Mycoscience 35: 279–285, 1994

Stromatinia cryptomeriae sp. nov., the teleomorph of *Gloeosporidina cryptomeriae* causing twig blight of Japanese cedar

Takanori Kubono[1] and Tsuyoshi Hosoya[2]

[1] Tohoku Research Center, Forestry and Forest Products Research Institute, Shimo-kuriyagawa, Morioka, Iwate 020–01, Japan (The United Graduate School of Agricultural Sciences, Iwate University, Ueda, Morioka, Japan)
[2] Sankyo Tsukuba Research Laboratories, 33 Miyukigaoka, Tsukuba-shi, Ibaraki 305, Japan

Accepted for publication 3 August 1994)

A new species, *Stromatinia cryptomeriae*, is described based on a specimen collected in Iwate Prefecture, Japan. It was found on fallen dead branches and twigs of Japanese cedar, *Cryptomeria japonica*. The morphology of isolates on potato-dextrose agar (PDA), which were obtained from single ascospores of *S. cryptomeriae*, was identical with *Gloeosporidina cryptomeriae*, the causal fungus of Japanese cedar twig blight. In an inoculation test using single ascospore isolates, many minute black spots (sclerotioid bodies; sclerotules) and acervuli of *G. cryptomeriae* were formed on the necrotic lesions, which developed into typical symptoms of Japanese cedar twig blight. These results show that *Stromatinia cryptomeriae* is the teleomorph of *G. cryptomeriae*. On PDA, the fungus grew over a range of about 1 to 25°C, with the optimum growth at about 15–20°C.

Key Words——*Cryptomeria japonica; Gloeosporidina cryptomeriae*; Japanese cedar twig blight; *Stromatinia cryptomeriae*; teleomorph-anamorph relationship.

Introduction

The twig blight (*kokuten-edagarebyo*) of Japanese cedar (*Sugi; Cryptomeria japonica* D. Don) is distributed widely throughout Japan and is a serious disease in cedar plantations. When the cambial zones of the primary branches are girdled by this pathogen, the branches die quickly, turning red from autumn to early spring (Kubono, 1990). The sites of onset of this disease are the twig buds and male strobilus (Kubono, 1994).

Through isolation and inoculation experiments, the coelomycete *Gloeosporidina cryptomeriae* Kubono (Kubono, 1993) was shown to be the cause of Japanese cedar twig blight (Kubono et al., 1987, 1988; Kubono, 1990, 1994). In March 1993, during a survey for the teleomorph of *G. cryptomeriae*, a discomycete was found on fallen dead branches of Japanese cedar at cedar stands in Morioka, Iwate Prefecture, in the northern part of Honshu, Japan. The same fungus was also repeatedly collected in Ibaraki Prefecture. The fungus seemed to be widely distributed in Japan and was considered to be a possible teleomorph of *G. cryptomeriae*.

Accordingly, the fungus was examined thoroughly as to its taxonomy and pathogenicity in association with *G. cryptomeriae*.

Materials and Methods

Materials and Isolation Materials were collected by the first author in an experimental forest at Tohoku Research Center, Forestry and Forest Products Research Institute (FFPRI) in Morioka, and by the second author in Tsukuba, Ibaraki. Fallen dead branches of Japanese cedar were observed carefully to find apothecia. The apothecia attached to the substrate were brought to the laboratory. A portion of the materials was examined immediately after the collection. The rest was dried at room temperature and kept as dried materials. The dried materials were rehydrated with 3% KOH and examined under both a dissecting microscope and a light microscope. For observation of apothecia, freeze sectioning was employed. Single ascospore isolates were obtained by using Skerman's micromanipulator (Skerman, 1968). The isolates were incubated in the dark at 10°C for about 50 days and then kept on a laboratory desk under natural light at room temperature. The morphological characteristics of the culture were examined under a light microscope.

