# Enumeration of Remarkable Japanese Discomycetes (4): First Records of One Operculate and Two Inoperculate Discomycetes in Japan

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**Abstract** Three remarkable discomycetes (one operculate and two inoperculate) are first records in Japan and are described and illustrated: *Cheilymenia granulata* (Pyronemataceae, Pezizales), *Mollisia amenticola* (Dermateaceae, Helotiales), and *Phialina pseudopuberula* (Hyaloscyphaceae, Helotiales).

**Key words**: Cheilymenia granulata, Mollisia amenticola, mycobiota, Phialina pseudopuberula, taxonomy.

### Introduction

This is the fourth part of the series on remarkable Japanese discomycetes following Hosoya (2009). Two inoperculate and one operculate discomycetes with minute apothecia, first reported from Japan are described and illustrated.

#### **Materials and Methods**

Collection and observation procedures follow Hosoya and Otani (1997) and Hosoya (2004). Color codes follow the Pantone color code adopting RGB system referring to a Pantone color bridge (Anonymous, 2005). For known distribution, the database of global biodiversity information facility (GBIF, http://data.gbif.org/welcome. htm) was searched, and counties with occurrence of the given species are shown with an asterisk (\*). Distributions known only in literature are shown with double asterisks (\*\*). Those with both information are shown with triple asterisks (\*\*\*)

# **Descriptions**

# 1. Cheilymenia granulata

Figs. 1 and 2

Peziza granulata Bull., Herb. Fr. 10: tab. 438, fig. 3 (1790)

Ascobolus granulatus (Bull.) Fuckel, Jb. nassau. Ver. Naturk. 23–24. 1870. [1869–70]

Ascophanus granulatus (Bull.) Speg., Michelia 1 (no. 2): 235. 1878.-Seaver, North American Cup-fungi (Operculate) p. 116. 1928.

Aleuria granulata (Bull.) Gill., Champ. Fr., Discom. 56. 1879.

Humaria granulata (Bull.) Sacc., Syll. fung. (Abellini) 8: 129. 1889.

Coprobia granulata (Bull.) Boud., Hist. Class. Discom. Eur. (Paris) 1: 69. 1907. -Le Gal, Discomycètes de Madagascar. p. 97. 1953.—Eckblad, Nytt Magasin For Botanikk. p. 53. 1968—Rifai, Australian Pezizales p. 138, 1968.

Humarina granulata (Bull.) Nannf., Fungi Exsiccati Suecici: no. 1363. 1946.

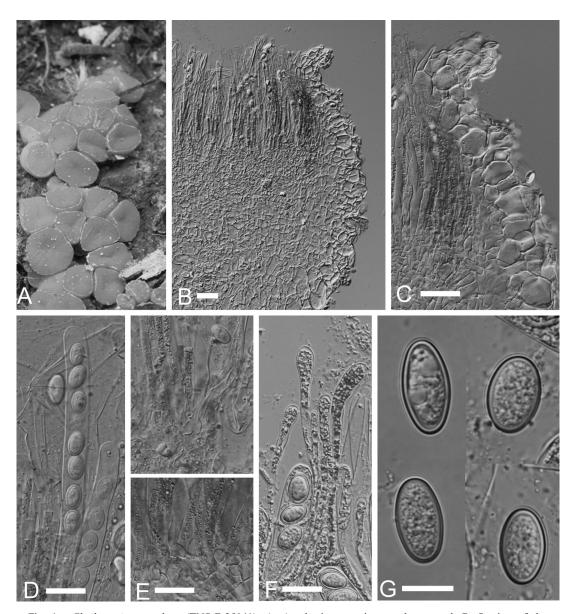


Fig. 1. Cheilymenia granulosa (TNS-F-35041). A. Apothecia occurring on the ground. B. Section of the apothecium showing the hymenium, ectal and medullary excipulum. Note orange granules are concentrated in the paraphyses at the hymenium. C. Close up of the section of the apothecium at the margin showing the ectal excipulum. D. Ascus. E. Ascal base with crosiers. F. Paraphyses containing carotenoid granules. G. Ascospores. Note either spumose or containing large oil drops. B-G, Mounted in lactic acid. Scales. B-G, 10 μm.

Cheilymenia granulata (Bull.) J. Moravec, Mycotaxon 38: 474. 1990. var. granulata Peziza granulosa Pers., Syn. meth. fung. (Göttingen) 2: 667. 1801.

