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Molecular identification of *Trichophyton benhamiae* in Strasbourg, France: a 9-year retrospective study

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Abstract

Trichophyton benhamiae is a zoophilic dermatophyte transmitted to humans mostly from guinea pigs and occasionally other animals. It presents two distinct phenotypes: yellow and white. *T. benhamiae* was formerly known as *Trichophyton* species of *Arthroderma benhamiae*; it was considered part of the *T. mentagrophytes* species complex, and some authors have incorrectly described the yellow phenotype of *T. benhamiae* as *T. mentagrophytes* var. *porcellae*. Identification of *T. benhamiae* has been difficult, as it was described under more than three names, two phenotypes, and in several different possible host species. During the past 15 years, human infections due to this dermatophyte have been increasingly reported all over the world. In order to better understand the local epidemiology of *T. benhamiae* and to compare it to other European countries, we performed a 9-year retrospective study in the Strasbourg University Hospital. We studied 41 dermatophytes (38 isolated from humans and 3 from guinea pigs) identified as *T. mentagrophytes* var. *porcellae* or *A. benhamiae* from January 2008 to December 2016 and verified their identification by ITS (Internal Transcribed Spacer) sequencing. ITS sequencing

was performed in 35 of the 41 strains, and they were identified as *T. benhamiae* (33), *T. bullosum* (1), and *T. eriotrephon* (1). The other six remaining strains were identified according to morphology as *T. mentagrophytes* var. *porcellae*, name incorrectly used since 2010 for the yellow phenotype of *T. benhamiae*. ITS sequencing is recommended for accurate identification of this dermatophyte and the culture phenotype (yellow or white) should be specified.

Key words: *Trichophyton benhamiae*, *Arthroderma benhamiae*, guinea pig, zoophilic dermatophyte, *T. mentagrophytes* var. *porcellae*, *T. bullosum*.

Introduction

Dermatophytes are a group of keratinophilic filamentous fungi that can infect animals and humans. Dermatophytosis, commonly called ringworm, is usually limited to hair, nails, and the keratinized layers of the skin.[1] These mycoses are transmissible diseases and can clinically range from mild to severe, depending on the host's immune status, the strain virulence, and other environmental factors.[1]

According to their habitat, dermatophytes can be divided into anthropophilic (human), zoophilic (animal) and geophilic (soil)[1]. The latter two types tend to cause more inflammatory human lesions that the former.

Dermatophytes belong to four genera: *Epidermophyton*, *Trichophyton*, *Microsporum*, and *Nannizzia*, with only the first two being truly anthropophilic.[2] The genus *Trichophyton* is the one most frequently isolated from humans, especially in regions with a temperate climate.

Trichophyton benhamiae (comb. nov.)[2] is a zoophilic species transmitted to humans mostly from guinea pigs and occasionally rabbits, cats and dogs. During the past 15 years, it has been described in animals in Japan, Europe, and the United States.[3,4,5,6,7]

Two phenotypes have been described for *T. benhamiae*: yellow and white.[8,9,10,11,12,13,14] The yellow phenotype strains are downy, with a pleated mycelium, a yellow-orange reverse, and a slow growth rate. Sporulation is poor on Sabouraud agar, with rare microconidia and no macroconidia or spiral hyphae. Subcultures on other media (potato dextrose agar, diluted Sabouraud agar or M40Y) can enhance sporulation.[8,10] The main differential diagnosis is *Microsporum canis*, also often macroscopically yellow, but presenting 6–12 celled macroconidia, with thick cell walls and thinner septa.[15]

The white phenotype strains are powdery to floccose, with a yellow, orange, or brown reverse and a rapid growth rate. Microconidia are numerous, spherical to clavate; macroconidia are sparse, 3–8 celled, smooth- and thin-walled, clavate to cigar-shaped; spiral hyphae are occasionally present.[2,10] The main differential diagnosis for the *T. benhamiae* white phenotype is *T. mentagrophytes*, which presents numerous spherical microconidia and frequent spiral hyphae, aside from the clavate to cigar-shaped macroconidia.

In the past years, the nomenclature of dermatophytes has undergone some changes. Initially, species were defined according to clinical data, and morphological and physiological characteristics. Thus, *T. benhamiae* was initially known as *Trichophyton* sp. of *Arthroderma benhamiae*[20,21,22] and it was considered to be part of the *T. mentagrophytes*[9,21,22,23,24,25,26,27,28,29] species complex. But dermatophytes presenting different phenotypes can have the same genotype, or *vice versa*. This is perfectly illustrated by the fact that in 2010 Contet-Audonneau and Leyer invalidly introduced the name *T. mentagrophytes* var. *porcellae* for the already described yellow phenotype *Trichophyton* sp. of *A. benhamiae*.[8]

The rapid development of molecular methods in the past 20 years has revolutionized the dermatophyte taxonomy. Based on sequencing the ITS ribosomal DNA region, seven clades have been described. The upper clade A comprises the *Trichophyton* species, with clade A-1 corresponding to *T. mentagrophytes*. *Trichophyton* sp. of *Arthroderma benhamiae* is no longer considered part of the *T. mentagrophytes* species complex; it became *T. benhamiae* (comb. nov.), which formed the A-2 clade together with *T. schoenleinii* and *T. verrucosum*. Clade A-3 is represented by the zoophilic species *T. bullosum*.[2]

Having more than three different names, two different phenotypes, and several different possible hosts rendered difficult the identification of *T. benhamiae*. This dermatophyte has been reported since 2001 from humans in Japan,[3,16] Switzerland,[4,10,17] Germany,[5,14,18] France,[8] Belgium,[9] and the United Kingdom[19]. In recent years in Japan, *T. benhamiae* has become the second most frequent dermatophyte after *M. canis* and a study performed in Germany between March 2010 and March 2013 showed that *T. benhamiae* had already become the most frequent zoophilic dermatophyte responsible of human infections, with a prevalence of 2.9%.[13,14]

In the Medical Mycology laboratory of the Strasbourg University Hospital we look for dermatophytes in 1400 to

1750 samples per year, and rare or interesting strains are stored in a mycology bank since 2008. We performed a 9-year retrospective study, in order to better understand the local epidemiology of *T. benhamiae* and to compare it to that of the other European countries or regions.

Methods

Strains

Strains were isolated in our laboratory from clinical samples sent to our diagnostic laboratory in sterile Petri dishes, or from strains sent to us for identification by external sources (private laboratories or smaller hospitals).

For each sample of sufficient quantity, a direct examination with KOH 30% and culture on in-house slant media (Sabouraud Chloramphenicol Dextrose Agar and Sabouraud Chloramphenicol Dextrose Cycloheximide Agar) were performed. The cultures were incubated at 27°C for 4 to 6 weeks and examined twice a week. No direct examination was performed for strains sent to us for identification.

