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Parathyridaria percutanea, *Subramaniula asteroides*, and *Albifimbria verrucaria* as Uncommon Causes of Keratitis in Mexican Patients: A Challenge in Their Identification and Treatment

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Abstract

Fungal keratitis is most frequently associated with ocular trauma. Predisposing factors have also been observed in many cases. Treatment outcomes are not always successful owing to antifungal drug resistance or other patient conditions. *Aspergillus* and *Fusarium* are the most frequent fungal genera causing keratitis. However, there are many other uncommon genera or species that are difficult to identify by morphology or to select an effective treatment without *in vitro* antifungal drug susceptibility testing. One, two, and three cases of *Parathyridaria percutanea*, *Subramaniula asteroides*, and *Albifimbria verrucaria*, respectively, have been reported in the literature. Here, we report one case each diagnosed in Mexican patients by PCR-sequencing, with variable treatment results.

Keywords

Fungal Keratitis, *Parathyridaria*, *Subramaniula*, *Albifimbria*, Corneal Ulcers

1. Introduction

Owing to the availability of molecular tools worldwide, reports of infections caused

by uncommon fungi are increasing daily. *Parathyridaria* is a fungus belonging to the family Thyridaceae and the order Pleosporales [1]. This microorganism is an environmental phytopathogen; however, under risky conditions, it can cause human infections. It is associated with subcutaneous infections in patients undergoing renal transplantation [2] [3]. Yadav *et al.* [4] reported a human endophthalmitis case. *Subramaniula asteroides* is an ascomycete belonging to the Chaetomiaceae family. It has been described as a potentially opportunistic fungus that causes traumatic eye infections such as corneal ulcers [5]. *Albifimbria verrucaria* is a saprophytic fungus belonging to the family Stachybotryaceae. It is a ubiquitous plant pathogenic organism that exhibits bioherbicidal, nematicidal, and antimalarial activities [6].

In cases of uncommon fungi causing human infections, morphological studies are not sufficient to identify the fungal genera or species. Here, we report three cases of keratitis caused by uncommon fungi, the identities of which were confirmed by PCR-sequencing. The Institutional Ethics Committee (Hospital Asociacion para Evitar la Ceguera en Mexico "Dr. Sanchez Bulnes IAP") determined that the presentation of three cases does not constitute research involving human subjects and does not require review by the Committee itself.

2. Clinical Cases

The patients described here were treated at the Cornea Service (Hospital Asociacion para Evitar la Ceguera en Mexico) at different times: Case 1 in July 2017, Case 2 in November 2024, and Case 3 in August 2025. The period for identifying the fungal species was one month.

Case 1

A fifty-nine-year-old man from Guerrero, Mexico, was dedicated to agricultural activities. The patient had a history of recent ocular trauma to the left eye with vegetative matter (day 0). One month later (day 30), the patient came for consultation (Cornea Service, Hospital Asociacion para Evitar la Ceguera en Mexico) because of acute pain in his left eye and decreased visual acuity. On slit-lamp examination, the patient showed conjunctivitis, central opacity, and a 2.2 × 2.6 mm central ulcer, and a 1 mm hypopyon (**Figure 1(A)**). A sample of the lesion was obtained by corneal scraping for smearing and culturing. Medical treatment consisted of 5% natamycin drops every 2 h for 3 days and oral itraconazole 100 mg every 12 h for 15 days. A week later (day 37), good improvement was observed; the patient had no hypopyon and no pain in his eye. Slit-lamp examination and ocular echography ruled out endophthalmitis. The patient was lost to follow-up because he did not return for his medical appointments (day 44).

The PAS-stained smear showed pigmented hyphae with an irregular diameter (**Figure 1(B)**). After five days, the culture on blood agar and chocolate agar showed many white and downy colonies, which became brown, with a cerebriform aspect, and a raised center with age (**Figure 1(C)**). Microscopic examination of the colony revealed septate brown hyphae without any conidial structures. The fungus was grown on several media (**Figures 1(D)-(F)**): oatmeal agar (OA), Borelli agar (BoA),

malt extract agar (MEA), V8 agar, Sabouraud dextrose agar (SDA), SDA with antibiotics, and potato dextrose agar (PDA), all incubated at 28˚C for 1 month, but periodically examined by microscopy. After 3 weeks, only on PDA several pigmented globose pycnidia (Figure 1(G)) with a maximum size of 320 µm (Figure 1(H)) were observed, forming numerous ellipsoidal conidia of average size 4 × 2 µm (Figure 1(I)).

