UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

The neurotropic black yeast Exophiala dermatitidis has a possible origin in the tropical rain forest

Sudhadham, M.; Prakitsin, S.; Sivichai, S.; Chaiyarat, R.; Dorrestein, G.M.; Menken, S.B.J.; de Hoog, G.S.

DOI
10.3114/sim.2008.61.15

Publication date
2008

Document Version
Final published version

Published in
Studies in Mycology

Link to publication

Citation for published version (APA):
Sudhadham, M., Prakitsin, S., Sivichai, S., Chaiyarat, R., Dorrestein, G. M., Menken, S. B. J., & de Hoog, G. S. (2008). The neurotropic black yeast Exophiala dermatitidis has a possible origin in the tropical rain forest. *Studies in Mycology*, *61*, 145-155. https://doi.org/10.3114/sim.2008.61.15

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:09 Mar 2023

available online at www.studiesinmycology.org
doi:10.3114/sim.2008.61.15

Studies in Mycology 61: 145–155. 2008.

The neurotropic black yeast *Exophiala dermatitidis* has a possible origin in the tropical rain forest

M. Sudhadham[1,2], P. Sihanonth[3], S. Sivichai[4], R. Chaiyarat[5], G. M. Dorrestein[6], S.B.J. Menken[2], and G.S. de Hoog[1,2]*

[1]*Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands,* [2]*Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands,* [3]*Department of Microbiology, Chulalongkorn University, Bangkok, Thailand,* [4]*Biotec-Mycology Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand.,* [5]*Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand ,* [6]*Dutch Research Institute for Avian and Exotic Animals (NOIVBD), Veldhoven, The Netherlands*

*Correspondence: G.S. de Hoog, de.hoog@cbs.knaw.nl

Abstract: The black yeast *Exophiala dermatitidis* is known as a rare etiologic agent of neurotropic infections in humans, occurring particularly in East and Southeast Asia. In search of its natural habitat, a large sampling was undertaken in temperate as well as in tropical climates. Sampling sites were selected on the basis of the origins of previously isolated strains, and on the basis of physiological properties of the species, which also determined a selective isolation protocol. The species was absent from outdoor environments in the temperate climate, but present at low abundance in comparable habitats in the tropics. Positive outdoor sites particularly included faeces of frugivorous birds and bats, in urban as well as in natural areas. Tropical fruits were found *E. dermatitidis* positive at low incidence. Of the human-made environments sampled, railway ties contaminated by human faeces and oily debris in the tropics were massively positive, while the known abundance of the fungus in steam baths was confirmed. On the basis of the species' oligotrophy, thermotolerance, acidotolerance, moderate osmotolerance, melanization and capsular yeast cells a natural life cycle in association with frugivorous animals in foci in the tropical rain forest, involving passage of living cells through the intestinal tract was hypothesized. The human-dominated environment may have become contaminated by ingestion of wild berries carrying fungal propagules

Key words: Black yeasts, *Exophiala dermatitidis*, frugivorous animals, human faeces, intestinal colonization, neurotropism.

INTRODUCTION

The black yeast *Exophiala dermatitidis* is an uncommon etiologic agent of fatal infections of the central nervous system in otherwise healthy, mainly adolescent patients in East Asia (Hiruma *et al*. 1993, Matsumoto *et al*. 1993, Chang *et al*. 2000). The species shows neurotropism in animal experiments (Dixon *et al*. 1989, 1992). In the U.S.A., cases have been reported where inoculation of patients with medical fluids containing contaminated water led to nosocomial common-source outbreaks with sometimes fatal neurological implications (Woollons *et al*. 1996, Engemann *et al*. 2002). More frequently than brain infection, asymptomatic colonization is observed in protected body sites, e.g. in the mucus of lungs in 2-8 % of patients with cystic fibrosis (CF) (Haase *et al.* 1991, Horré *et al.* 2003), in the intestinal tract in 0.3 % of the European population (de Hoog *et al.* 2005) and occasionally in the wax of human external ear canals (Kerkmann *et al.* 1999, G. Haase unpublished data). The fungus is a constitutive producer of melanin (Langfelder *et al.* 2003), is consistently able to grow at temperatures above 37 °C (Padhye *et al*. 1978) and produces extracellular polysaccharide capsules (Yurlova *et al.* 2002), which all are regarded to be virulence factors.

The route of infection is still a mystery. The species is known to occur in the environment, but is not among the commonly encountered saprobes. It is practically absent from dead plant material or soil, and has never been reported from outdoor air (Matos *et al*. 2002). The somewhat odd spectrum of main sources of isolation of strains presently available in culture collections (fruit surfaces, steam baths, faeces, and human tissue) suggests that a hitherto unknown, quite specific natural niche must be concerned.

Particularly the occurrence in steam rooms of public bathing facilities is consistent and with high colony counts (Nishimura *et al.* 1987, Matos *et al.* 2002). The artificial environment of the steam bath apparently provides a novel environmental opportunity for this fungus. The transition from the hitherto unknown natural niche to the human-dominated environment may be accompanied by selection and/or adaptation to the new habitat, facilitated by the stress protection provided by melanin. Given the nature of the fungus as an opportunistic agent of potentially fatal infections in humans, this process may have clinically relevant consequences. The present article documents a possible natural habitat of the fungus and on processes taking place during transition from nature to the domestic environment.

MATERIAL AND METHODS

Samples

Varying numbers of replicates each containing 0.1−0.5 g of fresh and dry faeces of wild or semi-wild birds, chickens, bats, flying foxes, jackdaws, rats and elephants were collected from different localities in the Netherlands and/or in Thailand. (Fig. 1) Forty-four bathing facilities, toilets, hot springs and railway ties (Tables 4−9) were sampled, either by using sterile cotton swabs, or by collecting 0.5−1.0 g (wet weight) bottom soil or aliquots of 10 L were filtered over a 0.8 µm pore size, sterile membrane cellulose acetate filter (Göttingen, Germany). In addition, 731 intestinal samples (~1 g)

Copyright 2008 CBS Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands.

You are free to share - to copy, distribute and transmit the work, under the following conditions:
Attribution: You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).
Non-commercial: You may not use this work for commercial purposes.
No derivative works: You may not alter, transform, or build upon this work.
For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

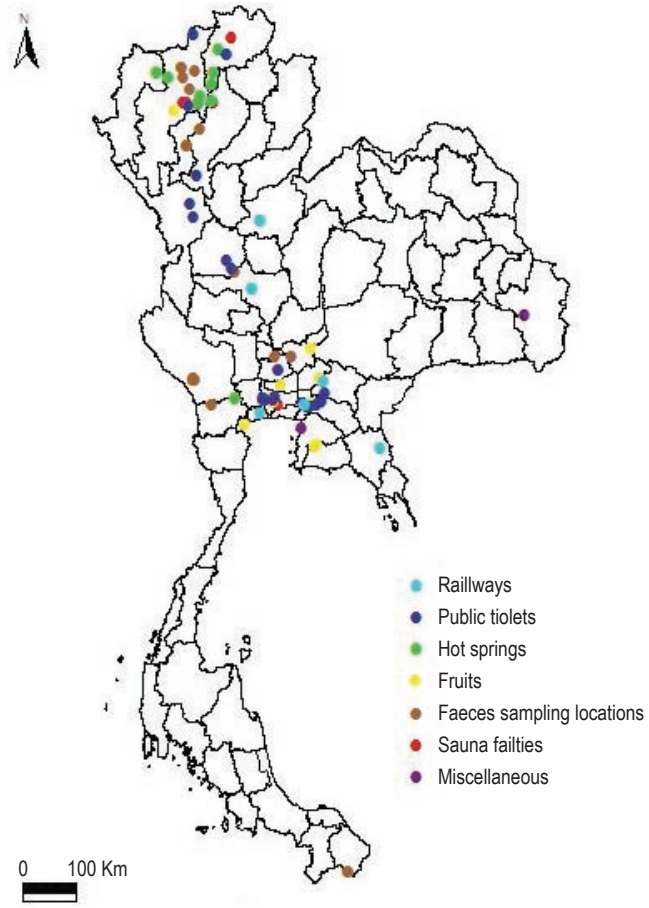

Fig 1. Map of Thailand showing the sampling sites.

