

## Biological species of *Armillaria* and their mycoparasitic associations with *Rhodophyllus abortivus* in Hokkaido

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Accepted for publication 17 November 1995

Specimens of basidiomes and/or rhizomorphs of *Armillaria mellea* complex and basidiomes of *Rhodophyllus abortivus*, developing on the same decaying stumps or stems of forest trees, were collected in three forests in Hokkaido. Normal basidiomes of *R. abortivus* were found near to, but free from, the rhizomorphs and/or basidiomes of *Armillaria*, while abnormal basidiomes, as carpophoroid forms, were developed on the rhizomorphs of *Armillaria*. Of three mycoparasitic *Armillaria* isolates found with *R. abortivus*, one was identified as *A. gallica* and two as *A. jezoensis*. The isolates of *R. abortivus* showed excellent mycelial growth and rhizomorph formation on PDA. However, on MDA, RMDA and BMDA, they showed poor aerial mycelia growth and no rhizomorphs. In the contrapositional cultures, the growth of *A. gallica* was completely inhibited by *R. abortivus* on PDA but only slightly inhibited on MDA and RMDA. On the other hand, mutual inhibition at a distance was observed on BMDA. The mycelial growth and rhizomorph formation in *A. jezoensis* were severely inhibited by the colony of *R. abortivus* on PDA, but only slightly inhibited on MDA. On RMDA and BMDA, the colonies of two *Armillaria* species and *R. abortivus* showed mutual inhibition at a distance and apparent rhizomorph formation by both *Armillaria* species.

Key Words—agaricoid form; *Armillaria gallica*; *Armillaria jezoensis*; carpophoroid form; *Rhodophyllus abortivus*.

*Armillaria mellea* (Vahl: Fr.) Kummer complex is well known as a forest pathogen as well as an orchid symbiont, and an excellent edible mushroom. The parasitic association of *A. mellea* complex with *Rhodophyllus abortivus* (Berkeley & Curtis) Singer has been reported by Watling (1974). Rhizomorphs of *A. mellea* complex invade the developing basidiomes of *R. abortivus*, and the subsequent mycelial development induces aberrant host morphology as carpophoroids (Watling, 1974). This phenomenon appears relatively common in eastern North America as well as in Japan (Watling, 1974; Imazeki and Hongo, 1987; Igarashi, 1988).

Recently, several studies have shown that *Armillaria* consists of a complex of several reproductive isolate groups or biological species in North America (Anderson and Ullrich, 1979; Anderson et al., 1980), Europe (Korhonen, 1978) and Australia (Kile and Watling, 1983). Based on mating behavior, six biological species of the fungus in Honshu and six in Hokkaido, Japan have been reported (Nagasawa et al., 1991; Cha et al., 1992, 1994, 1995; Cha and Igarashi, 1994, 1995a). However, reproductive studies of *Armillaria* as a biological species have not been carried out in relation to its mycoparasitic association with *R. abortivus*. The specificity of the relationship between *A. mellea* complex and *R. abortivus* has also not been studied.

This study was conducted to identify the biological species of *A. mellea* complex and to clarify the relationship between *Armillaria* and *R. abortivus*.

### Materials and Methods

**Collection** Basidiomes of *R. abortivus* and its mycoparasitic *A. mellea* complex, developing on the same decaying stumps or stems of forest trees, were collected in September and October 1993 in stumps forests in Hokkaido. Collection dates, hosts and habitats are recorded in Table 1.

**Isolation** Fragments of internal tissue, free from contaminants, of basidiomes of both *Armillaria* and *R. abortivus* were transferred to Petri dishes containing 1.5% water agar and incubated at 25°C for 1 wk in the dark. Rhizomorphs were washed with tap water and then sterilized with sodium hypochlorite solution (a.i.; 5% chlorine) for about 1–2 min. The sterilized rhizomorphs, cut to 1–1.5 cm in length, were transferred to Petri dishes and incubated in the same way as in tissue culture. Stock cultures from both *Armillaria* and *R. abortivus* were maintained on MA medium (30 g malt extract + 15 g agar in 1000 ml of distilled water) in Petri dishes at 25°C in the dark.

