A new *Ophiostoma* species associated with bark beetles infesting Norway spruce

R. Kirschner and F. Oberwinkler

Abstract: During a survey of fungi associated with bark beetles in Germany, an undescribed species of *Ophiostoma* was isolated that differs from the other species of the genus by having pigmented, aseptate, convergent ostiolar hyphae, cucullate, sheathed ascospores, and a *Hyalorhinocladiella* anamorph. The species is described as ***Ophiostoma neglectum*** Kirschner & Oberwinkler. It is rarely associated with primary bark beetles but often associated with secondary bark beetles mainly infesting Norway spruce.

Key words: *Ophiostoma neglectum*, *Hyalorhinocladiella*, secondary bark beetles, *Picea abies*, *Pinus sylvestris*, conidial development.

Résumé : Au cours d'un survol des champignons associés aux insectes des écorces en Allemagne, les auteurs ont isolé une espèce d'*Ophiostoma* qui diffère des autres espèces du genre par la présence de pigments, d'hyphes ostiolaires aseptés et convergents, de spores cucullées et enveloppées et d'un anamorphe de type *Hyalorhinocladiella*. Les auteurs décrivent cette espèce comme l'***Ophiostoma neglectum*** Kirschner and Oberwinkler. Elle est rarement associée avec les insectes primaires de l'écorce, mais souvent associée avec les insectes secondaires de l'écorce, surtout ceux qui infestent l'épinette de Norvège.

Mots clés : *Ophiostoma neglectum*, *Hyalorhinocladiella*, insectes secondaires de l'écorce, *Picea abies*, *Pinus sylvestris*, développement conidial.

[Traduit par la Rédaction]

Introduction

Bark beetles are known to be associated with specific fungi. Many of these are ophiostomatoid fungi, mainly species of *Ophiostoma* H. & P. Sydow, *Ceratocystis* Ellis & Halst., and *Ceratocystiopsis* Upadhyay & Kendrick, which are vectored by bark beetles to new host trees (Mathiesen-Käärik 1953; Solheim 1986). *Ceratocystis* spp. differ morphologically from the two other mentioned genera by their *Chalara* (Corda) Rabenh. anamorphs (de Hoog and Scheffer 1984). *Ceratocystiopsis* spp. differ from species of the genus *Ophiostoma* by having falcate ascospores (Upadhyay and Kendrick 1975). Species of *Ophiostoma* have ascospores of different shapes and often have anamorphs that can be accommodated in the following genera: *Hyalorhinocladiella* Upadhyay & Kendrick, *Sporothrix* Hektoen & Perkins, *Leptographium* Lagerberg & Melin, and *Graphium* Corda (Seifert et al. 1993).

Some ophiostomatoid fungi are virulent plant pathogens and assist aggressive bark beetles in overwhelming host tree resistance (Christiansen and Solheim 1990), whereas others are weak plant pathogens or saprophytes. They all depend on dispersal by bark beetles and sporulate in the bark beetles' galleries in the phloem and sapwood of the infested trees.

The diversity of ophiostomatoid fungi associated with some bark beetle species in Europe was investigated by Grosmann (1931) in Germany, Siemaszko (1939) in Poland, Mathiesen-Käärik (1953) in Sweden, Kotynková-Sychrová (1966) in the former Czechoslovakia Republic, Solheim (1986) in Norway, Harding (1989) in Denmark, Lévieux et al. (1989) in France, and Pashenova et al. (1995) in Russia. Nevertheless, bark beetle galleries are still a poorly explored habitat of the fungi.

During a recent survey of fungi associated with bark beetles in Germany, several new species were isolated (Kirschner 1998). One was described as *Phialocephala trigonospora* by Kirschner and Oberwinkler (1998). Another new species is presented here as a member of *Ophiostoma*.

Materials and methods

From autumn 1994 to autumn 1996, bark samples of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) containing adult bark beetles were collected near Bad Waldsee, Langenau, Schluchsee, and Tübingen in Baden-Württemberg, and near Riedlhütte (Bayrischer Wald) and Oberjoch in Bavaria, all in southern Germany. The beetles were identified as *Crypturgus cinereus* (Hrbst.), *C. pusillus* (Gyll.), *Dryocoetes autographus* (Ratz.), *Hylurgops palliatus* (Gyll.), *Ips typographus* (L.), *Pityogenes chalcographus* (L.), and *Trypodendron lineatum* (Olivier). Living beetles were individually placed in Petri dishes containing autoclaved pieces of spruce phloem embedded in water agar. Pure cultures of the new fungal species were obtained by transferring ascospores extruded at the tip of the fruit bodies with a sterile needle to Petri dishes with autoclaved pieces of spruce twigs

Received June 3, 1998.

