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Waterfoam Mycobiota collected in Kita-Iwojima Island, Ogasawara Islands

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Abstract A water foam sample formed in an ephemeral stream was obtained in Kita-Iwojima Island, one of the Volcano Islands south of the Ogasawara Islands, and its mycobiota was enumerated on a morphological basis. In total, 15 species among 13 genera and five unidentified fungi were recognized. The sample was characterized by the paucity in biodiversity, and the majority of species producing coiled spores, partially due to the lack of stable aquatic environment. This is the first report of Ingoldian and other water foam-associated microfungi from the Island.

Key words: Aquatic fungi, Bonin Islands, distribution, Ingoldian fungi, microfungi.

Introduction

Kita-Iwojima Island (latitude +25.4333, longitude +141.2833) is a small island with an area of 5.57 km^2, 3.3 km from south to north, 2.1 km from east and west, and 8 km in the periphery. The island is one of the three islands to form Volcano Island, under the jurisdiction of the Ogasawara Islands, and is about 1,200 km away from the main islands of Japan (Fig. 1). While Minami-Iwojima has been uninhabited since recorded history, Kita-Iwojima was once colonized in 1899, but uninhabited since 1944 due to the evacuation during the war. Because of their geographical isolation and the relative lack of human activity, the natural life on both islands has been receiving attention from researchers. However, due to severe geographical and environmental conditions, approaching these islands has been extremely difficult. Nevertheless, some explorations have been carried out on both islands. Fortunately, the authors of this paper were able to accompany one biota assessment exploration activity in Kita-Iwojima Island, and part of the result has been published (Hosoya *et al.*, 2011).

Aquatic hyphomycetes are the ecological group of fungi that have characteristic morphology like tetraradiate or coiled spores, considered to be an adaptation to aquatic habitats. The group is also known as "Ingoldian fungi" or "freshwater hyphomycetes". Because of the characteristic morphology of the spores, they accumulate in water foams, which makes the collection of samples easy. Within the water foams, however, spores of terrestrial fungi associated with humid environments are sometimes found due to the physical properties of the water foam, which capture particles.

Although the study of Ingoldian fungi started in Europe (Ingold, 1975), the fungal group is distributed worldwide, and the fungal diversity of this group has been enthusiastically studied in tropical areas (e.g. Nawawi, 1985; Goh, 1997; Marvanová,1997; Hosoya *et al.*, 2006; Graça *et al.*, 2016), but never in Volcano Islands. In Japan, Miura (1974) provided a survey, but large parts of Japan remain unsurveyed. In the Kita-Iwojima

