

Enumeration of Remarkable Japanese Discomycetes (11): Notes on Three Species of Lachnaceae New to Japan with One New Combination

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Abstract Three species of Lachnaceae new to Japan are described and illustrated with one new combination: *Brunnipila clandestina*, *B. pseudocannabina* (new combination), and *Incrucipulum capitatum*.

Key words: *Brunnipila clandestina*, *Brunnipila pseudocannabina*, *Incrucipulum capitatum*, mycobiota, taxonomy.

Introduction

This is the eleventh part of the series of remarkable Japanese discomycetes following Hosoya and Zhao (2016) to elucidate Japanese cup-fungi mycobiota. Three species of Lachnaceae Raitv. are newly documented from Japan, including one new combination. Descriptions, micrographs and line drawings of three species are provided.

Materials and Methods

Collection, isolation and observation procedures mainly followed Hosoya and Otani (1997) and Hosoya (2004). Examination of micromorphology was carried out using cotton blue dissolved in water (CB/W) or cotton blue dissolved in lactic acid (CB/LA). Color codes followed the Pantone color code adopting CYMK system referring to a Pantone color bridge (Anonymous, 2005).

For previously known distribution, the database of Global Biodiversity Information Facility (GBIF,

<http://www.gbif.org/occurrence>, as of January 26, 2019) was searched, and countries with occurrences of the given species are shown with an asterisk (*). Distributions known only from the literature are shown with double asterisks (**). Those with both information are shown with triple asterisks (***)

All the specimens used in this study are housed in the Department of Botany, National Museum of Nature and Science (TNS), Tsukuba, Japan.

DNA extraction and sequencing followed Hosaka and Castellano (2008). Obtained isolates will be deposited in NITE National Bioresource Center (NBRC). The obtained DNA vouchers are deposited in the Center for Molecular Biodiversity Research, National Museum of Nature and Science (Tsukuba, Ibaraki, Japan) and available for molecular phylogenetic researches.

Descriptions

1. *Brunnipila clandestina* (Bull.) Baral, Beih.

