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Diversity of conidial fungi and some abiotic variables of the water after the reopening of the Pirarungaua stream in the Jardim Botânico, São Paulo, São Paulo State, Brazil

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ABSTRACT - (Diversity of conidial fungi and some abiotic variables of the water after the reopening of the Pirarungaua stream in the Jardim Botânico, São Paulo, São Paulo State, Brazil). To evaluate the fungal diversity in the Pirarungaua stream reopened after remaining channeled about 40 years in the Botanical Garden of São Paulo, Brazil, two experiments in the cold and dry season and two in the hot and rainy season were performed. Leaves of *Tibouchina pulchra* Cogn and mixed leaf litter were confined in nylon litter bags, submerged and collected monthly, together with free leaf litter samples. Some abiotic factors were measured in the water. The leaves were fragmented, incubated in sterile water at 22 ºC and microscopically analyzed. We identified 33 conidial fungal taxa, among which, 23 are new to this area. Comparing seasons, the similarity of the mycota of confined *T. pulchra* leaves corresponded to 39% and to 56% regarding the confined mixed leaf litter. Similarities of 48-50% occurred between the mycota of confined and free leaf litter samples. Confined leaves showed a tendency to support higher numbers of species, whereas some rare fungi like *Ulocoryphus mastigophorus* Michaelides L. Hunter & W.B Kendr were more frequent in free leaf litter. Based on the results, it is recommended to monitor the limnological conditions of the stream and to promote awareness among the local population and visitors about the importance of a water body, even artificial, for the establishment of microclimatic features, and for the biodiversity of fungi.
Keywords: aquatic Hyphomycetes, conidial fungi, fungal diversity, stream recovery

RESUMO - (Diversidade de fungos conidiais e algumas variáveis abióticas da água após a reabertura do córrego Pirarungaua no Jardim Botânico, São Paulo, SP, Brasil). Para avaliar a diversidade de fungos no córrego Pirarungaua, reaberto após ter permanecido canalizado por cerca de 40 anos no Jardim Botânico de São Paulo, Brasil, foram realizados dois experimentos nas estações frias e secas e duas nas estações quentes e chuvosas. Folhas de *Tibouchina pulchra* Cogn e folhedo misto foram confinados em sacos de tela de nylon, submersas e coletadas mensalmente, juntamente com amostras de folhedo livre submerso. Alguns fatores abióticos foram medidos na água. As folhas foram fragmentadas, incubadas em água esterilizada, a 22 ºC e microscopicamente analisadas, identificando-se 35 táxons de fungos conidiais, entre os quais 23 são novos para a área. Comparando as estações climáticas, a similaridade da micota confinada de *T. pulchra* correspondeu a 38% e a 56% com relação ao folhedo misto confinado. As folhas confinadas mostraram a tendência de oferecer suporte a maior número de espécies, enquanto alguns fungos raros como *Ulocoryphus mastigophorus* Michaelides L. Hunter & W.B Kendr foram mais frequentes no folhedo livre. Com base nos resultados é recomendado o monitoramento das condições limnológicas do córrego e conscientização da população local e dos visitantes sobre a importância que um corpo d' água, mesmo artificial, possui no estabelecimento das características do microclima e representa para a biodiversidade de fungos.
Palavras-chave: diversidade fúngica, fungos conidiais, Hyphomycetes aquáticos, recuperação de córregos

Introduction

According to former research reports of the Botanical Garden in São Paulo, Brazil (Hoehne 1943), the Pirarungaua stream joins with the Córrego dos Simões stream, forming the River Ipiranga, whose importance lies in the historical fact that the independence of the country was declared on its margins in the nineteenth century.

Since the 1940 decade, of the 1380 m in length of the stream, only 500 m were flowing in its natural bed, from the springhead through the exuberant plateau

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Atlantic rainforest, being the remainder completely channeled (Hoehne 1943).

However, in August 2007 the underground gallery suffered a severe breakdown, which resulted in a hole of 5.00 m wide by 10.00 m long and 2.50 m deep. The underground channel was demolished and in its place, a ditch of 230 m long by 14 m wide was built, with smooth, inclined slopes composing a new profile for the new channel designed to remain in the open and integrated with the landscape.

Once the stream was opened, communities of aquatic plants, algae, mollusks and fishes were settling and thus probably fungi that fulfilling one of its main functions, may have become important decomposers of new sources of organic matter. As practically all major limnological characteristics of the stream were drastically modified and gradually the riparian vegetation has been established, it can be considered that the Pirarungaua is a waterbody in revitalization, constituting a "natural laboratory" (Tundisi & Tundisi 2008) to accompany changes in those communities.

The aquatic mycota of the State Park Fontes do Ipiranga - PEFI, where the Botanical Garden is located, is fairly well known and studies about fungal succession on leaves of *Ficus microcarpa* L. and *Tibouchina pulchra* Cogn. (Schoenlein-Crusius & Milanez 1989, Moreira 2006), surveys of aquatic facultatives and ingoldian fungi in mixed litter collected from different water bodies with several levels of eutrophication, in dry, cold and rainy and hot seasons (Schoenlein-Crusius *et al*. 2009). Several conidial fungi, specifically ingoldian fungi have been observed in lotic and also in lenthic waters in urban parks, with richness and diversity higher than expected for an anthropic environment (Schoenlein-Crusius *et al*. 2014). Revisions about the main surveys of ingoldian fungi in Brazil and South America may be consulted in Schoenlein-Crusius & Grandi (2003) and Schoenlein-Crusius & Malosso (2007). More recently outstanding conidial species found in very dry regions in the Northeast region of Brazil have been cited and excellently taxonomically described by Barbosa & Gusmão (2011), Almeida *et al*. (2011; 2012), among others.

Zoosporic organisms were also studied in several taxonomic surveys in the Botanical Garden of São Paulo (Milanez *et al*. 2007, Pires-Zottarelli & Rocha 2007); most recently including free mixed litter collected in the Pirarungaua stream (Jesus *et al*. 2013).

The present study proposes to add new knowledge about the diversity and richness of conidial fungi, which approaches aquatic facultatives and ingoldian fungi, taking the opportunity to study, during a revitalization process, a stream that is increasingly integrating nature. Some abiotic factors are presented as first measurements in the reopened lotic system.

Material and methods

Characterization and selection of sampling points at the Pirarungaua Stream - The present study was divided into four experiments conducted in Pirarungaua stream in the stretch phase of revitalization and comprising a length of approximately 229 m, with 2.80 to 3.00 m in width and minimum depth of 15 cm. The distance between the input stream and the first waterfall corresponds to 62 m. From this place to the second fall comprises 59 m, and until the third fall more 55.7 m. The distance of 25.4 m separates the third and fourth waterfall and more 27 m has to be transverse from this point until the river mouth. The following six collection points were marked with wooden stakes: Point 1 - water entrance in the stream, Point 2 - under the first bridge after entry, Point 3 - near the first waterfall; Point 4 - near the second waterfall, under the third bridge; Point 5 - near to the third waterfall, under the fourth bridge and Point 6 - near the river mouth of the channel.

Characterization of climatic conditions during the experiments - Data regarding rainfall, average relative humidity and air temperature during the experiments was provided by the Astronomical and Geophysical Institute, University of São Paulo, located within the Parque Estadual das Fontes do Ipiranga - PEFI.

Measurement of some abiotic factors in the water - During the monthly sampling, using Multiprobe equipment U_{10} from Horiba, temperature, pH, dissolved oxygen and conductivity were measured at 20 cm of water depth at each collection point.

