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Diversity and novel lineages of black yeasts in *Chaetothyriales* from freshwater sediments in Spain

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Abstract Black yeasts comprise a group of *Ascomycota* of the order *Chaetothyriales* with highly variable morphology, a great diversity of ecological niches and life cycles. Despite the ubiquity of these fungi, their diversity in freshwater sediments is still poorly understood. During a survey of culturable *Ascomycota* from river and stream sediments in various sampling sites in Spain, we obtained 47 isolates of black yeasts by using potato dextrose agar supplemented with cycloheximide. A preliminary morphological study and sequence analyses of the internal transcribed spacer region (ITS) and the large subunit (LSU) of the nuclear rDNA revealed that most of the isolates belonged to the family *Herpotrichiellaceae*. We have confidently identified 30 isolates representing the following species: *Capronia pulcherrima*, *Cladophialophora emmonsii*, *Exophiala equina*, *Exophiala pisciphila*, *Exophiala radicis*, and *Phialophora americana*. However, we encountered difficulty in assigning 17 cultures to any known species within *Chaetothyriales*. Combining phenotypic and multi-locus phylogenetic analyses based on the ITS, LSU, β-tubulin (*tub*2) and translation elongation factor 1-α (*tef*1-α) gene markers, we propose the new genus *Aciculomyces* in the *Herpotrichiellaceae* to accommodate the novel species *Aciculomyces restrictus*. Other novel species in this family include *Cladophialophora denticulata*, *Cladophialophora heterospora*, *Cladophialophora irregularis*, *Exophiala candelabrata*, *Exophiala dehoogii*, *Exophiala ramosa*, *Exophiala verticillata* and *Phialophora submersa*. The new species *Cyphellophora spiralis*, closely related to *Cyphellophora suttonii*, is described, and the phylogeny of the genus *Anthopsis* in the family *Cyphellophoraceae* is discussed. By utilizing these four markers, we were able to strengthen the phylogenetic resolution and provide more robust taxonomic assessments within the studied group. Our findings indicate that freshwater sediments may serve as a reservoir for intriguing black yeasts, which warrant further investigation to address gaps in phylogenetic relationships, particularly within *Herpotrichiellaceae*.

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INTRODUCTION

The *Chaetothyriales* is a species-rich and ecologically diverse order of dematiaceous fungi that includes the so-called black yeasts and their filamentous relatives. Species within this order display a broad diversity of life modes, highlighting their diverse ecological strategies. These include saprophytes inhabiting soil and decaying plant material (Iwatsu 1984, Decock et al. 2003, Badali et al. 2008), as well as species associated with living plants as phytopathogens (Crous et al. 2006, 2007, Gueidan et al. 2014) or being apparently harmless endo- or epiphytes (Narisawa et al. 2007, Chomnunti et al. 2012). Some species are recognized as pathogens affecting both humans and cold-blooded animals (Badali et al. 2008, Feng et al. 2014a, b, De Hoog et al. 2011, 2020, Quan et al. 2020). Black yeasts are often associated with extreme habitats; e.g., on rock surfaces in hot, arid climates or toxic niches with hydrocarbons and heavy metals (Prenafeta-Boldú et al. 2001, Badali et al. 2011, Teixeira et al. 2017). In more recent discoveries, several species have been observed engaging in mutualistic interactions with ant species. They play a significant role in the formation of the ant's cardboard-like material used for constructing nesting spaces in the host plant (Voglmayr et al. 2011, Nepel et al. 2014, Quan et al. 2020, 2021, 2022). Due to their ability to adapt to various extreme living conditions, black yeasts have been defined as polyextremotolerant fungi (Gostinčar et al. 2012, 2018, Moreno et al. 2018).

The order comprises at least 10 families (Batista & Ciferri 1962, Réblová et al. 2013, Wijayawardene et al. 2020, Tian et al. 2021), but DNA data is fragmentary and not available for all of them (Quan et al. 2020). Recent phylogenetic analyses combining sequences of the large subunit (LSU) with the internal transcribed spacer (ITS) of the nuclear rDNA of *Chaetothyriales* (available mainly in the GenBank database) revealed that they were distributed across nine statistically supported lineages, seven of which were representatives of formally described families in the literature (viz., *Chaetothyriaceae*, *Coccodiniaceae*, *Cyphellophoraceae*, *Epibryaceae*, *Herpotrichiellaceae*, *Paracladophialophoraceae* and *Trichomeriaceae*) (Quan et al. 2020, Tian et al. 2021). Among them, the family *Herpotrichiellaceae* is the best represented. It includes a great number of species described from isolates obtained in culture, with *Cladophialophora*, *Exophiala*, *Fonsecaea*, *Phialophora* and *Rhinocladiella* as the most diverse genera (Teixeira et al. 2017, Quan et al. 2020). Most species in the *Herpotrichiellaceae* reproduce asexually

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with conidia generated by a filamentous phase, while some members, especially those of the genus *Exophiala*, can produce yeast-like budding cells. Although the *Herpotrichiellaceae* are phylogenetically supported by the ITS and LSU rDNA barcodes (Untereiner & Naveau 1999, Tian et al. 2021), the taxonomic structure of this family remains unresolved. This is primarily due to the polyphyletic nature of most of the aforementioned genera (Quan et al. 2020, Phukhamsakda et al. 2022). According to De Hoog et al. (2011), the family was divided into six clades (the *bantiana*-clade, *carrionii*-clade, *salmonis*-clade, *europaea*-clade, *dermatitidis*-clade and *jeanselmei*-clade) with different ecological trends. Nevertheless, its phylogeny is still considered unstable, and therefore, it is not yet feasible to replace morphologically based taxonomy with solely molecular data (Quan et al. 2023). Currently, traits associated with morphologically defined genera, combined with multi-locus analyses based primarily on the ITS barcode, and fragments of the translation elongation factor 1-α (*tef*1-α) and β-tubulin (*tub*2) genes, have allowed to investigate phylogenetic relationships of closely related fungi and delineate novel lineages. Following such approach, the members of the *europaea*-clade were proposed as the family *Cyphellophoraceae* (Réblová et al. 2013), and new *Phialophora* species have been more recently described in the so-called *P. verrucosa* complex in *Herpotrichiellaceae* (Li et al. 2017).

The presence of black yeasts in aquatic environments has been widely reported and, as already mentioned, some species are even involved in infections of aquatic animals such as frogs, toads, fish or crabs (Badali et al. 2008, De Hoog et al. 2011, 2020, 2022, Vicente et al. 2012). However, their diversity in freshwater sediments still remains understudied (Lepère et al. 2019). It is important to highlight that the slow growth of these fungi poses challenges when isolating them from environmental samples. Their development is frequently influenced by the rapid proliferation of fast-growing moulds on commonly used agar media. This can impede the isolation and cultivation of the target fungi, requiring careful consideration and specialized techniques to mitigate the impact of competing moulds and facilitate the growth and study of the desired fungal species. In this sense and considering the polyextremotolerance of the *Chaetothyriales*, special isolation techniques have been used by different authors, such as the high incubation temperatures (Sudhadham et al. 2008), the extraction with mineral oil (Satow et al. 2008, Vicente et al. 2008), the enrichment on atmospheres with aromatic hydrocarbons (Prenafeta-Boldú et al. 2001, Zhao et al. 2010) and direct plating with media containing cycloheximide (Salgado et al. 2004, Madrid et al. 2016, Wang et al. 2018). In this work, we combined different culture media, including potato dextrose agar (PDA) supplemented with cycloheximide, in order to explore the diversity of culturable *Chaetothyriales* from freshwater sediment samples collected in Spain. The chosen methodology allowed us to study a rich set of unknown taxa with affinity to the families *Cyphellophoraceae* and *Herpotrichiellaceae*. They have been delineated by using sequence analyses of the above-mentioned gene markers, morphological and ecological data.

MATERIALS AND METHODS

Sampling areas and fungal isolation

Freshwater sediment samples were collected between February 2018 and March 2021 from rivers and streams in natural rural areas placed in three Spanish regions (Table 1); i.e., Aragon (Remáscaro Stream, Huesca), Baleares (Pont d'en Blai Stream, Mallorca) and Catalonia (Llobregat River, Barcelona; Segre River, Lleida; Ter River, Barcelona/Girona; Torrent de la Coma Stream, Lleida; Les Obagues Stream, Tarragona). While sediments samples from the streams were collected randomly, sediments from the rivers Llobregat, Segre and Ter were collected at two points, located in the upper and middle sections of rivers. Only the River Ter was additionally sampled in the lower section, near the Mediterranean coast (Fig. 1).

The upper sections of the rivers and most of the streams sampled were allocated in subalpine areas, characterized by a Mediterranean mountain climate of the Pyrenees and Pre-Pyrenees, with average annual temperatures ranging between 8.5 and 11 °C, average annual rainfalls between 900 and 1 200 mm, altitudes between 650 and 1 540 m, and vegetation dominated by *Abies alba*, *Buxus sempervirens*, *Fagus sylvatica*, *Pinus* (*Pi.*) *sylvestris*, *Pi. uncinata*, *Quercus* (*Q.*) *faginea*, *Q. ilex* and *Q. pubescens*. The middle sections of the rivers were in natural areas characterised by a continental Mediterranean climate with average annual temperatures between 12.5 and 14 °C, average annual rainfalls of 400–900 mm and altitudes between 400 and 500 m. These fluvial areas were formed by riparian forest dominated by *Juncus* spp., *Populus (P.) alba*, *P. nigra*, *Quercus coccifera*, *Rubus* (*R.*) *caesius*, *R. ulmifolius*, *Salix alba* and *Typha latifolia*. The lower section of the River Ter was characterised by a Mediterranean coast climate with an average annual temperature of 16 °C, an average annual precipitation of 550 mm, an altitude of 30 m and a riparian forest with *Crataegus monogyna*, *Fraxinus angustifolia*, *Phragmites australis*, *Pi. alba*, *R. ulmifolius* and *Spartium junceum* as the most representative vegetation. These data were mainly obtained from https://www.meteo.cat/wpweb/climatologia/el-clima/ and http://biodiver.bio.ub.es/biocat/ (accessed 23 April 2023) for the sampled areas in Catalonia and Baleares and from https://www.aragon.es/-/parque-natural-de-posets-maladeta (accessed 22 April 2023) for the sampled area in Aragon.

Sediment samples were collected and subjected to treatment procedures outlined in the studies conducted by Ulfig et al. (1997) and Torres-Garcia et al. (2022a). Briefly, four samples were obtained at each sampling point using sterile 100 mL plastic containers. Sediments were collected c. 10 cm below the surface layer of the bunk beds or edges of the rivers and streams selected. Once in the laboratory, samples were shaken vigorously in the same containers, and after leaving them for 1 min to settle, the water was decanted and the sediment poured onto several layers of sterile filter paper on plastic trays to remove any water excess. In order to obtain a wide range of fungal species, each sample was cultured in dichloran rose-bengal-chloramphenicol agar (DRBC; 2.5 g peptone, 5 g glucose, 0.5 g KH_2PO_4, 0.25 g $MgSO_4$, 12.5 mg rose-bengal, 100 mg chloramphenicol, 1 mg dichloran, 10 g agar, 500 mL distilled water), DRBC supplemented with 0.01 g/L benomyl, and PDA (Condalab, Madrid, Spain) supplemented with 2 g/L of cycloheximide. Each sample was cultured in duplicate in each medium, as follows: 0.5 or 1 g of sediment was mixed with melted medium at 45 °C in the same plate, and once solidified, it was incubated at room temperature (22–25 °C) in the dark. Cultures were examined weekly with a dissecting microscope for up to 4–5 wk. To achieve pure cultures of the black yeasts detected in the primary cultures, fragments of the colonies were transferred to fresh PDA plates and incubated at 25 °C in the dark. These PDA axenic cultures were used for a preliminary morphological identification and for DNA extraction.

Black yeasts isolated in this study were accessioned to the fungal culture collection of the Medicine Faculty at the Rovira i Virgili University (FMR; Reus, Spain), and those which resulted putative novel taxa were also deposited at the fungal collection of the Westerdijk Fungal Biodiversity Institute (CBS; Utrecht, The Netherlands), including dry cultures for holotypes. In addition, two *Cladophialophora* strains from the latter collection were also included in the study for comparison (Table 1).

Table 1 Strain information and GenBank/EMBL accession numbers of the species investigated in this study.

Species	Strain number*	Origin (coordinates)	GenBank accession numbers			
			ITS	LSU	*tub*2	*tef*1-α
Aciculomyces restrictus	FMR 18994^{T}	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27967° E2.00535°)	ON009870	ON009950	ON667802	ON667773
Capronia pulcherrima	FMR 18999	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27967° E2.00535°)	OP037946	OP037920	OP081036	OP081033
Cladophialophora denticulata (*Cladophialophora* sp. III)	FMR 18992^{T}	Llobregat River, Guardiola del Berguedà, Barcelona, Spain (N42.24012° E1.93742°)	ON009845	ON009925	ON667783	ON667754
Cladophialophora emmonsii	FMR 18631	Ter River, Torroella de Montgrí, Girona, Spain (N42.03005° E3.13468°)	ON009846	ON009926	ON667782	ON667753
Cladophialophora heterospora (*Cladophialophora* sp. I)	FMR 18641^{T}	Ter River, Vilanova de Sau, Barcelona, Spain (N41.97084° E2.38108°)	ON009847	ON009927	ON491592	ON667752
	CBS 142309	Non-potable water sample, USA	LT799460	OQ448833	LT799457	OQ466313
	CBS 142308	Non-potable water sample, USA	OQ293978	OQ294002	OQ308777	OQ466314
Cladophialophora irregularis (*Cladophialophora* sp. II)	FMR 18642^{T}	Ter River, Vilanova de Sau, Barcelona, Spain (N41.97084° E2.38108°)	ON009848	ON009928	ON491589	–
	FMR 18643	Ter River, Torroella de Montgrí, Girona, Spain (N42.03005° E3.13468°)	ON009849	ON009929	ON491587	–
Cyphellophora spiralis	FMR 18548^{T}	Ter River, Les Masies de Voltregà, Barcelona, Spain (N42.02951° E2.25359°)	ON009850	ON009930	ON667784	–
Exophiala candelabrata (*Exophiala* sp. I)	FMR 18336^{T}	Segre River, La Granja d'Escarp, Lleida, Spain (N41.42754° E0.35020°)	ON009851	ON009931	ON491591	ON667762
Exophiala dehoogii (*Exophiala* sp. IV)	FMR 19001^{T}	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27967° E2.00535°)	ON009858	ON009938	ON491588	–
Exophiala equina	FMR 18335	Segre River, Aitona, Lleida, Spain (N41.48444° E0.46446°)	ON009852	ON009932	ON491590	ON667757
Exophiala pisciphila	FMR 18640	Ter River, Torroella de Montgrí, Girona, Spain (N42.03005° E3.13468°)	ON009854	ON009934	ON667790	ON667761
Exophiala radicis	FMR 17547	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037947	OP037921	–	–
	FMR 17548	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037948	OP037922	–	–
	FMR 17549	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037949	OP037923	–	–
	FMR 17556	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037950	OP037924	–	–
	FMR 17557	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037951	OP037925	–	–
	FMR 17570	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037952	OP037926	–	–
	FMR 17572	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037953	OP037927	–	–
	FMR 17573	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037954	OP037928	–	–
	FMR 17578	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037955	OP037929	–	–
	FMR 17580	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037956	OP037930	–	–
	FMR 17581	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037957	OP037931	–	–
	FMR 18644	Remáscaro Stream, Cerler, Huesca, Spain (N42.58698° E0.54993°)	OP037959	OP037933	–	–
	FMR 17630	Pont d'en Blai Stream, Mallorca, Illes Balears Spain (N39.76907° E2.88418°)	OP037958	OP037932	–	–
	FMR 18040	Torrent de la Coma Stream, Gòsol, Lleida, Spain (N42.21889° E1.65159°)	ON009855	ON009935	ON667788	ON667759
	FMR 18549	Ter River, Sant Joan de les Abadesses, Girona, Spain (N42.24138° E2.28830°)	ON009856	ON009936	ON667787	ON667758
	FMR 18645	Ter River, Ripoll, Girona, Spain (N42.17258° E2.19372°)	ON009857	ON009937	ON667789	ON667760
	FMR 18646	Ter River, Sant Joan de les Abadesses, Girona, Spain (N42.24138° E2.28830°)	OP037960	OP037934	–	–
	FMR 18989	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27298° E1.99177°)	OP037961	OP037935	OP081037	OP081032
	FMR 18995	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27298° E1.99177°)	OP037962	OP037936	OP081038	OP081034
	FMR 19000	Llobregat River, Cercs, Barcelona, Spain (N42.10512° E1.88044°)	OP037963	OP037937	OP081039	OP081035
Exophiala ramosa (*Exophiala* sp. III)	FMR 18632^{T}	Ter River, Les Masies de Voltregà, Barcelona, Spain (N42.02951° E2.25359°)	ON009853	ON009933	ON667786	ON667756
Exophiala verticillata (*Exophiala* sp. II)	FMR 18551^{T}	Ter River, Llanars, Girona, Spain (N42.32383° E2.32989°)	ON009859	ON009939	ON667785	ON667755
Phialophora americana	FMR 18550	Ter River, Ripoll, Girona, Spain (N42.17258° E2.19372°)	OP037964	OP037938	–	–
	FMR 18552	Ter River, Sant Joan de les Abadesses, Girona, Spain (N42.24138° E2.28830°)	ON009860	ON009940	ON667793	ON667765
	FMR 18630	Ter River, Vilanova de Sau, Barcelona, Spain (N41.97084° E2.38108°)	ON009861	ON009941	ON667794	ON667774

Table 1 (cont.)

Species	Strain number*	Origin (coordinates)	GenBank accession numbers			
			ITS	LSU	*tub*2	*tef*1-α
Phialophora americana (cont.)	FMR 18983	Llobregat River, Guardiola del Berguedà, Barcelona, Spain (N42.24012° E1.93742°)	ON009862	ON009942	ON667791	ON667763
	FMR 18987	Llobregat River, La Pobla de Lillet, Barcelona, Spain (N42.24294° E1.96998°)	ON009863	ON009943	ON667792	ON667764
	FMR 18988	Llobregat River, La Pobla de Lillet, Barcelona, Spain (N42.24294° E1.96998°)	OP037965	OP037939	–	–
Phialophora submersa	FMR 17150$^{\mathrm{T}}$	Les Obagues Stream, Cornudella del Montsant, Tarragona, Spain (N41.26672° E0.90985°)	ON009864	ON009944	ON667800	ON667771
	FMR 18984	Llobregat River, Guardiola del Berguedà, Barcelona, Spain (N42.24012° E1.93742°)	ON009865	ON009945	ON667796	ON667767
	FMR 18985	Llobregat River, Guardiola del Berguedà, Barcelona, Spain (N42.24012° E1.93742°)	ON014042	ON015649	ON667795	ON667766
	FMR 18986	Llobregat River, Guardiola del Berguedà, Barcelona, Spain (N42.24012° E1.93742°)	ON009866	ON009946	ON667801	ON667772
	FMR 18996	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27298° E1.99177°)	ON009867	ON009947	ON667798	ON667769
	FMR 18997	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27298° E1.99177°)	ON009868	ON009948	ON667799	ON667770
	FMR 18998	Llobregat River, Castellar de n'Hug, Barcelona, Spain (N42.27298° E1.99177°)	ON009869	ON009949	ON667797	ON667768

* FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.
Ex-type strains are designated by a superscript 'T'. Novel species proposed are in **bold**.

Phenotypic characterization

Macroscopic features of the representative isolates of putative novel black yeasts were studied on malt extract agar (MEA; Oxoid, Madrid, Spain), oatmeal agar (OA; 30 g filtered oat flakes, 20 g agar, 1 L distilled water), potato carrot agar (PCA; potato 20 g, carrot 20 g, agar 13 g, 1 L distilled water) and PDA after 14 d at 25 °C in the dark, according to previous studies (Badali et al. 2008, Madrid et al. 2016, Li et al. 2017). Colour notations in descriptions were assigned according to Kornerup & Wanscher (1978). Because of some isolates did not sporulate in any of the culture media mentioned above, Synthetic Nutrient Deficient Agar (SNA; KH_2PO_4 1 g, KNO 1 g, $MgSO_4 \times 7H_2O$ 0.5 g, KCl 0.5 g, glucose 0.2 g, sucrose 0.2 g, agar 14 g, distilled water 1 L) and water agar (agar 15 g, distilled water 1 L), both supplemented with partially immersed sterile plant material (i.e., leaves of *Dianthus caryophyllus* and *Saccharum officinarum*), were used to induce sporulation.

The microscopic characterization was performed by direct wet mounts with Shear's solution (3 g potassium acetate, 60 mL glycerol, 90 mL ethanol 95 %, 150 mL distilled water; Chupp 1940) from colonies growing primarily on OA and MEA after 14–21 d at 25 °C in darkness or by using slide cultures with the same agar media and incubation conditions. Size ranges of relevant structures in species descriptions were derived from at least 30 measurements. Photomicrographs were obtained using a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity digital camera. Photo plates were assembled from separate photographs using PhotoShop CS6 (Adobe Systems, San Jose, CA, USA).

In addition, we determined the ability of the isolates to grow at different temperatures (i.e., 5 °C to 40 °C at 5 °C intervals, including 37 °C) on MEA by measuring colony diameter after 14 d in the dark.

DNA extraction, sequencing and phylogenetic analysis

Colonies of the isolates growing on PDA after 7–14 d at 25 °C were used for the DNA extraction following a modified protocol of Müller et al. (1998). The DNA extraction consisted in scratching mycelium from the colonies with sterile scalpel and transferring it to homogenization tubes containing 500 µL of lysis buffer (100 Mm Tris pH 8.0, 50 Mm EDTA, 1 % SDS) and beads. The tubes were immediately processed using a FP 120 FastPrep cell disrupter (Thermo Salvat, Holbrook, New York) for 40 s and then they were centrifuged for 10 min at 13 000 rpm. The resuspended material was transferred to new 2 mL plastic tubes, mixed with 275 µL of ammonium acetate 7 M (pH 7.0), and incubated at 65 °C for 5 min and then at 5 °C for 5 min. After incubations, 500 µL of chloroform was added to the mixtures, and the tubes were centrifuged again at 13 000 rpm for 5 min. The resulting upper layers were transferred to new 2 mL plastic tubes, to which 1 mL of isopropanol was added, and then incubated at 5 °C for 1 h. After that, the tubes were centrifuged at 13 000 rpm for 10 min and the supernatants were decanted. The resulting pellets were resuspended in 70 % ethanol and centrifuged at 13 000 rpm for 5 min. The resulting supernatants were again decanted, and the pellets were resuspended in 50 µL of sterile miliq water. The DNA was quantified by using Nanodrop 2000 (Thermo Scientific, Madrid, Spain).

