

Enumeration of Remarkable Japanese Discomycetes (7): Notes on One Operculate Discomycete and One Inoperculate Discomycete

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Abstract Two remarkable discomycetes (one operculate and one inoperculate) are described and illustrated: *Plicaria trachycarpa* (Pezizaceae, Pezizales), and *Lachnum hainanense* (Lachnaceae, Helotiales). Detailed description is given for the first time in Japan for *P. trachycarpa* based on wide range of collection in Japan preserved in the herbarium. The latter is a new record to Japan.

Key words: *Lachnum hainanense*, mycobiota, *Plicaria trachycarpa*, taxonomy.

Introduction

This is the seventh part of the series on remarkable Japanese discomycetes following Hosoya *et al.* (2012) to extend the knowledge of the Japanese mycobiota. One operculate discomycete not reported since its first record and one inoperculate discomycete recorded for the first time in Japan with minute apothecia are documented.

Materials and Methods

Observation procedures followed Hosoya and Otani (1997) and Hosoya (2004). 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://data.gbif.org/welcome.htm>) was searched, and countries with occurrence of the given species are shown with an asterisk (*). Distributions known

only from the literature are shown with double asterisks (**). Distribution records from both sources are shown with triple asterisks (***). DNA samples were obtained from fruiting bodies of *Plicaria trachycarpa*. DNA extraction, PCR and sequence procedure followed Hosoya *et al.* (2010). Briefly, a small piece of the apothecia was excised and kept in preservation buffer (20% DMSO, 250 mM EDTA, 100 mM Tris (pH 8.0), 0.1 M Na₂SO₃, NaCl to saturation; Hosaka and Castellano, 2008). DNA was extracted using Plant Mini Kit (Qiagen, Mississauga, ON, Canada) following the manufacturer's instruction. Sequence amplification was performed for large subunit of ribosomal RNA using the primer set LR0R and LR5. PCR and sequencing procedures followed Hosoya *et al.* (2010). Amplification of the second largest subunit of RNA polymerase II (RPB2) was obtained using the primer set RPB2-P6f and RPB2-7R. DNA vouchers are deposited in the Center for Molecular Biodiversity Research, National Museum of Nature and Science, and available for collaborative research.

Descriptions

1. *Plicaria trachycarpa* (Curr.) Boud., Hist. Class. Discom. Eur. p. 50. 1907.

[Figs. 1, 2]

Curreyella trachycarpa (Curr.) Masee, Brit. Fung.-Fl. 4: 401. 1895.

Detonia trachycarpa (Curr.) Sacc., Syll. fung. 8: 105. 1889.

Galactinia trachycarpa (Curr.) Le Gal, Bull. Trimest. Soc. Mycol. Fr. 78: 212. 1962.

Peziza trachycarpa Curr., Trans. Linn. Soc. London 24: 493. 1864.

Plicariella trachycarpa (Curr.) Velen., Monogr. Discom. Bohem. p. 342. 1934.

Apothecia scattered to gregarious; attached to the substrate at the center, at first cupulate with well defined margin, becoming flat to cupulate with irregularly waving margin, undulate to plicate; disc 0.5 mm up to 3 cm in diameter with indistinct margin, pale brown (C30M60Y80K0) to dark brown (C80M100Y100K0), paler from the reverse when fresh, very brittle, drying black (C0M0Y0B90) and hard. **Ectal excipulum** textura globulosa to textura angularis, composed of thin-walled cells of $8\text{--}20 \times 8\text{--}25 \mu\text{m}$, mixed with interwoven hyphae with variously inflated intercalary cells. **Asci** c. $210\text{--}245 \times 12.5\text{--}15\text{--}(17.5) \mu\text{m}$, 8-spored, cylindrical for upper 1/3 where occupied by the ascospores, gradually tapered to the base, thin-walled; apex thin-walled, round; upper part of the asci diffusely stained by MLZ +, but most strongly stained at the apex without KOH pretreatment; base truncate or pedicelate with croziers. **Ascospores** $13\text{--}14 \mu\text{m}$ in diameter, $(13.5 \pm 0.61 \mu\text{m})$ on average $\pm$ SD, $n = 20$) including the warts, globular, thick-walled (1.5–2 mm), ornamented by blunt warts stained in CB/LA, spumous containing small oil globules visible in LA, occasionally containing one De Bary bubble, hyaline when young, becoming pale brown at maturity, stained pale brown in MLZ mount, occupying upper 1/3 portion of the asci; warts coalescent, equal in height. **Paraphyses** cylindrical, straight, simple, multi-septate, $3\text{--}4 \mu\text{m}$ wide

