Developments in Fungal Taxonomy

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INTRODUCTION

The last 20 years or so has seen a growing number of fungal infections coincident with a dramatic increase in the population of severely immunocompromised patients. These infections are due mainly to impairments in host defence mechanisms as a consequence of viral infections, especially the human immunodeficiency virus epidemic, hematological disorders such as different types of leukemia, organ transplants, and more intensive and aggressive medical practices. Many clinical procedures and treatments, such as surgery, the use of catheters, injections, radiation, chemotherapy, antibiotics, and steroids, are risk factors for fungal infections. However, these procedures are necessary, and so the incidence of fungal infections will increase accordingly.

Until only a few years ago, pathogenic fungi were a well-
defined group, some of which were limited to geographical regions and were well known by clinicians. However, the situation has changed considerably, and new infectious agents are continually appearing, around 20 species yearly. These new opportunistic pathogens have increased the base of knowledge of medical mycology, and unexpected changes have been seen in the pattern of fungal infections in humans. It is also possible that most of the recently reported taxa have caused infections which previously passed unnoticed due to inadequate diagnostic expertise. This situation and the rapid appearance of such a wide range of new pathogens have created a growing interest in fungal systematics. Fungal taxonomy is a dynamic, progressive discipline that consequently requires changes in nomenclature; these changes are often difficult for clinicians and clinical microbiologists to understand. Another difficulty for microbiologists inexperienced in mycology is that fungi are mostly classified on the basis of their appearance rather than on the nutritional and biochemical differences that are of such importance in bacterial classification. This implies that different concepts have to be applied in fungal taxonomy. Generally, medical mycologists are familiar with only one aspect of pathogenic fungi, i.e., the stage that develops by asexual reproduction. Usually microbiologists ignore or have sparse information about the sexual stages of these organisms. However, the sexual stages are precisely the baseline of fungal taxonomy and nomenclature. It seems evident that in the near future, modern molecular techniques will allow most of the pathogenic and opportunistic fungi to be connected to their corresponding sexual stages and integrated into a more natural taxonomic scheme. The aim of this review is to update our present understanding of the systematics of pathogenic and opportunistic fungi, emphasizing their relationships with the currently accepted taxa of the phyla Ascomycota and Basidiomycota.

THE CONCEPT OF SPECIES IN FUNGI

The basic rank in taxonomy is the species. However, exactly what different mycologists consider to be a species can vary widely, and there are different approaches for delineating them. Attempts to reach a consensus for a universal definition of species have been unsuccessful, and consequently several very different concepts have been used. However, the genetic basis for some of these concepts is largely unknown. The widespread occurrence of asexual reproduction by asexual propagules (conidia) and of hyphal anastomosing can cause confusion because a mycelium in its natural environment seems to be a single physiological and ecological unit but in reality is a genetic mosaic (74). Therefore, in mycology, the distinction between a population and an individual is not always easy, and this can create confusion in genetic studies (87).

Different concepts have been used by mycologists to define the fungal species. The morphological (phenetic or phenotypic) concept is the classic approach used by mycologists; in this approach, units are defined on the basis of morphological characteristics and ideally by the differences among them. The polythetic concept is based on a combination of characters, although each strain does not have to have the same combination. The ecological concept, which is based on adaptation to particular habitats rather than on reproductive isolation, is often used for plant-pathogenic fungi. The biological concept, which was developed before the advent of modern phylogenetic analysis, emphasizes gene exchange (i.e., sexual and parasexual reproduction) within species and the presence of barriers that prevent the cross-breeding of species (111). A species is considered to be an actual or potential interbreeding population isolated by intrinsic reproductive barriers (13). However, application of the biological-species concept to fungi is complicated by the difficulties in mating and in assessing its outcome (302). Also, whether a cross is considered fertile or sterile depends on the frame of reference. In this sense, published accounts of crosses between different species are often difficult to interpret because authors have failed to specify the type of infertility and its severity (428). The biological-species concept cannot be applied to organisms that do not undergo meiosis (484). It is applied only to sexual fungi, whereas asexual ones need only possess similar characteristics to each other. However, in asexual fungi, genetic exchange through somatic hybridization is a theoretical possibility, although it is limited by vegetative incompatibility (87). For asexual dermatophytes, the cohesion-species concept, based on a demographic exchangeability of phenotypes, has been used to explain the proliferation of disjunct phenotypes. The demographic exchangeability would be the ecological analogue of the genetic exchangeability of sexual species (523).

Two recent and important developments have greatly influenced and caused significant changes in the traditional concepts of systematics. These are the phylogenetic approaches and incorporation of molecular biological techniques, particularly the analysis of DNA nucleotide sequences, into modern systematics. Molecular techniques, which were previously used only in research laboratories, are now commonplace. These developments have provided new information that has caused the biological-species concept to come under criticism in favor of the phylogenetic-species concept. This new concept has been found particularly appropriate for fungal groups in which no sexual reproduction has been observed (deuteromycetes). Hence, new concepts, specifically formulated within the field of phylogenetics, are becoming familiar to mycologists. Population studies and molecular data are increasingly showing that many widely used morphospecies actually comprise several biological or phylogenetic species. One of the problems for morphologists involves deciding how many base differences are required for strains to be considered different species. This has been partly solved by the phylogenetic-species concept, especially when based on cladistic analysis of molecular characteristics, which offers consistency in the delineation of species. Cladogram topology indicates the existence of monophyletic groups, which may represent species or supraspecific taxa (302, 484). Peterson and Kurtzman (430) correlated the biological-species concept with the phylogenetic-species concept by comparing the fertility of genetic crosses among heterothallic yeasts. They demonstrated that the D₂ region, a variable region of the 25S rDNA gene, is sufficiently variable to recognize biological species of yeasts and that conspecific species generally show less than 1% nucleotide substitution.

However, the definition of the phylogenetic-species concept is also complex, and several different definitions have been proposed. There appear to be two main approaches. The character-based concept, or diagnostic approach, defines a species as a group of organisms that have common observable attributes or combinations of attributes. In contrast, the history-based concept insists that organisms must be historically related before they can be considered members of any given species (23). Some of the different species concepts currently in use are difficult to distinguish, and so mycologists should be familiar with them and clearly define which they are using to recognize species.

PHYLGENY AND EVOLUTION

Little is known about evolutionary relationships among fungi. Only recently have some data become available, al-
though they are still sparse. The simple morphology, the lack of a useful fossil record, and fungal diversity have been major impediments to progress in this field (35). Classically, studies on fungal evolution have been based on comparative morphology, cell wall composition (19, 20), cytologic testing (538), ultrastructure (174, 242, 243), cellular metabolism (308, 562), and the fossil record (238). More recently, the advent of cladistic and molecular approaches has changed the existing situation and provided new insights into fungal evolution.

The phylogenetic relationships among higher fungal taxa remain uncertain, mainly because of a lack of sound fossil evidence, and remains a source of much controversy. The proposed phylogenetic relationships among the Animalia, Plantae, and Fungi kingdoms depend on the molecular regions and methods used by different investigators. Phylogenetic analysis has shown that the fungal kingdom is part of the terminal radiation of great eukaryotic groups (Fig. 1) which occurred one billion years ago (499, 540). Surprisingly, although mycology has been classically considered a branch of botany, there is also evidence that the kingdom Fungi is more closely related to Animalia than to Plantae (390). The analysis of amino acid sequences from numerous enzymes indicates that plants, animals, and fungi last had a common ancestor about a billion years ago and that plants diverged first (135). Another former hypothesis, i.e., that fungi are derived from algae, has been definitively abandoned (238).

Berbee and Taylor (37), following Hennig’s criteria (247), used geological time to calculate the appearance of nonsister taxa in an evolutionary tree. Just as sister lineages are equivalent units because they are equal in age, other lineages should receive equal rank because they diverged at about the same time. On this basis, it has been hypothesized that all the ranks from classes to families have successively appeared between the Cambrian and Tertiary periods.

The number of nucleotide substitutions in DNA sequences is directly proportional to the time passed, and so the number of base changes can be used to estimate the date of evolutionary radiation (238). On this basis, and using reference points such as the appearance of fossilized fungal clamp connections from the fossil record, the absolute timing of the origin of fungal groups has been estimated (36). The three main fungal phyla, Zygomycota, Ascomycota, and Basidiomycota, are thought to have diverged from the Chytridiomycota approximately 550 million years ago. The Ascomycota-Basidiomycota split occurred about 400 million years ago, after plants invaded the land. Many ascomycetes have evolved since the origin of the angiosperms in the last 200 million years. These results, with a few exceptions, are broadly supported by fossil evidence (36).

**NOMENCLATURE**

To be formally recognized by taxonomists, an organism must be described in accordance with internationally accepted rules and given a Latin binomial. The rules that control the binomenclature are very diverse and depend on the type of organism. Biological nomenclature is regulated by five different codes devoted to plants (197), cultivated plants (64), bacteria (496), viruses (168, 335), and animals (454). However, in an attempt to harmonize the different codes, some efforts are being made to find a unified system (236, 237, 239). Meanwhile, the nomenclature of fungi (including yeasts) is governed by the *International Code of Botanical Nomenclature* (ICBN) (197) as adopted by each International Botanical Congress. Any proposed changes to the Code are published in *Taxon*, the official journal of the International Association for Plant Taxonomy, and then debated in the Congress for approval. The Code aims to provide a stable method for naming taxonomic groups, avoiding and rejecting names which may cause error,
ambiguity, or confusion. However, strict application of the Code frequently leads to name changes for nomenclatural rather than scientific reasons (235). This causes annoyance among users, who do not usually understand the reasons for the changes. The taxonomy of some pathogenic fungi is particularly unstable and controversial at present. Changes to the names of taxa and their consequent diseases are potentially confusing (340). For example, the name of the fungus *Allescheria boydii* (so called in the early 1970s) was changed to *Petriellidium boydii* and then to *Pseudallescheria boydii* within a very short period. Consequently, infections caused by this organism were referred to as allescheriasis, allescheriosis, petriellidiosis, and pseudallescheriosis in the medical literature (403). To help clarify the confusion that changes in fungal names can cause, the Nomenclature Sub-Committee of the International Society for Human and Animal Mycology published its recommendations for mycosis nomenclature (402, 403). According to these recommendations, a disease name should ideally describe a disease, whereas many mycosis names indicate only a causative fungal genus. Disease nomenclature based on the traditional structure “fungus + sis,” which can be highly influenced by the taxonomic changes, was discouraged. In addition, the value of names of the “pathology A due to fungus B” construction was emphasized (402), e.g., “subcutaneous infection due to *Alternaria longipes*.”

The dual modality of fungal propagation, i.e., sexual and asexual, has meant that since the last century (463) there has been a dual nomenclature. The fungus, as a whole, comprises a teleomorph (sexual state) and one or more anamorphs (asexual states) (246). The term “holomorph” has been reserved for fungi with teleomorphic sporulation together with all their sporulating or vegetative anamorphs (180). The anamorph and teleomorph generally develop at different times and on different substrates, although in zygomycetes they often occur together. Since each phase has been described in total ignorance of the existence of the other in many cases, the ICBN maintains that it is legal to give them separate binomials. For a long time, the anamorphs that occurred alone have been grouped into anamorph genera because they share some morphological features. These anamorphs have been placed in a separate major high-level taxon called Deuteromycotina or Deuteromycetes. With the advent of molecular approaches in fungal taxonomy, some mycologists have advocated abandoning the dual system of naming because unified classification of all fungi may be possible on the basis of the rDNA sequences of the anamorphs (47, 71, 452). Other authors do not agree with this proposal and have considered it absolutely necessary to conserve deuteromycete taxon names, at least for identification purposes (180, 484). During the Holomorph Conference (453), it was agreed to maintain the term deuteromycetes with a lowercase “d” and not to formally recognize this group of organisms at a particular rank.

Another controversy resulted from the replacement of the terms “anamorph” and “telemorph” with “mitosporic fungus” and “meiosporic fungus”, respectively, in the last edition of *Ainsworth and Bisby’s Dictionary of the Fungi* (238); this source is considered a fundamental framework for fungal terminology and taxonomy. These changes have not been accepted by numerous authors (179, 180, 286), who consider that the anamorph and teleomorph phases of a fungus are determined not simply by the type of cellular processes (meiosis or mitosis) that precede sporulation but also by morphological features. Additionally, it has been argued that the cytological events preceding sporulation have not been investigated in sufficient depth to correlate teleomorph morphology with sexual recombination (179).

These problems particularly involve clinical fungi. The majority of pathogenic fungi are ascomycetes, which only rarely show their teleomorphs in the diagnostic laboratory. In contrast, these fungi frequently develop their anamorphs in routine culture media, and for some species there are at least two anamorphs (synanamorphs). Thus, the application of nomenclatural rules to the complicated life cycle of fungi can create some confusion among the users of fungal systematics. Clinical users have usually had little formal training in the fundamental principles of nomenclature and taxonomy, and they have a limited understanding and appreciation of these concepts. In addition, current reductions in financial support of hospital services means that the rapid identification of pathogenic fungi is an important concern (241). Consequently, a special effort should be made to develop reliable taxonomic systems which are easy to use and do not require complicated and expensive equipment (340).

**CURRENT MYCOLOGICAL TYPING METHODS**

The correct identification of fungi is of great practical importance not only in the clinical setting but also in plant pathology, biodeterioration, biotechnology, and environmental studies. An enormous number of species of fungi are already known, and so taxonomists are being kept very busy with recognizing and describing new species and grouping taxa. Hence, most species have received only limited study, so that classification has been mainly traditional rather than numerical and has been based on readily observable morphological features. However, some groups of fungi, because of their economical or pathological importance, have been studied more extensively. Other features beside morphology, such as susceptibilities to yeast killer toxins, susceptibilities to chemicals and antifungal drugs, the use of morphograms, molecular techniques, physiological and biochemical tests, secondary metabolites, ubiquinone systems, fatty acid composition, cell wall composition, and protein composition, have been used in classification and also in identification. The increased use and availability of modern techniques have opened up many new areas within systematics and have enabled more traditional ones to be developed further (67). Some of these approaches are detailed below.

**Morphology**

Classification systems of organisms are historically based on observable characteristics. This is the phenotypic approach. The classification and identification of fungi, unlike other important pathogens such as bacteria or viruses, relies mainly on morphological criteria. The fungi of medical importance are microscopic, and the study of their morphology requires the use of the light microscope. The classical light microscopic methods have been enhanced by Nomarski differential interference contrast, fluorescence, cytochemistry, and the development of new staining techniques such as those for ascus apical structures (461). Unfortunately, during infections most pathogenic fungi show only the vegetative phase (absence of sporulation); in host tissue, only hyphal elements or other nonspecific structures are observed. Although the pigmentation and shape of these hyphae and the presence or absence of septa (Fig. 2, structures a, h, and l) can give us an idea of their identity, fungal culture is required for accurate identification. Species-specific antibodies and the use of probes can be very useful in such cases. Although some commercial probes exist, these techniques are not yet available or convenient for routine use in medical mycology. Therefore, the growth of isolates in
appropriate culture media, enabling their most characteristic features to be recognized, is still the most common procedure used in practice.

As mentioned above, clinical microbiologists are used to seeing only deuteromycetes (Fig. 2) in cultures of clinical specimens. For the identification and classification of these fungi, the type of conidia (Fig. 2, structure p) and conidiogenesis (the process involved in conidium formation) (100) are considered the most important sets of characteristics to be observed. Cells that produce conidia are conidiogenous cells (Fig. 2, structure o). Often a different structure which bears one or more conidiogenous cells is present; this structure is the conidiophore (Fig. 2, structure n). There are two basic types of conidiogenesis, blastic and thallic (118). In blastic conidiogenesis, there is a small spot on the conidiogenous cell from which the conidia are produced. In thallic conidiogenesis, the entire conidiogenous cell is converted into one or more conidia. Primarily based on ultrastructural observations, Minter et al. (356–358) gave a personal interpretation of the detailed events involved in the formation of conidia and proliferation and regeneration of the cells bearing them. However, the terminology of the conidiogenesis events for the description of the anamorphic species proposed by Minter et al. is not widely followed. Hennebert and Sutton (245) described a set of basic unitary parameters which can aid in the study of the characteristics involved in conidiogenesis, although recognition of some of these parameters is difficult for inexperienced scientists. When sporulation is absent, there are still a number of morphological features to assist in classification. Other structures such as sclerotia, chlamydospores, or the presence of particular hyphal elements, as in anthropophilic dermatophytes, are sometimes very useful in the identification of anamorphs.

In the rare instances that opportunistic fungi develop the teleomorph in vitro (this happens in numerous species of Ascomycota and in a few species of Zygomyctota and Basidiomyctota), there are many morphological details associated with sexual sporulation which can be extremely useful in their classification. The type of fruiting body (basidioma in basidiomycetes, ascoma in ascomycetes) (Fig. 2, structures e and i) and the type of ascus (a microscopic, unicellular, frequently globose, sacate, or cylindrical structure which often contains eight ascospores produced after meiosis, and is usually developed in the cavity of fruiting bodies) (Fig. 2, structures j and k) are vital for classification. The shape, color, and the presence of an apical opening (ostiole) in the ascomata are important features in the recognition of higher taxa. Variations in ascus structure are currently important in the classification of these fungi, especially at the level of family and above. Ascospores are particularly subject to environmental selection and are consequently of value at lower ranks than are ascus structures. Pigmentation is a metabolic process occurring rather late in ascosporogenesis; as with ascospore septation, it is therefore of most value at lower taxonomic levels.

Electronic microscopy techniques allow the recognition of several details of special taxonomic significance. Cross sections of cell walls observed by transmission electronic microscopy (TEM) reveal significant differences between ascomycetous and basidiomycetous yeasts (296). The value of differences in the ultrastructure of the septum at the base of the ascus and in ascogenous hyphae has become apparent as a result of the studies of Kimbrough (277). In general, the correlation between septal type and family is high in discomycete groups but still requires investigation and analysis in other ascomycetes (277).