For identification purposes and molecular characterization, four nuclear loci were amplified: i.e., the ITS barcode, the D1/D2 domains of the LSU rRNA gene, and fragments of the *tub*2 and *tef*1-α genes. Primer pairs used for amplification were ITS5/LR5 for ITS and D1/D2 regions (White et al. 1990), EF1–728F/EF–986R for *tef*1-α (Carbone & Kohn 1999) and Bt2a/Bt2b for *tub*2 (Glass & Donaldson 1995). PCR reactions were carried out in a final volume of 25 µL that contained 30 µL/mL of genomic DNA, 10× PCR buffer (200 Mm Tris-HCl pH 8.4, 500 mM KCl), forward and reverse primers in a concentration of 10 µM, $MgCl_2$ 50 mM, dNTP's 0.2 mM (of each nucleotide), Taq DNA polymerase 5 U/µL (Invitrogen, The Netherlands) and all diluted in sterile Milli-Q water. Briefly, PCR conditions for ITS, LSU and *tub*2 were set as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C, 45 s at 56 °C, and 60 s at 72 °C, and a final extension step at 72 °C for 10 min. PCR conditions for *tef*1 consisted in an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 40 s at 95 °C, 60 s at 58 °C and 90 s at 72 °C, and a final extension step at 72 °C 7 min. PCR products were purified and sequenced at Macrogen Corp. Europe (Madrid, Spain) with the same pairs of primers that were used for amplification. Consensus sequences were assembled using SeqMan v. 7.0.0 (DNASTAR Lasergene, Madison, WI, USA).

The ITS and LSU sequences were compared with existing sequences of other species available in GenBank using the

Fig. 1 View of some of the river sections sampled. a, b. Upper and middle sections, respectively, of the River Llobregat; c, d. upper and lower sections, respectively, of the River Ter; e. middle section of the River Segre; f. upper section of the Stream Remáscaro. — Photo's by: a, b. Josepa Gené; c–e. Daniel Torres-Garcia; f. Jose F Cano-Lira.

Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi). A maximum similarity level of ≥ 98 % was used for species-level identification. Lower similarity values were considered as putative unknown fungi. Based on the BLAST results, which indicated a relationship between our isolates and members of the families *Cyphellophoraceae* and *Herpotrichiellaceae*, single-gene and concatenated ITS and LSU sequence datasets were constructed. These datasets included known genera and species of both families; their analyses were conducted to determine the generic placement and identify our set of isolates. Taxon sampling for our phylogenetic analyses was based primarily on the species included in previous published phylogenies for the *Chaetothyriales* (Feng et al. 2014a, b, Quan et al. 2020). Sequences of the ex-type strains and/or reference strains of all accepted species and genera of the above-mentioned families were included in the analyses, as well as representatives of the families *Chaetothyriaceae*, *Epibryaceae*, *Phaeosaccardinulaceae* and *Trichomeriaceae* used as outgroups. These sequences were retrieved from GenBank (Table S1); most have been previously published in Badali et al. (2008), De Hoog et al. (2011), Reblová et al. (2013), Feng et al. (2014 a, b), Madrid et al. (2016), Li et al. (2017), Quan et al. (2020) and Sun et al. (2020). Additional concatenated analyses were carried out using, when possible, sequences of the four gene markers to assess the species boundaries of our isolates and their phylogenetic relationships with species of *Cladophialophora* and *Exophiala* s.lat., species of the genus *Phialophora* and those of the family *Cyphellophoraceae*.

Datasets for each locus were aligned individually in MEGA (Molecular Evolutionary Genetics Analysis) software v. 6.0 (Tamura et al. 2013), using the ClustalW algorithm (Thompson et al. 1994) and refined in the same platform manually or using MUSCLE (Edgar 2004). Phylogenetic concordance of the different locus datasets was tested individually in each single-locus phylogeny through visual comparison and using the Incrongruence Length Difference (ILD) implemented in the Winclada programme (Farris et al. 1994). Once confirmed the lack of incongruence, individual alignments were concatenated into a single data matrix with SequenceMatrix (Vaidya et al. 2011). The best substitution model for all gene matrices was estimated using MEGA software for Maximum Likelihood (ML) analysis, while for the Bayesian Inference (BI) analysis it was estimated using jModelTest v. 2.1.3 according to the Akaike criterion (Guindon & Gascuel 2003, Darriba et al. 2012). The phylogenetic reconstructions were performed with the combined genes using ML under RAxML-HPC2 on XSEDE v. 8.2.12 (Stamatakis 2014) in CIPRES Science gateway portal (Miller et al. 2010), and BI with MrBayes v. 3.2.6 (Ronquist et al. 2012).

For ML, phylogenetic support for internal branches was assessed by 1 000 ML bootstrapped pseudoreplicates and bootstrap support (bs) ≥ 70 was considered significant (Hillis & Bull 1993). The phylogenetic reconstruction by BI was performed using 5 million Markov chain Monte Carlo (MCMC) generations, with four runs (once cold chain and three heated chains), and samples were stored every 1 000 generations. The 50 % majority-rule consensus tree and posterior probability (pp) values were calculated after discharging the first 25 % of samples. A pp value of ≥ 0.95 was considered significant (Hespanhol et al. 2019). The resulting trees were plotted using FigTree v. 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). The DNA sequences and alignments generated in this study were deposited in GenBank (Table 1) and in Zenodo (https://doi.org/10.5281/zenodo.7664024, accessed 22 February 2023).

RESULTS

We recovered 47 isolates of black yeasts from sediment samples, all plated on PDA medium amended with cycloheximide (Table 1). They were isolated from sediment samples collected in the rivers Llobregat (n = 16), Segre (n = 2) and Ter (n = 15), and in Remáscaro (n = 12), Torrent de la Coma (n = 1) and Pont d'en Blai (n = 1) streams. Through BLAST search and sequence analysis of the LSU and ITS gene regions, we confirmed that 46 isolates belonged to the *Herpotrichiellaceae* and one to the *Cyphellophoraceae*. At the species level, we could confidently identify 30 (63.83 %) of the isolates obtained,

Table 2 Overview and details used for phylogenetic analyses included in this study.

Dataset		*Chaetothyriales*	*Cyphellophoraceae*	*Cladophialophora*	*Exophiala*	*Phialophora*
ITS	Lenght (bp)	708	733	610	535	614
	Pvar	504	370	633	333	120
	Pi	443	286	307	296	83
	Model*	GTR+G+I	GTR+G+I	TN93+G+I	GTR+G+I	K2+G
	Model**	GTR+G+I	GTR+G+I	GTR+G+I	SYM+G+I	K2+G
LSU	Lenght (bp)	904	864	876	916	768
	Pvar	437	200	219	331	36
	Pi	274	134	174	235	19
	Model*	K2+G+I	K2+G+I	K2+G+I	K2+G+I	K2+G
	Model**	GTR+G+I	SYM+G+I	GTR+G+I	GTR+G+I	K2+I
*tub*2	Lenght (bp)	–	526	537	480	470
	Pvar	–	330	318	288	171
	Pi	–	270	276	239	138
	Model*	–	K2+G	K2+G+I	TN93+G+I	K2+G
	Model**	–	HKY+G+I	K2+G+I	HKY+G+I	K2+G
*tef*1-α	Lenght (bp)	–	–	232	246	225
	Pvar	–	–	145	204	101
	Pi	–	–	118	168	73
	Model*	–	–	K2+G	K2+G	K2+G
	Model**	–	–	K2+G	HKY+G	K2+I
Concatenated	Lenght (bp)	1612	2123	2255	2177	2077
	Pvar	941	900	1048	1156	428
	Pi	717	690	875	938	313
	Model*	GTR+G+I	GTR+G+I	GTR+G+I	K2+G+I	K2+G
	Model**	SYM+G+I	SYM+G+I	SYM+G+I	SYM+G+I	K2+G

Pvar = variable sites; Pi = phylogenetic informative sites; * = substitution model for Maximum Likelihood; ** = substitution model for Bayesian inference; GTR = General Time Reversible; HKY = Hasegawa-Kishino-Yano; K2 = Kimura 2-parameter; SYM = Symmetrical; TN93 = Tamura-Nei; G = Gamma Distributed; I = Invariant Sites.

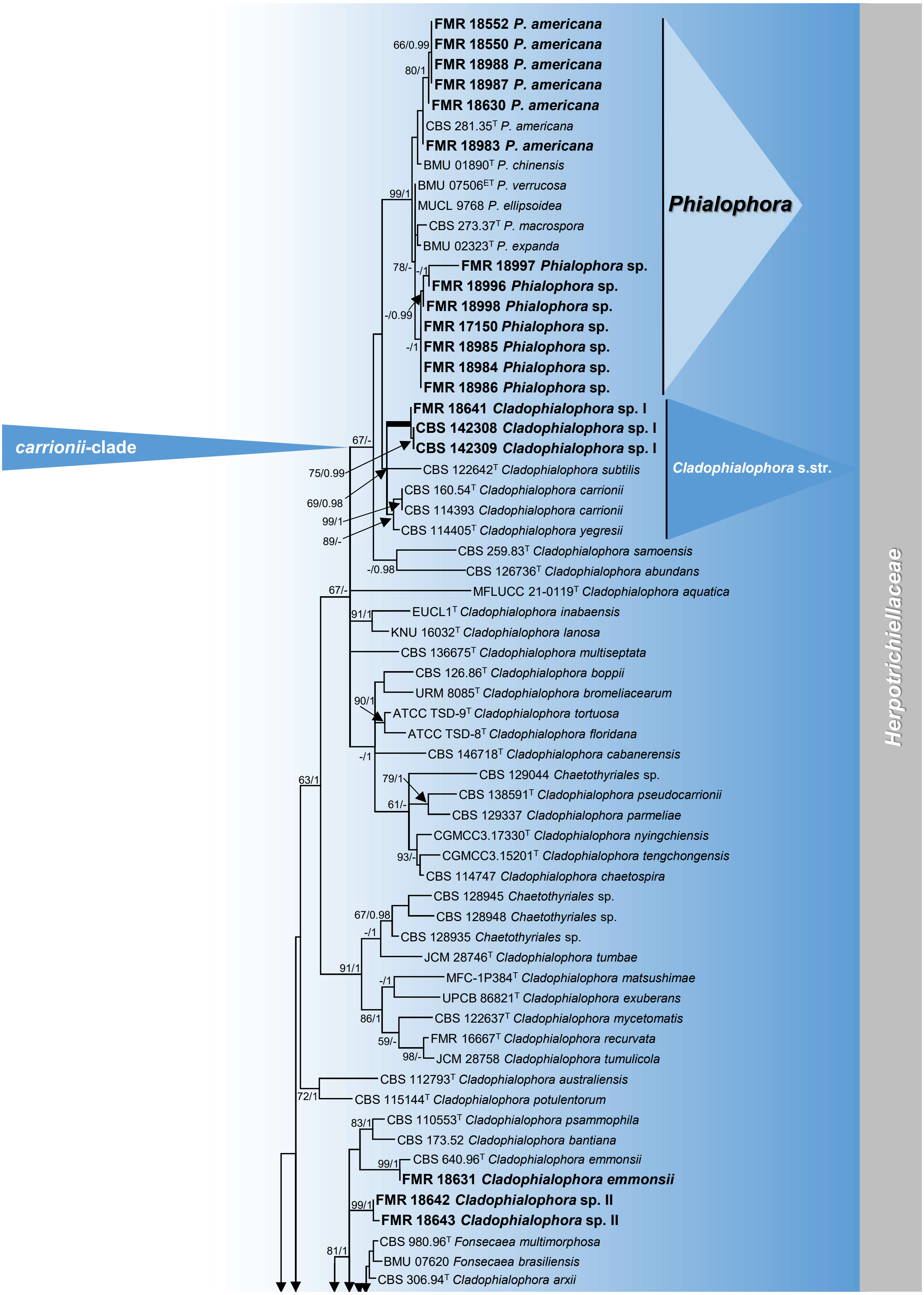

Fig. 2 Phylogenetic tree inferred from a Maximum Likelihood (ML) analysis based on the combined ITS and LSU sequence alignment of members of the families *Herpotrichiellaceae* and *Cyphellophoraceae* and outgroups. Branch lengths are proportional to phylogenetic distances. RAxML bootstrap support values above 60 % are shown at the nodes. The tree is rooted to several members of the families *Chaetothyriaceae*, *Epibryaceae*, *Phaeosaccardinulaceae* and *Trichomeriaceae*. Sediment isolates identified in this study and new species described are in **bold**. ^{T} = Ex-type strain.

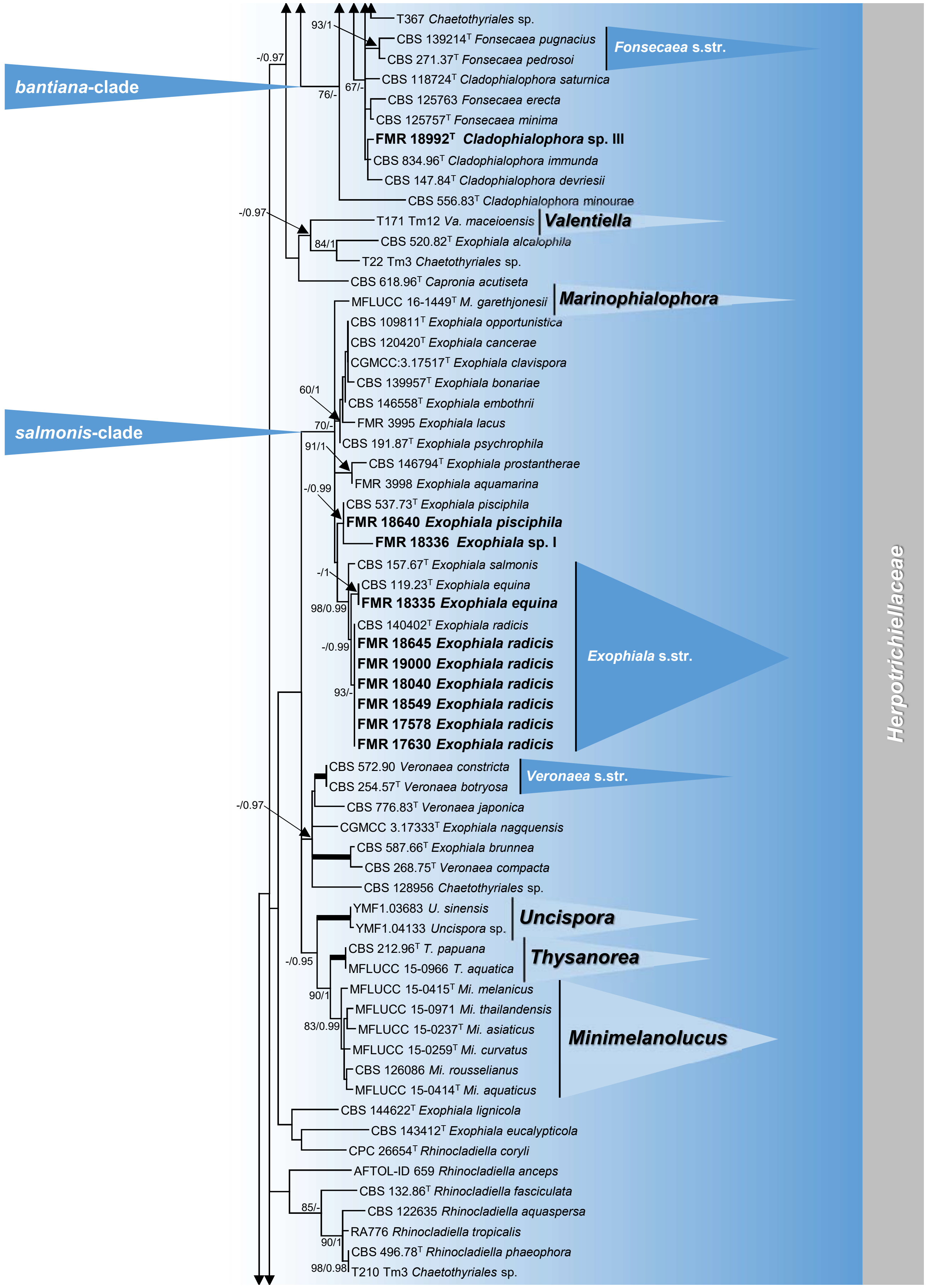
T367 *Chaetothyriales* sp.
93/1
CBS 139214^{T} *Fonsecaea pugnacius*
CBS 271.37^{T} *Fonsecaea pedrosoi*
Fonsecaea s.str.
-/0.97
CBS 118724^{T} *Cladophialophora saturnica*
bantiana-clade
67/-
76/-
CBS 125763 *Fonsecaea erecta*
CBS 125757^{T} *Fonsecaea minima*
FMR 18992^{T} *Cladophialophora* sp. III
CBS 834.96^{T} *Cladophialophora immunda*
CBS 147.84^{T} *Cladophialophora devriesii*
CBS 556.83^{T} *Cladophialophora minourae*
-/0.97
T171 Tm12 *Va. maceioensis*
Valentiella
84/1
CBS 520.82^{T} *Exophiala alcalophila*
T22 Tm3 *Chaetothyriales* sp.
CBS 618.96^{T} *Capronia acutiseta*
MFLUCC 16-1449^{T} *M. garethjonesii*
Marinophialophora
CBS 109811^{T} *Exophiala opportunistica*
CBS 120420^{T} *Exophiala cancerae*
CGMCC:3.17517^{T} *Exophiala clavispora*
CBS 139957^{T} *Exophiala bonariae*
60/1
CBS 146558^{T} *Exophiala embothrii*
salmonis-clade
70/-
FMR 3995 *Exophiala lacus*
CBS 191.87^{T} *Exophiala psychrophila*
91/1
CBS 146794^{T} *Exophiala prostantherae*
FMR 3998 *Exophiala aquamarina*
-/0.99
CBS 537.73^{T} *Exophiala pisciphila*
FMR 18640 *Exophiala pisciphila*
FMR 18336 *Exophiala* sp. I
CBS 157.67^{T} *Exophiala salmonis*
-/1
CBS 119.23^{T} *Exophiala equina*
98/0.99
FMR 18335 *Exophiala equina*
CBS 140402^{T} *Exophiala radicis*
-/0.99
FMR 18645 *Exophiala radicis*
FMR 19000 *Exophiala radicis*
Exophiala s.str.
FMR 18040 *Exophiala radicis*
93/-
FMR 18549 *Exophiala radicis*
FMR 17578 *Exophiala radicis*
FMR 17630 *Exophiala radicis*
Herpotrichiellaceae
CBS 572.90 *Veronaea constricta*
CBS 254.57^{T} *Veronaea botryosa*
Veronaea s.str.
CBS 776.83^{T} *Veronaea japonica*
-/0.97
CGMCC 3.17333^{T} *Exophiala nagquensis*
CBS 587.66^{T} *Exophiala brunnea*
CBS 268.75^{T} *Veronaea compacta*
CBS 128956 *Chaetothyriales* sp.
YMF1.03683 *U. sinensis*
YMF1.04133 *Uncispora* sp.
Uncispora
CBS 212.96^{T} *T. papuana*
-/0.95
MFLUCC 15-0966 *T. aquatica*
Thysanorea
MFLUCC 15-0415^{T} *Mi. melanicus*
90/1
MFLUCC 15-0971 *Mi. thailandensis*
83/0.99
MFLUCC 15-0237^{T} *Mi. asiaticus*
Minimelanolucus
MFLUCC 15-0259^{T} *Mi. curvatus*
CBS 126086 *Mi. rousselianus*
MFLUCC 15-0414^{T} *Mi. aquaticus*
CBS 144622^{T} *Exophiala lignicola*
CBS 143412^{T} *Exophiala eucalypticola*
CPC 26654^{T} *Rhinocladiella coryli*
AFTOL-ID 659 *Rhinocladiella anceps*
CBS 132.86^{T} *Rhinocladiella fasciculata*
85/-
CBS 122635 *Rhinocladiella aquaspersa*
RA776 *Rhinocladiella tropicalis*
90/1
CBS 496.78^{T} *Rhinocladiella phaeophora*
98/0.98
T210 Tm3 *Chaetothyriales* sp.

Fig. 2 (cont.)

