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Enumeration of Remarkable Japanese Discomycetes (6): Notes on Two Inoperculate Discomycetes new to Japan and One Operculate Discomycete

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Abstract Three remarkable discomycetes (two inoperculate and one operculate) are described and illustrated: *Hymenoscyphus immutabilis* (Helotiaceae, Helotiales), *Lachnum rachidicola* (Lachnaceae, Helotiales) and *Sphaerosporella brunnea* (Pyronemataceae, Pezizales). The first two species are documented for the first time in Japan. Detailed microscopic description is provided for *Sphaerosporella brunnea* for the first time for Japanese material.

Key words: *Hymenoscyphus immutabilis*, *Lachnum rachidicola,* mycobiota*, Sphaerosporella brunnea,* taxonomy.

Introduction

This is the sixth part of the series on remarkable Japanese discomycetes following Hosoya *et al.* (2011) to contribute the knowledge of mycobiota in Japan. Two inoperculate discomycetes with minute apothecia are documented for the first time from Japan. Microscopic examination for the confirmation on identification is carried out for *Sphaerosporella brunnea* and description is provided.

Materials and Methods

Collection and observation procedures followed Hosoya and Otani (1997) and Hosoya (2004). Color codes followed the Pantone color code adopting RGB system referring to a Pantone color bridge (Anonymous, 2005). For previously known distribution, the database of Global Biodiversity Information Facility (GBIF, http://data.gbif.org/welcome.htm) was searched, and countries 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 (***). To obtain sequence of *L. rachidicola*, cultivation, DNA extraction, PCR and sequence procedure followed Hosoya *et al.* (2010). The obtained extracted DNA samples are deposited in the Center for Molecular Biodiversity Research, National Museum of Nature and Science, and available for collaborative research.

Descriptions

1. ***Hymenoscyphus immutabilis*** (Fuckel) Dennis, Persoonia 3: 76. 1964.

[Figs. 1, 2]

Basionym: *Helotium immutabilis* Fuckel, Jb.

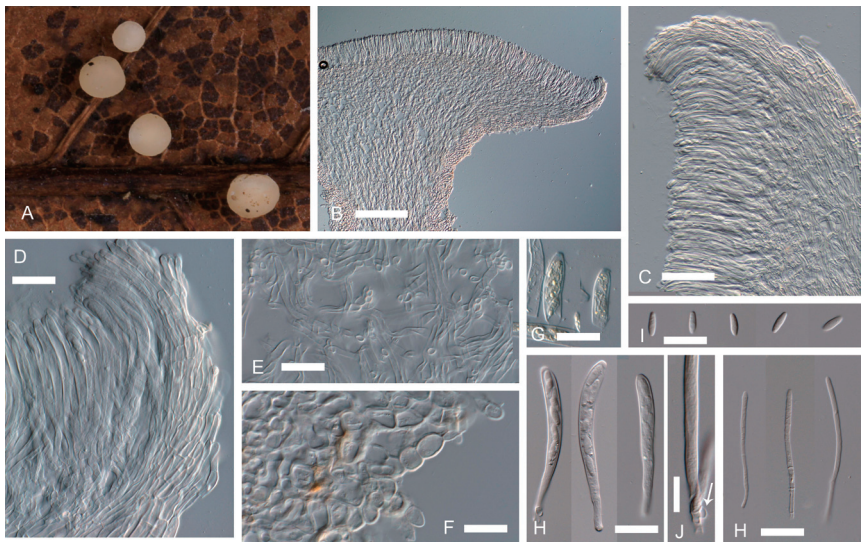

Fig. 1. *Hymenoscyphus immutabilis* (TNS-F-44242). A. Fresh apothecia. B. Section of an apothecium showing the ectal and medullary excipulum. C. Close up of the hymenium and the margin slightly elavated. D. Close up of ectal excipulum at the margin, showing textura prismatica tissue type. E. Close up of medullary excipulum. F. Close up of ectal excipulum near the stipe. Note the ticker-walled, globose cells. G. Ascal apex showing MLZ+ reaction in Melzer's reagent. H. Asci. I. Ascopores. J. Crozier at the base of ascus (arrow). K. Fragments of paraphyses showing the upper part. B–F, I–H, mounted in lactic acid. G, mounted in Melzer's reagent. Scales. B, 200 μm; C, 100 mm; D–I, H, 20 μm; J. 10 μm.

