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Note

Pyrenochaeta keratinophila sp. nov., isolated from an ocular infection in Spain

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ABSTRACT

The new coelomycete *Pyrenochaeta keratinophila*, isolated from corneal scrapings of a case of keratitis in Spain, is described and illustrated. This fungus is morphologically characterized by grey-olivaceous to greenish olivaceous colonies, scarce pycnidial setae placed mainly near the ostiole and production of phialoconidia from the aerial mycelium. The latter feature is unknown in any other species of the genus *Pyrenochaeta*. Sequencing of the ITS rDNA region of this clinical strain confirmed this proposal and revealed its close genetic relationship with the Leptosphaeriaceae.

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Pyrenochaeta keratinophila sp. nov., aislado de una infección ocular en España

RESUMEN

Se describe e ilustra el nuevo celomicete *Pyrenochaeta keratinophila*, aislado de un raspado corneal procedente de un caso de queratitis en España. Este hongo se caracteriza morfológicamente por tener colonias de color gris oliváceo a verdoso, setas picnidiales escasas principalmente situadas cerca del ostiolo, y producir conidios filídicos a partir del micelio aéreo. Este último carácter no se ha observado en ninguna otra especie del género *Pyrenochaeta*. La secuencia de la región ITS de ADN_r de esta cepa clínica confirma nuestra propuesta y pone de manifiesto su estrecha relación con la familia Leptosphaeriaceae.

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Although fungal infections of the cornea are mainly produced by species of the genera *Candida*, *Aspergillus* and *Fusarium*, the number of other fungal species that are able to cause keratitis is constantly increasing.^{1,3,4} The objective of this note is to describe a new species of *Pyrenochaeta* that was isolated from a case of keratitis in a Spanish, 77-year-old diabetic woman. Even though the patient was treated with topical natamycin and oral ketoconazole for 1 month and the lesion improved considerably, it was not possible to save the cornea and the patient required an optical penetrating keratoplasty. The detailed clinical case and mycological methods for fungal isolation were reported recently by Ferrer et al.² Briefly, the diagnosis was performed by direct

examination of corneal scrapings of the patient (Fig. 1), direct PCR from the clinical specimen and isolation of the fungus on different culture media. For identification purposes, the fungus was sent to the Faculty of Medicine of the Rovira i Virgili University in Reus (Spain) and to the Centraalbureau voor Schimmelfcultures in Utrecht (The Netherlands), where living cultures of the clinical strain were kept as FMR 9444 and CBS 121759, respectively.

The only fungus that grew in the culture media was morphologically identified as a *Pyrenochaeta* species. However, its main morphological features did not match with any other described species of this genus and consequently it is proposed as a new species below. This proposal was supported by analysis of the ITS sequence (GenBank accession number EU885415).

The description of the sporulating structures at 20 °C in 10 days on the laboratory bench (diffuse daylight) was as follows (colours

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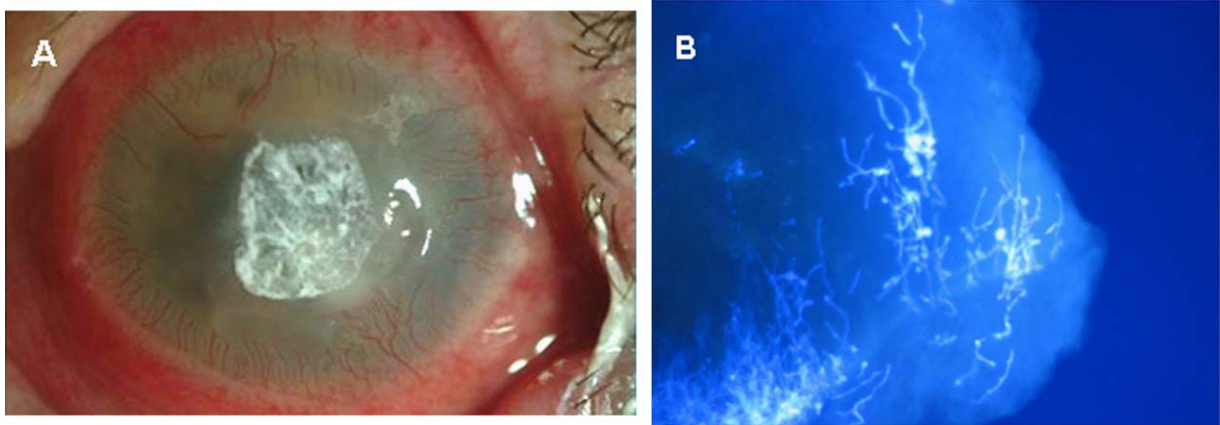


