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Minireview

The cell morphological diversity of *Saccharomycotina* yeasts

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Abstract

The ∼1 200 known species in subphylum *Saccharomycotina* are a highly diverse clade of unicellular fungi. During its lifecycle, a typical yeast exhibits multiple cell types with various morphologies; these morphologies vary across *Saccharomycotina* species. Here, we synthesize the evolutionary dimensions of variation in cellular morphology of yeasts across the subphylum, focusing on variation in cell shape, cell size, type of budding, and filament production. Examination of 332 representative species across the subphylum revealed that the most common budding cell shapes are ovoid, spherical, and ellipsoidal, and that their average length and width is 5.6 µm and 3.6 µm, respectively. 58.4% of yeast species examined can produce filamentous cells, and 87.3% of species reproduce asexually by multilateral budding, which does not require utilization of cell polarity for mitosis. Interestingly, ∼1.8% of species examined have not been observed to produce budding cells, but rather only produce filaments of septate hyphae and/or pseudohyphae. 76.9% of yeast species examined have sexual cycle descriptions, with most producing one to four ascospores that are most commonly hat-shaped (37.4%). Systematic description of yeast cellular morphological diversity and reconstruction of its evolution promises to enrich our understanding of the evolutionary cell biology of this major fungal lineage.

Keywords: evolutionary cell biology; cell size; cell shape; budding; hyphae; pseudohyphae; cell type; *Saccharomycotina*

Introduction

Yeasts are unicellular fungi and have evolved multiple times independently across the fungal kingdom (Nagy et al. 2014, Li et al. 2021). Yeasts are free-living organisms that inhabit diverse terrestrial, aquatic, and marine environments on every continent, forming associations with many plant, fungal, and insect species (Kurtzman et al. 2011). The most species-rich lineage of yeasts is that of the ∼1200 species in the subphylum *Saccharomycotina* (phylum *Ascomycota*), which we will hereafter refer to as yeasts. Yeast species display a wide diversity of ecological lifestyles (Opulente et al. 2018), partaking in mutualistic, competitive, opportunistic, parasitic, or pathogenic relationships with other organisms (Kurtzman et al. 2011). Several yeast species are of importance to diverse industries and human affairs, such as the baker's yeast *Saccharomyces cerevisiae* (baking, brewing, wine-making, biotechnology); the human pathogens *Candida albicans*, *Candida auris*, and *Nakaseomyces glabratus* (syn. *Candida glabrata*); and the plant pathogens in the genus *Eremothecium*.

Yeast species in the *Saccharomycotina* can propagate both through mainly mitosis and often meiosis, generating different cell types (Herskowitz 1988, Fischer et al. 2021). We refer to this type of cellular morphological variation as developmental variation. Developmental variation of cellular morphology can be observed in cell type differentiation that occurs during a yeast life cycle, as well as at various phases of cell cycle progression in mitosis and meiosis of either haploid or diploid cells (Fig. 1). Yeasts undergo cellular division and reproduction through mechanisms of division, germination, and filamentous growth to result in various cell types, such as budding cells, ascospores, and (pseudo)hyphae, in which cells utilize polarization for successful growth (Bi and Park 2012).

The developmental variation exhibited by yeast species is often dependent on environmental conditions, such as nutrient availability and temperature. For example, certain species primarily grow as unicellular cells, but they can switch to a multicellular state through filamentous growth under the influence of specific environmental stressors (Ruiz-Herrera and Sentandreu 2002, Cullen and Sprague 2012, Rupert and Rusche 2022). Mitosis of haploid or diploid daughter cells enables yeasts to continue budding and replicating so long as nutrients continue to be available. Some species of *Saccharomycotina* can undergo mitosis to result in filamentous growth through the production of hyphae and pseudohyphae. Budding and pseudohyphae cells undergo polarized growth during the G1 phase of the cell cycle, while hyphal growth does

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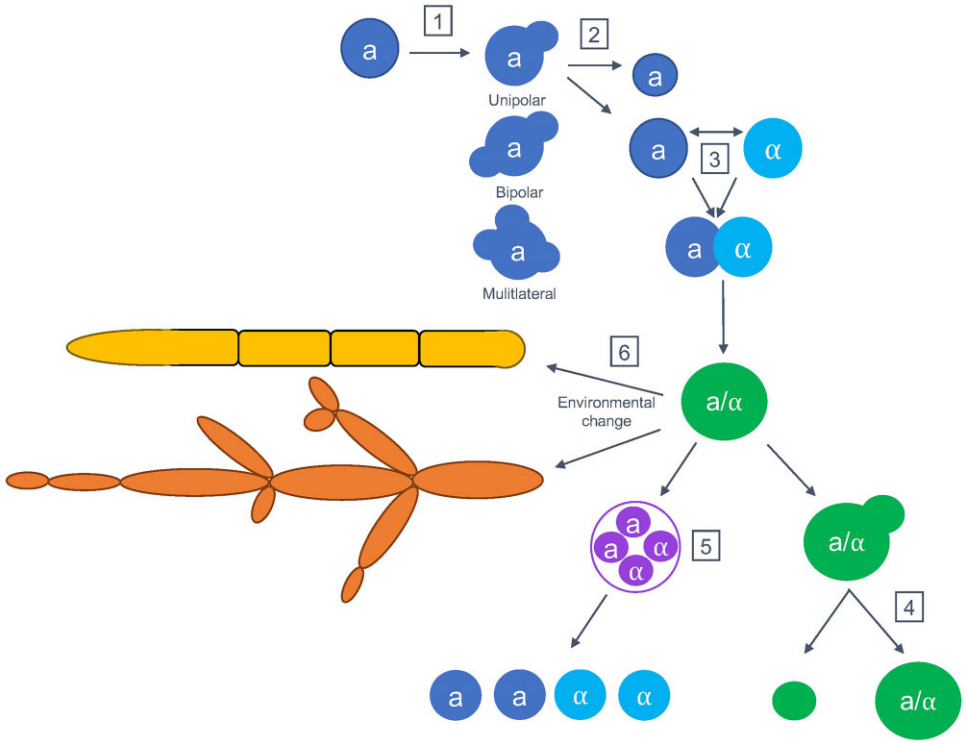

