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Alterations in ocular fungal microbiota in patients with fungal keratitis: a comparative study from coastal regions of Eastern China

Xudong Zhao[1†], Zhichao Ren[2†], Wenfeng Li[3], Qing Liu[1], Yanling Dong[1] and Yusen Huang[1*]

Abstract

Objective The understanding of the ocular microbiota, particularly in fungal keratitis (FK), is evolving with advancements in high-throughput sequencing technologies. Traditional culture-based methods may not fully capture the microbial diversity present in keratitis, highlighting the need for more comprehensive approaches to explore microbial dysbiosis in corneal infections. This study aimed to reveal the dysbacteriosis of the ocular fungal microbiome associated with FK.

Methods We analysed 105 samples, including conjunctival swabs from healthy eyes (HE) and conjunctival swabs (SW), as well as corneal scrapings (SC), from FK eyes. Positive results were observed in 58 samples, and detailed taxonomic categorization was carried out across multiple levels—phylum, class, order, family, and genus—using high-throughput ITS sequencing. Alpha and beta diversity indices were computed, and interaction networks at the genus level were predicted to elucidate changes in microbial communities. The analyses also included assessments of functional groups within the fungal microbiome.

Results Among the samples, the HE, SW, and SC groups presented differences in positivity rates and diversity indices. Compared with HE eyes, infected eyes (SW and SC) presented significantly greater Good's coverage estimator and lower Chao1, Shannon, and Simpson diversity indices, indicating reduced species richness and evenness. At multiple taxonomic levels, various taxa were significantly downregulated in the FK eyes. Functional analyses revealed differences, notably, an increase in the number of litter saprotrophs in FK eyes. *Ascomycota* and *Basidiomycota* were identified as core phyla in the ocular microbiota interaction network.

Conclusion Fungal keratitis significantly alters the ocular surface microbiome, which is characterized by decreased microbial richness and evenness. High-throughput sequencing revealed a complex interaction network with significant variability between healthy and infected eyes. Additionally, these findings suggest potential benefits from early and aggressive debridement in managing FK due to its impact on functional microbial groups.

Keywords Fungal keratitis, Ocular Microbiome, High-throughput sequencing, Microbial diversity, Dysbiosis

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Introduction

The ocular surface is known to harbour a diverse collection of microorganisms, constituting the ocular microbiota, which play essential roles in human health and disease [1, 2]. An imbalance in this microbiota can potentially facilitate the proliferation of opportunistic filamentous fungi or yeasts, leading to fungal keratitis (FK), an inflammatory corneal pathology [3, 4]. Compared with bacterial keratitis, FK is often associated with more severe clinical consequences [5]. FK affects over a million people annually, with roughly three-quarters at risk of losing their eyesight [6, 7]. Moreover, there has been a dearth of innovative treatments for FK since the introduction of topical natamycin in the 1960s [5].

The initiation of the Human Microbiome Project and the Ocular Microbiome Project has focused considerably on the microbiome of the human ocular surface [8]. Both projects aim to understand the microorganisms inhabiting healthy human bodies and to identify any microbial changes associated with disease states [4]. Numerous global studies suggest that microbial communities play integral roles in maintaining eye health and are important for understanding the pathophysiological mechanisms of ocular diseases [4, 9–12].

Metagenomic sequencing applied to corneal preservation environments has revealed previously undetectable microbial components, demonstrating that changes in the ocular surface microbiota are overlooked by conventional diagnostic methods [13]. The categorization of microbial profiles into eye community state types (ECSTs) has helped define baseline characteristics in healthy individuals [14]. In clinical settings, shotgun metagenomics has enabled the identification of unculturable pathogens in infectious keratitis, addressing a key limitation of traditional diagnostics [15]. Additionally, microbial diversity analyses have revealed the complexity and variability of ocular infections, suggesting the need for deeper investigations using multiple approaches [16]. These include cross-regional comparisons, the integration of clinical and animal models, and both static and longitudinal observations. The internal transcribed spacer (ITS), a unique barcode for fungal identification, has allowed high-throughput ITS sequencing to become a fundamental research approach to fungal microbiota [17]. This technique enables researchers to reveal the relative composition of almost all fungal microorganisms within a given sample [6]. However, although the ocular bacterial microbiota has been extensively characterized, studies focusing on the fungal component are relatively limited, particularly in the context of fungal keratitis [18]. Within this broader context, employing ITS sequencing to investigate the fungal component of the ocular surface microbiota is equally necessary.

Understanding the alterations in the ocular fungal microbiota associated with FK is highly important, as it may illuminate the pathogenesis of the disease, facilitate early detection, and bolster the development of more targeted and effective treatment strategies. This study aimed to fill this knowledge gap by elucidating the alterations in ocular fungal microbiota observed in FK patients. Through a comparative analysis of the fungal microbiota between the FK-affected eye and the unaffected contralateral eye, this study enhances our understanding of the potential roles of diverse fungal species in either predisposing an individual to FK or assisting in resistance against it.

Materials and methods

Primary information

This study was approved by the Ethics Committee of Shandong Eye Institute (Approval No. 2019-26) and was registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR1900023720) on June 8, 2019. The procedure was entirely interpreted, and informed consent was acquired from all patients. All procedures abided by the tenets of the Declaration of Helsinki.

A total of 35 patients diagnosed with FK in one eye were included in this prospective study. None of the participants had any other eye diseases, previous experience with wearing contact lenses, a history of ophthalmic surgery, or received any treatment prior to this research.

Sample collection

In a disinfected room, all patients were placed in a supine position to facilitate the collection of corneal scrapings and conjunctival samples. Prior to the procedure, the eyelids were carefully wiped with iodophor, while the remaining facial area was covered with a sterile surgical drape. To ensure anaesthesia, a single drop of oxybuprocaine hydrochloride was administered to the affected eye before corneal scrapings were collected [6].

The palpebral conjunctiva, bulbar conjunctiva, and fornical conjunctiva were lightly rubbed with sterile swabs. The swabs obtained from healthy eyes were labelled the HE group, whereas those obtained from the affected eye were labelled the SW group.

To collect the corneal scrapings, the corneal lesions of the affected eye were carefully scraped using an ophthalmic microsurgical knife (Cat. No. MR-G137A, Suzhou Mingren Medical Equipment Co., Suzhou, China) under a microscope. This group of samples was labelled group SC.

DNA extraction

The extraction of total genomic DNA from the samples was carried out via a DNA extraction kit (D3096-100T, Omega Biotek, USA) according to the manufacturer's

instructions. All the DNA samples were subsequently sent to Qingdao OeBiotech. Co., Ltd. (Qingdao, China) for high-throughput ITS sequencing. Further information regarding the sequencing process can be found at https://www.qdoebiotech.com.

Internal transcribed spacer sequencing

DNA amplification

The quality and quantity of the DNA were assessed using NanoDrop, Qubit, and agarose gel analyses. DNA samples that exhibited no signs of degradation or minimal degradation were diluted to a concentration of 1 ng/μl and used as templates for PCR amplification. The amplification of the ITS I variable regions was achieved using the universal primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3').

