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Zygomycetes in Human Disease

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INTRODUCTION

Organisms of the class *Zygomycetes* were first noted to cause disease in humans in publications from the 1800's. Platauf (369) is credited with the first description of zygomycosis in humans in his paper entitled Mycosis Mucorina. His descriptions, in German, are detailed enough to suggest that this first case of disseminated disease in a cancer patient was caused by *Absidia corymbifera*. The information that emerged over the next several decades was based predominantly on tissue morphology and rarely confirmed by culture. As a result, many of the early cases, and some of the cases still reported today, relied on the morphologic tissue findings of coenocytic, angioinvasive hyphae suggesting infection with one of the *Mucorales*. The majority of all cases reported had no culture identification; instead, the infection was identified as a "mucormycosis," or *Mucor* infection, despite this lack of culture confirmation. This was further promoted because most of the pathogenic zygomycetes were originally classified as members of the genus *Mucor*. These organisms were later reassigned, and continue to be reassigned, into different genera and families within the order *Mucorales*. Even with the poor showing with culture results, it soon became obvious that *Rhizopus* spp., and not *Mucor* spp., were the predominant organisms causing disease (286, 321). Other important information was also being collected by astute clinicians and researchers regarding the association of zygomycosis with cancer (28, 209, 297), antibiotic or prednisone use (463), diabetes (181, 260), deferoxamine and desferrioxamine therapy (48), transplantation (308), and the associated forms of immunosuppressive therapies. With the development of diagnostic tools that allowed earlier diagnosis, with better surgical and antifungal interventions, and with more sophisticated laboratory methods for identifying these agents, more patients are surviving these previously fatal infections. The variety of organisms causing disease has also expanded. In addition to *Rhizopus*, *Mucor*, and *Absidia*, human diseases due to *Rhizomucor*, *Apophysomyces*, *Saksenaea*, *Cunninghamella*, *Cokeromyces*, and *Syncephalastrum* spp. have all been confirmed. The manifestations of disease have also evolved from primarily rhinocerebral, pulmonary, and disseminated disease to include gastrointestinal, cutaneous/subcutaneous, allergic disease, and even asymptomatic colonization.

The *Entomophthorales* are a very interesting order of the *Zygomycetes* that produce subcutaneous and mucocutaneous mycoses. The *Entomophthorales* derive their name from the Greek word "*Entomon*," meaning insect, reflecting their original identification as pathogens or parasites infecting insects. The human pathogens in this order include *Basidiobolus* and *Conidiobolus* species. *Basidiobolus ranarum* was described from animal and environmental sources as early as 1886 (87, 97, 128, 138, 338, 343, 460). It was not until 1956, however, that the first human case was described in a patient from Indonesia (218). Although the organism is found worldwide, there are estimated to have been only a few hundred cases of infection (320). *Basidiobolus* infections have historically been limited to tropical and subtropical areas, occurring as a chronic subcutaneous mycosis of the trunk and extremities in immunocompetent hosts, primarily children (175, 320, 413, 442). In recent years, not only has the geographic distribution of basidiobolomycosis expanded but also the etiology of the disease and the range of infected hosts have broadened. Infection with *Basidiobolus* is now reported in immunocompromised as well as immunocompetent hosts (133, 397). In addition to subcutaneous infection, basidiobolomycosis has expanded to involve other tissues including the gastrointestinal tract, lymph nodes, and muscles (41, 110, 225, 228, 320, 359). Angioinvasive disease has also been seen in one culture-confirmed case (228).

The first cases of infection caused by *Conidiobolus* spp. were described in the early 1960s. *Conidiobolus coronatus* was identified first as an agent of nasal granulomatous disease in a horse in Texas in 1961 (147), while the first human infection was reported in Jamaica in 1965 (57). As of 1991, it was estimated that at least 150 cases of chronic sinusitis conidiobolomycosis had been seen world wide (99). Of the 27 known species of *Conidiobolus* (486), only 4 are known to infect vertebrates (175, 214, 392).

In this review, the diversity of the zygomycetes and their disease manifestations are explored and the taxonomy and the relationship of the zygomycetes to other fungal pathogens are discussed. The epidemiology, pathogenesis, disease manifestation, diagnosis, and treatment of infections caused by the *Mu-*

corales are presented in detail, with exhaustive discussions of the individual species implicated in causing human zygomycosis; diagnostic features are summarized in table form for quick reference.

Although the *Entomophthorales* have some morphologic and reproductive characteristics in common with the *Mucorales*, this review documents that there are more differences than similarities between the *Entomophthorales* and the *Mucorales* producing disease in humans. The taxonomy of the *Entomophthorales* and the distinguishing features between the organisms of the *Mucorales* and *Entomophthorales* and their disease manifestations are reviewed. A detailed discussion of the epidemiologic, pathologic, and diagnostic characteristics of *Basidiobolus ranarum*, *Conidiobolus coronatus*, and *Conidiobolus incongruus* is also presented.

TAXONOMY OF THE *ZYGOMYCETES*

The taxonomic classification of living organisms seems to be in a state of constant flux. Early scientists first divided life into two broad groups; the kingdom Animalia, composed of motile organisms that derive nutrition by ingestion of food, and the kingdom Plantae, which was composed of nonmotile organisms that predominantly but not exclusively synthesized their own nutrition. Under this two-kingdom classification, fungi had been placed into the kingdom Plantae since, like plants, they were clearly nonmotile, with many having portions of their mycelium firmly implanted into their growth medium. However, they were considered to be "lower plants," since they lacked leaves and roots and failed to produce chlorophyll. This classification was suggested despite their clearly different methods of acquiring nutrients (photosynthesis in plants versus assimilation or absorption in the fungi) (246, 503).

As a result of the difficulties in assigning the various types of life-forms into these two limited kingdoms, a taxonomic classification dividing living organisms into five kingdoms was suggested (503). The kingdoms were designated Monera (prokaryotes, including bacteria, actinomycetes, and blue-green algae), Protista (single-celled eukaryotes including the protozoa and a variety of colonial organisms such as the slime molds), Plantae (eukaryotes that develop from embryos), Animalia (eukaryotes that develop from a blastula), and Fungi (eukaryotes that develop from spores). The assignment of various life forms into these five kingdoms helped in the placement of organisms that did not clearly meet the definition of either plant or animal. The assignment of fungi into their own kingdom allowed the separation of life on the basis of both their mode of reproduction and their method of deriving nutrition (503). As molecular techniques are increasingly used to investigate relatedness on the basis of the DNA sequences, it has become apparent that the kingdom Fungi is extremely diverse and that some organisms previously believed to be fungi are being assigned to other kingdoms while organisms originally assigned to other kingdoms are now being identified as fungi (446).

Fungi are eukaryotes, having a nucleus with an RNA-rich nucleolus and cytoplasmic organelles including mitochondria, vacuoles, endoplasmic reticulum, ribosomes, Golgi apparatus, and other cytoplasmic inclusions. Fungi do not have chloroplasts and do not produce chlorophyll. These organisms are delineated within the eukaryotes by their lack of flagella (nonmotile), the development of spores during asexual reproduction, and their predominantly aerobic growth requirements (251, 391, 503). The fungi produce an ergosterol-rich cell membrane and a cell wall composed of a mixture of polysaccharides including chitin, glucan, and glycoproteins. The cell wall is similar but not identical for each fungus, allowing variations in the cell wall composition to be used to differentiate one fungus form another (11, 251, 391).

The kingdom Fungi is further divided into three phyla on the basis of differences in the mode of sexual reproduction of the organisms and on the basis of morphologic features (Fig. 1). The phylum *Basidiomycota* is delineated by the formation of sexual basidiospores on the surface of a club-shaped basidium. These spores are formed by either sexual (meiosis) or asexual (mitosis) mechanisms. This phylum contains mushrooms, toad stools, puffballs, rusts, smuts, and other related organisms. It also includes human pathogens including the sexual stage of *Cryptococcus neoformans*, *Filobasidiella neoformans*. The *Ascomycota* includes the higher fungi that reproduce sexually by the production of ascospores. This phylum contains several pathogens important to humans, including the teleomorphs of the dermatophytes and *Histoplasma capsulatum* and *Blastomyces dermatitides*. A variety of other yeast and filamentous human pathogens and nonpathogens also fall into this category. The third phylum, *Zygomycota*, is composed of fungi that form coenocytic hyphae and reproduce sexually by the production of zygospores (251, 253, 392, 503). Further details about the *Zygomycota* are presented below. A catch-all category of mitosporic fungi (formerly the form phylum *Deuteromyces*) represents the "holding cell" for fungi whose sexual (teleomorph) phase has not yet been identified (11, 446). Since these fungi were identified only by their asexual phase ("mitosporic" indicating reproduction by mitosis only), they have been designated Fungi Imperfecti, or imperfect fungi. A large number of fungi, and most of the human fungal pathogens, fall into this category. Included in this group are the yeast-like fungi including the human pathogens *Candida* spp. and other related yeasts. Many filamentous fungi with septate mycelium which reproduce by formation of conidia are also thrown into this group. Included in this former "form class *Coelomycetes*" are both hyaline and dematiaceous fungi. Important members of this group include the agents of aspergillosis, penicillin producers, the agents of subcutaneous mycosis and chromoblastomycosis, and other fungi. It is believed that with the use of molecular techniques, these organisms will eventually be linked with their sexual phase and reassigned into their correct phyla of the kingdom Fungi (446). Neither the form phylum *Deuteromyces* nor its form classes are recognized taxonomic designations any longer (11, 446).

The zygomycetes fall into a distinctive phylum, the phylum *Zygomycota*. It is composed of the organisms that are characterized by the formation of wide ribbon-like aseptate hyaline hyphae (coenocytic hyphae) and sexual reproduction with the formation of zygospores. This phylum is divided into two classes, the *Trichomycetes*, which are obligate symbionts of arthropods and contain no human pathogens (11), and the *Zygomycetes*, the class containing the human pathogens. This class is subdivided into two orders, which contain the agents of human zygomycosis, the *Mucorales* and *Entomophthorales* (251) (Fig. 1).

Traditionally, the *Mucorales* are divided into six families of significance in causing human or animal disease: *Mucoraceae*, *Cunninghamellaceae*, *Saksenaea*, *Thamnidiaceae*, *Syncephalastraceae*, and *Mortierellaceae* (Fig. 1). Under this classification system, the vast majority of human zygomycotic disease is caused by the members of the family *Mucoraceae*. Members of this family include zygomycetes that produce asexual sporangiospores in a sack-like structure called sporangia. A more recently proposed reclassification of the *Mucorales* by von Arx (481) places the mucoraceous zygomycetes into seven families containing human pathogens. In addition to the six families

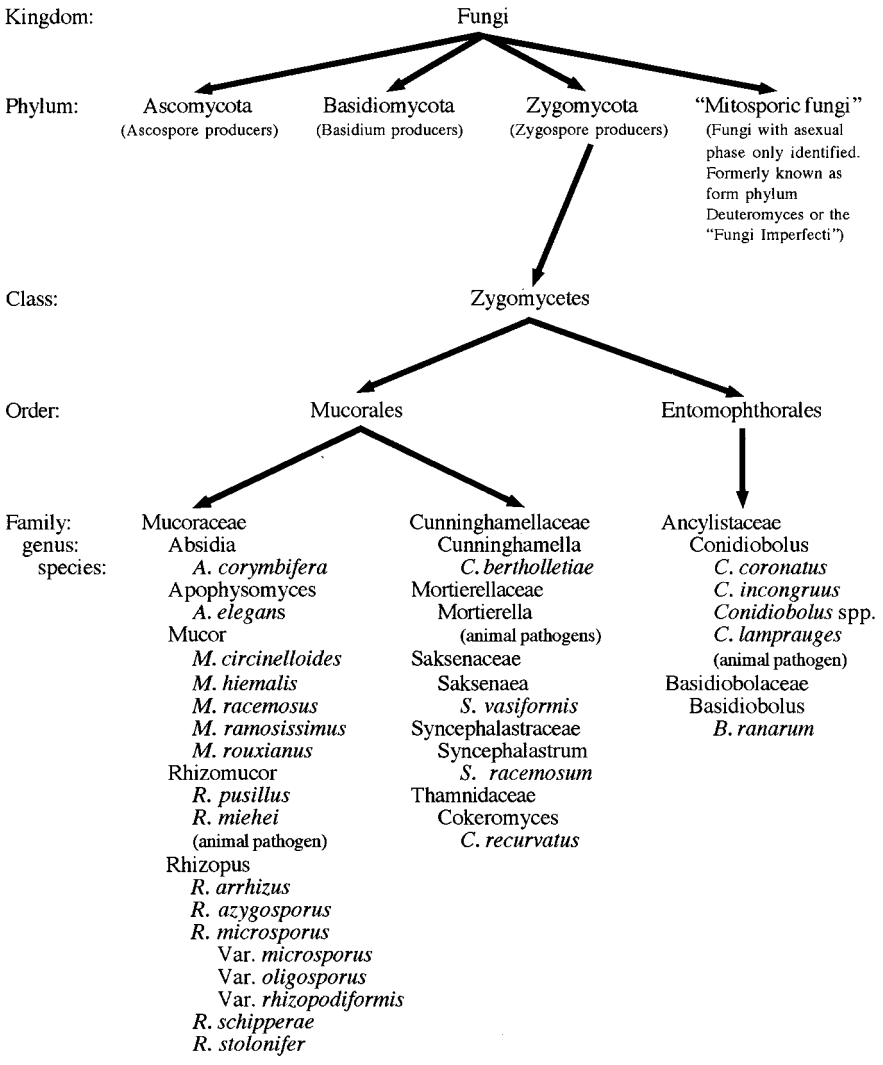


FIG. 1. Taxonomic organization of the zygomycetes.

mentioned in the traditional classification system, the family *Absidiaceae* is added based upon the presence of an apophysis, the widening of the terminal portion of the sporangiophore during sporangium formation (see Fig. 6). von Arx defined the family *Mucoraceae* as nonapophysate sporangium producers that may or may not produce stolons and rhizoids and includes in this family members of the genera *Mucor* and *Rhizomucor* (481). The proposed family *Absidiaceae* contains the zygomycetes that produce apophysate sporangia with deliquescent (dissolving) or persistent sporangial walls, produce both stolons and rhizoids, and produce zygospores with opposed suspensors. The most common pathogens in this family are in the genera *Rhizopus* and *Absidia*. Most of the recent texts on mycology adhere to the traditional taxonomic scheme in Fig. 1 (251, 446), with only a very few authors supporting the suggested reclassification scheme (11). The reader is alerted to the possibility that this reclassification may become the accepted nomenclature in time, particularly with better dissemination of its proposal.

In the family(ies) *Mucoraceae/Absidiaceae*, members of the genera *Rhizopus*, *Mucor*, *Absidia*, *Rhizomucor*, and *Apophysomyces* have all been implicated in causing human disease (251). Overall, *Rhizopus* species are the most commonly implicated organisms causing zygomycosis in humans (253, 279, 392, 455). In the family *Cunninghamellaceae*, only one species, *Cunninghamella bertholletiae*, has so far been proven to infect humans (495, 496). The monotypic genus *Saksenaea* contains *Saksenaea vasiformis* as its only member (400). *Cokeromyces* is likewise monotypic. *Cokeromyces recurvatus* is an unusual clinical isolate which can colonize the human colon and genitourinary tract (13, 25, 232, 289, 387). *Syncephalastrum racemosum* and *Mortierella wolfii* are two zygomycete isolates whose designations as human pathogens are tenuous. Of the few reports of these organisms causing human disease, several have been refuted as misidentifications of the organism involved and others lack complete substantiating evidence of correct identification. *S. racemosum* still has a single case report supporting some minimal human pathogenicity (226), while *Mortierella* should no longer be credited as causing human disease. We present *M. wolfii* in this paper to clarify the conflicting information on its pathogenicity seen in the literature.

The order *Entomophthorales* has two families that contain human pathogens, *Ancylistaceae* and *Basidiobolaceae* (Fig. 1) (11, 252). Similar to all zygomycetes, the *Entomophthorales* are

characterized by the production of coenocytic hyphae and by their sexual reproduction by production of zygospores (11, 252). The *Entomophthorales* are distinguished from the *Mucorales* by their production of actively expelled asexual sporangioles and by their markedly compact and glabrous mycelial morphology. Both of these features define this order within the class *Zygomycetes* (11, 252). Although several species of *Basidiobolus* exist in nature, all cases of human disease are now known to be caused by *Basidiobolus ranarum* (35, 175). *Conidiobolus* contains several species that are pathogenic to mammals. *Conidiobolus coronatus* is the major human pathogen (240, 252). *C. incongruus* has also been implicated in several relatively invasive infections in humans (63, 136, 487). *C. lamprauges* is pathogenic only to horses (208, 392). A single human case of a *Conidiobolus* infection by another member of the species has also been described (214).

Initial designation of the diseases associated with the *Zygomycetes* reflected the predominance of the *Mucorales* in causing disease in humans. The term "mucormycosis" was commonly used to describe disease caused by these agents. This term, however, ignored the important role that the *Entomophthorales* play in causing disease. The use of the term "mucormycosis" additionally led physicians to identify the zygomycetes found in tissue sections as *Mucor* when culture confirmation was lacking. Indeed, most of the human pathogens have, at one time or another, been considered to be of the genus *Mucor* (*M. corymbifera*, *M. rhizopodiformis*, *M. pusillus*, and *M. ramousus*). With the ever-changing taxonomy of these fungi, organisms causing zygomycosis have since been placed into the genera *Rhizopus*, *Absidia*, and *Rhizomucor*, so that *Mucor* spp. no longer represent the majority of the human pathogens. A subsequent designation of "phycomycosis" was transiently employed to encompass the members of both the orders *Mucorales* and *Entomophthorales*. The currently accepted designation is "zygomycosis," reflecting all disease processes caused by the members of the class *Zygomycetes* (141), a term that unfortunately ignores the diversity of disease caused by the organisms in these two orders.

RELATIONSHIP OF THE *ZYGOMYCETES* TO OTHER FUNGI CAUSING DISEASE

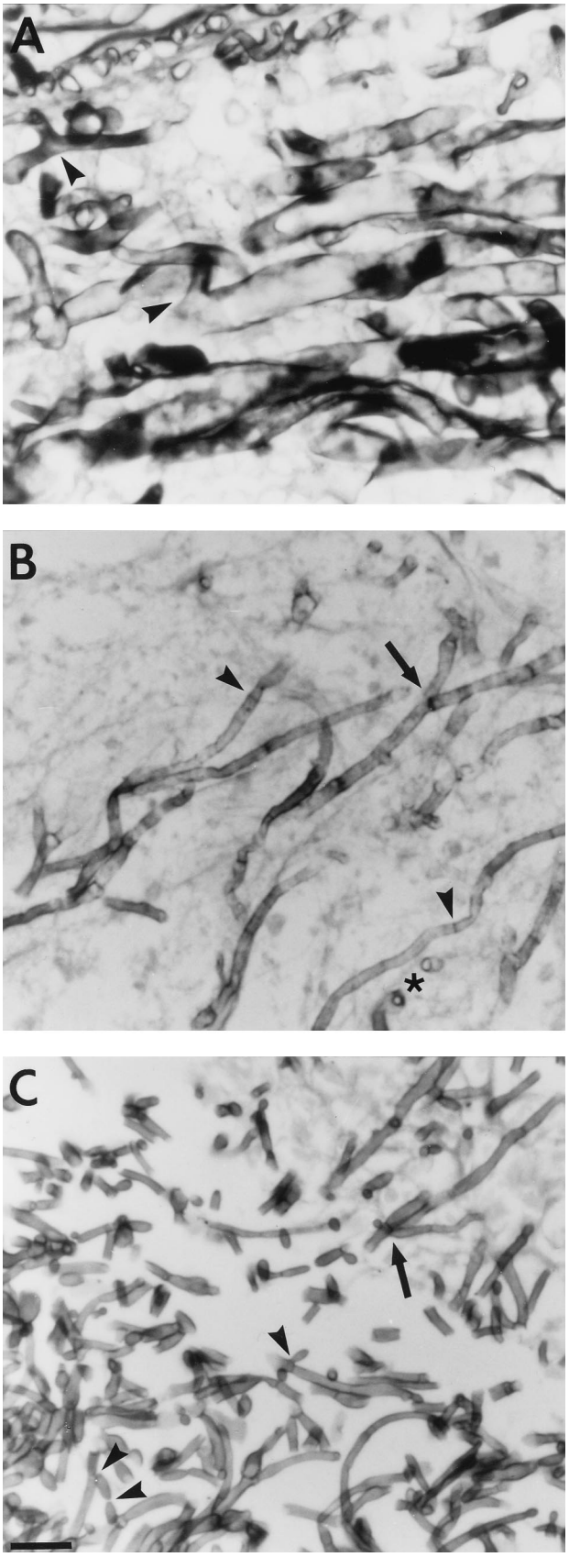
The class *Zygomycetes* contains hyaline fungi that produce wide ribbon-like, coenocytic hyphae in human tissues. Their asexual reproductive phase is characterized by the production of sporangiospores in sack-like structures. Sporangiospore formation occurs as a result of cleavage of the protoplasm inside the sporangium (free cell formation). This is distinguished from the process of conidiation, where hyphal elements are converted into conidial spores (412). Their sexual reproductive phase is marked by the production of zygospores. Sexual reproduction may occur within a single isolate (homothallic) or may require mating between oppositely oriented mating strains (heterothallic). Heterothallic sexual reproduction predominates among the members of the class *Mucorales*. Zygospore formation occurs when specialized (sexually oriented) hyphal branches called zygophores are attracted to one another. Zygophores develop near the ends of rapidly growing mycelia which secrete chemical attractants. These two elements contact one another and swell, forming the progametangia. These fuse to produce the gametangium, which undergoes plasmogamy (the mixing of cytoplasm) and karyogamy (the fusion of nuclei). The wall becomes thickened, with multiple layers, and forms the zygosporangium. It is the zygosporangium that accounts for the coloration and surface ornamentation that is characteristic for each isolate (11).

Like all fungi, the zygomycetes are eukaryotes. They lack flagella and are thus nonmotile, and they are predominantly aerobic (251, 503). While most of these organisms are considered to be saprophytic, the *Mucorales* may also be parasitic and predaceous, especially in regard to causing disease in humans and animals. In all cases, the fungi absorb their nutrients rather than synthesizing them. They require no light for growth (251, 503). Dimorphic conversion has been identified in some species (25, 30, 289, 363, 387, 443).

In comparison to the dimorphic fungal pathogens and agents causing chromoblastomycosis, the presence of the zygomycetes in clinical specimens need not always represent clinically significant isolates. The spores from asexual reproduction are easily airborne and may be demonstrated during sampling of both outdoor and indoor air (27, 51, 107, 265, 329). The small sporangiospore size (mean size, 6.6 μm) allows easy dissemination by the airborne route. Particles of this size have a settling rate that is very low and, as a result, may remain airborne even with very slight movements in air (329). The *Mucorales* may be seen in the laboratory as clinical contaminants, presumably as a result of airborne contamination of the culture medium, or they may be seen in clinical specimens as a result of oral or nasal ingestion in food or air prior to sample collection. Growth of a zygomycete in culture may therefore not represent clinically significant invasive disease. Demonstration of invasive disease by these organisms generally requires the identification of fungal elements directly in the clinical specimen or organism growth from more than one specimen obtained from a normally sterile site (497). When isolates are obtained from nonsterile sites such as sputa, culturing the same organism from multiple specimens or culturing large numbers of colonies from these specimens might suggest the diagnosis; however, these results might also reflect superficial transient colonization. A positive culture linked to a hyphal identification in cytologic specimens or tissue sections, however, is considered diagnostic (497).

The zygomycetes may be easily differentiated from other fungal agents of infection on examination of cytologic specimens or tissue sections. Zygomycetes in respiratory specimens are distinguished from the dimorphic fungal pathogens and yeasts since they do not produce a yeast phase in this site. They may be differentiated from dematiaceous fungi in clinical specimens by their lack of both darkly pigmented vegetative mycelium and septate hyphae. The major differentiation must be made between the zygomycetes, the other hyaline filamentous fungi, and members of the genus *Candida*. The morphology of the hyphae is important in making this distinction (Fig. 2). Zygomycetes produce wide, coenocytic, ribbon-like hyphae with wide-angle branching (Fig. 2A), while the other filamentous fungi (often times *Aspergillus* spp.) present as septate hyphae (Fig. 2B). *Candida* spp. produce pseudohyphae and blastoconidia in clinical specimens (Fig. 2C). Features that help to differentiate these fungal pathogens microscopically are summarized in Table 1.

For the most part, the *Mucorales* are considered to be opportunistic pathogens. They require a breakdown in the immune defenses, particularly disease processes that lead to neutropenia or neutrophil dysfunction. Although neutrophil dysfunction induced by ketoacidosis underlies the majority of cases of human zygomycosis, neutropenia induced by bone marrow suppression during chemotherapy or immunosuppression induced following transplantation is causing a growing proportion of cases (60, 160, 279, 308, 455). Disease is occasionally seen in competent hosts, however, usually associated with antibiotic use or a breakdown in the mucocutaneous barrier. The *Mucorales* are relatively uncommon causes of invasive



human infections, falling far behind *Aspergillus* spp., *Candida* spp., and other opportunistic yeasts. In several large studies of the occurrence of fungal infections in high-risk populations, infections with the agents of zygomycosis represented only 5 to 12% of all fungal infections (24, 182, 482). In an occasional study, zygomycosis represents up to 25 to 44% of all invasive fungal disease (318, 358). The zygomycetes should be in the differential diagnosis for causing disease in the immunocompromised host. Similar to *Aspergillus* spp. and *Candida* spp., the zygomycetes will cause a spectrum of disease, often leading to angioinvasion and systemic dissemination.

EPIDEMIOLOGY OF THE *ZYGOMYCETES*

Modes of Transmission

The major mode of disease transmission for the zygomycetes is presumed to be via inhalation of spores from environmental sources. Experimentally, when rabbits were infected by nasal instillation of a spore solution, they developed upper and lower respiratory disease with subsequent spread to the central nervous system (32, 382, 485). Inhalation of spores in dust likewise provides the exposure seen in the allergic interstitial pneumonitis or alveolitis syndrome seen in employees of both the malt and lumber industries (12, 34, 333, 509) and in outbreaks of rhinocerebral or pulmonary zygomycosis linked to excavation, construction, or contaminated air conditioning filters (113, 151, 271, 313).

Percutaneous routes of exposure are also very important in causing infection by the zygomycetes. Traumatic implantation of spores in dirt has been seen in a number of patients (6, 9, 66, 77, 93, 171, 178, 207, 210, 276, 280, 309, 342, 350, 453). Needlestick exposures have been implicated in zygomycotic infections occurring at the site of medicine injection (77, 158, 215, 361, 474), catheter insertion sites (160, 234, 237, 262, 332), injection sites for illicit drug use (7, 163, 189, 205, 365, 401, 426, 465, 476, 514), and tattooing (357). Insect bites or stings have also been implicated in disease transmission in cases of cutaneous and subcutaneous zygomycosis (33, 152, 159, 199, 203, 233, 372, 494). The development of wound zygomycosis has been seen with a variety of adhesive products used in the hospital setting (52, 53, 168, 234, 292, 353, 423, 479).

The ingestion of fermented milk with dried bread products (259, 321) or fermented porridges and alcoholic drinks derived from corn may play a role in promoting gastric zygomycosis (445). Spore-contaminated herbal or homeopathic remedies have likewise been linked to gastrointestinal disease (344). A series of cases were presumably transmitted orally by spore contaminated tongue depressors used for oropharyngeal examinations in a hematology/oncology clinic (261). Consump-

FIG. 2. Microscopic morphology of *Rhizopus* spp., *Aspergillus* spp., and *Candida* spp. in tissue. (A) *Rhizopus* spp. in tissue section stained with GMS. The mucoraceous zygomycetes produce wide ribbon-like aseptate hyphae in tissues. There is a great deal of variation of hyphal width. Branching occurs at wide angles nearing 90° (arrowheads). A frothy or bubbly tissue appearance may be seen in areas of tissue where the hyphae are cross-sectioned (upper left hand corner of the frame). (B) *Aspergillus* spp. in tissue section stained with GMS. *Aspergillus* spp. produce thin hyphae with relatively consistent diameters. Hyphae are septate, with no constriction of the fungus seen at the point of septation (arrowheads). Blastoconidia are not produced, although areas where hyphae are cross-sectioned may be confused with yeast cells (asterisk). Hyphae branch at acute angles of about 45° (arrow). (C) *Candida* spp. in tissue section stained with GMS. Fungal elements in tissue appear as pseudohyphae with blastoconidia. Fungal elements constrict or "bud" at sites of septation (arrowheads). Branching occurs at acute angles (arrow). Pseudohyphae are thin, and their diameter is very similar to that seen for the true hyphae of the *Aspergillus* spp. All three panels are the same magnification. Bar, 10 μm.

TABLE 1. Differentiating features of the zygomycetes, *Aspergillus* spp., and *Candida* spp. in tissue sections

Morphologic characteristic	Zygomycetes	*Aspergillus* spp.	*Candida* spp.
Hyphal type	Aseptate or nearly aseptate hyphae; may also present as gnarled or "crinkled cellophane" balls in specimens	Septate hyphae	Pseudohyphae
Hyphal width	Variable and wide (6–16 μm wide)	Consistently thin (2–3 μm wide)	Consistently thin (2–3 μm wide)
Blastoconidia	Absent	Absent	Present
Sporulation or conidiation	Absent in tissue	May be present if infected space communicates with air	Not applicable
Angioinvasion	Present	Present	May be present

tion of moldy hay or grains is the most likely way in which infection is acquired in animals (8, 402).

Risk Factors for Developing Zygomycosis

Although healthy humans have a strong natural immunity to infection with the zygomycetes, risk factors for developing zygomycosis were recognized several decades ago. Very early on, diabetes mellitus was seen as the major risk factor for developing rhinocerebral zygomycosis (181). In the original publication by Gregory et al., two out of the three patients reported had presented with diabetic ketoacidosis and the third patient was thought to have an undiagnosed case of diabetes. This publication served as the first description of fulminant rhinocerebral zygomycosis (181).

The mechanism behind this association of diabetic ketoacidosis and the development of zygomycosis has been extensively studied in diabetic animal models using *Rhizopus arrhizus* as the representative zygomycete. The strongest association occurs during diabetic ketoacidosis. In the diabetic-rabbit model, subcutaneous inoculation of *R. arrhizus* into acutely diabetic animals leads to disseminated disease and death whereas metabolically normal rabbits contained the infection at the sites of inoculation and spontaneous healing was seen (424). Intranasal infection of animals with *R. arrhizus* spores likewise led to disseminated zygomycosis in experimentally induced diabetic animals but not in normal controls (32, 381, 483, 484). The increased risk for developing zygomycosis seems to involve two main processes: failure to suppress the germination of spores and subsequent failure to kill proliferating hyphal elements. In normal hosts, macrophages prevent the initiation of infection by phagocytosis and oxidative killing of the spores. In hosts with diabetes, the monocytes/macrophages are dysfunctional and fail to suppress this spore germination process (264, 483, 484). In the diabetic-mouse model, serum also permits spore germination, while normal sera are relatively inhibitory (483). Once infection is established, neutrophils play a pivotal role in fighting fungal infections in the normal host. Despite the large size of the hyphal elements and their subsequent inability to be ingested by the inflammatory cells, neutrophils are still able to mediate fungal killing. Neutrophils are chemotactically attracted to the hyphae on which they attach and spread. Using their oxidative cytotoxic system, neutrophils damage and kill the fungal elements without accompanying phagocytosis (119, 264). In diabetes, each of the four phases of neutrophil activation is impaired (26). Chemotaxis, the phagocytic functions (adherence and spreading), and finally the oxidative burst are all inhibited in the ketoacidotic state, essentially inducing functional neutropenia (26, 64, 310, 424).

Immune system compromise also was linked to the development of zygomycosis in the mid-1900's. Many of the original observations were made in patients with solid tumors, leukemias, and lymphomas (64, 182, 209, 297). Researchers linked the acquisition of fungal disease to the onset of neutropenia due to either the progression of the underlying disease or the chemotherapy used in the treatment of the cancerous state (60, 166). The presence of neutropenia with an absolute neutrophil count of less than 1000/μl for 1 week or more poses the major risk for these patients (60). This association was nicely demonstrated for pulmonary zygomycosis by Tedder et al. in a retrospective review of published cases (455). Prior to the 1950s, there were relatively few cases of pulmonary zygomycosis. By the 1980s, chemotherapy represented the underlying condition associated with 13% of the cases reported (455). The widespread use of steroids had also resulted in an increased incidence of zygomycosis in this population of patients chronically or acutely treated with this class of drugs (166, 438, 463). The mechanism by which the corticosteroids enhance susceptibility to developing zygomycosis is probably twofold. First, steroids suppress the normal inflammatory cell response that would otherwise occur, and second, they may induce a diabetic state (291). Other immunocompromised states such as organ or bone marrow transplantation also contribute to the underlying risk factors for developing disease with these opportunistic infections (54, 160, 190, 196, 308). The use of broad-spectrum antibiotics has also been associated with an increased risk of developing zygomycosis. Presumably, elimination of the normal flora by antibiotic use allows the fungi to establish an infection in the absence of bacterial competition (166, 291, 463). For patients in whom myelosuppressive therapies have been administered and neutropenia and fever have persisted for longer than 7 to 10 days despite antibiotic therapy, a diagnosis of a fungal infection, including zygomycosis, should be suspected (60).

In the late 1980s, physicians began to notice the occurrence of zygomycosis in patients on dialysis who were receiving deferoxamine/desferrioxamine for iron or aluminum overload (48, 102, 139, 317, 374, 510). The more liberally the iron chelator was used, the more likely zygomycosis was to develop (48). A large body of evidence supports the theory that the zygomycetes are able to utilize iron bound to iron chelators to enhance their growth (49, 50, 470, 473). The effect is most prominent in patients with chronic renal failure on dialysis, a situation which leads to the presence of ferrioxamine in the serum for an extended period (50). Even in the absence of renal failure, deferoxamine/desferrioxamine therapy carries a risk for developing zygomycosis. In the guinea pig model, ad-

ministration of deferoxamine and iron in experimentally infected animals lead to decreased survival and decreased response to amphotericin B (470). The presence of acidosis together with deferoxamine therapy may also be a fatal combination (15). By inhibiting the binding and sequestration of iron by transferrin, acidosis also serves to keep the concentrations of iron in the plasma high, allowing its use as a growth factor by the zygomycetes (23). It has also been demonstrated that iron overload states, such as hemochromatosis, even in the absence of chelator usage, may pose a slightly increased risk for the development of zygomycosis in man (153, 212, 260, 274).

Percutaneous risks for developing cutaneous and subcutaneous disease with these fungi include several methods of breakdown of the cutaneous barrier. Burn wounds have emerged as a significant risk. Aside from the obvious risk incurred by the breach in cutaneous integrity, burn wound victims are at additional risk for developing zygomycosis due to the administration of Sulfamylon cream and broad-spectrum antibiotics to prevent the occurrence of *Pseudomonas* wound infections. An overall 10-fold increase in fungal infections in burn wounds was seen since the introduction of topical antibacterial preparations in 1964 (318). Also implicated in the increased risk for developing zygomycosis in the burn patient is the development of a condition called "burn stress pseudodiabetes," which is marked by the occurrence of persistent hyperglycemia and glucosuria (22, 375).

The association between intravenous drug use and zygomycosis has also been well described (7, 189, 205, 365, 476, 514). Whether this represents the introduction of spores into the patient from the cutaneous puncture or the injection of spores contained in the illicit drugs remains a point of conjecture. The fact that many of these infections do occur at sites remote from the needle stick (heart valves and the brain are often involved) suggests that the latter theory is probably correct. The role of deficient T-cell immunity in this process is likewise unknown, but notably, several of these cases involve individuals with human immunodeficiency virus (HIV) infection (205, 476).

The risk factors associated with gastrointestinal zygomycosis are varied. Protein calorie malnutrition (261, 306, 321, 432, 445, 491), diarrhea, typhoid fever, and gastric or intestinal ulcers (5, 67, 103, 111, 222, 259, 281, 307, 321, 432), and amebic colitis (321) have all been associated with the development of gastrointestinal disease by the zygomycetes. Gastrointestinal zygomycosis only occasionally occurs in the setting of diabetes (103, 111, 222).

General Disease Manifestations

The main categories of human disease with the *Mucorales* are sinusitis/rhinocerebral, pulmonary, cutaneous/subcutaneous, gastrointestinal, and disseminated zygomycosis. Other disease states occur with a much lower frequency and include cystitis, vaginal or gastrointestinal colonization, external otitis, and allergic disease.

Rhinocerebral disease represents one-third to one-half of all cases of zygomycosis (367, 438). The process originates in the sinuses following inspiration of fungal spores. It is estimated that 70% of the cases of rhinocerebral zygomycosis occur in the setting of diabetic ketoacidosis (291). Disease starts with symptoms consistent with sinusitis. Sinus pain, drainage, and soft tissue swelling are initially seen. The disease may become rapidly progressive, extending into neighboring tissues. Involved tissues become red, then violaceous, and finally black as vessels are thrombosed and the tissues undergo necrosis. Extension into the periorbital region of the face and ultimately into the orbit are often found, even at presentation (181, 260). Periorbital edema, proptosis, and tearing are early signs of tissue involvement. Ocular or optic nerve involvement is first suggested by pain, blurring, or loss of vision in the infected eye. Cranial nerve palsies may also be seen. Extension from the sinuses into the mouth often occurs, producing painful, black, necrotic ulcerations into the hard palate. A bloody nasal discharge is generally the first sign of that the disease has invaded through the terbinates and into the brain. Patients may demonstrate an altered mental state due either to ketoacidosis or to central nervous system invasion (243). Once the eye is infected, fungal disease can readily progress up the optic nerve, again gaining access to the central nervous system. Angioinvasion is often seen and may result in systemically disseminated disease (181, 243, 260, 291, 367). Decidedly uncommon forms of rhinofacial disease published in the literature include isolated sinusitis (172) and calcified fungal ball of the sinus (176). Early cases with rhinocerebral zygomycosis were almost uniformly fatal (181, 260, 356). There is still a high mortality rate with rhinocerebral disease, but curative interventions have been made with early diagnosis and aggressive surgical and antifungal treatment (61, 356). The nature of the underlying disease is the most important determinant of survival. In a study of 179 patients with rhinocerebral zygomycosis, 75% of patients with no underlying immune compromise survived therapy while 60% of those with diabetes and only 20% of patients with other systemic disease were cured of their disease (45).

Pulmonary disease is also a common manifestation with this group of organisms. Leukemia, lymphoma, and diabetes mellitus underlie the majority of cases with primary pulmonary involvement (455). A wide variety of pulmonary disease manifestations exist. Isolated solitary nodular lesion (167), lobar involvement (40, 374, 379, 455), cavitary lesions (192, 235, 279, 301, 354, 440), and disseminated lesions (48, 286, 455) have all been described. Cases of fatal hemoptysis associated with erosion of the fungus into the pulmonary artery have also been described (192, 315, 351). Upper respiratory disease may present as tracheal involvement (17, 40) and chronic endobronchial zygomycosis (202). In many cases of pulmonary zygomycosis, the diagnosis is missed, at least initially, and the patient is treated for a bacterial pneumonia. Chest X-rays may present a lobar picture, only to reveal a granulomatous process later on. Wedge infarcts of the lung may be seen, particularly following thrombosis of the pulmonary vessels with fungal angioinvasion (279). Extensive necrosis and subsequent bleeding into the involved tissues are often seen. For isolated pulmonary disease, the death rate is lower than for zygomycosis overall (65 and 80%, respectively) (455). This is due to the availability of good surgical and medical treatments. Localized disease is more likely to be curable by surgery, providing a large survival advantage for this group of patients (117, 455). This requires early identification of disease before dissemination occurs. In a large study of patients with pulmonary zygomycosis, however, only 44% were diagnosed premortem with a 20% overall survival (455). In a study where premortem diagnosis was improved to 93%, survival rates improved but were still only 73% (356). In patients who receive no treatment, death usually results from the manifestations of disseminated disease before pulmonary failure occurs (455). The notable exception to this are those rare cases of massive hemoptysis (192, 315, 351).

Cutaneous disease may occur from primary inoculation or as a result of disseminated disease. The clinical presentation will be quite different for these two disease processes. Growth of the fungus in a preexisting lesion produces an acute inflammatory response with pus, abscess formation, tissue swelling, and

necrosis. The lesions may appear red and indurated but often progress to form black eschars. Necrotic tissue may slough off and produce large ulcers. Infections may be polymicrobic and are generally rapidly aggressive even in the face of appropriate debridement and medical treatment. Occasionally, cutaneous lesions will produce an aerial mycelium that may be visible to the naked eye. Primary cutaneous disease may be very invasive locally, involving not only the cutaneous and subcutaneous tissues but also the fat, muscle and fascial layers beneath. Necrotizing fasciitis may occur secondary to cutaneous or subcutaneous zygomycosis. When present, necrotizing fasciitis due to the *Mucorales* has a very high mortality rate (80% in one study) (362). Direct extension into adjacent bone and other tissues has also been described. With vessel invasion, frankly disseminated disease may arise. Due to the superficial site of infection, this disease manifestation is fairly likely to be diagnosed and appropriately treated. Curative surgical intervention may, however, be quite disfiguring or may require amputation of the involved limb (89, 362). Individuals with operable sites of involvement, especially limbs, are more likely to survive than those with trunk or head involvement (89). In burn patients, both superficial colonization of the eschar and invasive disease may be seen. Superficial colonization may serve as the precursor to truly invasive disease and is thus an important process to identify and treat in these patients (318). While cutaneous or wound zygomycosis may be seen with most of the zygomycetes, *Apophysomyces elegans*, *Saksenaea vasiformis*, the *Mucor* spp., *Basidiobolus ranarum*, and *Conidiobolus* spp. tend to have the greatest predilection for these sites. Cutaneous disease arising from disseminated disease, in comparison to the wound zygomycosis, usually presents as nodular subcutaneous lesions which may ulcerate.