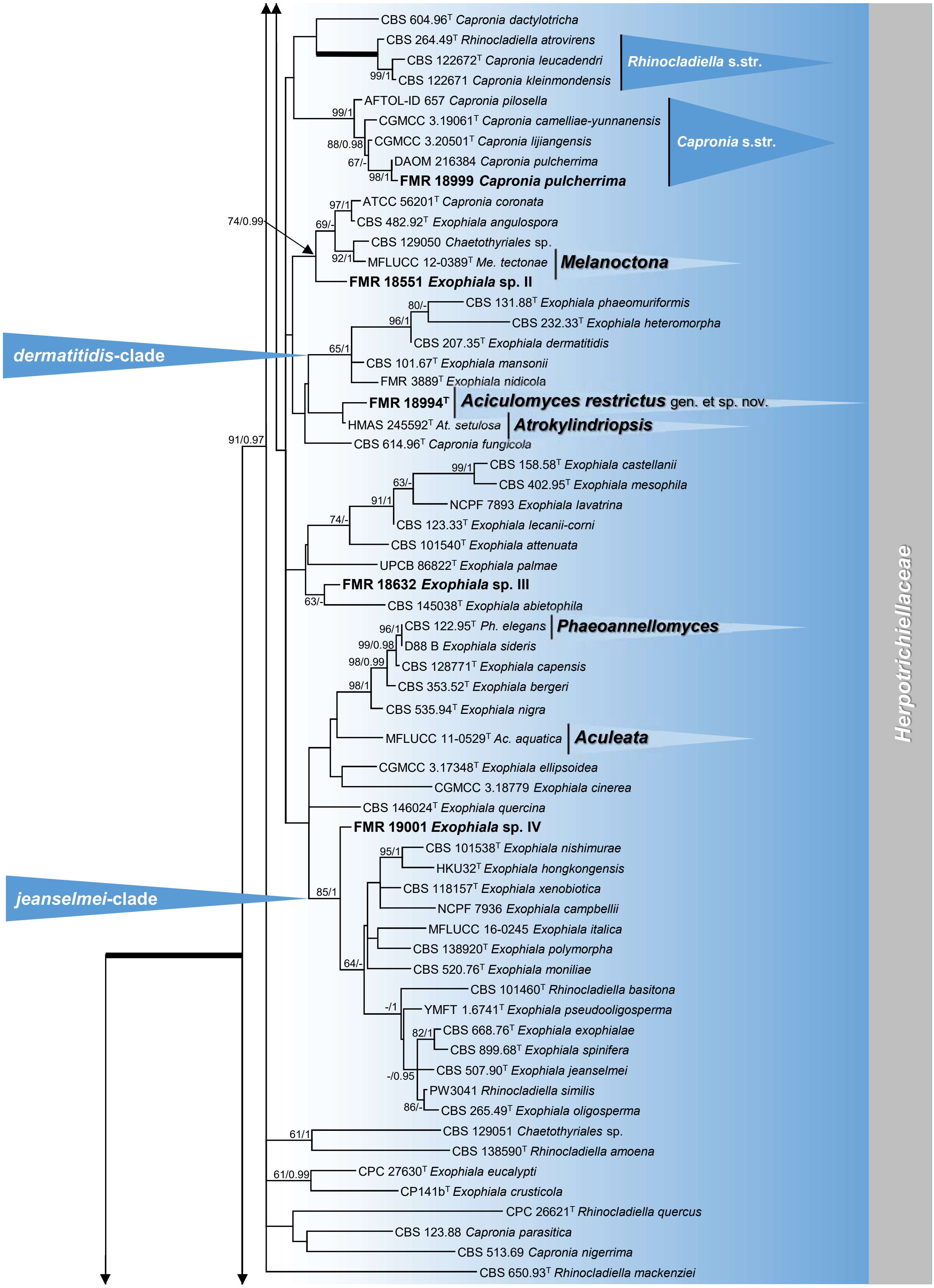
CBS 604.96^{T} Capronia dactylotricha
CBS 264.49^{T} Rhinocladiella atrovirens
CBS 122672^{T} Capronia leucadendri
99/1
CBS 122671 Capronia kleinmondensis
Rhinocladiella s.str.
99/1
AFTOL-ID 657 Capronia pilosella
CGMCC 3.19061^{T} Capronia camelliae-yunnanensis
88/0.98
CGMCC 3.20501^{T} Capronia lijiangensis
67/-
DAOM 216384 Capronia pulcherrima
98/1
FMR 18999 Capronia pulcherrima
Capronia s.str.
97/1
ATCC 56201^{T} Capronia coronata
74/0.99
69/-
CBS 482.92^{T} Exophiala angulospora
CBS 129050 Chaetothyriales sp.
92/1
MFLUCC 12-0389^{T} Me. tectonae
Melanoctona
FMR 18551 Exophiala sp. II
80/-
CBS 131.88^{T} Exophiala phaeomuriformis
96/1
CBS 232.33^{T} Exophiala heteromorpha
CBS 207.35^{T} Exophiala dermatitidis
dermatitidis-clade
65/1
CBS 101.67^{T} Exophiala mansonii
FMR 3889^{T} Exophiala nidicola
FMR 18994^{T}
Aciculomyces restrictus gen. et sp. nov.
HMAS 245592^{T} At. setulosa
Atrokylindriopsis
91/0.97
CBS 614.96^{T} Capronia fungicola
99/1
CBS 158.58^{T} Exophiala castellanii
CBS 402.95^{T} Exophiala mesophila
63/-
91/1
NCPF 7893 Exophiala lavatrina
74/-
CBS 123.33^{T} Exophiala lecanii-corni
CBS 101540^{T} Exophiala attenuata
UPCB 86822^{T} Exophiala palmae
FMR 18632 Exophiala sp. III
63/-
CBS 145038^{T} Exophiala abietophila
96/1
CBS 122.95^{T} Ph. elegans
Phaeoannellomyces
99/0.98
D88 B Exophiala sideris
98/0.99
CBS 128771^{T} Exophiala capensis
98/1
CBS 353.52^{T} Exophiala bergeri
CBS 535.94^{T} Exophiala nigra
MFLUCC 11-0529^{T} Ac. aquatica
Aculeata
CGMCC 3.17348^{T} Exophiala ellipsoidea
CGMCC 3.18779 Exophiala cinerea
CBS 146024^{T} Exophiala quercina
FMR 19001 Exophiala sp. IV
95/1
CBS 101538^{T} Exophiala nishimurae
HKU32^{T} Exophiala hongkongensis
jeanselmei-clade
85/1
CBS 118157^{T} Exophiala xenobiotica
NCPF 7936 Exophiala campbellii
MFLUCC 16-0245 Exophiala italica
CBS 138920^{T} Exophiala polymorpha
64/-
CBS 520.76^{T} Exophiala moniliae
CBS 101460^{T} Rhinocladiella basitona
-/1
YMFT 1.6741^{T} Exophiala pseudooligosperma
82/1
CBS 668.76^{T} Exophiala exophialae
CBS 899.68^{T} Exophiala spinifera
-/0.95
CBS 507.90^{T} Exophiala jeanselmei
PW3041 Rhinocladiella similis
86/-
CBS 265.49^{T} Exophiala oligosperma
61/1
CBS 129051 Chaetothyriales sp.
CBS 138590^{T} Rhinocladiella amoena
61/0.99
CPC 27630^{T} Exophiala eucalypti
CP141b^{T} Exophiala crusticola
CPC 26621^{T} Rhinocladiella quercus
CBS 123.88 Capronia parasitica
CBS 513.69 Capronia nigerrima
CBS 650.93^{T} Rhinocladiella mackenziei
Herpotrichiellaceae

Fig. 2 (cont.)

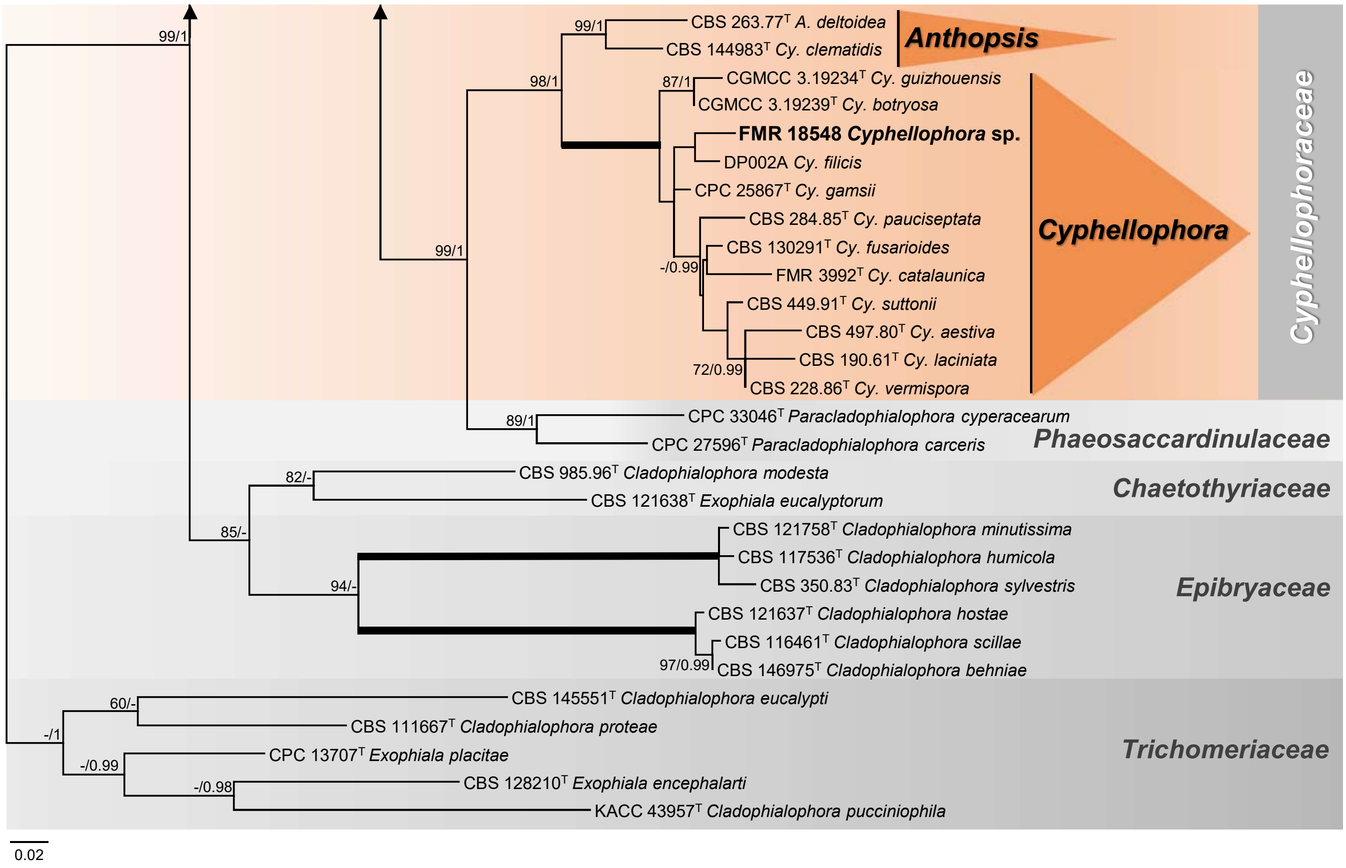

Fig. 2 (cont.)

which were distributed among the following species of *Herpotrichiellaceae* genera: *Capronia* (*Ca.*) *pulcherrima* (n = 1), *Cladophialophora* (*C.*) *emmonsii* (n = 1), *Exophiala* (*E.*) *equina* (n = 1), *E. pisciphila* (n = 1), *E. radicis* (n = 20) and *Phialophora* (*P.*) *americana* (n = 6). However, 17 of the 47 isolates (36.17 %) did not exhibit molecular matches with any known species in the above-mentioned families, although were morphologically determined to be members of *Cladophialophora* (FMR 18641, FMR 18642, FMR 18643, FMR 18992), *Cyphellophora* (FMR 18548), *Exophiala* (FMR 18336, FMR 18551, FMR 18632, FMR 19001), *Phialophora* (FMR 17150, FMR 18984, FMR 18985, FMR 18986, FMR 18996, FMR 18997, FMR 18998) and a rhinocladiella-like isolate (FMR 18994).

For all sediment isolates, phylogenetic analyses were performed using a combination of LSU, ITS, *tub*2 and/or *tef*1-α sequences. These analyses were conducted to confirm the identification and assess the phylogenetic position of the unidentified specimens. Dataset characteristics and substitution models for each data partition are summarised in Table 2. Since tree topologies were similar and congruent between the ML and BI analyses in all cases, we selected ML trees to represent the results obtained (Fig. 1–5).

Phylogeny

The first dataset (Fig. 2) shows the LSU/ITS phylogeny of the families *Herpotrichiellaceae* and *Cyphellophoraceae* and the distribution of the sediment isolates within them. Both families were resolved as monophyletic entities with strong statistical supports. This dataset represents an updated phylogeny recently published for both families of black yeasts (Crous et al. 2020, Quan et al. 2020, Phukhamsakda et al. 2022) and includes exclusively species for which sequences of the two gene markers were available (Table S1). To make it easier to understand the taxonomic position of our isolates and their phylogenetic relationships, basal nodes of the species clades defined in previous studies (Badali et al. 2008, De Hoog et al. 2011, Feng et al. 2014a, b) for *Cladophialophora* s.lat. (i.e., *bantiana* and *carrionii*) and *Exophiala* s.lat. (i.e., *dermatitidis*, *jeanselmei* and *salmonis*), including recently described species for all those clades, were highlighted in the tree. We also stand out all the monophyletic (i.e., *Anthopsis*, *Cyphellophora*, *Minimelanolocus*, *Phialophora*, *Thysanorea* and *Uncispora*) and monotypic genera (i.e., *Aculeata*, *Atrokylindriopsis*, *Marinophialophora*, *Melanoctona*, *Phaeoannellomyces*, and *Valentiella*) currently accepted in both families. The phylogeny of the sediment isolates are introduced below according to their position in this first dataset.

Datasets for the Herpotrichiellaceae

Phialophora isolates were all included in a monophyletic clade (99 % bs/1 pp) representing the genus *Phialophora* (Fig. 2). However, this analysis did not show enough discriminatory power to resolve the identity of the accepted species in *Phialophora*. Therefore, we performed a phylogenetic analysis combining LSU, ITS with *tub*2 and *tef*1-α sequences to delineate *Phialophora* species with precision, including also more sequences of representatives of *Phialophora* species mostly published in Li et al. (2017). This multi-locus analysis (Fig. 3) confirmed the identification of four representative sediment isolates (FMR 18552, FMR 18630, FMR 18983 and FMR 18987) as *P. americana*. Of those, only FMR 18983 matched the well-supported (97 % bs/0.99 pp) terminal clade of the strain CBS 281.35, selected by Li et al. (2017) as a reference strain of the species, and other strains of *P. americana* from different origins (Table S1). The other three isolates (FMR 18552, FMR 18630 and FMR 18987) clustered as an independent clade (99 bs/1 pp) with a Chinese isolate (BMU 04541), but without sufficient distance to be considered a different species. In fact, the above-mentioned Chinese isolate was formerly identified as *P. americana* by Li et al. (2017). The unidentified *Phialophora*

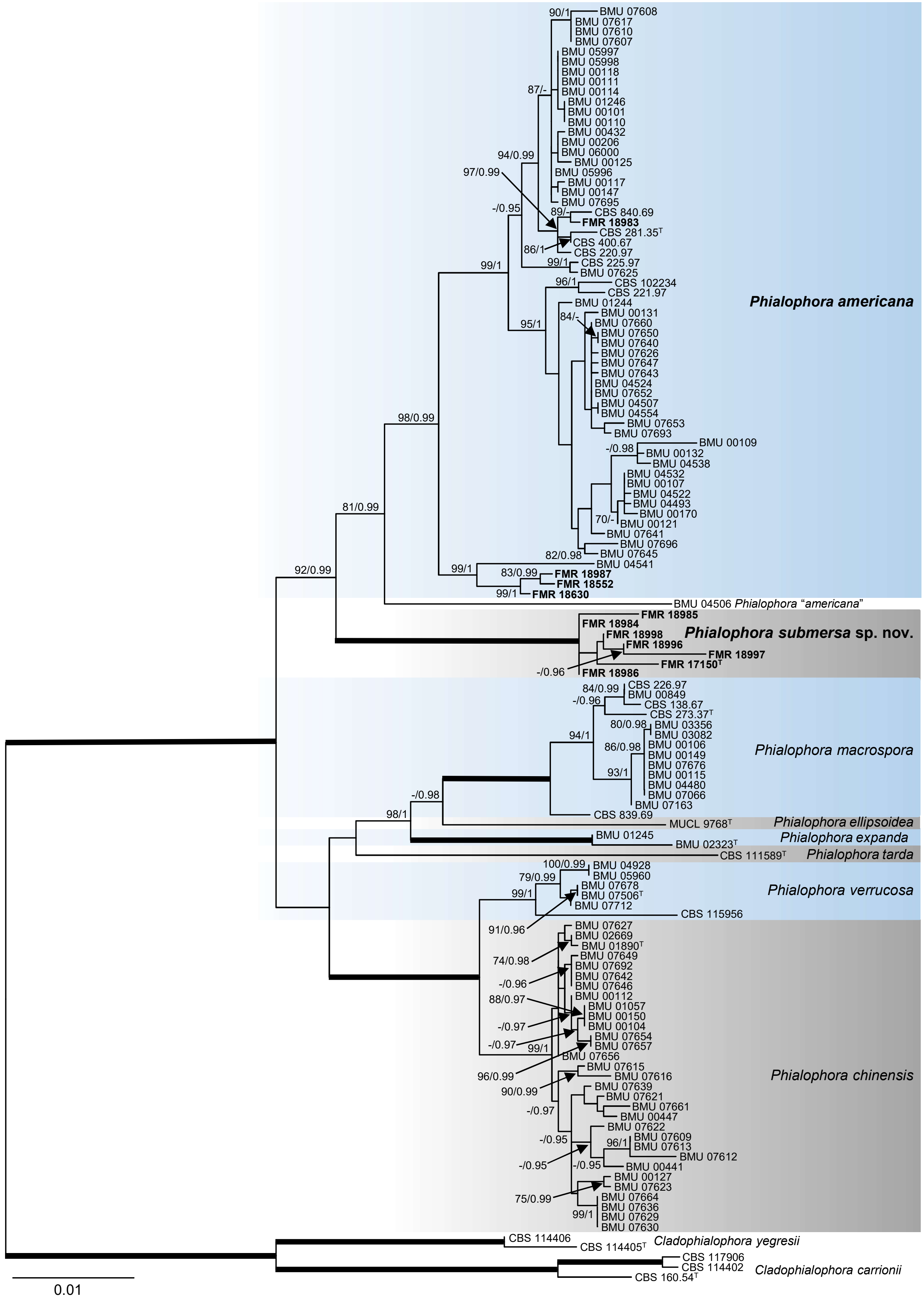

Fig. 3 Phylogenetic tree inferred from a Maximum Likelihood (ML) analysis based on the combined ITS, LSU, *tub*2 and *tef*1-α sequence alignment of *Phialophora* species and outgroups. Branch lengths are proportional to phylogenetic distances. RAxML bootstrap support values/Bayesian posterior probability scores (bs/pp) above 70 % / 0.95 are shown at the nodes. Bold branches represent bs/pp values 100/1. The tree is rooted to *Cladophialophora yegresii* (CBS 114406, CBS 114405) and *Cladophialophora carrionii* (CBS 117906, CBS 114406 and CBS 160.54). Sediment isolates identified in this study and new species described are in **bold**. ^{T} = Ex-type strain.

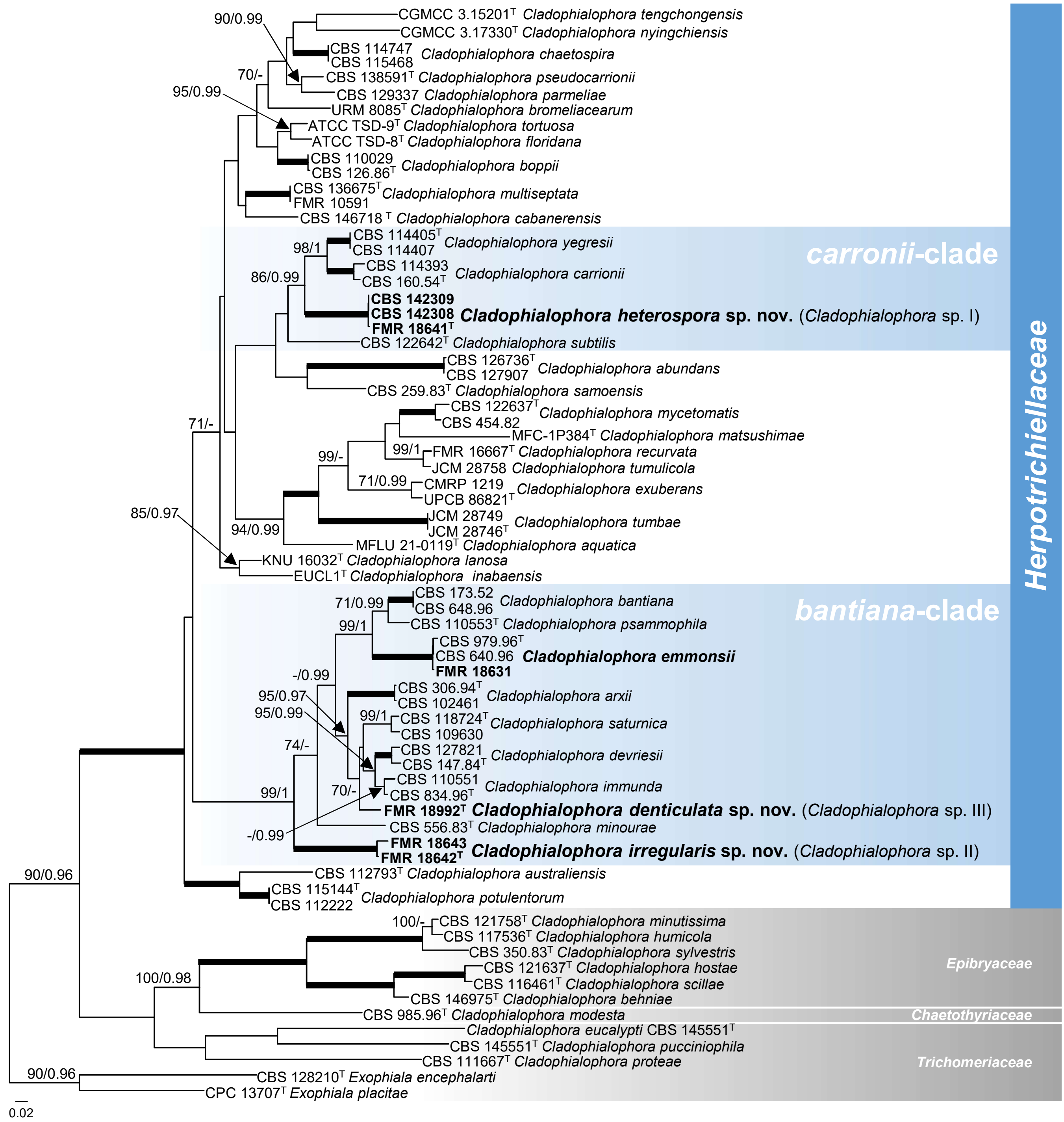

Fig. 4 Phylogenetic tree inferred from a Maximum Likelihood (ML) analysis based on the combined ITS, LSU, *tub*2 and *tef*1-α sequence alignment of *Cladophialophora* species and outgroups. Branch lengths are proportional to phylogenetic distances. RAxML bootstrap support values/Bayesian posterior probability scores (bs/pp) above 70 % / 0.95 are shown at the nodes. Bold branches represent bs/pp values 100/1. The tree is rooted to *Exophiala placitae* (CPC 13707) and *Exophiala encephalarti* (CBS 128210). Sediment isolates identified in this study and new species described are in **bold**. ^{T} = Ex-type strain.

isolates (FMR 17150, FMR 18984, FMR 18985, FMR 18986, FMR 18996, FMR 18997 and FMR 18998) were assigned to a fully supported monophyletic clade (100 % bs/1 pp) basal to the *P. americana* clade. Percentages of identity of this clade vs the *P. americana* clade ranged from 95.3–97 %, 95–96.6 % and 90.3–94.3 % for ITS, *tub*2 and *tef*1-α, respectively. Despite the subtle morphological differences observed in the unidentified isolates compared to *P. americana*, the phylogenetic analysis revealed a significant genetic distance between the respective clades. This supports the proposal of the new species *P. submersa*, to account for these isolates.

