Mycoscience (2009) 50:442–447
DOI 10.1007/s10267-009-0505-1

SHORT COMMUNICATION

Yuko Ota · Kozue Sotome · Eri Hasegawa

Seven *Armillaria* species identified from Hokkaido Island, northern Japan

Received: February 16, 2009 / Accepted: May 15, 2009

Abstract Sixty-two isolates from basidiocarps of *Armillaria* spp. were obtained from Hokkaido Island, northern Japan. Six species (*Armillaria cepistipes*, *A. gallica*, *A. nabsnona*, *A. ostoyae*, *A. sinapina*, and an undescribed species, "Nag. E") were identified by pairing tests with known tester strains and one subspecies (*Armillaria mellea* subsp. *nipponica*, a non-heterothallic form of *A. mellea*) was identified by its macro- and micro-morphological characters of the basidiocarps. This is the first case of "Nag. E" being reported from Hokkaido Island.

Key words Ecology · Morphology · Pairing tests

Armillaria species (Physalacriaceae, Agaricales, Basidiomycota) are widely distributed around the world, and some of them are well known as a cause of root rot (Hood et al. 1991). More than 600 species of woody and non-woody plants have been identified as hosts of fungi belonging to this genus (Shaw and Kile 1991; Fox 2000). About 40 species of *Armillaria* have been considered to exist all over the world (Volk and Burdsall 1995).

In Japan, nine annulate species including one subspecies (*A. cepistipes* Velen., *A. gallica* Marxm. & Romagn., *A. jezoensis* J.Y. Cha & Igarashi, *A. mellea* (Vahl) P. Kumm. subsp. *nipponica* J.Y. Cha & Igarashi, *A. nabsnona* T.J. Volk & Burds., *A. ostoyae* (Romagn.) Herink, *A. sinapina* Bérubé & Dessur., *A. singula* J.Y. Cha & Igarashi, and one undescribed species, "Nag. E"), and two exannulate species (*A. ectypa* (Fr.) Lamoure and *A. tabescens* (Scop.) Emel) have been reported so far (Nagasawa 1991; Cha et al. 1992; Ota et al. 1998b; Kudo and Nagasawa 2003). *Armillaria jezoensis* and *A. singula* are species so far known only from Hokkaido (Cha et al. 1994), and *A. sinapina*, which was originally described from North America, has been reported only from Hokkaido in Japan. Nag. E, which is considered as a new taxon, and *A. ectypa* have been reported only from Honshu, and *A. tabescens* was reported only in Honshu and Kyushu (Ota et al. 1998b).

Hokkaido is the second largest island and located in the most northern part of the Japan archipelago. Because of its geographical location and history, Hokkaido has more species in common with North America, Eurasia, and Honshu. Except for the studies conducted by Cha et al. (1992, 1994), there is little information on *Armillaria* in Hokkaido. In addition, the relationship between *Armillaria* species collected from Hokkaido and those from Honshu and Kyushu has not been thoroughly studied.

Pairing tests based on sexual behavior are the traditional and useful tool for identifying *Armillaria* spp., except for *A. mellea* subsp. *nipponica*, which is known as a non-heterothallic taxon (Cha and Igarashi 1995; Ota et al. 1998a). The objectives of this study were to obtain specimens and isolates of *Armillaria* spp. throughout Hokkaido and identify the species by pairing tests using known tester strains.

