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Lambertella advenula, a new combination proposed for *Moellerodiscus advenulus*, new to Japan

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***Moellerodiscus advenulus*, a sclerotiniaceous fungus new to Japan, was collected and its ascospore morphology and cultural characteristics were studied. Ascospores possess previously unreported characters, such as gelatinous polar appendages and a sheath, and become brown and one-septate after discharge, prior to germination. The stromata produced in culture have a thin black rind typical of *Lambertella*. The taxonomic position of *M. advenulus* is discussed, and a new combination, *L. advenula*, is proposed.**

Key Words——ascospore appendage; *Lambertella advenula*; *Moellerodiscus advenulus*; Sclerotiniaceae; stroma.

The discomycete genus *Moellerodiscus* P. Hennings, Sclerotiniaceae, is known as a heterogeneous assemblage (Dumont, 1976; Korf, 1990). The members of the genus occur on leaves and stems, and are characterized by the presence of an ectal excipulum of *textura globulosa* in the apothecium and small ascospores. Six species were accepted and monographed by Dumont (1976).

This genus has been treated variously by previous authors. Dennis (1962), not knowing *Moellerodiscus*, "reluctantly" proposed *Ciboriopsis* Dennis as a generic concept corresponding to *Moellerodiscus*. He included 6 species, which he transferred from *Helotium* Pers. and *Phialea* (Fr.) Gillet. Spevak and Korf (1966), having examined *Ciboriopsis simulata* (Ellis) Dennis in culture, supported Dennis's decision to segregate the genus *Ciboriopsis* from other genera. Dumont (1976) found that *Ciboriopsis* is a later name for *Moellerodiscus*. However, *Ciboriopsis* (=*Moellerodiscus*) has recently also been reduced to synonymy with *Ciboria* Fuckel, which now includes some 70 species (Spooner, 1987; Eriksson and Hawksworth, 1988). Through these treatments, the heterogeneity of *Moellerodiscus* was pointed out, and reconsideration of the genus was recommended (Korf, 1990).

Among the heterogeneous elements that differ from the typical species of *Moellerodiscus* (or *Ciboria*) is *Moellerodiscus advenulus* (W. Phillips) Dumont (Dumont, 1976). The fungus was originally described as a member of the genus *Helotium*, then placed in *Hymenoscyphus* Gray, *Phialea*, *Ciboriopsis* (Dennis, 1962), and *Moellerodiscus* (Dumont, 1976), but it was claimed to have "very different asci from other species" in *Moellerodiscus* (Dumont, 1976). The gross morphology and coloration of the apothecia of *M. advenulus* are similar to those in *Hymenoscyphus* (Leotiaceae), but the presence of a stroma and the excipular structure of *textura globulosa* in the apothecium places this species in Sclerotiniaceae. *Moellerodiscus advenulus* is also characterized by its habitat on *Larix* needles and extremely small apothecia (Dennis, 1956, 1962; Dumont, 1976). There is no previous report on cultural studies of this fungus.

The senior author collected *M. advenulus* in Sugadaira Montane Research Center, Sugadaira, Nagano Pref., Honshu, Japan. Its morphological features agreed almost perfectly with those described by Dumont (1976). It is a new record from Japan. In the process of identification and cultural studies, new information was obtained on its cultural characteristics, ascospore morphology and morphological changes through the germination process of ascospores, which was sufficient to allow segregation of this fungus from *Moellerodiscus*. In the present paper, taxonomic consideration of the placement of the fungus is presented with a description of the Japanese material.

Materials and Methods

Isolation and cultural studies Single apothecia were removed from the host, affixed with a block of water agar to the inner surface of the lids of Petri dishes, and left for 15–20 min for ascospores to be discharged onto the agar surface. After confirming the discharge of ascospores under × 10 objective lens, the apothecia were removed and air-dried. Three kinds of agar plates were used: potato dextrose agar (PDA, Nissui, Tokyo), half-diluted corn meal agar (1/2CMA, Nissui, Tokyo), and 2% water agar (WA). Multi-spore isolates were obtained by excising the agar on which the discharged ascospores were germinating and transferring it onto PDA slants as stock cultures. All cultures used in this study were deposited in the culture collection of the Sankyo Tsukuba Research Laboratories (SANK). For observation of stromatal formation, 80 ml of PDA was poured and solidified in glass bottles 8 cm in diam with screw caps, inoculated with a

piece of agar from a stock culture, and incubated at 17°C for up to 4 mo. Color indications followed Kornerup and Wanscher (1978).

