Antonie van Leeuwenhoek **80:** 149–161, 2001.

Cuniculitrema polymorpha (Tremellales, gen. nov. and sp. nov.), a heterobasidiomycete vectored by bark beetles, which is the teleomorph of *Sterigmatosporidium polymorphum*[a]

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Received 27 December 2000; accepted 7 May 2001

Key words: dimorphic heterobasidiomycetes, molecular phylogenetic analysis, mycoparasitism, large subunit ribosomal RNA gene, stalk-forming yeasts, *Tremellina*

Abstract

In a study of the mycobiota associated with bark beetles, a dimorphic fungus producing longitudinally septate basidia of the *Tremella*-type and yeast cells budding off from stalks, was collected. Detailed morphological, physiological and molecular studies revealed that this fungus represents the teleomorph of *Sterigmatosporidium polymorphum*. Consequently, a new genus, *Cuniculitrema* gen. nov., and a new species, *C. polymorpha* sp. nov., are proposed. Comparative morphological and molecular studies indicated that the new taxon belongs to a group that also comprises species of the stalk-forming anamorphic genera *Fellomyces* and *Kockovaella*. The new family Cuniculitremaceae is proposed for this group.

Introduction

In a study of the mycobiota associated with bark beetles, a dimorphic fungus producing tremelloid basidia and yeast cells budding off from stalk-like outgrowths of yeast or hyphal cells was collected (Kirschner 1998). Due to the uniqueness of this combination of characteristics a new genus, *Cuniculitrema* gen. nov., is proposed. Comparative morphological and molecular studies indicate that the new taxon belongs to a subgroup of the Tremellales comprising species of *Fellomyces* Yamada & Banno, *Kockovaella* Nakase Banno & Yamada, and *Sterigmatosporidium* Kraepelin & Schulze. The members of this group differ from most other species of the Tremellales by the formation of conidia on stalks that arise from yeast or hyphal cells. Within this group, *Sterigmatosporidium* was originally described as a teleomorphic genus (Kraepelin & Schulze 1982). Based on observations on nuclear staining with HCl-Giemsa, the authors assumed that karyogamy and meiosis took place in cells separated from clamp connections in the mycelium. Our results, based on comparative morphological and molecular studies, challenge this view and indicate that *Cuniculitrema* represents the teleomorphic stage of *Sterigmatosporidium*.

Materials and methods

Bark samples of *Picea abies* (L.) Karst., *Pinus sylvestris* L., and *Larix decidua* Mill. containing bark beetles and bark beetle galleries were collected between 1994 and 1996 in Germany, near Bad Waldsee, Langenau, Schluchsee, and Tübingen in Baden-Württemberg, near Oberjoch and Riedlhütte in Bavaria, near Darmstadt and in the Odenwald in Hessen, near Bremerhaven in Niedersachsen, and in

[a] Part 189 in the series, 'Studies in Heterobasidiomycetes' from the Botanical Institute, University of Tübingen.

Table 1. Frequencies of transportation of *Cuniculitrema polymorpha* by bark beetles collected from conifers near Bad Waldsee, Darmstadt, Langenau, Oberjoch, and Tübingen in Germany, and near Schwanden in Switzerland

Bark beetle species	No. of beetles carrying *C. polymorpha*	% beetles carrying *C. polymorpha*	Total no. of beetles examined
Crypturgus pusillus (Gyll.)	3	1	332
Dryocoetes autographus (Ratz.)	13	4	334
Gnathotrichus materiarius (Fitch)	1	5	20
Hylurgops palliatus (Gyll.)	18	4	437
Ips sexdentatus (Boern.)	4	6	70
Ips typographus (L.)	29	3	1071
Orthotomicus laricis (F.)	82	17	495
Pityogenes chalcographus (L.)	15	2	976
Polygraphus poligraphus (L.)	2	3	67
Tomicus piniperda L.	1	4	27

Switzerland near Schwanden in Glarus. Bark beetle galleries were directly examined for the presence of fungi. Living adult bark beetles were identified (Table 1), removed from the galleries and individually placed into Petri dishes containing autoclaved pieces of inner bark of *Picea abies* embedded in 4% water agar. Fungi vectored by the beetles and growing on this medium were transferred to 2% malt extract agar (MEA) and to Petri dishes containing autoclaved pieces of twigs of *Picea abies* embedded in 1.5% water agar. For light microscopy, material was mounted in water. The techniques described by Yarrow (1998) were used for physiological characterisation. Additional assimilation tests were performed using aldaric acids and aromatic compounds, as described by Fonseca (1992) and Sampaio (1999), respectively. Assimilation tests were performed in liquid medium at 22°C under continuous agitation. Due to the slow growth of the cultures, incubation periods were extended to 6 weeks and the results observed after 1, 3 and 6 weeks of incubation. For studying the ultrastructure of the septal pores, transmission electron microscopy was conducted as described by Kirschner et al. (1999).

For sequence analysis, DNA was isolated using an SDS method as described by Weiß et al. (1998). The D1/D2 region of the nuclear gene coding for the 26S RNA subunit was amplified using the polymerase chain reaction (Mullis & Faloona 1987) with the primers NL1 and NL4 (O'Donnell 1993) and the touch down thermo protocol described in Weiß et al. (1998). The PCR products were purified using the QIAquick^{TM} kit (QIAGEN, Hilden, Germany), followed by an ethanol precipitation. The dsDNA was sequenced using the ABI PRISM^{TM} Dye Terminator Cycle Sequencing Kit (Applied Biosystems) on an automated sequencer (ABI 373A, Applied Biosystems). An alignment of 545 bp was created with MEGALIGN of the Lasergene package (DNASTAR, Inc., 1997). DNA sequences used for alignments are listed in Table 2. To construct a phylogenetic hypothesis, we used the PHYLIP package, version 3.572 (Felsenstein 1995) to perform a neighbor-joining analysis (Saitou & Nei 1987; Kimura two-parameter distances, transition/transversion ratio 2.0), followed by a bootstrap analysis (Felsenstein 1985) with 1000 replicates. PAUP* 4.0b8 (Swofford 2001) was used for maximum parsimony analysis (heuristic search with 1000 rounds of random addition and TBR branch swapping; gaps treated as missing data, steepest descent and 'MulTrees' options in effect, 'collapse' option not in effect), also combined with bootstrapping (1000 rounds of heuristic search, each implementing 10 rounds of random addition and TBR branch swapping with the 'MulTrees' option in effect, steepest descent and 'collapse' options not in effect).