from small zoo animals from the Netherlands and autopsied at the Veterinary Faculty of the University of Utrecht, The Netherlands were included. Also large numbers of wild fruits and berries from the Netherlands and Thailand were analyzed (Table 3). Solid specimens were incubated in 5.5 mL Raulin's solution (Booth 1971) in test tubes for 2-3 d at 25 ° C in nearly horizontal position and shaken at 10 r.p.m. Subsequently 0.5 mL was transferred using a glass Drigalski spatula to Erythritol Chloramphenicol Agar (ECA) (de Hoog & Haase 1993) and Sabouraud's Glucose Agar (SGA) (de Hoog *et al.* 2000) – both media containing the same concentration of chloramphenicol – and incubated for up to 40 d at 40 °C. Small, blackish-brown colonies were transferred to new growth media on PDA using a loop, or were separated from contaminating yeasts and aspergilli by repeatedly washing agar blocks with black yeast cells in 0.1 % Tween 80 in sterile water followed by dispersing the visually clean blocks over fresh agar plates.

Validation of isolation procedure

Three strains were used to test the efficiency of recovery by the above method: CBS 207.35 (genotype A, capsular), CBS 116014 (genotype B, capsular) and CBS 109143 (genotype B, non-capsular). Strains were pre-cultured in Potato Dextrose Broth (PDB) for 3 d at 30 °C. Suspensions were adjusted to 10^2 cells/mL and recovery verified by plating on ECA and SGA at 25 °C and 40 °C. *Aureobasidium pullulans,* CBS 584.75 was used as positive control at 25 °C. Suspensions in Raulin's solution with a final concentration of 10^6 cells/mL were incubated for 3 d under conditions specified above and the recovery rate was counted on ECA, SGA and Potato Dextrose Agar (PDA) at 25 °C and 40 °C.

Identification of accompanying biota

White yeasts were identified physiologically by testing fermentation and carbon and nitrogen assimilation. C-assimilative capabilities were tested using API-ID 32 C strips (bioMérieux, Marcy-l'Étoile, France). Single colonies were grown at 25 °C for 3 d as a maximum. Suspensions were made and the densities were scored by McFarland turbidity standard point 2. Strips were inoculated according to specifications provided by the manufacturer and incubated at 25 °C. For nitrogen assimilation the substrates nitrate, ethylamine, L-lysine, cadaverine, D-glucosamine, HCl and tryptophane were used. Peptone was used as positive control. Suspensions were applied in N-auxanograms in culture plates. After the medium was cooled and solidified, a small amount of each N-source was put on the medium. For fermentation, D-glucose, D-galactose, maltose, sucrose, lactose and raffinose were tested. Sugars solutions were 2 %, except for raffinose which 4 % solution was used. These solutions were sterilized in tubes with Durham inserts and suspensions of McFarland turbidity standard point 2 were added. The identification software BIOLOMICS was used to score combined physiological results. Morphology was investigated to confirm the identifications (Kurtzman & Fell 1998).

Diagnostics

Strains were recognised as black yeasts were provisionally identified at the species level by colony appearance, morphology, and temperature tolerance. Species-identification and ITS-genotype attribution (Matos *et al.* 2002) was done on the basis of ITS rDNA, either by restriction length polymorphism (Sudhadham *et al*. 2009a) or by sequencing ITS1 and 2 (Sudhadham *et al*. 2009b) using standard methodology (de Hoog & Gerrits van den Ende 1998). The ITS-genotypes were recognized on the polymorphic sites listed in Table 2. A small number of isolates was genotype-attributed by similarity of patterns of amplified fragment length polymorphism (AFLP) to strains of which to ITS-genotype was known.

RESULTS

Recovery efficiency

The recovery rate of *Exophiala dermatitidis* suspensions (10^6 cells/mL) was not significantly altered by three days of incubation in Raulin's solution when strain CBS 116014 (genotype B) was used. In contrast, cell counts increased with a factor 4.3 in CBS 207.35 (genotype A), while those of the non-capsular strain CBS 109143 (genotype B) was reduced to about 9.5 % (Table 1). *Aureobasidium pullulans* showed an 83.3 % reduction in colony-forming units (CFU) at 25 °C and did not grow at 40 °C. Average recovery rate in 0.1−0.5 g (wet weight) of faeces was between 0−3 colonies per culture plate, which corresponded to 0−3 CFU per gram original material for genotype B and 0−0.69 for genotype A, judging from isolation efficiency described above.

The isolation protocol applied to animal faeces and to fruits and berries collected in temperate and tropical climates proved to be highly selective, judging from the very few ubiquitous saprobes that were recovered. The rarity of *E. dermatitidis* was proven by selective isolation from a large diversity of environments in temperate (The Netherlands) and tropical (Thailand) climates, supplementing data

of Matos *et al*. (2002) which involved leaves, fruits and berries, animal faeces and soil in a temperate climate.

Genotyping

Nuclear rDNA ITS sequencing of 86 reference strains confirmed the existence of two major genotypes differing in three positions in ITS1 (Table 2). Genotype A was more common than B (A:B = 57:29). Two out of nine animal faeces samples from Thailand belonged to genotype A, while seven were B. Two sets of samples from bird guano in Khao Khaew Zoo (where occasional mechanic cleaning is carried out) and flying fox faeces at a temple complex in Thailand contained genotypes A and B, of which the latter was isolated more frequently.

Isolation in temperate climate

Fruits and berries

Two areas in The Netherlands were chosen for isolation from berries, namely Boswachterij Noordwijk, in a dune area near the Northsea coast near Leiden, and a lane planted with shrubs in a rural area in Maartensdijk in the central part of the country. In Noordwijk, samples were taken in Autumn, when berries were predomantly eaten by migratory frugivorous birds such as *Turdus pilaris* (fieldfare). Twenty-one samples were taken from berries of *Rosa pimpinellifolia*, 345 samples from *Hippophae rhamnoides* and 187 samples from *Ligustrum vulgare* (Table 3). The shrubs near Maartensdijk were predominantly frequented by sedentary birds such as *Corvus monedula* (jackdaw) and *Sturnus vulgaris* (European starling). Thirty samples were taken from *Crataegus monogyna*, 92 samples from *Viburnum opulus*, 61 samples from *Ilex aquifolium*, 22 samples from *Rosa canina*, 20 samples *Rosa rubiginosa*, 36 samples *Prunus spinosa*, 19 samples *Ligustrum vulgare* and 51 samples from *Taxus baccata***.** With our isolation protocol plates mostly remained blank, or white yeasts were encountered. No black yeast was isolated.

Faeces of omnivorous birds

Faeces mixed with soil under *Thuja* conifers harbouring a large combined roosting site of *Corvus monedula* (jackdaw) and *Sturnus vulgaris* (European starling) in a park near Hilversum, The Netherlands, was sampled, as well as a roosting site of jackdaw alone. All samples were negative for *E. dermatitidis* (Table 4).