Observations on ascospore morphology The plates with discharged ascospores were kept at 17°C in the dark, and ascospore morphology was observed after 18, 24, 30 and 42 h under ×20 and ×40 long working distance objective lenses. After 48 h, a cover slip was applied onto the agar surface where the ascospores were germinating. Ascospore morphology and apothecial anatomy were also observed with an Olympus BH2 microscope equipped with Nomarski interference, phase-contrast optical system.

For SEM observation of ascospores, a piece of Millipore filter was placed on PDA to catch discharged ascospores. The filters with ascospores were fixed in 3% glutaraldehyde-1% osmium tetroxide for 1 h, in 2% osmium tetroxide in 0.1 M cacodylate sodium buffer (pH 7.2) for 1 h at 4°C. The material was then dehydrated in a graded ethanol series: 30, 50, 70, 80, 95, and 100% (15 min at each step). Following dehydration by ethanol and substitution by using the same graded series of isoamylacetate, the material was critical point dried in a Hitachi Critical Point Drier and gold-coated in a Eiko IB3 Ion Coater. Observations were carried out on a Hitachi S-510 scanning electron microscope operated at 20 kV.

Results and Discussion

Ascospore morphology The ascospores were found to possess a gelatinous polar appendage at each end and a gelatinous sheath (Figs. 1B, C, 2A–D), characters previously unreported. The appendage is sac-like to irregular in shape. The sheath envelops the whole ascospore with equal thickness, 0.5–1 μm.

The appendages are best observed in water mount. They can be observed faintly even on young ascospores in asci, but not on older ascospores. Mounting media greatly influence the appendages. Melzer's reagent (MLZ), 3% KOH, and lactophenol all dissolve the appendages and the sheath. Once the specimen had been dried, the appendages and the sheath were not recovered with rehydration and could not be observed. The appendages and gelatinous sheath degrade during germination or post-discharge maturation.

SEM observation of the discharged spores revealed that the appendages are determinate in structure to some extent, and the sac-like shape is retained well after discharge. In some cases, the edge of the appendages spread flatly on the surface of the substrate due to collision with the substratal surface (Figs. 2B, D). The edge of the appendages becomes fibrous and corasely granulate (Fig. 2D), while more intact appendages (Figs. 2A, C) have a smoother surface. The texture of the sheath is coarsely granulate and superficially continuous with the appendages under SEM (Figs. 2A–D).

The appendages appear to be formed initially as sac-like structures at both ends of the spore. As the ascospore matures, the sac deforms to extrude mucilaginous substances. The primary role of these appendages may