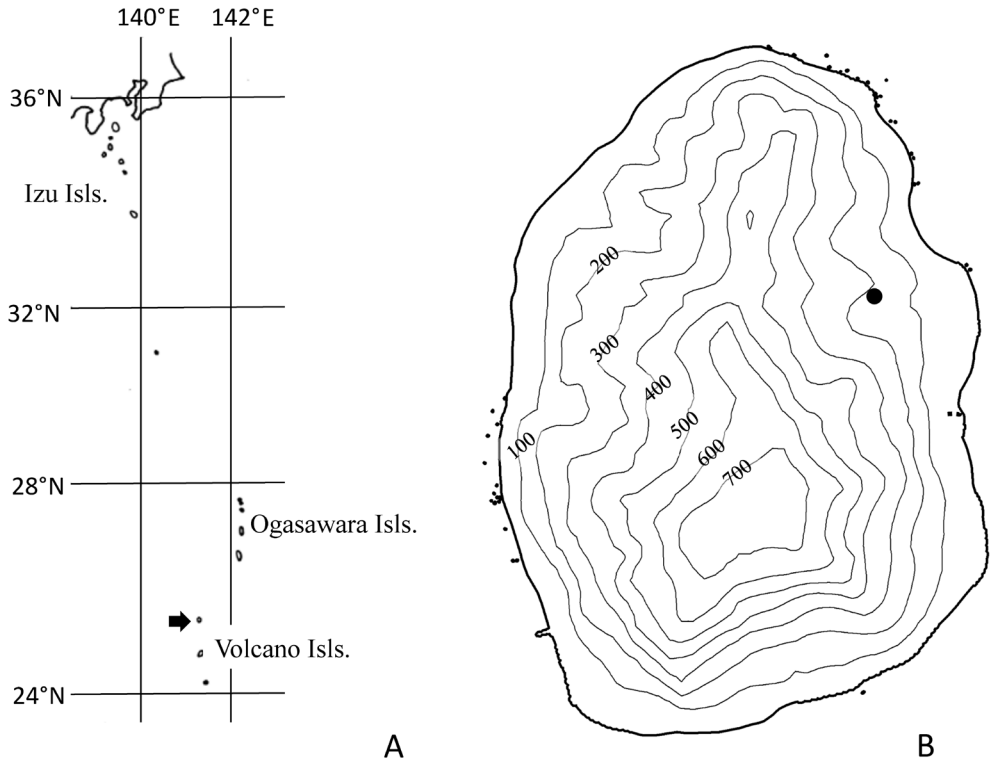

Fig. 1. Map of Kita-Iwojima Island and the collection site. A. Large-scale map showing the location of Kita-Iwojima Island (arrow). B. Map of Kita-Iwojima Island showing the collection site (●). The numbers in the figure shows the elevation in meters.

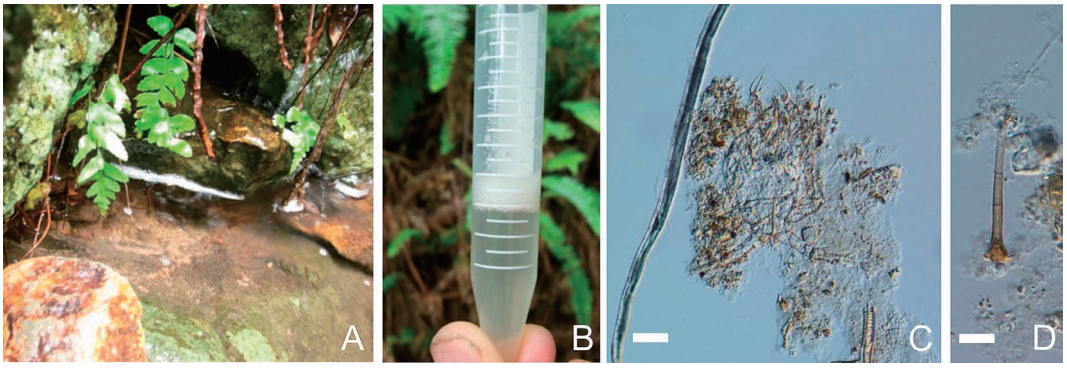

Fig. 2. Materials observed in the present study. A. Water foam formed in the tentative lotic water. B. Water foam sample collected in the site. C. Insect exoskeleton and filamentous plant material captured in the foam. D. Conidiophore of a dematiaceous fungus observed in the water foam. Scales = 10 µm.

Island, no perennial streams are known, with only ephemeral streams forming after heavy rain. The island also receives severe sunlight and high temperature. Despite such a harsh environment, the authors fortunately found an ephemeral stream where water foam was found. The present paper reports the analysis of the fungal elements in the water foam sample.

Materials and Methods

The sample was obtained on June 19, 2009, in an ephemeral stream (latitude +25.43700, longitude +141.2873889, elevation 186 m) that formed due to the heavy rain in the previous night (Fig. 2). The site was just below the campsite in the exploration from June 16–19 (Hosoya *et al.*, 2011). Several spoonfuls of water foam were collected by a spoon and immediately fixed by formalin in a 50 ml plastic vial and brought back to the laboratory. Part of the sample was transferred to a 1.5 ml Eppendorf tube and centrifuged (12,000 rpm, 30 sec) to obtain the concentrated sediment. A drop of the sediment was added to a drop of Hoyer's medium (distilled water 50 ml, Arabic gum 30 g, chloral hydrate 200 g and glycerin 20 g; Martin and Alexopulos, 1969) on the slide glass, and examined for fungal spores under light microscopy using an Olympus BX51 microscope. Identification of the fungi was made based on morphology.

Because some spores have complex three-dimensional structure, adjusting focus in a single frame in the photograph was difficult. To obtain photo image of these spores, digital photographs were taken by Nikon Digital Sight DS-Fi2 and depth synthesized using Combine ZP software (http://www.hadleyweb.pwp.blueyonder.co.uk). For supportive purpose, line drawings were prepared using the Olympus BX-51 microscope equipped with U-DA drawing tubes and 40× or 100× objective lens.

