Bull. Natn. Sci. Mus., Tokyo, Ser. B, **31**(4), pp. 117–126, December 22, 2005

Three *Coprinus* Species Occurred on the Animal Dungs Collected at Yatsugatake Range, Central Honshu, Japan

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Abstract Three *Coprinus* species i.e., *Coprinus candidolanatus*, *C. stercoreus* and *C. ephemeroides* occurred on animal dungs collected in Yatsugatake were documented. Production of basidiomata of *Coprinus stercoreus* and *C. candidolantus* started in 8 and 10 days, respectively, followed by *C. ephemeroides*, and lasted for about 40 days. At the maximum, 30 basidiomata were produced from ten dung particles. Descriptions and illustrations together with proper binomials to the two species and comments on the third species are given.

Key words: *Coprinus candidolanatus*, *Coprinus ephemeroides*, *Coprinus stercoreus*, dung fungi, mycobiota.

Introduction

Dung of herbivorous animal is a good substrate for various fungi, the life cycles of which largely depend on dung. These fungi are called "dung fungi" or "coprophilous fungi" (Bell, 1983; Furuya, 1990; Richardson, 2001; Webster, 1970). Dung fungi are composed of myxomycetes, zygomycetes, ascomycetes, basidiomycetes, and anamorphic fungi, and they occur in succession on dung (Richardson, 2002). Although dung fungi have been investigated for more than 100 years in Europe (Cailleux, 1971; Massee and Salmon, 1901, 1902; Moreau, 1953) and North America (Cain, 1934; Griffiths, 1901), still new members, including new taxa are being added (Uljé and Keizer, 2003; Uljé and Noordeloos, 2000; Uljé *et al.*, 2000).

In Japan, coprophilous zygomycetes (Indoh, 1962, 1965), yeasts (Soneda, 1959, 1962), discomycetes (Minoura and Yamada, 1976; Minoura *et al.*, 1978; Otani, 1973; Otani and Kanazawa, 1970a, 1970b), pyrenomycetes (Furuya and Udagawa, 1972a, 1972b, 1973; Udagawa and Furuya, 1974, 1977) and anamorphic fungi (Tubaki, 1954) have been studied. In spite of rich fungal diversity in Japan, research on dung fungi has relatively shorter history than that in Europe, and still studies of mycobiota are required. In particular, studies on coprophilous basidiomycetes are scarce.

Coprinus Pers. is the most dominant genus among the coprophilous basidiomycetes. A large number of *Coprinus* species are known to occur on low C/N ratio materials, such as ammonium treated soils (Sagara, 1975, 1992), compost, and dung (van Waveren, 1968), dung being one of the best substrates. Because the basidiomata of *Coprinus* degrades very quickly in the field, it is usually difficult to obtain specimens in good condition. On the other hand, occurrence of *Coprinus* on dung can be monitored under controlled condition by continuous observation, and specimens in good conditions can be obtained and preserved. This gives an advantage in taxonomical studies.

About 40 species of *Coprinus* are hitherto known in Japan, which include 7 coprophilous species (Hongo, 1987). However, literature sur-

vey suggests that 16 species of them may be included as coprophilous members in *Coprinus* (Breitenbach and Kränzlin, 1995; Moser, 1983).

During the taxonomic research of flora in area with remarkable biodiversity in Japan, Yatsugatake area, Nagano prefecture was surveyed for the occurrence of coprophilous basidiomycetes. In the present paper, the authors provided proper binomials, detailed descriptions and illustrations for two *Coprinus* species in Japan, and comments on the third species.

Materials and Methods

Dung of sika deer (*Cervus nippon*) and Japanese serow (*Capricornis crispus*) were collected in two sites at the foot of Yatsugatake range, Chino, Nagano Prefecture on Aug. 12 and Oct. 13 in 2004. Six specimens were collected for each species. The collected specimens were brought to the laboratory in paper bags, divided to two portions, and each was incubated on moistened moss in tall Petri-dishes at 15°C or 22°C under a 12 hours light/12 hours dark light regime. The specimens were examined every 3–4 days for the occurrence of fungi under dissecting microscope (Fig. 1). Basidiomata were counted for the frequency data, collected for identification and used for isolation. To isolate culture, a small portion of the young basidioma was taken by a sterilized needle, and transferred onto a medium prepared in a test tube. Cultures were grown at 22°C under a 12 hours light/12 hours dark light regime on MYC medium (malt extract 10 g, yeast extract 2 g, casamino acid 2 g, agar 12 g, D. W. 1L; Difco Laboratories) for about 2–3 weeks, and matured basidiomata were collected for identification and freeze-dried for herbarial specimens. Morphological observations of the anatomy and measurements were made from freeze-dried specimens mounted in 25% aqueous ammonia. Descriptions of macro- and microscopic characters were prepared based on basidiomata both cultivated on artificial medium and on the dung specimens. Specimens examined are deposited in the National Science Museum, Tokyo (TNS) and Natural History Museum and Institute, Chiba (CBM).