In-house media plates (potato-dextrose agar, water agar, Borelli's lactrimel agar, and diluted Sabouraud dextrose agar) were used for subcultures of all the strains. They were incubated at 27°C for 3 to 10 days and examined twice a week.

The urea hydrolysis activity was tested using Christensen's urea broth test.[30] Subcultures of the strains were inoculated in 1 ml of ready-to-use Christensen's urea broth (Sigma-Aldrich) incubated at 27°C and examined after 3 and 7 days.

All 41 strains were identified morphologically (*T. mentagrophytes* var. *porcellae*), and 35 were confirmed by DNA sequencing (33 *A. benhamiae/T. benhamiae*, 1 *T. bullosum* and 1 *T. eriotrephon*). The remaining six strains were not available for sequencing at the time of this study.

DNA extraction, amplification, and sequencing

Confirmation of the initial identification was performed retrospectively by sequencing the Internal Transcribed Spacer (ITS) region of the ribosomal DNA using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as previously described.[31] Briefly, DNA was extracted and purified directly from fungal colonies with a Qiagen QIAamp® DNA mini kit according to manufacturer's instructions. The polymerase chain reaction (PCR) mixture (40 μl) included 3 mM MgCl2 (Qiagen, Germany), 200 μM of each deoxynucleoside triphosphate (dNTP) (Euromedex, France), 0.2 μM of each primer, and 0.2 μM of Hotstar Taq DNA polymerase (Qiagen, Germany). The thermal cycler (Applied BioSystems, Foster City, CA, USA) was set for initial denaturation at 95°C for 15 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing for 1 min at 54°C, and extension for 2 min at 72°C. A final extension step at 72°C for 7 min was included at the end of the amplification. The PCR products were electrophoresed in 2% agarose (Eurogentec, Belgium) for 30 min at 150 V and viewed in gel documentation Gel Doc EZ System (BioRad, France) and stored at −20°C until they were sent (to GATC Biotech, Germany) for ITS sequencing. Resulting sequences were compared to GenBank, CBS, ISHAM and EMBL databases.

Phylogenetic analysis

ITS sequences from our strains were aligned with reference strains belonging to *Arthroderma benhamiae* complex using MUSCLE (Mega® 6.0 software), and the best model for phylogenetic analyses was identified using default setting. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura 3-parameter model.

The percentage of trees in which the associated taxa clustered together is shown above the branches (10000 replicates). Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable. Evolutionary analyses were conducted in MEGA6. *Trichophyton rubrum* strains IHEM13800 (JQ407179) and ATCC28188 (AF170472) were used as outgroup.

Results

Between 2008 and 2016 we received 1390 (2009) to 1732 (2015) samples per year for dermatophytes analysis. About 50% had positive cultures for dermatophytes, moulds, and yeasts. About 10% of the samples were positive for zoophilic dermatophytes, mostly *T. mentagrophytes, M. canis* and *T. benhamiae* (Table 1).

In our 9-year survey, 41 strains isolated from separate samples (38 human and 3 animal) and identified at that time as *T. mentagrophytes* var. *porcellae* or *A. benhamiae* were diagnosed in our laboratory by morphology or molecular identification. As this study is retrospective, some information is missing in some cases (Table 2).

No *M. canis* were included in this study because all of the strains had typical macroconidia that made them easy

Table 1. Main zoophilic dermatophytes identified in our laboratory from 2008 to 2016.

Year	Total samples	Positive samples (%)	*T. mentagrophytes* (%)	*T. benhamiae* (%)	*M. canis* (%)	*T. verrucosum* (%)
2008	1698	996 (58.65)	98 (9.84)	3 (0.3)	4 (0.4)	1 (0.1)
2009	1390	787 (56.61)	97 (12.32)	1 (0.13)	5 (0.63)	1 (0.12)
2010	1432	794 (55.44)	71 (8.94)	3 (0.37)	6 (0.75)	1 (0.1)
2011	1577	846 (53.44)	64 (7.56)	9 (1.06)	7 (0.82)	1 (0.12)
2012	1382	701 (50.72)	68 (9.7)	6 (0.85)	4 (0.57)	0
2013	1452	717 (49.38)	75 (10.46)	3 (0.41)	14 (1.95)	2 (0.28)
2014	1602	763 (47.62)	60 (7.86)	9 (1.18)	3 (0.4)	0
2015	1732	797 (46)	63 (7.9)	2 (0.25)	7 (0.87)	0
2016	1720	786 (45.69)	63 (8.01)	3 (0.38)	15 (1.65)	1 (0.12)
2008–2016	13985	7187 (51.4)	658 (9.15)	40 (0.55)	65 (0.9)	7 (0.09)

to differentiate from the yellow phenotype of *T. benhamiae*. We had no *T. concentricum* or *T. eriotrephon* in our collections, and all of our *T. verrucosum* isolates were already confirmed by ITS sequencing.

Only 35 of 41 strains were available for retrospective study in our mycology bank; for these, the morphological identification was verified by sequencing the ITS region of the ribosomal DNA. The six remaining strains had been identified according to morphology as *T. mentagrophytes* var. *porcellae*, name used incorrectly for the yellow phenotype *Trichophyton* sp. of *A. benhamiae* (Figs 1 and 2).

Strain 3 had a white phenotype and was identified by ITS sequencing as *T. bullosum* (Table 2, Fig. 1, Table 3). This result was confirmed by the phylogenetic analysis presented in Figure 3. Strain 3 had been isolated in 2008 from a thigh lesion of a 15-year-old girl, presented a white phenotype and a sterile microscopy; no information concerning animal contact was known.

Strain 6 had a white phenotype, and ITS sequencing showed 99–100% identity with *A. benhamiae* / *T. erinacei* strains (Table 3). However, the phylogenetic analysis with high bootstrap values presented in Figure 3 showed that it belongs to the *T. eriotrephon* clade. This strain had been isolated from a beard sycosis of a 24-year-old man in 2009 and no information concerning animal contact was given. Microscopically, it presented numerous microconidia and some macroconidia; urease activity was weekly positive (Table 2).

The remaining 33 sequenced strains were confirmed as *A. benhamiae* or *T. benhamiae*. One animal strain (strain 27) only had 97% identity with three strains of *T. benhamiae* (Table 3). The other 32 strains all have 99–100% identity with strains of *T. benhamiae*, including the type strain.

In sum, 36 of the total 39 *T. benhamiae/T. mentagrophytes* var. *porcellae* strains were isolated from humans and three from guinea pig samples (Fig. 1). And 35 of the 39 *T. benhamiae/T. mentagrophytes* var. *porcellae* strains (89.7%) belong to the yellow phenotype and four (10.3%) to the white phenotype. All white phenotype strains were isolated from human samples (strains 1, 2, 9, and 19); they all clustered together and separately from the yellow ones in the phylogenetic analysis presented in Figure 3.