For determination of molar percentage G+C and the extent of DNA–DNA reassociation, DNA was extracted and purified using the procedures described by Giménez-Jurado et al. (1990). The nuclear DNA base composition was determined using the method of Marmur & Doty (1962) with a Gilford Response UV-VIS Spectrophotometer and its thermal programming software, using nDNA from *Candida parapsilosis* (Ashford) Langeron & Talice IGC 2545^{T} (CBS 604) (mol% G+C=40.2%) as reference. For DNA–DNA reassoci-

Table 2. Species used in the molecular analysis and their respective GenBank accession numbers

Species DNA sequences determined for this study	BenBank accession no.	Strain
Cuniculitrema polymorpha R. Kirschner & J. P. Sampaio	AY 032662	IGC 5647
Fellomyces thailandicus Prillinger, Kraepelin & Lopandic	AY 032663	HB 49
DNA sequences taken from GenBank	Accession no.	Reference
Bullera armeniaca Buhagiar	AF 189883	Fell et al. (2000)
Bullera crocea Buhagiar	AF 075508	Fell et al. (1999)
Bullera dendrophila van der Walt & Scott	AF 189870	Fell et al. (2000)
Bullera globispora Johri & Bandoni	AF 075509	Fell et al. (1999)
Bullera oryzae Nakase & Suzuki	AF 075511	Fell et al. (1999)
Bullera pseudoalba Nakase & Suzuki	AF 075504	Fell et al. (1999)
Bulleromyces albus Boekhout & Fonseca	AF 075500	Fell et al. (1999)
Cryptococcus flavus (Saito) Phaff & Fell	AF 075497	Fell et al. (1999)
Cryptococcus heveanensis (Groenewege) Baptist & Kurtzman	AF 075467	Fell et al. (1999)
Cryptococcus laurentii (Kufferath) Skinner	AF 075469	Fell et al. (1999)
Cryptococcus skinneri Phaff & do Carmo-Sousa	AF 189835	Fell et al. (2000)
Cystofilobasidium bisporidii (Fell, Hunter & Tallman) Oberw. & Bandoni	AF 075464	Fell et al. (1999)
Cystofilobasidium capitatum (Fell, Hunter & Tallman) Oberw. & Bandoni	AF 075465	Fell et al. (1999)
Fellomyces borneensis Prillinger, Kraepelin & Lopandic	AF 189877	Fell et al. (2000)
Fellomyces chinensis Prillinger, Kraepelin & Lopandic	AF 189878	Fell et al. (2000)
Fellomyces fuzhouensis (Yue) Yamada & Banno	AF 075506	Fell et al. (1999)
Fellomyces horovitziae Spaaij, Weber & Oberw.	AF 189856	Fell et al. (2000)
Fellomyces penicillatus (Rodrigues de Miranda) Yamada & Banno	AF 177405	Sampaio et al. (1999)
Fellomyces polyborus (Scott & van der Walt) Yamada & Banno	AF 189859	Fell et al. (2000)
Fellomyces sichuanensis Prillinger, Kraepelin & Lopandic	AF 189879	Fell et al. (2000)
Filobasidium globisporum Bandoni & Oberw.	AF 075495	Fell et al. (1999)
Kockovaella imperatae Nakase, Banno & Yamada	AF 189862	Fell et al. (2000)
Kockovaella thailandica Nakase, Banno & Yamada	AF 075516	Sampaio et al. (1999)
Sirobasidium magnum Boedijn	AF 042240	Chen (1998)
Sterigmatosporidium polymorphum Kraepelin & Schulze	AF 075480	Fell et al. (1999)
Tremella encephala Pers.: Bref.	AF 042220	Chen (1998)
Tremella exigua Desm.	AF 042248	Chen (1998)
Tremella foliacea Pers.: Fr.	AF 042235	Chen (1998)
Tremella fuciformis Berkeley	AF 042227	Chen (1998)
Tremella giraffa C.-J. Chen	AF 042271	Chen (1998)
Tremella globispora Reid	AF 042243	Chen (1998)
Tremella mesenterica Retz.: Fr.	AF 042252	Chen (1998)
Tremella microspora Lloyd	AF 042253	Chen (1998)
Tremella moriformis (Fr.) Smith ex Berk.	AF 042244	Chen (1998)
Tremella mycophaga Martin	AF 042249	Chen (1998)
Tremella neofoliacea C.-J. Chen	AF 042236	Chen (1998)
Tremella nivalis C.-J. Chen	AF 042232	Chen (1998)
Tremella simplex Jacks. & Martin	AF 042246	Chen (1998)
Tremella taiwanensis C.-J. Chen	AF 042230	Chen (1998)
Tsuchiyaea wingfieldii (van der Walt, Yamada & Ferreira) Yamada, Kawasaki, Itoh, Banno & Nakase	AF 177404	Sampaio et al. (1999)
Ustilago hordei (Pers.) Lagerh.	L 20286	Berres et al. (1995)
Ustilago maydis (DC.) Corda	L 20287	Berres et al. (1995)

Table 3. Physiological characterisation of strains IGC 5647 of *Cuniculitrema polymorpha* and strain CBS 8088 of *Sterigmatosporidium polymorphum*. (+) positive results after one week; (D) delayed: positive results after three or six weeks; (−) negative results