R. Kirschner[1] and F. Oberwinkler. Lehrstuhl Spezielle Botanik/Mykologie, Universität Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.

[1]Author to whom all correspondence should be addressed. e-mail: roland.kirschner@uni-tuebingen.de

Table 1. Infestation by *Ophiostoma neglectum* of bark beetles collected from *Picea abies* and *Pinus sylvestris* in southern Germany from 1994 to 1996.

Species	No. infested	% infested	Total no. examined
Crypturgus cinereus	1	0.8	127
Crypturgus pusillus	49	15	332
Dryocoetes autographus	130	39	334
Hylurgops palliatus	130	30	437
Ips typographus	6	0.6	1071
Pityogenes chalcographus	8	0.8	976
Trypodendron lineatum	7	5	137

embedded in 1.5% water agar and to Petri dishes containing 2% malt extract agar (20 g Difco malt extract per 1 L water).

Growth diameters were measured from three cultures growing on malt extract agar in the dark at 23°C. For light microscopy, fresh material was mounted in water. To compare the new fungus with a similar species, a culture of *Ophiostoma brevicolla* (Davidson) de Hoog & Scheffer (CBS 795.73) was examined by light microscopy. For scanning electron microscopy (SEM), material was removed from a pure culture of CBS 100596 (from ex-type strain of the new *Ophiostoma* species) on autoclaved spruce twigs embedded in water agar, fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for several days, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, washed with distilled water, and dehydrated in a graded ethanol series (Rieder and Schmidt 1987). The material was subsequently critical point dried, coated with gold–palladium, and examined using a Cambridge Stereoscan 250 MK 2 scanning electron microscope.

Results

Originating from individual bark beetles that were placed in Petri dishes containing autoclaved spruce phloem pieces embedded in water agar, different fungi developed and sporulated after about 1 month. Among these, a previously undescribed *Ophiostoma* species was found. The new species has a *Hyalorhinocladiella* anamorph and cucullate ascospores and is therefore included in the genus *Ophiostoma*. The species was obtained from bark beetles mainly infesting *Picea abies*. At least 5% of the examined individual beetles of the four bark beetle species *Crypturgus pusillus*, *Dryocoetes autographus*, *Hylurgops palliatus*, and *Trypodendron lineatum* were associated with the new *Ophiostoma* sp. (Table 1). This *Ophiostoma* sp. was detected from less than 1% of the individuals from the other three species of bark beetles, *Crypturgus cinereus*, *Ips typographus*, and *Pityogenes chalcographus*. A description of the fungus as a new *Ophiostoma* species follows.

Ophiostoma neglectum Kirschner & Oberwinkler sp.nov. (Figs. 1–10)

Perithecia superficialia, bases globosae, irregulariter ornata angustatis (1–1.5 μm diametro) hyphis, pallide brunneae, 55–100 μm diametro, colla atrobrunnea, 65–130 μm longa, ad basim 21–35 μm, ad apicem 12–18 μm lata, hyphae ostiolares convergentes, rigidae, brunneae, aseptatae, parietibus crassis, 20–59 μm longae, ad basim 2–3 μm latae, sensim acuminatae. Asci clavati, ad 16 μm longi, 5 μm lati, evanescentes. Ascosporae curvatae, cucullatae, cum vagina hyalina cucullata, 3.5–4 × 1.5–2 μm a latere visae, circulares, 1–1.5 μm diametro, cum vagina hyalina triangulari aspectu terminali, in filo gelatinoso extrusae. Coloniae in agaro malto 10 mm diametro post 12 dies ad 23°C, albae, sine mycelium aerium. Conidiophorae in substrato naturali saepe aggregatae, hyalinae, erectae, mononematosae, stipes brevissimus, apparatus conidiogenus ex usque tribus seriebus metularum et cellularum conidiogenarum compositus. Metulae 5–6 × 2 μm, cellulae conidiogenae annellidicae, cylindricae, 20–27 × 1–2 μm. Conidia hyalina, aseptata, clavata, 3–4.5 × 1–1.5 μm, in massa hyalina aggregata. Conidiophorae in agaro malto simplices vel ex stipe brevissimo et cellulis conidiogenis compositae, conidia hyalina, aseptata, ellipsoidea, obovoidea vel clavata, 3–6 × 1–3 μm.