Figure 1. Generalized life cycle of yeast species in the subphylum Saccharomycotina. (1) Budding, including unipolar, bipolar, and multilateral budding types. Note that the shapes and sizes of budding cells vary across species (see Fig. 2). (2) Haploid mitosis (dark blue = type a haploid, light blue = type α haploid). (3) Haploid mating of opposite types, possibly after mating-type switching (Krassowski et al. 2019). (4) Diploid mitosis (green = diploid a/α). (5) Diploid meiosis (purple = ascus with four ascospores). Note that the shapes and sizes of asci and ascospores vary across species (see Fig. 3). (6) Filamentous growth as a result of an environmental change, such as a temperature increase and/or nutrient limitation (orange = pseudohyphae, yellow = true hyphae). Note that the known life cycles of several species in the subphylum differ from this generalized version (e.g. several species are not known to have a sexual stage).

not occur during the cell cycle, and instead depends on continuous polarized growth without cell separation (Diepeveen et al. 2017).

In addition to developmental variation, yeasts exhibit evolutionary variation (Kurtzman et al. 2011). Evolutionary variation of cellular morphology is characterized by the different morphologies of the same cell type across yeast species (Fig. 2). Population-level variation in cellular morphology between individual cells within a species has also been observed (Skelly et al. 2013, Yvert et al. 2013, Jung et al. 2016). Although it is well known that different species and clades (e.g. taxonomic orders) exhibit distinct morphologies and that the genomes of yeasts are fast-evolving and highly diverse (Shen et al. 2018, 2020, Groenewald et al. 2023) (e.g. at the level of protein sequence divergence, *S. cerevisiae* is as distantly related to *C. albicans* as humans are to sponges), whether this genomic variation is associated with cellular morphological variation remains poorly understood.

Species in the *Saccharomycotina* exhibit extensive developmental (Fig. 1) and evolutionary (Figs 2–4) variation in their cell sizes and shapes. Examination of cellular phenotypes across yeast species and orders reveals diverse morphologies that are atypical of or absent from *S. cerevisiae*, the premier model organism not just for yeasts, but for unicellular eukaryotes in general. This evolutionary variation in cell shape and size may stem from stochasticity in the form of genetic and environmental variance (i.e. growth conditions) (Lynch et al. 2014). Thus, full understanding of the evolutionary variation of cellular morphology requires also examining genomic variation, variation in gene/protein networks, and variation in environmental conditions involved in its determination.

Although the *Saccharomycotina* subphylum harbors abundant evolutionary variation of cell shapes and sizes that allows for phylogenetic comparison across both closely related and highly divergent taxa, this diversity of cell morphology has not been systematically characterized. This review aims to fill this gap by synthesizing the phenotypic diversity of cell morphology of evolutionary variation of yeasts across the subphylum.

Defining variation in yeast cell morphology

There are several attributes of the cellular morphology of yeasts that vary between species and can be measured across one or more cell types, including cell shape, cell size, budding type, and filament production. Individual cell types of each yeast species typically exhibit one or more distinct shapes (e.g. ovoid, spherical, apiculate, and bacilliform). The cells of different species also differ in their sizes, which are described by measuring their length and width. Cell size averages are determined by averaging the smallest and largest lengths and widths recorded.

Yeast species also vary with respect to budding type. In optimal growth conditions, some species divide during mitosis by budding at one of the poles of the cell at a time, which is termed unipolar budding. Other species can bud from both poles of the cell, which is termed bipolar budding. In some species, cell division by budding can also occur without the use of the poles (i.e. budding can occur at any region of the cell), which is termed non-polar or multilateral budding. Some species can grow continuously in

