

# Myxomycete diversity on *Cryptomeria japonica* bark varies with land-use type along montane rivers

Kazunari Takahashi<sup>1,\*</sup>

<sup>1</sup> Kurashiki City College, 160 Hieda-cho, Kurashiki city, Okayama 711-0937, Japan

\* Corresponding author:  
kumakusu03@yahoo.co.jp

**Keywords:** Corticolous myxomycetes, Forest depletion, Land-use type, Moist chamber culture, River basin, Species diversity

## Article info:

Received: 27 February 2021

Accepted: 13 June 2021

Published online: 23 August 2021

Corresponding Editor: Riikka Linnakoski

## Abstract

Myxomycetes occur globally, but little is known about the mechanism by which myxomycete diversity and community structure respond to environmental gradients and human activity at local scales. The present study assessed the distribution of corticolous myxomycetes living on the bark of *Cryptomeria japonica* trees along three rivers originating in the Chugoku Mountains in western Japan. Bark samples were collected from 14 sites along each river, which encompass the river sources as well as the upper, middle, and lower reaches. The environmental characteristics of each site were assessed for three variable types: geography, climate, and land-use. A 1 km<sup>2</sup> grid was superimposed onto each survey site, consisting of 100 cells of 1 ha each, and each cell was classified using aerial imagery into a broad land-use type. Of these, three land-use types (forest, farmland, and residential) were regularly used as a comprehensive indicator of landscape. The bark samples were cultured using the moist chamber technique, and the resulting myxomycete fruiting bodies were identified. Sporophores formed on 96% of the 1,490 moist chamber cultures and

were classified into 27 taxa (26 species and one variety). Species diversity was highest in natural forests near river sources and decreased with proximity to downstream estuaries. Community similarities between survey sites were analysed using non-metric multidimensional scaling (NMDS). The first NMDS axis was negatively correlated with distance from estuary and forest coverage. Indicator species were identified for environmental changes along river basins. The relative abundances of *Macbrideola argentea*, *Hemitrichia velutina*, and *Physarum nutans* var. *rubrum* were positively correlated with forest coverage. In contrast, the relative abundances of *Clastoderma debaryanum*, *Diderma chondrioderma*, and *Echinostelium minutum* were positively correlated with residential area coverage. The distribution of corticolous myxomycetes on *C. japonica* trees was associated with local landscape changes along the river environments. This is the first report on myxomycetes assemblages along river basins (acting as ecological corridors) and indicates that forest degradation and land-use types strongly affect myxomycete diversity on the bark of living *C. japonica* trees.

---

## Introduction

Myxomycetes are eukaryotic fungus-like protists that engulf bacteria as their primary source of nutrients. Many species are associated with wood and plant debris, and play important roles as scavengers or decomposers of decaying plant debris (Stephenson 2011), which is important in controlling the material cycle in terrestrial ecosystems. As myxomycetes mainly disperse as spores by wind, they are thought to occur globally; however, myxomycete species actually only colonise preferred habitats (Schnittler & Tesmer 2008). The corticolous myxomycetes are an ecological group of myxomycetes that exclusively live on tree bark surfaces (Gilbert & Martin 1933), where they complete their entire life cycle (Keller & Brooks 1973).

Recent studies have suggested that geographic ranges of myxomycetes are limited by regional climate and vegetation (Schnittler et al. 2000; Rollins

& Stephenson 2011; Lado et al. 2016; Dagamac et al. 2017), and that myxomycetes may follow the moderate endemism model (Stephenson et al. 2008). Some myxomycetes are likely to have restricted ranges based on preferences for different habitats with varied climates and substrates (Estrada-Torres et al. 2013; Aguilar et al. 2014). For example, *Physarum pseudonotabile* Novozh., Schnittler & Okun has been found in extreme habitats and described as being a xerotolerant species that inhabits the bark of living plants and ground litter in arid regions (Novozhilov et al. 2013). Myxomycete geographic distribution has been examined in a few studies at local scales (Macabago et al. 2017; Novozhilov et al. 2018; Takahashi et al. 2020), but there remains a lack of detailed insight about how their association with a region is affected by local environmental factors. The local climate, geographical features, and impacts from human activity could all affect myxomycete distribution and abundance.

The Japanese archipelago has many geological and topographic features creating various local environments, making it an ideal region to investigate myxomycete distribution patterns. Rivers in Japan create deep valleys, which are accompanied by varied natural environments and vegetation. They run from upper montane regions to estuaries, with the longest river in the country only 210 km in length. The environments along the rivers and associated streams produce varied habitats for many organisms as well as human occupation. Land use has historically changed the natural environment and influenced species distribution and biodiversity along rivers. For example, *Cryptomeria japonica* (Thunb. ex. L. f.) D. Don trees, which originally preferred humid locations, have been widely planted in the mountains and along rivers because *C. japonica* trees are useful for timber production; the forestry industry has extended many planting areas.

Corticolous myxomycetes grow abundantly on the bark of *C. japonica* trees in the Japanese archipelago: approximately 30 species have been identified in previous studies (Takahashi & Harakon 2018; Takahashi et al. 2018). The trunks of *C. japonica* are flat with fibrous and furrowed bark, which is suitable for trapping wind-dispersed spores and providing habitat for myxomycetes; however, little is known about how local geographical and climatic

---

environments, including land-use types, influence the community structure and species distribution of myxomycetes. A river basin is an ecological corridor from river source to estuary and is composed of various natural environmental factors and human activities. The objective of this study was to identify myxomycete distribution patterns and diversity on the bark of *C. japonica* trees growing along rivers in western Japan.

## Materials and methods

### Survey sites

Three class A rivers, the Yoshii River, the Asahi River and the Sendai River, in the Chugoku district in western Japan, were set for basin survey (Fig. 1) because they flow toward the north or south from the Chugoku Mountains (approximately 1,200 m altitude) to estuaries downstream of, and near to, urban

areas. Information on the river environment was obtained from the Ministry of Land, Infrastructure, Transport, and Tourism ([https://www.mlit.go.jp/river/toukei\\_chousa/kasen/jiten/nihon\\_kawa/07\\_chugoku.html](https://www.mlit.go.jp/river/toukei_chousa/kasen/jiten/nihon_kawa/07_chugoku.html), accessed April 2019). There is preserved natural forest of *C. japonica* at the source of each river. The Sendai River extends 52 km, running through Tottori Prefecture in the north to the Sea of Japan. To the south, the Yoshii River extends 130 km and the Asahi River extends 142 km. Both run through Okayama Prefecture to the Setouchi Inland Sea. The various landscapes surrounding these three rivers contain natural *C. japonica* forests in their reaches and *C. japonica* plantations scattered along the river basins.

