Three new species of *Ophiostoma* and notes on *Cornuvesica falcata*

G. Hausner, G.G. Eyjólfsdóttir, and J. Reid

Abstract: Sampling of beetles, beetle galleries, and stained tree tissues by ourselves and others to obtain isolates of ophiostomatoid fungal species yielded three *Ceratocystiopsis*-like entities. Using partial rDNA sequences, these were previously identified as being different from both each other and all other described species of *Ceratocystiopsis* and *Ophiostoma*. As *Ceratocystiopsis* Upadhyay et Kendrick has been reduced to synonymy with *Ophiostoma* Syd. et P. Syd., and sufficient dried material is now available, these are described herein as ***Ophiostoma carpenteri*** sp. nov., ***Ophiostoma rollhansenianum*** sp. nov., and ***Ophiostoma manitobense*** sp. nov. We found *O. carpenteri* to be closely related to *Ophiostoma retusum* (R.W. Davidson et T.E. Hinds) Hausner et al., and that both species may actually be fungal symbionts and could represent a discrete genus. Although morphologically *O. rollhansenianum* appears similar to *Ophiostoma minutum* Siemaszko, a clearly variable species, and *O. manitobense* to *Ophiostoma minus* (Hedgc.) Syd. et P. Syd., earlier rDNA data indicate that *O. rollhansenianum* and *O. manitobense* are actually more closely related to *Ophiostoma coliferum* (Marmolejo et Butin) Hausner et al., and *Ophiostoma ranaculosum* (J.R. Bridges et T.J. Perry) Hausner et al. We also comment on some morphological features that have previously been overlooked or misreported in *Cornuvesica falcata* (E.F. Wright et Cain) C.D. Viljoen et al., such as the ascospores actually being hyaline and the presence of two distinct *Chalara* anamorphs.

Key words: Ophiostoma, molecular and morphological criteria, *Ceratocystiopsis, Cornuvesica*.

Résumé : L'échantillonnage d'insectes, de galeries d'insectes et de tissus ligneux colorés, effectué par les auteurs et d'autres chercheurs, dans le but d'obtenir des isolats d'espèces fongiques ophiostomatoïdes, a conduit à trois entités ressemblant à des *Ceratocystiopsis*. À l'aide de séquences partielles de rADN les auteurs avaient déjà pu constater qu'elles diffèrent l'une de l'autre, aussi bien que des autres espèces décrites de *Ceratocystiopsis* et d'*Ophiostoma*. Comme *Ceratocystiopsis* Upadhyay et Kendrick a été mis en synonymie avec *Ophiostoma* Syd. et P. Syd., et qu'on possède maintenant suffisamment de matériel séché, on décrit les espèces en question comme ***Ophiostoma carpenteri*** sp. nov., ***Ophiostoma rollhansenianum*** sp. nov. et ***Ophisotoma manitobense*** sp. nov. Les auteurs ont constaté que l'*O. carpenteri* se situe près de l'*Ophiostoma retusum* (R.W. Davidson et T.E. Hinds) Hausner et al. et que les deux espèces peuvent en fait agir comme symbiotes fongiques; elles pourraient ainsi représenter un genre distinct. Bien que morphologiquement l'*O. rollhansenianum* apparaisse similaire à l'*Ophiostoma minutum* Siemaszko, une espèce nettement variable, et l'*O. manitobense* à l'*Ophiostoma minus* (Hedgc.) Syd. et P. Syd., des données d'ADN antérieures indiquent que l'*O. rollhansenianum* et l' *O. manitobense* sont en fait plus apparentés à l'*Ophiostoma coliferum* (Marmolejo et Butin) Hausner et al. et à l'*Ophiostoma ranaculosum* (J.R. Bridges et T.J. Perry) Hausner et al. Les auteurs commentent également certaines caractéristiques morphologiques qui ont été précédemment passées sous silence ou mal rapportées chez le *Cornuvesica falcata* (E.F. Wright et Cain) Viljoen et al., tels que le fait que les spores soient en fait hyalines, ainsi que la présence de deux anamorphes distincts du *Chalara.*

Mots clés : Ophiostoma, critères moléculaires et morphologiques, *Ceratocystiopsis*, *Cornuvesica*.

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Introduction

In a previous study (Hausner et al. 1993*a*) we included, among many others, isolates of three undescribed fungi in the genus *Ceratocystiopsis* Upadhyay et Kendrick (Upadhyay and Kendrick 1975). These were designated *Ceratocystiopsis* species 1, 2, and 3, and we also included isolates of *Cornuvesica falcata* (E.F. Wright et Cain) Viljoen et al. [≡*Ceratocystiopsis falcata* (E.F. Wright et Cain) Upadhyay] (Upadhyay 1981) because of peculiarities in the latter's nature. The data we obtained suggested that while the majority of the species investigated could be accommodated in *Ophiostoma* Syd. et P. Syd. (Sydow and Sydow 1919), they represented a polyphyletic group exhibiting convergent evolution. Indeed, several species, e.g., *Cornuvesica falcata*, *Ceratocystiopsis proteae* M.J. Wingfield et al. (Wingfield et al. 1988), and *Ceratocystiopsis alba* (Devay et al.) Upadhyay (Upadhyay 1981), had at best very

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distant relationships to typical *Ophiostoma* species. Therefore, we formally reduced *Ceratocystiopsis* to synonymy with *Ophiostoma*, and made necessary new combinations in the latter genus. However, this left several species we had studied without satisfactory placement, i.e., *Ceratocystiopsis alba*, *Cornuvesica falcata*, and *Ceratocystiopsis proteae*; it also left *Ceratocystiopsis* tax. spp. 1, 2, and 3 both undescribed and unplaced.

Our partial rDNA sequence data had linked *Ceratocystiopsis proteae* to *Ceratocystis* Ellis et Halst. sensu stricto (Ellis and Halstead 1890) at a bootstrap confidence level of 87.2%, and subsequently, Marais et al. (1998) employed restriction mapping to link closely *Ceratocystiopsis proteae* and *Ophiostoma capense* M.J. Wingfield et Van Wyk (Wingfield and Van Wyck 1993). They also showed that while these two were allied phylogenetically to *Ceratocystis* s. str., they were sufficiently distinct to warrant being placed in a new genus, *Gondwanamyces* Marais et M.J. Wingfield, type species *Gondwanamyces proteae* (M.J. Wingfield, Van Wyk et Marasas) Marais et M.J. Wingfield. *Ophiostoma capense* was included as *G. capensis* (M.J. Wingfield et Van Wyk) Marais et M.J. Wingfield.

