

# Diversity of corticolous myxomycetes in the last glacial refugia of *Cryptomeria japonica* in Japan

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## Abstract

*Cryptomeria japonica*, commonly known as Japanese cedar, is now widely distributed from glacial refuges to the entire Japanese archipelago, after the last ice age. The bark surface provides a habitat for many corticolous myxomycetes. Although corticolous myxomycetes are known to prefer tree species, the association between myxomycete distribution and host tree (*C. japonica*) divergence across the refuges has not been investigated. In this study, myxomycete communities in five refuges were assessed and compared with those in 14 peripheral areas. Bark samples were collected from at least 10 trees per site and were subjected to the moist chamber culture method (10 Petri dishes per tree) to examine the myxomycete fruiting bodies strictly. Environmental variables such as geographical location, climate condition, and bark traits (tree size, bark pH, and electric conductivity) were measured. Fruiting bodies appeared in 91% of the cultures, and 32 taxa (31 species and one varie-

ty) were recorded. Comparison of the communities between refuges and peripheral sites showed six myxomycete species, *Arcyria cinerea*, *Macbrideola argentea*, *Cribraria minutissima*, *Clastoderma debaryanum*, *Physarum viride* and *Physarum pusillum*, were significantly more abundant in the refuges and these communities preserved higher species diversity. By nonmetric multidimensional scaling, the communities in the Pacific side and the Sea of Japan side were ordered based on snow cover depth, in a pattern similar to the phylogenetic distribution of the host tree. Myxomycete groups were identified in the northern region, the Sea of Japan region, and the southern region (including Yakushima Island) of Japan. Thus, the refugial tree populations preserved the myxomycete species diversity on their bark and functioned as an important hotspot for myxomycetes. The distribution of corticolous myxomycetes was associated with the diversification and biogeographical distribution history of their host tree, *C. japonica*.

## Introduction

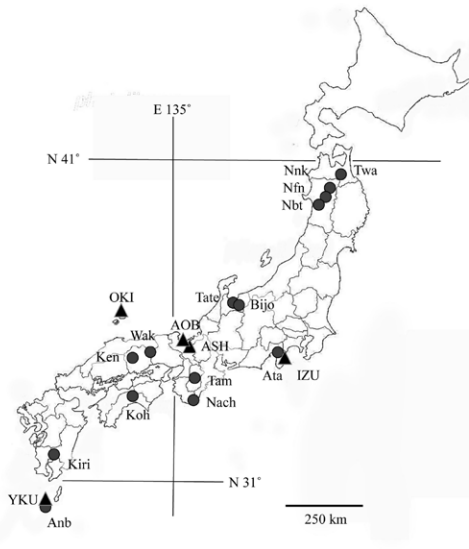
Myxomycetes (myxogastrids or plasmodial slime molds) are a group of amoeboid protists characterized by a stage of fruiting body formation in their life cycle (Stephenson & Rojas 2017). Their tiny and ephemeral fruiting bodies release spores that are mainly dispersed by the wind across long distances; they can be found in almost all terrestrial ecosystems (Stephenson et al. 2008). Myxomycetes inhabit various substrates, such as coarse woody debris, plant detritus, and living trees' bark surface. A group of myxomycetes called corticolous myxomycetes is found on the bark of living trees; they inhabit various tree species' bark and reproduce within a short life cycle in a dry environment (Keller & Brooks 1973).

Many corticolous myxomycete species have been shown to prefer the Japanese cedar (*Cryptomeria japonica* (Thunb. ex L. f.) D. Don) (Takahashi 2014) because the bark surface of this tree has a fibrous smooth carpet structure with abundant vertical grooves that provide a microhabitat for myxomycetes. Thirty-six myxomycete taxa have been reported on *C. japonica* bark across Japan

(Takahashi et al. 2018, Takahashi & Harakon 2018). However, the tree bark of *C. japonica* has distinctive protective and biological activities and is involved in defense of the tree; for instance, compounds with antifungal properties (e.g., ferruginol) have been identified in *C. japonica* bark (Kofujita et al. 2001), and the strongly acidic bark is found to demonstrate antibacterial properties (Cheng & Chang 2014). Despite these antibiotic properties, many myxomycetes live on the bark surface. As myxomycete species preference for different tree species has been reported in previous studies (Takahashi 2014, Vaz et al. 2017), a specific relationship is also presumed to exist between corticolous myxomycetes and a particular tree species.

Pollen analysis has shown that during the last glacial period (approximately 18,000 years ago), *C. japonica* was restricted to specific refuges such as the Wakasa Bay coast and Okinoshima Island along the Sea of Japan side, and the Izu Peninsula and Yakushima on the Pacific side of the Japanese archipelago (Tsukada 1982, Tsukada 1986). As the climate warmed after the glacial period, the distribution range of *C. japonica* extended from the original forest refuges but was limited to the Japanese archipelago and remained an endemic species over time. Two phylogenetic populations of *C. japonica* were distinctively formed along the Pacific and Sea of Japan side (Yasue et al. 1987). Currently, their range extends from Yakushima in Kagoshima Prefecture in the south to Serigasawa in Aomori Prefecture in the north (a distance of approximately 1,500 km). Tree expansion from the refuges occurred from approximately 7,000 to 3,000 years ago through the Japanese Islands (Sugita & Tsukada 1983). Phylogenetic DNA analysis of allelic variation in natural populations of *C. japonica* revealed that the original refuge populations have been less affected by genetic drift than other populations and demonstrate high allelic variation (Takahashi et al. 2005). Another study indicated that its long-term isolation from other populations could explain the divergence of the *C. japonica* population of Yakushima Island and that its population in northern Tohoku district is likely to have been established by cryptic northern refugia on the coast of Japan Sea to the west of the archipelago (Kimura et al. 2014).

The present study investigated the distribution



**Fig. 1.** Survey sites of corticolous myxomycetes on *Cryptomeria japonica* tree bark in the Japanese archipelago. ▲: Sites in glacial refuges ●: Peripheral sites

of myxomycete communities in *C. japonica* trees' original refuge populations and compared their community structures between glacial refuges and peripheral regions. The study aimed to reveal species diversity of corticolous myxomycetes in the host tree refugia and the host-related phylogenetic associations between *C. japonica* and myxomycetes.

## Materials and methods

### Survey sites

Corticolous myxomycetes were assessed on *C. japonica* trees' bark, an endemic species in the Japanese archipelago, and one of the most important forest trees with broad use its functional attributes, for example, in construction and housing materials. Although forests cover approximately 70% of the Japanese archipelago, natural populations of *C. japonica* are limited in remote and specific places be-

cause of historical over-exploitation. Consequently, natural populations are now protected to conserve their genetic resources.

In the present study, survey sites were selected in refuge forests comprising long-lived natural forests and old-growth artificially planted forests were found at the periphery of the refuges. Five sites in the refuges and fourteen sites in the periphery are listed in **Table 1**, describing the forest type, latitude, longitude, altitude, and climate conditions, and the 19 survey sites were geographically located from the southwest to northeast, from 30.30°N to 40.43° N and 130.56°E to 140.89°E. Their altitudinal locations ranged from 90 to 1,134 m in the Japanese Islands (**Fig. 1**).