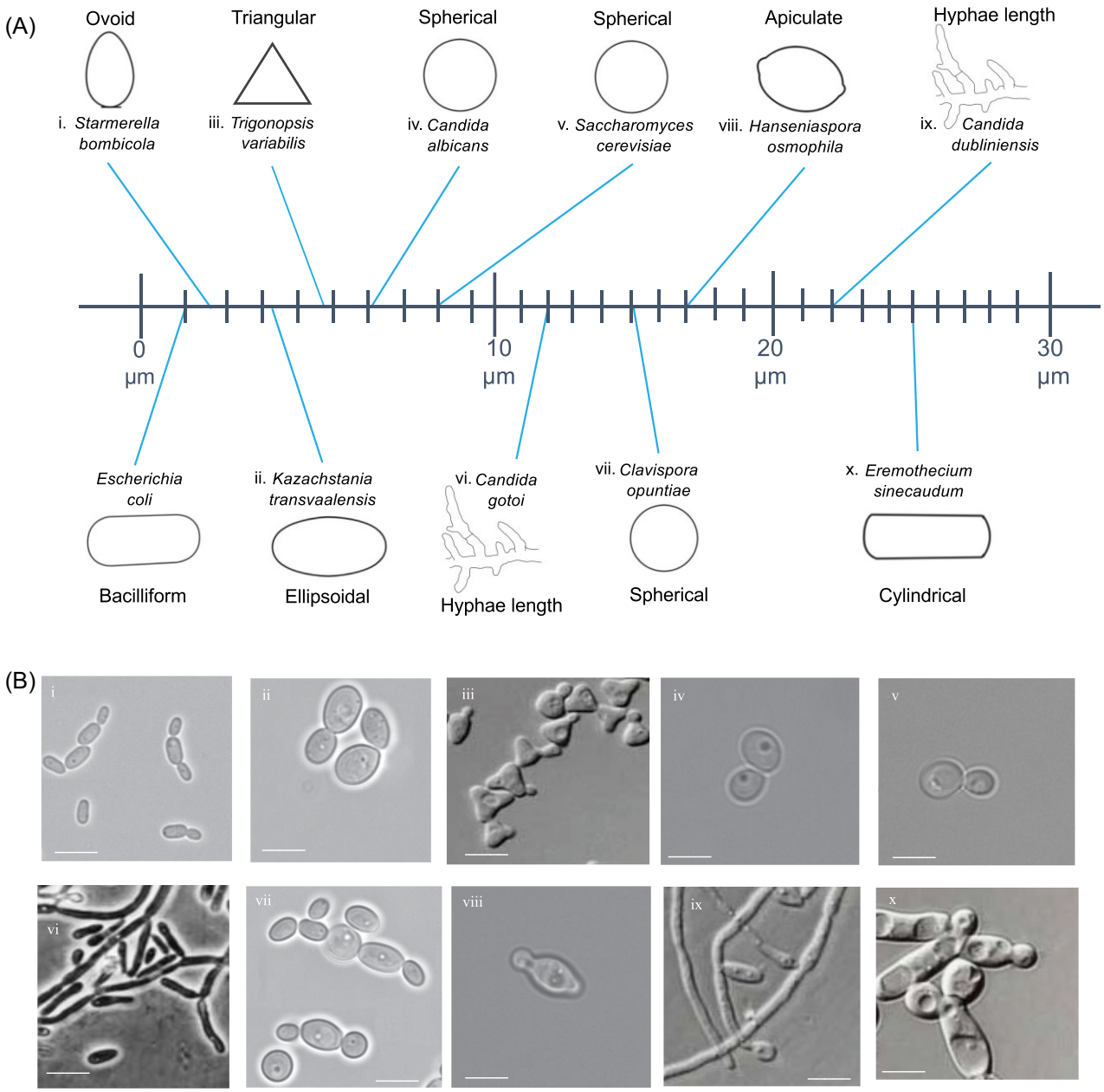

Figure 2. Variation in budding yeast cell size and shape in the subphylum *Saccharomycotina*. (A) For comparison, the cell shape and length of the model bacterium *Escherichia coli* is also provided (cell shape is bacilliform with a cell length average 1–2 µm). *Starmerella bombicola* (order *Dipodascales*) budding cell shape is ovoid or elongate with a cell size average of 3 × 1.5 µm. *Kazachstania transvaalensis* (order *Saccharomycetales*) budding cell shape is allantoid, apiculate, ovoid, or ellipsoidal with a cell size average of 5.25 × 4.25 µm. *Trigonopsis variabilis* (order *Trigonopsidales*) budding cell shape is spherical or triangular with a cell size average of 4.45 × 4 µm. *Candida albicans* (order *Serinales*) budding cell shape is spherical or ovoid with a cell size average of 6 × 4.75 µm. *Saccharomyces cerevisiae* (order *Saccharomycetales*) budding cell shape is spherical or ovoid with a cell size average of 7.5 × 5.5 µm. *Candida gotoi* (order *Serinales*) budding cell shape is spherical, ovoid, or elongate with a cell size average of 5.25 × 5 µm, and can produce hyphae that has an average size of 12 × 2.5 µm. *Clavispora opuntiae* (order *Serinales*) budding cell shape is spherical with cell size average of 9.5 × 3.5 µm. *Hanseniaspora osmophila* (order *Saccharomycodales*) budding cell shape is apiculate with cell size average of 12.7 × 4.75 µm. *Candida dubliniensis* (order *Serinales*) budding cell shape is apiculate with a cell size average of 7 × 4.9 µm and produces hyphae as large as 22 µm in length. *Eremothecium sinecaudum* (order *Saccharomycetales*) budding cell shape is cylindrical with a cell size average of 16 × 4.5 µm. (B) (i) Ovoid and elongate budding cells of *Starmerella bombicola*. (ii) Ellipsoidal budding cells of *Kazachstania transvaalensis*. (iii) Triangular budding cells exhibited by *Trigonopsis variabilis*. (iv) Spherical budding cells exhibited by *Candida albicans*. (v) Spherical budding cells exhibited by *Saccharomyces cerevisiae*. (vi) True hyphae exhibited by *Candida gotoi*. (vii) Spherical budding cells exhibited by *Clavispora opuntiae*. (viii) Apiculate bipolar budding cell exhibited by *Hanseniaspora osmophila*. (ix) True hyphae exhibited by *Candida dubliniensis*. (x) Cylindrical budding cells exhibited by *Eremothecium sinecaudum*. Taxonomic type strains are shown, except for *S. cerevisiae* S288C. Images i, ii, iii, vi, vii, ix and x were taken from theyeasts.org. Yeasts in images iv, v, and viii were taken by Amanda Hulfachor grown at room temperature in YPD (yeast extract, peptone, dextrose) medium until visible growth was observed. Size bar = 5 µm.