*Peziza fulva* Huds., Fl. Angl., Edn 2 2. 1778. *Humaria gollmeri* P. Henn., Hedwigia 36: 233. 1897. (teste Dennis, Kew Bull. 14: 428. 1959.)

**Apothecia** gregarious, sessile, broadly attached to the substrate; 1–2 mm in diameter, patellate to turbinate when fresh, 0.5–1.5 mm in diameter when dry; disc slightly pruinose, drying more granular, orange (1505C=R255 G145 B51)

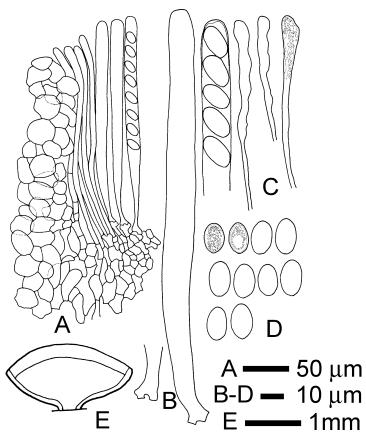


Fig. 2. Camera lucida illustration of *Cheilymenia granulosa* (TNS-F-35041). A. Section of the apothecium at the margin showing the ectal excipular structure. B. Asci. C. Paraphyses. One at the right shows the carotenoid granules. D. Ascospores. Two at the top left showing the cellular contents. E. Schematic drawing of the apothecial structure showing the distinction of the hymenium, ectal and medullary excipulum. Line drawings prepared in lactic acid mount.

when fresh, paler orange (1485C=R255 G176 B122) when dried. **Ectal excipulum** textura globulosa, composed of globular to angular cells,  $13-20\times20-30~\mu\text{m}$ . **Asci**  $180-230\times12-14~\mu\text{m}$ , cylindrical, thin-walled, 8-spored, arising from crosiers; apex not stained by MLZ with or without KOH pretreatment. **Ascospores** (12)15–16× (8-)9–10  $\mu$ m, ellipsoid, aseptate, spumose, smooth, some containing one to two large vacuoles surrounded by fine globules, hyaline, uniseriate in the asci. **Paraphyses** cylindrical, simple, irregularly enlarged at the apex up to 8  $\mu$ m wide, 2  $\mu$ m below, filled with orange, granular to acicular contents staining blue in MLZ.

Specimens examined. HONSHU: TNS-F-

**35041**, Gokayama, Nanto-shi, Toyama Pref. (36°25′34.8″N, 137°1′7.9″E, alt. 719 m) on bear soil, 13-VI-2010, col. T. Hosoya; **TNS-F-25566**, **30887**, Higashiagatsuma-cho, Agatsuma-gun, Gunma Pref. (36°33′20.3″N, 138°42′42.9″E, alt. 503 m), on plant debris compiled and decaying in abandoned human dunghill, 19-V-2009, col. T. Hosoya.

Known distribution. EUROPE: Denmark\*, Germany\*, Netherland\*, Norway\*\* (Eckblad, 1968), Slovenia\*, Spain\*, Sweden\*, Switzerland\*\* (Breitenbach and Kränzlin, 1984), UK\*. NORTH AMERICA: USA\* (Seaver, 1942). ASIA: Philippines\*. OCEANIA: Australia\*\* (Rifai, 1968). AFRICA: Madagascar\*\* (Le Gal, 1953).

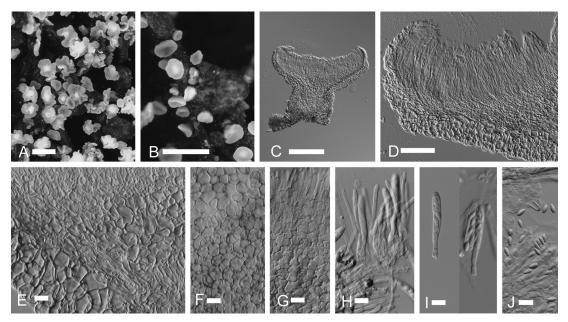


Fig. 3. Mollisia amenticola (TNS-F-30411). A, B. Fresh apothecia. C. Longitudinal section of the apothecium. Note brown-walled ectal excipulum and medullary excipulum. D. Section of the apothecium at the margin. E. Close up of the ectal excipulum. Note cells arranged right angle to the margin. F. Ectal excipular structure in the crush mount. G. Ectal excipular structure at the margin in crush mount. Note marginal hairs (upper side). H. Paraphyses. I. Asci. J. Ascospores. C-I, Mounted in lactic acid. J, Mounted in Melzer's reagent. Scales. A, B, 1 mm; C,100 μm; D, 50 μm; E-J, 10 μm.

