**Ophiostoma europhioides and Ceratocystis pseudoeurophioides, synonyms of O. piceaperdum**

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**Ophiostoma piceaperdum** and *O. europhioides* are well-known, first described from conifers in Canada. They have been considered to be synonymous. After studying the type material, as well as a collection of isolates of *O. piceaperdum* and *O. europhioides*, we have concluded that they cannot be distinguished from each other, so we support the synonymy of *O. europhioides* and *O. piceaperdum*. We provide a description for the *Leptographium* anamorph of *O. piceaperdum*. *Ceratocystis pseudoeurophioides* has previously been distinguished from *O. europhioides* based on differences in anamorph morphology. This species has also been reduced to synonymy with *O. penicillatum*, which was reported to have cucullate ascospores. Later descriptions of *O. penicillatum* reported it to have allantoid ascospores. We conclude that *C. pseudoeurophioides* cannot be distinguished from *O. piceaperdum* and is, therefore, reduced to synonymy with that species.

*Ophiostoma* is an economically important group of fungi, best known for an ability to cause diseases of trees (Gibbs, 1978; Brasier, 1991). Many species cause considerable economic losses due to sapstain of lumber (Solheim, 1986; Gibbs, 1993). Most are effectively dispersed by insects, especially bark beetles (Coleoptera: Scolytidae) (Münch, 1907; Lagerberg, Lundberg & Melin, 1927; Leach, Orr & Christensen, 1934; Upadhyay, 1981). This accounts for the extensive damage they can cause to plantation and forest trees (Solheim, 1986, 1992a, b).

*Ophiostoma* spp. are characterized by dark, flask-shaped ascomycetous ascocarps with ascospores that accumulate in slimy masses at the tips of ascocarp necks (Upadhyay, 1993; Wingfield, Seifert & Webber, 1993). Their anamorphs are found in many different genera including *Leptographium*, *Graphium*, *Sporothrix* and *Hyalorhinocladiella* (Mouton, Wingfield & Van Wyk, 1993, 1994; Wingfield et al., 1993). Most of these anamorphs are characterized by conidia that accumulate in mucilaginous masses at the apices of conidiophores, facilitating insect dispersal.

*Ophiostoma piceaperdum* (Rumbold) Arx was first described from sapwood of *Picea glauca* (Moench) Voss., associated with blue-stain and infestation by the bark beetle *Dendroctonus picea* perda* Hopkins (Rumbold, 1936). Rumbold (1936) found that this fungus resembled the recently described *Ceratostomella penicillata* (= *O. penicillatum*) Grosmann. She could, however, distinguish the two species by the smaller conidia and conidiophores in *O. piceaperdum*.

*Ophiostoma europhioides* (E. F. Wright & Cain) H. Solheim was described as *Ceratocystis europhioides* from Canada by Wright & Cain (1961) from *Picea* and *Pinus* spp. Wright & Cain (1961) noted the similarity with *O. penicillatum*, but could separate them because the allantoid ascospores and conidia of *O. penicillatum* (Grosmann, 1931, 1932) were unlike the cucullate ascospores and ellipsoid to obovoid conidia of *O. europhioides* (Wright & Cain, 1961).