Disseminated zygomycosis may originate from any of the primary sites of infection. Lung involvement is the single most common site of infection associated with disseminated disease, however. In the infected host, the zygomycetes rapidly invade vessels and may metastasize wildly by the hematogenous route. There is a very high death rate associated with this disease manifestation, with 96% mortality noted in one study (455) and 100% in another (438).

Gastrointestinal disease caused by the zygomycetes is relatively uncommon. Two syndromes have been described with gastric involvement. Gastric colonization occurs with superficial involvement in ulcerative lesions. These colonized ulcers often have a velvety surface texture. This syndrome is marked by a lack of vessel invasion and a good survival rate (259). Invasive zygomycosis has been identified more commonly and presents with fungal invasion into the mucosa, submucosa, and vessels. Necrotic gastric (5, 103, 139, 222, 259, 314, 491) or intestinal (67, 263, 306, 307) ulcers are produced, which may rupture, causing peritonitis. This disease manifestation is often fatal, with only 2% of patients surviving the infection (281, 511).

The zygomycetes may cause infection in virtually any body site. Brain involvement in the absence of sinus involvement has also been demonstrated, primarily in the intravenous drug abusers (7, 189, 514). Isolated renal involvement has been described in several cases, again associated with intravenous drug use (476). Cardiac infections may occur as a manifestation of disease disseminated from the lungs (358, 478) or secondary to intravenous drug use (7, 189) or may occur as an isolated cardiac mycosis (478). Of 60 cases of fungal cardiac infection, 12% represented disease by the zygomycetes (24). Several cases of postcardiac surgery zygomycosis have also been noted (24, 478). Isolated pleural involvement following a surgical intervention has also been seen (180).

Other disease manifestations are decidedly uncommon and involve predominantly noninvasive infection. Oncomycosis has been seen with infections due to *Mucor circinelloides* (443), while external otitis has been seen with infections due to *Mucor* spp. (78, 209, 355) and *Absidia corymbifera* (200).

TREATMENT

Treatment of zygomycosis requires several simultaneous approaches: surgical intervention, antifungal therapy, and medical management or correction of the underlying condition that is predisposing the patient to the disease. A retrospective study of 255 cases of pulmonary disease compared patient survival for individuals treated with or without surgical interventions. Surgical resection for patients with isolated pulmonary disease greatly improved survival compared to that of patients who received antifungal therapy alone (455). Response to surgery is best in cases of localized disease without dissemination (117, 455). Although there are several case reports of a single treatment modality producing cure, these are the exception and not the rule. There are notable cases of zygomycosis being cured by surgery only (77, 373, 455, 462). Similarly, antifungal therapy alone has been used successfully when surgical intervention is not possible or not preferable due to the site of the infection (159, 272, 293, 324, 357, 372, 477, 498). The vast majority of cases where successful treatment has been administered link aggressive surgical intervention with antifungal therapy and rigorous medical management of the patient.

Amphotericin B is the first-line drug of choice for most cases of zygomycosis caused by the *Mucorales*. Amphotericin mediates its antifungal action by modifying fungal cell walls. This drug binds to ergosterol and causes increased cell wall permeability. With permeabilization, ions leak from the cell and the membrane depolarizes. Lethal effects of amphotericin B occur at concentrations of drug higher than that causing increased permeability (55, 360). Secondary or indirect mechanisms responsible for its lethal antifungal action include the stimulation of the oxidative pathway in the immune response. Monocyte/macrophage stimulation by amphotericin B increases production of hydrogen peroxide and free radicals, which may then have killing action on the fungal elements, again mediated by cell wall changes (55). Amphotericin B is not effective in treatment of all cases, particularly if the patient presents late in the disease course and has inoperable or disseminated disease. The therapeutic activity of amphotericin B is limited by its potentially severe side effects. Impaired renal function often leads to cessation of therapy. The liposomal preparation of amphotericin B may help to alleviate this problem and allow for higher doses of medication to be administered (269). Although synergism of amphotericin B and rifampin in treating zygomycosis has been suggested by some authors (84, 324, 398), this has not been conclusively demonstrated in clinical trials.

Antifungal therapy has produced variable results, dependent upon the organism and the drug selected. It has become evident that the azoles should not be used in treating zygomycosis due to the lack of both in vitro and in vivo susceptibility (149, 348, 434). Early successful attempts at antifungal therapy for infection with the *Mucorales* included the use of oral saturated potassium iodide and local applications of tincture of iodide for cutaneous sites (98, 221, 398). This mirrors the treatment regimen successfully used to treat infections with *Basidiobolus* and *Conidiobolus* spp. (454). Similarly, topical treatment of *Mucor*-associated otomycosis has been optimal with mercurochrome applications (preferable to clotrimazole and miconazole) after adequate cleaning of the auditory canal (78). Oils

applied topically into the auditory canal have also been reported to be sporostatic (216). All the zygomycetes tested demonstrated resistance to saperconazole (347), 5-fluorocytosine, and naftitfine (348, 434). Assessing the impact that isolated pharmacological therapy has had on patient outcome is impossible since multiple simultaneous interventions are used for most patients. With *Rhizopus* spp. as the representative for the zygomycete infections, in vitro studies have demonstrated a lack of effect by pneumocadin L-743,872 (114), 5-fluorocytosine, and the echinocadins (364) in inhibiting fungal growth.

Hyperbaric oxygen treatments have been touted as an appropriate addition to standard surgical, medical, and antifungal therapy, particularly for rhinocerebral disease. Unfortunately, there has not been any extensive experience with this treatment modality. In a retrospective analysis of the cases seen in a single medical center, the addition of hyperbaric oxygen treatments to the standard treatments improved survival in the patients who received them. While four of the seven patients not receiving hyperbaric oxygen therapy died, only two of the six patients who received this treatment died (157). The mechanism behind the success of this therapy rests upon the presumed improved neutrophilic killing achieved by the higher oxygen delivery achieved in these patients (100). Additionally, the direct growth-suppressive effects of 10 atm of oxygen may play a role in successful treatment. Hyperbaric oxygen either delays or totally inhibits the growth of fungal spores and mycelium in vitro (393). The addition of hyperbaric oxygen treatment to systemic drug therapy could aid in direct fungal killing or at least diminish the fungal growth rate, allowing the natural host immune defenses to recover. Other scattered case reports also support the use of hyperbaric oxygen (178, 322, 342) and suggest that further trials with this treatment modality may be warranted.

Treatment of the underlying disease process placing the patient at risk for opportunistic infections with the zygomycetes can not be underestimated. Correction of diabetic ketoacidosis helps to restore neutrophil function that is temporarily impaired by the acidotic environment (64, 310). Occasionally, extraordinary measures such as continuous infusion of insulin have been utilized (504). Anecdotal reports of improved recovery from infection by correcting neutropenia either with granulocyte transfusions (47, 398) or by enhancing endogenous neutrophil production using growth factors (174, 262, 266) have been published, but the data are insufficient to suggest that this should be the standard of care. Discontinuation of iron chelation therapy or immunosuppressive therapy, particularly steroids, is often warranted in patients receiving these therapies when a diagnosis of zygomycosis is made.

PREVENTIVE MEASURES

Measures to decrease the incidence of zygomycosis in patients at risk are difficult at best. There is no routine antifungal prophylaxis available, and with the low prevalence of zygomycosis, there is no real indication to provide it. The most common preventive interventions attempted regard modifications and controls in the environment that reduce the risk of exposure to airborne spores. Most of these control measures are focused on easily identified patients at risk, i.e., those expected to be profoundly neutropenic for prolonged periods. Transplantation and chemotherapeutic wards are often isolated with Hepafilter treatment of the air supply and positive pressure to exclude the recruitment of dust into the ward. Dust should be kept to a minimum in the environment that houses these neutropenic patients. Additionally, flower arrangements and live plants are often excluded from such wards since they may harbor a variety of fungal agents. Patients when neutropenic below 1,000/ml are asked to wear masks when leaving the cancer or transplant wards, particularly when going outside. The monitoring of air quality, particularly during times of building renovation and excavation in the vicinity of transplant centers, is also important on infection control measure.

Preventive measures for patients other than the transplant and chemotherapy population require addressing the underlying risk factors for developing zygomycosis. Adequate control of diabetes, the use of iron chelators other than deferoxamine, limiting the use of aluminum-containing buffers in dialysis, and aggressive direct and culture-based detection of zygomycosis are among the best preventive measures that may be taken. Keeping a high level of suspicion for zygomycosis in patients who are at risk can aid an early diagnosis and implementation of appropriate therapy.

GENERAL DIAGNOSTIC FEATURES OF THE *ZYGOMYCETES*

Microscopic Examination of Clinical Specimens

Demonstration of fungal elements from cytologic preparations (i.e., sputa, inflammatory fluid aspirates from abscesses or sinusitis infection, and genitourinary and gynecologic specimens) may be quite difficult due to the difficulty in extracting fungal elements from invaded tissues. Fungal elements may be rare in cytologic specimens and when present are often fragmented. Additionally, hyphae may be very focal and may appear in only part of the specimen. The key features associated with the *Zygomycetes* on direct examination of cytologic specimens is the presence of wide, ribbon-like aseptate, hyaline hyphal elements, often in the setting of extensive necrotic debris. The width of the hyphal element varies substantially. Branching of the hyphae is seen, with wide-angle (generally around 90°) bifurcations noted. Yeast formation (blastoconidial formation) has been observed with *Cokeromyces recurvatus*, which presents as budding yeast that may be confused with *Paracoccidioides braziliensis* (102, 289, 387). In addition, some *Mucor* species are dimorphic, producing budding yeast at 37°C (443).

The stains most commonly used in identifying yeast directly from patient cytologic specimens include calcofluor white stain, Gomori methenamine silver stain (GMS), periodic acid shiff (PAS), Gram's stain, and Papanicolaou stain (PAP stain). Although the zygomycetes may be demonstrated on either PAP or Gram's stain, these are not generally the stains of choice. Demonstration of the fungal elements with fungal specific stains such as calcofluor white or GMS is recommended. Phase-contrast microscopy, although not widely used, has also been successful in identifying fungal elements in cytologic clinical specimens (394).

The diagnosis of zygomycosis is easily made on tissue section. Involved tissue demonstrates focal areas of infection and may appear nodular or may produce extensive areas of necrosis with accompanying hemorrhage into the tissue. Abscess formation with central tissue necrosis, acute inflammatory exudate, and peripheral tissue invasion by hyphal elements is quite common. An acute inflammatory exudate often accompanies these infections in nonneutropenic patients (Fig. 3A). Invasion of the blood vessels (angioinvasion) by hyphal elements is generally seen in infections with the *Mucorales* (Fig. 3) but usually not with the *Entomophthorales*. The hallmark of a zygomycosis includes the demonstration of wide, ribbon-like,

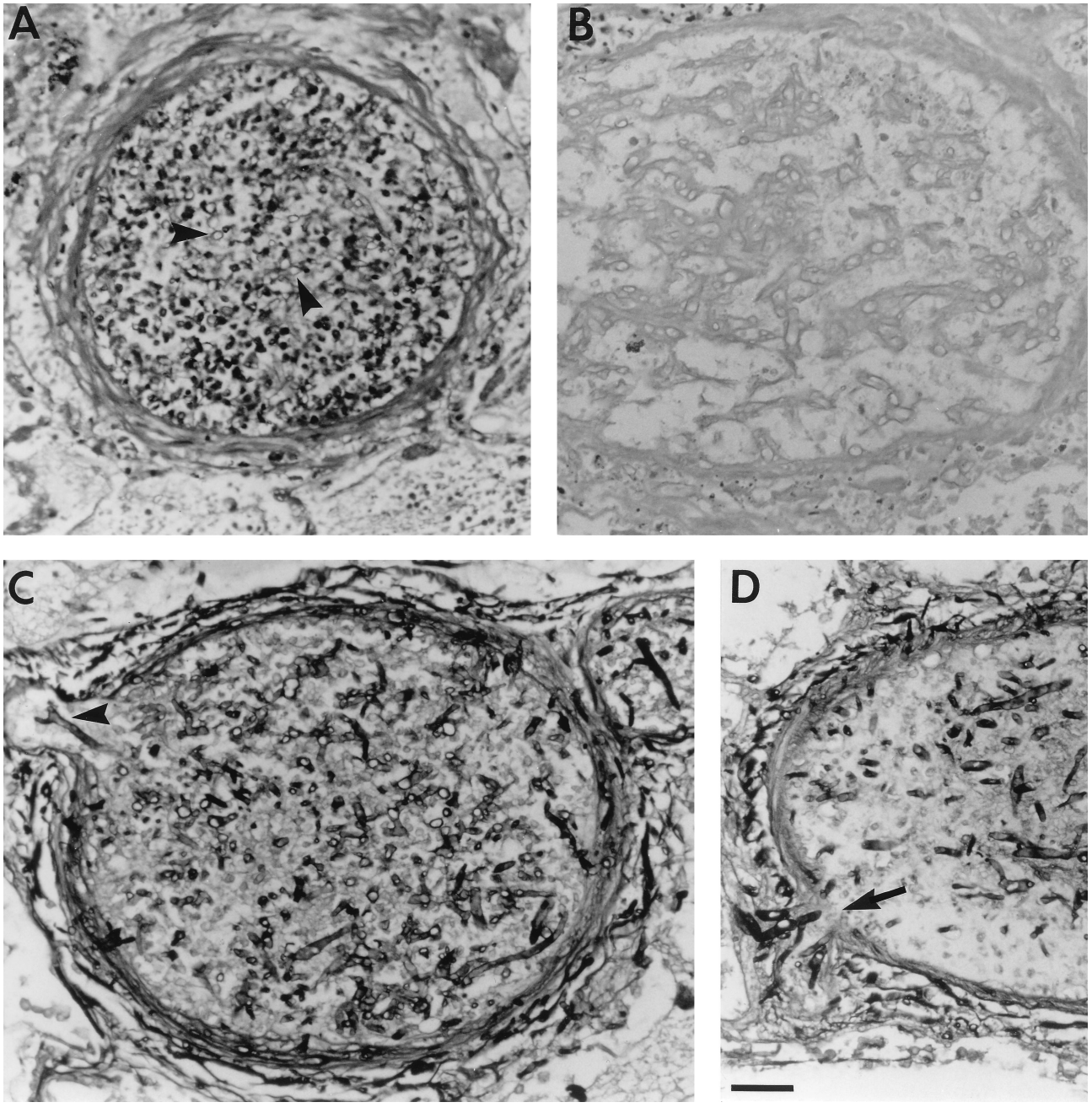


FIG. 3. Angioinvasion by mucoraceous zygomycetes. (A) H&E-stained section of a vessel filled with hyphae and inflammatory cells. Tissues infected with the mucoraceous zygomycetes often demonstrate extensive angioinvasion by fungal elements. Neutrophils are the predominant inflammatory cells responding to an infection with these agents. The inflammatory cells stain basophilic (dark), while the hyphae demonstrate an inconspicuous hyaline staining that can often be overlooked. Pale-staining hyphae (arrowheads) often look like hole or bubbles in the tissue. The hyphal elements are nearly obscured on this H&E section due to the inflammatory process seen. For these reasons, it is recommended that a fungus-specific stain such as GMS or PAS be performed to better characterize fungal elements in tissues having thrombosed vessels, extensive tissue necrosis, or hemorrhage. (B) H&E-stained section of a vessel with typical zygomycete hyphal elements in a patient with neutropenia. In contrast to panel A, the hyphal elements are much more prominent in this H&E preparation despite their hyaline staining, due largely to the absence of the inflammatory cells. (C) GMS-stained section of a vessel containing coenocytic fungal hyphae typical of the zygomycetes. Wide ribbon-like hyphae with broad-angle branching is seen (arrowhead). GMS provides excellent contrast to help distinguish hyphae, which stain black or dark gray, from the tissue elements or inflammatory cells, which stain with the pale counterstain. (D) GMS-stained section of a vessel with fungal hyphae penetrating the vessel wall. Infections originate in mucocutaneous sites, from which they may spread hematogenously. Fungal elements invade through the vessel walls from their tissue sites. Mycotic emboli disseminate and may thrombose small vessels in which they are lodged. Fungal elements may invade normal or devitalized tissues at these remote sites by directly penetrating the vessel wall (arrow). All four panels are the same magnification. Bar, 50 μm.

hyaline, predominantly aseptate hyphae with wide-angle (45 to 90°) branching (Fig. 2A and 3C). The hyphae often are not preserved well and may become crinkled or gnarled in the tissue sections. This is often referred to as a "crinkled cellophane" appearance of the hyphal elements. To the inexperienced observer, these artifactual folds in the hyphae may be confused with septations. Cross sections of hyphal elements often give tissues a vacuolated appearance. These cross sec-

tions vary in diameter and may be confused with yeast cells (Fig. 2A). In hematoxylin and eosin (H&E)-stained tissue section, the *Entomophthorales* demonstrate hyphal encasement by eosinophilic material. This Splenore-Hoeppli material may be the first indication that a patient has an infection with either *Basidiobolus* or *Conidiobolus* instead of one of the *Mucorales*, which rarely demonstrate this phenomenon in tissues.

In comparison to the other hyaline molds that cause disease in man, the zygomycetes stain more poorly or inconsistently with the stains typically used. In H&E-stained tissue sections, zygomycetes generally stain pink with empty or clear lumina. The infected tissue may appear frothy due to these clear spaces. Hyphae may be difficult to demonstrate convincingly using this stain. The diagnosis is more likely to be suggested by the bubbly and frothy appearance generated by the nonstaining hyphal interior than by the hyaline staining of the hyphae themselves (Fig. 3). Variability in staining with H&E may be seen. Although the zygomycete elements usually stain pink (eosinophilic) with H&E, purple (hematoxylyniophilic) staining may also be noted. The H&E stain should always be confirmed with a more fungus-specific tissue stain such as GMS or PAS. Although either of these stains will readily demonstrate zygomycete elements, the relative staining may be less intense than would be seen with other hyaline fungi. There is also some variability in staining intensity within a single clinical specimen. Sporulation in tissues, if it truly does occur, is exceedingly rare.

General Culture Characteristics of the *Mucorales*

Growth characteristics. The *Mucorales* grow well on both nonselective and fungal selective media. When fungal infections are suspected, it is recommended that fungal selective media be used to suppress the growth of bacterial elements potentially present in the sample (e.g., Inhibitory mold agar). The growth of the *Mucorales* tends to be rapid, with mycelial elements expanding to cover the entire plate in only a few (1 to 7) days. Organisms of the order *Mucorales* are characterized by an erect aerial mycelium that is described as fibrous or "cotton candy-like." The mycelium tends to be quite high, with some isolates reaching the lid of the petri dish at mature growth. It is this vigorous growth characteristic that is responsible for the group being designated "lid lifters" (Fig. 4A and C). These organisms are hyaline, with the reverse side of the plate demonstrating light coloration (tan to yellow for most species) (Fig. 4D). A great deal of inter- and intraspecies variation may be seen in height, rate of growth, and degree of pigmentation.

The recovery of zygomycetes from tissues may be problematic. Countless reports of negative culture results on both autopsy and premortem cultures are scattered throughout the literature. The reason for these negative cultures appears to be the aggressive processing of the specimen that occurs before plating. The practice of tissue grinding has largely been replaced by the "stomaching," process which is less likely to render the fragile zygomycete elements nonviable. Better recovery is seen if slices of minimally manipulated tissue are placed onto the culture medium or baited with bread to promote mycelial growth (386). When stomached specimens are plated, one or more pieces of tissue should be included on each culture plate to increase the culture yield. Despite aggressive invasion of vessels by the zygomycetes, blood cultures are rarely positive (317), especially with the liquid culture systems.

Sporulation characteristics. The sporulating surface of the colonies may demonstrate variable degrees of coloration. Depending upon the order, species, or individual isolate, zygomycetes will demonstrate surface coloration varying from pure white to tan, brown, grey, or even black (see Tables 2 through 4). Since it is the presence of the sporulating structures and their contents that generally imparts the coloration, areas of growth that lack the sporangia and related structures will remain light-colored. The production of pigmented zygospores may also have an influence on the overall pigmentation of an isolate. Some *Rhizopus* spp. may additionally produce lightly pigmented mycelia in culture, which will influence the overall colony pigmentation. While most members of the *Mucoraceae* readily sporulate on standard media such as Sabouraud dextrose, cornmeal, or potato dextrose agar, *Apophysomyces elegans*, *Saksenaea vasiformis*, and *Mortierella wolfii* may produce only sterile hyphae under these routine culture conditions. Sporulation with these isolates may be stimulated using the technique described by Ellis and Ajello (145). Cultures initially grown on cornmeal-sucrose-yeast extract agar are starved by being transferred to 1% agar in water. After growth under these minimal-nutrient conditions, the mycelium is stimulated to sporulate and a definitive microscopic identification can be made. Other minimally nutritive support media may also be used to stimulate sporulation; these include hay, straw, grass, and soil infusion agars. Inoculation of these organisms at temperatures ranging from room temperature up to 37°C has also been used. Even under optimal growth conditions, some isolates will fail to sporulate or may take several weeks to do so.

Preparation of specimens for microscopy. Definitive diagnosis and speciation of the zygomycetes requires microscopic identification of the mycelium and its associated sexual and asexual reproductive elements. This is done in most laboratories by the preparation of lactophenol cotton blue-stained slides. Although the "tape prep" is used in many laboratories for its ease of preparation and good preservation of the fungal morphology, it is being replaced by the "tease prep" for biological safety reasons. For this reason, we will focus on the tease prep as the rapid preparation of choice. Handling the cultures under a biological safety cabinet, a sterile wire is used to gouge a sample of mycelium from the variously pigmented areas of the colony. The fungal elements are placed on a glass slide with lactophenol cotton blue stain and gently teased apart with a second wire or needle. When adequately dispersed, the mycelium is covered with a coverslip and viewed by light microscopy. Due to the process of teasing, many of the characteristic morphologic features may be lost, so care must be taken to not be too aggressive with this step in the preparation.

An alternative to the quick tease prep is the traditional slide culture. Slide cultures remain a valuable diagnostic tool despite being labor-intensive and requiring prolonged growth. Slide cultures are often prepared when excellent-quality morphologic preservation of the fungus is required for either diagnosis or photography. For this technique, cultures are grown on cornmeal or potato dextrose agar. Cubes of agar are placed in a clean petri dish and inoculated with the organism. The agar is covered with a sterile coverslip. During incubation, new growth, in part, adheres to the coverslip. When sufficient new growth is seen, the coverslip is carefully transferred to a clean glass slide with lactophenol cotton blue stain. This technique may be used for the microscopic analysis of the zygomycetes with some success, although the adherence of the mycelium to the coverslip may be problematic in some cases.

Morphologic identification by sporulation characteristics. The *Mucorales* use several techniques for reproduction (Fig. 5) (412). The family *Mucoraceae* may be divided into three groups on the basis of the morphology of the predominant asexual spore-producing structures; sporangium producers, sporangiola producers, and merosporangium producers. Sporangia are defined as sack-like structures from which asexually pro-

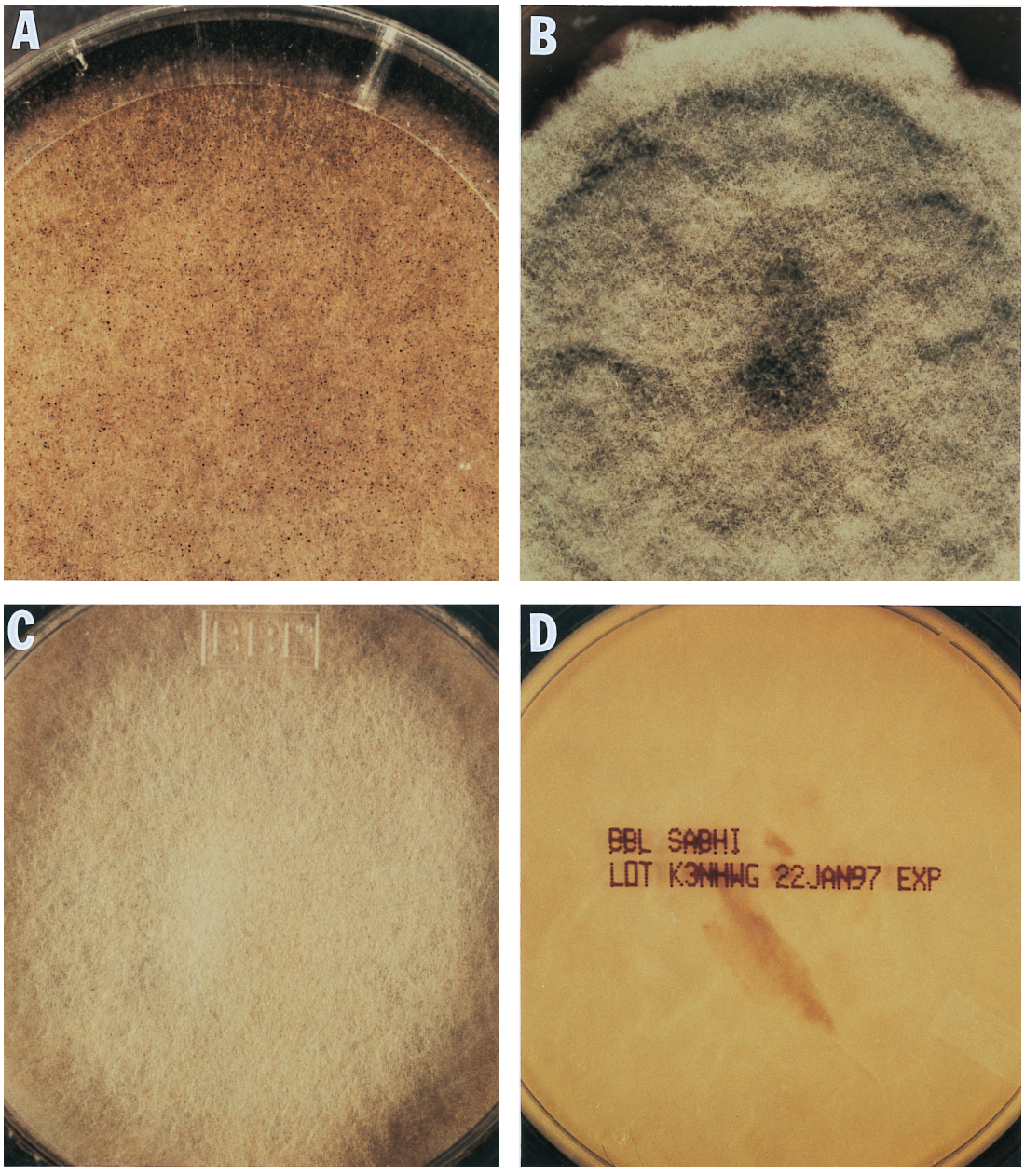


FIG. 4. Gross morphology of *Rhizopus*, *Mucor*, and *Absidia* isolates in culture. (A) *Rhizopus* gross morphology on SABHI medium. *Rhizopus* spp. typically produce a very high, fibrous colony that rapidly fills the entire petri dish. This isolate has expanded to the lid (known as a "lid lifter"). It has produced abundant pigmented sporangia, which are seen as the dark areas peppering the otherwise pale mycelium. This morphology is characteristic of the *Rhizopus* spp. (B) Low-growing *Mucor* variant gross morphology on SABHI medium. *Mucor* spp. will show variation from culture to culture. This particular isolate has produced a low-growing, fibrous colony morphology that readily demonstrates the "woolly" or floccose growth characteristic of the *Mucoraceae*. Pigmentation is also variable, both within and among the *Mucor* spp. Increased pigmentation is generally reflective of areas of the mycelium that are rich in sporangia. Depending on the individual isolate, *Mucor* may be extremely floccose or low growing and may range from pure white to shades of gray or brown. (C) *Absidia corymbifera* gross colony morphology on SABHI medium. *A. corymbifera* produces a light-colored mycelium, generally cream or gray. The peppered appearance seen in the *Rhizopus* spp. is lacking despite the production of abundant sporangia. The mycelium of this isolate is firmly plastered to the lid of the petri dish, consistent with the lid-lifting properly of this fungus. (D) *A. corymbifera*, reverse side of the culture plate shown in panel C. The *Mucoraceae* are hyaline molds that produce a pale reverse in culture on standard media such as SABHI agar. Although some isolates such as *Rhizopus* spp. may have lightly pigmented hyphal elements, this is generally reflected as a pale yellow or brown reverse and not the darkly pigmented reverse of the dematiaceous fungi.

duced sproangiospores are passively released (Fig. 5A). Sporangiole producers are characterized by the production of single-celled (Fig. 5C) or multiple-celled (Fig. 5D) asexual structures which form on stalks borne from swollen vesicles. Merosporangium producers are characterized by spore formation within a tubular sack called a merosporangium (Fig. 5B). These are produced surrounding a swollen vesicle similar to the sporangiole producers. Some isolates may additionally reproduce asexually by the production of chlamydoconida (Fig. 5F), gemmae (Fig. 5G), and budding-yeast forms (Fig. 5E). The production of zygospores (Fig. 5H) during sexual reproduction is also an important feature of the zygomycetes, hence

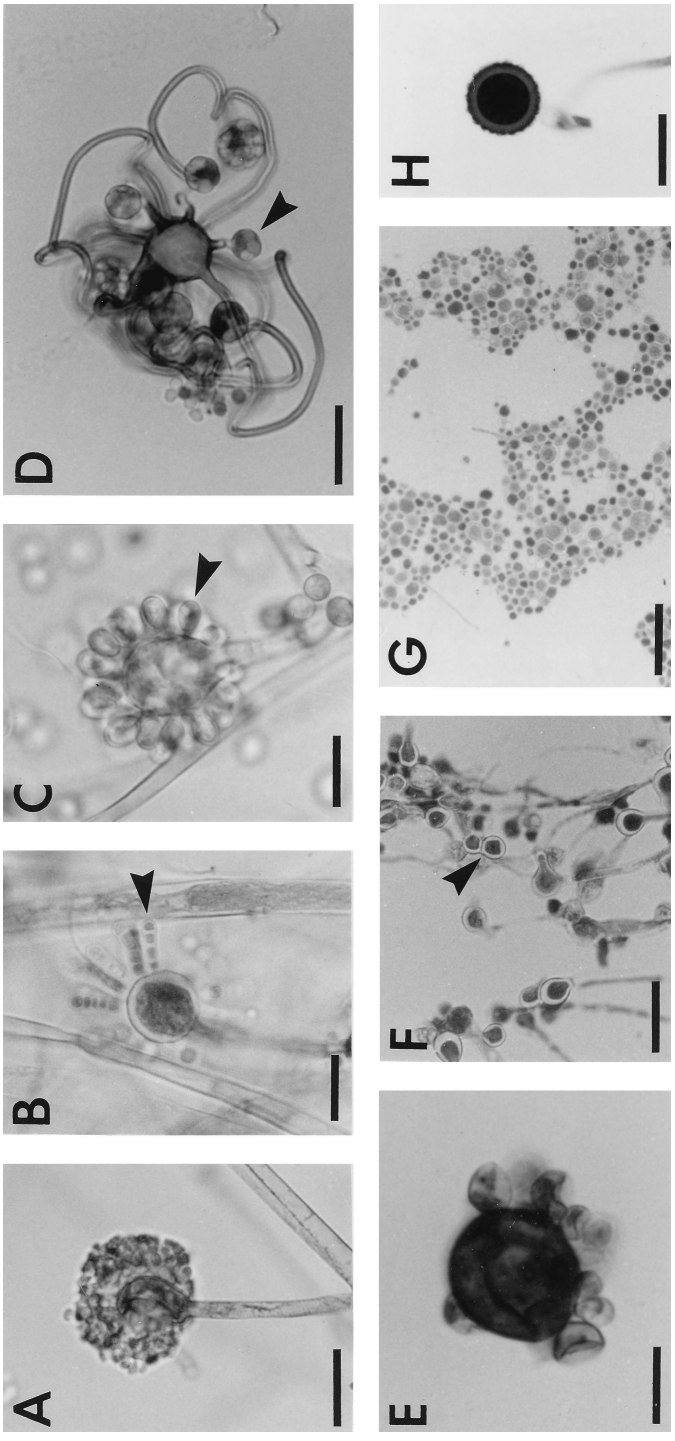


FIG. 5. Reproductive structures of the *Mucorales*. (A) Sporangium. Development of asexual sporangiospore in sacks called sporangia is characteristic of the families *Mucoraceae* and *Saksenaea*. The aerial mycelium of these organisms terminate in swollen structures that develop into sporangial sacks and columellae. Sporangiospores develop asexually by free-cell cleavage within the sporangial membrane. At maturity, the sporangium of the *Mucorales* becomes deliquescent, releasing the sporangiospores. A related mechanism where sporangiospores are released from the sporangiospore by dissolution of a gelatinous plug is seen for *Saksenaea vasiformis*. The fungus pictured here demonstrates the sporangium produced by *Mucor* spp. Bar, 20 μm. (B) Merosporangia. Cylindrical or finger-like projections surround a swollen vesicle in this isolate of *Syncephalastrum racemosum*. A single row of sporangiospores form inside these tubular merosporangia (arrowhead). Spores may be released as entire merosporangial units or singly as the merosporangial membrane dissolves. This form of reproduction is characteristic of *S. racemosum*. Bar, 20 μm. (C and D) Sporangiola. Sporangiola or conidia develop singly around a swollen vesicle on stalks called sterigmata. Single celled sporangiola (arrowhead), typically produced by members of the genus *Cunninghamella* (C), are often echinate and form on short sterigmata. Those produced by *Cokeromyces recurvatus* (D) are multicelled (arrowhead) and occur on long recurving stalks. Bar, 20 μm. (E) Yeast. Several members of the order *Mucorales* are dimorphic. Yeast forms have been identified in vivo for some *Mucor* spp. as well as *Cokeromyces recurvatus* (pictured here). Yeast production in vitro often requires increased temperature of incubation, high carbon dioxide tension, or anaerobic culture conditions. Bar, 20 μm. (F and G) Chlamydospores and gemmae. These asexual reproductive structures are derived from the vegetative hyphae of certain species of the *Mucorales*. Chlamydospores may be formed intercalated with the mycelium (endogenous formation) (arrowhead in panel F), while gemmae are separated from the mycelium and often demonstrate yeast-like budding (exogenous formation) (G). Chlamydospores and gemmae are often considered together, since they may be difficult to differentiate and are similarly derived. These are produced by some but not all members of the *Mucorales*. Their morphology may vary substantially but is not particularly useful for species determination (355). Bar, 40 μm. (H) Zygospores. This is the only form of sexual reproduction employed by the zygomycetes. Zygospores may form within a single isolate without mating (homothallic reproduction) or may require mating with an appropriately oriented mating strain (heterothallic reproduction). Zygospore morphology is often characteristic for an organism when color, size, shape and surface decoration are taken into account. Mating of two isolates to produce mature zygospores provides definitive taxonomic identification of an unknown isolate. The zygospores pictured here are from *Cokeromyces recurvatus*. Bar, 40 μm.

their name. Differentiating and determining the species of these fungi on the basis of their zygospore production are discussed separately.

(i) Differentiation of the sporangium-producing *Mucorales*. The sporangium producers include members of the species *Rhizopus*, *Rhizomucor*, *Mucor*, *Absidia*, *Mortierella*, *Apophysomyces*, and *Saksenaea*. These organisms may be differentiated by identifying the presence, absence, location, or specific morphology of the structures designated in Fig. 6.

In all species of the *Mucorales*, the vegetative mycelium is composed of wide, predominantly aseptate hyaline hyphae. The stolon, when present, makes up part of the vegetative mycelium of wide, ribbon-like hyphal elements that extends between two groups of rhizoids. The stolon grows laterally and contacts the growth medium in vitro and extends the colony laterally for expansive growth. In contrast, the sporangiophore is a wide aerial hyphal element that is produced erect to the vegetative mycelium (11). Sporangiophores may be either branched or unbranched. The rhizoids are root-like structures that also originate from the stolon. These must be character-

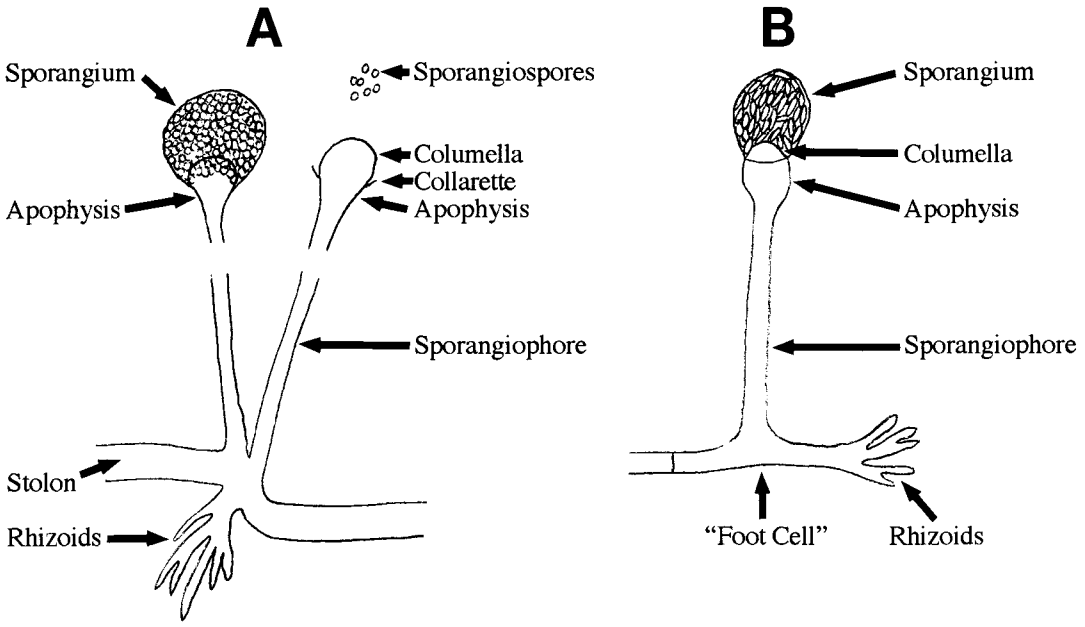


FIG. 6. Schematic diagram labeling the morphologic structures seen in the sporangium-producing *Mucorales* (not drawn to scale). (A) *Rhizopus* spp. (B) *Apophysomyces elegans*.

ized for their presence or absence, morphology (well developed or primitive), and location (directly below or between the sporangiophores). At the aerial end of the sporangiophore are the sporangia. The sporangiophore may widen as it interfaces with the sporangial sack. This widening or flaring of the sporangiophore is known as the apophysis. The sporangiophore may additionally protrude into the sporangial sack as the columella. This columellar membrane separates the end of the sporangiophore from the contents of the sporangial sack. Sporangiospores form within the sporangium by the process of free cell cleavage. Spores are released from the sporangium as the sporangial membrane dissolves or deliquesces. *Saksenaea vasiformis* has the additional feature of having a gelatinous plug occluding the apical opening of its sporangium (400). Sporangiospore release in this isolate is mediated by the dissolution of the gelatinous plug, allowing the spores to flow out from the sporangium.