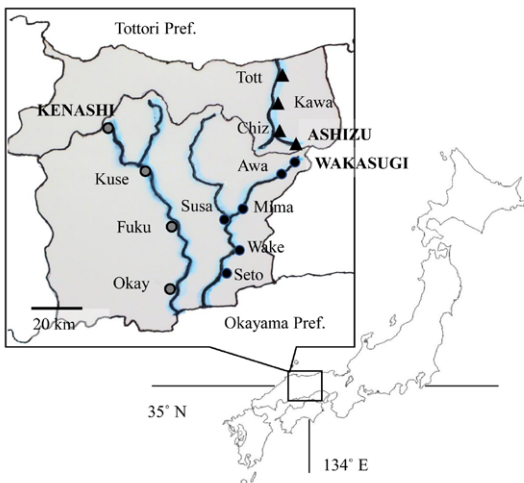
Survey sites were selected at several points along each river that encompass the river sources as well as the upper, middle, and lower reaches. Sample sites included native forests in the Chugoku Mountains and plantations, shrines, and green parks along the river basins. Four sites were established along the Sendai River, six along the Yoshii River, and four along the Asahi River (Fig. 1). Survey site information, including geographical locations, climate variables, and landscape types, is described in Table 1.

Temperature and precipitation data for each survey location were obtained from the Japanese Meteorological Agency (<http://www.data.jma.go.jp/obd/stats/etrn/index.php?sess>, accessed on March 2020) observations from neighbouring areas, and calculated for study sites based on a temperature decrease rate of  $-0.55\text{ }^{\circ}\text{C}$  per 100 m gain in elevation. The mean annual temperature across the study area ranged from  $7.6\text{--}16.2\text{ }^{\circ}\text{C}$  and the mean lowest monthly temperature ranged from  $-4.7\text{--}4.9\text{ }^{\circ}\text{C}$ . Regional mean annual precipitation ranged from 1,106–1,924 mm.

### Environmental gradients and land-use types

The 14 survey sites were assessed for three environmental variable types: geography [distance from estuary (km), latitude ( $^{\circ}$ north), longitude ( $^{\circ}$ east), and altitude (m)], climate [annual mean temperature ( $^{\circ}\text{C}$ ), lowest month mean temperature ( $^{\circ}\text{C}$ ), and annual precipitation (mm)], and land-use.

Land-use types were assessed using aerial im-



**Fig. 1.** Locations of 14 survey sites along three rivers in Okayama Prefecture and Tottori Prefecture in western Japan. Code names for survey sites are provided in Table 1. Symbols indicate three rivers, ▲: Sendai River, ●: Yoshii River, ●: Asahi River. The source of each river is in the Chugoku Mountains.

agery and an associated classification scheme from the Geographical Survey Institute (<http://maps.gsi.go.jp/>, accessed September 2019). Each survey location was overlaid by a 1 km<sup>2</sup> grid, consisting of 100 cells of 1 ha each, and each grid cell was categorised into one of six broad land-use types: forest, farmland, residential, empty space (wasteland, vacant lots, and roads), water, and industrial. Each cell was assigned a land-use type if >50% of the cell was occupied by that type, and cumulatively counted within each 1 km<sup>2</sup> grid. Land-use types that occupied ≤3% of the grid were excluded from the analysis, as they were considered insignificant and without meaningful association with the distance from estuary metric. In this study, three land-use types (forest, farmland, residential) were regularly used as a comprehensive indicator of landscape, as indicated in **Table 1**.

### Bark sampling

Bark was sampled over two to three days between May and November of 2019 along each river. Samples were collected from 10 to 13 trees at each site (149 trees in total). Trees with stem diameters at breast height (DBH) over 20 cm were selected for sampling. The outer bark was peeled off by hand from the stem surface at a height of 50–200 cm from the ground and placed in a paper bag. Bark that was attached to the surface by moss or lichen was excluded from sampling.

### Myxomycete cultures

Moist chamber (MC) cultures were prepared using plastic Petri dishes (9 cm diameter) following the protocol of Takahashi et al. (2018). Ten cultures were prepared per tree, resulting in a total of 1490 MC cultures. Bark samples were air-dried for 1–2 weeks at ambient temperature then cut into pieces 3–8 cm in length. Approximately 5 g of bark (dry weight) was placed on clean filter paper in each dish; outer surface of the bark was upper side, and soaked with approximately 25 mL distilled water (pH 6.9) for 3 days. The chemical properties of the bark were assessed by measuring the pH and electrical conductivity (EC) of the resulting exudate, using a compact pH and EC meter (Horiba, Kyoto, Japan).

The EC and pH values per tree were respectively estimated as the mean and median of five exudate samples in a random manner, and then the average values per site were calculated.

Next, excess water from each MC dish was drained and the dishes were incubated for three weeks at 23 °C to stimulate sporulation. Then, the dishes were kept with their lids halfway open to dry the bark samples. When fruiting bodies appeared in the MC, they were observed using a stereomicroscope. Myxomycete species were identified through morphological characteristics of the fruiting body and spore characterisation as described by Yamamoto (1998), and followed the nomenclature of the most recent literature (Lado 2005–2020). Voucher specimens were prepared using separate paper boxes for each species. Bark with myxomycete fruiting bodies were glued to the bottom of each box, and collection boxes were stored in the laboratory.

### Data analyses

Species richness and abundance were determined for each survey site. Sampling adequacy (percentage of exactitude) was assessed by dividing the number of species observed ( $S_{obs}$ ) within a site by the estimated number of species ( $S_{est}$ ) using the Chao1 estimator (Chao 1984) and PAST software (Hammer et al. 2001). To quantify myxomycete species diversity at different sites, the Shannon–Wiener diversity index,  $H'$ , (Shannon & Weaver 1963) and equitability,  $J'$ , were calculated, following previous studies of myxomycetes (Stephenson 1989).

