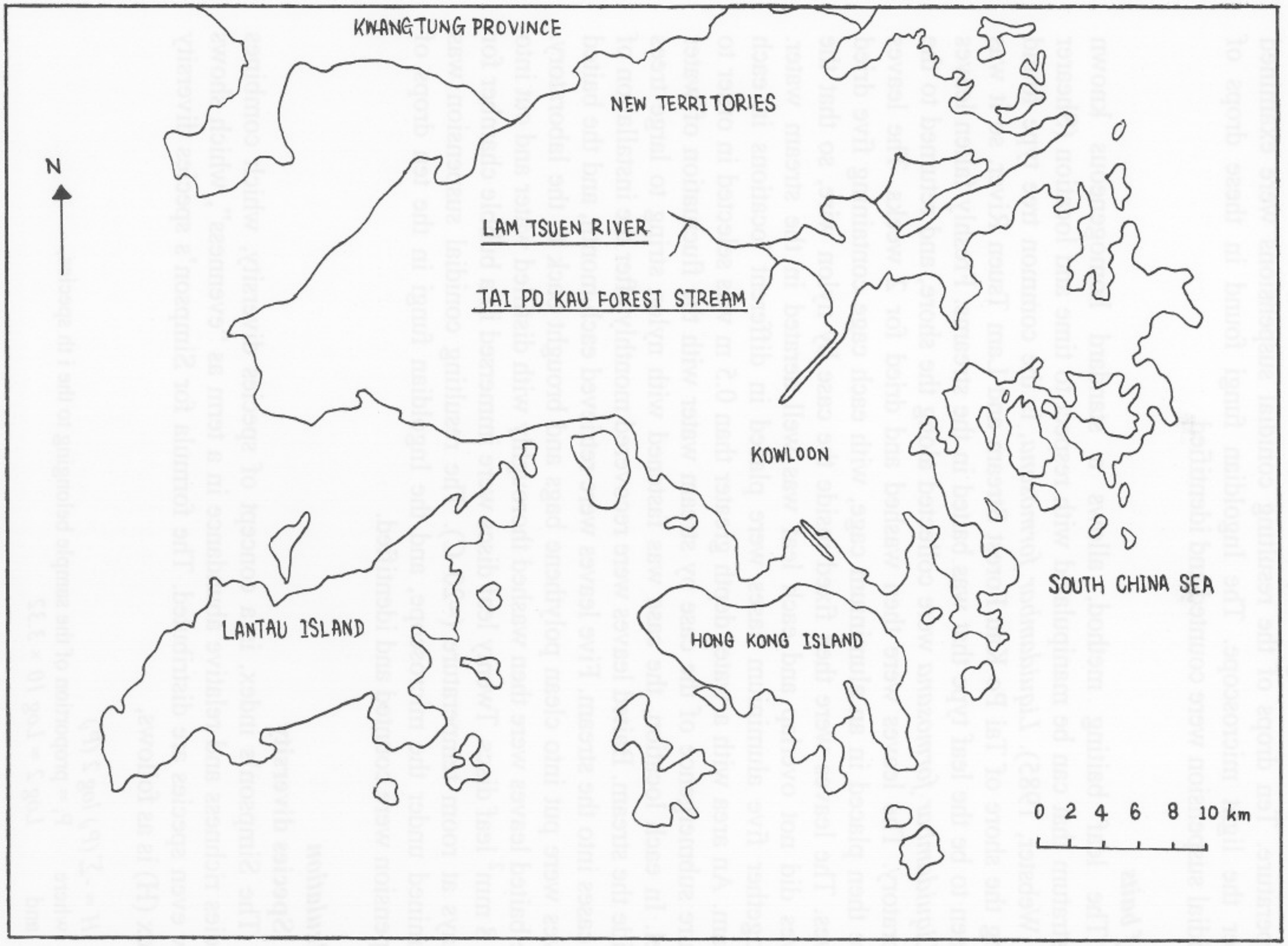

Fig. 1. Location of the Lam Tsuen River and the Tai Po Kau Forest stream in Hong Kong.

