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Arthrocladium, an unexpected human opportunist in *Trichomeriaceae* (*Chaetothyriales*)

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ABSTRACT

The family *Trichomeriaceae* (*Chaetothyriales*) mainly comprises epiphytic and epilithic organisms. In some species elaborate ascomata are formed, but for the great majority the species no asexual conidium formation is known other than simple fragmentation of the thallus. The present paper re-establishes the genus *Arthrocladium* with three non-sporulating species. One of these is described for a strain causing a fatal infection in a human patient with a rare genetic immune disorder.

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Introduction

The *Chaetothyriales* is an order of ascomycetes comprising black yeasts (anamorph genus *Exophiala*) and its filamentous relatives. At first glance the members of this order show very different ecological traits, regularly occurring in hydrocarbon-containing environments (Zhao *et al.* 2010), bathing facilities (Sudhadham *et al.* 2008), dead plant material (Vicente *et al.* 2008, 2014), as agents of disease in cold-blooded vertebrates (de Hoog *et al.* 2011) and in humans (Chowdhary *et al.* 2015), and in ant nests (Voglmayr *et al.* 2011). However, common factors in these dissimilar habitats are found in several types of extremotolerance (Gostinčar *et al.* 2012): oligotrophism, osmo- and thermotolerance, tolerance of irradiation and toxicity, and growth under acidic and alkaline conditions (Gümral *et al.* 2015). The order is unique in the significant role of monoaromatic hydrocarbon assimilation (Prenafeta-Boldú *et al.* 2006; Isola *et al.* 2013).

The best known family is the *Herpotrichiellaceae*, containing numerous human-pathogenic opportunists: about one-third of members of the family have been described from diseases of the human host, and occasionally infections are disseminated or systemic taking a fatal course (Li *et al.* 2010). Of special significance is that the majority of infected hosts seem to have no underlying metabolic or immune disorder (Bonifaz *et al.* 2013). The *Cyphellophoraceae* are closely related, and are mainly found as human skin colonizers with enigmatic environmental habitat (Feng *et al.* 2012). Of the family *Chaetothyriaceae* only very few members have been sequenced (Chomnunti *et al.* 2012a). The *Epibryaceae* contain minute intracellular pathogens of liverworths (Döbbeler 1980) and was recently suggested to belong to the *Chaetothyriales* on the basis of sequence similarities (Gueidan *et al.* 2014).

The family *Trichomeriaceae* was recently added to the *Chaetothyriales* (Chomnunti *et al.* 2012a). It accommodates the ascosporulating genus *Trichomerium*, characterized by small, setose ascocarps with eight transversely septate ascospores which indeed resemble those of *Capronia*, the teleomorph genus in *Herpotrichiellaceae*. *Trichomerium* species lack anamorph sporulation. In nature they grow together with sooty mould in plant exudates or the sugary honeydew secreted by insects (Chomnunti *et al.* 2012a). Recently several authors revealed that some genera of extremotolerant fungi growing on exposed surfaces were phylogenetically related to *Trichomerium* (Isola *et al.* 2015). Genera involved were *Bradymyces, Knufia*, and *Lithophila*. Most members of these genera inhabited bare rock and were slow-growing without recognizable sporulation. The taxonomic position of the non-sporulating fungi is ambiguous as they have variously been assigned to *Trichomeriaceae* and *Chaetothyriaceae* on the basis of sequence similarity only (Tsuneda *et al.* 2011, Réblová et al., 2013, Hubka *et al.* 2014; Isola *et al.* 2015).

A genus *Arthrocladium* was introduced by Papendorf (1969) to accommodate a single strain from *Acacia* leaf litter in South Africa. Sequencing of the type strain of *Arthrocladium caudatum* revealed that the genus is related to *Knufia*; phenotypically it is similar by absence of recognizably differentiated sporulation. The present paper introduces three further species that cluster with *Arthrocladium*. Two of these were isolated as inhabitants of rotten wood, whereas the third caused a fatal disseminated infection in a human with a GATA-2 immune defect (Egenlauf *et al.* 2015).

Material and methods

Strains analysed

Strains from woody substrates were obtained using the oil flotation method (Iwatsu *et al.* 1981; Vicente *et al.* 2008). A clinical isolate was isolated from a brain biopsy sample. Reference strains were obtained from the Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS) reference collection and are listed in Table 1. Stock cultures were maintained on slants of malt extract agar (MEA; Oxoid, UK) and potato dextrose agar (PDA; Oxoid). Taxonomic information for new species was deposited in MycoBank (www.MycoBank.org).

Morphology

Strains were cultured on MEA and PDA and incubated at 24 °C for 3 weeks. Microscopic preparations were performed by slide cultures on MEA and PDA using lactic acid, lactic acid-cotton blue and Shears as mounting fluids. Micrographs were taken using a Nikon Eclipse 80i microscope and DS Camera Head DS-Fi1/DS- 5 m/DS-2Mv/DS-2MBW using NIS-Element freeware package (Nikon Europe, Badhoevedorp, The Netherlands).

Physiology

Cardinal growth temperatures were determined on MEA plates incubated in the dark for 2 weeks at temperatures of 18–36 °C at intervals of 3 °C; growth was also recorded at 37 °C and at 40 °C with two replicates for each isolate. Laccase enzyme activity was tested according Feng *et al.* (2012), using ABTS agar medium containing 0.03 % ABTS (2, 2-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid diammonium salt). ABTS plates were incubated at room temperature for 1 week.

DNA extraction

Approximately 1 cm^2 of 14-day-old cultures was transferred to a 2 mL screw-capped tubes filled with 490 μL 2 % cetyltrimethylammonium bromide (CTAB) buffer and 6–10 acid-washed glass beads (diam 1.5–2.0 mm, Sigma). 10 μL Proteinase K were added and mixed on a MoBio vortex for 10 min, the mixture was incubated at 60 °C for 30 min. After incubation, 500 μL Chloroform: isoamyl alcohol (24:1) was added and shaken for 2 min. The tubes were centrifuged for 10 min at 20 400 g force value, supernatants were collected in new 1.5 mL Eppendorf tubes, ~270 μL of ice-cold iso-propanol was added followed by centrifugation again at 14 000 r.p.m. for 10 min. Pellets were washed with 1 mL ice-cold 70 % ethanol, dried using a vacuum dryer and re-suspended in 50 μL TE-buffer. DNA concentrations were measured with NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, U.S.A.). Extracted DNAs were stored at −20 °C until use.