Notes. The present fungus was known as *Coprotus granulata* for a long time (Corner, 1929; Le Gal, 1953; Eckblad, 1968; Rifai, 1968; Breitenbach and Kränzlin, 1984). However, based on the presence of hairs in the ectal excipulum, the genus *Coprotus* was reduced to a section in *Cheilymenia* (Moravec, 1990).

Cheilymenia granulata is known as a wide-spread coprophilous fungus. It has been reported by various authors from various places (Le Gal, 1953; Seaver, 1942; Eckblad, 1968; Rifai, 1968; Breitenbach and Kränzlin, 1984), always on dung of herbivorous animals. On the contrary, the habitat of TNS-F-35041 may be atypical for the present fungus. The soil was exposed to direct sunlight, and found to be rich in plant debris. TNS-F-35041 was associated with *Scutellinia* sp., also occurring on the soil surface, even more frequently than *C. granulata*. The composition of the soil and physical stimulation by the sunlight might have affected the germination of

ascospores and production of the apothecia.

The apothecia discharged the ascospores vigorously, but no discharged spores germinated.

Eckblad (1968) and Korf (1973) reported a pellicular sheath detached by heating in lactic acid for this fungus. In the Japanese specimens, however, no such pellicular sheath was observed.

## 2. Mollisia amenticola

Figs. 3 and 4

Mollisia amenticola (Sacc.) Rehm, in Rabenhorst, Rabenh. Krypt.-Fl. (Leipzig) 1: 540. 1891. [1896]

**Apothecia** gregarious to scattered, short stipitate; shallow cupulate when young, becoming irregularly undulate at the margin, 0.3 mm in diameter, up to 0.3 mm high when mature, totally white to grayish white, becoming brown toward the base when fresh; disc white to grayish white when fresh, grayish orange (Pantone 7401C=R 241 B 227 B 187), 0.25–0.75 mm in diameter

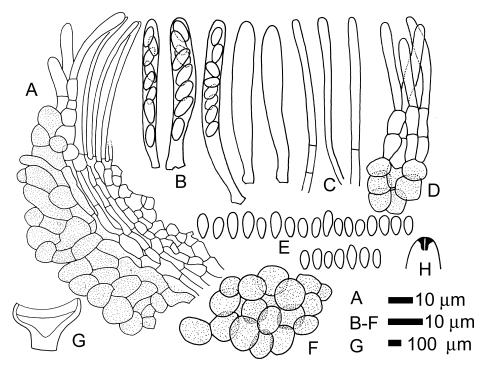


Fig. 4. Camera lucida illustration of *Mollisia amenticola* (TNS-F-30411). A. Section of the apothecium at the margin. B. Asci. C. Paraphyses. D. Ectal excipulum and hyphoid hairs. E. Ascospores. F. Ectal excipulum. G. Section of the apothecium showing the distinction of ectal and medullary excipulum, and the hymenium. H. Ascal apex showing the reaction to Melzer's reagent. Line drawings prepared in lactic acid mount except for H mounted in Melzer's reagent.

when dried; margin well defined, often slightly elevated. Ectal excipulum textura globulosa, composed of two to three layers of brown-walled cells, globular in middle flank, becoming angular, arranged nearly right angle to the surface, becoming hyaline and hyphoid at the margin (marginal hairs),  $8-10\times5-8 \mu m$  in crush mount; marginal hairs  $20-25\times2.5-3.0 \,\mu\text{m}$ , Medullary excipulum textura angularis, composed of thin-walled, elongated, pale brownwalled cells,  $4-10\times3-5 \mu m$ . Asci  $42-55\times4-6$  $\mu$ m (49.4 $\pm$ 4.09 $\times$ 5.18 $\pm$ 0.61  $\mu$ m in average $\pm$ SD, n=18 measured in lactic acid, based on 3 specimens), cylindrical-clavate, 8-spored, arising from crosiers; apex stained blue by MLZ without KOH pretreatment. Ascospores  $5-8\times2.5-3.0$   $(6.53\pm$  $1.09 \times 2.89 \pm 0.211 \,\mu \text{m}$  in average  $\pm \text{SD}$ , n=30 measured in lactic acid), ellipsoid, occasionally tapered to the bottom, aseptate, smooth, hyaline.