The abundance of a species was estimated using the number of positive culture dishes in which sporangia appeared on the bark. The percentage of positive cultures was calculated by dividing the number of dishes with successful cultures (i.e. those with sporangia) by the total number of dishes. Relative abundances were calculated by dividing a given species' abundance by the cumulative abundance of the site's community (Stephenson et al. 1993). We defined common species as those that appeared at every site, and dominant species as those with > 10% relative abundance at the site community level. The influence of environmental factors on relative abundances was examined with correlation analysis.

**Table 1.** Geography, climate and land-use types at survey sites along three class A rivers flowing from the Chugoku Mountains. Site names written in capital letters are river sources.

	Yoshii river					
Code of Survey site <sup>*</sup>	WAKA	Awak	Mima	Susa	Wake	Seto
<b>Geography</b>						
Distance from estuary (km)	133	116	68	66	41	10
North latitude (°)	35.2518	35.1503	34.9349	34.9181	34.8067	34.6948
East longitude (°)	134.3932	134.3312	134.1258	134.0961	134.1588	134.1012
Altitude (m)	1050	298	58.8	48.3	23.9	9.9
<b>Climate</b>						
Mean temperature (°C)	8.4	12.5	14.2	14.2	14.0	14.8
Lowest month temperature (°C)	-4.4	-0.2	1.9	1.9	2.5	3.9
Annual precipitation (mm)	1647	1647	1416	1416	1174	1086
<b>Land-use types (%)</b>						
Forest coverage	100	80	54	42	40	30
Farm coverage	0	18	16	23	21	44
Residence coverage	0	2	15	15	22	21

\*Codes for survey site names are provided.

WAKA: Wakasugi natural forest at Yoshii river source, Awa: Nishiawakura, Mima: Fukumoto at Mimasaka shi, Susa: Susai at Akaiwa shi, Wake: Wake chyo, Seto: Osafune chyo at Setouchi shi, KENA: Mt. Kenashi of Shinjou son at Asahi river source, Kuse: Kuse at Maniwa shi, Fuku: Fukuwatari at Okayama shi, Okay: Tsushima at Okayama shi, ASHI: Ashizu valley at Sendai river source, Chiz: Chizu chyo, Kawa: Kawahara chyo, Tott: Uemachi at Tottori shi.

Asahi river				Sendai river			
KENA	Kuse	Fuku	Okay	ASHI	Chiz	Kawa	Tott
142	99	41	16	52	38	22	10
35.2290	35.0872	34.8641	34.6861	35.2815	35.2660	35.3921	35.4998
133.5226	133.7443	133.9026	133.9194	134.3843	134.2469	134.2071	134.2452
782	189	69	4.2	1145	259	40	22
9.3	13.3	13.8	16.2	7.6	12.5	14.7	14.8
-4.7	1.8	2.3	4.9	-3.7	1.2	3.8	3.9
1726	1432	1240	1106	1924	1924	1914	1914
96	55	53	9	100	63	31	59
0	11	15	0	0	13	44	1
4	19	30	74	0	18	10	37

## Ordination of myxomycete communities

Non-metric multidimensional scaling (NMDS; Kenkel & Orlóci 1986) was performed for the 14 myxomycete communities based on Bray-Curtis dissimilarities (Bray & Curtis 1957), wherein higher values (to a maximum of 1.0) indicate greater dissimilarity between communities in terms of species composition and abundance. The NMDS was performed using PAST software (Hammer et al. 2001) based on its effectiveness in a similar study (Takahashi & Harakon 2016). Then, correlation analyses were conducted between the first two NMDS axes and environmental variables, such as environmental gradients, latitude, longitude, altitude, annual mean temperature, lowest monthly mean temperature, annual precipitation, tree traits (DBH, bark pH, and EC), and land-use types to identify important environmental factors for understanding myxomycete ecological features. Significant environmental variables affecting the community ordination were further assessed by correlation with species relative abundances of the 14 communities to determine distribution patterns of species corresponding to environmental variables.

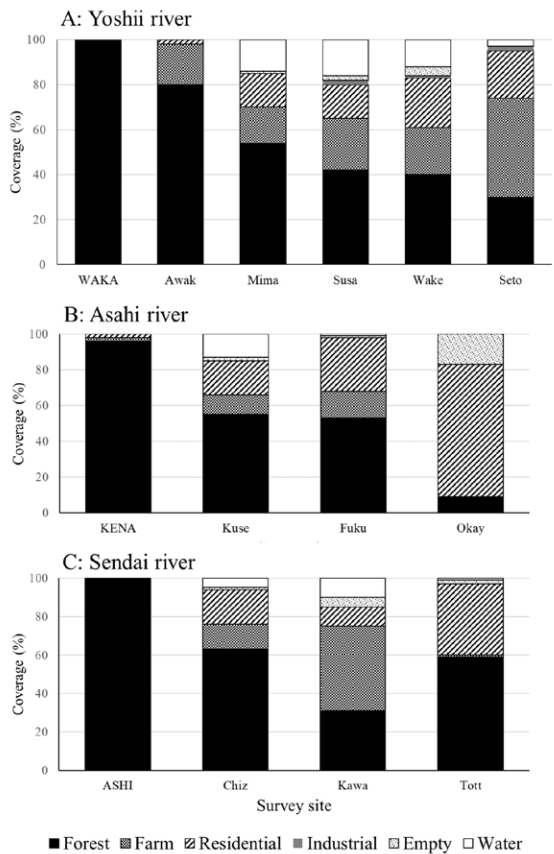
## Results

### Environmental gradients along rivers

The environment factors present at each river are presented in Table 1, in order from the site at the river source to the site closest to the estuary. Distance from estuary positively correlated with altitude ( $r = 0.603, p < 0.05$ ) and was also significantly correlated with annual mean temperature ( $r = -0.655, p < 0.05$ ), lowest monthly mean temperature ( $r = -0.791, p < 0.01$ ), forest coverage ( $r = 0.717, p < 0.01$ ), and residential coverage ( $r = -0.599, p < 0.05$ ). Landscape coverage with forest was greatest in montane sites but decreased closer to the estuaries with increasing residential and farmland coverage (Fig. 2).

Traits of sampled trees are shown in Table 2. The DBH ranged from 39–104 cm and did not significantly correlate with altitude ( $r = 0.537$ ), bark

pH ( $r = -0.392$ ), or EC ( $r = -0.084$ ). Bark pH ranged from 3.5–4.1 and EC ranged from 92–493  $\mu\text{S}/\text{m}$  (Table 2). There was no correlation between pH and EC ( $r = -0.089$ ); however, bark pH had a significant negative correlation with forest coverage ( $r = -0.731, p < 0.01$ ). The environmental variances selected as affective factors for environmental gradients were distance from estuary, altitude, annual mean temperature, forest coverage, and residential coverage, in addition to bark pH.