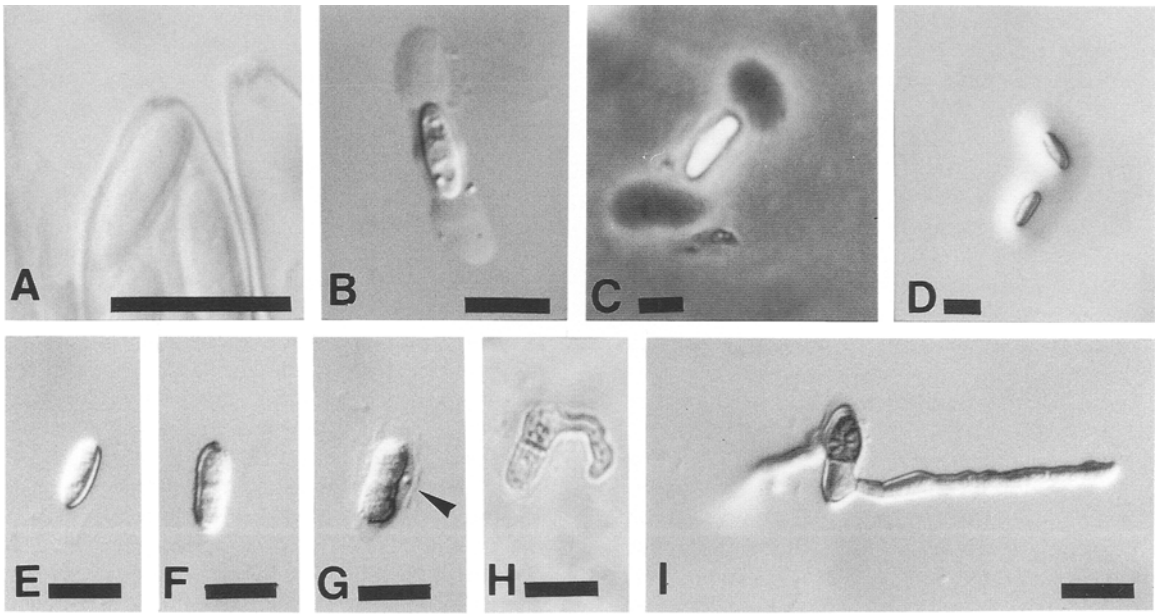

Fig. 1. Ascus and ascospore morphology in *Lambertella advenula* (A, D–I: TRL-1432; B, C: TRL-535).
A. Close-up of the ascal apex, mounted in MLZ. Note MLZ+ ring at the apex. B. Ascospore with gelatinous polar appendages from fresh specimen in water mount, observed under Nomarski phase interference. C. Ascospore with gelatinous polar appendages from fresh specimen in water mount, observed under phase-contrast microscope. D. Discharged ascospores observed under ×20 long working distance objective lens. Note appendages spread to envelope the ascospores. E–H. Changes in ascospore morphology during germination process. Note septation, coloration, granulation, and degradation of the sheath (G, arrowhead) and appendages. I. Germinating ascospore. Scales 10 μm.

