The Colacosomes: New Structures at the Host-parasite Interface of a Mycoparasitic Basidiomycete*

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Abstract

The ultrastructure of mycoparasitic interactions in heterobasidiomycetes is diverse. A previously unknown cell structure, involved in a specific parasitic interaction, is described and illustrated. Cells of the parasite *Platygloea peniophorae* (Auriculariales s.l., Basidiomycetes) which attach to host cells of *Hyphoderma praetermissum* (Aphyllophorales, Basidiomycetes) form distinct marginal vesicular bodies with electron-opaque cores and electron transparent sheaths. Finally, the vesicular content projects through the cell wall of the parasite and then interacts with the cell wall of the host. Ontogenetic stages are described and illustrated in detail.

Key words

Platygloea peniophorae, heterobasidiomycetes, host-parasite interaction, mycoparasitism, colacosomes.

Introduction

The ultrastructure of the host-parasite interaction in heterobasidiomycetous mycoparasites has only been studied in a few species. In *Tetragoniomyces uliginosus* (Oberwinkler and Bandoni, 1981) and *Carcinomyces effibulatus* (Oberwinkler and Bandoni, 1982) haustorial filaments attach to their host cells, *Rhizoctonia* sp., and *Collybia dryophila*, respectively. *Christiansenia pallida* haustoria penetrate the host cells of *Phanerochaete cremea* (Oberwinkler and Bandoni, 1982; Oberwinkler et al., 1984). Another type of host-parasite interaction has recently been described from *Phragmoxenidium mycophilium*, a basidiomycetous parasite of *Uthatobasidium fusisporum* (Oberwinkler et al., 1990). Micropores protrude through cell walls of the parasite and the host. Cytoplasmic connections through the pores were not found. However, a direct cytoplasm-cytoplasm connection between host and parasite cell is known from *Tetragoniomyces uliginosus* (Bauer and Oberwinkler, 1990).

In this study, we describe the ultrastructural characteristics of the interaction between the two basidiomycetous species *Platygloea peniophorae* and *Hyphoderma praetermissum*. A hitherto unknown mycoparasitic structure is documented.

Materials and Methods

The strain of *Platygloea peniophorae* Bourd. & Galz. used for the investigations reported here, was collected on *Hyphoderma praetermissum* (Karst.) Eriksss. & Strid in the Schönbuch near Tübingen, Baden-Württemberg, West Germany; (leg. F. Oberwinkler 36343, 24 November 1984). Living and untreated material of different developmental stages was studied with a Zeiss photoscope III, using phase optics and Nomarski's interference contrast optics. For transmission electron microscopy samples were 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 postfixed in 1 % osmium tetroxide in the same buffer for 2 hours in the dark, washed in distilled water, and prestained in 1 % uranyl acetate solution for 1 hour in the dark. After 5 washes in distilled water, the material was dehydrated in acetone, using 10 minute changes at 25 %, 50 %, 70 %, 95 % and 3 × 100 % acetone. The material was embedded in Spurr's resin (Spurr, 1969). Series of sections were cut on a Reichert ultramicrotome using a diamond knife, mounted on Formvar-coated single slot copper grids, stained with lead citrate (Reynolds, 1963) at room temperature for 3 to 5 minutes, and washed again with water. The thin sections were examined with a Zeiss EM 109 transmission electron microscope at 80 kV.

Results

Platygloea peniophorae grows internally in fructifications of *Hyphoderma praetermissum* and develops pulvinate to effused basidiocarps on the surface of host hymenia. Internal hyphal cells of the parasite, which are attached to host cells, form distinct vesicular bodies with electron-opaque cores and electron-transparent marginal regions (Figs. 1, 5–7, 12–13). For these bodies, the name colacosome (greek: colax-sycophant, parasite, and soma-body) is proposed. Colacosomes are developed by the parasite *Platygolea peniophorae* at the contact area between the parasite and the host fungus *Hyphoderma praetermissum* (Fig. 1). They are positioned at the inner surface of the parasite cell outside of the cytoplasm, but in-