More than 100 collections were obtained throughout Hokkaido, from 1994 to 2005, mostly during September to November in 2005. The morphological characters of basidiocarps, hosts, and origins were recorded. Tissue and/or single-spore isolates were obtained from a basidiocarp in each collection. A total of 62 isolates were selected and used in this study (Table 1, and isolates annotated in Table 2). Specimens examined were deposited at the Mycological Herbarium of the Forestry and Forest Products Research Institute (TFM), Tsukuba, and all Japanese isolates used in this study were stored at the same institute. To obtain new tester isolates, some basidiocarps collected from Hokkaido were selected based on morphological differences. At least 12 single-spore isolates obtained from each basidiocarp were paired in all combinations, and 2 to 4 isolates, each

Y. Ota (✉)
Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan
Tel. +81-29-829-8245; Fax +81-29-873-1543
e-mail: yuota@ffpri.affrc.go.jp

K. Sotome
Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan

E. Hasegawa
Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto, Japan

Table 1. *Armillaria* isolates from Hokkaido used in this study

Species	Isolate no.	Host/ substrate	Location	Collection date	Specimen no. (TFM)
Armillaria cepistipes	96-19-1	Unknown	Shirakawa, Kamikawa-cho, Kamikawa-gun, Hokkaido	23-Sep-96	26880
	04-4-1	Unknown	Asahi-machi, Kamikawa-cho, Hokkaido	10-Sep-04	26956
	04-12-1	Unknown	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	24-Sep-04	26962
	04-16-1	Stump of hardwood	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	25-Sep-04	26966
	05-11-2	Soil surface	Nagahashi, Otaru, Hokkaido	16-Sep-05	26990
	05-24-1	Unknown	Dominno-mori, Tobetsu-cho, Isikari-gun, Hokkaido	17-Sep-05	27002
	05-36-1	Unknown	Kikusui, Kamikawa-cho, Hokkaido	17-Sep-05	27012
	05-37-1	Unknown	Kikusui, Kamikawa-cho, Hokkaido	17-Sep-05	27013
	05-47-2	Soil surface	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	20-Sep-05	27022
	05-58-2	Decayed wood	Mt. Hokurei, Pippu-sho, Kamikawa-gun, Hokkaido	25-Sep-05	27033
	05-73-1	*Betula platyphylla* var. *japonica*	Toyoura-cho, Abuta-gun, Hokkaido	29-Sep-05	27047
	05-87-1	Soil surface	Tonebetu forest park, Iwamizawa, Hokkaido	5-Oct-05	27060
	05-91-1	*Tilia japonica*	Mt. Muroran, Muroran, Hokkaido	6-Oct-05	27064
A. gallica	96-6[a]	Soil surface	Tomakomai-shi, Hokkaido	14-Sep-96	26871
	96-9-1	Soil surface	Tomakomai-shi, Hokkaido	16-Sep-96	26873
	96-32-1	*Abies sachalinensis*	Asyoro-cyo, Asyoro-gun, Hokkaido	19-Sep-96	26890
	05-9-12	Soil surface	Mt. Muroran, Muroran, Hokkaido	13-Sep-05	26988
	05-10-1	Soil surface	Nagahashi, Otaru, Hokkaido	16-Sep-05	26989
	05-33-2	hardwood	Tonebetu forest park, Iwamizawa, Hokkaido	17-Sep-05	27010
A. mellea subsp. *nipponica*	05-84-1	Hardwood	Mt. Muroran, Muroran, Hokkaido	2-Oct-05	27058
	05-100-2	Conifer	Appenai, Tomakomai, Hokkaido	16-Oct-05	27073
A. nabsnona	02-1-1	Unknown	Kitamacni, Kamikawa-cho, Kamikawa-gun, Hokkaido	25-Aug-02	26934
	02-2-1	Unknown	Asahi-machi, Kamikawa-cho, Hokkaido	25-Aug-02	26935
	02-3-1	Unknown	Kitamacni, Kamikawa-cho, Kamikawa-gun, Hokkaido	26-Aug-02	26936
	05-8-1	*Quercus mongolica* var. *grosseserrata*	Mt. Muroran, Muroran, Hokkaido	13-Sep-05	26987
	05-41-1	Decayed wood	Tsukigata-cho, Kabato-gun, Hokkaido	18-Sep-05	27017
	05-45-1	Hardwood	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	20-Sep-05	27020
	05-50-1	Soil surface	Assabu-cho, Hiyama-gun ,Hokkaido	23-Sep-05	27025
	05-60-1	Soil surface	Mt. Hokurei, Pippu-sho, Kamikawagun, Hokkaido	25-Sep-05	27035
A. ostoyae	96-20-1	*Acea mono*	Esashi district forest office, Esasi-cho, Esasi-gun, Hokkaido	23-Sep-96	27086
	96-25[a]	*Abies sachalinensis*	Ashibetsu district forest office, Ashibetsu, Hokkaido	21-Sep-96	–
	96-28b-1	*Picea jezoensis*	Esashi district forest office, Esasi-cho, Esasi-gun, Hokkaido	25-Sep-96	26886
	04-7[a]	Trunk of *Tsuga diversifolia*	Sounkyo, Kamikawa-cho, Hokkaido	18-Sep-04	26959
	05-18[a]	Stump of *Abies sachalinensis*	Oketocho, Tokorogun, Hokkaido	16-Sep-05	26996
	05-30-1	Hardwood	Toyosatoko forest park, Kitami, Hokkaido	17-Sep-05	27007
	05-78[a]	Fallen trunk of *Quercus mongolica* var. *grosseserrata*	Arashiyama park, Asahikawa, Hokkaido	2-Oct-05	27052
	05-101-1	Stump of *Picea glehnii*	Appenai, Tomakomai, Hokkaido	16-Oct-05	27074
	05-102-1	Unknown	Obihiro, Hokkaido	17-Oct-05	27075
A. sinapina	96-8-1	Unknown	Tomakomai-shi, Hokkaido	16-Sep-96	26872
	04-3[a]	Unknown	Touun, Kamikawa-cho, Hokkaido	7-Sep-04	26955
	04-6[a]	Stump of *Acer tschonoskii* var. *tschonoskii*	Sounkyo, Kamikawa-cho, Hokkaido	16-Sep-04	26958
	05-7-11	Fallen trunk of *Sorbus commixta*	Mt. Muroran, Muroran, Hokkaido	13-Sep-05	26986
	05-12-1	Decayed wood of *Quercus* sp.	Kuriyama-cho, Yubari-gun, Hokkaido	16-Sep-05	–
	05-13-2	Soil surface	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26991
	05-15-1	Soil surface	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26993
	05-16-9	Soil surface	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26994
	05-17-1	Soil surface	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26995

