

# A new *Chionosphaera* species associated with conifer inhabiting bark beetles\*

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Received 3 March 2000; accepted 15 July 2001.

An undescribed species of the heterobasidiomycetous genus *Chionosphaera* is carried by the bark beetles *Dryocoetus autographus*, *Hylurgops palliatus*, *Ips acuminatus*, *Ips sexdentatus*, *Ips typographus*, *Orthotomicus laricis*, *Pityogenes chalcographus*, *Pityokteines spinidens*, and *Polygraphus poligraphus* infesting conifers in Europe (*Abies alba*, *Larix decidua*, *Picea abies*, and *Pinus sylvestris*). *Chionosphaera cuniculicola* sp. nov. differs morphologically from *C. apobasidialis* in the more slender basidiospores. The segregation of the two species is supported by comparison of partial sequences of the large subunit of the ribosomal gene. Isotype material of *Chionosphaera lichenicola* was re-examined. In contrast to *C. cuniculicola* and *C. apobasidialis*, *C. lichenicola* exhibits clamps at the septa. *Fibulostilbum phylacicola* is considered as a clamp-bearing species of *Chionosphaera*.

## INTRODUCTION

*Chionosphaera* Cox 1970 is a heterobasidiomycetous genus characterised by minute, pale, stipitate-capitate fruit bodies with gasteroid holobasidia, simple septal pores without associated organelles, and a yeast stage (Cox 1970, Oberwinkler & Bandoni 1982, Oberwinkler & Bauer 1989). Three species were hitherto described in this genus: *C. apobasidialis* Cox 1970 (Cox 1970), *C. lichenicola* Alstrup, Sutton & Tønsberg 1993 (Alstrup 1993), and *C. coppinsii* P. Roberts 1997 (Roberts 1997). *C. apobasidialis* was found on bark of deciduous trees and on lichens (Cox 1970, Diederich 1996). *Chionosphaera lichenicola* and *C. coppinsii* were exclusively found on lichens growing on deciduous trees (Alstrup 1993, Diederich 1996, Roberts 1997). A further fungus similar to *C. apobasidialis* was found on an ascomycete and described as *Fibulostilbum phylacicola* Seifert & Bandoni (Seifert, Oberwinkler & Bandoni 1992).

During a survey on fungi associated with bark beetles infesting conifers in central Europe, several undescribed species of heterobasidiomycetous fungi were detected (Kirschner & Oberwinkler 2000, Kirschner, Bauer & Oberwinkler 1999). One of the species collected could be assigned to *Chionosphaera* and is treated here.

## MATERIAL AND METHODS

Bark samples of Silver fir (*Abies alba*), European larch (*Larix decidua*), Norway spruce (*Picea abies*), and Scots pine (*Pinus sylvestris*) containing bark beetles and bark beetle galleries

were collected in Germany, Switzerland, and Italy. Collections in Germany were made near Bad Waldsee, Langenau, Albstadt-Lautlingen, and Tübingen in Baden Württemberg, near Darmstadt in Hessen, and near Oberjoch and Riedlhütte in Bavaria. In Switzerland, material was collected near Schwanden, Glarus. Additional bark samples were collected near Udine, Friuli-Venèzia Giulia, Italy, by M. Faccoli. Collections were made during spring to autumn, 1994–2000. The beetles were identified as *Crypturgus cinereus*, *C. pusillus*, *Dryocoetes autographus*, *Hylurgops palliatus*, *Ips acuminatus*, *Ips sexdentatus*, *Ips typographus*, *Orthotomicus laricis*, *Pityogenes chalcographus*, *Pityokteines spinidens*, and *Polygraphus poligraphus* using the keys by Grüne (1979) and Schedl (1981). Samples of each beetle species were deposited in the private collection of R. Kirschner. Living adult beetles were individually placed into Petri dishes containing autoclaved pieces of inner bark of Norway spruce embedded in 4% water agar (Kirschner *et al.* 1999). The fungus described herein was detected in the form of basidiomata developing in mixed cultures of microorganisms growing out from propagules disseminated by the beetles. Bark beetle galleries were also examined for the presence of this fungus with a dissecting microscope, and basidiospores were transferred with a fine needle from sporocarps in the galleries to Petri dishes containing 2% (Difco) malt extract agar. Specimens of the iso-type of *C. lichenicola* deposited in C and of the fungus associated with bark beetles were mounted in 10% KOH and investigated by light microscopy. Ultrastructural studies on the undescribed species were performed as described by Kirschner *et al.* (1999).