The shape of the sporangium provides important clues to the identity of the mucoraceous organism. Several morphologies are seen in the *Mucorales* that cause human disease, globose (round), pyriform (pear or tear-drop shaped), and vasiform (vase shaped) (Fig. 7). For the globose sporangium producers, differentiation to the genus level is accomplished by observing, most importantly, the production and location of rhizoids and the production of branched or unbranched sporangiophores. These two features alone will allow the distinction of *Rhizopus* spp. on the basis of nodal rhizoid production and the presence of unbranched sporangiophores, *Rhizomucor* or *Absidia* spp. on the basis of branched sporangiophores and the production of internodal rhizoids, and *Mucor* spp. on the basis of branched

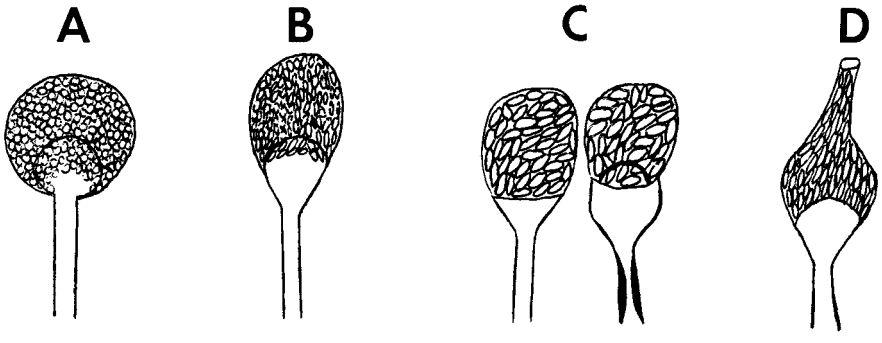


FIG. 7. Morphologic variation seen in the sporangia produced by the *Mucorales* (not drawn to scale). (A) Globose sporangium. Nearly round or spherical sporangia represent the predominant morphology produced by the *Mucorales*. Globose sporangia are produced by *Rhizopus*, *Mucor*, *Rhizomucor*, and *Mortierella* spp. Although *Absidia* spp. generally produce pyriform-shaped sporangia, occasional isolates superficially appear to have round sporangia. Care must be taken to look for the presence of a prominent apophysis in these isolates. *Rhizopus* spp. may also have slight, inconspicuous apophysis, while *Rhizomucor*, *Mucor*, and *Mortierella* spp. do not. All but the *Mortierella* spp. will produce a columella. The size of the sporangial sack is also important in differentiating the various isolates from one another. (B and C) Pyriform sporangium. Pyriform or teardrop-shaped sporangia are typically produced by *Absidia* (B) and *Apophysomyces elegans* (C). The apophysis is very prominent in both of these genera. *Absidia* spp. produce a flask-shaped apophysis with a large columella producing the overall teardrop-shaped fruiting structures. *A. elegans* produce both a "martini glass" and "bell-shaped" apophyseal swelling. The large columella produced by this organism is often obscured by the spores in the sporangial sack. (D) Vasiform sporangium. Vase-like sporangia are produced by *Saksenaea vasiformis*. The apophysis is prominent and flask-shaped, ending in a large dome-shaped columella which protrudes into the sporangial sack. The sporangium swells and then tapers into a tubular neck, similar to what one sees with a bud vase, hence its name "*vasiformis*," meaning vase like. At the apical end of the sporangial neck, a gelatinous plug is seen, which ultimately dissolves, releasing the sporangiospores.

TABLE 2. Differentiating features of the globose sporangium-producing *Mucorales*

Organism name	Colony morphology	Sporangium morphology	Apophysis and columella morphology
Rhizopus spp.	Floccose; rapidly growing	Globose; variation in size with species and variety	Together are globose; apophysis is small, may be inconspicuous
Mucor spp.	Floccose; rapidly growing; white, yellow, gray or brown	Globose; 15–80 μm wide; deliquescent	Columella present, but no apophysis; collars may also be present
Rhizomucor pusillus	Floccose mycelium 2–3 μm high; colorless to white, turning grey with age	Globose with smooth, opaque walls; usually 40–60 μm, may be up to 80 μm	Pyriform, subglobose, to spherical; 20–45 μm wide
Mortierella wolfii	Little aerial mycelium; white, gray, or yellow-gray	Globose and small (15–50 μm); often fails to sporulate	Little or no columella; large colarette left after deliquescence
Absidia corymbifera	Floccose; first white, turning brown to greenish brown with age	May be globose, but more often pyriform, 20–80 μm in diameter	Apophysis: flask shaped Columella: dome shaped, occupying about 50% of sporangium

sporangiophores but no rhizoids. Maximum-temperature growth may also been helpful in differentiating *Mucor* spp. (nonthermophilic) from *Rhizomucor* spp. (extremely thermophilic). The small size of the sporangium and the difficulty in stimulating sporulation should be additional clues to the identification of *Mortierella* in clinical specimens. Although *Absidia* may produce nearly round sporangia, pyriform sporangia are also usually present. In contrast to *Rhizomucor* spp., to which they are morphologically most similar, *Absidia* spp. are the only ones that have a significant apophysis. The differentiating features of the globose sporangium producers and those of the nonglobose sporangium producers are summarized in Tables 2 and 3, respectively. Species determination and more complete morphologic descriptions of the individual genera of *Mucorales* that produce disease in man are presented below (see "*Mucorales* causing zygomycosis in humans").

(ii) Differentiation of the sporangiole- and merosporangium-producing *Mucorales*. The other two major nonsporangium types of asexual reproductive structures seen in the *Mucorales* are the sporangioles and the merosporangia (Fig. 5B to D). Sporangioles are reproductive spores that may be either single celled or multicelled. They form on vesicles formed at the end of branched or unbranched sporangiophores. Each sporangiole is borne directly from the vesicle on a stalk called a sterigmata. Two *Mucorales* isolates, *Cunninghamella bertholletiae* and *Cokeromyces recurvatus*, produce sporangioles. These two organisms may be differentiated from one another on the basis of three main characteristics, the number of cells in the sporangiole, the length and morphology of the sterigmata, and the production of zygospores. *Cunninghamella* produces single-celled sporangioles on short sterigmata (Fig. 5C) and zygospores only after mating with appropriate mating strains. This is discussed below. In comparison, *Cokeromyces recurvatus* produces multicelled sporangioles on long recurving stalks (Fig. 5D). It additionally produces zygospores without requiring a mating strain and demonstrates dimorphic growth. The differentiating features for these two organisms are summarized in Table 4, and further detailed discussions of these organisms are presented by genus below.

Merosporangia are tubular sacks in which a single column of spores is formed. These structures, similar to the sporangioles, are produced on swollen vesicles that form at the end of aerial sporangiophores. The only merosporangium producer known to cause disease in humans is *Syncephalastrum racemosum*. The morphologic features characteristically observed for this organism are summarized in Table 4, and more detailed mor-

TABLE 3. Differentiating features of the nonglobose sporangium-producing *Mucorales*

Organism name	Colony morphology	Sporangium morphology	Apophysis and columella morphology	Sporangiospore morphology
Absidia corymbifera	Floccose; first white, turning brown to greenish brown with age	Pyriform, 20–80 μm in diameter	Apophysis: flask shaped Columella: dome shaped, occupying about 50% of the sporangium	Round to oval, 2–3 by 3–5 μm
Apophysomyces elegans	Floccose, rapidly growing colonies; pale gray to yellowish, turning brown at 37°C	Pyriform, 20–58 μm in diameter	Apophysis: prominent bell or "champagne glass" shaped Columella: dome shaped, 18–28 μm	Oval and light brown, 5.4–8.0 by 4.0–5.7 μm
Saksenaea vasiformis	Floccose; white colonies	Flask-shaped sporangium 20–50 by 15–45 μm; apical end has a gelatinous plug which dissolves	Apophysis: flask shaped Columella: dome shaped, 11–15 μm	Oval, 1.4–1.2 by 2.8–4.2 μm

TABLE 2—*Continued*

Sporangiospore morphology	Rhizoids	Sporangiophore morphology	Zygospores	Other
Round to oval, often 4–6 μm wide (may be larger); ridged	Abundant, often well developed	Predominantly unbranched; occur above rhizoidal tufts	Heterothallic	Most species with good growth at 37°C
Smooth walled; oval to round; most 3–5 μm, some larger	Absent	Predominantly branched	Heterothallic; chlamydoconidia may be present	Rarely grows above 37°C
Smooth walled, globose to broadly ellipsoidal; 3–5 μm	Primitive; rare	Extensively and irregularly branched; more typically monopodial; occur between rhizoidal tufts	Heterothallic; round to oval with warts; red, brown, or black; 45–70 μm wide	Growth from 20 to 60°C; assimilates sucrose, glycine, phenylalanine, and β-alanine
Cylindrical or kidney shaped, 6–10 by 3–5 μm	Primitive	Short and tapered, 80–250 μm; occur above rhizoidal tufts	Zygospores not seen; chlamydoconidia 35 μm; ameboid appendages	Good growth at >40°C; no growth at >48°C
Round to oval, 2–3 by 3–5 μm	Primitive; rare	Tall, 450 μm long, arising from stolon; branched, in whorls; occur between rhizoidal tufts	Heterothallic; globose to flattened, rough walled, brown, 50–80 μm wide; 1–5 equatorial ridges	Optimal growth at 37°C; maximum temperature of growth, 45–52°C

phologic, pathologic, and epidemiologic discussions are presented below.

Species identification by zygospore production. Zygospore production may occur without mating (homothallically) or only following mating with an appropriately oriented mating stain (heterothallically). The results of mating studies provides definitive identification of an isolate, since zygospores that are fertile or complete are produced only within a single species. Zygospore-like structures that result from matings between unrelated species will not be viable. For heterothallic organisms, zygospore production has been called "the last word" in species determination (499). Formation of zygospores homothallically is characteristic of only a few members of the order *Mucorales*. Homothallic zygospore production may be seen in *Rhizomucor miehei* (412) and in *Cokeromyces recurvatus* (289). The presence of zygospores in a pure culture of a *Rhizomucor* isolate should help to differentiate *R. miehei* from *R. pusillus*, which requires mating for sexual reproduction.

General Culture Characteristics of the *Entomophthorales*

Growth and sporulation characteristics. The *Entomophthorales* produce colony morphologies distinct from those of the *Mucorales* (Fig. 8). This class of molds is characterized by the production of a compact mycelium that actively expels asexually produced spores. *Basidiobolus* spp. produce waxy, glabrous, and furrowed colonies, which may be pigmented (Fig. 8A). *Conidiobolus* spp. produce colonies that are at first waxy but become powdery with age (Fig. 8C and D). Expelled spores may affix to the petri dish lid or tube wall (Fig. 8D) or may land on adjacent medium to produce characteristic satellite colonies.

Morphologic identification by sporulation characteristics. Microscopically, the *Entomophthorales* produce a coenocytic mycelium that develops moderate septation with age. Asexual sporangiole formation may be characteristic for speciation. Zygospore formation and morphology may also be useful in speciation. Like the *Mucorales*, the *Entomophthorales* may produce zygospores heterothalically or homothallically depending on the species involved. Differentiation of homothallically produced zygospore morphology (Fig. 9) or the performance of mating studies to produce zygospores heterothalically may be required for a definitive identification of isolates. The distinguishing characteristics of the *Entomophthorales* that cause disease in humans is summarized in Table 5.

TABLE 3—*Continued*

Rhizoids	Sporangiophore morphology	Zygospores	Other
Primitive; rare	Tall, 450 μm long, arising from stolon; branched, often in whorls; occur between rhizoidal tufts	Heterothallic; globose to flattened, brown, rough walls, 50–80 μm in diameter; 1–5 equatorial ridges	Optimal growth at 37°C; maximum temperature of growth, 45, 48 or 52°C
Tuft of rhizoids originates from a "foot cell" at base of sporangiophore	Unbranched; 200–300 μm long ending in a "foot cell"; pigmented, thickened wall just below the apophysis	Not seen	Good growth at 24–42°C; sporulation improved at 37°C on nutritionally deficient medium
Tuft of dematiaceous rhizoids form from a "foot cell" at base of sporangiophore, 3–5 μm long	Short and unbranched, 25–65 μm	Not seen	Optimal growth at 24°C; sporulation improved at 37°C on nutritionally deficient medium

TABLE 4. Differentiating features of the Sporangiole- and merosporangium-producing *Mucorales*

Organism name	Colony morphology	Fruiting-structure morphology	Sporangiophore morphology
Cunninghamella bertholletiae	Tall, 0.5–2 cm high; white, yellow, or gray	Sporangiophores end in vesicles 15–59 μm wide; lateral branches produce smaller vesicles 3–30 μm wide; sporangioles produced on short, straight stalks	Branched, 7–130 μm long
Cokeromyces recurvatus	Low mycelium; tan to gray with concentric zones of color; radial folds form with age	Sporangiophores terminate in vesicles 13–31 μm wide; sporangioles borne on recurving stalks	Mostly unbranched, 300–500 μm long
Syncephalastrum racemosum	Floccose mycelium 0.5–1.5 cm high; White, green, olive, gray, or almost black	Sporangiophores end in a terminal vesicle (30–80 μm); tubular sacks or merosporangia containing spores originate from the vesicle surface	Branched

Serologic Diagnosis of Infections with the *Zygomycetes*

Serologic diagnostic tests for detecting zygomycosis are not clinically useful. The experimental use of serologies to detect invasive zygomycosis has been attempted in several laboratories (154, 198, 220, 230, 275, 365, 417, 429, 517, 522). Although several serologic modalities have been tested, the enzyme-linked immunosorbent assays (ELISA) seem to have the best sensitivity and specificity for detecting antibodies produced during invasive zygomycosis (230, 365). These serologically based tests have demonstrated that the zygomycetes share several antigenic determinants with one another. This antigenic sharing makes it impossible for the various species and even genera to be reliably distinguished from one another (198, 230, 365). In addition to detecting antibody production due to invasive disease, double-diffusion and ELISA techniques have been used to detect antibodies produced as a result of allergic alveolitis (404). The lack of specificity, the inability to determine the species implicated in causing disease, and the unavailability of routine testing make serologic diagnosis of zygomycosis relatively useless at this time.

Several antigen detection systems have been developed using antisera developed against individual isolates. Since the genera of *Mucorales* do have some unshared antigenic determinants, determination of the species of fungal elements in tissues section has been successfully performed using selected fluorescent-antibody stains (154). Exoantigen testing has been developed for the sterile mycelium producers *Apophysomyces elegans* and *Saksenaea vasiformis* (232). In cultures where a positive test is obtained, this test is helpful, but due to the high false-negative rate, all negative exoantigen tests require confirmation by sporulating morphology for definitive identification.

Molecular and Antigen Detection Techniques Used in Diagnosis of Zygomycosis

There are very few molecular techniques currently in use for the diagnosis of zygomycosis, and those that are available are still experimental. PCR amplification of 18S rRNA sequences together with single-strand conformational polymorphism pattern identification has been successfully employed to help differentiate *Rhizopus* infections from those caused by other pathogenic fungi (488). Although these tools may be useful in epidemiologic studies for outbreaks of zygomycosis, little is currently being offered in the way of primary diagnosis. Molecular techniques are being employed more extensively for determining taxonomic assignments. The use of DNA complementarity studies (146) and PCR or other molecular techniques is providing the basis for the assignment of fungi into their appropriate class, family, genus, species, and variety (446).

MUCORALES CAUSING ZYGOMYCOSIS IN HUMANS

Rhizopus Species

Natural habitat. The various species of *Rhizopus* have a worldwide distribution. *Rhizopus (oryzae) arrhizus* is the most common environmental *Rhizopus* species seen and has been identified in India, Pakistan, New Guinea, Taiwan, Central and South America (155), Africa, Iraq, Somalia, Egypt, Libya, Israel, Turkey, Spain, Italy, Hungary, Czechoslovakia, Germany, Ukraine, the British Isles, and the United States (125). It has been found in soils from cultivated grassland and forest locations and also been cultured from volcanic mud (362). This organism has been isolated from hay (8), decaying grass and leaf mold, and a variety of food stuffs including barley, sorghum, wheat, corn (3), oat, rice, onions, cotton, groundnuts, sweet potatoes, pecans, brazil nuts, and tomatoes (125). Strains of this mold are responsible for the fermentation of various oriental foods and Indonesian alcoholic drinks (408).

R. stolonifer is likewise a commonly seen member of the zygomycetes. It occurs in a similar environmental distribution to *R. arrhizus*, with preference for the tropical and subtropical regions of the world. It is found in soil samples from forests, deserts, salt marshes, grasslands, and cultivated fields and has been isolated from hay (8), decaying vegetable matter, peat, garden compost, municipal waste, sewage (125), lumber, sawdust, and wood pulp (34, 509). It has been cultured from foodstuffs including barley, corn, sorghum, wheat (3), soybeans (142), rice, beans, tomatoes, groundnuts, pecans, brazilnuts, bananas, and cotton (125). *R. stolonifer* has also been implicated in causing soft rot in both stored sweet potatoes and strawberries (11).

The fungi of the *R. microsporus* group are relatively uncommon. This group is made up of closely related varieties of *Rhizopus*. They have been isolated from soil collected in forests and garbage (352) and from skin scrapings from otherwise healthy fowl (8). *R. microsporus* has also been isolated from moldy lumber in Norwegian sawmills (404) and other wood products (193, 261, 305, 475).

Rhizopus azygosporous is found in Indonesian tempeh, as one of the molds responsible for producing this fermented

TABLE 4—*Continued*

Sporangiole or merosporangium morphology	Rhizoids	Zygospores	Other
Sporangioles are golden brown, round to oval, 6–14 by 6–11 μm; smooth- and rough-wall forms both seen	Primitive; rare	Heterothallic; zygospores are brown, rough walled, and round, 35–50 μm wide	Growth between 24 and 45°C
Sporangioles are round, each containing 12–20 oval, smooth-walled sporangiospores	Absent	Homothallic; zygospores are readily produced and are round, rough walled, golden brown, 33.5–54.5 μm in diameter	Dimorphic, with yeast phase stimulated by anaerobiosis and 37°C incubation; yeasts are 30 to 90 μm wide, with multiple small buds similar to *Paracoccidioides braziliensis*
Tubular merosporangia (13–25 by 4–7 μm) contain round to oval sporangiospores 3–7 μm wide	Rare	Heterothallic; zygospores are rough walled, round, 50–90 μm wide	Thermophilic, with growth above 37°C

soybean food product (410, 519). It is a rare cause of human disease (410).

Rhizopus schipperae is a very uncommon organism, having been associated twice from disease in humans (19, 500). The natural habitat of this fungus is unknown (500).

The spores of the *Rhizopus* species are often isolated from air samples. In the home environment, the use of air conditioning, rugs, pillows, drapes and other cloth, and wooden furniture provides suitable environments for the proliferation and sequestration of fungal spores. *Rhizopus* spores have been identified both in house dust and from air plates placed in kitchen sites, notably consistent with this mold's status as "bread mold" (51). Spores from *R. arrhizus* (151, 335), *R. stolonifer* (27, 51, 125, 333), and *R. microsporus* (51) have all been collected from air samples or dust collected from air conditioning systems. *Rhizopus* spp. have also been found as part of the microbiota of dog hair, peaking in occurrence during the summer months (65). *R. stolonifer* has been found associated with healthy fingernails and toenails of Egyptian students (1).

Transmission. Infection by *Rhizopus* spp. has been transmitted by the respiratory, percutaneous, and gastrointestinal or oral routes. Most infections caused by *Rhizopus* involve the rhinocerebral and pulmonary sites. These are thought to occur secondary to inhalation of spores into the lungs or sinuses. Two temporally related cases of *Rhizopus* zygomycosis have been traced back to contaminated air conditioner filters in a physician office building, supporting this theory (151). An outbreak of fungal sinusitis in hospitalized children, including a case due to *Rhizopus* spp., was linked to a period of hospital construction where fungal spores were stirred up during excavation (238). *Rhizopus* sinusitis has also been linked to sniffing garden compost to assess its state of decomposition. Both the compost pile and clinical specimens from a patient's sinuses contained identical strains of *R. arrhizus* (313).

The detection of many cases involving percutaneous transmission was seen as a dramatic change in disease manifestation with *Rhizopus* spp. (279). Underlying this change in frequency of primary cutaneous infection was the association of disease with the use of spore-contaminated adhesive bandages. These bandages were used to hold dressings in place over surgical wounds, which subsequently became infected with either *R. (oryzae) arrhizus* (234, 292) or *R. microsporus* var. *rhizopodiformis* (52, 168, 279, 423). *R. microsporus* var. *oligosporus* has been seen in cutaneous infection following surgery (461), and *R. microsporus* var. *rhizopodiformis* has been seen in a surgical wound covered by an adhesive ostomy bag (353), both representing iatrogenic transmission. Sporadic cases of transmission by an insect bite (88, 152, 199, 233), intramuscular injection (215), and catheter insertion site infections (234, 237) have all been described. Intravenous drug use has also been linked to *Rhizopus* zygomycosis (163, 205, 365, 401). An outbreak of neonatal zygomycosis caused by *Rhizopus microsporus* was transmitted by spore-contaminated tongue depressors used to splint limbs of the newborns in the intensive care unit. Infections arose at intravenous or other catheter sites near the splints (305).

Wooden tongue depressors were also implicated as the source of spores that resulted in positive stool cultures in pediatric patients seen in a hematology/oncology clinic. Spores were presumably transmitted into the mouth during oropharyngeal examinations (261). Oral transmission is thought to underlie the gastrointestinal mucormycosis seen in humans (321), and has been associated with the ingestion of old bread or grain products together with fermented milk and cereal preparations in parts of Africa (445). Oral transmission, probably via small lacerations of the mouth, is probably responsible for disease in domesticated animals (402).

A pseudo-epidemic of *Rhizopus* infections was linked to spore-containing tongue depressors used in the collection of stool specimens. Other specimens collected in the cups in which the wooden tongue depressors were stored also produced positive cultures (193). Another pseudo-outbreak of *Rhizopus* in hospitalized patients was traced back to the laboratory's use of nonsterile wooden sticks during the homogenization or mixing step in stool processing. The sticks, which were later shown to be contaminated with *Rhizopus microsporus* var. *rhizopodiformis* spores, were responsible for several positive surveillance stool cultures in immunocompromised patients (475).

Host characteristics. *Rhizopus* spp. are predominantly opportunists when causing human disease. Diabetes is the single most common predisposing illness associated with the development of *Rhizopus* zygomycosis (299, 354, 507). Hyperglycemia has also been seen as a risk factor (151).

Immune system compromise associated with profound neutropenia at the time of infection has also been seen in a number of cases (299, 330, 398, 512). Leukemia (237, 271, 279, 299, 356), lymphoma (199), other hematologic malignancies (102, 374, 500), and organ or bone marrow transplantation, with their accompanying immunosuppressive regimens (188, 235, 308, 313, 330, 425, 440, 511) have likewise been associated with

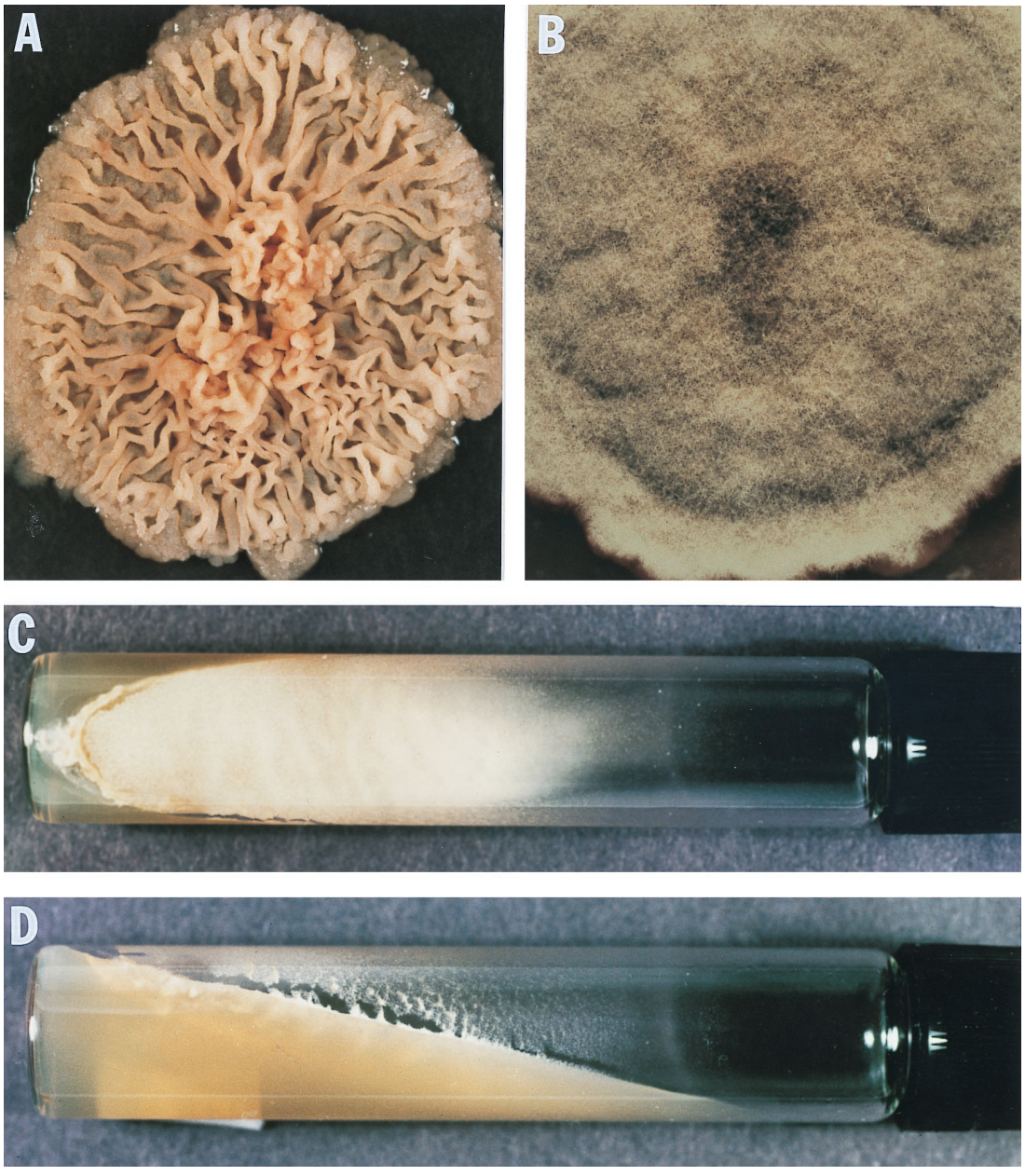


FIG. 8. Gross morphology of *Basidiobolus*, *Conidiobolus*, and *Mucor* colonies in culture. (A) *Basidiobolus microsporus* colony morphology on SABHI agar. Dense, waxy, folded, or furrowed colonies are produced on standard culture media. Colonies are low growing, lacking the floccose morphology seen in the typical isolate of the *Mucorales* (see *Mucor* morphology in panel B). This isolate produced a brownish orange colony that was pale on reverse. (B) *Mucor* colony morphology on SABHI agar. The floccose or "woolly" colony morphology produced by most of the *Mucorales* is well demonstrated in this *Mucor* isolate. Contrast the fibrous aerial mycelium with the glabrous morphology seen for both *Basidiobolus* (A) and *Conidiobolus* (C). This difference in colony morphology is one of the distinguishing features between the *Mucorales* and the *Entomophthorales*. (C) *Conidiobolus coronatus* colony morphology on Sabouraud dextrose agar slant. Similar to *Basidiobolus* spp., *Conidiobolus* spp. produce low-growing waxy (or sometimes powdery) folded and furrowed colonies that lack aerial mycelium. Satellite colonies arising from germination of ejected sporangiospores has lead to confluent growth in this tube containing a 6-week-old culture. (D) *Conidiobolus coronatus* tube culture, side view of the tube in panel C. Note the cloudy appearance of the glass surface of the tube. This is produced by the collection of a plethora of forcibly expelled sporangiospores which have become encrusted on the inside surface of the tube. This opacification of the culture tube (or petri dish lid) is characteristic of this organism.

Rhizopus infections. Many cases have described the use of steroids or broad-spectrum antibiotics as additional risk factors (213, 215, 308). Additionally, deferoxamine/desferrioxamine treatment of patients with iron or aluminum overload increases the risk of developing zygomycosis with *Rhizopus* spp. (48, 102). Iron overload alone has also been suggested as a risk factor for developing disease (212). Immaturity and low birth weight have also been implicated as risk factors for developing zygomycosis with *Rhizopus* spp. (305, 410). When the case reports are reviewed, most patients with zygomycosis due to the *Rhizopus* spp. have two or more of these risk factors for developing disease.

Infections with *Rhizopus* spp. rarely afflict immunocompetent hosts. In cases where this does occur, noninvasive or minimally invasive disease is generally seen. Notably, allergic pulmonary disease does occur in competent hosts and reflects an

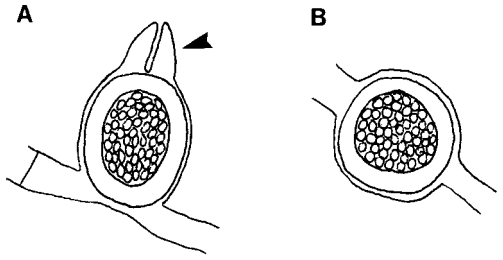


FIG. 9. Schematic diagram of characteristic zygospore morphology of *Basidiobolus ranarum* and *Conidiobolus incongruus*. (A) *B. ranarum* zygospores. Smooth, thick-walled zygospores are produced homothalically and demonstrate prominent conjugation beaks. These beaks represent the remnants of the conjugation tubes formed between the mating hyphal elements. (B) *C. incongruus* zygospore. Smooth, thick-walled zygospores are produced homothalically and may be distinguished from those produced by *B. ranarum* by the absence of the conjugation beaks.

acute hypersensitivity immune system-mediated illness rather than an invasive infection (12, 34, 333, 509). Other competent hosts have developed zygomycosis with *Rhizopus*, but invasive disease in these individuals has occurred following a disruption of the mucocutaneous barrier. Most of these cases involve underlying trauma (362, 469) or surgical wound infections (52, 168, 191, 286, 292, 423, 461). It is also noteworthy that cases reported in immunologically normal patients occur in the setting of additional risk factors for developing zygomycosis such as antibiotic or steroid use (191, 353, 379).

General disease manifestations. The hallmarks of disease with these organisms is angioinvasion, thrombosis, infarction, and necrosis of involved tissues. In patients who are not neutropenic, an acute inflammatory response is generally seen. Thick, necrotic fluids may be aspirated from areas of abscess formation. *Rhizopus* spp. are by far the most common organisms isolated from patients with zygomycosis, representing about 90% of all infections with the zygomycetes. *R. arrhizus* and *R. microsporus* var. *rhizopodiformis* make up the vast majority of pathogens. As of 1984, 60% of all cases and 90% of all rhinocerebral cases of zygomycosis were caused by *R. arrhizus*. About 15% of all cases of zygomycosis were due to *R. microsporus* var. *rhizopodiformis* (412). The remaining *Rhizopus* pathogens cause disease in humans only rarely. A series of three fatal cases of *R. azygosporus* infection in infants (410), two cases of *R. schipperae* infection (one nonfatal and one of unknown outcome) (19, 500), and one nonfatal case of *R. microsporus* var. *oligosporus* infection (461) have been published in the medical literature. Two cases of *R. microsporus* var. *microsporus* (233, 502) and a handful of cases due to *R. microsporus* (variety unknown) (261, 305) have also been reported. Although Scholer states explicitly in his 1983 chapter that *R. stolonifer* is not a pathogen (412), there are two reports of disease with this organism (*R. nigricans*) from the pre-1900 literature summarized in Hutter's review (209). Current literature supports the role of *R. stolonifer* in stimulating a hypersensitivity pneumonitis (509) but not actual invasive disease.

Nearly every site and type of infection seen with the zygomycetes has been described with one or more of the *Rhizopus* spp. The sites most often involved are sinuses and rhinocerebral structures (61, 151, 188, 299, 308) and the lungs (with or without dissemination) (235, 379, 440). Pulmonary disease is the second most common manifestation of disease with the *Rhizopus* spp., with about 47% of these cases involving disseminated infection (455). Most cases of disseminated disease involve the lung (48, 286, 317, 374, 425) or cutaneous and/or subcutaneous sites (469, 512) as their probable primary sources. Disseminated disease can involve virtually any organ in the body, with the skin, central nervous system, liver, spleen and kidney being most common. Myocardial disease has also been described (212).

A variant of pulmonary disease is seen with several members of the genus *Rhizopus*. Inhalation of spores leads to a hypersensitivity response rather than actual invasive infection. Lung damage is more prominent with prolonged exposure to the allergens and manifests itself as thickened alveolar walls, development of granulomas and the appearance of an interstitial infiltrate (12). This has become a tremendous problem in the sawmill ("woodcutter's disease") and malt industries and has led to the implementation of appropriate respiratory precautions when performing procedures where fungal allergens are aerosolized (34, 509). Likewise, a farmer whose closed cab tractor's air-conditioning filter became contaminated with *Rhizopus* spores developed hypersensitivity after prolonged respiratory exposure (333). *R. stolonifer*, *R. microsporus* var. *rhizopodiformis*, and nonspecific *Rhizopus* spp. have all been implicated in causing this disease process.

Primary cutaneous disease with *Rhizopus arrhizus* and *R. microsporus* var. *rhizopodiformis* have been reported in association with the use of nonsterile adhesive bandages (52, 168, 191, 234, 279, 292, 423). Spores were either introduced directly into the surgical wound or introduced into the traumatized skin at the time of bandage removal. Vesicular pustules, wound zygomycosis, gangrene, and necrotizing fasciitis were all seen as part of these out breaks. Similar cutaneous manifestations were seen in neonates infected by tongue depressors used to splint catheterized limbs (305). Primary cutaneous disease may occur following traumatic implantation of spores (362, 469). Cutaneous disease by *Rhizopus* spp. may occur at virtually any site, including the vulva (330). It is also an uncommon cause of inflammatory tinea capitis (118).

R. (oryzae) arrhizus has been implicated in causing gastrointestinal disease. Winkler et al. (511) described a gastric ulcer with invasive *Rhizopus* in a transplant patient who was receiving steroids and cyclosporin. While very few of the original descriptions of gastrointestinal zygomycosis actually have confirmatory cultures, six of the cases do demonstrate *Rhizopus* as the causal agent (321). Gastrointestinal disease is also reported with disseminated zygomycosis (286, 410, 425, 510). A series of three fatal cases of *R. azygosporus* were seen in premature infants. In each case, angioinvasion, tissue infarction, and granulomatous inflammation with the production of giant cells was seen. The neonates died due to peritoneal, gastrointestinal, kidney, or liver involvement by the fungus (410).

Other more unusual sites of involvement of zygomycosis with *Rhizopus* spp. include an additional case of peritonitis (56), an inguinal abscess (501), bone involvement (461), renal involvement (405, 410), and a case of rapidly progressive genitourinary disease originating from a traumatic lesion of the penis (506). One case of *Rhizopus* mastitis associated with silicone breast implants has also been described (452).

Virulence factors. *Rhizopus* species are primarily opportunists, requiring some breakdown in immune system defenses to cause disease in humans. Once infection is established, *Rhizopus* spp. spread readily due to their angioinvasive nature and their ability to grow at or above core body temperature. The thermotolerant nature of these organisms has been linked to their ability to produce infections in mice (242) and in diabetic rabbits (381). *Rhizopus* spp. can survive drying conditions of 80 to 82°C for at least 72 h (125). Dormant spores can withstand the extremes of temperature, with viable spores having been isolated from both arctic and desert climates (125).

TABLE 5. Differentiating features of the *Basidiobolus* and *Conidiobolus* species that cause disease in humans

Organism name	Colony morphology	Hyphal morphology
Basidiobolus ranarum	Flat furrowed colonies with a waxy texture; yellowish to gray	Wide vegetative mycelium (8–20 μm wide), increasing in septation with age; sporangiophores are short and either narrow or with inflated apices
Conidiobolus coronatus	Flat, waxy colonies, becoming powdery, with a short aerial mycelium with age; petri dish lid becomes covered with conidia ejected from older cultures; colonies are white, buff, tan or brown	Wide vegetative mycelium (6–15 μm); conidiophores are short and unbranched
Conidiobolus incongruus	Similar to *C. coronatus*; dry colonies are produced, becoming more aerial with increased humidity	Wide vegetative mycelium (10–22 μm); conidiophores are 30–130 μm tall and inflate subapically
Conidiobolus species	Not known	Coenocytic

Rhizopus spp. produce a variety of enzymes, proteins, and by-products that have pathogenic potential. *R. stolonifer* produces the metabolites agroclavin, ergosine, and ergosamine, all ergot alkaloids. Likewise, *R. (oryzae) arrhizus* produces agroclavin, which is toxic to sheep, cattle, and humans (125). *R. microsporus* produces rhizonin A, a mycotoxin which causes hepatitis and death in the Peking duck and rat animal models (508). *R. stolonifer* produces glycosidic, proteolytic, and lipolytic enzymes (125). Substrate-specific proteolytic-enzyme production has also been noted for *R. oligosporus* (489). *Rhizopus* spp. may also produce substances shown to have antibacterial activity (125). *R. arrhizus* actively produces hydroxamate siderophores which are used to acquire iron under conditions of low iron availability (204). *Rhizopus* spp. reportedly have an active ketone reductase system (15). This may act as an additional virulence factor for these organisms, permitting them to grow well in the acidic and glucose-rich environment seen in ketoacidotic states. Some species also produce antigenic extracellular polysaccharides (116). Whether these serve as virulence factors in human disease is unknown.

Diagnosis. Colonies of the genus *Rhizopus* are all characterized by rapid growth, coarse and floccose aerial mycelia, the presence of stolons, pigmented rhizoids, brown pigmented sporangiophores that arise singly or in groups, and multispored globose sporangia that are both apophysate and columellate (Fig. 10). Sporangiophores are usually unbranched, but when branching occurs, it is generally seen as a single bifurcation near the tip of the sporangiophore. *Rhizopus* spp. produce round terminal sporangia containing sporangiospores. Zygospores form between oppositely oriented strains within the aerial mycelium on opposite suspensors (125, 408, 409, 412). *Rhizopus* spp. all assimilate ethanol, glycerol, and adonitol; however, neither lactose nor nitrate is utilized (125). Additional features seen in all *Rhizopus* spp. are the production of striated or grooved sporangiospores. This is useful in differentiating *Rhizopus* spp. from *Absidia*, *Mucor*, and *Thamnidum* spp., all of which produce smooth sporangiospores. Surface striation patterns are not particularly useful in differentiating the various species of *Rhizopus* from one another, however, due to the tremendous variation and overlap seen (418). Gross colony characteristics and temperature-dependent growth likewise provide no useful basis on which to speciate *Rhizopus* isolates (418). Currently, there are considered to be seven distinct species and varieties of *Rhizopus* that cause human disease (Fig. 1). While most laboratories identify these molds to the genus level only, species may be identified with some difficulty by observing several differentiating features (Table 6).

The predominant human pathogen of the *Mucorales* is *R. arrhizus*. This mold is often cited in the medical literature as *R. oryzae*, a synonym. It may be recognized by its very rapid growth on standard media, producing colonies about 1 cm tall. Colonies are at first white and become yellow, pale brown, dark brown, or gray with age. The mycelium tends to collapse upon itself (125). This mold grows readily at 36°C but fails to grow at 46°C (412). Microscopically, *R. arrhizus* is characterized by the presence of wide, hyaline stolons and the production of abundant brown pigmented rhizoids having four to eight branches. Sporangiophores originate singly or in groups from the stolon directly above rhizoidal tufts and may reach a length of 750 to 2,000 μm (generally 1,500 μm). The sporangiophores are usually unbranched, terminating in round, powdery-appearing, grayish black sporangia measuring 100 to 200 μm in diameter. *R. arrhizus* produces lightly pigmented gray columella that are ellipsoidal to subglobose in shape and up to 130 μm high. An apophysis is always present but may be inconspicuous. Collarettes, if present, are short. Striated ellipsoidal to oval sporangiospores are produced in abundance and measure up to 8 μm in length. Zygospores are produced heterothallically and are globose or flattened, measuring 60 to 140 μm (usually 80 to 100 μm). They are reddish brown and decorated with stellate conical projections. Zygospores form on opposite, unequal suspensors (125, 408, 412).

The *R. stolonifer* group are rarely implicated as human pathogens, occurring instead as environmental contaminants which utilize rotting fruit as their predominant growth medium. Since *R. (nigricans) stolonifer* has been implicated as a cause of allergic alveolitis and since the *R. stolonifer* group may enter into the laboratory as contaminants, it is important to be

TABLE 5—*Continued*

Sporangiole (conidia) morphology	Zygospore production characteristics	Other
Round, single-celled conidia (7–15 by 6–12 μm) have flattened apices and retain a portion of their sporangiophore wall; they produce no papillated sporangioles; spores may be propelled by an ejected fluid stream or may be passively released; sporulation ability is rapidly lost in vitro	Homothallic; thick, smooth-walled zygospores (20–50 μm in diameter) are produced; conjugation "beaks" are often present; chlamydospores (20–24 μm in diameter) are produced by older cultures	Moderately fast growth at 30°C, slower growth at 37°C, and poor growth at 15°C; colonies often produce a musty odor; disease seen most often in children
Round to pyriform single-celled conidia (25–45 μm wide) are bluntly papillated; villous sporangioles, covered with short hair-like projections, are made in abundance; conidia may sporangiolate, producing a "corona" of sporangioles; spores are forcibly ejected from hyphal elements	Heterothallic production of zygospores	Grows well when incubated from 25 to 37°C; no musty odor is produced; disease seen most often in adult men
Round to pyriform, single-celled conidia (16–30 by 20–34 μm) have prominent, pointed papillae and are produced in abundance; no villous sporangioles are produced	Homothallic, occurring within 2 days of planting; zygospores are round, thick walled (20–21 μm), with no conjugation beaks	Good growth at 37°C, little growth seen at room temperature
Forcibly discharged conidia measure 16 by 16.9 μm	Homothallic; round, thick-walled zygospores (20–21 μm)	Single case report of human disease

able to differentiate these from the other *Rhizopus* spp. *R. stolonifer* grows on standard mycology media as a very tall, whitish erect mycelium studded with black spots representing the sporangia. It grows best with good sporulation between 15 to 30°C; it fails to grow above 33°C, supporting its lack of invasive pathogenicity at human core body temperature. Rhizoids are well developed and extensively branched and arise from the stolon directly below the pigmented sporangiophores. Sporangiophores are long and unbranched, measuring up to 2,000 μm, and generally occur in groups of one to three. Sporangia are large (up to 275 μm wide) and black with a globose morphology. The apophysis and columella together form an oval to subglobose structure that measures 70 to 120 μm. Sporangiospores are large, measuring up to 13 μm. They are round to oval and are black with prominent striations. Large black zygospores are produced heterothallically and are formed best under warm moist conditions (reflecting their occurrence in nature on rotting fleshy fruit in the summer). Mature zygospores measure 150 to 200 μm wide, form on equal suspensors, and are decorated with irregularly shaped surface projections (125, 408, 412).

