Part II

Importance of Yeasts

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Chapter 2

Yeasts Pathogenic to Humans

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s0010 1. INTRODUCTION TO THE MEDICALLY IMPORTANT YEASTS

p0010 Prior to global emergence of the human immunodeficiency virus (HIV), which is the causative agent of acquired immunodeficiency syndrome (AIDS), approximately 200 fungal pathogens were recognized from among the more than 100,000 then-known fungal species (Kwon-Chung and Bennett 1992, Rippon 1988). About 50 of these species were regularly associated with fungal disease (mycosis). Since then, there has been a concurrent dramatic increase in both the number of known fungal species and the incidence of mycoses that they cause. Moreover, the spectrum of pathogenic fungi has changed radically. Though HIV infection has been noted to be a significant factor in these changes, it is only one of many diseases that have encompassed the dynamic transformation of human affairs over the past several decades. The developments that have played noteworthy roles in the increased frequency of mycoses include the relative ease of international travel, an expanding and aging population, a breakdown in public health measures, rising immigration rates, heightened awareness of disease, industrialization, prolongation of life due to medical advances, and, sadly enough, politics (Cooper 2002, Lederberg et al. 1992, Smolinski et al. 2003). The collective result of these changes markedly influenced the diversification of etiological agents by permitting virtually any fungus capable of growing at or near body temperature (37°C) to eclipse a critical first hurdle in ascending to the level of pathogen.

p0015 Yeasts are among the most prominent of the disease-causing fungi, especially *Candida* spp., *Cryptococcus* (*Cr.*) *neoformans*, and *Cr. gattii*. There also exists a spectrum of unicellular fungi that have emerged as significant infectious agents (Table 2.1). Moreover, while the term "yeast infection" tends to elicit an image of an agent that exists mainly in a monomorphic state, other fungi also produce yeast cells or yeast-like forms *in vivo*. These include several thermally dimorphic fungi that exist as saprotrophic molds when grown at moderate temperatures (22°C–28°C), but convert to a yeast phase upon tissue invasion or *in vitro* culture at 37°C. In addition, a number of members grouped within the form-family Dematiaceae exhibit a yeast form both in culture and *in vivo*. Moreover, the enigmatic fungus *Lacazia loboi*, the agent of lobomycosis, presents chains of yeasts in tissue although this organism has yet to be isolated from nature. Hence, in medical mycology, the above examples support using the term "yeast" to strictly define a morphological feature without ascribing a taxonomic significance. However, many clinicians commonly use this term to refer mainly to the pathologies designated as candidiasis and cryptococcosis.

p0020 As an introduction to this text, the present review is meant to serve as a brief, descriptive compendium of mycoses caused by yeasts. Yet, it is not the author's intent to suggest that yeast biologists will regularly encounter the organisms described below. In fact, many medical mycologists spend entire careers without direct clinical exposure to many of these fungi. Rather, the purpose of this review is to enlighten the non-medical mycologist as to the diversity of yeast and mold species regularly associated with human and animal disease that also, at least in part, present a unicellular mode of growth *in vivo*.

p0025 The following descriptions present a concise overview of the key biological and clinical features of these fungi. Where appropriate, references to recent reviews of particular disease agents and their pathologies are provided. For a global perspective of fungal diseases, including in-depth clinical discussions of specific pathologies, diagnoses, and treatments, the reader is referred to several outstanding and recently published texts (Anaissie et al. 2003, Dismukes et al. 2003, Kauffmann 2006, Merz and Hay 2005). In addition, brief summaries of mycotic diseases and their etiological agents, including currently accepted binomials for these fungi, can be found on the internet web page; Doctor Fungus (www.doctorfungus.com). Moreover, the narratives below provide brief taxonomic descriptions for each species and, if known, its teleomorph. In some instances, the particular classification of the sexual state was retrieved from the National Library of Medicine (www.ncbi.nlm.nih.gov/genomes/static/euk_o.html). Finally, of those fungi presented below, with the possible exception of *Histoplasma*, all can be handled using Biosafety Level 2 practices (www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s7b.htm).

2. ASCOMYCETOUS YEASTS OF CLINICAL SIGNIFICANCE s0015

p0030 Overall, ascomycetous yeasts comprise the largest group of pathogenic fungi. Most of these pathogens are members of the anamorphic genus *Candida*. To a far lesser extent, fungi classified within the genera *Saccharomyces, Pichia* (including species formerly assigned to *Hansenula*), and *Magnusiomyces* (formerly *Dipodascus, Blastoschizomyces*) also cause human infections.

2.1. *Candida* s0020

p0035 A number of *Candida* species exist in a commensal relationship with humans as normal residents of the gastrointestinal tract, mucocutaneous tissues, and skin. The most notable of these species is *Candida albicans*. However, being opportunistic pathogens, *Candida* spp. can exploit local or systemic weaknesses in host resistance, to cause disease in virtually any part of the body. Such infections, termed candidiasis, have become increasingly common during the past several decades for a number of reasons, which include the use of immunosuppressive therapies for other diseases as well as the unfortunate

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t0010 **TABLE 2.1 Selected Yeast and Yeast-Like Pathogens for Humans and Animals**

Anamorph	Known Teleomorph
Ascomycetous Fungi	
Candida albicans	None
Candida dubliniensis	None
Candida glabrata	None
Candida nivariensis	None
Candida bracarensis	None
Candida guilliermondii	*Meyerozyma .Pichia guilliermondii*
Candida krusei	*Pichia kudriavzeii*
Candida lusitaniae	*Clavispora lusitaniae*
Candida parapsilosis	None
Candida metapsiloisis	None
Candida orthopsilosis	None
Candida tropicalis	None
—	*Saccharomyces cerevisiae*
—	*Saccharomyces boulardii*
—	*Wickerhamomyces (Pichia) anomala*
—	*Ogataea (Pichia) polymorpha*
Blastoschizomyces capitatus	*Dipodascus capitatus*
Basidiomycetous Fungi	
Cryptococcus neoformans var. *neoformans*	*Filobasidiella neoformans*
Cryptococcus neoformans var. *grubii*	*Filobasidiella neoformans*
Cryptococcus gattii	*Filobasidiella bacillispora*
Malassezia spp.	None
Trichosporon spp.	None
Rhodotorula spp.	None
Sporobolomyces spp.	None
Pseudozyma spp.	*Ustilago* spp.

prevalence of HIV infection. Other factors, such as increased awareness and improved diagnostic tools, have contributed to the sense that candidiasis has risen in importance as an affliction of humans and animals.

p0040 Evidence supporting the significance of *Candida* spp. as infectious agents might be readily noted by the number of publications cited in the medical literature that document case studies and research reports featuring these fungi. Using the words "*Candida albicans*," the present author searched for citations in the PubMed database supported by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). From 1970 through 1974, 1124 publications included the topic "*Candida albicans*." Over the next thirty years, this number rose by more than 334% with exponential increases from 1985 to 2004. Data for the past two years suggest that such publications will continue to proliferate exponentially over the next several years. Therefore, if the number of citations is indeed representative of the significance of *C. albicans* to public health, and by extrapolation to other *Candida* spp. as well, it is clear that these yeasts and the infections they cause have commanded much attention from clinical and experimental mycologists.

p0045 In addition to the human body, *Candida* spp. can be found in a wide variety of environmental habitats. Like those typically considered non-pathogenic, disease-causing species can be recovered from many of the same sources including air, water, foodstuffs, clothing, toothbrushes, etc. However, the environmental isolation of *Candida* spp. that are regularly associated with infections is often the result of contamination by humans or animals rather than a reflection of a true primary habitat for such fungi. This is a particularly disturbing fact that coincides with reports showing health care facilities to be a major location for contracting candidiasis (Jarvis 1995). Collectively, *Candida* spp. are the fourth most common cause of hospital-acquired (nosocomial) infections in North America.

p0050 A detailed description of the various clinical manifestations of candidiasis, as well as clinically relevant biological investigations of *Candida* spp., can fill entire books. Indeed, several books (Bodey 1993, Calderone 2002, Odds 1988, Segal and Baum 1994), chapters within medical mycology texts (Anaissie et al. 2003, Dismukes et al. 2003, Heitman 2006, Kauffmann 2006, Kwon-Chung and Bennett 1992, Merz and Hay 2005), and numerous reviews (Pappas 2006, San-Blas and Calderone 2004, Sims et al. 2005, Spellberg et al. 2006) have been published over the past two decades covering these topics. As such, the reader is referred to these resources. The remainder of the present discussion will focus on brief portrayals of the more significant pathogenic species of *Candida*. Suffice to note, however, candidiasis encompasses a broad range of infections. Mucocutaneous forms of the disease (e.g., vaginitis) are very common and rarely life threatening to the immune competent host. Rather, such mucosal and superficial skin infections are typically troublesome in terms of physical comfort, cosmetic concern, and occasional relapse of the condition. In contrast, mucocutaneous candidiasis in immunocompromised hosts (e.g., HIV-infected individuals with esophageal candidiasis) is more serious and subject to frequent relapses. The more serious cases of systemic candidiasis, either in immune compromised persons or others with predisposing conditions (e.g., burn patients), can produce a multitude of symptoms and involved organ systems. Highly significant mortality rates accompany these forms of candidiasis.