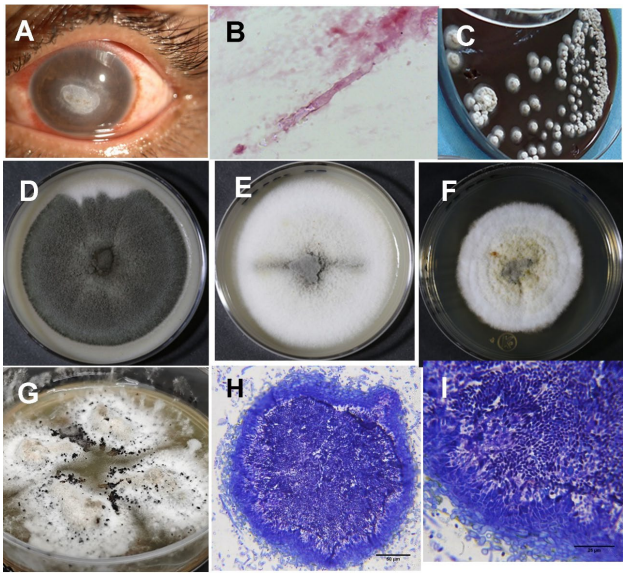

Figure 1. Clinical aspect and laboratory studies of a keratitis case caused by *Parathyridaria percutanea*. (A) Conjunctivitis and corneal ulcer. (B) PAS-stained smear showing irregular diameter and pigmented hypha. (C) Primary growth of numerous grayish colonies on blood agar after 10 days of incubation at 28˚C. (D) - (F) Growth on OA, BoA, and SDA, respectively. (G) Culture on PDA after six weeks, showing numerous pycnidia (black structures). (H) and (I) Transversal cut of a blue toluidine-stained, globose pycnidium. Bar scale: E: 50 µm; F: 25 µm.

Case 2

Eighty-two-year-old male from Guerrero, Mexico. The patient reported that three months ago, while cutting wood, sawdust fell into his left eye (day 0), causing progressive vision loss. Eight days prior (day 83), due to acute pain, the patient sought a private consultation, and topical 0.3% netilmycin and 5% natamycin suspensions were prescribed without a good response. During examination at our Cornea Service (day 90), his visual acuity was limited to hand movements; hyperemic conjunctiva with vascularization around the corneal limbus in 360 degrees, a central ulcer with high-risk perforation, and satellite lesions were observed (Figure 2(A)). At different times, two samples obtained by corneal scraping were subjected to microscopic examination (both negative) and culture. The first culture showed only a few bacterial colonies identified by Vitek 2 (BioMerieux, France) as *Gemella morbillorum* and without fungal growth. Therefore, 0.5% moxifloxacin, 0.3% netira, and 5% natamycin drops (due to a history of trauma) were prescribed. After four days, the second culture showed two filamentous and pigmented

colonies on SDA, microscopically showing hyphae and several brown hyphal clusters without visible reproductive structures. After 30 days of treatment, the patient did not respond well; therefore, tectonic keratoplasty was performed (day 120). PAS-stained corneal tissue showed abundant hyphae and vesiculous structures in the middle of a wide leukocyte infiltration zone (Figure 2(B)). After corneal surgery, medical treatment consisted of 1% voriconazole, one drop hourly, oral itraconazole 100 mg every 12 h, and 0.3% moxifloxacin every two hours. After 30 days of the surgical procedure, one stitch was lost, and it was removed (day 150). The ocular pressure was 8 mmHg. An anterior chamber narrow, opaque lens, and synechiae between the endothelium and iris were also observed (Figure 2(C)). Patient response to antifungal drugs was assessed as a poor prognosis. He is currently under observation.

Isolate was grown on different media and at temperatures 30, 35, and 40˚C, for four weeks (Figures 2(D)-(F)). All three temperatures promoted good growth, with 40˚C being the optimal temperature. The microscopic structures are shown in Figure 2(G). After two weeks, only EMA-scarce phialides producing small, ovoid, and hyaline phialoconidia (Figure 2(H), Figure 2(I)) were observed.