Cultural characteristics Single ascospore isolates were used throughout the present study. Mycelial growth was measured on eight kinds of agar media at 18°C. These media were potato-dextrose agar (PDA), malt-extract agar, YpSs agar (Udagawa et al., 1978), Czapek

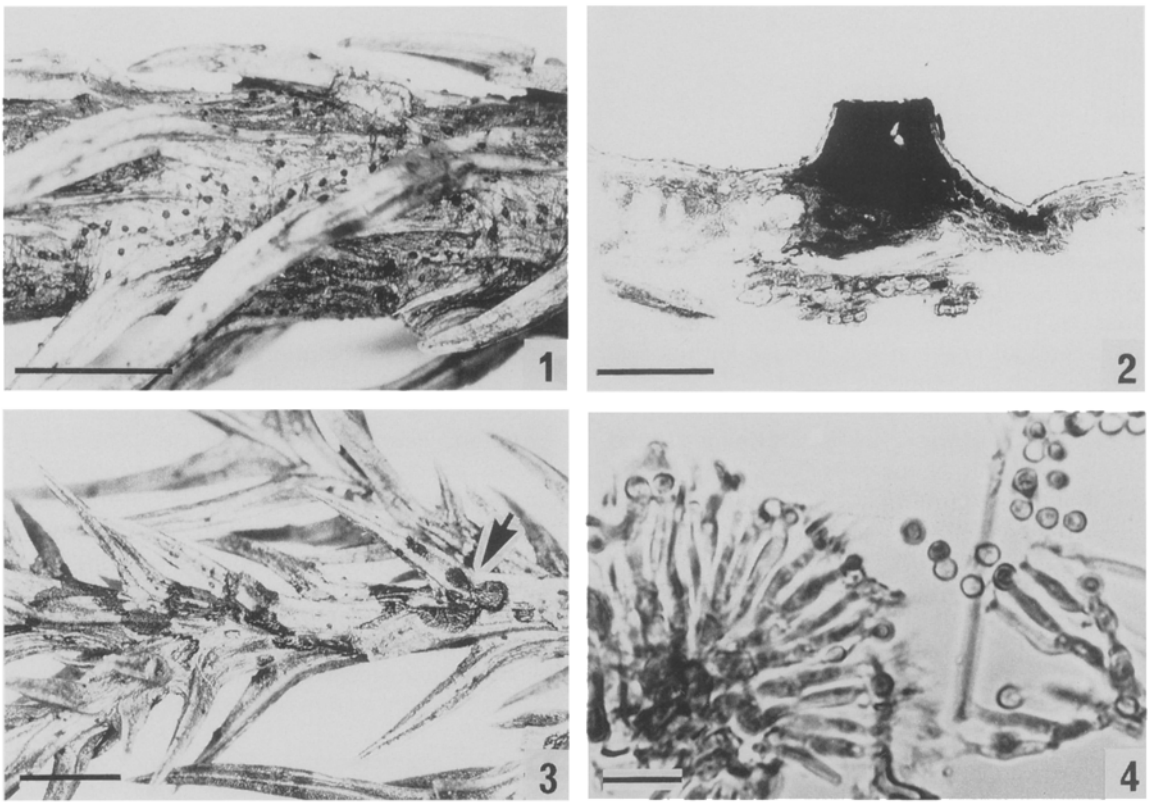
agar, Richards agar (Asuyama et al., 1963), Hopkins agar (Asuyama et al., 1963), needle leaves extract agar (fresh or dried needle leaves 50 g/1 L distilled water), and male strobilus extract agar (fresh or dried male strobili 50 g/1 L distilled water). Colony diameters originated from a single point inoculation on PDA plates were measured at 1, 5, 10, 15, 18, 20, 25, 27 and 30°C after 25 days' incubation. The average of five plates for each medium and temperature was calculated.

Inoculation experiment Inocula were prepared as described by Zinno (1979). Cultures on a PDA plate were cut into 2-3 mm blocks and transferred into a 500-ml flask containing 100 ml of liquid medium (Difco's potato extract powder, 4 g; glucose, 10 g; distilled water, 1,000 ml). After shaking incubation for 15 days at 18°C at 100 r.p.m., agar blocks with mycelia developed into sclerotium-like bodies of 4-5 mm in diam. These sclerotium-like bodies were inoculated onto male strobili of a 20-year-old Japanese cedar tree planted in the nursery of the Tohoku Research Center, FFPRI in April 1993. Twenty twigs with male strobili were inoculated without wounding by putting inocula on the male strobili. For a control, twenty other twigs with male strobili were inoculated by sterile PDA blocks without wounding. The inoculated twigs were observed for six months.

Results and Discussion

Pathogenicity of the isolates Twelve of 20 twigs with male strobili were infected, showing typical symptoms of Japanese cedar twig blight. Many minute black spots (sclerotioid bodies; sclerotules) (Whetzel, 1945) formed on the necrotic lesions in July through August (Figs. 1, 2), together with scab-like black acervuli with globose to ovoid conidia (Figs. 3, 4). The morphological characteristics of the conidial stage were completely identical with those of *Gloeosporidina cryptomeriae* Kubono (Kubono, 1993). This discomycete was therefore considered to be the teleomorph of *G. cryptomeriae*.

