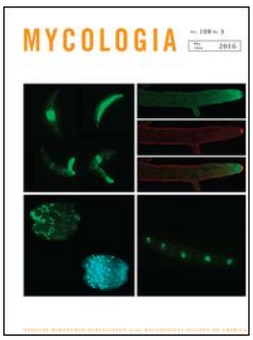

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Morphology, phylogeny and biology of *Gliocephalis hyalina*, a biotrophic contact mycoparasite of *Fusarium* species

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Abstract: *Gliocephalis hyalina,* a rarely seen microfungus with a morphology similar to the hyphomycete genus *Aspergillus* but with slimy conidia was found in a mixed microbial culture from soybean roots. This species has been reported sporadically since 1899, each time in association with other fungi or bacteria. *Gliocephalis hyalina* has not been maintained in monoxenic culture and requires other fungi to grow. Light and scanning electron microcope studies indicate that it is a biotrophic contact parasite of *Fusarium* species. The fungus may penetrate the cells but has no apparent deleterious effect on the growth or plant pathogenicity of its host. Phylogenetic analyses of partial nuclear small subunit rDNA sequences place *G. hyalina* near the Laboulbeniales, an order of obligate insect parasitic microfungi, and the related mycelial genus *Pyxidiophora. Gliocephalis hyalina* is mycoparasitic along with many *Pyxidiophora* species. These discoveries suggest that some ''unculturable'' microorganisms or ''cryptic DNA'' recovered from environmental DNA samples might represent obligate biotrophs that could be cultured and studied with simple techniques.

Key words: anamorphic ascomycete, evolution, Laboulbeniales

INTRODUCTION

Gliocephalis hyalina Matruchot (1899) is a poorly understood hyphomycete first discovered on beet roots collected in France. After its initial description, the fungus was not mentioned again until Arnaud (1952) recorded *G. hyalina* on cones and branches of *Pinus* and on stems and roots of *Cucumis melo.* Barron (1968) made the most recent published collection of *G. hyalina* from a rotten potato tuber in soil from Ontario. A second species, *Gliocephalis pulchella* (Penz. & Sacc.) D. Hawksw., appears to be a saprobe on lichens (Hawksworth 1979).

Both species bear a remarkable morphological similarity to species of the much better known genus *Aspergillus,* but the conidia are produced in slime rather than in dry chains. In this respect, *Gliocephalis* has some similarity with species of *Goidanichiella* Barron ex Gams (Gams et al 1990), but in contrast to the latter genus both species of *Gliocephalis* lack septa in their conidiophore stipes and the dematiaceous pigments characteristic of *Goidanichiella barronii.* In fact, the fungus reported by Embree (1963) as *G. hyalina* in potting soil in San Francisco in all likelihood was *G. barronii* because the report notes the presence of pigmented, septate conidiophores.

In these reports attempts at in vitro culturing of *G. hyalina* were unsuccessful and it was assumed to be a parasite of soil bacteria or other fungi. Matruchot (1899) was unable to grow it in monoxenic culture. He eventually succeeded in growing it in co-culture with bacteria and hypothesized that it lived on bacterial waste metabolites. Barron (1968) was unable to establish pure cultures but maintained it for a limited period of time in association with a *Fusarium* species. Gams et al (1990) concluded that *G. hyalina* could not be grown without bacteria. When we isolated this fungus from soybean (*Glycine max*) roots collected in Ottawa, Canada, we also failed to maintain a living culture using standard monoxenic microbiological techniques. However, like previous investigators, we noticed that other fungi were present on the isolation plate, which in our case consisted of a species of *Fusarium.* The notion that *G. hyalina* might be a parasite of *Fusarium* was intriguing. The genus *Fusarium* (asexual stage of *Gibberella,* Hypocreales) includes some of the most economically important plant pathogens. Contamination of agricultural commodities with *Fusarium* toxins, such as zearalenone, deoxynivalenol (vomitoxin) and fumonisins, is monitored and regulated internationally (Summerell et al 2001).

We report here on the successful establishment of *G. hyalina* in dual culture with species of *Fusarium* and the successful preservation of the dual culture. The availability of living material let us study the in-

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teraction between *G. hyalina* and its host using light, scanning and transmission-electron microscopy. We also established the phylogenetic placement of this species using parsimony analysis of nuclear small subunit rDNA sequences and compared *G. hyalina* to *Aspergillus terreus* and other *Aspergillus*-like fungi such as *Goidanichiella baronii, Custingophora olivacea, Escovopsis aspergillioides* and *Gondwannamyces proteae* (anamorph: *Knoxdaviesia proteae*).

MATERIALS AND METHODS

Morphology.—*Gliocephalis hyalina* was maintained on cornmeal agar (CMA) (BBL Microbiology Systems, Cockeysville, Maryland) in dual culture with an unidentified species of *Fusarium*. To maintain viability, transfers were made every 14 d to new medium. Dual cultures also were preserved in 10% glycerol at −80 C. All measurements and microscopic observations were made from strains grown on CMA or oatmeal agar (OA) (Gams et al 1998) and incubated in the dark or in incident light at 25 C. Fungal structures were mounted on slides in 85% lactic acid and photographed using phase or differential interference contrast microscopy. *Gliocephalis hyalina* (DAOM 229465, Canadian Collection of Fungal Cultures) was compared to several other *Aspergillus*-like fungi, including *Goidanichiella baronii* (DAOM 145402, DAOM 145918), *Custingophora olivacea* (CBS 335.68, Centraalbureau voor Schimmelcultures), *Escovopsis aspergilioides* (CBS 423.93) and the anamorph of *Gondwannamyces proteae* (CMW 3757, Tree Pathology Cooperative Programme Culture Collection, MJ Wingfield).