The five sites in the refuge natural forests were as follows (**Table 1**): Yakusugi land (30.3090°N, 130.5690°E, 1,134 m altitude, sampled on August 27, 2019) in Yakushima-cho of Kagoshima Prefecture and Izu Shiranuta (34.8535°N, 139.0093°E, 813 m altitude, sampled on April 29, 2019) in Izu Peninsula of Shizuoka Prefecture on the Pacific side; Ashyu (35.2856°N, 135.7267°E, 789 m altitude, sampled on November 11, 2019) in Miyama-cho of Kyoto Prefecture, Aobayama (35.5051°N, 135.4814°E, 625 m altitude, sampled on June 2, 2019) in Takahama-cho of Fukui Prefecture, and Shizenkaikinomori (36.2678°N, 133.3217°E, 422 m altitude, sampled on June 8, 2019) in Okinoshima Dougo island of Shimane Prefecture on the Sea of Japan side.

The fourteen peripheral sites surveyed in this study were listed in **Table 1** and ordered according to latitude, from south to north. These sites located along the Pacific side and the Sea of Japan side (**Fig. 1**) were Ambo (plantation, 249 m, sampled on August 27, 2019) in Yakushima-cho, Kirisimayama (plantation, 870 m, sampled on August 29, 2019) in Kirishima city of Kagoshima Prefecture, Shenbon-yama (natural forest, 880 m, sampled on November 29, 2019) in Umaji-mura of Kochi Prefecture, Nachi (shrine, 142 m, sampled on November 19, 2019) in Wakayama Prefecture, Tamaki shrine (natural forest, 963 m, sampled on November 18, 2019) in Tot-sukawa-mura of Nara Prefecture, and Ashitakayama (plantation, 768 m, sampled on December 8, 2018) in Susono-city of Shizuoka Prefecture on the Pacific side; Kenashiyama (natural forest, 782 m, sampled on September 29, 2019) in Shinjo-son, Wakasugi natural forest (natural forest, 1050 m, sampled on

May 6, 2019) in Nishiwakura-son in Okayama Prefecture, Bijodaira (natural forest, 1043 m, sampled on July 15, 2019) and Oyama shrine-Maetateshadan (shrine, 181 m, sampled on July 15, 2019) in Tateyama-cho of Toyama prefecture, Nibetu (natural forest, 221 m, sampled on May 1, 2019) in Akita-city, Nifuna Mizusawa (natural forest, 155 m, sampled on May 2, 2019) in Futatsui-cho, and Nanakurayama (natural forest, 91 m, sampled on May 2, 2019) in Futatsui-cho of Akita Prefecture close to the Sea of Japan side; and Towada shrine (shrine, 412 m, sampled at May 3, 2019) in a mountain lake area in Towada-city of Aomori Prefecture.

The geographical locations (latitude, longitude, altitude) were obtained from the Geospatial Information Authority of Japan (<https://www.gsi.go.jp/tizu-kutyu.html>, accessed on March 5, 2020). Climate data (annual mean temperature, coldest month temperature, annual precipitation, and maximum snow cover depth) were obtained from the Japan Meteorological Agency (<https://www.data.jma.go.jp/obd/stats/etrn/index.php>, accessed on March 5, 2020) (Table 1). Climatic characters at survey sites were indicated using the average data for 1981–2010 from the neighboring observation points; the temperature for the survey sites was adjusted based on a temperature decrease rate of  $-0.55\text{ }^{\circ}\text{C}$  per 100 m elevation. The mean annual temperatures across the survey sites ranged from  $7.5\text{ }^{\circ}\text{C}$  to  $19.2\text{ }^{\circ}\text{C}$ , and the mean temperature of the coldest month ranged from  $-3.8\text{ }^{\circ}\text{C}$  to  $11.5\text{ }^{\circ}\text{C}$ . The regional mean annual precipitation ranged from 1,563 to 4,477 mm, and maximum snow cover depth ranged from 0 to 171 cm.

### Bark sampling

The average diameter at breast height (DBH) of the sampled trees was 95 cm and ranged from 44 to 133 cm (Table 1). Among the peripheral sites, there were eight natural forest sites, three mature plantation forest sites, and three sites with long-lived trees in shrine areas. The average DBHs of sampled trees in the natural forest sites, mature plantation forest sites, and shrine sites were 125 cm, 65 cm, and 126 cm, respectively. Barks were sampled from 10–16 trees per site (total  $n = 220$  trees), with the bark stripped in approximately  $800\text{ cm}^2$  area around the tree stem's entire diameter. The bark without epi-

phytes at 50–200 cm above the ground was sampled manually and then placed in a paper bag.

Bark sampling was performed year-round because corticolous myxomycetes in moist chamber cultures were not affected by seasonal cues (Härkönen & Ukkola 2000); thus, myxomycete fruiting bodies were found in the moist chamber culture whenever the bark was sampled.

### Moist chamber cultures

The moist chamber culture method is useful for observing corticolous myxomycetes (Stephenson & Rojas, 2017). Bark samples were air-dried for approximately one week at room temperature and then placed in moist chamber cultures with 10 plastic Petri dishes (9 cm in diameter) per tree, as described by Takahashi et al. (2018). Bark samples were cut into 3–8-cm-long pieces. Approximately 5 g bark (dry weight, around  $50\text{ cm}^2$ ) was placed on a clean filter paper in each dish. Each dish was filled with approximately 25 mL distilled water (pH 6.9) and covered, and the bark was soaked for 3 days. The pH and electric conductivity (EC) of the resulting exudate were measured with a compact pH and EC meter, respectively (Horiba, Kyoto, Japan). The bark pH and EC were determined as the median and mean of five exudate samples per tree, respectively. At least 100 cultures were incubated for each survey site, with a total of 2,200 cultures.

The dishes were continually drained of excessive water and incubated for 3 weeks at  $23\text{ }^{\circ}\text{C}$  to develop myxomycetes. Next, the cultures were stored with their lids half-open to dry the bark samples. As the corticolous myxomycetes have a short lifecycle, the sporulation typically developed in 10–70 days from moist chamber cultures (de Basanta 2000). The fruiting bodies were assessed at 30–50 days using a dissecting stereomicroscope, and the internal structures were checked to identify the species by microscopic observation. All samples within a culture batch were assessed within a 2-week period. Successful cultures (i.e., those with sporangia) were recorded as positive cultures. The percentage of positive cultures was obtained by dividing the number of dishes with sporangia by the total number of cultured dishes. The species abundance was estimated from the number of the positive culture

