Review

Draft Genome Analysis of Trichosporonales Species That Contribute to the Taxonomy of the Genus *Trichosporon* and Related Taxa

Masako Takashima¹ and Takashi Sugita²

¹ Japan Collection of Microorganisms, RIKEN BioResource Research Center
² Department of Microbiology, Meiji Pharmaceutical University

ABSTRACT

Many nomenclatural changes, including proposals of new taxa, have been carried out in fungi to adapt to the “One fungus = One name” (1F = 1N) principle. In yeasts, while some changes have been made in response to 1F = 1N, most have resulted from two other factors: i) an improved understanding of biological diversity due to an increase in number of known species, and ii) progress in the methods for analyzing and evaluating biological diversity. The method for constructing a backbone tree, which is a basal tree used to infer phylogeny, has also progressed from single-gene trees to multi-locus trees and further, to genome trees. This paper describes recent advances related to the contribution of genomic data to taxonomy, using the order Trichosporonales as an example.

Key words: backbone tree, classification, identification, *Trichosporon*, Trichosporonales

Introduction

In *The Yeasts, A Taxonomic Study, 5th ed.*, which was published in 2011, the genus *Trichosporon* (Trichosporonales, Basidiomycota) included 37 species¹. Some species of the highly polyphyletic genus *Cryptococcus* (10 of 70 species listed in *The Yeasts, A Taxonomic Study, 5th ed.*) were found among *Trichosporon* species upon construction of a phylogenetic tree. The clinically important species, *Cryptococcus curvatus* (present name, *Cutaneotrichosporon curvatum*) and *Cryptococcus humicola* (present name, *Vanrija humicola*), were two of these.

In 2015, Liu et al.² reclassified species assigned to the genus *Trichosporon*, resulting in an increase from 37 to 45. In their paper, species formerly assigned to the genus *Trichosporon* were divided into five genera, *Apiotrichum* (21 species), *Cutaneotrichosporon* (10 species), *Effuseotrichosporon* (1 species), *Haglerozyma* (1 species), and *Trichosporon* (12 species). Most of the species formerly assigned to *Trichosporon* were reclassified into three genera, *Apiotrichum*, *Cutaneotrichosporon*, and *Trichosporon*, while two species were each assigned to the monotypic genera, *Effuseotrichosporon* (formerly *Trichosporon vanderwaltii*) and *Haglerozyma* (formerly *Trichosporon chiarellii*). *Cryptococcus curvatus* and *C. humicola* were transferred to *Cutaneotrichosporon* and *Vanrija*, respectively. According to the classification proposed by Liu et al. (2015), species formerly identified as *Trichosporon* belonged to one family, the Trichosporonaceae. Sugita et al.³ summarized this reclassification, focusing on medically important yeast species.

In the light of the recent advances in sequencing techniques, namely, next-generation sequencing, the importance of correct classification has increased greatly because it represents the foundation for the correct assignment of taxa for which genomic information becomes available. Accordingly, a robust backbone tree and the identification of phenotypic traits that can be used to distinguish taxa are required to build a taxonomic system that takes full advantage of genomic data. In this paper, we introduce the contributions of genomic data to taxonomy, using our data from the Trichosporonales as an example.

Single-gene tree, multigene tree, and draft genome tree

Most Trichosporonales species can be identified using one
or a combination of the sequences of the D1/D2 domain of the LSU rRNA gene (hereafter, D1/D2) and the internal transcribed spacer (ITS) region (Fig. 1). However, delineation based on the D1/D2 and ITS region is sometimes not possible in the case of closely related species, as shown in Table 1. In addition, some species, such as *C. curvatus*, could not be positioned with confidence in phylogenetic trees using these regions, as in our previous studies.

Liu et al. used seven genes to construct a backbone tree of tremellomycetous yeasts. Construction of backbone trees
Curvatus formed a clade with 173 amino acid sequences showed the same topology. Concatenated alignments based on 72,531 nucleotide and 24,306 protein-coding DNA sequences from the genomic data, extracted the same regions of the RPB1, RPB2, and TEF1-α genes from genomic data and constructed a phylogenetic tree. We found that the concatenation of these three genes is insufficient to resolve its phylogenetic position. For example, the phylogenetic position of Cutaneotrichosporon guehoae remained unstable in the seven-gene tree, leading to some nodes. For example, the phylogenetic position of Cutaneotrichosporon guehoae remained unstable in the seven-gene tree, leading to some nodes.

