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Survey of Stream Spora in Maliau River in Borneo

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Abstract Stream spora collected from Maliau River in Borneo Island were recorded with illustrations. Fourteen species among 11 genera and eight unidentified taxa were recognized.

Key words: freshwater hyphomycetes, fungal biodiversity, Ingoldian fungi, Malaysia, stream spora.

Introduction

Fungal diversity in tropical areas where enormous biodiversity is known, is one of the fascinating topics in mycology. Much attention has been paid to accumulate data on local mycobiota in Southeast Asia, where the fungal inventory is still insufficient (Watling *et al.*, 2002a, 2002b).

Aquatic hyphomycetes are a remarkable group of fungi that are characterized by tetra-radiating or coiled conidial morphology, which is considered to be an adaptation to aquatic habitats. Most of this group of fungi are also known as "Ingoldian fungi" or "freshwater hyphomycetes". The morphological adaptation of conidia results in their accumulation in water foams, which allows for convenient and easy sampling.

As a part of the elucidation of mega diversity of fungi in Malaysia, aquatic hyphomycetes were enthusiastically studied (e.g. Nawawi, 1985; Goh, 1997; Marvanová, 1997; Graça *et al.*, 2016). There are still a number of unidentified species (Nawawi, 1985), but less attention has been paid partially due to the low number of mycologists in the country (Bärlocher, 2016).

The Maliau Basin Conservation Area (MBCA) comprises tremendous biodiversity, for example $>$1,010 tree species and $>$86 mammal species (http://maliaubasin.org/research/). However, despite extensive biodiversity research, so far intensive mycological studies have not been carried out (Yayasan Sabah, 2014). In 2017, with permission from both the Maliau Basin Management Committee [YS/MBMC/2016/184 (to HT)] and the Sabah Biodiversity Council [JKM/MBS.1000-2/2 JLD.5(23) (to HT)], HT and MS participated in a botanical survey at the Maliau Basin Conservation Area (Sabah, Borneo, Malaysia), which covers ca. 59,000 ha and is surrounded by sandstone ridges. During the expedition, HT collected water foam samples in Maliau River.

The Maliau River is characterized by its tea-color water and is well-known for its foaming nature (Harun *et al.*, 2010), probably due to the saponin and humic composition originated from peat from the river basin. For the further elucidation of the local mycobiota in Borneo Island, a preliminary observation on the spore diversity in

the fixed water foam samples are presented in the present paper.

Materials and Methods

Water foam samples were collected beside the river bank of the Maliau River along Seraya Trail, Maliau Basin, Sabah, Borneo, Malaysia (+4.7425, +116.9638, Fig. 1) on August 29, 2017. The foam samples were scooped up by a spoon and collected in a 15 ml plastic container, and fixed with alcohol immediately after collection (Fig. 2). The sample was kept in room temperature until observation. Due to the lack of appropriate facilities, isolation was not attempted in this study.

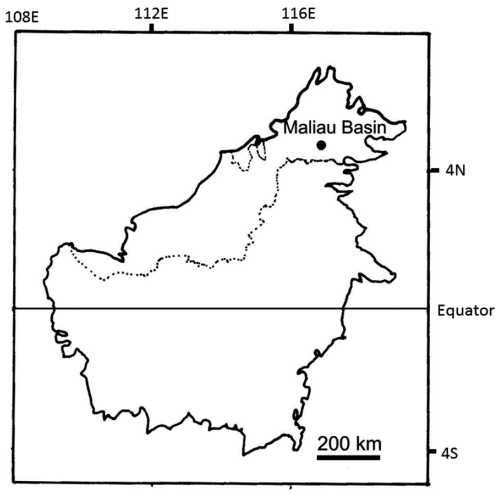

Fig. 1. Map of Borneo Island and collection sites. The mid line shows the equator. Dotted line shows the boundary of Indonesia and Brunei.

Part of the sample was transferred to a 1.5 ml Eppendorf tube and centrifuged (5000 g, 30 sec) to obtain concentrated sediment. A drop of the sediment was placed on a slide glass using a Pasteur pipet, and scanned by light microscopy using an Olympus BX51 microscope for fungal spores.