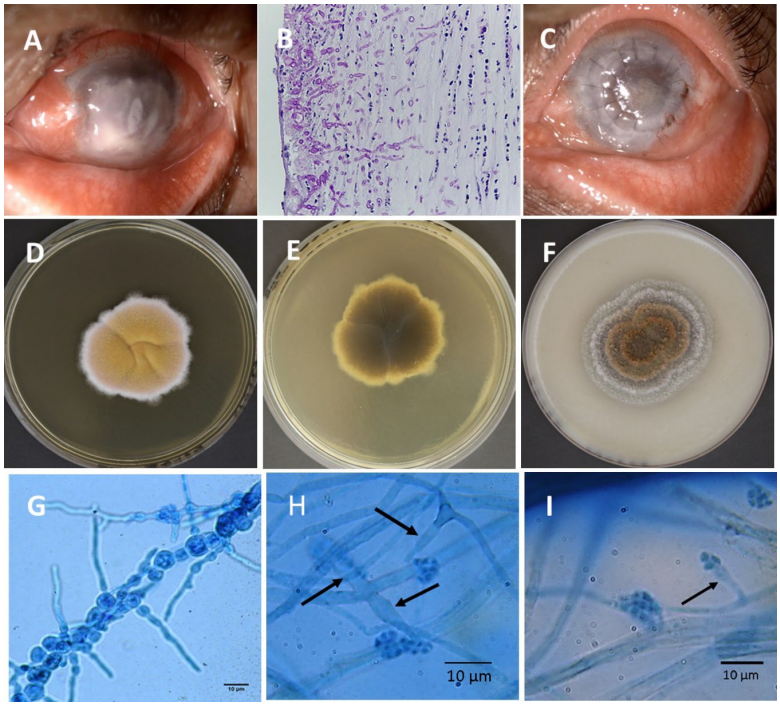

Figure 2. Clinical aspect and laboratory studies of a keratitis case caused by *Subramaniula asteroides*. (A) Keratitis consistent with a hyperemic conjunctiva and central ulcer. (B) Histopathological study of PAS-stained corneal tissue after tectonic keratoplasty, showing numerous hyphae and vesiculous structures. (C) Clinical aspect 30 days after surgery, showing a clear lack of response to antifungal treatment. (D) and (E) Anverse and reverse of culture on MEA, respectively. (F) Anverse of culture on OA. All cultures were grown for seven days at 40˚C. (G) Microscopic morphology observed on MEA (14 days at 40˚C), showing chlamydospore-like structures (100×). (H) and (I) Phialidic structures (arrows) with small ovoid or triangular conidia (1 × 2 µm) (100×). Bar scale: 10 µm.

Case 3

Male, 59 years old, from Guerrero, Mexico, presented with ocular trauma to the left eye (day 0) while drilling. Subsequently, the patient had low visual acuity. A general physician (day 7) indicated 0.5% moxifloxacin, 0.3% netilmicin drops, and ketoconazole, without specifying the dose and administration route. In our Cornea Service (day 15), a central corneal ulcer of 1.7 mm, with peripheral edema of 7.7 mm over the visual axis, and an inflammatory membrane over the pupil were observed (**Figure 3(A)**). The patient's visual acuity was 10/100. Corneal scraping for microscopic observation and culturing was performed. In a PAS-stained smear, one septate hyphal cell was observed (**Figure 3(B)**). For the medical treatment, 1% voriconazole drops every 2 h for 2 days (day and night), and then every 3 h for 21 days, were prescribed. After 5 days of incubation, a cottony white-grey colony appeared on the SDA. After 21 days of treatment (day 36), diffuse leukoma with no signs of active infectious infiltrative keratitis, a thin paracentral inferior zone, a persistent inflammatory membrane, a well-defined anterior chamber without inflammatory cells, and a refractive iris were observed. These data were defined as improvements (**Figure 3(C)**) and topical voriconazole was continued for 21 days. The patient did not attend the next consultation (day 57) and was lost to follow-up.

