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Assessment of myxomycete species diversity on twig litter on Yakushima Island, World Natural Heritage site, Japan

Kazunari Takahashi[1], Akira Tominaga[2] and Koutaro Minami[3]

[1] Member of the Japanese Society of Myxomycetology, Rokujoin-naka, Kamogata-chyo, Asakuchi City, Okayama 719-0252, Japan
[2] Tsushima Fukui, Kita-ku, Okayama City, Okayama 700-0080, Japan.
[3] Graduate school of Integrated Arts and Sciences, Kochi University, 2-5-1 Akebono-cho, Kochi City, Kochi, 780-8520, Japan.

Email: kumakusu03@yahoo.co.jp

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Abstract: Our knowledge of the ecological features of myxomycetes of the Yakushima Island World Natural Heritage site is poor. The present study assessed myxomycetes living on twig litter in Yakushima Island using the moist chamber culture method and focused on their distribution and association with different forest types. The survey was conducted along an altitudinal gradient from the coast to the montane forest (1360 m). Fallen twigs were sampled from the forest floor surface beneath representative dominant tree species at each survey site. Nineteen twig samples from seven tree species were cultured in dishes for a total of 380 cultures, of which 54% culture dishes yielded myxomycete fruiting colonies. In total, 35 myxomycete species in 15 genera were identified from 19 myxomycete communities. Ordination using non-metric multidimensional scaling indicated a significant correlation between community structure and twig pH. Four different forest types classified according to elevation separated into different myxomycete community structures, with species richness and abundance decreasing with increasing elevation. Species diversity was the highest in the coastal subtropical vegetation community, while the montane forest that comprised *Cryptomeria japonica* trees had lower species diversity. Subtropical vegetation characteristically involved four species, *Comatricha tenerrima*, *Lamproderma scintillans*, *Perichaena depressa*, and *Ophiotheca chrysosperma*, but *Cribraria microcarpa* preferentially occurred in the montane forests. The dominant trees in the forests apparently affected the myxomycete communities on twig litter. The new distribution data generated through this study provide a better understanding of the spatial composition of myxomycetes on Yakushima Island.

Keywords: community ecology, elevation gradient, forest ecology, tree species, pH

Introduction

Myxomycetes are protists that nourish at the amoeba and plasmodium stages, and then form fruiting bodies. Their spores are dispersed in a similar way to those of fungi. They mainly live on coarse woody debris, fallen twigs, leaf litter, and living tree bark in forests. Their nutrition is mainly sourced from substrate-dwelling bacteria and fungi or organic matter (Smith and Stephenson 2007; Fukasawa et al. 2018), which is related to material cycling in forests (Urich et al. 2008), thus maintaining a stable cycle and balance in terrestrial ecosystems (Gessner et al. 2010).

The decomposition rate of plant debris varies between dead trees, fallen twigs, and leaf litter (Yoneda 2000), and accordingly, they provide different niches for myxomycetes (Rollins and Stephenson 2012; Cedeño et al. 2014). Fallen twigs are ecologically considered as a distinctive substrate for myxomycetes in forests (Stephenson 1989; Stephenson et al. 2008). The falling of twigs occurs suddenly because of strong winds and irregular death of tree branches, and its decomposition takes several years (Guo et al. 2007), depending on the tree species (Matsumoto and Niijima 1993). However, ecological studies on myxomycetes inhabiting twig litter have not been extensively conducted.

The fruiting bodies of most myxomycete species are rather sporadic in their occurrence and are minute and fragile. Moreover, they are also relatively ephemeral because they also represent a source of nutrition for soil fauna (Dudka and Romanenko 2006; Kataoka et al. 2020). As they do not persist in nature for very long, it is not easy to detect them in the field and accumulate data. We assessed the myxomycetes on Yakushima Island in the present study using the moist chamber culture method, which can be used for producing myxomycete fruiting bodies on twig litter collected from the forest floor and allows for microscopic observations in a laboratory (Stephenson 1989).

Takahashi et al. (2022) found many myxomycetes on the twigs of 10 tree species in the forests of West Japan using moist chambers. Yakushima Island, which is a World Natural Heritage site, is also called the Ocean Alps, and both vertically and geographically comprise different forest vegetation types. The forests harbor diverse vegetation, from subtropical vegetation to cold temperate forest, likely in the subarctic zone (Ministry of the Environment 2022). Although Yakushima has diverse forest and rich ecosystems, knowledge of its myxomycetes is limited with only 29 known species within 17 genera from field studies and moist chamber cultures made from bark of living trees and dead leaves in a previous study (Matsumoto and Hagiwara 2007). In the present study, we investigated myxomycetes living on the twigs of dominant trees that constituted a forest crown in various forest vegetation types, to clarify the diversity and distribution patterns of myxomycetes on the island.