The *R. microsporus* group represents, as its name suggests, the diminutive members of the genus. This is an important group in causing human disease, with an estimated 15% of all culture-proven cases of human zygomycosis having been caused by *R. microsporus* var. *rhizopodiformis* (412). Two other members of this group, *R. microsporus* var. *microsporus* and *R. microsporus* var. *oligosporus*, have also been implicated in causing disease in humans (193, 233, 261, 305, 502). In contrast to *R. stolonifer* and *R. arrhizus*, the varieties of *R. microsporus* have sporangiophores that do not exceed 800 to 1,000 μm in length. Likewise, the sporangia are small, generally measuring 100 μm in diameter. Columella are pleomorphic in shape and size, demonstrating pyriform, ellipsoidal, cylindrical, conical, globose, and subglobose morphologies. A discrete apophysis is generally noted. The rhizoids are simple, without secondary branching. Zygospores measure 80 to 100 μm in diameter and are reddish brown, forming on unequal suspensors (409). The differentiating features of the *Microsporus* group are compared to the other pathogenic *Rhizopus* spp. in Table 6. It is more of a challenge to differentiate the varieties of *R. microsporus* and the other small species of *Rhizopus* that cause disease in humans due to the subtle differences in their morphology and growth characteristics (Table 7).

The major human pathogen in the *Microsporus* group is *R. microsporus* var. *rhizopodiformis*. This mold grows in culture as a floccose aerial mycelium (a "lid lifter") which is dark gray, gray-brown, or black in surface coloration, often with a speckled pattern. It is very thermophilic, growing at temperatures up to 50 to 52°C. Rhizoids and sporangiophores originate from stolons opposing each other. Rhizoids are intensely branched or may be rudimentary and measure 80 to 100 μm in length. Sporangiophores are brown and occur in clusters from one to four. They are 200 to 1,000 μm long and terminate in a single round sporangium measuring 40 to 130 μm in diameter (predominantly 50 to 60 μm). Columella are elongated to pyriform. Apophyses are well defined and angular. Sporangiospores are very regular in size and shape, measuring 4 to 6 μm. They have an overall subglobose to rhomboidal shape and definite surface striations and spines. Zygospores have not been demonstrated (409, 412).

R. microsporus var. *microsporus* has been implicated in causing human disease occasionally. The morphology of this mold is very similar to the other members of this group, but it produces colonies that are light brown to gray. Sporangia are gray-black and measure up to 80 μm in diameter. Sporangiophores originate from the stolon most often in pairs. This variety may be differentiated by its production of angulated and distinctly striated sporangiospores and lack of growth at 50°C (409, 502). Mating studies may also be performed to definitively identify the mold. *R. microsporus* var. *microsporus* produces mature zygospores heterothallically when mated with the properly oriented stain of the same variety. Zygospores are red-brown, occur on unequal suspensors, and measure 60 to 100 μm in diameter on the average (range, 40 to 120 μm) (409, 412).

R. microsporus var. *oligosporus* is a decidedly uncommon human pathogen, having caused only a single case of wound zygomycosis (461). This mold grows with a low aerial mycelium

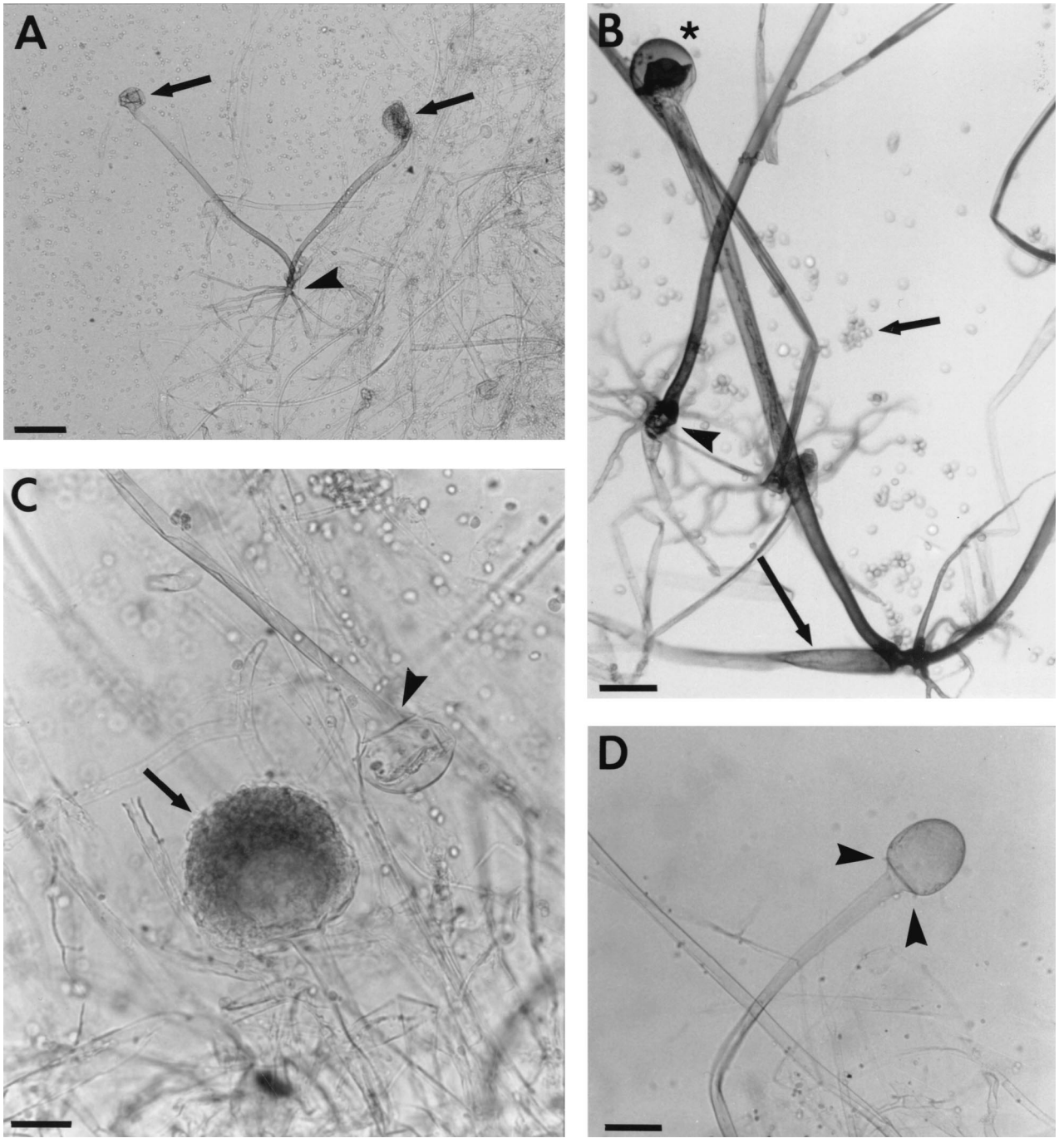


FIG. 10. Microscopic features of *Rhizopus* spp. in culture. (A) Low-power magnification (×4) of *Rhizopus* demonstrates the nodal occurrence of well-developed rhizoids in this genus (arrowhead). Sporangiophores are long and unbranched, usually terminating in large globose sporangia. In this figure, the sporangia have become deliquescent, releasing a plethora of sporangiospores seen dispersed in the background. The naked, somewhat collapsed columellae remain at the distal end of the sporangiophores (arrows). The sporangiophore themselves are pigmented light brown. Bar, 200 μm. (B) Rhizoids originate directly at the base of the sporangiophores, often from a gnarled, pigmented knot at the node (arrowhead). Stolons extend laterally from the node (long arrows). The naked columella has partially collapsed back on the sporangiophore (asterisk). At this higher power of magnification, the round to oval sporangiospore morphology is readily appreciated (short arrows). Bar, 25 μm. (C) An intact, mature globose sporangium is seen in this photomicrograph (arrow). The spherical columella is nearly obscured by the presence of the sporangiospores in the sporangial sack. A flattened, bare columella is also present, illustrating well the slight apophysis that is often seen in the *Rhizopus* spp. (arrowhead). Bar, 50 μm. (D) Upon deliquescence, small sporangial membrane remnants may adhere at the interface between the apophysis and the columella. These remnants are referred to as the collarette and are well illustrated in this photomicrograph (arrowheads). Note also the very slight apophysis at the top of the sporangiophore. This columella is intact and demonstrates the oval shape that may be seen in *Rhizopus* isolates. Bar, 25 μm.

that is pale yellowish brown to dark grey. Features that help to differentiate this variety from the others in the *Microsporus* group include identification of poorly developed rhizoids and growth up to 48°C with good sporulation seen at 40°C. Sporangiophores arise in groups from one to three and measure up to 300 μm in length. Sporangia are black and generally measure 50 to 60 μm (up to 100 μm) in diameter. This mold produces almost smooth, pleomorphic sporangiospores mea-

suring 7 to 10 μm. Zygospores have not been described for this variety of *Rhizopus* (412, 461).

R. schipperae is closely related morphologically to the *Microsporus* group by virtue of its small size. It grows in culture as a white, thin aerial mycelium. Optimal growth and development of spores are seen between 30 and 35°C, although the mold will grow when incubated up to 45°C. No growth is seen at 48 to 50°C. *R. schipperae* has rather fastidious growth requirements and fails to sporulate on most standard media. Sporangia may be produced on Czapek agar, while chlamydoconidia and sterile hyphae are produced on most other media. Under optimal growth conditions, *R. schipperae* produces tufts of up to 10 sporangiophores arising from a rhizoidal cluster. Sporangiophores are brown and unbranched and measure 100 to 410 μm in length and 5 to 15 μm in width. Globose, gray-black sporangia (<80 μm in diameter) are produced at the aerial end of each sporangiophore. These are both columellate and apophysate. The columella is subglobose to conical and nonpigmented. Sporangiospores are oval and faintly striated, measuring 5.8 to 6.8 μm by 4.8 to 5.0 μm. The rhizoidal complexes are well developed, with brown pigmented, unbranched rhizoids. Even under optimal growth conditions, abundant chlamydoconidia are produced. These occur as both terminal and intercalated oval structures measuring 20 μm in diameter. Zygospore production is unknown (500).

R. azygosporus is also closely related to the *Microsporus* group and is characterized by diminutive stature. Yuan and Jong (519) described this mold as a new species based on the complete absence of true zygospore production and abundant production of azygospores. *R. azygosporus* grows rapidly as a dense, white aerial mycelium that gradually turns grey or black with incubation. Optimal growth is seen between 28 and 45°C, although the fungus may demonstrate poor growth up to about 50°C. Both sporangium and azygospore formation are optimal between 25 to 37°C. Microscopically, dark brown sporangiophores originate from simple, pale brown rhizoidal complexes. Sporangiophores measure 6 to 14 μm in diameter and may be 350 to 500 μm long. At the aerial end of the sporangiophore, black, globose sporangia (35 to 100 μm in diameter) are produced, which are rapidly deliquescent. These are prominently columellate with a hyaline oval columella. Sporangiospores are oval, measuring 4 to 6 by 6 to 7 μm. Pleomorphic chlamydospores and azygospores are produced in abundance. Round azygospores (30 to 70 μm in diameter) are pale to dark brown and have conical spines. Superficially, these look like zygospores, but they are produced on one well-developed suspensor, not two (410, 519). Azygospores are not sexually formed but instead result from parthenogenesis rather than plasmogamy or karyogamy (11). Azygospore formation in this organism occurs best at 28°C (range, 25 to 37°C) (410, 519).

Other nonculture techniques have also been developed for the diagnosis of disease caused by *Rhizopus*. Detection of antibodies produced during an invasive infection (198, 230, 365, 517) or secondary to an allergic response to inhaled fungal elements (404) has been successful. Sandvren and Edwards (404) were able to detect antibodies to *Rhizopus* in sawmill workers by both double-diffusion and ELISA techniques, although the ELISA method proved to be more sensitive. Similarly, antibodies stimulated by subcutaneous exposures to *R. (oryzae) arrhizus* have been detected using crossed immunoelectrophoresis and counterimmunoelectrophoresis (198). ELISA (230) and immunodiffusion assays (230, 365) using *R. arrhizus* antigens detect antibodies produced during an infection with the zygomycetes. Both the sensitivity and specificity of the ELISA are superior to those of immunodiffusion. Neither test is able to determine the species responsible for disease, however (230, 365). In the assays developed by Yankey and Abraham (517), antibodies produced during disease with a zygomycete could be detected using either counterimmunoelectrophoresis or indirect immunofluorescence techniques. The immunofluorescence methods proved to be more sensitive and specific for detecting disease serologically. The limited availability and lack of specificity of serologic tests used to detect zygomycosis make these tests of very limited clinical value. PCR amplification of 18S rRNA and single-strand conformational polymorphism pattern identification have also been used to distinguish *Rhizopus* spp. from other medically significant fungal infections (488).

Treatment. Amphotericin B is the only clinically useful antifungal drug used to treat zygomycosis caused by the *Mucorales* (348). Numerous case reports have indicated successful therapy with intravenous amphotericin B, either alone or together with other interventions. The vast majority of in vitro testing with other antifungal agents has been investigated using *Rhizopus* spp. as the representative zygomycete. Itraconazole provides no survival benefit when used to treat experimental infections in animals (334). Ketoconazole, miconazole, and saperconazole have likewise proven ineffective against *Rhizopus* in in vitro studies (347). A recent review of the use of azole drugs in treating fungal infections concludes that there is no indication for the use of this class of drugs in treating zygomycosis (457). Pneumocadin L743,872 (114) and the echinocandin derivatives (364) have also been without efficacy in in vitro susceptibility studies.

Experience in successfully using hyperbaric oxygen treatments as an additional therapy is anecdotal (157). Growth of *Rhizopus* spp. may be inhibited by prolonged exposure to hyperbaric oxygen in vitro. In experiments where the fungi were exposed to 10 atm of oxygen at 24°C for 1 to 2 weeks, fungicidal activity was demonstrated (393), suggesting that hyperbaric oxygen may prove useful in combination with antifungal therapy.

Surgical intervention and appropriate medical management are also required for optimal treatment results. The underlying risk factors for developing zygomycosis should be corrected if possible. Despite aggressive debridement or amputation, however, some cases of zygomycosis are not curable. Zygomycosis caused by *Rhizopus* spp. still carries a high mortality rate, and cases are still left undiagnosed until after death, obviating the ability to intervene.

Relationship to other fungi causing infections. The *Rhizopus* species are closely related to one another. As is true for many of the *Mucorales*, initial attempts to determine the species of these organisms on the basis of morphologic features and effect of growth temperatures resulted in subspecies identification, which later was refuted due to the wide variability of these results (418). As a result, the medical literature is littered with a plethora of names that have been reduced to synonymy with time. Some of the more common synonyms are listed in Table 8. The status of *R. arrhizus* and *R. oryzae* as separate species remained until the work of Ellis (146) demonstrated 95% homology by DNA hybridization studies. Immunologically, *R. arrhizus* and *R. oryzae* demonstrate a close antigenic relationship, also suggesting that they are synonymous (198). The correct designation for *R. arrhizus*/*oryzae* has been widely disputed. The lack of type material and the indefinitive description and illustrations have led some individuals to conclude that *R. oryzae* is most appropriate, with *R. arrhizus* being reduced to synonymy (408). Performing DNA complementarity studies on type material from the Blakeslee Collection, Ellis (146) demonstrated that these species were indeed synonymous. Ellis further suggested that the mold be named *R. ar-*

TABLE 6. Differentiating features of the main *Rhizopus* species and groups causing disease in humans

Organism name	Colony morphology	Sporangium morphology	Apophysis and columella morphology	Sporangiospore morphology
R. stolonifer	"Lid-lifting" floccose white mycelium with black dots; rapid growth	Globose; black; large (up to 275 μm)	Together are oval to subglobose, 70–120 μm in diameter; pale brown; apophysis is distinct	Round to oval; black; prominent striations
R. arrhizus	Floccose, yellowish-brown colonies, darkening and collapsing with age; rapid growth	Globose; powdery and gray to black; intermediate in size, 100–200 μm in diameter	Together are globose to oval, 40–75 by 60–130 μm; apophysis is inconspicuous; often collapses at sporulation	Oval to ellipsoidal, 6–8 by 4.5–6 μm; striated
R. microsporus group	Moderately floccose mycelium; color varies with variety	Globose; small (usually about 100 μm)	Collumellar shape and size vary among varieties; apophysis is discrete	Variable size and shape depending on variety
R. azygosporus	Floccose white mycelim, turns grey to black with age	Globose; small (50–100 μm in diameter)	Oval collumella	Oval, faintly striated, 4–6 μm
R. schipperae	Thin white nonsporulating mycelium on routine media	Globose; small (<80 μm in diameter)	Subglobose to conical columella; distinct apophysis	Oval and faintly striated, 4–7 μm

rhizus var. *arrhizus* and that *R. oryzae* be reduced to synonymy under *R. arrhizus* (146), agreeing with the order of precedence. In reality, the literature is still littered with references to this mold under both designations, in addition to 30 or so others (408), and so one must realize that they are indeed the same organism.

R. schipperae is closely related to the *Microsporus* group on a morphologic basis. It is most closely related to *R. microsporus* var. *microsporus*, with similar sporangiophore, sporangium, sporangiospore, and columellar morphology and a similar temperature growth dependence. Distinguishing features that set this organism apart from the *Microsporus* species include its fastidious sporulation requirements and its distinctive organization of sporangiophores in large clusters of up to 10 sporangiophores. *R. schipperae* fails to produce zygospores when mated with *R. microsporus* var. *microsporus* isolates, however. Due to these distinctive features, Weitzman et al. (500) concluded that this organism deserved its own species designation. *R. schipperae* also has some limited morphologic similarities to *R. caespitosus*, which also produces large clusters of sporangiophores. Similar to *A. rouxii*, *R. schipperae* produces abundant chlamydospores. These two organisms fail to produce zygospores when mated with the *R. schipperae* isolate, however (500).

Antigenic studies on the *Rhizopus microsporus* group demonstrated that the varieties *microsporus*, *rhizopodiformis*, and *chinensis* are all very closely related (370). *Rhizopus azygosporus* is also closely related to the *R. microsporus* group. When *R. microsporus* var. *microsporus* is mated with *R. microsporus* var. *rhizopodiformis*, azygosporus strains are produced which have very similar morphology to *R. azygosporus*. This suggests that *R. azygosporus* resulted in nature from the spontaneous mating between these two varieties of *R. microsporus* (354). Due to its obligate azygosporic nature, *R. azygosporus* was given its own species designation despite its morphologic similarity to the *R. microsporus* group (519).

Mucor Species

Natural habitats. Members of the genus *Mucor* are saprophytes that are ubiquitous in nature. They are found in soil and environmental samples worldwide, from the Arctic to the tropics (123, 155, 177, 352). Spores have been demonstrated in air or dust samples obtained from both the home and hospital

TABLE 7. Differentiating features of the *R. microsporus* group and related small *Rhizopus* species

Organism name	Sporangiophore grouping and morphology	Sporangiospore morphology
R. microsporus var. *rhizopodiformis*	Occur in singly or in clusters of up to 4	Subglobose to rhomboid shape; regular size 4–6 μm; striated and spiny
R. microsporus var. *microsporus*	Occur singly, in pairs, or clusters of 3	Rhomboidal shape; regular size 5–6 μm; distinctly striated
R. microsporus var. *oligosporus*	Generally occur in clusters	Pleomorphic shapes; variable size 7–10 μm; almost smooth
R. azygosporus	Generally occur in pairs or clusters	Round to oval shape; regular size 4–7 μm; faintly striated
R. schipperae	Occur in large clusters of up to 10	Subglobose to oval shape; regular size 5 by 7 μm; faintly striated

TABLE 6—*Continued*

Rhizoids	Sporangiophore morphology	Zygospores	Other
Abundant; intensely ramified, 300–350 μm long	Long (up to 2 mm), pigmented, and unbranched; occur in groups of 1–3	Heterothallic; black, round zygospores with irregular surface decorations; 150–200 μm wide; equal suspensors	No growth at >33°C; good sporulation at 15–30°C
Abundant; brown, with 4–8 branches	Intermediate length (750–2,000 μm, generally 1,500 μm); occur singly or in groups	Heterothallic; reddish brown round to flattened with conical projections; usually 80–140 μm	No growth at >46°C
Simple, no branching	Short (usually 800–1000 μm or less)	Heterothallic; Small (80 to 100 μm) red-brown	Maximum temperature of growth varies with variety
Simple, pale brown	Short (usually 350–500 μm)	Azygospores 30–70 μm in diameter are abundant	Optimal growth and sporulation at 25–30°C
Simple, brown, rarely branched	Short (100–410 μm) in clusters of 10	Not known; produces many chlamydospores	No growth at >50°C

settings (27, 51, 107, 265, 329). *Mucor* species have also been seen as part of the microbiota of dog fur, showing seasonal fluctuations during the wet and temperate fall and spring months (65). Spores of *Mucor* species have also been demonstrated in a variety of food and medicinal products. *M. circinelloides* and *M. hiemalis* have been isolated from cereals, nuts, and flour (187, 368). *M. racemosus* is the most common *Mucor* species found in wheat grains (3), and it has also been found in stored Egyptian soybean seeds (142). Both *M. circinelloides* and *M. rouxianus* have been identified in herbal or naturopathic remedies (137, 344). *M. hiemalis* has been identified in stored white cabbage (169), while *M. circinelloides* has been isolated from oranges associated with an outbreak of onychomycosis (443, 444). Both *M. racemosus* and *M. hiemalis* have been found to contaminate hay (8).

Several species of *Mucor* cause disease in humans. These include *M. circinelloides*, *M. hiemalis*, *M. rouxianus*, *M. ramosissimus*, and *M. racemosus. M. racemosus* is an occasional cause of animal mycosis (8).

Transmission. Several modes of transmission have been identified for *Mucor* species. As is true for the other *Mucorales*, cases of rhinocerebral and pulmonary disease have been attributed to inhalation of spores. Infection of the alimentary tract is most probably acquired through ingestion of fungal spores in food products or herbal preparations (344, 386). Cutaneous disease has resulted from percutaneous exposures due to injections (130), insect bites (372; R. J. Fetchick, M. G. Rinaldi, and S. H. Sun, Abstr. 86th Annu. Meet. Am. Soc. Microbiol. 1986, abstr. F-42, p. 404, 1986), and other forms of traumatic implantation of spores (98, 210, 520). Nosocomial infections have resulted from implantation of spores into an erosive wound caused by the removal of an adhesive bandage (274) or introduction of spores subcutaneously by placement of an intravenous catheter (160). An outbreak of fungal nail infections occurred in workers who handled *M. circinelloides*-infected oranges. Workers contracted the nail infection while squeezing cull oranges with their bare hands (443, 444).

Host characteristics. *Mucor* spp. are opportunists. Similar to the other members of the family *Mucoraceae*, infections are seen with a variety of disease states that cause immunosuppression. Cases have been identified associated with leukemia (179, 265, 293, 299, 344, 456; Fetchick et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986), aplastic anemia (61, 498), organ or bone marrow transplantation (344), diabetes mellitus (61, 70, 98, 157, 299), renal disease (70, 156), iron overload (274), asthma (520), gastric cancer (127), burns (520), and

TABLE 7—*Continued*

Rhizoid morphology	Zygospore morphology	Other characteristics
Rudimentary to intensely ramified	Not known	Good growth at 50°C
Simple	Seen with mating	No growth at 50°C
Simple, with blunt ends	Not known	No growth at 50°C
Simple	Azygospores formed readily within a single isolate; no true zygospores produced; chlamydospores usually produced in abundance	Glabrous growth without sporulation seen at 50°C; optimal sporulation and azygospore formation between 25 and 37°C
Simple, rarely branched	Not known	No growth at 50°C; produces abundant chlamydospores and fails to sporulate on routine fungal media

TABLE 8. Most common synonyms for *Rhizopus* species in the medical literature

Species	Synonyms
Rhizopus arrhizus	*R. oryzae* *R. racemoosus* *R. japonicus* *R. rouxii* *R. chinensis* *Mucor arrhizus*
Rhizopus microsporus var. *rhizopodiformis*	*R. rhizopodiformis* *Mucor rhizopodiformis*
Rhizopus stolonifer	*Mucor stolonifer* *Mucor niger* *Rhizopus nigricans* *Rhizopus niger*

prednisone therapy (45, 157, 210, 293, 299, 344, 520). Immunoimmaturity may also have been a predisposing feature for a newborn (R. Sharma, R. R. Prem, A. A. Padhye, and R. Carzoli, Abstract Pediatr. Res. **34:**303A, 1994) and a 7-week-old infant (156). Hepatitis (498) and possibly HIV infection (499) may also have been underlying risks for infection. Artificial surfaces such as a prosthetic heart valve (81) and a deep venous line (160) served as the site of infection in two additional patients. Cases have also been seen in competent hosts and have been noninvasive or locally invasive without dissemination (130, 210, 372, 443, 444, 477, 490; Fetchick et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986).

General disease manifestations. The disease manifestations for *Mucor* are roughly divided according to the immune status of the patient. Immunocompromised hosts tend to have invasive solid-organ disease, while competent hosts demonstrate predominantly cutaneous or nail infections. Pulmonary (108, 265, 293) rhinocerebral (45, 157, 290, 299, 520), rhino-orbital (61), and gastrointestinal (127, 344, 456, 499) cases predominate. Gastrointestinal diseases include abdominal pain with hepatic abscesses (344, 456), necrotic ulcerations of the stomach wall (127), and diarrhea, paralytic ileus, and an appendiceal mass as evidence of the gastrointestinal involvement (456). Rare cases of cerebral disease (210; Sharma et al., Abstract, Pediatr. Res. **34:**303A, 1994), septic arthritis (Sharma et al., Abstract, Pediatr. Res. **34:**303A, 1994), peritonitis (156), severe myonecrosis (371), and endocarditis (81) have also been described.

Cutaneous infections with these organisms show a great deal of variability. Necrotic and hemorrhagic lesions with black eschars or gangrenous cellulitis may be seen (61, 160, 520; Fetchick et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986). Erythema is often noted surrounding the lesions. Erythematous papules, nodules, and plaques without superficial necrosis have also been described (130, 274, 372, 490). A single case described the formation of verrucous lesions (98). Shallow ulcerations have also been described (490). Some patients have demonstrated chronic infections that persisted for months or years (477, 490).

Nail infections with *M. circinelloides* are superficial, typically characterized by punctate erosions along the entire length of the nail. The free borders of the nails are somewhat friable and exhibit a yellowish discoloration but no thickening. Although the cuticle and skin may be red, slightly swollen, tender, and productive of a small amount of pus, the fungal infection does not appear to extend into adjoining surfaces of the skin. Of note, after removal of infected nails, infected nails regrew, suggesting involvement of the nail bed with the fungal infection (380). Asymptomatic colonization of nails by *M. hiemalis* has also been described (1).

Several cases of *Mucor* infection or the external ear canal have been described in the pre-1900 literature (209). More recently, a review of 110 patients with otomycosis in India documented that *Mucor* spp. account for 1.2% of the fungal infections of the external ear (78), while a study of 100 patients with external otitis in Greece demonstrated a 4% occurrence of otomycosis caused by the zygomycetes (355). The risk for developing *Mucor* otomycosis increases following administration of broad spectrum antibiotics, use of steroids and chemotherapy. Whether this represents a true disease process is debatable. These earlier reports may not be valid by current diagnostic criteria and may not reflect actual disease, since the *Mucorales* may be isolated from both normal and diseased skin and outer ear canals (386). Transient colonization of skin and wounds with the zygomycetes is not infrequent (386) and may pose a tremendous risk for developing into actual invasive disease in patients with large cutaneous defects such as those with burns (318, 520).

M. racemosus has been associated with mastitis in cattle, lung infections in rabbits and mink, and mycoses in pigs, chickens, turkeys, and dogs (8).

Virulence factors. *Mucor* spp. are relatively avirulent. In contrast to most of the other zygomycetes, *Mucor* species are not particularly thermotolerant. Although *M. rouxianus* grows optimally at 37°C, *M. ramosissimus* and *M. circinelloides* grow only slowly and *M. hiemalis* does not grow at all at this temperature. In their studies of zygomycosis in diabetic rabbits, Reinhardt et al. demonstrated the inability of *M. circinelloides* to cause either pulmonary or cerebral disease. These authors related this to their inability to grow in vitro at temperatures above 39°C (the rabbit core body temperature) (381). *M. hiemalis*, whose optimal growth temperature is only 32°C, has had its pathogenicity for deep invasive disease challenged (175, 412). It has been definitively linked to disease in cases of chronic skin infections. Its pathogenicity may therefore be limited to cutaneous infections, where the temperature may be cooler than core body temperature (98, 372).

The only other virulence factors for *Mucor* spp. are those identified in relation to plants. *M. hiemalis* produces fungal metabolites that strongly suppress cell division in germinating grains, while *M. circinelloides* produces chromosomal aberrations in dividing plant cells (4).

Diagnosis. H&E-stained sections of the affected tissue demonstrates the typical findings of zygomycosis. Morphologic variation in tissue involvement may be seen. In a patient with *M. hiemalis*, subcutaneous lesions showed aseptate hyphae immersed in an intense acute inflammatory exudate of neutrophils, eosinophils and fibrosis (98). Focal collections of epithelioid and Langhans multinucleated giant cells were also observed. Oval spores and coenocytic and sparsely septate hyphae were present in the cystic structures of the dermis. The hyphae were sometimes aggregated into fungus balls (98). Epidermal involvement in another case has shown mild spongiosis with superficial and deep nodular granulomatous dermatitis consisting of lymphocytes, histiocytes, plasma cells, and foreign-body giant-cell reaction (372). Pleocytosis and persistently elevated protein levels in the cerebrospinal fluid have been seen in a patient with central nervous system involvement with *M. ramosissimus*. Inflammatory response may produce either purulent or serous exudates (Sharma et al., Abstract, Pediatr. Res. **34:**303A, 1994). Yeast forms were identified in nail specimens infected with *M. circinelloides* (443).

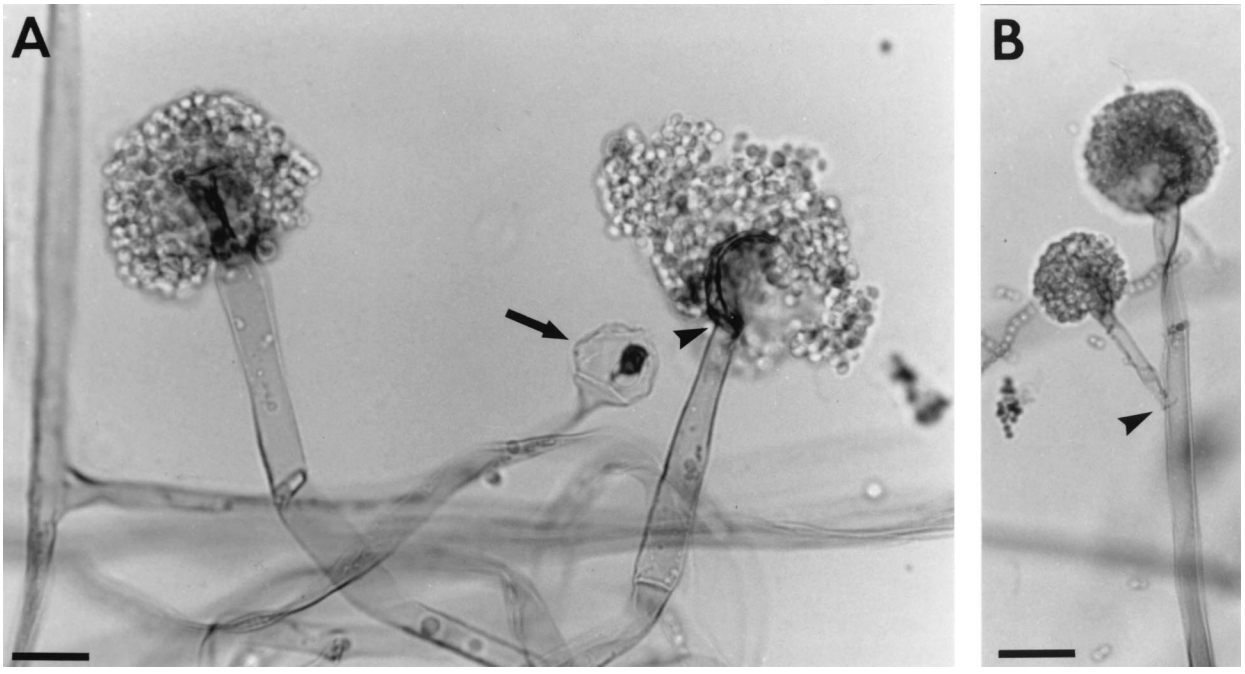


FIG. 11. Microscopic features of *Mucor* spp. in culture. (A) Stages of sporangial development. To the left, the mature, nearly globose sporangium is intact. The columella is partly obscured by the presence of sporangiospores within the sporangium. The sporangium to the far right is deliquescent. As the sporangial membrane dissolves, clumps of sporangiospores are released. The sporangiophore terminates without an apophyseal swelling (arrowhead). A bare, dome-shaped columella (arrow) is all that remains of the middle sporangium. Bar, 20 μm. (B) *Mucor* spp. produce branched sporangiophore (arrowhead). Also seen in *Mucor* spp. is a lack of both stolons and rhizoids. Bar, 30 μm.

Mucor spp. typically are rapid growing, producing globose sporangia on sporangiophores that are either solitary or branched. The sporangia contain the entire columella and spores that are mucus bound. The sporangial wall collapses irregularly, if at all. Sporangia may also be deliquescent (dissolving). Rhizoids and stolons are absent. These features distinguish the *Mucor* spp. from the other producers of globose sporangia (Fig. 11; Table 2). Occasionally, determination of *Mucor* species may be warranted, such as in cases of outbreaks or where linkage of a clinical isolates to an environment or food product source is sought (Table 9).

Colonies of *M. circinelloides* are pale gray or yellowish and are rapid growing. The organism grows at 25 to 37°C. Colonies appear more brown at 37°C. Sporangiophores are sympodially branched and often circinate. Occasional cultures fail to produce the circinate branches, however. Sporangiophores bear spherical sporangia that are variable in size (25 to 80 μm) and are deliquescent. Columellae are somewhat spherical but may vary in shape. They measure up to 50 μm in diameter. Collars may be present depending on the form. Sporangiospores are smooth walled, 4.5 to 7 μm in length, ellipsoidal, and hyaline. Chlamydoconidia occasionally are produced in some cultures and are spherical, cylindrical, or irregular in shape (175, 392). Mating studies with the appropriate mating strains will produce round, reddish brown to dark brown zygospores (up to 100 μm diameter) decorated with characteristic stellate spines. Cultures of *M. circinelloides* are positive for nitrate assimilation using Salkin's nitrate agar (499).

M. hiemalis produces grayish colonies in culture which rapidly spread and often fill the entire petri dish. Growth is sparse, and the reverse colony is pale. The maximum growth temperature is 32°C (412). Microscopically, *M. hiemalis* is similar to *M. circinelloides* but produces larger sporangiospores which are smooth-walled, 5 to 11 μm long, hyaline, ellipsoidal, or sometimes reniform. Sporangiophores are usually unbranched, although they may be sympodially branched. Sporangia are up to 60 μm in diameter with ellipsoidal columellae that are 15 to 30 μm in diameter. Chlamydoconidia may be up to 15 μm in diameter and spherical to cylindrical, but they are not common. *M. hiemalis* is heterothallic. Zygospores are not formed in pure culture (175).

M. rouxianus produces white, yellow, or gray colonies on standard fungal media. The optimum growth temperature is 37°C, with a range of 9 to 45°C. Colonies are delicate and low, at most 4 mm high. Bright yellow or golden brown sporangia, usually approximately 50 μm in diameter, are borne on sporangiophores that are weakly sympodially branched. Columellae up to 40 μm high are globose or flattened and often have a colored membrane. Sporangiospores are 4 to 5 μm long and oval. Chlamydoconidia are located on aerial hyphae and are numerous, black, variable in size, and up to 100 μm in diameter. Budding yeast-like cells may appear on submerged hyphae. Zygospores are not present. When the organism is grown on starch substrates, abundant fat globules that are deep yellow may develop in the mycelium (175).

M. racemosus produces colonies that are low to moderately raised and are light brown to mid-brown in color due to the sporangia and chlamydoconidia. The mycelium is colorless. Sporangiophores arise from the surface or aerial mycelium and are branched in a mixed sympodial and monopodial fashion. Sporangia are 80 μm in diameter and light brown and have encrusted walls. Sporangiospores are 5 to 8 μm in diameter, broadly ellipsoidal to subspheroidal, smooth walled, and hyaline to pale brown. The columellae, which are up to 40 μm long, are ellipsoidal to pyriform. Chlamydoconidia and arthroconidia have a long axis and are formed abundantly. Zygospores are produced heterothallically. The optimal growth temperature seems to be between 32 to 35°C (175, 412). Poor growth is obtained at 37°C (175). *M. racemosus* is one of a few dimorphic *Mucor* spp., along with *M. rouxii*, and *M. amphibi-*

TABLE 9. Differentiating features of the *Mucor* species pathogenic to humans

Organism name	Colony morphology	Sporangium morphology	Columella morphology
Mucor circinelloides	Floccose with rapid growth; pale gray to yellowish, brown at 37°C	Globose, 25–80 μm	Spherical, up to 50 μm in diameter; collars may be present
Mucor hiemalis	Sparse growth; gray	Globose, up to 60 μm	Ellipsoidal; 15–30 μm in diameter
Mucor rouxianus	Delicate low colonies (4 mm high); white, yellow, or gray	Globose, gold-brown to yellow, usually 50 μm in diameter	Flattened or round, up to 40 μm high, colored membrane
Mucor racemosus	Low to medium-high colonies; light to medium brown	Globose, light brown, encrusted walls, up to 80 μm	Ellipsoidal to pyriform, up to 40 μm long
Mucor ramosissimus	Rapidly growing low colonies; gray to buff	Globose, 15–70 μm	Round to flattened, 20–37 by 17–30 μm; collars may be seen; smaller sporangia lack columella

orum. Yeast phase conversion in vitro may be stimulated by incubation in conditions of high carbon dioxide concentrations (30, 31, 363).

M. ramosissimus grows rapidly on standard fungal media, producing low-growing, grayish olive to cinnamon-buff colonies. This fungus is hyaline, with a grayish reverse that is slightly yellow at the point of inoculation. The colony grows rapidly at 25°C but demonstrates restricted colonial spread when grown at 37°C. Sporangiophores always arise from the substrate mycelium, are up to 17 μm in diameter, and become progressively smaller in diameter, sometimes ending in a sterile filament. They are branched regularly in a sympodial fashion, typically roughened and erect, and somewhat constricted below the sporangia. The first branches (15 to 33 μm in length) bear the larger sporangia. Each successive branch is shorter and smaller in diameter and may have racquet-shaped regions toward the top and often successively above one another. The larger sporangial walls are deliquescent, but the majority are extremely persistent, roughened, encrusted, and transparent and remain in one or two pieces when crushed. Sporangia 15 to 70 μm in diameter contain a variable number of sporangiospores that are irregularly ovoid to globose and range in size from 3.3 to 5.5 by 3.5 to 8 μm. Sporangiospores are hyaline when seen singly and brownish when seen in a mass. Smooth hyaline columellae are 20 to 37 μm in diameter, 17 to 30 μm in length, and flattened to almost globose with collars. Smaller sporangia lack columellae and resemble those produced by *Mortierella* species. Specialized cells called ouida (6.5 to 13 μm in diameter) arise at the ends of hyphae or alongside the substrate hyphae and are hyaline with dense cytoplasm that are compressed to globose to elongate. Neither zygospores nor chlamydoconidia are produced. The temperature growth range is 15 to 37°C (197, 412).

Species identification is best left to the experts, since it requires extensive experience with the morphologic characteristics, physiological and biochemical tests, and growth temperatures. Most laboratories do not identify beyond the genus level. Zygospores may be used to identify species in some fungi, but this is not practical in routine practice because of the need for tester strains and thus is limited to reference laboratories.