Although Wingfield (1993) suggested that *Ceratocystiopsis falcata* (as it was then known) might best be placed in *Ceratocystis* s. str., our molecular data showed it was not related to members of either *Ophiostoma* or *Ceratocystis* (Hausner et al. 1993*a*, 1993*b*). Subsequently Viljoen, M.J. Wingfield, and Jacobs in Viljoen et al. (2000) erected the genus *Cornuvesica* to accommodate the sole species *Cornuvesica falcata* (E.F. Wright et Cain) Viljoen et al.

Herein, we name and formally describe the three *Ceratocystiopsis*-like fungi noted above as species of *Ophiostoma* and comment on *Cornuvesica falcata.*

Materials and methods

Strains studied

Details of the strains employed are given under the individual species. Living cultures and dried specimens of all newly described species have been deposited at the University of Alberta Microfungus Collection and Herbarium (UAMH), Devonian Botanic Garden, Edmonton, Alta.

Culturing procedures

Culture descriptions are based on colony morphologies of strains grown in the dark in a controlled environment chamber at 20°C. The primary medium employed was malt extract agar (MEA; 20 g malt extract, Difco, Mich.; 20 g bacteriological agar, Gibco, Paisley, U.K.; containing 1 g yeast extract (YE) (Gibco), all per litre (MEA–YE). Agar was poured into 9-cm plastic nonvented Petri dishes, and the dishes were inoculated by aseptically removing approximately 4 mm diameter plugs from the margin of a 1-week-old culture of the test organism and placing one plug centrally, original surface side down, on the surface of a fresh MEA–YE plate.

We also used the following media when growing selected isolates of *Cornuvesica falcata*. (*i*) Rabbit food agar (RFA) was prepared by boiling 25 g of commercial rabbit food (based on alfalfa pellets) for 1 h in 1.0 L of distilled water and then straining it through cheesecloth. To the filtrate, 20 g of bacteriological agar (Gibco) was added and then made up to 1.0 L with distilled water, autoclaved for 20 min at 121°C and dispensed as required. (*ii*) A complex agar containing 17 g corn meal agar (BBL™, Becton, Dickinson and Company, Cockeysville Md.), 10 g malt extract agar (Difco), 1 g yeast extract agar (Difco), 1 g protease peptone (Difco), all per litre, autoclaved for 20 min at 121°C before dispensing. (*iii*) A mixture of 20 g potato dextrose agar (BBL™) and 8.5 g cornmeal agar (BBL™), both per litre (PDCA), autoclaved for 20 min at 121°C before dispensing.

Cultures were also grown on wood discs cut from main stems and branches of small specimens of *Pinus banksiana* Lamb., *Pinus strobus* L., *Picea abies* (L.) H. Karst., or *Picea glauca* (Moench) Voss, depending on the strain under test or tree availability. Discs with bark ranged between 5 and 7 cm in diameter and were from 1.5 to 2 cm thick. Discs were placed in airtight plastic bags and frozen until required. They were then submerged in distilled water and autoclaved at 121°C for 2.5 h, then placed singly in deep pyrex dishes (Corning 3250 storage dishes, Corning Glass Works, Corning, New York). Distilled water was added to a depth of half the thickness of individual discs. Dishes were then autoclaved at 121°C for 30 min and stored in a refrigerator at 3°C until inoculated. Inoculations were made aseptically as described above, and the discs were then incubated in a controlled environment at 20°C in the dark for up to 60 days. Periodically, if required, dishes were aseptically recharged with sterile distilled water to the original level. Wood discs were used to more closely approximate the natural substrate in the hope it would ensure a more accurate production of reproductive structures than might otherwise occur on agar.

Growth rates for two of the new species were determined by inoculating five replicate Petri dishes of MEA–YE, as described above, for each organism tested. Plates were incubated in the dark at 20°C for 20 days, and growth was then measured with two readings being taken perpendicular to each other per dish; the average of 10 readings for each treatment was used to indicate rate of growth. For *Ophiostoma carpenteri*, growth was measured after 7 days of incubation at 20°C, as lateral growth was very slow and had essentially ceased after 7 days.

Morphological structures of importance in identifying the strains were mounted in 85% lactic acid (Fisher Scientific, Fair Lawn, N.J.) on slides, which were then placed on a slide warmer set at 40°C for at least 45 h before ringing with nail varnish. Names for colours are based on the mycological colour chart found in Rayner (1970). We attempted to make at least 50 measurements of each of the relevant morphological structures from both agar and wood disc cultures and, for each measurement set, both ranges and averages were computed. However, when material was sparse or difficult to mount, only 30 structures were measured.

To visualize ascospore sheaths for photography, spores were mounted in an aqueous solution of phloxine (1.0 $g \cdot L^{-1}$).

Molecular studies and cycloheximide sensitivity

No new molecular data nor phylogenetic analyses are presented, nor were new cycloheximide sensitivity tests undertaken. When such are discussed, the appropriate references are cited.

Taxonomy and discussion

Ophiostoma carpenteri J. Reid et Hausner sp. nov. Figs. 1–7.

ETYMOLOGY: Named in honour of Dr. Stephen J. Carpenter who collected the isolates studied and provided them to us for study.

Perithecia 45.0–102.5 μm alta, 45.0–100.0 μm lata, varia colore et forma; collis variis, 112.5–212.5 μm (hyphis ostiolaribus exclusis) longis, basi apiceque 32.5–62.5 μm medio 42.5–82.5 latis; hyphis ostiolaribus 35.0–49.0 μm longis, 1.1–2.0 μm latis obscure septatis. Asci non visi. Ascosporae unicellulares, hyalinae, 17.0–34.0 × 1.0–3.0 μm anguste clavatae, nunc strictae nunc curvatae nunc sigmoideae; vagina non visa. Conidiophora semi-macronemata, sympodularia, singularia vel laxe fasciculata. Conidia unicellularia, hyalina. Coloniae in agaro cum extracto malti fermentique post 7 dies ad 20°C in obscuritate 2.6–2.8 mm diametro, albidae, adpressae, sparsae. Hyphae hyalinae, laeves.

HOLOTYPUS: U.S.A. Oregon. Andrews Experimental Forest. Isolated from a specimen of *Trypodendron lineatum* (Olivier) by whole wash technique by S.E. Carpenter (SC0048 V). Dried agar cultures on MEA–YE and PDA as UM853. Ex-type culture UM 853 (UAMH 9695).