p0055 As yeasts, *Candida* spp. are generally small, ovoid, thin-walled fungi that reproduce mainly by budding. Relatively few species are routinely isolated from human and animal infections (Table 2.1). Clinical isolates are often distinguished from one another by using one of several simple methods. For example, as discussed below, yeast cells of several etiological agents of candidiasis produce germ tubes in serum at 37°C, thereby reducing the number of possible identifications. Also, only a few species produce chlamydospores, again restricting identification to a few yeasts. Perhaps one of the quickest means to a presumptive identification is the use of CHROMagar *Candida* media. Based upon the color and morphology of colonies growing on this proprietary medium, the clinical mycologist can distinguish among yeasts of *C. albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, and other non-*C. albicans* species (Jabra-Rizk et al. 2001, Odds and Bernaerts 1994, Pfaller et al. 1996). However, the traditional method of employing commercially available carbohydrate fermentation and assimilation assays (e.g., API 20C, Vitek 2 ID-YST, etc.) remains key to making more precise identifications when ambiguous or equivocal phenotypic results are obtained. Hopefully, some of the ambiguity will be resolved as progress is made in the development and application of molecular methods in which DNA-based technology will provide the means to more rapidly and accurately identify pathogenic yeasts. For a review of traditional and commercial yeast identification methods, as well as capsule summary of genotypic techniques, the reader is referred to a recent publication by Pincus et al. (Pincus et al. 2007).

p0060 The following discussions are limited to those species that are well-documented *Candida* pathogens. Regarding the taxonomy of these species, to note that this topic has been a source of tremendous study would be a gross understatement. For a comprehensive review of the taxonomic aspects of these fungi, the reader is referred to the pertinent sections of the present publication. To summarize, the following fungi are considered ascomycetes due to the teleomorphic state exhibited by some species as well as other cellular and molecular properties. Physiologically, these yeasts fall within Group VI

described by Meyer et al. (Meyer et al. 1998), in that all grow at 40°C, but they do not assimilate nitrate, erythritol, or inositol.

s0025 2.1.1. Candida Albicans

p0065 This fungus is the most common cause of candidiasis, but is not readily isolated from the environment. This apparent absence from the environment may be due to its adaptation to a parasitic life cycle with the concomitant loss of the properties permitting it to easily survive outside a host. In addition to a yeast phase, *C. albicans* produces true hyphae and pseudohyphae when it is cultured on the appropriate media and incubated under suitable environmental conditions. *In vivo*, both hyphal-like elements and yeast cells can be observed. The hyphae generate grape-like clusters of blastoconidia. The latter can grow as budding yeasts or germinate as hyphae. Also, *C. albicans* is well known for the ability to form thick-walled entities, termed chlamydospores, at the terminal ends of hyphae. This structure is often used as a diagnostic feature for this species, although *C. dubliniensis* also produces chlamydospores. An additional diagnostic feature of *C. albicans* is the ability of yeasts grown at 37°C in serum to form germ tubes. These hyphal initials differ from the outgrowth of other *Candida* spp. in that a constriction is absent at the germ tube base. Finally, on CHROMagar *Candida*, this species produces distinctive green colonies.

p0070 A true teleomorph of *C. albicans* has yet to be established although recent advances have demonstrated that this yeast possesses mating type genes (Hull and Johnson 1999). Genetically modified strains provided evidence of "mating" within a mammalian host (Hull et al. 2000). In the laboratory setting, appropriate strains can undergo a type of cytoplasmic fusion followed by karyogamy that strongly resembles that exhibited by *Saccharomyces cerevisiae* (Bennett and Johnson 2005, Miller and Johnson 2006, Soll 2006). A key difference is that the mating strains are naturally diploid and the daughter cell product of this mating is a tetraploid. Subsequent loss of chromosomes during vegetative growth of the daughter cell eventually results in the re-establishment of a diploid state. This entire process is highly regulated and is associated with a genetically controlled morphological phenomenon known as phenotypic switching.

s0030 2.1.2. Candida Dubliniensis

p0075 In 1995, a new species of *Candida* was isolated from the oral cavity of HIV-infected patients. This new species was designated as *C. dubliniensis*. Subsequently, like *C. albicans*, *C. dubliniensis* proved to be readily recovered from HIV-positive patients across the world. This yeast has also been isolated from bone marrow transplant patients and those persons on broad-spectrum antibiotics. A recent review highlights the status of this pathogen since its discovery (Sullivan et al. 2005).

p0080 *Candida dubliniensis* is morphologically similar to *C. albicans* in that it forms germ tubes and chlamydospores. These two properties are only exhibited by these two species among all members of the genus *Candida*. However, a published report suggests that Staib agar supports chlamydospore formation only by *C. dubliniensis*, thereby providing a possible diagnostic tool for differentiating these species (Staib and Morschhauser 1999). A more recent study suggests that the regulatory signals that control chlamydospore formation differ between *C. dubliniensis* and *C. albicans* (Staib and Morschhauser 2007). Such data may help decipher the biological function of this curious structure. In addition to the morphological and developmental resemblance of *C. dubliniensis* and *C. albicans*, both of these species are physiologically similar bearing only subtle differences. However, the two species can be distinguished from one another by incubating strains at 45°C. At this temperature, *C. dubliniensis* will not grow, whereas *C. albicans* readily forms colonies under the same conditions. On CHROMagar *Candida*, *C. dubliniensis* also forms green colonies like *C. albicans*, though on newer formulations of the medium the colonies are darker. There are differences in karyotypes and rDNA sequences, but the applications that would be employed to assess these characteristics are usually beyond the purview of a typical clinical laboratory.

2.1.3. Candida Glabrata s0035

p0085 From a medical viewpoint, the significance of this species of *Candida* can be found in its increased incidence worldwide, compounded by an apparent increase in its resistance to commonly applied antifungal agents. *Candida glabrata* is the second most common cause of blood stream infections following *C. albicans*, with the upsurge in frequency being due to the larger population of immunocompromised individuals and the widespread use of antimycotics. This species is also one of the most prevalent isolates from cases of oral infections and vaginitis. For further information regarding the biological and clinical properties of this yeast, the reader is referred to recently published reviews (Bialkova and Subik 2006, Kaur et al. 2005).

p0090 Isolates of *C. glabrata* can be readily recovered from clinical specimens using routine medical mycological media. A presumptive identification can be made using CHROMagar *Candida* media upon which colonies of *C. glabrata* appear purple to pale pink. Recent clinical findings, however, suggest that some diagnoses of *C. glabrata* infections may be missed since the growth of particular isolates is dependent upon exogenously supplied cholesterol (Bard et al. 2005, Hazen et al. 2005, Rezusta et al. 2007). Routine isolation media that are not supplemented with a source of cholesterol will not support the growth of these strains, which appear to have arisen from lipid-enriched therapies associated with the antifungal treatment. Apparently, these isolates are able to scavenge and use exogenous cholesterol in place of ergosterol for their plasma membrane structure. Because the mode of action of certain antifungals used to treat *C. glabrata* infections is related to the production of ergosterol (e.g., terbinafine, fluconazole, etc.) or its presence in the plasma membrane (e.g., amphotericin B) (see selected chapters within Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005), the antifungal drug may have facilitated the selection of cholesterol-dependent strains following their acquisition of spontaneous mutations in ergosterol biosynthesis (Bard et al. 2005). Recently, an amphotericin B-resistant, cholesterol-dependent isolate of *C. glabrata* has been recovered (Rezusta et al. 2007). Isolating such strains might have been predicted based upon the mechanism of action of amphotericin B. Moreover, this observation further suggests that cholesterol-dependent isolates of *C. glabrata* that are simultaneously resistant to certain azole drugs might be encountered in the near future.