Table 1. *Continued*

Species	Isolate no.	Host/ substrate	Location	Collection date	Specimen no. (TFM)
	05-19-1	Living *Tilia japonica*	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26997
	05-20-1	On the moss of living *Picea glehnii*	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26998
	05-21-1	On the moss of living *Picea glehnii*	Ashoro-cyo, Ashoro-gun, Hokkaido	16-Sep-05	26999
	05-46-1	On the root of *Quercus mongolica* var. *grosseserrata*	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	20-Sep-05	27021
	05-48-2	Soil surface	Assabu-cho, Hiyama-gun, Hokkaido	23-Sep-05	27023
	05-49-1	Soil surface	Assabu-cho, Hiyama-gun, Hokkaido	23-Sep-05	27024
Nag. E	05-77-1	*Acer tschonoskii* var. *tschonoskii*	Arashiyama park, Asahikawa, Hokkaido	2-Oct-05	27051
	05-95-1	*Salix* sp.	Otaki-mura, Soubetsu-gun, Hokkaido	7-Oct-05	27068
	05-96-1	Unknown	Otaki-mura, Soubetsu-gun, Hokkaido	7-Oct-05	27069
	05-97[a]	*Salix* sp.	Otaki-mura, Soubetsu-gun, Hokkaido	7-Oct-05	27070
	05-98-4	*Ulmus davidiana* var. *japonica*	Fukagawa-shi, Hokkaido	16-Oct-05	27071