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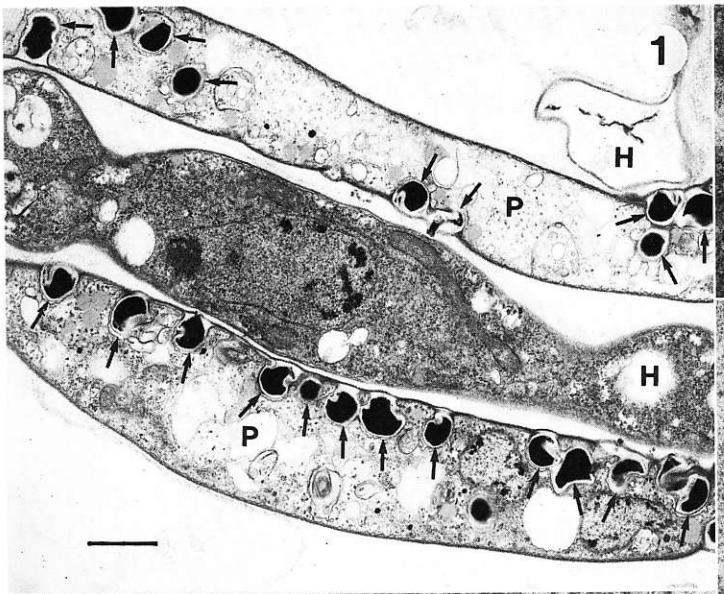
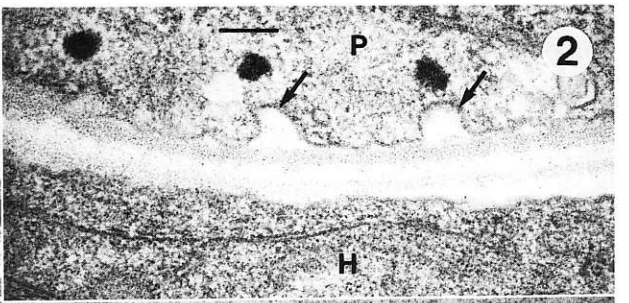