With regards to the 36 human *T. benhamiae* strains, 25 (69.4%) were from children with ages ranging from 2 to 15 years; the lesions were mostly inflammatory and located on the thorax or abdomen (seven), scalp (six), face (five), arms (two), leg (one), and groin (one). At least two children had more than one lesion (face and thorax, face and scalp). For four children, the clinical data were not precise (only "skin lesion" was specified).

The 11 adults (30.6% of *T. benhamiae* strains) had ages ranging from 20 to 89 and presented lesions on arms (six), legs (four), thorax (one), lip (one), scalp (one), face and neck (one).

Only two human strains come from two members (children) of the same family (strains 31 and 32). Both strains were of the *T. benhamiae* yellow phenotype, presented weakly positive urease activity but did not cluster next to one another in the dendrogramm presented in Figure 3.

In nine of the 36 human *T. benhamiae* infections (25%), contact with an animal could not be established at the time of diagnosis, mostly because the interrogatory had been performed out of our department and no detailed information was transmitted to our laboratory. In sum, 27 out of 36 *T. benhamiae* patients (75%) had been in contact with an animal. And 21 of these 27 patients had been in contact only with guinea pigs; three patients had been in contact with other animals besides guinea pigs (guinea pig, pony, and cat for patient 8; guinea pig, hamster, gerbil, and kitten for patient 17; and guinea pig, rabbit, cow, dog, and horse for patient 19). Seven of the 24 guinea pigs were symptomatic, but we did not receive samples from these animals. Three patients with no guinea pig contact had been in

Table 2. List of strains used in this study.

No.	Sex	Age	Year	Lesion localization	Contact with animals	Animal lesion	Pheno-type	Microscopy	Urea broth	Initial identification	ITS sequencing	GenBank Accession no.	Culture collection ID	Source
1	F	9	2008	Thorax (back)	NI	NI	White	μ and spirals	++	*A. benhamiae*	*T. benhamiae*	KY885203	0801m210208	Our department
2	F	8	2008	NI	Rabbit	NI	White	abundant μ	+	*A. benhamiae*	*T. benhamiae*	KY885204	0802m180906	Our department
3	F	15	2008	Thigh	NI	NI	White	sterile	NP	*A. benhamiae*	*T. bullosum*	KY885205	0805m150877	External source
4	M	9	2008	Cheek	Cat	NI	Yellow	few μ	+	*A. benhamiae*	*T. benhamiae*	KY885206	0810m040057	Our department
5	F	12	2009	Arm	NI	NI	Yellow	few μ	NP	*A benhamiae*	*T. benhamiae*	KY885207	0906m180356	External source
6	M	24	2009	Beard sycosis	NI	NI	White	μ and macro	+	*A. benhamiae*	*T. eriotrephon*	KY885208	0912m230081	Our department
7	M	8	2010	NI	NI	NI	Yellow	few μ	+	*A. benhamiae*	*T. benhamiae*	KY885209	1001m210429	External source
8	F	4	2010	Face	Guinea pig, pony, cat	NI	Yellow	few μ	+	*A. benhamiae*	*T. benhamiae*	KY885210	1005m220254	Our department
9	F	8	2010	Scalp kerion	Guinea pig	NI	White	μ and spirals	++	*A. benhamiae*	*T. benhamiae*	KY885211	1012m200864	Our department
10	F	15	2011	Scalp kerion	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885212	1102m170903	External source
11	F	12	2011	Shoulder	Guinea pig	NI	Yellow	few μ	±	*T. m.* var *porcellae*	*T. benhamiae*	KY885213	1102m250627	External source
12	F	5	2011	Thorax (sternum)	Guinea pig	Yes	Yellow	few μ	NP	*T. m.* var *porcellae*	*T. benhamiae*	KY885214	1102m280391	External source
13	F	5	2011	Pubis, groin	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885215	1104m210816	External source
14	F	20	2011	Arm	Guinea pig	Yes	Yellow	few μ	NP	*T. m.* var *porcellae*	*T. benhamiae*	KY885216	1105m260453	External source
15	F	10	2011	Face, thorax	Guinea pig	Yes	Yellow	few μ	NP	*T. m.* var *porcellae*	*T. benhamiae*	KY885217	1105m270378	External source
16	F	11	2011	Thorax	Guinea pig	Yes	Yellow	few μ	NP	*T. m.* var *porcellae*	NP	NA	1108m030060	Our department
17	F	12	2011	Thorax	Guinea pig, hamster, gerbil, kitten	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885218	1110m250836	External source
18	M	12	2011	Abdomen	Rabbit	NI	Yellow	few μ	+	*A. benhamiae*	*T. benhamiae*	KY885219	1111m220427	External source
19	F	7	2012	Scalp kerion	Guinea pig, rabbit, cow, dog, horse	NI	White	μ and macro	+	*A. benhamiae*	*T. benhamiae*	KY885220	1204m110484	Our department
20	F	36	2012	Thorax (nipple)	NI	NI	Yellow	some μ	++	*T. m.* var *porcellae*	*T. benhamiae*	KY885221	1205m290936	External source
21	F	33	2012	Calf, knee	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885222	1206m110884	Our department
22	F	22	2012	Arm	Guinea pig	NI	Yellow	few μ	±	*T. m.* var *porcellae*	NP	NA	1208m080872	Our department
23	F	26	2012	Arm	Guinea pig	Yes	Yellow	few μ	−	*T. m.* var *porcellae*	*T. benhamiae*	KY885223	1208m170694	Our department
24	M	60	2012	Forearm	NI	NI	Yellow	few μ	±	*T. m.* var *porcellae*	*T. benhamiae*	KY885224	1211m220440	External source
25	M	4	2013	Scalp kerion	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885225	1308m120592	Our department
26	F	43	2013	Leg	NI	NI	Yellow	few μ	+	*T. m.* var *porcellae*	NP	NA	1310m090513	External source
27	A	NA	2013	NI	NA	Yes	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885226	1310m160506	External source
28	A	NA	2014	NI	NA	Yes	Yellow	few μ	++	*T. m.* var *porcellae*	*T. benhamiae*	KY885227	1401m280504	External source
29	F	12	2014	Thorax (back)	Guinea pig	Yes	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885228	1403m070728	Our department
30	F	49	2014	Face, neck	Guinea pig	Yes	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885229	1405m100253	Our department
31	F	8	2014	Scalp, face	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885230	1405m170464	Our department
32	F	6	2014	Thorax	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885231	1405m191015	Our department
33	F	15	2014	NI	Guinea pig	NI	Yellow	few μ	++	*T. m.* var *porcellae*	*T. benhamiae*	KY885232	1410m030306	External source
34	F	2	2014	Scalp	NI	NI	Yellow	few μ	−	*T. m.* var *porcellae*	NP	NA	1410m160928	Our department
35	F	15	2014	Thigh	NI	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885233	1411m170810	Our department
36	A	NA	2014	NI	NA	Yes	Yellow	few μ	NP	*T. m.* var *porcellae*	NP	NA	GRA Sév Spik	Our department
37	M	9	2015	NI	Guinea pig	NI	Yellow	few μ	NP	*T. m.* var *porcellae*	NP	NA	1506m020295	External source
38	F	54	2015	Arm	Guinea pig	NI	Yellow	few μ	+	*T. m.* var *porcellae*	*T. benhamiae*	KY885234	1507m100694	Our department
39	F	11	2016	Eyelid	Guinea pig	NI	Yellow	few μ	++	*T. m.* var *porcellae*	*T. benhamiae*	KY885235	1607m060843	Our department
40	F	52	2016	Lip	Guinea pig	NI	Yellow	few μ	++	*T. m.* var *porcellae*	*T. benhamiae*	KY885236	1608m220245	Our department
41	M	43	2016	Elbow, knee	NI	NI	Yellow	few μ	++	*T. m.* var *porcellae*	*T. benhamiae*	KY885237	1611m220253	Our department