The increased availability of scanning electronic microscopy (SEM) has resulted in a number of significant findings and, in
some ascomycetes, has greatly facilitated identification at the species level by enabling differences in the surface detail of the ascospores to be clearly visualized (118). Freeze fracturing has revealed fine details of outer wall layers of conidia or ascospores (164, 531).

In recent years, morphological techniques have been influenced by modern procedures, which allow more reliable phenotypic studies to be performed. Numerical taxonomy, effective statistical packages, and the application of computer facilities to the development of identification keys offer some solutions and the possibility of a renaissance of morphological studies. Automated image analyzers, electronic particle sizing, and fractal geometry may have a lot to offer in the analysis of fungal morphology (94, 484).

The phenotypic approach has been largely criticized for its lack of standardized and stable terminology and for its high subjectivity. Moreover, some phenotypic characteristics have been considered to be unstable and dependent on environmental conditions, as with growth in artificial culture. A clear limitation of phenotypic approaches is that they cannot be applied to fungi that do not grow in culture. Consequently, there are many fungi that will remain unclassified as long as taxonomists rely solely on phenotypic characteristics. Another notable problem of classification based on morphological criteria is the above-mentioned dual system of classification, with no consistent correlation between the taxonomies of the ascomycetes and basidiomycetes (245). This is an important difficulty in establishing the taxonomic concept of the fungus as a whole.

**Molecular Techniques**

Since the distinguishing morphological characteristics of a fungus are frequently too limited to allow its identification, physiological and biochemical techniques are applied, as has been routinely done for the yeasts. However, for poorly differentiated filamentous fungi, these methods are laborious, time-consuming, and somewhat variable and provide insufficient taxonomic resolution. In contrast, molecular methods are universally applicable. Comprehensive and detailed reviews of the use of molecular techniques in fungal systematics have been provided by Bruns et al. (71), Hibbett (249), Kohn (284), Kurtzman (295), Maresca and Kobayashi (325), and Weising et al. (575).

Two important technical advances have stimulated the use of molecular techniques. Firstly, the advent of PCR has allowed the analysis of small numbers of fungal cells or even single spores, dried herbarium material (87), or extinct organisms (192). Second, the selection of universal oligonucleotide primers specific to fungi (244, 474, 559, 582) has provided easy access to nucleotide sequences.

The aim of molecular studies in biodiversity is fourfold: (i) phylogenetic studies, i.e., tracing back the most probable course of evolution and the historic coherence between groups at higher taxonomic ranks; (ii) taxonomic studies, mostly at the level of genera and species; (iii) diagnostic applications, i.e., recognition of defined taxonomic entities; and (iv) epidemiology and population genetics, i.e., monitoring outbreaks of sub-specific entities with respect to the analysis of populations and their mode of reproduction. Each of these broad aims and levels of diversity has its own set of optimal techniques. In this review, only phylogenetic and taxonomic studies are discussed.

One of the groups of genes which is most frequently targeted for phylogenetic studies is the one that codes for rRNA. Introns of several protein-encoding genes, such as the β-tubulin (405, 543), actin (101), chitin synthase (59, 526), acetyl coenzyme A synthase (42), glyceraldehyde-3-phosphate dehydrogenase (230), lignin peroxidase (377) or orotidine 5′-monophosphate decarboxylase (443) genes, can also be applied and can provide important information. The main reasons for the popularity of rDNA are that it is a multiple-copy, non-protein-coding gene, whose repeated copies in tandem are homogenized by concerted evolution, and it is therefore almost always treated as a single-locus gene. Furthermore, ribosomes are present in all organisms, with a common evolutionary origin.

Parts of the molecule are highly conserved (551, 552) and serve as reference points for evolutionary divergence studies. The conserved regions alternate with variable regions or divergent domains (232). The 5.8S, 18S, and 25S rDNAs are transcribed as a 35S to 40S precursor, along with internal and external transcribed spacers (ITS and ETS). All spacers are spliced out of the transcript. Between each cluster is a nontranscribed or intergenic spacer (NTS or IGS) that serves to separate the repeats from one another on the chromosome. A SS gene takes a variable position and is transcribed in the opposite direction. The total length of one DNA repeat is between 7.7 and 24 kb (249).

Comparisons of the 18S (also called the small-subunit [SSU]) rRNA sequences have been performed to assess the relationships of the major groups of living organisms (591, 592). For phylogeny of filamentous fungi, the 18S sequence is mostly used completely or in subunits of over 600 bp (70, 225, 233, 411, 502, 525, 586). In the yeasts, the D1 and D2 variable regions of 25S rDNA regions are almost exclusively used (220, 296). This technique is currently being extended to Heterobasidiomycetes (27) and sometimes also to filamentous ascomycetes (199, 329). In only a limited number of fungi have both regions been sequenced. Due to this different choice of target regions, comparison of fungi to all possible relatives is hampered. The 25S variable domains are very informative and allow comparisons from high taxonomic levels down to the species level, although only a limited number of variable positions remain (212). In the 18S gene, the variable domains mostly provide insufficient information for diagnostic purposes (116), and thus large parts of the molecule must be sequenced to obtain the resolution required (225). The ITS regions are much more variable, but sequences can be aligned with confidence only between closely related taxa. These regions are generally used for species differentiation but may also demonstrate patterns of microevolution (196). In contrast, 5.8S rDNA is too small and has the least variability. SS has been used mainly to infer relationships at the ordinal level (571), where differences could be traced back to the secondary structure of the molecule (48).

The evolutionary distance is generally displayed in the form of trees, and a wide diversity of algorithms are available to construct them. Two basic methods are available: distance matrix methods, resulting in phenograms, and maximum-parsimony methods, resulting in cladograms. Several papers have reported comparisons between the two methods with the same data set (329). If the difference between compared taxa is moderate, the results of the two algorithms are similar, but when dissimilarity values are higher, the number of evolutionary substitutions may be underestimated (509). The statistical significance of the tree found is tested by using resampling algorithms such as the bootstrap method. This subject has been reviewed by Avise (12) and Hillis et al. (250). The tree is usually rooted with an organism at a moderate distance (outgroup) that still can be aligned with confidence. For groups which have unexpected heterogeneity, such as the fission yeasts and the black yeasts, the correct choice of outgroup may be quite important (512).

Among the classical DNA-based methods is the determina-
The G+C content of nDNA has been established for many fungi, primarily yeasts. A difference of 2% in the G+C content has been considered to indicate that two strains should be assigned to different species (295). In some insufficiently resolved fungal groups, a difference of 8% has been allowed within species (50). In more precise recent studies with ecologically defined taxa, this difference was reduced to 1% (210). The G+C content is determined by using the $T_m$ from the S-shaped melting curve of the DNA. Occasionally the shape of the curve is deviant; by determination of the first derivative, this could be traced back to the presence of DNAs with different melting velocities. Guéhé et al. (214) found that these differences may be characteristic of subspecific entities.

The identity or nonidentity of closely related strains can be determined by DNA-DNA hybridization, estimating the velocity of heteroduplex formation compared to the standard kinetics of the individual strains. A relative hybridization value of over 80% is generally regarded as indicating membership in the same species, whereas values of less than 20% are proof of nonidentity (558). Intermediate values have increasingly been found recently, and these probably indicate subspecific entities (495).

In recent years, the methods most widely used for taxonomy at the species level have been sequencing and electrophoretic methods. Many authors have sequenced closely related species to investigate the relationship of the taxa. Such studies have been carried out with larger genera such as Penicillium (310, 311), Fusarium (429), and Trichoderma (292). Teleomorph and anamorph variation is not always congruent (292, 451). The speed of evolution seems heterogeneous and is characterized by different rates of variation between groups (127). In general, about 2% intraspecific variability is maintained within species (195). Occasionally the use of ITS is problematic due to the occurrence of two different types in a single organism (405).

Among the electrophoretic methods, restriction fragment length polymorphism (RFLP) is particularly significant for taxonomy. This technique involves digesting DNA samples with a panel of restriction enzymes. The patterns can be tabulated and compared (546), or phenetic trees can be constructed (73, 142). The first RFLP technique widely used in taxonomy compared patterns of mitochondrial DNA (mtDNA). However, some authors have sequenced the mtSSU rDNA instead (568, 597). mtDNA is generally indicative of differences somewhat below the species level (268), but in groups where microspecies are currently distinguished, such as in the dermatophytes, the differences seem to correspond to teleomorph species (360). RFLP-based typing methods have been used to reveal anamorph-teleomorph connections (185). Most commonly, the RFLP of PCR-amplified rDNA is used. This technique is also known as amplified rDNA restriction analysis (554) and provides a quick insight into relationships between moderately distant fungi (116). Therefore, homogeneity of ITS profiles corresponds well to final ITS sequencing diversity (547). The method is primarily confirmatory; i.e., new strains are quickly assigned to sequenced strains with the use of restriction maps (559). This strategy was used, for example, by Yan et al. (598) to study Phialophora. Amplified rDNA restriction analysis is particularly useful as an inexpensive and simple alternative to SSU rDNA sequencing when broad relationships have already been determined. However, the frequent occurrence of introns in SSU rDNA (183) may hamper quick comparison of strains.

Random primed methods (226) are particularly useful to determine relationships below the level of species, but depending on the length of the primers and the recognized taxonomic diversity of the group under study, the method may help to discriminate species. For such aims, a comparison of several unlinked molecular methods is overdue (545). A popular technique is random amplified polymorphic DNA (RAPD) with 10-mer primers. However, this method is gradually being abandoned because of poor reproducibility. Microsatellites are a special class of tandem repeats, which have a base motif of up to 10 bp that is frequently repeated (up to about 100 times); they are found in many genomic loci with an almost ubiquitous distribution (536). A general profile comparison of microsatellites enables species recognition (195, 389). In addition, due to the high level of polymorphism, individual bands can be informative for the characterization of strains (163, 314, 482).

Karyotyping, i.e., the migration of chromosomes under the influence of an electric field, has been commonly used for a long time in plant taxonomy and also in mammal and bacterial taxonomy. However, some problems have complicated the use of this technique in fungal taxonomy (e.g., the smallness of the fungal chromosomes and the difficulty of observing condensed chromosomes during meiosis) (284). Techniques such as electron microscopy, pulsed-field gel electrophoresis (354, 413, 493), and its recent modification, contour-clamped homogeneous electric field electrophoresis (535) can contribute to the precise determination of karyotypes. The high intraspecific variability of the electrophoretic karyotypes of fungi makes this technique especially applicable for studies of populations in pathogenic yeasts (133, 279, 318, 421, 427) and filamentous fungi (68, 355).

Other Techniques

**Physiological and biochemical techniques.** Because numerous fungi grow relatively rapidly in pure culture, it is possible to use physiological and biochemical techniques to identify and classify them (65, 424, 426). These techniques have been successfully used in the study of black yeasts (115, 117, 120, 128, 600). The different ranges of growth temperature have been used as a complementary tool in the identification both of asexual (339) and sexual (353) fungi. Growth rates on defined media under controlled conditions are also valuable in studies of complex genera such as Penicillium (431). Commercially available kits such as the API system have also been used to identify filamentous fungi (155, 450, 573). Paterson and Bridge (426) have published a compilation of the physiological techniques used in the identification of filamentous fungi. They list a variety of biochemical methods which range from simple agar-based tests to more sophisticated chromatographic and electrophoretic methods (426).

**Secondary metabolites.** Secondary metabolites are compounds neither essential for growth nor key intermediates of the organism’s basic metabolism but presumably playing some other role in the life of fungi. They are usually found as a mixture of closely related molecules with a peculiar and rare chemical structure (238). The most common are steroids, terpenes, alkaloids, cyclic peptides, and coumarins, and some of these are mycotoxins. The advent of thin-layer chromatography, especially the simple technique of directly spotting thin-layer chromatography plates with small samples of culture cut from petri dishes, has made it possible to qualitatively assess secondary metabolites much faster than by conventional extraction, purification, and concentration techniques. This improvement has resulted in huge amounts of new secondary-metabolite data, which is now being incorporated in databases (67, 424). The pattern of secondary-metabolite production has for a long time been of great use in the identification and classification of lichens. This pattern has been used less in the taxonomy of ascomycetes and basidiomycetes, although it is
well known that these organisms produce a vast array of such compounds (87). The use of this method in fungal taxonomy has been questioned because the production of these compounds can be affected by environmental conditions and the detection procedure presents some difficulties. However, its potential in ascomycete systematics is well illustrated by the chemotaxonomic studies performed in Eurotiales (170–172) and in Xylariales (581). In these orders, individual species can often be recognized on the basis of particular metabolite profiles. Integrated approaches involving morphology, physiology, and secondary metabolites have been used in several attempts to clarify the systematics of some fungi (65, 66, 361, 366, 371).

**Ubiquinone systems.** Besides the use of secondary metabolites in taxonomy, some use has also been made of other compounds which play an essential role in metabolism and which are primary rather than secondary metabolites, e.g., ubiquinones (coenzyme Q) (87). These compounds are important carriers in the electron transport chain of respiratory systems. The number of isoprene units attached to the quinone nucleus varies, and such differences in ubiquinone structure are excellent indicators in the classification of genera and subgeneric taxa in bacteria and yeasts. Although less common, these techniques are also being used in the taxonomy of black yeasts (596) and filamentous fungi (595). The results provided by these techniques sometimes correlate with those provided by molecular techniques, although conclusions based purely on ubiquinone systems are debatable and, depending on the method used, can provide different sets of data (466).

**Fatty acid composition.** Cellular fatty acid composition is routinely determined in bacterial systematics (370, 557). Both the type of fatty acid present and its relative concentration are useful characteristics for separating taxa. Until recently, these techniques were only rarely used in fungal taxonomy. Although fewer different fatty acids are produced by fungi than by bacteria (306), these analyses are increasingly used for differentiating fungi (6, 10, 49, 69, 510). Pyrolysis gas chromatography, pyrolysis mass spectrometry, gas chromatography, and partition aqueous polymer two-phase systems are among the numerous methods used to determine these compounds (49). Recently, gas chromatography, combined with methods of multivariate statistical analysis, has successfully been used to study the fatty acids of numerous and varied filamentous fungi, including oomycetes, zygomycetes, basidiomycetes, and even sterile mycelia (49, 510). These techniques have also proved to be useful at intraspecific level (510). However, several technical aspects of the procedure used must be highly controlled to minimize sources of variation, which can influence the results enormously. The culture conditions and temperature are among the most important factors to be standardized (510).

**Cell wall composition.** Numerous studies have shown differences in the structure and composition of the cell wall of fungi which have been used in the definition of high fungal taxa. For example, cellulose is a particular component of the alkali-insoluble fraction of oomycetes, while ascomycetes and basidiomycetes contain both chitin and glucan in these fractions. In contrast, the zygomycetes have chitosan and polyglucuronic acid. Significant variations in the sugar composition of cell walls have been observed in the different genera of dermatophytes (200b, 396, 397). The walls of yeast cells that have been subjected to hydrolysis yield substantial amounts of glucose and mannose, but species differ in the presence or absence of smaller amounts of other polysaccharides (fucose, galactose, rhamnose, and xylose). This feature has also been used in classification of fungi (87). Bartnicki-Garcia (20) has reviewed the biochemical and physiological characteristics of the cell wall components which have been used to delimit high taxa.

### Table 1. Phyla and classes of the tree kingdoms where fungi are placed

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Phylum</th>
<th>Class</th>
</tr>
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<tbody>
<tr>
<td>Chromista</td>
<td>Hyphochytriomycota</td>
<td>Basidiomycetes</td>
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<tr>
<td></td>
<td>Labyrinthulomycota</td>
<td>Telosporomycetes</td>
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<td></td>
<td>Oomycota</td>
<td>Oomycetes</td>
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<tr>
<td>Fungi</td>
<td>Ascomycota</td>
<td>Ustomycetes</td>
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<tr>
<td></td>
<td>Basidiomycota</td>
<td>Trichomycetes</td>
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<td></td>
<td>Zygomycota</td>
<td>Zygomycetes</td>
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<tr>
<td>Protozoa</td>
<td>Acrasiomycota</td>
<td>Myxomycetes</td>
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<tr>
<td></td>
<td>Dictyostelomycota</td>
<td>Protostelomycetes</td>
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<tr>
<td></td>
<td>Myxomycota</td>
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<tr>
<td></td>
<td>Plasmodiophoromycota</td>
<td></td>
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</tbody>
</table>

* Accepted by the Dictionary of Fungi (238).

### Protein composition

Isoenzyme patterns produced by electrophoretic techniques (zymograms) have determined generic relationships and differentiated species (87, 349, 375). Apart from electrophoresis, immunological techniques and protein sequencing also have suitable resolution for interspecific characterization (173, 284, 334). Isozyme analysis is considered to be an economical and practical technique for screening large populations. What is more, the characteristics determined by this technique are generally accepted to be of independent genetic origin (284). Allozyme (allelic isozyme) data are commonly used in phylogenetic studies (284).

### THE FUNGAL SYSTEM

Before DNA-sequencing methods became available, it was practically impossible to infer a reliable evolutionary tree containing all forms of life. Whittaker, in the context of Five Kingdoms (animals, plants, fungi, protists, and monera) (326), summarized evolutionary thought (583). The four multicellular forms of these traditional taxonomic kingdoms were considered to be eukaryotes, and only the monera were identified with the preceding prokaryotes. These two categories of organisms, defined mainly by the presence or absence of nuclear membranes, are considered to be independent and coherently related groups. For a long time, fungi were regarded as a single kingdom belonging to the aforementioned five-kingdom scheme. However, the organisms usually considered to be fungi are very complex and diverse; they include multicellular and filamentous absorptive forms and unicellular assimilative forms, among others, and can reproduce by different types of propagules or even by fission. This general concept cannot be based on a single phylogenetic line but, rather, on a way of life shared by organisms of different evolutionary backgrounds. This has recently been confirmed by ultrastructural, biochemical, and especially molecular biological studies. Hence, there has been controversy about the higher fungal taxa; i.e., some authors consider that fungi should be assigned to two kingdoms, Prototistida and Eumycota (274), and others consider that they should be assigned to three, Chromista, Fungi, and Protozoa (238) (Table 1). When it is useful to refer to fungi in this polyphyletic sense, the name can be used nonitalicized and noncapitalized to differentiate them from the kingdom Fungi.

