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A new *Ophiostoma* species with a *Graphium* anamorph from *Larix laricina* in eastern North America

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Abstract: The ophiostomatoid fungi are an economically important group that include various destructive plant pathogens. Knowledge regarding the occurrence of these fungi on larch (*Larix* spp.) and their relationship with insects associated with this host is limited. Recent isolations from *Larix laricina* in eastern North America infested with *Dendroctonus simplex*, revealed an apparently new *Ophiostoma* species. This taxon superficially resembles *O. cainii* but could be distinguished from the latter species, based on its robust perithecia that were much larger than those of *O. cainii*. The *Graphium* anamorph of this fungus was also substantially smaller than that of *O. cainii*. After thorough comparison of this fungus with the type specimen and the original description of *O. cainii*, we conclude this fungus represents a new taxon and it is thus described here as *O. simplex*.

Key Words: *Dendroctonus*, *Larix*, *Ophiostoma cainii*, systematics

INTRODUCTION

Members of the genus *Ophiostoma* Syd. & P. Syd. represent an economically important group of fungi that includes many plant pathogens. Perhaps the best known of these destructive pathogens are *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier (Gibbs, 1978; Brasier, 1991). The success of these and other ophiostomatoid fungi as plant pathogens can possibly be attributed to their association with insect vectors, especially bark beetles (Münch, 1907; Lagerberg et al., 1927; Nelson, 1934). However, the nature of this association and the role that these fungi play in killing trees has been a source of speculation and controversy (Lagerberg et al., 1927; Graham, 1967; Berryman, 1972; Harrington, 1993).

Species of *Ophiostoma* have long been known to be associated with bark beetle (Coleoptera:Scolytidae) vectors (Münch, 1907; Nelson, 1934; Lagerberg et al., 1927; Craighead, 1928). This insect vector association is especially well-known on conifers and has been intensively studied in the Northern hemisphere and most notably in Europe and North America (Bramble and Holst, 1940; Mathiesen 1950; Mathiesen-Kääarik, 1953; Mathiesen-Kääarik, 1960; Mathre, 1964; Solheim, 1986; 1992a,b; 1993; Harrington, 1993). This association can be very specific, as in the case of *O. penicillatum* (Grosmann) Siemaszko that is almost always associated with *Ips typographus* L. on spruce (Davidson et al., 1967; Solheim, 1986; 1992a,b; 1993). Also, it can be rather casual such as in the case of *O. piceae* (Münch) Syd. and P. Syd., which is known to be associated with many different bark beetle-fungus complexes (Mathiesen 1950; Mathiesen-Kääarik, 1953, 1960).

The occurrence of ophiostomatoid fungi on conifers such as pine (*Pinus* spp.) and spruce (*Picea* spp.) in various parts of the world has been well documented (Lagerberg et al., 1927; Mathiesen-Kääarik, 1960; Aoshima, 1965; Griffin, 1966; Olchowecki and Reid, 1974; Solheim, 1986; Hutchinson and Reid, 1988). Surprisingly few studies have considered these fungi on larch (*Larix* spp.) and the bark beetles that infest them. Some of the species known to occur on larch include *C. laricicola* Redfern & Minter, causing die-back and death of *L. decidua* Miller in Scotland and Japan (Redfern et al., 1987; Harrington et al., 1996), *C. allantospora* Griffin on *L. laricina* (Du Roi) K. Koch in Canada (Griffin, 1966), *C. autographa* Bakshi and *O. galeiformis* (Bakshi) Math.-Kääarik causing sapstain on *L. kaempferi* (Lamb) Carr. in Britain (Bakshi, 1951; Hunt, 1956), *Ceratocystiopsis falcata* (Wright & Cain) H. P. Upadhyay on *Larix* sp. in New Zealand (Hutchinson and Reid, 1988) and *O. piceae* on *Larix* sp. in New Zealand as well as in Japan (Aoshima, 1965, Hutchinson and Reid, 1988). Other species from Japan include *C. coerulescens* (Münch) Bakshi, *O. brunneo-ciliatum* Math.-Kääarik, *O. minus* (Hedgc.) Syd. & P. Sydow, *O. olivaceum* Math. (Aoshima, 1965) and *O. laricis* Van der Westh. et al. (Van der Westhuizen et al., 1996) on various species of *Larix*.

