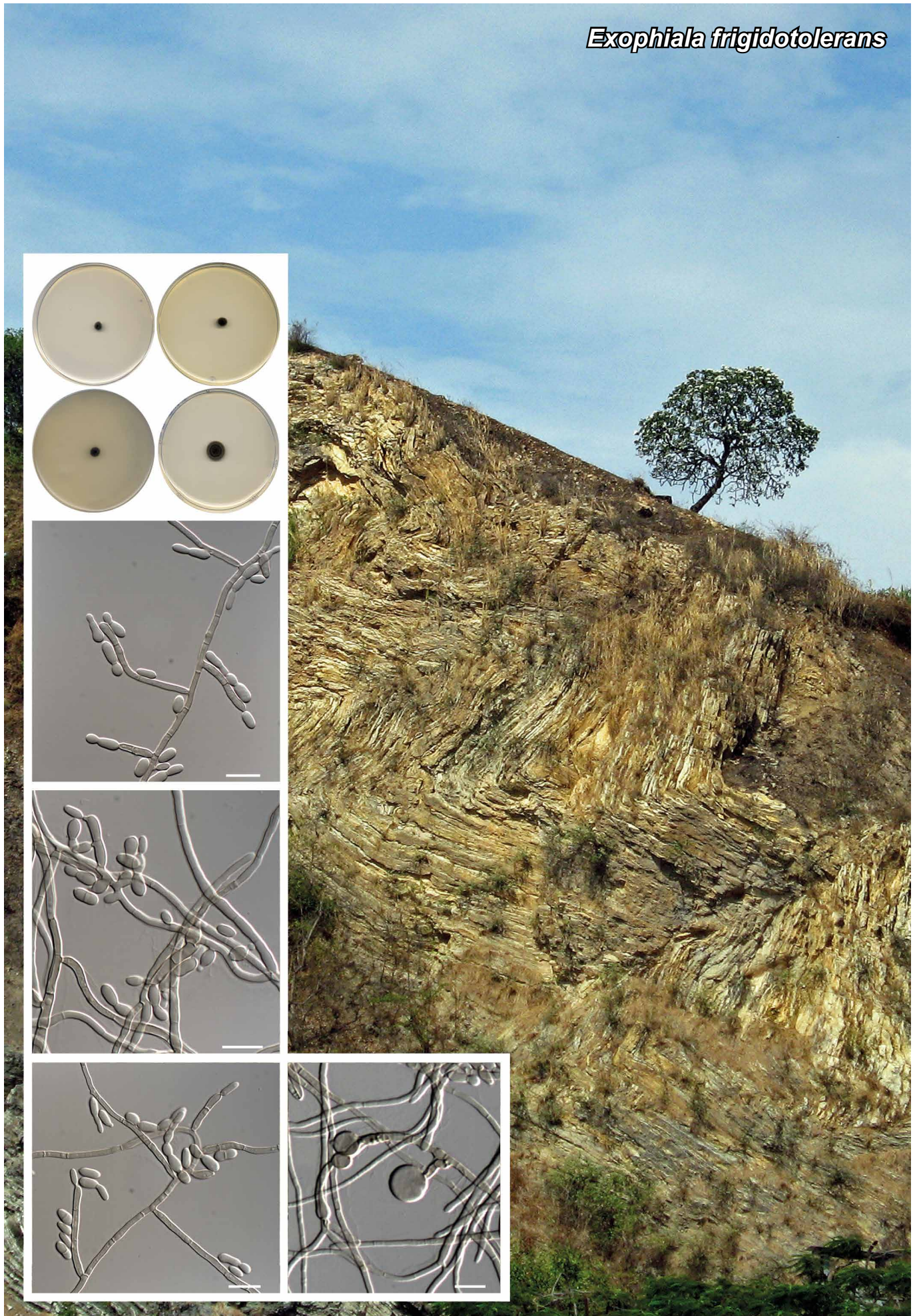


*Exophiala frigidotolerans*





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***Exophiala frigidotolerans* Rodr.-Andr., Cano & Stchigel, sp. nov.**

*Etymology.* From Latin *frigus*-, cold, and - *tolerans*, tolerant, referring to its ability to grow fast at lower temperatures than 20 °C.

