

Phragmoxenidium mycophilum sp. nov., an Unusual Mycoparasitic Heterobasidiomycete*

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Summary

Descriptions are provided of new heterobasidiomycetous taxa, *Phragmoxenidiaceae*, *Phragmoxenidium*, and *Phragmoxenidium mycophilum*. The type species is characterized by intrahymenial growth in fructification of the host *Uthatabasidium fusisporum*, without developing a basidiocarp. Hyphae and basidia of the parasite attach to host cells and develop a unique host-parasite-interaction. Hyphae are simple septate, the septal dolipores lacking parenthesomes. Transversely septate basidia are 4-celled, each cell provided with one sterigma. Basidiospores germinate by secondary spores or globose microconidia. The interphase spindle pole body resembles the *Dacrymyces*-type. The combination of taxonomically most important characters, i. e. septal pores, spindle pole bodies, and basidial type, is unique. Therefore, the family is only tentatively included in the *Tremellales*.

Key words: *Phragmoxenidium mycophilum* – *Uthatabasidium fusisporum* – *Phragmoxenidiaceae* – *Heterobasidiomycetes* – Mycoparasitism – Dolipore – Spindle pole body

Introduction

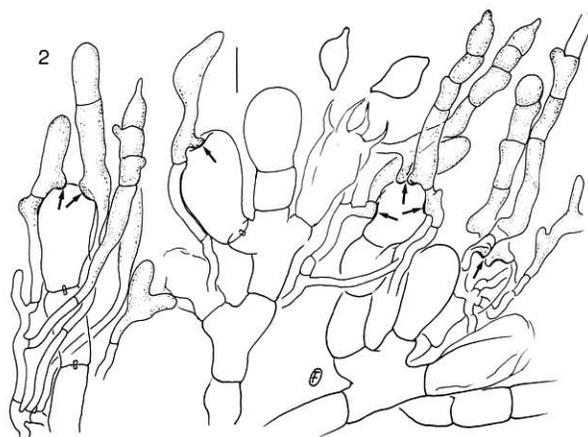
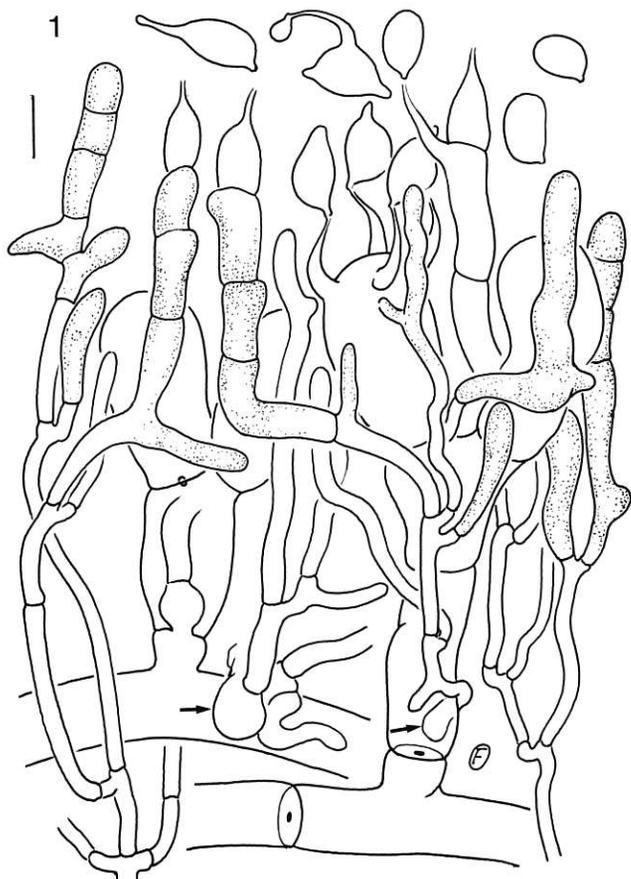
A recent collection of an unusual parasitic auricularioid heterobasidiomycete was identified as *Stypinella hypochnoides* von Höhnel (1905). In the original description, host and parasite were not recognized, and the material was considered to represent a single species. Therefore *Stypinella hypochnoides* is a *nomen confusum* which has to be replaced by a new name. Morphological and ultrastructural studies revealed a unique set of characteristics, justifying proposal of a new genus and family in the heterobasidiomycetes.