ETYMOLOGY: The epithet *neglectum* refers to the inconspicuous perithecia growing in a cryptic habitat.

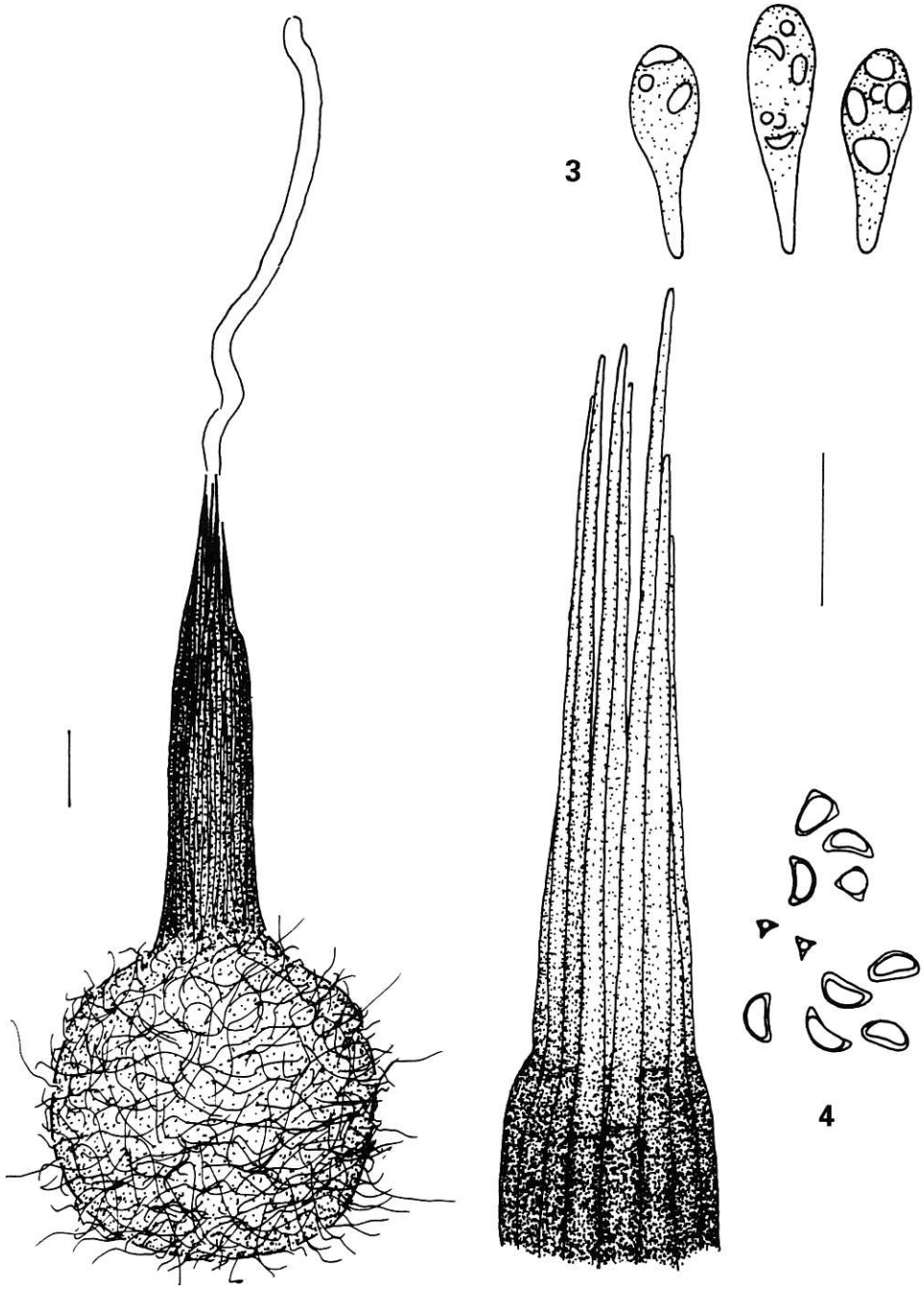
HOLOTYPUS: CBS 100596 (cultura sicca), de *Hylurgope palliato* ex trunco *Piceae abietis* lecto isolata, Germania, Baden-Württemberg, Tübingen, 10.09.1995, R. Kirschner.

Ophiostoma neglectum CBS 100597, de *Pityogene chalcographo* ex trunco *Piceae abietis* lecto isolata, Germania, Baden-Württemberg, Bad Waldsee, 12.09.1994, R. Kir schner.