The genome tree showed good resolution, with 100% bootstrap support for all branches, evidence that the topology is not affected by the sampling error associated with smaller sequences. Internode certainty was analyzed using Randomized Accelerated Maximum Likelihood (RAxML)\(^{16}\), which showed that the tree, for the most part, is well-supported by the majority of ortholog groups.\(^{15}\) The internode certainty of clades of the genera Apiotrichum, Cutaneotrichosporon, and Trichosporon are shown in Table 2. Notably, although Trichosporon guehoae was transferred to Cutaneotrichosporon by Liu et al.\(^{17}\), it did not cluster with other Cutaneotrichosporon species in the genome tree. Medelhoven et al.\(^{17}\) described C. guehoae as T. guehoae and positioned it between Trichosporon loubieri (present name, Apiotrichum loubieri) and Trichosporon asahii, although this arrangement lacked bootstrap support. In the most part, is well-supported by the majority of ortholog groups.\(^ {15} \) The internode certainty of clades of the genera Apiotrichum, Cutaneotrichosporon, and Trichosporon are shown in Table 2. Notably, although Trichosporon guehoae was transferred to Cutaneotrichosporon by Liu et al.\(^ {17} \), it did not cluster with other Cutaneotrichosporon species in the genome tree. Medelhoven et al.\(^ {17} \) described C. guehoae as T. guehoae and positioned it between Trichosporon loubieri (present name, Apiotrichum loubieri) and Trichosporon asahii, although this arrangement lacked bootstrap support. In the most part, is well-supported by the majority of ortholog groups.\(^ {15} \) The internode certainty of clades of the genera Apiotrichum, Cutaneotrichosporon, and Trichosporon are shown in Table 2. Notably, although Trichosporon guehoae was transferred to Cutaneotrichosporon by Liu et al.\(^ {17} \), it did not cluster with other Cutaneotrichosporon species in the genome tree. Medelhoven et al.\(^ {17} \) described C. guehoae as T. guehoae and positioned it between Trichosporon loubieri (present name, Apiotrichum loubieri) and Trichosporon asahii, although this arrangement lacked bootstrap support. In

### Table 1. Pairs of species that cannot be differentiated with a simple combination of sequences of the D1/D2 domain of LSU rRNA and the ITS region*

| Clade                                      | Base differences in D1/D2 domain | ITS region | Recommended method for differentiation          |
|--------------------------------------------|----------------------------------|------------|------------------------------------------------|
| Trichosporon asahii, T. coremiiforme, and T. faecale | 1–2                              | 1–2        | Sequencing of IGS region** (> 10% divergence)   |
| Trichosporon caseorum and T. lactis        | 2                                | 1          | Assimilation tests with melezitose and ribitol   |
| Apiotrichum domesticum and A. montevideense | 2                                | 0          | Sequencing of IGS region** (> 5% divergence)    |

* Data from Sugita\(^ {11} \)

** Intergenic spacer region

### Table 2. Internode certainty (IC) for three genera, Apiotrichum, Cutaneotrichosporon, and Trichosporon, in the genome tree

| Clade                                      | Number of species used                                                                 | IC      |
|--------------------------------------------|----------------------------------------------------------------------------------------|---------|
| Apiotrichum                                | 8 (A. brassicaceae, A. domesticum gamsii, A. gracile, A. laibachii, A. montevideense, A. porosum, A. veenhuissi) | 0.619   |
| Cutaneotrichosporon                        | clade for whole Cutaneotrichosporon species including C. curvatum                      | 0.552   |
|                                            | 8 (C. arboriforme, C. curvatum, C. cutaneum, C. cyanovorans, C. dassewskae, C. dermatis, C. mucoides, C. oleaginosus) |        |
|                                            | clade for formerly identified as Trichosporon + C. arboriforme                         | 0.804   |
|                                            | 5 (C. arboriforme, C. cutaneum, C. dermatis, C. mucoides, C. oleaginosus)               |         |
| Trichosporon                               | 5 (T. asahii, T. coremiiforme, T. faecale, T. inkin, T. ovoides)                        | 0.982   |

Data from Takashima et al.\(^ {15} \)

