The Yeast Handbook

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Biodiversity and Ecophysiology of Yeasts

With 40 Figures and 43 Tables

Springer

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Preface

Biological diversity or biodiversity, according to the United Nations Convention on Biological Diversity, "means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part" and includes genetic diversity, species diversity, and ecological diversity. Authors trying to estimate the number of extant yeast species usually come to the conclusion that, similarly to other groups of microorganisms, a very small proportion of the yeasts, possibly only 1% of the species, have been described.

Studies on "yeast biodiversity" are more focused on taxonomic inventories, with emphasis on the description of novel species. Approximately 30% of known yeast species were described from a single strain, and information about the ecology and the genetic and physiological variability of these yeasts is missing or incomplete.

In the last few decades an increasing number of new yeast habitats have been explored. As a result, a large body of ecological information has been accumulated and the number of known yeast species has increased rapidly. The volume "Biodiversity and ecophysiology of yeasts" provides a comprehensive and up-to-date overview of several areas in the field of yeast biodiversity and ecology. The chapters are written by respected experts in various fields. The first chapters approach yeast biodiversity from different points of view, including phylogenetics and genomics. Some aspects of sugar and nitrogen metabolism are also discussed. Separate chapters are devoted to stress responses of yeasts, to environmental factors influencing them, to antagonistic interactions among them, to methods used for investigating yeast biodiversity, and to the role of culture collections in handling the ever-increasing number of yeast strains and relevant data.

The chapters dealing with yeast communities from different habitats include reviews on yeasts from invertebrates, the phylloplane, soil, freshwater and marine ecosystems, cactophilic communities, as well as Antarctic and tropical forest ecosystems. In some chapters the effect of human activity on yeast communities is also considered. The black yeasts are treated in a separate chapter, and finally the role of yeast biodiversity in biotechnology is reviewed.

We gratefully acknowledge the contributors to this book. We hope that it will provide a useful overview of the biodiversity and ecophysiology of yeasts, and that it will stimulate increasing efforts in yeast biodiversity research.

Carlos Rosa
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Contents

Chapter 1

Yeast Biodiversity: How Many and How Much?

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1.1 Introduction

Biodiversity is now a common word. Harper and Hawksworth (1995) tabulated the frequency of use of the term in *Biosis* and reported its first occurrence in 1988 followed by an increase to approximately 900 by 1994. A similar search of the PubMed database yielded a cumulative total of 1,361 hits by the end of 2003. By comparison, the number of articles using the word "yeast" is approaching 100,000. If the present trend continues, by the year 2016 searches for either word will produce in excess of 36,000 hits for that year only. The task at hand is to make similar predictions about yeast biodiversity.

Biodiversity means different things to different individuals. Gaston (1996) reviewed several definitions and concluded that the concept is an abstract expression of all aspects of the variety of life. Recent publications dealing with yeast *diversity*, had they appeared only 15 years earlier, might have used instead such terms as *taxonomy*, *ecology*, or *survey* (Nout et al. 1997; Buzzini and Martini 2000; Fell et al. 2000; Poliakova et al. 2001; Gadanho et al. 2003; Granchi et al. 2003; Lachance et al. 2003a; Ganga and Martinez 2004; Renker et al. 2004) or even *enzymology* (Lamb et al. 1999). The Convention on Biological Diversity (Anonymous 1992) defines biological diversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems." As with most things in our society, biodiversity became a tangible reality when it could be assigned a significant economic value. And as with most things in science, the recognition of biodiversity as a worthy research topic is predicated on measurability and the generation of testable hypotheses. The current urgency of the scientific study of biodiversity stems from the realization that only a small fraction (approximately 8%) of the total diversity of life is known (Stork 1999) and that species extinction is occurring at a measurable and increasing rate (Purvis and Hector 2000).

1.2 Measurement and Significance of Biodiversity

1.2.1 Levels of Diversity

The inclusion of any level of biological variation in the definition of biodiversity could lead to a trivialization of the concept, as variation is the very essence of biology. A more restrictive circumscription should limit the term to ecological and evolutionary variation. Theoretical ecologists, following the model of Whittaker (1960), often subdivide species diversity into three hierarchical components, namely within-sample (α), between-samples (β), and global (γ) diversity. These components may be considered additive ($\gamma = \alpha + \beta$, Crist et al. 2003). The main units of measurement are richness (simple species count) and heterogeneity (relative abundance of each species in a community). The two measures can be examined simultaneously in relative abundance plots, which contrast the number of species in a sample as a function of the number of individuals representing each species. Considerable interest in the underlying causes of such distributions was stimulated by the pivotal publication of MacArthur and Wilson's (1967) treatise on island biogeography. A recent model (Hubbell 2001) attributes a large portion of the species composition of a community to chance. Implicit to this view (but perhaps not sufficiently explicit) is that membership of a species in a community depends initially on its fundamental niche, in other words, the sum of its intrinsic properties. For example, the community of floral nectar rarely contains basidiomycetous yeasts. This is not due to chance, but to the fact that such a habitat favours fermentative, osmotolerant, copiotrophic species, which are found most often in the Saccharomycetales. In the neutral model, a community is seen as an assemblage of ecologically equivalent species, where the abundance of each species within a local community is not so dependent on the fundamental niche. Instead, species composition is affected by speciation in the metacommunity, the rate of influx of species, the size of the local community, and the local rate of extinction. This is almost entirely analogous to Kimura's (1983) neutral model of evolution. Natural selection remains the preliminary screen that causes rapid elimination of deleterious mutations and rapid fixation of adaptive alleles, just as the environment determines whether or not a species can enter a community. The majority of species in the community have already "passed the test" of selection, and are equally adapted. As is the case for selectively neutral mutations, the relative abundance of a species will be due not so much to some intrinsic property, but to chance.

Application of such theories to yeast communities is not yet completely practical. Most models of community ecology were developed for communities where members can be identified and enumerated rapidly, e.g., forest trees and insect biota. The recent development of identification methods based on DNA sequencing (Kurtzman and Robnett 1998; Fell et al. 2000) has not yet resulted in practical means of identifying yeasts instantly, in the field, but such technologies are no doubt forthcoming. An attempt to explore the factors that underlie community structure was made recently (Lachance et al. 2003a). The yeast biota of morning glory flowers and associated nitidulid beetles was characterized in a "forest island" (*kīpuka*) on the slope of the Mauna Loa volcano in Hawaii. The yeast community is highly

specialized, consisting almost entirely of members of two clades with affinities to *Metschnikowia* and *Wickerhamiella*: the former clade is vectored primarily by the beetles, and the latter by drosophilid flies. Although the resulting community is a mixture, each clade can be studied separately with selective media. The *Metschnikowia* clade members in the community consists of six species and whose frequencies follow the expected log series distribution, from abundant to rare. Two of the species (*Metschnikowia hawaiiensis* and *Candida kipukae*) are probably Hawaiian endemics. The others have also been found in Central America and are thought to have reached Hawaii in recent history. The six species are similar physiologically, suggesting that they might be mutually neutral with respect to niche. However, their distribution within the *kīpuka* is not random and follows closely the distribution of the host beetles. The latter consist of two major species, one Hawaiian endemic and one that was introduced in the early twentieth century. Maximum growth temperature and insect choice may be important factors in the local distribution, such that a completely neutral model would have to be ruled out. The study is in progress, and increased sampling is hoped to provide a test of the neutral hypothesis.

