A genome-informed higher rank classification of the biotechnologically important fungal subphylum *Saccharomycotina*

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Abstract: The subphylum *Saccharomycotina* is a lineage in the fungal phylum *Ascomycota* that exhibits levels of genomic diversity similar to those of plants and animals. The *Saccharomycotina* consist of more than 1 200 known species currently divided into 16 families, one order, and one class. Species in this subphylum are ecologically and metabolically diverse and include important opportunistic human pathogens, as well as species important in biotechnological applications. Many traits of biotechnological interest are found in closely related species and often restricted to single phylogenetic clades. However, the biotechnological potential of most yeast species remains unexplored. Although the subphylum *Saccharomycotina* has much higher rates of genome sequence evolution than its sister subphylum, *Pezizomycotina*, it contains only one class compared to the 16 classes in *Pezizomycotina*. The third subphylum of *Ascomycota*, the *Taphrinomycotina*, consists of six classes and has approximately 10 times fewer species than the *Saccharomycotina*. These data indicate that the current classification of all these yeasts into a single class and a single order is an underappreciation of their diversity. Our previous genome-scale phylogenetic analyses showed that the *Saccharomycotina* contain 12 major and robustly supported phylogenetic clades; seven of these are current families (*Lipomycetaceae, Trigonopsidaceae, Alloascoideaceae, Pichiaceae, Phaffomycetaceae, Saccharomycodaceae*, and *Saccharomycetaceae*), one comprises two current families (*Dipodascaceae* and *Trichomonascaceae*), one represents the genus *Sporopachydermia*, and three represent lineages that differ in their translation of the CUG codon (CUG-Ala, CUG-Ser1, and CUG-Ser2). Using these analyses in combination with relative evolutionary divergence and genome content analyses, we propose an updated classification for the *Saccharomycotina*, including seven classes and 12 orders that can be diagnosed by genome content. This updated classification is consistent with the high levels of genomic diversity within this subphylum and is necessary to make the higher rank classification of the *Saccharomycotina* more comparable to that of other fungi, as well as to communicate efficiently on lineages that are not yet formally named.

Key words: Higher rank classification, Novel taxa, Orthologous groups (OGs), *Saccharomycotina*, Taxonomy, Yeasts

Taxonomic novelties: New classes: *Alloascoideomycetes* M. Groenew., Hittinger, Opulente & A. Rokas, *Dipodascomycetes* M. Groenew., Hittinger, Opulente & A. Rokas, *Lipomycetes* M. Groenew., Hittinger, Opulente, A. Rokas, *Pichiomycetes* M. Groenew., Hittinger, Opulente & A. Rokas, *Sporopachydermiomycetes* M. Groenew., Hittinger, Opulente & A. Rokas, *Trigonopsidomycetes* M. Groenew., Hittinger, Opulente & A. Rokas.

New orders: ***Alloascoideomycetes:*** *Alloascoideales* M. Groenew., Hittinger, Opulente & A. Rokas; ***Dipodascomycetes:*** *Dipodascales* M. Groenew., Hittinger, Opulente & A. Rokas; ***Lipomycetes:*** *Lipomycetales* M. Groenew., Hittinger, Opulente & A. Rokas; ***Pichiomycetes:*** *Alaninales* M. Groenew., Hittinger, Opulente & A. Rokas, *Pichiales* M. Groenew., Hittinger, Opulente & A. Rokas, *Serinales* M. Groenew., Hittinger, Opulente & A. Rokas; ***Saccharomycetes:*** *Phaffomycetales* M. Groenew., Hittinger, Opulente & A. Rokas, *Saccharomycodales* M. Groenew., Hittinger, Opulente & A. Rokas; ***Sporopachydermiomycetes:*** *Sporopachydermiales* M. Groenew., Hittinger, Opulente & A. Rokas; ***Trigonopsidomycetes:*** *Trigonopsidales* M. Groenew., Hittinger, Opulente & A. Rokas.

New families: ***Alaninales:*** *Pachysolenaceae* M. Groenew., Hittinger, Opulente & A. Rokas; ***Pichiales:*** *Pichiaceae* M. Groenew., Hittinger, Opulente & A. Rokas; ***Sporopachydermiales:*** *Sporopachydermiaceae* M. Groenew., Hittinger, Opulente & A. Rokas.

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This study is dedicated to the memory of Cletus P. Kurtzman (1938–2017), a pioneer of yeast taxonomy.

INTRODUCTION

Saccharomycotina yeasts are metabolically diverse, with great potential for use in biotechnological processes (Kurtzman *et al.* 2015). Furthermore, yeast species that are related often share biotechnology-relevant properties (Garay *et al.* 2016, 2017, Riley *et al.* 2016, Heistinger *et al.* 2022). For example, Riley *et al.* (2016) showed that some biotechnologically important traits, such as methylotrophy, lipogenesis, and xylose fermentation, as well as cellulose and hemicellulose hydrolysates utilisation, are mostly restricted to single phylogenetic clades. Currently, several *Saccharomycotina* species beyond *Saccharomyces cerevisiae,* so-called “non-conventional yeasts” (Spencer *et al.* 2002, Abbas 2003, Johnson 2013), serve as microbial cell factories for biotechnological applications, such as food and beverage fermentations (Bourdichon *et al.* 2012, Capece *et al.* 2018), biopharmaceutical molecules, biofuels and bioproducts from biomass, and compounds for the food, feed, pharmaceutical, and cosmetic industries (Groenewald *et al.* 2014, Hittinger *et al.* 2018, Spagnuolo *et al.* 2019, Gassler *et al.* 2020, Srinivasan & Smolke 2020, Solieri 2021, Abeln & Chuck 2021). Development of synthetic biology approaches, including CRISPR-Cas technologies, is expected to continue expanding the range of yeast species useful for biotechnology (Raschmanová *et al.* 2018, Cai *et al.* 2019, Patra *et al.* 2021). Historic classification and naming of yeast species were mostly restricted to analytical technologies and best practices and have resulted in classifications that do not always represent their evolutionary relationships. The revisions in this work are aimed to better align yeast species classification with evolutionary distance.

Traditionally, yeasts are separated from their filamentous counterparts based on a reproduction mode by budding or fission contrary to the production of hyphae (Flegel 1977). This delimitation has deep historical roots that date back to the early ages of mycology and to the separate study of yeasts and filamentous fungi (Lodder & Kreger-van Rij 1952). Although it is now well-established that many yeasts are dimorphic (*i.e*., also able to produce a filamentous stage), this fundamental separation is still widely used given its practicality. The definition that is best suited for yeasts is that they are ascomycetous or basidiomycetous fungi whose asexual growth predominantly results from budding or fission, with or without pseudohyphae and hyphae, and that they form sexual morphs that are not enclosed in fruiting bodies (Kurtzman *et al.* 2011a). The contemporary view acknowledges that the yeast stage has evolved multiple times across the fungal kingdom and, thus, should be viewed mainly as an ontological development and not as the diagnostic feature of any fungal group (Nagy *et al.* 2014). The so called “true yeasts”, by opposition to “yeast-like” (yeasts that form conspicuous mycelium often melanized and that are phylogenetically closer to filamentous taxa), are fungal lineages in the *Ascomycota* and *Basidiomycota* that adopted the yeast lifestyle, an assimilative stage predominantly based on unicellularity (Lodder & Kreger-van Rij 1952, Flegel 1977). A large portion of the known yeast taxa are found in the phylum *Ascomycota* and are divided into two subphyla, *Saccharomycotina* and *Taphrinomycotina*. The third subphylum in *Ascomycota*, *Pezizomycotina*, includes the ascomycetous filamentous fungi and some dimorphic yeast-like taxa (Spatafora *et al.* 2006, Kurtzman *et al.* 2011b, Nagy *et al.* 2014, James *et al.* 2020, Shen *et al.* 2020).

Most known ascomycetous yeast species belong to the subphylum *Saccharomycotina* (Shen *et al.* 2020), which is a lineage that exhibits levels of genetic diversity comparable to those of plants or animals (Dujon 2006, Kurtzman *et al.* 2011b, Shen *et al.* 2018, 2020, Li *et al.* 2021). *Saccharomycotina* yeasts inhabit a wide range of diverse habitats, such as plants, humans, animals, and terrestrial and aquatic ecosystems (Buzzini *et al.* 2017).

Many of the opportunistic human pathogens in *Saccharomycotina* are or were members of the genus *Candida* that grouped essentially all budding yeasts lacking sexual reproduction and other unique characteristics, most notably *Candida albicans*, *C. tropicalis*, *Nakaseomyces glabratus*, the *C. parapsilosis* species complex, and the emerging pathogen *C. auris* (de Hoog *et al.* 2020). Taxonomic revisions have placed *Candida* species in genera that better reflect their natural relationships, namely *Clavispora lusitaniae* (syn. *Candida lusitaniae*), *Pichia kudriavzevii* (syn. *Candida krusei*), *Meyerozyma guilliermondii* (syn. *Candida guilliermondii*), *Nakaseomyces glabratus* (syn. *Candida glabrata*), or will do so soon. To a lesser extent, species classified in the genera *Saccharomyces, Kodamaea, Galactomyces*, and *Magnusiomyces* have also been implicated as agents of fungemia, onychomycosis, and systemic disease, usually linked to immunocompromised patients, new-borns, and the elderly (Cooper 2011, de Hoog *et al.* 2020). Notably, human pathogenicity has evolved multiple times independently in the *Saccharomycotina*, including in *N. glabratus*, *C. albicans*, and *C. auris* (Gabaldon *et al.* 2016, Opulente *et al.* 2019, Rokas 2022).

An overview of the earlier classifications and trends can be found in the monographic series *The Yeasts: A Taxonomic Study* (Lodder & Kreger-van Rij 1952, Lodder 1970, Kreger-van Rij 1984, Kurtzman & Fell 1998, Kurtzman *et al.* 2011b). Before the advent of molecular sequencing the criteria for classifying yeasts were based on micro-morphological features of asexual and sexual reproduction and physiological / biochemical characteristics, with a strong emphasis placed on the ability to utilize different carbon and nitrogen compounds. These criteria divide the *Saccharomycotina* into two groups, yeasts which are known in its asexual (anamorphic) state and reproduce by forming asexual propagules (budding or fission), and yeasts which also show sexual (teleomorphic) morph and can reproduce sexually by forming ascospores (Kurtzman *et al.* 2011b). Often, names of sexual morphs served as the basis for high-rank classifications. The *Saccharomycotina*, including most of the cultivatable genera of ascomycetous yeasts, currently contains a single class, *Saccharomycetes*, and a single order, *Saccharomycetales* (Kurtzman *et al.* 2011b). Thus, the inclusion of all budding yeasts in the *Saccharomycetes* and/or the *Saccharomycetales* was due to the broad definition that has been used for more than 140 years.

From the 1990's onward, genetic sequence information became increasingly popular and immensely useful as a taxonomic criterion (Peterson & Kurtzman 1991, Kurtzman & Robnett 1997, 1998, 2013a, Kurtzman *et al.* 2003, Boekhout *et al.* 2021). Cletus Kurtzman gained prominence among yeast researchers by pioneering the use of molecular phylogenetic evidence to revise the taxonomy of the *Saccharomycotina.* He used sequences of the ribosomal RNA genes to group together genetically similar sexual and asexual species and genera of the *Saccharomycotina* yeasts, ensuring that clear affinities could be established (Kurtzman & Robnett 1997, 1998). During his career, he placed numerous *Candida* species and other asexual yeasts in existing sexual groups in the *Saccharomycetales*, making a great effort to address the problem of polyphyletic genera within this class.