Intestinal contents from sectioned zoo animals

A total of 731 samplings from dead animals originating from different zoos in the Netherlands, were received at the Veterinary Faculty at Utrecht for autopsy. In addition to routine analysis, the contents of the intestines with visible disorders such as discoloration or halfway digested food was subjected to selective isolation for *E. dermatitidis*. Results are available from 16 reptiles, 406 birds, 183 mammals, 8 fishes, 3 turtles, 2 amphibians, 5 lizards and 11 snakes; these data will not be included in this article, but are available as attachment at www.cbs.knaw.nl. Culture plates mostly remained blank. White yeasts were common in the intestinal tract of frugivorous animals. A single strain of *E. dermatitidis* (genotype A) was obtained from a bonobo monkey (*Pan paniscus*) with diarrhoea in the Apeldoorn Zoo, The Netherlands. Bonobo's are omnivorous with a marked preference of fruit.

Isolation in tropical climate

Fruits and berries

On grapes (*Vitis vinifera*) numerous white yeasts were isolated, which were not identified down to the species level. The only black fungus obtained was a *Cladosporium* species. On green papaya fruits (*Carica papaya*) mainly white yeasts occurred. Few filamentous fungi were obtained, mainly biverticillate *Penicillium* species, among which was *Eupenicillium cinnamopurpureum* (anamorph: *P. phaeniceum*). Three types of fruit were found positive on isolation for *E. dermatitidis*: papaya, pineapple (*Ananas comosus*) and mango (*Mangifera indica*). In papaya and pineapple, genotypes A and B were found, while in mango only genotype B was encountered. Mango fruits further contained white yeasts, a *Rhizopus* species, a recurrent *Aspergillus* species, a biverticillate *Penicillium* and *Talaromyces intermedius* (dH 13728). On lemon fruits (*Citrus* sp.) white yeasts were common, and a single colony of a white filamentous fungus was obtained. On fruits of tamarind (*Tamarindus indica*), Queen's flower (*Lagerstroemia calyculata*) and *Ficus lacor*, only *Aspergillus* species were obtained. In contrast, fruits of *Ficus annulata* contained much more white yeasts in addition to *Aspergillus* species; three times a hitherto undescribed species of *Munkovalsaria* species was isolated. On fruits of yellow santol (*Sandoronicum indicum*) the main filamentous fungi acquired were *Aspergillus* species, and infrequently white yeasts were encountered. Fruits of rambutan (*Nephelium lappaceum*), pomelo (*Citrus maxima***)** and mangrove palm (*Nypa fruticans*) contained many white yeasts only. Rose apple fruits (*Syzygium jambos*) contained biverticillate *Penicillium* species (Table 3).

Faeces of frugivorous birds

Samples were taken from faeces of a number of frugivorous birds from two zoos in Thailand. Bird species are described as follows. Columbiformes: *Ducula bicolor* (pied imperial-pigeon); Coraciiformes: *Aceros undulatus* (wreathed hornbill)*, Anorrhinus galeritus* (bushy-crested hornbill)*, Anorrhinus tickelli* (rusty-cheeked hornbill)*, Anthracoceros albirostris* (oriental pied-hornbill)*, Anthracoceros malayanus* (black hornbill)*, Berenicornis comatus* (white-crowned hornbill), *Buceros bicornis* (great hornbill)*, Buceros hydrocorax* (rufous hornbill)*, Buceros rhinoceros* (rhinoceros hornbill), *Rhinoplax vigil* (helmeted hornbill); Passeriformes: *Acridotheres tristis* (common myna)*, Sturnus burmannicus* (vinous-breasted starling)*, Pycnonotus finlaysoni* (stripe-throated bulbul); and Piciformes*: Megalaima virens* (great barbet). White yeasts were predominant on isolation media. Some of these were selected to be identified by ID32C and they invariably turned out to be *Candida tropicalis,* while a single strain was identified as *Trichosporon loubieri. Exophiala dermatitidis* (genotype B) was isolated from fresh faeces of *Acridotheres tristis* (common myna). In a feeding area for birds by fruits of papaya (*Carica papaya*), banana (kluai namwa; *Musa* 'ABB'), two isolates of *Exophiala dermatitids*, genotype B were found (dH 13132, dH 13134) and genotype A (dH 13135) (Fig. 2 D,E). Frugivorous bird faeces were also analyzed in the national park HalaBala Wildlife Sanctuary, established in 1996. Samples were taken in the Hala portion of the Sanctuary, approximately 22 km west from the Malaysian border, comprising a mix of forest broadly classified as tropical lowland evergreen forest, and small-scale agricultural land. The area is particularly famous for harbouring nine species of hornbill birds being one of richest areas for these birds in Southeast Asia (Fig H). *Exophiala dermatitidis* (dH 13148 genotype B) was found from the faeces of *Buceros rhinoceros* (rhinoceros hornbill) (Table 4).

Table 1. Efficiency of live recovery of *E. dermatitidis* after Raulin's incubation, verified on ECA, PDA and SGA at 25 °C and 40 °C.

	Pre-Raulin:	**Post-Raulin:**
CBS 207.35 (genotype A, capsular)	10^6	0.43 x 10^7
CBS 109143 (genotype B, non-capsular)	10^6	0.95 x 10^5
dH 13133 (genotype B, capsular)	10^6	0.83 x 10^6

Table 2. Genotypes within *E. dermatitidis* based on polymorphisms in rDNA Internal Transcribed Spacer 1.

	ITS1 (210)			**ITS2 (220)**
	162	**184**	**196**	**-**
Genotype A:	T	-	A	-
Genotype B:	C	T	C	-
Genotype C: deletion 74-99	T	-	A	-

Table 3. Overview of fruit and berry sampling locations in Thailand and The Netherlands*.

No.	**Plant**		**Province/ country**	**No. of samples**	***Exophiala dermatitidis***	
	Common name	**Scientific name**			**Genotype A**	**Genotype B**
1	Grape	*Vitis vinifera*	Nakornratchasima; Thailand	41	-	-
2	Yellow papaya	*Carica papaya*	Bangkok; Thailand	25	-	-
	Green papaya	*Carica papaya*		17	-	-
	Papaya with antrachnose disease	*Carica papaya*		30	-	-
	Lemon	*Citrus aurantifolia*		40	-	-
3	Bo-tree fruit	*Ficus religiosa*	Chachoengsao; Thailand	54	-	-
	Tamarind	*Tamarindus indica*		5	-	-
	Queen's flower	*Lagerstroemia calyculata*		1	-	-
	Liap	*Ficus lacor*		6	-	-
4	Papaya	*Carica papaya*	Rayong; Thailand	6	-	-
	Rose apple	*Syzygium jambos*		15	-	-
	Yellow sentol	*Sandoricum indicum*		9	-	-
	Breadfruit	*Artocarpus altilis*		12	-	-
	Jackfruit	*Artocarpus heterophyllus*		15	-	-
	Pineapple	*Ananas comosus*		20	3	1
6	Mango	*Mangifera indica*	Bangkok; Thailand	6	-	5
7	Lychee	*Litchi chinensis*		2	-	-
8	-	*Ficus annulata*		59	-	-
9	Mangrove palm	*Nypa fruticans*	Samutsakorn; Thailand	40	-	-
10	Autumn sampling from berries eaten by mainly migratory birds, e.g.	*Rosa pimpinellifolia*	Leiden; The Netherlands	21	-	-
	Turdus pilaris (fieldfare)	*Hippophae rhamnoides*		345	-	-
		Ligustrum vulgare		187	-	-
11	Autumn sampling from berries eaten by mainly resident birds , e.g.	*Crataegus monogyna*	Leiden; The Netherlands	30	-	-
		Viburnum opulus		92	-	-
	Corvus monedula (jackdaw),	*Ilex aquifolium*		61	-	-
	Sturnus vulgaris (European starling)	*Rosa canina*		22	-	-
		Rosa rubiginosa		20	-	-
		Prunus spinosa		36	-	-
		Ligustrum vulgare		19	-	-
		Taxas baccata		51	-	-

**Exact localities available upon request.*

Table 4. Overview of animal faeces sampling in Thailand*.