Analysis of the SSU rDNA sequence shows that fungal or-
organisms belong to four monophyletic groups: Acrasiomycota, Myxomycota, Oomycota, and Fungi (71). It also shows that the first two groups diverged separately prior to the terminal radiation of eukaryotes (231, 499, 540), which is consistent with morphology and function, because these two groups include slime molds, which are at present in the kingdom Protozoa. Similarly, phylogenetic analysis shows that Oomycota grouped with different types of algae (41, 167, 540), which is also consistent with their morphology and structure. At present, Oomycota are included in the kingdom Chromista (238). The rank “kingdom” has also been questioned, arguing that this term has no molecular definition (415). In addition, it has been argued that if animals, plants, and fungi are considered to be taxonomic kingdoms, we must recognize at least a dozen other eukaryotic groups as kingdoms. These groups have at least as much independent evolutionary history as that which separates the three traditional kingdoms (415). Above the rank of kingdom, the now-recognized three primary lines of evolutionary descent termed “urkingdoms” or “domains,” i.e., Eucarya (eukaryotes), Bacteria (initially called eubacteria), and Archaea (initially called archeobacteria), have been proposed (591, 592). In this context, the term “kingdom” indicates the main lines of radiation in the particular domain.

The kingdom Fungi is organized into phyla and then into classes and orders. However, some authors prefer to avoid particular categories above orders. Mycologists previously used the term “division” instead of “phylum” because it is more inherent to botany, but in the last 5 years the term “phylum,” used mainly by zoologists, has been used as an alternative and has practically replaced “division.” The four phyla presently accepted in Fungi are Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (Table 1). However, the inclusion of Chytridiomycota in the Fungi has been controversial because these organisms possess flagella. On this basis, they have been included in the Protoctista (326), but comparison of cell wall polysaccharides (19) and lysine synthesis (562) linked them firmly with the other classical phyla of Fungi. Analysis of 18S rDNA sequences also showed that chytridiomycetes cluster with representatives of Ascomycota and Basidiomycota (61) in a separate clade from ciliate protists. Subsequent analysis of many fungal 18S rDNA sequences (70) showed that Chytridiomycota and Zygomycota are difficult to separate and are basal to the Ascomycota and Basidiomycota. Although Chytridiomycota form the basal branch in the kingdom Fungi, flagella may have been lost more than once during the evolution of the ancestors through to Zygomycota, Ascomycota, and Basidiomycota. It has been demonstrated that among the non-flagellated fungi, the Zygomycota diverged first, and the branch defining the Ascomycota and Basidiomycota as terminal sister groups is strongly supported (33, 70) (Fig. 1).

There are thought to be so many species of fungi that insects are the only group with more varieties. Currently, approximately 1.4 million living species of microorganisms, fungi, plants, and animals have been recorded (198, 478). Most of these species are insects (950,000) and plants (250,000). On the basis of the current rate of discovery, it has been estimated that the number of undiscovered and undescribed species ranges from 1 million to more than 10 million (478). Fungi by far outnumber bacteria and viruses. In 1995, approximately 70,000 fungal species were accepted (236) compared to only 3,100 known bacteria species and 5,000 viruses (478). However, the number of fungal species so far discovered is probably only a small proportion of those that actually exist, since few habitats and regions have been intensively studied. Many extreme environments are still unexplored or not adequately explored. It has been conservatively argued that the total number of fungal species in the world must be at least 1 to 1.5 million (234, 478), meaning that we have so far recognized only about 5 to 7% of the world’s mycobiota. Other authors indicate that less than 20% of the terrestrial and marine fungi have been discovered (283). Since all proposed new species are catalogued in the Index of Fungi (edited twice yearly by the International Mycological Institute, Egham, United Kingdom), we can calculate that 800 to 1,500 new species are described annually. It is worth mentioning that some of the recently described new species have been found only in humans; these include Distitiramuris exedras, Calyptromyza arxii, Hormographiella spp., Onychochora canadensis, Ramichloridium mackenziei, Polycyrtella hominis, Pyrenochaeta unguis-hominis, Phoma cruris-hominis, Phoma oculo-hominis, Botryomyces caespitosus, and some dermatophytes.

The huge number of fungi constitutes a potentially serious problem to public health. An increasing number of fungi that are normally harmless to the immunocompetent host are able to invade the neutropenic host and cause severe infections.

**CLINICALLY RELEVANT FUNGI**

There are three main groups of pathogenic fungi which are quite different from one another. Firstly, dermatophytes are a group of obligate parasites which attack human skin, nails, and hair. Secondly, dimorphic saprobes are a group of normally soil-borne fungi which have developed a different morphology in order to adapt to the hostile environment of the human body. These organisms can cause disease in healthy people. The third group, which is the most numerous, consists of opportunistic saprobes, which can attack humans whose immune systems are deficient in some way or artificially suppressed (274). The pathogenic and opportunistic fungi are distributed among three phyla of the kingdom Fungi (Fig. 2), i.e., Zygomycota, Ascomycota, and Basidiomycota, although the phylum Chromista (Oomycota and Hypochytriomycota) also contains two human pathogens and several animal pathogens. Some of the most relevant taxonomic developments concerning the members of medical interest belonging to such phyla are addressed below.

**OOMYCOTA AND HYPHOCHYTRIOMYCOTA**

The phylum Oomycota comprises approximately 700 species. Cellulose and β-glucan are the main components of their cell walls. Some of them are virulent plant pathogens, while others are aquatic species and fish pathogens. Only a few members of this phylum, particularly species of Phytophthora and Pythium, have been the focus of molecular studies aimed at species level and below (24, 71, 328). Only the species Pythium insidiosum, belonging to the order Peronosporales, is an opportunistic pathogen in warm-blooded animals (118). It only rarely causes infection in humans (561, 572). Its life cycle was described by Mendoza et al. (346). An immunodiffusion test, which can be an important aid for its diagnosis, was developed by Pracharktam et al. (439). Rhinosporidium seeberi, which belongs to the phylum Hypochytriomycota, causes rhinosporidiosis in humans and is characterized by the formation of cysts in the subepithelial tissue of nasal mucosa. These infections occur in India and South America. It is not a well-known fungus because it cannot be grown on routine laboratory media.
**ZYgomycota**

The Zygomycota are a group of lower fungi whose thalli are generally nonseptate (coenocytic) (Fig. 3, structures I and m). After the fusion of isogamic sex organs (gametangia) (Fig. 3, structure c), they produce a single, dark, thick-walled, often ornamented sexual spore, called zygospore (Fig. 3, structure b). In host tissue, they are recognized by their broad, aseptate, hyaline, randomly branched hyphal elements. Zygomycota are divided into two classes: Table 1 and 11 orders (258). Fungi of clinical interest are found in only two of them: the small group of Entomophthorales, which have forcibly ejected spores, and the Mucorales, whose spores arise by cleavage of the sporangial plasma and are passively liberated (404, 602). In culture, these fungi show typical characteristics described below.

The order Mucorales is the most clinically important. Its members are widely distributed and are found in food, soil, and air. Several species are thermophilic. Eleven genera, containing 22 species of medical interest, have been described and illustrated by de Hoog and Guarro (118). The most frequent, significant mucoralean genera in the clinical laboratory are *Rhizopus* and *Absidia*. *Mucor* is abundant as a contaminant but is very rarely an etiologic agent. However, in recent years, some rare genera have been associated with human infections, e.g., *Cokeromyces* (273), *Saksenaea* (330), *Apophysomyces* (140), and *Chlamydosabia* (79). The clinical picture is rather similar throughout the whole group. Most rhinocerebral mycoses are found in patients with diabetes mellitus, although other types of infection (e.g., gastrointestinal, pulmonary, cutaneous and systemic) are also frequently reported. Disseminated and severe infections are encountered mainly in patients with immunological disorders (156, 304, 481).

Recognition of a clinical isolate which belongs to mucoralean fungi is easy and can be done by observing only the macroscopic features. Lax, woolly colonies which spread over the whole petri dish in a few days and which have small dots (sporangia) (Fig. 3, structures a and d) on the periphery are sufficient to identify them. This identification can be microscopically confirmed by the presence of nonseptate, wide, hyaline hyphae with the typical fertile structures of mucorales. Although serological tests have been developed (129, 267), the identification of the species is rather more complicated. The procedure is both time-consuming and labor-intensive and usually requires special media, some of which are not commercially available. Some species have considerable morphological variation, and in these cases it is important to culture the fungi under optimal conditions to determine the variability of characteristics (29, 480, 481, 578). The species is identified almost exclusively by careful microscopic observation and measurements of several morphological characteristics which are typical of asexual reproduction. This process takes place by means of sporangiospores (Fig. 3, structure h) produced in sporangia (multispored) (structure d), in sporangioles (with one or very few spores) (structure e), or in merosporangia (spores in rows) (structure f). A sporangium generally has a central columella (structure g), which may extend and be visible below the sporangium as a swelling known as the apophysis. Other structures such as zygospores, chlamydospores, or a combination of them are produced only in some organisms. The value of zygospore morphology is limited because the sexual spore has never been seen (29). However, the production of zygospores was considered to be very useful in clinical zygomycete taxonomy, especially for the identification of rare, heterothallic zygomycetes (578). An important limitation to this technique is the need for test strains to try to stimulate zygospore production (578). Several species creep over the agar surface with stolons (Fig. 3, structure j), and the substrate can be penetrated by means of rhizoids (structure k). These structures also have a certain diagnostic value. Fatty acid composition has been investigated in *Montierella* (6). A variety of biochemical and physiological tests (carbon and nitrogen assimilation, fermentation, requirement for thiamine, maximum temperature of growth, etc.) are also used by different authors (481, 578). Both SEM and TEM are important aids for the detailed observation of numerous structures and allow the morphology of surface ornamentations to be discerned. They can also resolve aspects of the ontogeny of sporangiospores (29, 404).

Benny (29) suggested exploring some traditional characteristics of phylogenetic studies. He considered it useful to investigate possible differences in sporangiospore ontogeny and distribution of chemical compounds and to search for unknown zygospores. It seems logical to expect that molecular studies will provide more consistent data. Molecular studies have only very rarely been performed in the Mucorales. Radford (443) compared sequences of the rDNA 5’-monophosphate dehydrogenase gene and showed that Mucorales was a sister group of basidiomycetes. As a result of a preliminary cladistic analysis based on morphology and SSU rDNA sequences (407), Benny (29) pointed out that many characteristics used to define mucoralean families probably do not indicate relationships but are still useful for identification. The lack of agreement between molecular data and morphological observations could indicate that many of the characteristics which are considered advanced in the Mucorales have arisen several times in the order (29).

In the order Entomophthorales, there are only three species occasionally pathogenic to humans: *Conidiobolus incongruus* (261, 569), *Basidiobolus ranarum* (112, 276), and *Delacroixia coronata* (Conidiobolus coronatus) (385). These species are characterized by forcibly discharged spores borne on tubular sporophores. There are very few molecular studies of the taxonomy of this order (383).

**Ascomycota**

Ascomycota is the largest phylum of Fungi. It comprises almost 50% of all known fungal species and approximately 80% of the pathogenic and opportunistic species. As a reference framework for the systematic of ascomycetes, the University of Umea and the International Mycological Institute publish jointly the periodically revised Outline of the Ascomycetes, which is developed through the journal Systema Ascomycetum. It is published about every 4 years and is based on data provided by numerous specialists (153). Proposals for changes at the generic level and above are compiled in twice-yearly issues of that journal. The classification scheme included in these outlines is followed by the majority of mycologists involved in ascomycetes systematics.

The basic characteristic which differentiates ascomycetes from other fungi is the presence of asci (Fig. 4, structures e to g) inside the ascomata (structures c and d). However, even in the absence of these important diagnostic characteristics, the ascomycetes can be recognized by their bilayered hyphal walls with a thin electron-dense outer layer and a relatively electron-transparent inner layer (238). The arrangement of the asci has played a major role in supraordinal systematics, and for a long time the ascomycetes have been grouped into six classes, i.e., Hemiascomycetes, Plectomycetes, Pyrenomycetes, Discomycetes, Laboulbeniomycetes, and Loculoascomycetes (372). The Plectomycetes are characterized by the presence of closed, more or less spherical fruiting bodies (cleistothecia) (Fig. 4,
FIG. 3. Typical characteristics of zygomycetes. a, mycelial colony; b, zygospore; c, gametangium; d, sporangium; e, sporangiole; f, meroспорangia; g, columella; h, sporangiospores; i, meroспорes; j, stolon; k, rhizoids; l, coenocytic hypha; m, section of a hypha; n, sporangiophore.
FIG. 4. Typical characteristics of ascomycetes. a, mycelial colony; b, yeast colonies; c, peritheciurn; d, cleistothecium; e, ascus; f, unitunicate asci; g, bitunicate ascus; h to m, anamorph types; n, conidiophore; o, conidiogenous cell; p, hyphae; q, detail of a uniporate septum; r, detail of a multiporate septum.
structure d), with an irregular distribution of the asci in the cavity, and the ascospores are released after the disintegration of the thin walls of the asci. The dimorphic pathogens (Histoplasma, Coccidioides, Emmonsia, etc.) are included in this group, as are the teleomorphs of the dermatophytes and of the frequent and cosmopolitan Penicillium and Aspergillus among others. The Pyrenomycetes have pyriform (flask-shaped) fruiting bodies (perithecia) (Fig. 4, structure c) with usually saccate or cylindrical asci. The ascospores are forcibly extruded from the ascus. The teleomorphs of several opportunistic fungi are also included in this class. The class Loculoascomycetes comprises species with bilayered asci, sometimes enclosed in stromatic ascomata. The sexual states of numerous pigmented (dematiaceous) pathogenic fungi are included in this latter class. The class Hemiascomycetes comprises the yeasts. In the remaining two classes there are no fungi of medical interest. An alternative, long-accepted classification scheme placed the ascomycetes into the classes Hymenoascomycetes and Loculoascomycetes, depending on whether the ascus wall was single (Fig. 4, structure f) or bilayered (structure g), respectively (17, 18, 317). Several other taxonomic schemes have been established over the years and have been accepted to greater or lesser extents (238).

The traditional systems of ascomycete classification have been much criticized due to their artificial nature. It has been repeatedly argued that the supposed similarities among these major groups may not reflect homology. Another much questioned aspect has been the confusing terminology. One example is the term “plecromycete,” which has been defined as a closed ascoma (162) and also as scattered asci within the ascomatal cavity (317). Another problem inherent in traditional classifications is the convergence of fruiting bodies (77, 320). It has been demonstrated that species which normally produce flask-shaped fruiting bodies can be induced to form closed fruiting bodies under certain environmental conditions (563). Some species, such as the recently reported opportunistic fungi, Microascus spp., have both the fruiting-body type of the Pyrenomycetes and the ascus arrangement characteristic of the Plectomycetes. For these reasons, it has been considered that there are too few stable morphological features which are useful for inferring relationships among higher taxa. Therefore, many mycologists currently deemphasize supraordinal groupings and the relationships that they define (150) while others support the use of these supraordinal rankings as reflecting natural groupings.

Molecular data are adding a new dimension to the understanding of the relationships among the different groups of ascomycetes. One of the first phylogenetic trees to depict the evolutionary history of ascomycetes was published by Berbee and Taylor (35), who studied morphological convergence in true fungi. They recognized three main groups of ascomycetes: (i) the basal ascomycetes, which included the Schizosaccharomycetales (fission yeasts) and *Pneumocystis*; (ii) the true yeasts, i.e., filamentous or unicellular ascomycetes without fruiting bodies; and (iii) the filamentous ascomycetes with fruiting bodies. Numerous authors subsequently provided sequences of these fungi which have been useful for filling some of the gaps in the evolutionary history of ascomycetes. Soon it may be possible to build a phylogenetic tree for all ascomycetes. Meanwhile, it is evident that some traditional classes such as the Pyrenomycetes (Hypocreales, Microascales, Diaporthales, and Sordariales) and the Plectomycetes (Eurotiales and Oxygenales) are also represented by well-supported monophyletic clades. However, the monophyly of the classes Hymenoascomycetes and Loculoascomycetes was rejected (501, 505). There are examples of perithecial and cleistothecial ascomycetes that do not group with these well-supported clades. Despite this, we consider that some of these old class names are useful, at least until sequences of more ascomycetes become available and the boundaries within these groups are defined. Therefore, in this article, and for the sake of simplicity, the taxa are arranged into five morphological groups, i.e., basal ascomycetes, unifuniculate Pyrenomycetes, bituniculate Pyrenomycetes, Plectomycetes, and budding yeasts. The relationships among these groups are inferred from the analysis of 18S rDNA and suggest an early radiation of Schizosaccharomycetales and *Pneumocystis carinii*, which represent a basal branch in the Ascomycota (basal ascomycetes). This was followed shortly by a bifurcation leading to budding yeasts on one branch and filamentous fungi with fruiting bodies on the other. Among the latter, there is another radiation comprising the unifuniculate Pyrenomycetes followed by the Loculoascomycetes (bitunicate Pyrenomycetes) and Plectomycetes (Fig. 5). However, the delimitations between these two groups are not well defined.