Nassau. Ver. Naturk. 25–26: 50. 1871.
See Lizoň (1992) for synonyms.

Apothecia scattered, occurring on decaying leaves, especially on the veins, short-stipitate to subsessile; disc flat to slightly convex, 0.6–1.5 mm in diameter when dry; hymenium white when fresh, still white or becoming dark yellow to pale brown (Pantone 721PC = C0 M31 Y43 K2) in drying; receptacle smooth, white; stipe concolorous with the receptacle, 0.2–0.6 mm long when dry, with smooth surface. **Ectal excipulum** of textura prismatica to textura globulosa, composed of subhyaline to pale-brown, thin-walled, prismatic cells of 4.0–11.0 × 3.0–7.0 μm, with slightly refractive, 7.0–11.0 μm, globulose cells mixed in the receptacle and stipe. **Medullary excipulum** of textura intricata, composed of hyaline, smooth, loosely interwoven hyphae of 2.0–5.0 μm wide. **Asci** 67.5–77.5 × 5.0–7.0 μm, clavate, 8-spored, croziers obscure and observed only for immature asci; apex rounded, slightly thickened, pore slightly blue in Melzer's reagent even with KOH pretreatment. **Ascospores** 9.0–13.0 × 3.5–4.0 (11.0 ± 1.2 × 3.9 ± 0.2, n = 20) μm, uniseriate or irregularly biseriate, fusoid to subellipsoid, non-septate, eguttulate. **Paraphyses** filiform, septate, hyaline, simple or branched near the base, occasionally slightly expanded at the apex of 2.0–3.0 μm wide.

Speciemens examined. HONSHU: TNS-F-16614, Mizunokizawa, Yozuku, Yamakita-cho, Ashigarakami-gun, Kanagawa Pref., 3-VII-2005. col. R. Sasagawa (culture FC-2176); TNS-F-16628, Oaza-Hara, Kikuchi-shi, Kumamoto Pref., 10-X-2005. col. R. Sasagawa; TNS-F-44242, Iryuda, Odawara, Kanagawa Pref. (57 m alt., 35°14′30.02″N, 139°7′11.87″E), 12-XI-2011. col. T. Hosoya (culture FC-2837); TNS-F-37001, Fukiage gyoen, Kokyo, Chiyoda-ku, Tokyo (23 m alt., 35°41′9.73″N, 139°45′3.79″E), 5-X-2010. col. T. Hosoya; TNS-F-44253, Fukiage gyoen, Kokyo, Chiyoda-ku, Tokyo (13 m alt., 35°41′9.64″N, 139°45′3.13″E), 21-XI-2011. col.

