

Species diversity of myxomycetes inhabiting twig litter of dominant native trees and exotic trees in the forests of the oceanic Ogasawara Island

Kazunari Takahashi¹, Kotaro Mimani², Kyoko Tateishi³

¹5792 Rokujoin-naka, Kamogata-cho, Asakuchi City, Okayama, 719-0252 Japan

²Kochi university graduate school. 2-5-1 Akebono-cho, Kochi City, Koch, 780- 8072 Japan

³533-6 Nakakitakami Tsuyama City, Okayama, 709-4606 Japan

E-mail: kumakusu03@yahoo.co.jp

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Abstract: The Ogasawara Islands (Bonin Islands) are ocean islands in the Japanese archipelago, where forest ecology in inherently natural environments is important for nature conservation. However, knowledge of myxomycete diversity in the forest ecosystems of these islands is limited and under threat from exotic tree invasion. The present study focused on myxomycetes living on twig litter from nine different tree species, representing five native and four exotic tree species, including an aerial leaf litter. A moist chamber culture method positively yielded fruiting bodies in 50% of 260 petri dish cultures for twigs and 8% of 100 cultures for aerial leaf litter. A total of 36 myxomycete species belonging to 21 genera were identified, including 21 species updated in the Ogasawara. Twig litter of the native species *Calophyllum inophyllum* yielded the highest diversity with 19 species, but only six species were found on the aerial leaf litter. The twigs of native trees yielded 30 species, and those of exotic trees yielded 18 species, including 14 species common in both communities. The most abundant species was *Arcyria cinerea*, followed by *Stemonitis fusca* var. *rufescens* and *Lamproderma scintillans*. *Willkommlangea reticulata* and *Ophiotheca pedata* characteristically appeared on twigs. Additionally, *Craterium concinnum* species are native vs. *Cribraria violacea* on exotic tree twigs. The ten myxomycete communities ordinated by multidimensional scaling were arranged according to substrate pH. Twig litter exhibited higher diversity, and the species diversity on native trees was higher than that on exotic trees on the Ogasawara Islands.

Keywords: exotic tree twigs, leaf litter, moist chamber culture, native tree twigs, ocean island.

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Introduction

Myxomycetes (plasmodial slime molds) have a unique life cycle, with the majority of it spent as myxoamoebae and plasmodia, the latter subsequently transforming into tiny sporocarps. They feed on bacteria, yeasts, and other microorganisms (Novozhilov et al. 2017) and play an important role in balancing soil microbial communities in forests (Gao et al. 2022). Myxomycetes are the most numerous

microorganisms constituting the soil protist communities (Stephenson et al. 2011). Myxomycetes typically produce sporocarps, which release large quantities of airborne spores that are dispersed long-distance globally (Foissner 2007). This suggests that the minute size and abundance of spores facilitate the dispersal and inhabitation of myxamoeba everywhere; however, myxomycete species generally do not produce fruiting bodies in a cosmopolitan distribution (Dahl et al. 2019). Fruiting body morphospecies have restricted distribution in different climatic zones or major ecosystem types (Schnittler et al. 2022). Myxomycetes are important members of the humus ecosystem and play an essential role in ecoregional systems (Novozhilov et al. 2017). The study of the biological diversity in humus ecosystems has become an important basis for understanding forest ecosystems to conserve and maintain nature (McLaren 2014). The short life cycle of myxomycetes serves as a valuable model for exploring adaptive microbial dynamics within humus ecosystems.

Island biogeography can be studied in myxomycetes, and its diversity is fascinating, as hundreds of islands worldwide are isolated locations that constitute a closed ecosystem. However, the biogeography of myxomycetes on islands is poorly understood (Stephenson 2023). Studies on these typical islands are limited, such as those on Madagascar Island (Wrigley de Basanta et al. 2013), the oceanic Galápagos Islands in the subtropics (Eliasson 1971) and the Hawaii Islands (Eliasson 2004) in the tropics. Among the thousands of islands in the Philippines, recent research has been conducted on several islands (e.g., Macabago et al. 2020a; 2020b; Pecundo et al. 2023). As the occurrence of myxomycete species is limited not only by geographical allopatric speciation but also by habitat suitability in a particular region, restricted distribution patterns as well as geographic barriers are assumed (Foissner 2007). In this context, the island biogeography of myxomycetes is recognized as an interesting study from ecological and evolutionary perspectives.