**Fig. 2.** Composition of land-use types in survey sites along the three rivers. A: Six sites along the Yoshii River, B: Four sites along the Asahi River, C: Four sites along the Sendai River. Land-use was categorised into the following six types: forest, farmland, residential, industrial, empty space, and water. Land-use type is indicated by percentage of area in 1 km<sup>2</sup>. Code names for survey sites are provided in table 1.

## Myxomycete species diversity

Myxomycete fruiting bodies developed in 82–100% of the sites and in 96% of the 1,490 total MC cultures. Species richness per tree ranged from 3.9–7.8, which was positively correlated with distance from estuary ( $r = 0.800$ ,  $p < 0.01$ ), altitude ( $r = 0.698$ ,  $p < 0.01$ ), and forest coverage ( $r = 0.689$ ,  $p < 0.01$ ). Species richness per site ranged from 8–15 species, comparable to the number of species estimated by Chao-1, which indicated 95% accuracy. Species richness was negatively correlated with latitude ( $r = -0.620$ ,  $p < 0.05$ ). Species diversity ( $H'$ ) ranged from 1.62–2.27, which was significantly correlated with the distance from estuary ( $r = 0.552$ ,  $p < 0.05$ ), and correlated with equitability ( $J'$ ) values ranging from 0.74–0.88 ( $r = 0.820$ ,  $p < 0.01$ ).

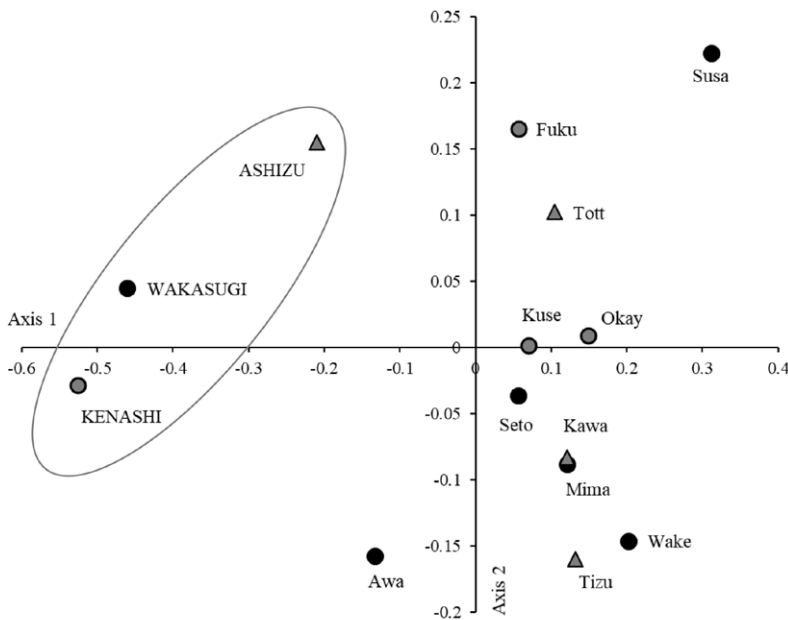
Twenty-seven taxa (26 species and one variety) were recorded and are arranged in order of relative abundance in Table 3. The most abundant species, which were recorded at every site and contributed to > 10% of the relative abundance, were *Paradi-*

*acheopsis rigida* (Brändä) Nann.-Bremek. (22%), *Cribraria confusa* Nann.-Bremek. & Y. Yamam. (17%), *Arcyria cinerea* (Bull.) Pers. (13%), and *Enerthenema melanospermum* T. Macbr. & G.W. Martin (11%). *Physarum nutans* Pers. also appeared at every site with a relative abundance of 6%. Thirteen taxa had relative abundances of 1–6% and there were nine rare taxa, with < 1% relative abundance.

## Ordination of myxomycete communities

Similarities among 14 myxomycete communities (i.e., site-level communities) were evaluated using NMDS analysis (Fig. 3). In the ordinations, the upriver communities were arranged on the negative side of the first axis, and the downstream communities were on the positive side. The six communities in the Yoshii River extended along the widest range of the first axis, following those of the Asahi River and the Sendai River.

Correlation coefficients between the first



**Fig. 3.** Non-metric multidimensional scaling (NMDS) ordination plots of myxomycete communities from 14 survey sites. Symbols indicate three rivers, ▲: Sendai River, ●: Yoshii River, ●: Asahi River. Codes for survey site names are provided in Table 1. Site names written in capital letters and surrounded by an oval are river sources. Stress = 0.154, coefficient of determination,  $r^2 = 0.754$  for axis 1 and  $r^2 = 0.050$  for axis 2.



**Table 2.** Tree traits, myxomycete occurrences and species diversity at survey sites. Codes for survey site names are provided in Table 1. Site names written in capital letters are river sources. Italics indicate data in a entire river.

Code	Yoshii river						Entire
	WAKA	Awak	Mima	Susa	Wake	Seto	
<b>Tree traits</b>							
Tree samples	13	10	11	10	10	10	64
Diameter of breast height (cm)	73	40	59	54	63	39	55
Bark pH	3.6	3.8	3.7	3.8	3.9	3.8	3.8
Electric conductivity ( $\mu\text{S}/\text{cm}$ )	320	268	354	344	305	338	321
<b>Myxomycetes</b>							
Positive culture (%)	100	100	98	100	100	99	100
Mean species richness per tree	7.5	7.1	5.5	6.1	5.2	5.4	6.1
Total species richness	13	13	12	13	11	15	25
Chao-1	13	13	13	13	11	16	28
Species diversity ( $H'$ )	2.27	2.19	2.02	2.17	1.84	2.16	2.51
Equitability ( $J'$ )	0.88	0.85	0.79	0.84	0.77	0.80	0.78

two NMDS axes (NMDS1 and NMDS2) and 11 environmental parameters (geographical, climate, landscape factors, and tree traits) are given in Table 4. NMDS1 was significantly correlated with distance from estuary ( $r = -0.725$ ,  $p < 0.01$ ), altitude ( $r = -0.852$ ,  $p < 0.01$ ), annual mean temperature ( $r = 0.838$ ,  $p < 0.01$ ), lowest monthly mean temperature ( $r = 0.877$ ,  $p < 0.01$ ), and forest coverage ( $r = -0.815$ ,  $p < 0.01$ ). NMDS2 was not significantly correlated with any environmental parameter.

