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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 ºC, whereas those able to grow until 33(−36) ºC 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*.

Key words: Black yeasts, *Exophiala*, *Chaetothyriales*, waterborne fungi, pathogenicity, fish disease, amphibian disease, lethargic crab disease.
Taxonomic novelties: *Exophiala cancerae* de Hoog, Vicente, Najafzadeh, Harrak, Seyedmousavi & Boeger, sp. nov., *E. aquamarina* de Hoog, Vicente, Najafzadeh, Harrak, Seyedmousavi & Nyaoke, sp. nov., *E. opportunistica* de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, sp. nov., *E. halophila* de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, sp. nov., *E. lakei* de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, sp. nov., *E. equina* (Pollacci) de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, comb. nov.

INTRODUCTION

Exophiala is an anamorph genus defined by annellidic conidiogenesis 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 recognizable 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 the disseminated species *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 the *Chaetothyriales*, also being expressed in several pathogenic *Cladophialophora* species (Badali *et al*. 2008). However, during the last decades many *Exophiala* isolates were recovered which consistently lacked thermotolerance, but nevertheless were involved in animal disease. Infections were particularly found in fish, and amphibians, occasionally also in invertebrates. This indicates that intrinsic virulence factors other than temperature tolerance are shared by members of *Chaetothyriales* enabling animal infection.

Among the early reports of infections in fish 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 growing over several years in farmed Atlantic salmon (*Salmo salar*) in Norway, with mortalities up to 50 %, caused by *Exophiala psychrophila* (Pedersen & Langvad 1989). Infections took place when smolts were transferred to seawater, leading to visceral symptoms with predilection for the kidney. Identical features with 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 predilection for the kidney, and the 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 with 18 species of fish infected by members of *Exophiala* at 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 fish 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*. (2000) corrected the infective organism as *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, 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 melanised 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 as yet difficult to acquire insight into the magnitude of the problem.

Numerous reports on infections concern also 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*, which in recent taxonomy would be more likely to have been *Cladophialophora* species close to *C. devriesii* (G.S. de Hoog, unpublished results). 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) 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.* (2008) 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.* 2003AB), 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 were 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 insufficiently been studied. Given the above described pressing questions on human and animal health, a revision of this group is overdue. In the present paper, 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 involved SSU and ITS rDNA, and partial β-tubulin (*BT2*) and 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.* 2011). In this order another ecological trend was observed, with opportunism and pathogenicity to plants rather than to animals. It is the aim of the present paper to describe the chaetothyrialean counterpart of the waterborne black yeast biota.

MATERIAL AND METHODS

Strains and culture conditions

Strains analyzed 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 2657 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) slants at 4 ºC. 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 wks.

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 days, and subsequent periodical measurements of colony diameters during four weeks. 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

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

Name	CBS / status	Cross reference numbers	Source	Geography	GenBank							Reference
					SSU	LSU	*RPB1*	*ITS*	*TEF1*	*BT2*	*ACT1*	
C. coronata	617.96 (T)	ATCC 56201	Wood	New Zealand	*			*	*	*	*	Müller et al. 1987
E. alcalophila	520.82 (T)		Soil	Japan, Wako-shi, Hirose	*			*	*	*	*	Goto et al. 1981
E. alcalophila	521,82		Soil	Japan, Wako-shi, Hirose				*	*	*	?!	Goto et al. 1981
E. alcalophila	118723	ISO13G	Soil	Brazil				*				
E. alcalophila	118722	ISO13	Soil	Brazil				*				
E. alcalophila	122256	dH 17077	Human, skin	Denmark				*	*	*	*	
E. alcalophila		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				*	*	*	*	
E. angulospora	482.92 (T)		Drinking water	Japan	*			*	*	*	*	Iwatsu et al. 1991
E. angulospora	120272		Drinking water	Japan				*	*	*	*	
E. angulospora	109906	dH 11628, IWW 324	Drinking water	Germany				*	*	*		
E. angulospora	109905	dH 11626, IWW 327	Drinking water	Germany				*	*	*		
E. angulospora	121503	dH 12621, LE 212405	Fish	Russia				*	*			
E. angulospora		dH 13563, VANADIJ-CI	Fish nursery	Russia, Stravropol Kraj				*	*	*	*	
E. angulospora	119911	UTHSC 05-3397, dH 16409	Weedy seadragon	U.S.A., Boston				*	*	*	*	Nyaoke et al. 2009
E. angulospora		Frasca 06-4543, UTHSC R-3889	Lumpfish, skin	U.S.A.				*				
E. angulospora		UTHSC R-3890	Lumpfish, spleen	U.S.A.				*				
E. angulospora		UTHSC 07-871 R-3925	Lumpfish	U.S.A.				*				
E. angulospora	441,92		Human, nail	The Netherlands				*	*	*	*	
E. angulospora	122264	dH 17026, Saunte 83	Human, leg	Denmark, Copenhagen				*	*	*	*	
E. angulospora	146,93		Tilia wood	Germany				*	*	*	*	
E. angulospora		dH 18649	Polluted soil, petrol refinery	Brazil, Paulinia City				*				
E. aquamarina	119918 (T)	dH 16401, UTHSC 00-1181	Leafy seadragon, skin	U.S.A.	*			*	*	*	*	
E. aquamarina	119916	dH 16404, UTHSC 04-3445	Leafy seadragon, necrotic tissue	U.S.A.				*	*	*	*	
E. aquamarina	119919	dH 16403, UTHSC 02-852	Leafy seadragon, skull	U.S.A.				*	*	*	*	
E. aquamarina	120417	dH 17512, UTHSC 06-3123	Leafy seadragon, bone	U.S.A.				*	*	*	*	

Table 1. Continued

Name	CBS / status	Cross reference numbers	Source	Geography	GenBank							Reference
					SSU	LSU	*RPB1*	*ITS*	*TEF1*	*BT2*	*ACT1*	
E. aquamarina	119917	dH 16402, UTHSC 02-554	Leafy seadragon	U.S.A.				*	*	*	*	
E. aquamarina	119921	dH 16412, UTHSC 05-3314, R-3673	Weedy seadragon	U.S.A.				*	*	*	*	
E. aquamarina		UTHSC R-3685	Weedy seadragon	U.S.A.				*				
E. aquamarina	119912	dH 16408, UTHSC 05-3142, R-3669	Winter flounder	U.S.A.				*	*	*	*	
E. aquamarina	119915	dH 16405, UTHSC 05-32	Little tunnyfish	U.S.A.				*	*	*	*	
E. aquamarina		UAMH 10488	Lumpfish	Canada				*				
E. aquamarina		UTHSC R-4110	Sandlance, aquarium outbreak	U.S.A.				*				
E. aquamarina		UTHSC R-4111	Sandlance	U.S.A.				*				
E. aquamarina		UTHSC 05-3605 R-3678	Sandlance	U.S.A.				*				
E. brunnea	587.66 (T)	ATCC 32288, PRE 43729	Acacia karoo, litter	South Africa, Potchefstroom	*			*	?!	*	?!	
E. cancerae	120532	dH 17408, Vicente EXO1	Mangrove crab	Brazil				*			?!	
E. cancerae	120420 (T)	dH 17409, Vicente HF 16/08	Mangrove crab	Brazil	*			*	*	*	*	
E. cancerae	119920	dH 16425, IMI 380731, Cunningham 179/99	Green toad, liver	Israel				*	*	*	*	
E. cancerae		Det 154M / 2005	Human					*				
E. cancerae		Det. M154/ 2007	Human, nail	The Netherlands				*				
E. cancerae		UTHSC 87-269 (EC001), dH 13414	Human					*	*	*	*	
E. cancerae		UWFP 724	Human	U.S.A., Washington				AY213652				Rakeman et al. 2005
E. cancerae		GHP 2409		Germany				*				
E. cancerae		GHP 2419	Human, diabetic, skin	Germany				*				
E. cancerae		Det 127/2002 8, dH 12901	Water	Germany				*				
E. cancerae		Det 127-2 2002, dH 12895	Water	Germany				*				
E. cancerae		DTO Tm 01.00, dH 12673	Water					*	*	*	*	
E. cancerae	117491	dH 13595, DTO Tm 04.045 M13	Clean water from CIP tank	The Netherlands, Bodegraven				*	*	*	*	
E. cancerae	115142	CPC 11044, DQ008139	Fruit drink	Australia				*				
E. castellanii	122325	dH 16683, Saunte 30	Human, foot	Denmark, Copenhagen				*	*	*		
E. castellanii	122265	dH 17085, Saunte 142	Human, hand	Denmark, Copenhagen				*	?!	*		
E. castellanii	158.58 (T)	ATCC 1865, IFM 4702, MUCL 10097	Human, skin	Sri Lanka	*			*	*	*		Iwatsu et al. 1984

Table 1. Continued

Name	CBS / status	Cross reference numbers	Source	Geography	GenBank							Reference
					SSU	LSU	*RPB1*	*ITS*	*TEF1*	*BT2*	*ACT1*	
E. castellanii	662,76		Nematode, cyst	United Kingdom				*	*			
E. castellanii	110025	dH 12071, IWW 970	Drinking water	Germany				*	*			
E. castellanii		IWW 778, dH 12065	Drinking water	Germany				*	*			
E. castellanii	109915	dH 11634, IWW 502	Drinking water	Germany				*	*	*	?!	
E. castellanii	121496	dH 12245, IWW 694	Drinking water	Germany				*	*	*		
E. castellanii	109812	dH 12246, IWW 493	Drinking water	Germany				*	*			
E. castellanii	109914	dH 11627, IWW 326		Germany				*	*	*		
E. castellanii	120913	dH 17747-2, DTO Tm 06.131 20/10 A	Ice water for cooling	The Netherlands, Oosterwolde				*	?!	*		
E. equina	109913	dH 11629, IWW 544	Drinking water	Germany				*	*	*		
E. equina	121501	dH 12466, det 175-01	Drinking water	The Netherlands				*	*	*	*	
E. equina	122977	dH 19905, DTO 57-E4 S	Drinking water	The Netherlands				*				
E. equina		Det 221 / 2006	Drinking water	The Netherlands				*				
E. equina	120278	dH 17390, DTO 06.095-1.3	Drinking water, after water meter	The Netherlands, Geldermalsen				*	*	*		
E. equina		GHP R53	Drinking water	Germany				*				
E. equina	115143	CPC 11047	Bottled water	Australia				DQ008140				Avila de la Calle et al. 2006
E. equina	120904	dH 13558, det 36/2004 h	Water from water machine	The Netherlands, Joure				*	*			
E. equina	121513	dH 14518, DTO M 14A Tm 05.033	Water system of packaging machine	The Netherlands				*				
E. equina	124181	dH 20175	Bathroom-flask	The Netherlands				*				
E. equina	124180	dH 20174	Bathroom-flask	The Netherlands				*				
E. equina		dH 19902	Bathroom-flask	The Netherlands				*				
E. equina	124173	dH 20043	Bathroom-plate	The Netherlands				*				
E. equina		Hamada 1238	Bathroom	Japan, Osaka				*				
E. equina	109789	dH 12503, Det 239/01	Human, dialysis					*	*	*		
E. equina	121283	dH 14520, DTO Tm 05.033 / V85A	Waste water	The Netherlands				*		*		
E. equina	120905	dH 13762, UTHSC 04-526	Human, ulcer cornea	U.S.A., Falmouth				*	*	*		
E. equina	122267	dH 17015, Saunte 72	Human, finger nail	Denmark, Statens Serum Institut				*	*			
E. equina	121285	dH 13080, Det M-116 / 2003	Human, skin flakes	The Netherlands				*	*	*		