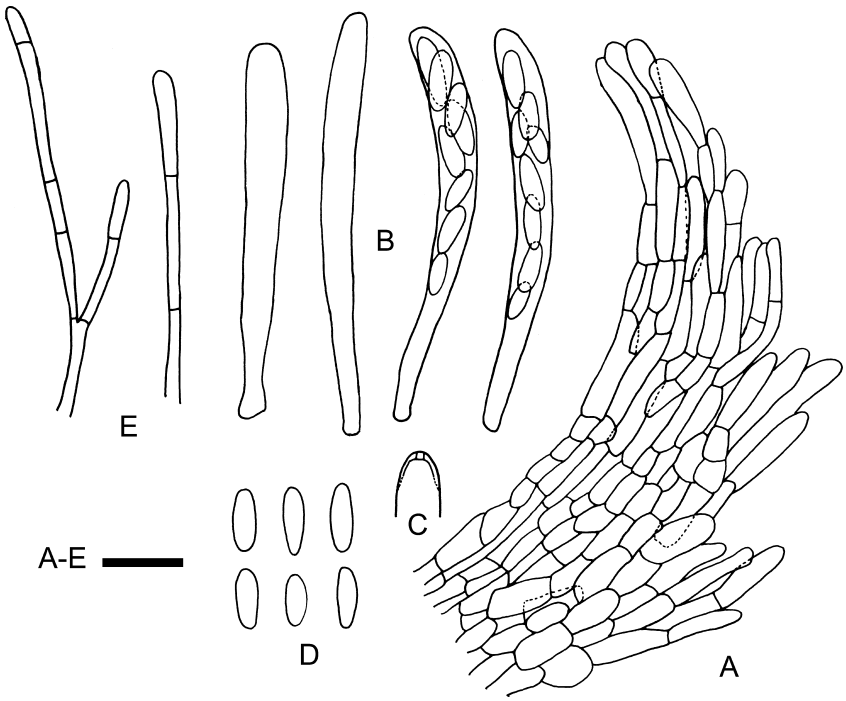

Fig. 2. Camera lucida illustration of *Hymenoscyphus immutabilis* (TNS-F-44242). A. Vertical section of an apothecium through the margin showing the ectal excipulum of textura prismatica. B. Asci. Two at the right showing the ascospores. C. Ascal apex showing the reaction in Melzer's reagent. D. Ascospores. E. Paraphyses. Line drawings prepared in lactic acid mount except for C in Melzer's reagent. Scales. A–E, 10 μm.

T. Hosoya (culture FC-2840). All the specimens occurring on *Zelkova serrata* leaf.

Previously known distribution. Denmark*, Germany*, North America***, Spain*, Sweden*, UK*** (White, 1943; Dennis, 1956; Seaver, 1961).

Japanese name: Ochiba-shiro-byoutake

Notes. The genus *Hymenoscyphus* Gray belongs to the family Helotiaceae, order Helotiales with 155 species commonly accepted (Kirk *et al.*, 2008). The generic characteristics are: sessile to stipitate, light colored, white to yellow apothecia, ectal excipulum of textura porrecta to prismatica (rarely textura angularis to textura globulosa), medullary excipulum of textura intricata, cylindric-clavate, 8-spored, asci with rounded or conical apex; ellipsoid to fusoid or turbinate, hyaline ascospores with no septa or 1–2-septa at maturity (Dennis, 1956, 1981; Dumont, 1981; Lizoň, 1992). Nine species have been known from Japan (Otani, 1966, 1987; Otani *et al.*, 1991; Tubaki, 1966; Katumoto, 2010).

Hymenoscyphus immutabilis is widely distributed in Europe, mainly occuring on fallen leaves of broad-leaved trees, such as *Alnus, Betula, Carpinus, Fagus, Populus, Quercus, Robinia, Salix* and *Ulmus* (White, 1943; Dennis, 1956; Lizoň, 1992). *Zelkova serrata* is a new host to this species. The specimens examined in the present study shared most morphological characteristics with previous descriptions (White, 1943; Dennis, 1956; Lizoň, 1992), except for smaller asci (cf. 80.0–105.0 × 8.0–10.0 μm in European and North American specimens) (White, 1943; Dennis, 1956; Dumont, 1981; Lizoň, 1992) which may have been caused by the different mounting fluid. As a previous record from Japan, a specimen (CUP-JA 201, conserved in the Herbarium of Cornell University) collected from Kyoto in 1957 is known (Lizoň, 1902). However,

Lizoň (1992) indicated CUP-JA 201 had ellipsoid to ovoid, small sized ascospores (7.2–8.4 × 4.0 μm). CUP-JA 201 also seems to be different from the specimens examined for the present study, and may represent other taxa close to *H. immutabilis*. So the present paper is the first documentation of *H. immutabilis* in Japan.