Myxomycete communities along NMDS1 were arranged according to species diversity ( $H'$ ) ( $r = -0.526$ ,  $p = 0.057$ ), equitability ( $J'$ ) ( $r = -0.631$ ,  $p < 0.05$ ), and species richness per tree ( $r = -0.776$ ,  $p < 0.01$ ). The upriver sites had higher species diversity than the downriver sites.

### Species spatial patterns

Nine species were found to be indicators of five environmental gradients (Table 5). Distance from estuary was positively correlated with the relative abundances of *Cribraria microcapa* (Schrad.) Pers. ( $r = 0.581$ ,  $p < 0.05$ ), *Cribraria minutissima* Schw. ( $r = 0.674$ ,  $p < 0.01$ ), *Macbrideola argentea* Nann.-Bremek. & Y. Yamam. ( $r = 0.630$ ,  $p < 0.05$ ), and *Phy-sarum nutans* var. *rubrum* ( $r = 0.565$ ,  $p < 0.05$ ), and negatively correlated with *P. rigida* ( $r = -0.590$ ,  $p < 0.05$ ) and *Clastoderma debaryanum* A. Blytt. ( $r = -0.579$ ,  $p < 0.05$ ). Altitude was positively correlated with *Hemitrichia velutina* Nann.-Bremek. & Y. Yamam. ( $r = 0.886$ ,  $p < 0.01$ ), *M. argentea* ( $r = 0.940$ ,  $p < 0.01$ ), and *P. nutans* var. *rubrum* ( $r = 0.816$ ,  $p < 0.01$ ), and negatively with *P. rigida* ( $r = -0.637$ ,  $p < 0.05$ ). Annual mean

Asahi river					Sendai river				
KENA	Kuse	Fuku	Okay	Entire	ASHI	Chiz	Kawa	Tott	Entire
10	11	10	12	43	11	10	10	11	42
104	72	70	40	71	79	57	46	86	67
3.6	3.5	3.6	3.6	3.6	4.1	3.5	3.7	3.9	3.8
179	155	123	175	158	92	493	126	94	201
100	90	88	94	93	97	100	96	82	94
7.8	4.4	4.8	4.6	5.4	6.2	4.7	4.7	3.9	4.9
14	12	15	13	20	12	8	10	10	18
15	15	15	16	26	12	9	10	11	19
2.22	1.89	2.09	2.08	2.42	2.11	1.62	1.73	1.70	2.11
0.84	0.76	0.77	0.81	0.81	0.85	0.78	0.75	0.74	0.73

temperature was positively correlated with *C. debaryanum* and *P. rigida* and negatively correlated with *H. velutina*, *M. argentea*, and *P. nutans* var. *rubrum*.

Forest coverage was positively correlated with *M. argentea* ( $r = 0.796, p < 0.01$ ), *H. velutina* ( $r = 0.731, p < 0.01$ ), and *P. nutans* var. *rubrum* ( $r = 0.651, p < 0.01$ ), whereas it was negatively correlated with *C. debaryanum* ( $r = -0.608, p < 0.05$ ), *Diderma chondrioderma* (De Bary & Rostaf.) G. Lister ( $r = -0.547, p < 0.05$ ), and *Echinostelium minutum* de Bary ( $r = -0.547, p < 0.05$ ). The latter three species were positively correlated with residential cover [*E. minutum* ( $r = 0.809, p < 0.01$ ), *D. chondrioderma* ( $r = 0.764, p < 0.01$ ), and *C. debaryanum* ( $r = 0.547, p < 0.05$ )]. The landscape change from forest coverage to residential area strongly influenced the abundances of these three species.

## Discussion

The dominant and common species of myxomycetes on *C. japonica* bark were identified across three river basins. There were two dominant species, *P. rigida* and *C. confusa*, which have previously been found to be common on *C. japonica* bark (Takahashi et al. 2018, 2020). *Arcyria cinerea* and *P. nutans* also frequently appeared in almost all survey sites, which was also observed in previous surveys (Takahashi et al. 2018, 2020).

The spatial distribution pattern of myxomycetes have suggested that it may relate to the ecological differences on a local scale (Stephenson et al. 2008). *Cryptomeria japonica* trees are distributed across river environments, from river source to estuary, and provide microhabitats for corticolous

**Table 3.** Corticolous myxomycete species and abundances on the bark of living *C. japonica* trees at each survey sites. Numbers written in italics are the relative abundances in the total community. Codes for survey site names are provided in Table 1. Site names written in capital letters are river sources. The relative abundance of each species was indicated by rounded values.

Species	Code	Yoshii river					
		WAKA	Awak	Mima	Susa	Wake	Seto
<i>Paradiacheopsis rigida</i> (Brândză) Nann.-Bremek.		38	68	75	41	77	64
<i>Cribraria confusa</i> Nann.-Bremek. & Y. Yamam.		72	63	20	74	36	52
<i>Arcyria cinerea</i> (Bull.) Pers.		31	60	30	28	8	45
<i>Enerthenema melanospermum</i> T. Macbr. & G. W. Martin		41	25	36	12	63	49
<i>Physarum nutans</i> Pers.		74	50	31	20	8	12
<i>Macbrideola argentea</i> Nann.-Bremek. & Y. Yamam.		91	4				
<i>Hemitrichia velutina</i> Nann.-Bremek. & Y. Yamam.		31	3				
<i>Cribraria microcarpa</i> (Schrad.) Pers.		22	13	2	1		20
<i>Physarum nutans</i> var. <i>rubrum</i> (Nann.-Bremek. & Y. Yamam.) Chao H. Chung		65					10
<i>Cribraria minutissima</i> Schwein.		16	47			5	1
<i>Comatricha pulchella</i> (C. Bab.) Rostaf.		10		2	41	9	
<i>Comatricha laxa</i> Rostaf.			4	21		13	9
<i>Clastoderma debaryanum</i> Blytt				17	9	7	19
<i>Enerthenema papillatum</i> (Pers.) Rothtaf.					18		8
<i>Diderma chondrioderma</i> (de Bary & Rostaf.) G. Lister		3		6			
<i>Comatricha elegans</i> (Racib.) G. Lister			14		3	7	2
<i>Macbrideola confusa</i> Nann.-Bremek. & Y. Yamam.			17		7		
<i>Enerthenema berkeleyanum</i> Rostaf.					21		
<i>Licea variabilis</i> Schrad.					3		
<i>Echinostelium minutum</i> de Bary				1			2
<i>Paradiacheopsis solitalia</i> Nann.-Bremek. & Y. Yamam.			5				
<i>Lycogala exigium</i> Morgan				2			1
<i>Licea kleistobolus</i> G. W. Martin		1					
<i>Stemonitopsis curiosa</i> Nann.-Bremek. & Y. Yamam.							1
<i>Hemitrichia minor</i> G. Lister						1	
<i>Macbrideola cornea</i> (G. Lister & Cran) Alexop.							
<i>Paradiacheopsis fimbriata</i> (G. Lister & Cran) Hertel ex Nann.-Bremek.							
<b>Total abundance</b>		<b>495</b>	<b>373</b>	<b>243</b>	<b>278</b>	<b>234</b>	<b>295</b>