Table 1. Continued

Name	CBS / status	Cross reference numbers	Source	Geography	GenBank							Reference
					SSU	LSU	*RPB1*	*ITS*	*TEF1*	*BT2*	*ACT1*	
E. equina	121282	dH 13350, UTHSC 97-1647	Human	USA, San Antonio				*	*	*	*	
E. equina	121286	dH 13330, Det M327 / 2003	Human, sputum	The Netherlands				*		*		
E. equina	120906	dH 13647, UTHSC 89-386	Stool	U.S.A.				*	*	*	*	
E. equina	119.23 (T)		Horse	Italy	*			*	*	*	*	Pollacci 1923
(Haplographium debellae-marengoi v. equinum)												
E. equina	116009	dH 13221, F1090	Galapagos turtle	U.S.A., Chicago, Zoo Aquarium				*	*			Manharth et al. 2005
E. equina	150,93		Washed Tilia root	Germany				*		*		
E. equina	116922	DTO Tm 04.136	Silica gel	The Netherlands				*	*			
E. equina		DTO Tm 04.114	Tube of gelly installation	The Netherlands				*				
E. equina	121504 (T)	dH 12647, det M360/2002 Brasch	Tinea on leg of child (18 mo)	Germany, Kiel	*			*		*	*	
E. equina		GHP 2426	Human, diabetic, skin	Germany				*				
E. equina		GHP 2411	Human, diabetic, skin	Germany				*				
E. equina	122263	dH 17045, Saunte 102	human, foot	Denmark, Copenhagen				*		*	*	
E. equina	120387	dH 16674, Saunte 21	human, toe nail	Denmark, Copenhagen				*	*	*	*	
E. equina	122270	dH 16692, Saunte 39	human, foot					*		*	*	
E. equina	515,76	dH 12615	soil	Canada				*		*		
E. equina	661,76		cyst Heterodera	Germany				*	*	*	*	
E. equina	160,89		washed root Hordeum	The Netherlands				*		*		
E. equina	159,89		washed root	The Netherlands				*	*	*		
E. equina		Selosse Isolate 1	root mycorrhiza, Cephalanthera damasonium					AY833042				Julou et al. 2005
E. equina		Det 238-1855, dH 12507	Olea twig					*				
E. equina	121502	dH 12489, det 209-01 F	Olea, twig	Italy, Bari				*	*	*	*	
E. equina		Ms16Mb14	Phragmitis australis	Germany, Lake Constance				AJ875365				Neubert et al. 2006
E. halophila	123150	Det 08-017-20	salty water					*				
E. halophila	121512 (T)	dH 13757, UTHSC 03-2191	human, skin axilary	U.S.A.	*			*	*	*	*	
E. halophila	121499	dH 12324, Mayser 2151/99	nail	Germany				*	*	*	*	
E. lakei	117497 (T)	dH 13711	lake water, 1 m depth	The Netherlands, Loosdrecht				*	*	?!	*	

Table 1. Continued

Name	CBS / status	Cross reference numbers	Source	Geography	GenBank							Reference
					SSU	LSU	*RPB1*	*ITS*	*TEF1*	*BT2*	*ACT1*	
E. mesophila	402.95 (T)		shower joint	Germany	*			*	*	*		Listemann & Freiesleben 1996
E. mesophila	836,95	dH 16276	swimming pool	Germany				*	*	*		
E. mesophila	119910	dH 16410, UTHSC R-3282	dental waterline	U.S.A.				*	*	*		
E. mesophila	109147	dH 11838, Matos T-20	bathroom	The Netherlands				*	*	*		
E. mesophila		dH 18626	bathroom	The Netherlands, Hilversum				*		?!		
E. mesophila	121498	dH 12261, M415-10-320/01						*	*	*		
E. mesophila	121509	dH 13436, UTHSC R-1444 (EC001)	phaeohyphomycotic cyst					*	*			
E. mesophila	121508	dH 13400, UTHSC 91-270 (EJ001)	human, finger	U.S.A.				*	*	*		
E. mesophila	120910	dH 13763, UTHSC 04-611	human, sinus	U.S.A.				*	*	*		
E. mesophila	120907	dH 13765, UTHSC 04-1300	human, hip joint	U.S.A.				*	*	*		
E. mesophila	121507	dH 13387, UTHSC 96-1493 (EJ001)	human, hair	U.S.A.				*	*	*		
E. mesophila	121497	dH 12260, M415-08-966/01	human, immunosuppressed, bronchial endoscopy	France, Rouen				*	*			
E. mesophila	121511	dH 13460, UTHSC 92-1021 (EJ005)	human, nasal tissue	U.S.A.				*	*	*	?!	
E. mesophila		GHP R28		Germany				*				
E. opportunistica	109811 (T)	dH 12243, IWW 720	drinking water	Germany	*			*	*	*	*	
E. opportunistica	122269	dH 16680, Saunte 27	human, nail	Denmark, Copenhagen				*	*	*	*	
E. opportunistica	122268	dH 16705, Saunte 52	human, foot	Denmark, Copenhagen				*	*	*	*	
E. opportunistica	660,76	dH 16144	rhizosphere,Triticum aestivum	West Australia				*	*	*		
E. opportunistica	637,69	dH 16111	polyvinyl alcohol					*	*	*	*	
E. opportunistica	631,69		unknown	The Netherlands				*	*			
E. pisciphila	121505	dH 13077, Det 100 / 2002	swimming pool	Germany				*	*	*		
E. pisciphila	101610	dH 11173	water pipe	Germany				*	?!	?!	*	
E. pisciphila	537.73 (T)	WUC 137	catfish	U.S.A.	*			*	*	*	*	Fijan 1969; McGinnis & Ajello 1987

Table 1. Continued

Name	CBS / status	Cross reference numbers	Source	Geography	GenBank							Reference
					SSU	LSU	*RPB1*	*ITS*	*TEF1*	*BT2*	*ACT1*	
E. pisciphila	119913	dH 16407, UTHSC 05-656, 05-460	potbelly seahorse					*	*	*	*	
E. pisciphila	119914	dH 16406, UTHSC 05-173, 5-317	potbelly seahorse					*	*	*	*	
E. pisciphila	121500	dH 12328, Mayser 1748/00	nail	Germany				*	*	*		
E. psychrophila	191.87 (T)		Salmo salar in fish farm	Norway	*			*	*	*	*	Pedersen & Langvad 1989
E. psychrophila	256,92		salmon	Ireland				*	?!	*	?!	
E. salmonis	157.67 (T)	BMU 00834	trout, brain	Canada	*			*		*	*	Carmichael 1966
E. salmonis	120274	dH 17392, DTO 06.095 nr.2.2	drinking water tap	The Netherlands, Geldermalsen				*	?!	*	*	
E. salmonis	110371	dH 12699, det 405-01-8862/01	drinking water	The Netherlands				*		*	*	
V. botryosa		Jorg Mayer M 218, dH 11516	frog	U.S.A., Rhode Island, Park Zoo				*	*	*	*	
V. botryosa	121506	dH 13325, Padhye 2004-000517	human, wrist skin	Japan				*	*	*	*	
V. botryosa	101462	dH 11373	human, skin					*		*	*	
V. botryosa	102593	dH 11917, CDC 5937	human, disseminated in child (12 y)	China, Jiangsu Province				*	*	*	*	
V. botryosa	254.57 (T)		sansa olive slag	Italy, Toscana, Pisa	*			*	*	*	*	Ciferri & Montemartini 1957
V. botryosa		dH 18628	Eucalyptus treated with creosote 20 y ago	Brazil, Rio Claro				*				
V. botryosa		dH 18639	Eucalyptus	Brazil, Rio Claro				*				
V. botryosa		dH 18642	Eucalyptus	Brazil, Rio Claro				*				
V. botryosa		dH 18630	Eucalyptus	Brazil, Rio Claro				*				
V. botryosa		dH 18640	Eucalyptus	Brazil				*				

Abbreviations used: C = Capronia, Cl = Cladophialophora, E = *Exophiala*, V = Veronaea, T = ex-type strain.
ATCC = American Type Culture Collection, Manasas, U.S.A.; BMU = Beijing Medical University, Beijing, China; CBS = Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre, Utrecht, The Netherlands; CDC = Centers of Disease Control, Atlanta, U.S.A.; CPC = Pedro Crous collection, Utrecht, The Netherlands; DAOM = National Mycological Herbarium, Ottawa, Canada; Det = CBS Identification Division, Utrecht, The Netherlands; dH = G.S. de Hoog working collection, Utrecht, The Netherlands; DTO = CBS Applied Division, Utrecht, The Netherlands; GHP = Gerhard Haase Collection, Aachen, Germany; IFM = Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba, Japan; IWW = Rheinisch-Westfälisches Institut für Wasserforschung, Duisburg, Germany; NCPF = National Collection of Pathogenic Fungi, Bristol, U.K.; PRE = National Herbari

sides. The block was subsequently covered with a sterile cover slip (~2 cm2). 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 days. 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 et al. (1999). About 1 cm2 fungal material of 3-4 weeks 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 sodiumdodecylsulphate (SDS) in 80 ml ultrapure water, pH 8.0). Cells were grinded 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 NH4-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) 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 V3.3.0 software (Coleman Technologies, Wilmington, DE, U.S.A.). 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* (Table 2) 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 degenerated 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 45 sec (denaturation), 52 °C 30 sec (annealing) and 72 °C 120 sec (extension), with a final delay of 7 min and using the maximum ramp speed for ITS amplification. For SSU the annealing temperature 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 9800 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 %) and 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* (Table 2), 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-x µL ultra pure water to 10 µL final volume. Reactions were performed in a GeneAmp in twenty-five cycles of: 95 °C for 20 sec, 50 °C for 15 sec, 60 °C for 60 sec and stopped with cooling tot 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 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

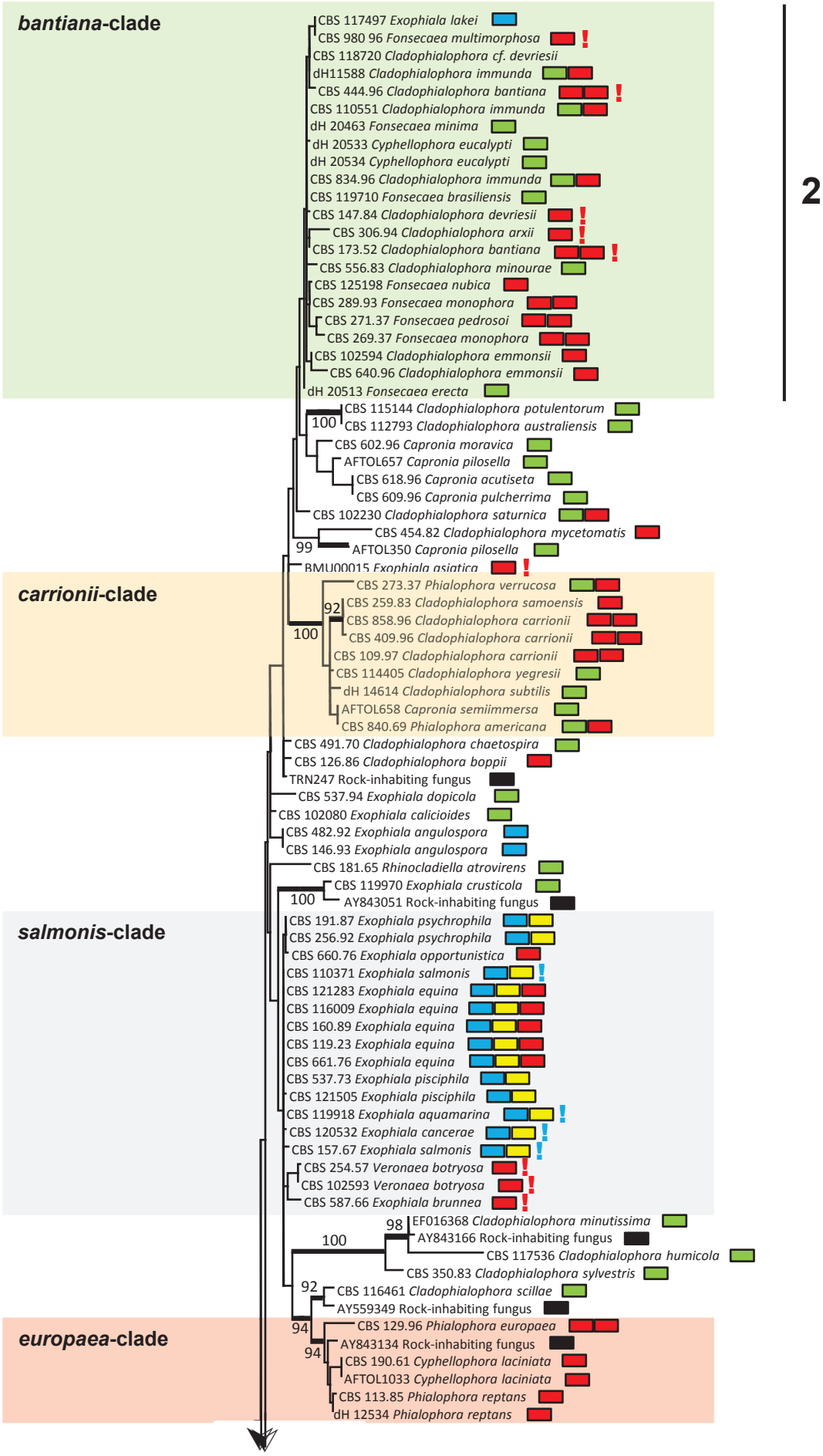
For genealogical concordance analysis, four genes ITS, *TEF1*, *BT2* and *ACT1* were first analyzed separately. Alignment was performed automatically and adjusted iteratively by hand with BioNumerics v. 4.61 (Applied Maths, Kortrijk, Belgium). Topological conflicts were evaluated visually and by using the partition homogeneity test implemented in PAUP* v. 4.Ob10. 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 146 waterborne strains of *Exophiala* and two related species (Table 1), *Exophiala castelanii* and *E. mesophila*, as outgroups in the SSU tree. For species circumscribed by genealogical concordance, a single strain (mostly the ex-type) was selected for sequencing conserved genes. Sequences were compared in a database in BioNumerics containing all described members of *Chaetothyriales* and which was regularly updated with GenBank and AFTOL submissions. SSU (1100 comparable sites) trees were generated with the Parsimony option of PAUP after removal of introns