The flasks were connected to an air pump to form bubble chambers. Each bubble chamber containing twenty leaf discs was aerated for two days at room temperature. Ten drops of the resulting conidial suspensions were examined under the light microscope. The Ingoldian fungi found in these drops of conidial suspension were counted and identified.

Leaf baits

The leaf baiting method, allows a standard homogeneous known substratum that can be manipulated with respect to time and location (Shearer and Webster, 1985). *Liquidambar formosana*, is the common tree type found along the shore of Tai Po Kau Forest Stream and Lam Tsuen River, so it was chosen to be the leaf type that was baited in the streams. Freshly fallen leaves of *Liquidambar formosana* were collected along the shore, and returned to the laboratory. The leaves were then washed and dried for 2 weeks. The leaves were then placed in an aluminium cage, with each cage containing five dried leaves. The leaves were then fixed inside the case by nylon wire, so that the leaves did not overlap and each leaf was well aerated in the stream water. Altogether five aluminium cases were placed in different locations in each stream. An area with a water depth greater than 0.5 m was selected in order to ensure submergence of the case by stream water with the fluctuation of water level. In each location, the case was fastened with nylon string to large trees beside the stream. Baited leaves were recovered monthly after the installation of the cases into the stream. Five leaves were retrieved each month, and the baited leaves were put into clean polythene bags and brought back to the laboratory. The baited leaves were then washed thoroughly with distilled water and cut into $8 \times 8\ mm^2$ leaf discs. Twenty leaf discs were immersed in a bubble chamber for 2 days at room temperature (~20 C). The resulting conidial suspension was examined under the microscope, and the Ingoldian fungi in the ten drops of suspension were counted and identified.

Calculation

Species diversity

The Simpson's index, is a concept of species diversity, which combines species richness and relative abundance in a term as "evenness", which shows how even species are distributed. The formula for Simpson's species diversity index (H) is as follows,

$$H = -\sum (P_i) \log 2 (P_i)$$

where P_i = proportion of the sample belonging to the i th species,

and $Log\ 2 = Log\ 10 \times 3.32$

Efficiency

Three sampling techniques were applied in this study. Ingoldian fungal communties, based on the data generated by the three sampling techniques used simultaneously, was compared to the Ingoldian fungi communities found by a single sampling method. The efficiency of each sampling technique was tested by comparing the percentage of the total number of species found by individual technique with the total number of species found by all three methods.

$$\text{Efficiency (\%)} = \frac{\text{Number of species found by individual technique}}{\text{Total number of species found by using three techniques simultaneously}} \times 100\%$$

ANOVA and Hsu's MCB test

ANOVA and Hsu's MCB test were carried out by using computer software JMP. Significant differences in Simpson's species diversity index (H) of Ingoldian fungi between the streams, months and methods were found when comapred by One-way ANOVA test. By using further statistical analysis Hsu's MCB test, the best collecting methods can also be found.

Results and discussion

The species of Ingoldian fungi found in this study are listed in Table 1. A total of 41 species belonging to 26 genera were found. Among the 41 species found in the two streams, *Anguillospora longissima*, *A. pseudolongissima*, *Flagellospora curvula*, *Triscelophorus acuminatus*, and *T. ponapensis* were the top five species.

A total of 28 species belonging to 15 genera were found in Lam Tsuen River between September 1998 to December1998. The five dominant species found in Lam Tsuen River were *Anguillospora longissima*, *A. pseudolongissima*, *Clavatospora tentacula*, *Triscelophorus acuminatus*, and *T. ponapensis*. *Clavatospora tentacula* was only found in Lam Tsuen River.

A total of 26 species belonging to 19 genera were found in Tai Po Kau Forest Stream. The top five rankings of species were *Anguillospora longissima*, *A. pseudolongissima*, *Condylospora spumigena*, *Triscelophorus acuminatus*, and *T. ponapensis*.