The distinguishing characteristics of *H. immutabilis* are the fusoid to ellipsoid ascospores and the globose cells mixed in the receptacle and stipe section. The most closely related species is *Hymenoscyphus epiphyllus* (Pers.) Rehm ex Kauffman (White, 1943; Dumont, 1981). Both species can be found on fallen leaves of broad-leaved trees and share common hosts, and both with the globose cells mixed in ectal excipulum (White, 1943; Dennis, 1956; Dumont, 1981). However, the hymenium color of *H. epiphyllus* is bright yellow to orange both in fresh and dry, while *H. immutabilis* is white when fresh and becoming dark colored when dry. *Hymenoscyphus epiphyllus* have somewhat oblong-fusoid and larger (15.0–23.0 × 3.0–5.0 μm) ascospores. The present fungus is classified in Helotiaceae, Helotiales.

2. *Lachnum rachdicola* J. G. Han, Raitv. & H. D. Shin, Mycotaxon 107: 456. 2009.

[Figs. 3, 4]

Basionym: *Lachnum rachidicola* J. G. Han, Raitv. & H. D. Shin, Mycotaxon 107: 456. 2009.

No other synonyms.

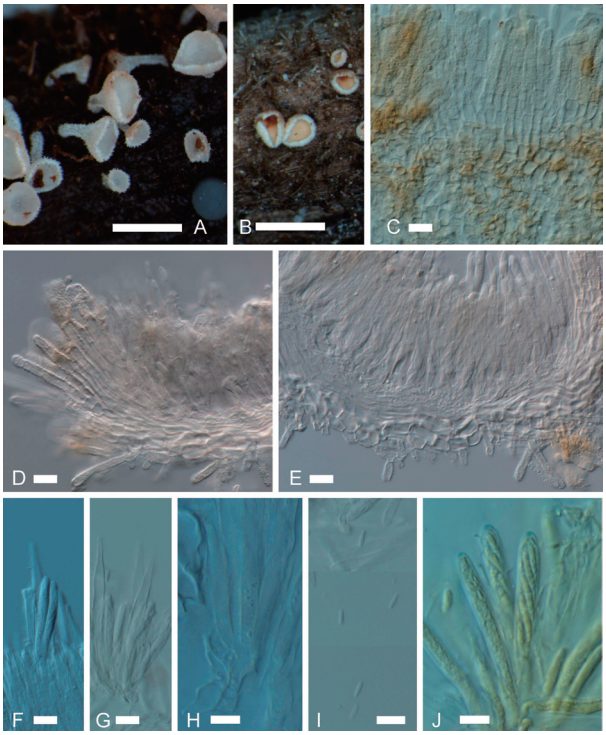

Fig. 3. *Lachnum rachidicola* (A. TNS-F-35017; B–I. TNS-F-41104). A. Fresh apothecia. Note reddish part in wounded part. B. Dried apothecia. Note more strongly reddish part in the apothecia. C. Hairs and ectal excipular cells in external view. D. Vertical section through the margin showing the hairs and ectal excipulum. E. Vertical section through the middle receptacle showing short hair-like protrusions at the bottom. F. Asci. G. Paraphyses exceeding the asci. H. Ascal base showing the simple septa. I. Ascospores. J. Ascal apex stained blue in Melzer's reagent. Scales A, B, 1 μm; C–J = 10 μm. C–I, mounted in lactic acid, J, mounted in Melzer's reagent.