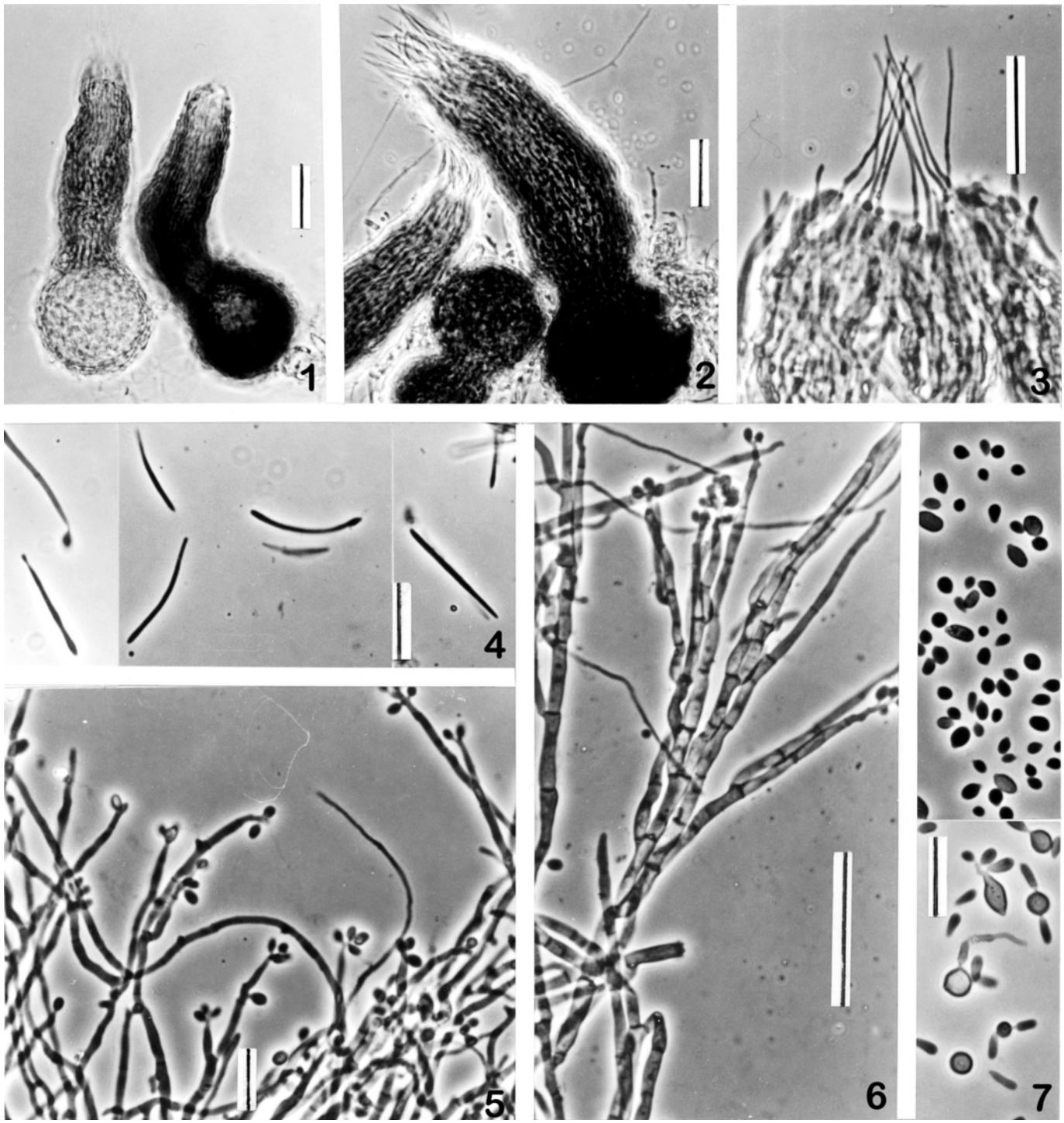
Perithecia are variable in colour, with either the base or the neck being pale to ochraceous in colour; frequently, by incident light, the necks appear to be translucent and, while the upper part of the base is of similar appearance, the lower portion of the base gradually becomes pale to mid-brown. Other ascoma may be more uniformly pale or ochraceous, but even then the terminal 1/4 to 1/3 of the neck is much paler to translucent. In a few cases, the majority of the neck is mid-brown while its base is much lighter in colour; forming on aging MEA–YE in 10–20 days at 20°C. Bases variable in outline; spherical to slightly flattened dorsi-ventrally; height 45.0–102.5 ($\bar{x}$ = 74.27, SD = 12.76) μm; width 45.0–100.0 ($\bar{x}$ = 73.46, SD = 11.41) μm. Necks unusually variable in outline (Figs. 1 and 2); they may be parallel-sided and tapering only slightly at their apices, or broader towards their apex and tapering towards their bases; or broader towards the base and tapering gradually towards the apex; or broadest in the middle and tapering gradually towards both the base and the apex. Regardless of outline, almost all are markedly constricted at the base of the neck at the point of attachment to the perithecial base (Fig. 2). The necks are also frequently curved in various ways. Neck length (excluding ostiolar hyphae) 112.5–212.5 ($\bar{x}$ = 164.79, SD = 32.57) μm; base width 32.5–62.5 ($\bar{x}$ = 42.92, SD = 7.06) μm; midpoint width 42.5–82.5 ($\bar{x}$ = 55.1, SD = 11.56) μm; tip width (circ. 0.5 μm behind the apex) 32.5–62.5 ($\bar{x}$ = 44.08, SD = 11.33) μm. Ostiolar hyphae (Figs. 2 and 3) are 35.0–49.0 ($\bar{x}$ = 39.67, SD = 11.1) μm long and 1.1–2.0 ($\bar{x}$ = 1.48, SD = 0.22) μm wide; indistinctly septate; often appearing to arise from a slightly swollen cell that forms at the terminus of various hyphal elements forming the neck (Fig. 3). Asci not seen; presumed deliquescent. Ascospores (Fig. 4) narrowly clavate; straight, and curved or sigmoid; widest at the apparent apex and tapering gradually to their bases; rounded to slightly tapered at their apices. Occasionally, what may be spores are almost parallel sided and slightly swollen at each end, and straight or curved; other more normal-shaped spores may have what appears to be a small vesicle attached at their narrow end. Sheath not seen; length 17.0–34.0 ($\bar{x}$ = 25.19, SD = 4.05) μm and 1.0–3.0 ($\bar{x}$ = 1.55, SD = 0.46) μm. Conidiophores semi-macronematous, consisting of erect, single (Fig. 5) to clustered (Fig. 6) branching conidiophores bearing conidia in a sympodular fashion at the various apices. Conidia are hyaline; 1-celled; shape variable (Fig. 7) being globose to oval, turbinate, obovoid, and clavate; often appearing apiculate or truncate; capable of budding or germinating in a sympodular fashion (Fig. 7; lower center) to produce secondary conidia; 2.0–8.0 ($\bar{x}$ = 5.33, SD = 1.67) μm × 2.0–5.0 ($\bar{x}$ = 3.53, SD = 0.92) μm; they do not appear to be associated with mucilaginous material. Colonies attaining a diameter of 2.6–2.8 ($\bar{x}$ = 2.73, SD = 0.07) mm in darkness on MEA–YE in 7 days at 20°C; white; appressed with quite sparse growth; hyphae hyaline and chiefly up to 5.0 μm in diameter, but they frequently form swollen intercalary cells along their lengths. Viewed by incident light with a dissecting microscope, only the fine conidiophores can be seen arising from the surface and, later, the perithecia.