DNA isolation, sequencing and analysing of the 5' region of the nuclear large subunit of the ribosomal RNA gene were conducted as described by Begerow, Bauer & Oberwinkler

\* Part 191 in the series 'Studies in Heterobasidiomycetes' of the Botanical Institute, University of Tübingen.

**Table 1.** GenBank accession numbers of strains.

Species	Strain <sup>a</sup>	GenBank accession no.	Reference
<i>Agaricostilbum hyphaenes</i>	CBS 7811	AF177406	Sampaio <i>et al.</i> (1999)
<i>Chionosphaera apobasidialis</i>	F 93	AF393470	
<i>Chionosphaera cuniculicola</i>	IGC 5683	AF393473	
<i>Chionosphaera cuniculicola</i>	IGC 5685	AF393471	
<i>Chionosphaera cuniculicola</i>	IGC 5686	AF393472	
<i>Kurtzmanomyces insolitus</i>	IGC 5510	AF17708	Sampaio <i>et al.</i> (1999)
<i>Kurtzmanomyces nectairei</i>	CBS 6405	AF17709	Sampaio <i>et al.</i> (1999)
<i>Kurtzmanomyces tardus</i>	IGC 4529 <sup>T</sup>	AF393467	
<i>Sporobolomyces lactophilus</i>	IGC 5366 <sup>T</sup>	AF393468	
<i>Sporobolomyces xanthus</i>	IGC 5356 <sup>T</sup>	AF393469	

<sup>a</sup> CBS, Centraalbureau voor Schimmelcultures; F, culture collection of the Botanical Institute, University of Tübingen, Germany; IGC, Portuguese Yeast Culture Collection.

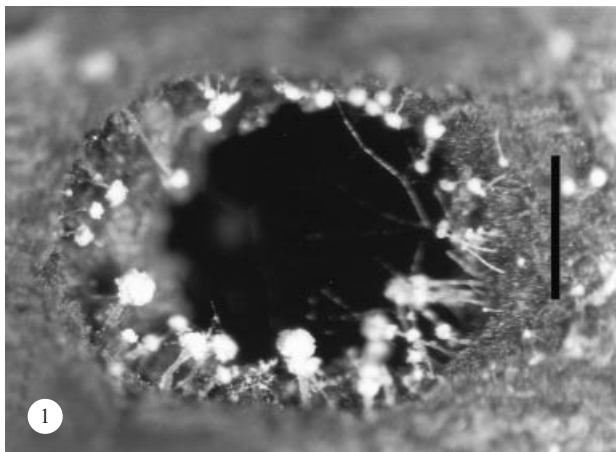
(1997). An alignment of 494 bp was produced with MEGALIGN of the Lasergene package (DNASTAR 1997) using DNA sequences deposited in GenBank (accession numbers in Table 1) and TreeBASE (accession number S641; matrix accession number M998). The following strain of *C. apobasidialis* was used for molecular analyses: **USA: Connecticut:** Litchfield Co., Butternut Brook, woods of White Memorial Foundation, on dead bark of *Carpinus caroliniana*, 10 Jan. 1978, C. T. Rogerson [cult. R. J. Bandoni, F 93] (culture collection of the Botanical Institute, University of Tübingen).