No.	Animal's faeces	Location	Province/ country	No. of samples	*Exophiala dermatitidis*	
					Genotype A	Genotype B
1	Rats	University	Bangkok; Thailand	4	-	-
2	Elephants	Domesticated elephant	Lum Pang; Thailand	27	-	-
3	Corn-eating animals	Chicken farm	Lum Poon; Thailand	58	-	-
4	Frugivorous birds	Dusit zoo	Bangkok; Thailand	20	-	-
5	Granivorous birds	Dusit zoo	Bangkok; Thailand	15	-	-
6	Frugivorous birds	Khao Khaew open zoo	Chonburi; Thailand	49	1	1
7	Granivorous birds	Khao Khaew open zoo	Chonburi; Thailand	105	-	-
8	Five different species of hornbill	Hala-Bala wildlife sanctuary	Narathiwat; Thailand	96	-	2
9	*Pteropus scapulatus* (flying fox): fresh, dry and dry faeces mixed with soil	Temple	Ayutthaya; Thailand	112	-	-
10	*Pteropus scapulatus* (flying fox): faeces mixed with soil	Temple	Ayutthaya; Thailand	62	-	-
11	*Pteropus scapulatus* (flying fox)	Temple	Saraburi; Thailand	23	-	-
12	*Pteropus scapulatus* (flying fox): fresh, dry and dry faeces mixed with soil	Temple	Chachoengsao; Thailand	88	1	2
13	Insectivorous bat: mix of old and fresh faeces	Temple	Chiang Mai; Thailand	14	-	-
14	Insectivorous bat: fresh and old faeces and soil	Cave near temple	Chiang Mai; Thailand	28	-	-
15	Insectivorous bat: mix of old and fresh faeces	Temple	Chiang Mai; Thailand	44	-	-
16	Insectivorous bat: old faeces mixed with soil	Temple	Chiang Mai; Thailand	5	-	-
17	Insectivorous bat: mix of old and fresh faeces	Temple	Chiang Mai; Thailand	10	-	-
18	Insectivorous bat: mix of old and fresh faeces	Bat-inhabited newly-built house	Lumpoon; Thailand	10	-	-
19	Insectivorous bat: fresh faeces	Temple	Nakornsawan; Thailand	20	-	-
20	Insectivorous bat: mix of old and fresh faeces	Temple	Kanchanaburi; Thailand	36	-	-
21	Insectivorous bat: mix of old and fresh faeces	Temple	Ratchaburi; Thailand	10	-	-
22	Omnivorous birds	Park	Hilversum, The Netherlands	60	-	-
23	Frugivorous bats	Blijdorp zoo artificial cave	Rotterdam, The Netherlands	110	-	-

*Exact localities available upon request.

Faeces of frugivorous bats

On leaves and branches of boh tree (*Ficus religiosa*) contaminated with faeces of Lyle's flying foxes (*Pteropus lylei*) we repeatedly encountered *Exophiala dermatitidis.* Sequencing showed that three of these were genotype A and B. Soil samples at from a temple in Chachoengsao were mixed with faeces of flying foxes provided white yeasts, among which were *Candida tropicalis* and *C. guillermondii.* Two colonies of an *Aspergillus* species were found and two isolates of *Penicillium* which were sequenced and proved to be *P. islandicum* and *P. pupurogenum* (Tables 4, 9) (Fig. 1 A–C).

Faeces of granivorous birds

Faeces samples were collected from two zoos in Thailand and involved the bird species Galliformes: *Pavo cristatus* (Indian peafowl); Psittaciformes: *Cacatua ducorpsii* (Ducorps' cockatoo), *Cacatua goffini* (Goffin's cockatoo), *Cacatua moluccensis* (Moluccan cockatoo), *Cacatua sulphurea* (yellow-crested cockatoo), *Cacatua tenuirostris* (long-billed corella), *Calyptorhynchus magnificus* (red-tailed black cockatoo), *Electus auratus* (eclectus parrot), *Probosciger aterrimus* (palm cockatoo), *Psittacula alexandri* (red-breasted parakeet), *Psittacula eupatria* (Alexandrine parakeet), *Psittacus erithacus* (African grey parrot), and *Psittrichas fulgidus* (Pesquet's parrot). Very few white yeasts were encountered, but filamentous fungi were relatively common. No *Exophiala* was isolated (Table 4).

Faeces of insectivorous bats

Thirteen mostly limestone caves were chosen for sampling of insectivorous bat faeces from different geographical regions in Thailand. Most of them were located in montane rain forest, about 1 000–1 900 meters above sea level. Others were touristic places surrounded by agricultural land, or were part of a temple complex. Numerous filamentous fungi were obtained (Table 4), most of these being rapidly growing *Aspergillus* species, biverticillate penicillia and zygomycetes. No black yeasts were detected (Table 4) (Fig. 2 F, G).

Public toilets

Public toilets of gas stations can be found along many highways in Thailand. They differ only slightly with the company with respect to building structure, hygienic level and intensity of warding. Most of them are pedestal squat toilets, which means that there is no

Table 5. Overview of public toilet sampling locations in Thailand*.

No.	Public toilets	Source	Province / country	No. of samples	*Exophiala dermatitidis*	
					Genotype A	Genotype B
1	Toilet in gas station	Wall , squat toilet, tap zink	Ayutthaya; Thailand	6	-	-
2	Toilet in gas station	Wall , squat toilet, tap zink	Nakhonsawan; Thailand	6	-	-
3	Toilet in gas station	Wall , squat toilet, tap zink	Nakhonsawan; Thailand	6	-	-
4	Toilet in gas station	Wall , squat toilet, tap zink	Tak; Thailand	6	-	-
5	Toilet in gas station	Wall , squat toilet, tap zink	Tak; Thailand	6	-	-
6	Toilet in gas station	Wall , squat toilet, tap zink	Tak; Thailand	6	-	-
7	Toilet in gas station	Wall , squat toilet, tap zink	Lumpoon; Thailand	6	-	-
8	toilet in hotspring	Wall , squat toilet, tap zink	Chiangmei; Thailand	6	-	-
9	toilet in hotspring	Floor in bath room, wall in toilet room	Chiangrai; Thailand	6	-	-
10	Toilet in gas station	Floor , squat toilet, tap zink	Bangkok; Thailand	6	-	-
11	Toilet in gas station	Floor , squat toilet, tap zink	Chachoengsao; Thailand	6	-	-
12	Toilet in gas station	Floor , squat toilet, tap zink	Chachoengsao; Thailand	6	-	-
13	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
14	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
15	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
16	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
17	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
18	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
19	Toilet in gas station	Squat toilet, tap zink	Nakhon Pathom; Thailand	5	-	-
20	Toilet in gas station	Squat toilet, tap zink	Chachoengsao; Thailand	5	-	-
21	Toilet in gas station	Squat toilet, tap zink	Chachoengsao; Thailand	6	-	-
22	Toilet in gas station	Squat toilet, tap zink	Prachinburi; Thailand	6	-	-
23	Toilet in gas station	Squat toilet, tap zink	Prachinburi; Thailand	6	-	-
24	Toilet in gas station	Squat toilet, tap zink	Chachoengsao; Thailand	6	-	-

*Exact localities available upon request.