Field experiments - From May/2010 to April/2014, four experiments were performed: the first and the third initiated in the dry and cold season, and the second and forth experiment in the rainy and hot season. To accumulate *Tibouchina pulchra* Cogn. fallen leaves, during 30 days, avoiding their contact with the ground, two nylon nets (1mm diam. Mesh), with the size of fairly 3 × 5 m, were tied on four trees in the Botanical Garden of São Paulo, at 1 m above the soil surface. The entire area of the extended nets (15 m^2), from one edge to the other retained leaves of the canopy of at least 10 trees of *T. pulchra* in June

(dry and cold season) and in November (rainy and hot season) of 2010.

In May (dry and cold season) and in October (rainy and hot season) of 2011, about 10 kg of fallen mixed leaf litter from one site of the Pirarungaua stream was collected. For each experiment the amount of leaves was divided in samples of 10 g each, which were confined in 120 nylon net litter bags (10 × 10 cm with 1 mm diam. mesh) and submerged in the six collection points along the stream.

The leaves of *T. pulchra* remained submerged from July to November of 2010 (1st. Experiment, 5 months, dry and cold season) and from December of 2010 to May of 2012 (2nd. Experiment, 6 months, rainy and hot season). The mixed leaf litter samples remained submerged in the stream from June to November of 2011 (3rd. Experiment, 6 months, dry and cold season) and from November of 2011 to April of 2012 (4th. Experiment, 6 months, rainy and hot season).

Monthly, one nylon net litter bag was taken from each collection point. Concomitantly during the 3rd and 4th. Experiments, two samples of 10-20 leaves of submerged free mixed leaf litter were taken at 20 to 40 cm deep at each point, totalizing 12 monthly samples from the stream.

Fungal isolations - At the laboratory, the leaves were taken from the nylon net litter bags and from the collection flasks, disposed on a strainer of 120 µm mesh and gently washed with tap water to get rid of adhered debris.

The confined leaves and also the free mixed leaf litter samples were fragmented in 1 cm diam. Aliquots, which were incubated in Petri dishes containing sterile distilled water (Ingold 1975). The number of Petri dishes obtained at this phase depended on the decomposition rate of the leaves, because in each dish the leaf fragments should remain submerged, not overlaying, but free, in a mostly similar way to what would occur in the field. From the 5th day on of incubation of the Petri dishes at a temperature about 20 °C, slides of the leaf fragments were prepared, using only drops of sterile distilled water and covered with coverslip, to be observed under an optical microscope, in order to look for taxonomical relevant features, which allow the identification of the species according to current literature (Nilsson 1964, Ingold 1975, Marvanová 1997, Santos-Flores & Betancourt-Lopez 1999, Gulis *et al*. 2005).

Statistical treatment of the data - In order to verify the existence of significant differences between the abiotic factors such as temperature, pH, dissolved oxygen and conductivity of the stream water, a Kruskal-Wallis test was employed. Calculations were made by the PAST Program, version 2.17 (Paleontological statistics software package for education and data analysis) of Hammer *et al*. (2001).

To compare the mycota observed in the different months and collection points, the Similarity Index of Sörensen (S) was used, expressed by the formula $S = 2xc/a + b$, where a is the total number of fungal taxa in a given situation, b is the number of fungal taxa in a situation to be compared and c is the number of fungal taxa that are common to the two situations which are being compared. The similarity is expressed in percentual values, multiplying the S value by 100 (Müller-Dombois & Ellenberg 1974).

Results and Discussion

Characterization of climatic conditions during the experiments - According to table 1, the climatic data confirm that the 1st and 3rd experiments were performed in periods with lower rainfall, lower number of rainy days and cooler (lower monthly average temperatures) and the 2nd and 4th experimentsin more rainy periods (higher values of volumetric rain fall and number of rainy days) and hot (higher rain fall and average monthly temperatures). The relative air humidity remained between 74.5 and 86.9% during the entire study.

Some abiotic factors in stream water during the collections - Table 2 shows the individual values, means and table 3 the results of the Kruskal-Wallis test regarding the temperature of stream water measured during the experiments. The Kruskal-Wallis test showed no significant differences between the sampling points, but between collections. As the experiments were performed subsequently, approaching cold and dry seasons and also hot and rainy seasons, the water temperature followed climatic variations, reaching minimum average values in July/10 (19 ºC, 1st experiment), May/11 (17,8 ºC, 2nd experiment), June/11 (16,1 ºC, 3rd experiment) and April/12 (21.2 ºC, 4th experiment), whereas the maximum mean values were registered in October/10 (1st experiment), December/10 (24.7 ºC, 2nd experiment), November/11 (22.9 ºC, 3rd experiment) and in February and March of 2012 (24.9 ºC, 4th experiment).

The mean values found in this study are similar to those cited by Bicudo *et al*. (2002) in the Lago das Ninféias, which is the reservoir that supplies the Pirarungaua stream. Also within the range of 26.6 ºC (February 2005) and minimum 12.6 °C (July 2004) cited in previous study conducted in several collection sites in the Parque Estadual das Fontes do Ipiranga (Schoenlein-Crusius *et al*. 2009).

Table 4 shows the individual values, means, and table 5, the result of the Kruskal-Wallis test related to the pH of the water stream measured during the experiments.

As with temperature, the Kruskal-Wallis test revealed significant differences only between collections.

The maximum average values of the pH of the water were recorded in October/10 (6.6 -1st experiment), May/11 (5.9-2nd experiment), October/11 (5.5 -3rd experiment) and April/12 (6.4-4th experiment). The average minimum values were recorded in November/10 (5.9-1st experiment), January/11

Table 1. Monthly total rainfall (mm), monthly mean values of temperature (ºC), and relative air humidity (%) from July 2010 to April 2012. Data provided by the Astronomical and Geophysical Institute, University of São Paulo.

Months and seasons	Monthly total rainfall (mm),	Nº rainy days/ month	Monthly mean values of temperature (ºC)	Relative air humidity (%)
1st Experiment				
1st collection - 29/07/10	89.6	6	17.4	79.7
2nd collection - 31/08/10	3.7	8	16.0	75.1
3rd collection - 29/09/10	96.7	18	18.5	76.9
4th collection - 26/10/10	76.6	22	17.7	82.5
5th collection - 17/11/10	159.2	18	20.1	80.1
2nd Experiment				
1st collection - 21/12/10	280.6	22	22.0	84.1
2nd collection - 18/01/11	332.8	22	23.2	83.4
3rd collection - 22/02/11	255.2	19	23.3	80.7
4th collection - 28/03/11	150.2	14	21.0	86.9
5th collection - 18/04/11	176.6	16	20.7	81.7
6th collection - 23/05/11	49.2	10	16.7	82.7
3rd Experiment				
1st collection - 20/06/11	191.4	14	14.8	81.1
2nd collection - 27/07/11	88.0	9	16.4	79.3
3rd collection - 24/08/11	1.8	3	17.2	77.3
4th collection - 19/09/11	22.0	8	17.1	76.7
5th collection - 24/10/11	91.7	14	19.3	80.0
6th collection - 28/11/11	163.0	15	18.9	79.3
4th Experiment				
1st collection - 28/11/11	163.0	15	18.9	79.3
2nd collection - 28/12/11	345.0	20	21.0	78.5
3rd collection - 23/01/12	332.8	22	20.7	74.5
4th collection - 27/02/12	255.2	19	23.4	78.7
5th collection - 26/03/12	150.2	14	21.6	79.0
6th collection - 23/04/12	49.2	10	20.5	83.6

(4.9-2nd experiment) November/11, August/11, September/11 (5.9-3rd experiment) and February/12 (5.7-4th experiment).

