## Notes on three Japanese Agaricales

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Three species of Agaricales from Japan are treated here. 1) From the examination of the types and additional specimens, *Lyophyllum nigrescens* is conspecific with *Hydropus nigrita* from Cuba. 2) *Hydropus kansaiensis* sp. nov. from Otsu appears close to "*Trogia*" mellea from Malaysia. 3) *Alnicola lactariolens* sp. nov. from Otsu is a distinctive species in this genus because of the purple color of its spore print.

Key Words—*Alnicola lactariolens; Hydropus kansaiensis; Hydropus nigrita; Lyophyllum nigrescens;* taxonomic position.

During Clémençon's stay in the Faculty of Education, Shiga University, Otsu, Japan from 1988 to 1989, several noteworthy species of agarics were collected by us in Otsu and the suburbs. In the present paper, one of these, *Lyophyllum nigrescens* Hongo, is discussed from the anatomical and taxonomical standpoints; and *Hydropus kansaiensis* and *Alnicola lactariolens* are described as new to science. The holotypes of the new taxa are kept in the Herbarium of the National Science Museum, Tokyo (TNS), and the isotypes in the Herbarium of the University of Lausanne (LAU).

1) The taxonomic position of *Lyophyllum nigrescens* Hongo, Mem. Shiga Univ. **25**: 56, 1975.

The protologue describes the basidiospores as being "very slightly amyloid or inamyloid," and underlines the "well-marked...black staining of all parts when bruised" and the "minutely pruinose stem." It does not mention any siderophilous granules in the basidia and does not describe the anatomy of the pileipellis. The revision of the type specimen by Clémençon (1982) lead to the conclusion that this species probably does not belong to *Lyophyllum* since the basidia are devoid of siderophilous granules, but the taxonomic position has not been studied.

Other collections and fresh material of this rare fungus collected in 1989 allowed a critical reexamination. The absence of siderophilous granules in the basidia is confirmed, and it is now well established that the spore walls are clearly amyloid, giving a blue-gravish color reaction with Melzer's solution. As is often the case, the amyloid reaction of whole, intact spores mounted directly in Melzer's solution is difficult to see in the light microscope, because the high refractive index of the spore content results in a narrow dark halo around the spore, which obscures the blue-gray color of the spore wall. This was probably the cause of the uncertainty expressed in the protologue about this character, and also of the wrong statement by Clémençon (1982) who indicated inamyloid spores. If the spores are first squeezed in KOH between slide and cover slip and then mounted in Melzer's solution, many empty spores can be seen whose walls show a clear and unmistakable dark blue-gray reaction.

The pileipellis is a slightly gelatinized, almost coherent hymeniderm of erect, pyriform cells with intracellular, brown pigment. These cells are slightly separated from each other by a gelatinous matrix which also covers the hymeniderm (Fig. 1). Beneath the pyriform cells is a layer of loosely interwoven, cylindrical, repent hyphae, 3- $5 \,\mu$ m thick, some with incrusting pigment. In the underlying context of the pileus are numerous cylindrical, 5-10  $\mu$ m thick hyphae with dark brown to black, mottled content.

The context of the stipe and the gill trama is clearly sarcodimitic. The spindle-like cells measure  $2-5 \,\mu\text{m}$  at the clamped septa and gradually enlarge their diameter to  $15-25 \,\mu\text{m}$ . They are at least 700–1500  $\mu\text{m}$  long, and probably even longer, but this is very hard to measure. The wall of these hyphae is slightly thickened and measures  $0.5-1 \,\mu\text{m}$ . It is smooth and inamyloid.

The amyloid spore wall, the hymeniform pileipellis and the sarcodimitic structure of the stipe and gill trama place our fungus in the genus *Hydropus*, section *Hydropus*, and the blackening context puts it in subsection *Hydropus*, where it agrees well with *Hydropus nigrita* (Berk. et Curt.) Singer.