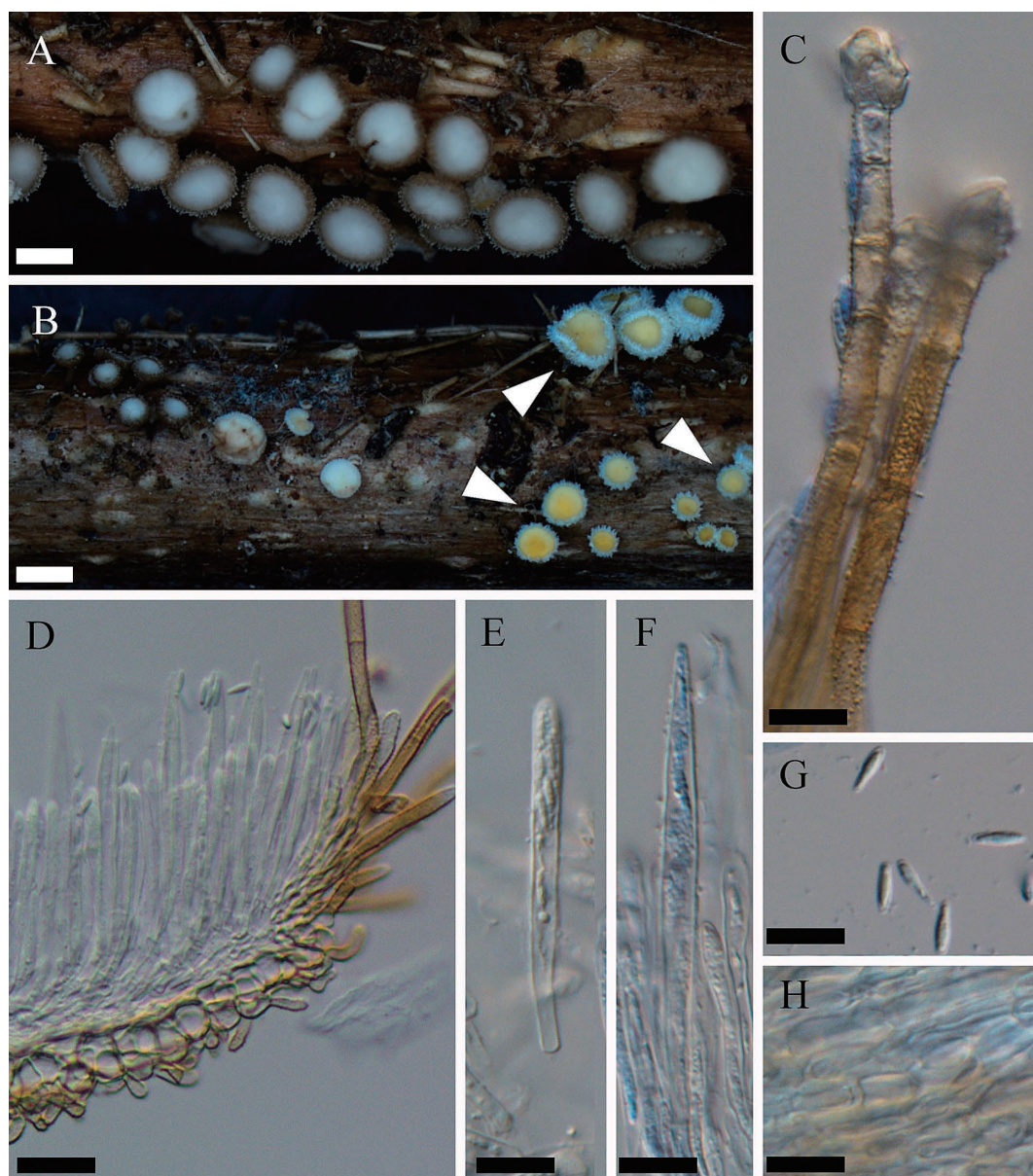


Fig. 1. *Brunnipila clandestina* (TNS-F-81486). A. Fresh apothecia. B. *Brunnipila clandestina* with *Capitotricha rubi* (arrow heads) on the same substrate (when fresh). C. Hairs with crystals. D. Vertical section of an apothecium showing the ectal and medullary excipulum. E. Ascus. F. Paraphysis. G. Ascospores. H. Ectal excipular inner cells. Scales. A, B, 1 mm; C, E–H, 10 µm; D, 20 µm. C, E–H, mounted in CB/W; D, CB/LA.

Z. Mykol. 6: 49. 1985.

[Figs. 1, 2]

Mycobank. MB104069

Peziza clandestina Bull., Herb. Fr. Champ., Hist. Champ. Fr. (Paris) 1: 251. 1791.

Peziza clandestina var. *clandestina* Bull., Herb. Fr. Champ., Hist. Champ. Fr. (Paris) 1: 251. 1791.

Dasyscyphus clandestinus (Bull.) Fuckel, Jb. nassau. Ver. Naturk. 23–24: 305. 1870 [1869–70].