A tendency of lower values to occur in more rainy and hot seasons, and the contrary in the opposite climatic seasons may be verified. However, in view that not even the highest pH values exceeded 7.0, probably the stream presents the tendency to have more acid waters. This result is consistent with what Bicudo *et al.* (2002) found in the Lago das Ninféias, where the pH of the water ranged from 5.5 to 6.5 between 1997 and 1998. It is also consistent with the amplitude of the pH of the waters of PEFI, cited by Schoenlein-Crusius *et al.* (2009).

Table 6 shows the individual values and averages and table 7, the result of the Kruskal-Wallis test regarding the content of dissolved oxygen (mgO_2L^{-1}) of stream water measured during the experiments.

Table 2. Individual values and means of the water temperature (°C) measured during the four experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brasil.

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Means
1s Experiment							
1st collection - 29/07/10	19.0	19.0	19.0	19.0	19.0	19.0	19.0
2nd collection - 31/08/10	20.0	21.0	22.0	22.0	20.0	21.0	21.0
3rd collection - 29/09/10	20.0	20.0	21.0	22.0	22.0	22.0	21.2
4th collection - 26/10/10	21.5	21.7	21.9	22.1	22.8	23.3	22.2
5th collection - 17/11/10	20.0	20.4	20.9	20.8	21.0	21.0	20.7
means	20.1	20.4	20.9	21.2	20.9	21.3	
2nd Experiment							
1st collection - 21/12/10	24.0	24.4	25.0	24.8	25.0	25.3	24.7
2nd collection - 18/01/11	21.9	22.0	22.1	22.3	22.7	22.8	22.3
3rd collection - 22/02/11	22.9	23.3	23.7	24.2	24.7	25.0	23.9
4th collection - 28/03/11	23.9	23.8	24.0	24.4	24.6	25.1	24.3
5th collection - 18/04/11	23.6	24.1	24.1	24.2	23.9	23.9	23.9
6th collection - 23/05/11	18.3	18.1	17.9	17.7	17.6	17.6	17.8
Means	22.4	22.6	22.8	22.9	23.1	23.3	
3rd Experiment							
1st collection - 20/06/11	17.1	16.6	16.2	15.8	15.5	15.5	16.1
2nd collection - 27/07/11	17.8	17.6	17.4	17.3	17.0	16.6	17.3
3rd collection - 24/08/11	16.6	17.1	17.4	17.2	17.3	17.4	17.2
4th collection - 19/09/11	19.6	20.9	21.6	22.5	22.1	22.4	21.5
5th collection - 24/10/11	21.7	22.3	23.1	22.8	23.3	23.6	22.8
6th collection - 28/11/11	22.7	22.9	22.9	22.8	23.0	23.0	22.9
Means	19.3	19.6	19.8	19.7	19.7	19.7	
4th Experiment							
1st collection - 28/11/11	22.7	22.9	22.9	22.8	23.0	23.0	22.8
2nd collection - 28/12/11	22.3	22.4	22.4	22.4	22.2	22.1	22.3
3rd collection - 23/01/12	21.5	22.0	22.3	22.4	22.7	22.9	22.3
4th collection - 27/02/12	24.5	24.6	24.9	25.1	25.2	25.3	24.9
5th collection - 26/03/12	23.9	24.4	24.9	25.2	25.5	25.7	24.9
6th collection - 23/04/12	21.1	21.3	20.9	21.4	21.1	21.2	21.2
Means	22.6	22.9	23.0	23.2	23.3	23.4	

In September/10 occurred the maximum mean value - 13.5 mgO_2L^{-1} and in November/10 the lowest, corresponding to 7.2 mgO_2L^{-1}. With exceptions of point 1, which mean value of oxygen corresponded to 5.72 mgO_2L^{-1}, the remaining points presented means above 7 mgO_2L^{-1} during the 1st experiment. In the 2nd experiment, initiated in the rainy and hot season, when according to the literature, the oxygen levels are usually lower (Esteves 1998), the mean values were significantly higher in the 6th collection made in May of 2011, in relation to other collections. In the 3rd experiment**,** started in the dry and cold season, the average values of the levels of dissolved oxygen were higher in July/11 (8.83 mgO_2L^{-1}) and significantly lower in October/11 (6.19 mgO_2L^{-1}; 5thcollection).

In the last experiment, started in the rainy and hot season, the average dissolved oxygen concentration was significantly higher in January/12 (7.54 mgO_2L^{-1} - 3rd collection) and significantly lower in April/12 (6.41 mgO_2L^{-1} - 6th collection), when a sharp decrease of the rainfall was recorded (table 1). These results are consistent with the aforementioned climatic variations in the literature (Esteves 1998, Tundisi & Tundisi 2008). The variation of the values found during the entire study is larger than the range between 0.4 and 5.6 mgO_2L^{-1} cited by Bicudo *et al*. (2002) and Schoenlein-

Table 3. Kruskal-Wallis test related to water temperature (°C) measured during the four experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brazil. Values in gray shades indicate significance ($p < 0.05$).

	1st collection	2nd collection	3rd collection	4th collection	5th collection	6th collection
1st Experiment						
1st collection	0	0.002619	0.002515	0.002778	0.002725	-
2nd collection	0.02619	0	0.798	0.06414	0.4591	-
3rd collection	0.02515	1	0	0.1697	0.3682	-
4th collection	0.02778	0.6414	1	0	0.004998	-
5th collection	0.02725	1	1	0.04998	0	-
						-
2nd Experiment						
1st collection	0	0.004998	0.09041	0.1712	0.01959	0.004922
2nd collection	0.07497	0	0.005075	0.005075	0.004922	0.004998
3rd collection	1	0.07612	0	0.4712	1	0.004998
4th collection	1	0.07612	1	0	0.3743	0.004998
5th collection	0.2938	0.07383	1	1	0	0.004847
6th. collection	0.07383	0.07497	0.07497	0.07497	0.0727	0
3rd Experiment						
1st collection	0	0.01594	0.01244	0.004998	0.004998	0.004847
2nd collection	0.239	0	0.5711	0.005075	0.005075	0.004922
3rd collection	0.1865	1	0	0.004998	0.004998	0.004847
4th collection	0.07497	0.07612	0.07497	0	0.04533	0.004922
5th collection	0.07497	0.07612	0.07497	0.6799	0	0.8721
4th Experiment						
1st collection	0	0.004624	0.02347	0.004922	0.004922	0.004847
2nd collection	0.06936	0	0.9349	0.004772	0.004772	0.004698
3rd collection	0.3521	1	0	0,005075	0.005075	0.004998
4th collection	0.07383	0.07158	0.07612	0	0.936	0.004998
5th collection	0.07383	0.07158	0.07612	1	0	0.004998
6th collection	0.0727	0.07047	0.07497	0.07497	0.07497	0

Crusius *et al*. (2009) for other water bodies located in PEFI. Although the statistical test did not pointed mathematically significant differences, it is notable that the average levels of dissolved oxygen tended to rise (with some exceptions) from the point 1 to 6, probably due to the turbulence of the waters, favored by the artificial falls built along the channel, thus fulfilling its goal to keep the water moving and sufficiently aerated.

Table 8 shows the individual values, means, and table 9, the results of the Kruskal-Wallis test concerning conductivity (μScm^{-1}) of the water stream measured during the experiments. According to the Kruskal-Wallis test, there are significant differences only between collections.

In the 1st experiment, initiated in the dry and cold season, the average conductivity of the water was higher

Table 4. Individual values and means of the water pH measured during the four experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brasil.