	Strains			Strains			Strains	
	CBS 8088	IGC 5647		CBS 8088	IGC 5647		CBS 8088	IGC 5647
Carbon compounds			D-Glucitol	+	+	Growth without vitamins	+	+
D-Glucose	+	+	D-Mannitol	+	+	Growth with 0.01% cycloheximide	+	+
D-Galactose	−	−	Galactitol	D	D	Growth with 0.1% cycloheximide	D	+
L-Sorbose	+	+	Inositol	+	+	Growth at 30°C	+	+
D-Glucosamine	+	+	Glucono-δ-lactone	+	+	Growth at 35°C	−	−
D-Ribose	+	+	D-Gluconic acid	+	+			
D-Xylose	+	+	D-Glucuronic acid	+	+			
L-Arabinose	D	D	D,L-Lactic acid	−	−			
D-Arabinose	−	−	Succinic acid	+	+			
L-Rhamnose	+	+	Citric acid	+	+			
Sucrose	+	+	L-Malic acid	+	+			
Maltose	+	+	L-Tartaric acid	−	−			
α,α-Trehalose	D	D	D-Tartaric acid	−	−			
Methyl-α-D glucoside	+	+	*m*-Tartaric acid	−	−			
Cellobiose	+	+	Saccharic acid	−	−			
Salicin	+	+	Mucic acid	D	D			
Arbutin	+	+	Methanol	−	−			
Melibiose	D	D	Ethanol	+	+			
Lactose	D	D	Nitrogen compounds					
Raffinose	+	+	Potassium nitrate	−	−			
Melezitose	+	+	Sodium nitrite	−	−			
Inulin	−	−	Ethylamine	+	+			
Soluble starch	+	+	L-Lysine	+	+			
Glycerol	−	−	Cadaverine	−	−			
Erythritol	−	−	Creatine	−	−			
Ribitol	D	D	Creatinine	−	−			
Xylitol	−	−						

ation experiments the same instrument was used and the methods of Kurtzman et al. (1980) were followed.

Results

Microorganisms vectored by the beetles, which had been placed into Petri dishes containing autoclaved *Picea abies* bark, originated mixed microbial populations. The cultures formed slimy masses composed of bacteria, yeasts, filamentous ascomycetes, and hyphomycetes, but rarely included filamentous basidiomycetes. In such aggregates, a fungus producing hyphae with clamp connections, haustorial cells, and conidiogenous cells with conidia seceding from basal clamps was found (Fig. 1). This type of conidiophores also developed in pure cultures on MEA. Haustorial cells were attached to hyphae of the same organism and to hyphae of *Hormonema dematioides* Lagerberg & Melin (Fig. 1G). Additionally, conidia and yeast cells originating on stalks, produced either from hyphal or yeast cells were found (Fig. 2). The conidiophores with conidia seceding from clamps and from stalks were also found in situ in bark beetle galleries. In older cultures on medium containing *Picea abies* bark, septate basidia of the *Tremella*-type developed on the same hyphae as the conidiogenous cells (Figs. 3, 4). Basidiocarps were not present, but the tips of the hyphae bearing basidia sometimes protruded from the slimy colonies and were visible with the dissecting microscope. The septal pores were found to be dolipores