Library construction

The quality of the amplicon was assessed through gel electrophoresis. The amplicon was subsequently refined via AMPure XP beads (Agencourt), followed by a second round of PCR amplification with the ITS1F and ITS2 primers. The final amplicon was then purified once again using the AMPure XP beads. The quantification of the final amplicon was performed via a Qubit dsDNA assay kit (Cat. No. Q32854; Invitrogen, Carlsbad, CA, USA). The desired final amplicon, characterized by a single band of 457 bp and a concentration exceeding 20 ng/μl, was deemed suitable for sequencing. Equal amounts of the purified amplicons were pooled together for subsequent sequencing using the Illumina MiSeq pe300 platform.

Bioinformatic analysis

The raw sequencing data were saved in FASTQ format. To preprocess the paired-end reads, Trimmomatic software (version 0.35) [19] was used. This software was utilized to identify and remove ambiguous bases (N) and trim sequences with an average quality score below 20 via a sliding window trimming approach. Following preprocessing, the paired-end reads were assembled using FLASH software (version 1.2.11) [20]. The assembly parameters were set as follows: a minimum overlap of 10 bp, a maximum mismatch rate of 20%, and a maximum overlapping length of 200 bp [6].

QIIME software (version 1.8.0) [21] was used for two subsequent data arrangements. First, the sequences were further denoised by removing reads with homologous, ambiguous sequences or sequences less than 200 bp in length. Additionally, reads with at least 75% of bases having a quality score greater than Q20 were retained. Second, reads containing chimeric sequences were eliminated.

Clean reads were then subjected to primer sequence removal and clustering using Vsearch software (version 2.4.2) [22] to generate operational taxonomic units (OTUs) with a 97% similarity cut-off. For each OTU, representative reads were selected using the QIIME package. Finally, these representative reads were aligned and annotated using the Unite database (ITS rDNA) [23, 24].

Microbial community parameters

Good's coverage estimator is utilized to determine whether the sequencing depth adequately captures all species within a given sample. A value closer to 1 indicates sufficient sequencing depth [25]. The Chao1 index is used to estimate the number of unique OTUs present in a community [26, 27]. The Shannon index, also known as the Shannon–Wiener index, considers both species richness and evenness; however, it is more strongly influenced by species richness than by evenness [27, 28]. The Simpson index, specifically Simpson's index of diversity (1-D), is another metric used to assess species richness and evenness. In comparison with species richness, it is more sensitive to variations in species evenness [25].

Results

Sample details

Among the 105 samples analysed, 58 yielded positive results. Specifically, conjunctival swabs from healthy eyes (HE) accounted for 16 positive samples (45.71%), whereas conjunctival swabs from FK eyes (SW) and corneal scrapings from FK eyes (SC) yielded 22 (62.86%) and 20 (57.14%) positive samples, respectively.

For the HE samples, the number of clean tags ranged from 34,679 to 74,723, with an average count of 68,174. For the SW samples, the number of clean tags ranged from 50,581 to 74,725, with an average of 70,517. Similarly, for the SC samples, the number of clean tags ranged from 49,880 to 74,328, with an average count of 70,098.

Following the removal of chimaeras, the number of valid tags, which were used for the final bioinformatics analysis, varied among the sample groups. In the HE samples, the number of valid tags ranged from 34,560 to 74,456, with an average count of 67,595. In SW samples, the number of valid tags ranged from 50,523 to 74,667, with an average of 70,440 tags. For the SC samples, the number of valid tags ranged from 49,850 to 74,279, with an average count of 70,029. The average length of the valid tags ranged from 219.27 to 279.84 bp.

The number of OTUs in the HE samples exhibited a range of 23 to ranged from 23,639, with an average count of 182. In the SW samples, the number of OTUs ranged from 9 to 91, with an average count of 32. Similarly, for the SC samples, the number of OTUs ranged from 9 to 99, with an average of 35. The content of each OTU in

each sample, along with the representative sequence for each OTU, can be found in Table S1.

Finally, a total of 7 phyla, 27 classes, 78 orders, 144 families and 246 genera were annotated in all 58 positive samples, and the relative contents of these taxonomic categories are shown in Tables S2-6.

Common alterations in the ocular fungal Microbiome induced by FK

Traditionally, the identification of pathogenic microorganisms in keratitis has relied on culture-based methods, with cultured microorganisms being defined as the causative pathogens [29]. However, our previous research [29] reported low concordance between fungal culture results and high-throughput ITS sequencing findings, suggesting that culture methods may preferentially report fungal genera that are more adapted to growth on the chosen media. Furthermore, our separate high-throughput 16 S rRNA sequencing study revealed a high prevalence of polymicrobial infections in bacterial keratitis, challenging the conventional notion that a single dominant pathogen is responsible for the majority of cases [30]. In light of these findings, and with the increasing prevalence of high-throughput sequencing technologies, it is imperative to reassess our understanding of keratitis from the perspective of microbial dysbiosis. This approach may provide a more comprehensive and nuanced view of the microbial ecology associated with corneal infections.

Since the ocular surface microbiota does not significantly differ between the eyes of individual subjects [6, 31], the comparison between healthy and infected eyes of FK patients reflects the fungal composition alterations caused by FK. As shown in Fig. 1, the relative composition of the top 30 fungal genera in all the samples

