

Tremelloid haustorial cells with haustorial filaments and potential host range of *Tremella mesenterica*

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In vitro and in vivo haustorial cells of *Tremella mesenterica*, consisting of a subglobose basal part with one or more thread-like haustorial filaments, were examined using light and electron microscopy. The haustorial cells developed from clamp connections of hyphal septa or intercalarily. Mostly such haustorial cells were monokaryotic, but in a few cases also dikaryotic haustorial cells were observed. In vitro, the host range of *Tremella mesenterica* was tested with the corticiaceous homobasidiomycetes *Peniophora erikssonii*, *P. quercina* and *Phanerochaete cremea*. The in vitro interaction of *Tremella mesenterica* with all these fungi was principally the same as in vivo with *Peniophora laeta*. A single micropore connected the cytoplasm of the haustorial filament with that of the respective host cell. The pore domain at the host side was delimited by curved ER cisternae only in *Peniophora quercina*, but not in *Peniophora erikssonii* and *Phanerochaete cremea*.

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Introduction

Tremella mesenterica Retz.: Fr. is a mycoparasite of the corticiaceous homobasidiomycete *Peniophora laeta* (Fr.) Donk (Zugmaier et al. 1994). Haustorial cells with haustorial filaments develop on *Tremella* hyphae and often haustorial filaments become attached to host cells (Zugmaier et al. 1994). In a parasitic interaction of *Tremella mesenterica* with *Peniophora laeta* the haustorial filament penetrates the host cell wall and a continuous micropore connects the cytoplasm of both cells (Zugmaier et al. 1994). In vitro, haustorial filaments of *Tremella mesenterica* attack the corticiaceous fungi *Peniophora laeta*, *P. incarnata* (Pers.: Fr.) P. Karst., *Phlebia radiata* Fr., *Schizopora paradoxa* (Schrad.: Fr.) Donk, *Stereum hirsutum* (Willd.: Fr.) Gray and *Diatrype stigma* (Hoffm.: Fr.) Fr. (Zugmaier et al. 1994). Micropores with direct cytoplasm to cytoplasm connections could be found however only in interactions of *Tremella mesenterica* with *Peniophora laeta*, *P. incarnata* and *Phlebia radiata* (Zugmaier et al. 1994). The pore domain at the host side is

delimited by curved ER cisternae in *Peniophora laeta* and *P. incarnata* but not in *Phlebia radiata* (Zugmaier et al. 1994).

This publication presents new data on tremelloid haustorial cells and the potential host range of *Tremella mesenterica*. Nuclear behavior of haustorial cells have been studied and one mycelial strain of *Tremella mesenterica* was cultivated together with *Peniophora erikssonii* Boid., *P. quercina* (Fr.) Cooke and *Phanerochaete cremea* (Bres.) Parm.

