

## A note on *Armillaria mellea* subsp. *nipponica* subsp. nov. in Japan

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Basidiomes of homothallic *Armillaria mellea* complex were discovered in the forests of Hokkaido. Their monosporous isolates showed partial compatibility with North American *A. mellea* s. str. haploid testers. The morphological characteristics of basidiomes differed from other temperate *A. mellea* s. str. in several aspects. Here, Japanese *A. mellea* s. str. is reclassified as *A. mellea* subsp. *nipponica* and its basidiome morphologies and habitats are described.

Key Words—*Armillaria mellea* s. str.; *Armillaria mellea* subsp. *nipponica*; basidiome morphology; heterothallic; homothallic.

Taxonomical studies have been conducted in several countries on *Armillaria mellea* (Vahl: Fr.) Kummer based on its reproductive features (Korhonen, 1978; Anderson and Ullrich, 1979; Anderson et al., 1980; Kile and Watling, 1983; Mwangi et al., 1989; Sung et al., 1990; Nagasawa et al., 1991; Sung et al., 1992). Moreover, the morphological characteristics of basidiomes of several biological species of *Armillaria* have been described in detail (Kile and Watling, 1983; Guillaumin et al., 1985; Roll-Hansen, 1985; Motta and Korhonen, 1986; Bérubé and Dessureault, 1988, 1989). We have proved that five biological species of *Armillaria* are distributed in Hokkaido and that their macro- and microscopical characteristics differ from each other (Cha et al., 1992, 1994; Cha and Igarashi, 1994).

Most *Armillaria* species have a heterothallic life cycle with tetrapolar sexuality. In particular, *A. mellea* s. str. was reported to have a heterothallic life cycle in European and North American biological species (Korhonen, 1978). African and Japanese *A. mellea* s. str. have a homothallic life cycle, however (Mohammed et al., 1989; Guillaumin et al., 1991; Kile et al., 1994; Cha et al., 1995). Moreover, Japanese *A. mellea* s. str. showed partial compatibility with North American haploid testers (Cha et al., 1994b). Matsushita et al. (1994) reported that Japanese *A. mellea* s. str. intersterility group Nag.-AM showed different band patterns on isozyme analysis from North American *A. mellea* s. str. On the other hand, Shiga et al. (1994) reported that Japanese *A. mellea* s. str. intersterility group Nag.-AM is differed from European and North American biological species in its life cycle. Japanese species in particular show different basidiome morphology from other temperate *A. mellea* s. str.

Therefore, in this study, Japanese *A. mellea* s. str. is reclassified as *A. mellea* ssp. *nipponica* and its basidiome morphologies and habitats are described.

