RESEARCH ARTICLE

Waterborne *Exophiala* species causing disease in cold-blooded animals

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Abstract   The majority of mesophilic waterborne species of the black yeast genus *Exophiala* (*Chaetothyriales*) belong to a single clade judging from SSU rDNA data. Most taxa are also found to cause cutaneous or disseminated infections in cold-blooded, water animals, occasionally reaching epidemic proportions. Hosts are mainly fish, frogs, toads, turtles or crabs, all sharing smooth, moist or mucous skins and waterborne or amphibian lifestyles; occasionally superficial infections in humans are noted. Cold-blooded animals with strictly terrestrial life styles, such as reptiles and birds are missing. It is concluded that animals with moist skins, i.e. those being waterborne and those possessing sweat glands, are more susceptible to black yeast infection. Melanin and the ability to assimilate alkylbenzenes are purported general virulence factors. Thermotolerance influences the choice of host. *Exophiala* species in ocean water mostly have maximum growth temperatures below 30 °C, whereas those able to grow until 33(−36) °C are found in shallow waters and occasionally on humans. Tissue responses vary with the phylogenetic position of the host, the lower animals showing poor granulome formation. Species circumscriptions have been determined by multilocus analyses involving partial ITS, *TEF1*, *BT2* and *ACT1*.

INTRODUCTION

Exophiala is an anamorph genus defined by annellidic conidio-genesis producing slimy heads of conidia, and a phylogenetic affiliation to the ascomycete order *Chaetothyriales*. Where known, teleomorphs belong to *Capronia*. Nearly all species are characterized and recognisable within the order by their production of budding cells, and the yeast/hypha transition mostly proceeds via torulose hyphae. The *Exophiala* ecotype of *Chaetothyriales* is therefore morphologically characteristic, despite its polyphyletic position within the order. Some *Exophiala* species produce phialidic, catenate or sympodial synanamorphs, reflecting dynamic life cycles.

The genus *Exophiala* contains numerous potential opportunists or pathogens of immunocompetent humans. The most serious pathogens, eventually leading to disseminated, fatal infections are the neurotrope *Exophiala dermatitidis* (Sudhadham et al.

2008), the osteotrope *E. spinifera* (Li et al. 2008), and a species tending to cause disseminated infection, *E. asiatica* (Li et al. 2009). These species are able to grow at 37–40 °C, which is taken to be one of the main virulence factors in *Chaetothyriales*, also being expressed in several pathogenic *Cladophialophora* species (Badali et al. 2008). However, in the last few decades many *Exophiala* isolates have been recovered that consistently lacked thermotolerance, but nevertheless were involved in animal disease. Infections were particularly found in fish and amphibians, but occasionally also in invertebrates. This indicates that, in addition to thermotolerance, other intrinsic virulence factors enabling animal infection are shared by members of *Chaetothyriales*.

Among the early reports of fish infections caused by melanized fungi was that of Reichenbach-Klinke (1956). Carmichael (1966) introduced the genus *Exophiala* with a report of *E. salmonis* from cerebral lesions in cut-throat trout (*Salmo clarkii*). Infections by this species repeatedly took epizootic proportions with up to 40 % mortality in fish hatcheries in Calgary, Canada, where the fish were grown in water drawn from underground springs with a temperature of 12–14 °C. Otis et al. (1985) described visceral infections in Atlantic salmon (*Salmo salar*) after fishes from Canadian hatcheries were transported to an aquaculture centre. Langvad et al. (1985) reported epizootics occurring over several years in farmed Atlantic salmon (*Salmo salar*) in Norway. Mortalities of up to 50 % were caused by *Exophiala psychrophila* (Pedersen & Langvad 1989). Infections took place when smolts were transferred to seawater, leading to visceral invasion with a predilection for the kidney. Identical pathologies linked to visceral symptomatology were described by Richards et al. (1978) in Scotland, but ascribed to *Exophiala salmonis*.

Fijan (1969) reported an epizootic in channel catfish (*Ictalurus punctatus*) in a private pond. Lesions were cutaneous and visceral, with a predilection for formation in the kidney. The

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etiologic agent was later described as *Exophiala pisciphila* (McGinnis & Ajello 1974). Langdon & McDonald (1987) reported this species from fifteen cranial mycoses in Atlantic salmon (*Salmo salar*) in Australia. Gaskins & Cheung (1986) described *Exophiala pisciphila* from brain and skin lesions in a smooth dogfish (*Mustelis canis*); this concerned a single infection in the New York Aquarium. These authors also provided an overview of the 18 species of fish known to have become infected by members of *Exophiala* up to that time (1986). Reuter et al. (2003) reported an epidemic in captured King George whiting (*Sillaginodes puntuta*) in Australian seawater tanks due to an unidentified *Exophiala* species. Kurata et al. (2008) reported ulcerative skin lesions in the Japanese Flounder (*Paralichthys olivaceus*). Cutaneous ulcers in captive American plaice (*Hippoglossoides platessoides*) were reported by Strongman et al. (1997). The infections were ascribed to *Hormoconis resinae* but de Hoog et al. (2009) corrected the identification of the infective organism to *Exophiala pisciphila*.

Infections by black yeast-like fungi in cold-blooded animals thus appear to be relatively frequent, at least in captive and farmed fish and amphibians. Many of the etiologic agents above have not been ITS-sequenced (or DNA-barcoded sensu Schoch et al. in prep.), which is a prerequisite for correct identification of *Exophiala* species (Zeng & de Hoog 2007), and the case reports are scattered in medical, veterinary and environmental literature. More infections, of which the etiologic agent has been preserved and was identified by current standards, are listed in the text below. Outbreaks of infections by melanized fungi in farmed fish and aquarium animals may cause severe losses in aquaculture and fishery industries, but due to the scattered nature of reports it is difficult to estimate the magnitude of the problem.

Numerous additional reports of *Exophiala* infection concern other kinds of cold-blooded animals. A classical study was that of Beneke (1977) on infections in laboratory-housed frogs. Cicmanec et al. (1973), Velázquez & Restrepo (1975) and Bube et al. (1992) reported spontaneous neurological disorders in marine toads (*Bufo marinus*). Agents were frequently identified as *Fonsecaea pedrosoi*; our unpublished analyses suggest that, in recent taxonomy, the causal isolates would be more likely to have been *Cladophialophora* species close to *C. devriesii* (G.S. de Hoog, unpubl. data). Manharth et al. (2005) described a disseminated infection in a Galapagos tortoise (*Geochelone nigra*), caused by an *Exophiala* species, while Joyner et al. (2006) described a subcutaneous inflammatory mass in an eastern box turtle (*Terrapene carolina carolina*) and Stringer et al. (2009) reported an infection of bone and carapace in Aldabra tortoise (*Geochelone gigantea*). Elkan & Philpot (1973) described an *Exophiala* species (as '*Phialophora*') with septate conidia, thus strongly resembling *E. salmonis* or *E. pisciphila*, from a systemic infection in a frog (*Phyllobates trinitatis*). Nyaoke et al. (2009) described several disseminated infections in weedy and leafy sea dragons (*Phyllopteryx taeniolatus* and *Phycodurus eques*, respectively).

Black yeasts also occur in invertebrates. From 1998, an epidemic took place in mangrove crabs (*Ucides cordatus*) along the Brazilian coast. This epidemic was caused by a hitherto undescribed *Exophiala* species, while sometimes co-infection was noted with a *Cladophialophora* species (Boeger et al. 2005), another member of the order *Chaetothyriales*. Vakali (1993) reported infection in earthworms (*Octolasian tyrtaeum*) and was able to reproduce the disease by artificial inoculation and recovery of the organism from cocoons. Dover et al. (2007) reported on a large epizootic of mussels (*Bathymodiolus brevior*) in the Fiji Basin; the etiologic agent was a relative of *Capronia moravica*.

Until today, only very few human infections caused by fish-associated species have been reported. A rare example is *Exophiala pisciphila* in a liver transplant recipient presenting skin papules, which eventually drained (Sughayer et al. 1991). However, in the course of our study we encountered numerous cutaneous cases, which will be discussed below. Recent isolation data suggest that *Exophiala* species may be dispersed via municipal drinking water (Göttlich et al. 2002, Porteous et al. 2003a, b), where they were hypothesized to be stimulated by the presence of amoebae (Cateau et al. 2009). Several black yeasts known to cause superficial infections in humans have been suggested to have an environmental reservoir in bathing facilities (Hamada & Abe 2009, Lian & de Hoog 2010). This finding raises serious questions concerning safety of tap water for the users.

The taxonomy of the psychrophilic, waterborne *Exophiala* species has not been sufficiently studied. Given the pressing questions on human and animal health mentioned above, a revision of this group is overdue. In the present paper, the phylogeny, taxonomy and ecology of relevant waterborne *Exophiala* species is analysed in a multi-locus study using the concept of 'Genealogical Concordance Phylogenetic Species Recognition' (GCPSR) (Taylor et al. 2000). Sequence analysis was based on the SSU and ITS rDNA, the partial β-tubulin (*BT2*) and the translation elongation factor 1-α genes (*TEF1*).

All waterborne *Exophiala* species were confirmed to belong to the order *Chaetothyriales*. Another group of black fungi reported to be common in municipal drinking water (Göttlich et al. 2002) was the genus *Cadophora*, anamorphs of *Pyrenopeziza* in the *Helotiales* (Nauta et al. in prep.). In this order another ecological trend was observed, featuring opportunism and pathogenicity to plants rather than to animals. It is the aim of the present paper to describe the chaetothyrialean component of the waterborne black yeast biota.

MATERIAL AND METHODS

Strains and culture conditions

Strains analysed are listed in Table 1. Reference strains were taken from the CBS culture collection, and eventually supplemented with published materials sent upon request. Strains from drinking water had been isolated between October 1998 and September 1999 by a pour-plate method, using 2 657 1.0 mL water samples from 700 sampling points at 29 separate locations in North Rhine-Westphalia, Germany (Göttlich et al. 2002). Sampling locations were ground water wells, waterworks and storage tanks, hydrants in the distribution network, water taps after water meters or elsewhere in house installations. In most cases the water was unchlorinated. Antarctic strains included were derived from an EU-funded Micromat project (www.sciencepoles.org). Arctic strains were collected by N. Gunde-Cimerman (Ljubljana, Slovenia). Strains were maintained on MEA (2 % Malt Extract Agar) or PDA (Potato-Dextrose Agar) (Crous et al. 2009) slants at 4 °C. Prior to analysis, small pieces from mature colonies were suspended in 4.5 mL sterile water to obtain conidial suspensions. Aliquots of 0.5 mL were plated on PDA in culture plates and incubated at 24 °C for 2–4 wk. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/treebase/index.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004).

Physiology

Fungal strains were cultured in duplicate. Growth was monitored on four different media in culture plates. Growth velocities were measured with subtraction of a baseline defined after 1–3 d, and subsequent periodical measurements of colony diameters

Table 1 Strains analysed of mesophilic waterborne *Exophiala* species.

Name	CBS	Other reference numbers	Source	Geography	GenBank					Reference
					SSU	ITS	TEF1	BT2	ACT1	
Capronia coronata	617.96 (T)	ATCC 56201	Wood	New Zealand	JN856009	JF747040	JN128782	JN112422	JN112378	Müller et al. 1987
E. alcalophila	520.82 (T)		Soil	Japan, Wako-shi, Hirose	JN856010	JF747041	JN128771	JN112423	JN112379	Goto et al. 1981
	521.82		Soil	Japan, Wako-shi, Hirose		JF747042	JN128772	JN112424	JN112380	Goto et al. 1981
	118723	ISO13G	Soil	Brazil						
	118722	ISO13	Soil	Brazil		JF747043				
	122256	dH 17077	Human, skin	Denmark		JF747044	JN128773	JN112425		
		GHP R18	Soap container washing machine	Germany						
E. angulospora	120272	dH 17395, DTO 06.095 nr.6.1 / 2006	Drinking water tap	The Netherlands, Geldermalsen		JF747045	JN128781	JN112427	JN112382	Iwatsu et al. 1991
	482.92 (T)		Drinking water	Japan	JN856011	JF747046	JN128780	JN112426	JN112383	
	109906	dH 11628, IWW 324	Drinking water	Germany		JF747047	JN128777	JN112428		
	109905	dH 11626, IWW 327	Drinking water	Germany		JF747048	JN128778	JN112429		
	121503	dH 12621, LE 212405	Fish	Russia		JF747049	JN128779			
	119911	dH 13563, VANADIJ-CI	Fish nursery	Russia, Stravropol Kraj		JF747050	JN128784	JN112430	JN112384	Nyaoke et al. 2009
		UTHSC 05-3397, dH 16409	Weedy seadragon	USA, Boston						
		Frasca 06-4543, UTHSC R-3889	Lumpfish, skin	USA						
		UTHSC R-3890	Lumpfish, spleen	USA						
		UTHSC 07-871 R-3925	Lumpfish	USA						
	441,92		Human, nail	The Netherlands		JF747051	JN128785	JN112431	JN112385	
	122264	dH 17026, Saunte 83	Human, leg	Denmark, Copenhagen		JF747052	JN128786	JN112432	JN112386	
	146.93		Tilia wood	Germany		JF747053	JN128787	JN112433	JN112387	
E. aquamarina	119918 (T)	dH 18649	Polluted soil, petrol refinery	Brazil, Paulinia City	JN856012	JF747054		JN112434	JN112388	
	119916	dH 16401, UTHSC 00-1181	Leafy seadragon, skin	USA		JF747055		JN112435	JN112389	
	119919	dH 16404, UTHSC 04-3445	Leafy seadragon, necrotic tissue	USA		JF747056		JN112436		
	120417	dH 16403, UTHSC 02-852	Leafy seadragon, skull	USA		JF747057		JN112437		
	119917	dH 17512, UTHSC 06-3123	Leafy seadragon, bone	USA		JF747058		JN112438	JN112390	
	119921	dH 16402, UTHSC 02-554	Weedy seadragon	USA		JF747059		JN112439	JN112391	
		dH 16412, UTHSC 05-3314, R-3673	Weedy seadragon	USA						
		UTHSC R-3685	Weedy seadragon	USA						
	119912	dH 16408, UTHSC 05-3142, R-3669	Winter flounder	USA		JF747060		JN112440	JN112392	
	119915	dH 16405, UTHSC 05-32	Little tunnyfish	USA		JF747061		JN112441		
		UAMH 10488	Lumpfish	Canada						
		UTHSC R-4110	Sandlance, aquarium outbreak	USA						
		UTHSC R-4111	Sandlance	USA						
		UTHSC 05-3605 R-3678	Sandlance	USA						
E. brunnea	587.66 (T)	ATCC 32288, PRE 43729	*Acacia karoo*, litter	South Africa, Potchefstroom	JN856013	JF747062	JN128783	JN112442	JN112393	Rakeman et al. 2005
E. cancerae	120532	dH 17408, Vicente EXO1	Mangrove crab	Brazil		JF747063	JN128746	JN112443	JN112394	
	120420 (T)	dH 17409, Vicente HF 16/08	Mangrove crab	Brazil		JF747064	JN128800	JN112444		
	119920	dH 16425, IMI 380731, Cunningham 179/99	Green toad, liver	Israel		JF747065	JN128801	JN112445	JN112395	
		Det 154M / 2005	Human	The Netherlands						
		Det. M154/ 2007	Human, nail	The Netherlands						
		UTHSC 87-269 (EC001), dH 13414	Human	USA, Washington						
		UWFP 724	Human							
		GHP 2409		Germany						
		GHP 2419		Germany						
		Det 127/2002 8, dH 12901	Human, diabetic, skin	Germany						
		Det 127-2 2002, dH 12895	Water	Germany						
		DTO Tm 01.00, dH 12673	Water	Germany						
E. castellanii	117491	dH 13595, DTO Tm 04.045 M13 CPC 11044, DQ008139	Clean water from cip tank	The Netherlands, Bodegraven		JF747066	JN128799	JN112446	JN112396	
	115142		Fruit drink	Australia		JF747067				
	122325	dH 16683, Saunte 30	Human, foot	Denmark, Copenhagen		JF747068	JN128763	JN112447		
	122265	dH 17085, Saunte 142	Human, hand	Denmark, Copenhagen		JF747069	JN128749	JN112448		
	158.58 (T)	ATCC 1865, IFM 4702, MUCL 10097	Human, skin	Sri Lanka	JN856014	JF747070	JN128766	JN112449		Iwatsu et al. 1984
	662.76		Nematode, cyst	UK						
	110025	dH 12071, IWW 970	Drinking water	Germany		JF747072	JN128770			
		IWW 778, dH 12065	Drinking water	Germany						
	109915	dH 11634, IWW 502	Drinking water	Germany		JF747073	JN128764	JN112450		
	121496	dH 12245, IWW 694	Drinking water	Germany		JF747074	JN128768	JN112451	JN112397	