In the LSU/ITS phylogeny (Fig. 2), the *Cladophialophora* isolates under study were related with species of the *carrionii*-clade (i.e., *Cladophialophora* sp. I = FMR 18641, CBS 142308, CBS 142309) and *bantiana*-clade (i.e., FMR 18631 as *C. emmonsii*; *Cladophialophora* sp. II = FMR 18642 and FMR 18643; *Cladophialophora* sp. III = FMR 18992). While *Cladophialophora* sp. I matched in the clade (69 bs/0.98 pp) representative of *Cladophialophora* s.str., including the ex-type strain of *C. carrionii*, *Cladophialophora* sp. II and III did not show relevant phylogenetic relationships with any species of the *bantiana*-clade. To resolve the taxonomy of the putative undescribed species, a multi-locus analysis of *Cladophialophora* species s.lat. was conducted with four phylogenetic markers (Fig. 4). In the tree, *Cladophialophora* sp. I clustered in a strongly supported terminal clade (100 % bs/1 pp) with a high statistical support (86 % bs/0.99 pp), related to the monophyletic lineages of *C. carrionii*

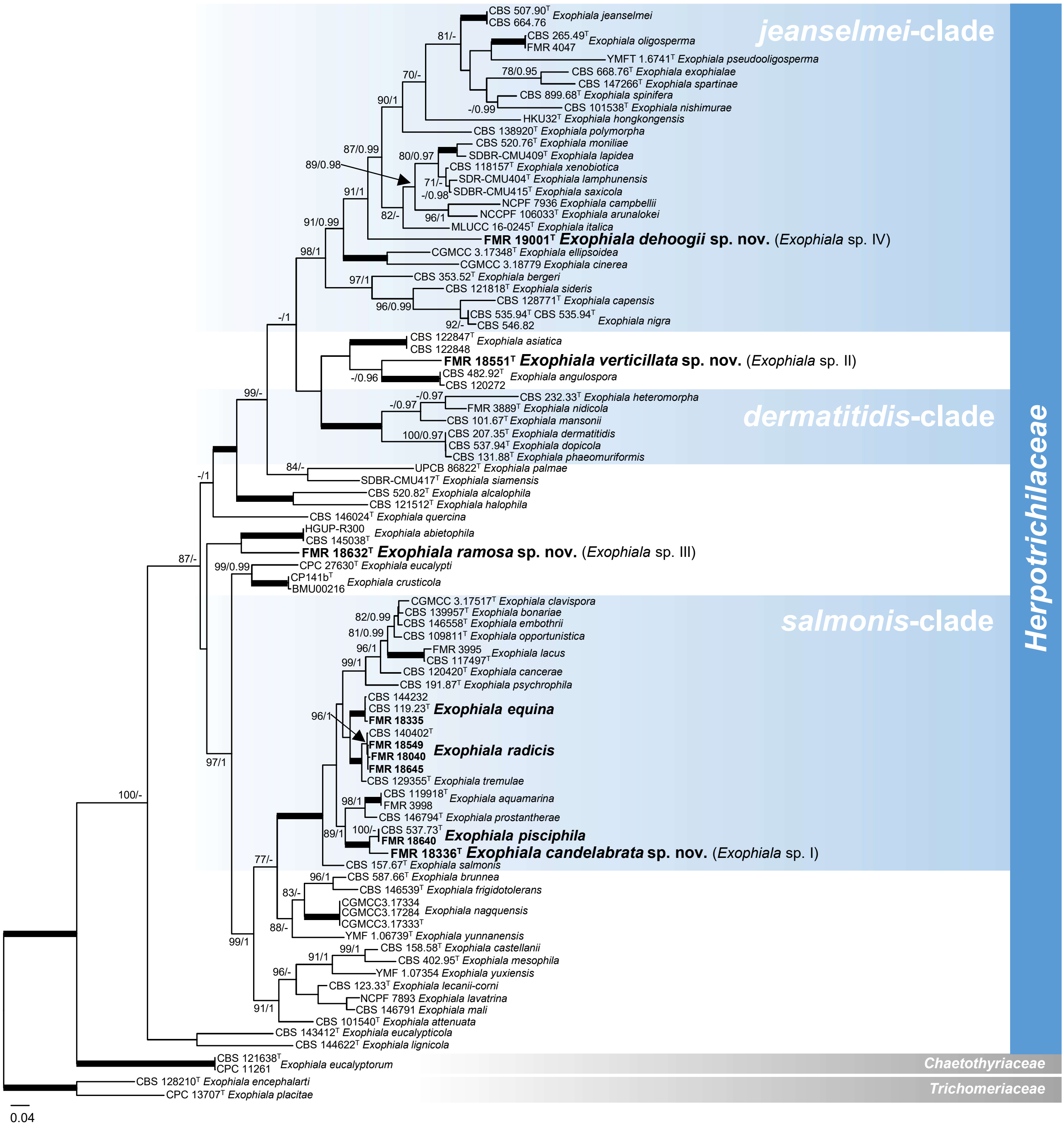

Fig. 5 Phylogenetic tree inferred from a Maximum Likelihood (ML) analysis based on the combined ITS, LSU, *tub*2 and *tef*1-α sequence alignment of *Exophiala* species and outgroups. Branch lengths are proportional to phylogenetic distances. RAxML bootstrap support values/Bayesian posterior probability scores (bs/pp) above 70 % / 0.95 are shown at the nodes. Bold branches represent bs/pp values 100/1. The tree is rooted to *Exophiala placitae* (CPC 13707) and *Exophiala encephalarti* (CBS 128210). Sediment isolates identified in this study and new species described are in **bold**. T = Ex-type strain.

and *C. yegresii*. *Cladophialophora carrionii* was the closest relative, but showing a sequence similarity of 99.15–99.38 % for LSU, 92.02–93.08 % for ITS, and ≤ 84.96 % for the other two gene markers. Therefore, the lineage of *Cladophialophora* sp. I is introduced below as *C. heterospora*. It differs from its counterparts primarily by morphological variation in conidia.

In the same multi-gene analysis (Fig. 4), FMR 18631 clustered in a fully supported terminal clade (100 % bs/1 pp) with the ex-type strain of *C. emmonsii* (CBS 979.96) and a reference strain (CBS 640.96) of this species. *Cladophialophora* sp. II formed a monophyletic lineage distantly related to other species of the *bantiana*-clade, representing an undescribed species which is proposed below as *C. irregularis*. Its closest relative was *C. minourae* but with a similarity of 98.6 % and 86.6–87.3 % for the LSU and ITS markers, respectively, and lower than 73.75 % for the two protein-coding loci. *Cladophialophora* sp. III formed a singleton distant branch which was placed in a clade moderately supported only by ML analysis (70 % bs/ - pp), along with three well-supported species of *C. devriesii*, *C. immunda* and *C. saturnica*. Although *Cladophialophora* sp. III can be distinguished by subtle phenotypic traits such as the cardinal growth temperatures and conidial size, its low sequence similarity with the different gene markers (93.1–95.6 % for ITS, 93 % for *tub*2 and 92–93 % for *tef*1-α) compared to the sequences of the three aforementioned *Cladophialophora* species allows us to introduce the new species *C. denticulata*.

According to the LSU/ITS phylogeny (Fig. 2), the sediment isolates that displayed *Exophiala* asexual morphs, including the one known to be *Ca. pulcherrima*, were distributed as follows: *Exophiala* sp. I (= FMR 18336), and the set of *Exophiala* isolates identified as *E. pisciphila* (FMR 18640), *E. equina* (FMR 18335), and representative isolates of *E. radicis* (FMR 17578, FMR 17630, FMR 18040, FMR 18549, FMR 18645, FMR 19000) were related to *salmonis*-clade. These two latter species together with the ex-type strain of *E. salmonis* formed a well-supported terminal clade (98 bs/0.99 pp) that we interpreted as representative of *Exophiala* s.str. since it included the type species of the genus. FMR 18999, identified as *Ca. pulcherrima*, was closely related to *Ca. camelliae-yunnanensis*, *Ca. pilosella* and *Ca. lijiangenesis*, forming a strongly supported clade (99 bs/1 pp) that could represent the genus *Capronia* since it included the sequence of *Ca. pilosella* AFTOL-ID 657 selected by Quan et al. (2020) as reference for the family *Herpotrichiellaceae* in absence of molecular data from the type of *Capronia*, *Ca. sexdecimspora*. *Exophiala* sp. II (= FMR 18551) clustered in a taxonomically heterogeneous terminal clade (74 bs/0.99 pp) with the ex-type strains of *E. angulospora*, *Ca. coronata*, and that of the monotypic genus *Melanoctona*. *Exophiala* sp. IV (= FMR 19001) formed a single branch into the well-supported clade of *jeanselmei* species complex (85 bs/1 pp). While *Exophiala* sp. III (= FMR 18632) did not exhibit relevant phylogenetic relationships with any species in the family.

An additional multi-gene analysis constructed exclusively for *Exophiala* species with all gene markers (Fig. 5) confirmed the identity of *E. equina*, *E. pisciphila* and *E. radicis* from our sediment isolates into the strongly supported *salmonis*-clade (100 % bs/1 pp). In the same clade, the unnamed *Exophiala* sp. I was shown as a sister to the lineage of *E. pisciphila* in a fully supported terminal clade (100 % bs/1 pp). However, those two lineages showed sufficient genetic distance to be considered separate species. Sequence similarities between members of the *E. pisciphila* clade vs our unidentified isolate were 96.5–96.7 % for ITS, 93.2 % for *tef*1-α and 92.6–93.3 % for *tub*2. These results support the proposal of *Exophiala* sp. I as a novel species, *E. candelabrata*. *Exophiala* sp. II formed a monophyletic divergent branch related to the terminal clade of *E. angulospora*, but this relationship was only supported by BI analysis (- bs/0.96 pp). The significant genetic distance (97.78 % for LSU, 94.2 % for ITS, 80.06 % for *tub*2 and 95.35 % for *tef*1-α sequence similarity) and the relevant morphological features observed in comparison with *E. angulospora*, such as the presence of only ellipsoidal conidia and conidiogenous cells arranged in verticils on branched conidiophores, allow us to describe the new species *E. verticillata*. *Exophiala* sp. III formed a divergent branch allocated in an unsupported clade together with the fully supported lineage of *E. abietophila*. Both fungi are distantly related to *Exophiala* species included in this analysis. The phylogenetic distance of *Exophiala* sp. III with similarity values ranging between 82.7–85.0 % for the ITS region against both *E. abietophila* strains (*tub*2 and *tef*1-α were not available for *E. abietophila*), allows us to propose the new species *E. ramosa*. *Exophiala* sp. IV was placed on a separate branch unrelated to other species of *jeanselmei*-clade and is therefore described below as *E. dehoogii*. Its closest related species was *E. ellipsoidea*, but with a sequence similarity of 97.8 % and 88.4 % for the LSU and ITS barcodes, respectively.

Finally, the phylogenetic relationships and taxonomy of the rhinocladiella-like isolate (FMR 18994) were assessed only through the LSU/ITS dataset (Fig. 2), primarily due to the lack of sequences for other gene markers of its relatives in *Herpotrichiellaceae*. The analysis revealed that FMR 18994 formed an unsupported terminal clade with the ex-type strain of the genus *Atrokylindriopsis* (*At.*), *At. setulosa* (HMAS 245592). Both fungi showed a sequence similarity of 97.58 % and 93.16 % for the LSU and ITS markers, respectively. According to BLAST searches, other close hits for LSU were *E. angulospora* (GenBank LC192082 and NG070601), *E. alcalophila* (GenBank AB192876 and MH873269), *Ca. coronata* (GenBank AF050242) and *E. attenuata* (GenBank KT013094) with sequence similarities of 97.33 %, 96.83 %, 96.79 % and 96.26 %, respectively. Meanwhile, the ITS sequence of our isolate matched only a few unidentified *Chaetothyriales* isolates with a similarity of ≤ 91.68 % (GenBank MZ016273, OM832969, OM833054 and ON555811). These relationships revealed that FMR 18994 represents an undescribed lineage in the *Herpotrichiellaceae*. Since *Atrokylindriopsis* exhibits very different morphological features (i.e., production of pigmented, transversally septate and setulate conidia from phialidic conidiogenous cells; Ma et al. 2015) as opposed to our isolate (i.e., production of subhyaline, one-celled conidia from sympodial and denticulate conidiogenous cells), we therefore propose the novel genus *Aciculomyces* to accommodate *Aciculomyces* (*Ac.*) *restrictus* for the sediment isolate.

Datasets for the Cyphellophoraceae

In the LSU/ITS phylogram, the family *Cyphellophoraceae* was divided in two well-supported lineages representatives of the genera *Anthopsis* and *Cyphellophora*, being the unidentified isolate FMR 18548 allocated in this latter genus (Fig. 2). However, the taxonomic position of this fungus was resolved with an additional multi-locus phylogeny in which all members of the genus *Cyphellophora* were included (Fig. 6). The latter dataset was constructed using LSU, ITS and *tub*2 markers because *tef*1-α sequences were not available for most species of the two genera included in the family. The unidentified *Cyphellophora* isolate was closely related (99 % bs/1 pp) to the *Cyphellophora* (*Cy.*) *suttonii* clade that included its ex-type strain (CBS 449.91), but formed a singleton lineage with significant genetic distance from *Cy. suttonii* strains (94.8–95.5 % for ITS and 96 % for *tub*2 sequence similarity). The phylogenetic distance and differences based primarily on the conidial morphology (see Taxonomy section) support the proposal of the novel species *Cy. spiralis*. In addition, two recently described species of *Cyphellophora* were included in our analyses (Fig. 1, 5); i.e., the ex-type strain of *Cy. clematidis* (CBS 144983) isolated from *Clematis vitalba* in Austria (Crous et al. 2019), and the ex-type and a reference strain of *Cy. neerlandica* (CBS 149512 and CPC 42641, respectively) isolated from lichens on a brick wall in the Netherlands (Crous et al. 2023). In the multi-gene phylogeny (Fig. 6) both fungi clustered as a well-supported lineage (90 % bs/ 0.95 pp) with the ex-type strain of *Anthopsis* (*A.*) *deltoidea* (CBS 263.77), the type species of the genus. Although our phylogeny provides support for the lineage of *Anthopsis* as a sister to *Cyphellophora* (91 % bs/1.0 pp), the inconsistent morphological features observed in both aforementioned *Cyphellophora* species hinder the introduction of taxonomical changes at this time (see also Discussion). Further studies are needed to elucidate and confirm the taxonomic status of these species.

Distribution of the isolates identified

When comparing the rivers and streams where the identified black yeasts were recovered, it is interesting to note that 91.5 % of the isolates were found in the Llobregat, Ter and Remáscaro Rivers (34 %, 32 % and 25.5 %, respectively). But in terms of diversity, the former two rivers showed the highest diversity, with seven (*Ac. restrictus*, *Ca. pulcherrima*, *C. denticulata*, *E. dehoogii*, *E. radicis*, *P. americana* and *P. submersa*) and nine (*C. emmonsia*, *C. heterospora*, *C. irregularis*, *Cy. spiralis*,

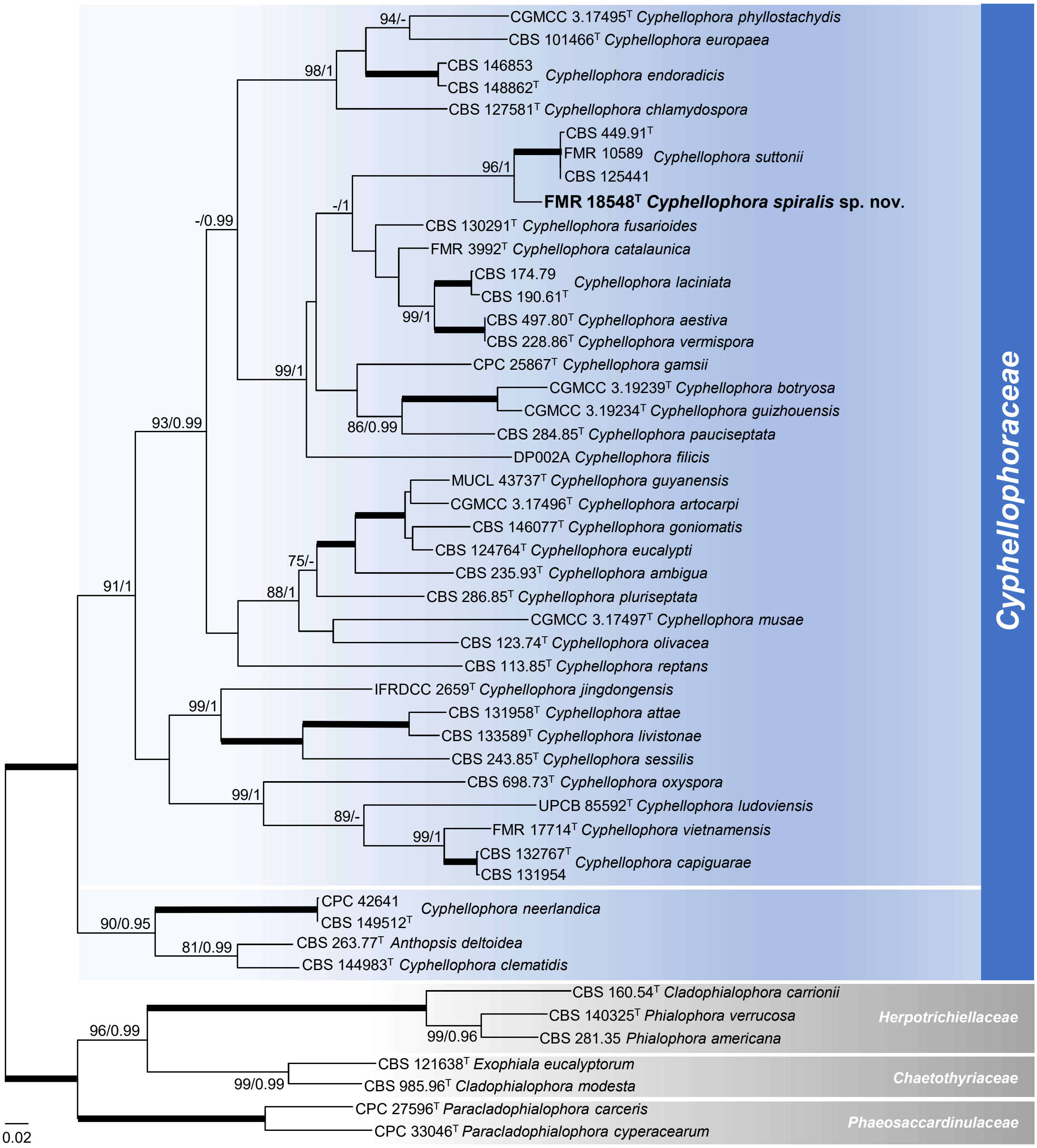

Fig. 6 Phylogenetic tree inferred from a Maximum Likelihood (ML) analysis based on the combined ITS, LSU and *tub*2 sequence alignment of *Cyphellophoraceae* members and outgroups. Branch lengths are proportional to phylogenetic distances. RAxML bootstrap support values/Bayesian posterior probability scores (bs/pp) above 70 % / 0.95 are shown at the nodes. Bold branches represent bs/pp values 100/1. The tree is rooted to several members of the families *Chaetothyriaceae*, *Herpotrichiellaceae*, *Phaeosaccardinulaceae*. New species described in this study is in **bold**. ^{T} = Ex-type strain.

E. pisciphila, *E. radicis*, *E. ramosa*, *E. verticillata* and *P. americana*) species identified, respectively, while in the Remáscaro River only *E. radicis* was recovered (Table 1). No other special correlation was found regarding river distribution of the fungi identified.

Taxonomy

Aciculomyces Torres-Garcia, Gené & Dania García, *gen. nov.* — MycoBank MB 846803

Etymology. Latin, *acicŭla*, diminutive of needle, referring to the resemblance of the conidiophores to small needles.

Type species. *Aciculomyces restrictus* Torres-Garcia, Dania García & Gené.

Colonies growing slowly, olivaceous green to dark green, villose to fasciculate. *Mycelium* partly superficial, partly immersed, composed of branched, septate, brown to dark brown, smooth-walled hyphae. *Conidiophores* erect, straight or flexuous, unbranched, continuous or with few septa, dark brown at the base, paler towards the apex, smooth- and thick-walled. *Conidiogenous cells* integrated, terminal or intercalary, polyblastic, with sympodial proliferation, denticulate. *Conidia* solitary, aseptate, subhyaline to pale brown, smooth-walled, rather thin-walled, obovoid or subglobose, with somewhat prominent and slightly pigmented scars; conidial secession schizolytic. *Sexual morph* unknown.

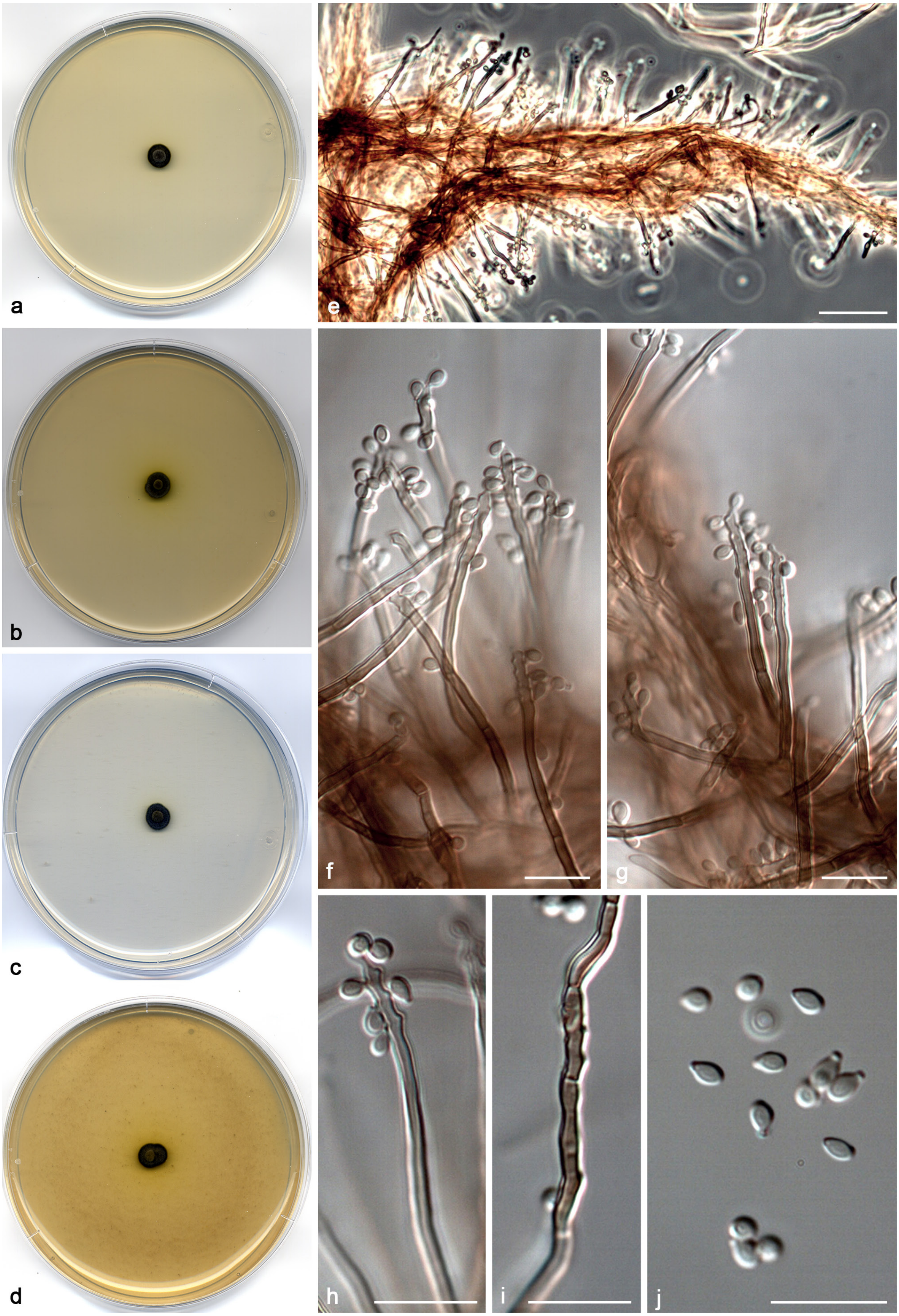

Fig. 7 *Aciculomyces restrictus* (ex-type FMR 18994). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–g. conidiophores from OA; h–i. detail of terminal and intercalary conidiogenous cells; j. conidia. — Scale bars: e = 25 μm, f–j = 10 μm.

