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Enumeration of Remarkable Japanese Discomycetes (10): Two Helotiales and a Helotialean Anamorph New to Japan

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Abstract Wild anamorph of *Bisporella discedens* (Helotiales), *Mniaecia jungermanniae* (Helotiales), *Lambertella corni-maris* (Helotiales), are described and illustrated. The first is a new record of anamorphic fungi with reference of barcode sequences almost identical to those from previously known teleomorph. The second is a new record with a possibility of a pathogen of a bryophyte. Two new hosts are reported for the third fungus.

Key words: *Bisporella discedens*, *Bloxamia* anamorph, *Lambertella corni-maris*, *Mniaecia jungermanniae*, mycobiota.

Introduction

This is the 10 th part of the series on remarkable Japanese discomycetes following Zhao and Hosoya (2015) to extend the knowledge of the Japanese mycobiota. The present paper documents two new occurrence of Helotiales, one in anamorphic form elucidated from the barcoding sequence. Two new hosts are added to another fungus.

Materials and Methods

Observation procedures followed Hosoya and Otani (1997). Color codes followed the Pantone color code adopting CYMK system referring to a Pantone color bridge (Anonymous, 2005). To examine previously known distribution, the occurrence database of Global Biodiversity Information Facility (GBIF, http://www.gbif.org/occurrence, as of June 16, 2016) was searched. To examine the known host, the Systematic Botany and Mycology Laboratory, Agricultural Research Service, US Department of Agriculture (SBML Database, http://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm, as of June 16, 2016) was searched.

Isolates were obtained from ascospores discharged from the fresh apothecium or conidial mass. DNA was extracted from the culture as previously described (Hosoya *et al.*, 2010). Selected culture has been deposited in the National Institute of Technology and Evaluation, Biological Resource Center (NBRC).

Primer pairs ITS1F and ITS4 (White *et al.*, 1990) were used to amplify internal transcribed spacer (ITS1 and ITS2) and 5.8S ribosomal (ITS–5.8S), the barcoding region of fungi. The obtained sequences were deposited to DNA Data Bank of Japan (DDBJ). The extracted DNA samples were deposited in the Center for Molecular Biodiversity Research, National Museum of Nature and Science, Japan, and available for collaborative research.

Descriptions

1. Anarmoph of ***Bisporella discedens*** (P. Karst.) S.E.Carp. Mycotaxon 2: 124. 1975. [Fig. 1]

Sporodochia pustulate, surrounded by irregularly shaped rim, scattered on the substrate, flat, grey when fresh, drying white, composed of palisade of conidiogenous cells, arising from dichotomously branching system at the base. Conidiogenous cells (14–)18–23 × 2 μm, cylindrical, brown, long-collared with truncate opening. Conidia 2–3 × 2–2.5 μm, phalidic, produced in basipetal order continuously, cubic to elongate rectangular shape, becoming more cubic and rounded due to maturation, hyaline.

Specimens examined. TNS-F-24589, on unidentified decaying wood, Amami-ohshima, Amami-shi, Kagoshima Pref. (28.3770, 129.4175), 2009–II–25, culture FC-1847 = NBRC 109678.

Notes. When the barcoding sequence (LC169493) obtained from NBRC 109678 was compared with previously obtained corresponding sequences from the *Bisporella discedens*, it differed only 6 bp (1.06%) and 1 bp (0.176%) from LC169491 (from *B. discedens* TNS-F-17895) and LC169492 (from *B. discedens* TNS-F-37025), respectively, in the total length (568 bp) compared. The conspecificity of the anamorphic isolate and *B. discedens* was confirmed in molecular basis.

On the other hand, the sequence (LC169493) presented only one candidate in BLAST result with identitity ＞95%: LC015694, Uncultured *Pezizella* genes with 99% identification (Floren *et al.*, 2015; Query coverage 96%, E = 0.0), and apparently shows the paucity of the sequences currently databased.