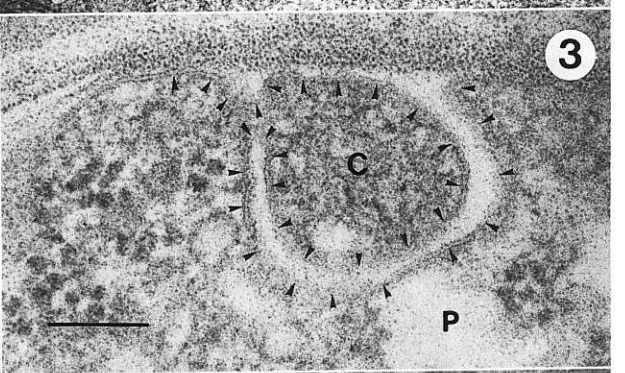
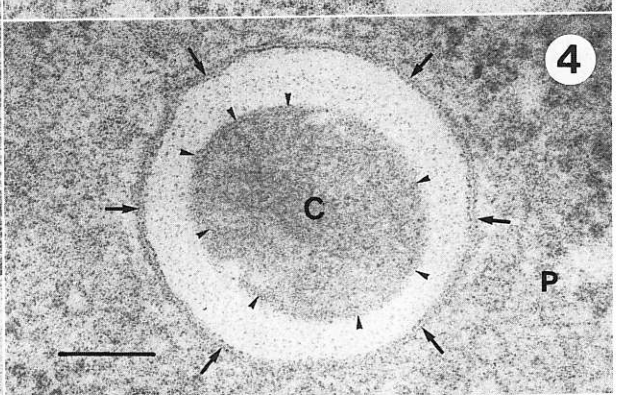
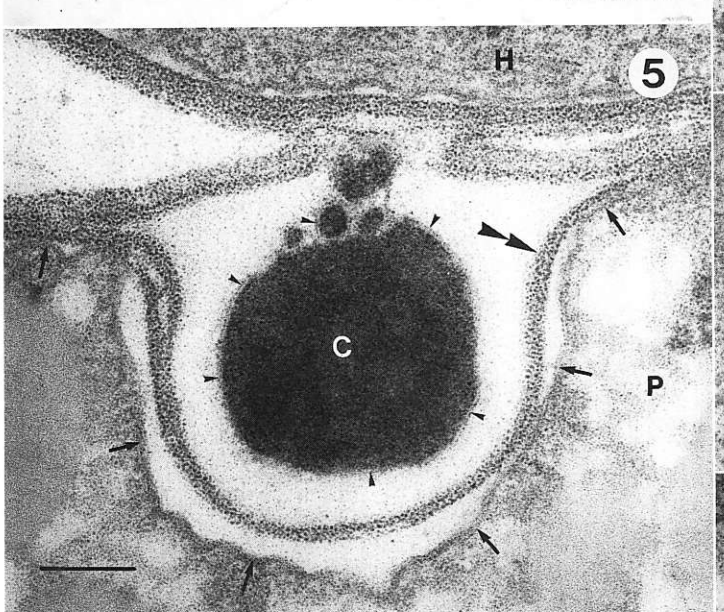
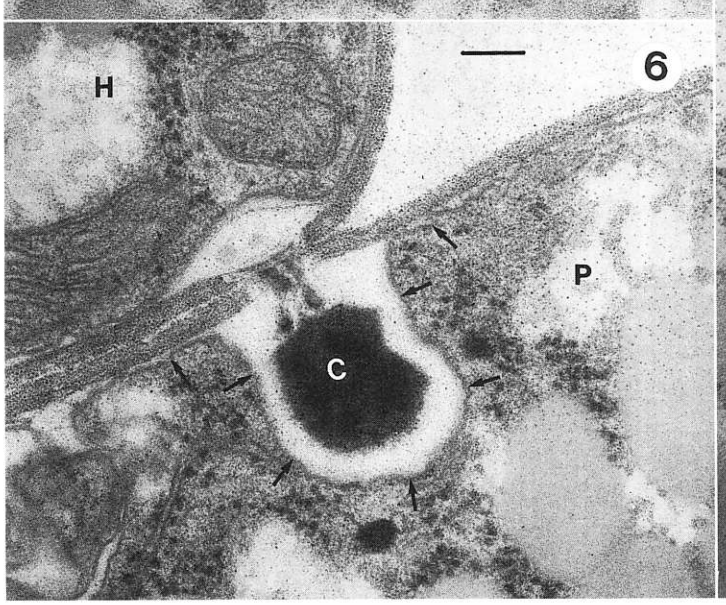


