Biological Species of *Armillaria* in Japan

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ABSTRACT

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Pairing tests with isolates of *Armillaria* in culture were used to identify species of *Armillaria* and their distribution throughout Japan. The existence of 10 intersterile groups of *Armillaria* was determined by pairing haploid single spore isolates obtained from 20 basidiocarps from a wide geographic distribution in Japan. Two to four haploid tester isolates from each intersterile group were then paired to biological and morphological species with haploid tester isolates from Europe and North America identified in previous studies. Japanese haploid tester isolates were then paired with 190 haploid or diploid isolates and compatibility reactions were used to identify these to species. Of the 10 intersterile groups identified in Japan, 7 were authenticated as *A. gallica*, *A. nabsnona*, *A. ostoyae*, *A. cepistipes*, *A. mellea*, *A. sinapina*, or *A. tabescens*. Three of the groups were not compatible with any of the tester species. The distribution and host relationships of the Japanese biological species are also discussed.

Fungi belonging to the genus *Armillaria* (Fr.:Fr.) Staude cause root diseases of forest trees, resulting in extensive economic losses to a range of crop plants all over the world (13). In Japan, *Armillaria* spp. are widely distributed throughout the country and are well-known as a cause of root rots (25).

Armillaria root disease was first reported on *Castanea crenata* in 1903 (22). Since then, *Armillaria* root rot has been reported in many cultivated tree species. Damage was particularly severe in pure *Larix kaempferi* plantations in Hokkaido island, the northern area of Japan, in the 1960s (23). Recently, *Armillaria* root rot has been reported in plantations of young Hinoki (*Chamaecyparis obtusa*), which is one of the most important cultivated tree species in Japan (20).

Pairing tests based on sexual behavior and in vitro somatic compatibility of isolates of *Armillaria* have been widely used to assess interspecific incompatibility in the genus *Armillaria*. Haploid monosporous cultures exhibit a white, fluffy colony morphology. After compatible mating, the culture morphology becomes crustose, which is characteristic of diploid mycelium. Pairing tests have distinguished biological species in Europe, North Amer-

ica, and Australasia (1,9,15,17). Except for the North American species X and XI, all of these biological species either correspond to known morphological species or are described as new species (3,4,9,15-17,29). In annulate *Armillaria* spp., 5 species have been established in Europe, 9 in North America, and 5 in Australasia (9). In exannulate *Armillaria* spp., 2 species have been reported in Europe and 1 in North America (9). All are heterothallic with a bifactorial mating system, with the exception of the European exannulate species *A. ectypa* (9).

In Japan, Terashita and Chuman (27) recognized 5 biological species from *Galeola septentrionalis*, an achlorophyllous orchid. *A. borealis*, *A. gallica* (as *A. bulbosa*), *A. cepistipes*, *A. mellea*, and *A. tabescens* were isolated from Kyushu, and 2 biological species, *A. gallica* and *A. cepistipes*, were isolated from Honshu. Nagasawa (21) recorded 6 intersterility groups (designated as A, B, C, D, E, and AM) in Honshu. Based on the morphological characters of their basidiocarps, he considered that groups C and AM were identical with *A. ostoyae* and *A. mellea*, respectively; groups A and D were similar to *A. gallica* (as *A. lutea*) and *A. cepistipes*, respectively; and B and E represented new species. The 6 intersterility groups were recognized as distinct by Matsushita et al. (18) using isozyme analysis. From pairing tests with European and North American biological species, Suzuki (25) and Suzuki et al. (26) have suggested that Nagasawa's (Nag.) A, Nag. B, Nag. C, Nag. D, and Nag. AM are equivalent to *A. gallica*, NABS IX (= *A. nabsnona*), *A. ostoyae*, *A.*

cepistipes, and *A. mellea*, respectively. The occurrence of biological species *A. gallica* and *A. mellea* in Honshu and Kyushu was recently recognized by Mohammad et al. (19). Also, 8 biological species, *A. gallica* (as *A. bulbosa*), *A. cepistipes*, *A. ostoyae*, *A. sinapina*, NABS IX, *A. mellea* subsp. *nipponica*, *A. singula*, and *A. jezoensis*, have been reported from Hokkaido, of which the latter three are new taxa (6-8,19).

