Discussion

The present isolate could be differentiated from: (i) *B. australiensis* by a thinner cell wall, (ii) *B. hawaiiensis* by its production of swollen conidia with 3 to 7 septa and (iii) *B. papendorfii* by being curved and broadest at the second cell. However, the isolate was very difficult to identify from morphological characteristics when first isolated because of the granular colony characteristics. Furthermore, it took more than six months to observe representative conidia of *B. spicifera*. Therefore, molecular biological identification seems to be the most rapid and convenient method of identification of the etiologic agent in this case. According to criteria proposed by Kurtzman and Robnett, more than 99% of the LSU rDNA sequence could be identified as identical with ascomycetous species [12]. The LSU rDNA sequence from the present isolate showed 99.3% identity to *B. spicifera* and less than 99% to other related species, allowing its identification as *B. spicifera*.

Our patient was diagnosed as having a disseminated infection due to *Bipolaris specifera* on the basis of the following features. First, inflammatory changes were seen in numerous organs, the distribution of which suggested dissemination of pathogens through the circulation. Second, the same fungal morphologic features were found in the lymph node, lung, and liver biopsy specimens. Third, matching DNA sequence between the cultured fungus and tissue extracts revealed that the isolated *Bipolaris spicifera* was related to the pathogenesis and was not simply a contaminant. Our patient had no history of immunocompromised factors. Although he was positive for lupus anticoagulant, no other findings suggest the presence of a collagen vascular disease such as systemic lupus erythematosus.

Marked elevations of eosinophils and IgE seem to be characteristics of diseases associated with *Bipolaris* species. It is reported that almost all allergic diseases and eosinophilia caused by agents of phaeohyphomycosis are due to two genera, i.e., *Bipolaris* and *Curvularia* [2]. Low-density areas distributed along the portal vein are a unique presentation on liver CT scan images in the case of disseminated fugal infections. The dissemination of pathogens via systemic circulation usually results in a diffuse distribution without any relation to the portal vein [14]. The precise mechanism is unknown, although it might reflect fungal dissemination from the digestive system through the portal vein. The patient also showed signs of anti-phospholipid antibody syndrome and hemolytic anemia. Although these findings have not been reported in previous studies of *Bipolaris* infections, a possible association between APS and several microbial pathogens has been indicated [15].

In disseminated *Bipolaris* infections, immunocompetent patients were successfully treated with ITCZ and/or AMPH-B [9–11], while the prognosis of immunocompromised patients was poor [8]. Our patient responded to AMPH-B and VRCZ. When receiving oral ITCZ, 300 mg daily, the trough serum concentrations of ITCZ and its active metabolite, hydroxy-ITCZ, were relatively high. Nevertheless, eosinophilia and prolonged APTT recurred. Re-enlargements of the low-density area along the portal vein and liver nodules were also seen. These findings might be due to ITCZ drug allergy. However, it is more likely that they reflect the recurrence of *Bipolaris* infection because enlargement of liver nodules cannot be explained by drug allergy, and these findings improved after treatment was switched to VRCZ. The reason why our patient had a recurrence during ITCZ treatment is speculated to be that the granular form of the fungus in the tissues of our patient might have been more resistant to drugs. An alternative explanation is that ITCZ was metabolized by cytochrome P450 3A4 in the liver, therefore, lower concentrations in local areas led to an insufficient effect.

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Case Reports

An intrafamilial transmission of *Arthroderma benhamiae* in Canadian porcupines (*Erethizon dorsatum*) in a Japanese zoo

HIDEO TAKAHASHI*, HIROSHI TAKAHASHI-KYUHACHI†, YOKO TAKAHASHI*, KYOKO YARITA*, AKIKO TAKAYAMA*, TOMO INOMATA‡, AYAKO SANO*, KAZUKO NISHIMURA* & KATSUHIKO KAMEI*

**Medical Mycology Research Center (formerly Research Center for Pathogenic Fungi and Microbial Toxicoses), Chiba University, Chiba, †Omoriyama Zoological Garden, Hamada, Akita, and, and ‡Department of Experimental Animals, Faculty of Veterinary Medicine, Azabu University, Kanagawa, Japan*

An intra-familial transmission of *Arthroderma benhamiae* in Canadian porcupines (*Erethizon dorsatum*) housed in a Japanese zoo was studied. The family consisted of an adult couple and two offspring (a male and a female). The porcupettes, born in Japan, showed severe hair loss while the parent animals, imported from the USA. (male) and Canada (female), showed mild symptoms or were asymptomatic. Morphologically identical *Tricophyton* spp. isolates were recovered within seven days from quills of all animals on chloramphenicol-supplemented potato dextrose agar plates incubated at 37°C. Two representative colonies from each animal were identified as *Arthroderma benhamiae* Americano-European race based on mating type (+) and the internal transcribed spacer (ITS) 1-5.5S-ITS 2 region of the rRNA gene sequences (AB236404-AB236408). The present cases constituted the second isolation of dermatophytes from porcupines. There were two different ITS types, i.e., the predominant one isolated from all animals and a secondary one recovered from only the mother porcupine. The sequences have never been recorded in Japan or in the GenBank database to the best of our knowledge. In addition, they were located at a cluster involving the type strain and mating strains of *A. benhamiae* Americano-European race and its F1 progeny. In contrast, 28 rodents (eight species) and three insectivora (1 species) exhibited in the petting zoo were negative for any dermatophytes as determined by culture.

Keywords *Arthroderma benhamiae* Americano-European race, Canadian porcupine, *Erethizon dorsatum*. *Trichophyton* sp.

Introduction

Infections in humans by *Arthroderma benhamiae*, a teleomorph of *Trichophyton* spp. [1], have recently emerged in Japan. More specifically, the incidence of patients with zoonotic infections caused by *A. benhamiae* has been increasing, following a boom in exotic pets such as rabbits, hamsters, chinchillas and four-toed hedgehogs [2–8]. Isolation of *A. benhamiae* was not recorded in Japan until 1997 [3]. It has been thought that *A. vanbreusegehemii* was endemic in Japan since 1980 [9]. Therefore, *A. benhamiae* might have been brought over and spread by importation of exotic animals from foreign countries between 1980 and 1996 and has since become a widespread zoophilic

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Correspondence: Ayako Sano, Medical Mycology Research Center (formerly Research Center for Pathogenic Fungi and Microbial Toxicoses), Chiba University, 1-8-1, Inohana, Chuo-Ku, 260-8673, Chiba, Japan. Tel: +81 43 226 2786; Fax: +81 43 226 2486; E-mail: aya1@faculty.chiba-u.jp

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dermatophyte species in Japan [2,6]. Furthermore, *A. benhamiae* infections constitute serious problems for humans regardless of contact with animals, as evidenced by an outbreak of indirect transmissions of the fungal species among schoolchildren who had no history of contact with exotic and/or any other household animals. The literature indicates that the fungal species might be spread by human activity as some *A. benhamiae* strains would seem to have a strong ability to be transmitted and survive among humans [10]. However, *A. benhamiae* has not been previously isolated from animals in zoological gardens in Japan.

The present study describes an intrafamilial transmission of *A. benhamiae* in Canadian porcupines (*Erethizon dorsatum*) imported from the USA and Canada, and their two offspring born in Japan. The study also involves a survey for dermatophytes in 31 rodents (eight species) and three insectivora (one species) exhibited in the petting zoo of the same institution.

Materials and methods

Cases

Case 1 was a young male Canadian porcupine (*Erethizon dorsatum*), born in Japan on July 17, 2004, that started to show hair loss on the eyelids at 1 month of age. During the four months following onset, the hair loss gradually spread over the neck to all four limbs, as well as to the abdomen, tail and dorsal part of the skin and was accompanied by severe dandruff (Fig. 1). Only the shoulders and back of the animal were unaffected. While the animal was treated with a topical application of antiseptics and ketoconazole lotion using spray bottles, this treatment was ineffective. Inoculation in December, 2005 of the hair and dandruff from the animal on chloramphenicol (100 mg/l)-supplemented potato dextrose agar (CPDA; Difco, Detroit, MO, USA) plates which were incubated at 37°C for seven days, resulted in the recovery of abundant white mycelial colonies that were identified as *A. benhamiae* Americano-European race by mycological and molecular analyses using the internal transcribed spacer (ITS) 1-5.8S-ITS 2 ribosomal RNA (ITS region of rRNA) gene sequences. The isolate was deposited as IFM 54326 (Institute of Food Microbiology, Chiba University which is the new name of the Medical Mycology Research Center, one of the official worldwide culture collection centers for pathogenic fungi and actinomycetes). The animal was treated with an oral administration of griseofulvin at 25 mg/kg *semel in die* (SID; single administration per a day). After treatment with the antifungal agent for one month, the animal had recovered its hair. However, a subsequent culture inoculated with samples from the animal was positive and the isolate was deposited as IFM 54328. After one additional month of treatment, negative culture results were obtained.