Currently, molecular phylogenetic analyses based on multiple genes play a vital role in the taxonomy of yeasts included in the *Saccharomycotina*, not only in the delineation of novel species, but also in revising the classification of current asexual species (Daniel *et al.* 2014, Kurtzman 2016, Kurtzman *et al.* 2016, Yamazaki *et al.* 2020). In view of the requirement that a fungal species or higher rank taxon be assigned only a single valid name under the new International Code of Nomenclature for algae, fungi, and plants (Turland *et al.* 2018), the competing generic names of asexual/sexual forms (Kurtzman 2011, Kurtzman *et al.* 2011b), such as *Blastobotrys*/*Trichomonascus*, *Botryozyma/Ascobotryozyma, Brettanomyces/Dekkera, Geotrichum/Dipodascus/Galactomyces, Kloeckera/ Hanseniaspora*, *Myxozyma/Lipomyces,* and *Saprochaete/Magnusiomyces,* as well as *Candida* species have been or are currently being revised to make genus membership consistent with phylogenetic affinities.

Analyses of multi-gene datasets that include representatives of all known genera and unresolved polyphyletic groups will facilitate stable generic and higher rank assignments and the placement of novel species, leading to an increase in the number of names and units to appropriately classify the given biological diversity. Currently, (novel) species and their classifications can be found on *The Yeasts* website (https://theyeasts.org/), the successor to *The Yeasts: A Taxonomic Study* book series. This website ensures that taxonomic changes are freely available as soon as possible after a classification is published.

The *Saccharomycotina* consists of more than 1 200 known species and 99 genera. They are currently divided into 15 recognised families (*Alloascoideaceae, Ascoideaceae, Cephaloascaceae, Debaryomycetaceae, Dipodascaceae, Lipomycetaceae, Metschnikowiaceae, Phaffomycetaceae, Pichiaceae* (invalid), *Saccharomycetaceae*, *Saccharomycodaceae*, *Saccharomycopsidaceae, Trigonopsidaceae, Trichomonascaceae*, and *Wickerhamomycetaceae*); *Endomycetaceae* is also listed in Kurtzman *et al.* (2011b) as a speculative family within the *Saccharomycotina*. All these families are comprised in one order (Kurtzman *et al.* 2011b). Recent phylogenetic studies utilising genome-scale data have divided the *Saccharomycotina* into 12 major clades (Shen *et al.* 2017, 2018). These 12 clades have been referred to as the *Lipomycetaceae, Trigonopsidaceae, Dipodascaceae/Trichomonascaceae, Alloascoideaceae, Sporopachydermia*, CUG-Ala, *Pichiaceae*, CUG-Ser1, CUG-Ser2, *Phaffomycetaceae, Saccharomycodaceae*, and *Saccharomycetaceae* clades. Shen *et al.* (2018) also proposed the presence of additional higher order lineages within these major clades.

Recent phylogenomic analyses of ascomycetous fungi and yeasts, including comparisons of the evolutionary age and genomic divergence of taxonomic categories between the *Saccharomycotina* and other subphyla, suggest that current taxonomic circumscriptions do not fully account for the high level of evolutionary divergence in the *Saccharomycotina* (Shen *et al.* 2020, Li *et al.* 2021). Comparisons of genomic properties between the *Saccharomycotina*, consisting of one class, and its sister subphylum, *Pezizomycotina*, consisting of 16 classes, revealed that the two subphyla exhibited substantial divergence in several genomic properties (Shen *et al.* 2020). The *Saccharomycotina* have smaller genomes, lower numbers of protein-coding genes, lower numbers of DNA repair genes, lower GC contents, higher numbers of tRNA genes, and higher levels of genomic diversity and evolutionary rates than the *Pezizomycotina* (Shen *et al.* 2020). Still, the subphylum *Saccharomycotina* consists of only one class compared to the 16 classes in *Pezizomycotina*. The taxonomic placement of Ascomycete budding yeasts into a single class, *Saccharomycetes*, means that the last common ancestor of this class is much more ancient than the last common ancestors of any of the nine classes studied by Shen *et al.* (2020) in the subphylum *Pezizomycotina*. When comparing the fission yeasts (*Taphrinomycotina*) with the *Saccharomycotina*, the genome sizes of species belonging to these two subphyla are similar, but the number of species belonging to the *Taphrinomycotina* (~140 species) is nearly 10-fold lower than that of the *Saccharomycotina*, even though the *Taphrinomycotina* consists of six classes and six orders (Čadež *et al.* 2021).

In the study of Li *et al.* (2021), relative evolutionary divergence (RED) analyses showed that one of the most serious instances of under-classification within the Fungal kingdom concerns the *Saccharomycotina*. Simply put, there is little correspondence between the current poor taxonomic resolution and the observed very high evolutionary divergence within the *Saccharomycotina*. The present higher rank classification system is imbalanced on two levels, the under-classified class *Saccharomycetes* and the under-classified order *Saccharomycetales*.

Saccharomycotina species continue to be described in the *Saccharomycetes* and the

Saccharomycetales. Given the high levels of evolutionary divergence detected in this group and considering the conclusions of previous phylogenomic studies (Shen *et al.* 2018, 2020, Li *et al.* 2021), it is clear that the higher taxonomic groups within the *Saccharomycotina* are due to be revisited and revised. A reclassification would be useful to communicate the evolutionary divergence more efficiently across biological disciplines. As the biotechnological industry currently uses only a small fraction of the more than 1 200 potentially exploitable *Saccharomycotina* species, reclassification of the higher ranks of the subphylum is expected to enhance the prospecting and discovery of novel biotechnological interesting yeasts and traits, as the focus of the search for traits of interest and novel species can then be more efficiently directed to clearly demarcated orders and classes.

Here we propose a new higher rank classification system that includes ten new orders and six new classes of the subphylum *Saccharomycotina*. Novel type families are described in the two cases for which it is essential for the description of the new orders and classes. The intent of our study is to focus on establishing higher level taxonomic boundaries that are evolutionarily consistent with the boundaries in other fungal lineages, as well as to set the groundwork for future studies that will address family- and genus-level circumscriptions. Using data from comprehensive genome-scale phylogenetic analyses, in combination with RED analyses, we propose an updated classification of the *Saccharomycotina* that includes the following seven classes: *Saccharomycetes, Pichiomycetes, Sporopachydermiomycetes, Alloascoideomycetes, Dipodascomycetes, Trigonopsidomycetes,* and *Lipomycetes*. We similarly propose the following 12 orders: *Saccharomycetales, Saccharomycodales, Phaffomycetales, Ascoideales, Serinales, Alaninales, Pichiales, Sporopachydermiales, Alloascoideales, Dipodascales, Trigonopsidales*, and *Lipomycetales*.

MATERIALS AND METHODS

RED analyses

To evaluate whether fungal taxonomy is consistent with evolutionary genomic divergence, we calculated relative evolutionary divergence (RED) values using PhyloRank (v. 0.0.37, https://github.com/dparks1134/PhyloRank/) from the annotated tree as described previously (Li *et al.* 2021). Briefly, the NCBI taxonomy associated with every fungal genome was obtained from the NCBI Taxonomy FTP site on January 17, 2020. PhyloRank linearly interpolates the RED values of every internal node according to lineage-specific rates of evolution under the constraints of the root being defined as zero and the RED of all present taxa being defined as one (Parks *et al.* 2018). The RED intervals for each rank were defined as the median RED value ± 0.1 to serve as a guide for the normalization of taxonomic ranks from genus to phylum.

To evaluate whether the newly proposed taxonomic categories of *Saccharomycotina* were more consistent with evolutionary genomic divergence, we ran the RED analysis again with the proposed class and order names of *Saccharomycotina*.

Genome-scale phylogeny of the kingdom Fungi

The fungal phylogeny used in this study is derived from a recent phylogenomic study that used maximum likelihood analysis with a concatenation single-model (LG+G4) approach on a data matrix of 1 672 taxa (1 644 fungi and 28 outgroups) and 290 BUSCO genes (Li *et al.* 2021). See also Table S3 for more information on the species used (Li *et al.* 2021).

Identification of class- and order-specific genes

To identify class- and order-specific genes, we used all annotated protein sequences in a previously published dataset of the genomes of 332 representative yeast species (Shen *et al.* 2018) to build orthologous groups of genes (OGs) using OrthoFinder v. 2.5.2 (Emms & Kelly

2019) with default parameters. For a given taxon of interest, we screened all 34 247 OGs and only retained those OGs with species occupancy ≥ 95 %, that is those OGs that were present in at least 95 % of species that were included in that taxon. In summary, we identified a total sum of 260 OGs and 325 OGs that were specifically present in the 7 proposed classes and 12 proposed orders, respectively. To further examine the specificity of those class- and order-specific OGs, we carried out TBLASTN searches of protein sequences of OGs against a custom database, consisting of the Nucleotide (nt) database downloaded from NCBI on December 11, 2021 and 1 644 fungal genomes from Li *et al.* (2021), with an e-value cut-off of 1e-6. Lastly, we annotated the screened order-/class-specific OGs with Gene Ontology (GO) and Pfam domain families using the eggNOG-mapper v. 2.0 (Cantalapiedra *et al.* 2021).

Data availability Statement

All data associated with this study are provided in the main article and supplementary materials. The class- and order-specific orthologous groups (OGs) and their DNA and protein sequences will become available on the figshare repository upon publication (http://dx.doi.org/10.6084/m9.figshare.19374350). For review purposes only, these files can be accessed via the private link: https://figshare.com/s/2396c452d2f214869cf2.

RESULTS AND DISCUSSION

RED analyses

The availability of a genome-scale phylogeny for fungi provides a unique opportunity to evaluate if current taxonomy truly reflects the evolutionary relationships and rates of genome evolution of the *Saccharomycotina*. Previous taxon-rich phylogenomic analyses yielded a robustly supported phylogeny of the *Saccharomycotina* (Shen *et al.* 2018, 2020, Li *et al.* 2021). The new phylogeny divides the subphylum into 12 major clades, each of which is strongly supported by different approaches of maximum likelihood phylogenetic inference (e.g., concatenation and coalescence).

The RED (relative evolutionary divergence) approach was developed to revise taxonomic ranks in Bacteria and Archaea so that they reflect evolutionary divergence (Parks *et al.* 2018, Rinke *et al.* 2020). We recently applied this approach to fungi and found that fungal taxonomy is broadly aligned with both genome sequence divergence and divergence time (Li *et al.* 2021, Fig. 1a). However, we also determined that nearly 40 % (22 of 49 categories, including 1 order, 5 families, and 16 genera) of the under-classified categories in the taxonomy of fungi were within the *Saccharomycotina* (Fig. 1b), suggesting that the lack of taxonomic and evolutionary correspondence of the subphylum *Saccharomycotina* hinders the utility of taxonomy as a yardstick for comparative biology.

To improve the taxonomic classification of the *Saccharomycotina*, we propose new *Saccharomycotina* higher categories based on the current understanding of evolutionary relationships and rates of genome evolution of *Saccharomycotina*. In contrast to the single class and single order present in the previous *Saccharomycotina* higher rank classification, our proposed taxonomic revision divides the subphylum into seven classes and 12 orders.

Of the proposed seven classes and 12 orders examined, we found that the majority (five of seven classes, nine of 12 orders) of proposed categories fell within ±0.1 of the median RED value of taxa at that rank (a threshold previously used by Li *et al.* (2021)) as a guide to evaluate the degree to which the evolutionary divergences of taxonomic ranks across fungi were similar), suggesting the new categories better reflect the evolutionary divergence within the subphylum (Figure 1b).