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There has been no previous study of fungi associated with *Dendroctonus simplex* LeConte (eastern larch beetle) infesting *L. laricina* in North America. This is surprising given the fact the insect is a serious pest of *L. laricina* throughout Northern America. The aim of the study was to isolate and identify ophiostomatoid fungi from *D. simplex* and/or from its galleries in order to expand our knowledge of these ophiostomatoid fungi in North America.

MATERIALS AND METHODS

Samples were collected by D. R. Bergdahl and M. J. Wingfield from *L. laricina* infested with *D. simplex* in northern Vermont, USA. Ascospore masses were collected from the apices of perithecia, cultured on 2% MEA (20 g Difco malt extract, 20 g Difco agar, 1000 mL distilled water), incubated at 25°C and examined at the onset of sporulation. All isolates used in this study are maintained in the culture collection of M. J. Wingfield (CMW 3412, CMW 3413, CMW 3414 and CMW 3415) and the Centraalbereau voor Schimmelcultures (CBS 505.96, CBS 506.96, CBS 507.96, CBS 508.96)

Herbarium material of *O. cainii* (Olchow. & J. Reid) T. C. Harr. (WIN(M)71–13 and WIN(M)69–18) housed in the herbarium of the University of Manitoba, Winnipeg, Manitoba, Canada was supplied by Prof. J. Reid.

All measurements were done on fungal structures produced in culture on the media described above. Slides representing teleomorph and anamorph structures were mounted in lactophenol amended with cotton blue. Fifty measurements of each relevant morphological structure were made and ranges and averages computed.

Isolates were also examined using scanning electron microscopy (SEM). Small blocks of agar cut from sporulating colonies were fixed in 3% glutaraldehyde and 0.5% osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded acetone series and critical-point dried. Specimens were mounted and coated with gold palladium alloy and examined using a JSM 6400 scanning electron microscope.

The rates of growth of isolates (CBS 505.96; CBS 506.96, CBS 507.96, CBS 508.96) in culture were determined at temperatures ranging from 5 to 40°C at 5°C intervals. Petri dishes containing 20 mL of agar were inoculated in the center with a 5 mm diam disc taken from the edge of an actively growing colony. Five replicate plates were prepared for each isolate and incubated at each temperature. Colony diameters were measured twice perpendicular to each other and growth was computed as an average of ten readings (five plates with two measurements per plate).

Cycloheximide tolerance, a characteristic used to differentiate between *Ophiostoma* and *Ceratocystis* (Harrington, 1981), was determined by measuring growth at different concentrations (0, 0.05, 0.1, 0.5, 1, 2.5%) of this antibiotic. Cycloheximide tolerance was determined in the same manner as in the growth studies using colonies incubated in the dark at 25°C.

RESULTS

The fungus predominantly isolated from *L. laricina* possessed robust dark perithecia, hat-shaped ascospores, and a relatively nondescript *Graphium* anamorph. This combination of characters led us to conclude that the isolates from larch were similar to *O. cainii* (FIGS. 1–4), a species reported from *Picea mariana* (Olchowecki & Reid, 1974). However, *O. cainii* has not been associated with any species of bark beetles, whereas the fungus from larch was found in trees infested with *Dendroctonus simplex*. Isolates of the species from larch could also be distinguished from *O. cainii* on the basis of morphological criteria. *Ophiostoma cainii* possess smaller perithecia (850 vs. 1600 μm neck lengths for the species from larch) and a *Graphium* anamorph with significantly larger synnemata (300–650 μm vs. 77–350 μm for the species from larch). Based on these results we concluded the fungus from larch represents a distinct taxon and it is described as follows:

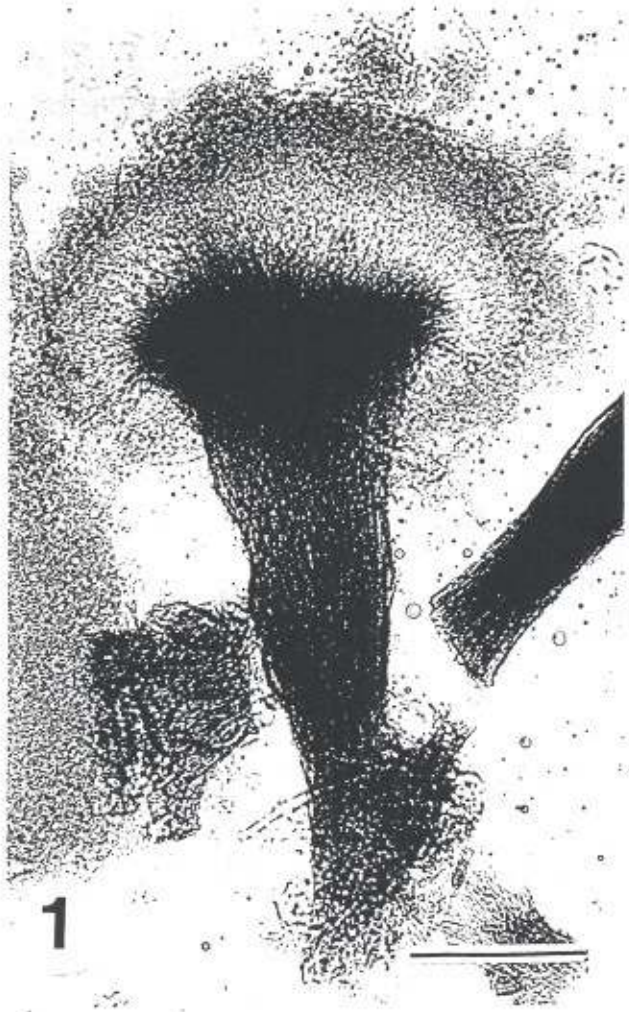
Ophiostoma simplex Jacobs et M. J. Wingf., sp. nov. FIGS. 5, 6, 9

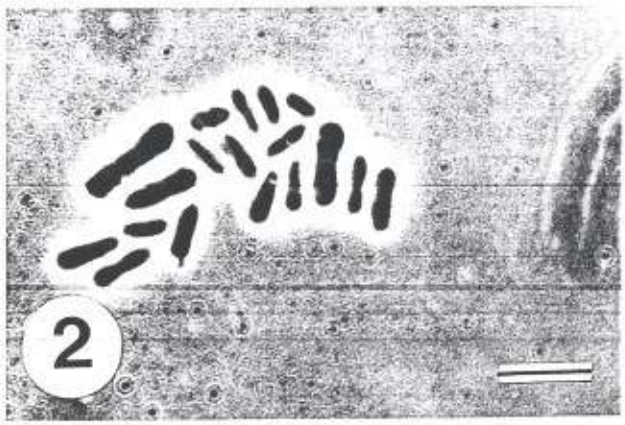
Perithecia evenientia singulatim, superficialia, bases atrae, globosae, glabro-tunicatae ornatione abundante in inferioribus dimidiis basium perithecialium et deminuente in superioribus dimidiis basium. Bases 170–460 μm diametro, colla atra, cylindrica exigua angustatione apicali versus apicem, levia, 270–1600 μm longa, 20–40 μm lata ad apicem, 50–140 μm lata supra basim globosam, hyphae ostiolares absentes. Asci prototunicati, hyalini, evanescentes. Ascosporae aseptatae, cucullatae cum a latere visae sunt, vaginatae, 3–4 × 2–2.5 μm. Status anamorphicus *Graphium simplex* Jacobs & M. J. Wingf.