## TAXONOMY

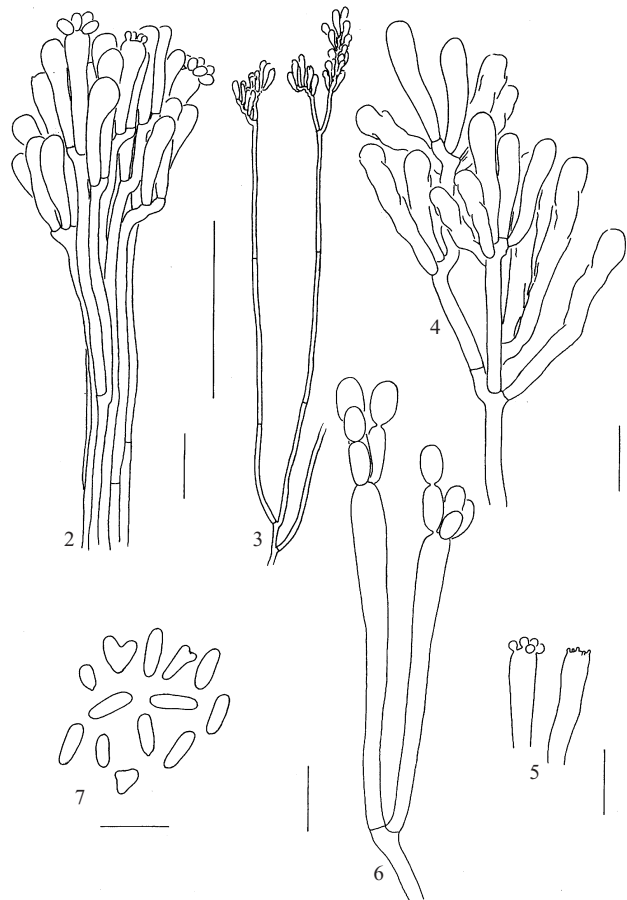
***Chionosphaera cuniculicola*** R. Kirschner, D. Begerow & Oberw., **sp. nov.** (Figs 1–10)

*Etym.*: The species name refers to the bark beetle galleries (Lat.: *cuniculus*, mine, in this case excavated by beetles) colonised by the fungus.

Fructificationes albae, stipitaticapitatae, ad 720 µm longae. Stipes albus, ex hyphis parallelis, efibulatis, 2–3 µm latis compositus, ad 18 µm latus, sine ramificationibus. Capitulum ex hyphis curtis

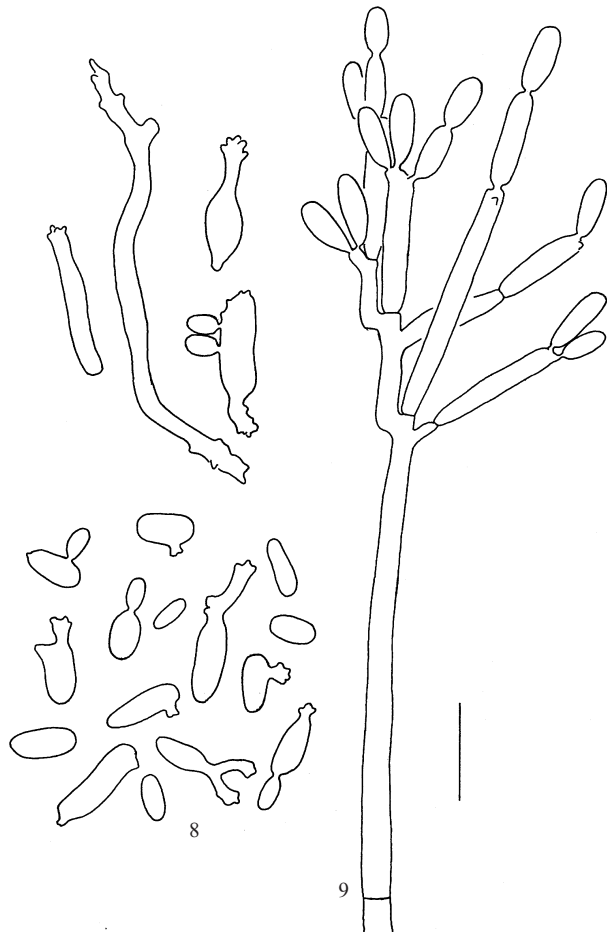


**Fig. 1.** Photograph of basidiomata of *Chionosphaera cuniculicola* (holotype) growing in a gallery of a bark beetle. Bar = 0.5 mm.