Because of the three dimensional morphology of the conidia, adjusting focus in a single frame in the photograph was difficult. Hence, digital photographs were taken using Nikon Digital Sight DS-Fi2 camera system and depth synthesis of the photographs was processed by Combine ZP software (http://www.hadleyweb.pwp.blueyonder.co.uk). Line drawings were prepared to support photographic data and drawn using the Olympus BX-51 microscope equipped with U-DA drawing tubes and 40× or 100× objective lens.

Results and Discussion

The observed and identified spores are listed

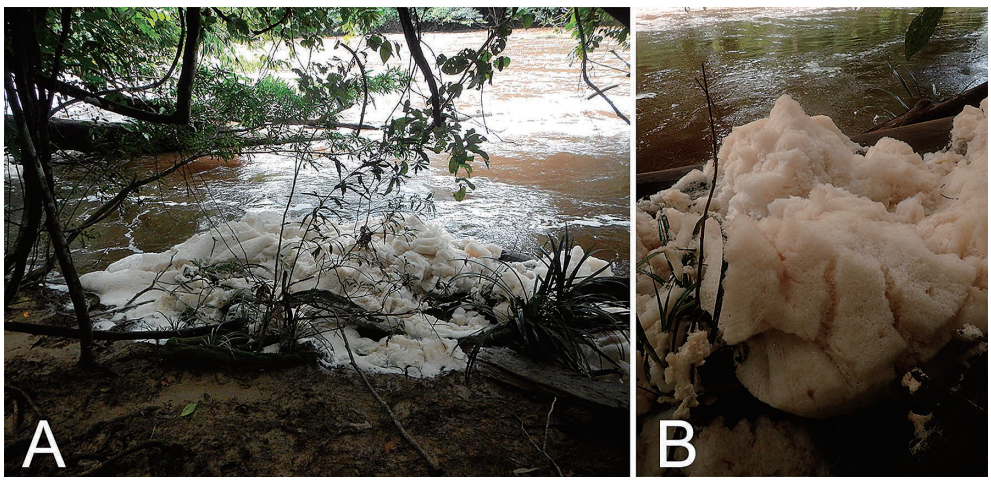

Fig. 2. Collecting the sample. A. Water foam produced and accumulated along the river bank. B. Close up of the water foam. Note the tea-colored foam due to the colored water.

Table 1. List of species obtained in the present study with the comparisons with previous studies

Present study	Nawawi (1985)	Hosoya *et al.* (2006)
Alatospora sp.		
Anguillospora sp.	−	+
Campylospora chaetocladia Ranzoni	+	+
Clavatospora filiformis (Nawawi) Marvanová	+	+
Condylospora gigantea Nawawi & Kuthub.	−	+
Ingoldiella fibulata Nawawi	−	−
Ingoldiella hamata D.E. Shaw	−	−
Nawawia filiformis (Nawawi) Marvanová	+	+
Scutisporus brunneus K. Ando & Tubaki	+	+
Subulispora malaysiana Nawawi & Kuthub.	+	−
Tricladium brunneum Nawawi	+	+
Tridentaria sp.		
Triscelophorus acuminatus Nawawi	+	−
Triscelosporium verrucosum Nawawi & Kuthub.	−	−
Wiesneriomyces javanicus Koord.	−	−
Unknown-Nawawi (1985) #75	+	+
unidentified (Fig. 4F)		
unidentified (Fig. 3U)		
unidentified (Fig. 3W)		
unidentified (Fig. 3X)		
unidentified (Fig. 3Y)		

Nawawi (1985) includes two sites (Gambak and Cameron Highlands). Hosoya *et al.* (2006) includes four sites (Tiki Kerawang Waterfall, Penang Island, Bukit Gatang, and two sites in Bukit Larut). + shows that the given species is listed in the literature, while — shows not listed. No marks shows that the given species is not compared for its uncertainty in morphological character. *Clavatospora filiformis* was cited as *Nawawia filiformis* in Hosoya *et al.* (2006).

in Table 1, with representatives shown in Figs. 3 and 4. In total, 15 species from 14 genera and six unidentified taxa (one of which have been repeatedly collected, but no names has been given; Nawawi, 1985) were recognized.

