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Biological species and morphological characteristics of *Armillaria mellea* complex in Hokkaido: *A. sinapina* and two new species, *A. jezoensis* and *A. singula*

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Three intersterility groups of *Armillaria mellea* sensu lato were discovered by examining all pairwise combinations of monosporous isolates of basidiomes collected in Hokkaido. One of them, group IV, was identified as *A. sinapina* by mating it with tester strains. Two new species, groups III and V, were named *A. jezoensis* and *A. singula*, respectively. Their morphological forms and the ecology of their basidiomes are described.

Key Words—*Armillaria mellea* complex; *Armillaria sinapina*; *Armillaria jezoensis*; *Armillaria singula*; morphological forms.

Introduction

Armillaria mellea (Vahl: Fr.) Kummer is an important forest pathogenic fungus with an extremely wide host range and variability of culture and basidiome morphology (Smith and Griffin, 1971; Pegler and Gibson, 1972; Bérubé and Dessureault, 1988; Shaw and Loopstra, 1988). In Japan, this fungus has caused severe damage in plantations of Japanese cypress, Japanese larch, and Japanese red pines (Ito, 1974). Studies of mating behaviour among single-spore isolates have revealed that this fungus consists of several biological species in North America (Anderson and Ullrich, 1979; Anderson et al., 1980), Europe (Korhonen, 1978), Australia (Kile and Watling, 1983), Africa (Mwangi et al., 1989) and Asia (Sung et al., 1990; Nagasawa et al., 1990; Cha et al., 1992; Sung et al., 1992). European and Australian biological species have been described in detail regarding their taxonomy and basidiome morphology (Kile and Watling, 1983; Roll-Hanson, 1985; Guillaumin et al., 1985). Of nine North American biological species, seven have been described in terms of the morphological characteristics of their basidiomes (Bérubé and Dessureault, 1988, 1989; Motta and Korhonen, 1986).

Although studies of the biological species constituting the *A. mellea* complex have recently been conducted in Japan, morphological characteristics of basidiomes were not described in detail. We have identified biological species and described the basidiome morphology of *A. ostoyae* (Romagnesi) Herink and *A. gallica* Marxmüller & Romagnesi (synonym, *A. bulbosa* (Barla) Kile & Watling) in Hokkaido (Cha et al., 1992). Moreover, basidiomes of different morphological forms of *A. mellea* complex were found in Hokkaido. The objectives of this study were to identify the biological species occurring in Hokkaido and describe their basidiome morphology and habitats.

Materials and Methods

Collection Fifteen basidiomes of *A. mellea* complex were collected from the fall of 1991 to 1992 in forests at various locations throughout Hokkaido, where they occurred in conifer forests, broad-leaved forests and mixed forests, in association with various hosts. Table 1 lists hosts, locations and habitats. Dried voucher specimens were preserved and stored.

Isolation In order to obtain haploid isolates, plugs measuring 2 cm × 2 cm × 0.5 cm were cut from the pileus of each basidiome with a knife. The upper surface of the pileus was attached with double-faced tape to the inside of a petri dish cover, and the cover was placed on a petri dish containing 1.5% water agar. Basidiospores were allowed to be discharged for 3–5 h or until several spores could be observed scattered on the surface of the medium with a stereomicroscope. After incubation for 12–24 h at 22°C, individual germinated spores were selected and each was transferred with a fine metal needle to a tube slant containing 1.25% MA (1.25% malt extract (Difco) and 1.5% agar). Diploid isolates were made from the tissue of the basidiomes and placed on tube slants containing 1.25% MA. Haploid isolates showed white or sometimes brownish fluffy aerial mycelium, but diploid isolates produced flat crustose mycelium in culture (Hintikka, 1973; Peabody and Peabody, 1984).

Intersterility groups Haploid isolates from each basidiome were mated in all possible pairwise combinations. Pairings were made by placing inocula 1 mm apart on a 1.25% MA and incubated at 22°C for 3 weeks. Incompatible pairings produce fluffy aerial

Table 1. Specimens of biological species of *Armillaria* in Hokkaido.