Cultural characteristics Colony characteristics varied among media. On PDA, colonies reached 8 cm in diameter after 20 days' incubation and become woolly, greenish gray to dark gray. Radial mycelial growth was poor on Czapek, Richards and Hopkins agars. On needle leaves extract and male strobilus extract agars, colonies were thinner than those on PDA, YpSs agar and malt extract agar. No apothecium was produced on any agar medium. Conidia of *G. cryptomeriae* were produced on PDA, needle leaves extract agar and male strobilus extract agar after about 55 days. Conidial drops were formed in PDA plate, recognized as black patches scattered in the medium. On PDA, a gelatinous layer, which was similar to those shown by Spevak and Korf (1966) for *Moellerodiscus* species, developed on the surface and



Figs. 1-4. Conidial stage, *Gloeosporidina cryptomeriae* and sclerotioid body formed on the necrotic lesions.
1. Sclerotioid bodies. 2. Vertical section of sclerotioid body. 3. Acervuli (arrow head). 4. Conidia and conidiogenous cells. (Scales: 1, 5 mm; 2,200 μm; 3, 5 mm; 4, 10 μm.)

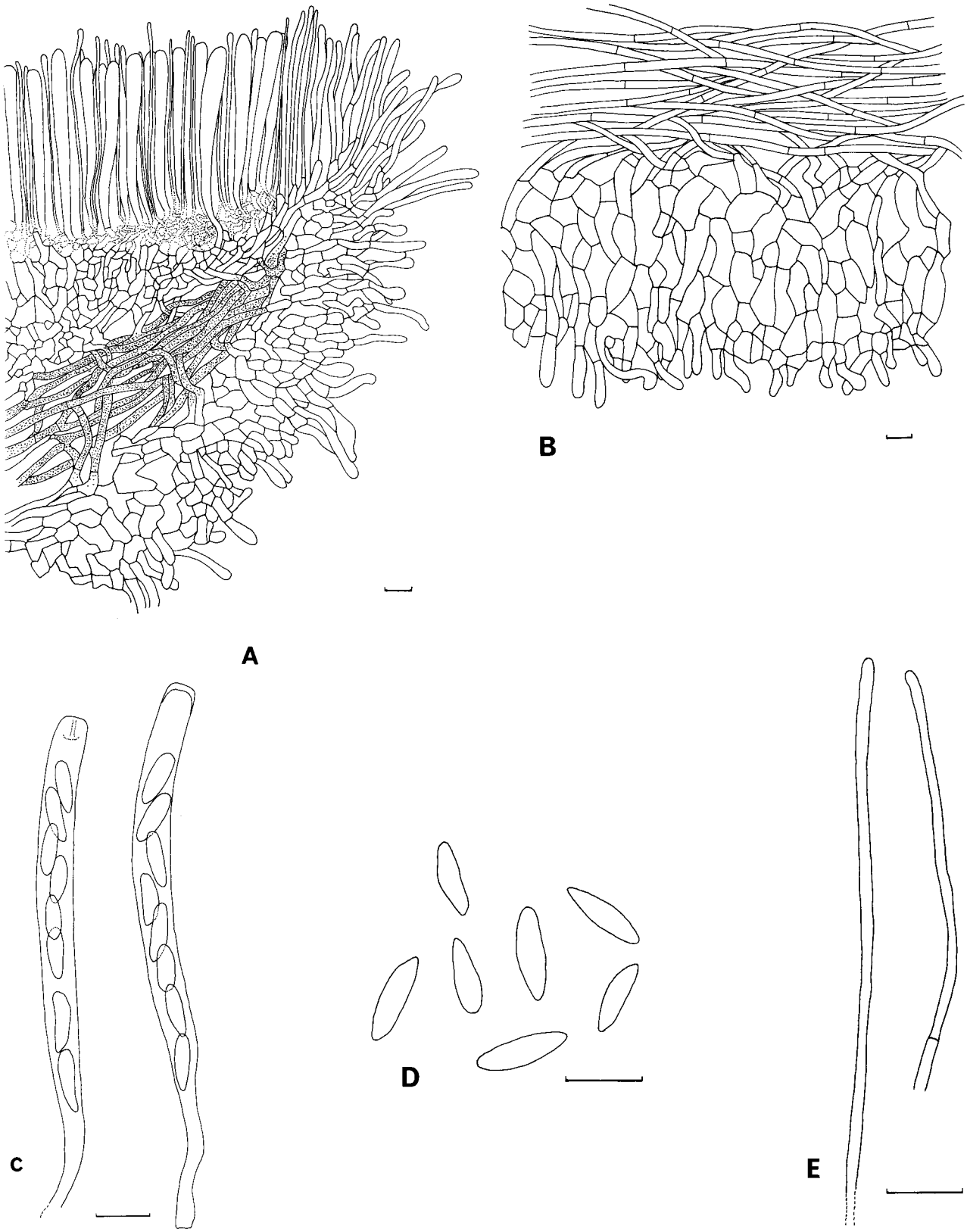


Fig. 5. *Stromatinia cryptomeriae*.
A. Vertical section of marginal part of apothecium.
B. Vertical section of ectal excipulum showing ectal and medulary excipulum.
C. Asci. D. Ascospores. E. Paraphyses. (Scales: A–E, 10 μm.)