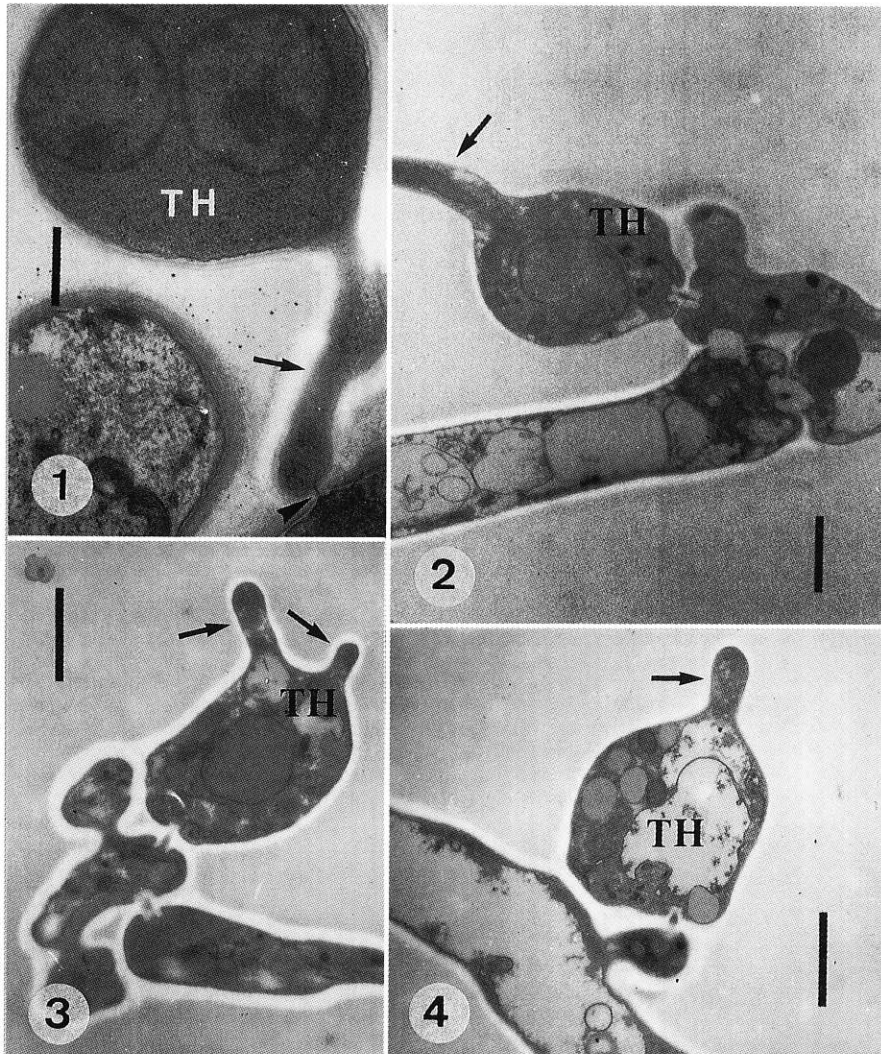
Materials and methods

Specimens used

Tremella mesenterica. Germany. Baden-Württemberg: Stuttgart, Haldenwald near Sonnenberg, on *Carpinus betulus* L. adjacent to *Peniophora laeta*. 8 June 1987, W. Zugmaier 97. Stuttgart, Haldenwald near Sonnenberg, on *Carpinus betulus*, 15 Sept. 1988, W. Zugmaier 117. Stutt-

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Figs 1–4. Transmission electron micrographs illustrating tremelloid haustorial cells with haustorial filaments of *Tremella mesenterica*. – Fig. 1. Dikaryotic tremelloid haustorial cell (TH) with haustorial filament (arrow) contacting a *Peniophora laeta* cell. Note micropore (arrowhead) between the apex of the haustorial filament and the host cell. Bar = 0.5 μm . – Figs 2, 3. Monokaryotic tremelloid haustorial cells (TH) with haustorial filaments (arrows) developing from clamp connections. The basal parts of the haustorial cells are separated from the clamp connections of the mother cells by additional clamps. Note two haustorial filaments in Fig. 3. Fig. 2: bar = 0.5 μm , Fig. 3: bar = 1 μm . – Fig. 4. Intercalary tremelloid haustorial cell subtended by a clamp. Arrow, haustorial filament. Bar = 1 μm .

gart, Schwäblesklinge near Sonnenberg, 5 May 1989, W. Zugmaier 153.

Peniophora erikssonii. Germany. Baden-Württemberg: Schönbuch near Bebenhausen, on *Alnus glutinosa* (L.) Gaertn., 8 Oct. 1992, W. Zugmaier 165.