Eriksson (152) recently also used these groups to demonstrate the value of the signature sequences in the taxonomy of ascomycetes. Signatures are short sequences in moderately conserved areas of DNA, RNA, or protein. They are characteristic of taxa of various ranks and give phylogenetic information that is not provided by other methods. Studying the terminal part of stem-loop E23-1 in the V4 region of 18S rRNA according to the terminology of Neeffs et al. (381), Eriksson found that in most cases it consisted of a short helix of 3 or 4 bp and an end-loop of usually 6 bases. Above the helix, there is almost always a bulge of a single C, and below a bulge of usually 3 bases. Supposed signatures, end-loops, and a larger bulge in this helix were discussed by Eriksson for different groups of ascomycetes and, for comparison, for some basidiomycetes. He found that when numerous taxa of the Plectomycetes and Pyrenomycetes were examined, they practically all had the loop CTCACC, which was not found in any other group of either ascomycetes or basidiomycetes, and they all had a helix of 3 bp. Other noteworthy findings were found in the signature sequences of the other groups examined such as the budding yeasts. For example, a higher A+T content was found in these sequences than in other ascomycetes and basidiomycetes. Surprisingly, a great variation was found in stem-loop E23-1 of the basidiomycetes compared with ascomycetes.

At present, 46 orders are accepted in Ascomycota (238) but only 9 of them include fungi of clinical interest (see Tables 2 to 8).

**Basal Ascomycetes**

*Pneumocystis carinii* is regarded as the only member of the order Pneumocystidales (238), although immunological and molecular data (14, 143, 187, 309, 514, 568), and host species specificity (5, 176, 188, 352) support the existence of different species or varieties (76). The organism has been isolated from a wide range of unrelated mammalian hosts, including humans. It has been known since the beginning of this century (92) and for nearly 70 years has been reported only as the agent of mild infections, against which residual antibodies are developed. It also causes occasional epidemic pneumonia under conditions of overcrowding and malnutrition (76). During the last 15 years, the organism has emerged as one of the most common pulmonary infections in AIDS patients, and its presence has become one of the first indications of the disease. This organism cannot be cultivated in routine laboratory media (108). In vivo, in alveolar tissue, it is characterized by the absence of hyphal elements. The vegetative cells are thin-walled, irregularly shaped, uninucleate, divided by fission and transformed.
into a thick-walled cyst-like structure (asci), with up to eight internal cells (ascospores) which are at first globose but become falcate. The crescent-shaped cysts are thought to liberate these endospores (238). The detection of *P. carinii* is enhanced by cellofluor staining (21) or by immunofluorescent staining with monoclonal antibodies (386). PCR-mediated detection has been developed on the basis of thymidylate synthase sequences (412).

For a long time, *P. carinii* was considered a Protozoan, but morphological studies have demonstrated its fungal affinities (556) and molecular studies have confirmed this (144, 515, 574). First, analysis of the mitochondrial 24S rDNA gene has shown a close relationship between *P. carinii* and the simple-septate, red basidiomycete yeasts (568). Morphological characteristics such as the absence of laminated cell walls (599) typical of basidiomycetes and the presence of a double-membrane which delimits each developing intracystic body (ascospore) (333), as well as molecular data (540), apparently confirm that *P. carinii* belongs to the Ascomycota. On this basis, a change in the terminology seems reasonable for descriptions of *P. carinii* infections. The terms “parasite,” “intracystic bodies,” “trophozoites,” and “infestation” (commonly reserved for parasites) should be abandoned and a terminology which is applicable to fungi should be adopted (483).

**Unitunicate Pyrenomycetes**

The wall structure of the asci determines two basic kinds of Pyrenomycetes: those with a single wall are called unitunicate (Fig. 4, structure f), and those with a bilayered wall are bitunicate (structure g). The unitunicate ascus sometimes has an operculum (a small lid), which opens to liberate the ascospores when the ascus is mature. These asci are called unitunicate-operculate. No pathogenic fungi belong to this group. Other asci have no operculum but have an apical pore and/or a ring-like structure at the tip, acting as a valve, or sphincter, through which the ascospores are violently discharged and are dispersed by the air. These forcibly ejaculated ascospores can reach long distances. Such asci are called unitunicate-inoperculate (274) and are present, for example, in *Neurospora* (see Table 3). There are still other perithecial fungi with unitunicate asci which have no obvious mechanism for ascospore release. In this case, the ascospores are released into the cavity of the ascomata, when the ascus wall disintegrates. These ascospores are never forcibly discharged, but they can be extruded in a dark, sticky mass or cirrhus. These fungi are adapted to dispersal by insects or other animals and develop in soil, in cavities, or on the under surface of the substrate (566). *Microascus* (see Table 2) and *Chaetomium* (see Table 3) are examples of this last type. The mechanism for the violent discharge of spores is practically absent in human pathogenic fungi, and, rather than being representative of an evolutionary origin, it seems to be merely coincidental. Berbee and Taylor (35) studied the phylogeny of several Pyrenomycetes and concluded that the ability to discharge ascospores forcibly has probably been lost, convergently, several times. However, the pyrenomycete asci that forcibly discharge ascospores have general morphological similarities, including an apical pore and often an apical ring structure, and this is consistent with a single evolutionary origin (35). Apart from the Pyrenomycetes, cylindrical, thin-walled asci with apical pores are rare. Within the unitunicate Pyrenomycetes, the orders Hypocreales, Microscales, Ophiostomatales, and Sordariales comprise species which cause human infections (Tables 2 to 4). By analyzing SSU rDNA sequences, Spatafora and Blackwell (503, 504) demonstrated a clear monophyly of unitunicate Pyrenomycetes and two lines of evolution within the group, represented by two subclades. Ophiostomatales and Sordariales were revealed to be polyphyletic; the Hypocreales were inferred to be a paraphyletic components of one of the two subclades; and
Microascales, although represented in that study by only two species, seemed to be monophyletic.

**Microascales.** According to von Arx et al. (566), fungi belonging to the family Microascaceae of the order Sphaeriales often have immersed, ostiulate or nonostiulate ascomata, with relatively small, obovate or nearly spherical asci arranged in vertical rows (basipetal chains) or irregularly in the cavity. They also have relatively small, pale, aseptate ascospores with one or two germ pores. In contrast, Hawksworth et al. (238) consider that these are the main characteristics of the order Microascales. The members of Microascales are uniformly benomyl resistant, unlike other morphologically similar ascomycetous fungi (521). This order, divided into two families, comprises about 80 species which are mainly coprophilous or isolated from animals. von Arx et al. (566) reviewed this group of fungi and provided synoptic keys for the identification of the genera and species. The genera *Microascus* and *Pseudallescheria* (Table 2), which easily develop ascomata in artificial cultures, are clearly related to human infections. However, their anamorphs, which belong to *Scopulariopsis*, *Scedosporium*, and *Graphium* (Table 2), are frequently predominant. *Pseudallescheria boydii* is a well-known pathogen which is commonly associated with mycetoma (2) and is frequently found as a soil saprobe and in many other natural substrates (102). This species is now emerging as an opportunistic fungus causing severe systemic infections in immunocompromised patients (39, 139). It is characterized by the production of globose, brown to black cleistothecia in culture and pale yellow to golden brown, lemon-shaped ascospores. Strains without ascomata can be recognized by their two typical anamorphs: *Scedosporium apiospermum*, with long, cylindrical conidiogenous cells producing slimy heads of subhyaline to brown, subspherical to elongated conidia, and *Graphium eumorphum*, with erect synnemata (a bundle of conidiophores sporulating in the apical part) (118). *P. boydii* is usually recognized by its morphological features, but to facilitate its identification, several serological techniques have been developed (316, 355, 365), which can even recognize the pathogen in tissue (259). Several physiological properties of *P. boydii* have also been investigated for use as diagnostic tools to differentiate it from similar species such as *Scedosporium prolificans* (102). These tests include cycloheximide tolerance and assimilation of glucose, fructose, sucrose, raffinose, inulin, biotin, thiamin, nitrates, higher alcohols, and sugar acids (90, 123, 464). Strains isolated from superficial infections showed isoenzymatic profiles that were different from those of strains isolated from disseminated infections (72). Analysis of partial large-subunit (LSU) rDNA (307) and of the entire SSU

<table>
<thead>
<tr>
<th>Genera</th>
<th>Anamorphs</th>
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<tr>
<td><em>Microascus</em></td>
<td><em>Scopulariopsis</em></td>
</tr>
<tr>
<td><em>Pseudallescheria</em></td>
<td><em>-graphium</em></td>
</tr>
<tr>
<td><em>Ophiostoma</em></td>
<td><em>Scedosporium</em></td>
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<td><em>Sporothrix</em></td>
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**Table 2. Ascomycetes of clinical interest and their anamorphs: Microascales and Ophiostomatales**
rDNA sequences (258) demonstrated that there is a close relationship between *S. apiospermum* and *S. prolificans*.

*Microascus* (Table 2) is a similar genus, which can be differentiated from *Pseudallescheria* by its ostiolate ascoma and its reniform, heart-shaped or triangular, and yellowish to straw-colored ascospores, which are extruded as a sticky, dark mass. In culture, *Microascus* spp. usually produce numerous ascoma and *Scopulariopsis* anamorphs. Three species, *M. cinctus, M. cirrosum*, and *M. mangini*, have been reported to be pathogenic to humans (118). The recent new genus *Ascobus subrenatum* is reported to cause chromoblastomycosis in India and with a supposed *Fonsecaea*-like anamorph (446), is also without doubt a species of *Microascus* with a typical *Scopulariopsis* anamorph (200a).

Severe anamorphic pathogenic species without teleomorph connections are closely related to this order. They are *Scedosporium prolificans* and *Scopulariopsis* spp. *S. prolificans* is an emerging pathogen which was named *Scedosporium inflatum* (208) until relatively recently. In the first cases described it showed an evident predilection for cartilage and joints (587). Of note, since 1991, numerous severe cases of disseminated infections were noticed in immunocompromised patients and resulted in death in most patients (58, 202, 327). In Spain at least 20 cases have been reported in the last 5 years, and there have been numerous unpublished cases. In 1997 our unit received six clinical strains for identification, all of which were from cystic fibrosis and leukemia patients. *S. prolificans* is very similar to *S. apiospermum*, but the conidiogenous cells are inflated at the base and are frequently in a brush-like arrangement. Also, it does not assimilate ribitol, xylitol, and L-arabinitol. On the basis of SSU rDNA sequences, Issakainen et al. (207) pointed out that the teleomorph of *S. prolificans* could be a species of *Petriella*. San Millán et al. (475) developed a RAPD technique to differentiate clinical isolates which can be applied to epidemiological studies and also can be used to differentiate this species from *S. apiospermum*.

*Scopulariopsis* spp. (Table 2) are different from *Scedosporium* spp. because they produce dry, hydrophobic conidia in chains rather than sliny conidia in heads (118). *Scopulariopsis* is a large genus comprising mainly soil species which are frequently isolated from food, paper, and other materials; they also occur as laboratory contaminants. Several species may be involved in human onychomycosis or pulmonary mycoses, and they have recently been associated with invasive human infections (118). There are eight pathogenic species, which differ from each other mainly in the size of the conidiogenous cells and the colony colors (118).

**Ophiostomatiales.** The ophiostomatoid fungi are important plant pathogens, some of which are adapted to insect dispersal (589). In this order there are only a few species of clinical interest which belong to the genus *Ophiostoma* (Table 2). *Ophiostoma stenoceras* has occasionally been reported to cause onychomycoses (522).

The most important pathogen in this group is the dimorphic fungus *Sporothrix schenckii*, which causes human sporotrichosis. In particular, it causes characteristic lesions at regional lymph nodes which often suppurate, ulcerate and drain (457), although other types of lesions are also occasionally produced. This fungus is characterized by unicellular conidia on clustered denticles (Table 2) and by the development of a yeast-like state at 37°C. It is able to assimilate starch, creatinine, and nitrate, and it requires thiamine (118). *S. schenckii* can be differentiated from the *Sporothrix* anamorph of *O. stenoceras* by its brownish colonies and brown conidia, whereas colonies of *O. stenoceras* are white and the conidia are hyaline. *S. schenckii var. lutei* (4) is different because it produce large, often septate budding cells and is unable to assimilate creatine and creatinine. A fatal infection caused by this variety was reported by Padhye et al. (418). Serological methods (394), pulsed-field gel electrophoresis analyses of the karyotypes (535), and RFLPs of mtDNA (529) have been used to identify strains. Although no teleomorph has been associated with this species, molecular studies demonstrated that *S. schenckii* is related to *Ophiostoma* (34, 307). *Sporothrix* is considered a heterogeneous genus, because some species have been associated with phylogenetically unrelated teleomorphs such as *Ophiostoma* spp. and *Pseudotrainiella ovale* (Eurotiales), the latter of which has caused two reported cases of onychomycoses (149). *Ceratosporium cyanescens* (syn. *Sporothrix cyanescens*) is a morphologically similar species, which is also occasionally pathogenic to humans, but the conidial scars are very small and its cultures produce a diffusible violet pigment; it probably belongs to the Basidiomycota (494). Until relatively recently, there was a tendency to place *Ophiostoma* in synonymy with *Ceratocystis*. They are morphologically related and produce similar long-necked ascoma, although it seems that they are phylogenetically very distant (233). Barr (18) placed *Ophiostoma* in the Microascales and *Ceratocystis* in the Sordariales. This placement is not currently accepted (238), and the relationship, or lack of relationship, between the two genera is not yet resolved (460).

**Sordariales.** Approximately 120 genera and 700 species have been included in the order Sordariales (238). The six genera that comprise the opportunistic species are *Chaetomium*, *Ar- niutium*, *Achaetomium*, *Corynascus*, *Neurospora* and *Thielavia* (118) (Table 3). The inclusion of other genera with opportunistic species such as *Ascochitra* and *Glomerella* (Table 3) is doubtful. The genus *Chaetomium* is one of the largest genera of saprobic ascomycetes and includes about 80 species (565). The distinctive characteristic of nearly all *Chaetomium* species is the presence of long, usually coiled, hair-like appendages covering the ascomata. Some *Chaetomium* species are commonly found in warm, dry areas on dung, straw, paper, textiles, seeds, plant debris, bird feathers, and many other substrates; others are mainly soil borne. Many species show cellulolytic activity, and cellulose is required for good growth and abundant fructification of some species. The ascospores are dispersed by insects and other animals and are very resistant. Three or four species of *Chaetomium* are opportunistic pathogens of humans (118, 207). *Chaetomium globosum* is the most common species and has been isolated in at least 10 clinical cases (207). *Neurospora sitophila* is the teleomorph of *Chrysosporium sitophila* (syn. *Monilia sitophila*), a common laboratory contaminant that is usually known as “bread mold,” which has caused several cases of human infection (253, 444, 541). This and other species of the genus have been the subject of a surprising number of genetic and molecular studies. It is considered the “Drosophila” of the fungi.

**Hypocreales.** The order Hypocreales is divided into three families and approximately 900 species (238). These species are characterized by having fleshy, usually brightly colored perithecial ascomata, occasionally cleistothecial or stromatic, and their ascospores are hyaline to pale brown and usually elongated and septeate. The teleomorphs, with the exception of *Neocosmospora* (Table 4), which was recently reported as causing infections in humans (93, 264), are rarely found in culture, although their anamorphs grow easily in routine culture media and are frequently isolated from clinical sources. Several of them, such as some species of the genera *Fusarium* (57, 204), *Acremonium* (165, 201), and, less frequently, *Trichoderma* (373) and *Cylindrocarpon* (262) (Table 4), are parasites of plants or saprobes and may infect humans. The species of *Fusarium* (Table 4) are characterized by the
<table>
<thead>
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<th>Genera</th>
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<tbody>
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<td>Achaetomium</td>
<td>—</td>
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<tr>
<td>Arnium</td>
<td>—</td>
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<tr>
<td>(?) Ascotricha</td>
<td>Dicyma</td>
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<tr>
<td>Corynascus</td>
<td>Myceliophthora</td>
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<tr>
<td>Chaetomium</td>
<td>—</td>
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<tr>
<td>(?) Glomerella*</td>
<td>Colletotrichum</td>
</tr>
<tr>
<td>Neurospora</td>
<td>Chrysonilia</td>
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<tr>
<td>Thielavia</td>
<td>Acremonium</td>
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*The fruiting bodies of this genus are not usually found in cultures of clinical isolates.*
production of falcate macroconidia, with several transverse septa and often with a beaked apical cell and a pedicelate basal cell, produced in dense cushion-like structures (sporodochia). Many species also form conidia in the aerial mycelium on phialidic or blastic conidiogenous cells. Pascoe (423) called those formed on blastic conidiogenous cells “mesoconidia,” in contrast to macroconidia and microconidia, which are both borne on phialides. Guarro and Gené (203) considered the presence or absence of mesoconidia to be a useful characteristic for identifying species of clinical interest. However, Nirenberg (393) argued that the term “mesoconidia” should not be used since it implies that the size of the conidium lies between those of microconidia and macroconidia. He suggested using the term “blastoconidia.” Teleomorphs of some *Fusarium* species occur in the genera *Gibberella* and *Nectria* (Table 4) (182, 291). *Gibberella* has ascospores with two or more septa and violet-blue or blue-black perithecia. *Nectria* forms ascospores with one or more septa, and its perithecia are white, pale orange, or bright red to brown.