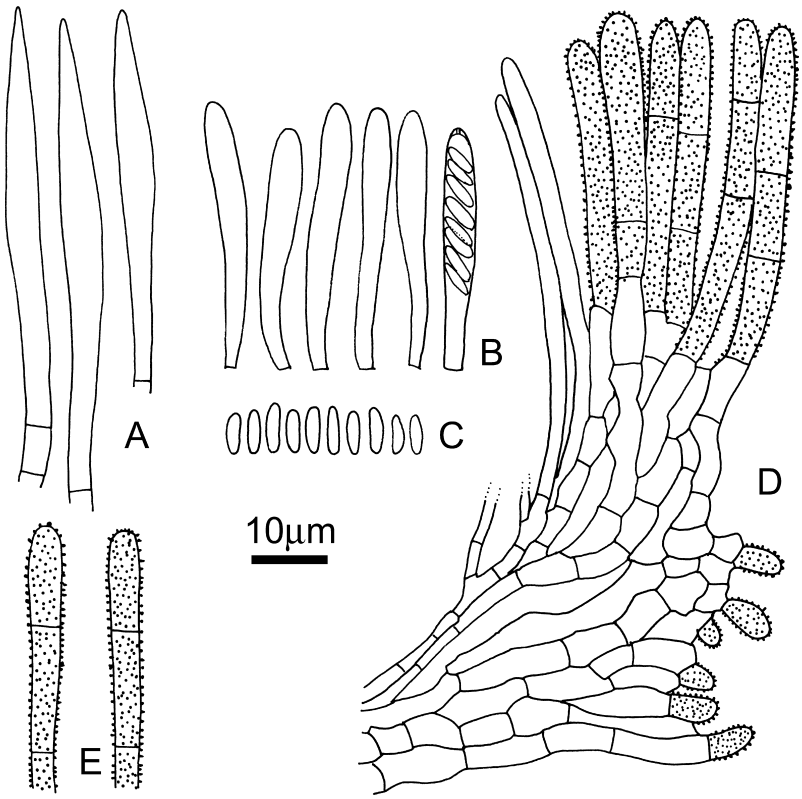

Fig. 4. Camera lucida illustration of *Lachnum rachidicola* (TNS-F-41104). A. Paraphyses. B. Asci. One at the right contains the ascospores, showing the MLZ + apex. C. Ascospores. D. Vertical section through the margin, showing the hairs, ectal excipulum, external protrusions with granulations. E. Hairs. Scales. 10 μm.

Apothecia gregarious, cupulate to shallow-cupulate, pure white when fresh, slightly reddening if wounded, pale brown (171PC = C0 M61 Y70 K0) when dry with partially prominent reddish tint; disc 0.5–2 mm in diameter, white to pale cream-colored when fresh, yellow to reddish brown (Pantone 113PC = C0 M4 Y71 K0 to 1655PC = C0 M68 Y90 K0) when dry. **Hairs** cylindrical with slightly clavate apex, hyaline, 2–3(-4)-septate, thin-walled, granulate, with no crystals, 35–55 × 4 μm, occasionally apically swollen up to 5 μm wide. **Ectal excipulum** textura prismatica composed of thin-walled cells, 8–15 × 3–5 μm. **Asci** 34–38 × 4–5 μm, cylindrical clavate, arising from simple septa; apical pore blue in MLZ. **Ascospores** 6–7 × 1.5–2 μm, elliptic to cylindrical clavate, straight to slightly curved, hyaline, aseptate, rarely containing bipolar guttules. **Paraphyses** narrowly lanceolate to lanceolate, exceeding the asci by 5–15 μm, up to 6 μm at the widest point.

Specimens examined. HONSHU: TNS-F-16645 (culture FC-2241), TNS-F-16647 (culture FC-2242), TNS-F-16648 (culture FC-2276), and TNS-F-16649 (culture FC-2238), Hananomaki-shi, Iwate Pref. collected on 23-V-2006. col. R. Sasagawa. TNS-F-16801 (culture FC-2333), TNS-F-16830 (culture FC-2348), TNS-F-16832 (culture FC-2343), TNS-F-35017 (culture FC-2592), and TNS-F-41104 (culture FC-2742), Tsukuba University Sugadaira Montane Research Center, Ueda-shi, Nagano Pref. (1340 m alt., 36°31′29.4″N, 138°20′57″E) collected on 7-VI-2007, 10-VI-2007, 10-VI-2007, 31-V-2010, 1-VII-2011, respectively. col. T. Hosoya. All the specimens were collected on pinnately com-

pound leaves of *Juglans mandshurica*.

Previously known distribution. Korea** (Han *et al*., 2009).