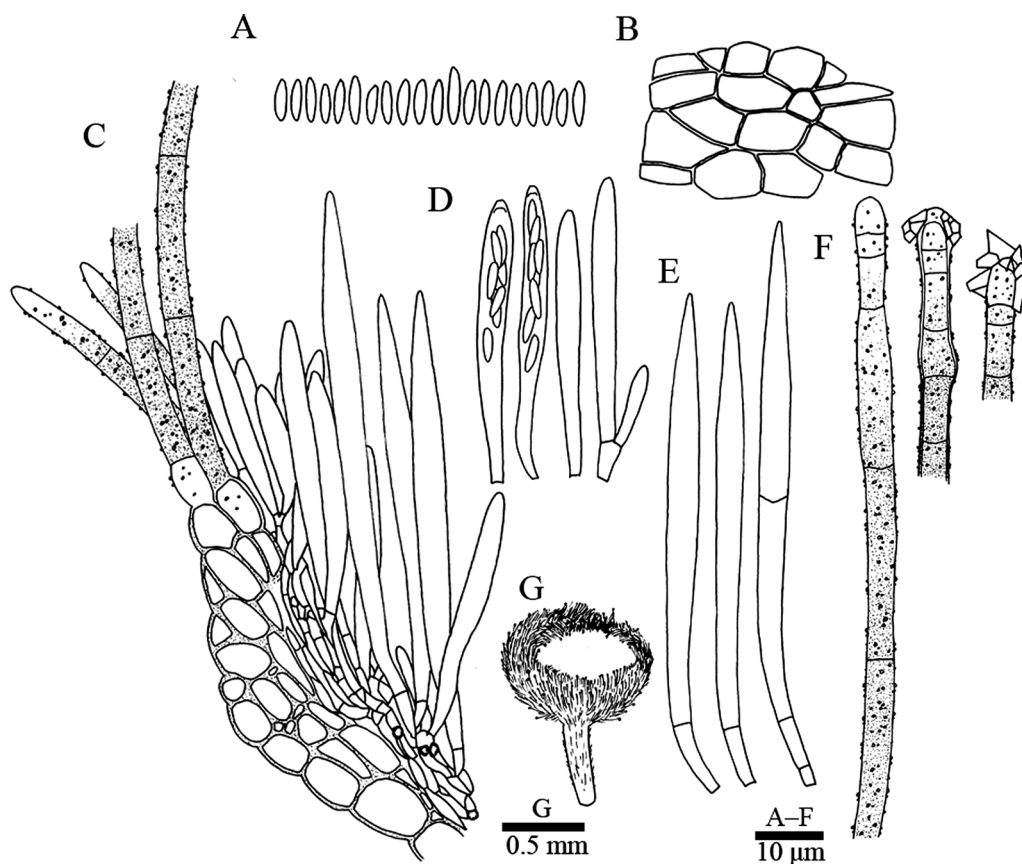


Fig. 2. *Brunnipila clandestina* (TNS-F-81486). A. Ascospores. B. Ectal excipular outer cells. C. Vertical section through the margin, showing the hairs and ectal excipulum. D. Asci. E. Paraphyses. F. Hairs with and detached (left) crystals. G. Apothecium.

Lachnea clandestina (Bull.) P.Karst., Not. Sällsk. Fauna et Fl. Fenn. Förh. 11: 250. 1870 [1871].

Erinella clandestina (Bull.) Quél., Enchir. fung. (Paris): 302. 1886.

Lachnella clandestina (Bull.) W. Phillips, Man. Brit. Discomyc. (London): 236. 1887.

Atractobolus clandestinus (Bull.) Kuntze, Revis. gen. pl. (Leipzig) 3 (3): 445. 1898.

Peziza clandestina var. *connivens* Alb. & Schwein., Consp. fung. (Leipzig): 326. 1805.

Peziza clandestina var. *patula* Alb. & Schwein., Consp. fung. (Leipzig): 326. 1805.

Peziza clandestina var. *discoidea* Pers., Mycol. eur. (Erlanga) 1: 263. 1822.

Apothecia gregarious, superficial, forming urce-

olate cup to almost flat disc, 0.3–1 mm in diameter, stipitate, up to 1 mm high, dark brown (4635PC = C13 M53 Y68 K40), externally covered with brown hairs mostly capitate. **Disc** concave, white to pale yellow (7499PC = C1 M2 Y20 K0) when fresh and dry. **Ectal excipulum** *textura angularis* to *prismatica* composed of hyaline to pale brown (4655PC = C6 M38 Y42 K18) cells; cells 3–17 × 3–12 µm, slightly thick-walled. **Medullary excipulum** *textura intricata* of hyaline hyphae up to 2.5 µm wide. **Hairs** straight, cylindrical with slightly rounded apices, up to 120 × 3.5–5 µm, brown (471PC = C5 M70 Y97 K20) with pale brown or hyaline apices, totally granulate, thin-walled except for the apical cell slightly thick-walled, septate every 10–30 µm except for one or two apical cells with

3–5 µm septation; apex crowned by crystal masses easily detached in squash mounts. **Asci** 41–47 × 3.2–4.5 µm, 8-spored, cylindrical-clavate; apical pore blue in Melzer's reagent without 3% KOH pretreatment; croziers absent at the basal septa; basal cells usually form more than two asci. **Ascospores** 5.8–9 × 1.2–2 µm, ellipsoid to fusiform, aseptate. **Paraphyses** straight, lanceolate, up to 5 µm wide, septate, exceeding the asci 20–30 µm.

Specimen examined. HOKKAIDO: TNS-F-81486, Engaru-cho on dead twigs of *Rubus idaeus* subsp. *melanolasius*. 2018-VII-14. coll. Y. Tochihara.

Ecology. Saprophytic on dead twigs of *Rubus idaeus*, sometimes on dead twigs or stems of other plants.

Previously known distribution. Europe^{***}, North America^{***}, Kazakhstan*, Kyrgyzstan*, Tajikistan*, Morocco*, New Zealand*, Russia^{***} (including Far East).

Notes. Baral and Krieglsteiner (1985) reported *B. clandestina* coexisted with *Capitotricha rubi* (Bres.) Baral and *Lachnum virgineum* (Batsch) P. Karst., on the same substrate (*R. idaeus*). We also observed *B. clandestina* shared the twig of *R. idaeus* with *C. rubi* in TNS-F-81486 (Fig. 1B).

Brunnipila clandestina, the type species of *Brunnipila* Baral, is widely distributed corresponding to the distribution of its host plant *Rubus idaeus*. In Japan, two occurrences of *B. clandestina* (TNS-F-50486 and TNS-F-50462, registered as '*Dasyscyphus clandestinus*') collected by Yoshio Otani, were found in GBIF. However, as a result of our re-examination, TNS-F-50486 and 50462 were found to be *Trichopeziza* sp. and *Lachnum pseudocannabinum* (Raitv.) Raitv. respectively. TNS-F-81486 is therefore the first record of *B. clandestina* from Japan.