* Cutaneotrichosporon guehoae is not included in this table as it did not cluster with Cutaneotrichosporon species.
our genome tree, *C. guehoae* was placed at the base of three genera formerly classified as *Trichosporon*, *Cutaneotrichosporon*, and *Apiotrichum*, with a 100% bootstrap value. We found that the taxonomic position of a species for which the phylogenetic position was supported by high bootstrap values in trees derived from D1/D2, SSU, and ITS regions were corroborated in the analysis using a larger dataset. We assume that if a newly isolated strain belongs to such a clade in a tree based on D1/D2 or ITS region, its identification (at least to the genus level) can be made with certainty. However, for some species, the phylogenetic position appeared unstable, and the reliability varied depending on the genes and analytical conditions used (Table 3). Determining the draft genome is a good choice for inferring a species’ phylogenetic position with confidence, as exemplified by *C. curvatus* and *T. guehoae*.

In addition, when choosing sequences for multigene analysis or draft genome analysis to construct a backbone tree, we found that target strains should be selected after careful consideration based on published trees. Our findings also suggest that, when constructing a backbone tree, inclusion of species located near the early-emerging position of the published tree is crucial. The optimal number of taxa for such an examination remains unclear, but may depend upon the phylogenetic range of the dataset. We also noted that monotypic taxa, namely those that do not join with other species to form cohesive clades, will continue to be detected even if draft genomes of every known species are provided.

### On species delineation

In prokaryotes, genomic data are used to determine whether a strain belongs to a new species. Generally, the DNA–DNA hybridization (DDH) value is used to distinguish new species; a strain with more than 70% DNA–DNA relatedness with another is treated as the same species, with some exceptions. Formulae have been developed to infer DNA–DNA reassocia-
tion values from genome sequence data. The conversion factor for estimating the DNA relatedness using genomic data has been investigated and proposed based on the correlation between DNA–DNA relatedness and genomic identity; 70% DDH for species delineation corresponds to 95% in the case of Average Nucleotide Identity (ANI) and to 70% in the case of Genome-to-Genome Distance Calculator (GGDC), respectively. For details, please see the following papers: Goris et al.\(^{18}\), Meier-Kolthoff et al.\(^{19}\), Rodriguez-R and Konstantinidis\(^{20}\), and Chun et al.\(^{21}\).

In fungi, because validation of the methods used to infer DNA relatedness from genome data has not yet been performed, it remains unclear whether the methods used in bacteriology can be used for species delineation. Nevertheless, the introduction of such a method for species delineation using genomic data is an attractive prospect. A validation study is necessary to introduce a similar method for delineation in yeast taxonomy; such a study will not be difficult, as there are existing DDH data for a large number of yeasts. It is reasonable to assume that Average Nucleotide Identity (ANI) can be transposed to yeasts, as the methods of data

### Table 3. The topology of species located at the basal position may change depending on the regions and number of genes used for analysis

| Regions used (reference) | Scientific name at the time of publication | Trichosporon guehoae |
|--------------------------|------------------------------------------|----------------------|
| D1/D2 domain of LSU rRNA gene (Sugita\(^{1}\)) | Not supported (bootstrap < 50%) | Not supported (bootstrap < 50%) |
| D1/D2 domain of LSU rRNA gene (Scorzetti et al.\(^{4}\)) | Not supported (bootstrap < 50%) | Not supported (bootstrap < 50%) |
| ITS region (Scorzetti et al.\(^{4}\)) | Joined the cutaneum clade (bootstrap 86%) | Basal position to cutaneum, gracile, and hyalodendron clades (bootstrap 63%) |
| 7-gene concatenation (Liu et al.\(^{10}\)) | Formed a clade with *Cryptococcus daszewskae* (bootstrap 61%). This clade was located at the basal position of clade cutaneum and haglerorum (bootstrap 87 and 92% with maximum likelihood and neighbor-joining methods, respectively) | Not analyzed |
| 30-gene concatenation (Takashima et al.\(^{13}\)) | Joined the clade of *C. cutaneum* and *C. dermatis* (bootstrap 100%, only two species were used in\(^{4}\)) | Not analyzed |
| Draft genome tree (Takashima et al.\(^{15}\)) | Joined the *Cutaneotrichosporon* clade (bootstrap 100%) | Located at the base of three genera, *Trichosporon*, *Cutaneotrichosporon*, and *Apiotrichum* (bootstrap 100%) |
treatment in ANI resemble those applied to the use of DNA–DNA reassociation experiments in delineating yeast species.