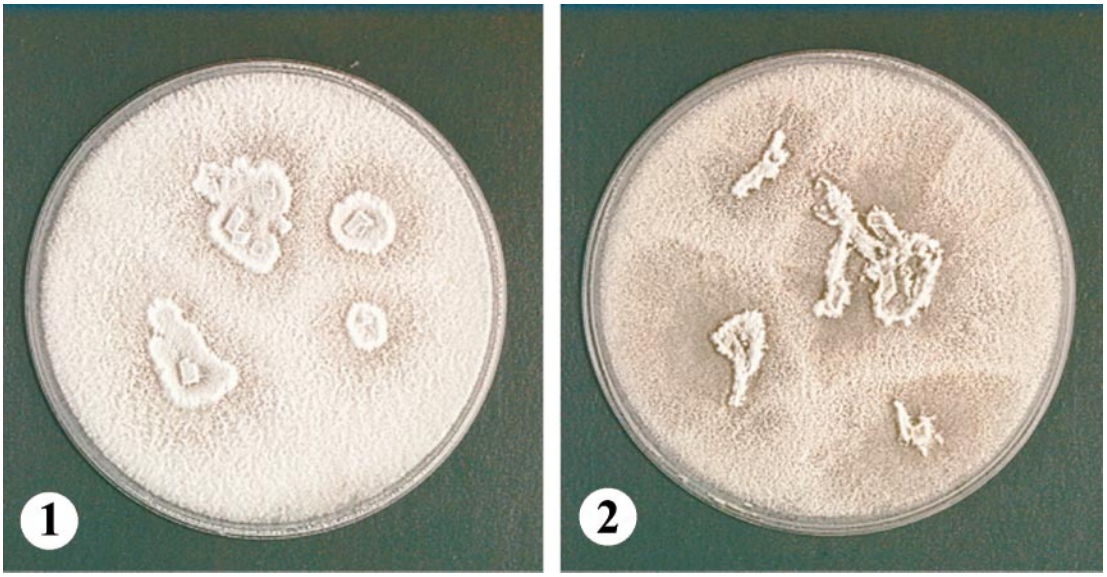
For scanning electron microscopy (SEM), blocks of agar about 5 mm across were cut from sporulating colonies and fixed in 4% glutaraldehyde and 0.5% osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded ethanol series, then critical-point dried (Tousimis SAMDRI PVT-3). Specimens were mounted and coated with gold palladium alloy (Technics Sputter Coater) and examined using a Phillips XL30 environmental scanning electron microscope. Preparations for transmission electron microscopy (TEM) were made by embedding the specimens in LR White and Spurr low viscosity embedding medium. Specimens were cut using a glass or diamond knife mounted in a microtome, and the thin sections were stained with uranyl acetate and lead citrate. These sections were examined using a Zeiss EM 902 analytical TEM microscope.

Infection studies were made on slide cultures (Cole et al 1969). Slides were sterilized by cleaning with 95% ethanol. Molten 10% water agar was dripped onto the slides and allowed to solidify. Conidia of *Fusarium* spp. were transferred to the slides and allowed to germinate. After ca. 12 h, *Gliocephalis* conidia were added to the slides and allowed to germinate. A cover slip was placed on the culture and the behavior of the germ tubes of *G. hyalina* was followed by light microscopy and pictures were taken at 1 h intervals.

Phylogenetic analysis.—The entire nuclear ribosomal small subunit (SSU) and internal transcribed spacer region (ITS) was amplified by the polymerase chain reaction using NS1 and ITS4 primers (White et al 1990) directly from spore suspensions of *G. hyalina* without DNA extraction. Amplicons were purified using Wizard fast-preps (BIO/CAN Scientific, Ontario, Canada). The NS1-NS4 sequence of the *G. hyalina* SSU rDNA was obtained using standard primers (White et al 1990) and direct sequencing of the PCR product on an ABI PRISM 310 automatic sequencer (Perkin Elmer Applied Biosystems, California). The alignment of the *G. hyalina* and SSU sequences of species representing different orders of the fungal kingdom was calculated using the Pileup algorithm of GCG 10.1 (Canadian Bioinformatics Resource http://www.cbr.nrc.ca/ with a gap weight of 5 and a gap length penalty of 1) and adjusted by eye. The final alignment contained 1083 bases. For phylogenetic analysis, a region with only single-stranded sequence for *G. hyalina* data was excluded (85 bp). The aligned dataset consisted of 988 unweighted characters, with gaps treated as a fifth base resulting in 468 constant, 156 parsimony uninformative and 364 parsimony informative characters. Phylogenetic relationships were inferred using heuristic searches in PAUP* 4.0b8 (Swofford 2001), using tree-bisection-reconnection (TBR) branch swapping. Starting trees were obtained through simple stepwise addition. Confidence levels were estimated using a bootstrap analysis (1000 replicates). The confidence levels of the different nodes in the tree also were evaluated with Bayesian analysis of the dataset using the Markov Chain Monte Carlo algorithm (MrBayes 3, Huelsenbeck and Ronquist 2001). The analysis was run for 200 000 generations with every 10th tree sampled. The first 2000 trees were discarded because these were generated before convergence of the chains. Four cold chains were run simultaneously. The posterior probability of each node was calculated. The SSU sequence of *G. hyalina* was determined in duplicate and is deposited in GenBank under accession numbers (AF505620 [5′ end] and AF505621 [3′ end]).