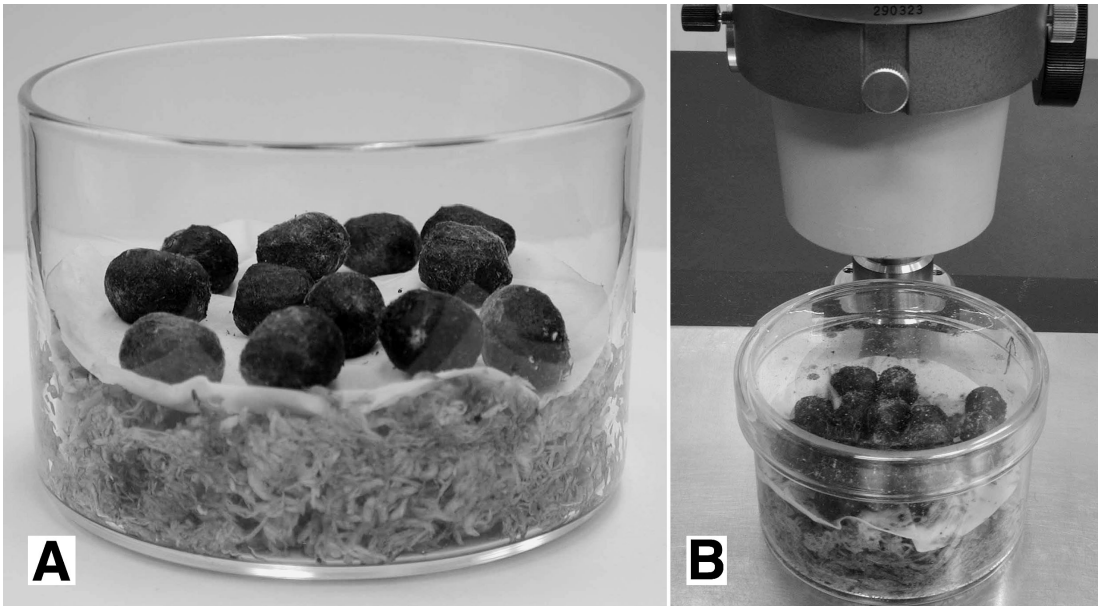

Fig. 1. Moist chamber for dung incubation. A. Dungs in a moist chamber. Moistened moss were seated under the dung to keep the chamber in moist condition. B. Dung fungi were examined under dissecting microscope.

Results and Discussion

Before the appearance of the basidiomycetes, zygomycetes, anamorphic fungi, and ascomycetes occurred in this order on dung specimens. The successive occurrence of fungi lasted for up to 10 weeks and 14 weeks at 22 and 15°C, respectively. All the basidiomycetes occurred belonged to the genus *Coprinus*. The occurrence and the occurring period differed by the species and incubation condition. Seven taxa of *Coprinus* were recognized, the three of which were identified: *Coprinus candidolanatus*, *C. stercoreus*, and *C. ephemeroides*. The frequency of occurrence and incubation condition are listed in Table 1. Production of basidiomata of *Coprinus stercoreus* and *C. candidolantus* started in 8 and 10 days, respectively, and lasted for about 40 days. At the maximum, 30 basidiomata were produced from ten dung particles (Fig. 6). *Coprinus candidolanatus* and *C. stercoreus* were observed only under 22°C while *C. ephemeroides* occurred at both temperatures. The identification of the remaining 4 taxa are in process. Two to three species of *Coprinus* species occurred from each specimen, but overlapping species were few.

Descriptions

Because *Coprinus candidolanatus* and *C. stercoreus* are identified and provided proper binomials for the first time in Japan, detailed descriptions are given as follows.

1. *Coprinus candidolanatus* Doveri & Uljé in Uljé, Doveri & Noordeloos, Persoonia **17**: 465. 2000. Figs. 2, 3

Pileus 1–2 mm broad, 2–3 mm high when closed, ellipsoid to ovoid when young, becoming convex to plane, 3–6 mm broad when expanded, turning applanate at maturity or even revolute at deliquescence; ground color at first white, soon becoming pale grayish ochre, surface densely covered with a fibrous-wooly white universal veil when young (Fig. 2B), sometimes with cortinate veil in very young stage (Fig. 2C), later almost glabrous or veil remaining only at the center. **Flesh** very thin, fragile, white, taste mild, odorless. **Lamellae** free, dispersed (number of lamellae reaching stipe=25–30), narrow (0.4–0.7 mm breadth), at first white, then grayish, finally blackish, deliquescent. **Stipe** up to 2–5 cm× 0.5 mm (Fig. 2A), cylindrical, equal, sometimes with clavate base, not rooting, fragile; surface white, at first with white fibrillose or squarrose scales, soon becoming smooth. **Basidiospores** black in mass, dark red-brown under the microscope, ovoid to ellipsoid, smooth, 8.5–10.0 (9.17±0.51: mean±SD, n=40) μm long, 4.7–5.9 (5.43±0.27, n=20) μm broad in face view, 4.7–5.9 (5.41±0.26, n=20) μm in side view, a central germ pore 1.2–1.5 μm wide (Figs. 2H, 3D). **Cheilocystidia** 35–70 μm long, 20–30 μm broad, ellipsoid to obovoid, numerous, thin-walled, hyaline (Fig. 2F, 3B). **Pleurocystidia** 45–65 μm long, 24–27 μm broad, ellipsoid, thin-walled, hyaline (Figs. 2G, 3C). Veil on the pileal surface composed of two type of thin-walled hyaline hyphal element: chain of sausage-shaped hyphae without branch (20–200×10–35 μm) and cluster of diverticulate hyphae with small side-branches and branchlets (Figs. 2D, 2E, 3A). **Clamp-connection** not recognized.