p0095 The taxonomy of this species had been one of controversy for a number of years. The primary argument focused on the inability of *C. glabrata*, previously designated as *Torulopsis glabrata*, to form pseudohyphae as opposed to *Candida* spp. that are characteristically noted to possess this property. However, the use of pseudohyphal formation as a diagnostic tool was demonstrated to be unreliable (Odds et al. 1997). Both molecular and phenotypic observations clearly support placing this species within the genus *Candida* (Fidel et al. 1999). In addition, laboratory investigations indicate that *C. glabrata* undergoes a mating process, similar to that recently discovered in *C. albicans*, and apparently analogous to that of *S. cerevisiae* (Brockert et al. 2003, Dodgson et al. 2005, Soll 2006, Srikantha et al. 2003). Recently, two additional species, *C. nivariensis*

and *C. bracarensis*, have been isolated from clinical specimens and these species are closely related to *C. glabrata*. However, analyses of 26S rRNA gene sequences support the differentiation of these two organisms as separate species (Alcoba-Florez et al. 2005, Correia et al. 2006, Wahyuningsih et al. 2008).

s0040 2.1.4. Candida Guilliermondii

p0100 This yeast species [teleomorph *Meyerozyma* (*Pichia*) *guilliermondii*] has been isolated from a wide number of environmental sources, e.g., fresh and salt water, soil, sand, amphibians, birds, and humans. It is also a noted source of nosocomial infections. Overall, the incidence of infections due to *C. guilliermondii* is low, but cases of candidemia, endocarditis, and invasive disease have been recorded (Girmenia et al. 2006).

p0105 Morphologically, pseudohyphal formation varies in abundance from strain to strain. True hyphae, however, are not produced by this species. The blastoconidia of *C. guilliermondii* may be found in short chains or clusters. Colonies of *C. guilliermondii* appear pink to lavender on CHROMagar *Candida*.

s0045 2.1.5. Candida Krusei

p0110 This species (teleomorph *Pichia kudriavzevii*) is the fifth most common cause of candidemia, but probably is most noteworthy for its innate resistance to the antifungal agent fluconazole in addition to somewhat reduced susceptibility to other drugs (Pelletier et al. 2005). Most commonly isolated from neutropenic patients, *C. krusei* has sometimes been inadvertently selected as a pathogen in some patients receiving prophylactic fluconazole therapy. This yeast, which is commonly recovered from various environmental sources, is a significant etiological agent of vaginitis although it is not typically recovered from mucosal surfaces of healthy persons.

p0115 The blastoconidia of *C. krusei* are typically elongate reaching up to 25 μm in length. These cells often take on a "match-stick" like appearance. In stationary liquid cultures, *C. krusei* forms a pellicle on the surface of the medium and on agar media the colonies often appear wrinkled and flat. Physiologically, *C. krusei* can grow on vitamin-free media and differs from other *Candida* spp. in a number of properties. Colonies of *C. krusei* appear pink and have a rough texture on CHROMagar *Candida*.

s0050 2.1.6. Candida Lusitaniae

p0120 Studies have shown *C. lusitaniae* (teleomorph *Clavispora lusitaniae*) to be part of the normal mycobiota of animals, though its prevalence among isolates from clinical samples is low. In health care settings, the possible transmission of this yeast from hospital personnel can lead to nosocomial colonization of the digestive and urinary systems. However, the medical importance of *C. lusitaniae* resides in the intrinsic resistance of some strains to the polyene antifungal agent, amphotericin B (Hawkins and Baddour 2003). Acquired resistance by *C. lusitaniae* to this drug has also been noted. Serious infections by *C. lusitaniae* typically involve patients with hematological malignancies as well as other types of individuals being treated in intensive care units.

p0125 Strains of *C. lusitaniae* produce pseudohyphae upon which chains of blastoconidia develop. Colonies of this species appear pink to lavender on CHROMagar *Candida* and some produce a waxy texture on this medium.

s0055 2.1.7. Candida Parapsilosis

p0130 This yeast is one of the most common causes of candidemia, especially in neonatal intensive care units (Bendel 2003, Chapman 2003). Patients with intravenous catheters and prosthetic devices are frequently at risk of infection by *C. parapsilosis*. This fungus produces an adhesive slime layer that enables the transmission to patients from environmental sources and hospital personnel.

p0135 In culture, *C. parapsilosis* produces long, branching pseudohyphae that present a "pine forest" appearance. However, the degree of pseudohyphal formation varies among strains. On CHROMagar *Candida*, colonies of this species appear ivory to pink to lavender and some are wrinkled.

p0140 To date, no teleomorph for *C. parapsilosis* has been documented. Within this species, though, a variety of previous typing studies was able to discern three different groups of isolates, designated I, II, and III. Using a multilocus typing scheme, however, investigators established two new species, *C. orthopsilosis* and *C. metapsilosis*, to replace the existing designations of *C. parapsilosis* groups II and III, respectively (Tavanti et al. 2005).

2.1.8. Candida Tropicalis s0060

p0145 As a frequent isolate from blood cultures, *C. tropicalis* mainly afflicts individuals suffering from leukemia, prolonged neutropenia, or extended hospitalization in intensive care units. This yeast is also found to be a frequent isolate from the oral cavities of asymptomatic persons. Like many other cases of candidiasis, infections due to *C. tropicalis* can be endogenous, i.e., from within the normal mycobiota of the patient, or transmitted from hospital personnel. On CHROMagar *Candida*, steel blue to dark gray colonies are formed by *C. tropicalis* that also often exhibit a brown to purple halo. No documented teleomorph has been observed for *C. tropicalis*.

2.2. *Saccharomyces* s0065

p0150 This genus is comprised of eight species. One, *S. cerevisiae*, has been documented to cause human infection (Enache-Angoulvant and Hennequin 2005, Munoz et al. 2005). Strains of *S. cerevisiae* appear to be more pathogenic, especially when used as a probiotic preparation in immune compromised patients. However, with appropriate treatment, such individuals tend to have a better prognosis than persons infected by other routes.

p0155 Commonly known as baker's or brewer's yeast, *S. cerevisiae* may colonize the mucosal surfaces of persons with underlying illness. Such infections tend to be superficial (e.g., thrush, esophagitis, vaginitis). Conceivably, cases of vaginitis caused by *S. cerevisiae* may be mistakenly attributed to *C. albicans* due to their similar symptoms. This may result in empirical treatment without performing a culture. In addition, serious invasive infections and fungemia due to *S. cerevisiae* have been recorded. Typically, such patients possess profoundly compromised immunity and infections are often associated with risk factors such as surgery, burns, malignancies, central catheters, hyperalimentation, and broad-spectrum antibiotic use. Because *S. cerevisiae* is a common colonizing fungus, histopathological examination of tissues is necessary to diagnose and confirm infection.

p0160 Most individuals tend to think of *S. cerevisiae* as being solely monomorphic. However, it does form pseudohyphae and chains of budding yeasts under the appropriate conditions. For example, nitrogen-poor media induces pseudohyphal growth in *S. cerevisiae* (Gagiano et al. 2002). This alternate growth form of *S. cerevisiae* is the result of a well-developed nutritional sensing mechanism that impacts cellular morphogenic programs (Gimeno et al. 1992).

2.3. *Pichia* and Derived Genera s0070

p0165 Many medical mycology texts and journal publications cite two species formerly classified in the genera *Hansenula (H.)* and *Pichia* as

causes of human infection. One, *Wickerhamomyces anomala (H. anomala)* is encountered more than the second, *Ogataea polymorpha (H. polymorpha*). The following brief description will employ the current binomials *W. anomala* and *O. polymorpha*, respectively.

p0170 The incidence of mycoses due to *W. anomala* and *O. polymorpha* has been relatively small. Documented infections include pediatric pneumonia, endocarditis, urinary tract infection, fungemia, and a "thrush-like" condition. One case of invasive disease was associated with chronic granulomatous disease in a child. In all cases, the predisposing factors to infection appear to coincide with those most often associated with encouraging colonization by opportunistic fungi. Clinical distinction between disease caused by *W. anomala* and *O. polymorpha* is not well established. Isolates of these two species are distinguished from similar yeasts by their sugar fermentation patterns.

s0075 *2.3.1. Magnusiomyces (Dipodascus) Capitatus (Blastoschizomyces Capitatus=Geotrichum Capitatum)*

p0175 *Dipodascus capitatus* is the teleomorph of the fungus commonly known as *Blastoschizomyces capitatus* and occasionally as *Geotrichum capitatum*. This species has been routinely isolated from the environment, particularly woody areas and from poultry feces. However, an environmental source is often not associated with infections. Infections can involve a single organ or multiple organs. Fungemia is common. Disseminated disease is similar in pathology to that evoked by infections due to *Candida* species. Most disease occurs in individuals with hemotologic abnormalities including leukemia and neutropenia (Anaissie et al. 2003, Christakis et al. 2005, Dismukes et al. 2003, Gadea et al. 2004, Levy et al. 2006, Martino et al. 2004, Merz and Hay 2005, Pimentel et al. 2005). Cultures of *D. capitatus* grow as hyaline mycelia that are septate and produce arthroconidia.

