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A new basidiomycetous anamorph genus with cruciform conidia

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An undescribed hyphomycete with clamped septa, conidiophores aggregated in sporodochia, and cruciform, aseptate conidia apically borne on clamps was found on a branch of a deciduous tree lying on the ground. The septal pores are dolipores with perforate parenthesomes indicating a homobasidiomycetous relationship. The morphology and ecology of the fungus are discussed in relation to morphologically similar anamorphic basidiomycetes. Because the fungus could not be classified in any known genus, the new genus and new species *Cruciger lignatilis* is proposed to accommodate this species.

Key Words——aquatic fungi; staurospores; ultrastructure.

In freshwater habitats, hyaline, branched conidia of aquatic hyphomycetes are abundant (Ingold, 1975). Branched spores (staurospores) have convergently evolved in different groups of fungi colonizing aquatic habitats (Webster and Descals, 1981). In comparison to spores of other shapes, staurospores are more effectively attached to substrata under submerged conditions (Webster, 1959). However, many staurosporous species are sometimes or even mostly found in terrestrial habitats (Bandoni, 1972). In these cases, the staurospores may be held up by the surface-tension on thin water layers temporarily covering terrestrial substrata, and spread by movements of the water film (Bandoni, 1972). In the present study, a basidiomycetous hyphomycete with staurospores was found in a terrestrial environment.

Materials and Methods

A rotting branch of a deciduous tree lying on the ground was collected near Tübingen in Germany in May 1997, brought to the laboratory, and examined with a dissecting microscope. For light microscopy, fresh material was mounted in water. For transmission electron microscopy (TEM), fresh material was fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer for several weeks, postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, then washed with distilled water, stained in 1% uranyl acetate, washed with water again, dehydrated in an acetone series, and embedded in ERL (Spurr, 1969). Sections of 60 nm were cut with a diamond knife, mounted on copper grids coated with Formvar, and stained in lead citrate (Reynolds, 1963) for 10 min. The sections were examined with a Zeiss EM 109 transmission electron microscope.

We attempted to cultivate this fungus by preparing suspensions of conidia from single sporodochia, each of them shaken in approx. 1 ml of sterile water, and subsequently plating these suspensions onto 2% (Difco) malt extract agar in Petri dishes. Whole sporodochia were removed from the natural substratum and placed in Petri dishes containing autoclaved pieces of twigs of *Picea abies* (L.) Karst. embedded in 1.5% water agar.

Results

Cruciger Kirschner & Oberwinkler gen. nov.

Genus Hyphomycetum. Sporodochia alba vel pallide colorata. Conidiophora hyalina, septata, fibulata, irregulariter ramosa. Cellulae conidiogenae cylindricae, conidia in fibulis apicalibus proliferantibus parientes. Conidia hyalina, aseptata, cum axe principali cylindrico rectoque et brachiis lateralibus. Septa hyphorum doliporis et parenthesomatibus perforatis.