Treatment. In the treatment of *Mucor* infections, amphotericin B is the drug of choice. In vitro testing has shown that both *M. circinelloides* and *M. hiemalis* are susceptible to amphotericin B (130, 372). *M. hiemalis* is inhibited by a MIC of 2.5 mg/ml, which is attainable in vivo (492). Although Sharma et al. have reported that *M. ramosissimus* is susceptible to miconazole in their in vitro susceptibility testing system (422), other authors have demonstrated that the azoles 5-flurocytosine and naftifine are ineffective in inhibiting the growth of *Mucor* spp. in vitro (348). Likewise, saperconazole had no inhibitory effect on the growth of *M. circinelloides* or *M. racemosus* in vitro (347). Other reported treatments include potassium iodide (1 g three times per day) for the treatment of subcutaneous lesions (98) and treatment of superficial nail infections with continuous moist dressings soaked with a 1:10,000 solution of mercuric chloride for 3 to 4 days.

Relationship to other fungi causing infections. Until relatively recently, it was believed that *Mucor* spp. were the second most common causes of zygomycosis. This is no longer the case for two main reasons. First, many publications describing zygomycosis fail to provide culture data, identifying "mucormycosis" on tissue sections only. Many authors arbitrarily assigned the name "*Mucor*" to the fungal elements despite the lack of culture confirmation. Second, the nomenclature of the mucoraceous fungi has changed substantially over the last century. When *Mucor corymbifera* was reassigned to the genus *Absidia* as *A. corymbifera*, this eliminated many if not most of the cases that had previously been attributed to *Mucor* spp. Likewise, the cases of *Mucor pusillus* infection, although relatively few were attributed the genus *Rhizomucor* with the reclassification of these thermophilic organisms into their own genus. These reassignments of organisms leave a relatively small number of infrequent clinical isolates attributed to the *Mucor* spp., thereby demoting *Mucor* to a distant third as a cause of infection by the *Mucoraceae*, well behind the *Rhizopus* spp. and *Absidia corymbifera* in occurrence.

Differentiation of *Mucor* from the other zygomycetes depends upon identification of globose sporangia, branched sporangiophores, and a lack of rhizoids. Temperature dependence, biochemicals, and mating studies may be performed to subspeciate isolates, although in reality, this is rarely done.

TABLE 9—*Continued*

Sporangiospore morphology	Rhizoids and apophyses	Sporangiophore morphology	Zygospores and chlamydoconidia	Other
Smooth walled and oval; 4.4–7 μm	Absent	Sympodially branched and circinate	Heterothallic; round, red to dark brown, with stellate spines up to 100 μm wide; chlamydoconidia sometimes seen	Growth from 5 to 37°C
Hyaline; smooth walled and oval or reniform; 5–11 μm	Absent	Branched or unbranched	Heterothallic; chlamydoconidia rarely produced	Growth up to 32°C
Oval, smooth walled; 4–5 μm	Absent	Minimally branched	No zygospores; many black chlamydoconidia up to 100 μm wide	Growth from 9 to 45°C; optimal growth at 37°C
Oval to subspherical, smooth walled; 5–8 μm	Absent	Branched	Heterothallic; abundant chlamydoconidia produced	Optimal growth at 25°C, poor or no growth over 32°C
Oval to round, smooth walled, brownish; 3.3–5.5 by 3.5–8 μm	Absent	Sympodially branched; may have racquet-shaped swellings	Zygospores and chlamydoconidia not seen; oidia may be present	Optimal growth at 24°C, poor growth at 37°C

Rhizomucor pusillus

Natural habitats. *Rhizomucor* species are found worldwide, with the *Compendium of Soil Fungi* listing references of their isolation from eastern Europe, the British Isles, North America, Japan, Indonesia, India, and Africa (124). *Rhizomucor* is commonly seen contaminating air, soil and organic matter. It has been isolated from decaying or composting garden and municipal wastes, composted wheat straw, self-heated hay and corn, cultivated mushroom beds, manure, guano, leaf mold, and grass (124). *Rhizomucor pusillus* is the most common species seen and has been detected in a variety of food items including grains, seeds, nuts, and beans (124). It has been cited as the most common thermophilic fungus to be isolated from soybeans in Egypt (142). Spores from *Rhizomucor* are easily airborne due to their small size and have been isolated from air samples collected outdoors (335) and in hospitals (113, 329, 434).

Transmission. Two modes of transmission have been suggested for *R. pusillus*: inhalation of spores and percutaneous introduction of spores into a susceptible host. Several cases have resulted from probable nosocomial exposures (113, 398, 417, 434, 504), with one series of iatrogenic cases being linked to *R. pusillus* spore-positive air and facility surface cultures (113). Cases of disseminated disease and endocarditis probably arose from primary pulmonary infections (153, 174). Cutaneous disease caused by this fungus is acquired by two mechanisms. Disease may result from direct traumatic implantation of spores into subcutaneous tissues (398, 504) or may occur as a manifestation of disseminated infection (249).

Host characteristics. Nineteen cases of *Rhizomucor* infection in humans have been described in the literature to date. The first reported case of *R. pusillus* (then called *Rhizomucor parasiticus*) infection occurred in a woman with pulmonary tuberculosis and was published in the French literature (209, 270). Most of the remaining cases have been associated with profound neutropenia. Although most of these cases were seen in patients with leukemia (29, 113, 249, 298, 398, 417, 434, 499), *Rhizomucor* infections have also been seen associated with neutropenia caused by aplastic anemia (174) and myelofibrosis (153). Treatment with steroids probably contributed to disease risk for a number of patients (29, 174, 249, 257, 398, 417, 434, 499, 504). Antibiotic use was seen in nearly every case of *Rhizomucor* cited in the literature. Immunosuppressive therapy following renal transplantation and diabetes mellitus were risk factors for the development of a zygomycosis in an additional patient (257). Diabetes alone was the risk factor for an additional patient with cellulitis (504). One patient had extensive hemosiderosis as an additional risk factor (153). A questionable case of pulmonary *Rhizomucor* occurred in an 86-year-old man with congestive heart failure and pneumonia (209, 325).

General disease manifestations. The majority of cases of *R. pusillus* present as pulmonary or disseminated disease (29, 174, 209, 249, 257, 270, 298, 325, 417, 434, 499). Primary cutaneous involvement has been described following trauma in two immunocompromised patients (398, 504). Rhinofacial disease and mycotic endocarditis have each been seen in a single patient (113, 153).

The *Rhizomucor* species have been identified as significant pathogens in animals. Pulmonary disease and mycosis affecting other solid organs has been described for *R. pusillus* in pigs, horses, cow, dogs, rabbits, ferrets, ducks, and seals (124). This organism is one of the most common causes of mycotic abortion and mastitis in cattle (412). In addition to its association with bovine mastitis and disease in the newborn calf, *R. pusillus* has been associated with cases of bovine infertility (8). *Rhizomucor miehei*, although never demonstrated to be a human pathogen, has also been described as a significant cause of bovine mycotic abortion and bovine mastitis (124, 412).

Virulence factors. *R. pusillus* is a rare cause of human disease and occurs as an opportunistic infection in a susceptible host. This, coupled with its widespread occurrence in nature, suggests that *Rhizomucor* has a low pathogenic potential. It has, however, been frequently isolated as a cause of disease in a variety of domesticated and wild animals (124, 412). *R. pusillus* is angioinvasive and may disseminate widely. Pathogenicity in this organism has been linked to its thermotolerance, which presumably allows it to grow in febrile patients. This ability to grow at temperatures above 39 to 40°C has been used as a marker for potential human pathogenicity for other thermophilic zygomycetes (382). Compared to many other zygomycetes, however, *R. pusillus* appears to be less pathogenic. Rienhardt et al. found that *Rhizomucor pusillus* produces fewer proteolytic enzymes that may be responsible for virulence than do *Rhizopus* spp. (382). This fungus produces lipases, acid

proteases, acids, and ethanol. Extracts from fungal mycelia contain mycotoxins with activity against chicken embryos and brine shrimp (124). Little is known about virulence factors associated with human disease.

R. miehei is extremely pathogenic in experimentally infected mice (412). Similar to *R. pusillus*, this fungus is also very thermotolerant, demonstrating enhanced growth above 40°C (124). No cases of human disease with this species have been noted.

Diagnosis. Similar to the other zygomycetes, *Rhizomucor* produces typical coenocytic hyphae in clinical specimens. Tissue infiltrates often appear nodular and may demonstrate necrosis, tissue and vessel invasion, and thrombosis. Culture is required for identification to the species level.

R. pusillus demonstrates a temperature growth range from 20 to 60°C. Colonies are initially colorless to white, becoming gray with age. Cultures have a floccose mycelium 2 to 3 mm high. Sporangiophores are branched irregularly and extensively but sometimes are unbranched. Poorly formed rhizoids may be present but occur between and not at the base of the sporangiophores. Spherical, brown or gray sporangia are usually 40 to 60 μm in diameter but may be up to 80 μm, with walls that are opaque and glittering. The sporangia rupture at maturity releasing the enclosed sporangiospores. The smooth-walled, hyaline sporangiospores measure 3 to 5 μm in diameter and may be globose to broadly ellipsoidal. Columellae are slightly pyriform to subglobose, measuring 20 to 45 μm in diameter. Chlamydospores are not produced. *Rhizomucor pusillus* is heterothallic, requiring opposite-orientation mating strains for the production of zygospores. Zygospore formation may be stimulated at higher temperatures of incubation (30 to 40°C). Zygospores measure 45 to 70 μm in diameter and may appear reddish brown to black in color. They are globose to broadly ellipsoidal with a textured surface resembling flat irregular warts, or "stellate warts" (124, 175, 499). Weitzman et al. suggest that mating studies are the "last word" in species determination for the zygomycetes (499). Sucrose, melezitose, and methyl-D-glucose are all assimilated. Potassium nitrate is also assimilated on potassium nitrate agar. Isolates do not require thiamine supplements for growth (434). In addition to culture, serologic assays have been developed to detect *Rhizomucor*-specific antibodies. Kaufman et al. have developed an *R. pusillus* homogenate that is capable of detecting antibodies produced in response not only to *Rhizomucor* but also to the other zygomycetes found causing rhinocerebral, pulmonary, and disseminated infections (230). Serology using antigens from other zygomycetes has likewise been useful in detecting antibodies produced in response to *Rhizomucor* infections (417).

R. miehei resembles *R. pusillus* in many respects. These two organisms may be differentiated from one another by several morphological and biochemical properties (Fig. 12, Table 10). *R. miehei* fails to grow below 22°C and grows only slowly at 25 to 30°C. Colonies tend to be more darkly pigmented than *R. pusillus*, being described as dirty gray or brown gray. The branching of the sporangiophore is looser, and the sporangia are 50 to 60 μm in diameter with spiny walls. Columellae are rarely larger than 30 μm. *R. miehei* is homothallic, readily producing zygospores without mating. Thus, reddish brown to blackish brown zygospores, up to 50 μm in diameter, are produced by every strain. The zygospore surface is decorated with the same "stellate warts" seen in *R. pusillus* and is held by suspensors that are elongate, slightly conical, and equal in length (175, 412). *R. pusillus* and *R. miehei* may also be differentiated on the basis of carbohydrate and amino acid utilization. Sucrose, glycine, phenylalanine, and beta-alanine are all assimilated by *R. pusillus* but not *R. miehei*. Studies of the genetic polymorphisms in these two species demonstrate a high level of dissimilarity between them, as large as the differences seen between *Rhizomucor* and the other genera of *Mucorales*, *Rhizopus*, *Mucor*, and *Absidia* (471).

Treatment. Only 6 of the 19 patients with *Rhizomucor* infection described in the literature have responded to treatment. Of historical interest, the first case described in 1901 by Lucet and Constantine reportedly responded to treatment with arsenic and iodides (209), a treatment certainly not considered appropriate by today's standards! The more recent treatment successes employed some combination of systemic antifungal, surgical intervention, and correction of underlying diseases seen as risk factors for zygomycosis. Systemic amphotericin B and its liposomal form are the drugs most often cited in successful treatment (113, 149, 174, 398, 504). Limited available in vitro susceptibility testing demonstrates *R. pusillus* susceptibility to amphotericin B (149, 434) but resistance to flucytosine (434), fluconazole (434), miconazole (149), and ketoconazole (149).

Relationship to other fungi causing infections. *Rhizomucor* is a markedly thermophilic genus of the family *Mucoraceae*, containing three known species: *R. pusillus*, *R. miehei*, and *R. tauricus*. The nomenclature of the species has changed substantially over this century. Now correctly designated *Rhizomucor pusillus*, this organism was originally cited in the literature as *Mucor pusillus* and *Rhizomucor parasiticus*. Both of these designations are now obsolete. *Rhizomucor miehei* is likewise synonymous with *Mucor miehei*, and the former is the preferred designation. *Rhizomucor tauricus* has reportedly been isolated only once and is not associated with human disease (124).

The genus *Rhizomucor* is differentiated from the other genera of the *Mucoraceae* on the basis of microscopic morphology and degree of thermophilic growth (Table 2). The diagnostic differential is generally between *Rhizomucor* and the other *Mucoraceae* that produce globose sporangia, predominantly *Rhizopus* and *Mucor* species. *Rhizomucor* spp. are easily differentiated from *Rhizopus* spp. by the presence of extensively branched sporangiophores and lack of well-developed nodal rhizoids. They may be distinguished from *Mucor* spp. by the presence of primitive internodal rhizoids and extreme thermotolerance. *Rhizomucor* species may also be differentiated from the genera *Rhizopus*, *Mucor*, and *Absidia* on the basis of isoenzyme analysis (471). *Rhizomucor*, *Mucor*, *Rhizopus*, and *Absidia* species have been shown by serologic techniques to have both species-specific and shared antigenic determinants. The highest degree of antigenic sharing is seen between *Absidia corymbifera* and *Rhizomucor* species. These appear to represent primarily carbohydrate similarities (198). *R. pusillus* is the only *Rhizomucor* species known to cause disease in humans. It is a rare cause of zygomycosis, falling far below *Rhizopus* in occurrence. It is an uncommon isolate to be seen in the laboratory, even as a contaminant. Its differentiation from the nonhuman pathogen *R. miehei* is discussed in detail above.

Absidia corymbifera

Natural habitats. *Absidia corymbifera* is a saprophytic organism that is isolated primarily from soil and decaying vegetation (121, 435). This fungus is distributed worldwide, with environmental isolates having been collected from Europe, the Middle East, Indonesia, North America, Africa (121), the British Isles (8, 107, 121), South America (155) and India (352). The organism has also been recovered from various foodstuffs and spices. *A. corymbifera* is seen as a colonizer of wheat straw compost and has been isolated from many different grains,

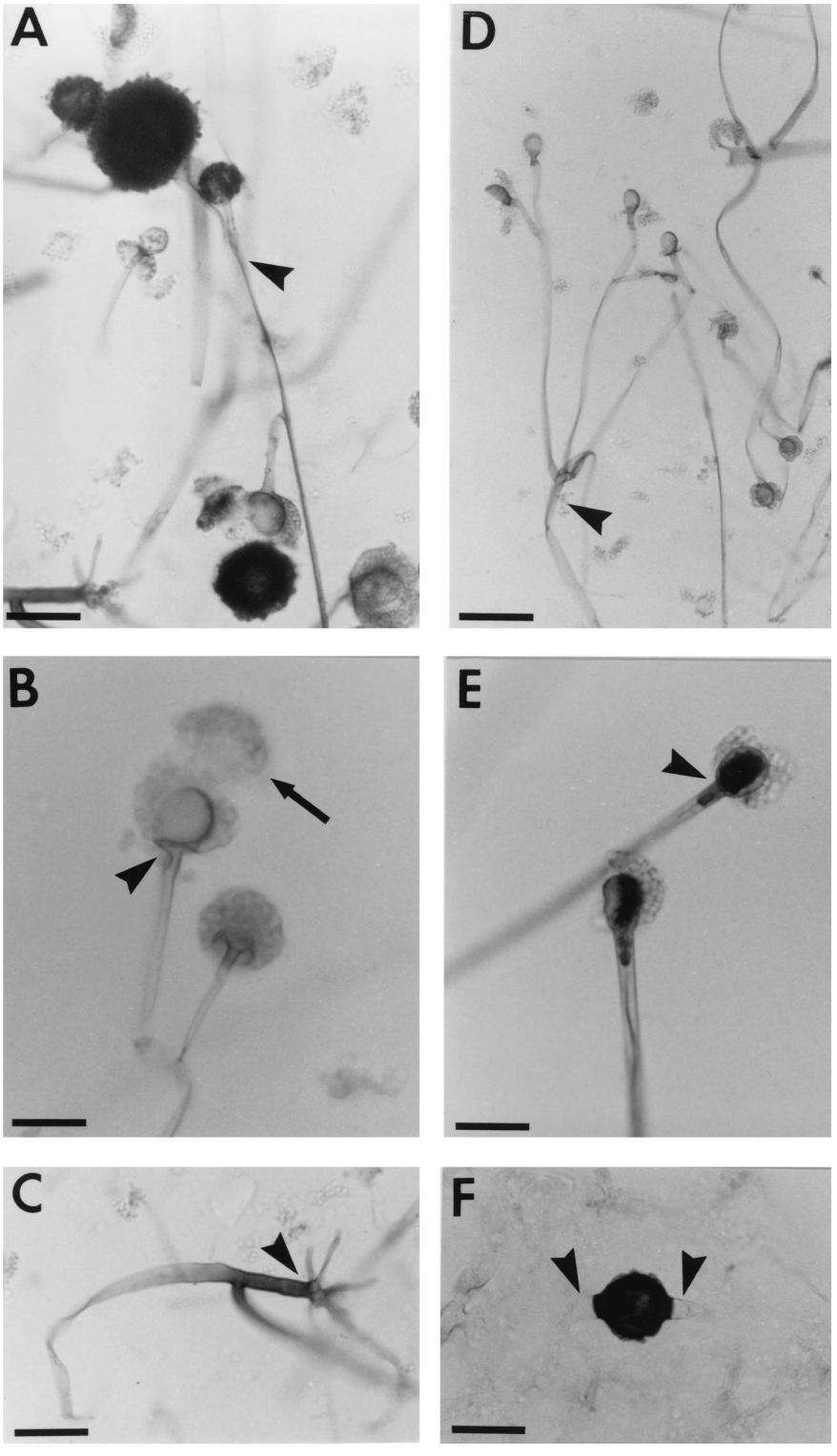


FIG. 12. Microscopic features of *Rhizomucor pusillus* and *Rhizomucor miehei* in culture. (A) *R. pusillus* produces branched sporangiophores (arrowhead) with globose sporangia often clustering at the aerial end. Bar, 50 μm. (B) Higher-power magnification of *R. pusillus* demonstrates a deliquescent sporangium (arrow) with an oval columella and no appreciable apophysis (arrowhead). The sporangiophore is again branched. Bar, 20 μm. (C) *Rhizomucor* spp. produce rhizoids that are primitive and inconspicuous (arrowhead). When they are present, they occur internodally, not at the base of the sporangiophore. Bar, 50 μm. (D) *R. miehei* produces sympodially branched sporangiophores that are very loose and open in morphology (arrowhead). The sporangia in this photomicrograph are all deliquescent, leaving little except the bare columellae behind. Bar, 100 μm. (E) Higher magnification of two mature sporangia of *R. miehei* demonstrates the oval columella, lack of apophysis (arrowhead), and small round sporangiospores. Bar, 25 μm. (F) Zygospores of *R. miehei*. Zygospores are produced homothalically in *R. miehei* but not *R. pusillus*. This is an important differentiating feature between these two isolates. These zygospores of *R. miehei* are spherical and thick walled and have a rough and/or "warty" surface decoration. The hyphal suspensors, the remnants of the conjugating mycelium, are still attached (arrowheads). When produced as a result of mating with an appropriate strain, the zygospores produced by *R. pusillus* are identical. Bar, 25 μm.

TABLE 10. Differentiating features of *Rhizomucor pusillus* and *Rhizomucor miehei*

Organism name	Colony morphology	Sporangium morphology	Columella morphology	Sporangiospore morphology
Rhizomucor pusillus	Floccose mycelium 2–3 μm high; colorless to white, becoming grey with age	Globose with smooth, opaque walls, usually 40–60 μm, may be up to 80 μm	Pyriform, subglobose, or spherical; 20–45 μm wide	Smooth walled, globose to broadly ellipsoidal, 3–5 μm
Rhizomucor miehei	Low-growing colonies, darker than *R. pusillus*; dirty gray to brown gray	Globose with spiny walls, 50–60 μm	Pyriform to subglobose, rarely over 30 μm wide	Smooth walled, globose to broadly ellipsoidal, 3–5 μm

nuts, seeds, and cotton (121). It has also been cultured from dust samples collected in British homes (107). It grows well under moist, humid conditions, having been cultured from waterlogged grasslands, swamps, mangrove mud, human sewage, animal dung, and bird and bat guano (121). *A. corymbifera* has been identified in hay and animal fodder in British farms (8). It is noteworthy that *A. corymbifera* has also been recovered from dried grass and the right boot that accompanied the frozen, well-preserved prehistoric corpse, Ice Man, with an age of approximately 5,300 years (194)!

Transmission. The modes of transmission for cases of pulmonary, rhinocerebral, and gastric absidiomycosis are consistent with those seen for the other zygomycetes. Inhalation of spores, ingestion of food contaminated with fungal spores, and traumatic implantation of spores probably underlie most cases of *A. corymbifera* infection. *A. corymbifera* infection has been reported to occur as a result of injuries with spore-contaminated farm equipment (178), of corneal laceration with a galvanized nail (280), and in a patient with meningitis that resulted from a strike to the left frontal region of the head with the sharp end of a pickaxe (276). Inoculation of spores into disrupted skin probably occurred in one patient who handled animal material or possibly moldy hay or feedstuffs (206). Intravenous inoculation of fungi contaminating drugs, skin, or inoculating needles is thought to be the route of infection in intravenous drug users (426, 465).

A. corymbifera infection may be transmitted nosocomially. *A. corymbifera* infection in a liver transplant patient was introduced from under an electrode monitoring vital signs (479), and it was introduced percutaneously in another patient at an atrial catheter exit site (262). Transplantation patients may become infected from contaminated surgical equipment, cadaver organs, or even live organs used for transplantation (431).

Host characteristics. Infections with *A. corymbifera* usually occur in immunocompromised hosts. Both the underlying host condition and the portal of entry dictate the form of mycotic disease, with cutaneous, rhinocerebral, pulmonary and gastrointestinal sites being affected most often. Depending on the status of the host, dissemination may occur from these sites (175, 268, 392, 431). Host characteristics associated with the published cases of absidiomycosis include immunocompromise from a variety of causes. Several cases of *A. corymbifera* infection have occurred in bone marrow or solid-organ transplant recipients (102, 188, 217, 262, 278, 431, 433, 479). *A. corymbifera* infections in cancer or leukemia patients have also been described (47, 140, 188, 268, 399). The widespread use of chemotherapeutic agents, immunosuppressive therapy (including prednisone), and broad-spectrum antibiotics probably play a contributory role in promoting fungal infections in these patients. Hospitalized patients, particularly those undergoing major procedures, are at increased risk since the organism may be spread nosocomially by various means (262, 431, 479). Among patients undergoing organ transplantation, orthotopic liver graft recipients are at highest risk for developing infections because of the prolonged operation time, need for reoperation and reintubation, massive use of antibacterial agents, immunosuppressive therapy, and possibly previous colonization (278, 479). Diabetes, cystic fibrosis, and malnutrition have been seen as other more unusual underlying disease processes for patients with *A. corymbifera* infections (200, 433, 499).

Coinfection with other organisms may also be predisposing factors for infection with *A. corymbifera*. For example, Andrews et al. (18) have suggested that cytomegalovirus may potentiate fungal growth, and gastrointestinal zygomycosis is associated with infections, including amebic colitis, pellagra, and typhoid (175). Indeed, *A. corymbifera* infections have been found together with a number of important pathogens. HIV infection and AIDS has been the predisposing risk for *A. corymbifera* infection in several patients (206, 426, 465). *A. corymbifera* infections have been found in patients with hepatitis C (479), other fungal infections (178, 479), and pulmonary tuberculosis (209). Many patients either have, or are treated for, concomitant bacterial infections.

Although infection with *A. corymbifera* usually occurs in immunosuppressed individuals, *Absidia* infections in immunocompetent hosts have also been described (178, 255, 280).

General disease manifestations. *A. corymbifera* is a relatively uncommon pathogen of the order *Mucorales* compared to *Rhizopus* spp. Analysis of some of the larger series of culture proven zygomycosis demonstrates that *A. corymbifera* represents 0 to 33% of all cases of zygomycosis. The statistics, when combined for these series, estimate the overall proportion of *A. corymbifera* infections to be only 2 to 3% of all zygomycete infections (154, 188, 209, 253, 279, 306, 321). Still, over 30 cases of disease with this organism have been recorded in the literature. Although Furbringer is credited with describing the first case of zygomycosis due to *A. corymbifera* as a cause of pulmonary zygomycosis in 1876 (154, 412), Rippon (392) noted that the organism may have been *Aspergillus niger*, not *A. corymbifera*. Platauf described an agent of zygomycosis in 1885 which was also responsible for the first disseminated case and was compatible with *A. corymbifera* (369, 392). The first case described in the United States was likewise attributed to *A.* (*Mucor*) *corymbifera* on the basis of expert review of the tissue section morphology alone (181). However, many cases of culture-confirmed absidiomycosis have been reported. Infections with *A. corymbifera* encompass the entire spectrum of zygomycete disease manifestations including cutaneous and subcutaneous, rhinocerebral, pulmonary, gastrointestinal, disseminated, and other more uncommon forms of disease (175).

Cutaneous infections with *A. corymbifera* manifest as grey-black plaques that rapidly increase in size over a 12- to 24-h period (178). Lopes et al. (268) described an unusual case in

TABLE 10—*Continued*

Rhizoids	Sporangiophore morphology	Zygospores	Other
Primitive; rare	Extensively and irregularly branched; more typically monopodial; occur between rhizoidal tufts	Heterothallic; zygospore formation best from 30 to 40°C; round to oval with stellate warts, red, brown or black; 45–70 μm wide	Growth from 20 to 60°C; assimilates sucrose, glycine, phenylalanine, and β-alanine
Primitive; rare	Branched sympodially, and more loosely than *R. pusillus*; occur between rhizoidal tufts	Homothallic; round to oval zygospores with stellate warts, red, brown or black; 50 μm wide	Slow growth at 25–30°C with growth up to 58°C; does not assimilate sucrose, glycine, phenylalanine, or β-alanine

which the infection probably resulted from latent osteomyelitis. The infection formed an extensive necrotic area but did not become systemic. When Lopes et al. (268) reviewed the literature, they learned that this case was the eighth case of cutaneous infection with *A. corymbifera*, including those reported in 1874 (200) of external ear infections; skin, subcutaneous tissue, and bone necrosis in a malnourished child; a granulomatous ulcer of the foot; facial cellulitis; and lymphomatoid pappulosis resembling ecthyma gangrenosum. Following the report by Lopes et al., primary cutaneous zygomycosis has been reported in a AIDS patient (206) and in two patients who had undergone bone marrow transplantation (217, 262).

Rhinocerebral and facial zygomycosis with *A. corymbifera* has been found in several patients (278, 279, 399, 433). Rhinocerebral disease with this fungus often presents as swelling or inflammation of the periorbital region and cheek, accompanied by facial pain, epistaxis, cranial nerve palsies, and blurred vision. A black necrotic nasal turbinate is typical (399). The outcome for patients with rhinocerebral zygomycosis due to *A. corymbifera* is variable. Stevens et al. (433) described an immunosuppressed renal transplant patient with steroid-induced diabetes mellitus who survived the infection, while both patients reported by Ryan and Warren (399) died. Brain involvement, aside from the cases spread from sinusitis lesions, may also result from disseminated disease (140, 188, 365) or direct trauma involving the central nervous system (276). It is an uncommon cause of brain abscess, with only one case having been reported in a study at the Fred Hutchinson Cancer Research Center covering the years 1984 to 1992 and including 58 culture-proven or histology-proven brain abscess diagnoses (188). Of note, however, the *A. corymbifera* infection represented one out of only three infections with zygomycetes (33%), the other two cases having been caused by *Rhizopus* spp.

Although 7% of all mucoraceous infections involve the gastrointestinal tract (253), absidiomycosis is an uncommon isolate from these sites. The only culture-confirmed case of gastrointestinal *A. corymbifera* infection occurred in an 11-year-old boy who was impaled on the tines of a rotating drum of a silage wagon (178). Because his cutaneous and abdominal wounds were coinfected with *Rhizopus*, *Aspergillus*, and *Stenotrophomonas* (*Xanthomonas*) *maltophilia*, consistent with contamination of these wounds at the time of the farm injury, the infection is better classified as a wound infection than as a true gastrointestinal infection.

Six cases of primary pulmonary absidiomycosis have been published to date. Nodular infiltrates or lobar pneumonias have been seen. A pulmonary infection was reported in Australia, an uncommon geographic site for *A. corymbifera* infection (255). This case is particularly unusual because the patient did not have an underlying disease. The patient responded to a combination of surgery and antifungals.

Several disseminated infections have now been reported with *A. corymbifera*, including disseminated zygomycosis involving the brain, lungs, and heart in a 50-year-old woman with metastatic carcinoma (140), posttraumatic infections followed by systemic dissemination (276), a brain abscess after bone marrow transplantation (188), two cases of renal involvement in patients with a history of intravenous drug abuse and AIDS (426, 465), and three cases of posttransplantation *A. corymbifera* infection (431, 479). One case of posttransplantation absidiomycosis was localized only in the kidney which had been transplanted from an unrelated living donor. The kidney was bought and transplanted in India.

As the most frequent agent of zygomycosis among lower animals, *A. corymbifera* may be isolated in cases of mycotic bovine abortion, bovine mastitis, gastric disease in swine, and avian zygomycosis (8, 121, 392, 412). Bovine fetal cerebral absidiomycosis has also been reported (8, 244). *Absidia* spp. are the predominant fungi cultured from skin scrapings of nondiseased farm animals (8) and have been found to cause zygomycosis in guinea pigs, horses, and sheep.

Virulence factors. *A. corymbifera* is an opportunist that rarely infects immunocompetent persons. Virulence factors associated with *A. corymbifera* include the ability to survive adverse conditions, such as extremes in temperature and harsh environmental conditions (194), and then germinate under ideal conditions. Conditions favorable to germination include an environment of low pH, high glucose (154), increased iron (23), and decreased phagocytic defense by leukocytes (94, 96). Iron is a known growth factor required by *Absidia* spp. Siderophore production by this fungus is considered to be a virulence factor (204).

In the immunocompetent individual, *A. corymbifera* is susceptible to human sera (150) and to the functions of leukocytes which prevent in vivo spores from germinating (154). Corbel and Eades (94) injected mice with various agents including cortisone azathioprine, cyclophosphamide, antithymocyte serum, zymosan, aggregated gamma globulin, cortisone acetate, and Fe(II) salts. The investigators found that these treatments increased the proportion of mice developing lethal mycoses of the central nervous system and, in the case of cortisone acetate, also promoted disseminated infection. Smith (428) infected noncortisone-treated mice with *A. corymbifera* and found nongerminated spores in lung tissues. Since the spores grew when cultured in vitro, Espinel-Ingroff et al. (154) suggested that the absence of hyphae is due to germination inhibition and not spore destruction. Once infection is established, however, it can be lethal, especially in the compromised individual because of the angioinvasive property of the organism (79, 392). *A. corymbifera* is considered to be thermophilic and psychrotolerant (21, 121). It has had its pathogenicity linked to its ability to grow in vitro at temperatures above core body temperature, even at temperatures up to 48 to 52°C (435). Of

note, nonpathogenic *Absidia* species are generally unable to grow at or above 37°C (435).

A. corymbifera appears to be less virulent than other members of the *Mucorales* that have been examined for pathogenicity. Rienhardt et al. (382) found that *A. corymbifera* caused cerebral infection in ketotic rabbits but that the pathogenicity was more marked with strains of thermotolerant *Rhizopus* spp. Eng et al. (150) showed that *Absidia* was more susceptible to human sera than was *Rhizopus*.

Additional virulence factors may be attributed to *A. corymbifera*. It is possible that the organism has proteolytic properties that allow it to penetrate intact skin. This property has been demonstrated for other fungi (92), and there are reports of cutaneous zygomycosis in patients with intact cutaneous barriers (392). Proteolytic activity has been demonstrated for another species of *Absidia*. Rajak et al. (377) showed that *A. cylindospora* degrades keratin, with human scalp hair being the most favored keratin substrate.

Absidia hyalospora is not a proven agent of mucormycosis but is considered potentially pathogenic because of its ability to grow at 37°C and because pathogenicity in mice has been demonstrated experimentally with this organism (412).

Diagnosis. *A. corymbifera* may be detected in tissue sections with the typical coenocytic hyphal elements seen most often in tissue sections. The angioinvasiveness of the organisms makes it a relatively uncommon finding in noninvasively obtained cytologic specimens. Hyphal elements of *A. corymbifera* are indistinguishable from those of the other *Mucoraceae* in clinical specimens.

A. corymbifera, the only *Absidia* species recognized as a human pathogen, grows readily on routine mycology medium and does not require enrichments for cultivation, although growth is stimulated with thiamine. Sabouraud dextrose agar with 1 to 2% dextrose, 1% peptone, and 2 to 3% agar plus chloramphenicol and gentamicin is an appropriate choice of agar for this organism (412). *A. corymbifera* grows more rapidly at 37 than 25°C and is capable of growth up to 48 to 52°C, a range that is inhibitory to most *Absidia* species. At 37°C, *A. corymbifera* produces a woolly colony which may cover the entire petri dish within 24 h. The colony is initially white and turns grayish brown to greenish beige on the surface with an uncolored reverse (35, 175, 412, 435).

A. corymbifera hyphae are large, aseptate, or with a few adventitious septa. *A. corymbifera* produces aerial sporangiophores arising from the stolon. The sporangiophores are branched and long (450 μm in length and 4 to 8 μm in diameter) and often are arranged in whorls or clusters called corymbs, hence the species name. Numerous small, irregularly shaped sporangiophores may be produced. *Absidia* produces a flask- or funnel-shaped apophysis beneath the sporangiophore. Sporangia are pear shaped and have a prominent conical columella. Rhizoids, which are usually scarce, are situated between the sporangiophores on the stolons. The rhizoids are hyaline and are approximately 370 μm long and 12 μm in diameter. Produced within the sporangia, asexual sporangiospores are round to elliptical and measure 2 to 3 by 3 to 4 μm in size (35, 175, 412, 435). Zygospores may form between appropriately oriented mating strains. Zygospores are round to somewhat flattened, measuring 50 to 80 μm in diameter. They are brown with rough walls, with several equatorial ridges. Weitzman et al. believe that mating of strains and production of zygospores provide definitive identification and should be employed for atypical zygomycetes isolated from clinical specimens (499). *A. corymbifera* is nonfermentative, assimilates neither sucrose nor ethanol, but does assimilate lactose, melibiose, raffinose, and nitrate (412). The morphologic features seen in culture are demonstrated in Fig. 13 and summarized in Tables 2 and 3.

In addition to colonial growth patterns and requirements, microscopic appearance, and carbohydrate and nitrate usage, other techniques may be used to identify *A. corymbifera*. Jones and Kaufman (220) described a serological diagnostic method that is 73% sensitive and 100% specific when using homogenate antigens. Double-diffusion precipitin tests (276, 365) and complement fixation antibodies to *A. corymbifera* have been described (276). Counterimmunoelectrophoresis as a mycological procedure was described by Mackenzie and Philpot (275) and Smith (429), who investigated the use of the procedure for detecting serum antibodies capable of precipitating fungal antigens. Levy et al. (265) described an ELISA method for the detection of zygomycosis. Immunofluorescence techniques are used by the Centers for Disease Control for detection and identification in formaldehyde-fixed tissue sections (154).

Absidia hyalospora, a potential pathogen due to its thermophilic growth characteristics, grows at 37°C, producing a dark gray pigment. Its maximum growth temperature is 40°C (412). This organism has a requirement for supplemental thiamine in the growth medium. In culture, *A. hyalospora* produces gray sporangiophores with a dark gray apex; they are either unbranched or poorly branched. Sporangia are nearly black and pyriform, with a transverse diameter of up to 40 to 80 μm. Columellae are dark gray, hemispherical to spatulate, and often with projections. The apophysis is long and conical, and sporangiospores are gray to nearly black and subglobose, averaging 6 to 10 μm in length. It is not known if this species produces zygospores (412). *A. hyalospora* is nonfermentative, does not assimilate ethanol, but does assimilate sucrose, lactose, and nitrate. Sucrose assimilation distinguishes *A. hyalospora* from *A. corymbifera*.

Treatment. Treatment for absidiomycosis infection includes control of the underlying disease, surgical debridement, hyperbaric oxygen therapy, and amphotericin B (1.0 mg/kg/day) or combinations of these treatments. Amphotericin B has been used in the treatment of zygomycosis since 1958. In vitro studies support its use in patients since *A. corymbifera* isolates are almost uniformly sensitive to this drug (348). Dosages must be individualized (154), and infections with the zygomycetes tend to need higher levels of drug than are used for other fungi (360). Liposomal amphotericin B is indicated in patients with renal insufficiency (217). The liposomal form has different pharmacokinetics, allowing for higher dosing, higher efficacy, and reduced toxicity (161, 360). No oral chemoprophylaxis has been recommended to prevent zygomycosis in cancer patients (154).

Other antifungals have been tested in vitro in addition to amphotericin B, including saperconazole (347), nystatin, pimaricin, 5-fluorocytosine, ketoconazole, itraconazole, miconazole, clotrimazole, and naftifine (307). Of the above list, other than itraconazole, which inhibits *A. corymbifera* at a very low concentration, amphotericin B has the highest activity. The organism is resistant to 5-fluorocytosine and naftifine. In vitro studies with ketoconazole and miconazole show both sensitive and resistant strains (149, 348). There is little evidence to suggest that the azole drugs are effective in vivo against the zygomycetes including *A. corymbifera* (457).

Debridement of infected tissues and surgical resection are important components of treatment in some *A. corymbifera* infections and have been part of the treatment since 1957 (154). Gordon et al. (178) recommend debridement every 12 to 24 h until the progression of mucormycosis is controlled.

Jain and Agrawal (216) have demonstrated that spore formation of *A. corymbifera* is inhibited by oils, including coconut,

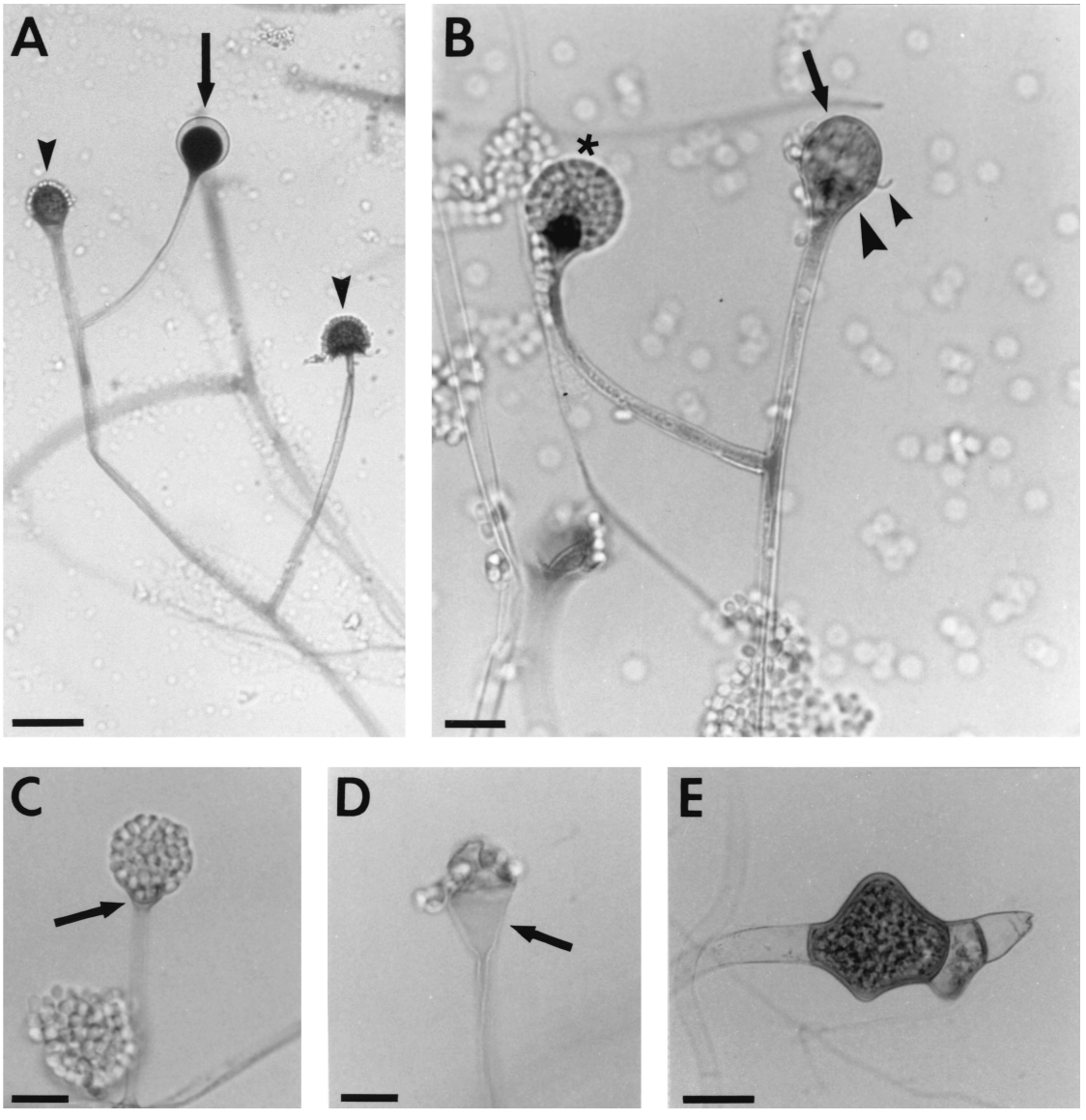


FIG. 13. Microscopic features of *Absidia corymbifera* in culture. (A) Branched sporangiophores terminate in sporangia (arrow) or the columellar remnants after sporangiospore release (arrow heads). The intact sporangium is immature. The cytoplasm has not yet been divided to form the asexually produced sporangiospores. Bar, 60 μm. (B) This branched sporangiophore demonstrates an intact mature sporangium (asterisk) and the columellar remnants after deliquescence (arrow). Prominent collorettes (small arrowhead) are often seen after the dissolution of the sporangium. The columella (long arrow) is dome shaped with a flask-shaped apophysis (large arrowhead), both of which are clearly depicted in this photomicrograph. Bar, 20 μm. (C) This intact sporangium appears almost globose. Note, however, the prominent apophysis (arrow) that helps to differentiate *Absidia* from *Rhizopus*, *Rhizomucor*, and *Mucor* spp. Bar, 20 μm. (D) This deliquescent sporangium clearly demonstrates the flask-shaped apophysis (arrow). Bar, 20 μm. (E) Giant cells are produced by some but not all *Absidia* isolates. Giant cells are found intercalated within the vegetative mycelium and may be very pleomorphic. They may be round, oval, irregular or club shaped. They often appear in clusters. These are specialized forms of chlamydoconidia. Bar, 30 μm.

mustard, soybean, alma, and groundnut oils. Alternative approaches such as oil treatments may be of questionable value for some forms of infection caused by *A. corymbifera* but certainly are not the standard of care.