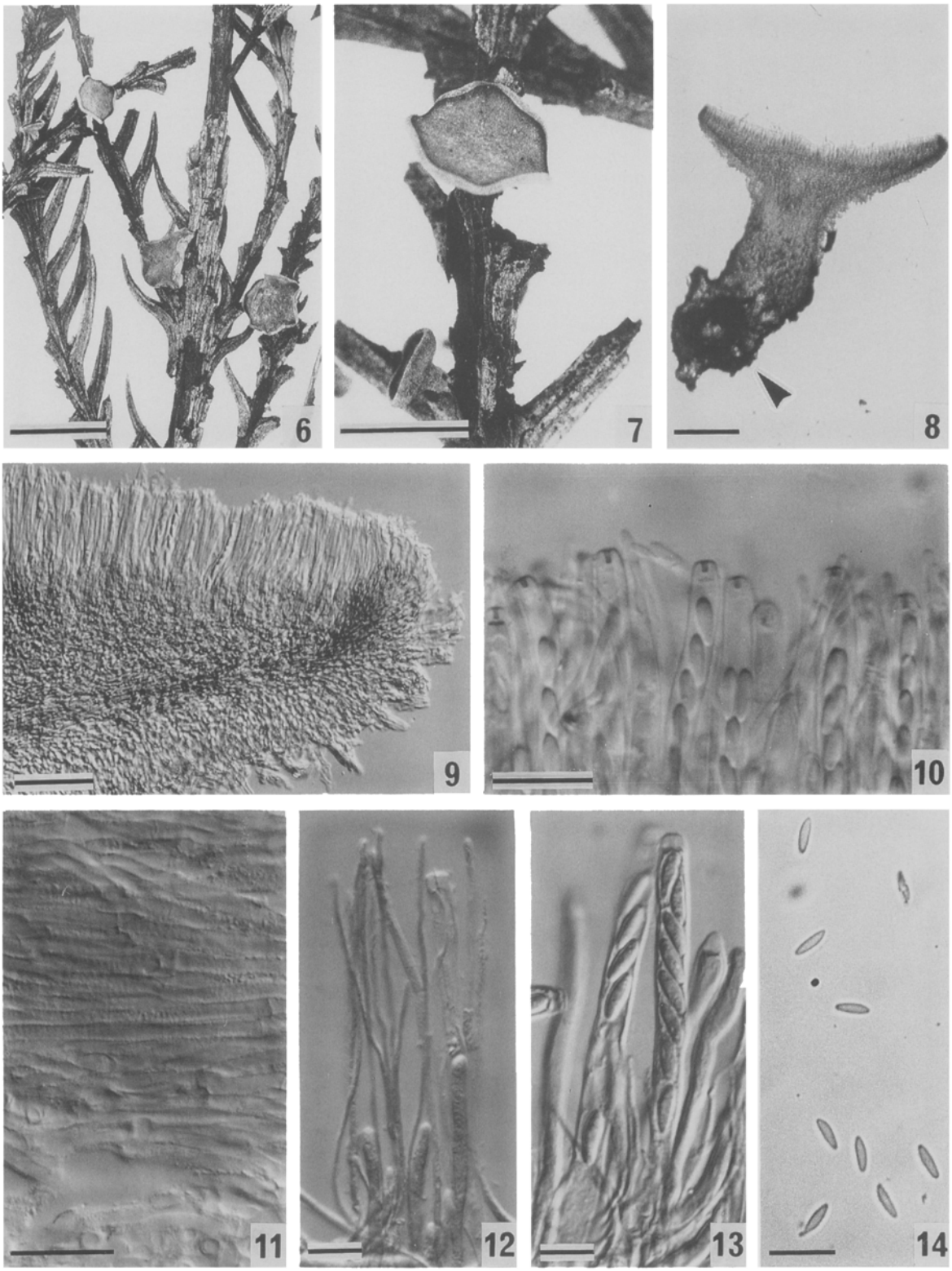


Fig. 6. Apothecia on branches of *Cryptomeria japonica* when dried. (Scale: 1 cm.)
Fig. 7. Apothecia, magnified. (Scale: 5 mm.)
Fig. 8. Vertical section through the apothecium. Arrow head shows stromatized area. (Scale: 1 mm.)
Fig. 9. Vertical section of apothecium showing hymenium, medulary and ectal excipulum. (Scale: 100 μm.)
Fig. 10. Ascus pores stained with Melzer's regent. (Scale: 20 μm.)
Fig. 11. Granulate hyphae in inner ectal excipulum. (Scale: 10 μm.)
Fig. 12. Paraphyses and young asci. (Scale: 10 μm.)
Fig. 13. Asci and paraphyses. (Scale: 10 μm.)
Fig. 14. Ascospores. (Scale: 20 μm.)

in the medium. Colonies were formed at temperatures ranging from 1 to 25°C (Fig. 16). The optimum temperature for growth was 15-20°C, producing dark green to dark gray colonies. Mycelial growth occurred slightly at 1-5°C. At lower temperature, cultures were greenish-white to green. The culture died at over 27°C.

Taxonomy *Stromatinia cryptomeriae* Kubono et Hosoya, sp. nov. Figs. 5-14

Status anamorphus: *Gloeosporidina cryptomeriae* Kubono, Trans, Mycol. Soc. Japan 34, 264, 1993.