Fig. 1 Hair loss accompanied by severe dandruff in a young male Canadian porcupine (*Erethizon dorsatum*).

Cases 2 and 3 were the parent animals. The male, presumed to be 4–5 years of age and imported from the USA on June 14, 2002, had been showing mild hypotrichosis since its arrival in Japan. The female, presumed to be 6–7 years of age and imported from Canada on March 17, 2000, was asymptomatic. Cultures inoculated with the quills from both parent animals were positive and the isolates were identified as *A. benhamiae* Americano-European race. Two isolates from the adult female were deposited as IFM 54330 and IFM 54333, and two from the adult male as IFM 54335 and IFM 54336. These animals were also treated with griseofulvin at 25 mg/kg SID for one month, after which cultures started with samples from both were negative.

Case 4 was a female porcupine born on April 14, 2005, that developed hypotrichosis at 3 months of age. A culture from the quills was positive and the isolate was identified as *A. benhamiae* Americano-European race on the basis of the ITS region of the rRNA gene sequence. Two representative isolates were deposited as IFM 54337 and IFM 54338. After treatment with griseofulvin at 25 mg/kg SID for four weeks, the animal had recovered its hair and subsequent cultures were negative.

Isolation of strains

A few quills, with or without scales, from each porcupine were cultured on two CPDA plates and

incubated at 37°C. Even when no colonies were noted within seven days, observations of the cultures for growth continued for up to four weeks. Two colonies were selected from each animal sample. Isolates from the young male animal were collected in December 2004 and January 2005. Those from the parent animals were selected on the basis of reddish diffusible pigment in January 2005, and from a sister culture in July 2005. When the colonies were almost identical in their appearance, we selected the darkest and palest ones.

The other animals studied were kept in the petting zoo corner of the zoological garden and handled by the same staff as the Canadian porcupines. Hair, quills, scales and desquamations from these animals were cultured on CPDA, incubated at 37°C, and observed for growth for up to four weeks. The tested animals were three prairie dogs (*Cynomys ludovicianus*), one Japanese flying squirrel (*Pteromys momonga*), two American beavers (*Castor canadensis*), two African crested porcupines (*Hystrix cristata*), seven capybaras (*Hydrochaerus hydrochaeris*), one chinchilla (*Chinchilla lanigera*), 10 guinea pigs (*Cavia porcellus*) chosen randomly from a colony consisting of more than 100 animals, five rabbits (*Oryctolagus cuniculus* forma *domestica*) chosen from 50 animals, and three four-toed hedgehogs (*Atelerix albiventris*). The survey was performed with the permission of Omoriyama Zoological Garden, Akita, Japan.

Mycological studies

These investigations included colonies on Sabouraud dextrose agar (SDA) containing 2% dextrose (Wako Chemical Co. Ltd, Osaka, Japan), 1% neopeptone (Difco) and 1.5% Bacto agar (Difco) and potato dextrose agar (PDA, Difco) plates that had been incubated at 25°C for 21 days and microcultured on PDA blocks under the same conditions.

The isolates were crossed with tester strains of both the (+) and (−) mating types of *A. benhamiae* Americano-European race on salt-added 1/10-diluted Sabouraud agar medium containing 0.2% glucose (Wako Chemical Co. Ltd.), 0.1% neopeptone (Difco), 0.1% $MgSO_4$-$7H_2O$, 0.1% KH_2PO_4, and 2% agar (Bacto agar, Difco), and incubated at 25°C for eight weeks. The tester isolates used included, as classified by Takashio [11], IFM 48142 (+) (=RV, i.e., the abbreviation of culture collection of Institute of Tropical Medicine, Antwerp, Belgium and transferred into Belgian coordinated Collections of Micro-Organisms in 1987, Brussels, Belgium, BCCM/IHEM), 26678) and IFM 48143 (−) (=RV 26680), as well as IFM 48144 (+) (=RV 30000) and IFM 48145 (−) (=RV 30001).

Urease activity on Christensen's urea agar slant (Eiken Co. Ltd., Tokyo, Japan) was assessed every day during incubation at 25°C for seven days. The maximum growth temperatures were determined by incubating cultures at 37, 40 and 42°C [5].

Hair perforation tests were performed using human infant hairs kindly supplied by a staff member. The hairs were cut into approximately 1.5 cm lengths and sterilized by autoclaving. Three milliliters of a culture medium containing 0.1% peptone and 0.2% glucose was decanted into 3 cm plastic Petri dish and inoculated with approximately 10 hairs and a loop of fungal mass. The plates were incubated at 25°C for three months in a plastic box. After this period of time, several hairs were removed and observed under a light microscope [5].

Molecular identification

The sequences of the ITS 1-5.8S-ITS 2 region of the rRNA gene were obtained by routine methods [12]. Briefly, DNA was extracted with a DEXPAT® kit (TaKaRa, Ohtsu, Japan) using a modified procedure. Approximately 100 μl of fungal cells from cultures incubated at 25°C for one month on PDA slants were placed in a sterilized microtube (1.5 ml), then 0.5 ml of DEXPAT® solution was added, and the mixture was homogenized with a plastic pestle. It was then incubated at 100°C for 10 min, centrifuged at 12,000 rpm (13,201 *g*) for 10 min and the supernatants used as DNA samples. Although this type of kit is designed for extracting DNA from paraffin-embedded tissue samples, we routinely use it for the isolation of genes from fungal cultures because of its convenience [13].

We mixed 2.5 μl of the DNA extract with a Ready-to-Go bead (Amersham Pharmacia Tokyo, Japan), 2.5 μl of 10 pM of the primers ITS-5 (5′- GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS-4 (5′- TCC TCC GCT TAT TGA TAT GC-3′) [14], and 17.5 μl of distilled water. The reaction mixture was subjected to 1 cycle of denaturation at 95°C for 4 min, 30 cycles of amplification at 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, and a final extension cycle at 72°C for 10 min with a PCR Thermal Cycler MP (TaKaRa, Ohtsu, Japan).

The PCR products were visualized by electrophoresis on 1.0% agarose in 1 × TBE buffer (0.04 M Tris-boric acid, 0.001 M EDTA [pH 8.0]) followed by ethidium bromide staining. The PCR samples were purified using a PCR purification kit (QIAquick, Qiagen Co. Ltd., Tokyo, Japan), and labeled with BigDye Terminator Ver. 1.1 (Applied Biosystems, Foster City, CA., USA) following the manufacturer's protocol. The labeled

samples were directly sequenced on an ABI PRISM® 3100 sequencer (Applied Biosystems, Foster City, CA., USA) using the primers ITS-5, ITS-4, ITS-2 (5′- GCT GCG TTC TTC ATC GAT GC-3′) and ITS3 (5′- GCA TCG ATG AAG AAC GCA GC-3′) [13]. DNA sequences were aligned using GENETEX-MAC genetic information processing software (Software Development Co., Ltd., Tokyo, Japan). The sequences were analyzed using a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) and closely related sequences were obtained through an analysis of the tree of results via http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD =GetSaved&RECENT_RESULTS =on, with ID: J5J481PK015.

Seventy-four nucleotide sequences from *Arthroderma benhamiae* and its related species deposited in GenBank were selected and aligned using the computer program ClustalX (Version 1.8) [15] followed by manual adjustments with a text editor together with the present nucleotide sequences. Phylogenetic analyses were performed with PAUP v4.0b10 [16] using a heuristic search for maximum parsimony trees. Base-pair composition and transition and/or transversion patterns of the dataset were estimated by PAUP v4.0b10. Bootstrap values were calculated over 1000 replicates to assess branch topology. A phylogenetic tree was selected from 3 unrooted trees and was drawn by Tree View PPC [17] (Roderic D. M. Page, Glasgow, Scotland, UK, 1998; http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). Clades were supported by bootstrap values above 50%.