**Paraphyses** cylindrical,  $2-3 \mu m$  wide, even in width to the apex, septate at the base.

Specimens examined. HONSHU: TNS-F-17420, Taraigoyazawa, plot No. T4, Susugaya, Kiyokawa-mura, Aiko-gun, Kanagawa Pref. (35°27′2.1″N, 139°13′6″E), on *Alnus* female cones lying on the ground, 16-IV-2005. col. T. Hosoya; TNS-F-30411, Osaka, Shijounawateshi, Osaka-fu (34°43′43″N, 135°40′5″E), on *Alnus* female cones lying on the ground, 25-II-2010. col. K. Shimohara. TNS-F-32118, Motomachi, Ikoma-shi, Nara Pref. (34°41′28″N, 135°41′22″E), on *Alnus* female cones lying on the ground, 10-III-2010. col. K. Shimohara.

Known distribution. EUROPE: Austria\*, Denmark\*, Poland\*, Switzerland\*\* (Breitenbach and Kränzlin, 1984). NORTH AMERICA: USA (Kanouse, 1947).

Notes. Two other species of discomycetes are

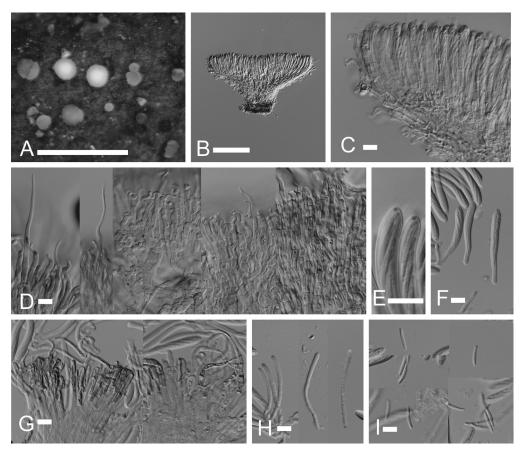


Fig. 5. *Phialina pseudopuberula* (TNS-F-31300). A. Fresh apothecia. B. Longitudinal section of the apothecium. C. Close up of the ectal excipulum and the hymenium. Note short hairs protruding from the outermost cells of the ectal excipulum. D. Various aspects of hairs. The left two shows longer hairs while the right two shows the shorter hairs. Note some parts of the hairs are stained blue in cotton blue in lactic acid. E. Ascal apex stained by Melzer's reagent. F. Asci. G. Hair contents stained golden yellow in Melzer's reagent. H. Paraphyses. I. Ascospores. B-D, H, I. Mounted in lactic acid stained by cotton blue. E-G. Mounted in Melzer's reagent. Scales. A, 1 mm; B, 100 μm; C-I, 10 μm.

known to occur on *Alnus* fruit: *Pezizella alniella* (Nyl.) Dennis and *Ciboria viridifusca* (Fuckel) Höhn. *Mollisia amenticola* is distinguished from these fungi in shorter asci and brown colored, globular ectal cells. The color of ectal excipular cells varies from almost hyaline to brown, typical of Dermateaceae. Although it is not described in detail in the recent literature (Kanouse, 1947), it can be distinguished based on characteristic habitat and ectal excipular cells.

# 3. Phialina pseudopuberula

Figs. 5 and 6

*Phialina pseudopuberula* (Graddon) Raitv., Folia cryptog. Estonica 9: 1. 1977.

*Hyaloscypha pseudopuberula* Graddon, Trans. Br. Mycol. Soc. 58: 151. 1972.

Hamatocanthoscypha tenuipila Graddon, Trans. Br. Mycol. Soc. 87: 329. 1986.

Calycellina pseudopuberula (Graddon) Baral, Z. Mykol. 59: 4. 1993.

Phialina plowrightii Arendh., Morphologischtaxonomische Untersuchungen an blattbewohnenden Ascomyceten aus der Ordnung der Helotiales (Ph.D. thesis, University of Hamburg) (Hamburg): 53. 1979.