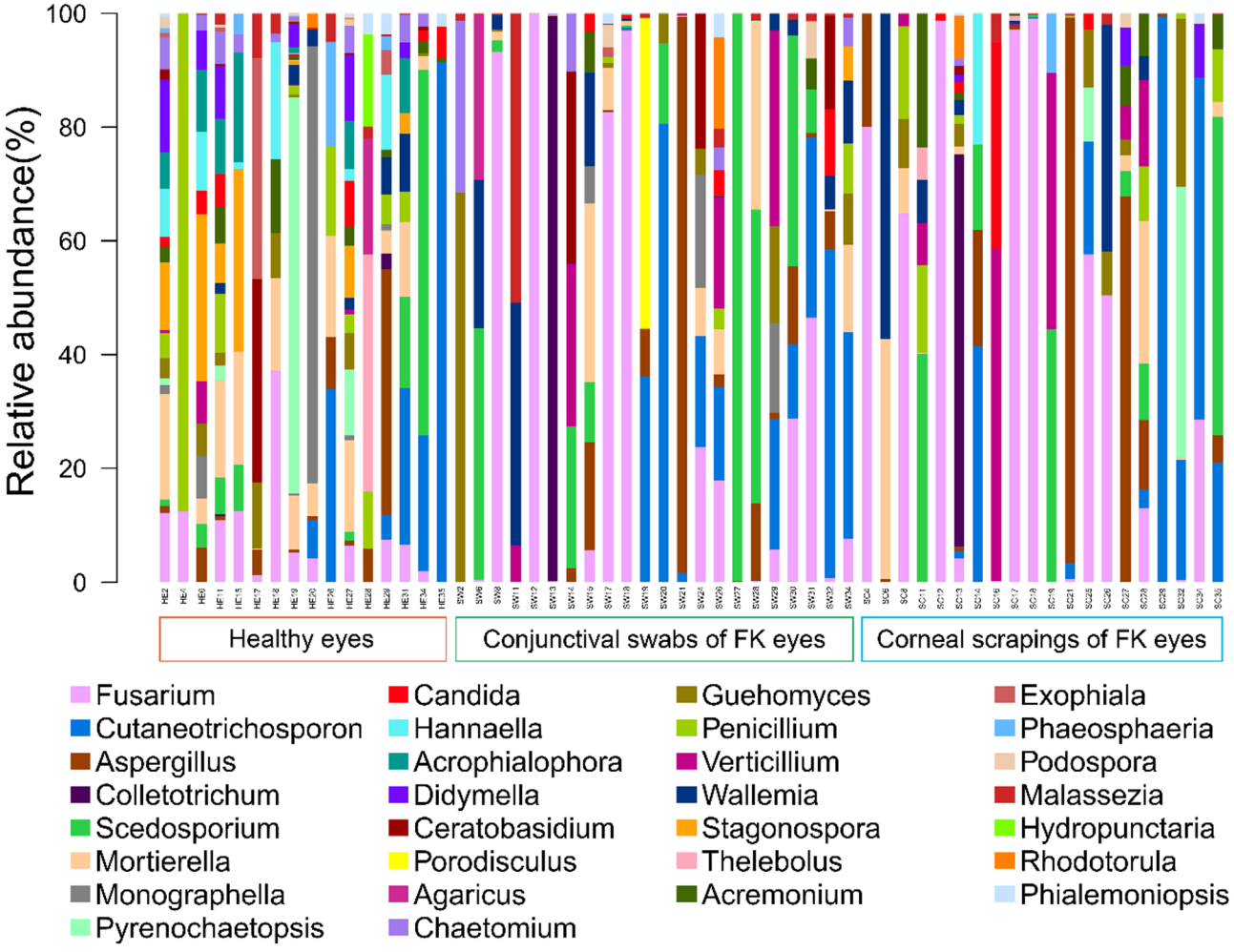

Fig. 1 The relative composition of the top 30 fungal genera in all the samples. Each bar represents a sample, various colour portions represent different genera, and the length of a coloured portion represents the relative content of a genus. Among the 105 samples, 16 (45.71%) from conjunctival swabs of healthy eyes were positive, 22 (62.86%) from conjunctival swabs of eyes with fungal keratitis were positive, and 20 (57.14%) from corneal scrapings of eyes with fungal keratitis were positive. Different samples possess diverse ocular fungal microbiota. The positive rate of high-throughput internal transcribed spacer sequencing seems to increase when fungal keratitis occurs

revealed that FK strongly affected the ocular surface microbiota.

Alterations in alpha diversity indices of ocular fungal microbiomes induced by FK

Alpha diversity represents the species diversity of individual samples. This measure encompasses two key aspects of species diversity: richness (the number of distinct species present) and evenness of species abundance distribution. These components collectively provide insight into the complexity and structure of microbial communities within each sample. Various alpha diversity metrics were employed in this study. Good's coverage estimator served as an indicator of sequencing depth, with values approaching 1 signifying nearly comprehensive species identification within the sample. The observed species metric quantified the count of detected OTUs, whereas the Chao1 index provided an estimate of the OTUs that existed in the community. The Shannon–Wiener index and Simpson's diversity index (1-D) both measure species richness and distribution; however, the former is more closely related to changes in richness, whereas the latter is more responsive to variations in evenness.

As shown in Fig. 2, Good's coverage estimator ($P = 0.00035$, Kruskal–Wallis) of infected eyes was significantly greater than that of healthy eyes, whereas the Chao1 index of infected eyes was significantly lower. These findings suggested that the microbiota richness of fungi in eyes with FK was significantly decreased ($P = 0.00027$, Kruskal–Wallis test). In addition, the Shannon index ($P = 4.44581e\text{-}05$, Kruskal–Wallis) and Simpson's index ($P = 0.00012$, Kruskal–Wallis) were significantly lower in infected eyes than in control eyes, which suggested a significant decline in microbiota evenness. No significant differences were detected between the SW and SC groups, suggesting that the fungal OTU quantity and abundance in the corneal scrapings and conjunctival swabs collected from patients' affected eyes are virtually identical, regardless of the respective methods employed (conjunctival swab and corneal scraping).

Alterations in beta diversity indices of ocular fungal microbiomes induced by FK

Beta diversity is employed to compare differences between sample groups and is often based on OTU sequence similarity, community structure (i.e., species abundance and distribution), or a combination of OTU sequence evolutionary relationships and community structure. This study focuses primarily on community structure, thus utilizing the Euclidean distance algorithm for principal coordinate analysis (PCoA).

PCoA is a visualization method for examining data similarities or differences. It operates by ordering a series of eigenvalues and eigenvectors, selecting the most significant eigenvalues, and identifying the principal coordinates within the distance matrix. This approach allows for the observation of differences between individuals or groups. As shown in Fig. 3, the beta diversity of the fungal communities of the three groups suggested that samples from healthy eyes were gathered in a specific region; however, samples from conjunctival swabs of eyes with FK and corneal scrapings of eyes with FK were difficult to differentiate from each other (Euclidean distance algorithm, $P = 0.999999$). These findings suggest that the ocular surface microbiota in FK eyes is more variable than that in healthy eyes.

Alterations in the ocular fungal microbiomes induced by FK at different levels

To elucidate the impact of FK on the ocular surface mycobiome across various taxonomic levels, we conducted a comprehensive analysis at the phylum, class, order, family, and genus levels. For each taxonomic rank, we identified and visualized the differentially abundant taxa using bar graphs.

At the phylum level (Fig. 4a), the abundances of *Basidiomycota*, *Cercozoa*, *Glomeromycota*, and *Zygomycota* were significantly decreased in FK eyes. At the class level (Fig. 4b), *Dothideomycetes*, *Glomeromycetes*, *Lecanoromycetes*, *Leotiomycetes*, *Mortierellomycotina cls Incertae sedis*, *Mucoromycotina cls Incertae sedis*, *Orbiliomycetes*, *Pezizomycotina cls Incertae sedis*, and *Tremellomycetes* were significantly downregulated in FK eyes. At the order level (Fig. 5a), *Capnodiales*, *Chaetothyriales*, *Filobasidiales*, *Glomerales*, *Helotiales*, *Mortierellales*, *Orbiliales*, *Pezizomycotina ord Incertae sedis*, *Pleosporales*, and *Xylariales* were significantly downregulated in FK eyes. At the family level (Fig. 5b), *Agaricaceae*, *Herpotrichiellaceae*, *Hypocreales fam Incertae sedis*, *Lasiosphaeriaceae*, *Mortierellaceae*, *Pezizomycotina fam Incertae sedis*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Tremellales fam Incertae sedis*, and *Xylariales fam Incertae sedis* were significantly downregulated in FK eyes. At the genus level (Fig. 5c), *Acrophialophora*, *Chaetomium*, *Didymella*, *Exophiala*, *Hannaella*, *Monographella*, *Mortierella*, and *Penicillium* were significantly downregulated in FK eyes. At the genus level, 55 genera were significantly different among the three groups, as shown in Figure S1.