The Ogasawara Islands are typical oceanic islands in the Japanese archipelago, located about 1000 km south of the mainland. They were separated from the continent during the geographical evolution of the earth, forming superior scenery and a distinct ecosystem (Shimizu 2010), which was designated as UNESCO world natural heritage in 2011. However, its inherent nature is influenced by the invasion and colonization of exotic species (Ogasawara World Heritage Center 2023). However, research on myxomycetes in the Ogasawara Islands is only limited to Hosoya et al. (2017). Therefore, the comprehensive and ecological features of myxomycetes on the Ogasawara Islands are particularly unclear. This study was performed to conduct a rapid survey of myxomycetes focusing on twig litter and fallen aerial leaves (not touching the soil) in forests to provide an update on the myxomycetes in Ogasawara Island, following the study of Hosoya et al. (2017). The primary goal was to explore the myxomycete communities residing on twig litter of dominant tree species, distinguishing between native and exotic trees. This distinction is crucial as plant invasions can significantly affect litter quality, decomposition, and nutrient cycling within native ecosystems (Mack et al. 2000).

Materials and methods

Survey sites and sampling

The survey sites were two oceanic islands, Chichijima and Hahajima, belonging to the Ogasawara group of islands (Bonin Islands, Fig. 1), located approximately 1000 km away from Tokyo city to the south in a subtropical oceanic climate. Chichijima (27.071346°N, 142.2109771°E) has an area of 24 km²,

320 m above sea level, whereas Hahajima (26.660128°N, 142.155764°E) has an area of 20.2 km², 463 m above sea level. The annual mean temperature in Chichijima is 23.0°C, and the yearly average precipitation is 1276.7 mm (1971–2000). These two islands have a population of more than 2600 (Ogasawara-mura official site, 2023).

The vegetation of the Ogasawara Islands comprises three dominant natural forest types, i.e., (1) dry shrubby forest, (2) humid tall-tree forest, and (3) shore forest, where several different trees dominate and construct the forest canopy. Dry shrubby, in which *Schima wallichii* (DC.) Korthals subsp. *mertensiana* (Sieb. & Zucc.) Bloem. adapted to a dry environment, was predominant in Chichijima, but undesirable alien trees, such as *Casuarina stricta* Ait. affect the natural vegetation. Humid tall-tree forests can reach heights of over 20 m, with *Elaeocarpus photiniifolius* Hook. & Arn. predominantly developed in the cloud belt of a high-altitude mountain in Hahajima, where exotic trees of *Bischofia javanica* Blume influence the growth of conventional plants and affect the forest ecosystem in the Ogasawara Islands (Kanto Forest Administration Bureau, 2023). The expansion of the shore forest comprised deciduous broad-leaved *Terminalia catappa* L. trees and evergreen broad-leaved trees such as *Hernandia nymphaeifolia* (Presl.) Kubitzki. trees and *Calophyllum inophyllum* L. (Fig. 1A), which are distributed at the northern limit of the Ogasawara Islands (Hanaoka et al. 2014). These trees grow in coastal regions and lowland forests and can reach a height of approximately 15 m.