PARATYPES: U.S.A. Oregon. Andrews Experimental Forest. All of the following live isolates were obtained from individual striped-ambrosia beetles, *Trypodendron lineatum* (Olivier) by Dr. S.E. Carpenter and are deposited in UAMH. Isolated from pronotum, June 24, 1986, SC0048III, UM 852. Dried agar cultures on MEA–YE and PDA. Isolated from pronotum, no date, SC0045, UM 854. Dried agar cultures on MEA–YE; isolated from pronotum, June 24, 1986, SC0047III, UM855; isolated from pronotum, June 24, 1986, SC0047I, UM856. Dried agar culture on PDA; isolated from pronotum July 1, 1986, SC0048 IV, UM857; isolated from pronotum, June 24, 1986, SC0047 IV, UM858.

These strains, sent to us many years ago by Dr. Carpenter, were isolated during a survey of fungi associated with ambrosia beetles in Andrews Experimental Forest; however, we have never been able to place them satisfactorily.

Reminiscent of members of *Ceratocystiopsis*, we included *O. carpenteri* as *Ceratocystiopsis* tax. sp. 1, in a study reappraising that genus using molecular criteria (Hausner et al. 1993*a*). While we found that *O. carpenteri* clustered in a potentially monophyletic group of ophiostomatoid fungi at a confidence level of 100%, it did not cluster closely with the core group of *Ophiostoma* spp. centered around *Ophiostoma minutum* Siemaszko [≡ *Ceratocystiopsis minuta* (Siemaszko) Upadhyay et Kendrick] (Upadhyay and Kendrick 1975) (see Hausner et al. 1993*a*, Fig. 1). Also it was insensitive to cycloheximide, as was *Ophiostoma retusum* (R.W. Davidson et T.E. Hinds) Hausner et al. (Hausner et al. 1993*a*), while all species in the core group were sensitive by the standards we then employed. In Hausner et al. (1993*a*) an unfortunate error of omission occurred. In Table 3 a fifth footnote was missed that was intended to indicate that *O. carpenteri* (then as *Ceratocystiopsis* tax. sp. 1) grew so slowly at 25°C, its sensitivity test had to be carried out at 20°C. This reflects the fact that *O. carpenteri*, similar to several other *Ophiostoma* species previously assigned to *Ceratocystiopsis*, does not grow very well on MEA–YE at 25°C, or even 20°C. And, because of the latter, the growth of this species

Figs. 1–7. Phase contrast micrographs of conidia, conidiophores, ascospores, and perithecia of *Ophiostoma carpenteri* (UM853). Fig. 1. Perithecia showing colour variation in parts of mature perithecia. Scale bar = 30 µm. Fig. 2. Whole perithecium and parts of two others showing ostiolar hyphae and the pronounced constriction at the neck base. Scale bar = 30 µm. Fig. 3. The tip of a slightly flattened neck. Note the slight inflations commonly found at the bases of the ostiolar hyphae. Scale bar = 40 µm. Fig. 4. Ascospores. Scale bar = 20 µm. Fig. 5. Conidiophores with conidia. Scale bar = 30 µm. Fig. 6. Branching conidiophores bearing conidia. Scale bar = 80 µm. Fig. 7. Conidia showing variations in form and patterns of secondary development. Scale bar = 20 µm.



was measured after 7 days, as all lateral growth had essentially ceased by then.

Ophiostoma carpenteri and *O. retusum* were grouped together at a 100% confidence level by our earlier molecular analysis, supporting their apparent close relatedness based on morphological characters. They both have perithecial necks and bases that are variable pale to ochraceous in colour, and ostiolar hyphae that are long, divergent and possibly septate. They both have elongate and quite similarly shaped ascospores, although those of *O. carpenteri* are significantly longer, and they also have similarly shaped conidia. Unfortunately, Upadhyay's (1981) description of the ascospores of *O. retusum* is markedly different from that of Hinds and Davidson (1972). The latter state "ascospores hyaline, elongate filiform, nearly cyclindrical, slightly curved or curved at one end, 13–15 µm long and 1.0–1.5 µm wide", and no mention was made of a sheath; Upadhyay (1981) states the ascospores are falcate and have sheaths. Also, similarly, they both grow slowly in culture, but *O. carpenteri* is the slowest, with meagre production of mycelium.

The above characteristics, e.g., long divergent ostiolar hyphae, plus the production of conidia on sterigmata (i.e., small hyphal projections) from the conidiogenous cell, along with a lack of sensitivity to cycloheximide and molecular data (Hausner et al. 1993*a*), clearly segregate *O. carpenteri* and *O. retusum* from the "core group" of former *Ceratocystiopsis* spp. that was transferred to *Ophiostoma* Syd. et P. Syd. However, some of the characteristics they share are also found in "core group" species, e.g., elongate ascospores in *O. coliferum* (Marmolejo et Butin) Hausner et al. (Hausner et al. 1993*a*). Therefore the best placement for these species at this time is probably within *Ophiostoma*, although this may change when additional criteria become available for assessing relationships in this group of fungi.

The possible ecology of these two species should be addressed here. *Ophiostoma retusum* was initially isolated from perithecia developing in the ambrosia fungus *Ambrosiella ferruginea* Batra that was growing in the galleries of *Trypodendron retusum* (Lec.). This, coupled with the fact that it grows feebly on both agar and wood discs, suggests that it is not really a blue-stain fungus, but that it might actually be a symbiont of the ambrosia fungus. And as *O. carpenteri* also grew feebly on both agar and wood, it too might not be a true blue-staining organism, but have some other type of association in the niche it occupies naturally. Neither fungus produced any significant pigment in culture, and this fact adds to the possibility proposed above.

Ophiostoma rollhansenianum J. Reid, Eyjólfsdóttir et Hausner, sp. nov. Figs. 8–13.