Japanese name: Kurumi-shirohinano-chawan-take

Notes. *Lachum radicicola* was described recently (Han *et al*., 2009). It is characterized by it host, absence of crosiers and reddening apothecia. The seven sequences obtained from FC-2241, 2242, 2276, 2238, 2333, 2348, 2343, and two

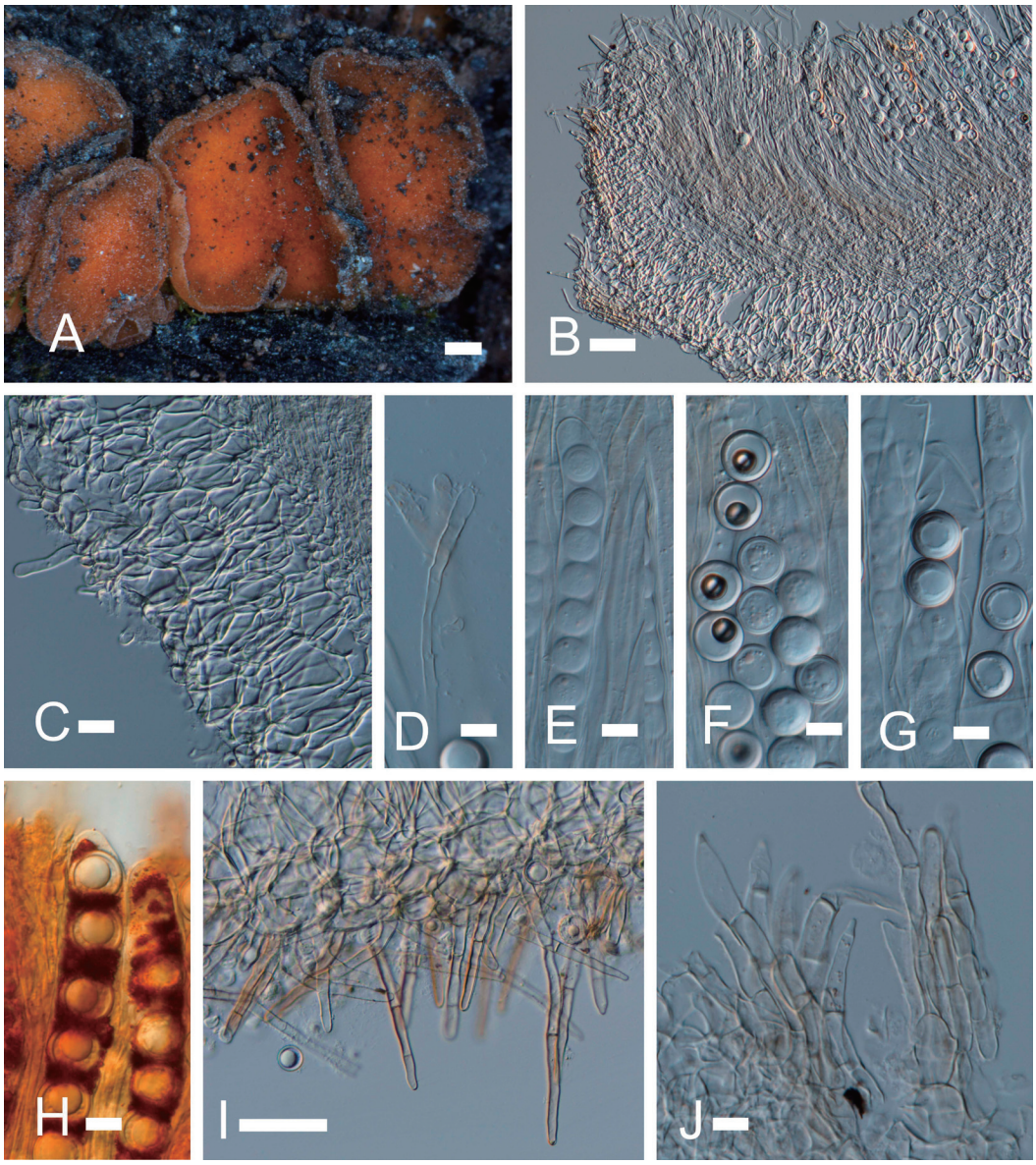

Fig. 5. *Sphaerosporella brunnea* (TNS-F-46870). A. Fresh apothecia occurring on burnt ground. B. Section of an apothecium showing the ectal and medullary excipulum. C. Close up of ectal excipulum at the margin. D. Paraphyses. E. Upper portion of asci. F–G. Ascospores. Note thick wall and air bubble and oil drops contained in the ascospore. H. Ascus observed in Melzer's reagent. Note ascal content stained brown in Melzer's reagent. I. Hairs at the bottom of ectal excipulum. J. Hairs near the margin. Note acicular, solidified apices. Scales. A. 1 μm. B, 20 μm; C, 100 μm; D–J, 10 μm. B–G, I–J, mounted in lactic acid, H, mounted in Melzer's reagent.

sequences from Korea (JGH52152, and JGH52679, provided by one of the author (JGH)) shared high similarity (＞99%), and supported our identification. The specific concept seemed to be well-defined. The present fungus is classified in Lachnaceae, Helotiales.

3. *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička, Česká Mykol. 15: 65. 1961.

[Fig. 5]

Basionym: *Peziza brunnea* Alb. & Schwein., Consp. fung. (Leipzig). p. 317. 1805.

See Rifai (1968) for synonyms.

Apothecia gregarious, sessile, saucer shaped or shallow cup shaped, externally pubescent to spiny due to hairs, 3–6 mm in diameter when fresh; disc concave, reddish brown (173PC = C0 M80 Y94 K1) or paler when fresh, becoming darker colored. **Hairs** at the margin or upper ectal excipulum acicular, thin walled, 2–3 celled, with pointed, frequently solidified (thick-walled) apex, 37–63 μm in length, 5–10 μm at the base; hairs on middle or lower ectal excipulum becoming