2. *Brunnipila pseudocannabinina* (Raitv.) Tochihara, Sasagawa & Hosoya *comb. nov.*

[Figs. 3, 4]

Dasyscyphus pseudocannabinus Raitv., Folia Cryptog. Estonica 9: 6. 1977.

Lachnum pseudocannabinum (Raitv.) Raitv., Folia Cryptog. Estonica 17: 3. 1985.

Apothecia gregarious, superficial, cup-shaped, 0.6–1.4 mm in diameter, stipitate, up to 1 mm high, dark brown (Panton 4635PC = C13 M53 Y68 K40), externally covered with capitate hairs. **Disc** concave, white when fresh and dry. **Ectal excipulum** *textura prismatica* to *angularis*, with cells 2–13 × 2–12 µm, hyaline, thick-walled up to 2 µm thick. **Medullary excipulum** *textura intricata* of hyaline hyphae up to 3 µm wide. **Hairs** straight, cylindrical with slightly rounded apices, up to 200 × 4–5 µm, brown (471PC = C5 M70 Y97 K20) with pale brown or hyaline apices, totally granulated, thin-walled, slightly thick-walled in one or two cells at the apices; apex crowned by crystal masses easily detached in squash mounts. **Asci** 57–70 × 5.5–6 µm, 8-spored, cylindrical-clavate; pore blue in Melzer's reagent without 3% KOH pretreatment; croziers present at the base. **Ascospores** 10–15 × 2–3 µm, slightly long fusiform, sometimes irregularly curved, aseptate or rarely one-septate, containing some small lipid bodies. **Paraphyses** straight, lanceolate with acute apices, up to 5 µm wide, septate, exceeding the asci for 25–30 µm.

Specimens examined. HOKKAIDO: TNS-F-50462, Zenibako, Otaru-city on dead herb stems. 1967-VI-1. coll. Y. Otani, previously registered as '*Dasyscyphus clandestinus*'. TNS-F-81252, Engaru-cho on dead stems of *Filipendula camtschatica* 2017-VII-12. coll. Y. Tochihara.

HONSHU: TNS-F-16690 (Culture NBRC 104374) and TNS-F-16691 (Culture NBRC 104382), Kosaka-machi, Akita Pref. on dead stems of *Fallopia*, 2006-V-26. coll. R. Sasagawa. TNS-F-17748 and 17751, Sugayu, Aomori-city, Aomori Pref. 2005-VI-23. coll. T. Hosoya. TNS-F-18049, Lake Towada, Towada-city, Aomori Pref. on dead herb stems. 2006-V-26. coll. T. Hosoya.

Ecology. Saprophytic on dead stems of tall herbaceous plants.