Materials and methods

Survey sites in forests

Yakushima Island has an essentially round shape and covers an area of 503 km^2 that includes the Miyanouradake peak (1936 m). The forest vegetation is roughly divided into five types, corresponding to an increase in altitude. These include subtropical forests in the coastal parts, which characteristically harbor the dominant trees *Ficus subpisocarpa* Gagnep. and *F. macrocarpa* L. f. The area up to approximately 500 m altitude comprises warm temperate forest dominated by *Castanopsis sieboldii* (Makino) Hatus. ex T.Yamaz. et Mashiba subsp. *sieboldii* and *Quercus salicina* Blume. Mixed forests are distributed in the range between 500 and 1200 m and consist of *Q. salicina*, *Distylium racemosum* Siebold et Zucc., and *Cryptomeria japonica* (Thunb. ex L.f.) D.Don. trees. The cold temperate forests are located in the 1000 – 1600 m range, where protected natural forests develop and are dominated by *C. japonica* trees, coexisting with *Abies firma* Siebold et Zucc. or *Tsuga sieboldii* Carrière and *Trochodendron aralioides* Siebold et Zucc. Bamboo grass areas are located at altitudes higher than 1600 m (Kyushu Forest Administration Bureau 2022a).

According to the distribution of the different forest types by elevation, survey points were selected in ten sites (Fig. 1) that were easy to access and retained natural vegetation along the Yakusugi-land line (Route 429). The sites are listed in Table 1, indicating the altitude and tree species of which twigs were sampled. Survey sites were separated into the following four groups according to the vegetation type: sites in subtropical zone, 75 m and 110 m; lowland evergreen zone, 210 m, 300 m and 400 m; upland evergreen, 650 m and 840 m; conifer zone at higher altitudes, 1020 m, 1200 m, and 1360 m.

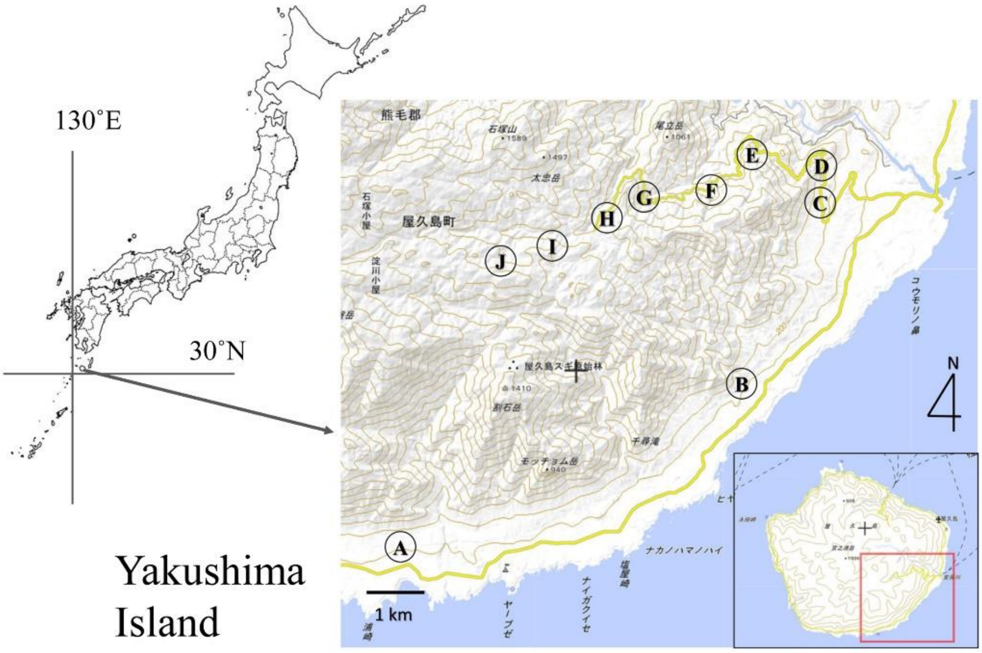

Figure 1. Sampling sites. A) Hirauchi-Hatiman Shrine (75–110 m), B) Woods of *Ficus microcarpa* in Sarukawa (100–110 m), C) Forest site along Yakusugiland-line (210 m), D) Forest site along Yakusugiland-line (300 m), E) Forest site along Yakusugiland-line (400 m), F) Forest site along Yakusugiland-line (650 m), G) Forest site along Yakusugiland-line (840 m), H) Forest site neighborhood of Yakusugi-land (1020 m), I) Forest site in the neighborhood of Kigensugi (1240 m), and J) Forest site at Yodogawa starting point of a mountain climb (1360 m). The Geographical Survey Institute map used herein was edited; from https://maps.gsi.go.jp.