[a] Isolate derived from tissue of basidiocarp

Table 2. Tester isolates of *Armillaria* spp. used in this study

Species	Isolate no.	Host/substrate	Location	Collection date	MAFF no.	Specimen no. (TFM)	Source
Japanese species							
A. cepistipes	01-14-3	Hardwood	Sashiro, Kasama, Ibaraki	19-Oct-01		27093	This study
	01-14-4	Hardwood	Sashiro, Kasama, Ibaraki	19-Oct-01		27093	This study
	05-11-3[a]	Soil surface	Nagahashi, Otaru, Hokkaido	16-Sep-05		26990	This study
A. gallica	96-34-3	Unknown	Kiyomizu Higashiyamaku, Kyoto, Kyoto	29-Sep-96		26892	Ota et al. (1998a,b)
	96-34-4	Unknown	Kiyomizu Higashiyamaku, Kyoto, Kyoto	29-Sep-96		26892	Ota et al. (1998a,b)
	04-13-13[a]	Unknown	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	25-Sep-04		26963	This study
A. nabsnona	00-3-1	Hardwood	Tashirodaira, Aomori, Aomori	19-Sep-00	420659	27089	Ota (2001)
	00-3-2	Hardwood	Tashirodaira, Aomori, Aomori	19-Sep-00	420660	27089	Ota (2001)
	05-44-1[a]	Hardwood	Nopporo prefectural Natural Park, Ebetsu, Hokkaido	20-Sep-05		27019	This study
A. ostoyae	94-8-12	*Pinus densiflora*	Tohoku-machi, Aomori	Oct-94	420662		Ota et al. (1998a,b)
	05-32-1[a]	*Betula platyphylla* var. *japonica*	Dominno-mori, Tobetsu-cho, Isikari-gun, Hokkaido	17-Sep-05		27009	This study
A. sinapina	96-7-1[a]	Decayed wood	Chitose-shi, Hokkaido	14-Sep-96	420657	27085	Ota (2001)
	96-7-2[a]	Decayed wood	Chitose-shi, Hokkaido	14-Sep-96		27085	Ota (2001)
	05-15-1[a]	Soil surface	Asyoro-cyo, Asyoro-gun, Hokkaido	16-Sep-05		26993	This study
Nag. E	94-2-1	Hardwood	Agematsu-machi, Nagano	12-Oct-94	420665	27081	This study
	00-15-1	Unknown	Mt. Kurikoma, Hanayama-mura, Kurikoma-gun, Miyagi	19-Oct-00			Ota (2001)
North American species							
A. sinapina	A-5	*Acea* sp.	Ithaca, NY, USA				
	A-16	Hardwood stump	Babine Lake, B. C., Canada				
A. calvescens	A-2	*Acer rubrum*	Underhill, VT, USA				
	A-1	*Acer saccharum*	Underhill, VT, USA				
A. gemina	A-14	Unknown	Smuggler's Notch, VT, USA				
NABS X	A-17	Conifer	Southeastern B.C., Canada				

Japanese materials examined here were deposited in the Mycological Herbarium of Forestry and Forest Products Research Institute (TFM)
[a] Tester isolates obtained from Hokkaido

with different mating types, were selected as the tester isolates. They were paired with the known tester strains (*A. cepistipes*, *A. gallica*, *A. nabsnona*, *A. ostoyae*, *A. sinapina*, and Nag. E), and then were identified (see annotated isolates in Table 2). Haploid tester strains used in this study are shown in Table 2. Some of them were previously identified by Ota et al. (1998b) and Ota (2001).

Pairings were conducted by the methods described by Korhonen (1978) and Guillaumin et al. (1991). Two 3-mm-diameter mycelium plugs, one from each of the two isolates,

were placed 5 mm apart in a plate containing potato dextrose agar medium (Nissui, Tokyo, Japan) and incubated in the dark for 2–3 weeks at 25°C. Individual pairings were performed at least two times. The compatibility was determined on the basis of the macroscopic mycelial appearance: In pairings between a single-spore isolate and a tester strain of the same species, cottony mycelium changes into the crustose type (compatible reaction). In pairings between different species, the formation of a dense line separating the two single-spore cultures was observed (incompatible reaction). In pairings between an isolate from tissue (diploid) and a tester strain (haploid) belonging to the same species, the appearance of the haploid tester strain usually changed into a crustose mycelial type.