These previous studies of biological species in Japan were conducted using some isolates from a limited area of Japan. The objectives of this study were to expand the survey for biological species of *Armillaria* in Japan by obtaining isolates from localities throughout Japan and identifying them to species, and to discuss their distribution and host ranges.

MATERIALS AND METHODS

Haploid isolates obtained from 20 basidiocarps were used for pairing tests to determine the intersterile groups in Japan. These isolates of Nag. A, B, C, D, E, and AM were obtained from the Tottori Mycological Institute Culture Collection (TMIC) and corresponded to Nagasawa's 6 intersterile groups A, B, C, D, E, and AM (21), respectively. Other monosporous isolates were prepared from single basidiocarps of additional collections. Details of 20 basidiocarps are shown in Table 1. The mating types of monosporous cultures from each of these 20 basidiocarps were determined by pairing 12 isolates from each basidiocarp in all combinations. The two or four mating types from each basidiocarp were paired with those of the other basidiocarps in all combinations, and the number of intersterile groups was determined. Tester strains from each intersterile group were then selected for pairing with European and North American tester strains from known biological or taxonomic *Armillaria* spp.

Haploid tester strains from known species of *Armillaria* in Europe and North America, their hosts, and geographic source are indicated in Tables 2 and 3, respectively. Two to four tester strains were used from each biological species. Japanese testers were paired with them in all combinations. Individual pairings were performed at least three times. The other 190 isolates of *Armillaria* spp., which were collected in Japan from single spore, stipe

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Table 1. Sources of the Japanese tester isolates of *Armillaria* spp.

Biological species	Stock number	Nuclear phase ^a	Host	Location	Original stock number
Nag. A	NA 4	H	Soil surface	Tateiwa-mura, Fukushima	TMIC 31063
Nag. A	NA 10	H	Soil surface	Tottori-shi, Tottori	TMIC 30984
Nag. A	NA 13	H	Soil surface	Aomori-shi, Aomori	TMIC 31525
Nag. B	NB 3	H	Soil surface	Tottori-shi, Tottori	TMIC 30987
Nag. C	NC 2	H	Unknown	Hirama-machi, Aomori	TMIC 30966
Nag. C	NC 3	H	<i>Quercus crispula</i>	Appi, Iwate	TMIC 30969
Nag. C	NC 8	H	Soil surface	Hiranai-cho, Aomori	TMIC 31491
Nag. D	ND 1	H	<i>Fagus crenata</i>	Towadako-machi, Aomori	TMIC 30968
Nag. D	ND 3	H	<i>Betula</i> sp.	Appi, Iwate	TMIC 30970
Nag. D	ND 11	H	<i>Quercus crispula</i>	Mt. Hakkoda, Aomori	TMIC 31069
Nag. E	NE 1	H	Decayed wood	Mt. Hakkoda, Aomori	TMIC 31061
Nag. E	NE 7	H	Unknown	Kawahara-cho, Tottori	TMIC 31510
Nag. E	NE 9	H	<i>Castanea crenata</i>	Tottori-shi, Tottori	TMIC 31515
Nag. AM	NAM 5	U	Hardwood	Tottori-shi, Tottori	TMIC 32415
Nag. AM	NAM 6	U	<i>Pyrus pyrifolia</i>	Aoya-cho, Tottori	TMIC 32417
Nag. AM	NAM 7	D	<i>Quercus serrata</i>	Kokufu-cho, Tottori	TMIC 32416
F	96-7	H	<i>Lalix kaempferi</i>	Chitose-shi, Hokkaido	
G	H-5	H	Unknown	Kamishihoro-cho, Hokkaido	
H	H-16	H	Unknown	Asahikawa-shi, Hokkaido	
T	NT 1	H	<i>Quercus myrsinaefolia</i>	Tsukuba-shi, Ibaraki	

^a D = diploid (isolated from stipe tissue or mycelial mat); H = haploid (isolated from single spore); U = single spore culture appear crustose.