Host range experiments.—Host specificity was tested by inoculating growing cultures of host fungi with 1 spore drop of *G. hyalina* in the center of the colony. Cultures were checked by light microscopy after 5 d for sporulation and formation of contact cells. The experiment was run in duplicate. Several species of *Fusarium* and some common soil fungi were tested as potential hosts for *G. hyalina,* namely *Fusarium sporotrichioides* DAOM 213383, *F. oxysporum* DAOM 197539, *F. poae* DAOM 13714, *F. verticillioides* KAS 99M-6, *F. merismoides* DAOM 167040, *F. culmorum* DAOM 211723, *F. tumidum* BBA 63572, *F. sambucinum* DAOM 214958, *F. venenatum* DAOM 64537, *F. solani* DAOM 193421, *F. torulosum* BBA 64988, *Epicoccum purpurascens* DAOM 185649, *Cladosporium cladosporioides* DAOM 196948, *Botrytis cinerea* DAOM 189076, *Trichoderma viride* JBT1003 and *Alternaria alternata* DAOM 216376. To ensure that pure spore drops of *G. hyalina* were used for host-range experiments, each aquaeous spore suspension was plated on CMA without any host to check that no mycelial growth occurred.

Pot culture experiments.—A bioassay originally designed to study the interaction of *Fusarium graminearum* and roots of wheat seedlings (Chongo et al 2001) was adapted to determine whether *G. hyalina* would reduce the pathogenic ef-



FIGS. 1–2. Sporulation of *Gliocephalis hyalina* on oatmeal agar (OA) and cornmeal agar (CMA) using a *Fusarium* sp. as host. 1. Growth of *G. hyalina* on OA using a *Fusarium* sp. as host. 2. Growth of *G. hyalina* on CMA using a *Fusarium* sp. as host.

fects of *Fusarium* on wheat seedlings grown in a sterile, environment without soil. Wheat seeds with a low incidence of natural *Fusarium* infection (variety AC Brio, collected at New Liskeard, Ontario) were surface sterilized using 10% chlorine bleach (5 min), 100% ethanol (5 min) and water (10 min). Thirty seeds per treatment were soaked in concentrated spore suspensions of (i) *F. graminearum* (DAOM 180378), (ii) *F. graminearum* and *Gliocephalis hyalina* and (iii) water. Seeds were planted in approximately 30 mL of a presterilized, soilless mix in potting trays, and plants were grown in a growth chamber with 12 h of light/dark cycles at 22 C and were watered as required. Seed germination and growth were assessed 16 d after planting, and stem and root lengths as well as stem dry weights were measured. The experiment was done in duplicate. The data were analysed using ANOVA as implemented in SAS 6.0.

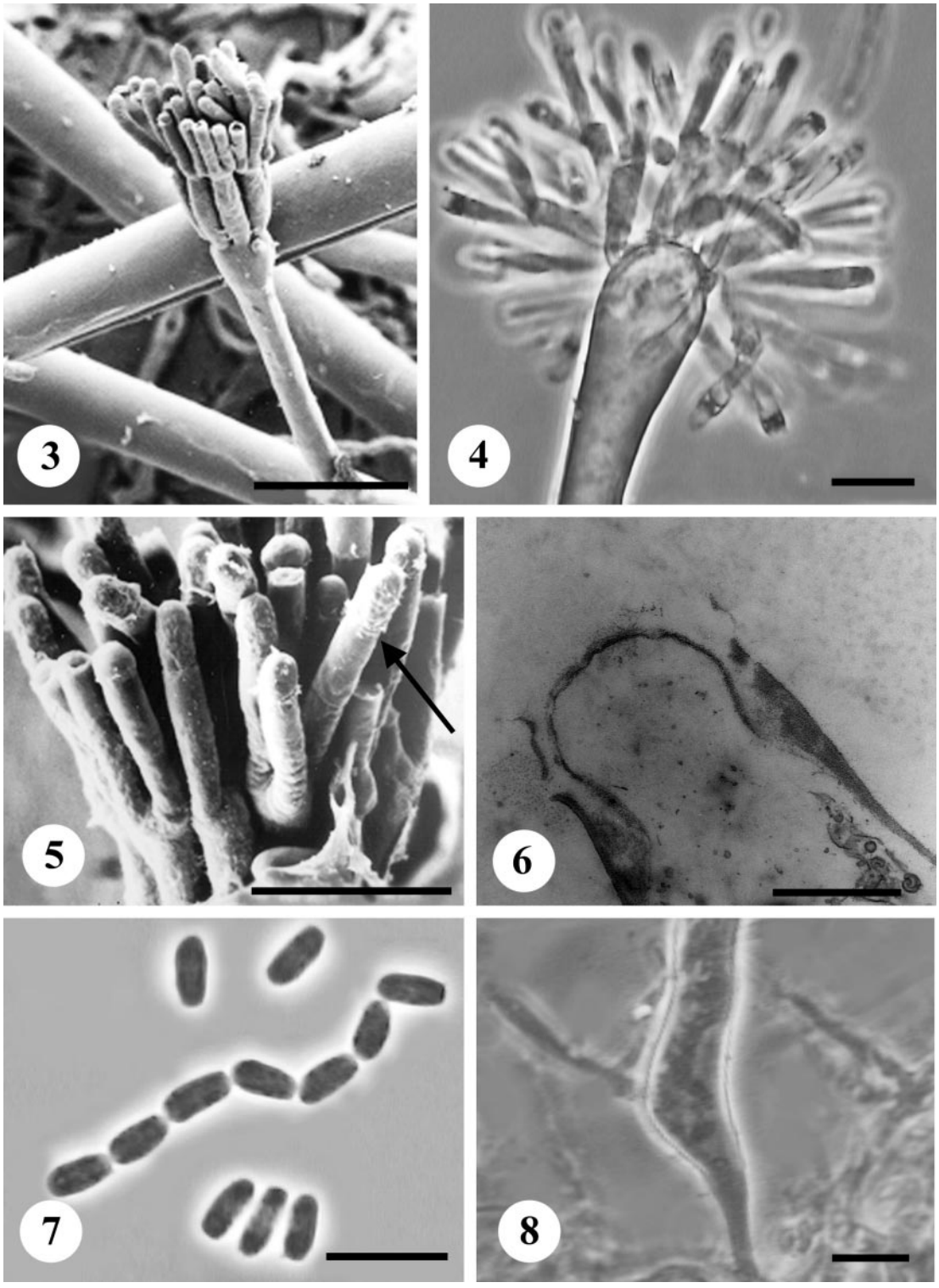
RESULTS

Morphology.—*Gliocephalis hyalina* sporulated abundantly in the presence of most *Fusarium* spp. The density of sporulation was similar on OA and CMA (FIGS. 1–2). Best results were obtained with *G. hyalina* in a mixed culture with an unidentified *Fusarium* sp. (DAOM 229465) on CMA (FIG. 2). *Fusarium* spp. sporulated sparsely on this medium and the conidiophores of the parasite were easily observed and studied. Colonies of *G. hyalina* were colorless and conidiophores were produced randomly over the host colony (FIGS. 1–2).