CULTURAE VIVAE: *Ophiostoma neglectum* CBS 100596.

Perithecia superficial on the substratum, bases globose, irregularly covered with thin hyphae (1–1.5 μm thick), pale brown, 55–100 μm in diameter, necks dark brown, 65–130 μm long, 21–35 μm wide at the base, 12–18 μm wide at the apex (Fig. 1), ostiolar hyphae convergent, stiff, brown, aseptate, thick-walled, 20–59 μm long, 2–3 μm wide at the base, gradually tapering to the acute apex (Fig. 2). Asci clavate, up to 16 μm long and 5 μm wide, evanescent (Fig. 3). Ascospores curved, cucullate, with hyaline, cucullate sheath, 1.5–2 × 3.5–4 μm in side view (including sheath), circular, 1–1.5 μm in diameter, with hyaline, triangular sheath in end view (Fig. 4), extruded as a gelatinous filament (Fig. 1). Colonies on malt extract agar 10 mm in diameter in 12 days at 23°C, white, without aerial mycelium and without perithecia. Conidiophores on natural media often aggregated, hyaline, erect, mononematous, with a very short stipe and up to three series of metulae and conidiogenous cells (Fig. 5*b*). Conidiogenous cells annellidic, cylindrical, 1–2 × 20–27 μm. Conidia (Fig. 5*a*) hyaline, one-celled, clavate, 1–1.5 × 3–4.5 μm, aggregated in a slimy head. Conidiophores in malt extract agar simple or composed, with a very short

Figs. 1–4. Teleomorph of *Ophiostoma neglectum*. Fig. 1. Perithecium with ascospores extruded in a filament. Scale bar = 20 μm. Fig. 2. Convergent, pigmented, aseptate ostiolar hyphae. Fig. 3. Clavate, young asci. Fig. 4. Ascospores with cucullate sheaths in side view and triangular sheaths in end view. Figs. 2–4. Scale bar = 10 μm.



stipe and conidiogenous cells (Fig. 6*b*), conidia hyaline, one-celled, ellipsoidal, obovoid or clavate, 1–3 × 3–6 μm (Fig. 6*a*).

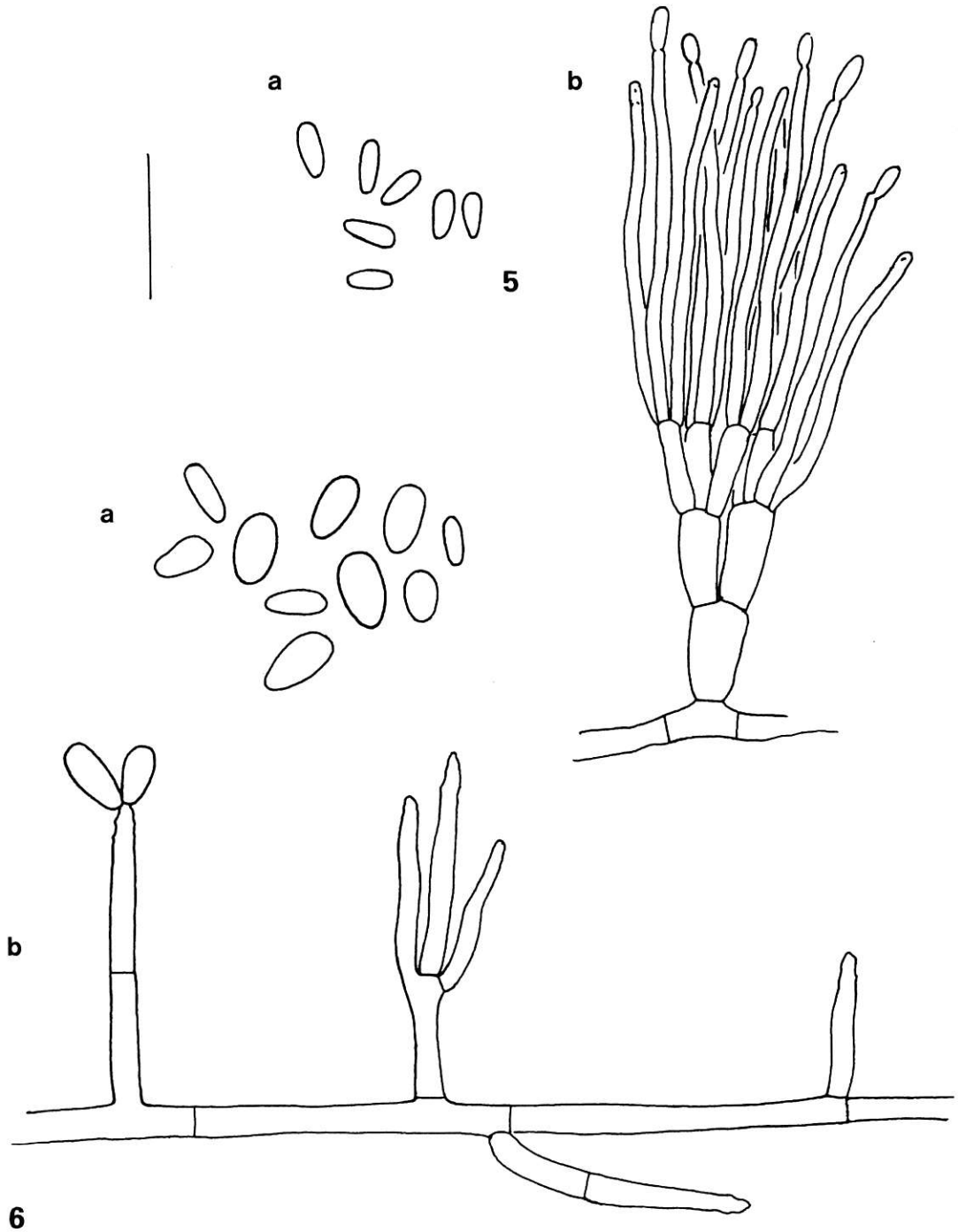
The mode of conidiogenesis was not clearly visible using light microscopy. Some very inconspicuous scars seemed to be present at the tips of the conidiogenous cells (Figs. 5*b*, 6*b*). Denticles indicating a sympodial conidiogenesis were not found by either light or electron microscopy. By SEM, annellations at the distal parts of the conidiogenous cells were visible (Figs. 7–10). The segments between the annellations are irregularly swollen, causing distortions of the conidiogenous cell.