**Identification of biological species of *Armillaria* isolates** Diploid isolates were mated with haploid tester strains, as listed in Table 2, of Hokkaido biological species. Mating pairing was made by placing inocula 1 mm apart on 1.25% MA in a Petri dish and incubating at 22°C for about 4 wk. Haploid testers in incompatible pairing produced fluffy aerial mycelium, while the haploid testers in compatible pairing produced flat crustose mycelia (Anderson and Ullrich, 1979; Korhonen, 1978; Ullrich and

Table 1. Origin of *Armillaria* and *R. abortivus* used in this study.

Specimens	Dates	Locations	Hosts <sup>a)</sup>	Habitats <sup>a)</sup>
HUA93106	9/27/93	Fukushima, Matsumae Gun	Un	Cj <sup>a)</sup> plantation, solitary on decaying trunk, with <i>R. abortivus</i> (R93106)
HUA93116	10/13/93	Tomakomai	Qm	Broad-leaved forests, rhizomorph in decaying stem, with <i>R. abortivus</i> (R93116)
HUA93122	10/21/93	Otoineppu, Nakagawa Gun	Uj	Mixed forests, rhizomorph in dead stem, with <i>R. abortivus</i> (R93122), swampy valley

a) Cj, *Cryptomeria japonica* D. Don; Qm, *Quercus mongolica* Fisch. var. *grosseserrata* Rehd. & Wils.; Uj, *Ulmus japonica* Sarg.; Un, unidentified broad-leaved tree.

Anderson, 1978).

**Cultures** The culture media were MDA (30 g malt extract + 20 g dextrose + 15 g agar in 1000 ml of distilled water), PDA (39 g potato-dextrose agar, Nissui) in 1000 ml of distilled water), RMDA (30 g malt extract + 20 g dextrose + 15 g agar in 1000 ml of rhizomorph decoction) and BMDA (30 g malt extract + 20 g dextrose + 15 g agar in 1000 ml of oak-bark decoction) for *R. abortivus* and *Armillaria*. The decoctions of *Armillaria* rhizomorph and of oak-bark were prepared by boiling 200 g each of raw rhizomorph and oak-bark, respectively, in 1000 ml of distilled water in a flask for 3 h, then filtering the resultant liquid through gauze. Fungal materials are listed in Table 1. For the pure cultures of *R. abortivus*, the mycelia from stock cultures were placed on the surface of 15 ml of media in Petri dishes and incubated at 25°C for 4 wk in the dark. For the contrapositional cultures of *R. abortivus* and *Armillaria*, two mycelial plugs were inoculated 1 cm apart from each other on the media. All cultures were conducted in triplicate using mycelial inoculum plugs of 5 mm diam cut from stock cultures. After incubation at 25°C for 4 wk in the dark, the colony characteristics of *R. abortivus* and the growth relationships between *Armillaria* and *R. abortivus* were observed on each medium.

## Results

**Habitats** Three specimens of *R. abortivus* basidiomes were collected with the basidiomes or rhizomorph of *A. mellea* complex on decaying stumps or stems in Hokkaido forests (Fig. 1). *Armillaria* rhizomorphs and *R. abortivus* mycelia or rhizomorphs were formed on and in-

side the barks of host trees. Normal basidiomes of *R. abortivus* were found near to, but free from, the rhizomorphs and/or basidiomes of *Armillaria* (Figs. 2-4). Abnormal basidiomes of *R. abortivus* contained *Armillaria* rhizomorphs enclosed in the bottom of the basidiomes. These *Armillaria* rhizomorphs were generally old and black or dark black in color (Fig. 5). No mycelia or rhizomorphs of *R. abortivus* were found in the mycelial fans or young rhizomorphs of *A. mellea* complex (Figs. 6, 7).