RESULTS

DNA 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.


bantiana-clade
CBS 117497 Exophiala lakei
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

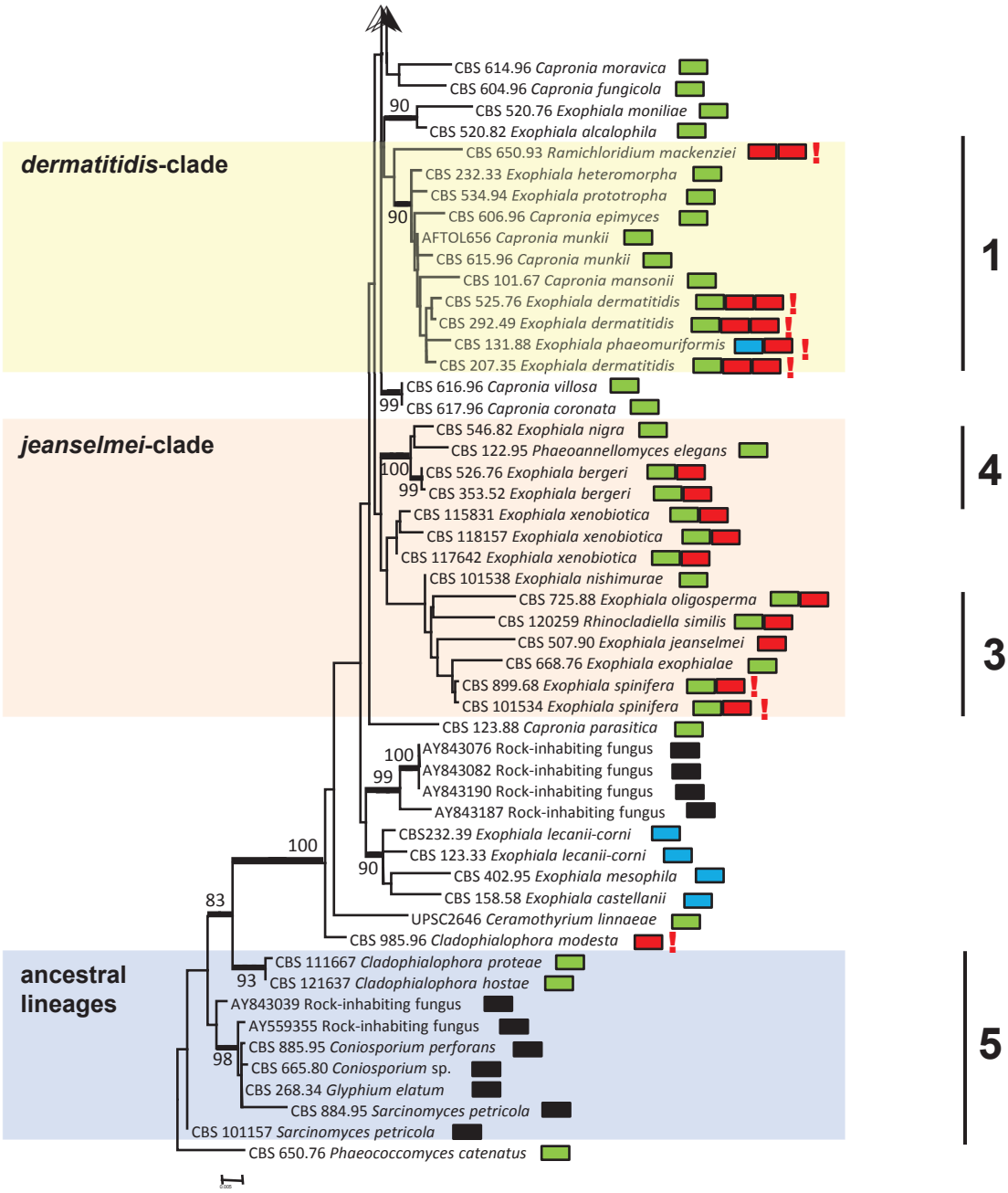


Fig. 1. Phylogeny of all members of *Chaetothyriales* described to date, obtained from a ML analysis based on SSU rDNA sequences. Bootstrap support was calculated from 100 replicates; values >80 % are shown with the branches. Supported branches are drawn in bold. The tree was rooted with *Phaeococcomyces catenatus*, CBS 650.76. Groups 1–5 represent supported clades indicated by Haase *et al.* (1999). Coloured boxes represent species complexes recognized in this paper. Prevalent species ecologies are summarized 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.

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 prevalent isodiametric morphology (group 5 of Haase *et al*. 1999) and the plant-inhabiting species *Cladophialophora hostae.* The remainder of the tree, at rather large distance and with 100 % bootstrap support is supposed to represent the ascomycete family *Herpotrichiellaceae*. Teleomorphs, when present in this group, are *Capronia* species while also *Ceramothyrium linnaeae* is found in this part of the tree.

Overall only 12 bootstrap-supported (i.e. > 80 % in Fig. 1) 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) recognized by Haase *et al.* (1999). Group Haase-1 is recognized below as the *dermatitidis*-clade, Haase-2 is the *bantiana*-clade, Haase-3 and -4 correspond with two clusters summarized 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*.

Plotting main sources of isolation on the tree (habitats: rock, plant, water / cold-blooded animal, warm-blooded animal) no aggregation was evident, except for a clustering of waterborne species in a group referred to as the *salmonis*-clade (Fig. 1).

However, in detail different trends are apparent. The *bantiana*-clade contained thermotolerant systemic pathogens, such as *C. bantiana* and *C. arxii*, as well as *Fonsecaea* agents of chromoblastomycosis. The clade comprised a single *Exophiala* species from water, known from a two strains, with CBS 117497 as the ex-type. The *carrionii*-clade had 100 % bootstrap support and contained agents of chromoblastomycosis. The *dermatitidis*-clade (Haase group 1), with 90 % bootstrap support, comprises 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 was strongly clustered. A large clade (Fig. 1, as *salmonis*-clade) contained almost exclusively waterborne species; only the drinking water species *Exophiala angulospora* took an isolated position. The *salmonis*-clade comprised ten waterborne *Exophiala* species (*E. psychrophila* CBS 256.92, *E. halophila* CBS 121512, *E. pisciphila* CBS 537.73, *E. aquamarina* CBS 119918, *E. equina* CBS 119.23, *E. superficiale* CBS 121504, *E. salmonis* CBS 157.67, *E. opportunistica*, CBS 109811, *E. cancerae* CBS 120420, *E. brunnea* CBS 587.66)**,** with the sympodial species *Veronaea botryosa*, a potential agent of disseminated infections in humans, at some distance.

The *salmonis*-clade was analyzed 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. 3), three groups at high bootstrap support are recognizable: 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 recognized 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. 3), among which seven have not formally been described as *Exophiala* species. The sympodial species *Veronaea botryosa*, which is morphologically very different from the annellidic *Exophiala* species, is found adjacent to the waterborne species in all genes analyzed (Figs 1–3). Partition-homogeneity test with heuristic search of four genes (ITS, TEF1, BT2, ACT1) with 100 replicates and 167 parsimony-informative characters of 2254 total characters revealed conflict (significant heterogeneity) among the genes (p = 0.01). The *salmonis*-clade comprised 10 groups in ITS / multilocus trees of prevalently waterborne *Exophiala* species with bootstrap support in both trees, as follows: *Exophiala equina* (75 / 100), *E. salmonis* (99 / 100), *E. pisciphila* (98 / 100), *E. aquamarina* (92 / 100), *E. psychrophila* (100 / 100), *E. opportunistica* (- / 99), E. cancerae (88 / 100); *Veronaea botryosa* and *Exophiala* 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* lakei is SSU a member of the *bantiana*-clade and consequently had a long branch with members of the *salmonis*-clade.

Outside the *salmonis*-clade two clusters of strains with waterborne species were recognizable in the SSU tree (Fig. 1) at 100 % ITS bootstrap support (Fig. 3). Nearly all species within these clusters (*Exophiala alcalophila, E. halophila, E. angulospora, E. castellanii* and *E. mesophila*) were strongly supported. Several species showed significant intraspecific variability.

TAXONOMY

Exophiala J.W. Carmich., Sabouraudia 5: 122. 1966. MycoBank MB8233.

The genus *Exophiala* was described as an anamorph taxon by Carmichael (1966), with *E. salmonis* J.W. Carmich. as its type species. The genus represents an anamorph member of the order *Chaetothyriales.* Note that the type species has among its synonyms the name *Aureobasidium salmonis* (J.W. Carmich.) Borelli, *Aureobasidium* being a member of the order *Dothideales* (Schoch *et al.* 2009*)*. Teleomorph relations of *Exophiala* are in the genus *Capronia*, typified by *Capronia sexdecimspora* (Cooke) Sacc. *Exophiala* is the main genus of black yeasts found as opportunists of vertebrates. It is characterized by annellidic conidiogenesis. Some cultures are entirely yeast-like (synanam. *Phaeococcomyces*), or form phialidic collarettes (synanam. *Phialophora*), sympodial conidiophores (synanam. *Rhinocladiella*) or dry conidial chains (synanam. *Cladophialophora*). Chlamydospores or sclerotial bodies may also be formed, occasionally leading to entirely meristematic mutants (synanam. *Sarcinomyces*). Several of these morphologies are represented in species of the waterborne *salmonis*-clade (B).

Thermophilic species of *Exophiala* have been described from systemic infections in humans, e.g. *E. dermatitidis* and *E. spinifera*. 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, attributed to a diversity of *Exophiala* species. However, morphology is not helpful for species identification due to variable appearance. Therefore in the present paper we will only include the case reports where voucher strains have been preserved and sequenced.

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

Teleomorph: Capronia coronata G.J. Samuels, in Müller *et al*., Trans. Br. 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 one-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, one-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.

Ex-type culture anamorph: **Japan**, Yokohama-shi, 18 April 1989, K. Arai, from drinking well water, CBS 482.92 = NHL 3101.

Ex-type culture teleomorph: **New Zealand**, Westland County, Nemona State Forest, 5 May 1983, G.J. Samuels, from decorticated wood, CBS 617.96 = ATCC 56201 = PDD 35308.

Additional material examined: Table 1.