For each dominant tree species, we sampled twigs from the forest floor in the different vegetation types at all survey sites and collected them in paper bags (A4 size). Nineteen samples were used to prepare moist chamber cultures from seven tree species, two subtropical trees, three evergreen broad-leaved trees, and two conifers (Table 1). *C. japonica* trees grow naturally in mountainous areas but were planted in lowlands. Therefore, *C. japonica* trees were sampled at all the survey sites.

Yakushima has a mean annual precipitation of 4652 mm and a mean annual air temperature of 19.6 °C (1991–2020, at the observatory of the Meteorological Agency). Records for the annual precipitation were 10499 mm (2015) at Yakusugi-land (1000 m), and 10519 mm (2017) at Yodogawa, the starting point of the mountain climb (1380 m), according to the Kyushu Forest Administration Bureau 2022b).

Table 1. Survey site information on vegetation type, elevation, tree species sampled, number of cultures.

Vegetation	Elevation (m)	Tree species	Site code	Number of cultures
Coastal subtropical				
	75	*Ficus subpisocarpa*	A75	20
	100	*Cryptomeria japonica*	S100	20
	110	*Ficus microcarpa*	G110	20
	110	*Ficus subpisocarpa*	A110	20
Lowland evergreen				
	210	*Cryptomeria japonica*	S210	20
	210	*Castanopsis sieboldii*	B210	20
	300	*Castanopsis sieboldii*	B300	20
	400	*Cryptomeria japonica*	S400	20
	400	*Castanopsis sieboldii*	B400	20
Upland evergreen				
	635	*Quercus salicina*	B650	20
	650	*Cryptomeria japonica*	S650	20
	840	*Cryptomeria japonica*	S840	20
	840	*Quercus salicina*	B840	20
Mountain conifer				
	1020	*Cryptomeria japonica*	S1020	20
	1240	*Cryptomeria japonica*	S1240	20
	1240	*Abies firma*	M1240	20
	1360	*Cryptomeria japonica*	S1360	20
	1360	*Abies firma*	M1360	20
	1360	*Trochodendron aralioides*	B1360	20
			Total	380

Myxomycete cultures

The moist chamber culture technique has been previously described as useful for observing the small and ephemeral fruiting bodies of myxomycetes on twigs (Stephenson et al. 2008). Twig samples of the predominant trees were air-dried for one week at room temperature (20–25 °C). Moist chamber cultures (Fig. 2A) were then established in 20 plastic Petri dishes (9 cm in diameter and 2 cm in height) per tree at a site. Twig samples were cut into 3–8 cm long pieces, and approximately 10 g of twigs (dry weight) were placed on clean filter paper in each Petri dish. Each dish was then filled with approximately 30 mL of distilled water (pH 6.9), covered, and soaked for four days at 23 °C.

Next, water was slowly poured out from the dishes, and the dishes were incubated in humid conditions for 50–60 days at 23°C to stimulate sporulation. The cultures were then maintained with their lid half open to slowly dry twig samples. The fruiting bodies that sporulated incrementally in moist chamber cultures were assessed after 50–70 days using a dissecting stereomicroscope (Nikon SMZ1000). The percentage of positive cultures (i.e., those with plasmodia or sporangia) was calculated by dividing the series of culture dishes. The number of myxomycete species identified per tree species was recorded at a survey site and accumulated per forest. The abundance of each species was estimated as the number of positive culture dishes in which sporangia appeared.

Myxomycete species classification was performed as described by Yamamoto (2021), based on microscopic observation of the external form or internal spores and the capillitium of sporangia. The nomenclature is based on the most recent literature (Lado 2005–2022) except for *Stemonitis nigrescens*. Voucher specimens were prepared using separate paper boxes for each species. The twig containing the myxomycete fruiting bodies was glued to the bottom of each box, and the collection boxes were stored in the laboratory.