A partial sequence of the mRNA of the urease gene (*URE*) was also analyzed. The primer set, Ab-Ure-F1 (5′- CAT GCC CCC GAC ATC ATC TCC GTC TG - 3′) and Ab-Ure-R1 (5′- GCC CAC ATA GGT CTT GAC AGA ATC G -3′), was designed from AB069970, which is the *A. benhamiae* URE mRNA for urease; complete cds, deposited by Kano and Hasegawa, appear only in the GenBank database. The defined sequences corresponded to the 504th through the 1,155th base pair of AB069970. The PCR conditions consisted of one cycle of denaturation at 95°C for 4 min, 30 cycles of amplification at 94°C for 1 min, at 55°C or 59°C for 1 min and at 72°C for 2 min, and a final extension cycle at 72°C for 10 min with a PCR Thermal Cycler MP (TaKaRa, Ohtsu, Japan). The purified PCR products by a PCR purification kit (QIAquick®) were sequenced using the above primer set, and aligned using GENETEX-MAC genetic information processing software (Software Development Co., Ltd., Tokyo, Japan).

Both the ITS 1-5.8S-ITS 2 regions of rRNA and the partial sequences of mRNA for the *URE* genes were deposited to the GenBank through the DNA Data Bank of Japan (DDBJ, Mishima, Shizuoka, Japan). In addition, when the two sequences derived from one animal were identical, only one sequence was deposited, except for the isolates derived from the mother animal.

Results

White mycelial colonies, 2–5 mm in diameter, with reddish reverse diffusible pigmentation were recovered on CPDA at 37°C within seven days from the quills or

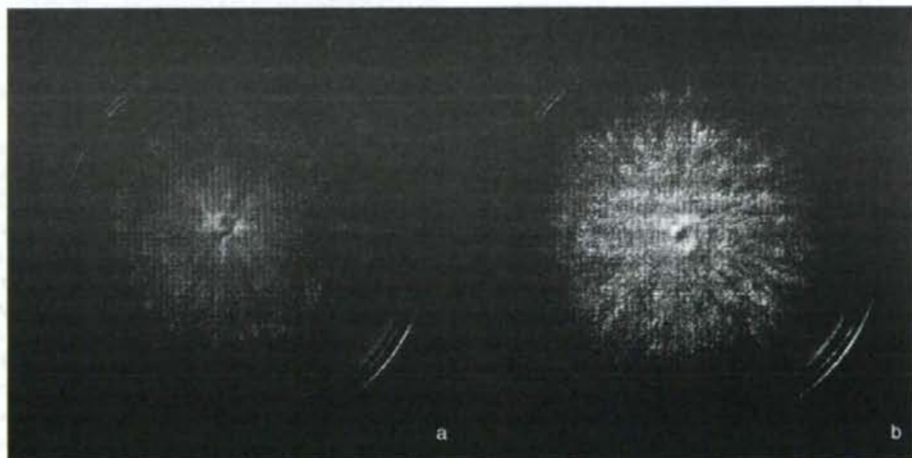

Fig. 2 Colonies of a porcupine isolate IFM 54326. (a) SDA at 25°C for 21 days and (b) PDA at 25°C for 21 days.

scales of all four porcupines. Two representative isolates from one of the porcupines are shown in Fig. 2. On the other hand, no dermatophytes were isolated from samples of any of the animals in the petting section of the zoo.

The colony and microscopic features of the porcupine isolates were identical. The obverse of the colonies on SDA plates cultured at 25°C for 21 days showed a lacy elevated center (Fig. 2a), and a yellow, lacy and flattened texture in the surrounding area. The reverse of the colonies had a yellowish brown center, and orange-yellow pigment that diffused uniformly in a ring shape in the surrounding area. The obverse of the colonies on PDA plates cultured at 25°C for 21 days were powdery in texture (Fig. 2b), while the reverse showed a reddish pigment that diffused uniformly throughout the medium.

Abundant pear-shaped or more elongated microconidia, 1–1.5 μm wide and 1–4 μm long, were microscopically observed attached at right-angles to the sides of the mycelium on PDA agar blocks incubated at 25°C for 21 days. In addition, a few macroconidia (Fig. 3a) and several spiral bodies (Fig. 3b) were noted in these cultures.

The Christensen's urea agar slant was positive after two days incubation at 25°C. All isolates had a maximum growth temperature of 40°C and were unable to perforate hair.

Many gymnothecia containing ascospores were produced when the *A. benhamiae* Americano-European race (−) tester strain was crossed with the isolates recovered from the porcupines, indicating that they were all (+) mating types. The sizes of gymnothecia on the sides of the cultures containing the animal isolates were smaller than on the tester strain side (Fig. 4). Matings with *A. benhamiae* African race were negative.

The GenBank accession numbers of the ITS 1-5.8s-its 2 region of the rRNA (704 bps) gene and the partial sequence of mRNA for the *URE* (652 bps) gene are shown in Table 1. All but one of the sequences derived from the 4 porcupines, IFM 54326, IFM 54328, IFM 54330, IFM 54335, IFM 54336, IFM 54337 and IFM 54338, were identical in the ITS 1-5.8s-its 2 region of the rRNA gene, while IFM 54333 originating from the mother animal showed a different sequence in two bases at the 203rd pair, showing 'C' and 'T', and the 648th pair, showing 'G' and 'T'. The partial sequences of mRNA for the *URE* derived from all isolates were identical.

The phylogenetic status of the two different sequences of the ITS 1-5.8S-ITS 2 region of the rRNA gene derived from the porcupine isolates were located at a cluster consisting of sequences from

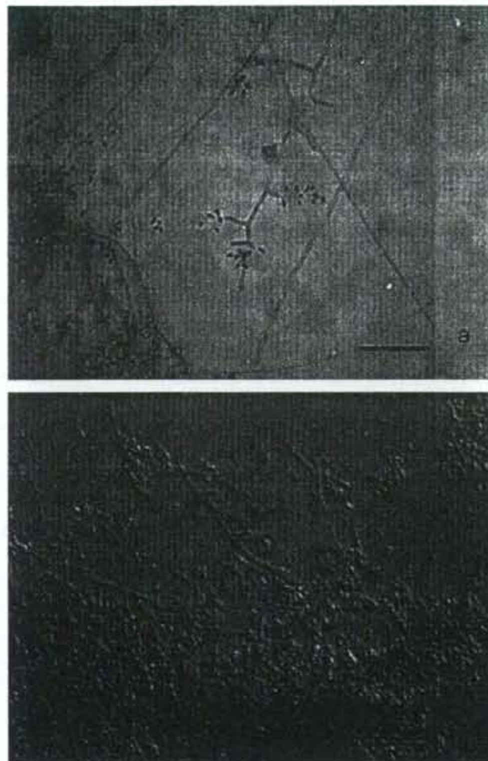

Fig. 3 (a) Pear-shaped or more elongated microconidia attached with a right-angle arrangement to the sides of the mycelium cultured in a microculture system on PDA at 25°C for 21 days (IFM 52326), and (b) a few spiral bodies in a microculture system on PDA at 25°C for 21 days (IFM 52330). The bar indicates 20 μm lactophenol cotton blue staining, ×400.