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	means
1st Experiment							
1st collection - 29/07/10	7.40	6.30	6.10	5.70	5.70	5.70	6.1
2nd collection - 31/08/10	6.10	5.40	5.30	5.50	5.60	5.70	5.6
3rd collection - 29/09/10	6.20	5.60	5.30	5.50	5.60	5.60	5.6
4th collection - 26/10/10	6.10	6.43	6.64	6.65	6.84	6.82	6.6
5th collection - 17/11/10	5.92	5.44	5.61	6.15	6.16	6.31	5.9
Means	6.34	5.83	5.79	5.90	5.98	6.03	
2nd Experiment							
1st collection - 21/12/10	4.79	5.50	5.10	5.34	5.41	5.41	5.2
2nd collection - 18/01/11	4.60	4.48	4.60	5.28	5.27	5.36	4.9
3rd collection - 22/02/11	4.64	4.96	5.00	5.00	5.15	5.29	5.0
4th collection - 28/03/11	6.46	5.36	5.66	5.97	5,83	6.16	5.9
5th collection - 18/04/11	5.20	5.18	5.71	5.91	6.04	6.10	5.7
6th collection - 23/05/11	6.17	5.35	5.96	5.72	6.03	6.05	5.9
Means	5.31	5.14	5.34	5.54	5.62	5.73	
3rd Experiment							
1st collection - 20/06/11	6.09	5.75	5.87	5.96	5.99	6.02	5.9
2nd collection - 27/07/11	7.29	6.72	5.68	5.91	6.08	6.31	6.3
3rd collection - 24/08/11	5.42	5.80	6.03	6.12	6.27	6.35	5.9
4th collection - 19/09/11	5.78	5.72	6.16	6.1	6.05	6.07	5.9
5th collection - 24/10/11	6.62	6.38	6.34	6.51	6.51	6.61	6.5
6th collection - 28/11/11	6.82	6.01	5.59	6.21	6.21	6.30	6.2
Means	6.34	6.06	5.95	6.13	6.18	6.27	
4th Experiment							
1st collection - 28/11/11	6.82	6.01	5.59	6.21	6.21	6.30	6.2
2nd collection - 28/12/11	6.18	5.96	6.14	6.25	6.12	6.50	6.2
3rd collection - 23/01/12	5.91	5.92	6.07	6.02	6.13	6.18	6.0
4th collection - 27/02/12	5.33	5.45	5.75	5.81	5.94	6.01	5.7
5th collection - 26/03/12	7.02	6.49	5.97	5.86	6.10	5.83	6.2
6th collection - 23/04/12	7.12	5.83	6.3	6.5	6.43	6.42	6.4
Means	6.39	5.94	5.97	6.11	6.15	6.21	

in October/10 (0.051 µScm^{-1}, 4th collection) and lower in November/10 (0.026 µScm^{-1}, 5th collection). In the 2nd experiment, the highest mean value was registered in December/10 (0.053 µScm^{-1}, 1st collection) and the lowest in May/11 (0.043 µScm^{-1}, 6th collection). In the 3rd experiment, the highest mean value occurred in October/11 (0.055 µScm^{-1}, 5th collection) and the lowest in July/11 (0.035 µScm^{-1}, 2nd collection). In the last experiment, the highest mean value occurred in December/12 (0.051 µScm^{-1}, 2nd collection) and the lowest in February/12 (0.042 µScm^{-1}, 4th collection).

The values of the water conductivity of the Pirarungaua stream are in the range of the variation of this parameter cited by Bicudo *et al*. (2002), Moreira (2006) and Schoenlein-Crusius *et al*. (2009) for other collection sites in the PEFI. As no abiotic variables measured before the reopening of the stream were found, it seems interesting to present some of them in the present paper for historical purposes.

Diversity of aquatic Hyphomycetes - During the entire study period, 33 taxa of fungi associated with submerged decaying substrates were obtained. Among these, eight (*Beltrania rhombica*, *Beltraniopsis* sp., *Beltraniella* sp., *Dictyochaeta* sp., *Pestalotiopsis*-like., *Fusarium oxysporum*, *Pestalotiopsis* sp. and *Ulocoryphus mastigophorus*) are more common in terrestrial environments, been considered

Table 5. Kruskal-Wallis test related to water pH measured during the four experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brazil. Values in gray shades indicate significance ($p < 0.05$).

	1st collection	2nd collection	3rd collection	4th collection	5th collection	6th collection
1st Experiment						
1st collection	0	0.04123	0.02834	0.1062	0.8089	-
2nd collection	0.4123	0	0.87	0.006392	0.0927	-
3rd collection	0.2834	1	0	0.007796	0.1705	-
4th collection	1	0.06392	0.07796	0	0.02024	-
5th collection	1	0.927	1	0.2024	0	-
2nd Experiment						
1st collection	0	0.09155	0.09155	0.02002	0.1275	0.02002
2nd collection	1	0	0.8095	0.0063	0.06508	0.008127
3rd collection	1	1	0	0.004998	0.01291	0.004998
4th collection	0.3003	0.0945	0.07497	0	0.4712	0.9362
5th collection	1	0.9762	0.1936	1	0	0.3785
6th collection	0.3003	0.1219	0.07497	1	1	0
3rd Experiment						
1st collection	0	0.298	0.3785	0.4712	0.004998	0.1275
2nd collection	1	0	0.4712	0.3785	0.3776	0.8099
3rd collection	1	1	0	0.6889	0.008127	0.5745
4th collection	1	1	1	0	0.004998	0.229
5th collection	0.07497	1	0.1219	0.07497	0	0.06461
6th collection	1	1	1	1	0.9692	0
4th Experiment						
1st collection	0	0.8099	0.229	0.03671	0.8099	0.1986
2nd collection	1	0	0.1087	0.008239	0.4712	0.1994
3rd collection	1	1	0	0.03064	0.9362	0.06555
4th collection	0.5506	0.1236	0.4596	0	0.04533	0.01307
5th collection	1	1	1	0.6799	0	0.4225
6th collection	1	1	0.9833	0.196	1	0

aquatic facultatives. New citations for Brazil are *Helicoon spirale* Boedjin, *Triscelophorus curviramifer* Matsushima and *Ulocoryphus mastigophorus* Michaelides, L. Hunter & W.B. Kendr.

Tables 10 to 15 present the occurrences of the aquatic Hyphomycetes in the four experiments and table 16 summarizes the similarity indexes of Sörensen calculated between the different situations analyzed in the present study.

Table 10 presents the fungal taxi of the 1st experiment, initiated during the dry and cold season, with leaves of *T. pulchra* confined in nylon net litter bags. During the 1st experiment 14 taxa were obtained, totalizing 48 occurrences. The highest number of taxa (8) was registered in the second and the lowest in the first collection. Point 1 presented the highest number of occurrences, whereas in point 2 the highest number of taxa (8). *Endophragmiella* sp. occurred

Table 6. Individual values and means of the dissolved oxygen (mgO_2L^{-1}) content of the water measured during the four experiments in the Pirarungaua stream, in the Botanical Garden in São Paulo, Brazil.