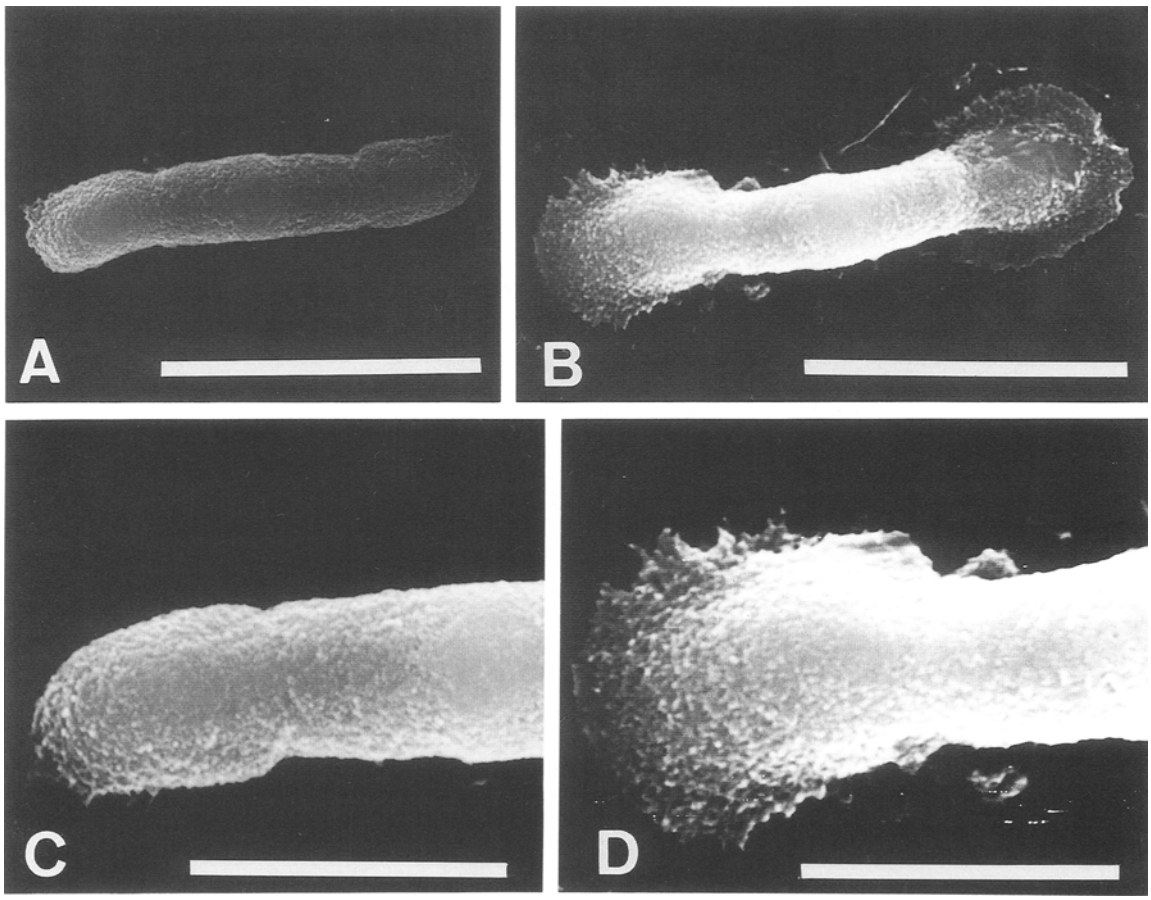

Fig. 2. Discharged ascospores of *Lambertella advenula* (TRL-535) observed under SEM.
A, B. Ascospore with appendages, discharged onto Millipore filter. A. Ascospore with appendage having rather intact edge. B. Ascospore with appendage expanded flatly due to the collision to the substratal surface. C, D. Close up of the appendage in A and B, respectively. Note the difference in edge of appendages in C and D. Scales. A, B, 10 μm; C, D, 5 μm.

be to help attachment of the ascospores to the substrate, as pointed out for some other fungi (Rees and Jones, 1984; Jones, 1994). However, this function may not always operate since the appendages and sheath are influenced chemically and easily lost on older ascospores (Fig. 3C).

The ascospores never germinate within the asci or when remaining attached to the surface of apothecia. All such ascospores remain hyaline and smooth. On the other hand, the ascospores discharged onto agar become more rounded in the first 18 h (Fig. 1E). In the next 24 h, they become transversely one-septate at the middle, dark colored, and the surface becomes coarse (Figs. 1F–H). The gelatinous appendages are apparent just after discharge (Figs. 1B, C), recognized by a massive water drop around the discharged ascospores (Fig. 1D), but degrade as the germination process proceeds, leaving only membranaceous vestiges (Fig. 1G). Such changes took place within 42 h in all agar plates tested. Ascospores germinate by germ tubes, often produced from both cells of the ascospores. Germination occurs in the absence of nutrients or in the presence of minimal nutrients, since ascospores on WA germinated not long after ascospores collected at the same time germinated on PDA or 1/2 CMA.

Cultural characteristics SANK 19596 grows very slowly on PDA, attaining a mat diameter of ca. 35 mm in 14 d at 17°C. Mat umbonate at the center, the surface Brownish Orange (6C3), becoming darker in age, slightly floccose. Context tough and glutinous. Aerial hyphae a little developed, white, forming short mycelial strands. Sectors and zonation present, becoming distinct as stromatal formation proceeds.

After incubation at 17°C for 3 mo, the texture of the mat surface becomes granular to glandular, darker-colored or blackened, forming sectors due to formation of stromata (Figs. 4A, B). Stromata form at first in irregular patches, dark brown, ellipsoid to irregular (Fig. 4C), later become confluent to cover large area of agar surface. The rind ca. 20 μm thick, vertically not well developed even after 4 mo at 17°C, *textura globulosa* to *t. angularis* in section, but seen as *t. epidermoidea* to *t. globulosa* in surface view, composed of thin-walled, dark brown, smooth-walled cells 8–13 μm across (Fig. 4E); in section, mainly one cell thick, partially two to several cell layers, wall typically darker toward the outside (Fig. 5). Medul-