Genomic data have also been used as with phenotype data to delineate species. An ortholog dataset for commonly shared genes in eukaryotes was published by Simão et al.22. In a recent paper by Haase et al.23, the new species *Yamadazyma laniorum* was proposed based on the relationship between physiological and biochemical characteristics and the presence/absence of particular genes. Using genomic data from 55 strains of *Metschnikowia*, Lachance et al.24 showed that phylogeny based on a presence–absence matrix of annotated genes was remarkably congruent with sequence-based phylogenies, and that all conspecifics had synapomorphies (unique genes that were shared or absent). We assume that discussions of such phenotype and genotype relationships will increase in the future. The inclusion of genes or gene clusters in the descriptions of new taxa may also be introduced. Delineation of closely related species such as *Trichosporon asahii*, *T. coremiiforme*, and *T. faecale* (see Table 1) will become easier as data accumulate.

**Meta-ITS analysis**

In 2012, a DNA barcode region was proposed for fungi25, and several primers for meta-ITS study of fungal diversity were described26. Discussions for identification of a more appropriate region have also been carried out to resolve accuracy problems27. A threshold of 97% identity, which is usually used in such meta-ITS analyses, corresponds to the sequence divergence between genera in the Trichosporonaceae; for example, the sequence identity of the ITS region including the 5.8S rRNA gene between *Trichosporon asahii* and *T. inkin* is 98%. Selection of genus- or species-specific genes will contribute to future metagenomic and metatranscriptomic analyses. As such, genes can be used for identification to the genus or species level, for selection of relatives at the species level, and for estimation of properties of that species.

**Hybrid species and taxonomy**

Hybrid strains and aneuploidy are well known in yeasts based on karyotyping and Amplified Fragment Length Polymorphism (AFLP) analysis. Studies of interspecies hybrid genomes have recently increased, as such organisms have potential applications in industrial fermentations. In particular, the fermentation history and domestication of lager beer yeasts have been investigated28,29. In the clinical field, the serotype AD strain of *Cryptococcus neoformans* is a famous example30,31 of species with hybrid genomes, along with *Malassezia furfur*32 and *Candida orthopsilosis*33. In our study of the Trichosporonales, we reported that *T. coremiiforme*, *Trichosporon ovoides*, and *Cutaneotrichosporon mucoides* contained hybrid genomes34,35. Gabaldón et al.35 reviewed the relationship between genome hybridization and pathogenicity using *Candida glabrata* and *Candida parapsilosis* and related species. The acquisition of aneuploidy may confer antifungal drug resistance36. Studies of inter- or intra-species hybridization, including its mechanism and genomic stability, may clarify fungal pathogenicity37,38.

Regarding nomenclature for hybrid species, Hagen et al.39 suggested the use of a hybrid formula; for example, the hybrid serotype AD strains *C. neoformans* should be referred to as *C. neoformans × C. deneoformans*. Nguyen and Boekhout27 also proposed a hybrid formula for well-characterized hybrid strains in the genus *Saccharomyces* (e.g., *S. eubayanus × S. cerevisiae* for *S. pastorianus* Weihenstephan 34/70). “Names of hybrids” is listed in the main body of the Shenzhen Code (Chapter H, International Code of Nomenclature for algae, fungi, and plants, https://www.iapt-taxon.org/nomen/main.php), while it was included as an Appendix in the former version of the code. Article H.2 of the Shenzhen Code states “A hybrid between named taxa may be indicated by placing the multiplication sign × between the names of the taxa; the whole expression is then called a hybrid formula.” When the parent species are known, the name of a hybrid strain can be indicated using this hybrid formula. In other words, it will be necessary to describe how the authors have identified the parent species when they use a hybrid formula. For example, a strain produced artificially in the laboratory can readily be named using this hybrid formula, as its parental species are known.