s0080 3. BASIDIOMYCETOUS YEASTS OF CLINICAL SIGNIFICANCE

p0180 The following section provides a relatively brief review of those basidiomycetous yeasts that have been isolated from diseased individuals. The major pathogenic genera, *Cryptococcus* and *Malassezia*, afflict significantly more persons on a regular basis that those yeasts belonging to the genera *Sporobolomyces, Rhodotorula, Trichosporon*, and *Ustilago*. Nonetheless, the latter are included to provide a broad view of the spectrum of infectious fungi. For in-depth descriptions beyond that presented below, the reader is referred to several clinical mycology texts (Anaissie et al. 2003, Dismukes et al. 2003, Kauffmann 2006, Merz and Hay 2005).

s0085 3.1. *Cryptococcus*

p0185 The genus *Cryptococcus* is comprised of at least 70 species that have been isolated from various habitats and animals on every continent. Though the species *Cryptococcus (Cr.) laurentii, Cr. curvatus,* and *Cr. albidus* have caused occasional infections, termed cryptococcosis, only two species, *Cr. neoformans* and *Cr. gattii*, have been routinely documented as pathogenic for humans. The biology and clinical significance of these two species have been recently reviewed (Campbell and Carter 2006, Chayakulkeeree and Perfect 2006, Lin and Heitman 2006, Perfect 2006).

p0190 Cryptococcosis is caused by basidiomycetous yeasts within the "*Cr. neoformans* species complex" (Lin and Heitman 2006). Phenetic, biologic, and phylogenetic analyses suggest that this complex is comprised of two distinct species, *Cr. neoformans* and *Cr. gattii* (Kwon-Chung et al. 2002, Kwon-Chung and Varma 2006). Both are encapsulated yeasts, but *Cr. gattii* colonies on agar media tend to be more mucoid. Also, subtle morphological differences exist between the yeast cells of *Cr. neoformans* and *Cr. gattii*. For example, *Cr. neoformans* produces colonies of ovoid to spherical cells. In contrast, *Cr. gattii* yeasts tend to be more ellipsoid in appearance. Also, these two species can be differentiated via their biochemical differences. Most notable is that *Cr. gattii* reacts positively on CGB agar, whereas *Cr. neoformans* does not. Furthermore, *Cr. neoformans* appears to consist of two varietal states (*var. neoformans* and *var. grubii*), whereas *Cr. gattii* seems to be a distinct species possessing pronounced intraspecific genetic diversity. Not all taxonomists agree with this position, however. Different experts have championed the establishment of up to eight distinct species within the *Cr. neoformans* species complex (Coenjaerts 2006, Lin and Heitman 2006). Much of this debate has been prompted by advances in the development and application of molecular epidemiological methods as well as the phylogenetic and genotypic analyses of various clinical, environmental, and hybrid isolates (Boekhout et al. 1997, 2001, 2007, Bovers et al. 2006, 2007, Campbell et al. 2005, Diaz et al. 2000, Escandon et al. 2006, Kidd et al. 2004, Meyer et al. 2003, Meyer et al. 1999, Trilles et al. 2003). The collective data from these various studies show that the *Cr. neoformans* species complex is highly divergent at the genomic level and comprises at least nine molecular types. Taken alone, the genotypic variation supports the contention that the complex is in the process of evolving new species. However, what is not clear is at which point does this genotypic variation define the emergence of separate species, particularly if phenetic and biologic information is not considered. Hence, based upon a species concept involving phenetic and biologic evidence, in addition to cladistic data, Kwon-Chung and Varma (2006) argued for two distinct species within the *Cr. neoformans* species complex. The following is a brief summary of this argument.

p0195 Prior to 1950, cryptococcosis was considered to be a mycosis caused by the single, homogeneous species, *Cr. neoformans*. However, investigations of the polysaccharide capsule of this species revealed the existence of four capsular epitopes which were designated A, B, C, and D. Apparent hybrid strains possessing serotype AD were also noted. Subsequently, in 1975, heterothallism in *Cr. neoformans* was demonstrated in laboratory experiments (Kwon-Chung 1975, 1976). To date, the teleomorph of *Cr. neoformans* has not been observed in nature. Laboratory crosses of appropriate mating type strains produce dikaryotic hyphae that form true clamp connections characteristic of basidiomycetous fungi. Some hyphal apices differentiate into basidia wherein meiosis occurs followed by the formation of uninucleate basidiospores. From matings attempted with the various different serotypes, two different biological species were defined. Both can be distinguished on the basis of basidiospore morphology. One species, designated *Filobasidiella neoformans*, produced spherical basidiospores. This teleomorph was initially observed between compatible serotype D strains and later demonstrated between appropriate strains bearing serotypes A and D. Correspondingly, the anamorphic strains comprising these serotypes were designated *Cr. neoformans*. In contrast, mating of suitable strains exhibiting serotypes B and C produced meiospores that are ovoid and bacilliform. Therefore, based upon the morphological differences, the teleomorph resulting from the serotype B and C crossings was defined as a second species, *Filobasidiella bacillispora*. Concomitantly, the anamorphic strains of the B and C serotypes were given a new epithet, *Cr. bacillisporus*, which was later changed to *Cr. gattii* (Kwon-Chung et al. 2002). Subsequent biochemical analyses, e.g., glycine metabolism, canavanine resistance, etc., (Kwon-Chung et al. 1982, Min and Kwon-Chung 1986, Polacheck and Kwon-Chung 1980, 1986),

further supported the phenetic distinction of *Cr. neoformans* and *Cr. gattii* as separate species. Moreover, with the development of molecular methodologies, phylogenetic analysis using nucleotide sequences from ribosomal RNA genes, various housekeeping genes, and virulence-associated genes demonstrated the genetic relatedness of all serotypes (Butler and Poulter 2005, Diaz et al. 2005, Diaz et al. 2000, Diaz and Fell 2005, Fan et al. 1995, Fell et al. 2000, Litvintseva et al. 2003, Xu et al. 2000). That is, serotypes A, D, and AD tended to cluster together as did serotypes B and C. Such observations are consistent with the phenetic and biological analyses that demonstrate the existence of two species.

p0200 With regard to pathogenesis, *Cr. neoformans* and *Cr. gattii* tend to afflict different types of individuals. Infections by *Cr. neoformans* are markedly more frequent in immunocompromised individuals, such as HIV-infected patients, with *var. grubii* (= serotype A) being the major etiological agent. In contrast, *Cr. gattii* appears to more commonly cause disease in persons with competent immune systems. Moreover, these species appear to differ in their ecological niches. Whereas *Cr. neoformans* appears to be distributed worldwide, *Cr. gattii* tends to be found mainly in tropical and subtropical regions. However, a recent and ongoing outbreak of *Cr. gattii* infections in Vancouver, Canada and the Northwestern region of the U.S. suggests that this species has disseminated beyond those artificial boundaries (Hoang et al. 2004, Kidd et al. 2004, 2007a, 2007b, Lindberg et al. 2007, MacDougall and Fyfe 2006, MacDougall et al. 2007, Upton et al. 2007).

p0205 Cryptococcosis can present a number of clinical manifestations (Chayakulkeeree and Perfect 2006). Infection presumably begins with the inhalation of the fungus via the aerosolization of particles (e.g., basidiospores) from bird dung (Sukroongreung et al. 1998). Such infections may cause asymptomatic lung colonization or a range of symptomatic pulmonary afflictions up to and including fulminant disease resulting in respiratory failure. In immune competent individuals, a coordinated cellular immune response eliminates the fungus or induces a quiescent state within pulmonary foci and lymph nodes. Subsequent debilitation of the immune system may permit such latent organisms to spread to other body locations. In immune compromised persons, however, colonization of the airways by *Cr. neoformans* typically leads to dissemination from the lungs to the central nervous system causing subacute or chronic meningitis. In a significant number of infected individuals, especially HIV-infected patients, skin manifestations occur. Less common are infections of the bone and other organs.

p0210 Various attributes of *Cr. neoformans* and *Cr. gattii* contribute to their virulence, including the presence of a polysaccharide capsule, the ability to synthesize melanin, thermotolerance, and the secretion of various metabolites (e.g., mannitol) and enzymes (e.g., proteases). In addition, host response via cellular immunity plays a crucial role in determining if infection is limited to transient colonization of the airways or subsequent establishment of diverse clinical manifestations. Also, the capsule serves as a diagnostic feature that is readily visible when specimens are stained with India ink. The presence of encapsulated yeasts from normally sterile body locations (e.g., spinal fluid) is a presumptive identifying characteristic for cryptococcosis.