Previously known distribution. Kunashir

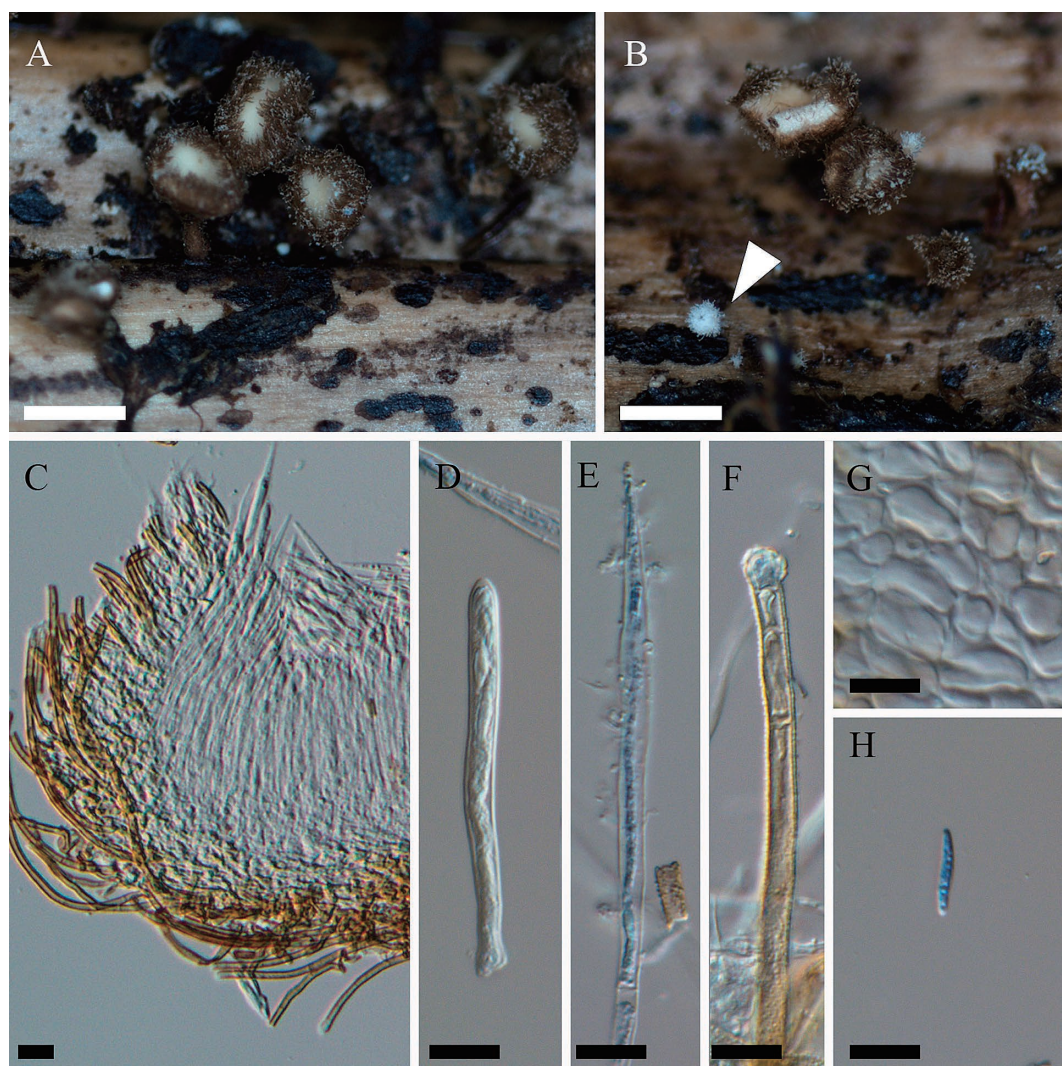


Fig. 3. *Brunnipila pseudocannabinina*. A. Dried apothecia. B. *Brunnipila pseudocannabinina* with *Lachnum nudipes* dried (arrowhead) on the same substrate. C. Vertical section of an apothecium showing the ectal and medullary excipulum. D. Ascus. E. Paraphysis. F. Hair with a crystal cap. G. Ectal excipular cells. H. Ascospore. A–F, H. TNS-F-81252. G, TNS-F-16690. Scales. A, B, 1 mm. C, 20 µm. D–H, 10 µm. D–F, H, mounted in CB/W; C, G, CB/LA.

Island (type locality) ***, Sakhalin**, Kamchatka *** (Raitviir, 1977; 1991).

Notes. TNS-F-16691 was examined in Hosoya *et al.* (2010) and determined as an unidentified lachnaceous species '*Lachnum* sp. 4'. Our re-examination revealed the six specimens of '*Lachnum* sp. 4' were *B. pseudocannabinum*, in agreement with the original description of Raitviir (1977).

Raitviir (1991) included *L. pseudocannabinum*

in section *Brunnipila* (Baral) Raitv., subgenus *Belonidium*, genus *Lachnum* Retz. together with *L. cannabinum* (= currently *B. cannabinum*), *L. clandestinum* (= currently *B. clandestinum*) and *L. fusciculatum* focusing on stipitate apothecia and brown, hairs equipped with crystals. Since molecular phylogenetic analysis (Hosoya *et al.*, 2010) concluded that '*Lachnum* sp. 4' was positioned in the '*Brunnipila* clade', and we propose