The *T. ovoides* and *T. coremiiforme* hybrid genomes were first discovered through genome analysis. The hybridization events occurred approximately 14–22 million years ago (Mya)40, assuming that the evolutionary rate has been constant and that *Saccharomyces* and *Kluyveromyces* diverged 100–150 Mya41. According to a recent report of the evolutionary diversification timeline of Saccharomycotina42, the divergence of *S. cerevisiae* from *Saccharomyces paradoxus* and of *Candida albicans* from *Candida dubliniensis* occurred 4.0–5.8 Mya and 5.0–14.0 Mya respectively. This result showed that hybridization of the genomes in *T. ovoides* and *T. coremiiforme* occurred at a similar or earlier time as the divergence of *C. albicans* and *C. dubliniensis*. The parent species of those hybrid genomes have not been identified and are assumed to have diverged sufficiently to be treated as distinct species. Accordingly, for these two species, the names *T. ovoides* and *T. coremiiforme* are maintained, as discussed in Takashima et al.43. For the *C. mucoides* hybrid genome, further analysis is required to estimate the time of hybridization.

The “natural hybrid” genomes of a strain for which the
parent species have not been identified (or a hybrid genome that is recognized by genomic analysis) should be recognized as a species in its own right. We assume that reports of hybrid genomes will further increase in response to the progress of genomic analysis.

The taxonomic position of such “natural hybrid” strains should be identified correctly, and thus the taxonomy of the genomic era should include such “natural hybrid” species.

Acknowledgement

We thank Prof. Marc-André Lachance, Department of Biology, University of Western Ontario, Canada, for his valuable comments and suggestions that improved this manuscript.

Conflicts of interest

None declared.

References

1) Sugita T: Trichosporon Behrend (1890). In The Yeasts, A Taxonomic Study, 5th ed. (Kurtzman CP, Fell JW, Boekhout T, ed), pp. 2015-2061, Elsevier, Amsterdam, 2011.

2) Liu XZ, Wang QM, Góker M, Groenewald M, Kachalkin AV, Lumbsch HT, Millanes AM, Wedin M, Yurkov AM, Boekhout T, Bai FY: Towards an integrated phylogenetic classification of the Tremellomycetes. Stud Mycol 81: 85-147, 2015.

3) Sugita T, Cho O, Takashima M: Current status of taxonomy of pathogenic yeasts: Cryptococcus neoformans/Cryptococcus gattii and the genus Trichosporon. Med Mycol J 58: 377-381, 2017. [Article in Japanese]

4) Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A: Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Res 2: 495-517, 2002.

5) Tamura K, Nei M: Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10: 512-526, 1993.

6) Felsenstein J: Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791, 1985.

7) Kumar S, Stecher G, Tamura K: MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874, 2015.

8) Sugita T, Takashima M, Ikeda R, Nakase T, Shinoda T: Phylogenetic and taxonomic heterogeneity of Cryptococcus humicolus by analysis of the sequences of the internal transcribed spacer regions and 18S rDNA, and the phylogenetic relationships of C. humicolus, C. curvatus, and the genus Trichosporon. Microbiol Immunol 44: 455-461, 2000.

9) Takashima M, Nakase T: Molecular phylogeny of the genus Cryptococcus and related species based on the sequences of 18S rDNA and internal transcribed spacer regions. Microbiol Cult Collect 15: 35-47, 1999.

10) Liu XZ, Wang QM, Theelen B, Groenewald M, Bai FY, Boekhout T: Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses. Stud Mycol 81: 1-26, 2015.

11) Kurtzman CP, Robnett CJ: Relationships among genera of the Saccharomycotina (Ascomycota) from multigene phylogenetic analysis of type species. FEMS Yeast Res 13: 23-33, 2013.

12) Lutzoni F, Kaufl F, Cox CJ, et al: Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. Am J Bot 91: 1446-1480, 2004.

13) Takashima M, Manabe R, Iwasaki W, Ohyama A, Ohkuma M, Sugita T: Selection of orthologous genes for construction of a highly resolved phylogenetic tree and clarification of the phylogeny of Trichosporonales species. PLoS One 10: e0131217, 2015.

14) Rokas A, Williams BL, King N, Carroll SB: Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425: 798-804, 2003.

15) Takashima M, Sriswasdi S, Manabe RI, Ohyama A, Sugita T, Iwasaki W: A Trichosporonales genome tree based on 27 haploid and three evolutionarily conserved ‘natural’ hybrid genomes. Yeast 35: 99-111, 2018.

16) Salichos L, Stamatakis A, Rokas A: Novel information theory-based measures for quantifying incongruence among phylogenetic trees. Mol Biol Evol 31: 1261-1271, 2014.