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Means
Collections - 1st Exp.							
1st collection - 07/29/10	-	-	-	-	-	-	
2nd collection - 08/31/10	-	-	-	-	-	-	
3rd collection - 09/29/10	2.03	7.08	10.13	15.16	17.25	29.30	13.5
4th collection - 10/26/10	7.02	7.30	6.15	8.37	6.21	8.77	7.3
5th collection - 11/17/10	8.12	7.46	7.34	6.14	6.86	7.30	7.2
Means	5.72	7.28	7.87	9.89	10.11	15.12	
Collections - 2nd Exp.							
1st collection - 12/21/10	9.02	7.56	7.25	6.76	8.21	8.00	7.80
2nd collection - 01/18/11	7.93	8.06	8.15	7.80	8.11	8.26	8.05
3rd collection - 02/22/11	7.98	7.65	7.40	8.00	8.11	8.56	7.95
4th collection - 03/28/11	7.74	8.15	7.62	7.53	7.81	8.36	7.87
5th collection - 04/18/11	8.21	8.32	6.43	7.36	6.64	7.07	7.34
6th collection - 05/23/11	10.82	9.89	8.52	8.76	8.52	9.15	9.27
Means	8.62	8.27	7.56	7.70	7.90	8.23	
Collections - 3rd Exp.							
1st collection - 06/20/11	8.57	7.89	8.20	8.11	7.88	9.25	8.31
2nd collection - 07/27/11	8.15	8.62	9.47	8.48	9.40	8.86	8.83
3rd collection - 08/24/11	8.72	7.42	5.63	7.33	7.65	7.88	7.44
4th collection - 09/19/11	7.02	8.00	6.67	6.76	7.58	8.44	7.41
5th collection - 10/24/11	6.28	6.31	4.93	6.51	6.51	6.61	6.19
6th collection - 11/28/11	8.59	7.02	4.50	5.60	6.11	7.86	6.61
Means	7.88	7.54	6.56	7.13	7.52	8.15	
Collections - 4th Exp.							
1st collection - 11/28/11	8.59	7.02	4.50	5.60	6.11	7.86	6.61
2nd collection - 12/28/11	6.56	7.41	7.17	7.30	7.46	8.14	7.34
3rd collection - 01/23/12	7.62	7.38	7.24	7.36	7.80	7.86	7.54
4th collection - 02/27/12	7.20	7.02	6.98	7.16	7.48	7.32	7.19
5th collection - 03/26/12	6.56	7.20	7.39	7.86	7.91	8.14	7.51
6th collection - 04/23/12	7.21	7.75	4.80	7.12	5.33	6.24	6.41
Means	7.29	7.29	6.35	7.06	7.01	7.59	

18 times, being the most frequent taxa, followed by *Margaritispora aquatica* (8 occurrences) and *Camposporium pellucidum* (6 occurrences).

Table 11 presents the fungal taxa obtained during the 2nd experiment, initiated in the rainy and hot season, with *T. pulchra* leaves too. In relation to the 1st experiment, the number of taxa was slightly higher, corresponding to 17. The number of occurrences ranged from 13 in 5th collection, to only one recorded occurrence in the last collection. Point 2 in this experimental presented the highest number of taxa (8), while point 1 had the highest number of occurrences (13). The number of taxa remained the same during all collections, except for the last, when only one taxa was isolated. However, the similarity index of Sörensen between the collections remained around 50%, demonstrating that, although the number of taxa had been equal, the taxonomical composition of the mycota changed according to the collections.

Camposporium pellucidum occurred 12 times, followed by *Triscelophorus monosporus*, registered 10 times and *Endophragmiella* sp., present 9 times during the experiment.

Table 7. Kruskal-Wallis test related to the dissolved oxygen (mgO_2L^{-1}) during the four experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brazil. Values in gray shades indicate significance ($p < 0.05$).

	1st collection	2nd collection	3rd collection	4th collection	5th collection	6th collection
2nd Experiment						
1st collection	0	0.4712	0.7483	0.8102	0.4225	0.02002
2nd collection	1	0	0.5211	0.3358	0.298	0.004998
3rd collection	1	1	0	0.8102	0.2298	0.01291
4th collection	1	1	1	0	0.2298	0.004998
5th collection	1	1	1	1	0	0.004998
6th collection	0.3003	0.07497	0.1936	0.07497	0.07497	0
3rd Experiment						
1st collection	0	0.0927	0.05424	0.06555	0.004998	0.04533
2nd collection	1	0	0.02024	0.008239	0.004998	0.01307
3rd collection	0.8136	0.3036	0	0.8102	0.04495	0.298
4th collection	0.9833	0.1236	1	0	0.004998	0.4225
5th collection	0.07497	0.07497	0.6743	0.07497	0	0.8099
6th. collection	0.6799	0.196	1	1	1	0
4th. Experiment						
1st collection	0	0.3785	0.3358	0.5211	0.2615	0.9362
2nd collection	1	0	0.4712	0.4712	0.6879	0.1282
3rd collection	1	1	0	0.03064	0.7483	0.03064
4th collection	1	1	0.4596	0	0.1994	0.3785
5th collection	1	1	1	1	0	0.06555
6th collection	1	1	0.4596	1	0.9833	0

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6
4th Experiment						
Point 1	0	0.8721	0.3776	0.9361	0.9361	0.376
Point 2	1	0	0.1727	0.8099	0.5745	0.1712
Point 3	1	1	0	0.3785	0.1282	0.04458
Point 4	1	1	1	0	0.5752	0.1249
Point 5	1	1	1	1	0	0.2281
Point 6	1	1	0.6686	1	1	0

Both in the first and in the second experiment, it was verified that some taxa occurred exclusively at certain collection point, and also some taxa occurred only incertain collections. According to Sörensen index (table 16), there is 39% similarity between the mycota of the experiments with *T. pulchra* leaves, that represents one of the lowest values found in the entire study, indicating that the composition of mycota differed considerably in climatically distinct seasons.

Regarding the fungal succession, the presence of higher number of taxa and occurrences of aquatic Hyphomycetes in the 2nd and 3rd collections of the 1st experiment (table 11), followed by the single presence of species was also reported by Moreira (2006) for leaves of *T. pulchra* submerged in the Lago das Ninféias. In the 2nd experiment (table 11), initiated in the rainy and hot season, with a greater number of fungal taxa, this occurrence pattern was not so clear,

Table 8. Individual values and means of the conductivity (μScm^{-1}) of the water measured during the experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brazil.

	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Means
Collections - 1st exp.							
1st collection - 07/29/10	0.030	0.030	0.030	0.030	0.030	0.030	0.030
2nd collection - 08/31/10	0.040	0.010	0.040	0.030	0.020	0.090	0.038
3rd collection - 09/29/10	0.030	0.030	0.030	0.030	0.030	0.030	0.030
4th collection - 10/26/10	0.052	0.052	0.058	0.050	0.048	0.047	0.051
5th collection - 11/17/10	0.001	0.001	0.005	0.053	0.050	0.050	0.026
Means	0.031	0.025	0.033	0.038	0.036	0.049	
Collections - 2nd exp.							
1st collection - 12/21/10	0.049	0.050	0.054	0.056	0.055	0.055	0.053
2nd collection - 01/18/11	0.047	0.049	0.050	0.052	0.052	0.050	0.050
3rd collection - 02/22/11	0.044	0.044	0.050	0.049	0.049	0.049	0.047
4th collection - 03/28/11	0.044	0.045	0.046	0.046	0.046	0.045	0.045
5th collection - 04/18/11	0.043	0.043	0.046	0.043	0.046	0.042	0.044
6th collection - 05/23/11	0.046	0.046	0.044	0.042	0.041	0.042	0.043
Means	0.045	0.046	0.048	0.048	0.048	0.047	
Collections - 3rd exp.							
1st collection - 06/20/11	0.044	0.044	0.042	0.040	0.043	0.040	0.042
2nd collection - 07/27/11	0.030	0.030	0.030	0.040	0.040	0.040	0.035
3rd collection - 08/24/11	0.050	0.050	0.050	0.050	0.040	0.040	0.046
4th collection - 09/19/11	0.048	0.046	0.042	0.056	0.043	0.043	0.046
5th collection - 10/24/11	0.055	0.055	0.061	0.055	0.059	0.046	0.055
6th collection - 11/28/11	0.056	0.050	0.049	0.046	0.045	0.042	0.048
Means	0.047	0.046	0.046	0.048	0.045	0.042	
Collections - 4th exp.							
1st collection - 11/28/11	0.056	0.05	0.049	0.046	0.045	0.042	0.048
2nd collection - 12/28/11	0.046	0.046	0.048	0.05	0.056	0.060	0.051
3rd collection - 01/23/12	0.046	0.046	0.048	0.046	0.049	0.049	0.047
4th collection - 02/27/12	0.039	0.042	0.041	0.045	0.044	0.044	0.042
5th collection - 03/26/12	0.045	0.042	0.046	0.045	0.044	0.040	0.044
6th collection - 04/23/12	0.042	0.043	0.048	0.047	0.050	0.047	0.046
Means	0.046	0.045	0.047	0.046	0.048	0.047	

although the total number of occurrences was also higher in the 2nd and 3rd collections.