Table 6. Overview of sauna facility sampling locations in Thailand*.

No.	Sauna facilitites	Source	Province / country	Number of samples	*Exophiala dermatitidis*	
					Genotype A	Genotype B
1	Steam bath	Floor, wall, seat	Bangkok; Thailand	12	-	10
2	Steam bath	Floor, wall, seat	Chiangrai; Thailand	20	8	53
3	Dry sauna	Floor, wall, seat	Bangkok; Thailand	12	-	-
4	Dry sauna	Floor, wall, seat	Bangkok; Thailand	12	-	-
5	Dry sauna	Floor, wall, seat	Bangkok; Thailand	12	-	-
6	Dry sauna	Floor, wall, seat	Bangkok; Thailand	55	-	-
7	Dry sauna	Floor, wall, seat	Bangkok; Thailand	30	-	-
8	Dry sauna	Floor, wall, seat	Lampang; Thailand	12	-	-
9	Dry sauna	Floor, wall, seat	Narathiwat; Thailand	13	-	-
10	Dry sauna	Floor, wall, seat	Chiangmai; Thailand	3	-	-
11	Dry sauna	Floor, wall, seat	Chiangmai; Thailand	12	-	-

*Exact localities available upon request.

flushing system installed to clean the toilet after use. Instead, water is collected from a tap into a bucket placed close to the squat toilet. Due to this situation, most of the time floors in the toilets were wet, and the hygienic level was low. Samples were taken at different points, such as the floor in the toilet room, the wall, as well as swabs taken from the bowl above the water level. The occurrence of fungi which grew after incubation of swabs in Raulin's solution and incubation on ECA at 40 °C was similar in all toilets: we found *Aspergillus* species and some white yeasts, but no *Exophiala* (Table 5) (Fig. 2 J, K).

Sauna facilities

The saunas in Thailand that were chosen for this experiment were dry sauna with continuous heating. The room was located inside the building complex with other steam baths and Gyms for sport entertainment. Inside the sauna room, seats and walls were made of wood. The steam rooms were also included in this experiment; these were mostly located on the same floor as the sauna room. The walls and floors were made of tiles while the seats were made from polyvinyl chloride. Black yeasts appeared abundantly in the

Table 7. Overview of railway sampling locations in Thailand*.

No.	Railw tiess	Source	Province/ country	Number of samples	*Exophiala dermatitidis*	
					Genotype A	Genotype B
1	Railway station	Stone on railway	Samut Sakhon; Thailand	30	-	-
2	Railway station	Stone on railway	Chachoengsao; Thailand	10	-	-
3	Railway station	Wood stained with petroleum oil	Prachinburi; Thailand	11	108	5
4	Railway station	Stone stained with petroleum oil	Srakaew; Thailand	10	31	-
5	Railway station	Stone stained with petroleum oil	Nakornsawan; Thailand	39	176	29
6	Railway station	Stone stained with petroleum oil	Pitsanulok; Thailand	40	59	-

*Exact localities available upon request.

Table 8. Overview of hot spring water sampling locations in Thailand*.

No.	Water sampling	Source	Province / country	Number of samples	*Exophiala dermatitidis*	
					Genotype A	Genotype B
1	Water from hot srping	Natural hot spring	Chiang Mai; Thailand	20 liters	-	1
2	Water from hot srping	Natural hot spring	Chiang Mai; Thailand	20 liters	-	-
3	Water from hot srping	Natural hot spring	Chiang Mai; Thailand	10 liters	-	-
4	Water from hot srping	Natural hot spring	Chiang Mai; Thailand	30 liters	-	-
5	Water from hot srping	Natural hot spring	Chiangrai; Thailand	10 liters	-	-
6	Water from hot srping	Natural hot spring	Chiangrai; Thailand	10 liters	-	-
7	Water from hot srping	Natural hot spring	Chiangrai; Thailand	10 liters	-	-
8	Water from hot srping	Natural hot spring	Chiangrai; Thailand	30 liters	-	-
9	Water from hot srping	Natural hot spring	Mae Hong son; Thailand	10 liters	-	-
10	Water from hot srping	Natural hot spring	Mae Hong son; Thailand	10 liters	-	-
11	Water from hot srping	Natural hot spring	Lampang; Thailand	10 liters	-	-
12	Water factory	Raw material pond	Ubonratchathani; Thailand	25 liters	-	-
13	Water from pond in the zoo	Dusit zoo	Bangkok; Thailand	1 liter	-	-
14	Water from pond in the zoo	Khao Khaew open zoo	Chonburi; Thailand	3 liters	-	-

*Exact localities available upon request.

Table 9. Overview of hot spring soil sampling locations in Thailand*.

No.	Soil	Source	Province / country	Amount	*Exophiala dermatitidis*	
					Genotype A	Genotype B
1	Soil from hot spring	Natural hot spring	Chiang Mai; Thailand	40 g	-	-
2	Soil from hot spring	Natural hot spring	Chiang Mai; Thailand	40 g	-	-
3	Soil from hot spring	Natural hot spring	Chiang Mai; Thailand	40 g	-	-
4	Soil from hot spring	Natural hot spring	Chiang Mai; Thailand	40 g	-	-
5	Soil from hot spring	Natural hot spring	Chiang Rai; Thailand	40 g	-	-
6	Soil from hot spring	Natural hot spring	Chiang Rai; Thailand	40 g	-	-
7	Soil from hot spring	Natural hot spring	Chiang Rai; Thailand	40 g	-	-
8	Soil from hot spring	Natural hot spring	Chiang Rai; Thailand	40 g	-	-
9	Soil from hot spring	Natural hot spring	Mae Hong son; Thailand	40 g	-	-
10	Soil from hot spring	Natural hot spring	Mae Hong son; Thailand	40 g	-	-
11	Soil from hot spring	Natural hot spring	Lampang; Thailand	40 g	-	-
12	Soil from spring in zoo	Dusit zoo	Bangkok; Thailand	40 g	-	-
13	Soil from spring in zoo	Khao Khaew open zoo	Chonburi; Thailand	130 g	1	2

*Exact localities available upon request.

Fig 2. A. Temple complex at Chachaosao province, Thailand, with colony of flying foxes positive for *E. dermatitidis*; B. Roosting flying foxes; C. Faeces of flying foxes; D. Cage in the zoo of Chonburi province, Thailand, with indoor feeding area (E) positive for two genotypes of E. dermatitidis (dH 13132, dH 13133, dH 13134); F. Mountain at Nakornsawan province, Thailand, with numerous caves harboring insectivorous bats and surrounded by agricultural land; G. Collecting bat faeces in cave at Chiengmai province, Thailand, with bat faeces (insert); H: Hala-Bala Wildlife Sanctuary, Narathiwat province, Thailand, with hornbill nesting sites positive for dH 13183; I. Public hot sping in Chiangmai province, Thailand, positive for dH 13145; J. Public toilet in Chiangmai province, Thailand; K. Squat toilet in public toilet; L. Railway ties in Prachinburi province, Thailand, with collection sites (a) outside ties contaminated with petroleum oil, and (b) between ties (arrows).

isolation step. Only *Exophiala dermatitidis* could be found in these samples, no other fungi were encountered. Sequencing results showed that *E. dermatitidis* could be assigned to both genotypes A and B (Table 6).

