

Enumeration of Remarkable Japanese Discomycetes (5): First Records of one Operculate and two Inoperculate Discomycetes in Japan

Tsuyoshi Hosoya^{1,*}, Jae-Gu Han², Yukiko Saito¹, Zhao Yan-jie³,
Makoto Kakishima³ and Hyeon-Dong Shin²

¹Department of Botany, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan

²Department of Environmental Science & Ecological Engineering, Korea University,
Anam-Dong, Seongbuk-Gu, Seoul 136–701, Korea

³Faculty of Life and Environmental Science, Tsukuba University,
1–1–1 Tennodai, Tsukuba, Ibaraki 305–8522, Japan

* E-mail: hosoya@kahaku.go.jp

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Abstract Three remarkable discomycetes (one operculate and two inoperculate) documented for the first time in Japan are described and illustrated: *Bisporella discedens* (Helotiaceae, Helotiales), *Proliferodiscus alboviridis* (Hyaloscyphaceae, Helotiales), and *Ramsbottomia asperior* (Pyrone-mataceae, Pezizales). Sequences of their ITS-5.8S region (barcoding region) were obtained and BLAST searched to confirm and evaluate their taxonomic position.

Key words: *Bisporella discedens*, mycobiota, *Proliferodiscus alboviridis*, *Ramsbottomia asperior*, taxonomy.

Introduction

This is the fifth part of the series on remarkable Japanese discomycetes following Hosoya *et al.* (2010a). 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 CMYK 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 (***)). Isolates obtained from single spores were used to obtain DNA from *Proliferodiscus alboviridis* and *Bisporella discedens*. A single apothecium was used for DNA extraction for *Ramsbottomia asperior*. DNA extraction, PCR, and direct sequence procedure followed Hosoya *et al.* (2010b). The obtained DNA sequences were BLAST searched (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome), to evaluate their taxonomic and phylogenetic affinities. The obtained extracted DNA samples are deposited in the Center for Molecular Biodiversity Research, National Museum of Nature and Science, Japan, and available for collaborative research.