A. benhamiae Americano-European race, *A. benhamiae* and *Trichophyton concentricum* via BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) and the distance tree of the results (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=GetSaved&RECENT_RESULTS=on,ID; J5J481PK015). The tree consisting of 72 related and one out-group sequences is shown in Fig. 5. The neighboring clusters mainly consisted of *A. benhamiae* derived from French animals [18], *A. benhamiae* having a *Trichophyton erinacei* anamorph, *Trichophyton verrucosum* and *A. benhamiae* African race. The clusters consisting of *A. vanbreuseghemii*, *A. simii* and *A. benhamiae* related to human infections [18] were located at completely different clusters (tree image data not shown). The identities between the present two different sequences

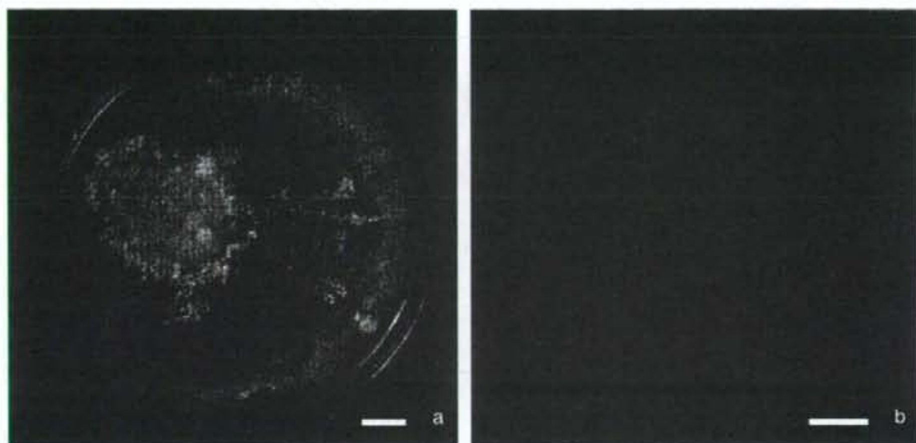

Fig. 4 (a) Gymnothecia between the Americano-European race (−) strain of *Arthroderma benhamiae* (right) and the animal isolate IFM 54330 (left) cultured on salt-added 1/10-diluted Sabouraud agar medium at 25°C for 8 weeks; the bar indicates 1 cm. (b) An enlarged image of gymnothecia; the bar indicates 300 μm (×40).

and the closely related ones in the cluster are shown in Table 2.

Discussion

The causative agent for the present intrafamilial transmission in the Canadian porcupine family was identified as *A. benhamiae* Americano-European race based on mycological and molecular studies. Our case was similar to that of Marais and Oliver who reported in 1965 the second isolation of *Trichophyton* spp. from a porcupine [19]. However, it is impossible to find the first isolates for mycological and molecular comparisons in any international culture collection, such as American Type Culture Collection (ATCC, Manassas, VA, USA), Belgian Co-ordinated Collections of Micro-Organisms, (BCCM/IHEM, Brussels, Belgium), CBS (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), German Collection of Microorganisms, Cell Cultures (DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen: Braunschweig, Germany), and the University of Alberta Microfungus Collection and Herbarium (UAMH, Edmonton, Canada).

The negative results in the hair perforation tests with the isolates in our case indicated that they lacked the ability to digest human hair, but the reasons for these results are not presently apparent.

To the best of our knowledge, the present sequences of the ITS 1-5.8S-ITS 2 region of the rRNA gene have never been recorded in Japan or in the GenBank database. Two different sequences of the present isolates in the ITS 1-5.8S-ITS 2 region of the rRNA

Table 1 Isolates and DDBJ accession numbers of *Arthroderma benhamiae* Americano-European race originated from Canadian porcupines (*Erethizon dorsatum*).

Strain	IFM number	Accession number of genes (bases)	
		rRNA (704 bps)	*URE* (652 bps)
Porcupette-1-2004-37-1	IFM 54326	AB236404	AB304409
Porcupette-1-2005-37	IFM 54328	ND	ND
Porcupine-Mother-37-1	IFM 54330	AB236405	AB304410
Porcupine-Mother-37-9	IFM 54333	AB236406	AB304411
Porcupine-Father-1	IFM 54335	AB236407	AB304412
Porcupine-Father-2	IFM 54336	ND	ND
Porcupette-2-1	IFM 54337	AB236408	AB304413
Porcupette-2-2	IFM 54338	ND	ND

rRNA, the internal transcribed spacer (ITS) 1-5.8S-ITS 2 ribosomal RNA gene; *URE*, A partial sequence of the mRNA of the urease gene; ND, Not deposited because of 100% identity to the isolate from the same animal.

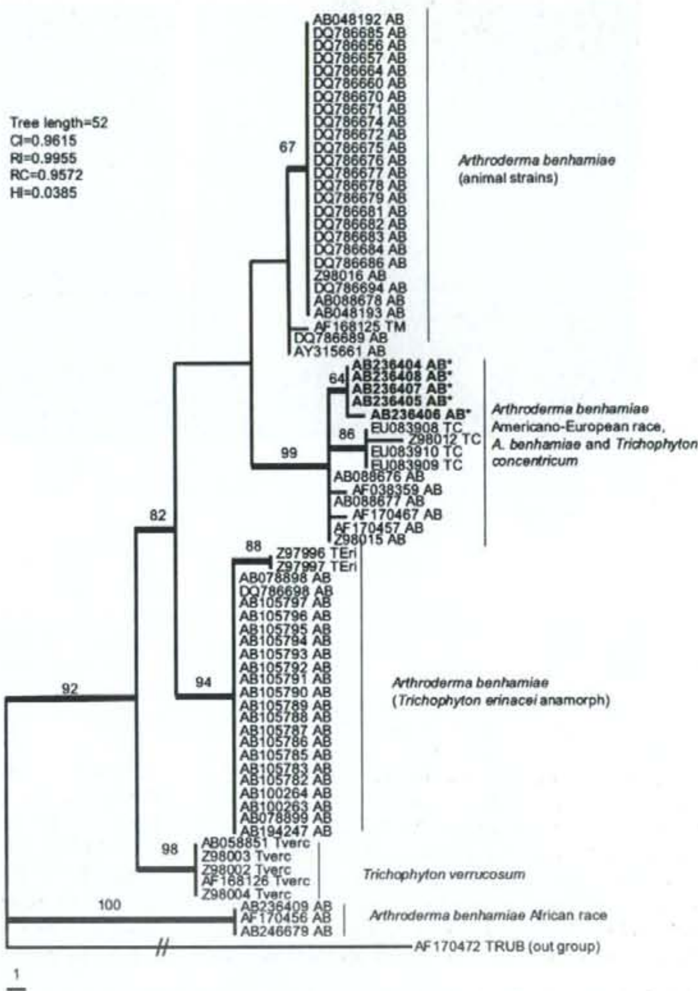

Fig. 5 One of the three most-parsimonious trees obtained from heuristic searches based on ITS 1-5.8s-ITS 2 rRNA gene sequences using 598 bases. Bootstrap support values above 50% are indicated at the nodes. The bar indicates 1 base difference. Data are shown with accession numbers and fungal species. AF170472 derived from *Trichophyton rubrum* was used as an outgroup sequence. *sequence analyzed at the present study; CI, consistency index; RI, retention index; RC, rescaled consistency index; HI, homoplasy index; AB, *Arthroderma benhamiae*; TM, *Trichophyton mentagrophytes*; TC, *T. concentricum*; TEri, *T. erinacei*; Tverc, *T. verrucosum*; TRUB, *T. rubrum*.

gene were located at a cluster involving the type strain and mating strains of *A. benhamiae* Americano-European race and its F1 progeny [20] and were different from the sequences derived from French animals [18]. Interestingly, one of the sequences in the ITS 1-5.8S-ITS 2 region of the rRNA gene of *A. benhamiae* was found in all tested porcupines, while the other one was limited to only the mother animal, indicating that the isolates having the predominant sequence might be more virulent than that derived only from the mother. On the other hand, the partial sequences of mRNA for the *URE* were identical in all isolates, indicating that the sequences were not useful for individual differentiation of the isolates.

Recently, petting zoos in zoological gardens have become popular attractions and could be the source of infectious zoonoses [21]. Outbreaks of simultaneous and/or plural dermatophytoses caused by zoophilic and anthropophilic dermatophytes have been reported [22–34]. Outbreaks of *Microsporum canis* caused by indirect contact with a Bengal tiger (*Panthera tigris tigris*) cub kept in a petting zoo [22] which spread to staff, related persons, and other animals [23] are marked evidence of this risk.

Table 2 Identities of *Arthroderma benhamiae* isolated from porcupines to related sequences.