Table 2. Species, host, geographic location, and source of European haploid testers of *Armillaria* spp.

Species	Isolate number	Substrate / host	Geographic location	Original isolate number	source
<i>A. borealis</i>	G-1	<i>Betula</i> sp.	Ruovesi, Finland	KA1	K. Korhonen
	G-2	<i>Betula</i> sp.	Hartola, Finland	KA2	K. Korhonen
<i>A. cepistipes</i>	G-3	<i>Alnus</i> sp.	Tampere, Finland	KB2	K. Korhonen
	G-4	<i>Acer pseudoplatanus</i>	Helsinki, Finland	KB3	K. Korhonen
	G-5	<i>Tilia platyphyllos</i>	Puy-de-Dôme, France	T21	J. J. Guillaumin
<i>A. ostoyae</i>	G-6	<i>Pinus sylvestris</i>	Nurmijärvi, Finland	KC2	K. Korhonen
	G-7	<i>Pinus sylvestris</i>	Nurmijärvi, Finland	KC4	K. Korhonen
	G-8	<i>Abies pectinata</i>	Puy-de-Dôme, France	SF2.5	J. J. Guillaumin
<i>A. mellea</i>	G-9	Unknown	Paris region, France	KD1	K. Korhonen
	G-10	Soil surface	Eure, France	KD4	K. Korhonen
	G-11	<i>Prunus persica</i>	Pyr6n6es-Orientales, France	PM8	J. J. Guillaumin
<i>A. gallica</i>	G-12	Unknown	Near Paris, France	KE1	K. Korhonen
	G-13	<i>Rubus fruticosus</i>	Puy-de-Dôme, France	R2	J. J. Guillaumin
	G-14	<i>Corylus avellana</i>	Puy-de-Dôme, France	ME 80-15-1	J. J. Guillaumin
<i>A. tabescens</i>	G-15	Unknown	Puy-de-Dôme, France	MT 80-42-3	J. J. Guillaumin
	G-16	<i>Quercus robur</i>	Randan Puy-de-Dôme, France	MT 83-10-8	J. J. Guillaumin
	G-17	Unknown	Puy-de-Dôme, France	MT 67-6-5	J. J. Guillaumin

Table 3. Species, host, geographic locations, and source of North American haploid testers of *Armillaria* spp.

Species	Isolate number	Substrate / host	Geographic location	Original isolate number	Source
<i>A. ostoyae</i>	A-3	<i>Abies balsamea</i>	Mt. Mansfield, VT	28-4	J. B. Anderson
	A-7	Soil surface	Orleans Co., VT	70-1	J. B. Anderson
	A-21	Hardwood sap.	Upper Peninsula, MI	430-5	J. B. Anderson
<i>A. gemina</i>	A-4	Unknown	Camel's Hump, VT	35-5	J. B. Anderson
	A-14	Unknown	Smuggler's Notch, VT	160-8	J. B. Anderson
<i>A. calvescens</i>	A-1	<i>Acer saccharum</i>	Underhill, VT	11-1	J. B. Anderson
	A-2	<i>Acer saccharum</i>	Underhill, VT	21-2	J. B. Anderson
<i>A. sinapina</i>	A-5	<i>Acer</i> sp.	Ithaca, NY	48-6	J. B. Anderson
	A-16	Hardwood stump	Babine Lake, B.C.	Sp 81-1-3	D. Morrison
<i>A. mellea</i>	A-6	Unknown	Boston area, MA	49-8	J. B. Anderson
	A-9	<i>Acer rubrum</i>	Provincetown, MA	97-1	J. B. Anderson
<i>A. gallica</i>	A-8	<i>Fraxinus</i> sp.	Burlington, VT	90-10	J. B. Anderson
	A-11	Unknown	Ann Arbor, MI	137-1	J. B. Anderson
	A-20	Hardwood sap.	Upper Peninsula, MI	427-4	J. B. Anderson
	A-23	Hardwood sap.	Upper Peninsula, MI	454-2	J. B. Anderson
<i>A. nabsnona</i>	A-10	<i>Acer macrophyllum</i>	Vancouver, B.C.	121-1	J. B. Anderson
	A-12	Soil surface	Moscow, ID	139-1	J. B. Anderson
NABS X	A-13	<i>Abies grandis</i>	Moscow, ID	140-6	J. B. Anderson
	A-17	Conifer	Southeastern B.C.	Sp 81-20-1	D. Morrison
NABS XI	A-18			Sp 82-14-8	D. Morrison
	A-19			Sp 82-14-9	D. Morrison
<i>A. tabescens</i>	A-15			202-1	J. B. Anderson