Phylogeny

Data from comprehensive phylogenomic analyses suggest that we can propose an updated classification of the *Saccharomycotina*. Specifically, we propose the following 7 classes: *Saccharomycetes, Pichiomycetes, Sporopachydermiomycetes, Alloascoideomycetes, Dipodascomycetes, Trigonopsidomycetes,* and *Lipomycetes*. Similarly, we propose the following 12 orders: *Saccharomycetales, Saccharomycodales, Phaffomycetales, Ascoideales, Alaninales, Pichiales*, *Serinales, Sporopachydermiales, Alloascoideales, Dipodascales, Trigonopsidales*, and *Lipomycetales* (Figs 2, S1, Table S3).

Class- and order-specific gene families

As described in the Materials and Methods, we identified a total of 260 OGs and 325 OGs that were specifically (≥ 98 % specificity) present in the seven proposed classes and 12 proposed orders respectively (Table 1). From the TBLASTN searches of protein sequences, we found that all 260 class-specific OGs and all 325 order-specific OGs had no significant blast hits elsewhere in the dataset (Table S1). Results are presented in Tables 1, S1, and S2.

Updated higher rank taxonomy of the *Saccharomycotina*

Table 2 gives an overview of all the existing and newly proposed classes, orders, families, and genera of yeasts of the subphylum *Saccharomycotina*. Tables 1, S1, and S2 list all the class and order-specific protein families (OGs). See these tables for detailed information on these proteins.

Taxonomy

Alloascoideomycetes M. Groenew., Hittinger, Opulente & A. Rokas, ***class. nov.*** MycoBank MB 847278.

Diagnosis: Class-specific protein families OG0009556 and OG0024318. Multilateral budding and formation of pseudohyphae and septate hyphae. Blastoconidia form on hyphae and may be sessile or arise from denticles. Sugars are not fermented. Phylogenetic analyses using DNA sequences encoding LSU rDNA, SSU rDNA, *EF-1*α, *Rpb1*, and *Rpb2* (Kurtzman & Robnett 2013b).

Description: With the characteristics of *Alloascoideaceae* (Kurtzman & Robnett 2013b).

Type order: *Alloascoideales* M. Groenew., Hittinger, Opulente & A. Rokas *ord. nov.*, this study.

Included order: *Alloascoideales*.

Notes: Although the number of species available for the orders *Alloascoideales* and *Sporopachydermiales* are limited, we propose the inclusion of these two orders in separate classes due to their long branches. See more information in the description of the class *Sporopachydermiomycetes*.

Alloascoideales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847279.

Diagnosis: Order-specific protein family OG0009556 and OG0024318. Phylogenetic analyses using DNA sequences LSU rDNA, SSU rDNA, *EF-1*α*, Rpb1*, and *Rpb2* sequences (Kurtzman & Robnett 2013b). Multilateral budding and formation of pseudohyphae and septate hyphae. Blastoconidia form on hyphae and may be sessile or arise from denticles.

Description: With the characteristics of *Alloascoideaceae* (Kurtzman & Robnett 2013b).

Type family: *Alloascoideaceae* Kurtzman & Robnett, FEMS Yeast Res. 13: 429. 2013. MycoBank MB 802503.

Type genus: *Alloascoidea* Kurtzman & Robnett, FEMS Yeast Res. 13: 426. 2013. MycoBank MB 802504.

Type: *Alloascoidea hylecoeti* (L.R. Batra & Francke-Grosm.) Kurtzman & Robnett, FEMS Yeast Res. 13: 427. 2013. MycoBank MB 802506.

Family included in the *Alloascoideales*: *Alloascoideaceae*.
Genus included in the *Alloascoideales* based on the current study: *Alloascoidea*.

Notes: Although only one species is included in these analyses, considering the long branches observed by Kurtzman & Robnett (2013b) and Shen *et al.* (2018), we have decided to split off *Alloascoidea* from *Sporopachydermia* for inclusion in the new order *Alloascoideales*.

Dipodascomycetes M. Groenew., Hittinger, Opulente & A. Rokas, ***class. nov.*** MycoBank MB 847281.

Diagnosis: Class-specific protein families OG0005588, OG0005810, and OG0006132. This class contains dimorphic yeasts that can also produce arthroconidia. Phylogenetic analyses using DNA sequences encoding LSU rDNA, SSU rDNA, mtSSU rDNA, and *EF-1*α (Kurtzman *et al.* 2007).

Description: With the characteristics of *Dipodascaceae* (Engler & Gilg 1924).

Type order: *Dipodascales* M. Groenew., Hittinger, Opulente & A. Rokas *ord. nov*., this study.

Included order: *Dipodascales*.

Dipodascales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847282.

Diagnosis: Order-specific protein families OG0005588, OG0005810, and OG0006132. Phylogenetic analyses using LSU rDNA, mtSSU rDNA, and *COX2* sequences (Kurtzman & Robnett 2007). Contains dimorphic yeasts that can also produce arthroconidia.

Description: With the characteristics of *Dipodascaceae* (Engler & Gilg 1924).

Type family: *Dipodascaceae* Engl. & E. Gilg, Syllabus der Pflanzenfamilien: 59. 1924. MycoBank MB 80709.

Type genus: *Dipodascus* Lagerh., Jahrb. Wiss. Bot. 24: 549. 1892. MycoBank MB 1632.

Type: *Dipodascus albidus* Lagerh., Jahrb. Wiss. Bot. 24: 549. 1892. MycoBank MB 246592.

Families included in the *Dipodascales*: *Dipodascaceae*, *Trichomonascaceae*.
Genera included in the *Dipodascales* based on the current and previous studies: *Crinitomyces, Deakozyma*, *Diddensiella*, *Dipodascus* (asexual morph *Geotrichum*), *Galactomyces* (asexual morph *Geotrichum*), *Groenewaldozyma*, *Limtongella*, *Magnusiomyces* (asexual morph *Saprochaete*), *Middelhovenomyces*, *Nadsonia* (asexual morph *Schizoblastosporion*), *Spencermartinsiella*, *Starmerella*, *Sugiyamaella*, *Trichomonascus* (asexual morph *Blastobotrys*), *Wickerhamiella*, *Yarrowia*, *Zygoascus*.

Notes: Genera that were not included in this study are *Limtongella* (Sakpuntoon *et al.* 2020b) and *Crinitomyces* (Sakpuntoon *et al.* 2022), but based on their phylogenetic placement, they are sister genera to *Deakozyma* and *Dipodascus*, respectively, which justifies the inclusion of both as genera of the *Dipodascales*. Based on our analyses, several genera that were previously included in the *Saccharomycetales incertae sedis* (Kurtzman *et al.* 2011b) are also included in this order, together with the genera of the families *Dipodascaceae* and *Trichomonascaceae*. Although the genus *Trichomonascus* was not included in the analyses, the asexual morph *Blastobotrys* was. Based on previous studies (Kurtzman & Robnett 2007, Kurtzman *et al.* 2011b) and due to the fact that *Trichomonascus* is the type genus of *Trichomonascaceae*, it is also included in the *Dipodascales*.

Our RED analyses showed low relative evolutionary divergence values, indicating possible continued under-classification for this group. However, when *Yarrowia* and *Nadsonia* were excluded from *Dipodascales*, this order's RED value only shifted from 0.482 to 0.479, and it was still not located within the order boundaries. This led to the decision to include *Yarrowia* and *Nadsonia* in *Dipodascales* for now. However, description of more species in this group may eventually indicate that a split is necessary.

Biotechnological relevance: This order includes the biotechnologically important oleaginous yeast *Yarrowia lipolytica*, which is being considered for multiple industrial applications, such as a protein source for livestock feeding, a production host for organic acids or hydrophobic substances, for heterologous production of pharmaceutical and industrial proteins and enzymes, and for the production of bioproducts and biofuels (reviewed by Groenewald *et al.* 2014). Certain *Blastobotrys* species are used in biotechnology, including *Blastobotrys mokoenaii*, a species with a high secretion of xylanase, which was used industrially in bio-bleaching and bioprocessing of wood pulps (Techapun *et al.* 2003). *Blastobotrys adeninivorans* has major stress tolerant properties and an unusual metabolic flexibility that allows this species to use a wide range of carbon and nitrogen sources, which makes it a potentially important species for industrial purposes (Kunze *et al.* 2014). *Starmerella* species, especially those related to *S. magnoliae*, can produce erythritol (Yang *et al.* 1999) and D-mannitol (Song *et al.* 2002), which can be used as sweeteners. *Starmerella bombicola* is an excellent producer of sophorolipids (glycolipids), which are biodegradable and non-toxic biosurfactants, with a wide range of biotechnological applications in the food, health, and cosmetic industries. (Takahashi *et al.* 2011, Qazi *et al.* 2022). Several species of *Sugiyamaella* are xylanase producers, and other species are reported to ferment D-xylose to ethanol, traits with biotechnological interest for the production of lignocellulosic ethanol (Sena *et al.* 2017).

Geotrichum klebahnii has several reported biotechnological characteristics and abilities, such as the rapid degradation of lignin from corn stover, good growth on hemicellulose from sugar-cane bagasse, and the secretion of propectinases with polygalacturonase activities. *Geotrichum candidum* is used to produce different varieties of cheeses and meat products (Ropars & Giraud 2022).

Clinical relevance: There have been reports of *Galactomyces candidus*, *Magnusiomyces capitatus*, and *Yarrowia lipolytica* causing human infections and the first two species are increasingly found in the blood of immunocompromised patients (see overview in Kurtzman *et al.* 2011b, Groenewald *et al.* 2014, de Hoog *et al.* 2020).

Lipomycetes M. Groenew., Hittinger, Opulente, A. Rokas, ***class. nov.*** MycoBank MB 847285.

Diagnosis: Class-specific protein families OG0010973, OG0011052, OG0008472, and OG0009553. Phylogenetic analyses using either LSU rDNA or DNA sequences encoding SSU rDNA, LSU rDNA, mtSSU rDNA, and *EF-1*α (Kurtzman *et al.* 2007). Strains produce intracellular lipids and extracellular starch-like compounds, fermentation is absent, nitrate is not assimilated.

Description: With the characteristics of *Lipomycetaceae* (Novák & Zsolt 1961).

Type order: *Lipomycetales* M. Groenew., Hittinger, Opulente & A. Rokas *ord. nov*., this study.

Included order: *Lipomycetales*.

Notes: It is known that the higher levels of genomic diversity in *Saccharomycotina* stem from an acceleration of the evolutionary rate that occurred within the subphylum, after the divergence of *Lipomycetaceae* from the rest of the *Saccharomycotina*. This acceleration can also be seen in the genetic divergence that exists between the *Lipomycetes* and the rest of the *Saccharomycotina* classes presented here. See clustering presented in the phylogenomic tree in Shen *et al.* (2018), as well Figure S1, which includes all groups of *Saccharomycotina*.

Lipomycetales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847286.

Diagnosis: Order-specific protein families OG0010973 OG0011052, OG0008472, OG0097159, and OG0009553. Phylogenetic analyses using LSU rDNA or DNA sequences encoding SSU rDNA, LSU rDNA, mtSSU rDNA, and *EF-1*α sequences (Kurtzman *et al.* 2007). Ability to produce intracellular lipids and extracellular starch-like compounds. Fermentation is absent, nitrate is not assimilated.

Description: With the characteristics of *Lipomycetaceae* (Novák & Zsolt 1961).

Type family: *Lipomycetaceae* E.K. Novák & Zsolt, Acta Bot. Acad. Sci. Hung. 7: 97. 1961. MycoBank MB 80960.