at the middle, even or sometimes enlarged at the clave to globular apex of $5\text{--}6 \mu\text{m}$ wide.

Specimens examined. HOKKAIDO: TNS-F-13851, Lake Shikotsu, VIII-1968. Col. Y. Otani; TNS-F-172098, on *Abies sachalinensis* forest ground, Furano, 21-VIII-1967, col. Y. Otani (specimen damaged by the insects); TNS-F-172099, on forest ground, Yufutsu, 29-VIII-1967, col. Y. Otani (specimen damaged by the insects). HONSHU: TNS-F-24786, on tree fern slab *Cyathea spinulosa* for horticultural use in green house, imported from Indonesia, in Tsukuba Botanical Garden, 1-V-2009. col. T. Hosoya. NEPAL: TNS-F-51432, on soil (probably on a burnt patch), Dunche-Sing Gompa, 18-20-IX-1980, col. Y. Otani.

Known distribution. Denmark*, Germany*, Nepal (reported for the first time based on the specimen in TNS.), North America***, Spain*, Sweden*, UK*** (Rifai, 1968; Seaver, 1961).

Japanese name: Zaratsuki-marumi-no-chawan-take

Notes. Besides morphology, the identity of the newly collected specimen TNS-F-24786 was also confirmed on the basis of the homology of sequence data available from GenBank. The sequences obtained in the present study from TNS-F-24786 (AB828284 for LSU, AB828285 for RPB2) showed 100% and 98% similarity, respectively to the corresponding region of the registered sequences (AY5000554 for LSU from *P. trachycarpa* voucher KH-97-93 (C); compared length = 909 bp, coverage 100%; AY500478 for RPB2 from the same voucher specimen; compared length = 710 bp, coverage 40%). These data supported the identification at the specific level.

Rifai (1968) indicated the possible affinity of *Plicaria* to *Peziza*, but recognized the common morphology of *Plicaria* different from *Peziza* (e.g. paraphyses show a tendency to adhere to each other; at complete maturity their spores become pale brown; spores always contain oil globules; apothecia invariably dark colored). Hansen *et al.* (2001, 2005) discussed the molecular phylogeny of *Plicaria* and noted that the