Functional analysis of the fungal ocular microbiomes

The functional group refers to an approach used to classify species according to their different ways of absorbing and utilizing environmental resources rather than their evolutionary relationships. Figure 6a shows the relative compositions of various functional groups in each sample. In addition, Fig. 6b shows that the fungal microbiota of healthy eyes and FK eyes were significantly different in litter saprotrophs. In soil, saprophytic fungi are well

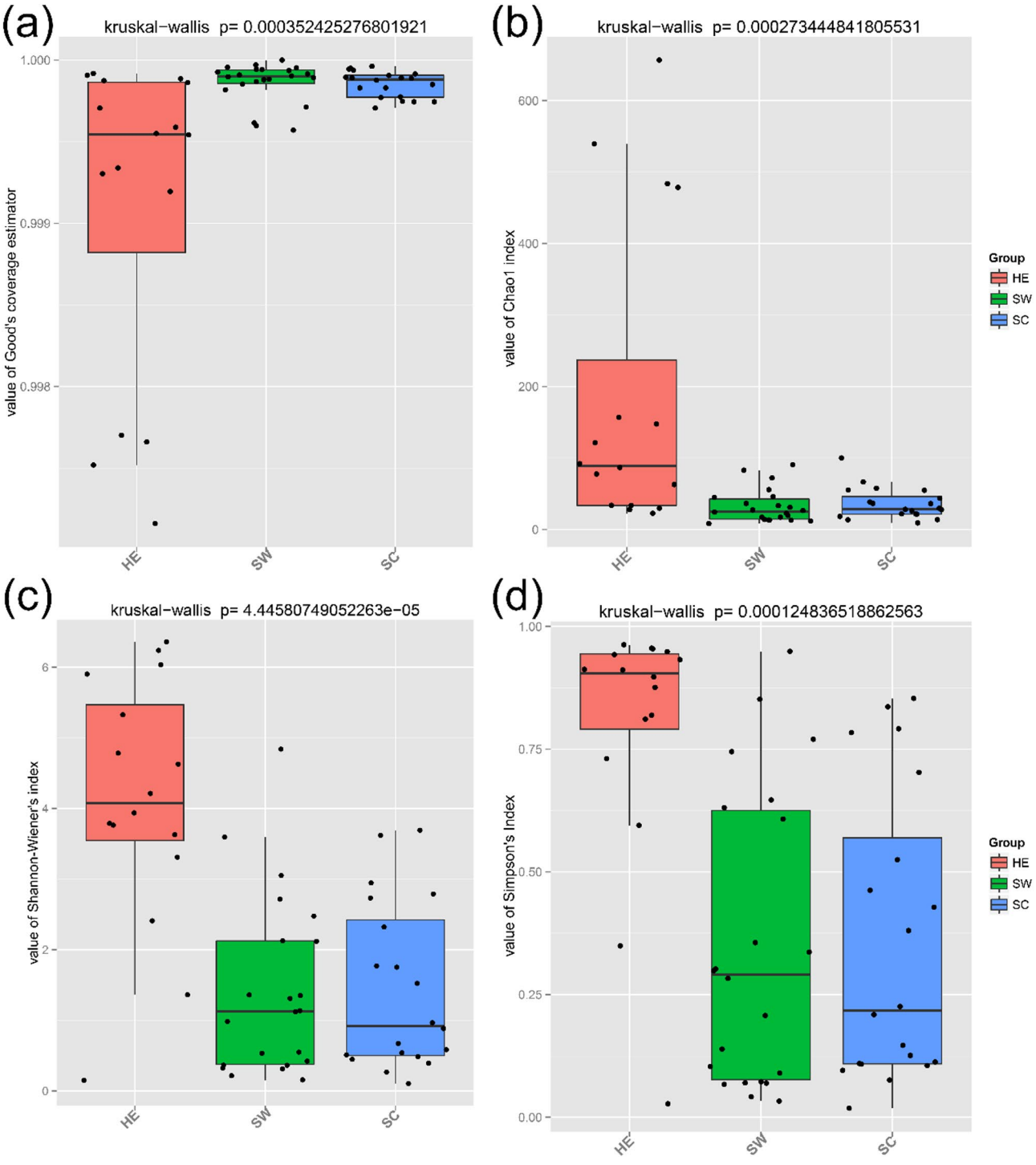

Fig. 2 The four kinds of alpha diversities of each group. (**a**) Good's coverage estimator reflects whether the sequencing depth has basically covered all species in a sample. The closer the estimator is to 1, the greater the sequencing depth. **(b)** The Chao1 index is used to estimate the number of OTUs present in the community. **(c)** The Shannon index, an estimator of species richness and species evenness, is more sensitive to species richness than evenness. **(d)** Simpson's index (which refers to Simpson's index of diversity, namely, 1-D), an estimator of species richness and species evenness, is more sensitive to evenness than species richness. In summary, when fungal keratitis occurs, the estimated actual number of OTUs, species richness and evenness of the ocular microbiota decrease (HE: conjunctival swabs of healthy eyes; SW: conjunctival swabs of eyes with fungal keratitis; SC: corneal scrapings of eyes with fungal keratitis)