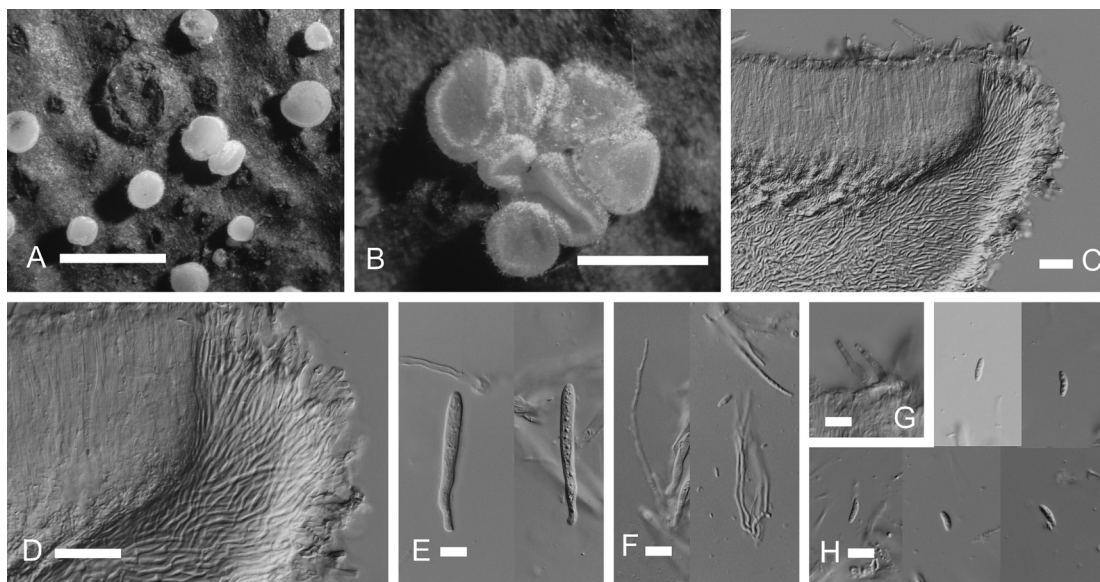


Fig. 1. *Bisprella discedens* (TNS-F-17895). A. Fresh apothecia. B. Close up of the fresh apothecia occurring in a group. Note brown hair-like structures on the margin are conidiophores. C. Section of an apothecium showing the hymenium, ectal and medullary excipulum. D. Close up of ectal excipulum at the margin. E. Ascus, F. Paraphyses. G. Conidiophores occurring on the ectal excipulum. H. Ascospores. C-E, G, mounted in lactic acid. F, H, mounted in cotton blue/lactic acid. Scales. A, 1 mm; B, 0.5 mm; C, D, 50 μ m; E-H, 10 μ m.

Descriptions

1. *Bisprella discedens* (P.Karst.) S.E.Carp.

[Figs. 1, 2]

Basionym: *Helotium discedens* P.Karst., Hedwigia 28: 191. 1889.

See Carpenter (1975) for synonyms.

Apothecia solitary to gregarious, patellate, shallow cup shaped or irregular due to crowded occurrence, disc up to 0.3 mm in diameter, white when fresh, pale yellow (Pantone 600PC=C1 M0 Y34 K0) when dried; margin distinct, elevated and surrounding the disc, lachnose, white with brown particles found to be phialides in higher magnification, shallow concave, white when dried. **Ectal excipulum** textura intricata, composed of thick-walled interwoven hyphae agglutinated to each other to make each hyphal element indistinguishable, leaving 1–2 μ m wide lumen, mostly arranged parallel to, but occasionally at a low angle to the margin. The outermost cells becoming protru-

sions, short cylindrical with rounded apex or clavate, thin-walled, occasionally differentiated into brown conidiophores. **Conidiophores** short, simple or in groups, reduced to one to two cubic cells, brown, bearing concolorous, *Chalara*-like phialides at the apex; phialides 3–4 μ m at the base, 2 μ m at the apex, producing hyaline, cubic conidia. **Asci** 46–56 \times 4.5–5 μ m, eight-spored, cylindrical to clavate, arising from indistinct croziers; apex conical, pore MLZ–, with or without KOH pretreatment. **Ascospores** 8.5–9 \times 2.5–3 μ m, ellipsoid or cuneate, hyaline, often containing several oil globules. **Paraphyses** filiform, 1.5–2 μ m thick, simple or branched at the base.

Specimens examined. HONSHU: TNS-F-17895, 17897, Kikuchi Canyon, Kikuchi-shi, Kumamoto Pref. (33°0'28.2"N, 130°56'26.3"E, alt. 612 m), on unidentified decaying wood. 10-X-2005 col. T. Hosoya (culture FC-1228 from TNS-F-17895); TNS-F-37025, Oyabu Keikoku, Hijikawa-cho, Ozu-shi, Ehime Pref. (33°27'15.56"N, 132°40'26.55"E, alt. 45 m), on unidentified