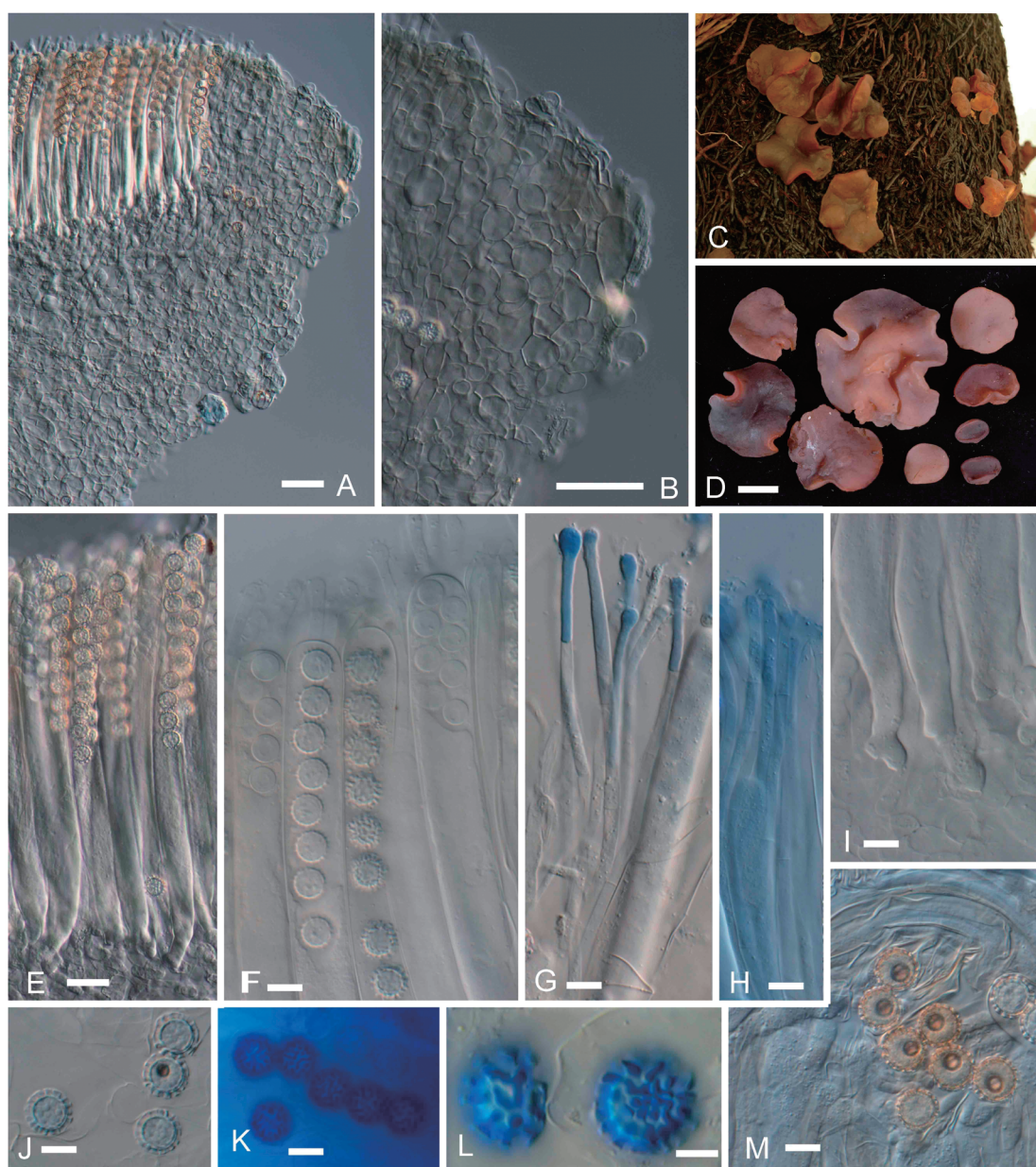


Fig. 1. *Plicaria trachycarpa* (TNS-F-24786). A. Vertical section at the margin showing the ectal excipulum. B. Close up of the ectal excipulum at the margin. C. Apothecia when fresh on wood fern slab made of *Cyathea spinulosa*. D. Close up of apothecia. E. Hymenium. Note brown ascospores. F. Ascospores at various stages of maturation within the asci. G. Paraphyses. Note apical enlargement. H. Paraphyses. I. Close up of the ascial base. J. Ascospores. Note one at the middle right having an air bubble. K. Ascospores in cotton blue in lactophenol (CB/LA). L. Ascospores under high magnifications (CB/LA). Note the warts are strongly stained by CB. M. Matured ascospores with De Bary's bubble. Scales. A–B. 50 μ m. D. 1 cm. E, F. 50 μ m. G–M. 10 μ m. All in cotton blue/lactic acid mount.