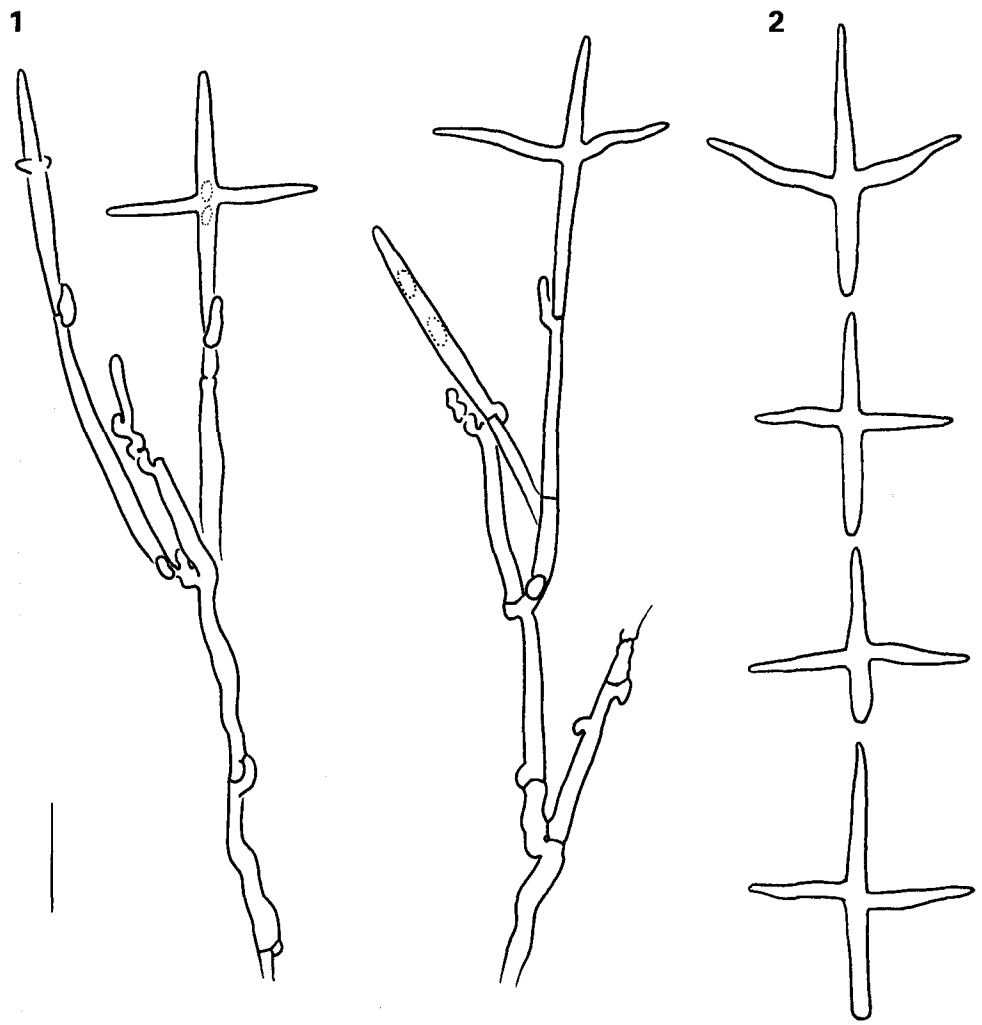
Typus generis: *Cruciger lignatilis* Kirschner & Oberwinkler, in opere ipso descriptus.

Etymology: The name refers to the conidiophores bearing cruciform conidia.

Hyphomycetous genus. Sporodochia white or pale-coloured. Conidiophores hyaline, septate, with clamps at the septa, irregularly branched. Conidiogenous cells cylindrical, producing conidia on apical, percurrently proliferating clamps. Conidia hyaline, aseptate, with cylindrical and straight main axis and lateral branches. Hyphal septa with dolipores and perforate parenthesomes.

Cruciger lignatilis Kirschner & Oberwinkler sp. nov.
Figs. 1–3

Sporodochia superficialia in ligno putrescenti, pulvinata, alba vel pallide violacea, 120–150 μm diametro. Conidiophora hyalina, tenuiter tunicata, fibulata, irregulariter ramosa. Cellulae conidiogenae cylindricae,



Figs. 1, 2. *Cruciger lignatilis*. Scale bar = 10 μm.
Fig. 1. Two conidiophores with conidia at different stages of development (nuclei marked by dotted lines). Fig. 2. Conidia.

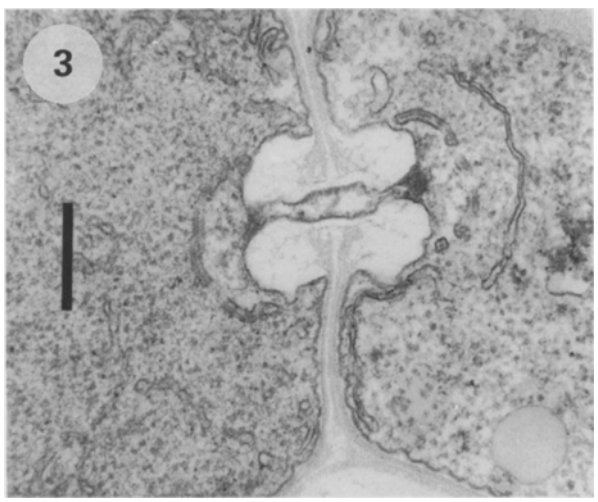


Fig. 3. *Cruciger lignatilis*. Median section through a dolipore with perforate parenthesomes. Scale bar = 0.25 μm.

10–15 × 1.5–2 μm, conidia in fibulis apicalibus parientes. Conidia hyalina, aseptata, cruciformia, binucleata, 1–2 μm crassa, axibus principalibus 16–27 μm longis, cum 2 brachiis lateralibus oppositis 8–12 μm longis.