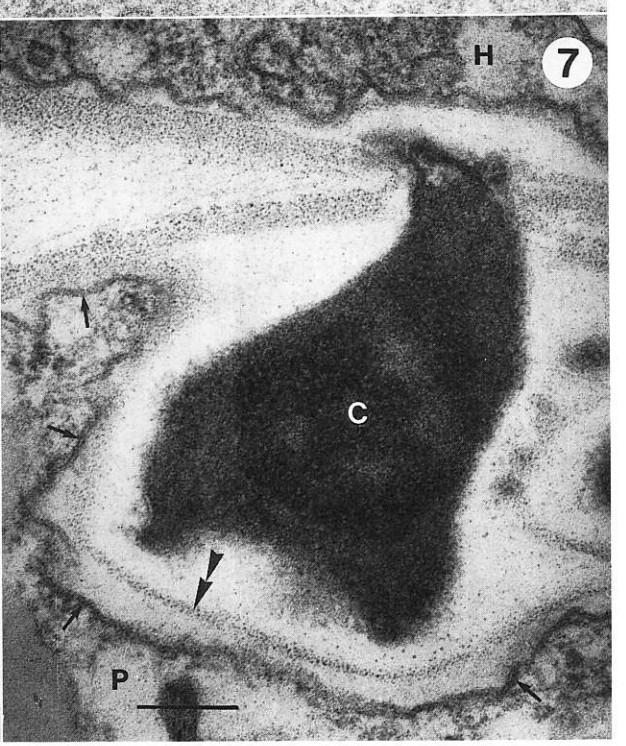












Figs. 1–7

side of the cell wall. Their shape is globular, subglobular or beaked (Figs. 1, 5–7, 12–13). The central part of the colacosome is electron-opaque, 0.3–0.4 µm in diameter, enclosed by a membrane (Fig. 5) and surrounded by an electron-transparent, unstructured sheath of approximately 0.05 µm diameter. The colacosome is covered by the plasmalemma (Figs. 5–7, 12–13). A thin secondary cell wall layer is often present along the plasmalemma covering the colacosome (Figs. 5, 7, 12–13). The presence or absence of this secondary cell wall layer could be correlated with developmental stages. The electron-opaque content projects through the cell wall of the parasite and then interacts with the cell wall of the host organism (Figs. 5–7, 12–13).

Colacosomes develop in great numbers close together (Fig. 1). It is evident that, in most cases, hyphae possessing colacosomes and their host hyphae lie side by side, closely attached to one another for relatively long distances (Fig. 1). Furthermore, the host hyphae often form one or two spirals around the colacosome-possessing hyphae (not illustrated).

Early developmental stages of colacosomes are hard to find. Obviously, the colacosomes develop rapidly. Initial stages were found in contact areas of hyphal tips of the parasite with host cells or vice versa. However, they are never located directly at the tips of the parasite hyphae. They may be discovered directly behind the "Spitzenkörper" of actively growing hyphae (not illustrated). Initially, the plasmalemma of the parasite is folded into the cytoplasm (Figs. 2, 8), then recurves (Figs. 3, 9), and finally fuses with itself at a distance of 0.2–0.3 µm from the original outgrowth (Figs. 3, 10). Thus, a more or less globose compartment is formed (Figs. 4, 10–11). Consequently, it is surrounded by a membrane derived from the plasmalemma (Figs. 4, 10–11, compare Fig. 5). The globose compartment is now separated from the cytoplasm by an electron-transparent, intermembranaceous space (Figs. 4, 10–11). After separation from the cytoplasm, the vesicular core becomes homogeneous (Fig. 4) and finally more and more electron-opaque (Figs. 5–7, 11–13). Simultaneously, the intermembranaceous space between the central part of the colacosome and the cytoplasm increases slightly in thickness (compare Fig. 3 with Fig. 4, and Fig. 10 with Fig. 11). The plasmalemma covers the colacosome (Figs. 4–7, 10–13). Cell to cell interaction starts with intrusion of electron-opaque core material of the colacosome into the cell wall of the parasite (Figs. 5, 12). The cell wall close to the intrusion peg becomes electron-transparent and indistinct in substructure (Fig. 5). Intrusion then continues through the closely attached cell wall of the host into an electron-transparent protuberance formed between the cell wall and the plasmalemma of the host (Figs. 6–7, 12–13). We assume that the stage illustrated in Fig. 7 really reflects the final development of the colacosome, because we have not observed any further stages in older hyphae.

Figs. 1–7 Transmission electron micrographs of interaction stages between the parasite *Platygloea peniophorae* (P) and the host fungus *Hyphoderma praetermissum* (H). Illustrations of colacosomes are arranged in a series of inferred developmental stages (Figs. 2–7).

Fig. 1 Colacosomes (arrows) are located at the contact zone between the parasite (P) and the host (H). Magnification bar: 1 µm.

Fig. 2 Initial stage of colacosome development with invaginating plasmalemma (arrows). Note neighboring electron-opaque bodies. Magnification bar: 0.1 µm.

Fig. 3 Developmental stage prior to fusion of the plasmalemma of the parasite (arrowheads) with itself and delimitation of the central part (C) of the colacosome. Magnification bar: 0.1 µm.

Fig. 4 Transverse section of a young developmental stage with a semi-transparent central part (C) separated from the plasmalemma of the parasite (arrows) by an electron-transparent zone. A tripartite membrane (arrowheads), derived from the plasmalemma, encloses the central part (C) of the colacosome. Magnification bar: 0.1 µm.