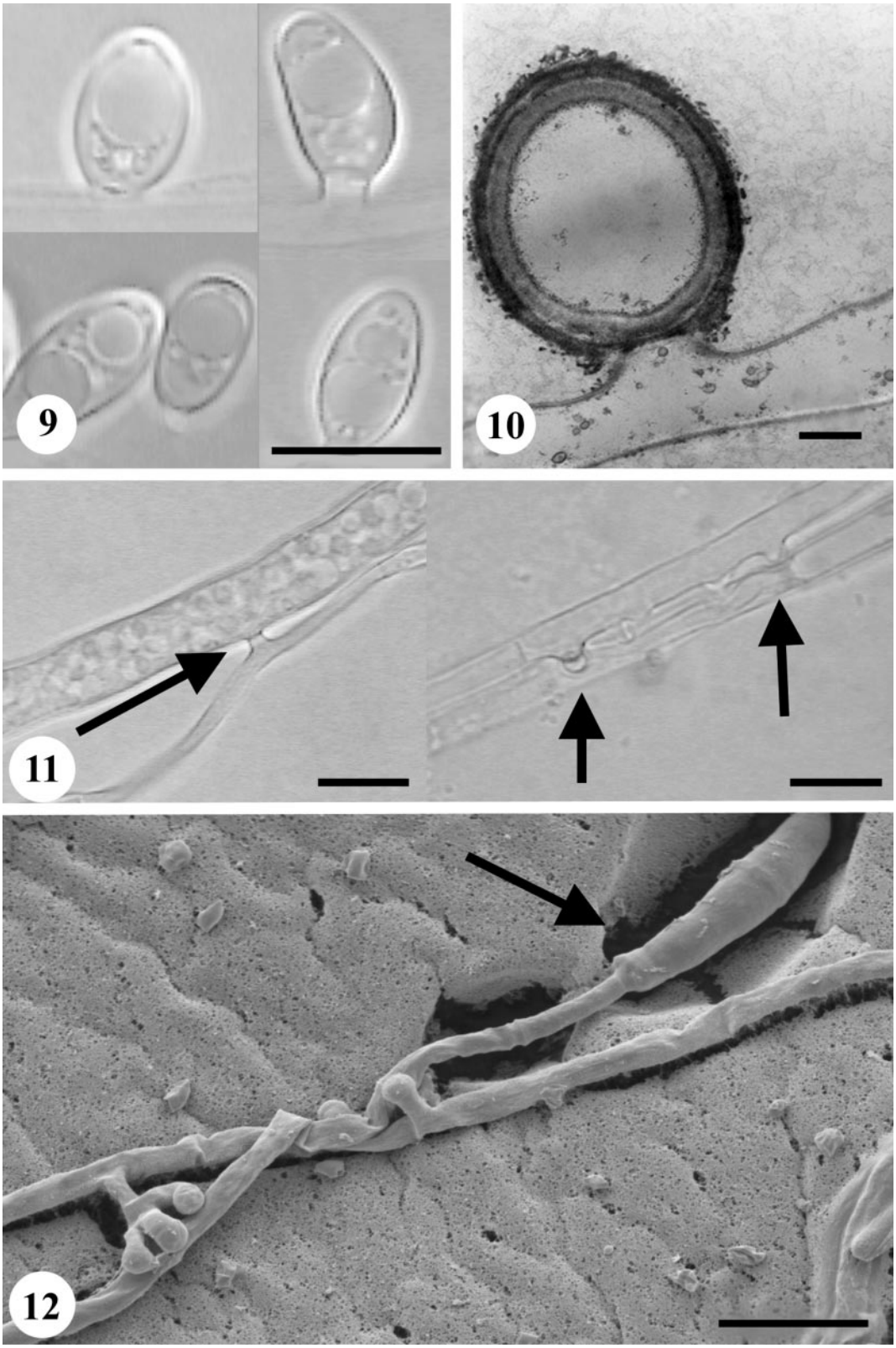
Gliocephalis hyalina superficially resembles *Aspergillus* (FIGS. 3–4), having an unbranched, aseptate, hyaline conidiophore terminating in a swollen vesicle, (8–)9–14(–17) μm, giving rise to 1–2 series of metulae, bearing conidiogenous cells (FIGS. 4–5). Conidiophores are (154–)226–408(–476) μm long. Each metula terminates in 2–3 cylindrical conidiogenous cells, (10–)12–16(–20) μm long, 2–3 μm wide, that produce conidia in basipetal chains (Cole and Samson 1979) (FIGS. 5–6). The conidia are hyaline, cylindrical with rounded apices and have slightly truncate bases, 6–9 μm × 2–3 μm (FIG. 7). They accumulate in clear, watery masses at the apices of the conidiophores; they become white as the culture ages. The bases of the conidiophores are slightly swollen (FIG. 8). In older colonies lateral, aseptate cells are produced directly on the hyphae. These cells resemble chlamydospores, and we speculate that they are survival structures (FIGS. 9–10).