Relationship to other fungi causing infections. Review of the literature with regard to *Absidia* spp. has been complicated significantly by repeated reclassification of its various species. Originally identified as a species of *Mucor* (*Mucor corymbifera*) (392), it was later reclassified into its own genus, *Absidia*. *Absidia corymbifera* has 48 synonyms (412). Early reports listed the species *A. ramosa* and *A. corymbifera*. These have since been shown by mating studies to be the same species (331). *A. corymbifera* is now the accepted name according to the rules of priority (426). It is now thought that only *A. corymbifera* is a human pathogen, and so reports of other *Absidia* species causing disease in humans should be considered to be *A. corymbifera*.

Absidia hyalospora (412) remains a separate species with possible significance in causing disease in humans as a result of its thermophilic nature. Distinction of *A. hyalospora* and *A. corymbifera* on the basis of culture characteristics has been discussed in detail in the diagnosis section of this review.

Hessian and Smith (198) studied the antigenic profiles of several mucoraceous fungi, including *A. corymbifera*. They found that unique and common antigens were demonstrable among the mucoraceous organisms, with the exception of *Mor-*

tierella wolfii, which was not antigenically similar to the others. As noted previously by Jones and Kaufman (220), *A. corymbifera* and *Mucor pusillus* (now *Rhizomucor pusillus*) showed considerable sharing of antigens. Cross-reactivity between *A. corymbifera* antigens and *R. pusillus* antiserum was obtained predominantly in material, possibly carbohydrate, bound to concanavalin A. *A. corymbifera* was not antigenically related to either *Candida albicans* or *Aspergillus fumigatus*.

Apophysomyces elegans

Natural habitats. The genus *Apophysomyces* was first described by Misra et al. in 1979 as an isolate from soil samples obtained in India (304). *Apophysomyces elegans* was subsequently identified from soil and air filter dust samples in North Australia in association with human infections (93, 415). Its distribution in tropical and subtropical climates is further suggested by the occurrence of human infections with this agent. Cases have been confirmed in Texas (207, 258, 342), Arizona (349, 493), Florida (134, 494), Illinois (145), Missouri (349), Mississippi (349), Oklahoma (316), India (76, 77, 85, 256, 284), Australia (93, 415), Venezuela (66), Aruba (295), and Mexico (376).

Transmission. Infection with *A. elegans* is predominantly the result of the introduction of spore-containing soil or vegetation into traumatic wounds (6, 66, 134, 207, 288, 316, 322, 342, 376, 493) or burns (93). In the two cases from Australia, the origin of infection was confirmed by culturing *A. elegans* from soil or dust samples at the site of exposure (93, 415). Percutaneous routes of infection are also suggested by several other cases including postoperative surgical wound infections (256, 284), an infection following an injection (77), and an infection following a spider or insect bite (494). Four cases of infection have no known mode of exposure, including one case where *A. elegans* was isolated from a bronchial washing (145), two cases with renal involvement with the fungus (85, 342), and one case of osteomyelitis (295). Of the two cases of rhinocerebral infection with *Apophysomyces*, one was probably the result of traumatic implantation of spores (376) and the other probably resulted from inhalation of spores into the sinus (76).

Host characteristics. Soil-contaminated wounds represent the single most common host risk factor. Most patients, by far, demonstrate no underlying immune system dysfunction. In the patients having risk factors for developing a zygomycosis, three had diabetes (295, 316, 342). Several patients were immunocompromised: one with severe burns (93), one following renal transplantation (316), one with myelofibrosis (76), and three receiving steroids (295, 316, 342). Thirteen patients were receiving broad-spectrum antibiotics. Although the administration of antibiotics may be considered a risk factor for zygomycosis (463), several of these patients received the drugs well into the disease process. The use of the antibiotics in this situation may not have been a risk factor for acquiring the initial infection but may have played a role in promoting fungal growth in a preexisting infection. There was no mention of risk factors or patient disease characteristics for the patient from whom the bronchial wash isolate was obtained (145) or for two additional patients with subcutaneous abscesses (349).

General disease manifestations. By far the most common site of disease manifestation in *A. elegans* infections is the cutaneous and subcutaneous tissue, with local invasion seen into neighboring tissues (necrotizing cellulitis). Painful swelling, edema, and redness or blackening of the involved tissues is often seen (256, 284, 295, 493). Blisters or bullae (93, 493, 494), abscesses (77, 93, 203, 258, 349), ulcers (203, 316), and sinus tract formation (93, 494) have been prominent features in some of these patients. Thrombosis with extensive necrosis of the involved tissues is common (66, 93, 256, 316, 376, 493). Lesions may appear black (316, 376, 493), hemorrhagic (493), or white and friable (76) or may even demonstrate white fungal growth in the infected tissues (66, 322). Exudates seen with these infections have likewise shown tremendous variation. Both acute and chronic inflammatory exudates have been noted (295, 494). Drainage characteristics often include necrotic debris and thick "anchovy paste-like" pus (93, 256, 284, 295). Exudates may also be serous, with few inflammatory cells and little necrotic debris seen (316). Cases of cutaneous and subcutaneous *A. elegans* tend to be locally invasive into adjacent tissues, particularly fat (66, 93, 207, 258, 322, 493), muscle (66, 77, 93, 207, 295, 493), nerves (258, 322), and bone (134, 207, 258, 295, 494). Necrotizing fasciitis (284, 288, 322, 376) and renal and bladder infections with this agent have also been described (85, 258, 342). Of the 18 patients with cutaneous infection, 17 have survived with appropriate treatment; the outcome for 3 additional patients is unknown (145, 349). *A. elegans* is an uncommon cause of rhinocerebral disease in humans (76, 376). Infection is generally considered to originate in the sinuses, from which the fungus may extend to involve cutaneous and subcutaneous tissues, palate, muscle, and orbital tissue and even the central nervous system (76, 376).

Virulence factors. *A. elegans* is a thermotolerant fungus capable of rapid growth at 24 to 43°C in vitro (295, 304). It is believed that this thermotolerance permits fungal proliferation in deep tissues, even in febrile patients (494). Although no specific virulence factors have been identified for this organism, it produces disease in much the same manner as the other *Mucoraceae*, with tissue and angioinvasion. In contrast to many of the other members of this family, *Apophysomyces* infections do not have a high death rate. It is thought that this does not reflect a lack of virulence in this genus but probably reflects its presentation in easily operable cutaneous sites, the availability of antifungal therapies, and its occurrence in individuals with intact immune systems (203).

Diagnosis. The microscopic morphology seen in tissues infected with *A. elegans* is similar to that of other zygomycetes. Pleomorphic, thin-walled, aseptate hyphal elements 5 to 25 μm in diameter invade both tissues and vessels (258). Hyphae may be twisted and collapsed, demonstrating bulbous vesicles and irregular nondicotomous branching (93). Fruiting structures are not seen (258, 493). Hyphae may be either hematoxylinophilic or amphophilic on H&E-stained specimens and are easily detected (258, 493). PAS and GMS staining may be weak (258). The inflammatory response seen with *A. elegans* infection is quite variable. Most patients show acute inflammation with necrosis and abscess formation (93, 134, 256, 258, 295, 493). Chronic inflammation and foreign-body reaction may be seen occasionally (134). Vessels are often invaded by hyphae and thrombosed (76, 93, 493). Tissue infarction and necrotizing vasculitis are often noted (93, 256, 258, 493). Hemorrhage into infected tissue has also been described (258).

In culture, *A. elegans* grows as a floccose aerial mycelium, demonstrating confluent growth in 2 days on standard medium. Its aggressive growth may be seen similar to that of other "lid-lifting" *Mucorales*. Although most authors describe this mold as white (76, 93, 134, 203, 256, 304, 493), other variations have been seen including cream (76, 258), grey (295), and yellow (134). Older colonies tend to have more surface pigmentation, with the cream, pale or bright yellow, or brownish grey colors developing with age (93, 258, 304). *A. elegans* is a hyaline mold, demonstrating a light or pale yellow reverse (203, 295, 304). It is a thermophilic fungus, demonstrating

rapid growth at a variety of temperatures ranging from 25°C to at least 43°C (76, 93, 134, 258, 295, 304, 493).

Although excellent growth is seen on standard culture media, *A. elegans* fails to sporulate, instead producing sterile coenocytic hyphae ranging in diameter from 3.4 to 8.0 μm (76, 93, 134, 145, 207, 256, 258, 295, 304, 493). This mycelium grows both aerially and submerged in the agar. Only with tremendous effort, using special nutrient-deficient growth medium, high temperatures of incubation, and sometimes prolonged incubation, can these isolates be forced to sporulate. A variety of different nutritionally deficient agars and culture conditions have been shown to variably induce sporulation. A 1% agar block in water stimulated sporulation in 3 weeks when incubated at 25°C (207). This time could be decreased to 10 to 14 days when isolates were incubated at 30°C (145, 207, 256, 258, 295, 493). Weiden et al. (493) reported excellent sporulation in only 5 to 7 days under these growth conditions. Some authors supplemented 1% agar with a small amount of yeast extract to promote optimal sporulation. Padhye and Ajello (349) demonstrated a temperature and time dependence of sporulation that was optimal at 37°C and 10 to 12 days of incubation. Smaller numbers of sporangia were even detected in some isolates as early as 4 to 7 days (76, 134, 284, 349). Lowering the incubation temperature to 25 or 30°C resulted in few or no sporangia being formed by 10 days. A cornmeal glucose-sucrose yeast extract agar block in water produced adequate sporulation in 2 weeks when incubated at 26°C (80, 119) or only 5 to 7 days when incubated at 32°C (145).

Microscopically, *A. elegans* has a distinctive morphology when sporulating (Fig. 14). Unbranched sporangiophores arise singly, usually from the ends of stolons or aerial hyphae. They generally measure 200 to 300 μm in length (295) but can be as long as 532 μm (304). Sporangiophores sometimes end in a "foot-cell-like" structure from which arises a tuft of colorless rhizoids. These sporangiophores are slightly tapered, taking on a light grey or brown color at both the bottom and top of the structure. Just below the apophysis, the sporangiophore becomes thicker and more darkly pigmented; this is particularly noticeable in older cultures. The apophyses are thick-walled and light brown and have a prominent bell or flask shape. These structures measure 10 to 40 μm high and 11 to 40 μm in greatest diameter. Sporangia are borne singly off of the sporangiophore. They are pyriform (20 to 58 μm in diameter) and arise above a dome-shaped columella. A translucent, smooth, thin sporangial wall surrounds multiple oval light brown sporangiospores (5.4 to 8.0 by 4.0 to 5.7 μm). This sporangial wall becomes deliquescent with age, releasing the asexually formed spores. Sexual reproduction with zygospore production has not been seen (304). The morphologic features seen in culture are summarized in Table 3.

An exoantigen test has been developed by Lombardi et al. (267) which produced precipitant bands for each of the five strains of *A. elegans* against which it was tested. The authors suggest that this is a very effective and rapid method of detecting *A. elegans* directly from sterile hyphal cultures, obviating the need for time-consuming and problematic culture of this organism.

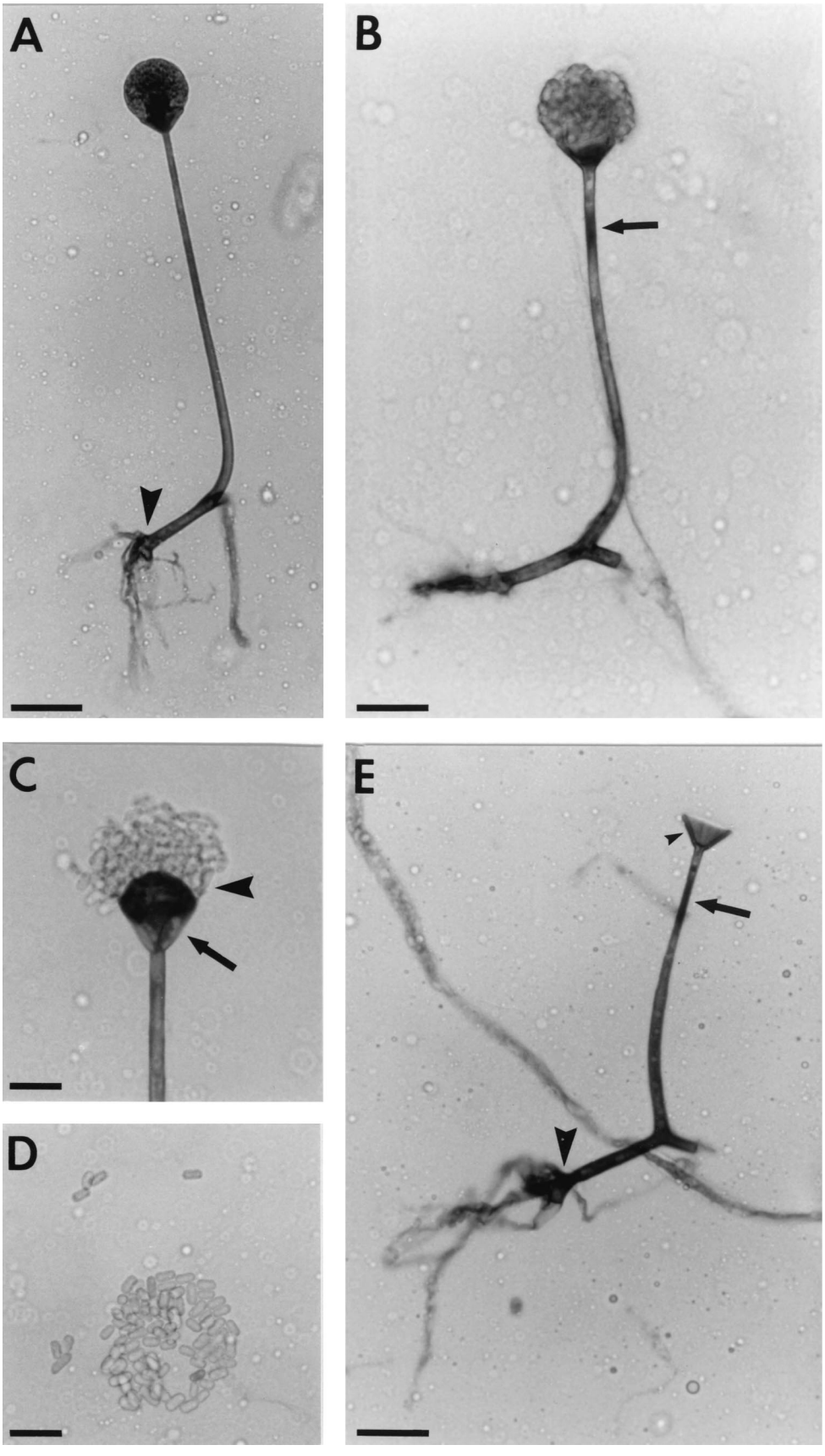
Treatment. Infections with *A. elegans* require aggressive therapy. Of the 20 patients with known outcomes, only 4 died as a direct result of the infection (76, 85, 256, 288). The vast majority of successfully treated patients were given amphotericin B treatment together with extensive surgical intervention. The importance of appropriate surgical intervention is underscored by a single case that was cured by debridement alone (77). Furthermore, four patients failed local debridement and systemic amphotericin B therapy and ultimately required amputation of the involved limbs for cure (6, 93, 207, 295, 493) and two patients receiving amphotericin B also required nephrectomy to eliminate the infection (258, 342). This suggests that the surgical interventions provide a more definitive treatment, with response to amphotericin B being variable at best. As anticipated by the responsiveness of other zygomycetes, *Apophysomyces* infections failed to respond to other antifungals such as itraconazole, fluconazole, and nystatin (66, 207). The roles that hyperbaric oxygen and gamma interferon may play in successful therapy cannot be adequately evaluated since the number of patients given these treatments is very small (322, 342). Despite the good survival rate seen overall in patients with *Apophysomyces* infections, it should be noted that significant morbidity is associated with effective treatment. In addition to the four patients requiring amputations, many of the patients underwent disfiguring debridements, requiring wide margins for complete fungal elimination. The extent of debridement has been so great as to require skin grafting for complete healing in several cases (76, 134, 284, 316, 322, 494).

Relationship to other fungi causing infections. The genus *Apophysomyces* falls within the family *Mucoraceae*. It is a monotypic genus, containing only the single species *A. elegans* (304). *A. elegans* has morphologic similarities to *Absidia* spp., with both demonstrating prominent apophyses and pyriform sporangia in sporulating cultures. Other morphologic features differ between these two fungi and help to distinguish them from one another. The apophyses are distinctive in each case. *A. elegans* has a very prominent flask or bell-shaped apophysis, while *Absidia* spp. have a less prominent flask-shaped one. Additionally, just below the apophysis, *Apophysomyces* has a brown-pigmented thickening in the sporangiophore. This is lacking in *Absidia*. Antigenically, these two molds are also quite distinct (267). It would also be uncommon for these two fungi to be confused since *Apophysomyces* has very fastidious culture requirements for sporulation while *Absidia* spp. do not.

A. elegans is often associated with *Saksenaea vasiformis* in the medical literature due to their similarities in culture requirements and in disease manifestation. Similar to *A. elegans*, the zygomycete *S. vasiformis* generally produces sterile hyphae on routine culture media. Both fungi require low-nutrient stress conditions to promote sporulation. In sporulating cultures, *A. elegans* and *S. vasiformis* produce rhizoidal complexes from foot-cell-like structures at the base of the sporangiophore. These rhizoids are submerged into the culture medium, anchoring the fungus onto the substrate. The morphology of the sporangia and the manner of sporangiospore formation in these two genera are, however, quite different. The two fungi have marked similarity in their disease presentation in humans, both primarily infecting cutaneous and subcutaneous tissues. Despite the several similarities between these fungi, *Apophysomyces* and *Saksenaea* fall into separate families of the phylum *Zygomycota*, class *Zygomycetes*, order *Mucorales*. *A. elegans* belongs to the family *Mucoraceae* (304), while *S. vasiformis* belongs to the family *Saksenaceae* (400).

Saksenaea vasiformis

Natural habitats. *Saksenaea vasiformis* was first described by Saksena in 1953 as the only species of a new genus of zygomycete from forest soil in India (400). Since then, the fungus has been isolated from soil samples in Panama (155), Israel (219), Honduras (177), and the southern United States (201). Cases of human disease occur in the warmer, tropical and subtropical climates and have been found in Mississippi (9, 112), Texas (464), Louisiana (332), Utah (229), California (33), Spain (75),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Australia (143, 173, 203, 272, 350, 357, 373), Colombia (361), Israel (171, 248), Thailand (453), India (77), and Iraq (195).

Transmission. The vast majority of cases of *S. vasiformis* infection resulted from trauma, with introduction of spore-containing dirt into open lesions. The first reported human case occurred in a previously healthy 19-year-old individual who suffered soil contaminated cranial and facial wounds in an automobile accident (9, 112). Soil contamination was likewise the probable source of infection in additional patients who sustained various crush or open injuries (9, 75, 173, 350, 366, 453). One patient developed necrotizing fasciitis after being gored by a bull (77). Another patient developed *S. vasiformis* cutaneous infection in soil-contaminated burn lesions (171). The apparent implantation of spores into subcutaneous tissue by a scratch from a tree branch (272), needle injection (361), tattooing (357), spider bite (203), or possible insect sting (33, 77) probably initiated infection in six additional patients. A case of suspected nosocomial *S. vasiformis* occurred in a motorcycle accident victim. This infection was thought to be related to an indwelling catheter rather than to his original trauma since the affected limb had not been injured in the accident (332). In one case, the role of herbal salve and oil treatments and the use of nonsterile bamboo splints could not be excluded as sources of the fungal infection in a patient who had sustained traumatic injuries (453). Two cases of sinusitis with *S. vasiformis* are thought to have originated either by inhalation of spores into the sinuses (229, 350) or by direct inoculation into facial wounds or sinuses by contaminated water (173). Five additional patients with cutaneous *S. vasiformis* had no known trauma or other exposures (195, 203, 248, 373, 464).

Host characteristics. The predisposing features to infection included some combination of trauma with open wounds (9, 75, 77, 112, 173, 272, 332, 453, 464), needle stick trauma (357, 361), insect or spider bites (33, 77, 203), immune system compromise due to steroid treatment (9, 112, 332, 464), antibiotic therapy (9, 33, 112, 195, 332, 464) or the presence of an indwelling catheter (332). Immune system compromise due to underlying disease also is thought to be a risk factor. Cases of *S. vasiformis* have been seen in patients with preleukemia (464), bladder cancer (143, 350), acute lymphoblastic leukemia with neutropenia (173), diabetes mellitus (33, 77), and thalassemia with splenectomy (453). In 13 of the 22 cases in the literature where the information is available, the hosts had no prior risk factors for immune system compromise. Of the seven patient deaths associated with this organism, four were seen in immunocompetent hosts (9, 195, 229, 361), two were associated with malignancies (173, 464), and one was in a diabetic (77).

General disease manifestations. Most cases of *S. vasiformis* infection described in the literature involve cutaneous sites, the exception being two cases of primary sinusitis (173, 229, 350). Cutaneous lesions with *S. vasiformis* may present as painful red blisters (332, 373), peeling skin with purulent eschar (453), black necrotic ulcers (75, 248, 464) indurated granulomatous ulcerative lesions (357) or raised red to purple lesions (33, 272). Often, the lesions demonstrate seropurulent drainage (9, 112, 361). Sinus tract or abscess formation has been found in a number of patients (350, 366, 453). Cheesy necrosis or friable tissue is grossly evident in some patients (77, 171, 332, 361, 373, 453). In contrast to most of the common zygomycoses, *S. vasiformis* infections seem to be much more indolent and localized, with only two cases of dissemination (195, 464) and three cases of widely invasive disease (9, 77, 361) described in the literature, all five of which were fatal. Rhinocerebral disease with this fungus is likewise uncommon, with only two cases seen to date (173, 229, 350). Both cases of *S. vasiformis* sinusitis were unable to be treated with debridement and did not respond to antifungal therapy. Both patients died with local progression of disease to the soft and hard palate, eyes, and central nervous system (173, 229, 350).

Virulence factors. A break in the cutaneous barrier provides *S. vasiformis* with the opportunity to cause disease, even in an immunocompetent host. In this regard, *S. vasiformis* is quite different from the typical opportunistic zygomycetes. No specific virulence factors have been identified for this organism. The relatively high survival rate for infections with this fungus probably reflects its occurrence as localized cutaneous lesions in otherwise immunocompetent hosts together with the availability of good surgical and medical treatments (203).

Diagnosis. Tissue section morphology in *S. vasiformis* infections is consistent with the typical findings of a zygomycosis, with tissue and vessel invasion by coenocytic, ribbon-like hyphal elements (3.25 to 5.0 μm in diameter). Microscopic analysis of infected tissues demonstrates an acute or chronic inflammatory infiltrate, tissue necrosis, and angioinvasion with vascular thrombosis (9, 77, 112, 229, 464). Inflammatory infiltrate may be minimal (248, 453). Giant-cell reaction (195) and hemorrhage into affected tissues (464) have also been noted. *S. vasiformis* infection in a tattoo demonstrated marked granulomatous inflammation with multinucleate giant-cell, plasma cell, lymphocyte, and eosinophil infiltration together with microabscess formation (357).

Cultures of this organism grow rapidly on standard fungal growth media, producing hyaline aerial mycelium. Colonies are white and floccose and demonstrate temperature-dependent growth rates. Growth is most rapid at 25°C, with confluent growth seen at 2 days (453). Confluent growth is seen within 4 days at 30°C (195, 332, 464), slow growth is seen at 37°C (195), and no growth is seen at 43°C (9). In contrast, Kaufman et al. (229) demonstrated rapid growth up to 40°C. The colonies produced on standard fungal media are generally composed of sterile hyphae measuring 3.2 to 6.4 μm in diameter (400). Sparse sporulation on direct culture is occasionally seen (357, 366, 464). Sterile hyphae may be stimulated to sporulate by stressing the fungus under low-nutrient growth conditions. Czepek's solution agar (195, 229, 272, 332, 373, 453), hay infusion agar (9, 171), 1% distilled water agar (145, 350), Borelli's Lactritmel agar (272), 1% agar with grass clippings (171), and saline agar (33) have all been used to stimulate

FIG. 14. Microscopic features of *Apophysomyces elegans* in culture. (A) *A. elegans* sporulates only with a great deal of effort on the part of the microbiologist. This isolate grew as sterile mycelium on both SABHI and potato dextrose agars. Sporulation was stimulated on hay, straw, and grass infusion agars incubated at 37°C. Sporangiophores are unbranched ending in a "foot cell"-like structure with an adjacent tuft of rhizoids (arrowhead). The aerial portion of the sporangiophore widens into a very prominent apophysis and a pyriform sporangium. Bar, 20 μm. (B) Sporangiophores often have a hyperpigmented region of thickening just below the apophysis (arrow). This finding is peculiar to *A. elegans*. Bar, 20 μm. (C) This high-power (×60) photomicrograph demonstrates the prominent flask- or bell-shaped apophysis (arrow) and dome-shaped columella (arrowhead) produced by *Apophysomyces*. Similar to the other sporangium-forming zygomycetes, sporangiospores are released passively from the sporangium by dissolution of the sporangial membrane, as demonstrated in this figure. Bar, 20 μm. (D) Sporangiospores are large (average 5 by 5 to 8 μm) and are rectangular to oval. Bar, 20 μm. (E) After deliquescence, the typical "martini glass" apophysis is left (small arrowhead), with the columella having collapsed. The thickened pigmented portion of the sporangiophore is again well demonstrated (arrow). A prominent tuft of rhizoids is produced from the foot cell, adjacent to the sporangiophore (large arrowhead). Bar, 20 μm.

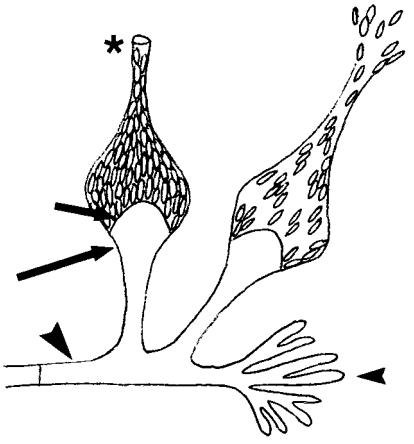

FIG. 15. Schematic diagram of *Saksenaea vasiformis* culture characteristics. This schematic diagram demonstrates the morphologic features produced by *S. vasiform* in culture. Similar to *Apophysomyces elegans*, unbranched sporangiophores arise from a "foot cell"-like hyphal element (large arrowhead). Rhizoidal tufts arise lateral to the sporangiophores (small arrowhead). At the aerial end of the sporangiophore, a prominent flask-shaped apophysis forms (long arrow), giving rise to a dome-shaped columella (short arrow). The sporangium first swells and then tapers to a thin neck. The distal end of the sporangium is occluded by a gelatinous plug (asterisk), which dissolves with maturity, allowing the sporangiospores to be passively liberated.

sporulation. The most rapid sporulation (4 days) is seen on Borelli's Lactritmel agar incubated at 37°C (272). Ellis and Ajello describe rapid sporulation (5 to 7 days) when water agar is incubated at 32°C (145). The growth of this fungus on grass clipping agar in water at 25°C produces sporulation in several weeks (400). Due to the difficulties in reliably inducing sporulation, inoculation of several different agars and incubation at several temperatures between 24 and 37°C is warranted (272).

Once sporulated, the fungus is easily identified by its characteristic flask-shaped sporangium and rhizoid complex, originally described by Saksena (400). Flask-shaped sporangia form on short sporangiophores (24 to 64 μm in length) (Fig. 15). The sporangiophore terminates in a dome-shaped columella. The sporangium (54 to 200 μm long) forms above the columella, with a tapered base that extends into a thin neck portion, giving the sporangium the overall flask- or "vase"-like shape for which the organism is named. Oval spores (1.4 to 1.2 by 2.8 to 4.2 μm) are produced within the sporangium by free-cell formation. A gelatinous plug develops at the apical or neck region of the sporangium. With age, this plug dissolves, permitting the mature sporangiospores to escape (400). At the base of the sporangiospore, a compact tuft of rhizoids is produced. This rhizoidal complex is dematiaceous (400). Definitive diagnosis of the fungus on the basis of this unique morphology may take several weeks due to the finicky nature of the sporulation. It is thought that the number of human cases of *S. vasiformis* infection reported in the literature underrepresent its true occurrence, since many laboratories do not have the capability or patience to perform the specialty culture techniques required for definitive identification (9, 145, 203, 267, 366). To underscore the difficulty in inducing sporulation, in the preparation of this review, four different minimal media, two temperatures of incubation, and several months of culture resulted in no sporulation using a known isolate of *S. vasiformis*. Diagnostic characteristics are summarized in Table 3.

An exoantigen test has been developed to help identify *S. vasiformis* from cultures producing only sterile hyphae (267). This test is limited by its low sensitivity (60%), requiring morphologic identification of all exoantigen-negative isolates. Antibodies to *S. vasiformis* may also be detected in cerebrospinal fluid and serum using an ELISA as developed by Kaufman et al. (229, 230).

Treatment. The majority of successfully treated patients received aggressive surgical debridement or amputation of infected tissue together with systemic treatment with amphotericin B (9, 75, 112, 171, 366, 453). Two additional patients who originally failed to respond to treatment with either potassium iodide or ketoconazole did respond to subsequent amphotericin B treatment (272, 357). One child and one adult responded to debridement alone (77, 373). The remaining seven patients all died despite receiving surgical and pharmacological interventions (9, 173, 229, 361), with some receiving no appropriate antifungal therapy (77, 195, 464). Both cases of primary sinusitis due to *S. vasiformis* were inoperable and progressed to invasive rhinocerebral disease and death despite treatment with amphotericin B (173, 230, 350). Of the 20 patients with primary cutaneous disease, 5 also died as a direct result of the infection (9, 77, 195, 361, 464). These five patients had disseminated disease or extensively destructive local invasion.

Relationship to other fungi causing infections. *S. vasiformis* is the only species in the genus *Saksenaea* (400). This monotypic fungus was placed in the family *Saksenaeaceae* within the order *Mucorales* by Ellis and Hesseltine on the basis of its production of aseptate hyphae, aerial mycelium, and spores and columellae similar to the *Mucorales* (144). It was considered to be substantially different from the other zygomycetes because of its flask-shaped sporangium and release of spores after dissolution of a gelatin plug at the sporangial apex. The only other member of the family *Saksenaeaceae* is another monotypic genus, *Echinosporangium*, which is not a human pathogen (144).

Due to similar difficulties in inducing sporulation in culture, *S. vasiformis* is often lumped together with *Apophysomyces elegans*, a member of the family *Mucoraceae* (304). Although they produce sterile mycelia under routine growth conditions, both fungi may be induced to sporulate under low-nutrient growth conditions. Interestingly, both *S. vasiformis* and *A. elegans* demonstrate similar disease patterns in humans: (i) infection with both of these organisms results predominantly from percutaneous exposure to spore-infected soil; (ii) cutaneous and subcutaneous tissues are most often involved; and (iii) many of the cases are reported in previously immunocompetent hosts. These two organisms have been reported together by several authors due to their unique culture requirements and disease characteristics (77, 145, 203, 267, 350).

S. vasiformis has antigenic similarities to *Rhizopus arrhizus* and *Rhizomucor pusillus*. An ELISA developed with antigens to these two fungi has been shown to identify an immunologic response in a patient with systemic *S. vasiformis*, suggesting the presence of shared antigenic determinants among these fungi (230).

Cunninghamella bertholletiae

Natural habitats. *Cunninghamella* spp. are well-known environmental organisms, having been isolated from soil, peat, sewage, water, air, seeds, nuts, flowers, and other vegetation worldwide, predominantly in more temperate climates. The various species of *Cunninghamella* have been isolated from soil in India (352), Panama (155), the former USSR, the Netherlands, Great Britain, Japan, Java (300), Germany, France, Croatia, Canada, Cuba, and various sites throughout the United States (101). They have been isolated from sewage in Egypt (2) and camel dung in French Sudan (285). They have

been found in dried or decaying flowers in China, Venezuela, and Puerto Rico and in dead wood in France (101). They have also been cultured from various seeds and nuts including Brazil nuts, peas, and the seeds of spinach, parsley, flax, peppers, pumpkins, lettuce, carrots, and musk melons (101, 300). Fungi of this family have been isolated from air samples collected in Japan (101) and in a British hospital (329). *C. bertholletiae*, the pathogenic member of this genus, is most often isolated from oil-rich brazil nuts, while the other nonpathogenic organisms are found most commonly in soil. Due to its widespread occurrence in nature, this organism, when seen in the laboratory, is often considered to be a clinical contaminant (10, 403). Although there are five species of *Cunninghamella* (101), only *C. bertholletiae* has been definitively shown to cause disease in humans (495).

Transmission. Evidence that *Cunninghamella* infections are acquired predominantly by inhalation of sporangioles into the upper respiratory tract is twofold. First, most cases of *Cunninghamella* involve the lungs or sinuses as the primary sites of infection (19 of 23 cases). Second, in the two studies in which it was sought, *Cunninghamella* has been found in air samples (101, 329). Particles the size of a single sporangiole may be kept airborne with very small air movements, keeping the risk of exposure high where the sporangioles contaminate the environment (286).

Acquisition of infection through the gastrointestinal tract is suggested by the prominent involvement of these organs in three patients (209, 245, 277). *C. bertholletiae* has been found in a wide variety of nuts, seeds, and vegetation, and so opportunity for ingestion of sporangioles certainly exists (101, 300).

Transmission by direct percutaneous implantation of the sporangioles is suggested by two additional cases of primary cutaneous and subcutaneous disease (53, 309). The patient studied by Mostaza et al. (309) probably acquired his infection during open trauma to his thigh, resulting in a large hematoma and abscess. The patient described by Boyce et al. (53) likewise sustained traumatic injuries: both opened and closed fractures of his legs. This patient developed significant pain under his cast, which was proven to be related to a polymicrobic infection. Cultures of the patient's wounds and the nonsterile cast padding demonstrated several of the same organisms. Although *C. bertholletiae* was not recovered from the padding material, it is thought that either the padding or environmental sporangioles implanted at the time of original injury were the most likely sources of infection (53).

Host characteristics. The overwhelming majority of patients (22 of 23) with *C. bertholletiae* infections have risk factors for immune system compromise that are known to predispose a patient to infections with zygomycetes. The first case described in the literature occurred in a patient with lymphosarcoma, who was further immunosuppressed by chemotherapy and steroid therapy (209). Other underlying diseases associated with *C. bertholletiae* infections include hematologic malignancies, often with neutropenia (57% of cases) (14, 58, 90, 115, 238, 247, 250, 287, 324, 380, 385, 396, 472), nonmalignant hematologic conditions requiring chronic transfusions (277, 403), diabetes mellitus or other causes of hyperglycemia and acidosis (53, 58, 83, 209, 380, 396), and transplant receipt (245, 328). Three patients were asplenic (250, 385, 403). *Cunninghamella* infections are often seen together with other infectious diseases. They have been seen with *Pneumocystis carinii* pneumonia (309, 380, 396), hepatitis (287, 385), cytomegalovirus infection (14, 247, 472), and HIV infection and AIDS (309). This fungal infection often occurs in the face of broad-spectrum antibiotic therapy (53, 58, 90, 238, 247, 277, 287, 309, 328, 380, 385, 396, 403). A single case was seen in a host with alcohol abuse but otherwise normal immune system functions (522).

Immunosuppressive therapies also pose an increased risk for disease with *Cunninghamella*. Chemotherapy (90, 115, 209, 238, 245, 247, 277, 328, 380), and treatment with steroids (14, 53, 83, 209, 245, 247, 250, 277, 287, 328, 380, 396, 403, 472) have been frequently implicated in predisposing patients to infections with this opportunistic fungus. Iron overload and chelation therapy with deferoxamine also have been seen as risk factors for infection (58, 277, 385, 403). Robinson et al. (396) further suggest that since most cases of *C. bertholletiae* occur in men, male hormones may also play a role in establishing or propagating infections.

General disease manifestations. Since the first description of disseminated *Cunninghamella* in 1958 by Hutter, 23 additional cases have been published in the literature. It is estimated that between 1986 and 1991, *Cunninghamella* zygomycosis reported in the literature worldwide represented 8% of all zygomycoses (324). The vast majority of these cases presented as either isolated pulmonary disease (90, 115, 238, 247, 380, 385, 396, 522) or pulmonary disease disseminated to other organs (14, 209, 245, 247, 250, 277, 288, 328, 396, 472). The lungs are the most common sites of tissue involvement, with the organism being found in this tissue in 17 of 24 patients. Only five cases of rhinocerebral (58, 83, 157, 247, 324) and two cases of primary cutaneous (53, 309) *Cunninghamella* infections have been described. In all, tissues infected with *Cunninghamella* have included the mediastinum or other lymph nodes (245, 328, 403), thymus (285), eye or brain (58, 83, 209, 245, 328, 380, 396), spleen (14, 247, 277, 287, 472), neck organs (209, 277), pancreas (277), various portions of the gastrointestinal tract (209, 245, 277, 380), heart (209, 245, 287, 396), sinuses (83, 247, 324), hard palate (324), skin (53, 58, 90, 247, 309, 324, 403), joints (309, 403), liver (14, 245, 247, 287, 472), kidneys (14, 245, 247, 287, 472) and inner ear (58).

The disease caused by *Cunninghamella* tends to follow a malignant course, with 19 of 24 cases (79%) ending in death as a direct result of the infectious process. Diagnosis of a fungal infection was initially missed in several of these cases, having been picked up at autopsy or by culture of clinical specimens only after death (14, 115, 209, 245, 247, 472). Pulmonary artery thrombosis has been seen in several cases of sudden death with this organism (238, 250, 287, 385).

Virulence factors. *C. bertholletiae* is the only species of *Cunninghamella* known to infect humans (495, 496). With its ubiquity in nature, it is interesting that it is an uncommon cause of human zygomycosis. Its low pathogenic potential is affirmed by the occurrence of infections predominantly in severely immunocompromised individuals. Its potential for pathogenicity is associated with its thermotolerant growth capabilities (496). In an experimental diabetic-animal model, invasive pulmonary and cerebral disease was linked to thermotolerance, with none of the *Cunninghamella elegans*-challenged animals developing disease. Although no *C. bertholletiae* isolates were used for comparison, the majority of thermotolerant zygomycetes tested established both invasive pulmonary and cerebral infections (381). Similar to many of the other zygomycetes, under appropriate conditions of immune system compromise, acidotic conditions, or antibiotic or deferoxamine therapy, *C. bertholletiae* may act as an opportunistic pathogen and propagate an infection by virtue of its angioinvasive propensities.

Diagnosis. Direct examination of cytologic specimens and tissue sections with *C. bertholletiae* demonstrates the typical findings of zygomycosis. Although the hyphal elements have been described as thinner than those of most of the other zygomycetes (average, 8.8 μm; range, 7 to 12 μm), there is

sufficient overlap and variability in width to make this not useful for differentiation (380). Culture is still required for species determination.