Fig. 5 The electron-opaque content of the colacosome intrudes into the cell wall of the parasite. Note the tripartite membrane (arrowheads) around the central part (C). The colacosome is covered by a thin secondary cell wall layer (double arrowhead) and the plasmalemma (arrows) of the parasite. Magnification bar: 0.1 µm.

Fig. 6 Longitudinal section through a colacosome. The electron-opaque material of the central part (C) interacts with the host cell wall. Arrows mark the plasmalemma of the parasite. Note the invagination of the host plasmalemma opposite the colacosome. Magnification bar: 0.1 µm.

Fig. 7 Final stage of host-parasite interaction with the content of the electron-opaque central part of the colacosome (C) penetrating the host cell wall. The colacosome is still covered by a thin secondary cell wall layer (double arrowhead) and the plasmalemma of the parasite (arrows). Magnification bar: 0.1 µm.

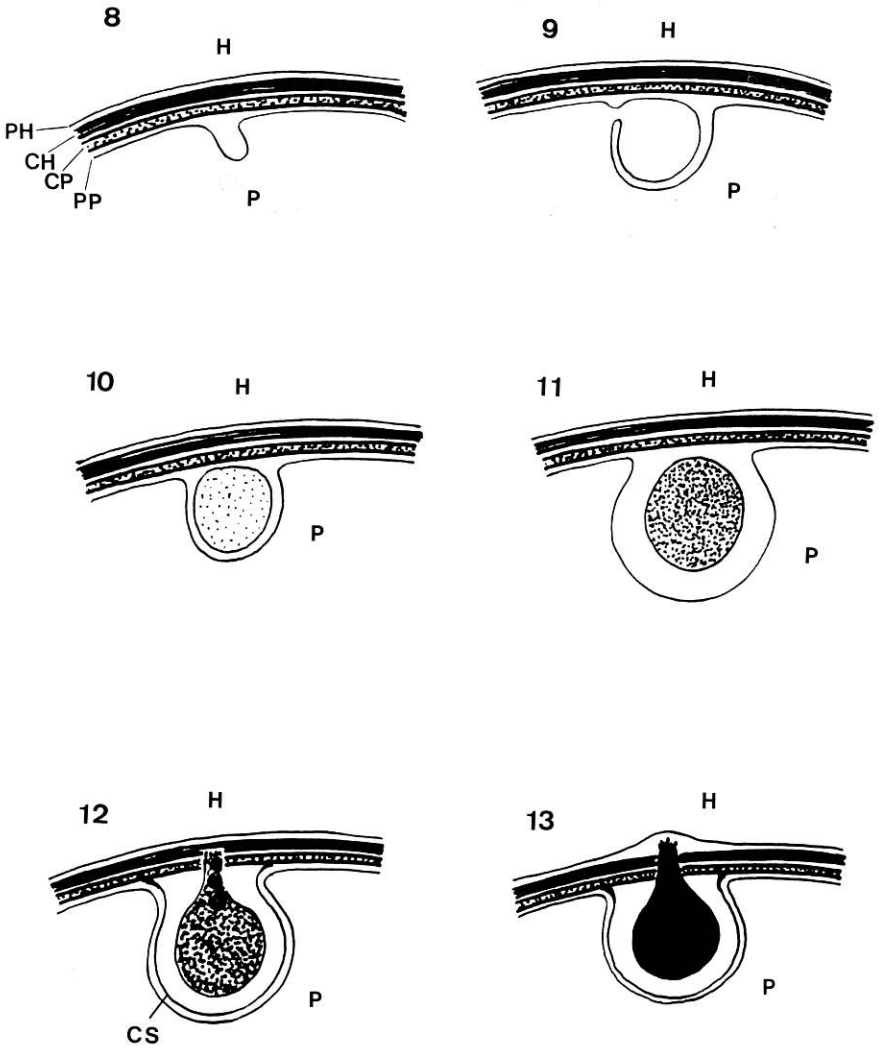
Discussion

Mycoparasitism is widespread in heterobasidiomycetes (Bandoni, 1984; Oberwinkler and Bandoni, 1981, 1982; Oberwinkler et al., 1984, 1990). The majority of the species included in the Auriculariales s.l. are parasites of fungi, mosses, ferns, and flowering plants (Bandoni, 1984). Among auricularioid taxa, the genera *Cystobasidium, Mycogloea, Platygloea* and *Phragmoxenidium* contain mycoparasitic species (Lagerheim, 1898; McNabb, 1965; Bandoni, 1956, 1984; Olive, 1951; Oberwinkler et al., 1990). *Platygloea peniophorae* grows in and on fructifications of basidiomycetes, mainly *Hyphoderma praetermissum* (Corticiaceae), but it is also reported as a parasite from species of the genera *Dacrymyces, Odontia, Peniophora*, and *Poria*. It is unclear whether all of these reports definitely refer to *P. peniophorae* s.str. (Bandoni, 1956).

The host-parasite interaction of *Platygloea peniophorae* and *Hyphoderma praetermissum* has not been studied by previous workers. Several stages in the development of parasitic interaction can be observed:

1. contact of parasite and host cell,
2. development of colacosomes at the contact area, and
3. intrusion of core material of the colacosome into the host cell wall.

Platygloea peniophorae represents a mycoparasite, lacking intracellular hyphae or haustoria, as in *Tetragoniomyces uliginosus* (Oberwinkler and Bandoni, 1981; Bauer and Oberwinkler, 1990) and *Carcinomyces effibulatus* (Oberwinkler and Bandoni, 1982). In contrast, haustoria of *Christiansenia pallida* penetrate the host cells



Figs. 8–13 Diagram of colacosome development, based on serial sections, and with reference to the original transmission electron micrographs of this article. Abbreviations and symbols: CH = cell wall of the host; CP = cell wall of the parasite; CS = secondary cell wall layer: H = cell of the host fungus *Hyphoderma praetermissum*; P = cell of the parasite *Platygloea peniophorae*; PH = plasmalemma of the host; PP = plasmalemma of the parasite.

Fig. 8 Initial stage of invagination of the plasmalemma of the parasite (compare Fig. 2).

Fig. 9 The plasmalemma of the parasite recurves (compare Fig. 3).

Fig. 10 Delimitation of the young colacosome from the cytoplasm (compare Figs. 3 and 4).