Species	Strain no.	Other strain codes	Source	Origin	(JN856 / FJ)	JF747…	JN128…	JN112…	Reference
E. equina	109812	dH 12246, IWW 493	Drinking water	Germany		JF747075	JN128769		
	109914	dH 11627, IWW 326	Drinking water	Germany		JF747076	JN128765		
	120913	dH 17747-2, DTO Tm 06.131 20/10 A	Ice water for cooling	The Netherlands, Oosterwolde		JF747144	JN128750	JN112506	
	109913	dH 11629, IWW 544	Drinking water	Germany		JF747145	JN128817	JN112507	
	121501	dH 12466, det 175-01	Drinking water	The Netherlands		JF747077	JN128806	JN112453	
	122977	dH 19905, DTO 57-E4 S	Drinking water	The Netherlands		JF747078		JN112398	
	120278	dH 17390, DTO 06.095-1.3	Drinking water, after water meter	The Netherlands, Geldermalsen		JF747079	JN128803	JN112454	
	115143	GHP R53	Drinking water	Germany		JF747080	JN128807		
	120904	CPC 11047	Bottled water	Australia		JF747081			Avila de la Calle et al. 2006
	121513	dH 13558, det 36/2004 h	Water from water machine	The Netherlands, Joure		JF747082			
	124181	dH 14518, DTO M 14A Tm 05.033	Water system of packaging machine	The Netherlands		JF747083			
	124180	dH 20175	Bathroom-flask	The Netherlands		JF747084			
	124173	dH 20174	Bathroom-flask	The Netherlands		JF747085			
		dH 19902	Bathroom-flask	The Netherlands		JF747086	JN128808		
		dH 20043	Bathroom-plate	The Netherlands		JF747087	JN128809		
		Hamada 1238	Bathroom	Japan, Osaka		JF747088	JN128810		
	109789	dH 12503, Det 239/01	Human, dialysis	The Netherlands		JF747089			
	121283	dH 14520, DTO Tm 05.033 / V85A	Waste water	USA, Falmouth		JF747090	JN128811		
	120905	dH 13762, UTHSC 04-526	Human, ulcer cornea	Denmark, Statens Serum Institut		JF747091	JN128804		
	122267	dH 17015, Saunte 72	Human, finger nail	The Netherlands		JF747092	JN128812		
	121285	dH 13080, Det M-116 / 2003	Human, skin flakes	USA, San Antonio		JF747093	JN128813		
	121282	dH 13350, UTHSC 97-1647	Human	The Netherlands					
	121286	dH 13330, Det M327 / 2003	Human, sputum	USA				JN112399	
	120906	dH 13647, UTHSC 89-386	Stool					JN112400	
	119.23	(T of *Haplographium debellae-marengoi* v. *equinum*)							
	116009	dH 13221, F1090	Horse	Italy	JN856017	JF747094	JN128814	JN112401	Pollacci 1923
	150.93		Galapagos turtle	USA, Chicago, Zoo Aquarium		JF747095	JN128805		Manharth et al. 2005
	116922		Washed *Tilia* root	Germany		JF747096	JN128815		
		DTO Tm 04.136	Silica gel	The Netherlands		JF747097	JN128816		
		DTO Tm 04.114	Tube of gelly installation	The Netherlands					
	121504 (T)	dH 12647, det M360/2002 Brasch	Tinea on leg of child (18 mo)	Germany, Kiel		JF747098	JN128820	JN112402	
		GHP 2426	Human, diabetic, skin	Germany		JF747099	JN128821		
		GHP 2411	Human, diabetic, skin	Germany		JF747100	JN128822		
	122263	dH 17045, Saunte 102	Human, foot	Denmark, Copenhagen		JF747101	JN128818	JN112381	
	120387	dH 16674, Saunte 21	Human, toe nail	Denmark, Copenhagen		JF747102	JN128819	JN112403	
	122270	dH 16692, Saunte 39	Human, foot			JF747103	JN128823		
	515.76	dH 12615	Soil	Canada		JF747104	JN128824		
	661.76		Nematode cyst, *Heterodera*	Germany		JF747105	JN128825		
	160.89		Washed root, *Hordeum*	The Netherlands				JN112404	
	159.89		Washed root	The Netherlands					
		Selosse Isolate 1	Root mycorrhiza, *Cephalanthera damasonium*						
E. halophila	121502	Det 238-1855, dH 12507	*Olea*, twig	Italy		JF747106	JN128826	JN112405	Julou et al. 2005
		dH 12489, det 209-01 F	*Olea*, twig	Italy, Bari					
		Ms16Mb14	*Phragmitis australis*	Germany, Lake Constance	FJ665274				
	123150	UAMH 10998	*Populus tremuloides*, root	Canada, Alberta		JF747107			Neubert et al. 2006
E. lacus		Det 08-017-20 (T of *Exophiala tremulae*)	Salty water	USA					
	121512 (T)	dH 13757, UTHSC 03-2191	Human, skin axilary	USA	JN856015	JF747108	JN128774	JN112406	
	121499	dH 12324, Mayser 2151/99	Human, nail	Germany		JF747109	JN128775	JN112407	
	117497 (T)	dH 13711	Lake water, 1 m depth	The Netherlands, Loosdrecht		JF747110	JN128776		
E. mesophila	402.95 (T)		Shower joint	Germany	JN856016	JF747111	JN128761		Listemann & Freiesleben 1996
	836.95	dH 16276	Swimming pool	Germany		JF747112	JN128752	JN112477	
	119910	dH 16410, UTHSC R-3282	Dental waterline	USA		JF747113	JN128753	JN112478	
	109147	dH 11838, Matos T-20	Bathroom	The Netherlands		JF747114	JN128754	JN112479	
		dH 18626	Bathroom	The Netherlands, Hilversum		JF747115	JN128755	JN112480	
	121498	dH 12261, M415-10-320/01	Human, phaeohyphomycotic cyst	USA		JF747116	JN128762	JN112481	
	121509	dH 13436, UTHSC R-1444 (EC001)	Human, finger	USA		JF747117	JN128756	JN112482	
	121508	dH 13400, UTHSC 91-270 (EJ001)	Human, sinus	USA		JF747118	JN128757	JN112483	
	120910	dH 13763, UTHSC 04-611	Human, hip joint	USA		JF747119	JN128758	JN112484	
	120907	dH 13765, UTHSC 04-1300	Human, hair	USA		JF747120	JN128759		
	121507	dH 13387, UTHSC 96-1493 (EJ001)	Human, immunosuppressed, bronchial endoscopy	France, Rouen		JF747121	JN128760		
	121497	dH 12260, M415-08-966/01	Human, immunosuppressed	USA		JF747122	JN128751	JN112485	
	121511	dH 13460, UTHSC 92-1021 (EJ005)	Human, nasal tissue	Germany					
		GHP R28							

Table 1 (cont.)

Name	CBS	Other reference numbers	Source	Geography	GenBank					Reference
					SSU	ITS	TEF1	BT2	ACT1	
E. opportunistica	109811 (T)	dH 12243, IWW 720	Drinking water	Germany		JF747123	JN128792	JN112486	JN112408	
	122269	dH 16680, Saunte 27	Human, nail	Denmark, Copenhagen		JF747124	JN128795	JN112487	JN112409	
	122268	dH 16705, Saunte 52	Human, foot	Denmark, Copenhagen		JF747125	JN128794	JN112488	JN112410	
	660.76	dH 16144	Rhizosphere, *Triticum aestivum*	West Australia		JF747126	JN128793	JN112489		
	637.69	dH 16111	Polyvinyl alcohol			JF747127	JN128796	JN112490		
	631.69		Unknown	The Netherlands		JF747128	JN128797			
E. pisciphila	121505	dH 13077, Det 100 / 2002	Swimming pool	Germany		JF747129	JN128790	JN112491		
	101610	dH 11173	Water pipe	Germany		JF747130		JN112492	JN112411	
	537.73 (T)	WUC 137	Catfish	USA	JN856018	JF747131	JN128788	JN112493	JN112412	Fijan 1969; McGinnis & Ajello 1974
	119913	dH 16407, UTHSC 05-656, 05-460	Potbelly seahorse			JF747132		JN112494	JN112413	
	119914	dH 16406, UTHSC 05-173, 5-317	Potbelly seahorse			JF747133		JN112495		
	121500		Human, nail	Germany		JF747134	JN128791	JN112496	JN112414	
E. psychrophila	191.87 (T)	dH 12328, Mayser 1748/00	*Salmo salar* in fish farm	Norway	JN856019	JF747135	JN128789	JN112497		Pedersen & Langvad 1989
	256.92		Salmon	Ireland		JF747136	JN128798	JN112498		
E. salmonis	157.67 (T)	BMU 00834	Trout, brain	Canada	JN856020	JF747137	JN128747	JN112499	JN112415	Carmichael 1966
	120274	dH 17392, DTO 06.095 nr.2.2	Drinking water tap	The Netherlands, Geldermalsen		JF747138	JN128802	JN112500	JN112416	
	110371	dH 12699, det 405-01-8862/01	Drinking water	The Netherlands		JF747139	JN128748	JN112501	JN112417	
V. botryosa	121506	Jorg Mayer M 218, dH 11516	Frog	USA, Rhode Island, Park Zoo		JF747140		JN112502	JN112418	
	101462	dH 11373	Human, wrist skin	Japan		JF747141		JN112503	JN112419	
	102593	dH 13325, Padhye 2004-000517	Human, skin	China, Jiangsu Province		JF747142		JN112504	JN112420	
		dH 1917, CDC 5937	Human, disseminated in child (12 y)							
	254.57 (T)		Sansa olive slag	Italy, Toscana, Pisa	JN856021	JF747143		JN112505	JN112421	Ciferri & Montemartini 1957
		dH 18628	*Eucalyptus* wood treated with creosote 20 y ago	Brazil, Rio Claro		*				
		dH 18639	*Eucalyptus*	Brazil, Rio Claro		*				
		dH 18642	*Eucalyptus*	Brazil, Rio Claro		*				
		dH 18630	*Eucalyptus*	Brazil, Rio Claro		*				
		dH 18640	*Eucalyptus*	Brazil						

during 4 wk. Incubation temperatures were between 4 and 40 °C with 3 °C intervals. Averages of two to three measurements were calculated.

Microscopy

Agar blocks (MEA) of ~1 cm^2 were placed on a sterile object glass supported by a V-shaped glass bar and inoculated at the four sides. The block was subsequently covered with a sterile cover slip (~2 cm^2). Growth was allowed in a closed glass Petri dish; the bottom was covered with sterile paper filter soaked with 5 mL sterile water to ovoid drying of the culture. The chambers were incubated at room temperature for 5, 10 or 14 d. Slides were made in lactic acid. Permanent slides were sealed with polyvinyl alcohol. Micrographs were taken using a Nikon Eclipse 80i microscope and DS Camera Head DS-Fi1/DS-5m/DS-2Mv/DS-2MBW using NIS-Element freeware package (Nikon Europe, Badhoevedorp, the Netherlands).

DNA extraction

Methods were outlined by Gerrits van den Ende & de Hoog (1999). About 1 cm^2 of fungal material from 3–4 wk old cultures was transferred to a 2 mL Eppendorf tube containing about 80 mg of a silica mixture (silica gel H, Merck 7736 / Kieselguhr Celite 545, Machery, 2 : 1, w/w) and 300 µL TES buffer (1.2 g Tris, 0.38 g Na-EDTA and 2 g sodium dodecylsulphate (SDS) in 80 mL ultrapure water, pH 8.0). Cells were ground mechanically with a tight-fit pestle for 1–2 min. Subsequently 200 µL TES was added. After the mixture was vortexed, 10 µL Proteinase K was added and the mixture incubated at 65 °C for 10 min. 140 µL 5 M NaCl and 65 µL 1 % CTAB (cetyltrimethylammonium bromide) were added, and the solution incubated at 65 °C for 30 min. Subsequently 700 µL SEVAG was added and carefully mixed by hand for about 1 min and incubated during 30 min at 0 °C (on ice water). The solution was centrifuged for 10 min at 4 °C at 20,400 *g*. The upper water phase was transferred to a clean Eppendorf tube to which 225 µL 5 M NH_4-acetate were added and gently mixed. The mixture was incubated again on ice water for at least 30 min and centrifuged for 10 min at 4 °C at 20,400 *g*. The supernatant was transferred to a clean Eppendorf and supplemented with 510 µL isopropanol, mixed carefully and directly centrifuged for 5 min at 20,400 *g*. The supernatant was decanted and the pellet washed twice with 70 % ethanol. The pellet with DNA was vacuum dried in a DNA Speed Vac (New Brunswick Scientific, Nijmegen, the Netherlands) for 10–15 min at a medium Drying Rate stand. Finally, the DNA was resuspended in 50 µL TE buffer including 1.5 µL RNAse and incubated for 15–30 min at 37 °C. DNA quality was verified by NanoDrop® ND-1000 Spectrophotometer using ND-1000 v. 3.3.0 software (Coleman Technologies, Wilmington, DE, USA). Samples were stored at –20 °C.

Amplification

Fragments of rDNA were amplified using the universal primers V9G and LS266 for rDNA ITS (Gerrits van den Ende & de Hoog 1999), NS1 and NS24 for rDNA SSU or alternative primer combinations (NS1 and Oli16, NS1 and NS8), Ef1-728F and Ef1-986R for *TEF1*, Bt2a and Bt2b for *BT2* and Actfw and Actbw or otherwise combined with EspActbw for *ACT1* in a reaction mixture containing 30 µL sterile water, 5 µL PCR buffer 10×, 10 µl dNTP (1 mM), 1 µL of each of the primers (50 pmol/µL, or otherwise for degenerate primers), 1 µL DNA polymerase (1 U/µL) and 1 µL fungal DNA. Thirty-five cycles were performed in a GeneAmp PCR System 9700 (Applied Biosystems), with 5 min delay, and 35 cycles of 94 °C for 45 s (denaturation), 52 °C for 30 s (annealing) and 72 °C for 120 s (extension), with a final delay of 7 min and using the maximum ramp speed for ITS amplification. For SSU the annealing tem-

perature was lowered to 48 °C and for *TEF1* amplification raised to 55 °C. Five µL of each PCR product, with 2 µL loading buffer, was electrophoresed in 1 % agarose gels with 0.5×10^{-5} (v/v) ethidium bromide, in TAE 1× buffer (200 ml TAE 50×) (BioRad: 242 g Tris, 57.1 mL acetic acid, 100 mL, 0.5 M EDTA) mixed with 9 800 mL ultrapure water) at 80–100 V for 90 min, and using 5 µL Smart Ladder (Eurogentec, Seraing, Belgium) as marker. Amplicon quality and concentration were estimated on agarose gels (1.0–1.2 %), which were photographed. Amplicons were cleaned using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences). Sequencing reactions were performed using ITS1 and ITS4 for ITS sequences. BF83, Oli9, BF963, BF1419 and Oli1, BF951, BF1438, NS24 (or Oli3) for SSU sequencing, EF1-728F, EF1-986R for *TEF1*, Bt2a and Bt2b for *BT2*, and Actfw and Actbw eventually combined with EspActbw for *ACT1*, following protocols for DYE-ET terminator cycle sequencing. Reaction mixtures varied with the sample, as follows: 1 µL template DNA (0.1 pmol), 1 µL primer (4 µM), 1 µL sequencing reagent premix, 3 µL dilution buffer completed with 5.5-× µL ultra pure water to 10 µL final volume. Reactions were performed in a GeneAmp in 25 cycles of: 95 °C for 20 s, 50 °C for 15 s, 60 °C for 60 s and stopped with cooling to 4 °C. Samples were purified with Sephadex G-50 Superfine into a 96 wells of a MultiScreen HV plate and recovered in a standard 96-well microtiter plate. This eluting plate is covered with aluminium foil tape (3M Scotch 431, 75 mm) and can directly be loaded on the ABI 3700 machine for sequences reading or stored at –20 °C. Sequences were analysed using SeqMan II software (DNASTAR).

Alignment and phylogenetic reconstruction

For genealogical concordance analysis, four genes ITS, *TEF1*, *BT2* and *ACT1* were first analysed separately. Alignment was performed automatically and adjusted iteratively by hand with BioNumerics v. 4.61 (Applied Maths, Kortrijk, Belgium). Topological conflicts were evaluated visually and by using the partition homogeneity test implemented in PAUP v. 4.0b10. ITS and multilocus trees were constructed with maximum likelihood and with 100 bootstrap replicates using RAxML v. 7.2.3 (Stamatakis et al. 2008) as implemented on the Cipres Portal v. 1.10, and edited with MEGA4 software (Tamura et al. 2007).

A phylogenetic approach was used to investigate relationships between 147 waterborne strains of *Exophiala* and related species (Table 1), with *Exophiala castelanii* and *E. mesophila* as outgroups in the SSU tree. For species circumscribed by genealogical concordance, a single strain (usually the ex-type) was selected for sequencing conserved genes. Sequences were compared in a database in BioNumerics containing all described members of *Chaetothyriales*. The database was regularly updated with recent GenBank and AFTOL submissions. SSU (1 100 comparable sites) trees were generated with the Parsimony option of PAUP after removal of introns.

RESULTS

Phylogeny

A general tree for a large set of representatives of the order *Chaetothyriales* was constructed using SSU rDNA data (Fig. 1), with *Phaeococcomyces catenatus* CBS 650.76 as outgroup. The SSU alignment had 562 distinct patterns; frequency pi(A) = 0.261153, pi(C) = 0.209808, pi(G) = 0.263550, pi(T) = 0.265489. ML was calculated with RAxML v. 7.2.3 with Gamma correction and GTR substitution matrix.