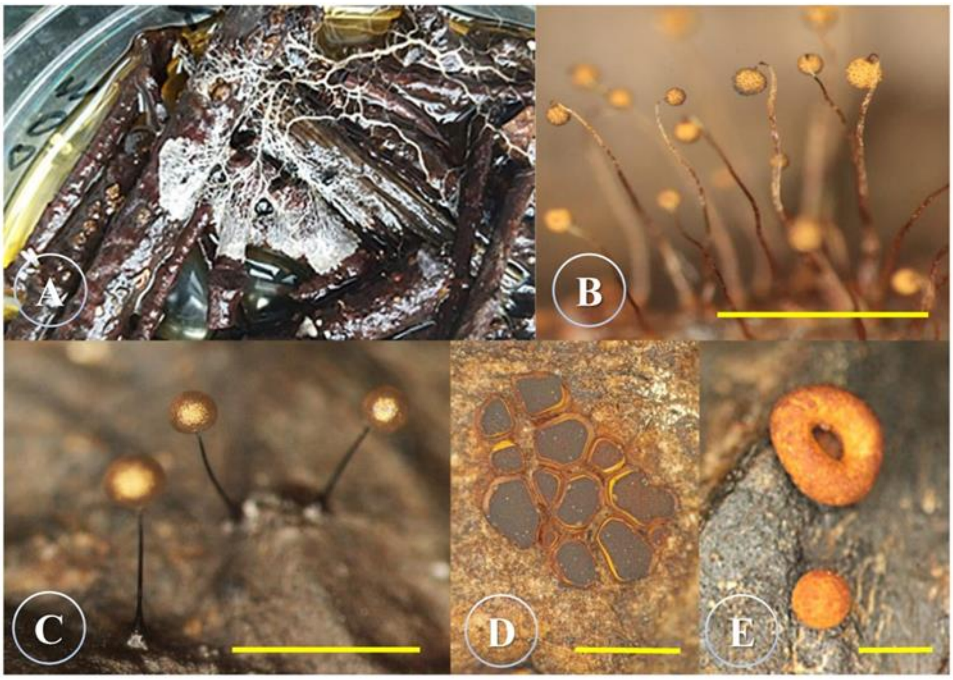

Figure 2. Moist chamber culture of twigs and myxomycete plasmodia, and fruiting bodies that appeared. A) Plasmodium on twigs, B) Fruiting bodies of *Cribraria microcarpa*, C) Fruiting bodies of *Lamproderma scintillans*, D) Fruiting bodies of *Perichaena depressa*, E) Fruiting bodies of *Ophiotheca chrysosperma*. Scale bars were 1 mm.

Data analyses

Species richness and abundance were recorded for each sample tree and summed according to the survey site. Sampling completeness was assessed by dividing the number of taxa observed (S_{obs}) within a tree species by the estimated number of taxa (S_{est}) (Chao 1, Chao 1984) using PAST software (Hammer *et al.* 2001), using the formula, $S_{obs} / S_{est} \times 100$.

To quantify the myxomycete α diversity in each forest, the Shannon–Wiener diversity index *H′* (Shannon and Weaver 1963) and the equitability index *J′* (Pielou 1966) were determined for tree species or vegetation types, as described in previous studies (Stephenson 1989). Species relative abundance in a community was calculated by dividing a given species abundance by the cumulative abundance of the community for that forest and was similarly calculated for the total of all communities. We defined dominant species as those with ≥ 10% relative abundance at the total community level, and frequent species as those with ≥ 1.8% relative abundance (five abundances).

The similarity of myxomycete communities was analyzed using non-metric multidimensional scaling (NMDS; Kenkel and Orlóci 1986) using the PAST software. This software has been used effectively in a similar study (Takahashi et al. 2018). 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 scores of the first two NMDS axes were then checked for correlations with biogeographical and abiotic variables, including elevation and bark pH, to identify the influential factors. Correlation analysis was performed using Excel Statistics (version 7.0; Esumi Co., Ltd, Tokyo, Japan) to estimate the association between myxomycete communities and biogeographical trends. Cluster analysis (Ward’s method) was performed to group the myxomycete communities based on NMDS scores.

Species that characteristically appeared at a given vegetation 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

Myxomycete communities on twigs

From 19 samples of twigs obtained from seven tree species, a total of 380 cultures were incubated, of which 245 cultures (64% of total cultures) contained plasmodia (Fig. 2A) and 205 cultures contained fruited sporangia (54% of total cultures, Table 2). A series of 20 cultures per tree sample comprised an average of 5.9 myxomycete species with a range of 2–11 species and overall accumulated 35 species belonging to five genera from 291 fruiting colonies (Table 3). The number of species estimated by Chao-1 was 43 taxa, which indicated an overall 81% sample completeness in this survey, with a range of 50–100% for each community overall (Table 2).

