Mycoparasitism of some *Tremella* species[1]

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Abstract: The parasitic interaction of *Tremella mesenterica, T. encephala* and *T. mycophaga* was studied by light and electron microscopy. The host range of *T. mesenterica* was tested with *Peniophora laeta, Peniophora incarnata, Phlebia radiata, Schizopora paradoxa, Stereum hirsutum* and *Diatrype stigma*. It was demonstrated that *T. mesenterica* is, at least in vivo, a mycoparasite of the corticiaceous homobasidiomycete *Peniophora laeta*. Specialized interactive cells of *T. mesenterica, T. encephala* and *T. mycophaga,* designated as tremelloid haustorial cells with haustorial filaments, penetrated the cell walls of their respective hosts. A single micropore connected the cytoplasm of the haustorial filament with that of the host cell. The pore membrane appears to be continuous with the plasmalemma of both cells. In older interaction structures, the micropore was often overgrown on the host side by secondary wall formation. In vitro such micropores were present in the interaction of *T. mesenterica* with *Peniophora laeta, P. incarnata* and *Phlebia radiata* but could not be found in the interaction of *T. mesenterica* with *Schizopora paradoxa, Stereum hirsutum* and *Diatrype stigma*. However, only in *Peniophora laeta* and *P. incarnata* was the pore domain delimited by a more or less circular arrangement of ER cisternae. Wall thickening may be responsible for the nonmicropore interaction.

Key Words: Heterobasidiomycetes, host-parasite interaction, mycoparasitism, *Peniophora laeta, Tremella mesenterica, Tremella encephala, Tremella mycophaga,* tremelloid haustorial cells

INTRODUCTION

While association with other fungi is common among *Tremella* species, it is not clear that true mycoparasitism occurs (Bandoni, 1987). Specialized interactive cells, designated here as tremelloid haustorial cells,

are often present. Each tremelloid haustorial cell is subtended by a clamp and consists of a subglobose basal part with one or more thread-like haustorial filaments that occasionally become attached to host cells. Such tremelloid haustorial cells have been described from a variety of tremelloid fungi (Olive, 1946; Bandoni, 1961, 1984, 1985, 1987; Bezerra and Kimbrough, 1978; Oberwinkler and Bandoni, 1981, 1983; Metzler et al., 1989) and from species with affinities to the Tremellales *sensu* Bandoni (1984) including *Szygospora alba* G. W. Martin (Oberwinkler and Lowy, 1981), *Christiansenia pallida* Hauerslev (Oberwinkler and Bandoni, 1982; Oberwinkler et al., 1984), *Filobasidium floriforme* L. Olive (Olive, 1968) and *Filobasidiella neoformans* Kwon-Chung (Kwon-Chung, 1976).

The ultrastructure of the host-parasite interface has only been studied in three species. Bezerra and Kimbrough (1978) noted that tremelloid haustorial cells of *Tremella rhytidhysterii* Bezerra & Kimbr. do not actually penetrate the cell wall of *Rhytidhysterium rufulum* (Spreng.) Speg. No ultrastructural differentiation was observed. A single micropore with direct cytoplasm-cytoplasm connection is present, however, between the haustorial filament of *Tetragoniomyces uliginosus* (Karst.) Oberw. & Bandoni and the host cell of *Rhizoctonia* sp. (Bauer and Oberwinkler, 1990a). Furthermore, the haustorial filament of *Christiansenia pallida* penetrates to the cell of *Phanerochaete cremea* (Bres.) Parm. Several micropores with direct cytoplasm-cytoplasm connections develop between the haustorial filament and host cell (Bauer and Oberwinkler, 1990b).