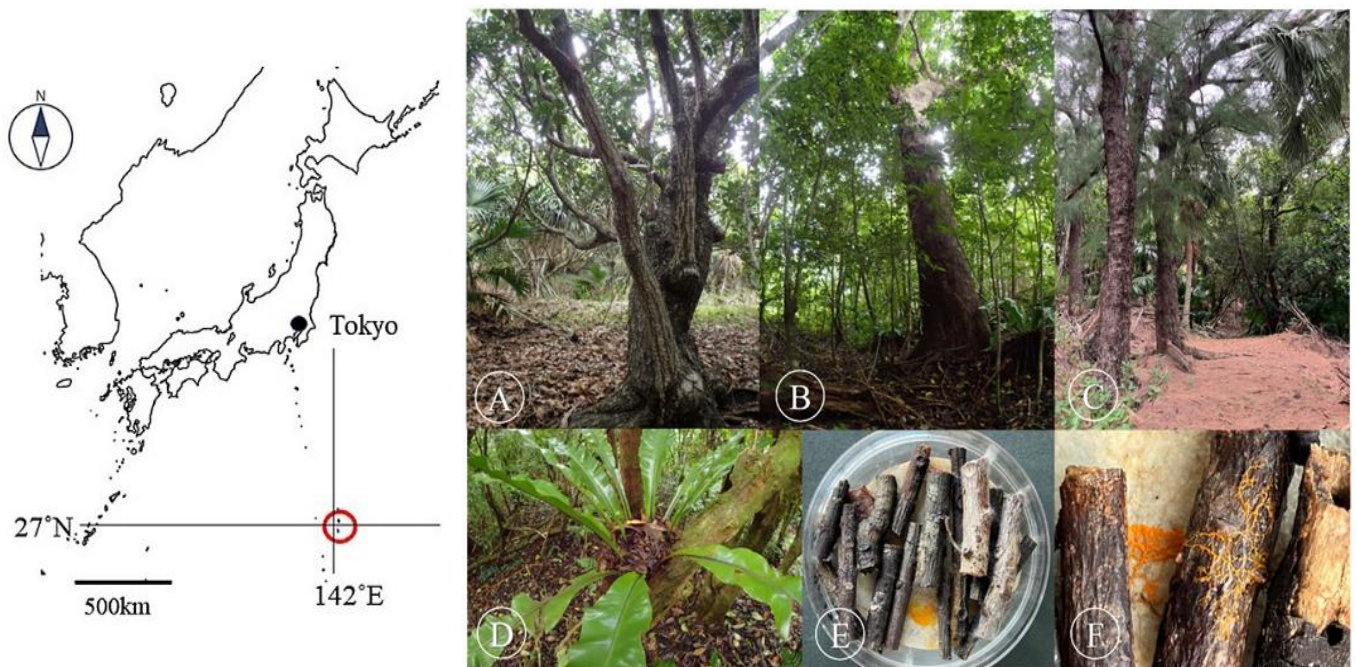


Figure 1. Geographic location of assessed Ogasawara Island (left) and sampled vegetation (right). Native and exotic trees, aerial leaf litter, and moist chamber culture of twigs. native tree A: *Calophyllum inophyllum* and B: exotic tree, *Bischofia javanica*, C: exotic tree *Casuarina equisetifolia*, D: aerial leaf litter on leaves of *Asplenium nidus*, E: moist chamber culture of twigs, F: plasmodium generated from twigs.

The exotic trees have invaded the Ogasawara Islands and are of three types: (1) invasion to native vegetation, such as *Bischofia javanica* (Fig. 1B), having a major impact on the ecosystem; (2) invasion to dry shrub forests with species such as *Pinus luchuensis* Mayr and *Casuarina equisetifolia* L. (Fig. 1C) forming pure wood on steep slopes and exposed rocky areas; and (3) invasion to wind-swept areas and ruins of cleared land flourishing with tree species such as *Leucaena leucocephala* de Wit (Shimizu 1989), although it is difficult for them to invade native forests.

The present study selected five native tree species: *Schima wallichii* subsp. *Mertensiana*, *Elaeocarpus photiniifolius*, *Terminalia catappa*, *Calophyllum inophyllum*, and *Hernandia nymphaeifolia*, as well as exotic tree species: *Bischofia javanica*, *Ficus microcarpa*, *Pinus luchuensis*, and *Casuarina stricta* in the forests of Chichijima and Hahajima (Table 1).

Table 1. Survey tree species and collection location in the Ogasawara Islands. Altitudes of collection points are indicated in parentheses (meters above sea level).

	Tree species	Location	
		Chichijima island	Hahajima island
Native tree	<i>Schima wallichii</i> subsp. <i>mertensiana</i>	Higashidaira (200 m)	
	<i>Elaeocarpus photiniifolius</i>	Asahiyama (190 m)	
	<i>Terminalia catappa</i>		Chichibusayama climbing course (100 m), Motochi (10 m)
	<i>Calophyllum inophyllum</i>	Buta coast (20 m)	Wakihama (10 m)
	<i>Hernandia nymphaeifolia</i>	Kominato coast (10 m)	
Exotic tree	<i>Bischofia javanica</i>		Kuwanokiyama (150 m)
	<i>Ficus microcarpa</i>	Heart lock trekking course (200 m)	Chichibusayama climbing course (200 m)
	<i>Pinus luchuensis</i>		Chichibusayama climbing course (200 m)
Aerial leaf litter	<i>Casuarina equisetifolia</i>	Yoakeyama (270 m)	Chichibusayama climbing course (300 m)

Twigs in forest litter are favorable and preferred habitats for many species that appear as plasmodia and fruiting bodies on the twigs (Stephenson et al. 2008; Takahashi et al. 2022). Myxomycetes inhabiting twig litter are an ecologically defined group in forests that exist as a result of forest litter and soil formation (Takahashi 2011; Takahashi 2015). Fallen twigs of the predominant trees scattered on the soil surface under tall trees of each species were collected. Fresh twigs and those with advanced decay were excluded during sampling. Twigs of each tree species with 5–15 mm diameter and 10–100 cm lengths were randomly selected, placed in paper bags, and transferred to the laboratory. Aerial leaf litter was collected on the leaves of *Asplenium nidus* L., that grew on native large tree trunks in the Hahajima cloud belt forest. The samples were placed in 20 paper bags and transferred to the laboratory.