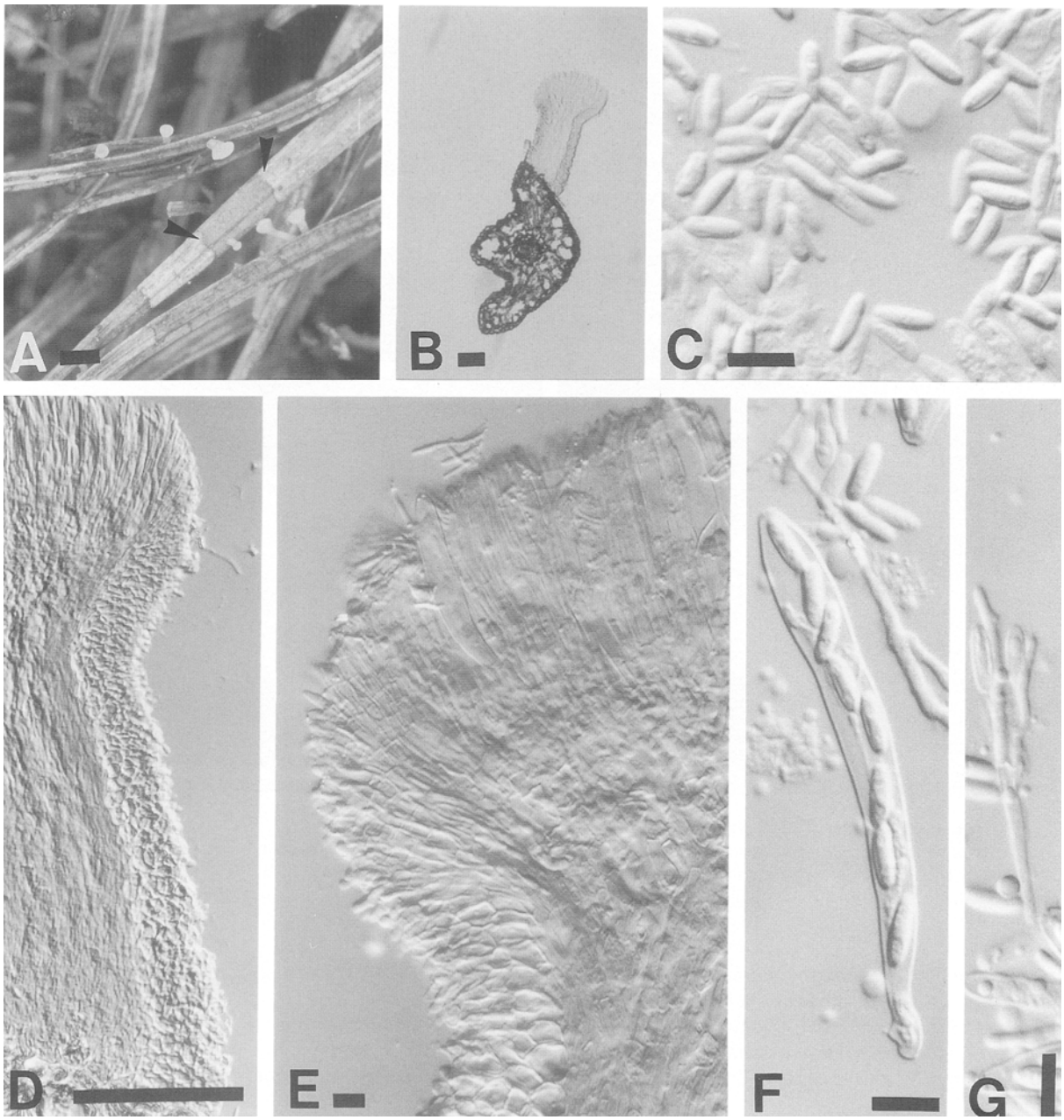

Fig. 3. Apothecial morphology of *Lambertella advenula* (A, B, D, E: TRL-535; C, F, G: TRL-1432).
A. Fresh apothecia on the host. Note black lines indicating the presence of rind in the host plant (arrowheads). B. Section through an apothecium and the host plant. C. Ascospores in MLZ mount. Appendages not observed. D. Close-up of apothecial section in Fig. 1B. Note ectal excipulum well developed along the stipe and outside the receptacle. E. Close-up of apothecial margin. F. An ascus with ascospores. G. Paraphysis with apical branches. Scales. A, 1 mm; B, D, 100 μm; C, E–G, 10 μm.

la basically *textura intricata*, composed of thin-walled, hyaline cells, 4.5–6 μm wide, tightly entangled to form a *textura angularis* near the rind, but partially retaining hyphal structure, often containing cells with very thick, hyaline, almost coalescent walls up to 2 μm thick, highly refractive when mounted in MLZ or in water, not reactive in MLZ; giving a *textura globulosa* of very thick-walled cells when viewed in section (Figs. 4D, F, 5). Neither spermatia nor conidial anamorphs observed.

Taxonomy In the Sclerotiniaceae, brown coloration of the ascospores prior to germination is the most important unique characteristic of *Lambertella* (Whetzel, 1943; Dumont, 1971; Korf and Zhuang, 1985) and its allies. Brown coloration is reported for only one other monotypic genus, *Lambertellinia* Korf et Lizon (Kimbrough and Atkinson, 1972; Korf and Lizon, 1994), not dealt with here for comparison because it is clearly distinct from *M. advenulus*.