Asahi river				Sendai river				Total	Relative abundance (%)
KENA	Kuse	Fuku	Okay	ASHI	Chiz	Kawa	Tott		
30	65	70	51	55	83	85	54	856	22
52	30	24	44	18	49	61	47	642	17
30	29	48	29	46	15	32	48	479	12
3	26	7	43	35	47	28	11	426	11
3	3	8	13	17	1	6	1	247	6
72		5		46		1	2	221	6
33	3	7		55	1			133	3
39	19	3			12			131	3
23				14				112	3
33	1	2	4				1	110	3
7	7	6					14	96	2
			1		21	19	3	91	2
	1	8	13	6		2	6	88	2
	6	30	6					68	2
1		4	19			5		38	1
						4		30	1
								24	1
								21	1
6		2		4				15	0.4
	1	2	5	1				12	0.3
				2				7	0.2
								3	0.1
			1					2	0.1
								1	0.0
								1	0.0
1								1	0.0
			1					1	0.0
<b>333</b>	<b>191</b>	<b>226</b>	<b>230</b>	<b>299</b>	<b>229</b>	<b>243</b>	<b>187</b>	<b>3856</b>	

**Table 4.** Correlation coefficients between the first two NMDS axes scores and environmental variables, tree traits, and myxomycete diversity.

	Axis1		Axis2
<b>Geographic factors</b>			
North latitude	-0.385		0.006
East longitude	0.097		-0.067
Distance from estuary	-0.725	**	-0.078
Altitude	-0.852	**	0.195
<b>Climatic factors</b>			
Annual mean temperature	0.838	**	-0.148
Precipitation	-0.348		-0.024
<b>Land-use types</b>			
Forest coverage	-0.815	**	0.102
Farm coverage	0.468		-0.287
Residence coverage	0.511		0.106
<b>Tree traits</b>			
Bark pH	0.072		0.210
Electrical conductivity	0.178		-0.445
<b>Myxomycete diversity</b>			
Species richness per tree	-0.776	**	-0.015
Total species richness	-0.251		0.285
Species diversity ( $H'$ )	-0.526		0.318
Equitability ( $J'$ )	-0.631	*	0.192

Significance \*\* $p < 0.01$ , \* $p < 0.05$

myxomycetes. Thus, it was revealed in this study that diverse climates and ecological differences exist along a river basin due to topographical and geographical differences influenced myxomycete distribution along river basins. It has been reported that altitudinal differences influence myxomycete distribution on *C. japonica* bark (Takahashi 2017). The distribution of three species specialised for mountain forests in the present study, *M. argentea*,

*H. velutina*, and *P. nutans* var. *rubrum*, were negatively affected by forest depletion that occurred as the rivers reached lower elevations (i.e., highly populated areas or farmland). Conversely, *P. rigida* preferred the lower altitudes found downstream. Decreasing forest and increasing residential area resulted in an increase in *C. debaryanum*, *D. chondrioderma*, and *E. minutum* abundance, which suggests that these species prefer urban environments. These species are likely to be sensitive to local geography and topography. The present study revealed that myxomycetes are not ubiquitously distributed across river basins but inhabit different regions depending on geographical features, including land-use changes and human activities. The river basins likely function as an ecological corridor, creating habitat diversity and affecting species diversity for myxomycetes in those environments.

The distribution of corticolous myxomycetes on *C. japonica* bark may have been influenced by a number of environmental factors, such as topology, geographical location, temperature, and land-use type (i.e., artificial environments). In this study, forest coverage along the river basins had the greatest effect on myxomycetes. The highest species diversity was found in the natural forests in the mountains, which provided habitats for specialists, but degradation of forest coverage affected the myxomycete community structure and influenced the distribution of several species. As landscape types changed from forest to urban or farmland, the abundance of corticolous myxomycetes decreased, resulting in lower species densities on trees. Natural forests along river reaches maintained higher species diversities of corticolous myxomycetes on *C. japonica* barks, whereas the downstream degradation of forest correlated with changes to dwelling myxomycete species. It is likely that the barks of *C. japonica* in natural forests that existed as refuges of the last ice age preserved higher myxomycete diversity than those in the peripheral planted forests (Takahashi 2020).

The variation in landscape composition affected the inhabitation of myxomycete species and the community structure. The land uses were quite different near the estuary for each river, i.e., the Seto site on the Yoshii River was dominated by farmland, the Okay site on the Asahi River was dominated by urban areas, and the Tott site on the Sendai River

**Table 5.** Correlation coefficients between the relative abundances of dominant species and environmental variables along rivers.