Apothecia ramifera, stipitata, disco usque ad 36 mm diametro plano vel paulo concavo, cineraceo-aurantiaco, receptaculo cupulato coriaceo fusco externe capillato. Stipites graciles, usque ad 20 mm longi. Asci 86.5-106×3.5-8.5 μm, inoperculati, cylindraceo-clavati, pedicellati, apice incrassati, jodo caerulescentes. Ascosporae 10-13.5×2.5-3.5 μm, ellipticae vel clavatae, uni-cellulares, hyalinae. Paraphyses 1.5 μm crassae, filiformes, simplices, basi ramosae. Excipulum medullosum 50-90 μm crassum, "textura intricata", ex cellulis aliquantum crassitunicatis in parte fere horizontale dispositis pallide brunneis compositum. Excipulum ectale bistratosum, strato exteriore "textura globulosa" vel "textura prismatica" plus minusve perpendiculare versus paginam receptaculi, strato interiore "textura porrecta" subtiliter granulato. Capilli e cellulis extimis excipuli ectali procurrentes, usque ad 40 μm longi. Stromata in substrato cum cortice intermixta, fusco-brunnea, arcte intertexta, pro linea atra visa in sectione verticali. Sclerotula nigra, 0.2-0.3 mm diametro, apothecium nullum faciens.

Holotype: On dead branches of *Cryptomeria japonica* D. Don, Shimo-kuriyagawa, Morioka-shi, Iwate Pref., Japan, 15 March 1993, T. Kubono, TNS-F 52063 (National Science Museum, Tokyo). Isotype IMI 360556.

Other specimens examined: TNS-F 52064, Makabe-machi, Ibaraki Pref., 7 April 1990, T. Hosoya; TNS-F 52065, Makabe-machi, Ibaraki Pref., 1 March 1992. T. Hosoya.