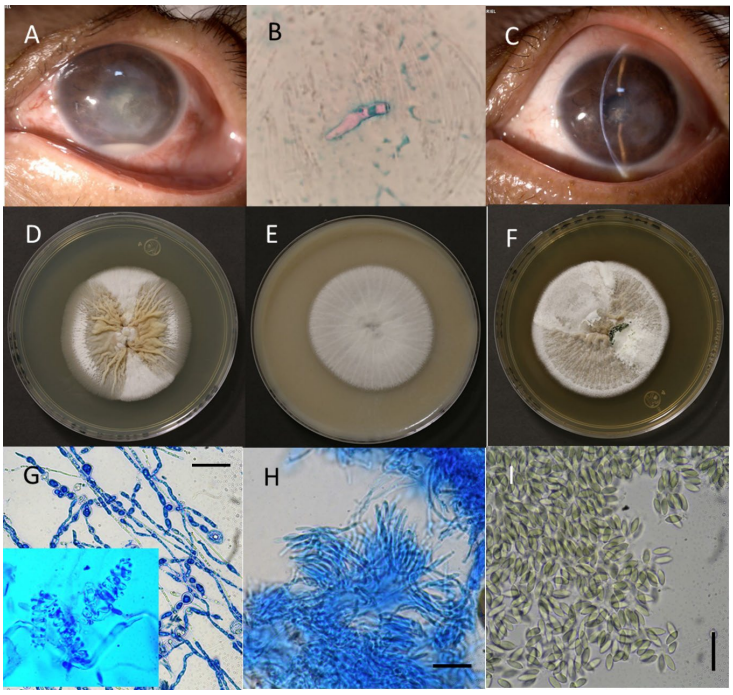

Figure 3. Clinical aspect and laboratory studies of a patient infected with *Albifimbria verrucaria*. (A) Ocular lesion consisting of peripheral edema and an inflammatory membrane over the pupil. (B) PAS-stained smear showing a fragment of hypha. (C) Ocular aspect showing clinical improvement. (D) - (F) Culture on SDA, OA, and MEA, respectively. (G) Microscopic examination of culture showing vesiculous hyphae. Insert: Spiral hyphae. (H) Sporodochia. (I) Abundant pigmented fusiform conidia. (G) - (I) Scale bars: 10 μm.

For the morphological analysis, the isolated fungus was processed as described in Case 1 (**Figure 3(D)**, **Figure 3(E)**). On the colonies grown on the EMA, small black points, suggesting reproductive structures, were observed (**Figure 3(F)**). Microscopic examination revealed vesiculous and curling hyphae (**Figure 3(G)**). Black points corresponded to sporodochia (**Figure 3(H)**), which produce abundant one-celled fusiform conidia (**Figure 3(I)**).

For the molecular identification of the three isolates, DNA extraction was performed using the ExgeneTM Plant SV Mini Kit (GeneAll Biotechnology Co., Ltd., Seoul, South Korea). PCR was performed, testing different targeting regions (internal transcribed spacer (ITS), calmodulin, D1/D2 domain, translation elongation factor 1 alpha gene, beta-tubulin gene) and to select the best sequence. The amplicons were purified using the DNA Clean & ConcentratorTM-5 kit (Zymo Research, CA, USA) and sent for sequencing in both forward and reverse directions to Instituto de Fisiologia Celular, UNAM. Sequences were compared with GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and MycoBank (https://www.mycobank.org) databases. These isolates were conserved in the Mycology Unit Collection under numbers 29985 (*P. percutanea*), 38930 (*S. asteroides*), and 39582 (*A. verrucaria*).

For isolate 1, Briefly, the PCR conditions targeting the ITS region were: primers: ITS1 5'-TCC GTA GGT GAA CCT GCG G-3' ITS4: TCC TCC GCT TAT TGA TAT GC-3'; thermal cycling: denaturation 96˚C, 5 min; 25 cycles of 95˚C, 30 s, 59˚C, 30 s, 72˚C, 30 s; final extension 72˚C, 5 min [7]. The size of the amplicon was approximately 650 bp. Sequence analysis of 523 nucleotides revealed 100% identity with *Parathyridaria percutanea* (LT796903.1; SH0984947.09), and 98% query cover. The sequence was registered in the GenBank database with the accession number PV425920.