17) Middelhoven WJ, Scorzetti G, Fell JW: Trichosporon guehoae sp.nov., an anamorphic basidiomycetous yeast. Can J Microbiol 45: 686-690, 1999.

18) Goris J, Konstantinidis K, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM: DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57: 81-91, 2007.

19) Meier-Kolthoff JP, Auch AF, Klenk HP, Góker M: Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14: 60, 2013.

20) Rodriguez-R LM, Konstantinidis KT: Bypassing cultivation to identify bacterial species. Microbe Magazine 9: 111-118, 2014.

21) Chun J, Oren A, Ventosa A, Christensen H, Arahal D, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME: Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68: 461-466, 2018.

22) Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM: BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31: 3210-3212, 2015.

23) Haase M, Kominek J, Langdon QK, Kurtzman CP, Hittinger CT: Genome sequence and physiological analysis of Yama- dazyma laniorum f. a. sp. nov. and a reevaluation of the apocyphal xylose fermentation of its sister species, Candida tenuis. FEMS Yeast Res 17: fox019, 2017.

24) Lachance MA, Hurtado E, Hsiang T: A stable phylogeny of the large-spored Metschnikowia clade. Yeast 33: 261-275, 2016.

25) Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium; Fungal Barcoding Consortium Author List: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA 109: 6241-6246, 2012.
26) Ihrmark K, Bödeker I, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE, Lindahl BD: New primers to amplify the fungal ITS2 region—evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol Ecol 82: 666-677, 2012.

27) Větrovský T, Kolafík M, Žifčáková L, Zelenka T, Baldrian P: The rpb2 gene represents a viable alternative molecular marker for the analysis of environmental fungal communities. Mol Ecol Resour 16: 388-401, 2016.

28) Gonçalves M, Pontes A, Almeida P, Barbosa R, Serra M, Libkind D, Hutzler M, Gonçalves P, Sampaio JP: Distinct domestication trajectories in top-fermenting beer yeasts and wine yeasts. Curr Biol 26: 2750-2761, 2016.

29) Gallone B, Steensels J, Prahl T, et al: Domestication and divergence of Saccharomyces cerevisiae beer yeasts. Cell 166: 1397-1410, 2016.

30) Sun S, Xu J: Chromosomal rearrangements between serotype A and D Strains in Cryptococcus neoformans. PLoS ONE 4: e5524, 2009.

31) Hagen F, Khayhan K, Theelen B, Koleccka A, Polacheck I, Sionov E, Falk R, Parmen S, Lumbsch H, Boekhout T: Recognition of seven species in the Cryptococcus gattii/Cryptococcus neoformans species complex. Fungal Genet Biol 78: 16-48, 2015.

32) Wu G, Zhao H, Li C, et al: Genus-wide comparative genomics of Malassezia delineates its phylogeny, physiology, and niche adaptation on human skin. PLoS Genet 11: e1005614, 2015.

33) Pryszcz L, Németh T, Gács G, Gabaldón T: Genome comparison of Candida orthopsilosis clinical strains reveals the existence of hybrids between two distinct subspecies. Genome Biol Evol 6: 1069-1078, 2014.

34) Sríswasdí S, Takashima M, Manabe R, Ohkuma M, Sagita T, Iwasaki W: Global deceleration of gene evolution following recent genome hybridizations in fungi. Genome Res 26: 1081-1090, 2016.

35) Gabaldón T, Naranjo-Ortiz M, Marcet-Houben M: Evolutionary genomics of yeast pathogens in the Saccharomycotina. FEMS Yeast Res 16: fox064, 2016.

36) Selmecki AM, Dulmage K, Cowen L, Anderson JB, Berman J: Acquisition of aneuploidy provides increased fitness during the evolution of antifungal drug resistance. PLoS Genet 5: e1000705, 2009.

37) Nguyen H, Boekhout T: Characterization of Saccharomyces uvarum (Beijerinck, 1898) and related hybrids: assessment of molecular markers that predict the parent and hybrid genomes and a proposal to name yeast hybrids. FEMS Yeast Res 17: fox014, 2017.

38) Wolfe KH, Shields DC: Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387: 708-713, 1997.

39) Shen XX, Opulente DA, Kominek J, et al: Tempo and mode of genome evolution in the budding yeast subphylum. Cell 175: 1533-1545, 2018.