Discussion

Taxonomy

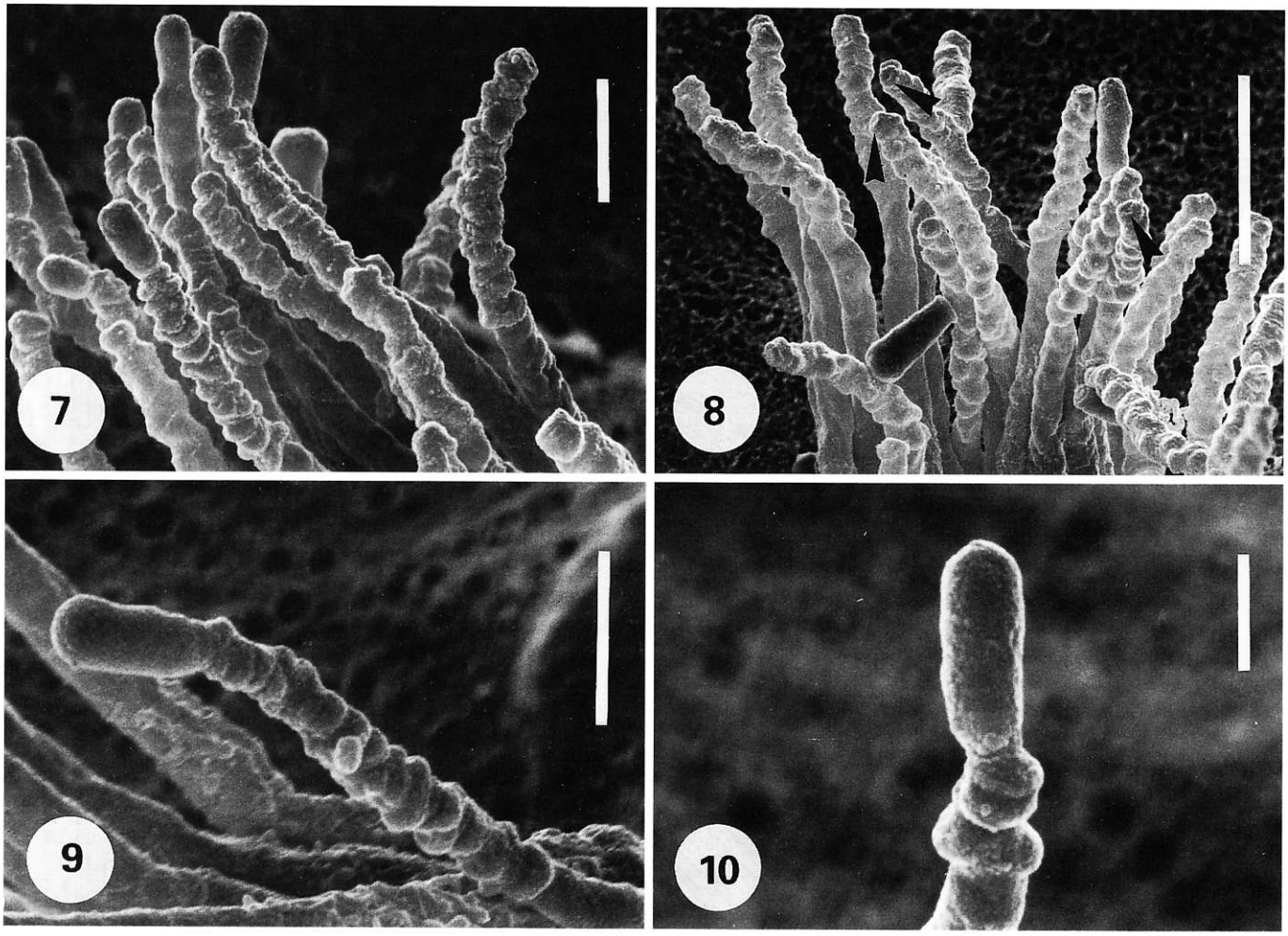
The anamorph of the new species has hyaline, mononematous conidiophores. The annellated conidiogenous cells of this anamorph, seen by SEM, differ from annellated conidiogenous cells without distortions known from other