For isolate 2, the D1/D2 domain [8] was the best-targeting region. The PCR conditions were: primers: Fw 5'-CAT ATC AAT AAG CGG AGC AAA AG-3'/Rev 5'-GCT CCG TGT TTC AAG ACG-3'; thermal cycling: denaturation 94˚C, 5 min; 35 cycles of 94˚C, 1 min; 63.3˚C, 1 min, and 72˚C, 1 min; final extension, 72˚C, 5 min, resulting in an amplicon of approximately 700 bp. The sequence of 410 nucleotides corresponded to *Subramaniula asteroides* with 100% identity (NG_069273.1; CBS128685), and 100% query cover. The sequence was registered in the GenBank database with the accession number PV425923.

For the molecular identification of isolate 3, the PCR targeting the D1/D2 domain showed the best performance. Primers, PCR conditions, and product size were the same as those for isolate 2. The sequence analysis of 536 nucleotides corresponded to *Albifimbria verrucaria*, 100% identity (KU845907.1; SH1878998.10FUMF826684) and 100% query cover. The sequence was registered in the GenBank database with the accession number PZ514106.

The three isolates were subjected to susceptibility testing for antifungal drugs (amphotericin B (AMB), caspofungin (CAS), fluconazole (FLU), isavuconazole (ISA), itraconazole (ITR), posaconazole (POS), voriconazole (VRC), and cyclo-

piroxolamine (CPO)) using the broth microdilution method [9] [10], with variable results for each isolate (Table 1). Considering that there are no cutoff points for these fungi, we can mention that high MICs were observed: *P. percutanea* to FLU, CPO, and CAS; *S. asteroides* to FLU and AMB; and *A. verrucaria* showed only low MICs to POS and ISA.

Table 1. Minimal inhibitory concentrations (μg/mL) found in etiological agents of fungal keratitis from Mexican patients.

Antifungal drug	*Parathyridaria percutanea* (MIC: μg/mL)	*Subramaniula asteroides* (MIC: μg/mL)	*Albifimbria verrucaria* (MIC: μg/mL)
Fluconazole	**16.0**	**>256**	**64**
Itraconazole	0.5	0.19	**4.0**
Voriconazole	0.25	0.19	**2.0**
Posaconazole	0.06	0.5	0.5
Isavuconazole	0.03	0.094	1.0
Ciclopiroxolamine	**4.0**	-	**4.0**
Caspofungin	**8.0**	-	**8.0**
Anfotericin B	0.5	**2.0**	**2.0**
Terbinafine	1.0	-	**4.0**
Ketoconazole	-	0.25	-

In bolds, high MICs.

3. Discussion

In 2014, Ahmed *et al*. [11] reported a novel agent of subcutaneous mycosis different from known species, and after phylogenetic analysis, the authors introduced a new taxon named *Roussoella percutanea*. In 2016, Jaklitsch and Voglmayr [1] applied a multigene analysis of sequences from isolates with Thyridaria-like morphology, and based on the results, proposed *Parathyridaria* as a new genus, therefore emerging *P. percutanea*. On oatmeal agar, this species forms a floccose, dark greyish colony. The hyphae are initially hyaline and turn dark with age. After 8 weeks, black, solitary, globose to subglobose pycnidia (59 - 102 × 54 - 96 μm) are observed. Hyaline to pale brown, unicellular, and ellipsoid conidia are formed from hyaline phialides [11]. Recently, several authors have reported cases of subcutaneous human infection caused by *P. percutanea*, mainly associated with renal transplantation [12]-[14]. To our knowledge, only one case of ocular infection (endophthalmitis) caused by this fungus has been reported [4], which showed a remarkable improvement with VRC after three months of treatment. Our patient showed improvement after one week of treatment with ITR and natamycin, but was lost to follow-up.