tissue, rhizomorphs, decayed wood, and infected roots, were identified by pairing with the Japanese tester strains. Pairings were made on 1.5% malt extract agar or potato dextrose agar medium, and compatibility was determined on the basis of the macroscopic mycelial appearance (17). Pairing between two compatible cottony haploid mycelium changes the morphology of haploid mycelia into the crustose type. When a cottony haploid mycelium is paired with a crustose diploid mycelium belonging to the same species, it usually changes to the crustose mycelial type (17).

For observation of the presence or absence of clamp connection and basidia, and the number of nuclei, gills were fixed with ethanol:acetic acid solution (3:1, vol/vol) and were stained with 500 ng/ml 4',6-dia-

Fig. 1. The culture morphology of Nag. AM (Japanese *Armillaria mellea*) after 4 weeks' inoculation at 25°C on potato dextrose agar. (A) Culture from stipe tissue; (B) culture from a single spore.

Table 4. Results of pairing tests between Japanese and European *Armillaria* isolates

	G	<i>A. gallica</i>			<i>A. ostoyae</i>			<i>A. cepistipes</i>			<i>A. mellea</i>			<i>A. borealis</i>		<i>A. tabescens</i>		
		12	13	14	6	7	8	3	4	5	9	10	11	1	2	15	16	17
Nag. A																		
NA10-3		+ ^a	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10-11		+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13-2		+	+?	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13-7		+	+?	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13-3		+	+?	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13-9		+	+?	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nag. B																		
NB3-2		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3-14		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3-3		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3-4		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nag. C																		
NC3-6		—	—	—	+?	—	+?	—	—	—	—	—	—	—	—	—	—	—
3-12		—	—	—	+?	—	+?	—	—	—	—	—	—	—	—	—	—	—
8-7		—	—	—	+?	—	+?	—	—	—	—	—	—	—	—	—	—	—
8-9		—	—	—	+?	—	+?	—	—	—	—	—	—	—	—	—	—	—
Nag. D																		
ND1-1		—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—
1-5		—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—
11-1		—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—
11-3		—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—
Nag. E																		
NE7-1		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7-2		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7-8		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7-11		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9-8		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9-9		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nag. AM																		
NAM5-1		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5-2		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6-1		—	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—
7		—	—	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—
F																		
96-7-1		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7-2		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7-3		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7-8		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G																		
H5-4		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5-8		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5-3		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5-6		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H16-4		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16-17		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
T																		
T1-8		—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
1-10		—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
1-9		—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
1-12		—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+

^a + = pairing clearly compatible; +? = pairing not clear but interpreted as compatible; - = pairing clearly incompatible.

midino-phenylindol (DAPI) and observed under light and fluorescence microscopes. *A. mellea* and *A. ectypa* are known to have clampless uninucleate basidia (9,21). Nagasawa (21) reported that group AM also had clampless basidia. These cytological characteristics can help to identify species.

All isolates used in this study are deposited in the Forestry and Forest Products Institute and the Japanese testers of Nag. A, B, C, D, E, and AM are also deposited in the TMIC.

RESULTS

Pattern of sexuality. The pattern of sexuality for almost all isolates was clearly bifactorial. Those isolates which did not

exhibit bifactorial sexuality possessed a crustose monosporous culture morphology typical of diploid isolates (Fig. 1) and had clampless uninucleate basidia and a similar basidiocarp morphology. Consequently, they were considered to belong to a single group. From this group, 1 or 2 monosporous isolates were randomly selected as tester strains from each basidiocarp. Their compatibility was similarly determined by haploid-diploid pairings.