A, animal; F, female; M, male; NA, not applicable; NI, no information; NP, not performed; μ, microconidia; macro, macroconidia; −, negative; ±, equivocal; +, weakly positive; ++, positive.
T. m., T. mentagrophytes.

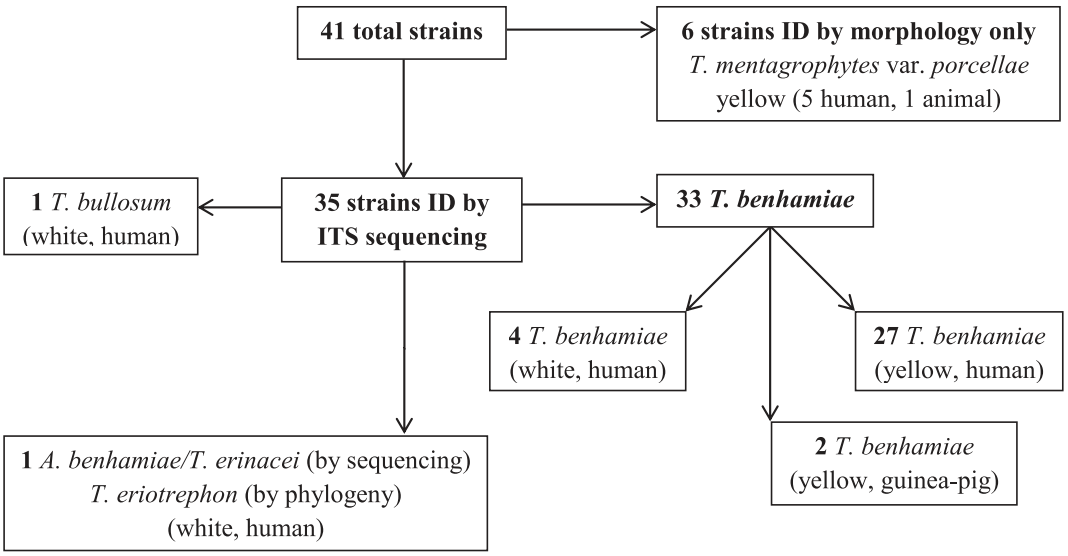

Figure 1. Flow chart of the 41 strains included in this study.

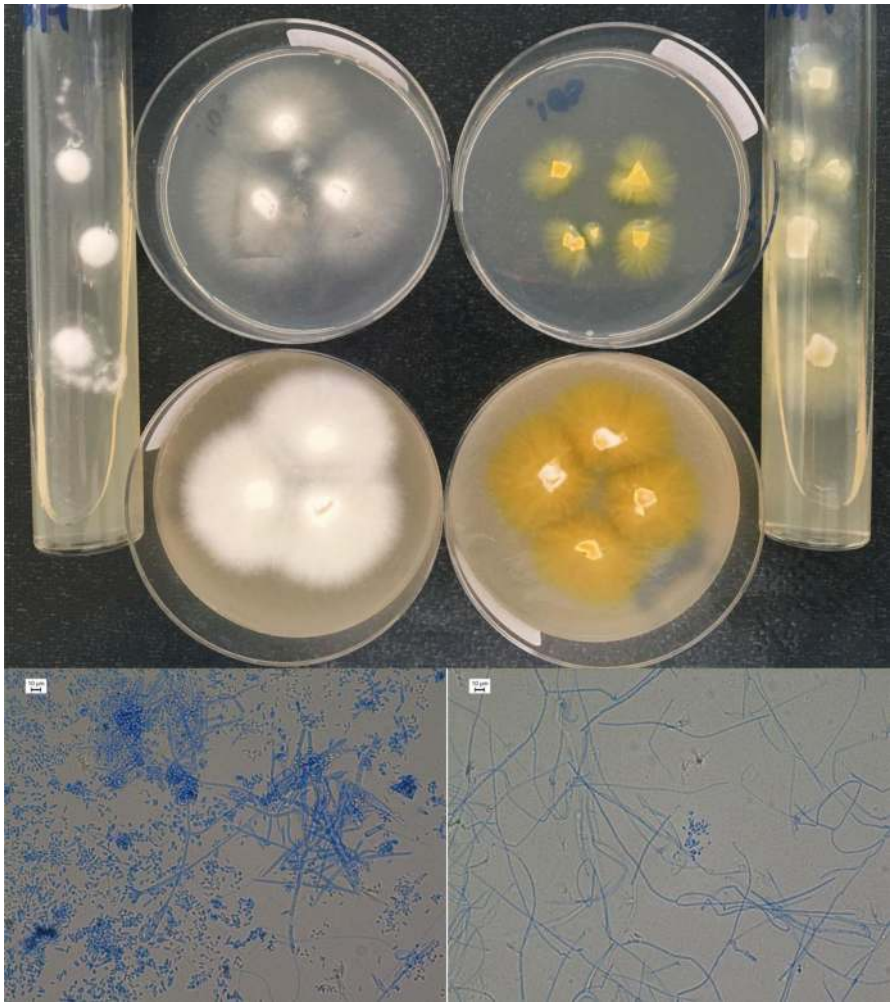

Figure 2. Macroscopy and microscopy of *T. benhamiae*. **Up:** Macroscopy of white (left) and yellow (right) phenotypes of *T. benhamiae.* Potato-dextrose-agar slant media, diluted Sabouraud dextrose agar (two upper Petri dishes) and Borelli's lactrimel agar (two lower Petri dishes). **Low-left:** Microscopy of the white phenotype of *T. benhamiae* (magnification 400 ×): numerous micro- and macroconidia. **Low-right:** Microscopy of the yellow phenotype of *T. benhamiae* (magnification 400 ×): absent macroconidia, few microconidia.

Table 3. Results of GenBank and CBS comparison for our 35 ITS sequenced strains.