1.2.2 Diversity Within Species

Even if one agrees that species abundance is central to the characterization of biodiversity, genetic diversity is an essential feature of the species itself. Even orthodox proponents of the phylogenetic/autapomorphic species concept would have to agree that a "species" that is completely devoid of variation can hardly be regarded as a species (Wheeler and Meier 2000). Variation among members of a species has long plagued pragmatic systematists in their search for stable diagnostic (autapomorphic) characters (Lodder and Kreger-van Rij 1952). As DNA sequence analysis took the study of yeast diversity by storm, the recurring dream of an invariant species trait was temporarily rekindled (Kurtzman and Robnett 1998). However, the sequencing approach has in some instances brought to light considerable variability among individuals that share a common gene pool and thus are members of the same biological species. One response might be to denounce the biological species concept as antiquated and inoperable (Wheeler and Meier 2000). Another would be to accept that the genes that are most amenable to phylogenetic construction are not necessarily involved in conferring a common evolutionary destiny to members of a species, and that species cannot be defined on the basis of invariance in gene sequences.

In a study of the distribution of yeasts in seawater, Gadanho et al. (2003) subjected 234 isolates to microsatellite-primed PCR fingerprinting and demonstrated that in most cases multiple isolates of various basidiomycetous yeast species contain a substantial amount of genomic variation. Ascomycetous species recovered in that habitat exhibited less variation.

Intraspecific variability has been examined in the two species in the genus *Clavispora*, both of which occur in nature as heterothallic, haploid mating types. This offers the advantage that species boundaries can be assessed by mixing of compatible strains and observation of ascospores. *Clavispora opuntiae* has so far

been isolated exclusively from necrotic tissue or tunnels of moth larvae found in cacti. Hundreds of specimens have been recovered globally and preserved for study. Although the growth responses of most isolates are generally constant, polymorphisms have been detected at the level of the ribosomal DNA (rDNA) gene cluster (Lachance et al. 2000b). In some 500 isolates examined by restriction mapping, over 40 variants were recognized. These correlated to a large degree with geography, host plant species, and insect vectors. Most of the variation was shown by sequencing to be located in the intergenic spacer region, although a small amount of polymorphism was also detected in the large subunit rRNA gene. Strains representing the extremes of that variation had been shown previously to exhibit a lower degree of interfertility (Lachance et al. 1994) and perhaps represent the beginning of a speciation event.

C. lusitaniae is similar morphologically and physiologically to *C. opuntiae*, but exhibits much less habitat specificity, having been recovered in cactus fruit, agave rots, industrial wastes, clinical specimens, and several other sources. Mating compatibility and large subunit rDNA sequences were determined in 37 strains (Lachance et al. 2003c). The sequences could be assigned to ten types belonging to two families that differed by as much as 32 substitutions in the D2 domain. The variation was not correlated with mating intensity or abundance of mature asci.

Although these studies do not allow generalizations about the evolutionary or ecological significance of genetic diversity within yeast species, they would seem to support the view that variability is an intrinsic property of species.

1.2.3 Species Diversity

From the first to the current edition of the *The yeasts, a taxonomic study*, the number of species described has grown from 164 in 1952, to 349 in 1970, to 500 in 1984, and to 700 in 1998 (Lodder 1970; Kurtzman and Fell 1998). Extrapolation of these numbers leads to the prediction that an eventual 2016 edition would contain approximately 1,000 species. However, this number may very well be exceeded in the forthcoming fifth edition, planned for 2005. The increase is due to several factors, including methodology and species concepts. In the first edition (Lodder and Kreger-van Rij 1952), species were circumscribed on the basis of morphology and a small number of growth tests. The doubling in the number of species found in the second edition was due in part to the use of a much larger battery of nutritional properties. Early application of molecular approaches had a considerable impact on the third edition, but was not entirely accountable for species proliferation, as the shift to a genomic basis for species delineation also caused the merger of physiological or morphological variants into larger and more diverse species. The publication of the fourth edition coincided with early application of DNA sequencing in yeast identification and phylogenetic reconstruction, although the full impact of this approach came later. Again, the result is a mixture of species fusions and subdivisions.

The definition of species is fundamental in the generation of meaningful estimations of biodiversity, which accounts in part for the heartiness of the debate on that subject (Wheeler and Meier 2000). The species problem as it applies to bacterial and

fungal diversity has been discussed by O'Donnell et al. (1995), who pointed out the lack of a common standard. Although species concept controversies are not alien to yeast systematics, many practitioners agree that species should, whenever possible, represent cohesive evolutionary units. Individual researchers may disagree on how best to document the boundaries of such units, but the result is nonetheless a relatively stable consensus. As the issue is far too complex to be examined here in detail, it will be expedient to assume, rightly or wrongly, that taxa which are recognized at any given time constitute genuine and meaningful species.

Sequence analysis resulted in an enormous increase in the ease and speed of identification, making intense biodiversity surveys almost manageable. Many species descriptions currently being published come from material collected in the past and stored in collections in the hope that new technologies would eventually facilitate meaningful species assignments. The sequencing approach has fulfilled this need. Unfortunately, the clarifications brought forward by sequencing have done little to improve our understanding of the natural history or ecology of the species being described. Unless the ecological context of species is also documented, Linnean binomials will remain no more than mere labels of little relevance to biodiversity. By their very nature as unicellular heterotrophs, yeasts are inexorably dependent on other fungi, bacteria, animals, and plants for their existence, and ideally species descriptions should include data on these interactions. The old precept, "everything is everywhere", although no longer tenable, sadly continues to influence yeast taxonomy. An inordinate amount of energy is devoted to transforming sequence data into "correct" trees, at the expense of the yeasts themselves and their biology. Another important consequence of the dependence of yeasts on other life forms is the urgency of documenting their natural history before their very habitats disappear. Unless conservation efforts are intensified, it will become easier to determine the rate of extinction of yeasts than to estimate the number of extant species. The fact that the construction of a comprehensive inventory of life on Earth is seen as a priority by an increasing number of researchers, governments, and granting agencies (Mulongoy et al. 1999) should be viewed with optimism. Equally encouraging is the emergence of more frequent studies aimed at characterising whole yeast communities in relation to their insect vectors. A case in point is a recent description of 16 closely related species originating from fungivorous beetles and their habitats (Suh et al. 2004). Members of the Coleoptera associated with tree decay have long been known to harbour numerous yeast species, as evidenced by the work of pioneers such as L.J. Wickerham, J.P. van der Walt, and H.J. Phaff. These yeasts are suspected to engage in intimate symbiotic relationships with insects, although the nature of the interaction remains elusive in most cases. Recent studies of yeasts found in tropical bees led to the discovery of the genus *Starmerella* (Rosa and Lachance 1998), the nucleus being a growing clade whose membership has increased from 12 described species in 1998 to 29 putative species at the last published count (Rosa et al. 2003). Studies of nitidulid beetles associated with ephemeral flowers have resulted in the near doubling of described species of *Metschnikowia* (Lachance et al. 2003b) and a significant expansion of the formerly monotypic genera *Kodamaea* (Lachance et al. 1999) and *Wickerhamiella* (Lachance et al. 2000a).

1.3 Predicting the Number of Yeast Species

Hawksworth (1991) attempted to predict the number of fungal species on the basis of an estimated 69,000 described species and the ratio of fungi to other life forms. By reference to vascular plants, he proposed a conservative estimate of 1.5 million fungal species, and a comparison with insect species extended the range to three million. The proportion of fungi described (in 1991) was thus thought to be approximately 5% of the total fungal biota. A hasty transposition of Hawksworth's reasoning to the yeasts generates a forecast of approximately 12,000 species.