Examination of the holotype of *Gliocephalis pulchella* (PAD235) confirmed that this fungus seems to be correctly classified in *Gliocephalis,* producing tall, aseptate, vesiculate conidiophores. In contrast to *G. hyalina, G. pulchella* has a reddish swollen apex and slightly rugose conidia (Hawksworth 1979).

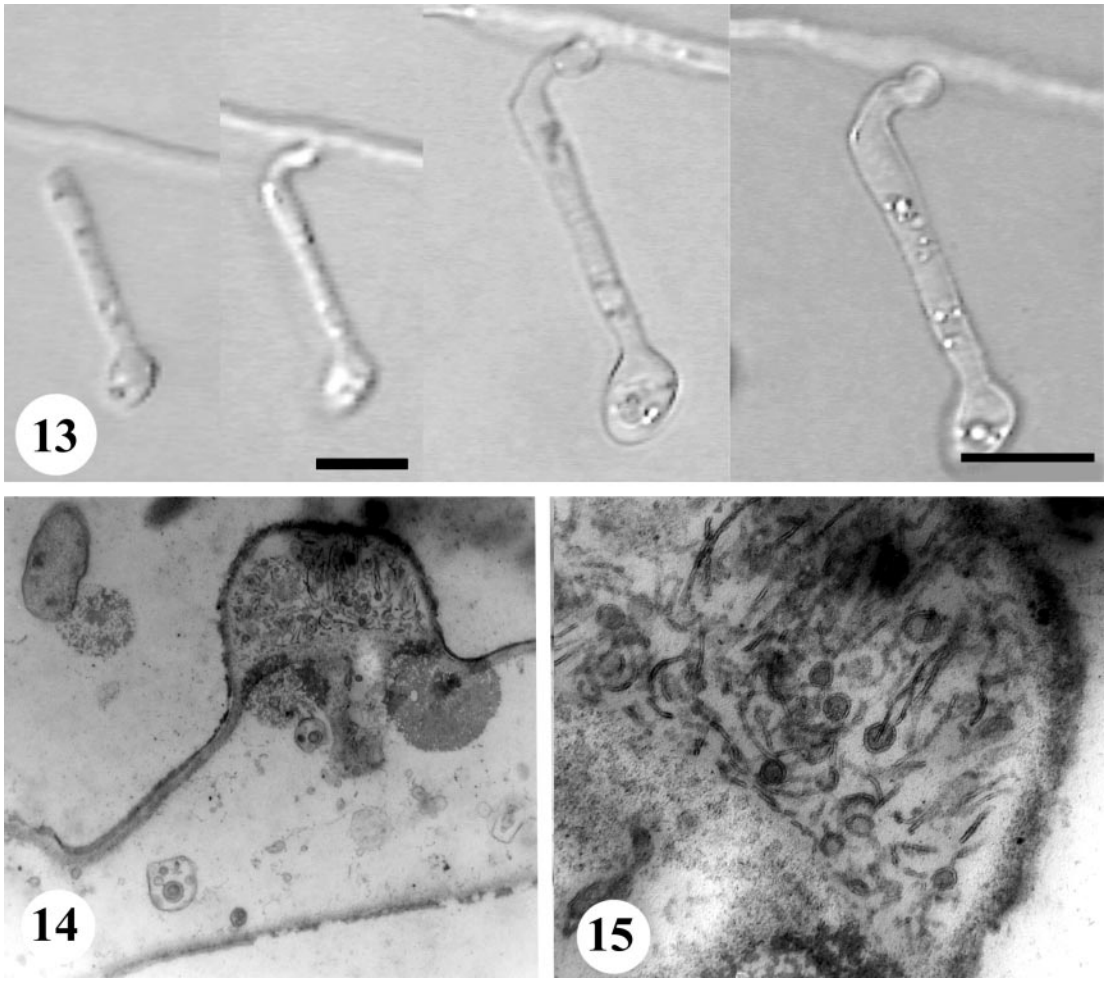
Parasitic interaction.—Time-lapse photography of slide cultures using light microscopy (LM) showed that spores of *G. hyalina* would germinate only when mycelium of *Fusarium* was present. Then the spores swelled and sent out single germ tubes, which grew directly toward the *Fusarium* hyphae. At contact the *Gliocephalis* hyphae seemed to attach to the *Fusarium* cells (FIG. 13). Multiple lateral contact points developed between the narrow growing hyphae of *Gliocephalis* and the broader *Fusarium* hyphae. No evidence of specialized attachment or penetration struc-



FIGS. 3–8. Morphology of *Gliocephalis hyalina*. 3. SEM of Aspergillus-like conidiophore (Bar = 20 μm). 4. Light micrograph of swollen apex of conidiphore with tubular conidiogenous cells (Bar = 10 μm). 5. Detail of tubular conidiogenous cells (arrow) producing barrel-shaped conidia (Bar = 10 μm). 6. TEM of the conidiogenous cells showing typical phialidic conidium development indicated by the periclinal thickening of the cell wall. 7. Barrel-shaped conidia (Bar = 10 μm). 8. Slightly swollen base of the conidiophore. (Bar = 10 μm).



FIGS. 9–12. Survival and infection structures of *G. hyalina*. 9. Lateral cells produced directly on the mycelium. (Bar = 10 μm). 10. Thin section through lateral cell showing a thick wall consistent with those of other survival spores. 11. Attachment points between *G. hyalina* and its *Fusarium* host (arrows). No evidence of penetration was observed. 12. SEM of contact cells formed by *G. hyalina* close to *Fusarium* conidiophore with a developing *Fusarium* conidium (arrow).



