Enumeration of Remarkable Japanese Discomycetes (1): Three Helotialean Members New to Japan

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Abstract Three helotialean discomycetes new to Japan are described., i. e. *Parachnopeziza miniopsis* (Hyaloscyphaceae), *Claussenomyces atrovirens* (Helotiaceae), and *Tapesia rosae* (Dermateaceae).

Key words: discomycetes, Japan, mycobiota.

Introduction

In comparison to basidiomycetes with large fruiting bodies, usually recognizable in naked eyes, less attention has been paid to discomycetes with small fruiting bodies. However, due to its climatic diversity, Japan embraces rich mycobiota of discomycetes, and still is "relatively unexplored paradise" (Korf, 1958). In spite of inventorial work of Otani (1989), further enumerative studies are required for comprehensive understanding of the biodiversity and achievement of inventory of Japanese discomycetes. In a series of papers, the author will describe remarkable discomycetes from Japan including new records and new to science. The first paper describes three discomycetes new to Japan.

Materials and Methods

Collection. Collection procedure followed Hosoya and Otani (1997).

Observation. Dried materials were rinsed in a drop of 70% ethanol, rehydrated in water, embedded in mucilage (Tissue Tek II; Miles laboratories, Inc., Naperville, Illinois, USA), and sliced at $15-25 \,\mu$ m with a microtome (SM2000R, Leica) equipped with an electric freezer (EF-10, Leica). The sliced materials were mounted in Melzer's reagent (MLZ; 0.5 g of iodine, 1.5 g of

KI, 20 g of chloral hydrate, 20 ml of distilled water), cotton blue dissolved in lactic acid (CB-LA) or in plain lactic acid (LA) for observation. When KOH pretreatment was required, specimens were treated with 3% KOH before exposure to MLZ. Color codes followed CMYK system referring to a publication (Anonymous, 2002).

Descriptions

- **1.** *Claussenomyces atrovirens* Figs. 1–2
 - Peziza atrovirens Pers., Syn. Meth. Fung. p. 635. 1801. Calloria atrovirens (Pers.) Fr., Summa Veg. Scand., p. 359. 1849.
 - *Ombrophila atrovirens* (Pers.) P. Karst., Mycol. Fenn. **1**: 92. 1871.
 - *Chlorosplenium atrovirens* (Pers.) de Not. apud Karst., Mycol. Fenn. 1: 93. 1871.
 - *Tympanis atrovirens* (Pers.) Rehm, Hedwigia **21**: 70. 1882.
 - *Coryne atrovirens* (Pers.) Sacc., Syll. Fung. 8: 641. 1889.—Dennis, Mycol. Pap. 62: 169. 1956.
 - Claussenomyces atrovirens (Pers.) Korf & Abawi, Can. J. Bot. 49: 1882. 1971.
 - Coryne virescens Tull., Sel. Fung. Carp. 3: 193. 1865.

Apothecia scattered, superficial; pulvinate to shallow discoid with ill-defined margin, olivaceous green (C10M0Y30K30) when fresh; becoming discoid to shallow discoid with well-defined margin, mostly $500 \,\mu$ m in diameter, black (C0M0Y0K100) when dry; pale yellow pigment



Fig. 1. Claussenomyces atrovirens (TNS-F-11166). C, G: mounted in MLZ; D–F, H: mounted in CB-LA) A, Fresh apothecia. B, Dried apothecia. C, Vertical section of an apothecium. D, Close-up of ectal excipulum. E, Ascus with ascospores. Note thick-walled apex. F, Ascus filled with ascoconidia. G, Paraphyses embedded in gelatinous matrix. H, Ascospores. One at the middle producing ascoconidia. I, Ascoconidia. Lower figure showing yeast-like growth initiated from the discharged ascoconidia in a patch. Scales. A, B, 1 mm; C, 50 μm; D–I, 10 μm