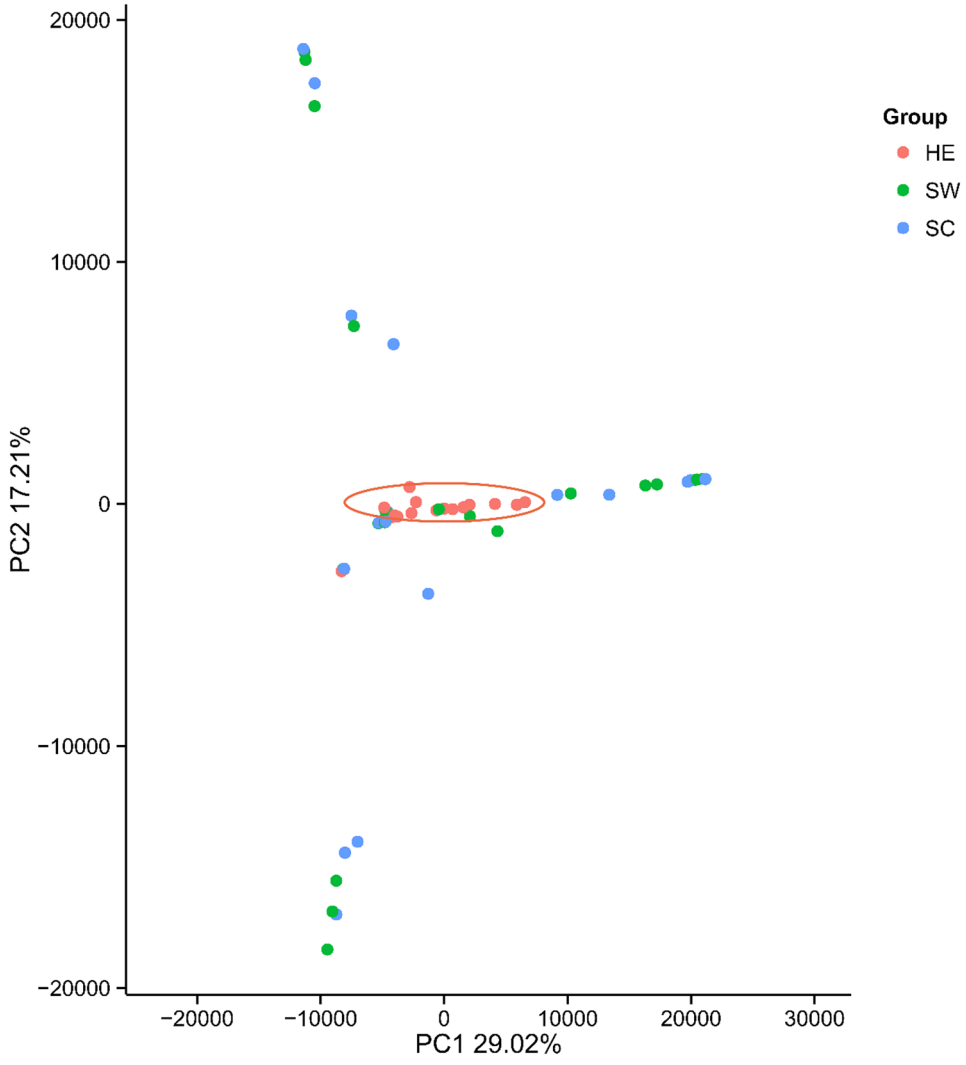

Fig. 3 The beta diversity of each group. The PCoA plot shows that the HE (conjunctival swabs of healthy eyes) group is significantly different from the SW (conjunctival swabs of eyes with fungal keratitis) group and the SC (corneal scrapings of eyes with fungal keratitis) group, while the latter two groups have considerable overlap

known for producing secondary metabolites, which play crucial roles in the initial destruction of complex organic compounds [32]. These results suggest that early and aggressive debridement may improve the outcome of FK.

Interaction network of ocular fungal microbiota

Figure 7 shows a predicted interaction network of the ocular fungal microbiota at the genus level. This interaction network was constructed on the basis of the Spearman correlation coefficient ($P < 0.05$) of the top 50 fungal genera among various fungal genera. A pairwise and multigenera interaction network was finally obtained. In our study, the core phyla of the fungal microbiome interaction network structure were identified as *Ascomycota* and *Basidiomycota*. Prashanthi et al. [32] reported that the abundance of fungal phyla on the ocular surface of fungal keratitis patients differed from that of healthy individuals, with an increase in *Ascomycota* or a decrease in *Basidiomycota*. These findings further highlight the pivotal role of these two phyla in the ocular fungal community.

Discussion

Corneal trauma is a chief risk factor for FK. However, many FK patients do not have a history of trauma [12]. Conversely, corneal trauma can lead to FK, bacterial keratitis, or Acanthamoeba keratitis [33]. This led Elisabeth Karsten et al. [18] to posit that FK occurrence may, to some extent, be related to imbalances in the ocular microbial community. Therefore, uncovering the pathophysiological mechanisms of FK, particularly from a dysbiosis perspective, is critically important. Our team previously characterized the normal fungal microbiota on the healthy ocular surface [34] and noted alterations in the ocular bacterial microbiota between healthy eyes

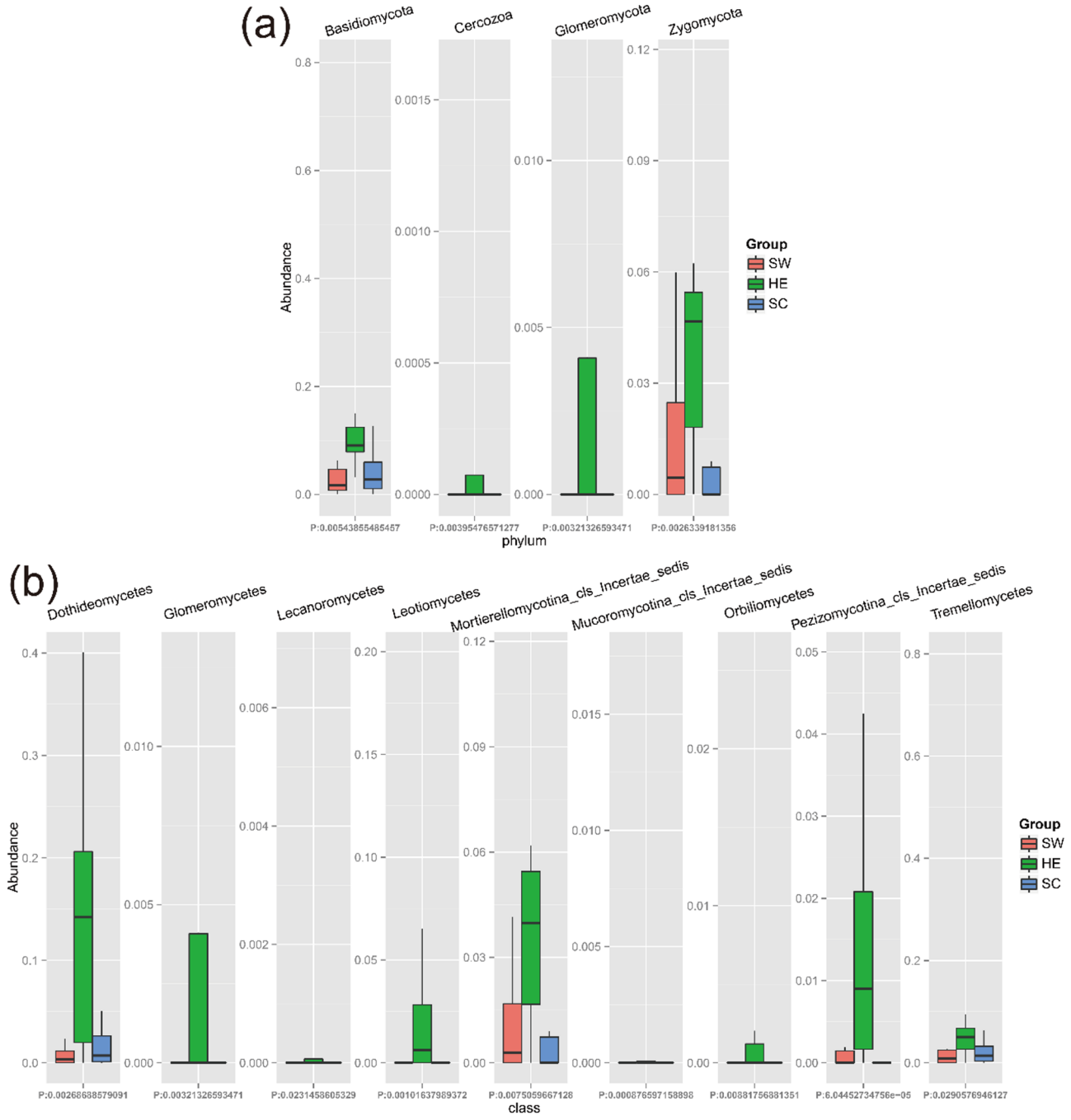

Fig. 4 Component differences in fungal microbiota at the phylum (**a**) and class (**b**) levels among various groups. Different colours represent different groups, and the ordinate represents the relative abundance

and those affected by FK [12]. Consequently, this study aimed primarily to reveal the dysbiosis of ocular fungal microbiota associated with FK.