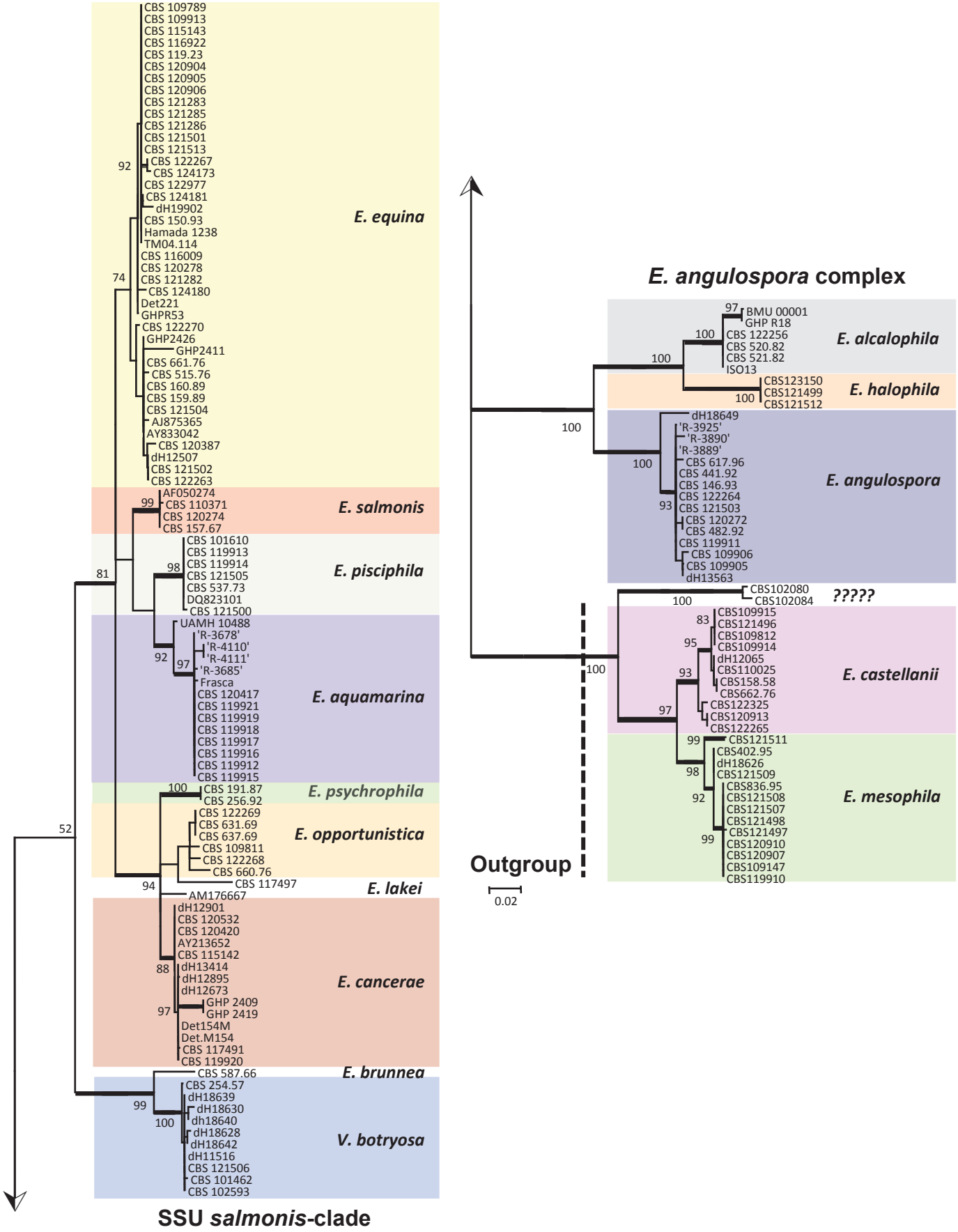


Fig. 2. Phylogeny of the SSU-based *salmonis*-clade, obtained from a ML analysis based on ITS rDNA sequences. Bootstrap support was calculated from 100 replicates; values >80 % are shown with the branches. Supported branches are drawn in bold. The tree was rooted with *Exophiala mesophila* and *E. castellanii.*

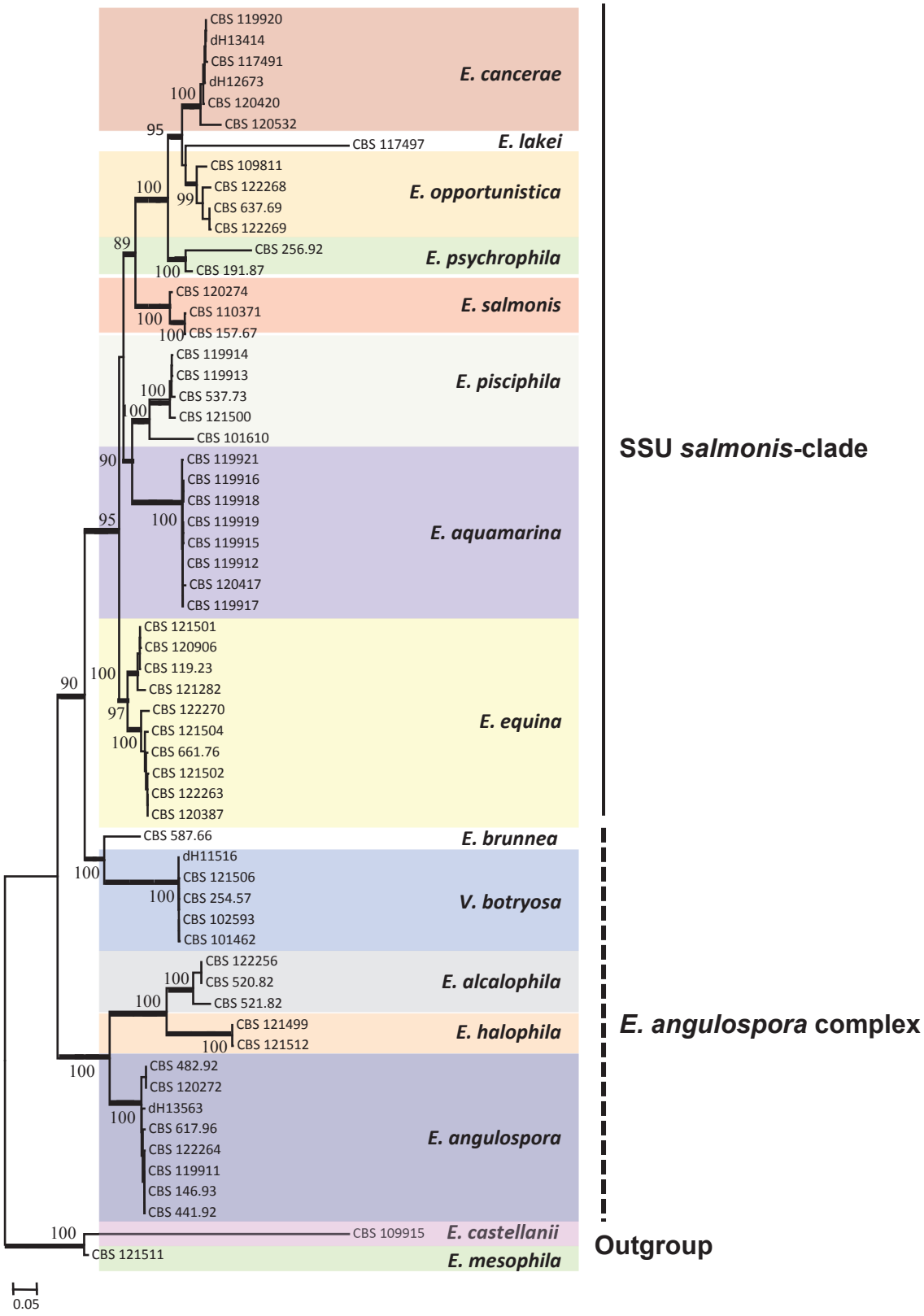


Fig. 3. Phylogeny of the SSU-based *salmonis*-clade, obtained from a ML analysis based on ITS, *ACT1, BT2* and and *TEF1* sequences. Bootstrap support was calculated from 100 replicates; values >80 % are shown with the branches. Supported branches are drawn in bold. The tree was rooted with *Exophiala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

Notes: With SSU sequences (Fig. 1) *Exophiala mesophila and E. castellanii* is found outside the *salmonis*-clade, and is therefore selected as outgroup in the ITS and multi locus trees (Figs 3, 4) **.** Yamada *et al.* (1989) demonstrated that *Exophiala salmonis* had a coenzyme Q 10($H_{2)}$ ubiquinone system, whereas *E. alcalophila* had Co-Q 10.

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, U.S.A., and also repeatedly in the marine lumpfish (*Cyclopterus lumpus*). Strain CBS 121503 was isolated affecting the freshwater fish *Scenodus leucichtys* in a fish nursery in Stravropol Kraj near Kislovodsk, southern Russia (V.A. Mel'nik, pers. comm.). Human isolates such as CBS 441.92 and CBS 122264 came from skin and nails and thus did not require growth abilities above 33 °C (D. Saunte Linhardt, pers. comm.). The species was once isolated 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 are insignificant, involving the outermost body parts only. This pathology is likely to be determined by its relatively low maximum growth temperature.

Exophiala psychrophila Pedersen & Langvad, Mycol. Res. 92: 153. 1989.

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 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. Condiogenous 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 x 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 are: minimum 0 °C (growth present after 6 months at 0 °C); optimum 17−21 °C; maximum 23 °C.

Ex-type culture anamorph: **Norway**, March 1987, F. Langvad, from Atlantic salmon smolt (*Salmo salar*), CBS 191.87 = dH15499 = CBS H-20009 **=** MBSPEC1293.

Notes: The species was originally isolated from farmed Atlantic salmon smolt (*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). Supplementary strains from salmon in different countries were available in this study (Table 1).

Exophiala halophila de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, **sp. nov.** MycoBank MB515715. Figs 5, 6.

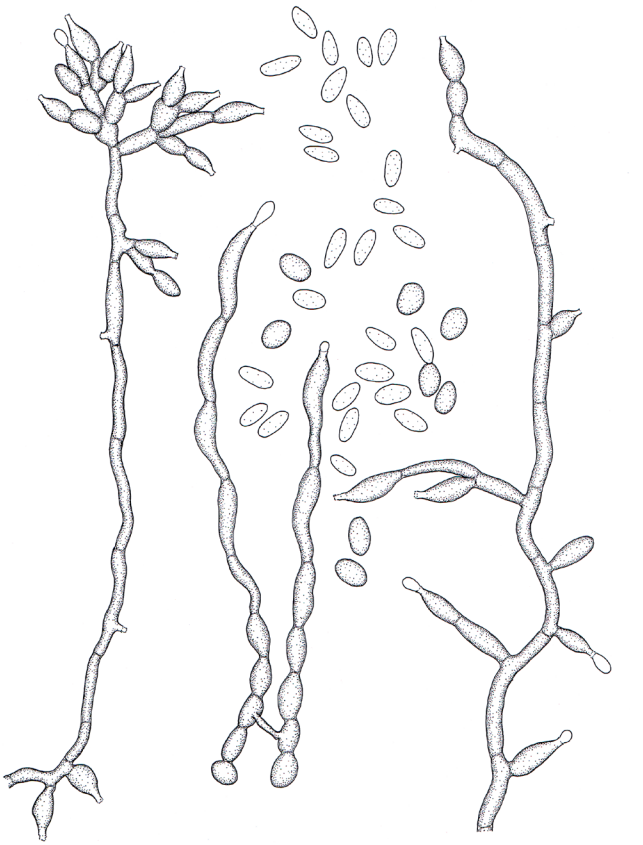


Fig. 5. *Exophiala halophila,* CBS 121512. Conidial apparatus, conidia and anastomosing torulose hyphae.

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.

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 ºC, optimum 24−27 °C, maximum 30−33 °C. No growth at 37 °C. Teleomorph unknown.