Type genus: *Lipomyces* Lodder & Kreger, The Yeasts: A Taxonomic Study: 669. 1952. MycoBank MB 2888.

Type: *Lipomyces starkeyi* Lodder & Kreger, The Yeasts: A Taxonomic Study: 669. 1952. MycoBank MB 299844.

Family included in the *Lipomycetales*: *Lipomycetaceae*.
Genera included in the *Lipomycetales* based on this study and previous studies: *Babjevia*,

Dipodascopsis, *Kockiozyma, Lipomyces* (asexual morph *Myxozyma*).

Notes: Although the genera *Dipodascopsis*, *Babjevia,* and *Kockiozyma* were not included in these analyses, based on previous analyses (Kurtzman *et al.* 2007, Yamazaki *et al.* 2020, Jindamorakot *et al.* 2012, Kurtzman *et al.* 2011a), it is clear that these genera belong to the family *Lipomycetaceae* and are therefore also included in the *Lipomycetales*.
Biotechnological relevance: There are many *Lipomyces* species with important biotechnological capabilities that enable them to play a role in the bioindustry. *Lipomyces kononenkoae, L. lipofer*, and *L. starkeyi* can hydrolyze dextran; produce δ-12-desaturase, which is an enzyme that converts oleic acid into linoleic acid; produce dextranases; and show extracellular α-amylase activity, a key characteristic for the hydrolysis of industrial starch (Kurtzman *et al.* 2011b). Various *Lipomyces* species such as L. doorenjongii, *L. kalimantanensis*, *L. lipofer*, *L. mesembrius*, *L. starkeyi*, *L. tetrasporus*, *L. tropicalis* and *L. yarrowii* have been reported to be the oleaginous yeasts with cabable of accumulate high lipid, therefore, they have potential for microbial lipid production (Sapsirisuk et al 2022, Poontawee et al 2023).

Pichiomycetes M. Groenew., Hittinger, Opulente & A. Rokas, ***class. nov.*** MycoBank MB 847268.

Diagnosis: Class-specific protein family OG0000547.

Type order: *Pichiales* M. Groenew., Hittinger, Opulente & A. Rokas, *ord. nov*., this study.

Orders included in *Pichiomycetes*: *Alaninales, Pichiales, Serinales*.

Alaninales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847270.

Diagnosis: Order-specific protein family OG0016373. The CUG codon is translated into an alanine, instead of the nearly universal leucine. Phylogenetic analyses using DNA sequences encoding LSU rDNA, SSU rDNA, EF-1α, and mtSSU rDNA. Budding is multilateral and on a narrow base, ascospores are hemispherical or hat-shaped.

Type family: *Pachysolenaceae* M. Groenew., Hittinger, Opulente & A. Rokas, *fam. nov*., this study.

Pachysolenaceae M. Groenew., Hittinger, Opulente & A. Rokas, ***fam. nov.*** MycoBank MB 847271.

Diagnosis: Family-specific protein family OG0016373. The CUG codon is translated into an alanine, instead of the nearly universal leucine. Phylogenetic analyses using DNA sequences encoding LSU rDNA, SSU rDNA, *EF-1*α, and mtSSU rDNA (Kurtzman & Robnett 2010). Budding is multilateral and on a narrow base, ascospores are hemispherical or hat-shaped.

Type genus: *Pachysolen* Boidin & Adzet, Bull. Trimestriel Soc. Mycol. France 73: 340. 1958 [1957]. MycoBank MB 3676.

Type: *Pachysolen tannophilus* Boidin & Adzet, Bull. Trimestriel Soc. Mycol. France 73: 340. 1958 [1957]. MycoBank MB 302177.

Family included in the *Alaninales*: *Pachysolenaceae.*
Genera included in the *Alaninales* based on this study: *Nakazawaea*, *Pachysolen, Peterozyma.*

Notes: All genera assigned now in this order were previously included in the *Saccharomycetales incertae sedis* (Kurtzman *et al.* 2011b). For many years, the taxonomic placement of taxa in *Nakazawaea*, *Pachysolen*, and *Peterozyma* was unclear. Recent studies have shown that these genera form a group in which the CUG codon of species in these genera is translated into an alanine, instead of the nearly universal leucine. The CUG-Ala clade is monophyletic (Mühlhausen *et al.* 2016, Riley *et al.* 2016, Shen *et al.* 2016, 2017, 2018, Krassowski *et al.* 2018).

Biotechnological relevance: *Nakazawaea peltata* produces a β-glucosidase that can help, in combination with purified cellulase, in the degradation of cellulose; it is also an efficient producer of xylitol, a substance used increasingly as an artificial sweetener (Saha & Bothast 1996,1999). *Pachysolen tannophilus* can convert crude glycerol feedstocks into ethanol (Liu *et al.* 2012), and it was also shown that strains from this species can switch from glucose to efficient xylose fermentation (Slininger *et al.* 2011).

Pichiales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847273.

Diagnosis: Order-specific protein families OG0005494 and OG0005374. Phylogenetic analyses using DNA sequences encoding LSU rDNA, SSU rDNA, *EF-1*α*,* mtSSU rDNA (Daniel *et al.* 2014).

Type family: *Pichiaceae* M. Groenew., Hittinger, Opulente & A. Rokas, *fam. nov*., this study.

Pichiaceae M. Groenew., Hittinger, Opulente & A. Rokas, ***fam. nov.*** MycoBank MB 847274.
Synonym: '*Pichiaceae*' Zender [as '*Pichiacées*'], Bull. Soc. Bot. Genève sér. 2, 17: 290. 1925. MycoBank MB 81172, *nom. inval.*, Art. 32.1(c), see Art. 18.4 (Shenzhen).

Diagnosis: Family-specific protein families OG0005494 and OG0005374. Phylogenetic analyses using DNA sequences encoding LSU rDNA, SSU rDNA, *EF-1*α, mtSSU rDNA (Daniel *et al.* 2014).

Type genus: *Pichia* E.C. Hansen, Centralbl. Bakteriol. Parasitenk., 2. Abth. 12: 538. 1904. MycoBank MB 4095.

Type: *Pichia membranifaciens* (E.C. Hansen) E.C. Hansen, Centralbl. Bakteriol. Parasitenk., 2. Abth. 12: 538. 1904. MycoBank MB 227217.

Family included in the *Pichiales*: *Pichiaceae*
Genera included in the *Pichiales* based on the current and previous studies: *Allodekkera, Ambrosiozyma*, *Brettanomyces* (teleomorph *Dekkera*), *Citeromyces*, *Komagataella*, *Kregervanrija*, *Kuraishia*, *Martiniozyma*, *Ogataea*, *Pichia, Saturnispora*.

Notes: Several genera that were previously included in the *Saccharomycetales incertae sedis* by Kurtzman *et al.* (2011b), together with the genera of the family *Pichiaceae* and the recently introduced genus *Allodekkera* (Jutakanoke *et al.* 2017), are now included in the *Pichiales*. *Komagataella* was previously included in the family *Phaffomycetaceae* (Kurtzman *et al.* 2011b), but genome-scale analyses support its reassignment to *Pichiales* (see also the description of *Phaffomycetales*).

Biotechnological relevance: Several species in the *Ogataea* clade were found to have characteristics that make them good candidates for biotechnological applications, with *O. parapolymorpha* as a model organism. *Komagataella pastoris*, *Komagataella phaffii*, *Ogataea polymorpha*, and *Ogataea methanolica* are the most frequently used yeasts for heterologous protein production (Ravin *et al.* 2013, Love *et al.* 2016, Riley *et al.* 2016, Liu *et al.* 2022). Strong regulatory promoters from *Ogataea polymorpha, O. methanolica*, and *Candida boidinii* were shown to be superior to endogenous promoters for recombinant protein expression in *Komagataella pastoris* (Mombeni *et al.* 2020, Vogl *et al.* 2020). Genome-scale metabolic models have have been constructed and metabolic engineering is being implemented in this clade, including protein production platform species in *Komagataella* and *Ogataea* (Patra *et al.* 2021, Vogl *et al.* 2020). *Pichia kluyveri* is common in natural fermentations of agricultural products, such as coffee beans and in beverages (Wang *et al.* 2020) and its potential importance in aroma production was also illustrated (Karaalioğlu & Yüceer 2021, Vicente et al 2021). *Candida arabinofermentans* (Kurtzman & Dien 1998) and *C. succiphila* (Dien *et al.* 1996) can ferment L-arabinose into moderate concentrations of ethanol, and *C. maris* can perform the enantioselective reduction of 5-acetylfuro[2,3-c]pyridine to (S)-5-(1-hydroxyethyl)furo[2,3-c]-pyridine, which can be useful in the pharmaceutical industry (Kawano *et al.* 2003). Tiukova *et al.* (2019) suggested that *Brettanomyces naardenensis* has great biotechnological potential by providing evidence through functional genome annotations, for stress tolerance, and its ability to ferment D-xylose well. *Brettanomyces bruxellensis* is associated with fermentation processes in which it can also be considered a beneficial microorganism, specifically due to its unique nutrient metabolism and peculiar flavour production hold great potential in food processes, such as production of specific beers (Steensels et al 2015, Tiukova *et al.* 2019) and as a bioethanol producer (Steensels et al 2015, da Silva *et al.* 2020).

Clinical relevance: *Pichia kudriavzevii* (asexual morph *Candida krusei*) is often isolated with humans and animals and was indicated to be the fifth most common cause of candidemia (see overview in Kurtzman *et al.* 2011b, de Hoog *et al.* 2020). Several *Pichia* species can grow at temperatures of 37 °C or higher, such as *P. mandshurica, P. inconspicua*, and *P. norvegensis*, and these have been suggested to have the potential to infect humans and cause disease (Majoros *et al.* 2003, Kurtzman *et al.* 2011b).

Serinales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847269.

Diagnosis: Order-specific protein family OG0004743. The CUG codon is translated into a serine, instead of the nearly universal leucine.

Type family: *Metschnikowiaceae* Kamieński ex Doweld, Index Fungorum 33: 1. 2013. MycoBank MB 550284.

Type genus: *Metschnikowia* Kamieński, Trudy Imp. S.-Peterburgsk. Obshch. Estestvoisp., Vyp. 3, Otd. Bot. 30(1): 364. 1900. MycoBank MB 3147.

Type: *Metschnikowia bicuspidata* (Metschn.) Kamieński. Trudy Imp. S-Peterburgsk. Obshch. Estestvoisp., Vyp. 3, Otd. Bot. 30(1): 363. 1900. MycoBank MB 100690.

Families included in the *Serinales*: *Cephaloascaceae, Debaryomycetaceae*, *Metschnikowiaceae.*

Genera included in the *Serinales* based on the current and previous studies: *Aciculoconidium, Babjeviella, Candida, Cephaloascus, Clavispora, Danielozyma*, *Debaryomyces, Diutina*, *Hemisphaericaspora*, *Hyphopichia, Kodamaea, Kurtzmaniella, Limtongozyma*, *Lodderomyces, Metahyphopichia, Metschnikowia, Meyerozyma, Millerozyma, Nematodospora*, *Priceomyces, Scheffersomyces, Schwanniomyces, Spathaspora, Suhomyces*, *Teunomyces*, *Wickerhamia*, *Yamadazyma.*

Notes: This group is represented by species that translate their CUG codon into a serine, instead of the nearly universal leucine, and were included in the CUG-Ser1 clade (Shen *et al.* 2016, 2017, 2018, Riley *et al.* 2016, Krassowski *et al.* 2018). Based on these analyses, the medically important *Candida* species *C. albicans, C. tropicalis, C. parapsilosis, C. orthopsilosis, and C. auris* are included in the *Serinales*. *Diutina, Hemisphaericaspora, Limtongozyma, Metahyphopichia*, *Nematodospora*, and *Schwanniomyces* were not included in this study, but previous studies (Kurtzman & Suzuki 2010, Hui *et al.* 2014, Khunnamwong *et al.* 2015, Gouliamova *et al.* 2016, Boontham *et al.* 2020, Khunnamwong *et al.* 2022) placed these genera in *Debaryomycetaceae* or *Metschnikowiaceae*, two of the current families included in the *Serinales*.