Figs. 5 and 6. Anamorph of *Ophiostoma neglectum*. Scale bar = 10 μm. Fig. 5. *Hyalorhinocladiella* anamorph on spruce twig pieces embedded in water agar. Conidia (*a*) and penicillately branched conidiophore (*b*). Fig. 6. *Hyalorhinocladiella* anamorph on malt extract agar. (*a*) Conidia. (*b*) Reduced conidiophores.



hyphomycetes with annellidic conidiogenesis, e.g., *Leptographium* spp. (Wingfield 1985) and the *Hyalorhinocladiella* anamorph of *Ophiostoma ips* (Rumb.) Nannf. (Benade et al. 1995). A very similar type of annellidic conidiogenous cells was found in the *Hyalorhinocladiella* anamorphs of *Ceratocystiopsis minuta-bicolor* (Davidson) Upadhyay & Kendrick and *Ophiostoma minus* (Hedgcock) H. & P. Sydow (Benade et al. 1996). Similar annellidic conidiogenous cells were also detected in the *Sporothrix* anamorph of *Ophiostoma nigrocarpum* (Davidson) de Hoog, but conidiogenous cells with denticles were predominant (Benade et al. 1997). The deviating type of annellidic conidiogenous cells was regarded as an intermediate stage between exclusively sympodial and exclusively annellidic in which most conidia are produced by annellidic conidiogenesis and few conidia by sympodial conidiogenesis (Benade et al. 1996, 1997). Although intermediates exist between annellidic and sympodial conidiogenesis in both the anamorph genera *Hyalorhinocladiella* and *Sporothrix*, the two genera can be separated by predominantly annellidic

Figs. 7–10. *Hyalorhinocladiella* anamorph of *Ophiostoma neglectum* (from ex-type culture) seen by SEM. Fig. 7. Distal parts of several conidiogenous cells producing terminal conidia. Scale bar = 2 μm. Fig. 8. Conidiogenous cells with the sites of last conidium secession visible (arrowheads). Scale bar = 4 μm. Fig. 9. Distal part of a conidiogenous cell with distorted annellations. Scale bar = 2 μm. Fig. 10. Apex of young conidiogenous cell terminally producing a conidium. Scale bar = 1 μm.



conidiogenesis in *Hyalorhinocladiella* and predominantly sympodial conidiogenesis in *Sporothrix*. Because no conidiogenous cells with denticles were detected in the anamorph of the new *Ophiostoma* species, annellidic conidiogenesis is apparently predominant. Therefore, this anamorph is accommodated in *Hyalorhinocladiella*.