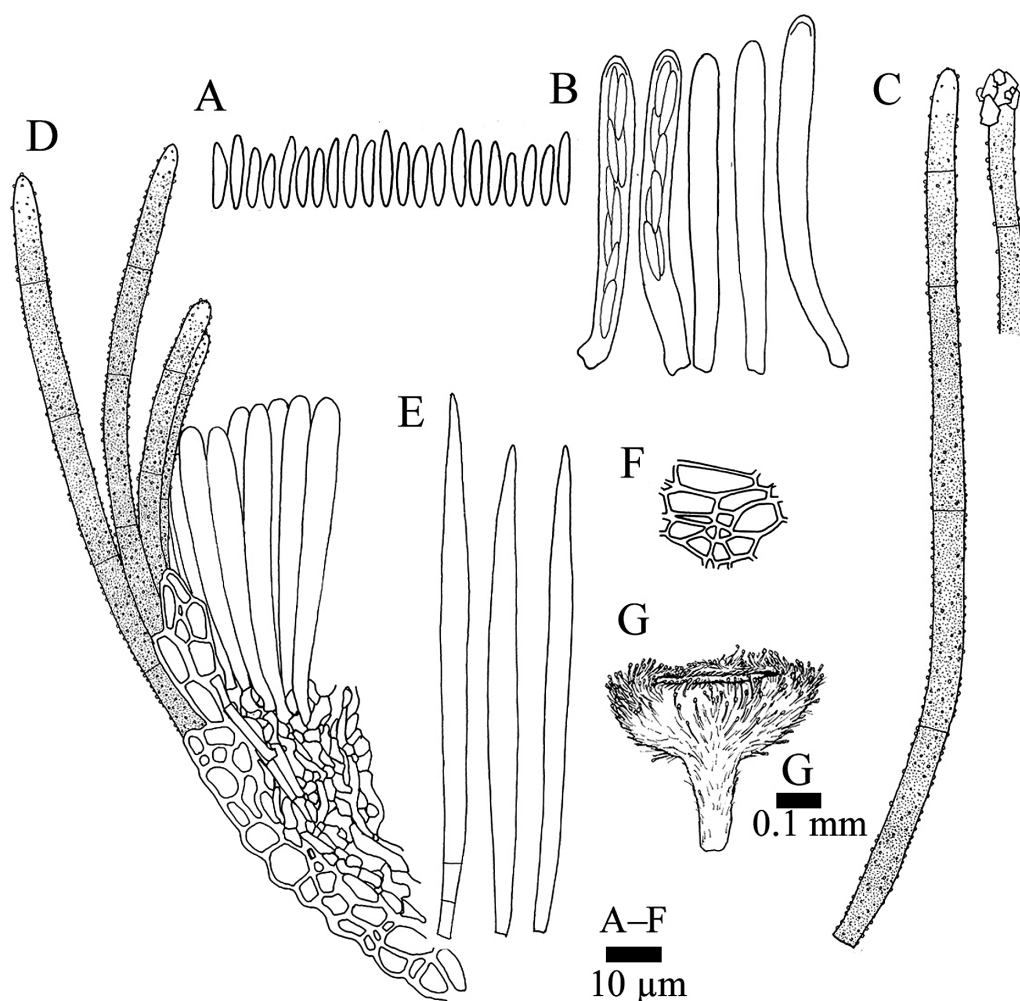


Fig. 4. *Brunnipila pseudocannabina* (TNS-F-16624, 16771). A. Ascospores. B. Asci. C. Hairs with and detached (left) crystals. D. Vertical section through the margin, showing the hairs and ectal excipulum. E. Paraphyses. F. Ectal excipular cells. G. Apothecium. Scales.

to transfer *L. pseudocannabinum* to the genus *Brunnipila*. As we have not examined specimens of *L. fuscidulum*, we hesitate to propose a new combination for *L. fuscidulum*.

Brunnipila pseudocannabina differs from *B. cannabina* (Rehm) Raitv. & Järvi, in having larger asci $>55\mu\text{m}$ (Raitviir, 1991). Raitviir (1979, 1991) pointed out this fungus was endemic to the Far East while *B. cannabina* was widely distributed throughout Russia and Europe. *Brunnipila pseudocannabina* distribution probably includes also the subarctic zone, from north of Central Honshu to Kamchatka.

Brunnipila pseudocannabina usually shares the same substrate with other lachnaceous fungi, such as *Lachnum nudipes* (Fuckel) Nannf. (Fig. 3B), which is found on decaying stems of tall herbs. Species of *Trichopezizella* are similar to *B. pseudocannabina* at first glance, but differ in having sessile to substipitate apothecia and smooth hairs lacking crystals.

For Japan, *B. fuscescens* (Pers.) Baral and *B. palearum* (Desm.) Baral have been included in the genus *Brunnipila* (Katsumoto, 2010). *B. pseudocannabina* transfer is made in this study.