Grouping of Japanese isolates. From the results of pairing tests among the monosporous isolates from the 20 basidiocarps, the isolates were grouped into 10 biological species: 9 annulate sepcies (Nag. A, Nag. B, Nag. C, Nag. D, Nag. E,

Nag. AM, F, G, and H) and 1 exannulate species (T).

Identification of Japanese groups by haploid-haploid pairing tests with known European and North American testers. The results of pairing tests with European testers and North American testers are shown in Tables 4 and 5, respectively. Three testers (A-6, A-16, and G-7) were considered to be debilitated, because they did not show any morphological change when paired with another tester belonging to same species. In annulate groups, Nag. A was compatible with both European and North American *A. gallica*. Nag. B was compatible with the North American species *A. nabsnona*. Nag. C

Table 5. Results of pairing tests between Japanese and North American *Armillaria* isolates

A-	<i>A. gallica</i>				<i>A. nabsnona</i>		<i>A. ostoyae</i>			<i>A. mellea</i>		<i>A. gemina</i>		<i>A. calvaescens</i>		<i>A. sinapina</i>		NABSX		NABSXI		<i>A. tabescens</i>
	8	11	20	23	10	12	3	7	21	6	9	4	14	1	2	5	16	13	17	18	19	15
Nag. A																						
NA13-12	+ ^{2a}	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13-7	+?	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13-3	+?	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13-9	+?	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nag. B																						
NB3-2	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-14	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-3	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3-4	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nag. C																						
NC3-6	-	-	-	-	-	-	+	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-
3-12	-	-	-	-	-	-	+	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-
8-7	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
8-9	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Nag. D																						
ND11-1	-	-	-	-	-	...	-	...	...	-	-	-	...	...	-	-	...	...	-	-	-	-
11-3	-	-	-	-	-	...	-	...	...	-	-	-	...	...	-	-	...	...	-	-	-	-
13-1	-	-	-	-	-	...	-	...	...	-	-	-	...	...	-	-	...	...	-	-	-	-
13-4	-	-	-	-	-	...	-	...	...	-	-	-	...	...	-	-	...	...	-	-	-	-
Nag. E																						
NE7-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7-11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9-8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nag. AM																						
NAM5-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6-1	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
F																						
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7-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
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1-12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^a + = pairing clearly compatible; +? = pairing not clear but interpreted as compatible; - = pairing clearly incompatible; -? = pairing not clear but interpreted as incompatible; ? = pairing too unclear to give an interpretation; ... = no pairing made.

was compatible with both European and North American *A. ostoyae*. Nag. D was compatible with the European species *A. cepistipes*. Nag. AM was partially compatible with European and North American *A. mellea*; NAM6 and NAM7 were compatible with European and North American testers but NAM5 was incompatible with both. Nag. E, group G, and group H were incompatible with all European and North American testers. Group F was compatible with the North American species *A. sinapina*. In the exannulate group, group T was compatible with the European *A. tabescens* but not with North American testers of the same species.

From the results of the pairing tests with Japanese testers, all isolates, with the exception of isolates belonging to Nag. AM, were identified. The isolates belonging to Nag. AM were identified from basidiocarp morphology, monosporous culture colony morphology, and the presence of clampless uninucleate basidia.

Distribution and hosts of Japanese *Armillaria* spp. The distribution of the isolates of each biological species is shown in Figure 2. *A. gallica* (Nag. A) was found throughout Japan. *A. nabsnona* (Nag. B) and Nag. E were found only on Honshu island. *A. ostoyae* (Nag. C) was found in Hokkaido, and the northern and central parts of Honshu island. *A. cepistipes* (Nag.

D) was found in Hokkaido and throughout Honshu island. *A. sinapina* (F), *A. singula*, (G) and *A. jezoensis* (H) were found only in Hokkaido. *A. tabescens* (T) was found in Kyushu and the central and southern parts of Honshu.