### Identification of biological species of *Armillaria* isolates

Vegetative isolates of *Armillaria* showed dark-brownish crustose mycelia (flat and lacking aerial mycelia), which are diploid (Hintikka, 1973; Korhonen, 1978; Ullrich and Anderson, 1978; Peabody and Peabody, 1984, 1985). The results of mating tests of the isolates with the haploid testers of Hokkaido *Armillaria* are detailed in Table 3. One isolate was identified as *A. gallica* (HUA93106) and two as *A. jezoensis* (HUA93116,

Table 2. Origin of haploid testers from Hokkaido *Armillaria*.

Biological species	Stock No. of isolates	Host trees
<i>A. ostoyae</i>	HUA9112	<i>Betula ermanii</i>
	HUA9113	<i>Betula ermanii</i>
<i>A. gallica</i>	HUA9102	<i>Fraxinus mandshurica</i> var. <i>japonica</i>
	HUA9125	<i>Prunus ssiiori</i>
<i>A. jezoensis</i>	HUA9116	<i>Ulmus japonica</i>
	HUA9127	<i>Quercus mongolica</i> var. <i>grosseserrata</i>
<i>A. sinapina</i>	HUA9115	<i>Salix sachalinensis</i>
	HUA9124	<i>Ulmus japonica</i>
<i>A. singula</i>	HUA9101	<i>Fraxinus mandshurica</i> var. <i>japonica</i>
	HUA9109	<i>Abies sachalinensis</i>

Table 3. Results of mating tests between diploid isolates and haploid testers originating from Hokkaido *Armillaria*.

Stock No. of diploid isolates	Haploid testers <sup>a)</sup>									
	<i>A. ostoyae</i>		<i>A. gallica</i>		<i>A. jezoensis</i>		<i>A. sinapina</i>		<i>A. singula</i>	
	1	2	3	4	5	6	7	8	9	10
HUA93106	-	-	+	+	-	-	-	-	-	-
HUA93116	-	-	-	-	+	+	-	-	-	-
HUA93122	-	-	-	-	+	+	-	-	-	-

a) Number of strains; 1, HUA9112; 2, HUA9113; 3, HUA9107; 4, HUA9125; 5, HUA9116; 6, HUA9127; 7, HUA9115; 8, HUA9124; 9, 9101; 10, HUA9109. +, compatible pairing; -, incompatible pairing.

Table 4. Cultural characteristics of *R. abortivus* isolate (R93106) on various media.

Medium <sup>a)</sup>	Mean diam of colony (mm)	Colony form
MDA	35.7	Somewhat whitish aerial mycelium on the surface, no rhizomorph formation
PDA	50.7	Submerged mycelium, rhizomorph formation on the surface
RMDA	37.0	Whitish aerial mycelium on the surface, no rhizomorph formation
BMDA	33.2	Whitish aerial mycelium on the surface, no rhizomorph formation

a) MDA, 30 g malt extract + 20 g dextrose + 15 g agar in 1000 ml of distilled water; PDA, 39 g potato-dextrose agar, Nissui in 1000 ml of distilled water; RMDA, 30 g malt extract + 20 g dextrose + 15 g agar in 1000 ml of rhizomorph decoction; BMDA, 30 g malt extract + 20 g dextrose + 15 g agar in 1000 ml of oak-bark decoction.

HUA93122). These isolates were clearly compatible with the haploid testers of the corresponding species.

**Cultural characteristics of *R. abortivus* isolates (R93106)**

The observations on mycelial growth and colony forms are described in Table 4. *Rhodophyllus abortivus* showed excellent mycelial growth and rhizomorph formation on PDA, but poor growth of aerial mycelia and no rhizomorphs on MDA, RMDA and BMDA (Fig. 8).