### Materials and Methods

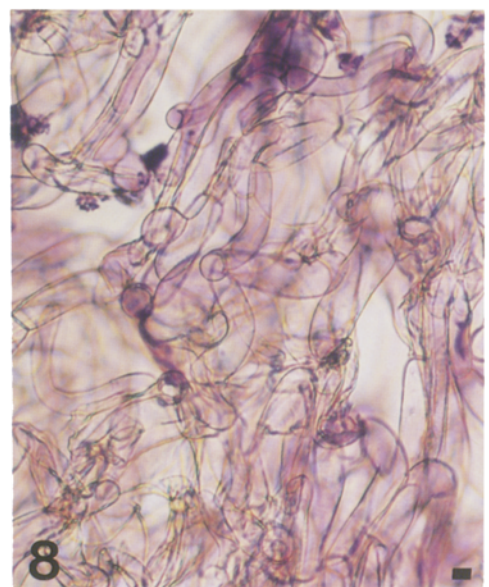
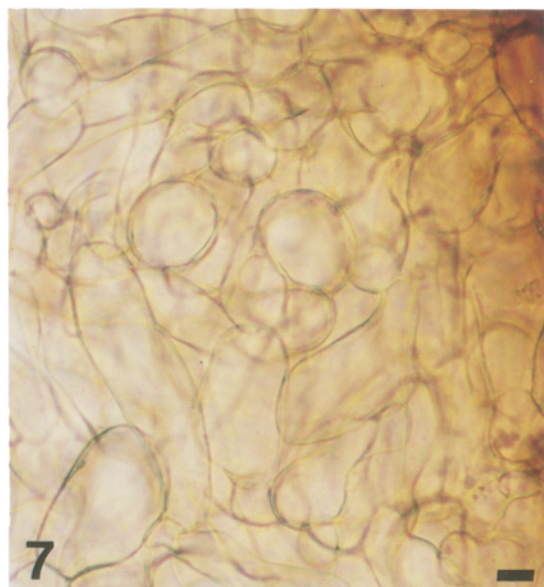
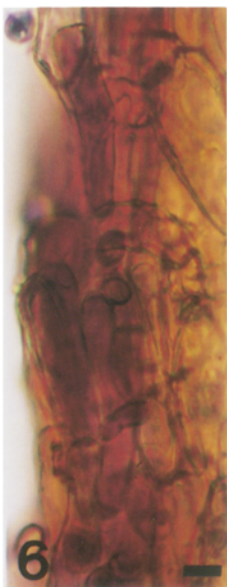
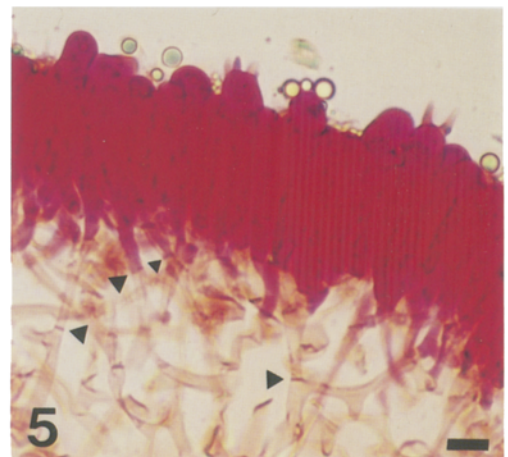
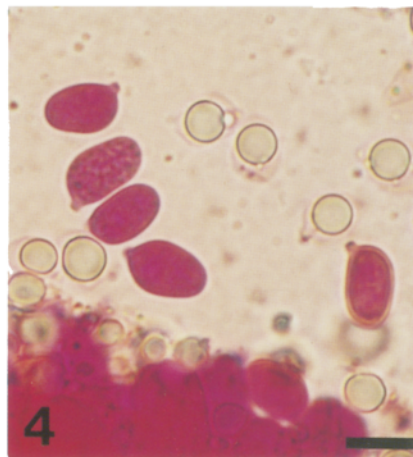
Basidiomes of *Armillaria* were collected from the fall of 1993 to the summer of 1994 in the forests of Hokkaido. Specimens, macroscopical features and habitats were described. By using a calibrated eyepiece micrometer, the sizes of spores and basidia were determined in material mounted with 4% KOH and 1% congo red plus 1% phloxine. 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. To observe the nuclear condition of subhymenial hyphae, materials were stained with fluorochrome 4'-6-diamidino-2-phenylindole (DAPI) (Coleman et al., 1981). Stained materials were observed with an Ortholux 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). Dried voucher specimens were preserved and stored.

### Results and Discussion

*Armillaria mellea* (Vahl: Fr.) Kummer subsp. *nipponica* Cha & Igarashi, subsp. nov. Figs. 1, 2

Pileus 1.6–7.5 cm diam, primum conicus vel convexus, postremo planoconvexus vel planus. Superficies sicca, flava vel flavo-brunnea, centro nigro-flavobrunnea, glabra vel raro cum fibrillis nigro-flavobrunneis. Lamellae decurrentes vel falcato-decurrentes, primum albae vel flavae, postremo flavobrunneae. Stipes centralis, 3.6–10×0.4–1 cm, aequalis vel sursum attenuatus, apice flavidus, infra annulus fuseus. Annulus membranaceus, albus. Basidiosporae obtuse ellipsoideae 10–13.5×6–8 μm, haud amyloideae, modice crassae, in cumulo albae.

Holotypus HUA93110: Super radicibus emortuis *Aceris mono* Maxim., Tomakomai, Hokkaido, Japonia, 6



Oct. 1993, J. Y. Cha leg., in Herbario Facultatis Agriculturae Universitatis Hokkaidoensis (SAPA) conservatus.

Pileus 1.6–7.5 cm in diam, abruptly conic to convex when young, planoconvex, finally plane; surface dry, yellow (10YR-8/6) to olive brown (2.5Y-5/6), dark yellowish brown (10YR-4/4) to dark olive brown (2.5Y-4/4) at center, sometimes very dark grayish brown (10YR-3/2) pigments scattered on the surface, somewhat covered with dark yellowish brown (10YR-4/4) fine fibers toward the center. Margin usually inrolled when young then acute later, concolorous with cap or somewhat darker later. Flesh firm, thin to thick at center; context white (2.5Y-8/2).

Lamellae 0.6–3.8 × 0.2–0.8 cm, white (10YR-8/1) to yellow (10YR-8/6) toward the margin when young then yellow (10YR-7/8) to yellowish brown (10YR-5/8) in age, close, decurrent to falcate-decurrent.

Stipe central, 3.6–10 × 0.4–1 cm, cylindrical, equal to slightly tapered from base to apex, pale yellow (2.5Y-8/3), yellow (2.5Y-7/8) at the apex and very dark grayish brown (2.5Y-3/2) to very dark brown (10YR-2/2) toward the base, longitudinally fibrillose-striate, covered with fibrous scales of yellow (2.5Y-8/8) at the apex and pale yellow toward the base, solid. Annulus membranous, thick, usually unbroken and attached to the stipe, white (10YR-8/1) with yellow (2.5Y-7/8) fibers on back-side.