Specimen	Date	Intersterility group	Locality & region	Host*	Habitat
HUA9106	9/13/91	III	Nakagawa Otoineppu	Qm	Solitary, on decaying trunk, mixed forest, e. ** 400
HUA9116	9/26/91	III	Horokanai Moshiri	Uj	Caespitose, solitary, scattered on decaying trunk, broad-leaved forest, e. 220
HUA9120	10/ 7/91	III	Tomakomai	Uj	Solitary, decaying stump, broad-leaved forest, e. 65
HUA9122	10/ 7/91	III	Tomakomai	Ps	Solitary, decaying trunk, mixed forest, e. 10
HUA1927	10/17/91	III	Tomakomai	Uj	Caespitose, on decaying stump up to 1.2 m, broad-leaved forest, e 50
HUA9108	9/17/91	IV	Tomakomai	Qm	Caespitose, decaying root, broad-leaved forest, e. 50
HUA9110	9/17/91	IV	Horokanai Moshiri	Vf	Caespitose, living stump, mixed forest, e. 200
HUA9115	9/26/91	IV	Horokanai Moshiri	Ss	Solitary, clustered on living stump, broad-leaved forest, e. 250
HUA9117	9/26/91	IV	Horokanai Moshiri	Qm	Caespitose, clustered on dead stump, broad-leaved forest, e. 250
HUA9121	10/ 7/91	IV	Tomakomai	Tj	Caespitose, solitary, decaying trunk broad-leaved forest, e. 60
HUA9124	10/ 8/91	IV	Tomakomai	Uj	Caespitose, clustered on decaying trunk and wood debris, broad-leaved forest, e. 50
HUA9126	10/11/91	IV	Tomakomai	Un	Caespitose, on decaying trunk, mixed forest, e. 50
HUA9233	7/12/92	IV	Kamikawa	Fm	Caespitose, solitary on the decaying root, conifer, e. 395
HUA9101	8/30/91	V	Tomakomai	Fm	Solitary, on soil, riversides of forest park, e. 20
HUA9109	9/17/91	V	Tomakomai	As	Solitary, on soil, riversides of forest park, e. 20

* Abbreviations: As, *Abies sachalinensis*; Fm, *Fraxinus mandshurica* var. *japonica*; Ps, *Prunus sargentii*; Qm, *Quercus mongolica* var. *grosseserrata*; Ss, *Salix sachalinensis*; Tj, *Tilia japonica*; Uj, *Ulmus japonica*; Un, unidentified broad-leaved; Vf, *Viburnum furcatum*. ** Elevation in meters above sea level.

mycelium, while compatible pairings produce flat crustose mycelium (Ullrich and Anderson, 1978) (Fig. 4, 5). The *A. mellea* complex has a bifactorial incompatibility system; two population are judged according to the criteria of Bérubé and Dessureault (1987).

Biological species status Haploid and diploid isolates from each of the basidiomes were mated with tester strains of each North American and European biological species. Tester strains were kindly supplied by Dr. T. C. Harrington, Dr. K. Korhonen and Dr. D. J. Morrison (Table 2).

Cultural characteristics In order to ascertain rhizomorph forms in culture, diploid isolates were incubated on 3% MA at 22°C and observed 3 weeks later.

Microscopical observation Sizes of spores and basidia were determined in material mounted with 4% KOH and 1% congo red plus 1% phloxine, using a calibrated eyepiece micrometer. Pileipellis layers were stained with 0.05% toluidine blue in 1% sodium borate (Bérubé and Dessureault, 1988). Materials were prepared by hand section with a knife and observed by microscope. In order to observe the nuclear condition of subhymenial

Table 2. Origin of haploid tester strains of biological species of *Armillaria*.

Biological species	No. of strain	Collector
A. mellea	M25, 274	D. C. Morrison, T.C. Harrington
A. ostoyae	E12, M1	K. Korhonen, D.C. Morrison
A. borealis	A1	K. Korhonen
A. cepistipes	E6, M29	K. Korhonen, D.C. Morrison
A. gallica	M19, 474	D. C. Morrison, T.C. Harrington
A. sinapina	A53	T. C. Harrington
A. calvescens	A77	T. C. Harrington
A. gemina	A78, A80	T. C. Harrington

hyphae, materials were stained with the fluorochrome 4'-6-diamidino-2-phenylindole (DAPI) (Coleman et al., 1981). Stained materials were observed with an Orthoplux II microscope fitted with an epifluorescence attachment and by utilizing the appropriate filter combinations.

Color names and codes in parentheses are indicated from Munsell (1990).

Results

The fifteen basidiomes consisted of three intersterility groups, III, IV and V. These groups were divided by examination of all pairwise combinations in a haploid × haploid mating system. The compatible pairings produced the crustose mycelium, as seen in vegetative isolates from the flesh of basidiomes, mycelial fans, and rhizomorphs. The incompatible pairings continued to show the fluffy aerial mycelium of single-spore isolates. All pairings showed complete interfertility within groups III, IV and V, respectively, but intersterility between groups. All isolates were paired with tester strains of North American and European biological species using haploid × haploid and diploid × haploid mating systems for identification of species. The Buller phenomenon occurs in the diploid × haploid mating system of *Armillaria* (Korhonen, 1978). When a fluffy haploid mycelium is paired with a crustose diploid isolate of the same species, the morphology of the former becomes crustose. We observed no exceptions to this reaction. Group IV showed complete interfertility with *A. sinapina* Bérubé & Dessureault, but groups III and V were not interfertile with any tester strain in either mating system. We named III *A. jezoensis* and V *A. singula*. The morphological characteristics of the three species identified in this study are described in detail.