Accession No.	Identity (%)		Strain	Species
	AB236404	AB236406		
AB236404	–	99.7	IFM 54326	*Arthroderma benhamiae* AE
AB236406	99.7	–	IFM 54333	*Arthroderma benhamiae* AE
AB088676	99.8	99.5	KMU 5-46	*Arthroderma benhamiae*
AB088677	99.9	99.6	RV 26678	*Arthroderma benhamiae* AE
AF038359	99.7	99.4	UAMH 6256	*Arthroderma benhamiae*
AF170457	99.9	99.6	RV 26680	*Arthroderma benhamiae* AE
AF170467	99.7	99.4	UAMH 7339	*Arthroderma benhamiae*
Z98015	99.3	99.0	CBS 623.66	*Arthroderma benhamiae*
EU083908	99.6	99.3	IHEM 22406	*Trichophyton concentricum*
EU083909	99.5	99.4	IHEM 22407	*Trichophyton concentricum*
EU083910	99.6	99.3	IHEM 22408	*Trichophyton concentricum*
Z98012	98.9	98.6	CBS 196.26	*Trichophyton concentricum*
Z98016	98.7	98.6	CBS 280.83	*Arthroderma benhamiae* (located at the neighbouring cluster)

IFM, the Institute of Food Microbiology, Chiba University, the former name of the Medical Mycology Research Center, which is recognized as one of official culture collection centers for pathogenic fungi and actinomycetes worldwide); KMU, Kanazawa Medical University, Kanazawa, Ishikawa, Japan; RV, culture collection of Institute of Tropical Medicine, Antwerp, Belgium and transferred into BCCM/IHEM (Belgian Co-ordinated Collections of Micro-Organisms, Brussels, Belgium) in 1987; UAMH, the University of Alberta Microfungus Collection and Herbarium Edmonton, Canada; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; AE, Americano European race.

The present intrafamilial transmission of *A. benhamiae* was not spread to other animals in the petting zoo or staff. However, it seems hasty to make this conclusion as a student was infected with *T. mentagrophytes* during an investigation of the first isolation of the fungal species from porcupines [19].

As to the route of infection, it was assumed that the parent porcupines might have carried *A. benhamiae* from their places of origin. They might have been exposed to the fungal species, maintained it asymptomatically for several years, and then transmitted it to the porcupettes. On the other hand, the possibility of their being infected after introduction in the zoo should not be ignored. It seemed to be impossible to prevent infestations of wild animals in zoological gardens. Rodents have been thought to be carriers of dermatophytes [35]. Furthermore, imported animals have become very popular as household pets in Japan, and some of them have become wild in our natural environment [5]. It is a very well-known fact that raccoons (*Procyon lotor*) imported from North American countries were released and became wild all over Japan [36]. Wild raccoons might be one of the reservoirs of *A. benhamiae*, based on a recorded *Trichophyton mentagrophytes* infection in a raccoon [37], although such a case has not yet been reported in Japan.

In conclusion, the outbreak of *A. benhamiae* Americano-European race in a family of Canadian porcupines in a zoo in Japan was caused by imported species with two different sequences of the ITS region of the rRNA gene.

Acknowledgements

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Three isolations of *Chaetomium globosum* from erythematous epilation of canine skin

KAZUTOSHI SUGIYAMA*‡, AYAKO SANO†, MASARU MURAKAMI‡, TAKASHI OGAWA§, HIROYUKI MISHIMA§, HIROYUKI OTAKE#, KATSUHIKO KAMEI† & SHUNITI SUGIYAMA*

**Sugiyama Veterinary Clinic, Shizuoka, †Medical Mycology Research Center (formerly; Research Center for Pathogenic Fungi and Microbial Toxicosis), Chiba University, ‡Faculty of Veterinary Medicine, Azabu University, Kanagawa, §Ogawa Animal Hospital, Shizuoka, and #Shikatani Animal Hospital, Shizuoka, Japan*

Chaetomium globosum is commonly found in natural environments worldwide and is known to be a causative agent for emerging fungal infections. The present study describes a case of erythematous epilation of a dog caused by *C. globosum*. A mixed-breed young dog, a 4-months-old male, weighing 7.25 kg, showed depilation, scales, and dermatitis with slightly itchiness on his skin. The main symptom was an erythematous epilation on the left subocular skin 7.5 cm in diameter, accompanied by elephantiasis-like hyperplasia and scales. Similar lesions were observed on the skin on both sides of the ear lobes, the heels, tail, and left angulus oris. The scales from the crusted lesion were cultured on chrolampheni-cole-added potato dextrose agar plates at the first visit, as well as followed by ambulatory practices. The isolates at the first visit, 1 and 3 weeks after treatment, were identified as *C. globosum* by mycological study and the D1/D2 domain of the large subunit rRNA gene sequence. The patient dog was treated by ketoconzole both orally and externally. The lesions were cured, showing new hair growth 9 weeks later. In addition, the susceptibilities to antifungal agents for the present *C. globosum* isolate were as follows: amphotericin B, 4.0 μg/ml; 5-FC 64.0 μg/ml; itraconazole, 0.5 μg/ml; miconazole, 1.0 μg/ml; fulconazole, 16.0 μg/ml; ketocona-zole, 0.25 μg/ml; and micafungin, 16.0 μg/ml.

Keywords *Chaetomium globosum*, dog, erythematous epilation

Introduction

Chaetomium species are commonly found in natural environments worldwide [1] and are known to be mycotoxicin-producing fungal species [2–4]; they are also, however, recognized as industrially important fungal species because of their ability to induce cellulose degeneration [5] and to be used in the production of medically useful compounds [6,7]. On the other hand, the fungal species are known to be causative agents for emerging fungal infections [8]. More than 20 human cases of *Chaetomium* spp.caused by *C. globosum*, *C. atrobrunneum*, *C. strumarium*, *C. perlucidum*, *C. funicolum*, and *C. murorum* [9] have been found to cause onycomycosis, superficial and deep mycoses, sinusitis, pneumonia, and fatal disseminated infection [8–19]. Furthermore, *C. globosum* has been recorded as one of the cutaneous fungal flora of dogs [20], though there are no documented cases of *Chaetomium* spp. infection in animals. The present study describes a cutaneous infection of *C. globosum* in a young dog showing erythematous epilation having repeated isolations of the fungal species from the cutaneous lesions.

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Correspondence: Kazutoshi Sugiyama, Sugiyama Veterinary Clinic, 9-1 60, Nagasaki Minami, Shimizu-ku, 424-0068, Shizuoka, Japan. Tel: +81 54 345 4669; Fax: +81 54 346 1896; E-mail: ks8081@msh.biglobe.ne.jp

Case report

A 4-month-old male mixed-breed dog from Shizuoka prefecture, the middle part of Japan, weighing 7.25 kg,

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received vaccinations for rabies, canine distemper, parvovirus, adenovirus type 2, parainfluenza virus, and leptospira on a routine schedule, and was fed commercial dry chow. The dog had a very intimate relationship with the owners, being kept indoors and sharing a bedroom with the family.

The main symptom was an erythematous epilation on the left subocular skin, 7.5cm in diameter, accompanied by elephantiasis-like hyperplasia and scales (Fig. 1a). According to the owner, scratching behavior was slightly increased and the lesion was expanding 10 days before the first visit. Similar lesions were observed on the skin on both sides of the ear lobes, the heels, tail, and left angulus oris. The dog's appetite was normal and physiological and biochemical data for the blood were within normal values. Direct microscopic observation of skin scales in 20% potassium hydroxide (KOH) showed mycelial components. The wood-light test showed a change to a slightly pinkish color, though the results were indeterminate. In order to avoid contamination, the scales from the lesions were collected by a sterilized brush after the surface was scrubbed with a cotton mass immersed in 70% ethanol. White cottony colonies with slight yellowish reverse pigment were sprouted from the scales cultured on Sabourand's glucose agar (SDA) supplemented with antibiotics at 25°C for 3 days. We identified the fungal sprouts as *Mycrosporum canis* and began to treat the skin lesions by topical application of antifungal and antiseptic agents such as ketoconazole cream and chlorhexidine, once to 3 times daily.