Holotypus: RoKi 209, depositus in M, in ligno putrescenti, Germania, Baden-Württemberg, Tübingen, Heuberger Tor, leg. R. Kirschner, 01 May 1997.

Sporodochia superficial on rotting wood, pulvinate, white to pale violet, 120–150 μm diam. Conidiophores hyaline, thin-walled, with clamps at the septa, irregularly branched. Conidiogenous cells cylindrical, 10–15 × 1.5–2 μm, producing conidia on apical, proliferating clamps. Conidia hyaline, aseptate, cruciform, binucleate, 1–2 μm thick, with the main axis 16–27 μm long, and two opposing lateral branches of 8–12 μm in length. The main axis develops first, and the two opposing lateral branches subsequently grow from the centre of the main axis.

With the naked eye, fruitbodies of *Exidia glandulosa* (Bull. ex St-Am.) Fr. were detected on the rotting branch brought to the laboratory. Using a dissection micro-

scope, we also found *Cruciger lignatilis*, old fruitbodies of a corticioid basidiomycete, pyrenomycetes, and conidiophores of hyphomycetes (mainly a species of *Ramichloridium* Stahel ex de Hoog).

The culture experiments failed because no colonies of *C. lignatilis* developed. In most cases, colonies arising from single spore isolations on malt extract agar represented a dematiaceous hyphomycete (*Ramichloridium* sp.).

Discussion

Genera producing staurosporous conidia on clamped conidiophores are *Arcispora* Marvanová & Bärlocher, *Ingoldiella* Shaw, *Microstella* Ando & Tubaki, *Taeniospora* Marvanová, *Titaeella* Arnaud, *Crucella* Marvanová & Suberkropp, *Cyrenella* Gochenaur, and *Naiadella* Marvanová & Bandoni (Table 1). As in *C. lignatilis*, the staurospores of species of *Arcispora* and *Taeniospora*, for example, develop in a special manner: Initially, the main axis of the conidium develops to almost its full length, then lateral branches grow out from the main axis (Marvanová and Bärlocher, 1998; Marvanová and Stalpers, 1987). In other genera, the main axis elongates after the initiation of lateral branching (Marvanová, 1977; Marvanová and Bandoni, 1987; Marvanová and Suberkropp, 1990; Nawawi, 1973). Species of *Ingoldiella*, *Taeniospora*, and *Titaeella* differ from *C. lignatilis* by their septate conidia (Ando and Tubaki, 1985; Marvanová and Stalpers, 1987; Nawawi, 1985). The conidia develop solitarily in *Ingoldiella* (Marvanová, 1977; Nawawi, 1973) and *Titaeella* (Ando and Tubaki, 1985), whereas several conidia are produced on percurrently proliferating conidiogenous cells in *Arcispora* and *Taeniospora* (Marvanová, 1977; Marvanová and Bärlocher, 1998; Marvanová and Stalpers, 1987) and in *C. lignatilis*.

The conidiophores are simple in *Ingoldiella fibulata* Nawawi (Nawawi, 1973), species of *Taeniospora* (Marvanová, 1977; Marvanová and Stalpers, 1987), and *Titaeella capnophila* Arnaud (Ando and Tubaki, 1985), rarely branched in *Arcispora bisagittaria* Marvanová & Bärlocher (Marvanová and Bärlocher, 1998); but mostly branched in *Ingoldiella hamata* Shaw (Nawawi, 1973) and *C. lignatilis*. Conidium dehiscence in *I. hamata* results from a rupture of an empty separating cell immediately below the conidium (Nawawi, 1973). The conidiogenous cells of *Crucella*, *Cyrenella*, and *Naiadella* are emptied during the transfer of the cytoplasm to the conidium and then collapse (Gochenaur, 1981; Marvanová and Bandoni, 1987; Marvanová and Suberkropp, 1990), whereas such a transfer of cytoplasm and following collapse do not occur in conidiogenous cells of *C. lignatilis*. In contrast to *C. lignatilis*, sporodochia were not described in species of *Microstella*, *Taeniospora* and *Titaeella*, but *I. hamata* develops clusters of conidiophores arising from cushion-shaped masses of hyphae (Nawawi, 1973). Distinct sporodochia are known in *Fibulotaeniella canadensis* Marvanová & Bärlocher, an aquatic anamorphic basidiomycete which develops unbranched conidia (Marvanová and Bärlocher, 1988).