*Classification* — *Herpotrichiellaceae*, *Chaetothyriales*, *Chaetothyriomycetidae*, *Eurotiomycetes*.

*Mycelium* composed of pale olivaceous brown, septate, branched, smooth- and thin-walled hyphae, 1–3 µm wide; older hyphae being more strongly pigmented. *Spirally twisted hyphae* present. *Moniliform cells* scarce, globose to ellipsoidal, in short chains (–5 cells). *Conidiophores* semi-micronematous, pale olivaceous brown, smooth- and thin-walled, mostly laterally disposed on the vegetative hyphae, sometimes terminally disposed, erect, rarely once branched near the base, cylindrical, with a rounded or pointed apex, 0–4-septate, with a terminal conidiogenous locus, sometimes with additional conidiogenous loci, 8–85 × 2–4 µm. *Conidiogenous cells* enteroblastic, mono- or polyblastic, integrated to the conidiophores, on vegetative hyphae or well-developed, in the latter case ellipsoidal, ovoid or flask-shaped, 5–11 × 2–3 µm, conidiogenous loci cylindrical or conic-cylindrical, with small percurrent proliferations. *Conidia* aseptate, occasionally 1-septate, pale olivaceous brown, smooth- and thin-walled, ellipsoidal to reniform, 4–7 × 2–4 µm, sometimes with a truncate base, solitary. *Budding cells* scarce, ellipsoidal, ovoid or barrel-shaped, 7–11 × 3–4 µm, in chains up to 5 elements. *Chlamydo-spores* scarce, olivaceous, globose, 5–15 µm diam.

*Culture characteristics* — *Colonies* on potato dextrose agar (PDA) reaching 5–6 mm diam after 2 wk at 25 °C, slightly raised, velvety, margins regular, brownish grey (M. 5E2; Komerup & Wanscher 1978), sporulation absent, exudate absent; reverse brownish grey (M. 5E2), diffusible pigment absent. *Colonies* on oatmeal agar (OA) reaching 6–7 mm diam after 2 wk at 25 °C, morphologically similar to those on PDA, with sparse sporulation. *Colonies* on malt extract agar (MEA) reaching 5–7 mm diam after 2 wk at 25 °C, slightly raised, velvety, margins regular, olive brown (M. 4E4), sporulation absent, exudate absent; reverse olive brown (M. 4F3), diffusible pigment absent. *Colonies* on potato carrot agar (PCA) reaching 4–6 mm diam after 2 wk at 25 °C, slightly raised, velvety, margins regular, olive brown (M. 4E4), sparse sporulation, exudate absent; reverse brownish grey (M. 4F2), diffusible pigment absent. *Colonies* on PDA reaching 10–11 mm diam after 2 wk at 15 °C slightly raised velvety, margins regular, brownish grey (M. 5E2), sporulation absent, exudate absent; reverse brownish grey (M. 5E2), diffusible pigment absent. Minimum, optimal and maximum temperature of growth, 10 °C, 15 °C, and 25 °C, respectively.