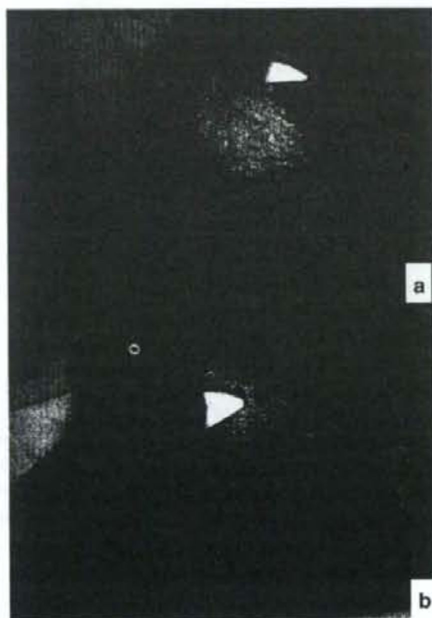

Fig. 1 The skin lesion showing erythematous epilation with scales at the first visit (a), and 9 weeks after treatment (b).

The redness and hypertrophy of the skin at the left subocular lesion showed remission after 3 weeks, but the diameter was expanded and other lesions appeared at the right subocular, the oral angular, inside the ears, on both tarsal joints, and the root of the tail. The dog continued to scratch intensely. Direct microscopic observation of scales mounted by 20% KOH also showed fungal elements. The wood-light test was slightly positive. The culture on SDA plates also produced a white floccose colony having a slightly yellowish color on the reverse, the same as the appearance of the first isolate.

We then began systemic administration of 50 mg *semel in die* (SID; single administration per a day) of ketokonazole followed by reference datum of minimum inhibitory concentration (MIC) ketokonazole [18] in parallel with topical administration of antifungal and antiseptic agents. The lesions entered remission and showed signs of hair growth after 9 weeks (Fig. 1b). The skin lesions had no fungal elements, as observed by microscopy. The wood-lighting test and culture on SDA became negative.

The systemic and topical administration of ketoconazole was continued for two weeks, at which time the lesions were completely healed; no relapse of infection has since been observed.

Colonies on SDA and potato dextrose agar (PDA, Difco, Detroit, MO, USA) plates at 25°C, 37°C, and 42°C were as follows. The colony on SDA after 4 weeks at 25°C was floccose and grey at the center and yellowish-grey at the margin; colonies at 37°C and 42°C after 4 weeks on SDA were white velvety. The diameters at 25, 37, and 42°C after 4 weeks on SDA were 9.0, 6.7, and 3.7 cm, respectively. That on PDA after 4 weeks at 25°C was deep green, having a granulated texture and a white flocosse margin on the surface, and dark brown on the reverse. The textures of colonies on PDA at 37°C were light brown flocosse at the center and white lobate cottony at the margin without ascomata, and at 42°C were ashy green and wrinkled. The diameters at 25, 37, and 42°C after 4 weeks on PDA were 9.0, 7.3, and 2.3 cm, respectively (Fig. 2a, 2b). The morphology of fungal colonies isolated at 3 and 6 weeks after treatment were equivalent to the first isolate.

Structures of ascomata on the PDA plate and slants at 25°C after 4 weeks under stereoscopy and light microscopy were as follows. Spherical ascomata with coiled hair were observed on the PDA plate under a stereomicroscope. The diameters of the ascomata were approximately 200 µm. The perithecium was filled with asci, including 8 ascospores. The matured ascospores were flat, brown, and lemon-shaped, with both sides being 10–12 µm in diameter, and had germination pores (Fig. 3a–3f).

The maximum growth temperature of the isolate was 42°C on the PDA slants using a series of different temperatures; 37, 42, 45, 48, and 50°C.

A partial sequence of the large subunit ribosomal DNA gene (LSU rDNA) sequence was processed by a standard method described by Kurtzman and Robnet [21] using DNA extracted from a culture on a PDA slant at 25°C after 2 weeks. The sequence was compared through BLAST search to the GenBank database [http://www.ncbi.nlm.nih.gov/BLAST/]. A partial sequence of the large subunit ribosomal DNA gene (LSU rDNA) sequence of the isolate had more than 99% identity in the GenBank database, located at a cluster of *C. globosum* by a distance tree of results, and was deposited as AB292591.

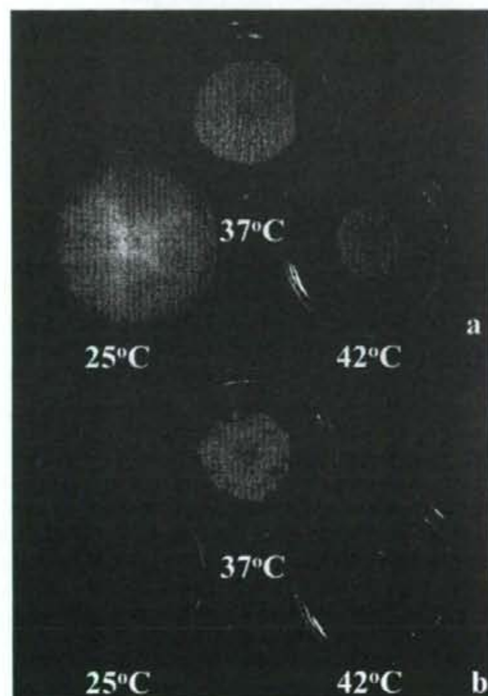

Fig. 2 Colonies on SDA (a) and on PDA (b) after 4 weeks at a 25, 37, and 42°C.

Antifungal susceptibility tests on conidia of the present isolate cultured on a PDA slant at 25°C at 4 weeks were performed according to the broth microdilution modified method of the CLSI M38-A [22] approved standard using RPMI 1640 medium (Sigma, Poole, UK) buffered to pH 7.0 with 3-(N-Morpholino) propanesulfonic acid (MOPS) (Sigma) using a kit (Dryplate, Eiken, Tokyo Japan) containing antifungals: amphotericin B, flucytosine, itraconazole, miconazole, fluconazole, and micafungin. Isolates of *Candida albicans* IFM 40213 equal to ATCC 90028 were included as quality-control strains for susceptibility testing. The microdilution plates were incubated in air. Readings were made after 48 h of incubation at 37°C (the *Candida* control strain was examined at 24 h) in RPMI medium. The minimal inhibitory concentration (MIC) endpoints for amphotericin B and itraconazole were read visually as the lowest drug concentration that prevented any discernible growth. The MIC endpoints for other antifungal drugs were read visually and taken as that which reduced growth by 80% compared with the drug-free control. The susceptibilities to antifungal agents for the present *C. globosum* isolate were as follows: amphotericin B, 4.0 µg/ml; 5-FC, 64.0 µg/ml; itraconazole ITC, 0.5 µ g/ml; miconazole, 1.0 µg/ml; fulconazole, 16.0 µg/ml; ketoconazole, 0.25 µg/ml; and micafungin, 16.0 µg/ml. The values were not changed after 72 hours.

The owner of the patient dog agreed to publish this paper.

Discussion

The skin disease of the dog was diagnosed as dermatitis caused by *C. globosum* identified as *Chaetomium globosum* based on mycological [23] and molecular biological techniques. The systemic administration of 50 mg SID of ketokonazole with a combination of topical treatment of ketoconazole cream for 12 weeks was effective. The present case was the first documented canine case of *C. globosum* infection in Japan, although *Chaetomium* spp. have been reported as species of a normal fungal flora in a foreign country [20].