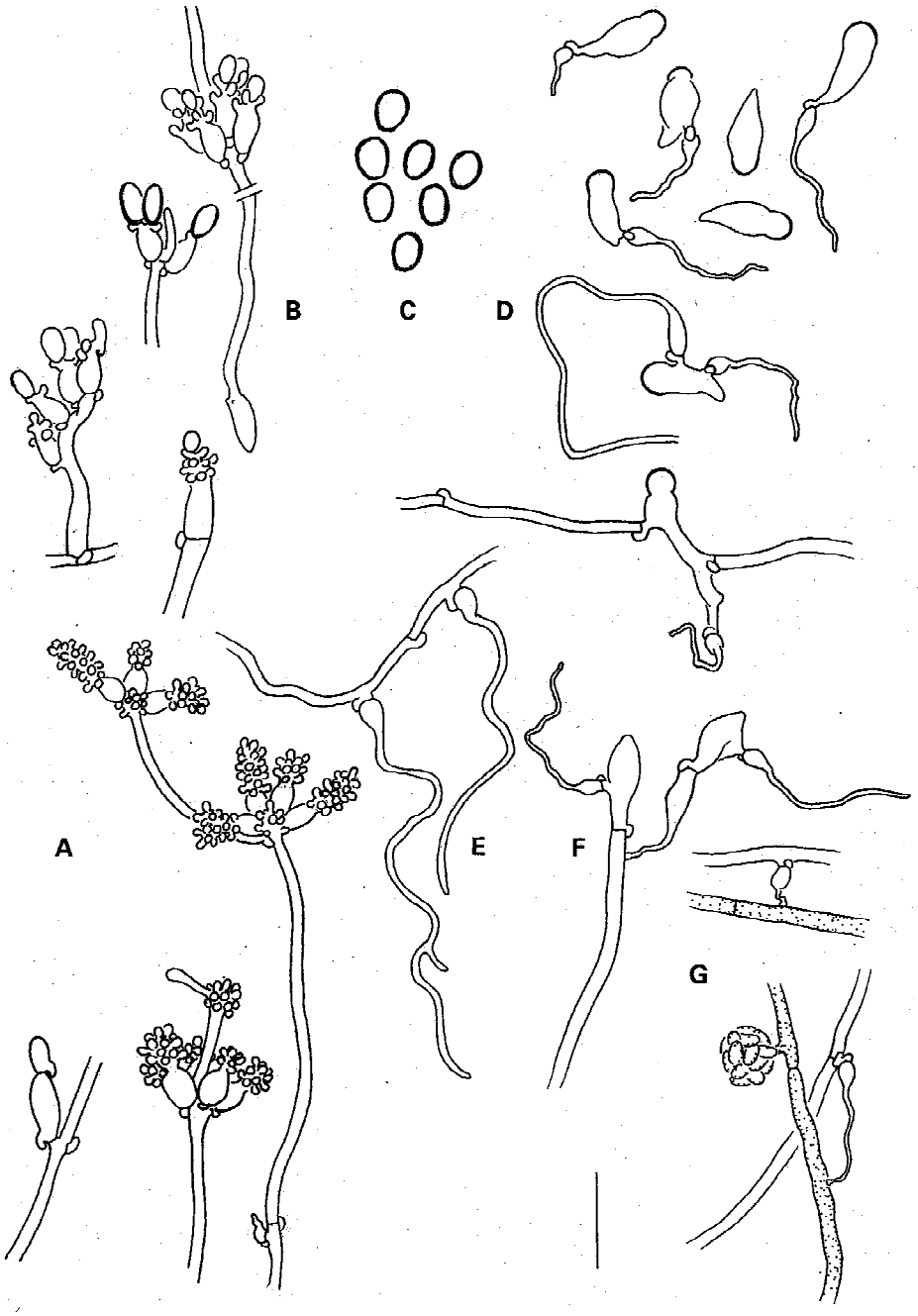

Figure 1. Cuniculitrema polymorpha: *Tremellina*-like conidiophores, conidia, and haustoria. (A) Conidiophores. (B) Conidiophore arising from a single propagule. (C) Conidia. (D) Germinating conidia producing haustoria. (E) Haustoria arising from a hypha. (F) Two germinating conidia. One haustorium apically attached to the germ tube of the other conidium. (G) Haustoria arising from hyphae attached to hyphae of *Hormonema dematioides*. Scale bar: 10 μm.

associated with cupulate parenthesomes (without illustration). A cluster, comprising this fungus, species of *Fellomyces*, *Kockovaella*, and *Sterigmatosporidium*, that was separated from the clusters containing species of *Tremella* Pers. was detected in both molecular phylogenetic analyses of the rDNA alignment: It is present both in the neighbor-joining tree (Fig. 5) and in the strict consensus of the five most parsi-

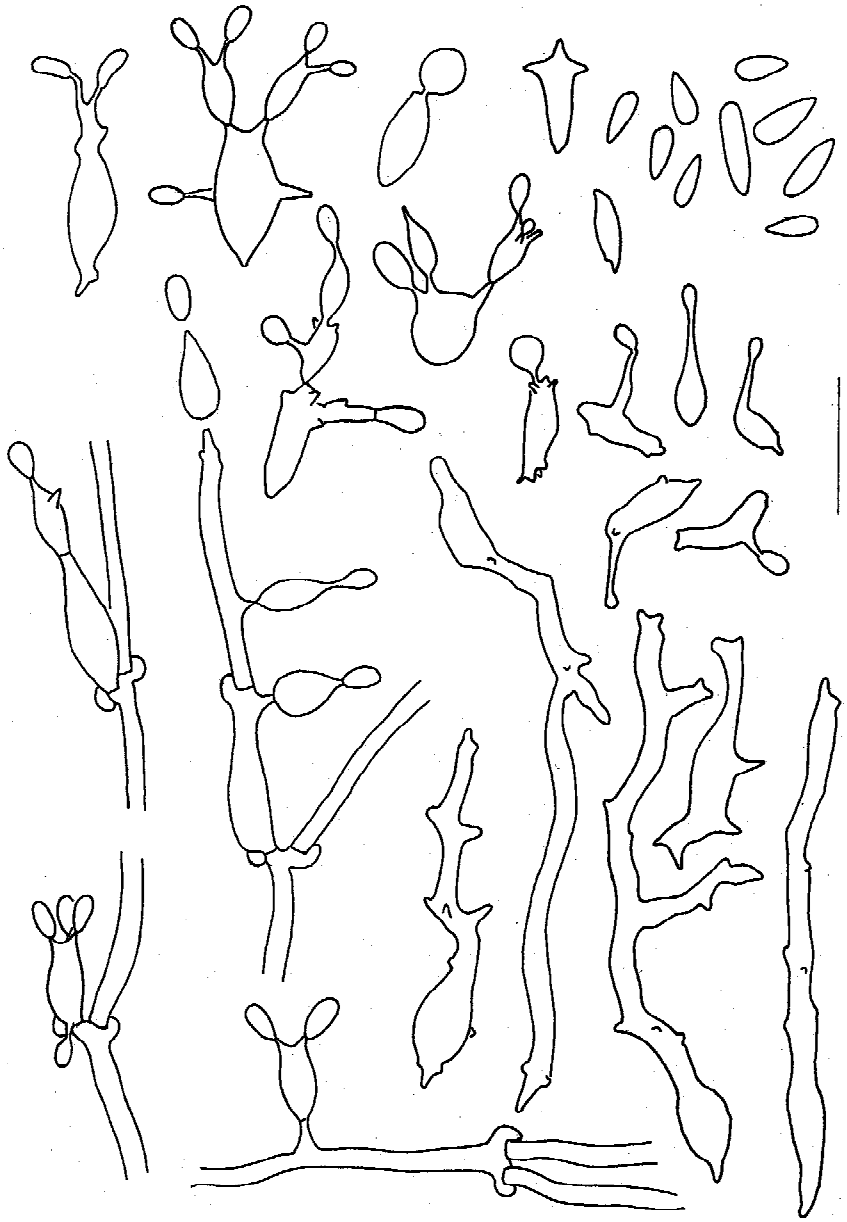

Figure 2. Cuniculitrema polymorpha: stalk forming anamorphic stage (yeast and hyphal cells). Scale bar: 10 μm.

monious trees found by heuristic search (not shown). Within the cluster consisting of stalk-forming yeasts, the fungus isolated from bark beetles (IGC 5647) and *S. polymorphum* CBS 8088 were clustered with a support of 100% both in the neighbor-joining and in the maximum parsimony bootstrap analysis. Two nucleotide differences between IGC 5647 and CBS 8088 were detected in the D1/D2 region. Due to the morphological similarities of the anamorphic stages and to the resemblance of the nutritional profiles (Table 3), nuclear DNA–DNA reassociation experiments were conducted between strains CBS 8088 and IGC 5647. Homology values of 84±2% (three determinations) were obtained, which indicates that the two strains are conspecific. A new teleomorphic taxon, *Cuniculitrema* gen. nov., is described below for the teleomorph of *Sterigmatosporidium*.