Hughes et al. (2001) pointed out that microbes may be too diverse to enumerate exhaustively and argued for a statistical approach. One tool used in reaching this objective is the accumulation curve, where species abundance is plotted as a function of sampling intensity. Species sampling follows a rarefaction pattern in which the rate of increase in the detection of species obeys the law of diminishing returns. Well-sampled habitats produce curves that can be fitted to saturation models such as the Michaelis–Menten equation or the negative-exponential function, characterized by growth towards an asymptotic maximum. Poorly sampled habitats produce nearly linear curves. Gadanho et al. (2003) applied this approached to yeasts in seawater and estimated that the 31 species recovered represented approximately 60% of the existing species in their study site. In order to extrapolate beyond a single site, Lachance (2000a) used random internal sampling of collection records to generate accumulation curves. The data were fitted to trend line functions available in Microsoft Excel and the curves were extrapolated to large sample sizes. For insects associated with ephemeral flowers, data from eight localities worldwide (26 yeast species in total) led to the prediction that sampling from 50 localities would raise the number to 42 species. Simulations based on yeasts from tree fluxes were validated by predicting, from eight samples, the total number of yeast species (45) present in 47 actual samples. Extrapolation to 1,000 samples predicted that the number of species would rise to approximately 500. In Fig. 1.1, the same data were analysed using the Michaelis–Menten model. Linear regressions of the double-reciprocal plots of species richness as a function of sampling intensity predict asymptotic maxima of 40 species for floricolous insects and 182 species for tree fluxes, which is not entirely inconsistent with the previous predictions. Using these numbers and an estimate of 1,000 currently described yeast species, a simple rule of 3 predicts that the number of yeast species on Earth is in the order of 1,500 on the basis of the insect model, and 15,000 on the basis of the flux model. The lower value comes from a highly specific yeast community, whereas the upper boundary is characteristic of a more generalistic community. Other well-sampled, highly specific communities follow a conservative pattern similar to that of flower insects. Calderone (2002) recognised 13 *Candida* species as human pathogens compared with eight in 1988. The highly specialized nature of yeasts associated with humans, combined with the extremely high sampling intensity makes the current number of species a good approximation of the saturation point. In the case of the moderately specific community of yeasts associated with necrotic cacti, 3,701 samples yielded fewer than 80 species (Starmer et al. 1990).

The broad range of predictions from 1,500 to 15,000 yeast species in total reflects the fact that the average degree of specificity for all yeast communities is not known.

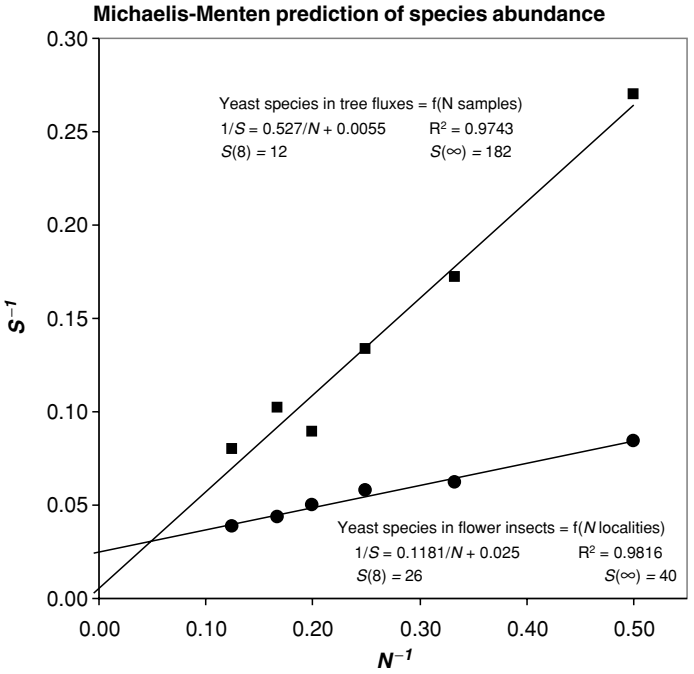

Fig. 1.1. Prediction of yeast species abundance in tree sap fluxes and in insects associated with morning glories based on pseudoreplicated collections from N=8 localities. As the data follow Michaelis–Menten kinetics, double reciprocal plots were used to predict $S(\infty)$, the number of species that would be found in similar habitats in an infinite number of localities. The corresponding linear equations and their regression coefficients are given. The equation S (total) = 1000($S(\infty)$/$S(8)$) was used to estimate of the total number of extant yeast species assuming that the global depletion curve is similar, which of course may not be the case

Furthermore, the numbers and kinds of habitats remaining to be studied are unknown. As floricolous insect and tree flux communities are greatly affected by the activity of members of Coleoptera, whose number of described species is in the order of 350,000, one might predict similar numbers for yeasts; however, the proportion of beetles that harbour yeasts remains to be established. Although tree-boring species and floricolous nitidulids frequently contain yeasts, the very speciose family Chrysomelidae seldom does. It is therefore not reasonable to assume that the number of yeasts is commensurate to that of beetles as a whole.

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Yeast Systematics and Phylogeny – Implications of Molecular Identification Methods for Studies in Ecology

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2.1 Introduction

A major factor that determines the validity of studies in yeast ecology is the correct identification of species in the ecosystem. Before the present era of yeast taxonomy, which uses gene sequences and other molecular criteria, identifications were of necessity based on phenotypic tests. Although phenotype can sometimes be used to correctly identify species, molecular comparisons have shown that many earlier identifications based on phenotype have been incorrect. While this does not mean that earlier work in yeast ecology is invalid, it does say that conclusions drawn from this work may need to be reexamined following more accurate identification of species. In particular, the often-asked question "Is everything everywhere?" cannot be adequately addressed until taxa are correctly identified. In this chapter, we will discuss molecular methods now used for identification of yeasts, what we perceive of their genetic resolution, their impact on systematics, and finally a description of some of the rapid molecular methods that are applicable to the large species populations often examined in ecological studies.

2.2 Molecular Identification of Species

The transition from phenotypic identification of yeasts to molecular identification began with determination of the mole percent guanine (G) plus cytosine (C) ratios of nuclear DNA. These analyses demonstrated that ascomycetous yeasts range from approximately 28 to 50 mol% G+ C, whereas basidiomycetous yeasts range from approximately 50 to 70 mol% G+ C. Depending on the analytical methods

used, strains differing by 1–2 mol% are recognized as separate species (Price et al. 1978; Kurtzman and Phaff 1987). The need for quantitative assessment of genetic similarity between strains and species was satisfied, in part, by the technique of nuclear DNA reassociation or hybridization. DNA from the species pair of interest is sheared, mixed, made single-stranded, and the degree of relatedness determined from the extent of reassociation. Many different methods are used to measure this process, which can be done spectrophotometrically or through use of radioisotopes or other markers (Kurtzman 1993a).

A major question has been how to interpret DNA reassociation data. Measurements of DNA complementarity are commonly expressed as percent relatedness. This usage can be misleading because DNA strands must show at least 75–80% base sequence similarity before duplexing can occur and a reading is registered on the scale of percent relatedness (Bonner et al. 1973; Britten et al. 1974). Experimental conditions can greatly influence the extent of duplex formation, but under optimum conditions, different methods of assessing DNA relatedness do give essentially the same result (Kurtzman 1993a). Percent DNA relatedness provides an approximation of overall genome similarity between two organisms, but the technique does not detect single gene differences or exact multiples of ploidy, although aneuploidy can sometimes be detected (Vaughan-Martini and Kurtzman 1985).