Mycoparasitic interactions, with emphasis on the type species *Tremella mesenterica* Retz. : Fr., were studied. The species occurs mainly on hardwoods with basidiomata often developing adjacent to fructifications of *Peniophora* spp. (Corticiaceae), especially those of the subgenus *Gloeopeniophora*, e.g., *Peniophora laeta* (Fr.) Donk, *Peniophora incarnata* (Pers. : Fr.) Karst. and *Peniophora aurantiaca* (Bres.) v. Höhn. & Litsch. (Bourdot and Galzin, 1928; Jahn, 1979; Wong et al., 1985; Bandoni, 1987). Therefore, Jahn (1979), Wong et al. (1985) and Bandoni (1987) suggested that *T. mesenterica* could be a mycoparasite, but clear evidence is lacking. *Tremella encephala* Pers.: Pers. grows on *Stereum sanguinolentum* (Alb. & Schw. : Fr.) Fr.; the basidiocarp consists of a firm, fleshy core surrounded by a gelatinous layer. Bandoni (1961) demonstrated that the fleshy core belongs to *Stereum sanguinolentum* and *T. encephala* forms
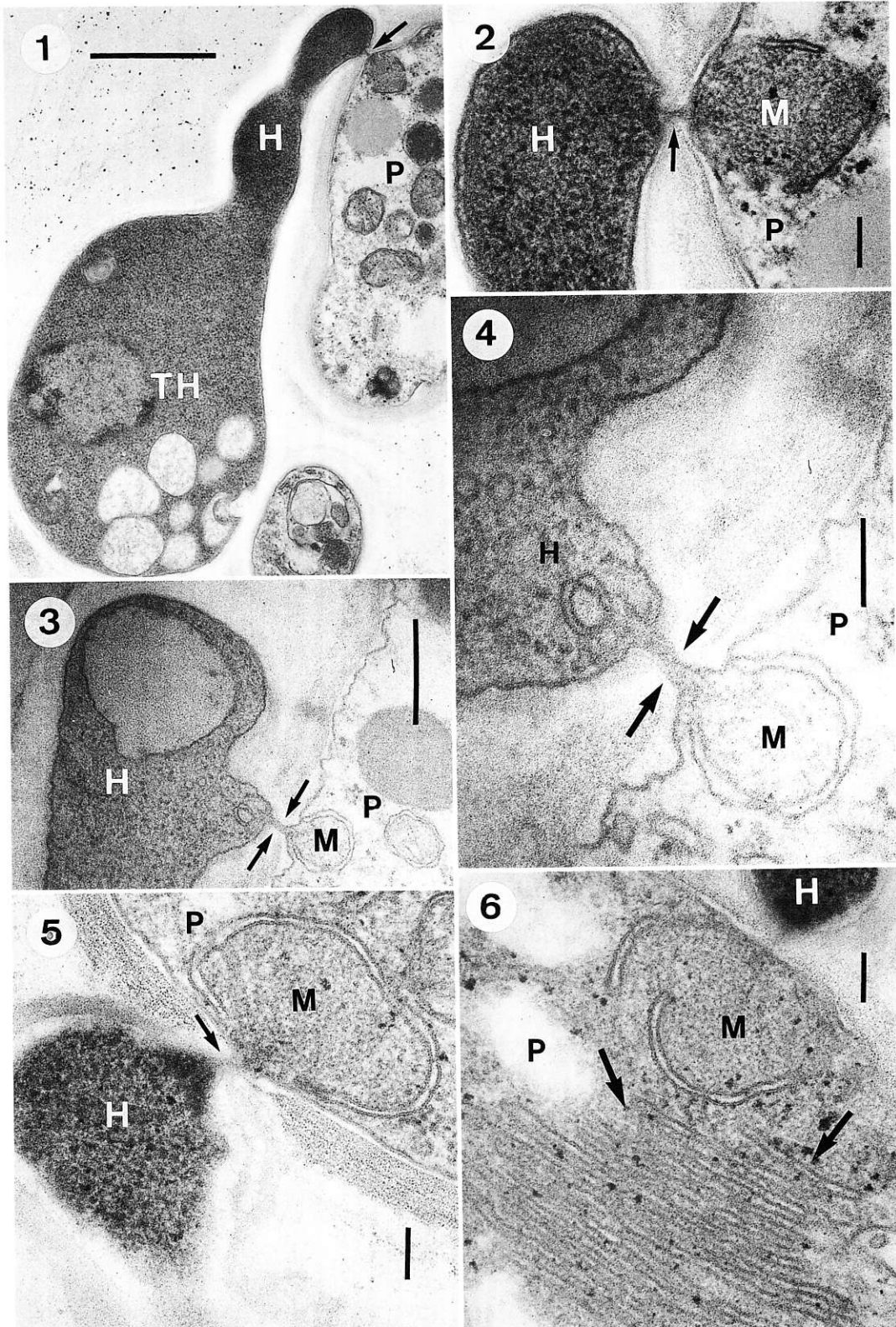
FIGS. 1–6.   Transmission electron micrographs of interaction stages between *Tremella mesenterica* and the host *Peniophora laeta*. 1, 2. Tremelloid haustorial cell (TH) with haustorial filament (H) attached to host cell (P). Note median section of micropore (arrow) between the apex of the haustorial filament and the host cell. 2. The micropore of FIG. 1 at higher magnification. M, pore domain with ER cisterna. In FIG. 1, bar = 1 µm; in FIG. 2, bar = 0.1 µm. 3, 4. Median section through a micropore. The arrows indicate the probably continuous plasma membrane through the pore. Note that the pore domain (M) at the host side (P) is delimited by a curved ER cisterna. H, haustorial filament. 4. The micropore of FIG. 3 at higher