**Figs 2–7.** Basidia and basidiospores of *Chionosphaera cuniculicola* (from culture from a gallery of *Dryocoetes autographus* in *Picea abies*, Germany, Bad Waldsee, 16 Oct. 1996, except for Fig. 3). **Fig. 2.** Apical part of a basidiome. **Fig. 3.** Details of hymenium and stipe (holotype). **Fig. 4.** Cluster of old basidia. **Fig. 5.** Apical part of two basidia showing development of basidiospores. **Fig. 6.** Abnormally developed long basidia with basidiospores budding while still attached to the basidia. **Fig. 7.** Basidiospores. Bars: Figs 2, 4–6 = 10 µm; Fig. 3 = 100 µm.

ramificantibus et basidiis compositum, album. Cystidia hyphidiaque desunt. Basidia clavata, aseptata, plerumque 15–20 × 3–4 µm, raro ad 55 × 5 µm magna, sine fibulis basalibus, cum sterigmatibus apicalibus inconspicuis, hexaspora ad octospora. Basidiosporae hyalinae, tenuiter

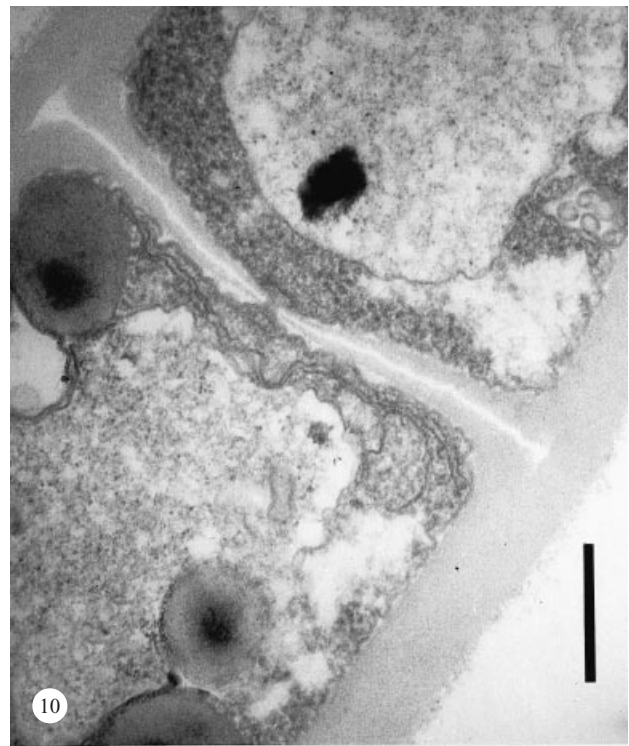


**Figs 8–9.** Anamorph of *Chionosphaera cuniculicola*. Bar = 10  $\mu$ m. Fig. 8. Free yeast cells (upper fig.: from the same isolate as in Fig. 10; lower fig.: isolate from a gallery of *Pityogenes chalcographus* from *Picea abies*, Germany, Bad Waldsee, 5 Oct. 1994). Fig. 9. Budding cells attached to a hypha (from the same culture as in Fig. 2).

tunicatae, ellipsoideae vel cylindricae, raro v- vel y-formes, 4–7.5(–10)  $\times$  1.5–2.5(–3)  $\mu$ m, blastosporas parientes.

**Typus:** **Germany:** *Hessen:* between Darmstadt-Eberstadt and Malchen, in galleries of the bark beetle *Orthotomicus laricis* in the inner bark of *Pinus sylvestris*, 28 Febr. 1998, R. Kirschner, TUB holotypus).