Armillaria sinapina Bérubé & Dessureault, Can. J. Bot. 66: 2027, 1988 Fig. 1

Pileus 1.5-10.5 cm diam, convex and slightly umbonate when young, plano-convex, finally plane to somewhat uplifted and sometimes irregularly undulating in age; surface dry, very pale brown (10YR-8/4), reddish brown (5YR-4/3) at center, reddish brown (5YR-4/3) to yellowish red (5YR-5/8) toward the margin, covered with fine fibers usually dark grayish brown (10YR-4/2), dark brown (7.5YR-3/2), dark reddish brown (5YR-3/2) scales, dense toward the center. Margin usually inrolled when young, then acute later, slightly translucent-striate, concolorous with cap or somewhat darker later. Flesh firm, thin to thick; context white (10YR-8/2).

Lamellae white when young, then light brown (7.5YR-6/4) to pinkish white (7.5YR-8/2), sinuate, subdecurrent, close, thick.

Stipe 4.7-6.8 × 0.5-0.8 cm, cylindric, with an abruptly bulbous base, concolorous with lamellae at the apex, usually grayish brown (10YR-5/2) to reddish brown (5YR-4/3) toward the base, longitudinally fibrillose-striate, without flocci but olive brown (2.5Y-4/3) fibers at the base, fragile, solid when young, stuffed when old. Annulus membranous, thin, usually unbroken or sometimes broken and attached to the stipe, white (10YR-8/1), brown (7.5YR-5/3) later.

Spores white (10YR-8/1) in mass, subglobose, broadly elliptic, with an apiculus, 6.7-10 × 4.9-6.9 μm, smooth, hyaline, nonamyloid. Basidia clavate, 37.9-44.9 × 7.2-9.4 μm, with 4 sterigmata, clamped at the base. Pleurocystidia absent. Suprapellis made up of parallel, thin-walled, hyaline hyphae of cells 35.4-90.8 × 9.6-19.5 μm, with the brownish pigmented uppermost layer, at septa without clamps (Fig. 9). Mediopellis composed of tight entangled structure with irregularly elliptical and globose thick-walled cells of 9.1-50.2 × 6.1-21.1 μm (Fig. 10). Subpellis made up of loosely net-like structure with longitudinal filamentous hyphae of cells 55.1-108.1(200) × 11.8-24.5 μm, staining in toluidine blue (Fig. 11). Subhymenial hyphae filamentous, sometimes with a clamp, binucleate. Lamellar trama bilateral.

Intersterility group: IV.

Specimens examined: Collected by J. Y. Cha, HUA9108, HUA9115, HUA9117, HUA9121, HUA9126, HUA9133; Collected by T. Miyamoto, HUA9110. All are deposited at SAPA (herbarium, Faculty of Agriculture, Hokkaido University, Sapporo, Japan). Table 1 lists host, habitat, collection date and location.

Japanese name: Hotei-naratake (n. nov.).

The basidiomes in Hokkaido forests developed from the middle of July to early October. They occurred mainly caespitose to sometimes solitary on dead or living stumps and roots. Hosts are composed of dead broad-leaved trees such as *Quercus mongolica* Fisch. ex Turcz. var. *grosseserrata* (Bl.) Rehd. & Wils., *Ulmus japonica* (Rehd.) Sarg. and *Fraxinus mandshurica* Rupr. var. *japonica* Maxim. and living stumps of *Viburnum furcatum* Bl. and *Salix sachalinensis* Fr. Schm. Rhizomorphs are discovered beneath the bark of hosts and in the soil. In terms of morphology, rhizomorphs are cylindric, have sharp tips and are of the monopodial branching type in pure culture. The collection sites are from 50 m to 395 m above sea level. It was reported that *Armillaria sinapina* in North American biological species may occur in the same location as *A. ostoyae, A. mellea* s. str. and *A. gallica*, but can be differentiated by its general brownish color often with tile red tinges and golden yellow universal veil (Bérubé and Dessureault, 1988). In our collections, it was shown to have wide local ranges and times. Moreover, HUA9233 specimen was discovered with *A. ostoyae* in the same place and period, but could be distinguished by morphological characteristics of the basidiomes. Basidiomes of *A. sinapina* had reddish brown pileus, small scales and a thin membranous annulus, while *A. ostoyae* was dark brown in color, being dark reddish brown to brownish gold, and had large conspicuous scales and a thick membranous annulus. In rhizomorph forms, *A. ostoyae* is belt-shaped and of the dichotomous branching type, but *A. sinapina* is cylindric and monopodial. *Armillaria mellea* s. str., which has no clamp on the basidium and is uninucleate in subhymenial hyphae, can be distinguished from *A. sinapina*. *Armillaria sinapina* has not been discovered in Europe but is widely distributed in Quebec (Bérubé and Dessureault, 1988),

British Columbia (Morrison et al., 1985) and New York State (Ullrich and Anderson, 1978). It has not been discovered in Japan.