The genus *Fusarium* has possibly attracted more interest

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**TABLE 4. Ascomycetes of clinical interest and their anamorphs: Hypocreales**

<table>
<thead>
<tr>
<th>Genera</th>
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<tr>
<td><em>Gibberella</em></td>
<td><em>Fusarium</em></td>
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<td><em>Hypocrea</em></td>
<td><em>Trichoderma</em></td>
</tr>
<tr>
<td><em>Nectria</em></td>
<td><em>Acremonium</em></td>
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<tr>
<td><em>Cylindrocarpon</em></td>
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<tr>
<td><em>Fusarium</em></td>
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<tr>
<td><em>Neocosmospora</em></td>
<td><em>Acremonium</em></td>
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* The fruiting bodies of these genera are not usually found in cultures of clinical isolates.
from a wider range of scientists and technologists than any other group of fungi because of its worldwide distribution and its involvement in plant pathology, human and animal toxicoses, and severe infections in neutropenic patients. It is no surprise, therefore, that numerous taxonomic schemes have been proposed in an attempt to make identification easier. Most clinical mycologists who do not have extensive knowledge of mycology are commonly unable to identify unusual strains of Fusarium and very frequently require the services of experts. Hence, a practical and reliable system for Fusarium identification that can be used under most laboratory circumstances is needed. Such a system has not been forthcoming, although a number of keys and laboratory manuals are now available. The most important contributions are those of Wollenweber and Reinking (593), Booth (55), Gerlach and Nirenberg (186), and Nelson et al. (382), among others, and those of Guarro and Gené (203) and de Hoog and Guarro (118) for the exclusive clinical species of clinical interest.

Due to the complexity of the genus, no international agreement on the systematics of Fusarium has been reached. The different number of taxa accepted within the genus by the Fusarium specialists is clear evidence of the confusion. A typical example of this chaos is the pathogenic species F. moniliforme, which is considered to be a valid species by Nelson et al. (382) and a synonym of F. verticillioides by Nirenberg (391) and Gams (178), who use criteria of name priority. Both names are widely used.

The taxonomy of Fusarium is based on numerous morphological features. Primary characteristics include the shape of the macroconidia, the presence or absence of microconidia and their shape, and whether the microconidia are borne in chains. Occasionally, some specimens develop only microconidia, but this problem can often be attributed to mutation or to growth of cultures under nonstandard conditions. This is relatively common in clinical isolates after several subcultures. In such cases, some confusion with other similar opportunistic genera such as Cylindrocarpon and Acremonium can occur. Gams and Nirenberg (182) proposed a polythetic definition for the genus that takes into account numerous variations and that enables the identification of isolates that form only microconidia. The type of conidiophores and conidiogenous cells and the presence or absence of sclerotia or sporodochia are of interest. Some macroscopic characteristics such as the texture of the colonies, pigmentation, and growth rate can be useful if they are determined by standardized methods. In contrast, the size and septation of conidia, which are determinative in other genera, are of limited value here (588). Some of the Fusarium classification systems currently used are based on establishing “sections,” which are taxa formally recognized by the ICBN. Each section within Fusarium contains species that have common morphological characteristics, although some taxonomists have placed a given species in different sections. To further complicate the delineation of sections, some of the recently described species such as F. napiforme and F. nygami have some characteristics of both the Elegans and Liseola sections. Both species were also isolated recently from clinical samples (290, 343). A fundamental problem inherent to Fusarium identification is that its species can have a wide variety of morphological and nonmorphological characteristics (588). Single-ascospore cultures have been used to study the range of variation within a species (497).

To aid in the identification of isolates and stimulate the sporation process, several media with low concentrations of nutrients have been developed. Two of these media, the synthetic medium SNA (392) and carnation leaf agar (166), constitute important methodological advances. The macroscopic aspect has still to be observed on other media, mainly potato dextrose and oatmeal agars, for the species to be completely described (467).

Another problem in the identification of Fusarium species is the frequency of mutation both in nature and in culture. The ideal phenotype of Fusarium in culture is the sporodochial. However, cultures from stressed strains or from patients treated with different antifungal agents frequently express different mutational phenotypes. These appear as sectors or throughout the colony, consisting of abundant production of white aerial mycelium and the almost complete absence of sporodochia. Sometimes the mutant cultures are more intensively pigmented than the sporodochial type (588). To avoid such mutation, it is recommended that cultures be initiated from single conidia (382, 588). Mutations in pathogenic isolates, at least those isolated from plant diseases, result in a decrease or loss of virulence (382, 588).

The numerous drawbacks which are inherent in the morphological criteria of Fusarium taxonomy have encouraged alternative approaches for evaluating taxonomic, phylogenetic, or pathogenic relationships between or within Fusarium species. Physiological (56, 256, 573), genetic (56, 442), and molecular (8, 142, 199, 278, 313, 315, 321, 387, 406, 408) techniques have been attempted. These tools can be very valuable in defining and confirming the boundaries and relationships of classical sections and species. Whether these or similar techniques will replace microscopic examination of cultures and make identification easier for nonspecialists is doubtful because, in our experience, this is still the best system for identifying species. However, molecular techniques have provided interesting data which could be useful, particularly for resolving concepts of confusing species. Certain polymorphism has been observed in ITS regions in Fusarium; e.g., some discordance in the nuclear rDNA-ITS2 was detected in a recent analysis of the phylogenetic relationships of the Gibberella fujikuroi and F. oxysporum species groups which, apart from F. solani, are the most common species of clinical interest. The species tested had two highly divergent nonorthologous ITS2 types (405, 408), which could explain the divergence observed in strains of F. oxysporum in other studies (8, 142). O’Donnell and Cigelnik (408) demonstrated by cladistically analyzing DNA sequences obtained from multiple unlinked loci (nSSU rDNA, nuclear 28S rDNA, and β-tubulin gene) that the morphological concepts of species which are commonly applied to these groups are often artificial. By comparing sequences of the divergent domains D1 and D2, eight Fusarium species have been differentiated (142, 199). Analyzing these regions, O’Donnell (406) argued that Gibberella is monophyletic. These results support the taxonomic scheme of Gerlach and Nirenberg (186) rather than that of Nelson et al. (382), because some of Nelson’s varieties are phylogenetically distinct. O’Donnell (406) also reported that many of the sections within Fusarium are artificial and suggested that they should be abandoned or redefined. A rapid molecular technique based on the digestion of nDNA with EcoRI and subsequent hybridization analysis distinguished three closely related species, F. moniliforme (F. verticillioides), F. nygami, and F. napiforme (315). These three opportunistic pathogens are difficult to distinguish if only morphological criteria are taken into account.

Acremonium (Table 4) is a cosmopolitan, morphologically simple genus (177), characterized by solitary, slender phialides or weakly branched conidiophores that arise from vegetative hyphae and bear a wet cluster or dry chains of mostly small and one-celled conidia. The hyphae are sometimes bound together into “strings”. Species of Acremonium are common in substrates such as soil, plant debris, and rotting mushrooms. Te-
The teleomorphs of *Acremonium* are found in several genera of ascomycetes such as *Emericellopsis*, *Hapidospora*, *Nectria*, *Nectriella*, *Neocosmospora*, *Providencia* (Table 4) and *Thielavia* (Sordariales) (Table 3). In the medical literature, the generic name *Cephalosporium* and especially the species name *C. acremonium* is still used for many different *Acremonium* spp. (201).

The role of *Acremonium* species in human infections has been known for a long time. In the old medical literature, cases such as mycetoma and ocular infections caused by *Acremonium* (*Cephalosporium*) spp. in tropical countries were frequently reported. However, the species involved were not usually reliably identified. In recent years, the number and diversity of the infections caused by *Acremonium* species have increased (165, 201) and numerous species have been implicated. Aggressive modern medical techniques and the appearance of new diseases and conditions which affect the immune system are the main predisposing factors. In a review of opportunistic fungi, de Hoog and Guarro (118) provided a key to help identify the 10 species of *Acremonium* which caused infection in vertebrates. In view of the high degree of similarity between *Acremonium* and some other genera such as *Phialoconidia*, *Lecythidium*, *Cylindrocarpon*, and *Fusarium*, it is possible that some clinical isolates were not correctly identified, especially in the last two genera, when the distinctive macroconidia are not otherwise poorly defined and may be heterogeneous. The same applies for the even less well-defined and more common *A. strictum*, although its involvement in human infection is uncertain (201).

Analysis of partial sequences of 18S rDNA clearly shows that the genus *Acremonium* is indeed polyphyletic, as the teleomorph connections had suggested (191). Affiliation of the type species, *A. alternatum*, and other common species such as *A. kilense*, *A. strictum*, *A. chrysogenum*, *A. murorum*, and *A. rutillum* to the Hypocreaceae suggests that the name *Acremonium* should be restricted to only the anamorphs of this family. A new genus has been proposed to accommodate other species of *Acremonium*, which are usually grass endophytes and are related to the *Clavicipitaceae*. For species that do not produce the teleomorph in culture, DNA sequence analysis would be needed for their correct placement. *Acremonium alabamense*, which was reported to be the etiologic agent of one case of human infection (579), is the anamorph of *Thielavia terrestris*, a member of Sordariales.

The species of *Trichoderma* are often isolated from decaying plants and from soil and are not generally regarded as important human pathogens, but there are scattered reports indicating occasional pathogenicity of some of them. *Trichoderma viride*, *T. longbrachiatum*, *T. pseudokoningii*, *T. koningii*, and *T. harzianum* were reported in recent years as occurring in humans (175, 184, 223, 260).

*Trichoderma* species are characterized by powdery colonies which grow quickly in routine culture media, and which have abundant green conidia and poorly defined conidiophores (Table 4) (43–46, 455). A closely related genus is *Tobysporodium*, the fungus that produces the powerful immunosuppressor cyclosporine; because of the similarity in arrangement of phialides, they have both been considered to be synonyms, although teleomorph connections seem to contradict this (473). The species of *Trichoderma* are defined on the basis of their morphology. However, the morphological characteristics usually used to recognize species in deuteromycetes are not useful in this genus. Riffai (455) used the concept of aggregate species and classified *Trichoderma* into nine species aggregates on the basis of colony growth and morphology of the conidiophores. Molecular and enzymatic studies demonstrated that some of the aggregate species are phylogenetically based (473, 511). Meyer and Plaskowtiz (348) described two types of conidial ornamentation in *T. viride*, and later, Meyer (347) found that mtDNA restriction fragment data distinguished between these two groups. He suggested that each group, characterized by conidial ornamentation and mtDNA type, could be a different species. Numerous studies summarized by Samuels (473) have demonstrated the usefulness of different macromolecular methods in the taxonomy of *Trichoderma*, mainly to establish groups within the different species aggregates. Muthumeenashkhi et al. (376) demonstrated the presence of polymorphism based on ITS variations. This strongly supports the two enzyme profiles found by Stasz et al. (511) in *T. harzianum*. Recently, Schickler et al. (479) developed an electrophoretic method for characterizing chitinases as a tool for the identifying *T. harzia-

**Bitunicate Pyrenomycetes**

The bitunicate Pyrenomycetes, also called Loculoascomycetes, are fungi that develop ascomata with bitunicate asci (Fig. 4, structure g). This sort of ascus has a distinctly bilayered wall, with the outer layer being rigid and the inner layer being expandable. As it matures, the thin outer layer splits and the thick inner layer absorbs water and expands upward. The ascus stretches up into the narrow neck of the ascoma, and the ascospores are expelled. These ascii with a “jack-in-the-box” design are also called fissitunicate. In contrast to the unuiticate Pyrenomycetes, which develop ascoma only after sexual stimulation, fertilization occurs after a solid primordium has developed in most bitunicate Pyrenomycetes. In the first case, the asci grow and mature inside an enlarged cavity. In bitunicate fungi, asci need room to grow. The asci can create this space themselves by either dissolving existing tissue as they grow or growing up between special sterile hyphae (pseudo-paraphyses) which grow down from the upper layer of the stroma (274). **Dothideales.** By focusing on the form of the fruiting body and the type of development, Barr (17) recognized 11 orders in the class Loculoascomycetes. Although this classification had originally been largely accepted, a more conservative scheme is used nowadays, with a wider concept of Dothideales (238). The main reason for this more cautious and provisional classification has been the lack of molecular data from which to infer the phylogeny of this group of ascomycetes (151), although molecular data seem to demonstrate the lack of monophyly of Dothideales. Loculoascomycetes appear to be paraphyletic in 18S rRNA trees of black yeasts (505, 548). Within the traditional Loculoascomycetes, the Pleosporales, an order not accepted presently (238), are represented as a monophyletic group with high bootstrap support when 18S RNA gene sequences are analyzed. This could mean that the presence of pseudoparaphyses, a typical feature of Pleosporales, is a good marker for a monophyletic group (32). Within the Pleosporales, the best-supported group was Pleosporaceae. A surprising aspect of Berbee’s study (32) was that species of black yeasts belonging to the family Herpotrichiellaceae clustered with members of the Plectomycetes rather than with other black yeasts. This suggests that either the bituniculate asci evolved independently into at least two lineages of filamentous ascomycetes or the Plectomycetes are phylogenetic Loculoascomycetes that have lost this characteristic. The Dothideales sensu lato is the most varied group of the Ascomycota, comprising 58 families and around 5,000 species (238). Most ascolocular as-
comycetes with bitunicate asci are included here, although molecular data suggest that this single broad order should be divided. The bitunicate teleomorphs of the pathogenic and opportunistic species are included in four families of the Dothideales (Table 5), i.e., Mycosphaerellaceae, Herpotrichiellaceae, Pleosporaceae, and Dothideaceae.

(i) Mycosphaerellaceae. The members of the family Mycosphaerellaceae are plant pathogens or saprobes. Some species of Cladosporium together with Penicillium spp. are the most abundant fungi present in the human environment and are particularly prevalent in air (78). However, human infections caused by these species are extremely rare, and the species are
regarded as typical opportunists, which occasionally infect animals but which have their normal ecological niche elsewhere (329). Masclaux et al. (329) performed a phylogenetic study of species of *Cladosporium* and *Xylohypha* which are responsible for subcutaneous and systemic mycoses. From the analysis of partial sequences of LSU rRNA, they demonstrated that human pathogenic and saprobic *Cladosporium* species were phylogenetically distinct from each other. On the basis of known teleomorph relationships, the two groups of species were considered to belong to the Herpotrichiellaceae and Mycosphaerellaceae, respectively. These species were therefore reclassified in different anamorphic genera. *Cladosporium* was restricted to plant-associated species and characterized by differentiated conidiophores with protruding dark conidial scars (Table 5) which were unable to grow at 37°C (329). The most pathogenic species of *Cladosporium* were transferred to the genus *Cladophialophora*, and the genus *Xylohypha* was considered unrelated (120, 329).

(ii) *Herpotrichiellaceae*. There are important human pathogens in the family *Herpotrichiellaceae* which cause invasive infections in immunocompetent hosts without eliciting a strong cellular immune response (114). In the genus *Cladophialophora*, in contrast to *Cladosporium*, each species causes its own characteristic infection (118). The species can be distinguished not only by their morphological characteristics but also by nutritional and physiological tests. *meso*-Erythritol, *l*-arabinose, ethanol, and growth at 40°C were found to be the most useful criteria (120). *Cladophialophora bantiana*, formerly known as *Cladosporium bantianum*, *Cladosporium trichoides*, and *Xylohypha emmonsii*, shows a remarkable predilection for causing fatal infections in the central nervous system (131, 147), although it is occasionally isolated from soil (130).

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<td>Mycosphaerella</td>
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* The fruiting bodies of these genera are not usually found in cultures of clinical isolates.
* Black nodule attached to the hair shaft.
Cladophialophora carrioni is a common etiologic agent of verrucous chromoblastomycosis and phaeomycotic cysts in warm, arid areas (457). The other three species of Cladophialophora, i.e., C. devriesii, C. arxii, and C. boppfi, are only rarely reported to cause human infections.

Exophiala (Table 5) is the main genus of black yeasts, and its taxonomy is very complicated because the species are highly pleomorphic and have anamorph life cycles with widely divergent types of propagation (126). Some cultures are entirely yeast-like (synanamorph Pheaeococcymyces) or form phialidic collarettes (synanamorph Phialophora) or sympodial conidiophores (synanamorph Rhinocladiella). Chlamydospores or scerolot bodies may also be formed. The species in this genus cannot be correctly separated only by morphological studies. These studies should be complemented by physiological tests, the most important of which measure assimilation of lactose, D-glucuronate, D-galacturonate, nitrite, creatine, and creatine, tolerance to cycloheximide, and growth to different temperatures (117, 118). Some species have been distinguished by approaches based on coenzyme Q analysis (516, 596), karyology (530), sequence analysis of the chiin synthase gene (59), serology (157, 352), and flow cytometry (528). Recently, Uijthof and de Hoog (546) used type strains to perform RFLP analysis on all the described taxa of the genus and showed that most of them are bona fide species. RFLPs of mtDNA were also found to be useful for delineation, identification, and epidemiology studies in Exophiala (257, 271, 272). It has been demonstrated that C. spinifera, which causes several types of infections mainly in tropical regions (388, 417, 445), is a complex species (257). De Hoog and Haase (121) studied the nutritional physiology of E. dermatitidis, an occasional agent of systemic infections (252, 331), and found it to be the only species of the genus which can grow at 40°C. RAPD can distinguish several populations within E. dermatitidis (545).

Phialophora is a heterogeneous genus with teleomorphs in Capronia (Herpotrichiellaceae) and Mollisia (Discomycetes). Small numbers of Phialophora-like conidiogenous cells are also produced under some circumstances in a variety of pathogenic dematiaceous fungi, such as Fonsecaea compacta, E. dermatitidis, and C. carrioni. Five species of Phialophora have been described as being pathogenic to humans (118) and cause a wide variety of infections. The three species most frequently found in humans, P. verrucosa, P. parasitica, and P. richardiae, were characterized by restriction enzyme mapping of the ITS and 5.8S rDNA region (598). Each species was placed in a well-supported clade which indicates monophyly. Phialophora americana and P. verrucosa, considered by some to be synonymous, were found to be closely related but in clearly different clades. P. parasitica was found to be the most genetically variable species (598). Recently, it has been proposed that P. parasitica and an additional small group of species which are associated with human infections and the disease symptoms of several woody hosts be included in the new genus Phaeacremomum (104). This genus is morphologically intermediate between Acrocomium and Phialophora. Phaeacremomum is distinguished from Phialophora by its aculeate conidiogenous cells and inconspicuous collarettes and from Acrocomium by its pigmented vegetative hyphae and conidiophores. In a comparative study involving PCR-ribotyping techniques of this family and Pleosporaceae, de Hoog (114) suggested that Herpotrichiellaceae evolved more dynamically because of the insertions in SSU and ITS rDNA and the variability of restriction patterns. This would explain the wide range of clinical pictures caused by the members of Herpotrichiellaceae. It should be pointed out that all dematiaceous neurotropic fungi, with the exception of Ochroconis gallopava, are members of a single clade in LSU phylogeny (329). The species Cladophialophora bantiana, Ramichloridium mackenziei, and E. dermatitidis were grouped with several Capronia teleomorphs, which are known members of Herpotrichiellaceae (548).