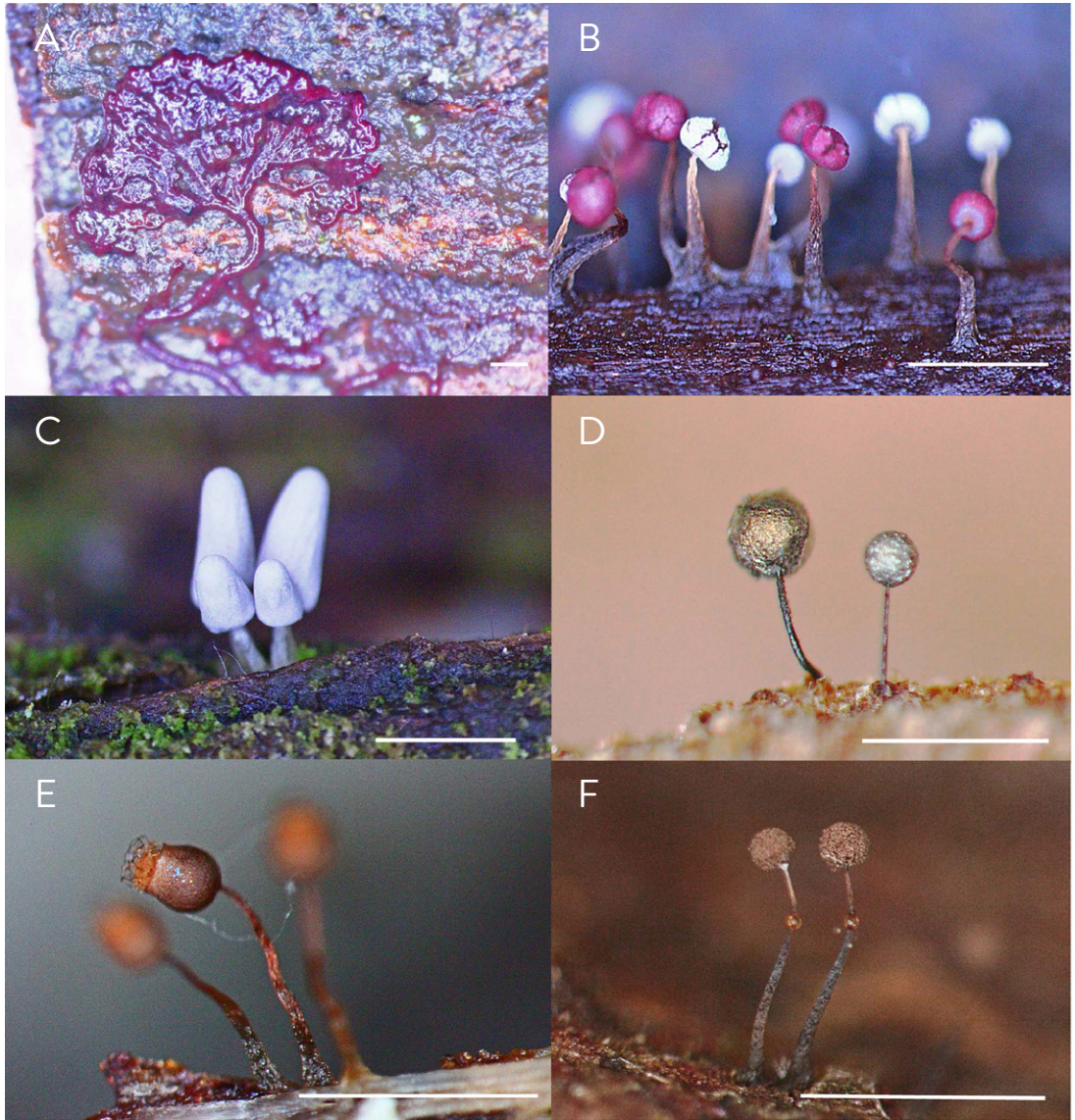
**Table 1.** Information of the 19 survey sites in the *Cryptomeria japonica* refuges, and the peripheral sites on the Pacific side and the Sea of Japan side including environmental variables such as geographic location, climate factors, tree traits, and myxomycete communities. a: natural forest, DBH: diameter at breast height, EC: electric conductivity

Geographical location	Code	Latitude (N°)	Longitude (E°)	Altitude (m)	Annual mean temperature (°C)	Colest month mean temperature	Annual precipitation (mm)	Maximum snow depth (cm)	Number of trees	DBH (cm)	Bark pH	EC (μS/cm)	Positive culture (%)	Number of species per tree	Species richness	Species diversity (H')	Equitability (J)	Chao-1
<b>Refuges</b>																		
Yakusugi-land <sup>a</sup>	YAK	30.3090	130.5690	1134	13.4	5.6	4477	0	14	133	3.7	154	74	5.8	16	2.18	0.786	18
Izu <sup>a</sup>	IZU	34.8535	139.0093	813	12.0	2.8	2779	1	11	44	3.8	92	100	9.2	17	2.29	0.808	18
Ashu <sup>a</sup>	ASH	35.2856	135.7267	789	9.7	-1.5	1752	55	16	108	3.6	176	98	8.0	19	2.44	0.828	20
Aobayama <sup>a</sup>	AOB	35.5051	135.4814	625	11.1	0.1	1827	35	11	70	3.7	171	100	9.9	18	2.52	0.872	18
Okinoshima <sup>a</sup>	OKI	36.2678	133.3217	422	12.1	2.0	1795	29	11	122	3.6	159	100	9.9	19	2.49	0.846	19
<b>The Pacific Ocean side</b>																		
Anbo	Anb	30.3138	130.6291	249	19.2	11.5	3246	0	10	49	3.9	94	97	6.5	17	2.23	0.787	18
Kirishima	Kiri	31.8885	130.8751	870	12.5	2.4	2642	0	10	73	3.3	192	81	4.2	9	1.73	0.786	9
Senbonyama <sup>a</sup>	Koh	33.6615	134.0928	880	11.2	0.6	2774	2	10	161	3.4	183	53	3.4	7	1.74	0.896	7
Nachi shrine	Nch	33.6710	135.8977	142	16.2	6.5	3127	0	10	179	3.9	98	67	4.0	10	2.10	0.913	10
Tamaki-shrine <sup>a</sup>	Tam	33.9249	135.8319	963	10.1	-0.4	3886	2	11	130	3.7	126	98	6.1	17	2.34	0.827	19
Ashtakayama	Ata	35.2368	138.8395	768	11.2	0.9	2819	0	12	74	3.8	210	100	7.7	18	2.47	0.856	18
<b>The Sea of Japan side</b>																		
Kenashiyama <sup>a</sup>	Ken	35.2290	133.5226	782	9.3	-1.8	1726	57	10	104	3.7	106	100	7.8	14	2.22	0.842	15
Wakasugi <sup>a</sup>	Wak	35.2518	134.3932	1050	8.4	-3.2	1647	27	13	73	3.5	166	100	7.5	13	2.27	0.883	13
Bijodaira <sup>a</sup>	Bijo	36.5824	137.4667	1043	7.5	-3.8	2970	171	13	181	3.5	152	95	5.2	15	2.17	0.801	16
Oyama-shrine	Iate	36.6079	137.3143	181	12.2	0.9	2970	62	11	92	3.9	82	97	6.5	14	1.99	0.754	14
Nibestu <sup>a</sup>	Nbt	39.8016	140.2626	221	9.6	-2.1	2185	37	11	103	3.2	631	91	8.5	16	2.30	0.830	16
Nifuna <sup>a</sup>	Nfn	40.0962	140.2445	155	9.5	-2.4	1995	115	14	112	3.2	457	93	6.3	14	1.93	0.731	16
Namakura <sup>a</sup>	Nnk	40.1997	140.2531	91	9.9	-2.0	1671	73	12	135	3.1	681	83	6.1	13	2.22	0.864	13
Towada-shrine	Twa	40.4327	140.8921	412	7.8	-3.8	1563	53	10	107	3.3	529	100	7.3	14	2.22	0.841	15

**Table 2.** Corticolous myxomycete species and abundance on *Cryptomeria japonica* tree bark in the glacial refuges and the peripheral sites of the Pacific side and the Sea of Japan side. Italics indicate relative abundance in total.