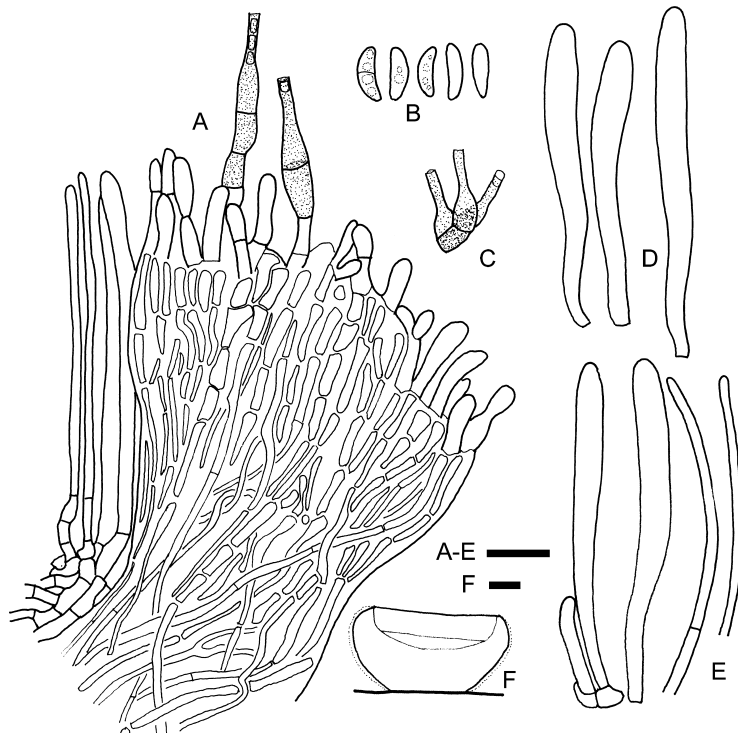


Fig. 2. Camera lucida illustration of *Bisporella discedens* (TNS-F-17895). A. Vertical section of an apothecium through the margin showing the ectal and medullary excipulum, and the conidia producing structures on the ectal excipulum. B. Ascospores. C. Conidia producing structures occurring in a group on the ectal excipulum. D. Asci. E. Paraphyses. F. Structural outline of the apothecium. Areas where short protrusions occur are delimited by dotted line. Line drawings prepared in lactic acid mount. Scales. A–E, 10 μm ; F, 100 μm .

decaying wood. 12-X-2010. Col. T. Hosoya (Culture FC-2660).

Previously known distribution. Brazil***, Dominica**, Guadeloupe**, Haiti**, Mexico*, New Zealand***, Philippines**, Puerto Rico**, Switzerland**, Venezuela**.

Japanese name: Tsutsu-ke-shiro-byotake

Notes. This fungus is quite distinctive for having a *Chalara* (Corda) Rabenh.-like structure on its ectal excipulum. The anamorph was first reported as *Cystodendron* Bubák (Carpenter, 1975), but later identified as *Bloxamia* Berk. & Broome (Johnston, 1988). The ectal excipulum composed of agglutinated thick-walled hyphae with glassy appearance is congruent with that of *Bisporella* Sacc. However, the senior author has never obtained an anamorph from other *Bisporella* isolates in Japan. As a result of

BLAST search of ITS-5.8S sequence from FC-1228, HQ630988 *Chalara* sp. TMS-2011 voucher MS3p_50-44 was found as the best matched sequence (Identities 530/575, 92%). The similarity of the ITS-5.8S sequence obtained from FC-1228 to known sequence of *Bisporella* identified to specific rank (*Bisporella citrina*, EU940164, AY789386, AF335454, AY833058, and GQ411507) was less than 90%. Although *Bisporella* is a large genus containing 19 species (Kirk *et al.*, 2008), only two species have been known in Japan (Katumoto, 2010). Apparently information is not sufficient to discuss the taxonomic affinity of *B. discedens*. The anamorphic state, *Bloxamia* was not previously documented in Japan.

This is the first documentation of the present fungus in Japan. The present fungus is classified