Holotype: **U.S.A.,** Texas, San Antonio, D.A. Sutton, from human skin, 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. Nearest

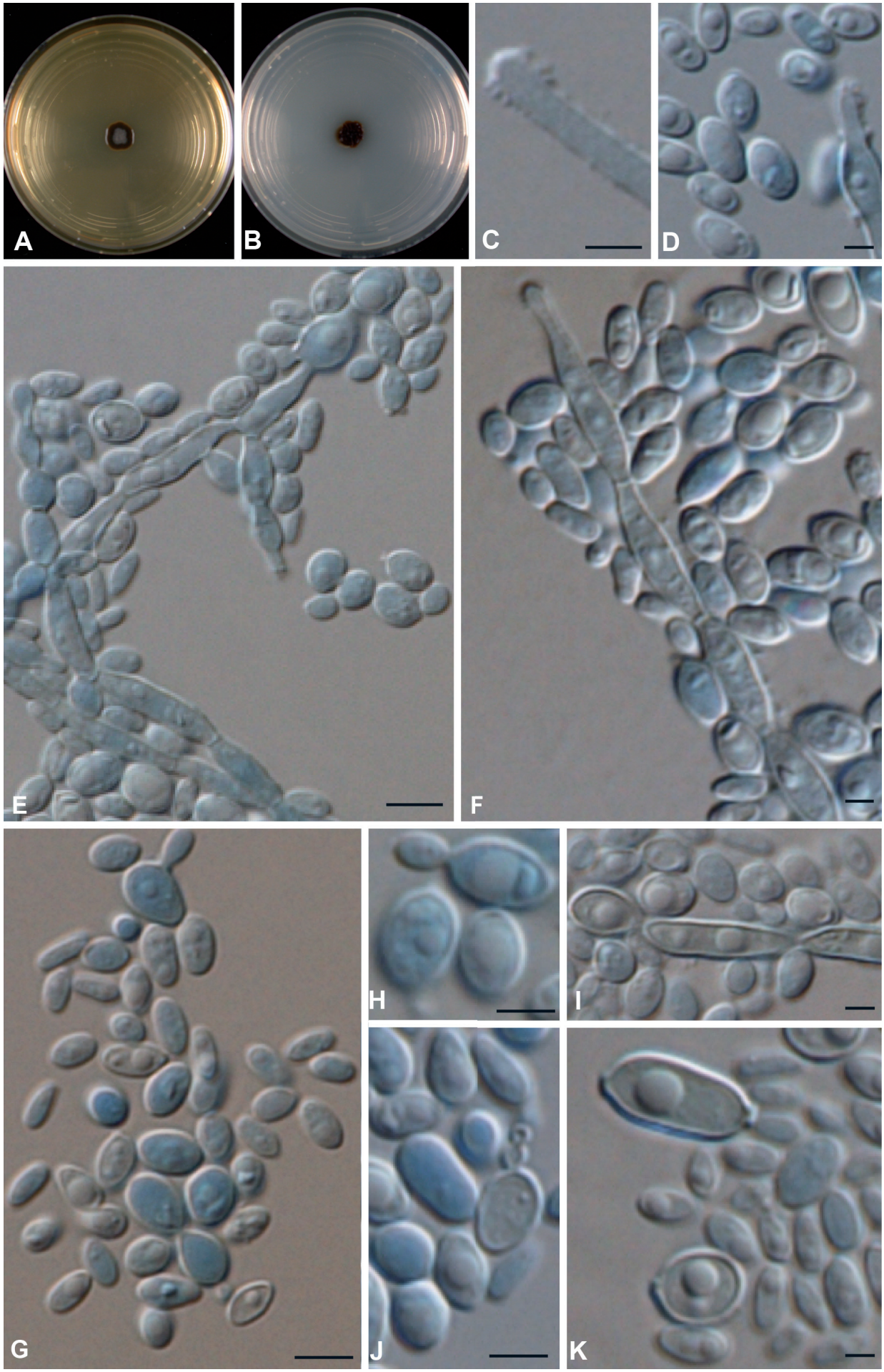


Fig. 4. *Exophiala halophila,* CBS 121512. A. Colony on MEA; B. Colony on PDA; C−E. Conidial apparatus with conidia; F−K. Budding cells and conidia; I. Tolurose hyphae.

neighbor is *Exophiala alcalophila* at a minimum distance of 8.4 % ITS distance, and thus its novelty is unambiguous (Fig. 3). The species has isolated from human skin of the armpit, and from human nails, but also from salty water. Strains were tolerant to 2.5, 5, 10 % $MgCl_2$ and to 2.5 and 5 % $NaCl_2$.

Exophiala alcalophila Goto & Sugiyama, in Goto *et al*., Trans. Mycol. Soc. Japan 22: 430. 1981. MycoBank MB110200.
= *Phaeococcomyces alcalophilus* Goto & Sugiyama, in Goto *et al.,* 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 one month) becoming slightly floccose at the centre and remaining slimy at the margin. Reverse brownish black, a rust brown pigment maybe 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 in 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 ºC, optimum 24−27 °C, maximum 36−40 °C. Teleomorph unknown.

Ex type culture: *Exophiala alcalophila,* **Japan,** Hirose, Wako-shi, Saitama pref., 23 April 1978, K. Horikoshi, from soil, IAM 12519 = CBS 520.82; herbarium CBS H-19960.

Ex type culture: *Phaeococcomyces alcalophilus,* **Japan,** Hirose, Wako-shi, Saitama pref., 23 April 1978, K. Horikoshi, from soil, 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 with two 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.

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 black yeast-like fungi in low-nutrient indoor water systems rich in soap such as bathrooms on the one hand, and cutaneous infection on the other. The authors repeatedly isolated several black yeast-like species from bathrooms which until then had only been known from human skin and nails, and hence suggested that maceration of human skin may provide a portal of entry for these fungi having moderate invasive capacity. *Exophiala alcalophila* apparently belongs to the same ecological group, although at low virulence. Yamada *et al.* (1989) reported a Coenzyme Q10 system in the species, different from species in the *salmonis*-clade.

Exophiala pisciphila McGinnis & Ajello [as *'pisciphilus*'], Mycologia 66: 518. 1974. MycoBank MB314043.

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. Cardinal temperatures: minimum ≤ 4−9 ºC, optimum 24−30 °C, maximum 30−33 °C. No growth at 37 °C. Teleomorph unknown.

Ex type culture: **U.S.A.,** Alabama, privately owned freshwater pond, April 1969, N. Fijan, from systemic mycosis in channel catfish (*Ictalurus punctatus*), CBS 537.73, dried culture CBS H-7135.

Additional material examined: Table 1.

Notes: This species was described as one of the first *Exophiala* species causing epizootics in cold-blooded vertebrates (Fijan 1969). Eighty percent of a probe of a population of freshwater channel catfish (*Ictalurus punctatus*) had cutaneous ulcers 2−15 mm diam and up to 5 mm deep, without inflamed margins. Numerous nodules were found in visceral organs, while 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 strain was not confirmed by sequencing. Strains causing an epizootic in the captive marine plaice (*Pleuronectes platessa*), published as *Hormoconis resinae* (Strongman *et al*. 1977) were re-indentified as *Exophiala pisciphila* (de Hoog *et al*. 2000). 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. 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 human patient in Germany (P. Mayser, unpublished data; Table 1). Yamada *et al.* (1989) reported a Coenzyme Q10($H_{2)}$ system in the species.

Exophiala aquamarina de Hoog, Vicente, Najafzadeh, Harrak, Seyedmousavi & Nyaoke, **sp. nov.** MycoBank MB 515716. Figs 7, 8.

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 spiralis. Conidiophora vix distinguenda, ramose vel simplicia, terminalia vel intercalaria; conidium annellidorum vel sympodiorum producens.

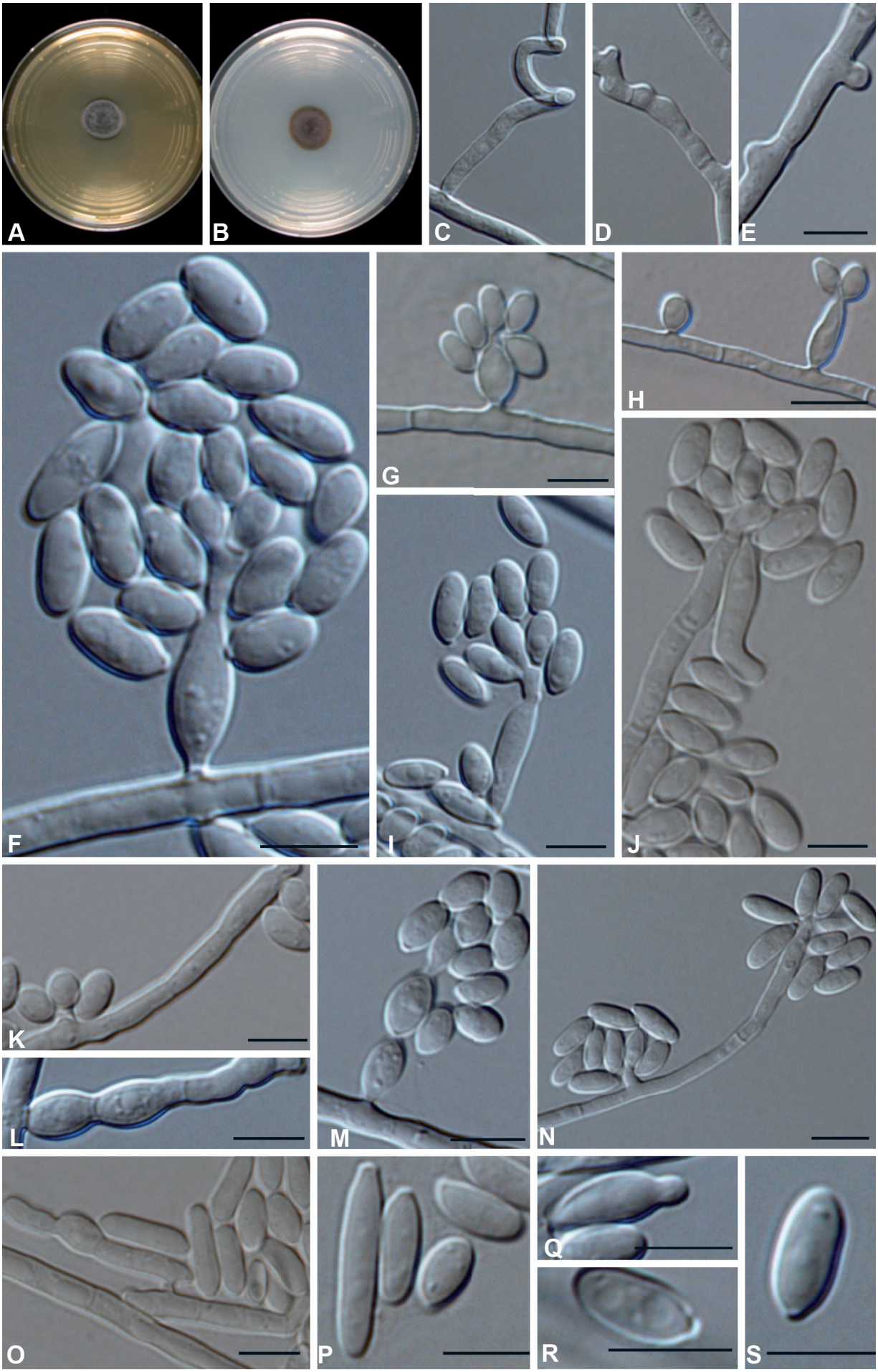


Fig. 6. *Exophiala aquamarina,* CBS 119918. A. Colony on MEA; B. Colony on PDA; C, D. Spirally twisted hyphae; E–N. Conidial apparatus with conidia; F–J. Annellidic conidiogeneses with sympodial conidiophores; O. Anastomosis between discrete cells; P–S. Conidia; Q. Budding cells.

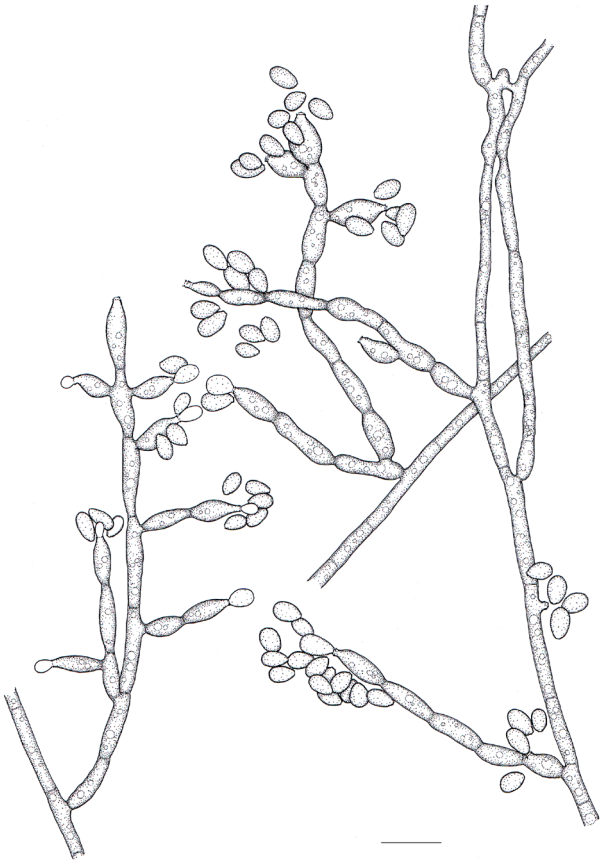


Fig. 7. *Exophiala aquamarina,* CBS 119918. Conidial apparatus and conidia

Conidia late ellipsoidea, levia, 6.7−19.2 × 4.0−4.8 µm. Temperatura maxima crescentiae 36 °C. Teleomorphe ignota.