**Basidiomata** white, stipitate-capitate (Figs 1–2), to 720  $\mu$ m high. Stipe simple, white, composed of parallel, efibulate, sparsely branched, 2–3  $\mu$ m thick hyphae (Figs 2–3), stipe to 18  $\mu$ m diam. **Capitulum** white, composed of short, branching hyphae and basidia. **Cystidia and hyphidia** lacking. Basidia (Figs 4–6) clavate, aseptate, mostly 15–20  $\times$  3–4  $\mu$ m, rarely to 55  $\times$  5  $\mu$ m, without basal clamps, with apical, inconspicuous sterigmata. Basidiospores (Fig. 7) 6–8 per basidium, hyaline, thin-walled, ellipsoidal or cylindrical, rarely formed like a ‘v’ or ‘y’, 4–7.5(–10)  $\times$  1.5–2.5(–3)  $\mu$ m (mean: 5  $\times$  2  $\mu$ m, calculated from 30 spores), not forcibly abstricted, often adhering in groups of 6–8 after dehiscence from the basidia in squash mounts, producing blastospores. Yeast colonies on 2% malt extract agar white or cream, slimy, glistening, composed of prolonged cells with sympodial budding (Figs 8–9). Septal pore simple, without any associated organelles (Fig. 10).



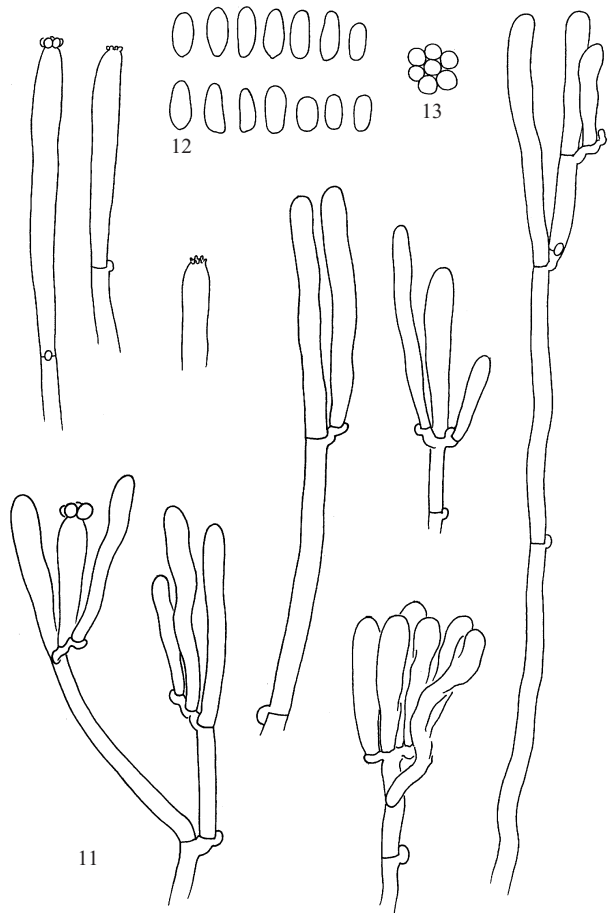
**Fig. 10.** Transmission electron micrograph with a longitudinal section through a hypha of *Chionosphaera cuniculicola* showing a simple septal pore (isolated from an adult individual of *Ips typographus*, Germany, Tübingen, Bebenhausen, 30 May 1996). Bar = 0.25  $\mu$ m.

**Habitat:** In galleries of conifer inhabiting bark beetles (*Dryocoetus autographus*, *Hylurgops palliatus*, *Ips acuminatus*, *Ips sexdentatus*, *Ips typographus*, *Orthotomicus laricis*, *Pityogenes chalcographus*, *Pityokteines spinidens*, *Polygraphus poligraphus*) in the cambium and secondary phloem of *Abies alba*, *Larix decidua*, *Picea abies*, and *Pinus sylvestris*.