The conidiophores of this anamorph genus were characterized as "simple, reduced or hardly distinguishable from the vegetative hyphae" (Upadhyay 1981). This is the case in cultures growing on rich media like malt extract agar (Fig. 6*b*). On natural media, however, the conidiophores of the *Hyalorhinocladiella* anamorph are more complex, i.e., the conidiophores are composed of a short, hyaline stipe and a penicillately branched conidiogenous head (Fig. 5*b*). Thus apart from the lack of pigmentation and the short stipe in comparison with the large conidiogenous head, these conidiophores exhibit a similar structure as those of *Leptographium*. The conidial development in both genera is identical (Benade et al. 1996). Similar, complex conidiophores were also described for the *Hyalorhinocladiella* anamorph of *O. minus* (Hedgcock) H. & P. Sydow developing on the natural substratum (Münch 1907).

Ophiostoma neglectum differs from other species of the genus by the combination of the following three characteristics: pigmented, aseptate, convergent ostiolar hyphae; cucullate, sheathed ascospores; and *Hyalorhinocladiella* anamorph. The species is similar to *Ceratocystiopsis minuta* (Siemaszko) Upadhyay & Kendrick in having aseptate, convergent ostiolar hyphae and a *Hyalorhinocladiella* anamorph, but the ascospores of the former are cucullate and those of the latter are falcate (Upadhyay 1981). *Ophiostoma neglectum* is similar to *O. brevicolla* because of the convergent ostiolar hyphae and curved, sheathed ascospores, but *O. brevicolla* differs by its septate ostiolar hyphae (R. Kirschner and F. Oberwinkler, personal observations on strain CBS 795.73) and its pigmented *Leptographium* anamorph (Upadhyay 1981). The ascospores of *O. brevicolla* are 6–7.5 μm long including sheath (Upadhyay 1981) and are therefore longer than those of *O. neglectum*.

Ecology

Ophiostoma neglectum was found to be associated with bark beetles (Table 1) that preferably infest *Picea abies* (Postner 1974). The fungus was not detected from bark bee-

tles predominantly infesting other trees, e.g., *Ips sexdentatus* (Boern.) and *Orthotomicus laricis* (F.) infesting *Pinus sylvestris* (Kirschner 1998). Therefore, *O. neglectum* seems to have a preference for *Picea abies* phloem and sapwood.

Among the spruce-infesting bark beetles associated with *O. neglectum*, *Ips typographus* and *Pityogenes chalcographus* are primary, i.e., aggressive bark beetles that can infest healthy living trees (Rudinsky 1962). The other beetle species in the list are secondary bark beetles that rarely infest living trees (Postner 1974). Table 1 shows that the fungus was rarely associated with the primary bark beetles *Ips typographus* and *Pityogenes chalcographus* because less than 1% of these beetles carried *O. neglectum*. In contrast, the fungus was very often associated with the secondary bark beetles *Dryocoetes autographus* and *Hylurgops palliatus*, being carried by at least 30% of the individual beetles. These beetles preferably colonize dead trees in a shady and moist environment (Grünwald 1986). Because of the comparatively close association of *O. neglectum* with *D. autographus* and *H. palliatus*, the fungus seems to have substratum requirements like those of these two bark beetle species. To a lesser extent, the fungus was also associated with the other secondary bark beetles *Crypturgus* spp. and *Trypodendron lineatum*. In most cases, *Crypturgus* spp. establish their galleries using galleries of other bark beetles (Postner 1974). In our collections, *Crypturgus* spp. were very often associated with primary bark beetles. *Crypturgus* spp. are assumed to carry a fungus flora similar to that of their host beetles and therefore are not regularly associated with *O. neglectum*. *Trypodendron lineatum* is an ambrosia beetle preferably associated with an ambrosia fungus (Postner 1974).

Because of the close association of *O. neglectum* with secondary bark beetles infesting spruce trees, this fungus appears to be a pure saprophyte predominantly colonizing dead trunks of Norway spruce, probably lacking plant pathogenic capabilities.

Acknowledgements

We thank H. Schoppmann for the work with SEM, F. Albrecht for helping with the photographic work, M. Piepenbring for critically reading the manuscript, and S. Wood and I. Reinhold for correcting the English text. C. Turnau and S. Harding kindly provided literature not available in Germany. The study was supported by the DFG-project Organismische Interaktionen in Waldökosystemen.

References

Benade, E., Wingfield, M.J., and van Wyk, P.S. 1995. Conidium development in the *Hyalorhinocladiella* anamorph of *Ophiostoma ips*. Mycologia, **87**: 298–303.