dissolved into the medium when rehydrated in 3% KOH. Ectal excipulum two-layered, internal layer textura intricata, of loosely interwoven, infrequently branched hyphae embedded in gelatinous matrix; external layer textura intricata of interwoven hyphae, $2-4 \,\mu m$ wide, less frequently branched and embedded in dark-colored, thicker gelatinous matrix. Medullary excipulum textura intricata, inconspicuous. Asci $93-100(-120)\times$ $(7.5-)9-11.5 \ \mu m \ (98.6\pm8.8\times9.8\pm0.8 \ \mu m \ on \ av$ erage \pm SD, n=21), cylindrical clavate with somewhat pedicellate base, 8-spored, often filled by secondary conidia (ascoconidia), eight-spored, arising from simple septa; apex rounded, thickwalled, pore MLZ- with or without KOH pretreatment. Ascospores $(14-)17-20\times 3-4 \mu m$ $(17 \pm 1.8 \times 3.6 \pm 0.4 \text{ on average} \pm \text{SD}, n=20) \,\mu\text{m},$ fusiform-clavate with narrowed base and rounded apex, transversely 4-7-septate, rarely vertically septate in the middle cells, often obscured by ascoconidia budding from the ascospore body still within the asci, constricted at the septa and disarticulating at the septa along with maturation. Ascoconidia $1.5 \times 1 \,\mu$ m, ellipsoid, filling up the ascus except for basal portion, discharged in mass, forming circular patches on the agar; germinating and initially proliferating in yeast-like manner. **Paraphyses** filiform, straight to flexuous, simple, branched or anastomosing, embedded in gelatinous matrix, $1-1.5 \,\mu$ m in width.

Specimens examined. TNS-F-11165, TNS-F-11166, Ura-Tsukuba, Mt. Tsukuba, Makabe-gun, Ibaraki Pref., on *Pinus* log, 8–VI–2002. col. T. Hosoya.

Known distribution. Europe (UK, Dennis, 1956); South America (Argentina, Romero, 1987).



Fig. 2. Claussenomyces atrovirens (TNS-F-11166).

A, Asci. One showing the contained ascospores, one showing the ascoconidia filing up the ascus. Note thick-walled apex. B, Vertical section of apothecium showing ectal excipulum and hymenium. Note hyphae in the ectal excipulum embedded in gelatinous matrix. C, Paraphyses. One at the right showing the anastomosis at the basal part. D, Ascospores. Some showing ascoconidial production. One at the right showing disarticulation. E, Diagrammatic drawing of the apothecial section showing the outline of the structure.



Fig. 3. Parachnopeziza miniopsis (TNS-F-11167). C–G: mounted in CB-LA. A, B, Fresh apothecia. C, Vertical section of the apothecium showing the outline of the structure. D, Vertical section of the apothecium showing the margin. E, Ascus with ascospores. F, Subicular hyphae. Note glassy texture. G, Hairs with various degree of curvature. Scales. A, B, 1 mm; C–G, 10 μm.

Notes. The genus Claussenomyces Kirschst. includes 14 species (Kirk et al., 2001). Although the genus is known to be widespread, occurrence of some members is reported from limited regions. The genus is divided into two major groups by the presence or absence of ionomidotic reaction (extracting dark pigment in KOH mount), and C. atrovirens belongs to the negative ionomidotic reaction group (Ouellette and Korf, 1979). However, pale yellow pigments were observed when mounted in KOH in the present specimens. Transversely septate ascospores are one of the characteristics of C. atrovirens, but occurrence of vertical septation is also reported, though infrequent (Ouellette and Korf, 1979). Fisher (1985) reported cultural characteristics of C. atrovirens, initial stage of which concurred with the result of the present study. Yeast-like growth at the initial stage of growth was also observed in the present study, but no further information was obtained due to the isolate depletion.