The sediment contained a number of small particles of insect bodies, plant debris and amorphous matter that caught stream spora (Fig. 3A–C). Some debris were accompanied with conidia produced by terrestrial fungi (e.g. *Beltrania*, *Fusarium* and *Pestalotiopsis*; Fig. 3F–I), suggesting active decomposition of these particles by hyphomycetes caught in stable bubbles. Frequent observation of conidiophores (Fig. 3E) also suggested higher ability of the foam to capture particles.

With the comparisons of the stream spora in the temperate zone (Miura, 1974; Ingold, 1975; Gulis *et al.*, 2005), we observed several interesting trends as follows. Larger spores occurred more frequently than in temperate zone. Coiled spores were more frequently observed. Coiled spores are known as an adaptation to intermediate environments between terrestrial and submerged areas (Michaelides and Kendrick, 1982). On the other hand, tetra-radiating spores were observed less frequently. A number of coiled spores are known in terrestrial fungi living in a wet environment (e.g. aero-aquatic fungi). This tendency agreed with the stream mycobiota in Kita-Iwojima Island (Hosoya *et al.*, 2018).

More dematiaceous spores were observed than in temperate zone. Dark-colored or melanised cells are known to be an adaptation to stronger sunlight intensity, and are a common character in tropical micro fungi.

Some spores were found to be germinated. In aquatic hyphomycetes, germination usually does not occur in lotic water, but in lentic water. These observation also suggest long-lasting bubbles provide stable habitat.

In the previous study, basidiomycetous anamorphs, relatively common in the tropics (Nawawi, 1973), were not observed (Hosoya *et al.*, 2006), but *Ingoldiella fibulata* and *I. hamata* were observed in the present study. Although the occurrence may be affected by seasonality and