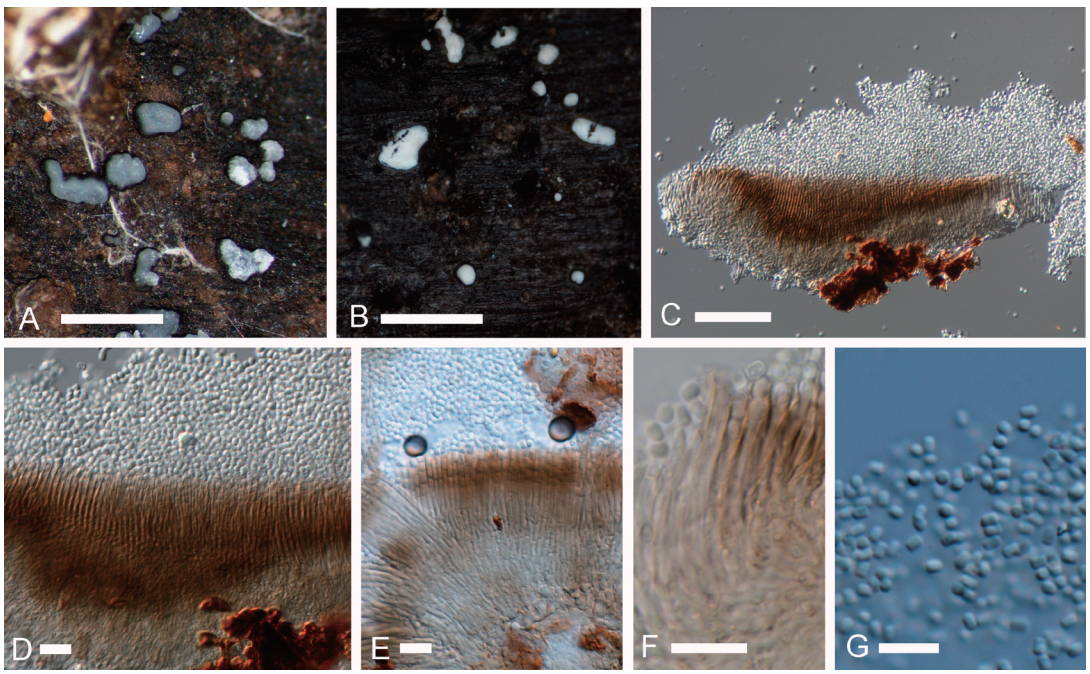

Fig. 1. *Bloxamia* anamorph of *Bisporella discedens* (TNS-F-24589). A. Pustulate sporodochia occurring in patches when fresh. B. Sporodochia becoming white when dried. C. Section of sporodochium. Note that the conidiophore openings ending up in the same height while the total length of the conidiophore are shorter at the edge. D. Close up of C in the middle showing the palisades of conidiophores. E. Close up of the crush mount of the sporodociha. Note pale colored basal area repeatedly branched to conidiogenous cell. F. Close up of the conidiogenous cell with long color bearing conidia in continuation. G. Conidia. Bars, A, B, 1 mm; C, 50 μm. D–G, 10 μm.

We documented the teleomorph of this fungus (Hosoya *et al.*, 2011) based on the collections from Kumamoto and Ehime for the first time from Japan. Since then, more specimens have been added (TNS-F-13516, 48430 from Kagoshima; TNS-F-57758, 57759, 57760, 57761, 57764, 57776, from Tokyo; and TNS-F-65470, from Ibaraki) to our herbarium, which broadened our knowledge for the wider distribution of this fungus. Most of the collection seems to be confined with laurel forest occurring in summer to autumn. The present specimen (TNS-F-24589) was collected in winter, and it is speculated that the fungus may survive in the asexual form during the winter.