Railway ties

Six creosote-treated oak railways ties in Thailand were chosen for this experiment. One of these was located at a fresh market, where heavy contamination and regular cleaning lead to nutritional enrichment, and yielded negative results. The remaining ties showed a line of blackish debris, probably a mixture of faeces and machine oil. These samples contained an enormous amount of *Exophiala dermatitidis* presenting both genotypes from all locations. Srakhaew railway ties yielded 31 isolates of *Exophiala dermatitidis* genotype A, Prachinburi railway ties had 108 genotype A and 5 genotype B, Nakornsawan railways had 176 genotype A, 29 genotype B and 2 genotype C, Pitsanulok railway yielded 61 genotypes A and 1 genotype C (Table 7) (Fig. 2 L).

Hot springs

In ten litre-samples of water from eleven hot springs, a few biverticillate *Penicillium* species were found: dH 13743 = *P. mineoluteum,* dH 13790 = *P. pinophilum* and dH 13744 = *P. funiculosum.* No white yeasts were detected, but one sample yielded a colony of *Exophiala dermatitidis*, genotype A. In the soil samples from the same hot springs, after incubation in Raulin's solution, analyzed with a dilution series and plated on ECA, few filamentous fungi were observed, but there was no evidence of black yeasts (Table 8; Fig. 2 I).

DISCUSSION

From our data, subjecting about 3 000 samples over a 3 yr period of collecting with a selective protocol, it has become apparent that *Exophiala dermatitidis* is not a ubiquitous fungus. Being rare in the environment, the baseline of occurrence is low, with small numbers of strains in all environment samples except for steam baths and creosote-treated and petroleum oil-polluted railway ties. Given its probable low competitive ability as an oligotroph (Satow *et al.* 2008), it may even be infrequent in its natural habitat. This makes it methodically difficult to detect this habitat, which should be indicated by small relative increase in frequency. The detection of even a few samples, contrasted with environments that are consistently negative, may therefore be significant. Matos *et al*. (2003) noted that in preferred habitats the fungus is in an active metabolic state, and grows on isolation plates within three day. Outside its natural habitat it is in a survival stationary state and may require wks before visible growth occurs.

During the present research we selected environments which are likely to be positive. Samples taken on the basis of strains already available in the reference collection of CBS, and on the basis of physiology. The species had previously recurrently been isolated from human sputum, particularly from CF patients, from deep infections, particularly from brain, from stool, from bathing facilities, from fruits and berries, and from creosote-treated wood (http://www.cbs.knaw.nl). This remarkably discontinuous spectrum of sources of isolation of *E. dermatitidis* suggests that the organism might be adapted to a particular, hitherto undiscovered habitat, rather than being a saprobe on dead plant material, as is frequently suggested in the literature (Gold *et al*. 1994). Based on its physiology, we hypothesized a niche containing a number of key elements. First, it must be dynamic, with simultaneously or consecutively occurring phases differing in environmental conditions, since the organism itself is polymorphic, exhibiting yeast-like, filamentous and meristematic phases (de Hoog *et al*. 1994). Second, the phases are likely to be nutritionally diverse, as is concluded from the fungus' consistent occurrence as an epiphyte on low-nitrogenous substrates such as fruit surfaces – promoted by its moderate osmotolerance – and bath tiles (Mayr 1999) combined with equally consistent occurrence in faeces (de Hoog *et al.* 2005). Third, the latter environment, combined with a consistent tolerance of *E. dermatitidis* of 40 °C (Padhye *et al*. 1978) and of very low pH values (de Hoog *et al.* 1994) led to the supposition of passage of the digestive tract of warm-blooded animals (G.S. de Hoog unpublished data). Fourth, the organism shows strong adhesion to artificial surfaces (Mayr 1999), probably promoted by production of sticky extracellular polysaccharides (Yurlova *et al*. 2002). An occurrence on wild fruits and berries that are subsequently ingested by frugivorous animals and dispersed via their faeces thus seems to provide a possible connection of the divergent sources of isolation. The hypothesis led to the successful development of a selective protocol used in this study, which involved an acidic enrichment step (Booth 1971) followed by a high temperature step on a nutritionally specific medium (de Hoog & Haase 1993). The protocol enabled the isolation of minute quantities of the fungus. Massive isolation studies further underlined that *E.dermatitidis* is a rare species in most outdoor environments. We believe our recovery data broadly reflect the actual presence of *E. dermatitidis* in the environment, for two reasons. (1) Environments known to harbor the species were indeed found to be positive at rates comparable to those published earlier (Matos *et al.* 2002). (2) Recently Zhao *et al.* (2008) applied a new, *Chaetothyriales*-specific isolation method based on toluene-enrichment at ambient temperature to the same shrubs near Maartensdijk and indeed found numerous mesophilic *Exophiala* species, but never the thermophilic species *E. dermatitidis*.

The recovery rate was tested experimentally and on average found to reflect the number of cells present prior to incubation in acid. However, slight differences were noted among the three strains analyzed. The representative of ITS-genotype A (CBS 207.35) was stimulated with a factor 4.3 by incubation in Raulin's solution, whereas genotype B (CBS 1160124) remained practically unaltered. The non-capsular strain (CBS 109143) was inhibited tenfold, but since such strains are extremely rare in the natural environment (Matos *et al*. 2002, Yurlova *et al*. 2002) we believe that this more vulnerable phenotype has little effect on the recovery rate of the species.

We analyzed a large diversity of substrates using a highly selective protocol, with accent on substrates bearing similarity to origins of reference strains (Table 2). Despite that, isolation was mostly unsuccessful outdoors in the temperate climate of The Netherlands (Table 3). Only a single strain from a berry of *Sorbus aucuparia* had been found (CBS 109142, genotype B) by Matos *et al*. (2002). The negative samples included berries commonly eaten by migratory and sedentary birds such as *Turdus pilaris, Corvus monedula* and *Sturnus vulgaris,* as well as faeces from several of these birds. From these extended environmental studies including 944 samples it may be concluded that *E. dermatitidis* does not occur naturally in temperate climates.

In contrast, the fungus was confirmed to reside consistently and abundantly from known foci in several artificial, indoor environments, such as steam rooms (Matos *et al*. 2002), in Slovenia, Austria, Thailand as well as in The Netherlands. Several of the negative

berry-sampling locations were only a few kilometers away from steam baths that proved to be highly positive. The species was recovered at high frequency (about 1 000 CFU.cm^{-2}) from thirteen bathing facilities. Steam baths (and not the adjacent sauna's) of public bathing facilities represent an artificial environment with conditions thought to be similar to parts of the natural habitat of *E. dermatitidis*, where high (body) temperature and epiphytie adhesion to (fruit) surfaces play a significant role.

In tropical Thailand, most fruits and berries were also negative (Table 3), although the species was encountered a few times on mango and pineapple. Positive samples were more regularly derived at low frequencies from animal faeces (Table 4). The consistent presence of *E. dermatitidis* in bat faeces and bird guano analyzed is demonstrated by the sample from guano-littered soil in the Khao Khaew Zoo in Chonburi, Thailand, and from flying fox faeces at the temple complex in Chachongsao, Thailand, where both genotypes A and B were recovered (Table 4), despite the overall environmental scarcity of *E. dermatitidis* (Fig. 2 A−C). Recovery rates were low, with a maximum of three colonies per culture plate. The isolation method used reflects the real frequency of genotype B in the original samples (Table 2), which means that positive samples contain maximally 3 CFU per gram faeces. Positive samples were obtained from birds as well as mammals such as flying foxes.