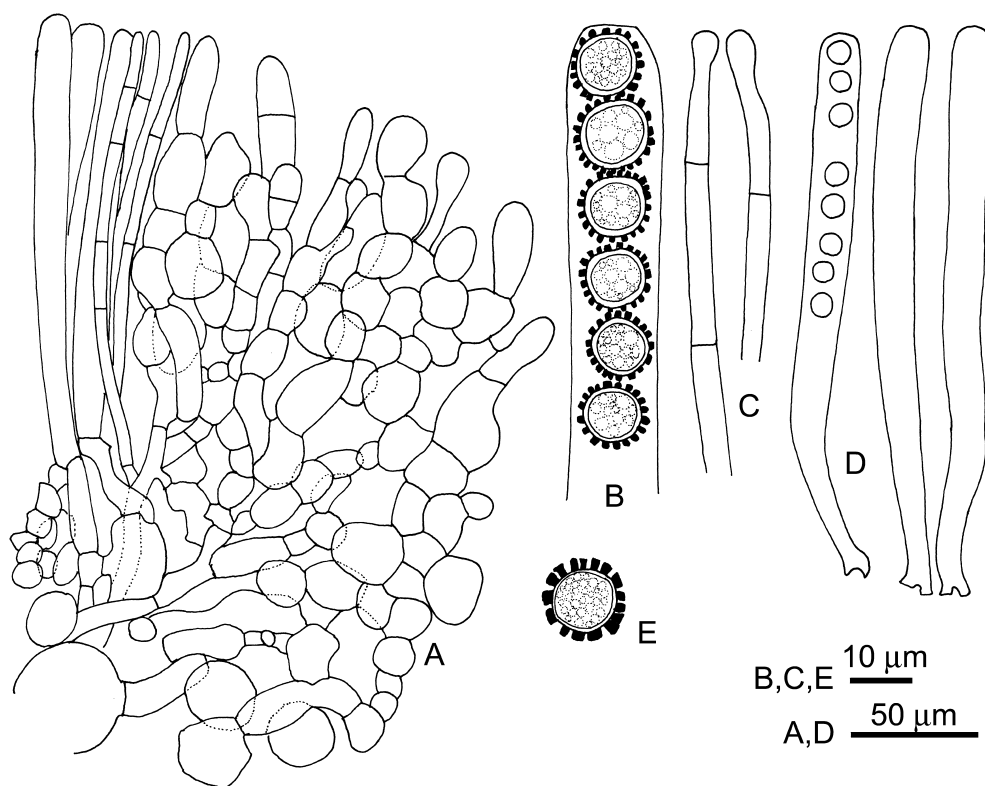


Fig. 2. *Plicaria trachycarpa* (TNS-F-24786). A. Vertical section at the margin. B. Upper part of ascus. C. Paraphyses. D. Asci. E. Close up of an ascospores. All mounted in lactic acid.

genus is close to *Hapsidomyces* and some species of *Peziza* which was turned out to be polyphyletic (Hansen *et al.*, 2005).

Three species of *Plicaria* (*P. leiocarpa* (Curr.) Boud., *P. suzukii* Henn. and *P. trachycarpa* have been reported from Japan (Imai, 1938; Katumoto, 2010), but none of them has been described in detail (Katumoto, 2010: 753). *Plicaria trachycarpa* was first collected as “unidentified fungus F.100”. in 1912 by Kumagusu Minakata, an amateur mycologist. The specimen was later identified as *P. trachycarpa* by Otani (1987). We also found several other collections hitherto undocumented in our herbarium collected from Hokkaido and Nepal by Otani. The current study shows the broad global distribution of *P. trachycarpa*.

Because the wood fern slab on which *P. trachycarpa* occurred derived from Indonesia, and it was not fumigated in the import process (con-

firmed with the manufacturer), micro-organisms or fungal spores might have been accidentally imported. Although it is difficult to prove, this may be a possible example of introduction of alien species. The impact of fungal invasions are poorly known (Fisher *et al.*, 2012), and may be ignored entirely except for pathogenic species. More awareness of non-pathogenic fungal invasions is necessary as globalization increases the exchange of biological materials around the world (Meyerson and Mooney, 2007; Desprez-Loustau *et al.*, 2007). To clarify the possible detection of alien species in the future, documentation of presently known mycobiota becomes more important.