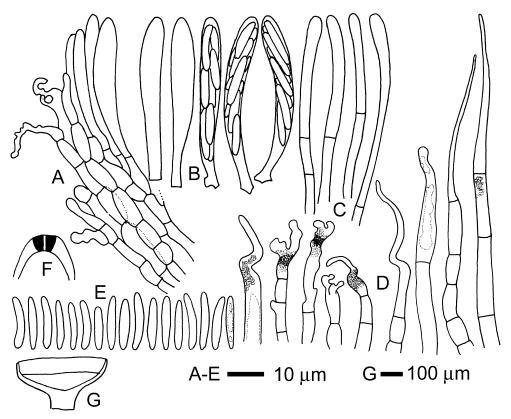


Fig. 6. Camera lucida illustration of *Phialina pseudopuberula* (TNS-F-31300). A. Longitudinal section of the apothecium at the margin. B. Asci. Three at the right show ascospores. C. Paraphyses. D. Hairs. Five at the left show aseptate to few septate hairs, four at the right with stained by cotton blue in lactic acid. E. Ascospores. F. Ascal apex stained by Melzer's reagent. G. Longitudinal section of the apothecium. F, Mounted in Melzer's reagent, the rest mounted in lactic acid.

**Apothecia** gregarious, superficial, short stalked; pure white to pale brown (720C=R233 G191 B155 or paler) when fresh, similar color when dried; 0.15-0.25 mm in diameter, 0.1 mm in height when dry; disc flat to shallow concave; margin slightly incurved when dry. Ectal excipulum textura prismatica, composed of several layers of prismatic cells,  $6-11\times2.5-5.0 \mu m$ . Hairs of various shape; longer hairs straight, gradually tapered to apex of 0.5-1.0 µm wide, multi-septate, up to  $100 \,\mu\text{m}$  long, c.  $4 \,\mu\text{m}$  at the base, protruding from the margin to the outside; shorter hairs straight to curved or circinate, simple to irregularly branched, aseptate to few septate, 13–30 µm long, gradually tapered or not tapered to the apex; long, septate hairs less frequently observed than shorter, branched hairs; cellular contents partially strongly stained by CB/LA, stained golden yellow in MLZ. **Asci** 40–50(-57)×5.0–7.0  $\mu$ m (44.8±3.80×5.85±0.56  $\mu$ m in average±SD, n=20, measured in MLZ without KOH pretreatment), cylindrical clavate, eight spored; apex stained blue in MLZ without KOH pretreatment; arising from croziers. **Ascospores** (11-)15–19×1.5–2.5  $\mu$ m (15.8±2.7×2±0.25  $\mu$ m in average±SD, n=18 measured in lactic acid), elongate-ellipsoid, straight or slightly curved, aseptate. **Paraphyses** straight, simple, cylindrical, gradually enlarged toward the apex, up to 3.5  $\mu$ m wide, 2–3  $\mu$ m below, septate; terminal cells 25–50  $\mu$ m long.

Colony on PDA in 20 days at 18°C 12 mm in

diameter, low and dense, irregularly wrinkled, radially sulcate with umbonate elevation at the center, brown (483C=R103 G51 B39) at the center, becoming reddish brown (486C=R231 G143 B119) and paler toward the margin, dark brown (476C=R76 G51 B39) from the reverse. Pale brown soluble pigment excreted into the agar. Margin distinct, filmy. Aerial mycelium little developed, velvety, hyaline. No anamorph observed.

Specimens examined. HONSHU: **TNS-F-31300**, Ushiku Nature Sanctuary, Kessokucho, Ushiku-shi, Ibaraki Pref. (35°57′47.5″N, 140°10′5.3″E), on decaying *Quercus myrsinifolia* leaf lying on the ground, 18-X-2009. col. T. Hosoya.

Known distribution. EUROPE: Belgium\*\*, Czechoslovakia\*\*, Denmark\*, UK\*\*\*, Netherland\*\*, Norway\*\*, USSR\*\*, ASIA: India\*\* (Huhtinen, 1989).

Notes. Because of its distinctive features of the hairs, having various shapes in particular short, aseptate to few septate hairs, and foliicolous habitat, this fungus was easily identified. *Phialina pseudopuberula* has been reported on *Quercus*, but also on *Acer*, *Betula, Castanea*, and *Fagus* (Huhtinen, 1989).

The genus *Phialina* seems to be rather rare in Japan. So far only *P. lachnobrachya* (Desm.) Raitv. and *P. lachnobrachyoides* have been previously reported (Huhtinen, 1989; Hosoya, 2005).

Huhtinen (1989) recognized various ascospore shapes including septate, equipped with cilia, but such variation depends on the collection. The specimen reported here did not show any of those variations.

Cultural characteristics is reported for the first time.

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