FIGS. 13–15. Infections structures of *G. hyalina*. 13. Germination and growth of a *G. hyalina* conidium during 4 h, at 1 h intervals. 14. Thin section through one of the contact cells. Cytoplasmic intrusion was observed into this host cell. 15. Virus-like particles also can be seen as in the case of similar biotrophic contact parasites.

tures using LM was found (FIG. 11). With SEM, we found clear evidence of specialized structures growing from *Gliocephalis* and ''grabbing'' the hyphae of *Fusarium* (FIG. 12). These attachment structures (haustoria), consisted of slightly swollen ends of otherwise normal-looking hyphae or hyphal branches. No evidence of hyphal collapse or erosion of the host cell wall was seen with SEM. Erosion of the host cell wall was seen in some ultrathin serial sections of the haustorium/host interface using trasmission electron microscopy (FIGS. 14–15), but no penetration structures or plasmodesmata were observed.

In dual culture experiments, *G. hyalina* grew and sporulated in the presence of *Fusarium sporotrichioides, F. oxysporum, F. poae, F. verticillioides, F. merismoides, F. culmorum, F. sambucinum, F. venenatum, F. tumidum, F. torulosum* and *F. solani* but exhibited no visible growth or sporulation when grown with *Epicoccum purpurascens, Cladosporium cladosporioides, Botrytis cinerea, Trichoderma viride* or *Alternaria alternata*. *Gliocephalis hyalina* grew considerably slower than its hosts and produced few hyphae in culture. However, conidiophores were readily produced in the presence of host fungi. No mycelial barriers or other barrage structures were observed when *G. hyalina* was grown in the presence of other fungi. Furthermore, we observed no differences in colony diameter of the host fungi in the presence of *G. hyalina*.

Given the apparent specificity of *G. hyalina* for a *Fusarium* host, we studied the interaction in a more natural system. *Fusarium graminearum* adversely affects germination of wheat seeds (Chongo et al 2001), so we inoculated a spore suspension of *G. hyalina* into sterile soil in small pots containing wheat grains, either alone or in mixture with a virulent strain of *F. graminearum* to determine the effectiveness of *G. hyalina* as a biocontrol agent. After 16 d we observed no statistically significant difference in seed survival and germination, seedling root length

or leaf length between experimental treatments and the control (FIG. 17). *Gliocephalis hyalina* was seemingly unable to protect wheat from the germination-inhibiting effects of *F. graminearum,* although a small but significant increase ($P = 0.0073$) in dry weight for plants treated with the mycoparasite was noted.

Phylogeny.—Amplification of the combined 18S and ITS rDNA resulted in products of approximately 2800 bp. The ITS region and part of the 18S sequence was omitted from the analysis to align it with other sequences from Genbank. A heuristic search of a dataset comprising 18S sequences from *G. hyalina* and species of different orders of the ascomycetes resulted in six most parsimonious trees. The shortest tree was 1551 steps (CI = 0.541, RI = 0.648). *Gliocephalis hyalina* clustered basal to the other orders of the ascomycetes and was related most closely to the Laboulbeniomycetes. This clade includes different members of the Laboulbeniales and Pyxidiophorales, where *G. hyalina* is placed, and is supported by high bootstrap values (FIG. 16).

DISCUSSION

Gliocephalis hyalina is an *Aspergillus*-like hyphomycete that is an obligate biotroph of other fungi and is phylogenetically related to, but still somewhat distant from, members of the order Laboulbeniales and the related mycoparasitic genus *Pyxidiophora.* The Laboulbeniales is a large order of more than 2000 species of obligate, biotrophic parasites of insects that produce specialized haustoria only in insect hosts and consist entirely of multicelled, spore-producing thalli attached to specific parts of the host bodies (Tavares 1985). The Laboulbeniales were considered phylogenetic orphans until their relationship with *Pyxidiophora* was discovered (Blackwell 1994, Weir and Blackwell 2001). The discovery of the relationship of *G. hyalina* to *Pyxidiophora* provides another link between the mycelial ascomycetes and the Laboulbeniales. *Gliocephalis hyalina* is a sister taxon of *Pyxidiophora* in this dataset. The placement of these taxa suggests that, although they are closely related to the Laboulbeniales, there is evidence for the separation of the Laboulbeniales, which lack hyphae, and the mycelial Pyxidiophorales. This relationship has an ecological consistency because many species of *Pyxidiophora* are considered mycoparasites (Lundqvist 1980, Blackwell and Malloch 1989). One species, *P. lundqvistii,* was discovered in cultures of *Fusarium poae* (Corlett 1986).

The conidiogenous cells of *G. hyalina* are phialides, and they are somewhat similar to those of the anamorphs of some species of *Pyxidiophora* (Lundqvist 1980) that have been ascribed to *Thielaviopsis* or *Chalara.* The phialides are hyaline and cylindrical and do not have the apical constriction that characterizes the phialides of some other *Aspergillus*-like genera. The phialides lack a well-developed collarette and conspicuous periclinal thickening. Despite the similarity in the conidiogenous cells and conidia between *G. hyalina* and the anamorphs of *Pyxidiophora,* none of the anamorphs of *Pyxidiophora* described by Lundqvist (1980) and Blackwell and Malloch (1989) have vesiculate conidiophores like those of *G. hyalina.*

The conidiophores of *Gliocephalis* spp. are similar to those of species of the genus *Aspergillus* (Matruchot 1899, Barron 1968). Species of both genera produce unbranched, aseptate conidiophores with swollen apical vesicles from which metulae and phialides develop. The genera are distinguished easily because conidia of *Aspergillus* species are produced in dry, basipetal chains and those of *Gliocephalis* spp. occur in slimy masses. The monotypic genus *Goidanichiella* also is similar to *Gliocephalis,* and the two have been confused sometimes. However, the conidiophores of *Gliocephalis* spp. are aseptate and hyaline, while those of *Goidanichiella barronii* are septate and darkly pigmented. Phylogenetic analysis confirms the significance of these characters. *Aspergillus*-like conidiophores have arisen several times in fungal evolution and occur in the Eurotiales (*Aspergillus*), Hypocreales (*Escovopsis*), Pyxidiophorales (*Gliocephalis*) and Microascales (*Custingophora, Knoxdaviesia* and *Goidanichiella*). A few remaining fungi with similar conidiophores, such as *Heterocephalum aurantiacum,* have uncertain phylogenetic affinities.