Habitat. On dungs of Japanese serow (*Capricornis crispus*), also on the dung of cow and pig (Aoki, 1981), on dungs of deer and sheep in the field (Uljé *et al.*, 2000).

Known distribution. Italy and Netherland (Uljé *et al.*, 2000), Kyoto and Saitama Prefecture (Aoki, 1981), Tokyo (Aoki, 1993).

Specimens examined. CBM-FB-34890= TNS-F-11630, CBM-FB-34950, CBM-FB-35061, CBM-FB-35062, CBM-FB-35768 (on the dung); CBM-FB-35441=TNS-F-11631 (basidiomata produced in culture). All specimens were from the dung of Japanese serow collected in Yatsugatake, Chino, Nagano Prefecture, alt. 2000 m in Aug. 12, 2004.

Japanese name. Shirage-ushiguso-hitoyotake (Aoki, 1981, 1993).

Notes. The isolate produced mature basidiomata on MYC medium. The fungal specimen obtained in the present study differed a little from

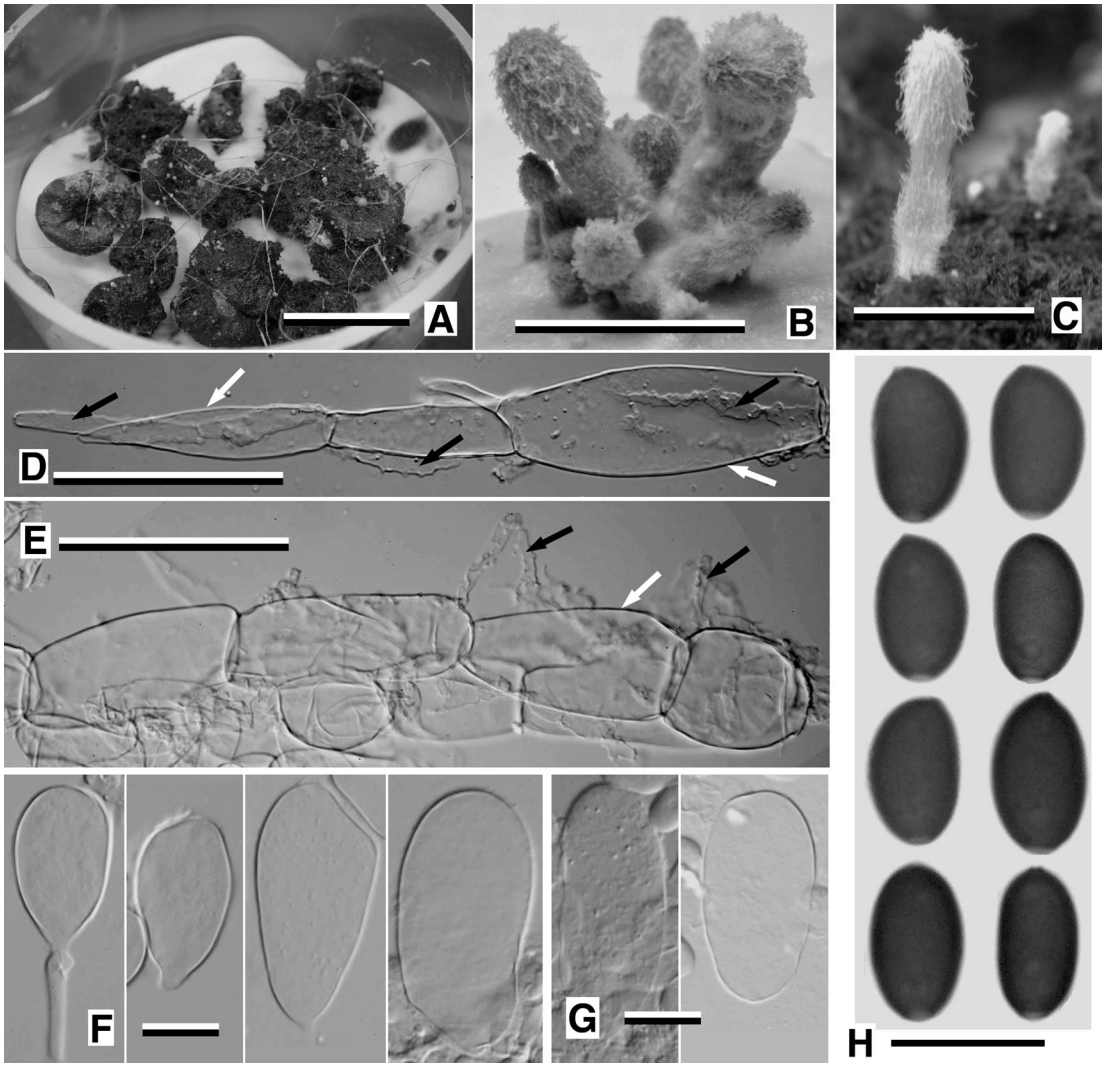

Fig. 2. *Coprinus candidolanatus*. A. Basidiomata on the dung specimens in moist chamber after 18 days of incubation. B. Basidiomata cultivated on MYC medium in 10 days, FB-35441 (CBM). C. Basidiomata on the dung in moist chamber of 9 days incubation. D, E. Veils on the pileipellis. The element of the veil was composed of two kind of hyphae: sausage-shaped elements (indicated by white arrows) and diverticulate elements (indicated by black arrows), FB-35441 (CBM). F. Cheilocystidia, FB-34890 (CBM). G. Pleurocystidia, FB-34890 (CBM). H. Basidiospores, FB-34950 (CBM). Scales. A=2 cm, B=5 mm, C=2 mm, D,E=50 μm, F, G=20 μm, H=10 μm.

the holotype in the size of basidiomata (holotype, pileus 3–6×2–4 mm when closed) and in the proportion of the basidiospore (length/bredth= ca. 1.5). However, conspicuous character of veil on the pileal surface, i.e. veil composed of two type of element, chain of sausage-shaped and cluster of diverticulate element with side-branches and branchlets are the unique characters to distinguish this species from others in this genus (Uljé *et al.*, 2000). Aoki (1981, 1993) documented this unique fungus as *Coprinus* sp. in his private communications in Japanese, giving a new Japanese name to it. Because the authors identified the present fungus as *C. candidolanatus* and recognized that it was identical to Aoki's *Coprinus* sp. (Aoki 1981, 1993), we document the fun-