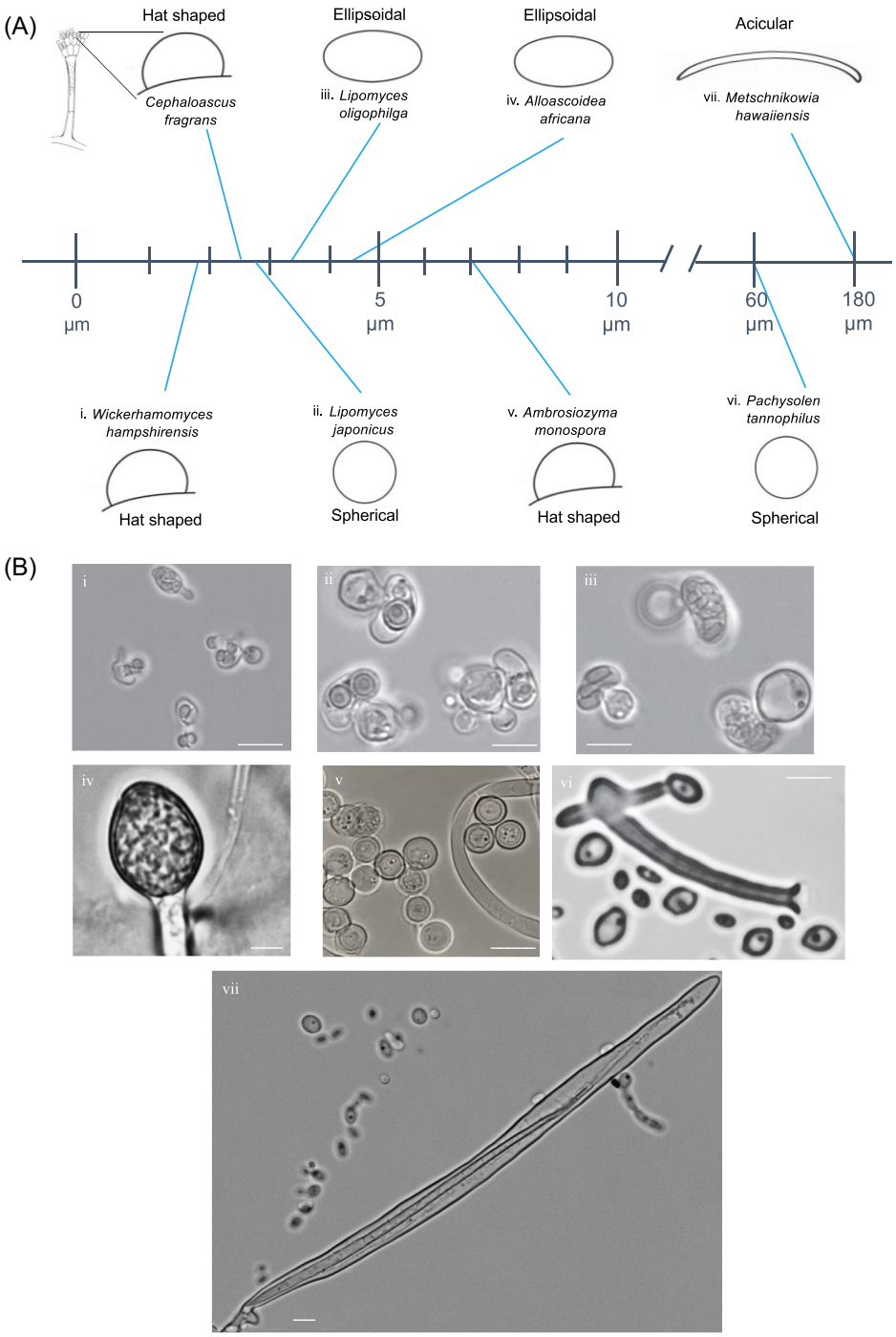

Figure 3. Variation in ascus and ascospore size and shape in the subphylum *Saccharomycotina*. (A) *Wickerhamomyces hampshirensis* (order *Phaffomycetales*) ascospores are hat-shaped and have average sizes of 1.9 × 1.2 µm; asci contain 1–4 ascospores and their average size is 6 × 4 µm. *Cephaloascus fragrans* (order *Serinales*) ascospores are hat-shaped and have average sizes of 2.5 × 2.15 µm; asci contain 2–4 ascospores and their average size is 6 × 3.5 µm; ascospores and asci can be contained inside an ascophore that is stout, tapered, and can be as long as 500 µm. *Lipomyces japonicus* (order *Lipomycetales*) ascospore shape is spherical and average length is 2.75 µm; asci contain 1–4 ascospores, have saccate (or sac-like) shapes, and have an average size of 10 × 4.5 µm. *Lipomyces oligophaga* (order *Lipomycetales*) ascospore shape is ellipsoidal and average size of 3.25 × 1.25 µm; asci contain 4 or more ascopsores, are saccate shaped, and have an average size of 16.3 × 7.3 µm. *Alloascoidea africana* (order *Alloascoideales*) ascospore cell shape is ellipsoidal and its average size is 4.5 × 3.25 µm; asci contains 16–70 ascospores, are ellipsoidal shaped, and their average size is 30 × 11 µm. *Ambrosiozyma monospora* (order *Pichiales*) ascospore cell shape is hat-shaped and average size of 7 × 3.5 µm; asci contain 1–2 ascopsores, and are spherical or ovoid in shape. *Pachysolen tannophilus* (order *Alaninales*) ascospore cell shape is spherical; asci contain up to 4 ascospores; ascospores and asci can be contained inside an ascophore that is curved, tube-shaped, and can be as long as 60 µm. *Metschnikowia hawaiiensis* (order *Serinales*) ascospore cell shape is acicular and average cell length of 160 µm; asci contain 2 ascospores, are wide and tube-shaped, and can be as long as 200 µm. (B) (i) Hat-shaped ascospores exhibited by *Wickerhamomyces hampshirensis*. (ii) Spherical ascospores exhibited by *Lipomyces japonicus*. (iii) Ellipsoidal ascospores exhibited by *Lipomyces oligophaga*. (iv) Ellipsoidal ascospores inside a large ascus exhibited by *Alloascoidea africana*. (v) Hat-shaped ascospores exhibited by *Ambrosiozyma monospora*. (vi) Spherical ascospores inside an ascus and tube-shaped ascophore exhibited by *Pachysolen tannophilus*. (vii) Acicular ascospore exhibited by *Me. hawaiiensis*. Taxonomic type strains are shown. Images i, ii, iii, v, and vii were taken from theyeasts.org, and image vi was adapted from (Kurtzman et al. 2011). Image iv was adapted from (Kurtzman and Robnett 2013). Size bar = 5 µm.