Materials and Methods

For descriptions and illustrations the following specimens were examined: *Dacrymyces stillatus* Nees: Fr., Deutschland, Baden-Württemberg, Tübingen, Schönbuch, Heubergertor, on *Abies alba* Mill., 470 m, leg. F. Oberwinkler 36415. *Stypinella hypochnoides* von Höhnel, Vorderer Sattelberg, Preßbaum, Wiener Wald, strongly decayed wood of *Fagus sylvatica* L., leg. F. von Höhnel, Oktober 1904; isotype, M. *Phragmoxenidium*

mycophilum Oberw. and Schneller, type, Switzerland, Küssnacht-Tobel below Allmend, on wood of *Fraxinus excelsior* L., 480 m, leg. J. Schneller, det. F. Oberwinkler. The type material is deposited in the Botanische Staatssammlung Munich (M), the isotype in Zürich (Z). Soaked material of the recent collection from Switzerland could be partly revived; these parts were used for light microscopy and transmission electron microscopy. Living and untreated material of different developmental stages was studied with a Zeiss photoscope III, using phase contrast 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% OsO₄ in the same buffer for 2 h in the dark, washed in distilled water, and stained 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 (1969) plastic. Series of sections were cut on a Reichert ultramicrotome using a diamond knife and, after mounting on Formvar coated single slot copper grids, stained with lead citrate (Reynolds, 1963) at room temperature for 3 to 5 min, and washed again with water. The thin sections were examined with a Zeiss EM 109 transmission electronmicroscope at 80 kV.

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Figs. 1, 2. Sections through fructifications of *Uthotobasidium fusisporum*, parasitized by *Phragmoxenidium mycophilum* with hyphae, basidia and basidiospores. *U. fusisporum* with broad hyphae, holobasidia and naviculate basidiospores, two germinating by repetition. *Ph. mycophilum* with small hyphae, transversely septate basidia and subglobose to ellipsoid basidiospores. Note tight attachments of host and parasite (arrows). Bars = 10 μm .

Results

Phragmoxenidium mycophilum lacks basidiocarps; it grows parasitically in the fructifications of *Uthotobasidium fusisporum* (Schroet.) Donk (Figs. 1, 2), a corticiaceous heterobasidiomycete. Hyphae of both species are clampless. They can be distinguished by hyphal diameter, those of the parasite measuring 2–4 μm , and of the host 8–15 μm . The septal pore in hyphae of the parasite is a dolipore without parentheses (Figs. 6–8). Faint striations are visible in the pore opening. Cystidia are lacking. Scattered and scarcely branched hyphidia occur. Basidia arise from terminal cells apically or laterally (Figs. 1, 2, 5). Quite often basidia are basally bent or curved. The mature basidium, (3)–5–8 \times 40–70 μm , is transversally 3-septate, the septa developing in the upper part of the meiosporangium. Basidial cells develop a conspicuous swelling on one side apically (Figs. 2, 5). These swellings predominantly develop into short, stout sterigmata (Fig. 5) with asymmetrically attached spores. Basidiospores are subglobose to ellipsoid in side view, 5–7 \times 9–11 μm , hyaline, inamyloid, thin-walled, smooth, germinating with secondary spores or microconidia (Figs. 3, 4, 5). In contrast, basidiospores of the host are naviculate (Figs. 1, 2) and taller (6–8 \times 11–15 μm).

The interphase spindle pole body (Fig. 13), ca. 0.5 μm long, has a conspicuous middle piece with oblique margins, each covered by a flattened, semi-subglobose body.