longer, cylindrical with rounded apex, 62–75 μm in length, 5–7.5 μm at the base. **Ectal excipulum** textura angularis, composed of thin-walled, elongate cells of 15–30 × 32–50 μm, arranged with their long axis almost perpendicular to the surface. **Medullary excipulum** textura intricata, composed of hyphae of 5–10 μm wide. **Asci** ca 220 × 15–20 μm, cylindrical, thin-walled, MLZ- with or without 3% KOH pretreatment, arising from crosiers; when observed in MLZ, the contents of asci stained brown. **Ascospores** (14–)15(–16.5) (15 ± 0.42 in average ± SD, n = 20) μm in diameter, globose, one-celled, moderately thick (1 μm thick) and smooth-walled, often containing a bubble when observed in lactic acid, uniseriate in the asci. **Paraphyses** cylindrical, slender, septate, simple, filled with brownish content stained brown by MLZ, 2.5–3 μm below, up to 5 μm at the apex.

Specimens examined. HONSHU: TNS-F-46870, Ogashiwa, Moriya-shi, Ibaraki Pref. on burnt ground. 2012-VI-19. col. K. Imamura.

Previously known distribution. Australia**, Austria*, Canada*, Denmark*, Finland*, Germany*, Ireland*, Norway*, Poland*, Spain*, Sweden*, USA*, UK* (Rifai, 1968).

Japanese name: Yakeato-marumi-chawantake

Notes. Rifai (1968) provided a detailed line-drawings for the microscopic structure, so we do not provide line-drawings here. He also mentioned the possible anamorph (*Botrytis*-like) obtained by other researchers. However, obtaining a culture was not tried. We could not find any *Botrytis*-like structure on the substrate, either.

Although *S. brunnea* is previously known in Japan (Otani, 1989; Imazeki *et al.*, 2011.), no description with microscopic features were provided before for Japanese material. The authors therefore confirmed its identification based on microscopic characteristics, and here provide the description for Japanese material. The present fungus is classified in Pyronemataceae, Pezizales.