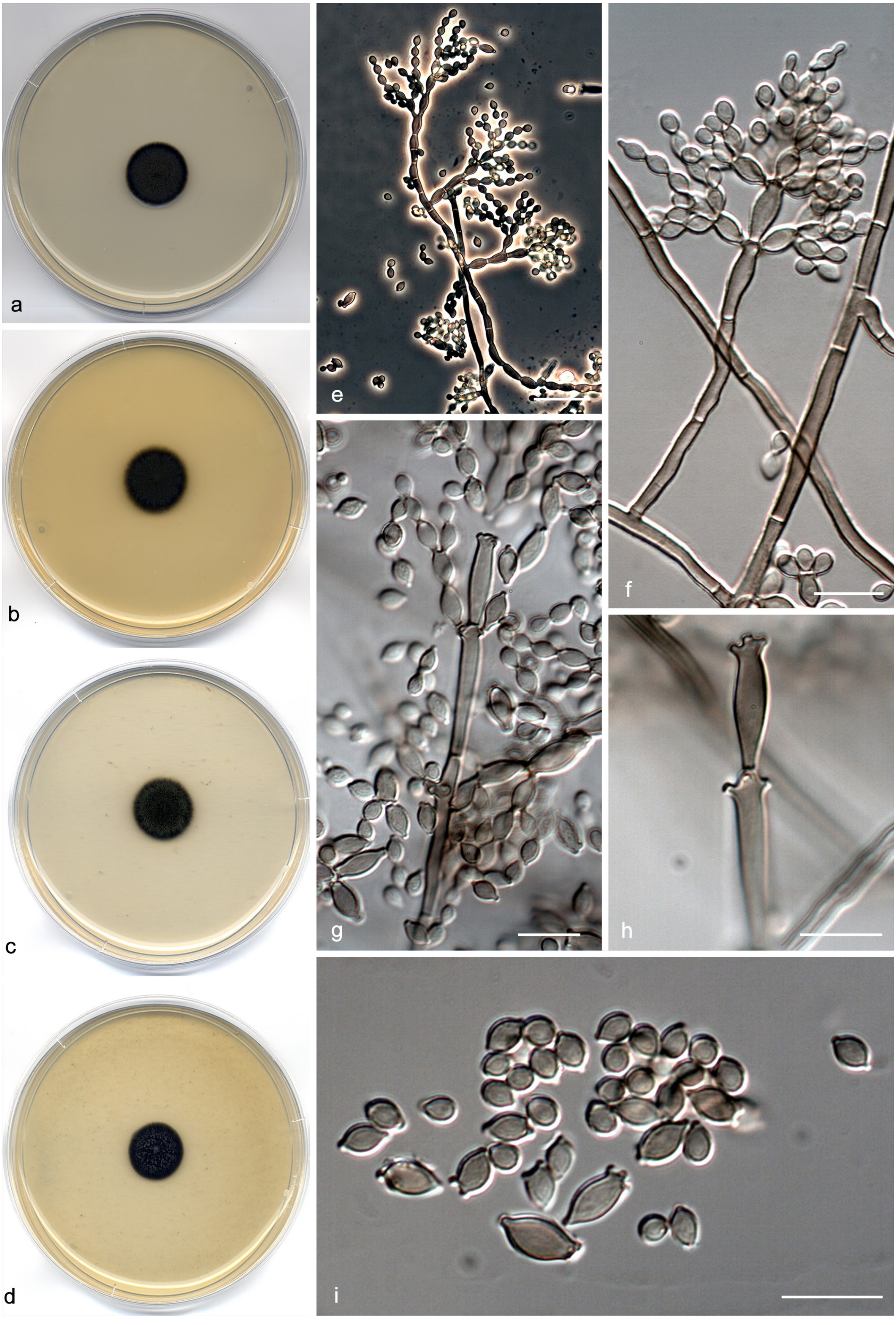

Fig. 8 *Cladophialophora denticulata* (ex-type FMR 18992). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–g. conidiophores from OA; h. denticulate conidiogenous cells; i. ramoconidia and conidia. — Scale bars: e = 25 μm, f–i = 10 μm.

Aciculomyces restrictus Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846804; Fig. 7

Etymology. Latin, *restrictus*, restricted, restrained, referring to the restricted growth of the fungus in all culture media tested.

Typus. Spain, Catalonia, Berguedà, Castellar de n'Hug, Llobregat River, N42.27967° E2.00535°, from fluvial sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25215, culture ex-type FMR 18994 = CBS 149850).

Mycelium composed of pale olivaceous to dark green, smooth-walled, septate, branched hyphae, 2.5–4 µm wide. *Conidiophores* often arising orthotropically from loose fascicles, straight or slightly flexuous, unbranched, continuous or 1–2-septate, dark brown, paler terminally, smooth- and thick-walled, somewhat cylindrical, 19–105.5 × 1.5–2.5 µm. *Conidiogenous cells* integrated, terminal or intercalary, proliferating sympodially and producing conidia from short denticles, brown to pale brown, subcylindrical, 14–40 × 1.5–2.5 µm; denticles scattered, slightly pigmented, up to 0.5 µm wide. *Conidia* solitary, subhyaline to pale brown, smooth-walled, obovoid or subglobose, occasionally ellipsoidal, 2–4 × 1.5–2.5 µm, with a prominent and slightly pigmented scar up to 0.5 µm wide. *Chlamydospores* and *budding cells* absent.

Culture characteristics (after 14 d at 25 °C) — Colonies were similar in all culture media tested, reaching 9–11 mm diam. On PDA, raised and cactus green (28E4) at centre, flat and dark green (29F8) towards periphery, velvety, margin entire, sporulation sparse; reverse dark green (28F8), diffusible pigment absent. On MEA, raised and leaf green (30D6) at centre, flat and grey green (29D4) towards periphery, velvety, margin entire, sporulation absent; reverse deep green (30E8), with a yellow green diffusible pigment. On PCA, raised and grass green (30E7) at centre, flat and dull green (30E4) towards periphery, velvety, margin entire, sporulation sparse; reverse dark green (28F8), diffusible pigment absent. On OA, raised and deep green (30E8) at centre, flat and grey green (30E5) towards periphery, velvety, margin entire, sporulation moderate; reverse parsley green (30F8), with a yellow diffusible pigment.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (2–3), optimum 25 °C (9–10), maximum 30 °C (2–3). No growth at 35 °C.

Notes — *Aciculomyces* resembles members of the genus *Ramichloridium* and *Rhinocladiella* in its conidiogenous apparatus characterised by brown, unbranched conidiophores with a denticulate rachis, and pale brown conidia with slightly thickened hila (Arzanlou et al. 2007). However, *Ramichloridium* is currently allocated in the family *Mycosphaerellaceae* (*Capnodiales*) and, although *Rhinocladiella* belongs to *Herpotrichiellaceae*, its type species *R. atrovirens* as well as other species of this genus are unrelated to *Aciculomyces*. In our phylogenetic analysis (Fig. 2), *Ac. restrictus* is placed in an unsupported clade together with *At. setulosa* and *Ca. fungicola*, with the former being the most closely related species. *Atrokylindriopsis* is a monotypic genus described from dead branches of a broadleaf tree in China and *At. setulosa*, its type species, is morphologically distinguished by phialidic conidiogenous cells that produce big, septate and setulous conidia (Ma et al. 2015). *Aciculomyces restrictus* resembles the asexual morph of *Ca. fungicola*. This species was originally described as *Berlesiella fungicola* and, in addition to the sexual morph (i.e., immersed, astromatic, black, globose ascomata, with bitunicate asci and phragmospores), it was characterised by branched, slender conidiophores with local annellations giving rise to small conidia (2.5–3.8 × 1.8–2.5 µm), and by the production of budding cells (Samuels & Müller 1978, De Hoog et al. 1983), features not observed in *Ac. restrictus*. *Capronia fungicola* was originally described on ascomata of *Trematosphaeria* sp. (Samuels & Müller 1978), but currently it has been reported worldwide as a commonly occurring species associated with other fungal taxa and, consequently, with a clear mycoparasitic lifestyle (Untereiner 1994).

Cladophialophora denticulata Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846238; Fig. 8

Etymology. Latin, *denticulātus*, denticulate, furnished with small teeth, referring to the conspicuous denticles present in conidiogenous cells and ramoconidia.

Typus. Spain, Catalonia, Berguedà, Guardiola de Berguedà, Llobregat River, N42.24012° E1.93742°, from fluvial sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25182, culture ex-type FMR 18992 = CBS 149643).

Mycelium composed of hyaline to olivaceous brown, smooth- and thin-walled, septate, branched hyphae, 1.5–3 µm diam. *Conidiophores* solitary, macronematous, erect, straight or slightly flexuous, olivaceous brown, branched, with terminal and lateral ramifications from conidiogenous cells and ramoconidia, up to 260 µm long. *Conidiogenous cells* terminal, lateral or intercalary, pale olivaceous, smooth-walled, sympodial, cylindrical to subcylindrical, 6–15.5 × 2.5–3.5 µm, with up to 3 conspicuous denticles, 0.5–1.5 µm wide. *Ramoconidia* one-celled, pale olivaceous, smooth-walled to finely roughened, subcylindrical, ellipsoidal to somewhat fusiform, 4–11.5 × 2.5–4.5 µm, with 2–5 conspicuous denticles at the apex, 0.5–1 µm wide. *Conidia* in acropetal chains with up to 5 conidia, one-celled, pale olivaceous, smooth-walled to finely roughened, ellipsoidal, lemon-shaped or obpyriform, 2.5–4.5 × 2–3.5 µm, terminal conidial scars narrow, denticulate and pale brown. *Budding cells* and *chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies on PDA, 22–24 mm diam, elevated, velvety, dull green (29E3) at centre to dark green (29F6) at periphery, margin slightly fimbriate, sporulation abundant; reverse deep green (28F8). On MEA, 23–26 mm diam, slightly raised at centre, velvety, cactus green (28E4) at centre to dark green (29F6) at periphery, margin fimbriate, sporulation abundant; reverse dark green (30F7) at centre to grass green (30E7) towards periphery. On PCA, 21–22 mm diam, slightly raised and grey green (28E5) at centre, parrot green (30E8) at periphery, velvety to slightly cottony, margin fimbriate, sporulation abundant; reverse dark green (28F8) at centre, grass green (30E7) towards periphery. On OA, 20–21 mm diam, flattened, dark green (30F7) to celadon green (30D3) at centre, dark green (29F8) at periphery, velvety to slightly cottony, margin slightly fimbriate, sporulation abundant; reverse olive brown (4F8) at center to parsley green (30F8) towards periphery. Diffusible pigment absent in all culture media tested.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 10 °C (6–7), optimum 30 °C (29–30), maximum 37 °C (5–6). No growth at 5 °C and 40 °C.

Notes — It was revealed that *C. denticulata* formed a singleton lineage within poorly supported clade comprising *C. devriesii*, *C. immunda* and *C. saturnica* (Fig. 4). The three species have been described as causal agents of human infections, and the latter two have also been found to be able to degrade polyaromatic hydrocarbons (Gonzalez et al. 1984, Satow et al. 2008, Badali et al. 2008, 2009, De Hoog et al. 2020). *Cladophialophora devriesii*, *C. immunda* and *C. saturnica* resemble *C. denticulata* in dry, lemon-shaped or obpyriform conidia arranged in branched acropetal chains on conspicuous denticles, and by its ability to grow at 36/37 °C (Badali et al. 2008, 2009). However, *C. denticulata* differs from *C. devriesii* in longer conidiophores (260 vs 60 µm), and shorter conidial chains (2–5 vs 3–8 conidia in *C. devriesii*). Although microscopic features

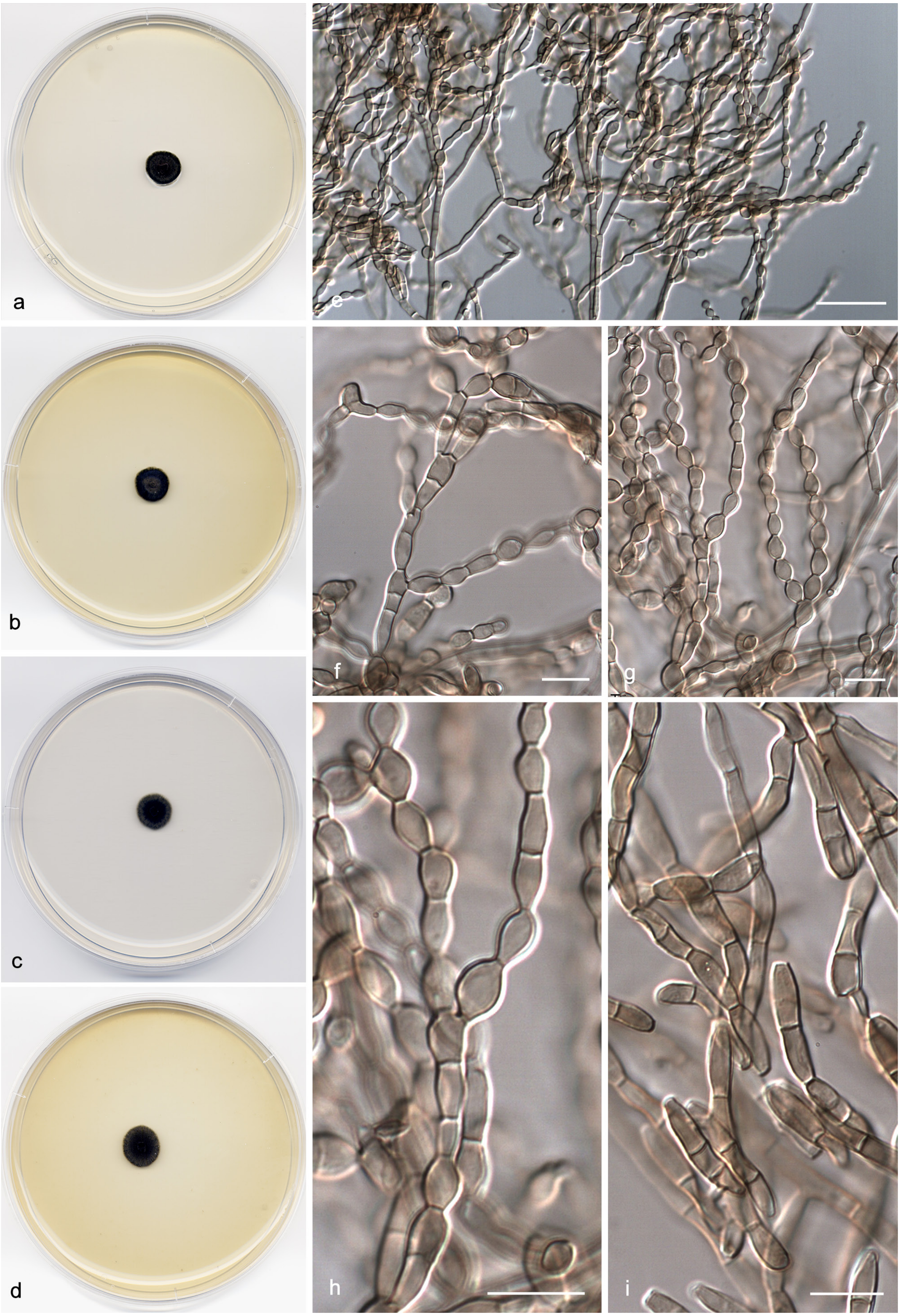

Fig. 9 *Cladophialophora heterospora* (ex-type FMR 18641). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–g. conidiophores from OA; h–i. conidial chains. — Scale bars: e = 25 µm, f–i = 10 µm.

of *C. denticulata* and *C. immunda* are very similar, they can be distinguished by their minimum growth temperature, which is 10 °C for the former and 15 °C for *C. immunda* (Badali et al. 2008). *Cladophialophora denticulata* and *C. saturnica* are phenotypically practically indistinguishable; they only differ in that the conidia of the former species tend to be finely roughened, while in *C. saturnica* they are smooth-walled. Unfortunately, the size of the conidiogenous apparatus, including conidia, is not included in the protologue of *C. saturnica* for comparison (Badali et al. 2009).

Cladophialophora heterospora Torres-Garcia, Jurjević, Réblová, Hubka & Gené, *sp. nov.* — MycoBank MB 846239; Fig. 9

Etymology. Latin, *hetero*-, different, other, and Latin, *spora*, spore, referring to the morphological variability of the conidia produced by the ex-type strain.

Typus. Spain, Catalonia, Osona, Vilanova de Sau, Ter River, Sau's Swamp, N41.97084° E2.38108°, from fluvial sediments, Sept. 2020, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25183, culture ex-type FMR 18641 = CBS 149644).

Mycelium composed of hyaline to pale olivaceous, smooth-walled, septate and branched hyphae, 2.5–4 μm wide. *Conidiophores* solitary, micronematous, pale brown, smooth-walled, consisting of ascending fertile hyphae, often apically branched giving conidial chains. *Conidiogenous cells* integrated, terminal or lateral, occasionally intercalary, pale brown, smooth-walled, with 1–2 conidiogenous loci, up to 1.5 μm wide, cylindrical, subcylindrical or ellipsoidal, 5–19 × 2.5–4 μm. *Ramoconidia* pale brown, smooth-walled, 0–2-septate, with 1–3 non-denticulate conidiogenous loci, cylindrical, fusiform or subglobose, 7–18 (–20.5) × 2.5–4.5 μm. *Conidia* subhyaline to pale olivaceous, smooth-walled, arranged in long branched acropetal chains, conidia of two types: conidia 0–1(–2)-septate, cylindrical to somewhat fusiform, 9.5–20(–25) × 3–4 μm (up to 3-septate and 35 μm long in CBS 142308), or conidia one-celled, subcylindrical, ellipsoidal, globose to subglobose, 3–8.5 × 2–5 μm, often remaining attached in chains. *Budding cells* absent. *Chlamydospores* absent. *Spiral hyphae* occasionally present only in CBS 142308. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies were similar in all culture media tested, reaching to 11–12 mm diam, and no diffusible pigment was produced. On PDA, slightly raised and dull green (30E3) at centre, flattened and dark green (28F7) towards periphery, velvety, margin entire, sporulation absent; reverse dark green (28F2) to parsley green (30F8). On MEA, slightly elevated and grey green (30E4) at centre, flattened and parsley green (30F8) towards periphery, velvety, margin regular and slightly fimbriate, sporulation sparse; reverse dark green (28F8). On PCA, slightly raised and dark green (28F4) at centre, flattened and cactus green (28E4) towards periphery, velvety, margin regular and slightly fimbriate, sporulation sparse; reverse dark green (28F4) to dull green (28E3). On OA, slightly raised, grey green (29E4) and often with rope-like structures at centre, flattened, velvety and dark green (29F7) towards periphery, margin regular and fimbriate, sporulation abundant in FMR 18641 (sparse in CBS 142308 and CBS 142309); reverse dark green (28F8).

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (3–4), optimum 30 °C (14–15), maximum 35/37 °C (2–7/0–3). No growth at 40 °C.

Additional specimens examined. USA, New Jersey, from a non-potable water sample, Sept. 2013, *V. Jurjević* (CBS 142309); ibid., from a non-potable water sample, Sept. 2013. *V. Jurjević* (CBS 142308).

Notes — The lineage of *C. heterospora* is represented by three isolates, all from aquatic environments, one obtained during our survey of microfungi inhabiting freshwater sediments, and the other two from non-potable water collected in the USA. Despite their distant geographical origin, the Spanish isolate showed a high percentage of similarity with the USA isolates (99.6 % for ITS, 99.2 % for *tub*2 and 99.3 % for *tef*1-α sequences). *Cladophialophora heterospora* is closely related to *C. carrionii* and *C. yegresii* in the *carrionii*-clade (Fig. 4). While the former is a well-known causal agent of human chromoblastomycosis, *C. yegresii* is a non-pathogenic species, associated with asymptomatic cactus (*Stenocereus griseus*) (De Hoog et al. 2007, 2020). All three species share poorly differentiated conidiophores with fertile hyphae ascending gradually over chains of pale olivaceous green conidia, and they are all able to grow at 35–37 °C (De Hoog et al. 2007, Badali et al. 2008). However, *C. heterospora* mainly differs by conidia that are differently shaped (i.e., cylindrical, subcylindrical, fusiform, ellipsoidal, globose or subglobose, and 0–2(–3)-septate) and larger (3–25(–35) × 2–5 μm) in comparison to their two counterparts (4.5–6 × 2.5 μm in *C. yegresii* and 2–3 × 1.5–2 μm in *C. carrionii*). Moreover, the phialidic synasexual morph described for *C. carrionii* was not observed in *C. heterospora* nor in *C. yegresii* (Honbo et al. 1984, De Hoog et al. 2007).

Cladophialophora irregularis Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846240; Fig. 10

Etymology. Latin, *irregularis*, irregular, referring to the irregularly shaped conidia produced by the ex-type strain.

Typus. Spain, Catalonia, Osona, Vilanova de Sau, Ter River, Sau's Swamp, N41.97084° E2.38108°, from fluvial sediments, Sept. 2020, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25184, culture ex-type FMR 18642 = CBS 149645).