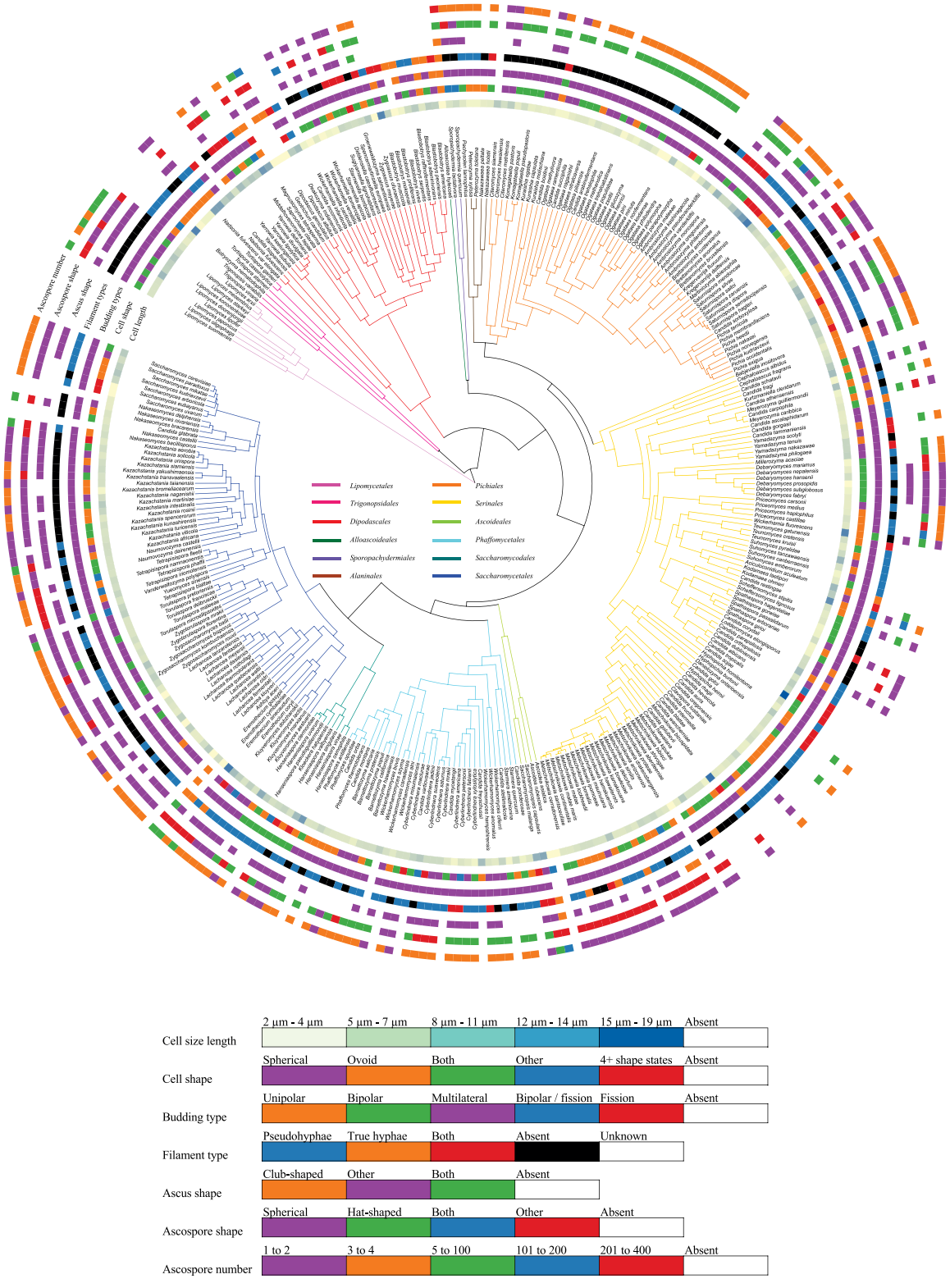

Figure 4. Cell morphology trait distributions within the subphylum *Saccharomycotina*. Phylogeny of 332 representative species of yeasts in the subphylum *Saccharomycotina*. The phylogeny is from the study by Shen et al. (2018). The branch colors of the phylogeny correspond to the subphylum's 12 taxonomic orders (Groenewald et al. 2023). Circles around the phylogeny display variation in select cell morphology traits. From inner to outer circle: cell length average (yellow to blue gradient); cell shape including spherical (purple), ovoid (orange), both spherical and ovoid (green), other shape (blue), four or more shape states (red), or absent (white); budding type including unipolar (orange), bipolar (green), multilateral (purple), bipolar and fission (blue), fission (red), or absent (white); filament type including pseudohyphae (blue), true hyphae (orange), both pseudohyphae and true hyphae (red), absent (black), or unknown (white); ascus shape including club-shaped (orange), other shape (purple), both club-shaped and other (green), or absent (white); ascospore shape including spherical (purple), hat-shaped (green), both spherical and hat-shaped (blue), other shape (red), or absent (white); ascospore number including 1 to 2 (purple), 3 to 4 (orange), 5 to 100 (green), 101 to 200 (blue), 201 to 400 (red), or absent (white). The trait data values used for the generation of this figure are provided in Table S1A.

the form of filaments, which are termed pseudohyphae or true hyphae. Pseudohyphae are characterized by the presence of filaments composed of chains or clusters of budding cells, while true hyphae are the result of continuous polarized growth that generates filaments of discrete cells separated by septa (Kurtzman et al. 2011).

There are a few caveats that are important to note regarding the cell morphology of *Saccharomycotina* species. The cell morphologies displayed by yeast species can vary by strain, by ploidy, and be influenced by growth conditions and cell age. For example, different culture media can induce slight variations in yeast cell morphologies; formation of pseudohyphae is prominent in media such as Dalmau plate culture on corn meal agar, but the same species will have reduced formation of pseudohyphae in other media such as glucose-yeast extract-peptone (Kurtzman et al. 2011). Time of incubation during cell culture can also have an influence on cell size, with higher incubation times leading to larger cell sizes. Cell cycle stage and phase of growth (e.g. lag vs. stationary phase) can also influence cell morphology. During the beginning stages of the cell cycle, a daughter cell is growing and therefore increasing in size, whereas at the end of the cell cycle the parent cell partakes in cellular quiescence in which replication and growth no longer occur (Sun and Gresham 2021). For the descriptions of cell size averages included in this review, most of the measurements were performed after three to five days of culture of the taxonomic type strain of each species.

Yeast cells can also undergo meiosis to form and release sexual spores known as ascospores. Although yeast species typically have both asexual (anamorphic) and sexual (teleomorphic) stages (Kurtzman et al. 2011), a considerable fraction of species are not known to have a sexual stage; for example, several species in the genera *Starmerella* of order *Dipodascales* and *Yamadazyma* of order *Serinales* are not known to produce sexual spores (or ascospores).

To synthesize available information on the variation of cell morphology of yeasts in the *Saccharomycotina* subphylum, we retrieved available data from taxonomic descriptions of individual species from *The Yeasts: A Taxonomic Study* (Kurtzman et al. 2011) and from *The Yeasts* website (https://theyeasts.org/), which is the successor to *The Yeasts: A Taxonomic Study* book series. Data were retrieved for 332 species recently compiled by the Y1000 + Project (http://y1000plus.org/) (Hittinger et al. 2015, Shen et al. 2018).