\*: natural forest. Significance  $p < 0.01$ ,  $p < 0.05$ .

Code	Refuges				The Pacific side				The Sea of Japan side				Total Refuges	Peripheral	Relative abundance (%)	Number of sites										
	YAK*	IZU*	ASH*	AOB*	OKI*	Anb	Kri	Koh*	Nch	Tam*	Asa	Ken*					Wak*	Bijo*	Tate	Nbt*	NFn*	Nnk*	Twa			
<i>Acyria cinerea</i> (Bull.) Pers.	48	97	109	89	62	27	27	3	13	32	36	30	31	15	49	104	120	70	62	405	**	619	16.1	19		
<i>Cibarioa confusa</i> Nann.-Bremek. & Y. Yamam.	28	51	91	79	44	10	44	29	22	24	43	52	72	10	89	27	94	70	36	293		622	14.4	19		
<i>Panarchaeopsis rigida</i> (Brändza) Nann.-Bremek.	24	63	54	45	34	29	8	8	5	17	38	30	38	68	57	40	69	66	63	220		536	11.9	19		
<i>Cibarioa microcarpa</i> (Schrad.) Pers.	35	1	8	28	100	76	34	8	20	9	83	39	22	2	88	56	13	6	6	172		456	9.9	18		
<i>Macbrideola argentea</i> Nann.-Bremek. & Y. Yamam.	6	54	68	51	65	13	1	11	17	17	17	72	91	46	12	29	40	12	5	244	**	361	9.5	17		
<i>Hemitrichia veltiana</i> Nann.-Bremek. & Y. Yamam.	36	32	37	19	39	58	18	14	8	60	32	33	31	29	21	16	10	12	5	163		347	8.0	17		
<i>Physarium nutans</i> Pers.	2	19	45	50	22	26		7	9	35		3	74	1	6	24	5	9	14	138		213	5.5	19		
<i>Eriotherisma melanospermum</i> T. Macbr. & G. W. Martin	4	49	21	12	31			7	9	10		3	41	21	27	14	15	14	42	117		194	4.9	15		
<i>Cibarioa minutissima</i> Schwein.	1	43	14	54	17	5		7	9	1		33	16	6	19	12	26	28	28	129	**	162	4.6	16		
<i>Physarium nutans</i> var. <i>rubrum</i> (Nann.-Bremek. & Y. Yamam.) Chao H. Chung	6		25	32	27		2		14	2		23	65	8	15	1	8	5	5	90		143	3.7	14		
<i>Comaricia pulchella</i> (C. Bab.) Rostaf.	7	4	11	5	7	1		3	3	4		7	10	5	2	18	1	36	1	56		88	2.3	14		
<i>Lecan variable</i> Schrad.						14	4	13	1	19		1	3	3	3	4	4	3	25	34		69	1.6	14		
<i>Dilema chionoderma</i> (de Bary & Rostaf.) Kuntze	1	8	1	8	15				4	2					15	15		1	1	51	**	25	1.2	9		
<i>Clastoderma debaryanum</i> A. Blytt	3	9	22	17	3	3			14					4	3	1				23	*	22	0.7	8		
<i>Physarium viride</i> (Bull.) Pers.		1	11	9	2							14								18		27	0.7	9		
<i>Echinostellum minutum</i> de Bary	5	7	3	2	1	14				3				9	1					18		27	0.7	9		
<i>Macbrideola comae</i> (G. Lister & Cran) Alexop.						3				35		3	1									39		0.6	3	
<i>Panarchaeopsis solitaria</i> (Nann.-Bremek.) Nann.-Bremek. Nann.-Bremek.	6													27						6		27		0.5	2	
<i>Colario elegans</i> (Racib.) Dhillon & Nann.-Bremek., ex Ing				2												3	3	17	9	2		29		0.5	4	
<i>Macbrideola confusa</i> Nann.-Bremek. & Y. Yamam.	4			3		3				14				4	1					7		22		0.5	6	
<i>Comaricia laxa</i> Rostaf.	2							2											17	2		19		0.3	3	
<i>Physarium pusillum</i> (Bierk. & Curtis) G. Lister			14						1											14	**	1			0.2	2
<i>Eriotherisma papillatum</i> (Pers.) Rostaf.	3		3			3				6										6		9			0.2	4
<i>Lycogala exiguum</i> Morgan		6		1					2						3	2				7		7			0.2	5
<i>Macbrideola marioni</i> (Alexop. & Benek.) Alexop.			9																	9					0.1	1
<i>Hemitrichia minor</i> G. Lister									4					1								5			0.1	2
<i>Clastoderma microcarpum</i> (Mey.) Kowaliki			1						1											1		1			0.03	2
<i>Lecan erecta</i> K. S. Thind & Dhillon														2								2			0.03	1
<i>Lecan kleisobolus</i> G. W. Martin						1							1									2			0.03	2
<i>Lecan minus</i> Fr.																						2			0.03	1
<i>Stemonitis smithii</i> T. Macbr.					2																	2			0.03	1
<i>Stemonaria gracilis</i> Nann.-Bremek. & Y. Yamam.	1																			1					0.02	1
<b>Total abundance</b>	210	447	539	546	501	288	141	80	102	222	393	333	495	247	287	420	429	356	314	2243		4107		100		



**Fig. 2.** Myxomycete plasmodium and fruiting bodies that appeared in the moist chamber culture of *Cryptomeria japonica* tree bark from a refuge site. **a:** Red plasmodium of *Physarum nutans* var. *rubrum*, **b:** Fruiting bodies of *P. nutans* and *P. nutans* var. *rubrum*, **c:** Fruiting bodies of *Arcyria cinerea*, **d:** *Macbrideola argentea*, **e:** *Cribraria minutissima*, **f:** *Clastoderma debaryanum*. Scale bar = 1 mm.

dishes in which sporangia appeared. The number of myxomycete species identified per tree and per site was recorded. Species richness and abundance were recorded for each tree and survey site.

According to Yamamoto (1998), myxomycete species classification was performed, and the nomenclature used in the most recent study (Lado 2005-2020) was followed. Voucher specimens were prepared using separate paper boxes for each species. Bark with myxomycete fruiting bodies was glued to the bottom of each box, and the collection boxes were stored at the Tottori Prefectural Museum.

### Data analysis

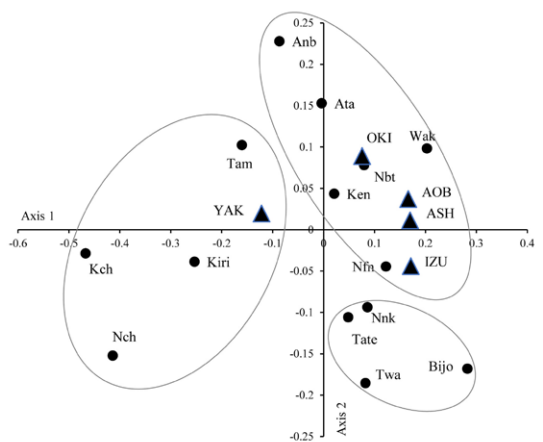
The observed number of taxa at each site was estimated by comparing the presumed number of taxa by Chao 1 (Chao 1984) using PAST software (Hammer et al. 2001). The number of taxa was counted, which comprised the number of species and varieties of species.