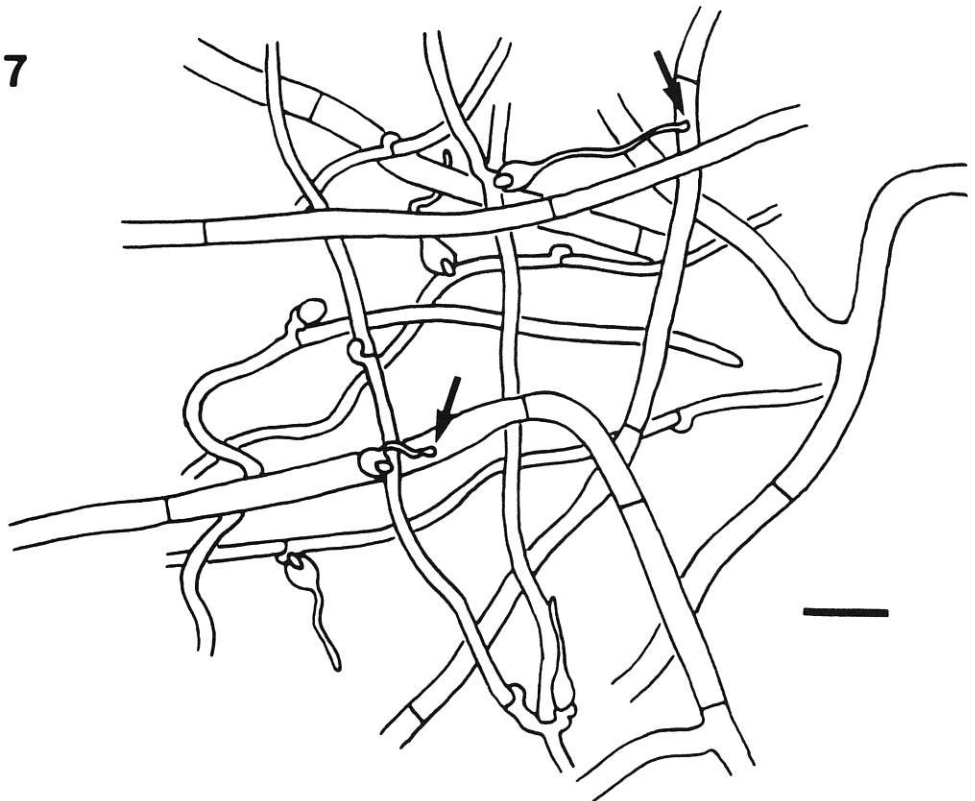
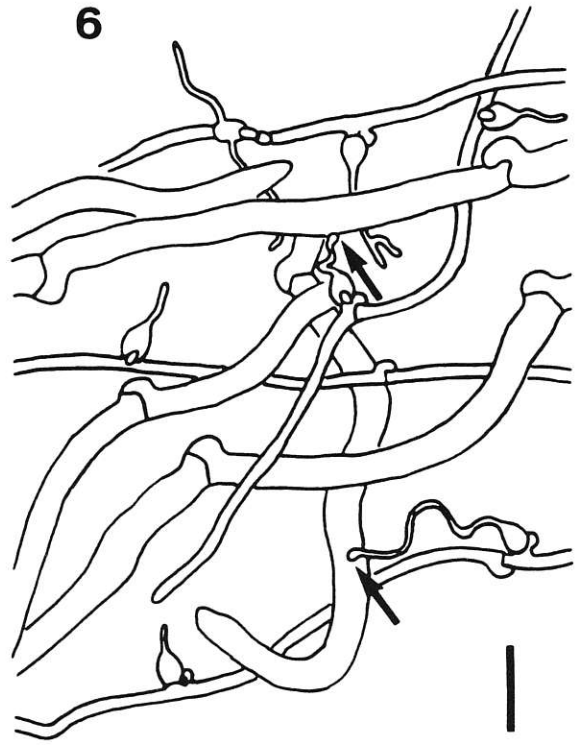
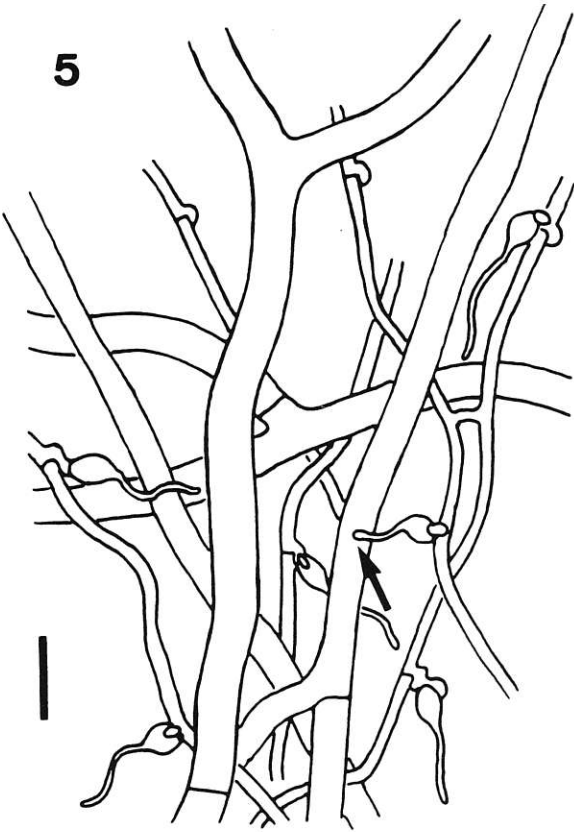
Peniophora quercina. Germany. Baden-Württemberg: Stuttgart, near Sonnenberg, on *Quercus robur* L., 12 Oct. 1992, W. Zugmaier 162.