2. *Lachnum hainanense* W.Y. Zhuang & Zheng Wang, Mycotaxon 67: 25. 1998.

[Figs. 3, 4]

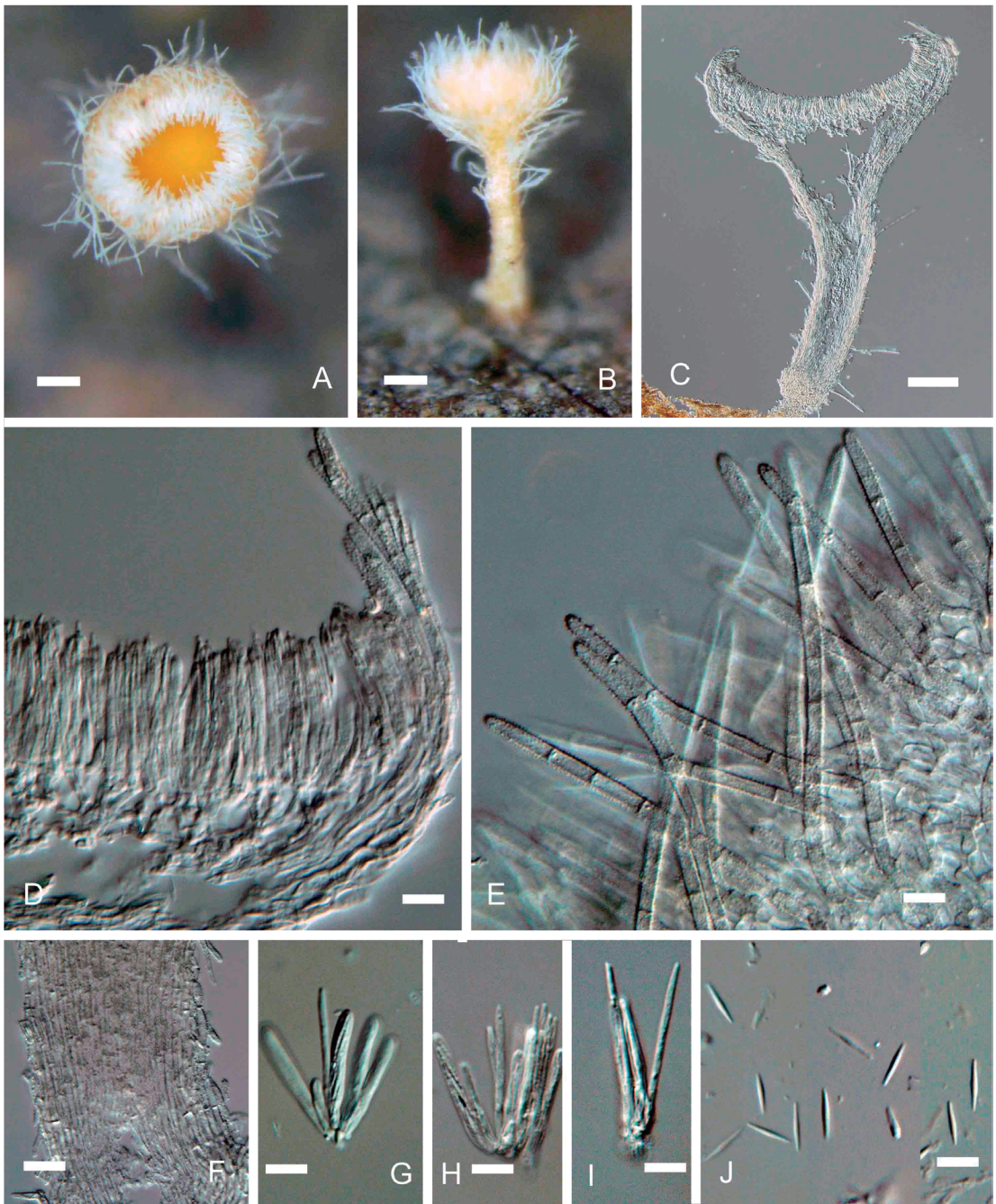


Fig. 3. *Lachnum hainanense* (TNS-F-38442). A, B. Dried apothecia. C. Vertical section of an apothecia. D. Vertical section of an apothecia showing the margin. E. Hairs. F. Vertical section of stipe. G–I. Asci and paraphyses. Note paraphyses extending beyond the asci. J. Ascospores. Scales. A–B, 100 μ m; C–E, G–J, 10 μ m. F, 20 μ m.