To analyze the tendency of spore frequency based on the spore morphology, numbers of the spores were counted by choosing 20 fields at 10× objective lens, and spores were counted on the morphological categories based on the typical spore morphology in aquatic fungi (see explanation of the categories in the legend of Fig. 6).

Results and Discussion

The spores observed and identified are listed in Table 1, the representative of which are shown in Figs. 3–5. In total, 15 species among 13 genera and five unidentified fungi were recognized.

Table 1. List of species found in the water foam

**Anguillospora crassa* Ingold	Fig. 3E, 5I
**Anguillospora longissima* (Sacc. & P. Syd.) Ingold	Fig. 3I, 5G
Camposporium sp.	Fig. 4H
Chaetospermum sp.	Fig. 4F
Cylindrocladium sp.	Fig. 4G
Diplocladiella scalaroides G. Arnaud ex M.B. Ellis	Fig. 4E, 5B
**Filosporella aquatica* Nawawi	Fig. 3H, 5A
**Flabellospora multiradiata* Nawawi	Fig. 4A
**Flabellospora verticillata* Alas.	Fig. 4D, 5C
Helminthosporium sp.	Fig. 4I
**Isthmotricladia laeensis* Matsush.	Fig. 4B, 5D
Pestalotiopsis sp.	Fig. 4J
Speiropsis pedatospora Tubaki	Fig. 3J, 5F
Wiesneriomyces javanicus Koord.	Fig. 3K, 5E
Unidentified #1 (Helicoid 1)	Fig. 3A, 5H
Unidentified #2 (Helicoid 2)	Fig. 3B
Unidentified #3 (Helicoid 3)	Fig. 3C, 5J
Unidentified #4 (Helicoid 4)	Fig. 3D
Unidentified #5 (Spore type #79 of Nawawi (1985))	Fig. 4C

Fungi with asterisk (*) are to be regarded as typical Ingoldian fungi.

All of them have been recorded for the first time from Kita-Iwojima Island.

The sediment also contained a number of small particles of insect exoskeletons, plant debris, and conidiophores (Fig. 2), because of the foam's physical ability to capture particles, but no diatoms were found indicating the water was lotic and tentatively formed.

The water sample had some remarkable features to be noted as below.

The majority of the spores were filiform and curved (Fig. 6). Coiled spores consisted of more than 50% of the observed spores. Together with sigmoid and curved spores, filiform spores occupied almost 80% of the Ingoldian fungal spores. Coiled spores are known as an adaptation to intermediate environments between terrestrial and submerged areas (Michaelides and Kendrick, 1982). A number of coiled spores are known in terrestrial fungi from highly humid environments (Ellis, 1971).

Some fungal spores derived from terrestrial environments were also recognized (Fig. 4). In fact, some of these have been recognized in aquatic environment, e.g. *Diploceras* and *Camposporium* (Gonczol and Revay, 2003). Yet, the specific diversity seemed low, again indicating the limited occurrence of aquatic environments on the island.