p0215 Determining the ecological niches of *Cr. neoformans* and *Cr. gattii* may help to identify crucial factors involved in virulence as well as in the dissemination of cryptococcosis. The source of *Cr. neoformans* in the environment has often been associated with pigeon droppings and infected soils. However, guano from other types of birds (e.g., chickens, parrots, turkeys, and canaries), and the soils contaminated by it, has also yielded *Cr. neoformans* upon culture. Both *var. neoformans* and *var. grubii* have been isolated from these sources worldwide. Prior speculation had suggested that bird (pigeon) guano actually selected for the growth *of Cr. neoformans var. neoformans* and *var. grubii* since *Cr. gattii* has never been cultured from this source. Nonetheless, it was recently demonstrated that media prepared from pigeon guano does support the growth of both *Cr. neoformans* and *Cr. gattii*, but only *Cr. neoformans* exhibits robust sexual reproduction and basidiospore formation under the same culture conditions (Nielsen et al. 2007). The results of these investigations help explain the cosmopolitan distribution of cryptococcosis due to *Cr. neoformans*, given pigeon migratory patterns and evidence that infection begins via the inhalation of basidiospores. In contrast, *Cr. gattii* is most often found in a unique and restricted habitat. This species has been regularly isolated from gum trees (*Eucalyptus spp.*) located in numerous tropical and subtropical areas across the world. Correspondingly, a significant number of cryptococcosis cases in areas harboring these trees have been due to *Cr. gattii* strains. Other trees in tropical areas may also be niches for this species. Hence, the localized nature of the ecological niche provided by eucalypts and other types of vegetation, which grow mainly in tropical and subtropical regions, helps to clarify the restricted endemicity of infections due to *Cr. gattii*. Curiously, however, the ongoing outbreak of cryptococcosis in the temperate zones of Vancouver, Canada and the Northwestern U.S. appears to be caused by a genetic subset of *Cr. gattii* strains (Kidd et al. 2004, 2007b, MacDougall et al. 2007). These strains appear to have developed the ability to grow in soil and thereby have expanded or altered the ecological niche in which they exist. Subsequently, dispersal of the organism was shown to be anthropogenic (via footwear and the wheels of vehicles) (Kidd et al. 2007a) suggesting that *Cr. gattii* infections will continue to reach beyond this geographical area.

3.2. *Malassezia* s0090

Malassezia spp. are considered part of the normal skin mycobiota of humans and animals (Ashbee 2006, Batra et al. 2005, Chen and Hill 2005, Crespo-Erchiga and Florencio 2006). They have, however, garnered a great deal of attention over the years mainly for their association with various dermatological afflictions. Among these conditions are pityriasis versicolor, seborrhoeic dermatitis, atopic eczema/dermatitis syndrome, psoriasis, dandruff, folliculitis and otitis. However, members of this genus have also been associated with invasive disease. The following discussion briefly highlights some of the salient features of this fungus. p0220

The taxonomy of the genus *Malassezia* has been, and continues to be, an area of intense investigation. No teleomorph is known for this genus. However, the basidiomycetous affinity of the genus *Malassezia* has been demonstrated phylogenetically using ribosomal RNA gene sequence analysis (Begerow et al. 2000, Fell et al. 2000). Collectively, the species used in these studies formed a separate clade (Malasseziales) within the class Ustilaginomycetes. Presently, physiologic and molecular methods have established the 13 species listed in the following studies (Ashbee 2006, Batra et al. 2005, Cabanes et al. 2007). All species of *Malassezia*, except *M. pachydermatis*, require an exogenous source of lipids for growth. The particular lipid requirements appear to vary among species. However, all grow as yeasts that bud in a repetitive, unipolar fashion. The buds are generated enteroblastically, usually forming a wide base between parent cell and buds. *Malassezia* species can also produce a mycelial form. p0225

Malassezia species are known to produce a diverse range of metabolites, including lactones that give rise to the fruity smell when the fungus is cultured (Ashbee 2006, Labows et al. 1979). These organisms also synthesize compounds (e.g., pityriarubins) that interfere with the respiratory burst of host immune cells (Kramer et al. 2005a). In addition, *Malassezia* species generate azelaic acid p0230

that inhibits reactive oxygen species (Akamatsu et al. 1991). These latter two compounds may be factors that contribute to the survival of the fungus within the harsh environment of the host skin. Moreover, melanin production by *Malassezia* species has been documented *in vivo* (Gaitanis et al. 2005). Melanin is a known fungal virulence factor that enhances survival of the invading organism on or within the host (Nosanchuk and Casadevall 2003). Hence, melanin formation may be another attribute that contributes to the ability of *Malassezia* species to colonize and survive on the skin.

p0235 Although considered to be generally benign members of the normal skin mycobiota, *Malassezia* spp. can pose clinical problems (Ashbee 2006, Batra et al. 2005). Under certain conditions, they initiate a superficial skin infection that, due to their lipid requirements, most often occur in the sebum-rich areas of the body, i.e., face, forehead, scalp, back and trunk. The major clinical conditions arising from *Malassezia* infections include pityriasis versicolor, seborrhoeic dermatitis, and dandruff. Additional types of superficial pathologies attributed to *Malassezia* include atopic eczema/dermatitis syndrome, otitis, folliculitis, and psoriasis among others. Traditionally, diagnosis of infection was based upon histopathological examination as well as biochemical and phenotypic characterization of clinical isolates. However, the advent of molecular biology has prompted the development of a number of nucleic acid-based methods for the diagnosis of *Malassezia* infection as well as the identification of the specific species involved, including techniques that are culture independent (Boekhout et al. 1998, Cafarchia et al. 2007, Diaz et al. 2006, Gaitanis et al. 2002, Gemmer et al. 2002, Guillot et al. 2000, Guillot and Guého 1995, Morishita et al. 2006, Sugita et al. 2001, Takahata et al. 2007, Theelen et al. 2001).

p0240 Perhaps one of the more notable clinical conditions caused by *Malassezia* species is pityriasis versicolor. This dermatologic manifestation is a chronic superficial infection characterized by round to oval lesions on the arms, trunk, and back. The lesions can be hypo- or hyperpigmented. Presumably, pityriasis versicolor occurs when the *Malassezia* species colonizing the skin converts to a mycelial form that subsequently invades the stratum corneum. Hypopigmentation of the lesions may result from the destruction of skin melanocytes caused by the release of malassezin by the invading fungus (Kramer et al. 2005b). Histopathological examination will confirm infection by noting the presence of characteristic yeast and mycelial forms in what is commonly referred to as "spaghetti and meatballs." Studies have suggested that *M. globosa* is the major pathogen causing this condition (Crespo Erchiga et al. 2000, Crespo-Erchiga and Florencio 2006), *although M. sympodialis* may also be a significant causative agent of this condition (Gupta et al. 2001, 2004). Two other conditions noted above, seborrhoeic dermatitis and dandruff, are frequently associated with *Malassezia* spp. It has been suggested that seborrhoeic dermatitis, and likely dandruff as well, are caused by an abnormal host response to the yeasts on the skin rather than overgrowth of the pathogen. The major species involved in these conditions appear to be *M. restricta* and *M. globosa*. Similarly, atopic eczema/dermatitis syndrome appears to result from the chronic inflammation of the skin due to various allergens released by *Malassezia*, perhaps in conjunction with other microbiota.

p0245 Finally, *Malassezia* spp. have been isolated as agents of deep-seated and systemic infections including abscesses, mastitis, and peritonitis. Usually, solid organ involvement does not occur. However, the most commonly reported systemic infection is fungemia, particularly in those patients receiving lipid infusions via a catheter. These cases have most often occurred in neonatal units in hospitals, but adult infections have been recorded (Ashbee 2006, Batra et al. 2005, Cannizzo et al. 2007, Chryssanthou et al. 2001, Curvale-Fauchet et al. 2004, Devlin 2006, Giusiano et al. 2006, Rosales et al. 2004). Left untreated, the fungus can disseminate to the lungs and brain. If the infection appears catheter related, simply removing this line helps alleviate the condition although antifungal treatment should still be considered in certain situations.