Mycelium composed of pale brown, smooth-walled, septate, branched hyphae, 2.5–4.5 μm wide. *Conidiophores* semi-macronematous to micronematous, pale to medium brown, dark brown in older cultures, smooth-walled, erect, straight to slightly flexuous, consisting of ascending fertile hyphae giving apically branched conidial chains, up to 75 μm long. *Conidiogenous cells* integrate, mono- to polyblastic, with terminal or lateral, non-denticulate conidiogenous loci, without darkened scars, pale to medium brown, smooth-walled, cylindrical to somewhat fusiform, 9.5–19 × 2.5–4 μm. *Ramoconidia* pale to medium brown, smooth-walled, 0–1-septate, with up to 2 non-denticulate conidiogenous loci, subcylindrical, often slightly curved or sinuous, 7.5–12.5 × 2–5 μm. *Conidia* pale to medium brown, darker in older cultures, produced in long chains, often curved, sinuous or irregularly shaped, in a minor proportion subcylindrical or ellipsoidal, 5.5–7.5 × 3–4.5 μm, with truncate and not darkened scars. *Budding cells* and *chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies on PDA, 16–17 mm diam, slightly raised, dark green (28F3) and cottony at centre, flattened, parsley green (30F8) and velvety towards periphery, margin regular and fimbriate, sporulation absent; reverse dark green (27F8). On MEA, 12–13 mm diam, slightly raised and green grey (28F2) at centre, flattened and dark green (28F5) towards periphery, margin regular and slightly fimbriate, sporulation absent; reverse dark green (28F5). On PCA, 13–14 mm diam, dark green (27F8), velvety, margin regular and fimbriate, sporulation absent; reverse dark green (28F8). On OA, 16–17 mm diam, slightly elevated and green grey (28E2) at centre, flattened and dark green (28F6) towards periphery, margin regular and fimbriate, sporulation absent (only sparsely produced on OA with plant material); reverse parsley green (30F8). Diffusible pigment absent in all culture media tested.

Fig. 10 *Cladophialophora irregularis* (ex-type FMR 18642). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–f. conidiophores from OA; g–h. conidial chains and conidia. — Scale bars: e = 25 μm, f–h = 10 μm.

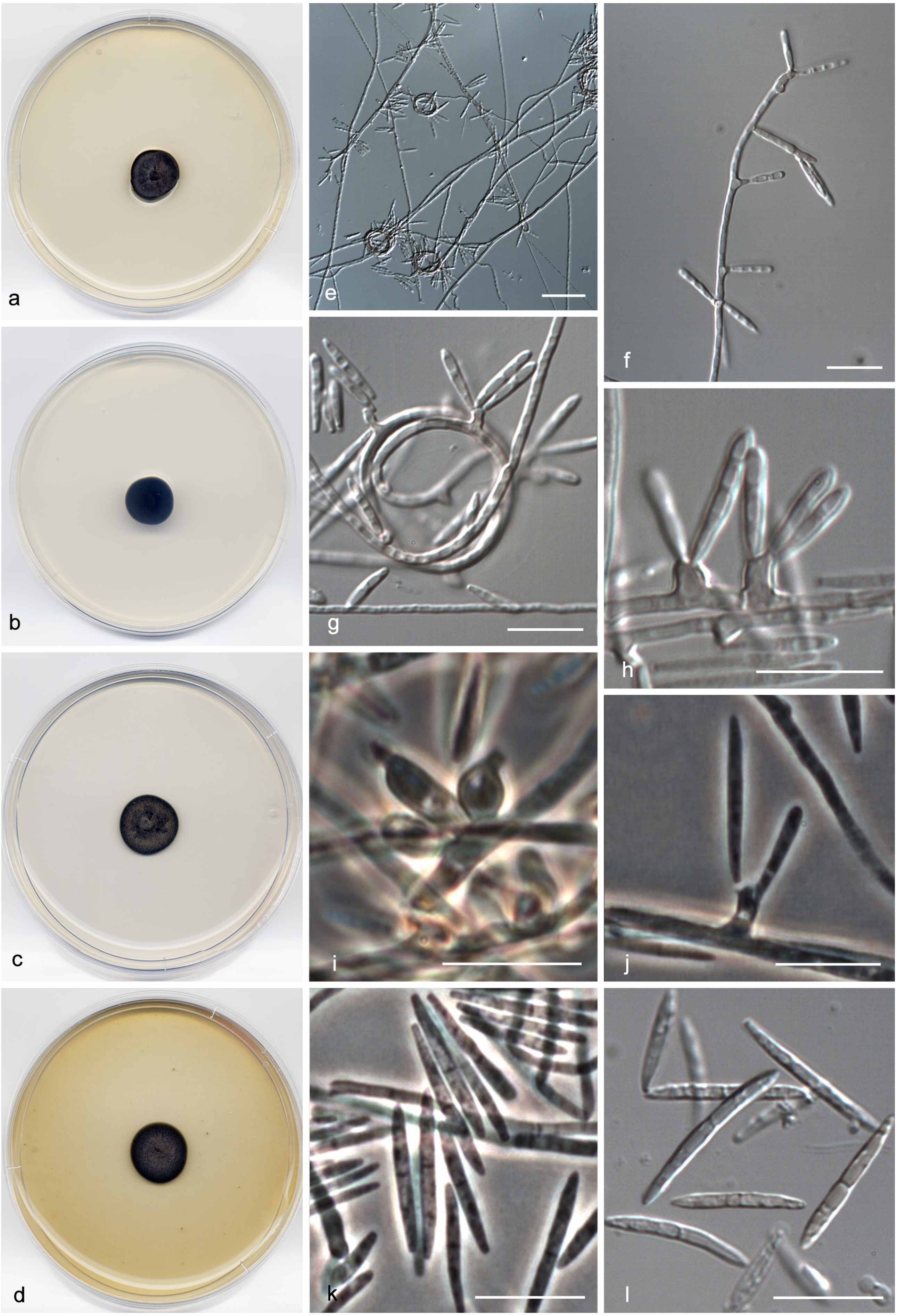

Fig. 11 *Cyphellophora spiralis* (ex-type FMR 18548). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–g. conidiophores and spiral hyphae from OA; h–j. conidiogenous cells; k–l. conidia. — Scale bars: e = 25 µm, f–l = 10 µm.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (3–4), optimum 30 °C (19–20), maximum 37 °C (2–3). No growth at 40 °C.

Additional specimen examined. Spain, Catalonia, Baix Empordà, Torroella de Montgrí, Ter River, N42.03005° E3.13468°, from fluvial sediments, Sept. 2020, *D. Torres-Garcia* & *J. Gené* (FMR 18643).

Notes — According to our phylogeny (Fig. 4), *C. irregularis* is allocated in a well-supported monophyletic basal subclade in the *bantiana*-clade, which includes other nine *Cladophialophora* species (i.e., *C. arxii*, *C. bantiana*, *C. denticulata*, *C. devriesii*, *C. emmonsii*, *C. immunda*, *C. minourae*, *C. psammophila* and *C. saturnica*). *Cladophialophora irregularis* resembles *C. bantiana*, *C. emmonsii* and *C. psammophila* in the production of poorly differentiated conidiophores and non-denticulate conidiogenous loci. This is in contrast to the rest of species of the *bantiana*-clade that mostly show macronematous conidiophores and conidiogenous cells and ramoconidia with protuberant denticles. In addition to the phylogenetic distance, those three species can be distinguished from *C. irregularis* by the following features: *C. bantiana* produces ellipsoidal to fusiform, longer (6–11 µm) conidia, and it is able to grow at 40 °C (De Hoog et al. 1995, 2020); *C. emmonsii* shows conidial chains composed of predominantly broadly ellipsoidal conidia and has a faster growth (30–32 mm in 2 wk) on PDA at 25 °C (Padhye et al. 1988, De Hoog et al 2020); and *C. psammophila* grows faster (30 mm diam in 2 wk) on PDA and MEA at its optimum temperature for growth (27–30 °C), and it was unable to grow below 9 °C (Badali et al. 2011).

Cyphellophora spiralis Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846245; Fig. 11

Etymology. Latin, *spiralis*, a spiral curve, shape, or pattern, referring to the abundant production of spiral hyphae by the ex-type strain.

Typus. Spain, Catalonia, Osona, Les Masies de Voltregà, Ter River, Gallifa's Fluvial island, N42.02951° E2.25359°, from fluvial sediments, Sept. 2020, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25185, culture ex-type FMR 18548 = CBS 149646).

Mycelium composed of subhyaline to pale olivaceous, smooth-walled, septate, branched hyphae, 1–2 µm diam. *Conidiophores* micronematous, often reduced to conidiogenous cells growing directly or on short supporting cells from vegetative hyphae. *Conidiogenous cells* intercalary, laterally or terminally on hyphae, pale-olivaceous, smooth-walled, mostly with inconspicuous collarets and producing conidia more or less sympodially; intercalary, cylindrical, with a lateral conidiogenous protrusion, conical, cylindrical or irregularly shaped, (1.5–)2–14.5 × (1–)1.5–5.5 µm; discrete phialidic conidiogenous cells present, subcylindrical or ampulliform, 3–6 × 1.5–3 µm. *Conidia* 0–2(–3)-septate, constricted at the septa, pale olivaceous, smooth-walled, subcylindrical to fusiform, straight or slightly curved, 9–18 × 1–2 µm, with slightly truncate base. *Spiral hyphae* abundant, growing laterally on vegetative hyphae. *Chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies were similar in all culture media tested, reaching to 16–18 mm diam, and no diffusible pigment was produced. On PDA, slightly raised at centre, radially sulcate, green grey (30E2) to dark green (29F7), velvety, margin entire, sporulation absent; reverse dark green (28F6). On MEA, flattened, grass green (30E7) at centre, parsley green (30F8) towards periphery, velvety, margin entire, sporulation absent; reverse parsley green (30F8). On PCA, slightly raised and lavender green (30D3) at centre, dark green (30F5) at periphery, slightly cottony to velvety, margin entire, sporulation sparse; reverse nickel green (27F3) to dark green (27F8). On OA, flattened, lavender green (30D3) and cottony at centre, parrot green (30E8) and velvety towards periphery, margin entire, sporulation abundant; reverse parsley green (30F8) to parrot green (30E8).

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (3–4), optimum 25 °C (16–18), maximum 30 °C (3–4). No growth at 35 °C.

Notes — Based on the multi-locus analysis (Fig. 6), *Cy. spiralis* is allocated in a terminal well-supported clade together with *Cy. suttonii*. The latter species was described as a causative agent of dog ear phaeohyphomycosis in the USA, but was also isolated from soil in Brazil and Spain (Nunes et al. 1999, Decock et al. 2003, Madrid et al. 2016). The new species differs from *Cy. suttonii* by the production of shorter conidia (9–18 µm vs (10–)15–20(–30) µm), with fewer septa ((0–2(–3) vs 3–8-septate), and by the pale olivaceous colour shades of conidia in contrast to the brown shades produced in *Cy. suttonii* (Feng et al. 2014b). Moreover, hyphal coils were not described in *Cy. suttonii* and the minimum temperature for growth in this species was 21 °C (Feng et al. 2014b), while *Cy. spiralis* is able to grow at 5 °C.

Exophiala candelabrata Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846246; Fig. 12

Etymology. Latin, *candelabrum*, chandelier, Latin, *-ata*, likeness, referring to the morphological resemblance of the conidiophore structure of the ex-type strain to a chandelier.

Typus. Spain, Catalonia, El Segrià, La Granja d'Escarp, Segre River, N41.42754° E0.35020°, from fluvial sediments, Dec. 2019, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25216, culture ex-type FMR 18336 = CBS 149778).

Mycelium composed of hyaline to pale brown, smooth-walled, branched, septate hyphae, 2.5–3 µm diam. *Conidiophores* macronematous to semi-macronematous, erect, septate, bearing conidiogenous cells in divergent branched systems, 14.5–97.5 µm long, or micronematous and reduced to conidiogenous cells born solitary or in loose clusters, laterally or terminally on hyphae. *Conidiogenous cells* discrete, terminal or lateral, pale brown, smooth-walled, flask-shaped, with inconspicuous, often subterminal annellated zones, 4.5–9 × 2.5–4.5 µm. *Conidia* one-celled, guttulate, pale brown, smooth-walled, broadly ellipsoidal to ellipsoidal, 3.5–6(–7.5) × 2.5–3 µm, often forming palisades alongside the hyphae or accumulating in slimy heads on the conidiogenous loci. *Budding cells* and *chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies on PDA reaching 22–23 mm diam, slightly raised and radially sulcate at centre, cactus green (28E4) to dark green (28F8), velvety, margin entire, sporulation sparse; reverse dark green (29F6). On MEA, 24–25 mm diam, flattened, velvety, parsley green (30F8) at centre, grass green (30E7) towards periphery, margin entire, sporulation sparse; reverse dark green (30F6) at centre to grass green (30E7) towards periphery. On PCA, 23–24 mm diam, flattened, velvety, parsley green (30F8) at centre, grass green (30E7) towards periphery, margin regular and slightly fimbriate, sporulation abundant; reverse dark green (30F5) to dull green (30E5). On OA, 26–27 mm diam, flattened, velvety, dark green (30F6) at centre, dull green (30E4) towards periphery, margin entire, sporulation abundant; reverse parsley green (30F8) at centre, dark green (30F5) towards periphery. Diffusible pigment absent in all culture media tested.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 10 °C (7–8), optimum 25 °C (24–25), maximum 30 °C (18–19). No growth at 5 °C and 35 °C.

Notes — *Exophiala candelabrata* is closely related to *E. pisciphila* (Fig. 5). The latter species was one of the first species of *Exophiala* described as the causative agent of epizootics in cold-blooded vertebrates from freshwater and marine environ-

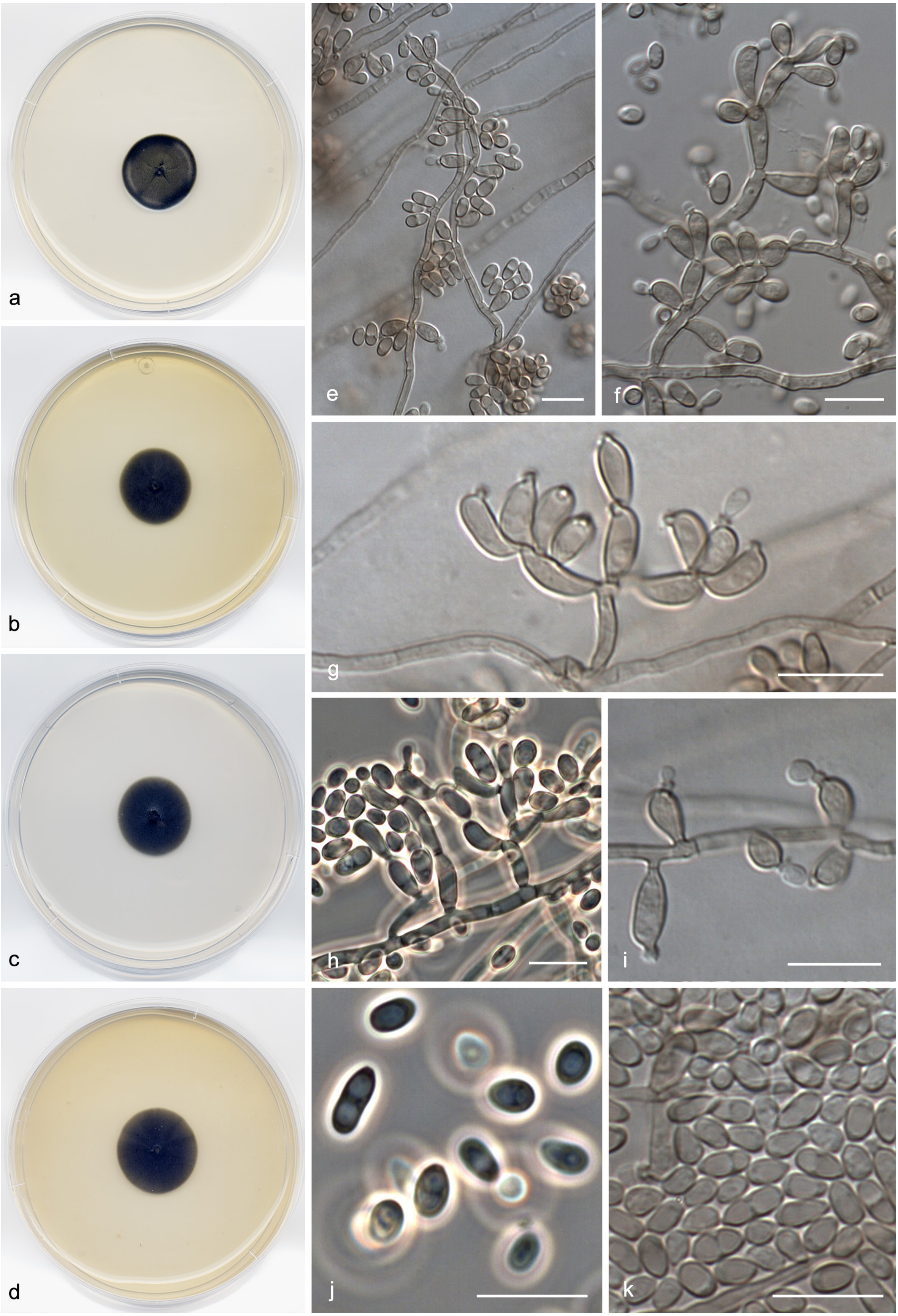

Fig. 12 *Exophiala candelabrata* (ex-type FMR 18336). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–h. conidiophores from OA; i. solitary conidiogenous cells; j–k. conidia. — Scale bars: e–k = 10 µm.

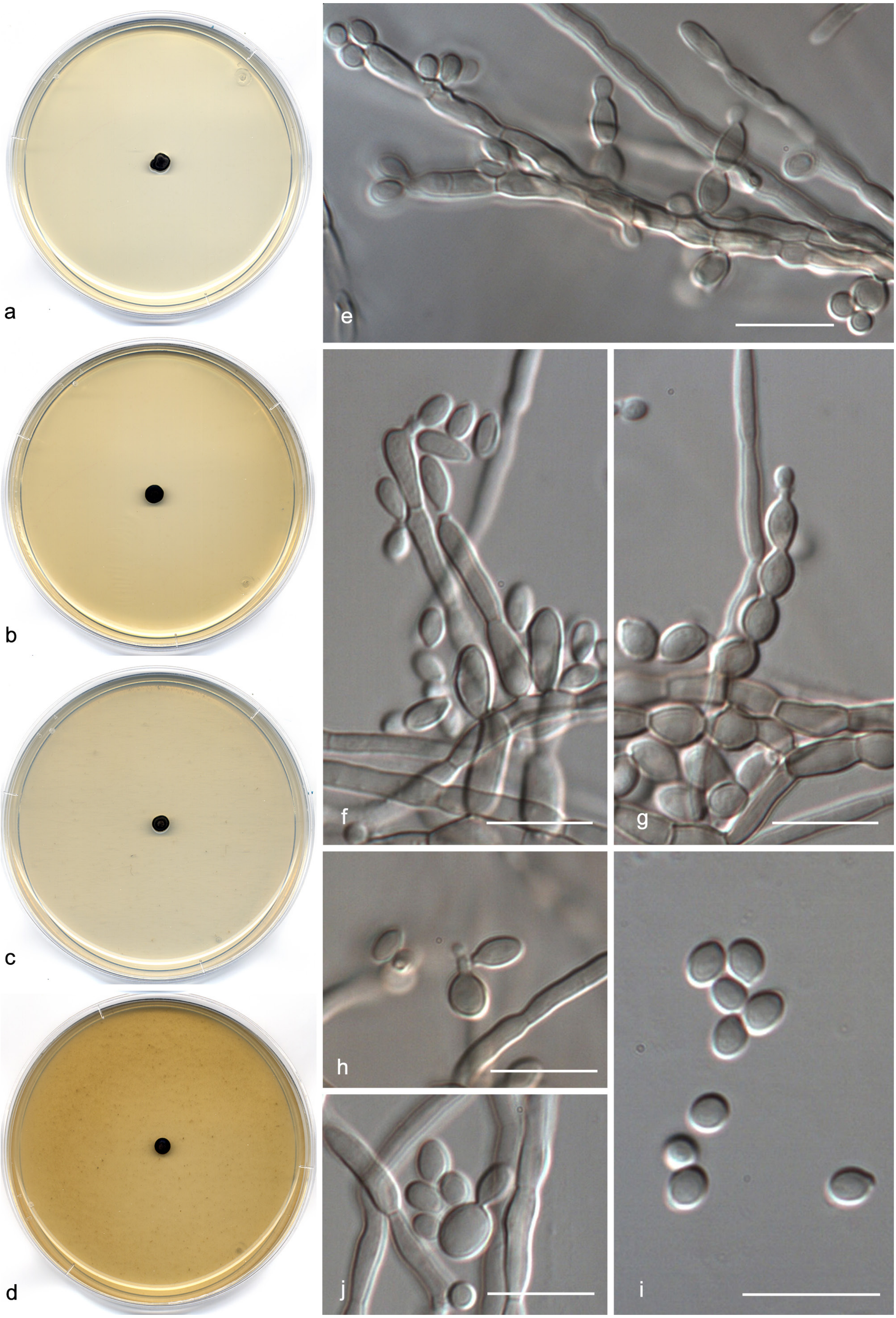

Fig. 13 *Exophiala dehoogii* (ex-type FMR 19001). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–g. conidiophores from OA; h–j. budding cells and conidia. — Scale bars: e–j = 10 µm.

ments, with a low degree of host specificity (Fijan 1969, De Hoog et al. 2011). The new species shares morphological similarities with *E. pisciphila* by forming similar flask-shaped conidiogenous cells in loose clusters or branched systems with inconspicuous annellated zones that forms ellipsoidal conidia. However, *E. candelabrata* can be distinguished by producing one-celled, pale brown and slightly smaller conidia (3.5–6(–7.5) × 2.5–3 µm), while in *E. pisciphila* these are 0–1-septate, hyaline and generally longer (6–8 × 2.5–4 µm) (De Hoog et al. 2011). Some conidia in *E. candelabrata* may appear septate, but this is a microscopic effect due to the presence of large guttules inside, since true outer layer separation is not present. In addition, although both species show a maximum temperature for growth at 30 °C, they differ in their minimum temperature that is 4 °C, even below in *E. pisciphila* (De Hoog et al. 2011), while *E. candelabrata* was unable to grow below 10 °C.

Exophiala dehoogii Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846247; Fig. 13

Etymology. Named in honour of G. Sybren De Hoog, for his expertise in black yeasts.

Typus. Spain, Catalonia, Berguedà, Castellar de n'Hug, Llobregat River, N42.27967° E2.00535°, from fluvial sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25217, culture ex-type FMR 19001 = CBS 149779).