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 ºC, optimum 24−30 °C, maximum 33−36 °C. No growth at 37 °C. Teleomorph unknown.
Type: **U.S.A.,** Boston, New England Aquarium, S. Frasca, from skin of leafy sea dragon (*Phycodures eques*), **holotype** CBS H-19950, ex-type strain CBS 119918 = UTHSC 00-1181 = dH 16401.

Additional material examined: Table 1.

Notes: The species repeatedly caused disseminated infections in several species of fish in the New England Aquarium in Boston, U.S.A., and in the Adventure Aquarium in Camden, U.S.A. (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 thus far restricted to fish, but is not host specific. Nyaoke *et al.* (2009) explain the pronounced susceptibility of sea dragons to *Exophiala* infection by their balanced habitat in algae-covered reefs and seagrass meadows.

Exophiala equina (Pollacci) de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, **comb. nov.** MycoBank MB515717. Figs 9, 10.
≡ *Haplographium debellae-marengoi* Pollacci var. *equinum* Pollacci, Revta Biol. 5: 370. 1923; Micosi Chirurg. II, p. 909. 1927 (basionym).

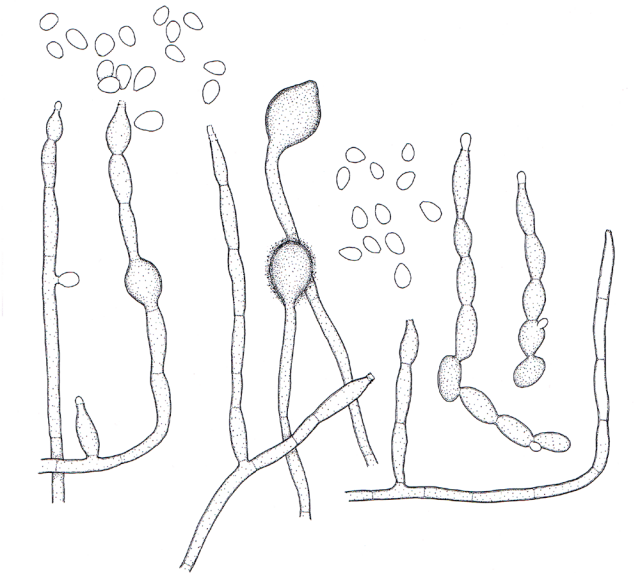


Fig. 9. *Exophiala equina*, CBS 119.23 . Combineer drawing.

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 × 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. Teleomorph unknown.

Type: **Italy,** Pavia, December 1923, G. Pollacci, subcutaneous infection of a horse, **holotype** CBS H-19957, ex-type strain CBS 119.23 = dH 15335.

Additional material examined: Table 1.

Notes: The 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 gelly installation, from silica gel and 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.
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 was a corneal ulcer and an onychomycosis (Table 1). The case caused by CBS 121504 concerned a 1-year-old child with circular, tinea-like lesions from

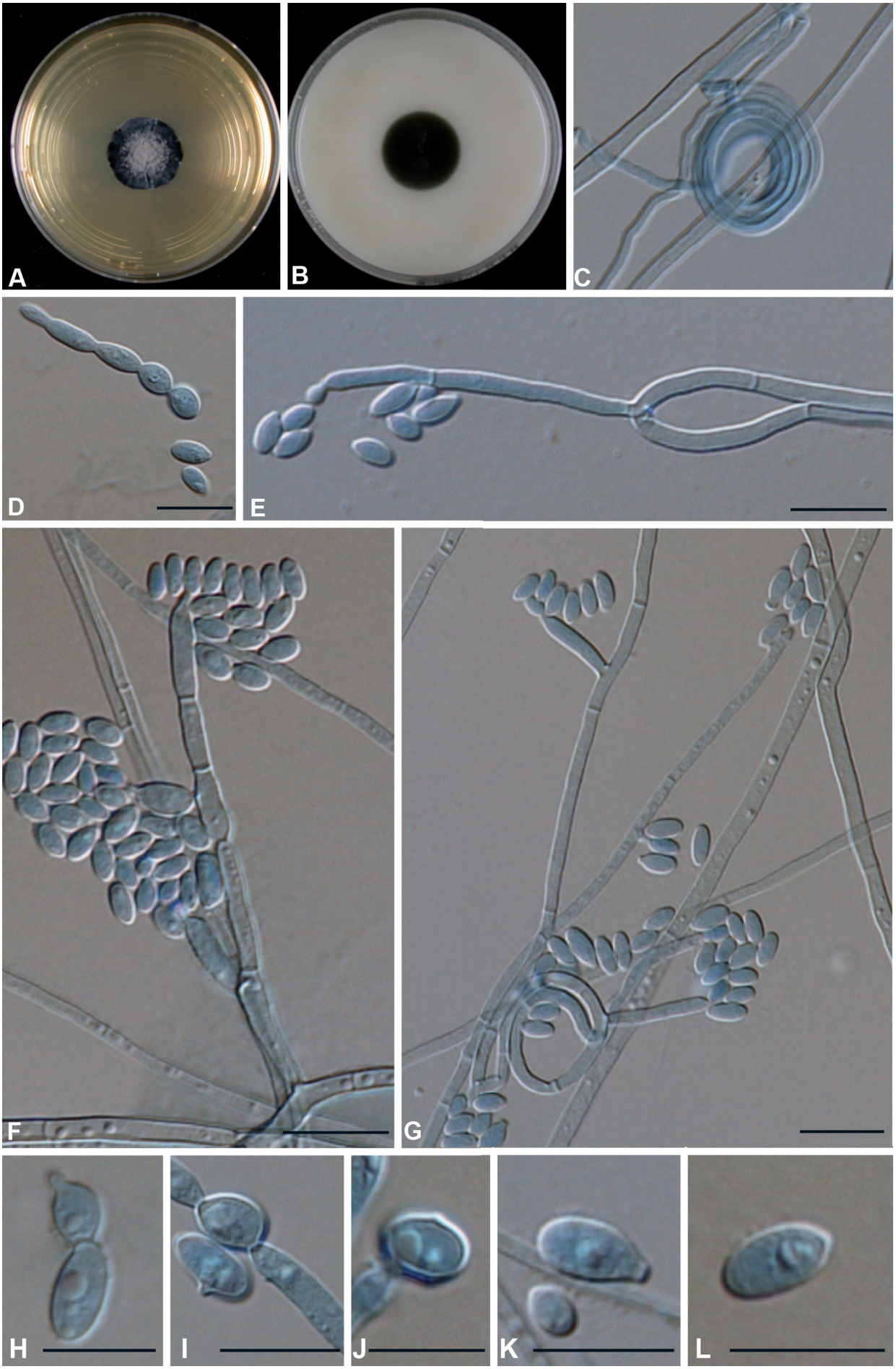


Fig. 8. *Exophiala equina*, CBS 119.23 CHECK same superficiale. A. Colony on MEA; B. Colony on PDA; C. Spirally twisted hyphae; D. Tolurose hyphae; E, G. Conidiophore with single conidiogenous cell; F. Conidiogenous subcylindrical cells flask shaped with ellipsoidal conidia; H. Budding cell; I, J. Chlamydospore; K, L. Ellipsoidal conidia.

which the fungus was isolated together with *Candida guilliermonii*. The lesion was successfully treated with ciclipirox. *Exophiala superficiale* 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 and P. Mayser, pers. comm.), which had a relatively low body temperature due to impaired circulation. This condition allows invasion by species that are unable to grow at temperatures below 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 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; annellated 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 ºC, optimum 18−24 °C, maximum 33−33 °C. No growth at 37 °C. Teleomorph unknown.

Type: **Canada,** Alberta, Calgary, Alberta hatchery; cerebral mycetoma of fingerling trout (*Salmo clarkii*), J.W. Carmichael, herbarium CBS H-12617, CBS H-7136, ex-type strain 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 frequently reported under this name, only very few confirmed isolates are available. The species was originally reported causing three epidemic episodes of cerebral mycetoma occurred in freshwater fingerling trout cod (*Maccullochella macquariensis*) at the Provincial Government Fish Hatchery in Galary, 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 & Wolke (1985) ascribed an infection in captive Atlantic salmon (*Salmo salar*) in the U.S.A. to the same fungus, but no sequencing data are available. 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-year-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 Q 10($H_{2)}$ system in *Exophiala salmonis.*

Exophiala opportunistica de Hoog, Vicente, Najafzadeh, Harrak & Seyedmousavi, **sp. nov.** MycoBank MB515719. Figs 11, 12.

Fig. 12. *Exophiala opportunistica,* CBS 109811. Hyphae with mostly intercalary conidiogenous cells with extended annellated zones, and 1−2-celled conidia.

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, 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.

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 ºC, optimum 21−24 °C, maximum 27−30 °C. No growth at 37 °C. Teleomorph unknown.

Type: **Germany,** Duisburg, from drinking water, E. Göttlich, **holotype** CBS-H 20383, ex-type strain CBS 109811 = dH 12243 = IWW 720.

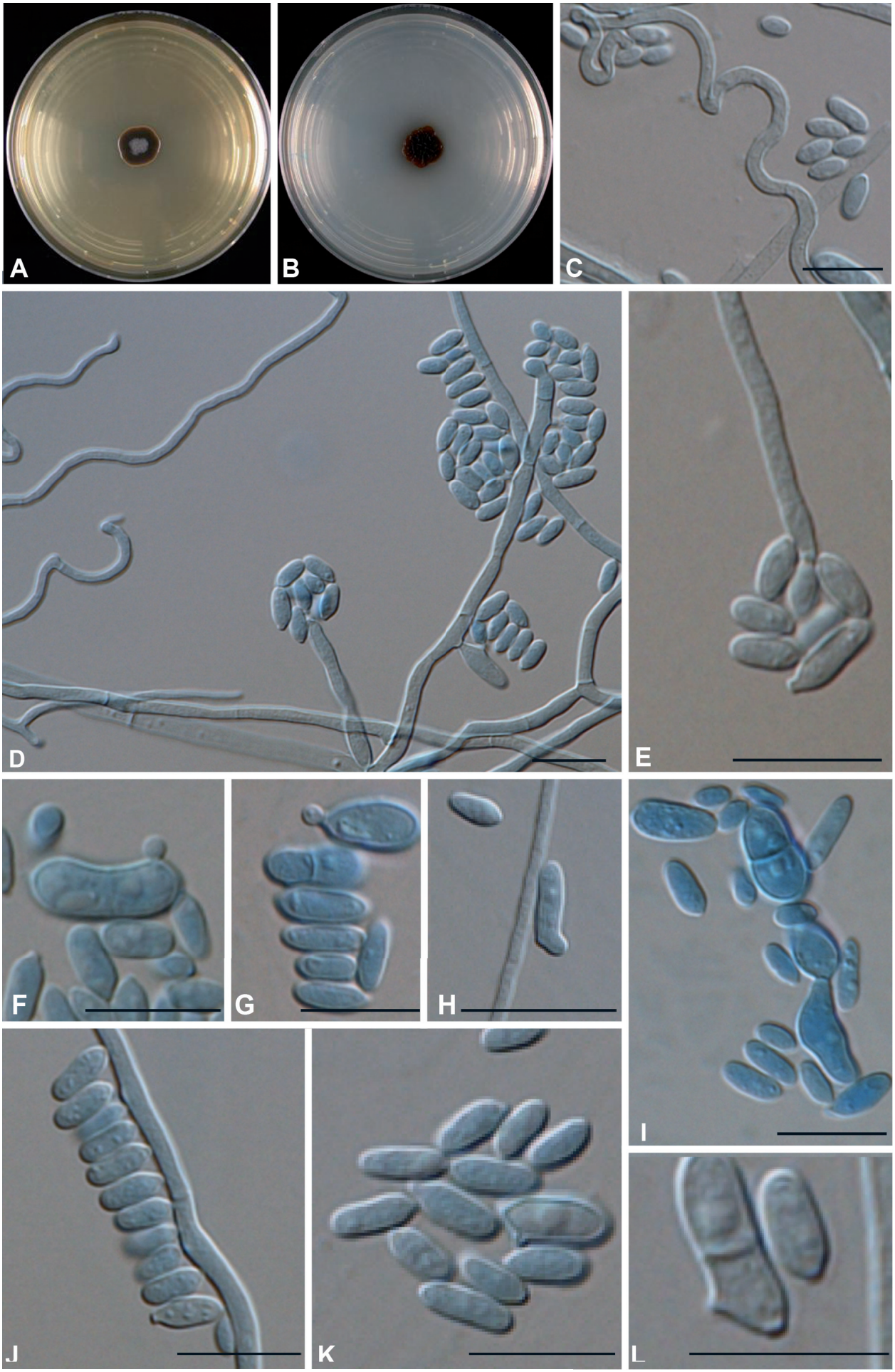


Fig. 11. *Exophiala opportunistica,* CBS 109811. A. Colony on MEA; B. Colony on PDA; C. Spirally twisted hyphae; D, E. Erect cilindrical multi-celled conidiophores; F−L. Yeast cells and conidia; I. Torulose hyphae.