Original descriptions of ascospore shapes suggest that *O. piceaperdum* and *O. europhioides* are distinct (Rumbold, 1936; Wright & Cain, 1961). Isolates of *O. piceaperdum* have been described as having ellipsoid ascospores, while ascospores of *O. europhioides* are cucullate. Ascospores of *O. piceaperdum* were not illustrated by Rumbold (1936). The importance of this fungus as an agent of blue-stain and its occurrence in various parts of the world has prompted us to reconsider its taxonomy. Olchowcki & Reid (1974) described *O. pseudoeurophioides* from spruce (*Picea* spp.) in Canada. The fungus can be distinguished from *O. europhioides* by apparently different anamorphs. *Ophiostoma pseudoeurophioides* was described as having a *Verticicladiella* anamorph and *O. europhioides* a *Leptographium* anamorph. In his monograph of *Ceratocystis* and *Ceratocystisps*, Upadhyay (1981) treated *O. pseudoeurophioides* as a synonym of *O. penicillatum*, and reported the anamorph...
to be a *Verticicladiella*. He also reported cuculate ascospores for *O. penicillatum*, in contrast to the allantoid ascospores reported for the neotype of this species by Solheim (1986). Wingfield (1985) reduced *Verticicladiella* to synonymy with *Leptographium*, thus eliminating the only obvious difference between *O. europhioides* and *O. pseudoeurophioides*. Harrington (1988) also treated these species as synonyms in his monographic study of species in *Leptographium*. In view of the confused taxonomy of *O. europhioides*, *O. piceaperdum* and *O. pseudoeurophioides*, this study was undertaken to review their status.

**MATERIALS AND METHODS**

Material for examination included all available herbarium specimens of *O. piceaperdum*, *O. europhioides* and *O. pseudoeurophioides*. In addition, isolates of *O. europhioides* and *O. piceaperdum* were obtained from a variety of culture collections and from various colleagues. Characterization of isolates was done on fungal structures produced on 2% malt extract agar (MEA, 20 g Biolab malt extract, 20 g Biolab agar and 1000 ml distilled water). For microscopy, relevant structures were mounted in lactophenol on glass slides. Herbarium specimens were examined by placing a drop of 1% KOH on the dried tissue. After 5 min, small pieces of fungal tissue were removed and mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made and ranges and means computed. Colours were determined using the charts of Rayner (1970).

Available isolates of the fungi under consideration were examined using 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 optimal growth temperatures for two representative isolates of *O. europhioides* and *O. piceaperdum* (CBS 366.75 and CMW 2811) were determined by inoculating eight MEA plates with 6 mm diam. agar discs taken from the actively growing margins of fresh isolates. The plates were incubated at temperatures in the range 5–20 °C at 5° intervals and 20–30 °C at 2.5° intervals. Colony diameters were measured after 8 d and growth was computed as means from eight readings. Cycloheximide tolerance of these two isolates was determined on MEA plates (eight per isolate) amended with 0.5 g l⁻¹ cycloheximide. The plates were incubated at 25 °C and colony diameters were measured on the eighth day.

**RESULTS**

Type specimens of *O. europhioides* (TRTC 45762, TRTC 36263, WIN(M) 71-18) and *O. piceaperdum* (BPI 595980, BPI 595981, BPI 595982), included both anamorph and teleomorph structures. Both species produced dark, olivaceous colonies and optimal growth occurred at 25 °C. Conidiophores developed abundantly on the surface of the mycelium in groups of 2–7. *Ophiostoma piceaperdum* and *O. europhioides*, have mean stipe lengths of 50–300 µm, and the conidiophores are characterized by the absence of rhizoid-like structures at the base. Both species have conidiophores with 2–3 primary branches and 3–4 series of branches. The conidia of *O. piceaperdum* and *O. europhioides* are ellipsoidal to obovoid with truncate ends and rounded apices. Conidia were also found to be of similar length, in the range 3–9 µm (Table 1).

No distinction could be made between the teleomorph structures of *O. piceaperdum* and *O. europhioides* in cultures. Perithecia of both species had neck lengths in the range 250–1130 µm, and the apices of the necks were characterized by the absence of ostiolar hyphae. Ascospores were distinctly cuculate in both species, and their sizes were in the range 4–6 µm long (Table 1).