Moist chamber cultures and species identification

Species on twigs and leaf litter mostly produce minute sporangia (approximately 1 mm in diameter) and ephemeral existence that are virtually impossible to detect under field conditions (Stephenson et al. 2008). Therefore, the moist chamber (MC) technique has proven useful for most survey projects, and specimens obtained from moist chamber cultures have been evaluated in the laboratory using a microscope (Stephenson 2023). Twig samples were air-dried for one week at room temperature (20–25 °C). The MCs were isolated in 20 plastic Petri dishes per tree (9 cm in diameter and 2 cm in height). For culture, small pieces of twigs (2–6 cm) were placed in a Petri dish (Fig. 1E), into which approximately 30 mL of distilled water (pH 6.9) was added and then kept for three days at 23°C. After five days, the pH of the resulting exudate was measured using a compact pH meter (Horiba, Kyoto, Japan), and most of the water was poured off, leaving the MC undisturbed (Stephenson 1989).

After approximately seven weeks, the Petri dishes were maintained with the lid half open to slowly dry twigs and to make fruiting bodies mature in a week. When the fruiting bodies of myxomycetes can be harvested and curated based on their morphological features, after eight weeks, all cultures were successively assessed using a dissecting stereomicroscope (Nikon SMZ1000). The number of cultures with plasmodia or sporangia was marked positive. To calculate the percentage of positive cultures per tree, divide it by the number of culture dishes. Myxomycete species were identified based on morphological traits by observing the external form, internal spores, and the capillitium of sporangia, as described by Yamamoto (2021). As for internal traits of sporangia, microscopic observation was performed by making slides of the fruiting bodies. The nomenclature is based on recent literature (Lado 2005-2023). The number of myxomycete species identified per tree species was recorded, and the abundance of each species was calculated as the number of positive culture dishes in which sporangia appeared. Twigs containing myxomycete fruiting bodies were glued to the bottom of each box, and voucher specimens were prepared using separate paper boxes for each species. The specimens were stored in the Wakayama Prefectural Museum's (WMNH) laboratory.

Data analyses

The estimated number of myxomycete species (*Sest*) for each tree surveyed was calculated using the Chao 1 estimator based on the individual data recorded for each species and the abundance (Chao 1984). PAST software was used for the calculations (Hammer et al. 2001). The number of species observed (*Sobs*) for a tree species was divided by the estimated number of species (*Sest*) using the formula $Sobs / Sest \times 100$ to determine sampling completeness. The Shannon–Wiener diversity index H' (Shannon and Weaver 1963) and the equitability index J' (Pielou 1966) were determined to quantify the myxomycete diversity in each community of tree species or tree types (native or alien), as described in previous studies (Stephenson 1989). The taxonomic diversity index was determined by calculating the ratio of the number of species to the number of genera (S/G ratio) as described previously (Stephenson et al. 1993; Macabago et al. 2020a). The value of this ratio is inversely proportional to taxonomic diversity, with a lower ratio indicating a more diverse biota (Stephenson et al. 1993). The relative abundance of species in a community was calculated by dividing the species abundance by the cumulative abundance of the community for the

tree species. Species with $\geq 10\%$ relative abundance at the whole community level were considered dominant, and those with $\geq 5\%$ abundance were considered frequent species.

The similarity among ten myxomycete communities was determined using non-metric multidimensional scaling (NMDS), which provides an effective analysis of community ordination (Takahashi 2013). The NMDS is based on Bray-Curtis dissimilarities (Doi and Okamura 2011), in which higher values (maximum value of 1.0) indicate greater dissimilarity between communities with respect to species composition and abundance. The PAST software was used because it has been used effectively in a similar study (Takahashi *et al.* 2023). The scores of the first two NMDS axes were then checked for correlations with species diversity and bark pH to identify factors related to community ordination. Correlation analyses were performed using Excel (version 7.0; Esumi Co. Ltd., Tokyo, Japan). Species that appeared unevenly in a given tree type were determined by their relative abundances using an independent *t*-test performed using ESUMI Excel Statistics 5.0 software (ESUMI Co. Ltd., Tokyo, Japan).

Results

Myxomycetes on twig litter

Twig pH varied between 5.0 (*C. stricta* twigs) and 6.8 (*T. catappa* twigs) observed on individual trees (Table 2). A total of 260 MCs of twigs from nine trees yielded 68% positive cultures and 50% formed fruiting bodies (Fig. 2). Out of 100 cultures of aerial leaf litter, sporangia were formed in only 8% of cultures. In total, fruiting colonies observed on individual trees or leaf litter reached 213 colonies (abundance), as shown in Table 2, yielding 36 species belonging to 21 genera. Native trees yielded 30 species from 20 genera, while exotic trees yielded 18 species from 13 genera, and six species appeared on aerial leaf litter. Myxomycete species per tree varied in the range of 6–19 species (supplementary table), and the maximum species were isolated from *C. inophyllum* tree twigs. Chao 1 estimated 45 species in total, indicating an overall 79% completeness of observation in this survey. In the case of each tree, the completeness was in the range of 48–96%. The species diversity of nine twig communities was in the range of 1.51–2.66 Shannon-Wiener diversity index (H'), with a maximum H' on *C. inophyllum* tree twig. The range of the equitability index (J') was 0.84–0.98 (Table 2).