**Interactions between *Armillaria* isolates and *R. abortivus* isolate (R93106)**

Contrapositional cultures between *Armillaria* isolates and *R. abortivus* showed different patterns of interaction on different media. For instance, growth of *A. gallica* was completely inhibited by *R. abortivus* colony on PDA but only slightly inhibited on MDA and RMDA (Fig. 9). On the other hand, mutual inhibition at a distance was observed on BMDA (Fig. 9). In particular, rhizomorphs of *R. abortivus* were formed on PDA, on which the growth of *A. gallica* was severely inhibited. *Rhodophyllus abortivus* formed sclerotia on all media tested when in contrapositional culture with *Armillaria*.

The results of contrapositional culture between *A. jezoensis* isolates HUA93116 and HUA93122 and *R. abortivus* are shown in Figs. 10 and 11. The mycelial growth and rhizomorph formation by *A. jezoensis* was severely inhibited by *R. abortivus* colony on PDA, but only slightly inhibited on MDA. On RMDA and BMDA, the colonies of the two *Armillaria* species and *R. abortivus* showed mutual inhibition at a distance. However, both *Armillaria* species formed rhizomorphs. *Rhodophyllus abortivus* produced rhizomorphs on PDA, and sclerotia on all media tested.

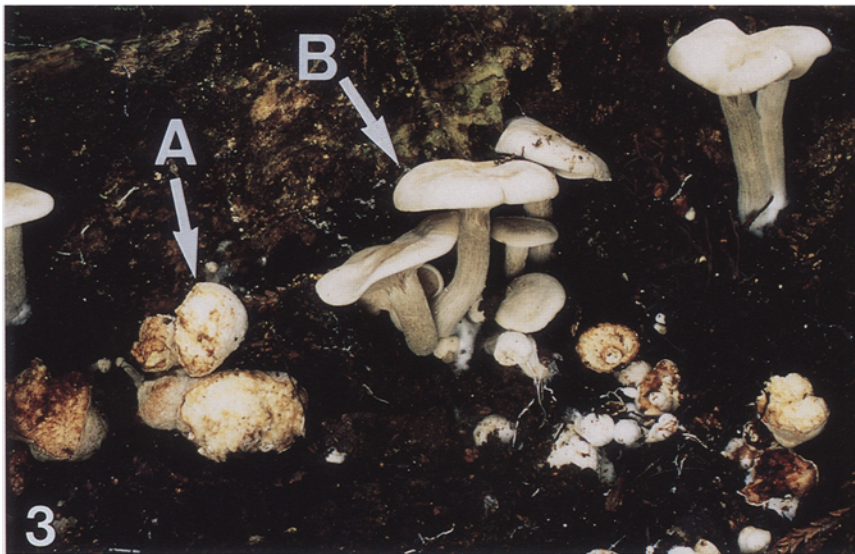
**Discussion**

Watling (1974) reported that the carpophoroid of *R. abortivus* is induced by an attack by the basidiomycete of *A. mellea* complex. No mating test for identification of biological species has yet been conducted on *Armillaria*. In

this study, the carpophoroid form was discovered on *Armillaria* rhizomorphs with a black or dark black color. The *Armillaria* isolates were identified by mating test as *A. gallica* and *A. jezoensis*. Watling (1987) suggested that *A. gallica* may be the most common mycoparasitic species, because this fungus produces copious rhizomorphs which not only permeate the soil around old trunks but also infect stumps and plants. The rhizomorphs of this fungus spread under and over bark forming a reticulate or net-like structure. The Hokkaido *A. gallica* produces abundant rhizomorphs in the cambium layer of decayed stumps or stems and in soil around them. *Armillaria jezoensis* has similar characteristics of rhizomorph formation to *A. gallica* (Cha, 1995). *Armillaria gallica* and *A. jezoensis* were frequently isolated from *Gastrodia elata* and *Galeora septentrionalis* of Orchidaceae (Terashita and Chuman, 1989; Cha and Igarashi, 1995b) Therefore, it can be considered that the well-developed ability of *Armillaria* to form rhizomorphs may play an important role in its associations with other basidiomycetous fungi or orchids.