In culture, *C. bertholletiae* demonstrates a great deal of pleomorphism depending on the substrate used. Colonies produce a tall, aerial mycelium ranging in height from 0.5 to 2 cm (496). Colonies vary in color, with white, yellow and light olive noted (101). Many of the case reports, however, indicate that a light gray color predominates in the clinical specimens (115, 245, 250, 328, 380). Cultures grow readily at room temperature and at 30, 37, 40, and 45°C (101, 496).

Investigators have reported some variation in the microscopic morphology of *C. bertholletiae* isolates. Cutter's early publication presents a description consistent with what is now considered typical *Cunninghamella* morphology (101). Hyphae are wide and ribbon-like with branched, erect sporangiophores. Sporangiophores terminate in swollen vesicles that are often filled with lipid drops. Sporangioles are borne off the vesicles on short stalks or sterigmata and are golden brown. Chlamydospores 30 to 40 μm in diameter are often seen in these cultures (101). There is significant variation in size and shape for these structures. Earlier reports confused *C. bertholletiae* for *C. elegans*, with some authors reporting descriptions that were inaccurate for *Cunninghamella* spp. (10).

The first comprehensive review of the microscopic morphology of *C. bertholletiae* was performed by Weitzman and Crist in 1980 in an attempt to systemically differentiate *C. bertholletiae* from *C. elegans* (496). They presented in detail the effect that various media had on the microscopic characteristics. On potato dextrose agar, *C. bertholletiae* produces large terminal vesicles 15 to 59 μm in diameter with smaller vesicles arising from the lateral branches (3 to 30 μm in diameter). Sporangioles form on short stalks from these vesicles. Sporangiophores are branched, measuring 7 to 130 μm in length. Brown sporangioles measuring 6 to 14 by 6 to 11 μm are either round, oval, or ellipsoidal. Smooth, rough, and echinulate sporangioles are seen. The morphologic features seen on Sabouraud dextrose agar are demonstrated in Fig. 16 and summarized in Table 4. Descriptions and measurements for fungal growth on synthetic mold agar and Czapek Dox agar are presented in the Weitzman and Crist paper (496).

C. bertholletiae is urease and gelatinase positive and assimilates glucose, sucrose, xylulose, cellobiose, maltose, trehalose, and raffinose. It does not utilize inositol, lactose, or melibiose. It is able to peptonize litmus milk and hydrolyze casein (496).

Cunninghamella spp. are heterothallic, forming the sexual zygospores when mated with the appropriately oriented (+ or −) strain of the same species. In mating studies, many brown rough-walled zygospores are supported by equal or unequal suspensors (495). Zygospores are round, measuring 35 to 50 μm in diameter (406).

An ELISA developed to detect zygomycosis has been effective in detecting antibodies to *C. bertholletiae*. In their case report, Zeilander et al. demonstrated an antibody titer of 1:12,800 for their case of pulmonary *Cunninghamella* (522). Kaufman et al. (230) likewise showed that an ELISA using *Rhizopus arrhizus* or *Rhizomucor pusillus* could detect antibodies produced in response to systemic *Cunninghamella* infections.

Treatment. *Cunninghamella* infections have not been particularly responsive to therapy. This may reflect several factors. Diagnosis of a zygomycosis is often delayed. Even when fungal cultures are obtained, culture results may be explained away as insignificant laboratory contaminants (403). Once diagnosis is made, surgical intervention may not be performed due to disseminated disease or inoperable sites. Finally, *C. bertholletiae* may not respond well to systemic antifungal therapy. Amphotericin B is believed to be the drug of choice for treatment in *Cunninghamella* infections (360). Not all cases have demonstrated good therapeutic response. Several reports where in vitro antifungal susceptibility testing was performed demonstrated a resistance to amphotericin B (406, 472, 522). In another paper, in vitro susceptibility studies to amphotericin B, ketoconazole, and miconazole demonstrated inhibition of growth only by amphotericin B and resistance to the other two drugs noted, as is typically expected for the zygomycetes. Furthermore, addition of either imidazole to amphotericin B treatment provided no additional benefit (149). Of the 20 patients who died of *Cunninghamella* infections, 13 had received

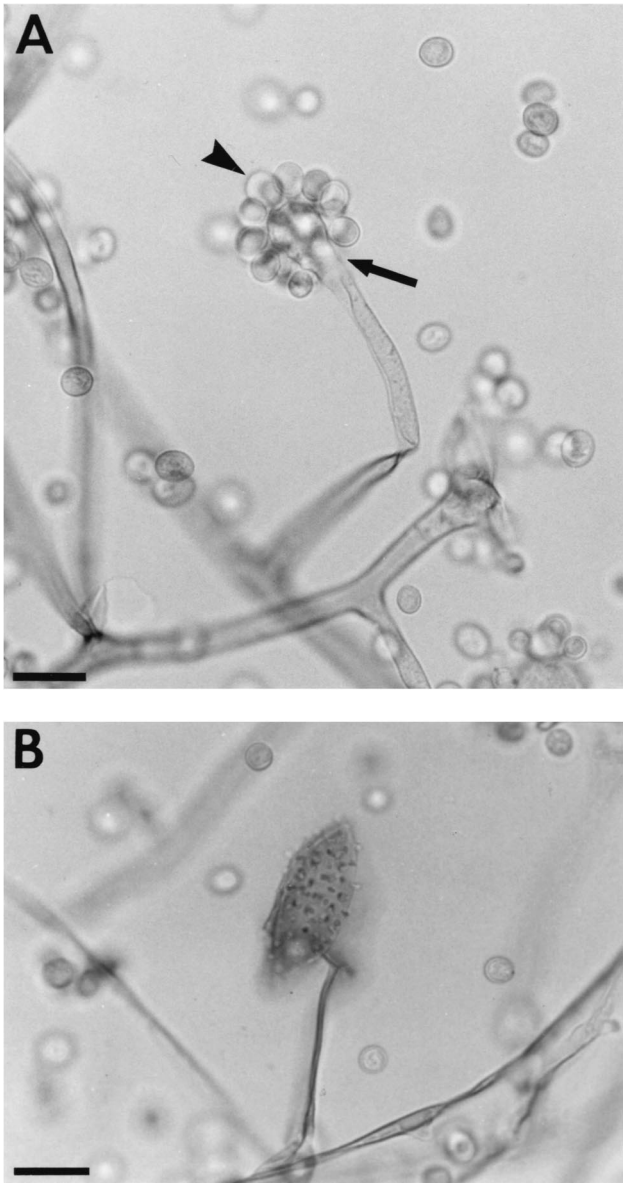

FIG. 16. Microscopic features of *Cunninghamella bertholletiae* in culture. (A) The branched sporangiophore of *C. bertholletiae* end in terminal swellings called vesicles (arrow). Single-celled sporangioles (arrowhead) are borne singly on short stalks called sterigmata, which entirely cover the vesicle. Bar, 20 μm. (B) This collapsed and disrupted vesicle bears the remnants of the sterigmata as short spines. Bar, 20 μm.

amphotericin B, but only 4 of these patients had significant debridement or resection of involved tissues.

Four patients were ultimately cured of the fungal infection (53, 90, 324, 403). For three of these, treatment combined extensive debridement, amputation, or lobectomy with topical and/or systemic amphotericin B antifungal therapy (53, 90, 403). A single patient responded to systemic therapy with rifampin and amphotericin B without surgical intervention (324).

Relationship to other fungi causing infections. The genus *Cunninghamella* was first described by Matruchot in 1903 upon his discovery of the sporangiolating fungus *C. africana* in camel dung (285). Matruchot assigned this fungus to the order *Mucorales* on the basis of its aseptate mycelium. Later, the genus was assigned to its own family, *Cunninghamellaceae*, in the order *Mucorales*. This assignment was based on the production of zygospores, the asexual production of sporangioles on short stalks from swollen vesicles, and the presence of typical *Mucorales* hyphal elements. Early case reports identified *C. elegans* as the organism responsible for human infection (209, 250). This identification was later refuted by experiments performed by Weitzman and Crist (495, 496). Through mating studies and temperature growth dependence studies, these authors were able to show that all cases of human disease with *Cunninghamella* were actually due to *C. bertholletiae*. Compared to other members of the family *Cunninghamella*, these two species are very difficult to distinguish from one another on the basis of morphology or biochemicals alone. Definitive identification of sporangiolating zygomycetes to the genus and species level requires mating studies with positive and negative strains of the possible organisms and demonstration of zygospore production (496). Additionally, temperature-dependent growth above 40°C is required. Although not all strains of *C. bertholletiae* are able to grow above 40°C, all isolates of *C. elegans* are inhibited in their growth above 40°C (101, 496). Thus, any organism with the characteristic morphology that grows at temperatures above 40°C can be assumed to be *C. bertholletiae*. Isolates that fail to grow at temperatures above 40°C, however, would require mating studies to determine whether they were *C. bertholletiae* or *C. elegans*.

Cokeromyces recurvatus

Natural habitats. *Cokeromyces* is an unusual monotypic genus of the family *Thamnidiaceae*. Isolation of *Cokeromyces recurvatus* has been reported only in North America. It was first cultured from rabbit dung collected in Illinois (420). Benny and Benjamin (38) further isolated this fungus from the dung of lizards, rabbits, and a variety of rodents including pack rats, squirrels, and mice. In nature, this zygomycete grows in great profusion as a felt-like mat on the excreta from these animals (38). The geographic distribution of these isolates seen in excreta covered parts of Mexico and the United States including California, Arizona, Illinois, Michigan, and Florida. An additional soil isolate was obtained in Texas. All cases of human colonization or infection have occurred in the United States (13, 25, 232, 289, 314, 387, 390, 467).

Transmission. The mode of transmission is unknown, although in the cases where symptomatic disease was present, prior colonization of the involved site was suspected (13, 25, 289, 314, 467).

Host characteristics. Of the six cases of human disease or colonization with *C. recurvatus*, two presented in essentially normal hosts (289, 387) and the remaining patients had risk factors for immune system compromise or dysfunction. One patient with chronic alcoholism and peptic ulcer disease presented with a ruptured ulcer (314). Another patient had insulin-dependent diabetes mellitus (232). One patient was receiving steroids to induce immunosuppression following bone marrow transplantation for multiple myeloma (13, 467). The remaining patient was being treated for esophageal cancer. His hemorrhagic cystitis occurred in the setting of functional and anatomic abnormalities in the urinary tract including bladder diverticula, squamous metaplasia, and prior prostatic surgery (25).

General disease manifestations. The vagina and cervix have been the most common sites from which *C. recurvatus* has been isolated in humans. Two cases were discovered on routine PAP smear (232, 387, 390), and a third case of vaginal *Cokeromyces* was discovered during evaluation of a girl with a chlamydial infection (289). In all three patients, no signs of tissue invasion by the fungus were seen, suggesting mere colonization by the organism.

A single case report described *C. recurvatus* in a man with chronic hemorrhagic cystitis. This patient presented with urinary urgency and frequency and gross terminal hematuria with pus. The bladder wall was reddened and edematous and demonstrated signs of acute and chronic inflammation, but no signs of fungal invasion were seen. *C. recurvatus* grew only from the urinary sediment but not the bladder tissue biopsy specimen. The mechanism for the production of cystitis in the absence of tissue invasion was not explained (25, 289).

Two additional cases of *Cokeromyces* infection involved gastrointestinal sites. One patient developed profuse mucus-laden and watery diarrhea 4 weeks after bone marrow transplantation. *Cokeromyces* was seen on direct examination of the stool and also on the intestinal mucosa in biopsy specimens. No signs of tissue invasion were noted (13, 467). The lack of red or white blood cells in the diarrheal stool supported the lack of tissue invasion seen microscopically. The final case of human *Cokeromyces* involved pleuritis and peritonitis in a patient with a ruptured peptic ulcer (314). *C. recurvatus* was cultured from three separate fluid specimens. The patient ultimately suffered multiorgan failure, became coagulopathic, and died. Tissue invasion by *C. recurvatus* was not recognized in this patient, and the role that the fungus played in his death is not clear. The authors hypothesized that the pleural and peritoneal seeding occurred after the ruptured ulcer, allowing contamination of these sites with *C. recurvatus* colonizing the upper gastrointestinal tract.

Virulence factors. The pathogenic potential of this fungus is unclear. In all reports, no tissue invasion is noted (13, 25, 232, 289, 314, 387, 467). In the four patients where symptomatic disease was reported, additional pathogens or disease processes existed that could explain the symptoms seen. In the patient with *Cokeromyces* infection and vaginitis (289), vaginal cultures were positive also for chlamydia. The case of diarrhea occurred in a patient experiencing graft-versus-host disease, a well-known cause of diarrhea in this setting (13). The case of peritonitis with *Cokeromyces* was associated with a perforated ulcer and peritonitis involving multiple bacterial agents in addition to the *Cokeromyces* (314). The only case where no additional pathologic process accompanied symptomatic disease was in the patient with hemorrhagic cystitis (25). The resolution of symptoms with antifungal therapy in both this patient and the patient with *Cokeromyces*-related diarrhea is the only evidence that *Cokeromyces* has pathogenic potential. Since no tissue invasion was seen, it seems likely that disease with this fungus is mediated by one or more extracellular mycotoxins.

Diagnosis. *C. recurvatus* is a dimorphic zygomycete. It is seen in the direct specimen as a large yeast with morphology similar to the yeast phase of *Paracoccidioides braziliensis*. The yeast are

thick walled, 30 to 90 μm in diameter. The large yeast cells are often surrounded by a crown of smaller yeast buds (13, 25, 232, 289, 387, 467). In cases of colonization, little if any inflammatory response is expected (13, 232, 387, 467). Hyphal elements have not been identified in direct specimens for any cases of infection with this fungus (13, 25, 232, 289, 387, 467).

Dimorphism in this fungus is dependent upon the culture medium, temperature of incubation, and degree of anaerobiosis. At room temperature in air, *C. recurvatus* grows as a filamentous fungus. The mycelium tends to be lower than that of most of the zygomycetes, and the colonial growth, overall, is slower and less aggressive. McGough et al. described a growth rate of 15 to 20 mm in 5 days (289), while Axelrod et al. observed 30 mm of growth in 3 days (25). Colonies are tan to gray, with concentric rings or zones of color seen in a single colony (289, 420). The gray portions of the colony represent areas of the culture containing a large number of black or gray sporangioles, while the lighter portions are composed of primarily vegetative mycelium. With age, the colonies become wrinkled with radial folds (289).

Microscopic morphology of the filamentous fungus demonstrates coenocytic ribbon-like hyphae, 5 to 15 μm in diameter, consistent with zygomycete morphology. Tall, predominantly unbranched sporangiophores (usually 300 to 500 μm long) bear a terminal vesicle (13 to 31 μm in diameter). Round sporangiola (8 to 11 μm in diameter) form from this vesicle and are supported on thin, recurving, pigmented stalks. Each sporangiolum contains 12 to 20 oval, smooth-walled sporangiospores. Cultures produce no rhizoids. Growth is seen up to 35°C, with profuse production of zygospores seen at the higher temperatures of incubation. These zygospores are round, rough-walled, golden brown structures measuring 33.5 to 54.5 μm in diameter (289, 420). Portions of the cultures where zygospore production predominates have a dark brown hue. Since *C. recurvatus* is homothallic (requiring only one mating type for sexual reproduction), zygospores are readily produced within a single isolate. Excellent discussions, photographs, and line diagrams of the growth characteristics of this unusual fungus are presented by Shanor et al. (420), Benny and Benjamin (38), McGough et al. (289), and Munipalli et al. (314). Characteristic features are also demonstrated in Fig. 17 and summarized in Table 4.

C. recurvatus may be forced into a yeast phase in culture (Fig. 17C). Axelrod et al. (25) linked the yeast production to yeast-glucose-peptone agar incubated at 37°C. Other authors found no dependence of yeast conversion on any particular growth medium but, rather, observed good conversion at the higher temperature of incubation (37°C) under anaerobic candle jar incubation conditions (232, 289, 314). The yeasts produced under these growth conditions are similar to those seen on direct examination, with the "mariner's wheel" pattern of multiple budding large yeasts being readily demonstrated.

Treatment. *C. recurvatus* has responded inconsistently to treatment with amphotericin B. Although bladder irrigation with amphotericin B was curative in the patient with chronic cystitis (25), the patient with complicated bacterial and fungal peritonitis (314) and a patient with vaginal colonization (387) failed to respond to systemic and topical treatment, respectively, with this agent. A 1-week course of topical miconazole likewise failed to eradicate the fungal colonization in the patient with vaginal *Cokeromyces* (289), but a subsequent 2-week course of terconazole resulted in negative cultures. In the patient described by Alvarez et al. (13) and Tsai et al. (467), a 10-day course of nystatin resulted in resolution of the diarrhea and elimination of the fungus from the bowel of a bone marrow transplant patient. On the basis of antifungal susceptibilities of the other zygomycetes, response to amphotericin B would be expected while response to the azoles would be unlikely.

Relationship to other fungi causing infections. *C. recurvatus* is the only species in the genus *Cokeromyces* (38, 420). Originally assigned to the family *Choanephoraceae* (420), it has since been designated a member of the family *Thamnidiaceae* (37). It is one of several zygomycetes that demonstrate dimorphism. Initial reports describing the yeast form of *Cokeromyces* in direct examinations of vaginal and colonic specimens identified the organism as a *Mucor* sp. or *Mucor circinelloides* (91, 387, 390). These were later cited as actual cases of *C. recurvatus* (289).

Syncephalastrum racemosum

Natural habitats. *Syncephalastrum racemosum* is a saprophytic organism that has been isolated from environmental sources worldwide. *Syncephalastrum* has been found in soil in India (352), southern United States (201), Panama (155), and Israel (219). The *Compendium of Soil Fungi* lists 17 tropical and subtropical locations where *S. racemosum* has been isolated from environmental sources, particularly sites rich with organic matter (126). Researchers have been able to collect airborne spores from outdoor air sources in Nigeria (335) and from air samples collected in a London hospital (329). *S. racemosum* has also been cultured from dust samples collected in British houses (107). It has been found to contaminate poultry feeds (4) and has been found in a variety of plants and foodstuffs including oats, wheat, soya, nuts, honeycombs, rice, sugar cane, corn, and barley. It has likewise been cultivated from a variety of water sources, bird guano, and composting plant debris (126).

Transmission. The only confirmed cases of *Syncephalastrum* infection in humans involved wound sites and probably represented contamination of open wounds with airborne or soilborne spores (226, 348). The isolation of *S. racemosum* in clinical specimens is uncommon, and the clinical significance of isolates from nonsterile sites is doubtful. Culture of this organism from stools or sputa generally cannot be linked to any signs of invasive disease and probably represents contamination of the specimen either before or after collection. Airborne spores may fall into the specimen or onto the culture medium or may be inhaled by the patient. Ingestion of spores in grains or other food products may also explain their presence in clinical specimens. Since most isolates are considered clinically insignificant, *S. racemosum* has been lumped into the category of saprophytes generally seen as clinical contaminants (254).

Host characteristics. Kamalam and Thambiah (226) described a case of cutaneous *Syncephalastrum* with progression to osteomyelitis in a 50-year-old diabetic man. Although it was not explicitly stated, a previous site of trauma with soil contamination is suggested by the location of infection in the finger of a man who was employed harvesting tea. Otcenasek and Buchta (348) did not provide clinical information on their patient from whom the wound isolate of *Syncephalastrum* had been obtained.

General disease manifestations. It is generally debated whether this fungus actually causes disease in humans. A case report of a *Syncephalastrum* fungus ball of the lung (241) is now believed to be due to *Aspergillus niger* (253). A case of cutaneous *Syncephalastrum* of the finger is the only remaining detailed case report published in the literature (226). The lesion was painful and swollen and contained several sinus tracts which drained bloody fluid with necrotic debris and fungal elements. Extension into the bone was seen on an X ray.

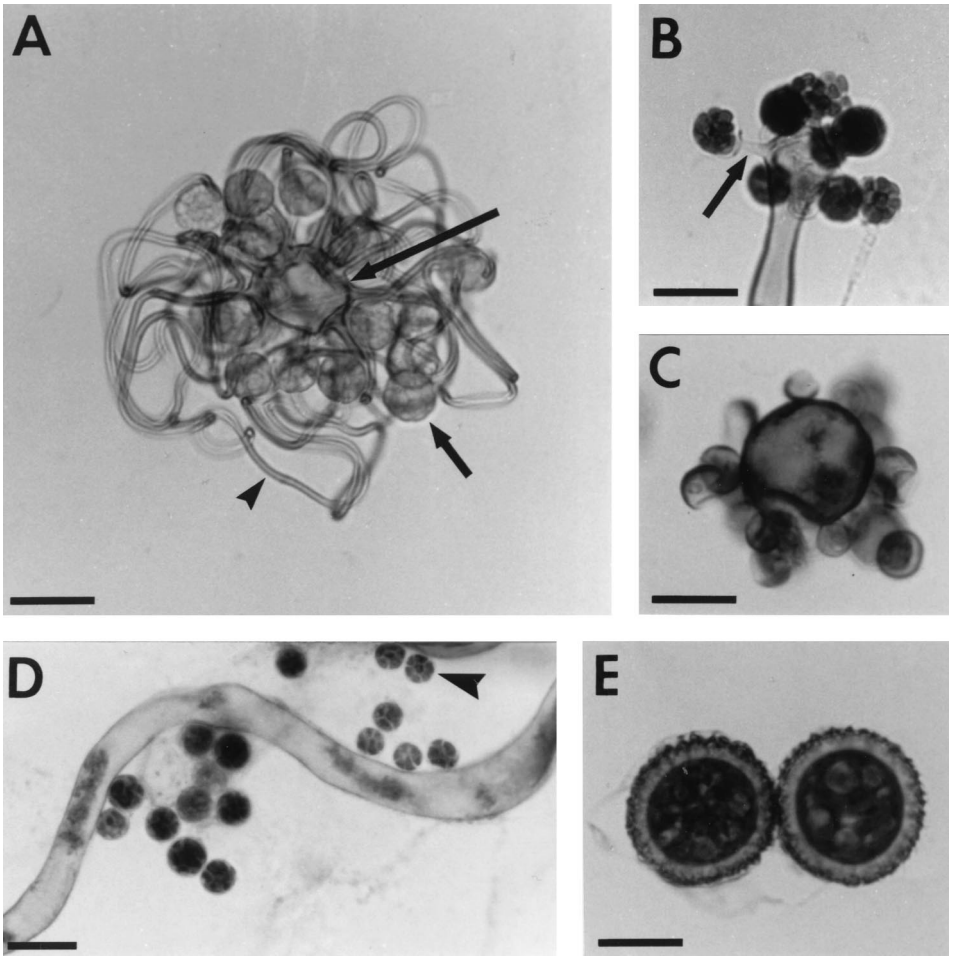

FIG. 17. Microscopic features of *Cokeromyces recurvatus* in culture. (A) Sporangiolating vesicle (mature). Swollen vesicles (long arrow) form at the end of unbranched sporangiophores. Multicelled sporangioles (short arrow) develop at the end of recurving stalks called sterigmata (arrowheads). Bar, 20 μm. (B) Sporangiolating vesicle (immature). Early in growth, the vesicle is marginally swollen. The sterigmata are short and straight (arrow). At this stage of maturation, the morphology is similar to that seen in *Cunninghamella* spp. These two genera may be differentiated at this stage by the presence of multicelled sporangioles. Bar, 20 μm. (C) Yeast phase. *C. recurvatus* is dimorphic. The yeast phase may be induced by anaerobiosis and increased temperature of incubation (37°C in this isolate) or by incubation in a high-carbon-dioxide atmosphere. The yeast are typically produced as a large central cell surrounded by a crown of smaller yeast cells. This morphology has been compared to that of the yeast produced by *Paracoccidioides braziliensis*. The yeast phase is the form that has been identified in vivo. Bar, 20 μm. (D) Hyphae and sporangioles. The hyphal morphology is typical for the *Mucorales*, consisting of wide ribbon-like aseptate elements. Sporangioles are spherical and multicelled. Bar, 20 μm. (E) Zygospores. Zygospores are produced homothalically in isolates of *C. recurvatus* and may be produced focally in great abundance in a given culture. Zygospores are round and thick walled with a rough or echinate surface decoration. Bar, 20 μm.

Although Otcenasek and Buchta reported isolating *Syncephalastrum* from a wound culture, no details of the case were provided (348).

Syncephalastrum has also been readily isolated from the normal finger- and toenails of several Egyptian students. These individuals, however, had no sign of actual disease due to this fungus (2). It has also been isolated in association with mycotic disease in a pig (8) and in an aborted calf (468).

Virulence factors. *S. racemosum* produces a variety of mycotoxins in vitro, including some that suppress the mitotic rate in plant root cells (4). It has been studied extensively as a plant pathogen. Since so few cases of human disease with *Syncephalastrum* have been seen, this fungus probably has a low pathogenic potential in a competent host and probably will cause disease only as an opportunistic pathogen. Immune system dysfunction, as in the diabetic patient described by Kamalam and Thambiah (226), probably played a pivotal role in promoting this case of cutaneous *Syncephalastrum*. The ability of *Syncephalastrum* to grow at or above 37°C (329) may also be important in its ability to cause disease in humans. In vivo studies in diabetic rabbits challenged with intranasal instillations of *S. racemosum* spores failed to detect pulmonary or cerebral invasive disease, again suggesting that the virulence of this organism is quite low (381).

Diagnosis. The definitive diagnosis of disease due to *S. racemosum* requires both the demonstration of zygomycete fungal elements in tissue sections or other direct clinical specimens and the diagnostic culture findings (497). In the case described by Kamalam and Thambiah (226), discharge from a draining sinus demonstrated necrotic debris and aseptate ribbon-like hyphae typical of the zygomycetes. Tissue sections demonstrated occlusive vasculitis. No fruiting bodies were produced.

In culture, *S. racemosum* grows rapidly, producing either low-growing or tall (0.5- to 1.5-cm) erect mycelia that cover the medium within 1 week. Cultures are hyaline, with surface coloration varying from nearly white to various shades of green, olive, and gray to almost black (36, 226, 407). The vegetative mycelium is aseptate. Sporulation occurs readily on routine medium at room temperature (407) and at temperatures above 37°C (329). Sporangiophores are branched and end in round or

oval terminal vesicles called ampullae (30 to 80 μm in diameter). The vesicles are often surrounded by rod- or club-shaped structures called merosporangia (13 to 25 μm long by 4 to 7 μm wide). The merosporangia represent an asexual reproductive "sack" in which the sporangiospores develop (162). The merosporangia contain multiple round to oval sporangiospores that are 3 to 7 μm in width. The sporangiospores may be smooth, rough, or verrucose, with all three morphologies produced by a single isolate under various culture conditions (36, 407). The terminal vesicles, merosporangia, and sporangiospores often are grey, brown, or black (36, 407). Dark brown zygospores are produced with mating. These round, rough-walled or echinate spores measure 50 to 90 μm in diameter. They are held by suspensors of nearly equal lengths (126). Figure 18 demonstrates typical culture morphology, and these features are summarized in Table 4.

Treatment. To attest to this organism's low pathogenic potential, the patient reported by Kamalam and Thambiah (226) responded to local debridement and better control of his diabetes mellitus. He recovered without amputation or specific antifungal therapy. In vitro susceptibility studies using antifungal agents demonstrate good susceptibility to amphotericin B (348, 450), nystatin (348), and primaricin (348) but resistance to saperconazole (347).

Relationship to other fungi causing infections. *S. racemosum* is now considered to be the only species of the genus *Syncephalastrum*, family *Syncephalastracaea* (407). Original studies reported several different species of *Syncephalastrum* on the basis of variation in the size and decorative features of the sporangiospores. As early as 1959, Benjamin questioned the species identifications of the various isolates of this family and was "inclined to recognize only the following wide spread and variable species of *Syncephalastrum: Syncephalastrum racemosum*." The species reported included *S. nigricans*, *S. elegans*, *S. cinereum*, *S. fuliginosum*, *S. avanicum*, and *S. racemosum* var. *paucisporum* (36). The status of the *Syncephalastrum* taxonomy by 1980 indicated that there were only two accepted species, *S. racemosum* and *S. verruculosum*, with the remaining species belonging to *S. racemosum* (126). Mating studies have subsequently demonstrated that *S. racemosum* and *S. verruculosum* represent natural biologic variation within a single species designated *S. racemosum* (351). The designations *S. javanicum*, *S. elegans*, and *S. nigricans* likewise are obsolete, and they are all now considered to be *S. racemosum* (36). It is not uncommon, however, to see *Syncephalastrum* isolates referred to as "*Syncephalastrum* spp." despite there being only a single species now recognized.

Although *S. racemosum* is a zygomycete, it may be confused with members of the genus *Aspergillus*, particularly *A. niger* (241, 253). Care must be taken to observe the aseptate, ribbon-like mycelium and the merosporangial sack surrounding the sporangiospores in *Syncephalastrum* cultures. If diagnosis is based only on identification of a vesicle surrounded by chains of spores, one could mistake *Syncephalastrum* for *A. niger*. Both fungi may produce low-growing colonies with black or dark gray/green surface pigmentation, again potentially causing confusion in identification.

Mortierella Species

Natural habitats. The various species of *Mortierella* are seen as environmental and soil isolates worldwide (122). *Mortierella wolfii*, the most common animal pathogen, was first described as an isolate from soil samples collected in India (294). Its geographic distribution in soil includes tropical areas (439) and warmer or subtropical regions of the world such as many of the southern United States (201). *M. wolfii* is found in moldy grass in silage and rotten hay but is not considered a common organism in the farm environment (8, 122).

Transmission. Although the exact mode is unknown, transmission of *Mortierella* spp. is assumed to be via spore-contaminated silage and infected farm animals. Although the spores of *Mortierella* become airborne (51), the relatively small number of cases of pulmonary disease in animals suggests that inhalation of spores is not the predominant route of transmission. Smith (427) suggests that in addition to inhalation of spores, disease transmission occurs via infected semen and via ulcerations in the alimentary tract after the ingestion of mold-containing silage.

Host characteristics. *Mortierella* spp. are considered to be primarily bovine pathogens and cause abortion, placentitis, encephalitis, and pulmonary infections in dairy cattle (72, 95, 120, 327, 392, 513). The status of the *Mortierella* spp. as true human pathogens has been refuted.

General disease manifestations. There are two cases of human *Mortierella* infection published in the literature, both of which represent cutaneous sites of infection. These reports of human disease with *Mortierella* spp. are unconvincing or inconclusive. This may be due to misclassifications or misidentification of *M. wolfii* since they all pre-date 1973, when *M. wolfii* was characterized as a human pathogen (412, 439). In these two cases where *M. wolfii* was implicated, hyphae were seen in tissue, cultures were gray or yellowish, and neither sporulated. As a result, identification was based on colony morphology and growth characteristics (148), prompting Ajello et al. to speculate that these two cultures may have been *Saksenaea vasiformis*, which requires special culture techniques (9). The first published case of *M. wolfii* subcutaneous infection in a human was reported by Ciferri and Ashford (86). Kwon-Chung and Bennett (253) suggest that the description of hyphal elements surrounded by eosinophilic Splendore-Hoeppli material more closely resembled *Basidiobolus* infection than *Mortierella* infection. The second report describes a leg ulcer with fungal morphology that is atypical of a zygomycosis (326). This report, written in French, provides a species description of *M. mycetomi*. Streekstra (439) suggests that this may in fact be *Madurella mycetomi* and that its identification and that continued referencing as a case of *Mortierella* zygomycosis resulted from a mistranslation of the original paper. Interestingly, Hutter, in his review of zygomycosis (209), categorizes this report as a "questionable" case of zygomycosis and did not include it in his statistics of true cases of zygomycosis in humans. In neither of the two cases was the organism described sufficiently well to definitively connect these strains with what are currently considered *Mortierella* spp. taxonomically (412).

M. wolfii is recognized as a true animal pathogen and is considered to be the most common cause of mycotic abortion in northern New Zealand (327, 392, 419). This fungus has also been reported as an agent of bovine mycotic abortion in both the United States (513) and Great Britain (95) and has been shown to cause pneumonia in cattle (120).

One additional species of *Mortierella*, *M. polycephala*, has been reported to cause disease in cattle (414). Animal infections previously attributed to other *Mortierella* spp. (*Mortierella alpina* and *Mortierella zychae*) (427) were probably due to *M. wolfii* (412).

Virulence factors. *M. wolfii* is not considered virulent relative to other species, except when given by the intracerebral route (95, 242). When spores were administered intravenously, intraperitoneally, intramuscularly, or subcutaneously to mice, no disease was produced. When the spores were injected in-

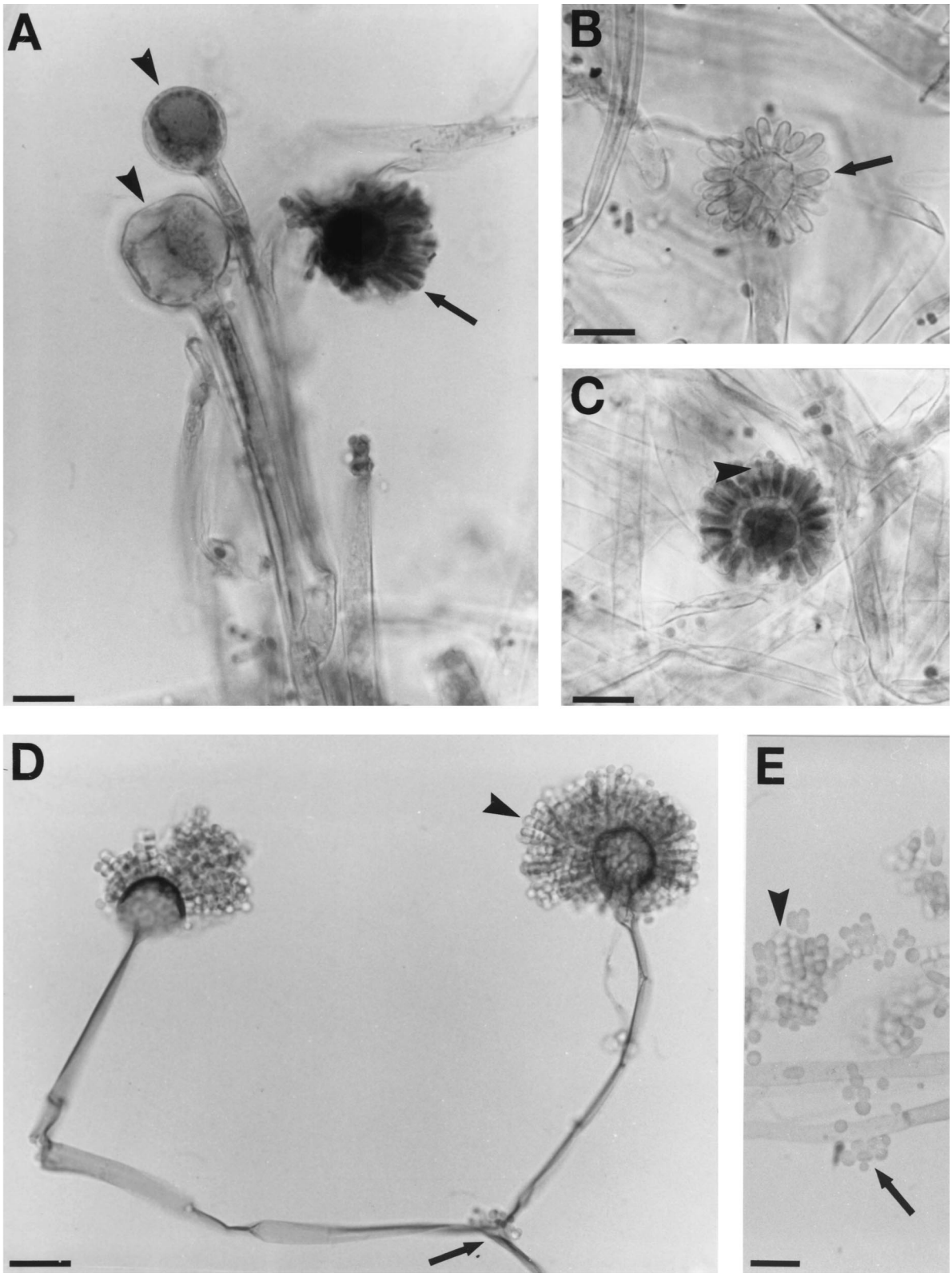

FIG. 18. Microscopic features of *Syncephalastrum racemosum* in culture. (A) Sporangial vesicles. *Syncephalastrum* produces an abundant aerial mycelium. Sporangiophores terminate in swollen vesicles (arrowheads). Tubular sacks called merosporangia then develop around the vesicle (arrow). Bar, 20 μm. (B and C) Merosporangia (immature). Merosporangial sacks develop as short finger-like projections from the vesicle surface (arrow in panel B). Initially, there is no internal detail (B), but with age, the protoplasm undergoes free cell cleavage to produce sporangiospores (arrowhead in panel C). At this stage of development, the merosporangial membrane is quite refractile. Note the abundant production of coenocytic vegetative mycelium. Bars, 20 μm. (D) Mature merosporangia. Sporangiospores form as a single column within the sporangial sack. With maturity, the merosporangial membrane becomes deliquescent. At this stage, the sporangiospores may appear like strings of conidia (arrowhead). Care must be taken to avoid misidentifying this organism as *Aspergillus* spp. The presence of branched sporangiophores (arrow) and the production of coenocytic hyphae together with the lack of metulae and phialides will aid in differentiating these two organisms. Bar, 20 μm. (E) Sporangiospores. Sporangiospores may be released from vesicles as entire merosporangial units (arrowhead) or as individual spores (arrow). Bar, 20 μm.

tracerebrally, however, a chronic granulomatous infection occurred which resulted in hydrocephalus and eventually in death (95). Reiss (383) has also described toxigenicity for *M. wolfii*. A water-soluble, heat- and trypsin-stable toxin has been isolated from this fungus that is nephrotoxic and capable of causing death in an experimental model. Since these were the same disease manifestations seen in experimental *Mortierella* infections, it is thought that the toxins are responsible for most of the pathogenicity of this organism (105, 106).

Diagnosis. *M. wolfii* is a thermophilic mold which grows well at 40°C, with a maximum growth temperature of 48°C. Colonies are white to gray or yellowish gray, fine and cottony. There are few aerial mycelia, and colonies frequently overlap, giving the appearance of rosettes. A characteristic garlic-like odor may be associated with the colony. Soil extract agar incubated above 25°C is necessary for sporulation, but some strains are difficult to identify because of poor spore formation even under the optimal growth conditions (175, 392, 412). Seviour et al. (419) described silage extract agar which contains filtered silage at pH 4.5 to 4.7 as a good medium for sporulation.

Microscopically, *M. wolfii* develops short (80- to 250-μm), tapering, delicate sporangiophores from rhizoids that are either simple or branched directly below the tip. Globose sporangia (15 to 50 μm wide) are rapidly deliquescent, leaving a large collarette and a very tiny or no columella. Sporangiospores are short and kidney-shaped or cylindrical (6 to 10 by 3 to 5 μm) and have a double wall. Stylospores, which are small, one-celled, spiny conidia, may also develop. Chlamydospores are formed and are up to 35 μm in diameter with ameboid appendages. It is not known if zygospores are produced (122, 175, 392, 412, 449). The microscopic and culture morphologic characteristics are summarized in Table 2 with the other globose sporangium-forming *Zygomycetes.*

Treatment. No in vitro susceptibility data are available for this organism. "Treatment" in animals generally consists of euthanasia. One report of successful treatment in a cow provided no details (327).

Relationship to other fungi causing infections. The family *Mortierellaceae* contains a single recognized genus, *Mortierella*. This genus contains four recognized species. The most commonly identified animal pathogen is *M. wolfii* (35, 412).

M. wolfii has rather fastidious growth requirements, failing to sporulate unless stressed under low-nutrient conditions. As a result, early identifications were based on the colony characteristics of otherwise sterile zygomycete mycelia. With regard to the difficulty in stimulating sporulation, *M. wolfii* has been compared to both *Apophysomyces elegans* and *Saksenaea vasiformis*, the latter of which also require special culture techniques, and is a well-known cause of cutaneous zygomycosis in humans (9).

Hessian and Smith (198) compared antigenic profiles of several fungi (*Absidia corymbifera*, *Mortierella wolfii*, *Rhizomucor* [*Mucor*] *miehei*, *Rhizomucor* [*Mucor*] *pusillus*, *M. racemosus*, *Rhizopus* [*oryzae*] *arrhizus*, *R. microsporus*, *R. microsporus* var. *rhizopodiformis*, and *R. stolonifer*, as well as *Candida albicans* and *Aspergillus fumigatus*). Unique and common antigens were demonstrable amongst the *Mucorales* with the exception of *M. wolfii*, which had little antigenic similarity to the others (198). The *Mortierellaceae* may be differentiated from the *Mucoraceae* by virtue of their very delicate features. Sporangia are small and have few or no columella. The mycelium is dichotomously branched. These delicate features provided the original grounds for their placement in a separate family from the *Mucoraceae*.