Armillaria jezoensis Cha & Igarashi, sp. nov. Fig. 2

Pileus 4.7-6.8 cm diam, primum hemisphaerico-parabolicus vel convexus, postremo plano-convexus vel planus, aliquando umbonatus, suberosus vel undulatus. Superficies sicca, centro rubro-brunneo, latere obscure rubro-brunneo vel brunneo, cum fibrillis vel squamis obscure brunnei obtecta. Lamellae sinuatae vel subdecurrentes, primum albae, postremo brunneae vel rubrobrnneae, densae. Stipes centralis, 3.9-6.1 × 0.7-1.1 cm, cylindricus, clavatus vel subclavatus, superne al-

Figs. 1-17. 1-3. Basidiomes of (1) *Armillaria sinapina*, (2) *A. jezoensis* and (3) *A. singula*. Bar = 1 cm. 4-5 Mating pairings (haploid × haploid) of (4) compatibility and (5) incompatibility. 6. Clamp on subhymenial hyphae. Bar = 10 μm. 7. Clamp on basidia. Bar = 10 μm. 8. Fluorescnce micrograph of DAPI-strained lamellar trama showing binucleate subhymenium. Bar = 10 μm. 9-11. (9) Suprapellis, (10) mediopellis and (11) subpellis of *A. sinapina*. 12-14. (12) Suprapellis, (13) mediopellis and (14) subpellis of *A. jezoensis*. 15-17. (15) Suprapellis, (16) mediopellis and (17) subpellis of *A. singula*. Bar = 20 μm.

bus, inferne brunneus. Annulus fibrosus vel submembranaceus, albus. Basidiosporae obtuse ellipsoideae, 6.3-10.3×4.8-6.3 μm, haud amyloideae, modice crassae, in cumulo albae. Rhizomorpha cylindrica, monopodialis.

Holotypus HUA9127: In ligno emortuo *Ulmi japonicae* (Rehd.) Sarg., Tomakomai, Hokkaido, Japonia, 17 Sept. 1991, J. Y. Cha leg., in Herbaio Facultatis Agrul-