Eight species exhibited abundance of five colonies each, including *Cribraria violacea* Rex (Fig. 2C), *Craterium concinnum* Rex (Fig. 2D), *Physarum lakhanpalii* Nann.-Bremek. & Y. Yamam. (Fig. 2E) and *Willkommlangea reticulata* (Alb. & Schwein.) Kuntze. (Fig. 2F). Twenty two species formed fewer than five colonies, and only one colony was detected for 12 species.

Compared with tree types, native tree twigs exhibited a higher diversity of myxomycetes (30 species of 20 genera, $H' = 2.91$, $J' = 0.85$) than that of alien tree twigs (18 species of 13 genera, $H' = 2.57$, $J' = 0.89$), compared with a hundred MCs or more. There were 14 common species between the native and alien tree types, with a dissimilarity index of 0.389. There were two species that unevenly occurred between those both tree types (i.e., *Craterium concinnum* on native and *Cribraria violacea* on alien trees, respectively). A comparison of the myxomycete communities between the two islands, Chichijima (27 species) and Hahajima (21 species) revealed 14 common species, and the Bray-Curtis dissimilarity index was 0.409.

Table 2. Number of moist chamber cultures, twig pH, and species diversity of myxomycetes on native trees, exotic trees, and aerial leaf litter in the Ogasawara Islands. NoC = number of cultures, NoS = number of species, Abund = abundance, Comp = completeness, SD = species diversity, EQ = equitability.

Tree	NoC	pH	NoS	Abund	Chao-1	Comp (%)	SD (H')	EQ (J')
Native tree								
<i>E. photiniifolius</i>	20	5.5	6	18	9.0	66.67	1.51	0.84
<i>S. wallichii</i> subsp. <i>mertensiana</i>	40	5.3	9	24	9.8	92.31	1.94	0.88
<i>C. inophyllum</i>	40	5.2	19	43	26.2	72.52	2.66	0.90
<i>T. catappa</i>	40	6.8	10	29	13.0	76.92	2.07	0.90
<i>H. nymphaeifolia</i>	20	5.8	7	12	10.0	70.00	1.82	0.94
Exotic tree								
<i>B. javanica</i>	20	6.7	6	11	9.0	66.67	1.54	0.86
<i>F. microcarpa</i>	40	6.6	11	34	16.0	68.75	2.14	0.89
<i>P. luchuensis</i>	20	5.2	9	24	9.3	96.43	2.08	0.95
<i>C. equisetifolia</i>	20	4.9	7	8	14.5	48.28	1.91	0.98
Total of native trees	160	–	30	126	34.5	86.96	2.91	0.85
Total of exotic trees	100	–	18	77	23.0	78.26	2.57	0.89
Aerial leaf litter	100	6.3	6	10	9.0	66.67	1.61	0.90
Total	360	–	36	213	45.4	79.24	2.94	0.82

The 36 myxomycete species are arranged in descending order of their relative abundance in the supplementary table. The most abundant dominant species was *Arcyria cinerea* (Bull.) Pers. (17.4%), followed by *Stemonitis fusca* var. *rufescens* Lister (11.7%), and *Lamproderma scintillans* (Berk. & Broome) Morgan (11.3%, Fig. 2A). The following three species were recorded in ten or more colonies: *Ophiotheca pedata* (Lister & G. Lister) Garcia-Cunch., J. C. Zamora & Lado (8.9%, Fig. 2B), *Diderma chondrioderma* (de Bary & Rostaf.) G. Lister (6.6%), and *Ophiotheca chrysosperma* Curr. (5.6%).