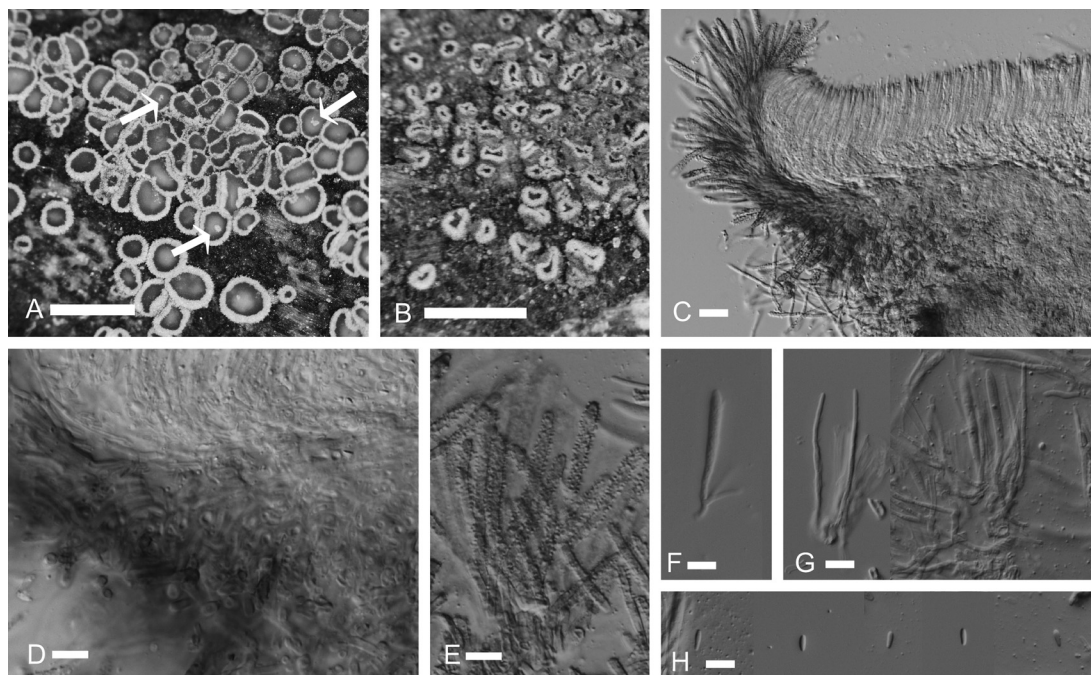


Fig. 3. *Proliferodiscus alboviridis* (TNS-F-17436). A. Fresh apothecia occurring on the substrate. Note proliferation of the daughter disc (at present only sterile elements are seen) from the disc (arrow). B. Dried apothecia. C. Section of an apothecium showing the hymenium, ectal and medullary excipulum. D. Close up of a section of an apothecium at the margin showing the thick-walled ectal excipulum. E. Hairs. F. Young ascus. G. Paraphyses. H. Ascospores. C, D, mounted in lactic acid, E–H, mounted in cotton blue/lactic acid. Scales. A, B, 1 mm; C, 20 μm ; D–H, 10 μm .

in Helotiaceae, Helotiales.

2. *Proliferodiscus alboviridis* (Sacc.) Spooner
[Figs. 3, 4]

Basionym: *Trichopeziza alboviridis* Sacc., Syll.

Fung. 8: 415 (1889) (ut “(Cooke) Sacc.”)

See Spooner (1987) for synonyms.

Apothecia solitary to gregarious, sessile, patellate, wedged to the substrate, infrequently proliferating from the disc; disc up to 0.5 mm in diameter, dark yellow (Pantone 123PC=C0 M21 Y88 K0) rimmed with a distinct, grayish-green hairy margin (Pantone 7542PC=C24 M4 Y8 K13) when fresh, concave to urceolate, obscured by elevated margin when dried. **Ectal excipulum** textura intricata, composed of interwoven hyphae 1.5–2 μm wide, arranged parallel to the margin, becoming thicker-walled and entangled closely,

agglutinated to form textura porrecta appearance toward the base of the apothecium. **Subicular hyphae** densely interwoven, 1–2 μm wide, smooth or partially granulate, hyaline to pale. **Hairs** simple, cylindrical, slightly enlarged toward the apex, strongly granulate by having granules of 1 μm in diameter, 3 μm wide near the base, up to 5 μm at the tip, 30–65 μm long, 1–3-septate, greyish green in mass; granules resistant to 10% KOH. **Asci** 36–45 \times 3–4 μm , eight-spored, cylindrical clavate, arising from simple septa; apex conical, pore minute, faintly MLZ+ without KOH pretreatment. **Ascospores** 4.5–6 \times 1–1.5 μm , elongate ellipsoid to cuneiform, hyaline, smooth, eguttulate, irregularly biseriolate in the asci. **Paraphyses** cylindrical to narrow lanceolate, 1.5–2 mm thick, simple or branched at the base. No anamorph was observed in culture.