Apothecia when fresh up to 36 mm in diam, mainly on branches, sometimes on twigs and needles, initially goblet-shaped, later opening to form flat to shallow cupulate disc with short to long stalk, leathery, dark brown all over when fresh, discoid to funnel-shaped, olive brown to grayish orange; outer wall scurfy due to the short hairs on the receptacle surface, with grayish orange hymenium when dried. Stipe slender, up to 20 mm. Hymenium about 100 μm thick, composed of asci and paraphyses. Asci 86.5-106×3.5-8.5 μm, cylindrical to clavate, pedicellate; apex thick, rounded to rather truncate, with a pore recognized as thin cylinder, clearly stained by iodine. Ascospores 10-13.5×2.5-3.5 μm, elliptical to clavate, one-celled, hyaline, monoseriate in the asci. Paraphyses 1.5 μm thick, filiform, simple or branched at the base, straight, septate, slightly thickened at the apex. Subhymenium about 25 μm thick, composed of closely interwoven short cells, compressed, pale brown to brown. Medullary excipulum 50-90 μm thick, "textura intricata" of very closely interwoven, rather thick walled cells, 3.5-5 μm wide with up to 1.5 μm thick wall, in part running almost horizontal, pale brown. Ectal excipulum two-layered; outer layer "textura globulosa" to "textura prismatica", mostly about 25×10 μm, wall thickened at inner part, arranged more or less perpendicular to the receptacle surface; inner ectal excipulum "textura porrecta" of elongated cells, 4 μm thick with finely granulate walls arranged almost parallel to the surface, pale brown colored. Hair-like structures produced from the outermost cells of ectal excipulum and the stalk, protruding outward, up to 40 μm, simple, sometimes enlarged at the apex, hyaline. Stromata substratal, rind composed of strongly brown-colored cells, very closely interwoven, forming black lines in vertical section. Sclerotioid bodies (black sclerotules) of 0.2-0.3 mm in diam, from which apothecia are not arising, produced on the needles.

The granular hyphae in the inner ectal excipulum and the gelatinous matrix produced in culture are similar to those observed in the genus *Moellerodiscus*, which is reduced under *Ciboria* as its synonym (Eriksson and Hawksworth, 1988; Spevak and Korf, 1966). The

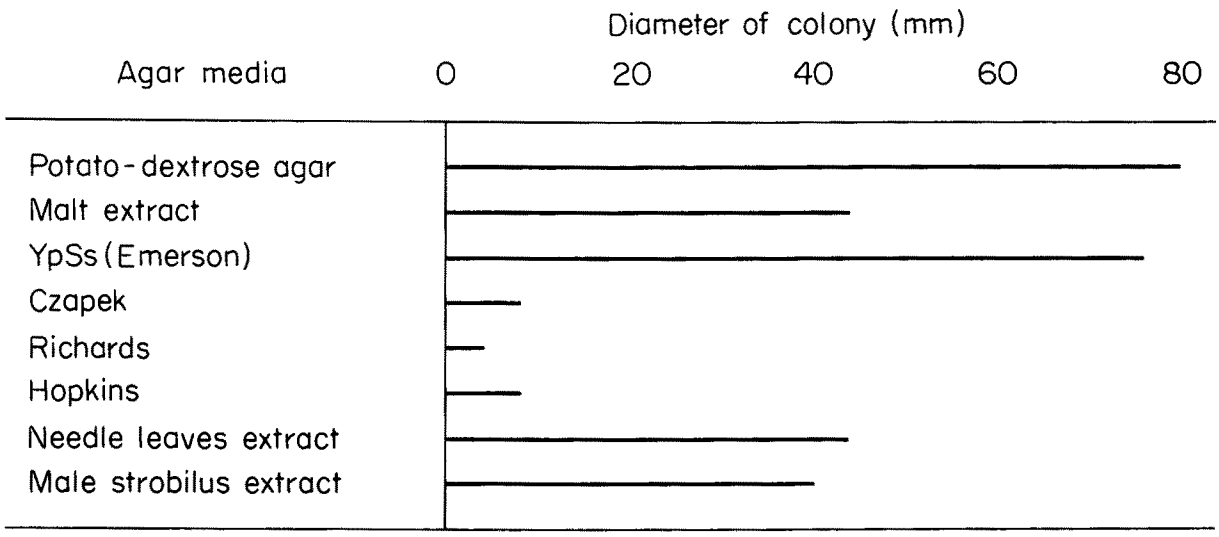


Fig. 15. Mycelial growth of *Stromatinia cryptomeriae* on various agar media at 18°C for 25 days.

Table 1. Comparison of some morphological characters among *Stromatinia* spp.