(iii) Dothideaceae. The family Dothideaceae comprises mainly epiphytic and plant-associated fungi (114), although there are several human pathogens such as Hortaee werneckii, the agent of tinea nigra, a mild cutaneous infection usually of the palm of the hand. This species was probably assigned to this family by analyzing the LSU rRNA sequences (329). PCR fingerprinting studies demonstrated that strains of human origin were grouped with strains from nonhuman sources (544). One feature which is important in distinguishing this species from other dematiaceous pathogenic fungi is its tolerance to high concentrations of NaCl (115, 265). Aureobasidium pullulans, another opportunistic species of this family, is commonly found in somewhat osmotic environments but also causes infection as a consequence of traumatic inoculation. Cases in immunocompromised patients have also been described (98, 118, 190). This fungus shows peculiar morphological characteristics such as the production of slimy colonies which vary in color during their development and different types of conidia, i.e., synchronously produced conidia, budding conidia, endoconidia, and chlamydospores. de Hoog and Yurlowa (128) demonstrated that reliable identification of this species requires a combination of conidiogenesis features, expansion growth, and assimilative abilities. Hormonema dematioides is a very similar fungus but can be differentiated from A. pullulans by the absence of synchronous conidiation (Table 5) and by different physiological profiles. It is also occasionally pathogenic to humans (99, 275).

This family is found to be monophyletic by analysis of both SSU (548) and with LSU (329) rRNA sequences. The molecular diversity in rDNA RFLP patterns found by de Hoog et al. (127) in this family was unexpected, given the poor morphological differentiation of its anamorph members. This heterogeneity might be explained by a process of rapid evolution and dynamic speciation. The family shows the greatest diversity in clinical pictures known for any group of fungi.

(iv) Pleosporaceae. The family Pleosporaceae comprises mainly pathogens on grasses (492), which are characterized by the production of large, darkly pigmented, multicelled conidia that leave a pore or a dark scar on the conidiophore after they are released. Their telemorphs are in the genera Cochliobolus, Lwia, and Setosphaeria (Table 5). Their ascomata are rather large and contain paraphyses; the asc are bitunicate and contain multilocular, pale or dark ascospores. When inhaled, conidia usually remain in the sinus due to their large dimensions. These species can cause severe invasive infections (477), mostly cerebral mycoses (338), but this should probably be interpreted as a complication of sinus colonization rather than as a primary pathogenicity (114). The genera involved in these processes are mainly Drechlera (Bipolaris), Exserohilum, Curvularia, and Alternaria (Table 5), which is probably the most common genus. The delineation of Alternaria species is complicated, and the species are identified only with difficulty by nonexperts. Simmons (491) has discussed the complexity of some of the species of the genus, e.g., A. alternata. Alternaria spp. are recognized and identified mainly by conidial morphology, which, it is argued, can be influenced by cultural conditions. Andersen and Thrane (7) used the combination of morphological characteristics, metabolite profiles, and color of colonies growing on dichloran rose bengal yeast extract sucrose agar to differentiate the two opportunistic fungi A. infectoria and A. alternata. Molecular studies also demonstrated the complexity of this group. For example, A. alternata, A. dianthicola,
and Ulocladium spp., despite being morphologically different, have identical digestion patterns (127). Botryomycetes caesitiosus is a rare agent known only by two strains isolated from subcutaneous infections (31, 124). Initially, this agent was considered a meristematic black yeast, but ITS RFLPs indicate a very close relationship to Alternaria chamydospora, another recently reported opportunistic fungus that causes superficial infections (95, 490). RFLP analysis of the total DNA obtained with probe-enzyme combinations was useful for discriminating species of Drechslera and Cuvulatula (378). de Hoog (114) found that anamorph species of Pleosporaceae, which are morphologically very different from each other, had very similar rDNA RFLP patterns. This could be because the Pleosporaceae is a family of evolutionary established plant pathogens. This family represents a robust monophyletic branch in the order Pleosporales (134, 225).

Plectomyces

Members of the Plectomyces are characterized by the production of cleistothecial ascomata, i.e., closed fruiting bodies with no paraphyses, more or less globose asci that are irregularly distributed in the ascomatal cavity (Fig. 4, structure d), and unicellular ascospores, which lack germ pores or germ slits and which are frequently bivalvate (162). The asci are uniloculate (Fig. 4, structure f) and have no active spore-shooting mechanism. Sometimes the ascus walls dissolve at maturity and release the ascospores, which can be extruded but are not forcibly discharged out of the ascma; alternatively, they may stay inside until the walls decay or rupture. These asci are sometimes called protuncinate. However, because these asci are found in several rather different orders, it seems very probable that they are a secondary condition and have evolved several times from uniloculate asci (410). Fennell’s (162) concept for the Plectomyces was reviewed by Malloch (320) and Benny and Kimbrough (30), who redefined the Plectomyetes on the basis of centrum development and the mode of asci discharge. Benny and Kimbrough (30) recognized six orders: Ascosphaerales, Elaphomyctesales, Eurotiales, Microasciales, Onygenales, and Ophiostomatales. However, the term Plectomyetes had fallen into disuse for the reasons explained above. In recent years, molecular data have added to our understanding of the relationships between the high taxonomic categories of ascomycetes, and Berbee and Taylor (33) used 18S rDNA sequence analysis to argue that the class Plectomyetes should be reinstated. This has not been widely accepted, the main argument being that their circumscriptions differ from those based only on ascomatal type (238).

Eurotiales. Approximately 10 genera comprise the 50 to 60 species of the Eurotiales, which have been associated with human infections, and practically all of them belong to the family Trichocomaceae. However, only a few species of Aspergillus (A. fumigatus, A. flavus, and A. terreus) and Penicillium marneffei are of real pathological significance. The remaining species only occasionally cause mycosis. Eurotiales are characterized by possessing cleistothecial ascomata with bright walls, thin-walled, evanescent, clavate or saccate asci sometimes forming chains, and ascospores which are very variable but usually small, aseptate, often ornamented, and with equatorial crests or furrows. The anamorphs are typically represented by the common hyphomycetes Penicillium, Aspergillus, and Paecilomyces (Table 6), which always predominate in culture, although ascomata are also frequently produced. These anamorphs form conidia which emerge from phialides (awl-shaped or flask-shaped conidiogenous cells) and form chains.

Eurotiales were recently comprehensively revised by von Arx (564), who proposed that the order Eurotiales sensu lato should include the Onygenales and the Eurotiales sensu stricto. This criterion has not been followed by other mycologists or by the Dictionary of Fungi (238). However, Berbee et al. (38), by analyzing 18S, 5.8S, and ITS rDNA sequences, demonstrated that Onygenales are basal to the Trichocomaceae and that together they form a strongly supported clade. This would confirm the classification scheme of von Arx (564). Berbee et al. (38) also demonstrated that Penicillium, Aspergillus, and Paecilomyces are assigned to the Trichocomaceae and that Penicillium is not monophyletic.

Eleven species of Penicillium have been reported to occur in vertebrates (118). However, the only pathologically significant one is P. marneffei, a species that occurs naturally in bamboo rats in southeast Asia. Because of the AIDS pandemic, this species has acquired considerable importance (137, 285). It possesses a particular characteristic, unique in the genus, which is a fission yeast-like phase when grown at 37°C. However, in culture it grows and sporulates poorly. Diagnosis of P. marneffei infections is usually difficult because the symptoms are very similar to those of infections caused by other fungal pathogens such as Histoplasma capsulatum. In vivo, the species differs from Histoplasma by the presence of some septate and slightly curved cells. Species-specific ITS-based primers have been developed by LoBuglio and Taylor (312). RAPD analysis demonstrated that strains isolated from AIDS patients form a genetically homogeneous group which differs only slightly from an isolate from a bamboo rat (342). The identification of the remaining Penicillium species that occasionally infect humans is difficult. The taxonomy of Penicillium has always been complex due to its great number of species (nearly 250), which have very few differences. Its colonies are usually green, and the conidiogenous cells are cylindrical to bottle-shaped and aggregated in compact penicilli. The first taxonomic review of the genus was made by Raper and Thom (449). Recently, the works of Samson et al. (472) and Pitt (432) have made important contributions to its taxonomy. Pitt (432) introduced a new approach based on gross physiology under standardized conditions; i.e., he used colony growth rate in response to parameters such as medium composition, temperature, and water activity. However, these procedures are not yet effective for all species of the genus (434).

The species of Aspergillus that cause human infections are more numerous than those of Penicillium, and their pathogenicity is also more important. The reference systematic works for the identification of the species are those of Raper and Fennell (448), Samson (465), Samson et al. (468), and van den Bossche et al. (550). Approximately 180 species are accepted in the genus Aspergillus (181). The circumscription and classification of these species have been based primarily on differences in cultural and morphological characteristics. The complexity of the genus is emphasized by the number and diversity of associated teleomorphs (12 in the Trichocomaceae) and by the heterogeneity of ubiquinone systems (293). As well as A. fumigatus, a common cause of pulmonary and other severe infections in immunocompromised patients, another 34 species have been isolated from humans. PCR-based molecular detection of A. fumigatus by using a fragment of 26S rDNA was described by Spreadbury et al. (508) and by using interrepeat PCR by van Belkum et al. (549). A combination of SEM techniques and mycotoxin profiles was also used to clarify the taxonomy of the genus Neosartorya (Table 6), which comprises the teleomorph of A. fumigatus and related species (469).

Although the identification by traditional morphological criteria is very effective in these two large and complex genera, the large number of species that exist has created a need for
alternative approaches. Secondary-metabolite production, particularly mycotoxins, provides metabolic profiles which can now also be useful (96, 145, 170–172, 424). Determination of isoenzyme patterns has permitted some difficult species to be classified (105). Numerous other approaches have been used to delineate species, including the presence of exocellular polysaccharides (113), biotyping methods by the yeast killer system (438), the ubiquinone system (292), serologic testing (173), pectinolytic enzymes (282), and rDNA restriction patterns (539). Molecular recognition of species by using the V7 to V9 variable domains of 18S rRNA was performed by Melchers et al. (345). RFLP by itself or with Southern blot analysis of mtDNA or nDNA or both has been used to indicate relationships among Aspergillus isolates (103, 194, 281, 287). Numerical taxonomic studies based on combinations of different types of biochemical, physiological, and morphological characteristics have also been used (65, 66, 425). Results obtained by different methods have not always agreed. For example, data obtained by RFLP from 18S rRNA sequences (517) disagreed with those obtained by the DNA-DNA reassociation method used by Kurtzman et al. (297, 303). To facilitate the handling of such a large number of species, several computer-assisted keys have been developed (280, 433, 585).

The International Commission on Penicillium and Aspergillus has organized three workshops (470, 471) which have been the main recent contribution to the systematics and nomenclatural stability of both genera. At these meetings, the most important aspects concerning the taxonomy of these genera were addressed. Topics such as nomenclature, terminology and methodology, taxonomic schemes, and new approaches to classification were debated, and the newest advances were presented. At the third meeting, held in May 1997 in Baarn, The Netherlands, a wide range of topics were discussed, in particular the application of molecular biology techniques to the taxonomy of these genera, including DNA sequencing, RFLPs, RAPDs, microsatellites, single-stranded conformation poly-

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morphisms, and heteroduplex analysis. The results and conclusions have not yet been published.

*Paecilomyces* (Table 6) is similar to *Penicillium*, but its colonies are not usually green and the conidiogenous cells are flask shaped with a strongly tapering tip, often rather pointed at the end, and usually aggregated in divergent clusters. It is also a heterogeneous genus whose teleomorphs clearly belong to different families (Trichocomaceae and Thermoascomycaceae) and they have significantly different response to antifungals (1). There are seven species which are described as opportunistic pathogens, but only *Paecilomyces varioti* and *P. italicus* have any relevant clinical importance (118). Here, too, the species are identified only by their morphology. The color of the colonies and the size and shape of the conidia are the most useful characteristics. *Paecilomyces fumosoroseus* is a highly variable species which has several RAPD fingerprint patterns and vegetative compatibility groups (86).

Recently, a very rare and interesting fungus, *Calyptrozyma arctic*, was isolated from a case of esophagitis. Although studies of the septal ultrastructure and rRNA sequences suggested that it belonged to Ascomycota, it could not be assigned to any of the present orders of that phylum. Its morphology suggested that it belonged to primitive Eurotiomycetes. However, a recent 5.8S rRNA sequence analysis suggests a close relationship with *Pezizales* (206). This fungus is typically pleomorphic, initially developing as a yeast and then producing hyphae. Both sexual and asexual reproductive structures are produced in the same thallus. Sexual reproduction is represented by naked ascii, and both blastic and thallic conidia are also produced (52).

**Onygenales.** The order Onygenales sensu Currah (107) comprises the four families Onygenaceae, Myxotrichaceae, Arthrodertaceae, and Gymnoascomycaceae and contains nearly 100 species. With exception of the black yeasts, most true pathogenic fungi such as the dermatophytes and the dimorphic fungi belong to this order (Table 7). They are characterized by their simple and rudimentary ascocarps formed by asci, which are surrounded by a more or less differentiated hyphal network, sometimes with complex appendages. Occasionally, the ascomata are reduced to clusters of asci. The asci are small, globose to ovate, and with evanescent walls. The ascospores are small, oblate (in circular in face view and broadly ellipsoidal in lateral view), and usually light brown. They sometimes have different types of ornamentations, and they frequently have equatorial crests or furrows. The anamorphs are constituted by species which possess unicellular and solitary conidia, named aleurioconidia (Fig. 4, structure k) (*Chrysosporium*, *Histoplasma*) or conidia in chains of alternately viable and nonviable cells, named arthroconidia (*Malbranchea*), or with multicellular conidia (Fig. 4, structure l). In the traditional medical literature, this group of fungi is probably best known under the name Gymnoascomycetes (486). On the basis of sequences of the LSU rRNA which included the two nondivergent domains D1 and D2, Leclerc et al. (307) confirmed that the distinction of the Onygenales into four families proposed by Currah (107) was not justified after noticing that members from different families clustered together. Molecular data provided by Bowman et al. (63) using SSU rDNA sequences were consistent with but did not prove the monophyletic nature of the families Onygenaceae, Gymnoascomycaceae, and Arthrodertaceae. As mentioned above, von Arx (564) proposed an alternative taxonomic scheme. He classified the Eurotiomycetes, Gymnoascales, and Onygenales in the single order Eurotiomycetes sensu lato, which also included four families, according to the shape, size, and symmetry of the ascospores.

In the family Onygenaceae, there are numerous pathogenic species, for example, *Aphanoascus* spp. (Table 7) which are occasionally agents of superficial infections (82) and whose anamorphs are *Chrysosporium* spp., and *Neoaeroclinotheca keratinophila*, which has recently been described as the teleomorph of *Myriodontium keratinophilum* (84) and which has been reported as causing human sinusitis (322). Other, more pathogenic fungi include *Ajellomyces* spp. (Table 7), which are the teleomorphs of *Blastomycetes dermatitidis*, *Emmonsia parva*, and *Histoplasma capsulatum*. *Coccidioides immitis* and *Para- coccidioides brasiliensis* also seem to have affinities with this family. *C. immitis* is the agent that causes coccidioidomycosis, which in its disseminated form is often fatal. Primary infection is through inhalation of airborne propagules, leading to non-specific fever with bronchopneumonia, but generally the disease resolves spontaneously. Dissemination may occur through the hematogenous spread of endocinidia, and this is associated with a high degree of fatality. The fungus is taxonomically confusing because in vivo it produces spherules with numerous endoconidia and in culture it develops arthroconidia at 24°C and a yeast phase at 37°C. Hence, this species has been placed in three fungal classes and also in the Protista (62). Its classification in Onygenales, very close to *Uncinocarpus reesi*, has received strong molecular support (59, 60). Bowman et al. (63) compared SSU rDNA sequences of the four closely related pathogens *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis* and *Trichophyton rubrum* and seven other nonpathogenic fungi and noticed that the pathogens did not constitute a clade as hypothesized but were interspersed among the nonpathogenic ones. Sequences of the partial 18S rDNA gene demonstrated a close relationship between *C. immitis* and the nonpathogenic *Malbranchea* (62). Bowman et al. (63) argued that pathogenicity could arise several times within this group. The study also revealed that the conidium types aleurioconidia and arthroconidia, used to distinguish between the genera *Chrysosporium* and *Malbranchea*, may be less distinct than these names suggest. DNA probes for the recognition of *C. immitis* have been developed (25, 420, 513). *Emmonsia parva* is the agent of adiaspiromycosis. This infection refers to the in vivo development of adiaspores following inhalation of conidia of *Emmonsia*. The adiaspores, which apparently do not reproduce, remain in the alveoli of the host and eventually becoming calcified. Such elements are only rarely found in sufficient numbers to cause disease in human or animal tissue (457). The host reaction is only slight, although its effect is greater in AIDS patients (141). *E. parva* var. *parva* produces uninucleate adiaspores and is endemic in the southwestern United States. *E. parva* var. *crescens* forms comparatively bigger and thicker-walled adiaspores, which are multinucleated; this fungus has a worldwide distribution in temperate zones (132). Differences in their tolerance to cycloheximide and their ability to form a teleomorph seem to justify the two varieties being treated as different species (487). The perfect state of *C. immitis* and the nonpathogenic *Malbranchea* (62), Bowman et al. (63) argued that pathogenicity could arise several times within this group. The study also revealed that the conidium types aleurioconidia and arthroconidia, used to distinguish between the genera *Chrysosporium* and *Malbranchea*, may be less distinct than these names suggest. 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Differences in their tolerance to cycloheximide and their ability to form a teleomorph seem to justify the two varieties being treated as different species (487). The perfect state of *E. crescens* has recently been described in the genus *Ajellomyces* as *A. crescens* (487). The teleomorphs of *B. dermatitidis* and *H. capsulatum* are also included in *Ajellomyces* as *A. dermatitidis* and *A. capsulatum*, respectively. A close relationship among these three species, which suggests that they should be included in the same genus, has been demonstrated by the analysis of 18S rDNA sequences (62).