Specimens examined. HONSHU: TNS-F-

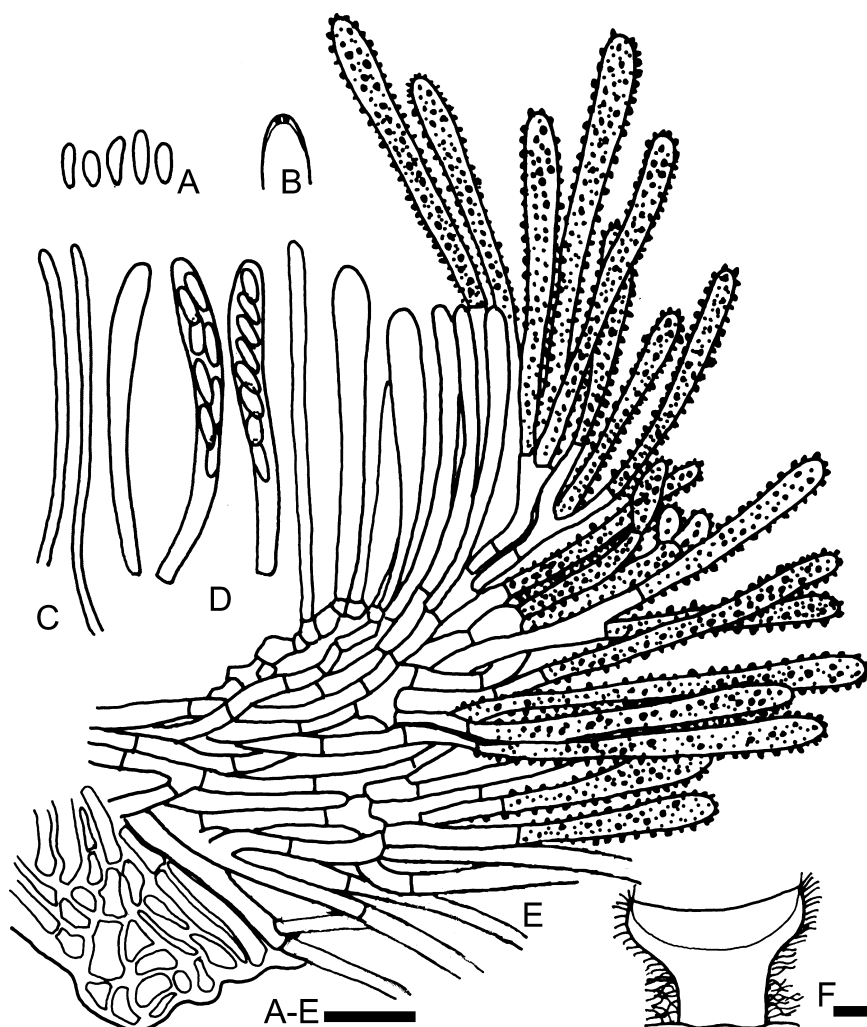


Fig. 4. Camera lucida illustration of *Proliferodiscus alboviridis* (TNS-F-17436). A. Ascospores. B. Ascus apex showing the reaction to Melzer's reagent. C. Paraphyses. D. Ascus. E. Section of an apothecium at the margin showing the ectal excipular structure. F. Structural outline of the apothecium. Line drawings prepared in lactic acid mount except for B mounted in Melzer's reagent. Scales. A–E, 10 μ m; F, 100 μ m.

17436, Riku-mura, Agazuma-gun, Gunma Pref. (36°38'56.1"N, 138°38'33.1"E), on unidentified decaying wood. 3-VII-2006. col. T. Hosoya (culture FC-1413); TNS-F-39240, Tsukuba Botanical Garden, Amakubo, Tsukuba, Ibaraki Pref. (38°15'23.0"N, 138°45'14.21"E), on unidentified decaying wood (probably *Prunus*). 29-IV-2011. col. T. Hosoya (culture FC-2681).