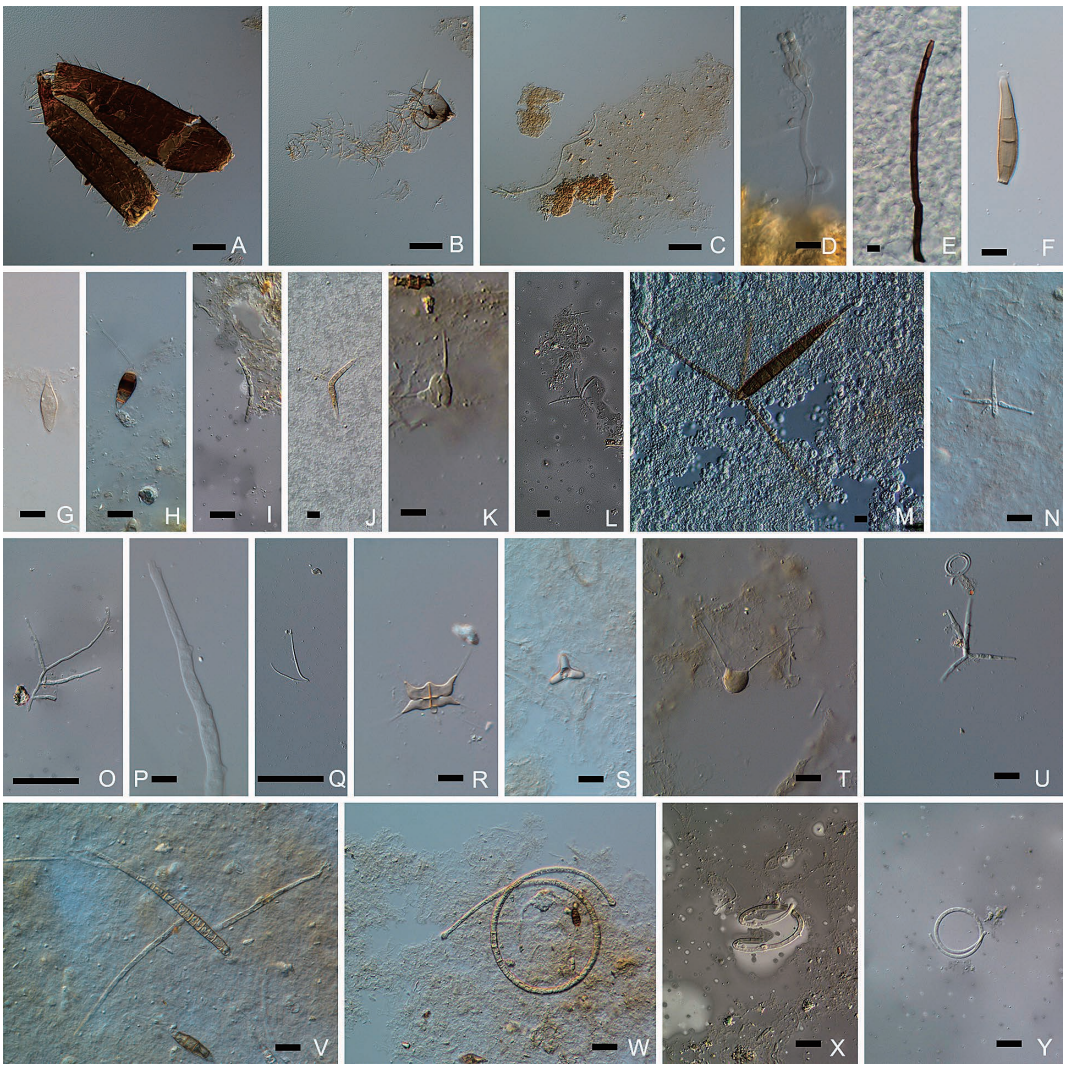

Fig. 3. Materials observed in water foam and some representatives of the stream spora. A. Part of the insect body observed in the foam sample. B. Residue of molted exoskeleton. C. Amorphous material capturing filamentous fungal spore. D. *Aspergillus* conidiophore producing conidia from plant debris captured in the foam. E. Conidiophore of a dematiaceous hyphomycete. F. *Pseudospiropes* sp. G. Damaged conidia of *Beltrania* sp. without appendages. H. *Pestalotiopsis* sp. I. *Wiesneriomyces javanicus*. J. *Tricladium brunneum*. K. *Campylospora chaetocladia* L. *Alatospora* sp. M. *Triscelosporium verrucosum*. N. *Triscelophorus acuminatus*. O. *Ingoldiella hamata*. P. Close up of conidia of *Ingoldiella hamata* bearing clamp connections at the septa. Q. *Condylospora gigantea*. R. *Scutisporus brunneus*. S. *Nawawia filiformis* [Unknown #71 of Nawawi (1985)]. T. *Clavatospora filiformis*. U. unidentified. V. *Tricladium brunneum*. W. unidentified coiled spore with two dimensional structure. X. unidentified coiled spore with three dimensional structure. G–L, R, X, depth synthesized. Scales. A–C, 100 µm. D–Y, 10 µm.

occasion, the comparison with the previous study shows that *Clavatospora filiformis* (Nawawi) Marvanová and *Tricladium brunneum* are the most widely distributed species in Malaysia.