In European literature this fungus is usually described as *Hydropus atramentosus* (Kalchbr.) Kotlaba et Pouzar (1962) or *Collybia fuliginaria* (Batsch ex Fr.) Bresadola. Singer (1982) gave a detailed description of the type

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specimen of *Agaricus nigrita* Berk. et Curt. (Wright 51 from Cuba, in K) and reduced *C. fuliginaria* and *H. atramentosus* to synonymy with *H. nigrita*. Singer notes amyloid spore walls, clamp connections and cystidia present, and spores measuring  $(3.8-)4.2-6(-6.5) \mu m$  long. One year later Pegler (1983) gave his own description of the same type material from Kew, but gives dextrinoid spore walls, clamp connections and pleurocystidia absent, and spores 5.5-7  $\mu m$  long. The considerable differences between the two descriptions prompted us to reinvestigate the type specimen. Our findings confirm Singer's description.

Additional collections of this species from the Antilles, the USA and Europe agree so well with the Japanese fungus that we have no hesitation in identifying *Lyophyllum nigrescens* Hongo with *Hydropus nigrita* (Berk. et Curt.) Singer.

There is one minor problem connected with the correct spelling of the epithet. In the original description

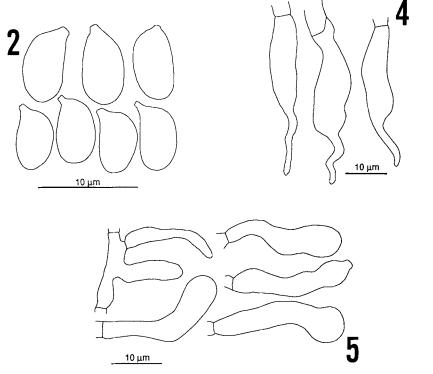
10 µm

this species is called *Agaricus nigrita*. To our knowledge there is no Latin adjective "*nigritus*," but there is the noun "*nigritia*" meaning "blackishness." *Nigrita* cannot be considered a misspelling of *nigritus*, since this latter word does not exist, but rather a variant spelling or a misspelling of *nigritia*. If this view is accepted, then the correct name for our fungus must be *Hydropus nigrita* or *H. nigritia*, but not *H. nigritus*.

Material studied: *Lyophyllum nigrescens*, Hongo 5058 (holotype, TNS) from Japan, *Lyophyllum nigrescens*, Hongo 6818 from Japan, *Lyophyllum nigrescens*, HC 89/214 (LAU) from Japan; *Mycena fuliginaria*, Bas 3620 (L) from Michigan USA; *Hydropus atramentosus*, Moser 75/173 (IB) from Poland; *Hydropus atramentosus*, coll. 1309-79 Mü (Naturmuseum Luzern) from Switzerland; *Hydropus nigritus*, Fiard 1581 and Fiard 781A (K) from Martinique; *Agaricus nigrita* Berk. & Curt., leg. Wright 51 (K) from Cuba (holotype).

2) *Hydropus kansaiensis* Clémençon et Hongo, sp. nov. Figs. 2-5, 13

10 µm



Figs. 1-5. Hydropus nigrita and H. kansaiensis.

1. *H. nigrita*. Pileipellis forming an interrupted, slightly gelatinized hymeniderm. Coll. Hongo 6818. 2-5. *H. kansaiensis*. 2. Basidiospores, 3. Basidia, 4. Cheilocystidia, 5. Covering of the stipe.