3.3. Less Common Basidiomycetous Yeast Pathogens s0095

p0250 A number of other yeasts with basidiomycetous affinities have been observed to cause mycoses. These include *Trichosporon* spp., *Rhodotorula* spp., and *Sporobolomyces* spp. In addition, two cases of infection due the corn smut fungus, *Ustilago*, have been recorded (Patel et al. 1995, Teo and Tay 2006). Collectively, these fungi do not cause large numbers of infections, yet they are often difficult to treat due to the condition of the patient or the low susceptibility of the etiological agent to antifungal regimens. A brief review of these fungi is provided below. For further details, the reader is referred to various clinical mycology texts and reviews (Anaissie et al. 2003, Boekhout and Guého 2003, Bouza and Munoz 2004, Dismukes et al. 2003, Girmenia et al. 2005, Groll and Walsh 2001, Kauffmann 2006, Kiken et al. 2006, Martino et al. 2004, Merz and Hay 2005).

p0255 Infections caused by members of the genus *Trichosporon* can be either superficial or deep. There are a variety of species within this genus, but the most well known pathogens are *T. cutaneum* and *T. asahii*. The superficial colonization of hair shafts, termed "white piedra", is caused by several species of *Trichosporon*, but is primarily due to *T. cutaneum* (Anaissie et al. 2003, Dismukes et al. 2003, Kiken et al. 2006, Merz and Hay 2005). Deep-seated infections caused by *Trichosporon* are being reported with increasing frequency (Chowdhary et al. 2004, Girmenia et al. 2005, Rodrigues Gda et al. 2006, Tokimatsu and Kadota 2006). The most common etiological agent is *T. asahii*. Most afflicted patients possess predisposing conditions such as catheterization, steroid use, immunosuppressive therapy, chemotherapy, granulocytopenia, surgical procedures, continuous ambulatory peritoneal dialysis, and HIV infection. Hypersensitivity pneumonitis due to *T. asahii* and *T. mucoides* has also been recorded (Ono et al. 2007, Sugiyama et al. 2005, Tokimatsu and Kadota 2006). The genus is characterized by septate hyphae that produce abundant arthroconidia. Budding cells are also produced but are less common.

p0260 The genus *Sporobolomyces* encompasses several species of yeast-like fungi commonly found in various environments. In culture, colonies are pink-orange in color much like *Rhodotorula rubra*. However, *Sporobolomyces* often can be discerned from other yeasts by formation of reproductive ballistoconidia. To date, only seven cases of disease have been recorded due to *Sporobolomyces* spp. (Anaissie et al. 2003, Bergman and Kauffman 1984, Dismukes et al. 2003, Merz and Hay 2005, Morris et al. 1991, Morrow 1994, Plazas et al. 1994, Sharma et al. 2006). These cases included dermatitis, formation of nasal polyps, skin blisters, eumycetoma, and endophthalmitis. Two others involved disseminated disease in two HIV-infected patients indicating the potential of this genus to produce invasive infection in immune compromised hosts.

p0265 Finally, *Rhodotorula* is another common environmental yeast that has been documented to cause infection (Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005). However, like Sporobolomyces, the number of cases is small. The most common condition is fungemia, but other conditions have been described including peritonitis, meningitis, endocarditis, and eye infections. A number of cases have occurred in HIV-infected patients (Kaur et al. 2007, Merkur and Hodge 2002). *Rhodotorula* grows as orange-pink colonies in culture and characteristically does not produce hyphae. Among the various species, some represent the anamorph of the teleomorphic genus *Rhodosporidium*.

4. MEDICALLY-IMPORTANT DIMORPHIC FUNGI

The dimorphic fungi that cause disease in humans typically exist as saprotrophic molds in nature (Anaissie et al. 2003, Dismukes et al. 2003, Kauffmann 2006, Merz and Hay 2005) (Table 2.2). Upon tissue invasion, many, but not all, undergo a morphological transition to a yeast form. To the non-medical mycologist, this can lead to confusion particularly when culture of clinical specimens results in growth of a mold form.

Another curious feature common to many of the clinically significant dimorphic fungi is their endemic nature. As described below, most of the infections caused by a particular species are geographically restricted. Nonetheless, diseases by these fungi are regularly diagnosed outside their endemic area. In some cases, infections are diagnosed in indigenous people from the region who have traveled outside its boundaries, whereas others have developed in non-native persons subsequent to visiting a particular endemic area.

The descriptions of the dimorphic fungi presented below mainly focus on their anamorphic state. In some cases, the teleomorphs of particular fungi have been demonstrated and each falls within the Ascomycota. For the remaining dimorphic fungi described below, despite the absence of a teleomorph, genetic and morphological evidence strongly suggests that all possess an ascomycetous nature.

4.1. *Histoplasma Capsulatum*

The most common endemic disease in the United States is histoplasmosis caused by the fungus *Histoplasma (Histo.) capsulatum var. capsulatum*. A second variety, *Histo. capsulatum var. duboisii*, is restricted to portions of the African continent. A third variety, *Histo. capsulatum var. farciminosum*, is a pathogen of mules and horses in parts of Asia and Africa. Although the latter variety is also thermally dimorphic, it will not be included in the present discussion. For more detailed information regarding histoplasmosis and the biology of Histoplasma, including virulence attributes, the reader is referred to recent reviews (Couppie et al. 2006, Wheat 2006, Woods 2006).

Briefly, *Histo. capsulatum var. capsulatum* mainly resides in the Mississippi and Ohio River valleys of the United States and in portions of Central and South America, *whereas Histo. capsulatum var. duboisii* is most often found between the Tropic of Cancer and the Tropic of Capricorn in Africa. Sources of exposure for both varieties include caves, decaying and rotting organic matter, and bird roosts or chicken coops. Moreover, the pathologies of these two etiologic agents are distinct. For further details, see Couppie et al. (2006), Wheat (2006), and Woods (2006). Morphologically, both varieties are indistinguishable in their mold phase. Yet the yeast forms *of Histo. capsulatum var. capsulatum* and *Histo. capsulatum var. duboisii* are different. The former appears as very tiny budding yeasts (2–4 μm) and are typically found residing within macrophages in vivo. By comparison, the budding yeast form of *Histo. capsulatum var. duboisii* is significantly larger (8–15 μm), having thick walls and a prominent bud scar. In addition, this latter variety often appears as short chains of yeasts within infected tissue. The ascosporic state of *Histo. capsulatum var. capsulatum* is *Ajellomyces capsulatus* (Class Eurotiomycetes, Order Onygenales, Family Ajellomycetaceae). Interestingly, although an ascosporic state for *var. duboisii* has not been observed, strains of this variety will mate with the *capsulatum* variety and form cleistothecia that are indistinguishable from those produced by *A. capsulatus*. The ascospores in this cross-variety mating do not germinate in vitro, but they will grow and cause disease in mice.

4.2. *Blastomyces Dermatitidis*

Blastomyces dermatitidis is the causative agent of the fungal disease termed blastomycosis (Bradsher et al. 2003, Bromel and Sykes 2005, Kauffman 2006). This mycosis is endemic to the eastern half of North America, thus earning it the ethnocentric name of North American blastomycosis. However, the disease has been documented in the continent of Africa as well as portions of central India.

Infection by *B. dermatitidis* is initiated via the inhalation of conidia. If suspected, diagnosis of blastomycosis can be readily made due to the relative ease of culture. The mold phase that grows at 25°C readily produces macroconidia, but these are quite similar to those formed by many other types of fungi. Instead, observation of the yeast phase of *B. dermatitidis*, either in vitro or in vivo, is a key diagnostic feature. Microscopically, yeast cells of this fungus are large (8–12 μm in diameter), multinucleate, and have a thick wall, often described as a "double wall". The most prominent attribute of the yeast form is that budding cells exhibit a wide base of attachment between a cell and its bud, i.e., broad-based budding. The ascosporic state of *B. dermatitidis* is *Ajellomyces dermatitidis* (Class Eurotiomycetes, Order Onygenales, Family Ajellomycetaceae).

4.3. *Paracoccidioides Brasiliensis*

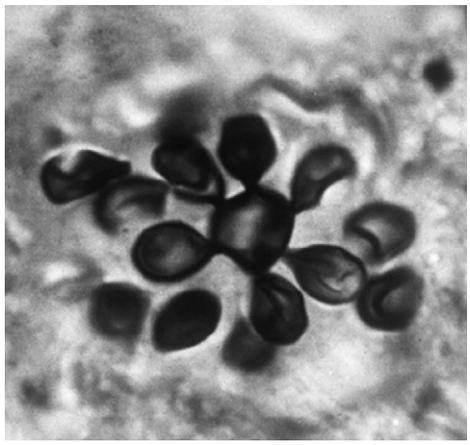
Paracoccidioidomycosis is the clinical pathology caused by the dimorphic fungus, *Paracoccidioides (Para.) brasiliensis* (San-Blas et al. 2002, Visbal et al. 2005). This fungus has exhibited no known teleomorph. However, based upon phylogenetic comparisons of rRNA gene sequences, *Para. brasiliensis* belongs to the order Onygenales, family Onygenaceae. The geographic distribution of *Para. brasiliensis* ranges from Mexico to South America, but it is more prevalent mainly on the southern continent rather than Central America.