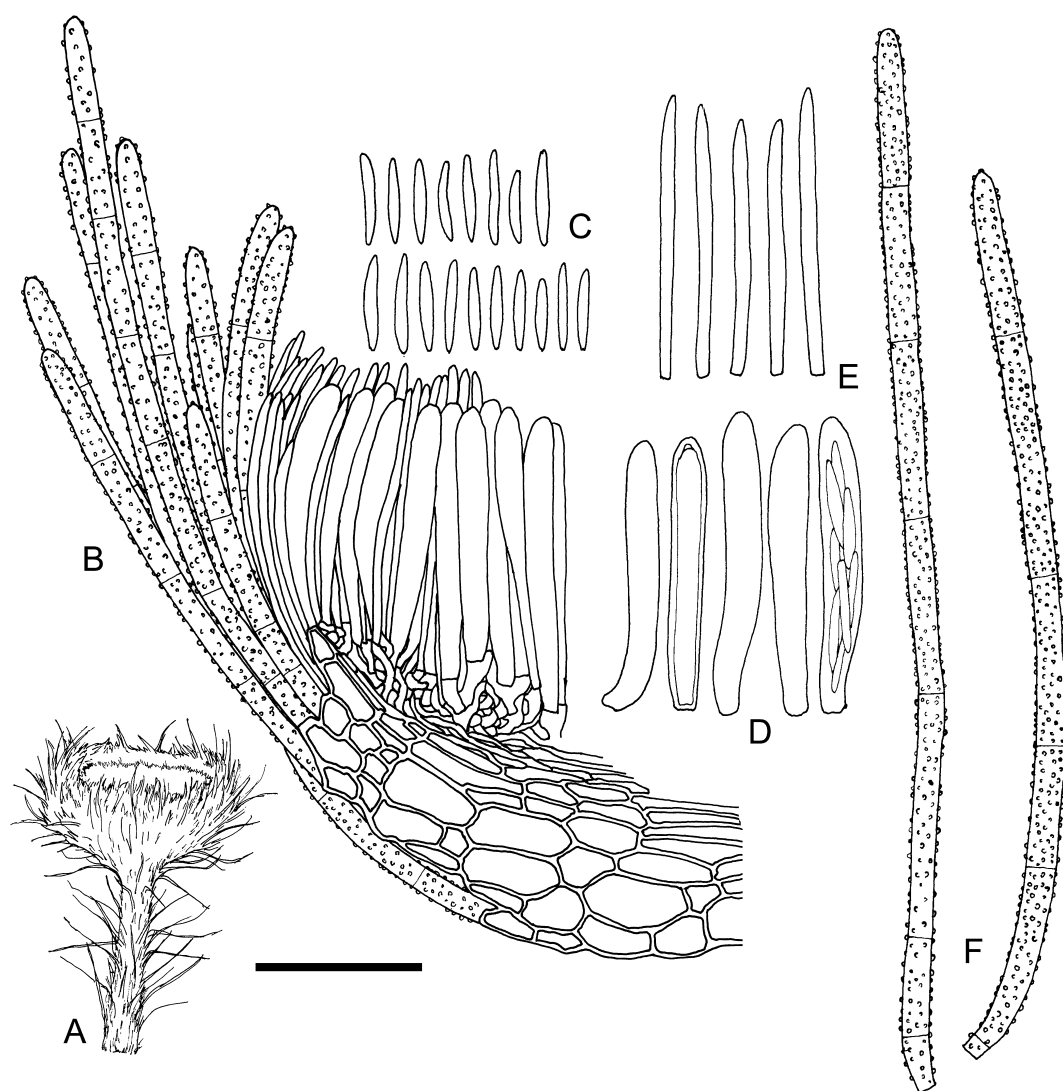


Fig. 4. *Lachnum hainanense* (TNS-F-38442). A. Apothecium. B. Vertical section showing ectal excipulum and part of hymenium. C. Ascospores. D. Paraphyses. E. Asci. F. Hairs.

Apothecia superficial, scattered or gregarious, stipitate, 400–850 μm high, 200–450 μm in diameter, externally light orange (155PC=C0M12 Y32K0); disc dark orange (135PC=C0M21 Y74K0) when dried, 550–750 μm in diameter, shallowly concave, pale orange when rehydrated, with somewhat inrolled margin furnished with white hairs. **Ectal excipulum** textura prismatica, composed of thick-walled cells of 10–15 $\times$ 3–7 μm , hyaline. **Medullary excipulum** textura intricata of dense interwoven hyphae,

1–1.5 μm wide, hyaline. **Hairs** cylindrical, straight or slightly curved, multi-septate; surface granular, obtuse at the apex, 2.0–2.5 μm wide, 44–99 μm (64.0 μm on average, $n=11$). **Asci** 30–42 $\times$ 3.5–5.5 μm (34.9 $\times$ 4.3 μm on average, $n=16$), cylindrical clavate, 8-spored; apex MLZ+ with or without KOH pretreatment, but faint; arising from croziers. **Paraphyses** narrowly lanceolate, aseptate, 34–42.5 μm (38.7 μm on average, $n=8$), 1.5–3.0 μm wide at the widest point, exceeding the asci in 5–10 μm . **Ascospores**

11.5–17.0 $\times$ 1.5–2.5 μ m (13.2 $\times$ 1.8 μ m on average, $n = 20$), fusiform to elongate ellipsoid, aseptate, hyaline.

Specimen examined. HONSHU: TNS-F-38442 on *Machilus* leaf, Takeyama, Miura peninsula, Kanagawa Pref., 28-V-1992, col. T. Hosoya.

Known distribution. China**

Japanese name: Hari-mi-no-shiro-hina-no-chawantake