Previously known distribution. Australia** (Questionable according to Spooner, 1987),

USA**. No record in GBIF.

Japanese name: Futasu-mi-shiro-hinano-chawantake

Notes. The genus *Proliferodiscus* J.H.Haines and Dumont was erected by Haines and Dumont (1983) in Polydesmiae, Arachnopezizoideae, Hyaloscyphaceae, because it had "crispated or cork screw-shaped" hairs and the presence of indeterminate, branched, subiculum-like hyphae at the base of their apothecia (Haines and Dumont,

1983). As indicated by Haines and Dumont (1983), the irregularly curved subiculum-like hyphae are not true subicular hyphae that fix the apothecia to the substrate as in *Arachnopeziza* Fuckel. Both Haines and Dumont (1983) and Spooner (1987) indicated the similarity in apothecial structure between *Lachnellula* P. Karst. and *Proliferodiscus*. However, Cantrell and Hanlin (1997) showed the two genera are distantly related. The BLAST search of ITS-5.8S sequence obtained from FC-1413 showed the high similarity to the registered sequence (U57990, *P. alboviridis*, Identities=535/545(98%)). This is the first documentation of the genus *Proliferodiscus* in Japan. The present fungus is classified in Hyaloscyphaceae, Helotiales.

3. *Ramsbottomia asperior* (Nyl.) Benkert & T. Schumach.

[Figs. 5, 6]

Basionym: *Peiziza asperior* Nyl., Not. Sällsk. Fauna Fl. Fenn., Förh. 10: 21. 1869.

See Benkert and Schmacher (1985) for synonyms.

Apothecia scattered to gregarious, patellate to convex, 1.5–2 mm in diameter, orange (Pantone 1495C=R255 G145 B51) when fresh, becoming discoid to shallow cupulate, brownish yellow (Pantone 135PC=C0 M21 Y74 K0). **Ectal excipulum** textura globulosa composed of globular to rectangular cells 30–50×20–40 μm, outermost cells protruding into hyphoid hairs up to 75 μm long, 10–15 μm wide. **Ascospores** globose, 13–