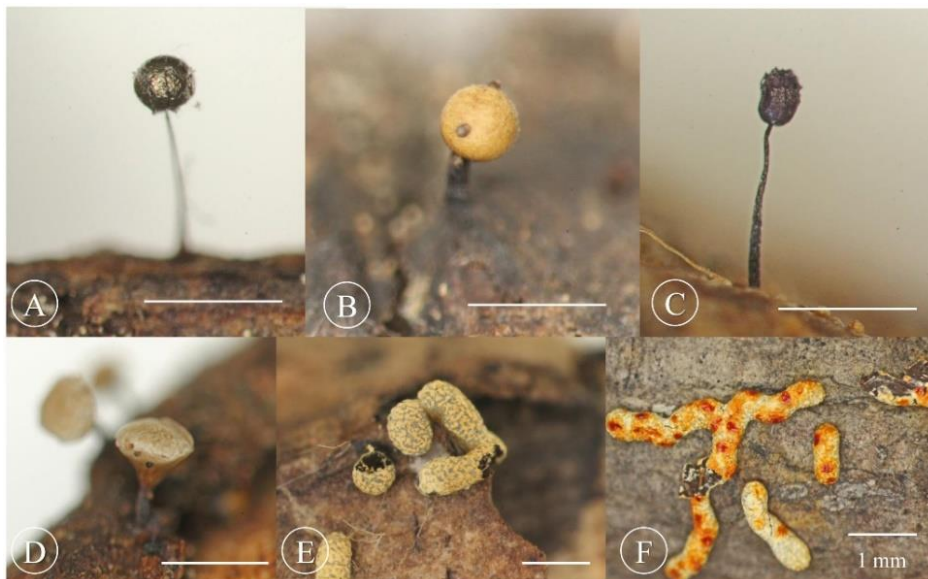


Figure 2. Myxomycete fruiting bodies. A: *Lamproderma scintillans*, B: *Ophiotheca pedata*, C: *Cribraria violacea*, D: *Craterium concinnum*, E: *Physaraum lakhanpalii*, F: *Willkommllangea reticulata*.

Similarity of myxomycete communities

Ten myxomycete communities were ordinated and plotted on the first two axes using non-metric multidimensional scaling (Fig. 3). The twig communities were located at lower scores, away from the aerial leaf litter, in the positive direction of the first axis. The native and exotic trees exhibited mixed arrangements among the twig communities, and no differences were observed between them, implying that there was no apparent relationship between the native and exotic tree myxomycete communities. The correlation analysis between the first two NMDS scores, myxomycete diversity, and twig pH is shown in Table 3. The first axis scores were inversely correlated with the number of colonies ($r = -0.785$, $p < 0.01$), whereas the second axis was positively correlated with twig pH ($r = 0.668$, $p < 0.05$). The myxomycete communities responded to the twig pH, which influenced the community structure.

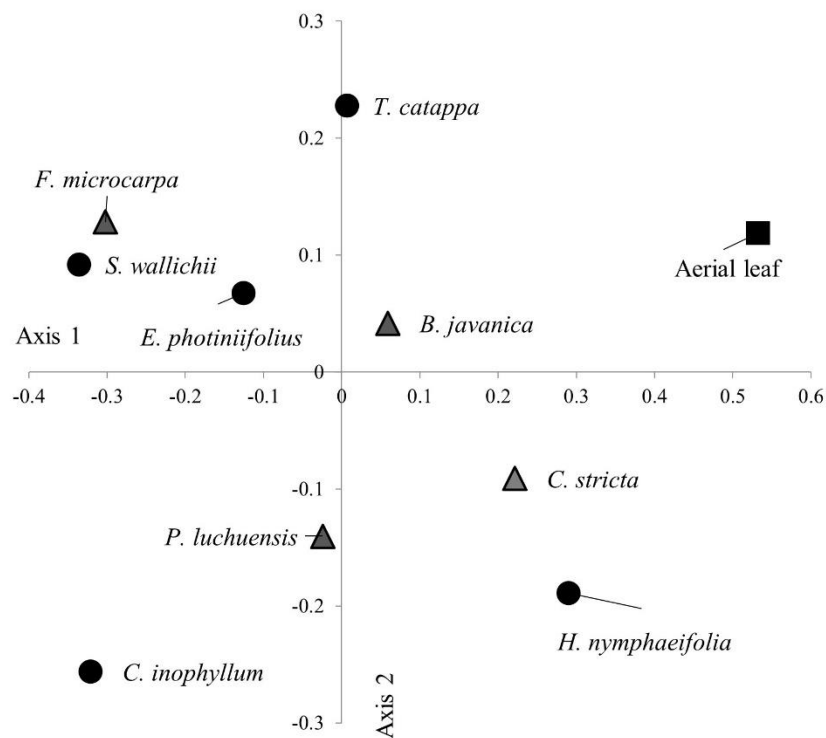


Figure 3. Similarity plots of the ten myxomycete communities analyzed using non-metric multidimensional scaling (NMDS). ●: Native trees, ▲: Exotic trees, ■: Aerial leaf litter. Stress value: 0.228, Determination coefficient R^2 axis1 $r^2 = 0.533$, axis 2 $r^2 = 0.050$. The data are shown in Table 2.

Discussion

The myxomycetes in the Ogasawara Islands are reportedly 59 species belonging to 21 genera, according to field surveys (Hosoya et al. 2017). Here, a survey focusing on twig litter using the MC method extended the microhabitat of myxomycetes and led to the discovery of new species. Common species from both studies were only eight because of differences in the substrate types surveyed and the survey method. Twenty-one species in the present study were subsequently updated in the Ogasawara Islands, resulting in the confirmation of 80 species in the Ogasawara Islands.

