

***Brefeldochium pruinorum*, gen. et sp. nov., the anamorph of
Polydesmia pruinosa (Hyaloscyphaceae, Helotiales, Ascomycota)**

by

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With 8 figures

Verkley, G.J.M. (2005): *Brefeldochium pruinorum* gen. et sp. nov., the anamorph of *Polydesmia pruinosa* (Hyaloscyphaceae, Helotiales, Ascomycota). - Nova Hedwigia 80: 503-509.

Abstract: A sporodochial anamorph was observed in single-ascospore cultures of the discomycete *Polydesmia pruinosa*. The hyaline, septate, falcate phialoconidia are reminiscent of *Fusarium* macroconidia, but lack a hilum and are only formed in sporodochia. Conidioma formation was followed *in vitro* by formation of well-differentiated and fertile apothecia. This is the first confirmed report of an anamorph for the discomycete genus *Polydesmia* since 1891, when the German mycologist Brefeld described a conidial morph which he observed in his isolates of *P. pruinosa*. Because the anamorph has not been found in nature, the formal description is based on conidiomata formed *in vitro*. As there is no genus in which to satisfactorily classify this conidial state, the new anamorph genus *Brefeldochium* is proposed.

Key words: culture, Helotiales, fungicolous fungus, *Fusarium*, ITS sequence, new anamorph genus

Introduction

The genus *Polydesmia* Boud. comprises inoperculate discomycetes that form apothecia with a downy to tomentose receptacle and a pruinose hymenium. Currently seven species are accommodated in the genus (Korf 1978, Raitviir & Galan 1995, Huhtinen & Santesson 1997, Zhuang 1999, 2000). Dennis (1978) classified *Polydesmia* in a monogeneric tribe of the family Helotiaceae, while Korf (1973) placed it in the monogeneric subfamily Polydesmioideae of the Leotiaceae. On the basis of structure of the sterile tissues, Korf (1978) concluded that the genus is akin to the Hyaloscyphaceae, where it is currently still classified. *Polydesmia pruinosa* (Jerdon) Boud., the type species of the genus, occurs primarily on stromata of pyrenomycetes. Although individual apothecia are very small, the species is often noticed in the field

with the naked eye due to the conspicuous aggregates of pure white fruiting bodies on the dark substratum. Brefeld (1891), who was one of the pioneers in culturing discomycetes, observed a conidial state with fusoid to bent, 3-6-septate conidia in ascospore isolates of *Pseudohelotium jerdonii* Sacc. (= *Polydesmia pruinosa*, Korf 1978). Korf (1978) mentioned Brefeld's observations, but was unable to observe conidia in his own isolates of *P. pruinosa*. No anamorphs have been reported for the other *Polydesmia* species.

Materials and methods

Fresh apothecia of *Polydesmia pruinosa* were collected during a foray of the Dutch Mycological Society in 2002 in De Lutte, in the eastern part of the Netherlands. In the laboratory, apothecia were placed above 2% malt extract agar (MEA) with 30 ppm penicillin and streptomycin to shoot off their ascospores. Germinating ascospores were transferred to fresh media and incubated on the laboratory bench and in an incubator at 15°C, under a regime of 12 h n-UV light, 12 h darkness. Subcultures of CBS 111544 and 111545, obtained from single ascospores, sporulated on oatmeal agar (OA), but remained sterile on potato-dextrose, cherry-decoction, or cornmeal agars, and MEA. The other CBS cultures of this species are sterile. Measurements of structures were obtained from material mounted in tap water. The material was also studied in Melzer's reagent (Mlz) and Lugol (IKI). Drawings were made using a drawing tube, and digital images were recorded with Nikon Coolpix 995.