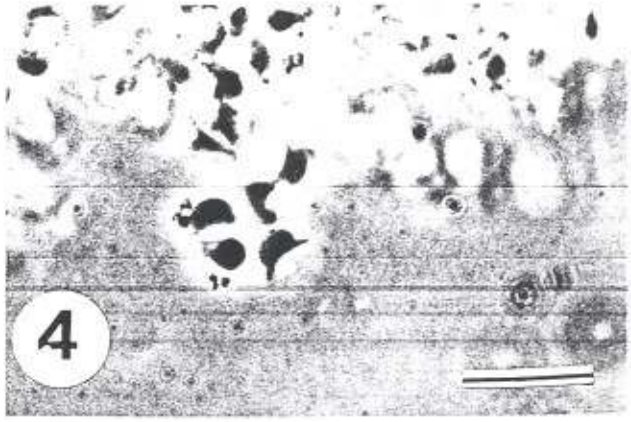
HOLOTYPUS. PREM 54684.

Graphium simplex Jacobs et M. J. Wingf., sp. nov. FIGS. 7–12, 14

Coloniae incremento optimo ad 25°C in MEA, attingentes 33 mm diametro octo diebus. Coloniae subbrunneae fusciorescentes dum senescunt. Incremento minimo ad 5°C et 35°C. Resistunt densissimo soluto "cycloheximide" incremento 40% deminuto in 2.5% cycloheximide post 8 dies ad