Table 3. Correlation coefficients between first two non-metric multidimensional scaling (NMDS) scores and species diversity of myxomycete communities and twig pH. Significance, ** $p < 0.01$, * $p < 0.05$.

	Axis 1	Axis 2
Number of species	-0.61	-0.39
Number of colonies	-0.78*	-0.08
Species diversity (H')	-0.54	-0.43
Equitability (J')	0.35	-0.52
Twig pH	0.17	0.67*

Species of *A. cinerea* commonly occur on various substrates (Pucundo et al. 2023), as well as litter twigs. This species appeared most dominantly, with a relative abundance of 17.4%. In the case of the myxomycete community on the twigs of Yakushima Island, Japan, the occurrence rate of *A. cinerea* was 23.4% (Takahashi et al. 2023). A survey in Virginia, USA, reported its abundance to be 35% on the twig litter (Cedeño et al. 2014). It is common for *A. cinerea* to predominantly inhabit twig litter. The characteristic species in Ogasawara was *Ophiotheca pedata* with a relative abundance of 8.9%, and preferred the twig litter of *Ficus microcarpa* as substrate instead of a rare species in Kanagawa Prefecture, Japan, owing to its rarity (Yano et al. 2016). Furthermore, the species of *W. reticulata* is considered specific to twig litter (Stephenson et al. 2008) and is distributed globally, but this species is rare despite its distribution in Honshu, Shikoku, and Ryukyu in Japan (Yamamoto 2021). It has not been observed on twigs in Yakushima (Takahashi et al. 2023) or western Japan (Takahashi et al. 2022). *W. reticulata*, however, frequently appeared on the twigs of native tree species on the Ogasawara Islands, with a relative abundance of 2.3%. Based on these findings, the myxomycetes on the Ogasawara Islands have been characterized, including rare species, such as *O. pedata* and *W. reticulata* on twig litter.

The distinctiveness of the myxomycete community is clearly affected by differences in climate zones and major ecosystems based on geographical location (Schnittler et al. 2022). A comparison of the myxomycete communities recorded on Christmas Island (68 myxomycete species) in the tropics with those recorded on Macquarie Island (26 species) in the sub-Antarctic showed that these two islands shared only seven species in common (Stephenson 2020). In the case of the Ogasawara and the Yakushima (Takahashi et al. 2023) found only 19 common species on twig litter, and the Bray-Curtis dissimilarity between them was 0.550, while the dissimilarity between Ogasawara and western Japan forests on the mainland (Takahashi et al. 2022) was 0.618. When comparing Chichijima and Hahajima on the Ogasawara Islands, the dissimilarity between these communities was 0.409. The higher dissimilarity between Ogasawara and the other two locations existed so that the biogeographical distance between the continental and oceanic islands, or between both islands (approximately 1200 km in straight-line distance) affects the distinctiveness of the community structure. The Ogasawara lacks the Japanese chinquapins, oaks, and cedars that dominate the forests of the continental island of Yakushima and the southwestern part of mainland Japan. The Yakushima and the southwestern Japan forests, which are similar in the evergreen vegetation in warm-temperate Japan, have a lower dissimilarity index of 0.429. The specific climate and vegetation in other areas suggest the distinctiveness of the community structure in Ogasawara.

Although the biogeography of myxomycetes is seriously understudied, the distribution of myxomycetes has been investigated on several islands in the Philippines (Macabago et al. 2020a, b). The study of four islands in the Caramoan Islands of the Philippines, which are geographically and ecologically distant, reported a total of 38 taxa belonging to 14 genera on ground leaf litter and twigs, using the MC

method, and their taxonomic diversity index (S/G ratio) was 2.7 (Macabago *et al.* 2020a). In the Japanese archipelago, the myxomycete community assessed on twigs in the continental island of Yakushima revealed 35 species of 15 genera, S/G ratio = 2.3, species diversity $H' = 2.74$, $J' = 0.770$, which were identified from 380 MCs of twig litter from seven tree species (Takahashi *et al.* 2023). In southwestern Japan, twig litter from ten tree species furnished 42 myxomycete species belonging to 19 genera, S/G ratio = 2.2, species diversity $H' = 2.73$, $J' = 0.730$, from 640 MCs. The present study identified 36 species belonging to 21 genera with S/G ratio = 1.7, species diversity $H' = 2.91$, $J' = 0.826$, from 360 MCs. The twig myxomycete community had comparatively higher diversity on the Ogasawara Islands than in the other study areas. The twig litter of *C. inophyllum* (pH = 5.2), which is distributed in tropical and subtropical coastal areas and the vulnerable north end in Ogasawara (Hanaoka *et al.* 2014), exhibit highest species diversity of myxomycetes (19 species, $H' = 2.66$, $J' = 0.90$) among the nine tree species on the island.