Two ancestral lineages, with 98 and 93 % bootstrap support, respectively, contained prevalently rock-inhabiting fungi (*Coniosporium* spp.) with prevalently isodiametric morphology (group 5
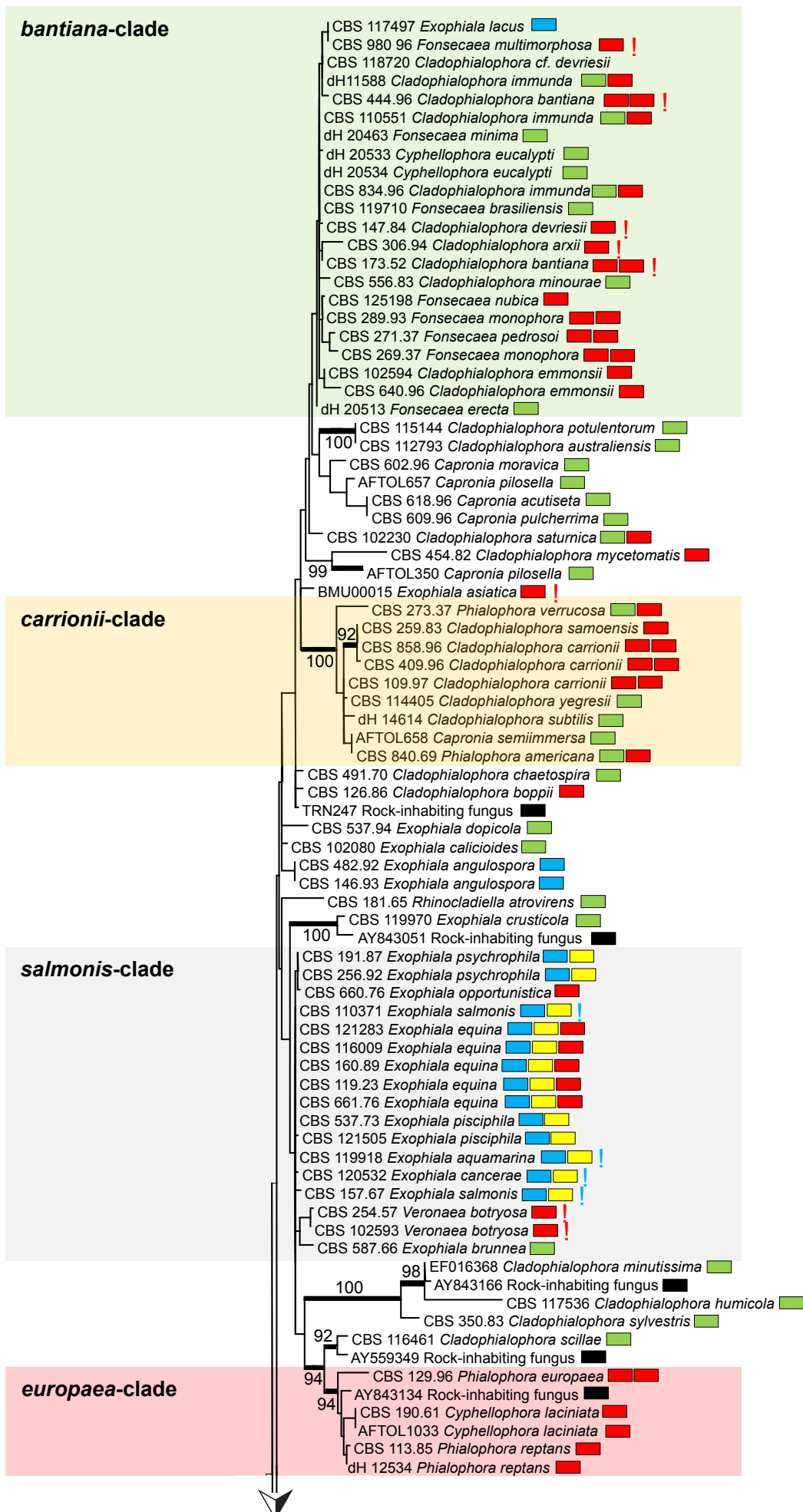
of Haase et al. 1999), plus the plant-inhabiting species *Cladophialophora hostae.* The remainder of the tree, at rather large distance and with 100 % bootstrap support, is considered to represent the ascomycete family *Herpotrichiellaceae*. Teleomorphs, when present in this group are *Capronia* species, except that *Ceramothyrium linnaeae* is also found in this part of the tree.

Overall 19 bootstrap-supported (i.e. > 80 %, indicated in bold in Fig. 1) branches were present in the *Herpotrichiellaceae*; the core structure was poorly resolved. Nevertheless, a number of approximate species complexes could be distinguished, some of which corresponded with SSU groups (1–4) previously recognised by Haase et al. (1999). The approximate groups have here been listed as *bantiana*-clade, *carrionii*-clade, *salmonis*-clade, *europaea*-clade, *dermatitidis*-clade, *jeanselmei*-clade, and some ancestral lineages (Fig. 1). Group Haase-1 is recognised below as the *dermatitidis*-clade, Haase-2 is the *bantiana*-clade, and Haase-3 and -4 correspond with two clusters, described in more detail below, as the *jeanselmei*-clade. None of the clades was morphologically homogeneous; the anamorph genera *Cladophialophora*, *Cyphellophora*, *Exophiala*, *Fonsecaea* and *Rhinocladiella* are all polyphyletic within the order *Chaetothyriales*.

When major sources of isolation were plotted on the tree (habitats included rock, plant, water/cold-blooded animal, warmblooded animal) no meaningful clustering was evident, except for a grouping of waterborne species in a group referred to as the *salmonis*-clade (Fig. 1). However, in detailed study, different trends become apparent. The *bantiana*-clade contained thermotolerant systemic pathogens, such as *C. bantiana* and *C. arxii*, as well as the *Fonsecaea* agents of chromoblastomycosis. The *bantiana*-clade included only a single *Exophiala* species, *E. lacus.* This species is known from two strains from water, and has CBS 117497 as the ex-type. The *carrionii*-clade had 100 % bootstrap support and contained many of the agents of chromoblastomycosis. The *dermatitidis*-clade (group Haase-1), with 90 % bootstrap support, includes some oligotrophic thermophiles with an invasive, neurotropic ability, in addition to environmental *Capronia* species. A group of superficial human pathogens clustered around *Phialophora europaea* (the *europaea*-clade had 94 % bootstrap support).

The majority of mesophilic, waterborne *Exophiala* species were strongly clustered. A large clade (seen in Fig. 1 as the *salmonis*-clade) contained almost exclusively waterborne species; only the drinking water species *E. angulospora* took an isolated position. The *salmonis*-clade comprised nine waterborne *Exophiala* species, *E. aquamarina*, *E. brunnea*, *E. cancerae*, *E. equina*, *E. halophila*, *E. opportunistica*, *E. pisciphila*, *E. psychrophila* and *E. salmonis*. The sympodially reproducing species *Veronaea botryosa*, a potential agent of disseminated infections in humans, was also found in the same clade at some distance from the *Exophiala* species.

The *salmonis*-clade was analysed in more detail using ribosomal ITS sequences (Fig. 2) and multilocus data (Fig. 3); *Exophiala castellanii* and *E. mesophila* were selected as outgroups for both trees. In the ITS tree (Fig. 2), three groups at high bootstrap support are recognisable: the *E. castellanii* / *E. mesophila* outgroup, the *E. angulospora* complex, and a large cluster (81 % bootstrap support) around the type species of *Exophiala*, *E. salmonis*, representing the *salmonis*-clade as recognised in SSU data. The majority of species in the *salmonis*-clade were waterborne *Exophiala* species, containing isolates causing disease on cold-blooded animals such as fish, turtles, crabs, sea horses and frogs. ITS and multilocus analyses distributed these strains over ten clusters (Fig. 2), among which seven have not formally been described as *Exophiala* species.
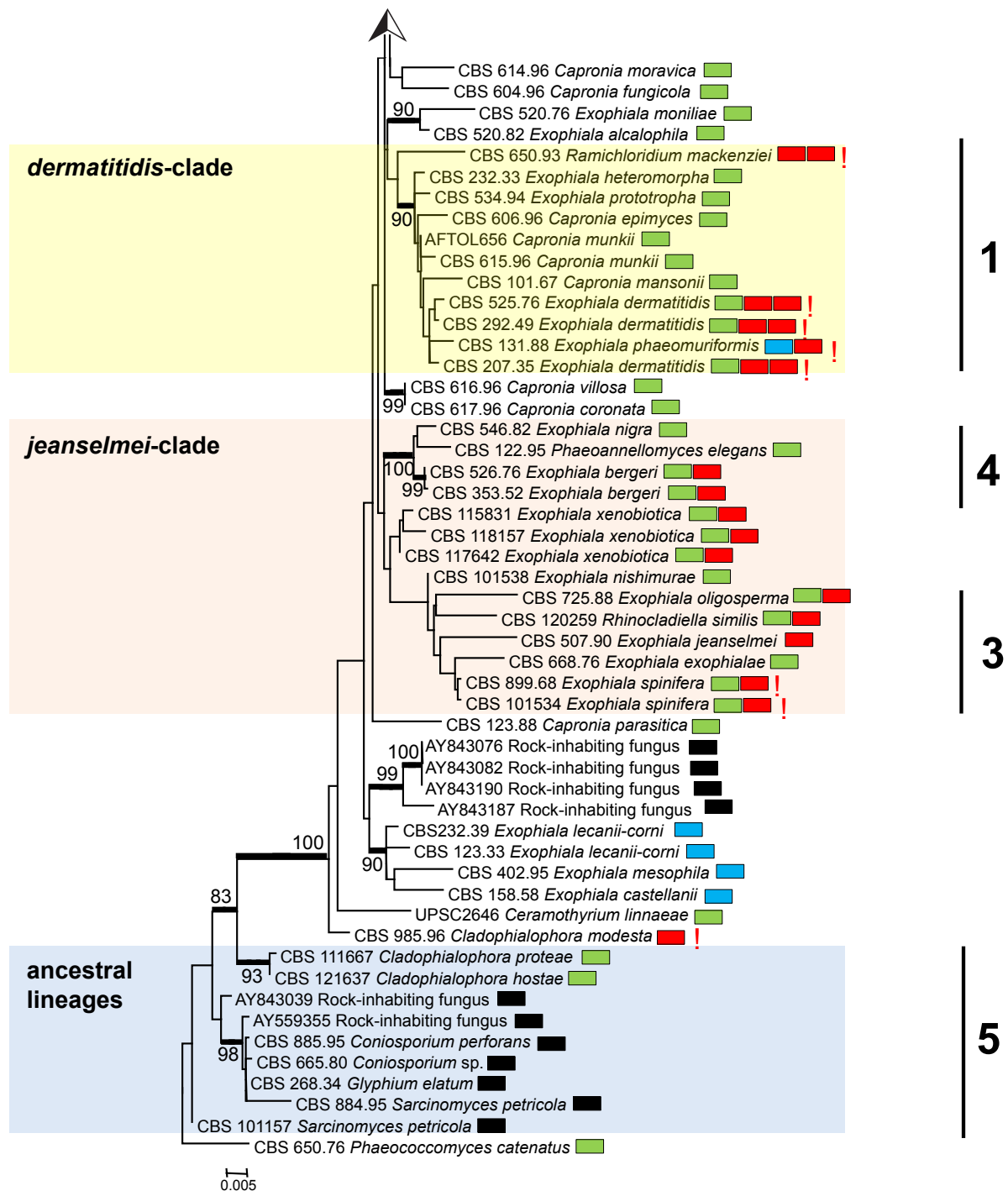
bantiana-clade

CBS 117497 *Exophiala lacus*
CBS 980 96 *Fonsecaea multimorphosa* !
CBS 118720 *Cladophialophora* cf. *devriesii*
dH11588 *Cladophialophora immunda*
CBS 444.96 *Cladophialophora bantiana* !
CBS 110551 *Cladophialophora immunda*
dH 20463 *Fonsecaea minima*
dH 20533 *Cyphellophora eucalypti*
dH 20534 *Cyphellophora eucalypti*
CBS 834.96 *Cladophialophora immunda*
CBS 119710 *Fonsecaea brasiliensis*
CBS 147.84 *Cladophialophora devriesii* !
CBS 306.94 *Cladophialophora arxii* !
CBS 173.52 *Cladophialophora bantiana* !
CBS 556.83 *Cladophialophora minourae*
CBS 125198 *Fonsecaea nubica*
CBS 289.93 *Fonsecaea monophora*
CBS 271.37 *Fonsecaea pedrosoi*
CBS 269.37 *Fonsecaea monophora*
CBS 102594 *Cladophialophora emmonsii*
CBS 640.96 *Cladophialophora emmonsii*
dH 20513 *Fonsecaea erecta*

2

100 CBS 115144 *Cladophialophora potulentorum*
CBS 112793 *Cladophialophora australiensis*
CBS 602.96 *Capronia moravica*
AFTOL657 *Capronia pilosella*
CBS 618.96 *Capronia acutiseta*
CBS 609.96 *Capronia pulcherrima*
CBS 102230 *Cladophialophora saturnica*
CBS 454.82 *Cladophialophora mycetomatis*
99 AFTOL350 *Capronia pilosella*
BMU00015 *Exophiala asiatica* !

carrionii-clade

CBS 273.37 *Phialophora verrucosa*
92 CBS 259.83 *Cladophialophora samoensis*
CBS 858.96 *Cladophialophora carrionii*
100 CBS 409.96 *Cladophialophora carrionii*
CBS 109.97 *Cladophialophora carrionii*
CBS 114405 *Cladophialophora yegresii*
dH 14614 *Cladophialophora subtilis*
AFTOL658 *Capronia semiimmersa*
CBS 840.69 *Phialophora americana*

CBS 491.70 *Cladophialophora chaetospira*
CBS 126.86 *Cladophialophora boppii*
TRN247 Rock-inhabiting fungus
CBS 537.94 *Exophiala dopicola*
CBS 102080 *Exophiala calicioides*
CBS 482.92 *Exophiala angulospora*
CBS 146.93 *Exophiala angulospora*
CBS 181.65 *Rhinocladiella atrovirens*
CBS 119970 *Exophiala crusticola*
100 AY843051 Rock-inhabiting fungus

salmonis-clade

CBS 191.87 *Exophiala psychrophila*
CBS 256.92 *Exophiala psychrophila*
CBS 660.76 *Exophiala opportunistica*
CBS 110371 *Exophiala salmonis* !
CBS 121283 *Exophiala equina*
CBS 116009 *Exophiala equina*
CBS 160.89 *Exophiala equina*
CBS 119.23 *Exophiala equina*
CBS 661.76 *Exophiala equina*
CBS 537.73 *Exophiala pisciphila*
CBS 121505 *Exophiala pisciphila*
CBS 119918 *Exophiala aquamarina* !
CBS 120532 *Exophiala cancerae* !
CBS 157.67 *Exophiala salmonis* !
CBS 254.57 *Veronaea botryosa* !
CBS 102593 *Veronaea botryosa* !
CBS 587.66 *Exophiala brunnea*

98 EF016368 *Cladophialophora minutissima*
100 AY843166 Rock-inhabiting fungus
CBS 117536 *Cladophialophora humicola*
CBS 350.83 *Cladophialophora sylvestris*
92 CBS 116461 *Cladophialophora scillae*
AY559349 Rock-inhabiting fungus

europaea-clade

94 CBS 129.96 *Phialophora europaea*
AY843134 Rock-inhabiting fungus
94 CBS 190.61 *Cyphellophora laciniata*
AFTOL1033 *Cyphellophora laciniata*
CBS 113.85 *Phialophora reptans*
dH 12534 *Phialophora reptans*

Fig. 1 Phylogeny of all members of *Chaetothyriales* described to date, obtained from a ML analysis based on SSU rDNA sequences. Bootstrap support was calculated from 100 replicates; values > 80 % are shown with the branches. Supported branches are drawn in **bold**. The tree was rooted with *Phaeococcomyces catenatus*, CBS 650.76. Coloured boxes represent species complexes recognised in this paper. Prevalent species ecologies are summarised in boxes at the right hand side of each strain. Black: rock-inhabiting; blue: waterborne; green: plant-associated; red: invasive in warm-blooded animals; yellow: invasive in cold-blooded animals; red exclamation mark: systemic in warm-blooded animals; blue exclamation mark: systemic in cold-blooded animals; double boxes indicate relative high frequency of the species in more than one category.

Veronaea botryosa, with sympodial conidiogenesis very different from the annellidic conidiogenesis of the *Exophiala* species, is found adjacent to the waterborne species in all genes analyzed (Fig. 1–3). Partition-homogeneity testing, based on heuristic searching of four genes (ITS, *TEF1*, *BT2*, *ACT1*) with 100 replicates and 167 parsimony-informative characters of 2 254 total characters, revealed conflict (significant heterogeneity) among the genes (*p* = 0.01). In ITS or multilocus trees, the *salmonis*-clade was composed of 10 distinct groups, mostly with bootstrap support in both trees. These groups were: *E. equina* (74 % bootstrap in ITS / 100 % in multilocus comparison),

E. salmonis (99 ITS / 100 multilocus), *E. pisciphila* (98 / 100), *E. aquamarina* (92 / 100), *E. psychrophila* (100 / 100), *E. opportunistica* (– / 99), and *E. cancerae* (88 / 100). *Veronaea botryosa* and *E. brunnea* are located separately within the clade, as was also visible in the SSU tree, at 99 % bootstrap support (Fig. 1). The single strain of *Exophiala lacus* is found in SSU analysis to be a member of the *bantiana*-clade and consequently it was connected by a long branch to members of the *salmonis*-clade.