Fig. 1. (A) Clinical picture of the left eye showing conjunctival hyperaemia, peripheral corneal pannus, corneal edema and a central ulcer with white infiltrates. (B) Calcofluor white staining of corneal biopsy showing fungi invading the cornea. Original magnification 400 ×.

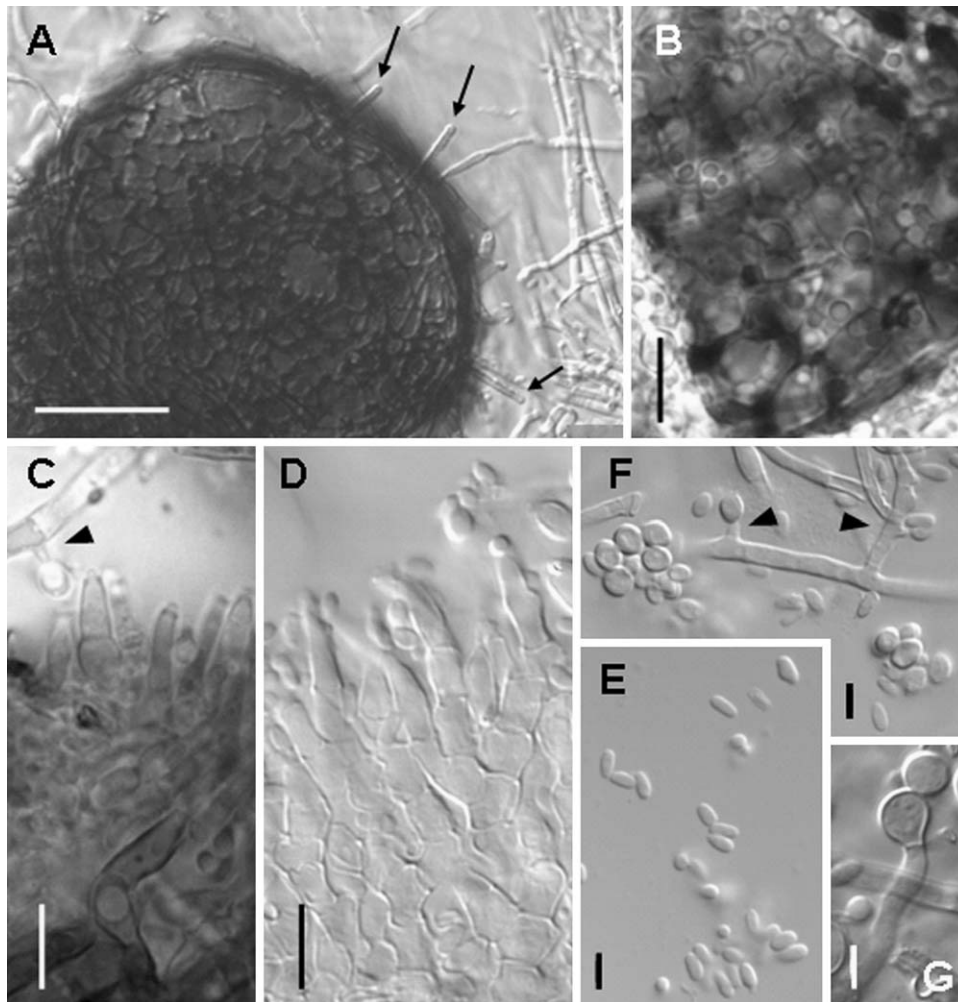


Fig. 2. Light microscopic images of the holotype of *Pyrenochaeta keratinophila* (CBS 121759) on OA at 20 °C. (A) A pycnidium with setae (arrows). Scale bar=50 µm. (B) Surface view of conidiomatal wall with dark intercellular material. Scale bar=10 µm. (C, D) Transverse view of pycnidial wall with conidiophores and a conidiogenous aperture in the aerial mycelium (head arrow). Scale bar=10 µm. (E) Conidia from pycnidia. Scale bar=5 µm. (F) Conidia produced from solitary apertures (head arrows) in the aerial mycelium. Scale bar=5 µm. (G) Chlamydoconidia-like structures. Scale bar=5 µm.