**Distribution:** Germany, Italy, Switzerland.

**Observations:** *Chionosphaera cuniculicola* was associated with all beetle species from the localities mentioned above. In collections made during 1994–1998, the fungus was carried by 12% of 127 individuals of *C. cinereus*, 4% of 332 individuals of *C. pusillus*, and 4% of 976 individuals of *Pityogenes chalcographus*, and by less than 4% in the other beetle species. Typical basidiomata usually developed in the presence of other fungi in mixed cultures, e.g. of *Cladosporium cladosporioides*, rarely in pure cultures on malt extract agar.

Neighbour-joining analysis of a partial region of the LSU ribosomal RNA gene shows that the sequences of three strains of *C. cuniculicola* clustered separately from a sequence of *C. apobasidialis*, which is supported by a bootstrap value of 100% (Fig. 14). There were approximately 16% bp differences between *C. apobasidialis* and each strain of *C. cuniculicola* in the alignment used for this analysis. Among the three strains of *C. cuniculicola* 0.6–1.0% sequence differences were found in the analysis. It might be investigated in future studies whether these differences reflect intraspecific genetic variability according to different geographic origins (Germany and Switzerland) and substrata (*Abies alba* and *Picea abies*) of the strains or merely originate from ambiguous base positions.



**Figs 11–13.** Basidia and basidiospores of *Chionosphaera lichenicola* (C-isotype). Bar = 10 µm. **Fig. 11.** Basidia. **Fig. 12.** Basidiospores in side view. **Fig. 13.** A cluster of basidiospores from one basidium in end view.

*Additional specimens deposited:* **Germany:** Baden-Württemberg: Tübingen, isolate IGC 5683 from *Crypturgus pusillus* from bark of *Picea abies*, 5 July 1996, R. Kirschner; Tübingen-Bebenhausen, isolate IGC 5684 from *Ips typographus* infesting a conifer stem, 30 Sept. 1996, R. Kirschner; Tübingen, isolate IGC 5686 from the bark beetle *Pityokteines spinidens* from bark of *Abies alba*, 30 Sept. 1996, R. Kirschner; Burgfelden near Albstadt-Lautlingen, isolate IGC 5687 from a gallery of the bark beetle *Ips acuminatus* infesting bark of *Pinus sylvestris*, 11 Sept. 2000, R. Kirschner, and dried material *in situ* in TUB. **Switzerland:** Kanton Glarus: Schwanden, isolate IGC 5685 from *Ips typographus* infesting *Picea abies*, 6 Sept. 1995, R. Kirschner.

***Chionosphaera lichenicola*** Alstrup, B. Sutton & Tønsberg  
1993 (Figs 11–13)

*Basidiomata* 100–200 µm long, white or beige, unbranched, hyphae with clamps, 2.5–3 µm diam, rarely branched. Basidia (Fig. 11) 20–46 × 3–5 µm, supported by basal clamps that proliferate to produce further basidia, sterigmata inconspicuous, basidia with 4–7 basidiospores, mostly developing 6 basidiospores. *Basidiospores* (Fig. 12) ellipsoidal or cylindrical, 5–7 × 2–3 µm (mostly 6–7 × 3 µm), often adhering in groups (Fig. 13) after dehiscence from the basidia in the squash preparation.

## DISCUSSION

Morphologically, *Chionosphaera cuniculicola* resembles *C. apobasidialis* and *C. lichenicola*. The basidiospores of *C. apobasidialis* are broader (3.5–5 µm; Oberwinkler & Bandoni 1982) than in *C. cuniculicola* and *C. lichenicola*. In *C. apobasidialis*, the stipes of some basidiocarps are branched (Oberwinkler & Bandoni 1982), whereas branched stipes were not found in *C. cuniculicola* and *C. lichenicola*. The differentiation between *C. cuniculicola* and *C. apobasidialis* is supported by the molecular analysis in which three isolates of *C. cuniculicola* form a cluster that is distinctly separated from *C. apobasidialis* and by the bp differences of 16% between both species. The topology of the species of *Chionosphaera* in relation to species of *Kurtzmanomyces* and *Sporobolomyces lactophilus* and *S. xanthus* illustrated by Sampaio *et al.* (1999) was reproduced here.