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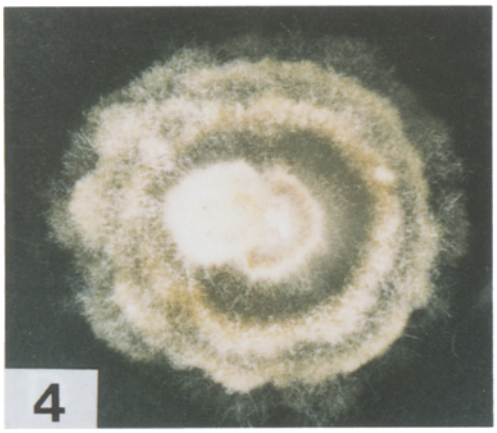
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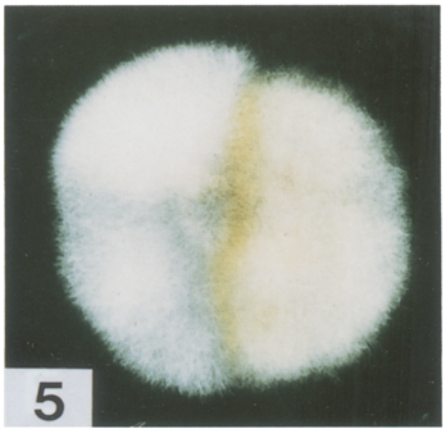
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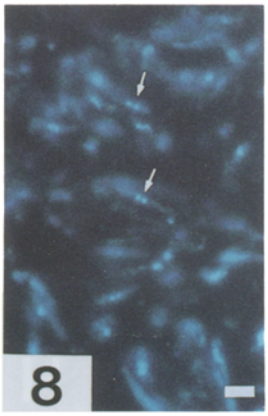
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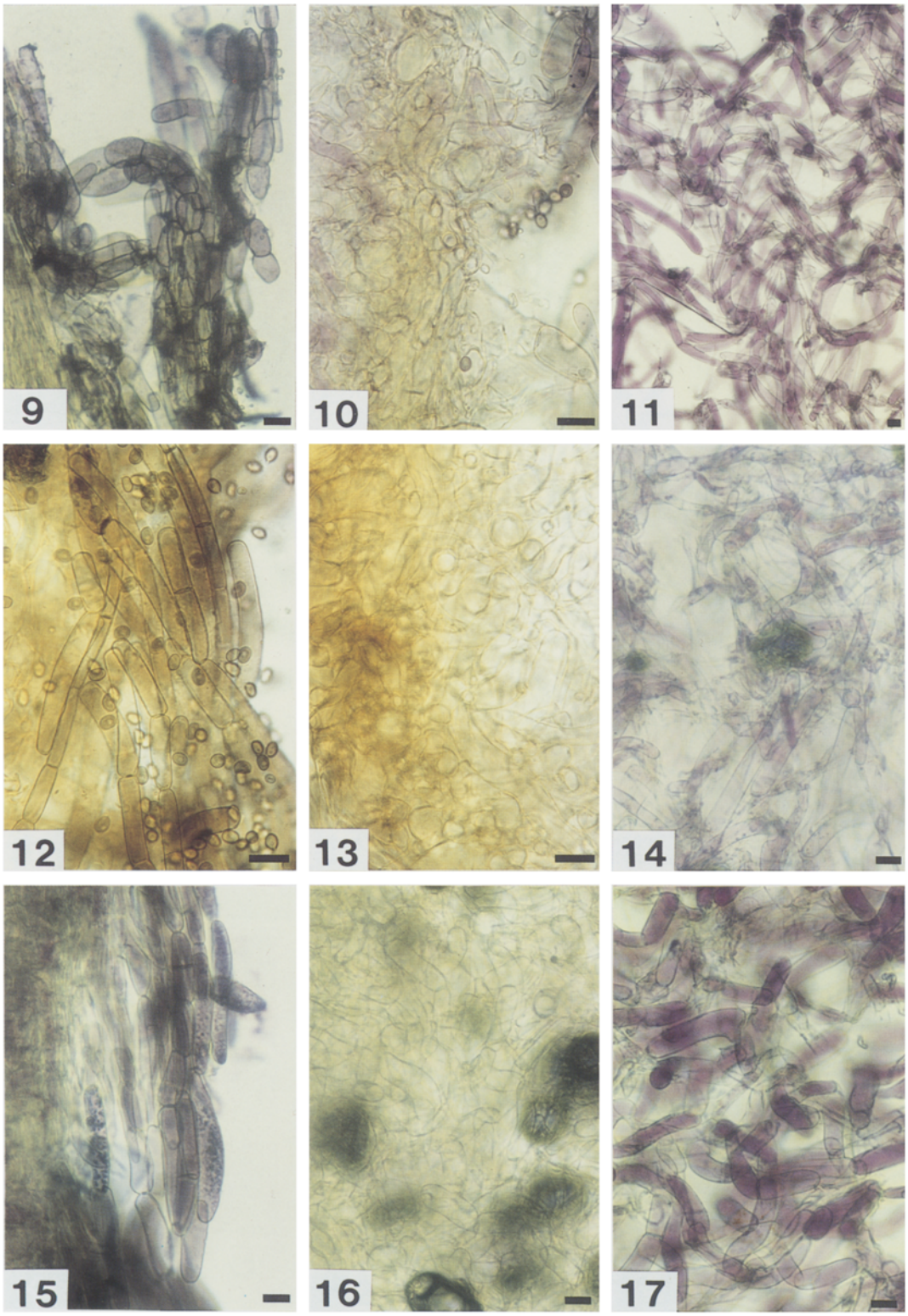
turae Universitatis Hokkaidoensis (SAPA) conservatus.

Pileus 4.7–6.8 cm diam, hemispherical-convex to convex when young, then plano-convex to plane, sometimes slightly umbonate, irregularly undulating toward the margin; surface dry, usually dark yellowish brown (10YR-4/6), strong brown (7.5YR-5/6), reddish brown (5YR-5/4), sometimes reddish brown (7.5YR-7/8) at center, covered with fine fibers or small scales of dark brown (7.5YR-4/2) to dusky red (2.5YR-3/2) at center, rarely with fine fibers dispersed toward the margin and reddish



brown (5YR-4/3) to brownish yellow (10YR-6/6). Margin inrolled at first, then acute or slightly incurved later, striate, concolorous with pileus, later darker, attached with a whitish partial veil. Flesh firm, thin to thick; context white (10YR-8/2).

Lamellae white (10YR-8/1) at young stage then reddish brown (5YR-5/4) to pink (7.5YR-7/4) in age, close, sinuate, subdecurrent, crenate, thick.