Histoplasmosis is a very common granulomatous disease that is caused by the inhalation of conidia of *H. capsulatum* and results in a wide range of clinical manifestations as a consequence of an intracellular infection of the monocyte-macrophage system. In vivo, *H. capsulatum* forms budding yeasts within these cells. When grown in culture, the fungus develops thick-walled and tuberculate, unicellular conidia, each of which...
emerges from a short, narrow, tubular conidiophore (Table 7). At 37°C, a yeast-like phase is produced. The African *H. capsulatum* var. *duboisii* differs by the production of larger budding cells in vivo. Vincent et al. (560) showed that mtDNA restriction patterns of both varieties are practically identical.

However, molecular data provided by Leclerc et al. (307) seem to confirm the varietal status of both *H. duboisii* and *H. farciminosum*, the latter of which is closely associated with lymphangitis in horses and mules (500), though this zoophilic species has diverged a little more significantly from both an-

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**TABLE 7. Ascomycetes of clinical interest and their anamorphs: Onygenales**

<table>
<thead>
<tr>
<th>Genera</th>
<th>Anamorphs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A inflictus</em></td>
<td><em>Blastomyces</em></td>
</tr>
<tr>
<td><em>Aphanoascus</em></td>
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<tr>
<td><em>Arachnomyces</em></td>
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<tr>
<td><em>Arthrodema</em></td>
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<tr>
<td><em>Emmonsia</em></td>
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<tr>
<td><em>Histoplasma</em></td>
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<tr>
<td><em>Chrysosporium</em></td>
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<tr>
<td><em>Onychocola</em></td>
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<tr>
<td><em>Keratinomyces</em></td>
<td></td>
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<tr>
<td><em>Microsporum</em></td>
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<tr>
<td><em>Trichophyton</em></td>
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throphophilic pathogens. Several probes have been developed to recognize the species (227, 255, 419, 513). Restriction patterns of mtDNA and RAPDs have been used to differentiate and typify intraspecific populations (324, 507, 560, 594).

*Paracoccidioides brasiliensis* is the agent of paracoccidioidomycosis. Initially a primary pulmonary infection that is usually subclinical, it can progress to a systemic, chronic granulomatous disease, occasionally occurring in otherwise healthy patients (40, 457). It is also a dimorphic fungus, producing only intercalary chlamydospores at 24°C whereas at 37°C it develops a yeast-like phase, which consists of spherical cells with synchronous thin-necked buds all over its surface. In an attempt to further delineate the taxonomy of this species, several nutritional and physiological studies have been performed (26, 85, 189, 422, 476), but attempts to correlate physiological patterns and virulence have failed (476). Serological techniques for the diagnosis of this species were described by Taborda and Camargo (527), and the karyotype analysis and genome sizing was determined by Nogueira et al. (398). RAPDs demonstrated intraspecific variations and characterized two different groups (498). Some controversy has existed about the taxonomic placement of this species. Biosynthesis reduction of tetrazolium salts and the canavaline-glycine-bromothymol blue reaction were similar to those caused by *Cryptococcus neoformans* (476), which suggested a basidiomycete relationship. Since RNA sequence comparisons revealed a close relationship with *H. capsulatum* and *B. dermatitidis*, *P. brasiliensis* is a probable member of the Onygenales (307).

The data of Leclerc et al. (307) emphasized the homogeneity of pathogenic species within the order Onygenales, with two clearly defined groups, i.e., the dimorphic fungi and the dermatophytes, included in the families Onygenaceae and Arthrodectarmataceae, respectively. The monophyly of the dermatophytes was also demonstrated in other molecular studies (229, 269). However, the phylogenetic distinction of three anamorphic genera, *Microsporum*, *Epidermophyton*, and *Trichophyton* (Table 7), within the dermatophytes seems unsustainable (270, 307, 360, 395). This would be in agreement with the combination of the two traditional dermatophyte teleomorphs *Nannizzia* and *Arthroderma* in the single genus *Arthroderma* (Table 7) (576). In recent years, numerous studies on dermatophytes, including their taxonomy, have been performed. Tanaka et al. (533) and Weitzman and Summerbell (577) have summarized these studies. These fungi are mainly classified now by using the taxonomic scheme proposed by Emmons (148), which stress the importance of the conidial morphology and accessory structures. On this basis, the three aforementioned anamorphic genera have been traditionally accepted (3, 457), although some authors have considered *Keratinomyces* (Table 7) to be a fourth genus (118, 219). The morphological characteristics used for differentiating some species and genera of dermatophytes, particularly between *Epidermophyton* and *Trichophyton*, seem to be inconsistent. Weitzman and Summerbell (577) also pointed out that some characteristics of these fungi overlapped. *Keratinomyces ceratianus*, considering its psychrophily (with optimum growth at 20°C) and its sensitivity to cycloheximide, appears to be one of the most different species (83, 441); molecular data seem to confirm this (200). However, this species has never been found in humans. Sequence data provided by Leclerc et al. (307) revealed the relationship of the common dermatophyte *Trichophyton rubrum* with *Ctenomyces serratus*, a member of Arthrodermataceae, as had been suggested by conidia morphology and mating-type reactions (251).

In the two remaining families of Onygenales, there are only a few pathogenic species (Table 7). *Myxotrichum deflexum*, a species of doubtful pathogenicity, is found in Myxotrichaceae. Gymnoascaceae contains *Gymnoascus dankaliensis*, which has caused some superficial infections (118), and *Arachnomyces nodosetosus*, which was recently proposed to be the teleomorph on *Onychocola canadensis*, a pathogenic species found exclusively in human nail and skin lesions. This agent is characterized by the production of long chains of arthroconidia and brown, sterile hyphae in old cultures (488).

### TABLE 7—Continued.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Anamorphs</th>
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<tbody>
<tr>
<td><strong>Gymnoascus</strong></td>
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</tr>
<tr>
<td><strong>Myxotrichum</strong></td>
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</tr>
<tr>
<td><strong>Neoarachnotheca</strong></td>
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</tr>
<tr>
<td><strong>Myrodonium</strong></td>
<td><img src="image.png" alt="Image" /></td>
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</tbody>
</table>

* The fruiting bodies of these genera are not usually found in cultures of clinical isolates.
<table>
<thead>
<tr>
<th>Genera</th>
<th>Anamorphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clavispora</td>
<td>Candida</td>
</tr>
<tr>
<td>Debaryomyces</td>
<td>Candida</td>
</tr>
<tr>
<td>Dipodascus</td>
<td>Geotrichum</td>
</tr>
<tr>
<td>Galactomyces</td>
<td>Geotrichum</td>
</tr>
<tr>
<td>Hansenula</td>
<td>Candida</td>
</tr>
<tr>
<td>Issatchenki</td>
<td>Candida</td>
</tr>
<tr>
<td>Kluyveromyces</td>
<td>Candida</td>
</tr>
<tr>
<td>Pichia</td>
<td>Candida</td>
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<tr>
<td>Saccharomyces</td>
<td>Candida</td>
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The term “yeast” is often taken to be a synonym for *Saccharomyces cerevisiae*, but the phylogenetic diversity of yeasts is illustrated by their assignments to diverse fungal taxa. Yeasts are neither a natural nor a formal taxonomic group but are a growth form shown by a range of unrelated fungi. In some cases, they are merely a phase of growth in the life cycle of filamentous fungi which takes place only under concrete environmental conditions. Different numbers of taxa have been estimated by the different authorities. In the most modern classification scheme, there are approximately 100 genera representing near 700 species (296), although the genetic relationship for some of the currently accepted taxonomic categories is still unknown. The teleomorphs of the yeasts of clinical interest are formally assigned to the Ascomycota, in the orders Saccharomycetales (see Table 8) and Pneumocystidales, or to the Basidiomycota in the orders Tremellales (see Table 9) and Ustilaginiales (see Table 10). Those not connected with teleomorphs are placed in the deuteromycetes.

The identification of yeasts has been traditionally based on morphological characteristics, but due to their poor differentiation, distinctive reactions in a standardized set of fermentation and assimilation tests have been more reliable. However, genetic crosses as well as molecular studies have demonstrated that those phenotypic characteristics are not adequate for defining taxa. Therefore, systematics have turned increasingly to molecular approaches to distinguish and identify yeast species and to develop a system of classification based on phylogeny. Kurtzman and colleagues analyzed partial sequences of SSU and LSU rRNAs and were the pioneers in yeast phylogenetic studies; they provided numerous and valuable data to assess taxonomic relationships between these fungi (296, 298, 301, 302).

Due to their simple morphology, the yeasts have been viewed as primitive fungi or reduced forms of more highly evolved taxa such as the Ophiostomatales (77). However, phylogenetic studies with different rRNA sequences clearly showed that the teleomorph yeasts, with the exception of the fission yeast genus *Schizosaccharomyces*, *Pneumocystis*, and a few more genera, are members of a monophyletic group which is separated from all filamentous fungi examined (16, 70, 233, 294, 295, 570). Since it is phylogenetically distant from the budding-yeast clade and from the euascomycetes, *Schizosaccharomyces* was reassigned to a separated order, the Schizosaccharomycetales (154, 294).

### Saccharomycetales

The order Saccharomycetales includes eight families, 75 genera, and 273 species (238), and its members are represented by vegetative cells which proliferate by budding or fission. They can also form a thallus with a poorly developed mycelium with a septum perforated by numerous pores (microporous) (Fig. 4, structure r) rather than by the single pore (structure q) typical of the ascomycetes. Their fruiting bodies are made up of single evanescent ascI or chains of such ascI. Usually ascI are morphologically identical to the vegetative cells (structure e). The ascospores are very varied in shape. The most important pathogenic species are in the genera included in Table 8.

*Candida* is a complex genus represented by 163 anamorphic species with teleomorphs in at least 13 genera (296). Some of these genera, such as *Pichia* (430) or *Debaryomyces* (299, 300) (Table 8), also seem to be polyphyletic. The genus *Candida* is divided into 12 physiological groups for identification purposes. The genetic origin of these groups is unknown (296). Thirty years ago, only a few pathogenic species of *Candida* were known (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. guilliermondii*). However, in recent years the number of emerging species related to human infections has increased considerably (110, 240, 519). Now nearly 20 species have been identified as being associated with human infections. Recently, Hazen (241) reviewed the main traditional characteristics used to identify these new pathogenic *Candida* spp. de Hoog and Guarro (118) described key physiological features useful for the identification of the clinically relevant species. PCR ribotyping for species recognition was developed by Niesters et al. (389) and Maiwald et al. (319). Jordan (263) developed probes based on chitin synthase genes. The role of molecular and genetic approaches used in the identification and typing of these species was discussed by Sullivan et al. (520). A technique for differentiating related species which is based on pyrolysis-mass spectrometry has recently been developed (542). The phylogeny and relationships of the most important pathogenic species of *Candida* and its relatives have been examined by various authors (16, 71, 75, 318, 586). Barns et al. (16) used the SSU rRNA to infer relationships among 10 pathogenic species of *Candida* and produced the first estimate of phylogenetic relationships within it; their results confirmed the heterogeneous nature of the taxon.

*C. albicans* is a well-known pathogen (400, 401) that is commonly found in the digestive tract. It can cause a variety of infections, including disseminated ones in neutropenic patients (337). It is characterized by the production of pseudomycelium, along with dense, spherical chlamydospores on rice agar. Germ tubes are formed in serum at 37°C. Two serotypes, A and B, are known. Numerous serological and molecular techniques have been developed for the diagnosis of this species (118, 296).

*Geotrichum* is another important pathogenic genus, which comprises 11 species with teleomorphs assigned to *Dipodascus* and *Galactomyces* (Table 8) (125, 209, 296). This yeast is characterized by the formation of hyaline hyphae, which fragment in arthroconidia (Fig. 6, structure i) and whose cell walls have three layers but no xylose or fucose and which have septa with micropores. de Hoog and Guarro (118) provided a key, based mainly on physiological characteristics for the identification of **TABLE 8—Continued**

<table>
<thead>
<tr>
<th>Genera</th>
<th>Anamorphs</th>
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<tbody>
<tr>
<td><em>Stephanoascus</em></td>
<td><em>Candida</em></td>
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* The sexual structures of these genera are not usually found in cultures of clinical isolates.
the three pathogenic species, *G. candidum*, *G. capitatum*, and *G. clavatum*, the first two of which are the most pathogenic for humans. *G. candidum* usually causes pulmonary infections (367) but also causes systemic infections (266). *G. capitatum*, formerly known as *Trichosporon capitatum* and *Blastoschizomyces capitatus* (209), has been involved in numerous localized (109, 437, 567) and disseminated (15, 409, 590) infections.

In recent years, *S. cerevisiae* has also caused opportunistic infections in immunosuppressed patients (414, 485, 537). It is characterized by budding cells with multilateral bud formation and ascospores with smooth walls (one to four per ascus) that are easily produced in culture. Pseudomycelium may be formed. McCullough et al. developed a method for genotyping strains (336).

**BASIDIOMYCOTA**

The phylum Basidiomycota comprises almost 23,000 species distributed in almost 1,500 genera, 40 orders, and three classes (Table 1) (238). The most characteristic feature of basidiomycetes is the formation of basidia (Fig. 6, structure f), although they are rarely produced in vitro. Basidia are usually aseptate structures, with four tiny projections, called sterigmata (structure g). Each sterigma bears a haploid meiospore (basidiospore) (structure h), which is shot away at maturity and dispersed by air (274). The basidiomycetes usually have a mycellial thallus (Fig. 6, structure a), but some are typical yeasts (structures b and k to m), and most basidiomycetous pathogenic species belong to the latter group. There are two general types of teleomorphs found among the basidiomycetous yeasts (51). In the first, teleomorphs are formed and germinate to produce a basidium that bears basidiospores (see Table 10, *Entyloma* and *Tilletia*). This type of sexual cycle is very similar to that of rust and smut fungi. The second type of sexual cycle has no telo-

spores. Basidia develop on hyphae or yeast cells and give rise to basidiospores in a manner similar to the jelly fungi (296). When these sexual structures are not present, basidiomycetous yeasts are morphologically indistinguishable from ascomy-

cetous yeasts, except that the morphology of the bud scars can sometimes be different. More useful diagnostic characteristics are the presence of clamp connections (Fig. 6, structures n and o), the red-stained colonies with diazonium blue B (colonies of ascomycetes remain unstained) (553), positive urease reaction, and a high G+C content. When there are no fruiting bodies, a reliable method for separating basidiomycetous yeasts from ascomycetous yeasts at the ultrastructural level is to observe thin sections under TEM. This technique demonstrates that the inner walls of the former are typically lamellar, in contrast to the uniform inner layer of ascomycetes (289). TEM is also useful to demonstrate dolipores (complex barrel-shaped structures formed in the septa, which are covered on both sides by a membrane called a parenthesome) (Fig. 6, structure p) in basidiomycetous yeast.

Supraspecific taxonomic ranks such as genus and family are classified according to the mode of vegetative reproduction, morphological characteristics found during sexual reproduction, and several physiological characteristics such as the ability to assimilate inositol. However, recent chemosystematic studies suggest that the taxonomic system based on these characteristics is not appropriate (379). The monosaccharide composition of the cells, especially the presence or absence of xylose (193), and the ubiquinone (coenzyme Q) systems are thought to be more reliable systematic markers than the mode of vegetative reproduction and morphological criteria found during sexual reproduction. However, coenzyme Q composition is often heterogeneous in many currently defined genera and will require additional study before its taxonomic significance is fully understood (379). The presence of carotenoids and bal-

listococconia (forcibly ejected vegetative cells) (Fig. 6, structure m) in some taxa has been used as a criterion for genus assign-

ment. However, both characteristics are found among many genera of the basidiomycetous yeasts, suggesting that these traits are ancestral but not always expressed. Thus, they are of little value for defining taxa (161, 380). Ultrastructural features correlate well with chemosystematic characteristics and are regarded as reliable systematic criteria in higher taxonomic ranks. However, at lower ranks the phenetic approaches seem to be less useful, e.g., rRNA sequence analysis shows that *Rhodotorula, Sporobolomyces* (see Table 10) and *Cryptococcus* (see Table 9) are polyphyletic, further confirming that commonly used phenotype characteristics are insufficient for defining anamorphic genera (379).

Guého et al. (215) presented an overview of the phylogeny of basidiomycetous yeasts from measurements of divergence among partial sequences of LSU and SSU rRNAs. Three major groups were resolved: teliospore formers with hyphae hav-
ing simple septal pores, teliospore formers with hypha having dolipore septa, and non-teliospore formers with hyphae having dolipore septa. Fell and Kurtzman (160) and Fell et al. (161) compared sequences from the D2 region of the LSU rRNA corresponding to numerous species and genera of basidiomycetous yeasts and obtained two major clades corresponding to the orders Ustilaginales and Tremellales, respectively. In general, molecular analysis supported the concept that taxa as-

signed to the Tremellales were characterized by dolipore septa and cellular xylose whereas taxa in the Ustilaginales form teliospores have simple septal pores and, with a few exceptions, lack cellular xylose. Blanz and Unserd (48) reached the same conclusions by using 5S rRNA. The same approach (161) demon-

strated that teleomorphs clustered with their respective ana-

morphic genera. *Sporidiobolus*/*Sporobolomyces* or *Rhodospora-

ridium/Rhodotorula* (see Table 10) and also allowed the separa-

tion of species and recognition of synonyms, e.g., the opportunistic pathogen *Rhodotorula mucilaginosa* and *Cryo-

coccus vishniacii* and their respective synonyms. In general, molecular biological results agreed with morphological criteria (159). On the basis of 18S sequence comparisons, Suh and Sugiyama (518) placed the smut fungus *Ustilago maydis*, a teliospore former, near the clade comprising representative genera of basidiomycetous yeasts. In turn, these yeasts appear to be a sister group of the Agaricales (36).