In the oceanic Hawaiian Islands, 102 species have been listed (Eliasson 2004), but their biogeographical characteristics are not clear, as many of the species are commonly distributed in temperate and tropical regions. Interestingly, two studies on myxomycetes recorded from the Galápagos Islands in the subtropics (Eliasson 1971) and Madagascar Island in the tropics (Wrigley de Basanta *et al.* 2013) revealed the presence of new species under the harsh environmental conditions that endemically exist on the two islands and are a major limiting factor for myxomycetes related to speciated plant vegetation. On the Galápagos Islands, 65 species, including two new species (*Diderma rimosum* U. Elisss. and Nann.-Brem., *Diderma scabra* U. Elisss. & Nann.-Brem.), have been reported in the forests of *Asteraceae* *Scalasia* (Eliasson and Nannenga-Bremekamp 1983), suggesting that several myxomycete species have adapted to special ecological niches. On the island of Madagascar, 124 species belonging to 22 genera with unique species compositions have been reported. A new species, *Perichaena madagascariensis* D. Wrigley, Lado, Estrada, & S.L. Stephenson, has been reported to have a strong relationship with the ecological distribution of substrates and myxomycete species (Wrigley de Basanta *et al.* 2013). These facts indicate that islands have ecologically unique vegetation and environments, and specific myxomycetes are expected to adapt to particular environments. Further surveys will yield new information on myxomycetes on the Ogasawara Islands.

According to the island biogeography theory (MacArthur and Wilson 1963), an island's size and isolation from other land masses impact its biodiversity and number of species. Larger, less-isolated islands had more species, whereas smaller, more-isolated islands had fewer species. The twig myxomycete community in Ogasawara, isolated far from continents in the Pacific Ocean, had higher species diversity, which was dissimilar from the less isolated island communities and landmasses. The island biogeography theory does not necessarily apply to the distribution of myxomycetes. It has been suggested that myxomycete spores are dispersed globally by air and form fruiting bodies in environments with favorable biogeographical features.

Forest vegetation types significantly affects the community composition and diversity of soil-inhabiting myxomycetes (Gao *et al.* 2019) and litter-inhabiting myxomycetes (Takahashi 2013; Takahashi 2015). On the Ogasawara Islands, the invasion of exotic species has affected the indigenous natural vegetation (Shimizu and Tabata 1985; Sugiura *et al.* 2013). Exotic trees such as *C. equisetifolia* (Fig.1C) and *P. luchuensis* have invaded and colonized coastal wind-swept areas and dwarf shrub forests, creating leaf litter layers on the forest floor that are difficult to decompose inhibiting the growth of other plants

(Forestry Agency (maff.go.jp, accessed 2023. 4. 10). Myxomycete communities on these exotic trees were less diverse (18 species, $H' = 2.57$) compared to native trees (30 species, $H' = 2.91$), with a dissimilarity index of 0.389. The pH levels of leaf litter from these exotic trees were lower (*C. equisetifolia* pH = 5.0, and *P. luchuensis* pH = 5.2) than those from native species (Table 2), affecting myxomycete populations as many species prefer neutral to slightly alkaline conditions (Everhart et al. 2008).

The spread of exotic tree species often leads to the formation of monocultures, as observed with *P. luchuensis* and *C. equisetifolia* (Shimizu and Tabata 1985; Sugiura et al. 2013), and the invasive *Bischofia javanica*, which releases chemicals inhibiting the growth of the native *Elaeocarpus photiniifolius* (National Institute for Environmental Studies 2023). While exotic trees and plants alter decomposition dynamics and nutrient cycling in ecosystems like Hawaiian montane forests (Rithstein et al. 2004; Funk 2005) and Ogasawara (Hata and Kachi 2012), their direct effect on myxomycetes remains uncertain. Nonetheless, the decreased diversity of myxomycetes on exotic trees contrasts with the higher diversity found on native tree species, underscoring the impact of exotic proliferation.

Myxomycetes feed on decomposing microorganisms such as bacteria (Smith and Stephenson 2007), making them important members of forest ecosystems. They are strongly associated with humus formation from twig litter and fallen leaves. However, research on myxomycete communities has been limited to twig litter. Since a humic ecosystem constitutes the foundation of forest vegetation, the myxomycete characteristics of litter are important for understanding forest ecosystems, and future studies on their distribution are necessary. Myxomycetes have the unique feature of forming macroscopic sporangia among all protists, an ecological feature of the myxomycete that makes it an appealing model for investigations of the humus microbiome.

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