The myxomycete species listed in Table 3 are shown in descending order of their relative abundance. *Arcyria cinerea* (Bull.) Pers. (23.4 %) was the most abundant, followed by *Clastoderma debaryanum* Blytt (13.7 %), *Diderma chondrioderma* (de Bary & Rostaf.) G. Lister (11.7 %), and *Cribraria microcarpa* (Schrad.) Pers. (6.9 %). Five additional species were recorded with five or more colonies: *Comatricha tenerrima* (M. A. Curtis), G. Lister (6.5%), *Lamproderma scintillans* (Berk. & Br.) Morgan (4.1%), *Perichaena depressa* Libert (3.8%), *Physarum psittacinum* Ditmar (3.8%), and *Ophiotheca chrysosperma* Curr. (3.4%). A further 26 taxa each had relative abundances of less than 3%.

The myxomycete communities of 19 samples were ordinated using non-metric multidimensional scaling and plotted on the first two axes (Fig. 3). Communities of *Cryptomeria japonica* twigs were mainly arranged on the lower value sides of both the first and second axes, with an average number of species in the community of 5.0, while subtropical trees were separately arranged far from them, of which the average number of species in a community was 9.0. Evergreen trees were situated mostly on the positive side of the second axis and on the negative side of the first axis, with an average number of species in a community of 6.3. Myxomycete communities tended to form three groups associated with tree types.

The first axis scores were significantly positively correlated with twig pH ($r = 0.625$, $p < 0.01$, Table 4), and the second axis had a significantly positive correlation with species richness ($r = 0.736$, $p < 0.01$). The myxomycete communities responded to pH, which increased with increasing species richness. Both species richness ($r = -0.589$, $p = 0.008$) and species diversity ($r = -0.547$, $p = 0.016$) of 19 myxomycete communities were correlated with elevation gradient, whereas there was no apparent correlation with twig pH ($r = -0.457$, $p = 0.053$). The species diversity of ten communities on a uniform substrate of *C. japonica* twigs tended to decrease as increasing elevation grade ($r = -0.601$, $p = 0.126$).

Table 2. Twig pH and myxomycetes occurrences: positive cultures, observed number of species, estimated number of species by Chao-1 and survey completeness of the communities on the distinctive trees in survey sites. Site codes were shown in Table1 according to elevation grade.

Site code	Twig pH	Positive cultures (%)	Observed species	Estimated species	Completeness (%)
Coastal subtropical					
A75	7.3	90	11	26	42
S100	5.3	65	8	11	73
G110	6.8	40	7	8	90
A110	7.6	90	9	19	47
Lowland evergreen					
S210	5.8	40	6	8	75
B210	4.7	60	6	12	50
B300	4.5	80	6	6	100
S400	4.5	25	4	6	73
B400	4.0	80	8	14	57
Upland evergreen					
B650	3.9	50	9	17	55
S650	4.7	40	4	4	100
S840	5.0	40	4	4	100
B840	5.0	55	3	4	75
Mountain conifer					
S1020	4.6	50	5	6	91
S1240	4.7	30	3	3	100
M1240	4.7	35	6	6	97
S1360	5.6	45	6	9	67
M1360	4.4	40	2	2	100
B1360	4.8	70	6	8	80
Mean value or Total	5.2	54	35	43	81

Table 3. Myxomycete taxa and abundances on twig litter at different vegetation types along an elevation gradient on Yakushima Island. Italics indicate relative abundance in total. Species are arranged in the order of relative abundance. ** Characteristic species in the vegetation types using an independent T-test for three or more occurrences, significance, $p < 0.01$