The genus *Subramaniula* was first described in 1985, and its morphology was

detailed in 1986 [15], highlighting the presence of ascospores. Because some phenotypic characteristics are variable and phylogenetic studies are unstable, this fungus has been misidentified as *Papulospora* sp. or *Chaetomium* sp. [5]. In contrast to Cannon's description, in the *S. asteroides* morphological study, Ahmed *et al*. [16] did not find ascomata but detected phialidic conidiophores with small ovoidal conidia and thick-walled chlamydospore-like structures. This finding is consistent with our results. On MEA, *S. asteroides* forms radially folded yellow-green colonies that become dark greyish with age and reverse dark grey [16]. Although this work included six isolates obtained from eye infection, the authors described a case of keratitis due to *S. asteroides*, treated with AMB and FLU, and recovery of ocular function. More recently, a clinical case of human keratitis caused by *S. asteroides* was reported [17], and the authors considered ISA to be the best antifungal drug for *S. asteroides* keratitis. Searching for the etiology of infectious keratitis in Malawi, Kalua *et al*. [18] found a case caused by *S. asteroides*. In a recent systematic review, Gautam *et al*. [19] reported a case of mycotic keratitis caused by *S. asteroides*, which responded well to ISA; additionally, they reported four cases of *Myrothecium* and two cases of *Roussoella*.

In our case, the patient infected with *S. asteroides* was initially treated with natamycin. Poor evolution induced the performance of a keratoplasty, after confirming the fungal etiology. In spite of additional treatment with VRC and ITR, which showed very low *in vitro* MICs, the clinical outcome was very poor. We believe that three months without medical attention, with a corneal compromise of 90%, and the pathogenic association of *G. morbillorum* with the infectious process were decisive in this unfortunate outcome. This gram-positive facultative anaerobic coccus frequently behaves as a commensal for some human endothelial tissues. However, several cases of infectious diseases, mainly endocarditis, caused by this bacterium have been reported. Severe cases increase the risk of death [20].

Albifimbria verrucaria (formerly *Myrothecium verrucaria*) is a highly attractive organism owing to its wide diversity in the production of biochemical compounds used in medicine, agriculture, and the development of new energy technologies [6] [21]. On PDA, it grew as a floccose white to rosy buff colony. Abundant conidia formed from pale olivaceous to black sporodochia are observed. Marginal hyphae are curling, hyaline, septate, and usually verrucose. Conidiophores arise from the knots of basal hyphae-bearing phialides, which are closely packed in a dense layer. Conidia are broadly fusiform, with one end pointed and the other protruding and truncate (6.5 - 8 × 2 - 3.5 μm) [22].

Recently, *A. verrucaria* has emerged as an opportunistic human pathogen. According to the literature, Refojo *et al*. [23] found an isolate corresponding to *Myrothecium* in a prospective study to identify molds causing keratitis. Later, other keratitis cases caused by this species have been described [24]-[26], and they have in common the use of VRC as a treatment, with good results. **Table 2** summarizes cases of keratitis caused by the three species found in this study that have been reported in other countries.

Table 2. Known reported clinical cases of human keratomycosis caused by *Parathyridaria percutanea*, *Subramaniula asteroides*, or *Albifimbria verrucaria*.

Reference	Patient (age) and risk factors	Symptoms/ Lesions	Identification method	Fungi (organism) identified	Treatment	Outcome
Yadav *et al*., 2022 [4]	Male (31) Car tyre blast	Open globe injury in the eye.	Culture. PCR-Seq	*Parathyridaria percutanea*	VRC	Favorable at 3 months.
Vanzzini-Zago *et al*., 2026	Male (59) Trauma with vegetative matter	Pain, low visual acuity.	Smear. Culture. PCR-Seq	*Parathyridaria percutanea*	ITR NAT	Improvement. The patient was lost.
Cultrera *et al*., 2021 [17]	Male (65) None	Photophobia, ocular pain.	Culture. PCR-Seq	*Subramaniula asteroides*	VRC, ISA	Cured after 6 weeks.
Kalua *et al*., 2024 [18]	Unknown	Corneal ulcer.	RNA-Seq	*Subramaniula asteroides*	Unknown	Unknown.
Vanzzini-Zago *et al*., 2026	Male (82) Trauma with sawdust	Pain, low visual acuity.	Culture. Vitek 2. PCR-Seq	*Gemella morbillorum* *Subramaniula asteroides*	NAT, MFC NM, VRC, ITR Keratoplasty	No good response. Poor prognosis.
Rameshkumar *et al*., 2019 [24]	Male (68) None	Pain, redness, irritation, low visual acuity.	Smear. Culture. PCR-Seq	*Albifimbria verrucaria*	NAT ECZ	Total healing in one week. Scar formation.
Liu *et al*., 2021 [26]	Male (53) Trauma with ashes	Redness, irritation, pain.	Smear. Culture. PCR-Seq	*Myrothecium verrucaria*	NAT, VRC, FLU	Healing in 45 days.
Moreno-Flores *et al*., 2020 [25]	Male (61) Strage material Diabetes mellitus	Pain.	Culture. Keratoplasty. PCR-Seq	*Albifimbria verrucaria*	Antibacterials VRC, Keratoplasty	Good evolution at 8 months.
Vanzzini-Zago *et al*., 2026	Male (59) Trauma meanhile drilling	Low visual acuity.	Smear. Culture. PCR-Seq	*Albifimbria verrucaria*	NM, KTZ, VRC	Improvement after 21 days. Following up lost.