Mycoparasites are classified either as necrotrophic parasites or biotrophic parasites (Barnett and Binder 1973). Necrotrophic parasites destroy their hosts (Barnett and Binder 1973) but biotrophic parasites are not destructive, instead interacting with their hosts in three different ways (Barnett and Binder 1973; Hoch 1977a, b). The parasites can live inside the host cell, as in the case of some chytrids. On the other hand they can produce haustoria on the host cell or parasitism can occur through contact points on the host cell without the production of haustoria or internal hyphae (Barnett and Binder 1973; Hoch 1977a, b). We conclude from our observations that *Gliocephalis hyalina* is a biotrophic contact parasite of *Fusarium* species because it does not destroy its host. In our experiments it grew with all the *Fusarium* species tested but did not grow with other soil fungi. According to Gams (personal communication), a culture of *G. hyalina* was preserved in 1993 as CBS 642.93 in co-culture with *Cylindrocarpon destructans,* an anamorphic species also classified in the Nectri-

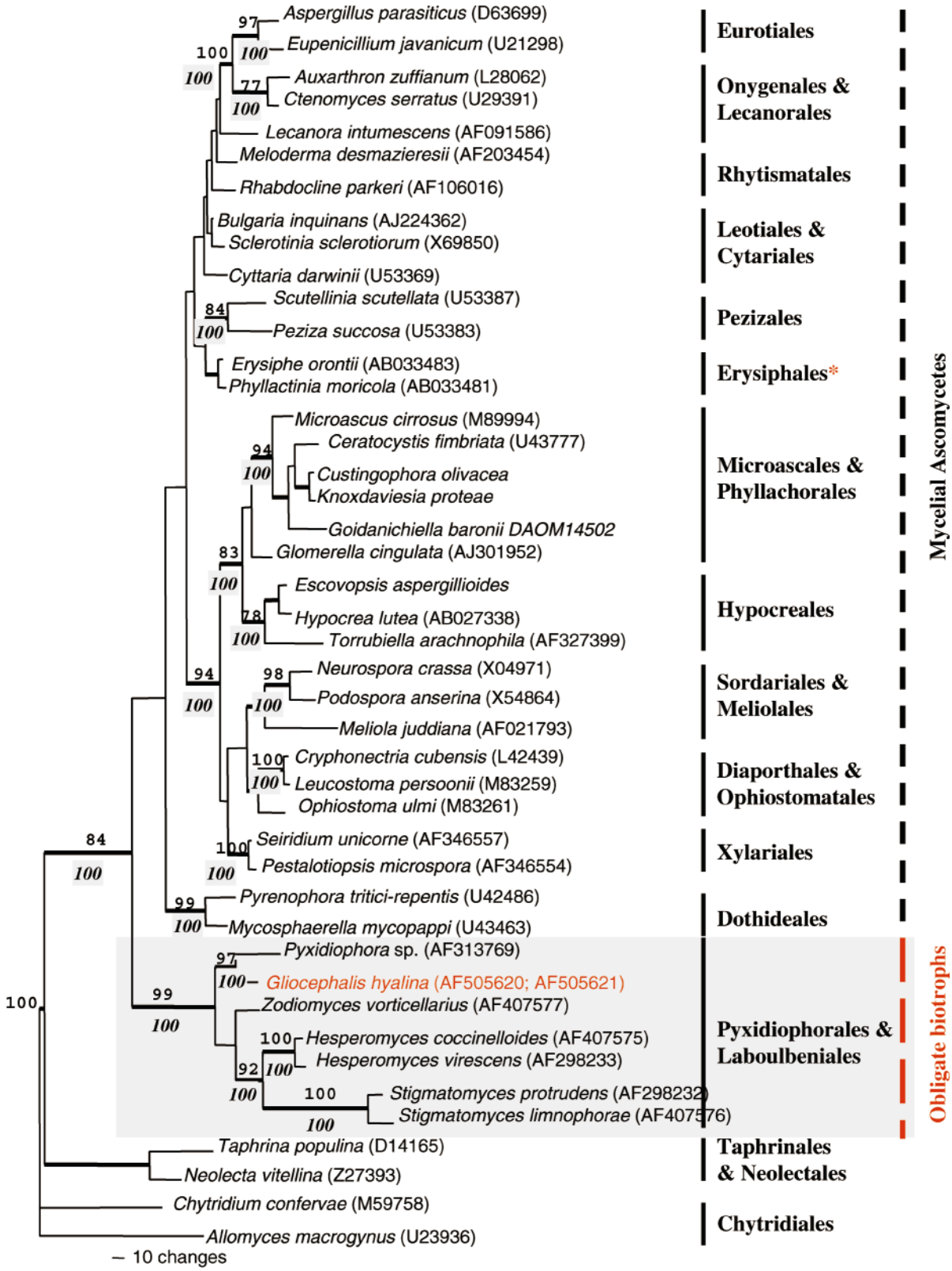


FIG. 16. Phylogenetic position of *Gliocephalis hyalina* in the Ascomycetes. Other taxa with *Aspergillus*-like conidiophores are in bold. Shown is one of the six most parsimonious trees obtained using a heuristic search from an alignment of ribosomal small subunit DNA. The shortest tree length was 1551 steps (CI = 0.541, RI = 0.648). Bootstrap values are indicated above branches and posterior probabilities of the node are indicated below the branches in shaded boxes. *Gliocephalis hyalina* falls into Pyxidiphorales clade, close to *Pyxidiophora*.