Pileo 4-10 mm lato, membranaceo, convexo, subumbonato, dein late convexo vel plano-convexo, centro depresso in adultis, pallide flavido-melleo, centro fuscobrunneo sparsis fibrillosogue, paullulum pellucido-striatu-Io. Lamellis breviter decurrentibus vel decurrentibus, interdum furcatis, distantibus, haud vel vix intervenosis, pileo concoloribus, in maturis acie fuscidulis. Stipite 3- $7 \times 0.7$ -1 mm, aequali sed basi leniter incrassato, cavo, pileo concolori vel leniter pallidiore, primitus minute albopruinoso et interdum fibrillis fusco-brunneis sparsis obtecto; mycelio basali parco, albo, strigoso. Carne superficiebus concolori; odore saporeque haud notabili. Basidiosporis in massa albis, ellipsoideis, 5.8-8.1  $\times$  3.7-4.5 μm, levibus, inamyloideis. Basidiis 30-40×5.5-7 µm, tetrasporis, haud siderophilis. Pleurocystidiis nullis. Cheilocystidiis numerosis, irregulariter fusiformibus, apice appendiculatis. Hyphis fibulatis. Ad truncos Metaseguoiae.

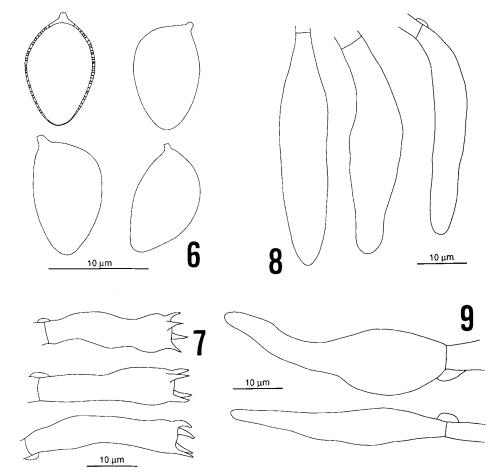
Young button-like basidiomata (diameter less than 1 mm) pale gray, becoming more and more yellowish brown with age.

Pileus 4-10 mm broad, membranaceous, only about 0.1 to 0.2 mm thick, convex and slightly umbonate when young, becoming broadly convex to plano-convex, with depressed center in age; pale yellow-beige 10C3 (Maerz

and Paul, 1930), 9G4 with darker brown center 12J7, sparsely covered with dark brown fibres especially toward the center, becoming more or less naked in age; dry, unpolished, minutely felty-uneven when young, becoming more or less glabrous in age; margin opaque when dry but faintly translucent-striate when wet, at first concolorous with the ground-color of the pileus, becoming darker in some aged specimens. Lamallae shortdecurrent to decurrent, sometimes forked; distant, L=13-18 in small specimens, 18-22 in bigger ones, 0.3 to 0.7 mm broad in mid-radius; not intervenose or only slightly so in age; concolorous with the pileus; edge concolorous with the sides, becoming darker brownish yellow in age, minutely fimbriate toward the stipe when young, soon naked, entire; lamellulae 0, 1 or 3 between two gills. Stipe  $3-7 \times 0.7-1$  mm, cylindrical with slightly swollen base, hollow; concolorous or slightly paler than the pileus, minutely white pruinose at first, soon becoming naked, sometimes loosely covered with the same dark brown fibres as the pileus, fibres disappearing in age; basal mycelium scarce, white, strigose. Context concolorous with the surface, odor and taste indistinctive.

Sporeprint pure white.

Basidiospores ellipsoid (Fig. 2), 5.8-8.1×3.7-



Figs. 6-9. Alnicola lactariolens. 6. Basidiospores, 7. Basidia, 8. Cheilocystidia, 9. Pleurocystidia.