Hyphae and basidia of *P. mycophilum* strongly attach to hyphae and basidia of *U. fusisporum* (Figs. 1, 2, arrows). Quite often cell to cell interactions show an outgrowth of the parasite and a depression of the host cell wall. These attachments appear to be mechanically strong; they can be broken only occasionally by heavily squashing the preparation under a cover slip. In TEM micrographs (Figs. 9–12) a close attachment of cell walls of host and parasite cells can be seen. Micropores of approximately 0.02 μm in diameter protrude through the cell walls of the host and the parasite. However, cytoplasmic fusions through micropores could not be found. All studied and illustrated stages showed host cells devoid of cytoplasm. Younger developmental stages could not be found.

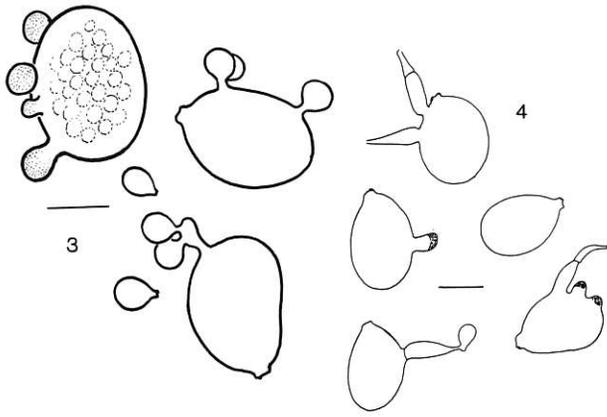
Description of *Phragmoxenidium* Oberwinkler, gen. nov.

phrag. mo. xe. ni'dium, (Gr.) *phragma* – septum, *xenos* – strange, *-idium* – suffix for basidium, basidiomycete.

Genus Heterobasidiomycetum. Carposoma nullum. Hyphae distinctae, hyalinae, tenuitunicatae, enodulosae, inter cellulas hospitalium crescunt. Ultrastructura septorum hypharum doliporis sine parenthesesomatism notata est. Cystidia desunt. Basidiosporae hyalinae, tenuitunicatae tunicisque levibus plerumque cellulis singulis germinant.

Typus generis: *Phragmoxenidium mycophilum* Oberw. and Schneller, opus ipsum.

Description of *Phragmoxenidium mycophilum* Oberwinkler and Schneller, sp. nov.



Figs. 3, 4. Basidiospores of *Phragmoxenidium mycophilum* germinating with microconidia and secondary spores. Bars = 5 μ m.

my. co. philum (Gr.) *mykes* – fungus, *philos* – friend. Carposoma nullum. Hyphae hyalinae, efibulatae, tenuitunicatae, tunicis levibus, 2–4 μ m in diam. Cystidia hyphidiaque desunt. Basidia longa, mature transverse septata, 5–8 \times 40–70 μ m. Basidiosporae hyalinae, tenuitunicatae, tunicis levibus, non amyloideis, apiculis prominentibus, 5–7 \times 9–11 μ m, frequenter per repetitionem germinantes vel cellulas parvas, 1–2 μ m in diam., germinando productum.

Typus: J. Schneller, 30.10.1988, M, Isotypus Z.

Discussion

Auricularioid mycoparasites with scanty or no basidiocarps are known in the genus *Platygløea* s.l. *P. peniophorae* var. *interna* (Olive, 1954) grows intrahymenially in *Dacrymyces* spp. Scattered hyphae develop single basidia or basidial clusters, but no hymenia and no basidiocarps. This species has a characteristic anamorph stage. Also *Platygløea arrhytidiae* is described as a dacrymycetaceous mycoparasite with conidiophores (Olive, 1951). The presence of hyphidia was reported from *P.*

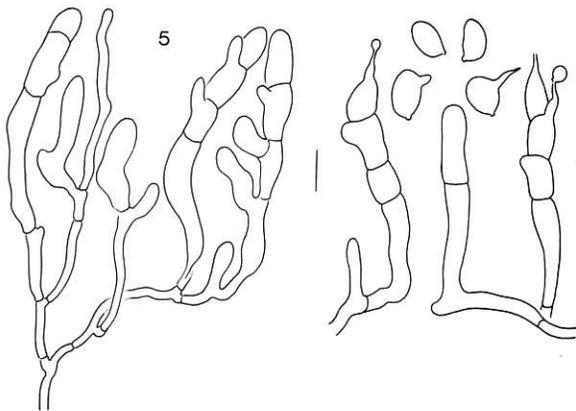


Fig. 5. Basidia of *Phragmoxenidium mycophilum* in different developmental stages and basidiospores. Bar = 10 μ m.