Biotechnological relevance: This order includes the agro-industrially important genus *Debaryomyces*, particularly the species *D. hansenii* and its close relatives, which are known as extremophilic yeasts with great biotechnological potential (Fröhlich-Wyder 2003, Breuer & Harms 2006, see overview in Kurtzman *et al.* 2011b). Several species in the genera *Debaryomyces* and *Metschnikowia* are associated with fermented foods and are thus candidates for food applications (Bourdichon *et al.* 2012). *Limtongozyma cylindracea* has been of considerable interest for the ability to produce lipases that have numerous applications in the biotransformation of various molecules in the food, pharmaceutical, pesticide, and other industries (Matten *et al.* 2022). Several *Scheffersomyces* species, such as *S. shehatae, S. insectosus*, *S. lignosus* and *S. stipitis* are known for their ability to ferment *D*-xylose (reviewed by Ruchala & Sibirny 2021). *Spathaspora passalidarum* and *S. arborariae* are efficient D-xylose fermenters (Cadete & Rosa 2018). Additional species in this order have biotechnological potential. For example, *Candida maltosa* can assimilate *n*-alkane and therefore could decontaminate soil polluted by petroleum products (Schmitz *et al.* 2000). *Candida mogii* and *C. tropicalis* have been used in the conversion of *D*-xylose into xylitol (de Mancilha & Karim 2003, Kumar *et al.* 2022), and the opportunistic human pathogen *C. tropicalis* is also known for its abilities to degrade hydrocarbons, perform lipid biotransformations, and degrade polyphenols in wastewater (Ettayebi *et al.* 2003).

Clinical relevance: *Candida albicans, C. tropicalis*, *C. auris*, and *C. parapsilosis* with its close relatives *C. metapsilosis* and *C. orthopsilosis* are broadly known as opportunistic human pathogens that have been isolated from various clinical material (Lockhart *et al.* 2008, Miranda *et al.* 2009, Silva *et al.* 2009, de Hoog *et al.* 2020). Additional species that are seen as opportunists with strains isolated from immunocompromised patients are *Candida dubliniensis* (included in the *Lodderomyces*-*Spathaspora* clade), *Clavispora lusitaniae, Meyerozyma guilliermondii, M. farinosa,* and *Kodamaea ohmeri* (Gargeya *et al.* 1990, Page *et al.* 2006, Pfaller *et al.* 2006, Taj-Aldeen *et al.* 2006, Kurtzman *et al.* 2011b, Hong *et al.* 2018, de Hoog *et al.* 2020, Zhou *et al.* 2021).

Saccharomycetes G. Winter, Rabenh. Krypt.-Fl. Ed. 2, 1(1): 32, 68. 1881. emended by M.

Groenew., Hittinger, Opulente & A. Rokas. MycoBank MB 90791.

Emended diagnosis: Class-specific protein families OG0004556 and OG0004235. See original taxonomic description in Winter (1881).

Type order: *Saccharomycetales*.

Included orders: *Ascoideales*, *Phaffomycetales*, *Saccharomycetales, Saccharomycodales.*

Ascoideales J.H. Schaffner, Ohio Naturalist 13 (4): 76 (1913), emended by M. Groenew., Hittinger, Opulente &, A. Rokas. MycoBank MB 90693.

Emended diagnosis: Order-specific protein families OG0018641, OG0018642, and OG0018656. CUG codons are preferentially translated into serine, instead of the nearly universal leucine. Cells are dimorphic, abundant development of true mycelium, often with blastoconidia. Asci are usually attached to hyphae. See original description in Schaffner (1913), which until now, has not been generally recognised by modern taxonomists.

Description: With the characteristics of *Ascoideaceae* (Engler 1892) and *Saccharomycopsidaceae* (von Arx & van der Walt 1987).

Type family: *Ascoideaceae* Engl., Syllabus: 26. 1892. MycoBank MB 816385.

Type genus: *Ascoidea* Bref., Untersuch. Gesammtgeb. Mykol. 9: 94. 1891. MycoBank MB 361.

Type: *Ascoidea rubescens* Bref., Untersuch. Gesammtgeb. Mykol. 9: 94. 1891. MycoBank MB 212818.

Families included in the *Ascoideales*: *Ascoideaceae*, *Saccharomycopsidaceae*.
Genera included in the *Ascoideales* based on this study: *Ascoidea, Saccharomycopsis*.

Notes: *Ascoideales* is represented by species that mostly translate their CUG codons into a serine, instead of the nearly universal leucine and were included in the CUG-Ser2 clade (Riley *et al.* 2016, Shen *et al.* 2016, 2017, 2018, Krassowski *et al.* 2018). One species, *Ascoidea asiatica*, translates CUG codons stochastically as either serine or leucine. All other studied species in the order, including *A. rubescens* and several *Saccharomycopsis* species, translate CUG only as serine (Riley *et al.* 2016, Krassowski *et al.* 2018, Mühlhausen *et al.* 2018, Junker *et al.* 2019). When the genome of only a single representative species was used in phylogenomic inference, the stability of the placement of the CUG-Ser2 clade in the phylogeny was influenced by the inclusion of an ortholog group consisting of orthologs of the *S. cerevisiae DPM1* gene (Shen *et al.* 2017). The inclusion of the genomes of three additional representative species from this clade eliminated the gene's disproportionate influence and stabilized this clade's placement within the *Saccharomycotina* phylogeny (Shen *et al.* 2018).

Biotechnological relevance: Several characteristics of *Saccharomycopsis fibuligera* can be further exploited, such as the ability to hydrolyse starch due to the presence of α-amylase and glucoamylase activities (Wickerham *et al.* 1944). The species can also actively accumulate trehalose when grown on starch (Chi *et al.* 2009). Many *Saccharomycopsis* species are capable of predation on fungi (Pimenta *et al.* 2008, Junker *et al.* 2018) and could be considered as biocontrol agents.

Phaffomycetales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847267.

Diagnosis: Order-specific protein families OG0006529 and OG0006543. Phylogenetic analyses using the DNA sequences encoding SSU rDNA, LSU rDNA, *EF-1*α, RNA polymerase II largest subunit (*RPB1*), and RNA polymerase II second largest subunit (*RPB2*) separate this order (Kurtzman & Robnett 2013a).

Description: With the characteristics of *Phaffomycetaceae* (Yamada *et al.* 1999) and *Wickerhamomycetaceae* (Kurtzman *et al.* 2008).

Type family: *Phaffomycetaceae* Y. Yamada *et al.*, Biosci. Biotechnol. Biochem. 63: 831. 1999. MycoBank MB 82097.

Type genus: *Phaffomyces* Y. Yamada, Bull. Fac. Agric. Shizuoka Univ. 47: 30. 1997. MycoBank MB 27889.

Type: *Phaffomyces opuntiae* (Starmer *et al.*) Y. Yamada, Bull. Fac. Agric. Shizuoka Univ. 47: 30. 1997. MycoBank MB 445220.

Families included in the *Phaffomycetales*: *Phaffomycetaceae*, *Wickerhamomycetaceae.* Genera included in the *Phaffomycetales* based on this study: *Barnettozyma, Cyberlindnera, Phaffomyces, Starmera, Wickerhamomyces.*

Notes: *Komagataella* was previously included in the family *Phaffomycetaceae* (Kurtzman *et al.* 2011b), but genome-scale analyses do not support its inclusion in the *Phaffomycetales* (this study, Shen *et al.* 2018), so *Komagataella* is included in the *Pichiales* (see the description of *Pichales*).

Biotechnological relevance: Species of the genus *Wickerhamomyces* have great biotechnological potential as many of them can grow under stressful environmental conditions, such as extremes of pH, low water activity, and anaerobic conditions (see overview in Kurtzman *et al.* 2011b, Chen *et al.* 2020, Sehnem *et al.* 2020). In particular *Wickerhamomyces anomalus* is appreciated for its production of metabolites useful for biopreservation of of fruit and cereals and for other purposes (Walker 2010). This species has also shown promising potential to be used as a biocontrol agent of various fungal diseases of plants (Hashem *et al.* 2014, Oro *et al.* 2018, Limtong *et al.* 2020). Several species in the genera *Cyberlindnera* and *Wickerhamomyces* are associated with food fermentations and are thus candidates for applications involving human consumption (Bourdichon *et al.* 2012, Karaalioğlu & Yüceer 2021). *Cyberlindnera jadinii* (asexual morph *Candida utilis*) is a well-known fodder yeast and industrial producer of single-cell protein (SCP) (Salazar-López *et al.* 2022).

Saccharomycetales C. Luerssen, Grundz. Bot.: 156. 1877, emended by M. Groenew., Hittinger, Opulente & A. Rokas. MycoBank MB 816369.

Emended diagnosis: Order-specific protein families OG0005235 and OG0005246. See original description in Winter (1881).

Type family: *Saccharomycetaceae* C. Luerssen, Grundz. Bot.: 160. 1877. MycoBank MB 816370.

Type genus: *Saccharomyces* Meyen, Arch. Naturgesch. 4 (2): 100. 1838. MycoBank MB 542401.

Type: *Saccharomyces cerevisiae* (Desm.) Meyen, Arch. Naturgesch. 4 (2): 100. 1838. MycoBank MB 492348.

Family included in the *Saccharomycetales*: *Saccharomycetaceae*.
Genera included in the *Saccharomycetales* based on this and previous studies: *Cyniclomyces, Eremothecium* (asexual morph *Ashbya*)*, Grigorovia, Hagleromyces*, *Kazachstania, Kluyveromyces, Lachancea, Nakaseomyces*, *Naumovozyma, Saccharomyces, Savitreea*, *Tetrapisispora, Torulaspora, Vanderwaltozyma, Yueomyces*, *Zygosaccharomyces*, *Zygotorulaspora.*

Notes: *Cyniclomyces, Grigorovia, Hagleromyces,* and *Savitreea* were not included in this study, but Kurtzman *et al.* (2011b) placed *Cyniclomyces* in the family *Saccharomycetaceae*, Sousa *et al.* (2014) showed that *Hagleromyces* is the sister genus to *Cyniclomyces*, *Grigorovia* is nested within *Kazachstania* (Gouliamova & Dimitrov 2020)*,* and *Savitreea* is the sister genus to *Lachancea* (Sakpuntoon *et al.* 2020a); therefore, we have assigned these genera to *Saccharomycetales*.