4.5  $\mu$ m, Q=1.40-1.94 (95% population limits based on the measurements of 25 spores), very thin-walled; wall smooth, inamyloid, acyanophilous, red in Congo red, faintly blue in toluidine blue; mostly binucleate, some with 3 nuclei. Basidia  $30-40 \times 5.5-7 \,\mu m$ , 4-spored, without siderophilous granules, clamped (Fig. 3). Pleurocystidia none. Cheilocystidia numerous, thinwalled, colorless, smooth, irregularly fusiform with an apical, wormlike appendix; main cell body 18-20×4.5-6  $\mu$ m, appendix 7-15  $\times$  1-1.5  $\mu$ m (Fig. 4). Gill trama sarcodimitic, spindle cells 70-350  $\mu$ m long, often free ending with only slightly tapered apex. The cross section (perradial) shows many transversely sectioned hyphae, giving a cellular impression, through which some spindle hyphae are winding. Hyphae of the gill trama with clamp connections, 2-15 µm wide, thin-walled, colorless, smooth, inamyloid. Pileipellis not differentiated, consisting of interwoven, repent hyphae 2-7  $\mu$ m wide, walls smooth, colorless, thin, inamyloid; some septa with

clamp connections, but clamps easily overlooked; some hyphae with a fuscous vacuolar pigment; some hyphal end cells erect; not gelatinized. Pileitrama interwoven, sarcodimitic, spindle hyphae 120-350  $\times$  8-14  $\mu$ m, wall 0.3-1  $\mu$ m thick, strongly staining in Congo red, inamyloid; some  $\pm$ cylindrical, turgescent hyphae also present. Stipitepellis a dry cutis of parallel, clamped hyphae 2-3 µm wide with colorless, smooth, thin, inamyloid walls. With a few scattered, short, semi-erect to erect, colorless end-cells with rounded or somewhat irregularly shaped apex,  $20-25 \times 4-6 \,\mu\text{m}$  (Fig. 5); some lateral, short cylindrical or narrowly cylindrical cells also present,  $10-17 \times 3-4 \mu m$ . Some superficial hyphae with the same fuscous vacuolar pigment as on the pileus. A few "oleiferous" hyphae present, but only 2-3  $\mu$ m wide. Stipitetrama nicely sarcodimitic with spindle hyphae up to 17  $\mu$ m wide and with walls up to 1  $\mu$ m thick, inamyloid.

Gregarious on the bark of living trees of Metasequoia

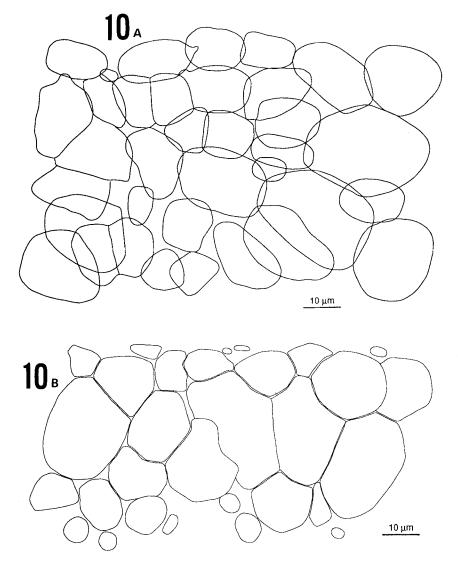


Fig.10. Alnicola lactariolens. A. Irregularly pseudoparenchymatic subsurface layer of the pileus. Tangential section. This layer is covered by cylindrical, incrusted hyphae 2-4 μm thick running all directions (not shown). B. Ditto. Cross section.

glyptostroboides Hu et Cheng.

Material studied: Shiga University Campus, Otsu-shi, Shiga-ken, Japan, HC 88/116, leg. H. Clémençon, Aug. 19, 1988 (Typus: holotype in TNS, isotypes in LAU). Further collections from the same site: HC 88/102; HC 88/115.

This species seems near "*Trogia*" mellea Corner (1966) from Malaysia, but differs by having smaller basidiospores, much smaller caulocystidia, by the absence of the conspicuous hyphal strands that connect the basidiomata of "*Trogia*" mellea, and by the much paler colors. Since the pileipellis of Corner's fungus is not described in detail, we do not know how the two specis compare with respect to this character.

3) Alnicola lactariolens Clémençon et Hongo, sp. nov.