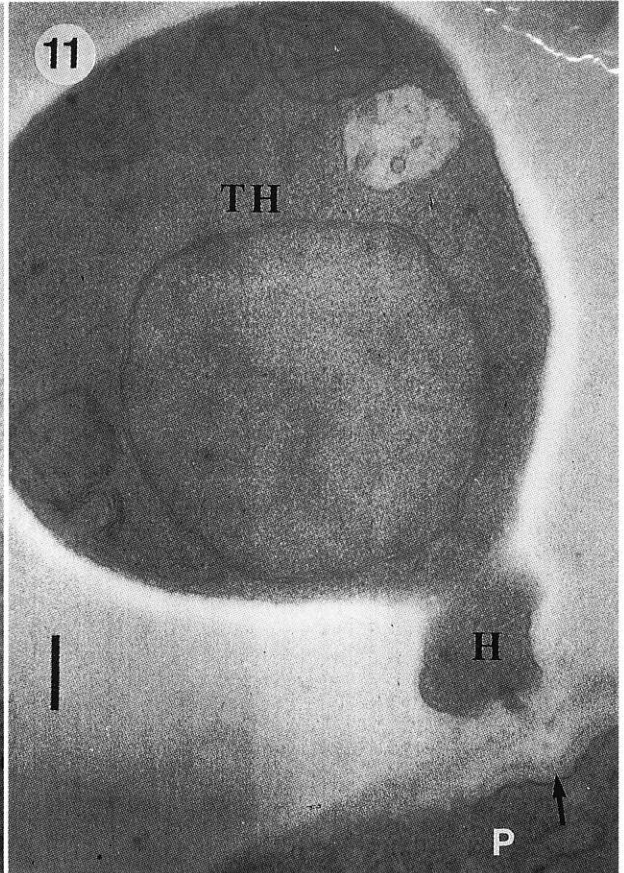
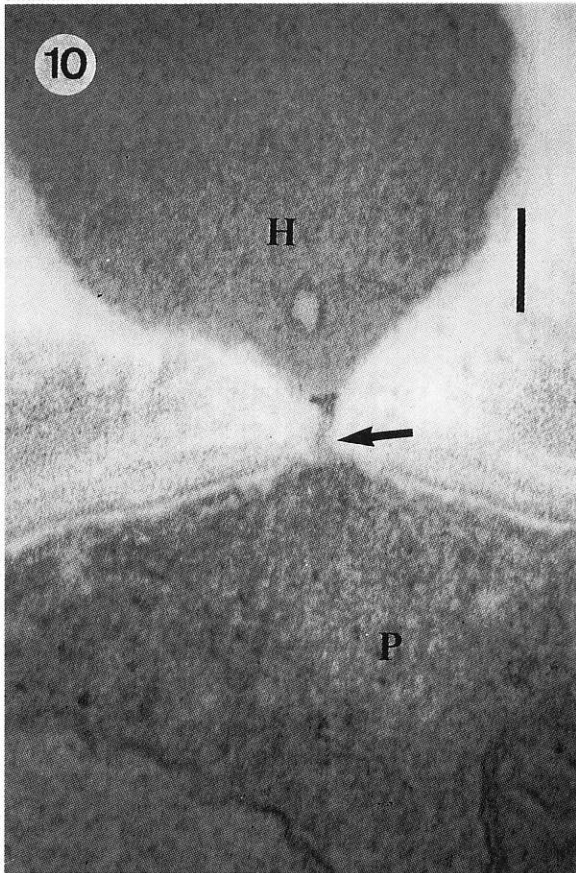
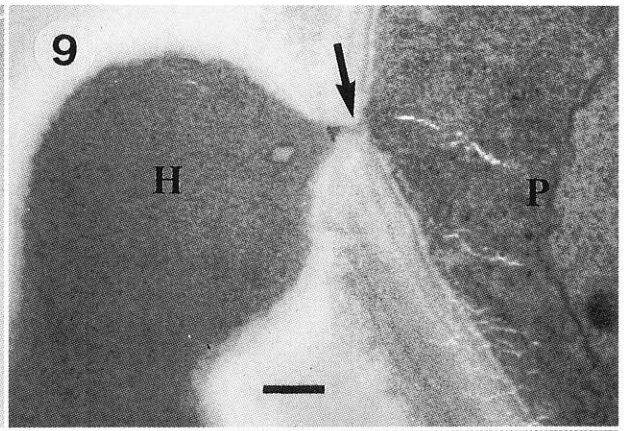
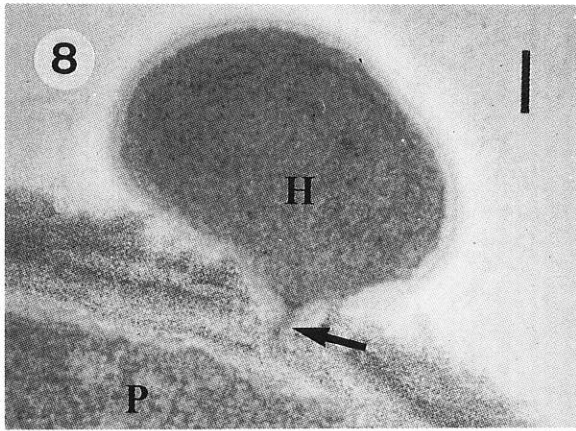
Phanerochaete cremea. Germany. Baden-Württemberg: Tübingen, Schönbuch near Hagelloch, on *Fagus sylvatica* L., 19 April 1985, F. Oberwinkler 36376 a.