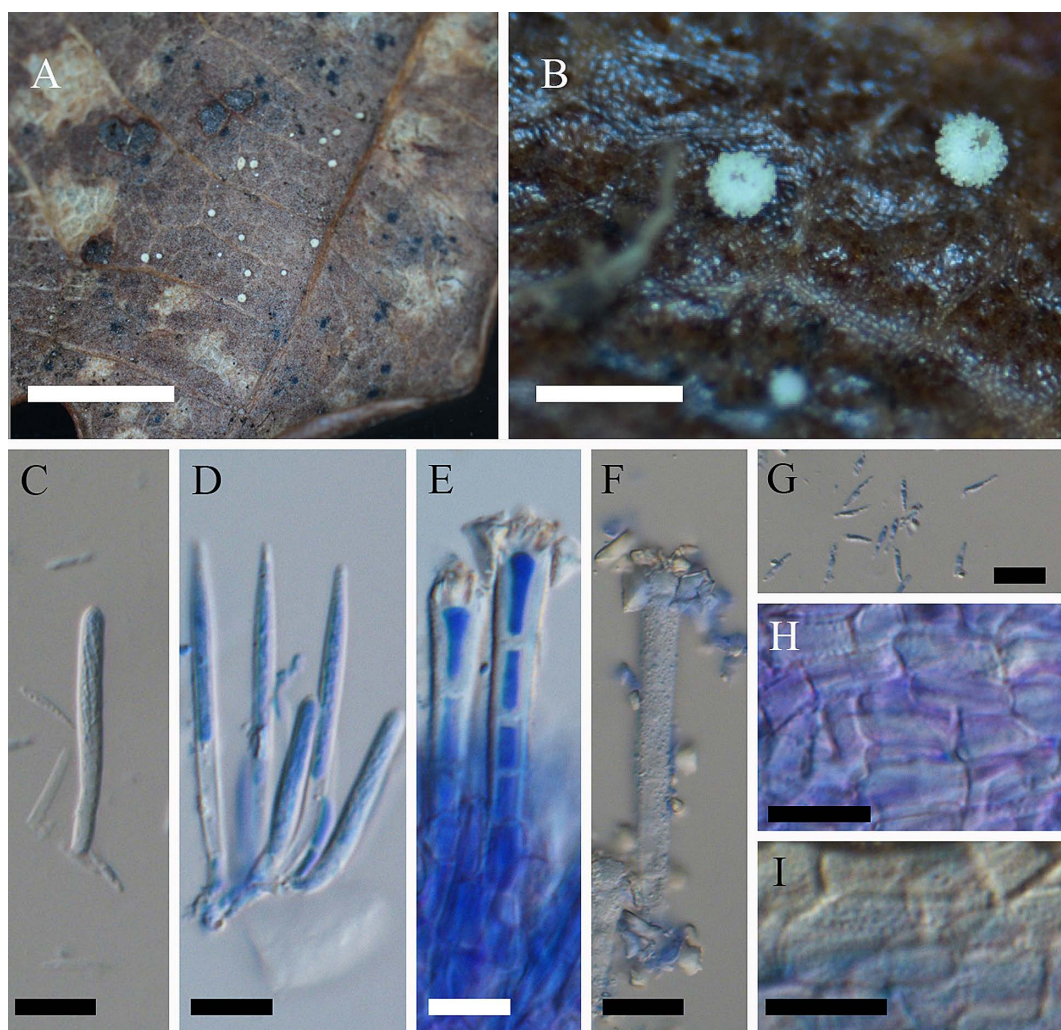


Fig. 5. *Incrucipulum capitatum* (TNS-F-81420). A. Scattered apothecia on *Quercus* leaf. B. Dried apothecia. C. Ascus. D. Paraphyses. E, F. Hairs. G. Ascospores. H. Ectal excipular cells. I. Ectal excipular cells with granulate surface. Scales. A, 1 cm. B, 1 mm. C–I, 10 μ m. C–I, mounted in CB/W.

3. *Incrucipulum capitatum* (Peck) Baral, Beih.
Z. Mykol. 6: 72. 1985.

[Figs. 5, 6]

Mycobank. MB104378

Peziza capitata Peck, Ann. Rep. N.Y. St. Mus. nat. Hist. 30: 60. 1878. (1877).

Trichopeziza capitata (Peck) Sacc., Syll. fung. (Abellini) 8: 417. 1889.

Dasyscyphus capitatus (Peck) Le Gal, Revue Mycol., Paris 4: 29. 1939.

Lachnella capitata (Peck) Seaver, North

American Cup-fungi (Inoperculates) (New York): 254. 1951.

Lachnum capitatum (Peck) Svrček, Česká Mykol. 39 (4): 214. 1985.

Atractobolus scintillans (Masse) Kuntze, Revis. gen. pl. (Leipzig) 3 (2): 446. 1898.

Dasyscyphus scintillans Massee [as '*Dasyscypha*'], Brit. Fung.-Fl. (London) 4: 328. 1895.

Apothecia scattered, superficial, very minute, at first spherical and later urceolate, 0.1–0.2 mm in diameter, subsessile to sessile, up to 0.2 mm high,