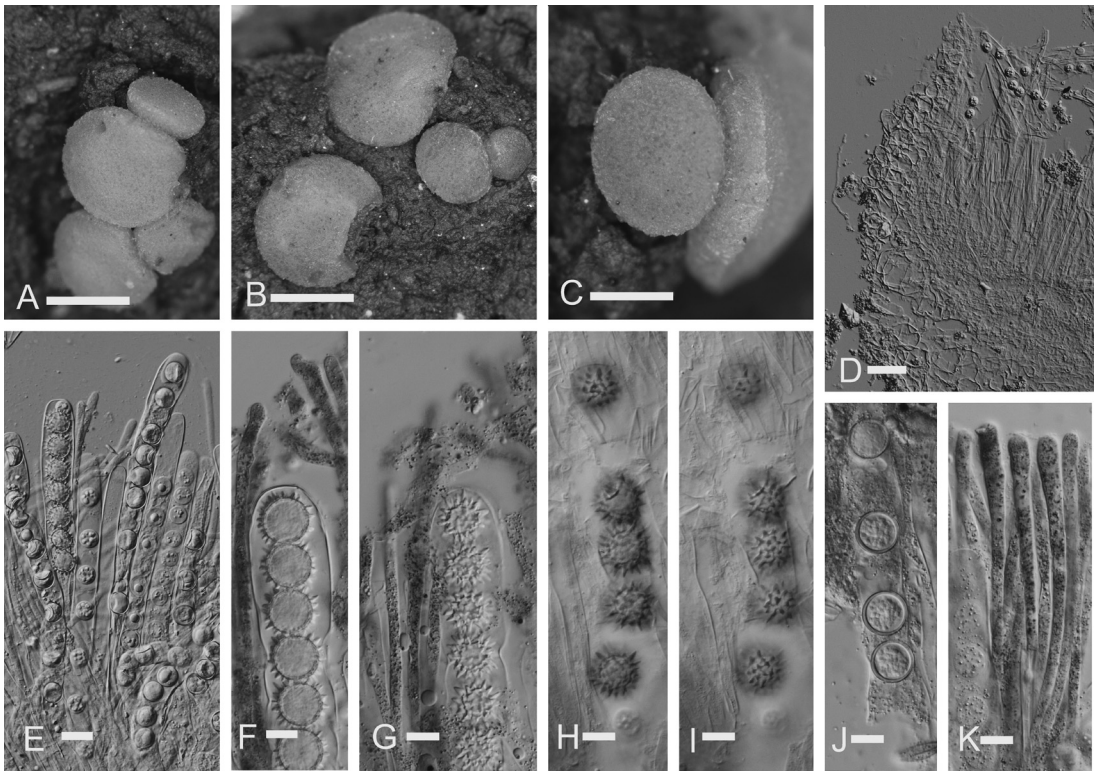


Fig. 5. *Ramsbottomia asperior* (TNS-F-36001). A–C. Fresh apothecia on ground. D. Vertical section of an apothecium at the margin showing the ectal excipulum. E. Asci. F. Ascospores. G. The same ascospores in F at different focus. Note spines on the ascospore wall. H–I. Ascospores in cotton blue/lactic acid. Note spines are stained by cotton blue. J. Young ascospores showing the globular cell content. K. Paraphyses in MLZ, showing the cellular contents stained. Scales. A, B, 1 mm, C, 2 mm, D, 50 μm; E, 20 μm. F–K, 10 μm.