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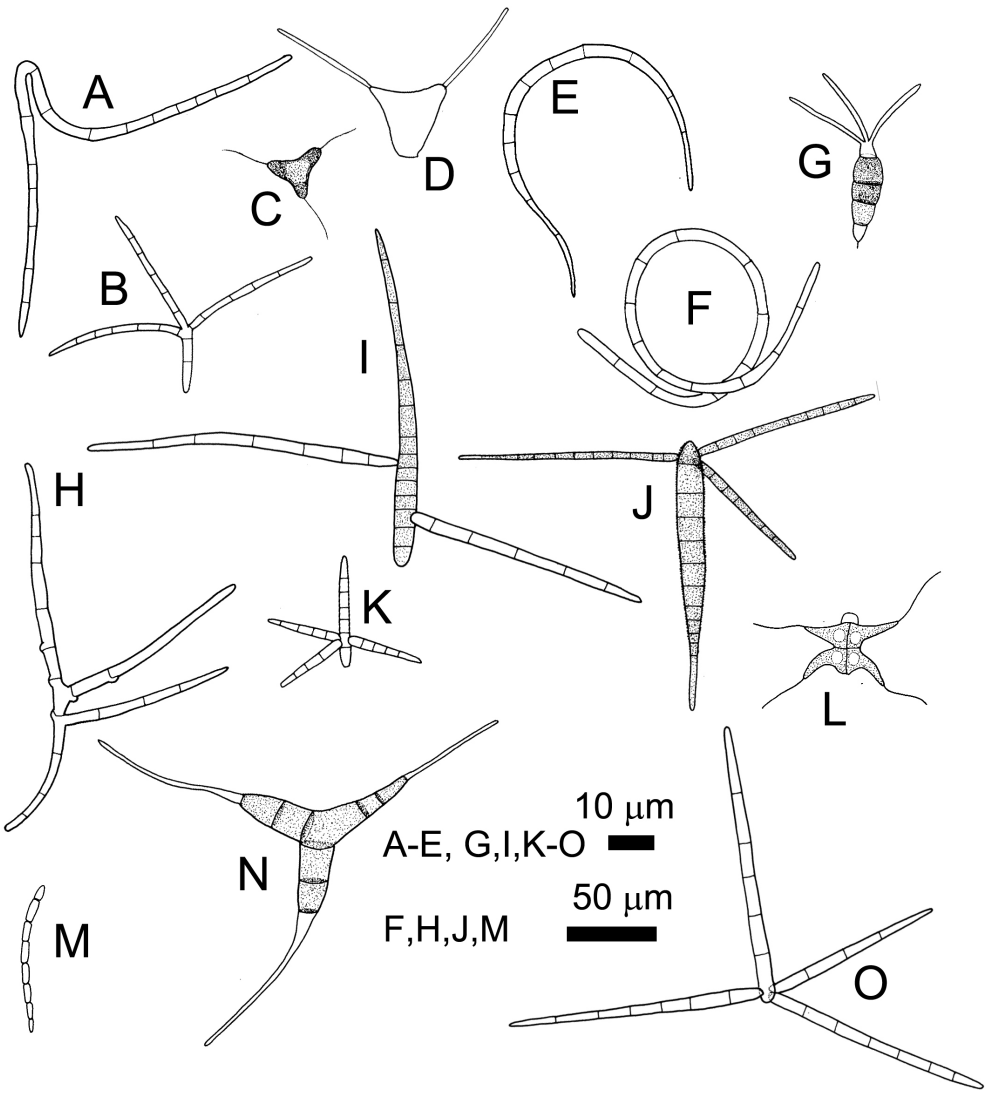

Fig. 4. Representatives of stream spora illustrated by line drawings. A. *Condylospora gigantea*. B. *Tridentaria* sp. C. *Nawawia filiformis* [Unknown #71 of Nawawi (1985)]. D. *Clavatospora filiformis*. E. *Anguillospora* sp. F. unidentified. G. *Pestalotiopsis* sp. H. *Ingoldiella fibulata*. I. *Tricladium brunneum* (?). J. *Triscelosporium verrucosum*. K. *Triscelophorus acuminatus*. L. *Scutisporus brunneus*. M. *Wiesneriomyces javanicus*. N. Unknown–Nawawi (1985) #75. similar to *Diplocladiella scalaroides* G. Arnaud ex M.B. Ellis, but differs in having third branch with appendage. O. *Triscelophorus acuminatus*

mittee [YS/MBMC/2016/184 (to HT)], Yayasan Sabah (Maliau Basin Conservation Area), and the Sabah Biodiversity Council [JKM/MBS. 1000-2/2 JLD.5 (23) (to HT)] for the present study. We also express our thanks to Dr. Joey Tanney, Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada for critically reviewing the manuscript.

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