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References

Anonymous. 2005. Pantone color bridge/coated. Pantone Inc, New Jersey.

Dennis, R. W. G. 1956. A revision of the British Helotiaceae in the Herbarium of the Royal Botanic Gardens, Kew, with notes on related European species. Mycological Papers 62: 1–216.

Dennis, R. W. G. 1981. British Ascomycetes. Revised edition. J. Cramer, Vaduz.

Dumont, K. P. 1981. Leotiaceae III. Notes on selected temperate species referred to *Helotium* and *Hymenoscyphus*. Mycotaxon 13: 59–84.

Han, J-G., Raitviir A., Shin, H-D. 2009. Three new species of *Lachnum* (Hyaloscyphaceae, Helotiales) from Korea. Mycotaxon 107: 455–461.

Hosoya, T. 2004. Enumeration of remarkable Japanese discomycetes (1): Three helotialean members new to

Japan. Bulletin of the National Science Museum, Series B 30: 155–163.

Hosoya, T. and Otani, Y. 1997. Hyaloscyphaceae in Japan (1): Non-glassy haired members of the tribe Hyaloscypheae. Mycoscience 38: 171–186.

Hosoya, T., Han, J-G., Saito, Y., Zhao, Y-J., Kakishima M. and Shin, H-D. 2011. Enumeration of remarkable Japanese discomycetes (5): First records of one operculate and two inoperculate discomycetes in Japan. Bulletin of the National Science Museum, Series B 37: 147–154.

Hosoya, T., Sasagawa, R., Hosaka, K., Hirayama, Y., Yamaguchi, K., Toyama, K. and Kakishima, M. 2010. Molecular phylogenetic studies of *Lachnum* and its allies based on the Japanese material. Mycoscience 51: 170–181.

Imazeki, R., Otani, Y. and Hongo, T. 2011. Fungi of Japan. Revisied and enlarged edition (in Japanese), Yama-kei Publishers Co., Tokyo.

Katumoto, K. 2010. List of fungi recorded in Japan. The Kanto Branch of the Mycological Society of Japan, Tokyo.

Kirk, P. M., Cannon, P. F., David, J. C. and Stalpers, J. A. 2008. Anisworth & Bisby's Dictionary of the Fungi, 9th ed. International Mycological Institute, Egham, Surrey.

Lizoň, P. 1992. The genus *Hymenoscyphus* (Helotiales) in Slovakia, Czechoslovakia. Mycotaxon 45: 1–59.

Otani, Y. 1966. Miscellaneous notes on Helotiales in Hokkaido I. Transactions of the Mycological Society of Japan 7: 174–180.

Otani, Y. 1987. Discomycetes In: Kobayasi, Y., Otani, Y. and Hagiwara, H. (eds.), Fungal enumeration by Kumagusu Minakata Vol. 1. pp. 27–74. Sanko Insatsu Publishing, Tokyo (in Japanese with English description).

Otani, Y. 1989. List of discomycete fungi recorded from Japan. Science Report of the Yokosuka City Museum 37: 61–81.

Otani, Y., Hosoya, T. and Furuya, K. 1991. Miscellaneous notes on Japanese discomycetes (II). Transactions of the Mycological Society of Japan 32: 315–322.

Rifai, M. A. 1968. The Australasian Pezizales in the herbarium of the Royal Btotanic Gardens Kew. Verhandelingen der Koninklijke Nederlandsche Akademie van Wettenschappen II 57: 1–295.

Seaver, F. J. 1961. The north American cup-fungi (Inoperculates). Hafner Publishing Co., Inc., New York.

Tubaki K. 1966. An undescribed species of *Hymenoscyphus*, a perfect stage of *Varicosporium*. Transactions of the British Mycological Society 49: 345–349.

White, W. L. 1943. Studies in the genus *Helotium*, III. History and diagnosis of certain European and North American foliicolous species. Farlowia 1: 135–170.