Interspecies variation of cellular morphology

Variation in asexual cell shape

Yeast species vary in cell shape within and between orders. Well-known yeasts, such as *S. cerevisiae* and *C. albicans*, produce spherical and ovoid budding cells, while *Hanseniaspora uvarum*, a species important to wine production, produces apiculate shaped cells. Examination of a representative set of 332 yeast species (Shen et al. 2018) showed that most budding cells have ovoid (63.8% of species or 212/332), spherical (59% or 196/332), and ellipsoidal (50.6% or 168/332) shapes (note that the numbers do not add up to 332 because some species exhibit two or more cell shapes). Less common shapes include elongate (19.6% or 65/332), cylindrical (19.5% or 65/332), apiculate (3.6% or 12/332), fusiform (1.5% or 5/332), and bacilliform (1.2% or 4/332). Other less common cell shapes that occur in two to four species include clavate (*Candida orba* of order *Phaffomycetales*, *Alloascoidea hylecoeti* of order *Alloascoideales*, *Cephaloascus albidus* and *Cephaloascus fragrans* of order *Serinales*), ogival (*Brettanomyces anomalus, Brettanomyces bruxellensis*, and *Brettanomyces custersianus* in order *Pichiales*), and aculeate (*Candida tammaniensis* and *Aciculoconidium aculeatum*, both in order *Serinales*). Shapes that occur in only one species in our dataset include triangular (*Trigonopsis variabilis* in order *Trigonopsidales*), rectangular (*Saprochaete clavata* in order *Dipodascales*), curved (*Sporopachydermia quercuum* in order *Sporopachydermiales*), lunate (*Candida golubevii* in order *Serinales*), and "bowling-pin" shaped (*Wickerhamia fluorescens* in order *Serinales*).

Examination of the distribution of cell shapes across the phylogeny of *Saccharomycotina* suggests that evolutionary relatedness is not always a good proxy for similarity of cell shape and that less common cell shapes are spread across the phylogeny (Fig. 4 and Table S1). For example, the curved-shaped species *Sp. quercuum* and the bacilliform-shaped species *Sporopachydermia lactativora* are closely related, whereas the distantly related *Al. hylecoeti* and *Ce. albidus* species are both clavate shaped. Similarly, organisms with cylindrical cell shapes are spread across different orders, such as *Teunomyces kruisii* (order *Serinales*), *Eremothecium sinecaudum* (order *Saccharomycetales*), and *Candida boidinii* (order *Pichiales*); ellipsoidal shaped cells are produced by *Kazachstania aerobia* (order *Saccharomycetales*) and *Zygoascus meyerae* (order *Dipodascales*); and tapered cells are produced by *Saccharomycopsis malanga* (order *Ascoideales*) and *Wickerhamomyces hampshirensis* (order *Phaffomycetales*).

Budding cell shape typically varies between species, but there is also variation within species. Almost all yeast species exhibit more than one type of budding cell shape (95.8% or 318/332). For example, budding cells of *Suhomyces canberraensis*, of order *Serinales*, are generally spherical, but some cells are cylindrical, ellipsoidal, or elongate. Species that exhibit four or more different budding shapes make up 7.5% of our dataset and are found in the orders *Pichiales*, *Serinales*, *Alaninales*, *Phaffomycetales*, *Dipodascales*, *Saccharomycetales*, and *Saccharomycodales* (Fig. 4 and Table S1). In some cases, this within species variation is conserved between species; budding cells of the sister taxa *Ce. albidus* and *Ce. fragrans* (order *Serinales*) can be ovoid, ellipsoidal, or clavate. However, other taxa exhibit a greater degree of conservation in their cell shape. For example, the ten species from the genus *Debaryomyces* (order *Serinales*) included in our dataset exhibit low levels of cell shape variation since all have mostly spherical and ovoid budding cells. Similarly, all *Hanseniaspora* species (order *Saccharomycodales*) in our dataset generate elongated and apiculate shaped cells, likely due to their specialized budding type (see "Budding type variation").

Variation in sexual cell shape

During sexual reproduction, ascomycetous yeast species typically undergo meiosis to generate ascospores that are enclosed within an ascus before release for fungal germination (Greig 2009). Of the 332 yeasts examined, 76.9% (230/332) have sexual cycle descriptions; this is likely an underestimate since examination of their genomes has revealed that 330 of the 332 species examined contain a mating type locus (Krassowski et al. 2019). The 230 yeast species with a known sexual cycle vary in the shape of ascospores and asci, as well as in the number of ascospores that each ascus contains (Figs 3 and 4). For example, a few species including *Al. hylecoeti* (order *Alloascoideales*) and *Vanderwaltozyma polyspora* (order *Saccharomycetales*) can produce large numbers of ascospores (~150–400 ascospores per ascus in the case of *Al. hylecoeti*), while most other species, such as those within the genera *Debaryomyces*, *Hanseniaspora, Kazachstania, Kluyveromyces, Lachancea, Priceomyces, Saccharomyces*, and *Torulaspora*, produce only one to four ascospores during meiosis. The average number of ascospores produced by species in our dataset is three.

Ascospores range in shape and can be spherical, ellipsoidal, hat-shaped, Saturn-shaped, acicular, and aculeate or needle-

shaped. Hat-shaped ascospores occur in 37.4% of species with a known sexual cycle in our dataset, including in some *Ambrosiozyma* (order *Pichiales*), *Barnettozyma* (order *Phaffomycetales*), and *Hanseniaspora* species (order *Saccharomycodales*), as well as in *Babjeviella inositovora* (order *Serinales*). About 17% of species in our dataset have spherical ascospores (e.g. *Kazachstania* species in order *Saccharomycetales*, *Citeromyces matritensis* in order *Pichiales*, *Starmerella bombicola* in order *Dipodascales*, and several *Hanseniaspora* species in order *Saccharomycodales*). *Debaryomyces* species (order *Serinales*) contain spherical ascospores that have a warty wall phenotype, although species, such as *Debaryomyces subglobosus*, have ascospores that contain a gear-like structure. *Lipomyces* species (order *Lipomycetales*) tend to have uncommon ascospore shapes, such as cymbiform, which is a shape that appears to be specific to this genus.