The current anamorph is disposed to form genus *Bloxamia*. However, the revised nomenclature (McNeill *et al.*, 2012) no longer support independent names for the anamorph, the authors refrain from calling this specimen in previously accepted anamorphic name. In the taxonomy of *Bloxamia*, Aramarri *et al.* (1992) paid attention whether the conidiogenous cells are cylindrical or lageniform. However, the plasticity of the character should be noted that the present fungus show long, cylindrical conidiogenous cells in the anamorph while the conidiophores on the teleomorph is shorter and lageniform (cf. Fig. 2 in Hosoya *et al.*, 2011 and Fig. 5 in the present paper). It is also probable that *Bloxamia* may be polyphyletic, but this should be examined in the light of molecular phylogeny.

The anamorphic morphology resembles *B. leucophtahalma* (Lév.) Höhn., and differs from most of the *Bloxamia* species in having white sporodochia (Spooren, 2014).

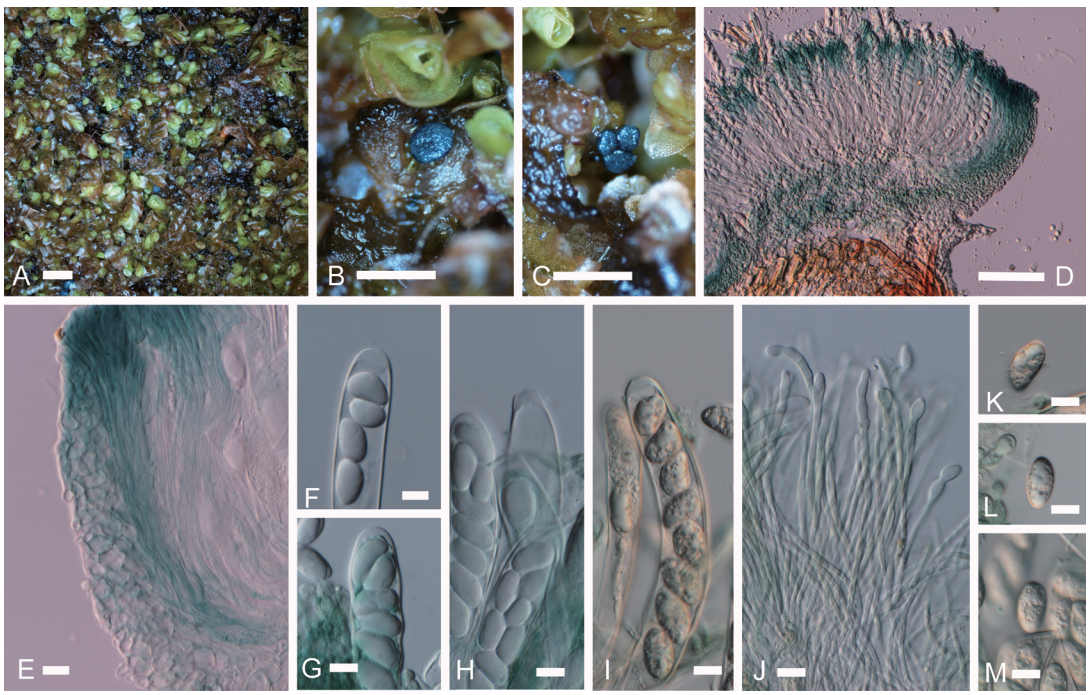

Fig. 2. *Mniaecia jungermanniae* (TNS-F-61776). A. Apothecia scattered on the host. B–C. Close up of the apothecia on the host. Note that the host tissue turning brown. D. Section of an apothecium showing the blue green excipulum and upper part of the hymenium due to the concentration of the pigment. E. Close up of the ectal structure lined by the paraphyses containing the blue green pigment. F–G. Thick-walled ascus apices in Melzer's reagent. H. Dehiscent ascus apex in Melzer's reagent. I. Thick-walled ascus apex and spumous ascospores in water. J. Paraphyses with irregularly enlarged to monilioid apices in water. K–M. Asospores with spumous content observed in water. Bars, A–C, 0.5 mm; D, 100 μm; E–M, 10 μm.