Anguillospora longissima and *Triscelophorus acuminatus* were the most common Ingoldian fungi found in this study. They are typical species of Ingoldian fungi, *A. longissima* spores being sigmoid in shape, and *T. acuminatus* spores being tetraradiate. Spores with these shapes are well adapted to the aquatic environment (Barlocher, 1992). *Triscelophorus acuminatus* was found to be one major common colonizers on different types of leaf litter in tropical Indian streams (Sridhar and Kaveriappa, 1988a,b, 1989). It was also

Table 1. List of Ingoldian fungi found in Lam Tsuen River and Tai Po Kau Forest Stream from September to December 1998 by all three methods.

Fungi	Lam Tsuen River	Tai Po Kau Forest Stream
Alatospora acuminata		+
Anguillospora crassa	+	
A. gigantea	+	+
A. longissima	+	+
A. pseudolongissima	+	+
Articulospora tetracladia		+
Beltrania rhombica	+	+
Brachiosphaera tropicalis	+	
Camposporium antennatum		+
Campylospora spp.	+	
C. filicladia	+	
Clavariana aquatica		+
Clavariopsis brachycladia		+
Clavatospora longibrachiata	+	
C. tentacula	+	
Condylospora spumigena		+
Dicranidon gracile	+	
Diplocladiella scalaroides	+	+
Flabellospora spp.		+
F. acuminata	+	+
F. crassa	+	+
F. verticillata		+
Flagellospora curvula	+	+
Helicomyces colligatus	+	
H. torquatus	+	
Helicomyces spp.	+	
Isthmolongispora spp.		+
Isthmotricladia spp.		+
Lemonniera spp.	+	
L. aquatica	+	
Lunulospora cymbisformis	+	+
Subulispora procurvata		+
Scutisporus brunneus		+
Tetracladium marchalianum	+	
T. setigerum	+	
Tricladium spp.		+
Tripospermum porosporiferum	+	+
Triscelophorus acuminatus	+	+
T. ponapensis	+	+
T. monosporus	+	+
T. magnificus	+	

Table 2. Comparison of H value of Ingoldian fungi between the streams.

Null Hypothesis, Ho : µLTR (Mean of H in Lam Tsuen River) = µTPK (Mean of H in Tai Po Kau Forest Stream)
Alternative Hypothesis, H1 : Ho is not true
From Oneway ANOVA, p-value = 0.9356
> x where x = 0.05
So Ho is accepted.
There is no significant difference between the abundance and species diversity of Lam Tsuen River and that of Tai Po Kau Forest Stream.

Table 3. Comparison of species diversity (H) of Ingoldian fungi between the months.

Null Hypothesis, Ho : µSept (Mean of H in September) = µOct (Mean of H in October) = µNov (Mean of H in November) = µDec (Mean of H in December)
Alternative Hypothesis, H1 : Ho is not true
From Oneway ANOVA, p-value = 0.0713
> x where x = 0.05
So Ho is accepted.
There is no significant difference between the abundance and species diversity of Ingoldian fungi in September, October, November and December.

Table 4. Comparison of species diversity (H) of Ingoldian fungi between the methods.

Null Hypothesis, Ho : µFoam (Mean of H in foam) = µSubmerged leaves (Mean of H in submerged leaves) = µBaited leaves (Mean of H in baited leaves)
Alternative Hypothesis, H1 : Ho is not true
From Oneway ANOVA, p-value < 0.0001
< x where x = 0.05
So Ho is rejected.
There is a significant difference between the abundance and species diversity found by foam collection, submerged leaves collection and leaf baiting.

Table 5. The values of the three sampling methods by Hsu's MCB test.

	F	L	B
Foam collection (F)	-0.16596	0.95405	1.09477
Collection of submerged leaves (L)	-1.39614	-0.26217	-0.13540
Leaf baitings (B)	-1.42669	-0.30668	-0.16596

If a column has any positive values, the mean is significantly less than the maximum.

found to be the prevalent summer species in Tai Po Kau Forest Stream (Au *et al.*, 1992b). Another Ingoldian fungi with tetraradiate spores common to this study was *Triscelophorus ponapensis*. Common Ingoldian fungi with sigmoid spores were *Anguillospora pseudolongissima* and *Flagellospora curvula*.