according to Rayner⁶): colonies on oatmeal agar (OA; 30 g oat flakes, 1 g MgSO₄, 1.5 g KH₂PO₄, 15 g agar, 1 L tap water) attained a diameter of 24–30 mm, margin even, colourless, flat; immersed

mycelium pale grey olivaceous to greenish olivaceous, aerial mycelium concolorous, diffuse woolly floccose; reverse olivaceous grey to olivaceous black in the centre. Colonies on malt extract

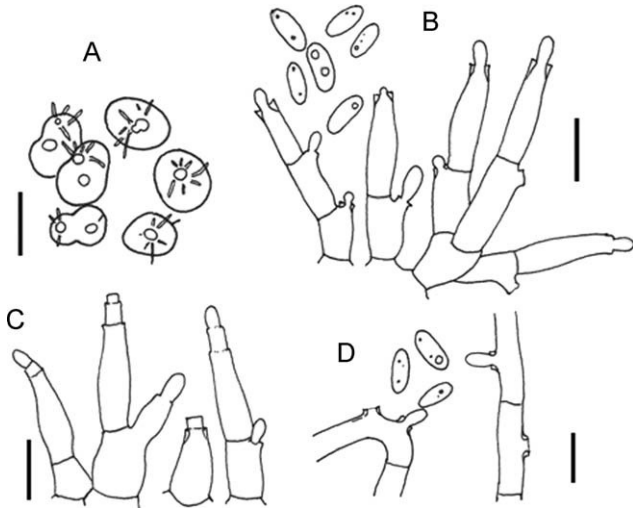


Fig. 3. Camera lucida drawings of the holotype of *Pyrenochaeta keratinophila* (CBS 121759) on OA. (A) Pycnidia in surface view. Scale bar=250 μ m. (B) Conidiophores showing phialidic apertures. Scale bar=5 μ m. (C) Conidiophores in an older fruiting body, showing percurrent proliferation. Scale bar=5 μ m. (D) Conidiogenous apertures in the aerial mycelium. Scale bar=5 μ m.