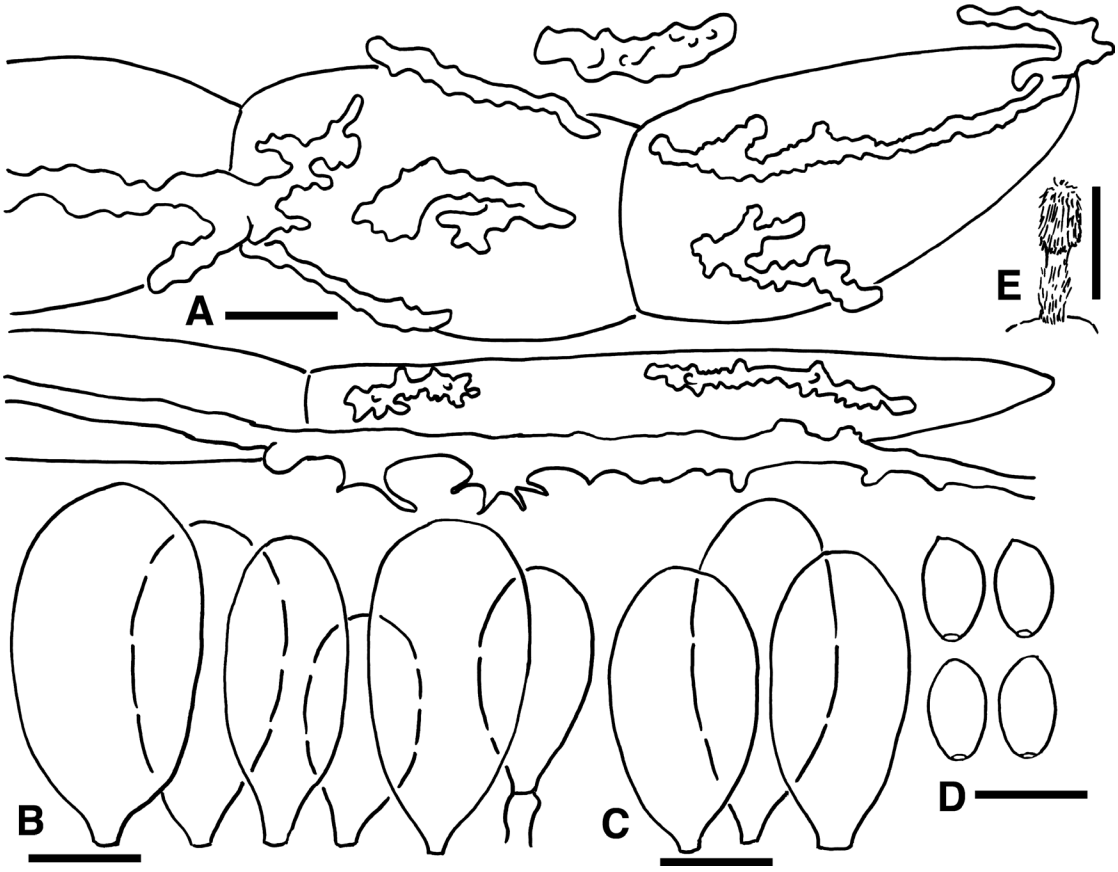

Fig. 3. *Coprinus candidolanatus*. A. Veils on the pileipellis, FB-35441, 34890 (CBM). B. Cheilocystidia, FB-34890 (CBM). C. Pleurocystidia, FB-34890 (CBM). D. Basidiospores, FB-34950 (CBM). E. Basidiomata on the dung in moist chamber of 9 days incubation. Scales. A–C = 20 μm, D = 10 μm, E = 2 mm.

gus here, adopting Aoki's Japanese name, and his observations were added to the previously known distribution in Japan. In his private communications, Aoki (1981, 1993) also described two other *Coprinus* spp. with two or three hyphal elemetnt in the pileal veil, but did not assign any name to them. These species differ from *C. candidolanatus* in the spore shape and veil element type.

2. *Coprinus stercoreus* Fr. Epicrisis systematis mycologici (Uppsala) p. 251. 1838.

Figs. 4, 5

Coprinus stercorarius (Bull.) Fr. Epicr. syst. mycol. p. 251. 1838.

Coprinus velox Godey *apud* Gillet, Cham. Fr. Hym. p. 614. 1878.

Coprinus evanidus Godey *apud* Gillet, Cham. Fr. Hym. p. 614. 1878.

Coprinus velox var. *stenosporus* Svrček, Česká Mycol. **10**: 176. 1956.

Coprinus stercoreus Fr. *sensu* Watling, Notes R. bot. Gdn Edinb. **28**: 48. 1967.

Coprinus velox Godey *sensu* Kits van Waveren, Persoonia **5**: 154. 1968.

Coprinopsis stercorea (Fr.) Redhead, Vilgalys & Moncalvo, in Redhead, Vilgalys, Moncalvo, Johnson & Hopple. Taxon **50**: 231. 2001.

Pileus 1.5–2 mm broad, 2–3 mm high when closed, when young ellipsoid to ovoid, later campanulate to plane, 3–4 mm broad when expanded, turning applanate at maturity or even revolute at deliquescence, ground color at first white, soon becoming pale greyish ochre, surface when young densely covered with a wooly white uni-