*Rhodophyllus abortivus* readily produced abundant mycelia as well as rhizomorphs on PDA. The mycelial growth and rhizomorph formation of both *A. gallica* and *A. jezoensis* were severely inhibited by *R. abortivus* on PDA. Furthermore, both *Armillaria* species were covered by the *Rhodophyllus* rhizomorphs. Although mutual inhibition between *Armillaria* and *Rhodophyllus* was observed on the media containing the rhizomorph (RMDA) and the bark decoction (BMDA), *Armillaria* isolates showed good mycelial growth and rhizomorph formation. At the early stage of wood decay, only the mycelial fans and rhizomorphs of *Armillaria* could be observed. However, where the cambium of the host tree showed advanced decay, both mycelia and rhizomorphs of *R. abortivus* and rhizomorphs of *Armillaria* could be found. These observations suggest that a dead tree that is initially occupied by *Armillaria* may favour the growth of *R. abortivus*.

Watling (1974) reported that the *A. mellea* complex apparently does not kill the basidiome, since actively growing mycelium of *R. abortivus* is always present and the carpophoroid does not putrefy any quicker than the agaricoid form. Therefore, it might appear that certain stimulants for mycelial growth are either induced by *Armillaria* or retained by *R. abortivus* (Watling, 1974). Moreover, Watling assumed that *R. abortivus* provides some attractant(s) to *Armillaria*. We assumed successive changes in the relationship between *Armillaria* and *R. abortivus* in the natural habitat. The following sequence can be suggested for these changes: 1) *Armillaria* is the primary decomposer, which may produce certain stimulative substance(s) for the growth of *R. abortivus*; 2) as a result, rhizomorphs of *Armillaria* and mycelia and rhizomorphs of *R. abortivus* develop at the same portion of the habitat; 3) the rhizomorphs of *Armillaria* may be enclosed by the primordia of *R. abortivus* basidiome; 4) the outermost layer of enclosed rhizomorph of *Armillaria* is destroyed; 5) the active inner mycelia of the enclosed *Armillaria* rhizomorph grow through the basidiome primordium