Commensal microorganisms can protect humans against a trail of sickness, such as by driving an interleukin-17 response from mucosal γδ T cells [10] and increasing the concentrations of immune effectors in the tear film [35]. However, commensal dysbiosis has also been proven to lead to a series of diseases, such as Sjogren-like lacrimal keratoconjunctivitis and meibomian gland dysfunction [11, 36, 37]. In a randomized controlled trial, 0.02% chlorhexidine reduced the preoperative bacterial load more effectively than did 0.6% povidone-iodine, with fewer symptoms [38]. In patients with anophthalmic sockets, taxonomic differences were observed compared with those in contralateral eyes

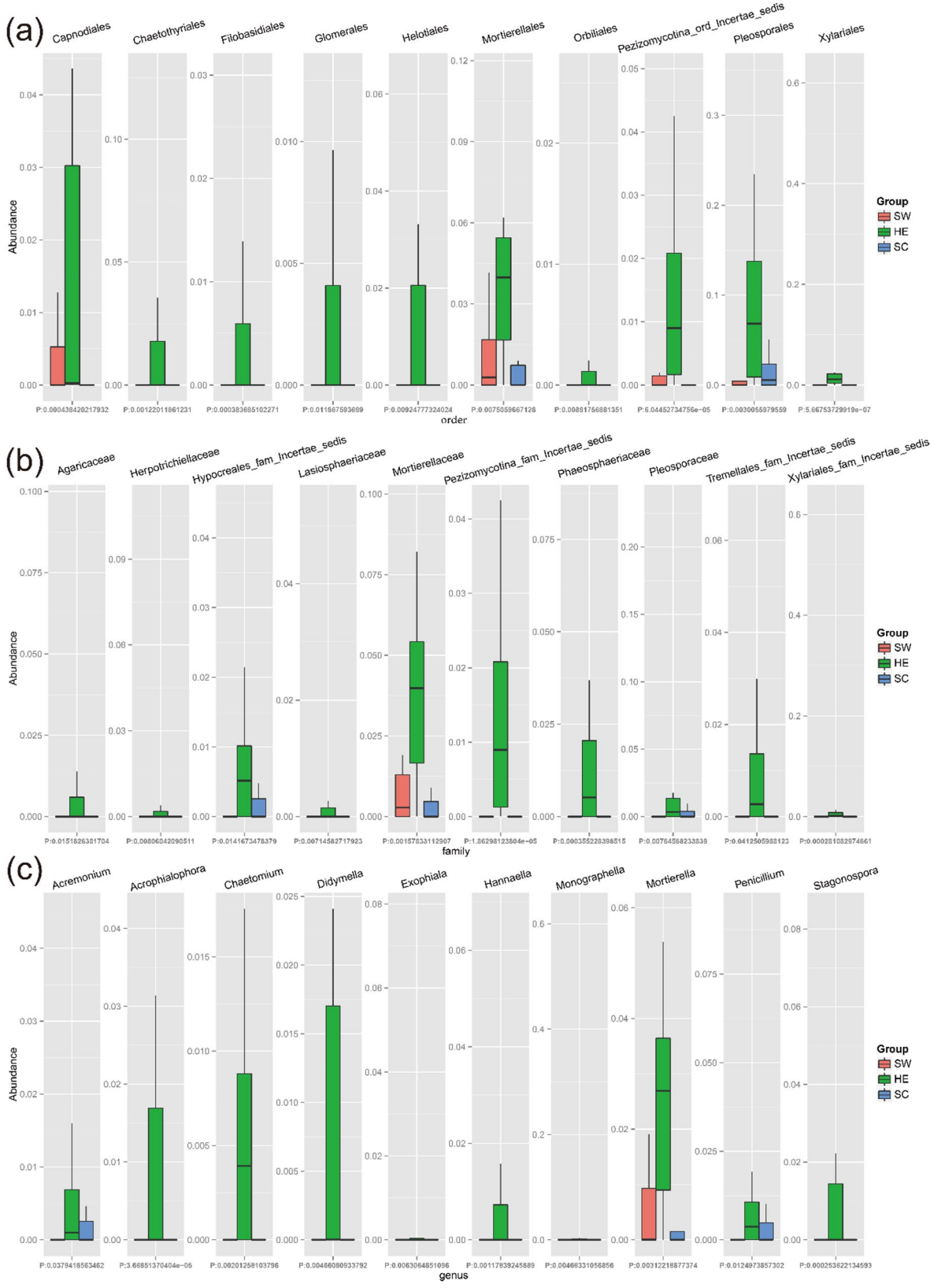

Fig. 5 Component differences in fungal microbiota at the order (**a**), family (**b**), and genus (**c**) levels among various groups. Different colours represent different groups, and the ordinate represents the relative abundance