Methods

Cultures of *Tremella mesenterica*, *Peniophora erikssonii*, *P. quercina* and *Phanerochaete cremea* were grown on malt yeast peptone agar (MYP; Bandoni 1972). Mycelium of *Tremella mesenterica* was obtained by crossing the same monospore cultures as in Zugmaier et al. (1994). Inocula, 2–4 mm in diam., of the potential host fungi were placed near the margin of a colony of *Tremella mesenterica* in a petri plate with MYP medium. Desired

Figs 5–7. *Tremella mesenterica* in dual culture with potential host fungi. – Light microscopical features. – Fig. 5. *Tremella mesenterica* in dual culture with *Phanerochaete cremea*. Note haustorial filament attached to a hypha of *Phanerochaete cremea* (arrow). Bar = 10 μm . – Fig. 6. *Tremella mesenterica* in dual culture with *Peniophora quercina*. Note haustorial filaments attached to cells of *Peniophora quercina* (arrows). Bar = 10 μm . – Fig. 7. *Tremella mesenterica* in dual culture with *Peniophora erikssonii*. Note haustorial filaments attached to cells of *Peniophora erikssonii* (arrows). Bar = 10 μm .





Figs 8–11. Transmission electron micrographs with interaction stages between *Tremella mesenterica* and *Peniophora erikssonii*. – Fig. 8. The apex of a haustorial filament (H) has invaded the outer cell wall layers of a *Peniophora erikssonii* hyphal cell (P) and a spur-like projection (arrow) penetrates the host cell wall. Bar=0.1 μm . – Figs 9, 10. median section through a micropore (arrows) between a haustorial filament (H) and a hyphal cell of *Peniophora erikssonii* (P). Fig. 10 shows the micropore of Fig. 9 in higher magnification. Fig. 9: bar=0.1 μm , Fig. 10: bar=0.1 μm . – Fig. 11. Invaginated plasmalemma and secondary wall formation (arrow) at the host side (P) of an interaction domain. TH, tremelloid haustorial cell. H, haustorial filament. Bar=0.2 μm .