the gelatinous part. *Tremella mycophaga* G. W. Martin grows on *Aleurodiscus amorphus* (Fr.) Schroet. The fructifications are pulvinate, discoid and can cover the hymenium of the host by confluence (Martin, 1940).

We present new data on the parasitic interaction of *T. mesenterica, T. encephala* and *T. mycophaga*. Further, the host range of *T. mesenterica* is tested with *Peniophora laeta, P. incarnata, Phlebia radiata* Fr., *Schizopora paradoxa* (Schrad. : Fr.) Donk, *Stereum hirsutum* (Willd. : Fr.) Gray and *Diatrype stigma* (Hoffm. : Fr.) Fr.

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, 20 Nov. 1989, *W. Zugmaier 97, 158*; Stuttgart, Haldenwald near Sonnenberg, on *Carpinus betulus,* 15 Sept. 1988, *W. Zugmaier 117*; Stuttgart, Schwälblesklinge near Sonnenberg, 5 May 1989, *W. Zugmaier 153. Tremella encephala.* GERMANY. BADEN-WÜRTTEMBERG: Kirnbachtal near Tübingen, on *Pinus sylvestris,* 20 Oct. 1988, *W. Zugmaier 134. Tremella mycophaga.* GERMANY. BADEN-WÜRTTEMBERG: Schwarzwald near Pfalzgrafenweiler, on *Aleurodiscus amorphus* on *Abies alba,* 14 Oct. 1988, *W. Zugmaier 133. Peniophora laeta.* GERMANY. BADEN-WÜRTTEMBERG: Stuttgart, Haldenwald near Sonnenberg, on *Carpinus betulus,* 5 May 1989, *W. Zugmaier 154. Peniophora incarnata.* GERMANY. BADEN-WÜRTTEMBERG: Stuttgart, Schwälblesklinge near Sonnenberg, on *Fagus sylvatica* L., 10 May 1989, *W. Zugmaier 155. Phlebia radiata.* GERMANY. BADEN-WÜRTTEMBERG: Stuttgart, Schwälblesklinge near Sonnenberg, on *Fagus sylvatica,* 10 Sept. 1989, *W. Zugmaier 157. Schizopora paradoxa.* GERMANY. BADEN-WÜRTTEMBERG: Goldersbachtal near Tübingen-Bebenhausen, on *Alnus glutinosa* (L.) Gaert., 21 Oct. 1989, *E. Langer 2096. Stereum hirsutum.* GERMANY. BADEN-WÜRTTEMBERG: Schönbuch, Bromberg, on *Quercus robur* L., 15 Jan. 1989, *W. Zugmaier 142. Diatrype stigma.* GERMANY. BADEN-WÜRTTEMBERG: Stuttgart, Haldenwald near Sonnenberg, on *Fagus sylvatica,* 12 Sept. 1989, *W. Zugmaier 156.*