The paucity of typical Ingoldian fungi having

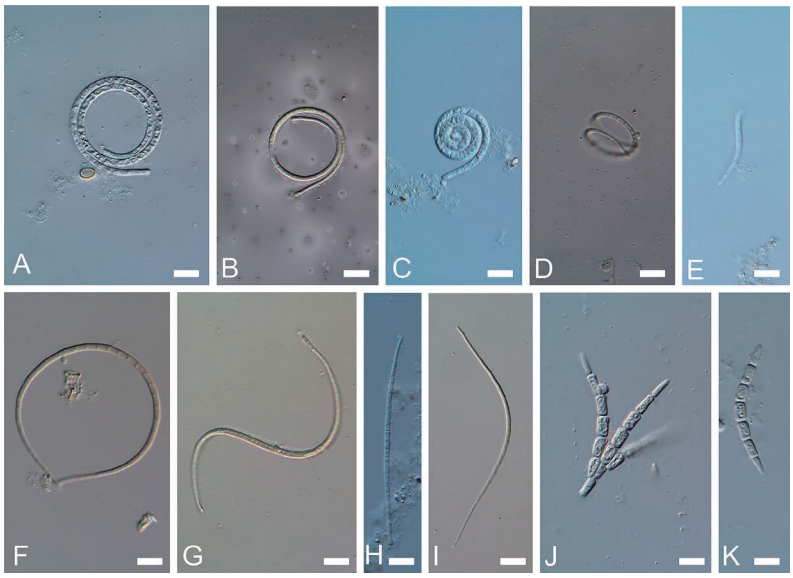

Fig. 3. Representative spores (helicosporous, sigmod or curved, and connected) observed in the water foam. A. Unidentified #1 (*Helicomyces*?). B. Unidentified #2 (*Helicomyces*?). C. Unidentified #3 (*Helicomyces*?). D. Unidentified #4 (*Helicomyces*?). E. *Anguillospora crassa.* F. *A. longissima* (?). G. *A. longissima* (?). H. *Filosporella aquatica.* I. *A. longissima*. J. *Speiropsis pedatospora.* K. *Wiesneriomyces javanicus*. B, D, E, G and J: depth synthesized. Scales = 10 µm.

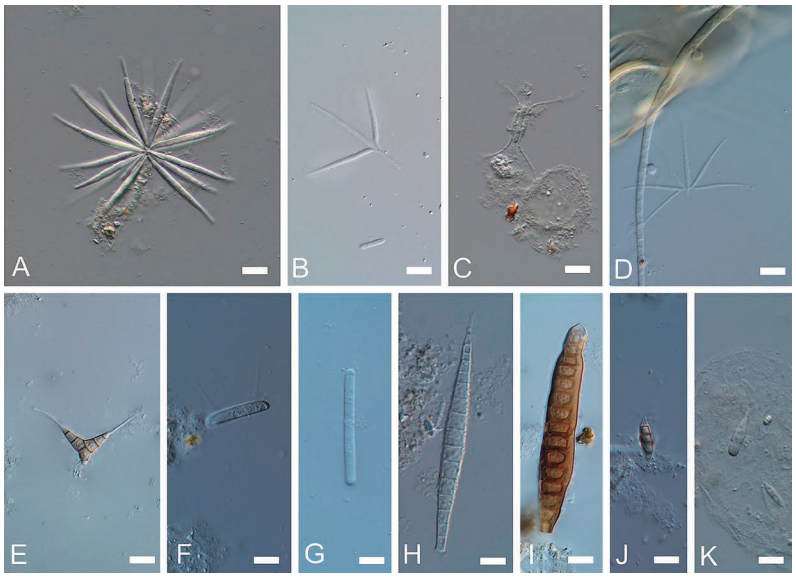

Fig. 4. Representative spores (radiating or terrestrial) observed in the water foam. A. *Flabellospora crassa.* B. *Isthmotricladia laeensis.* C. Unidentified #5 [Spore type #79 in Nawawi (1985)]. D. *F. verticillata*. E. *Diplocladiella scalaroides.* F. *Chaetospermum* sp. G. *Cylindrocladium* sp. H. *Camposporium* sp. I. *Helminthosporium* sp. J. *Pestalotiopsis* sp. K. *Jaculispora* sp. B, D and E: depth synthesized. Scales = 10 µm.