There appears to be greater variation of budding cell shapes than ascospore shapes within genera or orders. For example, *Metschnikowia* species (order *Serinales*) exhibit a diversity of budding cells (e.g. spherical, ovoid, elongate, ellipsoidal, and cylindrical), but most species produce acicular shaped ascospores. The same is true for *Eremothecium* species (order *Saccharomycetales*), which are known to produce spherical, ovoid, elongate, ellipsoidal, and cylindrical budding cells, but their ascospores tend to be elongated and acicular shaped.

Variation in asexual cell size

The cell size of the budding cells of different yeast species can vary from 2.5 × 1 µm in *Tortispora starmeri* (order *Trigonopsidales*) to as large as 28 × 7 µm in *Br. bruxellensis* (order *Pichiales*) and *Candida tropicalis* (order *Serinales*) (Fig. 2). Overall, yeast species produce budding cells that average 5.6 µm × 3.6 µm (average cell length and width of 332 yeast species across *Saccharomycotina*). There are species of yeast that have large budding cells, such as *Nakaseomyces bracarensis* (14 × 13.9 µm; order *Saccharomycetales*), and *Kurtzmaniella cleridarum* (15.8 × 11.1 µm; order *Serinales*). Budding cells whose maximum length is larger than 20 µm occur in species across the orders *Serinales*, *Dipodascales*, and *Pichiales*, including the species *Candida parapsilosis* (20 × 8 µm; order *Serinales*), *Magnusiomyces tetrasperma* (20 × 9 µm; order *Dipodascales*), *Kuraishia capsulata* (20 × 4 µm; order *Pichiales*), *Br. anomalus* (22 × 5.5 µm; order *Pichiales*), and *Blastobotrys muscicola* (22 × 2.5 µm; order *Dipodascales*). Species with small budding cells also occur across the yeast phylogeny. Examples include *Debaryomyces prosopidis* (2 × 2.25 µm; order *Serinales*), *Ogataea minuta* (2 × 1.8 µm) and *Ogataea nonfermentans* (2 × 1.8 µm) from order *Pichiales*, and *Wickerhamiella cacticola* (2.5 × 1.5 µm), and *Zygoascus ofunaensis* (2.05 × 3.45 µm) from order *Dipodascales*.

The relationship between budding cell size and evolutionary divergence is unknown, but it appears that similarly sized cells are more likely to be observed between closely related species than between distantly related ones. For example, budding cells that are 6 µm length on average (cell width average ranges from 2.5—5 µm) are observed in species within the orders *Serinales*, *Trigonopsidales*, *Pichiales*, and *Saccharomycetales*. However, average cell size can sometimes vary considerably between closely related species; for example, *Kazachstania bromeliacearum*, *Kazachstania kunashirensis*, and *Kazachstania martiniae* (all in order *Saccharomycetales*) have budding cells that are 3 × 2.5, 5 × 4, and 7.5 × 2.5 µm wide, respectively.

Variation in sexual cell size

Asci and ascospores are also highly variable in their sizes (Fig. 3). In some yeast species, a larger ascus can contain larger ascospores. For example, *Metschnikowia hawaiiensis* and *Metschnikowia bicuspidata* (both in order *Serinales*) produce large asci that have a maximum length of 200 µm and 60 µm, respectively, and also contain large ascospores that have a maximum length of 180 µm long and 50 µm, respectively. *Metschnikowia* species contain highly varied asci sizes, but the number of ascospores is conserved to one to two per ascus in the genus. In other cases, a yeast species can generate higher numbers of small ascospores within a large ascus. For example, *Al. hylecoeti* (order *Alloascoideales*) and *Ascoidea rubescens* (order *Ascoideales*) produce asci that can be at maximum as large as 400 µm × 24 µm or 150 × 30 µm, respectively, with ascospores that are 3.2 µm × 2 µm or 10 × 9 µm, respectively, such that each ascus can produce as many as 400 ascospores or 150 ascospores, respectively. *Ce. fragrans* (order *Serinales*) produces asci that are at maximum 7 µm × 3 µm and ascospores of 3 µm × 2 µm, making it one of the yeast species with the smallest asci and ascospores.

Variation in budding type

Yeasts can divide by budding in the following ways: utilizing one cell pole (side), or unipolar; utilizing both cell poles, or bipolar; and without relying on cell polarity, or multilateral (Fig. 1). Most species reproduce by multilateral budding (87.3% of species or 290/332); bipolar yeasts make up 3.9% (13/332), unipolar yeasts 4.5% (15/332), one species that only reproduces by fission (*Magnusiomyces tetrasperma* of order *Dipodascales*) and two yeasts that reproduce by both fission and bipolar budding (*Nadsonia fulvescens var. fulvescens* and *Nadsonia fulvescens var elongata* of order *Dipodascales*). As discussed previously, there are six species that have not been observed to produce budding cells, and there are 12 species that do not have budding cell information in Kurtzman et al. (2011). Unipolar budding is spread across the phylogeny of *Saccharomycotina* yeasts and is found in small numbers of species within orders *Saccharomycetales*, *Serinales*, *Pichiales*, and *Dipodascales*, whereas bipolar budding is highly conserved in the order *Saccharomycodales* (Fig. 4 and Table S1). Multilateral budding is also typically conserved, including in all species within the orders *Alaninales*, *Lipomycetales*, *Phaffomycetales*, and *Dipodascales*.

Variation in filament production

Many yeast species can grow filaments in the form of pseudohyphae or true hyphae; sometimes these occur during growth under stressful conditions (e.g. nutrient limitation, high temperature), but this is not always the case. Not every yeast species has been observed to generate pseudohyphae or true hyphae; approximately 36.7% (131/332) of species are not known to produce hyphae (Fig. 3). Filament morphological diversity is high among those species that can produce filaments, ranging from poorly developed pseudohyphae (rudimentary and poorly developed) to highly branched true hyphae (highly branched septate). For example, 12 species in the genus *Kazachstania* do not produce hyphae or pseudohyphae, while six other species do. Similarly, of the species included in our dataset, most *Barnettozyma* species (order *Phaffomycetales*) do not produce filaments, except for *Barnettozyma hawaiiensis* and *Barnettozyma populi*, while most *Ambrosiozyma* (order *Pichiales*) and *Hanseniaspora* (order *Saccharomycodales*) species generate hyphae, except for *Ambrosiozyma kashinagicola*, *Ambrosiozyma pseudovanderkliftii*, *Hanseniaspora pseudoguilliermondii*, and *Hanseniaspora vineae*.