Cultures of *Tremella mesenterica, Peniophora laeta, P. incarnata, Phlebia radiata, Schizopora paradoxa, Stereum hirsutum* and *Diatrype stigma* were grown on malt yeast peptone agar (MYP; Bandoni, 1972). To obtain mono-spore cultures of *T. mesenterica,* a small hymenal part of the basidiocarp was attached to the inside of a petri plate lid, which was then placed over the medium. When basidiospores could be observed on the medium, the lid was moved stepwise and finally replaced by a sterile lid (Brough, 1974). After a few days, yeast colonies from single spores were visible in a dissecting microscope and individually transferred to new plates. Mycelium of *T. mesenterica* was obtained by crossing monospore cultures from different basidiomata collected from *Carpinus betulus.* To determine the host spectrum, inocula 2–4 mm in diam of the potential fungal hosts were placed near the margin of a colony of *T. mesenterica* in a petri plate with MYP medium. The same culture of *T. mesenterica* was used for all experiments. Desired mixed stages were selected under the light microscope and fixed for electron microscopy.

Living fungi and semithin sections of embedded material were examined with phase contrast optics. For transmission electron microscopy, field 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 pre-stained in 1% uranyl 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 min, and washed again with distilled water. The thin sections were examined with a Zeiss EM 109 transmission electron microscope at 80 kV.

RESULTS

*Observations from field-collected material.—*Fructifications of *Tremella mesenterica* growing on *Carpinus betulus* adjacent to *Peniophora laeta* were found several times. Under optimal wet and cool atmospheric conditions, orange accumulations of *T. mesenterica* hyphae with conidia and very young fruitbodies were observed