On the basis of shared phenotype, strains that showed 80% or greater nuclear DNA relatedness were believed to represent members of the same yeast species (Martini and Phaff 1973; Price et al. 1978). This issue was also examined on the basis of the biological species concept (Dobzhansky 1976), asking what is the fertility between strains showing varying degrees of DNA relatedness (Kurtzman 1984a, b, 1987; Kurtzman et al. 1980a, b). In one of these studies, the heterothallic species *Pichia amylophila* and *P. mississippiensis*, which showed 25% DNA relatedness, gave abundant interspecific mating, but ascus formation was limited and no ascospores were formed. Similar results were found for crosses between *P. americana* and *P. bimundalis* (21% DNA relatedness) and between *P. alni* and *P. canadensis* (*Hansenula wingei*), the latter pair showing just 6% DNA relatedness. The varieties of *Issatchenkia scutulata*, which exhibit 25% DNA relatedness, behaved somewhat differently. Crosses between *I. scutulata* var. *scutulata* and *I. scutulata* var. *exigua* gave an extent of mating and ascospore formation comparable to that of intravarietal crosses. Ascospore viability from these intervarietal crosses was about 5%, but sib-matings of the progeny had 17% ascospore viability. However, backcrosses to the parentals gave poor ascosporulation and very low viability, which suggests that these two varieties represent separate species. *Williopsis saturnus* is a homothallic species with five varieties that range in DNA relatedness from 37 to 79% (Kurtzman 1987). Intervarietal fertility is reduced and varies depending on the strains crossed. Consequently, the preceding studies show that mating among heterothallic as well as homothallic taxa can occur over a wide range of DNA relatedness values, but that highly fertile crosses, which demonstrate conspecificity, seem to require 70–80% or greater DNA relatedness between strains. Because species barriers are complex and involve a number of factors, the numerical range of 70–100% DNA relatedness as indicative of conspecificity should be viewed as a prediction.

Nuclear DNA reassociation studies have had a marked impact on recognizing yeast species, but the method is time consuming and the extent of genetic resolution goes no further than that of closely related species. Gene sequencing offers a rapid method for recognizing species and resolution is not limited to closely related taxa. Peterson and Kurtzman (1991) determined that domain 2 of large subunit (26S) ribosomal RNA (rRNA) was sufficiently variable to resolve individual species. Kurtzman and Robnett (1998) expanded the preceding work by sequencing both domains 1 and 2 (approximately 600 nucleotides) of 26S ribosomal DNA (rDNA) for all known ascomycetous yeasts, thus providing a universally available database for rapid identification of known species, the detection of new species, and initial phylogenetic placement of the species. Fell et al. (2000) published the D1/D2 sequences of known basidiomycetous yeasts, thus completing the database for all known yeasts. Resolution provided by the D1/D2 domain was estimated from comparisons of taxa determined to be closely related from genetic crosses and from DNA reassociations. In general, strains of a species show no more than zero to three nucleotide differences (0–0.5%), and strains showing six or more noncontiguous substitutions (1%) are separate species. Strains with intermediate nucleotide substitutions are also likely to be separate species. One impact of the D1/D2 database has been to permit detection of a large number of new species, which has resulted in a near doubling of known species since publication of the most recent edition (fourth) of *The yeasts, a taxonomic study* (Kurtzman and Fell 1998). Another use is that the nontaxonomist can now quickly and accurately identify most known species, as well as recognize new species, by sequencing approximately 600 nucleotides and doing a BLAST search in GenBank.

The internal transcribed spacer regions ITS1 and ITS2, which are separated by the 5.8S gene of rDNA, are also highly substituted and often used for species identification, but for many species, ITS sequences give no greater resolution than that obtained from 26S domains D1/D2 (James et al. 1996; Kurtzman and Robnett 2003). However, Fell and Blatt (1999) were able to resolve cryptic species in the *Xanthophyllomyces dendrorhous* species complex that had been unresolved from D1/D2 sequence analysis, and Scorzetti et al. (2002) reported ITS sequences to provide somewhat greater resolution among many basidiomycetous species than was found for D1/D2, although, a few species were less well resolved by ITS than by D1/D2. Consequently, it appears useful to sequence both D1/D2 and ITS when comparing closely related species. The intergenic spacer (IGS) region of rDNA tends to be highly substituted and sequences of this region have been used with good success to separate closely related lineages of *Cryptococcus* (Fan et al. 1995; Diaz et al. 2000), *Xanthophyllomyces* (Fell and Blatt 1999), *Mrakia* (Diaz and Fell 2000) and *Saccharomyces* (Kurtzman et al., unpublished). Because of the occurrence of repetitive sequences and homopolymeric regions, the IGS region tends to be difficult to sequence for some species. Small subunit (18S) rDNA, which has been extremely important in broad-based phylogenetic analyses, is generally too conserved to allow separation of individual species (James et al. 1996; Kurtzman and Robnett 2003).

The focus of our discussion on species identification from gene sequences has been on rDNA. A major advantage of rDNA is that it is present in all living organisms, has a common evolutionary origin, occurs as multiple copies and is easy

to sequence because primer pairs for conserved regions can generally be used for all organisms. However, gene sequences other than those of the rDNA repeat have been used for separation of species from many kinds of fungi (Geiser et al. 1998; O'Donnell et al. 2000), including the yeasts. Belloch et al. (2000) demonstrated the utility of cytochrome oxidase II for resolution of *Kluyveromyces* species, Daniel et al. (2001) successfully used actin-1 for species of *Candida*, and Kurtzman and Robnett (2003) showed the usefulness of elongation factor 1-α and RNA polymerase II for resolution of *Saccharomyces* species. At present, the main impediment to widespread use of gene sequences other than rDNA is developing sequencing primers that are effective for essentially all species, and construction of databases that include sequences from all known species. Daniel et al. (2001) and Daniel and Meyer (2003) have made considerable progress in development of an actin sequence database for species identification, although no primer set has been effective for all species, thus requiring additional primers to obtain these sequences. The need for multiple primers seems to be a problem common to sequencing of protein encoding genes because of frequent nucleotide substitutions. Resolution of taxa from actin is somewhat greater than from D1/D2, but not surprisingly, clear separation of closely related species is not always certain.

Separation of species using single gene sequences is not always reliable. Different lineages may vary in their rates of nucleotide substitution for the diagnostic gene being used, thus confusing interpretation of genetic separation, and hybrids are common and appear to be part of the speciation process. For example, Vaughan-Martini and Kurtzman (1985) proposed from DNA reassociation studies that *Saccharomyces pastorianus* is a natural hybrid of *S. cerevisiae* and *S. bayanus*. Peterson and Kurtzman (1991) confirmed the proposal by showing that the D2 domain rRNA sequence of *S. pastorianus* is identical to that of *S. bayanus,* but divergent from *S. cerevisiae*. The three varieties of *Candida shehatae* may also represent hybrids, or are examples of a lineage with a slow rate of nucleotide substitution in the rDNA. From DNA reassociation, the varieties show approximately 50% relatedness, but they have essentially identical domain 2 large subunit sequences (Kurtzman 1990). Groth et al. (1999) discovered a natural chimeric isolate of *Saccharomyces* with genetic material from three species, and Nilsson-Tillgren et al. (1981) presented evidence that several natural and industrial yeast strains are hybrids. Kurtzman et al. (2005) reported that *Kazachstania heterogenica* appears to be a natural hybrid that shares an RNA polymerase II gene with *K. pintolopesii*. In an additional study, Lachance et al. (2003) found interfertile strains of *Clavispora lusitaniae* that are highly polymorphic in the D1/D2 domain. Detection of unexpected divergence in a gene sequence should be possible from its lack of congruence with other gene sequences. Single gene sequences are extremely useful for rapid species identification, but from the foregoing examples, caution in interpretation of species identity is required.