ITS sequences of strains CBS 111545, CBS 111544, CBS 160.92, and CBS 664.92 were determined as described by Verkley *et al.* (2003). These were found to be almost 100% identical, and have been deposited in GenBank under accession numbers AY775053-AY775056. BLAST searches revealed highest similarity to discomycete taxa of the family Dermateaceae, viz. species of *Neofabraea* H. S. Jacks., *Pezicula* Tul. & C. Tul., and *Dermea* Fr., as well as *Oidiodendron* spp. and several unidentified ericoid and epacrid mycorrhizal species. No close matches, however, were found for the relatively variable internal transcribed spacer 1 and 2 (results not shown), and therefore it was thought premature to produce a phylogenetic analysis at this time.

Taxonomy

Description of cultures, anamorph and teleomorph on OA.

Colonies on OA reaching 40-44 mm diam in 8 wk, spreading, glabrous (without aerial mycelium), with a slightly undulating, colourless margin, immersed mycelium colourless to buff; reverse concolorous.

Brefeldochium Verkley, anam. gen. nov.

Conidiomata sporodochia, circularia vel irregularia, solitaria vel aggregata, conidiophora paraphysisibus intermixta. Conidiophora acrogena, e stipite simplici, septato, cylindrico, hyalino, glabro, et 1-3 cellulis conidiogenis terminalibus, hyalinis, glabris, phialidicis, interdum collari brevi terminatis, constantia. Conidia falcata vel falcato-cylindrica, septata, ad septa saepe paulo constricta, hyalina, glabra, basim subtruncatam versus leniter attenuata, apicem obtusum versus curvata et attenuata.

Typus generis: *Brefeldochium pruinosum* Verkley

Etymology: Sporodochial genus named after Julius Oscar Brefeld (1839-1925), German mycologist, who for the first time studied and described many taxa *in vitro*.

Conidiomata sporodochial, circular or irregular, single or confluent, on the upper surface forming conidiophores intermixed with paraphyses. Conidiophores acrogenous, stipe simple, septate, cylindrical, hyaline, smooth-walled; conidiogenous cells terminal, hyaline, smooth-walled, phialidic, without visible proliferation, sometimes with a short collarete and an indistinct periclinal thickening; conidia falcate or falcate-cylindrical, septate, often slightly constricted around the septa, hyaline, smooth-walled, gradually attenuated towards the subtruncate base, curved and narrowed into a blunt tip.

Brefeldochium pruinoseum Verkley, sp. nov.

Figs 1-4, 8

Conidiomata sporodochia, circularia vel irregularia, solitaria vel aggregata, 100-300 μm diam., conidiophora paraphysibus intermixta. Conidiophora acrogena, e stipite simplici, 1-3-septato, cylindrico, hyalino, glabro, 15-50 \times 2.5-3.5(-4.0) μm et 1-3 cellulis conidiogenis terminalibus, hyalinis, glabris, phialidicis, interdum collari brevi terminatis, composita. Conidia falcata vel falcato-cylindrica, (1-)3-5-septata, ad septa saepe paulo constricta, hyalina, glabra, basim subtruncatam versus leniter attenuata, apicem obtusum versus curvata et attenuata, 18-45 \times 4-6 μm .

Etymology: Epithet taken from the teleomorph name; pruinoseus, referring to the hymenium of the apothecia which is covered with a pruina, i.e., a whitish powdery 'bloom'.

Conidiomata developing after 1-3(-6) weeks on the surface of OA, sporodochial, circular or irregular, single or confluent, 100-300 μm diam, white, composed of a sterile lenticular to irregular mass of isodiametric to irregular cells with hyaline, smooth and gelatinized walls, on the upper surface forming conidiophores intermixed with simple or branched paraphyses. Conidiophores acrogenous, stipe simple, 1-3-septate, cylindrical, hyaline, smooth-walled, 15-50 \times 2.5-3.5(-4.0) μm ; conidiogenous cells terminal, hyaline, smooth-walled, phialidic, without visible proliferation, sometimes with a short collarete and an indistinct periclinal thickening; conidia falcate or falcate-cylindrical, (1-)3-5-septate, often slightly constricted around the septa, hyaline, smooth-walled, widest just above the middle, gradually attenuated towards the subtruncate base, curved and narrowed into a blunt tip, 18-45 \times 4-6 μm , white, later pale yellowish in mass.