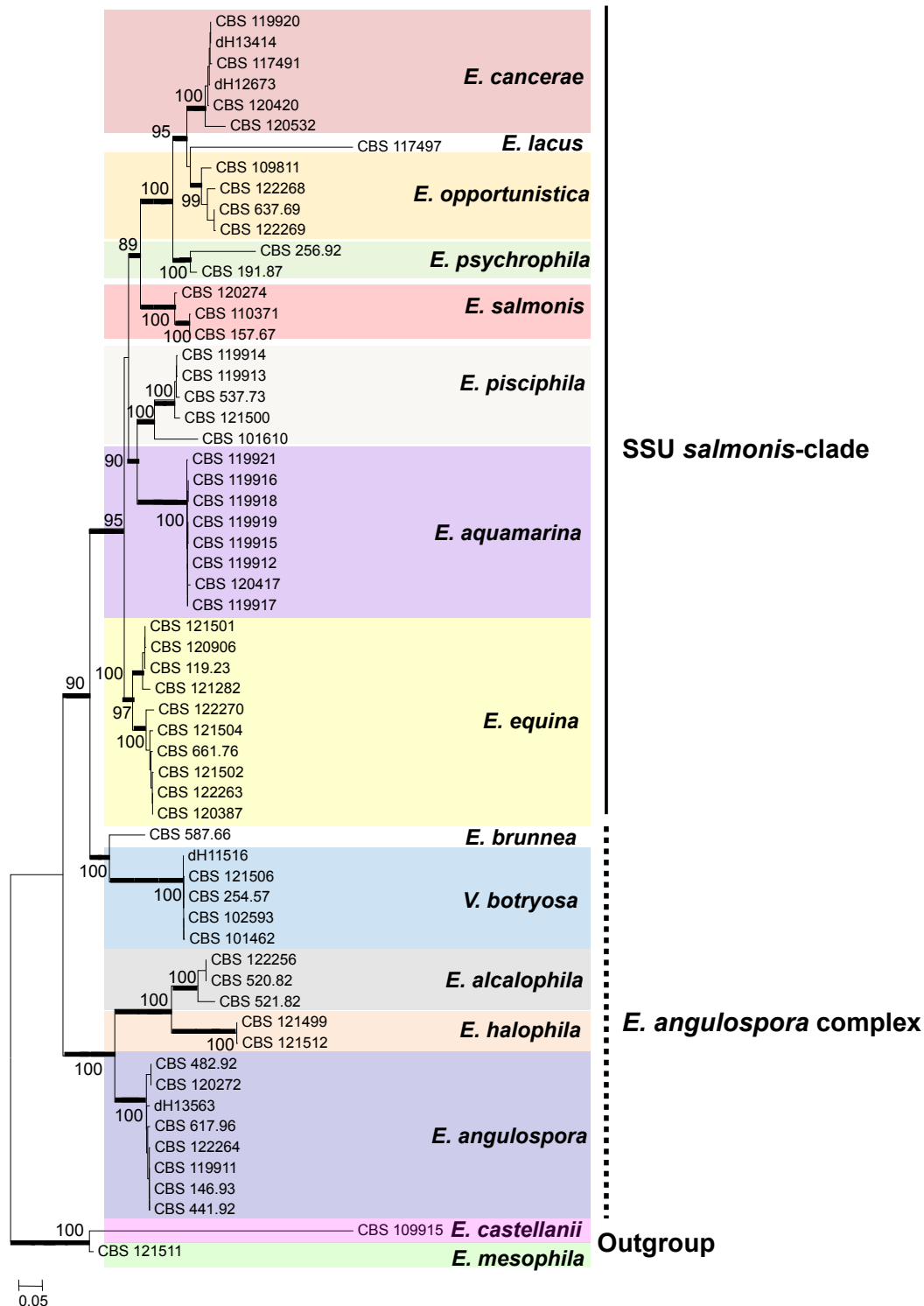Outside the *salmonis*-clade two clusters containing strains of waterborne species were recognisable in the SSU tree (Fig. 1)
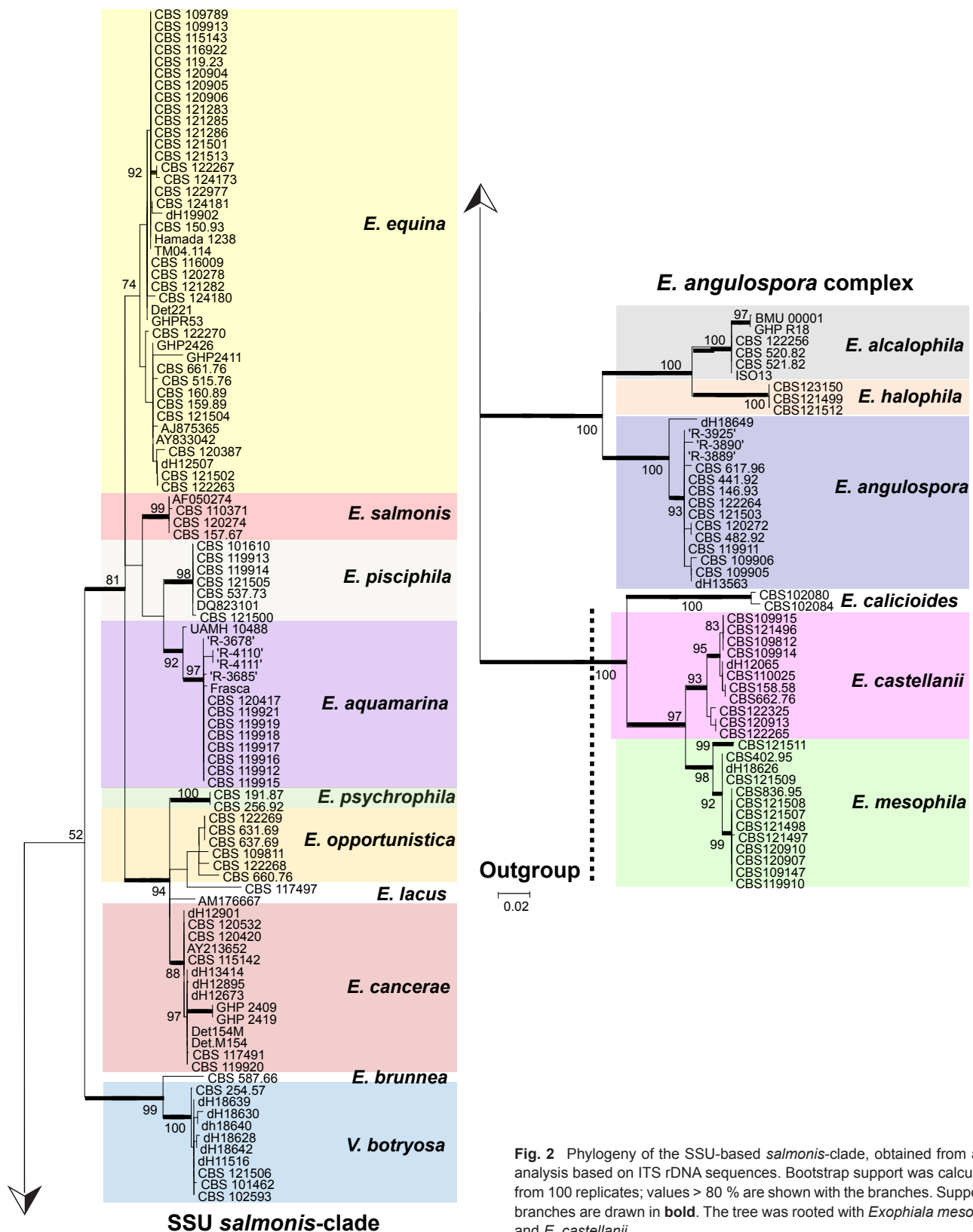
Fig. 2 Phylogeny of the SSU-based *salmonis*-clade, obtained from a ML analysis based on ITS rDNA sequences. Bootstrap support was calculated from 100 replicates; values > 80 % are shown with the branches. Supported branches are drawn in **bold**. The tree was rooted with *Exophiala mesophila* and *E. castellanii.*

and were also found at 100 % bootstrap support in the ITS tree (Fig. 2). Nearly all species within these clusters (*E. alcalophila*, *E. angulospora*, *E. castellanii*, *E. halophila* and *E. mesophila*) were strongly supported. Some showed significant intraspecific variability. The cardinal growth temperatures of the species of the *salmonis*-clade were determined. The maximum growth temperature was found to be 33 °C.

Taxonomy

Exophiala J.W. Carmich., Sabouraudia 5: 122. 1966. — Myco-Bank MB8233

The genus *Exophiala* was described as an anamorph taxon by Carmichael (1966), with *E. salmonis* as its type species. The genus represents an anamorphic element of the order *Chaetothyriales.* Note that the type species has among its synonyms the name *Aureobasidium salmonis. Aureobasidium*, however, is a member of the order *Dothideales* (Schoch et al. 2009).

Fig. 3 Phylogeny of the SSU-based *salmonis*-clade, obtained from a ML analysis based on ITS, *ACT1*, *BT2* and *TEF1* sequences. Bootstrap support was calculated from 100 replicates; values > 80 % are shown with the branches. Supported branches are drawn in **bold**. The tree was rooted with *Exophiala mesophila* and *E. castellanii*.

Teleomorphic relations of *Exophiala* lie in the genus *Capronia*, typified by *Capronia sexdecimspora*. *Exophiala* is the main genus of black yeasts found as opportunists of vertebrates. It is characterised by annellidic conidiogenesis. Some cultures are entirely yeast-like (synanamorph *Phaeococcomyces*), or form phialidic collarettes (synanamorph *Phialophora*), sympodial coni-diophores (synanamorph *Rhinocladiella*) or dry conidial chains (synanamorph *Cladophialophora*). Chlamydospores or sclero-tial bodies may also be formed, occasionally leading to entirely meristematic mutants (synanamorph *Sarcinomyces*). Several of these morphologies are represented in species of the water-borne *salmonis*-clade (B).

Thermophilic species of *Exophiala* have been described from systemic infections in humans, e.g. *E. dermatitidis* and *E. spini-fera*. In contrast, numerous species of the *salmonis*-clade (B) are mesophilic or psychrotolerant, and are regularly found as opportunists on cold-blooded vertebrates. Many such cases have been reported in the literature, and these cases have been attributed to a diversity of *Exophiala* species. However, the identification of etiologic agents in many of these cases must be questioned, since the morphological characters used have proven not to be reliable for species identification. This unreli-ability is attributable to variable morphologies within species and to overlapping characters among them. Therefore in the

present paper we will only reference the case reports where voucher strains have been preserved and sequenced.

Exophiala alcalophila Goto & Sugiy., in Goto et al., Trans. Mycol. Soc. Japan 22: 430. 1981. — MycoBank MB110200

= *Phaeococcomyces alcalophilus* Goto & Sugiy., Trans. Mycol. Soc. Japan 22: 432. 1981.

Description of CBS 520.82 after 2 wk incubation on MEA, 24 °C.

Colonies restricted, appearing slimy, smooth, soft, jet-black, convex with sharp margin. Initial growth with budding cells, later (after 1 mo) becoming slightly floccose at the centre and remaining slimy at the margin. Reverse brownish black; a rust brown pigment exuded into the agar. Budding cells abundant, smooth- and thin-walled, 1-celled, (sub)spherical to broadly ellipsoidal, 4–8 × 3–6 μm. Germinating cells present, (sub)spherical, 7–9 μm diam. Aerial hyphae smooth-walled, irregularly branched, 1.5–3.0 μm wide. *Conidiogenous cells* arising from undifferentiated hyphae, terminal or intercalary, with short annellated zones, mostly without discernible annellations; occasionally conidia produced apically in more or less sympodial order. *Conidia* hyaline, thin- and smooth-walled, 1-celled, spherical, ellipsoidal to slightly reniform, occasionally with truncate base, 3–7 × 2–5 μm, aggregated in slimy heads.

Cardinal temperatures — Minimum 4–9 °C, optimum 24–27 °C, maximum 36–40 °C.

Specimens examined. Japan, Hirose, Wako-shi, Saitama pref., 23 Apr. 1978, *K. Horikoshi*, from soil, holotype specimen of *Exophiala alcalophila*, CBS H-19960, culture ex-type IAM 12519 = CBS 520.82; Hirose, Wako-shi, Saitama pref., 23 Apr. 1978, *K. Horikoshi*, from soil, ex-type culture of *Phaeococcomyces alcalophilus*, IAM 12520 = CBS 521.82.
Additional material examined. Table 1.

Notes — The species was originally isolated with two morphotypes: one hyphal, and the other purely consisting of budding cells, for which reason the fungus was introduced under two generic names, *Exophiala* and *Phaeococcomyces*. This underlines the strongly dimorphic character of the species. Under the growth conditions applied in the present study, the yeast-like phase was pronounced during the first week, while hyphae later became more prevalent. The preponderance of either morphotype is unstable. Yamada et al. (1989) demonstrated that while *E. salmonis* had a coenzyme Q 10(H$_2$) ubiquinone system, *E. alcalophila* had Co-Q 10.

The original cultures were derived from soil on a minimal medium at a pH of 10.4 (Goto et al. 1981). Another strain (GHP R-18; Table 1) came from the soap container of a laundry machine. Nishimura et al. (1987) isolated the fungus repeatedly from bath water. Strain CBS 122256 was isolated from mildly symptomatic human skin in Denmark, but without precise clinical information. Lian & de Hoog (2010) recently noticed a possible link between cutaneous infection and the presence of potentially causal black yeast-like fungi in low-nutrient indoor water systems rich in soap, such as bathrooms. The authors repeatedly isolated several black yeast-like species from bathrooms, which until then had only been known from human skin and nails. They suggested that maceration of skin may provide a portal of entry during hygienic procedures for these fungi having moderate invasive capacity. *Exophiala alcalophila* apparently belongs to the same ecological group, although at low virulence.

Exophiala angulospora Iwatsu, Udagawa & Takase, Mycotaxon 4: 322. 1991. — MycoBank MB355245

Teleomorph. Capronia coronata Samuels, Trans. Brit. Mycol. Soc. 88: 65. 1967.

Description of CBS 482.92 after 2 wk incubation on MEA, 24 °C.

Colonies restricted, centrally mucous, velvety towards the outside, greyish green to olivaceous black. Germinating cells present, 6–10 × 2.4–4.0 μm. *Hyphae* pale olivaceous, smooth-walled, 1.5–3.0 μm wide. Budding cells present. *Conidiogenous cells* intercalary, lateral and terminal and then 1-celled, flask-shaped, 6–16 × 2.5–3.0 μm; conidia produced from a single short annellated zone per cell. *Conidia* aggregating in slimy heads, 1-celled, smooth- and thin-walled, subhyaline or pale olivaceous, mostly more or less triangular with rounded ends, 2.5–4.0 × 2–3 μm.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 24–27 °C, maximum 30–33 °C. No growth at 37 °C.

Specimens examined. Japan, Yokohama-shi, 18 Apr. 1989, *K. Arai*, from drinking well water, ex-type culture anamorph, CBS 482.92 = NHL 3101. – New Zealand, Westland County, Nemona State Forest, 5 May 1983, *G.J. Samuels*, from decorticated wood, ex-type culture teleomorph, CBS 617.96 = ATCC 56201 = PDD 35308.
Additional material examined. Table 1.

Notes — The species was originally repeatedly isolated from cold, low-nutrient drinking water (Iwatsu et al. 1991). The majority of strains sequenced originated from cold water, such as drinking water, aquaria and fish nurseries (Table 1). A teleomorph, *Capronia coronata*, originating from decorticated wood, was found to be identical in ITS sequence data. Nyaoke et al. (2009) noted a disseminated infection by CBS 119911 in a seawater-dependent weedy sea dragon (*Phyllopteryx taeniolatus*) in the New England Aquarium in Boston, USA, and also repeatedly in the marine lumpfish (*Cyclopterus lumpus*). Strain CBS 121503 was isolated from living freshwater fish *Scenodus leucichtys* in a nursery in Stravropol Kraj near Kislovodsk, southern Russia (V.A. Mel'nik, pers. comm.). Human isolates such as CBS 441.92 and CBS 122264 originated from skin and nail samples, but no proof of infection is available (Saunte et al. 2011). The species has been isolated once from hydrocarbon-polluted soil (Table 1).

In conclusion it appears that this fungus inhabits cold waters worldwide, where it has an invasive potential with fatal dissemination in cold-blooded vertebrates. Human infections have not been proven and may have concerned colonization only. This pathology is likely to be determined by its relatively low maximum growth temperature.

Exophiala aquamarina de Hoog, Vicente, Najafzadeh, Harrak, Badali, Seyedmousavi & Nyaoke, *sp. nov.* — MycoBank MB515716; Fig. 4, 5

Coloniae in agaro maltoso dicto 24 °C lente crescentes, velutinae, griseae; reversum olivaceo-nigrum. In agaro PDA pigmentum brunneum absens. Hyphae leves, dilute olivaeo-brunneae, intervallis regularibus septatae, nonnumquam spirales. Conidiophora vix distinguenda, ramose vel simplicia, terminalia vel intercalaria; conidium annellidorum vel sympodiorum producens. Conidia late ellipsoidea, levia, 6.7–19.2 × 4.0–4.8 μm. Temperatura maxima crescentiae 36 °C. Teleomorphe ignota.

Etymology. Name refers to sea water, the environment of most of the hosts of this species.

Description of CBS 119918 after 2 wk incubation on MEA, 27 °C.

Colonies restricted, olivaceous black, velvety with aerial mycelium at the centre. Reverse olivaceous black. No diffusible pigment produced. *Conidiogenous cells* flask-shaped, with short annellated zones, sometimes with sympodial conidiogenesis. Spirally twisted hyphae present. *Conidia* ellipsoidal to cylindrical, 6.7–19.2 × 4.0–4.8 μm. Yeast cells rarely present.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 24–30 °C, maximum 33–36 °C. No growth at 37 °C.

Specimen examined. USA, Boston, New England Aquarium, *S. Frasca*, from skin of leafy sea dragon (*Phycodures eques*), holotype CBS H-19950, ex-type culture CBS 119918 = UTHSC 00-1181 = dH 16401.
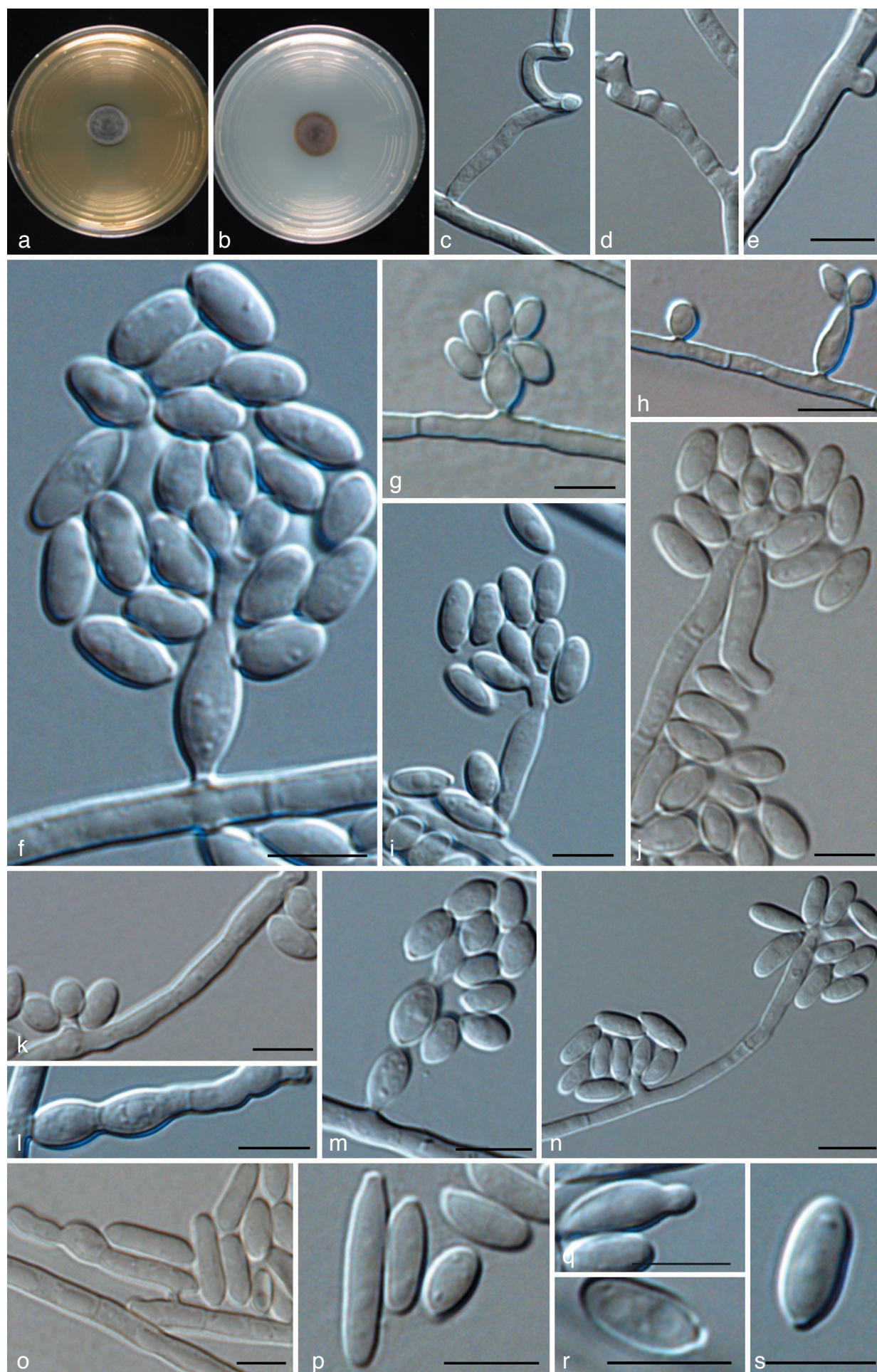*Additional material examined.* Table 1.