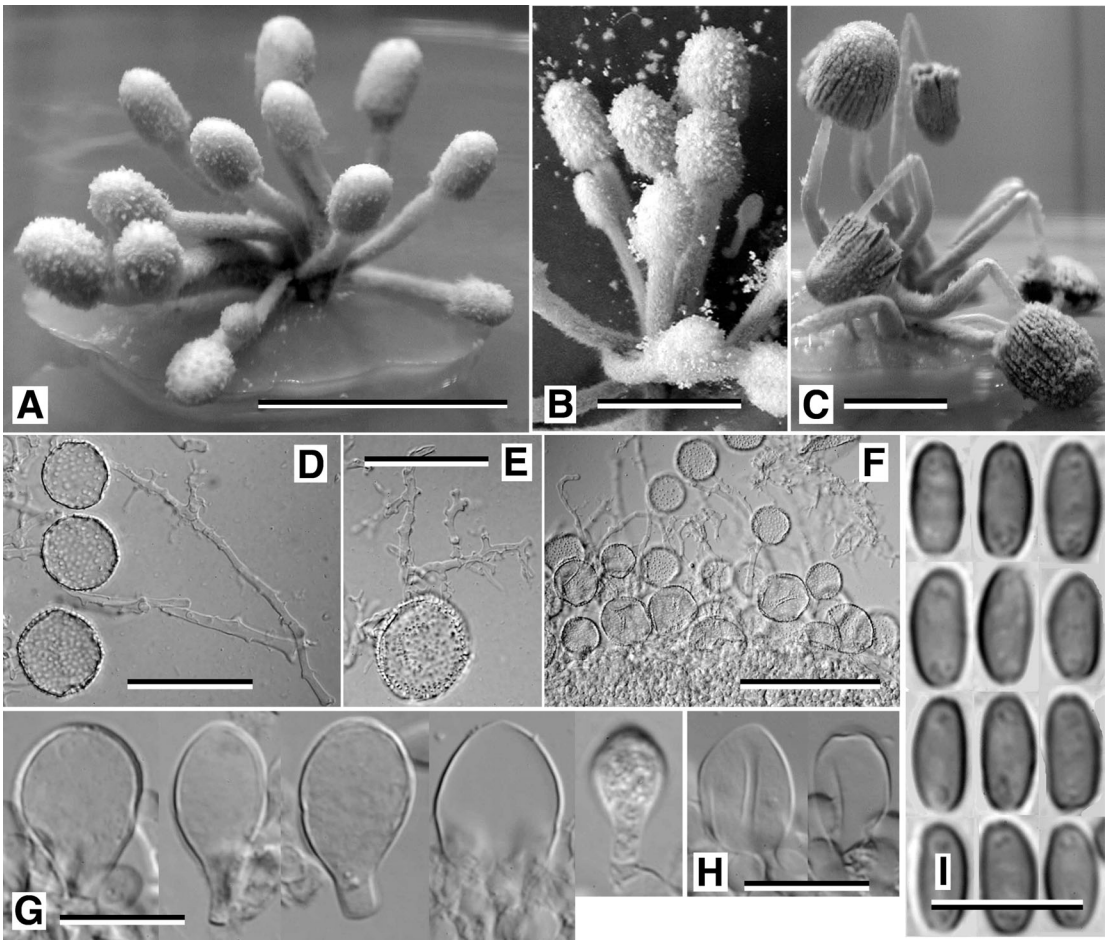

Fig. 4. *Coprinus stercoreus*. A, B. Basidiomata cultivated on MYC medium in 14 days, FB-35766 (CBM). C. Basidiomata cultivated on MYC medium in 17 days, FB-35415 (CBM). D, E, F. Veils on the pileipellis, the element of the veil was composed of two kind of hyphae, sphaerocyst elements and diverticulate elements, FB-35414 (CBM). G. Cheilocystidia, FB-35414 (CBM). H. Pleurocystidia, FB-35414 (CBM). I. Basidiospores, FB-35767 (CBM). Scales. A=1 cm, B=5 mm, C=5 mm, D, E=50 μm, F=100 μm, G, H=20 μm, I=10 μm.

versal veil (Fig. 4A, B), consisting of a whitish granules mixed with a wooly hairy mass of fibers particularly near the margin of the cap, later the veil remaining only in the center. **Flesh** very thin, fragile, white, taste mild, odorless. **Lamellae** free, dispersed (number of lamellae reaching stipe=20–30), narrow (0.3–0.6 mm breadth), at first white, then grayish, finally blackish, deliquescent. **Stipe** up to 2–5 cm×0.5 mm, cylindrical, equal, sometimes the base clavate, not rooting, fragile, surface white, at first with white fibrillose scales, soon becoming smooth. **Basidiospores** black in mass, dark red-brown under the microscope, ellipsoid, often phaseoliform, smooth, 6.3–7.5 (6.72±0.28: mean±SD, n=40) μm long, 2.8–3.8 (3.33±0.22, n=20) μm broad in face view, 3.1–3.4 (3.23±0.15, n=20) μm in side view, a central germ pore 1.1–1.3 μm wide, apiculus excentric on the adaxial face (Figs. 4I, 5D); perispore not observed. **Cheilocystidia** 20–35 μm long, 13–23 μm broad, ellipsoid to obovoid, numerous, thin-walled, hyaline (Fig. 4G, 5B), **Pleurocystidia** 17–23 μm long, 12–15 μm broad, subellipsoid to obovoid,