Notes: A number of *Lachnum* species shows substrate selectivity. Many *Lachnum* species occur on leaves, mostly on deciduous leaves. Vast areas with evergreen forests in Japan are not yet well-investigated for these fungi. *Lachnum hainanense* is characterized by fusiform ascospores, long hairs furnishing the flank and stalk, and faint MLZ+ reaction of the asci. *Lachnum hainanense* shows morphological similarity to other leaf inhabiting lachnoid fungi previously classified in *Lachnum* (Tanaka and Hosoya, 2001; Hosoya *et al.*, 2010), such as *Incrucipulum ciliare* (Schr.) Baral, *I. longispineum* (Hosoya & Issh. Tanaka) Sasagawa & Hosoya, and *I. radiatum* (Iss. Tanaka & Hosoya) Sasagawa & Hosoya in slender paraphyses and fusiform ascospores. However, it differs in lacking crystals or resinous materials at the apex of hairs and a more slender stalk. *Lachnum nipponicum* Haines & Kaneko (Haines and Kaneko, 1984), another species occurring on evergreen leaves (*Quercus glauca*) differs from *L. hainanense* in shorter hairs, shorter stalks, and multi-septate ascospores. *Lachnum fushanensis* M.L.Wu & J.H. Haines (Wu and Haines, 1999) is similar to the present fungus in having aseptate, fusiform ascospores, but differs in having much longer ascospores. *Lachnum hainanense* also resembles *L. confertum* Spooner (Spooner, 1987), but differs in having longer stalks, and shorter asci and ascospores. *Lachnum meifengense* Y.Z.Wang (Wang, 2003) is another similar fungus in gross morphology and host selectivity, but clearly differs in having much longer ascospores. Micro-morphology of hymenial elements of *L. hainanense* shares much similarity with *L. victoriani* S.A.Cantrell & J.H.Haines (Cantrell and Haines,

1997), but *L. victoriani* differs in reddish color on drying.