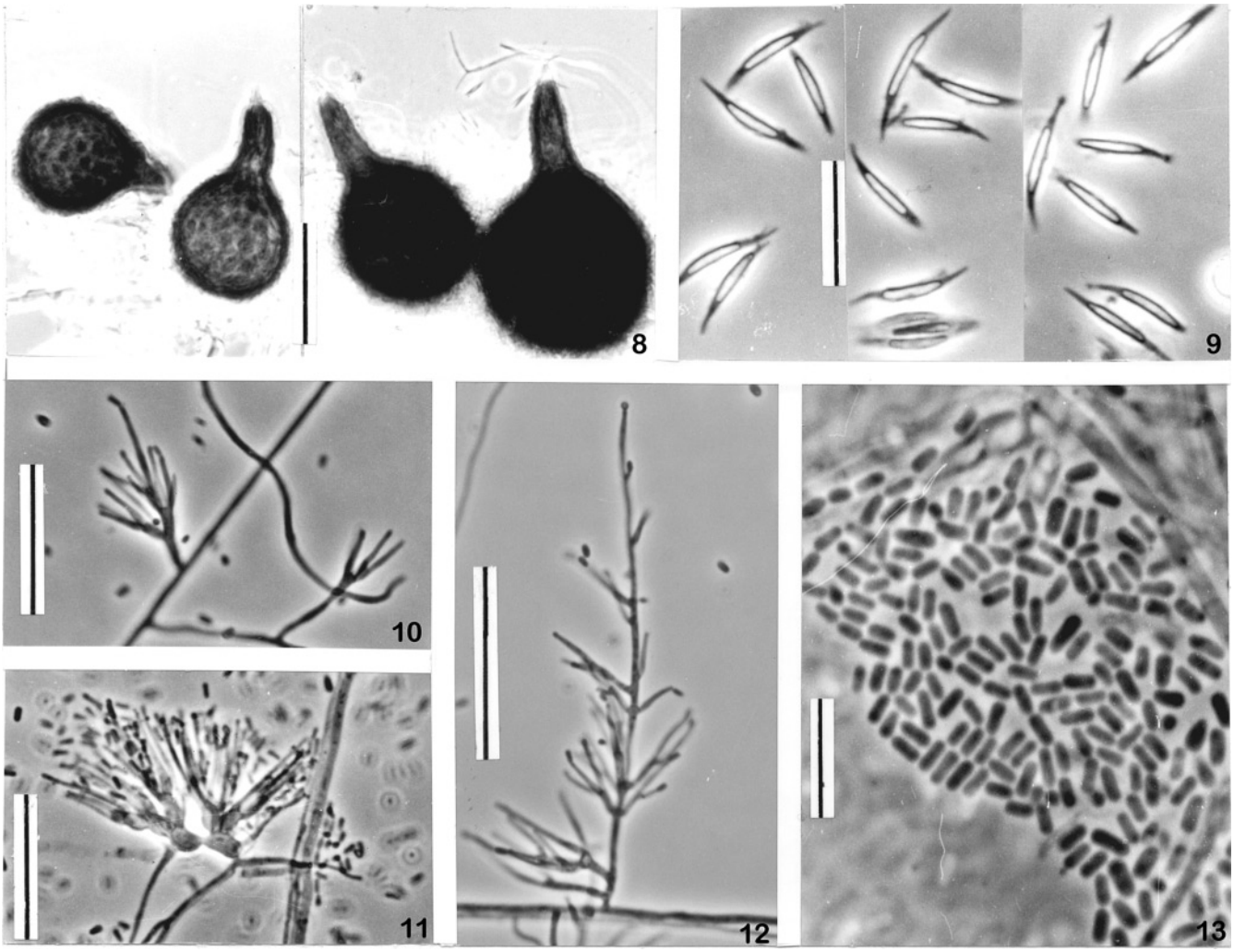
ETYMOLOGY: In honour of the distinguished Norwegian forest pathologist and mycologist, Professor Finn Roll-Hansen, for his contribution to science.

Perithecia varia, infrequentia in cultura sed ubera in ligno post 30–60 dies; basis atris, 70.0–112.5 µm altis, 70.0–100.0 µm latis; collis variis, pallidioribus, basi 17.5–40.0 µm, apice 5.0–22.5 µm latis, 12.5–125.0 µm (cum hyphis ostiolaribus) longis; hyphis ostiolaribus 5.0–20.0 µm longis, hyalinis. Asci non visi. Ascosporae unicellulares, hyalinae, 7.0–15.0 × 1.0–1.5 µm; vagina e latere falcata a fronte aciculari visa, ab extremo non visa. Conidiophora micronemata vel macronemata. Conidia unicellularia, hyalina, laevia. Coloniae in agaro cum extracto malti fermentique post 20 dies ad 20°C in obscuritate 52.0–60.0 mm diametro; albidae, adpressae cum agellis mucosis conidiorum. Hyphae hyalinae, laeves.

HOLOTYPUS: Norway. Akershus county. Highway #21, near Tangen, isolated from beetle galleries in an apparently dead, standing tree of *Pinus sylvestris* L., October 1973. J. Reid, and cultured on discs of *Pinus sylvestris* (73–13; UM 113). Dried cultures on MEA–YE. Ex-type culture UM113 (UAMH 9774).

Perithecia (Fig. 8) various in appearance, produced infrequently in agar culture, and then only after prolonged storage at 4°C; abundant on wood discs afer 30–60 days at 20°C. Bases black by incident light; globose, subglobose or slightly broader than high (slightly flattened dorsi-ventrally); height 70.0–112.5 ($\bar{x}$ = 86.52, SD = 10.55) µm; width 70.0–100.0 ($\bar{x}$ = 87.68, SD = 8.96) µm. Necks paler; short-conical to elongate and then tapering gradually to their blunted apices; base width 17.5–40.0 ($\bar{x}$ = 26.79, SD = 5.21) µm; tip width 5.0–33.5 ($\bar{x}$ = 15.89, SD = 3.29) µm; neck length (excluding ostiolar hyphae) 12.5–125.0 ($\bar{x}$ = 42.95, SD = 22.12) µm. Ostiolar hyphae are 5.0–20.0 ($\bar{x}$ = 9.06, SD = 3.44) µm in length, convergent, hyaline to very rarely buff coloured, and tapered slightly to blunted, rounded tips. Presumptive asci are seen chiefly as coherent aggregations of eight ascospores since the walls are extremely difficult to visualize. In outline these aggregations are chiefly broadest in the middle or about 1/3 along their length; they are long persistent, remaining visible in a mount for some time after the perithecia have been crushed; 12.0–22.0 ($\bar{x}$ = 15.92, SD = 2.29) × 3.0–5.0 ($\bar{x}$ = 3.3, SD = 0.5) µm. Ascospores 1-celled; hyaline; including the sheath falcate in side view, acicular in face view; end view not seen; when mounted in aqueous phloxine, it is clear the sheath is primarily responsible for the characteristic shape of the ascospores (Fig. 9), otherwise the body of the ascospore appears to be elongate orange-section shaped in side view, but somewhat acerose in face view; including sheath 7.0–15 ($\bar{x}$ = 10.59, SD = 1.34) × 1.0–1.5 ($\bar{x}$ = 1.01, SD = 0.07) µm. Conidophores micronematous or macronematous (Figs. 10–12). The micronematous conidiophores may be formed on both upright and prostrate hyphal elements. On upright elements they may consist of conidiogenous cells arising singly from the upright hypha or from a short basal cell bearing 2–4 conidiogenous cells (Fig. 12). Prostrate hyphae form short basal cells bearing three or more main branches, each of which can produce several conidiogenous cells (Fig. 10). Macronematous conidiophores arise from a single basal hypha and develop short chains of swollen, branching cells which then produce finer branches that bear conidiogenous cells at their apices (Fig. 11). Conidia are produced abundantly, and accumulate in slimy masses of various sizes; 1-celled, hyaline and smooth-walled; chiefly short-cylindric, but also slightly clavate (Fig. 13), they have obtuse apices and slightly rounded to truncate bases; 2.0–5.0 ($\bar{x}$ = 3.37, SD = 0.61) × 0.75–2.20 ($\bar{x}$ = 1.23, SD = 0.39) µm. Colonies attaining a diameter of 52.0–60.0 ($\bar{x}$ = 55.1, SD = 2.47) mm in darkness on MEA–YE in 20 days at 20°C; whitish,