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 cancerae de Hoog, Vicente, Najafzadeh, Badali, Seyedmousavi & Boeger, **sp. nov.** MycoBank MB515720. Figs 13, 14.

Fig. 14. *Exophiala cancerae,* CBS 120420. Conidial apparatus, conidia and torulose hyphae.

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.

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 ºC, optimum 24−27 °C, maximum 30−33 °C. No growth at 37 °C.

Holotype: **Brazil,** Pernambuco State, Goiana City, from diseased Mangrove crab (*Ucides cordatus*), W. Boeger, CBS-H 20382, ex-type strain CBS 120420 = dH 17409 = Boeger HF16/8.

Additional material examined: Table 1.

Notes: The 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 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 affected tissues are the epidermis, connective tissues, heart, and hepatopancreas. The fungus disseminates hematogenously. Despite the large scale of outbreak, locally with 50 % diseased crabs, we were unable to isolate *E. cancerae* from the environment (Boeger *et al.* 2010). The world wide 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 Lethargic crab disease (Boeger *et al.* 2010). 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).

Strain CBS 119920 was derived from the liver of a green toad (*Pseudepidalea viridis*) in Israel which was euthanased 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 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 U.S.A. (AY213652; Rakeman *et al*. 2005), a strain from a bathroom in Japan (AB 456581; Hamada & Abe 2009) and an isolated from healthy steam of corn in Australia (AM176667, Molnar & Prillinger, unpublished).

Exophiala lakei de Hoog, Vicente, Najafzadeh, Harrak, Seyedmousavi & Harrak, **sp. nov.** MycoBank MB515721. Figs 15, 16.

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.

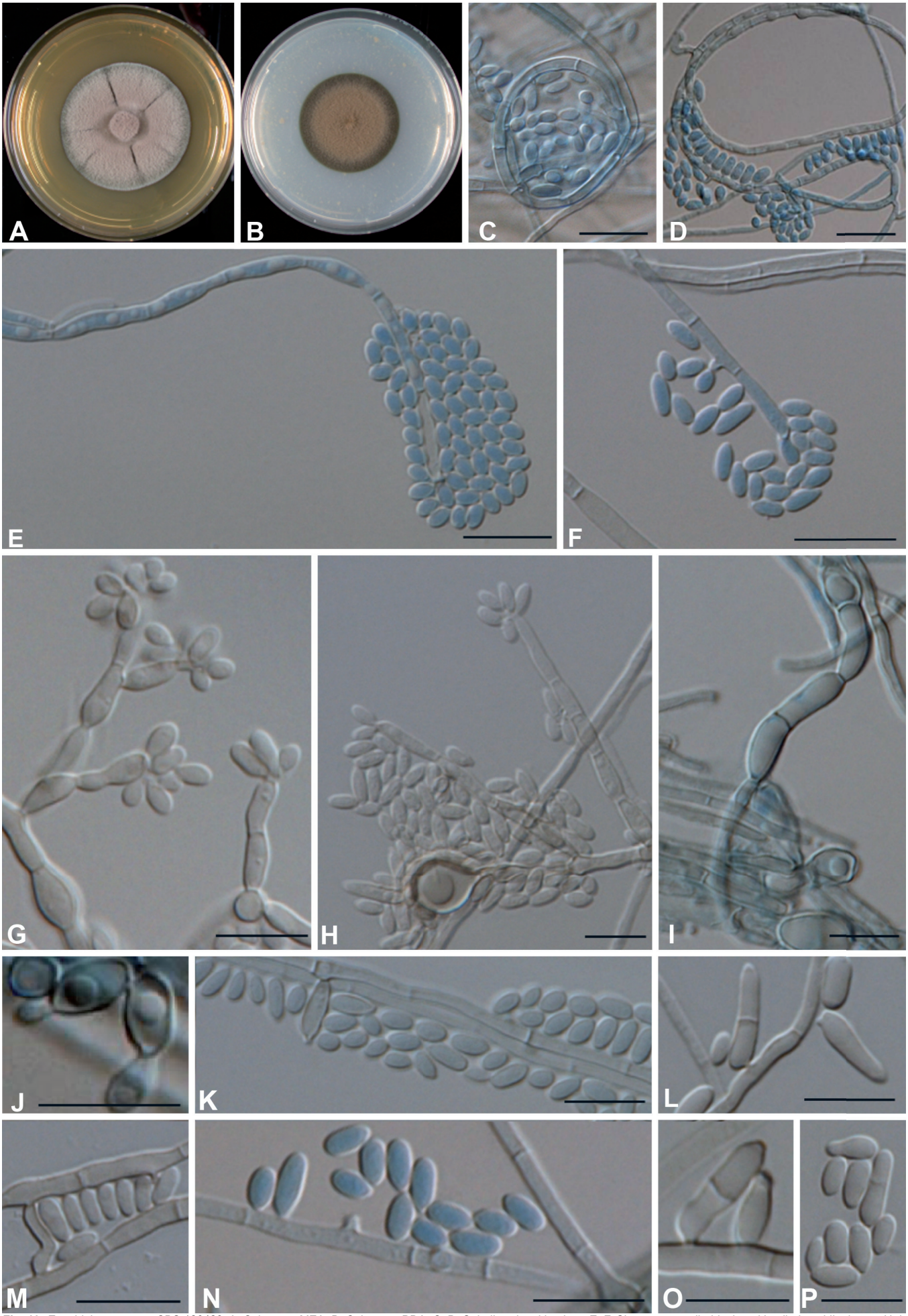


Fig. 13. *Exophiala cancerae,* CBS 120420. A. Colony on MEA; B. Colony on PDA; C, D. Spirally twisted hyphae; E, F. Short, erect, cylindrical, multi-celled conidiophores; H, I. Apical and intercalary chlamydospores; J. Budding cells; K, L. Intercalary conidigeneous cells; M. Hyphae and conidia with anastomoses, N–P. Conidia.

Fig. 16. *Exophiala lakei,* CBS 117497. Hyphae with intercalary conidiogenous cells; anastomoses leading network-like hyphae.

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 ºC, optimum 21−24 °C, maximum 27−30 °C. No growth at 37 °C. Teleomorph unknown

.

Holotype: **The Netherlands,** Loosdrecht, M.J. Harrak, from freshwater lake (1 m depth), 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 brunnea Papendorf, Trans. Br. 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 one or two 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 ºC, optimum 21−24 °C, maximum 30−33 °C. No growth at 37 °C.

***Type*: South Africa**, Potschefstroom, from leaf litter of *Acacia karroo*, ex-type culture CBS 587.66, herbarium CBS H-12618, CBS H-19966.

Notes: The species is known from a single strain isolated from top soil (leaf litter) of an *Acacia karroo* community (Leguminosae-Mimosoideae). The species was synonymized with *Exophiala salmonis* on morphological grounds (de Hoog 1977), but proved to be separate using molecular data.

Veronaea botryosa Ciferri & Montemartini, Atti Ist. Bot. Univ. 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 ºC, optimum 24−30 °C, maximum 33−36 °C. No growth at 37 °C.

***Type*: Italy,** from sansa olive slag, 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*, is found amidst the waterborne species in the *salmonis*-clade. The ex-type strain was isolated from sansa olive slag 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 12-year-old child in China (CBS 102593). The patient did not have any known immune disorder. A deep skin lesion in 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 e*t al.* 1989), Libiya (Ayadi *et al*. 1995), the U.S.A. (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 by this fungus.

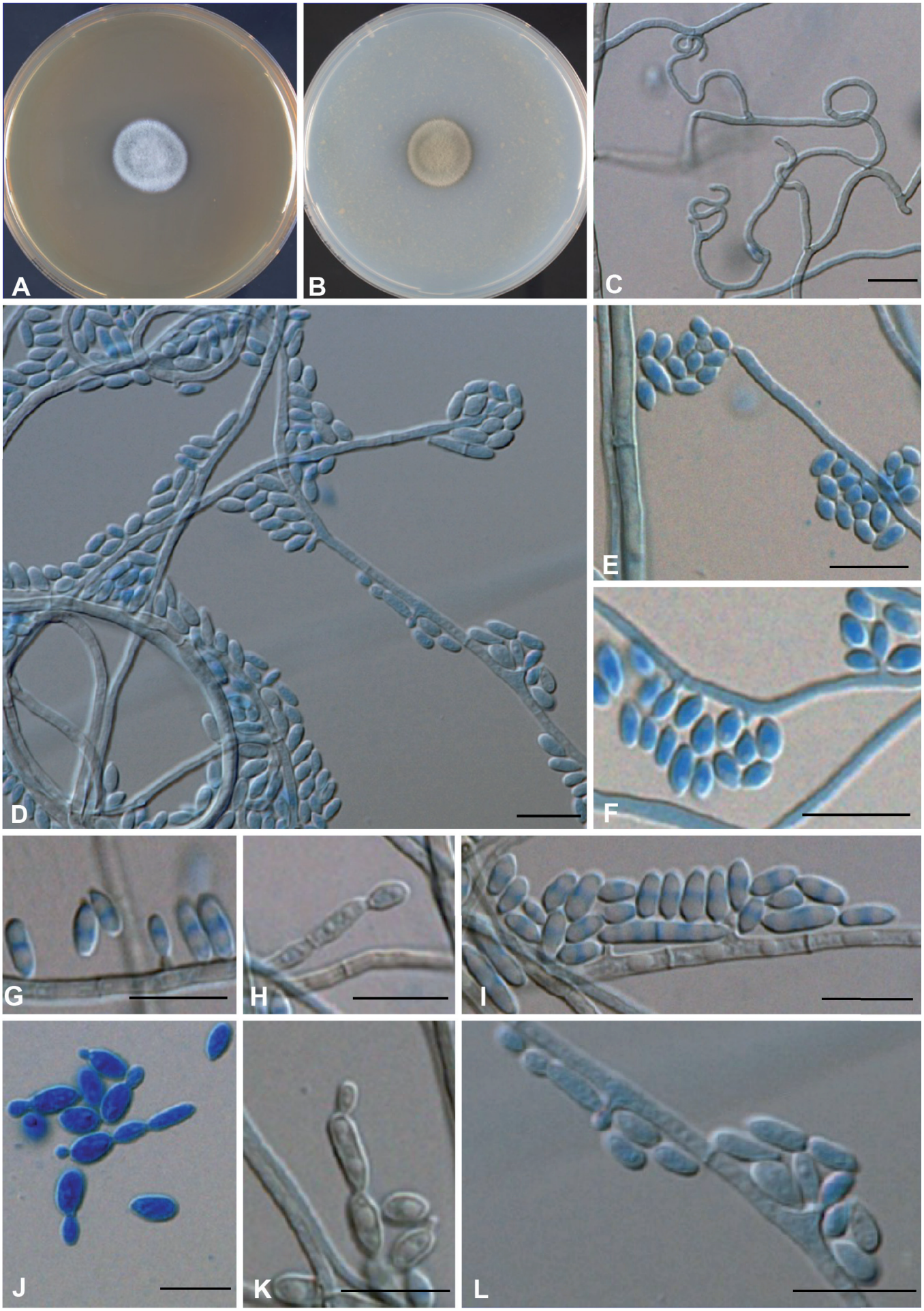


Fig. 15. *Exophiala lakei,* CBS 117497. A. Colony on MEA; B. Colony on PDA; C. Spirally twisted hyphae; D, E. Short, erect, cylindrical, multi-celled conidiophores; F, G. Hyphae with conidial heads; H. Chlamydospores; I, J. Budding cells; K. Conidia alongside hyphae; L. Conidia.