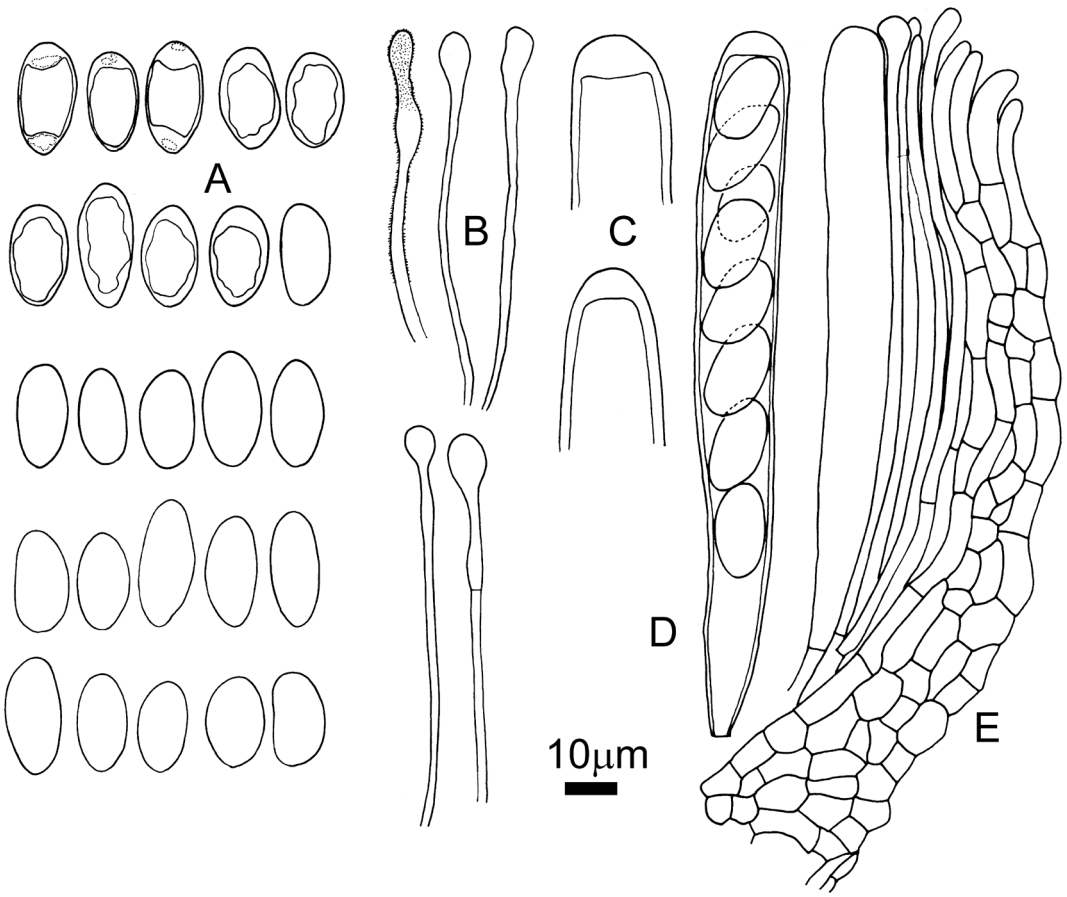

Fig. 3. Camera lucida illustration of *Mniaecia jungermanniae* (TNS-F-61776). A. Ascospores. The three ascospores at the top left show cell contents observed in water, followed by six ascospores containing irregular shaped lipid body in lactic acid. The remaining ascospores show only the external morphology in lactic acid. B. Paraphyses. Note one at the top right shows the fine granules covering the apex observed under water. C. Thick-walled apices of the ascus. D. Cylindrical ascus with eight ascospores. E. Ectal excipulum.