Like other endemic fungi, infection begins through breathing in an infectious propagule that may or may not result in a symptomatic

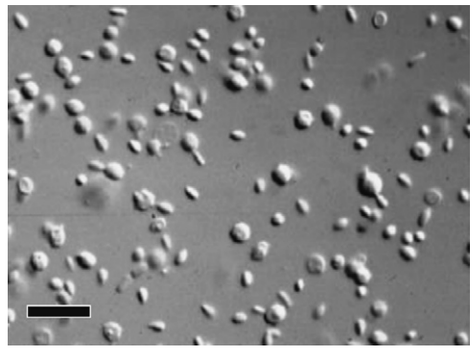
TABLE 2.2 Selected Dimorphic Fungi Pathogenic for Humans and Animals

Anamorph	Known Teleomorph	In Vivo Morphology
Histoplasma capsulatum var. *capsulatum*	*Ajellomyces capsulatus*	Small (2–4 μm) ovoid, budding yeasts
var. *duboisii*	*Ajellomyces capsulatus*[a]	Larger (8–15 μm), thick-walled budding yeast with a prominent bud/birth scar
Blastomyces dermatitidis	*Ajellomyces dermatitidis*	Large (8–15 μm), broad-based budding yeasts
Paracoccidioides brasiliensis	None	Multiply budding yeasts("Pilot's Wheel", 15–30 μm)
Sporothrix schenckii	None	Round to ovoid yeasts(4–6 μm, cigar-shaped)
Penicillium marneffei	None	Small (3–5 μm), globose to elongated fission yeast

[a]*Variety* **duboisii** *will mate with an appropriate strain from var.* **capsulatum** *and form a teleomorph indistinguishable from* **A. capsulatus**



f0010 **FIGURE 2.1** Multiple budding yeast form of *Paracoccidioides brasiliensis* in tissue. Gomori Methenamine Silver stain. Bar equals 10 μm. Figure adapted from image provided through the courtesy of www.doctorfungus.org © 2007.



f0015 **FIGURE 2.2** Budding yeast cells of *Sporothrix schenckii* grown in a 37°C broth culture. Differential interference contrast optics. Bar equals 12 μm. Figure adapted from image provided through the courtesy of www.doctorfungus.org © 2007.

response. Once established in the lungs, however, *Para. brasiliensis* undergoes a morphological transformation that gives rise to the yeast phase that is decidedly characteristic for the mycosis caused by this fungus. At 37°C or *in vivo*, spherical yeast cells develop over a wide range of sizes (3–30 μm in diameter). From attachment points all along the surface of a central yeast cell, lemon-shaped buds (2–10 μm in diameter) develop that are connected to the parent cell through a narrow-based, isthmus-like connection. In simple terms, this entity is often referred to as a "pilot's wheel" or "mariner's wheel" (Fig. 2.1).

s0120 4.4. *Sporothrix Schenckii*

p0315 The etiological agent of sporotrichosis, *Sporothrix schenckii*, causes a cosmopolitan disease that probably ranks as the world's most frequent subcutaneous mycosis (Bustamante and Campos 2001, Kauffman 2006, Pang et al. 2004). Sporotrichosis occurs in many areas of the world, but is most frequently encountered in the United States, Central and South America, Africa, and Japan. Most cases are sporadic, although rare epidemics have been well documented.

p0320 Transmission of *S. schenckii* typically occurs through traumatic implantation, such as a prick from a rose thorn or pine needle. In several documented epidemics, implantation was directly related to small skin injuries due to handling pine seedlings, shrubs, or other types of vegetation. However, several cases of sporotrichosis have been reported that resulted from non-implantation means of infection. Many of these were laboratory-acquired infections (Cooper et al. 1992). In addition, pulmonary disease has been traced to the inhalation *of S. schenckii* conidia.

p0325 Like the other dimorphic fungi described above, *S. schenckii* grows as a mold at room temperature forming unicellular, tear-shaped to clavate conidia that are darkly pigmented. When incubated at 37°C, though, this fungus undergoes mold-to-yeast conversion. The yeast phase is distinctive in that the budding yeast cells (4–6 μm in diameter) tend to exhibit a "cigar shaped" appearance (Fig. 2.2). Curiously, however, the yeast phase of this fungus is rarely seen in clinical specimens. Finally, a few sporotrichosis infections have been caused by S. *schenckii var. luriei*. This variety differs from *S. schenckii* in that the yeast form, which can exist at 25°C, is large and thick-walled.

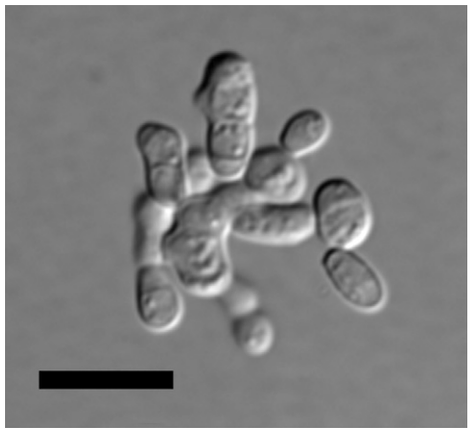
p0330 No known sexual phase has been observed for *S. schenckii* though evidence suggests it may be closely related to the genus *Ophiostoma*. In addition to *S. schenckii*, which is classified within the ascomycetous family Ophiostomataceae (Class Sordariomycetes, Order Ophiostomatales), cases of sporotrichosis have been ascribed to a far less common species, *S. cyanescens*. This species does not readily convert to a yeast phase *in vitro* and possesses septal structures consistent with basidiomycetous fungi. Recently, this species was transferred to the basidiomycetous genus *Fugomyces* (Sigler and Verweij 2003).

4.5. *Penicillium Marneffei* s0125

Infections by *Penicillium s*pp. (penicilliosis) are rare with one exception – those caused by *Penicillium (Pen.) marneffei* (Cooper 1998, Cooper and Haycocks 2000, Cooper and Vanittanakom 2008, Vanittanakom et al. 2006, Viviani and Vanittanakom 2005). The ability of this fungus to cause disease is directly associated with its formation of a yeast phase *in vivo*. This species emerged in the early 1980s as a significant pathogen of HIV-infected individuals residing or having traveled in Southeast Asia, the endemic region of disease caused by this fungus. Prior to this time, infections by *Pen. marneffei* were relatively rare. Penicilliosis due to *Pen. marneffei* typically disseminates systemically in immunocompromised patients and, left untreated, is universally fatal. p0335

Infection by *Pen. marneffei* is presumably initiated by the inhalation of conidia that are subsequently phagocytized by pulmonary histiocytes. There, the fungus grows as a very small yeast (2–3 μm × 2–7 μm) and is approximately the same size as yeast cells of *Histo. capsulatum* (Fig. 2.3). This may have been the factor by which many of the early cases were misdiagnosed as histoplasmosis. The distinguishing feature of *Pen. marneffei* yeast cells is that they divide by fission as opposed to the budding yeasts of *Histo. capsulatum*. Cultures of *Pen. marneffei* incubated at 37°C also reproduce as fission yeasts. p0340

Taxonomic studies have placed this fungus within the Phylum Ascomycota (Class Eurotiomycetes, Order Eurotiales, Family Trichocomaceae) (LoBuglio and Taylor 1995, Vanittanakom et al. 2006, Woo et al. 2003). Although no ascosporic state has been observed for *Pen. marneffei*, experimental studies have noted potential heterothallism in this fungus by documenting the existence of mating-type-like genes in its genome (Woo et al. 2006). Other studies have suggested that the profound asexual nature of this fungus has led it to develop a niche-adapted genotype, thus perhaps explaining its endemic nature (Fisher et al. 2005). p0345



f0020 **FIGURE 2.3** *Penicillium marneffei* growing as a fission yeast in a broth culture incubated at 37°C. Differential interference contrast optics. Bar equals 5 μm.

s0130 5. OTHER YEAST-LIKE MYCOTIC AGENTS

p0350 The following brief descriptions cover three distinct types of fungi that are not often given consideration as medically important yeasts. One, the dematiaceous (phaeoid) fungi, can exhibit growth as budding yeasts both in vitro and in vivo. A second, Lacazia loboi, is a noncultureable fungus that elicits keloidal lesions containing yeast cells often in chains. The remaining organism, Pneumocystis, is a major opportunistic pathogen of HIV-infected patients and for years was considered to be protozoan-like in nature, but is presently considered to belong to the Ascomycota, Taprhinomycotina, Taphrinomycetes (see Chapter 58, Pneumocystis).