C. globosum is one of the environmental saprophytic fungal species worldwide, and has been isolated from soil, woods, and wet walls [1]. Systemic infection of the fungal species has been thought to occur by inhalation of airborne spores [15]. The infection route of the

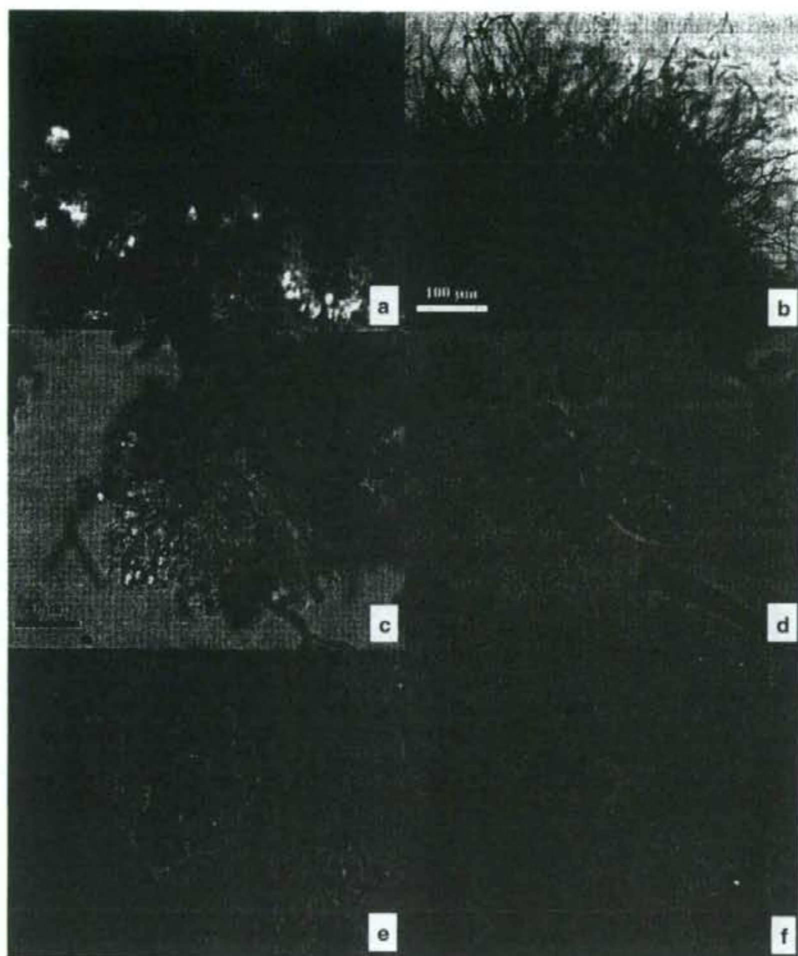

Fig. 3 Microscopic aspects of the isolate cultured on PDA plates or slants at 25°C for 4 weeks. Spherical ascomata with coiled hair on the plate (a) and on the slant (b), as seen by the stereomicroscope, perithecium (c), immature ascospores in a perithecium (d), maturing ascospores (e), and mature ascospores (f), as seen by light microscopy.

present case might be direct contact with the soil or other environments.

C. globosum is listed as one of the causative agents for emerging fungal infection in immunocompromised hosts [16–18]. Human cases in immunocompromised patients after transplantations have been reported [17,18], though the present case had no immunodeficient profile except for the dog's age of 4 months, indicating an under-developed immune system.

Morphological identification of the present *C. globosum* isolate on SDA plates was difficult. The colony on the SDA plate at room temperature within 4 days closely resembled *Microsporum canis* and lacked ascomata, even on the colony cultured at 25°C after 4 weeks. In contrast, PDA plates of slants were excellent media for observation of ascoma of *C. globosum* cultured at 25°C after 4 weeks, aiding in morphological identification. We supposed that considerable numbers of white flucosse mycelial colonies resembling *M. canis* might be misidentified as *M. canis*, and many cases of dermatitis diagnosed as *M. canis* infection in pet animals might involve *C. globosum* infection. In

addition, pseudo-positiveness in a wood-light reaction could also confuse the diagnosis between *M. canis* and *C. globosum* infection.

On the other hand, molecular biological identification of *C. globosum* on the basis of the D1/D2 domain of LSUrRNA was found to aid in rapid identification and orientation for treatments. The developments of rapid identification methods for PCR or other molecular biological techniques are eagerly anticipated. Antifungal susceptibility tests are also important for medical treatment. The present case was successfully treated with oral and topical applications of ketoconazole. The present isolate is susceptible to ketoconazole and shows equivalent susceptibilities to amphotericin B, itraconazole, and miconazole, as reported in human cases [18]. Itraconazole and miconazole might have been effective in the present case. In addition, other triazoles such as ravuconazole, voriconazole, and albaconazole effective for *C. globosum* infection [24] might be a further consideration when the drugs become commercially available in Japan.

Fungal species cause systemic infection depending on the host immune condition [17,18]. Therefore, hygiene control for owners holding infected animals with *C. globosum* seems to be important, especially for infants, the elderly and immunodeficient persons. The owners in the present case were advised to avoid intimate contact with the dog, to wash hands after touching, and to indefinitely keep the dog's environment very clean.

Recently, the number of emerging fungal infections has been increasing [25–27]. *C. globosum* infections in the veterinary field might not be an exception to this trend, and likely their incidence will increase in the near future, not only in cutaneous infections but also systemic ones such as in human cases.

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Short Communication

A new genotype of *Arthroderma benhamiae*

RUI KANO*, AYAKO SANO†, KOICHI MAKIMURA‡, SHINICHI WATANABE§, KAZUKO NISHIMURA†, HIDEO YAMAGUCHI‡ & ATUHIKO HASEGAWA*

*Department of Pathobiology, Nihon University School of Veterinary Medicine, Kanagawa, †Research center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, ‡Institute of Medical Mycology and Genome Research Center, Teikyo University, Hachioji, Tokyo, and §Department of Dermatology, Teikyo University School of Medicine, Tokyo, Japan

Nucleotide sequence analysis of chitin synthase 1 (*CHS1*) indicated 90% sequence similarities among human and animal isolates of *Arthroderma benhamiae*. In particular, greater than 99% similarity was noted in the nucleotide sequence among Americano-European race isolates, African race isolates, and five isolates from four-toed hedgehogs (*Atelerix albiventris*). The phylogenetic analysis indicated that the five isolates from hedgehogs were included in the same cluster and distinct from the Americano-European and the African race standard strains of *A. benhamiae*. These results were confirmed by ITS analysis. Therefore, it was proposed that genotypes could be established for the isolates of *A. benhamiae* in association with the mating behavior.

Keywords *Arthroderma benhamiae*, chitin synthase 1 gene, genotype

Introduction

Trichophyton mentagrophytes, which frequently causes human and animal dermatophytoses, has been proven to be a complex consisting of more than three teleomorphs, i.e., *Arthroderma benhamiae*, *A. simii* and *A. vanbreuseghemii* [1–3]. Hironaga and Watanabe reported in 1980 that none of the isolates of *T. mentagrophytes* recovered in Japan from human and animal dermatophytoses had ever been identified as *A. benhamiae* [4]. However, in 1998 we reported the first isolation of *A. benhamiae* in Japan from a case involving a rabbit [5]. More clinical isolates of *A. benhamiae* have since been recovered from humans, rabbits, guinea pig and hedgehogs in different areas of Japan. The isolates have been reported to divide on the basis of mating behavior into two races, i.e., the Americano-European race and the African race [3]. Strains of Americano-European race were frequently isolated from rabbits and guinea pigs, while African race strains were recovered from western European hedgehogs (*Erinaceus europaeus*) [6,7]. In recent years, the number of cases of dermatophytosis in Japan due to *A. benhamiae* in the four-toed hedgehog (*Atelerix albiventris*) has increased [6]. The hedgehogs are popular household pets and this particular fungus spreads easily to humans. Mating experiments have been conducted with clinical isolates from hedgehogs with the (+) and (−) standard strains of the Americano-European and African races of *A. benhamiae* (Table 1) [6]. The (+) and (−) isolates of the hedgehogs produced many more gymnothecia when crossed with themselves than with the standard strains of the indicated races, suggesting the establishment of the new race [6]. However, the hedgehog isolates have not been molecularly well characterized from an epidemologic perspective.

In order to understand phylogenetic relationships of *A. benhamiae*, we sequenced and analyzed partial DNA fragments of the chitin synthase 1 (*CHS1*) gene of several dermatophytes [8–10]. The results of these studies proved to be very useful in better understanding the evolution of the dermatophyte species, as well as in

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Correspondence: Rui Kano, Department of Pathobiology, Nihon University School of Veterinary Medicine, 1866, Kameino, Fujisawa Kanagawa, 252-8510, Japan. Tel: +81 466 84 3518; Fax: +81 466 84 3519. E-mail: kano@brs.nihon-u.ac.jp

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Table 1 Species and strains used in this study.

