Appendix S1.

Direct examination of the nail scrapings treated with dimethyl sulphoxide (DMSO) and 10% KOH showed the abundance of hyaline (2–3 μm wide) regularly septate hyphae, yet of irregular shape and without any arthroconidia. Short lengths of hyphae were mainly present, branching at acute angles. The micromorphology was thus unlike that of dermatophyte species (Fig. 1C). Similar picture was achieved in culture-derived preparations stained with lactophenol cotton blue (LPCB). Mycological cultures were carried out on both Sabouraud glucose agar (SGA) with chloramphenicol and gentamicin and SGA with cycloheximide. In addition, part of the nail scrapings was inoculated on dermatophyte test medium (DTM) with chloramphenicol and gentamicin, but without cycloheximide. The inoculated media were incubated aerobically at 25°C for up to 21 days. Starting from the third day post-inoculation white and flat colonies of one type were found on all the media, except for SGA with cycloheximide. After 14–18 days post-inoculation, the colonies were fully developed, albeit weakly sporulating. To promote sporulation, the fungal colonies were subcultured on potato dextrose agar (PDA), malt extract agar (MEA) and Leonian's agar (LA), all prepared as described elsewhere (S1). On all types of media applied, the fungal colonies were flat, white to greyish in colour, with darker grey patches, corresponding to zones of increased conidiation, and with iron-grey reverse. Lack of growth on SGA with cycloheximide and negative reaction for alkalinization of DTM allowed the exclusion of dermatophytes and other keratinophilic fungi as aetiological agents of the case described.

To investigate the micromorphology of the fungus cultured in greater detail, slide cultures were performed on PDA and LA, examined after 14 days of incubation at 25°C by microscopic observation of LPCB-stained mycelium preparations. Of particular note were pale-brown single-celled conidia with a clear brightly opalescent equatorial ring (Fig. 1E). Conidia, sympodially generated from poorly-developed, bottle-shaped and cluster-aggregated conidiogenous cells, were generally lenticular in side view, while convex or globose when seen from above or below, measuring approximately 3–4 or 5–7 μm in diameter, accordingly (Fig. 1E, F). Apart from typical conidia, the fungus sporadically produced sterile cells which were similarly coloured to the conidia, yet had rather irregular shape. The morphological characteristics of the fungus revealed by light microscopy were confirmed by scanning electron microscopy (SEM). Here, smooth conidial surface was clearly demonstrated (Fig. 1F). Based on the macro- and micromorphological features, the fungus was provisionally identified as representing *Arthrinium* spp.

To confirm this diagnosis, molecular speciation was performed. This involved chromosomal DNA extraction from fresh fungal mycelium (13), and PCR-sequencing of rDNA gene cluster and a part of the translation elongation factor 1-alpha (TEF). The primers ITS4 and ITS5 (S2) were used to amplify and sequence the rDNA fragment spanning the 3'-end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, and the second ITS region, whereas primers NL1 and NL4 were used to amplify and sequence the 5'-end of the 28S rRNA gene, including its D1/D2 variable domains (S3). Part of the *TEF* gene was amplified and sequenced using primers EF1-728F and EF-2. The 3 sequences: 604-bp, 602-bp, and 436-bp, obtained respectively were compared against the GenBank database using the BLAST algorithm (www.ncbi.nlm.nih.gov/blast/Blast.cgi). These sequences showed 99–100% identity and the closest match, with the corresponding sequences of *Arthrinium arundinis* NRRL 25634.

Drug susceptibility testing of the cultured fungus was performed with the Etest method, according to the protocol provided by the manufacturer (AB BIODISK, Solna, Sweden). An important modification was that the assay was carried out at 25°C instead of 35°C, as recommended in the original protocol (S4). This was forced by the fact that the isolate did not grow at ≥35°C. Among the drugs chosen for the evaluation were those most commonly used in the treatment of onychomycosis (S5) and especially in case of diabetic individuals (16). Fourth-generation antifungal agents, normally inadequate for the treatment of onychomycosis (16, S5), were also included in the testing panel. This was done to gain a more general view on the susceptibility pattern of the analysed fungus.

Nucleotide sequence accession numbers. The 604-bp ITS1/2 sequence, the 602-bp D1/D2 sequence, and the 436-bp TEF sequence were deposited in GenBank with accession numbers KX533933, KX533934 and KX533935, respectively.

SUPPLEMENTARY REFERENCES

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