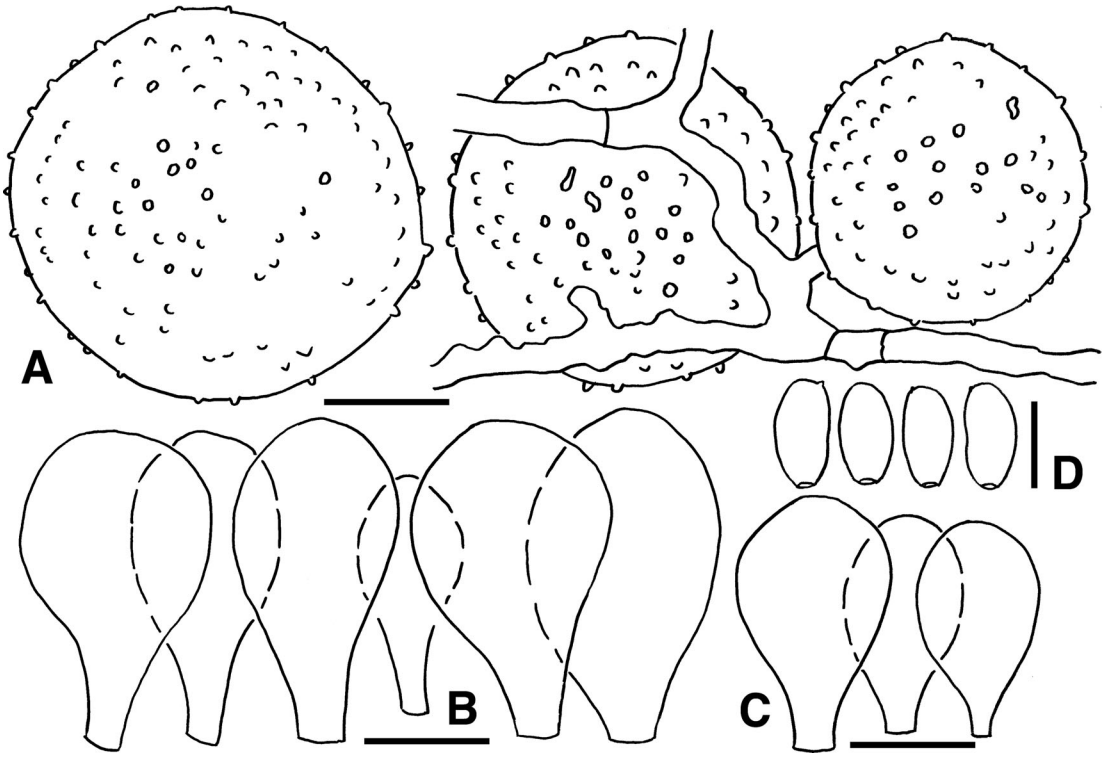

Fig. 5. *Coprinus stercoreus*. A. Veils on the pileipellis, the element of the veil was composed of two kind of hyphae, sphaerocyst elements and diverticulate elements, FB-35414 (CBM). B. Cheilocystidia, FB-35414 (CBM). C. Pleurocystidia, FB-35414 (CBM). D. Basidiospores, FB-35767 (CBM). Scales. A–C = 10 μm, D = 5 μm.

thin-walled, hyaline (Figs. 4H, 5C). **Universal** veil on the pileal surface composed of two type of element; verrucose globose to subglobose cells (diameter; 20–40 μm, young specimen) and cluster of diverticulate hyphae with side-branches and branchlets (diameter; 3–5 μm, Figs. 4D–F, 5A). **Clamp-connection** not recognized.

Habitat. On dung of Japanese serow (*Capricornis crispus*) or dung of rabbit (*Lepus brachyurus*, Aoki, 1980), on dung of cow, horse, sheep, rabbit, deer (Kits van Waveren, 1968).

Known distribution. Europe, North America, North Africa (Breitenbach and Kränzlin, 1995), Aichi (Aoki, 1981).

Specimens examined. CBM-FB-35079, CBM-FB-35080, CBM-FB-35767 (on dung of Japanese serow collected in Yatsugatake range, 2,000 m elevation on August 12, 2004); CBM-FB-35414, CBM-FB-35415 = TNS-F-11632, CBM-FB-35766 = TNS-F-11633 (basidiomata produced in culture).

Japanese name. Tofun-hitoyotake (Aoki, 1980).

Notes. *Coprinus stercoreus* belongs to the section *Vestiti* (Lange) Khner & Romagnesi (Moser, 1983; Singer, 1986) because of its sphaerocyst element in the pileal veil. In this section two species have been described: *Coprinus tuberosus* Qul. and *C. stercoreus* both of which have no or few perispore on the surface of the basidiospores, with two types of pileal veil element (sphaerocysts and filamentous hyphae with small side-branches and branchlets), spore length under 10 μm (Breitenbach and Kränzlin, 1995; Moser, 1983; Orton and Watling, 1979; van Waveren, 1968). *Coprinus stercoreus* is distinguished from *C. tuberosus* in the character of basidiospore (length under 9.5 μm, often phaseoliform) and

having no sclerotium under the stipe (Breitenbach and Kränzlin, 1995; van Waveren, 1968). The idea to transfer the present fungus to *Coprinopsis* (Redhead, 2001) is currently not widely accepted, and we followed the conventional taxonomy. Hongo (1987) documented *C. stercorarius* from Japan, but we determine that his specimen was conspecific with *C. tuberosus* based on

Table 1. Occurrence of the three *Coprinus* species documented in the present study from the dung specimens.

Origin of the dung	Incubated temperature (°C)	No. of specimens	*Coprinus candidolanatus*	*Coprinus stercoreus*	*Coprinus ephemeroides*
sika deer (*Cervus nippon*)	15	6	0	0	0
	22	6	0	0	1
Japanese serow (*Capricornis crispus*)	15	6	0	0	1
	22	6	1	1	0