Fig. 4 *Exophiala aquamarina*, CBS 119918. a. Colony on MEA; b. colony on PDA; c, d. spirally twisted hyphae; e−n. conidial apparatus with conidia; f−j. annellidic conidiogeneses with sympodial conidiophores; o. anastomosis between discrete cells; p−s. conidia; q. budding cells. — Scale bars = 10 μm.

Notes — The species repeatedly caused disseminated infections in several species of fish in the New England Aquarium in Boston, Mass., USA, and in the Adventure Aquarium in Camden, N.J., USA (Nyaoke et al. 2009), particularly in leafy sea dragon (*Phycodurus eques*) and in weedy sea dragon (*Phyllopteryx taebiolatus*), but also in winter flounder (*Pseudopleuronectes americanus*) and little tunnyfish (*Euthynnus alletteratus*). Necrotic skin lesions were observed with mild inflammatory response, with invasion of blood vessels, and infection of skull and bone, but no brain involvement. Massive amounts of hyphae were observed in tissue. Infections took place over a 5-year period (Nyaoke et al. 2009). *Exophiala aquamarina* is so far restricted to fish, but is not host specific.

Exophiala brunnea Papendorf, Trans. Brit. Mycol. Soc. 52: 487. 1969. — MycoBank MB330806

Description of CBS 587.66 after 2 wk incubation on MEA, 24 °C.

Colonies developing slowly, with mouse-grey aerial mycelium at the centre; peripheral area depressed, dark olivaceous; reverse greenish black. *Hyphae* poorly branched, smooth-walled, pale olive-brown, 1–3 μm diam. Budding cells absent. *Conidiogenous cells* lateral, slightly differentiated from vegetative hyphae, frequently with 1–2 septa, simple or branched, variable in shape, flask-shaped, ovoidal to elongate, pale brown, 8–350 μm long. Annellated zones inconspicuous or occasionally finely fimbriate, 6–20 × 2–4 μm, often inserted on intercalary cells of hyphae and conidiophores. *Conidia* forming a coherent mass, broadly ellipsoidal or ovoidal, with a broad truncate hilum, continuous or occasionally with a median septum and then slightly constricted, smooth-walled, pale brown, 4.5–10 × 2–3 μm.

Cardinal temperatures — Minimum 4–9 °C, optimum 21–24 °C, maximum 30–33 °C. No growth at 37 °C.
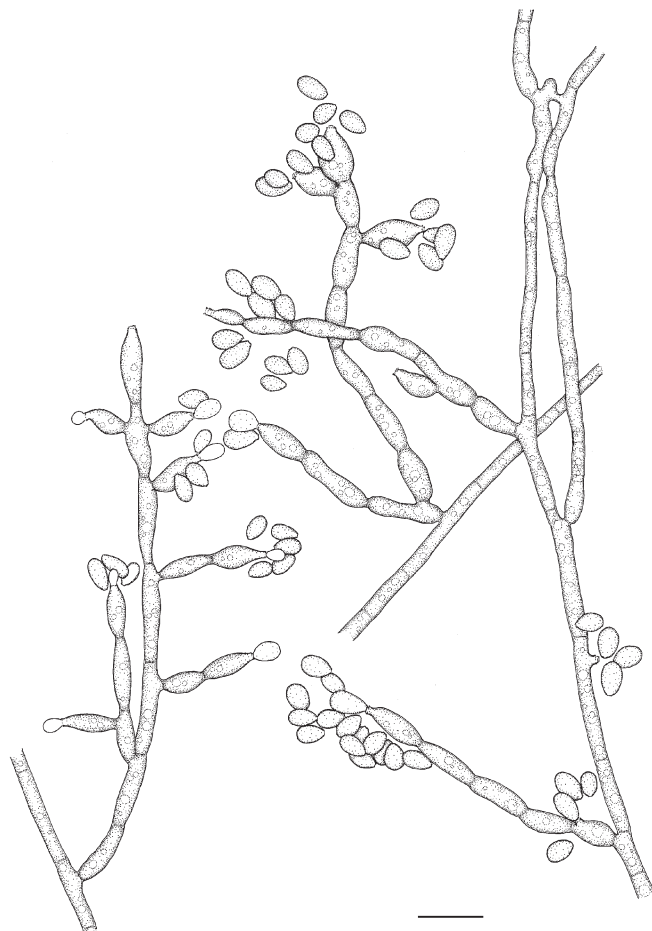

Fig. 5   *Exophiala aquamarina*, CBS 119918. Conidial apparatus and conidia. — Scale bar = 10 μm.

Specimen examined. South Africa, Potschefstroom, from leaf litter of *Acacia karroo*, specimens CBS H-12618, CBS H-19966, ex-type culture CBS 587.66.

Notes — The species is known from a single strain isolated from topsoil (leaf litter) of an *Acacia karroo* community (Leguminosae-Mimosoideae). The species was synonymised with *Exophiala salmonis* on morphological grounds (de Hoog 1977), but was later shown to be distinct based on molecular data.

Exophiala calicioides (Fr.) G. Okada & Seifert, Stud. Mycol. 45: 184. 2000. — MycoBank MB464794

Synonyms listed in Okada et al. (2000).

Notes — This species has fully been described and illustrated by Okada et al. (1998, 2000). It is morphologically remarkable by being synnematous. The fungus resides in rotten wood, eventually in association with bark beetles.

Exophiala cancerae de Hoog, Vicente, Najafzadeh, Harrak, Badali, Seyedmousavi & Boeger, *sp. nov.* — MycoBank MB515720; Fig. 6, 7

Coloniae in agaro maltoso dicto 25 °C lente crescentes, primum leves, deinde velutinae, griseo-olivaceae; reversum olivaceo-nigrum. In agaro PDA pigmentum brunneum absens. Hyphae leves, dilute olivaeo-brunneae, intervallis regularibus septatae. Cellulae zymoideum quasi absens. Conidiophora vix distinguenda, ramose vel simplicia, terminalia vel intercalaria. Annelloconidia nonnumquam septata, late ellipsoidea vel cylindrea, levia, 4.9–8.0 × 2.7–4.8 μm. Temperatura maxima crescentiae 33 °C. Teleomorphe ignota.

Etymology. Named after the crab, an arthropod host of this species.

Description of CBS 120420 after 2 wk incubation on MEA, 24 °C.

Colonies moderately expanding, circular, initially (on day 3) flat, olivaceous black, slimy with velvety, olivaceous grey centre and flat margin, later (on day 14) becoming velvety, dark olivaceous grey. Reverse olivaceous black, without diffusible pigment. Yeast cells nearly absent. *Conidiophores* short, erect, brown, cylindrical, multi-celled, poorly differentiated. *Conidia* 0–1-septate, subhyaline to pale brown, obovoidal to cylindrical, 4.9–8.0 × 2.7–4.8 μm.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 24–27 °C, maximum 30–33 °C. No growth at 37 °C.

Specimen examined. Brazil, Pernambuco State, Goiana City, from diseased Mangrove crab (*Ucides cordatus*), *W. Boeger*, holotype CBS-H 20382, ex-type culture CBS 120420 = dH 17409 = Boeger HF16/8.
Additional material examined. Table 1.

Notes — The ex-type strain was isolated from direct culture from tissue of moribund mangrove crabs (*Ucides cordatus*, *Brachyura: Ocypodidae*) with Lethargic Crab Disease (LCD). Since 1997 this systemic disease has caused extensive epizootic mortality of crabs along the Brazilian coast (Boeger et al. 2005). The histopathology of crabs in diverse stages of development of the disease shows that the most commonly affected tissues are the epidermis, connective tissues, heart and hepatopancreas. The fungus disseminates hematogenously. Despite the large scale of outbreak, locally causing 50 % of crabs to become diseased, we were unable to isolate *E. cancerae* from the environment (Boeger et al. 2007). The worldwide occurrence of the species (Table 1) suggests that it may have been present in Brazil prior to the beginning of the epizootic in 1997. Changes in host or in environmental conditions, rather than emergence of a virulent fungal genotype, are thus likely. This is underlined by the fact that also a second black yeast-like fungus was involved in LCD (Boeger et al. 2007). This was a *Cladophialophora* species close to *C. devriesii*, which was first described from a fatal disseminated infection in a human at the Caribbean Grand Cayman Island (Gonzalez et al. 1984).
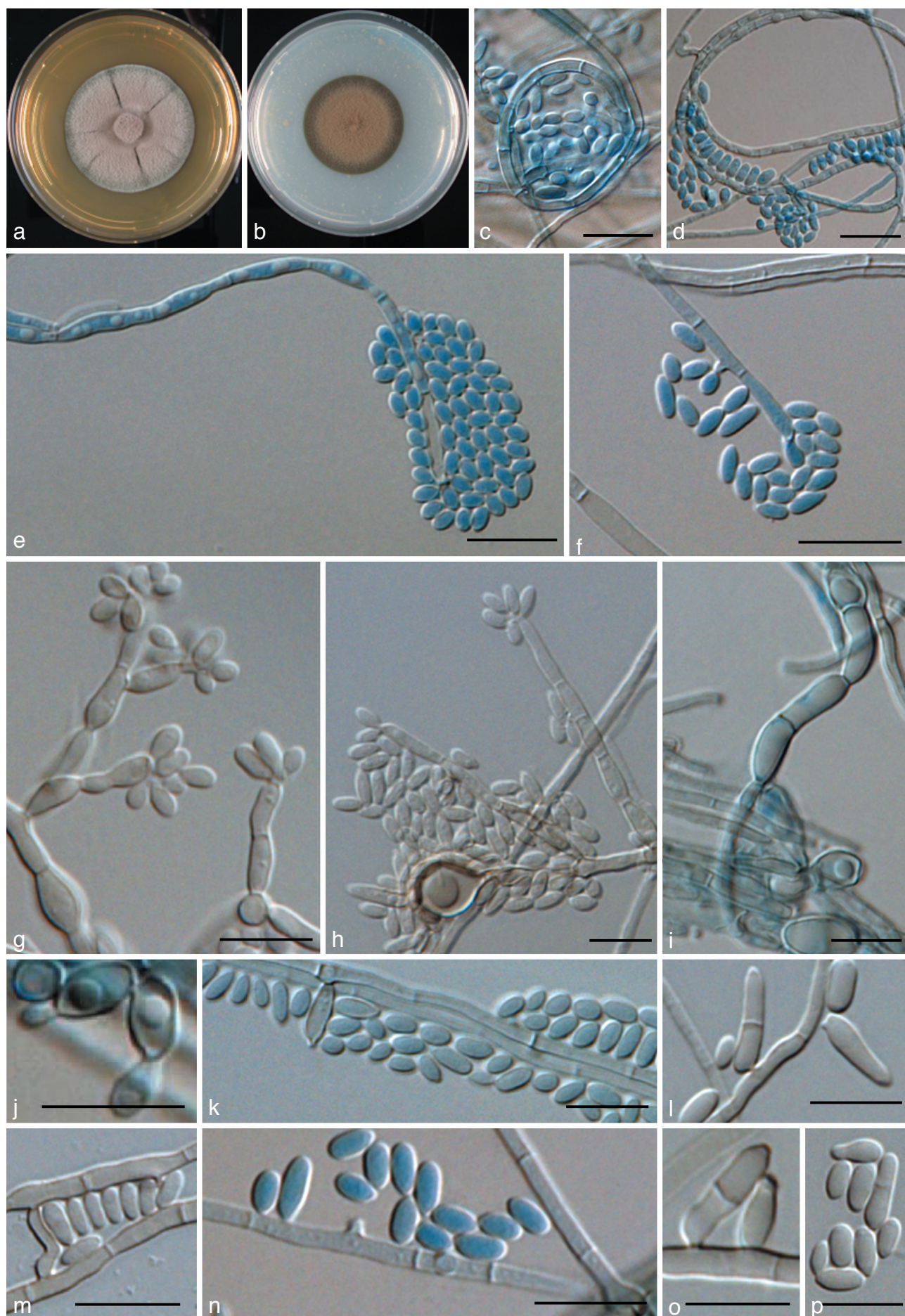
Fig. 6 *Exophiala cancerae*, CBS 120420. a. Colony on MEA; b. colony on PDA; c, d. spirally twisted hyphae; e, f. short, erect, cylindrical, multi-celled conidiophores; h, i. apical and intercalary chlamydospores; j. budding cells; k, l. intercalary conidiogenous cells; m. hyphae and conidia with anastomoses; n–p. conidia. — Scale bars = 10 μm.

Fig. 7 *Exophiala cancerae*, CBS 120420. Conidial apparatus, conidia and torulose hyphae. — Scale bar = 10 μm.

Strain CBS 119920 was derived from the liver of a green toad (*Pseudepidalea viridis*) in Israel, which was euthanised with clinical signs of systemic mycosis (A. Cunningham, pers. comm.). Additional strains were isolated on separate occasions from water in Germany (Table 1). The species was once isolated from clean water from a CIP (Clean-in-Place) tank (CBS 11749) in the Netherlands and once from fruit drink (CBS 115142) in Australia.

In humans, a skin infection was observed in a patient with diabetes in Germany (GHP 2419; G. Haase, pers. comm.); further human cases concern mild skin and nail infections. Despite its maximum growth temperature of 33 °C, the species possesses intrinsic virulence factors. These may be expressed particularly in external tissues of the extremities patients with reduced blood circulation, e.g. to underlying diabetes.

GenBank contains a number of accessions under the name *Exophiala salmonis*, which are now reidentified as *E. cancerae*: a clinical strain in the USA (AY213652; Rakeman et al. 2005), a strain from a bathroom in Japan (AB 456581; Hamada & Abe 2009) and an isolate from a healthy stem of corn in Australia (AM176667, Molnar & Prillinger unpubl. data).

Exophiala equina (Pollacci) de Hoog, Vicente, Najafzadeh, Harrak, Badali & Seyedmousavi, *comb. & stat. nov.* — MycoBank MB515717; Fig. 8, 9

Basionym. Haplographium debellae-marengoi Pollacci var. *equinum* Pollacci, Revta Biol. 5: 370. 1923.
= *Exophiala tremulae* W. Wang, Fungal Planet 70, Persoonia 26: 113. 2011.

Description of CBS 119.23 after 2 wk incubation on MEA, 27 °C.

Colonies restricted, circular, initially (on day 5) flat, olivaceous black, slimy with velvety, olivaceous grey centre and flat margin, later (on day 15) becoming umbonate, felty, greyish black, with velvety, grey centre. Reverse greyish black. No diffusible pigment produced. Yeast cells, when present, consisting of subspherical cells producing conidia. *Conidiogenous cells* flask-shaped, intercalary or terminal. *Conidia* ellipsoidal, $2.4-3.3 \times 4.8-5.2$ μm, with discernible scars. *Chlamydospores* ellipsoidal, up to 10 μm long and 5 μm wide; spirally twisted hyphae present.

Cardinal temperatures — Minimum ≤ 4 °C, optimum $24-30$ °C, maximum $33-36$ °C. No growth at 37 °C.

Specimen examined. ITALY, Pavia, Dec. 1923, *G. Pollacci*, subcutaneous infection of a horse, CBS H-19957, ex-type culture CBS 119.23 = dH 15335.
Additional material examined. Table 1.

Notes — The ex-type strain was isolated as the etiologic agent of a subcutaneous infection of the lower leg of a horse (Pollacci 1923). The majority of remaining strains sequenced of this species, however (Table 1), originated from different kinds of cold water, primarily drinking water but also from the cooling system of a packaging machine, the tubing of a instrument using silica gel and from washings of *Tilia* roots. One isolate came from a bathroom. Strain CBS 115143 was isolated from bottled spring water destined for human consumption in Australia (Avila de la Calle et al. 2006, Crous et al. 2007), while CBS 109789 came from dialysis tubing. Strain CBS 116009 caused a systemic infection in a Galapagos giant tortoise (*Geochelone nigra*), reported by Manharth et al. (2005). The tortoise presented with ocular lesions, but upon necropsy a widespread granulomatous inflammation was noted, probably resulting from hematogenous dissemination. *Exophiala tremulae* (from roots of *Populus tremuloides* seedings in Canada), is identical based on DNA sequence data, and represents a recent synonym (Crous et al. 2011).