Figure 3. Cuniculitrema polymorpha: Teleomorph. (A) Basidia and *Tremellina*-like conidiophores arising from the same hyphae. (B) Basidia. (C) Basidiospores. Scale bar: 10 μm.

Taxonomy

Cuniculitremaceae J. P. Sampaio, R. Kirschner & M. Weiß fam. nov.

Familia Tremellomycetidarum. Hyphae hyalinae, fibulatae, tenuiter tunicatae, haustoriis tremelloideis. Basidia longitudinaliter septata. Basidiosporae hyalinae, tenuiter tunicatae, aseptatae, sporas secundarias parientes. Blastosporae anamorphosis a spinis secedentes. Systema coenzymatis Q10.

Typus familiae: *Cuniculitrema* J. P. Sampaio & R. Kirschner, in opere ipso descripta.

Hyphae hyaline, thin-walled, tremelloid haustoria present. Basidia longitudinally septate. Basidiospores hyaline, thin-walled, aseptate, producing secondary spores. Anamorphic yeast stage with stalks. Coenzyme Q10.

Cuniculitrema J. P. Sampaio & R. Kirschner gen. nov.

Carposomata absentia. Hyphae hyalinae, fibulatae, tenuiter tunicatae, haustoriis tremelloideis. Basidia longitudinaliter septata, non catenata. Basidiosporae hyalinae, tenuiter tunicatae, aseptatae, sporas secundarias parientes. Status anamorphosium: *Fellomyces* Y. Yamada & I. Banno, *Sterigmatosporidium* G. Kraepelin & U. Schulze.

Typus generis: *Cuniculitrema polymorpha* R. Kirschner & J. P. Sampaio, in opere ipso descripta.

Etymology: *cuniculus*—mine, excavated by man in the ground as well as by insects in woody plants, refers to the known habitats of the fungus, *-trema* refers to the similarity with *Tremella*, like in *Sirotrema* described by Bandoni (1986a). (The compound *-trema* is used as a feminine form and does not refer to the neuter Greek noun *trema*—hole).

Basidiocarps lacking. Hyphae hyaline, with clamps and haustorial cells composed of a slender filament arising from a swollen base, thin-walled. Basidia becoming longitudinally septate, not arranged in chains. Basidiospores hyaline, thin-walled, aseptate, producing secondary spores. Anamorphs: *Fellomyces*

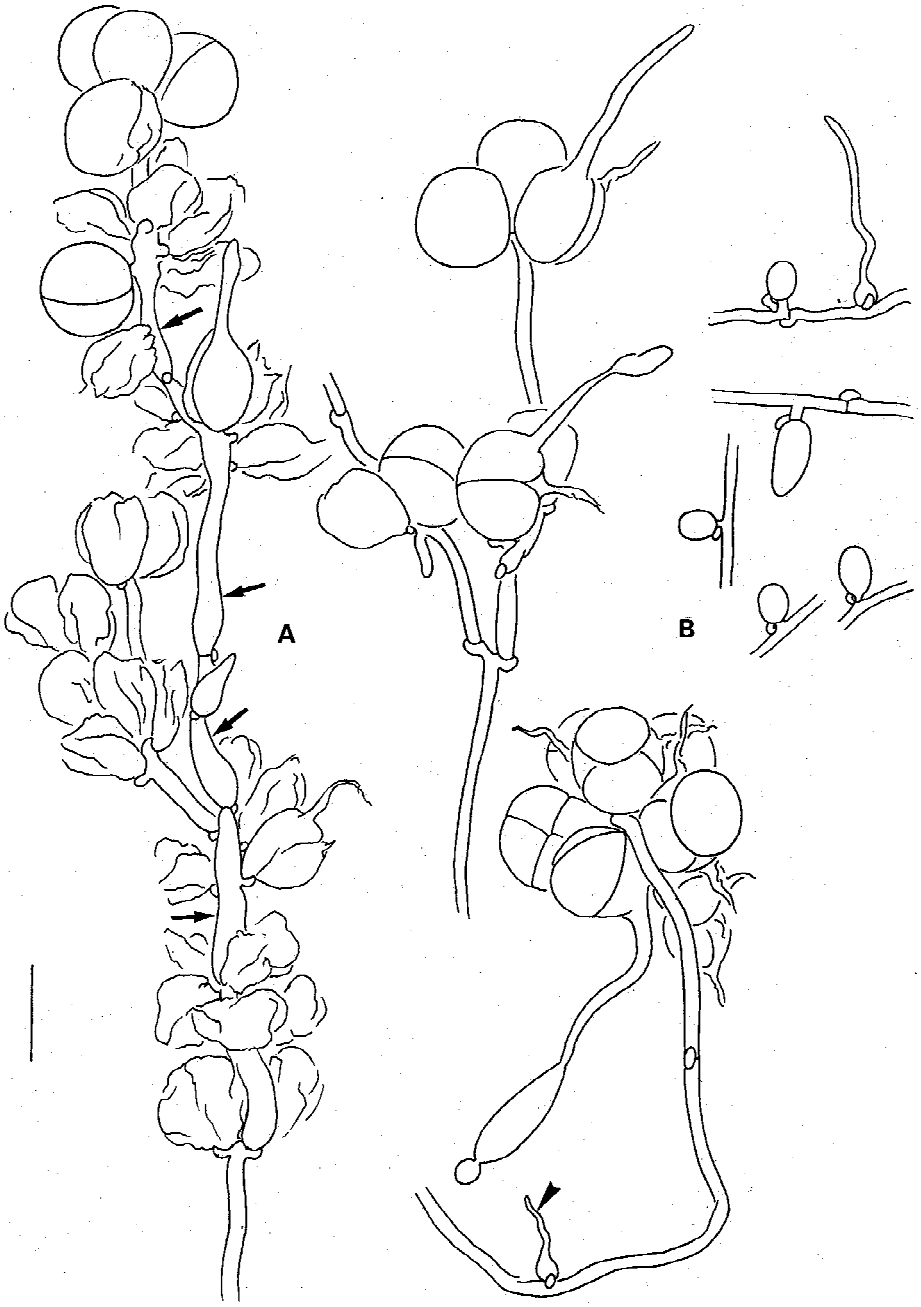

Figure 4. Cuniculitrema polymorpha: Teleomorph and chlamydospore-like structures. (A) Basidia. Note the catenulate arrangement of the basidia-supporting cells in the left figure (arrows), the absence of conidiophores and the presence of a haustorium (arrowhead). (B) Chlamydospore-like structures. Scale bar: 10 μm.