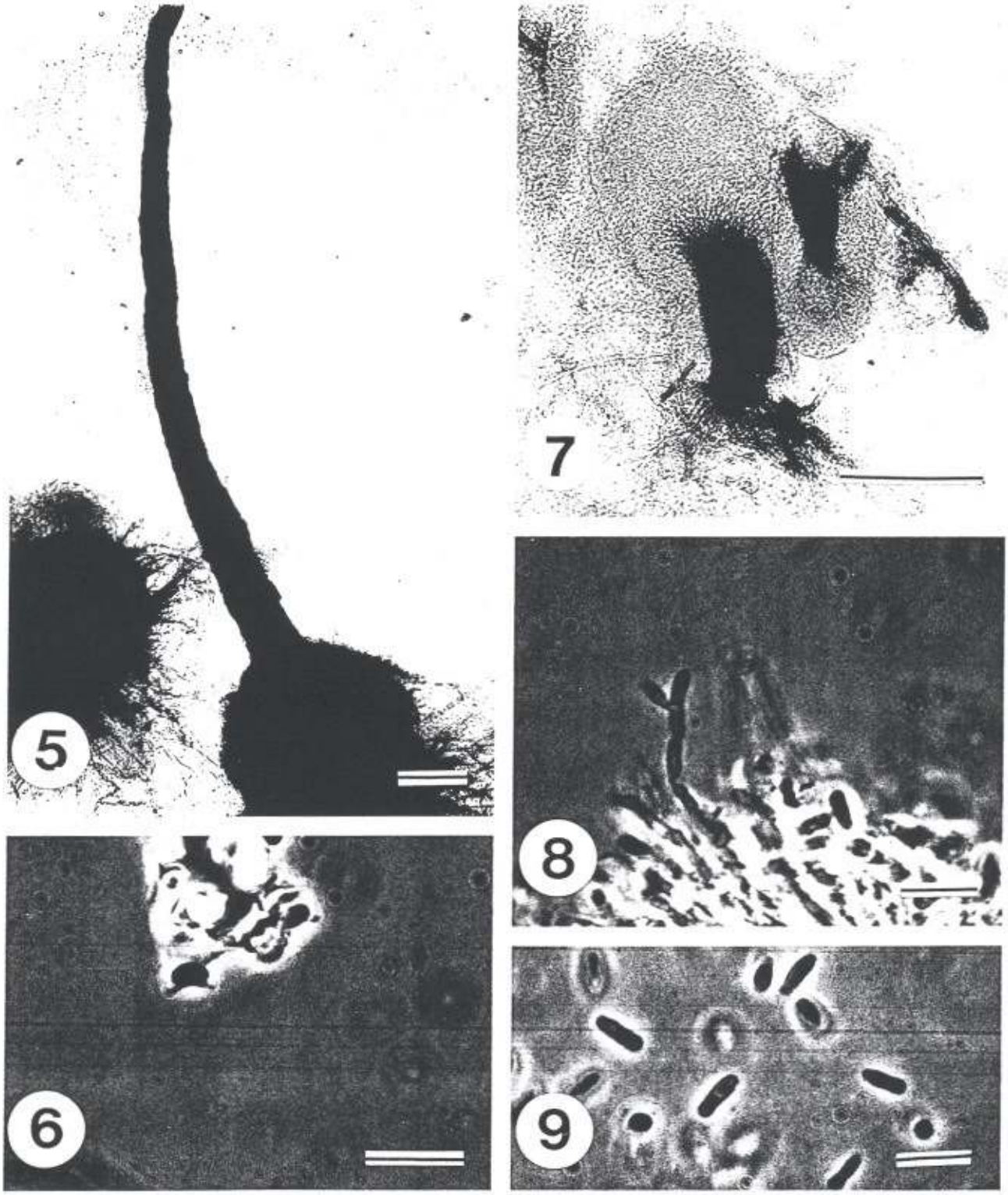




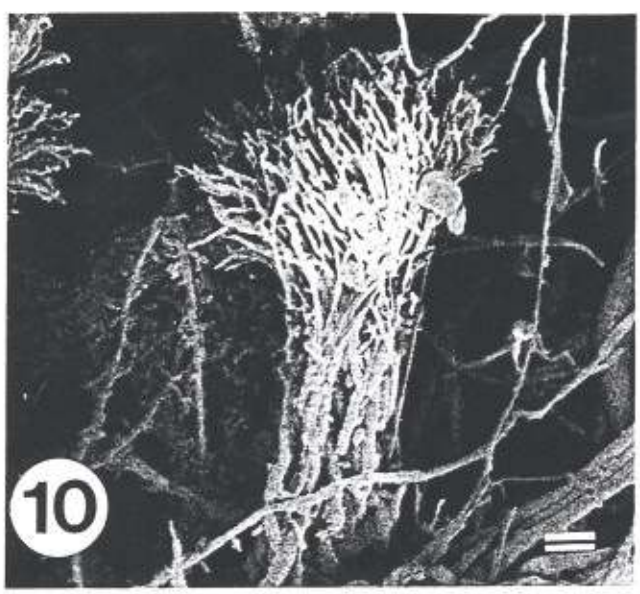


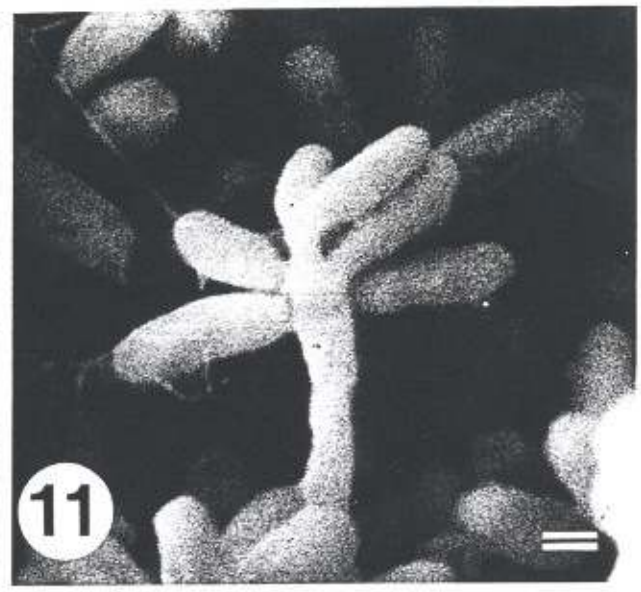
FIGS. 1–4. Teleomorph and anamorph characteristics of *Ophiostoma cainii* (WIN(M) 71–13). 1. *Graphium* synnemata with fan-shaped conidiogenous apparatus. 2. Oblong conidia with rounded apex and subtruncate base. 3. Perithecium. 4. Hat-shaped ascospores with sheaths. Scale bars: FIGS.1, 2, 4 = 10μm; 3 = 100μm.