The results of pairing tests are detailed in Table 3. Compatible reactions were clear in almost all combinations with a few exceptions. Six species (*A. cepistipes*, *A. gallica*, *A. nabsnona*, *A. ostoyae*, *A. sinapina,* and "Nag. E") were identified by pairing tests (see Table 1). One subspecies, *A. mellea* subsp. *nipponic*a, a non-heterothallic form of the species, was identified by its macro- and micro-morphological characters of the basidiocarps.

Armillaria cepistipes was collected from middle September to early October. The basidiocarps occurred most commonly in small fasciculate groups of two to five from the soil surface or from dead hardwoods. The existence of this species in Hokkaido was previously reported by Mohammed et al. (1994), Ota et al. (1998b), and Fukuda et al. (2003), but no basidiocarp has been obtained so far. This study suggests that *A. cepistipes* is common and widely distributed in broadleaf forests in Hokkaido.

Armillaria gallica was collected in September. It seemed to be the earliest fruiting annulate *Armillaria* species in Hokkaido. In Honshu, this species is the earliest fruiting annulate species (Nagasawa 1991; Ota et al. 1998b). The basidiocarps emerged most commonly singly or in small fasciculate groups of two or three from the soil surface or on dead hardwoods in the cool-temperate broad-leaved and mixed forests.

Armillaria mellea subsp. *nipponica* was the latest fruiting species that was collected in October. Only two specimens were collected in Hokkaido (from Muroran and Tomakomai) in this survey, and two other specimens had previously been collected in Hokkaido (from Tomakomai and Chitose) by Cha and Igarashi (1995). This species may not be common in Hokkaido.

Armillaria nabsnona was collected from late August to late September. This species commonly occurred in large or sometimes small groups on the soil surface or on dead broad-leaved trees in the cool-temperate broad-leaved and mixed forests. Before this survey, only two records of *A. nabsnona* were reported in Hokkaido (Mohammed et al. 1994; Sekizaki et al. 2008). This study showed that *A. nabsnona* was common and widely distributed in the southern part of Hokkaido. In Honshu, *A. nabsnona* is characterized by its bright yellowish and often hygrophanous pileus surface and finally plane to slightly depressed pileus with a small conical center (Nagasawa 1991; Kudo et al. 1998), and it usually occurs in riparian areas (Kudo et al. 1998; Ota et al. 1998b). However, in Hokkaido, it occurs more often in dry habitats and the pileus surface is also usually dry, and its color seems to be varied, from pale yellowish white to sometimes yellowish brown.

Armillaria ostoyae was collected from middle September to middle October on both conifer and hardwood species. This species has been known to be a cause of root rot of *Larix kaempferi* (Ota et al. 1998b). This study showed that *A. ostoyae* causes root rot also on *Abies sachalinensis* in Hokkaido. *Armillaria ostoyae* is distributed widely from lowlands to mountainous areas in Hokkaido.

Armillaria sinapina was the most frequently found species. It occurred in September, in the same season as *A. gallica* in Hokkaido. Mostly it occurred on the soil surface or on the broad-leaved trees, but also on conifers in the cool-temperate broad-leaved and mixed forests. *Armillaria sinapina* seems to distribute in more mountainous areas than *A. gallica*. This species is reported as a weak pathogen of hardwoods in China (Qin et al. 2007) and of conifers in North America (Bérubé and Dessureault 1988).