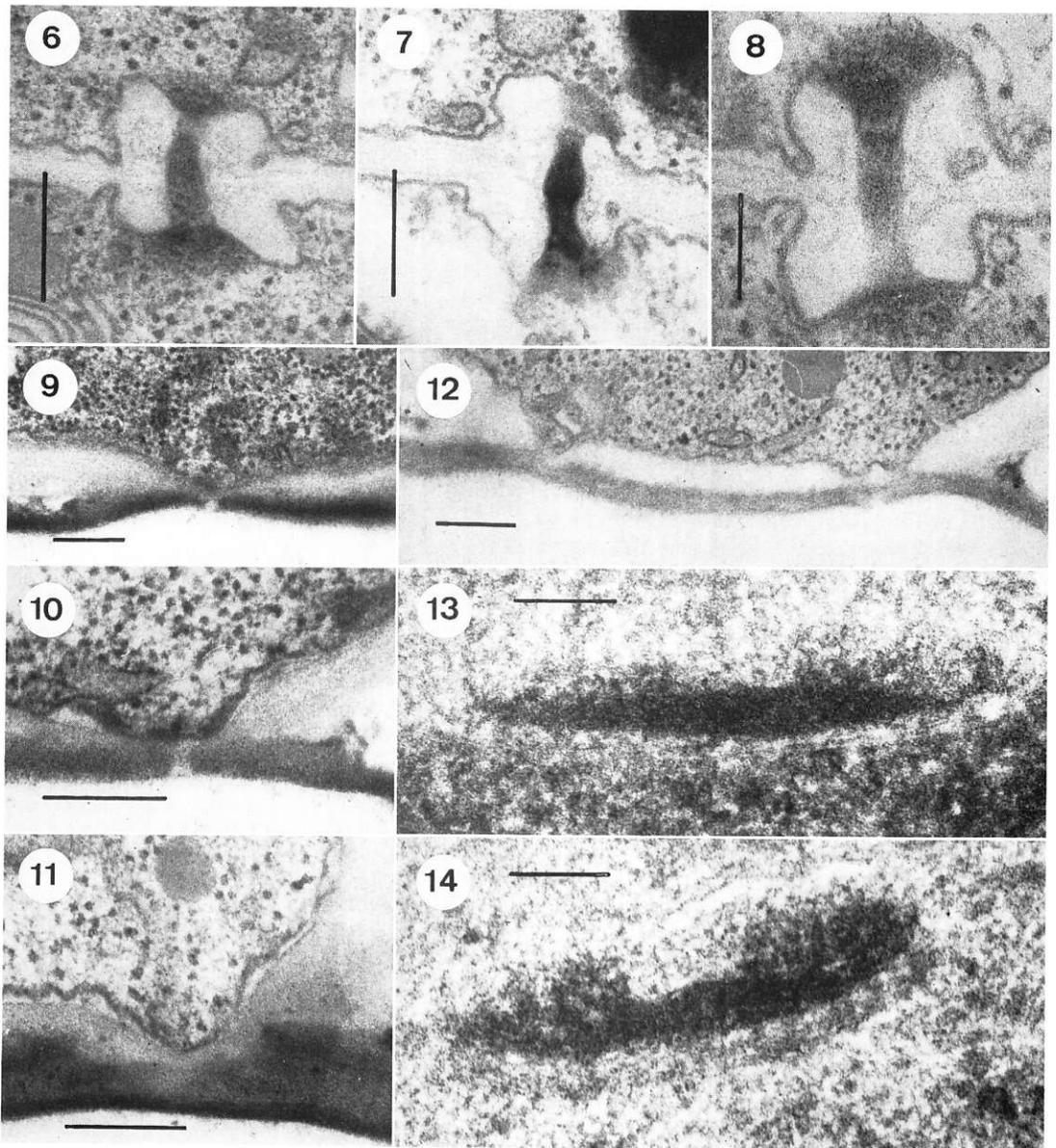
fungicola var. *interna* (Olive, 1958). However, conidia and hyphidia are lacking in *Phragmoxenidium mycophilum*. *Platygløea bispora* parasitizes *Tubulicrinis angustus* (Hauerslev, 1987) without producing basidiocarps. The species has two-sterigmate, curved or coiled basidia, features that are not found in *Phragmoxenidium mycophilum*. *Platygløea abdita* (Bandoni, 1959) and *P. subabdita* (Hauerslev, 1987), both growing in the fructifications of tremellaceous hosts, have conspicuous probasidia comparable to those of *Cystobasidium lasioboli* (Lagerheim, 1898). The latter species is another auricularioid mycoparasite, reported from the ascomycete *Lasiobolus ciliatus*. Basidia of *Phragmoxenidium mycophilum* lack probasidial swellings. Instead, quite often, pleurobasidia occur. Pleurobasidia are not reported, and, to our knowledge, do not occur in other heterobasidiomycetes. *Platygløea sebacea* (McNabb, 1965), with pulvinate basidiocarps, is associated with pyrenomycetes, but it differs from *P. mycophilum* in septal pore type (Bauer and Oberwinkler, personal observations). The basidia of this species are rather long and thin.