It is remarkable the high occurrence of *Endophragmiella* sp. that has not been previously observed by Moreira (2006) or by Schoenlein-Crusius *et al*. (2009) in PEFI, but has been frequently observed in urban waters, together with *Margaritispora* and *Pyramidospora*, as was verified by Moreira (2011) on leaves of *Caesalpinia echinata* Lam. and *Campomanesia pheae* (O. Berg.) Landrum submerged in a lake in the park Parque Municipal Alfredo Volpi in the city of São Paulo.

Table 12 presents the fungal taxa isolated during the 3rd experiment, initiated in the dry and cold season, using mixed leaf litter confined in nylon net litter bags. Twelve taxa were obtained, distributed in 42 occurrences, with the greatest number of taxa - 8 (15 occurrences) in the 5th collection and the lowest- two (two occurrences), in the first collection. The point 4 presented the highest number of taxa (8) and occurrences (11), whereas the opposite occurred at point 2, where only three occurrences of two fungal taxa were registered. The

Table 9. Kruskal-Wallis test related to the conductivity (μScm^{-1}) during the four experiments in the Pirarungaua stream in the Botanical Garden of São Paulo, Brazil. Values in gray shades indicate significance ($p < 0.05$).

	1st collection	2nd collection	3rd collection	4th collection	5th collection	6th collection
1st Experiment						
1st collection	0	0.6546	1	0.002725	0.9316	-
2nd collection	1	0	0.6546	0.06461	0.6874	-
3rd collection	1	1	0	0.002725	0.9316	-
4th collection	0.02725	0.6461	0.02725	0	0.2248	-
5th collection	1	1	1	1	0	-
2nd Experiment						
1st collection	0	0.105	0.01748	0.004624	0.004624	0.004847
2nd collection	1	0	0.09934	0.004551	0.004551	0.004772
3rd collection	0.2622	1	0	0.2867	0.02777	0.0416
4th collection	0.06936	0.06827	1	0	0.1542	0.1816
5th collection	0.06936	0.06827	0.4165	1	0	0.6792
6th collection	0.0727	0.07158	0.6239	1	1	0
3rd Experiment						
1st collection	0	0.01508	0.2122	0.1445	0.004624	0.02422
2nd collection	0.2262	0	0.01338	0.004408	0.004197	0.004479
3rd collection	1	0.2007	0	0.8062	0.02637	0.9337
4th collection	1	0.06612	1	0	0.05214	0.5189
5th collection	0.06936	0.06296	0.3955	0.782	0	0.07557
6th. collection	0.3633	0.06719	1	1	1	0
4th Experiment						
1st collection	0	0.3734	1	0.02977	0.08927	0.6874
2nd collection	1	0	0.3173	0.004922	0.007687	0.2265
3rd collection	1	1	0	0.004624	0.008856	0.7457
4th collection	0.4465	0.07383	0.06936	0	0.3289	0.07661
5th collection	1	0.1153	0.1328	1	0	0.1474
6th collection	1	1	1	1	1	0

Table 10. Occurrences of conidial fungi in the leaves of *Tibouchina pulchra* Cogn submerged in the Pirarungaua stream during the 1st experiment, from 29/07/10 to 17/11/10. 1: Point 1, 2: Point 2, 3: Point 3, 4: Point 4, 5: Point 5 and 6: Point 6.

Taxa/collections	1st collection 07/29/10	2nd collection 08/31/10	3rd collection 09/29/10	4th collection 10/26/10	5th collection 11/17/10	Total
Triscelophorus monosporus Ingold	1	1, 2		6		4
Margaritispora aquatica Ingold	1, 6	1, 2, 6	2, 3, 5			8
Endophragmiella sp.	3, 5, 6	1, 2, 3, 5, 6	1, 2, 3, 4, 5	1, 3, 4	3, 4	18
Camposporium pellucidum (Grove) S. Hughes		1, 2	1, 4	1	4	6
Triscelophorus acuminatus Nawawi		1	1, 2			3
Pyramidospora sp.		2				1
Tetrachaetum elegans Ingold		2				1
Tricladium angulatum Ingold		3				1
Tripospermum myrti (Lind.) Hughes				5		1
Anguillospora longissima (Sacc. & P. Syd.) Ingold				5		1
Tripospermum camelopardus Ingold, Dann & McDougall				6		1
Varicosporium elodeae Kegel					2	1
Pyramidospora casuarinea Nilsson					4	1
Anguillospora crassa Ingold					5	1
Total number of taxa	3	8	4	6	5	14
Total number of occurrences	6	16	12	8	6	48

Table 11. Occurrences of conidial fungi in the leaves of *T. pulchra* submerged in the Pirarungaua stream during the 2nd experiment, from 12/21/10 to 05/23/11. 1: Point 1, 2: Point 2, 3: Point 3, 4: Point 4, 5: Point 5 and 6: Point 6.

Taxa/collections	1st collection 12/21/10	2nd collection 01/18/11	3rd collection 02/22/11	4th collection 03/28/11	5th collection 04/18/11	6th collection 05/23/11	Total
Tricelophorus monosporus Ingold	1, 3	2, 6	2, 5	1, 4	3, 4		10
Beltrania rhombica Penzig	1						1
Margaristispora sp.	1		1	1	3, 4		5
Camposporium pellucidum (Grove) S. Hughes	1	5	4, 5, 6	2, 3, 4, 5	1, 2, 5		12
Varicosporium sp.	2						1
Helicoon spirale Boedjin	2						1
Anguillospora longuissima (Sacc. & P.Syd.) Ingold	4						1
Endophragmiella sp.	4	5	5, 6	5, 6	1, 2, 6		9
Lunulospora curvula Ingold		1, 4					2
Margaristispora aquatica Ingold		1, 5	1		3		4
Anguillospora crassa Ingold		4	2				2
Dendrospora erecta Ingold		5					1
Lemonniera aquatica Ingold		6					1
Pyramidospora casuarinae Nilsson			2				1
Tetrachaetum elegans Ingold			3				1
Tripospermum myrti (Lind.) Hughes					1, 2		2
Pestalotiopsis sp.						6	1
Total number of taxa	8	8	8	8	8	1	17
Total number of occurrences	9	11	12	9	13	1	55

Table 12. Occurrences of conidial fungi in the submerged mixed leaf litter in the Pirarungaua stream during the 3rd experiment, from 06/20/11 to 11/28/11. 1: Point 1, 2: Point 2, 3: Point 3, 4: Point 4, 5: Point 5 and 6: Point 6.