Although the species is unable to grow at 37 °C, some superficial infections in humans were noted, particularly from skin of the extremities. Among the infections was a corneal ulcer and an onychomycosis (Table 1). The case caused by CBS 121504 concerned a 1 yr old child with circular, tinea-like lesions from which the fungus was isolated together with *Candida guilliermondii*. The lesion was successfully treated with ciclipirox. *Exophiala equina* was judged to be a secondary invader (J. Brasch, pers. comm.). The species was also noted on the skin of patients with diabetes (G. Haase & P. Mayser, pers. comm.),
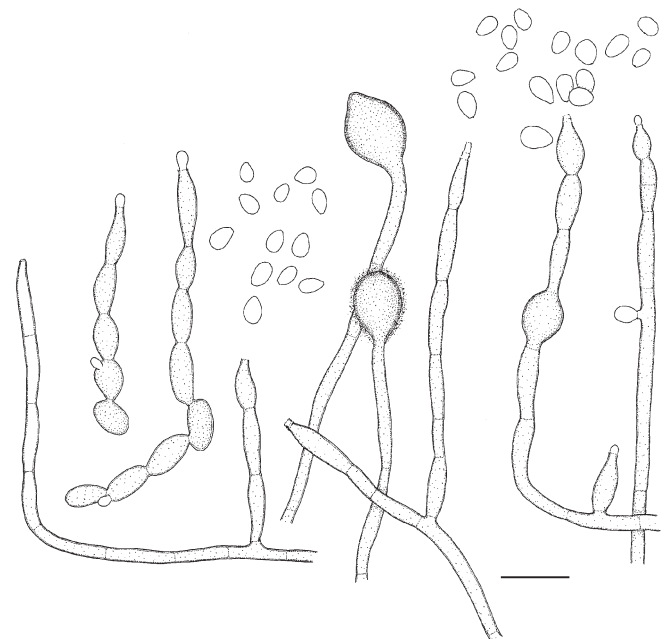
Fig. 8 *Exophiala equina*, CBS 121504. Conidial apparatus and conidia. — Scale bar = 10 μm.
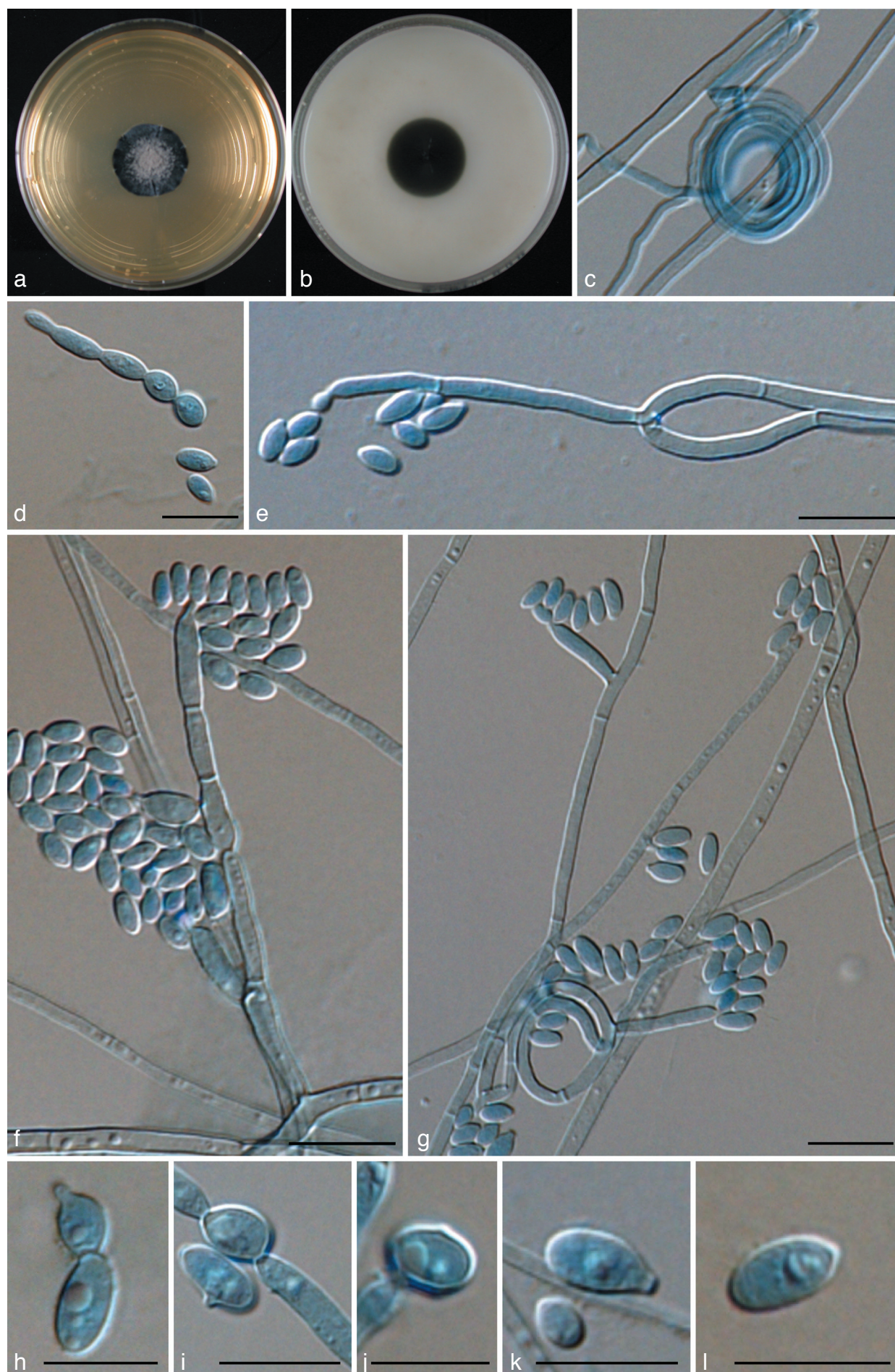
Fig. 9 *Exophiala equina*, CBS 119.23. a. Colony on MEA; b. colony on PDA; c. spirally twisted hyphae; d. tolurose hyphae; e, g. conidiophore with single conidiogenous cell; f. conidiogenous subcylindrical cells flask shaped with ellipsoidal conidia; h. budding cell; i, j. chlamydospore; k, l. ellipsoidal conidia. — Scale bars = 10 μm.
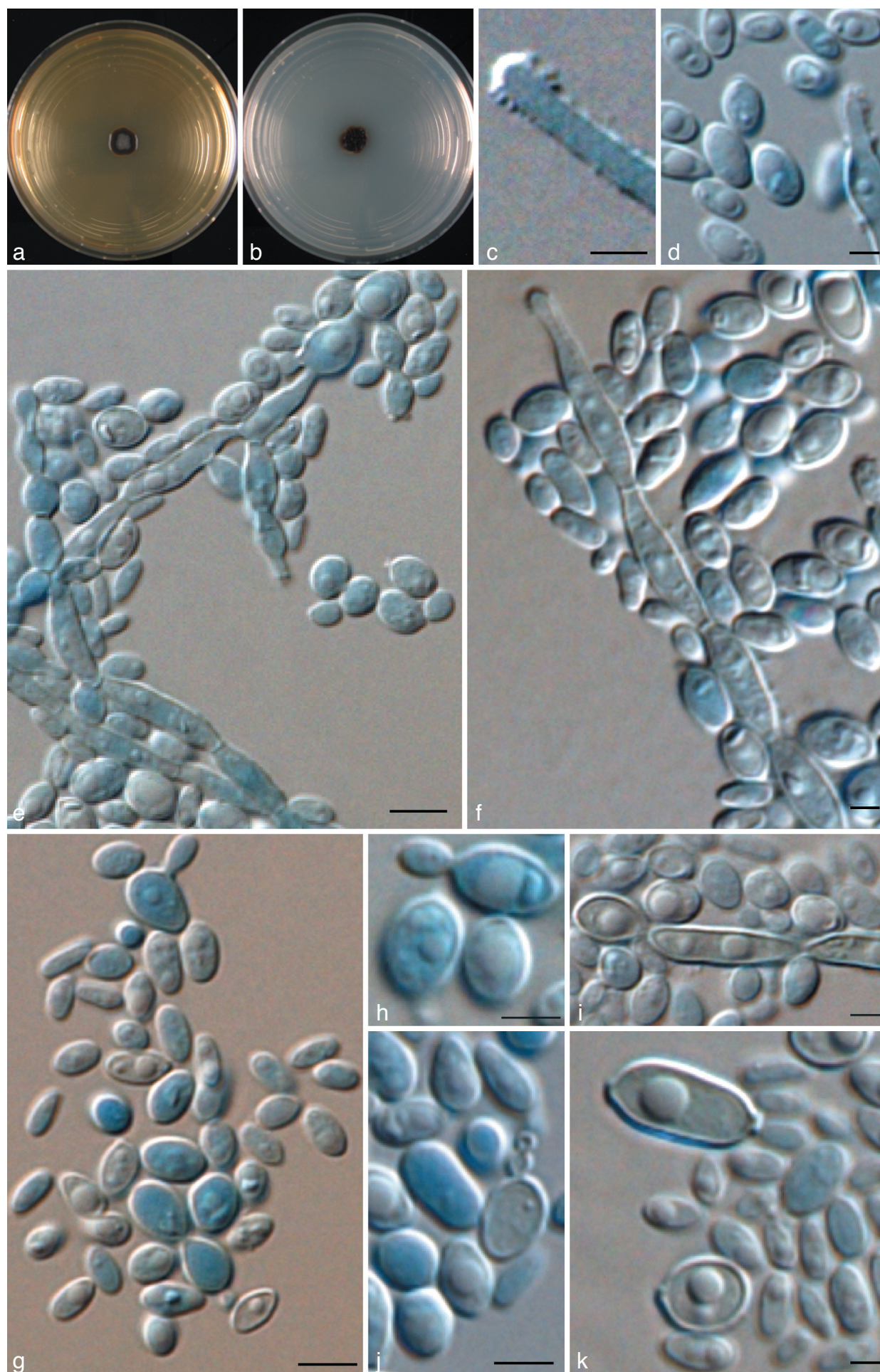
Fig. 10 *Exophiala halophila*, CBS 121512. a. Colony on MEA; b. colony on PDA; c–e. conidial apparatus with conidia; f–k. budding cells and conidia; i. tolurose hyphae. — Scale bars = 10 µm.

which had a relatively low body temperature due to impaired circulation. This condition allows invasion by species that are unable to grow at temperatures above 37 °C, particularly when infection takes place on the extremities. Further isolates were recovered from skin flakes, stool and sputum. A transmission route from bathing facilities, as hypothesized for other black yeasts (Lian & de Hoog 2010), seems probable for this species as well. The occurrence of the fungus in bottled water is of concern.

Additional strains sequenced originated from soil (CBS 515.76), from washed roots (CBS 160.89, CBS 159.89) and from a twig of *Olea* sp. (CBS 121502). Neubert et al. (2006) extracted DNA (GenBank AJ875365) from wetland reed in Germany and encountered the same species. All these environments have relatively low temperatures in common. Human infection seems to be coincidental.

Exophiala halophila de Hoog, Vicente, Najafzadeh, Harrak, Badali & Seyedmousavi, *sp. nov.* — MycoBank MB515715; Fig. 10, 11

Coloniae in agaro maltoso dicto 24 °C lente crescentes, primum leves et zymoideae, deinde elevatae, velutinae, griseae; reversum olivaceo-nigrum. In agaro PDA pigmentum brunneum exudens. Hyphae leves, dilute olivaeo-brunneae, intervallis regularibus septatae; mycelium torulosum proferentes. Conidiophora vix distinguenda, ramose vel simplicia, terminalia vel intercalaria; nonnumquam cellulae gemmantes etiam conidiogenae. Annelloconidia haud septata, late ellipsoidea, levia, 1.9–2.5 × 3.0–5.2 μm. Temperatura maxima crescentiae 33 °C. Teleomorphe ignota.

Etymology. Named after one of the sources of isolation of this species.

Description of CBS 121512 after 2 wk incubation on MEA, 24 °C.

Colonies restricted, compact, circular, olivaceous brown, initially (on day 5) moist and slimy, later (on day 14) becoming brownish grey, velvety at the centre. Reverse olivaceous black. Brown

pigment produced on PDA. Yeast cells abundant. Torulose hyphae present. *Conidiogenous cells* intercalary in undifferentiated hyphae, or discrete, then flask-shaped, lateral or terminal, with short annellated zones. *Conidia* subhyaline, ellipsoidal to subcylindrical 1.9–2.5 × 3.0–5.2 μm.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 24–27 °C, maximum 30–33 °C. No growth at 37 °C.

Specimen examined. USA, Texas, San Antonio, *D.A. Sutton*, from human skin, holotype CBS H-19967, ex-type culture CBS 121512 = UTHSC 03-2191 = dH 13757.
Additional material examined. Table 1.

Notes — Only three isolates of this species are available. The ex-type strain was isolated from asymptomatic human skin. The nearest phylogenetic neighbour is *E. alcalophila* at a minimum distance of 8.4 % (ITS), and thus the novelty of the new species is unambiguous (Fig. 3). The species has been isolated from human skin of the armpit, and from human nails, as well as from salt water. Strains were tolerant of 2.5, 5 and 10 % MgCl$_2$ and of 2.5 and 5 % NaCl$_2$.

Exophiala lacus de Hoog, Vicente, Najafzadeh, Harrak, Badali & Seyedmousavi, *sp. nov.* — MycoBank MB515721; Fig. 12, 13

Coloniae in agaro maltoso dicto 25 °C lente crescentes, velutinae, griseo-olivaceae; reversum olivaceo-nigrum. In agaro PDA pigmentum brunneum absens. Hyphae leves, dilute olivaeo-brunneae, intervallis regularibus septatae. Mycelium torulosum quasi absens. Conidiophora vix distinguenda, brevia, simplicia, preferentia intercalaria. Annelloconidia nonnumquam septata, late ellipsoidea, levia, 1.8–2.6 × 3.1–9.6 μm. Chlamydosporum praesens, maximum 5.2 × 2.6 μm. Temperatura maxima crescentiae 30 °C. Teleomorphe ignota.

Etymology. Named after the lake environment where the species was isolated.

Fig. 11 *Exophiala halophila*, CBS 121512. Conidial apparatus, conidia and anastomosing torulose hyphae. — Scale bar = 10 μm.
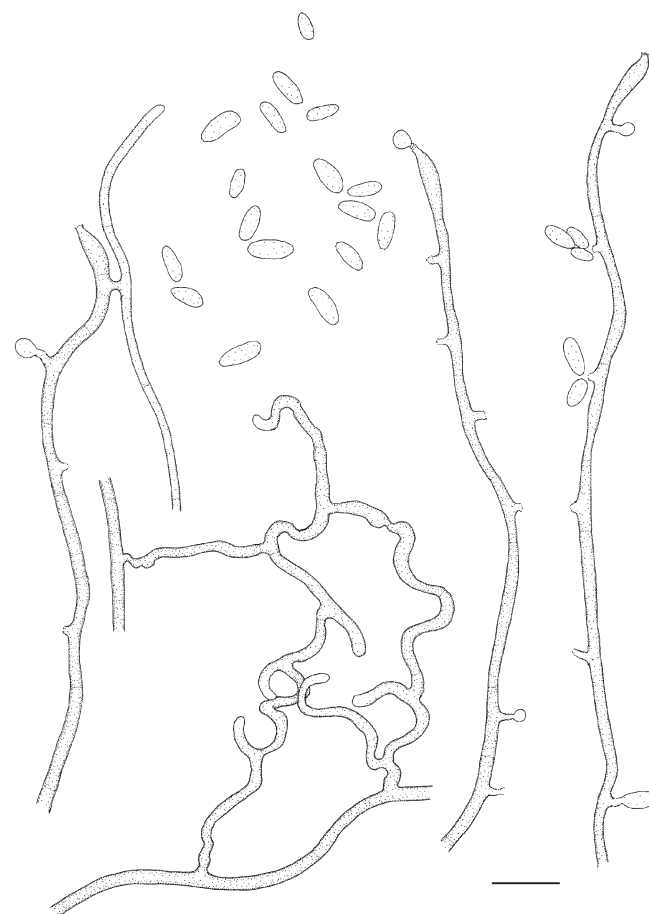
Fig. 12 *Exophiala lacus*, CBS 117497. Hyphae with intercalary conidiogenous cells; anastomoses leading network-like hyphae. — Scale bar = 10 μm.
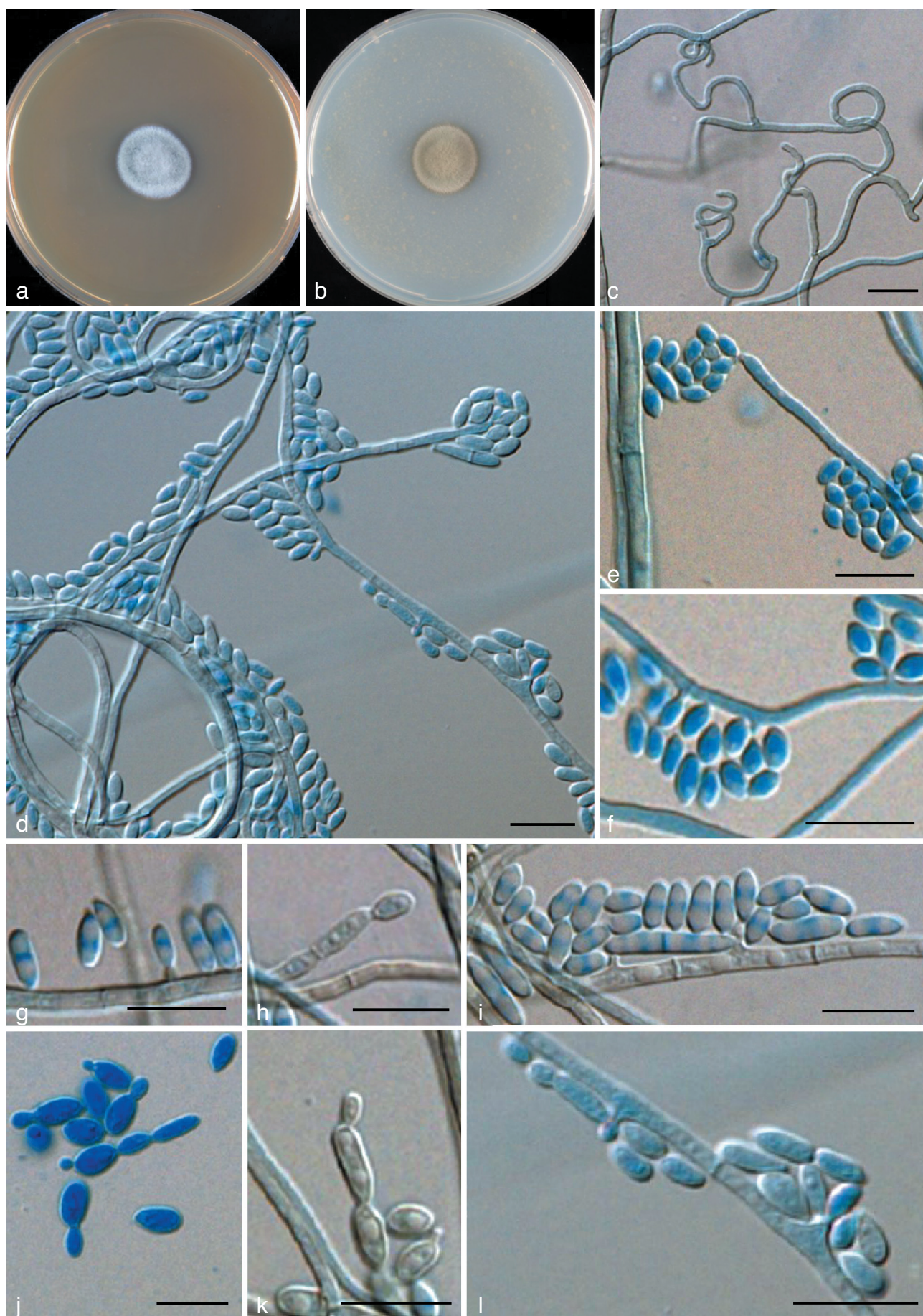
**Fig. 13**   *Exophiala lacus*, CBS 117497. a. Colony on MEA; b. colony on PDA; c. spirally twisted hyphae; d, e. short, erect, cylindrical, multi-celled conidiophores; f, g. hyphae with conidial heads; h. chlamydospores; i, j. budding cells; k. conidia alongside hyphae; l. conidia. — Scale bars = 10 µm.