The number of collections of each *Armillaria* species on each host species or substrate and their conditions are shown in Tables 6 and 7. *A. gallica*, *A. nabsnona*, *A. cepistipes*, *A. tabescens*, and Nag. E were found more frequently on hardwoods than on conifers. *A. ostoyae* and *A. mellea* were found on both hardwoods and conifers. *A. mellea* was strongly parasitic on *Chamae-*

cyparis obtusa. All *C. obtusa* hosts, from which 4 isolates of *A. mellea* were isolated, were either infected or killed (Table 7).

DISCUSSION

Japan consists of a group of islands extending north and south over a wide range of latitude. As a result, the climate ranges from cold-temperate in the north to subtropical in the south. Consequently, a great diversity of vegetational and mycological flora exists within Japan. From this study, it is clear that 9 annulate species and 1 exannulate species of *Armillaria* exist in Japan. This number should be recognized

Table 7. Number of collections on tree conditions of *Armillaria* species identified in Japan

Species	Conifers			Hardwoods			Others	Total ^b
	P ^a	S	U	P	S	U		
<i>A. gallica</i>	0	1	1	0	6	2	31	41
<i>A. nabsnona</i>	0	0	0	0	2	0	7	9
<i>A. ostoyae</i>	2	6	2	0	7	2	10	29
<i>A. cepistipes</i>	0	0	1	0	14	3	11	29
Nag. E	0	1	0	0	16	11	8	36
<i>A. mellea</i>	4	1	0	4	18	2	5	34
<i>A. sinapina</i>	0	0	0	0	0	1	2	3
G	0	0	0	0	0	0	5	5
H	0	0	0	0	0	0	15	15
<i>A. tabescens</i>	0	0	0	0	1	3	5	9

^a P = parasite (infection of the cambium of a live tree, mycelial fan penetrating into live cambium of root collar or roots); S = saprophyte (sap rot of a stump or log); U = unknown.

^b Total = 210 isolates.

Table 6. Number of collections on each host or substrate of *Armillaria* spp. identified in Japan

	<i>A. gallica</i>	<i>A. nabsnona</i>	<i>A. ostoyae</i>	<i>A. cepistipes</i>	Nag. E	<i>A. mellea</i>	<i>A. sinapina</i>	G	H	<i>A. tabescens</i>
Conifers										
<i>Abies sachalinensis</i>	1		4							
<i>Abies</i> spp.			2							
<i>Chamaecyparis obtusa</i>						4				
<i>Larix kaempferi</i>			1	1						
<i>Metasequoia glyptostroboides</i>					1					
<i>Picea jezoensis</i>			2							
<i>Pinus densiflora</i>	1		1							
<i>Pinus thunbergii</i>						1				
Hardwoods										
<i>Acer</i> spp.	1		1		1	1				
<i>Aphananthe aspera</i>					1					
<i>Aesculus turbinata</i>						1				
<i>Betula</i>										
<i>maximowicziana</i>	1		2							
<i>Betula</i> spp.			3	1						
<i>Castanea crenata</i>					1	1				
<i>Cercidiphyllum japonicum</i>				1	1	1				
<i>Fagus crenata</i>				8	5	1				
<i>Prunus</i> spp.						3				2
<i>Pyrus</i> spp.						1				
<i>Quercus crispula</i>			1	1	1					
<i>Quercus serrata</i>	2					2				
<i>Quercus</i> spp.	1	1	2	1	1	1				2
Other hardwoods	3	1		5	16	12	1			
Others										
Moss		1								
Soil surface	18	4	7	1						
<i>Galeola septentrionalis</i>	9			4		2				5
Unidentified hosts	4	2	3	6	8	3	2	5	15	
Total ^a	41	9	29	29	36	34	3	5	15	9

^a Total = 210 isolates.