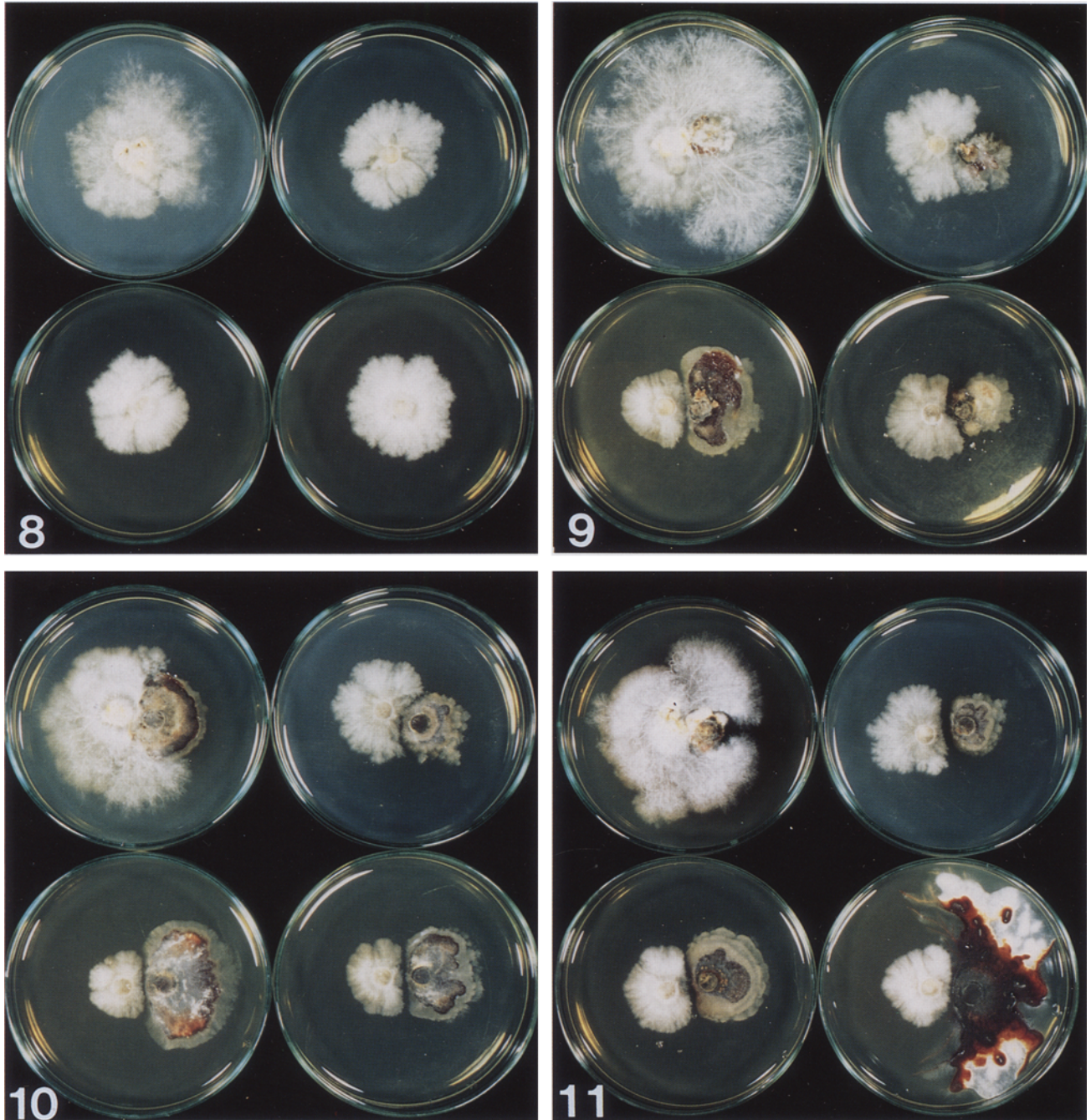


Fig. 1. Basidiome formation of *A. gallica* (A) and normal (B) and abnormal (C) forms of *R. abortivus* on a decaying stump.  
 Fig. 2. Normal form basidiomes of *R. abortivus*.  
 Fig. 3. Abnormal (A) and normal (B) form basidiomes of *R. abortivus*.  
 Fig. 4. Abnormal form basidiomes of *R. abortivus* formed on *Armillaria* rhizomorph (R).  
 Fig. 5. Rhizomorphs of *Armillaria* (A) and *R. abortivus* (B), and primordium (P) of *R. abortivus* basidiome formed on *Armillaria* rhizomorph in the bark of tree.  
 Figs. 6, 7. Mycelial fan (6) and young rhizomorph (7) of *Armillaria* formed in the bark of tree.  
 Fig. 8. Colonies form of *R. abortivus* on PDA (top left), MDA (top right), BMDA (bottom left) and RMDA (bottom right).  
 Figs. 9–11. Contrapositional cultures of *R. abortivus* with *A. gallica* HUA93106 (9), and with *A. jezoensis* HUA93116 (10) and HUA93122 (11) on PDA (top left), MDA (top right), BMDA (bottom left) and RMDA (bottom right). All pairings consist of *Rhodophyllus* (left) and *Armillaria* (right) isolates.

of *R. abortivus*, leading to abnormal basidiome production.