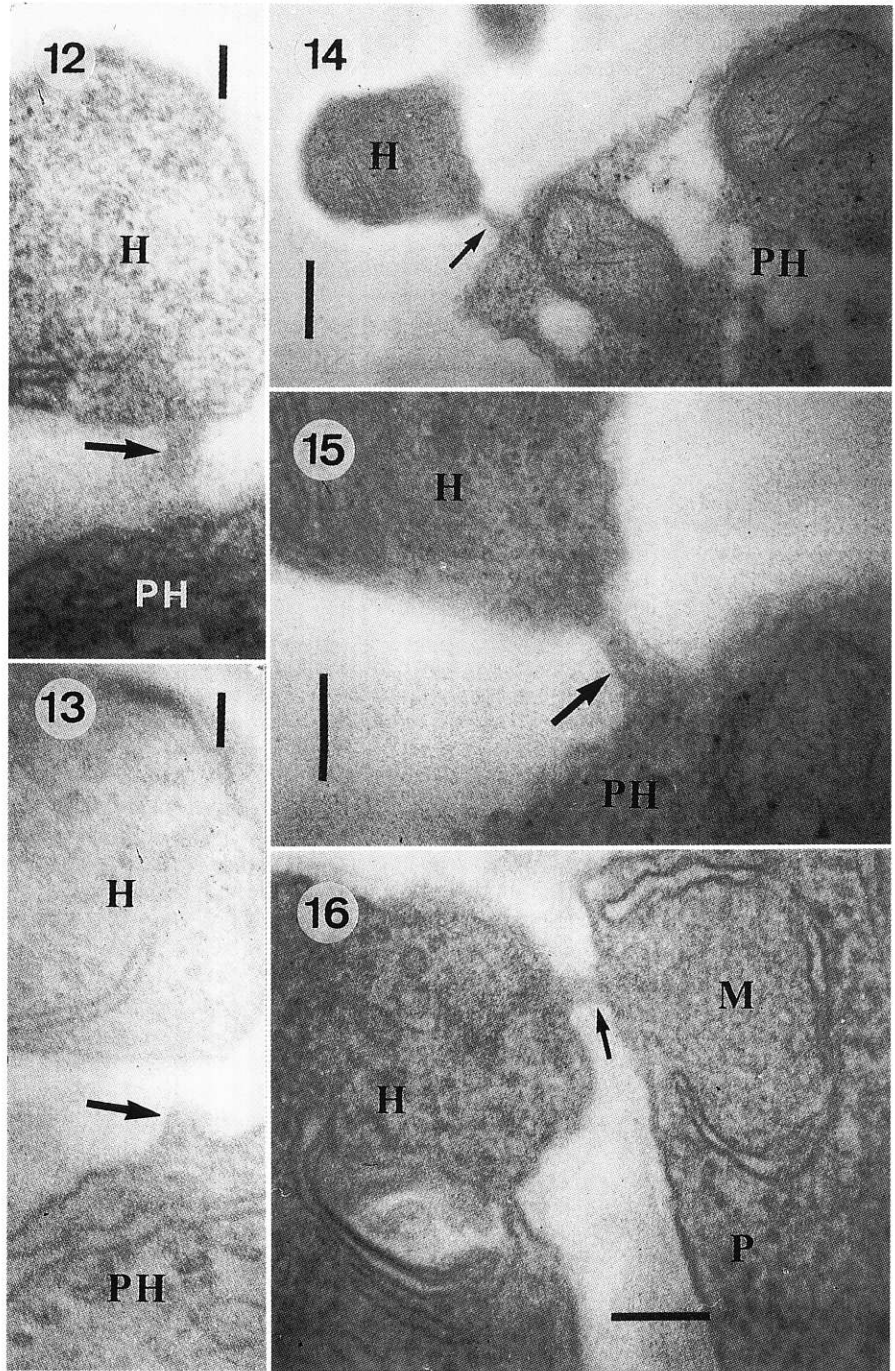
mixed stages were selected under the light microscope and fixed for electron microscopy.

Living fungi were examined with phase contrast optics.

HCL-Giemsa staining was used for studying the nuclear stages of tremelloid haustorial cells:

Slides containing freshly prepared samples were dried at room temperature and immediately afterwards fixed by

Figs 12–15. Transmission electron micrographs of *Tremella mesenterica* in interaction with *Phanerochaete cremea* (Figs 12–15) and *Peniophora quercina* (Fig. 16). – Figs 12, 13. Adjacent serial sections through a micropore (arrows) between a haustorial filament (H) and a hyphal cell of *Phanerochaete cremea* (PH). Note that the pore membrane (arrows) is continuous with the plasma membrane of both cells. Bars=0.05 μm . – Figs 14, 15. Median section through a micropore between a haustorial filament (H) and a hyphal cell of *Phanerochaete cremea* (PH). The arrows indicate the probably continuous plasma membrane through the micropore. Fig. 15 shows a detail from Fig. 14. Fig. 14: bar=0.2 μm , Fig. 15: bar=0.1 μm . – Fig. 16. Median section through a micropore between a haustorial filament (H) and a hyphal cell of *Peniophora quercina* (P). The arrow indicates the probably continuous plasma membrane through the micropore. Note that the pore domain (M) at the host side is delimited by a curved ER cisterna. Bar=0.1 μm .