2. Prachnopeziza miniopsis Figs. 3–4

- Peziza miniopsis Ellis, Bull. Torrey Bot. Club 8: 66. 1881.
- Erinella miniopsis (Ellis) Sacc., Syll. Fung. 8: 510. 1889.
- Dasyscyphella miniopsis (Ellis) Kirschst., Ann. mycol. 36: 384. 1938.—Korf, Lloydia 14: 152. 1951.
- Erioscypha miniopsis (Ellis) Kirschst., Ann. mycol. 36: 384. 1938.
- *Erinellina miniopsis* (Ellis) Seaver, North Amer. Cup Fungi (Inoperc.) p. 291. 1951.
- Lasiobelonium miniopsis (Ellis) Dennis, Persoonia 2: 185. 1962.
- Parachnopeziza miniopsis (Ellis) Korf, Mycotaxon 7: 469. 1983.

Apothecia scattered, seated on subiculum and with short stipe waged into the substrate, patellate to short turbinate, $50-100 \,\mu\text{m}$ in diameter when dry; disc flat to shallow, orange (C0M60Y100K0) with transparent texture, surrounded by white hairy margin when fresh, hidden by incurving margin when dry; receptacle





A, Vertical section of apothecium showing ectal excipulum and part of hairs and hymenium. B, Paraphyses. C, Ascospores. D, Hairs. E, Asci. F, Subicular hyphae. Note branching at right angles. G, Diagrammatic drawing of the apothecial section showing the outline of the structure.

covered by white, entangled hairs; stipe short, narrowed to the point at the base, waged into the substrate. Subiculum of finely granulate to smooth hyphae of 2.5–4 μ m thick, wall $1-1.5 \,\mu\text{m}$, few septate, mostly straight, occasionally waving or forming fascicules with several hyphae, branched at right angle. Ectal excipulum textura prismatica, composed of cells $6-10\times2.5-3\,\mu\text{m}$, becoming larger and angular toward the base, giving rise to hairs at the outermost layer. Medullary excipulum textura intricata, composed of densely entangled hyphae of 1.5–2 μ m. Hairs straight at the basal portion, waving to strongly curved, crisped, or spirally coiled toward the blunt apex, few-septate, even in width, having wall of variable thickness, hyaline, $2-3 \mu m$; narrow rumen of $1 \mu m$ conserved. Asci $125-140\times11-13 \,\mu\text{m}$, cylindrical clavate, eightspored, arising from crosiers; apex conical with flattened top, MLZ+without KOH pretreatment. Ascospores (50–)80–90 \times 3 μ m, filiform, straight to curved, 5-septate, with blunt apex, gradually narrowed toward the base. Paraphyses filiform, straight to waving, simple to branched, as long as

asci, $1-1.5(-2) \mu m$.

Specimens examined. HONSHU: TNS-F-11167, Sugadaira Montane Research Center, University of Tsukuba, Sanada Machi, Nagano Pref., on *Prunus* trunk, 20–V–2002. col. I. Tanaka.

Known distribution. North America (Korf, 1978).

Notes. Although 8 species are known in *Parachnopeziza* Korf (Kirk et al, 2001), so far no other members of the genus has been known from Japan. *Parachnopeziza miniopsis* is known on *Acer* and *Vitis*, and *Prunus* is a new host.

3. Tapesia rosae

Peziza rosae Pers., Obs. Myc. 2: 82. 1799.

Tapesia rosae (Pers.) Fuckel, Symb. myc., **1**: 301. 1869.—Aebi, 1972. Nova Hedw. **23**: 82.

Figs. 5-6

Mollisia rosae (Pers.) P. Karst., Myc. Fenn. 1: 208. 1871.

Lachnea rosae (Pers.) Gill., Discom. de Fr., p. 92.



Fig. 5. Tapesia rosae (TNS-F-11168). B-G: mounted in CB-LA.

Scales. A, 1 μ m; B 50 μ m; C–G, 10 μ m.