agar (Pronadisa, Torrejón de Ardoz, Spain) attained a diameter of 18–23 mm, margin even, buff; immersed mycelium olivaceous black or brown vinaceous, hidden under a dense mat of felty to woolly pale olivaceous grey aerial mycelium; reverse dark hazel. Superficial pycnidia were abundantly formed on both culture media after a few days. On OA, the pycnidia were olivaceous brown to almost black, globose or flask shaped, single or confluent, 100–400 μ m in diameter, with 1–3 ostioles of 10–25 μ m in diameter, displaying hyaline, thick-walled periphyses; pycnidial walls of textura angularis, composed of cells 4–9 μ m in diameter, with dark-brown intercellular material. The outer surface of the pycnidial wall displayed scarce, brown, slightly roughened, septate setae, 20–35 \times 2.5–4 μ m, with a blunt apex, mostly positioned near the ostiole (Figs. 2A, B and 3A). Conidiogenous cells arose from the entire inner surface of the pycnidial wall. They were rarely discrete, ampuliform to doliform, mostly cylindrical, 12–18(23) \times 2–3.5 μ m and integrated in conidiophores, which were branched at the base, acropleurogenous (i.e., having terminal and lateral apertures), phialidic, with a distinct periclinal thickening and sometimes with a short collarette (Figs. 2C, D and 3B). In older cultures, conidiogenous cells often showed several distinct percurrent proliferations (Fig. 3C). Conidia were whitish in mass, ellipsoid, (2)2.5–3(4) \times 1–2 μ m, straight or slightly curved, hyaline, continuous, smooth, with granular contents or few guttules, rounded at both ends (Fig. 2E). Phialoconidia produced directly from solitary apertures in the aerial mycelium were observed in 3-week-old cultures (Figs. 2C, F and 3D). At first, they were indistinguishable from conidia originating from pycnidia, but later became inflated unilaterally, globose or subglobose, 3–4 μ m in diameter, pale brown and slightly thick walled (Figs. 2F). Chlamydospores-like structures were also observed mainly on old OA cultures. They were terminal, hyaline, globose, 8–10 μ m in diameter, smooth and thick walled (Fig. 2G).

The sexual state of this fungus was not observed, but the molecular data confirm that the closest sexual state is *Leptosphaeria* (Ferrer et al.²) and that the newly proposed species can be regarded as a member of the family Leptosphaeriaceae.

Pyrenochaeta keratinophila Verkley, Ferrer et Gené, sp. nov. (Mycobank nr 512225). Conidiomata in agar OA pycnidialia, olivaceo-brunnea vel paene nigra, globosa vel ampulliformia, singularia vel confluentia, 100–400 μ m diametro, ostiolis 1–3 et periphysibus hyalinis crassitunicatis, 10–25 μ m diametro; parietes e textura angulari cellulis 4–9 μ m diametro compositi, extus setis paucis brunneis, minute verrucosis, 20–35 \times 2.5–4 μ m, apicibus obtusis, vulgo prope ostiolum locatis. Cellulae conidiogenae undique e cellulis interioribus exorientes, raro discretae, ampulliformes vel doliiformes, plerumque cylindricae et in conidiophoris acropleurogenis basibus ramosis integratae, phialidicae, tunica evidenter sursum perclinaliter incrassata collulo brevi, 12–18(23) μ m \times 2–3.5 μ m. Conidia ellipsoidea, recta vel leniter curvata, hyalina, continua, glabra, granulae vel paucas guttulas continentia, utrinque rotundata (2)2.5–3(4) \times 1–2 μ m. Synanamorphosis phialoconidia similaria e aperturis solitariis in mycelio exorientia.

Etymology refers to the fungus infecting matrices rich in keratin. The holotype is CBS H-20122, isolated from a corneal ulcer in March 2007, Alicante, Spain.

Up to now three species of *Pyrenochaeta* have been described from clinical samples: *Pyrenochaeta mackinnonii*, *Pyrenochaeta romeroi* and *Pyrenochaeta unguis-hominis*.⁵ *Pyrenochaeta keratinophila* and *P. unguis-hominis* have very similar conidiophores and conidia, but these species can be clearly distinguished by the colour of the colonies and the location of the setae on the pycnidia. In *P. keratinophila*, colonies on OA are grey olivaceous to greenish olivaceous, while those of *P. unguis-hominis* are brown vinaceous to fawn. The pycnidial setae of the former are scarce and placed mainly near the ostiole, while in the latter they are usually more abundant and more dispersed over the entire surface of the pycnidium. The new species also produces conidia from the mycelium, a feature unknown in *P. unguis-hominis* or any other species of the genus *Pyrenochaeta*. *P. mackinnonii* is distinguished from *P. keratinophila* by more restricted, raised or wrinkled colonies. *P. romeroi* is mainly distinguished by its greyish-sepia to fuscous-black colonies and the presence of discrete conidiogenous cells.

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