Abbreviations: DM: diabetes mellitus; AML: acute myeloid leukaemia; PCR-Seq: polymerase chain reaction followed by sequencing; PZ: posaconazole; VRC: voriconazole; ITZ: itraconazole; ISA: isavuconazole; FLU: fluconazole; NAT: natamycin; MFC: moxifloxacin; NM: netilmicin; ECZ: econazole; KTZ: ketoconazole.

In the last decade, the concept of DNA barcoding has emerged as the standardized analysis of an easily amplifiable PCR fragment for sequence-based identification of species [27]. Several molecular markers have been tested to reliably identify fungal species. The nuclear ribosomal Internal Transcribed Spacer (ITS) region is widely used as a DNA barcoding marker to characterize the diversity and composition of fungal communities. This marker is widely used in taxonomy and molecular phylogeny, giving a high resolving power for species discrimination due to key advantages such as its high degree of interspecific variability, conserved primer sites, and multiple copies within the fungal genome. Another alternative barcode region is the D1/D2 domain of the LSU rDNA gene, which was adopted for characterizing yeast species long before the concept of DNA barcoding was promoted [28]. However, in recent years, this region has been demonstrated to be use-

ful for the identification of filamentous fungi, and it has emerged as a promising barcode for identification up to the species level. Both molecular markers have limitations and challenges: low resolution in some groups, and secondary markers like protein-coding genes are needed; intragenomic variation within a single isolate can complicate taxonomic assignments; length and sequence variation across diverse phyla can make precise sequence alignment challenging [29]. The ITS and D1/D2 markers were used in this study for the reliable identification of three rare fungal species causing keratitis, whose identities could not have been determined based on morphology alone.

We observed highly variable CMIs for each of the three uncommon species involved in this study. Therefore, establishing a therapeutic drug or scheme for the treatment of keratitis caused by any of these species is difficult. A minimum of 1 million cases of fungal keratitis occurs globally annually. Fungal keratitis is more difficult to diagnose and has worse outcomes than other types of infectious keratitis [30].

At our hospital, we treat approximately 30 cases of fungal keratitis per year, with *Fusarium* spp., *Aspergillus* spp., and *Scedosporium* spp. predominating as causative agents.

As a conclusion, we report three non-consecutive cases of keratitis caused by uncommon fungal species whose morphological identification was uncertain, and the outcome of antifungal treatment was unpredictable. To the best of our knowledge, these are the first reported cases of keratitis caused by *P. percutanea*, *S. asteroides*, or *A. verrucaria* from Mexican patients. It is imperative for all exposed workers to use eye-protective equipment to avoid ocular trauma. Molecular identification and susceptibility tests are essential to determine the specific etiology and to establish or redirect the specific therapy for these infections involving unusual or emergent agents.

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Author Contributions

Virginia Vanzzini-Zago: Visualization, laboratory investigation, writing review, resources. **Ethel Guindo:** Clinical investigation and therapy, writing—review. **Emmanuel Cabrera**: Clinical investigation and therapy, writing—review. **Erika Córdova-Martínez**: Methodology, laboratory investigation, writing—review. **Patricia Manzano-Gayosso**: Writing—review & editing, writing original draft, laboratory investigation, formal analysis, resources. **Dolores Rios y Vallesa:** Laboratory investigation, writing—review. **Ivete Ayuso-Hernández:** Laboratory investigation, writing—review. **Francisca Hernández-Hernández**: Conceptualization, visualization, project administration, methodology, formal analysis, writing—review & editing, writing original draft.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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