Mycelium composed of hyaline to pale brown, smooth-walled, branched, septate, cylindrical or slightly torulose hyphae, 2.5–4 µm diam. *Conidiophores* micronematous, often reduced to single conidiogenous cells borne terminally or laterally from cylindrical or moniliform hyphae. *Conidiogenous cells* intercalary, lateral or terminal, pale brown, smooth-walled, broadly ellipsoidal to ellipsoidal, 4–10.5 × 2.5–5.5 µm. *Conidia* one-celled, pale olivaceous, smooth- and thin-walled, subglobose, ellipsoidal to broadly ellipsoidal, 4.5–9 × 2.5–4 µm, often accumulated in slimy heads on the conidiogenous loci. *Budding cell* present in all culture media. *Chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies on PDA reaching 7–8 mm diam, elevated, almond green (28E3) to dark green (28F8), velvety, margin irregular, slightly lobulate, sporulation sparse; reverse dark green (27F8). On MEA, 6–7 mm diam, slightly raised at centre, dark green (29F4) to dark green (29F8), velvety, margin entire, sporulation sparse; reverse dark green (28F8). On PCA, 6–7 mm diam, slightly elevated, dull green (29E3) to dark green (28F6), velvety, margin entire, sporulation sparse; reverse dark green (27F8). On OA, 4–5 mm diam, slightly raised, almond green (28E3) to dark green (28F2), velvety, margin entire, sporulation sparse; reverse dark green (28F8). Diffusible pigment absent in all culture media tested.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (2–3), optimum 25 °C (6–7), maximum 30 °C (3–4). No growth at 35 °C.

Notes — According to our phylogeny (Fig. 5), *E. dehoogii* forms a separate single branch in the *jeanselmei*-clade, one of the largest phylogenetically well-supported clades (98 % bs/1 pp) in *Exophiala* that includes more than 20 species. Although *E. dehoogii* is distantly located, based on a megablast search, *E. ellipsoidea* was the closest relative. It is a recently described *Exophiala* from rocks in China (Sun et al. 2020). *Exophiala dehoogii* differs morphologically from *E. ellipsoidea* in slightly longer conidiogenous cells (4–10.5 µm vs 4.7–8.6 µm), larger conidia (4.5–9 × 2.5–4 µm vs 2.1–6.4 × 1.1–4 µm), the presence of budding cells (absent in *E. ellipsoidea*) and a more restricted growth on MEA after 14 d at 25 °C (6–7 mm vs 23 mm diam).

Exophiala ramosa Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846248; Fig. 14

Etymology. Latin, *ramosus*, branched, referring to the presence of branched conidiophores.

Typus. Spain, Catalonia, Osona, Les Masies de Voltregà, Ter River, Galifa's Fluvial island, N42.02951° E2.25359°, from fluvial sediments, Sept. 2020, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25218, culture ex-type FMR 18632 = CBS 149851).

Mycelium composed of hyaline to subhyaline, smooth- and thin-walled, branched, septate hyphae, 1.5–4 µm wide. *Conidiophores* semi-macronematous to macronematous, brown, thick-walled, erect, commonly branched and bearing conidiogenous cells in groups of 2–5 on supporting metula-like cells, or solitary growing directly on the conidiophore stalk, 12.5–83.5 × 1.5–3 µm; metulae subcylindrical to ellipsoidal, 5.5–11.5(–14) × 2–3 µm. *Conidiogenous cells* discrete, terminal or lateral, occasionally intercalary, pale brown, smooth-walled; discrete annellides flask-shaped to ampulliform, 4–10.5 × 1.5–2.5 µm (without neck), usually extending terminally in a narrow and sinuous neck, 1–3.5 × 0.8–1 µm, with conspicuous annellidic scars; intercalary annellides, cylindrical or somewhat ellipsoidal, with a lateral neck up to 1.5 µm long. *Conidia* one-celled, guttulate, pale brown, smooth, thin-walled, subglobose, occasionally slightly claviform or ellipsoidal, 2.5–4(–5.5) × (1.5–)2.5–3 µm, often tapering into a narrow and truncate conidial scar, accumulating in slimy heads on the conidiogenous loci. *Budding cells* sparse. *Chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies were similar in all culture media tested, reaching to 15–17 mm diam, and no diffusible pigment was produced. On PDA, slightly raised and dull green (30E4) at centre, nickel green (27F3) to dark green (28F4) and slightly concentrically and radially sulcate towards periphery, velvety, margin slightly lobate and fimbriate, sporulation absent; reverse dark green (28F4). On MEA, slightly raised and dull green (29E3) at centre, slightly concentrically sulcate and dark green (30F6) towards periphery, velvety, margin fimbriate, sporulation sparse; reverse dark green (28F4). On PCA, flattened, almond green (28E3) at centre and dark green (29F7) towards periphery, velvety to somewhat cottony, margin fimbriate, sporulation abundant; reverse dark brown (7F8) to dark green (28F4). On OA, slightly elevated at centre, almond green (28E3) to dark green (29F4), velvety, margin regular and fimbriate, sporulation abundant; reverse dark green (28F5).

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (2–3), optimum 30 °C (19–20), maximum 35 °C (2–3). No growth at 37 °C.

Notes — Based on our multi-locus analysis (Fig. 5), *E. ramosa* is allocated in a terminal low-supported clade together with *E. abietophila* (Crous et al. 2019). Morphologically, the new species differs from *E. abietophila* in its differentiated and branched conidiophores, while conidiophores of *E. abietophila* are reduced to single conidiogenous cells (Crous et al. 2019). The conidiogenous cells and conidia produced by *E. ramosa* (5–14 × 1.5–2.5 µm and 2.5–4(–5.5) × (1.5–)2.5–3 µm, respectively) are larger than those of *E. abietophila* (4–6 × 2.5–3 µm and (2.5–)3(–3.5) × 1.5–2 µm, respectively). In addition, *E. abietophila* shows a more restricted growth at 25 °C (10 mm vs 15–17 mm in *E. ramosa*).

Exophiala verticillata Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846249; Fig. 15

Etymology. Latin, *verticillus*, whorl, referring to the arrangement of the conidiogenous cells in verticils on differentiated conidiophores.

Typus. Spain, Catalonia, Ripollès, Llanars, Ter River, N42.32383° E2.32989°, from fluvial sediments, Sept. 2020, *D. Torres-Garcia* & *J. Gené*, (holotype CBS H-25219, culture ex-type FMR 18551 = CBS 149780).

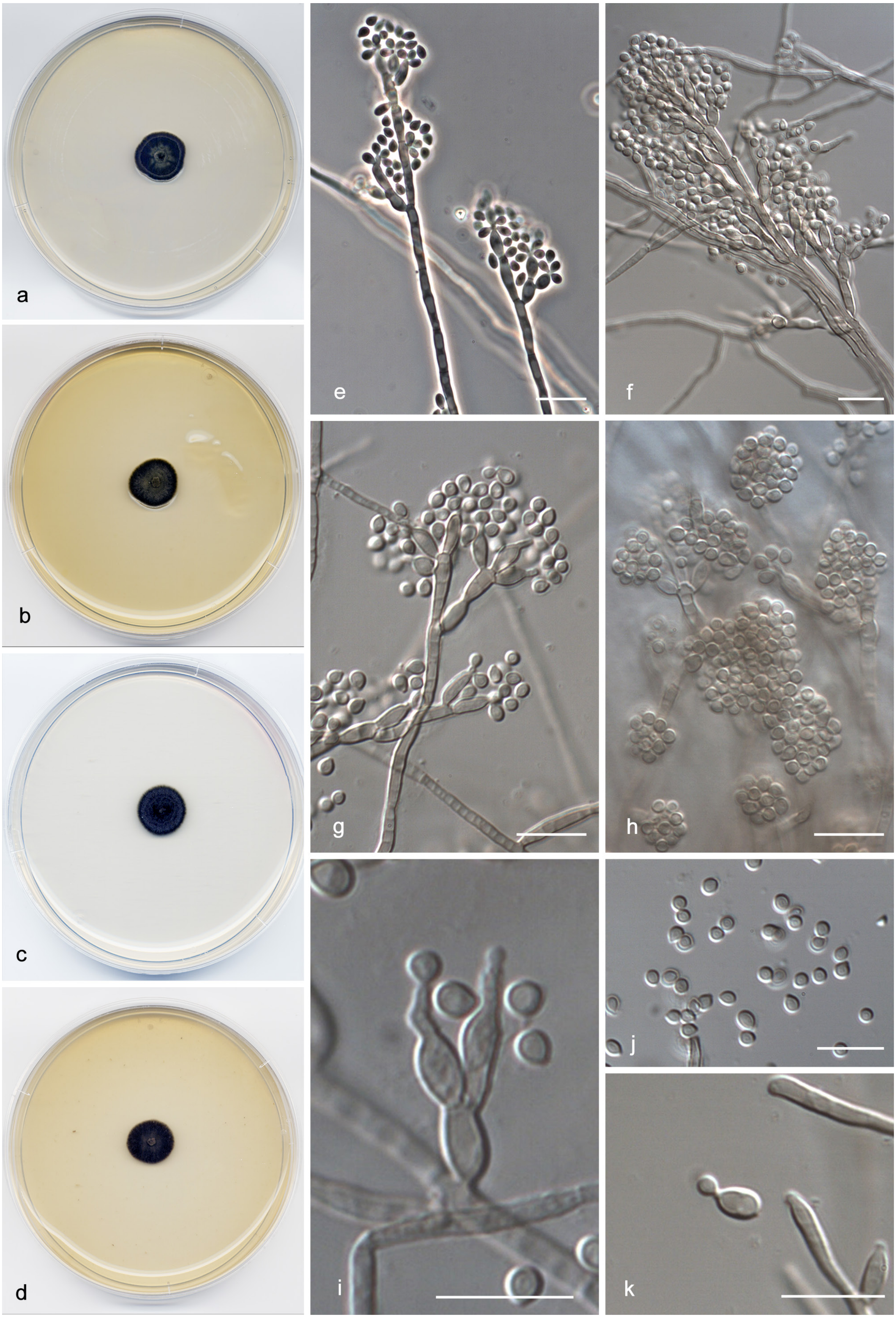

Fig. 14 *Exophiala ramosa* (ex-type FMR 18632). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–h. conidiophores from OA; i. conidiogenous cells; j. conidia; k. budding cell. — Scale bars: e–f = 25 µm, g–k = 10 µm.

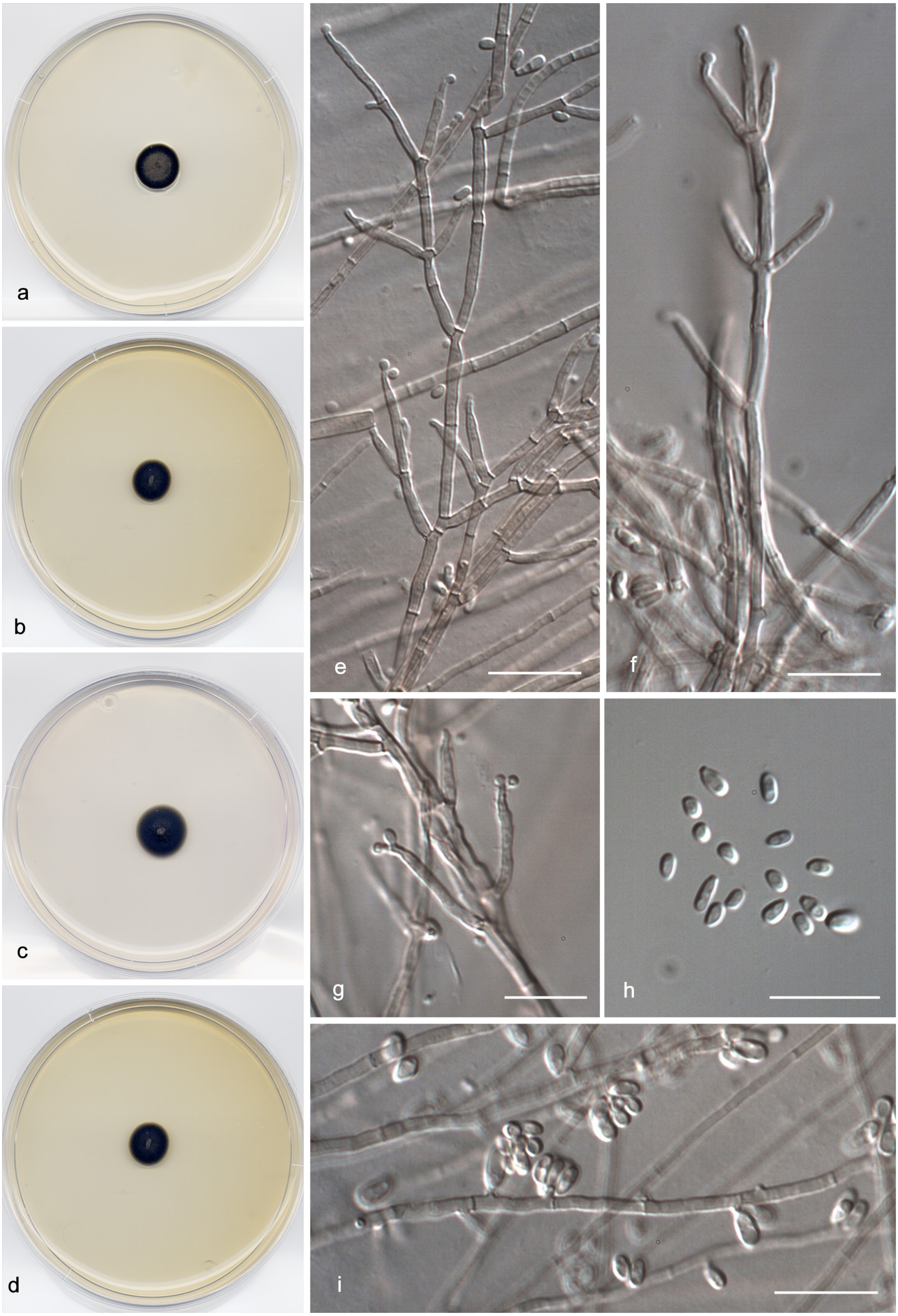

Fig. 15 *Exophiala verticillata* (ex-type FMR 18551). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–g. conidiophores from OA; h. conidia; i. intercalary conidiogenous cells producing conidia. — Scale bars: e–i = 10 µm.

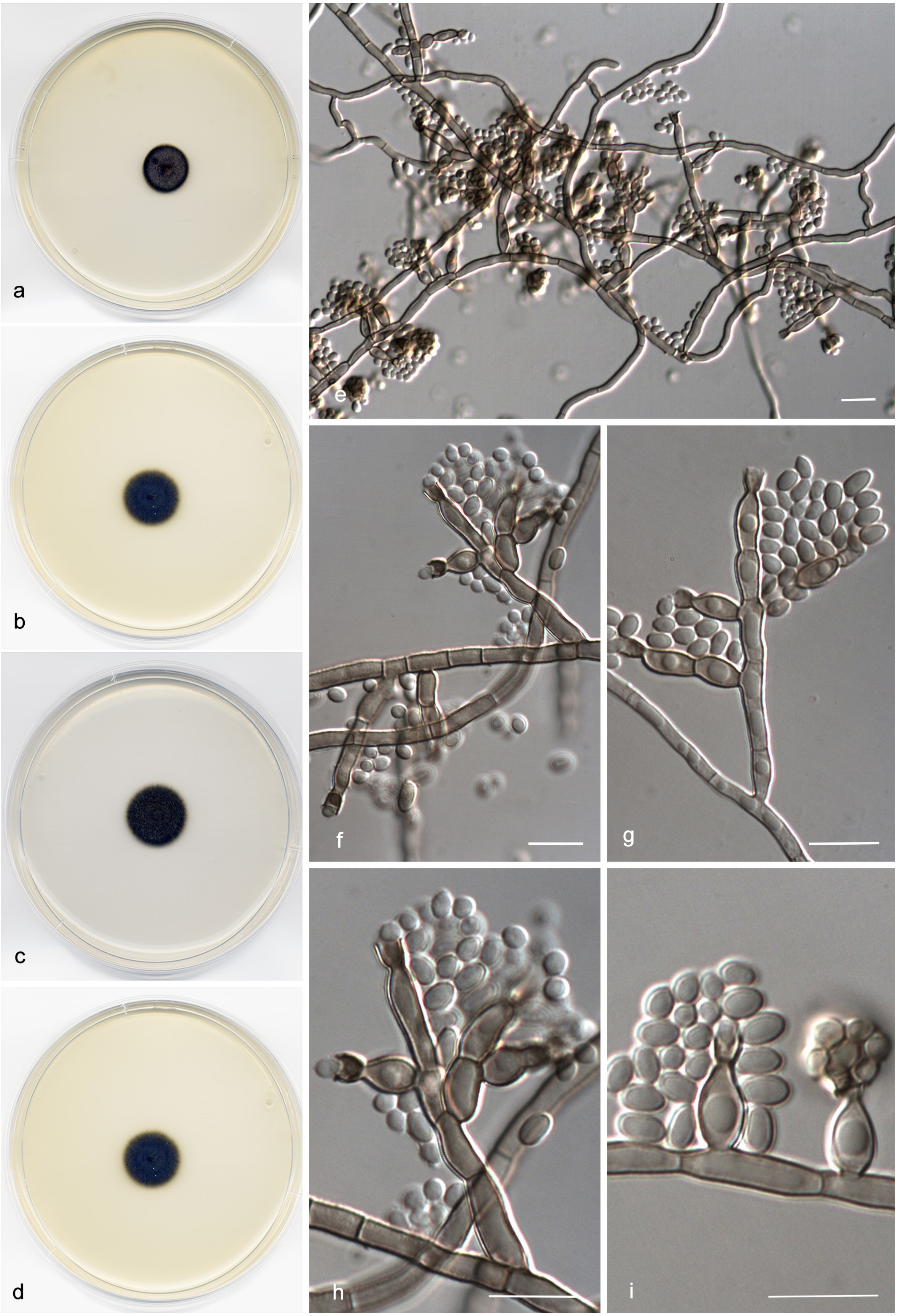

Fig. 16 *Phialophora submersa* (ex-type FMR 17150). a–d. Colonies on PDA, MEA, PCA and OA, respectively, after 14 d at 25 °C; e–h. conidiophores from OA; i. solitary phialides producing conidia. — Scale bars: e = 25 µm, f–i = 10 µm.

Mycelium composed of hyaline to pale brown, smooth-walled, branched, septate hyphae, 1–3 µm diam. *Conidiophores* semi-macronematous to macronematous, pale brown, smooth-walled, erect, branched, often bearing conidiogenous cells in verticils along the conidiophore, 27–239 × 1–2.5 µm, or micronematous reduced to intercalary conidiogenous cells in vegetative hyphae. *Conidiogenous cells* discrete, lateral or terminal, or intercalary, pale brown smooth- and thin-walled; discrete annellides arranged in verticils of 2–3 or solitary on the conidiophores, cylindrical, slightly apically tapering into an annellated neck, 5.5–15.5 × 1–2 µm; intercalary annellides cylindrical, with a lateral, protruding, annellated conidiogenous locus usually near the septa. *Conidia* one-celled, guttulate, pale olivaceous, smooth-walled, ellipsoidal to broadly ellipsoidal or subcylindrical, straight or sometimes curved, 1.5–4 × 0.5–2.5 µm, often with a narrow, truncate basal scar, forming slimy heads on the conidiogenous loci especially those produced from intercalary annellides. *Budding cells* and *chlamydospores* absent. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies were similar in all culture media tested, reaching to 14–17 mm diam, and no diffusible pigment was produced. On PDA, slightly raised and grey green (29E5) at centre, flat and dark green (28F8) at periphery, velvety, margin regular, sporulation sparse; reverse dark green (30F6) at centre to parsley green (30F8) towards periphery. On MEA, slightly elevated, slightly radially sulcate, dull green (30E3) at centre to dark green (28F8) and deep green (30E8) towards periphery, velvety, margin regular, sporulation sparse; reverse dark green (28F6). On PCA, slightly raised and dark green (28F8) at centre, flat and deep green (29E8) towards periphery, velvety, margin regular, sporulation abundant; reverse dark green (28F6) to grey green (30E5) at periphery. On OA, slightly elevated at centre, radially sulcate, grey green (30E4) at centre to olive brown (4E5) and brown grey (4D2) towards periphery, velvety, margin regular, sporulation abundant; reverse parsley green (30F8) to olive brown (4F6) at centre, sallow coloured (4D3) at periphery.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 5 °C (2–3), optimum 25 °C (14–15), maximum 30 °C (8–10). No growth at 35 °C.

Notes — Our multi-locus analysis (Fig. 5) showed that *E. verticillata* and *E. angulospora* are related, however such relationship is only supported by BI analysis (32 bs/0.96 pp). It is worth noting that *E. angulospora* was also found in the aquatic environment, specifically isolated from a water sample collected from a Japanese well (Iwatsu et al. 1991). In addition to the phylogenetic distance, both species can be easily differentiated by morphological features of their conidia, which are commonly angular (tri- or tetragonal) and smaller (1.5–4 × 0.5–2.5 µm) in *E. angulospora* and subcylindrical, ellipsoidal to broadly ellipsoidal and larger ((2.5–)4–6(–8) × (1.5–)2–3 µm) in *E. verticillata*. Furthermore, *E. angulospora* produce budding cells, whereas in *E. verticillata* they were not observed in any culture media tested.

Phialophora submersa Torres-Garcia, Gené & Dania García, *sp. nov.* — MycoBank MB 846241; Fig. 16

Etymology. Latin, *submersus*, sunken, drowned; referring to the submerged sediment sample from which the fungus was isolated.

Typus. Spain, Catalonia, Priorat, Cornudella del Montsant, Les Obagues stream, N41.26672° E0.90985°, from fluvial sediments, Feb. 2018, *E. Carvalho & J. Gené*, (holotype CBS H-25187, culture ex-type FMR 17150 = CBS 149782).

Mycelium composed of olivaceous, smooth- and thin-walled, branched, septate hyphae, 2.5–4 µm wide. *Conidiophores* micro- or semi-macronematous, erect, simples or poorly branched, 17–138.5 × 2.5–4 µm; micronematous conidiophores consisting in conidiogenous cells growing directly from vegetative hyphae, lateral or terminal. *Phialides* broadly flask-shaped or subcylindrical, with an apical conspicuous collarette, 8.5–19.5 × 2.5–4.5 µm; collarettes usually vase-shaped, darker than the rest of the phialide, 3.5–5 × 2.5–4 µm wide. *Conidia* hyaline to subhyaline, smooth-walled, usually broadly ellipsoidal to ellipsoidal, more rarely subglobose or obovoidal, 3–6 × 2–3 µm, accumulating in slimy heads or remaining aggregated around the phialides. *Sexual morph* unknown.

Culture characteristics (after 14 d at 25 °C) — Colonies on PDA reaching 14–15 mm diam, slightly raised and green grey (28D2) at centre, flat and dark green (28F8) towards periphery, margin entire, sporulation abundant; reverse dark green (28F8) to parsley green (30F8). On MEA, 16–17 mm diam, slightly raised and dark green (30F5) at center, flat and deep green (30E8) towards periphery, velvety, margin slightly fimbriate, sporulation abundant; reverse dark green (28F8) at centre to deep green (30E8) towards periphery. On PCA, 19–20 mm diam, slightly raised and grey green (30E5) at center, flat and dark green (28F8) towards periphery, velvety to floccose, margin slightly fimbriate, sporulation abundant; reverse dark green (28F8) to deep green (28E8). On OA, 14–16 mm diam, slightly raised and cactus green (28E4) at centre, flat and dark green (28F8) towards periphery, velvety or floccose, margin slightly fimbriate, sporulation abundant at centre and sparse at the rest of colony; reverse dark green (30F7). Diffusible pigment absent in all culture media tested.