Elevation range (m)	75–110	210–400	650–840	1020–1360		
Taxa	Coastal subtropical	Lowland evergreen	Upland evergreen	Mountain conifer	Total	Relative abundance (%)
Arcyria cinerea (Bull.) Pers.	10	30**	11	17	68	23.4
Clastoderma debaryanum Blytt	1	26**	1	12	40	13.7
Diderma chondrioderma (de Bary & Rostaf.) G. Lister	3	7	13**	11	34	11.7
Cribraria microcarpa (Schrad.) Pers.	4	2	4	10**	20	6.9
Comatricha tenerrima (M. A. Curtis) G. Lister	18**	1			19	6.5
Lamproderma scintillans (Berk. & Br.) Morgan	10**	2			12	4.1
Perichaena depressa Libert	10**			1	11	3.8
Physarum psittacinum Ditmar	2	6	3		11	3.8
Ophiotheca chrysosperma Curr.	9**			1	10	3.4
Physarum roseum Berk. & Br.		3	5**		8	2.7
Stemonitis nigrescens Rex	2	5			7	2.4
Arcyria insignis Kalchbr. & Cooke	3	1	2		6	2.1
Comatricha pulchella (C. Bab.) Rostaf.	5**			1	6	2.1
Physarum album (Bull.) Chevall.		1	2	1	4	1.4
Cribraria violacea Rex	1	2			3	1.0
Hemitrichia minor G. Lister				3**	3	1.0
Physarum sulphureum Alb. & Schw.	3**				3	1.0
Stemonitopsis reticulata (H. C. Gilbert) Nann.-Bremek. & Y. Yamam.				3**	3	1.0
Comatricha laxa Rostaf.		1	1		2	0.7
Hemitrichia intorta (Lister) Lister			2		2	0.7
Gulielmina vermicularis Schwein.) García-Cunch., J.C. Zamora & Lado	2				2	0.7
Stemonaria gracilis Nann.-Bremek. & Y. Yamam.	1		1		2	0.7
Stemonitis follicola Ing				2	2	0.7
Trichia erecta Rex	2				2	0.7
Ceratiomyxa fruticulosa (Mueller) T. Macbr.		1			1	0.3
Diderma hemisphaericum (Bull.) Hornem.	1				1	0.3
Diderma saundersii (Berk. & Broome ex Masse) Lado		1			1	0.3
Echinostelium minutum de Bary		1			1	0.3
Hemitrichia serpula (Scop.) Rostaf.	1				1	0.3
Physarum megalosporum T. Macbr.	1				1	0.3
Physarum rigidum (G. Lister) G. Lister			1		1	0.3
Physarum viride (Bull.) Pers.	1				1	0.3
Stemonaria laxa Nann.-Bremek. & Y. Yamam.				1	1	0.3
Stemonitis virginiensis Rex				1	1	0.3
Stemonitopsis curiosa Nann.-Bremek. & Y. Yamam.		1			1	0.3
Abundance	90	91	46	64	291	100
Species richness	21	17	12	13	35	35
Shannon *H*´	2.61	2.02	2.07	2.04	2.74	
Equitability *J*´	0.86	0.71	0.83	0.80	0.77	

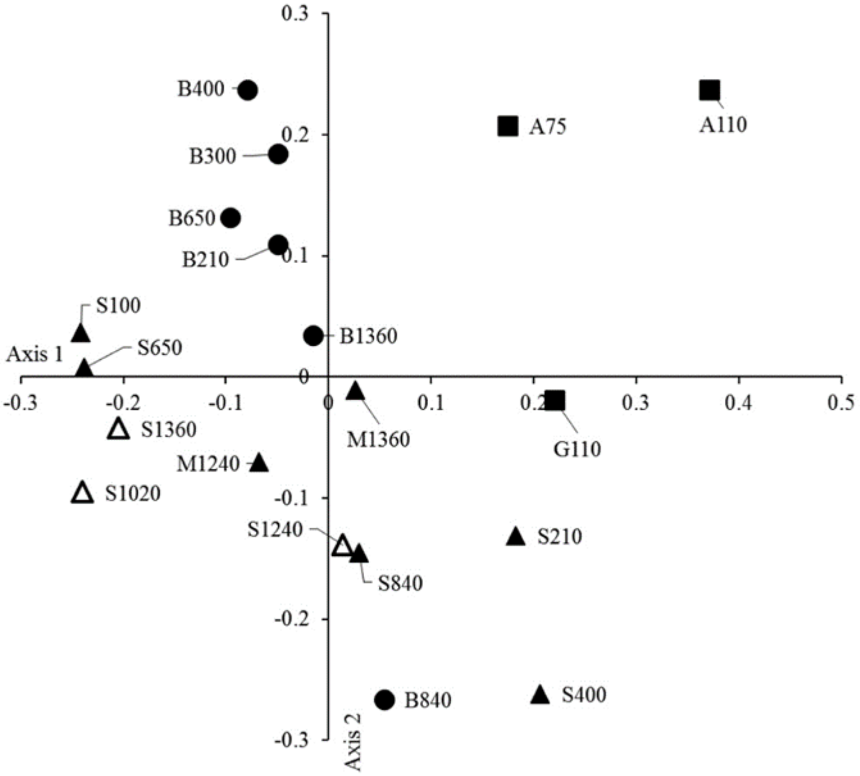

Figure 3. Similarity plots of the 19 myxomycete communities analyzed by non-metric multidimensional scaling. ■: Subtropical trees, ●: Evergreen broad-leaved trees, ▲: Coniferous trees, Δ: Montane coniferous trees. Stress value: 0.102, Determination coefficient R^2 axis1 $r^2 = 0.345$, axis 2 $r^2 = 0.792$. The legends were shown in Table 1.