Strains from this study	BLASTN first hits	Score	Identity	Query cover	Organism	GenBank definition
Strain 6	GenBank EU181452.1	1230 bits (666)	100%	99%	*T. erinacei*	*A. benhamiae* NCPF 431
672 bp	GenBank KJ606083.1	1229 bits (665)	99%	99%	*Trichophyton* cf. *erinacei ATCC 24552*	*Trichophyton* cf. *erinacei* ATCC 24552
ITS sequencing: ***A. benhamiae / T. erinacei***	GenBank JN134091.1	1203 bits (651)	99%	100%	*T. erinacei*	*T. erinacei* isolate 379
Phylogeny: ***T. eriotrephon***	GenBank EU622882.1	1203 bits (651)	99%	100%	*T. benhamiae*	*A. benhamiae* AMC 07101
	GenBank JX413539	1044.49	98.958%	100%	*T. erinacei*	*A. benhamiae* CS783
Strain 3	CBS 131645	1041.32	100%	100%	*T. bullosum* BCCM/IHEM 24321	
656 bp	CBS 363.35 GenBank FM992675.1	1038.32	100%	99.695%	*T. bullosum* LP 770	
ITS sequencing and phylogeny: ***T. bullosum***	GenBank LN589975.1	1197 bits (648)	99%	99%	*T. bullosum* CCF 4831	
	CBS 557.50 GenBank FM992676.1	958.02	100%	92%	*T. bullosum* No. 493	
	GenBank KU257463.1		99%	100%	*T. benhamiae* SU901378	
White phenotype	GenBank KJ606075.1		99%	100%	*A. benhamiae* ATCC MYA-4681	
Strains: 1, 2, 9, 19	GenBank JN134088.1		99%	100%	*T. benhamiae* 269	
T. benhamiae	CBS 112370		99.201%	100%	*A. benhamiae* CHUV 2352/02	
Yellow phenotype ***T. benhamiae***	GenBank KU257464.1		99%	100%	*T. benhamiae* SU209346	
	GenBank AB458216.1		99%	100%	*A. benhamiae* IFM 54424	
Strains: 4, 5, 7, 8, 10, 11, 12, 13,	GenBank AF170457.1		99%	100%	*A. benhamiae* RV 26680	
14, 15, 17, 18, 20, 21, 23, 24, 25,	CBS 624.66 GenBank JX122282.1		99%	100%	*A. benhamiae* ATCC 16782	
28, 29, 30, 31, 32, 33, 35, 38, 39, 40, 41	CBS 623.66 GenBank JX122281.1		99%	100%	*A. benhamiae* ATCC 16781	
Yellow phenotype ***T. benhamiae***	GenBank KU257464.1		97%	99%	*T. benhamiae* SU209346	
	GenBank KX092365.1		97%	99%	*T. benhamiae* ATCC 42873	
Strain 27	CBS 624.66		97%	99%	*A. benhamiae* ATCC 16782	

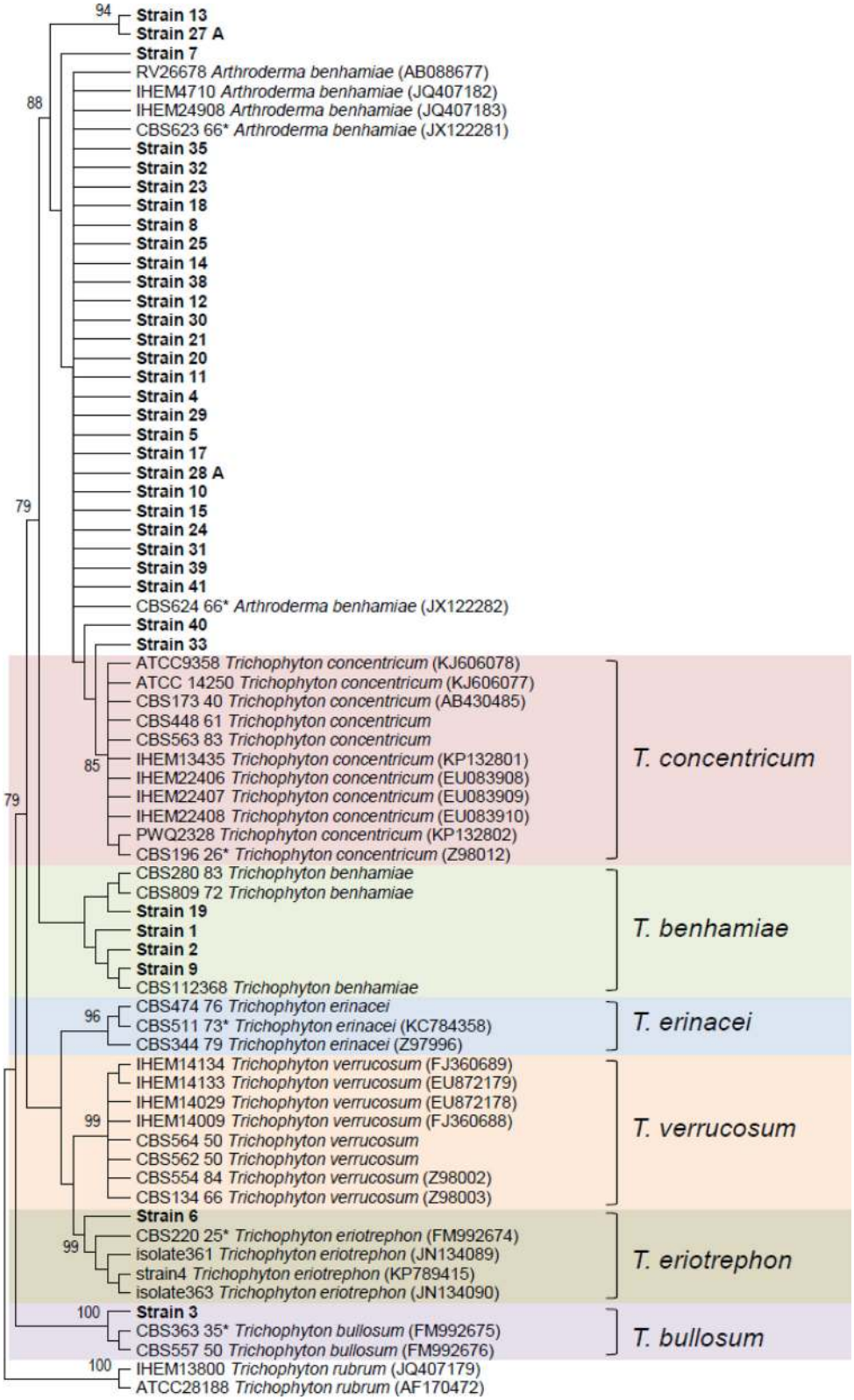

Figure 3. Maximum likelihood phylogenetic tree (MEGA® 6.0 software) based on ITS sequences of *Arthroderma benhamiae* complex using T92+G+I model, with 10,000 bootstrap replications. Bootstrap values above 70% are shown, *Trichophyton rubrum* was used as outgroup. A: animal strain (2); *: type strains.