Taxa/collections	1st collection 06/20/11	2nd collection 07/27/11	3rd collection 08/24/11	4th collection 09/19/11	5th collection 10/24/11	6th collection 11/28/11	Total
Margaritispora aquatica Ingold	1				4, 5		3
Ascomycota	3						1
Varicosporium elodeae Kegel		1, 2			3, 4	3	5
Camposporium pellucidum (Grove) S. Hughes		1, 4	4	6		1, 3	6
Triscelophorus monosporus Ingold		1	2, 4	1, 2, 3, 4	3, 6	3, 5	11
Camposporium antennatum Harkness			1				1
Lemonniera aquatica Ingold			4, 5		4		3
Anguillospora crassa Ingold			6		4, 5		3
Lunulospora curvula Ingold				5	3, 5, 6		4
Tripospermum myrtii (Lind.) Hughes					4	3	2
Triscelophorus curviramifer Matsushima					5, 6		2
Triscelophorus acuminatus Nawawi						4	1
Total number of taxa	2	3	5	3	8	5	12
Total number of occurrences	2	5	7	6	15	7	42

Table 13. Occurrence of conidial fungi in the submerged mixed leaf litter in the Pirarungaua stream during the 4th experiment, from 11/28/11 to 04/23/12. Legend: 1 (Point 1), 2 (Point 2), 3 (Point 3), 4 (Point 4), 5 (Point 5) and 6 (Point 6).

Taxa/collections	1st collection 11/28/11	2nd collection 12/28/11	3rd collection 01/23/12	4th collection 02/27/12	5thcollection 03/26/12	6thcollection 04/23/12	Total
Campylospora chaetocladia Ranzoni	1						1
Anguillospora crassa Ingold	1	1			2		3
Triscelophorus monosporus Ingold	1, 3, 6	4, 5		5	1	4	8
Lunulospora curvula Ingold	1						1
Margaritispora aquatica Ingold	2, 3, 4, 5	4	1, 4, 5, 6	1, 4, 6			12
Varicosporium elodeae Kegel		3					1
Dictyochaeta sp.		5					1
Lemonniera aquatica Ingold		5	3		4, 5		4
Flagellospora sp.		5					1
Beltraniopsis sp.			3				1
Fusarium oxysporum Schtdl.				5			1
Camposporium pellucidum (Grove) S. Hughes					2, 3	1	3
Pyramidospora casuarinea Nilsson					2, 5	2	3
Total number of taxa	5	7	3	3	5	3	13
Total number of occurrences	10	8	6	5	8	3	40

predominant taxa were *Triscelophorus mnonosporus* (11 occurrences), followed by *Camposporium pellucidum* (6 occurrences) and *Varicosporium elodeae* (5 occurrences).

The similarity index of Sörensen corresponded to 54% in the comparison of the 1st with the 3rd experiments, which were performed in similar seasons, but with different substrates (table 16).

Table 13 presents the fungal taxa isolated during the 4th experiment, started in the rainy and hot season, also using mixed leaf litter confined in nylon net litter bags. Thirteen taxa, distributed in 40

Table 14. Occurrences of conidial fungi in the submerged free leaf litter in the Pirarungaua stream during the 3rd experiment, from 06/20/11 to 11/28/11. 1: Point 1, 2: Point 2, 3: Point 3, 4: Point 4, 5: Point 5 and 6: Point 6.

Taxa / collections	1st collection 06/20/11	2nd collection 07/27/11	3rd collection 08/24/11	4th collection 09/19/11	5th collection 10/24/11	6th collection 11/28/11	Total
Triscelophorus monosporus Ingold	1,3	1,2,3,4,5,6	1,2,3,4,5,6		2	2	16
Beltraniella sp.	1						1
Dictyochaeta sp.	1						1
Lunulospora curvula Ingold		1,3,4					3
Anguillospora crassa Ingold				1,3	2,3	1, 2,4	7
Camposporium pellucidum (Grove) S. Hughes				1, 3			2
Triscelophorus sp. 1			1, 3	1, 3, 4	2, 3		7
Margarispora aquatica Ingold						2	1
Total number of taxa	3	2	2	3	3	3	8
Total number of occurrences	4	9	8	7	5	5	38

Table 15. Occurrences of conidial fungi in the free submerged leaf litter in the Pirarungaua stream during the 4th experiment, from 11/28/11 to 04/23/12. 1: Point 1, 2: Point 2, 3: Point 3, 4: Point 4, 5: Point 5 and 6: Point 6.

Taxa / collections	1st collection 11/28/11	2nd collection 12/28/11	3rd collection 01/23/12	4th collection 02/27/12	5th collection 03/26/12	6th collection 04/23/12	Total
Triscelophorus monosporus Ingold	2	3, 5, 6	6	4, 6	1, 3, 4	5, 6	12
Lunulospora curvula Ingold		1					1
Anguillospora crassa Ingold	1, 2,4		1				4
Anguillospora longuissima (Sacc. & P. Syd.) Ingold		1, 2, 6	1	1, 2, 5	2, 3, 4, 5	1, 2	13
Camposporium pellucidum (Grove) S. Hughes				4	1, 2	4	4
Margarispora aquatica Ingold	2						1
Ulocoryphus mastigophorus Michaelides, L. Hunter & W.B. Kendr.		5		4, 5			3
Pyramidospora sp.				1, 5, 6	1, 4, 6	1, 4, 6	9
Beltraniarhombica Penzig					5		1
Tetrachaetum elegans Ingold				4	4	5	3
Flabellosposra verticilata Alasoadura					3		1
Lemoniera aquatica Ingold					3, 4	3, 4	4
Pestalotiopsis sp.					3, 6	1, 5, 6	5
Total number of taxa	3	4	3	6	9	7	13
Total number of occurrences	5	8	3	12	19	14	61

occurrences were obtained, with predominance of *Margaritispora aquatica* (12 occurrences), followed by *Triscelophorus monosporus* (8 occurrences) and *Lemonniera aquatica* (4 occurrences). The greatest number of taxa was obtained in the first collection (10 occurrences) and the lowest in the 6th collection (three occurrences). At point 1 and point 5, ten occurrences, the highest number of taxa were registered, while at point 6, only three occurrences. The highest index of similarity (56%) were verified comparing the mycota of the 4th and 3rd experiments, where the same substrate was used, but in different seasons (table 16).

Excepting the 3rd experiment, a tendency of fungi to be present with larger number of taxa and occurrences in the first collections was observed. In the 3rd experiment these numbers were greater in the 5th collection, perhaps due to the impact of heavy rains after two months of drought.

Table 14 presents the fungal taxa that were isolated from free mixed leaf litter, collected concomitantly to the 3rd experiment, initiated in the dry and cold season. Eight fungal taxa were isolated, totalizing 38 occurrences. In the free leaf litter samples, the predominance of *Triscelophorus monosporus* (16 occurrences), especially in the 2nd and 3rd collections was observed. One more species of *Triscelophorus* sp.1 (species not identified) occurred seven times, as well as *Anguillospora crassa*. Seven taxa (11 occurrences) were registered at point 1 and only one taxon (two occurrences) at points 5 and 6. Comparing the mycota of the free leaf litter with the one of the confined leaves from the 3rd experiment, a similarity of 50% was verified.

Table 15 presents the fungal taxa obtained from free mixed leaf litter during the 4th experiment, started in the rainy and hot season. Thirteen taxa (61 occurrences) were isolated, with predominance of *Anguillospora longissima* (13 occurrences), *Triscelophorus monosporus* (12 occurrences), followed by one not identified species of *Pyramidospora* sp. (9 occurrences). The greatest number of taxa - nine (19 occurrences) was registered in the 5th collection and the lowest – three taxa (three occurrences) in the 3rd collection. The points 1, 4 and 5 presented equal total number of taxa (seven), being the highest number of occurrences (thirteen), registered for points 1 and 4. An index of 48% of similarity (table 16) was found comparing the mycota of the free leaf litter with the one of the confined leaves during the 4th experiment, as well as in the comparison with the 3rd experiment (mixed confined leaf litter, during the dry and cold season).