Table 4. Correlation coefficients between scores of the first two axes of the NMDS analysis of 19 myxomycete communities and elevation, twig pH and myxomycete species diversity. Significance $^*p < 0.05$, $^{**}p < 0.01$.

	Axis 1		Axis 2	
Elevation	−0.401		−0.416	
Twig pH	0.625	**	0.217	
Myxomycete community				
Species richness	0.166		0.736	**

Myxomycete communities on different vegetation types

Myxomycete communities were combined into four groups associated with different vegetation types along the elevation gradient. Cluster analysis of the four vegetation types indicated similarities among the groups (Fig. 4). The assemblages of upland evergreen and montane conifers had higher similarity than that at lower elevations. The subtropics were distinctively separated from the others. Bray-

Curtis dissimilarity was highest between the subtropics and montane conifers (β = 0.727), whereas the value was 0.724 between lowlands and 0.662 between uplands.

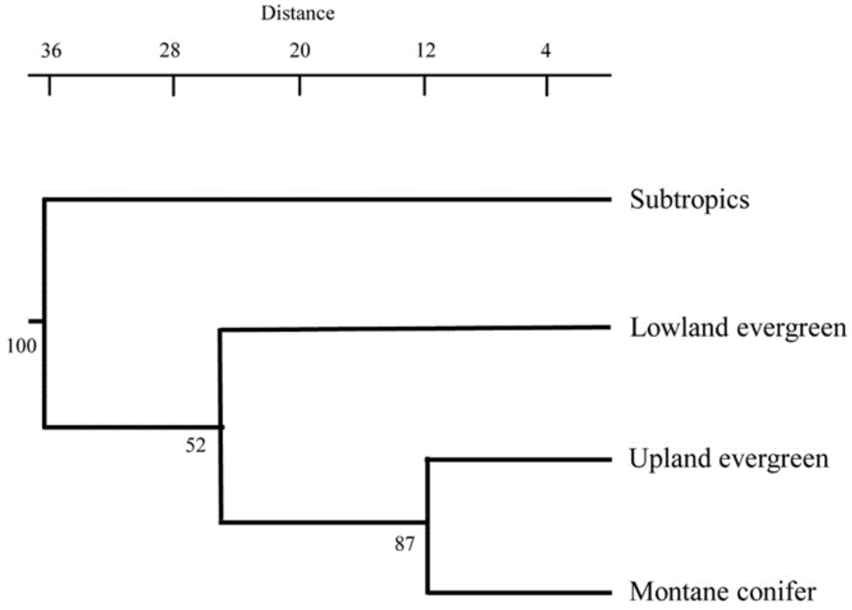

Figure 4. Grouping of the myxomycete communities in the different forest vegetation types corresponding to the elevation gradient based on cluster analysis (Ward's method). The numbers show reliability (%).

The subtropics (75–110 m) had a diversity index of 2.61 with equitability (*J*′) of 0.86, which appeared the richest of 21 taxa across vegetation types (Table 2). Fourteen taxa appeared on *Ficus subpisocarpa* twigs, of which diversity was the highest, with *H*′ = 2.61 and *J*′ = 0.858, across tree species. In lowland evergreen group (210–400 m), twigs of *C. sieboldii* and *C. japonica* furnished 17 taxa (*H*′ = 2.02, *J*′ = 0.713) in total. In the upland evergreen group (650–840 m), twigs of *Q. salicina* and *C. japonica* yielded 12 taxa (*H*′ = 2.07, *J*′ = 0.834). In the montane conifer group (1020–1360 m), 13 taxa were occurred on the twigs of *C. japonica*, *Abies firma*, and *Trochodendron aralioides* (*H*′ = 2.04, *J*′ = 0.797).

The distribution pattern of species was checked corresponding to vegetation types using the independence test (significance $p < 0.01$) among the nine dominant taxa with an abundance of ten or more, and relative abundance over 3% (Table 2). The taxa associated with specific vegetation were *Cribraria microcarpa* in conifer forest (1020–1360 m), *Diderma chondrioderma* in upland evergreen forest (650–840 m), *Arcyria cinerea* and *Clastoderma debaryanum* in lowland evergreen forest (210–400 m), and *Comatricha tenerrima*, *Lamproderma scintillans*, *Perichaena depressa*, and *Ophiotheca chrysosperma* in the coastal subtropical vegetation (75–110 m). The species richness per tree species in a vegetation type decreased on average for species with elevation grade, that is, coastal subtropics had 8.8, lowland had 6.0, upland had 5.0, and montane had 4.7 species.