Figure 4. Christensen's urea broth test. From left to right: negative control, equivocal reaction (strain 11), weakly positive reaction (strain 10), positive reaction (strain 40).

contact with rabbits (patient 2 and patient 18) and patient 4 with a cat (Table 2).

Three *T. benhamiae* strains isolated from guinea pigs were sent to us for identification. These animals were symptomatic but were not pets of any of the patients included in this article. They were all yellow type *T. benhamiae*.

Only 32 *T. benhamiae* strains of the total of 39 were tested for urease activity: eight were positive, 19 were weakly positive, five were equivocal, and two were negative (Table 2 and Fig. 4).

Regarding the incidence of *T. benhamiae* and *M. canis*, the former was more frequent than the latter in only 3 of the 9-year survey (Table 1): 2011 (1.06% vs 0.82%), 2012 (0.85% vs 0.57%), and 2014 (1.18% vs 0.4%).

Discussion

Most of the dermatophytes are cosmopolite, but some are confined to specific regions or areas of the globe or are associated with certain animals. Population migrations, changes in lifestyle, improvement of hygiene, and practice of more physical activity are changing the geographical distribution of dermatophytosis.

Another important factor influencing the epidemiology of these infections is the increasing number and variety of pets. Lately, under the influence of fashion and mediatisation, these are no longer limited to cats and dogs. Reptiles, mice, rabbits, guinea pigs or ferrets have become equally common[9,25,32].

Children and staff working in pet shops are populations especially at risk of contracting an infection transmitted by these no longer exotic animals.[32,33,34,35,36]

More than adults, children are at risk of developing an infection due to zoophilic dermatophytes because of their increased outdoors activity and preferred close contact with pets and other animals.[33] This was also evidenced by our cases, with infections concerning mostly children (25 out of 38 patients = 65.78%).

The adults' lesions were mostly situated on the exposed parts of the skin (arms and legs), whereas lesions on thorax, abdomen, scalp, face, groin, thigh, or shoulder, were predominant in children, probably due to petting and playing with their animals.

T. benhamiae is transmitted mainly by guinea pigs but also occasionally by other animals.[4,18,21,25,37] In our survey, three patients had been in contact with other animals besides guinea pigs (pony, cat, rabbit, cow, dog, and horse) and three patients had been in contact only with animals other than guinea pigs (rabbit and cat). These data correspond to those of the literature, since other hosts have recently been identified for *T. benhamiae*, such as cats, dogs, rabbits, mice, rats, foxes, and more rarely degus or porcupines.[6,10,13,38,39]

Lesions caused by *T. benhamiae* tend to be highly inflammatory. The animals are usually asymptomatic; when apparent, typical lesions are circumscribed areas of alopecia with erythema, scaling and crusting.[4] In 27 of our 36 *T. benhamiae* human cases (75%), contact with guinea pigs has been established. Out of these 27 cases, the animals were confirmed symptomatic in only seven cases (25.92%). Khettar et al. in 2012[9] and Bloch et al. in 2016[40] have investigated pet shops in the city of Nancy (Eastern France) and found that 1/2 of the guinea pigs in 2012 and 2/3 in 2016 were carriers of *T. benhamiae* (morphological identification), most of them asymptomatic. The percentages of carriers for hosts other than guinea pigs have not been investigated.

Transmission can take place directly by contact with the animals (even if asymptomatic) but also *via* soil, animal hairs, and scales. The infectivity could be as long as 2 years.[8] These are elements that add to the spreading of the infection, since the animal is neither identified as a potential source of infection for its entourage, nor isolated or treated.

In terms of incidence, in most regions with a temperate climate, *M. canis* remains the second most frequently isolated zoophilic dermatophyte[37] after *T. mentagrophytes*. It is mainly transmitted by cats and dogs[33,41] and determines lesions that are not very inflammatory, unlike most of the other zoophilic dermatophytes.

The observed frequency of *T. benhamiae* infection has been increasing recently in Japan,[11,16] Switzerland,[4,10,17] Germany,[5,14,18,42,43,44] France,[8] Belgium,[9] the

Netherlands,[37] and Chile[36]. In recent years in Japan[11,16] *T. benhamiae* has become the second most frequent dermatophyte after *M. canis*, and a study performed in Germany between March 2010 and March 2013 showed that *T. benhamiae* had already become the most frequent zoophilic dermatophyte responsible of human infections with a prevalence of 2.9%.[13,14]

In the past 9 years in our laboratory we diagnosed a total of 658 strains of *T. mentagrophytes*, 65 strains of *M. canis* and 39 strains of *T. benhamiae*/*T. mentagrophytes* var. *porcellae* (Tables 1 and 2). *T. mentagrophytes* remains the most frequent zoophilic dermatophyte (incidence around 9%). *M. canis* comes in second (incidence around 1%) and *T. benhamiae* third (incidence around 0.5%, varying between 0.25% and 1.18%).

From our 9-year survey we can conclude (Table 1) that *T. benhamiae* was the third most frequent zoophilic dermatophyte after *T. mentagrophytes* and *M. canis* in our laboratory, except for 2011 (1.06% vs 0.82%), 2012 (0.85% vs 0.57%), and 2014 (1.18% vs 0.4%), when it came second, surpassing *M. canis*. This seems to be mostly due to the increased number of guinea pigs as popular pets and to the fact that up to 2/3 of these animals can be carriers of *T. benhamiae*, even if asymptomatic.[9,40]

Microscopical differential diagnosis between *M. canis* and the yellow phenotype of *T. benhamiae* can be straightforward when rough-walled spindle-like macroconidia are present for the former and only few microconidia for the latter. Recently, Brasch and Wodarg[14] described loop or circuit-like mycelial junctions for *T. benhamiae*, which could make morphological identification easier. Easy and fast methods, like the one proposed by Mayser et al. in 2013[45] can be very useful. The authors used chromogenic media *Candi*Select™ 4 (Bio Rad, France), which allowed differentiation of the two dermatophytes after a few hours of incubation: the medium turned pink or purple for *M. canis* and turquoise-green for *T. benhamiae*.[45] The chromogenic medium used routinely in our laboratory is ChromID *Candida*™ (bioMérieux, France), but it does not allow the differentiation of *M. canis* and *T. benhamiae* described by Mayser et al. for *Candi*Select™ 4. When both *M. canis* and the yellow phenotype of *T. benhamiae* present as sterile mycelia, they are difficult to differentiate and ITS sequencing is recommended.

Differential diagnosis between *T. mentagrophytes* and the white phenotype of *T. benhamiae* can be problematic, considering their similar macroscopy and microscopy.[15] Since these two species also show overlapping host specificity, ITS sequencing is the only method that can provide reliable identification.