Typus: CBS 111545, two-week-old culture on OA, dried (CBS H-11004, holotype, in CBS herbarium sub *Brefeldochium*, ITS sequence deposited in GenBank as AY775054).

Teleomorphosis: *Polydesmia pruinosa* (Jerdon) Boud.

Figs 1, 5, 6

Apothecia developing in 10-13 weeks (15°C, n-UV 12 h rhythm), white, circular; disc convex, 0.2-0.75 mm diam, pruinose. Excipulum hyaline, composed of textura angularis, ectal and medullary tissues not well-differentiated, sometimes conidiophores also present on ascoma. Hairs undifferentiated, present as undulating hyphal projections on the surface of the receptacle. Asci 8-spored, cylindrical-clavate, apex conical-rounded, the apical apparatus blueing in iodine (IKI, MIz), arising from croziers, 85-125 \times 9-12.5 μm ; ascospores inaequilateral, ellipsoid-fusoid, (1-)3-septate, 13-19 \times 4-5 μm .

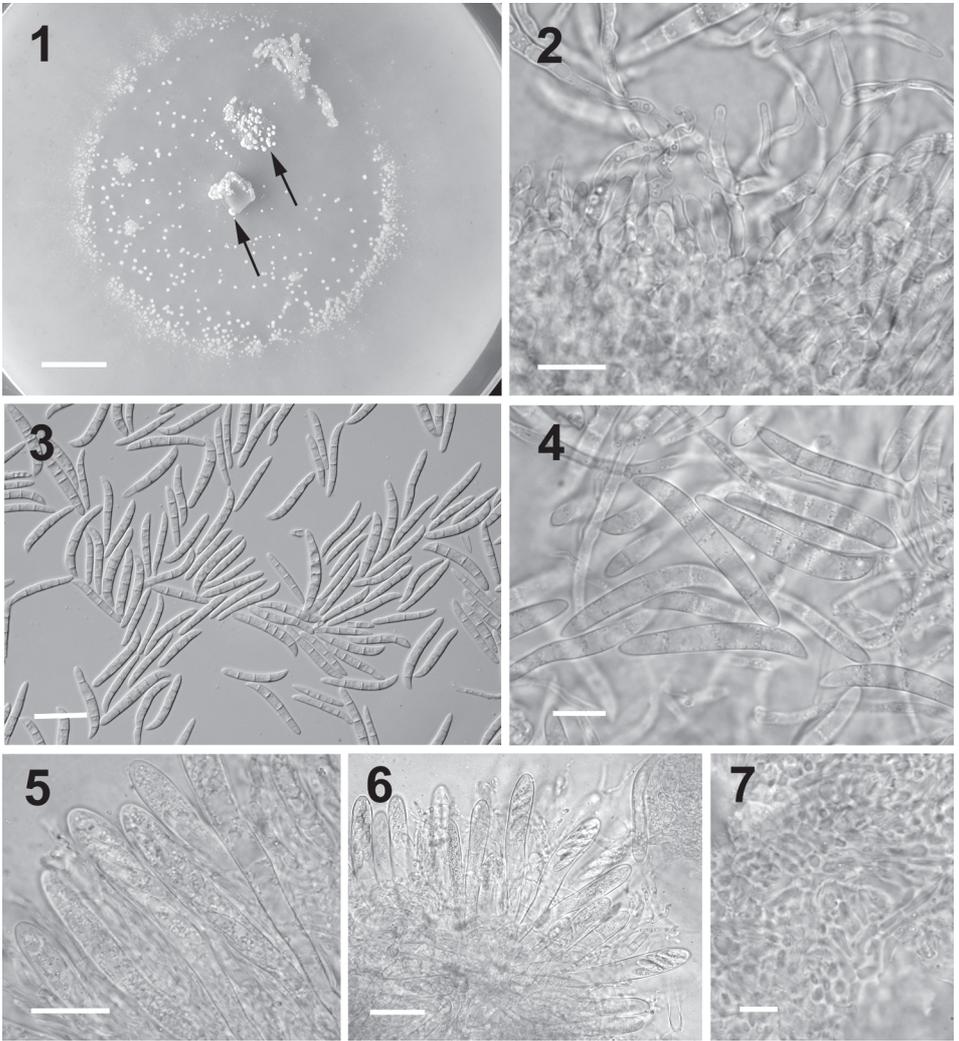