Description of CBS 117497 after 2 wk incubation on MEA, 24 °C.

Colonies moderately expanding, circular, velvety with olivaceous grey aerial mycelium. Reverse olivaceous black, without diffusible pigment. Yeast cells, when present, consisting of subspherical cells producing conidia. *Conidiophores* short, erect, cylindrical, multi-celled, poorly differentiated with submerged hyphae with conidial heads. *Conidia* arising alongside the hyphae; conidia 0−1-septate, ellipsoidal to cylindrical, 1.8−2.6 × 3.0−9.6 µm. *Chlamydospores* ellipsoidal, up to 5.2 × 2.6 µm; spirally twisted hyphae present.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 21−24 °C, maximum 27−30 °C. No growth at 37 °C.

Specimen examined. The Netherlands, Loosdrecht, *M.J. Harrak*, from freshwater lake (1 m depth), holotype CBS-H 20407, ex-type culture CBS 117497 = dH 13711.

Notes — Only a single strain of this species is available. It was isolated from a shallow freshwater lake, the lake bottom consisting of a few metres of plant material. The lake is unpolluted, being fed by seepage water from sandy hills.

Exophiala opportunistica de Hoog, Vicente, Najafzadeh, Harrak, Badali & Seyedmousavi, *sp. nov.* — MycoBank MB515719; Fig. 14, 15

Coloniae in agaro maltoso dicto 25 °C lente crescentes, velutinae, griseoolivaceae; reversum olivaceo-nigrum. In agaro PDA pigmentum brunneum absens. Hyphae leves, dilute olivaeo-brunneae, dense septatae. Mycelium torulosum quasi absens. Conidiophora vix distinguenda, ramose vel simplicia, terminalia vel intercalaria. Annelloconidia nonnumquam septata, late ellipsoidea, levia, 2.4−2.9 × 1.1−1.2 µm. Temperatura maxima crescentiae 30 °C. Teleomorphe ignota.

*Etymology*. Named after the apparent ability of the species to grow on human nails.

Description of CBS 109811 after 2 wk incubation on MEA, 17 °C.

Colonies restricted, olivaceous grey, velvety with floccose margin and with grey aerial mycelium at the centre. Reverse olivaceous black, without diffusible pigment. Yeast cells, torulose hyphae and spirally twisted hyphae present. Hyphae rather wide, profusely septate and strongly anastomosing. *Conidia* arising alongside the hyphae or on broadly ellipsoidal, poorly differentiated conidiophores; conidia (0−1)-septate, (sub)hyaline, obovoidal to ellipsoidal, 2.4−2.9 × 1.1−1.2 µm.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 21−24 °C, maximum 27−30 °C. No growth at 37 °C.

Specimen examined. Germany, Duisburg, from drinking water, *E. Göttlich*, holotype CBS-H 20383, ex-type culture CBS 109811 = dH 12243 = IWW 720.
Additional material examined. Table 1.

Notes — The original strain was derived from drinking water and also from rhizosphere of *Triticum aestivum* in Western Australia (CBS 660.76). One strain, CBS 637.69 originated from polyvinyl alcohol. We recently also noted presence of *E. opportunistica* on human nail and foot lesions in Denmark (Table 1).

Exophiala pisciphila McGinnis & Ajello (as '*pisciphilus*'), Mycologia 66: 518. 1974. — MycoBank MB314043

Teleomorph. Unknown.

Description of CBS 537.73 after 2 wk incubation on MEA, 24 °C.

Colonies moderately expanding, dry, floccose, olivaceous black. Yeast cells absent. *Conidiogenous cells* flask-shaped, mostly in loose clusters or branched systems, with inconspicuous annellated zones. *Conidia* 0(−1)-septate, (sub)hyaline, ellipsoidal, 6−8 × 2.5−4.0 µm.

Fig. 14 *Exophiala opportunistica*, CBS 109811. Hyphae with mostly intercalary conidiogenous cells with extended annellated zones, and 1–2-celled conidia. — Scale bar = 10 µm.

Cardinal temperatures — Minimum ≤ 4−9 °C, optimum 24−30 °C, maximum 30−33 °C. No growth at 37 °C.

Specimen examined. USA, Alabama, privately owned freshwater pond, April 1969, N. Fijan, from systemic mycosis in channel catfish (*Ictalurus punctatus*), ex-type culture CBS 537.73, dried culture CBS H-7135.
Additional material examined. Table 1.

Notes — This species was one of the first *Exophiala* species described as causing epizootics in cold-blooded vertebrates (Fijan 1969). Eighty percent of a sample of freshwater channel catfish (*Ictalurus punctatus*) in a pond had cutaneous ulcers. These were 2–15 mm diam and up to 5 mm deep, without inflamed margins. Numerous nodules were found in visceral organs. Hematogenous dissemination led to hemorrhagic peritonitis with purulent exudates. The isolated fungus killed the fish within 13 days after intraperitoneal inoculation, but no neurotropism was noted.

The species was later reported as an opportunistic invader in the marine coastal smooth dogfish (*Mustelus canis*) in the New York Aquarium (Gaskins & Cheung 1986), but the identity of this now unavailable strain cannot be confirmed by sequencing. Strains causing an epizootic in the captive marine plaice (*Pleuronectes platessa*), published as *Hormoconis resinae* (Strongman et al. 1977) were re-identified as *Exophiala pisciphila* (de Hoog et al. 2009). The fish were maintained in tanks with a continuous flow of pre-filtered seawater. Lesions were mainly cutaneous. The epizootic was thought to have been promoted by a relative high maintenance temperature of up to 15.2 °C.
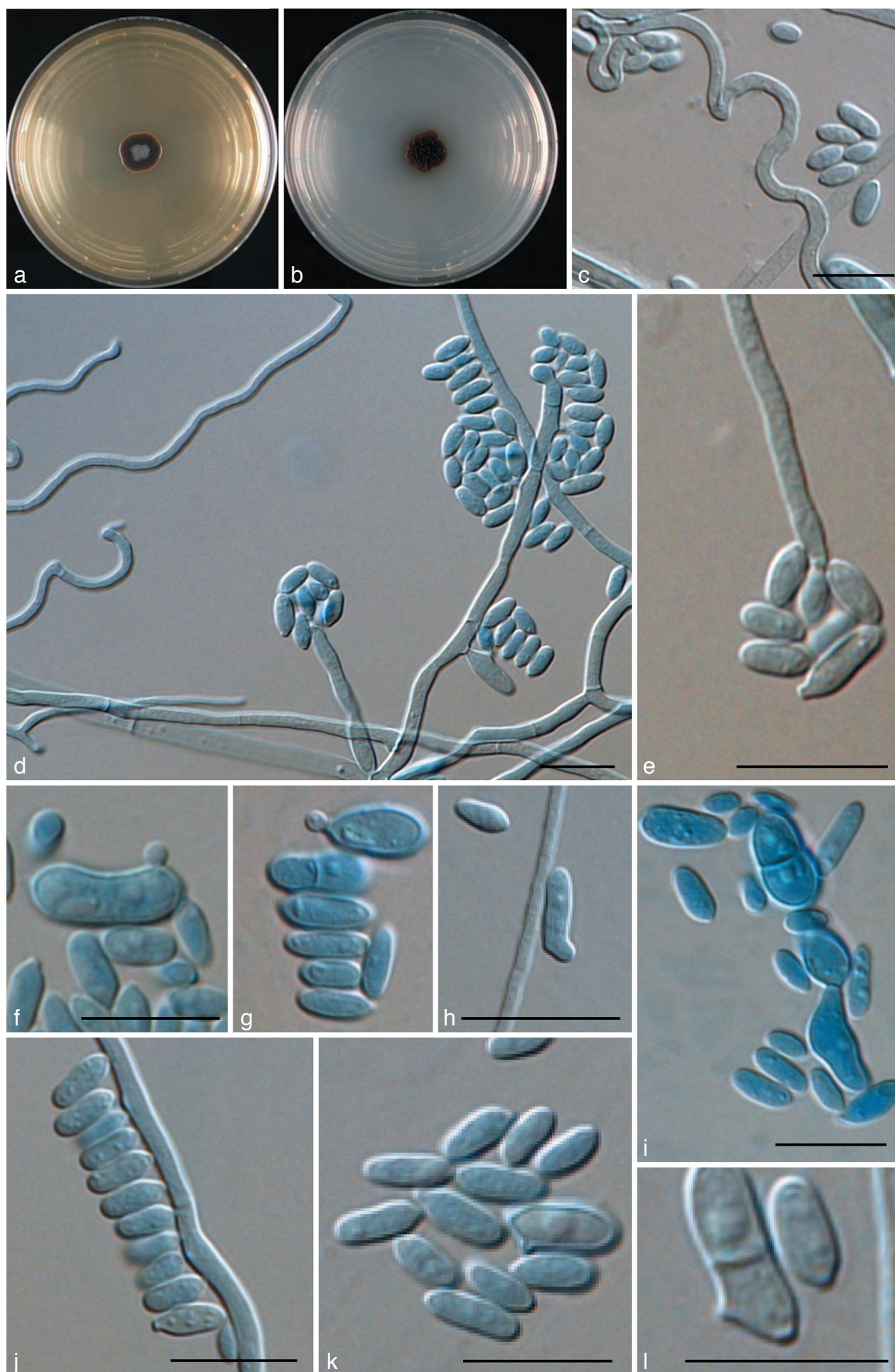
Fig. 15 *Exophiala opportunistica*, CBS 109811. a. Colony on MEA; b. colony on PDA; c. spirally twisted hyphae; d, e. erect cylindrical multi-celled conidiophores; f–l. yeast cells and conidia; i. torulose hyphae. — Scale bars = 10 µm.

Several isolates originated from disseminated infections in marine potbelly seahorses (*Hippocampus abdominalis*; Nyaoke et al. 2009). Additional strains sequenced (Table 1) originated from a swimming pool (CBS 121505) and from a water pipe (CBS 101610). The fungus thus occurs on fish living in freshwater as well as seawater, with a low degree of host specificity. In humans, a skin infection in an immunosuppressed patient from Brazil was reported by Sughayer et al. (1991), without sequence confirmation. We also recorded an isolate from the nail of a human patient in Germany (P. Mayser, unpubl. data; Table 1). Yamada et al. (1989) reported a Coenzyme Q10(H$_2$) system in the species.

Exophiala psychrophila O.A. Pedersen & Langvad, Mycol. Res. 92: 153. 1989. — MycoBank MB135481

Description of CBS 191.87 after 2 wk incubation on MEA, 24 °C.
Colonies initially yeast-like and black, gradually becoming effuse and dome-shaped. After 14 d at 18 °C colonies have a dark centre containing the bulk of the conidial mass surrounded by a felt of mouse grey mycelium. Hyphae pale brown, septate, sparingly branched, hyphae 1–3 μm wide. Moniliform cells very common, 3–6 × 5–15 μm, chains consisting of two to several hundred cells, 4–12 being the most common. *Conidiogenous cells* with several enteroblastic proliferations at the apices with 2.0–3.5 μm diam. *Conidia* holoblastic, aseptate, varying in shape from spherical to oblong, sometimes tapered, 1.5–2.5 × 3–6 μm, tending to accumulate in slimy balls at apex of conidiogenous cells. Lipid globules may sometimes give the false impression that the conidia are septate. Conidia may be produced from discrete conidiogenous cells, directly from hyphae, from moniliform cells and from conidia. Yeast-like cells also produce conidia.
   Cardinal temperatures — Minimum 0 °C (growth present after 6 mo at 0 °C); optimum 17–21 °C; maximum 23 °C.

   Specimen examined. Norway, Mar. 1987, *F. Langvad*, from Atlantic salmon smolt (*Salmo salar*), ex-type culture, CBS 191.87 = dH15499 = CBS H-20009 = MBSPEC1293.

   Notes — The species was originally isolated from farmed Atlantic salmon smolts (*Salmo salar*) in a farm in Western Norway. The disease led to very high mortality for four years with correspondingly great economic losses for the farmer (Langvad et al. 1985). Strains from salmon in other countries also became available for this study (Table 1).

Exophiala salmonis J.W. Carmich., Sabouraudia 5: 120. 1966. — MycoBank MB119468

Description of CBS 157.67 after 2 wk incubation on MEA, 24 °C.
Colonies moderately expanding, dry, depressed, hairy, olivaceous black. Yeast cells nearly absent. *Conidiogenous cells* poorly differentiated, intercalary or flask-shaped; annelated zones short, inconspicuous. *Conidia* 0–3-septate, subhyaline to pale brown, ellipsoidal to short cylindrical, 5.5–8.5 × 2.0–3.5 μm.
   Cardinal temperatures — Minimum ≤ 4 °C, optimum 18–24 °C, maximum 30–33 °C. No growth at 37 °C.

   Specimens examined. Canada, Alberta, Calgary, Alberta hatchery; cerebral mycetoma of fingerling trout (*Salmo clarkii*), *J.W. Carmichael*, specimens CBS H-12617, CBS H-7136, ex-type cultures CBS 157.67 = BMU 00834 = ATCC 16986 = IHEM 3405 = IMI 124165 = MUCL 10078 = UAMH 34 = VKM F-3000.
   Additional material examined. Table 1.

   Notes — This is the generic type species. Although isolates have frequently been reported under this name, only very few confirmed isolates are available. The species was origi-

nally reported as causing three epidemic episodes of cerebral mycetoma in freshwater fingerling trout cod (*Maccullochella macquariensis*) at the Provincial Government Fish Hatchery in Calgary, Alberta, Canada (Carmichael 1966). The hatchery drew its water from an underground spring which has a temperature range of 12–14 °C and sometimes had a high nitrate content. We sequenced two further isolates of *E. salmonis*, both from fresh water in The Netherlands. Otis et al. (1985) ascribed an infection in captive Atlantic salmon (*Salmo salar*) in the USA to the same fungus, but no isolates were available for sequencing. The authors observed interesting parallels to the Canadian case. Both mycoses were systemic in nature and occurred only in captive or hatchery-raised fishes. It appeared that the fishes were debilitated and hence predisposed to infection by opportunistic pathogens. The Atlantic salmon in this study had not been fed properly in captivity, and had just undergone an unsuccessful spawning period. Infections by cestodes (tapeworm, *Proteocephalidea*) could have weakened the fish, and provided a route of entry for the fungus with subsequent hematogenous spread to the kidney. Madan et al. (2006) reported on subcutaneous nodules in elbows and knees of a 64 yr old male under cyclophosphamide and prednisolone therapy for non-Hodgkin lymphoma ascribed to *E. salmonis*, but as no sequence data are available this report has to be regarded as doubtful.

Yamada et al. (1989) demonstrated the presence of a coenzyme Q10(H$_2$) system in *E. salmonis.*

Veronaea botryosa Cif. & A.M. Corte, Atti Ist. Bot. Lab. Crittog. Univ. Pavia, Ser. 5, 15: 68. 1958. — MycoBank MB307734

Description of CBS 254.57 after 2 wk incubation on MEA, 24 °C.
Colonies growing rapidly, velvety to lanose, greyish brown or blackish brown. *Conidiophores* erect, straight or flexuose, unbranched or occasionally loosely branched, sometimes geniculate, smooth-walled, olivaceous brown, up to 250 μm long, 2–4 μm wide. *Conidiogenous cells* terminal or lateral, often becoming intercalary, cylindrical in the apical part with numerous flat scars. *Conidia* smooth-walled or slightly verrucose, sometimes cylindrical, rounded at the apex and truncate at the base, pale brown, usually 1-septate, 5–12 × 3–4 μm.
   Cardinal temperatures — Minimum 4–9 °C, optimum 24–30 °C, maximum 33–36 °C. No growth at 37 °C.

   Specimen examined. Italy, from sansa olive slag, specimen CBS H-19962, ex-type culture CBS 254.57 = IMI 070233 = MUCL 9821.
   Additional material examined. Table 1.

   Notes — This sympodial species, which is morphologically very different from the annellidic genus *Exophiala* (Arzanlou et al. 2007), is found amidst the waterborne species in the *salmonis*-clade. The ex-type strain was isolated from sansa olive slag (olive presscake) in Italy (Ciferri & Montemartini 1957), a substrate rich in phenolic compounds. Additional environmental strains were isolated on separate occasions from *Eucalyptus* wood treated with creosote in Brazil (Table 1).

Otherwise the species is known to cause moderately severe to highly mutilating human infections. Matsushita et al. (2003) published a chronic disseminated mycosis of a 12-year-old child in China (CBS 102593). The patient did not have any known immune disorder. A deep skin lesion in a 37-year-old male patient from the Philippines (CBS 101462) was reported by Medina et al. (1998). Further cases have been reported from China (Nishimura et al. 1989), Libya (Ayadi et al. 1995), the USA (Sutton et al. 2004) and France (Foulet et al. 1999), mostly in immunocompromised patients. Several of these cases have not been verified by sequencing, but the species is morphologically sufficiently stable and characteristic for reliable classical

identification. Strain CBS 121506 was isolated from a lesion on the hand of a female patient and represents the first case in Japan caused by this fungus.