Y. Yamada & I. Banno, *Sterigmatosporidium* G. Kraepelin & U. Schulze.

Cuniculitrema polymorpha R. Kirschner & J. P. Sampaio sp. nov. (Figs. 1–4)

Status anamorphosis: *Sterigmatosporidium polymorphum* G. Kraepelin & U. Schulze. Carposomata absentia. Hyphae hyalinae, fibulatae, 1–2 μm diam., haustoriis. Basis haustorii 2–3 × 2–2.5 μm, filamentum haustorii 1 μm diam., ramosum vel non ramosum.

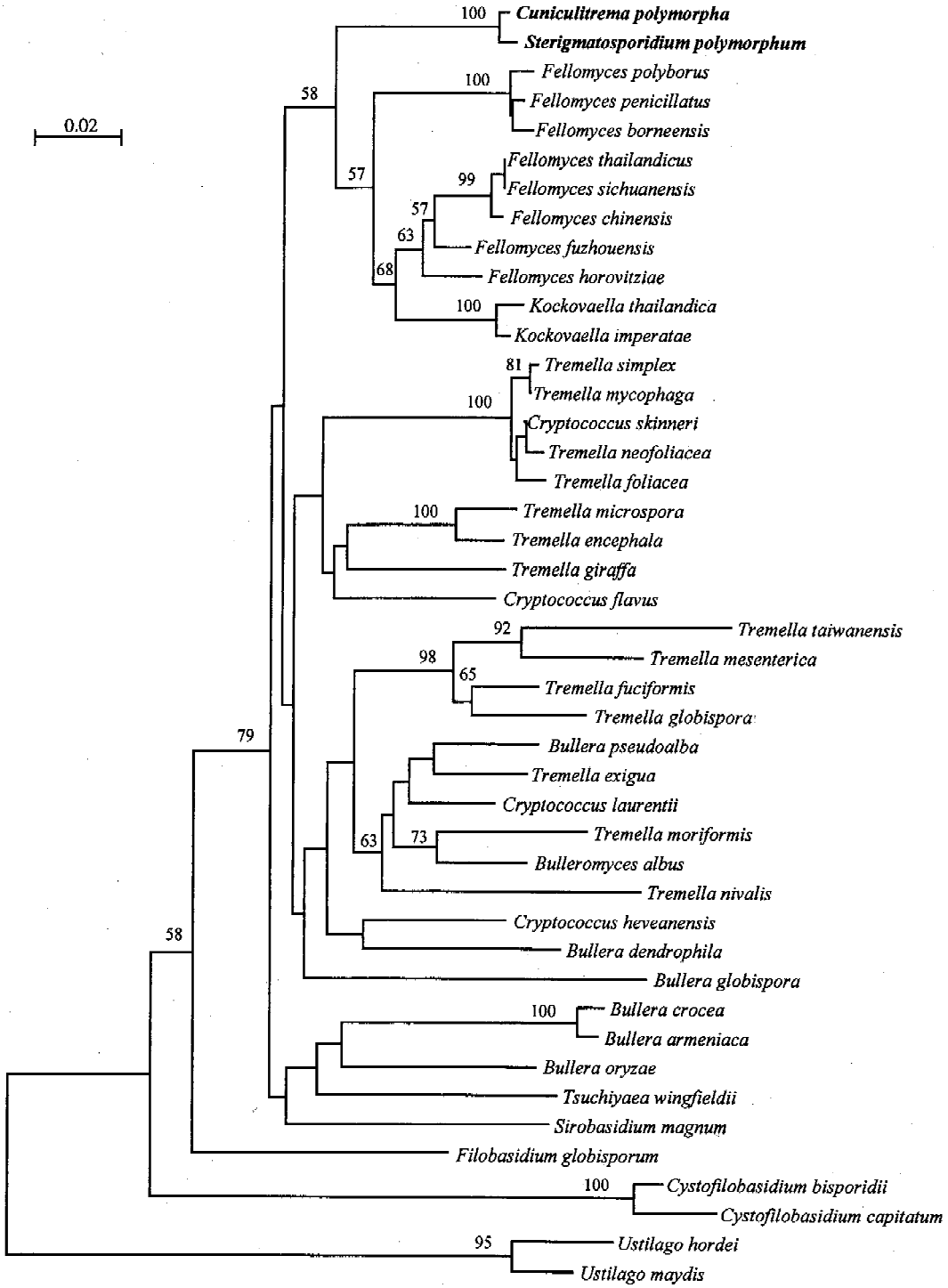

Figure 5. Neighbor-joining analysis of an alignment of the D1/D2 region of the large subunit ribosomal DNA using Kimura 2-parameter distances for tremellaceous fungi. Topology rooted with the cluster of *Ustilago hordei* and *U. maydis*. Bootstrap values given as numbers on branches (1000 replicates, values less than 55% not shown). Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site.

Basidia globosa vel subglobosa, longitudinaliter cruciatim septata, saepe aggregata, 9–11 μm diam., sterigmata 12–88×1–2 μm. Basidiosporae ellipsoideae vel allantoideae, 8–13×4–7 μm, sporas secundarias parientes.

Habitatio: In cuniculis insectorum in cortice *Piceae abietis*, *Pini sylvestris*, *Laricis deciduae*.