*Ophiostoma piceaperdum* and *O. europhioides* cannot be distinguished on morphology and are, therefore, considered to be synonyms. No anamorph names were provided in the descriptions of either *O. piceaperdum* or *O. europhioides*, although it was noted that a *Leptographium* anamorph was present (Rumbold, 1936; Wright & Cain, 1961). Given the fact

### Table 1. Comparison of *Ophiostoma piceaperdum*, *O. europhioides* and *O. pseudoeurophioides*.

<table>
<thead>
<tr>
<th>Host</th>
<th><em>O. europhioides</em></th>
<th><em>O. piceaperdum</em></th>
<th><em>O. pseudoeurophioides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Associated insect</td>
<td>Dendroctonus piceaferda</td>
<td>Picea abies; P. abies; P. mariana; P. jezeensis; Pinus glauca; P. resinosa; P. strobus; P. sylvestris; P. banksiana; Pseudotsuga menziesii</td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td>U.S.A.</td>
<td>Canada, USA</td>
<td>Canada</td>
</tr>
<tr>
<td>Anamorph</td>
<td>Leptographium</td>
<td>Leptographium</td>
<td>Absent</td>
</tr>
<tr>
<td>Rhizoids</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Conidiophore length</td>
<td>140–300 µm</td>
<td>60–300 µm</td>
<td>150–500 µm</td>
</tr>
<tr>
<td>Conidium shape</td>
<td>Obovoid</td>
<td>Obovoid</td>
<td>Obovoid</td>
</tr>
<tr>
<td>Conidium size</td>
<td>3–11 µm</td>
<td>3.2–8.5 µm</td>
<td>2.5–5 µm</td>
</tr>
<tr>
<td>Perithecum neck length</td>
<td>110–950 µm</td>
<td>256–1128 µm</td>
<td>300–850 µm</td>
</tr>
<tr>
<td>Perithecum base size</td>
<td>90–350 µm</td>
<td>136–360 µm</td>
<td>150–300 µm</td>
</tr>
<tr>
<td>Ascospore shape</td>
<td>Cuculate</td>
<td>Cuculate</td>
<td>Cuculate</td>
</tr>
<tr>
<td>Ascospore size</td>
<td>3.6–5.6 µm</td>
<td>2.8–5.5 µm</td>
<td>4.5–5 × 2–2.5 µm</td>
</tr>
</tbody>
</table>

* Data and information in this table are derived from publications of Rumbold (1936) (*O. piceaperdum*), Wright & Cain (1961) (*O. europhioides*) and Olchowecki & Reid (1974) (*O. pseudoeurophioides*).
that this fungus occurs commonly in the absence of the teleomorph, we provide a name for its Leptographium anamorph. A short Latin diagnosis for the anamorph of O. piceaperdum was provided by Rumbold (1936) and an additional Latin description is thus not required. The following emended description is provided to avoid further confusion.

**Leptographium piceaperdum** K. Jacobs & M. J. Wingf., sp. nov.


**Typus**: Canada: Nova Scotia, ex *Picea glauca*, C. T. Rumbold (BPI 59581-fungus anamorph holotypus).

*Colonies* with optimal growth at 25 °C on 2% MEA, reaching 34 mm diam after 8 d. No growth below 5 °C or above 30 °C. Able to withstand high concentrations of cycloheximide with no reduction in growth on 0.5 g l⁻¹ cycloheximide after 4 d at 20 °C in the dark. Colonies dark olive (21 § m) with smooth margins. Hyphae submerged on solid medium with little aerial mycelia, hyaline to light olivaceous (21 k), smooth, occasionally roughened by granular deposits, straight, not constricted at the septa, 1.5–6 (mean = 4) µm diam. Conidio-