Cardinal temperatures for growth on MEA after 14 d (mm) — Minimum 10 °C (8–9), optimum 25 °C (16–17), maximum 30 °C (7–9). No growth at 5 °C and 35 °C.

Additional specimens examined. Spain, Catalonia, Berguedà, Guardiola de Berguedà, Llobregat River, N42.24012° E1.93742°, from river sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené* (FMR 18984); ibid., N42.24012° E1.93742°, from river sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené* (FMR 18985); ibid., N42.24012° E1.93742°, from river sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené* (FMR 18986); Castellar de n'Hug, Llobregat River, N42.27298° E1.99177°, from river sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené* (FMR 18996); ibid., N42.27298° E1.99177°, from river sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené* (FMR 18997); ibid., N42.27298° E1.99177°, from river sediments, Mar. 2021, *D. Torres-Garcia* & *J. Gené* (FMR 18998).

Notes — *Phialophora submersa* is closely related to *P. americana* (Fig. 3), a species isolated from environmental substrates mainly from soil and plant material from different countries. It has also been described as human opportunist causing mainly subcutaneous infections (Li et al. 2017, De Hoog et al. 2020). The micromorphology of *P. submersa* is very similar to that of *P. americana*, having only subtle distinct traits between them. These are: i) the presence of differentiated conidiophores in the former, but absent in *P. americana*; ii) absence of collarettes growing laterally on vegetative hyphae, which are common in *P. americana*; and iii) the conidia in *P. submersa* are slightly smaller (3–6 × 2–3 µm) than those of *P. americana* (3.5–7 × 2–4 µm). However, they can be easily distinguished by their cardinal temperatures and colony colour shades. While *P. americana* shows the optimum and maximum temperature for growth at 30 and 37 °C, respectively (Li et al. 2017), in *P. submersa* these are 25 and 30 °C. While *P. americana* shows black olivaceous colonies with slightly pink margins on MEA, in *P. submersa* they are entirely dark green, and on OA the colonies of *P. americana* are pale grey and those of *P. submersa* are green to dark green. These phenotypic differences were also observed in the four isolates of *P. americana* obtained from our freshwater sediment samples. However, we found all these isolates to be tolerant to 0.2 % of cycloheximide, meanwhile *P. americana* was described as unable to grow at 0.1 % of such antimicrobial compound (De Hoog et al. 2020).

DISCUSSION

In the endeavour to enhance our understanding of the diversity of culturable *Ascomycota* in freshwater sediments, we successfully isolated several species belonging to the order *Chaetothyriales*. Although we used different culture media and conditions, all *Chaetothyriales* were isolated on PDA supplemented with 0.2 % cycloheximide (Ulfig et al. 1997, Madrid et al. 2016), confirming the efficacy of such a method to recover slow-growing moulds. This applies especially to black yeasts belonging to the *Herpotrichiellaceae*, as practically all our isolates (98 %) belong to this family. This result is not surprising, as several metagenomic studies focusing on fungal diversity in sediments and soil samples have already revealed that members of this fungal group are usually well represented in terms of abundance (Veach et al. 2015, Kavehei et al. 2021, Xu et al. 2021). In our case, the use of PDA with cycloheximide not only has allowed us to isolate several known herpotrichiellaceous species of the genera *Capronia* (*Ca. pulcherrima*), *Cladophialophora* (*C. emmonsii*), *Exophiala* (*E. equina*, *E. pisciphila*, *E. radicis*) and *Phialophora* (*P. americana*), which represent first reports from freshwater sediments in Spain, but also to find undescribed taxa in the families *Cyphellophoraceae* (*Cy. spiralis*) and *Herpotrichiellaceae* (*Ac. restrictus*, *C. denticulata*, *C. heterospora*, *C. irregularis*, *E. candelabrata*, *E. dehoogii*, *E. ramosa*, *E. verticillata* and *P. submersa*) (Table 1).

Identification of the Chaetothyriales: traditional vs molecular approach

The traditional identification of *Chaetothyriales* species has primarily been based on morphology. However, it is widely recognised that certain genera mentioned above, as well as others within the *Herpotrichiellaceae* family have a polyphyletic nature, while exhibiting homoplastic morphological features. This polyphyly extends within the *Chaetothyriales* order, as demonstrated by the LSU/ITS phylogeny presented in this study (Fig. 2). For instance, the genera *Cladophialophora* and *Exophiala* are not only distributed along distant clades in *Herpotrichiellaceae* but also belong to other families, such as *C. modesta* and *E. eucalyptorum*, which are classified in the *Chaetothyriaceae*; *C. behniae*, *C. hostae*, *C. humicola*, *C. minutissima*, *C. scillae* and *C. sylvestris* in the *Epibryaceae*; *C. eucalypti*, *C. proteae*, *C. pucciniophila*, *E. encephalarti* and *E. placitae* in the *Trichomeriaceae*. These species highlight the need for taxonomic adjustment in future studies (Quan et al. 2020, 2023).

Our data clearly demonstrate that sequence analyses, particularly of the LSU and ITS barcodes, are essential not only for the identification but also for the confirmation of taxonomy of many 'black yeasts', as previously mentioned (Quan et al. 2020, 2023, Tian et al. 2021, Phukhamsakda et al. 2022). These molecular approaches provide valuable insights and help to establish a more accurate and reliable classification system for these fungi. In our case, the phylogeny of both markers confirms that nine out of 10 novel lineages detected among sediment isolates belong to the *Herpotrichiellaceae* s.str. While some of the new taxa are placed in single unsupported branches like the new genus *Aciculomyces*, *E. verticillata* (*Exophiala* sp. II) and *E. ramosa* (*Exophiala* sp. III), others are included in moderate to well-supported terminal clades related to those overall clades delineated by Badali et al. (2008) and De Hoog et al. (2011) for *Cladophialophora*, *Exophiala* and relatives. Namely, *C. heterospora* (*Cladophialophora* sp. I) was placed in the *carrionii*-clade, *C. irregularis* (*Cladophialophora* sp. II) and *C. denticulata* (*Cladophialophora* sp. III) in the *bantiana*-clade, *E. candelabrata* (*Exophiala* sp. I) in the *salmonis*-clade, and *E. dehoogii* (*Exophiala* sp. IV) in the *jeanselmei*-clade. As previously mentioned, the naming of these clades was primarily aimed at establishing ecological relationships among species within the complex. It is noteworthy that some of these clades, such as the *bantiana*-, *carrionii*- or *dermatitidis*-clades, have consistently received significant statistical support values in successive phylogenetic studies (Feng et al. 2014a, b). However, with the introduction of novel taxa of different origins into those named clades, or, in general, into the family, the existence of the Badali and the De Hoog's clades can be appreciated, but they receive insignificant branch support, as in the case of the LSU/ITS phylogeny presented here or in recent phylogenetic studies for *Chaetothyriales* (Quan et al. 2020, 2023). Our phylogenetic analysis (Fig. 2) reveals the presence of several well-supported monophyletic clades that hold taxonomic significance. These clades clearly represent known genera, including *Phialophora*, *Minimelanolocus* or *Thysanorea*, as well as *Cladophialophora* s.str., *Exophiala* s.str., *Rhinocladiella* s.str. and *Veronaea* s.str. Additionally, there are putative undescribed genera, such as the *jeanselmei*-clade or other unnamed clades observed in the phylogenetic tree.

Considering the number of currently described species in *Herpotrichiellaceae* – more than 180 species according to Quan et al. (2023) – and that most of them are available in culture for their study, it is urgent to carry out a taxonomic re-evaluation of the family, at least to give formal taxonomic status to the well-supported monophyletic clades and, if necessary, to delineate novel taxa irrespective to their morphology. Although the combined LSU/ITS dataset has proven to be valuable in providing resolution within the clades (Sánchez et al. 2019, Quan et al. 2020, Phukhamsakda et al. 2022), incorporating the analysis of additional markers such as *tef*1-α, *tub*2 and actin genes has shown promising discriminatory power in distinguishing species within this group of fungi (Teixeira et al. 2017, Phookamsak et al. 2019, Sánchez et al. 2019, Thitla et al. 2022, Chang et al. 2023). The inclusion of these markers in future studies would contribute to establishing a more robust phylogenetic-taxonomic structure within the *Herpotrichiellaceae*. In our study, the combination of LSU, ITS, *tef*1-α and *tub*2 datasets proved to be valuable. Not only did it enable us to delineate novel species isolated from freshwater sediments, but it also enhanced the statistical support for the aforementioned clades, suggesting that these clades could represent distinct taxonomic entities (Fig. 4, 5).

Nevertheless, data on the whole genome sequencing of numerous herpotrichiellaceous species, combined with phenotypic and ecological insights, will be crucial to elucidate the taxonomy of this complex family.

Cyphellophoraceae, more than one genus?

While the family *Cyphellophoraceae* is well circumscribed using the LSU/ITS gene markers, the delineation of its individual genera remains a topic of controversy. Although several phylogenetic studies (Quan et al. 2020, Tian et al. 2021), and also here (Fig. 2), support that the family comprises at least the genera *Anthopsis* and *Cyphellophora*, the former genus has been recently synonymized with *Cyphellophora* (Crous et al. 2023). The monotypic genus *Kumbhamaya* is mentioned by Quan et al. (2020, 2023) as a third member of the family, but it was considered congeneric with *Cyphellophora* in previous studies (Decock et al. 2003, Réblová et al. 2013). The synonymy of *Anthopsis* with *Cyphellophora* was already suggested by Moussa et al. (2017) as the result of their ITS phylogenetic analysis. The ITS phylogeny presented by Crous et al. (2023) showed that the type species of *Anthopsis*, *A. deltoidea*, *Cy. clematidis* (Crous et al. 2020), and the newly described species *Cy. neerlandica* (Crous et al. 2023) formed a supported basal lineage in the *Cyphellophoraceae*, separated from the unsupported main clade that contained the rest of the *Cyphellophora* species analysed.

In this context, it is true that after the introduction of *Cy. neerlandica*, the *Anthopsis* lineage exhibits the same morphological conidial patterns that are present in *Cyphellophora* (i.e., small, aseptate conidia, and larger, elongate and septate conidia). However, the three species included in the *Anthopsis* lineage can also be distinguished by their ampulliform to ellipsoidal phialides with conspicuous collarettes, forming compact clusters on hyphae, and the presence of inverted phialides (i.e., discrete phialides with a conidiogenous opening placed near the base) as a distinctive feature of the genus *Anthopsis* (Marchisio et al. 1977). The protologue of *Cy. clematidis*, as described by Crous et al. (2020), did not mention the presence of inverted phialides, although they are clearly depicted in the accompanying illustrations. Additionally, *Cy. neerlandica* exhibits intermediate features of both genera. It displays long multiseptate conidia characteristic of *Cyphellophora*, as well as ampulliform phialides forming clusters on undifferentiated hyphae, which is typical of *Anthopsis*. However, it is important to note that inverted phialides have not been described or illustrated for *Cy. neerlandica*. The multi-gene phylogenies presented in this study (Fig. 1, 5), along with previous studies (Quan et al. 2020, 2023, Tian et al. 2021), provide further support for the distinction of the *Anthopsis* and *Cyphellophora* lineages as two taxonomic entities. Considering the current limitations and the need for more comprehensive morphological and phylogenetic studies, it is prudent to refrain from introducing taxonomical changes for the two *Cyphellophora* species mentioned above. It is advisable to await further studies that include a larger number of isolates, including the unidentified fungi related to the *Anthopsis* clade highlighted by Crous et al. (2023).

More insights on the black yeasts from Spanish freshwater sediments

Indeed, comprehensive studies specifically focused on the diversity of black yeasts from aquatic sediments in Spain are relatively scarce. Further research efforts in this area would contribute to filling this knowledge gap and providing a better understanding of the biodiversity and ecological significance of black yeasts in general. We can mention a study of Madrid et al. (2016) in which *E. aquamarina*, *E. lacus*, *E. oligosperma* and *Rhinocladiella similis* were isolated from sediments in different rivers in Catalonia (Spain), and the same authors described *Cy. catalaunica* from the same substrate (Crous et al. 2013). No such species have been identified in the present study. In our case, *E. radicis* was the most frequent species identified among the known species, accounting for 66.67 % of the isolates. It was found in sediments from almost every river and stream sampled in the current survey (Table 1). *Exophiala radicis* was also identified by Madrid et al. (2016) from soil samples collected in natural areas in Spanish provinces (i.e., Leon and Teruel) different from ours (i.e., Barcelona, Girona, Huesca, Illes Balears, Lleida). These results indicate that it is a widespread species in Spain, most likely inhabiting forest soils, and that its presence in river sediments is the result of the lixiviation of terrestrial surfaces such as soil and the vegetation of such areas. In fact, *E. radicis* was originally described as root endophyte of *Microthlaspi perfoliatum* and isolated from other plant materials collected in different European countries (Maciá-Vicente et al. 2016). This species is also found in human clinical specimens from skin lesions and nails, but its role as a pathogen has never been proven (Maciá-Vicente et al. 2016, Madrid et al. 2016).

On the contrary, other black yeasts identified in our samples, like *C. emmonsii* and *P. americana*, are well-known opportunistic pathogens of humans and other mammals (De Hoog et al. 2020). Several reports highlight the pathogenic potential of *C. emmonsii* in both human and animal hosts. *Cladophialophora emmonsii* has been reported as causative agent of human subcutaneous phaeohyphomycosis in the USA. It has also been associated with the subcutaneous and skin lesions in cats reported in Venezuela and the USA (Padhye et al. 1988, Badali et al. 2008, Kantarcioglu et al. 2017). As far as the authors are aware, the sediment isolate of *C. emmonsii* is the first specimen isolated from the natural environment. Nevertheless, according to metabarcoding data registered in Global Biodiversity Information Facility (GBIF.org, accessed 15 Dec. 2022), *C. emmonsii* has been detected in soil from Canada and the USA, as well as marine samples from Finland. To fully understand the global biogeography, genetic diversity and its role in the environment, additional studies using fresh isolates from different geographical regions are indeed necessary.

On the other hand, *P. americana* has been reported as a widespread species. It has been found in soil samples from Brazil, China and Colombia, and also from various plant material, including living and dead wood, roots, tree bark, leaves, bamboo and decaying timber in countries such as China, Czech Republic, Finland, France and the USA (Li et al. 2017). These findings suggest the ecological versatility of *P. americana* across different habitats and geographic regions. In our study, *P. americana* is the second most common (20 %) species detected. Although it has been reported mainly from the environment, it is a well-known opportunist capable of causing cutaneous and subcutaneous infections (i.e., phaeohyphomycosis and chromoblastomycosis) in immunocompromised animals and humans (Li et al. 2017, Huang et al. 2019, De Hoog et al. 2020, Ahmed et al. 2021, Borrás et al. 2022, Martini et al. 2022).

Two other pathogenic opportunists detected in our survey, although rarely reported to cause human infections, are *E. equina* and *E. pisciphila* (De Hoog et al. 2020). This finding is not surprising since both black yeasts are defined as waterborne species capable of causing infections in a variety of cold-blooded animals, particularly *E. pisciphila*, which has been reported to infect both marine and freshwater bony fish often living in captivity (De Hoog et al 2011, 2022, Řehulka et al. 2017). At any rate, *E. equina* and *E. pisciphila* have also been found inhabiting terrestrial environments. In a recent environmental prospecting study conducted on Brazil, using a culture-independent methodology and analysing various types of samples, including water along the Amazon River, both *Exophiala* species were exclusively detected in the rhizosphere and soil (Costa et al. 2020). These findings indicate specific ecological niches for these species and highlight the importance of considering the microhabitat and sample type when investigating the presence and distribution of fungal species in different ecosystems.

Capronia pulcherrima, although identified in freshwater sediments in our study, can be considered a transitory species in aquatic environments. It is commonly found associated with roots and wood of various trees and shrubs, including species such as *Acer*, *Betula*, *Erica*, *Fagus*, *Ilex*, *Pinus*, *Sambucus* and *Ulmus* spp., in forest areas across different countries (Argentina, Canada, Denmark, Germany, Russia, Spain and UK) (Sierra-López 2006, Friebes 2012, Gallo et al. 2014). Furthermore, according to metadata obtained from GBIF.org (accessed 22 Dec. 2022), this species has also been detected in soil from numerous European countries, Americas (Argentina, Canada, Guatemala, the USA) and Asia (Pakistan, Vietnam). Its presence in freshwater sediments may be a result of organic matter deposition or other ecological factors, but its primary habitat and ecological role are associated with terrestrial environments rather than aquatic ones. This information further emphasizes the ecological versatility and adaptability of *Ca. pulcherrima* across different ecosystems. Despite its widespread distribution and ubiquity, only a few specimens are preserved in culture and sequenced. The DNA data derived from the type material are not available for comparison, making the taxonomy of this species uncertain, and, therefore, epitypification would be required for its nomenclatural stabilization. Identification in our study is based on comparison with reference strain DAOM 216384 (= MUCL 39971) published by Untereiner & Naveau (1999). But also, the morphological features of the sediment isolate fit those of the *Exophiala* asexual morph described in *Ca. pulcherrima* (Munk 1953, Untereiner 1997). The phylogeny presented here (Fig. 2) agrees with previous published phylogenies for *Capronia*, in which *Ca. pulcherrima* is closely related to *Ca. camelliae-yunnanensis*, *Ca. pilosella* and *Ca. lijiangenesis* (Untereiner & Naveau 1999, Phookamsak et al. 2019, Sánchez et al. 2019, Quan et al. 2020, Phukhamsakda et al. 2022).

The novel taxa that were detected in our study exhibited unique phylogenetic positions as shown in Fig. 2–6. They can also be distinguished from their closest relatives based on a combination of features related to the conidiogenous apparatus, conidial morphology, and their cardinal temperatures for growth (see Taxonomy section). These distinguishing characteristics provide additional evidence for the novelty and distinctiveness of these taxa within their respective lineages. It is relevant to mention that all new species of *Cladophialophora* and *E. ramosa* showed optimal growth at 30 °C and were the only species described here able of growth at 35 °C and 37 °C, respectively. This is consistent with the predominant nature of these species as environmental saprobes, and with the potential to cause infections in animals and humans like other opportunists of this fungal group (Badali et al. 2008, De Hoog et al. 2011). Our phylogenetic analysis (Fig. 4) supports the inclusion of *C. heterospora* in the *carrionii*-clade, as closely related to *C. carrionii*. *Cladophialophora carrionii* has been frequently reported as a causative agent of chromoblastomycosis, a chronic fungal infection that affects the skin and subcutaneous tissues in humans. The close relationship between *C. heterospora* and *C. carrionii* suggests that *C. heterospora* may also have the potential to cause similar clinical manifestations in humans. This finding underscores the importance of further studies to investigate the pathogenicity and clinical significance of *C. heterospora*.

In any case, we can preface that the data on *C. heterospora* and *P. submersa* suggest that both inhabit aquatic environments. Although we found only one *C. heterospora* specimen in river sediments, two more strains of *C. heterospora* (CBS 142308 and CBS 142309) were examined and sequenced in the present study, both isolated by Ž. Jurjević et al. from water samples in the USA. Additionally, *C. heterospora* was found in the stomach content of a dead female Pacific dolphin captured off the Japanese coast (isolate IFM 58553 = CCF 5686; isolated by A. Sano in 2009; GenBank AB190399, AB190428), but the cause for the animal's demise could not be determined (Ž. Jurjević et al. unpubl. data). *Phialophora submersa* was isolated several times along the Llobregat River (Barcelona) as well as from a sample collected in the Les Obagues Stream (Tarragona), two remote rivers in Catalonia.

Cladophialophora denticulata and *C. irregularis* are species related to the *bantiana*-clade, in which reside several aetiological agents of human infections, such as *C. arxii*, *C. devriesii*, *C. emmonsia* and *C. modesta* (Fig. 4), although *C. bantiana* is the most virulent and well-known species in the complex (Badali et al. 2008, De Hoog et al. 2020).

All novel species described in this study showed a minimum temperature for growth at 5 °C (i.e., *A. restrictus*, *C. heterospora*, *C. irregularis*, *Cy. spiralis*, *E. dehoogii*, *E. ramosa* and *E. verticillata*) or 10 °C (*C. denticulata*, *E. candelabrata* and *P. submersa*), and most of them have a maximum growth at 30 °C (i.e., *A. restrictus*, *Cy. spiralis*, *E. candelabrata*, *E. dehoogii*, *E. verticillata* and *P. submersa*). These cardinal temperatures, particularly the ability to grow at low temperatures (5 °C), have been described in many members of the *Chaetothyriales* that are well adapted to extreme conditions, such as rock dwellers or waterborne species that can cause infections in cold-blooded animals in aquatic environments, as mentioned above (De Hoog et al. 2011, Sun et al. 2020, Thitla et al. 2022).

Freshwater sediments represent a unique and complex habitat that harbours diverse microbial communities, including fungi. These sediments are influenced by a range of factors, including water quality, sediment composition, and nutrient availability, which can shape the fungal communities present. The fungal group known as *Chaetothyriales*, isolated from freshwater sediments, has emerged as a subject of scientific interest. By delving deeper, we can address existing gaps in our understanding of the *Chaetothyriales*, and gain valuable insights into their ecological roles and broader contributions to the intricate web of life in aquatic ecosystems. Comprehensive phylogenetic analyses, utilizing multiple gene markers, can enhance our understanding of their taxonomic placement and evolutionary relationships.

CONCLUSIONS

We share the view expressed by Lepère et al. (2019) regarding the understudied nature of freshwater sediments, at least in terms of fungal diversity. The evidence supporting this claim is twofold: the findings presented in this study and the previously reported research conducted by our team on other groups of *Ascomycota* isolated from freshwater sediments (Torres-Garcia et al. 2022a, b). Nevertheless, such studies have often been overshadowed by investigations focused on other microbial groups or different environments. Hence, there is a compelling need for further investigation into the fungal communities inhabiting freshwater sediments.

Indeed, to fully understand the ecology and role of the novel fungi detected in our study in the aquatic environment, further research involving a larger number of isolates is necessary. This would enable a more comprehensive assessment of their distribution patterns, substrate preferences, and ecological interactions within the aquatic ecosystem. Additionally, in order to determine their potential virulence to warm- or cold-blooded animals, animal inoculation experiments would be valuable. Such experiments can provide insights into the pathogenicity of these fungi and to assess their risk to animal health.

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Supplementary material

Table S1 Strain information and GenBank/EMBL accession numbers of the species included in this study.