Fig. 11 The central part of the colacosome becomes homogeneous (compare Fig. 4) and more and more electron-opaque (without original illustration). The electron-transparent sheath of the colacosome increases in thickness (compare Fig. 4).

Fig. 12 The electron-opaque core material penetrates the cell wall of the parasite and begins to intrude into the cell wall of the host (compare Figs. 5 and 6).

Fig. 13 Final developmental stage with colacosome penetration through host cell wall (compare Fig. 7).

(Oberwinkler and Bandoni, 1982; Oberwinkler et al., 1984). It seems reasonable that haustorial production is the best way for the parasite to benefit from host cell metabolites. The development of haustoria is accompanied by an increase of the contact surface between the parasite and its host. But obviously it is not necessary for a parasite to invade its host cells. The hypothesis that biotrophic contact mycoparasites parasitize their respective hosts by simple contact and induce increased nutrient absorption by causing an increase in the permeability of the host plasma membrane has been proposed by some workers (Barnett and Binder, 1973; Shigo, 1960; Whaley and Barnett, 1963). The evidence for such a hypothesis has been based on the mode of parasitism observed by light microscopy and on nutritional studies of the various contact mycoparasites. From this study, it is clear that more than simple contact and plasmalemma permeability changes are involved in the parasitism of *Platygloea peniophorae*. A specialized parasitic structure, the colacosome, is produced by the parasite. As described above, the hyphae possessing colacosomes are attached over a relatively long distance to their host hyphae. In addition, the host hyphae are often slightly coiled around those hyphae. This situation may be explained as follows: Initially the parasite hypha grows loosely in the host fructifications. After a first, probably accidental contact of the hypha with a host hypha, colacosomes develop rapidly and in great numbers. The electron-opaque content of the colacosomes penetrates the host cell wall. Thus, the colacosomes combine both cells and the first contact remains stable. Furthermore, if the parasite and/or the host hypha continue to grow, additional colacosomes are rapidly developed. Consequently, the number of connections between both organisms is continually increased and both are forced to grow in close contact to each other. It follows then that the development of colacosomes is accompanied by an increase of the host-parasite interface. In this sense, the colacosomes could serve as connecting agents. It is unclear from the present data, however, whether or not the colacosomes are involved in host-parasite metabolic functions as no specific attempts to identify these were made. But the change in the electron density of the core material of the colacosome suggests that an alteration of the chemical composition occurs after separation of the colacosome from the cytoplasm. Furthermore, the penetration of the parasite and host cell wall appears to be enzymatic since both cell walls are not distorted at the site of penetration.