Christensen urea broth test for *T. benhamiae* has been described as negative,[8] weakly positive,[45] positive,[13] or variable.[14] Our results confirm the variability observed by Brasch and Wodarg[14]; 32 strains out of the 39 *T. benhamiae* strains were tested for urease activity: eight were positive, 19 were weakly positive, five were equivocal, and two were negative. Considering the subjective interpretation of this test, the different types of tests available and especially the strain variability of the urea hydrolysis activity, this test is less and less used and/or reported in the literature.

Recently, mass spectrometry has been used for the identification of *T. benhamiae*, with great correlations when compared to PCR.[28,46] This technique is now widely available in routine and hospital laboratories but some problems persist: sporulation of the mould is needed, extraction protocols are not standardized, databases need to be regularly updated, and most of them are expensive and/or contain a limited number of dermatophytes in general and of *T. benhamiae* isolates in particular.

Dermatophyte species identification can be performed or confirmed by DNA sequencing, most frequently targeting the ITS region of the ribosomal DNA.[2,21,25,27] Multilocus DNA sequencing has recently been used to revise the classification and taxonomy of dermatophytes.[2] De Hoog et al. showed that dermatophyte taxonomy has reached an acceptable level of stability. ITS sequencing can also reveal unexpected results: in our study, one of the strains we suspected of being a white phenotype of *T. benhamiae* proved to be *T. bullosum*, a zoophilic dermatophyte rarely isolated from the coat of horses and possibly donkeys. Only two cases have been described in the literature: one from a forearm lesion of a 21-year-old male in rural France[47] and one from a saddle-area lesion of a 6-year-old male horse in the Czech Republic.[48] As shown by the dendrogram presented in Figure 3, the ITS sequence of our *T. bullosum* strain clustered with the other two available strains. This newly described species is closely related to *T. verrucosum* and *T. eriotrephon* and systematic molecular identification of the dermatophytes comprised in these genera could give us more insight into the real prevalence of this species.

Another strain we suspected of being a white phenotype of *T. benhamiae* was strain 6, isolated from a beard sycosis of a 24-year-old man in 2009 (no information concerning animal contact was available). ITS sequencing showed a 99–100% identity with *A. benhamiae* and *T. erinacei* strains (Table 3), but the phylogenetic analysis with high bootstrap value presented in Figure 3 places this strain in the *T. eriotrephon* clade, next to the type strain. There are at least two limiting factors in our case: the sequence length

(672 bp) and having used only one marker for the phylogenetic analysis presented in Figure 3.

All four white phenotype *T. benhamiae* strains were isolated from human samples (strains 1, 2, 9, and 19); they all cluster together and separately from the yellow ones in the phylogenetic analysis presented in Figure 3.

The phylogenetic analysis shows clusters of *A. benhamiae* as well as *T. benhamiae*. We think this is due to ongoing updates taking place in the different databases following the new taxonomy of dermatophytes.[2] These efforts of the scientific community will be useful in deepening the knowledge of local epidemiologies and will improve communication between scientists.

T. benhamiae is a zoophilic dermatophyte diagnosed as an agent of human infection with increased frequency in the past years on three continents (Asia, Europe, South America). Our study shows that ITS sequencing is necessary for accurate identification of both phenotypes (white or yellow) of the species. The new taxonomy[2] should simplify identifying *T. benhamiae* and monitoring the epidemiology of this zoophilic dermatophyte.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Weitzman I, Summerbell RC. The dermatophytes. *Clin Microbiol Rev*. 1995; 8: 240–259.
2. de Hoog GS, Dukik K, Monod M et al. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. *Mycopathologia*. 2017; 182: 5–31.
3. Nakamura Y, Kano R, Nakamura E, Saito K, Watanabe S, Hasegawa A. Case report. First report on human ringworm caused by *Arthroderma benhamiae* in Japan transmitted from a rabbit. *Mycoses*. 2002; 45: 129–131.
4. Drouot S, Mignon B, Fratti M, Roosje P, Monod M. Pets as the main source of two zoonotic species of the *Trichophyton mentagrophytes* complex in Switzerland, *Arthroderma vanbreuseghemii* and *Arthroderma benhamiae*. *Vet Dermatol*. 2009; 20: 13–18.
5. Nenoff P, Schulze I, Uhrlaß S, Krüger C. [Kerion caused by the zoophilic dermatophyte *Trichophyton* species of *Arthroderma benhamiae* in a child: a new emerging pathogen of dermatomycoses in Germany]. *Hautarzt Z Dermatol Venerol Verwandte Geb*. 2013; 64: 846–849.
6. Sieklucki U, Oh S-H, Hoyer LL. Frequent isolation of *Arthroderma benhamiae* from dogs with dermatophytosis. *Vet Dermatol*. 2014; 25: 39–e14.
7. White SD, Guzman DS-M, Paul-Murphy J, Hawkins MG. Skin diseases in companion guinea pigs (*Cavia porcellus*): a retrospective study of 293 cases seen at the Veterinary Medical Teaching Hospital, University of California at Davis (1990–2015). *Vet Dermatol*. 2016; 27: 395–e100.
8. Contet-Audonneau N, Leyer C. [Emergence of a dermatophyte contracted from guinea pig and close to *Trichophyton mentagrophytes* var. *erinacei*: *T. mentagrophytes* var. *porcellae*]. *J Med Mycol*. 2010; 20: 321–325.
9. Khettar L, Contet-Audonneau N. [Guinea pigs and dermatophytosis]. *Ann Dermatol Venereol*. 2012; 139: 631–635.
10. Symoens F, Jousson O, Packeu A et al. The dermatophyte species *Arthroderma benhamiae*: intraspecies variability and mating behaviour. *J Med Microbiol*. 2013; 62: 377–385.
11. Hiruma J, Kano R, Harada K et al. Occurrence of *Arthroderma benhamiae* genotype in Japan. *Mycopathologia*. 2015; 179: 219–223.
12. Monod M, Fratti M, Mignon B, Baudraz-Rosselet F. [Dermatophytes transmitted by pets and cattle]. *Rev Med Suisse*. 2014; 10: 749–753.
13. Nenoff P, Uhrlaß S, Krüger C et al. *Trichophyton* species of *Arthroderma benhamiae*—a new infectious agent in dermatology. *J Dtsch Dermatol Ges*. 2014; 12: 571–581.
14. Brasch J, Wodarg S. Morphological and physiological features of *Arthroderma benhamiae* anamorphs isolated in northern Germany. *Mycoses*. 2015; 58: 93–98.
15. Atlas Online. http://atlas.clinicalfungi.org/AtlasOnline/. Accessed February 7, 2017.
16. Mochizuki T, Kawasaki M, Ishizaki H et al. Molecular epidemiology of *Arthroderma benhamiae*, an emerging pathogen of dermatophytoses in Japan, by polymorphisms of the non-transcribed spacer region of the ribosomal DNA. *J Dermatol Sci*. 2001; 27: 14–20.
17. Fumeaux J, Mock M, Ninet B et al. First report of *Arthroderma benhamiae* in Switzerland. *Dermatol Basel Switz*. 2004; 208: 244–250.
18. Kraemer A, Mueller RS, Werckenthin C, Straubinger RK, Hein J. Dermatophytes in pet guinea pigs and rabbits. *Vet Microbiol*. 2012; 157: 208–213.
19. El-Heis S, Borman AM, Szekely A, Godfrey KM. Tinea corporis caused by *Arthroderma benhamiae* in a child. *Clin Exp Dermatol*. 2016; 41: 955–957.
20. Ajello L, Cheng SL. The perfect state of *Trichophyton mentagrophytes*. *Sabouraudia*. 1967; 5: 230–234.
21. Heidemann S, Monod M, Gräser Y. Signature polymorphisms in the internal transcribed spacer region relevant for the differentiation of zoophilic and anthropophilic strains of *Trichophyton interdigitale* and other species of *T. mentagrophytes sensu lato*. *Br J Dermatol*. 2010; 162: 282–295.
22. Kawasaki M. Verification of a taxonomy of dermatophytes based on mating results and phylogenetic analyses. *Med Mycol*. 2011; 52: 291–295.
23. Takashio M. Observations on African and European strains of *Arthroderma benhamiae*. *Int J Dermatol*. 1974; 13: 94–101.
24. Makimura K, Mochizuki T, Hasegawa A, Uchida K, Saito H, Yamaguchi H. Phylogenetic classification of *Trichophyton mentagrophytes* complex strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. *J Clin Microbiol*. 1998; 36: 2629–2633.
25. Fréalle E, Rodrigue M, Gantois N et al. Phylogenetic analysis of *Trichophyton mentagrophytes* human and animal isolates based on MnSOD and ITS sequence comparison. *Microbiol Read Engl*. 2007; 153: 3466–3477.
26. Li HC, Bouchara J-P, Hsu MM-L, Barton R, Su S, Chang TC. Identification of dermatophytes by sequence analysis of the rRNA gene internal transcribed spacer regions. *J Med Microbiol*. 2008; 57: 592–600.
27. Symoens F, Jousson O, Planard C et al. Molecular analysis and mating behaviour of the *Trichophyton mentagrophytes* species complex. *Int J Med Microbiol*. 2011; 301: 260–266.
28. Packeu A, Hendrickx M, Beguin H, Martiny D, Vandenberg O, Detandt M. Identification of the *Trichophyton mentagrophytes* complex species using MALDI-TOF mass spectrometry. *Med Mycol*. 2013; 51: 580–585.
29. Chollet A, Cattin V, Fratti M, Mignon B, Monod M. Which fungus originally was *Trichophyton mentagrophytes*? Historical review and illustration by a clinical case. *Mycopathologia*. 2015; 180: 1–5.