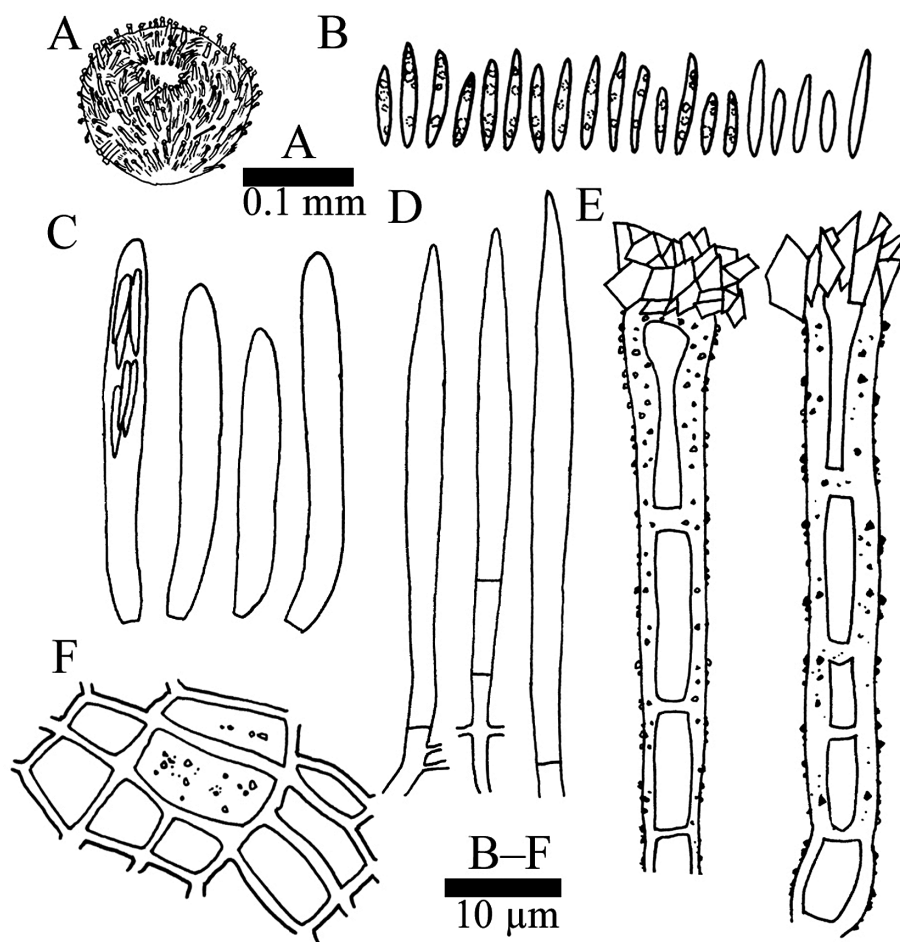


Fig. 6. *Incrucipulum capitatum* (TNS-F-81420). A. Apothecium. B. Ascospores. C. Asci. D. Paraphyses. E. Thick-walled hairs with crystals. F. Ectal exipular cells with granulate surface.

externally covered with short, white, stiff, capitate hairs. **Disc** concave, almost enclosed by an incurving margin when fresh and dry. **Ectal excipulum** *textura prismatica* composed of cubic cells like stone pavings with granulated surface, $3\text{--}10 \times 5\text{--}13\ \mu\text{m}$, hyaline, thick-walled up to $2\ \mu\text{m}$ wide; cells arranged in parallel rows. **Medullary excipulum** *textura intricata* of hyaline hyphae up to $2\ \mu\text{m}$ wide. **Hairs** straight, cylindrical with rounded apices, $55\text{--}80 \times 5.5\text{--}7.5\ \mu\text{m}$, 2–3-septate, hyaline, totally granulate, thin to slightly thick-walled towards the base, very thick-walled up to $3\ \mu\text{m}$ wide in one or two cells at the apices; apex crowned by crystal masses easily detached in squash mounts. **Asci**

$26.5\text{--}35 \times 2\text{--}3.8\ \mu\text{m}$, 8-spored, cylindrical-clavate; pore blue in Melzer's reagent without 3% KOH pretreatment; croziers absent at the basal septa. **Ascospores** $4.5\text{--}10 \times 1\text{--}1.2\ \mu\text{m}$, slightly long fusiform, aseptate, containing some small lipid bodies. **Paraphyses** straight, lanceolate, up to $4\text{--}(5)\ \mu\text{m}$ wide, aseptate, exceeding the asci by $20\ \mu\text{m}$.

Specimen examined. HOKKAIDO: TNS-F-81420, Jozankei, Sapporo-city on dead leaves of *Quercus crispula*. 2018-VI-17. coll. T. Hosoya and Y. Tochihara.

Ecology. Saprophytic on dead leaves of *Quercus*.

Previously known distribution. USA***,

Venezuela*, France*, Belgium*, Germany*, Estonia***, UK***, Netherlands*, Lithuania*, Armenia* (Dennis, 1949; Peck, 1878; Raitviir 1969; Seaver, 1951).

Notes. *Incrucipulum capitatum* is characterized by having lanceolate paraphyses, very-thick-walled and short hairs, and occurring on *Quercus* leaves. Morphology and the host plant correspond descriptions by Peck (1878), Dennis (1949), Seaver (1951) and Raitviir (1969). *Incrucipulum capitatum* is newly documented from Asia (including the Far East) in this study. In Japan, *Incrucipulum ciliare* (Schr.) Baral is present covering a wide area on *Quercus* leaves, while *I. capitatum* is rare. Since the host *Quercus* species are distributed in temperate zones, *I. capitatum* should be widely distributed in the temperate zones.

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