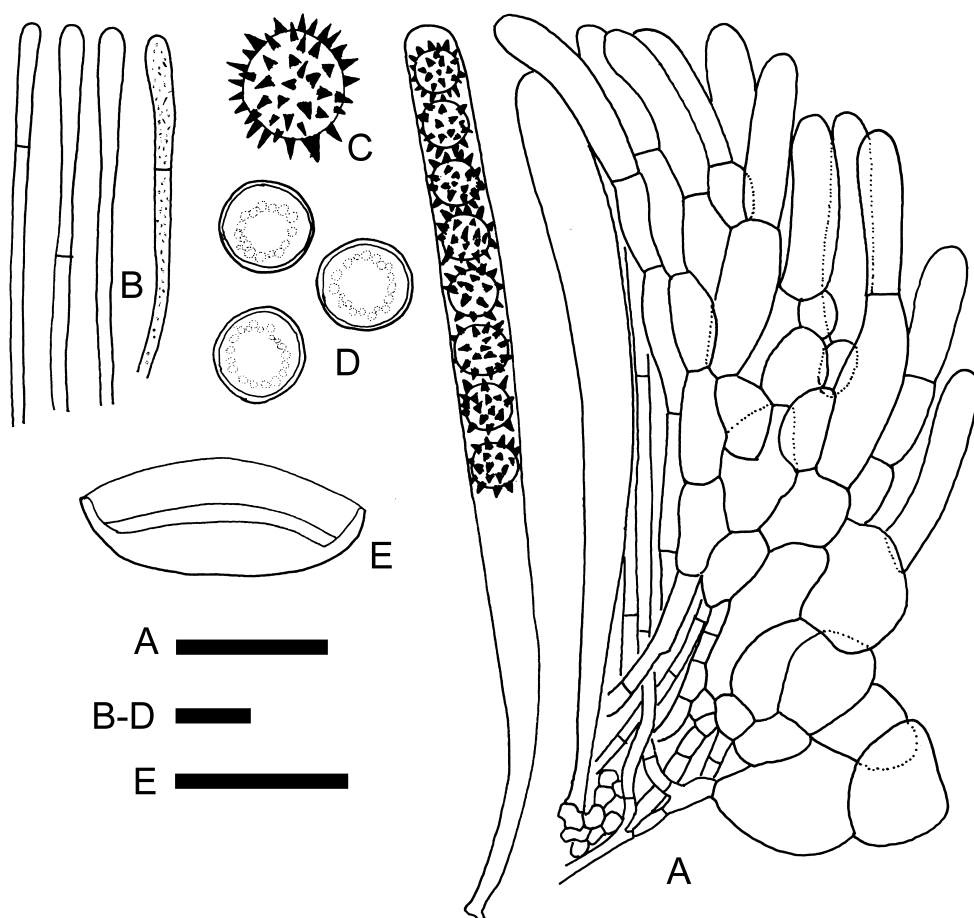


Fig. 6. Camera lucida illustration of *Ramsbottomia asperior* (TNS-F-36001). A. Vertical section of an apothecium at the margin showing the ectal and medullary excipulum and an ascus. B. Paraphyses. One at the right shows the cell contents stained in MLZ. C. Mature ascospore. D. Young ascospores showing globular cell contents. E. Structural outline of the apothecium. Line drawings prepared in lactic acid mount except for one at the right in photo B. Scales. A, 50 μm ; B–D, 10 μm ; E, 1 mm.

15 μm in diameter excluding the spines; smooth, spumose to multi-guttulate, stained brown in MLZ when young, becoming spiny, having remarkable, conical spines up to 3 μm long, stained blue in cotton blue in lactic acid; eguttulate when mature, uniseriate in the asci. **Asci** ca. $300 \times 18\text{--}20 \mu\text{m}$, eight-spored, thin-walled, MLZ—with or without KOH pretreatment, arising from croziers. **Paraphyses** straight, septate, occasionally slightly enlarged at the apex, up to 4–5 μm thick; apical cells 5–7.5(–10) long, having carotenoid granules stained blue in MLZ.

Specimens examined. HONSHU: TNS-F-

36001, Tsukuba Botanical Garden, Amakubo, Tsukuba, Ibaraki Pref. ($38^{\circ}15'23.0''\text{N}$, $138^{\circ}45'14.21''\text{E}$), on ground. 27-VI-2010. col. Y. Kondo.

Previously known distribution. Austria*, Brazil*, Finland**, Germany***, Iceland*, Morocco*, New Zealand*, Mexico*, Norway*, Scotland**, Sweden***, Switzerland*, UK*, USA***.

Japanese name: Togemino-daidai-saratake

Notes. The genus *Ramsbottomia* Buckley was erected by Buckley (1923) based on a single species *R. lamprosporoidea*. The generic concept was verified by Kullman and Brummelen (1992)

who justified its segregation from *Lamprospora* De Not., a genus with globose spores and bryophilous habitat. Yao and Spooner (1995) claimed the delimitation of *Ramsbottomia* and *Lamprospora* requires further investigation, and Rifai (1968) listed *Ramsbottomia* as a synonym of *Lamprospora*. However, Perry *et al.* (2007) concluded that *Ramsbottomia* can be segregated from *Lamprospora* as well as *Octospora* Hedw. and *Neottiella* (Cooke) Sacc. based on molecular phylogeny.

Currently, three more species are accepted in *Ramsbottomia*: *R. lamprosporoidea* W.D.Buckley, *R. crec'hqueraultii* (P.Crouan & H.Crouan) Benkert & T.Schumach. and *R. macrantha* (Boud.) Benkert & T.Schumach. (Kirk *et al.*, 2008). Benkert and Schmacher (1985) who emended the genus regarded *R. lamprosporoidea* synonymous to *R. asperior*. However, Yao and Spooner (1995) claimed it is distinguished by having longer spines (2–6 μm while the spines in *R. asperior* are up to 3.5 μm) on the ascospores and presence of colored hairs. The characteristics of the present specimen almost agree with the description given for *R. asperior* in Benkert and Schmacher (1985) except for the ascospores in the present specimen are almost globose while that in *R. asperior* are elliptic to globose.

Obtaining a culture was unsuccessful because the spores did not germinate.

This is the first documentation of the genus *Ramsbottomia* in Japan. The present fungus is classified in Pyronemataceae, Pezizales.

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