a 3:1 mixture of 92% ethanol and acetic acid for 30 minutes. After two washes with distilled water, samples were hydrolysed in 60°C HCL (1N) for 7 minutes. Then samples were rinsed two times with distilled water and afterwards two times with phosphate buffer pH 7.0. After staining for one hour in Giemsa stock solution and phosphate buffer (1:9) samples were rinsed with phosphate buffer and afterwards with distilled water. The stained

samples were embedded in Entellan (Merck) and mounted with a cover-glass.

For transmission electron microscopy, freshly collected and cultured material was fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 overnight or during several days. Following six transfers in 0.1 M sodium cacodylate buffer, the material was post-fixed in 1% osmium tetroxide in the same buffer for 2 h

in the dark, washed in distilled water, and prestained in 1% darkyl acetate solution for 1 h in the dark. After five washes in distilled water, the material was dehydrated in acetone, using 10 min changes at 25%, 50%, 70%, 95% and 3 times in 100% acetone. The material was embedded in Spurr's plastic (Spurr 1969). Series of sections were cut on a Reichert ultramicrotome using a diamond knife. Serial sections were mounted on Formvar coated single slot copper grids, stained with lead citrate (Reynolds 1963) at room temperature for 3–5 minutes, and washed again with distilled water. The thin sections were examined with a Zeiss EM 109 transmission electron microscope at 80 kV.

Results

In vivo and in vitro the haustorial cells of *Tremella mesenterica*, consisting of a subglobose basal part with one or more thread-like filaments, developed from clamp connections of the hyphal septa or often also intercalarily independent of the septa (Figs 2–7). In both cases the haustorial cell was separated from the original area by a clamp (Figs 2–7). The basal part of the haustorial cell mostly contains one nucleus (Figs 2, 3, 11), but in a few cases also two nuclei occurred (Fig. 1). As shown in Fig. 1, haustorial filaments of dikaryotic haustorial cells were also able to interact.

In dual cultures of *Tremella mesenterica* with *Peniophora erikssonii*, *P. quercina* and *Phanerochaete cremea* hyphae of both fungi grew between each other and haustorial filaments of *Tremella mesenterica* were often attached to hyphae of the respective potential host fungus (Figs 5–7). In interactions of *Tremella mesenterica* with all tested fungi the haustorial filament invaded the outer cell wall layers of a host cell (Figs 8–10, 12–16). Then a spur-like projection (Fig. 8), which developed from the haustorial apex, penetrated the rest of the host wall and finally a continuous micropore connected the cytoplasm of both cells (Figs 9, 10, 12–16). The diam of the intermembrane space within the micropore was 8–20 nm (Figs 9–10, 12–16). The pore membrane appeared to be continuous with the plasmalemmas of both cells and no separation of the cytoplasm was visible in the pore channel (Figs 10, 12, 13, 15, 16). Sometimes electron-opaque material could be observed in the apex of an interacting haustorial filament (Figs 8–10). Only in cells of *Peniophora quercina* however, curved cisternae of smooth endoplasmic reticulum delimited the pore domain (Fig. 16). Often ribosomes were nearly absent at the pore domain of the host side, especially when this area was surrounded by curved ER cisternae as in *Peniophora quercina* hyphae (Fig. 16). Stages in which the host plasma membrane was invaginated and the interaction area was closed by a secondary wall layer at the host side could be observed in cells of all tested potential host fungi (Fig. 11). The interaction of *Tremella mesenterica* in vitro with

Peniophora erikssonii, *P. quercina* and *Phanerochaete cremea* was principally the same as in vivo with *Peniophora laeta* (Zugmaier et al. 1994).