The relevance of the environmental factors described in **Table 1** was assessed by correlation analyses performed using the Bell Curve for Excel software (SSRI Co., Ltd., Tokyo, Japan). The relative abundance of different species was calculated by dividing the abundance of a given species by the site's community's cumulative abundance. We defined dominant species as those with >5% relative abundance at the site community level and recorded 10 or more sites. To quantify myxomycete species diversity at different sites, Shannon–Wiener diversity index ( $H'$ ) (Shannon & Weaver 1963) and equitability ( $J'$ ) were calculated.

The myxomycete community (i.e., of all 19 sites collectively) was analyzed by nonmetric multidimensional scaling (NMDS; Kenkel & Orlóci 1986), which is a method to examine the community structure and produces an ordination based on a dissimilarity matrix of communities, and is often used in the ecological analysis for community structure (e.g., Takahashi & Hada 2010, Park et al. 2014, Novozhilov et al. 2018). NMDS was performed using PAST software, based on Bray–Curtis dissimilarities (Bray & Curtis 1957). The communities were plotted and ordinated according to the NMDS scores of the first two axes in **Figure 3**. Grouping the communities was performed by cluster analysis (Ward's method) for the NMDS

scores of the first two axes. Correlation analyses were performed between the first two NMDS scores and environmental variables, such as latitude, longitude, altitude, annual mean temperature, the average temperature of the coldest month, annual precipitation, snowfall, and tree traits (DBH, bark pH, and EC) to identify important environmental factors.

Fisher's exact probability test of independence was used to compare the abundance of species between refuges and peripheral sites and the selected characteristic species in a site because of its observed efficacy in other studies (e.g., Takahashi & Hada 2010, Chytrý et al. 2002). When the test indicated that the abundance of a given community species was significantly greater ( $p < 0.01$ ) than zero, the species was considered to prefer this microhabitat. Species with fewer than 10 observations and three sites across all survey sites were excluded from the analysis. Differences in averages were evaluated using Tukey's honest significant difference test. Statistical tests were performed using Esumi Excel Statistics 5.0 software (Esumi Co. Ltd, Tokyo, Japan).



**Fig. 3.** Ordination plots of nineteen corticolous myxomycete communities analyzed by nonmetric multidimensional scaling (NMDS), of which stress value was 0.172. Coefficient of determination was for first axis,  $r^2 = 0.726$ , and second axis,  $r^2 = 0.091$ . ▲: Glacial refuge sites, ●: Peripheral sites. Ellipses indicate approximate groups of the communities as determined by the cluster analysis (Ward's method) of NMDS scores for the first two axes.



## Results

### Environmental factors

Latitudinal changes and geographical locations affected the climatic conditions, which tended to decrease the annual mean temperature ( $r = -0.667$ ,  $p < 0.01$ ), coldest month temperature ( $r = -0.748$ ,  $p < 0.01$ ), and precipitation ( $r = -0.663$ ,  $p < 0.01$ ), but increased the snow maximum accumulation ( $r = 0.602$ ,  $p < 0.01$ ) from the south to north. The maximum snow depth was greater on the Sea of Japan side than on the Pacific side. The latitudinal variance did not correlate significantly with variance in the longitude, altitude, and DBH, but was significantly correlated with bark pH ( $r = -0.640$ ,  $p < 0.01$ ) and EC ( $r = 0.772$ ,  $p < 0.01$ ). The bark pH was generally strongly acidic (average pH ranged from 3.1 to 3.9), and average EC ranged from 82 to 529  $\mu\text{S}/\text{m}$ . The bark pH was significantly negatively correlated with the EC ( $r = -0.841$ ,  $p < 0.01$ ). The average bark pH at refuge sites was not significantly different from that at the Pacific sites and Sea of Japan sites.

### Characteristics of myxomycete communities and species

Myxomycete fruiting bodies have developed in at least 53% of cultures at a site, with an average of 91% of cultures across all sites (Table 1). The observed species richness based on the sampling effort from at least 10 trees per site and the culture of 10 dishes per tree reached a stable number of species, which was estimated from Chao 1 (Table 1). The number of taxa comprising species and intraspecific variety was 7–19 taxa per site. The total number of taxa at all survey sites was 32 (Table 2), i.e., *Physarum nutans* Pers. and *Physarum nutans* var. *rubrum* (Nann.-Bremek. & Y. Yamam.) Chao H. Chung were synonyms of *Physarum album* (Bull.) Chevall. but only distinct taxa were counted in this list. The two taxa had different peridium colors (Fig. 2) (white or red) and ecologically different characters, i.e., *Physarum nutans* commonly distributed at almost all sites. In contrast, *Physarum nutans* var. *rubrum* was specifically distributed at higher montane sites in natural forests. The number of taxa at five refuge sites was

27, with a range of 16–19 taxa per site; at peripheral sites (Pacific sites and Sea of Japan sites), the richness of the taxa was 29, with a range of 9–18 taxa per site (Table 1). The taxa per tree were 5.8–9.9 at the refuge sites and 3.4–8.5 at the peripheral sites.

Species diversity ( $H'$ ) ranged from 2.18 to 2.52 at the refuge sites and 1.73 to 2.47 at the peripheral sites (Table 1). The mean value of species diversity at the refuge sites ( $H' = 2.38$ ) was significantly higher than that at the peripheral sites ( $H' = 2.14$ ) according to Tukey's test ( $p < 0.05$ ), but the equitability score was slightly lower of  $J' = 0.612$  at the refuge sites than at the peripheral sites ( $J' = 0.647$ ).

The four most abundant species were recorded at all sites were *Arcyria cinerea* (Bull.) Pers., *Cribraria confusa* Nann.-Bremek. & Y. Yamam., *Paradiacheopsis rigida* (Brândza) Nann.-Bremek., and *Hemitrichia velutina* Nann.-Bremek. & Y. Yamam. (Table 2). The relative abundances of these species were cumulatively over 50% of the total. An additional nine taxa were recorded at 13 or more sites: *Cribraria microcarpa* (Schrad.) Pers., *Macbrideola argentea* Nann.-Bremek. & Y. Yamam. (Fig. 2), *Physarum nutans* Pers., *Enerthenema melanospermum* T. Macbr. & G.W. Martin, *Cribraria minutissima* Schwein, *Physarum nutans* var. *rubrum*, *Comatricha pulchella* (C. Bab.) Rostaf., *Licea variabilis* Schrad., and *Diderma chondrioderma* (de Bary & Rostaf.) Kuntze. Additionally, 19 species temporarily appeared in less than ten sites and were rare, with a relative abundance below 1.4%.

Upon comparing populations between refuges and peripheral sites by Fisher's exact test, six species were detected to be significantly more abundant in the refuges than in the peripheral sites. These characteristic species in refuges are shown in Fig. 3 and include *Arcyria cinerea*, *Macbrideola argentea*, *Cribraria minutissima*, *Clastoderma debaryanum* A. Blytt, *Physarum viride* (Bull.) Pers., and *Physarum pusillum* (Berk. & M. A. Curtis) G. Lister (Table 2).