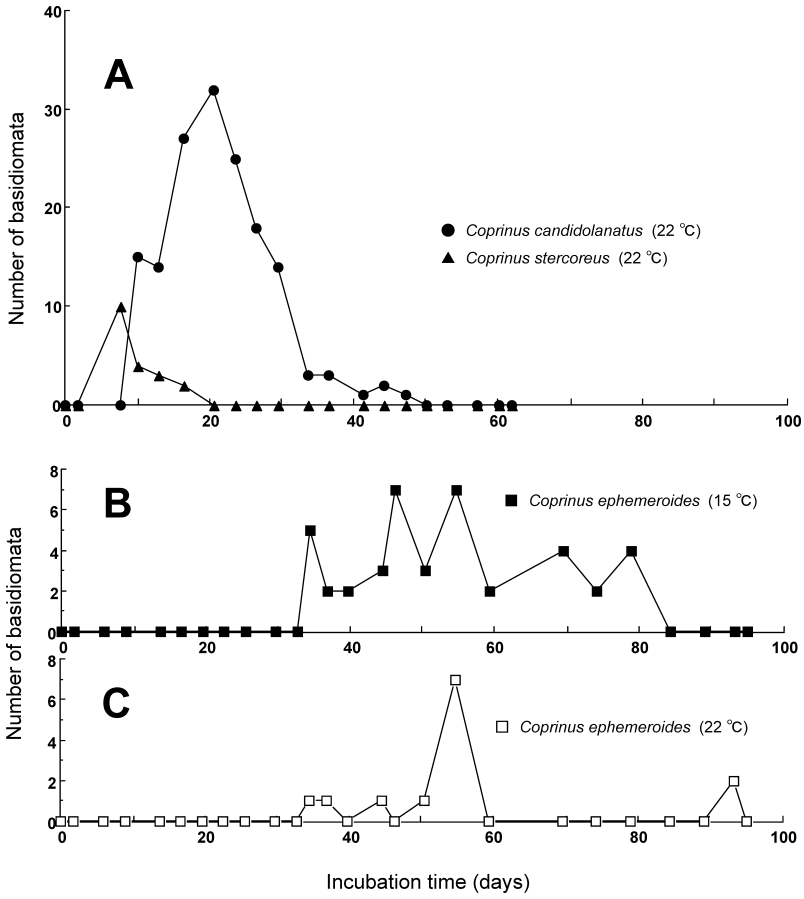

Fig. 6. Number of basidiomata of *Coprinus* spp. on the dung specimens. A. *Coprinus candidolanatus* (●) and *C. stercoreus* (▲) on the same dung of *Capricornis crispus*, incubated at 22°C for 9 weeks. B. *Coprinus ephemeroides* (■) on the dung of *Capricornis crispus*, incubated at 15°C for 14 weeks. C. *Coprinus ephemeroides* (□) on the dung of *Cervus nippon*, incubated at 22°C for 14 weeks.

his description. It seems that Aoki (1980) was the first to recognize the present fungus in Japan, but he identified it as *C. velox* Godey and published it in his private communication in Japanese with Japanese name. Because the authors recognized that the present fungus was identical to Aoki's description, we describe and illustrate it here under the current name, adopting Aoki's Japanese name, respecting his work. The present fungus is fairly common as a coprophilous fungus in the world (van Waveren, 1968).

3. *Coprinus ephemeroides* (Bull.) Fr.

Habitat. On dungs of various kinds (Orton and Watling, 1979); on dungs of cow and horse, Tatami mat disposed in the field (Hongo, 1987), on the dung of *Cervus nippon* and *Capricornis crispus*.

Known distribution. North America, Europe (Moser, 1983; Orton and Watling, 1979) ; Otu (Hongo, 1972), Tokorozawa (Aoki, 1976, 1995).

Specimens examined. On dung of sika deer (*Cervus nippon*); CBM-FB-35438, CBM-FB-35440, CBM-FB-35443=TNS-F-11634. On dung of Japanese serow (*Capricornis crispus*); CBM-FB-35439=TNS-F-11635, CBM-FB-35442, CBM-FB-35444, CBM-FB-35446=TNS-F-11636. All dung specimens were collected in Yatsugatake, Chino, Nagano Prefecture in Oct.13 2004.

Japanese name. Tsuba-hina-hitoyotake (Hongo, 1972)

Notes. Morphological characteristics agreed with those given by Hongo (1972, 1987). In the present study, *C. ephemeroides* occurred on dung of *Cervus nippon* (incubated at 22°C) and *Capricornis crispus* (incubated at 15°C). Isolation from the fruiting body was unsuccessful.

Coprinus ephemeroides belongs to the section Vestiti (Lange) Khner & Romagnesi (Moser, 1983; Singer, 1986) because of its sphaerocyst element in the pileal veil. *Coprinus ephemeroides* is relatively well-documented partly because it is easily distinguished from other species and can be identified by combination of obvious characters such as ring on the stipe, without perispore on the surface of the basidiospores which are pentagonal in face view.

Acknowledgements

The author expresses their gratitude to Dr. Keiji Ochiai, (Natural History Museum & Institute, Chiba) for identification of the dung. They thank Ms. Haruko Suga (Chiba mycological club) for her assistance in collecting the specimens. Mr. Ichiro Gomi, Suwa City Museum of Art is greatly appreciated for his excellent guiding in the field. The authors wishes to express their gratitude to Dr. Y. Hiratsuka for reviewing the manuscript.

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