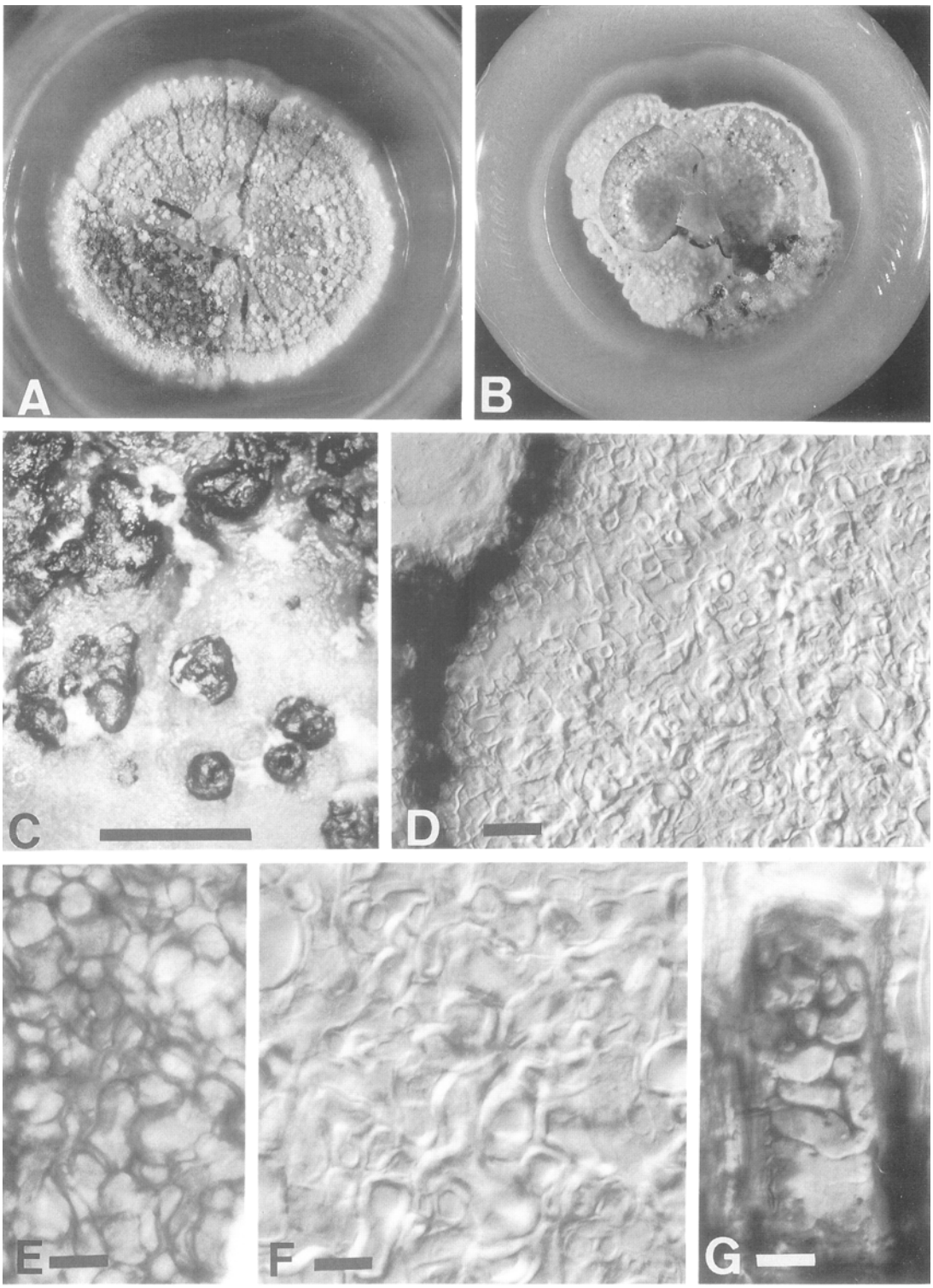

Fig. 4. *Lambertella advenula* in culture (SANK 19596).
A. Colony on PDA (17°C, 3 mo), from the surface. B. Colony on PDA (17°C, 3 mo), from the reverse. C. Close-up of the colony surface. Note formation of black sclerotia-like stromata. D. Section of a stroma. Note the black rind and partially refractive medulla. E. Close-up of the section of the rind. Note cells in *textura globulosa* arrangement. F. Close up of the section of the medulla. G. Surface view of the rind cells in host plant. Scales. C, 5 mm; D–G, 10 μm.