Biotechnological relevance: This order includes the most commonly known biotechnological important yeast genus *Saccharomyces*, with the species *S. cerevisiae* (Kurtzman *et al.* 2011b). The agro-industrially important yeast genera *Kazachstania*, *Kluyveromyces, Torulaspora* and *Zygosaccharomyces* are also part of this order (Kurtzman *et al.* 2011b, Urien *et al.* 2019, Karim *et al.* 2020, Fernandes *et al.* 2021, Karaalioğlu & Yüceer 2021, Solieri 2021). Several species in *Lachancea, Saccharomyces, Kazachstania, Kluyveromyces, Zygotorulaspora*, and *Zygosaccharomyces* are associated with fermented foods, and are thus candidates for food applications (Bourdichon *et al.* 2012, Karaalioğlu & Yüceer 2021). *Kluyveromyces marxianus*, a thermotolerant species, showes high ethanol fermentation at high temperature form sucrose, glucose and xylose, therefore, it has high potential for industrail ethanol production in tropical areas (Limtong *et al.* 2007, Nonklang *et al.* 2008, Nitiyon *et al.* 2016). Torulaspora indica reveals high antagonistic activity againt fungal phathogens cause of plant diseases, thus it has potential to be effective biocontrol agent (Konsue *et al.* 2020, Limtong *et al.* 2020). The metabolic functionalities of genera, including *Kluyveromyces*, *Lachancea* and *Ogataea*, have been mapped (Lertwattanasaku *et al.* 2015, Nanda *et al.* 2020, Kosaka *et al.* 2022, Lu *et al.* 2022).

Clinical relevance: *Nakaseomyces glabratus* is of great importance in clinical microbiology. This species is a major pathogen that accounts for an increasingly large proportion of nosocomial fungal infections, especially in immunocompromised patients (Fidel *et al.* 1999, de Hoog *et al.* 2020). In addition, *N. bracarensis* and *N. nivariensis*, are emerging human pathogens that are found in many patients in many countries. Although *S. cerevisiae* is classified as a safe organism, it has been found to colonize in the human body, in particular the human gut and genitourinary tracts (Belvoncikova *et al.* 2022), and several cases of *S. cerevisiae* fungemia have been reported, principally in immunocompromised people (see overview in Kurtzman *et al.* 2011b).

***Saccharomycetales incertae sedis* (*i.s.*)**

Five genera, *Coccidiascus, Endomyces, Helicogonium, Macrorhabdus,* and *Phialoascus* were indicated as *Saccharomycetales i.s.* by Kurtzman *et al.* (2011b) as no living type cultures of these genera are available. For *Endomyces*, partial rDNA sequences available from two non-type strains of *E. scopularum* indicate that this genus is part of the *Saccharomycetales*; together with *Helicogonium* and *Phialoascus*, *Endomyces* has been assigned to the family *Endomycetaceae* (Kurtzman *et al.* 2011b). However, additional work is necessary to clarify the status of that family.

Saccharomycodales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847266.

Diagnosis: Order-specific protein families present OG0011566, OG0011567, OG0011580, OG0011587, and OG0011592. Phylogenetic analyses using the following DNA sequences separate this order: mitochondrial small subunit rDNA (mtSSU rDNA), Internal transcribed spacer (ITS), Large Subunit of the rDNA (LSU rDNA), Translation elongation factor-alpha (*TEF-1*α), and cytochrome C oxidase subunit 2 (*COX2*) (Kurtzman *et al.* 2011b). Budding is bipolar, nitrate is not assimilated, the diazonium blue B reaction is negative, and coenzyme CoQ-6 is present.

Description: With the characteristics of *Saccharomycodaceae* (Kudryavtsev 1960).

Type family: *Saccharomycodaceae* Kudryavtsev, Die Systematik der Hefen: 270. 1960. MycoBank MB 81249.

Type genus: *Saccharomycodes* E.C. Hansen, Centralbl. Bakteriol. Parasitenk., 2. Abth. 12: 537. 1904. MycoBank MB 4814.

Type: *Saccharomycodes ludwigii* (E.C. Hansen) E.C. Hansen, Centralbl. Bakteriol. Parasitenk., 2. Abth. 12: 537. 1904. MycoBank MB 246456.

Family included in the *Saccharomycodales*: *Saccharomycodaceae*.
Genera included in the *Saccharomycodales* based on this and previous studies: *Hanseniaspora/Kloeckera*, *Saccharomycode*s.

Notes: *Saccharomycode*s was not included in this study, but Kurtzman *et al.* (2011b) placed *Saccharomycode*s in the family *Saccharomycodaceae*.

Biotechnological relevance: Several applications in biotechnological processes have been proposed for *Saccharomycodes ludwigii*, which can be investigated further. This includes production of (non-)alcoholic beverages (Romano *et al.* 1998, Methner *et al.* 2022, Vaštík *et al.* 2022), sulfidation of wine musts before fermentation (Gadzhiev *et al.* 1976), and production of vinegar due to its acetic acid resistance (Saeki 1990). *Hanseniaspora* species are common in many natural fermentations (Lleixà *et al.* 2016, Valera *et al.* 2021).

Sporopachydermiomycetes M. Groenew., Hittinger, Opulente & A. Rokas, ***class. nov.*** MycoBank MB 847275.

Diagnosis: Class-specific protein families OG0028621, OG0028581, OG0028722, and OG0028736. Phylogenetic analyses using LSU rDNA, mtSSU rDNA, and *COX2* sequences

(Kurtzman & Robnett 2007). Asexual reproduction is by multilateral budding on a narrow base. Glucose fermentation is absent or weak. Nitrate is not assimilated. *myo*-Inositol as sole carbon source is assimilated. Coenzyme Q-9 is formed.

Description: With the characteristics of *Sporopachydermiales*.

Type order: *Sporopachydermiales* M. Groenew., Hittinger, Opulente & A. Rokas, *ord. nov.*, this study.

Included order: *Sporopachydermiales*.

Notes: Several phylogenetic studies found that the clades including *Alloascoidea* and *Sporopachydermia* are situated on long branches indicative of substantial evolutionary divergence, and their divergence times are similar or greater than that of the orders *Pichiales* and *Alaninales* (Lachance 2011, Kurtzman & Robnett 2013b, Shen *et al.* 2018). Although only two *Sporopachydermia* and one *Alloascoidea* species were included in the current study, we propose to describe two separate sister classes: *Sporopachydermiomycetes* to accommodate the genus *Sporopachydermia*, and *Alloascoideomycetes* to accommodate the genus *Alloascoidea*. See also the description of *Alloascoideomycetes* below. Although the number of validly described species available for these two classes is limited, Lachance (2011) listed a fair number of species of the genus *Sporopachydermia* that are awaiting formal descriptions.

Sporopachydermiales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847276.

Diagnosis: Order-specific protein families OG0028621, OG0028581, OG0028722, and OG0028736. Phylogenetic analyses using LSU rDNA, mtSSU rDNA, and *COX2* sequences (Kurtzman & Robnett 2007). Asexual reproduction is by multilateral budding on a narrow base. Pseudohyphae and true hyphae are not formed. Glucose fermentation is absent or weak, nitrate is not assimilated, and *myo*-inositol is assimilated. Coenzyme Q-9 is formed.

Description: With the characteristics of *Sporopachydermia* (Rodrigues de Miranda 1978).

Type family: *Sporopachydermiaceae* M. Groenew., Hittinger, Opulente & A. Rokas, *fam. nov*., this study.

Sporopachydermiaceae M. Groenew., Hittinger, Opulente & A. Rokas, ***fam. nov.*** MycoBank MB 847277.

Diagnosis: Family-specific protein families OG0028621, OG0028581, OG0028722, and OG0028736. Phylogenetic analyses using LSU rDNA, mtSSU rDNA, and *COX2* sequences (Kurtzman & Robnett 2007). Asexual reproduction is by multilateral budding on a narrow base, and pseudohyphae and true hyphae are not formed. Glucose fermentation is absent or weak, nitrate is not assimilated, and *myo*-inositol as sole carbon source is assimilated. Coenzyme Q-9 is formed.

Description: With the characteristics of *Sporopachydermia* (Rodrigues de Miranda 1978).

Type genus: *Sporopachydermia* Rodr. Mir., Antonie van Leeuwenhoek 44: 440. 1978.

MycoBank MB 5165.

Type: *Sporopachydermia lactativora* Rodr. Mir., Antonie van Leeuwenhoek 44: 440. 1978. MycoBank MB 323856.

Family included in the *Sporopachydermiales*: *Sporopachydermiaceae.*
Genus included in the *Sporopachydermiales* based on the current study: *Sporopachydermia*.

Notes: Although only two species are included in these analyses considering the long branches observed by Kurtzman & Robnett (2013b) and Shen *et al.* (2018), we have decided to split off *Sporopachydermia* from *Alloascoidea* for inclusion in the new order *Sporopachydermiales*.

Trigonopsidomycetes M. Groenew., Hittinger, Opulente & A. Rokas, ***class. nov.*** MycoBank MB 847283.

Diagnosis: Class-specific protein families OG0008190 and OG0008482. Phylogenetic analyses using LSU rDNA, mtSSU-rDNA, and *COX2* sequences (Kurtzman & Robnett 2007).

Description: With the characteristics of *Trigonopsidaceae* (Lachance & Kurtzman 2013).

Type order: *Trigonopsidales* M. Groenew., Hittinger, Opulente & A. Rokas, *ord. nov*., this study.

Included order: *Trigonopsidales*.

Trigonopsidales M. Groenew., Hittinger, Opulente & A. Rokas, ***ord. nov.*** MycoBank MB 847284.

Diagnosis: Order-specific protein families OG0008190 and OG0008482. Phylogenetic analyses using LSU rDNA, mtSSU rDNA, and *COX2* sequences (Kurtzman & Robnett 2007, Lachance & Kurtzman 2013).

Description: With the characteristics of *Trigonopsidaceae* (Lachance & Kurtzman 2013).

Type family: *Trigonopsidaceae* Lachance & Kurtzman, Int. J. Syst. Evol. Microbiol. 63: 3113. 2013. MycoBank MB 803502.

Type genus: *Trigonopsis* Schachner, Z. Ges. Brauwesen 52: 137. 1929. MycoBank MB 10310.

Type: *Trigonopsis variabilis* Schachner, Z. Ges. Brauwesen 52: 137. 1929. MycoBank MB 281376.

Family included in the *Trigonopsidales*: *Trigonopsidaceae*.
Genera included in the *Trigonopsidales* based on this study and previous studies: *Botryozyma* (teleomorph *Ascobotryozyma*), *Tortispora, Trigonopsis*.

Notes: *Trigonopsis*, *Tortispora*, and *Botryozyma* were included in the family

Trigonopsidaceae (Lachance & Kurtzman 2013). Unfortunately, since *Trigonopsidales* contains only a single family, it could not be included in the RED analyses. Considering the exceptional early divergence of the basal branching point marking this order, it is possible that additional data may eventually support the division of this new order. However, due to the lack of species, we have decided to combine the genera *Trigonopsis*, *Tortispora*, and *Botryozyma* into one order, while acknowledging that future descriptions of more species in this group may lead to the split of *Tortispora* and *Botryozyma* from *Trigonopsis*.
Biotechnological relevance: The ability of *Trigonopsis variabilis* to produce D-amino acid oxidases that catalyse the oxidative deamination of α-amino acids to α-keto acids and ammonia (see overview in Kurtzman *et al.* 2011b) is of great importance to the pharmaceutical industry. Other uses for this enzyme include synthesis of chiral intermediates to produce antihypertensive drugs (Patel 2001) and the conversion of cephalosporin C to 7-amino cephalosporanic acid, which is a key intermediate for cephem antibiotics (Dib & Nidetzky 2008).