Typus: *Cuniculitrema polymorpha* (slide), from culture originating from propagules disseminated by the bark beetle *Orthotomicus laricis* from *Pinus sylvestris* bark, Germany, Hessen, Darmstadt-Eberstadt, leg. 05.IV.1996, R. Kirschner, holotypus, in M; isotypes: RoKi 170, RoKi 171, RoKi 172 (dried cultures), in M.

Reference culture: IGC 5647, isolated from the bark beetle *Gnathotrichus materiarius* from *Picea abies*, Germany, Hessen, Darmstadt-Eberstadt, leg. 09.IV.1996, R. Kirschner.

Basidiocarps lacking. Hyphae hyaline, thin-walled, 1–2 μm diam., with clamps, septa with dolipores and associated vesicles (not illustrated). Haustorial cells (Figs. 1, 4) arising from clamps, with a swollen base (2–3×2–2.5 μm) and with 1 μm thick, branched or unbranched haustorial filaments.

Basidia (Figs. 3, 4) sessile either on swollen cells or rarely on cells with the same width as the other hyphae, these cells often arranged in sparsely branched chains supporting clusters of up to approx. 15 basidia, sometimes also supporting conidiogenous cells. Basidia globose or subglobose, with four longitudinal, rarely transversal septa, 9–11 μm diam., sterigmata 12–88×1–2 μm. Basidiospores (Fig. 3C) allantoid, with one side flattened or slightly concave, 8–13×4–7 μm, producing secondary spores, apiculus conspicuous or inconspicuous.

Anamorph: *Sterigmatosporidium polymorphum* G. Kraepelin & U. Schulze.

Conidiogenous cells of the *Tremellina*-like stage (Fig. 1) arranged in whirls either on slender hyphae or on 4–7 μm long and 2–3 μm thick apical hyphal swellings, with an ellipsoidal, 3–4 μm long and 2–3 μm thick sterile base giving rise to a fertile, 2 μm broad and up to approx. 10 μm long apical part bearing numerous clamps that form vesicle-like remnants on the conidiogenous cell after conidium dehiscence. Conidia globose or broadly ellipsoidal, inconspicuously thick-walled in water mounting, hyaline, 2.5–4×2–3.5 μm, germinating with hyphae or haustorial cells.

Conidiogenous cells of the stalk-forming hyphal stage (Fig. 2) with or without a basal clamp, arising from septate hyphae, but not arranged in whirls, variable in size and shape, apically giving rise to one to several stalks, each apparently producing one conidium.

On MEA, the conidiogenous cells and conidia of the unicellular stage forming white or cream, brain-like wrinkled or smooth colonies with a shiny or powdery surface. Shapes and dimensions of the yeast cells vary (Fig. 2). Daughter cells budding off from up to 10 μm long stalks.

Single cells of the chlamydospore-like stage (Fig. 4B) produced on lateral clamps at the hyphae, ellipsoidal, 4–8×3 μm, with refractive content seen by phase contrast light microscopy. Subsequent development of these cells not observed.

The physiological properties of *C. polymorpha* are given in Table 3. Results of the utilisation of aromatic compounds are not listed in Table 3 because *C. polymorpha* was not able to grow with any of the 20 substrates investigated by Sampaio (1999). The urease and the DBB tests gave positive results and starch-like compounds were produced. The mol% of G+C (thermal denaturation method) was 51.5±3 (three determinations) for strain IGC 5647 of *C. polymorpha* and 51.4±2 (three determinations) for strain CBS 8088 of *S. polymorphum*.

Discussion

Intraspecific variability

The comparison of *C. polymorpha* and *S. polymorphum* revealed some discrepancies. The conidia of strain CBS 8088 of *S. polymorphum* measure 4.8–6.7×2.9–3.8 μm (Kraepelin & Schulze 1982) and are somewhat longer than those of *C. polymorpha*. With respect to the physiological characterisation no discrepant results were detected for the profiles of strain CBS 8088 of *S. polymorphum* and strain IGC 5647 of *C. polymorpha* (Table 3). Five discrepant results (assimilation of D-arabinose, glycerol, erythritol, xylitol, and D,L-lactic acid) were detected when our data were compared with the standard physiological profile of *S. polymorphum* listed by Barnett et al. (1990). In all five cases our results were negative and those of Barnett et al. (1990) were positive.

According to Fell et al. (2000) the difference in two nucleotides of the D1/D2 region between CBS 8088 and IGC 5647 suggests that these strains represent distinct species. According to Kurtzman & Robnett

(1998), however, strains with 0–3 nucleotide differences in the D1/D2 region may be conspecific, and identifications of species based on nucleotide differences in some cases have to be validated by DNA–DNA reassociation. The homology value of 84% determined in our DNA–DNA reassociation experiments is high enough to treat both strains as conspecific according to Kurtzman (1998).

Our DNA sequence analysis of the D1/D2 region of the LSU rDNA revealed that *F. thailandicus* Prillinger, Kraepelin & Lopandic and *F. sichuanensis* Prillinger, Kraepelin & Lopandic have identical sequences, which suggests that these strains might be conspecific.

Anamorph–teleomorph connection

Using the HCl-Giemsa staining method, Kraepelin & Schulze (1982) observed that the propagules separated from clamps contained one or two nuclei after secession from the conidiogenous cells and more than four nuclei prior to germination. Uninucleate and binucleate yeast cells were also found. The authors concluded that karyogamy and meiosis took place in those propagules and consequently that *S. polymorphum* should be regarded as a teleomorphic taxon.

Bandoni (1986b) described the heterobasidiomycetous anamorph genus *Tremellina*, in which propagules arise from clamps developing on inflated cells. In the description of *Tremellina pyrenophila* Bandoni, he illustrated propagules apparently containing one or two nuclei and reported that they contained four nuclei prior to production of the germ tube. He also found uninucleate and binucleate yeast cells. In contrast with Kraepelin & Schulze (1982), he regarded those propagules as conidia. Moreover, a similar mode of development was reported in the anamorphic stage of some species of *Tremella* and *Platygloea* Schröt. (Bandoni 1986b), *Christiansenia* Hauerslev (Hauerslev 1989) and *Occultifur* Oberwinkler (Oberwinkler 1990).