Nag. E collected in October was the latest fruiting species, similar to *A. mellea*, but it fruited also in early summer in Hokkaido (data not shown). It occurred most commonly in large groups only on hardwood species. This is the first record of "Nag. E" from Hokkaido. It is easily recognized by its fine small scales on the pileus and stipe and bright yellow to yellowish-brown pileus color. We conducted pairing tests between the isolates H-5-4 collected from Kamishihoro, Hokkaido, and H-16-4 collected from Asahikawa, Hokkaido, used by Ota et al. (1998b) and the new tester strains used in this study. H-5-4 and H-16-4 was reported to not show any compatible reactions with known European and North American tester strains and were assigned to new Japanese biological species groups G and H, respectively (Ota et al. 1998b). In the present study, H-5-4 also did not show any compatible reaction with tester strains used; however, the H-16-4 strain showed compatible reactions with the new tester strains of Nag. E, but only on the tester strain side (data not shown). Therefore, group H of Ota et al. (1998b) was identified as Nag. E. Haploid tester isolates sometimes degrade as they get older and lose the ability to mate (Guillaumin et al. 1991). The H-5-4 could have degraded already, or it may be truly incompatible with tester isolates used in this study. Identification using DNA sequences should be applied to determine the species of this isolate.

The morphological characteristics of *A. cepistipes*, *A. gallica*, and *A. sinapina* in Hokkaido were very similar to each other. However, *A. cepistipes* was distinguished from *A. gallica* and *A. sinapina* by its pileus, which remains convex even when older, and has finer scales on the pileus than those of *A. sinapina* and *A. gallica*. Pilei of *A. gallica* and *A. sinapina* become plane and sometimes undulate at the margin in age. The pileus surface color of *A. cepistipes* varied from yellowish brown to red brown or dark brown. *Armillaria gallica* is distinguished by its whitish, thin, delicate and fibrous annulus, which is collapsing and appressed to the stipe but sometimes completely lost in age. Sometimes the annulus of *A. gallica* is encircled by the brown

Table 3. Result of pairing tests between tester isolates and isolates from Hokkaido

Species	Isolate no.	04-12	04-16	05-24	05-36	05-37	05-47	05-58	05-73	05-87	05-91	96-6	96-9	96-32	05-9	05-10	05-32	05-33	02-1	02-2	02-3	05-8	05-41	05-45	05-50	05-60	04-7	96-20	96-25	96-28	05-30	05-78	05-101	05-102	96-19	96-8	04-3	04-6	05-7	05-12	05-13	05-16	05-17	05-19	05-20	05-21	05-46	05-48	05-49	05-77	05-95	05-96	05-97	05-98
Japanese species																																																							
A. cepistipes	01-14-3								+	+	+				-	-	-	-																																			
	01-14-4	+	+	+	+	+	+	+	+	+	+				-	-	-	-				-	-	-	-	-				-	-	-	-				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	05-11-3	+		+	+	+	+	+											-	-	-						-																										
A. gallica	96-34-3	–	–	–	–	–	–	–	–	–	-	+	+	+		+	+		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?		-	-
	96-34-4	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+		+				-	-	-	-	-		-	-	-	-				-				-					-	–	–	–	–	–	?	–		
	04-13-13														+	+	+	+									+																										
A. nabsnona	00-3-1	-	-	-	-	-	-	-	-	-	-								+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-		-	-	-					-	-	-	-	-	-	-	-		-		-
	00-3-2	–	–	–	–	–	–	–	–	–	–				-	-		-				+	+	+	+	+				-						-	-	-					-	-	-	-	-	-	-	-	-	-	-	-
	05-44-1														-	-	-	-	+	+	+	+	+	+	+	+																												
A. ostoyae	94-8-12		-		-							-	-	-								-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-				-	-	-	-	-	-					–
A. sinapina	96-7-1	–	–	–	–	–	–	–	–	–	–	–	–	–					-	-	-	-	-	-	-	-	-	-	-	-	-				-				-	-	-		-			-	-	-	-	-	-	-	-
	96-7-2								-	-	-				-	-	-	-										–	–	–					-	+	+	+					+	+	+	+	+	+	+	+	-	-	-	-
	05-15-1																		-	-	-																	+	+	+	+	+	+			+	+	+	+					
Nag. E	94-2-1	–	–	–	–	–	–	–	–	–	–	-	-	-			-					-	-	-	-	-				-				-	-	-	-	-				-	-	-	-	-	-	-	+	+	+	+	+
	00-15-1	–	–	–	–	–	–	–	-	-	-				-	-	-	-				–		–	–	–				-								-	-	-	-	-	-							+	+	+	+	+
North American species																																																					
A. sinapina	A5	–		-	-	-	-	-							-	-	-	-					–	–	–	–		–	–	–					-	+	+	+		+			+		+	+							-
	A16	–		-	-	-	-	-	-	-	-				-	-	-	-				-	-	-	-	-		-	-	-					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A. gemina	A2	–		-	-	-	-	-							-	-	-	-				-	-	-	-	-		-	-	-					-	-	-	-	-	-	-	-	–	–	–		–	-	-				
	A1	–		-	-	-	-	-							-	-	-	-				-	-	-	-	-		-	-	-					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A14	–		-	-	-	-	-		-							-					-	-	-	-	-		-	-	-	-				-	-	-	-	-	-	-	-	-	-	-	-	-	-				
NABS X	A17	–		-	-	-	-	-	-	-	-				-	-	-	-				-	-	-	-	-		-	-	-	-				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+, compatible reaction; –, incompatible reaction; ?, unclear reaction