In contrast to most *Exophiala* members of the *salmonis*-clade, combining waterborne ecology with an ability to invade cold-blooded animals and possibly cool human body sites, *Veronaea botryosa* has a strong predilection for human hosts, causing chronic, deep infections. The above-mentioned disseminated case in a Chinese adolescent, which developed over a six-year period (Matsushita et al. 2003), was a particularly impressive example.

DISCUSSION

Although the black yeast teleomorph genus *Capronia* was described in 1883 (Saccardo 1883), with the type species *C. sexdecimspora* even dating back to 1871 (Cooke 1871), its anamorph genus *Exophiala* was described only quite recently (Carmichael 1966). *Exophiala*-like anamorphs were occasionally encountered in older literature, demonstrated by the invalidly described genera *Foxia* (Castellani 1908) and *Nadsoniella* (Issatchenko 1914). In the present paper it was shown that *Haplographium debellae-marengoi* var. *equinum* (Pollacci 1923) could be reidentified as an *Exophiala* species. *Exophiala* species are difficult to recover from the environment as they grow very slowly. They are frequently overlooked. They show a remarkable association with monoaromatic hydrocarbons and may have a competitive advantage when these compounds are present (Prenafeta-Boldú et al. 2006). Many species require dedicated isolation methods (Prenafeta-Boldú et al. 2006, Sudhadham et al. 2008, Vicente et al. 2008, Zhao et al. 2010). When isolated, they are very difficult to diagnose based on morphology. This has hampered the accumulation of knowledge and understanding of these organisms.

A pathogenic potential towards immunocompetent animals is observed in nearly all major clades of the *Chaetothyriales*. The lowest, ancestral clade (Fig. 1) with uncertain affiliation contains predominantly rock-inhabiting species, with occasional taxa involved in mild cutaneous disease. The derived clades are considered to belong to the *Herpotrichiellaceae* (teleomorph genus *Capronia*; Fig. 1) and are pathogen-rich (Ruibal et al. 2008). Pathogenicity is particularly observed in fish, amphibians and humans, while infections in marine invertebrates are also known; in mammals, particularly humans are susceptible. Infections in reptiles and birds are absent. All chaetothyrialean fungi are obligatorily melanized, and thus melanin does not explain these predilections. Reptiles and birds are terrestrial animals with thick or protected, dry skin-lacking sweat glands. Infections in furred mammals are also rare; the majority of

mammal infections are observed in humans, who, as a species, have thin, predominantly nearly hairless skin provided with sweat glands for cooling.

If we compare this preponderance of potentially invasive species with another fungal order with numerous invasive species, the *Onygenales*, containing the dermatophytes and classical systemic pathogens, an entirely different picture emerges. Ancient onygenalean species are classified in *Chrysosporium* and are particularly found on the skin of snakes (Bertelsen et al. 2005), chameleons (Paré et al. 2006), crocodiles (Thomas et al. 2002) and other reptiles. The most derived species are anthrophophilic dermatophytes, which went through repeated host shifts from domesticated animals (Gräser et al. 2006). In the *Onygenales* we witness a consistent pathogenic evolution, paralleling the phylogeny of the host, whereas in *Chaetothyriales* all types of opportunism are scattered over the order. Chaetothyrialean opportunism tends, however, to manifest in consistent patterns within species, and thus is not a random process.

Waterborne animals are infected by Chaetothyrialean fungi much more frequently than terrestrial animals (Table 3). Host body temperatures can be correlated with maximum growth temperatures of invasive species in each of the clades. Roughly speaking, clades with species able to grow at temperatures well over 36–37 °C (*bantiana*, *dermatitidis* and *jeanselmei* clades) may cause systemic or disseminated infections in humans, while those with a maximum around 36–37 °C (*carrionii* and *europaea* clades) cause (sub)cutaneous and superficial infections. Species of the *salmonis* clade have maxima at 27–33 °C, exceptionally 36 °C, and cause superficial, barely invasive infections at most (Li et al. 2009, Saunte et al. 2011).

Animal hosts vary greatly in their immune responses to fungal infections. The invertebrate Crustaceans have poorly developed innate immunity and no adaptive immunity. In invasive infection, no or primitive granulomas are formed, and hyphae grow densely and regularly (Fig. 16a, b; Boeger et al. 2005). Primitive fish such as sea horses, with poorly developed innate as well as adaptive immunity, show similar histopathology (Fig. 16c, d; Nyaoke et al. 2009). True granulomas, with lymphocytes, granulocytes and giant cells are observed only in higher animals, which are equipped with fully developed innate and adaptive cellular immunity.

Several clades contain *Exophiala* species that are relatively common in low-nutrient aquatic environments, ranging from municipal drinking water and Arctic ocean water to hot springs and swimming pools. In all mentioned environments strains are encountered that cause infections in vertebrates, rarely in invertebrates. This apparent intrinsic pathogenic potency is generally recognised for thermophilic and mesophilic *Exophiala*

Table 2 Different pathogenic potentials in clades of *Chaetothyriales*: maximum growth temperature, synthesis of melanin and different levels of development of the immune system of potential hosts.

Clades of *Chaetothyriales*	Maximum growth temperature (°C)	Synthesis of melanin	Maximum disease in invertebrates		Maximum disease in vertebrates									
			Crustacean		Fish		Amphibian		Reptile		Bird		Mammal	
			Level of Immunity											
			Innate	Adaptive	Innate	Adaptive	Innate	Adaptive	Innate	Adaptive	Innate	Adaptive	Innate	Adaptive
			+	–	++	+	++	+	+++	+++	++++	++++	+++++	+++++
bantiana-clade	37–40	+	–		–		–		–		–		Disseminated	
dermatitidis-clade	36–42	+	–		–		–		–		–		Disseminated	
jeanselmei-clade	36–38	+	–		–		–		–		–		Disseminated	
carrionii-clade	36–37	+	–		–		–		–		–		Subcutaneous	
europaea-clade	37	+	–		–		–		–		–		Superficial, cutaneous	
salmonis-clade	27–33(36)	+	Disseminated		Disseminated		Disseminated		–		–		Superficial	
rock-clade	?	+	–		–		–		–		–		Superficial	

Table 3 Characteristics of species in the *salmonis*-clade: maximum growth temperature, main ecology, ability to cause infection in animals.

| *Salmonis*-clade | Maximum growth temperature (°C) | Main environmental habitat | Infections in animal kingdom | | | | | |
| | | | Invertebrate | Vertebrates | | | | |
				Fish	Amphibian	Reptile	Bird	Mammal
E. angulospora	30–33	Cold drinking water, sea water aquaria, fish nurseries	–	Sea dragon, marine lumpfish, unspecified fish	–	–	–	Human skin (colonizer?)
E. halophila	30–33	Salty water	–	–	–	–	–	Human skin (colonizer?)
E. alcalophila	36–40	Alkaline, bath waste water	–	–	–	–	–	Human skin (colonizer?)
E. pisciphila	30–33	Fresh water, seawater	–	Channel catfish, captive marine plaice, dog fish	Frog	–	–	Human nail (colonizer?)
E. aquamarina	33–36	Seawater, aquarium	–	Leafy & weedy sea dragon, winter flounder, tunnyfish, lumpfish	–	–	–	–
E. equina	33–36	Drinking water, waste water	–	–	–	Galapagos turtle	–	Horse; cutaneous in diabetic human
E. salmonis	30–33	Cold water (12–14 °C)	–	Trout cod, atlantic salmon	Frog	–	–	–
E. opportunistica	27–30	Drinking water?	–	–	–	–	–	Human skin and nail (colonizer?)
E. psychrophila	24–27	Cold water (12–14 °C)	–	Atlantic salmon	–	–	–	–
E. cancerae	30–33	Mangrove at Brazilian coast	Mangrove crab	–	Green toad	–	–	Human skin (colonizer?), cutaneous in diabetic human
E. calicioides	×	Otten wood, soil	–	–	–	–	–	–
V. botryosa	33–36	Sansa olive slag, treated wood	–	–	–	–	–	Disseminated and fatal in human
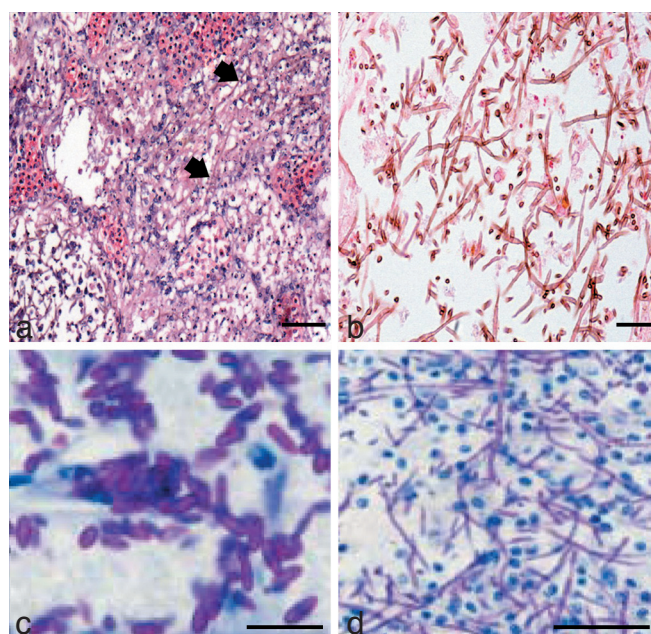
Fig. 16 Examples of histopathology of *Exophiala* species. – a, b. Disseminated phaeohyphomycosis by *E. aquamarina* in weedy seadragons (*Phyllopteryx taeniolatus*); a. abundant brown fungal hyphae (arrows) coursing through necrotic tubules, interstitium, and sinusoids in renal parenchyma. Stained with H & E. Reproduced from Nyaoke et al. 2009; b. fungal hyphae, kidney; weedy seadragon. Hyphae are slender, filamentous, and septate with occasional right-angled branches. Fontana-Masson staining; walls of hyphae stain brown, indicative of melanin. Reproduced from Nyaoke et al. 2009. – c. Light micrograph of transverse section of a gill of lethargic mangrove crab (*Ucides cordatus*) lamella with numerous conidia of *E. cancerae* in lacunae. Stained with PAS. Reproduced from Boeger et al. 2005. – d. Light micrograph of cardiac tissue of lethargic mangrove crab (*Ucides cordatus*) parasitized by hyphae of *E. cancerae.* Stained with PAS and counter-stained with H & E. Reproduced from Boeger et al. 2005. — Scale bars: a, d = 50 μm; b = 25 μm; c = 15 μm.

species that can be isolated from moist indoor environments. The best-known example is *E. dermatitidis* from Turkish steam baths. It causes neurotropic disseminated infections in humans (Hiruma et al. 1993, Chang et al. 2000). Mesophilic species such as *E. oligosperma* are also regularly encountered in human infections (de Hoog et al. 2009). Among the medical problems caused by *E. oligosperma* was a pseudoepidemic due to clinical use of contaminated hospital water (Nucci et al. 2001, isolates identified as *E. jeanselmei*).

Another factor discussed as a potential virulence factor is the ability to assimilate monoaromatic hydrocarbons by opening the benzene ring (Rustler et al. 2008). At this moment, insufficient data are available on physiological profiles of black yeasts in connection with the large diversity of alkylbenzenes. Nevertheless the relatively frequent infections of amphibians (e.g. Cicmanec et al. 1973, Elkan & Philpot 1973, Beneke 1977, Bube et al. 1992) that produce aromatic toxins in their skin such as epibatidine and bufotenin, is remarkable. A central role of alkylbenzene assimilation in the development of chaetothyrialean phylogeny seems highly probable. In summary, it may be stated that in *Chaetothyriales* the presence of melanin and the ability to assimilate alkylbenzenes generally enhance infection thermotolerance and physical skin properties determine host preferences, and cellular immunity determines the type of tissue response.

As noted above, the majority of preponderantly waterborne species are clustered in a single clade within the *Chaetothyriales*, the *salmonis*-clade, with the exception of scattered species elsewhere in the phylogenetic tree (Fig. 1). Most of the waterborne

Exophiala species are meso- or even psychrophilic (Table 3). Generally, a correlation is observed between the maximum growth temperature and the natural habitat of the host. Fungi with maximum growth temperatures below 33 °C are found causing diseases in animals in cold waters, such as the deep sea or the polar oceans. Examples are epizootics of *Exophiala psychrophila* in Atlantic salmon (Pedersen & Langvad 1989) and *Exophiala salmonis* in trout (Carmichael 1966). This is a remarkable feature, since elsewhere in the fungal Kingdom, species lacking thermotolerance tend to be saprobes without infectious abilities. Human infections caused by these non-thermotolerant fungi are rare. Some species with maximum growth temperatures around 33 °C, exceptionally up to 36 °C may cause zoonotics in cold-blooded animals living in shallow tidal zones in the subtropics. A striking example is the emerging Lethargic Crab Disease in crabs inhabiting mangrove areas along the east coast of Brazil (Boeger et al. 2005, 2007). Species of this group may be seen on human skin, although their role in pathology has not unambiguously been proven (Saunte et al. 2011; Table 1). Lian & de Hoog (2010) have shown that several members of *Chaetothyriales* known from superficial infections in humans are commonly isolated from bathrooms when appropriate isolation methods are applied.

The infection pattern of members of *Chaetothyriales* suggests the existence of intrinsic factors enhancing vertebrate invasion, but infection is probably not a prime factor in the natural habitat of these fungi. The different degrees and types of virulence to particular hosts is striking. The main susceptible groups are amphibians, fishes and humans, while other groups are significantly underrepresented; reptiles and birds are missing (Table 3). This may largely be due to the preponderant life styles of these hosts. While amphibians and fish are waterborne and have thin, mucous skins, reptiles and bird are predominantly terrestrial and are protected by thick, dry, water-repellent skin and, in birds, an envelope of water-repelling feathers. Most mammals have a hairy pelt which is difficult to penetrate, whereas the soft, naked skin of humans is more vulnerable to black yeast infection, particularly after maceration. Reports of *Exophiala* species infections in domestic and wild animals are very rare (Helms & McLeod 2000, Kano et al. 2000). Of note, black yeast infections in invertebrates all are recorded from moist animals, such as mussels (van Dover et al. 2007), earthworms (Vakali 1993) and mangrove crabs (Boeger et al. 2005), while terrestrial insects are never affected.

Besides integumental barriers, immune responses may be involved in host defence, since relatively few systemic infections in mammals other than humans are known. The immune response to waterborne *Exophiala* species varies with the host. The relative importance of specific innate versus adaptive defence mechanisms differs with the evolutionary position of metazoans (Muller & Muller 2003; Table 2). The involvement of phagocytic cells in engulfing foreign cells has been documented in virtually all metazoan organisms. Phagocytic cells possess a limited capacity to discriminate self from non-self, which is partly due to their use of a limited diversity of cell surface lectins for recognition. Although there is no evidence to suggest that invertebrate lectins and vertebrate immunoglobulins are homologous structures, sufficient diversity exists within lectins of certain species to indicate that these types of molecules and their cellular expression on phagocytes might serve as a primitive and universal recognition mechanism (Bosch et al. 2009). The presence of lymphocytes and circulating antibodies has been documented in all extant vertebrate species. However, the existence of induced, specific reactions homologous to the immune repertoire of vertebrates has not been clearly established in invertebrates (Frank 2002). All true vertebrates possess cells clearly recognisable as lymphocytes that can carry out T-cell

functions, and show the capacity of B-cells to synthesize and secrete immunoglobulins. True lymph nodes are not present in vertebrate species more primitive than mammals, but birds possess aggregates of lymphoid tissue probably serving a similar function (Davison et al. 2008).

Humans possess five major classes or isotypes of immuno-globulin: IgG, IgM, IgA, IgE and IgD. The IgM molecule is the first immunoglobulin to appear in ontogeny, and the first to appear in the phylogeny of the immune system (Davis & Hamilton 1998). Immunoglobulins of cyclostomes, sharks, rays and many teleost fishes consist of IgM polymers only. Some lungfish (*Dipnoi*) have a low-molecular-weight non-IgM immunoglobulin termed IgN (Magnadóttir 2006). Birds possess IgM and IgA immunoglobulins, but also possess a non-IgM immunoglobulin similar to that found in amphibians as their major immunoglobulin class. This immunoglobulin has been termed IgY (Davison et al. 2008). IgG immunoglobulins containing gamma chains and homologous to those of humans and other derived mammals are found only in the three subclasses of living mammals, namely Eutherians, Metatherians (Marsupials) and Monotremes (for example, the Echidna).

Although the precise nature of the precursors of elements of the immune system in evolution remains to be determined, the genetic and cellular events which lead to the ability of specific immune recognition, diversification, and reactivity are likely to have occurred early in vertebrate evolution (Abbas & Lichtman 2005). Nevertheless, different responses to comparable types of infection are known. For example, absence of advanced immune responses, such as granuloma formation or significant inflammatory response, is observed in fishes (Cooper & Alder 2006) and primitive metazoans (Du Pasquier 2001). This might be the result of inadequate or deficient immunological machinery. This is observed, for example, in the Lethargic Crab Disease (Boeger et al. 2005) and in systemic infections in seahorses (Nyaoke et al. 2009), where internal organs are homogeneously infected by black yeasts.

What is the nature of virulence in *Chaetothyriales*? The wide distribution of invasive abilities over members of the order is remarkable. Infections are generally regarded as being opportunistic, i.e. coincidental, not forming an essential part of the natural life cycle of the organism, and not conveying any evolutionary advantage because fitness is not increased. In this hypothesis, pathogenicity does not emerge over the course of evolutionary time. Pathogenicity is defined ecologically as a fitness advantage that is derived when an animal host is regularly used in any part of the fungal life cycle (de Hoog et al. 2009). If there is no self-reinforcing evolution in the infective ability of *Chaetothyriales*, one may wonder why opportunism is such a consistent feature throughout the order.

To our knowledge, infection is never caused by ingestion of one of the waterborne *Exophiala* species with food or water. Matos et al. (2003) and Hiruma et al. (1993) suggested an ingestive route of infection for the more virulent, systemic species *E. dermatitidis*, but this species has not been found in municipal water distribution networks. Therefore we consider the health risk of municipal water to be very low. In addition, since no pulmonary cases are known by any of the species treated, a route of infection through aerosols during showering or running tap water is unlikely. The main health risk of these fungi probably is cutaneous inoculation after moistening and abrasion of the skin, as suggested by Lian & de Hoog (2010).

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