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magnification. In FIG. 3, bar = 0.5 µm; in FIG. 4, bar = 0.1 µm. 5. Apex of a haustorial filament (H) with median section of micropore. Electron-opaque material is located in the haustorial apex and extends through the micropore. The pore domain (M) at the host side (P) is delimited by a curved ER cisterna. Bar = 0.1 µm. 6. Apex of a haustorial filament (H) with sectioned micropore attached to host cell (P). The pore domain (M) at the host side is surrounded by curved ER-cisternae. Note adjacent stacked ER-cisternae (arrows). Bar = 0.1 µm.
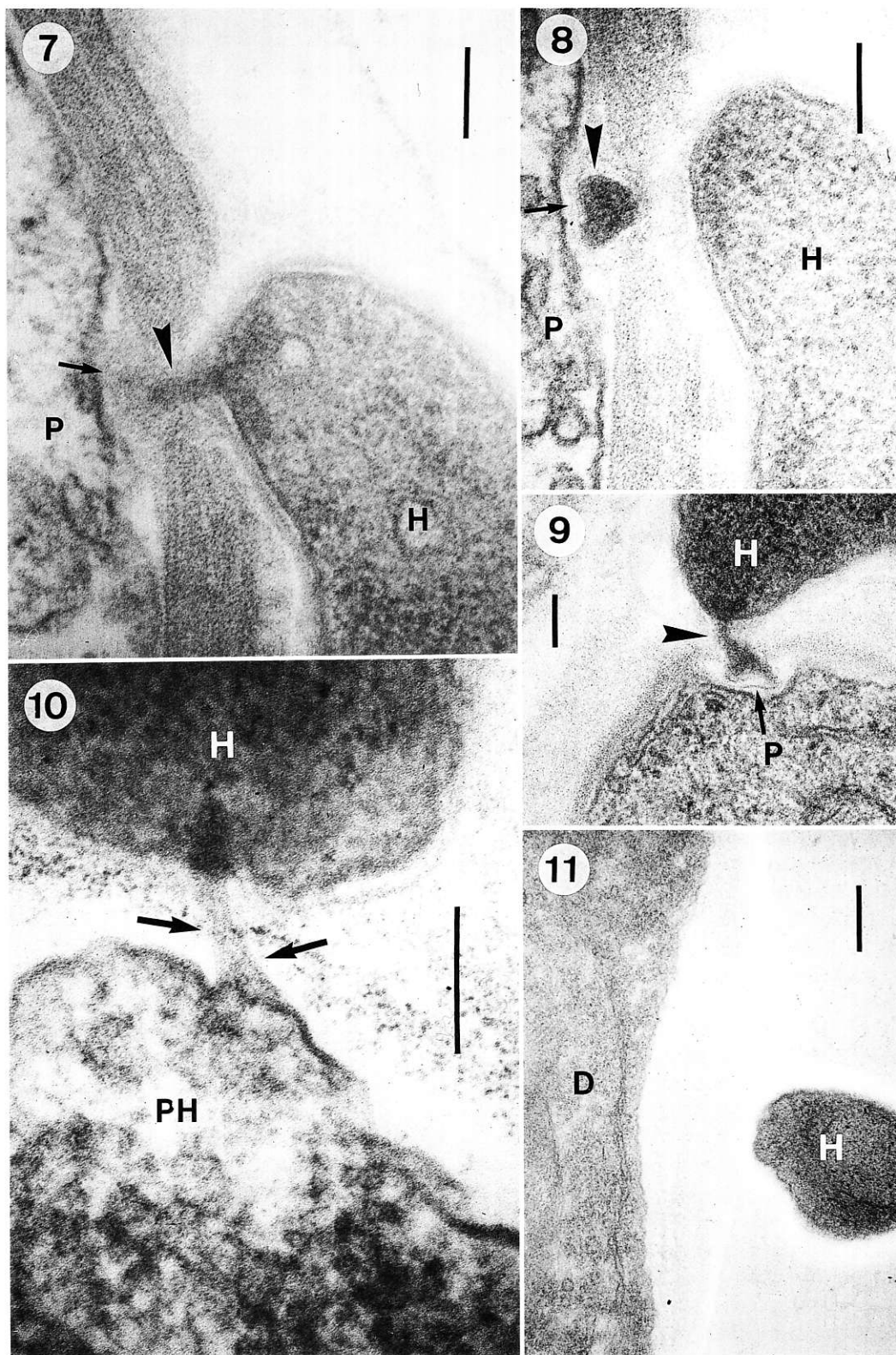
FIGS. 7–11. Transmission electron micrographs of interaction stages of *Tremella mesenterica*. 7, 8. Adjacent serial sections. Haustorial filament (H) with presumed old micropore (arrowhead) penetrating the cell wall of the host *Peniophora laeta* (P). The host plasma membrane is folded back adjacent to the presumed old micropore and a thin secondary wall layer has developed (arrow). Bar = 0.1 μm. 9. A presumed original micropore (arrowhead) is branched terminally. Note the thin secondary host wall (arrow). P, cell of *Peniophora laeta*. Bar = 0.1 μm. 10. Median section through a micropore between a

on *P. laeta*. In the rotting *Carpinus betulus* tissue and in fructifications of *P. laeta*, hyphae of *T. mesenterica* grew between host hyphae. The hyphae of *T. mesenterica* possessed numerous tremelloid haustorial cells and often the haustorial filaments were attached to host cells (FIG. 1), even in the zone of rotting *Carpinus betulus* tissue. In the white core of the basidiomata, hyphae of *Stereum sanguinolentum* were mixed with hyphae of *Tremella encephala* and many haustorial filaments were attached to host hyphae (FIG. 14). The hymenium of *Aleurodiscus amorphus* was the zone of interactions with many haustorial filaments of *Tremella mycophaga* attached to host cells (FIG. 12).