Other molecular-based methods commonly used for species identification include species-specific primer pairs and probes, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), and karyotyping. Species-specific primers are effective when used for PCR-based identifications involving a small number of species or

when a particular species is the subject of the search (Fell 1993; Mannarelli and Kurtzman 1998). Otherwise, there is the likelihood that PCR mixtures containing large numbers of species-specific primer pairs will lead to uncertain banding patterns. Microsatellite-primed RAPDs (Gadanho et al. 2003) and AFLP fingerprints (de Barros Lopes et al. 1999) have been effectively used in some laboratories. One concern in using these latter two techniques is reproducibility between laboratories because small differences in PCR conditions may impact the species-specific patterns that serve as a reference. Karyotyping with pulsed-field electrophoresis and RAPD on mitochondrial DNA can serve in the initial characterization and identification of yeast species. However, the interpretation of the chromosome band patterns and mitochondrial restriction fragments for taxonomic purposes is complicated by the high degree of polymorphism, such as chromosomal rearrangements, within some yeast taxa (Spírek et al. 2003).

2.3 Molecular Phylogeny and Systematics of the Yeasts – an Overview

In the previous section, we discussed various molecular methods for species identification. In addition, many phylogenetic relationships among the yeasts and other fungi have been resolved from analysis of gene sequence divergence. These studies presume that horizontal gene transfer among different lineages has been limited, which can be tested by comparing the congruence of phylogenies derived from different genes. Most of the analyses have used rDNA sequences, but there are generally no major differences in tree topologies whether the analyses are from rDNA sequences or from those of other genes (Geiser et al. 1998; Liu et al. 1999; O'Donnell et al. 2000; Kurtzman and Robnett 2003). Although phylogenetic trees derived from analyses of various genes are generally congruent, support for basal lineages from single gene analyses is often weak (Kurtzman and Robnett 2003; Rokas et al. 2003). Because of this weak support, branching order is uncertain, leading to ambiguity of what constitutes a genus, a family or an order. Hawksworth et al. (1995) addressed this issue in part by stating "there are no universally applicable criteria by which genera are distinguished, but in general the emphasis is now on there being several discontinuities in fundamental characters ...". Many systematists now regard these fundamental characters as gene sequences. However, a number of factors impact our recognition of genera and higher levels of classification. Phylogenetic trees determined from single genes are seldom robust, leading to uncertainty whether neighboring species groups are a separate genus or members of a more broadly based genus. Multigene analyses generally strengthen support for basal lineages. Kurtzman and Robnett (2003) examined relationships among the approximately 80 species of the "*Saccharomyces* complex" from multiple genes. Combined analysis of 18S, 26S, 5.8S/conserved ITS and mitochondrial small subunit rDNAs with elongation factor 1-α and cytochrome oxidase II gave high bootstrap support for moderately deep lineages, which were interpreted as genus-level, but not for more basal lineages.

Rokas et al. (2003) screened the published genome sequences from seven *Saccharomyces* species and that of *Candida albicans* and selected 106 widely distributed

orthologous genes for phylogenetic analysis. The resulting analyses showed that a dataset comprising a concatenation of a minimum of nearly any 20 genes gave well-supported trees that were comparable to those of a dataset comprising 106 genes. This work clearly illustrates that a much larger number of genes is required for reconstructing phylogenies than is currently being analyzed in most laboratories. Whether 20 gene sequences will strongly resolve species clades larger than *Saccharomyces* needs to be determined. However, partial genome sequence analysis appears sufficient to resolve phylogenetic relationships within different groups of yeasts. Another factor that impacts resolution, as well as circumscription of genera, is the issue of missing taxa. It seems likely that fewer than 1% of extant species are known, which can be inferred from the high frequency of long single-species branches in phylogenetic trees. Consequently, the majority of the yeasts are yet to be discovered and characterized, and their addition to future phylogenetic analyses is likely to influence our perception of genera, even those that are presently circumscribed from multigene analyses.

2.4 Ascomycetous Yeasts

The distinction between yeasts and dimorphic filamentous fungi has often been uncertain. Some authorities have viewed the yeasts as primitive fungi, whereas others perceived them to be reduced forms of more evolved taxa (Cain 1972; Redhead and Malloch 1977). Phylogenetic analyses of rDNA sequences demonstrated the ascomycetous yeasts, as well as yeast-like genera such as *Ascoidea* and *Cephaloascus*, to comprise a clade that is a sister group to the "filamentous" ascomycetes (euascomycetes). *Schizosaccharomyces*, *Taphrina*, *Protomyces*, *Saitoella, Pneumocystis,* and *Neolecta*, a mushroom-like fungus, form a divergent clade basal to the yeast-euascomycete branch (Hausner et al. 1992; Hendriks et al. 1992; Kurtzman 1993b; Nishida and Sugiyama 1993; Wilmotte et al. 1993; Kurtzman and Robnett 1994, 1995, 1998; Landvik 1996; Sjamsuridzal et al. 1997; Sugiyama 1998; Kurtzman and Sugiyama 2001). Nishida and Sugiyama (1994) have termed the basal ascomycete clade the "archiascomycetes." Some members of the yeast clade, such as certain species of *Ascoidea* and *Eremothecium,* show no typical budding, whereas budding is common among the so-called black yeasts in the genera *Aureobasidium* and *Phialophora*, as well as in certain other dimorphic euascomycete genera. Similarly, vegetative reproduction by fission is shared by *Dipodascus* and *Galactomyces*, members of the yeast clade, as well as by the distantly related genus *Schizosaccharomyces*. Consequently, yeasts cannot be recognized solely on the basis of the presence or the absence of budding, but with a few exceptions, ascomycetous yeasts can be separated phenotypically from euascomycetes by the presence of budding or fission and the formation of sexual states unenclosed in a fruiting body.

During the past 10 years, the widespread use of molecular taxonomic methods has resulted in the discovery and description of a large number of new taxa, bringing the total of ascomycetous species to nearly 1,000. Many of these new species are readily detected by sequencing a single species-resolving gene, such as domains D1/D2 of large subunit rDNA, keeping in mind the exceptions discussed in the previous section. If we accept that fewer than 1% of extant species are known and that current sequencing technologies allow rapid detection of new species, the limiting

factor for presenting new species is the time required for formal description, which includes information on vegetative and sexual states, fermentation and assimilation reactions, and ecology, where known.

From single gene analyses, such as the D1/D2 phylogenetic trees presented by Kurtzman and Robnett (1998), it is apparent that many of the ascomycetous yeast genera are not well circumscribed, but actual boundaries are often not clear. Multigene sequence analyses have been applied to just a few genera, such as those of the "*Saccharomyces* complex", which includes *Saccharomyces*, *Kluyveromyces*, *Tetrapisispora*, *Torulaspora*, and *Zygosaccharomyces*, as well as the neighboring genera *Eremothecium*, *Hanseniaspora*, and *Saccharomycodes* (Kurtzman and Robnett 2003). In this multigene study, approximately 80 species were compared from the combined signal of seven genes. The analysis gave 14 phylogenetically defined clades, most of which had strong bootstrap support. From this study, the major genera *Saccharomyces*, *Kluyveromyces*, and *Zygosaccharomyces* were shown to be polyphyletic, leading to reclassification of certain of the species in the new genera *Naumovia*, *Nakaseomyces*, *Vanderwaltozyma*, *Zygotorulaspora*, and *Lachancea*, and expansion of the earlier described genus *Kazachstania* (Kurtzman 2003) (Fig. 2.1). Lineages basal to the branches supporting the 14 clades generally had low bootstrap support, leaving uncertain the genetic relationships among the genera. The genus *Eremothecium* appears separate from the family *Saccharomycetaceae* and was maintained in the *Eremotheciaceae*. Similarly, the sister genera *Hanseniaspora* and *Saccharomycodes*, which reproduce by bipolar budding rather than multilateral budding typical of the *Saccharomycetaceae*, were retained in the family *Saccharomycodaceae*. As demonstrated from this analysis, as well as that of Rokas et al. (2003), a relatively large number of gene sequences will be required to understand phylogenetic relationships among the yeasts. Currently accepted ascomycetous yeast genera are listed in Table 2.1 with their proposed assignments to orders and families. Because of weak basal resolution in phylogenetic trees, many of the genera cannot be reliably assigned to families. Furthermore, on the basis of the large amount of phylogenetic divergence conveyed by present datasets, it seems likely that many new families will need to be described.