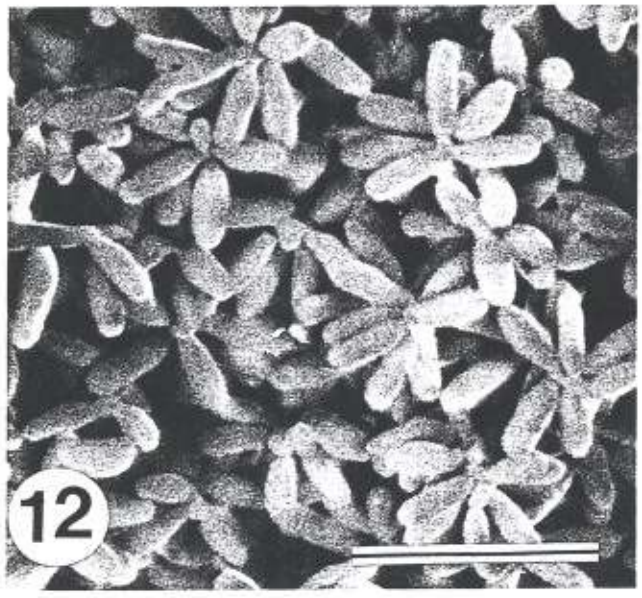
25°C in tenebris. Hyphae immersae in medio sparsis aeriis myceliis, hyalinae vel subbrunneae, leves, 3–7 μm diametro. Synnemata solitaria vel bina aggregata, exorientia directe ex mycelio, oriunda ex duabus vel pluribus cellis, erecta, macronematosa, synnematosa, levia, olivacea vel subbrunnea, saepe constricta ad septa, 77–356 μm longitudine, rhizoidea absentia. Synnemata flabelliformia, 32.5–187.5 μm loco latissimo. Conidiogenae cellae discretae, 1–4 per ramum, exigue attenuatae a basi ad apicem, cylindricae, rectae, 7–20 μm longae et 1–2 μm latae. Auctus conidii eveniens pariete reponendi causa constructo, holoblastica ontogenie et sympodiale proliferatione. Conidia hyalina, asep-



FIGS. 5–9. Teleomorph and anamorph characteristics of *Ophiostoma simplex* (PREM 54684). 5. Perithecium. 6. Hat-shaped ascospores with sheaths. 7. *Graphium* synnemata. 8. Conidiogenous cells showing sympodial proliferation. 9. Oblong conidia with rounded apex and subtruncate base. Scale bars: FIG. 6, 7, 8, 9 = 10 μm; 5 = 100 μm.