Since the host interactions of *T. mesenterica*, *T. encephala* and *T. mycophaga* are rather similar, they need not be described separately. In the initial stages of a parasitic interaction, a haustorial filament invaded the outer cell wall layers of a host cell (FIGS. 1–3, 5, 12, 14). A spur-like projection devoid of cell wall developed from the haustorial apex and penetrated the host wall (not illustrated). A single micropore finally connected the cytoplasm of the haustorial filament with that of the host cell (FIGS. 1–5, 13–16). The diam of the intermembrane space within the micropore was 12–15 nm (FIGS. 2–5, 13, 15, 16). Only under optimal sectioning conditions was it possible to observe the continuation of the plasma membrane through the micropore (FIGS. 3, 4, 15, 16). Sometimes the plasma membrane was slightly retracted on the pore openings (FIGS. 2, 13, 15, 16). Thus, occasionally, micropores had the appearance of simple dolipores (FIG. 15). Often electron-opaque material was located in the apex of the haustorial filament and apparently extended through the micropore into the host cell (compare FIGS. 5 and 13). In the interaction zone between *T. mesenterica* and *Peniophora laeta* curved cisternae of smooth endoplasmic reticulum were associated with the orifices of the micropore at the host side (FIGS. 2–6). These configurations of ER were observed already at the stage of haustorial penetration through the host cell wall prior to the formation of the micropore. Adjacent stacked ER cisternae were sometimes observed (FIG. 6). In all *Tremella* species studied stages were observed in which the host plasma membrane was invaginated and the presumed original micropore was overgrown by a thin secondary host wall layer (FIGS. 7–9). In some of these stages, the presumed original micropore was inflated or branched terminally (FIG. 9).

Cultural experiments with Tremella mesenterica.—To test the potential host spectrum of *T. mesenterica*, one mycelial strain of *T. mesenterica* was cultivated together with *Peniophora laeta*, *P. incarnata*, *Phlebia radiata*, *Schizopora paradoxa*, *Stereum hirsutum* and *Diatrype stigma*. Numerous haustorial filaments were attached to cells of all tested host fungi. Micropores with direct cytoplasm-cytoplasm connections were present in interactions of *T. mesenterica* with *P. laeta*, *P. incarnata* and *P. radiata* (FIG. 10). Stages in which the micropore was closed by a secondary wall layer at the host side were also observed. Thus, the interaction type was the same in vitro as in vivo. The pore domain at the host side, however, was delimited by curved ER cisternae only in *P. laeta* and *P. incarnata*.