2. ***Mniaecia jungermanniae*** (Fr.) Boud, Hist. Class. Discom. Eur. (Paris): 99. 1907. for synonyms, see Czarnota and Hernik (2013) [Figs. 2–3]

Apothecia occurring on decaying parts of the host, scattered, sessile, globular when young, convex to patellate when mature, 0.1–0.2 mm in diameter, with hymenium of granular appearance, blue green (5473PC = C83 N14 Y23 K50) allover. **Ectal excipulum** of thin-walled and green colored textura angularis, 5–16 × 5–10 μm, becoming narrowed and elongate at the margin, ending up in more elongate cells with rounded end at the margin. **Asci** cylindrical clavate, (125–)137–162.5(–167.5) × 17.5–20 μm; apex notably thick-walled, usually 5 μm, up to 8 μm thick; pore not stained by Melzer's reagent with or without KOH pretreatment. **Ascospores** 17–22(–25) × 9–12 (19.58 ± 2.14 × 10.5 ± 0.725 μm on average ± SD, n = 20 in lactic acid), elliptic, containing a large lipid body at the center with minute vesicles cumulated at the both end when observed in water, containing more irregular shaped lipid body at the center in lactic acid. **Paraphyses** filiform, simple, 1–1.5 μm wide at the base, often enlarged up to 7 μm thick more than once to give monilioid appearance, or irregularly curved, slightly green colored, having minute granules at

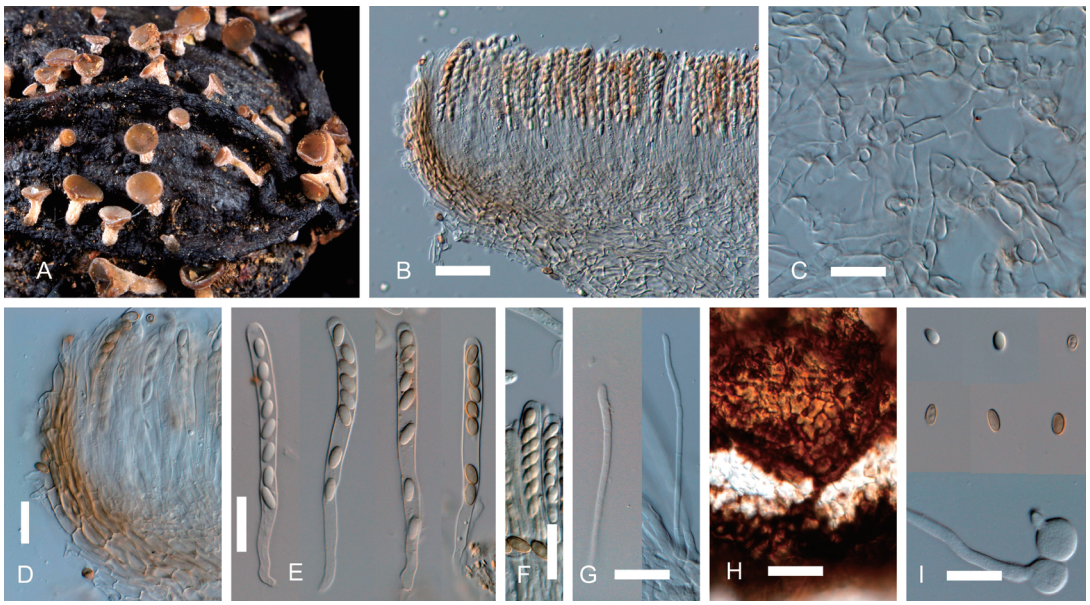

Fig. 4. *Lambertella corni-maris* (TNS-F-40083). A. Fresh apothecia on fallen fruits of *Torreya nucifera*. B. Vertical section of an apothecium. C. Close up of medullary excipulum. D. Close up of ectal excipulum at the margin. E. Asci. F. Reaction of ascal apex to MLZ. G. Paraphyses. H. Surface structure in blackened area on host substrate. I. Hyaline ascospores, brown ascospores and germinating ascospores. Bars, B, 40 μm; C–I, 20 μm.

the surface.