### Ordination of myxomycete communities

The corticolous myxomycete communities of the 19 sites were plotted using the first two axes scores of the NMDS analyses (Fig. 3). The NMDS plots illustrated divergence in the community composition

of myxomycetes between five refuges and fourteen peripheral sites. The myxomycete communities of the refuges were all found nearly in the central part of the NMDS plots, indicating the similarity of these communities; of these, three sites (Ashyu, Aobayama, and Okinoshima Island) around the Wakasa Bay coast along the Sea of Japan coast formed a core placement. The Izu refuge site, which is on the Pacific side, was situated close to the refuge sites on the Sea of Japan side. In contrast, the Yakushima Island refuge site was separated and located far in the first axis's negative domain. In the positive domain of two axes, the Nibetsu site of Akita Prefecture in northern Japan was plotted close to the Wakasugi and the Kenashiyama site in Chugoku montane of western Japan, adjacent to the refuge sites of Okinoshima Island and Wakasa-wan Bay along the Sea of Japan side. According to the first axis, all the investigated communities were divided into the Pacific side or the Sea of Japan side.

Cluster analysis results were mostly grouped into three assemblages, which were broadly represented by ellipses in Fig. 3 with a confidence level of 49%. The five sites on the Pacific side, including Yakushima island refuge, were marked in the negative dimension of the first axis (Group 1). Ten sites mostly plotted in the first and second axes' positive dimension included three refuges in the Sea of Japan side and one refuge in the Izu Peninsula (Group 2). Four sites in the northern region plotted positively in the first axis dimension and negatively in that of the second axis (Group 3).

The placement of these communities was associated with the species composition and community structure. The first axis scores were positively correlated with the number of species per tree and total species richness of a site ( $r = 0.697$ ,  $p < 0.01$  and  $r = 0.633$ ,  $p < 0.01$ , respectively). In addition, the index of species diversity was comparatively correlated with the first axis scores ( $r = 0.545$ ,  $p < 0.05$ ), and the equitability index ( $J'$ ) were weakly negatively correlated with the first axis scores ( $r = -0.460$ ,  $p < 0.05$ ) (Table 3).

### Relationships with environmental factors

Environmental factors influencing the myxomycete communities' NMDS ordination were examined by correlation analysis (Table 3). The NMDS score of

**Table 3.** Correlation coefficients between the NMDS scores of the first two axes for myxomycete species diversity and environmental variables.

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

	Axis 1		Axis 2
<b>Myxomycetes</b>			
Number of species per tree	0.697	**	0.382
Species richness	0.633	**	0.469
Species diversity ( $H'$ )	0.545	*	0.427
Equitability ( $J'$ )	-0.460	*	-0.122
<b>Geographical location</b>			
			≤
Latitude	0.519	*	-0.405
Longitude	0.426		-0.421
Altitude	0.009		0.163
<b>Climate factors</b>			
Annual mean temperature	-0.535	*	0.314
Coldest month mean temperature	-0.519	*	0.345
Annual precipitation	-0.483	*	0.111
Snow cover depth	0.605	**	-0.482
<b>Tree traits</b>			
DBH	-0.326		-0.518
Bark pH	-0.141		0.309
EC	0.223		-0.225

the first axis primarily showed a positive correlation with maximum snow cover depth ( $r = 0.605, p < 0.01$ ), annual mean air temperature ( $r = -0.535, p < 0.05$ ), and coldest month temperature ( $r = -0.519, p < 0.05$ ). There was also a negative correlation between the first axis and latitude ( $r = -0.519, p < 0.05$ ). Sites at the Sea of Japan and the Pacific were remarkably separated into distinctive groups associated with geographical and climatic conditions. The second axis did not show significantly high correlation coefficients for any environmental factors.

The relative abundances of myxomycete species were examined for their correlation with environmental factors, which were effective factors for the NMDS first axis, i.e. latitude, annual mean temperature, and snow cover depth (Table 4). An increase in latitude was negatively correlated with *H. velutina* ( $r = -0.734, p < 0.01$ ) and *Echinostelium minutum* de Bary ( $r = -0.674, p < 0.01$ ). The annual mean temperature was negatively correlated with the occurrence of *M. argentea* ( $r = -0.538, p < 0.01$ ) and was positively correlated with the occurrence of *C. microcarpa* ( $r = 0.576, p < 0.01$ ), *D. chondrioderma* ( $r = 0.649, p < 0.01$ ), and *E. minutum* ( $r = 0.876, p < 0.01$ ). The maximum snow depth was positively correlated with the occurrence of *P. rigida* ( $r = 0.774, p < 0.01$ ).

The altitudinal location further influenced the distribution of some species. Higher montane sites positively influenced the relative abundance of three species, i.e. *P. viride* ( $r = 0.613, p < 0.05$ ), *Lycogala exiguum* Morgan ( $r = 0.655, p < 0.05$ ), and *P. nutans* var. *rubrum* ( $r = 0.407, p < 0.05$ ), whereas the abundance of *D. chondrioderma* decreased with increasing altitude ( $r = -0.616, p < 0.01$ ).

## Discussion

Japanese forests have a dorsoventral distribution of vegetation on the Pacific side and the Sea of Japan side. Physical factors such as low temperature and snowslides or snowy pressure have a major impact on plant growth (e.g. Shimano 2006). The two separated populations of *C. japonica* have different traits in their regeneration systems (seedlings in the Pacific side and regeneration by laying in the Sea of

Japan side, Taira et al. 1997) and the diterpene components in the leaf (Yasue et al. 1987). After climate amelioration in the late-glacial period, *C. japonica* (Japanese cedar) populations expanded northward from their glacial refuges and upslope across Japan; currently, *C. japonica* is phytogeographically distributed on the Pacific side and the Sea of Japan side (Tsukada 1982, 1986). These distribution patterns of *C. japonica* are climatically influenced by snow cover depth (Kimura et al. 2013). Speciation of the Japanese cedar has resulted from adaptation to environmental differences; the Sea of Japan side is characterized by snowfall in the winter season. The Pacific side receives rain in the summer season. In the present study, the distribution pattern of corticolous myxomycete communities corresponded exactly to the divergence pattern of the host tree, *C. japonica*, and was thus divided into two groups, the Sea of Japan side and the Pacific side.

The refuge communities of myxomycetes had higher species diversity than the peripheral communities. The highest species diversity was in Aobayama ( $H' = 2.52$ ) in the vicinity of Wakasa Bay along the Sea of Japan. The Wakasa Bay communities (OKI, AOB, and ASH) were located adjacent to IZU on the Pacific side in the NMDS plot (Fig. 3). Thus, they are presumed to have a continuous relationship within the mainland, showing a higher similarity with each other. The distribution of *C. japonica* was originated in the Last Pleistocene (around one million years ago) and prospered in the Japanese archipelago (Taira 2001). The relationships with corticolous myxomycetes and host trees can be ongoing geographically and historically. After the last glacial period, historical geographic isolation from the mainland may have affected the community similarity as observed by the lower similarity of the Yakushima Island refuge site with the other refugia. In the ice age, *C. japonica* tree barks presume to provide microhabitats on the bark surface for many corticolous myxomycetes. The refuges communities today preserve the highest species diversity.