In the interactions of *T. mesenterica* with *Schizopora paradoxa*, *Stereum hirsutum* and *Diatrype stigma*, a continuous micropore between haustorial filament and host cell could not be found. An invagination of the host plasma membrane and a prominent wall thickening of the host cell wall adjacent to the attached haustorial filament was always present (FIG. 11).
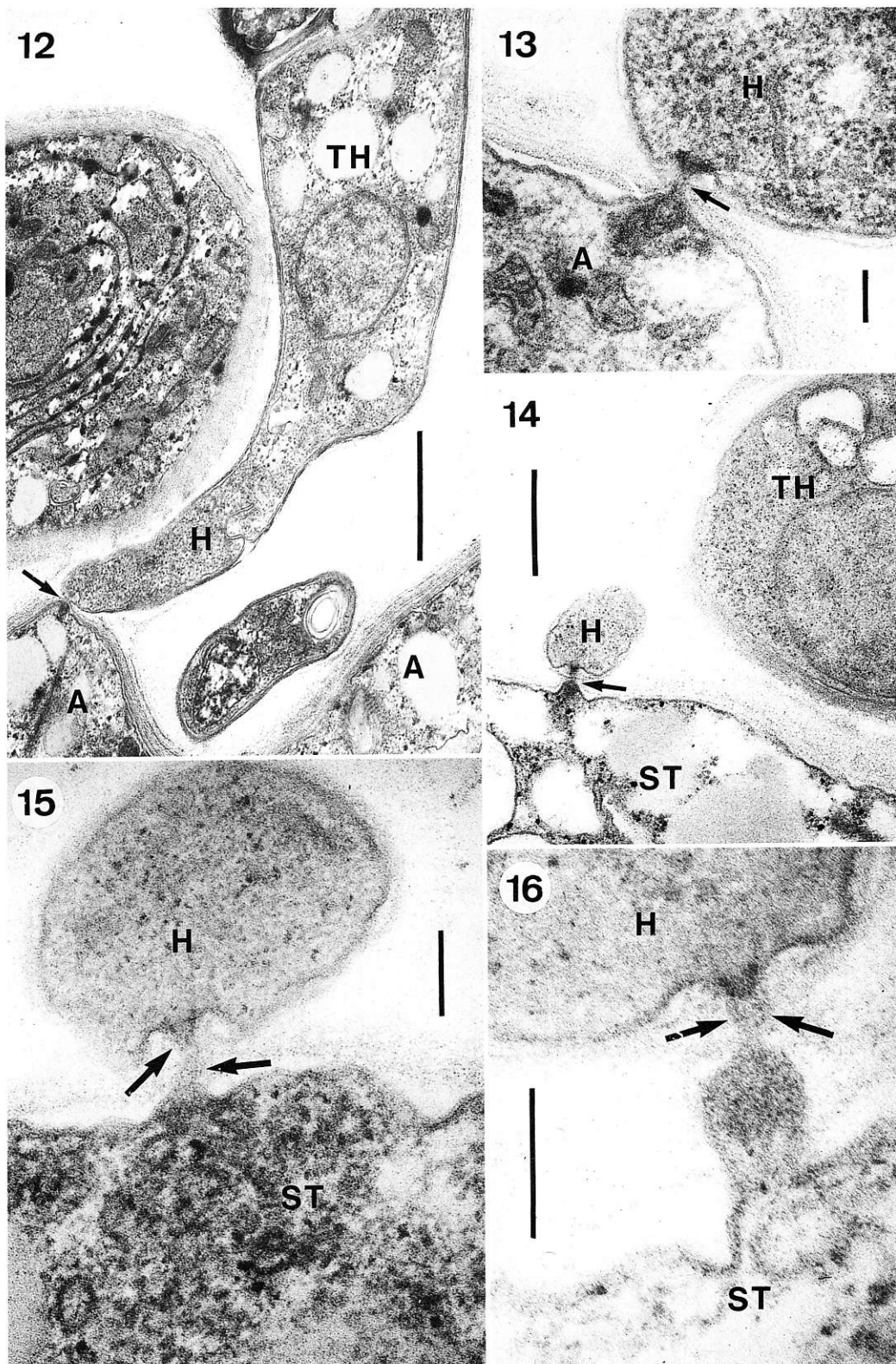
DISCUSSION

Micropore.—It is clear from our study that more than simple contact is involved in the mycoparasitism of *Tremella mesenterica*, *T. encephala* and *T. mycophaga*. Distinctive micropores with direct cytoplasmic contact are formed at the interface between the host and parasite. The type of host-parasite interface in the three *Tremella* species studied is essentially identical to that of *Tetragoniomyces uliginosus* (Bauer and Oberwinkler, 1990a). The similarities include the development and dimension of the micropore. As in *T. uliginosus*, an electron-opaque matrix is often present in the cytoplasm within the micropore in the three species of *Tremella* examined. Curved cisternae of ER are present in the host cytoplasm at the interface between *T. mesenterica* and host cells of *Peniophora laeta* and *Peniophora incarnata*. Since such cisternae are lacking at the interface between *T. mesenterica* and *Phlebia radiata*, their presence seems to be host dependent. *Peniophora laeta* and *P. incarnata* were grouped by Eriksson et al. (1978) in the subgenus *Gloeopeniophora*. Perhaps other species of this taxon react similarly in the interaction with *T. mesenterica*.