Specimens examined. TNS-F-61776, on *Jungermannia rosulans*, Shirataki, Okawa-mura, Kochi Pref. (33.8182, 133.467861, elev. 830 m), 2015-III-24, col. M. Higuchi, culture FC-5903 = NBRC 112050.

Known distribution. Known from Europe (Austria, Czech, France, Germany, Iceland, Irelands, Italy, Luxembourg, the Netherland, Norway, Sweden, UK), and USA.

Japanese name: Midori-koke-byoutake (newly proposed).

Notes. *Mniaecia jungermanniae* is a new record in Japan. Due to its characteristic habitat, the fungus may be easily recognized, but the small size of apothecia prevents the discovery. *Mniaecia jungermanniae* has been known mostly from Europe, more recently from US, but not from Asia (GBIF, as of July 16, 2016; Czarnota and Hernik, 2013). The fungus is characteristic in the morphology of ascus with notably thick-walled apex.

3. ***Lambertella corni-maris*** Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 127: 375 [47 repr.]. 1918.

For taxonomic synonyms, see Dumont (1971).

[Figs. 4–5]

Stroma substratal, visible as the blackened, shrinking areas on the substrate. Apothecia stipitate, occurring on fallen fruits; disc flat, discoid, or concave, 0.3–3 mm in diameter in dried specimen; hymenium beige to pale brown (1375PC = C0 M45 Y95 K0) when fresh, becoming reddish brown to dark brown (4695PC = C29 M79 Y71 K73) when dry; receptacle lighter than hymenium, beige, pruinose; stipe concolorous with receptacle, 0.4–1 mm long when dry, pruinose, darkish towards the base. **Ectal excipulum** two layered: outer layer textura prismatica, composed of thin-walled or slightly thick-walled, brick-shaped cells of 15–40 × 5–14 μm in middle flanks and 6–25 × 4–10 μm at the margin, with slightly granulate or smooth surface, becoming pale brown towards the margin; inner layer obscure but present, composed of thin walled, subhyaline