	Distance from estuary		Altitude	Annual mean temperature	Coverage				
					Forest	Residence			
<i>Clastoderma debaryanum</i>	-0.579	*	-0.514	0.577	*	-0.608	*	0.547	*
<i>Cribraria microcarpa</i>	0.581	*	0.277	-0.335		0.348		-0.306	
<i>Cribraria minutissima</i>	0.674	**	0.292	-0.326		0.455		-0.307	
<i>Diderma chondrioderma</i>	-0.307		-0.271	0.425		-0.547	*	0.764	**
<i>Echinostelium minutum</i>	-0.368		-0.269	0.407		-0.547	*	0.809	**
<i>Hemitrichia velutina</i>	0.357		0.886	**	-0.869	**	0.731	**	-0.448
<i>Macbrideola argentea</i>	0.630	*	0.914	**	-0.894	**	0.796	**	-0.485
<i>Paradiacheopsis rigida</i>	-0.590	*	-0.637	*	0.579	*	-0.515		0.298
<i>Physarum nutans</i> var. <i>rubrum</i>	0.565	*	0.816	**	-0.770	**	0.651	*	-0.448

Significance \*\* $p < 0.01$ , \* $p < 0.05$ .

was dominated by forest adjacent to residential areas (Fig. 2). Species richness along the Yoshi, Asahi, and Sendai Rivers were 26, 20, and 18 species, respectively, and the species diversity ( $H'$ ) was 2.51, 2.42, and 2.11, respectively. The Yoshii River had the highest species diversity in the basin, which exhibited a steady decrease in forest coverage from reach to estuary and consisted of heterogeneous landscapes. These geographical and landscape differences along a river basin likely have ecological effects for myxomycete species and the community. The river basin constitutes a corridor for myxomycete habitats.

Previously, the main factors thought to be affecting the occurrence of myxomycetes were temperature and moisture (Alexopoulos 1963). In a Japanese archipelago, geographical distribution of myxomycetes was primarily driven by air temperature (Takahashi & Hada 2010; Takahashi & Hara-kon 2018; Takahashi et al. 2018). In this study, the altitudinal difference from the lowest to the highest site was approximately 1,100 m, resulting in a temperature difference of approximately 7 °C. Large differences between the river source communities

and downstream communities were observed along NMDS1 scores, as shown in Fig. 3. The separated clusters may be due to the temperature differences of approximately 4 °C between river sources and neighbouring sites. This indicates the potential for climate warming to alter corticolous myxomycete community structures in these regions.

There have been several studies on the effects of human activities on myxomycete communities. Myxomycetes are influenced by air pollution in large European cities such as Helsinki (Härkönen & Vänskä 2004) and Madrid (Wrigley de Basanta 2000); however, it is unclear if urbanisation influences corticolous myxomycetes. In Sydney, myxomycete diversity is driven by factors at the substrate level in urban parks, but not by the park's location within the city (inner city or semi-urban) (Hosokawa et al. 2019). Differences in myxomycete communities have been observed between agricultural plantations (Redeña-Santos et al. 2017) and old-growth forests in the Philippines (Dagamac et al. 2015), and there is evidence that forest disturbance decreases myxomycete richness and fruiting in the

Amazon basin (Rojas & Stephenson 2013). In the current study, forest degradation had a major effect on myxomycete communities on *C. japonica* bark. This is likely not due to substrate loss or change, but rather to change of microenvironments in air flow, temperature, and humidity as a result of deforestation. It remains unclear whether bark-dwelling myxomycetes are affected by physical environmental factors or biotic interactions that result from forest loss. The association of myxomycete diversity with environmental changes in geography, climate, and anthropogenic activity require further research because myxomycetes play important functions in the ecological control of decomposition and nutrient cycling in local terrestrial ecosystems.

---

## Acknowledgements

The present study was supported by a grant from the River Environment Foundation (Issue number 2019-5411-002). I would like to thank the biology club members at Okayama Science University High School, Miss Ayane Fujikawa, Mr. Harumiti Jo, Miss Nanami Hori, and Mr. Yuya Yamasaki for their assistance with tree bark sampling and moist chamber culturing.

---

## References

- Aguilar, M., Fiore-Donno, A.M., Lado, C. & Cavalier-Smith, T. 2014: Using environmental niche models to test the "everything is everywhere" hypothesis for *Badhamia*. *The ISME Journal* 8: 737-745. doi: 10.1038/ismej.2013.183
- Alexopoulos, C.J. 1963: The myxomycetes II. *The Botanical Review* 29: 1-78.
- Bray, J.R. & Curtis, J.T. 1957: An ordination of upland forest communities of southern Wisconsin. *Ecological Monographs* 27: 325-349. doi: org/10.2307/1942268
- Chao, A. 1984: Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11: 265-270.
- Dagamac, N.H.A., Novozhilov, Y.K., Stephenson, S.L., Lado, C., Rojas, C., de la Cruz, T. E.E., Unterseher, M. & Schnittler, M. 2017: Biogeographical assessment of myxomycete assemblages from neotropical and Asian palaeotropical forests. *Journal of Biogeography* 44: 1524-1536. doi: org/10.1111/jbi.12985
- Dagamac, N.H.A., de la Cruz, T.E.E., Rea-Maminta, M.A.D., Aril-Dela Cruz, J.V. & Schnittler, M. 2015: Rapid assessment of myxomycete diversity in the Bicol Peninsula, Philippines. *Nova Hedwigia* 100: 31-46. doi: 10.1127/nova\_hedwigia/2015/0252
- Estrada-Torres, A., Wrigley de Basanta, D. & Lado, C. 2013: Biogeographic patterns of the myxomycete biota of the Americas using a parsimony analysis of endemism. *Fungal Diversity* 59: 159-177. doi: 10.1007/s13225-012-0209-2
- Gilbert, H. C. & Martin, G. W. 1933: Myxomycetes found on the bark of living trees. *University of Iowa Studies in Natural History* 15: 3-8.
- Hammer, Ø., Harper, D.A.T. & Ryan, P.D. 2001: PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 9. Downloaded from <http://folk.uio.no/ohammer/past/>. Date accessed: 1 May 2020.
- Härkönen, M. & Vänskä, H. 2004: Corticolous myxomycetes and lichens in the botanical garden in Helsinki, Finland: Comparison after decades of recovering from air pollution. *Systematics and Geography of Plants* 74: 183-187. doi: 10.2307/3668567
- Hosokawa, A., Reid, C.R. & Latty, T. 2019: Slimes in the city: The diversity of myxomycetes from inner-city and semi-urban parks in Sydney, Australia. *Fungal Ecology* 39: 37-44. doi: 10.1016/j.funeco.2018.11.004
- Keller, H.W. & Brooks, T.E. 1973: Corticolous myxomycetes I: Two new species of *Didymium*. *Mycologia* 65: 286-294. doi.org/10.1080/00275514.1973.12019438
- Kenkel, N.C. & Orlóci, L. 1986: Applying metric and nonmetric multidimensional scaling to ecological studies: Some new results. *Ecology* 67: 919-928. 10.2307/1939814
- Lado, C. 2005–2020: An online nomenclatural information system of Eumycetozoa. Downloaded from <http://eumycetozoa.com/data/index.php>. Date accessed: 1 May 2020.
- Lado, C., Wrigley de Basanta, D., Estrada-Torres, A. & Stephenson S.L. 2016: Myxomycete diversity in the coastal desert of Peru with emphasis on the lomas formations. *Anales del Jardín Botánico de Madrid* 73: e032. doi: 10.3989/ajbm.2436
-