fibrous margin. The annuli of *A. cepistipes* and *A. sinapina* were more yellowish, sometimes submembranous, but thin and delicate. In addition, their annulus was often circled by the brown fibrous margin, later collapsing and usually appressed to the stipe. *Armillaria sinapina* may be distinguished by its more plane pileus in age and scales that are more clear than those in *A. cepistipes*.

In this study, we could not obtain the exannulate *Armillaria* species, *A. tabescens* and *A. ectypa*, from Hokkaido. *Armillaria tabescens* occurs mainly from early summer to early autumn in Honshu Island, so we may have missed the fruit body season. The distribution of *A. ectypa* in Honshu is restricted to marshy habitats (Kobayasi 1954; Kudo and Nagasawa 2003; Ito 2004). We did not search such a habitat to find *Armillaria* in this study.

Unfortunately, we could not obtain any basidiocarps of *A. jezoensis* and *A. singula* in this survey in spite of our determined effort, which may suggest that they are rare in occurrence. Qin et al. (2007) reported 14 intersterile taxa from China, including 8 taxa so far unnamed. On the basis of basidiocarp morphology, Qin et al. (2007) suggested one of the unnamed taxa, CBS F (Chinese biological species F), could be conspecific with *A. singula*.

Acknowledgments The authors thank the following people for collecting and providing the specimens of *Armillaria* spp. used in this study: Dr. Y. Abe, Ms. Y. Ando, Ms. M. Hattori, Mr. A. Horoya, Dr. H. Kisanuki, Mr. M. Kitamura, Mr. S. Kudo, Mr. M. Nakagawa, Mr. M. Nakamura, Mr. Y. Nishihara, Mr. S. Nishiyama, Mr. S. Ogasawara, Mr. T. Ohsaka, Mr. J. Ohyama, Mr. S. Sato, Mr. H. Sasaki, Ms. H. Shirayama, Mr. S. Takehashi, Ms. S. Tokuda, Dr. I. Togashi, Mr. K. Toyosawa, Mr. Y. Yamada, and Mr. T. Yoshida. The authors thank Dr. T. Hattori and Dr. H. Neda for their helpful suggestions and comments.