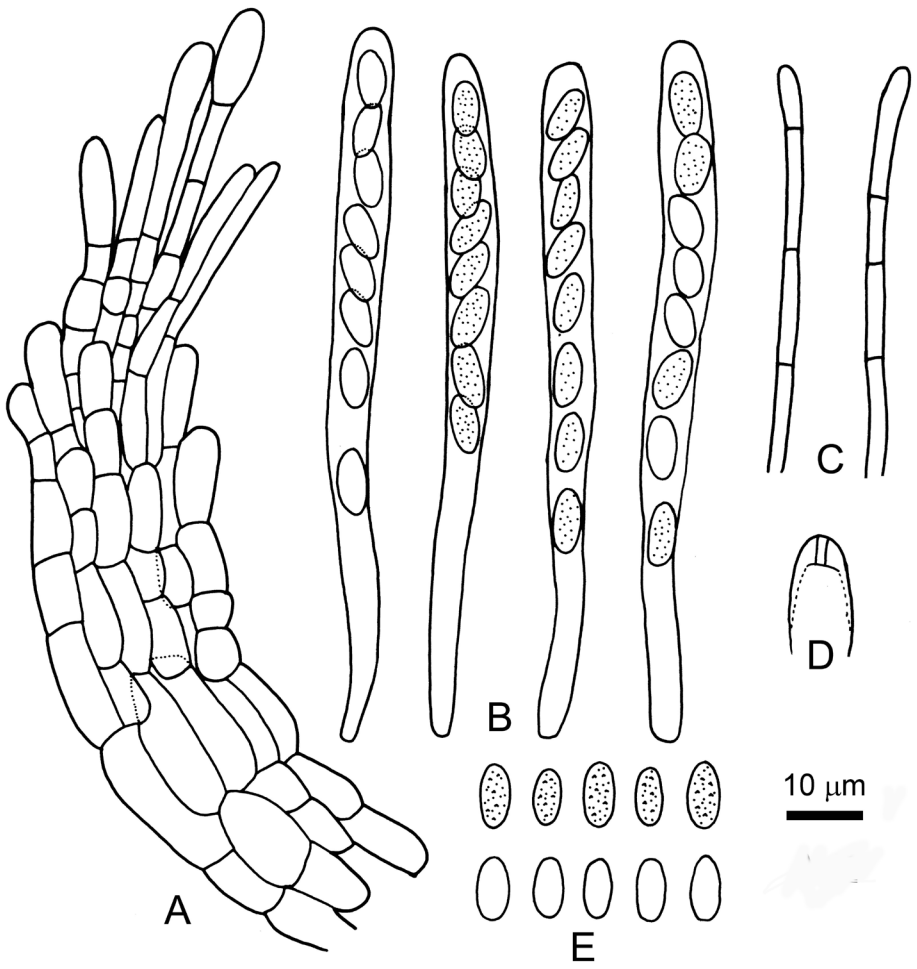

Fig. 5. Camera lucida illustration of *Lambertella corni-maris* (TNS-F-40083). A. Vertical section of an apothecium through the margin showing the ectal excipulum. B. Asci. C. Paraphyses. D. Reaction of ascal apex to MLZ. E. Ascospores.

to pale brown, granulate or smooth, separately ca. 5 μm wide hyphae. **Hairs** arising from the outermost layers of the ectal excipulum, cylindrical, septate, mostly hyaline, occasionally expanded up to 3–7 μm at the apex. **Medullary excipulum** textura intricata, hyaline to subhyaline, smooth, separate hypha of 3–5 μm wide. **Asci** 80–110 × 5.5–7 μm, cylindric to clavate, 8-spored, croziers absent or obscure; apex rounded to truncate, 2–3 μm thick; pore faintly stained by Melzer's reagent without 3% KOH pretreatment. **Ascospores** 7–10.5 × 3.5–4.5 μm (8.1 ± 0.9 × 4.1 ± 0.2 μm on average ± SD, n = 25 in lactic acid), uniseriate, broadly ellipsoid, non-septate; at first hyaline, then becoming pale brown, finally becoming yellow-brown to golden-brown within the ascus; hyaline walls smooth, eguttulate or with 1–2 polar guttules; brownish spores granulate and thickened surface, eguttulate or with 1–2 polar guttules. Germinated spores on PDA becoming paler colored to hyaline, inflated and becoming globose, 12–18 μm in diameter. Paraphyses filiform, septate, hyaline, up to 2–3 μm wide at the apex.

Specimens examined. TNS-F-30402, on decayed leaves of *Mallotus japonicus*, Sanshiro-ike, Tokyo University, Hongo, Bunkyo-ku, Tokyo (35.71176, 139.76248), 2009-XII-7, col. T.

Hosoya, culture FC-2389; TNS-F-40083, on fallen fruits of *Torreya nucifera* (Tsukuba Botanical Garden, Tsukuba City, Ibaraki Pref. (36.10265, 140.11222, elev. 44 m), 2011-X-14, col. T. Hosoya, culture FC-2821.

Japanese name. Chairomi-kinkaku-kin (newly proposed)

Notes. *Lambertella corni-maris* is known to occurs on *Carya ovata*, *Cornus mas*, *Malus domestica* (SBML Database) and *Pyrus* (Dumont, 1971) as plant hosts, and *Cyttaria gunni* as fungal host (SBML Database). In Japan, it has been known to occur as a hyper parasite of *Monilia* occurring on apples (Katumoto, 2010). No other host have been known in Japan, and the two hosts presented here are newly recorded hosts in nature. When the two ITS-5.8S sequences obtained (AB705239 from TNS-F-30402, and AB926069 from TNS-F-40083) were BLAST searched, both of them highly matched (99–100%) with previously known sequences of *L. corni-maris.*

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