Benade, E., Wingfield, M.J., and van Wyk, P.S. 1996. Conidium development in the *Hyalorhinocladiella* anamorphs of *Ceratocystiopsis minuta-bicolor* and *Ophiostoma minus*. Can. J. Bot. **74**: 891–897.

Benade, E., Wingfield, M.J., and van Wyk, P.S. 1997. Conidium development in *Sporothrix* anamorphs of *Ophiostoma*. Mycol. Res. **101**: 1108–1112.

Christiansen, E., and Solheim, H. 1990. The bark beetle-associated blue-stain fungus *Ophiostoma polonicum* can kill various spruces and Douglas-fir. Eur. J. For. Pathol. **20**: 436–446.

de Hoog, G.S., and Scheffer, R.J. 1984. *Ceratocystis* versus *Ophiostoma*: a reappraisal. Mycologia, **76**: 292–299.

Grosmann, H. 1931. Beiträge zur Kenntnis der Lebensgemeinschaft zwischen Borkenkäfern und Pilzen. Z. Parasitenkd. **3**: 56–102.

Grünwald, M. 1986. Ecological segregation of bark beetles (Coleoptera, Scolytidae) of spruce. J. Appl. Entomol. **101**: 176–187.

Harding, S. 1989. The influence of mutualistic blue stain fungi on bark beetle dynamics. Ph.D. thesis, Department of Zoology, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Kirschner, R. 1998. Diversität mit Borkenkäfern assoziierter filamentöser Mikropilze. Ph.D. thesis, Fakultät für Biologie Universität Tübingen, Tübingen, Germany.

Kirschner, R., and Oberwinkler, F. 1998. *Phialocephala trigonospora*, a new hyphomycete species associated with conifericolous bark beetles. Sydowia, **50**: 205–212.

Kotynková-Sychrová, E. 1966. Mykoflora chodeb kurovcu v Ceskoslovensku. Ceska Mykol. **20**: 45–53.

Lévieux, J., Lieutier, J., Moser, J.C., and Perry, T.J. 1989. Transportation of phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated mites. J. Appl. Entomol. **108**: 1–11.

Mathiesen-Käärik, A. 1953. Eine Übersicht über die gewöhnlichen mit Borkenkäfern assoziierten Bläuepilze in Schweden. Medd. Statens Skogsforskninginst. **43**: 1–74.

Münch, E. 1907. Die Blaufäule des Nadelholzes. Naturwiss. Land-Forstwirt. **5**: 531–573.

Pashenova, N.V., Vetrova, V.P., Matrenina, R.M., and Sorokina, E.N. 1995. Ophiostomataceae fungi in larch bark beetle galleries. [In Russian.] Lesovedenie, **6**: 62–68.

Postner, M. 1974. Scolytidae (= Ipidae), Borkenkäfer. *In* Die Forstschädlinge Europas. Band 2: Käfer. *Edited by* W. Schwenke. Paul Parey, Hamburg, Germany. pp. 334–482.

Rieder, N., and Schmidt, K. 1987. Morphologische Arbeitsmethoden in der Biologie. VCH Publishers, Weinheim, Germany.

Rudinsky, J.A. 1962. Ecology of Scolytidae. Annu. Rev. Entomol. **7**: 327–348.

Seifert, K.A., Wingfield, M.J., and Kendrick, W.B. 1993. A nomenclator for described species of *Ceratocystis*, *Ophiostoma*, *Ceratocystiopsis*, *Ceratostomella* and *Sphaeronaemella*. *In* *Ceratocystis* and *Ophiostoma*: taxonomy, ecology, and pathogenicity. *Edited by* M.J. Wingfield, K.A. Seifert, and J.F. Webber. APS Press, St. Paul, Minn. pp. 269–288.

Siemaszko, W. 1939. Zespoly grzybów towarzyszacych kornikom polskim. Planta Pol. **7**: 1–54.

Solheim, H. 1986. Species of Ophiostomataceae isolated from *Picea abies* infested by the bark beetle *Ips typographus*. Nord. J. Bot. **6**: 199–207.

Upadhyay, H.P. 1981. *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press, Athens, Ga.

Upadhyay, H.P., and Kendrick, W.B. 1975. Prodromus for a revision of *Ceratocystis* (Microascales, Ascomycetes) and its conidial states. Mycologia, **67**: 798–805.

Wingfield, M.J. 1985. Reclassification of *Verticicladiella* based on conidial development. Trans. Br. Mycol. Soc. **85**: 81–93.