Stipe central, 3.9-6.1 × 0.7-1.1 cm, cylindric, clavate to subclavate, fibrous, white (10YR-8/2) at the apex, very pale brown (10YR-7/4) to brown (7.5YR-5/3) toward the base, fragile, solid when young, stuffed when old. Annulus fibrillate, submembranous, thin and delicate, collapsing and appressed to stipe later, white (10YR-8/1).

Spores white (10YR-8/1) in mass, broadly elliptical to ovate, with an apiculus, 6.3-10.3 × 4.8-6.3 μm, surface smooth, hyaline, nonamyloid. Basidia clavate, 39.1-44.1 × 6-7.8 μm, with 4 sterigmata and clamped at the base. Pleurocysitidia absent. Suprapellis made up of filamentous, parallel, thin-walled hyphae of cells 24.4-57.5 × 6.9-18.1 μm, with the brownish pigmented uppermost layer, at septa without clamps (Fig. 12). Mediopellis composed of tight mosaic of irregular elliptic to globose thick-walled hyphae of cells 9.4-54.2(105) × 6.8-21.3 μm (Fig. 13). Subpellis made up of net-like with longitudinal, filamentous, loosely arranged, thin-walled hyphae of cells 27.9-169.2 × 4.2-18.3 μm and partially staining in toluidine blue (Fig. 14). Subhymenial hyphae filamentous, binucleate, sometimes with a clamp (Fig. 6). Lamellar trama bilateral.

Intersterility group: III.

Specimens examined: on decaying stump of *U. japonica*, Tomakomai, Hokkaido, Japan, Oct. 17, 1991, collected by J. Y. Cha, HUA9127 (holotype in SAPA); on decaying trunk of *Q. mongolica* var. *grosseserrata*, Otoineppu, Nakagawa, Hokkaido, Japan, Sept. 13, 1991, collected by J. Y. Cha, HUA9106 (isotype in SAPA); on decaying trunk of *U. japonica*, Moshiri, Horokanai, Hokkaido, Japan, Sept. 26, 1991, collected by J. Y. Cha, HUA9116 (isotype in SAPA); on decaying stump of *U. japonica*, Tomakomai, Hokkaido, Japan, Oct. 7, 1991, collected by J. Y. Cha, HUA9120 (isotype in SAPA); on *Prunus sargentii* Rehd., Tomakomai, Hokkaido, Japan, Oct. 7, 1991, Collected by J. Y. Cha, HUA9122 (isotype in SAPA).

Japanese name: Kobari-naratake (n. nov.).

Basidiomes were collected from the middle of September to late October in Hokkaido forests. The habitats were caespitose to solitary on decaying substrates such as stumps, trunks and roots. The host range was broad-leaved trees including *Q. mongolica* var. *grosseserrata, U. japonica* and *Prunus sargentii* Rehd. in mixed and broad-leaved forests. Rhizomorphs were found beneath the bark of decaying stumps and trunks, with conspicuous distributions between wood tissues. In nature and pure culture, rhizomorphs seemed to be monopodial and cylindrical in form. The collection sites ranged from 10 m to 400 m above sea level. The scales and annulus of *Armillaria jezoensis* can be distinguished from *A. ostoyae* by their morphology. The former has a submembranous annulus and is covered with fine fiber on the pileus, while the latter has a conspicuous and thick membranous annulus. The belt shape and dichotomous branches of rhizomorphs of *A. ostoyae* also serve to differentiate the two species. Unlike *A. mellea* s. str., *A. jezoensis* has clamp connections at the base of basidia. But *A. mellea* s. str. has not been discovered in Hokkaido. *Armillaria jezoensis* was also collected in the same local range as *A. gallica*, but it can be differentiated by its submembranous annulus and attached whitish partial veil in mature basidiomes, whereas *A. gallica* has an arachnoid and transient annulus on a yellowish stipe and does not show the partial veil when mature (Motta and Korhonen, 1986; Cha et al., 1992). *Armillaria jezoensis* also occurs in the range of *A. sinapina*, but can be easily differentiated because the latter has scale, a thin membranous annulus and a bulbous stipe in Hokkaido. *Armillaria jezoensis* may be widely distributed in Hokkaido, but it has not been discovered in North America and Europe.