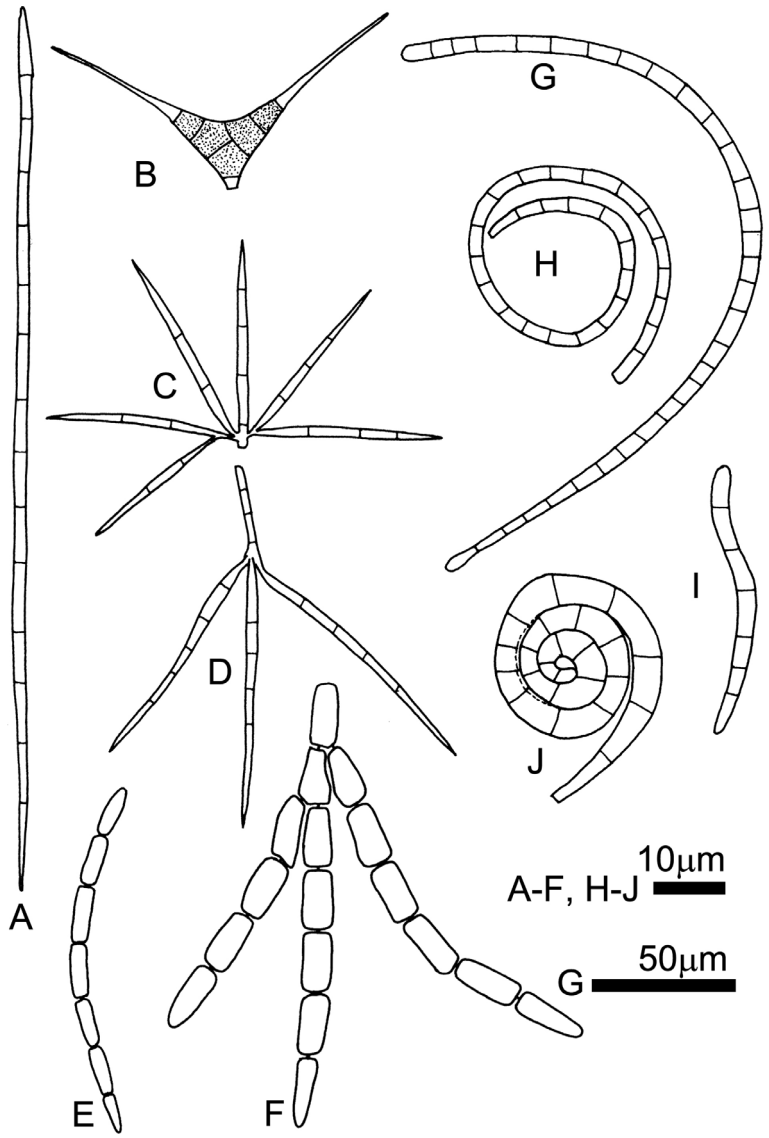

Fig. 5. Line drawings of representative spores observed in the water foam. A. *Filosporella aquatica*. B. *Diplocladiella scalaroides*. C. *Flabellospora verticillata*. D. *Isthmotricladia laeensis*. E. *Wiesneriomyces javanicus*. F. *Speiropsis pedatospora.* G. *Anguillospora longissima*. H. Unidentified #1 (Helicoid 1). I. Unidentified #2 (Helicoid 2). J. *A. crassa.*

radiating morphology was also remarkable. Only *Flabellospora* spp., *Isthimotricladia*, and unidentified #5 (Nawawi, 1985) possessed the typical radiating morphology of Ingoldian fungi.

It is also notable that the sample contained very few dematiaceous spores, basidiomycetous spores, and terrestrial-aquatic fungal spores. All these spores usually occur in samples in tropical environments, but not in this case, suggesting the paucity of fungal biodiversity in Kita-Iwojima.

Although Kita-Iwojima was once colonized in 1899, it has been uninhabited since 1944 when the inhabitants were forced to evacuate during the war. Some plants and animals were introduced to the island, but the vegetation in the island has remained relatively low diversity.

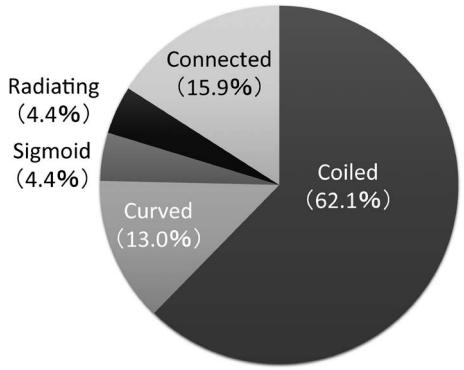

Fig. 6. Pie chart showing the frequency ratio of the observed spores based on the major morphological categories. Coiled: spores are curved >360 degrees in the same plain loosely or tightly or forming three-dimensional coils. Curved: spores are gently to strongly curved, but not reaching 360 degrees. Sigmoid: spores are curved twice to give "S"-like structure. Radiating: spores composed of linear hyphal elements that radiating into three or more directions. Connected: spores are constructed by small cells, connected by a thin connective structure (*Speiropsis pedatospora* and *Wiesneriomyces javanicus*). Note spore morphology of coiled, curved, and sigmoid are continuous.

Together with the extremely isolated environment in the middle of the ocean and its origin (volcanic island formed by mid-ocean eruption), inhabitation of fungi associated with freshwater aquatic environments may be limited.

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