Species	Apothecia(mm)	Asci(μm)	Ascospores(μm)	Habitat
Stromatinia a) *cryptomeriae*	5.0-36.0 dark brown	86.5-106×3.5-8.5	10.0-13.5×2.5-3.5	Branches, twigs and needle leaves on *Cryptomeria japonica*
S. rapulum b)	2.0-10.0 blackish brown	130-160×8-10	10-16×5.0-7.5	Rhizomes of *Polygonatum*
S. cepivorum c)	-	-	-	
S. gladioli d)	3.0-7.0 cinnamon-brown	190-235×8.5-9.0	10-17×5.5-9.5	Bulbs on *Acidanthera, Crocus, Freesia, Gladiolus, Laperirousia* and *Tritonia*
S. paridis b)	1.0-2.0 tawny-cinnamon	130-140×8-10	10-13×4-6	Root of *Paris quadrifolia*
S. smilacinae e)	10.0-30.0 bright cinnamon-brown	120-140×6-8	12-15×4-5	Dead rhizomes of *Smilacina racemosa*
S. narcissi f)	2.0-4.0 reddish brown	135-190×8.0-10.0	10.0-15.0×4.0-5.5	Outer papery scales of *Narcissus* and *Zephyranthes* bulbs

a) The authors; b) Boudier (1907); c) Whetzel (1945), the mantling stroma and apothecia are unknown in this species, but sclerotules are formed both in culture and under natural conditions; d) Dennis (1956); e) Seaver (1951); f) Drayton and Groves (1952).

present species, however, differs slightly from *Moellerodiscus* in the structure of the outer ectal excipulum. The outer ectal excipulum is composed of "textura angularis" to "textura globulosa" with a thin wall in *Moellerodiscus*, but "textura globulosa" to "textura prismatica" with gelatinized thick walls in *Stromatinia* (Dennis, 1956). As the two genera are very closely related in the structure of apothecia, this difference is rather subtle.

A distinguishing characteristic of the genus *Stromatinia* is the presence of the stromata in two different forms: a thin, subcuticular stroma mantling the substrate from which the apothecia arise, and smaller, sclerotioid bodies (black sclerotules) which do not produce apothecia (Drayton and Groves, 1952; Whetzel, 1945). From the descriptions of *S. rapulum, S. gladioli* (Drayton) Whetzel (Whetzel, 1945) and *S. narcissi* Drayton et Groves (Drayton and Groves, 1952) it is clear that the apothecia arise from the stroma but not from the sclerotioid bodies. As the apothecia of the present fungus also arise from the stromatic tissue, and not from the sclerotioid bodies, it is concluded that the present fungus should be placed in the genus *Stromatinia*.

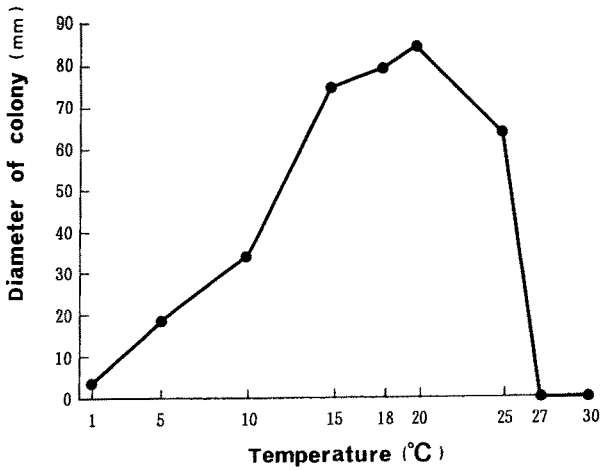


Fig. 16. Relation between temperature and mycelial growth on potato-dextrose agar after 25 days.

Since the establishment of the genus *Stromatinia* by Boudier (1907) with *S. rapulum* (Bull. ex Fr.) Boud. as its type, six species have been recognized (Hawksworth et al., 1983). The present species can be clearly distinguished from hitherto known *Stromatinia* species in the morphology of apothecia and asci (Table 1). It has the shortest asci. It is also clearly separable by the ascospore width, but not by ascospore length. It is further distinguished by formation of largest apothecia, which are dark brown in this species, but commonly cinnamon-brown in other species of the genus. Moreover, the present species has distinctive ecological aspects: its substrate is fallen needles and branches on the ground, while other *Stromatinia* species inhabit root's, rhizomes and bulbs of herbaceous plant under the ground.

In March 1993, apothecia of *S. cryptomeriae* were found on fallen dead branches in the experimental forest at Tohoku Research Center, just after the continuous snow cover had melted. Apothecia with mature ascospores were observed until the end of April. The monthly average temperature in the forest in March and April 1993 was 1.8 and 6.9°C, respectively. The examination of mycelial growth at various temperatures indicated that the fungus is fully able to grow at such low temperature's as 1 and 5°C (Fig. 16). In that over 90% of ascospores germinate on water agar within 48 h at 5 to 25°C (date omitted), it seems that *S. cryptomeriase* has a competitive advantage during cold periods. A similar feature was suggested by Wicklow and Malloch (1971) for *Thelebolus* species. *Stromatinia cryptomeriae* seems to be capable of growing vigorously and producing apothecia while lying beneath a continuous snow cover.