The index of similarities presented on table 16 varied from 39 to 56% and could be considered low when compared to the ones cited in studies about fungal succession such as in Schoenlein-Crusius & Milanez (1998) and Moreira (2006). Besides, according to Christensen (1989), in studies about fungi in tropical environments, similarity indexes around 70% are expected. Similarities below this value could be considered low.

Table 16. Sörensen Similarity Index calculated between the mycota obtained during the experiments in the Pirarungaua stream.

Experiment			1st	2nd	3rd	4th	3rd	4th
	Season		Dry and cold	Rainy and hot	Dry and cold	Rainy and hot	Dry and cold	Rainy and hot
		Type of substrate	*T. pulchra*	*T. pulchra*	Confined mixed leaf litter	Confined mixed leaf litter	Free lieaf litter	Free lieaf litter
1st	Dry and colda	*T. pulchra*	-	39%	54%	45%	36%	45%
2nd	Rainy and hot	*T. pulchra*	-	-	48%	47%	40%	53%
3rd	Dry and cold	Confined mixed leaf litter	-	-	-	56%	50%	48%
4th	Rainy and hot	Confined mixed leaf litter	-	-	-	-	48%	46%
3rd	Dry and cold	Free lieaf litter	-	-	-	-	-	48%
4th	Rainy and hot	Free leaf litter	-	-	-	-	-	-

Although the total number of fungal taxa obtained in each of the experiments in the present study may be considered modest in comparison to other studies, which includes the citation of 24 taxa of aquatic Hyphomycetes in the entire area of the Botanical Garden (Schoenlein-Crusius *et al.* 2009), the quantity of taxa in the different experimental situations was similar. The composition of the mycota, however, varied considerably in each situation, presenting the predominance of certain taxa in distinct collection and points.

The interpretation of the results of the present study is complex, because the confined leaves remained submerged and were gradually removed from the water, so that the isolated mycota followed a temporal sequence, whereas this was not the case for the free mixed leaf litter samples.

Added to this, the four experiments were conducted while the stream was in a regeneration phase, approaching increasingly to an situation of a natural water body, with new plants flanking the margins, along with new organisms as fishes and amphibians occupying new spaces in the forming environment.

The first two experiments were conducted with leaves of *Tibouchina pulchra*. The mycota of this leaf species was studied for the first time by Grandi & Gusmão (2002) in the Reserva Biológica do Alto da Serra de Paranapiacaba, in São Paulo State. In that study the authors obtained 22 fungal taxa employing the method of incubating leaf litter fragments in moist chambers. From their fungal list, only *Beltrania rhombica* is cited here for the *T. pulchra* leaves of the 2nd experiment, during the rainy and hot season.

Also in the Atlantic rainforest of Paranapiacaba, Moreira (2002) submerged leaves of *T. pulchra* confined in nylon net litter bags, in a stream, obtaining 40 fungal taxa, including six aquatic Hyphomycetes such as: *Lunulospora curvula* Ingold, *Triscelophorus monosporus* Ingold, *Lemonniera aquatica* de Wild., *Anguillospora crassa* Ingold, *Tripospermum myrti*, and *Margaritispora aquatica* Ingold. The presence of the taxa was more intensively observed after some weeks of decomposition of the leaves.

In the study of Moreira (2002), among the aquatic Hyphomycetes, *Margaritispora aquatica* was the most abundant taxa in *T. pulchra* leaves. In the present study, this taxon was the most abundant in the mixed leaf litter samples during the 4th experiment, not being the most common in *T. pulchra* leaves, although it was present mainly in the two initial collections of the two experiments.

Triscelophorus monosporus, in general, is one of the most common taxa mentioned in Brazilian studies about ingoldian fungi (Schoenlein-Crusius & Grandi, 2003). In the present study, the species showed a high number of occurrences during the 2nd experiment, was abundant during the 4th experiment and was one of the most frequent species in the free mixed leaf litter. The species was not the most frequent one in the study conducted by Moreira (2002). In a similar way, *Lunulospora curvula*, which is also very common in Brazilian studies, was not so frequent in the *T. pulchra* leaves in the present study and in the experiments mentioned by Moreira (2002).

The fungal succession and decomposition rate of submerged leaves of *T. pulchra* were studied by Moreira (2006) in the PEFI, at the Lago das Ninféias, which waters supplies the Pirarungaua stream. The mycota found in the lake, formerly characterized as meso-oligotrophic was compared to a hypertrophic lake called Lago das Garças. In the first lake, Moreira (2006) found 79 taxa, including two aquatic species. In that study and now, in the Pirarungaua stream, the following fungal taxa were found in common: *Anguillospora crassa*, *Dictyochaeta*, *Pestalotiopsis* and *Fusarium oxysporum.*

The fungal succession studied by Moreira (2006) in the PEFI included aquatic facultatives, zoosporic organisms and few ingoldian fungi, much less than occurred in the Atlantic rainforest of Paranapiacaba (Moreira 2002).The mycota composed by aquatic Hyphomycetes in the *T. pulchra* leaves in the present study approaches more the one found in Paranapiacaba than in the Lago das Ninféias in the Botanical Garden of São Paulo.

Considering that the methodology used to isolate the fungal species in these studies was similar, as well as the plant species used as substrate, one may assume that the type of aquatic environment and its intrinsic abiotic factors exert great influence on the composition of the mycota associated to decomposing plant substrates, what is in agreement with the literature (Bärlocher 1992).

However, Moreira (2006) verified a slower decomposition rate in the meso-oligotrophic lake than in the hypereutrophic lake, attributing this result to the massive presence of aquatic plants and probable presence of humic substances, which could have had a hampering effect on the diversity of ingoldian fungi.

Concomitantly to the study of fungal succession in the Lago das Ninféias (Moreira 2006), a survey of aquatic Hyphomycetes on submerged mixed leaf litter

was conducted in several water bodies in the entire area of the Botanical Garden, revealing 24 taxa, with predominance of *Anguillospora crassa*, *Lunulospora curvula*, *Tetrachaetum elegans* and *Camposporium pellucidum* (Schoenlein-Crusius *et al*. 2009). In the present study, the first three fungal taxa occurred almost in all experiments. However, the occurrence of *Tetrachaetum elegans* was registered only in the confined *T. pulchra* leaves in the 2nd experiment and in the free mixed leaf litter during the last experiment.

From the 24 total fungal taxa obtained in the survey mentioned above (Schoenlein-Crusius *et al*. 2009), 12 (50%) were no more observed in the present study. On the other hand, from the 35 total taxa registered in the experiments in the Pirarungaua stream, 12 were already cited for the Botanical Garden, but the remaining 23 are novelties for the area, including three new citations to Brazil.

Although the Pirarungaua stream is small and being in revitalization processes it is a strategic location for ecological studies of fungi, because the diversity is expressive. As the artificial environment will approach gradually the natural, probably other taxa may be revealed in the future, adding valuable information to the geographical distribution of fungi. Example of that is *Ulocoryphus mastigophorus* from the free mixed leaf litter (table 15), whose presence was only observed in *Agathis australis* leaves in New Zealand (Michaelides *et al.* 1982).

Initially much was asked how a taxon observed in such a distant continent suddenly was present in a small stream in São Paulo, but it has to be taken in account that the drastic modification of the watercourse from channeled to an open system that receives all the influence of environment, provides new niches and the opportunity for previously unknown species to become expressive in the coming taxonomic surveys.

A stream, even being small and designed primarily to compose an aesthetic landscape element, is still a part of the ecosystem where the biodiversity is established according to the biotic and abiotic conditions, deserving to be recovered, preserved and conserved for future generations.

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