Figs. 8–13. Phase contrast micrographs of conidia, conidiophores, ascospores and perithecia of *Ophiostoma rollhansenianum* (UM113). Fig. 8. Four perithecia. Scale bar = 60 µm. Fig. 9. Ascospores mounted in phloxine. Scale bar = 10 µm. Figs. 10–12. Various forms of conidiophores. Scale bars for Figs. 10 and 11 = 30 µm. Scale bar for Fig. 12 = 40 µm. Fig. 13. Conidia. Scale bar = 10 µm.



appressed with irregular white patches of slimy conidia. Appearing zonate when grown under light and darkness. Individual hyphae are hyaline, smooth-walled, and chiefly up to 4.0 µm in diameter; often funiculose; larger diameter hyphae may also be present.

PARATYPES: Norway. Hedemark. Highway #2, near Kongsvinger, in beetle galleries in a standing tree of *Pinus sylvestris* L., October 1973. J. Reid. Live culture UM 110 (UAMH 9797).

When first collected, it was presumed that *O. rollhansenianum* simply represented a slightly variant form of *Ophiostoma minutum* Siemaszko (Siemaszko 1939), a species long known from both spruce and pine in Scandinavia (Mathiesen 1951; Mathiesen-Käärik 1960; Solheim 1986, 1993) but also of worldwide distribution on various conifers (Siemaszko 1939; Mathiesen 1951; Mathiesen-Käärik 1960; Griffin 1968; Olchowecki and Reid 1974; Solheim 1986, 1993; Yamaoka et al. 1997, 1998). There is also an apparent report of its occurrence on *Ilex paraguariensis* St.-Hil. in Brazil (Mendes et al. 1998), but this may be of doubtful authenticity. In none of the above cases does *O. minutum* appear to be a serious blue-staining fungus, but rather a late invader and frequently associated with other fungal species. However, in several cases authors have drawn attention to minor character differences between their isolates and those normally reported for isolates from elsewhere. Yamaoko et al. (1998, p. 369) note isolates from Japanese larch had perithecia uniformly brown to dark brown in colour but isolates from Yezo spruce (Yamaoka et al. 1997) produced bicoloured perithecia in cultures where bark was placed on the agar surface. Upadhyay (1981), on the other hand, gives no indication of such a bicoloured nature of the perithecia, and neither did Mathiesen (1951) nor Davidson (1942). Clearly, others have also noted apparent minor variations similar to those that caused us to doubt that our species was actually *O. minutum*; comparative molecular analysis of their isolates might also be worthwhile.

The neck lengths and widths given above are for the strains grown on wood; on MEA–YE in the cold, the necks

of the perithecia are longer and wider: 120–220 µm long, 30–50 µm wide at the base and 20–25 µm wide at the apex. However, as those represented perithecia formed under significantly unusual growth conditions, they may not have been seen in field collections.

Ophiostoma manitobense J. Reid et Hausner sp. nov. Figs. 14–21.

ETYMOLOGY: From the provincial name, Manitoba.

Perithecia varia, infrequentia in cultura sed ubera in ligno post 30–60 dies; basis atris, 57.5–87.5 µm alta, 62.5–105.0 µm lata, collis nonnunquam aliquot, pallidioribus, basi 25.0–40.0 µm apice 12.5–27.5 latis, 11.5–82.5 µm (cum hyphis ostiolaribus) longis; hyphis ostiolaribus 7.0–14.0 µm longis, hyalinis, convergentibus. Asci non visi. Ascosporae unicellulares, hyalinae, 8.0–18.0 × 1.0–1.5 µm; vagina e latere falcata, a fronte aciculari visa, ab extremo non visa. Conidiophora micronemata vel macronemata, habitu variabilia. Conidia unicellularia, hyalina, laevia. Coloniae in agaro cum extracto malti fermentique post 20 dies ad 20°C in obscuritate 52.0–60.0 mm diametro; albidae, floccosae, cum acervis mucosis conidiorum. Hyphae hyalinae, laeves.

HOLOTYPUS: Canada. Manitoba. Sandilands Forest Reserve, in bark beetle galleries in *Pinus resinosa* Ait.; June 1985, J. Reid, and cultured on discs of *P. resinosa* UM 237. Ex-type culture (UAMH 9813).