Sampling of autopsied zoo animals was almost always negative for black yeasts. The great majority of these animals fed on corn and seeds, and also yielded very few white yeasts or filamentous fungi. The single black yeast-positive animal intestinal sample (www.cbs.knaw.nl) was a bonobo monkey, which is a largely frugivorous animal. A common factor linking this sample with positive samples elsewhere in the study is the diet of the animals: *E. dermatitidis* was almost exclusively found in animals that fed partially or entirely on wild fruits and berries. Herbivores have a large caecum, where digestion of food is enhanced by fermentation aided by a resident bacterial flora and white yeasts. Frugivorous animals, as those that feed on honey and nectar, may have problematic yeast overgrowth due to a high sugar content in the intestinal tract. *Exophiala dermatitidis* has a slight preference for osmotic environments. In clinical practice this was noted with its occurrence in the lungs of patients with cystic fibrosis, a disease characterized by an elevated salt content of tissues (de Hoog & Haase 1993).

De Hoog *et al*. (2005) found that *E. dermatitidis* occurs at a low incidence in the intestinal tract of humans. This matches with the abundant presence of *E. dermatitidis* on railway ties in Thailand, which are heavily contaminated by faeces (Fig. 2 L). Similar samples were taken in The Netherlands (data not shown), and these were positive for black yeasts other than *E. dermatitidis*. This situation is comparable with our isolation data from berries, which were negative in temperate but positive in tropical climates. Apparently the environmental temperature plays a significant role in the life cycle of *E. dermatitidis*. Public toilets in Thailand (Fig. 2 J, K) were, somewhat against expectations, also negative. This may be explained by competition of other, rapidly growing saprobes in this environment, such as white yeasts and *Aspergillus* species .

Exophiala dermatitidis, similar to other *Exophiala* species, is an oligotroph, as shown *in vitro* by Satow *et al*. (2008) on the basis of the ability of growth utilizing inoculum cells only. The property may be useful for growth on fruit surfaces, but is particularly expressed, in combination with thermotolerance, in abundant replication on the smooth surface of tiles and plastics of steam bath walls. The fungus was detected in large numbers in nearly all bathing facilities investigated located in temperate as well as in tropical climates. The species was also occasionally encountered in natural hot springs, which may be somewhat more difficult to colonize for the fungus due to their relative richness in nutrients. A single strain of *E. dermatitidis* was found in one of the hot springs but its presence might be explained by local people using the spring to clean and boil bamboo shoots after harvest from the forest. The sugary shoots may have been contaminated by *E. dermatitidis* and the fungus may have survived for a short period without significant colonization. A second sampling one year later without human activities was negative (Fig. 2 I).

Combining thermotolerance of the species with the knowledge that *E. dermatitidis* tolerates very acidic conditions (de Hoog *et al*. 1994), it is hypothesised that the fungus is able to pass through the intestinal tract of warm-blooded animals. About 80 % of positive hosts had diarrhoea at the moment of isolation of *E. dermatitidis,* a condition also encountered in positive bonobo. This suggests that the fungus may be present at a higher frequency but can only be isolated when the host has diarrhoea. De Hoog *et al.* (2005) reported isolation of the fungus at 3-wk-intervals from the faeces of a single hospitalized patient with diarrhoea, which indicates that maintenance in the intestines is possible. As a route of infection, translocation from the intestines may thus be supposed. For pulmonary cases an inhalative route would be more logical, but the apparent absence of *E. dermatitidis* from air remains in conflict with this explanation. Other similar phenomenon which support this hypothesis was the strain which was successful isolated from the bonobo from the zoo in the Netherlands. At that time , the sample was taken from the bonobo with the presence of diarrhoea (G. Dorrestein, personal information). Unfortunately, further sampling could not be continued due to the fact that the monkey had returned to the mother.

Reis & Mok (1979) and Muotoe-Okafor & Gugnani (1993) repeatedly found the species in internal organs of tropical frugivorous bats (*Phyllostomus discolor, Sturnira lilium* and *Eudolon helvum*) in American as well as African tropical rain forests. Representative isolates were verified to be *E. dermatitidis* genotype B (Table 4). Despite isolating the fungus from bat organs, Mok (1980) failed to isolate it from roosting sites. This may have been due to the use of inadequate isolation procedures. Reis & Mok (1979) also reported the species from two insectivorous bats (*Myotis albescens* and *M. molossus*), but the identity of these strains could not be verified.

Since in the reference set genotype A is about twice as common as genotype B and genotype A shows a higher recovery rate with the used isolation method (Table 2), our technique would expect to yield an A : B ratio of 10.4 : 1 would be expected in the fruit-eating animal faeces from Thailand. However, these samples showed a ratio A : B = 2 : 7, which would mean that the frequency of genotype B in tropical fruit-eating animal faeces samples deviates with a factor 36.4 from the average of all other sources of isolation.

This model study aims to prove the supposition that aspects of human behaviour i.e. the creation of environment that are extreme from a fungal perspective, by being hot, poor in nutrients, or poisonous, lead to the emergence of new, potentially virulent genotypes. The fungus under study causes a potentially fatal brain disease in otherwise healthy humans; this clinical picture is known in eastern Asia only (Horré & de Hoog 1999). Understanding the origin and course of this evolution may eventually lead to the development of measures which canalize speciation processes into a direction which is less harmful to humanity. The human community creates opportunities for adaptation and the emergence of pathogenic host races. Artificial, human-made environments may stimulate evolution and generate pathogenic genotypes which otherwise would not

have evolved. The source of contamination of a potentially harmful microorganism and its routes on infection and transmission will be studied, potentially leading to protocols for hygiene and prevention. This may be particularly significant in bathing facilities connected to hospitals, where susceptible populations of patients with cystic fibrosis or, in Asia, with immunosuppression are warded. Though the disease under study is extremely rare, our approach can be viewed as a model study for understanding emergence of new microbial pathogens in general and their translocation from the tropical rain forest to the human environment.

ACKNOWLEDGEMENTS

A. Mayr (Innsbruck, Austria) is thanked for data on the occurrence of black yeasts in bathing facilities in Austria, and M. de Boer (Rotterdam, The Netherlands) for help during sampling in an artificial bat cave. P. Puunsawat, S. ToaDam, Hala-Bala Wildlife Sanctuary, Dusit zoo and Khao Khaew Open Zoo are acknowledged for facilities during sampling, K. Assawachoung (Bangkok, Thailand) for water sampling, and T. Seelanan (Bangkok, Thailand) for background information for the manuscript. Thanks are also due to K. Sudhadham and T. Sudhadham for their help with isolation work elsewhere in Thailand. We are grateful to A. van Belkum (Rotterdam, The Netherlands) and R.C. Summerbell (Toronto, Canada) for comments on the manuscript and to M.Th. Smith (CBS) for help with yeast identification. H. Ansink, A.H.G. Gerrits van den Ende and A. Cegcel are thanked for technical assistance, and the technical service department at CBS for preparing large amounts of media. M. Buitelaar (Utrecht, The Netherlands) is thanked for collaboration with sectioning animals. This work was financially supported by WOTRO (Netherlands Foundation for the Advancement of Tropical Research, WB 93-421). We also acknowledge a travel grant from the Johanna Westerdijk Foundation (W0312).