The parasitic interaction of *Platygloea peniophorae* with *Hyphoderma praetermissum* represents a hitherto unique type. Colacosome-like structures have not previously been observed. Another, so far unique, type of mycoparasitic interaction has recently been described for *Tetragoniomyces uliginosus* (Bauer and Oberwinkler, 1990). A single micropore is produced between the haustorial filament and the host cell. The protoplasts of both, the haustorium and the host cell, are connected by the micropore. The interaction types of *Platygloea peniophorae* and *Tetragoniomyces uliginosus* are not comparable. Obviously, mycoparasitic basidiomycetes interact quite differently with their hosts.

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References

Bandoni, R. J. – A preliminary survey of the genus *Platygloea*. Mycologia 48 (1956), 821–840.

Bandoni, R. J. – The Tremellales and Auriculariales: an alternative classification. Trans. Mycol. Soc. Japan 25 (1984), 489–530.

Barnett, H. L. and Binder, F. L. – The fungal host-parasite relationship. Ann. Rev. Phytopathol. 11 (1973), 273–292.

Bauer, R. and Oberwinkler, F. – Direct cytoplasm-cytoplasm connection: an unusual host-parasite interaction of the tremelloid mycoparasite *Tetragoniomyces uliginosus*. Protoplasma (1990), in press.

Lagerheim, G. – Mykologische Studien. I. Beiträge zur Kenntnis der parasitischen Pilze, 1–3. [3. *Iola (Cystobasidium) lasioboli* nov. spec.]. Bihang Till K. Sv. Vet. – Akad. Handl. Afd. III. 24 (1898), 15–21.

McNabb, R. F. R. – Some auriculariaceous fungi from the British Isles. Trans. Brit. Myc. Soc. 48 (1965), 187–192.

Oberwinkler, F. and Bandoni, R. J. – *Tetragoniomyces* gen. nov. and Tetragoniomycetaceae fam. nov. (Tremellales). Canad. J. Bot. 59 (1981), 1034–1040.

Oberwinkler, F. and Bandoni, R. J. – Carcinomycetaceae: a new family in the Heterobasidiomycetes. Nord. J. Bot. 2 (1982), 501–516.

Oberwinkler, F., Bandoni, R. J., Bauer, R., Deml, G., and Kisimova-Horovitz, L. – The life-history of *Christiansenia pallida*, a dimorphic, mycoparasitic heterobasidiomycete. Mycologia 76 (1984), 9–22.

Oberwinkler, F., Bauer, R., and Schneller, J. – *Phragmoxenidium mycophilum*, an unusual mycoparasitic heterobasidiomycete. Syst. Appl. Microbiol. 13 (1990), 186–191.

Olive, L. S. – New or noteworthy Tremellales from the southern Appalachians. Bull. Torrey Bot. Club 78 (1951), 103–112.

Reynolds, E. S. – The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17 (1963), 208–212.

Shigo, A. L. – Parasitism of *Gonatobotryum fuscum* on species of *Ceratocystis*. Mycologia 52 (1960), 584–598.

Spurr, A. R. – A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26 (1969), 31–43.

Whaley, J. W. and Barnett, H. L. – Parasitism and nutrition of *Gonatobotrys* complex. Mycologia 55 (1963), 199–210.

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