The ultrastructure of the host parasite interaction in heterobasidiomycetous mycoparasites has been only fragmentarily studied. Haustorial filaments of *Tetragoniomyces uliginosus* (Oberwinkler and Bandoni, 1981) and *Carcinomyces effibulatus* (Oberwinkler and Bandoni, 1982) closely 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, so far unique type of host parasite interaction is described and illustrated here for *Phragmoxenidium mycophilum*.

Morphological and functional features of basidiospores of *P. mycophilum* are characteristically heterobasidiomycetous, smooth, thin-walled, hyaline, inamyloid, germinating by repetition or by microconidia. Conidia, not rare in species of auricularioid fungi, are commonly produced from conidiogenous cells which can arise from the same hyphae as the basidia. Germinating spores of *P. mycophilum* produce microconidia, morphologically similar to those of dacrymycetaceous fungi and of *Paullitorcticium pearsonii*. However, no further growth of *Phragmoxenidium mycophilum* microconidia occurred during our culture experiments. Therefore later ontogenetic stages are unknown.

In simple pored, auricularioid fungi, yeast stages are reported for *Cystobasidium lasioboli* (Lagerheim, 1898), *Platygløea peniophorae* (Bandoni, 1984), and *Mycogloea* spp. (Bandoni, 1984). However, no yeast stages were found in *Phragmoxenidium mycophilum*. The type species of the genus *Platygløea*, *P. disciformis* has been cultivated several times during the present studies. Mature spores always produced germination hyphae, and no yeasts have been observed. This confirms what was reported by Aoki et al. (1986) from Japanese *P. disciformis*.

As in *Phragmoxenidium mycophilum*, most species in the simple-pored, auricularioid fungi possess efibulate hyphae. In the *Herpobasidium* group, clampless species appear to be the rule, but in other taxa, both clamped and



Figs. 6–8. Dolipores of *Phragmoxenidium mycophilum*. Pore orifices with faint striations, parentheses lacking. Bars = 0.2 μm in Figs. 6, 7, and 0.1 μm in Fig. 8.

Figs. 9–12. Parasitic interactions of *Phragmoxenidium mycophilum* with *Uthatabasidium mycophilum*. Cytoplasmic, upper cell of the parasite, host cell below. Note depressions in the cell walls of the parasite (all Figs.) and micropores in Figs. 10, 11. Bars = 0.2 μm.