Cruciger lignatilis was compared with descriptions of the genera listed in Table 1. The three genera with heterobasidiomycetous nature, *Crucella*, *Cyrenella*, and *Naiadella*, differ from *C. lignatilis* in that they transfer cytoplasm from collapsing conidiogenous cells to the conidia. *Cruciger lignatilis* is more closely related to *Arcispora*, *Ingoldiella*, *Microstella*, *Taeniospora*, *Titaeella*, and *Fibulotaeniella*. Of these genera, *C. lignatilis* is most similar to species of *Taeniospora* in conidium shape and development. The conidia of species of *Taeniospora* are, however, septate, and sporodochia are absent. Sporodochia and aseptate conidia are present in *Fibulotaeniella*, but the conidia are unbranched.

The perforate parenthesomes associated with dolipores indicate the homobasidiomycetous nature of *C. lignatilis*. Teleomorphs of aquatic basidiomycetous anamorphs are known for species of *Ingoldiella* and

Table 1. Characteristics of *Cruciger* and similar basidiomycetous anamorph genera.

genus	conidia	conidiophores	conidium production	teleomorph-connections	known habitat
Cruciger	branched, aseptate	aggregated, branched	percurrent	probably homobasidiomycetous	terrestrial
Taeniospora	branched, septate	single, simple	percurrent	homobasidiomycetous	aquatic
Fibulotaeniella	unbranched, aseptate or septate	aggregated, branched	percurrent	probably homobasidiomycetous	aquatic
Ingoldiella	branched, septate	different	solitarily	homobasidiomycetous	aquatic
Microstella	branched, aseptate	single, branched	solitarily?	not known	probably terrestrial
Titaeella	branched, septate	single, simple	solitarily	not known	probably terrestrial
Arcispora	branched, septate	single, simple or sparsely branched	percurrent	homobasidiomycetous	aquatic
Crucella	branched, aseptate	single, simple or rarely branched	transfer of cytoplasm from collapsing cells	heterobasidiomycetous	aquatic
Cyrenella	branched, aseptate	single or aggregated, branched	transfer of cytoplasm from collapsing cells	probably heterobasidiomycetous	aquatic
Naiadella	branched, aseptate	single or aggregated	transfer of cytoplasm from collapsing cells	probably heterobasidiomycetous	aquatic

Taeniospora in the corticioid homobasidiomycetous genera *Fibulomyces* Jülich, *Leptosporomyces* Jülich, and *Sistotrema* Fr. (Marvanová and Stalpers, 1987; Nawawi and Webster, 1982), and for *Crucella* in the heterobasidiomycetous genus *Camptobasidium* Marvanová & Suberkropp (Marvanová and Suberkropp, 1990). In *Cyrenella elegans* Gochenaur, a yeast stage is developed in the culture (Gochenaur, 1981) indicating a heterobasidiomycetous relationship. *Naiadella fluitans* Marvanová & Bandoni was also assumed to be related to heterobasidiomycetous fungi because of the presence of tremelloid haustoria (Marvanová and Bandoni, 1987).

In some basidiomycetous aquatic hyphomycetes, teleomorphs are known which exclusively develop in terrestrial habitats (Nawawi et al., 1977). Experiments with cultures of species of *Taeniospora* showed that the conidia originate from submerged mycelium and that the teleomorph develops under emerse conditions (Marvanová and Stalpers, 1987; Nawawi et al., 1977). In contrast to the aquatic *Ingoldiella hamata* and the *Taeniospora* anamorph of *Leptosporomyces galzinii* (Bourd.) Jülich, found on submerged leaves (Nawawi, 1973; Nawawi et al., 1977), *C. lignatilis* was found on a decaying branch lying on the ground without any stream or pond nearby. *E. glandulosa*, an obviously terrestrial, wood-inhabiting basidiomycete, was also fruiting on this branch. The staurosporous hyphomycete *Digitodochium rhodoleucum* Tubaki & Kubono also produces sporodochia growing on fallen twigs without any discernible reference to an aquatic habitat (Tubaki and Kubono, 1989). *Titaeella capnophila* and *Microstella pluvioriens* Ando & Tubaki were isolated from rainwater on trees by Ando and Tubaki (1984, 1985). A fungus probably conspecific with *T. capnophila* was isolated from a stream and assumed to be washed from an epiphytic habitat (Descals, 1997). Rain or high atmospheric humidity may be sufficient to induce conidium development in some staurosporous hyphomycetes. In the present case, the conidia are distributed by temporary rainwater flows on decaying wood and soil rather than by permanent streams.

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