Perithecia (Fig. 14) are various in appearance, and are produced infrequently in agar culture; abundant on wood discs after 30–60 days. Bases black by incident light; globose or subglobose but flattened dorsiventrally; height 57.5–87.5 ($\bar{x}$ = 76.34, SD = 10.26) µm; width 62.5–105.0 ($\bar{x}$ = 74.5, SD = 12.02) µm. The necks are slightly lighter in colour; short conical to elongate with parallel sides and blunt apices; perithecia may produce up to three necks, but this appears to be rare. Neck base width 25.0–40.0 ($\bar{x}$ = 30.39, SD = 3.83) µm; tip width 12.5–27.5 ($\bar{x}$ = 18.29, SD = 6.18) µm; neck length (excluding ostiolar hyphae) 11.5–82.5 ($\bar{x}$ = 51.0, SD = 23.25) µm. Ostiolar hyphae 7.0–14.0 ($\bar{x}$ = 11.59, SD = 1.72) µm long; hyaline, convergent, and tapering slightly to a blunted tip. Asci apparently deliquescent; in eight consecutive mounts, only two were seen. Ascospores 1-celled; hyaline; including the sheath falcate in side view, acicular in face view; end view not seen; when mounted in aqueous phloxine, it is clear the sheath is primarily responsible for the characteristic shape of the ascospores (Figs. 15 and 16), otherwise the body of the ascospores appears elongate orange-section shaped in side view and cylindrical with rounded ends in face view; including sheath 8.0–18.0 ($\bar{x}$ = 12.83, SD = 2.00) µm long; 1.0–1.5 ($\bar{x}$ = 1.05, SD = 0.13) µm wide. Conidiophores micronemetous or macronematous (Figs. 18–21). The micronematous conidiophores may consist of conidiogenous cells arising singly from the vegetative hyphae (Fig. 18), or a short basal cell which continues to develop short lateral and terminal extensions with condiogenous sites at their apices (Fig. 19) or discrete basal cells that produce 2–4 branches, which then branch irregularly and form conidiogenous cells at their apices (Fig. 20). The macronematous conidiophores are much larger than the preceding forms (Fig. 20) and appear as loose structures, branching irregularly in a fan-like fashion, but they originate from a single basal cell and bear conidia at the branch apices. Conidia are hyaline; unicellular short oblong, with rounded ends, but some appear to be slightly constricted at the middle (Fig. 17); smooth-walled; 3.0–5.5 ($\bar{x}$ = 4.03, SD = 0.74) µm × 1.0–2.0 ($\bar{x}$ = 1.26, SD = 0.33) µm. Colonies attaining a diameter of 45.0–51.0 ($\bar{x}$ = 47.2, SD = 1.54) mm in darkness on MEA–YE in 20 days at 20°C; whitish, floculose, with irregular slightly depressed areas bearing slimy conidial masses. Individual hyphae are hyaline, smooth, and up to 5.0 µm wide, but occasionally funiculose; larger diameter hyphae may also be present.

Ophiostoma manitobense was collected only once from an obviously diseased red pine sapling exhibiting extensive development of basal bark-beetle galleries in which neither larvae nor adult beetles were to be found. Presumably the adults had emerged earlier in the spring. Although a number of trees of a similar size-class were present in the area, none of these appeared to have been infested. Red pine is not indigenous to the area, the trees present being a part of the general planting program, and it is impossible to speculate as to the beetle species involved.

Perithecia were observed in the galleries at the time of collection and, as *Ophiostoma minus* (Hedgc.) Syd. et P. Syd. (Sydow and Sydow 1919) is regularly collected from red pine plantings in the general area, it was again assumed we had another collection of the latter. This assumption was reinforced by the apparent size of the perithecia and the number of them that had slightly elongated necks. We were therefore surprised when laboratory examination and culturing showed we had what we then considered to be a probable new species of *Ceratocystiopsis*.

The conidia of *O. manitobense* are similar to those of *O. rollhansenianum* in size and shape (compare Figs. 13 and 17), but the presumptive asci in *O. rollhansenianum* appear to be more persistent then in *O. manitobense*, and the ascospores in the latter are longer. When the necks are elongated in *O. manitobense*, they appear to be more parallel-sided than is the case in *O. rollhansenianum* (compare Figs. 8 and 14). However, as several species formerly assigned to *Ceratocystiopsis* are, in fact, difficult to definitively separate solely on morphological criteria when they are grown comparatively on various substrates and under a variety of conditions, we had previously refrained from describing either strain as a new species. Although our molecular data (Hausner et al. 1993*a*) show clearly that while both of these species are part of the "core group" of strains formerly considered to be species of *Ceratocystiopsis*, both are unique and distinct from previously described species. This is supported by the consistent, although small, morphological differences that can be observed between these and other described species.

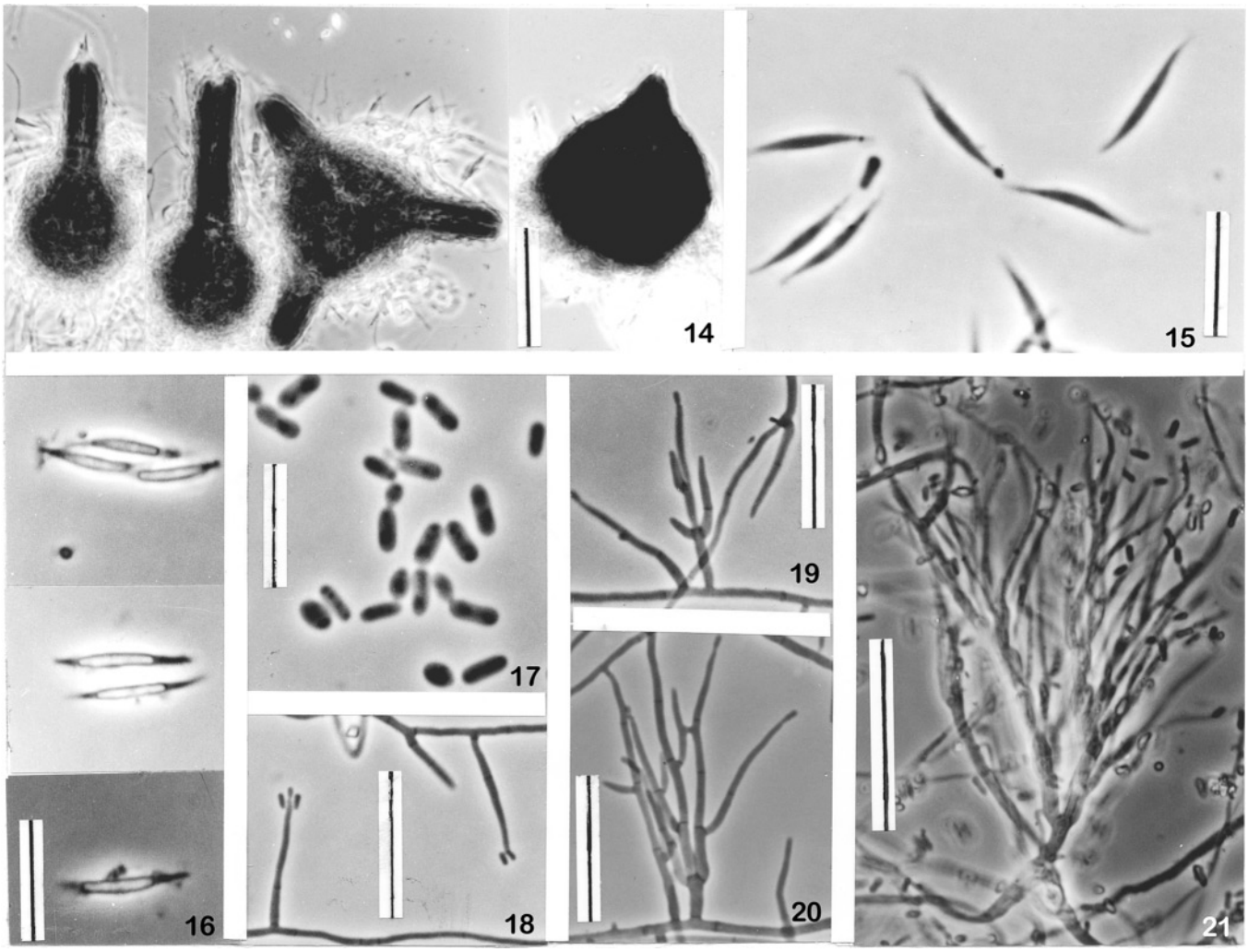
Cornuvesica falcata (E.F. Wright et Cain) C.D. Viljoen, M.J. Wingfield, et K. Jacobs. Mycol. Res. 104: 366–367. 2000.