Discussion

Previous studies found 23 taxa on the bark of living trees of *C. japonica* and suggested differences in communities between lowland and montane forests (Takahashi 2017). The present study found 35 taxa within 15 genera in twig habitats and clarified myxomycete diversity on litter twigs in Yakushima Island, consequently indicating the differences in myxomycete communities according to vegetation types. The communities apparently responded to changes in the elevational microenvironment involving vegetational differences, over an elevation range of approximately 1300 m. Myxomycete diversity decreased in the montane forests compared with coastal subtropical vegetation.

The distribution of myxomycetes on a local scale seems to be influenced by the ecological differences in particular habitats, such as forest types and substrates (Stephenson et al. 2008). However, knowledge of the influence of elevational grade on the distribution of myxomycetes remains unclear. In the tropics, in Mt. Arayat, Philippines, along the elevation gradient (200–800 m), the highest species diversity on leaf and twig litter was observed at 700 m. (Dagamac et al. 2014). The investigation of diverse sample substrates such as ground litter, aerial litter, and twigs in Costa Rica with increasing elevation within the range 100–1100 m indicated that elevation may not be as important a factor in shaping the distribution of myxomycetes (Rojas et al. 2016). Furthermore, investigations including diverse substrates suggested that species richness decreased among the four studied forest associations along the elevation and moisture gradients, and the Shannon index showed a similar trend in boreal forests in Russia (Novochirov et al. 2020). The present study showed that elevational gradient, accompanied by vegetation change and excessive humidity, tends to reduce myxomycete species richness and abundance in a local scale.

The important factors that determine myxomycete occurrence are collectively represented by temperature, moisture, pH, and the availability of decomposing plant material in nature (Stephenson 1989). A previous study has suggested that temperature influences myxomycete distribution in the Japanese archipelago (Takahashi et al. 2018). Furthermore, myxomycete communities depend on substrate differences between tree species (Takahashi et al. 2009, Takahashi et al. 2022). Myxomycete diversity decrease and is limited in the cloud forests located at high altitudes in Costa Rica, Central America, because of excess humidity (Schnittler and Stephenson 2000). The mountainous area on Yakushima Island may also display very high precipitation levels, which coincides with cool temperatures. Composite factors are assumed to influence and control the pattern of myxomycete distribution. The occurrence of myxomycetes was primary influenced by vegetation types along elevation and humidity gradients on Yakushima Island.

Substrate pH has a strong influence on the growth of myxomycetes (e.g., Everhart et al. 2008, Takahashi 2014). Myxomycete communities on twig litters of 10 tree species are affected by substrate pH in the forests of the Chugoku and Shikoku regions in western Japan (Takahashi et al. 2022). In this study, the difference in twig pH also affected a variety of myxomycetes, which tended to become richer with substrate pH increasing to neutral in the analysis of 19 communities from seven tree species. Acidic twigs (pH < 5.2) were preferred by *Cribraria microcarpa*, as reported in Takahashi et al. (2022), and the species was also abundant in the present study on *Cryptomeria japonica* twigs with a mean pH of 4.8. Species diversity tended to decrease at lower pH but became higher at a neutral pH associated with subtropical trees, where species of *Perichaena* and *Comatricha* occurred abundantly in the present study. The distribution pattern of myxomycetes are worth studying along islands scattered from temperate to subtropics in East Asia.

The myxomycete communities in leaf litter had higher diversity in mixed forest than broad leaved deciduous or evergreen forest in different vegetation types of warm temperate Japan (Takahashi 2013). Although the present study only investigated seven tree species using moist chamber cultures, a variety of myxomycetes were recorded. A higher diversity of myxomycetes is expected to be present on Yakushima Island, because of the variation in vertical distribution of different vegetation types and tree species occurring from the subtropical to subarctic climates. Further study of additional tree species or other substrates in various vegetation types will lead to further documentation of the myxomycete diversity in this World Natural Heritage site. Myxomycetes play an important role in maintaining the balance of material cycling in forest ecosystems because they consume decomposer microbes, such as bacteria (Smith and Stephenson 2007) and fungi (Fukasawa et al. 2018) and are strongly influenced by humus formation. The study of myxomycete diversity will considerably extend the importance of understanding terrestrial ecosystems.

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