The communities ordinated by NMDS were approximately divided into three groups, mostly indicating the myxomycetes' divergent region (Fig. 3). Group 1 in the negative domain of the NMDS first axis consisted of the southern region in the Pacific sites associated with the Yakushima Island refuge

**Table 4.** Correlation coefficients between the relative abundances of myxomycete species and environmental variables. Significance \*\* $p < 0.01$ , \* $p < 0.05$ .

	Latitude	Altitude	Annual mean temperature	Snow cover depth		
<i>Paradiacheopsis rigida</i>	0.497	-0.114	-0.415	0.774	**	
<i>Hemitrichia velutina</i>	-0.734	**	0.463	0.386	-0.386	
<i>Cribraria microcarpa</i>	-0.385	-0.137	0.576	**	-0.373	
<i>Macbrideola argentea</i>	0.162	0.361	-0.538	**	0.387	
<i>Diderma chondrioderma</i>	-0.180	-0.616	**	0.649	**	-0.453
<i>Echinostelium minutum</i>	-0.674	**	-0.234	0.876	**	-0.275
<i>Physarum viride</i>	-0.566	*	0.613	*	-0.278	-0.407
<i>Lycogala exiguum</i>	-0.470	0.655	*	0.127	-0.548	
<i>Physarum nutans</i> <i>var. rubrum</i>	-0.228	0.407	*	-0.230	-0.173	

site. Group 2 was plotted in the positive domain of the first two NMDS axes located along the coast of the Sea of Japan side and showed close similarity despite their large geographical distance from each other. Group 3 was located in the positive and negative domain of the first two NMDS axes, including communities in the northern part of the mainland, i.e., Tohoku district and Toyama Prefecture, and may likely correspond to the cryptic northern refugia of *C. japonica* (Kimura et al. 2014). Although the bark pH occasionally influenced the inhabitation of corticolous myxomycetes on *C. japonica* tree bark (Takahashi 2017, Takahashi et al. 2020), the traits of the bark did not induce any significant difference in the average values among these three groups. Natural forests of *C. japonica* have been recently preserved as genetic resources in various places in Japan. These areas are still spotted in smaller than 100 hectares,

except at locations such as Yakushima Island, Nibetu in Akita Prefecture, and Bijodaira in Tateyama in Toyama Prefecture (Tsumura 2011). These widely conserved natural forests and refuge forests of *C. japonica* were found to preserve the original myxomycete community. They could preserve a specific structure of the communities based on the geographical location.

The present study indicated divergence of the corticolous myxomycetes from the host tree refuges to the Japanese archipelago. A recent genetic study of *C. japonica* (Tsumura et al. 2012) indicates four gene pool groups, the Sea of Japan region, the Pacific region, the northern Tohoku region, and southern Yakushima Island. Corticolous myxomycetes showed three groups, and so, the Pacific community group was not distinct because the Izu community showed a high similarity with that at the Sea of Ja-

pan sites. Therefore, appropriate survey sites along the Pacific region need to be selected in further studies. Overall, *C. japonica*'s refuges are hotspots for corticolous myxomycetes, whose divergence suggested two distinctive patterns, from the Yakushima Island refuge into the southwestern Pacific area and from the refuges around Wakasa Bay in the Sea of Japan side into the coast of the Sea of Japan. Furthermore, a distinctive group of myxomycete communities may be broadly existing as a group in the northern part of the mainland, reflected by the cryptic northern refugia of *C. japonica*.

The ordination of the communities was affected by snow cover depth associated with annual mean temperature and latitude. This principally indicates the dorsoventral difference in location between the Sea of Japan side and the Pacific side. The sites at the Sea of Japan side are in the northern latitudes, corresponding to the Japanese archipelago's geographical and topographical features and a large amount of snow in the winter season on the Sea of Japan side. The geographical and climatic difference phytogeographically results in varying antibacterial properties (Yasue et al. 1987) and reproductive systems (regeneration by laying branches, Taira et al. 1997) of *C. japonica*. However, it remains unclear whether the snow cover depth influences corticolous myxomycete inhabitation directly as a climatic factor or indirectly through changes in the host tree's bark traits. The bark pH, which decreased with increasing bark exudates (indicated by the EC), has influenced the community structures in other studies (Takahashi 2017, Takahashi et al. 2020), but did not affect the myxomycete distribution in this study. Other environmental factors (e.g., wind direction and strength, storm system) might have influenced the distribution/diversity of corticolous myxomycetes but were not investigated here.

Snow cover is needed for an ecological group of myxomycetes called snowbank (nivicolous) myxomycetes (Stephenson & Stempen 1994). The myxomycete inhabitation is also affected by snow cover depth and the remaining period. The fruiting bodies appear on plant litter at the snow melting line in spring and the alpine snowbank in summer. Thus, they are associated with the amount of snowfall, which is likely explained by the presence of a stable and long-lasting snow cover (Dahl et al. 2018). Al-

though it is unclear how snow cover depth affects myxomycetes living on the bark of living trees, it remains a research subject that global warming and climate change will influence the distribution of corticolous myxomycetes.

*Cryptomeria japonica* trees can live for more than 1,000 years, and thus, myxomycete communities are persistent on the long-lived trees in refuges, especially on Yakushima Island. Yakushima island forms the southern limit of *C. japonica* distribution and exhibits a specific myxomycete community that is not closely related to that in the other refugia. Yakushima was historically separated from the mainland to a satellite island because of climate warming and volcanic activity in the region from approximately 10,000 years ago. Thus, specific isolation from that time may indicate the specificity of the myxomycete community. The present study suggested that the distribution of the corticolous myxomycete communities migrated and synchronized with the phylogenetic distribution of *C. japonica*, resulting in the expansion of myxomycetes from glacial refuges.

A biogeographical influence on myxomycete distribution is suggested by the difference in distribution between neotropical and Asian palaeotropical forests (Dagamac et al. 2017), which shows a historical and considerable biogeographical distance on the continent spatial scale. However, differentiation between myxomycete communities was regionally influenced by geographical and phylogenetic differences in the host tree. The last glacial refuges of trees and plants function as hotspots in the Mediterranean in Europe (Petit et al. 2003). It is likely that *C. japonica* population in the refuges demonstrate high allelic variation (Takahashi et al. 2005) and also preserve a hotspot for corticolous myxomycetes. The limited and protected refuges of *C. japonica* forests are thus valuable sanctuaries in nature. Further studies are needed to reveal the ecological and phylogenetic relationship between myxomycetes and their host trees with respect to biological interaction and evolution. It is the first finding that the similar distribution pattern between the host tree *C. japonica* and corticolous myxomycetes indicates a phytogeographical association between myxomycetes and the host tree species in biological evolution.