as a lower limit, because the subtropical regions of Japan (Nansei islands, Ogasawara islands, and so on) were not surveyed. Seven groups of *Armillaria* in Japan were identified by pairing tests with European and North American species. *A. gallica* (= Nag. A), *A. nabsnana* (= Nag. B), *A. ostoyae* (= Nag. C), *A. cepistipes* (= Nag. D), *A. mellea* (= Nag. AM), *A. sinapina* (= F), and *A. tabescens* (= T) were confirmed to exist in Japan. *A. nabsnana*, which has been reported in Hokkaido (19), was first recorded in Honshu. Terashita and Chuman (27) reported the European biological species, *A. borealis*, in Kyushu. In this study, the same isolate was paired with European and North American testers. The isolate was incompatible with *A. borealis* testers, but was compatible with *A. mellea* testers. Among the groups that were incompatible with all European and North American testers, we propose that Nag. E is a new

species due to its unique basidiocarp morphology (21). Groups G and H represent *A. singula* and *A. jezoensis*, respectively, because the morphological characteristics of their basidiocarps are in accord with those described by Cha et al. (7).

All groups have a bifactorial heterothallism except for Nag. AM. Nag. AM is partially compatible with *A. mellea* of both Europe and North America. Some features of basidiocarp morphology (21), such as the absence of clamped basidia and presence of uninucleate basidia, are the same as in its European and North American counterparts. However, the monosporous colony morphology is crustose, as is the parental diploid culture. The nonheterothallic life cycle of Japanese *A. mellea* was studied with regard to the nuclear behavior during basidiospore formation and germination, and DNA content per nucleus at different developmental stages (24). Based on culture morphology, Cha and Igarashi

(6) supposed homothallism in *A. mellea* from Hokkaido and named it *A. mellea* subsp. *nipponica*. The similarities in monosporous culture colony morphology and the presence of clampless basidia suggest that they are a single group. In this paper, we refer to this group as "Japanese *A. mellea*."

A. gallica was the most abundant species collected. It was reported as a species common at low altitudes and at southern latitudes in the northern temperate hemisphere, and was considered a weak pathogen (2,5,11,12). This also seems to be true in Japan (Fig. 2). In Japan, *A. gallica* is most commonly found on dead hardwoods and the soil surface.

A. nabsnana was reported as a species distributed on the west coast of North America (29). In this study, basidiocarps of *A. nabsnana* were collected at only 9 sites on Honshu island, central Japan (Fig. 2). The host range of *A. nabsnana* in North America is primarily hardwoods in riparian areas, particularly *Alnus* spp. (29). In Japan, basidiocarps were also collected from riparian areas.

A. ostoyae was reported as a species common in northern latitudes of the northern hemisphere (2,5,11). In Japan, basidiocarps of this species were collected from Hokkaido and the northern part of Honshu, in the cool temperate zone (Fig. 2). This species is parasitic on *Pinus densiflora* (28) and *Larix kaempferi* in the northern region. From previous reports of *Armillaria* root rot occurring in northern conifer forests, almost all of the causal pathogens can be considered to be *A. ostoyae*, based on basidiocarp morphology (23).

A. cepistipes was reported as a species distributed with an inverse relationship between altitude and latitude (10). In Japan, it is distributed widely from north to south (Fig. 2).

Armillaria sinapina is considered to be a species with a relatively northern distribution in North America (2,5,11). In Japan it is common in Hokkaido but not in the southern parts of Japan (Fig. 2).

A. mellea was reported to have a southern distribution in the northern hemisphere (5,11). In this study, *A. mellea* was collected from both Honshu and Kyushu islands (Fig. 2). Cha and Igarashi (6) reported *A. mellea* subsp. *nipponica* from the southern part of Hokkaido, but it may be rare in Hokkaido. Especially in the southern part of Japan, this species seems to be a strong pathogen of *C. obtusa*.

A. tabescens was reported as a species common at low altitudes and at southern latitudes in the northern temperate hemisphere (14). In this study, basidiocarps of *A. tabescens* were collected from Kyushu islands and the southern part of Honshu.

A. singula and *A. jezoensis* may be restricted to Hokkaido. Nag. E was found only in Honshu and had no strong relationship to either host or substrate type.

Fig. 2. Distribution of *Armillaria* spp. in Japan.

This study indicates that at least 10 biological species of *Armillaria* exist in Japan and perhaps explains some of the diverse parasitic activity noted in earlier studies of *Armillaria* root rot in Japan.

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