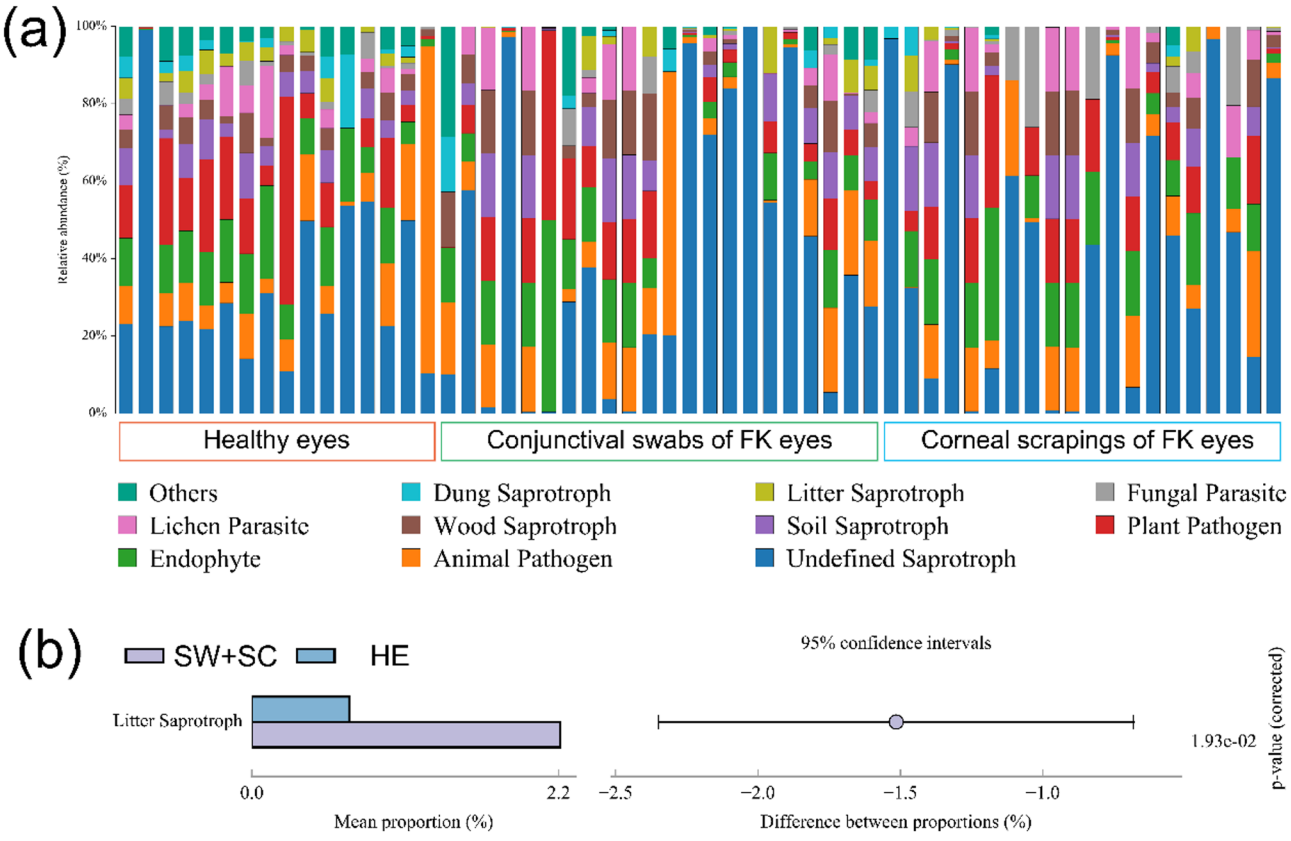

Fig. 6 Fungal composition of all the samples according to functional group. (**a**) Functional group refers to an approach to classify species according to their different ways of absorbing and utilizing environmental resources rather than their evolutionary relationships. Each bar represents a sample, various colour portions represent different functional groups, and the length of a coloured portion represents the relative content of a functional group. **(b)** The fungal microbiota of healthy eyes and eyes with fungal keratitis were significantly different for litter saprotrophs, rather than animal pathogens (HE: conjunctival swabs of healthy eyes; SW: conjunctival swabs of eyes with fungal keratitis; SC: corneal scrapings of eyes with fungal keratitis)

despite similar alpha diversity, indicating a structural impact on microbial composition [39]. Similarly, patients with keratoconus exhibit distinct ocular surface microbial signatures compared to healthy controls, suggesting that disease-specific alterations may influence microbiota structure and function [40]. Host factors such as nationality, lifestyle, and eyeglass use are also associated with microbial variation, underscoring the need to adjust for nondisease-related confounders in microbiome research [41]. The potential influence of systemic antibiotic therapies on ocular surface health warrants attention. In addition to its well-established antimicrobial and anti-inflammatory effects, doxycycline has been shown to modulate host immune responses and inhibit pathogen replication through mechanisms such as interferon pathway activation. While beneficial for controlling infection and inflammation, the impact of systemic antibiotics on the ocular surface microbiota should be carefully considered in future therapeutic strategies [42].

Compared with the bacterial microbiome, the ocular fungal microbiome has rarely been studied. This study revealed that the ocular fungal microbiome presented unique alterations in fungal corneal infections. When FK occurs, alpha diversity, the estimated actual number of OTUs, species richness and evenness of the ocular microbiota decrease. In addition, beta diversity was significantly different between healthy eyes and eyes with FK. Elisabeth Karsten et al. [43] summarized all articles that aimed to identify microorganisms related to keratitis between 1950 and 2012 and reported that 92 fungal genera seemed to be involved in microbial keratitis. Owing to the superiority of high-throughput ITS sequencing, 246 different genera that may be implicated in FK were annotated in our study, and our results are consistent with their findings.

The comparison of the fungal microbiota at the genus level showed that 55 genera were significantly different between healthy eyes and eyes with FK. After the fungi were clustered according to their different ways of absorbing and utilizing environmental resources, in comparison to healthy eyes, the fungal microbiota of eyes with fungal keratitis were found to be significantly enriched in fungi that are classified as litter saprotrophs. Therefore, fungi that act as litter saprotrophs may play