Spores white (10YR-8/1) in mass, subglobose, broadly elliptic, with an apiculus, 10–13.5 × 6–8 μm, smooth, hyaline, nonamyloid (Fig. 4). Basidia clavate, 48–52 × 10–14 μm, with 4 sterigmata, clampless at the base (Fig. 3). Pleurocystidia absent. Suprapellis made up of parallel, thin-walled, hyaline hyphae of cells 16–56 × 8–12 μm with the brownish pigmented uppermost layer, at the septa without clamps (Fig. 6). Mediopellis composed of tight entangled structure with globose and irregularly elliptical thin-walled cells of 36–68 × 22–36 μm (Fig. 7). Subpellis made up of loosely net-like structure with longitudinal filamentous hyphae of cells 56–160 × 16–20 μm, staining in toluidine blue (Fig. 8). Subhymenial hyphae filamentous, clampless septa (Fig. 5), uninucleate. Lamellea trama bilateral.

Intersterility group: VI.

Specimens examined: on decaying roots of *Acer mono* Maxim., Tomakomai, Hokkaido, Japan, 6 Oct. 1993, collected by J. Y. Cha, HUA93110 (holotype); on living stem of *Fraxinus lanuginosa* Koidz. and decaying roots of *Quercus mongolica* Fisch. ex Turcz. var. *grosseserrata* (Bl.) Red. & Wils., Chitose, Hokkaido, Japan, 23 June 1994, collected by T. Igarashi, HUA94135 (paratype). All are deposited at SAPA (herbarium, Faculty of Agriculture, Hokkaido University, Sapporo, Japan).

Japanese name: Naratake.

The basidiomes in the forests of Hokkaido developed from late June to early October. They occurred mainly caespitose on decaying roots and living stems. Hosts consisted of broad-leaved trees such as *A. mono*, *F.*

*lanuginosa* and *Q. mongolica* var. *grosseserrata*. Rhizomorphs and mycelial fan were formed into the tissue of decaying roots or cuticle layers. Their rhizomorphs have a belt shape, dichotomous branched forms and dull tips in pure culture (Cha et al., 1994). They have a distinctly yellowish pileus with an olive brownish central part and a glabrous or slightly scaly surface of caps. Moreover, their lamellae are yellowish in color in mature specimens and are distinctly decurrent in form, and their stipes are of equal forms or tapered from base to apex, and developed fibrillose scales exist at the apex. Their characteristics of basidiome morphology are similar to Honshu *A. mellea* s. str. described by Nagasawa et al. (1991). Based on the absence of clamped basidia at the base, *A. mellea* subsp. *nipponica* in particular was distinguished from other Japanese species.

European and North American *A. mellea* s. str. are similar to *A. mellea* subsp. *nipponica* in some respects, as the cap colors range from olive to yellow, the cap surface is glabrous and the annulus is prominent and persistent (Roll-Hansen, 1985; Motta and Korhonen, 1986). But, *A. mellea* subsp. *nipponica* is distinguished from European and North American *A. mellea* s. str. by its conspicuous yellowish lamellae in mature and decurrent to falcate-decurrent form. In microscopical features, *A. mellea* subsp. *nipponica* has monokaryotic subhymenial hyphae and clampless basidia, as does *A. mellea* s. str. The spores of *A. mellea* subsp. *nipponica* are larger than those of *A. mellea* s. str.

The life cycle of *A. mellea* s. str. was reported to be heterothallic in European and North American biological species. African *A. mellea* s. str. is closely similar in some respects, such as culture and basidiome morphology, poor fruiting in culture, and protein and esterase profiles, to European and North American *A. mellea* s. str. There even appears to be some partial compatibility between certain European and North American haploid *A. mellea* s. str. isolates and diploids of African *A. mellea* s. str. However, single spore isolates from African *A. mellea* s. str. cultured in vitro have been crustose, indicative therefore of homothallism (Kile et al., 1994). Kile et al. (1994) suggested that African *A. mellea* s. str. should be considered as a homothallic form of European and North American *A. mellea* s. str. or as a separate entity of sub-species level.

Like African *A. mellea* s. str., *A. mellea* subsp. *nipponica* also shows homothallism and partial compatibility with North American haploids. Moreover, the isozyme profiles of protein and several morphological characteristics of basidiomes differed from those of other temperate *A. mellea* s. str. We therefore consider *A. mellea* subsp. *nipponica* to be an unique biological species of *Armillaria*.

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Figs. 1–8. 1, 2. Basidiomes of *Armillaria mellea* subsp. *nipponica*. Bar=1 cm. 3–5. (3) Clampless basidium, (4) basidiospores, and (5) clampless subhymenial hypha of *A. mellea* subsp. *nipponica*. Bar=10 μm. 6–8. (6) Suprapellis, (7) mediopellis, and (8) subpellis of *A. mellea* subsp. *nipponica*. Bar=10 μm.



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