From D1/D2 sequence analysis, the greater than 100 species assigned to the genus *Pichia* are seen to be distributed across the *Saccharomycetales* (Kurtzman and Robnett 1998). Major species groups in *Pichia* are centered on *P. membranifaciens*, *P. anomala*, and *P. angusta* (*Hansenula polymorpha*), the latter species representing the majority of methanol-assimilating taxa. Some of the species will be maintained in *Pichia* and some will need to be placed in new genera as stronger datasets become available. A few of the outlying species have already been assigned to new genera. *P. pastoris*, the outlying member of the methanol-assimilating yeasts, was transferred to *Komagataella* (Yamada et al. 1995a), and support for this genus as a distinct clade recently increased with the discovery of two additional species of *Komagataella* (Dlauchy et al. 2003; Kurtzman 2005). *P. burtonii*, now transferred to *Hyphopichia*, is phylogenetically distant from the three main clades of *Pichia*, as are the D-xylose-fermenting species *P. stipitis* and *P. segobiensis*. An additional change was the assignment of *P. ohmeri* to the genus *Kodamaea* (Yamada et al. 1995b). Support for this genus has increased with the discovery of additional species closely related to

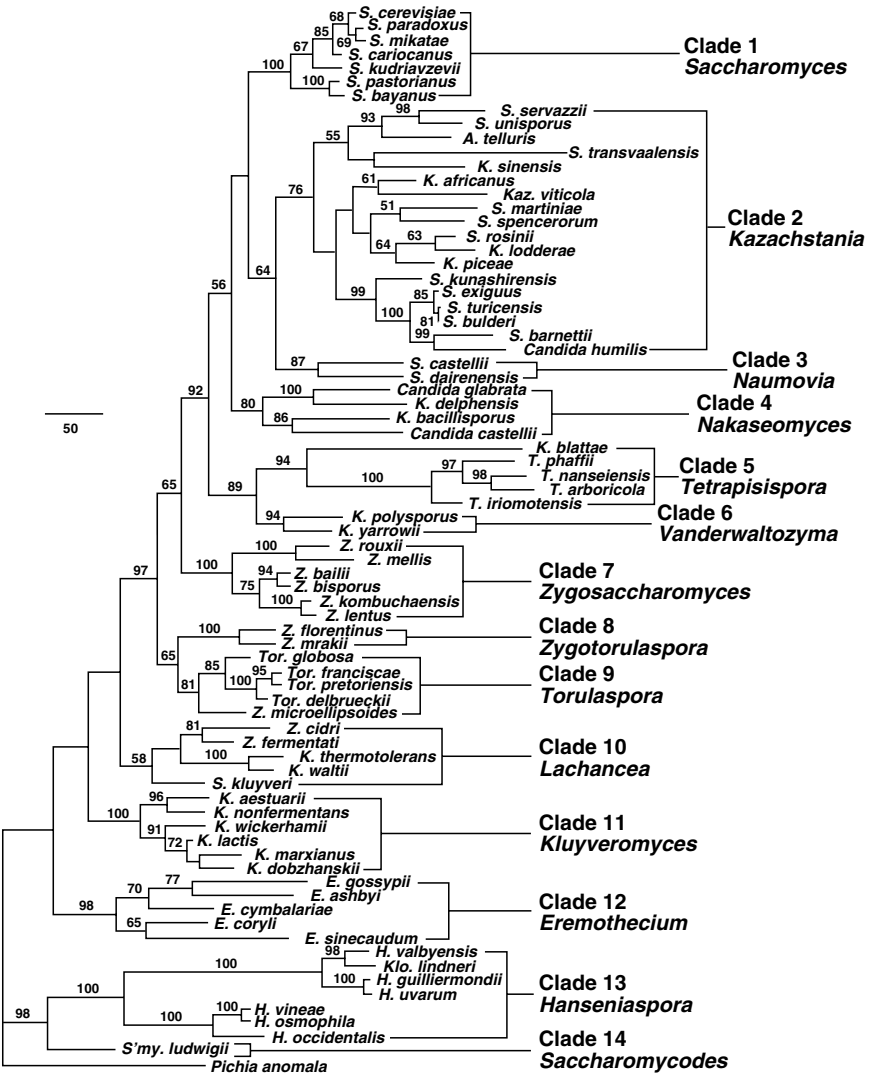

Fig. 2.1. Maximum parsimony tree resolving species of the "*Saccharomyces* complex" into 14 clades, which are interpreted as phylogenetically circumscribed genera. The analysis resulted in the description of five new genera. Earlier generic assignments are given for each species. This phylogenetic tree was derived from analysis of a dataset comprised of nucleotide sequences from 18S, 5.8S/alignable ITS, and 26S (three regions) *rDNAs*, elongation factor 1-α, mitochondrial small subunit rDNA and COXII. Branch lengths are based on nucleotide substitutions as indicated by the *bar*, and bootstrap values under 50% are not given. *Pichia anomala* is the outgroup species in the analysis. (Modified from Kurtzman 2003; Kurtzman and Robnett 2003)

Table 2.1 Classes, orders and families of yeasts and yeast-like genera of the *Ascomycota*

Neolectomycetes
Neolectales Landvik, O.E. Eriksson, Gargas & P. Gustafsson
Neolectaceae Redhead
Neolecta Spegazzini (T)

Pneumocystidomycetes
Pneumocystidales O.E. Eriksson
Pneumocystidaceae O.E. Eriksson
Pneumocystis P. Delanöe & Delanöe (A)

Schizosaccharomycetes
Schizosaccharomycetales Prillinger, Dörfler, Laaser, Eckerlein & Lehle ex Kurtzman
Schizosaccharomycetaceae Beijerinck ex Klöcker
Schizosaccharomyces Lindner (T)

Taphrinomycetes
Taphrinales Gäumann & C.W. Dodge
Protomycetaceae Gray
Burenia M.S. Reddy & C.L. Kramer (T)
Protomyces Unger (T)
Protomycopsis Magnus (T)
Saitoella S. Goto, Sugiyama, Hamamoto & Komagata (A)
Taphridium Lagerheim & Juel ex Juel (T)
Volkartia Maire (T)
Taphrinaceae Gäumann & C.W. Dodge
Lalaria R.T. Moore (A)
Taphrina Fries (T)