Discussion

Tremelloid haustorial cells with haustorial filaments were first described by Olive (1946) in *Tremella obscura* (Olive) M.P. Christ. He observed double clamp connections at the bases of such structures. Haustorial cells of *Tremella rhytidhysterii* Bezerra & Kimbr., *Tetragonomycetes uliginosus* (P. Karst.) Oberw. & Bandoni and *Christiansenia pallida* Hauerslev develop from clamp connections of the hyphal septa and are finally separated by another clamp (Bezerra & Kimbrough 1978, Oberwinkler et al. 1984, Bauer & Oberwinkler 1990). In *Tremella mesenterica* tremelloid haustorial cells could also originate often from intercalary positions independent of hyphal septa. In these cases no double clamp connections were present at the bases of haustorial cells. Metzler et al. (1989) have rarely observed such intercalary haustorial cells also in *Rhynchogastrema coronata* Metzler & Oberwinkler. Bezerra & Kimbrough (1978) and Oberwinkler et al. (1984) found only one nucleus in tremelloid haustorial cells. However, Bandoni (1986) could observe two nuclei in young haustorial cells of *Tremellina pyrenophila* Bandoni, a basidiomycetous anamorph possibly related to the Tremellaceae. In *Tremella mesenterica* we could find many monokaryotic haustorial cells in vivo and in vitro, but also some haustorial cells with two nuclei. In one case such a dikaryotic tremelloid haustorial cell was even in interaction with a host cell. There are different possibilities to explain the presence of one or two nuclei. If the tremelloid haustorial cells of *Tremella mesenterica* are primarily uninucleate, a secondary dikaryotic stage could be formed by division of the original nucleus. It is also possible that such haustorial cells are primarily dikaryotic.

Obviously numerous hardwood decaying fungi are potential hosts for *Tremella mesenterica*. Until now detailed knowledges about the host ranges of *Tremella* species are rare. Some *Tremella* species appear to be restricted to one host species, e.g. *Tremella aurantia* Schwein.: Fr. on *Stereum hirsutum* (Willd.: Fr.) S.F. Gray, *Tremella juniperina* P. Karst. on *Colpoma juniperi* (P. Karst.) Dennis or *Tremella subencephala* Bandoni & Ginns on *Acanthophysium lividoeruleum* (Torkelsen 1978; Bandoni & Ginns 1993). Others appear to be parasitic on several species of one host genus, e.g. *Tremella vesicolor* Berk. & Broome on *Peniophora incarnata*, *Peniophora nuda* (Fr.) Bres. and *Peniophora reidii* Boidin & Lanquetin (Bandoni & Ginns 1993). *Tremella mesenterica* is a potential mycoparasite of *Peniophora laeta* and tremelloid haustorial filaments are interacting with substrate hyphae and fruitbody hyphae of the host fungus (Zugmaier et al.

1994). Moreover, *Tremella mesenterica* basidiocarps occur not seldom adjacent to fructifications of other Basidiomycetes, especially those of *Peniophora* species (Bourdot & Galzin 1928; Jahn 1979; Wong et al. 1985; Bandoni 1987). In vitro dual cultural experiments with *Tremella mesenterica* and a number of corticiaceous fungi indicate, that the host range contains at least the following species of three genera: *Peniophora laeta*, *P. incarnata*, *P. erikssonii*, *P. quercina*, *Phlebia radiata* and *Phanerochaete cremea*. The genera *Peniophora*, *Phlebia* and *Phanerochaete* are considered to be related (Eriksson et al. 1978, 1981). The genus *Peniophora* is subdivided into the two subgenera *Gloeopeniophora* and *Peniophora* (Eriksson et al. 1978). *Peniophora laeta*, *P. incarnata* and *P. erikssonii* are in subgenus *Gloeopeniophora* while *Peniophora quercina* is placed in subgenus *Peniophora*. In parasitic interactions of *Tremella mesenterica* with *Peniophora laeta*, *P. incarnata* and *P. quercina* curved ER cisternae of smooth endoplasmic reticulum delimited the pore domain at the host side, while in cells of *Peniophora erikssonii*, *Phlebia radiata* and *Phanerochaete cremea* such ER cisternae were not associated with the interaction zone. Obviously only cells of some *Peniophora* species develop ER cisternae in case of being parasitised by haustorial filaments of *Tremella mesenterica*. If the presence or absence of such ER cisternae in host cells of *Tremella mesenterica* is a highly specific one, the subgenera *Gloeopeniophora* and *Peniophora* appear not to represent natural relationships.

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