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References

- Anonymous. 2005. Pantone color bridge/coated. Pantone Inc, New Jersey.
- Cantrell, S. A. and Haines, J. H. 1997. New red species of *Lachnum* from the tropics. *Mycological Research* 101: 1081–1084.
- Desprez-Loustau, M. L., Robin, C., Buée, M., Courtecuisse, R., Garbaye, J., Suffert, F., Sache, I. and Rizzo, D. M. 2007. The fungal dimension of biological invasions. *Trends in Ecology and Evolution* 22: 472–480.
- Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L. and Gurr, S. J. 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186–194.
- Haines, J. H. and Kaneko, S. 1984. A new foliicolous *Lachnum* from Japan. *Transactions of the Mycological Society of Japan* 25: 237–242.
- Hansen, K., Laessle, T. and Pfister, D. 2001. Phylogenetics of the Pezizaceae, with an emphasis on *Peziza*. *Mycologia* 93: 958–990.
- Hansen, K., LoBuglio, K. F. and Pfister, D. H. 2005. Evolutionary relationships of the cup-fungus genus *Peziza* and Pezizaceae inferred from multiple nuclear genes: RPB2, β -tubulin, and LSU rDNA. *Molecular Phylogenetics and Evolution* 36: 1–23.
- Hosaka, K. and Castellano, M. A. 2008. Molecular phylogenetics of Geastrales with special emphasis on the position of *Sclerogaster*. *Bulletin of the National Museum of Nature and Science, Series B* 34: 161–173.
- 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 Hyaloscyphae. *Mycoscience* 38: 171–186.
- 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.

- Hosoya, T., Zhao, Y. J., Han, J. G., Saito, Y. and Kaki-
shima, M. 2012. Enumeration of remarkable Japanese
discomycetes (6): Notes on two inoperculate discomy-
cetes new to Japan and one operculate discomycete.
Bulletin of the National Museum of Nature and Sci-
ence, Series B 38:139–146.
- Imai, S. 1938. Symbolae ad Floram Mycologicam Asiae
Orientalis. II. The Botanical Magazine, Tokyo 52: 357–
363.
- Katamoto, K. 2010. List of fungi recorded in Japan. The
Kanto Branch of the Mycological Society of Japan,
Tokyo.
- Meyerson, L. A. and Mooney, H. A. 2007. Invasive alien
species in an era of globalization. *Frontiers in Ecology
and Environment* 5: 199–208.
- 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 descrip-
tion).
- Rifai, M. A. 1968. The Australasian Pezizales in the her-
barium of the Royal Botanic Gardens Kew. *Verhan-
delingen der Koninklijke Nederlandsche Akademie van
Wetenschappen* II 57: 1–295.
- Seaver, F. J. 1961. The north American cup-fungi (oper-
culates). Hafner Publishing Co., Inc., New York.
- Spooner, B. M. 1987. Helotiales of Australasia: Geoglos-
saceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae.
Bibliotheca Mycologica 116: 1–711.
- Tanaka, I. and Hosoya, T. 2001. Hyaloscyphaceae in
Japan (4): New records of the genus *Lachnum*. *Myco-
science* 42: 597–609.
- Wang, Y. Z. 2003. A new species and a new record of
Lachnum from Taiwan. *Mycotaxon* 87: 137–140.
- Wu, M-L. and Haines, J. H. 1999. A new foliicolous
Lachnum from Taiwan. *Mycotaxon* 63: 45–49.
- Zhuang, W. Y. and Wang, Y. Z. 1998. Discomycetes of
tropical China. I. Collections from Hainan island.
Mycotaxon 67: 21–31.