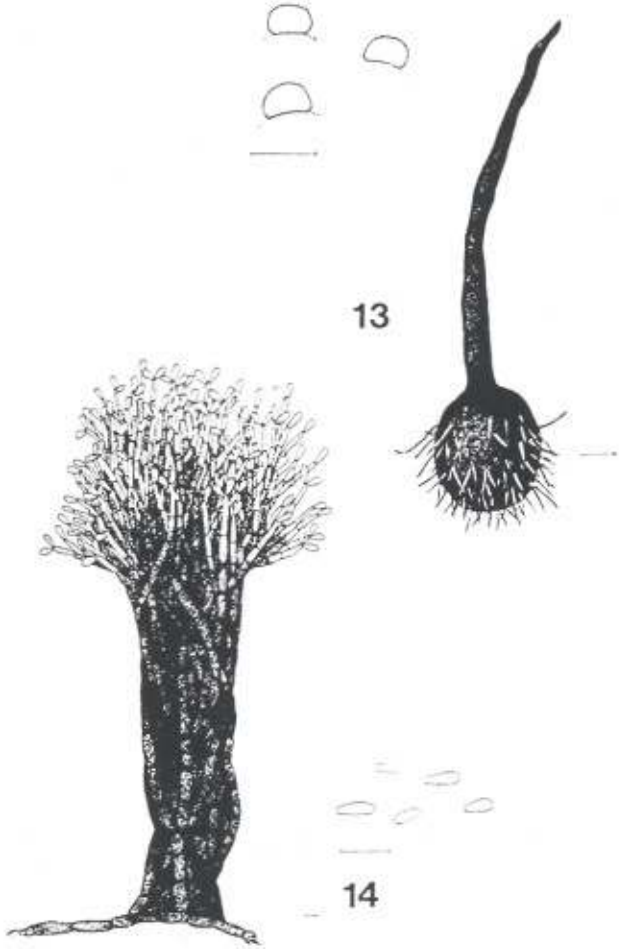
FIGS. 10–12. Scanning electron microscope view of anamorph characteristics of *Ophiostoma simplex* (PREM 54684). 10. *Graphium* anamorph. 11. Conidiogenous cell showing sympodial conidium development. 12. Oblong conidia with rounded apex and subtruncate base. Scale bars: FIG. 11 = 1 μm; 10, 12 = 10 μm.

tata, obovoidea apicibus rotundatis et subtruncatis basibus, 4–7 × 1–2.5 μm. Conidia accumulantur ad apices synnematum in massis pallidis et albis. Status teleomorphicus *Ophiostoma simplex* Jacobs & M. J. Wingf.

HOLOTYPUS. PREM 54684.

Perithecia occurring singly, superficial, black at base, globose, smooth walled with abundant hyphal ornamentation on the lower half of perithecial base and reducing in abundance in the upper half of the base; base 170–460 μm diam., neck black, cylindrical slightly tapering towards the apex, smooth, 270–1600 μm long, 20–40 μm wide at apex, 50–140 μm wide above the globose base, ostiolar hyphae absent (FIGS. 5, 13). *Asci* evanescent. *Ascospores* aseptate, cucullate in side view, sheathed, 3–4 μm × 2–2.5 μm (FIGS. 6, 13).

Colonies with optimal growth at 25°C on MEA, reaching 33 mm in diam. in eight days. Colonies light brown becoming darker with age. Minimal growth at 5°C and 35°C. Able to withstand high concentrations of cycloheximide with a 40% reduction in growth on 2.5% cycloheximide after 8 d at 25°C in the dark. *Hyphae* immersed in medium with sparse aerial mycelium, hyaline to pale brown, smooth, 3–7 μm diam. *Synnemata* single or in groups of two, arising directly from mycelium, originating from two or more cells,



FIGS. 13, 14. Teleomorph and anamorph characteristics of *Ophiostoma simplex* (CBS 505.96). 13. Perithecium and ascospores surrounded with sheaths of *Ophiostoma simplex*. 14. *Graphium* anamorph narrowly clavate. Scale bars: 10 μm.

erect, macronematous, synnematous, smooth, olivaceous to light brown, frequently constricted at septa, 77–356 μm in length, rhizoids absent. Synnemata fan-shaped at the apex, 32.5–187.5 μm at the broadest point (FIGS. 7, 10, 14). *Conidiogenous cells* discrete, 1–4 per branch, tapering slightly from base to apex, cylindrical, straight, 7–20 μm long and 1–2 μm wide (FIG. 8). Conidium development occurring holoblastic ontogeny and sympodial proliferation (FIG. 11). *Conidia* hyaline, aseptate, oblong to narrowly clavate with rounded apices and subtruncate bases, 4–7 × 1–2.5 μm (FIGS. 9, 12, 14). Conidia accumulating at the apices of synnemata in a pale white gelatinous mass.