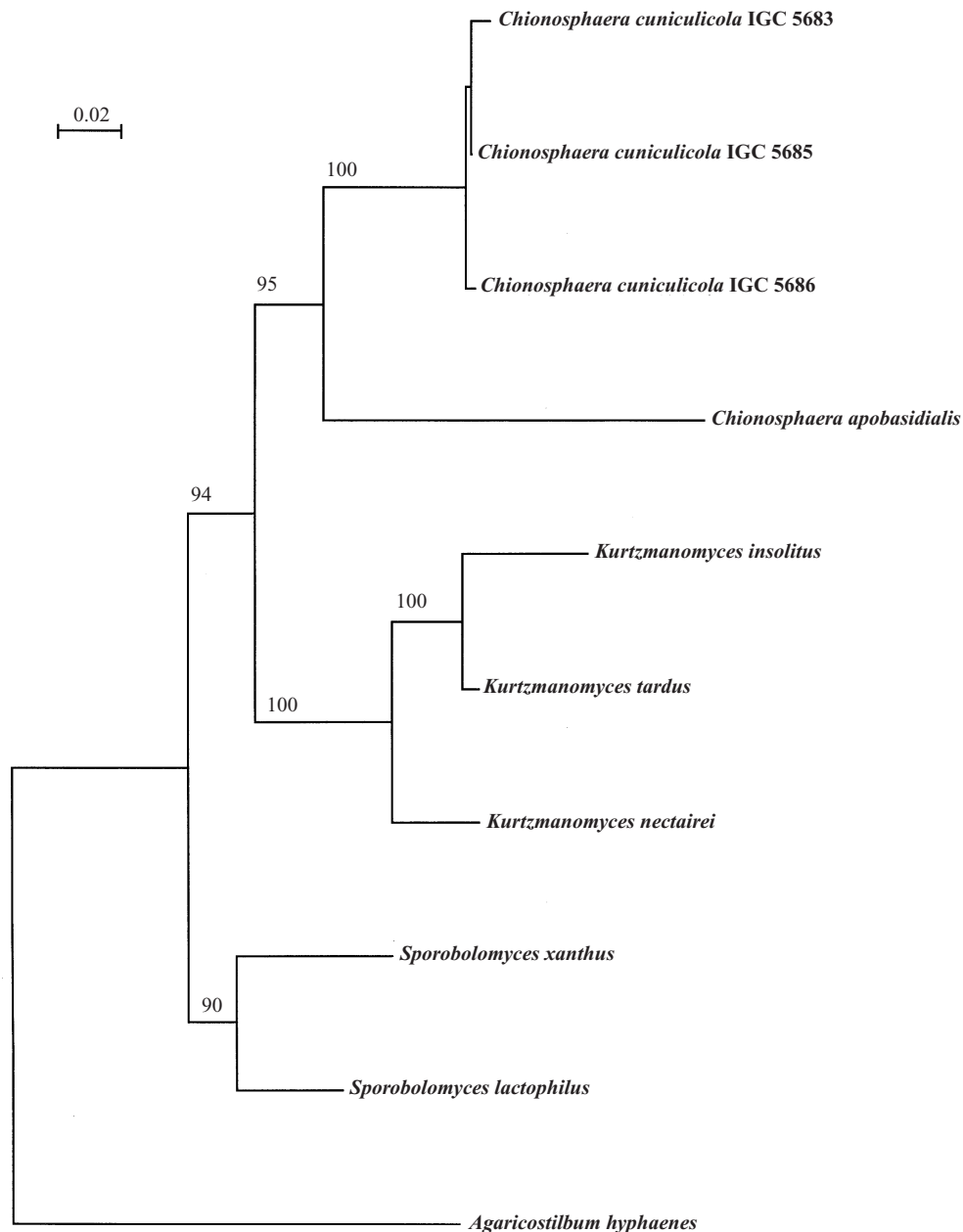
According to the descriptions of *C. lichenicola* by Alstrup (1993) and Diederich (1996), this species can hardly be distinguished from *C. cuniculicola* morphologically. In our re-investigation of the isotype of *C. lichenicola*, however, we found clamps at the septa and at the bases of the basidia, whereas Alstrup (1993) and Diederich (1996) did not observe them. Clamps on the base of basidia often proliferate and, therefore, become inconspicuous, but clamps at the hyphae of the stipes are conspicuous. *C. coppinsii* is a further clamp-bearing species that differs by its 60–70 µm long basidia from *C. lichenicola* (Roberts 1997). A specimen having shorter basidia and being designated as '*C. aff. coppinsii*' by Roberts (1997) can now be assigned to *C. lichenicola*.