Fig. 1-7. *Polydesmia pruinosa*, CBS 111545, on oatmeal agar. - 1. 14 weeks old colony, with sporodochial masses and apothecia (arrows), scale bar = 1 cm. 2. Conidiogenous cells, scale bar = 10 µm. 3. Conidia, scale bar = 25 µm. 4. Conidia, scale bar = 10 µm. 5, 6. Asci, scale bar = 25 µm. 7. Excipular tissue of apothecium, scale bar = 10 µm.

Specimens examined:

Netherlands: Prov. Overijssel, De Lutte, on old pyrenomycete fruitbodies on dead wood, G. Verkley 1463 (CBS herbarium), also living cultures CBS 111544, 111545 (single-ascospore isolates). Germany: Holzhaus near Schwandorf, ex dead fruitbodies of *Camarops polyspermum*, 11 Oct. 1987 (Herb. I. Nuss, REG M46/87), living culture CBS 160.92, isolated by W. Helfer 694; also from REG M46/87, living culture CBS 664.92, isolated by E. Weber 101/7.

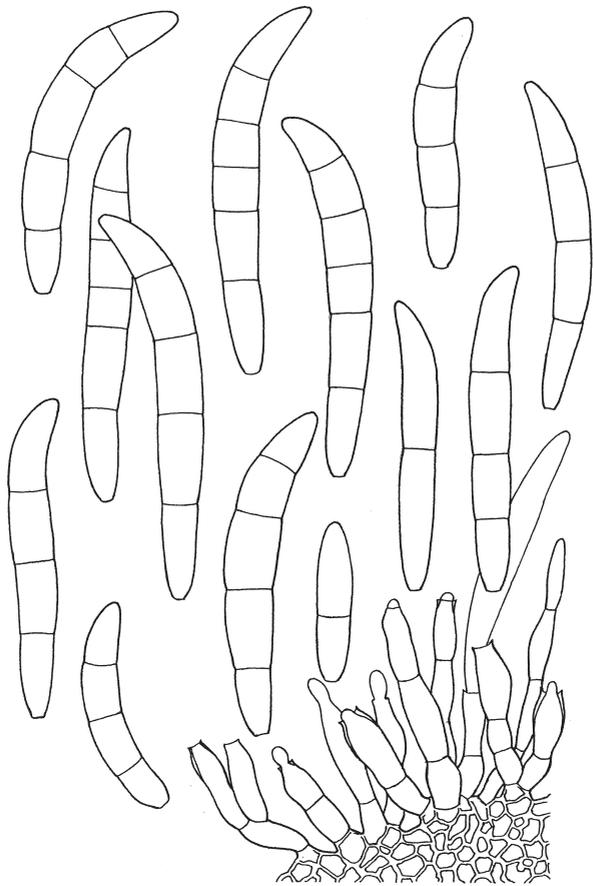


Fig. 8. *Brefeldochium pruinosum* state of *Polydesmia pruinososa*. Conidia and part of a conidioma of CBS 111545, after 15 days on oatmeal agar, scale bar = 10 μ m.