REFERENCES

Booth C (ed.) (1971). *Methods in Microbiology*, vol. 4. Academic, U.K.

Chang HL, Kim DS, Park DJ, Kim HJ, Lee CH, Shin HJ (2000). Acute cerebral phaeohyphomycosis due to *Wangiella dermatitidis* accompanied by cerebrospinal eosinophilia. *Journal of Clinical Microbiology* **38:** 1965−1966.

Dixon DM, Migliozzi J, Cooper CR, Solis O, Breslin B, Szaniszlo PJ (1992). Melanized and non-melanized multicellular form mutants of *Wangiella dermatitidis* in mice: mortality and histopathology studies. *Mycoses* **35:** 17−21.

Dixon DM, Polak A, Conner GW (1989). Mel$^-$ mutants of *Wangiella dermatitidis* in mice: evaluation of multiple mouse and fungal strains. *Journal of Medical and Veterinary Mycology* **27:** 335−341.

Engemann J, Kaye K, Cox G, Perfect J, Schell W, McGarry SA, Patterson K, Edupuganti S, Cook P, Rutala WA, Weber DJ, Hoffmann KK, Engel J, Young S, Durant E, McKinnon K, Cobb N, Bell L, Gibson J, Jernigan D, Arduino M, Fridkin S, Archibald L, Sehulster L, Morgan J, Hajjeh R, Brandt M, Warnock D, Duffus WA (2002). *Exophiala* infection from contaminated injectable steroids prepared by a compounding pharmacy-United States, July-November 2002. *Morbidity and Mortality Weekly Report.* **51:** 1109−1112.

Gold WL, Vellend H, Salit IE, Campbell I, Summerbell R, Rinaldi M, Simor AE (1994). Successful treatment of systemic and local infections due to *Exophiala* species. *Clinical Infectious Diseases* **19**: 339−341.

Haase G, Skopnik H, Groten T, Kusenbach G, Posselt H-G (1991). Long-term fungal cultures from patients with cystic fibrosis. *Mycoses* **34:** 373−376.

Hiruma M, Kawada A, Ohata H, Ohnishi Y, Takahashi H, Yamazaki M, Ishibashi A, Hatsuse K, Kakihara M, Yoshida M (1993). Systemic phaeohyphomycosis caused by *Exophiala dermatitidis. Mycoses* **36:** 1−7.

Hoog GS de, Gerrits van den Ende AHG (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41:** 183−189.

Hoog GS de, Guarro J, Gené J, Figueras MJ (2000). *Atlas of Clinical Fungi*, 2nd ed. Centraalbureau voor Schimmelcultures / Universitat Rovira i Virgili.

Hoog GS de, Haase G (1993). Nutritional physiology and selective isolation of *Exophiala dermatitidis. Antonie van Leeuwenhoek* **64:** 17−26.

Hoog GS de, Matos T, Sudhadham M, Luijsterburg KF, Haase G (2005). Intestinal prevalence of the neurotropic black yeast *Exophiala dermatitidis* in healthy and impaired individuals. *Mycoses* **48:** 142−145.

Hoog GS de, Takeo K, Yoshida S, Göttlich E, Nishimura K, Miyaji M (1994). Pleoanamorphic life cycle of *Exophiala (Wangiella) dermatitidis*. *Antonie van Leeuwenhoek* **65:** 143−153.

Horré R, Hoog GS de (1999). Primary cerebral infections by melanized fungi: a review. *Studies in Mycology* **43:** 176−193.

Horré R, Schröteler A, Marklein G, Breuer G, Siekmeier R, Sterzik B, Hoog GS de, Schnitzler N, Schaal KP (2003). Vorkommen von *Exophiala dermatitidis* bei Patienten mit zystischer Fibrose in Bonn. *Atemwegs- und Lungenkrankheiten* **29:** 373−379.

Kerkmann M-L, Piontek K, Mitze H, Haase G (1999). Isolation of *Exophiala (Wangiella) dermatitidis* in a case of otitis externa. *Clinical Infectious Diseases* **29:** 929−930.

Kurtzman, CP, Fell JW (eds) (1998). *The yeasts, a Taxonomic Study*, 4th ed. Elsevier, The Netherlands.

Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003). Biosynthesis of fungal melanins and their importance for human pathogenic fungi. *Fungal Genetics and Biology* **38:** 143−158

Matos T, Hoog GS de, Boer AG de, Crom I, Haase G (2002). High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. *Mycoses* **45:** 373−377.

Matos T, Haase G, Gerrits van den Ende AHG, De Hoog GS (2003). Molecular diversity of oligotrophic and neurotropic members of the black yeast genus *Exophiala*, with accent on *E. dermatitidis*. *Antonie van Leeuwenhoek* **83:** 293−303.

Matsumoto T, Matsuda T, McGinnis MR, Ajello L (1993). Clinical and mycological spectra of *Wangiella dermatitidis* infections. *Mycoses* **36:** 145−155.

Mayr A (1999). *Hyphomyceten, Zygomyceten und Hefepilze an Oberflächen von Saunaanlagen, mesophilen Wärmebädern und Dampfbädern.* Mimeographed, Leopold Franzens Univ., Innsbruck.

Mok WY (1980). Nature and Identification of *Exophiala werneckii*. *Pan American Health Organization Scientific Publication* **396:** 269−275.

Muotoe-Okafor FA, Gugnani HC (1993). Isolation of *Lecythophora mutabilis* and *Wangiella dermatitidis* from the fruit eating bat, *Eidolon helvum*. *Mycopathologia* **122:** 95−100.

Nishimura K, Miyaji M, Taguchi H, Tanaka R (1987). Fungi in bathwater and sludge of bathroom drainpipes. 1. Frequent isolation of *Exophiala* species. *Mycopathologia* **97:** 17−23.

Padhye AA, McGinnis MR, Ajello L (1978). Thermotolerance of *Wangiella dermatitidis*. *Journal of Clinical Microbiology* **8:** 424−426.

Reis NR, Mok WY (1979). *Wangiella dermatitidis* isolated from bats in Manaus, Brazil. *Sabouraudia* **17:** 213−218.

Satow MM, Attili-Angelis D, Hoog GS de, Angelis DF, Vicente VA (2008). Selective factors involved in oil flotation isolation of black yeasts from the environment. *Studies in Mycology* **61:** 157-163

Sudhadham M, Haase G, Menken SBJ, Belkum A van, Gerrits van den Ende AHG, Sihanonth P, Hoog GS de (2009a). Molecular diversity of the black yeast *Exophiala dermatitidis,* a neurotropic opportunist in humans. (in preparation).

Sudhadham M, Hoog GS de, Menken SBJ, Gerrits van den Ende AHG, Sihanonth P (2009b). Rapid screening for differentially virulent genotypes of the neurotropic black yeast *Exophiala dermatitidis* using PCR-RFLP. *Journal of Microbiological Methods* (in press).

Suntisuk T (2006). *Vegetation in Thailand.* Bangkok: Prachachon Co.,Thailand.

Woollons A, Darley CR, Pandian S, Arnstein P, Blackee J, Bul J (1996). Phaeohyphomycosis caused by *Exophiala dermatitidis* following intra-articular steroid injection. *British Journal of Dermatology* **135:** 475−477.

Yurlova NA, Hoog GS de (2002). Exopolysaccharides and capsules in human pathogenic *Exophiala* species. *Mycoses* **45:** 443−448.