*Fibulostilbum phylacicola* differs from clamp-bearing species of *Chionosphaera* only by its 0.75–2 mm tall basidiocarps and by its habitat (Seifert *et al.* 1992). We therefore consider *Fibulostilbum* as synonym of *Chionosphaera* and make the necessary new combination.<sup>1</sup>

The hitherto known species of *Chionosphaera* are apparently mycophilic. Basidiomata of *C. coppinsii* and *C. lichenicola* were found on lichens (Alstrup 1993, Diederich 1996, Roberts 1997), and *C. phylacicola* on stromata of the ascomycete *Phylacia poculiformis* (Seifert *et al.* 1992). In cultures, *C. apobasidialis* and *C. cuniculicola* usually develop basidiomata only in the presence of other fungi, e.g. species of *Cladosporium* according to Cox (1970) and our own observations. The hosts of species of *Chionosphaera* seem to be restricted to members of ascomycetes. Since neither the structures involved in mycoparasitic interactions nor a decline of the host were reported, the nature of this mycophilic behaviour is not clear.

Cox (1970) observed that the basidiospores of *C. apobasidialis* were passively released and assumed dispersal by insects or air currents. Since *C. cuniculicola* was isolated from bark beetles and their galleries, this fungus obviously benefits from transportation by the beetles and from the growth of the presumed ascomycetous hosts also introduced into the subcortical habitat by the beetles.

<sup>1</sup> *Chionosphaera phylacicola* (Seifert & Bandoni) R. Kirschner & Oberw., **comb. nov.** Basionym: *Fibulostilbum phylacicola* Seifert & Bandoni, *Bohn. Soc. Argent. Bot.* **28**: 215 (1992).



**Fig. 14.** Neighbour-joining analysis of an alignment of partial large subunit ribosomal RNA gene sequences using Kimura 2-parameter distances. Topology rooted with *Agaricostilbum hyphaenes*. Bootstrap values given as numbers on branches (1000 replicates). Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site.

## ACKNOWLEDGEMENTS

We thank Robert J. Bandoni and José P. Sampaio for providing cultures. The help of Beat Wermelinger and Massimo Faccoli in collecting bark beetles from Switzerland and Italy is highly appreciated. Magdalena Wagner-Eha and Robert Bauer kindly assisted in the ultrastructural studies. Technical assistance during the molecular studies by Markus Göker and Jacqueline Götze is gratefully acknowledged. We also thank Robert Bauer for critically reading the manuscript, Iris Reinhold and Stephen Wood for correcting the English text, and Friedhelm Albrecht for helping with the photographic work, and the curators of C and TUB for arranging loans of specimens. The study was supported by the DFG-projects 'Organismische Interaktionen in Wald-ökosystemen', 'OB 24/18-2', and 'KI 697/1-2'.

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Corresponding Editor: N. S. Hallenberg.