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## References

- Bray, J.R. & Curtis, J.T. 1957: An ordination of upland forest communities of southern Wisconsin. *Ecological Monographs* 27: 325-349.
- Chao, A. 1984: Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11: 265-270.
- Cheng, S.S. & Chang, S. 2014: Bioactivity and characterization of exudates from *Cryptomeria japonica* bark. *Wood Science and Technology* 48: 831-840.
- Chytrý, M., Tichý, L., Holt, J. & Botta-Dukát, Z. 2002: Determination of diagnostic species with statistical fidelity measures. *Journal of Vegetation Science* 13: 79-90.
- Dagamac, N.H.A., Novozhilov, Y.K., Stephenson, S.L., Lado, C., Rojas, C., dela Cruz, T.E.E., Unterseher, M. & Schnitler, M. 2017: Biogeographical assessment of myxomycete assemblages from neotropical and Asian palaeotropical forests. *Journal of Biogeography* 44: 1524-1536.
- Dahl, M.B., Shchepin, O., Schunk, C., Menzel, A., Novozhilov, Y.K. & Schnitler, M. 2018: A four year survey reveals a coherent pattern between occurrence of fruit bodies and soil amoebae populations for nivicolous myxomycetes. *Scientific Reports* 8: 11662
- de Basanta, DW 2000: Acid deposition in Madrid and corticolous myxomycetes. *Staphia* 73: 113-120.
- Hammer, Ø., Harper, D.A.T. & Ryan, P.D. 2001: PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 9. [https://palaeo-electronica.org/2001\\_1/past/issue1\\_01.htm](https://palaeo-electronica.org/2001_1/past/issue1_01.htm)
- Härkönen, M., & Ukkola, T. 2000: Conclusions on myxomycetes compiled over twenty-five years from 4793 moist chamber cultures. *Staphia* 73: 105-112.
- Keller, H.W. & Brooks, T.E. 1973: Corticolous myxomycetes I: Two new species of *Didymium*. *Mycologia* 65: 286-294.
- Kenkel, N.C. & Orlóci, L. 1986: Applying metric and nonmetric multidimensional scaling to ecological studies: Some new results. *Ecology* 67:919-928.
- Kimura, M.K., Kabeya, D., Saito, T., Moriguchi, Y., Uchiyama, K., Migita, C., Chiba, Y. & Tsumura, Y. 2013: Effects of genetic and environmental factors on clonal reproduction in old-growth natural populations of *Cryptomeria japonica*. *Forest Ecology and Management* 304: 10-19.
- Kimura, M.K., Uchiyama, K., Nakao, K., Moriguchi, Y., Jose-Maldia, L.S., & Tumura, Y. 2014: Evidence for cryptic northern refugia in the last glacial period in *Cryptomeria japonica*. *Annals of Botany*. 114: 1687-1700.
- Kofujita, H., Fujino, Y., Sasaki, T., Hasebe, M., Ota, M. & Suzuki, K. 2001: Antifungal activity of the bark of *Cryptomeria japonica* and its relevant components. *Journal of the Japan Wood Research Society*. 47: 479-486.
- Lado, C. 2005-2020: Nomen.eumycetozoa.com, an online nomenclatural information system of Eumycetozoa, Real Jardín Botánico, CSIC. Madrid. <https://eumycetozoa.com/data/genera.php> Accessed on April 1, 2020.
- Park, S., Hosoishi, S. & Ogata, K. 2014: Long-term impacts of Argentine ant invasion of urban parks in Hiroshima, Japan. *Journal of Ecology and Environment*. 37:123-129.
- Petit, R.J., Aguinagalde, I., de Beaulieu, J.-L., et al. 2003: Glacial refugia: Hotspots but not melting pots of genetic diversity. *Science* 300: 1563-1565.
- Novozhilov, Y.K., 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.
- Shannon, C.E. & Weaver, W. 1963: The mathematical theory of communication. University of Illinois Press, Urbana.
- Shimano, K. 2006: Differences in beech (*Fagus crenata*) regeneration between two types of Japanese beech forest and along a snow gradient. *Ecological Research* 21: 651-663.

- Stephenson, S.L. & Stempen, H. 1994: *Myxomycetes: a handbook of slime molds*. Timber Press, Inc. Hong Kong.
- 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.
- Stephenson, S.L. & Rojas, C. 2017: *Myxomycetes: Biology, Systematics, Biogeography and Ecology*. Academic Press: London.
- Sugita, S., Tsukada, M. 1983: Vegetation history around Nonbara bog in the San'in District for the past 17,000 years. *Japanese Journal of Ecology* 33: 225-230.
- Taira, H. 2001: A change of *Cryptomeria japonica* distribution from viewpoint of regeneration system and genetic diversity. *Journal of Phytogeography and Taxonomy* 49: 111-116.
- Taira, H., Tsumura, Y., Tomaru, Y. & Ohba, K. 1997: Regeneration system and genetic diversity of *Cryptomeria japonica* growing at different altitudes. *Canadian Journal of Forest Research* 27: 447-452.
- Takahashi, K. 2014: Influence of bark characteristics on the occurrence of corticolous myxomycetes in western Japan. *Journal of Japanese Botany* 89: 35-47.
- 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. & Hada, Y. 2010: Geographical distribution of myxomycetes on coniferous deadwood in relation to air temperature in Japan. *Mycoscience* 51: 281-290.
- Takahashi, K. & Harakon, Y. 2018: Biogeographical assessment of corticolous myxomycetes on *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: 379-385.
- Takahashi, K., Yamasaki, Y., Minami K. & Yabuki, N. 2020: Biogeographical assessment of corticolous myxomycetes on the living tree bark of *Cryptomeria japonica* in a region of western Japan. *Biogeography* 22: 35-45.
- Takahashi, T., Tani, N., Taira, H. & Tsumura, Y. 2005: Microsatellite markers reveal high allelic variation in natural populations of *Cryptomeria japonica* near refugial areas of the last glacial period. *Journal of Plant Research* 118: 83-90.
- Tsukada, M. 1982: *Cryptomeria japonica*: glacial refugia and late-glacial and postglacial migration. *Ecology* 63: 1091-1105.
- Tsukada, M. 1986: Altitudinal and latitudinal migration of *Cryptomeria japonica* for the past 20,000 years in Japan. *Quaternary Research* 26: 135-152.
- Tsumura, Y. 2011: *Cryptomeria*. In: Kole, C. (ed) *Wild Crop Relatives: Genomics and Breeding Resources: Forest Trees*, 49-64, Springer, Berlin.
- Tsumura, Y., Uchiyama, K., Moriguchi, Y., Ueno, S. & Ihara-Ujino, T. 2012: Genomic scanning for detecting adaptive genes along environmental gradients in the Japanese conifer, *Cryptomeria japonica*. *Heredity* 109: 349-360.
- Vaz, A.B.M., dos Santos, D.S., Cardoso, D., van den Berg, C., de Queiroz, L.P., Badotti, F., Fonseca, P.L.C., Cavalcanti, L.H. & Góes-Neto, A. 2017: Corticolous myxomycetes assemblages in a seasonally dry tropical forest in Brazil. *Mycoscience* 58: 282-289.
- Yamamoto, Y. 1998: *The myxomycetes biota of Japan*. Toyo Shorin: Tokyo, Japan (In Japanese).
- Yasue, M., Ogiwara, K., Suto, S., Tsukahara, H., Miyahara, F. & Ohba, K. 1987: Geographical differentiation of natural *Cryptomeria* stands analyzed by diterpene hydrocarbon constituents of individual trees. *Journal of the Japanese Forestry Society* 69: 152-156.