References

Bérubé JA, Dessureault M (1988) Morphological characterization of *Armillaria ostoyae* and *Armillaria sinapina* sp. nov. Can J Bot 66:2027–2034

Cha JY, Igarashi T (1995) A note on *Armillaria mellea* subsp. *nipponica* subsp. nov. in Japan. Mycoscience 36:143–146

Cha JY, Sung JM, Igarashi T (1992) Biological species and morphological characteristics of *Armillaria mellea* complex in Hokkaido: *A. ostoyae* and *A. bulbosa*. Res Bull Coll Exp For 49:185–194

Cha JY, Sung JM, Igarashi T (1994) Biological species and morphological characteristics of *Armillaria mellea c*omplex in Hokkaido: *A. sinapina* and two new species, *A. jezoensis* and *A. singula*. Mycoscience 35:39–47

Fox RTV (2000) Armillaria root rot: biology and control of honey fungus. Intercept Ltd., Andover

Fukuda M, Nakashima E, Hayashi K, Nagasawa E (2003) Identification of the biological species of *Armillaria* associated with *Wynnea* and *Entoloma abortivum* using PCR-RFLP analysis of the intergenic region (IGR) of ribosomal DNA. Mycol Res 107:1435–1441

Guillaumin JJ, Anderson JB, Korhonen K (1991) Life cycle, interfertility, and biological species. In: Shaw CG III, Kile GA (eds) Armillaria root disease. USDA Forest Service, Washington, DC, pp 10–20

Hood IA, Redfern DB, Kile GA (1991) *Armillaria* in planted hosts. In: Shaw CG III, Kile GA (eds) Armillaria root disease. USDA Forest Service, Washington, DC, pp 122–149

Ito S (2004) Occurrence of *Armillaria ectypa* in Aomori prefecture (in Japanese). Newsl Mycol Soc Jpn 2004-4:4–5

Kobayasi Y (1954) Sphagnicolous fungi found in the Ozegahara moor. In: Ozegahara-sougou-gakujyutsu-chosa-dan (ed) Scientific researches of the Ozegahara Moor. JSPS, Tokyo, pp 553–560

Korhonen K (1978) Interfertility and clonal size in the *Armillaria mellea* complex. Karstenia 18:31–42

Kudo S, Nagasawa E (2003) *Armillaria ectypa* rediscovered in Aomori Prefecture, northern Japan (in Japanese with English abstract). Rep Tottori Mycol Inst 41:26–34

Kudo S, Tezuka Y, Yonauchi H (1998) Fungi of Aomori. Graph Aomori, Aomori

Mohammed C, Guillaumin JJ, Berthelay S (1994) *Armillaria* species identified in China and Japan. Mycol Res 98:607–613

Nagasawa E (1991) Taxonomic reassessment of *Armillaria mellea* in Japan. Report for a Grant-in-Aid for Scientific Research, No. 63560155

Ota Y (2001) Identification of biological species of *Armillaria* in Japan using mating tests. MAFF Microorganism Genetic Resources Manual No. 10:1–7

Ota Y, Fukuda K, Suzuki K (1998a) The nonheterohtallic life cycle of Japanese *Armillaira mellea*. Mycologia 90:396–405

Ota Y, Matsushita N, Nagasawa E, Terashita T, Fukuda K, Suzuki K (1998b) Biological species of *Armillaria* in Japan. Plant Dis 82:537–543

Qin GF, Zhao J, Korhonen K (2007) A study on intersterility groups of *Armillaria* in China. Mycologia 99:430–441

Sekizaki H, Kuninaga S, Yamamoto M, Asazu SN, Sawa S, Kojoma M, Yokosawa R, Yoshida N (2008) Identification of *Armillaria nabsnona* in Gastrodia tubers. Biol Pharm Bull 31:1410–1414

Shaw CG III, Kile GA (eds) (1991) Armillaria root disease. USDA Forest Service, Washington, DC

Volk TJ, Burdsall HH Jr (1995) A nomenclatural study of *Armillaria* and *Armillariella* species. Synopsis Fungorum 8, Fungiflora, Oslo