The basidiomycetes of medical interest are placed in the classes Basidiomycetes (Table 9) and Ustomyces (Table 10) and in the orders Agaricales, Poriales, Schyzophyllales, Stere-

ales, Tremellales, and Ustilaginales.

**Tremellales**

Four anamorphic genera with pathogenic species have been placed in the order Tremellales. They are *Cryptococcus*, *Mo-

niliella*, *Trichosporon*, and *Cerinosteris*. The medically most important is *Cryptococcus*. This large genus has 34 species of diverse relationships. Its teleomorphs have been assigned to *Filobasidium*, *Filobasidiella* (Table 9), and *Cystofilobasidium* (296). The pathogenic species, *C. neoformans*, is phylogeneti-
cally rather well separated from the remaining species (54, 161, 212, 305). Two varieties have been described, *C. neoformans* var. *neoformans* (with serotypes A, D, and AD) and *C. neoformans* var. *gattii* (with serotypes B and C), their teleomorphs being *Filobasidiella neoformans* and *F. bacillispora*, respect-

ively. However, the teleomorphs of the *C. neoformans* complex were also reduced to varietal status (304). On the basis of
FIG. 6. Typical characteristics of basidiomycetes. a, mycelial colony; b, yeast colony; c, basidiomata; d, section of a basidiomata; e, detail of structure d; f, basidium; g, sterigma; h, basidiospores; i to l, different anamorph types; m, ballistoconidium; n, hypha with clamp connections (structure o); p, detail of structure n with a dolipore septum.
PFGE, RAPD, serotype, and killer toxin sensitivity patterns, Boekhout et al. (54) recently considered that they are two separate species rather than two varieties. In addition, there were no clear genetic or phenotypic differences among the clinical, saprobic and veterinary isolates within each taxon. Several other species such as *C. albidus*, *C. laurentii*, *C. ater*, *C. humicola*, and *C. uniguttulatus* have also been reported to be pathogens. However, it is possible that some of the cases of mycosis ascribed to non-*C. neoformans* cryptococci were erroneous or at least highly dubious and poorly evidenced (288). *C. neoformans* and *Candida albicans* are probably the two most extensively studied yeasts because of their pathogenic implications, mainly in AIDS patients, and their increasing antifungal resistance. In recent years, numerous physiological (28, 138, 436), serological (136, 169, 228), and molecular biological (11, 53, 101, 158, 359, 474, 532, 559, 584) techniques have been developed for diagnostic purposes and epidemiological studies. Boekhout et al. (54) suggested a combination of PFGE and RAPD analysis for distinguishing isolates.

*Trichosporon* is also an important pathogenic genus of the Tremellales. It is characterized by the production of arthroconidia and blastoconidia, and its species are able to assimilate many carbon compounds and degrade urea. The related genus *Geotrichum* also develops arthroconidia and sometimes blastoconidia, but it is urease negative and is able to assimilate only a few carbon compounds (211, 213). LSU rRNA sequences clearly demonstrate the basidiomycetous affinity of *Trichosporon* (215) and its relationship to the Filobasidiales, as had already been suggested by its dolipore ultrastructure and possession of a common capsular polysaccharide antigen with *C.*

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**TABLE 9. Genera of Basidiomycota of clinical interest and their anamorphs: Basidiomycetes**

<table>
<thead>
<tr>
<th>Order</th>
<th>Genera</th>
<th>Anamorphs</th>
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<tbody>
<tr>
<td>Agaricales</td>
<td><em>Coprinus</em></td>
<td><em>Homographiella</em></td>
</tr>
<tr>
<td>Poriales</td>
<td><em>Bjerkandera</em></td>
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<tr>
<td>Schizophyllales</td>
<td><em>Schizophyllum</em></td>
<td></td>
</tr>
<tr>
<td>Stereales</td>
<td><em>Phanerochaete</em></td>
<td><em>Sporotrichum</em></td>
</tr>
<tr>
<td>Tremellales</td>
<td><em>Filobasidella</em></td>
<td><em>Cryptococcus</em></td>
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* The fruiting bodies of these genera are not usually found in cultures of clinical isolates.
The clinical presentations caused by *Trichosporon* spp. vary widely from mild superficial infections such as “white piedra” to localized or disseminated infections in patients with hematological malignancies (248, 534, 555). This genus has traditionally been considered to have few species. However, recent molecular approaches involving partial sequences of the SSU and LSU rRNA have clearly demonstrated the existence of numerous *Trichosporon* species (210, 217). These species have very different habitats and usually occupy narrowly circumscribed ecological niches. Some of them are soil borne, while others are associated with animals, including humans. Six species are of clinical significance: *T. asahii*, *T. inkin*, *T. mucoides*, *T. cutaneum* (syn. *T. beigelii*), *T. ovoides*, and *T. asteroides*. *Tilletiopsis* should also be classified within *Trichosporon* (211, 213). The first three species are regularly isolated from clinical specimens, whereas the remaining species cause only occasional infections. A combination of features such as morphological characteristics, assimilation of carbon compounds, and tolerance at 37°C allow these species to be separated (211, 213, 248).

*Malassezia* (Pityrosporum) spp. are lipophilic yeasts which are frequently found in the skin of warm-blooded vertebrates. Except for one species, *M. pachydermatis*, these yeasts do not grow in routine culture media because they require exogenous sources of fatty acids. This and their inability to assimilate simple carbohydrates considerably hinder their differentiation and identification. These problems have meant that, traditionally, *Malassezia* species have been recognized by the clinical symptoms they produce rather than by other features. The two species classically recognized were *Pityrosporum ovale*, which has ovoid cells and causes pityriasis capitis and seborrheic dermatitis, and *P. orbiculare*, which has globose cells and causes pityriasis versicolor. Midgley (350) maintained the two species *M. furfur* (*P. orbiculare*) and *M. ovalis* (itself subdivided into three forms). Cunningham et al. (106) characterized the serovars A, B, and C within *M. furfur*. Later, these fungi were involved in more severe cases, such as superficial infections in AIDS patients and systemic infections in patients under parenteral alimentation supplemented with fatty acids (323). More recently, a morphological and molecular review, based on DNA-DNA complementarity and LSU rRNA sequence similarity, demonstrated that the genus is indeed more complex than had previously been thought, and seven species were recognized (216, 220, 221). *M. pachydermatis* was confirmed to...
be clearly associated with animals, and another six species were found on the skin of humans. They are *M. sympodialis*, the most common species, and *M. globosa*, *M. restricta*, *M. obtusa*, *M. furtur*, and *M. slooffiae*. The enlargement of the genus has made it more difficult to identify the species. However, Guillot et al. (222) developed a useful nonmolecular approach to recognizing the seven accepted species by using morphological characteristics, their ability to use several Tweenes, and their catalase reaction.

**Schizophyllales**

The common invader of rotten wood, *Schizophyllum commune*, is one of the few filamentous basidiomycetes able to colonize and cause severe infections in humans (22, 89, 456, 462). It is often easily recognized because on routine culture media, apart from the woolly spreading colonies with clamp connections, typical of basidiomycetes, it develops characteristic fruiting bodies (Table 9). These basidiomata are sessile, kidney shaped, and lobed, with split gills on the lower side which are lined with a dense palisade of basidia producing basidiospores (118). However, there may be some clinical isolates that do not have these characteristics in vitro. Therefore, other properties of the fungus, such as tolerance to the fungicide benomyl, susceptibility to high concentrations of cycloheximide, a dolipore-type septum, and mating tests, can be useful for its identification (489).

**Agaricales**

Coprinus spp. are the only members of the order Agaricales which cause human infections (384, 506). In recent years, several Hormographiella isolates have been found in clinical specimens (122, 580). Hormographiella is an anamorphic genus with three species, two of which were isolated from clinical sources (205). Hormographiella asperrigilata was found to be the anamorph of *Coprinus cinereus* (185). This fungus frequently develops mushroom fruiting bodies and conspicuous conidio- spores with cylindrical arthroconidia when grown in culture, which makes it easy to recognize (Table 9) (185). However, in a recent fatal case, the fungus failed to sporulate in culture and only RFLPs of PCR-amplified ITS and SSU rDNA enabled it to be identified (580).

**Ustilaginales**

*Rhodotorula*, *Sporobolomyces*, and *Tilletiopsis* (Table 10) are anamorphic genera with pathogenic species assigned to the order Ustilaginales. *Rhodotorula* is characterized by pink colonies and budding cells with narrow bud scars. A rare pseudomycelium may also be present. The teleomorph of the type species, *R. glutinis*, is *Rhodosporidium*, which can produce te- lospores in culture after mating compatible strains. Of the 34 accepted species (296), only 3, *R. glutinis*, *R. mucilaginosa*, and *R. minax*, have been reported to be pathogens (118, 224, 374, 399). These three species can be distinguished from each other by the assimilation or lack of assimilation nitrate and raffinose. *Sporobolomyces salmonicolor*, the anamorph of *Sporidiobolus johnsonii*, also develops salmon-pink colonies but can be differentiated from *Rhodotorula* spp. by the production of ballistoconidia on large sterrigmas (Table 10, *Sporobolomyces*). This agent has been reported as causing infection in AIDS patients (369, 435). *Tilletiopsis minor*, an etiologic agent of a recent subcutaneous infection in an immunocompromised patient (447), also produces ballistoconidia, but its colonies are whit- ish.

**DEUTEROMYCETES (MITOSPORIC FUNGI)**

All the fungi that do not have any connection with any ascomycetous or basidiomycetous taxa are included in the broad artificial group of deuteromycetes (Fig. 2). Numerous other terms such as “fungi imperfect,” “asexual fungi,” and “conidial fungi,” have been used to name these organisms, which produce only sterile mycelium or mycelium with conidia. Other fungi also form similar asexual structures (e.g., Oomy- cota and Zygomycota), but these have never been treated as deuteromycetes. The morphology of most of the deuteromy- cetes is very similar to that of the anamorph phases of some well-known taxa of Ascomycota and, to a lesser extent, of Basidiomycota. Therefore, we can assume, and it is frequently demonstrated by mating reactions and molecular studies, that the deuteromycetes represent conidial stages of members of these phyla, whose fruiting bodies are rarely formed in nature, have not been found, or have been dropped from the life cycle as these organisms evolved. It is not uncommon for the fruiting bodies of fungi to be detected in nature or in vitro many years after the fungi have been described as deuteromycetes. Under these circumstances, the fungi are placed in the genera represented by the fruiting bodies developed. While it is possible to use a separate name for each recognizable state of the fungus, many mycologists would regard it as unsatisfactory to have several names for a single fungus. According to Hawksworth et al. (238), there are about 15,000 species of deuteromycetes grouped in 2,600 anamorph genera. Emerging molecular tech- nology is allowing a more natural accommodation of many of these organisms. The remaining anamorph species, together with those not yet recorded, will still constitute an important permanent remainder. At present, deuteromycetes are the second largest group of fungi after the Ascomycota.

Although numerous deuteromycetes have also been found in aquatic habitats, the vast majority of these organisms are soil borne. They are the main components of the atmospheric mycobiota. The majority are either saprobes or weak plant parasites, and a few are parasites of other fungi. Deuteromycetes were once classically subdivided into three artificial orders, the Moniliales, Sphaeropsidales and Melanconiales; however, these terms are not currently used. It seems more practical to distinguish between hyphomycetes, which are the fungi which form sterile mycelium or bearing spores directly or on special branches of specialized hyphae (conidiophores) (Fig. 2 and 4, structure n), and coelomycetes, which have more complex reproductive structures.

Most of the molds found in the laboratory are hyphomycete- 

tes, a large portion of which are cultural states of Ascomycota, with asexual, mitotic propagation only. For routine identifica- tion, their biological relationship does not need to be deter- mined, but the mode of conidium formation must be exam- ined. As mentioned above, the process of conidiogenesis is very important in the identification of these fungi. Numerous hyphomycetes, including *Beauveria*, *Cephalotaphora*, *Engyod- 

nium*, *Corynespora*, *Dichomophilothora*, *Dictomophosphor- 

us*, *Metarhizium*, *Mycocentrospora*, *Nigrospora*, *Oidiodendron*, *Papulaspora*, *Phaeotrichoconis*, *Polypaecilum*, *Pseudomicrodo- 

chium*, *Stellula*, *Tetraloephora*, *Thermodiphyceae*, *Trichochladium*, *Triti- 

rachium*, *Veronaea*, *Volutella*, and *Wallemia*, have been report- 

ed as occasionally occurring in humans. Generally these genera are represented by only a single opportunistic species. A syn- 

optic key and the main diagnostic characteristics for the identi- 

fication of these fungi have been reported by Hoog and Guarro (118).

Approximately 1,000 species, found in a wide variety of ecological niches, are placed in the coelomycetes. Most are
saprobic or plant pathogens and are characterized as producing conidia in fruiting bodies (conidiomata). Fruiting bodies of coelomycetes are spherical (pycnidia), with conidigenous cells lining the inner cavity wall (Fig. 2, structure m) or are cup shaped (acervuli), in which case the conidigenous cells form a palisade on the conidiomatal surface. These two types of structure characterize the two artificial orders Sphaeropsidales and Melanconiales. When grown in culture, the pycnidia can be confused with fruiting bodies of ascomycetes. The distinction between a pycnidium and an ascoma should be verified by squashing the structure under a coverslip. In the case of ascomycetes, provided that the ascoma is suitably young, ascospores inside ascii are visible, whereas in the case of pycnidia, numerous free conidia are present, usually in mucous masses (Fig. 2, structure p). They are produced exogenously on conidiogenous cells. Eleven genera with approximately 20 species of coelomycetes were described as being pathogenic to humans as of 1995 (118). These taxa were isolated from clinical sources only very occasionally, but in the last 2 years a considerable number of infections caused by rare coelomycetes have been reported (9, 88, 91, 368, 416, 458, 601). Some of them have been found for the first time in human lesions; they include Pleurophomopsis lignicola (91, 416) and Microsphaeropsis subglobosa (206). Punithalingham (440) described the culture and microscopic characteristics of the pathogenic coelomycetes that had been isolated from humans, although over the last 20 years numerous other opportunistic coelomycetes have been reported. The most important manual for identifying coelomycetes is that of Sutton (524). This manual is not easy to use for nonexperts, because the identification of taxa is based primarily on the condidiogenesis pattern, which is not always easily observed in these fungi. Other approaches, based mainly on physiological and biochemical studies, have been used in an attempt to clarify the systematics of some complex genera of coelomycetes such as Phoma (36, 362).

The most common pathogenic species of coelomycetes is Nattrassia mangiferae, a plant pathogen but which also frequently causes nail infections in humans, especially in warmer areas (146, 363). Until recently, this species was known by the pycnidial synanamorph name of Hendersonula toruloidea. Another synanamorph of this species is Sclatyllum dimidiatum, which is characterized by a black mycelium with two types of arthroconidia, one of which is hyaline and the other is pigmented. In routine culture media, this species usually develops only the hyphomycetous synanamorph. Recently, Roeijmans et al. (459) used ITS RFLP patterns to demonstrate that other species, such as Sclatyllum hyalinum (80), also isolated from humans may be just a melanin-less culture mutant of S. dimidiatum. The two taxa have been previously related by using physiological tests (218), antigenic tests (364), and sterol composition (254). The other two species of Sclatyllum, S. infestans and S. japonicum, also found in clinical specimens, were clearly shown to be different species (118).

**GENERAL CONCLUSIONS**

Fungal systematics is still based mainly on morphological criteria, and pathogenic fungi are usually recognized and identified basically by their phenotypes. Numerous alternative approaches have been developed, including nutritional and physiological studies, serologic tests, secondary metabolites, ubiquinone systems, and fatty acids. Although some of these are very useful for identifying poorly differentiated fungi such as yeasts and black yeasts, they are only complementary tools of morphological data in most cases. Molecular biology techniques, especially the analysis of rRNA sequences, are currently used for reliable phylogenetic studies, which enable a more natural classification system to be established. However, despite the effective application of these techniques in PCR-mediated identification systems, they are not yet currently available in the routine clinical mycology environment. The constant discovery of new emerging mycotic agents in different clinical settings that often affect critically ill patients increases the need for the development of rapid and accurate identification systems, since delay in the diagnosis and initiation of therapy leads to a high mortality rate. On the other hand, if the isolates described in a report are discarded after a study finishes, the etiological data cannot then be checked in any further investigations (119). Therefore, contemporary practitioners and laboratorians should contact expert mycologists to identify the clinical isolates correctly and should deposit the strains in a reference culture collection. Most of these isolates must be reidentified by modern methods for a critical evaluation of the clinical cases. The problem of numerous undescribed species, together with the superficial level of knowledge that we have even for the fungi which have names, argues for a much greater emphasis on fungal biosystematics in the future. More attention to medical mycology and increased funding for training of medical mycologists are critical to address the current threats from new and reemerging fungal infections.

**ACKNOWLEDGMENTS**

Special thanks are due to Sybren de Hoog (Centraalbureau voor Schimmelcultures, Baarn, The Netherlands) for generously providing data and for invaluable comments. Two anonymous reviewers kindly improved the text.

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