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

GENERAL DISCUSSION

Pathogenicity in *Chaetothyriales*

Although the black yeast teleomorph genus *Capronia* already exists since 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) also appeared to be an *Exophiala* species. *Exophiala* species are difficult to recover from the environment as they grow very slowly and 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 (Vicente *et al.* 2008, Sudhadham *et al*. 2008, Prenafeta-Boldú *et al.* 2003, Zhao *et al.* 2010). When isolated, they are very difficult to diagnose by morphology. This has hampered the growth 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 supposed to belong to the *Herpotrichiellaceae* (teleomorph genus *Capronia*; Fig. 1) and are pathogen-rich (Ruibal *et al*. 2009). Pathogenicity is particularly observed in fish, amphibians and humans, while also infections in marine invertebrates are known; in the mammals, particularly humans are susceptible. Infections in reptiles and birds are missing. 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 and lacking sweat glands. Also infections in furred mammals are rare, the majority of mammal infections being observed in humans with thin, naked skin and cooling their body with the use of sweat glands.

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 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, in main traits following the phylogeny of the host, whereas in *Chaetothyriales* all types of opportunism are scattered over the order. Chaetothyrialean opportunism is, however, consistent within the species, and thus is not a random process.

Waterborne animals are infected much more frequently than terrestrial animals (Table 3). The choice of host can be correlated to maximum growth temperatures of species in each of the clades. Roughly, 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, those with a maximum around 36−37 °C (*carrionii* and *europaea* clades) cause (sub)cutaneous and superficial infections, whereas 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. At invasive infection, no or primitive granulomes are formed, with hyphae growing densely and regularly (Fig. 17A, B; Boeger *et al*. 2005). Primitive fish such as sea horses, with poorly developed innate as well as adaptive immunity, show similar histopathology (Fig. 17C, D; Nyaoke *et al*. 2010). True granulomes, 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 recognized for thermophilic and mesophilic *Exophiala* species that can be isolated from moist indoor environments. The best known example is *E. dermatitidis* from Turkish steam baths causing neurotropic disseminated infections in humans (Hiruma *et al*. 1993, Chang *et al*. 2000). But also mesophilic species such as *E. oligosperma* are regularly encountered in human infections (de Hoog *et al*. 2009); among infections caused by this species was a pseudoepidemic due to clinical use of contaminated hospital water (Nucci *et al.* 2001, 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 still insufficient data are available on physiological profiles of black yeasts of the large diversity of alkylbenzenes. Nevertheless the relative frequent infections of amphibians (e.g. Beneke 1977, Cicmanec *et al.* 1973, Bube *et al.* 1992, Elkan & Philpot 1973), producing aromatic toxins in their skin such as epibatidine and bufotenin, is remarkable. A central role of alkylbenzene assimilation in the 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 choice of host, while cellular immunity determines the type of tissue response.

The majority of preponderantly waterborne species are clustered in a single clade within the *Chaetothyriales*, called the *salmonis*-clade, with some scattered species elsewhere along the phylogenetic tree (Fig. 1). Most of this waterborne *Exophiala* species are meso- or even psychrophilic (Table 3). In main traits a correlation is observed between the maximum growth temperature and the natural habitat of the host. Fungi with maximum growth temperatures below 33 ºC are found causing diseases in animals in cold water, such as the deep or Arctic oceans. Examples are

Table 2. Pathogenic potentials in clades of *Chaetothyriales*: maximum growth temperature, synthesis of melanin and different levels of immune system.

Clades	Maximum growth temperature (°C)	Melanin	invertebrates		vertebrates							
			Crustacean		Fish		Amphibian		Reptile		Bird		Mammal	
			Level of Immunity											
			Innate	Adaptive	Innate	Adaptive	Innate	Adaptive	Innate	Adaptive	Innate	Adaptive	Innate	Adaptive
			+	-	++	+	++	+	+++	+++	++++	++++	+++++	+++++
			Maximum disease lavel											
***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	

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 without any thermotolerance are just saprobes without infective abilities. Human infections are rare by these fungi. Some species with maximum growth temperatures around 33 ºC, 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 mangroves along the east coast of Brazil (Boeger *et al.* 2005, 2010). Species of this group may be seen on human skin, causing mild superficial lesions or onychomycoses (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 the fungi concerned. The different degrees and types of virulence to particular hosts is striking. Main susceptible groups are amphibians, fishes and humans, while other groups are significantly underrepresented; reptiles and birds are missing (Table 4). This may largely be due to the preponderant life styles of these hosts. While amphibians and fish are waterborne and have thin, mocous skins, reptiles and bird are terrestrial and are protected by a thick, dry, water-repulsive skin or an envelope of feathers. Mammals have a hairy pelt which is difficult to penetrate, whereas the soft, naked skin is more vulnerable to black yeast infection, particularly after maceration. Reports of *Exophiala* species infections in domestic and field animals are very rare (Helms *et al.* 2000, Kano *et al.* 2000). Of note, black yeast infections in invertebrates all are noted in moist animals, such as mussels (van Dover et al. 2007), earthworms (Vakali 1993) or and mangrove crabs (Boeger 2005), while terrestrial insects are never affected.

Nevertheless immune responses may also be involved, 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 *et al.* 2003). The role 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 the presence of lectins on their surface. 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 recognizable as lymphocytes which 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 immunoglobulin: 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 *et al.* 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 of 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 *et al.* 2006) and primitive metazoans (Du Pasquier 2001). This might be the result of an 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, which is defined ecologically as fitness being increased when an animal host is used in any part of the fungal life cycle (de Hoog *et al.* 2009). If there is no further evolution in the nature of the infective ability of *Chaetothyriales*, one may wonder why opportunism is such a consistent feature throughout the order.

To our knowledge there is no infection 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 found in municipal water distribution networks. Therefore we consider the health risk of municipal water to be very low. 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 also unlikely. The main health risk of these fungi probably is cutaneous inoculation after moistening of the skin, as suggested by Lian & de Hoog (2010).

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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	Main ecology	Invertebrates	Vertebrates				
				Fish	Amphibian	Reptile	Bird	Mammal
E. angulospora	30–33 °C	Cold drinking water, sea water aquaria and fish nurseries	-	sea dragon, marine lumpfish	-	-	-	-
E. halophila	30–33 °C	Salty water	-	-	-	-	-	human
E. alcalophila	36–40 °C	Soil on a minimal medium ,bath waste water	-	-	-	-	-	human
E. pisciphila	30–33 °C	Freshwater ,seawater	-	channel catfish, captive marine plaice, dog fish	frog	-	-	human
E. aquamarina	33–36 °C	Sea Aquarium	-	sea dragon, winter flounder, tunny fish	-	-	-	-
E. equina	33–36 °C	Drinking and Waste water	-	-	-	Galapagos turtle	-	horse, human
E. superficiale	30–33 °C	Skin of patients With diabetes	-	-	-	-	-	human
E. salmonis	30–33 °C	Cold water with temperature range of 12–14 °C	-	trout cod, atlantic salmon	frog	-	-	human
E. opportunistica	27–30 °C	Drinking water	-	fish	-	-	-	-
E. psychrophila	24–27 °C	Cold water with temperature range of 12–14 °C	-	atlantic salmon	-	-	-	-
E. cancerae	30–33 °C	Brazilian coast	mangrove crabs	-	green toad	-	-	human
V. botryosa	33–36 °C	Sansa olive slag, wood treated	-	-	-	-	-	human

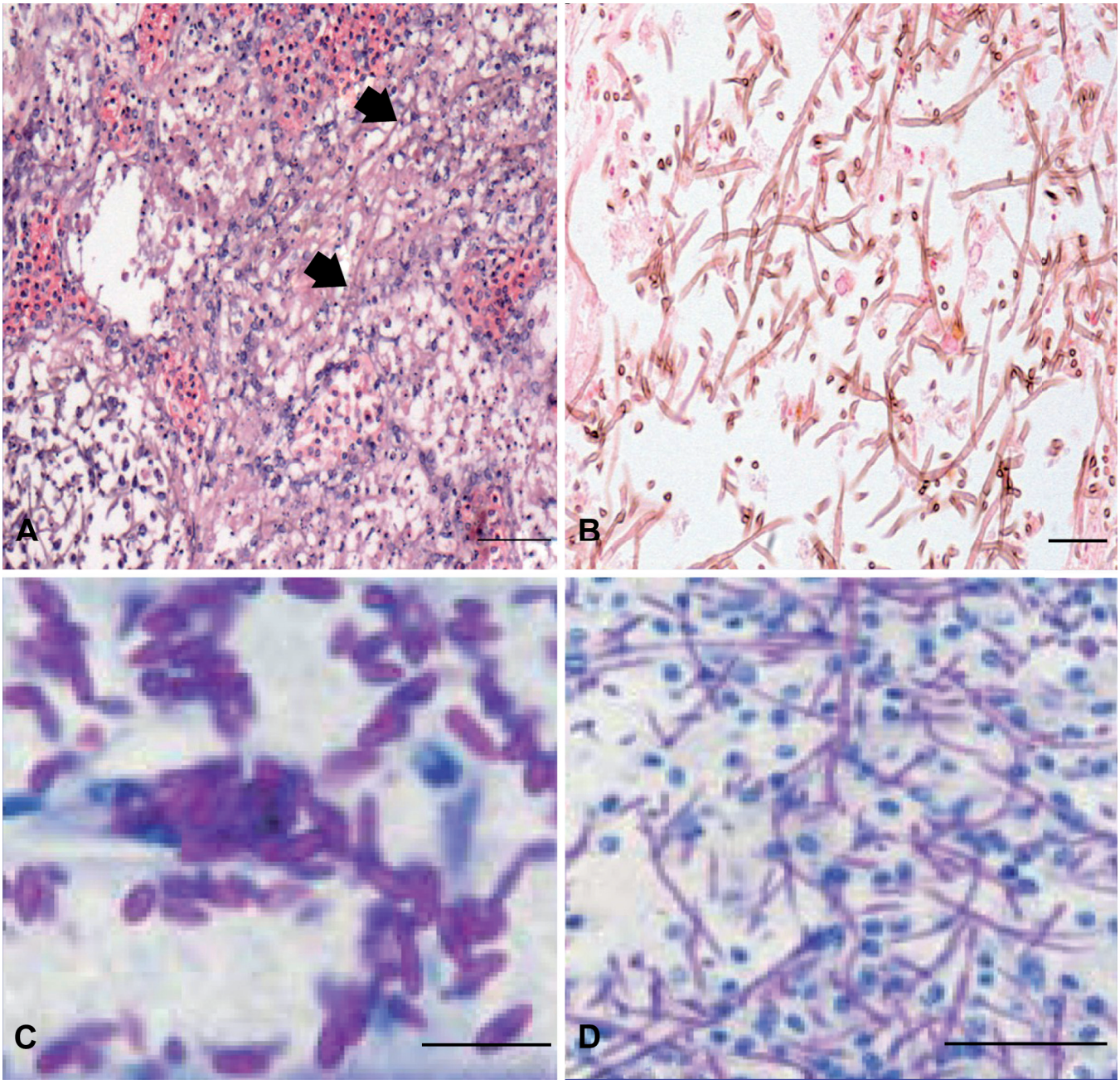

Fig. 17. Examples of histopathology of *Exophiala* species. A, B. Disseminated phaeohyphomycosis by *Exophiala aquamarina* in weedy seadragons (*Phyllopteryx taeniolatus*). A. Abundant brown fungal hyphae (arrows) coursing through necrotic tubules, interstitium, and sinusoids in renal parenchyma. Stained with H&E. Bar = 50 µm. 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. Bar = 25 µm. 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 *Exophiala cancerae* in lacunae. Stained with PAS. Bar = XX. Reproduced from Boeger *et al*. (2005). D. Light micrograph of cardiac tissue of lethargic mangrove crab (*Ucides cordatus*) parasitized by hyphae of *Exophiala cancerae*. Stained with PAS and counter-stained with H&E. Bar = XX. Reproduced from Boeger *et al*.

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