Species (mating type)	strain	origin
A. benhamiae (+)	VUT-77011 = RV26678	Americano-European race
A. benhamiae (−)	VUT-77012 = RV26680	Americano-European race
A. benhamiae (+)	RV 30000	African race
A. benhamiae (−)	RV 30001	African race
A. benhamiae (+)	VUT-97010	Rabbit (isolated at Hyogo)
A. benhamiae (−)	VUT-00001	Guinea pig (isolated at Hyogo)
A. benhamiae (−)	VUT-00002	Rabbit (isolated at Saitama)
A. benhamiae (−)	VUT-00003	Rabbit (isolated at Saitama)
A. benhamiae (−)	VUT-00003-2	Human (isolated at Saitama)
A. benhamiae (−)	KMU 4137	Rabbit (isolated at Tottori)
A. benhamiae (−)	KMU 4169	Human (isolated at Gifu)
A. benhamiae (−)	KMU 4170	Human (isolated at Gifu)
A. benhamiae (+)	IFM 50998	Hedgehog (isolated at Chiba)
A. benhamiae (−)	IFM 51499	Hedgehog (isolated at Chiba)
A. benhamiae (+)	IFM 51500	Hedgehog (isolated at Chiba)
A. benhamiae (+)	IFM 51502	Hedgehog (isolated at Ibaragi)
A. benhamiae (+)	IFM 51503	Hedgehog (isolated at Okayama)
A. simii (+)	VUT-77009	
A. simii (−)	VUT-77010	
A. vanbreuseghemii (+) VUT-77007		
A. vanbreuseghemii (−) VUT-77008		

KMU, Kanazawa Medical University; VUT, Veterinary Medicine, University of Tokyo, Japan; RV, Institute de Medecine Tropicale Prince Leopold, Antwerpen, Belgium; IFM, Research center for Pathogenic Fungi and Microbial Toxicoses, Chiba University.

their identification. In our previous investigation, the nucleotide sequences of *CHS1* from clinical isolates recovered in Japan from humans, rabbits and a guinea pig had proved to be genetically close to the Americano-European race [11]. These results indicated that only the Americano-European race of *A. benhaniae* was found in Japan.

In the present study, nucleotide sequences of the *CHS1* gene from clinical isolates of *A. benhamiae* recovered in Japan from hedgehogs (including four-toed ones), rabbits, a guinea pig and humans were compared with those from the standard strains belonging to both the Americano-European and African race of the fungus in order to better understand the molecular epidemiology of this dermatophyte.

Materials and methods

Strains

Strains studied are listed in Table 1 and were maintained by subculturing on 1/10 Sabouraud dextrose agar at the Department of Pathobiology, Nihon University School of Veterinary Medicine. Five strains of the *A. benhamiae* isolated from the four-toed hedgehogs were obtained from the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University [6]. The mating behavior of the hedgehog isolates was confirmed to be specific and different from that of the two races previously reported [6].

CHS1 and ITS (5.8 rRNA gene and ITS1 and ITS2) region sequence analysis

The dermatophyte isolates (Table 1) were cultured in Sabouraud liquid medium at 27°C for 5 days. The genomic DNA extraction from *A. benhamiae* isolates for *CHS1* sequence analysis was performed by the method previously described [11]. *CHS1* homology relationships and phylogenetic analysis of the isolates and standard strains of *A. benhamiae* were carried out as noted in a previous study [11].

The DNA sequences were compared by Clustal W multiple sequence alignment programs [12] and a phylogenetic tree was constructed by the TREEVIEW displaying phylogenies program [13]. Bootstrap analysis was performed on 1000 random samples and analyzed by the Clustal W programs [14].

To confirm *CHS1* sequence result, we also sequenced the 5.8 rRNA gene and ITS1 and ITS2 region of three clinical isolates (IFM 50998, IFM51499 and IFM 51501) from hedgehogs as described previously [6]. Phylogenetic analysis of the ITS region of the isolates and standard strains of dermatophytes were deposited in EMBL/GenBank as mentioned above.

Results and discussion

Amplification of dermatophyte DNAs with degenerate *CHS1* primers yielded fragments of about 443 bp, consistent with the sizes of fungal *CHS1* gene sequences reported previously [11]. Nucleotide sequence analysis of the *CHS1* gene fragments from the clinical isolates and standard strains of *A. benhamiae* (Americano-European race and African race), indicated that the sequence similarities were more than 90% among them (Fig. 1). Nucleotide sequence similarity

```
VUT-77011 Americano-European race TCGAGTACATGTGCTCGCGCACCAGCAGCAAGACATGGGGCAAAGAAGCCTGGAAGAAGA  60
RV 30000  African race            ************************************************************
IFM 50998 hedgehogs isolate       ************************************************************

VUT-77011 Americano-European race TTGTCGTTTGTATCGTCTCAGACGGTCGTGCAAAGATAAATCCACGTACGAGAGCTGTCC 120
RV 30000  African race            ***********C************************************************
IFM 50998 hedgehogs isolate       *****************T******************************************

VUT-77011 Americano-European race TTGCCGGTCTTGGTGTTTACCAGGACGGCATTGCCAAACAGCAGGTTAACGGCAAAGACG 180
RV 30000  African race            ***********A***********A**************G********T*****T******
IFM 50998 hedgehogs isolate       ***********C***********A**************G********C*****C******

VUT-77011 Americano-European race TCACTGCTCACATCTACGAATATACCACCCAGATAGGCATGGAGGTCAAGGGCACCCAGG 240
RV 30000  African race            *****************************************************C******
IFM 50998 hedgehogs isolate       *****************************************************A******

VUT-77011 Americano-European race TCATCCTCAAGCCGCGGCCGGGAATGCCGGTCCAGCTCCTCTTCTGTCTCAAAGAGAAGA 300
RV 30000  African race            *T**************A**A**G*************************************
IFM 50998 hedgehogs isolate       ****T*******************************************************

IFM 50998 hedgehogs isolate       ACCAGAAGAAGATCAACTCTCACAGATGGTTCTTCCAAGCCTTTGGTCGTGTCCTCGACC 360
RV 30000  African race            **************************C****************************T****
IFM 50998 hedgehogs isolate       **************************A*********************************

VUT-77011 Americano-European race CCAATATCTGTGTTCTCATCGACGCTGGAACAAAACCAGGCGGGCGAAGTATATACCAGC 420
RV 30000  African race            ***********************************A************************
IFM 50998 hedgehogs isolate       ***********************************G************************

VUT-77011 Americano-European race TCTGGCGTGCTTTTGACCTCGAG 443
RV 30000  African race            ***********************
IFM 50998 hedgehogs isolate       ***********************
```

Fig. 1 Alignment of *CHS1* gene fragments illustrating the sequence divergence among Americano-European race, African race and isolate from hedgehog. Asterisks symbolize identical nucleotides compared to the leader sequence (*A. benhamiae* strain VUT 77011).

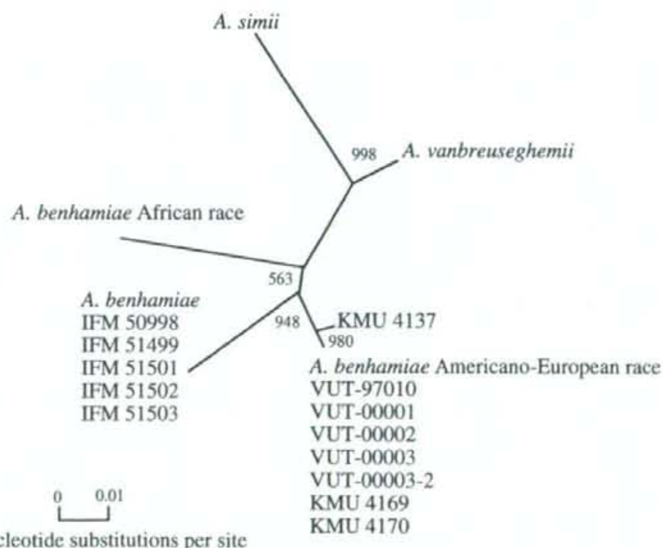

Fig. 2 A tree showing phylogenetic relationships of *CHS1* gene fragments of dermatophyte species. Numbers at branches were determined by the bootstrap analysis indicating the times in 1000 repeat sub samples in monophylogenic grouping.