A, Fresh apothecia. B, Vertical section of the apothecium. C, Vertical section of the apothecium showing the ectal excipulum and medullary excipulum. D, Close-up of the apothecial base showing the subicular hyphae extending from the ectal excipulum. E, Subicular hyphae. F, Paraphyses. Note cylindrical shape with septa. G, Ascospores.



Fig. 6. Tapesia rosae (TNS-F-11168).

A, Close up of hair-like protrusions, extending from the outermost cells of the ectal excipular cells. B, Subicular hyphae. Note fascicules and curving structure. C, Asci. D, Paraphyses. E, Ascospores. F, Vertical section of apothecium showing ectal excipulum and hair-like protrusions. G, Ectal excipular cells in squash mount. H, Diagrammatic drawing of the apothecial section showing the outline of the structure.

1879.

- Lachnella rosae (Pers.) Quel., Enchir. Fung. p. 312. 1886.
- *Tympanis obtexta* Wallr. var. *pezizaeformis* Wallr., Crypt. germ. **2**: 429. 1833.
- *Tapesia corni* Fuckel., Jahrb. Nass. Verein Naturk. **23–24**: 302. 1870.

Apothecia gregarious, globose when young, becoming urn-shaped by opening at the top, mostly 1-1.5 mm in diameter when dry, sessile, seated on subiculum; disc shallow, pale yellow (C0M0Y40K0), often obscured by the incurving margin when dry. Subiculum gray (C0M0Y0K70), covering the surface of the substrate, composed of brown (C30M60Y100K0) hyphae, $4-5 \,\mu\text{m}$ wide with $1-1.5 \,\mu\text{m}$ thick wall, septate every 10-15 mm, straight to strongly curved, with few branching, extending alone or forming a fascicles of 2-5 hyphae. Ectal excipulum textura globulosa to textura angularis, composed of dark-walled cells, $6-12 \times 5-8 \,\mu m$, arranged somewhat in oblique manner to the outside at the middle receptacle, ending up to hairlike, paler-colored protrusions of $35-80 \times 4-5 \,\mu m$ near the margin, connected to subicular hyphae at the middle receptacle and at the base of apothecia. Medullary excipulum textura oblita, of densely crowded small hyaline cells, $5-10 \times 2-3$ μ m. Asci 47–51×5–6 μ m, cylindrical clavate, eight-spored, from inconspicuous arising croziers, apex conical, pore MLZ+with or without KOH pretreatment. Ascospores $7-10\times$ $2-2.5 \,\mu m$, $(8.5\pm1.1\times2.0\pm0.1 \text{ on average}\pm\text{SD})$, n=27) ellipsoid, aseptate, hyaline, biseriate in the asci. Paraphyses cylindrical to slightly lanceolate with blunt end, simple or branched at the base, aseptate to septate, 3-5 mm wide.

Specimens examined. HONSHU: TNS-F-11168, Sugadaira Montane Research Center, University of Tsukuba, Sanada Machi, Nagano Pref., on *Rosa* branch, 20–V–2002. col. T. Hosoya.

Known distribution. Europe (Aebi, 1972).

Notes. So far no members of the genus Tapesia (Pers.) Fuckel has been reported in Japan, and the present paper includes the first report of its occurrence. The present fungus had been treated in various genera, partly because the genera were ill-defined. Tapesia is distinguished from Mollisia (Fr.) P. Karst. based on the presence of subiculum, but claimed to be continuous with Mollisia, because some Mollisia species have more or less subiculum-like hyphae. Because the limitation of Mollisia and its allied genera seems to be artificial and controversial (Korf, 1973; Baral, 1994), merging *Tapesia* under *Mollisia* has been proposed (Baral, 1994). However, distinguishing Tapesia from Mollisia is practical for identification purpose when the subiculum is remarkable. Because of this practical importance, I accept Tapesia, and dispose the present fungus in Tapesia. Close analysis incorporating molecular data will be required to understand any fundamental difference between Tapesia and Mollisia.

Tapesia rosae is characterized by textura oblita medullary excipulum, haired ectal excipulum.

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