#### Literature cited

- Anderson, J. B., Korhonen, K. and Ullrich, R. C. 1980. Relationships between European and North American biological species of *Armillaria mellea*. *Exp. Mycol.* **4**: 87–95.
- Anderson, J. B. and Ullrich, R. C. 1979. Biological species of *Armillaria mellea* in North America. *Mycologia* **71**: 402–414.
- Cha, J. Y. 1995. Taxonomy and ecology of *Armillaria mellea* complex in Hokkaido. Hokkaido University, Sapporo. (D. Agr. Thesis).
- Cha, J. Y. and Igarashi, T. 1994. Intersterility groups and cultural characteristics of *Armillaria mellea* complex in Hokkaido. In: Proceedings of the 8th Int. Conf. on Root and Butt Rots. IUFRO Working party S2. 06. 01, 1993 August 9–16; Wik, Sweden and Haikko, Finland, (ed. by Johansson, M. and Stenlid, J.), pp. 479–488. Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Cha, J. Y. and Igarashi T. 1995a. A note on *Armillaria mellea* subsp. *nipponica* subsp. nov. in Japan. *Mycoscience* **36**: 143–146.
- Cha, J. Y. and Igarashi T. 1995b. *Armillaria* species associated with *Gastrodia elata* in Japan. *Eur. J. For. Path.* **25**: 319–326.
- Cha, J. Y., Sung, J. M. and Igarashi, T. 1992. Biological species and morphological characteristics of *Armillaria mellea* complex in Hokkaido: *A. ostoyae* and *A. bulbosa*. *Res. Bull. Exp. For. Hokkaido Univ.* **49**: 185–194.
- Cha, J. Y., Sung, J. M. and Igarashi, T. 1994. Biological species and morphological characteristics of *Armillaria mellea* complex in Hokkaido: *A. sinapina* and two new species, *A. jezoensis* and *A. singula*. *Mycoscience* **35**: 39–47.
- Cha, J. Y., Sung, J. M. and Igarashi, T. 1995. *Armillaria mellea* (Vahl: Fr.) Kummer s. s. from Hokkaido. *J. Jpn. For. Soc.* **77**: 395–398.
- Hintikka, V. 1973. A note on the polarity of *Armillariella mellea*. *Karstenia* **13**: 32–39.
- Igarashi, T. 1988. Fungi of Hokkaido. Hokkaidosinbunshya. (In Japanese.)
- Imazeki, R. and Hongo, T. 1987. Colored illustrations of mushrooms of Japan. vol. I. Hoikusha. (In Japanese.)
- Kile, G. A. and Watling, R. 1983. *Armillaria* species from south-eastern Australia. *Trans. Br. Mycol. Soc.* **81**: 129–140.
- Korhonen, K. 1978. Interfertility and clonal size in the *Armillaria mellea* complex. *Karstenia* **18**: 31–42.
- Nagasawa, E., Komatsu, M. and Maekawa, N. 1991. Taxonomic reassessment of *Armillaria mellea* in Japan. Report for a Grant-in-Aid for Scientific Research No. 63560155, Ministry of Education, Science and Culture of Japan. (In Japanese.)
- Peabody, D. C. and Peabody, R. B. 1984. Microspectrophotometric nuclear cycle analysis of *Armillaria mellea*. *Exp. Mycol.* **8**: 161–169.
- Peabody, D. C. and Peabody, R. B. 1985. Widespread haploidy in monokaryotic cells of mature basidiocarps of *Armillaria bulbosa*, a member of the *Armillaria mellea* complex. *Exp. Mycol.* **9**: 212–220.
- Terashita, T. and Chuman, S. 1989. *Armillaria* species isolated from the wild orchid, *Galeola septentrionalis*. In: Proceedings of the 7th Int. Conf. on Root and Butt Rots. IUFRO Working party S2. 06. 01, 1988 August 9–16; Vernon and Victoria, BC, Canada, (ed. by Morrison, D. J.), pp. 27–44. Forestry Canada, Pacific Forestry Centre, Victoria, BC., Canada.
- Ullrich, R. C. and Anderson, J. B. 1978. Sex and diploidy in *Armillaria mellea*. *Exp. Mycol.* **2**: 119–129.
- Watling, R. 1974. Dimorphism in *Entoloma abortivum*. *Bull. Men. Soc. Linn. Lyon, Num. Spec.* **43**: 449–470.
- Watling, R. 1987. The occurrence of annulate *Armillaria* species in northern Britain. *Notes R. Bot. Gdn. Edinb.* **44**: 459–484.