Figs. 6-12, 14

Pileo 13-40 mm lato, hemisphaerico dein late convexo, elastico-cartilagineo, hygrophano, rubro-brunneo, margine pallide argillaceo, levi in juvenilibus dein ruguloso-venoso. Lamellis stipatis, adnexis, carneo-brunneis, acie albo-fimbriatis. Stipite usque ad 35×4 mm, aequali vel deorsum subincrassato, haud radicante, apicem versus stramineo floccoso-pruinosoque, basin versus pallide ochraceo-brunneo appresse fibrillosoque, e farcto fistuloso; rhizomorphis albis praesentibus. Velo fibrilloso, albo, in juvenilibus cortiniformi. Odore in mentem Lactarium porninsem revocante; sapore subamaro. Basidiosporis in massa purpureo-brunneis, amygdaliformibus, 8.0-10.5  $\times$  5.4-6.9  $\mu$ m, verruculosis. Basidiis 21- $29 \times 7-8 \,\mu m$ , tetrasporis. Cheilocystidiis numerosissimis,  $35-45 \times 6-10 \mu m$ , tenui-tunicatis, hyalinis, attenuatis vel cylindratis, summis rotundatis, obtusis. Pleurocystidiis sparsis,  $35-45 \times 7-12 \mu m$ , tenui-tunicatis, hyalinis, deorsum ventricosis, gracillimo rostro 2-3 µm. Hyphis fibulatis. Ad terram in silva mixta (Quercus et Pinus).

Pileus 13-40 mm broad, 1-2 mm thick in mid-radius, at first hemispherical then broadly convex, elastic-cartilaginous; hygrophanous, at first vivid red-brown Expo 23F (Cailleux and Taylor, no date), then paler red-brown 34F and margin reddish ocher beige to ocher beige 46E, 56C, in age or on drying pallescent to 63B, 63C, 62D; naked, dry, unpolished, smooth when quite young (when the caps are up to 4 mm broad), but soon becoming rugulose and radially venose, veins more or less anastomosing; margin smooth, opaque. Lamellae dense (L=20-40 for pileus diam. of 22-40 mm), I=3-5; 3-4 mm broad, adnexed; pinkish brown 32D, 32E, edge entire, straight, densely white fimbriate. Stipe up to 35 × 4 mm, cylindrical or slightly widening toward the base, base swollen, not radicating, but with whitish hyphal strands; white floccose-pruinose over a straw yellow surface under the gills, below appressed white fibrillose over a pale ocher to pale brown surface, scantily white strigose at the base; stuffed at first, then fistulose. Veil fibrillose, white, dry, at first cortinoid and covering the whole stipe and much of the pileus, leaving a few fibres on the stipe and thin, appressed patches of interwoven fibres on the margin of the pileus, disappearing in age. Context pale with reddish tone 34B; smell aromatic-acerb like Lactarius porninsis Rolland, sometimes weak but distinctive, usually quite strong; taste weakly bitter.

Spore print purple brown 23H, 23F.

Basidiospores amygdaliform (Fig. 6), 8.0-10.5  $\times$  5.4-6.9  $\mu$ m, F=1.34-1.66 (95% population limits, 30 spore measurements including the ornamentation), denselv verruculose without a plage (Fig. 12), with a weak papilla; wall dextrinoid, toluidine blue (after ammonia, without heating): wall of young, still smooth spores blue, young but already ornamented, immature walls with dark grayish blue warts, mature walls faintly bluish gray (not stained when not pretreated with ammonia), cotton blue ammonia): young spore walls strongly (after cyanophilous, mature ones acyanophilous, Congo red (1% in ammonia 1:5, 3 minutes, observed in KOH 5%): young spore walls red, mature ones not stained. In

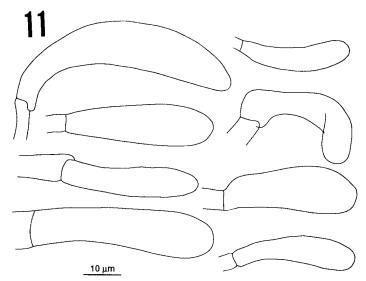


Fig.11. Alnicola lactariolens. Single celled hairs from the upper part of the stipe.

some spores the myxosporial ornamentation shows a moderate calyptra-like widening near the apiculus. Wall pigment unchanged in KOH, olive brown in concentrated sulfuric acid.