- Macabago, S.A., Dagamac, N.H.A., Cruz, T.E.D., & Stephenson, S.L. 2017: Implication of the role of dispersal on the occurrence of litter-inhabiting myxomycetes in different vegetation types after a disturbance: A case study in Bohol Islands, Philippines. *Nova Hedwigia* 104: 221-236.  
doi: 10.1127/nova\_hedwigia/2016/0391
- Novozhilov, Y. & Schnittler, M. 2008: Myxomycete diversity and ecology in arid regions of the Great Lake Basin of western Mongolia. *Fungal Diversity* 30: 97-119.
- Novozhilov, Y., Okun, M.V., Erastova, D.A., Shchepin, O.N., Zemlyanskaya, I.V. & Schnittler, M. 2013: Description, culture and phylogenetic position of a new xerotolerant species of *Physarum*. *Mycologia* 105: 1535-1546. doi: 10.3852/12-284
- Novozhilov, Y., Erastova, D.A., Shchepin, O.N., Schnittler, M., Alexandrova, A.V., Popov, E.S. & Kuznetsov, A.N. 2017: Myxomycetes associated with monsoon lowland tropical forest in southern Vietnam. *Nova Hedwigia* 104: 143-182.  
doi: 10.1127/nova\_hedwigia/2016/0395
- Novozhilov, Y., Shchepin, O.N., Alexandrova, A.V., Popov, E.S., & Dagamac, N.H.A. 2018: Altitudinal patterns of diversity of myxomycetes (*Myxogastria*) across tropical forests of southern Vietnam. *Protistology* 12: 73-80.  
doi: 10.21685/1680-0826-2018-12-2-2
- Redeña-Santos, J.C., Dunca, J.A.U., Thao, D.V., & Dagamac, N.H.A. 2017: Myxomycetes occurring on selected agricultural leaf litters. *Studies in Fungi* 2: 171-177. doi: 10.5943/sif/2/1/19
- Rollins, A.W. & Stephenson, S.L. 2011: Global distribution and ecology of myxomycetes. *Current Topics in Plant Biology* 12: 1-14.
- Rojas, C. & Stephenson, S.L. 2013: Effect of forest disturbance on myxomycete assemblages in the southwestern Peruvian Amazon. *Fungal Diversity* 59: 45-53.  
doi: 10.1007/s13225-012-0181-x
- Schnittler, M., Stephenson, S.L. & Novozhilov, Y.K. 2000: Ecology and world distribution of *Barbeyella minutissima* (Myxomycetes). *Mycological Research* 104: 1518-1523.  
doi: org/10.1017/S0953756200002975
- Schnittler, M. & Tesmer, J. 2008: A habitat colonisation model for spore-dispersed organisms—Does it work with eumycetozoans? *Mycological Research* 112: 697-707.  
doi: 10.1016/j.mycres.2008.01.012
- Schnittler, M., Dagamac, N.H.A. & Novozhilov, Y.K. 2017: Biogeographical patterns in myxomycetes. In: Stephenson, S.L. & Rojas, C. (eds). *Myxomycetes: Biology, systematics, biogeography, and ecology*, 299-331. Academic Press, London.
- Shannon, C.E. & Weaver, W. 1963: *The mathematical theory of communication*. University of Illinois Press: Urbana, Illinois.
- Stephenson, S.L. 1989: Distribution and ecology of myxomycetes in temperate forests. II. Patterns of occurrence on bark surface on living trees, leaf litters, and dung. *Mycologia*, 81: 608-621. doi: 10.2307/3760136
- Stephenson, S.L. 2011: From morphological to molecular: Studies of myxomycetes since the publication of the Martin and Alexopoulos (1969) monograph. *Fungal Diversity* 50: 21-34. doi: 10.1007/s13225-011-0113-1
- Stephenson, S.L., Kalyanasundaram, I. & Lakhanpal, T.N. 1993: A comparative biogeographical study of myxomycetes in the mid-Appalachians of eastern North America and two regions of India. *Journal of Biogeography* 20: 645-657.  
doi: org/10.2307/2845520
- Stephenson, S.L., Schnittler, M. & Novozhilov, Y.K. 2008: Myxomycete diversity and distribution from the fossil record to the present. *Biodiversity and Conservation* 17: 285-301.  
doi: 10.1007/s10531-007-9252-9
- Takahashi, K. 2017: Altitudinal distribution patterns of myxomycete species growing on bark of *Cryptomeria japonica* tree in warm temperate zone of Japan. *Hikobia* 17: 207-217.
- Takahashi, K. & Harakon, Y. 2016: Ecological patterns of wood-inhabiting myxomycetes in a natural forest of the Kamikochi, the Hida mountain range, central Japan. *The Journal of Japanese Botany* 91: 205-217.
- Takahashi, K. & Harakon, Y. 2018: Biogeographical assessment of corticolous myxomycetes on living *Cryptomeria japonica* in Japanese mainland and satellites. *Hikobia* 17: 285-297.
- Takahashi, K., Harakon, Y. & Fukasawa, Y. 2018: Geographical distribution of myxomycetes living on *Cryptomeria japonica* bark in Japan. *Mycoscience* 59: 379-2385. doi: org/10.1016/j.myc.2018.02.005
- Takahashi, K., Yamasaki, Y., Minami, K. & Yabumki, N. 2020: Biogeographical assessment of corticolous myxomycetes on the living tree bark of *Cryptomeria japonica* in a region of western Japan. *Biogeography* 22: 35-42. doi: org/10.11358/bioge.22.35
- Wrigley de Basanta, D. 2000: Acid deposition in Madrid and corticolous myxomycetes. *Stapfia* 73: 113-120.
- Yamamoto, Y. 1998: *The myxomycete biota of Japan*. Toyo Shorin Publishing Co.: Tokyo. (in Japanese)