Saccharomycetes
Saccharomycetales Kudryavtsev
Ascoideaceae J. Schröter
Ascoidea Brefeld & Lindau (T)
Cephaloascaceae L.R. Batra
Cephaloascus Hanawa (T)
Dipodascaceae Engler & E. Gilg
Dipodascus Lagerheim (T)
Galactomyces Redhead & Malloch (T)
Geotrichum Link:Fries (A)
Endomycetaceae J. Schröter
Endomyces Reess (T)
Helicogonium W.L. White (T)
Myriogonium Cain (T)
Phialoascus Redhead & Malloch (T)
Eremotheciaceae Kurtzman
Coccidiascus Chatton emend. Lushbaugh, Rowton & McGhee (T)
Eremothecium Borzi emend. Kurtzman (T)
Lipomycetaceae E.K. Novak & Zsolt
Babjevia van der Walt & M.Th. Smith (T)
Dipodascopsis Batra & Millner (T)
Lipomyces Lodder & Kreger van Rij (T)
Myxozyma van der Walt, Weijman & von Arx (A)
Zygozyma van der Walt & von Arx (T)
Metschnikowiaceae T. Kamienski
Clavispora Rodrigues de Miranda (T)
Metschnikowia T. Kamienski (T)

Continues

Table 2.1 Classes, orders and families of yeasts and yeast-like genera of the *Ascomycota—cont'd*

Pichiaceae Zender
Brettanomyces Kufferath & van Laer (A)
Dekkera van der Walt (T)
Pichia Hansen (*pro parte*) (T)
Saturnispora Liu & Kurtzman (T)
Saccharomycetaceae G. Winter
Kazachstania Zubkova (T)
Kluyveromyces Kurtzman, Lachance, Nguyen & Prillinger (T)
Lachancea Kurtzman (T)
Nakaseomyces Kurtzman (T)
Naumovia Kurtzman (T)
Saccharomyces Meyen ex Reess (T)
Tetrapisispora Ueda-Nishimura & Mikata (T)
Torulaspora Lindner (T)
Vanderwaltozyma Kurtzman (T)
Zygosaccharomyces Barker (T)
Zygotorulaspora Kurtzman (T)
Saccharomycodaceae Kudryavtsev
Hanseniaspora Zikes (T)
Kloeckera Janke (A)
Saccharomycodes Hansen (T)
Saccharomycopsidaceae von Arx & van der Walt
Saccharomycopsis Schiönning (T)

Saccharomycetales incertae sedis
Aciculoconidium King & Jong (A)
Ambrosiozyma van der Walt (T)
Arxula van der Walt, M.Th. Smith & Y. Yamada (A)
Ascobotryozyma J. Kerrigan, M.Th. Smith & J.D. Rogers (T)
Blastobotrys von Klopotek (A)
Botryozyma Shann & M.Th. Smith (A)
Candida Berkhout (A)
Citeromyces Santa María (T)
Cyniclomyces van der Walt & Scott (T)
Debaryomyces Lodder & Kreger-van Rij (T)
Hyphopichia von Arx & van der Walt (T)
Kodamaea Y. Yamada, T. Suzuki, Matsuda & Mikata emend. Rosa, Lachance, Starmer, Barker, Bowles & Schlag-Edler (T)
Komagataella Y. Yamada, Matsuda, Maeda & Mikata (T)
Kuraishia Y. Yamada, Maeda & Mikata (T)
Lodderomyces van der Walt (T)
Macrorhabdus Tomaszewski, Logan, Snowden, Kurtzman & Phalen (A)
Nadsonia Sydow (T)
Nakazawaea Y. Yamada, Maeda & Mikata (T)
Ogataea Y. Yamada, Maeda & Mikata (T)
Pachysolen Boidin & Adzet (T)
Phaffomyces Y. Yamada, Higashi, S. Ando & Mikata (T)
Schizoblastosporion Ciferri (A)
Sporopachydermia Rodrigues de Miranda (T)
Starmerella Rosa & Lachance (T)
Starmera Y. Yamada, Higashi, S. Ando & Mikata (T)
Stephanoascus M. Th. Smith, van der Walt & Johannsen (T)
Sympodiomyces Fell & Statzell (A)

Table 2.1 Classes, orders and families of yeasts and yeast-like genera of the *Ascomycota—cont'd*

Trichomonascus Jackson (T)
Trigonopsis Schachner (A)
Wickerhamia Soneda (T)
Wickerhamiella van der Walt (T)
Yamadazyma Billon-Grand emend. M. Suzuki, Prasad & Kurtzman (T)
Yarrowia van der Walt & von Arx (T)
Zygoascus M.Th. Smith (T)

[1](A) = Anamorphic genus, (T) = Telemorphic genus.
[2]Anamorphic and teleomorphic genera are placed together in the same family when relationships are known. For many anamorphic and teleomorphic genera, phylogenetic relationships are unclear and the genera are placed in Saccharomycetales *incertae sedis* until family relationships become known.

K. ohmeri (Rosa et al. 1999). On the basis of single gene analyses, species of the *Lipomycetaceae* and such genera such as *Yarrowia*, *Citeromyces*, and *Saccharomycopsis* appear to be natural groups. *Metschnikowia*, which is characterized by elongated, needlelike ascospores, is represented by a large number of phylogenetically divergent species, but molecular data are insufficient to determine if the genus is monophyletic. Consequently, multigene sequence analysis will be required to resolve relationships between the preceding genera as well as for determining relationships within the genera.

2.5 Basidiomycetous Yeasts

The division *Basidiomycota* is a group of approximately 30,000 described species, with a distinct sexual cycle that includes the production of spores on a clublike structure (basidium). The majority of the species, which are easily recognized as mushrooms, bracket fungi, rusts, and smuts, produce filamentous hyphae and do not have a yeast phase. The recognition of a phylogenetic connection between yeasts and basidiomycetes was slow to evolve. An initial observation of the presence of ballistoconidia led Kluyver and van Niel (1924, 1927) to suggest that *Sporobolomyces* was related to the basidiomycetes. An often overlooked basidiomycete connection was provided by Nyland's (1949) description of the teliosporic genus *Sporidiobolus*. Subsequently, Banno's (1967) description of a teliosporic life cycle in *Rhodosporidium toruloides* gave a solid recognition to the presence of basidiomycetes among the yeasts. That discovery was followed by descriptions of several teleomorphic genera, including *Filobasidium* (Olive 1968), *Leucosporidium* (Fell et al. 1969), *Filobasidiella* (Kwon-Chung 1975), *Cystofilobasidium* (Oberwinkler et al. 1983) and *Bulleromyces* (Boekhout et al. 1991). The phylogenetic relationship between the genera and to the anamorphic species remained open to conjecture until sequence analyses became readily available.

Many researchers explored basidiomycete phylogeny, and a particularly significant report (Swann and Taylor 1995) of 18S rDNA analysis found that basidiomycetous

yeasts occur in three classes: *Uredinomycetes*, *Hymenomycetes*, and *Ustilagenomycetes*. The *Hymenomycetes* are generally associated with the jelly fungi (*Tremellales*). Yeasts are found within four major clades of the *Hymenomycetes*: *Tremellales*, *Trichosporonales*, *Filobasidiales*, and *Cystofilobasidiales*. The *Uredinomycetes*, which are often linked with the rust fungi, include four major clades of yeasts and related genera: *Agaracostilbales*, *Microbotryales*, *Sporidiobolales*, and the *Naohidea* clade. The majority of the *Ustilaginales* are plant and fungal parasites, with the smuts as well-known examples. Sampaio (2004) reported three major groups in the Ustilaginales: Entorrhizomycetidae, Exobasidiomycetidae, and Ustilaginomycetidae. Yeasts are found in the latter two subclasses.