CONCLUSIONS

Since the currently used classification of the higher taxonomic categories within the *Saccharomycotina* is inconsistent with evolutionary relationships and distances, we have proposed an updated class and order classification. Increasing the numbers of classes and orders in the *Saccharomycotina* from one to seven and twelve, respectively, makes the higher rank lineages more consistent with the high level of genomic diversity that exists within this subphylum and more comparable to the higher-level taxonomic ranks in use for other fungi, which is especially important for environmental sequencing and comparative studies. Without this proposal, the current class and order classifications would continue to be nearly worthless for macro-evolutionary comparisons because all budding yeasts of the subphylum *Saccharomycotina* are currently included in the *Saccharomycetales* and the *Saccharomycetes*. Taxonomy should ideally reflect evolutionary relationships among organisms, and genome-based phylogenies are suitable to guide taxonomy because they allow to consider both evolutionary relationships and differing rates of evolution. The newly proposed classification accounts for monophyly and divergence time, making the main higher taxonomic ranks more informative. The newly proposed higher-level taxonomic categories of *Saccharomycotina* provide a more natural classification and improve the taxonomic and phylogenetic precision of higher taxonomic levels. Another important aspect of the improved taxonomy is that the cause of being a pathogen might be different in different clades. Currently, (novel) species and their classifications can be found on *The Yeasts* website (https://theyeasts.org/), the successor to *The Yeasts: A Taxonomic Study* book series. This website ensures that taxonomic changes are freely available as soon as possible after the new classification is published.

Current taxonomies scale consistently with many other features of biological interest, such as magnitudes of divergence in morphology, physiology, behaviour, or ecology. Most of the newly proposed orders are tied to unique genetic characters (unique OGs, (multi)gene sequences, codon translation), although in some cases, distinct morphological and physiological characters can be used as supplemental features. For example, the formal description of the newly proposed orders references diagnostic characters for each as follows: *Saccharomycetales* (unique OGs), *Saccharomycodales* (OGs, barcoding, morphology, physiology), *Phaffomycetales* (OGs, barcoding), *Alaninales* (OGs, codon translation CUG-Ala, barcoding, morphology, physiology), *Serinales* (OGs, codon translation CUG-Ser), *Ascoideales* (OGs, codon translation CUG-Ser, morphology), *Pichiales* (OGs, barcoding), *Lipomycetales* (OGs, barcoding, physiology), *Sporopachydermiales* (OGs, barcoding, morphology, physiology), *Alloascoideales* (OGs, barcoding, morphology), *Dipodascales*

(OGs, barcoding, morphology), and *Trigonopsidales* (OGs, barcoding, physiology). Although this current study only focused on the higher classifications of classes and orders, it is clear from the genome scale-phylogeny presented and earlier studies that the family *Wickerhamomycetaceae* is polyphyletic, and future genus and family-level reclassification within the *Phaffomycetales* is needed. Currently two formally described families are included in the *Dipodascales*, *Trichomonascaceae* and *Dipodascaceae*, but the *Yarrowia* and *Nadsonia* species have not yet been placed in either of the two families; thus, the proposal of a third family within the *Dipodascales* is a realistic possibility. The three families of the *Serinales* do no accommodate the genus *Babjeviella*, which remains of uncertain family assignment. Its highly divergent phylogenetic position prevents us from proposing a fourth family at this time as new related taxa may influence the taxonomic view on this.

Since the current classification does not fully meet the expectations of ecologists and biodiversity researchers for the subphylum *Saccharomycotina*, we expect that this new classification proposed here will be helpful and understandable for taxonomists, ecologists, industrial and biomedical users. This new *Saccharomycotina* classification offers great promise for classifying potentially uncultivable or novel species in different microbiomes, and it will be the key for reliable assignment of ecological and functional traits to taxa for further ecophysiological and biodiversity analyses.

To our knowledge, species that contain biotechnologically important traits are present in all the newly proposed orders, except *Sporopachydermiales* and *Alloascoideales*. However, only a handful of species are currently used as biotechnological production platforms, mostly those from the order *Saccharomycetales*. Since industry uses only a small fraction of the hundreds of species known in the *Saccharomycotina* linage, we expect that the introduction of this reclassification of the higher categories for this subphylum will make discovery and development of additional yeasts of biotechnological interest easier. For example, some biotechnologically important traits have been suggested to be restricted to single orders, such as the exceptional protein production systems of the *Pichiales*, and the new taxonomy can facilitate a more focused screening process. Alternatively, screens for traits of interest might choose a subset of taxonomically diverse yeasts.

Several opportunistic yeast species are primary agents of disease after a drop in patient immunity, while others cause secondary infections usually linked to patients suffering from immune deficiencies or are emerging human pathogens. Most of the major opportunistic pathogenic species belong to the order *Serinales*, but many of emerging opportunistic pathogens also belong to the *Saccharomycetales,* the *Pichiales*, and the *Dipodascales.* Therefore, a broad range of orders within the *Saccharomycotina* include yeast species that have the potential to emerge in the future as opportunistic human pathogens, especially in light of the increasing numbers of immunocompromised patients (Stavrou *et al.* 2019, de Hoog *et al.* 2020). The new classification will help to limit further reclassification and better reflects substantial evolutionary divergence of species. For example, in the current classification, the distantly related *N. glabratus* and *C. albicans* were in the same class, but the new scheme assigns them to different classes. This highlights the fact that the two species evolved the ability to cause human infections independently and have different clinically relevant properties, such as resistance to azoles.

The phylogeny-informed taxonomic classification that we are advocating separates phylogenetically distant taxa to highlight their diverse characteristics and potentials, which are sometimes hidden in their genotypes. The improved possibilities to communicate these characteristics and potentials by appropriate group names (class, order, family) may unlock otherwise obscure research opportunities in the fields that include evolution, biogeography, and conservation biology. It will also benefit researchers involved in bioprospecting by identifying genes that are specific to higher rank taxa and suggesting which taxa should be studied as fungal models of particular physiologies or mined as a source of biological parts (i.e., genes, promoters, chemicals) for applications in biotechnology.

Among fungal lineages, prior to this study, the *Saccharomycotina* were clearly under-classified in the higher taxonomic lineages. Many families and genera, previously included in the *Saccharomycetales incertae sedis* (Kurtzman *et al.* 2011b), are now placed within the newly proposed orders as it became clear that they did not fit into the sole order *Saccharomycetales*. This study provides a model for application of genome-scale datasets to higher rank taxonomy. This revised, genome-enabled classification within *Saccharomycotina* provides a foundation and a roadmap upon which future taxonomic studies can be built. In the near future, more than 1 000 genomes cataloguing nearly every known species of *Saccharomycotina* (Y1000+ Project: http://y1000plus.org) will be publicly available. The availability of these genomes will further allow the depth and breadth of family and genus circumscriptions to be evaluated to improve the classification of these lineages by integrating genomic approaches into taxonomy, an integrative process being called taxogenomics (Libkind *et al.* 2020). We predict the approach being taken in budding yeasts will pave the way for other fungi and, ultimately, taxonomy across the entire tree of life will enter the genomic era.

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DECLARATION ON CONFLICT OF INTEREST

Antonis Rokas is a scientific consultant for LifeMine Therapeutics, Inc.

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Table 1. An overview of the most unique (98–100 % unique) class- and order-specific groups of orthologous genes (OGs) to use as diagnostic characters for the newly proposed orders and classes.

Table 2. Classes, orders, families, and genera of yeasts of the subphylum Saccharomycotina. Blue fonts denote newly proposed ranks. Red fonts denote taxa of uncertain assignment (*incertae sedis = i.s.*).

Fig. 1. Relationship between relative evolutionary divergence (RED) and taxonomic rank between budding yeasts and other major fungal lineages. The RED intervals for each rank were defined as the median RED value ± 0.1 to serve as a guide for the normalization of taxonomic ranks from family to phylum. A) Data from Li *et al.* (2021) for the current order *Saccharomycetales* and class *Saccharomycetes* and other fungal lineages. B) Data for the newly proposed orders and classes. Note that when only a single lower rank is assigned to a higher rank (*e.g*. a single family is assigned to a single order), only the lower rank may be analyzed (*e.g*. *Trigonopsidaceae*, *Dipodascales*). *Dipodascomycetes/Dipodascales* (RED = 0.482) and *Trigonopsidomycetes/Trigonopsidales/Trigonopsidaceae* (RED = 0.439) still appear to be under-classified (i.e., had a much lower RED value than other ranks); whereas *Serinales* had a slightly lower RED value (RED = 0.633) compared to other newly proposed orders.

Fig. 2. A genome-scale phylogeny of *Taphrinomycotina*, *Pezizomycotina*, and *Saccharomycotina*. Maximum likelihood analysis with a concatenation single-model (LG+G4) approach on a data matrix containing 1 672 taxa (1 644 fungi and 28 outgroups) and 290 genes (Li *et al.* 2021). The orders and classes are indicted on the tree.

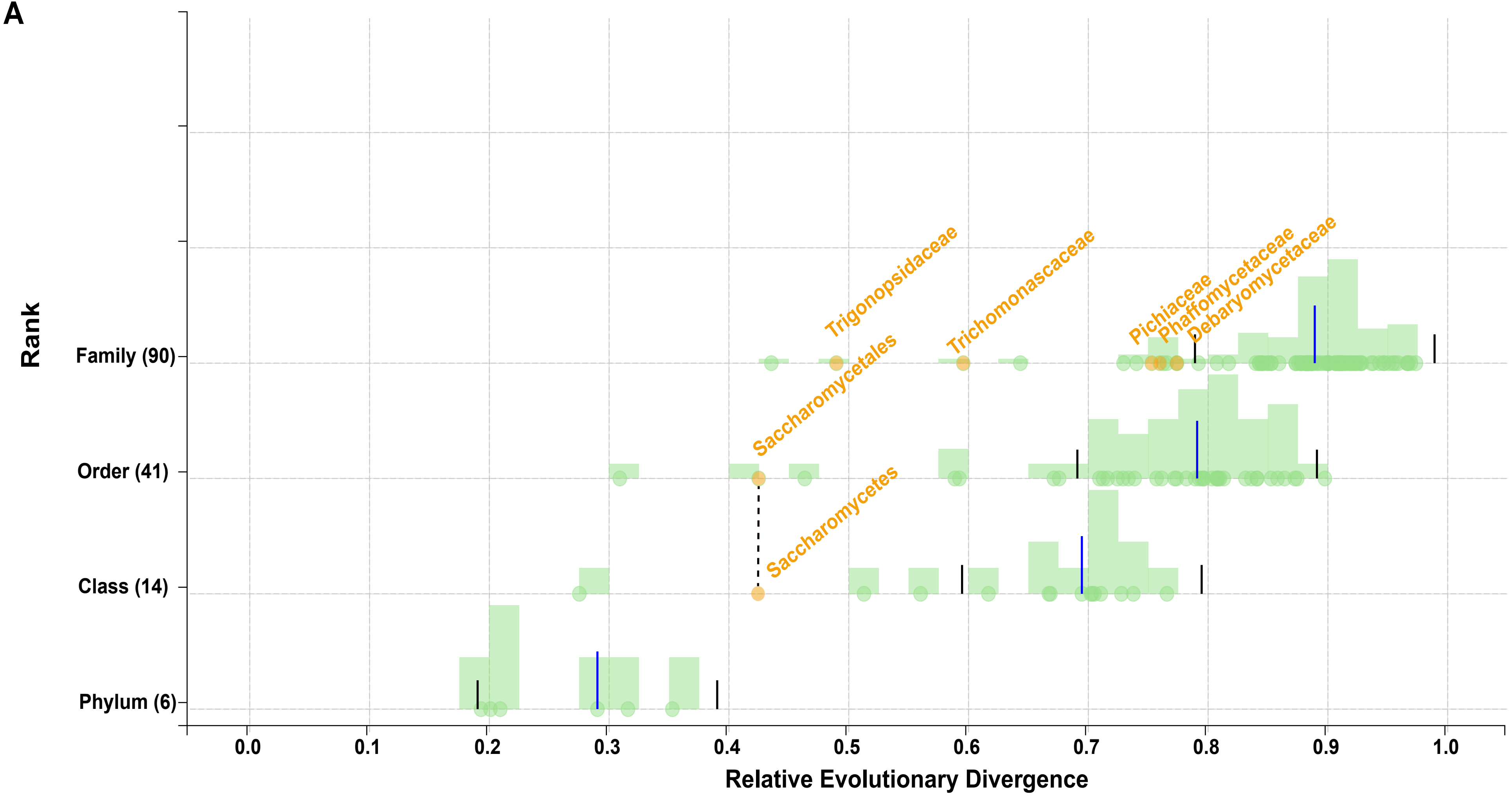
Table S1. Overview of the class- and order-specific genes. Detailed results of the best OGs (98–100 % unique) used as diagnostic characters for the newly proposed orders and classes.