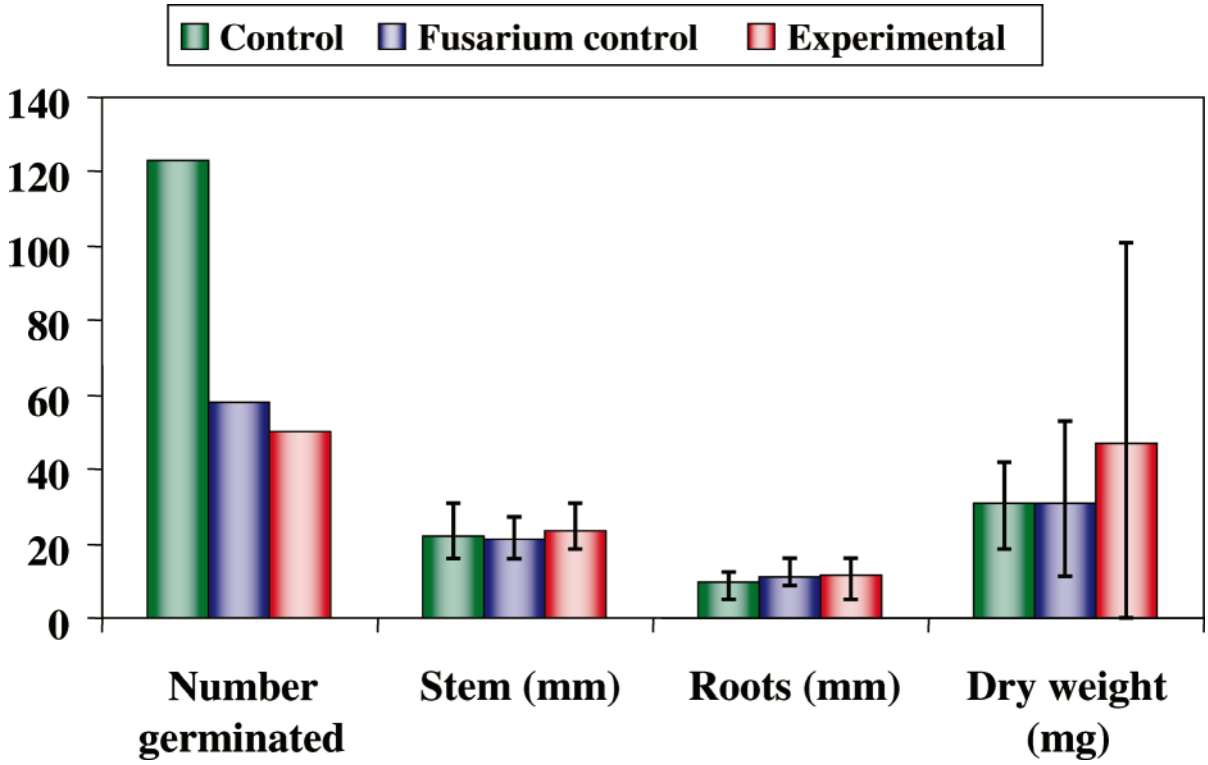


FIG. 17. The effect of *G. hyalina* on the ability of *Fusarium graminearum* to inhibit seed germination and growth. The y-axis indicates the number of seeds germinated in the experiment as well as the length (mm) and weight (g) of the seedlings. No statistical difference was noted between the seeds treated with *G. hyalina* and seeds not treated with the parasite.

aceae. Therefore it is possible that the host range of *G. hyalina* is slightly broader than we determined, including a broader range of species in the Nectriaceae. It also might be that different strains of the species have different host preferences.

Only 10 fungal species are reported as biotrophic contact parasites, namely *Melanospora zamiae* Zimm., *Harzia acremonioides* (Harz.) Cost., *Woronina pythii* Goldie-Smith, *Nematogonum ferrugineum* (Pers.) Hughes, *Calcarisporium parasiticum* Barnett, *Gonatobotrys simplex* Corda, *Gonatobotryum fuscum* Sacc., *Gonatorhodiella higheili* Smith, *Olpitrichum tenellum* (Berk. & M.A. Curtis) Hol.-Jech. and *Stephanoma phaeospora* Butler & McCain, (Hoch 1977a, b; Hoch 1978; Walker et al 1982; Dylewski and Miller 1983; Urbasch 1986; Jordan and Barnett 1978; Li and Shen 1996). As is the case with the other contact mycoparasites, *G. hyalina* has no visible effect on the growth and sporulation of the host fungi. Using TEM no infection structures or penetration of the host hyphae were found, although a slight invasion of the host cytoplasm was observed in some cases (FIGS. 14–15). This is similar to the parasitic behavior of *Stephanoma phaeospora,* also a biotrophic contact parasite on *Fusarium* (Hoch 1978). Biotrophic parasites of plant pathogenic fungi might seem like promising candidates for biological control agents because of their relative specificity, but it would be difficult to produce inoculum and separate it from the host mycelium on a commercial scale. In our pot experiments *G. hyalina* had little detectable effect on the pathogenicity of *Fusarium graminearum* to wheat seedlings (FIG. 17), so its possible benefits as a biocontrol agent are not obvious, at least in the assay that we used.

In this study we have shown that *G. hyalina* can be maintained in dual culture only by using *Fusarium* species as hosts among those tested. Some ''unculturable' micro-organisms detected by extracting environmental DNA (Hugenholtz et al 1998, Vandenkoornhuyse et al 2002) might be obligate biotrophs that could be isolated and propagated by growing in association with a compatible host. *Gliocephalis hyalina* is an example of an organism whose DNA would be considered novel if recovered from a soil sample. As mycologists continue the search for ''missing fungi' (Hyde 2001) and as ecologists explore environmental DNA, it is important to remember that sequence data are available currently for only about 10% of the fungal species now known to science and that many microfungi are known only from dried

herbarium specimens for which no living cultures or DNA samples exist.

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