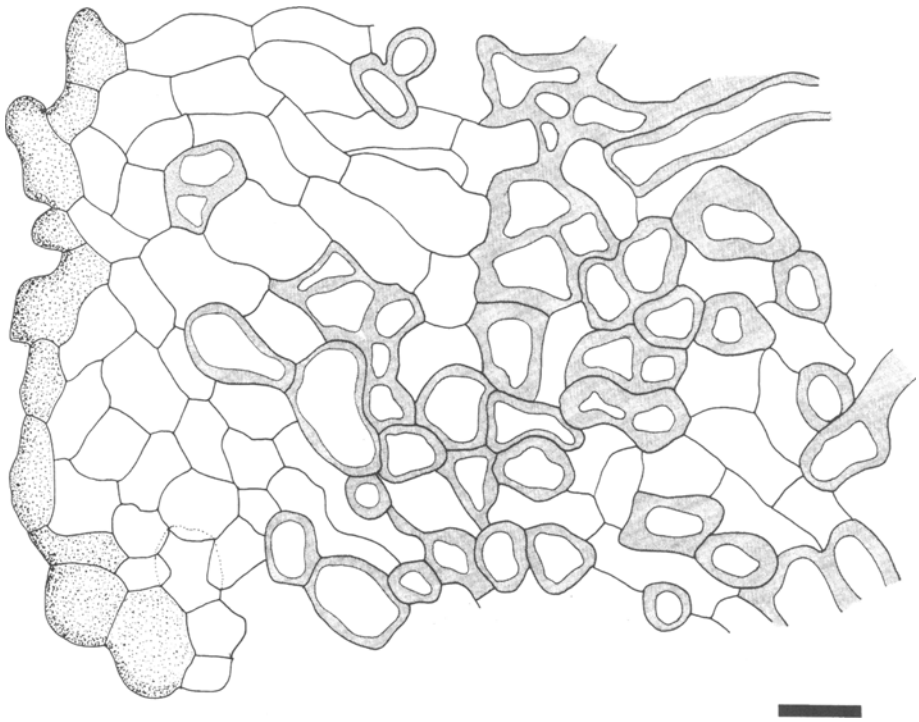

Fig. 5. Line drawing of section through the stroma formed in culture. Note the medulla containing cells with thickened walls, indicated by gray tones, and the thin rind, composed of dark-colored cells, which are darker at the outside. Scale 10 μm.

No *Moellerodiscus* species has been reported with brown-coloration in the ascospores. Ascospore coloration in *Lambertella* is sometimes overlooked because in some species, e.g., *L. albida* (Gillet) Korf (Hosoya and Otani, 1993), coloration takes place only after discharge.

Dumont (1976) reported that the general shape of the asci and ascal apex of this species "seem to be more like that of members of the Helotiaceae than other members of the Sclerotiniaceae." We agree with Dumont (1976), and take this to support the removal of *M. advenulus* from *Moellerodiscus*.

The stroma observed in *M. advenulus* differs from those in *Moellerodiscus lentus* (Berk. et Broome) Dumont (Spevak and Korf, 1966; Dumont, 1976) and *Moellerodiscus conocarpi* (Seaver et Waterston) Dumont (Dumont, 1976).

Although vertical development of the stromatal rind in culture, a typical generic characteristic of *Lambertella* in culture (Whetzel, 1943; Korf et al., 1966), was not observed clearly in the present fungus, rind morphology of small stromata in culture was within the range of variation in *Lambertella* (Korf et al., 1966). The rind observed in the host (Fig. 4G) had similar structure to that stressed for *Lambertella* by Whetzel (1943). The cultural characteristics and the germination process of ascospores place this fungus in *Lambertella*.

The presence of ascospore appendages is reported for some species in discomycetes, and in some cases it is treated as a generic character. *Dicephalospora* Spooner was established in the Sclerotiniaceae based on the presence of its characteristic ascospore appendages (Spooner, 1987). However, we believe that the presence of appendages alone cannot be accepted as a character to separate a genus. For example, in the genus *Arachnopeziza* Fuckel, Hyaloscyphaceae, a thread-like appendage is reported for the type species *Arachnopeziza aurelia* (Pers.: Fr.) Fuckel, but some species without an appendage have been accepted in it. Although the appendages in the present fungus are of determinate structure to some extent, they are less determinate than those in many marine fungi, because they are easily influenced by chemicals and not always present in all ascospores. No *Lambertella* species are known to have appendages on ascospores (Whetzel, 1943; Dumont, 1971). But appendages may have been overlooked because of the condition of the apothecia or the mounting media used. This study confirmed that observation of living material in water mounts, well-termed "vital taxonomy" (Baral, 1992), should be a standard protocol for description of ascomycetes and probably many other fungi. In apothecia of *Lambertella* species, ectal excipular structure is of typical *textura prismatica*, but some exceptions are known (Korf and Zhuang, 1985).