s0135 5.1. Dematiaceous (Phaeoid) Fungi

p0355 The dematiaceous fungi represent a heterogeneous collection of darkly pigmented organisms. (Cooper 2005). The term "dematiaceous", though not an appropriate description based upon its Greek root, has been used for so many years that it has found nearly complete acceptance in the mycological literature. Still, a better term to describe these pigmented fungi is "phaeoid." Both terms will be used interchangeably in the following discussion.

p0360 The dematiaceous fungi exist either as monomorphic or pleomorphic organisms capable of causing a wide range of mycoses (Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005). It is the pleomorphic nature of some of these fungi that presents many challenges to the clinical mycologist. Some species can grow in a variety of forms, among which includes mold, yeast, and pseudohyphal phases. To further complicate this issue, some species growing as molds can exhibit more than one type of conidium generated by different modes of conidiogenesis. In essence, a significant number of the phaeoid fungi express multiple synanamorphs. Of the most intriguing synanamorphs are those designated "black yeasts".

p0365 The phaeoid yeasts are anamorphs of certain darkly pigmented molds. They mainly reproduce by budding. However, not all dematiaceous molds produce a yeast phase. Of those that do, most exhibit ascomycetous characteristics, but a few appear more basidiomycetous in nature. Moreover, not all black yeasts are associated with human disease. Those that do cause infection represent two distantly related phylogenetic groups within the Ascomycota (de Hoog 1993, de Hoog et al. 2003, Matos et al. 2003, Spatafora et al. 1995, Uijthof 1996). Most of the infections caused by these fungi can be grouped into the pathological condition known as phaeohyphomycosis, but they can also elicit other conditions (Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005).

p0370 Clinical specimens of black yeasts can present a challenge in their identification (Dixon and Polak-Wyss 1991). Complicating this effort is that taxonomy of the phaeoid fungi appears to be in continual flux, thereby prompting seemingly constant nomenclatural changes. For an up-to-date synopsis of current nomenclature and obsolete synonyms of medically relevant fungi, including the black yeasts, the reader is urged to access the excellent web site, Doctor Fungus (www.doctorfungus.com). Despite these difficulties, however, Sanche et al. (2003) have presented a clear description of those factors that differentiate between the two groups of black yeasts previously defined by phylogenetic methods as well as the individual species comprising these groups. The reader is referred to this reference for details.

p0375 Within the scheme of Sanche et al. (2003), Group 1 consists of *Exophiala* species and *Wangiella dermatitidis*, both of which exhibit early growth as yeasts, but frequently convert to a mycelial phase in culture. There is a great deal of controversy regarding the correct taxonomy of *Wangiella dermatitidis* and its alternate binomial *Exophiala dermatitidis*. The issue basically comes down to two different interpretations on the mode of conidiogenesis in this fungus. Perhaps future genetic examination of the genomes of these putatively different fungi will settle the matter. Regardless of specific epithet, different species of these fungi are responsible for infections resulting in various forms of phaeohyphomycosis, eumycetoma, endocarditis, and chromoblastomycosis. However, it is primarily in phaeohyphomycosis that the yeast phase of these species is noted in vivo.

p0380 Group 2 of the black yeasts encompasses *Aureobasidium pullulans, Hormonema dematoides,* and *Phaeoannellomyces werneckii (= Hortaea werneckii).* Cases of phaeohyphomycosis, peritonitis, onychomycosis, and keratitis have been attributed to *A. pullulans* and *H. dematoides*. Both of these fungi are characteristically found in soils, waters, and fruits. Again, the yeast phase is primarily observed in phaeohyphomycosis. Infection in all these pathologies usually occurs via traumatic implantation of the fungus. In contrast, *P. werneckii* is the etiological agent of a superficial type of phaeohyphomycosis designated *tinea nigra.* This pathology is characterized by darkened areas of the palm or sole of the foot, in which yeast-like cells can be observed. The condition is strictly cosmetic and infection presumably occurs via skin abrasion in the presence of a suitable environmental source of the fungus.

5.2. *Lacazia Loboi* s0140

p0385 Lobomycosis is a rare, chronic subcutaneous infection of the skin. The infection is marked by the formation of keloidal, ulcerated, or verrucose lesions that contain the etiological agent, *Lacazia loboi* (Fonseca 2007, Talhari and Pradinaud 2005). The disease is confined to parts of Central and South America and also appears to be associated with seawater off the coasts of these areas. It is here and in other offshore tropical waters that infected dolphins have been documented (Haubold et al. 2000). However, the exact source of the organism is unknown, particularly since *L. loboi* has never been cultured from nature. Some evidence exists that the organism has been cultured in experimental animals using infected tissue (Talhari and Pradinaud 2005).

p0390 Diagnosis of infection is made by the histopathological observation of *L. loboi* in tissue. This fungus is present in large numbers mainly as chains of yeast cells connected by a small isthmus. Solitary yeast cells are also present, but hyphae have never been noted.

p0395 Despite the absence of an in vitro culture system for *L. loboi*, advances have been made to phylogenetically assess the lineage of

this fungus. Using novel methods to extract DNA from infected lesions, various gene fragments were amplified, cloned, and sequenced (Haubold et al. 1998, Herr et al. 2001, Mendoza et al. 2005, Vilela et al. 2005). The resulting data clearly show that a close relationship exists between *L. loboi* and *Paracoccidioides brasiliensis*, thereby placing the former species within the ascomycetous order Onygenales.

s0145 ## 5.3. *Pneumocystis*

p0400 Species of *Pneumocystis* are known pathogens of humans and animals. However, when first isolated in 1909, *Pneumocystis* was mistakenly identified as part of the life cycle of the protozoan parasite, *Trypanosoma cruzii*. This association relegated *Pneumocystis* to the protozoan realm, and virtual obscurity, until the 1950s when it was shown to be a cause of epidemic pneumonia. This prompted renewed interest in the pathogenicity of *Pneumocystis* as well as evoking studies directed towards its proper classification. Subsequent morphological and biochemical studies of this organism yielded some clues that suggested an affinity to the fungi, but these data were far from conclusive. Then, in 1988, Edman et al. (1988) demonstrated the relationship of *Pneumocystis* to the fungi based upon comparisons of ribosomal RNA gene sequences. Thereafter, a number of phylogenetic studies have been performed which include trees based upon SSU RNA gene sequences that infer a relationship with ascomyceteous yeasts (Gargas et al. 1995). For a more in-depth historical perspective of the taxonomy and phylogeny of *Pneumocystis*, the reader is referred to an excellent review by Cushion (2005), as well as chapter 58 in this book.

p0405 A complete description of the biology of this fungus, its host range, pathogenicity, and diagnosis are beyond the scope of this work. Therefore, the reader is directed to several reviews that address these and other topics (Cushion 2005, Frenkel 1999, Hui and Kwok 2006, Morris et al. 2004, Peterson and Cushion 2005, Redhead et al. 2006, Thomas and Limper 2007, Wazir and Ansari 2004). To summarize, however, there are currently four species of *Pneumocystis* recognized. *Pneumocystis jiroveci*, previously known as *P. carinii*, is a pathogen of humans, particularly those that are immunocompromised. Such conditions include HIV infection, leukemia, renal disease, co-infection by other viruses, and carcinoma. Moreover, *P. jiroveci* has been detected as a pathogen of neonates and pregnant women. Not all infections by *P. jiroveci* result in the pneumonia typically associated with HIV-infected patients. Extrapulmonary infections do occur in a variety of organ systems, though this is rare. The remaining species of *Pneumocystis* afflict other mammals, particularly rodents. Rats are infected by *P. carinii* and *P. wakefieldiae*, whereas mice are hosts for *P. murina*. Diagnosis of infection by culture is not possible given the absence of a continuous in vitro system for *Pneumocystis*. Instead, diagnosis relies on microscopic observations of stained specimens, serological tests, and molecular methods involving DNA amplification techniques.

6. SUMMARY s0150

p0410 The medically significant yeasts comprise more genera than the traditionally held pathogens in the genera *Candida* and *Cryptococcus*. Clinically important yeast forms can also be found among the endemic dimorphic fungi, the dematiaceous molds, and even a noncultureable fungus. Collectively, these fungal pathogens exhibit either teleomorphs of the Ascomycota or Basidiomycota, or their anamorphic forms express morphological and genetic features consistent with one of these taxa. They comprise a diverse spectrum of fungi that have found the means, primarily by exploiting a host organism's weakened immune system, to become noteworthy mycotic agents. Furthermore, they portend the potential for the spectrum of fungal pathogens to become wider and deeper in the number of etiological agents derived from normally benign species. Vigilance by the general as well as the medical mycologist must be maintained so as to better understand how to address the future challenges likely to be posed to public health by fungi, particularly the yeasts.