Table S2. Overview of the class- and order-specific genes. Detailed results of all OGs specific to the proposed orders and classes.

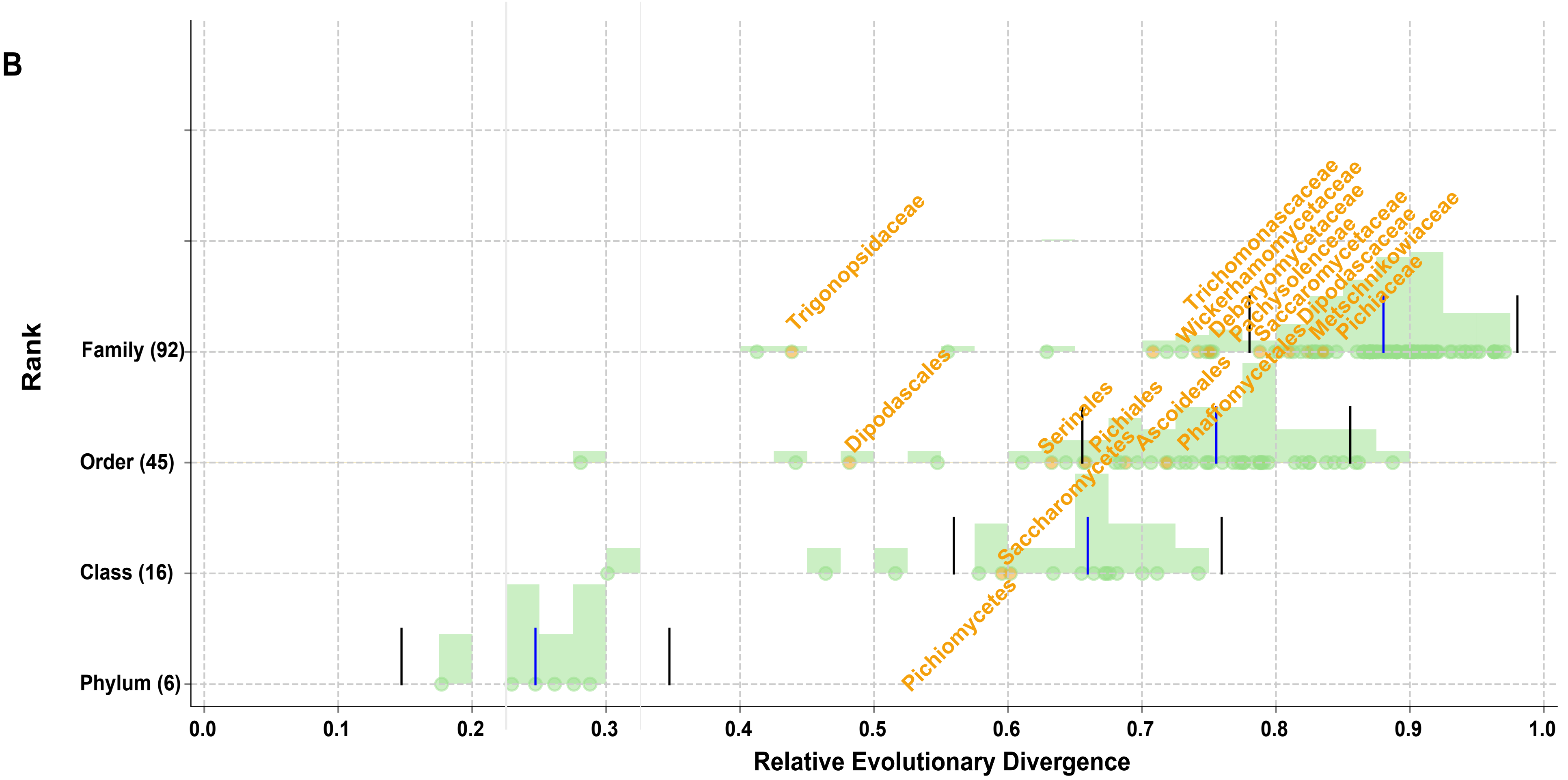
Table S3. Updated taxonomy as on 23 November 2022, strain ID, and source information of 1 672 fungal and outgroup genomes from the genome-scale phylogeny of the fungal kingdom constructed by Li *et al.* (2021).

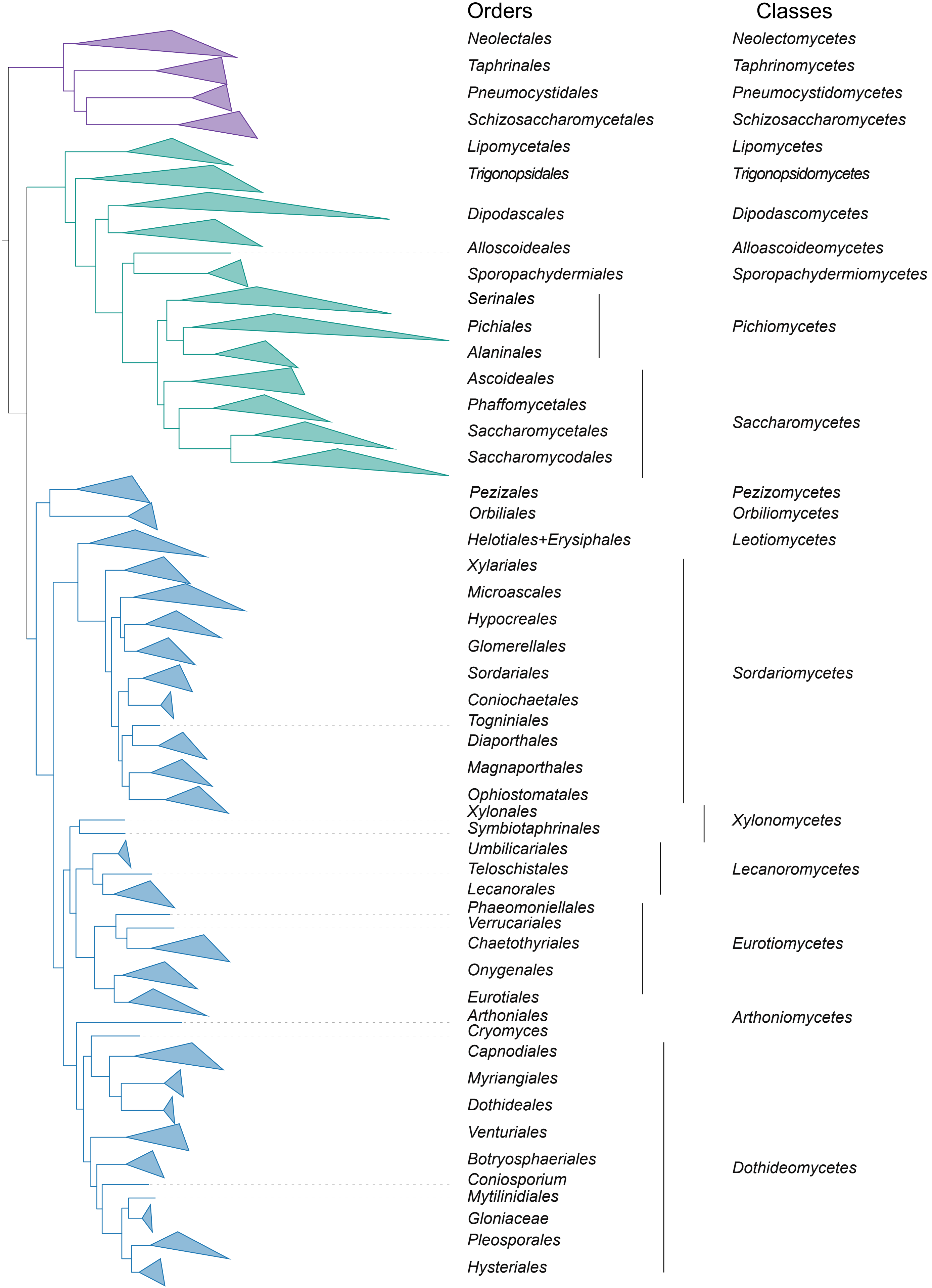
Fig. S1. The genome-scale phylogeny of 1 644 species in the fungal kingdom (with 28 outgroups) from Li *et al.* (2021). The tree of 1 672 fungal species was reconstructed from the maximum likelihood concatenation analysis of 290 single-copy BUSCO genes under a single LG+G4 model. See also Fig. 2 and Table S3 for the current names as on 23 November 2022.

A


Rank
Family (90)
Order (41)
Class (14)
Phylum (6)
Trigonopsidaceae
Trichomonascaceae
Pichiaceae
Phaffomycetaceae
Debaryomycetaceae
Saccharomycetales
Saccharomycetes
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relative Evolutionary Divergence

B


Rank
Family (92)
Order (45)
Class (16)
Phylum (6)
Trigonopsidaceae
Trichomonascaceae
Wickerhamomycetaceae
Debaryomycetaceae
Pachysolenceae
Saccaromycetaceae
Dipodascaceae
Metschnikowiaceae
Pichiaceae
Dipodascales
Serinales
Pichiales
Ascoideales
Phaffomycetales
Saccharomycetes
Pichiomycetes
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relative Evolutionary Divergence


Orders
Classes
Neolectales
Neolectomycetes
Taphrinales
Taphrinomycetes
Pneumocystidales
Pneumocystidomycetes
Schizosaccharomycetales
Schizosaccharomycetes
Lipomycetales
Lipomycetes
Trigonopsidales
Trigonopsidomycetes
Dipodascales
Dipodascomycetes
Alloscoideales
Alloascoideomycetes
Sporopachydermiales
Sporopachydermiomycetes
Serinales
Pichiales
Pichiomycetes
Alaninales
Ascoideales
Phaffomycetales
Saccharomycetales
Saccharomycetes
Saccharomycodales
Pezizales
Pezizomycetes
Orbiliales
Orbiliomycetes
Helotiales+Erysiphales
Leotiomycetes
Xylariales
Microascales
Hypocreales
Glomerellales
Sordariales
Sordariomycetes
Coniochaetales
Togniniales
Diaporthales
Magnaporthales
Ophiostomatales
Xylonales
Xylonomycetes
Symbiotaphrinales
Umbilicariales
Teloschistales
Lecanoromycetes
Lecanorales
Phaeomoniellales
Verrucariales
Chaetothyriales
Eurotiomycetes
Onygenales
Eurotiales
Arthoniales
Arthoniomycetes
Cryomyces
Capnodiales
Myriangiales
Dothideales
Venturiales
Botryosphaeriales
Dothideomycetes
Coniosporium
Mytilinidiales
Gloniaceae
Pleosporales
Hysteriales