≡*Ceratocystis falcata* E.F. Wright et Cain, Can. J. Bot. 39: 1226. 1961.

≡*Ceratocystiopsis falcata* (E.F. Wright et Cain) Upadhyay. A monograph of *Ceratocystis* and *Ceratocystiopsis*: 125–126. 1981.

SPECIMENS AND (OR) CULTURES EXAMINED: Canada, Ontario. On

Figs. 14–21. Phase contrast micrographs of conidia, conidiophores, ascospores, and perithecia of *Ophiostoma manitobense* (UM237). Fig. 14. Perithecia illustrating neck variations. Scale bar = 60 µm. Fig. 15. Ascospores. Scale bar = 10 µm. Fig. 16. Ascospores mounted in phloxine. Scale bar = 10 µm. Fig. 17. Conidia. Scale bar = 10 µm. Figs. 18–21. Conidiophores, with conidia, illustrating their differing degrees of complexity. Scale bars for Figs. 18–20 = 30 µm. Scale bar for Fig. 21 = 40 µm.



Pinus strobus L. Northwest of Nobleton, York Co., July 1, 1957. TRTC 33037 (holotype) UM (isotype); as *Ceratocystis minuta*, isolated from stumps of *Pinus resinosa* Ait., Turkey Point, Charlotteville Twp., Norfolk Co., Dec. 19, 1963. Coll. D. Punter, det. H.D. Griffin (dried tube culture). MFB7441 (SSMF755–80–98–01). New Zealand. On *Larix* sp., Compartment 5, Kaingaroa State Forest, Waiotapu, May 8, 1982. Coll. J. and W.S. Reid, det. J. Reid. Living cultures UM 487 (82–23) UAMH. On *Pinus radiata* D. Don, Compartment 1212, Kaingaroa State Forest, Taupo, Feb. 17, 1988. Col. J. and B. Reid, det. J. Reid. Living cultures UM 792 (88–305) UAMH; voucher specimen as dried agar cultures UM792. Compartment 1212, Kaingaroa State Forest, Taupo, Feb. 17, 1988. Coll. J. and B. Reid, det. J. Reid. Living cultures UM793 (88–306A), UAMH; voucher specimen as dried agar cultures UM793.

Earlier, Wingfield (1993) suggested that *Cornuvesica falcata* (as *Ceratocystiopsis falcata*) be assigned to *Ceratocystis* s. str. to clear up some anomalies that its placement in *Ceratocystiopsis* created. However, Hausner et al. (1993*a*, 1993*b*) showed that based on molecular data, the best solution was to separate *Ceratocystiopsis falcata* from both *Ophiostoma* and *Ceratocystis* s. str., and that *Ceratocystiopsis* should be reduced to synonymy with *Ophiostoma* (Hausner et al. 1993*a*). The problem of its placement was finally resolved when Viljoen et al. (2000) erected the monotypic genus *Cornuvesica* Viljoen et al. to accommodate this quite unique organism. But in doing so, these authors misreported some salient points of its morphology and overlooked interesting aspects of its apparent physiology.

Viljoen et al. (2000) state that the ascospores are dark, and although neither Wright and Cain (1961) nor Rayner and Hudson (1977) specifically mentioned the lack of pigmentation of the ascospores, these authors referred to them as being indistinctly septate, an observation not likely to be made with dark, very narrow spores. Also, both Upadhyay (1981) and Hutchison and Reid (1988) state specifically that the ascospores are hyaline. As we have repeatedly confirmed that the hyaline nature of the spores using bright light field microscopy and both lactic acid and aqueous phloxine as a mountant, we cannot rationalize this statement of Viljoen et

al. (2000). We examined the holotype, isotype, and the dried culture voucher material, and all mounts yielded the same results. Therefore *Cornuvesica falcata* must be described as having hyaline spores.

Hutchison and Reid (1988) stated two *Chalara* anamorphs were produced by this fungus, and while Viljoen et al. (2000) noted this observation, they did not do so in their formal description. The two classes of phialophores are clearly present in our voucher specimens and should be expected to occur on appropriate growth media.

Rayner and Hudson (1977) note that *Cornuvesica falcata* grew very poorly on malt extract agar in culture and did not colour the medium, but did produce perithecia sparsely on oatmeal agar. They also noted that it was stimulated markedly in both mycelial growth and perithecial production when grown in mixed cultures with *Acremonium butyri* (van Beyma) Gams and several *Trichoderma* spp. with which they had found it growing naturally. Hutchison and Reid (1988) also drew attention to this phenomenon of stimulation, although in this case the stimulator was *Gliocladium roseum* (Link) Bainier. The latter phenomenon was discussed in some detail by Kawchuk et al. (1993), who demonstrated that compound(s) extractable from *G. roseum* greatly enhanced mycelial growth, pigment and perithecial production of *Cornuvesica falcata*.

We found mycelial growth and perithecial production enhancement equal to, or greater than, that reported by Kawchuk et al. (1993) when we grew *Cornuvesica falcata* on RFA, CA, and PDCA media (voucher specimens UM 792 and 793). There was also greatly enhanced pigmentation on CA and PDCA, but because of the natural pigmentation of RFA, pigment enhancement of the fungus could not be determined visually. Thus, whatever the required stimulatory principle for growth, pigmentation and fruiting is in *Cornuvesica falcata*, it is clearly not solely of fungal origin, nor is direct contact with the agent's producer required as suggested by Rayner and Hudson (1977).

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