RELATIONSHIP OF THE *ENTOMOPHTHORALES* TO THE *MUCORALES*

Despite their relatedness as zygomycetes, the organisms of the orders *Mucorales* and *Entomophthorales* have glaringly different morphologic and pathogenic features that distinguish them from one another (Table 11). As mentioned above, taxonomically these two classes are differentiated on the basis of their gross morphology and by the identification of active expulsion of asexually produced spores. Contrasted to the floccose aerial mycelium or "lid-lifter" morphology of the *Mucorales* (Fig. 4A to C; Fig. 8B), the *Entomophthorales* produce dense, waxy, and deeply furrowed colonies (Fig. 8A and C). Although the *Entomophthorales* produce coenocytic hyphal elements, these tend to become moderately septate with age (252, 447). Multispored sporangioles, merosporangia, and sporangia are not produced. Asexually produced, single-celled sporangioles (conidia) are produced in sporangiophores within the vegetative mycelium, from which they are either actively or passively released (252, 447). The *Mucorales* release their sporangiospores by passive mechanisms only.

The epidemiology of human disease is also quite different between these two orders. Most of the *Mucorales* enjoy a worldwide distribution of both organism and human disease. In contrast, despite their occurrence as environmental organisms worldwide, *Basidiobolus* and *Conidiobolus* spp. cause human disease mostly in tropical climates, primarily in Africa (62, 68, 87, 184, 480, 505).

Disease transmission for the *Mucorales* falls into one of three broad categories: inhalation of spores, traumatic implantation of spores, or ingestion of spores. The disease manifestations reflect these modes of transmission. On the basis of experimental and epidemiologic data, inhalation of spores likely represents the major mode of transmission, underlying both the pulmonary and rhinocerebral forms of mucormycosis in humans (32, 40). Disseminated disease from pulmonary, cutaneous, and mucocutaneous sites is commonly seen with the *Mucorales* and is often fatal (438). In comparison, the majority of cases of *Basidiobolus* and *Conidiobolus* infections occur as a result of some form of minor traumatic implantation. While inhalation of spores may also play a role for disease transmission with *Conidiobolus*, intranasal trauma such as is seen with nose picking also probably contributes to the development of disease (184). Disseminated disease is uncommonly described with the *Entomophthorales* (63, 136, 228, 451, 486, 487).

The degree of invasiveness of disease is also markedly different for the *Mucorales* and *Entomophthorales*. While the hallmark of infection for the *Mucorales* is invasion of blood vessels, thrombosis, tissue necrosis, acute inflammation, and dissemination, the *Entomophthorales* typically lack these features (442). A chronic inflammatory response is generally seen. Hyphal elements may become phagocytized in monocytic cells or surrounded by Splendore-Hoeppli material (79, 170, 184), a finding rarely seen in *Mucorales* infection.

Disease caused by the *Mucorales* is summarized by the term "opportunistic infections." Disease in immunocompetent hosts represents a tiny minority of cases in the *Mucorales* (46, 74, 379). The exact reverse of this is true for *Conidiobolus* and *Basidiobolus* infections. Disease occurs primarily in normal, immunocompetent hosts (184, 442), with relatively few cases currently seen associated with immuncompromise (214, 411, 486, 487).

TABLE 11. Features distinguishing the *Mucorales* from the *Entomophthorales*

Characteristic	*Mucorales*	*Entomophthorales*
Geographic distribution of organisms	Most but not all species distributed worldwide	Worldwide distribution, but endemic in tropical climates
Geographic distribution of cases	Most species cause infections worldwide	Predominantly seen in tropical and subtropical regions
Mode of transmission	Majority of infections result from inhalation of spores or traumatic implantation	Majority of infections result from inhalation of spores, traumatic implantation, bug bites, or other percutaneous mechanisms
Host immune status	Predominantly immunocompromised, but some competent hosts also seen	Predominantly immunocompetent, only a few compromised hosts
Most common disease manifestations	Pulmonary disease most common; rhinocerebral, cutaneous/subcutaneous, gastric, and other forms also seen	Sinusitis disease predominates for *Conidiobolus coronatus*, while subcutaneous mycosis predominates for *Basidiobolus ranarum*
Invasive qualities	Primarily angioinvasive	Most infections are localized, demonstrating no angioinvasion
Organism colony morphology	Floccose aerial mycelium; often seen as "lid lifters"	Waxy, folded, and compact mycelium
Organism mycelium morphology	Coenocytic hyphae, predominantly aseptate; Splendore-Hoeppli phenomenon rarely seen	Coenocytic hyphae, becoming moderately septate with age; Splendore-Hoeppli phenomenon characteristically seen in tissue sections

ENTOMOPHTHORALES CAUSING ZYGOMYCOSIS IN HUMANS

Basidiobolus ranarum

Natural habitats. *Basidiobolus* species have been identified as saprobes and as parasites, living off of decaying vegetation, insects, woodlice, and feces of amphibians, reptiles, and other animals from which they have been isolated (87, 97, 338, 521). *Basidiobolus* is endemic in Uganda and certain other areas of Africa, India, and other parts of Asia but is found worldwide even in areas where the disease has not surfaced (442). It has been isolated from leaves and decaying plants in southern and northeastern states of the United States (128) and in Australia (521).

Basidiobolus ranarum was first described as an isolate from frogs in 1886 (138). It was later cultured from the intestinal contents (343) and ultimately the excreta (460) of frogs. The organism spends part of its life cycle in the intestine of the agamid lizard and is liberated as spores and mycelia in the lizard excrement. The spores germinate, and the organism grows saprophytically on lizard droppings, from which it may be picked up by individuals with traumatic lesions (135). In addition to being isolated from insects and reptiles, *Basidiobolus* has been isolated from several mammalian species. It has been found in bats (521), horses (302, 480), dogs (303), and humans (320). In horses, *Basidiobolus* is an uncommon pathogen associated with a subcutaneous mycosis called "kunckers" (303) that is associated with consumption of standing water. It is believed that humans and horses are the traditional mammalian hosts for *Basidiobolus* infections (303).

Despite the worldwide environmental distribution of this fungus, human disease is concentrated in tropical and subtropical regions. Following its identification as a human pathogen in Indonesia (218), cases were reported primarily in Africa, including Uganda, Sudan, Nigeria, Senegal, Ivory Coast, Cameroon, Ghana, Kenya, and Upper Volta (87). Additional cases were also identified in India, England, Burma, and Iraq (87). Since Clark's report (87), additional cases have been reported in Africa (20, 62, 68, 87, 311, 480, 505), India (104, 223), South America (primarily Brazil) (41), Thailand (80, 211, 459), Infect. Dis., abstr. 43-34A, 1970), the United States (411), Australia (109), and Pakistan (320). More than 300 cases of *Basidiobolus* infection have been reported (320). The largest number of cases have been from Uganda. The infection is found under varied climatic conditions. In Africa, most cases come from agricultural areas, some come from high-rainfall areas around Lake Victoria, and some come from hot, dry climates (69).

Transmission. The mode of transmission for *Basidiobolus* had not been confirmed but is assumed to be via minor trauma and insect bites (69, 87). Fungal spores are found on bristles of mites and are probably also carried by other insects. Infected insects are eaten by reptiles and amphibians, which subsequently pass the spores in their excreta (129). Clark (87) describes a case involving the hand of a patient bitten by a caterpillar. The patient had caught the insect and squeezed the "juice" onto the site of the bite, which served as the nidus for the fungal lesion.

The organism may also be transmitted from soil and vegetation that is contaminated with animal feces (480). Mugerwa (311) notes that *Basidiobolus* may be picked up on contaminated "toilet leaves" used for skin cleaning after a bowel movement, resulting in direct inoculation in the perineum. Consistent with this theory is the fact that the buttocks, thighs, and perineum are often the sites of infection in patients (69, 175).

The occurrence of rhinocerebral disease in a hyperglycemic host (133) suggests that inhalation of spores, as seen in many cases of zygomycosis with the *Mucorales*, may also play a role in infection for some patients with *Basidiobolus*.

Iatrogenic infection has been reported. Nazir et al. (320) report a case of possible inoculation during appendectomy,

and Kamalam and Thambiah (228) report inoculation from a needle injection.

Host characteristics. Basidiobolomycosis occurs predominantly in healthy individuals (442). It is hypothesized that host immunity is responsible for the small number of cases worldwide, since the organism is ubiquitous (442). Infection with *Basidiobolus* can occur at any age and in either sex (69, 359) but usually occurs in children younger than 10 years and occurs more frequently in males than in females (69, 392, 413, 441). No predisposing factors for infection are known. Scholtens and Harrison (413) predict that with increased HIV infection, it is possible that the infection will be seen more often in the future.

General disease manifestations. *B. ranarum* typically causes a chronic infection of the peripheral or subcutaneous tissue, usually on the arms, trunk, and buttocks (175). The most common presentation for basidiobolomycosis is on the thighs and buttocks in a "bathing suit" distribution (69). The infection is characterized by a hardened nodule which expands and spreads locally. Although the nodules will eventually ulcerate the overlying skin, dissemination usually does not occur (79). The nodular lesions contain inflammatory cellular material with many eosinophils, accounting for the associated erythema and warmth of the skin (44). The infection is slowly progressive without treatment but may heal spontaneously (44).

In addition to chronic subcutaneous disease reported worldwide (20, 41, 42, 44, 62, 87, 104, 109, 211, 218, 311, 413, 480; Thasnakorn et al., Abstr. 5th Int. Congr. Infect. Dis., 1970), other disease manifestations have been seen with a much lower frequency. *B. ranarum* has been reported to cause gastrointestinal infections (41, 110, 359). These infections have been reported primarily in healthy patients (four of five cases), but in one case the patient was diabetic and anergic (110, 411). The stomach, duodenum, and colon are the primary sites of gastrointestinal involvement, and granulomatous inflammation and fibrosis are limited to the muscularis propria with no vascular invasion in any of the patients (359). In one patient, however, the pancreas, liver, and biliary tract were involved. Symptoms of this disease presentation included fever, abdominal pain, diarrhea, constipation, weight loss, and sometimes chills and rigors. Blood in the stool was unusual. Three of the five infected patients died due to progression and obstruction. *Basidiobolus* has also been identified in patients with lymph node (224, 225, 228) and muscle (227, 228) involvement. Other reports of invasive disease with *B. ranarum* include a case of invasive retroperitoneal infection in an 8-year-old boy (320) and a patient with locally aggressive disease originating in the maxillary sinuses causing ulcerations in the hard palate (133). Infections involving additional body sites are cited in the literature, but these lack culture confirmation and should be classified as "zygomycosis" instead of basidiobolomycosis. Likewise, vessel invasion by this organism has been clearly identified in only one case (228). Additional cases credited with blood vessel invasion are based on tissue diagnosis alone and lack culture confirmation (273, 451).

Virulence factors. Low virulence of *Basidiobolus* has been postulated based on the small number (approximately 300) (320) of cases worldwide (442). This lack of virulence is also suggested by experiments in mice. Following experimental inoculation of *Basidiobolus* in mice, invasive disease was not seen (42). It has been suggested that some unknown abnormality must account for the disease (442) and that invasive and progressive infection in previously healthy individuals may result from transient immunosuppression during viral infections or following surgery (441). If immunosuppression indeed accounts for this mycosis, infection with *Basidiobolus* may become more prevalent due to HIV infection (320).

Yangco et al. (516) demonstrated production of exoantigens by *Basidiobolus*. In vitro production of proteases (339, 341) and lipases (339, 340) have also been seen. Echetebu and Ononogbu (135) found that production of extracellular proteinases and lipases allow *Basidiobolus haptosporus* to survive and thrive under various growth conditions. They noted that production of these enzymes on Sabouraud's medium nearly paralleled mycelial growth of the organism. They suggested that growth of the organism and release of enzymes in vivo may be related to the level of fat deposits under the skin. An analogous situation exists for *Achromobacter lipolyticum*, an organism which will produce increased amounts of extracellular lipase after the addition of different oils to growth medium (236). Echetebu and Ononogbu (135) reported that one of the lipases produced by *B. haptosporus* is phospholipase A. A hydrolytic product of phosphatidylcholine by phospholipase A is lysolecithin, a protein which is able to digest human serum proteins. The authors hypothesized that hydrolysis of lecithin to lysolecithin destroys cell membranes (blood, skin, and muscle cells) and may be the pathogenic mechanism used by *B. haptosporus*. They reasoned that protein components of liberated extracellular contents are digested by proteinases produced by *Basidiobolus* and serve as nutrients for organism growth.

Basidiobolus is relatively thermotolerant and is capable of growing, although poorly, at 37°C, similar to the pathogenic *Mucorales* (252, 436). It is hypothesized that thermotolerance is an important virulence factor for these organisms, allowing them to establish infections in vivo with those possessing growth capabilities above 37°C having the advantage of survival in the febrile patient (381).

Diagnosis. The definitive diagnosis of basidiobolomycosis requires an excellent physical examination together with both pathologic and microbiologic evaluation, since basidiobolomycosis may resemble other infections, especially other tropical infections, which present with subcutaneous lesions (442). *Basidiobolus* infection may resemble other fungal infections (pythiosis and sporotrichosis), parasitic infections (filarial elephantiasis and onchocerciasis), bacterial infections (*Mycobacterium tuberculosis* and *M. ulcerans*) (442), and other diseases, including Burkitt's lymphoma (42). While the direct examination may suggest the diagnosis, culture remains the "gold standard" for diagnosis.

Biopsy of subcutaneous (or submucosal) tissue demonstrates broad, thin-walled hyphae together with acute and/or chronic inflammatory cell infiltrates. The mycelium of *Basidiobolus* is more septate than the hyphae of other zygomycetes (35, 79, 442). Hyphae are easily visualized with H&E stain, not due to the staining of the actual hyphal elements but more due to the staining of the Splendore-Hoeppli material encasing the hyphae (79). Hyphae are not well demonstrated with PAS or GMS stain (359). It should be noted that although this is an important histopathologic finding for infection with *Basidiobolus*, eosinophilic deposition around hyphae can occur with other fungi, particularly *Conidiobolus*, and may also occur with parasitic infections. Additionally, the Splendore-Hoeppli phenomenon is not always present with entomophthoromycosis (442). The organism does not typically invade the blood vessel and tissue; necrosis and tissue infarction are therefore not present (442).

Basidiobolus can be grown on standard mycology medium such as Sabouraud, potato dextrose, and cornmeal agars (35). Growth is moderately rapid at 30°C and less rapid at 37°C. Colonies are flat and furrowed, are yellowish to grayish on the surface with a pale reverse, and have a waxy texture (Fig. 8A).

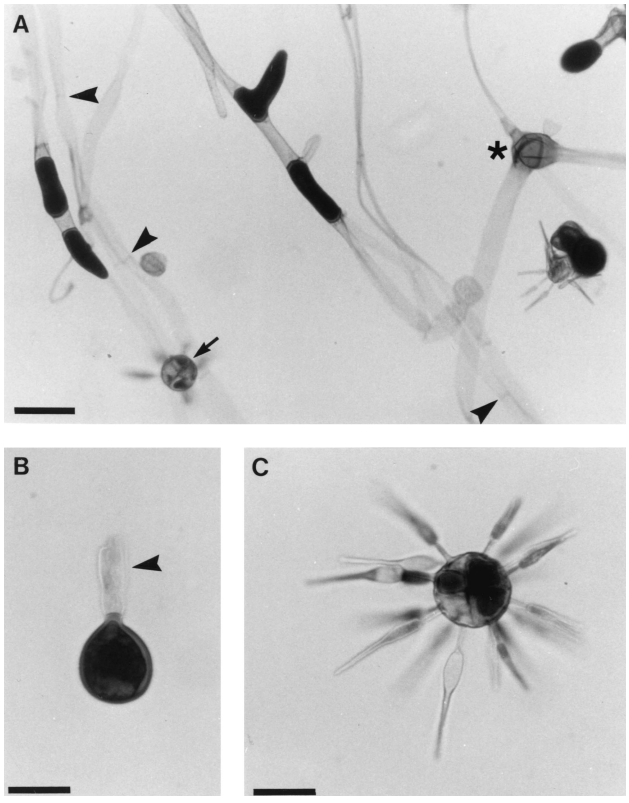

FIG. 19. *Basidiobolus* microscopic morphology on slide culture. (A) Low-power magnification of *B. microsporus. Basidiobolus* produces wide ribbon-like hyphae that are occasionally septate (arrowhead). An empty sporangiophore that has expelled its sporangiospore is seen (asterisk). Note the sporangiospore that has undergone cleavage to produce multiple meristospores (arrow). Bar, 50 μm. (B) Sporangiospore (ballitospore or conidiospore). Actively expelled, single-celled sporangiospores are characterized by a hyphal tag (arrowhead) that represents the remnent of their sporangiophore wall. These spores are generally round or may have flattened apices. Bar, 20 μm. (C) Sporangiolating sporangiospore. Sporangiospores may undergo cleavage to produce multicelled meristospores. Secondary sporangioles form a crown around the original sporangiospore. These represent the passively released population of sporangioles. Bar, 20 μm.

Satellite colonies sometimes form from the germination of ejected sporangioles. Colonies produce a musty odor (252). After 7 to 10 days, the colony will be overgrown and the mycelia will contain masses of conidia, chlamydoconidia, and zygospores (175, 436). *B. ranarum* is homothallic and thus requires no mating strain for sexual reproduction. Microscopically, colonies produce large vegetative hyphae (8 to 20 μm in diameter) which become increasingly septate with maturation (Fig. 19A) (35, 175, 436). *Basidiobolus* produces two types of sporangiophores. Sporangiophores with inflated apices produce single-spored sporangioles (ballistospores or conidia), measuring 7 to 15 by 6 to 12 μm in diameter (130) (Fig. 19B). Conidia are ejected from the inflated, apical portion of the sporangiophore when the subconidial vesicle or swelling emits a stream of fluid that propels the conidium. This propulsion of conidia is a characteristic of the genus. Narrow sporangiophores with adhesive apices also produce single-spored sporangioles, but these sporangioles are released passively (Fig. 19C) (175, 436). Sporangiospores may undergo cleavage to produce meristospores (Fig. 19A and C). They may also produce secondary sporangia (Fig. 19C) (71).

Sexual reproduction occurs via smooth-walled zygospores (20 to 50 μm in diameter). The zygospores show conjugation beaks which represent the remains of copulatory tubes. Zygospores with conjugation beaks distinguish *B. ranarum* from *Conidiobolus incongruus*, both of which produce zygospores homothalically (Fig. 9) (436). *Basidiobolus* often rapidly loses its ability to sporulate during passage in vitro (240). The differentiating features used in identifying *Basidiobolus ranarum* are summarized in Table 5.

In addition to culture, an infection with this agent may be diagnosed by detecting an immune response in an immunodiffusion test developed for the diagnosis of basidiobolomycosis (211, 231). This test has also been useful for monitoring infected patients. Serology has been useful in making a diagnosis of disease even in the absence of culture (359).

Treatment. A discussion on the treatment of *Basidiobolus* infection must include a warning regarding prevention, since the organism has been introduced iatrogenically (via needle injection) (228) and may be spread during diagnosis and treatment (69). Cameroon (69) suggests that because of the potential for spreading, surgery should be limited to biopsy only. Surgical resection and debridement may therefore not be routinely performed. In contrast, other researchers have found surgical resection and debridement appropriate for treatment and cure (109, 359).

Pharmaceutical agents that have been used to successfully treat *Basidiobolus* infection include most commonly potassium iodide (KI) (42, 228, 384, 480), trimethoprim-sulfamethoxazole (384), amphotericin B (133), oral azoles (109, 131, 359) and KI combined with oral azoles (44). Treatment of *Basidiobolus* is not always successful, and no single drug has proved effective in the treatment of all cases of basidiobolomycosis (320, 384). Yangco et al. (515) conducted in vitro studies to look at the activities of drugs used to treat *Basidiobolus*. They found that in vitro, amphotericin B was active against only 50% of *Basidiobolus* isolates and that both KI and 5-fluorocytosine failed to inhibit or kill at the maximum concentration. Since the action of KI is unknown and may not be mediated by direct fungal killing, these in vitro findings may not be significant (454). The imidazole derivatives, especially ketoconazole, were found to be active against the organism. Ketoconazole has demonstrated variable effectiveness against the *Entomophthorales* in vivo, sometimes requiring more than one course of therapy or months of continuous treatment for cure (131). The dosage and length of treatment must be taken into consideration. KI appears to be effective when given for at least 3 months (1.5 to 2 g/day), but patients often do not comply due to adverse effects. Trimethoprim-sulfamethoxazole, given for at least 6 months, is more effective and is without the side effects noted with KI (131, 384). Since *Basidiobolus* infection may resolve spontaneously and may not require treatment (441), other factors may be involved in cases where drug treatment was reported as being effective (515). To date, no single therapy is recommended. Restrepo (384) emphasizes that detection of infection in the early stages of this chronic disease is the best way to reduce morbidity.

Relationship to other fungi causing infections. *B. ranarum* is now recognized as the only human pathogen in the genus *Basidiobolus*. Previous articles describing this organism have used the synonyms *B. haptosporus*, *B. meristosporus*, and *B. heterosporus. Basidiobolus ranarum* is now considered the preferred designation (35, 175).

Basidiobolus infections resemble those caused by the other agents of subcutaneous mycoses. *Basidiobolus* shares antigens with *Conidiobolus* (231) and shares at least one antigen with *Pythium insidiosum* (211). *Basidiobolus* also demonstrates common antigenic determinants with the *Mucorales Rhizopus arrhizus* and *Rhizomucor pusillus*, although this is more limited

than the antigenic sharing between the *Mucorales*. Antibodies stimulated by *B. ranarum* infections may be identified using purified fungal antigens from each of these fungi (230).

Conidiobolus Species

Natural habitats. *Conidiobolus* is found worldwide in soils and plant detritus, with *C. coronatus* being the most common *Conidiobolus* species identified (130). Although the organism is found around the world, including the soils and beach litter in the United Kingdom (430) and the soils of the eastern United States and India (388), it is more concentrated in warm, wet climates, particularly countries in West Africa (184). Organism concentrations increase with peak periods of heavy rainfall (87). Germination of *C. coronatus* does not occur at humidity levels below 95% and is maximum at 100% humidity (518). This correlates with the majority of human infections being reported in individuals living and working out of doors in the wet, tropical forests of Africa (184, 442). *Conidiobolus* spp. have also been see parasitizing insects (59), horses (208, 392), and sheep (448). These fungi have also been isolated from the feces of lizards, frogs, reptiles, and other animals (35).

Transmission. The mode of transmission of *Conidiobolus* has not been established but is probably via inhalation of fungal spores that are implanted in the nasal mucosa or from a minor trauma such as an insect bite (87, 175, 184). Gugnani (184) notes that frequent picking of the nose may result in minor trauma and be a means of entry for the organism. This investigator (184) explored the possibility of snuff-taking as a contributing factor but did not isolate *Conidiobolus* from any of the tobacco samples studied in his laboratory. Mukhopadhyay et al. (312) reported a case of sinusitis zygomycosis caused by *C. coronatus* in a 72-year-old Brazilian woman with a history of chewing tobacco leaves, pond bathing, and cattle raising, suggesting that other possible contributing factors should be addressed.

Host characteristics. The majority of individuals infected with *Conidiobolus* are males from the tropical rain forests of West Africa. Agriculture and other outdoor workers have been infected most often, and cases in males outnumber those in females 8:1 (87, 184, 282, 336). Most cases involve adults; children and adolescents are rarely infected (184). Most infections occur in healthy patients (184), but immunocompromised individuals may also be infected (214, 487).

Although *Conidiobolus* occurs worldwide, the number of cases of infection is small (the 1991 estimate was 150 cases) (99). The majority of infections have been limited to tropical areas, particularly the African rain forest (184), but cases have been reported worldwide (283, 389), including Nigeria (87, 165, 183, 283, 336, 345), Congo (71, 283), Zaire (282), Cameroon (82, 165, 283, 378), Côte d'Ivoire (132, 165), Central Africa (71, 165, 283, 458), India (165, 283, 312), Brazil (16, 43, 99, 466), Jamaica (57), Costa Rica (416), Columbia (165), Puerto Rico (165), Thailand (63), Malaysia (323), and the United States (136, 165, 239, 319, 486, 487).

Conidiobolus species cause disease in animals, including horses (147, 296), mules (73), a dolphin (389), and a chimpanzee (397). It is interesting, however, that there have been no reported *C. coronatus* infections in animals in regions of Africa where human infections are frequent (184). Attempts at experimental infection have been unsuccessful in several laboratory animal models (165).

General disease manifestations. Infections with *Conidiobolus* present most commonly as chronic sinusitis zygomycosis. Sinusitis disease begins as swelling of the inferior nasal turbinates and extends to nearly all adjacent tissues and structures. The infection spreads to involve facial and subcutaneous tissues and the paranasal sinuses. As a result of swelling of the nose, mouth, and perinasal tissue, patients may experience nasal stuffiness, draining, sinus pain, and in some cases epistaxis (345, 346). Due to the swelling in the nasal area, patients may have a feeling of nasal obstruction (282). As the infection spreads, subcutaneous nodules, which are usually firm and painless, attach to underlying tissues and can be palpated through the skin (175, 184). Severe facial swelling may make the eyes unable to open (442), and development of subcutaneous nodules in the eyebrows, upper lip, and cheeks may give the patient the appearance of a hippopotamus or tapir (184, 378). The infection usually does not extend to the cerebral hemispheres (79), and distant dissemination is unusual but does occur (184). Infection in the pharynx and larynx, causing dysphagia and obstruction of the larynx, as well as extensive and chronic lymphedema, has been reported (184, 282, 337). Sinusitis zygomycosis with *C. coronatus* has been reported worldwide in patients considered to be physiologically and immunologically competent, with different lengths of illness and varying success with treatments (99, 312, 323, 416, 454, 466). Disseminated infections, including those with fatal outcomes, have been reported with *C. coronatus* in both immunocompetent and immunocompromised hosts. Lymph node invasion with severe disfigurement has been reported with *C. coronatus* infection (225). Walker et al. (486) report a case of disseminated *C. coronatus* with blood vessel invasion in a renal transplant patient, who was also infected with the pathogens *Histoplasma capsulatum* and cytomegalovirus.

Conidiobolus incongruus is also responsible for several invasive infections, including the first *Conidiobolus* case reported in the United States, which occurred in a 15-month-old child (136). Infection with this organism has been reported in immunocompetent patients (63, 136) and in immunocompromised patients (486, 487). The infection has proved fatal for patients independent of their immune system status (63, 486, 487). Busapakum et al. (63) reported on the first fatal case of disseminated infection due to *C. incongruus* in a previously healthy, 20-year-old female Thai student. The patient presented with fever, weight loss, cough with hemoptysis, and a subcutaneous mass. The symptoms were of 2 months duration. Skin, subcutaneous tissue, lung, lymph nodes, esophagus, liver, and jejunum revealed a granulomatous reaction with eosinophilic amorphous material and broad hyphae. Cultures identified the etiologic agent as *C. incongruus*. This patient died 6 weeks after admission, although 2 g of co-trimoxazole was given daily. The route of transmission was not determined, and no underlying disease or immunologic defect could be detected.

Walsh et al. (487) described culture-positive *C. incongruus* infection in an immunocompromised patient. This 32-year-old woman was granulocytopenic due to lymphocytic lymphoma, and she developed pulmonary and pericardial zygomycosis. In spite of treatment with amphotericin B and pericardiocentesis, the patient died on the third post-operative day.

Jaffey et al. (214) reported a case of disseminated *Conidiobolus* infection with endocarditis in a cocaine abuser. The *Conidiobolus* species was not one previously known to cause infection in vertebrates. The organism gained access to the bloodstream via skin abrasions and disseminated to the lungs, heart, skeletal muscles, kidneys, and brain. The authors noted that this fatal case of *Conidiobolus* infection resembled a mucoraceous infection, with its acute disease course, angioinvasive tissue involvement, and the lack of characteristic Splendore-Hoeppli phenomenon accompanying the infection.

Of the 27 known species of *Conidiobolus* (486), three are

known to infect vertebrates: *C. coronatus*, *C. incongruus* and *C. lamprauges* (175, 214, 392). *C. coronatus* has been identified as the agent of nasal granuloma in a horse in Texas (147), and infection with *C. lamprauges* is limited to the horse (208). Maxillofacial disease with *Conidiobolus* species is also seen in sheep (448).

Virulence factors. Despite the ubiquity of *Conidiobolus* spp., disease with these agents is relatively uncommon. This suggests that the organism is not very virulent and/or there is some, as yet unknown, mechanism that promotes infection and transient loss of immunological protection in previously healthy individuals. Clark (87) suggests low virulence for *Conidiobolus* because of certain patient histories. She reports that some cases heal spontaneously and that one patient had been working in an environment in a *C. coronatus*-endemic environment for at least 20 years before developing symptoms. Other patients, in rural areas, have a mild form of the infection and do not seek treatment. The Splendore-Hoeppli phenomenon in immunocompetent individuals is an expression of host resistance (505) and is often absent in infected patients who are immunocompromised. In the case reported by Jaffey et al. involving infection in a cocaine abuser, the authors suggest that the cocaine abuse rendered the patient immunocompromised and susceptible to the opportunistic *Conidiobolus* infection (214).

Conidiobolus species produce elastase, esterase, collagenase, and lipase in vitro, and *C. coronatus* produces more lipase and protease than do saprophytic strains (184, 339). A role for most of these enzymes in the infection process has been demonstrated or postulated. For example, it has been shown that serine protease plays a role in the discharge of *C. coronatus* conidia (39, 184). Gugnani (184) suggests that since proteases are secreted rapidly, they may be secreted into infected tissue at the initiation of infection and will then degrade protein components to make amino acids available for organism growth and that lipase, which is secreted later, would hydrolyze fatty materials in the subcutaneous tissues. Collagenase activity has been reported and would be important for muscle invasion, which has been reported in some cases (184, 214, 227). Jaffey et al. (214) suggest that the extensive rhabdomyalysis in the *Conidiobolus*-infected cocaine abuser discussed earlier could be due to proteases released by the fungus.

Conidiobolus spp. are also thermophilic, readily growing at 37°C. It has been noted that strains isolated from infected humans grow more rapidly at 37°C than do those obtained from environmental sources, suggesting that this might also be a virulence factor for these organisms (252, 239). King and Jong (239) suggest that enhanced growth at 37°C might indicate adaption for growth in the human body.

Diagnosis. The physical examination, especially in areas where infection is endemic, is an important feature of the diagnosis of *Conidiobolus* infection (442). Where features of subcutaneous mycoses, particularly involving the sinusitis site, are present, biopsy and culture of subcutaneous or submucosal tissue are required to establish the diagnosis. Broad, thin-walled hyphae are found in tissue, along with acute and/or chronic inflammatory cells (79, 442). The tissue reaction is similar to that seen in basidiobolomycosis and is both acute and chronic. In tissue sections, coenocytic hyphae are easily visualized with H&E stain due to the presence of the eosinophilic Splendore-Hoeppli material also seen in *Basidiobolus* infections. Fungal elements are not well demonstrated with PAS or GMS (442). The lesions (subcutaneous and sinusitis) are composed of cellular granulation tissue, rich in eosinophils. The acute reaction consists of lymphocytes and plasma cells, in addition to eosinophils, at the site of infection. The chronic reaction also contains eosinophils and lymphocytes plus epithelioid cells, giant cells, and histiocytes (79, 184). Hyphal fragments, often phagocytosed within giant cells, are scattered throughout the tissue, and each fragment is enveloped by an eosinophilic sheath. Since the hyphae are typically not angioinvasive, infarction and hematogenous dissemination are not observed (79). Gugnani (184) notes that healed granulomata with fibroblasts, histiocytes, and edema may also be seen. Some variation in the disease process exists, however. Not all *Conidiobolus* infections exhibit the Splendore-Hoeppli phenomenon, and this phenomenon is not at all specific to *Conidiobolus*. In some rare cases, *Conidiobolus* is also angioinvasive (442).

C. coronatus can be grown on standard mycology medium (35), including Sabouraud, potato dextrose, and cornmeal agars. Growth is rapid at 37°C. Colonies are white, on the surface, becoming beige to brown, with a pale reverse. They are waxy to powdery with folding and furrowing. Satellite colonies develop from ejected sporangioles, and older colonies become covered with short, aerial mycelia and conidiophores. Conidia are forcibly discharged and stick to the inside of the culture container, completely clouding the view into the culture with time (Fig. 8C and D) (35, 437).

Microscopic culture morphology demonstrates hyphae that are broad (6 to 15 μm wide) (147) and more or less septate (Fig. 20). The degree of septation increases with the age of the culture. The organism produces short erect conidiophores that are unbranched and are difficult to differentiate from vegetative hyphae. The conidiophores (sporangiophores) produce single-celled (one-spored) large conidia (25 to 45 μm in diameter), which may be discharged from the conidiophores when mounted on a slide. In contrast to *Basidiobolus*, the conidia, which are round to pyriform, have prominent papillae (Fig. 20) on the wall, which give rise to secondary conidia. This configuration gives the original spore a corona appearance, hence the species name, "*coronatus*." A conidium also may produce hairlike appendages called villae. In contrast to *Basidiobolus* and *C. incongruus*, *C. coronatus* is heterothallic and rarely produces zygospores (35, 437). Costa et al. (99) note that it may be difficult to produce villose resting spores, especially for human isolates.

C. incongruus is a homothallic species that produces a plethora of conidia with pointed papillae (16 to 30 by 20 to 34 μm), but unlike *C. coronatus* this species does not produce villous sporangioles (239). Like *Basidiobolus*, *C. incongruus* is homothallic, producing zygospores (21 to 32 μm) (239) that can be distinguished from those of *Basidiobolus* in the absence of conjugation beaks on these structures (Fig. 9B) (175, 437). In this species, hyphal segments become distended in older portions of the colony (99). Growth of *C. incongruus* is optimal at 37°C, producing little or no growth at room temperature (239).

The *Conidiobolus* isolate described by Jaffey et al. in a patient with a disseminated infection was a species not previously known to cause disease in vertebrates (214). The infection was acute, with hyphal invasion of blood vessels and absence of the Splendor-Hoeppli phenomenon. The organism produced sporangiola that were forcibly discharged (16 by 16.9 μm in diameter) and mature zygospores (20 to 21 μm in diameter) that were not compatible with either *C. incongruus* or *C. lamprauges*. This isolate was significantly different from several isolates of *Conidiobolus* (214).

Other culture characteristics that may be helpful in identifying *Conidiobolus* spp. is the inability of this genus to utilize nitrate or nitrite (175). Additionally, *Conidiobolus* cultures lack the musty, streptomyces-like odor that is often associated with the *Basidiobolus* isolates. In comparison to *Basidiobolus*, *Conidiobolus* spp. do not lose sporulation ability as rapidly on in

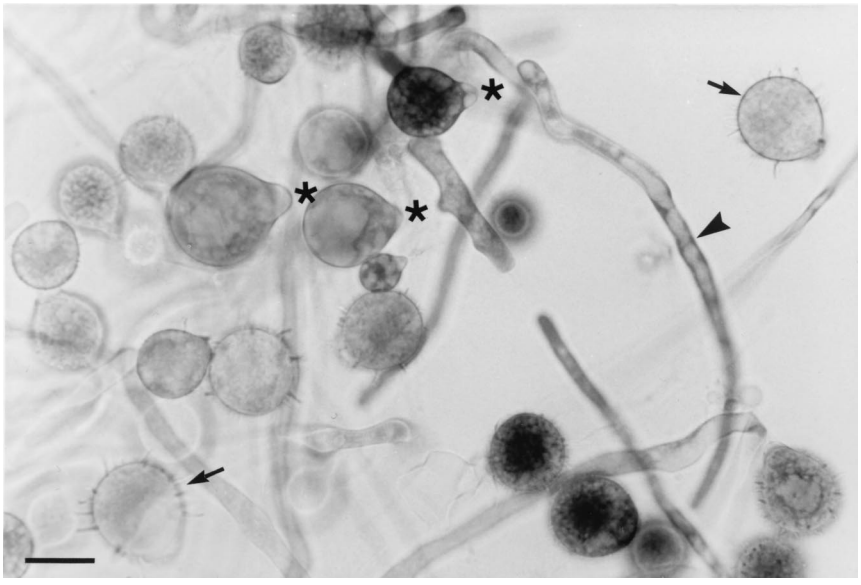

FIG. 20. *Conidiobolus coronatus* microscopic morphology on slide culture. Thin-walled hyaline hyphae are coenocytic in morphology. Occasional septations (arrowhead) are seen, increasing in number with culture age. Round and pyriform sporangiospores (conidiospores) are produced in abundance. Many have a prominent papilla at one side (asterisks). Some conidiospores are encircled by many hair-like processes called villae. These are referred to as villous conidiospores and are the structures from which this organism derives its name "*coronatus*," meaning crown (arrow). Bar, 25 μm.

vitro growth (240). The morphologic features used to distinguish *Conidiobolus* spp. are summarized in Table 5.

Serologic tests are available to detect conidiobolomycosis. Concentrated brain heart infusion culture filtrate antigen is useful for immunodiagnosis in cases of infection (184). An immunodiffusion test for *C. coronatus* has also been useful for diagnosis and for monitoring the response to treatment (231).

Treatment. No single drug has been shown to be effective for the treatment of all *Conidiobolus* infections. Potassium iodide, co-trimoxazole, amphotericin B, ketoconazole, itraconazole, and combinations of these agents have been used with different degrees of success (184). Potassium iodide has traditionally been used in treatment of *Conidiobolus* infection with both successes (388) and failures (82, 336, 466) reported in the literature. High in vitro doses of potassium iodide must be used (337), but Taylor et al. (454) warn that the method of action of KI is not known and that it may not have a direct antifungal effect, making in vitro testing less meaningful. Trimethoprim-sulfamethoxazole has given good results (184, 282, 336), but in vitro high doses have been required for organism inhibition with this drug as well (384). Yangco et al. (515) reported in vitro results for the imidazoles and found that ketoconazole was most effective. Favorable in vivo responses to ketoconazole have been reported in the literature (39, 82, 131, 458, 466), as have failures (131). Other imidazoles have been effective, including itraconazole (39, 458) and fluconazole (186). Amphotericin B is usually not the first choice for treatment but is often tried after other agents have failed (136, 297, 319, 384, 395, 454). Gugnani found amphotericin B more effective than imidazoles in the treatment of *C. coronatus* (H. C. Gugnani, Abstr. XII Congr. Int. Soc. Hum. Anim. Mycol., abstr. PO2 59, 1995). In vitro susceptibility testing using amphotericin B has demonstrated both sensitive (124) and resistent (134) findings. Anecdotal descriptions of combination drug therapy may warrant further study to determine the true efficacy. The combinations of sulfamethoxazole-trimethoprim and potassium iodide (184), ketoconazole and saturated potassium iodide (312), septrim and ketoconazole (51), and amphotericin B and terbinafine (164) have reportedly been effective in medically treating *Conidiobolus* infections where single-drug therapy had failed.

In some cases, *Conidiobolus* infection heals spontaneously, and some patients are cured by surgical treatments alone (87, 165). Surgery may be required to remove accessible nodules and permit reconstructive and cosmetic surgeries (442). The surgical approach is not always optimal, and relapses often are seen (252). There is no prophylactic antifungal usage for *Conidiobolus* infection (442).

Relationship to other fungi causing infections. Similar to most of the other zygomycetes, the *Conidiobolus* species have undergone a number of name changes. *Conidiobolus coronatus* was initially designated *Entomophthora coronatus*, linking this organism to the insect source from which it had originally been identified.

Conidiobolus shares antigens with *Basidiobolus. B. ranarum* and *C. coronatus* each have five specific antigens and share one antigen (231, 516). *C. coronatus* also shares one antigen with *Pythium insidiosum* (231). Morphologically, the species of *Basidiobolus* and *Conidiobolus* are more closely related to one another than to the other zygomycetes. The colony morphology and the production of actively propelled sporangioles clearly separate these organisms from the *Mucorales*. The predominant disease manifestations also separate *Conidiobolus* spp. not only from the *Mucorales* but also from *Basidiobolus* spp.

CONCLUSIONS

The *Zygomycetes* as a group represent two distinct and unusual orders of fungi, the *Mucorales* and the *Entomophthorales*. The more common of these fungi, the *Mucorales*, are an ever-expanding group of organisms which are capable of causing disease in humans. The scope of disease involvement with this diverse group of molds has expanded beyond their role as opportunists to also include them as causes of cutaneous or wound infections and asymptomatic colonization in hosts at

low risk for developing opportunistic infections. Although linked by their production of coenocytic hyphae, zygospore production, and often floccose colony morphology, these fungi display a wide range of beautiful and unusual morphologic variations.

The *Entomophthorales* represent much less common clinical isolates. Their possible role in human disease should be considered in cases of subcutaneous mycosis, sinusitis disease, and even disseminated disease when the Splendore-Hoeppli phenomenon is seen in tissue sections, particularly when a history of travel to a tropical climate is seen. Their isolation in the laboratory should be suggested by their glabrous and folded colonial growth, forcible expulsion of spores onto the petri dish lid, and characteristic microscopic morphology in culture. Lumping the *Entomophthorales* together with the *Mucorales* as causes of "zygomycosis" does not adequately reflect the distinct morphologic, epidemiologic, and pathogenic nature of these two fungal orders.

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