A list of the genera assigned to the three classes (Table 2.2) was modified from the information provided by Scorzetti et al. (2002) and Sampaio (2004). An observation of note is the presence of anamorphic genera *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* in more than one phylogenetic group. Historically, anamorphic genera were described on phenotypic characteristics. For example, the genus *Rhodotorula* was originally delineated by the characteristic red color of the colony, although species with white and cream colonies were subsequently included in the genus (Weijman et al. 1988). A cursory identification of a red yeast as *Rhodotorula* has a high probability of being correct, however, the color is not phylogenetically specific. These phenotypic names are temporarily being maintained, with conversion to teleomorphic nomenclature as sexual cycles and species relationships are determined. For example, Sampaio et al. (2004) found the complete sexual cycle of

Table 2.2 Classes and orders of yeasts and yeast-like genera of the *Basidiomycota*

Hymenomycetes
Cystofilobasidiales Boekhout & Fell
Cystofilobasidium Oberwinkler & Bandoni (T)
Cryptococcus Vuillemin (A)
Guehomyces Fell & Scorzetti (A)
Itersonilia Derx (A)
Mrakia Y. Yamada & Komagata (T)
Phaffia Miller, Yoneyama & Soneda (A)
Tausonia Bab'eva (A)
Udeniomyces Nakase & Takematsu (A)
Xanthophyllomyces Golubev (T)
Filobasidiales Julich
Cryptococcus Vuillemin (A)
Filobasidium Olive (T)
Trichsporonales Boekhout & Fell
Cryptococcus Vuillemin (A)
Trichosporon Behrend (A)
Tremellales Rea emend. Bandoni
Auriculibuller Sampaio (T)
Bullera Derx (A)
Bulleribasidium Sampaio, Weiss & Bauer (T)
Bulleromyces Boekhout & Fonseca (T)
Cryptococcus Vuillemin (A)

Table 2.2 Classes and orders of yeasts and yeast-like genera of the *Basidiomycota—cont'd*

Cuniculitrema Sampaio & Kirschner (T)
Dioszegia Zsolt emend.Takashima, Deak & Nakase (A)
Fellomyces Y. Yamada & Banno (A)
Filobasidiella Kwon-Chung (T)
Holtermannia Saccardo & Traverso (T)
Kockovaella Nakase, Banno & Y. Yamada (A)
Sirobasidium Lagerheim & Patouillard (T)
Sterigmatosporidium Kraepelin & Schulze (T)
Tremella Persoon (T)
Trimorphomyces Bandoni & Oberwinkler (T)
Tsuchiyaea Y. Yamada, Kawasaki, M. Itoh, Banno & Nakase (A)

Uredinomycetes

Agaricostilbales Oberwinkler & Bauer
Agaricostilbum Wright emend. Wright, Bandoni & Oberwinkler (T)
Bensingtonia Ingold emend. Nakase & Boekhout (A)
Chionosphaera Cox (T)
Kondoa Y. Yamada, Nakagawa & Banno emend. Fonseca et al. (T)
Kurtzmanomyces Y. Yamada, M. Itoh, Kawasaki, Banno & Nakase emend. Sampaio (A)
Sporobolomyces Kluyver & van Niel (A)
Sterigmatomyces Fell emend. Y. Yamada & Banno (A)
Microbotryales
Bensingtonia Ingold emend. Nakase & Boekhout (A)
Curvibasidium Sampaio & Golubev (T)
Leucosporidiella Sampaio (A)
Leucosporidium Fell, Statzell, Hunter & Phaff (T)
Mastigobasidium Golubev (T)
Reniforma Pore & Sorenson (A)
Rhodotorula Harrison (A)
Rhodosporidium Banno (T)
Sporobolomyces Kluyver & van Niel (A)
Naohidea clade
Bannoa Hamamoto (T)
Erythrobasidium Hamamoto, Sugiyama & Komagata (T)
Naohidea Oberwinkler (T)
Rhodotorula Harrison (A)
Sakaguchia Y. Yamada, Maeda & Mikata (T)
Sporobolomyces Kluyver & van Niel (A)
Sporidiobolales Sampaio,Weiss & Bauer
Rhodotorula Harrison (A)
Rhodosporidium Banno (T)
Sporidiobolus Nyland (T)

Ustilaginomycetes

Rhodotorula Harrison (A)
Sympodiomycopsis Sugiyama, Tokuoka & Komagata (A)
Malassezia Baillon (A)
Pseudozyma Bandoni emend. Boekhout (A)
Tilletiopsis Derx ex Derx (A)

[1](A) = Anamorphic genus, (T) = Telemorphic genus.
[2]Some genera, such as the anamorphic genus *Cryptococcus*, are presently polyphyletic as defined, and members of the genus are found in more than one teleomorphic order.

Rhodotorula fujisanensis, for which they described the genus and species *Curvibasidium cygneicollum*. Wholesale description of new anamorphic genera on the basis of clade relationships should be avoided. These nomenclatural changes would result in temporary taxonomic fixes that would be confusing and potentially phylogenetically incorrect.

The development of extensive basidiomycetous rDNA (ITS and D1/D2) databases (Fell et al. 2000; Scorzetti et al. 2002) provided a springboard for sizeable expansion in the rate of the descriptions of new species and genera. The number of genera increased from 33 (Kurtzman and Fell 1998) to approximately 55 (Sampaio 2004). The increase in number of species can be exemplified by the genus *Trichosporon*: 19 (Guého et al. 1998) to 36 (Fell and Scorzetti 2004). Importantly, the resulting phylogenetic trees indicate the extent of genetic diversity and the extent of relationships between species, including the anamorphic and teleomorphic species.

The definition of a basidiomycetous species, based on sequence analysis, needs considerable attention. As previously discussed, zero to three nucleotide differences among ascomycetous yeasts in the D1/D2 region generally signifies strains within a single species. This general concept is not always applicable among basidiomycetes. Several significant examples exist, which demonstrate that other genetic regions must be examined to distinguish taxa. *Mrakia gelida* and *M. frigida* are identical in the D1/D2 and significantly different in the ITS and IGS regions (Diaz and Fell 2000). Similarly, the pairs *Filobasidiella neoformans*: *F. bacillispora* and *Phaffia rhodozyma*:*Xanthophyllomyces* type strains differ by one base pair in the D1/D2 domains and significantly in the ITS and IGS regions (Fell and Blatt 1999; Scorzetti et al. 2002).

2.6 Rapid Identification of Yeasts from Ecological Studies

Prior to the existence of molecular phylogeny, ecological research on basidiomycetous yeasts was hampered by reliance on phenotypic characteristics. As a consequence, there was a generalized concept that many of the species have worldwide distributions in diverse environments. This concept was particularly true for species such as *Cryptococcus albidus* and *Rhodotorula glutinis*. Fonseca et al. (2000) dispelled this concept by demonstrating that *C. albidus* is a complex of 12 species. This concept is further dispelled as established species and their phenotypic synonyms are being examined, e.g., *Rhodosporidium* (Sampaio et al. 2001), and as new species are being described that are phenotypically indistinguishable from related and unrelated species, e.g., *Trichosporon* (Middelhoven et al. 2004).

A major ecological problem is that estimates indicate that only 1% of the yeast species in nature have been described. Yeast ecology, therefore, is at a stage of discovery. The ability to undertake biocomplexity studies, viz., environmental/population interactions, is difficult, if the individual players (species) are unknown. A case in point is an ongoing study of yeast populations in the Florida Everglades (Fell and Statzell-Tallman, unpublished). This study involves quarterly (seasonal) sampling in a subtropical Everglades watershed that ranges from freshwater marshes to seawater mangrove habitats. The number of cells ranges from 100 to 2,700 per liter of water. These variations in density correlate with sample location and season of the year.