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**Figs 1–4.** Light micrographs of the conidiophore and conidia of *L. piceaperdum* (CMW 660) (Bars = 10 µm). **Fig. 1.** Conidiophore. **Fig. 2.** Conidiogenous apparatus. **Fig. 3.** Conidiogenous cells. **Fig. 4.** Conidia.
Conidiophores occurring singly or in groups of 2–7, arising directly from the mycelium or in groups of 2–7, arising directly from the mycelium or aerial mycelia, erect, macronematous, mononematous, 140–300 (mean = 204) µm long, rhizoid-like structures absent (Figs 1, 5, 9). Stipe light olivaceous (21°k), smooth, cylindrical, simple, 3–8 septate, 70–195 (mean = 121) µm long (from first basal septum to below primary branches), 5–9 (mean = 6) µm wide below primary branches, apical cell not swollen; 6–12.5 (mean = 8) µm wide at base, basal cell not swollen. Conidiogenous apparatus 55–120 (mean = 84) µm long, excluding the conidial mass, with 2–5 series of cylindrical branches, 2–3 primary branches, light olivaceous (21°k), smooth, cylindrical, 0–1 septate 15.5–39 (mean = 21.0) µm long and 3–8 (mean = 5) µm wide, secondary branches light olivaceous (21°k), aseptate, 11–23 (mean = 12) µm long, 2–6 (mean = 4) µm wide, tertiary branches light olivaceous (21°k), aseptate, 9–22 (mean = 15) µm long, 2–5 (mean = 3) µm wide, quaternary branches, hyaline to light olivaceous (21°k), aseptate, 7–16 (mean = 11.5) µm long, 2–3 (mean = 2.5) µm wide (Figs 2, 10). Conidiogenous cells discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, 11–26 (mean = 17.5) µm long and 1.5–3 (mean = 2) µm wide. Conidium development occurring through replacement wall building with holoblastic ontogeny, percurrent proliferation and delayed secession giving the false impression of sympodial proliferation (Minter et al., 1982, 1983; Van Wyk, Wingfield and Marasas, 1988) (Figs 3, 6, 7). Conidia light grey olivaceous (19°°), aseptate, obovoid to ellipsoid with truncated ends and rounded apices, 3–9 × 1–3 (mean = 5 × 2) µm (Figs 4, 8, 11). Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus, hyaline at first, becoming cream coloured (19°f) with age.
cucullate (Wright & Cain, 1961), whereas those of
able based on morphological data. In the original description,
P district, Ophiostoma piceaperdum
Herbarium types:
Conidiophores and conidia of
Figs 9–11. [Image]
Synonyms of O glauca
Picea glauca
Peters, Cape Breton, 595981; (holotype); Canada, Nova Scotia, St Peters, Cape Breton, 660).
P Shabotik River, Algoma district,
Reid & W. Obust
MFB 7439; Canada, Ontario, Shabotik River, Algoma district,
mariana
P Sandilands Forest Reserve,
O WIN(M) 71-18;
Cultures
T as CBS 366.75);
479 (CMW 3314.

Cain: Canada, Nova Scotia, St
P: USA, Nippletop Mountain,

Ophiostoma piceaperdum and O. europhioides have both been
considered to be similar to O. penicillatum (Rumbold, 1936; Wright & Cain, 1961; Griffin, 1968). O. piceaperdum can, however, easily be distinguished from O. penicillatum based on ascospore and conidial morphology. Ophiostoma penicillatum is characterized by the presence of curved ascospores and large, allantoid conidia. This is in contrast to the cucullate ascospores and obovoid conidia of O. piceaperdum (Grossman, 1931, 1932; Rumbold, 1936; Wright & Cain, 1961).

The synonymy of C. pseudoeurophioides with O. penicillatum, proposed by Upadhyay (1981) is rejected. Ceratocystis pseudoeurophioides has been distinguished from O. europhioides based on the presence of a Verticicladiella state in the former species and a Leptographium state in the latter. When Wingfield (1985) synonymized Verticicladiella with Leptographium, this distinction became redundant. Examination of the type specimen [WIN]M 71-13 and the original description of C. pseudoeurophioides (Olchoweki & Reid, 1974), revealed that this species cannot be distinguished from O. piceaperdum and we, therefore, propose the following synonymy:

Ophiostoma piceaperdum (Rumbold) Arx, Antonie van Leuwenhoek 18: 211 (1952).


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REFERENCES