Armillaria singula Cha & Igarashi, sp. nov. Fig. 3

Pileus 2.5-3.8 diam, primum convexus vel hemisphaericus, postremo plano-convexus vel planus, obtuse umbonatmus. Superficies sicca, centro flavido, margine flavo vel brunnescento-flavo, fibrillis rubro-brunneis versus obscure griseis centro densiusculis ad marginem, plus minusve dispersis obtecta. Lamellae subdecurrentes, primum cremeae, dein pallide brunneae, densae. Stipes centralis, 4.2-6.0 × 0.4-0.6 cm, cylindricus vel clavatus, pallide brunneus, supra annulum fibrosus et albus. Annulus albus, membranaceus. Basidiosporae 6.2-10.6 × 3.6-6.2 μm, obtuse ellipsoideae, haud amyloideae, modice crassae, in cumulo cremeae. Rhizomorpha cylindrica, monopodialis.

Holotypus HUA9109: Ad terram humidiam, Tomakomai, Hokkaido, Japonia, 17 Sept. 1991, J. Y. Cha leg., in Herbario Facultatis Agriculturae Universitatis Hokkaidoensis (SAPA) conservatus.

Pileus globose, 2.5-3.8 cm diam, convex to hemispherical when young, plano-convex to plane later, obtusely umbonate; surface dry, pale yellow (2.5Y-8/3) to very pale brown (10YR-8/3) in center, yellow (10YR-7/8) to brownish yellow (10YR-6/6) toward the margin, covered with tufts of fine fibers which are dark reddish brown (5YR-3/2) to very dark gray (5YR-3/1), concentrated toward the center, more or less dispersed to marginal zone where with yellow (2.5Y-8/8) fibrils. Margin inrolled at first then acute later, transluscent-striate, usually concolorous with pileus or somewhat paler. Flesh firm, thin to thick at the apex of the stipe; context white (10YR-8/1).

Lamellae cream when young, then light brown (7.5YR-6/4) later, close, subdecurrent, thick at the apex, thin toward the margin.

Stipe central, 4.2-6.0 × 0.4-0.6 cm, cylindric, clavate, fibrous, white at the apex, usually very pale brown (10YR-7/3), reddish yellow (5YR-6/6) toward the base, bruising red (10R-4/8), fragile, solid when young, slightly hollow later. Annulus fibrous, membranous, very thin,

appressed to the stipe or sometimes lost later, white (10YR-8/1) to cream.

Spores cream to white in mass, broadly elliptical to ovate, with an apiculus, 6.2-10.6×3.6-6.2 μm, surface smooth, hyaline, nonamyloid. Basidia clavate, 33-37.8×5.4-7.5 μm, 4 sterigmata, clamped at the base (Fig. 7). Pleurocystidia absent. Suprapellis made up of parallel, filamentous, thin-walled, hyline hyphae of cells 32-65×10-20 μm, with uppermost layer brownish pigmented (Fig. 15). Mediopellis composed of tight mosaic of parallel and thick-walled hyphae with irregularly elliptical cells of 22.5-70×12.5-18.8 μm (Fig. 16). Subpellis made up of net-like with loosely arrayed filamentous hyphae of cells 50-100×10-50 μm, staining in toluidine blue (Fig. 17). Subhymenial hyphae filamentous, sometimes with a clamp connection, binucleate (Fig. 8). Lamellar trama bilateral.

Intersterility group: V.

Specimens examined: on swampy land, Tomakomai, Hokkaido, Japan, Sept 9, 1991, collected by J. Y. Cha, HUA9109 (holotype in SAPA); on swamy land, Tomakomai Hokkaido, Japan, Aug. 30, 1991, collected by J. Y. Cha, HUA9101 (isotype in SAPA).

Japanese name: Hitori-naratake (n. nov.).

Basidiomes collected from late August to the middle of September in Hokkaido forests. Habitat was solitary on swampy land of riversides in forest park. Hosts consist of *F. mandushrica* var. *japonica* and *Abies sachalinensis* (Fr. Schm.) Mast. Rhizomorphs extended to the litter layer and soil and formed basidiomes in fall. They are of the monopodial branching type and have cylindrical sharp tips. The collection sites were 20 m above sea level. *Armillaria singula* can be differentiated from *A. ostoyae* by the morphological characters of basidiomes, which have a very thick membranous annulus, with large scales and pilei. It has a pale yellowish to pale brownish pileus and monopodial, cylindrical rhizomorphs, while the latter shows the belt and dichotomous branching type. It can also be differentiated from *A. mellea* s. str. basidiome by have no clamp at the base on basidia and being uninucleate in subhymenial tissue. It may be collected in the same local range as *A. sinapina* but can be differentiated because the latter has a brownish cap and small conspicuous scales. *Armillaria singula* can also be distinguished by its ecological characteristics, such as always growing solitarily on soil and swampy land. It also occurs in the range of *A. gallica* but can be differentiated by cortinus annulus and scanty scales on pileus. *Armillaria sinapina* can easily be differentiated from *A. singula* by its brownish and larger pileus, and conspicuous scales. *Armillaria singula* can be distinguished from *A. jezoensis* by its submembranous annulus and whitish partial veil. *Armillaria singula* has not been discovered in North America and Europe.