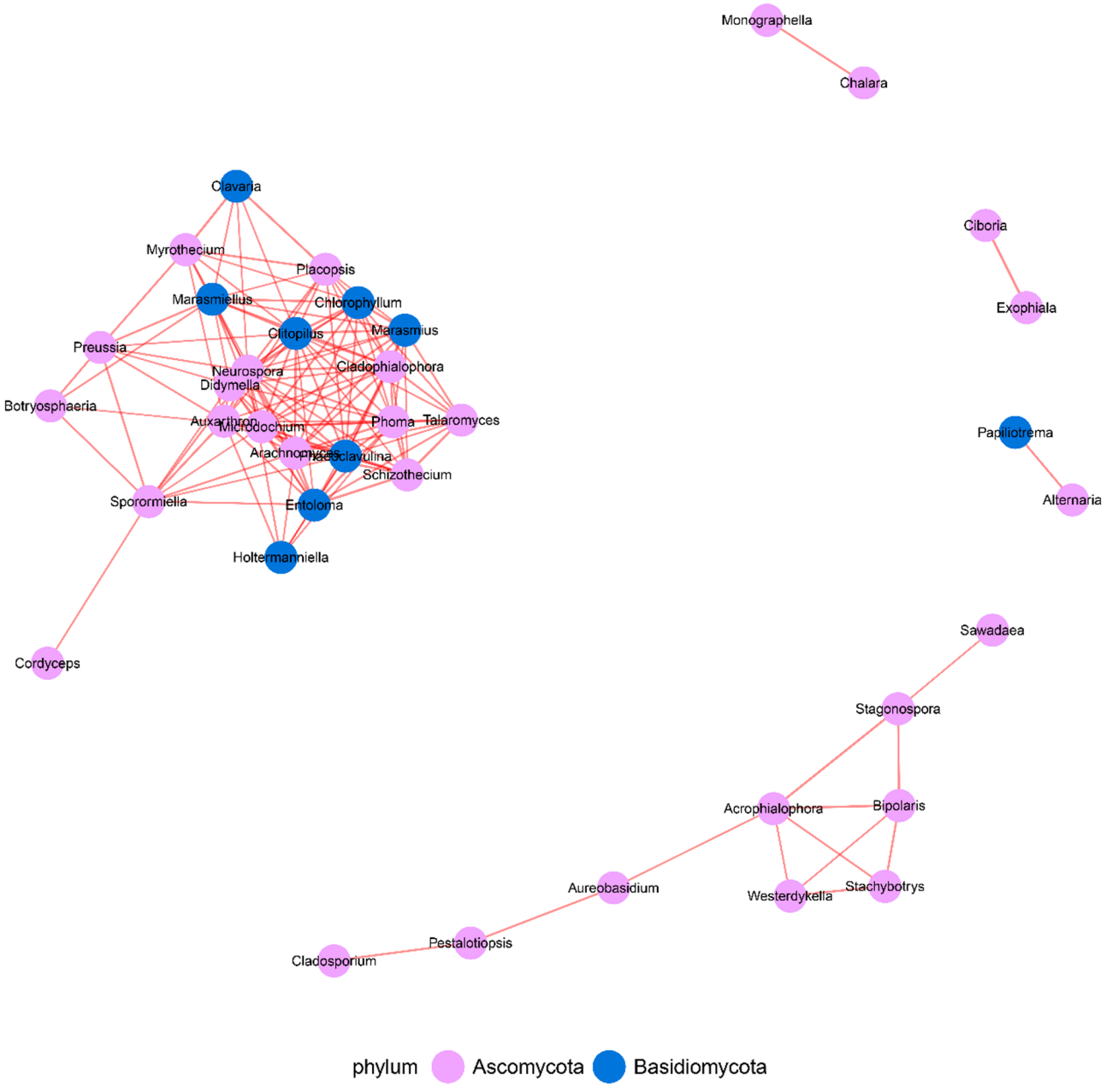

Fig. 7 Predicted interaction network of ocular fungal microbiota at the genus level. This interaction network was constructed on the basis of the Spearman correlation coefficient ($P < 0.05$) of the top 50 fungal genera. The red line indicates a positive correlation

important roles in the pathogenesis of FK. The functional category of litter saprotrophs primarily involves the degradation of dead host cells to obtain nutrients in natural environments. A significantly decreased biomass of litter saprotrophic fungi often occurs after litter inundation, and fungal production may also vary depending on the quality of plant litter (such as lignin and nutrient concentrations) [44]. The alteration of litter saprotrophic fungi could be related to a decrease in carbon sources and other nutrients following alterations in the ocular environment after fungal keratitis infection. Although their specific role on the ocular surface remains unknown, these results suggest that early and aggressive debridement may improve the outcome of FK. This has also been reported by Peter Zloty et al. [45] in a prospective randomized clinical trial. Our study substantially contributes to the understanding of FK by revealing the underlying mechanism involved.

The ocular microbiome engages in a series of interactions, such as commensalism, cometabolism, synergism, mutualism, competition, and amensalism. Thus, the differences among these fungi may imply complicated

biological connections that cannot be ignored. On the basis of the co-occurrence and coexclusion relationships among various fungal genera, an interaction network was used to assess the interactions of fungal genera in the ocular microbial community. This interaction network provided pairwise interactions and multigenera interactions, whereas Gumpili Sai Prashanthi et al. [18] mainly obtained higher-order interactions. The network developed in this study has not been fully verified in vitro, but it may still help to avoid blind investigations. In addition, which of the pairwise and higher-order interactions comprise the major dynamics of the ocular microbiome also needs further study. For gut microbiome communities, pairwise interactions are major drivers of multigenera community dynamics rather than higher-order interactions [46]. Our results differ from those of a previous study by Gumpili Sai Prashanthi et al. [18], who reported that at the phylum level, the abundance of *Ascomycota* and *Basidiomycota* significantly differed among the three groups, while our study showed that at the phylum level, the abundance of *Basidiomycota*, *Cercozoa*, *Glomeromycota* and *Zygomycota* significantly differed among the three groups [18]. The difference between our study and previous studies is due to the different control groups. They used samples from healthy people as the control group, whereas we used the healthy eyes of single-eye FK patients as the control group. Owing to the same degree of environmental stress, the composition of the microbiota does not differ between the right and left eyes [31]. Therefore, our study provides a stricter controlled trial (to some extent, a self-controlled study) to reveal alterations in the ocular microbiome caused by FK, as irrelevant factors that may influence the ocular microbiota were exactly offset.

This study has several limitations that should be acknowledged. First, there is a lack of sufficient and directly comparable literature on the ocular surface fungal microbiota, particularly studies utilizing ITS-based sequencing in similar clinical settings. This limits the extent to which our findings can be contextualized within existing research and constrains comparative interpretation. Second, the absence of standardized reference databases for ocular fungal communities hinders species-level annotation and reduces the cross-study reproducibility of results. Finally, given the current scarcity of large-scale, multicentre fungal microbiome datasets, the generalizability of our findings remains limited. These constraints underscore the need for broader collaborative efforts and methodological standardization in future studies to validate and expand upon the present results.

On the basis of the findings of this study and recent developments in ocular surface microbiome research, future strategies to prevent FK should emphasize microbiome-informed approaches. Integrating ITS-based fungal profiling with clinical risk assessment could help identify individuals at elevated risk owing to fungal dysbiosis or early microbial shifts. Longitudinal monitoring of ocular fungal communities, particularly in high-risk settings, may enable early detection of dysbiosis preceding clinical infection. Furthermore, microbiota-preserving antifungal strategies and targeted prophylactic interventions could support ocular surface ecological stability. Building on these insights, future research will focus on screening and developing specialized probiotic therapies aimed at restoring microbial balance and enhancing resistance to FK.

In conclusion, this study identified alterations in the diversity and abundance of the fungal microbiome between the healthy and infected eyes of single-eye FK patients. Significant alterations were observed from the phylum to the genus level. After clustering the fungi according to their different ways of absorbing and utilizing environmental resources, the fungal microbiota of healthy eyes was found to possess more fungi that belong to the category of litter saprotrophs, suggesting that early and aggressive debridement may improve the outcome of FK. In addition, this study revealed a reciprocal interaction network of ocular fungal microbiota. These findings will facilitate further comprehension of the pathophysiologic mechanism of FK from the perspective of dysbacteriosis.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12886-025-04133-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Author contributions

X.Z. contributed to the literature search and data analysis. Z.R. contributed to the design of this research, data analysis, and drafting of the manuscript (co-first author). W.L. contributed to the design of this research and revision of the manuscript. Q.L. contributed to sample collection and patient management. Y.D. contributed to clinical diagnosis and treatment. Y.H. contributed to the design of this research, literature search, data analysis, and funding support.

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Data availability
The authors declare that the main data supporting the results in this study are available within the paper and its Supplementary Information. All gene sequences will be deposited in the National Center for Biotechnology Information (NCBI) prior to the publication of this article, and the data will subsequently be made available in the database to facilitate further global research.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Shandong Eye Institute (Approval No. 2019-26) and registered on the Chinese Clinical Trial Registry (Registration No. ChiCTR1900023720) on June 8, 2019. The procedure was entirely interpreted, and informed consent was acquired from all patients. All procedures abided by the tenets of the Declaration of Helsinki.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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