Kubono (1994) clearly showed that the site of onset of twig blight was the male strobilus. The pollen in the male strobili begins to disperse in the middle of March in Morioka. This coincides with the period of vigorous production of apothecia in nature. As *S. cryptomeriae* grows luxuriantly in the male strobilus extract medium which contains pollen, the infection of *S. cryptomeria* may be enhanced by the presence of pollen grains as nutrients.

Acknowledgements——The authors would like to thank Dr. S. Kaneko for his advice in the present study. We are also grateful to Dr. Y. Otani for his suggestions concerning *Stromatinia cryptomeriae*. We wish to thank Dr. T. Shoji, Dr. N. Sahashi and Dr. K. Furuya for their kind support and cooperation.

Literature cited

Asuyama, H., Mukoo, H. and Suzuki, N. 1962. "Laboratory guide for plant pathologists," Japan Plant Protection Association, Tokyo. 843 p. (in Japanese).

Boudier, E. 1907. Histoire et classification des discomycetes d'Europe, Paris. 108 p.

Dennis, R. W. G. 1956. A revision of the British Helotiaceae in the Herbarium of the Royal Botanic Gardens, Kew, with notes on related European species. Mycol. Papers **62**: 1—216.

Drayton, F. L. and Groves, J. W. 1952. *Stromatinia narcissi*, a new sexually dimorphic discomycete. Mycologia **44**: 119–140.

Eriksson, O. E. and Hawksworth, D. L. 1988. Notes on ascomycete systematics. Systema Ascomycetum **7**: 59—102.

Hawksworth, D. L., Sutton, B. C. and Ainsworth, G. C. 1983. "Ainsworth & Bisby's Dictionary of the Fungi, 7th ed.," Commonwealth Mycol. Inst., Kew, 445p.

Kubono, T., Yokosawa, Y. and Kaneko, S. 1987. Isolation and morphological characteristics of Japanese cedar twig blight causal fungus. Trans. **39**th Meet. Tohoku Branch Jpn, For. Soc.: 223—225 (in Japanese).

Kubono, T., Yokosawa, Y. and Kaneko, S. 1988. On the twig blight of Japanese cedar and its causal fungus. 5th Int. Cong. Plant Pathol., Abstracts of Paper: 375.

Kubono, T. 1990. Pathology of Japanese cedar twig blight. In: "Recent research on foliage disease," pp. 71—79. Conference Proc. of IUFRO, USDA Forest Service General Technical Report WO—56.

Kubono, T. 1993. *Gloeosporidina cryptomeriae* sp. nov. causing twig blight of Japanese cedar (*Cryptomeria japonica*). Trans. Mycol. Soc. Japan **34**: 261—265.

Kubono, T. 1994. Symptom development of the twig blight of Japanese cedar caused by *Gloeosporidina cryptomeriae*. J. Jpn. For. Soc. **76**: 52—58.

Seaver, F. J. 1951. "North American cup-fungi," Hafner Publishing, New York., 89 p.

Skerman, V. B. D. 1968. A new type of micromanipulator and microforge. J. Gen. Microbiol. **54**: 287–297.

Spevak, M. B. and Korf, P. R. 1966. On *Ciboriopsis simulata* and the genus *Ciboriopsis*. Lloydia **29**: 130–135.

Udagawa, S., Tubaki, K., Horie, Y., Miura, K., Minoura, K., Yamazaki, K., Yokoyama, T. and Watanabe, S. 1978. "Kinrui-Zukan, vol. 2," Kodanshya, Tokyo, 556 p. (in Japanese)

Whetzel, H. H. 1945. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate discomycetes. Mycologia **37**: 648—714.

Wicklow, D. and Malloch, D. 1971. Studies in the genus *Thelebolus*: Temperature optima for growth and ascocarp development. Mycologia **63**: 118—131.

Zinno, Y. 1979. Studies on artificial sporulation of *Cercospora sequioae* Ellis et Everhart, the needle blight fungus of *Cryptomeria japonica*. Bull. For. Forest. Prod. Res. Inst. **302**: 1—77 (in Japanese).