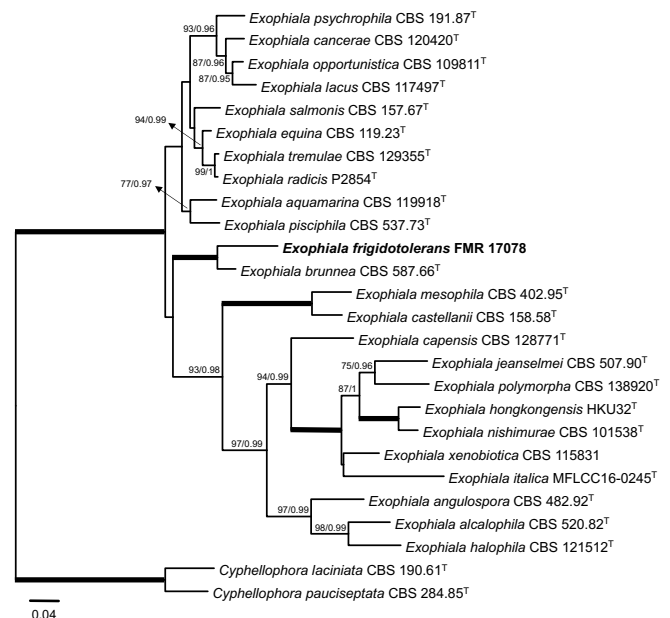
*Typus.* ECUADOR, Guayaquil, isolated from soil, Nov. 1996, *L. Zaror* (holotype CBS H-24326, cultures ex-type FMR 17078 = CBS 146539; ITS, LSU and *BenA* sequences GenBank LR699566, LR699567 and LR699568, MycoBank MB832466).

*Notes* — *Exophiala frigidotolerans* was recovered from a soil sample collected in Guayaquil, Ecuador. The genus *Exophiala* pertains to a group of fungi known as ‘black yeasts’, because of the production of yeast-like colonies and budding cells with dark,

*Colour illustrations.* Guayaquil, Ecuador (image credit Doug Moyer). Colonies growing on different culture media (PCA, MEA, OA at 25 °C and PDA at 15 °C; upper pictures); conidiogenous cells, conidia, budding cells and inflated cells. Scale bars = 10 µm.

melanised cell walls. The genus *Exophiala* is characterised by an annellidic conidiogenesis and the production of solitary conidia grouping in slimy masses, and its phylogenetic affiliation to the ascomycete order *Chaetothyriales* (De Hoog et al. 2011). This genus contains numerous potential opportunists or pathogens of immunocompetent humans (Sudhadham et al. 2008, Li et al. 2008, 2009) and are isolated from a broad spectrum of substrata, environments and geographic areas (De Hoog et al. 2011, Ferrari et al. 2011). As in *E. psychrophila*, *E. frigidotolerans* exhibited the ability to grow at low temperatures. However, *E. frigidotolerans* presents more developed conidiophores than *E. psychrophila* (which are reduced to a unique discrete conidiogenous cell in this latter species), and produces shorter chains of moniliform cells (scarce and of up to 5 cells in the former species, and very abundant and of up to several hundred of cells in the latter).

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hit using the ITS sequence is the ex-type strain of *Exophiala brunnea* CBS 587.66 (GenBank JF747062; Identities = 539/560 (96 %), 6 gaps (1 %)); and using the LSU sequence the ex-type strain of *Exophiala brunnea* CBS 587.66 (GenBank MH870554; Identities = 868/876 (99 %), 1 gap (0 %)). The ITS-LSU-*BenA* phylogenetic tree corroborated the placement of our isolate as a new species of *Exophiala*, being located phylogenetically close to *E. brunnea*. *Exophiala brunnea* is easily distinguished from *E. frigidotolerans* by the production of 2-celled conidia (mostly 1-celled in *E. frigidotolerans*) and absence of budding cells (formed in *E. frigidotolerans*).



Maximum likelihood tree obtained from the ITS-LSU-*BenA* alignment of our isolate and sequences retrieved from GenBank. The tree was built by using RAxML CIPRES ([http://www.phylo.org/sub\\_sections/portal/](http://www.phylo.org/sub_sections/portal/)) and the analysis of probability was run in MrBayes v. 3.2.1 (Ronquist et al. 2012). Bootstrap support values  $\geq 70\%$  and Bayesian posterior probability values  $\geq 0.95$  are presented at the nodes. Fully supported branches (100 % BS / 1 PP) are thickened. *Cyphellophora laciniata* CBS 190.61 and *Cyphellophora pauciseptata* CBS 284.85 were used as outgroup. The new species proposed in this study is indicated in bold. <sup>†</sup> represents the ex-type strains of the taxa employed in this analysis.