Micropore and nonmicropore interactions.—Tremelloid haustorial cells of *Tremella mesenterica* were attached

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haustorial filament of *T. mesenterica* (H) and a hyphal cell of *Phlebia radiata* (PH) showing the probably continous plasma membrane through the pore (arrows). Bar = 0.1 μm. 11. Haustorial filament of *T. mesenterica* (H) attached to a hyphal cell of *Diatrype stigma* (D). Note the prominent wall thickening adjacent to the haustorial filament. Bar = 0.2 μm.

FIGS. 12–16.   Transmission electron micrographs of *Tremella mycophaga* (FIGS. 12, 13) and *T. encephala* (FIGS. 14–16). 12. Haustorial filament (H) of *T. mycophaga* attached (arrow) to a hyphal cell of *Aleurodiscus amorphus* (A). TH, haustorial cell. Bar = 1 μm. 13. A micropore (arrow) connects the apex of a haustorial filament (H) of *T. mycophaga* and a hyphal cell of *A. amorphus* (A). Electron-dense material is situated in the pore and adjacent cytoplasmatic areas. Bar = 0.1 μm. 14. Haustorial filament (H) of *Tremella encephala* attached (arrow) to a hyphal cell of *Stereum sanguinolentum* (ST). TH, haustorial cell. Bar

to hyphae of all tested host fungi. A single micropore is present, however, only at the interface between the tremelloid haustorial cell of *T. mesenterica* and host cells of *Peniophora laeta*, *P. incarnata* and *Phlebia radiata*. On the other hand, prominent wall thickenings occurred adjacent to the attached haustorial filaments by *Schizopora paradoxa*, *Stereum hirsutum* and the ascomycete *Diatrype stigma*. These results may indicate that species of *Peniophora* and related taxa (see Eriksson et al., 1978, 1981) are at least potential hosts of *T. mesenterica*. Apparently *T. mesenterica* is capable of parasitizing several wood decaying fungi. Those resistant wood decaying species possibly are immune by virtue of their capacity to rapidly form secondary wall layers. The potential hosts of *T. mesenterica* and also the hosts of *T. encephala* and *T. mycophaga* are sometimes also able to stop or interrupt parasitic interactions by the formation of secondary wall layers.

Systematic implications.—Tremelloid haustorial cells are typical of many species of the Tremellales *sensu* Bandoni (1984), Carcinomycetaceae (Oberwinkler and Bandoni, 1982), and Filobasidiaceae (Olive, 1968; Kwon-Chung, 1976; Bandoni et al., 1991). Most of these species are dimorphic with dolipores and cupulate caps (Oberwinkler and Bandoni, 1982; Oberwinkler et al., 1984; Bandoni, 1987). The host-parasite interface of five species has been studied. At the interface between *Tremella mesenterica*, *T. encephala*, *T. mycophaga* and *Tetragoniomyces uliginosus* (Bauer and Oberwinkler, 1990a) with their hosts only a single micropore is known to occur. At the interface between *Christiansenia pallida* and the host several micropores have been detected (Bauer and Oberwinkler, 1990b). Otherwise the host-parasite interfaces in the species are similar. We interpret the "*Christiansenia* interaction type" as derived from the "*Tremella-Tetragoniomyces* interaction type" (Bauer and Oberwinkler, 1990b). Similar micropores at the host-parasite interface have not been reported in other basidiomycetes.

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= 0.5 μm. 15, 16. Median sections through micropores between haustorial filaments of *T. encephala* (H) and hyphal cells of *S. sanguinolentum* (ST). Note that the pore membrane (arrows) is continuous with the plasma membrane of both cells. Bars = 0.1 μm.

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