Discussion

Brefeldochium pruinosum is morphologically surprisingly similar to some members of the Section *Eupionnotes* of *Fusarium*, in particular *F. merismoides* var. *crassum* Wollenw., which can occur in the same environment as the discomycete (pers. comm. W. Gams). When isolating from ascospores of *P. pruinososa* in nature, one should always be aware of the risk of unwanted isolation of *Fusarium* and other hypocrealean fungi such as *Cosmospora episphaeria* (anamorph *F. aquaeductuum* var. *medium* Wollenw.). The identity of the *P. pruinososa* strains was confirmed by the development of the teleomorph *in vitro*, as well as by ITS sequencing. Blast searches using these sequences only yielded taxa from Dermateaceae of the order Helotiales. The diversity of ITS sequences of helotialean taxa available in GenBank is still very limited, and therefore no firm conclusions can be made at this time regarding the correct familial disposition of the genus *Polydesmia*. For this purpose, nuclear large and small subunit sequences would be more suitable.

In the cultures of *P. pruinosa* made by Brefeld (1891), conidium formation started after 10-14 d, and increased with time. Brefeld observed and also illustrated branched conidiophores which produced hyaline, first continuous, later 3-5-septate conidia, $26-34 \times 3-5 \mu\text{m}$. As far as is known, no material of his work has been preserved that could be re-examined. Korf (1978), who grew his cultures on MEA, did not observe the conidial morph in his isolates of *P. pruinosa*. He suggested that Brefeld might have been dealing with a contamination of one of these hypocrealean fungi that often grow together with *P. pruinosa* on the same substratum. There are, nonetheless, several indications that Brefeld successfully isolated *P. pruinosa* and that he was the first to observe and provide a description of *Brefeldochium pruinosum*. Firstly, he did not report any microconidia. Secondly, the shape and particularly the width of the conidia described by Brefeld suggest *B. pruinosum* rather than *Fusarium*. He did not mention or illustrate a pedicellate basal cell or hilum as seen in most *Fusarium* conidia, but *Fusarium merismoides* and its varieties also lack them. Thirdly, at the time Brefeld also described the anamorph of *C. episphaeria* and considered it as a true *Fusarium*; so he knew these anamorphs well and, as he himself noted, was aware of a possible contamination.

According to Gerlach & Nirenberg (1982), the cultures of *C. episphaeria* are also relatively slow-growing (reaching 25-35 mm diam in 10 d on PDA at 25°C), but start sporulating already after 2-4 d with small conidia. Later, macroconidia predominate in pionnotal or sporodochial slimy masses which cover the agar surface, and are subcylindrical, moderately to strongly curved, generally with a somewhat bent apical cell and a more or less distinct pedicellate basal cell, and 3(-5)-septate, about $20-78 \times 2.4-4.2$ in 3-5-septate conidia (Gerlach & Nirenberg, 1982), $20-66 \times 2-4 \mu\text{m}$ according to Wollenweber & Reinking (1935). The conidia of *B. pruinosum* are most similar to those of *F. merismoides* var. *crassum* (teleomorph unknown), which are relatively wide, almost allantoid, with a rounded apex, and without a distinct hilum or foot, 3-5-septate conidia $(29-)-37-65(-72) \times (3.8-)-4.5-5.8(-6.3) \mu\text{m}$. However, this *Fusarium* is capable of forming globose chlamydospores 7-12 μ diam, while the cultures grow much faster than those of *B. pruinosum*, in which chlamydospores are not formed (Gerlach & Nirenberg, 1982). The macroconidia can also become considerably longer than the conidia of *B. pruinosum*.

To date, *B. pruinosum* has only been observed in culture, and it is unclear what role the conidia play in the life cycle of this fungus in nature. From the formation of the apothecia in single-ascospore isolates *in vitro* it is clear that this species is homothallic. If the anamorph is formed in the same micro-environment as the teleomorph, conidia might play a role in short-distance dispersal and might infect new fruitbodies of the host.

Because the genus *Polydesmia* now comprises seven species that might have similar anamorphs, a separate binomial for this fungus does not seem superfluous (see Art. 59, Rec. 59A.3, ICBN). With the provision of a modern description and also a formal name, it is hoped that this anamorph may be noticed in its environment or in other habitats and that its role can be clarified in the future.

Acknowledgements

Prof. Pedro W. Crous and dr Richard C. Summerbell are kindly thanked for critical reading of the manuscript.

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Received 10 October 2004, accepted 18 October 2004.