From all these analyses, the present fungus should be removed from *Moellerodiscus*. Although somewhat atypical in ascospore morphology, the species should be transferred to *Lambertella* because of the brown coloration of ascospores prior to germination, supported by the stromatal rind structure observed under culture and in nature. Hence, a new combination is proposed.

Lambertella advenula (W. Phillips) Hosoya et Y. Otani, comb. nov. Figs. 1–5

≡*Helotium advenulum* W. Phillips in W. Phillips et Plowright, Grevillea **6**: 24. 1877.

≡*Hymenoscyphus advenulus* (W. Phillips) W. Phillips, British Discomycetes, p. 133. 1887.

≡*Phialea advenula* (W. Phillips) Sacc., Syll. Fung. **8**: 256. 1889.

≡*Ciboriopsis advenula* (W. Phillips) Dennis, Kew Bull. **16**: 319. 1962.

≡*Moellerodiscus advenulus* (W. Phillips) Dumont, Mycologia **68**: 235. 1976.

Stroma substratal, seen as black lines developed transversely on the host, or blackened areas developed in the superficial tissue surrounding the base of the apothecial stipe. Rind in section of *textura angularis*, (15–)20(–60) μm in thickness, composed of brown, thin-walled cells 6.5–10 × 5–7 μm; in surface view, *textura epidermoidea*. Apothecia scattered, arising from a stroma, mostly 0.4–0.6(–0.8) mm high; disc 0.2–0.3 mm in diam, flat to slightly convex, finely pruinose, Yellow (2A6) to Light Yellow (3A5) when fresh, Pastel Yellow (3A4) to Deep Yellow (4A8) when dry; receptacle concolorous, gradually tapered below to the stipe; stipe cylindrical, 0.1 mm thick, up to 0.7 mm long, concolorous with receptacle or darker, externally pruinose when fresh, almost smooth when dried. Ectal excipulum of *textura angularis* to *t. globulosa*, thin-walled, distinctly distinguished from medullary excipulum, well developed along the outside of the stipe and the receptacle, not stained by MLZ. Asci (70–)75–85 × 7–8.5 μm, cylindrical-clavate, thin-walled, 8-spored, arising from croziers; apex conical, somewhat flattened, pore MLZ+ without KOH pretreatment, ring well observed as two blue points in optical section in MLZ mounts. Ascospores 9–12 × 2.5–3 μm, ellipsoid, inconspicuously biguttulate to spumose, irregularly seriate in the asci, enveloped entirely by a gel sheath (ca. 0.5–1 μm thick just after discharge), with gelatinous appendages saccate to irregular in shape at the ends, one-celled, smooth, hyaline in the asci; becoming two-celled with a transverse septum at the middle, brown with somewhat to clearly rough walls 42 h after discharge; germinating by germ tubes formed at one or both ends; appendages and sheath degrading as spore germination proceeds. Paraphyses simple or branched, septate, 1.5–2.5 μm thick at the middle, becoming irregular in breadth in upper part, occasionally slightly expanded at the apex.

Specimens examined. Honshu: Sugadaira Montane Research Center, Tsukuba Univ., Nagano Pref., on decaying *Larix* needle, 22-VI-91, col. T. Hosoya, TRL-364; Sugadaira Montane Research Center, Tsukuba Univ., Nagano Pref., on decaying *Larix* needle, 9-VI-92, col. T. Hosoya, TRL-535; Sugadaira Montane Research Center, Tsukuba Univ., Nagano Pref., on decaying *Larix* needle, 20-VI-96, col. I. Tanaka, TRL-1432, TNS-F-181723 (culture SANK 19596).

Other illustrations. Dennis, Mycol. Pap. **62**: 101, fig. 96. 1956; Dumont, Mycologia **68**: 236, fig. 1. 1976.

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