The finding and characterisation of a fungus having a *Sterigmatosporidium* stage and also *Tremella*-like basidia, as reported in our study, strongly suggests that *Sterigmatosporidium* should be regarded as an anamorphic genus. *Sterigmatosporidium* differs from *Tremellina* by the additional development of conidia on stalks. In contrast with the apparently multiple budding-off of conidia from a single conidiogenous locus in some species of *Tremella* (Chen 1998), each conidiogenous locus produces a single conidium in the stalk-forming genera *Fellomyces*, *Kockovaella* and *Sterigmatosporidium* (Yamada & Banno 1984; Sampaio et al. 1999).

Systematic position and similar species

Microscopic characteristics of the basidial stage and the presence of dolipores with cupulate parenthesomes confirm the position of *C. polymorpha* within the Tremellales, as was previously shown for its anamorph *S. polymorphum* by using biochemical, physiological, and molecular characteristics (Boekhout et al. 1993; Fell et al. 2000). The basidia and haustoria of *Cuniculitrema polymorpha* resemble those of species of *Tremella* and similar genera such as *Bulleromyces* Boekhout & Fonseca or *Trimorphomyces* Bandoni & Oberwinkler. There are, however, only a few species that do not produce basidiocarps, namely *Tremella caloceraticola* Hauerslev, *T. giraffa* C.-J. Chen, *T. mycophaga* var. *obscura* Olive, *T. occultifuroidea* C.-J. Chen & Oberwinkler, *T. penetrans* (Hauerslev) Jülich, *Bulleromyces albus* Boekhout & Fonseca, and *Exidiopsis invisa* Hauerslev (which is a member of the Tremellales and not of the Auriculariales because of the presence of the tremelloid haustoria reported by Hauerslev (1993)). Conidia budding off from stalk-like outgrowths produced by yeast cells or originating from hyphae were not reported in these species. Furthermore, a *Tremellina* stage is not present in *B. albus* (Boekhout et al. 1991), *E. invisa* (Hauerslev 1993), *T. giraffa* (Chen 1998), and *T. mycophaga* var. *obscura* (Bandoni 1986b; 1987). *Tremella caloceraticola*, *T. occultifuroidea*, and *T. penetrans* differ by the two-celled basidia and parasitism in members of Dacrymycetales (Jülich 1983; Chen et al. 1999; Hauerslev 1999).

The distinction between *Cuniculitrema* and *Tremella* is also reflected in the topology of the DNA sequence analysis, which does not support the inclusion of *C. polymorpha* in any of the clusters containing species of *Tremella* (Fig. 5). On the contrary, it suggests that all tremellaceous species that produce conidia on stalks, presently classified in the genera *Cuniculitrema*, *Fellomyces*, *Kockovaella*, and *Sterigmatosporidium*, form a distinct clade (Fig. 5). A similar topology, using a different sampling of taxa, was also found in analyses of the D1/D2 region of the 26S ribosomal DNA (Fell et al. 2000), of 18S ribosomal RNA by Nakase et al. (1991, 1993) and of 18S ribosomal DNA by Cañete-Gibas et al. (1998) and Takashima & Nakase (1999), which justifies the erection of the family Cuniculitremaceae. The only

exception is *Tsuchiyaea wingfieldii*, a CoQ 9-equipped species related to *Cryptococcus amylolentus* (van der Walt, D.B. Scott & van der Klift) Golubev based on molecular studies (Fell et al. 2000). The micromorphology of *T. wingfieldii* was investigated by Sampaio et al. (1999) who illustrated cellular outgrowths being different from the typical stalks of the Cuniculitremaceae. Moreover, all members of this family possess a CoQ 10 system (Nakase et al. 1991).

Ecology

Cuniculitrema polymorpha was found to be carried by bark beetles infesting *Picea abies*, *Pinus sylvestris*, and *Larix decidua* in the localities mentioned in Table 1. The fungus developed within slimy microbial masses. The anamorph *S. polymorphum* was reported from a similar micro-habitat on wood in an old mine by Kraepelin & Schulze (1982). Since the fungus develops haustorial cells that can be attached to other fungal cells, it is probably a mycoparasite. In most of the mixed cultures containing *C. polymorpha*, other basidiomycetes were not detected. Ophiostomatoid ascomycetes, i.e. members of *Ophiostoma* H. & P. Sydow and similar genera, are the dominant fungi in bark beetle galleries (Kirschner 1998) and thus are probably the hosts of *C. polymorpha*. *Tremellina pyrenophila* is another presumably tremellaceous species that was reported as a parasite of an ophiostomatoid fungus (Bandoni 1986b).

Bandoni (1979, 1998) described two species of *Fibulobasidium* Bandoni growing in association with stromatic fungi beneath the bark of deciduous trees. Since the basidiocarps develop adjacent to insect tunnels and the basidiospores are sessile, these fungi are probably dispersed by insects (Bandoni 1998). For another heterobasidiomycete growing in bark beetle galleries, *Atractocolax pulvinatus* Kirschner, Bauer & Oberwinkler, transportation by bark beetles was shown by direct isolations of the fungus from bark beetles (Kirschner et al. 1999).

Acknowledgements

We thank Dr. S. Wood and Ms. I. Reinhold for correcting the English text, Dr. B. Wermelinger for his aid in collecting bark beetles in Switzerland, and Drs. D. Begerow and A. Riethmüller, and Ms. J. Götze for help in conducting the molecular analysis. The study was supported by the DFG-projects 'Organismische Interaktionen in Waldökosystemen' and KI 697/1–2. Work by J. P. Sampaio and M. Gadanho was supported by grants No PRAXIS/PCNA/C/BIA/174/96 and No PRAXIS/BTI/16804/98, respectively.

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