30. Kane J, Fischer JB. The differentiation of *Trichophyton rubrum* and *T. mentagrophytes* by use of Christensen's urea broth. *Can J Microbiol*. 1971; 17: 911–913.
31. White TJ, Bruns T, Lee S et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ et al., editors. *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, 1990: 315–322.
32. Quinet B. [Zoonoses in children from new pets]. *Med Mal Infect*. 2005; 35: S117–120.
33. Reichert-Pénétrat S, Contet-Audonneau N, Barbaud A, Schurra JP, Fortier B, Schmutz JL. Epidemiology of dermatophytoses in children living in northeast France: a 5-year study. *Pediatr Dermatol*. 2002; 19: 103–105.
34. Shiraki Y, Hiruma M, Matsuba Y et al. A case of tinea corporis caused by *Arthroderma benhamiae* (teleomorph of *Tinea mentagrophytes*) in a pet shop employee. *J Am Acad Dermatol*. 2006; 55: 153–154.
35. Noël F, Piérard-Franchimont C, Piérard GE, Quatresooz P. [Fungi, pets and their owners]. *Rev Med Liege*. 2011; 66: 589–595.
36. Thomson P, Monsalves P, Maier L, Silva V. [Dermatophyte colonization on guinea pigs (*Cavia porcellus*) kept in pet stores. First report from Santiago, Chile]. *Rev Iberoam Micol*. 2015; 32: 103–105.
37. Jaspers GJ, Werrij BG, Jagtman BA, Loza B. Severe kerion celsi due to *Trichophyton mentagrophytes*: a case report. *Acta Paediatr*. 2011; 100: e181–183.
38. Hiruma J, Kano R, Kimura U et al. Mating type gene for isolates of *Trichophyton mentagrophytes* from guinea pigs. *J Dermatol*. 2014; 41: 743–745.
39. Ziółkowska G, Nowakiewicz A, Gnat S, Trościańczyk A, Zięba P, Dziedzic BM. Molecular identification and classification of *Trichophyton mentagrophytes* complex strains isolated from humans and selected animal species. *Mycoses*. 2015; 58: 119–126.
40. Bloch M, Cavignaux R, Debourgogne A, Dorin J, Machouart M, Contet-Audonneau N. [From guinea pig to man: Tinea outbreak due to *Trichophyton mentagrophytes* var. *porcellae* in pet shops in Nancy (France)]. *J Mycol Medicale*. 2016; 26: 227–232.
41. Czaika VA, Lam P-A. *Trichophyton mentagrophytes* cause underestimated contagious zoophilic fungal infection. *Mycoses*. 2013; 56: 33–37.
42. Budihardja D, Freund V, Mayser P. Widespread erosive tinea corporis by *Arthroderma benhamiae* in a renal transplant recipient: case report. *Mycoses*. 2010; 53: 530–532.
43. Hiernickel C, Wiegand C, Schliemann S et al. [*Trichophyton* species of *Arthroderma benhamiae*: Clinical therapeutic aspects of a new pathogen in dermatology]. *Hautarzt Z Dermatol Venerol Verwandte Geb*. 2016; 67: 706–711.
44. Brasch J, Beck-Jendroschek V, Voss K, Uhrlaß S, Nenoff P. [*Arthroderma benhamiae* strains in Germany: Morphological and physiological characteristics of the anamorphs]. *Hautarzt Z Dermatol Venerol Verwandte Geb*. 2016; 67: 700–705.
45. Mayser P, Budihardja D. A simple and rapid method to differentiate *Arthroderma benhamiae* from *Microsporum canis*. *J Dtsch Dermatol Ges*. 2013; 11: 322–327.
46. de Respinis S, Tonolla M, Pranghofer S, Petrini L, Petrini O, Bosshard PP. Identification of dermatophytes by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Med Mycol*. 2013; 51: 514–521.
47. Sitterle E, Frealle E, Foulet F et al. *Trichophyton bullosum*: a new zoonotic dermatophyte species. *Med Mycol*. 2012; 50: 305–309.
48. Lyskova P, Hubka V, Petricakova A, Dobias R, Cmokova A, Kolarik M. Equine Dermatophytosis due to *Trichophyton bullosum*, a poorly known zoophilic dermatophyte masquerading as *T. verrucosum*. *Mycopathologia*. 2015; 180: 407–419.