Discussion

Basidiomes of *A. mellea* complex collected in Hokkaido from 1991 to 1992 were divided into 5 intersterility groups, I, II, III, IV and V, by mating test of haploid isolates. Groups I and II were identified as *A. ostoyae* and *A. gallica*, respectively, and the morphological forms of each basidiome were determined (Cha et al., 1992). Group IV corresponded to *A. sinapina*, and the morphological characteristics of basidiomes were described. Groups III and V are proposed as new species, *A. jezoensis* and *A. singula*, which have not been discovered in Europe and North America. The morphological characteristics of *A. sinapina* were a general reddish brown pileus color, small, dark brown scales, a predominant, more or less thin, membranous annulus, and an abrupt bulbousness at the base of the stipe. The counterpart North American species can be differentiated by its being clavate stipes and covered with a golden yellowish universal veil and having a mustard yellowish annulus (Bérubé and Dessureault, 1988). The microscopical features and enviromental habitats were similar to those of North American species. *Armillaria sinapina* may be a parasite on *V. furcatum* and *S. sachalinensis*, but it is generally a saprophyte on cut trunks, debris and litter on the forest floors. *Armillaria jezoensis* can be differentiated from European biological species such as *A. gallica* and *A. cepistipes* Velenovský by its submembranous annulus, brown color and clavate stipe. North American biological species such as *A. ostoyae, A. mellea* s. str., *A. sinapina, A. gemina* Bérubé & Dessureault and *A. calvescens* Béurbé & Dessureault can be distinguished from *A. jezoensis* by their morphological characteristics (Motta and Korhonen, 1986; Bérubé and Dessureault, 1988, 1989). *Armillaria jezoensis* may be saprophytic more than parasitic on tree hosts, because its rhizomorphs were equally distributed in soil and decaying wood. *Armillaria singula* can be distinguished from *A. gallica* and *A. cepistipes* by its arachnoid annulus and pinkish brown hairs on pileus. Motta and Korhonen (1986) reported that North American *A. gallica* has a solitary growth habit in soil, but the specimens of Hokkaido *A. gallica* were not limited to solitary growth but also showed a caespitose habit (Cha et al., 1992). However, the growth habits of Hokkaido *A. singula* were totally solitary in soil beside low riverside swampy land. Microscopical features such as basidiospore shapes and sizes and hymenial and pileipellis structure are not very important for distinguishing *A. ostoyae, A. gallica, A. sinapina, A. jezoensis* and *A. singula* in Hokkaido. Macroscopical features such as color and scales of pileus, partial veil, annulus forms and stipe are very useful for separating the species. Rhizomorph features were only useful to distinguish *A. ostoyae* from other species in Hokkaido. A key to Hokkaido biological species of the *A. mellea* complex is given below.

Key to species of *A. mellea* complex in Hokkaido

1. Pileus predominantly brown to reddish brown·····2
1. Pileus predominantly golden to reddish yellow ····· 4
2. Pileus covered with fibrous hairs; annulus submembranous, white; stipe clavate, brown; caespitose or solitary; rhizomorphs monopodially branching, cylindric; subhymenial tissue binucleate; clamp connection on basidia present; widely distributed in Hokkaido···III: *A. jezoensis*
2. Pileus covered with conspicuous scales ····· 3

3. Annulus membranous, very thick, white, with dark yellowish brown flocci; stipe clavate to more or less equal, with strong yellow fibers on base; generally caespitose; rhizomorphs dichotomously branching, belt-shaped, with dull tip; subhymenial tissue binucleate; clamp connections on basidia present; widely distributed in Hokkaido ····· I: *A. ostoyae*

3. Annulus membranous, thin, white, unpigmented; stipe abruptly bulbous, grayish brown; caespitose or solitary; rhizomorphs monopodially branching, cylindric; subhymenial tissue binucleate; clamp connection on basidia present; widely distributed in Hokkaido···IV: *A. sinapina*

4. Annulus arachnoid, unpigmented; stipe clavate, fibrillous, fine golden fibers at the base; clusters caespitose or solitary; rhizomorphs monopodially branching, with cylindrical sharp tips; subhymenial tissue binucleate; clamp connection on basidia present; widely distributed in Hokkaido·····II: *A. gallica*

4. Annulus submembranous to fibrous, unpigmented; stipe clavate, fibrillous, bruising red; solitary on soil; rhizomorphs monopodially branching, cylindric; subhymenial tissue binucleate; clamp connection on basidia present; often growing on swampy land in Hokkaido···V: *A. singula*

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