HOLOTYPE. UNITED STATES. VERMONT: Lamoille County, Wolcott, isolated from dead *Larix laricina* infested with *Dendroctonus simplex*, May 1994, *M. J. Wingfield and D. R. Bergdahl* (*PREM 54684*, dry culture on 2% malt extract agar; with both teleomorph and anamorph. Ex-type live culture deposited at Centraal Bureau voor Schimmelcultures, *CBS 505.96*).

Paratypes. Data as holotype (PREM 54685, PREM 54686, PREM 54687: dry cultures on 2% malt extract agar; with both teleomorph and anamorph. Live cultures deposited at Centraal Bureau voor Schimmelcultures, respectively *CBS 506.96, CBS 507.96, CBS 508.96*).

DISCUSSION

Ophiostoma simplex resembles four other species of *Ophiostoma*, viz. *O. cainii, O. davidsonii* (Olchow. and J. Reid) H. Solheim, *O. cucullatum* H. Solheim and *O. ainoae* H. Solheim. *Ophiostoma simplex*, however, occurs on larch while the others are found on spruce or, as in the case of *O. davidsonii*, on *Pseudotsuga menziesii* (Mirb.) Franco (Olchowecki and Reid, 1974; Solheim, 1986). *Ophiostoma cucullatum* and *O. ainoae* are both associated with *Ips typographus* in Europe while *O. cainii* and *O. davidsonii* have not been associated with any single insect vector (Olchowecki and Reid, 1974; Solheim, 1986).

Ophiostoma simplex has crescent-shaped ascospores and can thus easily be distinguished from *O. davidsonii* and *O. ainoae*, species that have cylindrical-shaped ascospores (Olchowecki and Reid, 1974; Solheim, 1986). The absence of ostiolar hyphae in *O. simplex* distinguishes it from *O. cucullatum*, which has well developed ostiolar hyphae (Solheim, 1986). *Ophiostoma cainii*, the species most similar to *O. simplex*, possesses smaller perithecia and larger synnemata.

Ophiostoma simplex occurs on larch infested with *D. simplex* whereas *O. cainii* is known to occur on black spruce (Olchowecki and Reid, 1974). Both species produce a *Graphium* anamorph but the synnemata of *O. simplex* are significantly smaller, than those of *O. cainii* . Olchowecki and Reid (1974) described the synnemata of *O. cainii* as fan-shaped structures arising from a single cell. *Ophiostoma simplex*, also produces fan-shaped synnemata but these arise from more than one cell and are not as broad as those found in *O. cainii*. Because conidia of both species are of a similar size and shape, these characters cannot be used to separate the species. Perithecia of *O. simplex* are larger and more robust than those of *O. cainii*, and the perithecial necks of *O. simplex* are twice as long as those of *O. cainii*.

In Japan, larch is commonly infested by the bark beetle *Ips cembrae* Heer and the predominant fungi associated with larch in Japan include *C. coerulescens, O. brunneo-cilliatum, O. minus, O. olivaceum, O. piceae* (Aoshima, 1965) and the newly described, *O. lar-*

icis (Van der Westhuizen et al., 1996). In Europe and Japan, *L. decidua* infested with *I. cembrae* is known to be infected with *C. laricicola* (Harrington et al., 1996), although this species is probably conspecific with *C. polonica* (Visser et al., 1995). It is perhaps not surprising that *O. simplex* has not been found on larch in Europe and Japan given that the insect species associated with this fungus infests unrelated tree genera.

Ophiostoma spp. on larch in North America and the insects associated with them deserve further investigation. Future studies are planned that will consider the distribution, vectoring and virulence of *O. simplex*. Our knowledge of the taxonomy of the ophiostomatoid fungi will increase as additional species such as *O. simplex* are collected and described. Little attention has been given to this group in many parts of the world and this situation will hopefully change in near future.

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