Fig. 13. Interphase spindle pole body of *Phragmoxenidium mycophilum* with large midpiece and oblique, flattened marginal elements. Bar = 0.1 μm.

Fig. 14. Interphase spindle pole body of *Dacrymyces stillatus* with midpiece and semiglobular marginal elements. Bar = 0.1 μm.

clampless species are known. Several species of *Mycogloea* have clamps only at the bases of basidia. Apart from species of the *Auricularia* group, with dolipores and continuous parenthesomes, auricularioid fungi have simple septal pores. In contrast, *Phragmoxenidium mycophilum* septa have dolipores without parenthesomes, a unique feature in these taxa.

Nuclear divisions and spindle pole body (SPB) cycles in *P. mycophilum* have not been studied. We can only compare interphase SPB ultrastructure with that of other Basidiomycetes. The figures are selected from pictures of serial sections. The morphology of spindle pole bodies (SPBs) is heterogeneous in heterobasidiomycetous fungi. Two major types can be distinguished, the disc-SPB and the diglobular SPB. Interphase and prophase SPBs with lateral discs connected by midpieces are known from *Basidiomycetes* only in the *Uredinales* (Heath, 1980, 1981; O'Donnell and McLaughlin, 1981a, b, c; Hoch and Staples, 1983; Heath and Heath, 1976; Bauer, 1987), *Helicobasidium mompa* (Bourett and McLaughlin, 1986), *Heterogastrium pycnidioideum* (Oberwinkler et al., 1990), and in the gasteroid, auricularioid taxa *Agaricostilbum pulcherrimum*, *Atractiella solani*, *Phleogena faginea*, *Stilbum vulgare*, *Chionosphaera apobasidialis*, and *Pachynocybe ferruginea* (Oberwinkler and Bauer, 1989). SPBs with discs in *C. apobasidialis* and *P. ferruginea* are the first records from holobasidiate taxa. In contrast to all other atractielloid taxa, the interphase SPB in *Atractogloea stillata* is diglobular (Oberwinkler and Bauer, 1989). An electron dense midpiece connects the homogeneous, non lamellated, globular elements. Similar SPBs have been described and illustrated from several *Homobasidiomycetes* (Wells, 1977), and for the heterobasidiomycetous *Bullera alba* (Taylor and Wells, 1979), *Auricularia fuscococcinea* (McLaughlin, 1980), and *Tremella globospora* (Berbee and Wells, 1988). However, the globular element in SPBs of *Leucosporidium scottii* (McCully and Robinow, 1972a), *Sporidiobolus salmonicolor* (McCully and Robinow, 1972b), and *Ustilago maydis* (O'Donnell and McLaughlin, 1984a, b) contains an electron dense layer. Apart from *Atractogloea stillata* (Oberwinkler and Bauer, 1989), SPBs with homogeneous globular elements were only known from *Basidiomycetes* with dolipores. The interphase SPBs of *P. mycophilum* are most like those of *Dacrymyces stillatus* (Fig. 14).

Interphase and prophase SPBs in *Basidiomycetes* show stable morphological features and they are therefore applicable to taxonomic evaluations. Since all other simple pored, auricularioid, except *Atractogloea stillata* (Oberwinkler and Bauer, 1989), have disc-SPBs, *Phragmoxenidium mycophilum* with a diglobular SPB cannot be classified with the cystobasidiaceous taxa. The unique characteristics discussed for *P. mycophilum* clearly indicate that the genus cannot be properly placed in one of the families or orders, already described. Therefore, a new family is proposed to accommodate the taxon. This family is only tentatively assigned to the *Tremellales*.

Description of *Phragmoxenidiaceae* Oberwinkler and Bauer, fam. nov.

Fungi mycoparasitici. Carposomata propria cystidiaque desunt. Heterobasidiomycetes hyphis hyalinis basidiisque cylindraceis-clavatis et transverse septatis, sterigmatibus brevibus. Basidiosporae cellulis singulis vel per repetitionem germinant.

Typus familiae: *Phragmoxenidium* Oberw. and Schneller, opus ipsum.

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