Comparison between streams

The number of species found in the streams was actually very similar. There was no significant difference in species diversity (H) of Ingoldian fungi between the streams, when compared by One-way ANOVA test (p-value = 0.9356, Table 2). However, it was quite unexpected that Lam Tsuen River could have a species diversity so close to that found in Tai Po Kau Forest Stream, which is located inside a natural reserve area. When compared with previous data, a greater number of speices was found on decomposing *Bauhinia purpurea* leaves in Tai Po Kau Forest Stream than that in Lam Tsuen River (Au *et al*., 1992a). The difference between the present result and the past result may be due to the improvement of water quality in Lam Tsuen River after the implementation of environmental measures, which aimed to control the stream water quality in 1987. Only small amounts of pollutants may now be discharged into the stream following these environmental controls.

Comparison between months

This research was carried out between September 1998 and December 1998. The study started in late summer and continued into early winter. In the first study of Ingold (1942), he noticed that most species of aquatic hyphomycetes were more common from late summer to early winter than during the rest of the year (Iqbal, 1994). The months chosen for collection in this study fitted the time of high occurrence of fungi in temperate region. Since the study lasted for four months from late summer to early winter, but not the whole year, occurrence of Ingoldian fungi from September to December instead of differences in the seasonal occurrence in the whole year were investigated.

A similar number of species were found over the four months. Referring to the ANOVA test, there was no significant difference in abundance and species diversity between the four months (p-value = 0.0713, Table 3). This may account for the consistent pattern of fungal communities occurring over the four months.

Comparison between methods

The efficiency in capturing conidia in foam collection, collection of submerged leaves and leaf baits were 100%, 11.6% and 11.6% respectively. Referring to the ANOVA test, the abundance and species diversity (H) of Ingoldian fungi found by the three methods were also significantly different (p-value < 0.0001, Table 4). Further statistical analysis was carried out using Hsu's MCB test (Table 5). Foam collection was found to be the most effective sampling technique among the three. The other two methods were found to be ineffective in detecting the fungal community. A previous study of freshwater hyphomycete communities in the Jabori Canal in Pakistan investigated the similarity indices between freshwater hyphomycetes detected by each sampling techniques used alone, which is the same as the efficiency used in this study. The highest index was observed in the use of foam collection, however, this was not as high a 100% in those studies (Iqbal, 1994). Species composition detected by foam collection in this study was the same as that by using all three methods simultaneously, indicating that only using foam collections can detect most of the Ingoldian fungal community.

The purpose of using collection of naturally submerged leaves and leaf baiting was to capture the fungal species other than those with high affinity to foam, and to measure the fungal communities accurately. The species detected by these two methods however, were more similar to those in foam samples, mostly with tetraradiate and sigmoid conidia such as *Anguillospora longissima*, *A. ponapensis*, *Triscelophorus acuminatus* and *T. pseudolongissima*. This confirmed these species were common in the streams, and not only in foam samples due to their affinity to foam.

Factors affecting the occurrence of fungal communities

There are a number of factors affecting the conidial concentration and species composition of aquatic hyphomycetes. These include the availability of autumn shed leaves from surrounding vegetation, changes in water temperature and inter- or intra-specific interactions, they are the variations in conidial concentration of species composition of the community (Au *et al.*, 1992b).

Several studies have been carried out to correlate aquatic hyphomycete abundance in streams with their surrounding riparian vegetation, and these have shown that conidial concentration of aquatic hyphomycete in streams was significantly connected with adjacent vegetation. No correlation, however, existed between the adjacent vegetation and the species richness (Iqbal and Webster, 1977, Bärlocher and Rosset, 1981, Wood-Eggenschwiler and Bärlocher, 1983). Since the study sites of Tai Po Kau Forest Stream banks are more well vegetated than that in Lam Tsuen River, the better development of fungi and the accompanying higher conidiophore abundance might explain usually comparatively higher conidial concentration in Tai Po Kau Forest Stream.

Although the effects of water temperature were not considered in this study, it may not be a vital factor in determining the aquatic fungal communities because no marked change in temperature was observed in the period from September to December 1998 in Hong Kong. On the other hand, biological inter- or intra-specific interaction was not considered neither in the present study, the relationships are complex and little seems to be known (Bärlocher, 1992).

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