Filament production can vary drastically within orders, even between closely related species. For example, in the order *Serinales*, *Me. hawaiiensis* can produce true hyphae with dark septa, *Metschnikowia cerradonensis* produces abundant pseudohyphae,

and *Metschnikowia hamakuensis* can only produce poorly developed pseudohyphae (Kurtzman et al. 2011). Similarly, species within the genus *Blastobotrys* (order *Dipodascales*) produce various forms of hyphae; for example, *Blastobotrys adeninivorans*, *Blastobotrys attinorum*, and *Blastobotrys parvus* produce true hyphae with distinct septa, while *Blastobotrys aristata, Blastobotrys nivea,* and *Blastobotrys proliferans* produce true hyphae that are hyaline or transparent (von Klopotek 1967, Sesma and Ramirez 1978; Kurtzman and Robnett 2007).

The dimensions of pseudohyphae and true hyphae are often uncharacterized, but data from 17 species suggest that the average width of hyphae is 3.5 µm. The width of hyphae and pseudohyphae ranges from 1 µm in *Ce. fragrans* (order *Serinales*) to up to 8 µm in *Al. hylecoeti* (order *Alloascoideales*). The diameter of hyphae does not vary as much as the size of other yeast cell types. For example, the size of budding cells of *Ce. albidus* and *Ce. fragrans* (order *Serinales*) range from 3 µm to 6 µm but generate hyphae with diameters of 1 µm to 3 µm.

Conclusions

We have characterized cell morphology traits of a representative set of 332 species in the subphylum *Saccharomycotina*, which has revealed extensive diversity of cell shapes and sizes, as well as budding types and filament production (Kurtzman et al. 2011, Shen et al. 2018). Model species of yeasts exhibit cellular morphological diversity that differs from the typical spherical and ovoid budding displayed by *S. cerevisiae*; their ascospore morphologies also differ from those observed in *S. cerevisiae*. Across the yeast phylogeny, budding cells are typically round (i.e. ovoid or spherical) with an average diameter twice the size of a typical bacterial species, such as *Escherichia coli* (Fig. 2). Most yeast species do not utilize the poles of the cells and instead reproduce asexually via multilateral budding. Furthermore, more than half of yeasts can generate pseudohyphae or true septate hyphae. The sexual cell morphology of yeasts includes ascospores that are typically spherical or hat-shaped, mostly found in pairs or quartets within an ascus (Fig. 3). The great interspecies diversity is nicely exemplified in the cell morphology traits of asexual cell shape and size, filament production, and sexual cell shape (Fig. 4 and Table S1).

An organism will display various cell morphology phenotypes, which are likely reflected in the collective interactions of protein components and their relative abundances (Chiou et al. 2017, Barber et al. 2020). Regarding cell morphology, cellular functions, such as division and reproduction, are controlled by the multiple pathways within the cell polarity network (CPN). In the baker's yeast *S. cerevisiae*, cell polarity is responsible for the localization of proteins to sites of division for successful budding, mating, and filament production. Highly conserved proteins in the CPN include the GTPase Cdc42, which recruits downstream proteins for polarization at the plasma membrane or can detach following polarization to diffuse freely (Chiou et al. 2017, Diepeveen et al. 2018). Cell size in *S. cerevisiae* is closely regulated during the cell cycle phases, with increased protein abundance of Whi5 correlated with larger cell sizes (Barber et al. 2020). Whi5 phosphorylation activity is maintained by two CPN proteins, Swi4 and Cln3; activation is mediated by Whi3, but how these and other proteins work together in *Saccharomycotina* yeasts to control cell morphology is unknown. Functional relationships between genes can be revealed by orthologous gene coevolution networks; for example, coevolutionary analysis of ∼2 400 orthologous genes across the 332 *Saccharomycotina* yeasts found that *CDC6*, a gene essential for replication, is connected to 96 other orthologs (Steenwyk et al. 2022). Furthermore, the genes that were found to coevolve with *CDC6* across *Saccharomycotina* showed substantial overlap with the genes found to genetically interact with *CDC6* in *S. cerevisiae* (Constanzo et al. 2010). An example of budding cell shape that is tightly associated with budding type occurs in the genus *Hanseniaspora*, in which all species reproduce by bipolar budding and exhibit apiculate-shaped budding cells. Interestingly, most *Hanseniaspora* species have lost over 700 genes in comparison to *S. cerevisiae*, including *WHI5* and many others involved in the cell cycle (Steenwyk et al. 2019).

Another approach for identifying candidate genes and pathways that have likely contributed to variation in yeast cellular morphology is experimental evolution. Experimental evolution studies have shown that both new cell morphologies, including multicellular structures, and considerable differences in existing traits (e.g. cell size) can arise quite rapidly and via diverse mutational routes (Bozdag et al. 2021, Farkas et al. 2022). Interestingly, experimental evolution of *S. cerevisiae* following the deletion of genes involved in the CPN can recover quickly and reproducibly. For example, deletion of the gene encoding the CPN protein Bem1 can be rescued by subsequent compensatory mutations in *BEM2* and *BEM3*, which encode Rho GTPase -activating proteins, after ∼1 000 generations (Laan et al. 2015). Future evolutionary experiments that focus on yeast cell morphologies should be performed to further understand the genetic mechanisms involved in their generation and evolution.

The cellular morphology of *Saccharomycotina* asexual and sexual cells is highly diverse, with various patterns observed within and across its taxonomic orders, but the association of morphological traits within and between species is not well characterized. For example, as mentioned previously, the genus *Hanseniaspora* reproduce asexually by bipolar budding which results in apiculate shaped cells, but it is unknown whether this is due to coincidence or whether it reflects functional constraints between the type of budding and specific cell shapes. Future studies of how the cell morphology network is evolving across yeasts could benefit with studies of association between and within the diversity of asexual cell traits and sexual cell traits of *Saccharomycotina* yeasts.

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Supplementary data

Supplementary data is available at *FEMSYR Journal* online.

Conflict of interest: Antonis Rokas is a scientific consultant for LifeMine Therapeutics, Inc.

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