Basidia  $21-29 \times 7-8 \mu m$ , four-spored, cylindrical with slightly swollen, round apex, or sometimes slightly constricted in the middle, as are most of the immature basidia, without siderophilous granules (Fig. 7). Cheilocystidia very numerous,  $35-45 \times 6-10 \mu m$ , projecting about 25-35 µm, thin-walled, colorless, smooth, tapering to a rounded tip or cylindrical with broadly rounded apex (Fig. 8). Pleurocystidia scattered, 35-45×7-12  $\mu$ m, tapering to a thin apex 2-3  $\mu$ m thick, thin-walled, colorless, smooth (Fig. 9). Gill trama regular, hyphae 5-12 µm thick, thin-walled, clamped, smooth, colorless; subhymenium thin, cellular. Pileipellis heteromerous, consisting of irregularly polyhedrical, more or less isodiametric cells several layers deep, composed of irregularly moniliform hyphae pressed into a compact structure; hyphal end cells often more elongate and narrower and sometimes erect, or changing into repent hyphae 2-4  $\mu$ m wide; uppermost surface of the pileus loosely covered with hyphae running in all directions, 2-4  $\mu$ m wide, clamped and incrusted with a brown pigment. At times the aspect of the pileipellis is reminiscent of the heteromerous structure of the Russula-trama, the polyhedrical cells being arranged in rosettes. Not gelatinized. Pileus trama interwoven of inflated, clamped hyphae 3-7  $\mu$ m broad. Stipitetrama from cylindrical to slightly inflated, parallel, smooth, colorless and clamped hyphae. Covering of the stipe below the gills consisting of nests of erect, club-shaped cells (Fig. 11). Hyphae of the veil cylindrical, 3-5 µm thick, clamped, colorless, smooth, thin-walled.

## On the ground in Quercus-Pinus forest.

Material studied: Tomikawa, Otsu-shi, Shiga-ken, Japan, HC 88/95, leg. T. Hongo & H. Clémençon, Aug. 15, 1988 (Typus: holotype in TNS, isotypes in LAU).

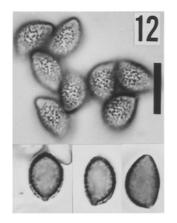


Fig.12. Alnicola lactariolens. Photomicrographs of the basidiospores. Above: Spore wall ornamentations. Below left and middle: Myxosporium loosening around the spicular part of the spore making the spore almost calyptrate. Below right: optical section showing thin spot at the apex of the spore. (Scale bar =  $10 \mu m$ ).



Fig.13. Hydropus kansaiensis. Basidiomata.



Fig.14. Alnicola lactariolens. Basidiomata.

Other collections from the same locality: HC 88/98, Aug. 17, 1988; HC 88/125, Aug. 26, 1988; HC 88/144, Sept. 21, 1988.

The dry heteromerous pileipellis, the spore wall structure and the cheilocystidia put this species into the genus *Alnicola* Kühner in the sense of Singer (1986).

The most striking feature is its dark purple-brown spore print, unlike that of any other known species of *Alnicola*. However, this species is to the rest of *Alnicola* what the subgenus *Porphyrospora* is to the rest of *Hebeloma*, both differing mainly by the purple color of the spore print. We therefore think that an analogous taxonomic situation exists in *Alnicola* which probably deserves recognition by the creation of a new subgenus, but we refrain from doing so now because taxonomic information is too limited.

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